A Rapid ICP-MS Method for the Quantitative Determination of Silicon and Heavy Metals in Biopharmaceutical Samples

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Overview

A rapid method has been developed for the quantitative determination of heavy metals and silicon in various biological matrices utilizing inductively coupled plasma mass spectrometry (ICP-MS) for detection.

Introduction

As the development of biopharmaceutical drug products has substantially grown over the past decade, it has become increasingly important to monitor inorganic impurities in biological matrices. In the past, toxic heavy metals have been examined using USP monograph <231>; however, this procedure has been found to be inadequate to accurately quantify these metals. A method developed for the analysis of heavy metals using ICP-MS allows for rapid, specific, sensitive, and accurate quantitation in a variety of biological matrices.

Methods

Six types of biological matrices (unfractionated heparin, human globulin proteins, bovine serum albumen (BSA), tryptic soy broth (TSB), phosphate buffer with 2% glycerol (formulation 1), and Tween 80 surfactant with 0.1 mM EDTA disodium (formulation 2) were prepared by microwave-assisted closed-vessel acid digestion using nitric acid. These samples were then screened for all metals with a 3-point calibration curve from 10-1000 ng/mL. Full metal screening was performed on a Perkin Elmer Elan DRC II ICP-MS. All other experiments were performed on a Thermo X7 series ICP-MS.

System			Thermo Electron
Plasma	RF Power	Forward	1404W
		Reflected	< 10W
	Gas Flows	Plasma	13 L/min
		Auxiliary	0.90 L/min
		Nebulizer	0.85 L/min
Nebulizer			Burgener Nebulizer
Spray Chamber	Type		Impact Bead Quartz
	Composition		Quartz
Ion Sampling	Sampling Cone		HP cone
	Skimmer Cone		HP cone
	Sampling Distance		10 mm from load coil
Vacuum	Expansion Range		2.0 mbar
	Analyzer		1.0 x 10^{-7} mbar
Sample Wash	Peristaltic Pump		30 rpm
	Uptake		30 sec
	Wash		60 sec
Main Run	Type		Peak Jumping
	Runs		3
	Resolution		Standard
	Dwell Time		10000 ms

System	Perkin Elmer Elan DRC II
Sample Uptake Rate	175 µL/min (self aspiration)
Spray Chamber	Cyclonic
RF Power	1500 W
Nebulizer Flow	Set for 1.5% oxides (CeO^+/Ce^+)
Nebulizer	PFA-100 (ESI, Inc., Omaha, NE)

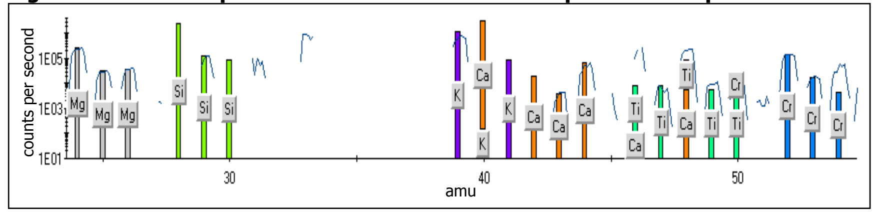
Results

Full metal screens were performed for a wide range of formulations as defined with results summarized in Table 1. The full metal screening was performed to evaluate for potential interferences from the analytes present in the sample matrices. A representative full metal screening spectrum from the Heparin analysis is presented in Figure 1. Accuracy, precision LOD/LOQ, and Linearity results are summarized in Tables 2, 3, and 4, respectively. All analytes were determined acceptably accurate and linear across the range tested with individual recovery results ranging from a low of 80.7% to a high of 108.6% and linearity correlation coefficients all greater than 0.996. Method precision was inferred from the %RSD of the accuracy results, which ranged from 1% to 9%. The method provides a detection limit of at least 0.2 ppb for all analytes.

