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L-CYSTINE DIHYDROCHLORIDE TESTING METHODS

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1. PURPOSE:

1.1. To provide Laboratory personnel with procedures for testing L-Cystine diHCl Raw Material, In-Process and Finished Goods.

2. SCOPE:

2.1. Applies to the testing of L-Cystine diHCl Raw Material, In-Process and Finished Goods in the Laboratory at both the Bangor, PA location and the Stroudsburg, PA location (if applicable instrumentation is present). Methods include testing for all types of L-Cystine diHCl sold by BioSpectra; only the specific tests required for the requested type must be tested.

3. RESPONSIBILITIES:

3.1. The Laboratory Manager is responsible for control, training, maintenance, and implementation of this procedure.

3.2. The Analysts are responsible for compliance with the terms of this procedure. This includes notifying the Laboratory Manager or designee if any analyses fail to meet their respective specifications.

4. EQUIPMENT:

4.1. Analytical Balance
4.2. Blue M Oven, or equivalent
4.3. Metrohm 907 Titrando Auto-Titrator
4.4. Muffle Furnace
4.5. Perkin Elmer NexION 350X ICP-MS
4.6. Perkin Elmer Spectrum Two UATR
4.7. MCP5300 Polarimeter
4.8. XL200 pH/Conductivity Meter or equivalent.
4.9. OPI-180 OD Handheld Colorimeter SOP

5. REAGENTS:

5.1. **0.1N $AgNO_3$:** Purchased Commercially.

5.2. **0.2% Polyvinyl Alcohol (PVA):** Dissolve 2.0 g of polyvinyl alcohol in approximately 800 mL of purified water while gently heating and stirring. Once dissolved, remove the stir bar and Q.S to 1000 mL with purified water.

5.3. **1M HCl:** Purchased Commercially.

5.4. **15% Nitric Acid:** Utilize the following calculation: $V1 = \frac{M1V1}{M2}$

5.4.1. M1 = CoA % value for Concentrated Nitric Acid
5.4.2. M2 = 15%
5.4.3. V2 = Total solution volume (Ex: 100 mL)
5.4.4. Pipette calculated Volume (V1) of concentrated nitric acid into the appropriate size volumetric flask containing a small amount of purified water. Dilute to volume with purified water.

5.5. **Glacial Acetic Acid:** Purchased Commercially.

5.6. **Methanol:** Purchased Commercially:

5.7. **Sulfuric Acid:** Purchased Commercially.

5.8. **0.25M Tris Base:** Purchased Commercially.

5.9. **EDTA:** Purchased Commercially.

5.10. **LAL Reagent Water:** Purchased Commercially

6. REFERENCES:

6.1. BSI-ATM-0085, Analytical Method for the Determination of ICH Q3D Elemental Impurities via ICP-MS in L-Cystine DiHCl
6.2. BSI-MEM-0130, Endosafe NexGen PTS Endotoxin Reader: Qualified Products
6.3. BSI-SOP-0019, Result Reporting
6.4. BSI-SOP-0090, Lambda 25 UV/Vis Operation and Calibration
6.5. BSI-SOP-0094, Muffle Furnace SOP and Calibration
6.6. BSI-SOP-0098, Balance SOP
6.7. BSI-SOP-0126, Laboratory Notebooks
6.8. BSI-SOP-0140, Standardization of Titrants
6.9. BSI-SOP-0143, Metrohm Titrando 907 Auto-Titrator SOP
6.10. BSI-SOP-0254, Spectrum Two UATR SOP
6.11. BSI-SOP-0255, XL200 pH/mV/Conductivity Meter SOP
6.12. BSI-SOP-0256, MP50 Melting Range Operation, Verification and Calibration SOP
6.13. BSI-SOP-0257, MCP 300 Polarimeter SOP
6.14. BSI-SOP-0303, NexION 350X ICP-MS SOP
6.15. BSI-SOP-0345, Endosafe nexgen-PTS Endotoxin Reader SOP
6.16. BSI-SOP-0490, MCP 5300 Polarimeter SOP
6.17. BSI-SOP-0668, OPI-180 OD Handheld Colorimeter SOP
6.18. Current USP

