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TRIS TESTING METHODS

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TABLE OF CONTENTS

1. PURPOSE:

1.1. To provide the Quality Control (QC) Laboratory personnel with a procedure for examining Tris.

2. SCOPE:

2.1. Applies to examination of Tris Raw Materials, In Process, Stability, and Finished Goods in the QC Laboratory. Methods include testing for all types of 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 Executive Director of Quality Control is responsible for the control, training, maintenance and implementation of this procedure.
3.2. The QC Analysts are responsible for compliance with the terms of this procedure. This includes notifying the Quality Assurance and Quality Control Managers, or designees, if any analyses fail to meet their respective specifications.

4. REFERENCES:

4.1. *ACS, Reagent Chemicals,* current edition
4.2. Analytical Method: Quantification of Formaldehyde by GC-MS
4.3. Analytical Method Verification Protocol: Elemental Impurities via ICP-MS
4.4. Analytical Method of Analysis: Trace Metal Impurities: Tris and THCl, 20-003601
4.5. Balance SOP
4.6. Bangor Portable Turbidimeter and Calibration
4.7. Blue M Convection Oven Operation and Calibration SOP
4.8. Analytical Method Validation Protocol: Limit of Tris (Hydroxylmethyl) nitromethane
4.9. *Current EP/BP*
4.10. *Current USP*
4.11. *Current USP General Chapter <791> pH*
4.12. Determination of Elemental Impurities by ICP-MS in Tris, DCN: 20-003602
4.13. DNase (Endonuclease) Assay
4.14. DNase (Exonuclease) Assay
4.15. Laboratory Notebooks
4.16. Lambda 25 UV/Vis Operation and Calibration
4.17. Metrohm 914 pH Conductometer Operation and Calibration
4.18. Metrohm Titrando 907 Auto-Titrator SOP
4.19. MP50 Melting Range Operation and Calibration SOP
4.20. Muffle Furnace SOP and Calibration
4.21. NexION 350X ICP-MS SOP
4.22. Portable Turbidimeter SOP and Calibration
4.23. Protease Assay
4.24. RNase (Ribonuclease) Assay
4.25. Spectrum Two UATR SOP
4.26. Standardization of Titrants
4.27. XL200 pH/mV/Conductivity Meter SOP
4.28. Tris Organic Impurities via UPLC
4.29. Tris Related Substances Analysis Method via HPLC, DCN: 21-003960

4.30. Perkin Elmer Flexar HPLC SOP

5. EQUIPMENT:

5.1. Analytical Balance
5.2. Blue M Oven, or equivalent
5.3. Hach Portable Turbidimeter
5.4. Lambda 25 UV/Vis Spectrophotometer
5.5. Metrohm 907 Titrando Auto-Titrator
5.6. MP50 Melting Point Apparatus
5.7. Muffle Furnace
5.8. Perkin Elmer NexION 350X ICP MS
5.9. Perkin Elmer Spectrum Two UATR
5.10. XL200 pH/Conductivity Meter or equivalent
5.11. Waters H Class HPLC/ UPLC or equivalent
5.12. Shimadzu QP2010 GC-MS w/ Headspace Sampler or equivalent
5.13. Perkin Elmer Flexar HPLC

6. REAGENTS:

6.1. **0.1N HCl:** Purchased Commercially.
6.2. **0.1N Silver Nitrate:** Purchased Commercially.
6.3. **1N Acetic Acid**: Dilute 60.0mL of glacial acetic acid with water to make 1000mL.
6.4. **200g/L Citric Acid Solution R**: Weigh 20g of citric acid and dilute to 100mL with Water R.
6.5. **2-Propanol R**: Purchased Commercially.
6.6. **500ppm Chloride Stock Solution**: Weigh 0.0824g of NaCl and dilute to 100mL with water R.
6.7. **5g/L Potassium Permanganate in a 10g/L Solution of Sodium Carbonate R**: Dissolve 1.0 of Potassium Permanganate and 2.0g of Sodium Carbonate in water R to make a total volume of 200mL.
6.8. **6N Ammonium Hydroxide**: Prepare by diluting 400mL of Ammonia Water, Stronger with water to make 1000mL.
6.9. **Acetonitrile:** Purchased Commercially.
6.10. **Ammonia R**: Purchased Commercially, see Ammonium Hydroxide.
6.11. **Ammonia TS**: See 6N Ammonium Hydroxide.
6.12. **APHA no. 500 Pt-Co Standard:** Purchased Commercially.
6.13. **Ceric Ammonium Nitrate in 2N Nitric Acid:** Dissolve 40g of Ceric Ammonium Nitrate in 2.0N Nitric Acid to make a total volume of 100mL.
6.14. **Citric Acid Solution R,** (20% w/v or 200g/L): Weigh 20g of citric acid and dilute to 100mL with water R.
6.15. **Cupric Sulfate TS:** Dissolve 12.5g of Cupric Sulfate in purified water to make 100mL.
6.16. **Dilute Acetic Acid:** Dilute 6 g of acetic acid (100) with water to make 100 mL (1 mol/L).
6.17. **Dilute Ammonia R1**: Dilute 41g of concentrated ammonia R to 100mL with water R.
6.18. **Dilute Nitric Acid R**: Dilute 20g of Nitric Acid to 100mL with Water R.
6.19. **Dilute Sulfuric Acid (JPC 1997)**: Cautiously add 5.7 mL of sulfuric acid to 10 mL of water, cool, and dilute with water to make 100mL (10%).
6.20. **Dilute Sulfuric Acid (EP)**: Add 5.5mL of sulfuric acid R to 60 mL of water R, allow to cool and dilute to 100mL with the same solvent.
6.21. **Glacial Acetic Acid**: Purchased Commercially.
6.22. **Glycerin:** Purchased Commercially.

