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Chemometrics approaches for the study of systematic error in inductively coupled plasma atomic emission spectrometry and mass spectrometry†‡

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Systematic errors observed when using inductively coupled plasma atomic emission spectrometry (ICP-AES) and mass spectrometry (ICP-MS) for the multi-element determination in acid digests of environmental samples (tea leaves) were evaluated. Two chemometric approaches, experimental design and principal component analysis, were used in order to establish the errors associated with each stage of the analytical method: sample digestion (effect of the "number of acid digestions") and measurement step (effect of the "number of replicates" and the "calibration"). The elements under study were Co, Cr, Cs, Cu, Ni, Pb, Rb and Ti by ICP-MS, and Ba, Ca, Fe, Mg, Mn, Sr and Zn by ICP-MS and ICP-AES. Flame atomic absorption spectrometry was used for comparative purposes. A Chinese tea certified reference material with certified concentration for most of the elements was employed as the sample matrix. Variance estimation was made from ANOVA outputs from a full factorial design (FFD) $4^1 \times 4^1 \times 2^1$.

Introduction

Any analytical method must offer not only adequate performance in terms of analyte sensitivity, but also good precision and accuracy. One of the simplest means of determining the accuracy of a method is to analyse a certified reference material (CRM) for which the concentrations of the analytes are known with high accuracy and precision.[1] The mean of replicate determinations obtained after application of the analytical method under study may differ from the known concentration present in the CRM. This difference observed between the certified and determined values can arise from both method bias and random errors and it is often important to characterise the type of error and, if method bias is detected, the causes of such errors. For a general procedure involving the analysis of a solid sample by ICP-AES or ICP-MS, such errors may be split into two groups: the first corresponding to errors during the sample pre-treatment (incomplete dissolution and/or loss of analytes of interest), and the second, to the measurement process itself (for instance, matrix interferences in the plasma). Because of the complexity of the problem and because more than one variable must be taken into account, it is evident that the study cannot be carried out with conventional univariate techniques, as the error obtained for a certain element and instrumental technique is related to the overall variables rather than one by one. Therefore the use of multivariate statistical methods is necessary to find out the relationship between such variables and the effect of them on the systematic error.

In order to evaluate the sources of variation in an analytical method, the analysis of variance (ANOVA) technique is a well-established methodology. This approach compares both systematic and random errors with the purpose of determining whether or not the factors (attributed to a sample pre-treatment or to a measurement procedure) have a significant effect. Many applications can be found in the literature aimed at solving a range of different problems, from the comparison of several procedures to the breakdown of the total precision into its components, such as between-days and within-days, between-laboratories and within-laboratories, *etc.*[2] Although an *n*-way layout ANOVA can be established for *n* factors, most commercial statistical packages offer only one and two-way layout ANOVA tests. This is a limitation when more than 2 factors must be studied. However, one of the more important applications of the ANOVA test is its use as a preliminary stage in the experimental design approach, for which it can be applied to a number of factors higher than 2.[3] In this sense, the use of ANOVA to evaluate the significance of certain factors in an analytical procedure, by an experimental design study, is the basis of assessing and establishing the ruggedness of that analytical procedure.[4]

Besides ANOVA and experimental design approaches, principal component analysis (PCA) and multivariate regression methods, such as multiple linear regression (MLR), principal component regression (PCR) and partial least squares (PLS), are known as useful multivariate techniques for many fundamental and applied studies.[5–7] Applications of these methods to atomic spectrometry can be found in the literature, such as the work of Vaughan and Horlick,[8] who successfully applied PCA to correct spectral overlap problems in the determination of rare earth metals by ICP-MS; van Veen *et al.*,[9] who studied and corrected spectral interferences in ICP-MS by PCA and MCA; and Brenner *et al.*,[10] who studied the depressive effects of nitric acid on the line intensities of rare earth elements in ICP-AES. Fernandez *et al.*[11] have applied an experimental design approach in order to evaluate the influence of the operating parameters on the effect of acid concentration

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Table 1 Operating parameters for ICP-MS

Forward power/W	1350
Gas flows/l min^{-1}	
Nebulizer	0.85
Auxiliary	1.0
Coolant	15.0
Nebulizer type	Ebdon, high solids
Data acquisition for quantitative analysis	Peak jump
Internal standard	In, 100 μg l^{-1}
Isotopes monitored	^{24}Mg, ^{48}Ti, ^{52}Cr, ^{54}Fe, ^{55}Mn, ^{59}Co, ^{60}Ni, ^{65}Cu, ^{66}Zn, ^{85}Rb, ^{88}Sr, ^{133}Cs, ^{138}Ba, ^{206}Pb, and ^{207}Pb

Table 2 Operating conditions for ICP-AES

Forward power/W		1000
Gas flows/l min^{-1}	Coolant	15.0
	Auxiliary	1.5
Nebulizer type		Cross flow
Nebulizer pressure/kPa		150
Pump speed/rev min^{-1}		20
PMT voltage/V		650
Stabilisation time/s		10
Detection wavelengths/nm	Ba	455.403
	Ca	393.366
	Cu	324.754
	Fe	259.940
	Mg	279.533
	Mn	257.610
	Sr	407.771
	Zn	213.856

for certain atomic and ionic emission lines in ICP-AES. Finally PCR and PLS methods have been applied by Rupprecht and Probst[12] to correct interferences in ICP-MS, while Tangen and Lund[13] have applied PLS for the multivariate study of nitric acid concentration and liquid flow rate on the signal from 13 elements by ICP-MS.

The aim of this study is to apply an experimental design to evaluate the significance of three different factors (“number of acid digestions”, “number of measurements” and “calibration”) in the determination of several elements (Ba, Ca, Cd, Co, Cr, Cs, Cu, Fe, Mg, Mn, Ni, Pb, Rb, Sr, Ti and Zn) by ICP-AES and ICP-MS. The mean element concentrations in the analysis of a well-characterised sample matrix of a Chinese tea reference material has been the response variable used in the study. In addition, scores and loadings obtained by principal component analysis (PCA) were also used in order to determine the optimum “number of replicates” and “number of acid digestions”.

Experimental

Instrumentation

A Liberty 200 ICP-AES (Varian, Walton on Thames, Surrey, UK) and a PlasmaQuad PQ2+ ICP-MS (Fisons Instruments, Winsford, Cheshire, UK) were used in the study.

A SpectrAA 50 FAAS (Varian), equipped with a N_2O–acetylene flame was used for Ca determinations, and a SpectrAA 300/400 FAAS (Varian), equipped with an air–acetylene flame, was used for Cu, Fe, Mg, Mn and Zn determinations. Hollow cathode lamps (Varian) operating at the manufacturers recommended current (Table 3) were used for all FAAS determinations.

Total digestion of the Chinese tea reference material was achieved using a hot plate (SH3, Stuart Scientific, UK) as described below.

A commercial chemometrics package (UNSCRAMBLER, 1998, CAMO ASA, Trondheim, Norway) was used throughout this study.

