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L-ARGININE HYDROCHLORIDE TESTING METHODS

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1. PURPOSE:

1.1. To provide Laboratory Personnel with procedures for testing L-Arginine Hydrochloride.

2. SCOPE:

2.1. Applies to the testing of L-Arginine Hydrochloride in the laboratory at all BioSpectra Facilities. Methods include testing for all types of L-Arginine Hydrochloride sold by BioSpectra; only the specific tests required for the desired type must be tested.

3. RESPONSIBILITIES:

3.1. The Director of Laboratory Testing is responsible for the control, 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 Quality Assurance / Laboratory Managers or designee if any analyses fail to meet their respective specifications.
3.3. Standard laboratory safety regulations apply. Before working with any chemical, read and understand the Safety Data Sheet (SDS).

4. REFERENCES:

4.1. BSI-RPT-1537, Analytical Method Verification Report: L-Arginine HCl Chloride Assay
4.2. BSI-RPT-1579, Analytical Method Verification Report: L-Arginine HCl Assay by Potentiometric Titration with 0.1N Perchloric acid
4.3. BSI-SOP-0069, Preparation of Samples for Outside Testing
4.4. BSI-SOP-0090, Lambda 25 UV/Vis Operation and Calibration
4.5. BSI-SOP-0094, Muffle Furnace SOP and Calibration
4.6. BSI-SOP-0098, Balance SOP
4.7. BSI-SOP-0126, Laboratory Notebooks
4.8. BSI-SOP-0134, Pipette SOP
4.9. BSI-SOP-0140, Standardization of Titrants
4.10. BSI-SOP-0143, Metrohm Titrando 907 Auto-Titrator SOP
4.11. BSI-SOP-0242, Bangor Portable Turbidimeter Operation and Calibration
4.12. BSI-SOP-0244, VWR Gravity Convection Oven Operation and Calibration (Model Number 414005-106)
4.13. BSI-SOP-0254, Spectrum Two UATR SOP
4.14. BSI-SOP-0255, XL200 pH/mV/Conductivity Meter SOP
4.15. BSI-SOP-0345, Endosafe nexgen-PTS Endotoxin Reader SOP
4.16. BSI-SOP-0420, 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 Cytidine, Uridine, L-Arginine HCl, and L-Glutamine
4.17. BSI-SOP-0490, MCP 5300 Polarimeter SOP
4.18. *Current USP/ NF*
4.19. *EP*
4.20. ICH Q1A
4.21. *JP*

5. EQUIPMENT:

5.1. 0.25mm Layer Thickness Chromatographic Silica Gel Mixture TLC Plate
5.2. 50mL Glass Burette
5.3. 50mL Nessler Color Comparison Tubes
5.4. Analytical Balance
5.5. Anhydrous Potentiometric Electrode

5.6. Bunsen Burner
5.7. Calibrated Pipettes
5.8. Calibrated Timer
5.9. 0.2µm PVDF filters
5.10. Desiccator
5.11. Endosafe nexgen-PTS Endotoxin Reader
5.12. Litmus Paper
5.13. MCP 5300 Polarimeter
5.14. Metrohm Titrando 907 Auto-Titrator
5.15. Muffle Furance
5.16. Perkin Elmer Lambda 25 UV/Vis Spectrophotometer
5.17. Perkin Elmer NexION 350X ICP-MS
5.18. Perkin Elmer Spectrum Two UATR
5.19. pH Probe
5.20. Portable Turbidimeter
5.21. Temperature Probe
5.22. VWR Gravity Convection Oven or equivalent
5.23. XL200 pH/Conductivity Meter or equivalent

