



GUANIDINE HYDROCHLORIDE 6M SOLUTION TESTING METHODS

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1. PURPOSE:

- 1.1. To provide the Laboratory personnel with a procedure for testing Guanidine Hydrochloride 6M Solution.

2. SCOPE:

- 2.1. This document applies to the testing of Guanidine Hydrochloride 6M Solution In-Process, Stability, and Finished Goods. The testing can be performed at BioSpectra, Inc at the Bangor and Stroudsburg, PA facilities.

3. RESPONSIBILITIES:

- 3.1. The Laboratory Manager is responsible for the implementation, control, training, and maintenance of this procedure.
- 3.2. All Laboratory analysts are responsible for complying with this procedure.

4. REFERENCES:

- 4.1. BSI-ATM-0071, Method of Analysis: Elemental Impurities by ICP-MS in Guanidine 6M
- 4.2. BSI-ATM-0089, Analytical Method of Analysis: Trace Metals in Finished Goods Products by ICP-OES
- 4.3. BSI-SOP-0019, Result Reporting <http://dm-svr-01/ensur/contentAction.aspx?key=Production.1007.S1R4E1A3.20180926.61.4671568>
- 4.4. BSI-SOP-0090, Lambda 25 UV/Vis Operation and Calibration
- 4.5. BSI-SOP-0098, Balance SOP
- 4.6. BSI-SOP-0126, Laboratory Notebooks
- 4.7. BSI-SOP-0133, Blue M Convection Oven Operation and Calibration SOP
- 4.8. BSI-SOP-0140, Standardization of Titrants
- 4.9. BSI-SOP-0143, Metrohm Titrando 907 Auto-Titrator SOP
- 4.10. BSI-SOP-0144, Metrohm 914 pH Conductometer Operation and Calibration <http://dm-svr-01/ensur/contentAction.aspx?key=Production.8534.S1R4E4A3.20180926.61.4697220>
- 4.11. BSI-SOP-0244, VWR Gravity Convection Oven Operation and Calibration (Model Number 414005-106)
- 4.12. BSI-SOP-0254, Spectrum Two UATR SOP
- 4.13. BSI-SOP-0255, XL200 pH/mV/Conductivity Meter SOP
- 4.14. BSI-SOP-0303, NexION 350X ICP-MS SOP
- 4.15. BSI-SOP-0350, Anton Paar DMA 35 Portable Density Meter Operation and Calibration
- 4.16. BSI-SOP-0362, Operation and Maintenance of the Perkin Elmer Avio 500 ICP-OES

5. EQUIPMENT:

- 5.1. Analytical Balance
- 5.2. Anton Paar DMA 35 Portable Density Meter
- 5.3. UV/VIS Spectrophotometer
- 5.4. Perkin Elmer UATR Spectrum Two
- 5.5. Metrohm Titrando 907
- 5.6. NexION 350X ICP-MS
- 5.7. pH/ Conductivity Meter
- 5.8. Calibrated Oven
- 5.9. Calibrated Timer
- 5.10. Calibrated Pipettes

6. REAGENTS:

- 6.1. **Acetate Buffer, pH 3.5:** Dissolve 62.5 g of ammonium acetate in 62.5 mL of purified water

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and add 47 mL of concentrated HCl. Adjust, if necessary, with ammonium hydroxide 6N to a pH of 3.5, dilute with purified water to 250 mL.

