BIOSPECTRA
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ANALYTICAL METHOD VALIDATION REPORT: DETERMINATION OF ELEMENTAL IMPURITIES BY INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY (ICP-MS) IN HEPES REVALIDATION

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1. PURPOSE:

1.1. The purpose of this validation report is to establish documented evidence that the validation protocol, BSI-PRL-0508 v. 2.1, for Elemental Impurities in HEPES products performs according to USP and BioSpectra requirements.

1.2. This protocol is a revalidation of the original HEPES elemental impurity test method for the addition of bismuth as an analyte of interest and to lower the detectability for several elements.

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

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

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

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

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

1.2.1.5. Class 4: Ca, Fe, K, Mg, Mn, and Zn

1.2.1.6. Other: Bi

2. SCOPE:

2.1. Applies to HEPES (HEPE) 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 by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) performed at BioSpectra Inc.

3. REFERENCES:

3.1. BSI-PRL-0508: Analytical Method Validation Protocol: Determination of ICH Q3D Elemental Impurities (Class 1, 2A, 2B, 3 & 4) by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in HEPES

3.2. BSI-RPT-0544: Analytical Method Validation Report: Determination of Elemental Impurities by ICP-MS in HEPES

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

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

3.5. BSI-SOP-0436: Analytical Methods Validation Master Plan

3.6. ICH Guideline for Elemental Impurities Q3D

3.7. NexION Operation with Syngistix Software Guide

3.8. USP <730> Plasma Spectrochemistry

3.9. USP <232>, <233>

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

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 (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	*150	1.5	4.5	7.5	15	22.5
Cu	3	*50	0.50	1.5	2.5	5.0	7.5
Sn	3	600	6.0	18	30	60	90
Cr	3	*50	0.50	1.5	2.5	5.0	7.5
Fe	4	^50	0.50	1.5	2.5	5.0	7.5
Mn	4	^50	0.50	1.5	2.5	5.0	7.5
Zn	4	^50	0.50	1.5	2.5	5.0	7.5
Ca	4	^500	5.0	15	25	50	75
K	4	^500	5.0	15	25	50	75
Mg	4	^50	0.50	1.5	2.5	5.0	7.5
Bi	Not Applicable	^50	0.50	1.5	2.5	5.0	7.5

*No PDE limits for Class 4 elements; limits derived from other internal product specifications.
^PDE limits set based on customer specifications.

5. MATERIALS AND EQUIPMENT:

TABLE 2: EQUIPMENT

Type	Supplier	Model	Serial Number	Cal. Due
Analytical Balance	Sartorius	MSE224S	36707108	10/31/23
Automatic Pipette	Rainin	E4-XLS (2-20 µL)	C249353491	01/03/24
Automatic Pipette	Rainin	E4-XLS (20-200 µL)	C249353515	01/03/24
Automatic Pipette	Rainin	E4-XLS (100-1000 µL)	C244197408	01/03/24
Automatic Pipette	Rainin	E4-XLS (0.5-5 mL)	C238841856	01/03/24
ICP-MS	Perkin Elmer	NexION 350X	85VN5093001	01/2024
Deionized water system	Millipore	IQ-7005/ Element POD	F9SA14284H	12/31/23

TABLE 3: REAGENTS

Type	Grade	Supplier	Catalog Number	Lot Number	Expiration
70% Nitric Acid	Trace Metal	SCP Science	250-038-175	23040044	10/13/24
36% Hydrochloric Acid	Trace Metal	SCP Science	250-038-155	22410207	11/03/24
Sulfuric Acid	Trace Metal	Spectrum	S1136	3121040	06/30/24
Deionized water	Type 1 Ultrapure	In-House	Not Applicable	Not Applicable	Not Applicable
Thiourea	99+% Pure	ACROS	220052500	A0407315	10/31/23
ICP-MS Setup Solution	Not Applicable	Perkin Elmer	N8145051	41-202GSX1	06/30/24
ICP-MS KED Setup Solution	Not Applicable	In-House	Not Applicable	ICP21P07	08/31/23
DigiSep SPE Cartridge	Green Label	SCP Science	010-700-034	SC6243885	Not Applicable

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 Finished Good Lot HEPE-0123-00056

