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

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1. PURPOSE:

1.1. The purpose of this validation report is to establish documented evidence that the test protocol, BSI-PRL-0521 v. 1.0, for Elemental Impurities in Dextran Sulfate performed 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 Dextran Sulfate (molecular weight 8000) 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 protocol validation for elemental impurities in Dextran Sulfate, by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) performed at BioSpectra Inc.

3. REFERENCES:

3.1. BSI-SOP-0303, NexION 350X ICP-MS SOP.

3.2. BSI-SOP-0304, NexION 350X ICP-MS Care and Maintenance SOP.

3.3. BSI-SOP-0426, Operation and Maintenance of CEM Mars 6 Digestion Microwave SOP.

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

3.5. BSI-PRL-0521, Dextran Sulfate Analytical Method Validation Protocol.

3.6. ICH Guideline for Elemental Impurities Q3D.

3.7. USP <730> Plasma Spectrochemistry.

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

3.9. NexION Operation with Syngistix Software Guide.

3.10. USP <232>, <233>

4. BACKGROUND:

4.1. This validation was executed using a parenteral PDE 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 DEXTRAN SULFATE (10 GRAM/DAY EXPOSURE)						
Elements	**ICH Class**	**Parenteral PDE Limits (µg/day)**	**0.3J LOQ (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	0.45	0.75	1.5	2.25
Cd	1	2.0	0.06	0.10	0.20	0.30
Hg	1	3.0	0.09	0.15	0.30	0.45
Pb	1	5.0	0.15	0.25	0.50	0.75
Co	2A	5.0	0.15	0.25	0.50	0.75
Ni	2A	20	0.60	1.0	2.0	3.0
V	2A	10	0.30	0.50	1.0	1.5
Tl	2B	8.0	0.24	0.40	0.80	1.2
Se	2B	80	2.4	4.0	8.0	12
Ag	2B	10	0.30	0.50	1.0	1.5
Au	2B	100	3.0	5.0	10	15
Pd	2B	10	0.30	0.50	1.0	1.5
Ir	2B	10	0.30	0.50	1.0	1.5
Os	2B	10	0.30	0.50	1.0	1.5
Pt	2B	10	0.30	0.50	1.0	1.5
Rh	2B	10	0.30	0.50	1.0	1.5
Ru	2B	10	0.30	0.50	1.0	1.5
Ba	3	700	21	35	70	105
Sb	3	90	2.7	4.5	9.0	13.5
Li	3	250	7.5	12.5	25	37.5
Mo	3	1500	45	75	150	225
Cu	3	300	9.0	15	30	45
Sn	3	600	18	30	60	90
Cr	3	1100	33	55	110	165
Fe	4	*150	4.5	7.5	15	22.5

*PDE 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	04/2022, 10/2022[1]
Automatic Pipette	Rainin	E4-XLS (20-200 µL)	C016314640	06/30/22
Automatic Pipette	Rainin	E4-XLS (100-1000 µL)	C016314969	06/30/22
Automatic Pipette	Rainin	E4-XLS (0.5-5 mL)	C023506909	06/30/22
ICP-MS	Perkin Elmer	NexION 350X	85VN5093001	01/2023
Deionized water system	Millipore	IQ-7005/ Element POD	F9SA14284H	06/2022
Digestion Microwave	CEM	Mars 6	MY2255	09/22/22

[1]Semi-annual calibration performed during validation.

