BIOSPECTRA

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HEPES TESTING METHODS

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1. PURPOSE:

1.1. To provide the Quality Control (QC) Laboratory personnel with procedures for testing of HEPES raw materials (RM), in-process (IP), finished goods (FG), and stability.

2. SCOPE:

2.1. Applies to the testing HEPES in the QC Laboratory. Methods include testing for all types of HEPES sold by BioSpectra; only the specific tests required for requested type must be tested for.

3. REFERENCES:

3.1. *ACS, Reagent Chemicals*, current edition.
3.2. *Current USP*
3.3. Balance SOP
3.4. Bangor Portable Turbidimeter and Calibration SOP
3.5. DNase (Endonuclease) Assay
3.6. DNase (Exonuclease) Assay
3.7. Laboratory Notebooks
3.8. Lambda 25 UV/Vis Operation and Calibration
3.9. Metrohm Titrando 907 Auto-Titrator SOP
3.10. Muffle Furnace SOP and Calibration
3.11. NexION 350X ICP-MS SOP
3.12. Protease Assay RNase (Ribonuclease)Assay
3.13. Spectrum Two UATR SOP Standardization of Titrants XL200 pH/mV/Conductivity Meter SOP
3.14. HEPES In-Process Analysis and Specifications

4. EQUIPMENT:

4.1. PerkinElmer Lambda 25 UV/Vis Spectrophotometer
4.2. Optically matched set of UV quartz cells, 10 mm path length
4.3. Laser Printer, or equivalent
4.4. Analytical Balance
4.5. PerkinElmer Spectrum Two UATR
4.6. PerkinElmer NexION 350X ICP-MS
4.7. XL200 pH/Conductivity Meter or equivalent pH / mv / Conductivity Meter
4.8. Nessler Color Comparison Tubes and Support Rack
4.9. Muffle Furnace
4.10. Blue M Oven, or equivalent
4.11. Metrohm 907 Auto-Titrator
4.12. Mortar and Pestle
4.13. Hach 2100Q Portable Turbidimeter, or equivalent

5. PROCEDURE:

5.1. MOTHER LIQUOR ABSORBANCE 1.000 a.u. max @ 250nm, 260nm, 280nm:

5.1.1. Prepare 10 mL of a 1:1 dilution by pipetting 5 mL of purified water and 5 mL of the Mother Liquor into an LOD vial or small beaker.
5.1.2. Swirl to homogenize the solution.

5.1.3. Refer to Lambda 25 UV/Vis Operation and Calibration to determine the absorbance of the sample. Record results at specified wavelengths in the appropriate laboratory documentation and Batch Record.

5.2. MOTHER LIQUOR ASSAY Monitor:

5.2.1. Standardize Metrohm pH electrode as per Metrohm Titrando 907 Auto-Titrator SOP
5.2.2. Standardize 0.1N NaOH as per Standardization of Titrants.
5.2.3. Accurately weigh 0.8g of sample and transfer to a beaker.
5.2.4. Dissolve in 50 mL of purified water. Determine the Assay concentration using the Metrohm Auto titrator.

$$\% \text{ HEPES} = \frac{(\text{mL} \times \text{N of NaOH})\ (23.831)}{\text{Sample Weight (g)}}$$

5.2.5. Record results in the appropriate laboratory documentation and Batch Record.

5.3. **ABSORBANCE (0.1 M) Refer to Summary Sheet:**

5.3.1. Accurately weigh 0.60g of sample.
5.3.2. Transfer accurately weighed sample to a graduated cylinder and dilute to 25 mL with purified water.
5.3.3. Swirl to dissolve completely.
5.3.4. Refer to Lambda 25 UV/Vis Operation and Calibration, to determine the Absorbance of the sample.

5.4. **APPEARANCE AND COLOR Refer to Summary Sheet:**

5.4.1. Place 25-50g of the sample in a clean, dry glass beaker.
5.4.2. In an area with sufficient lighting, view the sample from all sides.
5.4.3. The sample should be white in color and characteristic of powder.
5.4.3.1. If the sample does not conform to these specifications, notify a supervisor immediately.

5.5. **ASSAY AND pKa Refer to Summary Sheet:**

5.5.1. Standardize Metrohm pH electrode as per Metrohm Titrando 907 Auto-Titrator SOP
5.5.1.1. For pK_a ensure that the slope of standardization is 99.3-101.0% .
5.5.2. Standardize 0.1N NaOH as per Standardization of Titrants.
5.5.3. Accurately weigh 0.8g of sample and transfer to a beaker.
5.5.4. Dissolve in 50 mL of purified water. Determine the Assay concentration using the Metrohm Auto titrator.
5.5.5. The pK_a should be reported on the Assay printout from the Metrohm Auto titrator.

$$\% \text{ HEPES} = \frac{(\text{mL} \times \text{N of NaOH})\ (23.831)}{\text{Sample Weight (g)}}$$

5.6. **CHLORIDE 0.005% max:**

5.6.1. Weigh 2.0 g of sample and dissolve sample in 40 mL of purified water in a Nessler Color Comparison Tube. If necessary, neutralize the solution with nitric acid to litmus.
5.6.2. Pipette 0.141 mL of 0.02 N HCl and dilute to 40 mL with purified water in a Nessler Color Comparison Tube.

5.6.3. Add to each solution 1 mL of concentrated nitric acid and 1 mL of 0.1N silver nitrate. Dilute to 50 mL with purified water. Cover with parafilm and mix by inversion.
5.6.4. After 5 minutes, the turbidity of the sample prep does not exceed that produced by the standard when viewed against a dark background.
5.6.5. If a visible difference in the turbidity is not observed, then utilize the Turbidimeter to measure the turbidity of the standard and the sample solutions. Follow Bangor Portable Turbidimeter SOP and Calibration.

