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Detection of the origin of Brazilian wines based on the determination of only four elements using high-resolution continuum source flame AAS

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ABSTRACT

A method has been developed to determine 10 elements in Brazilian red wines using high-resolution continuum source flame atomic absorption spectrometry, a technique that allows the fast sequential determination of an essentially unlimited number of elements per sample, each one under previously optimized conditions. All measurements were made without sample preparation, using aqueous standard solutions for calibration. The results were in agreement within 99% of confidence (*t*-test) with those obtained by inductively coupled plasma optical emission spectrometry. The same grape, *Cabernet sauvignon*, was used in all experiments, and the wines from each region were prepared especially for this investigation in order to avoid any confusion due to grapes from other regions, which are often used in commercial wines. The elements K, Mn, Rb and Sr were found to be the best indicators for the origin of the wines, based on a Principal Component Analysis.

1. Introduction

Wine production and consumption have increased considerably over the last decade in Brazil [1]. To maintain the quality of wine, some properties have to be monitored regularly, such as pH, sugars, organic and inorganic compounds [2]. In this context, the determination of metals present in the wine is crucial; some elements are essential for our organism and wine is known as a good source for them [3]. The mineral composition of wine is based in the grape quality, which is directly related with the soil, the climatic conditions and the production procedures [4]. The organoleptic properties are also significantly influenced by the presence of some elements [5]; the most important ones are Be, Ca, Co, Cu, K, Li, Mn, Na, Rb and Sr [6–9].

Wine is known to have a complex matrix and many studies in the literature report the determination of metals in wine [5]. Atomic and mass spectrometric techniques, such as flame atomic absorption spectrometry (F AAS), graphite furnace atomic absorption spectrometry (GF AAS), inductively coupled plasma optical emission spectrometry (ICP OES) and inductively coupled plasma mass spectrometry (ICP-MS) are most frequently used for the wine analysis. The principal advantages of the AAS techniques are the high sensitivity in the case of GF AAS and simplicity and low cost in the case of F AAS. The disadvantage of AAS techniques is that they are typically designed for the determination of just one element at-a-time [3–5,10]. The plasma techniques have the advantages of high sensitivity and multi-element determination, with the disadvantage that compromised conditions have to be used, which are not optimum for each element, and the higher cost, mostly in the case of ICP-MS [3–5,11].

Santos et al. [12] evaluated different pre-treatment procedures of wine for the determination of iron and manganese by F AAS because of the relatively high concentration of these elements. They found no significant difference between acid digestion and

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Abbreviations: SGa, Serra Gaúcha; VVi, Vale dos Vinhedos; PBa, Pinto Bandeira; CCS, Campos de Cima da Serra; CGa, Campanha Gaúcha; SCa, Serra Catarinense; OPR, Oeste do Paraná; VSF, Vale do São Francisco; AAS, Atomic Absorption Spectrometry; CCD, Charge Coupled Device; F AAS, Flame Atomic Absorption Spectrometry; GF AAS, Graphite Furnace Atomic Absorption Spectrometry; HCA, Hierarchical Cluster Analysis; HR-CS F AAS, High-Resolution Continuum Source Flame Atomic Absorption Spectrometry; ICP-MS, Inductively Coupled Plasma Mass Spectrometry; ICP OES, Inductively Coupled Plasma Optical Emission Spectrometry; LOD, Limit of Detection; LOQ, Limit of Quantification; PCA, Principal Component Analysis; PC, Principal Component; RSD, Relative Standard Deviation.

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photo-oxidation of the wine samples. Sauvage et al. [13] used only the dilution of the wine samples to determine copper, iron, potassium, sodium, magnesium and calcium.

Moreno et al. [14] determined nickel and lead in commercial wine samples from Spain by GF AAS using dry ashing, followed by the addition of a 5% nitric acid solution. The results were used to classify Spanish wines according to their denomination of origin (DO). The same authors also determined Al, Ba, Cu, Fe, Mn, Sr, Zn, Ca, K, Na and Mg by ICP OES for the same purpose and using the same conditions. Dessuy et al. [15] developed a method to determine lead in wine via direct analysis using GF AAS, comparing different atomizers; the transversally heated filter atomizer showed better sensitivity and lower background absorption compared to a conventional transversely heated platform atomizer.

Bentlin et al. [16] evaluated wine samples from South America (Argentina, Brazil, Chile and Uruguay) by ICP OES and ICP-MS. They determined 45 elements after nitric acid digestion, and the results were used to classify each sample according to its country of origin using the Principal Component Analysis (PCA) and the Hierarchical Cluster Analysis (HCA). Šperková and Suchánek [17] determined 27 elements in Bohemian wines (Czech Republic) by ICP OES and ICP-MS. The samples were subjected to nitric acid digestion, after ethanol was eliminated by heating in open vessels.

High-resolution continuum source atomic absorption spectrometry (HR-CS AAS), which was introduced commercially less than a decade ago [18], appears to be an alternative technique for the determination of trace and minor elements in wine samples. The xenon short-arc lamp, used as a continuum source, provides the possibility to determine almost all known elements in a fast sequential manner when F AAS is used. The possibility of using the principal and secondary lines without loss in signal-to-noise ratio (SNR), the choice of the line wings to enhance sensitivity and/or to extend the linear working range are among the most important advantages brought about by this technique [10,18].

Some analytical techniques, such as ICP OES and ICP-MS provide a great number of results; however, the extraction of information might be difficult and time-consuming, even when using statistical tools, such as pattern recognition [19]. In wine samples, these tools can be used to determine their geographical origin, to verify frauds or to control the quality of the wine produced [20]. The most common pattern recognition techniques used in these cases are PCA and HCA [21].

The principal objective of this work was to develop a precise and accurate method for the determination of Be, Ca, Co, Cu, K, Li, Mn, Na, Rb and Sr in Brazilian red wine samples by HR-CS F AAS. With the results obtained by the developed method, techniques for pattern recognition were applied to verify some group formation. No commercially available wines were used in this study in order to avoid any confusion due to grapes from other regions, which are quite often added in this type of wines. Instead, only one kind of grape (*Cabernet sauvignon*) was used and the wine produced in small quantity directly at the winery under strict control, but following the regular winemaking procedure.

