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GUANIDINE HYDROCHLORIDE TESTING METHODS

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1. PURPOSE:

1.1. To provide the Quality Control (QC) Laboratory personnel with procedures for testing Guanidine Hydrochloride, Raw Material, In-Process, Finished Goods, and stability.

2. SCOPE:

2.1. Applies to the testing of Guanidine Hydrochloride Raw Material, In-Process, Finished Goods, and Stability in the QC 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 Executive Director of Quality Control is responsible for training, maintenance and implementation 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.

4. REFERENCES:

4.1. Balance SOP, DCN:16-000368
4.2. *Current USP*
4.3. *Current ACS Reagent Chemicals*
4.4. DNase (Endonuclease) Assay, DCN:16-000365
4.5. DNase (Exonuclease) Assay, DCN:16-000511
4.6. Laboratory Notebooks, DCN:16-000482
4.7. Lambda 25 UV/Vis Operation and Calibration, DCN:16-000359
4.8. Metrohm Titrando 907 Auto-Titrator SOP, DCN:16-000521
4.9. MP50 Melting Range Operation and Calibration SOP, DCN:16-001332
4.10. Muffle Furnace SOP and Calibration, DCN: 16-000364
4.11. NexION 350X ICP-MS SOP, DCN:16-001923
4.12. Protease Assay, DCN: 16-000512
4.13. Result Reporting, DCN:16-000063
4.14. RNase (Ribonuclease)Assay, DCN: 16-000366
4.15. Spectrum Two UATR SOP, DCN:16-001330
4.16. Standardization of Titrants, DCN: 16-000513
4.17. XL200 pH/Conductivity Meter SOP, DCN: 16-001331
4.18. Guanidine Hydrochloride Analytical Procedure, DCN: LAB19-000023
4.19. Analytical Procedure for Gel Assays, DCN: LAB19-000038
4.20. Analytical Procedure for Protease, DCN: LAB19-000060

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.

6. ANALYTICAL PROCEDURE:

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

6.1.1. Prepare 10mL of sample by pipetting 5 mL water and 5mL specified Mother Liquor into a 50mL 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 $AgNO_3$ as per Standardization of Titrants.

6.2.2. Accurately weigh 0.35g of sample.

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

6.2.4. Add 10mL of glacial acetic acid, 100mL of methanol, and 10 mL of a 0.2% polyvinyl alcohol solution.

6.2.5. Titrate with 0.1*N* $AgNO_3$ to a potentiometric end-point utilizing the Metrohm Titrando 907.

$$\%\ GHCl = \frac{mL_{AgN03} * N_{AgN03} * 9.553}{Sample\ Weight\ (g)} \qquad \%\ Cl = \frac{mL_{AgN03} * N_{AgN03} * 3.545}{Sample\ Weight\ (g)}$$

6.2.6. **Alternate Manual Assay Method:**

6.2.6.1. Refer to Standardization of 0.1N Silver Nitrate ($AgNO_3$) by hand as per Standardization of Titrants

6.2.6.2. Accurately weigh 0.35g of sample.

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

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

6.2.6.5. Titrate to a pink endpoint.

$$\%\ GHCl = \frac{mL_{AgN03} * N_{AgN03} * 9.553}{Sample\ Weight\ (g)}$$

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).

6.3.1.1. 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.

6.3.2. Refer to Lambda 25 UV/Vis Spectrophotometer Operation and calibration to determine the absorbance of the sample.

6.3.3. 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 $AgNO_3$ as per Standardization of Titrants.

