

Evaluation of Internal Standards for the Mass-shift Analyses of As, Fe, S, and Zn in Biological Reference Materials Using ICP-Reaction Cell -MS

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ABSTRACT: In inductively-coupled plasma – mass spectrometry (ICP-MS) analyses, internal standards are often used to correct for signal variability. Determining the best internal standard has historically involved matching certain characteristics of the internal standard element like mass or first ionization energy to the analyte of interest. However, advances in ICP-MS such as reaction cell (RC) technology, which can effectively modify the ion beam or chemically alter the analyte of interest, introduce new considerations for the choice of internal standard. The commonly used internal standard elements ${}^6\text{Li}$, ${}^{45}\text{Sc}$, ${}^{69}\text{Ga}$, ${}^{74}\text{Ge}$, ${}^{89}\text{Y}$, ${}^{115}\text{In}$, ${}^{159}\text{Tb}$, ${}^{165}\text{Ho}$, ${}^{187}\text{Re}$, ${}^{193}\text{Ir}$, and ${}^{209}\text{Bi}$ were analyzed under five different analytical conditions and displayed a wide range of RC behavior including suppressed signal, increased signal (collisional focusing) and product ion formation. The behavior of these elements is not correlated to first ionization energy or mass and the product ions formed with NH_3 or O_2 as a cell gas are not consistent for all elements. To evaluate their use as internal standards, several product ions were used to correct the mass-shifted analysis of As, Fe, S and Zn in the NIST standard reference materials SRM 1573a Tomato Leaves and SRM 1577c Bovine Liver. With few exceptions, both mass-shifted and on-mass internal standards were effective in correcting the analytes of interest. Poor performance of the internal standards could be explained by sample matrix specific interferences, highlighting the importance of understanding RC behavior and its influence on analyses.

INTRODUCTION

Inductively-coupled plasma – mass spectrometry (ICP-MS) is a well-established analytical tool for determining trace levels of elemental concentrations in a wide range of sample matrices and has been the topic of many reviews.^{1,2} Because of the sensitivity of the instrument, small changes in the ionization efficiency of the plasma torch, stability of the ion beam or discrepancies in sample matrix can lead to significant changes in analytical precision.³ The ionization within the torch can be affected by short term changes in the laboratory environment, sample intake system and gas flows. To correct for signal variation and matrix effects, methods such as isotope dilution, standard addition or internal standardization can be used during analyses. Isotope dilution and standard addition can

be expensive due to the cost of enriched isotopes or time intensive due to the need to prepare many standards. Internal standardization offers an advantage because it uses readily available single or multi-element solutions and can be added in-line during analysis requiring no additional prep. The recovery of the internal standard is monitored during the analysis and drift in the internal standard is used to adjust the measured concentration of the sample. A good internal standard is an element that is not present in high concentrations in the sample to be analyzed and will not interfere with the measurement of the analyte of interest (*i.e.*, same mass). Despite the importance of this relationship between analyte of interest and internal standard, there is uncertainty about how exactly to determine the best internal standard element for each analyte and sample matrix. The predominate approach is to choose an internal standard with a similar mass and/or first ionization

energy (IE) of the analyte of interest.^{4,8} Other considerations such as the ionization potential of the matrix and mean free path - in relation to collisions between the analyte of interest and matrix particles - have been used to develop a unique index for assigning internal standards in a carbon-rich sample matrix.⁹ More recently, several studies have investigated the choice of internal standard in relation to reaction cell (RC) technology in ICP-MS.^{10,11}

RC technology in ICP-MS and ICP-MS/MS instrumentation utilizes a reaction cell filled with cell gas that will undergo thermodynamically favorable reactions with the ion beam to reduce spectral interferences such as single (isobaric) or polyatomic ions overlapping with the analyte mass of interest.¹²⁻¹⁶ In order to address these two types of interferences, two different analytical modes may be used. First, dynamic reaction cell (DRC) mode uses a cell gas that would result in gas-phase ion/molecule reactions.^{17, 18} When reactions occur the new product ions or molecules will have a different mass than the original mass of interest. Interferent ions will change to higher or lower masses and be removed from the reaction cell and avoid detection. Alternatively, the analyte of interest undergoes an atom-addition reaction within the cell and the new molecule is analyzed at a higher mass/charge ratio (m/z), *i.e.* mass-shifted, which has less on-mass interferences. In the second analytical mode, the cell gas acts as a bombardment gas to inhibit large ions, such as polyatomic interferences from escaping the reaction cell based on kinetic energy discrimination (KED). This technique is effective for complex analyses with multiple analytes and is increasingly used as a default analyses mode because it will prevent many polyatomic ions from reaching the analyzer quadrupole without requiring consideration for different ion/molecule reactions. In addition to suppressing the analytical signal because of chemical reactions or KED gas bombardment, RC technology may also increase a signal due to collisional focusing which occurs when the signal intensity increases with increasing gas pressure due to more effective transfer of ions out of the cell.¹⁹

