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GUANIDINE HYDROCHLORIDE TESTING METHODS

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1. PURPOSE:

- 1.1. To provide the Laboratory personnel with procedures for testing Guanidine Hydrochloride, Raw Material, In-Process, Finished Goods, and Stability samples.

2. SCOPE:

- 2.1. Applies to the testing of Guanidine Hydrochloride Raw Material, In-Process, Finished Goods, and Stability samples in the Laboratory at all BioSpectra locations. Methods include testing for all types of Guanidine Hydrochloride 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 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 appropriate personnel if any analyses fail to meet their respective specifications.

4. REFERENCES:

- 4.1. BSI-ATM-0071, Method of Analysis: Elemental Impurities by ICP-MS in Guanidine HCl 6M
- 4.2. BSI-FRM-0716, Guanidine Hydrochloride Analytical Procedure
- 4.3. BSI-FRM-0728, Analytical Procedure for Gel Assays
- 4.4. BSI-FRM-0745, Analytical Procedure for Protease Assay
- 4.5. BSI-SOP-0019, Result Reporting
- 4.6. BSI-SOP-0090, Lambda 25 UV/Vis Operation and Calibration
- 4.7. BSI-SOP-0094, Muffle Furnace SOP and Calibration
- 4.8. BSI-SOP-0095, DNase (Endonuclease) Assay
- 4.9. BSI-SOP-0096, RNase (Ribonuclease) Assay
- 4.10. BSI-SOP-0098, Balance SOP
- 4.11. BSI-SOP-0126, Laboratory Notebooks
- 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-0254, Spectrum Two UATR SOP
- 4.17. BSI-SOP-0255, XL200 pH/Conductivity Meter SOP
- 4.18. BSI-SOP-0256, MP50 Melting Range Operation, Verification and Calibration SOP
- 4.19. BSI-SOP-0303, NexION 350X ICP-MS SOP
- 4.20. *Current USP*
- 4.21. *Current ACS Reagent Chemicals*

5. EQUIPMENT:

- 5.1. Lambda 25 UV/Vis Spectrophotometer, or equivalent
- 5.2. Analytical Balance
- 5.3. Perkin Elmer NexION 350X ICP-MS
- 5.4. Perkin Elmer Spectrum Two UATR
- 5.5. Blue M Oven, or equivalent
- 5.6. MP50 Melting Point Apparatus
- 5.7. XL200 pH/Conductivity Meter or equivalent.
- 5.8. Muffle Furnace
- 5.9. Metrohm 907 Titrando Auto-Titrator.

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6. ANALYTICAL PROCEDURE:

6.1. IN-PROCESS ML ABSORBANCE REFER TO BATCH RECORD:

- 6.1.1. Prepare 10 mL of sample by pipetting 5 mL water and 5 mL specified Mother Liquor into a 50 mL beaker. Swirl to dissolve completely.
- 6.1.2. Refer to Lambda 25 UV/Vis Operation and Calibration to determine the Absorbance of the sample.
- 6.1.3. Record results at specified wavelengths in the Guanidine hydrochloride In-Process Testing Log Book and the appropriate laboratory documentation.
- 6.1.4. Notify the appropriate personnel if any results do not meet the specifications.

6.2. IN-PROCESS ML ASSAY :

- 6.2.1. Standardize 0.1N AgNO₃ as per Standardization of Titrants.
- 6.2.2. Accurately weigh 0.35 g of sample.
- 6.2.3. Transfer to a 250 mL beaker and dissolve with 10 mL of purified water.
- 6.2.4. Add 10 mL of glacial acetic acid, 100 mL of methanol, and 10 mL of a 0.2% polyvinyl alcohol solution.
- 6.2.5. Titrate with 0.1N AgNO₃ to a potentiometric end-point utilizing the Metrohm Titrand 907.

$$\% GHCL = \frac{mL_{AgNO_3} \times N_{AgNO_3} \times 9.553}{Sample\ Weight\ (g)} \quad \% Cl = \frac{mL_{AgNO_3} \times N_{AgNO_3} \times 3.545}{Sample\ Weight\ (g)}$$

6.2.6. Alternate Manual Assay Method:

- 6.2.6.1. Refer to Standardization of 0.1N Silver Nitrate (AgNO₃) by hand as per Standardization of Titrants
- 6.2.6.2. Accurately weigh 0.35 g of sample.
- 6.2.6.3. Transfer to a 250 mL beaker and dissolve with 10 mL of purified water.
- 6.2.6.4. Add 10 mL of glacial acetic acid, 100 mL of methanol, and 0.5 mL of Eosin Y Indicator.
- 6.2.6.5. Titrate to a pink endpoint.