6.4.2. Accurately weigh 0.35g of sample that has been previously dried at 105°C for four hours.

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

6.4.4. Add 10mL of glacial acetic acid, 100mL of methanol, and 10 mL of a 0.2% polyvinyl alcohol solution.

6.4.5. Titrate with 0.1*N* $AgNO_3$ to a potentiometric end-point utilizing the Metrohm Titrando 907.

$$\%\ GHCl = \frac{mL_{AgNO3} * N_{AgNO3} * 9.553}{Sample\ Weight\ (g)} \qquad \%\ Cl = \frac{mL_{AgNO3} * N_{AgNO3} * 3.545}{Sample\ Weight\ (g)}$$

6.4.6. **Alternate Manual Assay Method:**

6.4.6.1. Refer to Standardization of 0.1N Silver Nitrate ($AgNO_3$) by hand as per Standardization of Titrants

6.4.6.2. Accurately weigh 0.35g of sample that has been previously dried at 105°C for four hours.

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

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

6.4.6.5. Titrate to a pink endpoint.

$$\%\ GHCl = \frac{mL_{AgNO3} * N_{AgNO3} * 9.553}{Sample\ Weight\ (g)}$$

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

6.5.1. Prepare a 6M solution of the specified sample (Solution may be scaled as needed).

6.5.1.1. Accurately weigh 57.2g of sample into a 100 mL volumetric flask and Q.S. to the mark with purified water.

6.5.1.2. Mix thoroughly.

6.5.2. Refer to Lambda 25 UV/Vis Operation and Calibration to determine the absorbance of the sample.

6.5.3. 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 20g of sample in 200 mL purified water.

6.6.2. Add 0.15mL of phenolphthalein indicator and titrate to a 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 <0.2%:**

6.7.1. Outside testing.

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

6.8.1. Place 25-50g 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. **ASSAY (Dried Basis) GHCl | Cl 99.5-101% | 36.92-37.48%:**

6.9.1. Standardize 0.1N $AgNO_3$ as per Standardization of Titrants.

6.9.2. Accurately weigh 0.35g of sample that has been previously dried at 105°C for four hours.

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

6.9.4. Add 10mL of glacial acetic acid, 100mL of methanol, and 10 mL of a 0.2% polyvinyl alcohol solution.

6.9.5. Titrate with 0.1*N* $AgNO_3$ to a potentiometric end-point utilizing the Metrohm Titrando 907.

$$\%\ GHCl = \frac{mL_{AgNO3} * N_{AgNO3} * 9.553}{Sample\ Weight\ (g)} \qquad \%\ Cl = \frac{mL_{AgNO3} * N_{AgNO3} * 3.545}{Sample\ Weight\ (g)}$$

6.9.6. **Alternate Manual Assay Method:**

6.9.6.1. Refer to Standardization of 0.1N Silver Nitrate ($AgNO_3$) by hand as per Standardization of Titrants

6.9.6.2. Accurately weigh 0.35g of sample that has been previously dried at 105°C for four hours.

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

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

6.9.6.5. Titrate to a pink endpoint.

$$\%\ GHCl = \frac{mL_{AgNO3} * N_{AgNO3} * 9.553}{Sample\ Weight\ (g)}$$

6.10. **ASSAY (Guanidine Basis) 99.5% Min:**

6.10.1. Standardize 0.1N Perchloric by hand as per Standardization of Titrants.

6.10.2. 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.

6.10.3. Accurately weigh 0.35g of Guanidine hydrochloride into an appropriate beaker.

6.10.4. Add stir bar.

6.10.5. Add 140mL of Glacial Acetic Acid.

6.10.6. Add 20mL of 5% mercuric acetate in glacial acetic acid solution.

6.10.7. Add 0.1mL of 100mg/10mL crystal violet in glacial acetic acid solution.

6.10.8. Dissolve sample while stirring.

6.10.9. Record Temperature of the Perchloric Acid.

6.10.10. Titrate to a blue green end point with 0.1N Perchloric Acid.

6.10.10.1. 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. $C_f = [1+1.07x10^{-3} (T_s-T_i)]$

6.10.10.1.1. T_s = temperature at standardization

6.10.10.1.2. T_i = temperature at sample titration

$$\%Guanidine = \frac{(EP_{Sample} - EP_{Blank})(N\ of\ Perchloric\ Acid)(9.55318)(C_f)}{\big(Sample\ weight\ (g)\big) - \big((Sample\ weight\ (g)\big)(\%LOD)\big)}$$

6.1. **CHLORIDE IDENTIFICATION Passes Test:**

6.1.1. Prepare a 6M solution of the specified sample (Solution may be scaled as needed). Solution previously prepared for Absorbance (6M) may be utilized.

