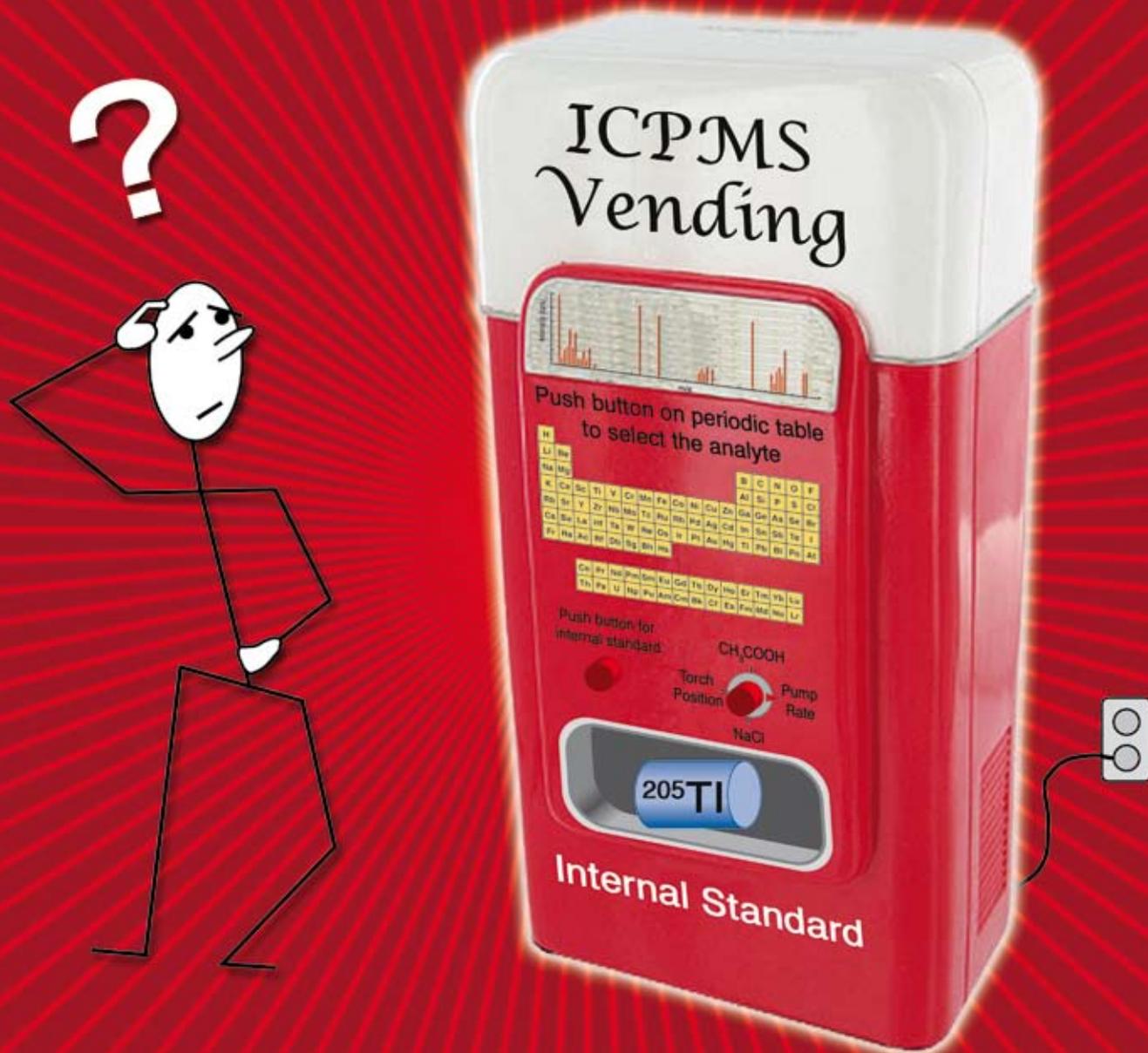


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The effect of hydrogen and nitrogen  
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# Choosing internal standards based on a multivariate analysis approach with ICP(TOF)MS†‡

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Internal standards are routinely used with inductively coupled plasma mass spectrometry (ICPMS) to minimize the impact of signal instability. Previous studies indicate that internal standard choice should be directed by similarities in mass, but they neglect to address the possibilities of exceptions or what impact a poor internal standard choice may have on quantitative results. A 51 element suite was run under varying instrument and matrix perturbations on an ICPMS instrument equipped with a time of flight mass spectrometer. Each element was ratioed to every other element for perturbed conditions that were intended to simulate variations in matrix composition or drift in operating parameters. These included changing sample delivery rate, matrices (NaCl and acetic acid) and horizontal torch position. The %RSDs of each analyte-to-internal standard ratio were used to rank the relative quality of each analyte-internal standard pair and their response to changing conditions. Multiple ordinary least squares equations were calculated to evaluate various chemical and physical properties that may be predictors of an optimal analyte-internal standard combination. Overall, similarity of masses was found to be the most important predictor of a good internal standard. However, exceptions did exist under various perturbations and among different elements. In a final study, nine analytes were run under perturbed experimental conditions and in two complex matrices (NaCl and acetic acid), but quantitation was attempted using simple aqueous standards. Accuracy and precision were evaluated for several internal standards for each of the nine elements as well for the nine analytes where no internal standard was employed. From this evaluation, it was clear that internal standard choice can have substantial effects on analytical accuracy and the improvement of analytical precision, but there is no single physical or chemical parameter that reliably allows *a priori* selection of a “good” internal standard for any given analyte.

