BIOSPECTRA
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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 Laboratory Manager 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-ATM-0073, Analytical Method for the Determination of ICH Q3D Elemental Impurities (Class 1, 2A, 2B, 3 & 4) by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in Guanidine Thiocyanate, MOPS, and Urea

4.2. BSI-ATM-0089, Analytical Method of Analysis: Trace Metals in Finished Goods Products by ICP-OES

4.3. BSI-ATM-0131, Analytical Method for the Determination of Trace Metals in BioTech Products

4.4. BSI-FRM-0728, Analytical Procedure for Gel Assays

4.5. BSI-FRM-0745, Analytical Procedure for Protease Assay

4.6. BSI-SOP-0019, Result Reporting

4.7. BSI-SOP-0090, Lambda 25 UV/VIS Operation and Calibration

4.8. BSI-SOP-0094, Muffle Furnace SOP and Calibration

4.9. BSI-SOP-0095, DNase (Endonuclease) Assay

4.10. BSI-SOP-0096, RNase (Ribonuclease) Assay

4.11. BSI-SOP-0098, Balance SOP

4.12. BSI-SOP-0126, Laboratory Notebooks

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

4.14. BSI-SOP-0134, Pipette SOP

4.15. BSI-SOP-0135, Laboratory Chemicals

4.16. BSI-SOP-0138, DNase (Exonuclease) Assay

4.17. BSI-SOP-0139, Protease Assay

4.18. BSI-SOP-0140, Standardization of Titrants

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

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

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4.21. BSI-SOP-0254, Spectrum Two UATR SOP
4.22. BSI-SOP-0255, XL200 pH/mV/Conductivity Meter SOP
4.23. BSI-SOP-0256, MP50 Melting Range Operation, Verification, and Calibration SOP
4.24. BSI-SOP-0303, NexION 350X ICP-MS SOP
4.25. BSI-SOP-0668, OPI-180 OD Handheld Colorimeter SOP
4.26. *ACS, Reagent Chemicals,* current edition
4.27. *Current EP/BP*
4.28. *Current JP*
4.29. *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. Muffle Furnace
5.6. OPI-180 OD Handheld Colorimeter SOP
5.7. Perkin Elmer Avio 500 ICP-OES
5.8. Perkin Elmer NexION 350X ICP-MS
5.9. Perkin Elmer Spectrum Two UATR
5.10. 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. **Barium Chloride TS –** Dissolve 30g of barium chloride dihydrate in water to make 250mL.
6.5. **Dibutyl Phthalate –** Purchased Commercially.
6.6. **Ferric Ammonium Sulfate Dodecahydrate –** Purchased Commercially.
6.7. **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.
6.8. **Methanol –** Purchased commercially.
6.9. **Nitric Acid (HNO_3), concentrated –** Purchased Commercially.
6.10. **HNO_3, USP Dilute –** Dilute 14.3 mL of nitric acid to 100 mL with purified water.
6.11. **Hydrochloric Acid 3N –** Add 25.75mL of hydrochloric acid carefully into ~50mL of purified water in a 100mL volumetric flask, dilute to 100mL and mix thoroughly. Scale as needed.
6.12. **Polyvinyl Alcohol –** Purchased Commercially.
6.13. **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.
6.14. **Silver Nitrate, 0.1N –** Purchased Commercially
6.15. **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.

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6.16. **Sulfuric Acid –** Purchased Commercially

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 $AgNO_3$ to a potentiometric endpoint utilizing the Metrohm Titrando 907.

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

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.

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

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.

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7.4.6. Calculate the loss according to the following calculation:

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

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.
7.8.5. 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.

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 ± 2°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.

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

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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 ± 2°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.

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

7.10. **ENZYME ACTIVITY:**

7.10.1. RNase, DNase, and Protease performed as per procedures outlined in section 4. Analysis should be performed in the Analytical Procedure for Gel Assays and Analytical Procedure for Protease Assay Packets.

7.11. **IDENTIFICATION (IR):**

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

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

7.12. **LOSS ON DRYING @ 105°C:**

7.12.1. 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.

7.12.2. 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.