The RC environment adds a layer of complexity in the selection of an internal standard for an analyte of interest. For one, internal standard performance in RC can suffer signal loss due to overlapping cluster formation, which is especially common with the use of NH_3 as a cell gas.¹¹ Only a few studies have focused on determining if it is more important that the internal standard and analyte of interest have a similar mass/^{1st} IE or RC behavior. Amaral *et al.* showed that the boron composition of plant material can be accurately determined by both mass-shifted and on-mass internal standards.²⁰ Bolea-Fernandez *et al.* recommends analyte/internal standard pairs that respond the same way within the RC for the best correction results.¹⁰ Both of these studies use ICP-MS/MS instrumentation which is equipped with a second mass filter after the reaction cell to reduce interferences.

In this work, we utilize the more readily available ICP-MS to

investigate how reactions taking place in the cell may affect the use of internal standards. We analyzed eleven different commonly used internal standards in five different analytical modes in order to characterize their prevalence for forming product ions. In many cases, these product ions differed from those described in the ICP-MS/MS studies and could shed light on how differing reaction cell behavior may influence analyte/internal standard compatibility. Both on-mass or mass-shifted internal standards were used in the mass-shifted analyses of As, Fe, S and Zn concentrations of two commonly used standard reference materials. These four elements are important analytes of interest in biological studies and have been successfully measured in mass-shifted analyses, previously.^{14,21-25}

EXPERIMENTAL

Standard solutions. All solutions were prepared in 2% (v/v) nitric acid (HNO_3) using ultrapure 18.2 M Ω water from a Milli-Q IQ7000 unit (MilliporeSigma, Burlington, MA, USA) and sub-boiled nitric acid from a DST-4000 acid purification system (Savillex, Eden Prairie, MN, USA). Single and multi-elemental standard solutions were sourced from certified reference solution suppliers, PerkinElmer (Waltham, MA, USA) and Inorganic Ventures (Christiansburg, VA, USA). All solutions were prepared volumetrically with calibrated manual pipettes (Gilson Pipettes, Middleton, WI, USA).

A stock solution of eleven commonly used internal standard elements was prepared that contained 100 $\mu\text{g/L}$ Bi, Ga, Ge, Ho, In, Ir, Li, Re, Sc, Tb, Y in 2% HNO_3 . All analytes were measured with the same parameters in each cell gas mode. Using the prepFast autosampler, the stock solution was analyzed at 40, 20, 10 and 5-fold dilutions. Roughly 30% of the sample volume is made up of an internal standard solution with the prepFast but during these runs a blank (2% HNO_3) was used in the internal standard line resulting in an additional dilution of 1.4x. Single element solutions were analyzed when necessary to confirm observed product ions. An acid blank was analyzed and used to subtract background cps from all measurements.

Calibration standards used for the determination of elemental concentrations in standard reference materials are summarized in [Table S1](#). A unique mix of internal standard elements was added in-line during each analysis at concentrations ranging from 5 to 6000 $\mu\text{g L}^{-1}$ to allow for a signal intensity of 0.2-1 M cps for each measurement condition.

Preparation of reference materials. Two National Institute of Standards and Technology (NIST; Gaithersburg, MD, USA) standard reference materials (SRMs) were used in this study. The reference materials were SRM 1573a Tomato Leaves and SRM 1577c Bovine Liver. Roughly 300 mg of material was weighed in

Table 1. NexION 2000 ICP-MS operating parameters

	Parameter	Value
ICP	Auxiliary gas flow (L min ⁻¹)	1.2
	Nebulizer gas flow (L min ⁻¹)	0.95
	Plasma gas flow (L min ⁻¹)	17
	ICP RF Power (W)	1600
Cell gas parameters	Cell gas flow (mL min ⁻¹)	Rpq
	Standard	0
	H ₂ /He KED	3.8
	H ₂ /He DRC	4.7
	NH ₃ DRC	1
	O ₂ DRC	1.2

Table 2. Important properties of potential internal standards

Analyte	Isotopic Abundance	1st Ionization Energy (eV)	Correction equation
⁶ Li	enriched	5.4	-
⁴⁵ Sc	1	6.5	-
⁶⁹ Ga	0.601	6	-0.116645 × ⁷⁷ Se
⁷⁴ Ge	0.363	7.9	-
⁸⁹ Y	1	6.4	-
¹¹⁵ In	0.957	5.8	-0.014038 × ¹¹⁸ Sn
¹⁵⁹ Tb	1	5.9	-
¹⁶⁵ Ho	1	6	-
¹⁸⁷ Re	0.626	7.9	-0.121362 × ¹⁸⁹ Os
¹⁹³ Ir	0.627	9.1	-
²⁰⁹ Bi	1	7.9	-

