BIOSPECTRA
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HEPES TESTING METHODS

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1. PURPOSE:

1.1. To provide the 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 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. RESPONSIBILITIES:

3.1. The Laboratory Manager is responsible for the control, training, maintenance and implementation of this procedure.

3.2. The Analysts are responsible for compliance with the terms of this procedure. This includes notifying the Laboratory Manager and Quality Assurance Managers, or designees,if any analyses fail to meet their respective specifications.

4. REFERENCES:

4.1. BSI-ATM-0054, Analytical Method for the Determination of ICH Q3D Elemental Impurities by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in HEPES

4.2. BSI-ATM-0089, Analytical Method of Analysis: Trace Metals in Finished Goods Products by ICP-OES

4.3. BSI-ATM-0121, HEPES Identity by HPLC with UV Detection

4.4. BSI-PRL-0338, Analytical Method Validation Protocol: HEPES Identity via HPLC

4.5. BSI-PRL-0508, Analytical Method Validation Protocol: Determination of ICH Q3D Elemental Impurities by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in HEPES

4.6. BSI-RPT-1758,Analytical Method Transfer Report: HEPES Identity by HPLC with UV Detection

4.7. BSI-SOP-0090, Lambda 25 UV/Vis Operation and Calibration

4.8. BSI-SOP-0094, Muffle Furnace SOP and Calibration

4.9. BSI-SOP-0095, DNase (Endonuclease) Assay

4.10. BSI-SOP-0096, RNase (Ribonuclease) Assay

4.11. BSI-SOP-0098, Balance SOP

4.12. BSI-SOP-0126, Laboratory Notebooks

4.13. BSI-SOP-0138, DNase (Exonuclease) Assay

4.14. BSI-SOP-0139, Protease Assay

4.15. BSI-SOP-0140, Standardization of Titrants

4.16. BSI-SOP-0143, Metrohm Titrando 907 Auto-Titrator SOP

4.17. BSI-SOP-0242, Bangor Portable Turbidimeter and Calibration

4.18. BSI-SOP-0254, Spectrum Two UATR SOP

4.19. BSI-SOP-0255, XL200 pH/mV/Conductivity Meter SOP

4.20. BSI-SOP-0303, NexION 350X ICP-MS SOP

4.21. BSI-SOP-0345, Endosafe nexgen-PTS Endotoxin Reader SOP

4.22. BSI-SOP-0422, Empower 3 General Procedure

4.23. *ACS, Reagent Chemicals*, current edition.

4.24. ACQUITY UPLC Quaternary Solvent Manager PLUS Series

4.25. ACQUITY UPLC TUV Detector Operator's Overview and Maintenance Guide
4.26. Analytical Method: Residual Solvents on the GC-FID
4.27. *Current USP*

5. EQUIPMENT:

5.1. PerkinElmer Lambda 25 UV/Vis Spectrophotometer
5.2. Optically matched set of UV quartz cells, 10 mm path length
5.3. Analytical Balance
5.4. Endosafe nexgen-PTS Endotoxin Reader
5.5. Waters Acquity UPLC
5.6. PerkinElmer Spectrum Two UATR
5.7. PerkinElmer NexION 350X ICP-MS
5.8. PerkinElmer Avio 500 ICP-OES
5.9. XL200 pH/Conductivity Meter or equivalent pH / mv / Conductivity Meter
5.10. Muffle Furnace
5.11. Blue M Oven, or equivalent
5.12. Metrohm 907 Auto-Titrator
5.13. Hach 2100Q Portable Turbidimeter, or equivalent
5.14. Shimadzu GC-2010, FID detector

