

# BIOSPECTRA

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

To Whom It May Concern,

**INTRODUCTION:** The following analyses are conducted for Trehalose, Dihydrate, product code TE3250, in accordance with the Trehalose Testing Methods DCN: 18-002375 v.2.0 and Certificate of Analysis DCN: 18-002600 v.6.0.

**1. APPEARANCE AND COLOR WHITE TO OFF-WHITE CRYSTALLINE POWDER:**

- 1.1. Place 25-50g of sample in a clean, dry glass beaker.
- 1.2. In an area with sufficient lighting, view the sample from all sides.
- 1.3. The sample should be white, to off white, in color and characteristic of crystalline powder. If the sample does not conform to these specifications, notify the appropriate personnel immediately.

**2. APPEARANCE OF SOLUTION (EP) CLEAR, COLORLESS:**

- 2.1. Clear (2.2.1.) Turbidimetry
  - 2.1.1. Rinse the sample bottle with the sample solution twice.
  - 2.1.2. Fill sample bottle with the sample solution S to the white line.
  - 2.1.3. Coat outside of bottle with a thin coat of silicon oil.
  - 2.1.4. Remove any air bubbles from the solution by using a syringe.
  - 2.1.5. Allow the sample to sit capped for 2-3 minutes.
  - 2.1.6. Follow the appropriate SOP as follows:
    - 2.1.6.1. Stroudsburg- Measure and record the turbidity of the sample according to Portable Turbidimeter Operation and Calibration.
    - 2.1.6.2. Bangor- Measure and record the turbidity of the sample according to Bangor Portable Turbidimeter SOP.
  - 2.1.7. The sample solution must be < 3 NTU.
- 2.2. Colorless (2.2.2, Method II)
  - 2.2.1. Add 10mL of Solution S into a Nessler Color Comparison Tube.
  - 2.2.2. Add 10 mL of *USP Purified Water* into a second Nessler Color Comparison Tube.
  - 2.2.3. Compare the colors in sufficient lighting, viewing vertically against a white background.
  - 2.2.4. In order for the sample solution to be colorless, it must have the appearance of *USP Purified Water*.

**3. ASSAY % W/W 98.0-101.0%:**

- 3.1. Solution Preparation:
  - 3.1.1. Mobile phase: Water
  - 3.1.2. Standard solution: 10 mg/mL of USP Trehalose, Dihydrate RS, calculated on the anhydrous basis
  - 3.1.3. Sample solution: 10 mg/mL of Trehalose, calculated on the anhydrous basis
- 3.2. Chromatographic system:
  - 3.2.1. (See Chromatography 621, System Suitability.)
  - 3.2.2. Mode: LC
  - 3.2.3. Instrument: HPLC or equivalent

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- 3.2.4. Detector: Refractive index
- 3.2.5. Column: 8-mm × 30-cm; packing L58
- 3.2.5.1. Column: 80°
- 3.2.6. Flow rate: 0.35 mL/min
- 3.2.7. Injection volume: 10 µL
- 3.3. System suitability:
- 3.3.1. Sample: Standard solution
- 3.3.2. Suitability requirements: Relative standard deviation: NMT 2.0%
- 3.4. Analysis:
- 3.4.1. Samples: Standard solution and Sample solution
- 3.4.2. Calculate the percentage of Trehalose (C<sub>12</sub>H<sub>22</sub>O<sub>11</sub>) in the portion of Trehalose taken:
- $$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$
- $r_u$  = peak response from the Sample solution
- $r_s$  = peak response from the Standard solution
- $C_s$  = concentration of USP Trehalose, Dihydrate RS in the Standard solution (mg/mL)
- $C_u$  = concentration of Trehalose, Dihydrate in the Sample solution (mg/mL)

