

# The determination of the authenticity of wine from its trace element composition

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Multi-element analysis of 112 Spanish and English wines by inductively coupled plasma mass spectrometry (ICP-MS) was undertaken to ascertain whether or not this method could provide data for determining the region of origin of wine. Flow injection analysis proved superior to continuous nebulisation. Quality assurance of these measurements proved entirely satisfactory and was typical of that previously established for this technique. An intercomparison exercise with another expert laboratory showed satisfactory agreement.

The data was examined using the statistical technique of discriminant analysis. This was able to unequivocally identify the region of origin of Spanish wines from three different regions. It was also possible to completely differentiate English and Spanish white wines. If red and rosé wines were included in the Spanish set, the English and Spanish populations could be distinguished with 95% accuracy.

This preliminary study has indicated the power which multi-element analysis can bring to determining the region of origin of wines. At present, no other technique has the ability to perform this categorisation. © 1997 Published by Elsevier Science Ltd

## INTRODUCTION

An indication that trace elements might be valuable for determining the region of origin of wine was provided by McCurdy *et al.* (1992). More recently, Stroh *et al.* (1994) have reported further preliminary data which indicate the usefulness of lanthanides in this regard. Thermal ionisation mass spectrometry has been used to provide precise data on <sup>87</sup>Sr/<sup>86</sup>Sr ratios (Horn *et al.*, 1993). Latorre *et al.* (1994) have used pattern recognition analysis for classifying wines from northwest Spain. By using Li and Rb as key features, they were able to differentiate between Rias Baixas and non-Rias Baixas wines in 41 out of 42 attempts. Thus, trace elements and their isotopes may be valuable for determining wine origin although a more extensive data base is required before a firm judgement can be made. There is now some evidence that trace elements can provide complementary information to other techniques. In a study of five Venetian wines (Moret *et al.*, 1994), the most important variables in their classification were: 1-hexanol, K, nitrogen compounds and total phenols. Day *et al.* (1994) have shown that the characterisation of the region of origin of wines using hydrogen-deuterium

ratios can be refined by including data on their trace element composition.

There are, therefore, a number of preliminary studies in the literature which herald the promise of trace elements as a means of determining the region of origin of wine. However, the number of samples which has been analysed has not always been sufficient to provide a statistically meaningful data base. We have therefore addressed this issue by obtaining a large number of authentic samples from two countries, analysing their trace element composition, and assessing the data by appropriate statistical techniques.

## EXPERIMENTAL

### Sampling

Samples of wine were obtained from English Vineyards by representatives of the Wine Standards Board. Samples of Spanish wine were obtained by the University of Zaragoza direct from Spanish vineyards and were certified as being authentic.

The following procedure was conducted for each wine sample. The neck and cork areas were wiped carefully with a dampened tissue to remove dust. Using a

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proprietary extractor, the cork was removed ensuring the screw did not penetrate through, and the rim was wiped again with a dampened tissue. Then, using a 10 ml pipettor fitted with a long pipette, two aliquots were removed to condition the pipette before finally transferring 20 ml to a clean Nunc vial (Life Technologies Ltd) pending analysis. The pipette was thoroughly rinsed with pure water before commencing the next sample. In all, 112 wines were measured by ICP-MS and subjected to subsequent statistical analysis.

The method of storage was monitored by placing aliquots of three reference wines in the Nunc vials for analysis at the end of the survey. The wines, a red, a white and a liquor, are Community Bureau of Reference (BCR) reference wines which are undergoing certification. Comparison of the data obtained from these with that from wine in the reference bottles showed no differences.

### Materials and apparatus

Nitric acid (Aristar) was obtained from BDH Ltd (Poole, Dorset). Ethanol (AR) was obtained from Hayman Ltd (Witham, Essex). Purified water was produced on-site using a Milli-Q system linked to the laboratory deionised supply. Standards (1000 and 10 000 ppm) were obtained from various companies; BDH Ltd, Aldrich Chemical Co. (Gillingham, Dorset), Sigma Chemical Co. Ltd (Poole, Dorset), and Johnson Matthey Ltd (Royston, Herts). The reference materials used throughout this survey were obtained from a BCR intercomparison trial, May 1992. A bulk in-house wine standard (red) was also prepared and stored in a chemically inert (fluorinated ethylene propylene) litre bottle, previously conditioned with a similar wine sample. Samples were measured using a PlasmaQuad PQII Plus Turbo (FI Elemental Ltd, Winsford, Cheshire). Solutions were introduced by flow injection using a FIAS 200 flow injection analysis system and AS 90 autosampler (Perkin-Elmer Ltd, Beaconsfield, Bucks).