TABLE 3: REAGENTS					
Type	**Grade**	**Supplier**	**Catalog Number**	**Lot Number**	**Expiration**
70% Nitric Acid	Trace Metal	VWR	87003-261	1121090	09/29/23
36% Hydrochloric Acid	Trace Metal	VWR	87003-253	4120110	12/23/23
Sulfuric Acid	Trace Metal	Fisher	A510-P212	3120012	04/13/24
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	37-147GSX1	03/30/23
ICP-MS KED Setup Solution	N/A	Perkin Elmer	N8145052	36-191GST1	08/30/22
SiliaPrep SPE Filter	Silica-Based AMPA	Silicycle	R85130B	175959	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

TABLE 4: REFERENCE STANDARDS				
Identification	**Manufacturer**	**Lot Number**	**Expiration**	**Concentrations / Elements**
Pharma-CAL Standard Parenteral STD# 1 IA 140-131-201	SCP Science	S210429002	05/2022	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	S210331019	07/2022	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	25-59FEY1	08/30/22	Fe (1,000 µg/mL)
Pharma-CAL Custom Standard AQ0-086-125 (Internal Standard)	SCP Science	S210104029	01/2022	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 Fe single source 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	0.150			15

* 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 5.33% HNO_3, 2.67% 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. Intermediate standards were not allowed to contact concentrated acids while preparing solutions.

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	4.0	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					15

6.7. 1.5J Calibration Standard Preparation

6.7.1. Prepared a solution containing the elements listed in Table 7 below in 5.33% HNO_3, 2.67% 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. Intermediate standards were not allowed to contact concentrated acids while preparing solutions.

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	4.0	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					45

6.8. 2.0J Calibration Standard Preparation

6.8.1. Prepared a solution containing the elements listed in Table 8 below in 5.33% HNO_3, 2.67% 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. Intermediate standards were not allowed to contact concentrated acids while preparing solutions.

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	4.0	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					60

6.9. **Calibration Blank**

6.9.1. Prepared a solution containing 5.33% HNO_3, 2.67% 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	4.0	1.0	50

6.10. **Method Blank Preparation**

6.10.1. Added 4.0 mL of Acid Digestion Mixture into a clean 20 mL digestion vessel, placed a plug on the vessel, and properly torqued the vessel cap. Placed vessel in the microwave carousel then digested and completed preparation according to Section 6.12 below.

6.11. **Sample Preparation**

6.11.1. Weighed approximately 100 mg of the sample into a clean 20 mL digestion vessel and added 4.0 mL of Acid Digestion Mixture.

6.11.2. Properly torqued vessel cap and placed in microwave carousel. Digested and completed preparation according to Section 6.12 below.

6.12. **Microwave Digestion Procedure**

6.12.1. Refer to BSI-SOP-0426 for general usage guidelines of the Mars 6 Microwave Digestion System.

6.12.2. Prepared at least one method blank per digestion run. Method blank was prepared in the same manner as the sample without the addition of the sample (see above).

6.12.3. Digested the sample using the program listed in Table 10.

TABLE 10: TEMPERATURE CONTROLLED MICROWAVE DIGESTION PROGRAM				
Power (Watts)	**Percent Power**	**Ramp (Minutes)**	**Temperature (°C)**	**Hold (Minutes)**
1800	100	15:00	150	10:00
1800	100	6:00	175	15:00

6.12.4. After digestion, placed the vessels into an ice bath and allowed the vessels to cool for approximately 40 minutes. Before opening, the vessels were turned sideways and slowly rotated in order to collect the condensation on the inside of the vessel walls.

6.12.5. Quantitatively transferred the vessel contents into a 50 mL Digitube® containing approximately 5 mL of deionized water and 1.0 mL of Internal Standard/Complexing Solution. Rinsed the bottom of the plug into the 50 mL Digitube® using deionized water.

6.12.6. Extracted any remaining volatile elements by adding 15 mL of a pre-mixed solution of 0.500 mL of 2% Thiourea diluted to 15 mL using deionized water to the digestion vessel. Added this directly to the 50 mL Digitube®.

6.12.7. Rinsed the vessel an additional two times using deionized water and transferred each rinse to the 50 mL Digitube®. Diluted to a final volume of 50 mL using deionized water and mixed well.

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:

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

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

6.13.3. 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 check prior to beginning the analytical sequence. Refer to NexION 350X ICP-MS SOP DCN 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 to be performed a minimum of once every 10 samples and at the end of the analytical run.