a 55 mL Teflon microwave digestion vessel, 5 mL of HNO₃ was added to each vessel, followed by 0.5 mL of Optima grade H₂O₂ (J. T. Baker, USA). The vessels were left to predigest overnight and then capped and sealed with a screw cap. A MARS 6 microwave digestion unit (CEM Corp, Matthews, NC, USA) was used for all sample digestions. The digestion method was as follows: Heat to 190 °C for 20 mins, hold at 190 °C for 10 mins. The vessels were cooled completely and the digestate was diluted gravimetrically to 50 mL with Milli-Q H₂O (52.25 ± 0.25 g). The SRMs were prepared in triplicate. Two method blanks were prepared and the average concentration was subtracted from the SRMs.

Instrumentation. All measurements were made with a NexION 2000 ICP-MS (PerkinElmer, Waltham, MA, USA) equipped with a PFA-ST MicroFlow nebulizer (Elemental Scientific, Omaha, NE, USA) and a glass cyclonic spray chamber cooled to 0 °C. The ICP-MS software was Syngistix (Ver 2.5). A prepFast autosampler (Elemental Scientific, Omaha, NE, USA) was used for sampling, inline addition of internal standard solution and performing dilutions. High purity argon (Ar, 99.999%) was sourced from Airgas (Holts Summit, MO, USA). The cell gasses were a hydrogen - helium mix (H₂/He, 8%/92%), anhydrous ammonia (NH₃, 99.995%) or research grade oxygen (O₂, 99.999%). H₂/He and O₂ were sourced from Airgas and NH₃ was sourced from Linde Gas (Columbia, MO, USA). The five analytical modes investigated in this study were standard mode with no cell gas used, KED mode with H₂/He and DRC mode with H₂/He, NH₃ or O₂.

The instrument parameters are given in Table 1. Gas flows were optimized according to Perkin Elmer recommendations. The RC was vented and pressurized with cell gas for ten minutes after each gas or gas flow change to allow for stabilization. Instrument performance was optimized daily according to the manufacturer's guidelines.

RESULTS AND DISCUSSION

Comparison of Std Mode and RC mode signal intensity. The eleven-element stock solution was analyzed for ⁶Li, ⁴⁵Sc, ⁶⁹Ga, ⁷⁴Ge, ⁸⁹Y, ¹¹⁵In, ¹⁵⁹Tb, ¹⁶⁵Ho, ¹⁸⁷Re, ¹⁹³Ir, and ²⁰⁹Bi. One or several of these elements are used as an internal standard in a typical ICP-MS procedure. Five of these eleven elements are monoisotopic which is an advantage for limiting isobaric interferences. The majority of these elements are rarely analytes of interest in ICP-MS studies (e.g. trace metal characterization) or abundant in natural samples. Additionally, these elements cover a wide range of masses and ionization energy which are important properties for internal standard selection. Properties and correction equations used for each analyte are listed in Table 2. Correction equations were used to subtract the signal contribution from an isobaric interference by measuring the signal of a non-interfering isotope. The solution was analyzed for all elements at four concentrations in five analytical modes resulting in 55 separate curves for interpretation. Every element shows a linear relationship between counts per second (cps) and concentration ($r^2 = 0.982-1.0$) with the exception of Sc in O₂DRC mode ($r^2 = 0.792$).

The slope of each curve is an indicator of the sensitivity for each element in each mode and in every case is positive, with the exception of Sc in O₂DRC mode which has a negative slope. In all but eight instances, the sensitivity is high (> 1.5 k cps (ug L⁻¹)⁻¹) showing good detection of those elements (Table S2). The most sensitive elements (> 250 k cps (ug L⁻¹)⁻¹) in descending order are Y in HeDRC, Tb in HeDRC and Std Mode, Ho in NH₃DRC and Std Mode and In in HeDRC and Std Mode. The relative change in sensitivity between STD mode and gas mode can be used to identify the behavior of analytes within the reaction cell (Table S3). If the sensitivity of an element decreases between STD mode and a cell gas mode, the ions must have less transmittance from the cell due to gas bombardment and KED or the element has interacted with the cell gas to create a new product ion, shifting the mass from the target mass. If the signal increases, we can assume the target analyte has more efficiently left the cell as a result of collisional focusing. However, an increase in the signal at a certain mass can also indicate the creation of a new on-mass interference as a result of reactions taking place within the cell. This is especially important when analyzing multi-element solutions like the one in this study and during the analyses of samples with complex, unknown matrices. With the exception of HeKED, there is no

apparent correlation between relative sensitivity and mass or ionization energy (Fig. S1).