6.23. **Glycerin Base TS:** To 200g of glycerol, add purified water to bring the total weight to 235g. Add 140mL of 1N Sodium Hydroxide and 50mL of purified water.
6.24. **Hydrochloric Acid, concentrated:** Purchased Commercially.
6.25. **Hydrochloric Acid (0.02N):** Slowly add 20mL of 0.1N Hydrochloric Acid to 80mL of purified water to make a total volume of 100mL.
6.26. **Lead Nitrate Stock Solution (USP/EP/JPC)**: Weigh exactly 159.8mg of Lead (II) nitrate and dissolve in 10mL of dilute nitric acid and add water to make exactly 1000mL. Prepare and store this solution using glass containers, free from soluble lead salts.
6.27. **LAL Reagent Water**: Purchased Commercially.
6.28. **Methanol R:** Purchased Commercially.
6.29. **Nitric Acid, Concentrated:** Purchased Commercially.
6.30. **pH 3.5 Acetate Buffer**: Dissolve 62.5g of ammonium acetate in 62.5mL of purified water, and add 47.0mL of concentrated hydrochloric acid. Adjust, if necessary, with 6N ammonium hydroxide or 6N hydrochloric acid to a pH of 3.5, dilute with purified water to 250mL.
6.31. **Phenolphthalein TS**: Dissolve 1g of phenolphthalein in 100mL of ethanol (95).
6.32. **Reference Solution B_9**. Prepare immediately before use. Transfer 1.0mL of Standard Solution B to 99.0mL of 1% HCl.
6.33. **Salicylaldehyde:** Purchased Commercially.
6.34. **Silver Nitrate Solution R2**: See 0.1N $AgNO_3$.
6.35. **Sodium Nitrite:** Purchased Commercially.
6.36. **Solution S** (may be scaled as needed): Weigh 2.5 grams of sample and dissolve in purified water. Dilute to a total volume of 50mL with purified water.
6.37. **Sulfuric Acid, concentrated**: Purchased Commercially
6.38. **Thioacetamide TS**: Dissolve 4.0g of thioacetamide in 100mL of purified water.
6.39. **Thioglycolic Acid R**: Purchased Commercially.
6.40. **Tris IR Reference Standard:** Prepare a vial at 105°C for 30 minutes. Allow to cool in desiccator and weigh a maximum 10.0g of Tris. Dry at 105°C for 3 hours. Cool and store in desiccator in a closed container. Perform a UATR analysis on the Reference Standard and compare it to a previously approved reference scan. The correlation must be 0.95 or greater between the two scans.
6.41. **Trometamol R/ Tromethamine CRS**: Purchased Commercially. Secondary reference standards may be used.

7. ANALYTICAL PROCEDURES:

7.1. **ABSORBANCE (MOTHER LIQUOR) 0.150 max. @ 400nm; 2.000 max. @ 280 and 2.200 max @ 260 nm:**
7.1.1. Prepare 10mL of a 1:1 dilution with purified water of the submitted ML sample. Prepare by pipetting 5mL of submitted ML into an LOD vial or small beaker. Add 5mL of purified water to the same beaker/vial. Mix thoroughly.
7.1.2. Refer to Lambda 25 UV/Vis Operation and Calibration to measure the Absorbance of the sample.
7.1.3. Record results at specified wavelengths in the In-Process Tris ML Absorbance Log Book
7.1.4. Notify appropriate personnel if the results are within the action limits listed below

