

The Determination of $^{11}\text{B}/^{10}\text{B}$ and $^{87}\text{Sr}/^{86}\text{Sr}$ Isotope Ratios by Quadrupole-Based ICP-MS for the Fingerprinting of South African Wine

C. Vorster, L. Greeff and P.P. Coetzee*

Department of Chemistry, University of Johannesburg, PO Box 524, Johannesburg, 2006, South Africa.

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ABSTRACT

The $^{11}\text{B}/^{10}\text{B}$ and $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratios in wines and soils of four major South African wine-producing regions have been determined by quadrupole-based ICP-MS in order to establish a fingerprint for origin verification of the wines. The $^{11}\text{B}/^{10}\text{B}$ isotope ratio was found to be a useful tool to distinguish among the wines of the selected wine regions. In addition, the use of B isotope ratios together with elemental concentrations of selected indicator elements as independent variables in a linear discriminant analysis procedure was shown to be a highly successful method to classify wine according to geographical origin. A good correlation between the B and Sr isotope ratios in wine and its provenance soil was found. Both wine and soil samples were prepared using microwave-assisted digestion followed by the isolation of boron and strontium from the sample matrix through element-specific ion exchange. Isotope ratio measurements with good precision, $\sim 0.1\%$ RSD, for both boron and strontium have been obtained. The $^{87}\text{Sr}/^{86}\text{Sr}$ ratio showed limited potential as an indicator of provenance in the wine-producing regions included in this study, since the wines of only one region could be distinguished from the others.

KEYWORDS

Boron isotope ratio, strontium isotope ratio, ICP-MS, wine analysis, fingerprinting, provenance determination.

1. Introduction

Food authenticity is of major concern in the food industry because fraudulent practices such as the adulteration of food and wine through addition of undeclared or harmful ingredients, deviant descriptions on labels, or fake statements about the geographical origin of a food product, can have negative economic consequences and can even be a health hazard. Various ways of food and wine authentication¹ are therefore applied or are in the process of being developed to help regulate matters. Wine fraud, in particular, is a common malpractice. In an attempt to combat this, wine authenticity is commonly addressed by a regulatory 'wine of origin' system in many countries. The system, however, does not always succeed in preventing the different manifestations of wine fraud. Therefore wine fingerprinting by chemical means as a method of characterization has been studied as an alternative, to support the authenticity of a wine through verification of its geographical origin. Various approaches to wine authentication^{1–3} by chemical analysis have been used including the analysis of organic wine components^{4,5}, multi-element analysis^{6–10}, rare earth element analysis^{11,12}, and isotope ratio analysis^{13–19}. The determination of stable isotope ratios of not only light elements^{20–22}, such as hydrogen, boron, carbon, nitrogen, oxygen, and sulphur, but also two heavy elements^{23,24} strontium and lead, has found application in the authentication of food and wine.

The choice of a suitable element to use for isotope ratio analysis for wine fingerprinting is influenced by various factors. Ideally, the element selected for provenance identification should indicate a link between the wine and the geochemistry of the provenance soil²⁵.

Since the $^{11}\text{B}/^{10}\text{B}$ ratio varies in rocks, natural waters and sedi-

ments, these variations in isotope composition have been investigated in environmental, geological and biological samples for various purposes²⁶. Variations in the $^{11}\text{B}/^{10}\text{B}$ ratio could be caused by the selective adsorption of ^{10}B compounds onto sediments and clay and fractional salt precipitation in brine water leading to increased $^{11}\text{B}/^{10}\text{B}$ ratios in natural waters, including interstitial water. The natural variation in $^{11}\text{B}/^{10}\text{B}$ ratios in soils, if also reflected in the grapevine, would therefore make it possible to use the isotope ratio for the provenance determination of wine¹⁵.

Strontium shows variations in isotope composition due to radioactive decay. Of strontium's four stable isotopes, ^{84}Sr , ^{86}Sr , ^{87}Sr and ^{88}Sr , ^{87}Sr is radiogenic²⁷ due to the β^- decay of the long-lived radio nuclide ^{87}Rb (half-life $t_{1/2} = 48.8 \times 10^9\text{y}$) to generate ^{87}Sr . The mean natural abundances²⁸ of ^{84}Sr , ^{86}Sr and ^{88}Sr remain constant, but ^{87}Sr gradually increases if ^{87}Rb is present in the soil, which is generally the case. The $^{87}\text{Sr}/^{86}\text{Sr}$ ratio was shown not to be affected by biological isotope fractionation processes^{29,30}. Accurate determination of the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio requires a measurement precision of 0.01% or better and Sr isotope ratio studies are therefore mostly done by high resolution mass spectrometry, such as thermal-ionization mass spectrometry (TIMS). The technique is known for its excellent precision (%RSD of $\pm 0.002\%$) and accuracy, but analyses are time-consuming, expensive and difficult to perform^{27–30}. Quadrupole-based ICP-MS, on the other hand, provides an alternative method for the determination of strontium isotope ratios, with better sensitivity and higher sample throughput, albeit with limited precision²⁷. Specific problems^{31–34} related to the determination of B and Sr isotope ratios with quadrupole-based ICP-MS, however, need to be addressed. In the case of B, these include the 'boron memory effect', interference of the large ^{12}C at mass 11, and matrix interferences peculiar to wine and digested wine

*To whom all correspondence should be addressed. E-mail: pccoetzee@uj.ac.za

Table 1 Sample codes and information for the wine samples sourced from four well-known South African wine regions.

