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GUANIDINE THIOCYANATE TESTING METHODS

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1. PURPOSE:

- 1.1. To provide the Laboratory personnel with a procedure for analyzing Guanidine Thiocyanate Raw Material, In-Process, Finished Goods, and Stability in the Laboratory at all BioSpectra facilities.

2. SCOPE:

- 2.1. Applies to the testing of Guanidine Thiocyanate Raw Material, In-Process, Finished Goods, and stability in the Laboratory at all BioSpectra facilities. Methods include testing for all types of Guanidine Thiocyanate sold by BioSpectra; only the specific tests required for the requested type must be tested.

3. RESPONSIBILITIES:

- 3.1. The Director of Laboratory Testing is responsible for the implementation, control, training and maintenance of this procedure.
- 3.2. The Laboratory Analysts are responsible for compliance with the terms of this procedure. This includes notifying the appropriate personnel if any analyses fail to meet their respective specifications.
- 3.3. All Laboratory personnel are responsible for reviewing the appropriate SDS's prior to handling any chemicals used in this procedure.
- 3.4. All Laboratory personnel are responsible for referring to the applicable summary sheets for specifications.

4. REFERENCES:

- 4.1. BSI-FRM-0728, Analytical Procedure for Gel Assays
- 4.2. BSI-FRM-0745, Analytical Procedure for Protease Assay
- 4.3. BSI-SOP-0019, Result Reporting
- 4.4. BSI-SOP-0090, Lambda 25 UV/VIS Operation and Calibration
- 4.5. BSI-SOP-0095, DNase (Endonuclease) Assay
- 4.6. BSI-SOP-0096, RNase (Ribonuclease) Assay
- 4.7. BSI-SOP-0098, Balance SOP
- 4.8. BSI-SOP-0126, Laboratory Notebooks
- 4.9. BSI-SOP-0133, Blue M Convection Oven Operation and Calibration SOP
- 4.10. BSI-SOP-0134, Pipette SOP
- 4.11. BSI-SOP-0135, Laboratory Chemicals
- 4.12. BSI-SOP-0138, DNase (Exonuclease) Assay
- 4.13. BSI-SOP-0139, Protease Assay
- 4.14. BSI-SOP-0140, Standardization of Titrants
- 4.15. BSI-SOP-0143, Metrohm Titrando 907 Auto-Titrator SOP
- 4.16. BSI-SOP-0244, VWR Gravity Convection Oven and Calibration (Model Number 414005-106)
- 4.17. BSI-SOP-0254, Spectrum Two UATR SOP
- 4.18. BSI-SOP-0255, XL200 pH/mV/Conductivity Meter SOP
- 4.19. BSI-SOP-0256, MP50 Melting Range Operation, Verification, and Calibration SOP
- 4.20. BSI-SOP-0303, NexION 350X ICP-MS SOP
- 4.21. *ACS, Reagent Chemicals*, current edition
- 4.22. *Current EP/BP*

4.23. *Current JP*

4.24. *Current USP*

5. EQUIPMENT:

- 5.1. Analytical Balance
- 5.2. Lambda 25 UV/VIS Spectrophotometer
- 5.3. Metrohm 907 Auto Titrator
- 5.4. MP50 Melting Range Apparatus
- 5.5. Perkin Elmer NexION 350X ICP-MS
- 5.6. Perkin Elmer Spectrum Two UATR
- 5.7. XL200 pH/mV/Conductivity Meter SOP

