

Comparison of ICP OES and LIBS Analysis of Medicinal Herbs Rich in Flavonoids from Eastern Europe

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Essential and toxic metals were determined in eighteen samples of medicinal herbs from Poland, Lithuania and Serbia by means of laser-induced breakdown spectroscopy (LIBS) and inductively coupled plasma optical emission spectrometry (ICP OES). Calcium, K and Mg concentrations were obtained in % (m/m) and other metals (Na, Co, Cu, Fe, Mn, Ni, Zn, Cd, Cr and Pb) in mg kg⁻¹. The fact that the herb samples analyzed belonged to specific plant species and represented different morphological plant parts explained the characteristic distribution in two-dimensional scores plots obtained by principal component analysis (PCA). A strong correlation of LIBS results was achieved in comparison to those obtained by ICP OES for Ca, K and Mg. Differences in the types of infusions were observed, in that leaves are related to Zn and Ni concentrations, leaves and flowers to Co, Ca and Mn concentrations and flowers to K, Na, Mg and Fe content.

Keywords: medicinal herbs, essential and toxic metals, LIBS, ICP OES, chemometrics

Introduction

Quantitative analysis of essential and toxic metals present in medicinal herbs is an important issue for researchers worldwide. Its significance is illustrated by the numerous studies to determine the levels of metallic and non-metallic elements, macro- and microelements, essential and toxic elements in plants used medicinally, which have employed a myriad of instrumental analytical techniques. The list includes several spectroscopic methods, for instance, atomic absorption spectrometry (AAS),^{1,2} and related techniques such as inductively coupled plasma optical emission spectrometry (ICP OES)^{3,4} and inductively coupled plasma-mass spectrometry (ICP-MS),⁵ as well as other analytical techniques, e.g., instrumental neutron activation analysis (INAA).^{6,7}

In recent years, laser-induced breakdown spectroscopy (LIBS)⁸ has found application in the analysis of elemental concentrations in samples of natural origin, including plant materials. The advantage of this technique is that LIBS does not involve sophisticated and time-consuming

sample preparation procedures and yet facilitates an enormous amount of spectral data with relatively high reproducibility.^{8,9} It was used recently in the analysis of Indian medicinal plants to study the concentrations of glycemic elements, namely C, Ca and Mg levels in a spectral range between 200 to 500 nm.9 The LIBS technique was also successfully applied in the elemental analysis of the Chinese medicinal herb Blumea balsamifera DC in order to detect similarities and variations in elemental composition based on C, Ca and Mg concentrations in samples originating from Hainan and Guizhou provinces.¹⁰ LIBS has been applied in the study of several other materials including polymers,¹¹ electronic waste,¹²⁻¹⁴ soils,¹⁵ cosmetics,16 and foods,17 all of which have confirmed its high utility in fast qualitative and quantitative analysis of diverse elements.18,19

Concentration levels of essential elements, both macroand microelements, in medicinal herbs can represent a different range of concentrations, and are dependent on many factors: the morphological part of the plant in question, origin from different botanical species and the impact of the environment in which plant was growing before harvest (soil, precipitate or dry season, year of

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harvest, among other aspects).²⁰⁻²² Recently, it has been discovered that differences in levels of the same metallic element can be attributed to plant species or botanical family,^{1,21} but also in several cases (e.g., Fe level) to origin within a specific region of Europe.²²

In order to interpret the huge databases of results obtained for determining elements in medicinal herbs or in other materials of natural origin, such as tea samples or honeys, multivariate statistical methods are very often required.²³⁻²⁷ Based on the experimental results obtained by high performance liquid chromatography (HPLC) technique with various detection systems, it was found that after the application of principal component analysis (PCA) and cluster analysis (CA), samples of medicinal plants representing three botanical species, *Equisetum*, *Polygonum* and *Viola*, were always grouped together. This observation indicates a similar chemical composition.²³

Therefore, the aim of this investigation was to explore the results after determining essential (Ca, Cu, Fe, K, Mg, Mn, Na and Zn) and toxic elements (Cd, Co, Cr, Pb and Ni) in medicinal herbs representing popular herbal remedies rich in flavonoids. These samples were harvested in Eastern Europe, and the particular focus was on comparing results obtained after using ICP OES (mineralization and infusion) with those obtained by the LIBS technique (directly solid sample analysis). In order to achieve this, several statistical methods were applied, such as factorial design for LIBS parameters optimization, PCA for exploratory analysis and partial least squares (PLS) for the multivariate regression model proposition.

