BIOSPECTRA
100 Majestic Way, Bangor, PA 18013 / www.biospectra.us

ANALYTICAL METHOD VALIDATION REPORT: DETERMINATION OF ICH Q3D ELEMENTAL IMPURITIES BY INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY (ICP-MS) IN HEPES SODIUM AND MES SODIUM

TABLE OF CONTENTS

1. PURPOSE:

1.1. The purpose of this validation report is to establish documented evidence that the validation protocol, BSI-PRL-0595 v. 1.0, for Elemental Impurities in HEPES Sodium and MES Sodium products performs according to USP and BioSpectra requirements.

1.1.1. Elements under USP <232> will be considered and are as follows:

1.1.1.1. Class 1: Hg, As, Cd, and Pb

1.1.1.2. Class 2A: Co, V, and Ni

1.1.1.3. Class 2B: Tl, Au, Pd, Ir, Os, Rh, Ru, Se, Ag, and Pt

1.1.1.4. Class 3: Li, Sb, Sn, Ba, Mo, Cu, and Cr

1.1.1.5. Class 4: Fe

2. SCOPE:

2.1. Applies to HEPES Sodium (HEPN) and MES Sodium (MESN) and related products manufactured at BioSpectra.

2.2. Applies to the NexION 350X S/N 85VN5093001 ICP-MS located in the Quality Control (QC) Laboratory at the BioSpectra Bangor, PA facility.

2.3. This report applies to the validation protocol for elemental impurities in HEPES Sodium and MES Sodium by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) performed at BioSpectra Inc.

3. REFERENCES:

3.1. BSI-PRL-0595: Analytical Method Validation Protocol: Determination of Elemental Impurities in MES Sodium and HEPES Sodium by ICP-MS

3.2. BSI-SOP-0303: NexION 350X ICP-MS SOP

3.3. BSI-SOP-0304: NexION 350X ICP-MS Care and Maintenance SOP

3.4. BSI-SOP-0436: Analytical Method Validation Master Plan

3.5. ICH Guideline for Elemental Impurities Q3D

3.6. USP <730> Plasma Spectrochemistry

3.7. USP <1730> Plasma Spectrochemistry—Theory and Practice

3.8. NexION Operation with Syngistix Software Guide

3.9. USP <232>, <233>

4. BACKGROUND:

4.1. This validation was executed using a parenteral PDE (permissible daily exposure) daily dose calculation of 10 grams per day and was performed as per ICH Q3D and USP General Chapters <232> and <233>, Elemental Impurities –Procedures, Validation of Quantitative Procedures. (See Table 1)

4.2. The test protocol validation report includes the following parameters:

4.2.1. Specificity

4.2.2. Linearity and Range

4.2.3. Limit of Quantification (LOQ)

4.2.4. Accuracy by “Spiked Recovery”

4.2.5. Precision (Repeatability)

4.2.6. Intermediate Precision (Ruggedness)

4.2.7. Standard and Sample Solution Stability

TABLE 1: LIMITS FOR HEPES SODIUM AND MES SODIUM (10 GRAM/DAY EXPOSURE)							
Elements	**ICH Class**	**Parenteral PDE Limits (µg/day)**	**0.1J LOQ (µg/g) in sample**	**0.3J Target (µg/g) in sample**	**0.5J Target (µg/g) in sample**	**1.0J Target (µg/g) in sample**	**1.5J Target (µg/g) in sample**
As	1	15	0.15	0.45	0.75	1.50	2.25
Cd	1	2.0	0.02	0.06	0.10	0.20	0.30
Hg	1	3.0	0.03	0.09	0.15	0.30	0.45
Pb	1	5.0	0.05	0.15	0.25	0.50	0.75
Co	2A	5.0	0.05	0.15	0.25	0.50	0.75
Ni	2A	20	0.20	0.60	1.0	2.0	3.0
V	2A	10	0.10	0.30	0.50	1.0	1.5
Tl	2B	8.0	0.08	0.24	0.40	0.80	1.2
Se	2B	80	0.80	2.4	4.0	8.0	12
Ag	2B	10	0.10	0.30	0.50	1.0	1.5
Au	2B	100	1.0	3.0	5.0	10	15
Pd	2B	10	0.10	0.30	0.50	1.0	1.5
Ir	2B	10	0.10	0.30	0.50	1.0	1.5
Os	2B	10	0.10	0.30	0.50	1.0	1.5
Pt	2B	10	0.10	0.30	0.50	1.0	1.5
Rh	2B	10	0.10	0.30	0.50	1.0	1.5
Ru	2B	10	0.10	0.30	0.50	1.0	1.5
Ba	3	700	7.0	21	35	70	105
Sb	3	90	0.90	2.7	4.5	9.0	13.5
Li	3	250	2.5	7.5	12.5	25	37.5
Mo	3	1,500	15	45	75	150	225
Cu	3	300	3.0	9.0	15	30	45
Sn	3	600	6.0	18	30	60	90
Cr	3	1,100	11	33	55	110	165
Fe	4	*200	2.0	6.0	10	20	30

*No PDE limits for Class 4 elements; limits derived from other internal product specifications.

5. MATERIALS AND EQUIPMENT:

TABLE 2: EQUIPMENT

Type	Supplier	Model	Serial Number	Cal. Due
Analytical Balance	Sartorius	MSE224S	36707108	04/2023
Automatic Pipette	Rainin	E4-XLS (2-20 µL)	C040200714	12/31/22
Automatic Pipette	Rainin	E4-XLS (20-200 µL)	C016314640	12/31/22
Automatic Pipette	Rainin	E4-XLS (100-1000 µL)	C016314969	12/31/22
Automatic Pipette	Rainin	E4-XLS (0.5-5 mL)	C023506909	12/31/22
ICP-MS	Perkin Elmer	NexION 350X	85VN5093001	01/2023
Deionized water system	Millipore	IQ-7005/ Element POD	F9SA14284H	06/2023

TABLE 3: REAGENTS

Type	Grade	Supplier	Catalog Number	Lot Number	Expiration
70% Nitric Acid	Trace Metal	VWR	87003-261	1122020	02/16/24
70% Nitric Acid	Trace Metal	SCP Science	250-038-175	22330073	02/15/24
36% Hydrochloric Acid	Trace Metal	VWR	87003-253	4121050	06/03/24
Sulfuric Acid	Trace Metal	Spectrum	S1136	3121040	06/30/24
Deionized water	Type 1 Ultrapure	In-House	N/A	N/A	N/A
Thiourea	99+% Pure	ACROS	220052500	A0407315	10/31/23
ICP-MS Setup Solution	N/A	Perkin Elmer	N8145051	38-31GSX1	04/30/23
ICP-MS KED Setup Solution	N/A	Perkin Elmer	N8145052	40-71GST1	07/30/23
SiliaPrep SPE Filter	Silica-Based AMPA	Silicycle	R85130B	178772	N/A

5.1. Consumable Supplies
- 5.1.1. SCP Digitubes® 15 mL, 50 mL and 100 mL
- 5.1.2. Pipette Tips of various sizes

5.2. Reagent Lots for validation analysis
- 5.2.1. HEPES Sodium Lot RMAT-1021-0143
- 5.2.2. HEPES Sodium Lots RMAT-1021-0144, RMAT-1021-0145, and RMAT-1021-0146 were run concurrently for elemental impurity analysis.
- 5.2.3. MES Sodium Lot 1220002172
- 5.2.4. MES Sodium Lots 1217003016, 1219002228, and 1220004175 were run concurrently for elemental impurity analysis.