### Measurement

A fresh multi-element stock solution was prepared daily and standards were created from this using sequential dilution with standard diluent (5.0% ethanol, 0.5% nitric, containing indium at 10 ppb). Wine samples (5.0 ml) were diluted 1:1 with sample diluent (1.0% nitric acid, containing indium at 20 ppb). Spiked samples for recovery determinations were prepared by adding 0.1 ml stock to 4.9 ml sample before the 1:1 dilution. The standard diluent was used for the reagent blank. A typical batch comprised 42 tubes; 20 samples, 5 reference, 4 blanks, 6 spikes and 7 standards. Single injections (0.5 ml) of all solutions were introduced into a carrier stream (5.0% ethanol, 0.5% nitric acid, containing indium at 10 ppb) whose flow rate was set at

1.0 ml/min. The Gilson Minipuls 3 peristaltic pump from the PlasmaQuad was used for the carrier flow to minimise signal fluctuation. Operating parameters for the FIAS 200 are shown in the table below:

Time (s)	Value	Pump 1	Pump 2
60	Fill	50	0
60	Inj.	0	0

A wash cycle of 120 s after each injection was performed in an auto rinsing well designed in-house. The rinse solution (5.0% ethanol, 0.5% nitric acid) was fed into and removed from the well via pump 1 on the FIAS 200.

The PQ2 was set up according to the following conditions:

Coolant gas (s.l.p.m.):	13.5	
Auxiliary gas (s.l.p.m.):	1.0–1.5	
Nebuliser gas (s.l.p.m.):	0.80	Cross-flow nebuliser used

Spray chamber temp (°C): 5

RF forward power (watts): 1500

Collector type: Pulse  
Acquire mode: Peak jump  
Points per peak: One  
Dwell time (μS): 10 240

Isotopes monitored:

<sup>7</sup> Li	<sup>9</sup> Be	<sup>27</sup> Al	<sup>45</sup> Sc	<sup>48</sup> Ti	<sup>51</sup> V	<sup>52</sup> Cr
<sup>55</sup> Mn	<sup>57</sup> Fe	<sup>59</sup> Co	<sup>62</sup> Ni	<sup>65</sup> Cu	<sup>66</sup> Zn	<sup>68</sup> Zn
<sup>69</sup> Ga	<sup>73</sup> Ge	<sup>75</sup> As	<sup>78</sup> Se	<sup>81</sup> Br	<sup>85</sup> Rb	<sup>86</sup> Sr
<sup>87</sup> Sr	<sup>88</sup> Sr	<sup>89</sup> Y	<sup>90</sup> Zr	<sup>93</sup> Nb	<sup>95</sup> Mo	<sup>102</sup> Ru
<sup>103</sup> Rh	<sup>106</sup> Pd	<sup>107</sup> Ag	<sup>111</sup> Cd	<sup>114</sup> Cd	<sup>115</sup> In	<sup>120</sup> Sn
<sup>121</sup> Sb	<sup>125</sup> Te	<sup>127</sup> I	<sup>133</sup> Cs	<sup>137</sup> Ba	<sup>139</sup> La	<sup>140</sup> Ce
<sup>141</sup> Pr	<sup>146</sup> Nd	<sup>152</sup> Sm	<sup>153</sup> Eu	<sup>154</sup> Gd	<sup>158</sup> Gd	<sup>159</sup> Tb
<sup>162</sup> Dy	<sup>165</sup> Ho	<sup>166</sup> Er	<sup>169</sup> Tm	<sup>174</sup> Yb	<sup>175</sup> Lu	<sup>178</sup> Hf
<sup>181</sup> Ta	<sup>182</sup> W	<sup>185</sup> Re	<sup>192</sup> Os	<sup>193</sup> Ir	<sup>195</sup> Pt	<sup>197</sup> Au
<sup>202</sup> Hg	<sup>205</sup> Tl	<sup>206</sup> Pb	<sup>207</sup> Pb	<sup>208</sup> Pb	<sup>209</sup> Bi	<sup>232</sup> Th
<sup>238</sup> U	<sup>239</sup> Pu					

The instrument was tuned on <sup>113</sup>In in the carrier solution.

