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## TREHALOSE TESTING METHODS

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## 1. PURPOSE:

- 1.1. To provide the Quality Control (QC) 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 QC Laboratory at both the Bangor, PA location and the Stroudsburg, PA location. 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  $\alpha$ ,  $\alpha$ -1, 1 configuration. It contains NLT 97.0% and NMT 102.0% of Trehalose (C<sub>12</sub>H<sub>22</sub>O<sub>11</sub>), calculated on the anhydrous basis.

## 3. RESPONSIBILITIES:

- 3.1. Quality Control Management is responsible for the control, 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 QA Manager and QC 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-0098, Trehalose Assay by HPLC with RI detection
- 4.2. BSI-ATM-0099, Determination of Related Substances for Trehalose by HPLC with RI Detection
- 4.3. BSI-SOP-0090, Lambda 25 UV/Vis Operation and Calibration
- 4.4. BSI-SOP-0091, Portable Turbidimeter SOP 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-0133, Blue M Convection Oven Operation and Calibration SOP
- 4.9. BSI-SOP-0135, Laboratory Chemicals
- 4.10. BSI-SOP-0140, Standardization of Titrants
- 4.11. BSI-SOP-0143, Metrohm Titrando 907 Auto-Titrator SOP
- 4.12. BSI-SOP-0242, Bangor Portable Turbidimeter and Calibration
- 4.13. BSI-SOP-0244, VWR Gravity Convection Oven Operation and Calibration
- 4.14. BSI-SOP-0254, Spectrum Two UATR SOP
- 4.15. BSI-SOP-0255, XL200 pH/mV/Conductivity Meter SOP
- 4.16. BSI-SOP-0257, MCP 300 Polarimeter SOP
- 4.17. BSI-SOP-0303, NexION 350X ICP-MS SOP
- 4.18. BSI-SOP-0345, Endosafe nexgen-PTS Reader SOP
- 4.19. BSI-SOP-0348, Waters Acquity H-Class Plus UPLC SOP
- 4.20. *ACS, Reagent Chemicals*, current edition
- 4.21. *Current EP*
- 4.22. *Current USP-NF*
- 4.23. *Current JP*

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## 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 300
- 5.7. Metrohm 907 Titrando Auto-Titrator
- 5.8. Calibrated Muffle Furnace
- 5.9. Perkin Elmer NexION 350X ICP MS
- 5.10. Perkin Elmer HPLC
- 5.11. Perkin Elmer Spectrum Two UATR
- 5.12. Waters Acquity H-Class Plus UPLC
- 5.13. XL200 pH/Conductivity Meter or equivalent
- 5.14. Calibrated Pipets

## 6. ANALYTICAL PROCEDURES:

### IN-PROCESS TESTING

#### 6.1. Mother Liquor Analysis (Refer to Batch Record for Specifications):

- 6.1.1. Microbial Analysis will be sent to MPL Laboratories (USP-NF <61> <62>).
- 6.1.2. Endotoxin Analysis:
  - 6.1.2.1. Dilute 0.3mL of mother liquor to 10mL with LAL reagent water in a sterile tube.
  - 6.1.2.2. Follow Endosafe nexgen-PTS Reader SOP using high sensitivity cartridge (0.5-0.005EU/mL).
- 6.1.3. Color and Clarity of Solution:
  - 6.1.3.1. Prepare a 1:1 dilution of sample by pipetting 30mL of Mother Liquor into 30mL 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.

#### 6.2. Wet Crystal Analysis (Refer to Batch Record for Specifications):

- 6.2.1. Microbial Analysis will be sent to MPL Laboratories (USP-NF <61> <62>).
- 6.2.2. Endotoxin Analysis:
  - 6.2.2.1. Weigh 300mg +/- 10mg and transfer to a sterile tube.
  - 6.2.2.2. Dissolve in ~5mL of LAL reagent water.
  - 6.2.2.3. Dilute to 10mL with LAL reagent water and mix thoroughly.
  - 6.2.2.4. Follow Endosafe nexgen-PTS Reader SOP using high sensitivity cartridge (0.5-0.005EU/mL).
- 6.2.3. Color and Clarity of Solution:
  - 6.2.3.1. Accurately weigh 33.0 g of sample. Transfer accurately weighed sample to a 150 mL beaker and add 67.0 g of recently boiled water to dissolve. 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.