6. REAGENTS:

6.1. **0.020N Sulfuric Acid:** Slowly add 20mL of 0.1N Sulfuric Acid to 80mL of purified water to make a total volume of 100mL.
6.2. **0.1N Perchloric Acid:** Purchased Commercially.
6.3. **0.1N Silver Nitrate / Silver Nitrate TS:** Purchased Commercially.
6.4. **0.1N Sodium Hydroxide:** Purchased Commercially.
6.5. **0.1N Sulfuric Acid:** Purchased Commercially.
6.6. **0.25M Tris Base:** Purchased Commercially.
6.7. **1 – 0.01 EU/mL Endotoxin Cartridge:** Purchased Commercially.
6.8. **2N Acetic acid:** Slowly add 12mL of Glacial Acetic Acid to 25mL of Purified Water in a 100mL volumetric flask. If solution feels warm, stopper and allow to cool to room temperature. Bring to final volume with purified water.
6.9. **3N Hydrochloric Acid:** Pipette 25.75mL of Concentrated Hydrochloric Acid and transfer to a 100mL volumetric flask that contains a small amount of Purified Water. Dilute to volume with Purified Water.
6.10. **6N Hydrochloric Acid:** Pipette 51.50mL of Concentrated Hydrochloric Acid and transfer to a 100mL volumetric flask that contains a small amount of Purified Water. Dilute to volume with Purified Water.
6.11. **98% Formic Acid:** Purchased Commercially.
6.12. **150g/L Sodium Hydroxide:** Dissolve 15.0g of Sodium Hydroxide in Purified Water, dilute to 100mL with Purified Water, and mix well.
6.13. **Ammonia TS:** To 40mL of Ammonia Solution (28%) add Purified Water to make 100mL.
6.14. **Ammonium Hydroxide / Ammonia Solution (28%):** Purchased Commercially.
6.15. **Arginine Hydrochloride Certified Reference Standard:** Purchased Commercially.
6.16. **Barium Chloride Dihydrate:** Purchased Commercially.
6.17. **Barium Chloride TS:** Dissolve 12g of Barium Chloride Dihydrate in Purified Water. Filter and dilute to make a total volume 100mL with Purified Water.
6.18. **Butyl Alcohol:** Purchased Commercially.
6.19. **Dichlorofluorescein:** Purchased Commercially.
6.20. **Dichlorofluorescein TS:** Dissolve 100mg of Dichlorofluorescein in 60mL of Reagent Alcohol, add 2.5mL of 0.1N Sodium Hydroxide, mix, and dilute with Purified Water to 100mL.

6.21. **Dilute Nitric Acid:** Dilute 10.5mL of Concentrated Nitric Acid with Purified Water to make 100mL.
6.22. **Eosin Y:** Purchased Commercially.
6.23. **Eosin Y Indicator:** Dissolve 50mg of Eosin Y in 10mL of Purified Water.
6.24. **EP Color Reference Solution BY_1:** Purchased Commercially.
6.25. **Glacial Acetic Acid:** Purchased Commercially.
6.26. **Isopropyl Alcohol:** Purchased Commercially.
6.27. **LAL Reagent Water:** Purchased Commercially.
6.28. **L-Lysine Hydrochloride Certified Reference Standard:** Purchased Commercially.
6.29. **Mercuric Acetate:** Purchased Commercially.
6.30. **Mercuric Acetate TS:** Dissolve 6.0g of Mercuric Acetate in Glacial Acetic Acid to make 100mL. Store in a tight container, protected from direct sunlight.
6.31. **Methanol:** Purchased Commercially.
6.32. **α-Naphthol (1-Napthol):** Purchased Commercially.
6.33. **α-Naphthol Solution R:** Dissolve 0.10g of α-naphthol in 3mL of 150g/L Sodium Hydroxide and dilute to 100mL with Purified Water. Prepare immediately before use.
6.34. **Ninhydrin:** Purchased Commercially.
6.35. **NIST Traceable Sodium Chloride:** Purchased Commercially.
6.36. **Nitric Acid, concentrated:** Purchased Commercially.
6.37. **Potassium Hydrogen Phthalate (KHP):** Prepare an appropriate sample container at 120°C for 30 minutes. Allow to cool in a desiccator. Crush and dry a suitable amount of Potassium Hydrogen Phthalate. Dry at 120°C for 2 hours. Cool and store in a desiccator in a closed container. Stable for 3 months.
6.38. **Potassium Iodide Starch Paper:** Purchased Commercially.
6.39. **Potassium Permanganate:** Purchased Commercially.
6.40. **Purified Water:** In-House or Purchased Commercially.
6.41. **Reagent Alcohol:** Purchased Commercially.
6.42. **Sodium Chloride:** Prepare a crucible at 450°C for 30 minutes. Allow to cool in a desiccator and weigh a maximum of 10.0g of NIST Sodium Chloride. Dry at 450°C for 24 hours. Cool in a desiccator, transfer to a previously dried vial, and store in a desiccator. Stable for 3 months.
6.43. **Sodium Hydroxide, pellets:** Purchased Commercially.
6.44. **Strong Sodium Hypochlorite Solution R:** Purchased Commercially.
6.45. **Sulfuric Acid, concentrated:** Purchased Commercially.