6.1.1.1. Accurately weigh 57.2g of sample into a 100 mL volumetric flask and Q.S. to the mark with purified water.

6.1.1.2. Mix thoroughly.

6.1.2. Transfer 2mL of the sample solution to a beaker and add 0.2mL of 0.1N Silver Nitrate.
6.1.3. A white, curdy precipitate that is insoluble after the addition of 1mL of Concentrated Nitric Acid is produced.
6.1.4. If no precipitate is produced, notify the appropriate personnel.
6.1.5. Add 4mL of 6N Ammonium Hydroxide. The precipitate should dissolve after mild agitation.

6.2. **CLARITY AND COLOR OF SOLUTION** **Clear, Colorless:**

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

6.3. **CYANIDE** **0.1 ppm max.:**

Primary Cyanide Method:

6.3.1. 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 0.45g of ferrous sulfate in 50 mL of 0.1N Hydrochloric acid and dilute to 100 mL with purified water.
6.3.2. 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 0.1mL 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).

Alternative Cyanide Method:

6.3.3. Equipment and Reagents:
6.3.3.1. Distillation apparatus: Use a suitable cyanide distillation apparatus.
6.3.3.2. 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.
6.3.3.3. 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.
6.3.3.4. Sodium Hydroxide Solution 0.2N- Dilute 8 grams of NaOH pellets to 1L with purified water.
6.3.3.5. Phosphate Buffer: Dissolve 138g of Sodium Phosphate in water. Add 70mL of Acetic Acid.
6.3.3.6. Chloramine T Solution: Dissolve 1.0g of white, water soluble Chloramine T in 100mL of water. Prepare fresh each use.

6.3.3.7. Stock Cyanide Solution: Prepare from Potassium Cyanide or purchase commercially.

6.3.4. Distillation:

6.3.4.1. Transfer 500mL of the test sample [250grams of crystals plus 250grams of water mixed until dissolved] to a 1-liter distillation flask.

6.3.4.2. Add several glass beads.

6.3.4.3. Turn on the laboratory vacuum.

6.3.4.4. 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.

6.3.4.5. Connect it to the condenser outlet tube.

6.3.4.6. Start cooling water through the condenser.

6.3.4.7. Apply heat.

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.

6.3.4.8. 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.

6.3.4.9. After 2 hours, turn off the heat and continue to vacuum flow for 15 minutes.

6.3.4.10. Disconnect the absorption bottle.

6.3.4.11. 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.

6.3.4.12. 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.

6.3.5. Determining CN- Concentration and Preparation:

6.3.5.1. 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.

6.3.5.2. Transfer 50.0mL of absorption liquid to 100mL volumetric flask.

6.3.5.3. Prepare a blank of 50.0mL of 0.2N NaOH in a 100mL volumetric flask.

6.3.5.4. To 50.0mL of 0.2N NaOH in a 100mL volumetric flask, transfer 0.005mg of Cyanide.

6.3.5.5. Add 15mL of phosphate buffer solution to each flask and mix. (The pH of the solution now should be 5.5-6.5.)

6.3.5.6. Add 2.0mL of Chloramine T solution to each flask and mix.

6.3.5.7. 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.

6.3.5.8. Allow 8 minutes for color development.

6.3.5.9. Any red color in the sample should be less than that of the standard. (0.1ppm maximum)

6.4. **ETHYL ALCOHOL SOLUBILITY** **Passes Test:**

6.4.1. Dissolve 5g of sample in 100 mL of reagent alcohol.

6.4.2. Sample solutions should dissolve and be a clear colorless solution to pass test.

6.5. **FREE ACID** **0.01% Max:**

6.5.1. Refer to Acidity.

6.6. **FREE CN** **0.01% Max:**

6.6.1. Outside Testing.

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

6.7.1. RNase, DNase, and Protease per SOPs listed in Section 3.

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

6.12.1 Refer to section 6.21: Trace Metals.

6.12.2 Alternate Wet Method:

6.12.2.1 Standard Lead Solution: On the day of use, dilute 10.0mL of Lead Nitrate Stock Solution with purified water to 100 mL.