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#### 6.2.4. Water (KF):

6.2.4.1. Standardize Composite 5 as per Standardization of Titrants. Immediately weigh 0.1g of sample (no grinding necessary) into the glass weighing spoon and tare it. 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. Do not leave the rubber septum open for long periods of time as this will allow moisture to enter the titration vessel. 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. 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. Check to ensure the sample is fully dissolved before the titration begins (i.e. before the stir command completes). The moisture content will then be determined by the Metrohm Titrando 907 Autotitrator.

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

#### 6.3. **Dry Crystal Analysis (Refer to Batch Record for Specifications):**

##### 6.3.1. Water (KF):

6.3.1.1. Standardize Composite 5 as per Standardization of Titrants. Immediately weigh 0.1g of sample (no grinding necessary) into the glass weighing spoon and tare it. 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. Do not leave the rubber septum open for long periods of time as this will allow moisture to enter the titration vessel. 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. 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. Check to ensure the sample is fully dissolved before the titration begins (i.e. before the stir command completes). The moisture content will then be determined by the Metrohm Titrando 907 Autotitrator.

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

## **FINISHED GOOD TESTING**

### **6.4. SOLUTIONS** :

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

### **6.5. APPEARANCE** :

- 6.5.1. Place 25-50g of sample in a clean, dry glass beaker.
- 6.5.2. 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.
- 6.5.3. If the sample does not conform to these specifications, notify the appropriate personnel immediately.

### **6.6. APPEARANCE OF SOLUTION** :

#### **6.6.1. Clear (2.2.1.) Turbidimetry**

- 6.6.1.1. Rinse the sample bottle with the sample solution twice.
- 6.6.1.2. Fill sample bottle with the sample Solution S to the white line.
- 6.6.1.3. Coat outside of bottle with a thin coat of silicon oil.
- 6.6.1.4. Remove any air bubbles from the solution by using a syringe.
- 6.6.1.5. Allow the sample to sit capped for 2-3 minutes.
- 6.6.1.6. Follow the appropriate SOP as follows:
  - 6.6.1.6.1. Stroudsburg - Measure and record the turbidity of the sample according to Portable Turbidimeter Operation and Calibration.
  - 6.6.1.6.2. Bangor - Measure and record the turbidity of the sample according to Bangor Portable Turbidimeter SOP.
- 6.6.1.7. The sample solution must be < 3 NTU.

#### **6.6.2. Colorless (2.2.2, Method II)**

- 6.6.2.1. Add 10mL of Solution S into a Nessler Color Comparison Tube.
- 6.6.2.2. Add 10 mL of *USP Purified Water* into a second Nessler Color Comparison Tube.
- 6.6.2.3. Compare the colors in sufficient lighting, viewing vertically against a white background.
- 6.6.2.4. In order for the sample solution to be colorless, it must have the appearance of *USP Purified Water*.

### **6.7. ASSAY (w/w%)** :

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

### **6.8. IMPURITIES** :

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

### **6.9. CHLORIDE** :

#### **6.9.1. USP-NF Analysis**

##### **6.9.1.1. Sample Preparation:**

- 6.9.1.1.1. Weigh 2.0g 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.

##### **6.9.1.2. 0.0125% Standard Preparation:**

- 6.9.1.2.1. 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.

6.9.1.3. **Procedure:**

6.9.1.3.1. 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.

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

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

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

6.9.1.5.1. Follow the appropriate SOP:

6.9.1.5.1.1. Stroudsburg: Portable Turbidimeter SOP and Calibration

6.9.1.5.1.2. Bangor: Bangor Portable Turbidimeter and Calibration

6.9.2. **CP Analysis**

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

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

6.9.2.3. Reference solution: Pipette 5.0mL of sodium chloride standard solution to a 50mL Nessler tube. Add 10mL of dilute nitric acid and sufficient water to produce 40mL and mix well.

