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Case study

Evaluation of the effects of residual carbon content matrix on the determination of pharmaceutically regulated elemental impurities by ICP-MS

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1. Introduction

In recent years, several changes to elemental impurities control strategy in active pharmaceutical ingredients (APIs), drug products (DPs) and excipients were proposed by the United States Pharmacopoeia (USP), European Pharmacopoeia/European Medicine Agency (EP/EMA) and the International Conference on Harmonisation (ICH). The wet chemistry colorimetric tests for heavy metals such as USP<231> and EP 2.4.8 have been replaced with instrumental methods, one of which being inductively coupled plasma mass spectrometry (ICP-MS) that provides fast, specific and quantitative determination of individual elemental impurities. ICP-MS has the capability to quantify the 24 regulated elemental impurities using a single method, whether present at ultra-trace levels or high concentrations, due to the instruments' wide dynamic range (up to 11 orders).

USP <233> defines different methods for sample preparation prior to ICP-MS analysis. The majority of pharmaceutical materials, including the most commonly used excipients, can be digested using either closed-vessel microwave digestion or room temperature digestion in concentrated acids. The choice of concentrated acid depends on the sample matrix, however the most used reagent for organic matrix decomposition is nitric acid. Only a limited number of pharmaceutical samples, such as polymers and heavy organic chemicals, cannot be digested directly in nitric acid and these require an initial char step with sulfuric acid.

Acid solutions combined with different additives (e.g. thiourea, d-mannitol) can cause matrix effects in ICP-MS analysis. Furthermore, direct dissolution of pharmaceutical samples can lead to relatively high residual carbon content (RCC) which may cause matrix effects as well. The chemical structure of the pharmaceutical samples contains elements other than C and H, such as N, O, S, P and Cl. The presence of these elements in digests can result in spectral interferences in ICP-MS due to the formation of polyatomic ions. In general, matrix effects in ICP-MS may be divided into two basic categories; matrix induced spectral overlap problems (due to polyatomic interferences) and matrix induced signal intensity changes. It is not unusual that both effects are present simultaneously and both of them can significantly impact the accuracy of the analytical data.

The RCC matrix effects on the regulated elements were assessed in this study. Two quantitative methods (external calibration and standard addition) were compared by analysing elemental impurities in sucrose, as a source of residual carbon content, to investigate the RCC induced bias and the efficiency of the two techniques in reducing it.

2. Residual Carbon Content (RCC) Matrix Effects

In this study, matrix effects resulting from sucrose were evaluated using solutions with concentrations ranging from 0.05 mg/mL to 10 mg/mL, corresponding to approximately 0.002% to 0.42% carbon content.

To assess matrix effects, single-element reference standards were mixed to create a stock standard containing all 24 ICH impurity elements at a concentration of 200 ng/mL. From this stock, multi-element test solutions were prepared at 5 ng/mL in both deionised water (UHQ water) and in each of the investigated sucrose matrices. The matrix content varied from a low concentration of 0.05 mg/mL to a high concentration of 10 mg/mL.

The results in Figure 1 and Figure 2 show that ion signals for almost all analysed elements (except for ^{105}Pd and ^{197}Au) were enhanced in the presence of sucrose in the matrix, thus indicating a positive matrix effect.

Figure 1 Spike recoveries of the 24 regulated elements in low and high sucrose matrices

