BIOSPECTRA
100 Majestic Way, Bangor, PA 18013 / www.biospectra.us

To Whom It May Concern,

INTRODUCTION: The following analyses are conducted for Tris Hydrochloride, product code TH3250, in accordance with the Tris Hydrochloride Testing Methods DCN: 16-000042 v.8.0 and Certificate of Analysis DCN: 19-002949 v.10.0. Specific details for the procedures were also obtained from Spectrum Two UATR SOP DCN: 16-001330 v.3.0, MP50 Melting Range Operation and Calibration SOP DCN: 16-001332 v.4.1, and NexION 350X ICP-MS SOP DCN: 16-001923 v.2.0.

1. ABSORBANCE (1M) REFER TO CERTIFICATE OF ANALYSIS:

1.1. Prepare a 1 M solution of the specified sample.
- 1.1.1. Accurately weigh 3.94g of sample.
- 1.1.2. Transfer accurately weighed sample to a graduated cylinder and Q.S. to 25 mL with purified water.
- 1.1.3. Swirl to dissolve completely.

1.2. Refer to Lambda 25 UV/Vis Operation and Calibration to determine the Absorbance of the sample.

2. APPEARANCE Passes Test:

2.1. Perform the test by placing approximately 10 grams of sample on a piece of white filter paper.
2.2. 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.

3. ASSAY (DRIED) ≥ 99.0%:

3.1. Standardize 0.1N $AgNO_3$ as per Standardization of Titrants.
3.2. Accurately weigh 0.5 g of sample that has been previously dried following LOD analysis.
3.3. Transfer to a 250-mL beaker and dissolve with 10 mL of purified water.
3.4. Add 10 mL of glacial acetic acid, 100 mL of methanol, and 10 mL of a 0.2% polyvinyl alcohol solution.
3.5. Titrate with 0.1N $AgNO_3$ to a potentiometric end-point utilizing the Metrohm Titrando 907.

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

3.6. Alternate Manual Titration Method:
- 3.6.1. Standardize 0.1N $AgNO_3$ as per Standardization of Titrants.
- 3.6.2. Accurately weigh 0.5 g of sample that has been previously dried following LOD analysis.
- 3.6.3. Transfer to a 250-mL beaker and dissolve with 10 mL of purified water.
- 3.6.4. Add 10 mL of glacial acetic acid, 100 mL of methanol, and 0.5mL of Eosin Y Indicator.

Printed On:	26-Aug-2020 03:25:13 PM	Hamelburg, Crystal	: Printed By
Authored By:	Baun, Cassie 25-Aug-2020 12:57:09 PM		
Approval:	Hamelburg, Crystal 26-Aug-2020 03:21:13 PM		
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3.6.5. Titrate to a pink endpoint.

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

4. BIOBURDEN (MICROBIAL ANALYSIS) ≤ 100 CFU/g:

4.1. Microbial Analysis will be performed by an approved outside testing facility.
4.2. Package and send 35 grams of sample to the approved outside testing facility.

5. ENDOTOXIN ≤ 2.5 EU/g.:

5.1. Accurately weigh 50-60mg of sample into a sterile tube. Add 50-60mg of suitable tris base (Molecular biology grade or better). Dilute to 10mL with LAL reagent water, dissolve completely. Follow Endosafe NexGen PTS Endotoxin Reader SOP for analysis.
5.2. Endotoxin analysis can also be performed by an outside laboratory.
5.2.1. Package and send 20 grams of sample to the approved outside testing facility.