$$\% GHCL = \frac{mL_{AgNO_3} \times N_{AgNO_3} \times 9.553}{Sample\ Weight\ (g)}$$

6.3. IN-PROCESS WET CRYSTAL ABSORBANCE REFER TO BATCH RECORD:

- 6.3.1. Prepare a 6M solution of the specified sample (Solution may be scaled as required).
- 6.3.2. Accurately weigh 14.3 g of sample. Transfer accurately weighed sample to a 50 mL graduated cylinder and q.s. to 25 mL with purified water. Swirl to dissolve completely.
- 6.3.3. Refer to Lambda 25 UV/Vis Spectrophotometer Operation and calibration to determine the absorbance of the sample.
- 6.3.4. Record results at specified wavelengths in the Guanidine Hydrochloride In-Process Testing Log Book, the appropriate laboratory documentation, and in batch record.

6.4. IN-PROCESS DRY CRYSTAL ASSAY REFER TO BATCH RECORD:

- 6.4.1. Standardize 0.1N AgNO₃ as per Standardization of Titrants.
- 6.4.2. Accurately weigh 0.35 g of sample that has been previously dried at 105°C for four hours.
- 6.4.3. Transfer to a 250 mL beaker and dissolve with 10mL of purified water.
- 6.4.4. Add 10 mL of glacial acetic acid, 100 mL of methanol, and 10 mL of a 0.2% polyvinyl alcohol solution.
- 6.4.5. Titrate with 0.1N AgNO₃ to a potentiometric end-point utilizing the Metrohm Titrand 907.

6.4.6. **Alternate Manual Assay Method:**

- 6.4.6.1. Refer to Standardization of 0.1N Silver Nitrate (AgNO₃) by hand as per Standardization of Titrants
- 6.4.6.2. Accurately weigh 0.35 g of sample that has been previously dried at 105°C for four hours.
- 6.4.6.3. Transfer to a 250 mL beaker and dissolve with 10 mL of purified water.
- 6.4.6.4. Add 10 mL of glacial acetic acid, 100 mL of methanol, and 0.5 mL of Eosin Y Indicator.
- 6.4.6.5. Titrate to a pink endpoint.

$$\% \text{ GHCL} = \frac{mL_{AgNO_3} \times N_{AgNO_3} \times 9.553}{\text{Sample Weight (g)}} \quad \% \text{ Cl} = \frac{mL_{AgNO_3} \times N_{AgNO_3} \times 3.545}{\text{Sample Weight (g)}}$$

6.5. **ABSORBANCE** **REFER TO SUMMARY SHEET:**

- 6.5.1. Prepare a 6M solution of the specified sample (Solution may be scaled as needed).
- 6.5.2. Accurately weigh 57.2 g of sample into a 100 mL volumetric flask and Q.S. to the mark with purified water.
- 6.5.3. Mix thoroughly.
- 6.5.4. Refer to Lambda 25 UV/Vis Operation and Calibration to determine the absorbance of the sample.
- 6.5.5. NOTE: This 6M solution preparation may be used to perform the following tests in this procedure: Chloride Identification, Clarity and Color of Solution, Melamine, pH (6M), and Solubility (6M). Solution must be covered and used within 24 hours.

6.6. **ACIDITY** **0.01% max.:**

- 6.6.1. Using an analytical balance, dissolve 20 g of sample in 200 mL purified water.
- 6.6.2. Add 0.15 mL of phenolphthalein indicator and titrate to a lasting pink endpoint with 0.01N NaOH.
- 6.6.3. Not more than 5.5 mL of titrant is required to obtain the pink endpoint (0.01% max).

6.7. **AMMONIUM CHLORIDE** **1.0% max.:**

- 6.7.1. Accurately weigh and dissolve 0.8 g of sample in 100 mL purified water in an Erlenmeyer flask and mix well.
- 6.7.2. Add ~3 drops of Methyl Red Solution and mix well.
- 6.7.3. Neutralize the solution to a yellow color with dropwise additions of 0.1N NaOH VS.
 - 6.7.3.1. Note: If solution is red; neutralize with 0.1N HCl.
- 6.7.4. Add 15mL of neutralized formaldehyde solution.
 - 6.7.4.1. Solution Preparation Reference; Neutralized Formaldehyde Solution - Transfer 100 mL of formaldehyde solution into a suitable container. Add 2.0 g of magnesium carbonate and shake for several minutes. Filter and use the clear filtrate.
 - 6.7.4.2. If any red color due to the indicator (Methyl Red Solution) is present, titrate the solution with 0.1N Sodium Hydroxide to a yellow endpoint.
- 6.7.5. Add 10 drops of phenolphthalein TS and mix well.
- 6.7.6. Titrate with 0.1N NaOH VS until a light pink endpoint which lasts 10 minutes is visible. Record volume of titrant delivered.
 - 6.7.6.1. Note: A timer and reference solution of water against a white background is recommended to help visually detect whether the visible pink color is still present after 10 minutes.
- 6.7.7. Not more than 1.50 mL of titrant is required to obtain the light pink endpoint (1.0% max).