Some isotopes were monitored to check for potential interferences. Thus, <sup>154</sup>Gd was measured to check for the presence of oxides such as <sup>138</sup>Ba<sup>16</sup>O, others for possible geographic isotopic abundance variation, e.g. Sr and Pb.

Data acquisition for each injection was started manually, monitoring the FIAS valve movement. This method was the only option available for linking the FIAS system to the PQ2 whilst monitoring large numbers of isotopes. For each injection, two acquisitions were performed, one pre-peak, one on-peak. From this, blank subtracted results could be obtained using the PQ2 software. This allowed correction for interferences

**Table 1. Limits of Detection (LOD) for Trace Elements in wine**  
 $\mu\text{gL}^{-1}$ 

Mass	Element	LOD	Mass	Element	LOD
7	Li	0.2	120	Sn	0.08
27	Al	1	121	Sb	0.02
48	Ti	1	133	Cs	0.03
51	V	0.1	137	Ba	0.3
53	Cr	8	139	La	0.007
55	Mn	0.1	140	Ce	0.005
57	Fe	5	141	Pr	0.005
59	Co	0.03	146	Nd	0.04
62	Ni	1	152	Sm	0.01
65	Cu	0.2	153	Eu	0.01
66	Zn	0.2	158	Gd	0.02
69	Ga	0.06	159	Tb	0.005
73	Ge	0.17	162	Dy	0.01
75	As	0.05	165	Ho	0.005
85	Rb	0.07	166	Er	0.01
86	Sr	0.2	169	Tm	0.005
87	Sr	0.10	174	Yb	0.01
88	Sr	0.03	175	Lu	0.007
90	Zr	0.03	181	Ta	0.01
93	Nb	0.02	182	W	0.07
95	Mo	0.4	205	Tl	0.02
102	Ru	0.02	207	Pb	0.1
103	Rh	0.007	232	Th	0.02
111	Cd	0.05	238	U	0.01

which originated from the plasma/diluent and offered better precision for scarce isotopes for which signal fluctuations (e.g. from previous peak-tailing) would be significant. All data were corrected for blanks and recovery before statistical analysis.

#### Quality assurance

Recovery of added analyte for all reported elements was good. All values were within  $100 \pm 25\%$ . The majority (83%) fell within  $\pm 20\%$ , which was in line with previous multi-element surveys performed by this laboratory.

The effects of varying ethanolic strength (3 to 12% v/v) on analyte signal produced only minor variations of  $\pm 10\%$ , thus validating the method for the range of alcohol contents of the wines analysed.

Limits of detection (LOD) are reported in Table 1. These were very good due to the minimal dilution (1:1) and limited sample preparation. The LODs for the majority of elements were in the parts per trillion range, from 10-100 ppt. Only three elements, Cr, Fe, and Ni were in the low part per billion range.

The reference wines chosen were three provisionally certified BCR RMs. They do not yet have certified values but were supplied with provisional values by Laboratoire Interregional De Bordeaux (LIDB, personal communication Dr B. Medina). A comparison of our data (A) with the provisional data (B) is shown in Table 2. Acceptable agreement is obtained within the tolerances supplied for all samples and analytes with the exceptions of iron and cadmium data for BCR E. The reasons for this have not yet been thoroughly investigated; however, differences between our data and the provisional values did not indicate systematic bias. For example, the copper values are either higher (BCR D) or lower (BCR E) but generally within the provisional range.

#### Statistical evaluation of data using discriminant analysis

In order to test the robustness of the data sets, two different statistical packages were used.

First, all trace elements were analysed (without normalisation) using the 'Discriminate' program from Genstat. Discriminant analysis is used with data in which each observation comes from one of several well-defined groups. A total of 112 wines were analysed with observations on 48 trace elements. This data set is too large for any type of regression analysis but fortunately the use of discriminant analysis provides a dimension reducing technique.

