BIOSPECTRA
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MES SODIUM TESTING METHODS

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1. PURPOSE:

1.1. To provide the Laboratory Technicians with a procedure for the analysis of MES Sodium Raw Material (RM) for repackaging or Finished Goods (FG) in the Laboratory at the Stroudsburg, PA and Bangor, PA facilities.

2. SCOPE:

2.1. These procedures apply to the analysis of RM for repackaging and all FG testing in the Laboratory at the Stroudsburg, PA and Bangor, PA facilities. Methods include testing for all types of MES Sodium sold by BioSpectra; only the specific tests required for the requested type must be performed.

3. REFERENCES:

3.1. BSI-ATM-0104, Analytical Method: Determination of Elemental Impurities in MES Sodium and HEPES Sodium by ICP-MS
3.2. BSI-SOP-0019, Result Reporting
3.3. BSI-SOP-0090, Lambda 25 UV/Vis Operation and Calibration
3.4. BSI-SOP-0094, Muffle Furnace SOP and Calibration
3.5. BSI-SOP-0095, DNase (Endonuclease) Assay
3.6. BSI-SOP-0096, RNase (Ribonuclease) Assay
3.7. BSI-SOP-0098, Balance SOP
3.8. BSI-SOP-0126, Laboratory Notebooks
3.9. BSI-SOP-0138, DNase (Exonuclease) Assay
3.10. BSI-SOP-0139, Protease Assay
3.11. BSI-SOP-0140, Standardization of Titrants
3.12. BSI-SOP-0143, Metrohm Titrando 907 Auto-Titrator SOP
3.13. BSI-SOP-0244, VWR Gravity Convection Oven Operation and Calibration (Model Number 414005-106)
3.14. BSI-SOP-0254, Spectrum Two UATR SOP
3.15. BSI-SOP-0255, XL200 pH/mV/Conductivity Meter SOP
3.16. BSI-SOP-0303, NexION 350X ICP-MS SOP
3.17. BSI-SOP-0668, OPI-180 OD Handheld Colorimeter SOP

4. EQUIPMENT:

4.1. UV/VIS Spectrophotometer
4.2. Analytical Balance
4.3. Convection Oven
4.4. pH/mV/Conductivity Meter
4.5. PerkinElmer Spectrum Two UATR
4.6. Muffle Furnace
4.7. Metrohm Auto Titrator
4.8. OPI-180 OD Handheld Colorimeter
4.9. Perkin Elmer NexION 350X

5. ANALYTICAL PROCEDURE:

5.1. **ABSORBANCE (10.0%):**

5.1.1. Weigh 2.5 grams of sample.
5.1.2. Transfer accurately weighed sample to a graduated cylinder and Q.S. to 25 mL with purified water.
5.1.3. Swirl to dissolve completely.
5.1.4. Refer to Lambda 25 UV/VIS Spectrophotometer Operation and Calibration to determine the Absorbance of the sample at the specified wavelengths.

5.2. **APPEARANCE AND COLOR:**

5.2.1. Place 25-50 g of the sample in a clean, dry glass beaker.
5.2.2. In an area with sufficient lighting, view the sample from all sides.
5.2.3. The sample should be white in color and characteristic of crystals.
5.2.4. If the appearance of 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.

5.3. **ASSAY:**

5.3.1. Standardize 0.1 N Sulfuric Acid as per Standardization of Titrants.
5.3.2. Accurately weigh 0.9g ±0.0005g of sample. Transfer to a 100-mL beaker.
5.3.2.1. **Note: If the Product code requires Assay, dried basis: utilize the LOD for analysis**.
5.3.3. Add 50 mL of purified water to sample beaker and stir to dissolve.
5.3.4. Titrate with 0.1N Sulfuric Acid to a potentiometric endpoint using utilizing the Metrohm Titrando 907.
5.3.5. Calculate according to the following:

$$\% \text{ MES Na} = \frac{\text{mL x N of } H_2SO_4 \text{ x } 21.72}{\text{Sample Weight (g)}}$$

5.4. **COLOR (1M, ALKALINE):**

5.4.1. Accurately weigh 21.7g of sample and transfer to a clean, dry beaker.
5.4.2. Dissolve in 100mL of purified water.
5.4.3. Adjust the pH of the sample solution to 12 by adding (dropwise) 42% sodium hydroxide.
5.4.4. Observe the color of the solution.
5.4.5. The solution must remain clear and colorless when compared against a common background to a clear and colorless reference solution to report clear.