2. Materials and methods

2.1. Instrumentation

A Model contrAA 700 high-resolution continuum source atomic absorption spectrometer (Analytik Jena, Jena, Germany), with a graphite furnace and a flame atomizer in two separate sample compartments, was used for most of the experiments. The spectrometer is equipped with a xenon short-arc lamp operating in a hot-spot mode, a prism pre-monochromator, an echelle grating monochromator for high resolution, and a charge-coupled device (CCD) array detector; the resolution is about 1.5 pm per pixel at 200 nm. An air-acetylene flame was used for the determination of Co, Cu, K, Li, Mn, Na and Rb, and a nitrous oxide-acetylene flame for Be, Ca and Sr under optimized conditions as specified in Table 1. High-purity acetylene and high-purity nitrous oxide (both 99.0% v/v, White Martins, Brazil) were used as fuel and oxidant gas, respectively. The aspiration rate used was 8 mL min^{-1}, and all measurements were carried out in triplicates. A Model SFS 6 injection valve (Analytik Jena) was used to reduce the sample consumption and to maintain the stability of the flame, as no air is aspirated when the standard or the sample solution is changed.

A Model MPX-CCD inductively coupled plasma optical emission spectrometer (Varian, Mulgrave, Australia) with radial configuration was used for the ICP OES measurements. Two different nebulization chambers were used in this equipment according to the concentration of the element to be determined. An ultrasonic nebulizer model U-5000 AT^{+} (CETAC Technologies, Omaha, NE) was used for the microelements Be, Co, Cu, Li, Mn, Rb and Sr, and a Sturman-Masters nebulization chamber (Varian) was applied for the macroelements Ca, K and Na. The operating conditions of the ICP are summarized in Table 2.

A microwave oven Model Microwave 3000 (Anton Paar, Graz, Austria), equipped with an eight-vessel capacity rotor and microwave-operated UV lamps was used for pre-treatment of the wine samples to evaluate if there is any need for sample digestion.

Table 2
Operational conditions for metals determination in wine samples by ICP OES.

Power (W)	1300
Auxiliary gas flow-rate(L min^{-1})	2.25
Plasma gas flow-rate (L min^{-1})	15
Measurement height (mm)	15
Pump rotation (rpm)	35
Nebulizer pressure (kPa)	240

Table 1
Instrumental parameters for HR-CS F AAS measurements.

Element	Wavelength (nm)	Relative sensitivity (%)[a]	Flame	C_2H_2 flow-rate (L h^{-1})	Burner height (mm)	Pixels evaluated
Be	234.861	100	C_2H_2–N_2O	240	6	3
Ca	239.856	1.1	C_2H_2–N_2O	210	6	3
Sr	460.733	100	C_2H_2–N_2O	210	6	1
Co	240.725	100	C_2H_2–Air	80	7	3
Cu	324.754	100	C_2H_2–Air	40	5	3
K	404.720	0.24	C_2H_2–Air	50	5	1
Li	670.785	100	C_2H_2–Air	45	4	3
Mn	279.827	67	C_2H_2–Air	60	6	3
Na	330.237	0.48	C_2H_2–Air	50	6	3
Rb	780.027	100	C_2H_2–Air	45	5	1

[a] Compared to the main resonance line for this element.

2.2. Reagents and solutions

High-purity de-ionized water with a resistivity of 18 MΩ cm from a Milli-Q system (Millipore, Bedford, MA) was used throughout. All plastic bottles and glassware were cleaned by soaking them in a 10% (v/v) HNO_3 solution for at least 24 h and rinsed abundantly with ultrapure water before use.

The standard solutions were prepared daily by appropriate dilution of the stock standard solutions of 1000 mg L^{-1} (Specsol, Brazil) for Be, Co, Cu, Li, Mn, Rb and Sr and of 10,000 mg L^{-1} for Ca, K and Na, which were prepared from CaCl, KCl and NaCl salts (Merck, Darmstadt, Germany). Hydrochloric acid, purified in a quartz sub-boiling still (Kürner Analysentechnik, Rosenheim, Germany), 10 g L^{-1} Cs (Sigma-Aldrich, USA) and 10% (v/v) ethanol (Merck) were added to all standards and blank solutions to stabilize the solutions, in order to control ionization interferences and to simulate the wine matrix, respectively.

2.3. Samples and sample preparation

The wine samples were obtained from eight different regions of Brazil. All samples were produced specially for this work, using *C. sauvignon* grapes, harvested in 2009. This provides more relevance to this study, since there is a guarantee regarding the origin of the samples. This, however, cannot be assured when evaluating samples of commercial wines. A total of 32 wine samples from eight regions of the south and the northeast of Brazil, as specified in Table 3, were evaluated. The location of the seven wine-growing areas in the south of Brazil is shown in Fig. 1; it should be noted that the distance between area 1 and area 7 is more than 1000 km. The eighth location is in the northeast of Brazil, more than 3000 km from Rio Grande do Sul, and is not shown in Fig. 1. All wine samples were stored in a refrigerator to preserve their properties. For direct analysis, 10 mL of wine were transferred to 15-mL volumetric flasks; appropriate volumes of HCl and Cs solutions were added to result in a final concentration of 1% (v/v) each of HCl and Cs, and the volume was completed with ultrapure water. All samples were prepared in triplicate.

For comparison the samples were also subjected to a microwave- and UV-assisted digestion. A closed-vessel system was used in the digestion procedure, with the addition of 5.0 mL of sample, 2.0 mL of 30% H_2O_2, 1.0 mL of 65% HNO_3 and 3.0 mL of ultrapure water. The UV lamps were inserted in the quartz vessels and the following optimized program was used: step 1—power of 900 W, ramp time of 10 min and ventilation of 127 $m^3 h^{-1}$; step 2—power of 900 W, hold of 20 min and ventilation of 127 $m^3 h^{-1}$; and step 3—power of 0 W and ventilation of 190 $m^3 h^{-1}$ (cooling). After digesting and cooling, the resulting digests were transferred to 15-mL volumetric flasks, 300 μL of a 500 g L^{-1} Cs solution was added, and the volume completed with ultrapure water.

Table 3
Acronyms and origin of the Brazilian wines investigated in this study; the numbers refer to the location of the wine-growing areas in Fig. 1.