TABLE 4: REFERENCE STANDARDS				
Identification	**Manufacturer**	**Lot Number**	**Expiration**	**Concentrations / Elements**
Pharma-CAL Standard Parenteral STD# 1 IA 140-131-201	SCP Science	S230203025	02/2024	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 140-131-211	SCP Science	S230706010	10/2024	Au (100 µg/mL); Ir, Os, Pd, Pt, Rh, & Ru (10 µg/mL)
USP232/ICH Q3D Parenteral STD# 2 IA 140-131-211	SCP Science	S220713001	10/2023	Au (100 µg/mL); Ir, Os, Pd, Pt, Rh, & Ru (10 µg/mL)
Pharma-CAL Custom Standard Parenteral STD# 3 AQ0-150-191	SCP Science	S230612005	06/2024	Ba (700 µg/mL), Cr (50 µg/mL), Cu (50 µg/mL), Li (250 µg/mL), Mo (150 µg/mL), Sb (90 µg/mL), Sn (600 µg/mL), Fe (50 µg/mL), Mn (50 µg/mL), Zn (50 µg/mL), Ca (500 µg/mL), K (500 µg/mL)
Bismuth Stock Standard N9303761	Perkin Elmer	25-164BIY1	09/30/23	Bi (1,000 µg/mL)
Magnesium Stock Standard N9300179	Perkin Elmer	26-98MGY1	11/30/23	Mg (1,000 µg/mL)
Beryllium Stock Standard 140-051-040	SCP Science	S220705011	06/24/24	Be (1,000 µg/mL)
Germanium Stock Standard 140-050-321	SCP Science	S221004010	10/2024	Ge (1,000 µg/mL)
Rhenium Stock Standard 140-050-750	SCP Science	S220517007	05/2024	Re (1,000 µg/mL)
Scandium Stock Standard 140-051-211	SCP Science	S221125001	04/30/24	Sc (1,000 µg/mL)
Tellurium Stock Standard 140-051-521	SCP Science	S221222019	11/07/24	Te (1,000 µg/mL)
Terbium Stock Standard 140-051-650	SCP Science	S220324017	03/16/24	Tb (1,000 µg/mL)
Yttrium Stock Standard 140-051-391	SCP Science	S220915001	06/01/24	Y (1,000 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. Prepared day of use.

6.3. Internal Standard Stock Solution

6.3.1. Prepared a standard solution containing the elements listed in Table 5 below using the standards listed in Table 4 above.

TABLE 5: INTERNAL STANDARD STOCK

Identification	Element	Stock Identification	Amount Added (mL)	Final Volume (mL)	Final Concentration (µg/mL)
Internal Standard Stock	Ge	1,000 µg/mL	0.05	10	5.0
	Tb		0.05		5.0
	Be		0.10		10
	Re		0.10		10
	Sc		0.10		10
	Y		0.10		10
	Te		0.25		25

6.4. Internal Standard/Complexing 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 DigiSEP Cation Solid Phase Extraction (SPE) cartridge.

6.4.4. Transferred 2.5 mL of Internal Standard Stock to the filtered solution and added 25 mL of hydrochloric acid.

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

6.4.6. Scaled proportionally as needed for use.

6.5. 2% Thiourea Solution

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

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

6.5.3. Filtered solution through a DigiSEP Cation Solid Phase Extraction (SPE) cartridge.

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

6.5.5. Scaled proportionally as needed for use.

6.6. Intermediate Standard Preparation

6.6.1. Prepared a standard solution containing the elements listed in Table 6 using the standards STD#1 IA, STD#2 IA, STD#3 IA, and individual single source 1,000 µg/mL standards. 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 6: 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 140-131-211	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 AQ0-150-191	1.0			70
	Sb					9.0
	Li					25
	Mo					15
	Cu					5.0
	Sn					60
	Cr					5.0
	Fe					5.0
	Mn					5.0
	Zn					5.0
	Ca					50
	K					50
	Mg	1,000 µg/mL Mg Std	0.050			5.0
	Bi	1,000 µg/mL Bi Std	0.050			5.0

6.7. 0.5J 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: 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					15
	Cu					5.0
	Sn					60
	Cr					5.0
	Fe					5.0
	Mn					5.0
	Zn					5.0
	Ca					50
	K					50
	Mg					5.0
	Bi					5.0