Discriminant analysis can be used for analysing observations from two or more groups. The user provides a training set for which group membership is known. On the basis of assumptions about the structure of the training set, rules are constructed for assigning future observations to one of the groups which minimise the probability of misclassification.

**Table 2. Data ( $\mu\text{gL}^{-1} \pm 1\text{SD}$ ) for reference wines, showing values obtained by the authors (A,  $n = 7$ ) and provisional values (B,  $n$  unknown)**

Wine Type:	BCR C White		BCR D Liquor		BCR E Red	
	A	B	A	B	A	B
Li	35 $\pm$ 5	45	8 $\pm$ 2	7	10 $\pm$ 3	13
Al	2000 $\pm$ 290	1660 $\pm$ 730	1300 $\pm$ 340	1270 $\pm$ 600	510 $\pm$ 130	580 $\pm$ 210
Cr	30 $\pm$ 10	25 $\pm$ 10	55 $\pm$ 20	25 $\pm$ 10	25 $\pm$ 10	20 $\pm$ 5
Mn	800 $\pm$ 190	950	1550 $\pm$ 430	1110	870 $\pm$ 250	850
Fe	4520 $\pm$ 1120	5310 $\pm$ 980	7070 $\pm$ 2360	6730 $\pm$ 1220	3640 $\pm$ 1060	6890 $\pm$ 1090
Cu	170 $\pm$ 60	270 $\pm$ 45	610 $\pm$ 370	1150 $\pm$ 230	410 $\pm$ 160	250 $\pm$ 70
Zn	150 $\pm$ 50	350 $\pm$ 120	490 $\pm$ 200	320 $\pm$ 115	420 $\pm$ 135	450 $\pm$ 100
As	12 $\pm$ 2	12 $\pm$ 10	11 $\pm$ 2	10 $\pm$ 12	4 $\pm$ 1	6 $\pm$ 4
Cd	0.3 $\pm$ 0.2	0.5 $\pm$ 0.4	0.6 $\pm$ 0.2	0.8 $\pm$ 0.8	0.4 $\pm$ 0.2	0.9 $\pm$ 0.1
Pb	90 $\pm$ 20	70 $\pm$ 10	185 $\pm$ 50	135 $\pm$ 40	50 $\pm$ 15	40 $\pm$ 10

The Genstat discriminate program finds linear combinations of the original variables (the trace element data) that maximise the ratio of the between-group to within-group variation. This gives functions of the original variables that can be used to discriminate between the groups. The wine data was tested in two ways. In the first test, three groups of wines were defined from three separate Spanish regions. The trace element data for each wine was then tested against this data set to determine its authenticity. In the second test, two authentic groups were defined representing English and Spanish wines. Again, individual wine data were then tested against this data set to determine the country of origin of the wine.

The second evaluation was undertaken, again using discriminant analysis, but within the SPSS (Statistics Package for Social Sciences) program. In this case, data were normalised and this permitted an evaluation of the importance of the contribution made by individual trace elements. The SPSS package was used to investigate the minimum number of trace elements needed to separate the three Spanish wine regions. In addition, it was used to ascertain whether or not the data allowed the separation of the three Spanish wine types (red, white and rosé). Finally, it was also used to evaluate whether the country of origin could be ascertained if the analysis was restricted to white wines from England and Spain.

## RESULTS AND DISCUSSION

### Multi-element results

The development of the best procedure to undertake a multi-element analysis of wine proved rather more challenging than was originally anticipated. Initial studies were undertaken using continuous nebulisation. This technique provides a continuous supply of sample to the nebuliser and the measurement is made under steady-state conditions. Considerable problems with signal instability were encountered and it was discovered that this was due to progressive blocking of the sampling and skimmer cones due to the concentrated nature of the sample matrix. Flow injection analysis resolved this difficulty. This procedure is suitable for automation although appropriate equipment was not available for this study.

Multi-element analysis of wines was possible with minimal sample preparation. Only sample dilution was required. This permitted the analysis of some elements e.g. tungsten, which, if present, would already be in solution in the wine samples. Normally, the dissolution of tungsten from solid samples is problematic and would not therefore be measured. Typical data found for a sample of red wine are presented in Table 3.