No.	Acronym	Origin	State	No. of samples
1	CGa	Campanha Gaúcha	Rio Grande do Sul	6
2	VVi	Vale dos Vinhedos	Rio Grande do Sul	4
3	PBa	Pinto Bandeira	Rio Grande do Sul	4
4	SGa	Serra Gaúcha	Rio Grande do Sul	6
5	CCS	Campos de Cima da Serra	Rio Grande do Sul	4
6	SCa	Serra Catarinense	Santa Catarina	4
7	OPR	Oeste do Paraná	Paraná	2
8	VSF	Vale do São Francisco	Bahia and Pernambuco	2

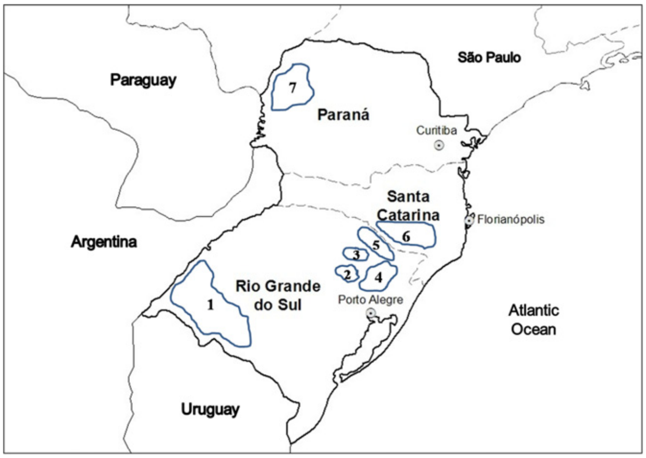

Fig. 1. Map of the south of Brazil, where seven of the eight wine growing areas are located; for the identification of the areas see Table 3.

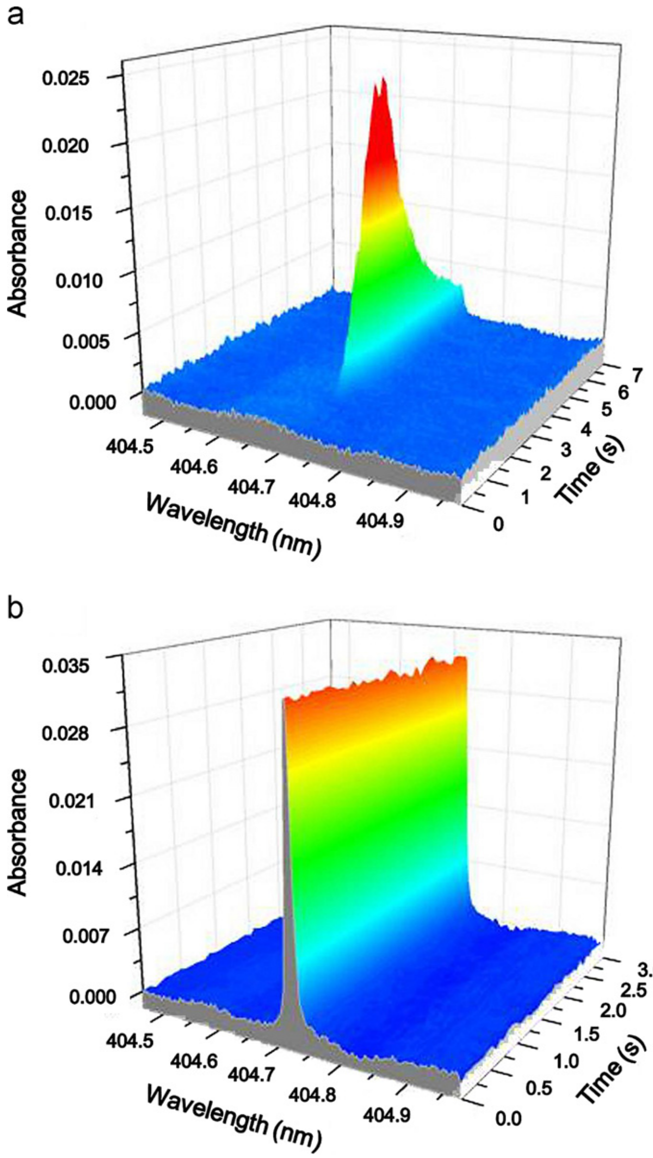

Fig. 2. Analytical signals for K in a wine sample using HR-CS FAAS; (a) flow injection and (b) continuous injection.

2.4. Analytical procedure

The determination of the 10 elements was carried out according to the sequence: (i) air-acetylene flame for Cu, Li, Rb, Na, K, Mn and Co, and (ii) nitrous oxide-acetylene flame for Ca, Sr and Be. This sequence was optimized according to the acetylene flow-rate; the flame conditions and the burner height were optimized automatically by the software using a wine sample, taking into account the maximum absorbance as criterion. The optimized conditions are summarized in Table 1. The sequence was repeated three times for each sample to obtain an average and standard deviation for each element and sample. In order to perform all determinations using the same sample solution without further dilution, the measurements were carried out at the most sensitive lines for Be, Co, Cu, Li, Rb and Sr, and at secondary, less sensitive lines for Ca, K, Mn and Na. In addition, the number of pixels used for each element was optimized as well and fixed at 3 pixels for Be, Ca, Co, Cu, Li, Mn and Na and 1 pixel for K, Rb and Sr.

Finally, the results were compared with those obtained by ICP OES as an independent technique. The ICP OES operational conditions (Table 2) were optimized by a mathematical algorithm, the modified Simplex method. This algorithm works with the equipment parameters searching for the maximum or the minimum response. Aliquots of 1000 mg L^{-1} yttrium (Specsol, Brazil) were used as an internal standard and added to all sample and standard solutions.

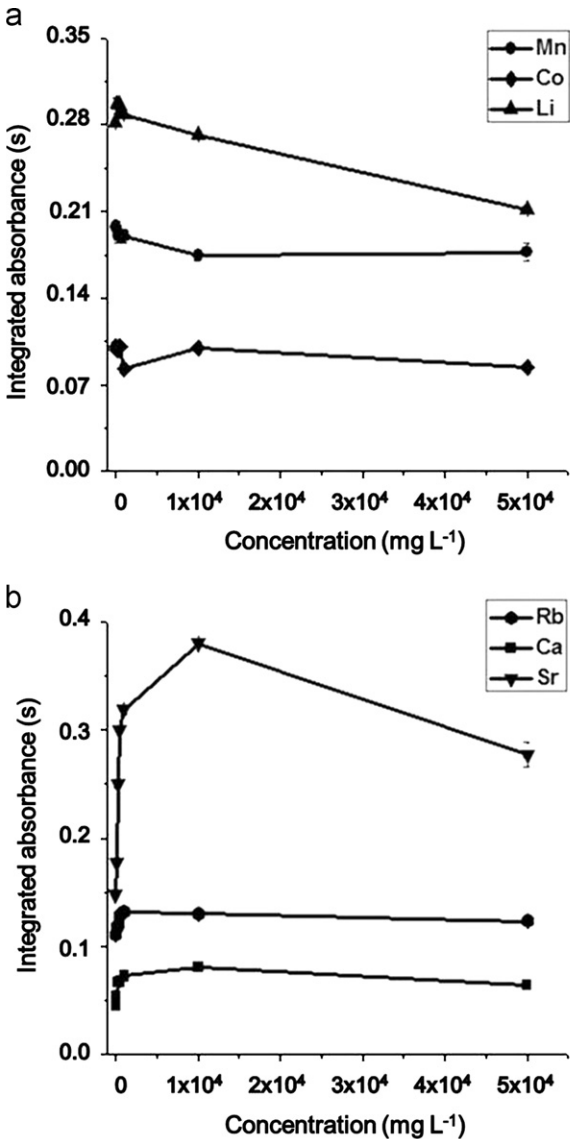

Fig. 3. Influence of the concentration of Cs added to a sample enriched with 1 mg L^{-1} of Co, Li, Mn, Rb and Sr and 10 mg L^{-1} of Ca. (a) Elements not significantly affected by the concentration of Cs and (b) elements affected by the concentration of Cs added.