6.12.2.2 Standard Preparation: Into a Nessler comparison tube pipette 2 mL of the Standard Lead Solution and QS to 40 mL in purified water.

6.12.2.3 Sample Preparation: Weigh 2g of sample, transfer to a Nessler comparison tube, and dissolve in 40 mL of purified water.

6.12.2.4 Procedure: Adjust pH to between 3 and 4 using acetic acid. To all solutions, add 2mL of pH 3.5 Acetate Buffer and 1.2mL 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 50mL 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).

6.9. **IDENTIFICATION (UATR)** **Passes Test:**

6.9.1. Follow Spectrum Two UATR SOP.

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

6.10.1. 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.

6.10.2. Place the vial on the analytical balance and tare the dried vial. Weigh 2.0 g of sample and record results.

6.10.3. Dry for 4 hours at 105˚C. Cool for 15 minutes in desiccator.

6.10.4. Retain Sample for Assay, dried basis.

6.10.5. Reweigh and calculate the % LOD.

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

6.11. **MELAMINE** **Passes Test:**

6.11.1. Prepare a 6M solution of the specified sample (Solution may be scaled as needed). Solution previously prepared for Absorbance (6M) may be utilized.

6.11.1.1. Accurately weigh 57.2g of sample into a 100 mL volumetric flask and Q.S. to the mark with purified water.

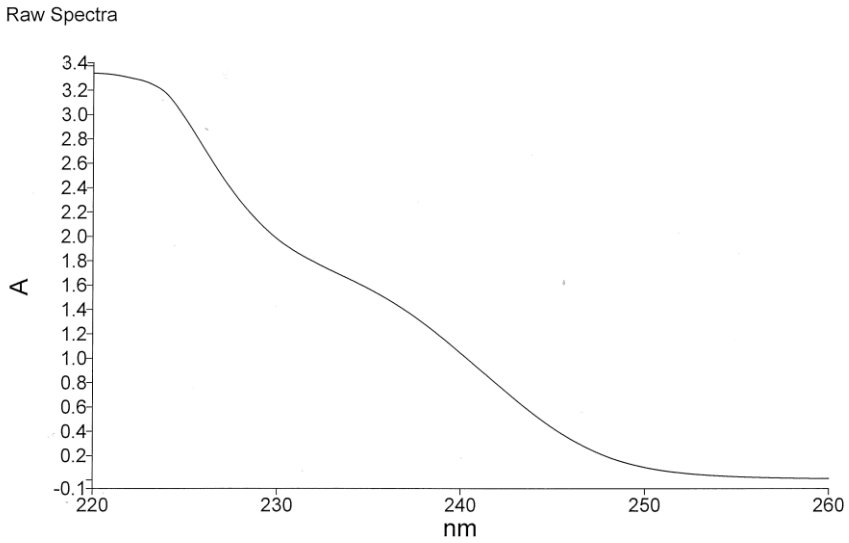
6.11.1.2. Mix thoroughly.

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

6.11.3. 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.

6.11.3.1. If any point exceeds an absorbance of 3 notify the QC Manager immediately.

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



6.12. **MELTING RANGE** **184 – 188°C:**

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

6.13. **NITRATE** **0.005% max.:**

6.13.1. Sample Preparation (Solution A):

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

6.13.2. Control Preparation (Solution B):

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

6.13.3. Blank Preparation (Solution C):

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

6.13.4. Procedure:

6.13.4.1. 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 10mm 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)". Remove blank solution C and scan sample

solution A and control solution B respectively at 410nm. Calculate the nitrate content as:

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

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

6.14.1. Dissolve 5.0 g of sample in 100mL of purified water.
6.14.2. Ensure the pH curve is stable before obtaining result.
6.14.3. Follow the appropriate SOP for calibration and pH measurement.

