

## Internal Standardization–Atomic Spectrometry and Geographical Pattern Recognition Techniques for the Multielement Analysis and Classification of Catalanian Red Wines

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Major and minor (K, P, Ca, Mg, Na, Fe, Mn, Zn, and Sr) and trace (Ba, Ni, Pb, V, Co, Cd, and Sb) elements from wine samples from the Denomination of Origin (DO) Empordà-Costa Brava (Catalonia, Spain) were analyzed by inductively coupled plasma atomic emission spectrometry (ICP-AES) and mass spectrometry (ICP-MS) respectively. Previously, a comparison of different calibration methodologies and sample digestion treatments had been carried out using ANOVA statistical tool. The obtained results demonstrated that internal standardization provides reliable results with the advantage that no further manipulation of the sample is needed. A principal component analysis of the concentration data was performed to differentiate the samples of DO Empordà-Costa Brava from wine samples from other wine-producing regions in Spain (i.e., Penedès, Somontano, and Rioja). It was found that Sr and Ba contents discriminate the two DO groups. Moreover, a discriminant analysis function involving both variables distinguishes the two groups with a 100% classification rate. At the level of the leave-one-out cross-validation, all of the Empordà-Costa Brava samples were well classified, whereas the other DOs presented two borderline misclassifications.

**KEYWORDS:** Wine; ICP-AES; ICP-MS; internal standard calibration; discriminant analysis; principal component analysis

### INTRODUCTION

Mineral contents of wines depend on several factors including type of grape, area of production, soil characteristics, and environmental conditions. Their determination has been considered to be of great interest because it allows the definition of a “fingerprint” for each of them and represents one of the criteria used for evaluating the identity and authenticity of wine. The importance of metals in wine is also related to the role they play as catalysts of biological systems or as promoters of some enzymes (Fe, Cu, Mn, and Zn). They can also modify the stability, color, and clarity of wines, and they may affect the organoleptic characteristics of the wine (Zn and Fe). Other metals have been studied for toxicity, as is the case of Pb.

Atomic spectrometric techniques (atomic absorption and atomic emission) have been most commonly used to perform multielement determination in wine. For routine analysis, inductively coupled plasma atomic emission spectrometry (ICP-AES) (1–4) and mass spectrometry (ICP-MS) (5–14) are preferred nowadays. Several works have been published dealing with their use for elemental characterization of wines: Baxter

et al. (6) established the elemental composition of several wines by analyzing their major, minor, and trace elements. Greenough et al. (12) demonstrated that wine multielemental composition is strongly influenced by the solubility of inorganic compounds from vineyard soil. Stroh et al. (13) and Augagneur et al. (14) determined rare earth elements in a few wine samples.

From an analytical point of view, wine is a fairly complex matrix owing to the content of its organic compounds, above all, ethanol, which can cause additional spectral and nonspectral interference in ICP-AES and ICP-MS. To overcome matrix-induced effects, which can change the signal intensity, some authors have simply diluted the sample (5, 6, 13) or removed the alcohol from the sample by evaporation to dryness and dissolution of the residue in nitric acid (12, 14). Others have applied microwave-assisted acid digestion of the wine (15). UV-irradiation-assisted digestion has also been used for sample preparation (16).

Fingerprint techniques based on chemical composition and multivariate statistical analysis can be used for characterizing or classifying products according to origin, quality, variety, type, or other features (17, 18). In particular, numerous applications of multivariate methods for wine classification are reported. These consider several classes of chemical variables and sensory properties: different enological parameters (19, 20), polypheno-

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nolic compounds together with volatile and amino acids components of musts (21), and trace element concentrations alone (22) or in conjunction with other data such as site-specific natural isotope fractionation studied by NMR (23) or Sr isotope ratios (24). Aroma compounds of wines (25, 26) have also been used for this purpose. The amount of generated data has traditionally been treated with different statistical methods. The most common procedures used for pattern recognition purposes include, for instance, cluster analysis, *K*-nearest neighbors, classification trees, and canonical variate analysis or linear methods such as principal component analysis, sometimes combined with the derived soft independent modeling of class analogies (SIMCA) and factor or discriminant analysis. Some of these methods have been employed in combination, as reflected in many publications (3, 6–9, 22, 27–30). On the other hand, nonlinear procedures are also very common, for instance, the quadratic discriminant analysis (31) or the use of neural networks (7, 27, 30, 32).