#### 4. CHLORIDE (NF) ≤ 0.0125%:

- 4.1. Sample Preparation:
- 4.1.1. 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.
- 4.2. Standard Preparation:
- 4.2.1. 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.
- 4.3. Procedure:
- 4.3.1. 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.
- 4.3.2. Mix and allow to stand for 5 minutes utilizing a calibrated timer.
- 4.4. Any turbidity produced in the sample solution should not exceed that produced by the standard.
- 4.5. If a visible difference in the turbidity is not observed, utilize the Turbidimeter to measure the turbidity of the sample and standard solutions
- 4.5.1. Follow the appropriate SOP:
- 4.5.1.1. Stroudsburg: Portable Turbidimeter SOP and Calibration
- 4.5.1.2. Bangor: Bangor Portable Turbidimeter and Calibration
- 4.5.2. 0.01M HCl: Dilute 0.85 mL of hydrochloric acid 37% with water to make 1000 mL.
- 4.5.3. 0.1N silver nitrate is purchased commercially.

#### 5. CHLORIDE (EP) ≤0.0125%:

- 5.1. Dilute 4mL of Solution S to 15mL with USP Purified Water. Prepare a 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.

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- 5.1.1. Chloride standard solution (5 ppm Cl): Immediately before use, dilute with purified water to 100 times its volume a solution containing sodium chloride R equivalent to 0.824 g of NaCl in 1000.0 mL.
- 5.1.2. Nitric Acid R: Dilute 20 g of nitric acid 70% to 100 mL with purified water.
- 5.1.3. 0.1N Silver nitrate is purchased commercially.

**6. CHLORIDE (JP) <0.018%:**

- 6.1. 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 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.
- 6.1.1. Nitric acid, dilute: Dilute 10.5mL of nitric acid with water to make 100mL.
- 6.1.2. 0.01mol/L hydrochloric acid: Dilute 0.85mL of hydrochloric acid 37% with water to make 1000mL.

**7. COLOR AND CLARITY OF SOLUTION (NF) A720 ≤0.050, A420-A720 ≤0.100:**

- 7.1. Accurately weigh 33.0 g of sample.
- 7.2. Transfer accurately weighed sample to a 150 mL beaker and add 67.0 g of recently boiled water to dissolve.
- 7.2.1. Note: If diluent water is ambient temperature the following exchange calculations can be 67.20mL at 25°C; 67.12mL at 20°C if done by volume) the following exchange calculations may be used.
- 7.3. Using the Lambda 25 UV/Vis, measure the absorbance of the sample solution at 420 and 720 nm in a **10-cm** cuvette.
- 7.4. Refer to Lambda 25 UV/Vis Operation and Calibration to determine the absorbance of the sample.
- 7.5. Determine the absorbance difference:
- $$Result = A_{420} - A_{720}$$
- $$A_{420} = \text{Absorbance of the Sample Solution at 420nm}$$
- $$A_{720} = \text{Absorbance of the Sample Solution at 720nm}$$
- 7.6. The absorbance difference should be  $\leq 0.100$  a.u.

**8. DEXTRIN, SOLUBLE STARCH, AND SULFITE Passes Test:**

- 8.1. 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.
- 8.1.1. Iodine TS (0.1N) is purchased commercially.
- 8.1.2. Starch TS is purchased commercially.

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**9. ENDOTOXINS ≤ 2.4 EU/g:**

9.1. Refer to Endosafe PTS Endotoxin Reader SOP for instrument operation for sample preparation and analysis.

**10. HEAVY METALS (JP) ≤5 ppm:**

- 10.1. 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 control solution with 2.5mL of Standard Lead Solution. Add 2 mL of dilute acetic acid and water to make 50mL.
- 10.2. 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.
- 10.2.1. Sodium sulfide TS: Dissolve 5g of sodium sulfide nonahydrate in a mixture of 10mL of water and 30mL of glycerin. Preserve in well-filled, light-resistant bottles. Amber bottle is sufficient. Use within 3 months.
- 10.2.2. Dilute acetic acid: Dilute 6 g of glacial acetic acid with water to make 100 mL (1 mol/L).
- 10.2.3. Standard Lead Stock Solution: Weigh exactly 159.8 mg of lead (II) nitrate, dissolve in 10mL of dilute nitric acid, and add water to make exactly 1000mL.
- 10.2.4. Standard Lead Solution: Measure exactly 10mL of Standard Lead Stock Solution, and add water to make exactly 100mL. Each mL of this solution contains 0.01 mg of lead (Pb). Prepare before use.