7. ANALYTICAL PROCEDURES:

7.1. **APPEARANCE AND COLOR :**

7.1.1. Place a suitable amount of sample on a white piece of filter paper.
7.1.1.1. Approximately 5 grams is suggested but more or less may be used.
7.1.2. In an area with sufficient lighting, spread the sample over white filter paper and observe color, crystalline form, and any foreign particulate matter.
7.1.3. If the appearance and color result is unable to be definitely determined visually, the sample may be analyzed using the Colorimeter. Refer to BSI-SOP-0668, OPI-180 OD Handheld Colorimeter SOP
7.1.4. Any non-conformance will be reported to the Laboratory Manager, or designee, immediately.

7.2. **ASSAY (DRIED BASIS) L-CYSTINE DIHCL |CL :**

7.2.1. Standardize 0.1N $AgNO_3$ as per Standardization of Titrants.
7.2.2. Accurately weigh ~0.4 g of sample that has been previously dried at 105°C for 3 hours.
7.2.2.1. LOD sample may be retained and used for assay.
7.2.3. Transfer to a 250 mL beaker and dissolve with ~10 mL of 15% nitric acid solution.
7.2.4. Add 10 mL of glacial acetic acid, 100mL of methanol, and 10 mL of a 0.2% polyvinyl alcohol solution.
7.2.5. Titrate with 0.1*N* $AgNO_3$ to a potentiometric end-point utilizing the Metrohm Titrando 907.

$$\%\ L-Cystine\ diHCl = \frac{(mL\ AgNO_3)(N\ AgNO_3)(31.32)(0.5)}{Sample\ Weight\ (g)}$$

$$\%\ Cl = \frac{(mL\ AgNO_3)(N\ AgNO_3)(3.545)}{Sample\ Weight\ (g)}$$

7.3. **BIOBURDEN :**

7.3.1. Microbial analysis will be performed by an outside testing laboratory.

7.3.2. Primary Provider: Mary Paul Laboratories (MPL).

7.3.3. Package and send NLT 35 grams of sample to MPL with a purchase order and analysis request form for TAMC and TYMC Analysis.

7.3.4. Analyses:

7.3.4.1. Total Aerobic Microbial Count (TAMC)

7.3.4.1.1. In accordance with USP<61>, the result for Bioburden will be reported from the TAMC result only.

7.3.4.1.2. If there is growth, Identification is required.

7.3.4.2. Total Yeasts and Molds Count (TYMC)

7.3.4.2.1. TYMC will be tested for Internal Information Only and will not be officially reported.

7.3.4.2.2. If there is growth, Identification is required.

7.4. **ENDOTOXIN :**

7.4.1. Analysis can be performed utilizing an internal method or be sent to an external lab (NAMSA) for testing.

7.4.1.1. For Internal Testing (RMAT-0165):

7.4.1.1.1. Accurately weigh 100 mg of sample into a sterile tube.

7.4.1.1.2. Add 5 mL of LAL reagent water and 1 mL of 1N HCl, dissolve and mix thoroughly.

7.4.1.1.3. Dilute to 10mL with LAL reagent water and mix thoroughly.

7.4.1.1.4. Transfer 0.5 mL of resulting solution into a separate sterile tube, add 5.5 mL of LAL reagent water, and adjust pH between 6-8 using 0.25M Tris Base Solution, mix thoroughly.

7.4.1.1.5. Dilute to 10 mL with LAL reagent water and mix thoroughly for a final concentration of 0.5 mg/mL.

7.4.1.2. For Outside Testing (For BSI Manufactured Product only): Prepare an outside testing sample of ~30 g in a sterile container, analysis request form,and ship to NAMSA for Endotoxin testing.