This study was undertaken in order to test the proposition that trace element data, with appropriate statistical treatment, can be used to provide information

on the origin of wine. These measurements were carried out with the same rigour which would be appropriate for a surveillance exercise except that only single determinations were performed. The analytical quality control data confirmed that the overall standard of the data acquisition was satisfactory (section 2.4).

Although some trace elements might arise from spray treatments (e.g. copper in Bordeaux mixture), these will not provide statistically meaningful data (unless they were only used in one region). The trace elements which are present in grape juice are likely to have arisen from uptake by the plant from the soil. Equally, it is likely

**Table 3. Trace Element data for a typical red wine sample ( $\mu\text{gL}^{-1}$ ) All data is corrected for blank values and recovery**

Mass	Element	Concentration $\mu\text{gL}^{-1}$
7	Li	32.5
27	Al	1451
48	Ti	240
51	V	10.4
53	Cr	30
55	Mn	1875
57	Fe	7768
59	Co	3.35
62	Ni	34
65	Cu	210.5
66	Zn	569.8
69	Ga	9.85
73	Ge	0.64
75	As	4.42
85	Rb	1414
86	Sr	909
87	Sr	853
88	Sr	829
90	Zr	6.34
93	Nb	0.47
95	Mo	3
102	Ru	0.43
103	Rh	0.038
111	Cd	< L.O.D.
120	Sn	5.27
121	Sb	5.55
133	Cs	10.61
137	Ba	588.2
139	La	0.336
140	Ce	0.78
141	Pr	0.059
146	Nd	0.22
152	Sm	0.06
153	Eu	0.01
158	Gd	< L.O.D.
159	Tb	< L.O.D.
162	Dy	0.06
165	Ho	0.012
166	Er	0.06
169	Tm	0.008
174	Yb	0.06
175	Lu	< L.O.D.
181	Ta	0.14
182	W	1.75
205	Tl	0.28
207	Pb	58.4
232	Th	0.53
238	U	0.31

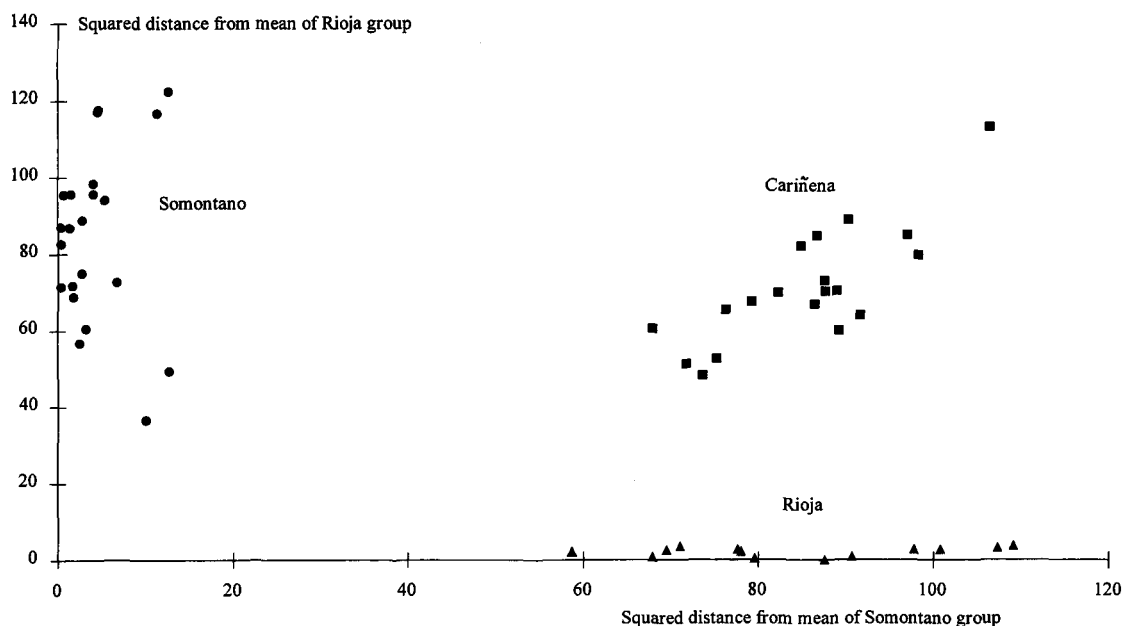


Fig. 1. Classification of Spanish wines according to region of origin using 48 elements with Genstat.

that dust from soil may adhere to the grape skins and contribute to the trace element load. However, much of this will be from the fields where the grapes were grown. This source of trace elements should be just as valid as a marker of wine region as those inherent in the grape juice.