7.6. Bracketing standard checks were to recover ± 20% of the calculated theoretical concentrations for multi-element analysis. Additionally, the drift (calculated as absolute difference) between the bracketing standard checks were 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 poly atomic 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	15-100	113Cd	125Te	KED	0.12-0.80
51V	45Sc	KED	0.60-4.0	118Sn	125Te	KED	36-240
52Cr	45Sc	KED	66-440	119Sn	125Te	KED	36-240
53Cr	45Sc	KED	66-440	120Sn	125Te	KED	36-240
56Fe	72Ge	H_2 DRC	9.0-60	121Sb	125Te	KED	5.4-36
57Fe	72Ge	KED	9.0-60	123Sb	125Te	KED	5.4-36
58Ni	72Ge	KED	1.2-8.0	135Ba	159Tb	KED	42-280
59Co	72Ge	KED	0.30-2.0	137Ba	159Tb	KED	42-280
60Ni	72Ge	KED	1.2-8.0	138Ba	159Tb	KED	42-280
62Ni	72Ge	KED	1.2-8.0	188Os	209Bi	KED	0.60-4.0
63Cu	72Ge	KED	18-120	189Os	209Bi	KED	0.60-4.0
65Cu	72Ge	KED	18-120	190Os	209Bi	KED	0.60-4.0
75As	72Ge	H_2 DRC	0.90-6.0	191Ir	209Bi	KED	0.60-4.0
75As	72Ge	KED	0.90-6.0	192Os	209Bi	KED	0.60-4.0
77Se	89Y	H_2 DRC	4.8-32	193Ir	209Bi	KED	0.60-4.0
78Se	89Y	H_2 DRC	4.8-32	194Pt	185Re	KED	0.60-4.0
95Mo	89Y	KED	90-600	195Pt	185Re	KED	0.60-4.0
97Mo	89Y	KED	90-600	196Pt	185Re	KED	0.60-4.0
98Mo	89Y	KED	90-600	197Au	209Bi	KED	6.0-40
99Ru	125Te	KED	0.60-4.0	199Hg	185Re	KED	0.18-1.2
101Ru	125Te	KED	0.60-4.0	200Hg	185Re	KED	0.18-1.2
103Rh	125Te	KED	0.60-4.0	202Hg	185Re	KED	0.18-1.2
105Pd	125Te	KED	0.60-4.0	203Tl	209Bi	KED	0.48-3.2
106Pd	125Te	KED	0.60-4.0	205Tl	209Bi	KED	0.48-3.2
107Ag	125Te	KED	0.60-4.0	206Pb	209Bi	KED	0.30-2.0
108Pd	125Te	KED	0.60-4.0	207Pb	209Bi	KED	0.30-2.0
109Ag	125Te	KED	0.60-4.0	208Pb	209Bi	KED	0.30-2.0
111Cd	125Te	KED	0.12-0.80				

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: (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.

7.9.1.1. Acceptance Criteria:

7.9.1.1.1. 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%.

TABLE 14: LINEARITY STANDARD PREPARATION

Description	Intermediate Standard (mL)	Acid Mix (mL)	Internal Standard/ Complexing Solution (mL)	Final Volume (mL)
Cal Blank Reference	N/A	4.0	1.0	50
0.3J Standard	0.030	4.0	1.0	50
0.5J Standard	0.050	4.0	1.0	50
1.0J Standard	0.100	4.0	1.0	50
1.5J Standard	0.150	4.0	1.0	50
2.0J Standard	0.200	4.0	1.0	50