6.8. 1.5J 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: 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					45
	Cu					15
	Sn					180
	Cr					15
	Fe					15
	Mn					15
	Zn					15
	Ca					150
	K					150
	Mg					15
	Bi					15

6.9. 2.0J Calibration Standard Preparation

6.9.1. Prepared a solution containing the elements listed in Table 9 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 9: 2.0J CALIBRATION STANDARD

Identification	Element	Intermediate Standard (mL)	Acid Mix (mL)	Internal Standard/ ComplexingSolution (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					60
	Cu					20
	Sn					240
	Cr					20
	Fe					20
	Mn					20
	Zn					20
	Ca					200
	K					200
	Mg					20
	Bi					20

6.10. Calibration Blank

6.10.1. 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 10 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 10: CALIBRATION BLANK

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

6.11. Method Blank Preparation

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

6.11.2. Added 3.75 mL of Acid Digestion Mixture.

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

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

6.12. Sample Preparation

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

6.12.2. Transferred approximately 30 mL of deionized water and swirled to dissolve sample.

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

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

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

6.13. Isobaric Overlap Corrections

6.13.1. 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:

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

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

6.15. 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 11.

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 11: 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 12: 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 13: 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	108Pd	185Re	KED	0.20-4.0
24Mg	45Sc	KED	1.0-20	109Ag	185Re	KED	0.20-4.0
39K	45Sc	KED	10-200	111Cd	125Te	KED	0.04-0.80
44Ca	45Sc	KED	10-200	113Cd	125Te	KED	0.04-0.80
51V	45Sc	KED	0.20-4.0	118Sn	125Te	KED	12-240
52Cr	45Sc	KED	1.0-20	119Sn	125Te	KED	12-240
53Cr	45Sc	KED	1.0-20	120Sn	125Te	KED	12-240
55Mn	72Ge	KED	1.0-20	121Sb	125Te	KED	1.8-36
56Fe	45Sc	H_2 DRC	1.0-20	123Sb	125Te	KED	1.8-36
57Fe	72Ge	KED	1.0-20	135Ba	89Y	KED	14-280
58Ni	72Ge	KED	0.40-8.0	136Ba	89Y	KED	14-280
59Co	72Ge	KED	0.10-2.0	137Ba	89Y	KED	14-280
60Ni	72Ge	KED	0.40-8.0	138Ba	89Y	KED	14-280
62Ni	72Ge	KED	0.40-8.0	188Os	185Re	KED	0.20-4.0
63Cu	72Ge	KED	1.0-20	189Os	185Re	KED	0.20-4.0
65Cu	72Ge	KED	1.0-20	190Os	185Re	KED	0.20-4.0
66Zn	72Ge	KED	1.0-20	191Ir	185Re	KED	0.20-4.0
67Zn	72Ge	KED	1.0-20	192Os	185Re	KED	0.20-4.0
68Zn	72Ge	KED	1.0-20	193Ir	185Re	KED	0.20-4.0
75As	72Ge	H_2 DRC	0.30-6.0	194Pt	185Re	KED	0.20-4.0
75As	72Ge	KED	0.30-6.0	195Pt	185Re	KED	0.20-4.0
77Se	89Y	H_2 DRC	1.6-32	196Pt	185Re	KED	0.20-4.0
78Se	89Y	H_2 DRC	1.6-32	197Au	185Re	KED	2.0-40
95Mo	89Y	KED	3.0-60	199Hg	185Re	KED	0.06-1.2
97Mo	89Y	KED	3.0-60	200Hg	185Re	KED	0.06-1.2
98Mo	89Y	KED	3.0-60	202Hg	185Re	KED	0.06-1.2
99Ru	89Y	KED	0.20-4.0	203Tl	185Re	KED	0.16-3.2
101Ru	89Y	KED	0.20-4.0	205Tl	185Re	KED	0.16-3.2
103Rh	125Te	KED	0.20-4.0	206Pb	185Re	KED	0.10-2.0
105Pd	185Re	KED	0.20-4.0	207Pb	185Re	KED	0.10-2.0
106Pd	185Re	KED	0.20-4.0	208Pb	185Re	KED	0.10-2.0
107Ag	185Re	KED	0.20-4.0	209Bi	185Re	KED	1.0-20

7.9. Linearity and Range

7.9.1. The ICP-MS linearity study included standards equivalent to the concentrations shown in Table 15 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 6.7 to Section 6.9. 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 14 below. The concentrations of each element analyzed is listed in Table 15. Percent recovery values and correlation coefficients for each isotope were tabulated in Tables 16 and 17, respectively.