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

6.9.2.4. Procedure: To each of the Nessler tubes add 1mL of silver nitrate TS, dilute with water to 50mL 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.

6.9.3. **EP Analysis**

6.9.3.1. Dilute 4mL of Solution S to 15mL with USP Purified Water. Prepare a 0.0125% standard in the same manner using 10mL of chloride standard solution (5ppm Cl) R and 5mL of purified water. To both the standard and sample add 1mL of dilute nitric acid R and add 1mL 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.

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

6.9.4. **JP Analysis**

6.9.4.1. Weigh 2.0g of sample dilute to 40mL with purified water. Add 6 mL of dilute nitric acid and purified water to make 50mL, and use this solution as the test solution. Prepare the 0.018% control solution with 1.0mL of 0.01mol/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.

## 6.10. **COLOR AND CLARITY OF SOLUTION** :

### 6.10.1. **USP-NF Analysis**

- 6.10.1.1. Accurately weigh 33.0 g of sample.
- 6.10.1.2. Transfer accurately weighed sample to a 150 mL beaker and add 67.0 g of recently boiled water to dissolve.
- 6.10.1.3. Using the Lambda 25 UV/Vis, measure the absorbance of the sample solution at 420 and 720 nm in a 10-cm cuvette.
- 6.10.1.4. Refer to Lambda 25 UV/Vis Operation and Calibration to determine the absorbance of the sample.
- 6.10.1.5. 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

- 6.10.1.6. The absorbance at 720nm is not more than 0.050 a.u.
- 6.10.1.7. The absorbance difference should be  $\leq 0.100$  a.u.

### 6.10.2. **CP Analysis**

- 6.10.2.1. To (33.0g anhydrous sample) or (36.5g dihydrate sample) in 100mL volumetric flask, add freshly boiled and cooled water, dissolve completely, and cool.
- 6.10.2.2. Measure the absorbances of the solution at 420 and 720nm <0401>.
- 6.10.2.3. Using the Lambda 25 UV/Vis, measure the absorbance of the sample solution at 420 and 720 nm in a 10-cm cuvette.
- 6.10.2.4. Refer to Lambda 25 UV/Vis Operation and Calibration to determine the absorbance of the sample.
- 6.10.2.5. 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

- 6.10.2.6. The absorbance at 720nm is not more than 0.033 a.u.
- 6.10.2.7. The absorbance difference should be  $\leq 0.067$  a.u.

## 6.11. **ENDOTOXINS** :

- 6.11.1. Weigh 300mg +/- 10mg and transfer to a sterile tube.
- 6.11.2. Dissolve in ~5mL of LAL reagent water.
- 6.11.3. Dilute to 10mL with LAL reagent water and mix thoroughly.
- 6.11.4. Follow Endosafe nexgen-PTS Reader SOP using high sensitivity cartridge (0.5-0.005EU/mL).

## 6.12. **HEAVY METALS** :

### 6.12.1. **CP Analysis**

- 6.12.1.1. Test Solution (2nd tube): Dissolve 4.0g in 23mL of purified water, add 2mL of

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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.

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

6.12.1.3. **Method 1:** use three 25mL Nessler tubes.

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

6.12.1.3.2. To the 2nd tube (test tube) add the test solution prepped in step 6.11.1.1 above.

6.12.1.3.3. To the 3rd tube (monitor tube) add the test solution prepped in step 6.15.1 above. Add 2mL of the lead standard solution.

6.12.1.3.4. To each tube add 2mL 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.

#### 6.12.2. **JP Analysis**

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

6.12.2.2. 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.

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

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

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

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

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

6.14.1.2. Dissolve sample in 5mL of purified water.

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

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

6.14.4. A violet color should be produced.

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

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

6.15.1.1. Accurately weigh 1.0 grams of sample and transfer to a suitable beaker.

6.15.1.2. Dissolve sample in 25mL of purified water.

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

- 6.15.3. To the sample solution, add 4mL of a 40g/L solution of sodium hydroxide TS and 2 mL of a 40mg/mL solution of glycine R and mix.
- 6.15.4. 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.