Table 1. Full Metal Screen (all values listed in PPM (ng/mL))

	BSA	TSB	Human Globulin	Formulation 1	Formulation 2	Heparin
B	1943	374	7318	63.0	68.8	ND
Mg	143	14211	84.6	21.8	27.5	48.8
Al	10.4	299	ND	27.0	39.5	ND
Si	7414	4966	921	392	1766	30.4
K	499	S	1455	463	923	120
Ca	434	5561	812	111	322	144
Ti	4.34	25.3	1.12	10.5	29.7	5.87
V	18.6	110	16.3	3.16	22.8	ND
Cr	4.98	20.3	5.02	17.9	73.1	14.6
Cu	11.2	13.4	5.14	4.43	5.17	ND
Zn	8.84	976	15.8	35.0	10.7	1.68
As	2.86	7.09	3.72	0.780	4.56	ND
Br	544	469	382	51.2	1357	ND
Rb	ND	187	ND	ND	ND	ND
Sr	4.05	41.5	ND	ND	7.55	2.91
Mo	0	17.0	0	2.24	2.31	ND
Ba	6.76	148	18.1	2.13	ND	7.72
Pb	ND	ND	ND	ND	ND	238
Cd	ND	ND	ND	ND	ND	75.0

Figure 1. Partial Representative Full Metal Screen Spectra for Heparin



Note: some elements outside the scope of this study were detected but are not labeled in this figure.

Table 2. Accuracy, Recovery, Precision Results

Spiked Levels	^{65}Cu	^{75}As	^{95}Mo	^{107}Ag	^{111}Cd	^{118}Sn	^{121}Sb	^{200}Hg	^{208}Pb	^{209}Bi	^{28}Si
TSB Accuracy and Recovery											
50%	91.2	96.6	95.6	90.5	90.8	97.9	98.0	97.0	98.9	100.5	93.2
100%	85.8	88.4	87.7	86.2	85.1	90.0	89.7	88.3	90.8	92.4	106.8
150%	85.7	87.1	86.0	80.7	82.5	88.1	88.0	87.1	88.5	84.7	108.6
Mean	87.6	90.7	89.8	85.8	86.1	92.0	91.9	90.8	92.7	92.6	102.9
%RSD	4%	6%	6%	6%	5%	6%	6%	6%	6%	9%	8%
Formulation 1 Accuracy and Recovery											
50%	91.7	90.4	94.5	97.7	92.8	97.2	96.8	95.5	98.9	102.0	84.4
100%	89.0	87.4	87.9	88.1	86.5	90.1	89.8	88.4	91.0	93.1	95.8
150%	92.3	91.7	91.7	90.0	89.5	94.3	93.8	92.9	94.5	91.2	100.7
Mean	91.0	89.8	91.4	91.9	89.6	93.9	93.4	92.3	94.8	95.4	93.7
%RSD	2%	2%	4%	5%	3%	4%	4%	4%	4%	6%	9%
Formulation 2 Accuracy and Recovery											
50%	88.9	88.8	87.7	86.4	88.2	101.1	90.0	94.1	96.3	97.2	93.4
100%	84.8	84.7	84.2	82.0	82.3	86.1	85.9	84.2	86.4	87.7	104.5
150%	87.7	89.3	86.6	84.3	84.7	89.9	89.7	89.0	90.5	86.1	108.0
Mean	87.1	87.6	86.2	84.2	85.1	92.3	88.5	89.1	91.1	90.3	102.0
%RSD	2%	3%	2%	3%	4%	8%	3%	6%	5%	7%	7%
BSA Accuracy and Recovery											
50%	91.8	95.7	95.6	96.2	88.5	98.4	98.2	97.5	99.5	102.1	94.7
100%	88.6	87.8	87.4	88.1	86.2	89.2	89.1	88.0	90.9	91.3	90.1
150%	91.6	91.9	91.3	88.8	89.1	93.9	93.1	92.5	94.9	91.9	105.6
Mean	90.7	91.8	91.4	91.0	87.9	93.8	93.5	92.7	95.1	95.1	96.8
%RSD	2%	4%	4%	5%	2%	5%	5%	5%	5%	6%	8%
Heparin Accuracy and Recovery											
50%	103.4	103.5	102.5	89.8	100.8	104.7	104.3	103.1	105.7	107.9	100.7
100%	101.9	102.7	101.7	93.2	102.7	103.8	103.4	101.6	105.0	105.1	94.9
150%	100.2	100.4	100.1	90.7	102.8	101.7	101.2	99.8	103.8	100.5	95.6
Mean	101.8	102.2	101.4	91.2	102.1	103.4	103.0	101.5	104.8	104.5	97.1
%RSD	2%	2%	1%	2%	1%	1%	2%	2%	1%	4%	3%

Note: 50% level: spiked 50 ppb; 100% level: spiked 100 ppb; 150% level: spiked 150 ppb

Limit of Quantitation (LOQ) and Limit of Detection (LOD)

Adequate sensitivity is critical for the safety evaluation of heavy metals in biopharmaceutical samples. LOD/LOQ for heavy metals and silicon is presented in Table 3.