## Introduction

Signal variations that are not representative of concentration changes in inductively coupled plasma mass spectrometry (ICPMS) can be caused by variations in instrument operating conditions and sample matrix. There are a number of procedures an analyst may use to overcome these instabilities, including isotope dilution, standard additions and internal standardization. The cost of isotopically enriched standards and the applicability restrictions on mono-isotopic species can limit the practical utility of isotope dilution. Standard additions can improve accuracy and correct for many matrix effects, but increased sample preparation time and sample size requirements can limit the application of this technique. Internal standardization generally increases precision, and when a nearly “ideal

internal standard” is employed, can improve accuracy. With an “ideal internal standard,” one can employ calibration curves prepared from simple standard solutions to determine analyte concentrations in complex matrix solutions, thus simplifying sample preparation and quantitation. In ICPMS, internal standards are routinely used to improve precision with hopes of also improving accuracy.

The ideal internal standard for any given analyte is one whose intensity changes are directly proportional to that of the analyte, *i.e.*, the analyte-to-internal standard intensity ratio remains constant for all changes in the sample composition and instrument performance. However, internal standard choice is limited to elements that are initially absent from the sample or whose concentrations remain constant within measurement uncertainty. This restriction is not trivial in samples with very complex matrices such as environmental and biological specimens. Chen *et al.* attempted to overcome this issue by utilizing polyatomics from metal-oxides and Ar as internal standards.<sup>1</sup> There are also a number of studies which indicate that one internal standard, such as <sup>115</sup>In, can be used to successfully improve the precision for a variety of analytes.<sup>2–4</sup> <sup>103</sup>Rh is another internal standard that is frequently used.<sup>5–7</sup> Several studies, however, have addressed the importance of choosing the correct internal standard for each specific analyte.<sup>1,8–10</sup> These studies attempted to elucidate the characteristics that might allow *a priori* selection of an internal

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‡ Electronic supplementary information (ESI) available: The prediction program (Excel); the OLS results for each element in table form (as PDF file); and a visual display using colored highlights on periodic tables (as ppt file). See DOI: 10.1039/b804048f

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standard for a given analyte. Thompson *et al.*<sup>8</sup> determined that both mass and ionization potential should be taken into consideration while Vanhaecke and colleagues<sup>10</sup> suggest that similarities in mass alone are the dominating factor for internal standard choice. A study by Sartoros *et al.* used cluster analysis to develop an automated system for determining the best internal standard.<sup>9</sup> This algorithm utilized chemical properties such as kinetic energy and electronegativity for determining analyte-internal standard pairs.

The majority of internal standard studies to date have used a quadrupole mass analyzer, which is limited in its multielement capabilities.<sup>11</sup> A smaller data set limits the opportunity for statistical analyses of trends when normal distributions are not present. In short, the quantitative, statistical evaluations of elemental properties that contribute to a good analyte-internal standard pair have been very limited. Although general trends have been determined in the previously cited studies, it is difficult to ascertain the number of and reasons for exceptions that may exist. From the previous work, the extent of the loss in precision and accuracy that would result from choosing a less than optimal internal standard or using no internal standard is unclear.

In ICPMS there are a large number of parameters that impact the relationship between analyte concentration in a sample and the signal recorded from the detector. They begin with nebulizer efficiency, and proceed through physicochemical processes in the plasma and ion pathways in the optics and spectrometer. Fundamental studies of individual processes continually appear and provide insights that ideally will lead to a master equation, allowing one to predict signal magnitude and dictate the practical selection of the nearly ideal internal standard for any given analyte. However, the complexity and interdependencies of these processes make this noble objective a daunting task.

The present study attempts to cover a diverse set of operating conditions and sample matrices for a large number of elements to generate a “database series” from which specific predictions and statistical evaluations regarding internal standard selection can be made. Ideally, the information will suggest characteristics of the analyte that will permit *a priori* selection of an internal standard, *e.g.*, similarities in mass, ionization potential, *etc.* Four different condition sets were evaluated: acetic acid (CH<sub>3</sub>COOH) matrices, sodium chloride matrices (NaCl), sample delivery rates to the nebulizer and torch position relative to the sampling cone. The acetic acid matrix (0–10%) simulates a relatively difficult to ionize matrix with a high carbon content. The NaCl matrix (0–500 ppm) emulates a common “salt matrix” with an easily ionizable element. The sample delivery rate perturbations simulate potential nebulizer blockage and other parameters affecting aerosol formation, *e.g.*, solution viscosity, surface tension. The alterations in torch-to-sampling cone distances simulate variability changes in plasma temperature at the skimmer, extent of elemental/ion diffusion, electron density at point of plasma sampling, *etc.*

One goal of the current study is to correlate a variety of elemental properties to the selection of optimal analyte-internal standard pairs. The first step in attempting to establish such a correlation that would permit *a priori* selection of an analyte-internal standard pair might be to evaluate all elements as potential internal standards for all possible analytes (*i.e.*, all other elements). The simultaneous multielement detection

capabilities of a time-of-flight based ICPMS system permit collection of all masses without sacrificing duty cycle for any given *m/z* being monitored. However, some restrictions are imposed by, for example, intense ion flux from plasma gases (*e.g.* Ar<sup>+</sup>, Ar<sub>2</sub><sup>+</sup>, ArO<sup>+</sup>, *etc.*).

The properties considered include mass and ionization potential,<sup>12</sup> various thermodynamic properties of formation,<sup>12</sup> electronegativity,<sup>13</sup> elemental charge in solution and ionic mobility in solution. For example, with an altered temperature or electron density in the plasma, analyte-internal standard similarities in enthalpies or ionization potentials may be parameters worth matching. Similarly, with altered matrices, inter-ionic interactions (*e.g.*, space charge effects) in the interconal regions or in the ion optics are known to exhibit dependencies on mass. Correlations to charge and ionic mobility of the elements in solution were evaluated to determine whether disproportional ionic distribution might exist within the aerosol droplets and alter the signals, possibly having a significant impact on internal standard performance, especially during the experiments in which sample delivery rate to the nebulizer was changed. A number of studies have addressed this phenomenon known as aerosol ionic redistribution (AIR) and its effect on the analyte signal.<sup>14–17</sup>

While it might not be possible to elucidate a definitive “rule” for choosing the best internal standard, the present study will show how to isolate and evaluate the efficacy of using general trends as a means of choosing the best internal standard for any given analyte.