#### 6.16. **IDENTIFICATION 3 (CP)** \_\_\_\_\_ :

- 6.16.1. Refer to Assay Section 6.7 for result.
  - 6.16.1.1. The retention time of the major peak of the sample solution corresponds to that of the reference solution as obtained in the Assay.

#### 6.17. **MICROBIAL CONTENT** \_\_\_\_\_ :

- 6.17.1. Package 40 grams into a sterile container and send to MPL Laboratories.
- 6.17.2. Refer to appropriate product code for specifications and analyses to be performed.

#### 6.18. **NITROGEN CONTENT** \_\_\_\_\_ :

- 6.18.1. Sample size: 5.0g
- 6.18.2. 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.
- 6.18.3. Procedure: Place an accurately weighed 5.0 g of sample in the digestion flask of the apparatus. 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. Add 30 mL of sulfuric acid, allowing it to rinse down the wall of the flask, then, while swirling the flask, add 1 mL of 30 percent hydrogen peroxide cautiously down the side of the flask. (Do not add hydrogen peroxide during the digestion.)
- 6.18.4. Heat the flask over a free flame or an electric heater until the solution has a clear blue color and the sides of the flask are free from carbonaceous material. Cautiously add to the digestion mixture 70 mL of water, cool the solution, and arrange for steam distillation. Add through a funnel 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, rinse the funnel with 10 mL of water, tightly close the apparatus, and begin the distillation with steam immediately. 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. Continue the distillation until the distillate measures 80 to 100 mL. Remove the absorption flask, rinse the end of the condensing tube with a small quantity of water, and titrate the distillate with 0.01 N sulfuric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.01 N sulfuric acid VS is equivalent to 140.1 µg of nitrogen.
- 6.18.5. Sulfuric Acid 0.01N reagent value: Run once to verify 0.01N sulfuric acid.
  - 6.18.5.1. Sulfuric 0.01N: Slowly add 10mL of 0.1N sulfuric acid to 80mL of USP purified water to make a volume of 100mL
- 6.18.6. Accurately weigh about 0.036g of NIST 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<sub>2</sub>SO<sub>4</sub> to a colorimetric endpoint.
- 6.18.7. Each 1.2114 mg of NIST Tromethamine is equivalent to 1mL of 0.01N H<sub>2</sub>SO<sub>4</sub>. N  
H<sub>2</sub>SO<sub>4</sub> = (g Tromethamine) / (0.12114 x mL x 0.01N H<sub>2</sub>SO<sub>4</sub>)
- 6.18.8. % Nitrogen = ((EP<sub>1</sub>-EP<sub>Blank</sub>)(1.41)(N<sub>H<sub>2</sub>SO<sub>4</sub>)) / Sample Weight (g)</sub>

#### 6.19. **pH @ 25°C/Acidity (CP)** \_\_\_\_\_ :

- 6.19.1. Sample Preparation (100mg/mL):
  - 6.19.1.1. Accurately weigh 10.0 g of sample. Transfer to a clean, dry 100mL volumetric flask.

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

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

## 6.20. **RESIDUAL ETHANOL, IPA, and METHANOL** :

6.20.1. Residual solvent analysis will be performed by an outside laboratory. Prepare a 10g sample for shipment.

6.20.1.1. Residual Solvent analysis can also be performed utilizing an internal validated method.

## 6.21. **RESIDUE ON IGNITION** :

6.21.1. Turn on muffle furnace and allow temperature to stabilize at 600°C. Follow muffle furnace SOP and calibration procedure for operation.

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

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

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

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

6.21.6. 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.

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

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

6.21.9. Ignite in the muffle furnace at  $600 \pm 50$  °C for 15 minutes or until all carbon has been removed.

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

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

6.21.11. 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  $600 \pm 50$  °C for 30 minutes until two consecutive weighings of the residue do not differ by more than 0.0005g or until the specification is met.

