BIOSPECTRA
100 Majestic Way, Bangor, PA 18013 / www.biospectra.us

TREHALOSE TESTING METHODS

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1. PURPOSE:

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

2. SCOPE:

2.1. Applies to the testing of Trehalose Raw Material, In-Process, Stability, and Finished Goods in the Laboratory at both the Majestic and Rockdale locations. Methods include testing for all types of Trehalose sold by BioSpectra; only the specific tests required for the requested type must be tested.

2.2. Trehalose is defined as a stable, non-reducing disaccharide with two glucose molecules linked in α, α-1, 1 configuration. It contains NLT 97.0% and NMT 102.0% of Trehalose ($C_{12}H_{22}O_{11}$), calculated on the anhydrous basis.

3. RESPONSIBILITIES:

3.1. Laboratory Management 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 Manager and Laboratory Management 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-ATM-0057, Analytical Method of Analysis: Elemental Impurities by ICP-MS in Trehalose

4.2. BSI-ATM-0084, Analytical Method of Analysis: Low Level Elemental Impurities in Trehalose Dihydrate via ICP-MS

4.3. BSI-ATM-0098, Trehalose Assay by HPLC with RI detection

4.4. BSI-ATM-0099, Determination of Related Substances for Trehalose by HPLC with RI Detection

4.5. BSI-ATM-0147, Residual Solvents Method for Trehalose

4.6. BSI-RPT-0885, Analytical Method Validation Report: Aqueous Soluble Residual Solvents USP 1467 – Trehalose

4.7. BSI-RPT-1066, Analytical Method Validation Report: Residual Solvents by Head Space GC FID – Trehalose, Dihydrate

4.8. BSI-SOP-0090, Lambda 25 UV/Vis Operation and Calibration

4.9. BSI-SOP-0091, Portable Turbidimeter SOP and Calibration

4.10. BSI-SOP-0094, Muffle Furnace SOP and Calibration

4.11. BSI-SOP-0098, Balance SOP

4.12. BSI-SOP-0126, Laboratory Notebooks

4.13. BSI-SOP-0133, Blue M Convection Oven Operation and Calibration SOP

4.14. BSI-SOP-0135, Laboratory Chemicals

4.15. BSI-SOP-0140, Standardization of Titrants

4.16. BSI-SOP-0143, Metrohm Titrando 907 Auto-Titrator SOP

4.17. BSI-SOP-0242, Bangor Portable Turbidimeter Operation and Calibration

4.18. BSI-SOP-0244, VWR Gravity Convection Oven Operation and Calibration (Model Number 414005-106)
4.19. BSI-SOP-0254, Spectrum Two UATR SOP
4.20. BSI-SOP-0255, XL200 pH/mV/Conductivity Meter SOP
4.21. BSI-SOP-0303, NexION 350X ICP-MS SOP
4.22. BSI-SOP-0345, Endosafe nexgen-PTS Reader SOP
4.23. BSI-SOP-0348, Waters Acquity UPLC H-Class Plus SOP
4.24. BSI-SOP-0490, MCP 5300 Polarimeter SOP
4.25. ACS, Reagent Chemicals, current edition
4.26. Current EP
4.27. Current USP-NF
4.28. Current *JP*

5. EQUIPMENT:

5.1. Analytical Balance
5.2. Calibrated Oven
5.3. Endosafe PTS Endotoxin Reader
5.4. Hach Portable Turbidimeter
5.5. Lambda 25 UV/Vis Spectrophotometer
5.6. MCP Polarimeter 5300
5.7. Metrohm 907 Titrando Auto-Titrator
5.8. Calibrated Muffle Furnace
5.9. Perkin Elmer NexION 350X ICP-MS
5.10. Perkin Elmer Flexar HPLC
5.11. Perkin Elmer Spectrum Two UATR
5.12. Waters Alliance HPLC
5.13. XL200 pH/Conductivity Meter or equivalent
5.14. Calibrated Pipets

6. REAGENTS:

6.1. **Acetic Acid:** Purchased commercially
6.2. **Acetic Acid R:** Dilute 30 g of glacial acetic acid to 100 mL with purified water.
6.3. **Acetate Buffer (pH 3.5):** Dissolve 62.5 g of ammonium acetate in 62.5 mL of purified water, and add 47.0 mL of concentrated hydrochloric acid. Adjust, if necessary, with 6N ammonium hydroxide or 6N hydrochloric acid to a pH of 3.5, dilute with purified water to 250 mL.
6.4. **Barium Chloride, dihydrate:** Purchased commercially
6.5. **Barium Chloride R:** Weigh 250 g of barium chloride and dissolve in a total volume of 1000 mL of purified water.
6.6. **Barium Chloride TS:** Dissolve 30 g of barium chloride dihydrate in water to make 250 mL.
6.7. **Boric Acid:** Purchased commercially
6.8. **Boric Acid Solution (1 in 25):** Weigh 4 g of boric acid. Transfer to a 100-mL volumetric flask. Dissolve and dilute to volume with purified water.
6.9. **Composite 5:** Purchased commercially
6.10. **Cupric Sulfate, powder:** Purchased commercially