Experimental

Samples and reagents

Eighteen samples of five herbal tea plant species from Lithuania (3 samples), Poland (10 samples) and Serbia (5 samples) were analyzed in this study (see details in Table 1). These samples represent 5 different species (*Sambucus nigra* L., *Hypericum perforatum* L., *Crataegus oxyacantha* auct. non L., *Rubus idaeus* L. and *Betula* species L.), with the plant parts analyzed being flower, leaves with flowers, fruits, leaves and herbs.

In the mineralization process for further ICP OES determination of the analytes, dried and milled samples were mixed with 65% v/v HNO₃ (Qhemis, Indaiatuba, SP, Brazil) and 30% v/v H_2O_2 (Synth, Diadema, SP, Brazil). The HNO₃ had been previously purified by sub-boiling distillation using DistillacidTM BSB-939-IR (Berghof, Eningen, Germany). Multi-element standard solutions for the calibration curve were prepared from stock standard

Table 1	. Herbal	tea	samples	description
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Sample	Plant species	Origin country	Plant parts
1	SAM	Serbia	flowers
2	HYP	Serbia	herbs
3	SAM	Serbia	flowers
4	SAM	Lithuania	flowers
5	HYP	Serbia	herbs
6	CRA	Lithuania	leaves and flowers
7	CRA	Poland	fruits
8	SAM	Serbia	flowers
9	RUB	Poland	fruits
10	HYP	Poland	herbs
11	CRA	Poland	flowers
12	HYP	Lithuania	herbs
13	SAM	Poland	flowers
14	BET	Poland	leaves
15	CRA	Poland	fruits
16	RUB	Poland	fruits
17	BET	Poland leaves	
18	HYP	Poland	herbs

Plant species: SAM: Sambucus nigra; HYP: Hypericum perforatum; CRA: Crataegus oxyacantha; RUB: Rubus idaeus; BET: Betula species.

solutions of 1000 mg L⁻¹ (Fluka Analytical, Switzerland) by subsequent dilutions with 2% v/v HNO₃. All volumetric flasks and glassware were washed, kept in 10% v/v HNO₃ for 24 h, rinsed with deionized water and dried before use. Deionized water (resistivity > 18.2 M Ω cm) was generated using a Milli-Q[®] Plus Total Water System (Millipore Corp., Bedford, MA, USA).

Sample preparation and mineralization

All herbal samples were dried beforehand in an oven at 60 $^{\circ}$ C up to constant mass, milled using a multi-processor and sieved in order to obtain particle size lower than 500 μ m.

A digester block with PFA closed vessels (perfluoroalkoxy, Savillex, MN, USA) was employed for sample mineralization. PFA vessels of 50 mL were used and samples digested in triplicate according to the following procedure: 200 mg of tea material was accurately weighed in PFA vessels and kept overnight (approximately 16 h) with 2.0 mL of 65% v/v HNO₃ at room temperature. One mL of 30% v/v H_2O_2 was added and the solution heated at 95 °C for 180 min. NIST 1515 (apple leaves), NIST 1573a (tomato leaves) and FO-01/2012 (*Brachiaria Brizantha* cv Marandu, EMBRAPA) reference materials were also digested for quality control of the measurements.

After digestion, the PFA tubes were cooled, the digests filtered through filter paper (Unifil, Germany) to 15 mL volumetric flasks and the volume made up to 10 mL with high purity deionized water. The final solutions were analyzed by ICP OES (iCAP 6000, Thermo Scientific, Waltham, MA, USA).