7. ANALYTICAL PROCEDURES:

7.1. **ABSORBANCE (1.4M)/(30.24% soln in water)**
7.1.1. Filter Milli-Q water through a 0.2µm PVDF filter prior to use.
7.1.2. Prepare a 1.4M solution of the specified sample.
7.1.2.1. Accurately weigh 7.51-7.61 grams of sample.
7.1.2.2. Transfer accurately weighed sample to a graduated cylinder, dissolve, and fill to a final volume of 25mL with purified water.
7.1.2.3. Mix Well.
7.1.2.4. Filter the solution through a 0.2µm PVDF filter, discarding the first 2mL of filtrate.
7.1.2.5. Collect the filtrate into a suitable colorless glass container.
7.1.3. Refer to the Lambda 25 UV/Vis Operation and Calibration SOP to measure the absorbance of the sample with a 1cm pathlength at 280nm and 400nm.

7.2. **AMMONIUM and NINHYDRIN-POSITIVE SUBSTANCES**
7.2.1. Note: Test results will be used to report USP specification Individual Impurities and Total Impurities.

7.2.2. Ammonium and Ninhydrin-Positive Substances will be performed by an outside testing laboratory.
- 7.2.2.1. Primary Provider: New Jersey Laboratories.
- 7.2.2.2. Package and send 5g of sample to New Jersey Laboratories with a purchase order and analysis request form.
- 7.2.2.3. Sample Submission form information is as follows:
  - 7.2.2.3.1. Storage Condition: Store at Room Temp.
  - 7.2.2.3.2. Controlled Substance: No
  - 7.2.2.3.3. Sample Disposition: Discard
  - 7.2.2.3.4. Turn Around Time: Standard Up to 12 Business Days
  - 7.2.2.3.5. Sample Name/Sample Size Submitted: L-Arginine HCl (5grams)
  - 7.2.2.3.6. Analysis Requested: Ammonium and Ninhydrin Positive Substances
  - 7.2.2.3.7. Method: NJL verified method, Report #VER-21-029
  - 7.2.2.3.8. Include the analysis and specification, below:

Table 1: Analysis and specification

Analysis	Specification
Ninhydrin-Positive Substance	For Each Impurity: NMT 0.2%
	Total Impurities: NMT 0.5%
Ammonium	NMT 0.02%

7.3. **APPEARANCE**
- 7.3.1. Place a suitable amount (~5-10g) of sample in a clean, dry, glass beaker.
- 7.3.2. In an area with sufficient lighting, view the sample from all sides.
- 7.3.3. The sample should be white or almost white in color and characteristic of a crystalline powder.