6.12. **Dilute Acetic Acid:** Dilute 6 g of glacial acetic acid with water to make 100 mL (1 mol/L).
6.13. **Dilute Hydrochloric Acid (HCl) R:** Dilute 23.6 mL of concentrated hydrochloric acid with purified water to make 100 mL. (10%)
6.14. **Dilute Nitric Acid (HNO3) R:** Dilute 20 g of nitric acid to 100 mL with purified water.
6.15. **Dilute Nitric Acid (HNO3):** Dilute 10.5 mL of nitric acid with purified water to make 100 mL.
6.16. **Dipotassium Sulfate:** Purchased commercially
6.17. **Endosafe nexgen-PTS Reader high sensitivity cartridges (0.5 – 0.005 EU/mL):** Purchased commercially
6.18. **Ethanol, 30% v/v:** Dilute 30 mL of Ethanol 95% to 100 mL with purified water.
6.19. **Ethanol, 95%:** Purchased commercially
6.20. **Formamide:** Purchased commercially
6.21. **Glycine R:** Purchased commercially
6.22. **Hydrogen Peroxide (H2O2):** Purchased commercially
6.23. **Hydrochloric Acid (HCl):** Purchased commercially
6.24. **3N Hydrochloric Acid (HCl):** Pipette 25.75 mL of concentrated hydrochloric acid and transferred to a 100 mL volumetric flask that contains a small amount of purified water. Dilute to volume with purified water.
6.25. **0.01M Hydrochloric Acid (HCl):** Slowly add 10 mL of 0.1 N HCl to a 100-mL volumetric flask containing a small amount of purified water. Dilute to volume with purified water. Or purchased commercially
6.26. **Iodine TS:** Purchased commercially
6.27. **LAL Reagent Water:** Generated in-house or purchased commercially
6.28. **Lead Nitrate:** Purchased commercially
6.29. **Litmus:** Purchased commercially
6.30. **Methanol:** Purchased commercially
6.31. **Methyl Orange:** Dissolve 0.10 g of methyl orange in 100 mL of Purified water. Filter if necessary.
6.32. **Methyl Red TS:** Dissolve 100 mg of methyl red in 100 mL of alcohol and filter if necessary.
6.33. **Methylene Blue TS:** Dissolve 125 mg of methylene blue in 100 mL of alcohol and dilute with alcohol to 250 mL.
6.34. **Methyl Red- Methylene Blue TS:** Add 10 mL of methyl red TS to 10 mL of methylene blue TS and mix.
6.35. **1-naphthol:** Purchased commercially
6.36. **Nitric Acid (HNO3), concentrated:** Purchased commercially
6.37. **Potassium Sulfate, powder:** Purchased commercially
6.38. **Purified Water:** Generated in-house or purchased commercially
6.39. **Silicon Oil:** Purchased commercially
6.40. **Silver Nitrate (AgNO3) TS:** Purchased commercially
6.41. **0.1N Silver Nitrate (AgNO3):** Purchased commercially
6.42. **Sodium Chloride:** Purchased commercially
6.43. **Sodium Hydroxide:** Purchased commercially
6.44. **Sodium Hydroxide TS:** Dissolve 40 g in sufficient purified water, Q.S to 1000 mL when cool.
6.45. **Sodium Hydroxide Solution (2 in 5):** Dissolve 200 g of sodium hydroxide in 500 mL of purified water.

6.46. **Sodium Sulfide TS:** Dissolve 5 g of sodium sulfide nonahydrate in a mixture of 10 mL of water and 30 mL of glycerin. Preserve in well-filled, light-resistant bottles. Use within 3 months.
6.47. **Starch TS:** Purchased commercially
6.48. **Sulfuric Acid (H2SO4):** Purchased commercially
6.49. **0.005M Sulfuric Acid (H2SO4):** Dilute 0.14 mL of sulfuric acid 96% to 1000 mL with purified water.
6.50. **0.01N Sulfuric Acid (H2SO4) VS:** Slowly add 10 mL of 0.1N sulfuric acid to a small amount of purified water. Dilute to 100 mL with purified water.
6.51. **0.1N Sulfuric Acid (H2SO4):** Purchased commercially
6.52. **Thioacetamide:** Purchased commercially
6.53. **Thioacetamide TS:** Dissolve 4.0 g of thioacetamide in 100 mL of purified water.
6.54. **Tromethamine (NIST):** Purchased commercially
6.55. **USP Purified Water**: Generated in-house or purchased commercially

7. ANALYTICAL PROCEDURES:

IN-PROCESS TESTING

7.1. MOTHER LIQUOR ANALYSIS

7.1.1. Microbial Analysis will be sent to MPL Laboratories (USP-NF <61> <62>).
7.1.2. Endotoxin Analysis:
7.1.2.1. Dilute 0.3 mL of mother liquor to 10 mL with LAL reagent water in a sterile tube.
7.1.2.2. Follow Endosafe nexgen-PTS Reader SOP using high sensitivity cartridge (0.5 - 0.005 EU/mL).
7.1.3. Color and Clarity of Solution:
7.1.3.1. Prepare a 1:1 dilution of sample by pipetting 30 mL of Mother Liquor into 30 mL of USP purified water. Using the Lambda 25 UV/Vis, measure the absorbance of the sample solution at 420 and 720 nm in a 10-cm cuvette. Refer to Lambda 25 UV/Vis Operation and Calibration to determine the absorbance of the sample.

7.2. WET CRYSTAL ANALYSIS

7.2.1. Microbial Analysis will be sent to MPL Laboratories (USP-NF <61> <62>).
7.2.2. Endotoxin Analysis:
7.2.2.1. Weigh 300 mg ± 10 mg and transfer to a sterile tube.
7.2.2.2. Dissolve in ~5 mL of LAL reagent water.
7.2.2.3. Dilute to 10 mL with LAL reagent water and mix thoroughly.
7.2.2.4. Follow Endosafe nexgen-PTS Reader SOP using high sensitivity cartridge (0.5 - 0.005 EU/mL).
7.2.3. Color and Clarity of Solution:
7.2.3.1. Accurately weigh and transfer 33.0 g of sample into a 150-mL beaker.
7.2.3.2. Add 67.0 g of recently boiled water to dissolve.
7.2.3.3. Using the Lambda 25 UV/Vis, measure the absorbance of the sample solution at 420 and 720 nm in a **10-cm** cuvette.