Sample code	Wine cellars	Wine	Sample code	Wine cellars	Wine
Robertson			Swartland		
R 1	McGregor	Shiraz	SW1	Paardeberg	Chenin Blanc
R 2	McGregor	Cabernet Sauvignon	SW 2	Paardeberg	Chenin Blanc
R 3	Rooiberg	Shiraz	SW 3	Paardeberg	Chenin Blanc
R 4	Rooiberg	Merlot	SW 4	Paardeberg	Cabernet Sauvignon
R 5	McGregor	Shiraz	SW 5	Paardeberg	Shiraz
R 6	McGregor	Chardonnay	SW 6	Paardeberg	Pinotage
R 7	McGregor	Sauvignon Blanc	SW 7	Paardeberg	Pinotage
R 8	Clairvaux	Chardonnay	SW 8	Paardeberg	Shiraz
R 9	Clairvaux	Merlot	SW 9	Paardeberg	Cabernet Sauvignon
R 10	Roodezandt	Chardonnay	SW 10	Riebeeck	Chardonnay
R 11	Roodezandt	Chardonnay	SW 11	Swartland	Chardonnay
R 12	Roodezandt	Cabernet Sauvignon	SW 12	Swartland	Shiraz
R 13	Roodezandt	Cabernet Franc	SW 13	Swartland	Merlot
R 14	Roodezandt	Shiraz	SW 14	Porterville	Chenin Blanc
R 15	Roodezandt	Cabernet Sauvignon	SW 15	Porterville	Ruby Cabernet
R 16	Bonnievale	Cabernet Sauvignon	SW 16	Porterville	Shiraz
R 17	Bonnievale	Shiraz	SW 17	Porterville	Cabernet Sauvignon
Stellenbosch			SW 18	Porterville	Pinotage
ST 1	L'Avenir	Chenin Blanc	SW 19	Kloovenburg	Shiraz
ST 2	Delheim	Sauvignon Blanc	SW 20	Kloovenburg	Cabernet
ST 3	Overgaauw	Malbec	Walker Bay		
ST 4	Overgaauw	Merlot	WB 1	Beaumont	Chenin Blanc
ST 5	Overgaauw	Chardonnay	WB 2	Beaumont	Malbec
ST 6	Overgaauw	Cabernet Sauvignon	WB 3	Beaumont	Petit Verdot
ST 7	Jordan	Chardonnay	WB 4	Beaumont	Pinotage
ST 8	Jordan	Merlot	WB 5	Newton Johnson	Shiraz
ST 9	Simonsig	Sauvignon Blanc	WB 6	Newton Johnson	Chardonnay
ST 10	Simonsig	Sauvignon Blanc	WB 7	Newton Johnson	Shiraz
ST 11	Simonsig	Sauvignon Blanc	WB 8	Hamilton Russell	Pinot Noir
ST 12	Simonsig	Semillon	WB 9	Hamilton Russell	Chardonnay
ST 13	Groot Constantia	Shiraz	WB 10	Hermanuspietersfontein	Cabernet Franc
ST 14	Groot Constantia	Merlot	WB11	Hermanuspietersfontein	Sauvignon Blanc
ST 15	Groot Constantia	Chardonnay	WB12	Hermanuspietersfontein	Merlot
ST 16	Groot Constantia	Sauvignon Blanc			
ST 17	Alexanderfontein	Chardonnay			
ST 18	Eikendal	Sauvignon Blanc			

matrices¹⁵. In the case of Sr, the isobaric overlap of ⁸⁷Rb and ⁸⁷Sr signals in ICP-MS analysis requires that a chemical separation of strontium and rubidium must be done prior to strontium isotope ratio measurements.

In this work the determination of the ¹¹B/¹⁰B and the ⁸⁷Sr/⁸⁶Sr isotope ratios in the wines and soils of four major wine-producing regions of South Africa using quadrupole-based ICP-MS was studied. The purpose was to obtain a wine fingerprint for each wine and to establish whether the wines of the four regions could be classified according to area of production using this information. A second objective was to ascertain whether a link between the B or Sr isotope ratio in a wine and that in its provenance soil could be demonstrated since this is an important prerequisite for a successful fingerprinting application. The use of isotope ratio data together with element concentration data for selected elements as independent variables in a multivariate statistical analysis to facilitate the classification of the wines according to geographical origin was also tested.

2. Experimental

2.1 Wine and Soil Samples

A total of 67 wine samples (29 white, 38 red) of the 2005 and 2006 vintages was collected from four wine-producing regions in the Western Cape: Robertson (16: 5 white, 11 red),

Stellenbosch (17: 11 white, 6 red), Swartland (22: 8 white, 14 red) and Walker Bay (12: 4 white, 8 red). All the wines were made from grapes produced in known vineyards from the region. Four wines sourced from the Groot Constantia cellar in the Constantia ward were considered part of the Stellenbosch region for this study because of similar soil types in both regions. Table 1 summarizes cellars and cultivars of the wines that were sampled for this project.

