BIOSPECTRA
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DEGRADATION AND IMPURITY PROFILE PROTOCOL: DEXTRAN SODIUM SULFATE 8000

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1. PURPOSE AND SCOPE:

1.1. The impurity profiling of Dextran Sodium Sulfate 8000 is intended to identify and potentially quantify impurities found in Dextran Sodium Sulfate 8000 (CAS 9011-18-1) product manufactured and purified at BioSpectra.

1.1.1. In the case where an impurity is found, a limit will be set to the maximum allowable without measurable compromise to predetermined critical quality attributes or toxicity. In the case where a limit cannot be set, a procedure will be written and followed, to identify if the possible impurity is present or not (i.e. an identity test, which is qualitative and not quantitative).

1.1.2. The profiling results and data will allow BioSpectra to understand the purity and characteristics of Dextran Sodium Sulfate 8000 through all stages of manufacturing.

1.1.3. The four stages of Dextran Sodium Sulfate 8000 that will be tested are Raw Material solution pre-filtration, Post-Purification Solution, 1st 10-gallon FG Spray Dried Increment Sample, and Finished Good Composite Sample.

1.1.3.1. If adjustment is required, the pre-filtration sample to be tested will be the sample after adjustment.

1.1.4. Tables will be generated to include all sample results in the Dextran Sodium Sulfate 8000 Degradation and Impurity Profile Report.

1.1.5. The tests that will be used to determine the presence of impurities and degradation products will be as follows: The 4 stages for evaluation are listed in section 1.1.3.

1.1.5.1. Appearance and Color

1.1.5.1.1. All four stages.

1.1.5.2. Total Bioburden (reported from TAMC result)

1.1.5.2.1. All four stages.

1.1.5.2.2. All individual Samples for Total Bioburden except for the Composite sample will be submitted in accordance with Process Validation Protocol.

1.1.5.3. Chloride Content

1.1.5.3.1. All four stages.

1.1.5.4. Clarity (20% Solution) at 360nm

1.1.5.4.1. All four stages.

1.1.5.5. Elemental Impurities

1.1.5.5.1. All four stages.

1.1.5.6. Endotoxin

1.1.5.6.1. All four stages

1.1.5.7. Free Sulfate

1.1.5.7.1. All four stages.

1.1.5.8. Glucose Content

1.1.5.8.1. All four stages.

1.1.5.9. Identification(s)

1.1.5.9.1. All four stages.

1.1.5.10. Iron:

1.1.5.10.1. All four stages.

1.1.5.11. Loss on Drying

1.1.5.11.1. All four stages.

1.1.5.12. Manganese

1.1.5.12.1. All four stages.

1.1.5.13. pH (1% Solution)

1.1.5.13.1. All four stages.

1.1.5.14. Residue on Ignition
1.1.5.14.1. All four stages.
1.1.5.15. Pyridine
1.1.5.15.1. All four stages.
1.1.5.16. Residual Solvents (Methanol and Isopropyl Alcohol)
1.1.5.16.1. All four stages.

1.2. All results will be recorded in the appropriate laboratory documentation. The results will be detailed and analyzed in the degradation and impurity profile report. This report will include all relevant data as well as references to the initial documented results. This report will discuss any impurities found in the product and include a specification for any limits on the impurities found when applicable.

2. RESPONSIBILITIES:

2.1. The Associate Director of Product Life Cycle is responsible for control, implementation, training, and maintenance of this procedure.
2.2. The QC Analysts (or qualified designees) are responsible for performing the testing stated in the protocol and recording all results.
2.3. The Associate Director of Product Life Cycle, or designee is responsible for completing the degradation and impurity testing report.
2.4. It is the responsibility of all personnel to read and understand the SDS and don the appropriate PPE for handling and disposing of chemicals in a safe manner.