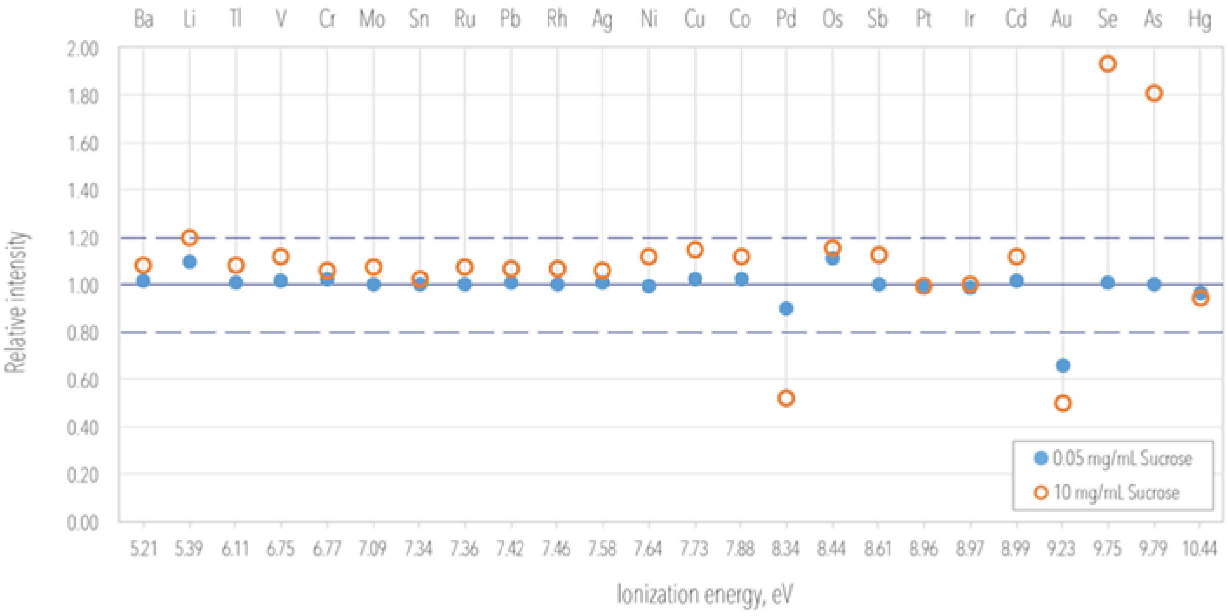


Figure 2 Relative intensities of the 24 regulated elements in low and high sucrose matrix contents

For ^{105}Pd and ^{197}Au, the nuclide signals were lower when sucrose was present in the solution and varying relative intensities were observed (Figure 3). No robust hypotheses can be drawn from this data regarding ^{105}Pd and ^{197}Au, however it is safe to conclude that these elements are not stable in a UHQ water and sucrose matrix.

A significant signal enhancement was observed for ^{52}Cr, ^{75}As, and ^{82}Se in the presence of 10 mg/ml sucrose. The high spike recovery for ^{52}Cr was expected since it is well known that this nuclide is affected by the carbon-based polyatomic interference ^{40}Ar^{12}C [1]. As can be seen from Figure 2, the relative signal intensity calculated using the blank-subtracted signal was 1.06, indicating that this nuclide was mainly affected by the polyatomic interferences rather than carbon-based ionisation enhancement. Relative intensities for ^{75}As and ^{82}Se however in the presence of solutions containing 10 mg/ml sucrose were 1.80 and 1.93, respectively. Therefore, it can be concluded that carbon-induced signal enhancement occurs for these nuclides in the presence of sucrose in the matrix.

Figure 3 shows that the influence of sucrose concentration (up to 10 mg/ml) on the relative intensities of ^{75}As, ^{82}Se, ^{105}Pd and ^{197}Au and the averaged relative intensity for the other remaining nuclides. The observed positive matrix effect is accentuated with increasing concentrations of sucrose. The mean relative intensity value of all nuclides except for ^{75}As, ^{82}Se, ^{105}Pd and ^{197}Au showed slightly enhanced ion signals increasing from 1.01 to 1.08 when sucrose concentration was changed from 0.5 mg/ml to 10 mg/ml. The same change in sucrose concentration resulted in an enhancement in the relative intensities from 0.99 to 1.80 for ^{75}As and from 1.01 to 1.93 for ^{82}Se.

In summary, our results highlight the complex interplay between sucrose concentration and nuclide signals, with implications for analytical measurements in the presence of matrix effects.

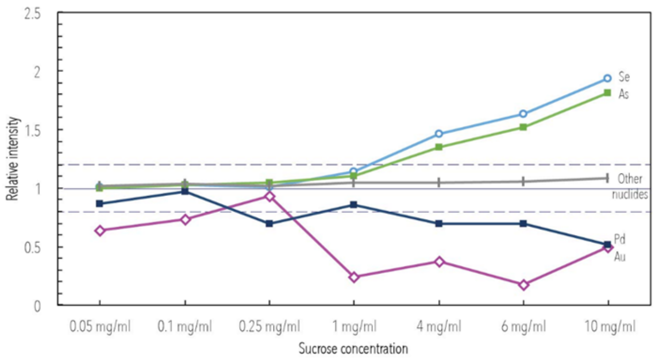


Figure 3. Influence of the sucrose concentration on the relative intensity value obtained for Se, As, Pd, Au and average relative intensity value for the remaining nuclides tested in this study

Figure 4 shows the spike recoveries obtained for ^{75}As and ^{82}Se under different ICP-MS instrument conditions; [No Gas], [He] and [HEHe] modes, all in the presence of 10 mg/ml sucrose matrix.