7.4. **APPEARANCE OF SOLUTION**
- 7.4.1. Test Solution:
  - 7.4.1.1. Dissolve 2.5g of sample in purified water, dilute to 50mL with purified water, and mix thoroughly.
- 7.4.2. Color:
  - 7.4.2.1. EP Color Reference Solution BY_6: Dilute 5.0mL of *EP Color Reference Solution* BY_1 to 100mL with purified water and mix well.
  - 7.4.2.2. In an area with sufficient lighting, compare the color of the *Test Solution* to *EP Color Reference Solution* BY_6.
  - 7.4.2.3. Acceptance Criteria:
    - 7.4.2.3.1. The color of the *Test Solution* may not be more intense than the color in *EP Color Reference Solution* BY_6 to report as Colorless.
- 7.4.3. Clarity:
  - 7.4.3.1. Analyze the *Test Solution* for turbidity using a calibrated turbidimeter.
  - 7.4.3.2. Acceptance Criteria:
    - 7.4.3.2.1. The turbidity result may not exceed 3NTU to report as Clear.
- 7.4.4. To report as Passes Test, the solution must be both Clear and Colorless.

7.5. **ASSAY (DRIED BASIS)**
- 7.5.1. Perform a daily check or standardization of 0.1N Perchloric Acid as per Standardization of Titrants.
- 7.5.2. Blank Analysis:
  - 7.5.2.1. Add 50mL of Glacial Acetic Acid, 3mL of 98% Formic Acid, and 6mL of Mercuric Acetate TS to a suitable beaker.

7.5.2.2. Titrate to a potentiometric endpoint with 0.1N Perchloric Acid using the Metrohm Titrando 907 and Anhydrous Potentiometric Electrode.

7.5.3. Sample Analysis:

7.5.3.1. Accurately weigh and transfer 0.24-0.26g of sample, dried per the Loss on Drying procedure, to a suitable beaker.

7.5.3.2. Completely dissolve the sample in 3mL of 98% Formic Acid.

7.5.3.2.1. Note: Ensure sample is completely dissolved before addition of Glacial Acetic Acid.

7.5.3.3. Add 50mL of Glacial Acetic Acid and 6mL of Mercuric Acetate TS.

7.5.3.4. Titrate to a potentiometric endpoint with 0.1N Perchloric Acid using the Metrohm Titrando 907 and Anhydrous Potentiometric Electrode.

7.5.3.5. Calculate % L-Arginine HCl using the following equation in the Metrohm® Tiamo™ software:

$$\%\ L\text{-}Arginine\ HCl = \frac{(Sample\ Endpoint\ (mL) - Blank\ Endpoint\ (mL))(Normality\ of\ Titrant)(10.53)}{Dried\ Sample\ Weight\ (g)}$$

7.6. **CHLORIDE CONTENT**

7.6.1. Perform a daily check or standardization of 0.1N Silver Nitrate as per Standardization of Titrants.

7.6.2. Accurately weigh and transfer 350mg of L-Arginine HCl sample into a porcelain casserole or a colorless glass beaker with a white background.

7.6.3. Add 140mL of purified water and 1mL of Dichlorofluorescein TS and mix well.

7.6.4. Titrate with 0.1N Silver Nitrate until the silver chloride flocculates and the mixture acquires a faint pink color.

7.6.5. Calculate the percentage of Chloride (Cl^-) in the sample using the following equation:

$$\%\ Chloride = \frac{(mL\ of\ Silver\ Nitrate)(Normality\ of\ Titrant)(3.545)}{Sample\ Weight\ (g)}$$

7.7. **CHROMATOGRAPHIC PURITY (TLC) / IDENTIFICATION C (EP)/RELATED SUBSTANCES (USP/JP)**

7.7.1. Solution Preparation:

7.7.1.1. Note: All solutions may be scaled as needed.

7.7.1.2. Development Solvent System (70% Isopropyl Alcohol: 30% Ammonium Hydroxide): Add 30mL of Ammonium Hydroxide to 70mL of Isopropyl Alcohol, mix well, and allow to come to room temperature.