6. REAGENTS:

6.1. **Acetate Buffer, pH 3.5:** Dissolve 62.5g of ammonium acetate in 62.5mL of purified water, and add 47.0mL of concentrated hydrochloric acid. Adjust, if necessary, with 6N ammonium hydroxide or 6N hydrochloric acid to a pH of 3.5, dilute with purified water to make 250mL.
6.2. **Acetic Acid, 1N:** Dilute 6 g of glacial acetic acid to 100 g with purified water.
6.3. **Ammonium Hydroxide, 6N:** Dilute 206.9mL of Ammonium Hydroxide 29% to a final volume of 500mL with purified water.
6.4. **Ammonium Peroxydisulfate:** purchased commercially.
6.5. **Ammonium Thiocyanate, 30%:** Weigh 30g and dilute to 100mL with purified water.
6.6. **Barium Chloride Dihydrate:** purchased commercially.
6.7. **Barium Chloride TS:** Dissolve 12 grams of barium chloride dihydrate in purified water. Filter and dilute to make a total volume of 100 mL with purified water.
6.8. **Composite 5:** purchased commercially
6.9. **Hydrochloric Acid, Concentrated:** purchased commercially.
6.10. **Iron Standard (10ppm):** Dissolve 863.4mg of ferric ammonium sulfate dodecahydrate to 100mL with purified water that contains 10mL of 2N Sulfuric Acid. Pipette 10mL of this solution into a 1L volumetric flask that contains 10ml of 2N sulfuric acid, then dilute to volume with purified water.
6.11. **Glycerin Base TS:** To 200g of glycerol, add purified water to bring the total weight to 235g. Add 140mL of 1N Sodium Hydroxide and 50mL of purified water.
6.12. **Hydrochloric Acid, 0.02N:** Dilute 10mL of 1N HCl to 500mL with purified water.
6.13. **Hydrochloric Acid 3N:** Pipette 25.75 mL of concentrated hydrochloric acid and transfer to a 100 mL volumetric flask that contains a small amount of purified water. Dilute to volume with purified water.
6.14. **LAL Water:** purchased commercially.

6.15. **Lead Nitrate Stock Solution:** Dissolve 0.1598g of lead nitrate in 100mL of purified water and add 1mL of nitric acid. Dilute with purified water to 1000mL. Store in a glass container free from soluble lead salts.
6.16. **Nitric Acid:** purchased commercially.
6.17. **Platinum Cobalt Color Standard (10 APHA):** Dilute 2mL of Platinum Cobalt Color Standard 500 to 100mL with purified water.
6.18. **Potassium Hydrogen Phthalate (KHP) Standard:** Prepare a vial at 120°C for 30 minutes. Allow to cool in a desiccator and weigh a maximum of 10.0g of NIST Potassium Hydrogen Phthalate. Dry at 120°C for 2 hours. Cool and store in a desiccator in a closed container. Stable for 3 months.
6.19. **Silver Nitrate, 0.1N:** purchased commercially.
6.20. **Sodium Hydroxide 1N:** purchased commercially.
6.21. **Sodium Hydroxide 0.1N:** purchased commercially
6.22. **Sulfuric Acid:** purchased commercially
6.23. **Sulfuric Acid 0.02N:** Slowly add 20 mL of 0.1N Sulfuric Acid to 80 mL purified water to make a total volume of 100 mL.
6.24. **Thioacetamide TS:** Dissolve 4.0g of Thioacetamide in 100mL of purified water.

7. PROCEDURE:

7.1. **MOTHER LIQUOR ABSORBANCE** **Refer to Batch Record:**
7.1.1. 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.
7.1.2. Swirl to homogenize the solution.
7.1.3. 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.

7.2. **MOTHER LIQUOR ASSAY** **Monitor:**
7.2.1. Standardize Metrohm pH electrode as per Metrohm Titrando 907 Auto-Titrator SOP.
7.2.2. Standardize or perform a daily check of 0.1N NaOH as per Standardization of Titrants.
7.2.3. Accurately weigh 0.8 g of sample and transfer to a beaker.
7.2.4. Dissolve in a suitable amount of purified water. Determine the Assay concentration using the Metrohm Auto titrator.

