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BIS-TRIS TESTING METHODS

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1. PURPOSE:

- 1.1. To provide Laboratory Personnel with a procedure for examining Bis-Tris.

2. SCOPE:

- 2.1. Applies to examination of Bis-Tris Raw Materials, In Process, Stability, and Finished Goods in the Laboratory. Methods include testing for all types of Bis Tris sold by BioSpectra; only the specific tests required for the desired type must be tested. This document applies to all BioSpectra facilities.

3. RESPONSIBILITIES:

- 3.1. The Laboratory Manager is responsible for the control, training, maintenance, and implementation of this procedure.
- 3.2. The Laboratory Personnel are responsible for compliance with the terms of this procedure. This includes notifying the Quality Assurance and Laboratory Managers, or designees, if any analyses fail to meet their respective specifications.
- 3.3. Laboratory personnel are responsible for referring to applicable summary sheet for all specifications.

4. EQUIPMENT:

- 4.1. Analytical Balance
- 4.2. Anton Paar DMA 35 Portable Density Meter
- 4.3. Calibrated Oven
- 4.4. Calibrated Pipettes
- 4.5. Calibrated Timer
- 4.6. Endosafe NexGen-PTS Endotoxin Reader
- 4.7. Hach Portable Turbidimeter
- 4.8. Lambda 25 UV/Vis Spectrophotometer
- 4.9. Metrohm 907 Titando Auto-Titrator
- 4.10. MP50 Melting Point Apparatus
- 4.11. MP90 Melting Point Apparatus
- 4.12. Muffle Furnace
- 4.13. OPI-180 OD Handheld Colorimeter
- 4.14. Perkin Elmer NexION 350X ICP MS
- 4.15. Perkin Elmer Spectrum Two UATR
- 4.16. XL200 pH/Conductivity Meter, or equivalent

5. REAGENTS:

- 5.1. **0.02N HCl:** Slowly add 20 mL of 0.1N hydrochloric acid to 80 mL of purified water to make a total volume of 100 mL or purchased commercially.
- 5.2. **0.1N HCl:** Purchased Commercially.
- 5.3. **0.1N Sulfuric Acid:** Purchased Commercially.
- 5.4. **0.1N Silver Nitrate:** Purchased Commercially.
- 5.5. **3N HCl:** Pipette 25.75mL of concentrated hydrochloric acid and transfer to a 100mL volumetric flask that contains a small amount of purified water. Dilute to volume with purified water.
- 5.6. **1 – 0.01 EU/mL LAL Test Cartridges:** Purchased Commercially.
- 5.7. **Alkaline Potassium Tetraiodomercurate Solution R:** Dissolve 11g of potassium iodide and 15g of mercury (II) iodide in purified water and dilute to 100mL.
- 5.8. **Barium Chloride TS:** Dissolve 30g of barium chloride dihydrate in water to make 250mL.
- 5.9. **Boric Acid:** Purchased Commercially.

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- 5.10. **Boric Acid Solution (1 in 25):** Weigh 4 g of boric acid. Transfer to a 100 mL volumetric flask. Dissolve and dilute to volume with purified water.
- 5.11. **Composite 5:** Purchased Commercially.
- 5.12. **Formamide Dry:** Purchased Commercially.
- 5.13. **LAL Reagent Water:** Purchased Commercially.
- 5.14. **Methanol Dry:** Purchased Commercially.
- 5.15. **Methyl Red:** Purchased Commercially.
- 5.16. **Methyl Red TS:** Weigh 0.1 g of methyl red into a 100 mL volumetric flask. Dissolve and dilute to volume with reagent grade alcohol (ethanol (95)).
- 5.17. **Methylene Blue:** Purchased Commercially.
- 5.18. **Methylene Blue TS:** Weigh 125 mg of methylene blue into a 250 mL volumetric flask. Dissolve in 100 mL of reagent grade alcohol (ethanol (95)) and dilute to 250 mL with reagent grade alcohol (ethanol (95)).
- 5.19. **Methyl Red – Methylene Blue TS:** Add 10 mL of methyl red TS to 10 mL of methylene blue TS and mix well.
- 5.20. **Nitric Acid, concentrated:** Purchased Commercially.
- 5.21. **Purified Water:** In-House or Purchased Commercially.
- 5.22. **Reagent Grade Alcohol (Ethanol (95)):** Purchased Commercially.
- 5.23. **Sodium Hydroxide:** Purchased Commercially.
- 5.24. **Sodium Hydroxide Solution (2 in 5):** Weigh 200 g of sodium hydroxide into a 500 mL volumetric flask. Dissolve and dilute to volume with purified water.
- 5.25. **Sodium Hydroxide Solution (25% w/v):** Weigh 25g of sodium hydroxide and dilute to 100mL with purified water.
- 5.26. **Sulfuric Acid, concentrated:** Purchased Commercially.
- 5.27. **0.02N Sulfuric Acid:** Slowly add 20mL of 0.1N Sulfuric Acid to 80mL of purified water to make a total volume of 100mL.

