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MES, MONOHYDRATE TESTING METHODS

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1. PURPOSE:

1.1. To provide the Laboratory personnel with procedures for analyzing MES,Monohydrate Raw Materials, Finished Goods, In-Process, and Stability.

2. SCOPE:

2.1. These procedures apply to the testing of MES, Monohydrate in the Laboratory.

3. RESPONSIBILITIES:

3.1. The Laboratory Manager or designee is responsible for 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 appropriate personnel if any analyses fail to meet their respective specifications.
3.3. Standard laboratory safety regulations apply. Read and understand the safety data sheet (SDS) before handling or working with any chemical.

4. EQUIPMENT:

4.1. Analytical Balance
4.2. Oven
4.3. Hach Portable Turbidimeter
4.4. Litmus paper
4.5. Metrohm Auto-Titrator
4.6. Muffle Furnace
4.7. OPI-180 OD Handheld Colorimeter
4.8. Perkin-Elmer NexION 350X
4.9. Perkin-Elmer Spectrum Two UATR
4.10. XL200 pH/Conductivity Meter, or equivalent
4.11. UV/Vis Spectrophotometer
4.12. Endosafe nexgen-PTS Endotoxin Reader

5. REAGENTS:

5.1. **Acetic Acid (1N):** Dilute 6 g of glacial acetic acid to 100 grams, with purified water.
5.2. **Ammonium Hydroxide (6N):** Dilute 206.9 mL of Ammonium Hydroxide 29% to 500 mL final volume with purified water.
5.3. **Barium Chloride TS:** Dissolve 12 g of barium chloride dehydrate in purified water. Dilute to make a total volume of 100 mL with purified water.
5.4. **Composite 5:** Purchased Commercially.
5.5. **Glacial Acetic Acid:** Purchased Commercially.
5.6. **Glycerin TS:** To 200 g of glycerol, add purified water to bring the total weight to 235 g. Add 140 mL of 1N sodium hydroxide (NaOH) and 50 mL of purified water.
5.7. **Glycerol:** Purchased commercially
5.8. **Hydrochloric Acid (HCl):** Purchased Commercially.
5.9. **Hydrochloric Acid (HCl) (6N):** Pipette 51.50 mL of concentrated HCl and transfer to a 100-mL volumetric flask that contains a small amount of purified water. Dilute to volume with purified water.
5.10. **Hydrochloric Acid (HCl) (3N):** Pipette 25.75 mL of concentrated hydrochloric acid and transfer to a 100 mL volumetric containing a small amount of purified water. Dilute to volume with purified water.
5.11. **Hydrochloric Acid (HCl) (0.1N):** Purchased Commercially.
5.12. **Hydrochloric Acid (HCl) (0.02N):** Purchased Commercially.

5.13. **IgG Antibody, 5 mg/mL:** Dissolve 15 mg of IgG from Human Serum in 3 mL of PRB Buffer. Vortex gently to dissolve. Scale as required.
5.14. **IgG from Human Serum:** Purchased Commercially.
5.15. **LAL Reagent Water:** Purchased Commercially.
5.16. **Lead Nitrate Stock Solution:** Dissolve 0.1598 g of lead nitrate in 100 mL purified water and add 1 mL of nitric acid (HNO_3). Dilute with purified water to 1000 mL. Store in a glass container, free from soluble lead salts.
5.17. **Nitric Acid (HNO_3), concentrated:** Purchased Commercially.
5.18. **PBS Buffer:** Purchased Commercially.
5.19. **pH 3.5 Acetate Buffer:** Dissolve 62.5 g of ammonium acetate in 62.5 mL of purified water and add 47.0 mL of concentrated HCl. Adjust, if necessary, with 6N ammonium hydroxide or 6N HCl to a pH of 3.5. Dilute with purified water to a volume of 250 mL.
5.20. **Poly (vinylsulfonic acid, sodium salt) (25%):** Purchased Commercially.
5.21. **Purified Water:** Generated in-house or purchased commercially.
5.22. **Silver Nitrate ($AgNO_3$) (0.1N):** Purchased Commercially.
5.23. **Sodium Hydroxide (NaOH) (50%):** Weigh 50 g of NaOH and dilute and dissolve to 100 mL with purified water.
5.24. **Sodium Hydroxide (NaOH) (42%):** Weight 42 g of NaOH and dissolve and dilute to 100 mL with purified water.
5.25. **Sodium Hydroxide (NaOH) (1N):** Purchased Commercially.
5.26. **Sodium Hydroxide (NaOH) (0.1N):** Purchased Commercially.
5.27. **Sulfuric Acid (H_2SO_4):** Purchased Commercially.
5.28. **Sulfuric Acid (H_2SO_4) (0.020N):** Slowly add 20 mL 0.1N sulfuric acid (H_2SO_4) to 80 mL of purified water to make a total volume of 100 mL.
5.29. **Thioacetamide:** Purchased commercially
5.30. **Thioacetamide-glycerin base TS:** Add 0.2 mL of Thioacetamide TS to 1 mL of Glycerin TS, heat gently, and use immediately.
5.31. **Thioacetamide TS:** Dissolve 4.0 grams of thioacetamide in 100 mL of purified water.

