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GUANIDINE THIOCYANATE TESTING METHODS

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1. PURPOSE:

1.1. To provide the Quality Control (QC) Laboratory personnel with a procedure for analyzing Guanidine Thiocyanate Raw Material, In-Process, Finished Goods, and Stability in the QC 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 QC 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 Executive Director of Quality Control is responsible for the implementation, control, training and maintenance of this procedure.
3.2. The QC 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 QC Laboratory personnel are responsible for reviewing the appropriate SDS's prior to handling any chemicals used in this procedure.

4. REFERENCES:

4.1. *ACS, Reagent Chemicals,* current edition
4.2. Balance SOP
4.3. Blue M Convection Oven Operation and Calibration SOP
4.4. *Current EP/BP*
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5. EQUIPMENT:

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

6. ANALYTICAL PROCEDURES:

IN-PROCESS TESTING

6.1. **MOTHER LIQUOR ASSAY MONITOR:**

6.1.1. Accurately weigh 0.36g of sample and transfer to a suitable beaker.
6.1.2. 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.
6.1.3. Titrate with 0.1N $AgNO_3$ to a potentiometric endpoint utilizing the Metrohm Titrando 907.

$$\%\ Guanidine\ Thiocyanate = \frac{mL\ of\ AgNO_3 \cdot N\ of\ AgNO_3\ 11.82}{Sample\ Weight\ (g)}$$

6.1.4. **Alternate Manual Titration**

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

$$\%\ Guanidine\ Thiocyanate = \frac{mL\ of\ AgNO_3 \cdot N\ of\ AgNO_3\ 11.82}{Sample\ Weight\ (g)}$$

6.2. **ML ABSORBANCE ≤1.00 a.u. @ 280, 300, and 340 nm:**

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

6.3. **WC ABSORBANCE 0.03 max. @ 340nm; 0.05max. @ 300; and 0.3max. @ 280 nm:**

6.3.1. Accurately weigh 5.0 g of sample and accurately transfer the weighed sample to a graduated cylinder.
6.3.2. Dilute to 25 mL with purified water.
6.3.3. Dissolve completely.
6.3.4. Refer to the Lambda 25 UV/VIS Operation and Calibration procedure to determine the absorbance of the sample.

6.4. **Dry Crystal LOSS ON DRYING @ 105°C 0.3% max.:**

6.4.1. 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.

6.4.2. 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.

6.4.3. Place the LOD vial containing the sample into the oven.

6.4.4. Dry the sample at 105°C ± 2°C for 3 hours.

6.4.5. Allow to cool to room temperature in a desiccator for at least 15 minutes before weighing.

6.4.6. Calculate the loss according to the following calculation:

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

FINISHED GOOD TESTING

6.1. **ABSORBANCE (1.7M) Refer to Summary Sheet:**

6.1.1. Accurately weigh 5.0 g of sample.

6.1.2. Transfer accurately weighed sample to a graduated cylinder and Q.S. to 25 mL with purified water.

6.1.3. Dissolve completely.

6.1.4. Refer to Lambda 25 UV/VIS Operation and Calibration procedure to determine the Absorbance of the sample.

6.2. **ABSORBANCE (70%) Refer to Summary Sheet:**

6.2.1. Accurately weigh 17.5 g of sample.

6.2.2. Transfer accurately weighed sample to a graduated cylinder and Q.S. to 25 mL with purified water.

6.2.3. Dissolve completely.

6.2.4. Refer to Lambda 25 UV/VIS Operation and Calibration procedure to determine the Absorbance of the sample.

6.3. **ACETONE TEST (20% W/W) Refer to Summary Sheet:**

6.3.1. Weigh 2 g of sample in a beaker.

6.3.2. Add 8 g of acetone to sample.

6.3.3. Mix to dissolve completely.

6.3.4. Solution should be clear and free of particles to pass the test.

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6.4. **APPEARANCE AND COLOR** **White / Crystals:**

6.4.1. Sample Size:
6.4.1.1. For Raw Material: Inspect the entire testing sample for appearance and color.
6.4.1.2. For Finished Goods: Use a suitable amount of sample.
6.4.2. Place sample into a clean, dry glass beaker.
6.4.3. 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.
6.4.4. The sample should be white in color and characteristic of crystals.

6.5. **ASSAY** **Refer to Summary Sheet:**

6.5.1. Note: Raw Material may be analyzed as-is. Refer to product code analysis required.
6.5.2. As-Is: Accurately weigh 0.36g of sample. Transfer to a beaker.
6.5.3. Dried Basis: Accurately weigh 0.36g of sample, previously dried at 105 ± 2°C for 3 hours (utilize LOD sample, if available). Transfer to a beaker.
6.5.4. 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.
6.5.5. Titrate with 0.1N $AgNO_3$ to a potentiometric endpoint utilizing the Metrohm Titrando 907.

$$\%\ \text{Guanidine Thiocyanate} = \frac{(\text{mL of } AgNO_3)(\text{N of } AgNO_3)(\ 11.82\ \text{mg})}{\text{Sample Weight (g)}}$$

6.5.1. **Alternate Manual Titration**

6.5.1.1. As-Is: Accurately weigh 0.36g of sample. Transfer to a beaker.

6.5.1.2. Dried Basis: Accurately weigh 0.36g of sample, previously dried at 105 ± 2°C for 3 hours (utilize LOD sample, if available). Transfer to a beaker.