In this work, the multielement content of a number of red wines from the Denomination of Origin (DO) Empordà-Costa Brava (an emerging wine-producing zone in Catalonia, north-eastern Spain) has been determined by ICP-AES and ICP-MS for major, minor, and trace elements, respectively. Different calibration methodologies and sample digestion treatments were compared: direct analysis using partial matrix matching calibration, internal standard calibration, standard addition, open vessel digestion, and microwave-assisted digestion. The accuracy of the method based on internal standardization was tested by analyzing a wine prepared by the Laboratoire Interregional de Bordeaux and intended to be a reference material. Finally, a chemometric analysis of the data obtained with the proposed method was undertaken to differentiate samples from the DO Empordà-Costa Brava from wine samples from other wine-producing regions in Spain.

## MATERIALS AND METHODS

**Apparatus.** An ETHOS PLUS Millestone microwave with an HPR-1000/10S high-pressure rotor (Sorisole, Bergamo, Italy) was employed for the acid digestion of samples.

A sequential inductively coupled plasma atomic emission spectrometer (ICP-AES, Liberty RL, Varian) in radial configuration was used for major and minor element determination.

A quadrupole-based ICP-MS system (Agilent 7500c, Agilent Technologies, Tokyo, Japan) equipped with an octapole collision reaction cell was used for trace element determination. In this work, the collision/reaction cell acts only as an ion focusing lens because it was not filled with any pressurized gas.

The operating conditions of both ICP-MS and ICP-AES equipment are summarized in **Table 1**.

**Reagents.** The reagents used were of analytical grade suprapur quality: nitric acid (Suprapur, Merck, Darmstadt, Germany) and hydrogen peroxide (Trace Select, Fluka, Gilligham, Dorset, U.K.). Moreover, water obtained from a Milli-Q purifier system (Millipore Corp., Bedford, MA) was used throughout the study. For matrix matching standards, ethanol absolute (UV-IR-HPLC) PAL, from Panreac (Barcelona, Spain) was used.

Monoelemental ICP standard solutions (1000 or 5000 mg L<sup>-1</sup> for Ba) from each element studied were purchased from Pure Chemistry, ROMIL, UKAS calibration.

**Sample Collection.** Different red wines (*n* = 34) generously donated by the DO Empordà-Costa Brava cellars were analyzed. Wine samples from other regions in Spain [La Rioja (R1, R2, R3, R4), Somontano (S1, S2, S3), and Penedès (P1, P2)] were purchased from a local store. All wines were produced during the year 2004.

**Sample Treatment.** Major and Minor Element Determination: Ca, Mg, Na, K, P, Sr, Zn, Cu, Al, Fe, and Mn. Procedure 1: Partial Matrix

**Table 1.** Operating Conditions

ICP-MS, Agilent 7500c	
RF power	1500 W
sampling cone	Ni, 1 mm aperture diameter
skimmer cone	Ni, 0.4 mm aperture diameter
plasma gas flow rate	15 L min <sup>-1</sup>
potential ion lenses	daily tuning
acquisition mode	three points per peak
nebulizer	Babington
spray chamber	Scott type double path
detector	electron multiplier
ICP-AES, Liberty series II, Varian	
RF power	1000 W
plasma gas flow rate	12 L min <sup>-1</sup>
torch configuration	radial
nebulizer	V-groove
spray chamber	double-pass cylindrical
detector	photomultiplier tube

**Table 2.** Analysis by ICP-AES: Elements Analyzed Together with Their Working Wavelength, the Internal Standard (IS) Used for Correction, and Concentration Ranges Used in Each Set of Calibrations

element	wave-length (nm)	IS (wave-length, nm)	concentration range (mg L <sup>-1</sup> )	concentration range (mg L <sup>-1</sup> ) with IS
Zn(II)	206.200	Ni(II) (231.604)	0.2–3	0.1–3
K(I)	769.896	Ni(I) (232.003)		20–160
Mg(I)	285.213	Ni(I) (352.454)	50–250	3–30
Na(I)	589.592	Ni(I) (352.454)		3–30
P(I)	214.914	Ni(I) (352.454)		1–25
Ca(II)	317.933	Y(II) (377.433)	50–300	3–30
Fe(II)	259.940	Y(II) (321.669)	2–25	0.4–25
Mn(II)	257.610	Y(II) (321.669)	0.5–8	0.5–8
Sr(II)	421.552	Y(II) (321.669)	0.5–3	0.1–3
Al(I)	396.152	Ni(I) (232.003)	1–8	1–8
Cu(I)	324.754	Ni(I) (232.003)	0.1–2	0.1–2

**Matching.** The calibration standards were prepared from individual standard solutions in 1% HNO<sub>3</sub>. The concentration ranges for each element are summarized in **Table 2**, together with the wavelength used for calibration. Different percentages of ethanol were added to the solutions so as to obtain three different calibration sets, that is, 0, 11, and 14% (v/v) ethanol.

**Procedure 2: Standard Addition.** The usual standard addition procedure was used. Twenty-five milliliters of wine was diluted to a 50 mL final volume.