Reagents

All chemicals used were of ultrapure grade, and diluted using ultrapure water of resistivity 18 MΩ cm obtained from a Milli-Q purification device (Millipore Co., Bedford, MA, USA). AnalaR nitric acid 70.0% was obtained from BDH (Poole, Dorset, UK). Stock standard solutions (1.000, or 10.000 g l^{-1}) were supplied by Merck (Poole, Dorset, UK). A cobalt stock standard solution was purchased from Aldrich (Gillingham, Dorset, UK). The Chinese tea reference material GBW 08505 was obtained from the Bureau of Analysed Samples (Middlesbrough, UK).

Tea acid digestion procedure

A Chinese tea reference material was digested using the method published previously.[14] Tea (0.2500 g) was weighed into a clean beaker and nitric acid (10 ml) was added. The beaker was covered with a watch-glass and the sample boiled gently on a hot-plate until digestion was complete (about 3 h). After cooling to room temperature, the acid digests were transferred quantitatively into 50 ml volumetric flasks. Indium (as an internal standard for ICP-MS measurements) was added to each digest to give a concentration of 100 μg l^{-1} after dilution to 50 ml. The tea acid digests were kept in polyethylene vials at room temperature prior to analysis.

ICP-AES, ICP-MS and FAAS measurements

The elements Ba, Co, Cr, Cs, Cu, Fe, Mg, Mn, Ni, Pb, Rb, Sr, Ti and Zn were measured by ICP-MS without dilution, using the operating parameters and the isotope masses shown in Table 1. In addition Ba, Ca, Fe, Mg, Mn, Sr and Zn were also measured by ICP-AES, also without dilution, using the

Table 3 Operating parameters for FAAS

	Wavelength/nm	Slit width/nm	Lamp current/mA	Air flow rate/l min^{-1}	C_2H_2 flow rate/l min^{-1}
Ca	239.9	0.2	10	10.00[a]	6.35
Cu	324.8	0.7	10	13.50	2.00
Fe	248.3	0.2	6	13.50	2.00
Mg	202.6	0.7	10	13.50	2.00
Mn	279.5	0.2	6	13.50	2.00
Zn	213.9	0.2	10	13.50	2.00

[a]N_2O flow rate.

Table 4 Certified or informative concentrations in the Chinese tea certified reference material GBW 08505 for the elements investigated and techniques employed

Element	Certified concentration/ μg g^{-1}	Technique
Ba	15.7 ± 1.9	ICP-AES, ICP-MS
Ca[a]	0.284 ± 0.021	ICP-AES, FAAS
Co[b]	0.2	ICP-MS
Cr[b]	0.8	ICP-MS
Cs[b]	0.13	ICP-MS
Cu	16.2 ± 1.9	ICP-MS, FAAS
Fe	373 ± 23	ICP-AES, ICP-MS, FAAS
Mg[a]	0.224 ± 0.019	ICP-AES, ICP-MS, FAAS
Mn	766 ± 28	ICP-AES, ICP-MS, FAAS
Ni	7.61 ± 0.48	ICP-MS
Pb	1.06 ± 0.10	ICP-MS
Rb	36.9 ± 1.3	ICP-MS
Sr	10.8 ± 1.8	ICP-AES, ICP-MS
Ti[b]	36	ICP-MS
Zn	38.7 ± 3.9	ICP-AES, ICP-MS, FAAS

[a]Concentration expressed in % (m/m). [b]Informative concentration.

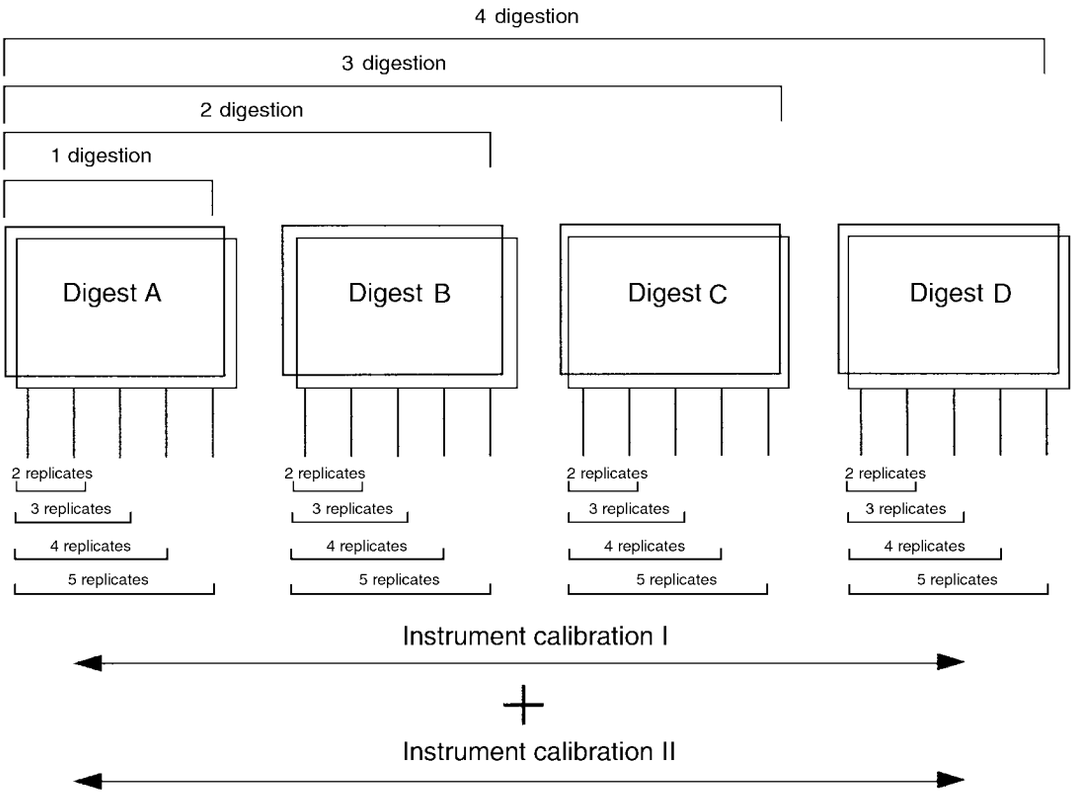

Fig. 1 Scheme showing the hierarchical relationship of sample pre-treatment and ICP-AES/MS measurement.

operating conditions and emission wavelength lines given in Table 2. Finally, for comparative purposes, some elements (Cu, Fe, Mg, Mn, Zn) were also determined by FAAS, using an air–acetylene flame. For Ca, an N_2O–acetylene flame was used. In order to avoid the necessity of sample dilution for the Ca and Mg FAAS measurements, less sensitive resonance lines (239.9 and 202.6 nm, for Ca and Mg, respectively) were used. The operating parameters and atomic absorption resonance lines used in this study for the FAAS measurements are summarised in Table 3.