TABLE 4: REFERENCE STANDARDS

Identification	Manufacturer	Lot Number	Expiration	Concentrations / Elements
Pharma-CAL Standard Parenteral STD# 1 IA 140-131-201	SCP Science	S220310005	03/2023	Ag (10 µg/mL), As (15 µg/mL), Cd (2 µg/mL), Co (5 µg/mL), Hg (3 µg/mL), Ni (20 µg/mL), Pb (5 µg/mL), Se (80 µg/mL), Tl (8 µg/mL), V (10 µg/mL)
USP232/ICH Q3D Parenteral STD# 2 IA 140-131-215	SCP Science	S210811029	11/2022	Au (100 µg/mL); Ir, Os, Pd, Pt, Rh, & Ru (10 µg/mL)
Pharma-CAL Standard Parenteral STD# 3 IA 140-131-221	SCP Science	S220705014	07/2024	Ba (700 µg/mL), Cr (1,100 µg/mL), Cu (300 µg/mL), Li (250 µg/mL), Mo (1,500 µg/mL), Sb (90 µg/mL), Sn (600 µg/mL)
Iron Stock Standard N9303771	Perkin Elmer	26-37FEY1	10/30/23	Fe (1,000 µg/mL)
Pharma-CAL Custom Standard AQ0-086-125 (Internal Standard)	SCP Science	S220825027	10/2023	Be, Sc, Y, Re (10 µg/mL); Te (25 µg/mL); Ge, Tb, Bi (5 µg/mL)

6. PROCEDURE:

6.1. All standards were prepared volumetrically from stock solutions purchased from certified vendors. If the vendor supplied stock standard was within 2% of the nominal value as per the certificate of analysis, then the nominal value was used to calculate the concentration of the standard. If the stock standard certificate of analysis value was greater than or less than 2% of the nominal value, then the certificate of analysis value was used for the stock standard concentration.

6.2. Acid Digestion Mixture (Acid Mix)
[2:1] Nitric Acid (HNO_3): Sulfuric Acid (H_2SO_4)

6.2.1. Added 50 mL of nitric acid to a 100 mL Digitube® and then slowly added 25 mL of sulfuric acid. Scaled as required.

6.2.2. Solution was placed in a cold-water bath to aid cooling and was prepared day of use.

6.3. Internal Standard/Complexing Solution

6.3.1. Weighed approximately 1.0 gram of Thiourea into a 50 mL Digitube®.

6.3.2. Added approximately 20 mL of deionized water and mixed to dissolve.

6.3.3. Filtered solution through a SiliaPrep Cation Solid Phase Extraction (SPE) cartridge.

6.3.4. Transferred 2.5 mL of Pharma CAL Custom Standard (Internal standard) Stock to the filtered solution and added 25 mL of hydrochloric acid.

6.3.5. Diluted to a final volume of 50 mL with deionized water and mixed well.

6.3.6. Scaled proportionally as needed for use.

6.4. 2% Thiourea Solution

6.4.1. Weighed approximately 1.0 gram of Thiourea into a 50 mL Digitube®.

6.4.2. Added approximately 20 mL of deionized water and mixed to dissolve.

6.4.3. Filtered solution through a SiliaPrep Cation Solid Phase Extraction (SPE) cartridge.

6.4.4. Diluted to a final volume of 50 mL with deionized water and mixed well.

6.4.5. Scaled proportionally as needed for use.

6.5. Intermediate Standard Preparation

6.5.1. Prepared a standard solution containing the elements listed in Table 5 using the standards STD#1 IA, STD#2 IA, STD#3 IA, and individual single source Iron 1,000 µg/mL standard. Prepared by adding stock standards to a 15 mL Digitube®. Added DI water to approximately 8 mL then added hydrochloric acid. Diluted to volume using DI Water.

TABLE 5: INTERMEDIATE STANDARD

Identification	Element	Stock Identification	Amount Added (mL)	HCl (mL)	Final Volume (mL)	Final Concentration (µg/mL)
Intermediate Standard	As	STD# 1 IA 140-131-201*	1.0	1.0	10	1.5
	Cd					0.20
	Hg					0.30
	Pb					0.50
	Co					0.50
	Ni					2.0
	V					1.0
	Tl					0.80
	Se					8.0
	Ag					1.0
	Au	STD# 2 IA 140-131-215*	1.0			10
	Pd					1.0
	Ir					1.0
	Os					1.0
	Pt					1.0
	Rh					1.0
	Ru					1.0
	Ba	STD# 3 IA 140-131-221*	1.0			70
	Sb					9.0
	Li					25
	Mo					150
	Cu					30
	Sn					60
	Cr					110
	Fe	1,000 µg/mL Fe Std	0.200			20

* SCP Science catalog numbers ending in 1 denote 125 mL bottle sizes and catalog numbers ending in 5 denote 500 mL bottle sizes.

6.6. 0.5J Calibration Standard Preparation

6.6.1. Prepared a solution containing the elements listed in Table 6 below in 5.0% HNO_3, 2.5% H_2SO_4, 1.0% HCl, and 0.04% (400 µg/mL) Thiourea. Added intermediate standard to a separate 50 mL Digitube® followed by addition of approximately 35 mL of DI Water. Added acid mixture then diluted to 45 mL using DI Water. Added internal standard/complexing solution and diluted to volume using DI Water.

TABLE 6: 0.5J CALIBRATION STANDARD

Identification	Element	Intermediate Standard (mL)	Acid Mix (mL)	Internal Standard/ Complexing Solution (mL)	Final Volume (mL)	Final Concentration (µg/L)
0.5J Calibration Standard	As	0.050	3.75	1.0	50	1.5
	Cd					0.20
	Hg					0.30
	Pb					0.50
	Co					0.50
	Ni					2.0
	V					1.0
	Tl					0.80
	Se					8.0
	Ag					1.0
	Au					10
	Pd					1.0
	Ir					1.0
	Os					1.0
	Pt					1.0
	Rh					1.0
	Ru					1.0
	Ba					70
	Sb					9.0
	Li					25
	Mo					150
	Cu					30
	Sn					60
	Cr					110
	Fe					20

6.7. 1.5J Calibration Standard Preparation

6.7.1. Prepared a solution containing the elements listed in Table 7 below in 5.0% HNO_3, 2.5% H_2SO_4, 1.0% HCl, and 0.04% (400 µg/mL) Thiourea. Added intermediate standard to a separate 50 mL Digitube® followed by addition of approximately 35 mL of DI Water. Added acid mixture then diluted to 45 mL using DI Water. Added internal standard/complexing solution and diluted to volume using DI Water.

TABLE 7: 1.5J CALIBRATION STANDARD

Identification	Element	Intermediate Standard (mL)	Acid Mix (mL)	Internal Standard/ Complexing Solution (mL)	Final Volume (mL)	Final Concentration (µg/L)
1.5J Calibration Standard	As	0.150	3.75	1.0	50	4.5
	Cd					0.60
	Hg					0.90
	Pb					1.5
	Co					1.5
	Ni					6.0
	V					3.0
	Tl					2.4
	Se					24
	Ag					3.0
	Au					30
	Pd					3.0
	Ir					3.0
	Os					3.0
	Pt					3.0
	Rh					3.0
	Ru					3.0
	Ba					210
	Sb					27
	Li					75
	Mo					450
	Cu					90
	Sn					180
	Cr					330
	Fe					60

6.8. 2.0J Calibration Standard Preparation

6.8.1. Prepared a solution containing the elements listed in Table 8 below in 5.0% HNO_3, 2.5% H_2SO_4, 1.0% HCl, and 0.04% (400 µg/mL) Thiourea. Added intermediate standard to a separate 50 mL Digitube® followed by addition of approximately 35 mL of DI Water. Added acid mixture then diluted to 45 mL using DI Water. Added internal standard/complexing solution and diluted to volume using DI Water.

TABLE 8: 2.0J CALIBRATION STANDARD

Identification	Element	Intermediate Standard (mL)	Acid Mix (mL)	Internal Standard/ Complexing Solution (mL)	Final Volume (mL)	Final Concentration (µg/L)
2.0J Calibration Standard	As	0.200	3.75	1.0	50	6.0
	Cd					0.80
	Hg					1.2
	Pb					2.0
	Co					2.0
	Ni					8.0
	V					4.0
	Tl					3.2
	Se					32
	Ag					4.0
	Au					40
	Pd					4.0
	Ir					4.0
	Os					4.0
	Pt					4.0
	Rh					4.0
	Ru					4.0
	Ba					280
	Sb					36
	Li					100
	Mo					600
	Cu					120
	Sn					240
	Cr					440
	Fe					80

6.9. Calibration Blank

6.9.1. Prepared a solution containing 5.0% HNO_3, 2.5% H_2SO_4, 1.0% HCl and 0.04% (400 µg/mL) Thiourea as per Table 9 below. Internal Standard Solution was not allowed to contact concentrated acids. To a separate 50 mL Digitube®, added approximately 35 mL of DI Water. Added acid mixture then diluted to 45 mL using DI Water. Added Internal Standard/Complexing Solution and diluted to volume using DI Water.