**Procedure 3: Open Vessel Digestion.** (a) Fifty milliliters of wine was taken into a Pyrex beaker and placed in a heater to reduce the volume of the sample by gentle warming.

When the volume was 25 mL, warming was stopped, the sample was allowed to cool, and it was diluted to the initial volume in a volumetric flask.

(b) Twenty milliliters of HNO<sub>3</sub> was added to 10 mL of wine, and the mixture was heated until a dark color appeared. Then 20 mL of HNO<sub>3</sub> was added and heated again until a volume of 5–10 mL was reached. Afterward, 15 mL of distilled water and 1–2 mL of H<sub>2</sub>O<sub>2</sub> were added, and the solution was heated again for 10–20 min. The final mixture was brought to a final volume of 25 mL with distilled water.

The intensity of the emission line for the selected elements was then measured using calibration standards prepared in distilled water.

**Procedure 4: Internal Standard (IS) Calibration.** Y and Ni were chosen as internal standards because they are present in wine at very low concentrations (micrograms per liter). The corresponding amount of an internal standard solution containing both metals (200 mg L<sup>-1</sup>) was added to 5 or 10 mL of wine sample, to obtain a final IS

**Table 3.** Analysis by ICP-MS: Isotope Analyzed with the Internal Standard Used and the Concentration Range in the Calibration Sets

isotope	internal standard isotope	concentration range (ng g <sup>-1</sup> )
<sup>7</sup> Li	<sup>103</sup> Rh	1–40
<sup>27</sup> Al	<sup>103</sup> Rh	40–1150
<sup>51</sup> V	<sup>103</sup> Rh	0.6–20
<sup>59</sup> Co	<sup>103</sup> Rh	0.4–14
<sup>60</sup> Ni	<sup>103</sup> Rh	3–80
<sup>65</sup> Cu	<sup>103</sup> Rh	5–140
<sup>88</sup> Sr	<sup>103</sup> Rh	7–200
<sup>111</sup> Cd	<sup>103</sup> Rh	0.04–1
<sup>121</sup> Sb	<sup>103</sup> Rh	0.03–1
<sup>137</sup> Ba	<sup>205</sup> Tl	3–100
<sup>208</sup> Pb	<sup>205</sup> Tl	2–60

concentration of 8 mg L<sup>-1</sup>. Then distilled water was added to the mark (50 or 25 mL, respectively). Ten times dilution was employed for K, Na, P, Mg, and Ca determination and 2.5 times dilution for Sr, Zn, Cu, Al, Fe, and Mn determination. A calibration set containing 8 mg L<sup>-1</sup> of Y and Ni was prepared, together with the target elements. IS correction was carried out by taking into account the intensity of the line for the measured element and for the internal standard. Additionally, due to the different behaviors traditionally observed for ionic and atomic lines (33, 34), each ionic line was corrected by a ionic line of the internal standard and each atomic line was corrected using an atomic line (see **Table 2**).

*Trace Element Determination: Pb, Ba, V, Ni, Co, Cd, and Sb (plus Li, Cu, Sr, and Al in the Reference Wine). Procedure 5: Internal Standard (IS) Calibration.* The isotopes <sup>103</sup>Rh and <sup>205</sup>Tl were used for internal standard correction after checking that they were not present in the wine samples at the concentration range studied (results from a semiquantitative analysis). <sup>103</sup>Rh was employed to correct the signal of all the measured elements except for Ba and Pb (signal corrected with <sup>205</sup>Tl). The concentration of the IS in the standard solutions prepared for calibration and in the samples was 5 ng g<sup>-1</sup> for Rh and 30 ng g<sup>-1</sup> for Tl.

From the commercial standard solutions, an intermediate solution in 1% HNO<sub>3</sub> containing the measured elements in the appropriate range of concentrations was prepared and used to obtain the calibration set (see **Table 3**).

As will be discussed in the following section, all samples were prepared with 3 g of wine, the necessary amount of the internal standard solution, and finally water, added to reach a final weight of 5 g.

*Procedure 6: Microwave Digestion.* Five grams of concentrated HNO<sub>3</sub> was added to a 3 g wine sample placed in a quartz reactor. This reactor was introduced inside the Teflon vessel, and a mixture of 2 g of H<sub>2</sub>O<sub>2</sub> plus 5 g of doubly distilled water was added to the space between both reactors. Once the vessel was capped, it was placed in the microwave oven and the following program was run (35): step 1, 10 min to reach 120 °C; step 2, 2 min at 120 °C; step 3, 10 min to reach 150 °C; step 4, 3 min at 150 °C; step 5, 15 min to reach 170 °C; step 6, 3 min at 170 °C; step 7, cooling down.