7.2.3.4. Refer to BSI-SOP-0090, Lambda 25 UV/Vis Operation and Calibration, to determine the absorbance of the sample.

7.2.4. Water (KF):

7.2.4.1. Perform a standardization of the titrant (Composite 5) as per Standardization of Titrants.

7.2.4.2. Immediately weigh 0.1 g of sample (no grinding necessary) into the glass weighing spoon and tare it.

7.2.4.3. Transfer the sample to the KF vessel by removing the rubber septum and adding the sample into the conditioned methanol and formamide solution in the titration vessel.

7.2.4.3.1. Do not leave the rubber septum open for long periods of time as this will allow moisture to enter the titration vessel.

7.2.4.4. Return the weighing spoon to the balance, making sure not to lose any sample that was left behind. Once the weight stabilizes, record the sample weight and transfer to the method.

7.2.4.5. Check to make sure there is no residual sample stuck to the sides of the titration vessel. If there is any sample stuck to the side, stop the stir bead from spinning before swirling the vessel to rinse the sides.

7.2.4.6. Check to ensure the sample is fully dissolved before the titration begins (i.e. before the stir command completes).

7.2.4.7. The moisture content will then be determined by the Metrohm Titrando 907 Auto-titrator, using the following equation:

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

7.3. DRY CRYSTAL ANALYSIS

7.3.1. Water (KF):

7.3.1.1. Perform a standardization of the titrant (Composite 5) as per Standardization of Titrants.

7.3.1.2. Immediately weigh 0.1 g of sample (no grinding necessary) into the glass weighing spoon and tare it.

7.3.1.3. Transfer the sample to the KF vessel by removing the rubber septum and adding the sample into the conditioned methanol and formamide solution in the titration vessel.

7.3.1.3.1. Do not leave the rubber septum open for long periods of time as this will allow moisture to enter the titration vessel.

7.3.1.4. Return the weighing spoon to the balance, making sure not to lose any sample that was left behind. Once the weight stabilizes, record the sample weight and transfer to the method.

7.3.1.5. Check to make sure there is no residual sample stuck to the sides of the titration vessel. If there is any sample stuck to the side, stop the stir bead from spinning before swirling the vessel to rinse the sides.

7.3.1.6. Check to ensure the sample is fully dissolved before the titration begins (i.e. before the stir command completes).

7.3.1.7. The moisture content will then be determined by the Metrohm Titrando 907, using the following equation:

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

FINISHED GOOD TESTING

7.4. **SOLUTIONS :**

7.4.1. Dissolve 10.0 g of sample in purified water and dilute to 100 mL.

7.5. **APPEARANCE :**

7.5.1. Place 25-50 g of sample in a clean, dry glass beaker.

7.5.2. In an area with sufficient lighting, view the sample from all sides. The sample should be white, to almost white, in color and characteristic of crystalline powder.

7.5.3. If the sample does not conform to these specifications, notify the appropriate personnel immediately.

7.6. **APPEARANCE OF SOLUTION :**

7.6.1. Clear *(2.2.1.)* Turbidimetry

7.6.1.1. Rinse the sample bottle with the sample solution twice.

7.6.1.2. Fill sample bottle with the sample Solution S to the white line.

7.6.1.3. Coat outside of bottle with a thin coat of silicon oil.

7.6.1.4. Remove any air bubbles from the solution by using a syringe.

7.6.1.5. Allow the sample to sit capped for 2-3 minutes.

7.6.1.6. Follow the appropriate SOP as follows:

7.6.1.6.1. Stroudsburg - Measure and record the turbidity of the sample according to Portable Turbidimeter Operation and Calibration.

7.6.1.6.2. Bangor - Measure and record the turbidity of the sample according to Bangor Portable Turbidimeter SOP.

7.6.1.7. The sample solution must be < 3 NTU.

7.6.2. Colorless (*2.2.2, Method II*)

7.6.2.1. Add 10 mL of Solution S into a Nessler Color Comparison Tube.

7.6.2.2. Add 10 mL of USP Purified Water into a second Nessler Color Comparison Tube.

7.6.2.3. Compare the colors in sufficient lighting, viewing vertically against a white background.

7.6.2.4. In order for the sample solution to be colorless, it must have the appearance of *USP Purified Water.*

7.7. **ASSAY (w/w%) :**

7.7.1. Refer to BSI-ATM-0098 for instrument, sample preparation, and analysis.

7.8. **Related SUBSTANCES :**

7.8.1. Refer to BSI-ATM-0099 for instrument, sample preparation, and analysis.

7.9. **CHLORIDE :**

7.9.1. **USP-NF Analysis**

7.9.1.1. Sample Preparation:

7.9.1.1.1. Weigh 2.0 g of sample and dissolve in ~30-40 mL of purified water in a 50-mL Nessler Color Comparison Tube. If necessary, neutralize the solution with nitric acid to litmus.

7.9.1.2. 0.0125% Standard Preparation:

7.9.1.2.1. Prepare a standard solution by pipetting 0.70 mL of 0.01 M HCl in a 50-mL Nessler Color Comparison Tube. Dilute to ~30-40 mL with purified water.