6. REFERENCES:

6.1. BSI-ATM-0065, Analytical Method: Elemental Impurities via ICP-MS in MES Monohydrate
6.2. BSI-ATM-0089, Analytical Method of Analysis: Trace Metals in Finished Goods Products by ICP-OES
6.3. BSI-ATM-0131, Analytical Method for the Determination of Trace Metals in BioTech Products
6.4. BSI-SOP-0019, Result Reporting
6.5. BSI-SOP-0090, Lambda 25 UV/Vis Operation and Calibration
6.6. BSI-SOP-0091, Portable Turbidimeter SOP and Calibration
6.7. BSI-SOP-0094, Muffle Furnace SOP and Calibration
6.8. BSI-SOP-0095, DNase (Endonuclease) Assay
6.9. BSI-SOP-0096, RNase (Ribonuclease) Assay
6.10. BSI-SOP-0098, Balance SOP
6.11. BSI,SOP-0126, Laboratory Notebooks
6.12. BSI-SOP-0133, Blue M Convection Oven Operation and Calibration SOP
6.13. BSI-SOP-0138, DNase (Exonuclease) Assay
6.14. BSI-SOP-0139, Protease Assay
6.15. BSI-SOP-0140, Standardization of Titrants
6.16. BSI-SOP-0242, Portable Turbidimeter Operation and Calibration
6.17. BSI-SOP-0244, VWR Gravity Convection Oven Operation and Calibration (Model Number 414005-106)
6.18. BSI-SOP-0254, Spectrum Two UATR SOP

6.19. BSI-SOP-0255, XL200 pH/mV/Conductivity Meter SOP
6.20. BSI-SOP-0303, NexION 350X ICP-MS SOP
6.21. BSI-SOP-0345, Endosafe nexgen PTS Endotoxin Reader SOP
6.22. BSI-SOP-0595, DNase (NICKase) Assay
6.23. BSI-SOP-0668, OPI-180 OD Handheld Colorimeter SOP
6.24. *ACS, Reagent Chemicals*, current edition
6.25. *Current USP*

7. PROCEDURES:

IN-PROCESS TESTING

Note: MES Monohydrate Mother Liquor will be submitted to the Laboratory to send for TAMC/TYMC if the Mother Liquor has been stored for over 30 days.

7.1. **MOTHER LIQUOR ABSORBANCE:**

7.1.1. Prepare a 1:1 dilution of purified water and the ML sample. Mix thoroughly.
7.1.2. Refer to Lambda 25 UV/VIS Spectrophotometer to determine the Absorbance of the sample.

7.2. **MOTHER LIQUOR ASSAY:**

7.2.1. Standardize 0.1 N NaOH in accordance with Standardization of Titrants procedure, BSI-SOP-0140, utilizing the Metrohm Auto Titrator.
7.2.2. Accurately weigh 0.8 g of MES, Monohydrate ML sample and transfer to a suitable beaker.
7.2.3. Add 50 mL of purified water and stir to dissolve.
7.2.4. Titrate to the potentiometric endpoint with 0.1N NaOH.

$$\%\, MES, Monohydrate = \frac{(mL \times N\ of\ NaOH)(21.325)}{Sample\ Weight\ (g)}$$

7.3. **WET CRYSTAL ABSORBANCE:**

7.3.1. Weigh 5.33 g of sample and accurately transfer the weighed sample to a graduated cylinder and Q.S. to 25 mL with purified water.
7.3.2. Swirl to dissolve completely.
7.3.3. Refer to Lambda 25 UV/VIS Spectrophotometer to determine the Absorbance of the sample.