## 6.22. **SOLUBLE STARCH** :

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

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

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

6.22.2. Add several drops of iodine TS to the *sample solution*.

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

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

6.22.4.1. Dissolve 1.0g of sample in 10mL 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.

## 6.23. **SPECIFIC ROTATION/OPTICAL ROTATION** :

6.23.1. Sample Preparation (100mg/mL):

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

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

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6.23.3. Follow MCP 300 Polarimeter SOP and analyze within 30 minutes of preparation at 20°C.

6.23.4. Result calculated on an anhydrous basis:

Specific Rotation for Dihydrate = Raw Result (1.105)

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

6.24.1. Sample Preparation:

6.24.1.1. Sample preparation is calculated on anhydrous basis.

6.24.1.2. Accurately weigh 6.33 grams of sample and transfer to a 100 mL volumetric flask.

6.24.1.3. Calculation for sample prep:

$$\text{Adjusted Concentration} = \frac{7 \times \left[ \frac{378.3 - 36.04}{378.3} \right]}{100 \text{ mL}} = 6.33 \text{ g}$$

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

6.24.2. Follow MCP 300 Polarimeter SOP and analyze within 30 minutes of preparation at 20°C.

6.24.2.1. Results calculated on an anhydrous basis.

**6.25. SULFATED ASH :**

6.25.1. Turn on muffle furnace and allow temperature to stabilize at 600°C. Follow muffle furnace SOP and calibration procedure for operation.

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

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

6.25.4. Ignite the quartz crucible at  $600 \pm 50$  °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.

6.25.5. 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.

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

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

6.25.8. Ignite in the muffle furnace at  $600 \pm 50$  °C for 15 minutes or until all carbon has been removed.

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

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

6.25.10. 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  $600 \pm 50$  °C for 30 minutes until two consecutive weighings of the residue do not differ by more than 0.0005g or until the specification is met.

**6.26. SULFATE :**

6.26.1. USP-NF Analysis

6.26.1.1. Sample Preparation:

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- 6.26.1.1.1. Weigh 2.0g of sample and dissolve in ~30-40 mL of purified water in a 50mL Nessler Color Comparison Tube. If necessary, neutralize the solution with hydrochloric acid to litmus.
- 6.26.1.2. 0.0200% Standard Preparation:
  - 6.26.1.2.1. Prepare a standard solution by pipetting 0.83mL of 0.005M H<sub>2</sub>SO<sub>4</sub> in a 50mL Nessler Color Comparison Tube. Dissolve in ~30-40 mL of purified water.
- 6.26.1.3. Procedure:
  - 6.26.1.3.1. To both the sample and standard solutions, add 1 mL of 3N HCl, 3 mL of Barium Chloride TS and sufficient water to make 50mL.
  - 6.26.1.3.2. Mix and allow samples and standard to stand for 10 minutes utilizing a calibrated timer.
- 6.26.1.4. Any turbidity produced in the sample solution should not exceed that produced by the standard.
- 6.26.1.5. If a visible difference in the turbidity is not observed, utilize the Turbidimeter to measure the turbidity of the sample and standard solutions
  - 6.26.1.5.1. Follow the appropriate SOP:
    - 6.26.1.5.1.1. Stroudsburg: Portable Turbidimeter SOP and Calibration
    - 6.26.1.5.1.2. Bangor: Bangor Portable Turbidimeter and Calibration SOP
- 6.26.2. CP Analysis
  - 6.26.2.1. Carry out the limit test for sulfate <0802>, using 1.0g. Any opalescence produced is not more pronounced than that of the reference solution using 2.0mL of Potassium sulfate standard solution.
  - 6.26.2.2. Test solution: weigh 1.0g of sample, dissolve it in about 40mL of water, neutralize this solution with hydrochloric acid and filter if necessary. Transfer the solution to a 50mL Nessler tube, add 2mL of dilute hydrochloric acid and mix well.
  - 6.26.2.3. Reference solution: Pipette 2.0mL of Potassium sulfate standard solution to a 50mL Nessler tube, dilute with purified water to 40mL, add 2mL of hydrochloric acid and mix well.
    - 6.26.2.3.1. Potassium sulfate standard solution: Dissolve 0.181g of potassium sulfate in water in a 1000mL volumetric flask. Dilute to volume with water and mix well (each mL of potassium sulfate standard solution is equivalent to 100µg of Sulfate (SO<sub>4</sub>)).
  - 6.26.2.4. Procedure: To each of the Nessler tubes, add 5mL of 25% barium chloride solution, dilute with water to 50mL 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.
- 6.26.3. EP Analysis
  - 6.26.3.1. Sample solution: Dilute 7.5mL of Solution S to 15mL with purified water (distilled water R equivalent).
  - 6.26.3.2. In a separate beaker, add 3mL of a 250 g/L solution of barium chloride R to 4.5mL of sulfate standard solution (10ppm SO<sub>4</sub>) R1. Shake and allow to stand for 1 min. To 2.5mL of this suspension, add the 15mL sample solution and 0.5 mL of acetic acid R. Prepare a 0.0200% standard in the same manner using 15mL of sulfate standard solution (10ppm SO<sub>4</sub>) R instead of the prescribed solution.