**Statistical analysis**

Of the 112 wines analysed, 55 were Spanish, of which 22 were from Somontano, 14 were from Rioja and 19 were from Cariñena. Data from all 48 elements were included without normalisation and, using the Genstat program, the mean squared distance from each observation to the three group means was calculated. Figure 1 shows a plot of this data. The axes chosen were the squared distance from the mean of the Rioja group and the squared

distance from the mean of the Somontano group. The Rioja and Somontano groups are separated by 60 units. Samples from Cariñena form a separate group toward the centre of the graph, separated by 40 units from the Rioja mean and by 60 units from the Somontano mean. In all cases, samples associated with a designated group are well separated from the other groups. The applicability of the Genstat program was further evaluated. Each wine sample was treated as an unknown. The program then evaluated its authenticity by comparison with the authentic data set. In every case the program placed the sample in the correct region of origin.

The SPSS program was used to evaluate the classification of wines from these three Spanish regions. Using this package, it was found that some trace elements did not contribute significantly towards classification and 13 were removed by Canonical Discriminant Analysis.

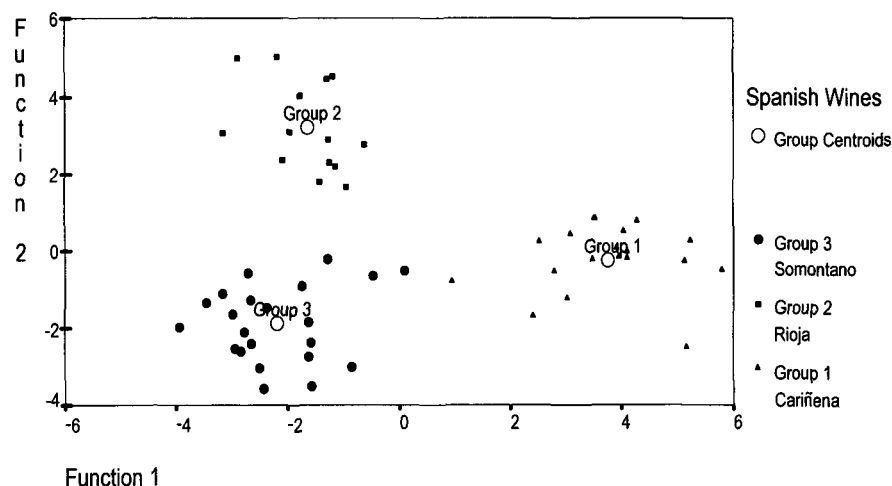


Fig. 2. Classification of Spanish wines according to region of origin using 35 elements with SPSS.

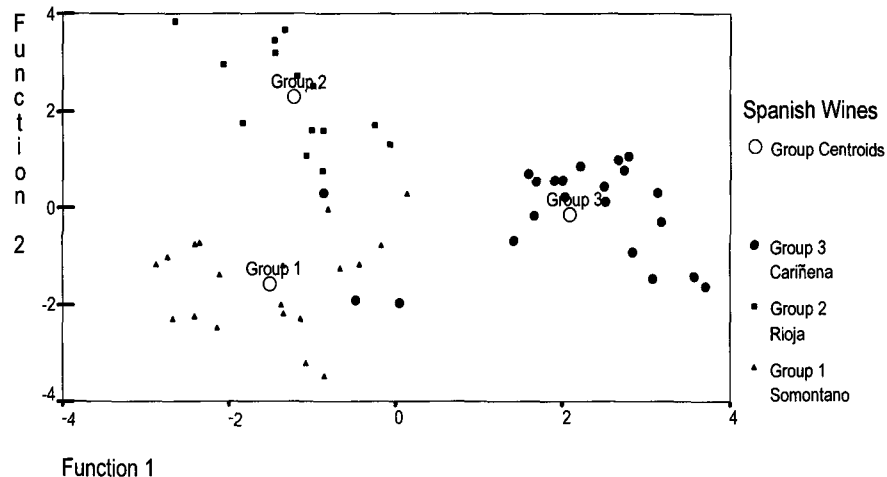


Fig. 3. Classification of Spanish wines according to region of origin using seven elements with SPSS.