Infusions

Infusions were prepared as follows: 200 mg of the sample were weighed into glass beakers. To this was added 10 mL of boiled deionized water, after which they were covered by watch glasses to extract sample components. After a twenty-minute extraction period, the tea infusion was filtered to 15 mL volumetric flasks, acidified with one drop of concentrated HNO₃ (65% v/v) and the final volume adjusted to 10 mL with deionized water. Tea infusion solutions were also analyzed by ICP OES.

LIBS instrumentation

Experiments with LIBS were carried out with a Nd:YAG laser (Model J200, Applied Spectra, California, USA) which emits a laser pulse at 1064 nm (maximum energy of 100 mJ). The laser was operated by Axiom 2.5 software in a single laser pulse of 8 ns duration at a frequency of 10 Hz. This instrument is equipped with an ablation chamber with an HEPA air cleaner to remove ablated particles, as well as an automated XYZ stage and a 1280 \times 1024 CMOS color camera imaging system. The

emission of the laser-induced plasma was collected using an optical fiber bundle coupled with a 6-channel CCD spectrometer covering wavelengths from 186 to 1042 nm (12288 variables).

LIBS analysis

The herbal samples (particle sizes lower than 500 μ m) were weighed (approx. 400 mg) on analytical balances (model AY 220, Shimadzu, Kyoto, Japan) in order to facilitate LIBS analysis. The samples were pressed under 10 t inch⁻¹ to form pellets with 12 mm in diameter. Pellets samples numbered 8, 12 and 16 (see Table 1 for details) were prepared in duplicate for preliminary studies using LIBS (optimization of the system). These plants belonged to the species *Sambucus nigra* (sample 8), *Hypericum perforatum* (sample 12) and *Rubus idaeus* (sample 16).

Doehlert design

The additional instrumental parameters of the LIBS were evaluated through the use of Doehlert design.²⁸ The variables chosen were delay time (0-2.0 μ s, varied in 5 levels), laser energy (30-80 mJ, varied in 7 levels) and spot size (50-150 μ m, varied in 3 levels). Table 2 shows the Doehlert experimental conditions. Spectral lines for Ca, K and Mg were monitored for regression model proposition in order to establish the commitment working condition for all analytes. Gate width of the measurements was 1.05 ms.

Table 2. Variables evaluated for LIBS and its levels (coded and real scale) in the Doehlert design

	Dela	y time	En	ergy	Spot size		
Experiment	Coded	Real / µs	Coded	Real / mJ	Coded	Real / µm	
1	0	1	0	55	0	100	
2	0	1	0	55	0	100	
3	0	1	0	55	0	100	
4	1	2	0	55	0	100	
5	0.5	1.5	0.866	80	0	100	
6	0.5	1.5	0.289	63	0.817	150	
7	-1	0	0	55	0	100	
8	-0.5	0.5	-0.866	30	0	100	
9	-0.5	0.5	-0.289	47	-0.817	50	
10	0.5	1.5	-0.866	30	0	100	
11	0.5	1.5	-0.289	47	-0.817	50	
12	-0.5	0.5	0.866	80	0	100	
13	0	1	0.577	72	-0.817	50	
14	-0.5	0.5	0.289	63	0.817	150	
15	0	1	-0.577	38	0.817	150	

ICP OES analysis

The aim of this study was to quantify by ICP OES macronutrients (Ca, K and Mg), micronutrients (Co, Cu, Fe, Mn, and Zn) and toxic elements (Cd, Cr, Ni and Pb) present in herbal plants in the form of digests and infusions. Table 3 shows the instrumental parameters and wavelengths chosen for each analyte. The ICP OES instrument used allows axial and radial views to be monitored sequentially.