5.7. **ENZYME ACTIVITY** **None Detected:**

5.7.1. DNase, RNase, and Protease as per SOP.

5.8. **HEAVY METALS** **Refer to Summary Sheet:**

5.8.1. Refer to NexION 350X ICP-MS SOP
5.8.2. This analysis may also be done by an outside testing laboratory.

5.9. **IDENTIFICATION TEST (UATR)** **Passes Test:**

5.9.1. Follow Spectrum Two UATR SOP.

5.10. **INSOLUBLE MATTER** **Refer to Summary Sheet:**

5.10.1. Accurately weigh 20.00g of sample and transfer to a 600 mL beaker.
5.10.2. Add 200 mL of purified water. If necessary, utilize a Teflon encapsulated magnetic stirring bar and electric stir plate to dissolve the sample.
5.10.3. Dry a filter crucible and filter paper at 105°C ± 2°C for 1 hour. Cool in ambient air for 15 minutes and weigh.
5.10.4. Filter sample solution through the filter crucible using a suitable vacuum pump.
5.10.5. Rinse sample vessel and filter crucible with 100 mL of purified water.
5.10.6. Dry the filter crucible and filter paper at 105°C ± 2°C for 1 hour. Cool in ambient air for 15 minutes and weigh.

$$\%\ \text{Insolubles} = \frac{\text{residue weight (g)}}{\text{sample weight (g)}}\ \text{x}\ 100$$

5.11. **LOSS ON DRYING** **Refer to Summary Sheet:**

5.11.1. Dry a Loss On Drying (LOD) vial in an oven at 105°C ± 2°C for 30 minutes. Cool for 15 minutes in a desiccator, weigh on the analytical balance, and record results.
5.11.2. Tare the dried vial and weigh 1 – 2 g of sample and record the results.
5.11.3. Dry for 3 hours at 105°C ± 2°C. Cool for 15 minutes in a desiccator.
5.11.4. Reweigh and calculate the % LOD.
5.11.5. Retain sample for Assay, dried basis.

$$\%\ LOD = \frac{initial\ sample\ weight\ (g) - final\ sample\ weight\ (g)}{initial\ sample\ weight\ (g)} * 100$$

5.12. **pH of a 5% SOLUTION** **Refer to Summary Sheet:**

5.12.1. Prepare a 5% solution of the sample.
5.12.1.1. Accurately weigh 5.0g of sample.
5.12.1.2. Transfer accurately weighed sample to a beaker and dissolve in 100mL of purified water.

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5.12.1.3. Swirl to dissolve completely.

5.12.2. Follow the appropriate SOP to measure and record the pH at 25°C at 25 ± 2°C.

5.13. **RESIDUE ON IGNITION/SULFATED ASH** **Refer to Summary Sheet:**

5.13.1. Turn on muffle furnace and allow temperature to stabilize at 600°C. Follow muffle furnace calibration procedure as per Muffle Furnace SOP and Calibration.

5.13.2. Utilize the 10 inch forceps to insert and remove crucible into the furnace.

5.13.3. Ignite the quartz crucible at 600 ± 50°C for 30 minutes. Cool in a desiccator for one hour and 30 minutes and weigh.

5.13.4. Weigh 1.0 g sample in the previously ignited quartz crucible. Moisten the sample with 0.5 mL of sulfuric acid.

5.13.5. 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 no sample is lost.

5.13.5.1. The rate of heating should be such that from 30 to 60 minutes is required to volatilize the sample.

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

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

5.13.7. Gently remove the ignited crucible with 10 inch forceps from the furnace.

5.13.8. Inspect the crucible for cracks, chips, or signs of damage such as discoloration- the muffle furnace insulation is made of rough ceramics and metal, care must be taken to not crack, chip, or rub the crucible against the lining.

5.13.9. Cool in a desiccator for an hour and a half and reweigh.

$$\%\ ROI = \frac{Residue\ weight\ (g)}{Sample\ weight\ (g)} * 100$$

5.14. **SULFATE** **50 ppm max.:**

5.14.1. Sample Preparation:

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

5.14.2. Standard Preparation:

5.14.2.1. Prepare a standard solution by pipetting 0.1 mL of 0.020 N H_2SO_4 in a 50 mL Nessler Color Comparison Tube. Dilute to 40 mL with purified water.

5.14.3. Procedure:

5.14.3.1. To both solutions add 1 mL of 3 N HCl and 3 mL of Barium Chloride TS. Dilute to 50 mL with purified water.

5.14.3.2. Cover with parafilm and mix by inversion.

5.14.3.3. Compare turbidity 10 minutes after addition of the barium chloride to the sample and standard solutions.

5.14.4. Any turbidity produced in the sample solution should not exceed that produced by the standard when viewed from above against a black surface.

5.14.5. If turbidity of the sample solution exceeds that of the standard, notify the QC Manager immediately.

5.15. **SOLUBILITY (5%)** **Refer to Summary Sheet:**

5.15.1. Weigh 5.0g into a clean glass beaker.

5.15.2. Add 100mL purified water and swirl to dissolve.

5.15.3. View sample from all sides under sufficient light noting any apparent color or undissolved particulate when compared to a clear and colorless reference.

5.16. **TRACE METALS (As, Cu, Fe, Pb)** **Refer to Summary Sheet:**

5.16.1. Refer to NexION 350X ICP-MS SOP