Finally, a set of nine analytes with varying physicochemical properties were determined under altered instrumental and matrix conditions using calibration curves prepared from optimized ICPMS operating conditions and simple standards in 1% HNO<sub>3</sub>. These data constituted an “evaluation series”. This second series consists of two separate goals. The first of which is to determine the utility of the original database series as a means of internal standard prediction. Internal standardization was performed on these analytes to determine the impact of internal standard choice on both analytical precision and accuracy. The prediction scheme created for the database series was used to select the internal standard, and these results were compared to the use of alternative internal standards as well as using no internal standard. In making these comparisons, the evaluation series will offer additional insight into the quantitative penalties of using a non-optimal internal standard.

## Experimental

### Instrumentation and reagents

All data were collected on a GBC Optimass 8000 (GBC Scientific, Hampshire, IL, USA) inductively coupled plasma orthogonal time-of-flight mass spectrometer using a Meinhardt concentric glass nebulizer and the GBC cyclonic spray chamber. Multi-element and single element standards were combined to make a 10 ppb standard containing 54 elements. Distilled, deionized water and ultra high purity HNO<sub>3</sub> (Fisher Scientific) were used in all cases. Trace metal grade acetic acid (Fisher Scientific) and NaCl (Sigma Chemical Company) were used in matrix laden samples.

**Table 1** Experimental design of database series, where one parameter is changed at a time to evaluate its impact on internal standardization of each evaluated analyte. Optimizing conditions with simple standards in 1% HNO<sub>3</sub> gave a flow rate of 1.6 mL min<sup>-1</sup> and relative torch position of 6 mm

Condition name	Matrix	Matrix conc.	Torch position <sup>a</sup> /mm	Sample delivery rate <sup>b</sup> /mL min <sup>-1</sup>
Sample delivery rate (mL min <sup>-1</sup> )	HNO <sub>3</sub>	1%	6	0.6, 0.9, 1.3, 1.6, 1.9, 2.2, 2.5, 2.9, 3.2
Torch position	HNO <sub>3</sub>	1%	6, 7, 8, 9, 10, 11, 12, 13, 14, 15	1.6
NaCl matrix	NaCl	0, 1, 500 ppm	6	1.6
Acetic matrix	CH <sub>3</sub> COOH	0, 0.1%, 1%, 10%	6	1.6

<sup>a</sup> Solutions are 1% HNO<sub>3</sub>. <sup>b</sup> The pump speeds corresponding to these delivery rates ranged from 4–20 rpm.

## Procedure

All glassware was washed in 4 M HNO<sub>3</sub> for at least 24 h and deactivated with 2,2-dimethyldichlorosilane. Class A polymethylpentene (PMP) volumetric flasks were used for the evaluation series.

The database series was prepared using dilutions of the multielement standards. In all cases, the analytes were present at 10 ppb for all 54 elements. The conditions employed to generate the database values are detailed in Table 1. Solutions in 1% HNO<sub>3</sub> were employed to study condition changes in torch position, sample delivery rate and long term drift. These 54 element standards were also made in varying concentrations of glacial acetic acid and NaCl to evaluate the effects of sample matrix on internal standard choice. From Table 1 it can be seen that ICPMS intensity information was obtained for 27 different “variations” (including optimized “baseline” runs) within five “conditions”. Initially, 224 masses were monitored for each variation, from which 102 *m/z* values, representing 51 elements, were subjected to detailed data analysis. (Criteria for data set reduction are given below in “Data analysis” section.)

An evaluation series was prepared to assess the internal standard selection criteria suggested by the database series results. The evaluation series consisted of nine elements covering a wide range of *m/z*, and were run at 50 ppb under varied matrix and instrument conditions to evaluate the effect of internal standardization on analytical accuracy and precision. Evaluation was based on the use of simple standards in 1% HNO<sub>3</sub> using optimized instrument settings to prepare a calibration curve for quantitation, independent of the test condition or matrix composition. Instrument settings were adjusted to optimize the collective signal strengths of 5 elements (Be, Co, In, Pb and Th). The test solutions employed several different perturbed conditions, *i.e.*, sample delivery rate of 0.6 mL min<sup>-1</sup> and 1.9 mL min<sup>-1</sup>; torch positions of 6 and 12 mm; 5% CH<sub>3</sub>COOH; and 10 ppm NaCl matrix.

## Data analysis

Initially, 224 masses were monitored and exported for further data analysis. Elimination of masses from the database series were based on: (a) an element's absence in the standard, (b) low signal due to low isotopic abundance, and (c) significant isobaric and Ar<sup>+</sup> or ArX<sup>+</sup> interferences. All isotopes for K were eliminated based on low signal and Ar<sup>+</sup> interferences. All isotopes of Si and Na were eliminated as a result of large background interferences in the blank. This initial data processing left 102 masses from 51 elements for further analysis. The ICPMS

produced data for the five standard conditions and 27 variations, yielding useful intensity information for 51 unique elemental-system situations. These were organized in Microsoft Excel with each worksheet containing the intensity ratio of each remaining mass to the 102 *m/z* values in the set. Thus, 5202 unique ratio values were available per condition. A Microsoft Excel macro written in Visual Basic for Applications (Microsoft Visual Basic 6.3) was used to calculate these ratios from the imported intensity data.‡ This macro was used to calculate the percent relative standard deviation (%RSD) of each ratio through a changing set of conditions for every analyte-internal standard pair. The calculated %RSDs were used in the data analysis as well as in the prediction of good analyte-internal standard pairs.

JMP6 (SAS) was used to calculate correlations and multiple ordinary least squares (OLS) regression equations to establish relationships between analyte-internal standard pairs and a variety of physical and chemical properties.

