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L-GLUTAMINE TESTING METHODS

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1. PURPOSE:

1.1. To provide the Laboratory personnel with procedures for testing L-Glutamine Raw Material, In-Process and Finished Goods.

2. SCOPE:

2.1. Applies to the testing of L-Glutamine in the Laboratory at both the Bangor, PA location and the Stroudsburg, PA location (if applicable instrumentation is present).

3. RESPONSIBILITIES:

3.1. The Director of Laboratory Testing is responsible for 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.

4. REFERENCES:

4.1. BSI-SOP-0019, Result Reporting
4.2. BSI-SOP-0094, Muffle Furnace SOP and Calibration
4.3. BSI-SOP-0098, Balance SOP
4.4. BSI-SOP-0126, Laboratory Notebooks
4.5. BSI-SOP-0140, Standardization of Titrants
4.6. BSI-SOP-0143, Metrohm Titrando 907 Auto-Titrator SOP
4.7. BSI-SOP-0254, Spectrum Two UATR SOP
4.8. BSI-SOP-0303, NexION 350X ICP-MS SOP
4.9. BSI-SOP-0420, Elemental Impurities via ICP-MS in Cytidine, Uridine, L-Arginine HCl, and L-Glutamine
4.10. BSI-SOP-0490, MCP 5300 Polarimeter SOP
4.11. *Current USP*

5. EQUIPMENT:

5.1. Analytical Balance
5.2. Anton Paar MCP 5300 Polarimeter
5.3. Blue M Oven, or equivalent
5.4. Metrohm 907 Titrando Auto-Titrator
5.5. Muffle Furnace
5.6. Perkin Elmer Flexar HPLC
5.7. Perkin Elmer NexION 350X ICP-MS
5.8. Perkin Elmer Spectrum Two UATR

6. ANALYTICAL PROCEDURES:

6.1. **Appearance and Color** **White Crystals or Crystalline Powder:**

6.1.1. Place a suitable amount (25-50 g is suggested, less may be used depending on sample size) of the sample in a clean, dry glass beaker.

6.1.2. In an area with sufficient lighting, view the sample from all sides. Observe color, crystalline form, and any foreign particulate matter.

6.1.3. Any non-conformance will be reported to the Quality Control Manager or designee, immediately.

6.2. **ASSAY (Dried Basis)** **98.5 – 101.5%:**

6.2.1. Sample: 150 mg of Glutamine Sample

6.2.2. Titrant: 0.1N Perchloric Acid VS

6.2.3. Endpoint Detection: Potentiometric

6.2.4. Blank: 3 mL of Formic Acid and 50 mL of glacial acetic acid

6.2.5. Analysis: Dissolve the sample in a mixture of 3 mL of formic acid and 50 mL of glacialacetic acid and titrate with 0.1N Perchloric Acid to a potentiometric end-point utilizing the Metrohm Titrando 907.

6.2.6. Calculation:

$$\% \text{ Glutamine} = \frac{(V-B)(N)(F)}{W} \times 100$$

V= Sample Titrant volume (mL)
B= Blank Titrant volume (mL)
N= Titrant normality
F =Equivalency factor: 146.10 mg/mEq
W= Sample Weight (mg)

6.3. **Chloride and Sulfate <*Chloride*>** **≤ 0.05%:**

6.3.1. Standard Preparation:

6.3.1.1. Pipette 0.50 mL of 0.02N HCl into a Nessler Color Comparison tube and dilute to approximately 40 mL with purified water.

6.3.2. Sample Preparation:

6.3.2.1. Weigh 0.7 g of sample and dissolve in approximately 40 mL of purified water. If necessary, neutralize the solution with nitric acid to litmus.

6.3.3. Procedure:

6.3.3.1. To each solution, add 1 mL of concentrated nitric acid and 1 mL of 0.1N silver nitrate. Dilute to 50 mL with purified water. Cover with parafilm, and mix by inversion. After 5 minutes, the turbidity of the sample preparation does not exceed that produced by the standard when viewed against a dark background.

6.4. **Chloride and Sulfate <*Sulfate*>** **≤ 0.03%:**

6.4.1. Standard Preparation:

6.4.1.1. Pipette 0.25 mL of 0.02N Sulfuric acid into a Nessler Color Comparison tube and dilute to approximately 40 mL with purified water.

6.4.2. Sample Preparation:

6.4.2.1. Weigh 0.8 g of sample and dissolve in approximately 40 mL of purified water. If necessary, neutralize the solution with hydrochloric acid to litmus.

