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ANALYTICAL METHOD VALIDATION REPORT: DETERMINATION OF MANGANESE BY INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY (ICP-MS) IN DEXTRAN SULFATE

TABLE OF CONTENTS

1. PURPOSE:

1.1. The purpose of this validation report is to establish documented evidence that the test protocol, BSI-PRL-0565 v. 1.0, for Quantification of Manganese 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 4: Mn

1.2. Dextran Sulfate has been previously validated for full Elemental Impurities Assessment and Total Sulfur Content. Rather than revalidate the full elemental impurities method for inclusion of Manganese, a separate manganese testing method was validated for low detection across a wider range using the same sample preparation as the Elemental Impurities method. Samples can be used interchangeably across the Elemental Impurities method and manganese methods using separate standard preparation.

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 quantification of manganese in Dextran Sulfate, by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) performed at BioSpectra Inc.

3. REFERENCES:

3.1. BSI-PRL-0521, Analytical Method Validation Protocol: Determination of ICH Q3D Elemental Impurities + Iron by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in Dextran Sulfate

3.2. BSI-PRL-0538, Analytical Method Validation Protocol: Sulfur Quantification in Dextran Sulfate by ICP-OES

3.3. BSI-PRL-0565, Analytical Method Validation Protocol: Determination of Manganese by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in Dextran Sulfate

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

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

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

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

3.8. ICH Guideline for Elemental Impurities Q3D

3.9. NexION Operation with Syngistix Software Guide

3.10. USP <232>, <233>

3.11. USP <730> Plasma Spectrochemistry

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

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)							
Element	ICH Class	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	2.0J Target (µg/g) in sample
Mn	4	5.0	0.05	0.15	0.25	0.50	1.0

PDE calculated based on customer specifications.

5.   MATERIALS AND EQUIPMENT:

TABLE 2: EQUIPMENT				
Type	Supplier	Model	Serial Number	Cal. Due
Analytical Balance	Sartorius	MSE224S	36707108	10/2022
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
Digestion Microwave	CEM	Mars 6	MY2255	09/22/22

TABLE 3: REAGENTS					
Type	Grade	Supplier	Catalog Number	Lot Number	Expiration
70% Nitric Acid	Trace Metal	VWR	87003-261	1122020	02/16/24
36% Hydrochloric Acid	Trace Metal	VWR	87003-253	4121050	06/03/24
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**
Manganese Stock Standard N9303783	Perkin Elmer	25-71MNY1	10/30/22	Mn (1,000 µg/mL)
Pharma-CAL Custom Standard AQ0-086-125 (Internal Standard)	SCP Science	S210827010	09/2022	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 (HNO₃): Sulfuric Acid (H₂SO₄)
    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 described in Table 5 using the single source Mn 1,000 μg/mL stock standard. Prepared by adding stock standard to a 50 mL Digitube®. Added DI water to approximately 40 mL then added nitric acid. Diluted to volume using DI Water.

TABLE 5: INTERMEDIATE STANDARD						
Identification	Element	Stock Identification	Amount Added (mL)	Nitric Acid (mL)	Final Volume (mL)	Final Conc. (μg/mL)
Intermediate Standard	Mn	1,000 μg/mL Mn Std	0.025	1.0	50	0.50

6.6. Calibration Standards Preparation

6.6.1. Prepared four standard solutions described in Table 6 below using a 5.33% HNO3, 2.67% H2SO4, 1.0% HCl, and 0.04% (400 μg/mL) Thiourea matrix. 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: CALIBRATION STANDARDS PREPARATION						
Identification	Element	Intermediate Standard (mL)	Acid Mix (mL)	Internal Standard/ Complexing Solution (mL)	Final Volume (mL)	Final Conc. (μg/L)
0.5J Calibration Standard	Mn	0.050	4.0	1.0	50	0.50
1.5J Calibration Standard		0.150				1.5
2.0J Calibration Standard		0.200				2.0
4.0J Calibration Standard		0.400				4.0

6.7. **Calibration Blank**

6.7.1. Prepared a solution containing 5.33% HNO3, 2.67% H2SO4, 1.0% HCl and 0.04% (400 µg/mL) Thiourea as per Table 7 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 7: CALIBRATION BLANK			
Description	**Acid Mix (mL)**	**Internal Standard/ Complexing Solution (mL)**	**Final Volume (mL)**
Cal Blank	4.0	1.0	50

6.8. **Method Blank Preparation**

6.8.1. 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 6.10 below.

6.9. **Sample Preparation**

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

6.9.2. Properly torqued vessel cap and placed in microwave carousel. Digested and completed preparation according to Section 6.10 below.

6.10. **Microwave Digestion Procedure**

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

6.10.2. 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).

6.10.3. Digested the sample using the program listed in Table 8.

TABLE 8: 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	5:00

6.10.4. 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.

6.10.5. 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.

6.10.6. 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®.

6.10.7. 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.

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 $\geq$ 0.99.

7.3. Set up the sequence as per Table 9.

7.4. Confirmed the calibration by analyzing the 1.5J standard after the calibration. The calibration check must recover ±10% of the calculated theoretical concentration 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 ± 10% of the calculated theoretical concentrations for single element analysis. Additionally, the drift (calculated as absolute difference) between the bracketing standard checks were to be NMT 10% for Manganese.

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 9: 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
4.0J Cal Std	Cal Std	Level 5
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%.