## Results and discussion

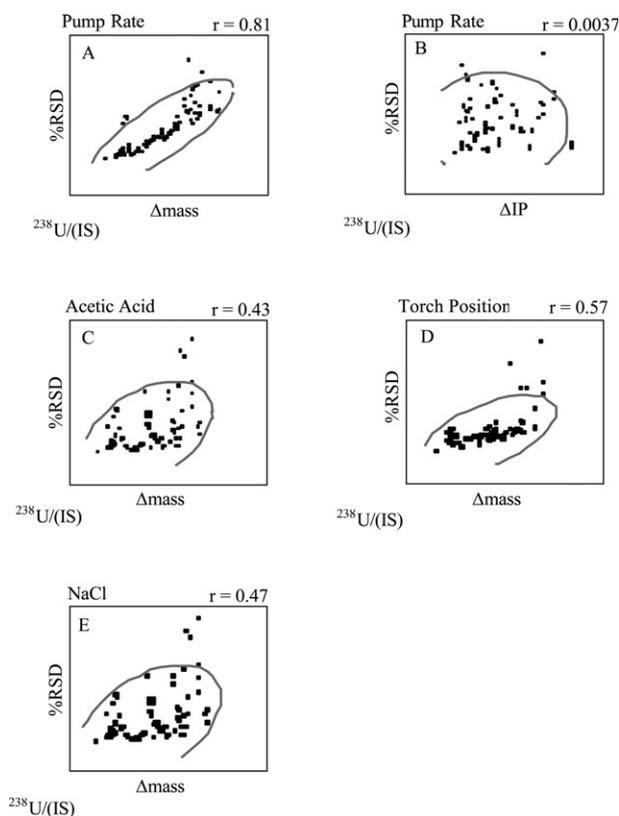
### RSD of internal standard ratio as an indicator of spread

A small RSD calculated over a range of conditions (*e.g.*, varied NaCl concentrations) suggests that internal standard signal is changing in proportion to that of the analyte in those instances where the analyte signal changes. Thus, a small %RSD should represent a good analyte-internal standard pair. If a correlation between %RSD and chemical property can be found, an *a priori* selection of “ideal” analyte-internal standard pairs may be possible. It should be noted that the data are not normally distributed and the calculated RSD should be viewed as a measure of relative spread of values rather than an actual uncertainty in the data. Using the RSD may not give an accurate assessment of how ideal an internal standard is in cases where only one particular parameter (*e.g.*, an extreme pump speed) caused a large change in the analyte-to-internal standard ratio.

### Correlation plots

Correlations between %RSD and changes in chemical properties were calculated to evaluate the presence of a relationship between good internal standards and similarities in chemical properties. Plots were prepared for each perturbation studied, for each analyte ratioed to all other recorded elements.

As an example, Fig. 1A shows a strong correlation between internal standard choice and the mass difference between internal standard and analyte, *viz.*, a small mass difference between analyte and internal standard correlates to a small %RSD. An example of a very weak correlation is represented in Fig. 1B



**Fig. 1** Example correlation scatter plots. Each point represents a ratio of  $^{238}\text{U}$  and one of approximately 100 internal standards considered during the pump rate perturbations. The solid lines are set to encompass 95% of the points.

where the change in ionization potential exhibits virtually no relationship to %RSD. As expected from the results observed by other authors,<sup>8,10</sup> mass typically offered the strongest correlation to %RSD for every condition. However, as shown in Fig. 1C–E, the level of correlation with mass for different perturbation sets and different analytes is highly variable. For the nearly 2000 correlation plots created, most correlations were not as distinctively differentiated as shown in Fig. 1A and B, but offered less definitive conclusions such as those data in Fig. 1C–E. Thus, using well correlated data or outliers (*i.e.*, poorly correlated) to draw general conclusions became difficult primarily because of the continuum of data between good and poor correlations.

### Multiple ordinary least squared (ols) regressions

While the correlation graphs provided a quick visualization of dependencies, a more quantitative approach was necessary to evaluate the relationships established by the correlation plots. Multiple OLS regressions offered a method that is not only quantitative, but takes the relative relationships between chemical properties into consideration and is unaffected by differences in units. Using the data from all 102 isotopes (51 elements), regression equations were calculated for the most abundant isotope of each element with no interfering isobars. Linearity, homoscedasticity and normality were needed for valid analysis by OLS, and each model was tested for these characteristics. It

should be noted, however, that some elements yielded more valid models than others. Normality was the most difficult specification to meet, although some elements did meet this requirement. The elements that did not have a normal distribution among the residuals were approximated as normal by the Central Limit Theorem since  $n$  is large. However, the tests for these elements are less predictive than the tests for elements that did not need to rely on this theorem. Therefore, this method was suitable for general trend analysis within a reasonable margin of error, but probably should not be used for individual predictions. Log-log normalization was used to improve some of these specifications. Quantitative  $t$ - and  $F$ - tests were used to establish and rank significance among the different chemical properties considered. Four separate regression equations were calculated for each major isotope from the four different perturbations considered. Therefore, a total of 204 equations were calculated using JMP6 (SAS) in the form illustrated in eqn (1).

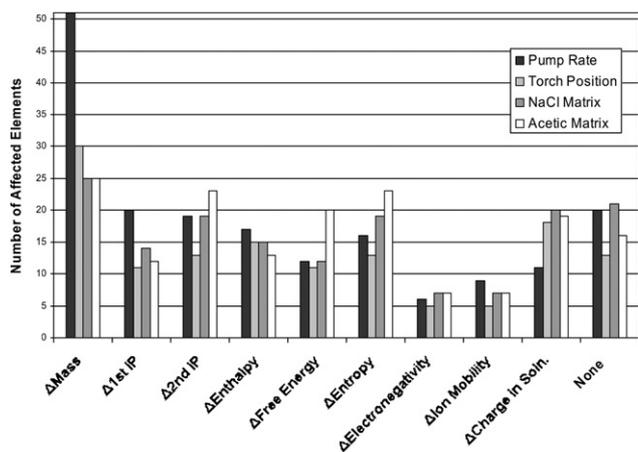
$$Y = \beta_1 x_1 + \beta_2 x_2 + \dots + \beta_n x_n \quad (1)$$

$Y$  represents a predicted %RSD,  $x$  represents a change in chemical property and  $\beta$  is the slope, or proportionality constant, that signifies the relationship between %RSD and each change in chemical property.