The sensitivity for every analyte is lower in HeKED mode compared to STD mode demonstrating a reduction in signal due to bombardment with the cell gas and decrease in kinetic energy. The signal decrease ranges from 54% to 100% of the STD mode signal intensity. Because lighter elements lose more kinetic energy within the cell, the drop in signal between STD mode and HeKED is lower with increasing analyte mass. The dramatic 85-97% drop in signal intensity for the lighter internal standard elements Li, Sc, Ga, Ge, Y, and In could cause a potential issue in the analyses of elements requiring a high bombardment gas flow to reduce isobaric interference. If these lighter elements are considered the ideal internal standard in these situations, it should be noted that internal standard recovery is going to be heavily influenced by small changes in RC conditions and higher concentrations will be required.

With the exception of ${}^6\text{Li}$, signal intensity remains nearly the same (within 2%) or increases in HeDRC compared to STD mode. An increase in the signal suggests more ions are making it out of the cell and to the detector as a result of collisional focusing. This behavior was investigated by analyzing the multi-element solution in DRC mode while varying the cell gas flow rate (Fig. 1). The signal intensity increases with increasing H_2/He flow until a peak is reached and then signal intensity levels out or starts to decline suggesting H_2/He first improves signal intensity by collisional focusing and then acts as a bombardment gas resulting in scattering. This pattern demonstrates that high gas flows of even reactive gases can effectively reduce the number of ions leaving the cell if the flows are high enough. Gas flows higher than 3 mL min^{-1} led to a decrease in the Sc signal and gas flows higher than 4 mL min^{-1} led to a decrease in signal for Ga, Ge, and Y. This experiment highlights that the analytical conditions for each analyte are not optimized and high signal intensity may be reached with H_2/He gas flows different than those used in this study.

In NH_3DRC and O_2DRC mode, nearly all internal standard elements show a drop in sensitivity compared to STD mode which can be partly explained by atom addition reactions mass-shifting the analyte of interest. The element/mode combinations with the lowest sensitivity are Sc, Ge, Y, and Ir in NH_3DRC mode and Sc, Y, Tb, and Ho in O_2DRC mode. The reduction in signal intensity in NH_3DRC mode can be explained by the formation of cluster product ions which is a well-known reaction pathway for cations and NH_3 cell gas.^{10, 11, 26} In O_2 mode Sc, Y, Tb and Ho have the potential to form oxides effectively shifting their mass $+16\text{ m/z}$. To confirm the formation of mass-shifted product ions, the multi-element solution was analyzed in either mass scanning mode to identify unknown product ions or at specific higher masses such as $+16\text{ m/z}$ (MO^+) to account for mass shift from the addition of oxygen. The literature for both experimental and theoretical data

Fig. 1 Internal standard elements analyzed in HeDRC mode with increasing cell gas flow.

agreed with many of the reactions (or lack thereof) observed in our experiments despite difference of analytical instrumentation.²⁶⁻³⁰ The reactions occurring during NH_3DRC and O_2DRC analyses are discussed further in the following sections.

Internal Standard elements in NH_3DRC mode. With the exception of Ho and Bi, all elements showed a decrease in signal in NH_3DRC mode compared to STD mode suggesting the formation of product ions with NH_3 resulting in a mass-shift for analytes of interest. To investigate which product ions are formed, the stock solution was measured at low (0.1 mL min^{-1}) and high (1 mL min^{-1}) NH_3 gas flows in mass scanning mode. Increases in cps at higher masses in the high gas flow measurements are interpreted as product ions. When product ions of different analytes may have overlapped with one another, single element solutions were analyzed to confirm product ions. Single element solutions were measured for all elements except Li because no product ions could be observed for Li due to interferences in the low mass range.

The observed product ions are listed in Table 3. The most common product ion was $\text{M}(\text{NH}_3)_x^+$ where M = analyte and $x = 1-2$. Other observed products included $\text{M}(\text{NH})_{2,3}^+$ and other cluster ions such

Table 3. Major observed product ions in NH₃DRC mode. References are listed where product ions have been previously described

Element	Product Ion	References
⁶ Li	-	-
⁴⁵ Sc	ScNHNH ₃	24, 25
⁶⁹ Ga	GaNH ₃ , Ga(NH ₃) ₂	25
⁷⁴ Ge	GeNH ₂ , GeNH ₂ NH ₃	10, 24
⁸⁹ Y	YNH(NH ₃) ₄ , YNH ₂ (NH ₃) ₄	25
¹¹⁵ In	InNH ₃ , In(NH ₃) ₂	25
¹⁵⁹ Tb	TbNHNH ₃ , TbNH(NH ₃) ₂	24
¹⁶⁵ Ho	HoNH ₃	24
¹⁸⁷ Re	Re(NH) ₂ , Re(NH) ₃ , ReNH ₃ (NH) ₂	25
¹⁹³ Ir	IrNH ₂ NH ₃ , Ir(NH ₃) ₂	25
²⁰⁹ Bi	BiNH ₃	25

Fig. 2 Signal intensity of internal standard elements measured on-mass and mass-shift in DRC mode with O₂ cell gas.