6. REAGENTS:

- 6.1. **Acetic Acid, Glacial** – Purchased commercially.
- 6.2. **Acetone** – Purchased commercially.
- 6.3. **Barium Chloride, 12%** - Dissolve 12.0 g of Barium Chloride in purified water. Filter into a 100 mL volumetric flask and dilute to volume with purified water.
- 6.4. **Dibutyl Phthalate** – Purchased commercially.
- 6.5. **Ferric Ammonium Sulfate Dodecahydrate** – Purchased commercially.
- 6.6. **Ferric Ammonium Sulfate, aqueous (10 g/100 mL)** – Dissolve 10.0 g of ferric ammonium sulfate dodecahydrate in purified water and dilute to a volume of 100 mL with purified water.
- 6.7. **HCl, concentrated** - Purchased commercially.
- 6.8. **HCL, 0.1N** – Purchased commercially.
- 6.9. **HCL, 0.02N** – Slowly add 20 mL of 0.1N HCl to 80 mL of purified water. Dilute to a volume of 100 mL, cap, and mix thoroughly.
- 6.10. **HCl (1 in 20)** – Dilute 5mL of Hydrochloric Acid to 100mL with purified water.
- 6.11. **Methanol** – Purchased commercially.
- 6.12. **Nitric Acid (HNO₃), concentrated** – Purchased commercially.
- 6.13. **HNO₃, USP Dilute** – Dilute 14.3 mL of nitric acid to 100 mL with purified water.
- 6.14. **Polyvinyl Alcohol** – Purchased commercially.
- 6.15. **Polyvinyl Alcohol, 0.2%** - 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 dilute to a final volume of 1000 mL with purified water.
- 6.16. **Silver Nitrate, 0.1N** – Weigh 1.7 g of Silver Nitrate (AgNO₃) and dilute to 100 mL with purified water.
- 6.17. **Sulfate Standard Solution (0.01 mg/mL)** – Dissolve 0.148 g of anhydrous sodium sulfate in purified water. Dilute to 100 mL with purified water, cap, and mix thoroughly. Dilute 10 mL of this solution, to a volume of 1000 mL with purified water, cap, and mix thoroughly.

7. ANALYTICAL PROCEDURES:**IN-PROCESS TESTING****7.1. MOTHER LIQUOR ASSAY**

- 7.1.1. Accurately weigh 0.36 g of sample and transfer to a suitable beaker.
- 7.1.2. Add 10 mL of purified water, 10 mL of glacial acetic acid, 10 mL of 0.2% polyvinyl alcohol, and 100 mL methanol to the sample beaker.
- 7.1.3. Titrate with 0.1N AgNO₃ to a potentiometric endpoint utilizing the Metrohm Titrand 907.

$$\% \text{ Guanidine Thiocyanate} = \frac{\text{mL of AgNO}_3 \times N \text{ of AgNO}_3 \times 11.82}{\text{Sample Weight (g)}}$$

7.1.4. Alternate Manual Titration

- 7.1.4.1. Accurately weigh 0.36 g of sample and transfer to a beaker and dissolve in 100 mL of purified water.
- 7.1.4.2. Add 5 mL of (10 g/100 mL) Ferric Ammonium Sulfate aqueous, 5 mL of USP Dilute Nitric Acid, and 5 mL of dibutyl phthalate.
- 7.1.4.3. Titrate to a white/colorless endpoint.

$$\% \text{ Guanidine Thiocyanate} = \frac{\text{mL of AgNO}_3 \times N \text{ of AgNO}_3 \times 11.82}{\text{Sample Weight (g)}}$$

7.2. ML ABSORBANCE

- 7.2.1. Prepare a 1:1 dilution of the mother liquor with purified water in a LOD vial or small beaker.
- 7.2.2. Swirl to homogenize.
- 7.2.3. Refer to the Lambda 25 UV/VIS Operation and Calibration procedure to determine the Absorbance of the sample.

7.3. WC ABSORBANCE

- 7.3.1. Accurately weigh 5.0 g of sample and accurately transfer the weighed sample to a graduated cylinder.
- 7.3.2. Dilute to 25 mL with purified water.
- 7.3.3. Dissolve completely.
- 7.3.4. Refer to the Lambda 25 UV/VIS Operation and Calibration procedure to determine the absorbance of the sample.

7.4. Dry Crystal LOSS ON DRYING @ 105°C

- 7.4.1. Tare an LOD vial that has been previously dried for 30 minutes under the same conditions to be employed in the determination. Cool in desiccator for at least 15 minutes before weighing.
- 7.4.2. Transfer approximately 3 g of the sample to be tested to the LOD vial, and accurately weigh the LOD vial and contents. By gentle, sidewise shaking, distribute the sample as evenly as possible.
- 7.4.3. Place the LOD vial containing the sample into the oven.
- 7.4.4. Dry the sample at 105°C ± 2°C for 3 hours.
- 7.4.5. Allow to cool to room temperature in a desiccator for at least 15 minutes before weighing.
- 7.4.6. Calculate the loss according to the following calculation:

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

FINISHED GOOD TESTING

7.5. ABSORBANCE (1.7M)

- 7.5.1. Accurately weigh 5.0 g of sample.
- 7.5.2. Transfer accurately weighed sample to a graduated cylinder and Q.S. to 25 mL with purified water.
- 7.5.3. Dissolve completely.
- 7.5.4. Refer to Lambda 25 UV/VIS Operation and Calibration procedure to determine the Absorbance of the sample.