Table 3. Instrumental parameters for ICP OES determination	le 3. Instrumental parameters for	ICP OES determination
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Instrument	parameter	Operational conditions	
Integration	time / s	15 for low and 5 for	
		nigh emission lines	
Sample intr	oduction flow rate	$/ (mL min^{-1})$	2.1
Pump stabi	lization time / s		5
RF applied	power / kW		1.15
Auxiliary g	as flow rate / (L m	in-1)	0.50
Nebulizer g	gas flow rate / (L m	in ⁻¹)	0.70
Argon gas f	flow rate / (L min-1)	12
	Elements	s / nm	
Ca	317.933°	393.366 ^b	396.847 ^b
Cd	226.502°	228.802°	
Co	228.616°	238.892°	
Cr	357.869°		
Cu	324.754°	327.396°	
Fe	238.204°	239.562°	259.940°
Κ	691.107ª	766.490°	769.896°
Mg	279.553°	280.270°	285.213°
Mn	257.610°	259.373°	260.569°
Na	589.592 ^b		
Ni	231.604°	341.476°	
Pb	216.999ª	220.353°	
Zn	202.548 ^b	206.200°	213.856°

View modes used: "axial; bradial; cboth.

Results and Discussion

ICP OES determinations

Three certified reference materials were analyzed in order to verify the accuracy of the method proposed and help in the wavelength and view modes selection. When the certified and obtained values were compared, the relative errors (%) for the macroelements (Ca, K, Mg and Na) ranged from 3 in the case of Mg to 24% in the case of Na (with an average of 8%). An unpaired *t*-test was calculated and the average *t* calculated value was 2.22 (tabulated value with two degrees of freedom and 95% of confidence level = 4.303). In the case of microelements the relative errors calculated were: 12 (for Co), 3 (Cu), 32 (Fe), 12 (Mn) and 44% (Zn). For the toxic elements the relative errors were 12 (Cd), 40 (Cr), 25 (Ni) and 25% (Pb). As can be observed, the relative errors for the micro and toxic elements were higher than those observed in the macroelements. This observation can be explained due to low elements concentration levels observed for micro and toxic elements. Actually, the concentrations of the macroelements are, in average, 300 times higher than those presented by the others.

Table 4 shows the concentration of elements determined in the mineralized samples. Calcium, K and Mg concentrations were expressed in % (m/m) and the remainder in mg kg⁻¹. The level of elements determined in this study by ICP OES is comparable to the results obtained by the same technique in other studies of the elemental composition of medicinal herbs and their infusions.³⁻⁵ In the case of infusions only Ca, K and Mg were observed in all samples. For the other analytes, in several cases, the concentrations were below the limits of detection (LOD) or quantification (LOQ) values.

In order to obtain a global vision of all analytes and their relations, PCA was calculated separately using Pirouette 4.5 (Infometrix, Bothell, USA) for each type of data set (infusions and mineralized, Table 4) and the data set autoscaled. Figures 1a and 1b plot the scores (1a) and loadings (1b) for the infusions, but it proved difficult to observe a clear difference among the different types of samples and their characteristics (see Table 1 for further details). Some elements (see Figure 1b, loadings) are positively correlated (Co, Ni, Zn, Fe, Na, Pb and Cu).

However, several characteristic herbs can be depicted in Figure 1a. For example, two samples (samples 14 and 17) of *Betulae folium* originating in Poland are located in the upper left corner of plot PC1 vs. PC2. Two samples of *Crataegi fructus* are also seen in the left-hand side of the plot (samples 7 and 15), and a group of herbs, mainly of the species *Sambucus nigra* (samples 1, 3, 4 and 13) in the lower left.