- 6.2. **Acetic Acid, 1N:** Dilute 57 mL of glacial acetic acid to 1 L with purified water.
- 6.3. **Ammonium Thiocyanate, 30%:** Weigh 30.0 g of ammonium thiocyanate and dilute to 100 mL with purified water.
- 6.4. **Ammonium Peroxydisulfate:** Purchased Commercially.
- 6.5. **Cyanide Stock Solution, 5 ppm:** Dissolve 4 mg of potassium cyanide in purified water and dilute to 1000mL with purified water.
- 6.6. **Ferric Chloride Solution, 1g/100mL:** Dissolve 1.0 grams of ferric chloride hexahydrate in purified water and dilute to 100 mL with purified water.
- 6.7. **Ferrous Sulfate:** Purchased Commercially.
- 6.8. **Glycerin Base TS:** To 200 g of glycerol, add purified water to bring the total weight to 235 g. Add 140 mL of Sodium Hydroxide, 1N and 50 mL purified water.
- 6.9. **Glacial Acetic Acid:** Purchased Commercially
- 6.10. **GHC1 6M IR Reference Standard:** Decant previously approved sample into a dropper bottle. Analyze the sample using the Spectrum Two UATR and compare to a previously approved GHC1 6M IR reference standard. The correlation must be ≥ 0.95 to be considered acceptable to use as a reference standard.
- 6.11. **Hydrochloric Acid 0.1N:** Purchased Commercially.
- 6.12. **Hydrochloric Acid, concentrated:** Purchased Commercially.
- 6.13. **Iron Standard, 10 ppm:** Dissolve 0.702 g of ferrous ammonium sulfate hexahydrate in 10 mL of 10% sulfuric acid reagent solution, dilute with water to 100 mL. To 10 mL of this solution, add 10mL of 10% sulfuric acid reagent solution, and dilute with water to 1L.
- 6.14. **Lead Nitrate Stock Solution:** Dissolve 0.1598 g of lead nitrate in 100 mL purified water and add 1 mL of nitric acid. Dilute with purified water to 1000 mL. Store in a glass container free from soluble lead salts.
- 6.15. **Methanol:** Purchased Commercially
- 6.16. **Polyvinyl Alcohol Solution 0.2%:** Dissolve 2 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.
- 6.17. **Silver Nitrate, 0.1N:** Purchased Commercially
- 6.18. **Sodium Hydroxide, Dilute (0.1N):** Purchased Commercially; Weigh 0.4 g of NaOH crystals and dilute to 100 mL with purified water.
- 6.19. **Sulfuric Acid, 10%:** In a well-ventilated fume hood, slowly add 30 mL of 96% sulfuric acid to 375 mL of purified water, cool and dilute with water to 500 mL
- 6.20. **Thioacetamide TS:** Dissolve 4g of thioacetamide in 100 mL purified water.

7. PROCEDURE:**7.1. ABSORBANCE (Neat) Refer to Summary Sheet:**

- 7.1.1. Pipette 3 mL of sample into a cuvette.
- 7.1.2. Refer to Lambda 25 UV/Vis Operation and Calibration, to determine the absorbance of the sample.

7.2. APPEARANCE & COLOR Refer to Summary Sheet:

- 7.2.1. Transfer approximately 25 mL of the solution into a Nessler tube and examine under sufficient light from different angles.
- 7.2.2. Solution should be clear and colorless when compared to water, and free of particulates.

7.3. ASSAY Refer to Summary Sheet:**7.3.1. Chloride Assay:**

- 7.3.1.1. Standardize 0.1N AgNO₃ as per Standardization of Titrants.
- 7.3.1.2. Accurately weigh 0.7 g of sample.
- 7.3.1.3. Add 10 mL of purified water, 10 mL of glacial acetic acid, 100 mL of methanol, and 10mL of a 0.2% polyvinyl alcohol solution.
- 7.3.1.4. Titrate with 0.1N AgNO₃ to a potentiometric end-point utilizing the Metrohm Titrand907.

$$\% \text{ Cl} = \frac{(\text{mL of AgNO}_3 \times \text{N of AgNO}_3)(3.545)}{\text{Sample Weight (g)}}$$

7.3.2. Dried Basis Chloride Assay:

- 7.3.2.1. Dry a watch glass in the Oven at 105 ±2°C for at least 30 minutes.
- 7.3.2.2. Cool in desiccator for 15 minutes, and then record its weight.
- 7.3.2.3. Accurately weigh and record the weight of 1 mL of sample in the tared watch glass and place in Oven at 105 ±2°C overnight.
- 7.3.2.4. Allow to cool in the desiccator for 15 minutes.
- 7.3.2.5. Remove and weigh the sample and watch glass.
- 7.3.2.6. Return the sample and watch glass to the Oven at 105 ±2°C for 4 additional hours.
- 7.3.2.7. Repeat the weighing, if not constant, repeat the drying and weighing until a constant weight is achieved.
- 7.3.2.8. Record the weight of the residue and perform the following calculations.

$$\% \text{ Cl in Residue} = \frac{\text{Residue Weight (g)} \times 100 \times 0.371}{\text{Sample Weight (g)}}$$

$$\% \text{ Assay} = \frac{\% \text{ Cl} \times 100}{\% \text{ Cl in Residue}}$$

7.4. CONDUCTIVITY (1:10) Refer to Summary Sheet:

- 7.4.1. Following the Metrohm 914 pH/ Conductometer Operation and Calibration SOP, calibrate the conductivity meter
 - 7.4.1.1. Use a 50,000 µS/cm Conductivity Standard Solution

- 7.4.2. Dilute 10 mL of sample to 100 mL with purified water.
 - 7.4.2.1. Swirl to mix completely.
 - 7.4.2.2. Allow sample solution to reach room temperature.
- 7.4.3. Measure and record the conductivity.