After cooling to ambient temperature, the reactor was opened and the resultant solution was transferred into a vial where the total weight was carefully measured. To prepare the samples for analysis, the necessary amount of IS solution was added to 5 g of the digested solution. The samples were measured by using the calibration set prepared as explained before.

**Statistical Analysis.** An exploratory principal component analysis (PCA) (36) and a linear discriminant analysis (LDA) (37) were performed. Data manipulations were carried out with the MINITAB program (38). The calculations were performed using the 16 variables (contents of Ca, Mg, Mn, Zn, Fe, Sr, P, Na, K, V, Co, Ni, Cd, Sb, Pb, and Ba) in standardized adimensional units.

## RESULTS AND DISCUSSION

To determine the elemental content of wines for authenticity purposes, it is imperative to have an analytical method that performs well in terms of accuracy, precision, and lineal range. When ICP-AES or ICP-MS is used for this purpose, we must take into account that wine is a complex matrix that contains many inorganic and organic substances which can affect signal intensity. A matrix effect arising from organic solvents, for example, ethanol, present at elevated levels is widely reported in the literature (39). Our purpose here was to evaluate the mineral contents of wines from the DO Empordà-Costa Brava by using the most appropriate calibration technique. Once these data were obtained, the statistical analysis was performed to show how the wines under study can be differentiated from other wines from different wine-producing regions in Spain.

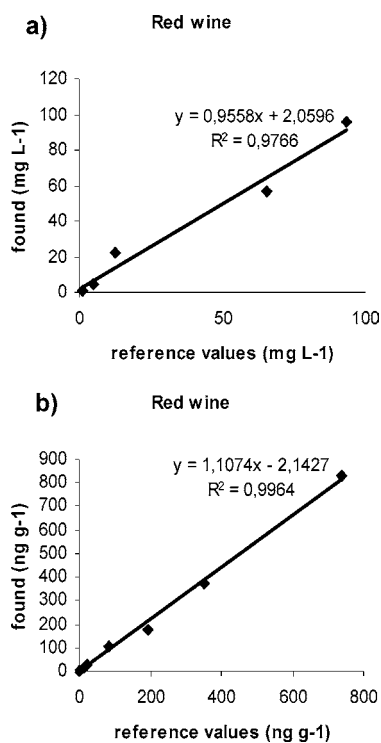
**Sample Treatments and Calibration in ICP-AES.** Different approaches were evaluated to guarantee the trueness of the results obtained for those elements present at higher concentrations: direct measurements of the samples without any treatment and using aqueous standards; direct measurement with standards containing 11 or 14% ethanol; results of standard addition; measurements of decomposed wine samples; and finally results obtained with the internal standard calibration technique. The comparison was made by analyzing in triplicate one sample of wine from the DO Empordà-Costa Brava. The results are summarized in **Table 4**. ANOVA was carried out to compare the different sets of results for each element: the one-tailed *F* test showed that the means given by the seven techniques differed significantly (*P* = 0.05) in all cases except for Cu. Visual inspection of these data allows us to reach some hypotheses about the differences observed: it is clear that the results obtained after direct measurements of the wine sample using a calibration graph obtained with aqueous standards are higher than the results obtained with other treatments, confirming the existence of a matrix effect. As Cocchi et al. reported (35), when using the direct method, apparent differences in intensity, caused by the influence of the organic components in the sample nebulization and measurement process, could be compensated by adding ethanol, the main organic component of wine, to the calibration standards. Regarding the results of the standard addition, it can also be observed that the concentrations are higher compared to the other treatments (for Mg, Mn, Sr, and Ca), as was pointed out by Thiel and Danzer (2). In addition, measuring the decomposed wines may provide a wide variability in the values found caused by losses during oxidation, evaporation, and contamination by instruments and chemicals. This is especially true in the case of Zn: extremely low concentrations were found when the digestion treatment was performed (see **Table 4**). The same effect was found by Castiñeira et al. (11), and it was explained by the oxidation process. In conclusion, direct measurement of wine should be avoided, and the same is true for sample decomposition or open vessel digestion due to Zn losses. As for standard addition, the results obtained for most of the elements were higher than those obtained for the other calibration techniques.

