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TRIS HYDROCHLORIDE STABILITY ANALYTICAL PROCEDURE

LOT NUMBER: _____

T = _____

PACKAGING CONFIGURATION: _____

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1. SPECIFICATIONS:

1.1. Reference the Tris Hydrochloride Stability Data Card for specifications (DCN: BSI-LST-0149).

2. SAFETY:

2.1. Standard laboratory safety regulations apply. Read and understand the material Safety Data Sheet before handling this product and all reagents specified in this procedure.

3. EQUIPMENT:

3.1. Analytical Balance
3.2. Calibrated Oven
3.3. Lambda 25 UV/Vis Spectrophotometer, or equivalent
3.4. Metrohm 907 Titrando Auto-Titrator, or equivalent
3.5. Calibrated Pipettes
3.6. Perkin Elmer Spectrum Two UATR, or equivalent
3.7. XL200 pH/Conductivity Meter, or equivalent
3.8. Metrohm 914 pH/Conductometer, or equivalent
3.9. MP50 or MP90 Melting Range Apparatus

4. REAGENTS:

4.1. **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.
4.2. **Glacial Acetic Acid** – Purchased commercially.
4.3. **Methanol** – Purchased commercially.
4.4. **0.2% PVA** – 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.
4.5. **0.1N Silver Nitrate** – Purchased commercially.
4.6. **Eosin Y Indicator** – Dissolve 50 mg of Eosin Y in 10 mL of purified water.
4.7. **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 in order to meet requirements.
4.8. **Hydranal Composite 5**: Purchased commercially.
4.9. **Hydranal Methanol Dry:** Purchased commercially.
4.10. **Hydranal Formamide Dry:** Purchased commercially.

5. REFERENCES:

5.1. BSI-ATM-0002, Tris Hydrochloride Testing Methods
5.2. BSI-FRM-0702, Tris Hydrochloride Analytical Procedure
5.3. BSI-LST-0149, Tris Hydrochloride Stability Data Card
5.4. BSI-PRL-0054, Analytical Method Validation Report: Silver Nitrate Assay Utilizing Metrohm 907 Auto-Titrator
5.5. BSI-SOP-0019, Result Reporting
5.6. BSI-SOP-0090, Lambda 25 UV/Vis Operation and Calibration

5.7. BSI-SOP-0098, Balance SOP
5.8. BSI-SOP-0126, Laboratory Notebooks
5.9. BSI-SOP-0133, Blue M Convection Oven Operation and Calibration SOP
5.10. BSI-SOP-0140, Standardization of Titrants
5.11. BSI-SOP-0143, Metrohm Titrando 907 Auto-Titrator SOP
5.12. BSI-SOP-0144, Metrohm 914 pH Conductometer Operation and Calibration
5.13. BSI-SOP-0244, VWR Gravity Convection Oven and Calibration (Model Number 414005-106)
5.14. BSI-SOP-0254, Spectrum Two UATR SOP
5.15. BSI-SOP-0255, XL200 pH/mV/Conductivity Meter SOP
5.16. BSI-SOP-0256, MP50 Melting Range Operation, Verification and Calibration SOP
5.17. BSI-SOP-0573, MP90 Melting Range Operation, Verification, and Calibration SOP
5.18. ACS Reagent Chemicals current edition
5.19. General standard operating procedures
5.20. USP-NF current edition

6. ANALYTICAL PROCEDURE:

6.1. ABSORBANCE (1M) :

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

Sample [_____g] Diluted to [_____mL] with purified water.

Balance S/N/Due Date [_____/_____]

Instrument S/N/Due Date [_____/_____]

Absorbance (1M) Results			
400 nm	280 nm	260 nm	250 nm
a.u.	a.u.	a.u.	a.u.

Analyzed By: [_____/_____] Reviewed By: [_____/_____]

6.2. APPEARANCE AND COLOR :

Perform the test by placing approximately 10 g of sample on a piece of white filter paper. 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.

Sample [_____g]

Balance S/N/Due Date [_____/_____]

Appearance and Color Result [_____]

Analyzed By: [_____/_____] Reviewed By: [_____/_____]

6.3. **ASSAY (DRIED BASIS)** :

Assay by Auto-titrator:

Standardize 0.1N $AgNO_3$ as per Standardization of Titrants.
Accurately weigh 0.5g of dried 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$AgNO_3$ to a potentiometric end-point utilizing the Metrohm Titrando 907.
Assay by Manual Titration:

Standardize 0.1N $AgNO_3$ as per Standardization of Titrants. Accurately weigh 0.5g of dried 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.

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

Sample weight [_____g] Dissolve in [_____mL] Purified Water

$AgNO_3$ Standardization Value [_____N]

Standardization Reference [_____]

Daily Check Reference [_____]

Metrohm Titrando 907 S/N/Due Date [_____/_____]

Balance S/N/Due Date [_____/_____]

Pipettes S/N/Due Date [_____/_____];[_____/_____]

Glacial Acetic Acid [_____/_____] Volume Added [_____mL]

Methanol [_____/_____] Volume Added [_____mL]

0.2% polyvinyl alcohol solution [_____/_____] Volume Added [_____mL]

Eosin Y Indicator [_____/_____] Volume Added [_____mL]

0.1N Silver Nitrate Lot Number/Due Date [_____/_____]

Titrant Added [_____mL]

Assay (Dried) [_____%]

Analyzed By: [_____/_____] Reviewed By: [_____/_____]

6.4. **IDENTIFICATION (IR)** :

Follow Spectrum Two UATR SOP for sample preparation and analysis. Analyze dried sample.