In [He] mode, the ICP-MS uses helium as the collision gas in the collision cell. This mode allows for efficient removal of interferences using an inert cell gas (helium) and kinetic energy discrimination (KED) with the octupole-based cell. It provides good interference removal for most elements. But some polyatomic ions may still be present. [HEHe] mode is an enhanced version of [He] mode. In [HEHe] mode, the helium flow is higher than in standard [He] mode, and some lenses are more negative. The increased helium flow and optimised lens settings improve interference removal, especially for polyatomic ions.

The use of [He] and [HEHe] modes however did not yield spike recovery values closer to 100%. Figure 4 shows an increase of the spike recovery over the modes.

Based on the above results, it can be concluded that sensitivity for the analysis of ^{75}As and ^{82}Se can be significantly enhanced by presence of sucrose in the matrix. This matrix effect can be explained in relation to an increase in the analyte ion population as a results of charge transfer reactions involving C+ species in the plasma [2].

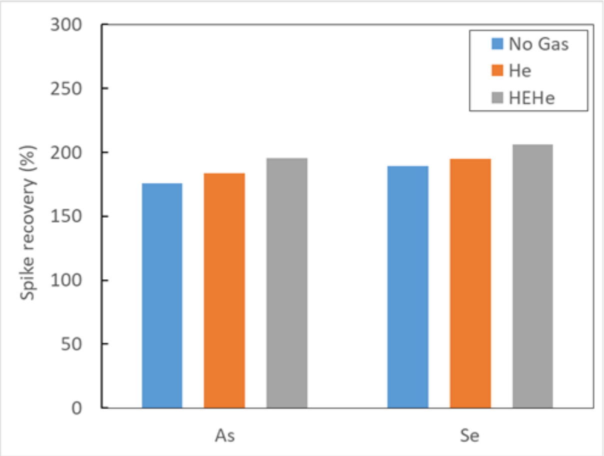


Figure 4. Spike recoveries obtained for As and Se in [No Gas], [He] and [HEHe] modes in the presence of 10 mg/ml of sucrose

To assess the impact of the observed sucrose matrix effects on the accuracy of determining the 24 regulated elements, two quantitation methods were investigated; external calibration and standard addition. The next section will describe the performance of these two developed methods and illustrate how carbon can introduce bias in the obtained recoveries.

3. Comparison of Two Quantitation Methods for Elemental Impurities Analysis in Sucrose by ICP-MS

The guidelines for regulating elemental impurities in pharmaceutical products and ingredients propose various options for calculating limits for each element. These limits are based on factors such as the drug's route of administration, whether it is a drug product or component, and the maximum daily dose. For this case study, we selected the lowest permitted concentrations of elemental impurities for inhaled products, as indicated in Table 1.

The "J" value represents the maximum permitted concentration limit for an analyte in a prepared sample. It is calculated based on the permitted daily exposure (PDE), total dilution, and maximum daily dose. Accurate recovery (as per USP <233>) must be demonstrated at concentrations corresponding to 50%, 100%, and 150% of the J value.

To determine elemental impurities, both unspiked and spiked sucrose samples were analysed using ICP-MS with three different modes: [No Gas], [He], and [HEHe].

Table 1. Limits for elemental impurities and the correspondent J values in the final testing solutions.

Element	Allowed limit in Sucrose powder µg/g (ppm)	J value for 10 mg/mL sucrose solution ng/mL (ppb)	J value for 20 mg/mL sucrose solution ng/mL (ppb)	Element	Allowed limit in Sucrose powder µg/g (ppm)	J value for 10 mg/mL sucrose solution ng/mL (ppb)	J value for 20 mg/mL sucrose solution ng/mL (ppb)
Cd	0.2	2	4	Rh	0.1	1	2
Pb	0.5	5	10	Ru	0.1	1	2
As	0.2	2	4	Se	13	130	260
Hg	0.1	1	2	Ag	0.7	7	14
Co	0.3	3	6	Pt	0.1	1	2
V	0.1	1	2	Li	2.5	25	50
Ni	0.5	5	10	Sb	2	20	40
Tl	0.8	8	16	Ba	30	300	600
Au	0.1	1	2	Mo	1	10	20
Pd	0.1	1	2	Cu	3	30	60
Ir	0.1	1	2	Sn	6	60	120
Os	0.1	1	2	Cr	0.3	3	6

Specifically, two unspiked samples and three spiked samples at each concentration level were examined. The sucrose content was 10 mg/ml, and the matrix for the calibration standards and samples consisted of 2% (v/v) hydrochloric acid and 2% (v/v) nitric acid.

