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TRIS HYDROCHLORIDE TESTING METHODS

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1. PURPOSE:

1.1. To provide the Quality Control (QC) Laboratory personnel with procedures for testing Tris Hydrochloride.

2. SCOPE:

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

3. RESPONSIBILITIES:

3.1. The Executive Director of Quality Control is responsible for training, maintenance and implementation of this procedure.
3.2. The QC Analysts are responsible for compliance with the terms of this procedure. This includes notifying the Executive Director or Quality Control if any analyses fail to meet their respective specifications.

4. REFERENCES:

4.1. Analytical Method Validation Report: Silver Nitrate Assay Utilizing Metrohm 907 Auto-Titrator, DCN: 16-001926
4.2. Analytical Method Validation Protocol: Trace Metal Impurities: Tris and Tris HCl
4.3. Analytical Method Validation Report Trace Metal Impurities: Tris HCl
4.4. Balance SOP, DCN:16-000368
4.5. Blue M Convection Oven Operation and Calibration SOP, DCN: 16-000502
4.6. *Current USP Tromethamine Monograph*
4.7. *Current EP Trometamol Monograph*
4.8. Endosafe PTS Endotoxin Reader SOP
4.9. Endosafe nexgen-PTS Endotoxin Reader SOP, DCN: 18-002735
4.10. DNase (Endonuclease) Assay, DCN: 16-000365
4.11. DNase (Exonuclease) Assay, DCN: 16-000511
4.12. Laboratory Notebooks, DCN: 16-000482
4.13. Lambda 25 UV/Vis Operation and Calibration, DCN: 16-000359
4.14. Metrohm 914 pH Conductometer Operation and Calibration, DCN: 16-000522
4.15. Metrohm Titrando 907 Auto-Titrator SOP, DCN: 16-000521
4.16. MP50 Melting Range Operation and Calibration SOP, DCN: 16-001332
4.17. Muffle Furnace SOP and Calibration, DCN: 16-000364
4.18. NexION 350X ICP-MS SOP, DCN: 16-001923
4.19. Protease Assay, DCN: 16-000512
4.20. Result Reporting, DCN: 16-000063
4.21. RNase (Ribonuclease) Assay, DCN: 16-000366
4.22. Spectrum Two UATR SOP, DCN: 16-001330
4.23. Standardization of Titrants, DCN: 16-000513
4.24. VWR Gravity Convection Oven Operation and Calibration (Model Number 414005-106), DCN:16-001319
4.25. XL200 pH/mV/Conductivity Meter SOP, DCN: 16-001331

5. EQUIPMENT:

5.1. Analytical Balance
5.2. Calibrated Oven

5.3. Endosafe PTS Endotoxin Reader, or equivalent
5.4. Lambda 25 Spectrophotometer, or equivalent
5.5. Metrohm 914 pH/ Conductometer
5.6. Metrohm Titrando 907 Auto-Titrator
5.7. MP50 Melting Point Apparatus
5.8. Muffle Furnace
5.9. NexION 350X ICP-MS
5.10. Spectrum Two UATR
5.11. XL200 pH/mV/Conductivity Meter

6. ANALYTICAL PROCEDURE:

6.1. **IN-PROCESS ML ABSORBANCE** **REFER TO BATCH RECORD:**

6.1.1. Prepare 10 mL of a 1:1 dilution by pipetting 5 mL of purified water and 5 mL of the specified Mother Liquor sample into a small beaker. Swirl to mix completely.
6.1.2. Refer to the Lambda 25 UV/Vis Operation and Calibration to determine the Absorbance of the sample.
6.1.3. Record the results at specified wavelengths in the Tris Hydrochloride In-Process Testing Log Book and in the batch record. If failing results are obtained, notify the Executive Director of Quality Control and Production immediately.

6.2. **IN-PROCESS ML ASSAY** **REFER TO BATCH RECORD:**

6.2.1. Assay by Auto-titrator:
6.2.1.1. Standardize 0.1N $AgNO_3$ as per Standardization of Titrants.
6.2.1.2. Accurately weigh 0.5 grams of as-is sample. Transfer to a 250mL beaker and dissolve with 10mL of purified water. Add 10 mL of glacial acetic acid, 100 mL of methanol, and 10 mL of a 0.2% polyvinyl alcohol solution. Titrate with 0.1N $AgNO_3$ to a potentiometric end-point utilizing the Metrohm Titrando 907.
6.2.2. Assay by Manual Titration:
6.2.2.1. Standardize 0.1N $AgNO_3$ as per Standardization of Titrants. Accurately weigh 0.5 g of sample. Transfer to a 250-mL beaker and dissolve with 10 mL of purified water. Add 10 mL of glacial acetic acid, 100 mL of methanol, and 0.5mL of Eosin Y Indicator. Titrate to a pink endpoint.