7.9.1.3. Procedure:

7.9.1.3.1. Add to each solution, 1 mL of concentrated nitric acid and 1 mL of 0.1N silver nitrate. Q.S. to 50 mL with purified water.

7.9.1.3.2. Mix and allow to stand for 5 minutes utilizing a calibrated timer.

7.9.1.4. Any turbidity produced in the sample solution should not exceed that produced by the standard.

7.9.1.5. If a visible difference in the turbidity is not observed, utilize the Turbidimeter to measure the turbidity of the sample and standard solutions.

7.9.1.5.1. Follow the appropriate SOP:

7.9.1.5.1.1. Stroudsburg: Portable Turbidimeter SOP and Calibration

7.9.1.5.1.2. Bangor: Bangor Portable Turbidimeter and Calibration

7.9.2. **CP Analysis**

7.9.2.1. Carry out the limit test for chloride <0801> using 0.40 g of sample. Any opalescence produced is not more pronounced than that of a reference solution using 5.0 mL of sodium chloride standard solution.

7.9.2.2. Test solution: Weigh 0.40 g and dissolve it in about 25 mL of water. Add 10 mL of dilute nitric acid and filter if necessary, transfer the solution to a 50-mL Nessler tube, add water to produce 40 mL and mix well.

7.9.2.3. Reference solution: Pipette 5.0 mL of Sodium Chloride Standard Solution to a 50-mL Nessler tube. Add 10 mL of dilute nitric acid and sufficient water to produce 40 mL and mix well.

7.9.2.3.1. Sodium Chloride Standard Solution: Dissolve 0.165 g of sodium chloride in water in a 1000-mL volumetric flask. Dilute to volume with water and mix well (stock solution). Immediately before use, transfer 10 mL of the stock solution, accurately measured, into a 100-mL volumetric flask, dilute to volume with water and mix well (each mL of sodium chloride standard solution is equivalent to 10 µg of chlorine).

7.9.2.4. Procedure: To each of the Nessler tubes add 1 mL of Silver Nitrate TS, dilute with water to 50 mL and mix well. Allow to stand in the dark for 5 minutes, and compare the opalescence produced by viewing down the vertical axis of the tubes against a black background. Any opalescence in the test solution is not more intense than that in the reference solution.

7.9.3. **EP Analysis**

7.9.3.1. Dilute 4 mL of Solution S to 15 mL with USP Purified Water. Prepare a 0.0125% standard in the same manner using 10 mL of Chloride Standard Solution (5 ppm Cl) R and 5 mL of purified water. To both the standard and sample add 1 mL of dilute Nitric Acid R and add 1 mL of 0.1N Silver Nitrate Solution. After 5 minutes protected from light, any opalescence in the test solution is not more intense than that in the standard.

7.9.3.2. Chloride standard solution (5 ppm Cl): Immediately before use, dilute with purified water to 100 times its volume a solution containing sodium chloride equivalent to 0.824 g of NaCl in 1000 mL.

7.9.4. **JP Analysis**

7.9.4.1. Weigh 2.0 g of sample dilute to 40 mL with purified water. Add 6 mL of dilute nitric acid and purified water to make 50 mL, and use this solution as the test solution. Prepare the 0.018% control solution with 1.0 mL of 0.01 mol/L hydrochloric acid. Add 6 mL of dilute nitric acid and water to make 50 mL, and use this solution as the control solution. When the test solution is not clear, filter both solutions by using the same procedure. Add 1 mL of Silver Nitrate TS to the test solution and to the control solution, mix well, and allow to stand for 5 minutes protecting from light. Compare the opalescence developed in both solutions against a black background by viewing downward or transversely. The opalescence developed in the test solution is not more than that of the control solution.

7.10. **COLOR AND CLARITY OF SOLUTION :**

7.10.1. **USP-NF Analysis**

7.10.1.1. Accurately weigh 33.0 g of sample.

7.10.1.2. Transfer accurately weighed sample to a 150-mL beaker and add 67.0 g of recently boiled water to dissolve.

7.10.1.3. Using the Lambda 25 UV/Vis, measure the absorbance of the sample solution at 420 and 720 nm in a 10-cm cuvette.

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

7.10.1.5. Determine the absorbance difference:

$$Result = A_{420} - A_{720}$$

$$A_{420} = Absorbance\ of\ the\ Sample\ Solution\ at\ 420nm$$
$$A_{720} = Absorbance\ of\ the\ Sample\ Solution\ at\ 720nm$$

7.10.1.6. The absorbance at 720 nm is not more than 0.050 a.u.

7.10.1.7. The absorbance difference should be $\leq$ 0.100 a.u.

7.10.2. **CP Analysis**

7.10.2.1. To (33.0 g anhydrous sample) or (36.5 g dihydrate sample) in 100-mL volumetric flask, add freshly boiled and cooled water, dissolve completely, and cool.

7.10.2.2. Measure the absorbances of the solution at 420 and 720 nm <0401>.

7.10.2.3. Using the Lambda 25 UV/Vis, measure the absorbance of the sample solution at 420 and 720 nm in a 10-cm cuvette.

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

7.10.2.5. Determine the absorbance difference:

$$Result = A_{420} - A_{720}$$

$$A_{420} = Absorbance\ of\ the\ Sample\ Solution\ at\ 420\ nm$$

$$A_{720} = Absorbance\ of\ the\ Sample\ Solution\ at\ 720\ nm$$

7.10.2.6. The absorbance at 720 nm is not more than 0.033 a.u.

7.10.2.7. The absorbance difference should be ≤ 0.067 a.u.

7.11. ELEMENTAL IMPURITIES :

7.11.1. Primary method of analysis: Refer to BSI-ATM-0084 for sample preparation and analysis for low-level elemental impurities.