TABLE 10: 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
Dilution Gas	Argon
Sample and Skimmer Cone	Platinum
Sample Rinses	Rinse-1: 60 sec at 45 rpm 5.0% HNO3, 2.5% HCl with 0.04% Thiourea (or as applicable to mitigate carry over)

TABLE 11: LINEAR RANGE AND CORRESPONDING TUNE MODE			
Isotope	**Internal Standard**	**Mode**	**Linear Range (µg/L)**
55Mn	72Ge	KED	0.10-4.0

7.9. Linearity and Range

7.9.1. The ICP-MS linearity study included standards equivalent to the concentrations shown in Table 12 and encompassed the following standards: (10%, 30%, 50%, 100%, 150%, 200%, and 400% of the Target Concentration). Each standard was prepared in triplicate and analyzed against the calibration curve prepared from calibration standards described in Section 6.6. 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 12: 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
0.1J Standard	0.010	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
4.0J Standard	0.400	4.0	1.0	50

TABLE 13: 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)**	**4.0J Standard (µg/L)**
Mn	0.10	0.30	0.50	1.0	1.5	2.0	4.0

TABLE 14: LINEARITY PERCENT RECOVERY RESULTS							
Element	**0.1J Mean**	**0.3J Mean**	**0.5J Mean**	**1.0J Mean**	**1.5J Mean**	**2.0J Mean**	**4.0J Mean**
55Mn	103	98	98	103	99	100	101

Manganese met 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 four spiking levels (30%, 50%, 100%, and 200% 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 four 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 6.9.

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 15 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 6.10.

   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 15: 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 Sample	100	N/A	4.0	1.0	50
0.1J Spiked Sample	100	0.010	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
2.0J Spiked Sample	100	0.200	4.0	1.0	50

TABLE 16: ACCURACY RESULTS FOR DEXTRAN SULFATE (Mean percent recovery of triplicate preparations)				
Element	0.3J Mean	0.5J Mean	1.0J Mean	2.0J Mean
55Mn	97	96	97	98

Manganese 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, low standard, 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 two calibration standards 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 17: SPECIFICITY RESULTS				
Element	Calibration Blank (CPS)	Method Blank (CPS)	0.5J STD (CPS)	4.0J STD (CPS)
55Mn	28	38	1011	7838

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 18: PRECISION RESULTS FOR DEXTRAN SULFATE (Mean recovery concentration of 6 preparations)		
Element	1.0J Mean Recovery Conc. N=6 (µg/kg)	% RSD N=6
55Mn	594	3

Manganese 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 19: RUGGEDNESS RESULTS FOR DEXTRAN SULFATE (Mean recovery concentration of 12 preparations)		
Element	1.0J Mean Recovery Conc. N=12 (µg/kg)	% RSD N=12
55Mn	604	3

Manganese 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 10% 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 spiked samples in Table 15 above.

7.14.2.1. Acceptance Criteria:

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

TABLE 20: LIMIT OF QUANTITATION RESULTS (Mean percent recovery of 3 preparations)	
Element	0.1J Mean % Recovery
55Mn	90

Manganese met LOQ acceptance criteria of 70% - 150%.

7.15. Sample and Standard Stability

7.15.1. The 50% and 400% 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 Ruggedness 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 21: SAMPLE AND STANDARD STABILITY (% RECOVERY)			
Element	0.5J Std (%)	4.0J Std (%)	1.0J Spike (%)
55Mn	102	96	101

Standard and Sample stability met the acceptance criteria of 80% to 120% recovery for Manganese.

8. DEVIATIONS:

8.1. No deviations were observed or noted during the execution of this validation protocol.

9. CONCLUSION:

9.1. The test method for Quantification of Manganese in Dextran Sulfate has been validated. All acceptance criteria set forth in the validation protocol were met. The Method was found to be:

9.1.1. Specific: The calibration blank and method blank did not show any significant interference as shown by the intensities compared against the standards.

9.1.2. Linear: 10% to 400% of working standard solution corresponding to 0.1J to 4.0J. Mean percent recovery ranged from 98% to 103%.

9.1.3. Sensitive: LOQ recovery was 90% at the 0.1J level or approximately an additional 0.05 ppm of spiked sample for dextran sulfate.

9.1.4. Accurate: From 30% (0.3J) to 200% (2.0J) of working standard concentration level, accuracy was successful with mean percent recoveries ranging from 96% to 98%.

9.1.5. Precise: Closeness of agreement demonstrated between six sample preparations by percent RSD of 3%.

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 was 3%.

9.1.7. Stable: With respect to stability of solutions, the sample solutions and standard solutions are to be considered stable for 24 hours (T=1 day) for quantification of manganese in dextran sulfate.

9.1.8. The concentration of manganese in unspiked dextran sulfate samples was determined to be 115 ppb, which falls within the range of the desired specification and the calibration range set forth in the validation protocol. In the final test method, a statement will be included to note that if samples exceed the upper limit of the calibration range at 4.0 ppm (2.0 ppm of manganese in the sample), a dilution will be performed to accurately quantitate the amount of manganese to fall within the calibration curve.

10. NOTEBOOK REFERENCE:

TABLE 22: NOTEBOOK REFERENCE		
STUDY		**NOTEBOOK REFERENCE**
Specificity		EIV-7/ pages 66-70
Linearity and Range		EIV-7/ pages 66-70
Accuracy/ Precision/LOQ by "Spiked" recovery		EIV-7/ pages 66-70, 74-77
Intermediate Precision (Ruggedness)		EIV-7/ pages 71-73
Solution Stability	Day-0	EIV-7/ pages 71-73
	Day-1	EIV-7/ pages 74-77