7.9.3.1. Acceptance Criteria:

7.9.3.1.1. 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%.

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

TABLE 14: 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 15: 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	3.0	9.0	15	30	45	60
Cu	1.0	3.0	5.0	10	15	20
Sn	12	36	60	120	180	240
Cr	1.0	3.0	5.0	10	15	20
Fe	1.0	3.0	5.0	10	15	20
Mn	1.0	3.0	5.0	10	15	20
Zn	1.0	3.0	5.0	10	15	20
Ca	10	30	50	100	150	200
K	10	30	50	100	150	200
Mg	1.0	3.0	5.0	10	15	20
Bi	1.0	3.0	5.0	10	15	20

TABLE 16: 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	85	88	89	107	106	109	108Pd	KED	102	104	104	103	103	104
24Mg	KED	110	106	109	106	108	109	109Ag	KED	99	103	104	104	102	105
39K	KED	104	107	104	102	103	105	111Cd	KED	102	87	99	103	99	99
44Ca	KED	105	96	97	98	98	101	113Cd	KED	97	105	101	101	101	101
51V	KED	97	99	101	102	101	101	118Sn	KED	102	103	103	104	104	104
52Cr	KED	104	103	101	100	100	100	119Sn	KED	101	103	103	103	104	104
53Cr	KED	99	102	97	97	98	97	120Sn	KED	99	101	101	102	103	102
55Mn	KED	102	104	108	102	106	102	121Sb	KED	100	100	99	100	100	99
56Fe	H_2 DRC	112	104	103	101	102	101	123Sb	KED	97	100	99	100	100	100
57Fe	KED	112	105	112	103	111	104	135Ba	KED	97	101	100	101	99	99
58Ni	KED	101	102	107	102	104	100	136Ba	KED	99	102	99	100	100	99
59Co	KED	104	104	106	102	103	101	137Ba	KED	97	100	98	100	99	99
60Ni	KED	104	103	109	104	107	104	138Ba	KED	89	92	90	90	91	107
62Ni	KED	96	100	107	105	105	100	188Os	KED	99	102	101	100	100	101
63Cu	KED	105	104	108	105	106	102	189Os	KED	99	101	102	100	99	100
65Cu	KED	103	103	107	103	104	102	190Os	KED	100	101	101	102	100	101
66Zn	KED	107	100	106	102	104	101	191Ir	KED	101	102	102	102	100	101
67Zn	KED	111	100	113	101	103	101	192Os	KED	100	102	101	101	99	100
68Zn	KED	107	101	107	102	104	101	193Ir	KED	101	102	102	102	99	100
75As	H_2 DRC	87	95	93	95	98	96	194Pt	KED	97	99	98	100	98	99
75As	KED	97	99	101	99	103	98	195Pt	KED	100	101	100	101	100	100
77Se	H_2 DRC	95	102	99	97	95	96	196Pt	KED	102	100	101	102	99	100
78Se	H_2 DRC	98	102	100	99	97	98	197Au	KED	99	99	99	99	97	97
95Mo	KED	99	101	99	101	99	97	199Hg	KED	102	97	100	98	97	98
97Mo	KED	96	103	100	103	99	99	200Hg	KED	96	101	100	99	96	98
98Mo	KED	99	102	100	101	99	99	202Hg	KED	95	99	94	97	92	94
99Ru	KED	103	100	104	104	102	101	203Tl	KED	103	102	102	102	99	101
101Ru	KED	104	101	98	103	99	100	205Tl	KED	101	101	101	101	99	100
103Rh	KED	102	104	104	107	105	104	206Pb	KED	101	102	100	100	98	99
105Pd	KED	100	103	103	105	103	104	207Pb	KED	101	103	101	101	100	100
106Pd	KED	103	102	101	102	103	104	208Pb	KED	101	101	100	100	99	99
107Ag	KED	101	101	102	101	102	103	209Bi	KED	100	101	100	100	98	99