Soil samples were collected from the corresponding vineyards, and therefore from the provenance soil, for each of the wine samples. Soil samples were taken from each vineyard at three different positions in the block associated with a particular wine. Samples were taken 30 cm below the surface with non-metallic grabs and sealed in Ziplock® bags. For the purpose of the isotope ratio studies, equal portions of the three soil samples per block were combined and thoroughly mixed. The soil samples were then partitioned using Tyler screens into three particle fractions (> 355 μm, > 180 μm, and < 180 μm). The fractions with particle size <180 μm were used for further sample preparation.

2.2. Sample Preparation

2.2.1. Microwave Digestion

Wine samples for Sr analysis were pre-concentrated before microwave digestion by reducing 10 mL wine to ca. 1 mL by

Table 2 Microwave digestion temperature programme employed for the digestion of wine and soil samples.

Duration/min	T/°C	Power/W	Temp. gradient/°C
10	180	1000	0–180
10	180	1000	180

evaporation in an oven at 70 °C. The analysis of B required no pre-concentration. The method for microwave digestion of wine and soil samples for isotope ratio measurement using a Milestone Ethos system, was: 1 mL of wine or 1 g of soil, 4 mL 65 % HNO₃ (Merck Suprapur), and 2 mL 30 % H₂O₂ (Merck pro analysi) were microwaved for 20 min at 180 °C. The temperature programme used for the digestion of both wine and soil samples is given in Table 2.

After digestion, the wine and samples were made up to a final volume of 100 mL with deionized water. Soil samples were filtered using 0.45 µm cellulose nitrate filters (Sartorius) in order to remove residual silicate particles from solution.

2.2.2. Ion Exchange Separations

The isobaric overlap of ⁸⁷Sr and ⁸⁷Rb requires the separation of these two elements prior to the instrumental measurements of ⁸⁷Sr/⁸⁶Sr ratios. Rb-free samples were obtained by applying an ion exchange method developed for this study and described elsewhere³⁵. The method used Dowex 50W-X8 (200–400 mesh) ion exchange resin (Sigma Aldrich) packed in columns and elution with DCTA (*trans*-1,2-diamino-cyclohexane-N,N,N',N'-tetraacetic acid) for the isolation of strontium from the digested wine and soil matrices. It was found to be effective in eliminating the majority of the Rb interference.

Matrix interference in B isotope ratio measurements required the isolation of B from interfering matrix components still present after microwave digestion. An ion exchange procedure³⁶, with Amberlite IRA 743 B-specific resin (Saarchem), was modified and used for the isolation of B compounds. The pH of a microwave-digested wine or soil sample (30 mL) was adjusted to 10.00 with 3 M NH₃ (Merck) to convert all of the boron present in the sample to the borate ion form, B(OH)₄⁻. The sample was then loaded onto the conditioned resin in its cationic form, and washed with 30 mL of Milli-Q water. Thereafter, the total B was eluted with 100 mL of 2 M HNO₃. The resin was regenerated by addition of 6 M HCl in order to be reused in further separations.

Pyrex Quickfit (UK) borosilicate glass columns of 1.0 cm inner diameter with a fused-in sintered glass frit of No. 0 porosity and Rotaflo[®] screw tap at the bottom were used as columns in both separations.

2.3 Instrumentation

The boron and strontium isotope ratios were determined using a Thermo Electron X Series 2 quadrupole ICP-MS equipped with a Meinhard concentric nebulizer, conical spray chamber with impact bead, Ni sampler and skimmer cones, and dual mode electron multiplier detector. Measurements were made in counting mode. A CETAC ASX-510 automatic sampler was utilized to aid in sample introduction. Instrumental conditions for the ICP-MS were optimized, after mass calibration and detector cross calibration, for maximum B or Sr count rates by following a manual tuning procedure. Thermo Tuning Solution A, containing the elements ⁷Li, ⁹Be, ⁵⁹Co, ¹¹⁵In, ¹³⁸Ba, ¹⁴⁰Ce, ²⁰⁶Pb and ²³⁸U at 10 µg L⁻¹, was used for tuning. The operating conditions are summarized in Table 3.

Detector dead time corrections are critically important for

Table 3 Quadrupole ICP-MS parameter settings chosen to acquire optimal operating conditions.

Parameter	Settings
RF power	1.4 kW
Plasma gas flow rate	14 L min ⁻¹
Auxiliary gas flow rate	0.7 L min ⁻¹
Nebulizer gas flow rate	0.71 L min ⁻¹
Sample uptake rate	1 mL min ⁻¹

Table 4 Optimized parameters for the measurement of the ¹¹B/¹⁰B and ⁸⁷Sr/⁸⁶Sr ratio in wine and soil samples with ICP-QMS.

Parameter	Settings	
	Boron	Strontium
Sample uptake time	30 sec	30 sec
Number of replicate readings	5	7
Dwell time	10 ms	10 ms
Number of sweeps	3000	3000
Wash time between samples	300 sec	60 sec
Detector dead time	35 ns	55 ns

accurate isotope ratio determinations. Accurate detector dead times were determined separately for B and Sr measurements because they are dependent on analyte mass³⁷. The different detector dead times were used in the instrument set-up to apply the appropriate count rate corrections during B and Sr isotope ratio measurements. Optimized acquisition parameters for the determination of B and Sr isotope ratios are given in Table 4. A long wash-out time of 300 s with 1 % HNO₃ was required to eliminate the B memory effect during instrumental analysis of B isotope ratios.