The method demonstrated good accuracy overall, with spike recoveries for most nuclides falling between 90% and 110%. However, exceptions were observed for ^{51}V and ^{52}Cr in [No Gas] mode, as well as ^{75}As and ^{82}Se across all three modes. The low spike recoveries for ^{51}V, ^{52}Cr, and ^{75}As in [No Gas] mode can be attributed to chloride-based interferences, which are common for these nuclides [4, 5]. Fortunately, the application of [He] and [HEHe] modes successfully mitigated these interferences, resulting in spike recoveries within the acceptable range of 70% to 150% for ^{51}V and ^{52}Cr.

Interestingly, the obtained results showed spike recoveries exceeding 180% for ^{75}As and over 200% for ^{82}Se. These high values correlated with the relative intensities of these nuclides in the presence of 10 mg/ml of sucrose. The presence of sucrose in the sample solutions led to an enhanced signal for both nuclides, resulting in overestimated spike recoveries.

To improve accuracy, matrix-matched calibration standards [6] were explored. A follow-up experiment performed the same analysis but added carbon-containing acetic acid at a concentration of 1% (v/v). The results, as shown in Table 2 and Table 3, revealed improved spike recoveries for both ^{75}As and ^{82}Se. Specifically, better accuracies were achieved for ^{75}As in [He] and [HEHe] modes, and for ^{82}Se across all three modes.

Table 2. Spike recoveries at three different spiking levels for Arsenic obtained in different experiments and analysed in different modes.

Element/ mode	Spike level	Spike recovery, %					
		External calibration				Standard addition	
		Experiment 1	Experiment 2	Experiment 3	Experiment 4	Experiment 5	Experiment 6
As [He]	50%	188	122	122	131	81	112
	100%	166	121	128	151	90	110
	150%	163	121	125	155	87	106
As [HEHe]	50%	193	131	126	151	90	94
	100%	183	127	121	177	94	102
	150%	183	128	121	148	90	97

Experiment 1: 2% HNO^3+2%HCl, 10 mg/ml sucrose, without AcOH, As limit: 0.2 ppm
Experiment 2: 2% HNO^3+2%HCl, 10 mg/ml sucrose, with AcOH, As limit: 0.2 ppm
Experiment 3: 2% HNO^3+2%HCl, 10 mg/ml sucrose, with AcOH, As limit: 0.01 ppm
Experiment 4: 2% HNO^3+2%HCl, 20 mg/ml sucrose, with AcOH, As limit: 0.005 ppm
Experiment 5: 2% HNO^3+2%HCl, 10 mg/ml sucrose, As limit: 0.01 ppm
Experiment 6: 2% HNO^3+2%HCl, 20 mg/ml sucrose, As limit: 0.005 ppm

[No Gas] mode is not suitable for As analysis at trace level therefore not presented.

Table 3. Spike recoveries for Selenium obtained in different experiments and analysed in three different modes.

Element/ mode	Spike level	Spike recovery, %					
		External calibration				Standard addition	
		Experiment 1	Experiment 2	Experiment 3	Experiment 4	Experiment 5	Experiment 6
Se [No Gas]	50%	218	123	126	143	98	98
	100%	210	123	123	147	98	105
	150%	209	119	123	124	97	97
Se [He]	50%	227	120	124	148	89	101
	100%	206	117	125	140	91	106
	150%	203	119	126	139	94	101
Se [HEHe]	50%	231	127	124	148	97	100
	100%	212	124	127	164	98	100
	150%	218	124	124	152	97	100