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

TABLE 17: LINEARITY STANDARD CORRELATION COEFFICIENTS					
Isotope	**Correlation Coefficient**	**Isotope**	**Correlation Coefficient**	**Isotope**	**Correlation Coefficient**
7Li	0.9987	78Se	0.9998	138Ba	0.9907
24Mg	0.9997	95Mo	0.9998	188Os	0.9998
39K	0.9996	97Mo	0.9997	189Os	0.9998
44Ca	0.9986	98Mo	0.9996	190Os	0.9998
51V	0.9997	99Ru	0.9995	191Ir	0.9999
52Cr	0.9998	101Ru	0.9995	192Os	0.9999
53Cr	0.9997	103Rh	0.9998	193Ir	0.9999
55Mn	0.9991	105Pd	0.9997	194Pt	0.9999
56Fe	0.9998	106Pd	0.9995	195Pt	0.9998
57Fe	0.9985	107Ag	0.9996	196Pt	0.9999
58Ni	0.9996	108Pd	0.9996	197Au	0.9999
59Co	0.9994	109Ag	0.9995	199Hg	0.9997
60Ni	0.9994	111Cd	0.9980	200Hg	0.9996
62Ni	0.9993	113Cd	0.9995	202Hg	0.9997
63Cu	0.9996	118Sn	0.9995	203Tl	0.9998
65Cu	0.9995	119Sn	0.9993	205Tl	0.9999
66Zn	0.9995	120Sn	0.9996	206Pb	0.9999
67Zn	0.9989	121Sb	0.9995	207Pb	0.9998
68Zn	0.9994	123Sb	0.9995	208Pb	0.9998
75As-DRC	0.9983	135Ba	0.9994	209Bi	0.9999
75As-KED	0.9990	136Ba	0.9994		
77Se	0.9996	137Ba	0.9995		

All analytes met 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 Table 19.

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

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 6.12.

7.10.3. Spike Recovery Sample Preparation

7.10.3.1. Weighed the appropriate amount of sample as per Table 18 into a 50 mL Digitube®.

7.10.3.2. Pipetted appropriate intermediate standard spike amount as per Table 18 and swirled to mix.

7.10.3.3. Transferred approximately 30 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 18: 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
0.1J 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 19: ACCURACY RESULTS FOR HEPES

(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	87	87	102	108Pd	KED	99	103	102
24Mg	KED	99	99	97	109Ag	KED	96	104	102
39K	KED	101	100	100	111Cd	KED	99	94	94
44Ca	KED	108	101	100	113Cd	KED	99	96	97
51V	KED	97	99	97	118Sn	KED	99	99	98
52Cr	KED	101	100	98	119Sn	KED	100	99	99
53Cr	KED	98	98	97	120Sn	KED	98	98	98
55Mn	KED	98	100	98	121Sb	KED	97	99	98
56Fe	H_2 DRC	98	100	98	123Sb	KED	99	98	98
57Fe	KED	98	102	99	135Ba	KED	96	100	99
58Ni	KED	99	101	97	136Ba	KED	97	99	99
59Co	KED	99	102	99	137Ba	KED	97	98	97
60Ni	KED	98	99	96	138Ba	KED	96	99	98
62Ni	KED	96	102	100	188Os	KED	98	99	97
63Cu	KED	99	101	97	189Os	KED	97	100	97
65Cu	KED	98	99	97	190Os	KED	99	99	97
66Zn	KED	92	96	95	191Ir	KED	99	99	99
67Zn	KED	87	102	95	192Os	KED	99	99	98
68Zn	KED	93	96	94	193Ir	KED	99	99	97
75As	H_2 DRC	97	98	97	194Pt	KED	98	99	98
75As	KED	100	98	94	195Pt	KED	97	98	95
77Se	H_2 DRC	101	97	97	196Pt	KED	98	99	97
78Se	H_2 DRC	96	96	96	197Au	KED	96	98	95
95Mo	KED	98	99	98	199Hg	KED	100	101	97
97Mo	KED	97	99	98	200Hg	KED	93	100	93
98Mo	KED	97	98	98	202Hg	KED	97	98	97
99Ru	KED	98	100	99	203Tl	KED	101	100	98
101Ru	KED	95	100	101	205Tl	KED	99	100	98
103Rh	KED	101	99	100	206Pb	KED	100	100	98
105Pd	KED	102	103	100	207Pb	KED	98	100	98
106Pd	KED	98	103	100	208Pb	KED	100	100	97
107Ag	KED	100	101	101	209Bi	KED	99	100	97