Absorbance	Action Limit
400 nm	0.100 a.u
280 nm	1.600 a.u

260 nm	1.750 a.u

7.1. **ML ASSAY** **Monitor:**

7.1.1. Perform a daily check or standardization of 0.1N HCl as per Standardization of Titrants.
7.1.2. Accurately weigh 0.5 grams of ML sample.
7.1.3. Transfer accurately weighed sample to a suitable beaker.
7.1.4. Add an appropriate amount of water.
7.1.4.1. Ensure that the electrode is covered, and/or the titration vessel will not overflow after titration.
7.1.5. Titrate with 0.1N HCl to a potentiometric endpoint using the Metrohm 907 Auto Titrator.
7.1.6. Each ML is equivalent to 12.114mg of Tris.
7.1.7. Calculate % Assay using the following equation:

$$\%\,\boldsymbol{Tris} = \frac{(\boldsymbol{mL\ x\ N\ of\ 0.1N\ HCl})(\mathbf{12.114})}{\boldsymbol{Sample\ Weight}\ (\boldsymbol{g})}$$

7.2. **ABSORBANCE (1M)** **Refer to Summary Sheet:**

7.2.1. Prepare a 1M solution of the specified sample.
7.2.1.1. Accurately weigh 3.03g of sample.
7.2.1.2. Transfer accurately weighed sample to a graduated cylinder and Q.S. to 25mL with purified water.
7.2.1.3. Swirl to dissolve completely.
7.2.2. Refer to Lambda 25 UV/Vis Operation and Calibration to measure the Absorbance of the sample.
Analyze at the following wavelengths: 260nm, 280nm, 400nm, and 430nm.

7.3. **ABSORBANCE (10% Solution)** **Refer to Summary Sheet:**

7.3.1. Prepare a 10% solution of the specified sample.
7.3.1.1. Accurately weigh 2.5g of sample.
7.3.1.2. Transfer accurately weighed sample to a graduated cylinder and Q.S. to 25mL with purified water.
7.3.1.3. Swirl to dissolve completely
7.3.1.3.1. Sonicate if necessary to accelerate dissolution. Allow to cool to room temperature before analysis, if applicable.
7.3.2. Refer to Lambda 25 UV/Vis Operation and Calibration to measure the Absorbance of the sample.
7.3.3. Analyze at the following wavelengths: 260nm, 280nm, and 430nm.

7.4. **ABSORBANCE (40% Solution)** **Refer to Summary Sheet:**

7.4.1. Prepare a 40% solution of the specified sample.
7.4.1.1. Accurately weigh 10g of sample.
7.4.1.2. Transfer accurately weighed sample to a graduated cylinder and Q.S. to 25mL with purified water.
7.4.1.3. Swirl to dissolve completely
7.4.1.3.1. Sonicate if necessary to accelerate dissolution. Allow to cool to room temperature before analysis, if applicable.
7.4.2. Refer to Lambda 25 UV/Vis Operation and Calibration to measure the Absorbance of the sample 290nm.

7.5. **APHA COLOR, 20% SOLUTION** **Refer to Summary Sheet:**

7.5.1. Sample Solution:

7.5.1.1. Accurately weigh 10 grams of sample. Transfer to a 50mL volumetric flask and mix thoroughly. Transfer to a 50 mL Nessler Color Comparison tube.

7.5.2. APHA 20 Standard Solution:

7.5.2.1. Pipette 2.00 mL of APHA no. 500 Pt-Co Standard into a 50 mL volumetric flask and mix thoroughly. Transfer to a 50mL Nessler Color Comparison tube.

7.5.3. View downward over a white surface. In order to report as < 20 APHA, the color of the sample solution must not be darker than that of the standard solution.

7.6. **APPEARANCE AND COLOR** **Refer to Summary Sheet:**

7.6.1. Place 25-50g of the 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 crystals. If the sample does not conform to these specifications, notify the QC Manager immediately.

7.6.3.1. Refer to raw material summary sheets for additional raw material requirements.

7.7. **APPEARANCE OF SOLUTION** **Refer to Summary Sheet:**

7.7.1. Clear *(2.2.1.)* Turbidimetry

7.7.1.1. Rinse the sample bottle with Solution S twice.

7.7.1.2. Fill sample bottle with approximately 15mL of Solution S to the white line.

7.7.1.3. Coat outside of bottle with a thin coat of silicon oil.

7.7.1.4. Remove any air bubbles from the solution by using a syringe.

7.7.1.5. Allow the sample to sit capped for 2-3 minutes.

7.7.1.6. Follow the appropriate SOP as follows:

7.7.1.6.1. Stroudsburg- Measure and record the turbidity of the sample according to Portable Turbidimeter SOP.

7.7.1.6.2. Bangor- Measure and record the turbidity of the sample according to Bangor Portable Turbidimeter and Calibration SOP

7.7.1.7. The sample solution must be ≤ 3 NTU, reference suspension I to pass as clear.

7.7.2. Colorless (*2.2.2. Method II*)

7.7.2.1. Pipette 2.0 mL of Solution S into a test tube.

7.7.2.2. Pipette 2.0 mL of purified water into a second test tube.

7.7.2.3. Compare the colors in diffused daylight, viewing vertically against a white background.

7.7.2.4. In order for Solution S to be colorless, it must have the appearance of purified water or the solvent used for the preparation of the solution to be examined or is not more intensely colored than reference solution B_9.