2.4 Isotope Ratio Measurements

Determination of the B isotope ratio, ¹¹B/¹⁰B, by means of quadrupole-based ICP-MS, is complicated by a large mass discrimination effect (because of the relatively large mass difference between the two B isotopes) and the drift in the mass discrimination during measurement which may lead to a concomitant drift in the measured isotope ratio. This problem, which to a lesser extent also occurs for Sr isotope ratio determination, was solved by using an external standard approach^{13,15} where samples and the isotope standard were measured alternately in the following 'bracketing' measurement sequence: standard-sample1-standard-sample2-standard. This method assumes that the drift in mass discrimination is linear with respect to time between the analyses of the standards bracketing a sample measurement. Mass discrimination correction is then achieved by applying the equation:

$$R_{i-\text{corr}}^s = R_i^s \times \frac{R_{\text{ref}}^{\text{std}}}{(R_{i-1}^{\text{std}} + R_{i+1}^{\text{std}})/2}$$

The isotopic ratios of the standard, R_{i-1}^{std} and R_{i+1}^{std} are measured as closely as possible in time before and after the measurement of the *i*th sample with isotope ratio R_i^s . $R_{\text{ref}}^{\text{std}}$ is the reference value for the isotope standard. A 25-µg L⁻¹ Sr isotope standard reference solution (NIST SRM 987) and a 100-µg L⁻¹ B isotope standard reference solution (NIST SRM 951) were used as isotope standards.

The determination of the ⁸⁶Sr/⁸⁷Sr isotope ratio by quadrupole-based ICP-MS requires the ⁸⁷Sr count rate to be corrected for the

Table 5 The $^{11}\text{B}/^{10}\text{B}$ and $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratios obtained for the wine and soil samples from the Robertson, Stellenbosch, Swartland, and Walker Bay wine regions.

Sample code	$^{11}\text{B}/^{10}\text{B}$		$^{87}\text{Sr}/^{86}\text{Sr}$		Sample code	$^{11}\text{B}/^{10}\text{B}$		$^{87}\text{Sr}/^{86}\text{Sr}$	
	Soil	Wine	Soil	Wine		Soil	Wine	Soil	Wine
Robertson					Swartland				
R 1	4.066	4.067	0.7133	0.7145	SW 1			0.7096	0.7075
R 2	4.077	4.067	0.7128	0.7136	SW 2	4.037	4.045	0.7107	0.7091
R 3	4.065	4.065	0.7144	0.7141	SW 3	4.044	4.044	0.7133	0.7093
R 4	4.045	4.068	0.7136	0.7154	SW 4	4.033	4.038	0.7127	0.7083
R 5	4.064	4.073	0.7134	0.7136	SW 5	4.039	4.033	0.7082	0.7135
R 6	4.072	4.076	0.7139	0.7125	SW 6	4.035	4.039	0.7149	0.7090
R 7			0.7130	0.7113	SW 7	4.031	4.045	0.7081	0.7079
R 8	4.075	4.068	0.7132	0.7129	SW 8	4.034	4.039	0.7115	0.7101
R 9	4.062	4.063	0.7144	0.7141	SW 9	4.043	4.030	0.7085	0.7094
R 10	4.054	4.066	0.7152	0.7147	SW 10	4.044	4.043	0.7148	0.7099
R 11	4.065	4.078	0.7147	0.7116	SW 11	4.035	4.041	0.7137	0.7101
R 12	4.058	4.071	0.7149	0.7125	SW 12	4.037	4.044	0.7132	0.7084
R 13	4.069	4.062	0.7142	0.7139	SW 13	4.035	4.040	0.7121	0.7115
R 14	4.060	4.074	0.7149	0.7113	SW 14	4.037	4.044	0.7126	0.7090
R 15	4.064	4.078	0.7155	0.7146	SW 15	4.040	4.040	0.7110	0.7110
R 16	4.053	4.075	0.7133	0.7132	SW 16	4.047	4.037	0.7140	0.7114
R 17	4.062	4.062	0.7153	0.7144	SW 17	4.034	4.038	0.7125	0.7094
Stellenbosch					Walker Bay				
ST 1	4.064	4.045	0.7119	0.7079	WB 1	4.059	4.053	0.7120	0.7099
ST 2	4.072	4.044	0.7159	0.7082	WB 2	4.059	4.060	0.7136	0.7094
ST 3	4.066	4.055	0.7128	0.7100	WB 3	4.055	4.060	0.7138	0.7089
ST 4	4.073	4.048	0.7089	0.7110	WB 4	4.045	4.057	0.7128	0.7093
ST 5	4.082	4.054	0.7158	0.7087	WB 5	4.061	4.049	0.7101	0.7098
ST 6	4.083	4.049	0.7159	0.7109	WB 6	4.040	4.058	0.7136	0.7105
ST 7	4.075	4.055	0.7091	0.7104	WB 7	4.057	4.067	0.7110	0.7131
ST 8	4.067	4.046	0.7100	0.7105	WB 8	4.044	4.049	0.7092	0.7110
ST 9	4.066	4.041	0.7129	0.7097	WB 9	4.054	4.068	0.7108	0.7088
ST 10	4.068	4.040	0.7107	0.7108	WB 10	4.047	4.050	0.7129	0.7110
ST 11	4.071	4.056	0.7082	0.7078	WB 11	4.051	4.063	0.7130	0.7078
ST 12	4.068	4.051	0.7107	0.7081	WB 12	4.050	4.063	0.7128	0.7101
ST 13	4.080	4.048	0.7137	0.7109					
ST 14	4.072	4.052	0.7129	0.7104					
ST 15	4.076	4.056	0.7137	0.7076					
ST 16	4.070	4.042	0.7121	0.7080					
ST 17	4.074	4.053	0.7129	0.7070					
ST 18	4.072	4.043	0.7131	0.7101					