3. REFERENCES:

3.1. BSI-ATM-0091, Dextran Sulfate 8000 MW (DS8) Testing Methods
3.2. BSI-ATM-0093 Analytical Method for the Determination of ICH Q3D Elemental Impurities (Class 1, 2A, 2B, 3, & 4) by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in Dextran Sulfate
3.3. BSI-ATM-0100, Analytical Method for the Determination of Manganese by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in Dextran Sulfate
3.4. BSI-ATM-0106, Dextran Sulfate Solution Testing Methods.
3.5. BSI-MEM-0130, Endosafe NexGen PTS Endotoxin Reader: Qualified Products
3.6. BSI-RPT-1296, Analytical Method Validation Report: Dextran Sulfate Glucose Content via UV/Vis Spectroscopy
3.7. BSI-RPT-1339, Analytical Method Validation report: Limit of Pyridine in Dextran Sulfate
3.8. BSI-SOP-0069, Preparation of Samples for Outside Testing.
3.9. BSI-SOP-0090, Lambda 25 UV/Vis Operation and Calibration.
3.10. BSI-SOP-0094, Muffle Furnace SOP and Calibration.
3.11. BSI-SOP-0098, Balance SOP.
3.12. BSI-SOP-0102, Degradation and Impurity Profiling SOP
3.13. BSI-SOP-0126, Laboratory Notebooks.
3.14. BSI-SOP-0134, Pipette SOP.
3.15. BSI-SOP-0244, VWR Gravity Convection Oven Operation and Calibration.
3.16. BSI-SOP-0255, XL200 pH/mV/Conductivity Meter SOP
3.17. BSI-SOP-0303, NexION 350X ICP-MS SOP.
3.18. BSI-SOP-0316, Shimadzu QP2010S GC/MS SOP
3.19. BSI-SOP-0345, Endosafe Nexgen-PTS Endotoxin Reader SOP.

4. EQUIPMENT:

4.1. Analytical Balance
4.2. Blue M Convection Oven
4.3. Bunsen Burner

4.4. Calibrated Pipette
4.5. Calibrated Timer
4.6. Endosafe NexGen PTS Endotoxin Reader
4.7. Hot Plate
4.8. Lambda 25 UV/Vis Spectrophotometer
4.9. Litmus Paper
4.10. Metrohm 914 pH Conductometer
4.11. Muffle Furnace
4.12. Perkin Elmer NexION 350X ICP-MS
4.13. pH Probe
4.14. Shimadzu QP2010S GC/MS
4.15. VWR Gravity Convection Oven
4.16. XL200 pH/mV/Conductivity Meter

5. REAGENTS:

5.1. **0.02N Hydrochloric Acid:** Slowly add 20mL of 0.1N hydrochloric acid to 80mL of purified water to make a total volume of 100mL or purchased commercially.
5.2. **0.1M Barium Chloride:** Dissolve 2.4g of barium chloride dihydrate in purified water and dilute with purified water to make 100mL.
5.3. **0.1N Hydrochloric Acid:** Purchased Commercially.
5.4. **0.1N Silver Nitrate:** Purchased Commercially.
5.5. **1% Acrinol:** Dissolve 1.0 grams of Acrinol Monohydrate in purified water and dilute with purified water to 100mL.
5.6. **1 – 0.01 EU/mL LAL Test Cartridges:** Purchased Commercially.
5.7. **2N Sodium Hydroxide:** Dissolve 8g of Sodium Hydroxide in purified water to make 100mL. Preserve in polyethylene bottles.
5.8. **Acrinol Monohydrate:** Purchased Commercially.
5.9. **Anhydrous Sodium Sulfate:** Purchased Commercially.
5.10. **Anthrone Solution:** Prepare immediately before use. Weigh 90 – 100mg of anthrone powder into a beaker, add 50mL of concentrated sulfuric acid, dissolve, and mix thoroughly.
5.11. **Anthrone Powder:** Purchased Commercially.
5.12. **Barium Chloride Dihydrate:** Purchased Commercially.
5.13. **Barium Chloride TS (~0.5M):** Dissolve 30g of barium chloride dihydrate in water to make 250mL.
5.14. **Dextrose (D-Glucose) Certified Reference Standard (CRS):** Purchased Commercially.
5.15. **Glacial Acetic Acid:** Purchased Commercially.
5.16. **Hydrochloric Acid, concentrated:** Purchased Commercially.
5.17. **Hydrochloric Acid, Dilute (~10%):** Dilute 23.6mL of concentrated hydrochloric acid with water to make 100mL.
5.18. **LAL Reagent Water (or equivalent):** Purchased Commercially.
5.19. **Nitric Acid, concentrated:** Purchased Commercially.
5.20. **Purified Water:** In-House or Purchased Commercially.
5.21. **Sulfate Standard Solution (0.2% SO42- Solution):** Dissolve 0.296g of anhydrous sodium sulfate in purified water and dilute with purified water to 100mL.
5.22. **Sulfuric Acid, concentrated:** Purchased Commercially.