6.8. APPEARANCE AND COLOR White / Crystals:

- 6.8.1. Place 25-50 g of the sample in a clean, dry glass beaker.
- 6.8.2. In an area with sufficient lighting, view the sample from all sides.
- 6.8.3. The sample should be white in color and characteristic of crystals. If the sample does not conform to these specifications, notify the appropriate personnel immediately.

6.9. APPEARANCE OF SOLUTION :

- 6.9.1. Prepare a 6M solution of the specified sample (Solution may be scaled as needed).
 - 6.9.1.1. Accurately weigh 57.2 g of sample into a 100 mL volumetric flask and Q.S. to the mark with purified water.
 - 6.9.1.2. Mix thoroughly.
 - 6.9.1.3. Solution for “Absorbance” test may be used.
- 6.9.2. Clear (2.2.1.) Turbidimetry
 - 6.9.2.1. Rinse the sample bottle with the sample solution twice.
 - 6.9.2.2. Fill sample bottle with the sample Solution S to the white line.
 - 6.9.2.3. Coat outside of bottle with a thin coat of silicon oil.
 - 6.9.2.4. Remove any air bubbles from the solution by using a syringe.
 - 6.9.2.5. Allow the sample to sit capped for 2-3 minutes.
 - 6.9.2.6. Follow the appropriate SOP as follows:
 - 6.9.2.6.1. Stroudsburg- Measure and record the turbidity of the sample according to Portable Turbidimeter Operation and Calibration.
 - 6.9.2.6.2. Bangor- Measure and record the turbidity of the sample according to Bangor Portable Turbidimeter SOP.
 - 6.9.2.7. The sample solution must be < 3 NTU.
- 6.9.3. Colorless (2.2.2, Method II)
 - 6.9.3.1. Add 10 mL of Solution S into a Nessler Color Comparison Tube.
 - 6.9.3.2. Add 10 mL of *USP Purified Water* into a second Nessler Color Comparison Tube.
 - 6.9.3.3. Compare the colors in sufficient lighting, viewing vertically against a white background.
 - 6.9.3.4. In order for the sample solution to be colorless, it must have the appearance of *USP Purified Water*.

6.10. ASSAY (Dried Basis) GHCl | Cl 99.5-101% | 36.92-37.48%:

- 6.10.1. Standardize 0.1N AgNO₃ as per Standardization of Titrants.
- 6.10.2. Accurately weigh 0.35 g of sample that has been previously dried at 105°C for four hours.
- 6.10.3. Transfer to a 250 mL beaker and dissolve with 10 mL of purified water.
- 6.10.4. Add 10 mL of glacial acetic acid, 100 mL of methanol, and 10 mL of a 0.2% polyvinyl alcohol solution.
- 6.10.5. Titrate with 0.1N AgNO₃ to a potentiometric end-point utilizing the Metrohm Titrand 907.

$$\% \text{ GHCl} = \frac{\text{mL}_{\text{AgNO}_3} \times N_{\text{AgNO}_3} \times 9.553}{\text{Sample Weight (g)}} \quad \% \text{ Cl} = \frac{\text{mL}_{\text{AgNO}_3} \times N_{\text{AgNO}_3} \times 3.545}{\text{Sample Weight (g)}}$$

6.10.6. Alternate Manual Assay Method :

- 6.10.6.1. Refer to Standardization of 0.1N Silver Nitrate (AgNO₃) by hand as per Standardization of Titrants.
- 6.10.6.2. Accurately weigh 0.35 g of sample that has been previously dried at 105°C for four hours.
- 6.10.6.3. Transfer to a 250 mL beaker and dissolve with 10 mL of purified water.