6. REFERENCES:

- 6.1. BSI-ATM-0075, Analytical Method for the Determination of ICH Q3D Elemental Impurities (Class 1, 2A, 2B, 3 & 4) by Inductively Coupled Mass Spectrometry (ICP-MS) in Bis-Tris and Bis-Tris Hydrochloride
- 6.2. BSI-ATM-0131, Analytical Method for the Determination of Trace Metals in BioTech Products
- 6.3. BSI-RPT-1083, Analytical Method Verification Report: Bis-Tris Water Determination via Karl Fischer Utilizing Metrohm 907 Auto Titrator
- 6.4. BSI-RPT-1086, Analytical Method Validation Report: Bis-Tris Assay by Potentiometric Titration
- 6.5. BSI-SOP-0019, Result Reporting
- 6.6. BSI-SOP-0069, Preparation of Samples for Outside Testing
- 6.7. BSI-SOP-0090, Lambda 25 UV/Vis Operation and Calibration
- 6.8. BSI-SOP-0091, Portable Turbidimeter SOP and Calibration
- 6.9. BSI-SOP-0094, Muffle Furnace SOP and Calibration
- 6.10. BSI-SOP-0095, DNase (Endonuclease) Assay
- 6.11. BSI-SOP-0096, RNase (Ribonuclease) Assay
- 6.12. BSI-SOP-0098, Balance SOP
- 6.13. BSI-SOP-0126, Laboratory Notebooks
- 6.14. BSI-SOP-0133, Blue M Convection Oven Operation and Calibration SOP
- 6.15. BSI-SOP-0134, Pipette SOP
- 6.16. BSI-SOP-0135, Laboratory Chemicals
- 6.17. BSI-SOP-0138, DNase (Exonuclease) Assay
- 6.18. BSI-SOP-0139, Protease Assay

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- 6.19. BSI-SOP-0140, Standardization of Titrants
- 6.20. BSI-SOP-0143, Metrohm Titrando 907 Auto-Titrator SOP
- 6.21. BSI-SOP-0144, Metrohm 914 pH Conductometer Operation and Calibration
- 6.22. BSI-SOP-0242, Bangor Portable Turbidimeter Operation and Calibration
- 6.23. BSI-SOP-0244, VWR Gravity Convection Operation and Calibration (Model Number 414005-106)
- 6.24. BSI-SOP-0254, Spectrum Two UATR SOP
- 6.25. BSI-SOP-0255, XL200 pH/mV/Conductivity Meter SOP
- 6.26. BSI-SOP-0256, MP50 Melting Range Operation, Verification and Calibration SOP
- 6.27. BSI-SOP-0303, NexION 350X ICP-MS SOP
- 6.28. BSI-SOP-0345, Endosafe nexGen-PTS Endotoxin Reader SOP
- 6.29. BSI-SOP-0350, Anton Paar DMA 35 Portable Density Meter Operation and Calibration SOP
- 6.30. BSI-SOP-0573, MP90 Melting Range Operation, Verification, and Calibration SOP
- 6.31. BSI-SOP-0668, OPI-180 OD Handheld Colorimeter SOP
- 6.32. ACS, Reagent Chemicals, current edition
- 6.33. Current USP
- 6.34. Current *USP General Chapter <791> pH*

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7. ANALYTICAL PROCEDURES:

7.1. MOTHER LIQUOR ABSORBANCE :

- 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 the Lambda 25 UV/Vis Operation and Calibration SOP to measure the absorbance of the sample at the required wavelengths in the batch record.