7.5. CYANIDE

Refer to Summary Sheet:

7.5.1. Primary Cyanide Method:

7.5.1.1. Prepare a 1.05 M sample solution:

- 7.5.1.1.1. Pipette 0.35 mL of the 6M solution to a beaker and add 1.65 mL purified water to prepare 2.0 mL of solution.

7.5.1.2. Prepare a standard solution:

- 7.5.1.2.1. Pipette 0.02 mL of 5 ppm Cyanide Standard Stock solution into 1.98 mL of purified water.

7.5.1.3. Ferrous Sulfate Solution Immediately before use:

- 7.5.1.3.1. Dissolve 0.45 g of ferrous sulfate in 50 mL of 0.1N hydrochloric acid and dilute to 100 mL with purified water.

7.5.1.4. Procedure:

- 7.5.1.4.1. To the sample and standard, add 0.1 mL of dilute sodium hydroxide (0.1N NaOH) and 0.1 mL of the ferrous sulfate solution.
- 7.5.1.4.2. Warm both the sample and standard then cool to room temperature.
- 7.5.1.4.3. Add 0.2 mL of ferric chloride solution.
- 7.5.1.4.4. The solution must not turn red or blue to report Passes Test.

7.5.2. Alternative Cyanide Method:

7.5.2.1. Equipment and Reagents:

- 7.5.2.1.1. Distillation apparatus: Use a suitable cyanide distillation apparatus.
- 7.5.2.1.2. Pyridine- Barbituric Acid Reagent: Place 60g of barbituric acid in a 1000mL volumetric flask and add just enough water to wash the sides of the flask and wet the barbituric acid. Add 300mL of pyridine and mix. Add 60mL of HCl, mix, and cool to room temperature. Dilute to 1000mL 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.
- 7.5.2.1.3. Sodium Hydroxide Solution, 1N- Dilute 160mL of 50% NaOH to 3L with purified water or dissolve 40g of NaOH pellets in purified water and dilute to 1L.
- 7.5.2.1.4. Sodium Hydroxide Solution 0.2N- Dilute 8 grams of NaOH pellets to 1L with purified water.
- 7.5.2.1.5. Phosphate Buffer: Dissolve 138g of Sodium Phosphate in water. Add 70mL of Acetic Acid.
- 7.5.2.1.6. Chloramine T Solution: Dissolve 1.0g of white, water soluble Chloramine T in 100mL of water. Prepare fresh each use.
- 7.5.2.1.7. Stock Cyanide Solution: Prepare from Potassium Cyanide or purchase commercially.

7.5.2.2. Distillation:

- 7.5.2.2.1. Transfer 440mL of the test sample to a 1-liter distillation flask.
- 7.5.2.2.2. Add several glass beads.
- 7.5.2.2.3. Turn on the laboratory vacuum.
- 7.5.2.2.4. Add 50mL 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.

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- 7.5.2.2.5. Connect it to the condenser outlet tube.
- 7.5.2.2.6. Start cooling water through the condenser.
- 7.5.2.2.7. Apply heat.
- 7.5.2.2.8. 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.
- 7.5.2.2.9. 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.
- 7.5.2.2.10. After 2 hours, turn off the heat and continue to vacuum flow for 15 minutes.
- 7.5.2.2.11. Disconnect the absorption bottle.
- 7.5.2.2.12. Transfer the contents of the absorption bottle (absorption liquid) to a 250mL 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.
- 7.5.2.2.13. 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.
- 7.5.2.3. Determining CN- Concentration and Preparation:
 - 7.5.2.3.1. 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.
 - 7.5.2.3.2. Transfer 50.0mL of absorption liquid to 100mL volumetric flask.
 - 7.5.2.3.3. Prepare a blank of 50.0mL of 0.2N NaOH in a 100mL volumetric flask.
 - 7.5.2.3.4. To 50.0mL of 0.2N NaOH in a 100mL volumetric flask, transfer 0.005mg of Cyanide.
 - 7.5.2.3.5. Add 15mL of phosphate buffer solution to each flask and mix. (The pH of the solution now should be 5.5-6.5.)
 - 7.5.2.3.6. Add 2.0mL of Chloramine T solution to each flask and mix.
 - 7.5.2.3.7. After 1 to 2 minutes, add 5.0mL of pyridine-barbituric acid reagent to each flask and mix. Dilute to the mark with water and mix again.
 - 7.5.2.3.8. Allow 8 minutes for color development.
 - 7.5.2.3.9. Any red color in the sample should be less than that of the standard. (0.1ppm maximum)