$$\%\boldsymbol{Tris\ HCl} = \frac{(\boldsymbol{mL\ of\ titrant})(\boldsymbol{N\ of\ Silver\ Nitrate})(\mathbf{15.76})}{\boldsymbol{Sample\ weight}}$$

6.3. **ABSORBANCE (1M)** **REFER TO SUMMARY SHEET:**

6.3.1. Prepare a 1 M solution of the specified sample.
6.3.1.1. Accurately weigh 3.94g of sample.
6.3.1.2. Transfer accurately weighed sample to a graduated cylinder and Q.S. to 25 mL with purified water.
6.3.1.3. Swirl to dissolve completely.
6.3.2. Refer to Lambda 25 UV/Vis Operation and Calibration to determine the Absorbance of the sample.

6.4. **ABSORBANCE (0.1M)** **REFER TO SUMMARY SHEET:**

6.4.1. Prepare a 0.1 M solution of the specified sample.
6.4.1.1. Accurately weigh 0.394g of sample.
6.4.1.2. Transfer accurately weighed sample to a graduated cylinder and Q.S. to 25 mL with purified water.

6.4.1.3. Swirl to dissolve completely.

6.4.2. Refer to Lambda 25 UV/Vis Operation and Calibration to determine the Absorbance of the sample.

6.5. **APPEARANCE AND COLOR** **WHITE / CRYSTALS:**

6.5.1. Perform the test by placing approximately 10 grams of sample on a piece of white filter paper.

6.5.2. Observe the sample for appearance. Test passes if the sample is colorless crystals to a white crystalline powder and is free from visual extraneous matter such as fibers or off-color specks.

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

6.6.1. Standardize 0.1N $AgNO_3$ as per Standardization of Titrants.

6.6.2. Accurately weigh 0.5g of sample.

6.6.3. Transfer to a beaker and dissolve with 10 mL of purified water.

6.6.4. Add 10 mL of glacial acetic acid, 100 mL of methanol, and 10 mL of a 0.2% polyvinyl alcohol solution.

6.6.5. Titrate with 0.1N $AgNO_3$ to a potentiometric end-point utilizing the Metrohm Titrando 907.

$$\%C_4H_{11}NO_3 \cdot HCl = \frac{(\text{mL x N of } AgNO_3)(15.76)}{\text{Sample Weight (g)}}$$

6.6.6. Alternate Manual Titration Method:

6.6.6.1. Standardize 0.1N $AgNO_3$ as per Standardization of Titrants.

6.6.6.2. Accurately weigh 0.5g of sample.

6.6.6.3. Transfer to a 250-mL beaker and dissolve with 10 mL of purified water.

6.6.6.4. Add 10 mL of glacial acetic acid, 100 mL of methanol, and 0.5mL of Eosin Y Indicator.

6.6.6.5. Titrate to a pink endpoint.

$$\%C_4H_{11}NO_3 \cdot HCl = \frac{(\text{mL x N of } AgNO_3)(15.76)}{\text{Sample Weight (g)}}$$

6.7. **ASSAY (DRIED BASIS)** **REFER TO SUMMARY SHEET:**

6.7.1. Standardize 0.1N $AgNO_3$ as per Standardization of Titrants.

6.7.2. Accurately weigh 0.5 g of sample that has been previously dried following LOD analysis.

6.7.3. Transfer to a 250-mL beaker and dissolve with 10 mL of purified water.

6.7.4. Add 10 mL of glacial acetic acid, 100 mL of methanol, and 10 mL of a 0.2% polyvinyl alcohol solution.

6.7.5. Titrate with 0.1N $AgNO_3$ to a potentiometric end-point utilizing the Metrohm Titrando 907.

$$\%C_4H_{11}NO_3 \cdot HCl = \frac{(\text{mL x N of } AgNO_3)(15.76)}{\text{Sample Weight (g)}}$$

6.7.6. Alternate Manual Titration Method:

6.7.6.1. Standardize 0.1N $AgNO_3$ as per Standardization of Titrants.

6.7.6.2. Accurately weigh 0.5 g of sample that has been previously dried following LOD analysis.

6.7.6.3. Transfer to a 250-mL beaker and dissolve with 10 mL of purified water.

6.7.6.4. Add 10 mL of glacial acetic acid, 100 mL of methanol, and 0.5mL of Eosin Y Indicator.

6.7.6.5. Titrate to a pink endpoint.

$$\%C_4H_{11}NO_3 \cdot HCl = \frac{(\text{mL x N of } AgNO_3)(15.76)}{\text{Sample Weight (g)}}$$

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

6.8.1. Accurately weigh 50-60mg of sample into a sterile tube. Add 50-60mg of suitable tris base (Molecular biology grade or better). Dilute to 10mL with LAL reagent water, dissolve completely. Follow Endosafe NexGen PTS Endotoxin Reader SOP for analysis.