7.2. MOTHER LIQUOR ASSAY :

- 7.2.1. Standardize the Metrohm pH electrode as per Standardization of Titrants.
- 7.2.2. Perform a daily check or standardization of 0.1N HCl as per Standardization of Titrants.
- 7.2.3. Weigh 0.8 – 1.2 g of sample directly into a clean glass beaker.
- 7.2.4. Dissolve in an appropriate amount of purified water (ensure that the sample dissolves/homogenizes, the electrode is covered, and/or the titration vessel will not overflow after titrant addition ~50 mL).
- 7.2.5. Titrate with 0.1N HCl to a potentiometric endpoint using the Metrohm 907 Auto-Titrator.
- 7.2.6. Each mL of 0.1N HCl is equivalent to 20.924 mg of Bis-Tris.

$$\% \text{ Bis-Tris} = \frac{\text{mL of Titrant} \times \text{Normality of Titrant} \times 20.924}{\text{Sample weight (g)}}$$

7.3. MOTHER LIQUOR DENSITY :

- 7.3.1. Refer to the Anton Paar DMA 35 Portable Density Meter Operation and Calibration SOP to measure the density of the Mother Liquor.

7.4. ABSORBANCE (0.1M) :

- 7.4.1. Prepare a 0.1M solution of the specified sample.
 - 7.4.1.1. Accurately weigh 0.52 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 measure the Absorbance of the sample at the required wavelength.

7.5. AMMONIUM :

7.5.1. Reagent Preparations:

7.5.1.1. Alkaline Potassium Tetraiodomercurate Solution R

- 7.5.1.1.1. Prepare *alkaline potassium tetraiodomercurate solution R* immediately before use.
- 7.5.1.1.2. Make a 1:1 solution of alkaline potassium tetraiodomercurate solution and 25% NaOH. Scale as needed.

7.5.1.2. Ammonium Standard Solution (1 ppm NH₄⁺):

- 7.5.1.2.1. Immediately before use, dilute 0.1 mL of a solution containing ammonium chloride R equivalent to 0.741 g of NH₄Cl in 1000 mL to 25mL with purified water.

7.5.1.3. Ammonium Standard Test Solution

- 7.5.1.3.1. Pipette 15 mL of the *Ammonium Standard Solution (1 ppm NH₄⁺)* into a 100 mL Nessler Tube.
- 7.5.1.3.2. Dilute standard solution to ~70 mL with purified water.
- 7.5.1.3.3. Make alkaline if necessary, using dilute sodium hydroxide solution R (~8.5% w/v or ~2M).

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7.5.2. Sample Test Preparation:

- 7.5.2.1. Dissolve 0.03 g of sample into ~70 mL of purified water in a 100 mL Nessler Tube.
- 7.5.2.2. Make alkaline if necessary, using dilute Sodium Hydroxide Solution R (~8.5% w/v or ~2M).

7.5.3. Analysis:

- 7.5.3.1. To both sample and standard test solutions, add 4.5 mL of *alkaline potassium tetraiodomercurate solution R*.
- 7.5.3.2. Dilute both the sample and standard test solutions to 100 mL using purified water.
- 7.5.3.3. Cover the Nessler Tubes and mix.
- 7.5.3.4. After 5 minutes any yellow color in the test solution is not more intense than any yellow color in the standard solution to report as < 0.05%.

7.6. **APPEARANCE AND COLOR** :

- 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 sides.
- 7.6.3. The sample should be white in color and characteristic of a crystalline powder.
- 7.6.4. If the appearance and 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.
- 7.6.5. If the sample does not conform to these specifications, notify the Laboratory Manager immediately.