3. Results and discussion

3.1. Optimization of the HR-CS F AAS method

The HR-CS F AAS technique makes possible that in one run, major and minor elements can be determined sequentially using a single sample dilution. The possibility of sequential multi-element determination by atomic absorption spectrometry came as an alternative because of the use of a single lamp for all elements and the versatility of the software to operate the experimental conditions. All elements are determined at optimized conditions, automatically adjusted by the software of the equipment or manually by the operator. Another advantage of HR-CS F AAS is the possibility to use secondary lines without deterioration of the SNR. In addition, the number of pixels can be adjusted to increase or decrease the sensitivity. These advantages permit samples with significantly different analyte concentrations to be evaluated without further dilution.

The flow injection valve SFS 6 is a special feature in this work. This accessory controls the solution injection, changing from sample to water and back, avoiding variations in the flame temperature, as no air

Table 4
Linear calibration equations and figures of merit for the determination of 10 elements in wine using HR-CS F AAS.

Element	$A_{int}=b+ac$ (mg L^{-1})	R	c_0 (mg L^{-1})	LOD (mg L^{-1})	LOQ (mg L^{-1})
Cu	$A_{int}=0.0070+0.1825c$	0.9995	0.02	0.04	0.15
Li	$A_{int}=0.0053+0.7907c$	0.9997	0.006	0.005	0.02
Rb	$A_{int}=0.0074+0.1492c$	0.9997	0.03	0.02	0.06
Na	$A_{int}=0.0050+0.0087c$	0.9997	0.51	0.9	3.0
K	$A_{int}=0.0052+0.0006c$	0.9997	7.3	4.4	15
Mn	$A_{int}=0.0162+0.1622c$	0.9995	0.02	0.04	0.14
Co	$A_{int}=0.0050+0.1070c$	0.9996	0.04	0.06	0.20
Ca	$A_{int}=0.0077+0.0074c$	0.9995	0.55	0.9	3.1
Sr	$A_{int}=0.0098+0.1155c$	0.9994	0.04	0.02	0.05
Be	$A_{int}=0.0033+1.089c$	0.9995	0.005	0.02	0.08

Table 5
Recovery of the four key elements added to three different wine samples.

Wine sample	Rb (%)	K (%)	Mn (%)	Sr (%)
Pinto Bandeira 2	103	103	104	96
Campanha Gaúcha 5	91	91	106	98
Pinto Bandeira 3	95	107	103	91

Table 6
Determination of metals in a wine sample from an interlaboratorial comparison by HR-CS F AAS; all values in mg L^{-1}.

	Cu	Li	Rb	Na	K	Mn	Co	Ca	Sr	Be
Obtained values	<LOQ	0.08	0.90	18	968	1.0	<LOQ	63	1.2	<LOQ
Reference values	0.13	ND[a]	ND	18	907	ND	ND	68	ND	ND

[a] ND: not determined (elements that were not determined in the interlaboratorial comparison).

Table 7
Results obtained for calcium, copper and sodium in Brazilian wine samples from the different regions by HR-CS F AAS and by ICP OES; all values in mg L^{-1}. All samples were prepared in triplicate and three measurements were taken for each sample; hence, all values are the average of $n=9$ measurements ±SD.

Region[a]	Ca		Cu		Na	
	F AAS	ICP OES	F AAS	ICP OES	F AAS	ICP OES
CGa 1	59±1	53±2	<LOQ	0.09±0.02	35±0.5	34±2
CGa 2	74±1	70±2	<LOQ	0.15±0.02	7.9±0.3	7.7±0.5
CGa 3	61±1	58±2	<LOQ	0.02±0.01	4.2±0.2	4.2±0.2
CGa 4	84±1	87±4	0.20±0.01	0.21±0.01	23±0.4	25±1
CGa 5	51±1	47±2	<LOQ	0.12±0.01	46±0.7	43±2
CGa 6	83±1	81±2	<LOQ	<LOQ	20±0.5	21±1
CCS 1	58±1	58±1	<LOQ	0.12±0.01	44±0.5	46±2
CCS 2	121±2	119±2	1.4±0.01	1.2±0.1	14±0.1	14±1
CCS 3	47±1	43±2	<LOQ	0.04±0.01	57±0.7	56±2
CCS 4	86±1	79±5	1.5±0.03	1.2±0.2	20±0.3	20±1
OPr 1	90±2	95±3	<LOQ	<LOQ	26±0.4	25±1
OPr 2	62±1	63±2	<LOQ	0.08±0.01	36±0.5	34±2
PBa 1	72±1	70±2	<LOQ	<LOQ	27±0.3	26±2
PBa 2	65±1	64±2	<LOQ	<LOQ	5.3±0.1	4.5±0.3
PBa 3	64±1	61±2	1.3±0.02	0.97±0.01	8.0±0.2	7.4±0.5
PBa 4	83±1	80±2	<LOQ	<LOQ	27±0.3	26±1
SCa 1	46±1	44±1	<LOQ	<LOQ	20±0.2	21±1
SCa 2	57±1	53±2	<LOQ	0.16±0.01	33±0.3	35±1
SCa 3	73±1	71±1	<LOQ	<LOQ	49±0.5	51±2
SCa 4	44±1	44±1	<LOQ	0.07±0.01	28±0.4	24±1
SGa 1	43±1	41±1	<LOQ	0.06±0.01	45±0.3	4.5±0.3
SGa 2	65±1	67±1	<LOQ	0.17±0.01	23±0.4	25±1
SGa 3	50±1	52±2	<LOQ	<LOQ	12±0.5	16±1
SGa 4	75±1	73±3	3.2±0.02	3.3±0.2	13±0.4	16±1
SGa 5	54±1	53±1	<LOQ	0.14±0.01	13±0.2	15±1
SGa 6	93±1	90±2	3.3±0.04	3.1±0.2	13±0.3	17±1
VSF 1	96±1	93±4	0.48±0.01	0.30±0.02	35±0.3	11±0.5
VSF 2	5.1±0.3	5.6±0.3	0.34±0.01	0.18±0.01	8.6±0.2	11±0.5
VVi 1	81±1	83±3	<LOQ	0.11±0.01	21±0.5	22±1
VVi 2	78±2	79±2	<LOQ	0.05±0.01	8.8±0.3	10±0.5
VVi 3	48±1	47±2	<LOQ	0.11±0.01	54±0.4	50±2
VVi 4	75±1	75±2	0.42±0.01	0.39±0.02	21±0.5	23±1

[a] For acronyms see Table 3.

