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TRIS HYDROCHLORIDE TESTING METHODS

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1. PURPOSE:

- 1.1. To provide Laboratory personnel with procedures for testing Tris Hydrochloride.

2. SCOPE:

- 2.1. This document applies to the testing of Tris Hydrochloride in the Laboratory at all BioSpectra, Inc Facilities. This test method includes testing for all product codes of TrisHydrochloride sold by BioSpectra.

3. RESPONSIBILITIES:

- 3.1. The Laboratory Manager is responsible for training, maintenance, and implementation of this procedure.
- 3.2. The Laboratory Technicians are responsible for compliance with the terms of this procedure. This includes notifying the Laboratory Manager if any analyses fail to meet their respective specifications.
- 3.3. Laboratory Technicians are responsible for referring to the applicable summary sheet or batch record for specifications.

4. REFERENCES:

- 4.1. BSI-ATM-0058, Analytical Method of Analysis: Determination of Trace Metal Impurities by ICP-MS in Tris and Tris Hydrochloride
- 4.2. BSI-ATM-0059, Analytical Method of Analysis: Determination if ICH Q3D Elemental Impurities by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in Tris API
- 4.3. BSI-ATM-0089, Analytical Method of Analysis: Trace Metals in Finished Goods Products by ICP-OES
- 4.4. BSI-SOP-0019, Result Reporting
- 4.5. BSI-SOP-0090, Lambda 25 UV/Vis Operation and Calibration
- 4.6. BSI-SOP-0094, Muffle Furnace SOP and Calibration
- 4.7. BSI-SOP-0095, DNase (Endonuclease) Assay
- 4.8. BSI-SOP-0096, RNase (Ribonuclease) Assay
- 4.9. BSI-SOP-0098, Balance SOP
- 4.10. BSI-SOP-0126, Laboratory Notebooks
- 4.11. BSI-SOP-0133, Blue M Convection Oven Operation and Calibration SOP
- 4.12. BSI-SOP-0138, DNase (Exonuclease) Assay
- 4.13. BSI-SOP-0139, Protease Assay
- 4.14. BSI-SOP-0140, Standardization of Titrants
- 4.15. BSI-SOP-0143, Metrohm Titrando 907 Auto-Titrator SOP
- 4.16. BSI-SOP-0144, Metrohm 914 pH Conductometer Operation and Calibration
- 4.17. BSI-SOP-0244, VWR Gravity Convection Oven Operation and Calibration (Model Number 414005-106)
- 4.18. BSI-SOP-0254, Spectrum Two UATR SOP
- 4.19. BSI-SOP-0255, XL200 pH/mV/Conductivity Meter SOP
- 4.20. BSI-SOP-0256, MP50 Melting Range Operation, Verification, and Calibration SOP
- 4.21. BSI-SOP-0303, NexION 350X ICP-MS SOP
- 4.22. BSI-SOP-0573, MP90 Melting Range Operation, Verification, and Calibration SOP

4.23. *Current EP Trometamol Monograph*

4.24. *Current USP Tromethamine Monograph*

5. EQUIPMENT:

- 5.1. Analytical Balance
- 5.2. Calibrated Oven
- 5.3. Lambda 25 Spectrophotometer, or equivalent
- 5.4. Metrohm 914 pH/ Conductometer
- 5.5. Metrohm Titrando 907 Auto-Titrator
- 5.6. MP50 or MP90 Melting Point Apparatus
- 5.7. Muffle Furnace
- 5.8. Perkin Elmer NexION 350X ICP-MS
- 5.9. Perkin Elmer Avio 500 ICP-OES
- 5.10. Spectrum Two UATR
- 5.11. XL200 pH/mV/Conductivity Meter