7.7. **ASSAY and pKa** :

- 7.7.1. Standardize the Metrohm pH electrode as per Standardization of Titrants.
- 7.7.2. Perform a daily check or standardization of 0.1N HCl as per Standardization of Titrants.
- 7.7.3. Accurately weigh 0.65 g of Bis-Tris sample in to a weigh boat or paper. Transfer accurately weighed sample to a suitable clean, glass beaker.
- 7.7.4. Dissolve in an appropriate amount of water (ensure that the sample dissolves, the electrode is covered, and/or the titration vessel will not overflow after titrant addition ~50mL).
- 7.7.5. Titrate with 0.1N HCl to a potentiometric endpoint using the Metrohm 907 Auto Titrator.
- 7.7.6. Each mL of 0.1N HCl is equivalent to 20.924 mg of Bis Tris.
- 7.7.7. The pK_a should be reported on the assay printout from the Metrohm Auto-Titrator.

$$\% \text{Bis-Tris (As-is)} = \frac{mL \text{ of Titrant} \times \text{Normality of Titrant} \times 20.924}{\text{Sample Weight (g)}}$$

$$\% \text{ Bis-Tris (Dried Basis)} = \frac{100 \times \% \text{ Bis Tris (As - Is)}}{100 - \% \text{ LoD}}$$

7.8. **BIOBURDEN** :

- 7.8.1. Microbial analysis will be performed by an outside testing laboratory.
 - 7.8.1.1. Primary Provider: Mary Paul Laboratories.
 - 7.8.1.2. Package and send NLT 35 grams of sample to Mary Paul Laboratories with a purchase order and analysis request form.
- 7.8.2. Analyses:
 - 7.8.2.1. Total Aerobic Microbial Count (TAMC)
 - 7.8.2.2. Total Yeasts and Molds Count (TYMC)

7.9. CHLORIDES :

7.9.1. Sample Solution:

- 7.9.1.1. Weigh 2.0 grams of sample and dissolve in approximately 40mL of purified water.
- 7.9.1.2. If necessary, neutralize the solution with nitric acid to litmus.

7.9.2. 0.005% Standard Solution:

- 7.9.2.1. Pipette 0.141 mL of 0.02N HCl into a Nessler Color Comparison Tube and dilute to approximately 40 mL with purified water.

7.9.3. Procedure:

- 7.9.3.1. To each solution add 1 mL of Concentrated Nitric Acid and 1 mL of 0.1N Silver Nitrate.
- 7.9.3.2. Q.S. to 50 mL with purified water. Cover with parafilm and mix by inversion.
- 7.9.3.3. After 5 minutes, the turbidity of the sample preparation does not exceed that produced by the 0.005% standard when viewed against a dark background.
- 7.9.3.4. If a visible difference in turbidity is not observed, then utilize the Turbidimeter to measure the turbidity of the standard and the sample solutions. Follow the appropriate Portable Turbidimeter SOP.

7.10. ENDOTOXIN :

7.10.1. Sample Preparation using Endosafe Nexgen PTS Endotoxin Reader:

- 7.10.1.1. Accurately weigh 100 mg of sample into a sterile tube. Dilute to 10mL with LAL reagent water, dissolve, and mix thoroughly.
- 7.10.1.2. Transfer 1.0 mL of the 0.01 mg/mL solution to a separate 10mL sterile vessel and dilute to 10mL for a final concentration of 0.001 g/mL (1.0mg/mL).
- 7.10.1.3. Refer to Endosafe NexGen PTS Endotoxin Reader SOP (DCN: BSI-SOP-0345) for further instrument instructions and sample analysis.

7.11. ENZYME ACTIVITY :

7.11.1. Analyses:

- 7.11.1.1. DNase: Refer to DNase (Exonuclease) Assay (BSI-SOP-0138) and DNase Endonuclease Assay (BSI-SOP-0095) for sample preparation and analysis.
- 7.11.1.2. RNase: Refer to RNase (Ribonuclease) Assay (BSI-SOP-0095) for sample preparation and analysis.
- 7.11.1.3. Protease: Refer to Protease Assay (BSI-SOP-0139) for sample preparation and analysis.

