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

ANALYTICAL METHOD FOR THE DETERMINATION OF MANGANESE BY INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY (ICP-MS) IN DEXTRAN SULFATE

TABLE OF CONTENTS

1. PURPOSE:

1.1. To provide a procedure for the quantification of manganese in Dextran Sulfate (molecular weight 8000) via the NexION 350X S/N 85VN5093001 ICP-MS. This procedure was assessed as a full quantitative procedure as per validation report BSI-RPT-1097 and follows the validation parameters for quantitation procedures as outlined in USP <233>.

1.2. Elements under validated for this test method are as follows:

1.2.1. Class 4: Mn

1.3. 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 of manganese 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.

3. RESPONSIBILITIES:

3.1. The Executive Director of Quality Control or other qualified designated individual, is responsible for the control, implementation, training, and maintenance of this method.

3.2. The QC Staff is responsible for complying with the requirements of this procedure.

3.3. If any abnormalities are determined during routine use of the ICP-MS or during calibration, the QC Manager shall be promptly notified. If necessary, the ICP-MS will be serviced and recalibrated by Perkin Elmer before being approved for use.

4. REFERENCES:

4.1. BSI-ATM-0093, Analytical Method of Analysis: Elemental Impurities in Dextran Sulfate by ICP-MS

4.2. BSI-PRL-0521, Determination of Elemental Impurities by ICP-MS in Dextran Sulfate

4.3. BSI-PRL-0565, Analytical Method Validation Protocol: Determination of Manganese in Dextran Sulfate.

4.4. BSI-RPT-0988, Analytical Method Validation Report: Elemental Impurities in Dextran Sulfate

4.5. BSI-RPT-1097, Analytical Method Validation Report: Determination of Manganese in Dextran Sulfate

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

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

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

4.9. ICH Guideline for Elemental Impurities Q3D Current

4.10. NexION Operation with Syngistix Software Guide

4.11. USP <730> Plasma Spectrochemistry

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

4.13. USP <232>, <233>

TABLE 1: LIMITS FOR DEXTRAN SULFATE (10 GRAM/DAY PATIENT 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 provided customer specification.

5. MATERIALS AND EQUIPMENT:

5.1. Equipment
- 5.1.1. Analytical Balance
- 5.1.2. NexION 350X ICP-MS S/N 85VN5093001
- 5.1.3. CEM Digestion Microwave S/N MY2255
- 5.1.4. Clean 20 mL Digestion Vessels
- 5.1.5. Micropipettes, Eppendorf or Rainin

5.2. Reagents
- 5.2.1. Nitric Acid, Trace metals grade or equivalent
- 5.2.2. Hydrochloric Acid, Trace metals grade or equivalent
- 5.2.3. Sulfuric acid, Trace metals grade or equivalent
- 5.2.4. Deionized (DI) water (Type 1 Ultrapure)
- 5.2.5. Thiourea, 99+ % grade
- 5.2.6. NexION Setup and KED Setup Solution

5.3. Consumable Supplies
- 5.3.1. SCP Digitubes® 15 mL, 50 mL and 100 mL
- 5.3.2. Pipette Tips of various sizes
- 5.3.3. SiliaPrep MB SPE Cartridges, Silica-Based AMPA, 500 mg, 4 mL, 40 - 63 µm, 60 Å

5.4. Personnel
- 5.4.1. All personnel that executed the protocol are trained on ICP-MS or are considered Subject Matter Experts. This test method will be assigned a mark as read training to QC analysts involved with the execution.

TABLE 2: REFERENCE STANDARDS

Identification**	Manufacturer	Concentrations / Elements
Manganese Stock Standard P/N: N9303783	Perkin Elmer	Mn (1,000 µg/mL)
Pharma-CAL Custom Std P/N: AQ0-086-125* (Internal Standard)	SCP Science	Be, Sc, Y, Re (10 µg/mL); Te (25 µg/mL); Ge, Tb, Bi (5 µg/mL)

* SCP Science catalog numbers ending in 1 denote 125 mL bottle sizes and catalog numbers ending in 5 denote 500 mL bottle sizes.
** Additional standards/custom standards can be used as long as the concentration remains the same in final preparations.

6. PROCEDURE:

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

6.2. Acid Digestion Mix

[2:1] Nitric Acid (HNO_3): Sulfuric Acid (H_2SO_4) (Prepare same day)

6.2.1. Caution: Combining nitric acid and sulfuric acid generates excessive heat. Never seal cap tightly before solution has completely cooled.

6.2.2. To prepare, add 50 mL of nitric acid to a 100 mL Digitube® and then slowly add 25 mL of sulfuric acid. Solution can be placed in a cold-water bath to aid cooling.

6.2.2.1. Scale as necessary for use (Prepare same day).

6.3. Internal Standard/Complexing Solution

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

6.3.2. Add approximately 20 mL of deionized water and mix to dissolve.

6.3.3. Filter solution through a SiliaPrep Cation Solid Phase Extraction (SPE) cartridge into a separate 50 mL digitube.

6.3.4. Add 2.5 mL of Internal Standard Intermediate followed by 25 mL of hydrochloric acid.

6.3.5. Dilute to a final volume of 50 mL with deionized water and mix well.

6.3.6. Scale proportionally as needed for use.

6.4. 2% Thiourea Solution

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

6.4.2. Add approximately 20 mL of deionized water and mix to dissolve.

6.4.3. Filter solution through a SiliaPrep Cation Solid Phase Extraction (SPE) cartridge into a separate 50 mL digitube.

6.4.4. Dilute to a final volume of 50 mL with deionized water and mix well.

6.4.5. Scale proportionally as needed for use.

6.5. Intermediate Standard Preparation

6.5.1. Prepare a standard solution described in Table 3, using single source Mn stock standard.

6.5.2. Prepare by adding stock standards to a 50 mL Digitube®.

6.5.3. Add DI Water to approximately 40 mL and pipette 1.0 mL Nitric Acid.

6.5.4. Dilute to volume using DI Water.

TABLE 3: 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. Prepare four standard solutions described in Table 4 below using a 5.33% HNO_3, 2.67% H_2SO_4, 1.0% HCl, and 0.04% (400 µg/mL) Thiourea matrix.