6. REAGENTS:

- 6.1. **6N Ammonium Hydroxide:** Dilute 206.9 mL of Ammonium Hydroxide 29% to 500 mL final volume with purified water.
- 6.2. **Composite 5:** Purchased commercially.
- 6.3. **Eosin Y Indicator:** Dissolve 50 mg of Eosin Y in 10mL of purified water.
- 6.4. **Formamide:** Purchased commercially
- 6.5. **Glacial Acetic Acid:** Purchased commercially
- 6.6. **Glycerin TS:** To 200 g of glycerol, add purified water to bring the total weight to 235 g. Add 140 mL of 1N sodium hydroxide (NaOH) and 50 mL of purified water.
- 6.7. **KHP:** Prepare a vial at 120 °C for 30 minutes. Allow to cool in desiccator and weigh a maximum of 10.0 g of an approved secondary lot of potassium hydrogen phthalate. Dry at 120 °C for 2 hours. Cool and store in desiccator in a closed container. Stable for 3 months.
- 6.8. **Lead Nitrate Stock Solution:** Dissolve 0.1598 g of lead nitrate in 100 mL purified water and add 1 mL of nitric acid (HNO₃). Dilute with purified water to 1000 mL. Store in a glass container, free from soluble lead salts.
- 6.9. **Methanol:** Purchased commercially
- 6.10. **Methanol, Dry:** Purchased commercially.
- 6.11. **Nitric Acid (HNO₃), Concentrated:** Purchased commercially
- 6.12. **pH 3.5 Acetate Buffer:** Dissolve 62.5 g of ammonium acetate in 62.5 mL of purified water and add 47.0 mL of concentrated HCl. Adjust, if necessary, with 6N ammonium hydroxide or 6N HCl to a pH of 3.5. Dilute with purified water to a volume of 250 mL.
- 6.13. **0.2% Polyvinyl Alcohol Solution:** Dissolve 2.0 g of polyvinyl alcohol in approximately 800 mL of purified water while gently heating and stirring. Once dissolved, remove the stir bar and Q.S. to 1000 mL with purified water.
- 6.14. **Purified Water:** Generated in-house or purchased commercially
- 6.15. **0.1N Silver Nitrate (AgNO₃):** Purchased commercially

- 6.16. **Sodium Chloride (NaCl):** Prepare a crucible at 450 °C for 30 minutes. Allow to cool in a desiccator and weigh a maximum of 10.0 g of an approved secondary lot of sodium chloride. Dry at 450 °C for 24 hours. Cool in a desiccator, transfer to a previously dried vial, and store in desiccator. Stable for 3 months.
- 6.17. **0.1N Sodium Hydroxide (NaOH):** Purchased commercially
- 6.18. **Sulfuric Acid (H₂SO₄):** Purchased commercially
- 6.19. **Thioacetamide:** Purchased commercially
- 6.20. **Thioacetamide-glycerin Base TS:** Add 0.2 mL of Thioacetamide TS to 1 mL of Glycerin TS, heat gently, and use immediately.
- 6.21. **Thioacetamide TS:** Dissolve 4.0 grams of thioacetamide in 100 mL of purified water.
- 6.22. **Tris Hydrochloride UATR Reference Standard:** Dry a purchased reference standard for 3 hours at 105°C. Compare to a previously approved reference standard. Correlation must achieve ≥ 0.95 to meet requirements.

7. ANALYTICAL PROCEDURE:

7.1. IN-PROCESS ML 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 specified Mother Liquor sample into a small beaker. Swirl to mix completely.
- 7.1.2. Refer to the Lambda 25 UV/Vis Operation and Calibration to determine the Absorbance of the sample.
- 7.1.3. Record the results at specified wavelengths in the Tris Hydrochloride In-Process Testing Log Book and in the batch record. If failing results are obtained, notify the Executive Director of Quality Control and Production immediately.

7.2. IN-PROCESS ML ASSAY REFER TO BATCH RECORD:

- 7.2.1. Assay by Auto-titrator:
 - 7.2.1.1. Standardize 0.1 N AgNO₃ as per Standardization of Titrants.
 - 7.2.1.2. Accurately weigh 0.5 g of as-is sample. Transfer to a 250-mL beaker and dissolve with 10 mL of purified water. Add 10 mL of glacial acetic acid, 100 mL of methanol, and 10 mL of a 0.2% polyvinyl alcohol solution. Titrate with 0.1N AgNO₃ to a potentiometric end-point utilizing the Metrohm Titrando 907.
- 7.2.2. Assay by Manual Titration:
 - 7.2.2.1. Standardize 0.1 N AgNO₃ as per Standardization of Titrants. Accurately weigh 0.5 g of sample. Transfer to a 250-mL beaker and dissolve with 10 mL of purified water. Add 10 mL of glacial acetic acid, 100 mL of methanol, and 0.5 mL of Eosin Y Indicator. Titrate to a pink endpoint.