7.4. **DRY CRYSTAL ASSAY:**

7.4.1. Standardize 0.1N NaOH in accordance with Standardization of Titrants procedure, BSI-SOP-0140, utilizing the Metrohm Auto Titrator.
7.4.2. Accurately weigh 0.8 g of MES, Monohydrate (sample is measured as-is) and transfer to a suitable beaker.
7.4.3. Add 50 mL of purified water and stir to dissolve.
7.4.4. Titrate to the potentiometric endpoint with 0.1N NaOH.
7.4.5. Submerge the probe in storage solution after analysis is completed to condition the glass electrode.
7.4.6. The pK_a should be reported on the Assay printout from the Metrohm Auto-Titrator.

$$\%\, MES, Monohydrate = \frac{(mL \times N\ of\ NaOH)(21.325)}{Sample\ Weight\ (g)}$$

FINISHED GOOD ANALYSIS

7.5. ABSORBANCE (1M):

7.5.1. Weigh 5.33 g of sample and accurately transfer the weighed sample to a graduated cylinder and Q.S. to 25 mL with purified water.
7.5.2. Swirl to dissolve completely.
7.5.3. Refer to Lambda 25 UV/VIS Spectrophotometer to determine the Absorbance of the sample.

7.6. ABSORBANCE (0.1M):

7.6.1. Weigh 0.53 g of sample and accurately transfer the weighed sample to a graduated cylinder and Q.S. to 25 mL with purified water.
7.6.2. Swirl to dissolve completely.
7.6.3. Refer to the Lambda 25 UV/Vis Spectrophotometer to determine the Absorbance of the sample.

7.7. APPEARANCE AND COLOR:

7.7.1. Weigh a suitable amount of the sample into a clean, dry glass beaker.
7.7.2. In an area with sufficient lighting, view the sample from all sides.
7.7.3. The sample should be white in color and characteristic of crystals.
7.7.4. If the appearance and color result is unable to be definitively determined visually, the sample may be analyzed using the Colorimeter. Refer to BSI-SOP-0668, OPI-180 OD Handheld Colorimeter SOP.

7.8. ASSAY AND pK_a:

7.8.1. Standardize 0.1N NaOH in accordance with the Standardization of Titrants procedure, BSI-SOP-0140, utilizing the Metrohm Auto Titrator.
7.8.2. Accurately weigh 0.8 g of MES, Monohydrate (sample is measured as-is) and transfer to asuitable beaker.
7.8.3. Add 50 mL of purified water and stir to dissolve.
7.8.4. Titrate to the potentiometric endpoint with 0.1N NaOH.
7.8.5. Submerge the probe in storage solution after analysis is completed to condition the glass electrode. The pK_a should be reported on the Assay printout from the Metrohm Auto-Titrator.

$$\%\ MES, Monohydrate = \frac{(mL \times N\ of\ NaOH)(21.325)}{Sample\ Weight\ (g)}$$

To calculate assay on the anhydrous basis, use below equation:

$$\%\ MES, Monohydrate\ (as-is, anhydrous\ basis) = \frac{(mL \times N\ of\ NaOH)(19.524)}{Sample\ Weight\ (g)}$$

$$\%\ Mes, Monohydrate\ (anhydrous) = \frac{As-Is\ Assay\ \%}{(100 - KF\ Value)} * 100$$

7.9. CHLORIDE:

7.9.1. Weigh 2.0 g of sample and dissolve sample in approximately 40 mL purified water. If necessary, neutralize the solution with HNO_3 to litmus.
7.9.2. Pipette 0.141 mL of 0.02N HCl into approximately 40 mL of purified water in a Nessler Color Comparison Tube.
7.9.3. Add to each solution, 1 mL of concentrated HNO_3 and 1 mL of 0.1N silver nitrate. Q.S. to 50 mL with purified water. Cover with parafilm and mix by inversion.

7.9.4. Allow to stand for 5 minutes utilizing a calibrated timer. View tubes against a dark background. The turbidity of the sample preparation does not exceed that produced by the standard.
7.9.5. If a visible difference in the turbidity is not observed, utilize the Turbidimeter to measure the turbidity of the standard and the sample solutions.
7.9.6. Follow the appropriate SOP.

7.10. **COLOR OF A 1M ALKALINE SOLUTION:**

7.10.1. Weigh 21.3 g of sample and transfer to a clean, dry 150-mL beaker.
7.10.2. Dissolve in 100 mL of purified water.
7.10.3. Adjust the pH of the sample solution to 12.0 by adding (dropwise) 42% NaOH.
7.10.4. Observe the color of the solution.
7.10.5. The solution must remain clear and colorless when compared against a common background to a clear and colorless reference solution to report clear.