Internal standardization has shown good results to compensate for matrix effects in ionic lines with radially viewed inductively coupled plasma atomic emission spectrometry (34). According to our results, if we compare the results obtained with internal standardization, those obtained using standards prepared in ethanol and those measured after sample digestion (the data for Zn was excluded) using ANOVA analysis (*P* < 0.05), we can conclude that they do not differ significantly. Moreover, internal standardization avoids tedious sample treatment such as diges-

**Table 4.** Metal Concentrations Obtained by ICP-AES in a Red Wine Using Different Calibration Methods and Sample Treatments [Data in Milligrams per Liter, Mean  $\pm$  SD ( $n = 2$ )]

	water <sup>a</sup>	ethanol 11% <sup>a</sup>	ethanol 14% <sup>a</sup>	std add. <sup>b</sup>	OV digestion <sup>c</sup>	OV acid digestion <sup>d</sup>	IS <sup>e</sup>
Al	5.07 $\pm$ 0.08	4.05 $\pm$ 0.11	4.08 $\pm$ 0.02	3.58 $\pm$ 0.21	3.72 $\pm$ 0.01	3.85 $\pm$ 0.35	4.56 $\pm$ 0.39
Ca	146.07 $\pm$ 1.57	110.68 $\pm$ 0.32	114.59 $\pm$ 0.01	131.12 $\pm$ 0.84	126.16 $\pm$ 2.24	124.39 $\pm$ 4.42	114.06 $\pm$ 4.52
Cu	0.67 $\pm$ 0.01	0.54 $\pm$ 0.01	0.56 $\pm$ 0.02	0.61 $\pm$ 0.02	0.55 $\pm$ 0.05	0.61 $\pm$ 0.08	0.57 $\pm$ 0.01
Sr	1.17 $\pm$ 0.01	1.02 $\pm$ 0.01	1.02 $\pm$ 0.01	1.26 $\pm$ 0.01	1.03 $\pm$ 0.08	0.99 $\pm$ 0.05	0.96 $\pm$ 0.13
Fe	19.23 $\pm$ 0.34	11.81 $\pm$ 0.02	13.29 $\pm$ 0.21	14.58 $\pm$ 0.87	13.66 $\pm$ 0.87	15.96 $\pm$ 1.66	16.02 $\pm$ 0.70
Zn	1.48 $\pm$ 0.02	1.08 $\pm$ 0.02	0.87 $\pm$ 0.01	0.96 $\pm$ 0.24	0.09 $\pm$ 0.02	0.08 $\pm$ 0.02	1.00 $\pm$ 0.03
Mn	3.69 $\pm$ 0.02	2.47 $\pm$ 0.05	2.42 $\pm$ 0.01	2.97 $\pm$ 0.05	2.54 $\pm$ 0.21	2.59 $\pm$ 0.10	2.65 $\pm$ 0.10
Mg	187.56 $\pm$ 1.61	124.21 $\pm$ 1.48	121.25 $\pm$ 0.30	136.96 $\pm$ 1.19	125.29 $\pm$ 5.92	128.50 $\pm$ 2.82	117.58 $\pm$ 0.33

<sup>a</sup> Procedure 1. <sup>b</sup> Procedure 2. <sup>c</sup> Procedure 3a. <sup>d</sup> Procedure 3b. <sup>e</sup> Procedure 4.

**Figure 1.** Comparison of concentration values for the RM and internal standardization method: (a) metals analyzed by ICP-AES; (b) metals analyzed by ICP-MS.

tion and allows the use of the same calibration set for the different wine samples, which is not possible with the standard addition technique. The proposed methodology was validated by analyzing two provisionally certified BCR RMs. They do not yet have certified values but were supplied with provisional values by the Laboratoire Interregional de Bordeaux (personal communication from Dr. B. Medina). A comparison of our data with the provisional data is shown in **Figure 1a**. Acceptable agreement is obtained within the tolerances supplied for all analytes with the exception of K (not plotted in the figure). It is well-known that ICP-AES is not the most convenient technique for K determination due to its low sensitivity. It was also observed that its emission peak was placed in a region of the spectrum with a variable background, which makes it difficult to measure. Moreover, the precipitation of this element can take place in a wine matrix.

Taking these results into account, internal standardization was chosen for the analysis of the different wine samples. The data obtained are given in **Table 6**.

**Sample Treatments and Calibration in ICP-MS.** Similarly to the comparison made with ICP-AES measurements, we also tested different procedures for the analysis of metals in a wine

**Table 5.** Metal Concentrations Obtained by ICP-MS in the Provisionally Certified Red Wine Using Different Sample Treatments and Comparison with the Provisional Values Given (Data in Nanograms per Gram)

element	MW digestion (with IS) <sup>a</sup>	quantitative direct analysis (with IS) <sup>b</sup>	Laboratoire Interregional de Bordeaux <sup>c</sup>
Li	14.20 $\pm$ 0.19	13.05 $\pm$ 0.29	12.90 $\pm$ 5.3
Al	925 $\pm$ 29	829.5 $\pm$ 6.6	740 $\pm$ 240
V	35.45 $\pm$ 0.72	33.67 $\pm$ 0.12	
Co	4.21 $\pm$ 0.05	3.91 $\pm$ 0.02	
Ni	32.2 $\pm$ 1.7	27.55 $\pm$ 0.50	20.3 $\pm$ 3.5
Cd	0.30 $\pm$ 0.02	0.37 $\pm$ 0.01	0.35 $\pm$ 0.21
Sb	0.99 $\pm$ 0.10	1.65 $\pm$ 0.04	
Ba	108.4 $\pm$ 1.8	107.9 $\pm$ 1.5	82 $\pm$ 12
Pb	22.89 $\pm$ 0.62	21.22 $\pm$ 0.11	16.5 $\pm$ 4.0