6. ANALYTICAL PROCEDURES:

6.1. APPEARANCE AND COLOR :

6.1.1. Finished Good:
- 6.1.1.1. Place 10 grams of sample in a clean, dry, glass beaker.
- 6.1.1.2. In an area with sufficient lighting, view the sample from all sides.
- 6.1.1.3. The sample should be white to light yellow in color and characteristic of a powder. If the sample does not conform to these specifications, notify the Lab Manager immediately.

6.1.2. Liquid:
- 6.1.2.1. Observe approximately 10mL of sample in a clean, colorless, glass vessel.
- 6.1.2.2. Report observed color and qualitative solution clarity characteristics. (Clear, Hazy, etc.)

6.1.3. **Solution requirements:** Monitor

6.1.4. **Finished Goods Requirements:** White to light yellow powder.

6.2. TOTAL BIOBURDEN :

6.2.1. Package no less than 35 grams of sample into a sterile container and send to Mary Paul Laboratories with a purchase order and analysis request form.

6.2.2. The Analysis Request form should include:
- 6.2.2.1. Total Aerobic Microbial Count (TAMC)
  - 6.2.2.1.1. Note: Total Bioburden will be reported from the TAMC result.
- 6.2.2.2. Total Yeasts and Mold Count (TYMC)
  - 6.2.2.2.1. Document result in report.

6.2.3. **Solution requirements:** Monitor

6.2.4. **Finished Good requirements:** ≤100 CFU/g for Total Bioburden

6.3. CHLORIDE CONTENT :

6.3.1. Finished Good: Refer to Dextran Sulfate 8000MW (DS8) Testing Methods (DCN: BSI-ATM-0091) for sample preparation and analysis.

6.3.2. Liquid: Refer to Dextran Sulfate Solution Testing Methods (DCN: BSI-ATM-0106) for sample preparation and analysis.

6.3.3. **Solution requirements:** Monitor

6.3.4. **Finished Goods requirements:** <1000ppm

6.4. CLARITY (20% SOLUTION AT 360nm) :

6.4.1. Finished Good: Refer to Dextran Sulfate 8000MW (DS8) Testing Methods (DCN: BSI-ATM-0091) for sample preparation and analysis.

6.4.2. Liquid: Refer to Dextran Sulfate Solution Testing Methods, BSI-ATM-0106 for sample preparation and analysis.

6.4.3. **Solution requirements:** Monitor

6.4.4. **Finished Goods requirements:** 0.9 OD Unit Max.

6.5. ELEMENTAL IMPURITIES :

6.5.1. Refer to Analytical Method for the Determination of ICH Q3D Elemental Impurities (Class 1, 2A, 2B, 3, & 4) by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in Dextran Sulfate (DCN: BSI-ATM-0093), for sample preparation and analysis.
- 6.5.1.1. There is no official preparation for the Solution defined, begin with finished good preparation and if required adjust sample preparation for analysis.

6.5.2. **Solution Requirements:** Monitor.

6.5.3. **Finished Good requirements:** Complies with USP <232> <233>.

6.6. **ENDOTOXIN**

6.6.1. Refer to Dextran Sulfate 8000MW (DS8) Testing Methods (DCN: BSI-ATM-0091) for sample preparation and analysis.

6.6.1.1. There is no official preparation for the Solution defined, begin with finished good preparation and adjust sample preparation (if applicable) to meet system suitability requirements.

6.6.2. **Solution requirements:** Monitor.

6.6.3. **Finished Good requirement:** ≤0.012 EU/mg.

6.7. **FREE SULFATE**

6.7.1. Finished Good: Refer to Dextran Sulfate 8000MW (DS8) Testing Methods (DCN: BSI-ATM-0091) for sample preparation and analysis.

6.7.2. Liquid: Refer to Dextran Sulfate Solution Testing Methods (DCN: BSI-ATM-0106) for sample preparation and analysis.

6.7.3. **Solution requirements:** Monitor.

6.7.4. **Finished Good requirement:** <0.2%

6.8. **GLUCOSE CONTENT**

6.8.1. Finished Good: Refer to Dextran Sulfate 8000MW (DS8) Testing Methods (DCN: BSI-ATM-0091) for sample preparation and analysis.

6.8.2. Liquid: Refer to Dextran Sulfate Solution Testing Methods (DCN: BSI-ATM-0106) for sample preparation and analysis.

6.8.3. **Solution requirements:** Monitor.

6.8.4. **Finished Good requirement:** 35% - 48%

6.9. **IDENTIFICATION TESTS**

6.9.1. Finished Good: Refer to Dextran Sulfate 8000MW (DS8) Testing Methods (DCN: BSI-ATM-0091) for sample preparation and analysis.