Table 8
Results obtained for manganese and strontium in Brazilian wine samples from the different regions by HR-CS F AAS and by ICP OES; all values in mg L^{-1}. All samples were prepared in triplicate and three measurements were taken for each sample; hence, all values are the average of $n=9$ measurements ±SD.

Region[a]	Mn		Sr	
	HR-CS F AAS	ICP OES	HR-CS F AAS	ICP OES
CGa 1	4.61±0.06	4.9±0.3	0.72±0.01	0.74±0.04
CGa 2	4.50±0.05	4.3±0.3	0.89±0.01	1.0±0.1
CGa 3	4.32±0.02	4.5±0.1	0.72±0.01	0.69±0.02
CGa 4	4.11±0.04	4.0±0.1	0.79±0.02	0.86±0.04
CGa 5	8.59±0.06	8.2±0.2	1.30±0.03	1.50±0.05
CGa 6	1.81±0.02	1.9±0.1	2.10±0.02	1.9±0.1
CCS 1	6.02±0.04	6.0±0.3	0.22±0.01	0.22±0.01
CCS 2	2.89±0.02	2.8±0.1	<LOQ	<LOQ
CCS 3	6.11±0.09	5.8±0.3	0.24±0.01	0.23±0.03
CCS 4	4.03±0.04	4.2±0.1	0.28±0.01	0.33±0.02
OPr 1	6.14±0.07	7.0±0.3	0.33±0.01	0.35±0.02
OPr 2	6.40±0.05	6.2±0.1	0.29±0.01	0.28±0.01
PBa 1	1.64±0.03	1.7±0.1	0.44±0.01	0.50±0.03
PBa 2	1.78±0.01	1.8±0.1	0.10±0.01	0.12±0.01
PBa 3	2.14±0.02	2.4±0.1	0.55±0.01	0.50±0.02
PBa 4	2.61±0.04	2.5±0.1	0.44±0.01	0.48±0.01
SCa 1	4.63±0.02	4.8±0.2	0.29±0.01	0.31±0.02
SCa 2	5.04±0.04	5.4±0.2	0.25±0.01	0.22±0.01
SCa 3	2.61±0.01	2.6±0.2	0.51±0.01	0.56±0.03
SCa 4	4.24±0.02	4.5±0.1	0.24±0.01	0.29±0.02
SGa 1	4.11±0.04	3.8±0.1	0.83±0.01	0.82±0.01
SGa 2	3.12±0.03	2.9±0.1	0.62±0.02	0.68±0.01
SGa 3	3.10±0.01	3.0±0.1	0.70±0.01	0.65±0.02
SGa 4	7.24±0.09	7.8±0.3	0.33±0.01	0.36±0.03
SGa 5	3.51±0.01	3.3±0.1	0.84±0.02	0.86±0.05
SGa 6	7.40±0.10	6.7±0.3	0.37±0.01	0.45±0.03
VSF 1	1.72±0.03	1.5±0.1	0.79±0.01	0.77±0.03
VSF 2	1.64±0.02	1.8±0.1	0.69±0.01	0.68±0.03
VVi 1	6.40±0.12	5.9±0.3	0.63±0.01	0.63±0.01
VVi 2	2.61±0.02	2.4±0.1	1.20±0.01	1.3±0.1
VVi 3	5.13±0.04	5.2±0.1	0.68±0.01	0.71±0.01
VVi 4	6.10±0.06	6.0±0.1	0.81±0.02	0.75±0.03

[a] For acronyms see Table 3.

Table 9
Results obtained for potassium and rubidium in Brazilian wine samples from the different regions by HR-CS F AAS and by ICP OES; all values in mg L^{-1}. All samples were prepared in triplicate and three measurements were taken for each sample; hence, all values are the average of $n=9$ measurements ±SD.

Region[a]	K		Rb	
	HR-CS F AAS	ICP OES	HR-CS F AAS	ICP OES
CGa 1	828±6	810±10	4.45±0.02	4.2±0.3
CGa 2	886±6	896±6	4.41±0.04	4.4±0.4
CGa 3	890±7	875±10	4.78±0.05	4.5±0.3
CGa 4	841±7	860±12	3.71±0.04	3.5±0.2
CGa 5	1613±9	1640±18	6.67±0.12	6.1±0.5
CGa 6	1351±12	1330±15	2.32±0.03	2.1±0.2
CCS 1	1361±10	1356±17	2.81±0.02	2.9±0.1
CCS 2	1028±4	1050±18	2.20±0.01	2.2±0.1
CCS 3	1362±18	1351±16	3.62±0.03	3.4±0.2
CCS 4	1311±10	1337±15	3.30±0.03	3.3±0.1
OPr 1	2541±7	2560±20	4.33±0.03	4.2±0.1
OPr 2	2457±24	2482±21	4.78±0.09	4.7±0.2
PBa 1	1024±8	1029±10	7.64±0.04	7.7±0.6
PBa 2	1542±10	1552±16	4.01±0.02	3.6±0.2
PBa 3	1049±12	1040±16	6.89±0.06	6.2±0.5
PBa 4	1141±13	1121±14	7.78±0.05	7.7±0.1
SCa 1	1515±7	1598±14	3.78±0.04	3.6±0.3
SCa 2	1935±20	1962±15	3.89±0.03	3.7±0.2
SCa 3	1361±9	1344±17	6.11±0.04	6.0±0.1
SCa 4	1629±13	1636±10	4.10±0.03	3.8±0.3
SGa 1	1452±11	1432±10	6.42±0.04	6.3±0.2
SGa 2	1361±23	1350±15	5.89±0.06	6.1±0.2
SGa 3	1312±20	1322±16	4.11±0.05	3.6±0.3
SGa 4	1863±15	1844±13	6.40±0.06	6.2±0.2
SGa 5	1272±8	1296±18	4.63±0.04	4.7±0.2
SGa 6	1557±16	1533±10	6.22±0.05	6.3±0.3
VSF 1	1901±19	1955±20	2.71±0.02	2.6±0.1
VSF 2	1874±25	1870±25	2.01±0.01	2.0±0.1
VVi 1	1313±10	1344±16	5.56±0.06	5.2±0.3
VVi 2	1848±22	1840±17	9.04±0.12	9.1±0.5
VVi 3	1353±10	1373±14	6.43±0.05	6.7±0.3
VVi 4	1326±17	1353±18	7.60±0.07	7.4±0.2

[a] For acronyms see Table 3.

is aspirated in between. Another important characteristic of the valve is the sample consumption. The injection volume can be controlled and significantly reduced when compared with the conventional F AAS procedures. This feature was important in this work, as only a small quantity of each wine was produced, and a number of other analyses had to be carried out in addition to the metals determination.