as MNH₂NH₃⁺ and MNHNH₃⁺. There is no evidence for the formation of MH⁺ or MOH⁺. In most cases, more than one product ion is observed. The signal intensity of the product ion is a small fraction of the on-mass signal in low gas flow mode and the combined signal intensity of all product ions cannot account for all on-mass signal loss which suggests an additional cause of signal loss such as collisional scattering. Ho and Bi showed slight formation of MNH₃⁺ but show an enhanced on-mass signal in the presence of NH₃ due to collisional focusing. One observation unique to NH₃DRC mode is the difference in Tb and Ho behavior. Tb exhibits product ion formation and Ho exhibits collisional focusing although the two analytes demonstrate very similar behavior in all other modes, suggesting a unique property of each element contributes to reaction preferences with NH₃.

Internal standard elements in O₂DRC mode. In O₂DRC mode, the signal sensitivity for all elements is lower than STD mode with the exception of Ir and Bi. To identify the product ions that are formed, the metal oxide (MO⁺) and higher order oxides (MO₂⁺, MO₃⁺) were analyzed at the expected mass-shifted values (MO⁺ = +16 m/z, MO₂⁺ = +32 m/z, *ect*) (Fig. 2). The greatest oxide (MO⁺)

formation is seen with Sc, Y, Tb, and Ho and shows signal strength 43-21000 times higher than the on-mass measurements. Higher order oxides MO₂⁺ and MO₃⁺ are also observed for these four elements although to a much lower extent at less than double the on-mass measurement signal. Tb is an exception which has 6700 and 190 times the signal intensity of Tb for TbO₂⁺ and TbO₃⁺, respectively. The high signal at TbO₂⁺ and TbO₃⁺ is a result of overlap from ¹⁹¹Ir and ¹⁹¹Ir¹⁶O, respectively, and analyses of a Tb only solution shows the actual signal for TbO₂⁺ is 72 times the on-mass signal. There is no observable formation of TbO₃⁺ in the single element analyses.

The signal for oxides of Li, Ga, Ge, In and Re was lower than on-mass analyses and was often very low (<1000 cps). The most significant single oxide signals were for Ge and Re. There was a significant signal for ⁶Li¹⁶O₂⁺ but the intensity was inconsistent ranging from 100-18852 cps for three consecutive measurements and likely influenced by ³⁸Ar⁺ interference. ReO₂⁺ and ReO₃⁺ are also observed in the measurements and at a higher intensity than ReO⁺. The preference for Re to form higher order oxides has been observed previously using Fourier-transform ion cyclotron resonance/MS³¹ and ICP/selected-ion flow tube tandem MS³² with even higher order oxides observed (ReO_n⁺; n = 2-6, 8). No considerable oxide formation is observed for Ga or In suggesting that the reduction in sensitivity from STD mode for Ga and In is likely due to O₂ acting as a bombardment gas.

The remaining two elements, Ir and Bi showed an increased on-mass signal compared to STD mode and the formation of several oxide product ions. Single element analyses of Ir in O₂DRC mode shows a collisional focusing affect for Ir resulting in an increased signal of 13% over STD mode. The formation of IrO was measured as roughly 3% of the on-mass measurement and higher order oxides IrO₂ and IrO₃ were less than 0.05%. Single element analyses of Bi confirmed a collisional focusing effect with a 37% increase over STD mode and insignificant formation of BiO⁺. Lancaster *et al.* showed that in ICP-MS/MS a small fraction of Bi reacts to form BiO⁺ with O₂ but did not show any evidence for collisional focusing.³³

Comparing corrections made by internal standards in varying analytical modes. The two reference materials used in this study were SRM 1573a Tomato Leaves and SRM 1577c Bovine Liver. Both of these SRM were released in 2018 and are used frequently as biological reference materials for elemental concentrations measured by ICP-MS. They differ from one another in their elemental composition with SRM 1573a having significantly higher concentrations of Ca, K and Mg and SRM 1577c having higher concentrations of Cu, Na and Zn.^{34, 35} Using both SRMs offers insight into how different sample matrices could influence the choice of internal standard. Iron, Zinc, Sulfur and Arsenic concentrations were chosen as the analytes of interest in this study because they are important analytes of interest in biological

Fig. 3 Fe, Zn and select product ions with varying NH₃ gas flow.

