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L-HISTIDINE MONOCHLORIDE MONOHYDRATE TESTING METHODS

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1. PURPOSE:

- 1.1. To provide Laboratory Personnel with procedures for testing L-Histidine Monochloride Monohydrate.

2. SCOPE:

- 2.1. Applies to the testing of L-Histidine Monochloride Monohydrate in the laboratory at all BioSpectra Facilities. Methods include testing for all types of L-Histidine Monochloride Monohydrate sold by BioSpectra; only the specific tests required for the desired type must be tested.

3. RESPONSIBILITIES:

- 3.1. The Laboratory Manager is responsible for the control, training, maintenance and implementation of this procedure.
- 3.2. The Laboratory Analysts are responsible for compliance with the terms of this procedure. This includes notifying the Quality Assurance / Laboratory Managers or designee if any analyses fail to meet their respective specifications.
- 3.3. Standard laboratory safety regulations apply. Before working with any chemical, read and understand the Safety Data Sheet (SDS).

4. REFERENCES:

- 4.1. BSI-SOP-0019, Result Reporting
- 4.2. BSI-SOP-0069, Preparation of Samples for Outside Testing
- 4.3. BSI-SOP-0098, Balance SOP
- 4.4. BSI-SOP-0126, Laboratory Notebooks
- 4.5. BSI-SOP-0134, Pipette SOP
- 4.6. BSI-SOP-0140, Standardization of Titrants
- 4.7. BSI-SOP-0143, Metrohm Titrando 907 Auto-Titrator SOP
- 4.8. BSI-SOP-0242, Bangor Portable Turbidimeter Operation and Calibration
- 4.9. BSI-SOP-0255, XL200 pH/mV/Conductivity Meter SOP
- 4.10. BSI-SOP-0490, MCP 5300 Polarimeter SOP
- 4.11. Current USP/ NF
- 4.12. EP
- 4.13. ICH Q1A
- 4.14. JP

5. EQUIPMENT:

- 5.1. Analytical Balance
- 5.2. Anton Paar MCP 5300 Polarimeter
- 5.3. Calibrated Pipettes
- 5.4. Hach Portable Turbidimeter
- 5.5. Metrohm Titrando 907 Auto-Titrator
- 5.6. pH Probe
- 5.7. XL200 pH/mV/Conductivity Meter, or equivalent

6. REAGENTS:

- 6.1. **Acetic acid:** Purchased commercially.
- 6.2. **Ammonia Water:** See Ammonia TS.
- 6.3. **Ammonia TS:** Purchased Commercially.
- 6.4. **0.1N Sodium Hydroxide:** Purchased Commercially.
- 6.5. **1M Sodium Hydroxide:** purchased commercially.