TABLE 15: LINEARITY STANDARD CONCENTRATIONS					
Element	**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	0.90	1.5	3.0	4.5	6.0
Cd	0.12	0.20	0.40	0.60	0.80
Hg	0.18	0.30	0.60	0.90	1.2
Pb	0.30	0.50	1.0	1.5	2.0
Co	0.30	0.50	1.0	1.5	2.0
Ni	1.2	2.0	4.0	6.0	8.0
V	0.60	1.0	2.0	3.0	4.0
Tl	0.48	0.80	1.6	2.4	3.2
Se	4.8	8.0	16	24	32
Ag	0.60	1.0	2.0	3.0	4.0
Au	6.0	10	20	30	40
Pd	0.60	1.0	2.0	3.0	4.0
Ir	0.60	1.0	2.0	3.0	4.0
Os	0.60	1.0	2.0	3.0	4.0
Pt	0.60	1.0	2.0	3.0	4.0
Rh	0.60	1.0	2.0	3.0	4.0
Ru	0.60	1.0	2.0	3.0	4.0
Ba	42	70	140	210	280
Sb	5.4	9.0	18	27	36
Li	15	25	50	75	100
Mo	90	150	300	450	600
Cu	18	30	60	90	120
Sn	36	60	120	180	240
Cr	66	110	220	330	440
Fe	9.0	15	30	45	60

TABLE 16: LINEARITY PERCENT RECOVERY RESULTS													
Isotope	**Mode**	**0.3J Mean**	**0.5J Mean**	**1.0J Mean**	**1.5J Mean**	**2.0J Mean**	**Isotope**	**Mode**	**0.3J Mean**	**0.5J Mean**	**1.0J Mean**	**1.5J Mean**	**2.0J Mean**
7Li	STD	102	112	111	101	103	113Cd	KED	107	100	100	103	104
51V	KED	97	101	99	96	97	118Sn	KED	102	99	100	104	104
52Cr	KED	99	99	98	96	97	119Sn	KED	100	99	99	104	104
53Cr	KED	100	100	99	97	97	120Sn	KED	100	98	100	103	104
56Fe	H_2 DRC	102	104	102	103	102	121Sb	KED	100	95	95	96	94
57Fe	KED	100	101	99	104	104	123Sb	KED	99	94	94	95	94
58Ni	KED	104	104	106	107	110	135Ba	KED	96	95	98	95	98
59Co	KED	102	105	108	110	114	137Ba	KED	95	95	96	95	96
60Ni	KED	103	102	106	107	109	138Ba	KED	95	94	96	95	96
62Ni	KED	105	106	105	112	113	188Os	KED	100	103	105	105	107
63Cu	KED	102	103	104	107	109	189Os	KED	100	102	104	106	104
65Cu	KED	103	104	107	109	112	190Os	KED	103	104	105	106	107
75As	H_2 DRC	102	91	89	87	82	191Ir	KED	99	100	103	103	104
75As	KED	101	96	94	96	90	192Os	KED	100	101	105	103	106
77Se	H_2 DRC	98	94	93	92	88	193Ir	KED	100	102	104	105	106
78Se	H_2 DRC	98	92	93	91	87	194Pt	KED	101	98	100	100	99
95Mo	KED	102	99	101	99	98	195Pt	KED	103	99	100	100	99
97Mo	KED	103	102	102	101	100	196Pt	KED	101	98	99	98	97
98Mo	KED	103	101	101	100	99	197Au	KED	102	99	99	98	96
99Ru	KED	101	102	105	109	114	199Hg	KED	101	94	94	91	92
101Ru	KED	104	105	106	113	115	200Hg	KED	98	95	96	94	92
103Rh	KED	103	104	107	112	113	202Hg	KED	95	95	87	94	89
105Pd	KED	100	103	105	108	110	203Tl	KED	98	100	102	102	104
106Pd	KED	103	104	105	109	112	205Tl	KED	100	100	102	102	104
107Ag	KED	104	103	104	111	114	206Pb	KED	99	107	100	101	103
108Pd	KED	108	104	106	111	113	207Pb	KED	100	107	102	102	103
109Ag	KED	105	104	107	112	115	208Pb	KED	101	108	103	102	103
111Cd	KED	109	98	100	103	111							

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

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.

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

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. Prepare as per section 6.11.

7.10.3. Spike Recovery Sample Preparation

7.10.3.1. Weighed 100 mg of sample into a 20 mL digestion vessel.

7.10.3.2. Pipetted appropriate intermediate standard spike amount as per Table 17 on top of the solid. All intermediate standards spikes were added prior to acid addition.