The magnitude of the slope corresponding to each change in chemical property was used as the basis for the significance testing. Both the  $t$ - and  $F$ -tests work under a null hypothesis of  $H_0: \beta = 0$  and an alternative hypothesis of  $H_1: \beta \neq 0$ . A slope of zero is indicative of no correlation between a given chemical property and %RSD. Significance was defined by a  $p$ -value of less than or equal to 0.1.

If two chemical properties were strongly correlated to one another (strong multicollinearity), the property offering a smaller contribution was removed. This removal was necessary because multicollinearity can affect further significance testing. The variance inflation factor (VIF) was used to quantitatively determine which properties were affected. For example, enthalpy of formation is linearly correlated to Gibb's free energy of formation. Therefore, these two properties have a strong multicollinearity, and one was always dropped based on relative significance.

Although OLS is often utilized for its forecasting capabilities, the equations derived in the present study are limited for a number of reasons. The lack of normal distribution among the residuals in  $Y$  that were previously discussed limits the validity of each model and its predictive capabilities. Another limitation lies in the  $R^2$  values, which varied widely and ranged from 0 to 0.7. Although excellent explanatory coverage was not expected, very low  $R^2$  values offer evidence that there are other potential chemical properties and parameters that may play a role and have not been incorporated into this study.  $R^2$  offers a quantitative measure of the improvement in the error sum of squares on  $Y$  resulting from the presence of predictors ( $X$ ).  $R^2$  can be expressed as a percent of this improvement, so if  $R^2 = 0.3$ , it can be said that 30% of the  $Y$ -values are explained by the given predictors. Consequently, the forecasting abilities for the study at hand vary widely with respect to both analyte and condition. Multiple OLS does, however, offer a very effective approach for determining the relative importance of each chemical

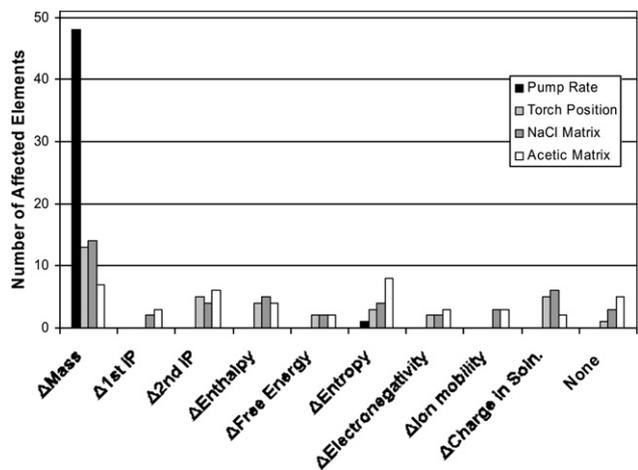


**Fig. 2** The number of elements whose %RSDs are significantly dependent ( $p$ -value < 0.1) on each chemical property as determined by a  $t$ - or  $F$ -test.

property on all analytes and over all conditions. For a more detailed discussion of multiple OLS regressions with chemical applications, please refer to the papers by Andrade *et al.*<sup>18</sup> and Leclerc *et al.*<sup>19</sup>

As shown by Fig. 2, mass is the most common predictor for all conditions, especially sample delivery rate. Although a factor for the largest number of elements, mass is not as dominant during other condition changes. For example, internal standard choice in an acetic acid matrix should possibly take 2nd ionization potential and charge into consideration along with mass. This clearly suggests that mass similarity is not the only property that needs to be considered. However, from Fig. 3, mass is the most significant as well as the most abundant predictor.

Fig. 2, in contrast to Fig. 3, is a plot of all the chemical property significances as opposed to the most significant chemical property. Mass shows a greater dominance in Fig. 3 for all the conditions represented than it does for Fig. 2. This implies that although many chemical properties play a role in internal standard compatibility, mass plays a more dominant role in comparison with the other properties considered. The  $t$ -scores



**Fig. 3** The number of elements whose internal standard pairs are most significantly related to each chemical property as determined by the  $t$ -values calculated for each model.

used to rank chemical properties indicate that for any condition, mass is not only the most abundant predictor, but it is often the strongest. This is especially apparent during the changes in sample introduction rate.

The study by Thompson and colleagues<sup>8</sup> concluded that ionization potential was an important factor for internal standard choice. However, the data collected in this study for 51 pairs indicate that there are only a select few elements that exhibit this dependency for any given condition. Analyte-internal standard pairs that either correlated well or correlated poorly spanned the entire range of ionization potentials, and no single element was dependent on this property for every condition. The change in chemical properties calculated for the purposes of linear regression were absolute. In the case of ionization potential, it may be important to consider the relative values since changes in matrix are expected to impact ionization efficiency.<sup>20</sup> At this time, however, there doesn't seem to be any logical trend as to which elements are significantly affected by ionization potential.

### Evaluation of analytical accuracy

The nine element evaluation series were analyzed, and the quantitative amounts of each analyte were determined using external calibration curves prepared from simple standards run under optimized instrument parameters as defined in Table 2. It should be noted that the slight variations in some of the operating parameters seen between conditions used for the database and evaluation series exist because independent optimization of the ICPMS system was performed before each study. The variations likely reflect the expected interdependencies on many of the adjustable torch and lens parameters and the expectation of arriving at a slightly modified set of optimized conditions.