Figure 2 shows that complete separation of the three Spanish wine groups is possible using only 35 trace element variables. Indeed, most of the separation is achieved on the basis of just 7 elements: Cd, Cr, Cs, Er, Ga, Mn,  $^{86}\text{Sr}$ . The separation they provided when used to calculate two discriminant functions is shown in Fig. 3. These functions produced a classification success rate of 94.7% for Cariñena, 100% for Rioja and 86.4% for Somontana.

The Genstat program was also used to determine whether or not it was possible to differentiate English and Spanish wines on the basis of their trace element composition. Fifty-five Spanish wines and 57 English wines were examined by obtaining the squared distance from the means for each group as previously described.

Figure 4 is a plot of the squared distance of each individual wine from the mean of the English group against the squared distance from the mean of the Spanish group. This shows no distinct clusters but a continuum from one group to another. When the classification of individual wines was performed, six wines were misclassified. Four Spanish wines were misclassified as English and two English wines were misclassified as Spanish. These are identified in Fig. 4. It is noteworthy that the misclassified Spanish wines were at the very edge of the Spanish data whereas the misclassified English wines were totally distinct from the English data set. The remaining 106 wines, representing 95% of the data, were classified correctly. A similar success rate was also achieved using the SPSS program (93%).

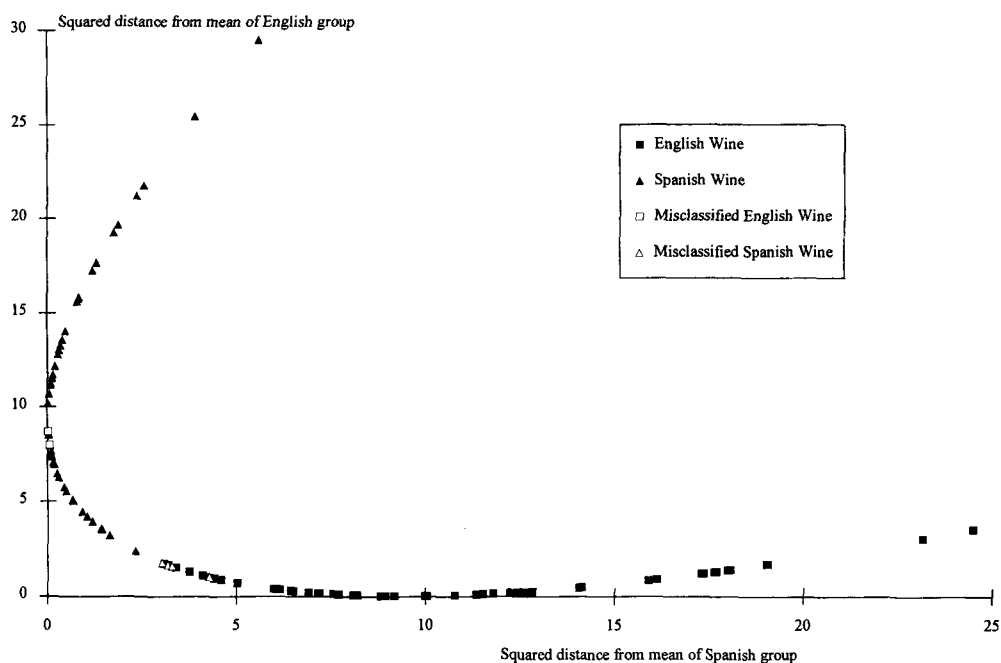


Fig. 4. Classification of English and Spanish wines according to region of origin using 48 elements with Genstat.