- 6.10.6.4. Add 10 mL of glacial acetic acid, 100 mL of methanol, and 0.5 mL of Eosin Y Indicator.
- 6.10.6.5. Titrate to a pink endpoint.

$$\% \text{ GHCL} = \frac{mL_{AgNO3} \times N_{AgNO3} \times 9.553}{\text{Sample Weight (g)}}$$

6.11. ASSAY (Guanidine Basis) 99.5% Min:

- 6.11.1. Standardize 0.1N Perchloric by hand as per Standardization of Titrants.
- 6.11.2. NOTE: Perform a blank determination utilizing all reagents below minus the product, there will be a blank for standardization and a blank for the assay.
- 6.11.3. Accurately weigh 0.35 g of Guanidine hydrochloride into an appropriate beaker.
- 6.11.4. Add stir bar.
- 6.11.5. Add 140 mL of Glacial Acetic Acid.
- 6.11.6. Add 20 mL of 5% mercuric acetate in glacial acetic acid solution.
- 6.11.7. Add 0.1 mL of 100 mg/10 mL crystal violet in glacial acetic acid solution.
- 6.11.8. Dissolve sample while stirring.
- 6.11.9. Record Temperature of the Perchloric Acid.
- 6.11.10. Titrate to a blue green end point with 0.1N Perchloric Acid.
 - 6.11.10.1. Note: Due to the high volumetric coefficient of expansion of glacial acetic acid a temperature correction factor is included in the analysis as C_f . $C_f = [1 + 1.07 \times 10^{-3} (T_s - T_i)]$

T_s = temperature at standardization. T_i = temperature at sample titration

$$\% \text{ Guanidine} = \frac{(EP_{\text{Sample}} - EP_{\text{Blank}})(N \text{ of Perchloric Acid})(9.55318)(C_f)}{[\text{Sample (g)} - \text{Sample Weight (g)}](\% \text{ LOD})}$$

6.12. CHLORIDE IDENTIFICATION Passes Test:

- 6.12.1. Prepare a 6M solution of the specified sample (Solution may be scaled as needed). Solution previously prepared for Absorbance (6M) may be utilized.
- 6.12.2. Accurately weigh 57.2 g of sample into a 100 mL volumetric flask and Q.S. to the mark with purified water.
- 6.12.3. Mix thoroughly.
- 6.12.4. Transfer 2 mL of the sample solution to a beaker and add 0.2 mL of 0.1N Silver Nitrate.
- 6.12.5. A white, curdy precipitate that is insoluble after the addition of 1 mL of Concentrated Nitric Acid is produced.
- 6.12.6. If no precipitate is produced, notify the appropriate personnel.
- 6.12.7. Add 4 mL of 6N Ammonium Hydroxide. The precipitate should dissolve after mild agitation.

6.13. CLARITY AND COLOR OF SOLUTION Clear, Colorless:

- 6.13.1. Prepare a 6M solution of the specified sample. Solution previously prepared for Absorbance (6M) may be utilized.
- 6.13.2. Accurately weigh 57.2 g of sample into a 100 mL volumetric flask and Q.S. to the mark with purified water.
- 6.13.3. Mix thoroughly.
- 6.13.4. Sample solution should be clear and colorless and comparable to the color and turbidity of a sample of water visually.

6.14. CYANIDE**0.1 ppm max.:****6.14.1. Primary Cyanide Method:**

- 6.14.1.1. Weigh 5 g of sample and transfer to a 50 mL beaker. Dissolve in 10 mL of purified water. Prepare ferrous sulfate solution immediately before use: Dissolve 0.45 g of ferrous sulfate in 50 mL of 0.1N Hydrochloric acid and dilute to 100 mL with purified water.
- 6.14.1.2. Prepare a standard solution by pipetting 0.1 mL of 5 ppm Cyanide Stock Solution and adding 9.9 mL of purified water. To the sample and standard, add 0.1 mL of 0.1N Sodium Hydroxide and 0.1 mL of the ferrous sulfate solution. Warm both the sample and standard then cool to room temperature. Add 0.2 mL of the ferric chloride solution (1g/100 mL). The sample solution must not develop more red or blue than that of the standard (0.5µg/10 mL).