All elements met 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	431	4520708	108Pd	8	13041
24Mg	8	2341	109Ag	3	22105
39K	517	10545	111Cd	1	38
44Ca	11	390	113Cd	5	375
51V	60	2688	118Sn	65	272194
52Cr	17	18187	119Sn	26	103721
53Cr	24	2287	120Sn	98	418163
55Mn	4	14386	121Sb	13	35031
56Fe	1402	80258	123Sb	-33	28899
57Fe	18	654	135Ba	8	108051
58Ni	15	18965	136Ba	22	135243
59Co	13	6476	137Ba	8	203661
60Ni	6	7952	138Ba	20	1383726
62Ni	1	1262	188Os	13	16257
63Cu	64	55466	189Os	19	20425
65Cu	57	28029	190Os	22	33166
66Zn	45	3590	191Ir	17	49379
67Zn	15	1053	192Os	32	54084
68Zn	12	4255	193Ir	26	84694
75As (DRC)	83	1883	194Pt	8	28381
75As (KED)	18	1613	195Pt	13	29630
77Se	43	5772	196Pt	9	22177
78Se	8	19388	197Au	35	428782
95Mo	7	65199	199Hg	1	951
97Mo	4	43204	200Hg	2	1319
98Mo	6	116544	202Hg	1	1698
99Ru	4	5248	203Tl	59	23466
101Ru	2	7854	205Tl	4	57599
103Rh	13	48147	206Pb	14	11183
105Pd	40	9637	207Pb	13	9699
106Pd	2	12319	208Pb	38	24273
107Ag	4	21288	209Bi	1	339159

7.12. Precision

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

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
(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	22414	5	108Pd	KED	1034	2
24Mg	KED	4926	3	109Ag	KED	1031	1
39K	KED	50805	1	111Cd	KED	191	7
44Ca	KED	50373	5	113Cd	KED	196	3
51V	KED	992	1	118Sn	KED	59353	2
52Cr	KED	5013	1	119Sn	KED	59747	1
53Cr	KED	4950	4	120Sn	KED	59111	1
55Mn	KED	5009	1	121Sb	KED	8910	1
56Fe	H_2 DRC	4945	2	123Sb	KED	8877	1
57Fe	KED	5045	5	135Ba	KED	69580	2
58Ni	KED	2015	2	136Ba	KED	68923	2
59Co	KED	510	1	137Ba	KED	68740	1
60Ni	KED	1990	1	138Ba	KED	69098	2
62Ni	KED	2031	5	188Os	KED	974	2
63Cu	KED	5038	1	189Os	KED	995	1
65Cu	KED	4986	2	190Os	KED	989	2
66Zn	KED	4943	3	191Ir	KED	990	2
67Zn	KED	5257	4	192Os	KED	990	1
68Zn	KED	4882	2	193Ir	KED	987	2
75As	H_2 DRC	1522	5	194Pt	KED	992	1
75As	KED	1463	2	195Pt	KED	981	2
77Se	H_2 DRC	7784	1	196Pt	KED	988	2
78Se	H_2 DRC	7747	1	197Au	KED	9733	2
95Mo	KED	14822	2	199Hg	KED	299	3
97Mo	KED	14958	2	200Hg	KED	297	3
98Mo	KED	14827	2	202Hg	KED	293	3
99Ru	KED	995	1	203Tl	KED	794	1
101Ru	KED	1000	3	205Tl	KED	797	1
103Rh	KED	992	1	206Pb	KED	496	2
105Pd	KED	1016	3	207Pb	KED	498	1
106Pd	KED	1027	0	208Pb	KED	499	1
107Ag	KED	1020	1	209Bi	KED	4964	2

All analytes met 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 Table 22.