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

7.2.5. Record results in the appropriate laboratory documentation and Batch Record, if applicable.

7.3. **ABSORBANCE (0.1 M)** **Refer to Summary Sheet:**
7.3.1. Accurately weigh 0.60 g of sample.
7.3.2. Transfer accurately weighed sample to a graduated cylinder and dilute to 25 mL with purified water.
7.3.3. Swirl to dissolve completely.
7.3.4. Refer to Lambda 25 UV/Vis Operation and Calibration to determine the Absorbance of the sample.

7.4. **ABSORBANCE (0.05 M)** **Refer to Summary Sheet:**

7.4.1. Accurately weigh 0.30 g of sample.
7.4.2. Transfer accurately weighed sample to a graduated cylinder and dilute to 25 mL with purified water.
7.4.3. Swirl to dissolve completely.
7.4.4. Refer to Lambda 25 UV/Vis Operation and Calibration to determine the Absorbance of the sample.

7.5. **ABSORBANCE (1M)** **Refer to Summary Sheet:**

7.5.1. Accurately weigh 6.0 g of sample.
7.5.2. Transfer accurately weighed sample to a graduated cylinder and dilute to 25 mL with purified water.
7.5.3. Swirl to dissolve completely.
7.5.4. Refer to Lambda 25 UV/Vis Operation and Calibration to determine the Absorbance of the sample.

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

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

7.7. **APPEARANCE OF SOLUTION (1% WATER)** **Refer to Summary Sheet:**

7.7.1. Prepare a 1% solution of the sample.
7.7.1.1. Weigh 1.0g of sample and transfer to a 100mL volumetric flask.
7.7.1.2. Dissolve sample in purified water and dilute to 100mL with purified water.
7.7.1.3. Swirl to dissolve completely.
7.7.2. Solution must be clear and colorless when compared to a clear and colorless reference standard.

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

7.8.1. Standardize Metrohm pH electrode as per Metrohm Titrando 907 Auto-Titrator SOP.
7.8.1.1. For pKa, ensure that the slope of the standardization is 99.3-101.0% and the pH (0) is between 6.8-7.2
7.8.2. Standardize 0.1N NaOH as per Standardization of Titrants.
7.8.3. Accurately weigh 0.8g of sample dried per LOD method and transfer to a beaker.
7.8.3.1. Raw material may be analyzed as-is. Refer to the assay requirement of the code being tested.
7.8.4. Dissolve in a suitable amount of purified water. Determine the assay concentration using the Metrohm Auto Titrator.
7.8.5. The pKa should be reported as the Assay printout from the Metrohm Auto Titrator.

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

7.9. **CHLORIDE** **Refer to Summary Sheet:**

7.9.1. Weigh 2.0g of sample and dissolve sample in 40mL of purified water in a Nessler Color Comparison Tube. If necessary, neutralize the solution with nitric acid to litmus.
7.9.2. Pipette 0.141mL of 0.02N HCl and dilute to 40mL with purified water in a Nessler Color Comparison Tube.
7.9.3. Add to each solution, 1mL of concentrated nitric acid and 1mL of 0.1N Silver Nitrate.
7.9.4. Dilute to 50mL with purified water. Cover with parafilm and mix by inversion.
7.9.5. After 5 minutes, the turbidity of the sample prep does not exceed that produced by the standard when viewed against a dark background.

7.9.6. 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.

7.10. **ENDOTOXINS** **Refer to Summary Sheet:**

7.10.1. Accurately weigh 0.100g of sample into a sterile tube. Add 170µL of 1N NaOH. Dilute to 10mL with LAL reagent water, dissolve, and mix thoroughly for a final concentration of 0.0100 g/mL.

7.10.2. Refer to Endosafe NexGen-PTS Endotoxin Reader SOP for further instrument instructions and sample analysis.

7.11. **ENZYME ACTIVITY** **Refer to Summary Sheet:**

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

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

7.12.1. Refer Section 7.26.

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

7.13.1. Follow Spectrum Two UATR SOP.

7.14. **IDENTIFICATION (TLC equivalent)** **Refer to Summary Sheet:**

7.14.1. Refer to DCN: BSI-ATM-0121 for Primary Method via HPLC

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

7.15.1. Accurately weigh 20.00 g of sample and transfer to a 600 mL beaker.

7.15.2. Add 200 mL of purified water. If necessary, utilize a Teflon encapsulated magnetic stirring bar and electric stir plate to dissolve the sample.