Table 3 shows the analytes and the respective internal standards that were employed for accuracy determination. As noted earlier, the analytes were selected for the diversity of properties represented, *e.g.*, mass, ionization potential, *etc.* For each condition (torch position, sample delivery rate, 10 ppm NaCl matrix and 5% acetic acid matrix) an internal standard for each analyte was chosen from the database series based on a requirement that they be one of the five internal standard elements showing the lowest %RSD values in the database series. These internal standards will be subsequently referred to as the

**Table 2** Optimized instrument operating parameters

	Database series	Evaluation series
Sample introduction time (s)	30.0	30.0
Optimized sample delivery rate (mL min <sup>-1</sup> )	1.6	1.4
Flush pump time (s)	N/A	20.0
Flush sample delivery rate (mL min <sup>-1</sup> )	N/A	4.8
Acquisition time (s)	5.000	20.000
Number of replicates	5	5
Optimized torch position X, Y, Z (mm)	6, 0.3, -0.5	8.5, 1.9, 0.2
Nebulizer gas flow (L min <sup>-1</sup> )	1.150	0.970
Plasma gas flow (L min <sup>-1</sup> )	10.000	11.000
Auxiliary gas flow (L min <sup>-1</sup> )	1.000	1.500
Generator set power (W)	1300	1200

**Table 3** Analytes and internal standards chosen for their relatively low %RSD for each condition

Analyte	Best internal standard from database series			
	Torch position	Sample delivery rate	NaCl matrix	CH <sub>3</sub> COOH matrix
<sup>9</sup> Be	<sup>7</sup> Li	<sup>55</sup> Mn	<sup>121</sup> Sb	<sup>121</sup> Sb
<sup>26</sup> Mg	<sup>55</sup> Mn	<sup>76</sup> Se	<sup>44</sup> Ca	<sup>44</sup> Ca
<sup>59</sup> Co	<sup>64</sup> Zn	<sup>85</sup> Rb	<sup>121</sup> Sb	<sup>133</sup> Cs, <sup>89</sup> Y
<sup>115</sup> In	<sup>109</sup> Ag	<sup>133</sup> Cs	<sup>69</sup> Ga	<sup>166</sup> Er
<sup>138</sup> Ba	<sup>152</sup> Sm	<sup>133</sup> Cs	<sup>178</sup> Hf	<sup>88</sup> Sr
<sup>140</sup> Ce	<sup>178</sup> Hf	<sup>153</sup> Eu	<sup>88</sup> Sr	<sup>166</sup> Er
<sup>205</sup> Tl	<sup>193</sup> Ir	<sup>193</sup> Ir, <sup>178</sup> Hf	<sup>202</sup> Hg	<sup>166</sup> Er

“predicted internal standard” for the sake of clarity. It should be noted that often there were more than 5 internal standard elements whose %RSD were sufficiently low to be statistically indistinguishable from the pool of 5 elements from which the predicted internal standard was chosen. This will be expanded on in subsequent sections. To explore the efficacy of using a single internal standard for a range of masses, <sup>103</sup>Rh was evaluated as an internal standard for all nine analytes.

All calibration curves exhibited  $R^2 \geq 0.999$ . Relative error in concentration was calculated based on the differences between the known concentration of each sample and the concentration calculated from the external calibration curves. A statistical *t*-test was used to determine whether the concentration under the perturbed conditions using the analyte-internal standard ratio and the calibration curve made from simple standards were statistically different from the “true value” of 50 ppb.

Fig. 4 gives the absolute % error for each evaluated analyte-internal standard pair under each condition. Each plot represents the accuracy results for one analyte under all the perturbed conditions. An asterisk (\*) above the bar indicates that there is no statistical difference between the determined and true concentrations. The error for each analyte includes (i) using the predicted internal standard, (ii) using the predicted internal standard for the other 3 perturbed conditions, (iii) using the “universal internal standard” (*i.e.*, <sup>103</sup>Rh) and (iv) the signal without an internal standard. A wealth of inferences can be drawn from these plots.

<sup>9</sup>Be has the highest ionization potential of the nine analytes in the evaluation series. In the presence of an acetic acid matrix, all <sup>9</sup>Be/internal standard pairs offer worse accuracy than no internal standardization except <sup>121</sup>Sb, which is closest to <sup>9</sup>Be in ionization potential. However, the use of <sup>9</sup>Be/<sup>121</sup>Sb as an analyte-internal standard pair or no internal standardization for this condition still results in significant analytical error. In the easily ionized NaCl matrix, there is no apparent relationship to ionization potential and all errors were less than 10%, except when using no internal standard. Interestingly, the predicted internal standard, <sup>9</sup>Be/<sup>121</sup>Sb, shows slightly poorer results than the other analyte-internal standard pairs tested in this NaCl matrix.

Using any internal standard was better than no internal standard for altered sample delivery rates in nearly all cases. This is reasonable since it would be expected that any change that primarily affects the amount or size distribution of transportable aerosol would affect all elements similarly. However, the two

lightest elements showed exception to this observation, but the cause is unknown at this time.