7.7.1.3. Spray Reagent (2 mg/mL Ninhydrin in 95% Butyl Alcohol: 5% 2N Acetic Acid): Dissolve 200mg of Ninhydrin in 95mL of Butyl Alcohol and 5mL of 2N Acetic Acid and mix well.

7.7.1.4. System Suitability Solution (0.4 mg/mL Arginine HCl; 0.4 mg/mL L-Lysine HCl): Dissolve 10mg of Arginine HCl CRS and L-Lysine HCl CRS in purified water and dilute to 25mL with purified water. Mix well.

7.7.1.5. Standard Stock Solution (0.1 mg/mL Arginine HCl): Dissolve 10mg of Arginine HCl CRS in purified water and dilute to 100mL with purified water. Mix well.

7.7.1.6. Standard Test Solution (0.05 mg/mL Arginine HCl): Pipette 5.0mL of *Standard Stock Solution* into a 10mL volumetric flask. Dilute to volume with purified water and mix well.

7.7.1.7. Sample Solution (10 mg/mL Arginine HCl Sample): Dissolve 250mg of L-Arginine HCl sample in purified water and dilute to 25mL with purified water. Mix well.

7.7.2. Chromatographic System:

Table 2: Chromatographic System

Parameter	Setting
Mode	Thin-Layer Chromatography (TLC)
Adsorbent	0.25mm layer of Chromatographic Silica Gel Mixture
Application Volume	5 µL
Developing Solvent System	70%:30% Isopropyl Alcohol: Ammonium Hydroxide
Spray Reagent	2 mg/mL Ninhydrin in 95%:5% Butyl Alcohol: 2N Acetic Acid

7.7.3. Analysis:

7.7.3.1. Spot 5µL of *System Suitability Solution*, *Standard Test Solution*, and *Sample Solution* onto the TLC plate.

7.7.3.2. Place the plate in the chamber, ensuring that the spots or bands are above the surface of the mobile phase.

7.7.3.3. Allow the mobile phase to ascend the plate until the solvent front has traveled three-quarters of the length of the plate.

7.7.3.4. Remove the plate, mark the solvent front with a pencil.

7.7.3.5. Dry the plate between 100°C and 105°C until the ammonia disappears.

7.7.3.6. Spray with spray reagent and heat the plate between 100°C and 105°C for about 15 minutes.

7.7.3.7. Immediately examine the plate under white light, photograph plate if a reviewer is not immediately available for verification as the color will dissipate over time.

7.7.3.7.1. Note: If the Standard Test Solution is not easily seen, Increasing Image Contrast may be done electronically using the National Institute of Health publicly available ImageJ software:

7.7.3.7.1.1. https://ij.imjoy.io/

7.7.3.7.1.2. Step 1: File, Open, Select Local file of the plate photograph and load the image.

7.7.3.7.1.3. Step 2: Select, Image from the Top Menu, Select Adjust, Click Brightness/Contrast.

7.7.3.7.1.4. Step 3: Increase the contrast, then increase the brightness, then increase the contrast again to enhance the visualization of the spots.

7.7.3.7.1.5. Step 4: Save the file and print.

7.7.3.8. Acceptance Criteria:

7.7.3.8.1. The *System Suitability Solution* exhibits two clearly separated spots.

7.7.3.8.2. Any secondary spot from the *Sample Solution* is not larger or more intense than the principle spot from the *Standard Test Solution*. To report "Passes Test".

7.7.3.8.3. Identification C (EP): The principle spot in the *Sample Solution* is similar in position, color, and size to the principle spot in the *Standard Test Solution*.

