BIOSPECTRA
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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 Director of Laboratory Testing is responsible for the control, training, maintenance and implementation of this procedure.

3.2. The Laboratory Analysts 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. REFERENCES:

5.1. BSI-ATM-0061, Method of Analysis: Determination of Elemental Impurities by ICP-MS in 2-MEA

5.2. BSI-PRL-0403, Analytical Method Validation Protocol: Aqueous Soluble Residual Solvents (2-MEA)

5.3. BSI-SOP-0091, Portable Turbidimeter SOP and Calibration

5.4. BSI-SOP-0098, Balance SOP

5.5. BSI-SOP-0126, Laboratory Notebooks

5.6. BSI-SOP-0133, Blue M Convection Oven Operation and Calibration SOP

5.7. BSI-SOP-0140, Standardization of Titrants

5.8. BSI-SOP-0143, Metrohm Titrando 907 Auto-Titrator SOP

5.9. BSI-SOP-0242, Bangor Portable Turbidimeter Operation and Calibration

5.10. BSI-SOP-0244, VWR Gravity Convection Oven Operation and Calibration (Model Number 414005-106)

5.11. BSI-SOP-0254, Spectrum Two UATR SOP

5.12. BSI-SOP-0303, NexION 350X ICP-MS SOP

5.13. *ACS, Reagent Chemicals,* current edition

5.14. *Current EP*

5.15. *Current USP*

6. EQUIPMENT:

6.1. Analytical Balance

6.2. Calibrated Oven

6.3. Color Wheel Tray

6.4. Hach Portable Turbidimeter

6.5. Perkin Elmer Flexar HPLC

6.6. Perkin Elmer NexION 350X ICP-MS

6.7. Perkin Elmer Spectrum Two UATR

6.8. Metrohm Titrando 907 or Equivalent

6.9. Gas Chromatograph Equipped with Head Space Autosampler and FID

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7. REAGENTS:

7.1. 0.1N $AgNO_3$: Purchased Commercially OR Dissolve about 17.5 g of silver nitrate in 1000 mL of purified water.
7.2. 30% Hydrogen Peroxide: Purchased Commercially.
7.3. Glacial Acetic Acid: Purchased Commercially.
7.4. Methanol: Purchased Commercially.
7.5. 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.
7.6. Eosin Y TS: Dissolve 50 mg of Eosin Y in 10 mL of purified water.
7.7. 1-Heptanesulfonic Acid Sodium Salt: Purchased Commercially.
7.8. Acetonitrile: Purchased Commercially.
7.9. 2-MEA Reference Standard: Purchased Commercially.
7.10. LAL Reagent Water: Purchased Commercially.

8. ANALYTICAL PROCEDURES:

8.1. APPEARANCE AND COLOR — REFER TO SUMMARY SHEET:

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 sample does not conform to these specifications, or if particulates are noted, notify the Laboratory Manager or Supervisor immediately.
8.1.5. If the sample fails or is suspect, a color wheel evaluation may be done to compare the suspect sample to multiple samples of material that are considered acceptable for Appearance and Color.
8.1.5.1. Place a suitable amount of the sample (approximately 5 to 10 g) in one well of the color wheel tray and label it.
8.1.5.2. Place a suitable amount of comparison materials (approximately 5 to 10 g each) in the other wells and label them. Comparison materials can be: Alternate lots with acceptable Appearance and Color results, finished goods retains, alternate timepoint stability samples (such as T=Extra), and raw materials.
8.1.5.3. Evaluate the suspect sample directly versus the comparison samples to determine if it is acceptable for Appearance and Color.

8.2. APPEARANCE OF SOLUTION — REFER TO SUMMARY SHEET:

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.

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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 REFER TO SUMMARY SHEET:

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 REFER TO SUMMARY SHEET:

8.4.1. Solution Preparations:

8.4.1.1. *Diluent*: HPLC Grade water or equivalent.

8.4.1.2. *Mobile Phase*: Weigh 0.81 g of 1-Heptanesulfonic Acid Sodium Salt and dissolve into 850 mL with HPLC Grade water or equivalent, add 1.18 mL of 85% o-phosphoric acid and add 150 mL of acetonitrile and mix thoroughly. Scale as required.

8.4.1.3. *Column Storage Solution*: Store column with mobile phase. Allow new column to equilibrate at least 24 hours before use. Column may NOT be used for any other mobile phase after it is paired with the ion pairing agent.

8.4.1.4. *Standard Solution (4.0 mg/mL 2-MEA, prepare in duplicate):* Accurately weigh 0.40 g of 2-MEA RS (Reference Standard) and transfer aliquot quantitatively to a 100.0 mL volumetric flask. Dissolve in diluent. Dilute to volume with diluent and mix thoroughly.