7.10.3.3. Pipetted 4.0 mL of Acid Mixture, placed plug on the vessel, properly torqued cap, and placed vessel in microwave carousel. Digested according to Section 6.12

7.10.3.4. After digestion, vessels were placed into ice bath for approximately 40 minutes. Before opening vessel cap, the vessels were turned sideways and slowly rotated to collect condensation on the inside of vessel walls.

7.10.3.5. Quantitatively transferred the contents into a 50 mL Digitube® containing approximately 5 mL of deionized water and 1.0 mL of Internal Standard/Complexing Solution.

7.10.3.6. Extracted any remaining volatile elements by adding 15 mL of pre-mixed solution of 0.500 mL of 2% Thiourea diluted to 15 mL using deionized water. Transferred to the 50 mL Digitube® after swirling in the vessel.

7.10.3.7. Rinsed the vessel an additional two times with deionized water and transferred each rinse to the Digitube®. Diluted to final volume of 50 mL and mixed well.

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	4.0	1.0	50
Unspiked	100	N/A	4.0	1.0	50
0.3J Spiked Sample	100	0.030	4.0	1.0	50
0.5J Spiked Sample	100	0.050	4.0	1.0	50
1.0J Spiked Sample	100	0.100	4.0	1.0	50
1.5J Spiked Sample	100	0.150	4.0	1.0	50

TABLE 18: ACCURACY RESULTS FOR DEXTRAN SULFATE
(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	91	95	95	113Cd	KED	89	95	93
51V	KED	91	99	95	118Sn	KED	91	95	92
52Cr	KED	83	91	87	119Sn	KED	92	97	93
53Cr	KED	83	91	88	120Sn	KED	91	96	93
56Fe[1]	H_2 DRC	63	73	67	121Sb	KED	95	99	97
57Fe	KED	92	104	99	123Sb	KED	95	99	96
58Ni	KED	81	93	90	135Ba	KED	111	103	107
59Co	KED	89	100	96	137Ba	KED	109	104	105
60Ni	KED	80	94	89	138Ba	KED	110	102	104
62Ni	KED	74	91	90	188Os	KED	97	100	98
63Cu	KED	84	91	88	189Os	KED	97	100	99
65Cu	KED	82	91	87	190Os	KED	96	100	98
75As	H_2 DRC	101	100	100	191Ir	KED	109	114	111
75As	KED	94	101	96	192Os	KED	97	99	99
77Se	H_2 DRC	99	95	95	193Ir	KED	109	114	112
78Se	H_2 DRC	96	95	95	194Pt	KED	90	92	90
95Mo	KED	90	95	92	195Pt	KED	89	91	91
97Mo	KED	90	95	91	196Pt	KED	91	91	90
98Mo	KED	89	94	91	197Au	KED	110	110	110
99Ru	KED	96	96	97	199Hg	KED	114	105	119
101Ru	KED	93	98	97	200Hg	KED	112	107	116
103Rh	KED	89	94	93	202Hg	KED	112	102	115
105Pd	KED	85	87	85	203Tl	KED	103	106	103
106Pd	KED	81	85	83	205Tl	KED	104	106	105
107Ag	KED	79	79	78	206Pb	KED	99	102	100
108Pd	KED	81	85	83	207Pb	KED	100	102	101
109Ag	KED	75	80	78	208Pb	KED	100	100	100
111Cd	KED	95	88	92					

[1]56Fe failed to meet acceptance criteria for accuracy and system suitability for bracketing check standard recovery. All other elements met accuracy acceptance criteria of 70% - 150%.

7.11. **Specificity**

7.11.1. Specificity was demonstrated by using a method blank and a calibration blank for ICP-MS analysis. A method blank was prepared as per the analytical protocol. The method blank was compared against the calibration blank and high standard for any matrix interference.