$$\% \text{ Tris HCl} = \frac{(\text{mL of Titrant})(N \text{ of Silver Nitrate})(15.76)}{\text{Sample Weight (g)}}$$

7.3. ABSORBANCE (1 M) :

- 7.3.1. Prepare a 1 M solution of the specified sample.
 - 7.3.1.1. Accurately weigh 3.94 g of sample.
 - 7.3.1.2. Transfer accurately weighed sample to a graduated cylinder and Q.S. to 25 mL with purified water.
 - 7.3.1.3. Swirl to dissolve completely.
- 7.3.2. Refer to Lambda 25 UV/Vis Operation and Calibration to determine the Absorbance of the sample.

7.4. ABSORBANCE (0.1 M) :

- 7.4.1. Prepare a 0.1 M solution of the specified sample.
 - 7.4.1.1. Accurately weigh 0.394 g of sample.
 - 7.4.1.2. Transfer accurately weighed sample to a graduated cylinder and Q.S. to 25 mL with purified water.
 - 7.4.1.3. Swirl to dissolve completely.
- 7.4.2. Refer to Lambda 25 UV/Vis Operation and Calibration to determine the Absorbance of the sample.

7.5. APPEARANCE AND COLOR :

- 7.5.1. Perform the test by placing approximately 10 g of sample on a piece of white filter paper.
- 7.5.2. Observe the sample for appearance. Test passes if the sample is colorless crystals to a white crystalline powder and is free from visual extraneous matter such as fibers or off-color specks.

7.6. ASSAY (AS-IS) :

- 7.6.1. Standardize 0.1N AgNO₃ as per Standardization of Titrants.
- 7.6.2. Accurately weigh 0.5 g of sample.
- 7.6.3. Transfer to a beaker and dissolve with 10 mL of purified water.
- 7.6.4. Add 10 mL of glacial acetic acid, 100 mL of methanol, and 10 mL of a 0.2% polyvinyl alcohol solution.
- 7.6.5. Titrate with 0.1 N AgNO₃ to a potentiometric end-point utilizing the Metrohm Titrand 907.

$$\%C_4H_{11}NO_3 \text{ HCl} = \frac{(mL \times N \text{ of } AgNO_3)(15.76)}{Sample \text{ Weight (g)}}$$

- 7.6.6. Alternate Manual Titration Method:
 - 7.6.6.1. Standardize 0.1N AgNO₃ as per Standardization of Titrants.
 - 7.6.6.2. Accurately weigh 0.5 g of sample.
 - 7.6.6.3. Transfer to a 250-mL beaker and dissolve with 10 mL of purified water.
 - 7.6.6.4. Add 10 mL of glacial acetic acid, 100 mL of methanol, and 0.5 mL of Eosin Y Indicator.
 - 7.6.6.5. Titrate to a pink endpoint.

$$\%C_4H_{11}NO_3 \text{ HCl} = \frac{(mL \times N \text{ of } AgNO_3)(15.76)}{Sample \text{ Weight (g)}}$$

7.7. ASSAY (DRIED BASIS) :

- 7.7.1. Standardize 0.1 N AgNO₃ as per Standardization of Titrants.
- 7.7.2. Accurately weigh 0.5 g of sample that has been previously dried following LOD analysis.
- 7.7.3. Transfer to a 250-mL beaker and dissolve with 10 mL of purified water.
- 7.7.4. Add 10 mL of glacial acetic acid, 100 mL of methanol, and 10 mL of a 0.2% polyvinyl alcohol solution.
- 7.7.5. Titrate with 0.1 N AgNO₃ to a potentiometric end-point utilizing the Metrohm Titrand 907.

$$\%C_4H_{11}NO_3 \text{ HCl} = \frac{(mL \times N \text{ of } AgNO_3)(15.76)}{Sample \text{ Weight (g)}}$$

- 7.7.6. Alternate Manual Titration Method:
 - 7.7.6.1. Standardize 0.1 N AgNO₃ as per Standardization of Titrants.
 - 7.7.6.2. Accurately weigh 0.5 g of sample that has been previously dried following LOD analysis.