small residual rubidium present, even after ion exchange separation of the Sr from Rb in the wine and soil samples. To apply this correction ^{88}Sr was measured in addition to ^{86}Sr and ^{87}Sr . The natural isotope abundance of ratio $^{87}\text{Sr}/^{86}\text{Sr}$ was then used to derive the corrected ^{87}Sr count rate with adequate accuracy^{27,28}. Sr isotope ratios differ only slightly from place to place and isotope ratio precisions of better than 0.1 % are therefore normally required for fingerprinting applications. An isotope ratio precision of ca 0.1 % with quadrupole ICP-MS is regarded as the best level achievable with this technique. Careful optimization is, however, required. In this work an overall strontium isotope ratio precision of ~0.1 % RSD was achieved by judicious adjustment of acquisition parameters: long integration times but short dwell times (10 ms), a large number of sweeps (3000) and seven replicate readings. Peristaltic pump tubing was regularly replaced and cones and nebulizer regularly cleaned.

3. Results and Discussion

3.1 Boron Isotope Ratios

The $^{11}\text{B}/^{10}\text{B}$ isotope ratios for the wine and soil samples of the four wine regions are listed in Table 5. The average relative standard deviation for the measured isotope ratios in the samples

was 0.08 % RSD for B and 0.09 % RSD for Sr. The overall variation in precision, based on measurement statistics, was relatively small, <30 %. Standard deviations for the isotope ratios of individual samples were therefore not included in Table 3 to make it easier to read. Box plots of the B isotope ratios for the wine and soil samples for each region are graphically represented in Figs 1 and 2, respectively. The box length represents the inter quartile range (IQR) and includes 50 % of the data. The median is shown by the horizontal line in the middle of box. The upper edge of the box plot is the 3rd quartile (Q_3) and the lower edge is the 1st quartile (Q_1). The position of the median line relative to the quartile lines, shows the skewness of the data. The whiskers represent the range of the data and is calculated from $Q_3 + 1.5 \times (\text{IQR})$ for the higher limit and $Q_1 - 1.5 \times (\text{IQR})$ for the lower limit.

The average $^{11}\text{B}/^{10}\text{B}$ isotope ratios for the wine samples of the four wine regions were found to be distinctly different for each region as is evident from the results displayed in Fig. 1. ANOVA results confirm that the averages are different with a P value <0.0001 at the 95 % confidence level. The highest $^{11}\text{B}/^{10}\text{B}$ isotope ratios were obtained for the wine samples from the Robertson (4.070) region. The $^{11}\text{B}/^{10}\text{B}$ isotope ratios of the samples from the Walker Bay region were slightly lower (4.058) than that of

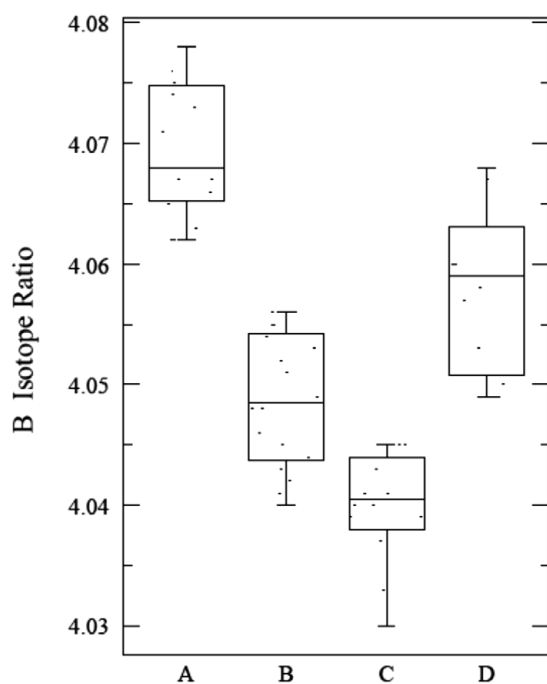


Figure 1 Box plots of the variation in the $^{11}\text{B}/^{10}\text{B}$ isotope ratios of wine samples originating from four wine regions of South Africa. A = Robertson, B = Swartland, C = Stellenbosch, D = Walker Bay.