Table 4. Interferences and correction equations for measured masses

Analyte	<i>m/z</i>	Interferences	Correction equation
SO	48	⁴⁸ Ti, ⁴⁸ Ca	$-0.1011 \times (^{47}\text{Ti}) - 0.289 \times (^{42}\text{Ca})$
ZnNH ₃	83	⁸³ Kr	none
GaNH ₃	86	⁸⁶ Sr	$-0.1194 \times ^{86}\text{Sr}$
Fe(NH ₃) ₂	90	⁹⁰ Zr	none
AsO	91	⁹¹ Zr	none
TbNH(NH ₃) ₂	208	²⁰⁸ Pb	$-2.371 \times ^{207}\text{Pb}$

materials, cover a wide range of concentrations within the chosen SRMs and have been previously measured by mass-shift analysis in ICP-RC-MS.^{14, 21-25}

The analytes of interest were analyzed using the mass-shift approach based on observed product ions. Fe and Zn were measured in NH₃DRC mode and S and As were measured in O₂DRC mode. The internal standards used for each experiment were measured on-mass or mass-shifted and were chosen based on similarities to the analyte of interest because they had similar mass, ionization energy or product ions. For example, to correct for a mass-shifted analyte with an added NH₃⁺ ion (*i.e.* mass shift = +17 *m/z*), a previously identified mass-shifted standard like InNH₃ was used. Additionally, several mass-shifted internal standards with different cell gas reaction pathways were chosen for comparison.

The analytical session involved measuring external standards as described in the methods to create a calibration curve, followed by three replicates of each SRM using one of the internal standards to correct the analyte signal. A select few of the previously examined internal standard elements were added in-line during analyses. The data was then reanalyzed within the software, using a different internal standard so that any variability in the instrument performance during the run is identical for each internal standard. The measured concentrations were compared to the accepted values for each SRM with a ± 10% acceptance criteria. This range was chosen as a test for the effectiveness of an internal standard

and is smaller than the acceptance criteria of several standard operating protocols used in industry such as EPA 6020b which allows for ± 20% recovery of SRM elemental concentrations.³⁶ The lower limit of quantification (LLOQ) was calculated for each internal standard from ten consecutive measurements of the blank and was nearly the same for each analyte/internal standard combination (Table S4). The product ions analyzed and the elemental correction equations used to correct for isobaric interferences is listed in Table 4.

With two exceptions, the internal standard recovery (cps_sample/cps_blank) ranged from 86-110%. Recovery within this range demonstrates that 1) the internal standard signal is stable over the course of the run and 2) there are no significant contributions of internal standard elements from the SRMs. The two internal standards that had poor recovery were GaNH₃ in NH₃DRC and Ga in O₂DRC mode. Despite 97-105% internal standard recovery during calibration and the measurement of blanks, these internal standards gave poor performance during the analyses of SRM 1573a. The unique matrix of SRM 1573a interferes with the measurement of GaNH₃ in NH₃DRC mode and reduces the signal by nearly a third. In O₂DRC mode, the recovery of Ga increases to 140% and 186% for S and As, respectively. The unique behavior of Ga during the analysis of SRM 1573a demonstrates the importance of understanding the sample matrices influence on an analytes signal. In these two cases, Ga would be a problematic internal standard for SRM 1573a. The potential causes of the interferences are discussed in the following sections.

Determination of Fe in NH₃DRC mode. The iron concentration of the SRMs was determined in NH₃DRC mode by analyzing Fe(NH₃)₂⁺ (*m/z* = 90). This product ion was found to be the predominant ion formed during mass scanning mode and the only isobaric interference was ⁹⁰Zr which was not corrected for due to the low abundance of zircon in the SRMs. Fe(NH₃)₂ can be an alternative analyte to ⁵⁶Fe which has significant polyatomic

interference from $^{40}\text{Ar}^{16}\text{O}$ as previously reported by ICP-MS/MS studies.^{10,23} Seven internal standards were used for the correction of Fe. ^{165}Ho and ^{209}Bi were measured on-mass and the mass-shifted internal standards were GaNH_3 ($m/z = 86$), $\text{YNH}(\text{NH}_3)_4$ ($m/z = 172$), $\text{TbNH}(\text{NH}_3)_2$ ($m/z = 208$), $\text{Re}(\text{NH}_3)_3$ ($m/z = 232$), $\text{Ir}(\text{NH}_3)_2$ ($m/z = 227$). Several mass-shifted internal standards suffer from on-mass interference which were corrected for as detailed in Table 4. The NH_3 cell gas flow was optimized for the formation of the product ions by adjusting the flow from 0.1 mL min^{-1} to 1.2 mL min^{-1} and monitoring both on-mass and mass-shifted product ions. The formation of $\text{YNH}(\text{NH}_3)_4$ most closely represents the formation of $\text{Fe}(\text{NH}_3)_2$ which may make it a more appropriate internal standard (Fig. 3). The ideal NH_3 gas flow was determined to be 0.8 mL min^{-1} .