Results and discussion

Table 4 lists the elements with certified (Ba, Ca, Cu, Fe, Mg, Mn, Ni, Pb, Rb, Sr and Zn) or informative (Co, Cr, Cs and Ti)

Table 5 Pb mean concentrations in the Chinese tea reference material GBW 08505, and relative standard deviations obtained by ICP-MS analysis using the isotope mass ^{206}Pb

^{206}Pb (ICP-MS) certified concentration of 1.06 ± 0.10 μg g^{-1}

	Digests					Number of digestions			
	Mean concentration/μg g^{-1}					Mean concentration/μg g^{-1}			
	A	B	C	D		1	2	3	4
I2	1.18	1.15	1.02	0.93	I2	1.18	1.16	1.11	1.07
I3	1.13	1.05	1.00	0.89	I3	1.13	1.09	1.06	1.02
I4	1.16	1.05	1.04	0.95	I4	1.16	1.11	1.08	1.05
I5	1.11	1.07	1.00	0.99	I5	1.11	1.09	1.06	1.04
II2	1.13	1.14	1.07	1.07	II2	1.13	1.14	1.12	1.11
II3	1.18	1.13	1.08	1.01	II3	1.18	1.16	1.13	1.10
II4	1.23	0.97	1.04	1.07	II4	1.23	1.10	1.08	1.08
II5	1.38	1.04	1.02	1.10	II5	1.38	1.21	1.14	1.13
	RSD (%)					RSD (%)			
	A	B	C	D		1	2	3	4
I2	8.8	2.9	8.5	10.0	I2	8.8	2.0	7.8	11.0
I3	8.8	2.9	8.5	10.0	I3	10.0	4.9	6.0	9.7
I4	10.0	2.8	6.9	9.7	I4	9.6	6.9	6.2	8.4
I5	9.6	2.2	6.4	13.2	I5	13.4	2.4	5.2	5.6
II2	13.4	2.0	5.7	14.6	II2	2.3	1.0	3.3	3.3
II3	2.3	4.0	7.8	7.2	II3	8.2	3.4	4.6	6.8
II4	8.2	3.9	5.5	12.7	II4	10.2	16.5	12.4	10.2
II5	10.2	8.6	9.4	15.0	II5	9.1	19.8	17.6	14.7

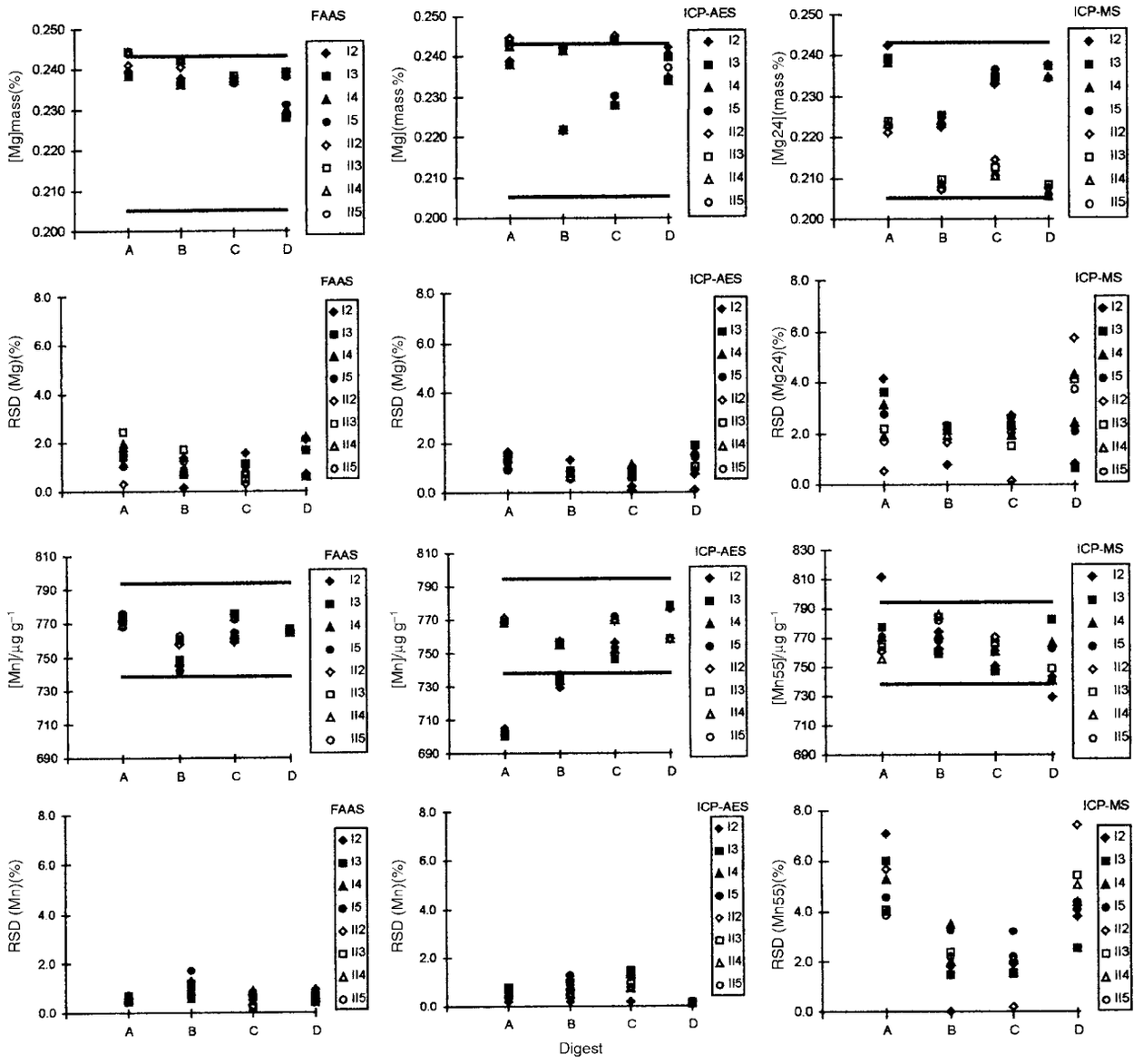

Fig. 2 Mean concentrations and RDSs for the 32 data points from four acid digests labelled as A, B, C and D (each acid digestion has been measured against two different aqueous calibration graphs and using 2, 3, 4 or 5 replicate measurements).

concentrations in the GBW 08505 Chinese tea reference material used in this study. The uncertainty is also listed in Table 4 for those elements which offer certified concentrations.