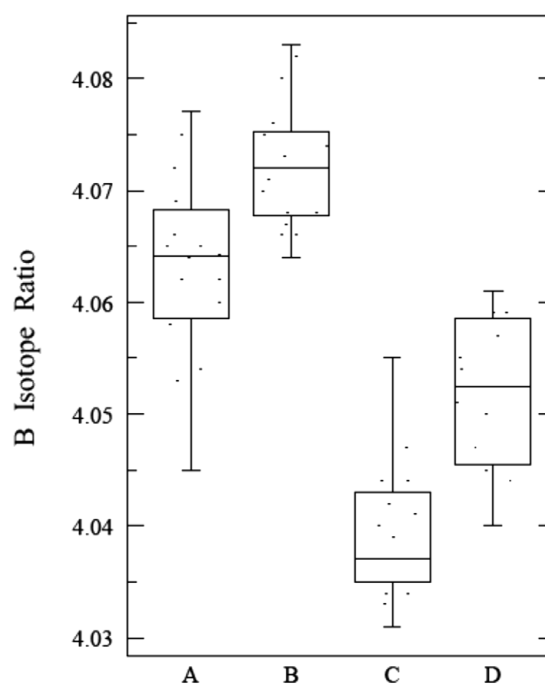


Figure 2 Box plots of the variation in the $^{11}\text{B}/^{10}\text{B}$ isotope ratios of soil samples originating from four wine regions of South Africa. A = Robertson, B = Swartland, C = Stellenbosch, D = Walker Bay.

Robertson. The Stellenbosch and Swartland samples had significantly lower $^{11}\text{B}/^{10}\text{B}$ isotope ratios.

The results in Fig. 2 indicate that the soil samples from the four different wine regions were also distinguishable with regard to their $^{11}\text{B}/^{10}\text{B}$ isotope ratios. ANOVA results confirm that the averages are different with a P value <0.0001 at the 95 % confidence level. The highest $^{11}\text{B}/^{10}\text{B}$ isotope ratios were obtained for the Stellenbosch (4.072) and Robertson (4.063) regions. The lowest $^{11}\text{B}/^{10}\text{B}$ isotope ratios were acquired for the Swartland region. The samples of the Walker Bay region had $^{11}\text{B}/^{10}\text{B}$ isotope ratios that were slightly higher than those of the Swartland region.

In order to determine whether a correlation exists between the boron isotope ratio obtained for the wine and soil of a specific region, the average ratio for the wines of a region was divided by the average ratio for the soils. A value close to 1 for this ratio, herein referred to as the w/s-ratio, indicates good wine–soil isotope ratio correlation. The w/s-ratio for each wine region is given in Table 6. The four wine regions studied showed characteristic $^{11}\text{B}/^{10}\text{B}$ isotope ratios for the wine and soil samples with excellent correlation between wine and soil, since a w/s-ratio ≈ 1 had been obtained for all four wine regions.

The difference in the $^{11}\text{B}/^{10}\text{B}$ ratios among the wine regions could be related to the geographical location and underlying geochemistry of the regions. The predominant soil type throughout the Robertson region, situated in the Breede River valley, is limestone. Limestone-containing soils show $^{11}\text{B}/^{10}\text{B}$ isotope ratios in the range³⁸ of 4.044–4.084. The average boron

isotope ratio obtained for the Robertson region of 4.063 falls well within this range. The granite, shale, and sand soils of the Stellenbosch area show the $^{11}\text{B}/^{10}\text{B}$ range³⁹ to be 4.035–4.071. The average value of 4.072 for Stellenbosch soils measured in this study lies to the upper edge of this range. The average B isotope ratio 4.039 obtained for the Swartland soils could be related to the isotope range⁴⁰ in sandstone (4.001–4.034) found in this region, as well as other metamorphic rocks with a boron isotope range⁴¹ of 3.890–4.125. The Walker Bay wine region has soils consisting mostly of weathered shale and marine sandstone. The average soil boron isotope ratio of 4.052 obtained for the Walker Bay region falls within the $^{11}\text{B}/^{10}\text{B}$ range of 4.038–4.064 obtained for evaporate minerals⁴². Since all the wine regions have a w/s-ratio close to 1, it could be concluded that the $^{11}\text{B}/^{10}\text{B}$ ratio of wine produced in the four regions correlates well to the B isotope ratio of the provenance soil.

The use of B isotope ratios together with elemental concentrations of selected indicator elements for the same wines, as independent variables in a multivariate statistical analysis, produced a highly successful classification of the wines according to geographical origin. The elements were selected on the basis of their proven ability as indicator elements in fingerprinting of wine⁶. The element concentrations were determined in a parallel study⁴³ on the multi-element analysis of the same wines by ICP-MS. By using stepwise discriminant analysis, canonical discriminant functions for the combined boron isotope ratio and multi-element wine and soil data have been derived using the statistical package SPSS. The determination of discriminant functions entails the derivation of linear groupings of independent variables in order to best differentiate between dependant variables (wine-producing regions). The isotope ratios and elemental concentrations were \log_e transformed to ensure that the varying elemental concentrations fall within the same numerical range so as not to distort the statistical analysis. The canonical discriminant functions enabled the classification of a sample into one of the four regions. The classification ability of the model is expressed as a percentage. The effectiveness of the classification model was

Table 6 The average $^{11}\text{B}/^{10}\text{B}$ ratios obtained for wine and soil samples together with the wine/soil ratio (w/s) for each wine region.