7.12. HEAVY METALS AS PB :

7.12.1. Refer to Section 7.21 Trace Metals for sample preparation and analysis.

7.13. IDENTIFICATION TEST (UATR) :

- 7.13.1. Follow Spectrum Two UATR SOP (DCN: BSI-SOP-0254).
- 7.13.2. Analyze sample as-is; if solvent peaks are present or interfere with reference spectra; homogenize and dry both the reference material and sample over desiccant for at least 16 hours and reanalyze.

7.14. IRON :

7.14.1. Refer to Section 7.21 Trace Metals for sample preparation and analysis.

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7.15. LOSS ON DRYING :

- 7.15.1. Note: Bis-Tris melting may occur during drying.
- 7.15.2. Dry an LOD vial in the oven at $105 \pm 2^\circ\text{C}$ for 30 minutes.
- 7.15.3. Cool for 15 minutes in a desiccator, weigh the LOD vial, and record results.
- 7.15.4. If the substance to be tested is in the form of large crystals, reduce the particle size to about 2mm by quickly crushing before weighing.
- 7.15.5. Transfer approximately 1-2g of the sample to the LOD vial and accurately weigh the vial and contents. By gentle, sidewise shaking, distribute the sample as evenly as possible in the LOD vial to a depth of about 5mm.
- 7.15.6. Place the LOD vial containing the sample into the oven and dry at $105^\circ\text{C} \pm 2^\circ\text{C}$ for 3 hours.
- 7.15.7. Remove LOD vial from the oven and allow to cool in the desiccator for 15 minutes.
- 7.15.8. Reweigh the LOD vial and sample.
- 7.15.9. Calculate the %LOD as follows:

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

7.16. MELTING POINT (RANGE) :

- 7.16.1. Sample Preparation (Finished Good and Stability Samples):
 - 7.16.1.1. **Note: Sample may not be dried at a temperature and time according to the LOD procedure.**
 - 7.16.1.2. Reduce sample to a fine powder in a mortar and pestle prior to drying the sample.
 - 7.16.1.3. Dry the sample over a suitable desiccant for a minimum of 16 hours.
- 7.16.2. Sample Preparation (Raw Material):
 - 7.16.2.1. Analyze the sample as-is; if melting range is OOS, notify management and dry the sample over a suitable desiccant and reanalyze.
- 7.16.3. Ensure the Heating Rate is set to $0.2\text{ }^\circ\text{C}/\text{min}$ for analysis.
- 7.16.4. Refer to BSI-SOP-0573, MP90 Melting Range Operation, Verification, and Calibration SOP, or BSI-SOP-0256, MP50 Melting Range Operation and Calibration SOP, for analysis.
- 7.16.5. Result Reporting:
 - 7.16.5.1. The beginning of melt temperature is reported as the Melting Point for Bis-Tris Finished Good and Bis-Tris Raw Material.

7.17. pH OF A 1% @ $25 \pm 2^\circ\text{C}$:

- 7.17.1. Accurately weigh 1.0 g of sample. Transfer to a suitable beaker.
- 7.17.2. Add 100 mL of purified water and dissolve.
- 7.17.3. Follow the appropriate SOP for calibration and pH measurement.

7.18. RESIDUE ON IGNITION :

- 7.18.1. Turn on muffle furnace and allow it to stabilize at 600°C . Follow muffle furnace calibration procedure for operation of furnace.
- 7.18.2. Inspect a quartz crucible for cracks, chips, and discoloration.
- 7.18.3. Utilize forceps to insert and remove the crucible from the furnace.
- 7.18.4. Ignite quartz crucible at $600 \pm 50\text{ }^\circ\text{C}$ for 30 minutes. Cool in a desiccator for 1.5 hours and weigh using an analytical balance.
- 7.18.5. Weigh 1.0 g sample in the previously ignited quartz crucible. Moisten the sample with a small amount of sulfuric acid (Between 0.2-1.0mL).
- 7.18.6. 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.