**11. IDENTIFICATION TEST: NF (A), EP (A), JP (ID 3) Conforms to Standard:**

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

**12. IDENTIFICATION TEST: NF (B) EP (B), JP (ID 1) Passes Test:**

- 12.1. Sample Solution (400mg/mL of Trehalose):
- 12.1.1. Accurately weigh 2.0g of sample and transfer to a suitable beaker.
- 12.1.2. Dissolve sample in 5mL of purified water.
- 12.2. To 1mL of the sample solution, add 0.4mL of a 1 in 20 solution of 1-naphthol in 95% ethanol and mix thoroughly.
- 12.3. Gently add 2mL of sulfuric acid to the sample solution.
- 12.4. A violet color should be produced.

**13. IDENTIFICATION TEST: NF (C), EP (C), JP (ID2) Passes Test:**

- 13.1. Sample Solution (40mg/mL of Trehalose):
- 13.1.1. Accurately weigh 1.0 grams of sample and transfer to a suitable beaker.
- 13.1.2. Dissolve sample in 25mL of purified water.
- 13.2. 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.
- 13.3. 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.
- 13.4. 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.

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13.4.1. Dilute Hydrochloric acid R: Dilute 23.6mL of hydrochloric acid with water to make 100mL (10%).

#### 14. **IMPURITIES (NF, EP)** **REFER TO C OF A :**

14.1. Mobile Phase and Chromatographic System: Proceed as directed in the Assay.

14.1.1. Sample Solution: 10 mg/mL of Sample

14.1.2. Standard Solution: 0.1mg/mL of Trehalose CRS

14.1.3. System Suitability Solution: Transfer 25mg of Trehalose CRS, 25 mg of maltotriose CRS, and 25 mg of glucose CRS to a 10-mL volumetric flask, and dilute with water to volume.

14.1.4. Standard Solution B: Dilute 5.0 mL of Standard Solution to 25.0 mL with water.

14.1.5. System Suitability:

14.1.5.1. Sample: System suitability

14.1.5.2. [Note—The relative retention times for maltotriose, Trehalose, and glucose are about 0.9, 1.0, and 1.2, respectively.]

14.1.6. Suitability requirements:

14.1.6.1. Resolution: NLT 1.5 between Trehalose and maltotriose

14.1.6.2. Relative standard deviation: NMT 2.0% for the Trehalose peak over the analysis of 5 consecutive runs.

14.1.7. Analysis:

14.1.7.1. Samples: Sample solution and Standard solution

14.1.7.2. Determine the peak areas for all peaks.

14.1.7.3. Acceptance criteria:

14.1.7.3.1. For the Sample Solution, the areas of any peaks corresponding to maltotriose and other polysaccharides and eluting before trehalose (RRT < 1.0) are not more than 0.5 times the area of the Trehalose peak in the chromatogram obtained with the Standard Solution (0.5%).

14.1.7.3.2. For the Sample Solution, the areas of any peaks corresponding to glucose and eluting after Trehalose (RRT > 1.0) are not more than 0.5 times the area of the Trehalose peak in the chromatogram obtained with the Standard Solution (0.5%).

14.1.7.3.3. For each unspecified impurity in the Sample Solution chromatogram, the peak is not more than the area of the principal peak in the chromatogram obtained with the Standard Solution B (0.2%).

14.1.7.3.4. The total area of all impurity peaks in the Sample Solution chromatogram is not more than the area of the principal peak in the chromatogram obtained with the Standard Solution (1.0%).

#### 15. **MICROBIAL CONTENT USP <61 and 62>** **REFER TO C OF A :**

15.1. Package 40 grams into a sterile container and send to an approved Outside Testing Facility. The analysis request form should include TAMC, TYMC, *Escherichia coli* Test for Absence per 1 gram and *salmonella* Test for Absence per 10 grams.