Region	$^{11}\text{B}/^{10}\text{B}$: Soil	$^{11}\text{B}/^{10}\text{B}$: Wine	w/s
Robertson	4.063 \pm 0.002	4.070 \pm 0.003	1.001
Swartland	4.039 \pm 0.003	4.038 \pm 0.004	1.000
Stellenbosch	4.072 \pm 0.004	4.049 \pm 0.003	0.994
Walker Bay	4.052 \pm 0.004	4.058 \pm 0.005	1.001

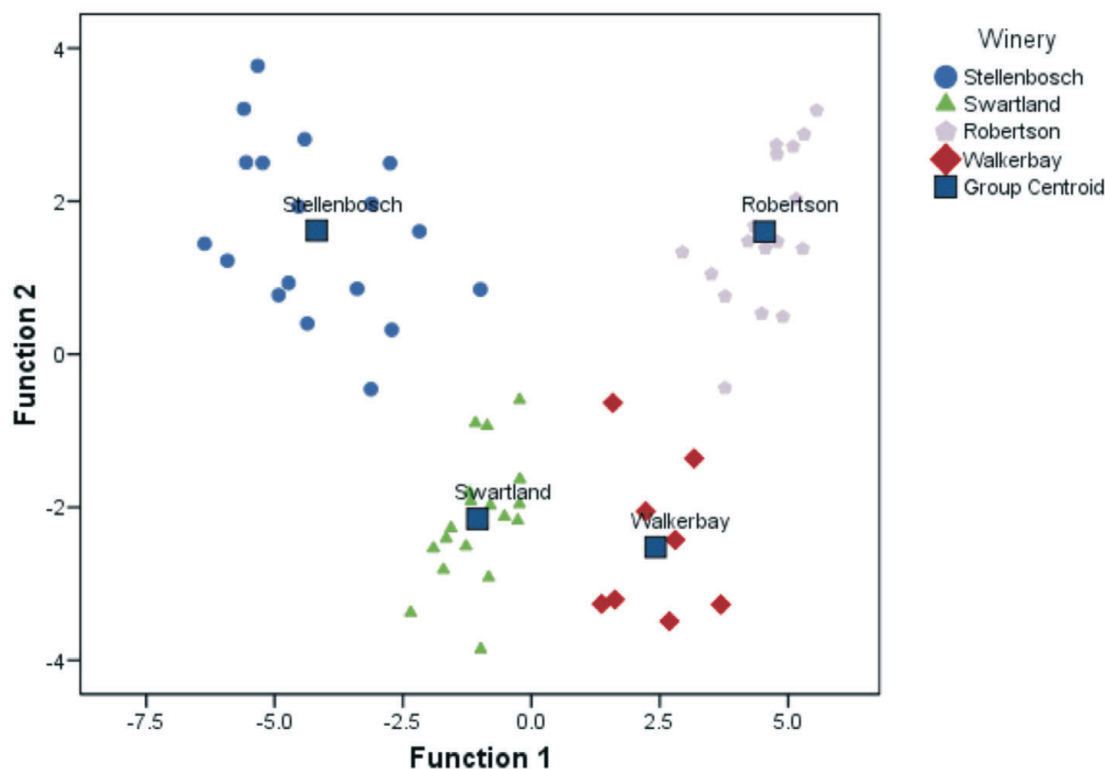


Figure 3 A graphical representation of the canonical discriminant functions used to classify the wine samples of the four wine regions using a combination of $^{11}\text{B}/^{10}\text{B}$ and multi-element concentration data as independent variables.

evaluated through a cross-validation procedure. This involves the removal of each sample in turn from the approximation of model constants, and determining its membership by using the resulting model. As for the discriminant function, the classification ability of the cross-validation model is also expressed as a percentage. The graphical representation of the results of discriminant analysis of $^{11}\text{B}/^{10}\text{B}$ isotope ratio and multi-element concentration data for the four wine regions, are shown in Fig. 3. No systematic difference in the isotope ratios for white and red wines for each region was observed.

The statistical data represented in Fig. 3 for the wine samples indicate that the four South African wine regions were distinguishable by means of the first two component functions arising from the elemental concentrations of Rb, Li, Sc, and B and the $^{11}\text{B}/^{10}\text{B}$ isotope ratio data. The data showed that the classification of the wine samples from Robertson, Swartland and Walker Bay was 100 % successful. For the Stellenbosch region, 94.4 % of the wine samples were classified successfully. One of the Stellenbosch wine samples was found to be closer to the group centroid of the Swartland region and was therefore classified to the Swartland region. An overall classification of 98.4 % was obtained. This was also the case after a cross validation procedure was performed.

3.2 Strontium Isotope Ratios

The Sr isotope ratios obtained for the wine and soil samples are listed in Table 5. Box plots of Sr isotope ratios for the wine and soil samples for each region are represented in Figs 4 and 5, respectively. By inspection of Fig. 4, and through the interpretation of multiple ANOVA comparisons of the data, it appears that the average Sr isotope ratio and the bulk of the wines from the Robertson region are distinguishable from wines from the remaining three wine regions. The same pattern is recognizable in Fig. 5 for the soil data. Table 7 summarizes the average Sr isotope ratio for wines and soils and w/s-ratio for each of the

regions. For the wine samples, a mean $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of 0.7134 had been obtained for Robertson compared to the respective mean $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of Stellenbosch, Swartland and Walker Bay of 0.7093, 0.7100 and 0.7100. The same trend could be seen for the soil mean $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of the four regions. The mean $^{87}\text{Sr}/^{86}\text{Sr}$ ratio for the Robertson soil was 0.7141, while the mean isotope ratio for the other three regions were 0.7123, 0.7120 and 0.7121 respectively. The w/s-ratio is close to 1 for all four regions,