The hierarchical scheme followed is shown in Fig. 1. In general, four acid digestions of the certified reference material were performed in parallel. This replication gave information about the effect of the acid digestion process on the uncertainty in the analysis. Each acid digest was analysed for each element by the techniques listed in Table 4, using two independent aqueous calibration graphs, prepared independently and covering an adequate range of concentrations, and the analyses were performed using 2, 3, 4 and 5 replicate measurements. The procedure for calibration when using the three techniques was based on a weighted least squares (weighted regression). This gave information about the effect of the calibration on the results, while the replicate measurements showed the repeatability, which is a part of the overall uncertainty due to the technique itself. It can be expected that the contribution of the instrumental measurement to the variation can be minimised simply by increasing the number of replicate measurements.[15]

Using this procedure, the sources of errors were replicated in the 32 measurements. The first 16 measurements corresponded to the first calibration, and are followed by the second 16 from the second calibration. An example of the 32 results obtained is given in Table 5 for the determination of Pb by ICP-MS using the isotope mass ^{206}Pb. The results for digests A, B, C and D correspond to mean concentration values for 2, 3, 4 and 5 replicate measurements (calibration 1 and 2) considering each digest individually. However, results under the heading 1, 2, 3 and 4 are related to one digestion, two digestions, three digestions, *etc.* The two different calibrations are listed as I and II, respectively, and the measurements are listed as I or II followed by the number of replicates (2, 3, 4 or 5). A complete set of data for all the elements and techniques investigated is available on the RSC web site under the title Table S1†.

Examples of the results obtained for a selection of the elements investigated using the various instrumental techniques are plotted in Fig. 2. A complete set of data is available on the RSC web site in file Fig. S1†. In these figures we can see two plots for each element; the mean concentration value, and the RSDs of the determination. In this case, both mean concentration and RSD values found for 2, 3, 4 and 5 replicate measurements and using the two different calibration graphs (denoted as I and II for calibration 1 and 2, respectively) are plotted against the digestion (digestion A, B, C or D). We can see that, in most cases, the mean concentration values are

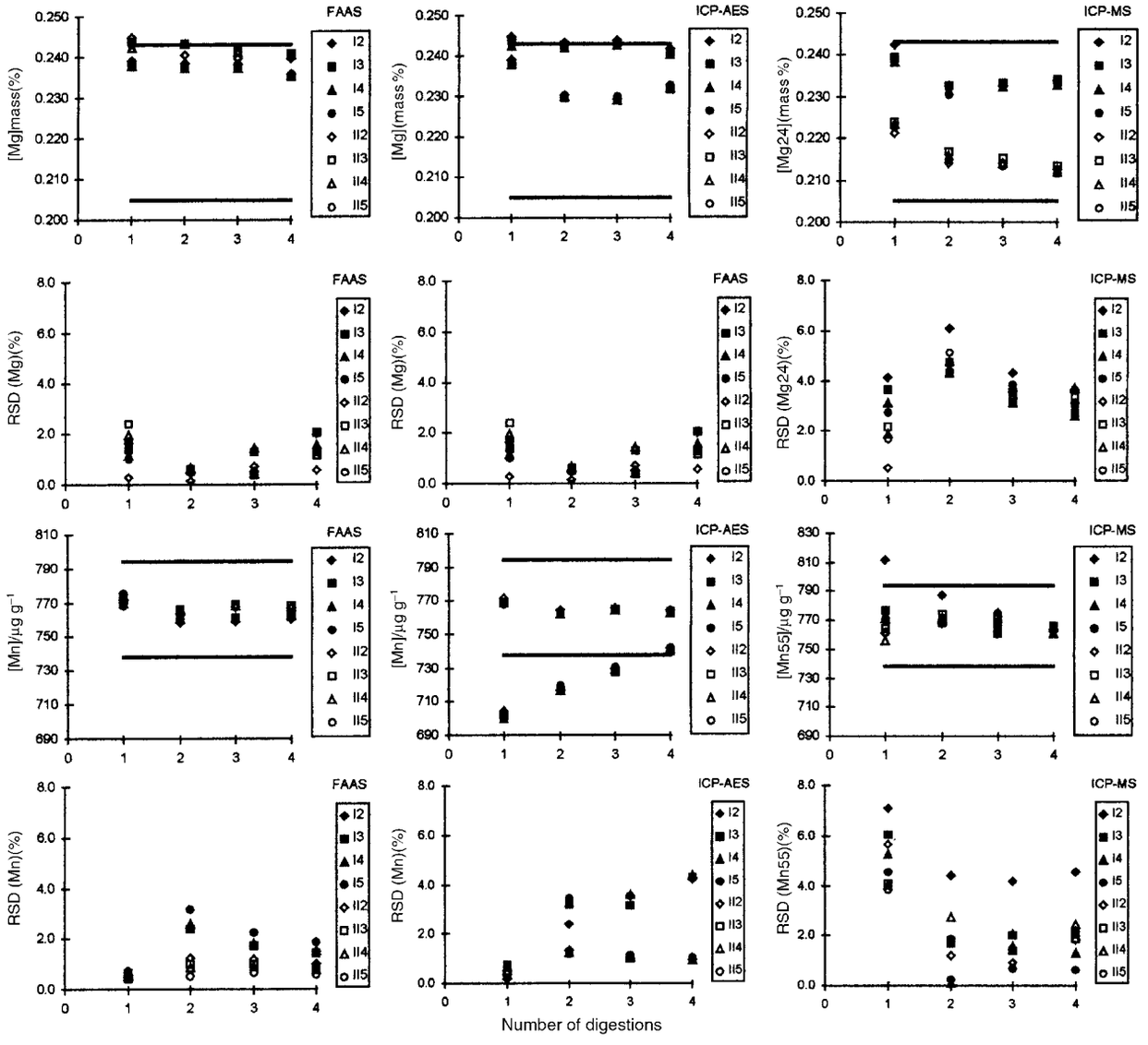

Fig. 3 Mean concentrations and RSDs for the 32 data points considering one, two, three and four digestions (the mean of one, two, three or four acid digestions is also dependent on the two different aqueous calibration graphs used and the number of replicates 2, 3, 4 or 5).

different for the first and second calibration. This difference is more important for trace elements measured by ICP-MS and is less important for ICP-AES determinations. In addition, for FAAS measurements (Fig. 2, for Mg and Mn) it can be seen that the influence of the calibration has less importance.

These results suggest that ICP-MS offers worse precision than ICP-AES and FAAS. This can be visualized easily in the RSD plots in Fig. 2, where the RSDs for the same element corresponding to ICP-AES and FAAS determinations are clearly less than those corresponding to ICP-MS. As the nebulizer type is a very important source of variability in atomic spectrometry, it must be said that some of these differences could be due to the use of different nebulizers (Ebdon high solid for ICP-MS and cross flow for ICP-AES measurements).

Fig. 3 shows the mean concentration and RSDs for 2, 3, 4 and 5 replicate measurements for the two different calibrations. The figure only contains data for a small numbers of elements for illustrative purposes. A complete set of plots is available on the RSC web site under the title Fig. S2†. However, Fig. 3 clearly shows a different situation to that shown in Fig. 2. It can be seen that the effect of the calibration is not so important, and that the similarity of the mean concentration values improves if the number of digestions is increased. However, the trends for the RSDs are similar for all number of digestions. The reduction in the difference between the two calibrations when a larger number of digestions are considered suggests that this parameter contributes significantly to the uncertainty of the results. This can be verified by further data treatment.