TABLE 9: CALIBRATION BLANK

Description	Acid Mix (mL)	Internal Standard/ Complexing Solution (mL)	Final Volume (mL)
Cal Blank	3.75	1.0	50

6.10. Method Blank Preparation

6.10.1. Added 35 mL of deionized water to a 50 mL Digitube®.

6.10.2. Added 3.75 mL of Acid Digestion Mixture.

6.10.3. Added deionized water to approximately 45 mL and then transferred 1.0 mL of Internal Standard/Complexing Agent Solution.

6.10.4. Diluted to a final volume of 50 mL using deionized water and mixed well.

6.11. Sample Preparation

6.11.1. Weighed approximately 100 mg of sample into a 50 mL Digitube®.

6.11.2. Transferred approximately 5.0 mL of deionized water and swirl to dissolve sample.

6.11.3. Added 3.75 mL of Acid Digestion Mixture and allowed to react with gentle swirling periodically to evolve any gasses produced during the reaction.

6.11.4. Added deionized water to approximately 45 mL and then transferred 1.0 mL of Internal Standard/ Complexing Solution.

6.11.5. Diluted to a final volume of 50 mL with deionized water and mixed thoroughly.

6.12. Isobaric Overlap Corrections

6.12.1. An isobaric interference results from equal mass isotopes of different elements present in the sample solution. Analysis sequences that are processed utilizing multi-element standards will require the use of correction equations to compensate for known isobaric overlaps originating from the elemental standard and sample. The following correction equations should be used.

KED Mode:

$M_c\,(58) = M_u\,(58) \times 1 - M_{(rm)}\,(57) \times 0.13208$
$M_c\,(98) = M_u\,(98) \times 1 - M_{(rm)}\,(99) \times 0.14655$
$M_c\,(106) = M_u\,(106) \times 1 - M_{(rm)}\,(111) \times 0.09766$
$M_c\,(108) = M_u\,(108) \times 1 - M_{(rm)}\,(111) \times 0.06953$
$M_c\,(120) = M_u\,(120) \times 1 - M_{(rm)}\,(125) \times 0.01273$
$M_c\,(123) = M_u\,(123) \times 1 - M_{(rm)}\,(125) \times 0.12588$
$M_c\,(190) = M_u\,(190) \times 1 - M_{(rm)}\,(195) \times 0.00036$
$M_c\,(192) = M_u\,(192) \times 1 - M_{(rm)}\,(195) \times 0.02315$
$M_c\,(196) = M_u\,(196) \times 1 - M_{(rm)}\,(202) \times 0.005023$

The correction equations can be derived from the following equation:
$Mc = M_u - [M_{(rm)} \times (A_{(ie)}/A_{(rm)})]$

Where:

M_c = Corrected Count Rate for the analyte
M_u = Uncorrected count rate for the analyte
$M_{(rm)}$ = Count Rate of Reference Mass (rm) for the Interfering Element
$A_{(ie)}$ = Percent Abundance of Interfering Element (ie) at the analyte mass
$A_{(rm)}$ = Percent Abundance of Interfering Element at the Reference Mass (rm)

Example:

$M_c\,(58) = M_u\,(58) \times 1 - M_{(rm)}\,(57) \times (0.28 / 2.12)$

6.13. All correction coefficients were calculated based on the Agilent Technologies 2016 Relative Isotopic Abundance Table.

6.14. Multiplier used in the correction equation may differ slightly from the multiplier used in the Syngistix instrument method due to rounding.

7. INSTRUMENT PROCEDURE:

7.1. Performed the ICP-MS daily performance checks prior to beginning the analytical sequence. Refer to NexION 350X ICP-MS SOP BSI-SOP-0303 for Daily Check procedures.

7.2. A calibration curve of no less than two standards and a blank was used. The calibration correlation coefficient (R) was ≥ 0.99.

7.3. Set up the sequence as per Table 10.

7.4. Confirmed the calibration by analyzing the 1.5J standard after the calibration. The calibration check must recover ± 20% of the calculated theoretical concentration for multi-element analysis and ± 10% for single element determinations.

7.5. The check standard was verified after each calibration. A re-analysis of the check standard was performed a minimum of once every 10 samples and at the end of the analytical run.

7.6. Bracketing standard checks were verified to recover ± 20% of the calculated theoretical concentration for multi-element analysis. Additionally, the drift (calculated as absolute difference) between bracketing standard checks was verified to be NMT 20% for each target element.

7.7. The sample concentration is calculated as:

$$\text{Conc. } (\mu g/g) = \frac{\text{Solution Conc. } (\mu g/L) \times \text{Solution vol. (L)} \times \text{Dilution Factor}}{\text{Sample Mass (g)}}$$

TABLE 10: EXAMPLE SAMPLE ANALYSIS SEQUENCE

ID	Type	Level
Cal Blank	Cal Blank	Level 1
0.5J Cal Std	Cal Std	Level 2
1.5J Cal Std	Cal Std	Level 3
2.0J Cal Std	Cal Std	Level 4
Cal Blank Check	QC Check	N/A
1.5J Check Std 1	QC Check	N/A
Method Blank	Sample	N/A
Sample(s) 10 or less	Sample	N/A
1.5J Check Std 2	QC Check	N/A

7.8. Instrument Setup and Parameters

7.8.1. Instrument settings are only listed as guidelines. Settings may be changed in order to accommodate changes in sample matrix or hardware configurations.

7.8.2. The AMS-II makeup gas was engaged during analysis using a minimum dilution gas ratio of 15%.

7.8.3. The elements arsenic, iron, and selenium were analyzed using hydrogen reaction gas in order to remove polyatomic interferences. A hydrogen DRC (Dynamic Reaction Cell) flow rate of approximately 4 mL/min was used.

TABLE 11: ICP-MS PARAMETERS

ICP-MS System	Perkin Elmer NexION 350X Inductively Coupled Plasma Mass Spectrometry (ICP-MS) with Syngistix Software Version 2.4
Sweeps/Readings	20
Replicates	3
Nebulizer Gas	Argon
Collision Cell Gas	Helium
Reaction Cell Gas	Hydrogen
Dilution Gas	Argon
Sample and Skimmer Cone	Platinum
Sample Rinses	Rinse-1: 60 sec at 45 rpm 5.0% HNO_3, 2.5% HCl with 0.04% Thiourea (or as applicable to mitigate carry over)