6.6.2. Add appropriate amount of intermediate standard to separate 50 mL Digitubes® followed by addition of approximately 35 mL of deionized water.

6.6.3. Add 4.0 mL of Acid Mixture then dilute to 45 mL using deionized water.

6.6.4. Add 1.0 mL of internal standard/complexing solution and dilute to volume using deionized water.

6.6.5. Do not allow intermediate standard to contact concentrated acids while preparing solutions. (Standards are stable for 24 hours.)

TABLE 4: 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. Prepare a solution containing 5.33% HNO_3, 2.67% H_2SO_4, 1.0% HCl, and 0.04% (400µg/mL) Thiourea as described in Table 5 below.

6.7.2. To a separate 50 mL Digitube®, add approximately 35 mL of DI Water.

6.7.3. Add 4.0 mL of Acid Mixture then dilute to 45 mL using DI Water.

6.7.4. Add 1.0 mL of internal standard/complexing solution and dilute to volume using DI Water.

6.7.5. Do not allow Internal Standard Solution to contact concentrated acids.

TABLE 5: 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. Add 4.0 mL of Acid Digestion Mixture into a clean 20 mL digestion vessel, place a plug on the vessel, and properly torque the vessel cap. Place vessel in the microwave carousel then digest and complete preparation according to Section 6.10 below.

6.9. Sample Preparation

6.9.1. Sample solutions analyzed for Manganese analyses are stable for 24 hours.

6.9.2. Samples prepared for the Elemental Impurities method, BSI-ATM-0093, can be utilized for the manganese quantification method.

6.9.3. Weigh approximately 100 mg of sample into a clean 20 mL digestion vessel and add 4.0 mL of Acid Digestion Mixture.

6.9.4. Properly torque vessel cap and place in microwave carousel. Digest and complete 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. Prepare at least one method blank per digestion. Method blank is prepared in the same manner as the sample without the addition of sample (see above).

6.10.3. Digest the vessels using the program listed in Table 6.

TABLE 6: 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

6.10.4. If fewer than 4 samples are to be digested, use extra place holder "dummy" samples to ensure at least four vessels are digested. The extra place holder "dummy" samples can be discarded after the digestion is complete.

6.10.5. After digestion, place the vessels into an ice bath and allow the vessels to cool for approximately 40 minutes. Before opening, turn the vessels sideways and slowly rotate in order to collect the condensation on the inside of the vessel walls.

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

6.10.7. Extract 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. Add this directly to the 50 mL Digitube®.

6.10.8. Rinse the vessel an additional two more times using deionized water and transfer each rinse to the 50 mL Digitube®. Dilute to a final volume of 50 mL using deionized water and mix well.

6.10.9. If the sample result is above 2.0 ppm Manganese, a dilution, described in section 6.10.10 will be performed to accurately quantitate the amount of manganese to fall within the calibration curve.

6.10.10. Pipette 5.0 mL of the sample above 2.0 ppm Manganese into a separate 50 mL Digitube®. Add deionized water to approximately 35 mL mark on tube and add 3.60 mL of Acid Digestion Mixture. Dilute to 45 mL using deionized water and add 0.900 mL of Internal Standard/Complexing Solution. Dilute to final volume of 50 mL and mix well. The additional acid mixture and internal standard is to match the standard preparations.

7. INSTRUMENT PROCEDURE:

7.1. Perform the ICP-MS daily performance check 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 must be used. The calibration correlation coefficient (R) must be ≥ 0.99.

7.3. Set up the sequence as per Table 7.

7.4. Confirm 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 must be verified after the calibration. A re-analysis of the check standard will be performed a minimum of once every 10 samples and at the end of the analytical run.

7.6. Bracketing standard checks must recover ± 10% of the calculated theoretical concentration for single-element analysis. Additionally, the drift (calculated as absolute difference) between the bracketing standard checks must 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 7: 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 must be engaged during analysis using a minimum dilution gas ratio of 15%.

7.8.3. The instrument method is stored under the Approved Test Method Folder labelled as "Dextran_Manganese.mth" for manganese single element testing.

TABLE 8: ICP-MS PARAMETERS

ICP-MS System	Perkin Elmer NexION350X Inductively Coupled Plasma Mass Spectrometry (ICP-MS) with Syngistix Software
Sweeps/Readings	20
Replicates	3
Nebulizer Gas	Argon
Collision Gas	Helium
Dilution Gas	Argon
Sample and Skimmer Cone	Platinum
Sample Rinse	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 9: LINEAR RANGE AND CORRESPONDING TUNE MODE

Isotope	Internal Standard	Mode	Linear Range (µg/L)
55Mn	72Ge	KED	0.10-4.0

8. REPORTING

8.1. Any result below the 0.1J LOQ concentration will be reported as less than the corresponding LOQ value listed in Table 1. Results above the LOQ concentration will be reported in µg/kg (ppb) to the nearest whole number. 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, as described in Section 6.10.10, to accurately quantitate the amount of manganese to fall within the calibration curve.