7.11.2. Secondary method of analysis: Refer to BSI-ATM-0057 for sample preparation and analysis.

7.12. ENDOTOXINS :

7.12.1. Weigh 300 mg +/- 10 mg and transfer to a sterile tube.

7.12.2. Dissolve in ~5 mL of LAL reagent water.

7.12.3. Dilute to 10 mL with LAL reagent water and mix thoroughly.

7.12.4. Follow Endosafe nexgen-PTS Reader SOP using high sensitivity cartridge (0.5 - 0.005 EU/mL).

7.13. HEAVY METALS :

7.13.1. CP Analysis

7.13.1.1. Test Solution (2nd tube): Dissolve 4.0 g in 23 mL of purified water, add 2 mL of acetate buffer (pH 3.5), carry out the limit test for Heavy metals <0821 method 1>. Prepare in duplicate for test solution and monitor solution.

7.13.1.2. Lead Standard Solution: Dissolve 0.1599 g of lead nitrate in 5 mL of nitric acid and 50 mL of purified water in a 1000-mL volumetric flask, dilute to volume with water, mix well as the stock solution. Transfer 10 mL of the stock solution, accurately measured, to a 100-mL volumetric flask, dilute with water to volume and mix well (each mL is equivalent to 10 µg of lead). This solution should be prepared on the day of use. All glassware used for standard prep should be free from lead.

7.13.1.3. **Method 1:** Use three 25-mL Nessler tubes.

7.13.1.3.1. To the 1st tube (standard tube) add 2 mL of lead standard solution. Add 2 mL of Acetate BS (pH 3.5), dilute with water to 25 mL.

7.13.1.3.2. To the 2nd tube (test tube) add the test solution prepped in step 7.13.1.1, above.

7.13.1.3.3. To the 3rd tube (monitor tube) add the test solution prepped in step 7.13.1.1, above. Add 2 mL of the Lead Standard Solution.

7.13.1.3.4. To each tube add 2 mL of Thioacetamide TS and mix well, allow to stand for 2 minutes, compare the color produced in all three tubes against a white background. The color produced in the 2nd tube is not more intense than the 1st tube and the color produced in the third tube is equal or more intense than that produced in the 1st tube.

7.13.2. **JP Analysis**

7.13.2.1. Weigh 5.0 g of sample. Dissolve in water to make 40 mL. Add 2 mL of dilute acetic acid and water to make 50 mL, and designate it as the test solution. Prepare the 5 ppm control solution with 2.5 mL of Standard Lead Solution. Add 2 mL of dilute acetic acid and water to make 50 mL.

7.13.2.2. Add 1 drop of Sodium Sulfide TS to each of the test solution and the control solution, mix thoroughly, and allow to stand for 5 minutes. Then compare the colors of both solutions by viewing the tubes downward or transversely against a white background. The test solution has no more color than the control solution.

7.14. **IDENTIFICATION TEST: USP-NF (A), EP (A), CP (ID 4), JP (ID 3) :**

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

7.15. **IDENTIFICATION TEST: USP-NF (B) EP (B), CP (ID 1) JP (ID 1) :**

7.15.1. Sample Solution (400 mg/mL of Trehalose):

7.15.1.1. Accurately weigh 2.0 g of sample and transfer to a suitable beaker.

7.15.1.2. Dissolve sample in 5 mL of purified water.

7.15.2. To 1 mL of the sample solution, add 0.4 mL of a 1 in 20 solution of 1-naphthol in 95% ethanol and mix thoroughly.

7.15.3. Gently add 2 mL of sulfuric acid to the sample solution.

7.15.4. A violet color should be produced.

7.16. **IDENTIFICATION TEST: USP-NF (C), EP (C), CP (ID2), JP (ID2) :**

7.16.1. Sample Solution (40 mg/mL of Trehalose):

7.16.1.1. Accurately weigh 1.0 g of sample and transfer to a suitable beaker.

7.16.1.2. Dissolve sample in 25 mL of purified water.

7.16.2. To 2 mL of the sample solution, add 1 mL of dilute Hydrochloric Acid R and mix. Keep the solution at room temperature for 20 minutes utilizing a calibrated timer.

7.16.3. To the sample solution, add 4 mL of a 40 g/L solution of Sodium Hydroxide TS and 2 mL of a 40 mg/mL solution of Glycine R and mix.

7.16.4. Heat the solution in boiling water (can utilize a water bath) for 10 minutes using a calibrated timer. No brown color should develop in order to report as passes test.

7.17. **IDENTIFICATION 3 (CP) :**

7.17.1. Refer to Assay Section 7.7 for result.

7.17.1.1. The retention time of the major peak of the sample solution corresponds to that of the reference solution as obtained in the Assay.

7.18. **MICROBIAL CONTENT :**

7.18.1. Package NLT 65 g into a sterile container and send to MPL Laboratories.

7.18.2. Refer to appropriate product code for specifications and analyses to be performed.

7.19. **NITROGEN CONTENT :**

7.19.1. Sample size: 5.0 g

7.19.2. Select an appropriate size Kjeldahl flask, from which the nitrogen is first liberated by acid digestion and then transferred quantitatively to the titration vessel by steam distillation.

7.19.3. Preparation Procedure:

7.19.3.1. Place an accurately weighed 5.0 g of sample in the digestion flask of the apparatus.

7.19.3.2. To the flask, add 1 g of a powdered mixture of potassium sulfate and cupric sulfate (10:1), and wash down any adhering material from the neck of the flask with USP purified water.