7.6. ABSORBANCE (70%)

- 7.6.1. Accurately weigh 17.5 g of sample.
- 7.6.2. Transfer accurately weighed sample to a graduated cylinder and Q.S. to 25 mL with purified water.
- 7.6.3. Dissolve completely.
- 7.6.4. Refer to Lambda 25 UV/VIS Operation and Calibration procedure to determine the Absorbance of the sample.

7.7. ACETONE TEST (20% W/W)

- 7.7.1. Weigh 2 g of sample in a beaker.
- 7.7.2. Add 8 g of acetone to sample.
- 7.7.3. Mix to dissolve completely.
- 7.7.4. Solution should be clear and free of particles to pass the test.

7.8. APPEARANCE AND COLOR

- 7.8.1. Sample Size:
 - 7.8.1.1. For Raw Material: Inspect the entire testing sample for appearance and color.
 - 7.8.1.2. For Finished Goods: Use a suitable amount of sample.
- 7.8.2. Place sample into a clean, dry glass beaker.
- 7.8.3. In an area with sufficient lighting, view the sample from all sides and gently sift through the crystals inspecting for nonconforming matter, color, and structure.
- 7.8.4. The sample should be white in color and characteristic of crystals.

7.9. ASSAY

- 7.9.1. Note: Raw Material may be analyzed as-is. Refer to product code analysis required.
- 7.9.2. As-Is: Accurately weigh 0.36 g of sample. Transfer to a beaker.
- 7.9.3. Dried Basis: Accurately weigh 0.36 g of sample, previously dried at $105 \pm 2^\circ\text{C}$ for 3 hours (utilize LOD sample, if available). Transfer to a beaker.
- 7.9.4. Add 10 mL of purified water, 10 mL of glacial acetic acid, 10 mL of 0.2% polyvinyl alcohol, and 100 mL methanol to the sample beaker.
- 7.9.5. Titrate with 0.1N AgNO_3 to a potentiometric endpoint utilizing the Metrohm Titrando 907.

$$\% \text{ Guanidine Thiocyanate} = \frac{(\text{mL of AgNO}_3)(N \text{ of AgNO}_3)(11.82 \text{ mg})}{\text{Sample Weight (g)}}$$

7.9.6. **Alternate Manual Titration**

- 7.9.6.1. As-Is: Accurately weigh 0.36 g of sample. Transfer to a beaker.
- 7.9.6.2. Dried Basis: Accurately weight 0.36 g of sample, previously dried at $105 \pm 2^\circ\text{C}$ for 3 hours (utilize LOD sample, if available). Transfer to a beaker.
- 7.9.6.3. Dissolve with 100 mL of purified water.
- 7.9.6.4. Add 5 mL of (10 g/100 mL) Ferric Ammonium Sulfate aqueous, 5 mL of USP Dilute Nitric Acid, and 5 mL of dibutyl phthalate.
- 7.9.6.5. Titrate to a white endpoint.

$$\% \text{ Guanidine Thiocyanate} = \frac{(\text{mL of AgNO}_3)(N \text{ of AgNO}_3)(11.82 \text{ mg})}{\text{Sample Weight (g)}}$$

7.10. **CHLORIDE** _____ :

- 7.10.1. Accurately weigh 14.3 g of sample, transfer to a Nessler Color Comparison Tube, and Q.S. to 50 mL using purified water.
- 7.10.2. Prepare a standard by pipetting 101 μ L of 0.02 N HCl into a Nessler Color Comparison Tube, and Q.S. to 50 mL using purified water.
- 7.10.3. To the sample and standard add 1 mL of concentrated nitric acid and 1 mL of 0.1N silver nitrate.
- 7.10.4. The sample should be less turbid than the standard.

7.11. **ENZYME ACTIVITY** _____ :

- 7.11.1. RNase, DNase, and Protease performed as per procedures outlined in section 3. Analysis should be performed in the Analytical Procedure for Gel Assays and Analytical Procedure for Protease Assay Packets.

7.12. **IDENTIFICATION (IR)** _____ :

- 7.12.1. Note: Raw Material may be analyzed as-is. Refer to product code analysis required.
- 7.12.2. Follow Spectrum Two UATR SOP to perform IR analysis.