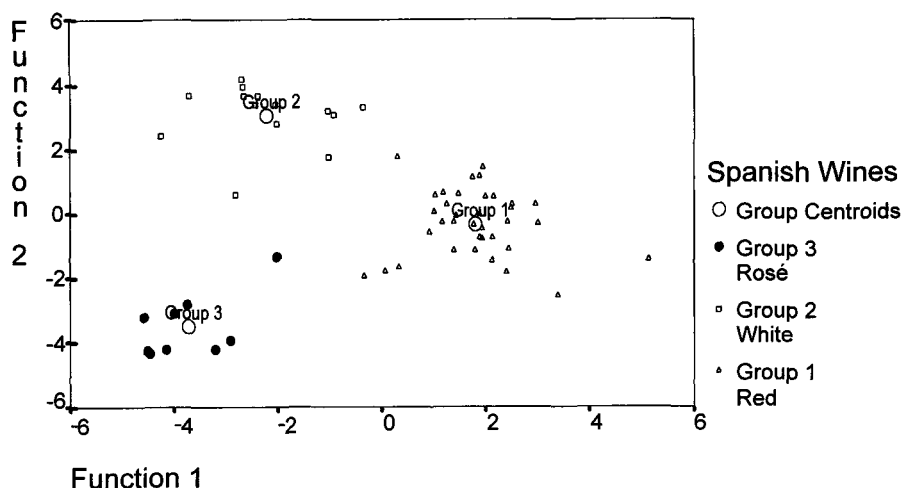


Fig. 5. Classification of Spanish wines according to wine type using 48 elements with SPSS.

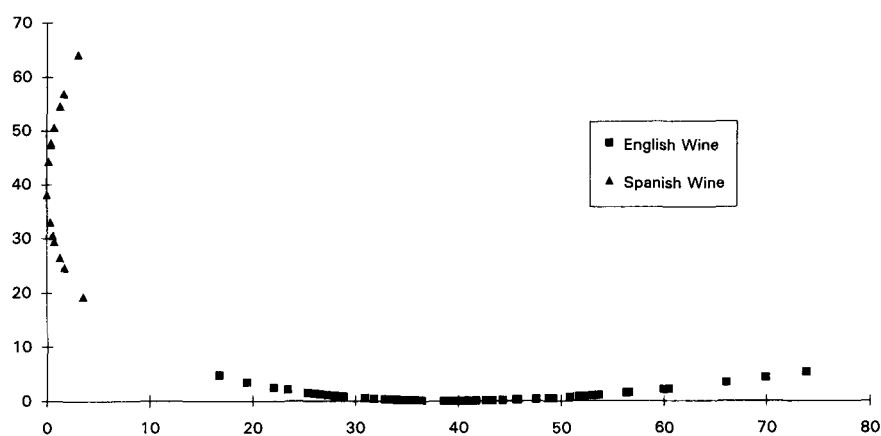


Fig. 6. Classification of English and Spanish white wines according to region of origin using 48 elements with Genstat.

These classifications of wine have been achieved without taking into account factors such as the type of wine. However, Fig. 5 indicates that red, white and rosé wines can be differentiated by their trace element composition. This effect may be due either to processing factors and/or grape variety. It may therefore be valuable to take into account wine type when classifications are attempted since this may provide an extra discriminatory factor.

This approach was taken by considering a data set comprised of white wines from England and Spain. The SPSS package was used on normalised data of all trace elements and the results are shown in Fig. 6. By comparing only the white wines from each country, a complete differentiation of country of origin was possible. However a note of caution is advisable since the removal of red and rosé wines from the data set reduced the number of wines in the Spanish group to only 14. A larger data base of Spanish white wines would be desirable in order to confirm this observation.

## CONCLUSIONS

Multi-element analysis of wines using FI-ICP-MS, when accompanied with appropriate quality assurance, was found to be a rapid method for obtaining good data with minimal sample preparation. Subsequent statistical analysis was carried out using Genstat and SPSS. The good agreement between data interpretation achieved by these packages provided a strong indication that the data were robust. It would be valuable to extend this evaluation further by obtaining completely new wine samples from these regions and seeing if the wines were correctly classified. In addition, the approach needs to be applied to wines from other countries to fully evaluate this procedure and this is currently underway.

This study has strongly indicated that trace element analysis provides an excellent prospect (indeed the best available so far) for determining the region of origin of wines.

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