7.15.3. Dry a filter crucible and filter paper at 105°C ± 2°C for 1 hour. Cool in ambient air for 15 minutes and weigh.

7.15.4. Filter sample solution through the filter crucible using a suitable vacuum pump.

7.15.5. Rinse sample vessel and filter crucible with 100 mL of purified water.

7.15.6. Dry the filter crucible and filter paper at 105°C ± 2°C for 1 hour. Cool in ambient air for 15 minutes and weigh.

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

7.16. **LOSS ON DRYING (LOD)** **Refer to Summary Sheet:**

7.16.1. 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.

7.16.2. Tare the dried vial and weigh 1 – 2 g of sample and record the weight.

7.16.3. Dry for 3 hours at 105°C ± 2°C. Cool for 15 minutes in a desiccator.

7.16.4. Reweigh and calculate the % LOD.

7.16.5. Retain sample for Assay, dried basis.

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

7.17. **MICROBIAL CONTENT** **Refer to Summary Sheet:**

7.17.1. Prepare ~65 g of sample in a sterile vessel to send to MPL Laboratories for analysis.

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

7.18.1. Prepare a 5% solution of the sample.

7.18.1.1. Accurately weigh 5.0 g of sample.

7.18.1.2. Transfer accurately weighed sample to a beaker and dissolve in 100 mL of purified water.

7.18.1.3. Swirl to dissolve completely.

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

7.19. **pH of a 1% SOLUTION** **Refer to Summary Sheet:**

7.19.1. Prepare a 1% solution of the sample.

7.19.1.1. Accurately weigh 1.0 g of sample.

7.19.1.2. Transfer accurately weighed sample to a beaker and dissolve in 100 mL of purified water.

7.19.1.3. Swirl to dissolve completely.

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

7.20. **RESIDUAL SOLVENTS (Methanol)** **Refer to Summary Sheet:**

7.20.1. Calibration and System Suitability:

7.20.1.1. Calibrate the GC-FID instrument using calibration standard levels 1,2,3,4, and 5 and diluent blank (Standard 0 ppm) by pipetting 10 mL of each standard to a headspace vial. Crimp to seal, mix thoroughly.

7.20.1.2. System Suitability Requirements: r^2 of NLT 0.95 is required for each solvent of interest.

7.20.1.3. Solution Preparation:

7.20.1.3.1. 0ppm (Blank): Purified water or equivalent

7.20.1.3.2. 10,000 ppm Residual Solvent Stock Solution:

7.20.1.3.2.1. Prepare a 10,000-ppm solution of Methanol in purified water by weighing approximately 0.50 g of standard directly into a 50 mL volumetric flask. Mix thoroughly.Calculate actual concentration based off CoA/purity.

7.20.1.3.3. Calibration Standard Preparation: Dilute (Refer to Table) mL of the 10,000 ppm Methanol Stock Solution to 100 mL with water. Mix thoroughly. Calculate (ppm) and report into laboratory notebook for each standard solution. Input the data into the calibration table of the method in the LabSolutions software.

Calibration Level	10,000 Methanol Stock (mL)	Final Volume (mL)
1	1.50	100
2	2.40	100
3	3.00	100
4	3.60	100
5	4.50	100

7.20.2. Sample Preparation:

7.20.2.1. Weigh 1.0 g of sample to a head space vial. Add 10 mL of purified water into a 20 mL headspace vial. Dissolve, crimp to seal, and mix thoroughly.