7.11.2. The solutions were analyzed as per the analytical method and the counts for the calibration blank, method blank, and high calibration standard are reported 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 19: SPECIFICITY RESULTS

Isotope	Blank (CPS)	Method Blank (CPS)	2.0J STD (CPS)	Isotope	Blank (CPS)	Method Blank (CPS)	2.0J STD (CPS)
7Li	2040	3402	3894700	113Cd	9	8	482
51V	113	103	4696	118Sn	45	74	339919
52Cr	43	254	686245	119Sn	12	37	126018
53Cr	49	64	86914	120Sn	49	92	492930
56Fe	7372	29063	1862217	121Sb	8	9	44232
57Fe	16	51	3270	123Sb	-51	-11	35669
58Ni	36	921	30345	135Ba	28	40	98136
59Co	6	33	11119	137Ba	39	72	180519
60Ni	23	456	13448	138Ba	396	555	1186140
62Ni	8	78	2197	188Os	20	17	13378
63Cu	75	175	525776	189Os	24	17	16375
65Cu	47	114	248778	190Os	24	25	26909
75As	70	88	11314	191Ir	63	171	41476
75As	23	21	2348	192Os	55	54	42593
77Se	66	213	20718	193Ir	94	281	70517
78Se	68	546	67263	194Pt	18	3	25053
95Mo	13	65	874359	195Pt	14	7	25728
97Mo	12	51	550050	196Pt	17	3	19408
98Mo	16	100	1409735	197Au	124	89	377038
99Ru	8	17	6322	199Hg	14	9	889
101Ru	18	9	8818	200Hg	18	10	1250
103Rh	23	29	51487	202Hg	9	5	662
105Pd	60	59	10218	203Tl	91	74	22536
106Pd	11	10	13316	205Tl	29	37	55985
107Ag	25	21	22059	206Pb	256	339	11397
108Pd	14	8	13592	207Pb	224	305	9968
109Ag	32	8	22632	208Pb	508	720	25018
111Cd	8	9	438				

7.12. **Precision**

7.12.1. Repeatability

7.12.2. All solutions for the Repeatability test were prepared by a single analyst.

7.12.3. 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.

7.12.3.1. 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 20: PRECISION RESULTS FOR DEXTRAN SULFATE
(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)	% RSDN=6
7Li	STD	22942	4	113Cd	KED	187	5
51V	KED	1088	3	118Sn	KED	55173	4
52Cr	KED	98118	3	119Sn	KED	55967	5
53Cr	KED	97839	4	120Sn	KED	55573	5
56Fe[1]	H_2 DRC	11961	5	121Sb	KED	8649	4
57Fe	KED	17382	4	123Sb	KED	8645	5
58Ni	KED	1937	6	135Ba	KED	72211	2
59Co	KED	487	5	137Ba	KED	72454	2
60Ni	KED	1946	6	138Ba	KED	71455	2
62Ni	KED	1927	6	188Os	KED	977	3
63Cu	KED	26821	4	189Os	KED	995	2
65Cu	KED	26624	4	190Os	KED	989	2
75As	H_2 DRC	1497	2	191Ir	KED	1113	3
75As	KED	1497	4	192Os	KED	974	2
77Se	H_2 DRC	7645	1	193Ir	KED	1126	2
78Se	H_2 DRC	7630	1	194Pt	KED	897	2
95Mo	KED	138346	3	195Pt	KED	899	2
97Mo	KED	139012	3	196Pt	KED	900	2
98Mo	KED	137288	3	197Au	KED	10807	2
99Ru	KED	941	4	199Hg	KED	329	4
101Ru	KED	948	5	200Hg	KED	331	4
103Rh	KED	914	3	202Hg	KED	319	4
105Pd	KED	831	6	203Tl	KED	827	3
106Pd	KED	820	4	205Tl	KED	836	2
107Ag	KED	772	4	206Pb	KED	507	3
108Pd	KED	820	4	207Pb	KED	506	3
109Ag	KED	773	4	208Pb	KED	502	2
111Cd	KED	177	6				

[1]56Fe failed to meet system suitability for bracketing check standard recovery. All elements 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").