TABLE 12: LINEAR RANGE AND CORRESPONDING TUNE MODE							
Isotope	**Internal Standard**	**Mode**	**Linear Range (µg/L)**	**Isotope**	**Internal Standard**	**Mode**	**Linear Range (µg/L)**
7Li	9Be	STD	5.0-100	113Cd	125Te	KED	0.04-0.80
51V	45Sc	KED	0.20-4.0	118Sn	125Te	KED	12-240
52Cr	45Sc	KED	22-440	119Sn	125Te	KED	12-240
53Cr	45Sc	KED	22-440	120Sn	125Te	KED	12-240
56Fe	45Sc	H_2 DRC	4.0-80	121Sb	125Te	KED	1.8-36
57Fe	72Ge	KED	4.0-80	123Sb	125Te	KED	1.8-36
58Ni	72Ge	KED	0.40-8.0	135Ba	159Tb	KED	14-280
59Co	72Ge	KED	0.10-2.0	137Ba	159Tb	KED	14-280
60Ni	72Ge	KED	0.40-8.0	138Ba	159Tb	KED	14-280
62Ni	72Ge	KED	0.40-8.0	188Os	185Re	KED	0.20-4.0
63Cu	72Ge	KED	6.0-120	189Os	185Re	KED	0.20-4.0
65Cu	72Ge	KED	6.0-120	190Os	185Re	KED	0.20-4.0
75As	72Ge	H_2 DRC	0.30-6.0	191Ir	185Re	KED	0.20-4.0
75As	72Ge	KED	0.30-6.0	192Os	185Re	KED	0.20-4.0
77Se	89Y	H_2 DRC	1.6-32	193Ir	185Re	KED	0.20-4.0
78Se	89Y	H_2 DRC	1.6-32	194Pt	185Re	KED	0.20-4.0
95Mo	89Y	KED	30-600	195Pt	185Re	KED	0.20-4.0
97Mo	89Y	KED	30-600	196Pt	185Re	KED	0.20-4.0
98Mo	89Y	KED	30-600	197Au	185Re	KED	2.0-40
99Ru	125Te	KED	0.20-4.0	199Hg	185Re	KED	0.06-1.2
101Ru	125Te	KED	0.20-4.0	200Hg	185Re	KED	0.06-1.2
103Rh	125Te	KED	0.20-4.0	202Hg	185Re	KED	0.06-1.2
105Pd	125Te	KED	0.20-4.0	203Tl	209Bi	KED	0.16-3.2
106Pd	125Te	KED	0.20-4.0	205Tl	209Bi	KED	0.16-3.2
107Ag	125Te	KED	0.20-4.0	206Pb	209Bi	KED	0.10-2.0
108Pd	125Te	KED	0.20-4.0	207Pb	209Bi	KED	0.10-2.0
109Ag	125Te	KED	0.20-4.0	208Pb	209Bi	KED	0.10-2.0
111Cd	125Te	KED	0.04-0.80				

7.9. Linearity and Range

7.9.1. The ICP-MS linearity study included standards equivalent to the concentrations shown in Table 14 and encompassed the following standards: 10%, 30%, 50%, 100%, 150%, and 200% of the Target Concentration. Each standard was prepared in triplicate and analyzed against the calibration curve described in Section 6.6 to Section 6.8. The average standard recovery for each level of the three replicates was then determined.

7.9.2. For all replicates of the linearity standards, intensity was plotted against concentration. Correlation coefficients were determined for each isotope and the data was analyzed as a linear regression model. In addition, each isotope was analyzed via statistical methods for measured concentration versus theoretical concentration. The data is uploaded as supporting information with the report.

7.9.3. The preparation for linearity standard is described in Table 13 below. The concentrations of each element analyzed is listed in Table 14. Percent recovery values and correlation coefficients for each isotope was tabulated in Tables 15 and 16, respectively.

7.9.4. Linearity study was performed once as the standard preparation in the protocol is the same regardless of the product analyzed.

7.9.4.1. Acceptance Criteria:

7.9.4.1.1. The mean standard recovery for each element at each of the spike levels, as per USP <233> requirement, must be in the range of 70% - 150%.

7.9.4.1.2. The correlation coefficient of each isotope for the linearity plots of signal versus concentration must be NLT 0.99.

TABLE 13: LINEARITY STANDARD PREPARATION

Description	Intermediate Standard (mL)	Acid Mix (mL)	Internal Standard/ Complexing Solution (mL)	Final Volume Deionized Water (mL)
Cal Blank Reference	N/A	3.75	1.0	50
0.1J Standard	0.010	3.75	1.0	50
0.3J Standard	0.030	3.75	1.0	50
0.5J Standard	0.050	3.75	1.0	50
1.0J Standard	0.100	3.75	1.0	50
1.5J Standard	0.150	3.75	1.0	50
2.0J Standard	0.200	3.75	1.0	50

TABLE 14: LINEARITY STANDARD CONCENTRATIONS						
Element	**0.1J Standard (µg/L)**	**0.3J Standard (µg/L)**	**0.5J Standard (µg/L)**	**1.0J Standard (µg/L)**	**1.5J Standard (µg/L)**	**2.0J Standard (µg/L)**
As	0.30	0.90	1.5	3.0	4.5	6.0
Cd	0.04	0.12	0.20	0.40	0.60	0.80
Hg	0.06	0.18	0.30	0.60	0.90	1.2
Pb	0.10	0.30	0.50	1.0	1.5	2.0
Co	0.10	0.30	0.50	1.0	1.5	2.0
Ni	0.40	1.2	2.0	4.0	6.0	8.0
V	0.20	0.60	1.0	2.0	3.0	4.0
Tl	0.16	0.48	0.80	1.6	2.4	3.2
Se	1.6	4.8	8.0	16	24	32
Ag	0.20	0.60	1.0	2.0	3.0	4.0
Au	2.0	6.0	10	20	30	40
Pd	0.20	0.60	1.0	2.0	3.0	4.0
Ir	0.20	0.60	1.0	2.0	3.0	4.0
Os	0.20	0.60	1.0	2.0	3.0	4.0
Pt	0.20	0.60	1.0	2.0	3.0	4.0
Rh	0.20	0.60	1.0	2.0	3.0	4.0
Ru	0.20	0.60	1.0	2.0	3.0	4.0
Ba	14	42	70	140	210	280
Sb	1.8	5.4	9.0	18	27	36
Li	5.0	15	25	50	75	100
Mo	30	90	150	300	450	600
Cu	6.0	18	30	60	90	120
Sn	12	36	60	120	180	240
Cr	22	66	110	220	330	440
Fe	4.0	12	20	40	60	80

TABLE 15: LINEARITY PERCENT RECOVERY RESULTS

Isotope	Mode	0.1J Mean	0.3J Mean	0.5J Mean	1.0J Mean	1.5J Mean	2.0J Mean	Isotope	Mode	0.1J Mean	0.3J Mean	0.5J Mean	1.0J Mean	1.5J Mean	2.0J Mean
7Li	STD	99	99	98	98	98	96	113Cd	KED	91	92	97	98	98	100
51V	KED	102	99	103	103	103	103	118Sn	KED	101	98	101	101	103	102
52Cr	KED	101	99	101	102	102	101	119Sn	KED	99	98	99	100	101	102
53Cr	KED	100	99	101	101	102	100	120Sn	KED	100	98	100	100	102	102
56Fe	H_2 DRC	100	98	99	101	101	107	121Sb	KED	97	97	99	101	102	102
57Fe	KED	101	96	94	95	100	106	123Sb	KED	98	97	100	101	101	102
58Ni	KED	99	96	96	96	99	101	135Ba	KED	104	99	101	101	103	103
59Co	KED	102	98	98	99	100	104	137Ba	KED	102	100	101	101	103	102
60Ni	KED	99	97	100	98	100	102	138Ba	KED	101	97	100	99	101	101
62Ni	KED	107	92	93	98	103	103	188Os	KED	101	100	98	99	101	101
63Cu	KED	101	97	97	97	99	102	189Os	KED	99	100	99	98	97	100
65Cu	KED	101	97	97	96	99	101	190Os	KED	100	99	97	100	99	101
75As	H_2 DRC	100	99	97	100	97	97	191Ir	KED	97	100	98	101	100	101
75As	KED	114	100	99	96	97	100	192Os	KED	98	99	99	99	99	101
77Se	H_2 DRC	98	97	99	100	101	100	193Ir	KED	99	101	99	100	101	101
78Se	H_2 DRC	99	98	99	98	99	100	194Pt	KED	98	101	99	101	101	102
95Mo	KED	98	99	99	101	100	100	195Pt	KED	102	102	100	102	102	102
97Mo	KED	98	100	99	101	100	100	196Pt	KED	100	101	99	102	101	104
98Mo	KED	98	99	99	101	101	100	197Au	KED	100	101	100	102	101	103
99Ru	KED	101	97	100	99	102	103	199Hg	KED	91	96	102	104	101	103
101Ru	KED	98	96	100	101	102	103	200Hg	KED	93	96	98	104	101	101
103Rh	KED	100	97	99	101	102	102	202Hg	KED	88	97	96	101	97	101
105Pd	KED	98	100	101	102	101	104	203Tl	KED	100	99	100	101	101	102
106Pd	KED	100	98	101	101	103	105	205Tl	KED	99	98	99	100	101	101
107Ag	KED	99	98	100	99	102	102	206Pb	KED	103	96	98	99	99	100
108Pd	KED	100	99	102	101	102	104	207Pb	KED	103	98	100	99	101	101
109Ag	KED	99	98	101	103	102	103	208Pb	KED	100	99	99	99	100	101
111Cd	KED	82	93	97	95	97	99								

All analytes meet Linearity acceptance criteria of 70% - 150%.