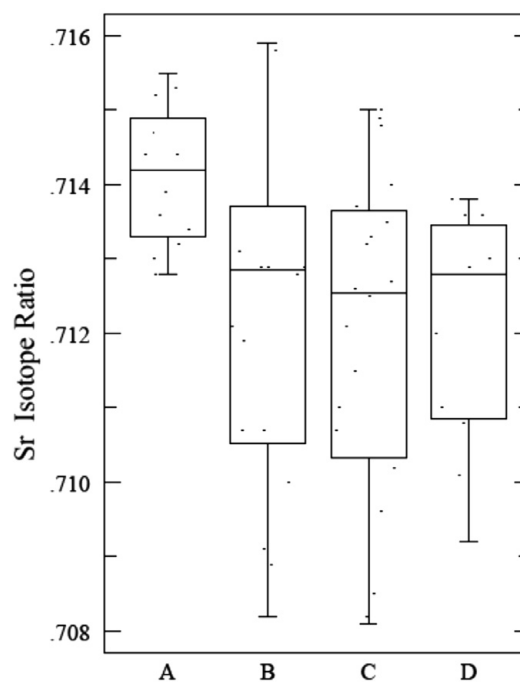


Figure 4 Box plots of the variation in the $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratio of wine samples originating from four wine regions of South Africa. A = Robertson, B = Swartland, C = Stellenbosch, D = Walker Bay.

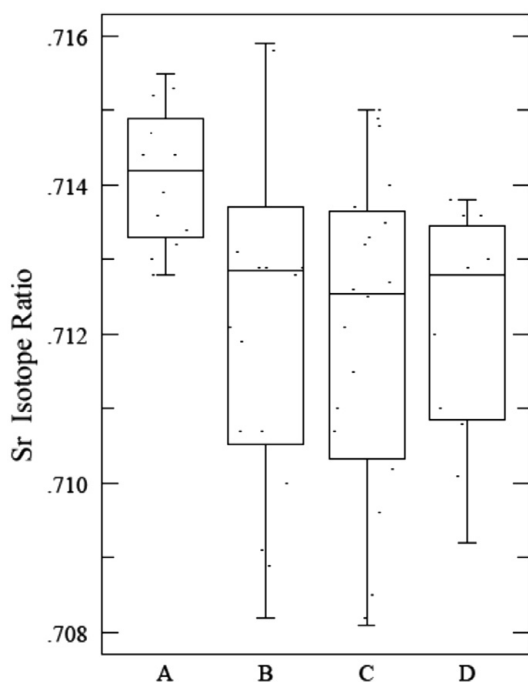


Figure 5 Box plots of the variation in the $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratio of soil samples originating from four wine regions of South Africa

Table 7 The average $^{87}\text{Sr}/^{86}\text{Sr}$ ratios obtained for wine and soil samples together with the wine/soil ratio (w/s) for each wine region.

Region	$^{87}\text{Sr}/^{86}\text{Sr}$: Soil	$^{87}\text{Sr}/^{86}\text{Sr}$: Wine	w/s
Robertson	0.7142 ± 0.0009	0.7134 ± 0.0012	0.9989
Swartland	0.7123 ± 0.0023	0.7093 ± 0.0014	0.9958
Stellenbosch	0.7120 ± 0.0022	0.7100 ± 0.0018	0.9972
Walker Bay	0.7121 ± 0.0015	0.7100 ± 0.0014	0.9971

confirming the soil–wine correlation in $^{87}\text{Sr}/^{86}\text{Sr}$ ratios, but unlike for boron isotope ratios, the average Sr isotope ratios for three of the four regions were similar, making it impossible to distinguish among these regions

4. Conclusions

The $^{11}\text{B}/^{10}\text{B}$ isotope ratio as determined by quadrupole-based ICP-MS, was found to be a useful tool to distinguish among the wines from four major South African wine regions. An essential requirement in provenance determination is that a link between the agricultural product and the provenance soil must exist. A good correlation between the B isotope ratio in wine and its provenance soil was found in this study and thus supports this requirement. B isotope ratio measurements with adequate precision were obtained confirming the use of quadrupole ICP-MS in fingerprinting applications with $^{11}\text{B}/^{10}\text{B}$ ratios. The isolation of total B from the sample matrix prior to the determination of the $^{11}\text{B}/^{10}\text{B}$ isotope ratio is, however, required. The combination of B isotope ratios and elemental concentrations of selected indicator elements in stepwise linear discriminant analysis, was shown to be a highly successful method to classify wine according to geographical origin.

The $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratios obtained for the wine and soil samples have limited merit as a provenance indicator in the wine regions included in this study, since only one wine-producing region could be distinguished from the rest. From an analytical point of view it was shown that the isobaric overlap of

^{87}Rb on ^{87}Sr could effectively be reduced by means of the newly-developed Sr-Rb ion exchange separation method. Relatively good precision of $\sim 0.1\%$ RSD for strontium isotope ratio measurements could be obtained using quadrupole-based ICP-MS.

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