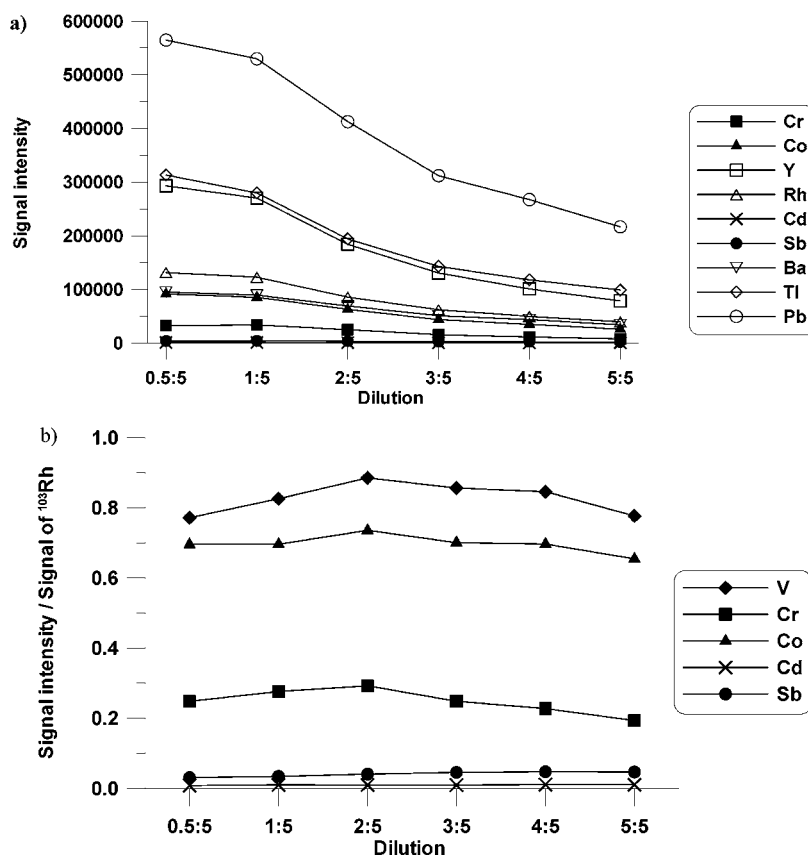
<sup>a</sup> Procedure 5; mean  $\pm$  SD ( $n = 3$ ). <sup>b</sup> Procedure 6; mean  $\pm$  SD ( $n = 3$ ). <sup>c</sup> Mean  $\pm$  SD, interlaboratory.

matrix in the case of metals present at trace and ultratrace levels and using ICP-MS as analytical technique. Only two methodologies were tested using internal standard in both cases: microwave digestion and direct analysis after dilution. To perform direct analysis, previous measurements were carried out to evaluate the most appropriate dilution of the wine sample. **Figure 2a** shows the signal intensity of the chosen elements, multiplied by the dilution factor, as a function of the mass of wine taken for the analysis (final mass of 5 g). The variation observed in the signal intensity can be compensated for by normalization to the intensity of Rh, the internal standard (**Figure 2b**). If IS correction is done, the response is more or less constant in the range of percent of ethanol studied. Taking into consideration that a high dilution would result in a lower sensitivity, the quantity of wine selected to perform the rest of the experiments was 3 g. This dilution is similar to that proposed by other authors, who use 1 + 1 diluted wine in ICP-MS analysis (5).