TABLE 16: LINEARITY STANDARD CORRELATION COEFFICIENTS					
Isotope	**Correlation Coefficient**	**Isotope**	**Correlation Coefficient**	**Isotope**	**Correlation Coefficient**
7Li	0.9999	99Ru	0.9998	189Os	0.9998
51V	0.9999	101Ru	0.9997	190Os	0.9999
52Cr	1.0000	103Rh	1.0000	191Ir	0.9999
53Cr	1.0000	105Pd	0.9997	192Os	0.9999
56Fe	0.9966	106Pd	0.9998	193Ir	0.9999
57Fe	0.9963	107Ag	0.9998	194Pt	0.9999
58Ni	0.9999	108Pd	0.9999	195Pt	0.9998
59Co	0.9998	109Ag	0.9999	196Pt	0.9999
60Ni	0.9999	111Cd	0.9976	197Au	1.0000
62Ni	0.9987	113Cd	0.9998	199Hg	0.9992
63Cu	1.0000	118Sn	0.9999	200Hg	0.9994
65Cu	1.0000	119Sn	1.0000	202Hg	0.9987
75As-DRC	0.9998	120Sn	0.9999	203Tl	0.9999
75As-KED	0.9998	121Sb	0.9999	205Tl	1.0000
77Se	0.9998	123Sb	0.9999	206Pb	1.0000
78Se	0.9999	135Ba	0.9999	207Pb	0.9999
95Mo	1.0000	137Ba	0.9999	208Pb	1.0000
97Mo	0.9999	138Ba	1.0000		
98Mo	0.9999	188Os	0.9998		

All analytes meet Linearity acceptance criteria of NLT 0.99 for correlation coefficients.

7.10. Accuracy

7.10.1. Three (N=3) unspiked samples were prepared for analysis. The unspiked sample preparations were used for spike recovery calculations. Samples were prepared in triplicate at three spiking levels (50%, 100%, and 150% of the 1.0J Target Concentration) as shown in Table 1. The solutions were analyzed by ICP-MS, as per the method, by a single analyst. Results are shown in Tables 18 and 19 for spike recovery for HEPES and MES Sodium.

$$\% \text{ Recovery} = \frac{(\text{Conc. of spiked replicate} - \text{Average Conc. of 3 unspiked samples}) \times 100}{\text{Expected spiked concentration}}$$

7.10.1.1. Acceptance Criteria

7.10.1.1.1. The mean spike recovery for each element at each of the three spike levels, as per USP <233> requirement, must be in the range of 70% - 150%.

7.10.2. Spiked Reference (Unspiked) Solution Preparation

7.10.2.1. Prepared as per section 6.11.

7.10.3. Spike Recovery Sample Preparation

7.10.3.1. Weighed the appropriate amount of sample as per Table 17 into a 50 mL Digitube®.

7.10.3.2. Pipetted appropriate intermediate standard spike amount as per Table 17 and swirled to mix.

7.10.3.3. Transferred approximately 5.0 mL of deionized water and mixed thoroughly to dissolve sample.

7.10.3.4. Pipetted 3.75 mL of Acid Mixture and allowed to react. Swirled solution to mix.

7.10.3.5. Added deionized water to 45 mL and transferred 1.0 mL of Internal Standard/ Complexing Solution.

7.10.3.6. Diluted to a final volume of 50 mL with deionized water and mixed well.

7.10.3.7. Prepared spiked sample solutions in triplicate and three preparations of unspiked sample solutions.

TABLE 17: ACCURACY SAMPLE SPIKES

Description	Sample Amount (mg)	Intermediate Standard Spike (mL)	Acid Mix (mL)	Internal Standard/ Complexing Solution (mL)	Final Volume (mL)
Method Blank	N/A	N/A	3.75	1.0	50
Unspiked	100	N/A	3.75	1.0	50
0.3J Spiked Sample	100	0.010	3.75	1.0	50
0.3J Spiked Sample	100	0.030	3.75	1.0	50
0.5J Spiked Sample	100	0.050	3.75	1.0	50
1.0J Spiked Sample	100	0.100	3.75	1.0	50
1.5J Spiked Sample	100	0.150	3.75	1.0	50

TABLE 18: ACCURACY RESULTS FOR HEPES SODIUM
(Mean percent recovery of triplicate preparations)

Isotope	Mode	0.5J Mean	1.0J Mean	1.5J Mean	Isotope	Mode	0.5J Mean	1.0J Mean	1.5J Mean
7Li	STD	86	83	85	113Cd	KED	89	90	93
51V	KED	95	97	97	118Sn	KED	90	90	90
52Cr	KED	91	90	91	119Sn	KED	91	90	91
53Cr	KED	91	89	90	120Sn	KED	91	90	91
56Fe	H_2 DRC	94	94	95	121Sb	KED	98	98	97
57Fe	KED	98	100	99	123Sb	KED	97	95	95
58Ni	KED	92	91	91	135Ba	KED	107	113	112
59Co	KED	98	97	96	137Ba	KED	106	110	110
60Ni	KED	92	92	91	138Ba	KED	105	110	109
62Ni	KED	89	90	89	188Os	KED	95	95	94
63Cu	KED	89	88	87	189Os	KED	96	96	95
65Cu	KED	89	88	87	190Os	KED	97	94	93
75As	H_2 DRC	105	105	104	191Ir	KED	94	93	93
75As	KED	104	102	100	192Os	KED	95	94	93
77Se	H_2 DRC	107	109	109	193Ir	KED	94	93	93
78Se	H_2 DRC	107	108	108	194Pt	KED	92	93	92
95Mo	KED	94	91	92	195Pt	KED	94	94	93
97Mo	KED	92	89	90	196Pt	KED	94	93	93
98Mo	KED	94	90	92	197Au	KED	96	96	95
99Ru	KED	92	94	96	199Hg	KED	86	90	92
101Ru	KED	92	93	94	200Hg	KED	86	90	93
103Rh	KED	87	87	87	202Hg	KED	88	88	94
105Pd	KED	82	82	83	203Tl	KED	103	102	102
106Pd	KED	81	82	81	205Tl	KED	102	102	103
107Ag	KED	79	77	79	206Pb	KED	99	99	100
108Pd	KED	81	82	81	207Pb	KED	100	100	100
109Ag	KED	76	76	77	208Pb	KED	99	100	100
111Cd	KED	77	85	85					

All elements meet Accuracy acceptance criteria of 70% - 150%.