7.8. CLARITY AND COLOR OF SOLUTION

7.8.1. Dissolve 1.0g of L-Arginine HCl sample in 10mL of purified water.

7.8.2. Solution should be clear and colorless.

7.9. **ENDOTOXIN**

7.9.1. Accurately weigh 100 mg of sample into a sterile tube.
7.9.2. Add 5 mL of LAL Reagent Water, dissolve, and mix thoroughly.
7.9.3. Adjust the pH between 6-8 with 0.25M Tris Base.
7.9.4. Dilute to 10 mL with LAL Reagent Water and mix.
7.9.5. Prepare a 1:1 dilution from the initial sample preparation and LAL Reagent Water for a final concentration of 0.0050 g/mL.
7.9.6. Refer to Endosafe nexgen-PTS Endotoxin Reader SOP for instrument analysis.

7.10. **IDENTIFICATION, (IR): A (USP), B (EP), 1 (JP)**

7.10.1. Follow Spectrum Two UATR SOP for sample preparation and analysis.

7.11. **IDENTIFICATION D, COLOR (EP)**

7.11.1. Dissolve about 20 - 30mg of sample in 2mL of purified water.
7.11.2. Add 1 mL of α-Naphthol Solution R.
7.11.3. Add 2mL of a mixture of equal volumes Strong Sodium Hypochlorite Solution R and Purified Water
7.11.3.1. In a separate vial, mix equal volumes of Strong Sodium Hypochlorite Solution R and Purified Water.
7.11.4. A red color develops to report as pass test.

7.12. **IDENTIFICATION E, CHLORIDES (EP), 2 (JP)**

7.12.1. Test Solution:
7.12.1.1. Dissolve 1g of sample in 10mL of Purified Water. Aliquot 3mL of sample to three separate test tubes.
7.12.2. Part 1:
7.12.2.1. To tube 1, add 1mL of Concentrated Sulfuric Acid and 100mg of Potassium Permanganate to the test solution and heat. The gas evolved turns moistened Potassium Iodide Starch Paper blue to pass test.
7.12.3. Part 2:
7.12.3.1. **(A)** To tube 2, add 1mL of Silver Nitrate TS., Add 1mL of Dilute Nitric Acid to the tube; the precipitate does not dissolve.
7.12.3.2. **(B)** To tube 3, add 1mL of Silver Nitrate TS. Add excess Ammonia TS to the tube; the precipitate dissolves.

7.13. **LOSS ON DRYING**

7.13.1. Dry an LOD vial in the oven at 105 ± 2˚C for 30 minutes.
7.13.2. Cool for 15 minutes in a desiccator, weigh the LOD vial, and record results.
7.13.2.1. If the substance to be tested is in the form of large crystals, reduce the particle size to about 2 mm by quickly crushing before weighing.
7.13.3. Transfer 1 - 2 g of the sample to the LOD vial, and accurately weigh the vial and contents. By gentle, sidewise shaking, distribute the sample as evenly as possible in the LOD vial to a depth of about 5 mm.
7.13.4. Place the LOD vial containing the sample into the oven and dry at 105°C + 2 °C for 3 hours.
7.13.5. Remove LOD vial from the oven and allow to cool in the desiccator for 15 minutes.
7.13.6. Reweigh the LOD vial and sample and retain the dried sample for other analyses, in necessary.
7.13.7. Calculate the %LOD as follows:

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

7.14. **OPTICAL ROTATION, SPECIFIC ROTATION / IDENTIFICATION A (EP)**

7.14.1. Accurately weigh 8.00 g of L-Arginine HCl sample and transfer to a 100 mL volumetric flask.

7.14.2. Dissolve the sample in 6N Hydrochloric Acid, fill to volume with 6N Hydrochloric Acid, and mix well.

7.14.2.1. NOTE: Plastic syringes are not recommended to transfer the sample solution as they can become stuck; a glass syringe is recommended. Tubing should be used to deliver the sample solution to the optical cell. Care must be taken to prevent splashing, when delivering the sample solution. If glass syringes are unavailable, contact the laboratory supervisor and draw the sample through tubing using a plastic syringe for vacuum on the other side of the measuring cell. Appropriate safety precautions including googles or splash shield should be worn when transferring the sample solution through tubing in case of unexpected leakage or breakage.