Finally, in order to identify either a dominance of systematic errors or random errors, Youden plots can be useful.[16] Each point, for instance, the mean obtained with two replicates and one digestion, three replicates and one digestion, and so on, has been plotted in the two-dimensional space formed by Calibration I (x-axis), and Calibration II (y-axis). Thus, each point (x,y) is the mean obtained with Calibration I (x-value) and with Calibration II (y-value). As examples, Youden plots for Ba determination by ICP-AES (a) and ^{206}Pb by ICP-MS (b) are given in Fig. 4. It can be seen that most of the points fall in a certain quadrant, for instance, the upper right and lower left for Ba and ICP-AES [Fig. 4(a)], or lower left for ^{206}Pb and ICP-MS [Fig. 4(b)]. This means that systematic errors dominate over random errors because, if random errors were dominating, an equal number of points would fall in the four quadrants. Similar results were obtained for other elements and techniques (plots not given), so it can be said that the variations can be attributed to systematic errors rather than random errors.

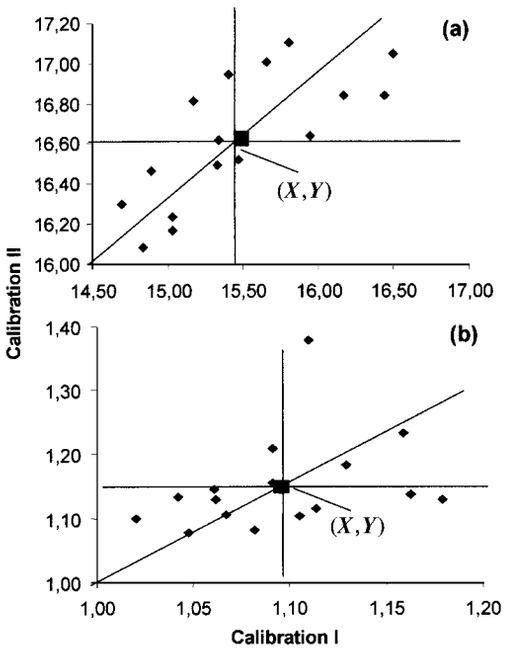

Fig. 4 Youden plots for Ba determination by ICP-AES (a) and ^{206}Pb determination by ICP-MS (b).

Experimental design

As described above, the ANOVA output from an experimental design is used to determine the significance of the variables, as well as the significance of the two-order interactions among them.

A full factorial design (FFD) $4^1 \times 4^1 \times 2^1$ was used to assess the significance of the three variables, number of replicates, number of digestions and calibration. The different levels of the variables are shown in Table 6. The number of samples in the FFD is $4^1 \times 4^1 \times 2^1 = 4 \times 4 \times 2 = 32$, as can be seen in the FFD matrix listed in Table 7. In this case, the response variables represent the mean concentration for each analyte, and those for ^{206}Pb are shown in Table 5 as an example. The remaining data are available on the RSC web site (Table S1). The higher order interaction effects (HOIE) significance testing method was used as part of the statistical package UNSCRAMBLER.[17] The significance level used was 95.0% ($p = 0.05$).

An ANOVA table obtained from Ba (ICP-AES) is shown in Table 8. The complete set of ANOVA tables, for each analyte and technique, is available on the RSC web site (Table S2†).

It can be seen that, at 95.0% significance, the variables, number of digestions and calibration are significant for the determination of Ba by ICP-AES, as is the two-order interaction number of digestions/calibration (*D*/*C*) and number of replicates/calibration (*R*/*C*) with *p*-values lower than 0.05 (Table 8). The multiple correlation as well as the R-Square of the model for each case are close to unity (higher than 0.90), except for the Mn determination by ICP-MS (data in Table S2†), for which a value of 0.888 for R-Square was obtained. This means that the model is not robust for Mn (ICP-MS).

Investigation of the ANOVA tables reveals that the three variables, number of replicates, *R*, number of digestions, *D*, and calibration, *C*, are significant in most cases, while the variables, number of digestions, *D*, and calibration, *C*, are significant for Mg and ^{206}Pb by ICP-MS, Mg by FAAS, and Ba by ICP-AES. In addition, the two-order interaction, *D*/*C*, was found to be significant in all cases, except for ^{206}Pb (ICP-MS), so it is apparent that the effect of the variable, number of digestions, can be affected by the variable, calibration. This is in agreement with the PCA results, where the effect of the calibration can lead to an incorrect interpretation of loadings (projections of the variable, number of digestions). One interpretation of this interaction is that results from the two calibration graphs are different, although both are within the certified concentration range, but, as can be seen in Figs. 2 and 3, the difference due to the calibration graph is reduced when the number of digestions is higher. Thus, it is evident that the increase in number of digestions reduces the differences arising from the use of two different calibration graphs. These results offer an important practical advantage because only an increase in the number of digestions is required to reduce the uncertainty of the measurements, and performing multiple calibrations is not required.

Table 6 Experimental field definition of the variables

Variables	Symbol	Levels
Number of replicates	*R*	2, 3, 4, 5
Number of digestions	*D*	1, 2, 3, 4
Calibration	*C*	I, II

Table 7 $4^1 \times 4^1 \times 2^1$ Full Factorial Design matrix

Experiment	Replicates	Digestions	Calibration
1	2	1	I
2	3	1	I
3	4	1	I
4	5	1	I
5	2	2	I
6	3	2	I
7	4	2	I
8	5	2	I
9	2	3	I
10	3	3	I
11	4	3	I
12	5	3	I
13	2	4	I
14	3	4	I
15	4	4	I
16	5	4	I
17	2	1	II
18	3	1	II
19	4	1	II
20	5	1	II
21	2	2	II
22	3	2	II
23	4	2	II
24	5	2	II
25	2	3	II
26	3	3	II
27	4	3	II
28	5	3	II
29	2	4	II
30	3	4	II
31	4	4	II
32	5	4	II

Table 8 ANOVA results for Ba (ICP-AES) from a FFD $4^1 \times 4^1 \times 2^1$

	SS[a]	DF[b]	MS[c]	*F*-ratio	*p*-value
Summary					
Model	14.4610	22	0.6570	348.277	0.0000
Error	0.0170	9	0.0019		
Adjusted total	14.4780	31	0.4670		
Variable					
Replicates (*R*)	0.0073	3	0.0024	1.287	0.3368
Digestions (*D*)	1.4750	3	0.4920	260.424	**0.0000**
Calibration (*C*)	12.1350	1	12.1350	6430.0	**0.0000**
R/*D*	0.0153	9	0.0017	0.901	0.5607
R/*C*	0.0505	3	0.0168	8.923	**0.0046**
D/*C*	0.7780	3	0.2590	137.416	**0.0000**
Multiple correlation		0.999			
R-Square		0.999			

[a]SS, sum of squares. [b]DF, degrees of freedom. [c]MS mean squares.