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 22: RUGGEDNESS RESULTS FOR HEPES
(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	22346	4	108Pd	KED	1023	2
24Mg	KED	4984	3	109Ag	KED	1013	2
39K	KED	50284	2	111Cd	KED	199	9
44Ca	KED	49853	5	113Cd	KED	202	4
51V	KED	977	2	118Sn	KED	60372	2
52Cr	KED	4948	2	119Sn	KED	61022	3
53Cr	KED	4864	4	120Sn	KED	60461	3
55Mn	KED	5012	2	121Sb	KED	9069	2
56Fe	H_2 DRC	5020	2	123Sb	KED	9079	3
57Fe	KED	4956	5	135Ba	KED	70302	2
58Ni	KED	2000	2	136Ba	KED	69503	2
59Co	KED	513	2	137Ba	KED	70169	2
60Ni	KED	2003	2	138Ba	KED	69713	2
62Ni	KED	2033	4	188Os	KED	991	2
63Cu	KED	5007	2	189Os	KED	997	1
65Cu	KED	4990	2	190Os	KED	994	1
66Zn	KED	4919	3	191Ir	KED	996	2
67Zn	KED	5035	6	192Os	KED	999	2
68Zn	KED	4899	3	193Ir	KED	993	2
75As	H_2 DRC	1514	5	194Pt	KED	993	1
75As	KED	1497	4	195Pt	KED	983	2
77Se	H_2 DRC	7972	3	196Pt	KED	995	1
78Se	H_2 DRC	7870	2	197Au	KED	9783	1
95Mo	KED	14939	2	199Hg	KED	299	2
97Mo	KED	15059	2	200Hg	KED	304	3
98Mo	KED	14919	2	202Hg	KED	291	3
99Ru	KED	1010	2	203Tl	KED	803	2
101Ru	KED	1013	3	205Tl	KED	803	1
103Rh	KED	1018	3	206Pb	KED	500	2
105Pd	KED	1012	2	207Pb	KED	500	2
106Pd	KED	1031	2	208Pb	KED	503	1
107Ag	KED	1016	2	209Bi	KED	5007	2

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 18 above. Results are reported in Table 23 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 23: LIMIT OF QUANTITATION RESULTS FOR HEPES

(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	90	88	108Pd	KED	98	101
24Mg	KED	101	100	109Ag	KED	96	97
39K	KED	94	100	111Cd	KED	93	107
44Ca	KED	112	110	113Cd	KED	101	97
51V	KED	87	97	118Sn	KED	100	102
52Cr	KED	102	102	119Sn	KED	99	101
53Cr	KED	97	100	120Sn	KED	98	100
55Mn	KED	101	97	121Sb	KED	98	100
56Fe	H_2 DRC	158	136	123Sb	KED	99	100
57Fe	KED	171	133	135Ba	KED	99	100
58Ni	KED	101	98	136Ba	KED	99	100
59Co	KED	98	98	137Ba	KED	100	100
60Ni	KED	102	100	138Ba	KED	99	99
62Ni	KED	87	105	188Os	KED	98	99
63Cu	KED	99	97	189Os	KED	99	100
65Cu	KED	99	98	190Os	KED	99	99
66Zn	KED	81	88	191Ir	KED	100	99
67Zn	KED	73	85	192Os	KED	101	99
68Zn	KED	86	91	193Ir	KED	99	100
75As	H_2 DRC	104	94	194Pt	KED	99	98
75As	KED	88	98	195Pt	KED	95	98
77Se	H_2 DRC	101	103	196Pt	KED	101	99
78Se	H_2 DRC	95	98	197Au	KED	97	98
95Mo	KED	98	99	199Hg	KED	94	96
97Mo	KED	101	100	200Hg	KED	98	96
98Mo	KED	98	99	202Hg	KED	108	101
99Ru	KED	95	97	203Tl	KED	100	102
101Ru	KED	101	100	205Tl	KED	100	101
103Rh	KED	99	103	206Pb	KED	102	102
105Pd	KED	99	102	207Pb	KED	103	101
106Pd	KED	97	100	208Pb	KED	100	101
107Ag	KED	98	102	209Bi	KED	99	100

Both isotopes for Fe fail the 0.1J level LOQ, but pass at the 0.3J level. All other analytes met LOQ acceptance criteria of 70% - 150% at 0.1J and 0.3J levels.

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. 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 24.