7.11. **COLOR OF A 1% ALKALINE SOLUTION:**

7.11.1. Weigh 1.0 g of sample and transfer to a clean, dry 150-mL beaker.
7.11.2. Dissolve in 100 mL of purified water.
7.11.3. Adjust the pH of the sample solution to 12.0 by adding (dropwise) 42% NaOH.
7.11.4. Observe the color of the solution.
7.11.5. The solution must remain clear and colorless when compared against a common background to a clear and colorless reference solution to report clear.

7.12. **ENDOTOXIN:**

7.12.1. Accurately weigh 20 mg of sample into a sterile tube.
7.12.2. Add 70 µL of 1N NaOH.
7.12.3. Dilute to 10 mL with LAL reagent water.
7.12.4. To 1 mL of this solution, add 4 mL of LAL reagent water. Mix thoroughly for a final concentration of 0.0004 g/mL.
7.12.5. Follow the Endosafe nexgen-PTS Endotoxin Reader SOP to analyze sample.

7.13. **ENZYME ACTIVITY:**

7.13.1. RNase, DNase, and Protease per procedures referenced in section 4.
7.13.2. If Applicable, NICKase per procedure referenced in section 4.

7.14. **HEAVY METALS:**

7.14.1. Refer to section 7.28: Trace Metals for primary method of analysis. Alternate Method:
7.14.2. Sample Preparation – Into a 50-mL Nessler color comparison tube, dissolve 10.0 g MES, Monohydrate in approximately 40 mL of purified water. Adjust with 1N acetic acid or 6*N* ammonium hydroxide to a pH between 3.0 and 4.0, using a pH meter or short-range pH indicator paper as external indicator, dilute with purified water to approximately 45 mL, and mix.
7.14.3. Standard Lead Solution – On the day of use, dilute 10.0 mL of Lead Nitrate Stock Solution with purified water to 100.0 mL in a volumetric flask.
7.14.4. Standard Preparation – Into a 50-mL Nessler comparison tube, pipette 2 mL of Standard Lead Solution and add approximately 40 mL of purified water. Adjust with 1 *N* acetic acid or 6*N* ammonium hydroxide to a pH between 3.0 and 4.0. Using a pH meter or short-range pH indicator paper as external indicator, dilute with purified water to approximately 45 mL, and mix.

7.14.5. Monitor Preparation – Into a 50-mL Nessler comparison tube, add 40 mL of a solution prepared as directed for Sample Preparation and add 2.0 mL of Standard Lead Solution. Adjust with 1 *N* acetic acid or 6N ammonium hydroxide to a pH between 3.0 and 4.0. Using a pH meter or short-range pH indicator paper as external indicator, dilute with purified water to approximately 45 mL and mix.

7.14.6. Procedure – To each of the Nessler tubes, add 2 mL of pH 3.5 Acetate Buffer, 1.2 mL of Thioacetamide-glycerin Base TS, dilute with purified water to 50 mL, cover with parafilm, and mix by inversion.

7.14.7. Allow to stand for 2 minutes using a calibrated timer.

7.14.8. View downward over a white surface; the color of the solution from the Sample Preparation is not darker than that of the solution from the Standard Preparation, and the color of the solution of the Monitor Preparation is equal to or darker than that of the Standard Preparation.

7.15. **IDENTITY (IR) (AS-IS):**

7.15.1. Follow Spectrum Two UATR SOP.

7.15.2. Analyze sample as-is.

7.16. **LOSS ON DRYING (105°C):**

7.16.1. Dry an LOD vial at 105°C ± 2°C for 30 minutes, cool for 15 minutes in a desiccator and record the weight utilizing an analytical balance.

7.16.2. Transfer approximately 1- 2 g of the sample to the LOD vial and analytically weigh the vial and contents. By gentle, sidewise shaking, distribute the sample as evenly as possible in the LOD Vial.

7.16.3. Place the LOD vial containing the sample into the oven. Dry the sample at 105°C ± 2°C overnight to constant weight.

7.16.4. Remove the LOD vial from the oven and allow to cool in desiccator for 15 minutes.

7.16.5. Reweigh and return sample to the oven for 2 additional hours.

7.16.6. Remove the LOD vial from the oven and allow to cool in desiccator for 15 minutes.

7.16.7. Reweigh and repeat the weighing process, if necessary, to obtain a constant weight.

7.16.7.1. If the weight is not constant, repeat the drying and weighing process until a constant weight is achieved.

7.16.8. Use the calculation below to determine %LOD:

$$\%\,LOD = \frac{\big(Initial\ Sample\ Weight\ (g) - Final\ Sample\ WEight\ (g)\big)x\ 100}{Initial\ Sample\ Weight\ (g)}$$

7.17. **LOSS ON DRYING (130°C):**

7.17.1. Dry an LOD vial at 130 ± 2°C for 30 minutes, cool for 15 minutes in a desiccator and record the weight utilizing an analytical balance.