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- 6.6. **0.1N Silver Nitrate:** purchased commercially.
 - 6.7. **1-Propanol:** purchased commercially.
 - 6.8. **Acetic Acid, Dilute:** 12g of glacial acetic acid diluted to 100mL with water.
 - 6.9. **Barium Chloride TS:** Dissolve 12 grams of Barium Chloride Dihydrate in purified water. Filter and dilute to make a total volume of 100mL with purified water.
 - 6.10. **Butanol:** purchased commercially.
 - 6.11. **Dilute Nitric Acid:** Dilute 20 grams of Concentrated Nitric Acid to 100mL with purified water.
 - 6.12. **Dipotassium Sulfate:** purchased commercially.
 - 6.13. **EP Reference Solution BY₆:** Purchased Commercially.
 - 6.14. **Hydrochloric Acid, concentrated:** Purchased Commercially.
 - 6.15. **Hydrochloric Acid R1:** Dilute 70 grams of concentrated Hydrochloric Acid to 100mL with Purified Water and mix well.
 - 6.16. **Hydrochloric Acid, 6N:** Pipette 51.5mL of concentrated hydrochloric acid into a 100mL volumetric flask containing a small amount of purified water. Dilute to volume with purified water.
 - 6.17. **Hydrochloric Acid, dilute (10%):** Dilute 23.6mL of hydrochloric acid with purified water to make 100mL.
 - 6.18. **LAL Reagent Water:** purchased commercially.
 - 6.19. **L-Histidine Monochloride Monohydrate CRS:** purchased commercially.
 - 6.20. **Ninhydrin:** purchased commercially.
 - 6.21. **Ninhydrin Solution:** Dissolve 0.2g of ninhydrin in 5 mL of dilute acetic acid and 95 mL of butanol.
 - 6.22. **Nitric acid, dilute:** Pipette 10.5mL of concentrated nitric acid into a 100ml volumetric flask containing a small amount of purified water. Dilute to volume with purified water.
 - 6.23. **Potassium Hydrogen Phthalate (KHP):** Prepare an appropriate sample container at 120°C for 30 minutes. Allow to cool in a desiccator. Crush and dry a suitable amount of Potassium Hydrogen Phthalate. Dry at 120°C for 2 hours. Cool and store in a desiccator in a closed container. Stable for 3 months.
 - 6.24. **Purified Water:** In-House or Purchased Commercially.
 - 6.25. **Reagent Alcohol:** purchased commercially.
 - 6.26. **Reagent Alcohol (30% V/V):** Pipette 30 mL of reagent alcohol into a 100 mL volumetric flask. Dilute to volume with purified water and mix.
 - 6.27. **Sodium Hydroxide, 200g/L:** Weigh 20g of sodium hydroxide and transfer into a 100 mL volumetric flask. Dissolve and dilute to volume with purified water.
 - 6.28. **Sodium Nitrite Solution:** Weigh 1g of Sodium Nitrite, transfer to a 10mL volumetric flask and dilute to volume with purified water. Prepare immediately before use.
 - 6.29. **Sulfanilic Acid:** Purchased Commercially.
 - 6.30. **Sulfuric Acid, concentrated:** Purchased Commercially.
 - 6.31. **Sulfuric Acid, 0.005M:** Dilute 10mL of 0.05M (0.1N) Sulfuric Acid to 100mL in a volumetric flask.
 - 6.32. **250g/L Barium Chloride:** Weigh 25.0 grams of barium chloride and transfer to a 100mL volumetric flask. Dissolve and dilute to volume with purified water.
 - 6.33. **0.01-1.0 EU/mL Endotoxin Cartridges:** purchased commercially.
- 7. ANALYTICAL PROCEDURES:**
- 7.1. **AMMONIUM (EP)** _____ :
 - 7.1.1. Refer to Section 7.15, Ninhydrin-Positive Substances.
 - 7.2. **APPEARANCE AND COLOR** _____ :
 - 7.2.1. Place ~10 grams of sample into a clean, dry, glass beaker.
 - 7.2.2. In an area with sufficient lighting, viewed the sample from all sides.

- 7.2.3. The sample should be white or colorless in color and characteristic of crystals or a crystalline powder.
- 7.2.4. Any non-conformance will be reported to the Laboratory Manager or designee, immediately.

7.3. APPEARANCE OF SOLUTION :

- 7.3.1. Test Solution:
 - 7.3.1.1. Dissolve 2.5 grams of sample in Purified Water, dilute to 50mL with Purified Water, and mix thoroughly.
- 7.3.2. Clarity:
 - 7.3.2.1. Analyze the *Test Solution* for turbidity using a calibrated turbidimeter.
 - 7.3.2.2. Acceptance Criteria:
 - 7.3.2.2.1. The turbidity result may not exceed 3NTU to report as clear.
- 7.3.3. Color:
 - 7.3.3.1. In an area with sufficient lighting, compare the color of the *Test Solution* to *EP Color Reference Solution BY₆*.
 - 7.3.3.2. Acceptance Criteria:
 - 7.3.3.2.1. The color of the *Test Solution* may not be more intense than the color in *EP Color Reference Solution BY₆* to report as colorless.
- 7.3.4. To report as Pass Test, the solution must be both Clear and Colorless.

7.4. ASSAY (DRIED SUBSTANCE) (EP/JP) :

- 7.4.1. **Note:** Loss on Drying value is required for analysis.
- 7.4.2. Perform a daily check or standardization of 0.1N Sodium Hydroxide as per Standardization of Titrants.
- 7.4.3. Accurately weigh 0.5 grams of sample into a suitable beaker.
- 7.4.4. Dissolve in 50mL of Purified Water.
- 7.4.5. Titrate to a potentiometric endpoint with 0.1N Sodium Hydroxide.
- 7.4.6. Calculate the %L-Histidine Monochloride Monohydrate using the following equation in the Metrohm Tiamo software:

$$\%L\text{-Histidine Monochloride Monohydrate} = \frac{(\text{Sample Titrant Volume (mL)})(\text{Titrant Normality})(19.16)}{\text{Sample Weight (g)}} \times \frac{(100 - LOD (\%))}{100}$$

7.5. BIOBURDEN :

- 7.5.1. Package and send NLT 35 grams to Mary Paul Laboratories for TAMC/TYMC.