7.19.3.3. Add 30 mL of sulfuric acid, allowing it to rinse down the wall of the flask.

7.19.3.4. While swirling the flask, carefully add 1 mL of 30% hydrogen peroxide down the inside of the flask. NOTE: Do not add hydrogen peroxide while the flask is being heated, or reaction may be violent.

7.19.4. Heating:

7.19.4.1. Place the flask in the bowl of the electric heater and loosely place thermal beads around the edges, as proper insulation will aid in speeding up the digestion process. NOTE: Avoid allowing the beads beneath the flask.

7.19.4.2. At the beginning of the digestion, monitor and adjust heat as needed. The solution should be bubbling but should remain below the neck of the flask. Heat the flask until the solution has a clear blue color and the sides of the flask are free from carbonaceous material.

7.19.4.3. Once the color and absence of carbonaceous material has been achieved, carefully add 70 mL of water to the digestion mixture, cool the solution, and arrange for steam distillation.

7.19.5. Steam Distillation:

7.19.5.1. Using a funnel, add 45 mL of Sodium Hydroxide Solution (2 in 5) in such manner as to cause the solution to flow down the inner side of the flask to form a layer under the acid solution.

7.19.5.2. Rinse the funnel with 10 mL of water, tightly close the apparatus, and begin the distillation with steam immediately.

7.19.5.3. Receive the distillate in 15 mL of Boric Acid Solution (1 in 25), to which has been added 3 drops of Methyl Red-Methylene Blue TS and sufficient water to cover the end of the condensing tube.

7.19.5.4. Continue the distillation until the distillate measures 80 to 100 mL.

7.19.5.5. Remove the absorption flask and rinse the end of the condensing tube with a small quantity of water.

7.19.6. Titrate the distillate with 0.01N Sulfuric Acid VS.

7.19.6.1. Perform a blank determination, and make any necessary correction. Each mL of 0.01N Sulfuric Acid VS is equivalent to 140.1 µg of nitrogen.

7.19.7. Sulfuric Acid 0.01N reagent value: Run once to verify 0.01N sulfuric acid.

7.19.8. Accurately weigh about 0.036 g of Tromethamine, previously dried at 105°C for 3 hours. Dissolve sample in 50 mL of purified water. Add 3 drops of methyl orange. Titrate with 0.01N H_2SO_4 to a colorimetric endpoint.

7.19.8.1. Each 1.2114 mg of NIST Tromethamine is equivalent to 1 mL of 0.01N H_2SO_4.

N H_2SO_4 = (g Tromethamine) / (0.12114 x mL x 0.01 N H_2SO_4)

$$\% \, Nitrogen = \frac{(EP_1 - EP_{Blank}) \times 1.41 \times N \; of \; H_2SO_4}{Sample \; Weight \; (g)}$$

7.20. **pH @ 25°C/Acidity (CP) :**

7.20.1. Sample Preparation (100 mg/mL):

7.20.1.1. Accurately weigh 10.0 g of sample. Transfer to a clean, dry 100-mL volumetric flask.

7.20.1.2. Dilute to 100 mL with purified water. Swirl to dissolve.

7.20.1.3. Follow the appropriate SOP to measure and record the pH.

7.21. **RESIDUAL ETHANOL, IPA, and METHANOL :**

7.21.1. Residual solvent analysis is validated by internal method. Refer to BSI-RPT-0885 for sample preparation and analysis of higher specification. Refer to BSI-ATM-0147 for sample preparation and analysis lower specification.

7.22. **RESIDUE ON IGNITION :**

7.22.1. NOTE: Residue on Ignition will be the primary method. Residue on Ignition results will be reported for Residue on Ignition and Sulfated Ash.

7.22.2. Turn on muffle furnace and allow temperature to stabilize at 600ºC. Follow muffle furnace SOP and calibration procedure for operation.

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

7.22.4. Utilize forceps to insert and remove a crucible into the furnace.

7.22.5. Ignite the quartz crucible at 600° ± 50 °C for 30 minutes minimum. Cool in a desiccator for one hour and 30 minutes and weigh on analytical balance.

7.22.6. Weigh 2.0 g sample in the previously ignited quartz crucible. Moisten the sample with ~1 mL of sulfuric acid.

7.22.7. 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.

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

7.22.9. Continue using the Bunsen burner to heat the sample until all excess sulfuric acid has been volatilized.

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

7.22.11. Cool in the desiccator for a minimum of an hour and a half and reweigh.

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

7.22.12. If the amount of the residue exceeds the limit specified, repeat the moistening with sulfuric acid using up to 1 mL, heat via Bunsen burner and 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.

7.23. **SOLUBLE STARCH :**

7.23.1. Sample Solution Preparation (10% Trehalose (w/v)):

7.23.1.1. Accurately weigh 10.0 g of sample and transfer to a clean, dry suitable beaker.

7.23.1.2. Dissolve in purified water and dilute to 100 mL.

7.23.2. Add several drops of iodine TS to the sample solution.

7.23.3. No blue color should develop in order to report as passes test.

7.23.4. **Dextrin, Soluble Starch, and Sulfite JP Analysis:**

7.23.4.1. Dissolve 1.0 g of sample in 10 mL of purified water and add 1 drop of Iodine TS: a yellow color appears, which is changed to blue on addition of 1 drop of Starch TS.