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7.18.6.1. The rate of heating should be such that from $\frac{1}{2}$ to 1 hour is required to volatilize the sample.

7.18.6.2. Continue to heat the sample until all the excess sulfuric acid has been volatilized.

7.18.7. Ignite in the muffle furnace at 600 ± 50 °C for 15 minutes or until all carbon has been removed.

7.18.8. Cool in a desiccator for the same amount of time employed in the preparation of the crucible and weigh on an analytical balance.

7.18.9. Calculate the %ROI as follows:

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

7.18.10. If the amount of the residue exceeds the limit specified, repeat the moistening with sulfuric acid using up to 1mL, heat to char, then ignite at 600 ± 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.

7.19. **SOLUBILITY 0.1M IN WATER**

7.19.1. Prepare a 0.1M solution of the specified sample.

7.19.1.1. Accurately weigh 2.09 grams of sample.

7.19.1.2. Transfer accurately weighed sample to a clean, dry glass beaker and dissolved sample in 100 mL of purified water.

7.19.2. View sample from all angles under sufficient lighting, the solution should be clear and complete.

7.20. **SULFATE**

7.20.1. Sample Solution:

7.20.1.1. Weigh 2.0 grams of sample and dissolve in approximately 40mL of purified water.

7.20.1.2. Neutralize the solution with nitric acid to litmus.

7.20.2. 0.05% Standard Solution:

7.20.2.1. Pipette 1.0 mL of 0.02N H_2SO_4 into a Nessler Color Comparison Tube and dilute to approximately 40 mL with purified water.

7.20.3. Procedure:

7.20.3.1. To each tube, add 1 mL of 3N HCl and 3 mL of Barium Chloride TS.

7.20.3.2. Q.S. to 50 mL with purified water. Cover with parafilm and mix by inversion.

7.20.3.3. Allow to stand for 10 minutes.

7.20.3.4. After 10 minutes, the turbidity of the sample preparation does not exceed that produced by the 0.05% standard when viewed against a dark background.

7.20.3.5. If a visible difference in turbidity is not observed, then utilize the Turbidimeter to measure the turbidity of the standard and the sample solutions. Follow the appropriate Portable Turbidimeter SOP (Rockdale: BSI-SOP-0091 or Majestic: BSI-SOP-0242).

7.21. **TRACE METALS**

7.21.1. Refer to NexION 350X ICP MS SOP (DCN: BSI-SOP-0303).

7.21.2. Available Methods dependent on Product Code Requirements:

7.21.2.1. Refer to Analytical Method of Analysis: Bis-Tris and Bis-Tris Hydrochloride vis ICP-MS (DCN: BSI-ATM-0075), for sample preparation and analysis.

7.21.2.2. Refer to Analytical Method for Determination of Trace Metals in BioTech Products, BSI-ATM-0131.

7.22. WATER (BY KARL FISCHER TITRATION) :

- 7.22.1. Perform a standardization of the titrant (Composite 5) as per Standardization of Titrants.
- 7.22.2. Grind the sample in a dry mortar into a fine powder utilizing a pestle.
- 7.22.3. Immediately weigh 1.0 g of sample into the glass weighing spoon and tare it.
- 7.22.4. Transfer the sample to the KF vessel by removing the rubber septum and adding the sample into the titration vessel.
 - 7.22.4.1. Do not leave the rubber septum open for long periods of time as this will allow moisture to enter the titration vessel.
- 7.22.5. 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.
- 7.22.6. Check to make sure there is no residual sample stuck to the sides of the titration vessel.
 - 7.22.6.1. If there is any sample stuck to the side, stop the stir bead from spinning before swirling the vessel to rinse the sides.
- 7.22.7. Once the method begins, check to ensure the sample is fully dissolved before the titration begins (i.e., before the stir command completes).
- 7.22.8. The moisture content will then be determined by the Metrohm Titrando 907.

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