6.8.2. Endotoxin analysis can also be performed by an outside laboratory.

6.8.2.1. Package and send 20 grams of sample to the approved outside testing facility.

6.9. **ENZYME ACTIVITY** **NONE DETECTED:**

6.9.1. Follow the RNase, DNase and Protease procedures referenced in section 4.

6.10. **HEAVY METALS** **REFER TO SUMMARY SHEET:**

6.10.1. Refer to section 6.30: Trace Elements

Alternative Method:

6.10.2. 0.001% test sample preparation:

6.10.2.1. Into a 50-mL Nessler color comparison tube, dissolve 2.0 g of sample in ~40 mL of purified water.

6.10.3. 5 ppm test sample preparation:

6.10.3.1. Into a 50-mL Nessler color comparison tube, dissolve 4.0 g of sample in ~40 mL of purified water.

6.10.4. 2ppm test sample preparation:

6.10.4.1. Into a 50-mL Nessler color comparison tube, dissolve 10.0 g of sample in ~40mL of purified water.

6.10.5. Standard Lead Solution:

6.10.5.1. On the day of use, dilute 10.0 mL of Lead Nitrate Stock Solution to 100 mL with purified water in a volumetric flask.

6.10.6. Standard Preparation:

6.10.6.1. Into a 50-mL Nessler color-comparison tube, pipette 2 mL of Standard Lead Solution, and add ~ 40 mL of purified water.

6.10.7. Monitor Preparation:

6.10.7.1. Place 40 mL of a solution as directed for the required Test Preparation and add 2.0 mL of Standard Lead Solution.

6.10.8. Procedure:

6.10.8.1. To each solution, add 2 mL of pH 3.5 Acetate Buffer and 1.2 mL of thioacetamide-glycerin base TS (1 mL of glycerin TS and 0.2 mL of thioacetamide TS. Heat gently and use immediately). Dilute to 50 mL with purified water, parafilm, and mix by inversion.

6.10.8.2. Allow to stand for 2 minutes using a calibrated timer.

6.10.8.3. View downward over a white surface; the color of the solution from the Test Preparation is not darker than that of the solution from the Standard Preparation, and the color of the solution of the Monitor Preparation is equal to or darker than that of the Standard Preparation.

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

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

6.12. **IDENTIFICATION (CHLORIDE)** **PASSES TEST:**

6.12.1. Accurately weigh 7.88 g of sample and transfer to a 100 mL volumetric flask.
6.12.2. Q.S. to volume with purified water.
6.12.3. The solution from pH 0.5M analysis may be used.
6.12.4. Transfer 2 mL of the sample solution to a beaker and add ~0.2 mL of 0.1N Silver Nitrate. A white, curdy precipitate that is insoluble after the addition of 1 mL of concentrated nitric acid is produced. If no precipitate is produced, notify the appropriate personnel.
6.12.5. Add 4 mL of 6N Ammonium Hydroxide. The precipitate should dissolve after mild agitation.

6.13. **INSOLUBLE MATTER** **0.001% MAX.:**

6.13.1. Weigh 60.0 g of sample and transfer to a beaker.
6.13.2. Add 600 mL of water and utilize a Teflon encapsulated magnetic stirring bar and electric stir plate to dissolve sample.
6.13.3. Heat to boiling and digest on a hot plate in a covered beaker for 1 hour.
6.13.4. Prepare a crucible and 10-15µm filter by drying at 105°C ± 2°C for 1 hour. Record weight of the crucible and filter after cooling in ambient air for at least 15 minutes.
6.13.5. Filter sample solution through the previously prepared crucible, and rinse thoroughly with at least 150 mL of hot purified water.
6.13.6. Dry the crucible in the oven at 105°C ± 2°C for 1 hour.
6.13.7. Cool in ambient air for at least 15 minutes and reweigh.
6.13.8. Calculate the % Insoluble Matter as follows:

$$\%\text{Insoluble Matter} = \frac{\text{Residue Weight (g) x 100}}{\text{Sample Weight (g)}}$$