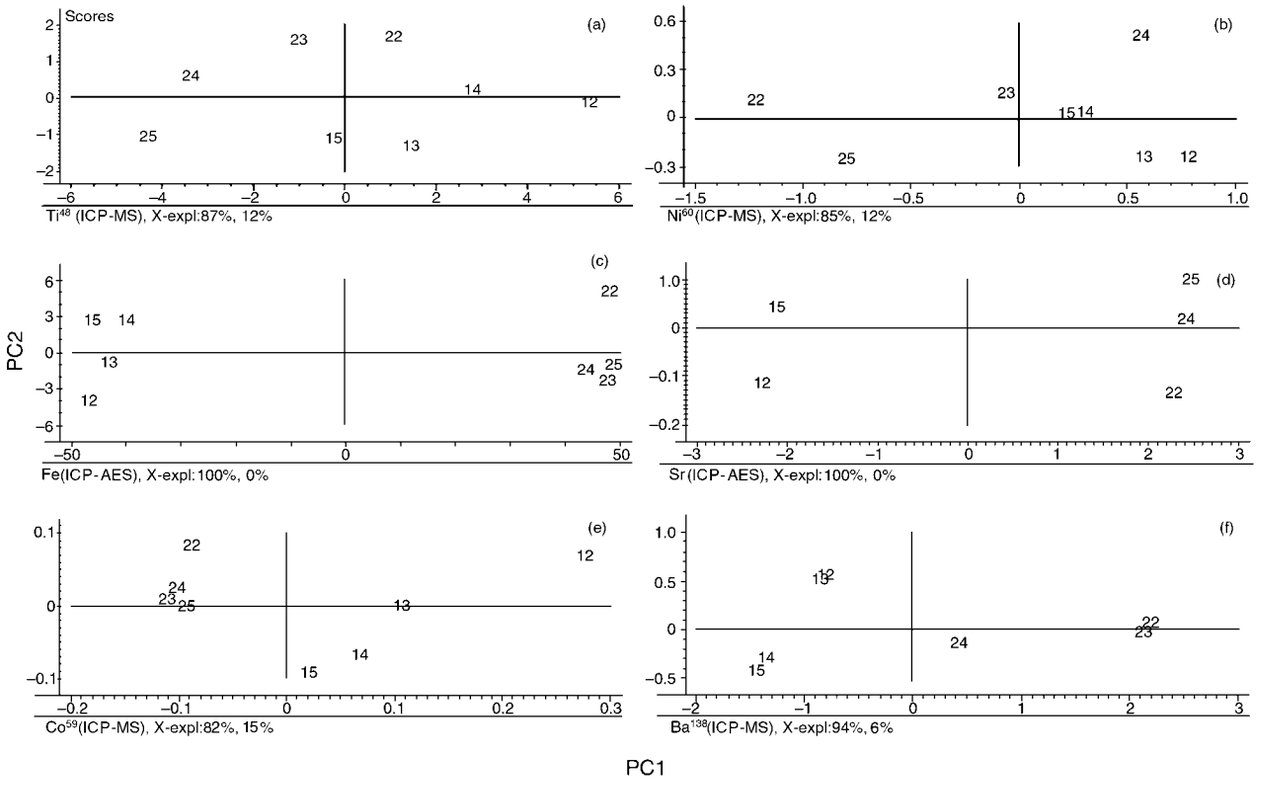

Fig. 5 2-D (PC1/PC2) scatter plots for the scores: ^{60}Ni ICP-MS (a), ^{48}Ti ICP-MS (b), Fe ICP-AES (c), Sr ICP-AES (d), ^{59}Co ICP-MS (e) and ^{138}Ba ICP-MS (f).

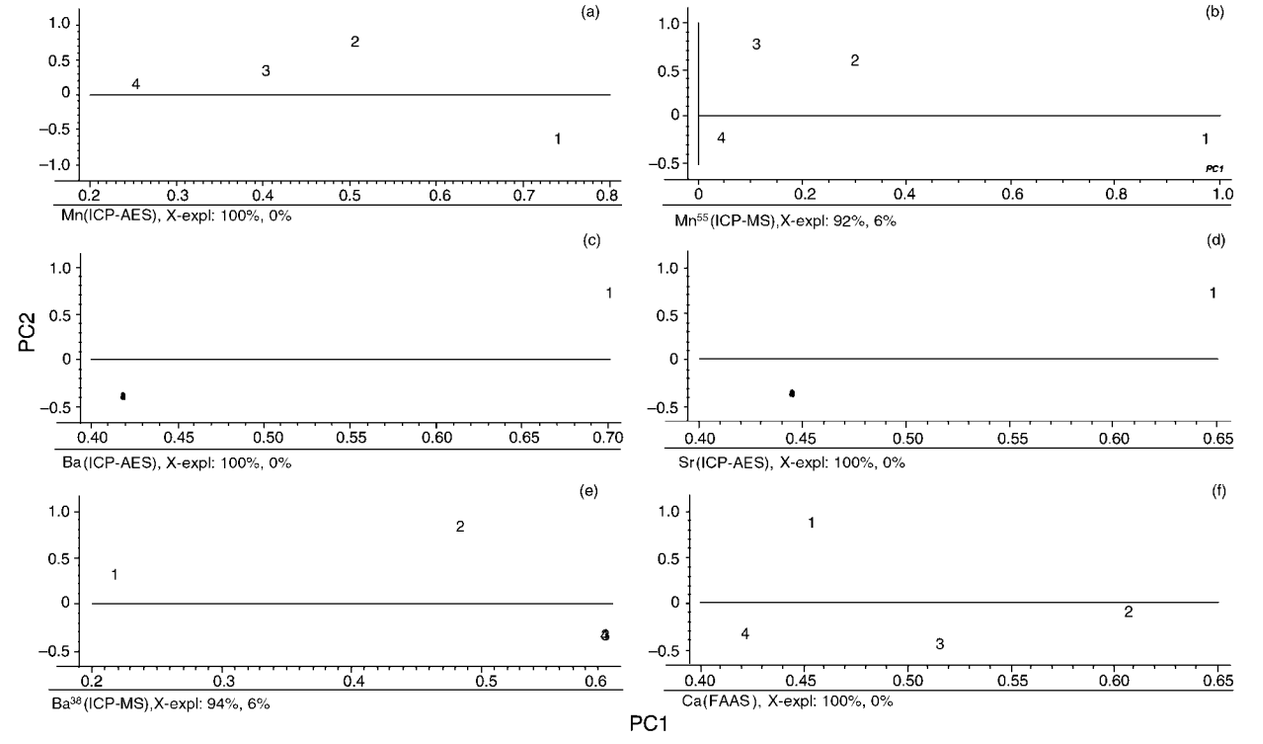

Fig. 6 2-D (PC1/PC2) scatter plots for the loadings: Mn ICP-AES (a), Mn ICP-MS (b), Ba ICP-AES (c), Sr ICP-AES (d), ^{138}Ba ICP-MS (e) and Ca FAAS (f).

Principal component analysis

Principal component analysis is a well known projection method that helps visualise all the information contained in a data set and identify in which respect one sample is different from another.[18] In addition, information about whether variables are truly independent of each other, and the detection of sample patterns, such as groupings, can also be obtained.