6.14.2. Alternative Cyanide Method:**6.14.2.1. Equipment and Reagents:**

- 6.14.2.1.1. Distillation apparatus: Use a suitable cyanide distillation apparatus.
- 6.14.2.1.2. Pyridine- Barbituric Acid Reagent: Place 60 g of barbituric acid in a 1000 mL volumetric flask and add just enough water to wash the sides of the flask and wet the barbituric acid. Add 300 mL of pyridine and mix. Add 60 mL of HCl, mix, and cool to room temperature. Dilute to 1000 mL with water and mix. This reagent is stable for approximately 6 months if stored in a cool, dark place. Filter when prepared or just before use, if insoluble form through Whatman No. 40 filter paper or equivalent.
- 6.14.2.1.3. Sodium Hydroxide Solution, 1N- Dilute 160 mL of 50% NaOH to 3L with purified water or dissolve 40 g of NaOH pellets in purified water and dilute to 1L.
- 6.14.2.1.4. Sodium Hydroxide Solution 0.2N- Dilute 8 grams of NaOH pellets to 1L with purified water.
- 6.14.2.1.5. Phosphate Buffer: Dissolve 138 g of Sodium Phosphate in water. Add 70 mL of Acetic Acid.
- 6.14.2.1.6. Chloramine T Solution: Dissolve 1.0 g of white, water soluble Chloramine T in 100 mL of water. Prepare fresh each use.
- 6.14.2.1.7. Stock Cyanide Solution: Prepare from Potassium Cyanide or purchase commercially.

6.14.2.2. Distillation:

- 6.14.2.2.1. Transfer 500 mL of the test sample [250 grams of crystals plus 250 grams of water mixed until dissolved] to a 1L distillation flask.
- 6.14.2.2.2. Add several glass beads.
- 6.14.2.2.3. Turn on the laboratory vacuum.
- 6.14.2.2.4. Add 50 mL of 1N NaOH to the absorption bottle, and enough water to obtain an adequate depth of liquid to insure complete scrubbing of the air stream.
- 6.14.2.2.5. Connect it to the condenser outlet tube.
- 6.14.2.2.6. Start cooling water through the condenser.
- 6.14.2.2.7. Apply heat.
- 6.14.2.2.7.1. Caution: Do not leave the apparatus unattended during this initial heating because the vacuum flow may need to be increased to prevent the solution from backing up into and overflowing from the air inlet tube or the absorber inlet.

- 6.14.2.3. Reflux for 2 hours. The rate of reflux should be such that the vapors rise to

about 3/4-7/8 of the length of the condenser.

- 6.14.2.4. After 2 hours, turn off the heat and continue to vacuum flow for 15 minutes.
- 6.14.2.5. Disconnect the absorption bottle.
- 6.14.2.6. Transfer the contents of the absorption bottle (absorption liquid) to a 250 mL volumetric flask and dilute to the mark with water washings from the absorption bottle (at least 3 washings) and connecting tubes. The final concentration of this solution is 0.2N sodium hydroxide.
 - 6.14.2.6.1. Note: If the following section cannot be done the same day as the distillation, transfer the solution from the volumetric flask to a clean plastic bottle for storage.
- 6.14.2.7. Determining CN- Concentration and Preparation:
 - 6.14.2.7.1. Prepare a spiking solution of 1ppm by: a) serial dilution of Potassium Cyanide diluted in 0.2N NaOH. b.) use a purchased standard solution diluted in 0.2N NaOH.
 - 6.14.2.7.2. Transfer 50.0 mL of absorption liquid to 100 mL volumetric flask.
 - 6.14.2.7.3. Prepare a blank of 50.0 mL of 0.2N NaOH in a 100 mL volumetric flask.
 - 6.14.2.7.4. To 50.0 mL of 0.2N NaOH in a 100 mL volumetric flask, transfer 0.005 mg of Cyanide.
 - 6.14.2.7.5. Add 15 mL of phosphate buffer solution to each flask and mix. (The pH of the solution now should be 5.5-6.5.)
 - 6.14.2.7.6. Add 2.0 mL of Chloramine T solution to each flask and mix.
 - 6.14.2.7.7. After 1 to 2 minutes, add 5.0 mL of pyridine-barbituric acid reagent to each flask and mix. Dilute to the mark with water and mix again.
 - 6.14.2.7.8. Allow 8 minutes for color development.
 - 6.14.2.7.9. Any red color in the sample should be less than that of the standard. (0.1 ppm maximum)

6.15. **ENDOTOXINS** _____ :

- 6.15.1. In-House Method:
 - 6.15.1.1. Weigh 25 mg +/-1 mg and transfer to a sterile tube.
 - 6.15.1.2. Dissolve in ~5 mL of LAL reagent water.
 - 6.15.1.3. Dilute to 10 mL with LAL reagent water and mix thoroughly.
 - 6.15.1.4. Follow Endosafe nexgen-PTS Reader SOP.
- 6.15.2. Outside Testing:
 - 6.15.2.1. Fill out applicable ARF in NAMSA Connect Portal.
 - 6.15.2.2. Send 5 g of sample to NAMSA (Irvine) with SDS.