Table 3. LOD and LOQ Results

^{65}Cu	^{75}As	^{95}Mo	^{107}Ag	^{111}Cd	^{118}Sn	^{121}Sb	^{200}Hg	^{208}Pb	^{209}Bi	^{28}Si
Limit of Quantitation (ppb)										
0.502	0.501	0.4985	0.500	0.502	0.501	0.5	0.500	0.501	0.501	5.025
Limit of Detection (ppb)										
0.167	0.167	0.166	0.167	0.167	0.167	0.167	0.167	0.167	0.167	1.675
USP Oral Limit (ppm)[1,2]										
50	1.5	25	NA	2.5	3000	2	1.5	1	NA	NA
USP Parenteral Limit (ppm)[1,2]										
5	0.15	2.5	NA	0.25	300	0.2	0.15	0.1	NA	NA

Linearity Results

The ICP-MS has a wide dynamic range for detection. The response is linear. The linearity plots for each analyte are shown in Figure 2. The equation used to fit the curves is summarized in Table 4.

Figure 2. Linearity Plots

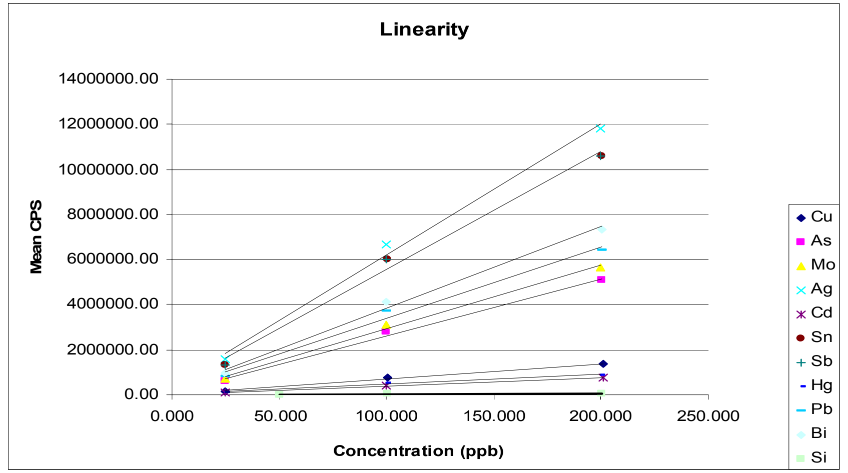


Table 4. Linearity Results

^{65}Cu	^{75}As	^{95}Mo	^{107}Ag	^{111}Cd	^{118}Sn	^{121}Sb	^{200}Hg	^{208}Pb	^{209}Bi	^{28}Si
Linearity Equation (First Order) Fit										
Y=mx+b										
Correlation Coefficient (r)										
0.9982	0.9983	0.9983	0.9977	0.9982	0.9975	0.9974	0.9982	0.9966	0.9978	0.9995

Conclusions

This paper presents a single, rapid ICP-MS method for evaluating heavy metals and silicon across a wide range of biopharmaceutical formulations. This method was demonstrated to be linear, precise, accurate, and sensitive down to 0.2 ppb level. This method can efficiently and effectively quantitate heavy metals with greater accuracy than traditional ICP-OES, AA, and USP <231> regardless of the actual formulation. The advantages of this ICP-MS method include greater specificity, sensitivity, accuracy, and its versatility to detect multiple elements for multiple sample types, which overcomes the limitation of qualitative procedures such as USP <231>, less sensitive techniques such as ICP-OES, and AA. This methodology also allows for a first pass evaluation of potential analytes including toxic heavy metals (USP class I, II, III metals) for various formulations and raw materials with minimal sample preparation optimization. The ultimate benefit is to provide more valuable and complete information to support the drug development process in regard to choosing an appropriate source for the raw materials and assures the quality and efficacy of the final drug products.

References

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