TABLE 19: ACCURACY RESULTS FOR MES SODIUM
(Mean percent recovery of triplicate preparations)

Isotope	Mode	0.5J Mean	1.0J Mean	1.5J Mean	Isotope	Mode	0.5J Mean	1.0J Mean	1.5J Mean
7Li	STD	86	83	87	113Cd	KED	92	89	86
51V	KED	101	97	98	118Sn	KED	94	89	89
52Cr	KED	94	88	89	119Sn	KED	93	89	89
53Cr	KED	95	88	89	120Sn	KED	95	89	88
56Fe	H_2 DRC	98	98	99	121Sb	KED	100	95	95
57Fe	KED	107	102	101	123Sb	KED	99	94	93
58Ni	KED	98	94	91	135Ba	KED	107	113	112
59Co	KED	102	97	95	137Ba	KED	106	111	111
60Ni	KED	100	95	93	138Ba	KED	104	109	109
62Ni	KED	93	90	86	188Os	KED	101	99	99
63Cu	KED	95	90	88	189Os	KED	99	95	95
65Cu	KED	96	90	89	190Os	KED	99	98	97
75As	H_2 DRC	108	106	105	191Ir	KED	97	95	94
75As	KED	112	108	105	192Os	KED	98	95	96
77Se	H_2 DRC	111	110	111	193Ir	KED	97	95	94
78Se	H_2 DRC	111	110	110	194Pt	KED	96	96	94
95Mo	KED	97	91	91	195Pt	KED	94	94	94
97Mo	KED	95	90	89	196Pt	KED	93	95	95
98Mo	KED	96	90	88	197Au	KED	99	97	95
99Ru	KED	96	89	89	199Hg	KED	104	96	98
101Ru	KED	96	90	91	200Hg	KED	104	97	94
103Rh	KED	89	82	83	202Hg	KED	109	99	100
105Pd	KED	85	78	78	203Tl	KED	105	104	103
106Pd	KED	84	77	77	205Tl	KED	105	103	103
107Ag	KED	81	75	74	206Pb	KED	102	100	101
108Pd	KED	82	76	76	207Pb	KED	103	100	101
109Ag	KED	78	71	72	208Pb	KED	102	100	100
111Cd	KED	95	84	88					

All elements meet Accuracy acceptance criteria of 70% - 150%.

7.11. Specificity

7.11.1. Specificity was demonstrated by using a calibration blank and spiked calibration blank for ICP-MS analysis. The calibration blank was prepared as per the analytical method protocol. A separate blank was spiked with a mixed standard solution which produced a spiked solution at a concentration equivalent to the 2.0J calibration standard.

7.11.2. The solutions were analyzed as per the analytical method and the intensities for the calibration blank and 2.0J calibration standard are reported in Table 20 below.

7.11.2.1. Acceptance Criteria:

7.11.2.1.1. The lack of a significant interference (as demonstrated by the spike recovery of 70% to 150%, as per the Accuracy requirement from USP <233>) or by any other element in the spiked blank solution or the solution matrix itself will indicate the specificity of the method.

TABLE 20: SPECIFICITY RESULTS

Isotope	Blank (CPS)	2.0J STD (CPS)	Isotope	Blank (CPS)	2.0J STD (CPS)
7Li	1981	3152923	113Cd	4	553
51V	119	4123	118Sn	65	444206
52Cr	60	612435	119Sn	31	164157
53Cr	43	76099	120Sn	115	634558
56Fe	4693	823273	121Sb	17	54440
57Fe	28	3964	123Sb	-13	44115
58Ni	42	24098	135Ba	63	137847
59Co	7	9101	137Ba	137	252982
60Ni	18	10458	138Ba	981	1683441
62Ni	7	1686	188Os	22	13588
63Cu	79	420395	189Os	25	16753
65Cu	88	200287	190Os	30	27548
75As (DRC)	58	5052	191Ir	35	37652
75As (KED)	33	2116	192Os	48	42977
77Se	57	11720	193Ir	78	62623
78Se	38	38373	194Pt	13	20864
95Mo	10	916340	195Pt	13	21824
97Mo	8	583831	196Pt	10	16121
98Mo	16	1495880	197Au	123	296359
99Ru	8	6480	199Hg	5	785
101Ru	8	9061	200Hg	4	1083
103Rh	13	54101	202Hg	9	1408
105Pd	76	10651	203Tl	81	22370
106Pd	7	13583	205Tl	15	54295
107Ag	29	22974	206Pb	299	11250
108Pd	6	14191	207Pb	255	9856
109Ag	39	23718	208Pb	644	24171
111Cd	2	536			

7.12. Precision

7.12.1. All solutions for the Precision (Repeatability) test were prepared by a single analyst for HEPES Sodium and MES Sodium samples and reported in Tables 21 and 22.

7.12.2. The value of the unspiked sample preparations from Section 7.10, "Accuracy," was used for spike recovery calculations. Six sample solutions were prepared at the 1.0J Target Concentration as shown in Table 1. For ICP-MS analysis, the Target Concentration spiked samples and the unspiked samples were used for the accuracy experiment.

7.12.3. Precision and %RSD results are reported to the nearest whole number, but values are calculated from data that contains nine decimal places.

7.12.3.1. Acceptance Criteria:

7.12.3.1.1. The %RSD for the spike recovery concentration must be NMT 20% for each element in each sample.

TABLE 21: PRECISION RESULTS FOR HEPES SODIUM
(Mean recovery concentration of 6 preparations)

Isotope	Mode	1.0J Mean Recovery Conc. N=6 (µg/kg)	% RSD N=6	Isotope	Mode	1.0J Mean Recovery Conc. N=6 (µg/kg)	% RSD N=6
7Li	STD	20501	3	113Cd	KED	179	4
51V	KED	962	2	118Sn	KED	53423	3
52Cr	KED	97598	2	119Sn	KED	53619	3
53Cr	KED	96861	1	120Sn	KED	53479	4
56Fe	H_2 DRC	18862	2	121Sb	KED	8686	3
57Fe	KED	20040	4	123Sb	KED	8524	3
58Ni	KED	1900	4	135Ba	KED	77829	2
59Co	KED	482	4	137Ba	KED	76467	2
60Ni	KED	1900	4	138Ba	KED	76559	2
62Ni	KED	1890	5	188Os	KED	941	3
63Cu	KED	25993	3	189Os	KED	947	3
65Cu	KED	25972	4	190Os	KED	929	2
75As	H_2 DRC	1570	2	191Ir	KED	921	2
75As	KED	1515	5	192Os	KED	929	2
77Se	H_2 DRC	8673	2	193Ir	KED	927	2
78Se	H_2 DRC	8545	2	194Pt	KED	926	2
95Mo	KED	134605	3	195Pt	KED	927	2
97Mo	KED	132139	2	196Pt	KED	922	3
98Mo	KED	132993	3	197Au	KED	9532	2
99Ru	KED	931	5	199Hg	KED	261	3
101Ru	KED	931	2	200Hg	KED	262	4
103Rh	KED	864	4	202Hg	KED	256	4
105Pd	KED	818	2	203Tl	KED	808	3
106Pd	KED	815	4	205Tl	KED	812	2
107Ag	KED	766	3	206Pb	KED	493	2
108Pd	KED	808	4	207Pb	KED	496	2
109Ag	KED	753	3	208Pb	KED	495	2
111Cd	KED	177	5				

All analytes meet Precision RSD% acceptance criteria of NMT 20%.

TABLE 22: PRECISION RESULTS FOR MES SODIUM
(Mean recovery concentration of 6 preparations)

Isotope	Mode	1.0J Mean Recovery Conc. N=6 (µg/kg)	% RSD N=6	Isotope	Mode	1.0J Mean Recovery Conc. N=6 (µg/kg)	% RSD N=6
7Li	STD	20766	1	113Cd	KED	178	3
51V	KED	991	1	118Sn	KED	53466	3
52Cr	KED	97426	2	119Sn	KED	53418	2
53Cr	KED	97609	2	120Sn	KED	53686	2
56Fe	H_2 DRC	19915	1	121Sb	KED	8539	2
57Fe	KED	20687	2	123Sb	KED	8489	3
58Ni	KED	1878	2	135Ba	KED	79308	2
59Co	KED	490	2	137Ba	KED	78604	2
60Ni	KED	1904	3	138Ba	KED	76358	2
62Ni	KED	1842	3	188Os	KED	997	2
63Cu	KED	26902	1	189Os	KED	962	2
65Cu	KED	27127	2	190Os	KED	982	2
75As	H_2 DRC	1599	3	191Ir	KED	947	2
75As	KED	1646	2	192Os	KED	968	2
77Se	H_2 DRC	8980	2	193Ir	KED	945	2
78Se	H_2 DRC	8935	1	194Pt	KED	970	1
95Mo	KED	137286	3	195Pt	KED	963	2
97Mo	KED	135094	3	196Pt	KED	966	2
98Mo	KED	135668	2	197Au	KED	9652	2
99Ru	KED	899	4	199Hg	KED	293	2
101Ru	KED	912	2	200Hg	KED	294	2
103Rh	KED	828	2	202Hg	KED	296	6
105Pd	KED	785	3	203Tl	KED	828	2
106Pd	KED	775	3	205Tl	KED	826	2
107Ag	KED	750	3	206Pb	KED	508	3
108Pd	KED	769	2	207Pb	KED	507	2
109Ag	KED	714	2	208Pb	KED	507	2
111Cd	KED	169	4				

All analytes meet Precision RSD% acceptance criteria of NMT 20%.