7.6. CLARITY AND COLOR OF SOLUTION (JP) :

- 7.6.1. Dissolve 1.0g of sample in 10mL of purified water. The solution must be clear and colorless.

7.7. ENDOTOXIN :

- 7.7.1. Accurately weigh 0.10 grams of sample into a sterile tube.
- 7.7.2. Dissolve in 5mL of LAL reagent water.
- 7.7.3. Add 0.4mL of 1M NaOH.
- 7.7.4. Dilute to 10mL with LAL reagent water for a final concentration of 0.010 g/mL.
- 7.7.5. Refer to Endosafe NexGen-PTS Endotoxin Reader SOP for instrument analysis.

7.8. IDENTIFICATION (IR): C (EP), 1 (JP) :

- 7.8.1. Follow the Spectrum Two UATR SOP for sample preparation and analysis.
- 7.8.2. Analyze sample as-is.

7.9. IDENTIFICATION 2, CHLORIDE (JP) :

- 7.9.1. Prepare a 1 in 10 sample solution by weighing 1 gram of sample and dissolving in 10mL of purified water. Mix thoroughly.

- 7.9.2. To the sample solution, add 0.2mL of 0.1N Silver Nitrate.
- 7.9.3. A white, curdy precipitate that is insoluble after the addition of 1mL of dilute Nitric Acid is produced.
- 7.9.4. If no precipitate is produced, notify Laboratory Management, or designee.
- 7.9.5. Add 4 mL of Ammonia TS. The precipitate should dissolve after minor agitation.

7.10. IDENTIFICATION E (EP) _____ :

- 7.10.1. Weigh 0.1g of sample, transfer to a suitable beaker and dissolve in 7mL of purified water.
- 7.10.2. Add 3mL of 200g/L sodium hydroxide.
- 7.10.3. In a separate beaker, weigh 50mg of Sulfanilic acid and dissolve in a mixture of 0.1mL Hydrochloric Acid and 10mL of purified water. Add 0.1mL of sodium nitrite solution.
- 7.10.4. Add the Sulfanilic acid solution to the sample solution and mix.
- 7.10.5. An orange-red color must develop in order to report as passes test

7.11. IDENTIFICATION F (EP) _____ :

- 7.11.1. Weigh 20mg of sample into a suitable vessel and dissolve in 2mL of purified water.
- 7.11.2. Acidify with dilute nitric acid.
- 7.11.3. Add 0.4mL of 0.1N Silver Nitrate.
 - 7.11.3.1. A curdled, white precipitate is formed.
- 7.11.4. Centrifuge and wash the precipitate with three, 1mL quantities of purified water.
 - 7.11.4.1. Carry out rapidly in subdued light, disregarding the fact that the supernatant may not become clear.
- 7.11.5. To the precipitate, add 2mL of purified water and 1.5mL of ammonia R.
 - 7.11.5.1. The precipitate dissolves easily with the possible exception of a few large particles which dissolve slowly in order to report as passes test.

7.12. HEAVY METALS _____ :

- 7.12.1. Refer to BSI-ATM-0076, Analytical Method Determination of ICH Q3D Elemental Impurities (Class 1, 2A, 2B, 3 & 4) By Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in L-Histidine Monochloride Monohydrate for sample preparation and analysis.

7.13. IRON _____ :

- 7.13.1. Refer to BSI-ATM-0076, Analytical Method Determination of ICH Q3D Elemental Impurities (Class 1, 2A, 2B, 3 & 4) By Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in L-Histidine Monochloride Monohydrate for sample preparation and analysis.

7.14. LOSS ON DRYING _____ :

- 7.14.1. Dry an LOD vial in an oven at 150°C for 30 minutes.
- 7.14.2. Cool for 15 minutes in a desiccator, weigh the LOD vial and record the weight.
 - 7.14.2.1. Note: If the substance to be tested is in the form of large crystals, reduce the particle size to about 2 mm by quickly crushing before weighing.
- 7.14.3. Transfer 1.000g of the sample to the LOD vial and accurately weigh the vial and contents. By gentle sideways shaking, distribute the sample as evenly as possible in the LOD vial to a depth of about 5mm.
- 7.14.4. Place the LOD vial containing the sample into the oven to dry at 150°C to a constant weight (NMT 0.5mg between weighings). Note: It is recommended to dry overnight to minimize the amount of times to reweigh the sample to ensure constant weight.
- 7.14.5. Remove the LOD vial from the oven and allow it to cool in the desiccator for 15 minutes.
- 7.14.6. Reweigh the LOD vial and sample and return to the oven for an additional 1 hour minimum.
- 7.14.7. Remove the LOD vial and sample from the oven and allow it to cool in the desiccator for 15 minutes.