The NIST certified value for Fe concentration in SRM 1573a is $367.5 \pm 4.3 \text{ mg kg}^{-1}$.³⁴ All internal standards resulted in concentrations that were within 10% of this range (Fig. 4). The highest concentration was given with GaNH_3 as an internal standard. During the analysis, GaNH_3 recovery was low at $\sim 78\%$ suggesting there may be some overcorrection, resulting in a higher apparent Fe concentration. The low recovery of GaNH_3 may be a result of the correction equation used on the analysis to correct for Sr which measures ^{88}Sr to correct for the ^{86}Sr contributing to the GaNH_3 signal. The intensity of the $m/z = 88$ signal is heightened due to $^{40}\text{Ar}^{48}\text{Ca}$ being formed in the plasma as a result of the high Ca concentration in SRM 1573a, effectively overcorrecting for ^{86}Sr and lowering GaNH_3 recovery. Analysis of a hundred-fold dilution of SRM 1573a had no signal repression for GaNH_3 (95% recovery) suggesting $^{40}\text{Ar}^{48}\text{Ca}$ is formed in the torch when there is a high concentration of Ca, increasing the signal at $m/z = 88$. The 2σ precision of the 3 replicates ranges from 22 to 57 mg kg^{-1} .

The NIST certified value for Fe concentration in SRM 1577c is $197.94 \pm 0.65 \text{ mg kg}^{-1}$.³⁵ With the exception of Ho, all internal standards produced concentrations within 10% of the accepted value (Fig. 4). The concentration determined using Ho internal standard is slightly out of range at 177.7 mg kg^{-1} Fe. The 2σ precision of the 3 replicates ranges from 2.6 to 7.7 mg kg^{-1} . Because all internal standards used result in similar concentration values that overlap in uncertainty and fall within the 10% acceptance criteria, there is no definitive indication that one is the best. Using $\text{Ir}(\text{NH}_3)_2$, which has the same reaction pathway as $\text{Fe}(\text{NH}_3)_2$, results in the measured value closest to the accepted value for SRM 1573a but the best precision is achieved when using GaNH_3 despite GaNH_3 analysis being problematic as described above. For SRM 1577c, the closest concentration and lowest precision are achieved when using $\text{YNH}(\text{NH}_3)_4$ which has no similarities to the analyte of interest except a similar formation rate (Fig. 3). The difference in the performance of the internal standards between the two reference materials, suggests the sample matrix plays a larger role than previously expected.

Fig. 4 Measured iron concentrations for SRMs using seven different internal standards. The NIST reported concentrations are shown as a solid horizontal line with a $\pm 10\%$ area shaded. Error bars represent 2σ precision, $n=3$.

Determination of Zn in NH_3DRC mode. The zinc concentration of the SRMs was determined in NH_3DRC mode by analyzing ZnNH_3^+ ($m/z = 83$). This product ion was found to be the predominant ion formed during mass scanning mode and the only isobaric interference was ^{83}Kr which was not corrected for because the largest source of Kr would be from the plasma gas and at consistent levels for all blanks and samples. Product ions of Zn and ammonia gas have been previously measured in at least one ICP-MS study focused on measuring Zn in sulfuric acid where interferences from S and O would be especially difficult to avoid for on-mass measurements of Zn.²⁴ The same seven internal standards and cell gas flow described for the analysis of Fe were used for Zn measurements (Fig. 3).

The NIST certified value for Zn concentration in SRM 1573a is $30.94 \pm 0.55 \text{ mg kg}^{-1}$.³⁴ All internal standards resulted in concentrations that were within 10% of this range (Fig. 5). As with the Fe analysis, the highest Zn concentration is given when using GaNH_3 as an internal standard due to the low recovery of GaNH_3 during SRM 1573a measurements. The 2σ precision of the 3 replicates ranges from 1.8 to 4.4 mg kg^{-1} (Fig. 5). The measured concentration closest to the certified value and the lowest uncertainty is determined when using Bi as an internal standard. The NIST certified value for Zn concentration in SRM 1577c is $181.1 \pm 1.0 \text{ mg kg}^{-1}$.³⁵ Concentrations determined with GaNH_3 and $\text{YNH}(\text{NH}_3)_4$ are within this range and all other measured concentrations are below. The 2σ precision of the 3 replicates ranges from 4.4 to 8.1 mg kg^{-1} . The best internal standard which produced the closest measured concentration and the lowest uncertainty is $\text{YNH}(\text{NH}_3)_4$. This was also the ideal internal standard determined by Fe measurements of NIST 1577c suggesting the internal standard may be ideally paired with a sample matrix as opposed to the analyte of interest.

Fig. 5 Measured zinc concentrations for SRMs using seven different internal standards. The NIST reported concentrations are shown as a solid horizontal line and a $\pm 10\%$ area is shaded. Error bars represent 2σ precision, $n=3$.

Fig. 6 Measured sulfur concentrations for SRMs using six different internal standards. The NIST reported concentrations are shown as a solid horizontal line and a $\pm 10\%$ area is shaded. Error bars represent 2σ precision, $n=3$.