7.6. **DENSITY**

Refer to Summary Sheet:

- 7.6.1. Primary Method:
 - 7.6.1.1. Measure the Density using the DMA 35 portable density meter. Refer to DCN:19-002946 for instrument operation and sample preparation.
- 7.6.2. Alternate Method:
 - 7.6.2.1. Tare a small watch glass or weigh boat on the analytical balance.
 - 7.6.2.2. Pipette 1 mL of room temperature sample onto the watch glass or weighboat.
 - 7.6.2.3. Record weight. Calculate density of the sample using the equation below:

$$\text{Density} = \frac{\text{Sample Weight (g)}}{\text{Volume of solution (mL)}}$$

7.7. HEAVY METALS**10 ppm max:**

- 7.7.1. Refer to Section 6.16 Trace Elements for Primary Analysis.
- 7.7.2. Alternate Wet Method:
- 7.7.3. Standard Lead Solution:
- 7.7.3.1. On the day of use, dilute 10.0 mL of Lead Nitrate Stock Solution with purified water to 100 mL.
- 7.7.4. Standard Preparation:
- 7.7.4.1. Pipette 2 mL of the Standard Lead Solution into Nessler Color Comparison tube, and add approximately 40 mL of purified water.
- 7.7.5. Sample Preparation:
- 7.7.5.1. Weigh 2g of sample, transfer to a Nessler Color Comparison tube. Then add approximately 40mL of purified water.
- 7.7.6. Procedure:
- 7.7.6.1. Adjust pH to between 3 and 4 using 1N Acetic Acid.
- 7.7.6.2. 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 gently heat immediately before use.) QS to 50 mL with purified water.
- 7.7.6.3. Let stand for 2 minutes using a calibrated timer.
- 7.7.6.4. Any brown color in the sample must not exceed that in the 0.01 mg standard (10 ppm).

7.8. IDENTITY IR**Refer to Summary Sheet:**

- 7.8.1. For UATR analysis, follow Spectrum Two UATR SOP.

7.9. IRON**3 ppm max:**

- 7.9.1. Refer to section 6.16 Trace Elements for Iron Analysis.
- 7.9.2. Alternate Iron method
- 7.9.2.1. Sample Preparation:
- 7.9.2.1.1. Pipette 4 mL of sample into a 100-mL beaker. Dissolve in 20 mL of purified water.
- 7.9.2.2. Standard Preparation:
- 7.9.2.2.1. Pipette 1.20 mL of 10 ppm Iron Standard. Dilute to 20 mL with purified water.
- 7.9.2.3. Procedure:
- 7.9.2.3.1. To both the standard and the sample add 2 mL of concentrated HCl and dilute to 50 mL with purified water. Then add 30-50 mg of ammonium peroxydisulfate and 3 mL of 30% ammonium thiocyanate. Any red color produced by the sample must not exceed that in the standard (3 ppm) in order to pass test.

7.10. MELAMINE RELATED**Refer to Summary Sheet:**

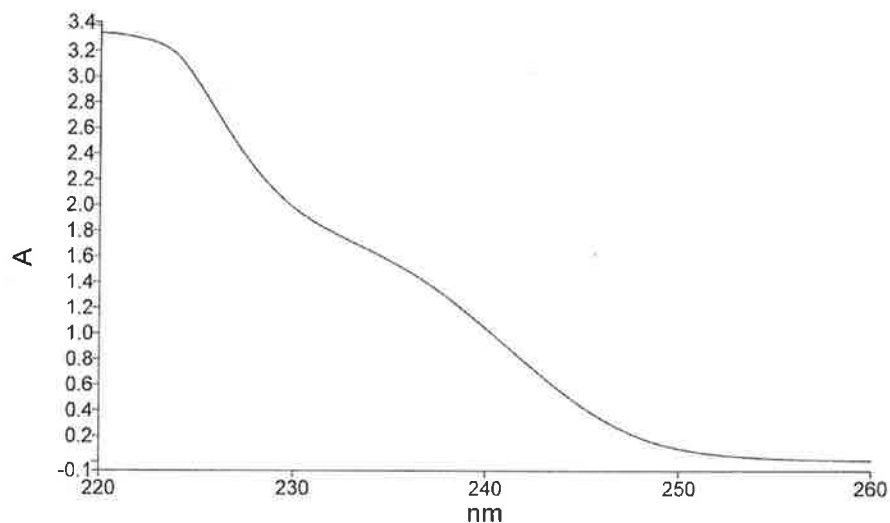
- 7.10.1. Refer to Lambda 25 UV/Vis Operation and Calibration to determine the Absorbance of the neat sample in the range of 220-260nm.