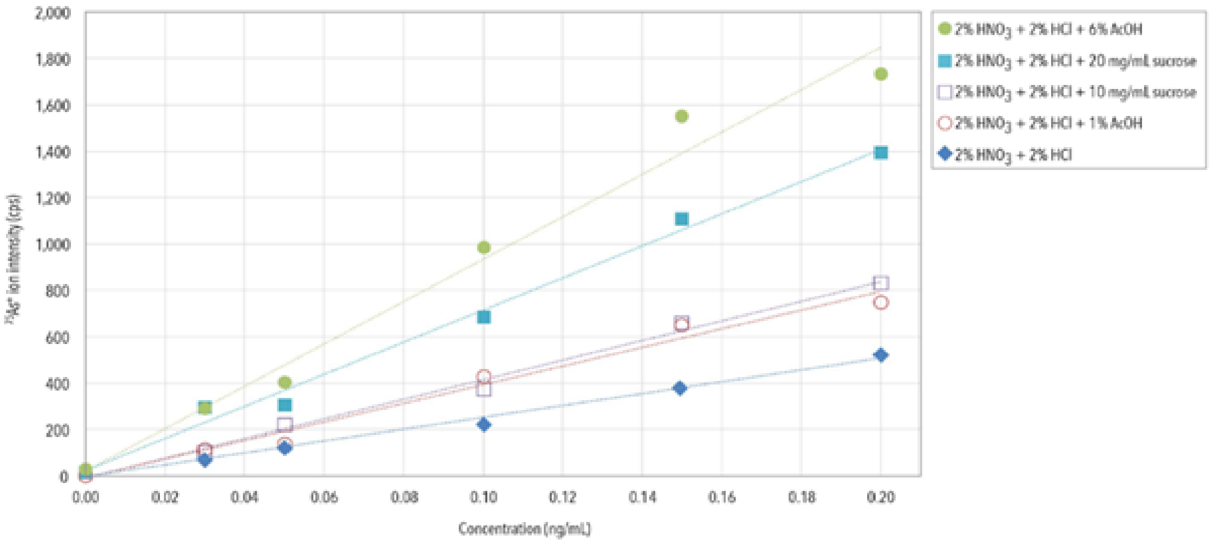
Experiment 1: 2% HNO^3+2%HCl, 10 mg/ml sucrose, without AcOH, Se limit: 13 ppm
Experiment 2: 2% HNO^3+2%HCl, 10 mg/ml sucrose, with AcOH, Se limit: 13 ppm
Experiment 3: 2% HNO^3+2%HCl, 10 mg/ml sucrose, with AcOH, Se limit: 0.650 ppm
Experiment 4: 2% HNO^3+2%HCl, 20 mg/ml sucrose, with AcOH, Se limit: 0.325 ppm
Experiment 5: 2% HNO^3+2%HCl, 10 mg/ml sucrose, Se limit: 0.650 ppm
Experiment 6: 2% HNO^3+2%HCl, 20 mg/ml sucrose, Se limit: 0.325 ppm

To assess the ICP-MS instrument's capability for detecting elemental impurities at trace and ultra-trace levels, the target limits and J values described in Table 1 were decreased by a factor of 20. Interestingly, no significant difference in accuracies was observed for ^{75}As and ^{82}Se in experiment 3 (Table 2 and Table 3) compared to the previous experiment.

However, in experiment 4, where higher concentrations of sucrose (20 mg/ml) were used, accuracies for both elements worsened across all modes. While theoretically increasing the concentration of acetic acid in the calibration standards could help overcome the matrix effect caused by sucrose, introducing high concentrations of acetic acid alongside 2% HCl and 2% HNO^3 is not desirable for the ICP-MS instrument. Acids other than nitric acid at higher concentrations can cause spectral interferences and their use should be limited to the smallest amount possible.

The initial matrix used for calibration purposes was 2% (v/v) hydrochloric acid and 2% (v/v) nitric acid. In Figure 5, a rotational effect (for other matrices) was observed. This effect occurs when the intensity of the signal derived from the analyte is affected by non-analyte constituents of the test solutions. The magnitude of the effect for a given matrix is usually proportional to the signal and is therefore sometimes called a 'proportional' effect [7]. This effect is characterised by a change in the slope of the calibration function, but not in its intercept.

Figure 5. 75As ion signal intensities obtained for different matrices as a function of Arsenic concentrations in the solutions. Values are average



Standard addition is a generally accepted calibration technique, devised to overcome a rotational type of matrix effect that would otherwise give rise to a biased result [7, 8]. Arsenic and Selenium were determined in unspiked and spiked sucrose samples by ICP-MS in [No Gas], [He] and [HEHe] modes via standard addition calibration technique. As can be seen from Table 2 and Table 3, the application of standard addition helped to overcome matrix effects caused by sucrose. The best results were obtained for ^{82}Se in all three modes, spike recoveries varied between 93% and 101%, showing good accuracy of the method in the presence of 10 mg/ml and 20 mg/ml sucrose in the solution. In [He] and [HEHe] modes, better accuracy for ^{75}As was observed using the standard addition calibration technique than with the usual external calibration, as the rotational effects are accounted for. Table 2 shows that spike recoveries for ^{75}As in [He] mode vary between 81% and 112% whereas in [HEHe] mode spike recoveries were from 90% to 102%.

4. Conclusion

In summary, the accurate analysis of all elements except As and Se in carbon containing matrix by ICP-MS can be achieved by using external calibration. Improved accuracy of As and Se can be achieved by using external calibration and matrix-matched standards. However even better accuracy can be obtained for As and Se by using standard addition calibration.

At Almac, we possess the requisite expertise and advanced instrumentation to tackle complex samples. We establish robust methods for analysing elemental impurities using ICP-MS, aligning with current regulatory guidelines.

We effectively apply the knowledge gained from this study to various method development and the application of these methods for elemental impurities analysis in pharmaceutical compounds.

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