6.16. **ETHYL ALCOHOL SOLUBILITY** _____ **Passes Test:**

- 6.16.1. Dissolve 5 g of sample in 100 mL of reagent alcohol.
- 6.16.2. Sample solutions should dissolve and be a clear colorless solution to pass test.

6.17. **FREE ACID** _____ **0.01% Max:**

- 6.17.1. Refer to Acidity.

6.18. **ENZYME ACTIVITY** _____ **None Detected:**

- 6.18.1. RNase, DNase, and Protease per SOPs listed in Section 4.

6.19. **HEAVY METALS** _____ **10 ppm max:**

- 6.19.1. Refer to section 6.34: Trace Metals.
- 6.19.2. Alternate Wet Method:

- 6.19.2.1. Standard Lead Solution: On the day of use, dilute 10.0 mL of Lead Nitrate Stock Solution with purified water to 100 mL.
- 6.19.2.2. Standard Preparation: Into a Nessler comparison tube pipette 2 mL of the Standard Lead Solution and QS to 40 mL in purified water.
- 6.19.2.3. Sample Preparation: Weigh 2 g of sample, transfer to a Nessler comparison tube, and dissolve in 40 mL of purified water.
- 6.19.2.4. Procedure: Adjust pH to between 3 and 4 using acetic acid. To all solutions, add 2 mL of pH 3.5 Acetate Buffer and 1.2 mL of thioacetamide-glycerin base TS (Mix 0.2 mL of thioacetamide TS and 1 mL of glycerin base TS, and heat in a boiling purified water bath for 20 seconds. Use immediately.). QS to 50 mL with purified water. Let stand for 2 minutes. Any brown color in the sample must not exceed that in the 0.01 mg standard (10ppm).

6.20. IDENTIFICATION (UATR) Passes Test:

6.20.1. Follow Spectrum Two UATR SOP.

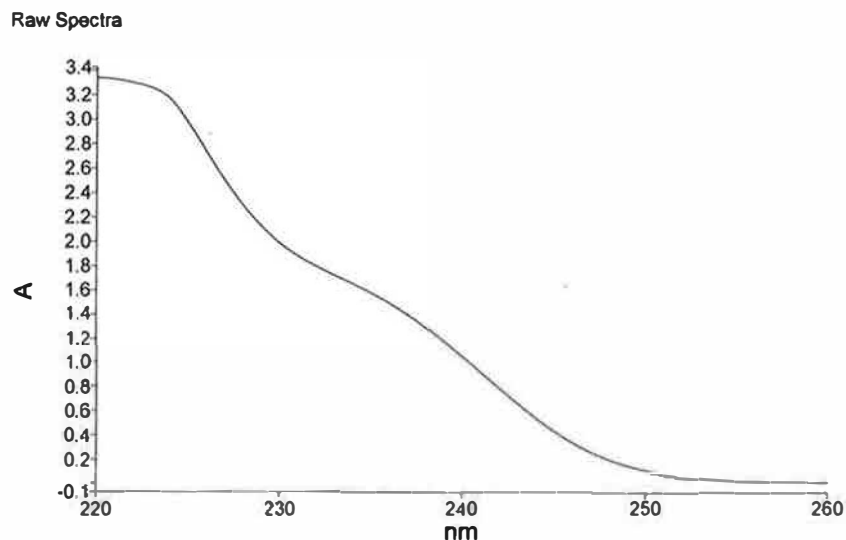
6.21. LOSS ON DRYING @ 105°C 0.5% max.:

- 6.21.1. Dry a LOD vial in an oven at 105 ± 2°C for at least 1 hour. Cool for 15 minutes in a desiccator, weigh, and record results.
- 6.21.2. Place the vial on the analytical balance and tare the dried vial. Weigh 2.0 g of sample and record results.
- 6.21.3. Dry for 4 hours at 105°C. Cool for 15 minutes in desiccator.
- 6.21.4. Retain Sample for Assay, dried basis.
- 6.21.5. Reweigh and calculate the % LOD.

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

6.22. MELAMINE Passes Test:

- 6.22.1. Prepare a 6M solution of the specified sample (Solution may be scaled as needed). Solution previously prepared for Absorbance (6M) may be utilized.
- 6.22.2. Accurately weigh 57.2 g of sample into a 100 mL volumetric flask and Q.S. to the mark with purified water.
- 6.22.3. Mix thoroughly.
- 6.22.4. Refer to Lambda 25 UV/Vis Operation and Calibration to determine the absorbance of the sample in the range of 220-260nm.
- 6.22.5. A shoulder in this part of the curve is acceptable but there should be no observable peak or plateau in this range to report as Passes Test.
If any point exceeds an absorbance of 3 notify the QC Manager immediately.
Below is an example of a Melamine containing sample's spectrum.