7.10.2. 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.

7.10.2.1. If any point exceeds 3 notify the QC Manager immediately.

7.10.2.2. Below is an example of a Melamine containing sample's spectrum.

Raw Spectra



7.10.2.3.

7.11. **MOLARITY**

Refer to Summary Sheet:

7.11.1. Standardize 0.1N AgNO₃ as per Standardization of Titrants.

7.11.2. Pipet 0.5 mL of sample into a beaker.

7.11.3. Add 10 mL of purified water, 10 mL of glacial acetic acid, 100 mL of methanol, and 10 mL of a 0.2% polyvinyl alcohol solution (PVA).

7.11.4. Titrate with 0.1N AgNO₃ to a potentiometric end-point utilizing the Metrohm Titrando 907.

$$\text{Molarity} = \frac{(\text{mL} * \text{N of AgNO}_3)}{\text{Sample Volume (0.5 mL)}}$$

7.11.5. **Alternate Manual Assay Method:**

7.11.5.1. Refer to Standardization of Titrants SOP for 0.1M Silver Nitrate.

7.11.5.2. Accurately weigh 0.5mL of sample

7.11.5.3. Transfer to a 250mL beaker and dissolve with 10mL of purified water.

7.11.5.4. Add 10mL of glacial acetic acid, 100mL of methanol, and 0.5mL of Eosin Y Indicator.

7.11.5.5. Titrate to a pink endpoint.

$$\text{Molarity} = \frac{\text{mL of AgNO}_3 \times \text{N of AgNO}_3}{\text{Sample Volume (mL)}}$$

7.12. **pH (Neat)**

Refer to Summary Sheet:

6.12.1 Decant 50 mL of sample into a 100-mL beaker. Measure and record the pH according to the appropriate SOP.

6.12.2 Set a calibrated timer for 15 minutes. Record the pH and temperature.

7.13. pH (1:10) **Refer to Summary Sheet:**

- 7.13.1. Transfer 10 mL of sample to a 100-mL graduated cylinder.
- 7.13.2. QS to 100 mL with purified water.
 - 7.13.2.1. Cover with parafilm and mix by inversion. Measure and record the pH according to the appropriate SOP.
 - 7.13.2.2. Ensure the pH reading is stable before obtaining result.

7.14. SOLUTIONS TEST **Refer to Summary Sheet:**

- 7.14.1. Transfer 50 mL of sample to a Nessler tube.
- 7.14.2. Examine the solution; it must be free of particulate matter.
- 7.14.3. If particulates are present:
 - 7.14.3.1. Dry a Gooch crucible and a 10 µm Millipore filter at 105° ± 2°C for 1 hour. Cool in ambient air for 15 minutes and weigh.
 - 7.14.3.2. Filter sample solution through the Gooch crucible using a suitable vacuum pump.
 - 7.14.3.3. Rinse sample vessel and crucible filter with 10 mL of purified water.
 - 7.14.3.4. Dry the crucible at 105° ± 2°C for 1 hour. Cool in ambient air for 15 minutes and weigh.
 - 7.14.3.5. Calculate the % residue as follows:

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

- 7.14.3.6. If greater than 0.2%, carefully remove and examine the filter paper using a microscope in order to determine the type of particulate matter present.
- 7.14.3.7. Record the amount and type of particulate matter observed.

7.15. TITRABLE ACID **Refer to Summary Sheet:**

- 7.15.1. Prepare a 5.23 M solution:
 - 7.15.1.1. Pipette 8.72 mL of the 6M solution into a beaker and add 1.28 mL of purified water.
- 7.15.2. Add 0.05 mL of phenol red TS.
- 7.15.3. The solution becomes yellow.
- 7.15.4. Titrate 0.1N Sodium Hydroxide, utilizing a 2-20µL pipette, until the solution turns red.
 - 7.15.4.1. Record amount of titrant required to turn the solution red.
- 7.15.5. Not more than 0.15 mL of 0.1N Sodium Hydroxide is required to report passes test.

7.16. TRACE ELEMENTS **Refer to Summary Sheet:**

- 7.16.1. Primary Method: Refer to Avio 500 ICP-OES SOP and Trace Metals in Finished Goods Products, BSI-ATM-0089, for the determination of As, Cu, Fe, & Pb.
- 7.16.2. Secondary Method: Refer to NexION 350X ICP-MS SOP, BSI-SOP-0303 and BSI-ATM-0071 for the determination of As, Cu, Fe, & Pb.

Signature Manifest

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Quality Approval

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