BIOSPECTRA
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BIS-TRIS HYDROCHLORIDE TESTING METHODS

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1. PURPOSE:

1.1. To provide the Laboratory personnel with a procedure for testing Bis-Tris Hydrochloride.

2. SCOPE:

2.1. This document applies to the testing of Bis-Tris Hydrochloride in the Laboratory at all BioSpectra, Inc Facilities. This test method includes testing for all product codes of Bis-Tris Hydrochloride tested at BioSpectra.

3. RESPONSIBILITIES:

3.1. The Director of Laboratory Testing is responsible for the control, training, maintenance and implementation of this procedure.
3.2. The Laboratory Technicians are responsible for compliance with the terms of this procedure. This includes notifying Quality Assurance and Laboratory Management, or designees, if any analyses fail to meet their respective specifications.
3.3. It is the responsibility of all personnel to read and understand the Safety Data Sheet (SDS) and don the appropriate PPE for handling and disposing of chemicals in a safe manner.

4. REFERENCES:

4.1. BSI-MEM-0130, Endosafe NexGen PTS Endotoxin Reader: Qualified Products
4.2. BSI-PRL-0431, Analytical Method Validation Protocol: Determination of ICH Q3D Elemental Impurities by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in Bis-Tris and Bis-Tris Hydrochloride
4.3. BSI-PRL-0569, DNase (Endonuclease) Assay Method Validation Protocol
4.4. BSI-PRL-0570, DNase (Exonuclease) Assay Method Validation Protocol
4.5. BSI-PRL-0571, Analytical Method Validation Protocol: RNase (Ribonuclease) Assay
4.6. BSI-PRL-0579, Analytical Method Validation Protocol: Bis-Tris HCl Assay by Potentiometric Titration
4.7. BSI-PRL-0646, Analytical Method Verification Protocol: Protease Assay
4.8. BSI-RPT-0803, Analytical Method Validation Report: Determination of ICH Q3D Elemental Impurities by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in Bis-Tris and Bis-Tris Hydrochloride
4.9. BSI-RPT-1179, Analytical Method Validation Report: DNase (Endonuclease) Assay
4.10. BSI-RPT-1200, Analytical Method Validation Report: DNase (Exonuclease) Assay
4.11. BSI-RPT-1258, Analytical Method Validation Report: RNase (Ribonuclease) Assay
4.12. BSI-RPT-1448, Analytical Method Verification: Bis-Tris HCl Water Determination Via Karl Fischer Utilizing Metrohm 907 Auto-Titrator
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4.15. BSI-SOP-0019, Result Reporting
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4.17. BSI-SOP-0090, Lambda 25 UV/Vis Operation and Calibration
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4.19. BSI-SOP-0096, RNase (Ribonuclease) Assay
4.20. BSI-SOP-0098, Balance SOP
4.21. BSI-SOP-0126, Laboratory Notebooks
4.22. BSI-SOP-0138, DNase (Exonuclease) Assay
4.23. BSI-SOP-0139, Protease Assay
4.24. BSI-SOP-0140, Standardization of Titrants

4.25. BSI-SOP-0143, Metrohm Titrando 907 Auto-Titrator SOP
4.26. BSI-SOP-0244, VWR Gravity Convection Oven Operation and Calibration (Model Number 414005-106)
4.27. BSI-SOP-0254, Spectrum Two UATR SOP
4.28. BSI-SOP-0255, XL200 pH/mV/Conductivity Meter SOP
4.29. BSI-SOP-0345, Endosafe nexgen-PTS Endotoxin Reader SOP

5. EQUIPMENT:

5.1. Analytical Balance
5.2. Calibrated Pipettes
5.3. Calibrated Timer
5.4. Endosafe Nexgen-PTS Endotoxin Reader
5.5. Lambda 25 UV/Vis Spectrophotometer
5.6. Metrohm 907 Titrando Auto-Titrator
5.7. Perkin Elmer Spectrum Two UATR
5.8. pH Probe
5.9. VWR Gravity Convection Oven, or equivalent
5.10. XL200 pH/Conductivity Meter or equivalent

6. REAGENTS:

6.1. **0.1N Sodium Hydroxide (NaOH):** Purchased Commercially.
6.2. **0.25M Tris Buffer:** Purchased Commercially.
6.3. **1 – 0.01 EU/mL LAL Test Cartridges:** Purchased Commercially.
6.4. **Composite 5:** Purchased Commercially.
6.5. **Formamide Dry:** Purchased Commercially.
6.6. **LAL Reagent Water:** Purchased Commercially.
6.7. **Methanol Dry:** Purchased Commercially.
6.8. **Purified Water:** In-House or Purchased Commercially.

7. ANALYTICAL PROCEDURE:

7.1. ABSORBANCE (0.1M) REFER TO SUMMARY SHEET:

7.1.1. Prepare a 0.1M solution of the specified sample.
  7.1.1.1. Accurately weigh 0.61 grams of sample.
  7.1.1.2. Transfer accurately weighed sample to a graduated cylinder and dilute to 25 mLwith purified water.
  7.1.1.3. Swirl to dissolve completely.
7.1.2. Refer to Lambda 25 UV/Vis Operation and Calibration to determine the Absorbance of the sample.

