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CYSTEAMINE HCL (2-MEA)
TESTING METHODS

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1. PURPOSE:

1.1. To provide the Laboratory personnel with a procedure for examining 2-MEA Raw Materials, In Process, Stability, and Finished Goods.

2. SCOPE:

2.1. Applies to examination of 2-MEA in the Laboratory. Methods include testing for all types of 2-MEA received, produced, or sold by BioSpectra; only the specific tests required for the desired type must be tested for. This document applies to both the Bangor, PA and Stroudsburg, PA BioSpectra facilities.

3. RESPONSIBILITIES:

3.1. The Laboratory Manager, or qualified designee, 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 the Laboratory Manager, or designees, if any analyses fail to meet their respective specifications.

4. SAFETY CONSIDERATIONS:

4.1. Read and understand the SDS for any chemical prior to use. Wear appropriate PPE and dispose of non-compatible chemicals in separate waste streams.

5. EQUIPMENT:

5.1. Analytical Balance
5.2. Calibrated Oven
5.3. Hach Portable Turbidimeter
5.4. Waters Alliance HPLC
5.5. OPI-180 OD Handheld Colorimeter
5.6. Perkin Elmer NexION 350X ICP-MS
5.7. Perkin Elmer Spectrum Two UATR
5.8. Metrohm Titrando 907 or Equivalent
5.9. Gas Chromatograph Equipped with Head Space Autosampler and FID

6. REAGENTS:

6.1. **0.1N $AgNO_3$:** Purchased Commercially.
6.2. **30% Hydrogen Peroxide:** Purchased Commercially.
6.3. **Glacial Acetic Acid:** Purchased Commercially.
6.4. **Methanol:** Purchased Commercially.
6.5. **Eosin Y TS:** Dissolve 50mg of Eosin Y in 10mL of purified water.
6.6. **2-MEA Reference Standard:** Purchased Commercially.
6.7. **LAL Reagent Water:** Purchased Commercially.
6.8. **0.1-1.0 EU/mL Endotoxin Cartridges:** Purchased Commercially.

7. REFERENCES:

7.1. BSI-ATM-0061, Method of Analysis: Determination of Elemental Impurities by ICP-MS in 2-MEA
7.2. BSI-ATM-0122, Cysteamine HCl (2-MEA) via HPLC with UV Detection
7.3. BSI-PRL-0403, Analytical Method Validation Protocol: Aqueous Soluble Residual Solvents (2-MEA)
7.4. BSI-RPT-1760, Analytical Method Transfer Report: Cysteamine HCl (2-MEA) via HPLC with UV Detection
7.5. BSI-SOP-0091, Portable Turbidimeter SOP and Calibration

7.6. BSI-SOP-0098, Balance SOP
7.7. BSI-SOP-0126, Laboratory Notebooks
7.8. BSI-SOP-0133, Blue M Convection Oven Operation and Calibration SOP
7.9. BSI-SOP-0140, Standardization of Titrants
7.10. BSI-SOP-0143, Metrohm Titrando 907 Auto-Titrator SOP
7.11. BSI-SOP-0242, Bangor Portable Turbidimeter Operation and Calibration
7.12. BSI-SOP-0244, VWR Gravity Convection Oven Operation and Calibration (Model Number 414005-106)
7.13. BSI-SOP-0254, Spectrum Two UATR SOP
7.14. BSI-SOP-0303, NexION 350X ICP-MS SOP
7.15. BSI-SOP-0422, Empower 3 General Procedure
7.16. BSI-SOP-0668, OPI-180 OD Handheld Colorimeter SOP
7.17. *ACS, Reagent Chemicals,* current edition
7.18. *Current EP*
7.19. *Current USP*

8. ANALYTICAL PROCEDURES:

8.1. APPEARANCE AND COLOR :

8.1.1. Place a suitable amount (10-20 g, if available) of sample in a clean, dry glass beaker.
8.1.2. In an area with sufficient lighting, view the sample from all sides.
8.1.3. The sample should be white or colorless crystals or powder that may contain lumps.
8.1.4. If the appearance and color result is unable to be definitively determined visually, the sample may be analyzed using the Colorimeter. Refer to BSI-SOP-0668, OPI-180 OD Handheld Colorimeter SOP.
8.1.5. If the sample does not conform to these specifications, or if particulates are noted, notify the Laboratory Manager or Supervisor immediately.

8.2. APPEARANCE OF SOLUTION :

8.2.1. Solution S Preparation
8.2.1.1. Prepare a 10% solution by weighing 10 g of sample and diluting to 100 mL with purified water.
8.2.1.2. Dissolved the sample and mix thoroughly.
8.2.1.3. Solution S should be clear and colorless.
8.2.1.3.1. Perform the appropriate test to determine if the sample meets requirements.
8.2.1.3.2. Turbidity Observed: Follow the appropriate SOP as follows:
8.2.1.3.2.1. Stroudsburg - Measure and record the turbidity of the sample according to Portable Turbidimeter Operation and Calibration.
8.2.1.3.2.2. Bangor - Measure and record the turbidity of the sample according to Bangor Portable Turbidimeter SOP.
8.2.1.3.2.3. The sample solution must be ≤ 3 NTU to pass test.
8.2.1.3.3. Color Observed:
8.2.1.3.3.1. Add 10 mL of Solution S into a Nessler Color Comparison Tube.
8.2.1.3.3.2. Add 10 mL of USP Purified Water into a second Nessler Color Comparison Tube.
8.2.1.3.3.3. Compare the colors in sufficient lighting, viewing vertically against a white background.
8.2.1.3.3.4. In order for the sample solution to be colorless, it must have the appearance of *USP Purified Water.*
8.2.2. If the sample does not conform to these specifications, notify the Laboratory Manager or Supervisor immediately.