A principal component analysis was performed for each data set used in this study (mean concentration levels designed as number of digestions in Table 5). The leverage correction (available in UNSCRAMBLER) was used as a validation

method, and four principal components were chosen to carry out the analysis. Raw data were used for all chemometric manipulations. The information incorporated into the two first principal components was higher than 95% for most cases. The information from a PCA is interpreted in terms of scores and loadings. The scores describe the data structure in terms of sample patterns, but more generally also show sample differences or similarities. The loadings describe the data structure in terms of variable correlations. In this case, the samples are a set of eight points (2, 3, 4 and 5 replicate measurements) for two calibrations ($4 \times 2 = 8$ samples) while the variables are the mean analyte concentrations for one digestion (first variable), two digestions (second variable), and three and four digestions (third and fourth variables, respectively).

Scores. Some examples of scores plots are given in Fig. 5 (again the complete set of data is available on the RSC web site, Fig. S3†). In most cases a grouping of the samples (calibration 1 and calibration 2) can be seen, which implies differences arising from the calibration graph. However, the calibration appears to be less important for Fe, Ni and Ti (measured by ICP-MS), and scores from calibration 1 or 2 are mixed in the PC1-PC2 space, as can be seen in Fig. 5 (a) and (b) for Fe and Ti (ICP-MS). These results agree with those previously plotted in Fig. 3, where it can be seen that, for the Fe, Ni, ^{206}Pb and Ti determinations by ICP-MS, the results from the two calibrations overlap with greater similarity when the number of digestions is larger. This is not so evident in Fig. 3 but becomes clear when we visualise the results following the PCA procedure.

Further useful information can be extracted from scores plots related to the precision of the measurements. Two different scenarios were found. The first, and more common, is that, where all scores for a certain calibration (2, 3, 4 and 5 replicate measurements) are very similar, it can be concluded that the variable, number of replicates, is not important, and hence the analysis of 2 or 5 replicates is equally valid. This occurs for Ba (ICP-AES), Ca (ICP-AES), Cr, Cs, ^{207}Pb and Rb (ICP-MS), Cu (FAAS and ICP-MS), Fe, Mg, Mn and Zn (FAAS, ICP-AES, ICP-MS), and Sr (ICP-AES and ICP-MS). In Fig. 5 (c) and (d) examples for Fe and Sr (ICP-AES) are given. The second scenario is when each score, also from the same calibration, is different from the other. In these cases it can be concluded that the number of replicates is important. This occurs for the determination of Ba and Co by ICP-MS. Here it can be seen that certain scores are close to the origin, for instance replicates 3, 4 and 5 with the calibration 1 and 2, for Co determination by ICP-MS. Thus, it can be concluded that the number of replicates must be higher than 2 for Co (Fig. 5). A similar situation can be established for ^{135}Ba (ICP-MS) (Fig. 5), although in this case the number of replicates can be higher than 3 or 4 if we use the results from calibration 1 or 2. These results agree with the observation that the contribution of the instrumental measurement to the variation can be reduced by using a large number of replicate measurements.

Also important to note from these plots is that the scores from ICP-MS measurements are more diverse than those corresponding to ICP-AES or FAAS (*e.g.*, compare scores plots for Cu, Fe, Mg, Mn and Zn and the three techniques). This indicates worse precision for the ICP-MS measurements, even when relatively high analyte concentrations were being determined.

Loadings. Despite the easy interpretation of the score plots, some consideration must be given to loadings. A 3-way problem (number of replicates, number of digestions and calibration) is being attempted when using the PCA, even though PCA is a 2-way chemometrics tool. Therefore, the third dimension of the problem (calibration) can mask the effects of the variables, number of replicates and number of digestions. An *N*-way decomposition method, such as PARAFAC,[19] would be preferred; however, useful information from loadings and scores plots can be obtained by application of PCA.

In Fig. 6 examples of some loadings plots are given (the complete set of data is available on the RSC web site, Fig. S4†). For each PC, the variable numbers of digestions with high loadings are those that explain the most variance in the data set, and hence indicate the highest variability of the results. The numbers of digestions with the small loadings are those that offer the best precision, and therefore these are the ones that should be selected. Three different situations have been identified. The first situation is plotted in Fig. 6 (a) and (b), where the loading is small (closer to the origin) and where the number of digestions is larger, *i.e.*, the results become less variable as the number of digestions increases. This situation has been found for Mn [ICP-AES and ICP-MS as in Fig. 6 (a) and (b), respectively] and also for Fe, Ni, ^{207}Pb, Rb, Sr and Zn (ICP-MS).

A second case, plotted in Fig. 6 (c) and (d) for Ba and Sr (ICP-AES), respectively, is obtained when the loadings are close to zero for the variables two digestions, three digestions, *etc.*, and where the highest loadings are obtained for the variable one digestion. This means that the variability is small and the precision is good for two digestions or more. This situation has also been obtained for Cu (FAAS) and Cr, and ^{206}Pb (ICP-MS). A third case is plotted in Fig. 6 (e) and (f) where the variables related to a small number of digestions (one digestion or two digestions) present the smallest loadings for Ba (ICP-MS) and Ca (FAAS), respectively. In such cases, it cannot be concluded that the smallest variability is reached for one or two digestions (as in the second case described above), because a higher number of digestions offers high loading values. If minimum variability is reached for a certain number of digestions, this parameter must remain constant for higher numbers of digestions, as is shown in the second case [Fig. 6 (c) and (d)]. This abnormality results from using PCA for a 3-way problem.

Variance estimation

The sample pre-treatment and analysis scheme showing the relationships among number of digestions, number of replicates and calibration is given in Fig. 1. In accordance with the hierarchical relationships among the three variables, each mean square and the various variance components can be expressed mathematically as follows:[20]

$$\mathrm{MS}_D = \mathrm{Var}_{\mathrm{error}} + 4\mathrm{Var}_R + 8\mathrm{Var}_D \quad (1)$$

$$\mathrm{MS}_R = \mathrm{Var}_{\mathrm{error}} + 4\mathrm{Var}_R \quad (2)$$

$$\mathrm{MS}_C = \mathrm{Var}_{\mathrm{error}} + 16\mathrm{Var}_C \quad (3)$$

where MS_D, MS_R and MS_C are the mean squares for the variables *D*, *R* and *C*, respectively (obtained from ANOVA tables); Var_D, Var_R and Var_C are the estimates of variance associated with each variable, *D*, *R* and *C*, respectively; and $\mathrm{Var}_{\mathrm{error}}$ is the estimate of variance of the error of the model.

The overall variance of the 32 measurements can be calculated according to:

$$\mathrm{Var} = \frac{\mathrm{Var}_D}{n_D} + \frac{\mathrm{Var}_R}{n_R} + \frac{\mathrm{Var}_C}{n_C} + \frac{\mathrm{Var}_{\mathrm{error}}}{n_{\mathrm{error}}} \quad (4)$$

where n_D, n_R, and n_C represent the number of digestions, replicates and calibrations denoted by the subscripts *D*, *R* and *C*, respectively, meanwhile, n_{error} is the total number of replicates. The denominators of eqn. (4) are therefore 4, 8, and 16 for *D*, *R*, and *C*, respectively, and 32 for the total number of replicates.