7.20.3. Refer to Analytical Method: Residual Solvents on the GC-FID for instrument parameters and sample analysis.

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

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

7.21.2. Utilize forceps to insert and remove crucible into the furnace.

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

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

7.21.5. Volatize the sample until the sample is thoroughly charred. Heat the sample slowly, so that the sample does not boil over and sample is not lost.
7.21.5.1. The rate of heating should be such that from ½ to 1 hour is required tovolatilize the sample.
7.21.5.2. Continue to heat the sample until all the excess sulfuric acid has been volatilized.
7.21.6. Ignite the quartz crucible in a muffle furnace at 600 ± 50°C for 15 minutes or until all carbon has been removed.
7.21.7. Gently remove the ignited crucible with forceps from the furnace.
7.21.8. 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.
7.21.9. Cool in a desiccator for an hour and a half and reweigh.

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

7.22. **SULFATE** **Refer to Summary Sheet:**
7.22.1. Sample Preparation:
7.22.1.1. 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.
7.22.2. 50 ppm Standard Preparation:
7.22.2.1. 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.
7.22.3. Procedure:
7.22.3.1. To both solutions add 1 mL of 3 N HCl and 3 mL of Barium Chloride TS. Dilute to 50 mL with purified water.
7.22.3.2. Cover with parafilm and mix by inversion.
7.22.3.3. Compare turbidity 10 minutes after addition of the barium chloride to the sample and standard solutions.
7.22.4. Any turbidity produced in the sample solution should not exceed that produced by the standard when viewed from above against a black surface.
7.22.5. If turbidity of the sample solution exceeds that of the standard, notify the Laboratory Managerimmediately.

7.23. **SOLUBILITY (1%)** **Refer to Summary Sheet:**
7.23.1. Weigh 1.0 g of sample into a clean glass beaker.
7.23.2. Add 100 mL of purified water and swirl to dissolve.
7.23.3. View sample from all angles under sufficient lighting. Solution should be clear and complete.
7.23.3.1. Note any insoluble matter present, if any, and refer to material TUPP and insoluble matter specification for disposition.

7.24. **SOLUBILITY (5%)** **Refer to Summary Sheet:**
7.24.1. Weigh 5.0 g of sample into a clean glass beaker.
7.24.2. Add 100 mL of purified water and swirl to dissolve.
7.24.3. View sample from all angles under sufficient lighting. Solution should be clear and complete.
7.24.3.1. Note any insoluble matter present, if any, and refer to material TUPP and insoluble matter specification for disposition.

7.25. **SOLUBILITY (0.05M)** **Refer to Summary Sheet:**

7.25.1. Weigh 1.19 g of sample 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 angles under sufficient lighting. Solution should be clear and complete.

7.25.3.1. Note any insoluble matter present, if any, and refer to material TUPP and insoluble matter specification for disposition.

7.26. **TRACE METALS (As, Ca, Cd, Co, Cu, Cr, Fe, Pb, Mg, Mn, Ni, K, Zn)** **Refer to Summary Sheet:**

7.26.1. For quantitative metal requirements, refer to the method DCN: BSI-ATM-0054 as primary method of analysis for instrument parameters, standard calibration, and sample analysis.

7.26.2. For product codes only requiring As, Cu, Fe, Pb, refer to Analytical Method of Analysis: Trace Metals in Finished Goods Products by ICP-OES, DCN: BSI-ATM-0089 as secondary method of analysis.

7.27. **WATER (BY KARL FISCHER TITRATION)** **Refer to Summary Sheet.:**

7.27.1. Standardize Composite 5 as per Standardization of Titrants.

7.27.2. Grind the sample in a dry mortar into a fine powder utilizing a pestle.

7.27.3. Immediately weigh 2.0 g of sample into the glass weighing spoon and tare it.

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

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

7.27.5. 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 instrument.

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

7.27.6.1. If there is any sample stuck to the side, stop the stir bead from spinning before swirling the vessel to rinse the sides.

7.27.7. Once the method begins, check to ensure the sample is fully dissolved before the titration begins (i.e. before the stir command completes).

7.27.8. The moisture content will then be determined by the Metrohm Auto Titrando 907.

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