BIOSPECTRA
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TRIS TR3251 TEST METHODS

1. ABSORBANCE (40% SOLUTION) 0.2 AU. MAX. @ 290NM:

1.1. Prepare a 40% solution of the specified sample.
  1.1.1. Accurately weigh 10.00g of sample.
  1.1.2. Transfer accurately weighed sample to a 50mL graduated cylinder and Q.S. to 25mL with purified water.
  1.1.3. Swirl to dissolve completely
    1.1.3.1. Sonicate if necessary to accelerate dissolution. Allow to cool to room temperature before analysis, if applicable.
1.2. Refer to Lambda 25 UV/Vis Operation and Calibration to measure the Absorbance of the sample.

2. APPEARANCE AND COLOR WHITE / CRYSTALS:

2.1. Place 25-50g of the sample in a clean, dry glass beaker.
2.2. In an area with sufficient lighting, view the sample from all sides.
2.3. The sample should be white in color and characteristic of crystals. If the sample does not conform to these specifications, notify the QC Manager immediately.

3. ASSAY (USP) (99.0 – 101.0%):

3.1. NOTE: The USP General Chapter will be followed for USP and EP/BP, unless a dispute arises in which case the appropriate compendia chapter will be followed.
3.2. Perform a daily check or standardization of the 0.1N HCl per Standardization of Titrants.
3.3. Accurately weigh 0.25g + 0.0005g of Tris previously dried at 105°C for 3 hours (use LOD sample).
3.4. Transfer accurately weighed sample to a suitable clean, glass beaker. 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).
3.5. Titrate with 0.1N HCl VS to a potentiometric endpoint using the Metrohm 907 Auto Titrator.
3.6. Each mL of 0.1N HCl is equivalent to 12.11mg of Tris:

$$\% \text{ Tris} = \frac{(mL \; x \; N \; of \; 0.1N \; HCL) * 0.12114}{Sample \; Weight \; (g)}$$

4. CHLORIDES (EP/BP) (0.01% MAX.):

4.1. Sample Solution:
  4.1.1. To 10 mL of Solution S add 2.5 mL of dilute nitric acid R in a test-tube.
  4.1.2. Dilute to 15 mL with purified water.
4.2. Standard Solution:
  4.2.1. Immediately before use, dilute 0.1mL of 500 ppm Chloride Stock Solution to a total of 10mL with purified water, in order to prepare chloride standard solution (5 ppm Cl) R.
  4.2.2. Transfer to a test tube and add 5 mL of purified water.
4.3. Procedure:
  4.3.1. To both the sample and standard solutions, add 1 mL of dilute nitric acid R and 1 mL of silver nitrate solution R2.

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4.3.2. Examine the tubes horizontally (laterally) against a black background.
4.3.3. Allow to stand for 5 minutes, using a calibrated timer, protected from light.
4.3.4. Any opalescence in the test solution is not more intense than that in the standard.

5. ENDOTOXIN CONCENTRATION (≤30 EU/G):

5.1. **Sample Preparation:** Weigh 0.008g of sample utilizing an analytical balance directly in to a 2.5mL micro centrifuge tube and record weight. Pipette 1mL of LAL Reagent Water (LRW) in the micro centrifuge tube and dissolve completely.
5.2. Refer to Endosafe PTS Endotoxin Reader SOP for instrument operation.

6. ENZYME ACTIVITY NONE DETECTED:

6.1. RNase, DNase, and Protease as per SOPs.

7. HEAVY METALS (PB) (EP/BP) (5PPM MAX.):

7.1. **EP/BP – Test sample preparation:**
7.1.1. Dissolve 2.0g Tris in 20 mL of purified water, and add 1.2 mL of concentrated hydrochloric acid to a 50-mL color-comparison tube.
7.2. **5ppm limit specification-Test sample Preparation:**
7.2.1. Dissolve 4.0g of Tris in 20mL of purified water and neutralize with 2.4mL of concentrated HCl to a 50-mL color-comparison tube.
7.3. Standard Lead Solution – On the day of use, dilute 10.0mL of Lead Nitrate Stock Solution with purified water to 100.0mL in a volumetric flask.
7.4. Standard Preparation – Into a 50-mL color-comparison tube, pipette 2mL of Standard Lead Solution prepared above, and dilute with purified water to 25mL.
7.5. Test Preparation – In the 50-mL color-comparison tube prepared above, dilute with purified water to 25mL.
7.6. Monitor Preparation – Into a third 50-mL color-comparison tube, place 25mL of a solution prepared as directed for Test Preparation and add 2.0mL of Standard Lead Solution.
7.7. Procedure:
7.7.1. Adjust all 50-mL color-comparison tubes to a pH between 3.0 and 4.0 using 1N acetic acid or 6N ammonium hydroxide. Use a pH meter or short-range pH indicator paper as an external indicator.
7.7.2. Dilute each with purified water to 40 mL and mix.
7.7.3. To all tubes add 2 mL of pH 3.5 Acetate Buffer and 1.2 mL of thioacetamide-glycerin base TS (1mL of glycerin TS and 0.2mL of thioacetamide TS gently heated for about 20 seconds). Dilute with purified water to 50 mL, parafilm and mix by inversion.
7.7.4. Allow to stand for 2 minutes.
7.7.5. View downward over a white surface. The color of the Test Preparation is not darker than the Standard Preparation, and the Monitor Preparation is equal to or darker than the Standard Preparation.