Table 9 Estimates of the variance for some elements: TOTAL is the estimate of the total variance according to eqn. (4); SE is the standard error and is equal to the square root of the overall variance (TOTAL)

Ba (ICP-AES)			
Source	Variance	*n*	Var/*n*
Replicates	1.36×10^{-4}	4	3.39×10^{-5}
Digestions	6.12×10^{-2}	4	1.53×10^{-2}
Calibration	7.58×10^{-1}	2	3.79×10^{-1}
Error	1.89×10^{-3}	32	5.90×10^{-5}
		TOTAL	3.95×10^{-1}
		SE	6.28×10^{-1}
^{138}Ba (ICP-MS)			
Source	Variance	*n*	Var/*n*
Replicates	1.71×10^{-1}	4	4.27×10^{-2}
Digestions	3.63×10^{-2}	4	9.06×10^{-3}
Calibration	6.42×10^{-1}	2	3.21×10^{-1}
Error	4.14×10^{-3}	32	1.29×10^{-4}
		TOTAL	3.73×10^{-1}
		SE	6.11×10^{-1}
Ca (FAAS)			
Source	Variance	*n*	Var/*n*
Replicates	1.87×10^{-5}	4	4.68×10^{-6}
Digestions	4.85×10^{-5}	4	1.21×10^{-5}
Calibration	2.98×10^{-5}	2	1.49×10^{-5}
Error	3.66×10^{-5}	32	1.14×10^{-7}
		TOTAL	3.18×10^{-5}
		SE	5.64×10^{-3}
Ca (ICP-AES)			
Source	Variance	*n*	Var/*n*
Replicates	1.39×10^{-5}	4	3.46×10^{-6}
Digestions	3.93×10^{-6}	4	9.83×10^{-7}
Calibration	1.81×10^{-4}	2	9.06×10^{-5}
Error	5.33×10^{-7}	32	1.67×10^{-8}
		TOTAL	9.51×10^{-5}
		SE	9.75×10^{-3}
Cu (FAAS)			
Source	Variance	*n*	Var/*n*
Replicates	5.84×10^{-3}	4	1.46×10^{-3}
Digestions	1.05×10^{-1}	4	2.62×10^{-2}
Calibration	9.82×10^{-2}	2	4.91×10^{-2}
Error	4.70×10^{-3}	32	1.47×10^{-4}
		TOTAL	7.69×10^{-2}
		SE	2.77×10^{-1}
^{65}Cu (ICP-MS)			
Source	Variance	*n*	Var/*n*
Replicates	6.93×10^{-2}	4	1.73×10^{-2}
Digestions	1.68×10^{-1}	4	4.19×10^{-2}
Calibration	1.93×10^{-1}	2	9.63×10^{-2}
Error	4.20×10^{-2}	32	1.31×10^{-3}
		TOTAL	1.57×10^{-1}
		SE	3.96×10^{-1}
Fe (FAAS)			
Source	Variance	*n*	Var/*n*
Replicates	17.7195	4	4.429 875
Digestions	2.066 75	4	0.516 688
Calibration	334.5324	2	167.2662
Error	2.482	32	7.76×10^{-2}
		TOTAL	172.2903
		SE	13.125 94
Fe (ICP-AES)			
Source	Variance	*n*	Var/*n*
Replicates	-1.3300	4	-0.3325
Digestions	30.0088	4	7.502 188
Calibration	999.6373	2	499.8187
Error	5.8030	32	0.181 344
		TOTAL	507.1697
		SE	22.520 43
^{54}Fe (ICP-MS)			
Source	Variance	*n*	Var/*n*
Replicates	290.3868	4	72.596 69
Digestions	-135.324	4	-33.831
Calibration	-0.506 75	2	-0.253 38
Error	8.453	32	0.264 156
		TOTAL	38.776 47
		SE	6.227 075

Assuming the term Var_{error} as the residual variance, MS of the Error in the ANOVA tables, eqn. (1)–eqn. (3) can be resolved to obtain the estimates of the variances associated with each variable. Estimates of variances are listed in Table 9 for a number of elements using the three techniques studied (the complete set of results is given on the RSC web site under Table S3†). From Table 9 it can be seen that the variances associated with the sample dissolution (number of digestions) and calibration are by far the largest sources of error. For such elements measured by the three techniques (FAAS, ICP-AES and ICP-MS) some comparative observations can be made. It can be seen that the variance associated with the three parameters is greater for ICP-MS than for either FAAS or ICP-AES, see results for Ba, Ca, Cu and Fe in Table 9, and also for Mg, Mn, Sr and Zn (Table S3†). The difference is higher for the parameter number of replicates, for instance 0.000 136 and 0.171 for Ba determination by ICP-AES and ICP-MS, respectively, indicating a worse precision by ICP-MS measurement. Similar results were also obtained for Cu, Fe, Mg, Mn, Sr and Zn. For Ca, the uncertainties in the ICP-AES and FAAS determinations were similar, but for Fe, the variance due to the calibration was higher for both ICP-AES and FAAS than for ICP-MS, although this last technique offers 20–300-fold higher variance for the number of replicates.

The uncertainty in the determination of Pb by ICP-MS is the same for both ^{206}Pb and ^{207}Pb since the precision is the same for both.

Conclusions

The uncertainty in analytical methods by ICP-MS and ICP-AES can be estimated taking into account all possible sources of error. If one or more of the variance components are negligible, they can be dropped from the analysis. Variance estimates can also help identify key sources of error and those factors that require most attention for better control. For ICP-AES measurements the number of replicates was not found to be a significant source of error in that the variance associated with this variable is very small in comparison with the total error. On the other hand, the variables, number of digestions and calibration, were significant. An increase in the number of digestions would therefore be desirable in order to reduce the uncertainty in a multi-element determination by ICP-AES of solid materials. For ICP-MS, all variables were significant, and the number of digestions must be increased. In addition, due to the relatively poor precision when compared with ICP-AES, the number of replicates must also be increased in order to reduce this source of error. It must be said that the significance of the variable, number of replicates, is not a function of the analyte concentration, and that the significance of this variable

is present for both major (Ba, Cu, Fe, Mg, Mn, Sr and Zn) and trace (Co and Cr) elements by ICP-MS.

The use of an experimental design has proved useful when assessing the significance of each variable and also to obtain the 3-way ANOVA. Additionally, this technique gives information about the two-order interactions amongst the variables, for example, interactions between the variables number of digestions and calibration were found to exist. This interaction is also evident in the PCA but it cannot be calculated using this technique, since a 3-way decomposition method is required.

Finally, although the variable "calibration" is significant, the two-order interaction number of digestion/calibration is also significant, so we can reduce the uncertainty by increasing the number of digestions. It is therefore only necessary to perform one calibration for the analysis.

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