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SODIUM HYDROXIDE 2N
TESTING METHODS

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1. PURPOSE:

1.1. To provide Laboratory personnel with a procedure for analyzing Sodium Hydroxide 2N In-Process, Stability, and Finished Good samples.

2. SCOPE:

2.1. Applies to the analysis of Sodium Hydroxide 2N In-Process, Stability, and Finished Goods in the Laboratory. Methods include testing for all grades of Sodium Hydroxide 2N sold by BioSpectra; only the specific tests required for the requested grade must be tested.

3. RESPONSIBILITIES:

3.1. The Laboratory Manager is responsible for training, maintenance and implementation of this procedure.
3.2. Laboratory Analysts are responsible for compliance with the terms of this procedure. This includes notifying the applicable Laboratory Management if any analyses fail to meet their respective specifications.

4. SAFETY:

4.1. Causes SEVERE skin burns and eye damage. Standard laboratory safety regulations apply. Before working with any chemical, read and understand the Safety Data Sheet (SDS).

5. REFERENCES:

5.1. BSI-FRM-0760, Sodium Hydroxide 2N Analytical Procedure
5.2. BSI-SOP-0019, Result Reporting
5.3. BSI-SOP-0098, Balance SOP
5.4. BSI-SOP-0126, Laboratory Notebooks
5.5. BSI-SOP-0135, Laboratory Chemicals
5.6. BSI-SOP-0140, Standardization of Titrants
5.7. BSI-SOP-0242, Portable Turbidimeter Operation and Calibration
5.8. BSI-SOP-0244, VWR Gravity Convection Over Operation and Calibration (Model Number 414005-106)
5.9. BSI-SOP-0303, NexION 350X ICP-MS SOP
5.10. BSI-SOP-0345, Endosafe nexgen-PTS Endotoxin Reader SOP
5.11. ACS Reagent Chemicals, current edition
5.12. USP-*NF* current edition

6. EQUIPMENT:

6.1. Analytical Balance
6.2. Hach Portable Turbidimeter Model 2100 Q, or equivalent
6.3. Endosafe PTS Endotoxin Reader, or equivalent
6.4. NexION 350X ICP-MS
6.5. Calibrated Oven
6.6. Calibrated Pipettes

7. REAGENTS:

7.1. **1N Acetic Acid:** Dilute 57 mL of glacial acetic acid to 1 L with purified water.
7.2. **10% Ammonium Hydroxide:** Dilute 35mL of 29% ammonium hydroxide to 100 mL with purified water.
7.3. **Ammonium Peroxydisulfate Crystals:** Purchased commercially.
7.4. **Ammonium Thiocyanate:** Purchased commercially.
7.5. **30% Ammonium Thiocyanate:** Dissolve 30 grams of ammonium thiocyanate in water, and dilute with water to 100 mL.
7.6. **Endosafe PTS Cartridge 1-0.01 EU/mL**: Purchased commercially.
7.7. **Glycerin**: Purchased commercially.
7.8. **Glycerin Base**: To 200 grams of glycerin, add water to a total weight of 235 grams. Add 140 mL of 1N NaOH and 50 mL of purified water. Mix thoroughly.
7.9. **Hydrochloric Acid (HCl) (concentrated):** Purchased commercially.
7.10. **Hydrochloric Acid (0.02 N HCl)**: Dilute 20 mL of HCl 0.1 N to 100 mL with purified water. Can be purchased commercially.
7.11. **LAL Reagent Water**: Purchased commercially.
7.12. **Methyl Orange**: Dissolve 0.10g of methyl orange in 100mL of purified water. Filter if necessary.
7.13. **Nitric Acid (HNO3) Concentrated (Reagent Grade)**: Purchased commercially.
7.14. **Phenolphthalein**: Purchased commercially.
7.15. **Phenolphthalein TS:** Dissolve 1.0g of phenolphthalein in 100mL of reagent grade alcohol.
7.16. **pH Paper / Litmus Paper**: Purchased commercially.
7.17. **Potassium Carbonate**: Purchased commercially.
7.18. **15% Potassium Carbonate**: Weigh 15.000 grams of potassium carbonate and transfer to a 100 mL volumetric flask. Dilute to volume with purified water.
7.19. **Potassium Hydrogen Phthalate (KHP)**: Purchased Commercially
7.20. **Potassium Hydrogen Phthalate (KHP)**: Prepare an appropriate sample container at 120 °C for 30 minutes. Allow to cool in desiccator. Crush and dry a suitable amount of potassium hydrogen phthalate. Dry at 120 °C for 2 hours. Cool and store in desiccator in a closed container. Stable for 3 months.
7.21. **Potassium Pyroantimonate TS:** Purchased commercially.
7.22. **Silver Nitrate, 0.1 N TS**: Purchased commercially.
7.23. **Sulfuric Acid (1N H2SO4)**: Purchased commercially
7.24. **Thioacetamide**: Purchased commercially.
7.25. **Thioacetamide TS**: Dissolve 4 grams of thioacetamide in purified water to make 100 mL.
7.26. **Tris Base Solution (0.25M):** Purchased commercially