8.4.1.4.1. **Label SS1and SS2, respectively.**

8.4.1.5. *Sample Solution (4.0 mg/mL 2-MEA)*: Accurately weigh 0.40 g of 2-MEA and transfer aliquot quantitatively to a 100.0 mL volumetric flask. Dissolve in diluent. Dilute to volume with diluent and mix thoroughly.

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8.4.2. Set up qualified HPLC equipped with UV detector with the following method parameters.

Parameter	Setting
Flow Type	Isocratic
Mobile Phase	Refer to Section 7.4.1.2.
Flow Rate	1.5 mL/min
Injection Volume	5 µL
Detector	UV- 215 nm
Detector Temperature	Ambient
Column Temperature	35°C
Run Time	10 minutes

8.4.3. Injection sequence.

8.4.3.1. Note: SS1 must be injected after the last sample in the sequence to monitor drift It will be used as a QC Check and must meet the NMT 1.0% criteria for the results to be reportable.

Sample ID	Number of Injections
System Suitability	
Diluent	≥ 2
SS1	5
SS2	2
Samples[1]	
Diluent	1
Samples[2]	≤ 10
SS1	1
[1]Repeat the sample injection sequence if additional samples are to be analyzed. [2]Samples may be substituted with diluent injections.	

8.4.4. Column: 250 x 4.6mm; Gemini 5µm C18 110Å

8.4.5. System Suitability:

8.4.5.1. %RSD of 2-MEA in the first 5 SS1 injections is NMT 1.0%.

8.4.5.2. %RSD of 2-MEA in all SS1 injections in NMT 1.0%

8.4.5.3. %Agreement between the average of the first 5 SS1s and average SS2s is 98-102%.

8.4.6. Processing chromatograms:

8.4.6.1. Enable Peak Detection T= 2.6 minute(s)

8.4.6.2. Area Threshold = 100

8.4.6.3. Noise Threshold = 10

8.4.6.4. Bunching Factor =2

8.4.6.4.1. Note: Variance to these parameters must be documented with sufficient justification and reporting of new integration parameters.

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8.4.7. **Calculations:**

8.4.7.1. **Calculation %w/w Assay:**

$$\% \text{ w/w Assay} = (r_u/r_s)(C_s/C_u)(100)$$

Where:

8.4.7.1.1. R_u = Peak response of 2-MEA from the *Sample Solution*
8.4.7.1.2. R_s= Average peak response of 2-MEA from the Standard Solution
8.4.7.1.3. C_s= Concentration of 2-MEA RS in the standard solution (mg/mL prepared * Purity of RS)
8.4.7.1.4. C_u= Concentration of 2-MEA in the *Sample Solution* (mg/mL)

8.4.7.2. **Calculation 2-MEA Purity:**

$$\text{Area \%} = (R_s/R_t)*100$$

Where:

8.4.7.2.1. R_s = 2-MEA (Cysteamine) Response in Sample Solution
8.4.7.2.2. R_t = Total Response of All Peaks Appropriately Detected

8.4.7.3. **Calculation Total Related Substances Impurity:**

$$\text{Area \%} = (R_i/R_t)*100$$

Where:

8.4.7.3.1. R_i= Total Area Response of All Appropriately Detected Impurities
8.4.7.3.2. R_t = Total Response of All Peaks Appropriately Detected in the Sample Solution.

8.4.7.4. **Calculation Minor Component (Cystamine):**

$$\text{Area \%} = (R_i/R_s)*100$$

Where:

8.4.7.4.1. R_i = Minor Component Response in Sample Solution
8.4.7.4.2. Cystamine relative retention time ~2.5, when specified.
8.4.7.4.3. R_s = Total Response of All Peaks Appropriately Detected in the Sample Solution.

8.4.8. **Result Reporting:**

Impurity Reporting: Total Related Substances and Minor Component 1 (Cystamine)	
Result	**Reporting**
Method LOQ	0.2% w/w
If < 0.2%	Report as < 0.2%
If ≥ 0.2%	Report to two (2) decimal places
If ≥ 1.0%	Report to one (1) decimal place

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

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 20g 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** **REFER TO SUMMARY SHEET:**

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 10mL.
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 0.0003g/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** **REFER TO SUMMARY SHEET:**

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)** **REFER TO SUMMARY SHEET:**

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 7.

8.9. **LOSS ON DRYING** **REFER TO SUMMARY SHEET:**

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:

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

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8.10. **METAL TRACE ANALYSIS** **REFER TO SUMMARY SHEET:**

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 %)** **REFER TO SUMMARY SHEET:**

8.11.1. Refer to section 7.4 for analysis.

8.12. **RESIDUAL SOLVENTS** **REFER TO SUMMARY SHEET:**

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** **REFER TO SUMMARY SHEET:**

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.

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