8.3. ARGENTOMETRIC TITRATION :

8.3.1. Manual Assay Method:
8.3.1.1. Standardize or perform a daily check of 0.1N AgNO3 by hand as per Standardization of Titrants.
8.3.1.2. Accurately weigh 0.40 g of sample into a suitable beaker.
8.3.1.3. Dissolve with 20 mL of 30% hydrogen peroxide, cover with a watch glass and digest with heat until the first sign of bubbles appears in the beaker. Remove from heat immediately. Do not boil. Rinse the sides of the beaker and watch glass with a small amount of water after digestion ensuring zero sample loss.
8.3.1.4. Add 5 mL of glacial acetic acid and 50 mL of methanol.
8.3.1.5. Add 1 mL of Eosin Y.
8.3.1.6. Titrate to a pink endpoint.

8.3.1.7. Calculate % Cl using the following equation:

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

8.4. **ASSAY/IMPURITIES :**

8.4.1. Refer to DCN: BSI-ATM-0122 for Primary Method via HPLC.

8.5. **BIOBURDEN :**

8.5.1. Microbial analysis will be performed by an Outside Testing Laboratory.
8.5.1.1. Primary provider Mary Paul Laboratories.
8.5.1.2. Package and send NLT 30 g of sample to Mary Paul Laboratories with a Purchase Order and Analysis Request Form.
8.5.2. Analyses:
8.5.2.1. Total Aerobic Microbial Count (TAMC)
8.5.2.2. Total Yeasts and Molds Count (TYMC)

8.6. **ENDOTOXINS :**

8.6.1. Accurately weigh 60 mg of sample.
8.6.2. Hygienically transfer to a sterile tube with a capacity of greater than 10 mL.
8.6.3. Dilute to 10 mL with LAL reagent water, dissolve and mix thoroughly.
8.6.4. Transfer 1.0 mL of this solution to a sterile tube with a capacity of greater than 10 mL.
8.6.5. Dilute to 10 mL with LAL reagent water for a concentration of 0.0006 g/mL.
8.6.6. Dilute this solution 1:1 with LAL reagent water for a final concentration of 0.0003 g/mL and mix thoroughly.
8.6.7. Refer to Endosafe nexgen-PTS Endotoxin Reader SOP for instrument operation and sample analysis.

8.7. **HEAVY METALS :**

8.7.1. Refer to Method of Analysis: Determination of Elemental Impurities by ICP-MS in 2-MEA: DCN BSI-ATM-0061 for sample preparation and analysis.

8.8. **IDENTIFICATION (IR, HPLC) :**

8.8.1. IR: Analyze as-is against an appropriate reference standard, if correlation does not meet specification of NLT 0.95, then the sample and standard should be dried following the Loss on Drying procedure. Follow the Spectrum Two UATR instrument SOP for instrument operation instructions.
8.8.2. HPLC: The major peak retention time of the sample solution corresponds to the major peak retention time of the system suitability solution as assessed in section 8.4.1.

8.9. **LOSS ON DRYING :**

8.9.1. Dry an LOD vial in the oven at 60 ± 2˚C for at least 30 minutes.
8.9.2. Cool for 15 minutes in a desiccator, weigh the LOD vial, and record results.
8.9.3. If the substance to be tested is in the form of large crystals, reduce the particle size by quickly crushing before weighing.
8.9.4. Transfer 1-2 grams of the sample to the LOD vial and accurately weigh the vial and contents. By gentle, sidewise shaking, distribute the sample as evenly as possible in the LOD vial.
8.9.5. Place the LOD vial containing the sample into the oven and dry at 60°C ± 2°C for 3 hours.

8.9.6. Remove LOD vial from the oven and allow it to cool in a desiccator for 15 minutes. Reweigh the LOD vial and sample.

8.9.7. Calculate the %LOD as follows:

$$\%\ LOD = \frac{initial\ sample\ weight\ (g) - final\ sample\ weight\ (g)}{initial\ sample\ weight\ (g)}\ x\ 100$$

8.10. **METAL TRACE ANALYSIS :**

8.10.1. Refer to Method of Analysis: Determination of Elemental Impurities by ICP-MS in 2-MEA: BSI-ATM-0061 for sample preparation and analysis.

8.11. **HPLC PURITY (AREA %) :**

8.11.1. Refer to section 8.4 for analysis.

8.12. **RESIDUAL SOLVENTS :**

8.12.1. Residual Solvents analysis is performed utilizing a validated in-house method.

8.12.1.1. In-house method: DCN BSI-PRL-0403 (Analytical Method Validation Protocol: Aqueous Soluble Residual Solvents (2-MEA)).

8.13. **SOLUBILITY :**

8.13.1. Solution Preparation

8.13.1.1. Prepare a 10% solution by weighing 10g of sample and diluting to 100 mL with purified water.

8.13.1.2. Dissolved the sample and mix thoroughly.

8.13.2. The resulting 10% sample solution should be clear and complete.

8.13.2.1. If any insoluble matter is present, refer to insoluble matter specification for material disposition.