6.4.3. Procedure:

6.4.3.1. To each solution, add 1 mL of 3N HCl and 3 mL of barium chloride TS. Dilute to 50 mLwith purified water. Cover with parafilm and mix by inversion. After 10 minutes, the turbidity of the preparation does not exceed that produced by the standard when viewed against a dark background.

6.5. **Identification UATR** **Conforms to Standard:**

6.5.1. Follow Spectrum Two UATR SOP.

6.6. **Iron** **≤ 30 ppm:**

6.6.1. Refer to BSI-SOP-0420 for sample preparation and analysis.

6.7. **Loss on Drying (105°C)** **≤ 0.3%:**

6.7.1. Dry a LOD vial in an oven at 105 ± 2°C for at least 1 hour. Cool for 15 minutes in adesiccator, weigh, and record weight.

6.7.2. Place the vial on the analytical balance and tare the dried vial. Weigh 1-2 g of sample and record weight.

6.7.3. Dry for 3 hours at 105°C. Cool for 15 minutes in desiccator.

6.7.3.1. Retain sample as needed for assay, dried basis.

6.7.4. Reweigh and calculate the % LOD.

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

6.8. **Optical Rotation, Specific Rotation @ 20°C** **+6.3° to +7.3°:**

6.8.1. Sample solution: 40 mg/mL in purified water, warm at 40°C to dissolve.

6.8.1.1. Accurately weigh 4.0 g of sample and transfer to a 100 mL volumetric flask.

6.8.1.2. Dissolve sample in purified water. Warm the sample to 40°C to dissolve.

6.8.1.3. QS to a final volume of 100 mL with purified water.

6.8.1.4. Measure at 20°C.

6.8.2. Refer to BSI-SOP-0490 for sample analysis.

6.9. **Related Compounds** **≤ 0.5%:**

6.9.1. TLC Chromatographic Method:

6.9.1.1. Solution Preparation:

6.9.1.1.1. Standard solution: 0.05 mg/mL of USP Glutamine RS in water

6.9.1.1.2. Sample solution: 10 mg/mL of Glutamine in water

6.9.1.2. Chromatographic System: (See Chromatography <621>, Thin-Layer Chromatography.)

6.9.1.2.1. Mode: TLC

6.9.1.2.2. Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

6.9.1.2.3. Application volume: 5 µL

6.9.1.2.4. Developing solvent system: Butyl alcohol, glacial acetic acid, and water (3:1:1)

6.9.1.2.5. Spray reagent: 2 mg/mL of ninhydrin in a mixture of butyl alcohol and 2 N acetic acid (95:5)

6.9.1.3. Analysis: Dry the plate at 80° for 30 min. Spray the plate with Spray reagent, heat at 80° for 10 min, and examine under white light.

6.9.1.4. Acceptance criteria: No secondary spot of the Sample solution is larger or more intense than the principal spot of the Standard solution (NMT 0.5%).

6.10. **Residue on Ignition** **≤ 0.3%:**

6.10.1. Turn on muffle furnace and allow temperature to stabilize at 600 ± 50 °C. Follow muffle furnace SOP and calibration procedure for operation.
6.10.2. Inspect crucible for discoloration or chips.
6.10.3. Utilize the 10-inch forceps to insert and place a crucible into the furnace.
6.10.4. Ignite the quartz crucible at 600 ± 50 °C for 30 minutes. Cool in a desiccator for 90
6.10.5. minutes and record weight utilizing an analytical balance.
6.10.6. Weigh 1-2 g sample in the previously ignited quartz crucible. Moisten the sample with 1 mL of sulfuric acid and then heat gently at a temperature as low as practicable until the sample is thoroughly charred.
6.10.7. Cool, then moisten the residue with 1 mL of sulfuric acid.
6.10.8. Volatilize the sample with a Bunsen burner or hot plate, such that it is heated gently until white fumes are no longer evolved. Keep the sample an appropriate distance from the heat, so that the sample does not boil over and sample is not lost.
6.10.8.1. The rate of heating should be such that from ½ to 1 hour is required tovolatilize the sample.
6.10.8.2. Continue using the Bunsen burner or hot plate to heat the sample until all excess sulfuric acid has been volatilized.
6.10.9. Ignite in the muffle furnace at 600 ± 50 °C for 15 minutes or until all carbon has been removed.
6.10.10. Cool in the desiccator for 90 minutes and reweigh.
6.10.11. Calculate %ROI using the following equation:

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

6.10.12. Additional volatilizations may be conducted in accordance with USP <281>.