8. ANALYTICAL PROCEDURES:

8.1. IN-PROCESS TESTING:

8.1.1. NORMALITY (CONFIRMATION 1 AND 2) REFER TO BATCH RECORD:

8.1.1.1. **Burette preparation:**

8.1.1.1.1. Allow the NaOH 2N sample to come to 25°C ± 2°C.

8.1.1.1.2. Prime the 50 mL burette by filling it with the NaOH 2N sample solution. Empty the burette and repeat.

8.1.1.1.3. Fill the burette to the required volume with the NaOH 2N sample solution.

8.1.1.2. **Sample preparation:**

8.1.1.2.1. Weigh 12.0 g of the previously dried KHP into a 250 mL beaker.

8.1.1.2.2. Add 100 mL of purified water down the sides of the beaker to avoid the loss of KHP.

8.1.1.3. **Analysis Procedure:**

8.1.1.3.1. To the KHP solution, add 150 µL of phenolphthalein indicator.

8.1.1.3.2. Titrate the KHP using the sample solution in the burette, to a pink endpoint.

8.1.1.3.3. Calculate the normality using the following equation:

$$N = \frac{(KHP\ Weight\ (g))(KHP\ Purity)}{(0.20423)(mL\ of\ NaOH\ sample\ solution)}$$

$$KHP\ Purity = Assay\ percent\ of\ KHP/100\ (from manufacturer's\ CoA)$$

$$0.20423 = Formula\ weight\ of\ KHP/1000$$

NOTE:

-If Confirmation 1 sample is in specification, perform Normality on the Confirmation 2 sample. Both must be in specification to release for packaging.

-If Confirmation 1 sample is out of specification, perform Assay % (w/w) and inform appropriate Laboratory Management of both results to make adjustment to the blend.

8.1.2. ASSAY REPORT:

8.1.2.1. NOTE: only required if Normality Confirmation sample is out of specification.

8.1.2.2. Perform a manual standardization or titrant check of 1N Sulfuric Acid per Standardization of Titrants.

8.1.2.3. Accurately weigh 10-15 g of sample and add 100 mL of purified water in a clean flask. Stopper the flask and allow to cool to room temperature.

8.1.2.4. Add 150 µL of phenolphthalein as the indicator and titrate using previously standardized 1N Sulfuric Acid to a colorless endpoint (V1).