7.24. **SPECIFIC ROTATION/OPTICAL ROTATION :**

7.24.1. Sample Preparation (100 mg/mL):

7.24.2. Accurately weigh 10.00 g of sample and transfer to a 100-mL volumetric flask.

7.24.2.1. Dissolve sample in USP purified water and QS to a final volume of 100 mL with purified water.

7.24.3. Follow MCP 5300 Polarimeter SOP and analyze within 30 minutes of preparation at 20°C.

7.24.3.1. Select the following method in the software: BSI-Specific Rotation (USP/NF; 20°C)

7.24.3.2. Enter the following information into the software:

7.24.3.2.1. Volume (dryness)(mL)

7.24.3.2.2. Mass (dryness)(mL)

7.24.3.2.3. Drying Loss (%) – KF Water Result

7.24.4. Result calculated on an anhydrous basis:

$$(Raw\ Result)x\ \frac{100}{100 - Karl\ Fisher\ \%}$$

7.25. **SPECIFIC ROTATION, (C=7 gm/100 mL water calculated on the basis of dihydrate, dried, 20°C): Customer Requested specification**

7.25.1. Sample Preparation:

7.25.1.1. Sample preparation is calculated on anhydrous basis.

7.25.1.2. Accurately weigh 6.33 g of sample and transfer to a 100-mL volumetric flask.

7.25.1.3. Calculation for sample prep:

$$Adjusted\ Concentration = \frac{7 \times \left(\frac{378.8 - 36.04}{378.3}\right)}{100\ mL} = 6.33\ grams$$

7.25.1.4. Dissolve sample in purified water and QS to a final volume of 100 mL with purified water.

7.25.2. Follow MCP 5300 Polarimeter SOP and analyze within 30 minutes of preparation at 20°C.

7.25.2.1. Results calculated on an anhydrous basis.

7.26. **SULFATED ASH :**

7.26.1. NOTE: Residue on Ignition will be the primary method. Residue on Ignition results will be reported for Residue on Ignition and Sulfated Ash.

7.26.2. Turn on muffle furnace and allow temperature to stabilize at 600ºC. Follow muffle furnace SOP and calibration procedure for operation.

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

7.26.4. Utilize forceps to insert and remove a crucible into the furnace.

7.26.5. Ignite the quartz crucible at 600° ± 50 °C for 30 minutes minimum. Cool in a desiccator for one hour and 30 minutes and weigh on analytical balance. Weigh 1.0 g sample in the previously ignited quartz crucible. Moisten the sample with ~1 mL of sulfuric acid.

7.26.6. 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.

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

7.26.8. Continue using the Bunsen burner to heat the sample until all excess sulfuric acid has been volatilized.

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

7.26.10. Cool in the desiccator for a minimum of an hour and a half and reweigh.

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

7.26.11. If the amount of the residue exceeds the limit specified, repeat the moistening with sulfuric acid using up to 1 mL, heat via Bunsen burner and 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.

7.27. **SULFATE :**

7.27.1. **USP-NF Analysis**

7.27.1.1. Sample Preparation:

7.27.1.1.1. Weigh 2.0 g of sample and dissolve in ~30-40 mL of purified water in a 50-mL Nessler Color Comparison Tube. If necessary, neutralize the solution with hydrochloric acid to litmus.

7.27.1.2. 0.0200% Standard Preparation:

7.27.1.2.1. Prepare a standard solution by pipetting 0.83 mL of 0.005M H_2SO_4 in a 50-mL Nessler Color Comparison Tube. Dissolve in ~30-40 mL of purified water.

7.27.1.3. Procedure:

7.27.1.3.1. To both the sample and standard solutions, add 1 mL of 3N HCl, 3 mL of Barium Chloride TS and sufficient water to make 50 mL.

7.27.1.3.2. Mix and allow samples and standard to stand for 10 minutes utilizing a calibrated timer.

7.27.1.4. Any turbidity produced in the sample solution should not exceed that produced by the standard.

7.27.1.5. If a visible difference in the turbidity is not observed, utilize the Turbidimeter to measure the turbidity of the sample and standard solutions.

7.27.1.5.1. Follow the appropriate SOP:

7.27.1.5.1.1. Stroudsburg: Portable Turbidimeter SOP and Calibration

7.27.1.5.1.2. Bangor: Bangor Portable Turbidimeter and Calibration SOP.

7.27.2. **CP Analysis**

7.27.2.1. Carry out the limit test for sulfate <0802>, using 1.0 g. Any opalescence produced is not more pronounced than that of the reference solution using 2.0 mL of Potassium Sulfate Standard Solution.

7.27.2.2. Test solution: weigh 1.0 g of sample, dissolve it in about 40 mL of water, neutralize this solution with hydrochloric acid and filter if necessary. Transfer the solution to a 50-mL Nessler tube, add 2 mL of dilute hydrochloric acid and mix well.

7.27.2.3. Reference Solution: Pipette 2.0 mL of Potassium Sulfate Standard Solution to a 50-mL Nessler tube, dilute with purified water to 40 mL, add 2 mL of dilute hydrochloric acid and mix well.

7.27.2.3.1. Potassium Sulfate Standard Solution: Dissolve 0.181 g of potassium sulfate in water in a 1000-mL volumetric flask. Dilute to volume with water and mix well (each mL of potassium sulfate standard solution is equivalent to 100 µg of Sulfate (SO_4).

7.27.2.4. Procedure: To each of the Nessler tubes, add 5 mL of 25% barium chloride solution, dilute with water to 50 mL and mix well. Allow to stand for 10 minutes and compare the opalescence in the test solution produced by viewing down the vertical axis of the tubes against a black background. Any opalescence in the test solution is not more intense than that in the reference solution.