6.9.2. Liquid: Refer to Dextran Sulfate Solution Testing Methods (DCN: BSI-ATM-0106) for sample preparation and analysis.

6.9.3. **Solution requirements:** Monitor.

6.9.4. **Finished Good requirements:** Passes Test

6.10. **IRON**

6.10.1. Refer to Analytical Method for the Determination of ICH Q3D Elemental Impurities (Class 1, 2A, 2B, 3, & 4) by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in Dextran Sulfate (DCN: BSI-ATM-0093) for sample preparation and analysis.

6.10.1.1. There is no official preparation for the Solution defined, begin with finished good preparation and if required adjust sample preparation for analysis.

6.10.2. **Solution requirements:** Monitor.

6.10.3. **Finished Good requirements:** 2% Max.

6.11. **LOSS ON DRYING**

6.11.1. Finished Good: Refer to Dextran Sulfate 8000MW (DS8) Testing Methods (DCN: BSI-ATM-0091) for sample preparation and analysis.

6.11.2. Liquid: Refer to Dextran Sulfate Solution Testing Methods (DCN: BSI-ATM-0106) for sample preparation and analysis.

6.11.3. **Solution requirements:** Monitor.

6.11.4. **Finished Good requirements:** 10.0% Max

6.12. **MANGANESE**

6.12.1. Refer to Analytical Method for the Determination of Manganese by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in Dextran Sulfate (DCN: BSI-ATM-0100) for sample preparation and analysis.

6.12.1.1. There is no official preparation for the Solution defined, begin with finished good preparation and if required adjust sample preparation for analysis.

6.12.2. **Finished Good requirements:** 1 ppm Max.

6.13. **pH (FG - 1% SOLUTION; SOLUTION - 5% SOLUTION)**:

6.13.1. Finished Good: Refer to Dextran Sulfate 8000MW (DS8) Testing Methods (DCN: BSI-ATM-0091) for sample preparation and analysis.

6.13.2. Liquid: Refer to Dextran Sulfate Solution Testing Methods (DCN: BSI-ATM-0106) for sample preparation and analysis.

6.13.3. **Solution requirements:** Monitor

6.13.4. **Finished Good requirements:** 5.0 – 7.5

6.14. **RESIDUE ON IGNITION**:

6.14.1. Refer to Dextran Sulfate 8000MW (DS8) Testing Methods (DCN: BSI-ATM-0091) for sample preparation and analysis for both the solutions and finished good.

6.14.2. **Solution requirements:** Monitor

6.14.3. **Finished Good requirements:** 35 – 50%

6.15. **PYRIDINE**:

6.15.1. Finished Good: Refer to Dextran Sulfate 8000MW (DS8) Testing Methods (DCN: BSI-ATM-0091) for sample preparation and analysis.

6.15.2. Liquid:

6.15.2.1. Accurately weigh 10 grams of Dextran Sulfate sample (as-is) and transfer to a suitable beaker.

6.15.2.2. Add 100mL of purified water and stir to dissolve.

6.15.2.3. Using a pH probe and burette or pipette, titrate to pH 2.8 with 0.1N hydrochloric acid.

6.15.2.4. Calculate Residual Pyridine using the following equation:

$$Residual\ Pyridine\ (\%) = \frac{mL\ of\ Titrant\ \times Normality\ of\ Titrant\ \times 0.00791}{Sample\ Weight\ (g) \times \left(\frac{100 - LOD\ (\%)}{100}\right)}$$

6.15.3. **Solution requirements:** Monitor

6.15.4. **Finished Good requirements:** NMT 2%

6.16. **RESIDUAL SOLVENTS**:

6.16.1. Methanol and IPA analysis may be performed by Advanced Analytical Testing Laboratories.

6.16.2. Analysis can be performed utilizing an in-house method, spectral identification via MS is recommended for any peaks detected greater than ICH residual solvent thresholds; (0.3% Methanol and 0.5% IPA).

6.16.3. **Solution requirements:** Monitor

6.16.4. **Finished Good requirements:** Monitor

7. REPORT:

7.1. The results will be detailed and analyzed in the Dextran Sodium Sulfate 8000 degradation and impurity report. The report will include all relevant data as well as references to the initial documented results. The report will discuss any impurities found in the product and include a specification for any limits on the impurities found when applicable. A copy of referenced testing should be available with the report.