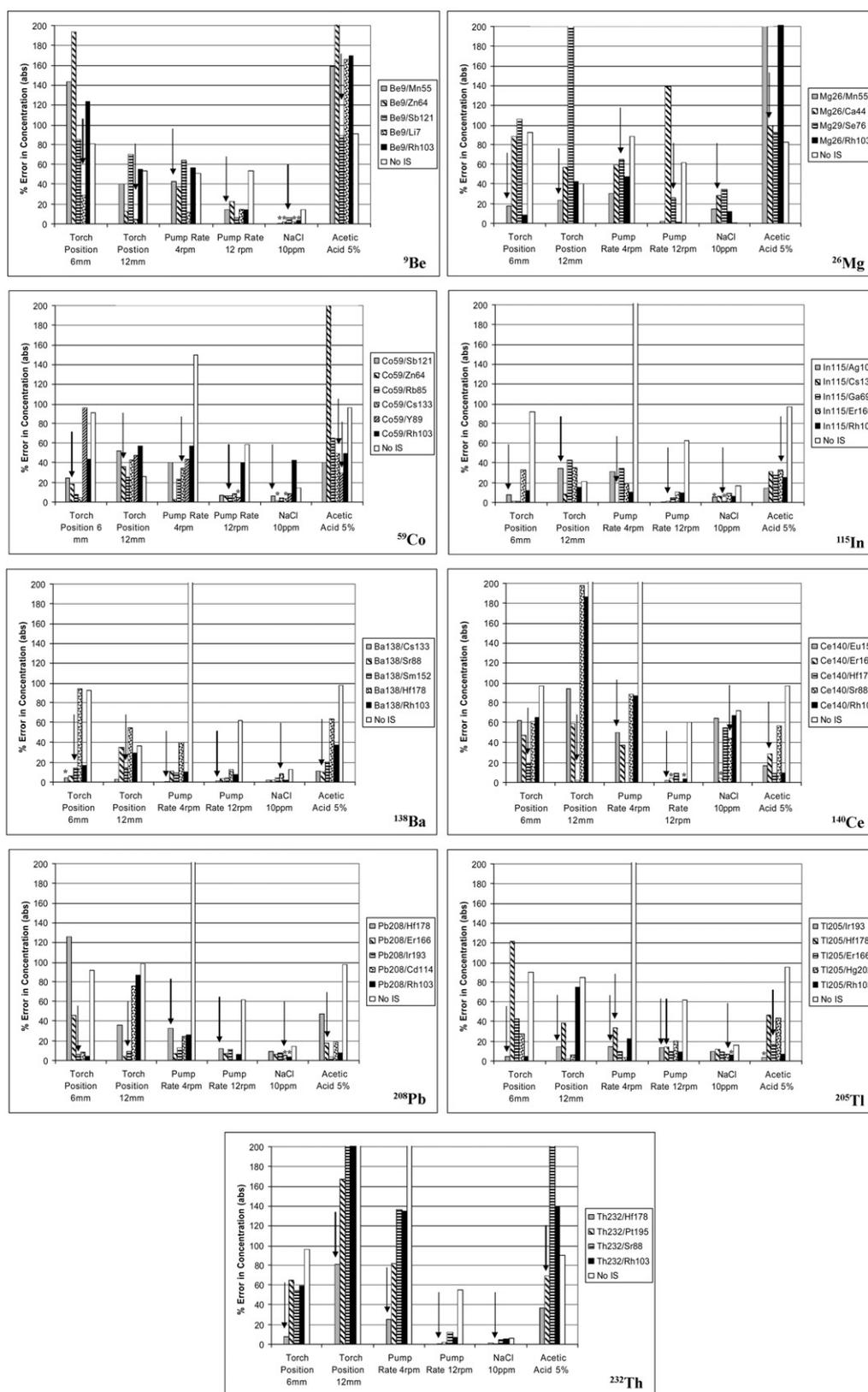
There are a number of circumstances in which the database series did not isolate the best internal standard. However, in most of these situations the predicted internal standard still provided relatively good accuracy. In the NaCl matrix, for example, the predicted <sup>208</sup>Pb/<sup>114</sup>Cd and <sup>115</sup>In/<sup>69</sup>Ga pairs yielded concentrations that were not statistically different from the true concentration. The same held true for the predicted <sup>140</sup>Ce/<sup>178</sup>Hf pair during a torch perturbation. The finite level of uncertainty due to counting statistics offers an explanation for many such cases, especially where low RSDs were observed, *e.g.*, sample delivery rate variations.

Table 4 summarizes which deviations from the database series predictions for the evaluation series can be explained by counting statistics. There are some cases in which the internal standard yielding the lowest % error follows an OLS trend previously discussed. For example, in the torch position condition for <sup>138</sup>Ba, <sup>152</sup>Sm was predicted to be the best internal standard by the database series. However, for both torch positions considered, <sup>133</sup>Cs offers the lowest error, which follows the expected mass trend for <sup>138</sup>Ba under perturbed torch positions. Although similarities in mass seem to correlate to a good analyte-internal standard pair, the data from this series would indicate that choosing the closest mass is not always the best choice. In fact, the results from this study indicate that only approximately 30% of the best performing analyte-internal standard pairs were also those that were closest in mass.

In some cases it is possible that the wide range of values for any given perturbation (*e.g.*, NaCl proceeding from 0–500 ppm) yielded a prohibitively high dispersion (%RSD); but for any individual perturbation value, there was very little difference. For example, it is conceivable that one element works better than any other as an internal standard over a large range of NaCl concentrations, but it may not be the best internal standard in the 10 ppm NaCl matrix, which is the specific matrix tested in the evaluation series. Since Table 4 shows that the matrix condition discrepancies are less completely explained by the uncertainties resulting from counting statistics, it is possible that matrix perturbations are more sensitive to the fact that the evaluation series was collected with a single perturbation value as opposed to a range. Finally, as previously mentioned, there may be uncertainty in the %RSD calculations due to the non-normal distribution of the data.

Some analysts choose to internal standardize with a single element for all analytes. This practice has been evaluated for the current study with <sup>103</sup>Rh. In the majority of cases presented, the use of any internal standard gave a more accurate result than when no internal standard was used. However, in approximately 18% of the samples used, <sup>103</sup>Rh showed a greater error than when no internal standard was used; and in 33% of the samples, at least one of the internal standard choices led to a larger error than was observed without any internal standard.

It should also be noted that some conditions gave consistently better accuracies than others (*e.g.*, NaCl matrix). Although a 10 ppm NaCl matrix did not appear to create any significant analytical problem, it is clear from the high %RSDs seen in the database series that larger concentrations of NaCl greatly deteriorate analytical accuracies.