5.5. **CHLORIDE:**

5.5.1. Sample Preparation:
5.5.1.1. Accurately weigh 2.0 g of sample.
5.5.1.2. Transfer to a Nessler tube, dilute to approximately 40 mL with purified water and swirl to dissolve.
5.5.2. Standard Preparation:
5.5.2.1. Add 0.282 mL of 0.02 N HCl to the Nessler tube.
5.5.2.2. Dilute to approximately 40 mL with purified water and swirl to dissolve.
5.5.3. Procedure:
5.5.3.1. Add 1 mL of 0.1 N Silver Nitrate and 1 mL of concentrated nitric acid to each Nessler tube.
5.5.3.2. Dilute to 50 mL with purified water.
5.5.3.3. Cover each Nessler tube with parafilm and mix by inversion.
5.5.3.4. Allow solutions to stand for 5 minutes using a calibrated timer.
5.5.3.5. Turbidity in sample should not exceed the standard.

5.6. **ENZYME ACTIVITY:**

5.6.1. RNase, DNase, and Protease tested as per procedures referenced in Section 3.

5.7. **HEAVY METALS :**

5.7.1. Primary Method: Refer to NexION 350X SOP, BSI-SOP-0303 for instrument use and BSI-ATM-0104 for sample analysis.

5.7.2. Alternative Method:

5.7.3. Sample Preparation:

5.7.3.1. Transfer 20.0 g of sample to a 50-mL Nessler tube and add approximately 25 mL of purified water.

5.7.4. Standard Lead Solution:

5.7.4.1. On the day of use, dilute 10 mL of Lead Nitrate Stock Solution with to 100 mL with purified water in a volumetric flask.

5.7.5. Standard Preparation:

5.7.5.1. Into a 50 mL Nessler tube, pipette 200 µL of Standard Lead Solution and dilute with Purified Water to approximately 25 mL.

5.7.6. Procedure:

5.7.6.1. Adjust the Standard and Sample solutions 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, if necessary.

5.7.6.2. To each solution, 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).

5.7.6.3. Dilute to 50 mL with purified water and mix by inversion.

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

5.7.6.5. 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.

5.8. **IDENTITY (IR) :**

5.8.1. Prepare sample by following the LOD procedure.

5.8.2. Follow Spectrum Two UATR SOP to perform IR analysis.

5.9. **IDENTITY (Na) :**

5.9.1. Accurately weigh 0.1 g of the sample.

5.9.2. Dissolve in 2 mL of purified water.

5.9.3. Add 2 mL of 15% Potassium Carbonate and heat to boiling.

5.9.4. Allow to cool in ice bath and as necessary, rub the inside of the test tube with a glass rod to initiate precipitation.

5.9.5. No precipitate is formed.

5.9.6. Add 4 mL Potassium Pyroantimonate TS and heat to boiling.

5.9.7. Allow to cool in ice bath and as necessary, rub the inside of the test tube with a glass rod to initiate precipitation.

5.9.8. A dense precipitate must form in order to pass.

5.10. **LOSS ON DRYING :**

5.10.1. Tare an LOD vial that has been previously dried for 30 minutes under the same conditions to be employed in the determination.

5.10.2. Cool in desiccator for at least 15 minutes and weigh the dried LOD vial.

5.10.3. Transfer approximately 2 g of the sample to the LOD vial, and accurately weigh the LOD vial and contents. By gentle, sidewise shaking, distribute the sample as evenly as possible in the LOD vial.

5.10.4. Dry the sample at 130°C ± 2°C overnight to a constant weight.

5.10.4.1. Cool in a desiccator at least 15 minutes, weigh, and place back into the oven for 2 additional hours.

5.10.4.2. Cool in a desiccator for at least 15 minutes and reweigh.
5.10.4.3. Repeat until a constant weight is achieved.

$$\text{Calculation: \%LOD} = \frac{\text{Initial Sample Weight (g)} - \text{Final Sample Weight (g)}}{\text{Initial Sample Weight (g)}}$$

5.11. **pH (1%)** :

5.11.1. Accurately weigh 1.0 g of sample and transfer to a clean, dry beaker. Dissolve in 100mL of purified water. Mix by inversion until thoroughly dissolved.
5.11.2. Follow the appropriate SOP for calibration and pH measurement.