6.14. **LOSS ON DRYING @ 105°C** **REFER TO SUMMARY SHEET:**

6.14.1. Tare an LOD vial that has been previously dried for 30 minutes in the oven at 105°C ± 2°C.
6.14.2. Allow to come to room temperature in a desiccator for at least 15 minutes before weighing.
6.14.3. Transfer 2-3 g of the sample to be tested to the LOD vial, and accurately weigh the vial and contents. By gentle, sidewise shaking, distribute the sample as evenly as possible in the weighing bottle.
6.14.4. Place the LOD vial containing the sample into the oven.
6.14.5. Dry the sample at 105°C ± 2°C for 3 hours.
6.14.6. Allow to come to room temperature in a desiccator for at least 15 minutes before weighing.
6.14.7. Calculate result using the equation below:

$$\%\text{LOD} = \frac{\text{Initial Sample Weight (g)} - \text{Final Sample Weight (g)}}{\text{Initial Weight (g)}} \text{ x } 100$$

6.15. **LOSS ON DRYING @ 110°C** **REFER TO SUMMARY SHEET:**

6.15.1. Tare an LOD vial that has been previously dried for 30 minutes in the oven at 110°C ± 2°C.

6.15.2. Allow to come to room temperature in a desiccator for at least 15 minutes before weighing.
6.15.3. Transfer 2-3 g of the sample to be tested to the LOD vial, and accurately weigh the vial and contents. By gentle, sidewise shaking, distribute the sample as evenly as possible in the weighing bottle.
6.15.4. Place the LOD vial containing the sample into the oven.
6.15.5. Dry the sample at 110°C ± 2°C for 3 hours.
6.15.6. Allow to come to room temperature in a desiccator for at least 15 minutes before weighing.
6.15.7. Calculate result using the equation below:

$$\%\text{LOD} = \frac{\text{Initial Sample Weight (g)} - \text{Final Sample Weight (g)}}{\text{Initial Weight (g)}} \text{ x } 100$$

6.16. **MELTING RANGE** **REFER TO SUMMARY SHEET:**
6.16.1. Refer to MP50 Melting Range Operation and Calibration SOP.

6.17. **MICROBIAL ANALYSIS** **REFER TO SUMMARY SHEET:**
6.17.1. Microbial Analysis will be performed by an approved outside testing facility.
6.17.2. Package and send 35 grams of sample to the approved outside testing facility.

6.18. **OPTICAL DENSITY AT 290NM** **REFER TO SUMMARY SHEET:**
6.18.1. Accurately weigh 20.0 grams of sample and dissolve in 50 mL of purified water.
6.18.1.1. If needed to dissolve, the sample may be gently heated. Allow the solution to return to room temperature.
6.18.2. Measure the absorbance of the sample at 290 nm using the Lambda 25 UV/Vis.

6.19. **PH (1:10) @ 25°C ±2°C** **REFER TO SUMMARY SHEET:**
6.19.1. Accurately weigh 10 g of sample. Transfer to a suitable beaker.
6.19.2. Dissolve in 100 mL of purified water. Cover with parafilm and mix until thoroughly dissolved.
6.19.2.1. Measure and record the pH using the appropriate SOP.

6.20. **PH (0.5M) @ 25°C ±2°C** **REFER TO SUMMARY SHEET:**
6.20.1. Accurately weigh 7.88 g of sample and transfer to a 100-mL volumetric flask.
6.20.2. Q.S. to volume with purified water. Mix until thoroughly dissolved.
6.20.2.1. Measure and record the pH using the appropriate SOP.
6.20.3. This solution can be utilized for the Identification (Chloride) test.

6.21. **PH (1.0M) @ 25°C ±2°C** **REFER TO SUMMARY SHEET:**
6.21.1. Accurately weigh 15.76 g of sample and transfer to a 100-mL volumetric flask
6.21.2. Q.S. to volume with purified water. Mix until thoroughly dissolved.
6.21.2.1. Measure and record the pH using the appropriate SOP.
6.21.3. This solution can be utilized for Solubility (1M Solution).

6.22. **pH (1%) @ 25°C ±2°C** **REFER TO SUMMARY SHEET:**
6.22.1. Accurately weigh 1.0 g of sample. Transfer to a suitable beaker.
6.22.2. Dissolve in 100 mL of purified water. Cover with parafilm and mix until thoroughly dissolved.
6.22.2.1. Measure and record the pH using the appropriate SOP.

