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Effective Date:	11-Jun-2020	11-Jun-2023	: Date of Next Review
Initiated By:	Uhlig, Mark W	4.0	: Supersedes
Reason for Print:	Placement on Website		

Approval:

Approvers

Date	Time	Group	Name
11-Jun-2020	11:33:03 AM	EDITOR	Bertsch, Amy M
11-Jun-2020	12:37:41 PM	QUALITY	Miller, Jenna M
11-Jun-2020	03:22:10 PM	MANAGEMENT	Uhlig, Mark W

MES, MONOHYDRATE TESTING METHODS

Printed On:	25-Oct-2021 02:19:32 PM	Ledergerber, Alissa L	: Printed By
Print Expiration:	This document expires on the Date of Next Review listed in the header of this document of upon next revision.		
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1. PURPOSE:

1.1. To provide the Quality Control (QC) 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 QC Laboratory.

3. RESPONSIBILITIES:

3.1. The Director of Quality Control or designee is responsible for 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 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. REFERENCES:

4.1. *ACS, Reagent Chemicals*, current edition.
4.2. Balance SOP
4.3. Bangor Portable Turbidimeter and Calibration SOP
4.4. Blue M Convection Oven Operation and Calibration SOP
4.5. *Current USP*
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4.12. NexION 350X SOP
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4.15. Result Reporting SOP
4.16. RNase (Ribonuclease) Assay
4.17. Spectrum Two UATR SOP
4.18. Standardization of Titrants
4.19. VWR Gravity Convection Oven Operation and Calibration (Model Number 414005-106)
4.20. XL200 pH/mV/Conductivity Meter SOP

5. EQUIPMENT:

5.1. Analytical Balance
5.2. Convection Oven
5.3. Hach Portable Turbidimeter
5.4. Metrohm Auto-Titrator
5.5. Muffle Furnace
5.6. Perkin-Elmer NexION 350X
5.7. Perkin-Elmer Spectrum Two UATR
5.8. pH/Conductivity Meter
5.9. UV/Vis Spectrophotometer

6. PROCEDURES:

IN-PROCESS TESTING

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

6.2. **MOTHER LIQUOR ABSORBANCE <1.00 a.u. @ 260nm and 280nm.:**

6.2.1. Prepare a 1:1 dilution of purified water and the ML sample. Mix thoroughly.

6.2.2. Refer to Lambda 25 UV/VIS Spectrophotometer to determine the Absorbance of the sample.

6.3. **MOTHER LIQUOR ASSAY Monitor:**

6.3.1. Standardize 0.1 N Sodium Hydroxide in accordance with Standardization of Titrants utilizing the Metrohm Auto Titrator.

6.3.2. Accurately weigh 0.8g of MES, Monohydrate ML sample and transfer to a suitable beaker.

6.3.3. Add 50 mL of purified water and stir to dissolve.

6.3.4. Titrate to the potentiometric endpoint with 0.1N sodium hydroxide.

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

6.4. **WET CRYSTAL ABSORBANCE <0.1000 a.u. @ 260 and 280nm:**

6.4.1. 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.

6.4.2. Swirl to dissolve completely.

6.4.3. Refer to Lambda 25 UV/VIS Spectrophotometer to determine the Absorbance of the sample.

6.5. **DRY CRYSTAL ASSAY 99.0% min:**

6.5.1. Standardize 0.1 N Sodium Hydroxide in accordance with Standardization of Titrants utilizing the Metrohm Auto Titrator.

6.5.2. Accurately weigh 0.8g of MES, Monohydrate (sample is measured as-is) and transfer to a suitable beaker.

6.5.3. Add 50 mL of purified water and stir to dissolve.

6.5.4. Titrate to the potentiometric endpoint with 0.1N sodium hydroxide.

6.5.5. Submerge the probe in storage solution after analysis is completed to condition the glass electrode.

6.5.6. The pK_a should be reported on the Assay printout from the Metrohm Auto-Titrator.

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

FINISHED GOOD ANALYSIS

6.6. **ABSORBANCE (1M) REFER TO SUMMARY SHEET:**

6.6.1. 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.

6.6.2. Swirl to dissolve completely.

6.6.3. Refer to Lambda 25 UV/VIS Spectrophotometer to determine the Absorbance of the sample.

6.7. **APPEARANCE AND COLOR White/Crystals:**

6.7.1. Weigh a suitable amount of the sample into a clean, dry glass beaker.
6.7.2. In an area with sufficient lighting, view the sample from all sides.
6.7.3. The sample should be white in color and characteristic of crystals.

6.8. **ASSAY AND pK_a 99.0% min. and 5.9 – 6.3:**

6.8.1. Standardize 0.1 N Sodium Hydroxide in accordance with Standardization of Titrants utilizing the Metrohm Auto Titrator.
6.8.2. Accurately weigh 0.8g of MES, Monohydrate (sample is measured as-is) and transfer to a suitable beaker.
6.8.3. Add 50 mL of purified water and stir to dissolve.
6.8.4. Titrate to the potentiometric endpoint with 0.1N sodium hydroxide.
6.8.5. 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.