Using this accessory a transient or a continuous analytical signal can be obtained, depending on how it is programed, as shown in Fig. 2. The injection time and the load time were optimized using a wine sample in order to obtain an almost Gaussian peak profile and relative standard deviation (RSD) values lower than 5%. Integrated absorbance (peak area) was used for signal evaluation. The results of this optimization were: 1.5 s for injection time and 15 s for load time.

The need of sample pre-treatment was also evaluated, comparing the microwave-assisted acid digestion procedure described in Section 2.3 with the results obtained by direct sample analysis. There were no significant differences in the results obtained with direct analysis and microwave-assisted digestion; the results were identical, within 95% of confidence. Hence, direct analysis was used for the following experiments, resulting in lower reagent consumption, lower risk of contamination and shorter total analysis time.

3.2. Ionization interference

A relatively high concentration of potassium was found in all of the investigated wine samples, and potassium is known as an ionization suppressor; hence, the behavior of the other elements was investigated with respect to the presence of this analyte. Calcium, Rb and Sr were influenced by the K concentration, whereas Be, Co, Cu, Li, Mn and Na were not significantly affected (<5%), which is according to expectation. As K was one of the analytes determined in this study, Cs was investigated as an ionization suppressor and the influence of its concentration on the other elements was evaluated, as is shown in Fig. 3. The Cs concentration was investigated in the range from 50 to 50,000 mg L^{-1}. From 10,000 mg L^{-1} Cs on, the analytical signals reached a plateau or were even decreasing (Sr and Li). The other elements did not exhibit any influence of the Cs concentration. For the following experiments 10,000 mg L^{-1} Cs was added in order to suppress ionization and to maintain the analytical signals.

Table 10
The relation between each component and its relative variance percentage.

Component	Eigenvalues	% of variance	Cumulative %
1	1.69	42.2	42.2
2	1.09	27.2	69.4
3	0.54	16.4	85.8
4	0.57	14.3	100

3.3. Figures of merit and validation of the method

After the optimization of the analytical conditions, the sequence of the steps and the sample preparation conditions, the calibration curves were established for each element and the figures of merit determined, as shown in Table 4. The calibration curves were established using aqueous standard solutions, according to the concentration range for each element and wavelength: 0.2–6.0 mg L^{-1} for Co, Cu, Mn, Rb and Sr, 0.02–0.60 mg L^{-1} for Be, 0.02–0.80 mg L^{-1} for Li, 5.0–100 mg L^{-1} for Ca, 5.0–80 mg L^{-1} for Na and 100–1500 mg L^{-1} for K. The limits of detection (LOD) and quantification (LOQ) for all analytes were calculated according to IUPAC recommendations as three times and 10 times, respectively, the standard deviation of 10 measurements of a blank, divided by the slope of the calibration curve [22]. The characteristic concentration, c_0, is defined as the concentration of an analyte that produces an integrated absorbance signal of 0.0044 s.

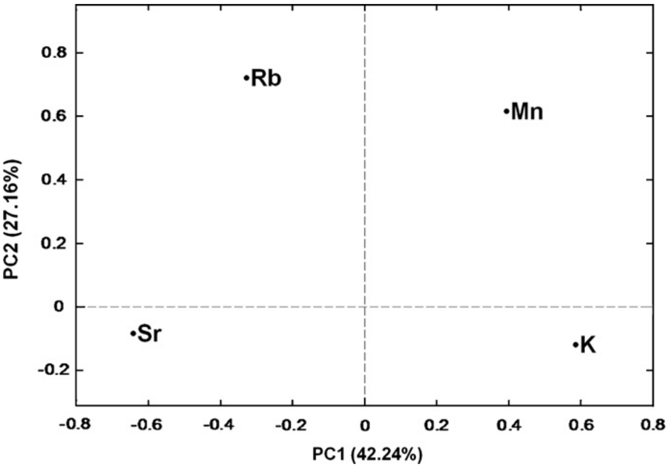

Fig. 5. Loadings evaluation observing PC1 and PC2, and verifying which variable had more influence in each group formation.

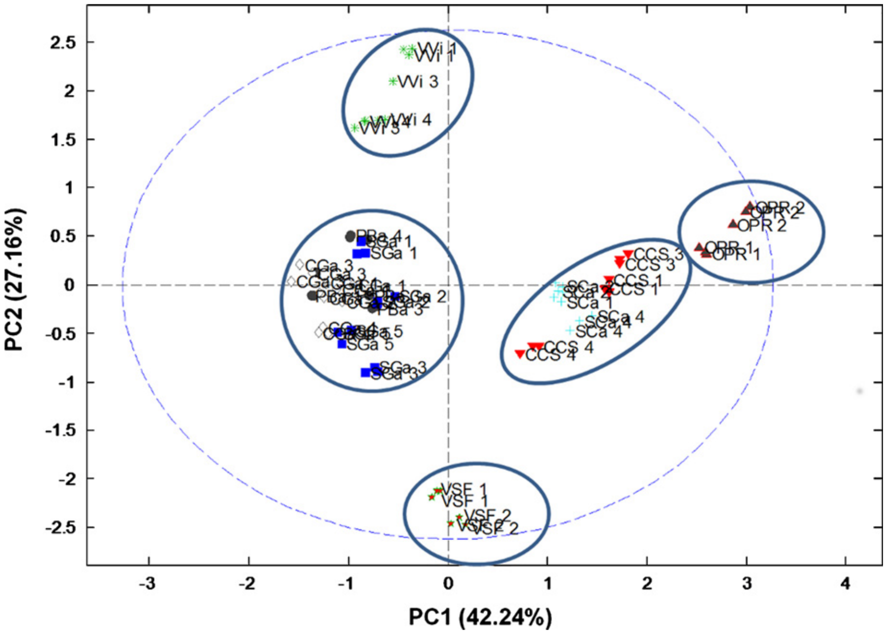

Fig. 4. Scores evaluation observing PC1 and PC2, and verifying the formation of five different groups. For acronyms see Table 3.