8.1.2.5. Add 150 µL of Methyl Orange as the indicator.

8.1.2.6. Titrate using previously standardized 1N Sulfuric Acid to a pink endpoint (V2).

8.1.2.7. Calculate the percentage of Sodium Hydroxide using the following equation:

$$\%\ NaOH = \frac{(V_2)(N\ H_2SO_4)(4.00)}{Sample\ Weight\ (g)}$$

8.2. **FINISHED GOOD TESTING:**

8.2.1. **APPEARANCE AND COLOR** **REFER TO SUMMARY SHEET:**

8.2.1.1. Transfer 2 mL of sample into a 4 mL (10-mm) glass comparison tube.

8.2.1.2. Transfer 2 mL of purified water into a separate 4 mL (10-mm) glass comparison tube.

8.2.1.3. View the tubes vertically against a color comparison plate with suitable lighting. In order to pass, the test solution is complete, clear, and colorless when compared to purified water.

8.2.1.4. For Stability Testing: If the sample does not pass specification when compared to purified water, it can be compared to another sample determined to be passing (such as the Finished Goods lot retain) as a direct comparison to make the qualitative determination for Appearance and Color.

8.2.2. **CHLORIDE** **REFER TO SUMMARY SHEET:**

8.2.2.1. Thoroughly rinse Nessler tubes using purified water prior to use.

8.2.2.2. **Sample Preparation:**

8.2.2.2.1. Weigh 2.0 g of sample and quantitatively transfer to a 50 mL Nessler Color Comparison Tube using purified water.

8.2.2.2.2. Dilute to ~20 mL with purified water.

8.2.2.2.3. Slowly, using extreme caution, acidify the sample with Nitric Acid to litmus.

8.2.2.2.4. Dilute to 40 mL with purified water.

8.2.2.3. **5 ppm Standard Preparation:**

8.2.2.3.1. Dilute 14.1 µL of 0.02N HCl to ~40 mL with purified water.

8.2.2.4. **Analysis:**

8.2.2.4.1. To both the sample and standard solutions, add 1 mL of concentrated nitric acid and 1 mL of 0.1N Silver Nitrate TS. Dilute each tube to 50 mL with purified water.

8.2.2.4.2. Mix and allow solutions to sit for 5 minutes using a calibrated timer.

8.2.2.4.3. After 5 minutes, the turbidity in the sample solution does not exceed the turbidity produced by the standard when viewed against a dark background. Analyze turbidity utilizing the turbidity meter and record the sample NTU results.

8.2.3. **ENDOTOXINS** **REFER TO SUMMARY SHEET:**

8.2.3.1. Pipet 0.200 mL of sample into a sterile vial and add 1.600 mL of LAL reagent water.

8.2.3.2. Add concentrated Hydrochloric acid to acidify.

8.2.3.3. Check the pH of the solution with pH paper: solution must be acidic.

8.2.3.3.1. If basic, add HCl in increments until acidic.

8.2.3.3.1.1. Add approximately 1-2 µL of HCl.

8.2.3.4. Once acidic add sufficient buffer of a pH range ~9-10 until the solution is between pH 6-8.

8.2.3.4.1. Add approximately 0.3 mL of buffer.

8.2.3.5. Dilute with LAL reagent water to a final volume of 10 mL.

8.2.3.6. Follow the Endosafe Nexgen PTS Endotoxin Reader SOP, BSI-SOP-0345, for sample analysis.

8.2.3.6.1. The dilution factor is 50.

8.2.4. **HEAVY METALS (PB) REFER TO SUMMARY SHEET:**

8.2.4.1. Primary Method:

8.2.4.1.1. Standard and Solution Prep:

8.2.4.1.1.1. Lead Standard Solution (0.01 mg of Pb in 1 mL): Dilute 10 mL of lead stock solution to 100 mL with purified water. This must be prepared at time of use.

8.2.4.1.1.2. Thioacetamide-Glycerin Base: Thoroughly mix 1 mL of thioacetamide with 5 mL of glycerin base. Heat in a boiling bath for 20 seconds. Prepare immediately before use.

8.2.4.1.2. Procedure:

8.2.4.1.2.1. **Note:** Prepare in a hood and use caution for standard and sample prep to avoid spattering of sample.