The comparison between direct analysis and microwave digestion was done with the provisionally certified red wine sample provided by the Laboratoire Interregional de Bordeaux. The results are shown in **Table 5**. ANOVA analysis of the results was carried out for the two sets of results using the one-tailed *F* test. In this case, the two techniques did not differ significantly ( $P = 0.05$ ) for all metals (Li, Al, V, Ni, Cd, Ba, and Pb) except Co and Sb. Lower concentrations found for Sb when the digestion treatment was performed can be explained by the known volatility of this metal (40). The differences found in the case of Co may be due to the extremely good repeatability attained for both methods. Good agreement can be observed between our results and those from the Laboratoire Interregional de Bordeaux. Unfortunately, the results of Co and Sb were not

**Table 6.** Concentration Results Obtained for ICP-AES (in Milligrams per Liter) and ICP-MS (in Nanograms per Gram) Analyses of the 43 Wines under Study

	wines											
	DO Empordà-Costa Brava (n = 34)			DO Penedès (n = 2)			DO Somontano (n = 3)			DO Rioja (n = 4)		
	min	max	median	min	max	median	min	max	median	min	max	median
Ca	57.67	160.97	90.20	62.07	112.88		48.82	75.81	66.76	73.40	82.13	78.74
Mg	88.75	153.28	114.61	109.07	113.27		71.19	113.04	97.74	94.63	117.36	106.56
P	94.89	333.44	172.42	125.31	154.73		113.24	138.02	128.51	88.51	140.02	105.23
Na	12.33	191.51	51.33	33.94	40.35		19.14	25.27	21.33	25.22	58.47	38.91
K	422.96	1375.16	926.01	856.34	875.96		586.51	1055.35	819.61	910.41	1147.70	1028.29
Mn	0.99	4.00	2.04	1.06	1.20		0.64	1.15	0.86	0.75	0.99	0.85
Zn	0.30	1.07	0.54	0.27	0.41		0.11	0.32	0.25	0.31	0.53	1.59
Fe	1.32	7.56	3.38	1.84	2.20		0.55	0.58	0.57	1.77	4.51	2.66
Sr	0.22	0.69	0.29	0.70	0.85		0.40	0.81	0.59	0.81	1.05	0.94
Pb	4.64	60.32	16.68	11.57	29.95		6.41	24.54	13.20	13.08	19.67	16.36
Ba	96.13	263.80	173.42	105.96	143.71		65.50	82.56	75.59	66.53	79.70	72.49
V	0.32	20.31	3.04	5.95	192.69		1.06	22.67	9.23	6.06	54.25	29.59
Co	1.96	11.05	5.47	1.92	2.95		0.93	2.19	1.57	2.40	3.26	2.83
Ni	18.85	73.24	40.17	19.08	22.92		12.73	15.88	14.22	17.40	44.80	26.39
Cd	0.21	2.65	0.98	0.17	0.20		0.11	0.19	0.15	0.19	0.31	0.22
Sb	0.21	0.93	0.45	0.96	0.99		0.39	37.44	12.83	0.49	1.04	0.70

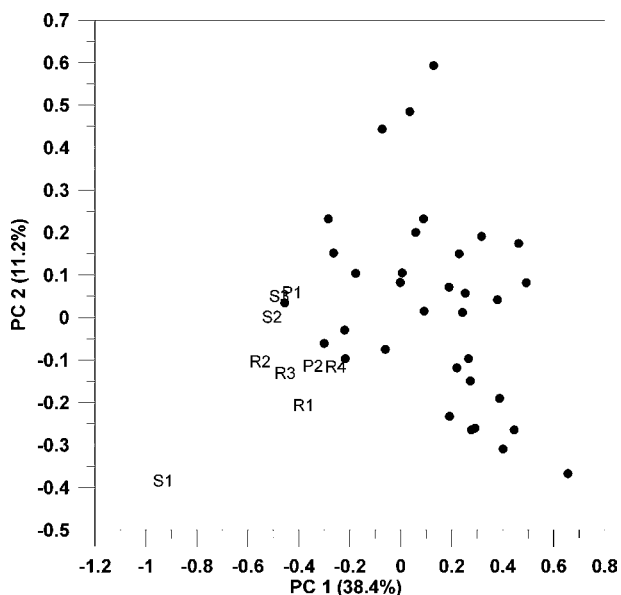
**Figure 2.** Effect of dilution factor in the signal obtained in ICP-MS for some selected elements: (a) without IS correction; (b) with IS correction.  $^{103}\text{Rh}$  signal was used as IS for correction.

available for this RM. This comparison is also shown in **Figure 1b**, where a slope of the curve close to 1 was obtained.

In conclusion, direct measurement with 3:5 dilution together with internal standardization demonstrated good performance in ICP-MS analysis of elements in wine samples. In addition, sample preparation time is shorter than the time needed with microwave digestion. For these reasons internal standardization was chosen to perform the elemental analysis of wine samples. The results obtained are presented in **Table 6**.