7.13.1.1. Acceptance Criteria:

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

TABLE 21: RUGGEDNESS RESULTS FOR DEXTRAN SULFATE
(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	23209	4	113Cd	KED	187	4
51V	KED	1112	3	118Sn	KED	56763	4
52Cr	KED	100117	3	119Sn	KED	56828	4
53Cr	KED	99935	4	120Sn	KED	56812	4
56Fe	H_2 DRC	12550	7	121Sb	KED	8981	5
57Fe	KED	17740	5	123Sb	KED	8990	5
58Ni	KED	2007	6	135Ba	KED	74067	4
59Co	KED	506	5	137Ba	KED	74517	4
60Ni	KED	2017	5	138Ba	KED	73205	3
62Ni	KED	2019	6	188Os	KED	1015	4
63Cu	KED	27572	4	189Os	KED	1005	2
65Cu	KED	27294	4	190Os	KED	1010	3
75As	H_2 DRC	1522	3	191Ir	KED	1156	4
75As	KED	1584	7	192Os	KED	1004	4
77Se	H_2 DRC	7994	5	193Ir	KED	1154	3
78Se	H_2 DRC	7936	4	194Pt	KED	945	6
95Mo	KED	144304	5	195Pt	KED	947	6
97Mo	KED	144196	5	196Pt	KED	946	5
98Mo	KED	142524	5	197Au	KED	11347	5
99Ru	KED	977	5	199Hg	KED	342	6
101Ru	KED	981	6	200Hg	KED	345	6
103Rh	KED	929	3	202Hg	KED	347	9
105Pd	KED	849	5	203Tl	KED	849	4
106Pd	KED	843	4	205Tl	KED	854	3
107Ag	KED	794	4	206Pb	KED	519	3
108Pd	KED	842	4	207Pb	KED	522	4
109Ag	KED	787	5	208Pb	KED	520	4
111Cd	KED	181	6				

All elements met 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 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 0.3J spiked samples in Table 17 above.

7.14.2.1. Acceptance Criteria:

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

TABLE 22: LIMIT OF QUANTITATION RESULTS
(Mean percent recovery of 3 preparations)

Isotope	Mode	0.3J Mean % Recovery	Isotope	Mode	0.3J Mean % Recovery
7Li	STD	92	113Cd	KED	91
51V	KED	90	118Sn	KED	93
52Cr	KED	85	119Sn	KED	93
53Cr	KED	84	120Sn	KED	93
56Fe[1]	H_2 DRC	65	121Sb	KED	97
57Fe	KED	98	123Sb	KED	97
58Ni	KED	87	135Ba	KED	111
59Co	KED	92	137Ba	KED	109
60Ni	KED	89	138Ba	KED	110
62Ni	KED	77	188Os	KED	95
63Cu	KED	86	189Os	KED	95
65Cu	KED	87	190Os	KED	98
75As	H_2 DRC	103	191Ir	KED	112
75As	KED	100	192Os	KED	98
77Se	H_2 DRC	96	193Ir	KED	109
78Se	H_2 DRC	95	194Pt	KED	91
95Mo	KED	93	195Pt	KED	91
97Mo	KED	92	196Pt	KED	90
98Mo	KED	91	197Au	KED	110
99Ru	KED	99	199Hg	KED	97
101Ru	KED	95	200Hg	KED	104
103Rh	KED	92	202Hg	KED	101
105Pd	KED	90	203Tl	KED	105
106Pd	KED	82	205Tl	KED	105
107Ag	KED	79	206Pb	KED	98
108Pd	KED	83	207Pb	KED	99
109Ag	KED	77	208Pb	KED	101
111Cd	KED		103		

[1]56Fe failed to meet acceptance criteria for LOQ and system suitability for bracketing check standard recovery. All other elements met 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 (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 experiment was 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).