- 7.14.8. Reweigh the LOD vial and sample and verify the weight has changed NMT 0.5mg between the last weighing.
- 7.14.9. Repeat steps 7.14.6-7.14.7 until a constant weight is achieved.
- 7.14.10. Retain the dried sample for dried analyses, if necessary.
- 7.14.11. Calculate the % LOD as follows:

$$\%LOD = \frac{\text{Initial Sample Weight (g)} - \text{Final Sample Weight (g)}}{\text{Initial Sample Weight (g)}} \times 100$$

7.15. NINHYDRIN-POSITIVE SUBSTANCES :

- 7.15.1. Package and send NLT 10g to New Jersey Labs for Ninhydrin-Positive substances.

7.16. OPTICAL ROTATION, SPECIFIC ROTATION @ 20°C; IDENTIFICATION A (EP) :

7.16.1. Sample Preparation:

- 7.16.1.1. Weigh and transfer 11 grams of sample to a 100mL volumetric flask, dissolve in 48mL of Hydrochloric Acid R1, fill to volume with purified water, and mix well.

7.16.2. Optical Zero Reference:

- 7.16.2.1. Pipette 48mL of Hydrochloric Acid R1 into a 100mL volumetric flask, fill to volume with purified water, and mix well.

7.16.3. The following information will be required to be entered into the software:

- 7.16.3.1. Volume (dryness) (mL), Mass (dryness) (g), Drying Loss (%)
 - 7.16.3.1.1. Utilize the sample's Loss on Drying result for drying loss.

7.16.4. Analysis: Perform at 20°C.

7.16.5. Refer to the MCP 5300 Polarimeter SOP for instrument analysis.

7.17. OPTICAL ROTATION, SPECIFIC ROTATION @ 20°C (JP) :

7.17.1. Sample Preparation:

- 7.17.1.1. Weigh and transfer 11 grams of sample to a 100mL volumetric flask and dissolve in 6N HCl. Dilute to volume with 6N HCl.

7.17.2. Optical Zero Reference:

- 7.17.2.1. 6N HCl

7.17.3. The following information will be required to be entered into the software:

- 7.17.3.1. Volume (dryness) (mL), Mass (dryness) (g), Drying Loss (%)
 - 7.17.3.1.1. Utilize the sample's Loss on Drying result for drying loss.

7.17.4. Analysis: Perform at 20°C

7.17.5. Refer to the MCP 5300 Polarimeter SOP for instrument analysis.

7.18. pH (5% SOLUTION); IDENTIFICATION B (EP) :

- 7.18.1. Weigh 2.5g of sample and transfer to a beaker,
- 7.18.2. Dissolve in 50mL of purified water and mix well.
- 7.18.3. Follow the appropriate SOP for pH calibration and measurement.

7.19. pH (10% SOLUTION) (JP) :

- 7.19.1. Weigh 10.0g of sample and transfer to a suitable beaker.
- 7.19.2. Dissolve in 100mL of purified water and mix well.
- 7.19.3. Follow the appropriate SOP for pH calibration and measurement.

7.20. RELATED SUBSTANCES; IDENTIFICATION D (EP) :

7.20.1. Solution Preparation:

- 7.20.1.1. Note: All solutions may be scaled as needed.
- 7.20.1.2. Developing Solvent System (20:20:60 Glacial Acetic Acid: Purified Water: Butanol): Pipette 20mL of purified water into a 100mL volumetric flask. Add 20mL of glacial acetic acid. QS to the final volume with butanol.

- 7.20.1.3. Spray Reagent: Ninhydrin Solution
- 7.20.1.4. Sample Solution: Accurately weigh 10mg of sample and transfer to a 50mL volumetric flask. Dissolve and dilute to volume with purified water. Mix well.
- 7.20.1.5. Standard Solution: Accurately weigh 10mg of L-Histidine Monochloride Monohydrate CRS and transfer to a 50mL volumetric flask. Dissolve and dilute to volume with purified water.