- 7.7.6.3. Transfer to a 250-mL beaker and dissolve with 10 mL of purified water.
- 7.7.6.4. Add 10 mL of glacial acetic acid, 100 mL of methanol, and 0.5 mL of Eosin Y Indicator.
- 7.7.6.5. Titrate to a pink endpoint.

$$\%C_4H_{11}NO_3 \text{ HCl} = \frac{(mL \times N \text{ of } AgNO_3)(15.76)}{\text{Sample Weight (g)}}$$

7.8. ENDOTOXIN :

- 7.8.1. Endotoxin analysis is to be performed by an outside laboratory.
- 7.8.1.1. Package and send NLT 20 g of sample to the approved outside testing facility.

7.9. ENZYME ACTIVITY :

- 7.9.1. Follow the RNase, DNase, and Protease procedures referenced in Section 4.

7.10. HEAVY METALS :

- 7.10.1. Refer to section 7.30: Trace Elements
Alternative Method:
- 7.10.2. 0.001% test sample preparation:
 - 7.10.2.1. Into a 50-mL Nessler color comparison tube, dissolve 2.0 g of sample in ~40 mL of purified water.
- 7.10.3. 5 ppm test sample preparation:
 - 7.10.3.1. Into a 50-mL Nessler color comparison tube, dissolve 4.0 g of sample in ~40 mL of purified water.
- 7.10.4. 2 ppm test sample preparation:
 - 7.10.4.1. Into a 50-mL Nessler color comparison tube, dissolve 10.0 g of sample in ~40 mL of purified water.
- 7.10.5. Standard Lead Solution:
 - 7.10.5.1. On the day of use, dilute 10.0 mL of Lead Nitrate Stock Solution to 100 mL with purified water in a volumetric flask.
- 7.10.6. Standard Preparation:
 - 7.10.6.1. Into a 50-mL Nessler color-comparison tube, pipette 2 mL of Standard Lead Solution, and add ~ 40 mL of purified water.
- 7.10.7. Monitor Preparation:
 - 7.10.7.1. Place 40 mL of a solution as directed for the required Test Preparation and add 2.0 mL of Standard Lead Solution.
- 7.10.8. Procedure:
 - 7.10.8.1. To each solution, add 2 mL of pH 3.5 Acetate Buffer and 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). Dilute to 50 mL with purified water, parafilm, and mix by inversion.
 - 7.10.8.2. Allow to stand for 2 minutes using a calibrated timer.
 - 7.10.8.3. View downward over a white surface; the color of the solution from the Test Preparation is not darker than that of the solution from the Standard Preparation, and the color of the solution of the Monitor Preparation is equal to or darker than that of the Standard Preparation.

7.11. IDENTIFICATION (IR) :

- 7.11.1. For UATR analysis, follow Spectrum Two UATR SOP.

7.12. IDENTIFICATION (CHLORIDE) :

- 7.12.1. Accurately weigh 7.88 g of sample and transfer to a 100-mL volumetric flask.
- 7.12.2. Q.S. to volume with purified water.
- 7.12.3. The solution from pH 0.5M analysis may be used.

- 7.12.4. Transfer 2 mL of the sample solution to a beaker and add ~0.2 mL of 0.1N Silver Nitrate. A white, curdy precipitate that is insoluble after the addition of 1 mL of concentrated nitric acid is produced. If no precipitate is produced, notify the appropriate personnel.
- 7.12.5. Add 4 mL of 6N Ammonium Hydroxide. The precipitate should dissolve after mild agitation.

7.13. INSOLUBLE MATTER :

- 7.13.1. Weigh 120 g of sample and transfer to a beaker.
- 7.13.2. Add 1200 mL of water and utilize a Teflon encapsulated magnetic stirring bar and electric stir plate to dissolve sample.
- 7.13.3. Heat to boiling and digest on a hot plate in a covered beaker for 1 hour.
- 7.13.4. Prepare a crucible and 10-15 µm filter by drying at 105°C ± 2 °C for 1 hour. Record weight of the crucible and filter after cooling in ambient air for at least 15 minutes.
- 7.13.5. Filter sample solution through the previously prepared crucible, and rinse thoroughly with at least 300 mL of hot purified water.
- 7.13.6. Dry the crucible in the oven at 105°C ± 2 °C for 1 hour.
- 7.13.7. Cool in ambient air for at least 15 minutes and reweigh.
- 7.13.8. Calculate the % Insoluble Matter as follows:

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

7.14. LOSS ON DRYING @ 105 °C :

- 7.14.1. Tare an LOD vial that has been previously dried for 30 minutes in the oven at 105 °C ± 2 °C.
- 7.14.2. Allow to come to room temperature in a desiccator for at least 15 minutes before weighing.
- 7.14.3. Transfer 2-3 g of the sample to be tested to the LOD vial, and accurately weigh the vial and contents. By gentle, sidewise shaking, distribute the sample as evenly as possible in the weighing bottle.
- 7.14.4. Place the LOD vial containing the sample into the oven.
- 7.14.5. Dry the sample at 105 °C ± 2 °C for 3 hours.
- 7.14.6. Allow to come to room temperature in a desiccator for at least 15 minutes before weighing.
- 7.14.7. Calculate result using the equation below:

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

7.15. LOSS ON DRYING @ 110 °C :

- 7.15.1. Tare an LOD vial that has been previously dried for 30 minutes in the oven at 110 °C ± 2 °C.
- 7.15.2. Allow to come to room temperature in a desiccator for at least 15 minutes before weighing.
- 7.15.3. Transfer 2-3 g of the sample to be tested to the LOD vial, and accurately weigh the vial and contents. By gentle, sidewise shaking, distribute the sample as evenly as possible in the weighing bottle.
- 7.15.4. Place the LOD vial containing the sample into the oven.
- 7.15.5. Dry the sample at 110 °C ± 2 °C for 3 hours.
- 7.15.6. Allow to come to room temperature in a desiccator for at least 15 minutes before weighing.

7.15.7. Calculate result using the equation below:

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

7.16. MELTING RANGE :

7.16.1. Refer to BSI-SOP-0256, MP50 Melting Range Operation and Calibration SOP, or BSI-SOP-0573, MP90 Melting Range Operation, Verification, and Calibration SOP.

7.17. MICROBIAL ANALYSIS :

7.17.1. Microbial Analysis will be performed by an approved outside testing facility.

7.17.2. Package and send 35 g of sample to the approved outside testing facility.

7.17.3. **FG MPL Suitability Number for ARF: 12H5792A**

7.17.4. **ML MPL Suitability Number for ARF: 12H5797A**

7.18. OPTICAL DENSITY AT 290NM :

7.18.1. Accurately weigh 20.0 g of sample and dissolve in 50 mL of purified water.

7.18.1.1. If needed to dissolve, the sample may be gently heated. Allow the solution to return to room temperature.

7.18.2. Measure the absorbance of the sample at 290 nm using the Lambda 25 UV/Vis.

7.19. PH (1:10) @ 25 °C ±2 °C :

7.19.1. Accurately weigh 10 g of sample. Transfer to a suitable beaker.

7.19.2. Dissolve in 100 mL of purified water. Cover with parafilm and mix until thoroughly dissolved.

7.19.2.1. Measure and record the pH using the appropriate SOP.

7.20. PH (0.5 M) @ 25 °C ±2 °C :

7.20.1. Accurately weigh 7.88 g of sample and transfer to a 100-mL volumetric flask.

7.20.2. Q.S. to volume with purified water. Mix until thoroughly dissolved.

7.20.2.1. Measure and record the pH using the appropriate SOP.

7.20.3. This solution can be utilized for the Identification (Chloride) test.

7.21. pH (1.0 M) @ 25 °C ±2 °C :

7.21.1. Accurately weigh 15.76 g of sample and transfer to a 100-mL volumetric flask

7.21.2. Q.S. to volume with purified water. Mix until thoroughly dissolved.

7.21.2.1. Measure and record the pH using the appropriate SOP.

7.21.3. This solution can be utilized for Solubility (1M Solution).

7.22. pH (1%) @ 25 °C ±2 °C :

7.22.1. Accurately weigh 1.0 g of sample. Transfer to a suitable beaker.

7.22.2. Dissolve in 100 mL of purified water. Cover with parafilm and mix until thoroughly dissolved.

7.22.2.1. Measure and record the pH using the appropriate SOP.

7.23. pH (5%) @ 25 °C ±2 °C :

7.23.1. Accurately weigh 5.0 g of sample. Transfer to a suitable beaker.

7.23.2. Dissolve in 100 mL of purified water. Cover with parafilm and mix until thoroughly dissolved.

7.23.2.1. Measure and record the pH using the appropriate SOP.

7.24. pH (10%) @ 25 °C ±2 °C :