6.23. MELTING RANGE **184 – 188°C:**

6.23.1. Refer to MP50 Melting Range Operation and Calibration SOP.

6.24. NITRATE **0.005% max.:**

Note: Prepare Brucine Sulfate at time of use.

6.24.1. Sample Preparation (Solution A):

6.24.1.1. Add 0.40 g of sample to 2.0 mL of water, dilute to 50 mL with brucine sulfate reagent solution, and mix thoroughly.

6.24.2. Control Preparation (Solution B):

6.24.2.1. Add 0.40 g of sample to 2.0 mL of 10 ppm standard nitrate solution and dilute to 50 mL with brucine sulfate reagent solution, and mix thoroughly.

6.24.3. Blank Preparation (Solution C):

6.24.3.1. Transfer 50 mL of the Brucine Sulfate reagent solution to a 50 mL volumetric flask.

6.24.4. Procedure:

6.24.4.1. Heat the three solutions in a preheated (boiling) water bath for 10 min. Cool rapidly in an ice bath to room temperature. Utilizing the Lambda 25, dispense blank solution C into one of the two matched 10 mm cuvettes and place into the second cell holder (closest to the front of the instrument) in order to complete a “100% / 0A Baseline (Auto Zero)”. **Note: The cell holder closest to the back of the instrument should remain empty throughout the entire analysis.** Remove blank solution C and scan sample solution A and control solution B respectively at 410 nm. Calculate the nitrate content as:

$$\% NO_3 = \frac{AU_{Solution A}}{AU_{Solution B} - AU_{Solution A}} \times \% \text{ maximum allowable}$$

6.25. pH 5% SOLUTION @25°C **4.5-6.0:**

6.25.1. Dissolve 5.0 g of sample in 100 mL of purified water.

6.25.2. Ensure the pH is stable before obtaining result.

6.25.3. Follow the appropriate SOP for calibration and pH measurement.

6.26. pH 0.5M SOLUTION @25°C **:**

6.26.1. Dissolve 4.8 g of sample in 100 mL of purified water.

- 6.26.2. Ensure the pH is stable before obtaining result.
- 6.26.3. Follow the appropriate SOP for calibration and pH measurement.

6.27. pH (6M) @ 25°C 4.0-6.0:

- 6.27.1. Prepare a 6M solution of the specified sample (Solution may be scaled as needed).
Solution previously prepared for Absorbance (6M) may be utilized.
- 6.27.2. Accurately weigh 57.2 g of sample into a 100 mL volumetric flask and Q.S. to the mark with purified water.
- 6.27.3. Mix thoroughly
- 6.27.4. Follow the appropriate SOP for calibration and pH measurement.
- 6.27.5. Set a calibrated timer for 15 minutes. Record the pH and temperature.

6.28. RELATED SUBSTANCES REFER TO SUMMARY SHEET:

- 6.28.1. Refer to BSI-RPT-0538 for HPLC method parameters and sample and standard preparation.

6.29. RESIDUE ON IGNITION 0.05% max.:

- 6.29.1. Turn on muffle furnace and allow temperature to stabilize at 600°C. Follow muffle furnace SOP and calibration procedure for operation.
- 6.29.2. Inspect a quartz crucible for cracks, chips and discoloration.
- 6.29.3. Utilize the 10-inch forceps to insert and remove a crucible into the furnace.
- 6.29.4. Ignite the quartz crucible at 600 ± 50 °C for 30 minutes. Cool in a desiccator for one hour and 30 minutes and weigh.
- 6.29.5. Weigh 5.0 g sample in the previously ignited quartz crucible. Moisten the sample with 2 mL of sulfuric acid.
- 6.29.6. Volatilize the sample with a suitable heating apparatus. Ensure that the sample does not boil over and sample is not lost.
- 6.29.7. The rate of heating should be such that from ½ to 1 hour is required to volatilize the sample.
- 6.29.8. Continue to heat the sample until all excess sulfuric acid has been volatilized.
- 6.29.9. Ignite in the muffle furnace at 600 ± 50 °C for 30 minutes or until all carbon has been removed.
- 6.29.10. Cool in the desiccator for a minimum of an hour and a half and reweigh.