7.2. APPEARANCE AND COLOR REFER TO SUMMARY SHEET:

7.2.1. Place 10 grams of sample on a piece of white filter paper.
7.2.2. In an area with sufficient lighting, view the sample from all sides.
7.2.3. The sample should be colorless crystals to a white crystalline powder
  7.2.3.1. If there is any visual extraneous matter such as fibers, off-color specks, or any particulate matter present, please notify Lab, QA, and Manufacturing management.

7.3. **ASSAY (AS-IS)** **REFER TO SUMMARY SHEET:**

7.3.1. Standardize 0.1N NaOH as per Standardization of Titrants.
7.3.2. Accurately weigh 0.75 grams of as-is sample to a suitable, clean glass beaker.
7.3.3. Add 50 mL of purified water.
7.3.4. Titrate with 0.1N NaOH to a potentiometric end-point utilizing the Metrohm Titrando 907.
7.3.5. Each mL of 0.1N NaOH is equivalent to 24.57 mg of Bis-Tris Hydrochloride.

$$\% \text{ Bis-Tris HCl} = \frac{(\text{mL NaOH})\ (\text{Normality of Titrant})\ (24.570)}{Sample\ Weight\ (g)}$$

7.4. **BIOBURDEN** **REFER TO SUMMARY SHEET:**

7.4.1. Microbial analysis will be performed by an outside testing laboratory.
7.4.1.1. Primary Provider: Mary Paul Laboratories
7.4.1.2. Package and send NLT 35 g of sample to Mary Paul Laboratories with a purchase order and analysis request form.
7.4.1.3. MPL Suitability Number to be used for analysis: 23H3126a
7.4.2. Analyses:
7.4.2.1. Total Aerobic Microbial Count (TAMC)
7.4.2.1.1. In accordance with USP <61>, the result for ***Bioburden*** will be reported from the TAMC result only.
7.4.2.1.2. If there is growth, Identification is required.
7.4.2.2. Total Yeasts and Molds Count (TYMC)
7.4.2.2.1. TYMC will be provided For Information Only and will not be officially reported.
If there is growth, Identification is required.

7.5. **ENDOTOXIN** **REFER TO SUMMARY SHEET:**

7.5.1. Accurately weigh 100 mg of sample into a sterile tube. Dilute with LAL Reagent Water to make 10mL, dissolve, and mix. Transfer 1.0 mL of the resulting solution to a separate sterile tube, add 150 µL of 0.25M Tris Buffer, and dilute to 10 mL with LAL Reagent Water for a concentration of 0.001 g/mL.
7.5.2. Refer to Endosafe nexgen-PTS Endotoxin Reader SOP for analysis (BSI-SOP-0345).

7.6. **ENZYME ACTIVITY** **REFER TO SUMMARY SHEET:**

7.6.1. DNase: Refer to DNase (Exonuclease) Assay (BSI-SOP-0138) and DNase (Endonuclease) Assay (BSI-SOP-0095) for sample preparation and analysis.
7.6.2. RNase: Refer to RNase (Ribonuclease) Assay (BSI-SOP-0096) for sample preparation and analysis.
7.6.3. Protease: Refer to Protease Assay (BSI-SOP-0139) for sample preparation and analysis.

7.7. **IDENTIFICATION (IR)** **PASSES TEST:**

7.7.1. For UATR analysis, follow Spectrum Two UATR SOP.

7.8. **pH (1%) @ 25°C ±2°C** **REFER TO SUMMARY SHEET:**

7.8.1. Accurately weigh 1.0 g of sample. Transfer to a suitable beaker.
7.8.2. Dissolve in 100 mL of purified water.
7.8.3. Measure and record the pH using the appropriate SOP.

7.9. **SOLUBILITY (0.1M SOLUTION)** **PASSES TEST:**

7.9.1. Weigh 2.46 g of sample and transfer to a graduated cylinder.
7.9.2. Q.S. to 100 mL with purified water. Cover with parafilm and mix until thoroughly dissolved.
7.9.3. Transfer solution into a clean beaker and observe under sufficient light.
7.9.4. Solution should be clear and colorless.

7.10. **WATER (BY KARL FISCHER TITRATION)** **1.0% MAX.:**

7.10.1. Standardize Composite 5 as per Standardization of Titrants.
7.10.2. Grind the sample in a dry mortar into a fine powder utilizing a pestle.
7.10.3. Immediately weigh 1.0 grams of sample into the glass weighing spoon and tare it.
7.10.4. Transfer the sample to the KF vessel by removing the rubber septum and adding the sample into the titration vessel.
7.10.4.1. Do not leave the rubber septum open for long periods of time as this will allow moisture to enter the titration vessel.
7.10.5. Return the weighing spoon to the balance, making sure not to lose any sample that was left behind. Once the weight stabilizes, record the sample weight and transfer to instrument.
7.10.6. Check to make sure there is no residual sample stuck to the sides of the titration vessel.
7.10.6.1. If there is any sample stuck to the side, stop the stir bead from spinning before swirling the vessel to rinse the sides.
7.10.7. Once the method begins, check to ensure the sample is fully dissolved before the titration begins (i.e. before the stir command completes).
7.10.8. The moisture content will then be determined by the Metrohm Auto Titrando 907.

$$\%\ Moisture = \frac{(mL\ of\ Composite\ 5)\left(\frac{mg}{mL}of\ Composite\ 5\right)(0.1)}{Sample\ Weight\ (g)}$$