6.15. **pH (6M) @25°C 4.0-6.0:**

6.15.1. Prepare a 6M solution of the specified sample (Solution may be scaled as needed). Solution previously prepared for Absorbance (6M) may be utilized.
6.15.1.1. Accurately weigh 57.2g of sample into a 100 mL volumetric flask and Q.S. to the mark with purified water.
6.15.1.2. Mix thoroughly.
6.15.1.3. Ensure the pH curve is stable before obtaining result.
6.15.1.4. Follow the appropriate SOP for calibration and pH measurement.

6.16. **RESIDUE ON IGNITION 0.05% max.:**

6.16.1. Turn on muffle furnace and allow temperature to stabilize at 600ºC. Follow muffle furnace SOP and calibration procedure for operation.
6.16.2. Utilize the 10 inch forceps to insert and remove a crucible into the furnace.
6.16.3. Ignite the quartz crucible at 600 ± 50 °C for 30 minutes. Cool in a desiccator for one hour and 30 minutes and weigh.
6.16.4. Weigh 5.0g sample in the previously ignited quartz crucible. Moisten the sample with 2 mL of sulfuric acid.
6.16.5. Volatilize the sample with a Bunsen burner. Keep the sample an appropriate distance from the flame, so that the sample does not boil over and sample is not lost.
6.16.5.1. The rate of heating should be such that from ½ to 1 hour is required to volatilize the sample.
6.16.5.2. Continue using the Bunsen burner to heat the sample until all excess sulfuric acid has been volatilized.
6.16.6. Ignite in the muffle furnace at 600 ± 50 °C for 30 minutes or until all carbon has been removed.
6.16.7. Cool in the desiccator for a minimum of an hour and a half and reweigh.

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

6.17. **SOLUBILITY (6M) Clear/Colorless:**

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

6.18. **SOLUBILITY IN WATER** **Clear/Colorless:**

6.18.1. Accurately weigh 60.0 g of sample and transfer to a 250 mL beaker.
6.18.2. Accurately measure 100mL of purified water in a graduated cylinder and transfer to the sample beaker.
6.18.3. Swirl to dissolve the sample. If necessary, utilize a stirrer plate and Teflon encapsulated magnetic stirring bar.
6.18.4. The sample completely dissolves in purified water to report as Clear/Colorless when compared to a clear and colorless reference solution.

6.19. **SULFATE** **0.005% max.:**

6.19.1. Sample Preparation:
6.19.1.1. Weigh 1.0g of sample and transfer to a 50 mL Nessler Color Comparison Tube. Dissolve in 40mL of purified water.
6.19.2. Standard Preparation:
6.19.2.1. Prepare a standard solution by pipetting 0.052 mL of 0.020 N H_2SO_4 in a 50 mL Nessler Color Comparison Tube. QS to 40 mL with purified water.
6.19.3. Procedure:
6.19.3.1. To both the sample and standard solutions, add 1 mL of 3 N HCl, 3 mL of Barium Chloride TS and QS to the line.
6.19.3.2. Cover with parafilm and mix by inversion.
6.19.3.3. Compare turbidity 10 minutes after addition of the barium chloride to the sample and standard solutions.
6.19.4. Any turbidity produced in the sample solution should not exceed that produced by the standard when viewed from above against a black surface.
6.19.5. If turbidity of the sample solution exceeds that of the standard notify the QC Manager immediately.

6.20. **TOTAL CN** **<0.005%:**

6.20.1. Outside Testing.

6.21. **TRACE METALS** **REFER TO SUMMARY SHEET:**

6.21.1. Refer to Perkin Elmer NexION 350X ICP-MS SOP.

6.22. **WATER INSOLUBLES** **REFER TO SUMMARY SHEET:**

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

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