Figures 1c and 1d show the scores and loadings for mineralization, respectively. Analysis of the scores plot (Figure 1c) indicates a difference among types of teas: leaves (open squares) are related to Zn and Ni concentrations, leaves with flower (gray triangle) to Co, Ca and Mn concentrations and flower (black squares) to K, Na, Mg and Fe content. In Figure 1c tendencies generally similar to Figure 1a can be discerned. Again, two samples (14 and 17) of *Betulae folium* are far removed from the remainder in the upper left area of the plot. On the other hand, it is possible to detect a group of herbs in the lower right from the species *Sambucus nigra* (samples 1, 3, 4 and 13). After combined interpretation of the results shown in Figures 1a and 1c, it can be concluded that the herb samples analyzed belonged to specific plant species and

Sample	Ca	К	Mg	Na	Cd	Со	Cr	Cu	Fe	Mn	Ni	Pb	Zn
1	0.62 ± 0.02	3.8 ± 0.4	0.42 ± 0.03	< LOD	< LOD	0.2 ± 0.1	LOD-LOQ	9 ± 1	115 ± 35	25 ± 2	LOD-LOQ	< LOD	LOD-LOQ
2	0.64 ± 0.04	1.4 ± 0.2	0.30 ± 0.02	37 ± 31	0.60 ± 0.06	0.22 ± 0.04	<lod< td=""><td>8.4 ± 0.4</td><td>78 ± 9</td><td>176 ± 3</td><td>4.3 ± 0.8</td><td><lod< td=""><td>36 ± 3</td></lod<></td></lod<>	8.4 ± 0.4	78 ± 9	176 ± 3	4.3 ± 0.8	<lod< td=""><td>36 ± 3</td></lod<>	36 ± 3
3	0.69 ± 0.05	0.3 ± 0.4	0.52 ± 0.05	LOD-LOQ	< LOD	0.2 ± 0.1	< LOD	11 ± 1	117 ± 10	29 ± 3	<lod< td=""><td><lod< td=""><td>LOD-LOQ</td></lod<></td></lod<>	<lod< td=""><td>LOD-LOQ</td></lod<>	LOD-LOQ
4	0.6 ± 0.1	3.1 ± 0.4	0.43 ± 0.04	58 ± 63	< LOD	0.3 ± 0.2	LOD-LOQ	11 ± 1	158 ± 17	48 ± 4	LOD-LOQ	<lod< td=""><td>LOD-LOQ</td></lod<>	LOD-LOQ
5	0.57 ± 0.02	1.3 ± 0.2	0.18 ± 0.01	LOD-LOQ	0.6 ± 0.1	0.20 ± 0.05	<lod< td=""><td>9.2 ± 0.3</td><td>67 ± 10</td><td>68 ± 2</td><td>2.1 ± 0.5</td><td><lod< td=""><td>37 ± 3</td></lod<></td></lod<>	9.2 ± 0.3	67 ± 10	68 ± 2	2.1 ± 0.5	<lod< td=""><td>37 ± 3</td></lod<>	37 ± 3
6	1.5 ± 0.1	1.7 ± 0.2	0.33 ± 0.03	64 ± 55	LOD-LOQ	0.7 ± 0.7	LOD-LOQ	10 ± 1	419 ± 287	34 ± 3	2 ± 1	<lod< td=""><td>LOD-LOQ</td></lod<>	LOD-LOQ
7	0.74 ± 0.03	1.2 ± 0.1	0.14 ± 0.01	LOD-LOQ	< LOD	0.11 ± 0.05	0.8 ± 0.9	LOD-LOQ	28 ± 5	LOD-LOQ	LOD-LOQ	<lod< td=""><td><lod< td=""></lod<></td></lod<>	<lod< td=""></lod<>