7.17.2. Transfer approximately 1 - 2 g of the sample to the LOD vial and analytically weigh the vial and contents. By gently, sidewise shaking, distribute the sample as evenly as possible in the LOD Vial.

7.17.3. Place the LOD vial containing the sample into the oven. Dry the sample at 130°C ± 2°C overnight to constant weight.

7.17.4. Remove the LOD vial from the oven and allow to cool in the desiccator for 15 minutes.

7.17.5. Reweight and return sample to the oven for 2 additional hours.

7.17.6. Remove the LOD vial from the oven and allow to cool in desiccator for 15 minutes.

7.17.7. Reweigh and repeat the weighing process, if necessary, to obtain a constant weight.

7.17.7.1. If the weight is not constant, repeat the drying and weighing process until a constant weight is achieved.

7.17.8. Use the calculation below to determine % LOD:

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

7.18. **MICROBIAL:**

7.18.1. Microbial analysis will be performed by an outside testing laboratory

7.18.1.1. Primary Provider: Mary Paul Laboratories (MPL)

7.18.1.2. Package and send NLT 35 g of sample to Mary Paul Laboratory

7.18.2. Analyses:

7.18.2.1. Total Aerobic Microbial Count (TAMC)

7.18.2.1.1. In accordance with USP <61>, the result for *Total Bioburden* will be reported from the TAMC result only.

7.18.2.1.2. If there is growth, Identification is required.

7.18.2.2. Total Yeast Microbial Count (TYMC)

7.19. **pH OF A 0.5 M SOLUTION:**

7.19.1. Weigh 5.33 g of sample. Transfer to a 50-mL graduated cylinder.

7.19.2. Q.S. to 50 mL with purified water. Cover with parafilm and mix by inversion.

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

7.20. **pH OF A 1.0M SOLUTION:**

7.20.1. Weigh 5.33 g of sample and accurately transfer the weighed sample to a graduated cylinder and Q.S. to 25 mL with purified water.

7.20.2. Dissolve completely.

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

7.21. **pH OF A 5% SOLUTION:**

7.21.1. Weigh 5.0 g of sample. Transfer to a suitable beaker.

7.21.2. Add 100 mL of purified water and stir to mix.

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

7.22. **pH OF A 1% SOLUTION:**

7.22.1. Weigh 1.0 g of sample. Transfer to a suitable beaker.

7.22.2. Add 100 mL of purified water and stir to mix.

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

7.23. **PVS CONTENT:**

7.23.1. Solution Preparation:

7.23.1.1. Sample: Dissolve 1.066 g of sample in approximately 80 mL of purified water. Adjust pH to 5.9-6.1 with 50% NaOH and dilute to 100.0 mL with purified water.

7.23.1.2. Prepare a blank solution of MES by dissolving 5.33 g of previously approved MES or Ultra-Pure MES in approximately 400 mL of purified water. Adjust pH to 5.9-6.1 with 50% NaOH. Dilute to 500.0 mL.

7.23.1.3. Prepare 5 mg/mL IgG Antibody solution by dissolving 15 mg of IgG from Human Serum in 3 mL of PBS Buffer. Vortex gently to dissolve. Scale as required.

7.23.1.4. Prepare a 1000 ppm PVS stock solution by diluting 1.0 mL of 25% poly (vinylsulfonic acid, sodium salt) dissolved and dilute in water to 250.0 mL.

7.23.1.5. Prepare a 50 ppm PVS Standard Solution by diluting 5.0 mL of the 1000 ppm PVS Stock Solution with the blank solution (50 mM MES Ultra-Pure Solution) to 100.0 mL. Mix thoroughly.

7.23.1.6. Prepare a 10 ppm PVS Standard Solution by diluting 10.0 mL of the 50 ppm PVS Stock Solution with the blank solution (50 mM MES Ultra-Pure Solution) to 50.0 mL. Mix thoroughly.

7.23.1.7. Prepare a 1ppm PVS Standard Solution by diluting 5.0 mL of the 10 ppm PVS Stock Solution with the blank solution (50 mM MES Ultra-Pure Solution) to 50.0 mL. Mix thoroughly.