7.13. **LOSS ON DRYING @ 105°C** _____

- 7.13.1. Tare an LOD vial that has been previously dried for 30 minutes under the same conditions to be employed in the determination. Cool in desiccator for at least 15 minutes before weighing.
- 7.13.2. Transfer approximately 3 g of the sample to be tested to the LOD vial, and accurately weigh the LOD vial and contents. By gentle, sidewise shaking, distribute the sample as evenly as possible.
- 7.13.3. Place the LOD vial containing the sample into the oven.
- 7.13.4. Dry the sample at 105°C \pm 2°C for 3 hours.
- 7.13.5. Allow to cool to room temperature in a desiccator for at least 15 minutes before weighing.
- 7.13.6. Calculate the loss according to the following calculation:

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

7.14. **MELTING RANGE** _____

- 7.14.1. Note: Raw Material may be analyzed as-is.
- 7.14.2. Follow MP50 Melting Range Operation and Calibration procedure to determine melting range.

7.15. **pH OF A 5% SOLUTION @ 25° \pm 2°C** _____

- 7.15.1. Accurately weigh 5.0 g of sample.
- 7.15.2. Dissolve the sample in 100 mL of purified water. If necessary, utilize a stir plate and Teflon encapsulated magnetic stirring bar to achieve solubility.
- 7.15.3. Follow the appropriate SOP to calibrate and record the pH measurement of the solution at the 25 \pm 2°C.

7.16. **SOLUBILITY OF A 6M SOLUTION** _____ :

- 7.16.1. Weigh 35.2 g of sample and transfer to a graduated cylinder.
- 7.16.2. Dissolve in and dilute to 50 mL with purified water.
- 7.16.3. Observe from all sides, under sufficient lighting.
- 7.16.4. Solution should be clear when compared to purified water.

7.17. SOLUBILITY OF A 35% SOLUTION

- 7.17.1. Weigh 17.5 g of sample and record weight and transfer to a graduated cylinder.
- 7.17.2. Dissolve and dilute to 50 mL with purified water.
- 7.17.3. Observe from all sides under sufficient lighting.
- 7.17.4. Solution should be clear when compared to purified water.

7.18. SOLUBILITY (COLOUR)

- 7.18.1. Prepare a 100 mg/mL sample, dissolve and dilute with purified water.
- 7.18.2. Observe from all sides under sufficient lighting.
- 7.18.3. Solution must be colorless when compared to purified water to pass test.

7.19. SOLUBILITY (TURBIDITY) 100mg/mL H₂O

- 7.19.1. Prepare a 100 mg/mL sample, dissolve and dilute with purified water
- 7.19.2. Observe from all sides under sufficient lighting.
- 7.19.3. Solution must be clear when compared to purified water to pass test.

7.20. SULFATE

- 7.20.1. Accurately weigh 2 g of sample.
- 7.20.2. Dissolve in ~5 mL of purified water.
- 7.20.3. Add 1 mL of HCl (1 in 20).
- 7.20.4. Dilute to 10 mL with purified water.
- 7.20.5. Prepare a standard using 1 mL of Sulfate Standard Solution (0.01mg/mL), 1 mL HCl (1 in 20), and dilute to 10 mL with purified water.
- 7.20.6. To the sample and standard add 1 mL of 12% barium chloride and allow to stand for 10 minutes utilizing a calibrated timer.
- 7.20.7. The sample should be less turbid than the standard.

7.21. TRACE METALS

- 7.21.1. Refer to NexION 350X ICP-MS SOP for sample preparation and analysis.

7.22. TRACE METALS

- 7.22.1. Refer to NexION 350X ICP-MS SOP for sample preparation and analysis.
- 7.22.2. Refer to summary sheet for specifications.

7.23. WATER INSOLUBLES

- 7.23.1. Accurately weigh 20 grams of sample utilizing an analytical balance.
- 7.23.2. Dissolve in 200 mL of purified water.
- 7.23.3. Heat to boiling and digest, covered, on a hot plate for 1 hour.
- 7.23.4. Prepare a Gooch filtering crucible and 10-15 µm filter by drying at 105 ± 2°C for 1 hour. Allow to cool in ambient air at least 15 minutes and weigh on analytical balance.
- 7.23.5. Filter solution through conditioned filtering crucible and 10-15 µm filter. Rinse thoroughly with hot purified water.
- 7.23.6. Dry the crucible at 105 ± 2°C for 1 hour.
- 7.23.7. Cool in ambient air for at least 15 minutes and reweigh.
- 7.23.8. Calculate the water insoluble content using the following calculation:

$$\% \text{ Water Insolubles} = \frac{\text{Residue Weight (g)}}{\text{Sample Weight (g)}} \times 100$$