8	0.52 ± 0.05	3.2 ± 0.5	0.38 ± 0.04	< LOD	< LOD	0.2 ± 0.1	<lod< td=""><td>8.1 ± 0.4</td><td>104 ± 15</td><td>34 ± 4</td><td>9 ± 13</td><td><lod< td=""><td>LOD-LOQ</td></lod<></td></lod<>	8.1 ± 0.4	104 ± 15	34 ± 4	9 ± 13	<lod< td=""><td>LOD-LOQ</td></lod<>	LOD-LOQ
9	0.17 ± 0.02	1.9 ± 0.2	0.19 ± 0.01	LOD-LOQ	< LOD	0.13 ± 0.06	<lod< td=""><td>LOD-LOQ</td><td>68 ± 6</td><td>29 ± 1</td><td>LOD-LOQ</td><td><lod< td=""><td>LOD-LOQ</td></lod<></td></lod<>	LOD-LOQ	68 ± 6	29 ± 1	LOD-LOQ	<lod< td=""><td>LOD-LOQ</td></lod<>	LOD-LOQ
10	0.71 ± 0.04	1.8 ± 0.2	0.32 ± 0.03	< LOD	0.6 ± 0.1	0.3 ± 0.2	LOD-LOQ	8.1 ± 0.5	179 ± 24	34 ± 2	LOD-LOQ	<lod< td=""><td>28 ± 3</td></lod<>	28 ± 3
11	1.5 ± 0.1	1.5 ± 0.2	0.32 ± 0.03	81 ± 57	LOD-LOQ	0.3 ± 0.1	LOD-LOQ	9.0 ± 0.5	143 ± 8	31 ± 2	LOD-LOQ	< LOD	LOD-LOQ
12	0.74 ± 0.04	1.2 ± 0.1	0.23 ± 0.02	LOD-LOQ	LOD-LOQ	0.13 ± 0.02	<lod< td=""><td>7.0 ± 0.4</td><td>53 ± 5</td><td>112 ± 5</td><td>LOD-LOQ</td><td><lod< td=""><td>29 ± 3</td></lod<></td></lod<>	7.0 ± 0.4	53 ± 5	112 ± 5	LOD-LOQ	<lod< td=""><td>29 ± 3</td></lod<>	29 ± 3
13	0.54 ± 0.04	3.3 ± 0.5	0.41 ± 0.04	51 ± 64	< LOD	0.2 ± 0.1	LOD-LOQ	10 ± 1	91 ± 14	35 ± 3	<lod< td=""><td><lod< td=""><td>LOD-LOQ</td></lod<></td></lod<>	<lod< td=""><td>LOD-LOQ</td></lod<>	LOD-LOQ
14	0.91 ± 0.05	1.0 ± 0.1	0.29 ± 0.02	LOD-LOQ	0.40 ± 0.06	0.35 ± 0.04	LOD-LOQ	LOD-LOQ	127 ± 19	932 ± 45	4.0 ± 0.7	<lod< td=""><td>86 ± 6</td></lod<>	86 ± 6
15	0.82 ± 0.04	1.3 ± 0.1	0.16 ± 0.01	99 ± 34	< LOD	0.11 ± 0.04	<lod< td=""><td>14 ± 2</td><td>30 ± 5</td><td>11 ± 2</td><td>LOD-LOQ</td><td><lod< td=""><td>LOD-LOQ</td></lod<></td></lod<>	14 ± 2	30 ± 5	11 ± 2	LOD-LOQ	<lod< td=""><td>LOD-LOQ</td></lod<>	LOD-LOQ
16	0.18 ± 0.02	1.4 ± 0.2	0.18 ± 0.01	LOD-LOQ	LOD-LOQ	0.13 ± 0.05	<lod< td=""><td>7 ± 1</td><td>40 ± 4</td><td>66 ± 3</td><td>4 ± 1</td><td><lod< td=""><td>LOD-LOQ</td></lod<></td></lod<>	7 ± 1	40 ± 4	66 ± 3	4 ± 1	<lod< td=""><td>LOD-LOQ</td></lod<>	LOD-LOQ
17	0.71 ± 0.05	0.9 ± 0.1	0.27 ± 0.02	< LOD	0.30 ± 0.05	0.2 ± 0.1	< LOD	LOD-LOQ	55 ± 4	930 ± 33	2.0 ± 0.4	<lod< td=""><td>93 ± 5</td></lod<>	93 ± 5
18	0.7 ± 0.1	2.0 ± 0.3	0.24 ± 0.02	< LOD	1.3 ± 0.1	0.14 ± 0.03	< LOD	13 ± 1	58 ± 6	132 ± 8	2.4 ± 0.5	< LOD	35 ± 3
LOD ^a	15	105	2	10	0.1	0.03	0.2	2.0	4.0	3.0	0.5	1.0	8.0
LOQ ^a	49	351	7	32	0.2	0.10	0.7	6.4	13	9.0	1.7	3.1	26