7.13. Intermediate Precision (Ruggedness)

7.13.1. A second analyst, on a different day from the performance of the Repeatability experiment, prepared and analyzed the Intermediate Precision solutions. Six sample solutions were prepared at the 1.0J Target Concentration level found in Table 1 for ICP- MS analysis (this fulfilled two events as "different day" and "different analyst").

7.13.2. Ruggedness and %RSD results are reported to the nearest whole number, but values are calculated from data that contains nine decimal places. Results are reported in Tables 23 and 24.

7.13.2.1. Acceptance Criteria:

7.13.2.1.1. The %RSD for the spike recovery concentration from both analysts (N=12) must be NMT 25% for each element.

TABLE 23: RUGGEDNESS RESULTS FOR HEPES SODIUM
(Mean recovery concentration of 12 preparations)

Isotope	Mode	1.0J Mean Recovery Conc. N=12 (µg/kg)	% RSD N=12	Isotope	Mode	1.0J Mean Recovery Conc. N=12 (µg/kg)	% RSD N=12
7Li	STD	20649	2	113Cd	KED	185	5
51V	KED	982	3	118Sn	KED	55354	4
52Cr	KED	99334	2	119Sn	KED	55042	4
53Cr	KED	98997	3	120Sn	KED	55133	4
56Fe	H_2 DRC	19908	6	121Sb	KED	8928	4
57Fe	KED	20508	4	123Sb	KED	8818	4
58Ni	KED	1954	4	135Ba	KED	78362	2
59Co	KED	496	4	137Ba	KED	77212	2
60Ni	KED	1961	4	138Ba	KED	76550	2
62Ni	KED	1960	6	188Os	KED	954	3
63Cu	KED	27069	5	189Os	KED	963	3
65Cu	KED	26748	4	190Os	KED	950	3
75As	H_2 DRC	1622	4	191Ir	KED	936	2
75As	KED	1583	6	192Os	KED	946	3
77Se	H_2 DRC	8845	3	193Ir	KED	939	2
78Se	H_2 DRC	8702	3	194Pt	KED	958	4
95Mo	KED	138995	4	195Pt	KED	952	4
97Mo	KED	137289	4	196Pt	KED	949	4
98Mo	KED	137345	4	197Au	KED	9702	2
99Ru	KED	953	4	199Hg	KED	256	5
101Ru	KED	953	3	200Hg	KED	254	6
103Rh	KED	885	4	202Hg	KED	247	6
105Pd	KED	839	4	203Tl	KED	825	3
106Pd	KED	842	5	205Tl	KED	824	2
107Ag	KED	790	4	206Pb	KED	503	3
108Pd	KED	819	3	207Pb	KED	501	2
109Ag	KED	781	4	208Pb	KED	505	3
111Cd	KED	187	7				

All analytes meet the Ruggedness %RSD acceptance criteria of NMT 25%.

TABLE 24: RUGGEDNESS RESULTS FOR MES SODIUM
(Mean recovery concentration of 12 preparations)

Isotope	Mode	1.0J Mean Recovery Conc. N=12 (µg/kg)	% RSD N=12	Isotope	Mode	1.0J Mean Recovery Conc. N=12 (µg/kg)	% RSD N=12
7Li	STD	20840	2	113Cd	KED	184	4
51V	KED	1012	3	118Sn	KED	55315	4
52Cr	KED	100171	4	119Sn	KED	54773	3
53Cr	KED	100680	4	120Sn	KED	55209	3
56Fe	H_2 DRC	20424	3	121Sb	KED	8829	4
57Fe	KED	20609	3	123Sb	KED	8777	4
58Ni	KED	1918	4	135Ba	KED	78296	3
59Co	KED	497	3	137Ba	KED	77286	3
60Ni	KED	1924	3	138Ba	KED	75677	3
62Ni	KED	1891	5	188Os	KED	979	2
63Cu	KED	27377	3	189Os	KED	972	2
65Cu	KED	27301	3	190Os	KED	979	2
75As	H_2 DRC	1640	4	191Ir	KED	952	2
75As	KED	1654	3	192Os	KED	968	2
77Se	H_2 DRC	8989	2	193Ir	KED	952	2
78Se	H_2 DRC	8899	2	194Pt	KED	978	2
95Mo	KED	143543	5	195Pt	KED	970	2
97Mo	KED	142090	6	196Pt	KED	968	2
98Mo	KED	142088	5	197Au	KED	9811	2
99Ru	KED	927	5	199Hg	KED	297	4
101Ru	KED	939	4	200Hg	KED	297	3
103Rh	KED	868	5	202Hg	KED	299	5
105Pd	KED	818	5	203Tl	KED	835	2
106Pd	KED	824	7	205Tl	KED	831	2
107Ag	KED	779	5	206Pb	KED	511	2
108Pd	KED	804	5	207Pb	KED	507	1
109Ag	KED	757	6	208Pb	KED	512	2
111Cd	KED	182	8				

All analytes meet the Ruggedness %RSD acceptance criteria of NMT 25%.

7.14. Limit of Quantitation (LOQ)

7.14.1. The limit of quantitation (LOQ) is demonstrated from spike recovery performed at the 10% and 30% Target Concentration spiking levels as shown in Table 1.

7.14.2. Samples were prepared in triplicate following Section 7.10.3 and using amounts listed for 0.1J and 0.3J spiked samples in Table 17 above. Results for HEPES and MES Sodium are reported in Tables 25 and 26 below.

7.14.2.1. Acceptance Criteria:

7.14.2.1.1. The mean percent spike recovery for each element at the 10% and 30% Target Concentration spiking levels, as per the USP <233> accuracy guideline, must be in the range of 70% - 150%.

TABLE 25: LIMIT OF QUANTITATION RESULTS FOR HEPES SODIUM

(Mean percent recovery of 3 preparations)

Isotope	Mode	0.1J Mean % Recovery	0.3J Mean % Recovery	Isotope	Mode	0.1J Mean % Recovery	0.3J Mean % Recovery
7Li	STD	82	81	113Cd	KED	104	99
51V	KED	101	100	118Sn	KED	94	96
52Cr	KED	91	90	119Sn	KED	96	95
53Cr	KED	92	91	120Sn	KED	95	95
56Fe	H_2 DRC	101	100	121Sb	KED	99	102
57Fe	KED	105	102	123Sb	KED	103	103
58Ni	KED	96	98	135Ba	KED	110	112
59Co	KED	100	101	137Ba	KED	108	111
60Ni	KED	101	100	138Ba	KED	110	111
62Ni	KED	90	101	188Os	KED	100	99
63Cu	KED	96	94	189Os	KED	99	96
65Cu	KED	94	94	190Os	KED	98	98
75As	H_2 DRC	111	108	191Ir	KED	95	95
75As	KED	105	110	192Os	KED	98	98
77Se	H_2 DRC	112	115	193Ir	KED	96	96
78Se	H_2 DRC	111	113	194Pt	KED	99	99
95Mo	KED	96	95	195Pt	KED	97	98
97Mo	KED	95	93	196Pt	KED	96	102
98Mo	KED	94	93	197Au	KED	100	100
99Ru	KED	107	100	199Hg	KED	80	97
101Ru	KED	103	99	200Hg	KED	97	96
103Rh	KED	94	94	202Hg	KED	76	86
105Pd	KED	95	89	203Tl	KED	104	103
106Pd	KED	88	86	205Tl	KED	102	103
107Ag	KED	88	85	206Pb	KED	103	104
108Pd	KED	88	87	207Pb	KED	110	102
109Ag	KED	82	82	208Pb	KED	107	102
111Cd	KED	74	93				

All analytes meet LOQ acceptance criteria of 70% - 150%.