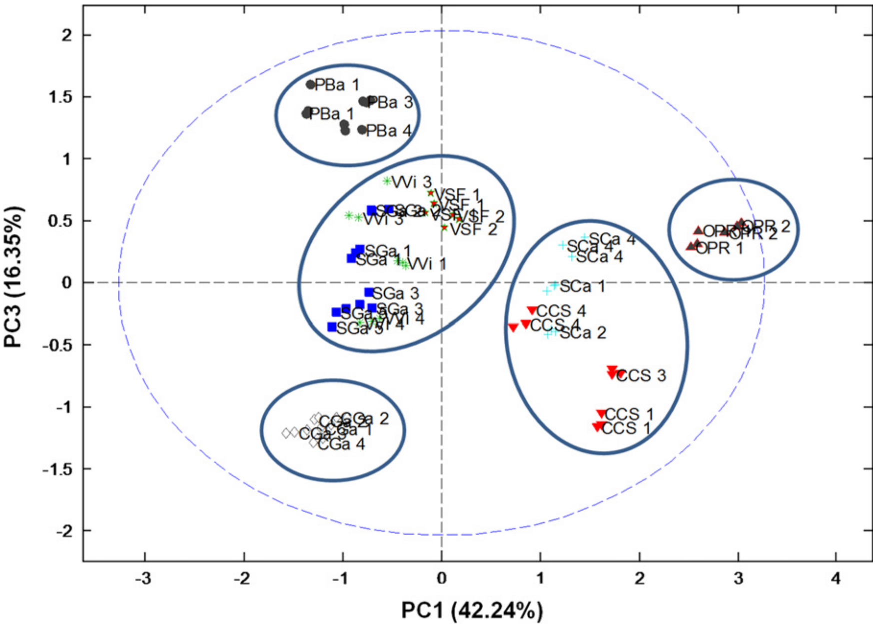

Fig. 6. Scores evaluation observing PC1 and PC3, and verifying the formation of three new groups: PBa, CGa and SGa. For acronyms see Table 3.

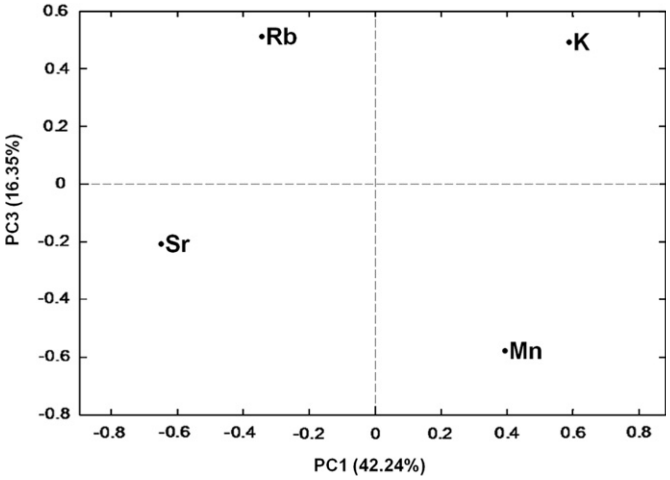

Fig. 7. Loadings evaluation observing PC1 and PC3, and verifying which variable had more influence in each new group.

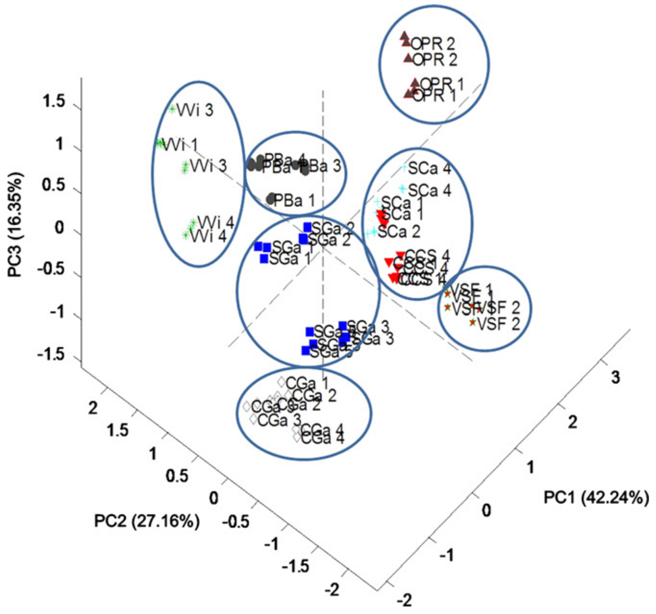

Fig. 8. A three-dimensional plot for PC1, PC2 and PC3, showing the formation of seven different groups. For acronyms see Table 3.

For all investigated elements the linear correlation coefficient was better than 0.999 and the limits of detection and quantification were appropriate to determine the elemental composition of the wine samples. Recovery tests for spiked samples were also carried out by adding appropriate aliquots of 1000 or 10,000 mg L^{-1} single-element stock standard solution to wine samples in order to obtain spikes of 1.0 mg L^{-1} of Co, Cu, Mn, Rb and Sr, 0.10 mg L^{-1} Be and Li, 20 mg L^{-1} of Ca and Na, and 100 mg L^{-1} of K. Recoveries of analytes added to wine samples varied between 91% and 111%, which might be considered appropriate for the purpose of this work. The recoveries found for the four key elements in three wine samples are shown in Table 5.

As there is no certified reference material available for wine, a sample was evaluated, which was produced in France with *C. sauvignon* grapes in 2011 and analyzed in an interlaboratorial comparison [23]. Some of the most significant wine properties, including organic and inorganic compounds were evaluated by a number of European laboratories, and the final results were presented as the mean and the standard deviations. The results, which are shown in Table 6, were in agreement with 95% of confidence (*t*-test) for the investigated elements.