5.12. **PVS CONTENT** :

5.12.1. Solution Preparation:
5.12.1.1. Sample: Dissolve 1.0g of sample in approximately 80mL of purified water.
5.12.1.2. Adjust pH to 5.9 -6.1 with Hydrochloric Acid and dilute to 100mL with purified water.
5.12.1.3. Prepare a blank solution of MES Sodium by dissolving 5.43g of previously approved MES Sodium or MES sodium Reference standard in approximately 400mL of purified water. Adjust pH to 5.9-6.1 with hydrochloric acid. Dilute to 500mL.
5.12.1.4. 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.
5.12.1.5. Prepare a 1000ppm PVS Stock solution by diluting 1mL of 25% poly (vinylsulfonic acid, sodium salt) dissolved and dilute in water to 250mL.
5.12.1.6. Prepare a 50ppm PVS Standard solution by diluting 5ml of the 1000ppm PVS Stock Solution with the blank solution (50mM MES Sodium Salt Solution) to 100mL. Mix thoroughly.
5.12.1.7. Prepare a 10ppm PVS Standard Solution by diluting 10mL of the 50ppm PVS Stock Solution with the blank solution (50mM MES Sodium Salt Solution) to 50mL. Mix thoroughly.
5.12.1.8. Prepare a 1ppm PVS Standard Solution by diluting 5mL of the 10ppm PVS Stock Solution with the blank solution (50mM MES Sodium Salt Solution) to 50mL. Mix thoroughly.
5.12.2. Blank, Standard, and Sample Analysis:
5.12.2.1. 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.
5.12.2.2. Let test mixtures stand for at least 5 minutes.
5.12.2.3. Using the portable turbidimeter, analyze the blank, 1ppm PVS Standard, and samples within 30 minutes after IgG is added. (Before timer goes off.).
5.12.2.4. Measure each sample in triplicate and average the results.
5.12.2.5. The turbidity of the sample solution should not exceed the 1ppm PVS standard to report as <1ppm PVS.

5.13. **SOLUBILITY** :

5.13.1. Accurately weigh 10 g of sample and transfer to a clean, dry beaker.
5.13.2. Dissolve in 100 mL of purified water, which has been boiled and cooled to room temperature.
5.13.3. Upon complete dissolution, observe the color of the solution, which must remain clear and colorless when compared against a common background to a clear and colorless reference solution.

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

5.14.1. Accurately weigh 5 g of sample and transfer to a clean, dry beaker.

5.14.2. Dissolve in 100 mL of purified water, which has been boiled and cooled to room temperature.

5.14.3. Upon complete dissolution, observe the color of the solution, which must remain clear and colorless when compared against a common background to a clear and colorless reference solution.

5.15. **SULFATE :**

5.15.1. Sample Preparation:

5.15.1.1. Accurately weigh 4.0 g of sample.

5.15.1.2. Transfer accurately weighed sample to a Nessler Tube.

5.15.1.3. Add approximately 40 mL of purified water, swirl to dissolve.

5.15.2. Standard Preparation:

5.15.2.1. Dilute 0.102 mL of 0.04 N sulfuric acid to approximately 40 mL with purified water in a Nessler Tube.

5.15.3. Test:

5.15.3.1. Add 1 mL of 10% Hydrochloric Acid and 1 mL of Barium Chloride T.S.

5.15.3.2. Dilute to 50 mL with purified water.

5.15.3.3. Allow solutions to stand for 10 minutes using a calibrated timer.

5.15.3.4. The turbidity in sample should not exceed the standard.

5.16. **TRACE ELEMENTS :**

5.16.1. Refer to NexION 350X SOP, BSI-SOP-0303 for instrument use. Refer to BSI-ATM-0104 for sample preparation and analysis.

5.17. **WATER (KF) :**

5.17.1. Perform a standardization of the titrant (Composite 5) as per Standardization of Titrants.

5.17.2. Weigh approximately 0.3g of sample into a glass weighing spoon and tare the balance.

5.17.3. Transfer the sample to the Karl Fischer vessel by removing the rubber septum and adding the sample into the titration vessel.

5.17.4. Do not leave the rubber septum open for longer than 20 seconds as this will allow moisture to enter the titration vessel.

5.17.5. Return the weighing spoon to the balance, making sure not to lose any sample that was left behind. Once the weight stabilizes, record the weight in the Tiamo software.

5.17.6. MES Sodium may not fully dissolve in the 50/50 Methanol/Formamide mix. Ensure that all sample that was added to the KF vessel is suspended in the solution.

5.17.7. The moisture content will then be determined by the KF titration using the Metrohm Titrando 907.

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