Table 4. Elements concentration (Ca, K and Mg in % m/m, the other elements in mg kg⁻¹) for the mineralization (n = 3)

aICP OES: LODs and LOQs are in mg kg-1. LOD: limit if detection; LOQ: limit of quantification.

represented different morphological plant parts, with these two factors causing their characteristic distribution in the two-dimensional plots obtained by PCA. This conclusion confirms earlier studies of elemental concentrations in medicinal herbs obtained using the AAS technique.^{1,20-23}

LIBS analysis

LIBS equipment parameters need to be optimized in order to obtain spectral signals with a high signal-tobackground ratio (SBR). In this context, three parameters were evaluated using a Doehlert design (see Table 2) and the following atomic (I) emission lines were monitored (SBR, area and height): Ca I 527.027 nm, K I 693.878 and Mg I 517.268 nm. These lines were monitored in samples 8, 12 and 16 (representative samples, see Table 1).

For the experiments presented in Table 2, regression models were calculated for samples 8, 12 and 16 (1 model for each sample). In these models desirability function was calculated to cover SBR, signal area and height for Ca, K and Mg individually. Using this approach, each response was converted into numbers from 0 (undesired response, low SBR, area and height) to 1 (desired response, high SBR, area and height). After this, the individual desirability was combined into one single response (D, global desirability).²⁹ Three models were calculated for global desirability (one for each sample type) and 10 coefficients were obtained: constant (b0), linear coefficients (b1, b2 and b3), quadratic coefficients (b11, b22 and b33) and interaction coefficients

(b12, b13 and b23). The significance of these coefficients was calculated using analysis of variance (ANOVA) table with 95% confidence level.

Figure 2 shows contour plots for sample 8 (Figure 2a) and 16 (Figure 2b). For sample 12 was not possible to obtain a significant model. A compromise condition for all types of samples was established with a delay time of 1.0 μ s and energy of 70 mJ (see circle in Figures 2a and 2b). Spot size was not relevant to the range studied and was fixed at 100 μ m. With this working condition, the predicted D values for samples 8 and 16 were 0.4 and 0.1, respectively. After testing commitment condition, obtained D values were 0.5 and 0.1 for samples 8 and 16, respectively, confirming a high level of concordance between both values.

This condition was tested for all 18 samples and the subsequent typical emission spectra are shown in Figure 3. Figure 3a (sample 6, leaves and flowers) shows strong emission lines for C, Ca, K, Mg, N and O (the symbols I and II represent atomic and emission lines, respectively). The same tendency was observed for the other samples: Figure 3b (sample 14, leaves), Figure 3c (sample 2, herbs), Figure 3d (sample 15, fruits) and Figure 3e (sample 13, flowers).

Calibration models (multivariate and univariate)

For Ca, Mg and K calibration models were calculated and two strategies tested: (*i*) multivariate using the whole signal profile (186 to 1042 nm, 12288 variables) and



Figure 1. Scores and loadings plots for the datasets obtained for (a and b) infusion and (c and d) mineralization.



Figure 2. Contour plots obtained for the models after LIBS parameters optimization for samples (a) 8 and (b) 16. The circle represents the optimum condition.

(*ii*) univariate. In this instance, the goal was to propose an expeditious method for direct determination of these three macronutrients in solid samples.