8.2.4.1.2.2. Sample Preparation: Weigh 30 grams of sample into a suitable beaker and carefully neutralize with 1 mL of nitric acid.

8.2.4.1.2.3. Standard Preparation: Weigh 10 grams of sample into a suitable beaker and add 0.3 mL of concentrated nitric acid. Add 2 mL of 0.01 mg of Lead Standard Solution.

8.2.4.1.2.4. Place both the standard and sample on a hot plate and evaporate to dryness. Cool and dissolve each residue with 20mL of purified water. Adjust the pH to between 3 and 4 utilizing a pH meter, with 1N Acetic acid or 10% ammonium hydroxide.

8.2.4.1.2.5. Transfer the solutions to separate Nessler Color Comparison tubes. Add 1.2 mL of thioacetamide-glycerin base to each of the solutions and mix. QA each tube to 50 mL and mix.

8.2.4.1.3. Any brown color produced in the sample solution must not exceed that in the standard solution to be reported as ≤1 ppm.

8.2.4.2. Alternate Method: Refer to NexION 350X ICP-MS SOP, BSI-SOP-0303.

8.2.5. **IDENTIFICATION (SODIUM) REFER TO SUMMARY SHEET:**

8.2.5.1. Pipette 1 mL of sample into a test tube containing 25 mL of purified water.

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

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

8.2.5.4. No precipitate should be formed at this stage of analysis.

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

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

8.2.5.7. A dense precipitate must form in order to pass test.

8.2.6. **IRON** **REFER TO SUMMARY SHEET:**

8.2.6.1. Primary Method:

8.2.6.1.1. Procedure:

8.2.6.1.1.1. Sample Preparation: To 20 grams of sample, add 0.1mL of phenolphthalein indicator solution, neutralize with hydrochloric acid and dilute with water to 40 mL.

8.2.6.1.1.2. 0.01 mg Iron Standard Preparation: Pipette 1 mL of Iron Standard (0.01 mg of Fe in 1 mL) and dilute with water to 40 mL.

8.2.6.1.1.3. To the sample and standard solutions, add 2 mL of hydrochloric acid and dilute with purified water to 50 mL. To both solutions, add 30-50 mg of ammonium peroxydisulfate crystals and 2 mL of Ammonium Thiocyanate reagent solution and mix.

8.2.6.1.2. Any red color in the sample must not exceed the 0.01 mg standard solution to report as <0.5 ppm.

8.2.6.2. Alternate Method: Refer to NexION 350X ICP-MS SOP, BSI-SOP-0303.

8.2.7. **NORMALITY** **REFER TO SUMMARY SHEET:**

8.2.7.1. **Burette preparation:**

8.2.7.1.1. Allow the NaOH 2N sample to come to 25°C ± 2°C.

8.2.7.1.2. Prime the 50 mL burette by filling it with the NaOH 2N sample solution. Empty the burette and repeat.

8.2.7.1.3. Fill the burette to the required volume with the NaOH 2N sample solution.

8.2.7.2. **Sample preparation:**

8.2.7.2.1. Weigh 12.0 g of the previously dried KHP into a 250 mL beaker.

8.2.7.2.2. Add 100 mL of purified water down the sides of the beaker to avoid the loss of KHP.

8.2.7.3. **Analysis Procedure:**

8.2.7.3.1. To the KHP solution, add 150 µL of phenolphthalein indicator.

8.2.7.3.2. Titrate the KHP using the sample solution in the burette, to a pink endpoint.

8.2.7.3.3. Calculate the normality using the following equation:

$$N = \frac{(KHP\ Weight\ (g))(KHP\ Purity)}{(0.20423)(mL\ of\ NaOH\ sample\ solution)n}$$

KHP Purity = Assay Percent of KHP/100 (from manufacture's CoA)

0.20423 = Formula Weight of KHP/1000