6. IDENTIFICATION (IR) PASSES TEST:

6.1. For UATR analysis, follow Spectrum Two UATR SOP for Instrument Set-Up and Use.
6.1.1. Perform a background scan prior to use each day and after every ten samples.
6.1.2. Each analyst must run a Reference Standard prior to analyzing a product. A Reference Standard may be compared to multiple lots of the corresponding product on that day.
6.1.3. Enter the Lot Number, Expiration Date, Date of Analysis, and Analyst Initials in the Sample ID.
6.1.4. Place the Sample on the UATR crystal using a static free scoop.
6.1.5. Align the swinging arm with the crystal and apply force by turning the green arm clockwise.
6.1.6. Press "Scan" on the top Toolbar. The program will preview the sample. Turn the green arm until the Force Gauge is approximately 125, or until the noise has subsided.
6.1.7. Once the Force Gauge is adjusted, press "Scan".
6.1.8. Once the scan is complete, release the swinging arm by turning it counterclockwise.
6.1.9. Clean the UATR crystal and the swinging arm with methanol and a Kim Wipe.
6.1.10. If the correlation is above 0.95. the comparison will be reported with Pass as the result.

7. IDENTIFICATION (CHLORIDE) PASSES TEST:

7.1. Accurately weigh 7.88 g of sample and transfer to a 100 mL volumetric flask.
7.2. Q.S. to volume with purified water.
7.3. The solution from pH 0.5M analysis may be used.
7.4. 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.
7.5. Add 4 mL of 6N Ammonium Hydroxide. The precipitate should dissolve after mild agitation.

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8. LOSS ON DRYING @ 105°C ≤ 0.5%:

8.1. Tare an LOD vial that has been previously dried for 30 minutes in the oven at 105°C ± 2°C.
8.2. Allow to come to room temperature in a desiccator for at least 15 minutes before weighing.
8.3. 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.
8.4. Place the LOD vial containing the sample into the oven.
8.5. Dry the sample at 105°C ± 2°C for 3 hours.
8.6. Allow to come to room temperature in a desiccator for at least 15 minutes before weighing.
8.7. Calculate result using the equation below:

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

9. MELTING RANGE 147 - 152°C:

9.1. Refer to MP50 Melting Range Operation and Calibration SOP for Instrument Set-Up and Use.
9.1.1. Reduce sample to a fine powder in a mortar and pestle prior to drying the sample
9.1.2. Dry the sample over a suitable desiccant for a minimum of 16 hours, or dry at a temperature and time according to the product's LOD procedure.
9.1.3. Place the prepared sample into a capillary tube. A clean packing rod can be used to push residual sample down the capillary tube but should not enter approximately 2 cm from the closed end of the capillary tube. The sample should then be packed down to a height of 2.5-3.0mm by gentle tapping on a solid surface.
9.1.4. Allow the instrument to reach the approximate start temperature for the current method. The instrument will beep once the initial temperature is reached.
9.1.5. Place the capillary tube containing the sample in the melting point apparatus.
9.1.6. Ensure that the packed sample is within the "min" and "max" lines on the instrument.
9.1.7. Do not force the capillary tube(s) into the apparatus; they should drop right in.
9.1.8. Select the correct method on the home screen, according to the product being analyzed.
9.1.9. Press the start button.
9.1.10. When prompted, entire the lot number into the "Analysis Comments".
9.1.11. Using the on-screen camera display follow these steps:
9.1.11.1. Record the initial temperature as the temperature at which the sample begins to collapse in on itself.
9.1.11.2. Record the final temperature as the temperature at which the sample is completely liquidated.
9.1.11.3. Bubbles may form with in the sample during melting. If the sample is completely liquidated and there are still bubbles present in the sample, the sample is still considered completely melted.

10. pH (1%) @ 25°C ± 2°C 4.0 – 5.0:

10.1. Accurately weigh 1.0 g of sample. Transfer to a suitable beaker.
10.2. Dissolve in 100 mL of purified water. Cover with parafilm and mix until thoroughly dissolved.
10.3. Measure and record the pH using the appropriate SOP.

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11. pH (0.5M) @ 25°C ± 2°C 3.5 – 5.0:

11.1. Accurately weigh 7.88 g of sample and transfer to a 100-mL volumetric flask.
11.2. Q.S. to volume with purified water. Mix until thoroughly dissolved.
11.2.1. Measure and record the pH using the appropriate SOP.
11.3. This solution can be utilized for the Identification (Chloride) test.