**Fig. 4** Evaluation of analytical accuracy measured with relative % error in concentration from the “true value” of 50 ppb. Each bar represents the absolute errors associated with the use of each respective internal standard considered. The arrow indicates the results from a good, general internal standard choice as predicted by the *database series*. The errors associated with  $^{103}\text{Rh}$  (as a “universal internal standard”) and no internal standard are denoted by black and white error bars, respectively. Asterisks indicate determined concentrations that were not statistically significantly different from the “true value”. Each of the nine analytes is depicted in a separate plot.

**Table 4** Summary of pairs where the analytical errors fall within what might be expected from statistical (counting) uncertainties. Cases where the predicted internal standard gave the most accurate result are noted by “—”. The blank cells showed less than optimal accuracy with the predicted internal standard but fell outside of accepted precision limits based on counting statistics

Analyte	Internal standard discrepancies explained by counting statistics			
	Torch	Pump	NaCl	Acetic acid
<sup>9</sup> Be	—	<sup>7</sup> Li, <sup>64</sup> Zn, <sup>121</sup> Sb	<sup>7</sup> Li, <sup>103</sup> Rh	—
<sup>26</sup> Mg	<sup>103</sup> Rh	<sup>55</sup> Mn	—	—
<sup>59</sup> Co	—	—	<sup>85</sup> Rb, <sup>133</sup> Cs	—
<sup>115</sup> In	<sup>69</sup> Ga	—	—	<sup>133</sup> Cs
<sup>138</sup> Ba	<sup>88</sup> Sr, <sup>133</sup> Cs	—	—	—
<sup>140</sup> Ce	—	<sup>166</sup> Er	—	—
<sup>205</sup> Tl	—	<sup>166</sup> Er, <sup>202</sup> Hg	—	—
<sup>208</sup> Pb	—	<sup>166</sup> Er, <sup>193</sup> Ir	—	—
<sup>232</sup> Th	—	—	—	—

### Evaluation of analytical precision

In many analytical techniques, the use of just about any internal standard should improve analytical precision by compensating for multiplicative enhancements/depressions, *e.g.*, variability of sample volume injected into a GC, slight variations in sample delivery rate to the ICP, *etc.* An F-test was used to compare the precisions of the analyte signal in the evaluation series with the precision when an internal standard was employed. The results indicated that any time there was a statistically significant precision difference, the use of an internal standard improved the precision. Some experimental or matrix condition sets saw as much as an order of magnitude improvement in precision from using internal standardization. In only ~35% of the samples, the internal standard that gave the highest accuracy also gave the highest precision for a given analyte. It is possible that there may be different internal standard characteristics that should be considered when high precision is needed over high accuracy. This, however, must be evaluated further.

### Conclusions

Although many elements show an internal standard choice based on properties other than mass, no other single property stands out among the OLS trends to the same extent. Therefore, minimizing mass differences between an analyte and its internal standard is important, especially in the case of variability in sample delivery rate, as simulated in this study by “pump speed” changes. In an analytical situation such altered delivery rates may result from partial blockage of nebulizer, peristaltic pump roller noise and possible changes between calibration standards and unknown solution viscosities, surface tensions, *etc.*

However, similarity of mass between analyte and internal standard is not the only property that should be taken into consideration since there were many situations where the internal standard with a mass closest to the analyte was not necessarily the most ideal internal standard. For example, carbon content as simulated using an “acetic acid matrix” seems to also rely on closeness of the 2<sup>nd</sup> ionization potential between analyte and internal standard for several elements, although the explanation for such a correlation is not understood at this time. When it

comes to determination of the ideal internal standard for a given analyte, these other properties may become important. As seen in the evaluation data set where simple standards were used for quantitation, the pairs that were closest in mass yielded the most accurate determination in only 30% of the samples analyzed. During the evaluation of analytical accuracy, it became clear that a judicious choice of internal standard can make a significant difference in the validity of concentration measurements. Although most of the results examining accuracy indicated that choosing any internal standard was more effective than calibrating with no internal standardization, a poor internal standard choice yielded analytical errors in excess of 100% in many cases.

In all cases, precision was generally improved using internal standardization and no loss in precision was observed in any case. Interestingly, the largest improvements in precision showed little correlation to the best internal standard that would be selected from the evaluation series.

In practice, the method used for collecting the database series is an effective approach for determining the best internal standard if the matrix or other conditions within the sample are unknown or extremely varied from sample to sample. While the best performing internal standard in the nine element evaluation series was not always the internal standard predicted from the database series, the database series prediction generally exhibited relatively little analytical error. If matrix conditions between samples are expected to be less varied and instrument operation relatively stable then it may be prudent to run a short experiment similar to the method used in collection of the evaluation series data for analytes of interest under the conditions that are expected to dominate signal instability.

It should also be noted that all the data presented were collected from one instrument with one torch and spectrometer design. Some trends (*e.g.*, sample delivery rate’s relationship to mass) are expected to be reproducible on other instruments. However, those signal fluctuations that are more dependent on torch design and/or ion optics may result in alternative specific internal standard recommendations. Such potential variability from one instrument to another remains to be determined.

It was hoped that one of the outcomes of this study would be the isolation of a small number of physical or chemical characteristics common to analyte and a good internal standard. These could then be used as predictors for internal standard selection. Similarly, isolation of parameters whose difference precluded the use of certain pairs would have been nearly as useful. While the study shows that similarities in mass are common to a large number of good analyte-internal standard pairs, there are a sufficiently large number of exceptions to this “rule” that it should be used with scepticism. These data may serve as a foundation to evaluate trends for a given analyte with respect to designing “rules” or an equation to yield a “quality factor” for potential internal standards for that specific element. However, this element-by-element detailing was not within the scope of this paper.

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