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 24: SAMPLE AND STANDARD STABILITY (% RECOVERY)

Isotope	0.5J Std (%)	2.0J Std (%)	1.0J Spike (%)	Isotope	0.5J Std (%)	2.0J Std (%)	1.0J Spike (%)
7Li	107	103	120	108Pd	101	104	101
24Mg	107	101	98	109Ag	102	105	99
39K	110	105	102	111Cd	187	113	117
44Ca	125	102	101	113Cd	163	103	107
51V	100	100	99	118Sn	97	103	101
52Cr	102	102	98	119Sn	98	103	100
53Cr	98	101	101	120Sn	98	101	101
55Mn	107	100	97	121Sb	96	98	96
56Fe	105	100	102	123Sb	95	99	96
57Fe	116	102	94	135Ba	96	95	95
58Ni	103	99	91	136Ba	97	96	97
59Co	107	101	92	137Ba	99	95	96
60Ni	104	99	94	138Ba	100	97	96
62Ni	105	102	92	188Os	103	97	100
63Cu	104	99	95	189Os	99	99	101
65Cu	104	99	95	190Os	102	98	100
66Zn	99	94	89	191Ir	99	99	98
67Zn	105	91	86	192Os	101	97	100
68Zn	101	94	94	193Ir	102	97	99
75As	102	95	102	194Pt	99	96	98
75As	98	90	87	195Pt	103	97	97
77Se	94	98	97	196Pt	101	97	98
78Se	95	95	97	197Au	96	95	96
95Mo	98	96	96	199Hg	122	97	99
97Mo	99	97	99	200Hg	122	92	96
98Mo	101	95	98	202Hg	110	94	101
99Ru	110	98	101	203Tl	101	98	100
101Ru	105	98	95	205Tl	100	98	99
103Rh	103	105	106	206Pb	104	98	100
105Pd	111	104	99	207Pb	92	98	98
106Pd	107	107	102	208Pb	103	98	98
107Ag	102	104	104	209Bi	98	97	98

Multiple elements failed standard stability at the 0.5J level standard. All analytes met Sample Solution Stability acceptance criteria of 80% - 120%.

8. DEVIATIONS:

8.1. Both isotopes for iron, ^{56}Fe and ^{57}Fe, failed the Limit of Quantitation study at 0.1J level. Since both isotopes were within acceptance criteria for 0.3J level, the LOQ will be set at 0.3J for iron in the final analytical test method, while every other element will have a LOQ at 0.1J level.

8.2. Solution stability failed for 0.5J standard for multiple elements. Solution stability will be noted to be prepared as fresh for standards in the final method. Sample stability passed acceptance criteria and will be noted at stable for 24 hours.

9. CONCLUSION:

9.1. The test method for Elemental Impurities in HEPES 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 85% to 113%.

9.1.3. Sensitive: LOQ recoveries were within 73% to 136% for the analytes that met acceptance criteria with the exception of iron at the 0.1J level. All other analytes met acceptance criteria established at 0.1J level.

9.1.4. Accurate: From 50% (0.5J) to 150% (1.5J) of working standard concentration level with mean percent recoveries ranging from 87% to 108%. 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 0% to 7%.

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 1% to 9%.

9.1.7. Stable: With respect to stability of solutions, the sample solutions for HEPES are shown to be stable for 24 hours for all elements analyzed using this protocol. The working standard preparations did not meet acceptance criteria for the low standard, thus the standards will be noted to be prepared as fresh in the final method.

10. NOTEBOOK REFERENCE:

TABLE 25: NOTEBOOK REFERENCE

STUDY		NOTEBOOK REFERENCE
Specificity		EIV-8/ pages 16-18, 26
Linearity and Range		EIV-8/ pages 7-10, 15
LOQ by "Spiked" recovery		EIV-8/ pages 16-18, 24
Accuracy/ Precision by "Spiked" recovery		EIV-8/ pages 11-14, 16-18, 23
Intermediate Precision (Ruggedness)		EIV-8/ pages 19-22
Sample Solution Stability	Day-0	EIV-8/ pages 11-13
	Day-1	EIV-8/ pages 16-18, 25
Standard Solution Stability	Day-0	EIV-8/ pages 11-13
	Day-1	EIV-8/ pages 16-18, 25

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