7.5. **HEAVY METALS AS PB :**

7.5.1. Refer to BSI-ATM-0085 for sample preparation and analysis. Method may be truncated down to analyze for Iron and Lead.

7.5.1.1. NOTE: Monitor sample for Iron .

7.6. **IDENTIFICATION UATR :**

7.6.1. Follow Spectrum Two UATR SOP.

7.7. **LOSS ON DRYING (105°C) :**

7.7.1. Dry a LOD vial in an oven at 105 ± 2˚C for at least 30 minutes. Cool for 15 minutes in a desiccator, weigh, and record weight.

7.7.2. Place the vial on the analytical balance and tare the dried vial. Weigh 1-2 g of sample and record weight.

7.7.3. Dry for 3 hours at 105˚C. Cool for 15 minutes in desiccator.

7.7.3.1. Retain sample as needed for assay, dried basis.

7.7.4. Reweigh and calculate the % LOD.

7.7.5. Calculate Loss on Drying as follows:

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

7.8. **pH 0.1% Solution :**

7.8.1. Weigh 0.10 g of sample 100 mL of purified water. Dissolve.

7.8.2. Follow the appropriate SOP for pH measurement.

7.9. **Residue on Ignition :**

7.9.1. Turn on muffle furnace and allow temperature to stabilize at 600 ± 50 °C. Follow muffle furnace SOP and calibration procedure for operation.

7.9.2. Inspect crucible for discoloration or chips.

7.9.3. Utilize the 10-inch forceps to insert and place a crucible into the furnace.

7.9.4. Ignite the quartz crucible at 600 ± 50 °C for 30 minutes. Cool in a desiccator for 90 minutes and record weight utilizing an analytical balance.

7.9.5. Weigh 1-2 g sample in the previously ignited quartz crucible. Moisten the sample with 1mL of sulfuric acid.

7.9.6. Volatilize the sample with a hot plate or equivalent heating source, such that it is heated gently until white fumes are no longer evolved. Keep the sample at the appropriate temperature, so that the sample does not boil over and sample is not lost.

7.9.6.1. The rate of heating should be such that from ½ to 1 hour is required to volatilize the sample.

7.9.6.2. Continue using the hot plate or equivalent heating source to heat the sample until all excess sulfuric acid has been volatilized.

7.9.7. Ignite in the muffle furnace at 600 ± 50°C for 15 minutes or until all carbon has been removed.

7.9.8. Cool in the desiccator for 90 minutes and reweigh.

7.9.9. Calculate ROI as follows:

$$\%\ ROI = \frac{Residue\ Weight\ (g)}{Sample\ Weight\ (g)}\ x\ 100$$

7.10. **Solubility :**

7.10.1. Prepare solution by dissolving 1 g in 10 mL of 1M HCl.

7.10.2. Swirl to dissolve completely.

7.10.3. Observe from all angles. Sample solution should be clear and complete.

7.11. **Specific Rotation @ 20°C :**

7.11.1. Accurately weigh 2.00 g of sample and transfer to a 100 mL volumetric flask.

7.11.2. Dissolve sample in 1M HCl and QS to a final volume of 100 mL with 1M HCl.

7.11.3. The MCP5300 or MCP300 may be used for analysis.

7.11.3.1. For the MCP5300, refer to MCP 5300 Polarimeter SOP, BSI-SOP-0490 and analyze within 30 minutes of preparation.

7.11.3.2. For the MCP300, refer to MCP 300 Polarimeter SOP, BSI-SOP-0257 and analyze within 30 minutes of preparation.

7.11.4. Result calculated on a free basis:

$$\text{Specific Rotation} = Result(1.303)$$

7.11.4.1. Calculation performed in MCP5300 Polarimeter Software.

7.11.4.2. Calculation performed by hand when using MCP300 Polarimeter.