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

6.9. **CHLORIDE 0.005% max.:**

6.9.1. Weigh 2.0 g of sample and dissolve sample in approximately 40 mL purified water. If necessary, neutralize the solution with nitric acid to litmus.
6.9.2. Pipette 0.141 mL of 0.02 N HCl into approximately 40 mL of purified water in a Nessler Color Comparison Tube.
6.9.3. Add to each solution, 1 mL of concentrated nitric acid and 1 mL of 0.1 N silver nitrate. Q.S. to 50 mL with purified water. Cover with parafilm and mix by inversion.
6.9.4. 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.
6.9.5. If a visible difference in the turbidity is not observed, utilize the Turbidimeter to measure the turbidity of the standard and the sample solutions.
6.9.6. Follow the appropriate SOP:
6.9.6.1. Stroudsburg: Portable Turbidimeter SOP and Calibration
6.9.6.2. Bangor: Bangor Portable Turbidimeter and Calibration SOP

6.10. **COLOR OF A 1M ALKALINE SOLUTION Clear/Colorless:**

6.10.1. Accurately weigh 21.3 g of sample and transfer to a clean, dry 150 mL beaker.
6.10.2. Dissolve in 100 mL of purified water.
6.10.3. Adjust the pH of the sample solution to 12.0 by adding (dropwise) 42% sodium hydroxide.
6.10.4. Observe the color of the solution.
6.10.5. The solution must remain clear and colorless when compared against a common background to a clear and colorless reference solution to report clear.

6.11. **ENZYME ACTIVITY** **None Detected:**

6.11.1. RNase, DNase, and Protease per procedures referenced in section 4.

6.12. **HEAVY METALS** **(0.0002% max.):**

6.12.1. Refer to section 6.23: Trace Metals for primary method of analysis.

Alternate Method:

6.12.2. 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 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.

6.12.3. 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.

6.12.4. 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.

6.12.5. 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 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.

6.12.6. Procedure—To each of the Nessler tubes, add 2 mL of pH 3.5 Acetate Buffer, 1.2 mL of thioacetamide-glycerin base TS (1 mL of glycerin TS and 0.2 mL of thioacetamide TS. Heat gently and use immediately), dilute with purified water to 50 mL, cover with parafilm and mix by inversion.

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

6.12.8. 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.

6.13. **IDENTITY (IR) (As-is)** **Passes Test:**

6.13.1. Follow Spectrum Two UATR SOP.

6.13.2. Analyze sample as-is.

6.14. **LOSS ON DRYING (105°C)** **REFER TO SUMMARY SHEET:**

6.14.1. 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.

6.14.2. 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.

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

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

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

6.14.6. Remove the LOD vial from the oven and allow to cool in desiccator for 15 minutes.
6.14.7. Reweigh and repeat the weighing process, if necessary, to obtain a constant weight.
6.14.7.1. If the weight is not constant, repeat the drying and weighing process until a constant weight is achieved.
6.14.8. Use the calculation below to determine %LOD:

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

6.15. **LOSS ON DRYING (130°C) REFER TO SUMMARY SHEET:**
6.15.1. 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.
6.15.2. Transfer approximately 1 – 2 grams 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.
6.15.3. Place the LOD vial containing the sample into the oven. Dry the sample at 130°C ± 2°C overnight to constant weight.
6.15.4. Remove the LOD vial from the oven and allow to cool in the desiccator for 15 minutes.
6.15.5. Reweight and return sample to the oven for 2 additional hours.
6.15.6. Remove the LOD vial from the oven and allow to cool in desiccator for 15 minutes.
6.15.7. Reweigh and repeat the weighing process, if necessary, to obtain a constant weight.
6.15.7.1. If the weight is not constant, repeat the drying and weighing process until a constant weight is achieved.
6.15.8. Use the calculation below to determine % LOD:

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

6.16. **pH of a 0.5 M SOLUTION REFER TO SUMMARY SHEET:**
6.16.1. Accurately weigh 5.33 g of sample. Transfer to a 50-mL graduated cylinder.
6.16.2. Q.S. to 50 mL with purified water. Cover with parafilm and mix by inversion.
6.16.3. Follow the appropriate SOP for calibration and pH measurement.

6.17. **pH of a 1.0M SOLUTION REFER TO SUMMARY SHEET:**
6.17.1. 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.
6.17.2. Dissolve completely.
6.17.3. Follow the appropriate SOP for calibration and pH measurement.

6.18. **pH of a 5% SOLUTION REFER TO SUMMARY SHEET:**
6.18.1. Accurately weigh 5.0 g of sample. Transfer to a suitable beaker.
6.18.2. Add 100 mL of purified water and stir to mix.
6.18.3. Follow the appropriate SOP for calibration and pH measurement.