12. TRACE ELEMENTS REFER TO CERTIFICATE OF ANALYSIS:

12.1. Refer to NexION 350X ICP-MS SOP for Instrument Set-Up and Use.
12.1.1. Sample Preparation:
12.1.2. General Notes:
12.1.2.1. Before use, all plasticware that is not rated as "metal-free", should first be rinsed with purified water, rinsed with 15% Nitric Acid, and then rinsed again with purified water. Plasticware rated as "metal-free" may be used as-is.
12.1.2.2. Glass should be avoided as it has high potential for metal and mineral contaminations.
12.1.2.3. Standard and sample solutions should be prepared in 50mL centrifuge tubes.
12.1.3. 1% Nitric Acid
12.1.3.1. Measure 14.5 mL of Trace Metal Grade Nitric Acid and transfer to a rinsed plastic 1000 mL volumetric flask. QS to 1000 mL with purified water.
12.1.4. 15% Nitric Acid
12.1.4.1. Dilute approximately 110 mL of Trace Metal Grade Nitric Acid to 500 mL with purified water.
12.1.4.2. The solution is only used to rinse glassware and plasticware.
12.1.5. BioSpectra Daily Method:
12.1.5.1. Sample Solutions:
12.1.5.1.1. Weigh 0.10g of sample on an analytical balance. Add 100 uL of Environmental Standard Mix 6 and QS to 50.0 with 1% Nitric Acid.
12.1.5.2. Standard Curve Preparation:
12.1.5.2.1. 2 ppm Stock
12.1.5.2.1.1. Weigh 1.00 g of Instrument Calibration Standard 2 and QS to 50.0 g with 1% Nitric Acid.
12.1.5.2.2. 100 ppb Stock
12.1.5.2.2.1. Weigh 2.50 g of 2 ppm Stock and QS to 50.0 with 1% Nitric Acid.
12.1.5.2.3. Blank
12.1.5.2.3.1. Pipette 100 uL of Environmental Standard Mix 6 into the centrifuge tube. QS to 50.0 g with 1% Nitric Acid.
12.1.5.2.4. 1 ppb Standard
12.1.5.2.4.1. Pipette 0.50 mL of the 100 ppb Stock, add 100 uL of Environmental Standard Mix 6 and QS to 50.0 g with 1% Nitric Acid.
12.1.5.2.5. 2 ppb Standard (also used as a Continuing Check Verification Sample (CCV))

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12.1.5.2.5.1. Pipette 1.00 mL of the 100 ppb Stock, add 100 uL of Environmental Standard Mix 6 and QS to 50.0 g with 1% Nitric Acid.

12.1.5.2.6. 4 ppb Standard

12.1.5.2.6.1. Pipette 2.00 mL of the 100 ppb Stock, add 100 uL of Environmental Standard Mix 6 and QS to 50.0 g with 1% Nitric Acid.

12.1.5.2.7. 6 ppb Standard

12.1.5.2.7.1. Pipette 3.00 mL of the 100 ppb Stock, add 100 uL of Environmental Standard Mix 6 and QS to 50.0g with 1% Nitric Acid.

13. WATER (BY KARL FISCHER TITRATION) ≤ 2.0%:

13.1. Standardize Composite 5 as per Standardization of Titrants.

13.2. Grind the sample in a dry mortar into a fine powder utilizing a pestle.

13.3. Immediately weigh 0.8g of sample into the glass weighing spoon and tare it.

13.4. Transfer the sample to the KF vessel by removing the rubber septum and adding the sample into the titration vessel.

13.4.1. Do not leave the rubber septum open for long periods of time as this will allow moisture to enter the titration vessel.

13.5. 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.

13.6. Check to make sure there is no residual sample stuck to the sides of the titration vessel.

13.6.1. If there is any sample stuck to the side, stop the stir bead from spinning before swirling the vessel to rinse the sides.

13.7. Once the method begins, check to ensure the sample is fully dissolved before the titration begins (i.e. before the stir command completes).

13.8. The moisture content will then be determined by the Metrohm Auto Titrando 907.

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

If there are any questions or concerns, please feel free to contact ra@biospectra.us.

Sincerely,

Cassie Baun

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