8. IDENTIFICATION TEST (B) (USP) PASSES TEST:

8.1. To 4.5mL of a saturated solution of Salicylaldehyde, add 0.5mL of glacial acetic acid and mix in a 50mL beaker.
8.2. Dissolve 1g of sample in 5mL of purified water.
8.3. Transfer 4.0mL of the sample solution to the above 50mL beaker and mix. A yellow color should be produced.

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9. IDENTIFICATION TEST (C) (USP) PASSES TEST:

9.1. Prepare a 4 in 10 solution of Ceric Ammonium Nitrate in 2 N Nitric Acid. Transfer 0.5mL of the resulting solution to a 50mL beaker and add 3mL of purified water.
9.2. Dissolve 1g of sample in 5mL of purified water.
9.3. Transfer 0.5mL of the sample solution to the above 50mL beaker and mix. The color should change from light yellow to orange.

10. IDENTIFICATION (IR) PASSES TEST:

10.1. Follow Spectrum Two UATR SOP.

11. INSOLUBLE MATTER 0.005% MAX.:

11.1. Accurately weigh 20.0g of sample and transfer to a 600mL beaker.
11.2. Add 200mL of purified water and utilize a Teflon encapsulated magnetic stirring bar and electric stir plate to dissolve sample.
11.3. Heat to boiling and digest on a hot plate in a covered beaker for 1 hour.
11.4. Prepare a Gooch filtering crucible and 6-15micron filter by drying at 105°C ± 2°C for 1 hour. Allow to cool in ambient air for 15 minutes and weigh.
11.5. Filter sample solution through conditioned filtering crucible and 6-15micron filter. Rinse thoroughly with at least 3 crucible volumes of hot purified water.
11.6. Dry the crucible at 105° ± 2°C for 1 hour.
11.7. Cool in ambient air for 15 minutes and reweigh.
11.8. Calculate the % Insoluble Matter as follows:

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

12. KARL FISCHER WATER (2% MAX):

12.1. Perform a standardization of the titrant (Composite 5) as per Standardization of Titrants.
12.2. Grind the sample in a dry mortar into a fine powder utilizing a pestle.
12.3. Immediately weigh ~0.800g of sample into the glass weighing spoon and tare it.
12.4. Transfer the sample to the KF vessel by removing the rubber septum and adding the sample into the titration vessel.
12.4.1. Do not leave the rubber septum open for long periods of time as this will allow moisture to enter the titration vessel.
12.5. Return the weighing spoon to the balance, making sure not to lose any sample that was left behind. Once the weight stabilizes, press the print button on the balance.
12.6. Check to make sure there is no residual sample stuck to the sides of the titration vessel.
12.6.1. If there is any sample stuck to the side, stop the stir bead from spinning before swirling the vessel to rinse the sides.
12.7. Once the method begins, check to ensure the sample is fully dissolved before the titration begins (i.e. before the stir command completes).
12.8. The moisture content will then be determined by the Metrohm Auto Titrando 907.

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

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13. LOSS ON DRYING (0.6% MAX.):

13.1. Dry an LOD vial in the oven at 105 ± 2˚C for 30 minutes.
13.2. Cool for 15 minutes in a desiccator, weigh the LOD vial, and record results.
13.3. 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.
13.4. Transfer approximately 1- 2g of the sample to the LOD vial, and accurately weigh the bottle and contents. By gentle, sidewise shaking, distribute the sample as evenly as possible in the LOD vial to a depth of about 5mm.
13.5. Place the LOD vial containing the sample into the oven and dry at 105°C ± 2°C for 3 hours.
13.6. Remove LOD vial from the oven and allow to cool in the desiccator for 15 minutes.
13.7. Reweigh the LOD vial and sample and retain the dried sample to perform the Assays.
13.8. Calculate the %LOD as follows:

$$\%LOD = \frac{[\text{initial sample weight (g)} - \text{final sample weight (g)}]}{\text{initial sample weight (g)}} x\ 100$$