In the case of univariate calibration, the signals depicted in Figure 4 were used. In both strategies (multivariate or univariate), 12 normalization signal modes were employed after obtained around 400 spectra for each sample: signal average, signal normalized by individual norm, area and maximum, signal sum, signal sum after normalization by individual norm, area and maximum and signal average



Figure 3. Emission spectra for samples (a) 6; (b) 14; (c) 2; (d) 15 and (e) 13.

and sum after normalization by C signals (I 193.090 and I 247.856 nm). 30

For Ca and Mg the best results (lower prediction error) were obtained with signal normalized by maximum and by norm, respectively. In order to evaluate the quality of the proposed models standard error of cross-validation (SECV), standard error of calibration (SEC) and standard error of validation (SEV) values were used for multivariate calibration (PLS).³¹ In the case of univariate models, the evaluation was conducted using SEC, SEV and LOD and LOQ. In both cases the data set was subdivided into calibration and validation sets. The models were then

calculated using the calibration data set (14 samples) and tested in 4 samples (validation data set).

For LOD and LOQ calculation using univariate calibration, the standard deviation was obtained using signal noise in the surrounding wavelengths near Ca, K and Mg signals (see Figure 4).³² Table 5 shows the parameters



Figure 4. Emission lines selected for (a) Ca; (b) K and (c) Mg to calculate the univariate regression models.

for the models and Figure 5 reference (obtained by ICP OES) and predicted concentrations for Ca (Figure 5a), K (Figure 5b) and Mg (Figure 5c). The univariate models



Figure 5. Reference *versus* predicted concentration for (a) Ca; (b) K and (c) Mg. Calibration and validation samples are represented by squares and circles, respectively.

	Parameters		Analyte	
		Ca	K	Mg
	normalization mode	normalized by maximum	sum	normalized by norm
	SECV / %	0.3	0.6	0.1
PLS models (whole signal	SEC / %	0.3	0.5	0.1
profile)	SEV / %	0.2	0.6	0.2
	latent variable	2	1	2
	accumulated / %	65	44	68
	spectral line / nm	527.027	693.878	517.268
	signal type	area	height	height
	SEC / %	0.2	0.4	0.04
Univariate models (see selected	SEV / %	0.1	0.4	0.2
emission lines in Figure 4)	linear model $(y = ax + b, r)$	$[Ca] = 8.21.10^{-2}x + 0.029$ r = 0.8898	$[K] = 5.39.10^4 x - 3.24.10^4 r = 0.9394$	[Mg] = 19.9x + 1.17 r = 0.9319
	LOD / %	0.1	0.4	0.07
	LOQ / %	0.5	1	0.2

Table 5. Regression models (multivariate and univariate) parameters for the models calculated for Ca, K and Mg

PLS: partial least squares; SECV: standard error of cross-validation; SEC: standard error of calibration; SEV: standard error of validation; LOD: limit of detection; LOQ: limit of quantification.

using signal area (Ca) and height (K and Mg) presented error values lower than the multivariate calibration models.

For Ca, the best results were obtained after data set normalization by the maximum of each spectrum. SEC values for PLS and univariate models (signal area) were 0.3 and 0.2%, respectively, and the LOD obtained was 0.1%. Reference and predicted concentration for univariate models (Figure 5a) showed a strong correlation at r = 0.8898. In the case of K, the best results were obtained after the sum of the signals. Error values ranged from 0.4 (SEC for univariate using signal height) to 0.6% (SEV using whole signal profile). LOD value was 0.4%. Magnesium, while presenting the highest relative intensity among the analytes studied, simultaneously presented the lowest LOD values (0.07%) and the best normalization model was normalization by individual norm. The correlation between the reference and predicted values was 0.9319 for univariate model (see Figure 5c).

Conclusions

The results obtained confirm that chemometric methods could be introduced to effectively extract spectral data, samples classification (PCA) and to solve multivariate problems like parameter optimization and multivariate simultaneous analysis (PLS). With the benefit of applying LIBS for the monitoring of multiple elements in medicinal herbs, PLS was combined with LIBS as a multivariate calibration method and a set of univariate models developed (Table 5). These models successfully predict high concentrations of Ca, Mg and K with high correlation coefficient and low SEC and LOD for univariate models.

It can be also inferred that the combination of LIBS, ICP OES and chemometric tools has significant potential for the development and implementation of methods towards exploring concentrations of essential and toxic elements in medicinal herbs.

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