3.4. Analysis of the wine samples

The 32 specially prepared Brazilian wine samples from eight different regions (Fig. 1 and Table 3) were analyzed using HR-CS F AAS and also by ICP OES. The values for Ca, Cu and Na are compiled in Table 7, and those obtained for Mn and Sr, and K and Rb in Tables 8 and 9, respectively. Beryllium, Co and Li were not quantified in these wine samples, as all values for these analytes were below the LOQ of both techniques used in this study. Comparing the results for the other seven elements using ICP OES and HR-CS F AAS, they appeared to be statistically identical,

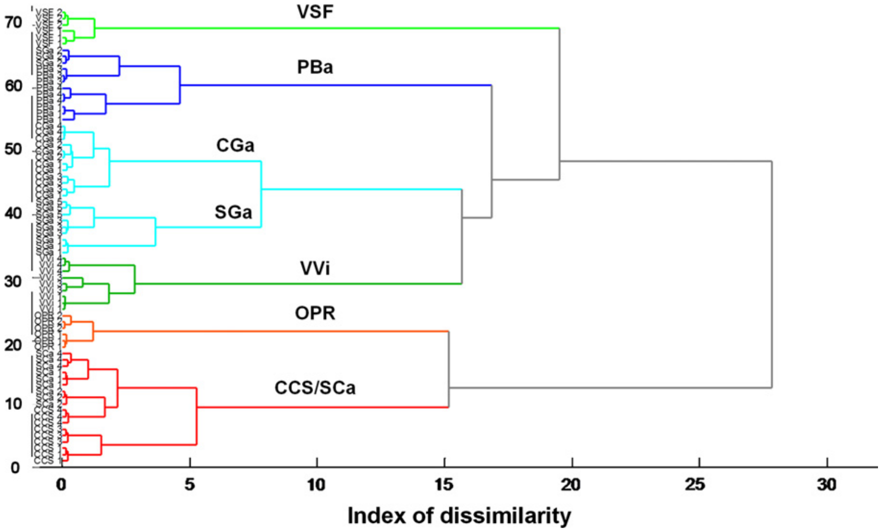

Fig. 9. Dendrogram for the Hierarchical Component Analysis. For acronyms see Table 3.

with 99% of confidence, by a *t*-test. This proves that the developed method has good accuracy and trueness, and can be used for metal determination in wine samples.

3.5. Statistical evaluation

With the obtained results for all the samples and elements a statistical evaluation was made. Pattern recognition techniques, PCA and HCA were used to extract more information from these results. Elements that presented concentration values below the limit of quantification (Be, Co and Li) obviously were not statistically evaluated. Also elements that had great variation in the concentration values (Ca, Cu and Na), considering different samples from the same region, which are shown in Table 7, were not statistically evaluated. Only K, Rb, Mn and Sr concentration values, shown in Tables 8 and 9, were used to evaluate the geographical identification of Brazilian wines through a Principal Component Analysis and a Hierarchical Cluster Analysis.

With the auto-scaled results for K, Mn Rb and Sr a matrix with 24 samples and 4 variables was constructed. Three principal components (PCs) were extracted, explaining 85.8% of the accumulated variance, as is shown in Table 10. The plot of scores in the plane defined by PC1 and PC2, depicted in Fig. 4, shows the eight evaluated regions, separated in five different groups, according to each mineral profile. The regions VVi in Rio Grande do Sul, VSF in Bahia and Pernambuco and OPR in the west of Paraná are clearly separated. This is no surprise as the VSF area, which is more than 3000 km north of Rio Grande do Sul, has a humid tropical climate and a soil composition completely different from the other areas. Wine production in this area started only a few years ago and has to be monitored as a new producing area. The OPR region is more than 1000 km north of the main wine growing area in Rio Grande do Sul and has different soil and climatic conditions as well. On the other hand, the regions CCS and SCa on the Serra of Rio Grande do Sul and Santa Catarina are forming one group as are the regions CGa, SGa and PBa, all in Rio Grande do Sul, forming another group. In Fig. 5, the plot of loadings in the plane PC1 versus PC2 shows which variable had more impact during the group formation. With this in mind it is possible to observe that Rb has clearly separated VFS and VVi (the first one with high concentration of Rb and the other one with a low concentration) and also indicates that OPR wines are rich in K and Mn.

Considering Fig. 6, the plot of scores by PC1 versus PC3 shows a different point of view, adding a third dimension to the system. In that figure it is possible to visualize the formation of three new groups: CGa, SGa and PBa, making it possible to separate the three wine growing areas in Rio Grande do Sul. The regions CCS and SCa on the Serra of Rio Grande do Sul and Santa Catarina remained like one single group, as they are very close to each other, indicating their geographical similarity regarding soil composition. In Fig. 7, the plot of loadings in the plane PC1 versus PC3 presents which variable had more influence during the new group formation. Again Rb showed to be most expressive in this context. The CGa wines have lower concentration of Rb, SGa wines have intermediate concentrations of Rb and PBa wines have the highest Rb concentration of all samples evaluated.

Finally, a three-dimensional plot of PC1 versus PC2 versus PC3 has been constructed, which is shown in Fig. 8, to give a better view of the groups' distribution, showing that seven groups are formed. The information obtained showed that two regions (CCS and SCa) have a very similar mineral profile. The other regions (VVi, VSF, OPR, CGa, SGa and PBa) can be successfully separated, by evaluating their mineral composition and applying a PCA. To confirm the information obtained by the PCA a HCA dendrogram has been constructed using the Ward's method and the auto-scale data, which is shown in Fig. 9. The dendrogram shows that one sample from SGa is similar to the PBa samples, which can also be confirmed in the 3D PCA of Fig. 8, but the other SGa samples do not show the same similarity, so that two different groups have been formed. This can be explained by the proximity of the regions PBa and SGa and the similarity in climate and soil composition. With that it is possible to observe the formation of seven different groups with high similarity index, confirming the information obtained by the PCA. So, according to the mineral composition it is possible to differentiate Brazilian wines by their origin using only the information about the content of four elements. The software used for all the statistical evaluation was Matlab 7.11.0/PLS_Toolbox 6.2.1.

4. Conclusion

The developed method for metal determination in wine samples by HR-CS F AAS via direct sample analysis showed to be fast and reliable, providing a method with no need of sample pre-treatment and with only little dilution. This is possible because secondary lines could be used for elements present at high concentration and selection of the appropriate number of pixels to increase or decrease the sensitivity for those in microconcentration. Another advantage was that all elements could be determined under optimal conditions of operation and the sample consumption was reduced significantly.

The results obtained by the developed method for the wine samples were used to observe groups with the same mineral profile using PCA and HCA as statistical tools. Potassium, manganese, rubidium and strontium were responsible for the group formation. The statistical evaluation showed that Brazilian wines can be separated according to their mineral profile, providing seven distinct groups within the eight areas studied.

This investigation has shown that there is no need for a large number of elements in order to clearly classify the origin of a wine even within a relatively short distance of the production areas, as long as the wines are not mixed, as it is often the case in commercial wines. It might even be concluded that the results of previous studies carried out with commercial wine samples using a great number of data, are irrelevant as grapes of different origins might have been mixed and caused a false and erroneous pattern. Obviously further studies should be carried out in order to confirm this supposition.

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