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

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

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

7.12.6. Calculate the loss according to the following calculation:

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

7.13. **MELTING RANGE:**

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

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

7.14. **pH OF A 5% SOLUTION @ 25° ±2°C:**

7.14.1. Accurately weigh 5.0 g of sample.

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

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

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7.15. **RESIDUE ON IGNITION :**

7.15.1. **Caution**: Ignition of guanidine thiocyanate produces hydrogen cyanide; a potentially fatal gas if inhaled. Take extreme care to perform all ignition steps in a closed fume hood with particular care to ensure the sash is completely shut and the ventilation is fully functional. DO NOT PERFORM IF FUME HOOD IS NOT OPERATIONAL OR ANOTHER TECHNICIAN IS PERFORMING TESTING IN THE SAME HOOD.

7.15.2. Turn on muffle furnace and allow it to stabilize at 600°C. Follow muffle furnace calibration procedure for operation of furnace.

7.15.3. Inspect a quartz crucible for cracks, chips, and discoloration.

7.15.4. Utilize forceps to insert and remove the crucible from the furnace.

7.15.5. Ignite quartz crucible at 600 ± 50 °C for 30 minutes. Cool in a desiccator for 1.5 hours and weigh using an analytical balance.

7.15.6. Weigh 0.5 g sample in the previously ignited quartz crucible. Moisten the sample with a small amount of sulfuric acid (Between 0.2-1.0mL).

7.15.7. Volatilize the sample until the sample is thoroughly charred and white fumes are no longer evolved. Heat the sample slowly, so that the sample does not boil over and sample is not lost.

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

7.15.7.2. Continue to heat the sample until all the excess sulfuric acid has been volatilized.

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

7.15.9. Cool in a desiccator for the same amount of time employed in the preparation of the crucible and weigh on an analytical balance.

7.15.10. Calculate the %ROI as follows:

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

7.15.11. If the amount of the residue exceeds the limit specified, repeat the moistening with sulfuric acid using up to 1mL, heat to char, then ignite at 600 ± 50 °C for 30 minutes until two consecutive weighings of the residue do not differ by more than 0.0005g or until the specification is met.

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.

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

7.19.1. Observe from all sides under sufficient lighting.

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

7.20. **SULFATE :**

7.20.1. Sample Solution:

7.20.1.1. Weigh 2.0 grams of sample and dissolve in 5mL of purified water in a test tube.

7.20.2. 50ppm Standard Solution:

7.20.2.1. Pipette 0.052 mL of 0.02N H_2SO_4 into a Nessler Color Comparison Tube and add 4.95 mL of purified water.

7.20.3. Procedure:

7.20.3.1. To each tube, add 0.2 mL of 3N HCl and 1.0 mL of Barium Chloride TS.

7.20.3.2. Cover with parafilm and mix by inversion.

7.20.3.3. Allow to stand for 10 minutes.

7.20.3.4. After 10 minutes, the turbidity of the sample preparation does not exceed that produced by the 50ppm standard when viewed against a dark background.

7.21. **TRACE METALS :**

7.21.1. Primary Method: Refer to Analytical Method for the Determination of Elemental Impurities in Guanidine Thiocyanate, MOPS, and Urea, BSI-ATM-0073.

7.21.2. Secondary Method: Refer to Analytical Method of Analysis: Trace Metals in Finished Goods Products by ICP-OES, DCN: BSI-ATM-0089.

7.21.3. For BioTech Product Analysis: Refer to Analytical Method for the Determination of Trace Metals in BioTech Products, BSI-ATM-0131.

7.21.4. Refer to summary sheet for specifications.

7.22. **WATER INSOLUBLES :**

7.22.1. Accurately weigh 20 grams of sample utilizing an analytical balance.

7.22.2. Dissolve in 200 mL of purified water.

7.22.3. Heat to boiling and digest, covered, on a hot plate for 1 hour.

7.22.4. 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.

7.22.5. Filter solution through conditioned filtering crucible and 10-15 µm filter. Rinse thoroughly with hot purified water.

7.22.6. Dry the crucible at 105 ± 2°C for 1 hour.

7.22.7. Cool in ambient air for at least 15 minutes and reweigh.

7.22.8. Calculate the water insoluble content using the following calculation:

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

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