☐ Utilized Sample from LOD method. Refer to Section 6.5.

☐ Sample dried as per LOD method for [_____ hours] at [_____°C] (if applicable).

☐ Sample crushed prior to analysis.

Tris Hydrochloride IR Reference Standard [_____/_____]

Spectrum Two UATR S/N/Due Date: [_____/_____]

Oven S/N/Due Date (if applicable) [_____/_____]

Calibrated Timer S/N/Due Date (if applicable) [_____/_____]

Correlation: [_____] Identification Result: [_____]

Analyzed By: [_____/_____] Reviewed By: [_____/_____]

6.5. **LOSS ON DRYING** :

Tare an LOD vial that has been previously dried for 30 minutes in the oven at 105°C ± 2°C.Allow to come to room temperature in a desiccator for at least 15 minutes before weighing. Transfer approximately 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 vial. Place the LOD vial containing the sample into the oven. Dry the sample at 105°C ± 2°C for 3 hours. Allow to come to room temperature in a desiccator for at least 15 minutes before weighing. Calculate result using the equation below:

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

Initial Vial Weight	Initial Sample Weight	Final Vial Weight	Final Sample Weight	Result
g	g	g	g	%

LOD Vial dried for [_____minutes] and cooled for [_____ minutes]

Sample Drying time [_____hours] and cooled for [_____ minutes]

Oven temperature [_____°C]

Oven S/N/Due Date [_____/_____]

Balance S/N/Due Date [_____/_____]

Calibrated Timer S/N/Due Date [_____/_____]

Analyzed By: [_____/_____] Reviewed By: [_____/_____]

6.6. **MELTING RANGE**:

Refer to MP50 or MP90 Melting Range Operation, Verification, and Calibration SOP.

☐ Sample ground and dried as per LOD method for [_____ hours] at [_____°C]

☐ Sample ground and dried for [_____ hours] over desiccant.

Start of Melt [_____°C] End of Melt [_____°C]

Result determined visually? ☐ Yes ☐ No If yes, Reason: _____

Result visually verified? ☐ Yes ☐ No If yes, Reason: _____

Melting Range Apparatus S/N/Due [_____/_____]

Oven S/N/Due Date [_____/_____]

Calibrated Timer S/N/Due Date [_____/_____]

Analyzed By: [_____/_____] Reviewed By: [_____/_____]

6.7. **pH (0.5M) @ 25±2°C**:

Accurately weigh 7.88 g of sample and transfer to a 100-mL volumetric flask. Q.S. to volume with purified water. Mix by inversion until thoroughly dissolved. Measure and record the pH using Metrohm 914 pH Conductometer Operation and Calibration or XL200 pH/mV/Conductivity Meter SOP.

Sample [_____g] diluted to [_____mL]

Balance S/N [_____/_____]

pH meter S/N [_____]

pH probe S/N [_____]

Temperature Probe S/N/Due Date [_____/_____]

Calibration Reference [_____]

Slope [_____@_____°C]

pH of 0.5M Result [_____@_____°C]

Analyzed By: [_____/_____] Reviewed By: [_____/_____]

6.8. **WATER BY KARL FISCHER :**

Standardize Composite 5 as per Standardization of Titrants. Grind the sample in a dry mortar into a fine powder utilizing a pestle. Immediately weigh 0.8 g of sample in the glass weighing spoon and tare it. Transfer the sample to the KF vessel by removing the rubber septum and adding the sample into the titrant vessel. Do not leave the rubber septum open for long periods of time as this will allow moisture to enter the titration vessel. Return the weighing spoon to the balance, making sure not to lose any sample that was left behind. Once the weight stabilizes, transfer the weight to the instrument. Check to make sure there is no residual sample stuck to the sides of the titration vessel. If there is any sample stuck to the side, stop the stir bead from spinning before swirling the vessel to rinse the sides. Once the method begins, check to ensure the sample is fully dissolved before the titration begins (i.e. before the stir command completes). The moisture content will then be determined by the Metrohm Auto Titrando 907.

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

Sample [_____g] mL of titrant added [_____mL]

mg/mL of Composite 5 [_____mg/mL]

Standardization Reference [_____]

Composite 5 Lot Number/Expiration Date [_____/_____]

Methanol Lot Number/Expiration Date [_____/_____]

Formamide Lot Number/Expiration Date [_____/_____]

Balance S/N/Due Date [_____/_____]

Metrohm Auto Titrando 907 S/N/Due Date [_____/_____]

Water Result [_____%]

Analyzed By: [_____/_____] Reviewed By: [_____/_____]

BALANCE PRINTOUTS

ANALYSIS	**ATTACHMENTS**
Absorbance 1M	Initial/date: _____
Assay	Initial/date: _____
UATR	Initial/date: _____
Melting Range	Initial/date: _____
Karl Fischer (KF)	Initial/date: _____

Applicable Audit Trails Performed Initial/Date_____

Reviewed By:_____Date:_____