6.23. **pH (5%) @ 25°C ±2°C** **REFER TO SUMMARY SHEET:**

6.23.1. Accurately weigh 5.0 g of sample. Transfer to a suitable beaker.

6.23.2. Dissolve in 100 mL of purified water. Cover with parafilm and mix until thoroughly dissolved.

6.23.2.1. Measure and record the pH using the appropriate SOP.

6.24. **pH (10%) @ 25°C±2°C** **REFER TO SUMMARY SHEET:**

6.24.1. Accurately weigh 10.0 g of sample. Transfer to a suitable beaker.

6.24.2. Dissolve in 100 mL of purified water. Cover with parafilm and mix until thoroughly dissolved.

6.24.2.1. Measure and record the pH using the appropriate SOP.

6.25. **pKa** **8.0-8.4:**

6.25.1. Weigh 0.5g of sample and add 50-100 mL of purified water.

6.25.2. Titrate to a potentiometric end-point utilizing the Metrohm Titrando 907 using 0.1N NaOH.

6.26. **RESIDUAL ETHANOL, IPA, and METHANOL** **REFER TO SUMMARY SHEET:**

6.26.1. Residual solvent analysis will be performed by an outside laboratory on validation batches and on one batch yearly. Prepare and send a 10g sample.

6.27. **RESIDUE ON IGNITION** **REFER TO SUMMARY SHEET:**

6.27.1. Turn on the muffle furnace and allow it to stabilize at 600°C.

6.27.2. Inspect a quartz crucible for cracks, chips and discoloration. Use the long 10 inch forceps to place the crucible in the furnace and to remove the crucible from the furnace. Do not touch any surface of the furnace, or you will get burned!

6.27.3. Ignite quartz crucible at 600 ± 50ºC for 30 minutes, cool in a desiccator for 1.5 hours and weigh.

6.27.4. Weigh 1.0 g sample in the previously ignited quartz crucible. Moisten the sample with 0.2 mL of sulfuric acid. Volatilize the sample with an appropriate heating apparatus. Keep the sample an appropriate distance and location from the heat source, so that the sample does not boil over and no sample is lost. The rate of heating should be such that from ½ to 1 hour is required to volatilize the sample.

6.27.5. Continue to heat the sample until all the excess sulfuric acid has been volatilized. Ignite in a muffle furnace at 600 ± 50ºC for 15 minutes or until all carbon has been removed.

6.27.6. Cool in a desiccator for 1.5 hours and reweigh.

6.27.7. The weight of residue should not exceed 0.001 grams (0.1 %)

$$\%RO = \frac{\text{Residue Weight (g)} * 100}{\text{Sample Weight (g)}}$$

6.28. **SOLUBILITY (1M SOLUTION)** **PASSES TEST:**

6.28.1. Dissolve 15.76 g of sample in 100 mL of purified water.

6.28.1.1. The solution made for pH (1.0M) may be utilized for this analysis.

6.28.2. Observe under sufficient light.

6.28.3. The solution should be clear and colorless.

6.29. **SOLUBILITY (35% SOLUTION)** **PASSES TEST:**

6.29.1. Weigh 35.0 g of sample and transfer to a graduated cylinder.

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6.29.2. Q.S. to 100 mL with purified water. Cover with parafilm and mix until thoroughly dissolved.
6.29.3. Transfer solution into a clean beaker and observe under sufficient light.
6.29.4. Solution should be clear and colorless.

6.30. **TRACE ELEMENTS** **REFER TO SUMMARY SHEET:**

6.30.1. Refer to NexION 350X ICP-MS SOP,
6.30.2. Refer to DCN:20-003510 and 20-003538 for quantitative method analysis.

6.31. **WATER (BY KARL FISCHER TITRATION)** **0.5% MAX.:**

6.31.1. Standardize Composite 5 as per Standardization of Titrants.
6.31.2. Grind the sample in a dry mortar into a fine powder utilizing a pestle.
6.31.3. Immediately weigh 0.8g of sample into the glass weighing spoon and tare it.
6.31.4. Transfer the sample to the KF vessel by removing the rubber septum and adding the sample into the titration vessel.
6.31.4.1. Do not leave the rubber septum open for long periods of time as this will allow moisture to enter the titration vessel.
6.31.5. 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.
6.31.6. Check to make sure there is no residual sample stuck to the sides of the titration vessel.
6.31.6.1. If there is any sample stuck to the side, stop the stir bead from spinning before swirling the vessel to rinse the sides.
6.31.7. Once the method begins, check to ensure the sample is fully dissolved before the titration begins (i.e. before the stir command completes).
6.31.8. The moisture content will then be determined by the Metrohm Auto Titrando 907.

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