Fig. 7 Measured arsenic concentrations for SRMs using six different internal standards. The NIST reported concentrations are shown as a solid horizontal line and a $\pm 10\%$ area is shaded. Error bars represent 2σ precision, $n=3$.

Determination of S in O₂DRC mode. The sulfur concentration

of the SRMs was determined in O₂DRC mode by measuring SO⁺ ($m/z = 48$). The analysis of SO⁺ ions instead of S is a common practice due to spectral overlap of all isotopes of S which has been summarized elsewhere.^{21,22,37} The O₂ flow was adjusted to 0.8 mL min⁻¹ which was the optimal flow for SO⁺ formation. Due to spectral overlap at mass 48 by ⁴⁸Ti and ⁴⁸Ca mathematical corrections were made to the SO signal (Table 4). Six internal standards were used to correct the signal and determine S concentrations in the SRMs (Fig. 6). Ga and In were measured on-mass. TbO, HoO, and IrO were measured at a +16 m/z mass-shift and ReO₃ was measured as a higher-order oxide mass shift (+48 m/z).

The NIST reported S concentration for SRM 1573a is 9600 mg kg⁻¹.³⁴ (informational value only) and with the exception of Ga, the S determined by each internal standard is within 10% of the accepted value. The S concentration as determined by Ga was only 74% of the expected value due to interference affecting the correction of Ga internal standard (⁶⁹Ga IS recovery = 140%). The increased counts suggest an interference present in the matrix of SRM 1573a that is not present in the blank or standard solutions and was not observed during analysis of SRM 1577c. This observation highlights not only that unique isobaric interferences in a sample matrix may influence recovery of analytes of interest, but also additional interferences can be introduced in ICP-RC-MS if elements react with the reaction gas. In this case isobaric interferences could be oxides formed from Cl, Cr or V which are all more abundant in SRM 1573a than SRM 1577c. The lowest 2σ uncertainty of 60 mg kg⁻¹ was seen when using TbO as an internal standard and was nearly double for all other internal standards. Corrections using TbO also produced the best measured concentration. Besides being a single oxide ion, TbO has no similarities to SO that would suggest it is the best internal standard (i.e. similar mass or first ionization energy).

The NIST certified value for S concentration in SRM 1577c is 0.749% \pm 0.034³⁵ and the measured concentration for every internal standard is within 10% of that value. The concentration calculated with Ga is the lowest value compared to other internal standards and there is no apparent interference at $m/z = 69$ during analysis (i.e. Ga recovery is $\leq 100\%$). The lowest 2σ uncertainty is ± 41 mg/kg with Ga and the closest measured concentration is determined with HoO although this measurement has the highest uncertainty at ± 165 mg kg⁻¹.

Determination of As in O₂DRC mode. The arsenic concentration of the SRMs was determined in O₂DRC mode by measuring AsO⁺ ($m/z = 91$). The mass shift approach is the recommended method for As determination as it avoids polyatomic interferences from chloride species and can increase signal sensitivity.^{14, 25, 38} The same five internal standards previously described for S determination were used for As analysis and the measured values were compared to the accepted values of the SRMs (Fig. 7).

The NIST certified concentration of As in SRM 1573a is $0.1126 \pm 0.024 \text{ mg kg}^{-1}$.³⁴ Measured As concentrations using Ga as an internal standard were significantly lower than the accepted values because Ga recovery was high as previously described for S measurements in SRM 1573a. All other internal standards resulted in values within the acceptable range. The lowest 2σ precision for the replicate digestions is 0.009 mg kg^{-1} when using ReO_3 . The NIST certified concentration of As in SRM 1577c is $0.0196 \pm 0.0014 \text{ mg kg}^{-1}$.³⁵ All internal standards resulted in a measured As value within 10% of the accepted value. The 2σ precision was similar for each internal standard and ranged from $0.001 - 0.003 \text{ mg kg}^{-1}$. The similarity between all internal standards for As suggests that similarity in RC behavior plays no role in the effectiveness of internal standard corrections (*i.e.* TbO does not make a better internal standard than In).

CONCLUSION

Eleven elements commonly used as internal standards were analyzed in five analytical modes by ICP-RC-MS and shown to behave independent of physical properties such as mass or first ionization energy. By comparing several internal standards to determine elemental concentrations in two SRMs, it was shown that multiple internal standards with differing RC behavior are suitable for mass-shift determinations. Additionally, an ideal internal standard for one element was not the same for both SRM suggesting that matrix effects play a large role in the variability of the analytical signal. The behavior of a chosen internal standard can be unpredictable because of the formation of product ions which may decrease analyte signal or because of the introduction of isobaric interferences from sample matrices. This highlights the importance of properly understanding the behavior of the internal standard element as well as the interferences that may arise when unique sample matrices interact with the reaction gas.

ASSOCIATED CONTENT

The supporting information (Fig. S1 and Tables S1–S4) is available at www.at-spectrosc.com/as/home.

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Notes

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