7.20.2. Chromatographic System:

Parameter	Setting
Mode	Thin-Layer Chromatography (TLC)
Adsorbent	0.25mm Layer of Chromatographic Silica Gel Mixture
Application Volume	5µL
Developing Solvent System	20:20:60 V/V/V Glacial Acetic Acid: Purified Water: Butanol
Spray Reagent	Ninhydrin R

7.20.3. Analysis:

- 7.20.3.1. Spot 5µL of *Sample Solution* and *Standard Solution* onto the TLC plate.
- 7.20.3.2. Place the plate in the chamber, ensuring the spots or bands are above the surface of the mobile phase.
- 7.20.3.3. Allow the mobile phase to ascent the plate until the solvent front has traveled two thirds of the length of the plate.
- 7.20.3.4. Remove the plate, mark the solvent front with a pencil.
- 7.20.3.5. Dry the plate at room temperature with air.
- 7.20.3.6. Spray with *Spray Reagent* and heat the plate at 105°C for 15 minutes.
- 7.20.3.7. Examine the plate under white light.
- 7.20.3.8. Acceptance Criteria: The principal spot for the test solution is similar in position, color and size to the principal spot for the standard solution.

7.21. RELATED SUBSTANCES (JP) _____ :

7.21.1. Solution Preparation:

- 7.21.1.1. Note: All solutions may be scaled as needed.
- 7.21.1.2. Developing Solvent System (67:33 1-Propanol: Ammonia Water): Add 67mL of 1-Propanol to 33mL of Ammonia Water.
- 7.21.1.3. Spray Reagent: Weigh 1 gram of ninhydrin and transfer to a 100mL volumetric flask. Dissolve and dilute to volume with a 97:3 mixture of methanol: acetic acid.
- 7.21.1.4. Sample Solution: Accurately weigh 0.50g of sample and transfer to a 50mL volumetric flask. Dissolve and dilute to volume with purified water. Mix well.
- 7.21.1.5. Standard Solution: Accurately weigh 0.10g of L-Histidine Monochloride Monohydrate CRS and transfer to a 10mL volumetric flask.
- 7.21.1.5.1. Pipette 1mL of the resulting solution into a 10mL volumetric flask and dilute to volume with purified water.
- 7.21.1.5.2. Pipette 1mL of the second dilution solution into a 50mL volumetric flask and dilute to volume with purified water.

7.21.2. Chromatographic System:

Parameter	Setting
Mode	Thin-Layer Chromatography (TLC)
Adsorbent	0.25mm Layer of Chromatographic Silica Gel Mixture
Application Volume	5 μ L
Developing Solvent System	67:33 1-Propanol: Ammonia Water
Spray Reagent	Ninhydrin in 97:3 Methanol: Acetic Acid (1 in 100)

7.21.3. Analysis:

- 7.21.3.1. Spot 5 μ L of *Sample Solution* and *Standard Solution* onto the TLC plate.
- 7.21.3.2. Place the plate in the chamber, ensuring the spots or bands are above the surface of the mobile phase.
- 7.21.3.3. Allow the mobile phase to ascend the plate until the solvent front has traveled approximately 10cm.
- 7.21.3.4. Remove the plate, mark the solvent front with a pencil.
- 7.21.3.5. Dry the plate at 80°C for 30 minutes.
- 7.21.3.6. Spray with *Spray Reagent* and heat the plate at 80°C for 10 minutes.
- 7.21.3.7. Examine the plate under white light.
- 7.21.3.8. Acceptance Criteria: Any secondary spot of the sample solution is not more intense than the primary spot of the standard solution.

7.22. **SULFATED ASH (EP), RESIDUE ON IGNITION (JP)** :

- 7.22.1. Turn on the muffle furnace and allow it to stabilize at 600°C. Follow the muffle furnace calibration procedure for operation of the furnace.
- 7.22.2. Inspect a quartz crucible for cracks, chips, and discoloration.
- 7.22.3. Utilize forceps to insert and remove the crucible from the furnace.
- 7.22.4. Ignite a quartz crucible at 600 \pm 50°C for 30 minutes. Cool in a desiccator for 1.5 hours and weigh using an analytical balance.
- 7.22.5. Weigh 1.0 grams of sample in the previously ignited quartz crucible. Moisten the sample with 1.0mL of concentrated Sulfuric Acid.
- 7.22.6. Volatilize the sample until the sample is thoroughly charred and white fumes are no longer evolved. Heat the sample slowly, so that the sample does not boil over and sample is not lost.
 - 7.22.6.1. The rate of heating should be such that from ½ to 1 hour is required to volatilize the sample.
 - 7.22.6.2. Continue to heat the sample until all excess sulfuric acid has been volatilized.
- 7.22.7. Ignite in the muffle furnace at 600 \pm 50°C for 15 minutes or until all carbon has been removed.
- 7.22.8. Cool in a desiccator for the same amount of time employed in the preparation of the crucible and weigh on an analytical balance.
- 7.22.9. Calculate the %ROI as follows:

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

- 7.22.10. If the amount of residue exceeds the limit specified, repeat the moistening with sulfuric acid using up to 1mL, heat to char, then ignite at 600 \pm 50°C for 30 minutes until two consecutive weighings of the residue do not differ by more than 0.0005g or until the specification is met.