7.15.2.1. Acceptance Criteria:

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

7.15.2.1.2. 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 23: 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	113	105	96	113Cd	100	99	94
51V	113	112	105	118Sn	101	102	95
52Cr	109	107	102	119Sn	99	102	92
53Cr	110	109	102	120Sn	98	100	92
56Fe	84	91	97	121Sb	105	105	100
57Fe	109	116	111	123Sb	103	103	99
58Ni	110	108	108	135Ba	116	120	118
59Co	104	107	113	137Ba	119	121	116
60Ni	108	108	109	138Ba	116	119	116
62Ni	119	108	118	188Os	110	107	107
63Cu	107	107	111	189Os	112	105	101
65Cu	105	106	112	190Os	110	106	99
75As (DRC)	101	104	112	191Ir	108	107	108
75As (KED)	111	107	134	192Os	107	106	104
77Se	112	112	120	193Ir	104	108	107
78Se	108	111	120	194Pt	118	111	123
95Mo	104	104	111	195Pt	120	109	122
97Mo	103	103	107	196Pt	119	113	121
98Mo	104	101	107	197Au	110	109	120
99Ru	104	107	96	199Hg	102	105	124
101Ru	115	109	98	200Hg	106	107	125
103Rh	109	107	95	202Hg	125	112	144
105Pd	102	105	93	203Tl	108	108	104
106Pd	111	104	97	205Tl	107	107	105
107Ag	105	104	96	206Pb	107	109	105
108Pd	106	104	95	207Pb	108	106	104
109Ag	100	100	93	208Pb	107	108	106
111Cd	112	89	113				

Standard stability fails for 202Hg at the 0.5J standard and for 137Ba at the 2.0J standard. Sample stability fails for multiple isotopes outside of the acceptance criteria of 80% to 120% recovery.

8. DEVIATIONS:

8.1. Linearity study was performed again for 7Li due to check standard failure in the final bracketing standard. This was considered justified as only the system suitability failed and acceptance criteria was met in initial run. Similar concentrations were obtained between two attempts and single element determinations criteria of 10% was met.

8.2. 56Fe results will be invalidated due to final bracketing check standard failure along with LOQ and accuracy acceptance criteria not being met either. This is considered acceptable as 57Fe met all acceptance criteria for the validation of dextran sulfate.

8.3. Standard T=1 stability failed for 137Ba and 202Hg and sample T=1 stability failed for 75As (KED) and all isotopes of Pt and Hg. Standards and samples will be noted to be prepared as fresh in the final test method for elemental impurities.

9. CONCLUSION:

9.1. The test method for Elemental Impurities in Dextran Sulfate 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: 30% to 200% of working standard solution corresponding to 0.3J to 2.0J. Mean percent recovery ranged from 82% to 115%.

9.1.3. Sensitive: LOQ recoveries were within 77% to 112% for dextran sulfate.

9.1.4. Accurate: From 50% (0.5J) to 150% (1.5J) of working standard concentration level with mean percent recoveries ranging from 74% to 119%. All masses analyzed with the exception of 56Fe 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 6%.

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 RSD's ranged from 2% to 7%.

9.1.7. Stable: With respect to stability of solutions, the sample solutions for dextran sulfate are shown to not be stable for 24 hours as 75As (KED), Pt, and Hg did not meet acceptance criteria. The working standard preparations are shown to not be stable for 137Ba and 202Hg. The standards and samples will be noted to be prepared fresh in the final test method for elemental impurities. If iron is the only analyte to be tested, the stability of solutions will be noted that standards and samples are stable for 24 hours.

10. NOTEBOOK REFERENCE:

TABLE 24: NOTEBOOK REFERENCE		
STUDY		**NOTEBOOK REFERENCE**
Specificity		EIV-7/ pages 30-34
Linearity and Range		EIV-7/ pages 22-25
Accuracy/ Precision/LOQ by "Spiked" recovery		EIV-7/ pages 30-39
Intermediate Precision (Ruggedness)		EIV-7/ pages 40-44
Solution Stability	Day-0	EIV-7/ pages 35-39
	Day-1	EIV-7/ pages 40-44