6.5.1.3. Dissolve with 100mL of purified water.

6.5.1.4. Add 5mL of (10g/100mL) Ferric Ammonium Sulfate aqueous, 5mL of USP Dilute Nitric Acid, and 5mL of dibutyl phthalate.

6.5.1.5. Titrate to a white endpoint.

$$\%\ \text{Guanidine Thiocyanate} = \frac{(\text{mL of } AgNO_3)(\text{N of } AgNO_3)\ (11.82\ \text{mg})}{\text{Sample Weight (g)}}$$

6.6. **CHLORIDE** **5 ppm max.:**

6.6.1. Accurately weigh 14.3 g of sample, transfer to a Nessler Color Comparison Tube, and Q.S. to 50 mL using purified water.

6.6.2. Prepare a standard by pipetting 101 µL of 0.02 N HCl into a Nessler Color Comparison Tube, and Q.S. to 50 mL using purified water.

6.6.3. To the sample and standard add 1 mL of concentrated nitric acid and 1 mL of 0.1N silver nitrate.

6.6.4. The sample should be less turbid than the standard.

6.7. **ENZYME ACTIVITY** **None Detected:**

6.7.1. 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.

6.8. **IDENTIFICATION (IR)** **Passes Test:**

6.8.1. Note: Raw Material may be analyzed as-is. Refer to product code analysis required.

6.8.2. Follow Spectrum Two UATR SOP to perform IR analysis.

6.9. **LOSS ON DRYING @ 105°C** **Refer to Summary Sheet:**

6.9.1. 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.

6.9.2. 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.

6.9.3. Place the LOD vial containing the sample into the oven.

6.9.4. Dry the sample at 105°C ± 2°C for 3 hours.

6.9.5. Allow to cool to room temperature in a desiccator for at least 15 minutes before weighing.

6.9.6. Calculate the loss according to the following calculation:

$$\%LOD = \frac{\big(Initial\ Sample\ Weight\ (g) + Vial(g)\big) - Final\ Vial\ Weight\ (g)}{Initial\ Sample\ Weight\ (g)} X100$$

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6.10. **MELTING RANGE** **Refer to Summary Sheet:**

6.10.1. Note: Raw Material may be analyzed as-is.

6.10.2. Follow MP50 Melting Range Operation and Calibration procedure to determine melting range.

6.11. **pH OF A 5% SOLUTION @25° ±2°C** **Refer to Summary Sheet:**

6.11.1. Accurately weigh 5.0 g of sample.

6.11.2. Dissolve the sample in 100mL of purified water. If necessary, utilize a stir plate and Teflon encapsulated magnetic stirring bar to achieve solubility.

6.11.3. Follow the appropriate SOP to calibrate and record the pH measurement of the solution at the 25 ± 2°C.

6.12. **SOLUBILITY OF A 6M SOLUTION** **Clear:**

6.12.1. Weigh 35.2 g of sample and transfer to a graduated cylinder.

6.12.2. Dissolve in and dilute to 50 mL with purified water.

6.12.3. Observe from all sides, under sufficient lighting.

6.12.4. Solution should be clear when compared to purified water.

6.13. **SOLUBILITY OF A 35% SOLUTION** **Clear:**

6.13.1. Weigh 17.5 g of sample and record weight and transfer to a graduated cylinder.

6.13.2. Dissolve and dilute to 50mL with purified water.

6.13.3. Observe from all sides under sufficient lighting.

6.13.4. Solution should be clear when compared to purified water.

6.14. **SOLUBILITY (COLOUR)** **Colorless:**

6.14.1. Prepare a 100mg/mL sample, dissolve and dilute with purified water.

6.14.2. Observe from all sides under sufficient lighting.

6.14.3. Solution must be colorless when compared to purified water to pass test.

6.15. **SOLUBILITY (TURBIDITY) 100mg/mL H_2O** **Clear:**

6.15.1. Prepare a 100mg/mL sample, dissolve and dilute with purified water.

6.15.2. Observe from all sides under sufficient lighting.

6.15.3. Solution must be clear when compared to purified water to pass test.

6.16. **SULFATE** **5 ppm max:**

6.16.1. Accurately weigh 2 g of sample.

6.16.2. Dissolve in ~5 mL of purified water.

6.16.3. Add 1 mL of HCl (1 in 20).

6.16.4. Dilute to 10 mL with purified water.

6.16.5. 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.

6.16.6. To the sample and standard add 1 mL of 12% barium chloride and allow to stand for 10 minutes utilizing a calibrated timer.

6.16.7. The sample should be less turbid than the standard.

6.17. **TRACE METALS** **Refer to Summary Sheet:**

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

6.18. **TRACE METALS** **Ba, K, Na:**

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

6.19. **WATER INSOLUBLES** **Refer to Summary Sheet:**

6.19.1. Accurately weigh 20 grams of sample utilizing an analytical balance.
6.19.2. Dissolve in 200 mL of purified water.
6.19.3. Heat to boiling and digest, covered, on a hot plate for 1 hour.
6.19.4. Prepare a Gooch filtering crucible and 10-15µm 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.
6.19.5. Filter solution through conditioned filtering crucible and 10-15 µm filter. Rinse thoroughly with hot purified water.
6.19.6. Dry the crucible at 105±2°C for 1 hour.
6.19.7. Cool in ambient air for at least 15 minutes and reweigh.
6.19.8. Calculate the water insoluble content using the following calculation:

$$\%\, Water\ Insolubles = \frac{Initial\ Crucible\ Weight_{(g)} - Final\ Crucible\ Weight_{(g)}}{Sample\ Weight_{(g)}} \cdot 100$$