6.19. **PVS CONTENT REFER TO SUMMARY SHEET:**
6.19.1. Solution Preparation:
6.19.1.1. Sample: Dissolve 1.066g of sample in approximately 80mL of purified water. Adjust pH to 5.9-6.1 with 50% NaOH and dilute to 100.0mL with purified water.

6.19.1.2. Prepare a blank solution of MES by dissolving 5.33g of previously approved MES or Ultra-Pure MES in approximately 400mL of purified water. Adjust pH to 5.9-6.1 with 50% sodium hydroxide. Dilute to 500.0mL.
6.19.1.3. Prepare 5mg/mL IgG Antibody solution by dissolving 15mg of IgG from Human Serum in 3mL of PBS Buffer. Vortex gently to dissolve. Scale as required.
6.19.1.4. Prepare a 1000ppm PVS stock solution by diluting 1.0mL of 25% poly(vinylsulfonic acid, sodium salt) dissolved and dilute in water to 250.0mL.
6.19.1.5. Prepare a 50ppm PVS Standard Solution by diluting 5.0mL of the 1000ppm PVS Stock Solution with the blank solution (50mM MES Ultra-Pure Solution) to 100.0mL. Mix thoroughly.
6.19.1.6. Prepare a 10ppm PVS Standard Solution by diluting 10.0mL of the 50ppm PVS Stock Solution with the blank solution (50mM MES Ultra-Pure Solution) to 50.0mL. Mix thoroughly.
6.19.1.7. Prepare a 1ppm PVS Standard Solution by diluting 5.0mL of the 10ppm PVS Stock Solution with the blank solution (50mM MES Ultra-Pure Solution) to 50.0mL. Mix thoroughly.

6.19.2. Blank, Standard and Sample Analysis:
6.19.2.1. In a clean test tube or other suitable vessel pipette 6mL of test aliquot solution and 0.4mL of the 5mg/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.
6.19.2.2. Let test mixtures stand for at least 5 minutes.
6.19.2.3. Using the portable turbidimeter, analyze the blank, 1ppm PVS standard, and samples within 30 minutes after IgG is added. (Before timer goes off.)
6.19.2.4. Measure each sample in triplicate and average the results.
6.19.2.5. The turbidity of the sample solution should not exceed the 1ppm PVS standard to report as <1ppm PVS.

6.20. **RESIDUE ON IGNITION/SULFATED ASH REFER TO SUMMARY SHEET:**

6.20.1. Turn on muffle furnace and allow it to stabilize at 600°C. Follow Muffle Furnace SOP and Calibration for operation of furnace.
6.20.2. Inspect a quartz crucible for cracks, chips and discoloration.
6.20.3. 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.
6.20.4. 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.
6.20.5. 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.
6.20.6. Cool in a desiccator for one and a half hours and reweigh.
6.20.7. The weight of residue should not exceed 0.001 g (0.05 %).

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

6.21. **SOLUBILITY (5%) REFER TO SUMMARY SHEET:**

6.21.1. Weigh 5.0 g into a clean glass beaker.
6.21.2. Add 100 mL of purified water and swirl to dissolve.
6.21.3. View sample from all sides under sufficient light noting any apparent color or undissolved particulate. Solution should be clear.

6.22. **SULFATE 50 ppm max.:**

6.22.1. Sample Preparation:
6.22.1.1. 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.
6.22.2. Standard Preparation:
6.22.2.1. 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.
6.22.3. Procedure:
6.22.3.1. 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.
6.22.3.2. Mix and allow to stand for 10 minutes utilizing a calibrated timer.
6.22.3.3. Any turbidity produced in the sample solution should not exceed that produced by the standard.
6.22.3.4. If a visible difference in the turbidity is not observed, utilize the Turbidimeter to measure the turbidity of the standard and the sample solutions.
6.22.3.4.1. Follow the appropriate SOP:
6.22.3.4.1.1. Stroudsburg: Portable Turbidimeter SOP and Calibration
6.22.3.4.1.2. Bangor: Bangor Portable Turbidimeter and Calibration SOP.

6.23. **TRACE METALS REFER TO SUMMARY SHEET:**

6.23.1. Refer to NexION 350X SOP.

6.24. **WATER BY KARL FISCHER REFER TO SUMMARY SHEET:**

6.24.1. Perform a standardization of the titrant (Composite 5) as per Standardization of Titrants.
6.24.2. Immediately weigh 0.1g of as-is sample into the glass weighing spoon and tare it.
6.24.3. Transfer the sample to the KF vessel by removing the rubber septum and adding the sample into the titration vessel.
6.24.3.1. Do not leave the rubber septum open for long periods of time as this will allow moisture to enter the titration vessel.
6.24.4. 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.
6.24.5. Check to make sure there is no residual sample stuck to the sides of the titration vessel.
6.24.6. Ensure the sample is fully dissolved before the titration begins (i.e. before the stir command completes).
6.24.7. The moisture content will be determined by the Metrohm Auto Titrando 907, using the following equation:

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