7.24.1. Accurately weigh 10.0 g of sample. Transfer to a suitable beaker.

7.24.2. Dissolve in 100 mL of purified water. Cover with parafilm and mix until thoroughly dissolved.

7.24.2.1. Measure and record the pH using the appropriate SOP.

7.25. pKa :

- 7.25.1. Weigh 0.5 g of sample and add 50-100 mL of purified water.
- 7.25.2. Titrate to a potentiometric end-point utilizing the Metrohm Titrando 907 using 0.1 N NaOH.

7.26. RESIDUAL ETHANOL, IPA, and METHANOL :

- 7.26.1. Residual solvent analysis will be performed by an outside laboratory on validation batches and on one batch yearly. Prepare and send a 10 g sample.

7.27. RESIDUE ON IGNITION :

- 7.27.1. Turn on the muffle furnace and allow it to stabilize at 600 °C.
- 7.27.2. Inspect a quartz crucible for cracks, chips and discoloration. Use the long 10-inch forceps to place the crucible in the furnace and to remove the crucible from the furnace. Do not touch any surface of the furnace or you will get burned!
- 7.27.3. Ignite quartz crucible at 600 ± 50 °C for 30 minutes, cool in a desiccator for 1.5 hours and weigh.
- 7.27.4. Weigh 1.0 g sample in the previously ignited quartz crucible. Moisten the sample with 0.2 mL of sulfuric acid. Volatilize the sample with an appropriate heating apparatus. Keep the sample an appropriate distance and location from the heat source, so that the sample does not boil over and no sample is lost. The rate of heating should be such that from ½ to 1 hour is required to volatilize the sample.
- 7.27.5. Continue to heat the sample until all the excess sulfuric acid has been volatilized. Ignite in a muffle furnace at 600 ± 50 °C for 15 minutes or until all carbon has been removed.
- 7.27.6. Cool in a desiccator for 1.5 hours and reweigh.
- 7.27.7. The weight of residue should not exceed 0.001 g (0.1 %)

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

7.28. SOLUBILITY (1 M SOLUTION) :

- 7.28.1. Dissolve 15.76 g of sample in 100 mL of purified water.
 - 6.26.1.1. The solution made for pH (1.0 M) may be utilized for this analysis.
- 7.28.2. Observe under sufficient light.
- 7.28.3. The solution should be clear and colorless.

7.29. SOLUBILITY (35% SOLUTION) :

- 7.29.1. Weigh 35.0 g of sample and transfer to a graduated cylinder.
- 7.29.2. Q.S. to 100 mL with purified water. Cover with parafilm and mix until thoroughly dissolved.
- 7.29.3. Transfer solution into a clean beaker and observe under sufficient light.
- 7.29.4. Solution should be clear and colorless.

7.30. TRACE ELEMENTS :

- 7.30.1. Primary Method of Analysis: Refer to BSI-ATM-0058 or BSI-ATM-0059 for quantitative method analysis.
- 7.30.2. Alternate Method of Analysis: Refer to BSI-ATM-0089 for sample preparation and analysis.

7.31. WATER (BY KARL FISCHER TITRATION) :

- 7.31.1. Standardize Composite 5 as per BSI-SOP-0140, Standardization of Titrants.
- 7.31.2. Grind the sample in a dry mortar into a fine powder utilizing a pestle.
- 7.31.3. Immediately weigh 0.8 g of sample into the glass weighing spoon and tare it.
- 7.31.4. Transfer the sample to the KF vessel by removing the rubber septum and adding the sample into the titration vessel.

- 7.31.4.1. Do not leave the rubber septum open for long periods of time as this will allow moisture to enter the titration vessel.
- 7.31.5. 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.
- 7.31.6. Check to make sure there is no residual sample stuck to the sides of the titration vessel.
 - 7.31.6.1. If there is any sample stuck to the side, stop the stir bead from spinning before swirling the vessel to rinse the sides.
- 7.31.7. Once the method begins, check to ensure the sample is fully dissolved before the titration begins (i.e. before the stir command completes).
- 7.31.8. The moisture content will then be determined by the Metrohm Auto Titrando 907.

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