7.23.2. Blank, Standard and Sample Analysis:

7.23.2.1. In a clean test tube or other suitable vessel, pipette 6 mL of test aliquot solution and 0.4 mL of the 5 mg/mL IgG antibody solution in to each tube. Cap or parafilm and mix gently by inversion ensuring no air bubbles are formed. Start timer for 30 minutes.

7.23.2.2. Let test mixtures stand for at least 5 minutes.

7.23.2.3. Using the portable turbidimeter, analyze the blank, 1 ppm PVS standard, and samples within 30 minutes after IgG is added. (Before timer goes off.)

7.23.2.4. Measure each sample in triplicate and average the results.

7.23.2.5. The turbidity of the sample solution should not exceed the 1 ppm PVS standard to report as < 1 ppm PVS.

7.24. **RESIDUE ON IGNITION/SULFATED ASH:**

7.24.1. Turn on muffle furnace and allow it to stabilize at 600°C. Follow Muffle Furnace SOP and Calibration for operation of furnace.

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

7.24.3. Wearing heat resistant gloves, use long 10-inch forceps to place the crucible in the furnace and to remove the crucible out of the furnace. Ignite quartz crucible at 600°C ± 50°C for 30 minutes, cool in a desiccator for one and a half hours and weigh.

7.24.4. Weigh 2.0 g sample in the previously ignited quartz crucible. Moisten the sample with 0.5 mL of sulfuric acid. Volatilize the sample with a Bunsen burner. The rate of heating should be such that from ½ to 1 hour is required to volatilize the sample.

7.24.5. Continue using the Bunsen burner to heat the sample until all the excess sulfuric acid has been volatilized. Ignite in a muffle furnace at 600°C ± 50°C for 15 minutes or until all carbon has been removed.

7.24.6. Cool in a desiccator for one and a half hours and reweigh.

7.24.7. The weight of residue should not exceed 0.001 g (0.05 %).

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

7.25. **SOLUBILITY (5%):**

7.25.1. Weigh 5.0 g into a clean glass beaker.

7.25.2. Add 100 mL of purified water and swirl to dissolve.

7.25.3. View sample from all sides under sufficient light noting any apparent color or undissolved particulate. Solution should be clear.

7.26. **SOLUBILITY (0.1M):**

7.26.1. Weigh 0.53 g of sample and quantitatively transfer the aliquot to a 25-mL volumetric flask and dissolve in ~15-20 mL of purified water.

7.26.2. Q.S. to 25 mL with purified water. Scale as required.

7.26.3. View sample from all sides under sufficient light noting any apparent color or undissolved particulate. Solution should be clear (complete) and colorless to pass test.

7.27. **SULFATE:**

7.27.1. Sample Preparation:

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

7.27.2. Standard Preparation:

7.27.2.1. Prepare a standard solution of 0.1 mL of 0.020 N H_2SO_4 in 40 mL purified water in a 50-mL Nessler Color Comparison Tube.

7.27.3. Procedure:

7.27.3.1. To both the sample and standard solutions, add 1 mL of 3 N HCl, 3 mL of Barium Chloride TS and Q.S. to 50 mL with purified water in Nessler color comparison tubes.

7.27.3.2. Mix and allow to stand for 10 minutes utilizing a calibrated timer.

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

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

7.27.3.4.1. Follow the appropriate SOP.

7.28. **TRACE METALS:**

7.28.1. Refer to Analytical Method: Elemental Impurities via ICP-MS in MES Monohydrate, DCN: BSI-ATM-0065 and NexION 350X ICP-MS SOP, DCN: BSI-SOP-0303.

7.28.2. Available Methods dependent on Product Code Requirements:

7.28.2.1. Refer to Analytical Method of Analysis: Trace Metals in Finished Goods Products by ICP-OES, for sample preparation and analysis.

7.28.2.2. Refer to Analytical Method for Determination of Trace Metals in BioTech Products, BSI-ATM-0131.

7.29. **WATER BY KARL FISCHER:**

7.29.1. Perform a standardization of the titrant (Composite 5) as per the Standardization of Titrants procedure, BSI-SOP-0140.

7.29.2. Immediately weigh 0.1 g of as-is sample into the glass weighing spoon and tare it.

7.29.3. Transfer the sample to the KF vessel by removing the rubber septum and adding the sample into the titration vessel.

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

7.29.4. Return the weighing spoon to the balance, making sure not to lose any sample that was left behind. Once the weight stabilizes, transfer the sample weight to the auto-titrator software.

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

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

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

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