TABLE 26: LIMIT OF QUANTITATION RESULTS FOR MES SODIUM

(Mean percent recovery of 3 preparations)

Isotope	Mode	0.1J Mean % Recovery	0.3J Mean % Recovery	Isotope	Mode	0.1J Mean % Recovery	0.3J Mean % Recovery
7Li	STD	81	83	113Cd	KED	92	95
51V	KED	91	98	118Sn	KED	91	97
52Cr	KED	87	90	119Sn	KED	91	95
53Cr	KED	88	91	120Sn	KED	89	94
56Fe	H_2 DRC	96	98	121Sb	KED	97	101
57Fe	KED	100	101	123Sb	KED	95	102
58Ni	KED	93	95	135Ba	KED	108	112
59Co	KED	99	99	137Ba	KED	108	109
60Ni	KED	91	94	138Ba	KED	108	110
62Ni	KED	83	93	188Os	KED	91	99
63Cu	KED	89	91	189Os	KED	96	98
65Cu	KED	88	89	190Os	KED	94	97
75As	H_2 DRC	106	107	191Ir	KED	91	95
75As	KED	110	101	192Os	KED	95	97
77Se	H_2 DRC	104	112	193Ir	KED	92	96
78Se	H_2 DRC	106	110	194Pt	KED	101	99
95Mo	KED	91	96	195Pt	KED	100	100
97Mo	KED	90	95	196Pt	KED	99	97
98Mo	KED	88	94	197Au	KED	96	99
99Ru	KED	94	101	199Hg	KED	107	101
101Ru	KED	89	98	200Hg	KED	87	104
103Rh	KED	89	93	202Hg	KED	77	99
105Pd	KED	82	88	203Tl	KED	100	103
106Pd	KED	79	86	205Tl	KED	100	105
107Ag	KED	77	83	206Pb	KED	97	103
108Pd	KED	80	85	207Pb	KED	95	102
109Ag	KED	77	82	208Pb	KED	97	103
111Cd	KED	87	95				

All analytes meet LOQ acceptance criteria of 70% - 150%.

7.15. Sample and Standard Stability

7.15.1. The 50% and 200% Target Concentration level calibration standards were analyzed as samples against calibration curves constructed from freshly prepared calibration standards at T=1 day from the date of preparation.

7.15.2. A spiked sample solution prepared at the 1.0J Target Concentration level in Table 1 from the Precision experiments were used for sample stability for both HEPES Sodium and MES Sodium. The spiked sample solution was analyzed against calibration curves constructed from freshly prepared calibration standards at time points T=0 (day of preparation) and T=1 (1 day from the date of preparation).

7.15.3. Both sample and standard solution stability results are reported in Table 27.

7.15.3.1. Acceptance Criteria:

7.15.3.1.1. The recovery of each element must be within the range of 80% to 120% recovery of the T = 0 results for the calibration standard.

7.15.3.1.2. The recovery of each element must be within the range of 80% to 120% recovery of the T = 0 results for the spiked sample solution.

TABLE 27: SAMPLE AND STANDARD STABILITY (% RECOVERY)

Isotope	0.5J Std (%)	2.0J Std (%)	HEPN 1.0J Spike (%)	MESN 1.0J Spike (%)	Isotope	0.5J Std (%)	2.0J Std (%)	HEPN 1.0J Spike (%)	MESN 1.0J Spike (%)
7Li	95	90	104	98	113Cd	99	107	111	111
51V	103	99	96	101	118Sn	106	103	107	111
52Cr	99	97	97	101	119Sn	106	105	108	110
53Cr	99	97	98	103	120Sn	106	105	107	107
56Fe	110	109	103	103	121Sb	108	105	105	108
57Fe	103	110	101	103	123Sb	105	104	108	111
58Ni	100	108	107	102	135Ba	110	111	98	99
59Co	103	110	102	110	137Ba	108	107	98	99
60Ni	97	111	100	103	138Ba	110	111	100	98
62Ni	92	103	109	101	188Os	100	106	97	96
63Cu	99	109	105	106	189Os	105	103	98	102
65Cu	100	107	102	100	190Os	103	104	101	98
75As (DRC)	105	104	98	100	191Ir	105	104	102	99
75As (KED)	97	104	100	98	192Os	105	104	101	99
77Se	107	108	95	103	193Ir	105	105	98	101
78Se	106	108	98	102	194Pt	107	105	102	102
95Mo	99	99	105	103	195Pt	111	105	102	104
97Mo	100	98	103	106	196Pt	105	106	102	104
98Mo	99	101	101	104	197Au	107	105	100	101
99Ru	117	116	116	118	199Hg	101	101	109	107
101Ru	110	110	110	111	200Hg	101	107	104	101
103Rh	110	110	111	113	202Hg	109	105	101	91
105Pd	109	111	109	114	203Tl	105	101	100	99
106Pd	106	107	111	118	205Tl	104	103	100	101
107Ag	115	110	110	111	206Pb	100	103	101	99
108Pd	110	109	111	112	207Pb	103	104	101	101
109Ag	108	106	111	113	208Pb	100	103	100	99
111Cd	101	112	115	115					

All analytes meet Sample and Standard Solution Stability acceptance criteria of 80% - 120%.

8. DEVIATIONS:

8.1. No deviations occurred during validation of elemental impurity assessment protocol for HEPES Sodium and MES Sodium.

9. CONCLUSION:

9.1. The test method for Elemental Impurities in HEPES Sodium and MES Sodium products has been validated. The Method was found to be:

9.1.1. Specific: The method blank did not show any significant interference for all analyzed masses.

9.1.2. Linear: 10% to 200% of working standard solution corresponding to 0.1J to 2.0J. Mean percent recovery ranged from 82% to 114%.

9.1.3. Sensitive: LOQ recoveries were within 74% to 115% for HEPES Sodium and 77% to 112% for MES Sodium. All analytes met acceptance criteria established.

9.1.4. Accurate: From 50% (0.5J) to 150% (1.5J) of working standard concentration level with mean percent recoveries ranging from 76% to 113% for HEPES Sodium and 71% to 113% for MES Sodium. All masses analyzed met acceptance criteria within the specified range.

9.1.5. Precise: Closeness of agreement demonstrated between six sample preparations by percent RSD's ranging from 1% to 5% for HEPES Sodium and 1% to 6% for MES Sodium.

9.1.6. Rugged: Satisfactory precision was demonstrated between two sets of six sample preparations performed on different days and by different analysts. The percent RSDs ranged from 2% to 7% for HEPES Sodium and 1% to 8% MES Sodium.

9.1.7. Stable: With respect to stability of solutions, the sample solutions for both HEPES Sodium and MES Sodium are shown to be stable for 24 hours for all elements analyzed using this protocol. The working standard preparations were shown to be stable for all analytes under the protocol as well for 24 hours. The samples and standards are to be noted as stable for 24 hours in the final analytical testing method.

10. NOTEBOOK REFERENCE:

TABLE 28: NOTEBOOK REFERENCE

STUDY		NOTEBOOK REFERENCE
Specificity		EIV-7/ pages 82-86
Linearity and Range		EIV-7/ pages 78-81
LOQ by "Spiked" recovery for HEPES Sodium		EIV-7/ pages 82-86
LOQ by "Spiked" recovery for MES Sodium		EIV-7/ pages 91-94
Accuracy/ Precision by "Spiked" recovery for HEPES Sodium		EIV-7/ pages 87-90
Accuracy/ Precision by "Spiked" recovery for MES Sodium		EIV-7/ pages 95-100
Intermediate Precision (Ruggedness) for HEPN and MESN		EIV-8/ pages 1-6
Sample Solution Stability	Day-0	EIV-7/ pages 87-90, 95-100
	Day-1	EIV-7/ pages 91-94 EIV-8/ pages 1-6
Standard Solution Stability	Day-0	EIV-7/ pages 78-81
	Day-1	EIV-7/ pages 82-86