**Classification of Wines from the Different Regions.** To check for similarities and differences among the distinct 43 wine samples, a PCA was performed to visualize the multivariate

data. A total of 13 standardized variables entered into the calculation (Ca, Mg, Mn, Zn, Fe, Sr, Na, K, Co, Ni, Cd, Pb, and Ba), whereas the concentrations of Sb, V, and P were discarded because for some samples the corresponding concentrations lie beyond the  $3\sigma$  range. The first eigenvector obtained accounts for more than a third of the data variance (38.4%), whereas the second one for 11.2% and the third for 10.8%. The other vectors contribute  $<10\%$  each. **Figure 3** shows the scores representation in the two first principal components plane and clearly reveals that the two groups of DOs can be differentiated. In this figure the dots stand for Empordà-Costa Brava samples, whereas the other DOs are labeled. Both point clouds remain



**Figure 3.** Representation of wine samples in the two first principal components. Points correspond to Empordà-Costa Brava denomination, whereas the other DOs are labeled.

well separated, and it can be seen how a Somontano sample (S1) is also discriminated from the remaining non-Empordà-Costa Brava samples. This is mainly due to the significantly higher contents of Sb ( $>6\sigma$  from the mean value). The first and second principal component loadings allow us to see that Ni, Co, Mn, Cd, and Ba (first component) and Sr (second component) play a prominent role in these eigenvector compositions. In particular, the Sr content is a dominant variable in the structure of the second component, presenting a coefficient of 0.61. This suggests that the content of Sr can be effective in discriminating the DO Empordà-Costa Brava from other DOs. This particular feature is reproduced in previous works, see, for instance, ref 8 and references cited therein.

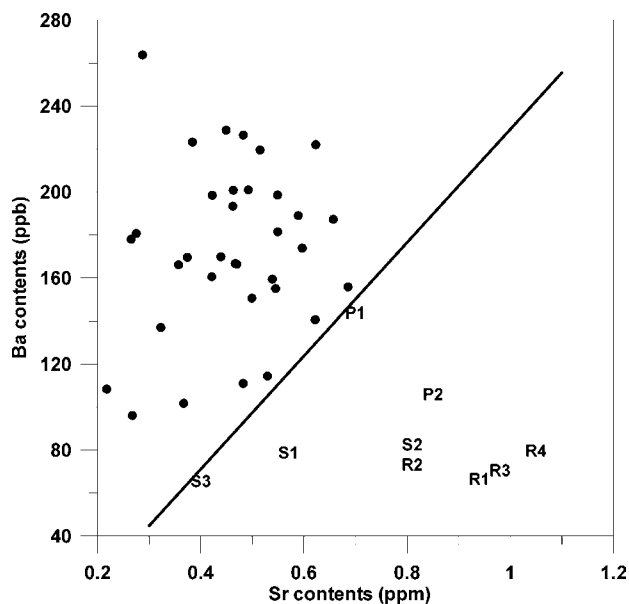
A representation of the wine samples with respect to all of the pairs of variables showed that the combination of Sr and Ba contents is capable of discriminating the two DOs. This is graphically visualized in **Figure 4**. The group separation is evident and suggests that an LDA involving these two variables will classify the two wine groups. The calculation was carried out with Minitab, obtaining a 100% classification rate. The assignment equations are

for Empordà-Costa Brava samples:

$$F_1 = 20.319c_{\text{Sr}} + 0.118c_{\text{Ba}} - 14.940$$

for the other DOs:  $F_2 = 38.762c_{\text{Sr}} + 0.048c_{\text{Ba}} - 17.340$

where the variables  $c_{\text{Sr}}$  and  $c_{\text{Ba}}$  are the contents of Sr and Ba expressed in  $\text{mg L}^{-1}$  and  $\text{ng g}^{-1}$ , respectively. As is known, Sr and Ba together with other elements (Al, B, Li, Mn, Mo, Rb, ...) came from the soil, and their concentration in wine is not at all influenced by the production cycle (9). This may possibly explain why these two elements allow the geographical classification of wines. The attached discriminant line is depicted in **Figure 4**. In this calculation, only two samples (P1 and S3, see figure) are near a borderline situation for misclassification (the probabilities assigned to the respective true groups were 0.59 in both cases). To check for the classification ability of this model, a leave-one-out calculation was also performed, using again only the two aforementioned variables. In this case, all 34 Empordà-Costa Brava wines were well classified and 2



**Figure 4.** Representation of the wine samples according to the contents of Sr and Ba. Points correspond to Empordà-Costa Brava denomination, and the other DOs are labeled.

foreign wines were misclassified. As expected, the misclassified samples were the former P1 and S3 ones.

In conclusion, the PCA clearly shows that the DO Empordà-Costa Brava wines can be differentiated from the wines of other DOs studied by their elemental content, Sr and Ba concentrations being essential to discriminate between wine samples. As for the multielemental analysis we have demonstrated that the direct measurement with adequate dilution together with internal standardization using ICP-AES and ICP-MS shows good performance in terms of accuracy and sample throughput.

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