7.23. **SULFATES (EP)** :

- 7.23.1. **Solution S:** Weigh 2.5g of sample and transfer to a 50mL volumetric flask. Dissolve and dilute to volume with purified water.

- 7.23.2. Test Solution: Dilute 10mL of Solution S to 15mL with purified water in a suitable beaker.
- 7.23.3. Sulfate Standard Solution (10ppm SO₄) R1: Immediately before use, weigh 0.181g of dipotassium sulfate into a 100mL volumetric flask and dilute to volume with reagent alcohol (30% V/V). Pipette 1mL of the resulting solution into a 100mL volumetric flask and dilute to volume with reagent alcohol (30% V/V).
- 7.23.4. Sulfate Standard Solution (10ppm SO₄): Immediately before use, weigh 0.181g of dipotassium sulfate into a 100mL volumetric flask and dilute to volume with purified water. Pipette 1mL of the resulting solution into a 100mL volumetric flask and dilute to volume with purified water.
- 7.23.5. Procedure: In a beaker, add 3mL of 250 g/L Barium Chloride to 4.5mL Sulfate Standard Solution (10ppm SO₄) R1. Shake and allow to stand for 1 minute. Pipette 2.5mL of the resulting solution into the 15mL of test solution and add 0.5mL of acetic acid. Prepare a standard in the same manner using 15mL of Sulfate Standard Solution (10ppm SO₄) previously prepared instead of the test solution. Allow solutions to sit for 5 minutes. Any opalescence in the test solution is not more intense than that of the standard in order to report as <300 ppm.

7.24. SULFATES (JP) _____ :

- 7.24.1. Standard Preparation:
 - 7.24.1.1. Pipette 0.35mL of 0.005M Sulfuric Acid into a 50mL Nessler Color Comparison Tube. Add ~40mL of purified water.
 - 7.24.1.2. Add 1 mL of dilute hydrochloric acid and dilute to 50mL with purified water.
- 7.24.2. Sample Preparation:
 - 7.24.2.1. Weigh 0.6 grams of sample and transfer to a 50mL Nessler Color Comparison Tube. Dissolve in ~40mL of purified water
 - 7.24.2.2. Add 1mL of dilute hydrochloric acid and dilute to 50mL with purified water.
- 7.24.3. Procedure:
 - 7.24.3.1. To both the standard and the sample, add 2mL of barium chloride TS.
 - 7.24.3.2. Mix well and allow to stand for 10 minutes utilizing a calibrated timer.
 - 7.24.3.3. Compare the turbidity produced in both solutions against a black background by viewing downward or transversely.
 - 7.24.3.4. Any turbidity produced in the sample solution should not exceed that produced by the standard in order to report as <0.028%.

7.25. WATER _____ :

- 7.25.1. Standardize Composite 5 as per Standardization of Titrants.
- 7.25.2. Grind the sample in a dry mortar into a fine powder utilizing a pestle.
- 7.25.3. Immediately weigh 0.3 g of sample into the glass weighing spoon and tare it.
- 7.25.4. Transfer the sample to the KF vessel by removing the rubber septum and adding the sample into the titration vessel.
 - 7.25.4.1. Do not leave the rubber septum open for long periods of time as this will allow moisture to enter the titration vessel.
- 7.25.5. 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 into the Tiamo software.
- 7.25.6. Check to make sure there is no residual sample stuck to the sides of the titration vessel.
 - 7.25.6.1. If there is any sample stuck to the side, stop the stir bead from spinning before swirling the vessel to rinse the sides.
- 7.25.7. Once the method begins, check to ensure the sample is fully dissolved before the titration begins (i.e. before the stir command completes).
- 7.25.8. The moisture content will then be determined by the Metrohm Titrando 907.

$$\%Moisture = \frac{(mL \text{ of Composite 5}) \left(\frac{mg}{mL} \text{ of Composite 5} \right) (0.1)}{Sample \text{ weight } (g)}$$