15.2. In order to pass, Total Aerobic Microbial Count must be less than 100 CFU/g, the Total Yeast and Mold Count must be less than 100 CFU/g, and all identifications must be noted as Absent.

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**16. NITROGEN CONTENT (NF) ≤ 0.005%:**

- 16.1. Sample size: 5.0g
- 16.2. 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.
- 16.3. 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.)
- 16.4. 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.
- 16.5. Sulfuric Acid 0.01N reagent value: Run once to verify 0.01N sulfuric acid.
- 16.5.1. Sulfuric 0.01N: Slowly add 10mL of 0.1N sulfuric acid to 80mL of USP purified water to make a volume of 100mL
- 16.5.2. Accurately weigh about 0.036g of NIST Tromethamine, previously dried at 105 °C for 3 hours.
- 16.5.3. Dissolve sample in 50 mL of purified water.
- 16.5.4. Add 3 drops of Methyl Orange.
- 16.5.5. Titrate with 0.01N H<sub>2</sub>SO<sub>4</sub> to a colorimetric endpoint.
- 16.5.6. Each 1.2114 mg of NIST Tromethamine is equivalent to 1mL of 0.01N H<sub>2</sub>SO<sub>4</sub>
- $$N \text{ H}_2\text{SO}_4 = (\text{g Tromethamine}) / (0.12114 \times \text{mL} \times 0.01N \text{ H}_2\text{SO}_4)$$
- 16.5.7. % Nitrogen = ((EP<sub>1</sub>-EP<sub>Blank</sub>)(1.41)(N<sub>H<sub>2</sub>SO<sub>4</sub></sub>)) / Sample Weight (g)

**17. pH @ 25°C 4.5-6.5:**

- 17.1. Sample Preparation (100mg/mL):
- 17.1.1. Accurately weigh 10.0 g of sample. Transfer to a clean, dry 100mL volumetric flask.
- 17.1.2. Dilute to 100mL with purified water. Swirl to dissolve.
- 17.1.3. Follow the appropriate SOP to measure and record the pH.

**18. RESIDUAL ETHANOL, IPA, and METHANOL REFER TO C OF A:**

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

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**19. RESIDUE ON IGNITION (NF) ≤ 0.1%:**

- 19.1. Turn on muffle furnace and allow temperature to stabilize at 600°C. Follow muffle furnace SOP and calibration procedure for operation.
- 19.2. Inspect a quartz crucible for cracks, chips and discoloration.
- 19.3. Utilize forceps to insert and remove a crucible into the furnace.
- 19.4. 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.
- 19.5. Weigh 2.0 g sample in the previously ignited quartz crucible. Moisten the sample with ~1 mL of sulfuric acid.
- 19.6. 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.
- 19.7. The rate of heating should be such that from ½ to 1 hour is required to volatilize the sample.
- 19.8. Continue using the Bunsen burner to heat the sample until all excess sulfuric acid has been volatilized.
- 19.9. Ignite in the muffle furnace at 600 ± 50 °C for 15 minutes or until all carbon has been removed.
- 19.10. Cool in the desiccator for a minimum of an hour and a half and reweigh.  

$$\% \text{ ROI} = \frac{\text{Residue Weight (g)}}{\text{Sample Weight (g)}} \cdot 100$$
- 19.11. 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 0.0005g or until the specification is met.

**20. SOLUBLE STARCH (NF) Passes Test:**

- 20.1. Sample Solution Preparation (10% Trehalose (w/v)):
  - 20.1.1. Accurately weigh 10.0 g of sample and transfer to a clean, dry suitable beaker.
  - 20.1.2. Dissolve in purified water and dilute to 100mL.
- 20.2. Add several drops of iodine TS to the *sample solution*.
- 20.3. No blue color should develop in order to report as passes test.
  - 20.3.1. Iodine TS is 0.1N Iodine purchased commercially.