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

6.30. SOLUBILITY (6M) Clear/Colorless:

- 6.30.1. Prepare a 6M solution of the specified sample (Solution may be scaled as needed).
Solution previously prepared for Absorbance (6M) may be utilized.
- 6.30.2. Accurately weigh 57.2 g of sample into a 100 mL volumetric flask and Q.S. to the mark with purified water.
- 6.30.3. Mix thoroughly.
- 6.30.4. Observe from all angles. Sample solution should be clear and colorless and comparable to the color and turbidity of a sample of water visually.

6.31. SOLUBILITY IN WATER Clear/Colorless:

- 6.31.1. Accurately weigh 60.0 g of sample and transfer to a 250 mL beaker.
- 6.31.2. Accurately measure 100 mL of purified water in a graduated cylinder and transfer to the sample beaker.

- 6.31.3. Swirl to dissolve the sample. If necessary, utilize a stirrer plate and Teflon encapsulated magnetic stirring bar.
- 6.31.4. The sample completely dissolves in purified water to report as Clear/Colorless when compared to a clear and colorless reference solution.

6.32. SULFATE 0.005% max.:

- 6.32.1. Sample Preparation:
 - 6.32.1.1. Weigh 1.0 g of sample and transfer to a 50 mL Nessler Color Comparison Tube. Dissolve in 40 mL of purified water.
- 6.32.2. Standard Preparation:
 - 6.32.2.1. Prepare a standard solution by pipetting 0.052 mL of 0.020 N H₂SO₄ in a 50 mL Nessler Color Comparison Tube. QS to 40 mL with purified water.
- 6.32.3. Procedure:
 - 6.32.3.1. To both the sample and standard solutions, add 1 mL of 3N HCl, 3 mL of Barium Chloride TS and QS to the line.
 - 6.32.3.2. Cover with parafilm and mix by inversion.
 - 6.32.3.3. Compare turbidity 10 minutes after addition of the barium chloride to the sample and standard solutions.
- 6.32.4. Any turbidity produced in the sample solution should not exceed that produced by the standard when viewed from above against a black surface.
- 6.32.5. If turbidity of the sample solution exceeds that of the standard notify the QC Manager immediately.

6.33. TOTAL CN <0.005%:

- 6.33.1. Outside Testing.

6.34. TRACE METALS REFER TO SUMMARY SHEET:

- 6.34.1. Refer to BSI-ATM-0071 for sample preparation and analysis.

6.35. WATER INSOLUBLES REFER TO SUMMARY SHEET:

- 6.35.1. Accurately weigh 50.0 g of sample and transfer to a 150 mL beaker.
- 6.35.2. Add 25 mL of room temperature purified water. If necessary, utilize a Teflon encapsulated magnetic stirring bar and electric stir plate to dissolve sample.
- 6.35.3. Dry a Gooch crucible and filter paper at 105° ± 2°C for 1 hour. Cool in ambient air for 15 minutes and weigh.
- 6.35.4. Filter sample solution through the Gooch crucible using a suitable vacuum pump.
- 6.35.5. Rinse sample vessel and crucible filter with at least 150 mL of purified water.
- 6.35.6. Dry the crucible at 105° ± 2°C for 1 hour. Cool in ambient air for 15 minutes and weigh.

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

6.36. WATER (by Karl Fischer Titration) REFER TO SUMMARY SHEET:

- 6.36.1. Perform a standardization of the titrant (Composite 5) as per Standardization of Titrants.
- 6.36.2. Grind the sample in a dry mortar into a fine powder utilizing a pestle.
- 6.36.3. Immediately weigh 2.0 g of sample into the glass weighing spoon and tare it.
- 6.36.4. Transfer the sample to the KF vessel by removing the rubber septum and adding the sample into the titration vessel.
 - 6.36.4.1. Do not leave the rubber septum open for long periods of time as this will allow moisture to enter the titration vessel.

- 6.36.5. Return the weighing spoon to the balance, making sure not to lose any sample that was left behind. Once the weight stabilizes, press the print button on the balance.
- 6.36.6. Check to make sure there is no residual sample stuck to the sides of the titration vessel.
 - 6.36.6.1. If there is any sample stuck to the side, stop the stir bead from spinning before swirling the vessel to rinse the sides.
- 6.36.7. Once the method begins, check to ensure the sample is fully dissolved before the titration begins (i.e. before the stir command completes).
- 6.36.8. The moisture content will then be determined by the Metrohm Auto Titrando 907.

$$\% \text{ Moisture} = \frac{(mL \text{ of Composite 5}) \left(\frac{mg}{mL} \text{ of Composite 5} \right) (0.1)}{\text{Sample Weight (g)}}$$