7.14.3. Analysis: Perform at 20°C.

7.14.4. Refer to MCP 5300 Polarimeter SOP for analysis.

7.15. **pH (1 in 10)**

7.15.1. Weigh out 10.0 g of L-Arginine HCl sample, transfer to a beaker, dissolve in 100 mL of purified water, and mix well.

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

7.16. **RESIDUE ON IGNITION / SULFATED ASH**

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

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

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

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

7.16.5. Weigh 1.0 g of sample in the previously ignited quartz crucible. Moisten the sample with 1.0 mL of concentrated sulfuric acid.

7.16.6. 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.

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

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

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

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

7.16.9. Calculate the %ROI as follows:

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

7.16.10. If the amount of the residue exceeds the limit specified, repeat the moistening with sulfuric acid using up to 1 mL, 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 0.0005 g or until the specification is met.

7.17. **SULFATES**

7.17.1. Note: Test written for tightest monograph compendia. Result will be reported for Sulfate USP, EP, and JP analysis.

7.17.2. Sample Preparation:

7.17.2.1. Weigh out 1.6 g of sample and transfer to a 50 mL Nessler Color Comparison Tube. Dissolve in 40 mL of Purified Water. If necessary, neutralize the solution with hydrochloric acid to litmus.

7.17.3. Sulfate Standard Solution:

7.17.3.1. Prepare a standard solution by pipetting 0.46 mL of 0.020N Sulfuric Acid into a Nessler Color Comparison Tube. Dilute to 40mL with Purified Water.

7.17.4. Procedure:

7.17.4.1. To both solutions, add 1mL of 3N Hydrochloric Acid and 3 mL of Barium Chloride TS.

7.17.4.2. Dilute to 50 mL with Purified Water.

7.17.4.3. Cover with parafilm and mix by inversion.

7.17.4.4. Compare turbidity 10 minutes after addition of the Barium Chloride to the sample and standard solutions.

7.17.5. Any turbidity produced in the sample solution should not exceed that produced by the standard when viewed from above against a black surface to report as $\leq 0.028\%$.

7.18. **TRACE METALS**

7.18.1. Note: Arsenic, Heavy Metals, and Iron will be reported from this analysis.

7.18.1.1. Refer to BSI-SOP-0420, 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 Cytidine, Uridine, L-Arginine HCl, and L-Glutamine for sample preparation and analysis.

8. COMPENDIAL METHOD REFERENCE:

8.1. Compendial Analytical Methods

Table 3: Compendial Analytical Methods

USP-NF Compendia	EP Compendia	JP Compendia
Analytical Test		
Chloride Content	Appearance of Solution	Clarity and Color of Solution
Chloride and Sulfate, *Sulfate*	Sulfates	Sulfate
		pH

8.2. Harmonized Compendial Analytical Methods

Table 4: Harmonized Compendial Analytical Methods

Analytical Test
Identification, IR (USP-A/EP-B/JP-1)
Identification, Specific Rotation (EP-A), Optical Rotation (USP/JP)
Identification C (EP), Chromatographic Purity (USP), Related Substances (JP)
Residue on Ignition (USP), Sulfated Ash (EP)
Loss on Drying (USP, EP, JP)

8.3. Validated Methods in accordance with Compendial Monograph or General Chapters

Table 5: Validated Methods in accordance with Compendial Monograph or General Chapters

Analytical Test
Ammonium and Ninhydrin-Positive Substances (USP/EP)
Arsenic, Heavy Metals, Iron
Assay (dried basis/dried substance)

8.4. Non-Compendial Methods

Table 6: Non-Compendial Methods

Analytical Test
Absorbance (1.4M), (30.24% soln in water)
Appearance and Color