**21. SPECIFIC ROTATION/OPTICAL ROTATION (NF) +197° to +201°@ 20°C:**

- 21.1. Sample Preparation (100mg/mL):
  - 21.1.1. Accurately weigh 10.00 g of sample and transfer to a 100 mL volumetric flask.
  - 21.1.2. Dissolve sample in USP purified water and QS to a final volume of 100 mL with purified water.
- 21.2. Follow MCP 300 Polarimeter SOP and analyze within 30 minutes of preparation at 20°C.
- 21.3. Result calculated on an anhydrous basis:  

$$\text{Specific Rotation for Dihydrate} = \text{Raw Result} (1.105)$$

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**22. SULFATE (NF) ≤ 0.0200%:****22.1. Sample Preparation:**

22.1.1. 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.

**22.2. Standard Preparation:**

22.2.1. 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.

**22.3. Procedure:**

22.3.1. 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.

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

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

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

22.5.1. Follow the appropriate SOP:

22.5.1.1. Stroudsburg: Portable Turbidimeter SOP and Calibration

22.5.1.2. Bangor: Bangor Portable Turbidimeter and Calibration SOP

22.5.1.3. 3N Hydrochloric Acid TS: Dilute 2.46g of 37% HCl to 10g with USP purified water.

22.5.1.4. 0.005M sulfuric acid TS: Dilute 0.14mL of sulfuric acid 96% to 1000mL with USP purified water.

22.5.1.5. Barium chloride TS: Dissolve 12g of barium chloride in water to make 100mL.

**23. SULFATE (EP) ≤0.0200%:**

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

23.2. 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 standard in the same manner using 15mL of sulfate standard solution (10ppm SO<sub>4</sub>) R instead of the prescribed solution.

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

23.4. Acetic acid R: Dilute 30g of acetic acid to 100mL with USP/EP purified water.

23.5. Sulfate standard solution (10ppm SO<sub>4</sub>) R1: Immediately before use, dilute with ethanol 30% R to 100 times its volume a solution containing dipotassium sulfate R equivalent to 0.181 g of K<sub>2</sub>SO<sub>4</sub> in 100.0mL of ethanol (30% V/V) R.

23.6. Sulfate standard solution (10ppm SO<sub>4</sub>) R: Immediately before use, dilute with USP/EP purified water to 100 times its volume a solution in USP purified water containing dipotassium sulfate R equivalent to 0.181g of K<sub>2</sub>SO<sub>4</sub> in 100.0mL.

**24. SULFATE (JP) ≤0.024%:**

24.1. 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 control solution with 1.0mL of 0.005mol/L sulfuric acid VS. Add 1mL of dilute hydrochloric acid and water to

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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.

- 24.1.1. 0.005M sulfuric acid TS: Dilute 0.14mL of sulfuric acid 96% to 1000mL with USP purified water.
- 24.1.2. Hydrochloric acid, dilute: Dilute 23.6mL of hydrochloric acid with water to make 100mL (10%).
- 24.1.3. Barium chloride TS: Dissolve 12g of barium chloride in water to make 100mL.

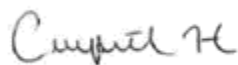
**25. WATER (By Karl Fischer Titration) (NF) 9.0-11.0%:**

- 25.1. Standardize Composite 5 as per Standardization of Titrants.
- 25.2. Immediately weigh 0.1g of sample (no grinding necessary) into the glass weighing spoon and tare it.
- 25.3. 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.
- 25.4. Do not leave the rubber septum open for long periods of time as this will allow moisture to enter the titration vessel.
- 25.5. 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.
- 25.6. Check to make sure there is no residual sample stuck to the sides of the titration vessel.
- 25.7. If there is any sample stuck to the side, stop the stir bead from spinning before swirling the vessel to rinse the sides.
- 25.8. Ensure the sample is fully dissolved before the titration begins (i.e. before the stir command completes).
- 25.9. The moisture content will then be determined by the Metrohm Titrando 907 Autotitrator.

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

If there are any questions or concerns, please feel free to contact [ra@biospectra.us](mailto:ra@biospectra.us).

Sincerely,



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