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- 6.26.3.3. After 5 min, any opalescence in the test solution is not more intense than that in the standard.
- 6.26.3.4. Acetic Acid R: Dilute 30g of glacial acetic acid (99%) to 100mL with purified water.
- 6.26.3.5. Sulfate standard solution (10ppm SO<sub>4</sub>)R1: Immediately before use, dilute with ethanol (30% V/V)R to 100 times its volume a solution containing dipotassium sulfate R equivalent to 0.181g of K<sub>2</sub>SO<sub>4</sub> in 100mL of ethanol (30%V/V)R.
- 6.26.3.6. Sulfate standard solution (10ppm SO<sub>4</sub>): Immediately before use, dilute with purified water to 100 times its volume a solution in purified water containing dipotassium sulfate equivalent to 0.181g of K<sub>2</sub>SO<sub>4</sub> in 100mL.

#### 6.26.4. **JP Analysis**

- 6.26.4.1. Weigh 2.0g of sample. Add water to make 40mL. Add 1mL of dilute hydrochloric acid and water to make 50mL, and use this solution as the test solution. Prepare the 0.024% control solution with 1.0mL of 0.005mol/L sulfuric acid VS. Add 1mL of dilute hydrochloric acid and water to make 50mL, and use this solution as the control solution. When the test solution is not clear, filter both solutions according to the same procedure. Add 2mL of barium chloride TS to the test solution and to the control solution, mix well, and allow to stand for 10 minutes. 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.

#### 6.27. **WATER (By Karl Fischer Titration)** :

- 6.27.1. Standardize Composite 5 as per Standardization of Titrants.
- 6.27.2. Immediately weigh 0.1g of sample (no grinding necessary) into the glass weighing spoon and tare it.
- 6.27.3. 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.
- 6.27.4. Do not leave the rubber septum open for long periods of time as this will allow moisture to enter the titration vessel.
- 6.27.5. 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.
- 6.27.6. Check to make sure there is no residual sample stuck to the sides of the titration vessel.
- 6.27.7. If there is any sample stuck to the side, stop the stir bead from spinning before swirling the vessel to rinse the sides.
- 6.27.8. Ensure the sample is fully dissolved before the titration begins (i.e. before the stir command completes).
- 6.27.9. The moisture content will then be determined by the Metrohm Titrando 907 Autotitrator.

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

## 7. COMPENDIAL DIFFERENTIATIONS:

### 7.1. Compendial Analyses

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

### 7.2. 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: <i>Escherichia Coli</i> , <i>Salmonella Species</i> , 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)

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

7.3.1. <1225> Validation of Compendial Procedures

7.3.2. <1467>Residual Solvents- Verification of Compendial Procedures and Validation of Alternative Procedures

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

### 7.4. In-house Methods for 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

### 7.5. Customer Requested Methods

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

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