7.27.3. **EP Analysis**

7.27.3.1. Sample solution: Dilute 7.5 mL of Solution S to 15 mL with purified water (distilled water R equivalent).

7.27.3.2. In a separate beaker, add 3 mL of a 250 g/L solution of Barium Chloride R to 4.5 mL of Sulfate Standard Solution (10 ppm SO_4) R1. Shake and allow to stand for 1 min. To 2.5 mL of this suspension, add the 15 mL sample solution and 0.5 mL of Acetic Acid R. Prepare a 0.0200% standard in the same manner using 15 mL of Sulfate Standard Solution (10 ppm SO_4) R instead of the prescribed solution.

7.27.3.3. After 5 min, any opalescence in the test solution is not more intense than that in the standard.

7.27.3.4. Sulfate Standard Solution (10 ppm SO_4) R1: Immediately before use, dilute with ethanol (30% v/v) R to 100 times its volume a solution containing dipotassium sulfate R equivalent to 0.181 g of K_2SO_4 in 100 mL of ethanol (30%v/v) R.

7.27.3.5. Sulfate standard solution (10 ppm SO_4): Immediately before use, dilute with purified water to 100 times its volume a solution in purified water containing dipotassium sulfate equivalent to 0.181 g of K_2SO_4 in 100 mL.

7.27.4. **JP Analysis**

7.27.4.1. Weigh 2.0 g of sample. Add water to make 40 mL. Add 1 mL of dilute hydrochloric acid and water to make 50 mL, and use this solution as the test solution.

7.27.4.2. Prepare the 0.024% control solution with 1.0 mL of 0.005 mol/L Sulfuric Acid VS. Add 1 mL of dilute hydrochloric acid and water to make 50 mL, and use this solution as the control solution.

7.27.4.3. When the test solution is not clear, filter both solutions according to the same procedure.

7.27.4.4. Add 2 mL of Barium Chloride TS to the test solution and to the control solution, mix well, and allow to stand for 10 minutes.

7.27.4.5. Compare the white turbidity produced in both solutions against a black background by viewing downward or transversely. The turbidity produced in the test solution is not thicker than that of the control solution.

7.28. **WATER (By Karl Fischer Titration) :**

7.28.1. Perform a standardization of the titrant (Composite 5) as per Standardization of Titrants.

7.28.2. Immediately weigh 0.1 g of sample (no grinding necessary) into the glass weighing spoon and tare it.

7.28.3. Transfer the sample to the KF vessel by removing the rubber septum and adding the sample into the conditioned formamide/methanol solution in the titration vessel.

7.28.4. Do not leave the rubber septum open for long periods of time as this will allow moisture to enter the titration vessel.

7.28.5. Return the weighing spoon to the balance, making sure not to lose any sample that was left behind. Once the weight stabilizes, record the sample weight and transfer to the method.

7.28.6. Check to make sure there is no residual sample stuck to the sides of the titration vessel.

7.28.7. If there is any sample stuck to the side, stop the stir bead from spinning before swirling the vessel to rinse the sides.

7.28.8. Ensure the sample is fully dissolved before the titration begins (i.e. before the stir command completes).

7.28.9. The moisture content will then be determined by the Metrohm Titrando 907 Auto-titrator.

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

8. COMPENDIAL DIFFERENTIATIONS:

8.1. Compendial Analyses

Table 1: Compendia Analysis

USP-NF Compendia	EP Compendia	JP Compendia	CP Compendia
Analysis Name	Analysis Name	Analysis Name	Analysis Name
Chloride Color and Clarity of Solution Sulfate	Appearance of Solution Chloride Sulfated Ash Sulfate	Chloride Heavy Metals Sulfate	Chloride Sulfate Heavy Metals Color and Clarity of Solution

8.2. Harmonized Methods: One Method utilized for analysis

Table 2: Harmonized Methods

Analysis Name
Endotoxin (USP-NF), (EP), (CP)
Identification A (USP-NF), Identification A (EP), Identification 3 (JP), Identification 4 (CP)
Identification B (USP-NF), Identification B (EP), Identification 1 (JP), Identification 1 (CP)
Identification C (USP-NF), Identification C (EP), Identification 2 (JP), Identification 2 (CP)
Microbial Content: *Escherichia Coli, Salmonella Species,* TAMC, TYMC (USP-NF), (EP), (CP)
Nitrogen Content (USP-NF), (JP)
pH @ 25°C (USP-NF), (EP), (JP), (CP) test for pH is Acidity
Residue on Ignition (USP-NF), (JP), (CP)
Soluble Starch (USP-NF), (EP), (CP)/ Dextrin, Soluble Starch, and Sulfite (JP)
Specific Rotation/Optical Rotation (USP-NF), (EP), (CP), (JP)
Water (USP-NF), (EP), (CP), (JP)

8.3. In-House Validated Methods in accordance with USP General Chapters:

8.3.1. <1225> Validation of Compendial Procedures

8.3.2. <1467> Residual Solvents- Verification of Compendial Procedures and Validation of Alternative Procedures

Table 3: In-House Validated Methods

Analysis Name
Assay (validated method utilizing a modified version of JP Assay Analysis)
Identification 3 (CP)
Related Substances: Impurities
Residual Solvents: Ethanol, IPA, Methanol

8.4. In-house Methods for Product Quality Description

Table 4: Product Quality Description

Analysis Name
Appearance and Color Descriptions that apply to passing product quality: White or almost white, crystalline powder. White to Off-White Crystalline Powder

8.5. Customer Requested Methods

Table 5: Customer Requested Methods

Analysis Name
Specific Rotation (C=7 gm/100 mL Water calculated on the basis of dihydrate, dried, 20°C)