14. MELTING RANGE (USP) (168 – 172°C):

14.1. Refer to MP50 Melting Range Operation and Calibration SOP.

15. MICROORGANISMS (TAMC/TYMC) ≤1000 CFU/G/≤100 CFU/G, ID: ABSENCE IN G:

15.1. Package approximately 20 grams into a sterile container and send to MPL Laboratories. The Analysis Request form should include TAMC, TYMC, *Escherichia coli* Test for Absence per 1 gram, *Salmonella* Test for Absence per 10 grams, *Pseudomonas aeruginosa* Test for Absence per 1 g, *Staphylococcus aureus* Test for Absence per 1 g, *Candida albicans* Test for Absence per 1 g, and Bile-Tolerant Gram Negative Bacteria Absence per 1 g.
15.2. In order to Pass, Total Aerobic Microbial count must be less than or equal to 1000 CFU/g, the Total Yeast and Mold Count must be less than or equal to 100 CFU/g and all identifications must be noted as Negative.

16. PH OF A 5% @ 25 ± 2°C (USP & EP/BP) (10.0 – 11.5):

16.1. Accurately weigh 5.0g of sample. Transfer to a suitable beaker.
16.2. Add 100g of purified water and dissolve.
16.3. Follow the appropriate SOP to measure and record the pH.

17. RELATED SUBSTANCES (TLC) (EP/BP) (1% MAX.):

17.1. Examine by thin-layer chromatography (2.2.27), using silica gel G R as the coating substance. Wash the plate with methanol R before applying the solutions.
17.2. Test Solution (a):
17.2.1. Dissolve 0.20g in 1mL of purified Water, with gentle heating, and dilute to 10mL with methanol R.
17.3. Test Solution (b):
17.3.1. Dilute 1mL of Test Solution (a) to 10mL with methanol R.
17.4. Reference Solution (a):
17.4.1. Dissolve 20mg of trometamol CRS in methanol R and dilute to 10mL with the same solvent.
17.5. Reference Solution (b):
17.5.1. Dilute 1mL of Test Solution (a) to 100mL with methanol R.

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17.6. Procedure:
- 17.6.1. Apply to the plate 10 µL of each solution. Develop over a path of 10cm using a mixture of 10 volumes of dilute ammonia R1and 90 volumes of 2-propanol R.
- 17.6.2. Dry the plate at 100°C to 105°C.Spray a 5g/L solution of potassium permanganate R in a 10g/L solution of sodium carbonate R.
- 17.6.3. After about 10 min examine in daylight. Any spot in the chromatogram obtained with Test Solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with Reference Solution (b) (1.0 per cent).

18. RESIDUE ON IGNITION/SULFATED ASH (USP, EP/BP) (0.1% MAX.):

18.1. NOTE: The USP General Chapter will be followed for USP, EP/BP, and JP testing, unless a dispute arises in which case the appropriate compendia chapter will be followed.

18.2. Turn on muffle furnace and allow it to stabilize at 600°C.Follow muffle furnace calibration procedure for operation of furnace.

18.3. Inspect a quartz crucible for cracks, chips and discoloration.

18.4. Utilize the10 inch forceps to insert and remove the crucible from the furnace.

18.5. Ignite quartz crucible at 600 ± 50 °C for 30 minutes. Cool in a desiccator and weigh on an analytical balance.

18.6. For specification of 0.1% max:
- 18.6.1. Weigh 1.0 g sample in the previously ignited quartz crucible. Moisten the sample with 0.2 mL of sulfuric acid.

18.7. Volatilize the sample with a Bunsen burner until the sample is thoroughly charred. Keep the sample an appropriate distance from the flame, so that the sample does not boil over and sample is not lost.
- 18.7.1. The rate of heating should be such that from ½ to 1 hour is required to volatilize the sample.
- 18.7.2. Continue using the Bunsen burner to heat the sample until all the excess sulfuric acid has been volatilized.

18.8. Allow the sample to cool, and then moisten with 0.2mL of sulfuric acid.

18.9. Volatilize the sample with a Bunsen burner until the sample is thoroughly charred and white fumes are no longer evolved. Keep the sample an appropriate distance from the flame, so that the sample does not boil over and sample is not lost.
- 18.9.1. The rate of heating should be such that from ½ to 1 hour is required to volatilize the sample.
- 18.9.2. Continue using the Bunsen burner to heat the sample until all the excess sulfuric acid has been volatilized.

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

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

18.12. Calculate the %ROI as follows:

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

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18.13. If the amount of the residue exceeds the limit specified, repeat the moistening with sulfuric acid using up to 1mL, heat via Bunsen burner and 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.

19. TRACE METALS 5 PPM MAX: AS, CA, CU, FE, PB, MG; 15 PPM MAX: NI

19.1.1. NOTE: The USP General Chapter will be followed for USP and EP/BP testing, unless a dispute arises in which case the appropriate compendia chapter will be followed.

19.1.2. Refer to NexION 350X ICP MS SOP.

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