7.8. **ARSENIC** **Refer to Summary Sheet:**

7.8.1. Refer to section 7.40: Trace Metals.

7.9. **ASSAY** **Refer to Summary Sheet:**

7.9.1. Perform a daily check or standardization of 0.1N HCl as per Standardization of Titrants.
7.9.2. Accurately weigh 0.5g of Tris previously dried at 105°C for 3 hours (use LOD sample).
7.9.3. 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).
7.9.4. Titrate with 0.1N HCl VS to a potentiometric endpoint using the Metrohm 907 Auto Titrator.
7.9.5. Each mL of 0.1*N* HCl is equivalent to 12.114mg of Tris:

$$\%\ \text{Tris} = \frac{(\text{mL x N of 0.1N HCl}) \cdot 12.114}{\text{Sample Weight (g)}}$$

7.10. **ASSAY (Ultrapure)** **Refer to Summary Sheet:**

7.10.1. If not being performed at the same time as the assay above, perform a daily check or standardization of 0.1N HCl as per Standardization of Titrants.
7.10.2. Accurately weigh 0.5g of Tris previously dried at 105°C for 3 hours (use LOD sample).
7.10.3. 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).
7.10.4. Titrate using 0.1N HCl to a pH of 4.7.

$$\%\ \text{Tris} = \frac{(mL\ x\ N\ of\ 0.1N\ HCl) * 12.114}{Sample\ Weight\ (g)}$$

7.11. **CHLORIDES** **Refer to Summary Sheet:**

EP Method (0.01% max)

7.11.1. Sample Solution:
7.11.1.1. To 10 mL of Solution S add 2.5 mL of dilute nitric acid R in a test-tube.
7.11.1.2. Dilute to 15 mL with purified water.
7.11.2. Standard Solution:
7.11.2.1. 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.
7.11.2.2. Transfer to a test tube and add 5 mL of purified water
7.11.3. Procedure:
7.11.3.1. To both the sample and standard solutions, add 1 mL of dilute nitric acid R and 1 mL of silver nitrate solution R2.
7.11.3.2. Compare the standard and samples solutions against a black background.
7.11.3.3. Allow to stand for 5 minutes, using a calibrated timer, protected from light.
7.11.3.4. Any opalescence in the test solution is not more intense than that in the standard.

USP Method (0.002% max)

7.11.4. Standard Preparation:
7.11.4.1. Pipette 0.057 mL of 0.02N HCl into a Nessler Color Comparison Tube and dilute to approximately 40 mL with purified water.
7.11.5. Sample Preparation:
7.11.5.1. Weigh 2.0 grams of sample and dissolve in approximately 40 mL of purified water. If necessary, neutralize the solution with nitric acid to litmus.

7.11.6. Procedure:
- 7.11.6.1. Add to each solution, 1mL of concentrated nitric acid and 1 mL 0.1N silver nitrate.
- 7.11.6.2. Q.S. to 50 mL with purified water. Cover with parafilm and mix by inversion.
- 7.11.6.3. After 5 minutes, the turbidity of the sample preparation does not exceed that produced by the 0.002% standard when viewed against a dark background.

7.12. **CLARITY AND COLOR OF SOLUTION (JPC 1997) Refer to Summary Sheet:**
- 7.12.1. Dissolve 1.0 g of sample in 10 mL of purified water. The solution must be clear and colorless.

7.13. **ENDOTOXIN Refer to Summary Sheet:**
- 7.13.1. Sample Preparation using Endosafe Nexgen PTS Endotoxin Reader**:**
  - 7.13.1.1. Accurately weigh 100 mg of sample into a sterile tube. Add 70µL of concentrated HCl. Dilute to 10mL with LAL reagent water, dissolve, and mix thoroughly for a final concentration of 0.0100g/mL.
  - 7.13.1.2. Refer to Endosafe Nexgen PTS Endotoxin Reader SOP for instrument operation.

7.14. **ELEMENTAL IMPURITIES Refer to Summary Sheet:**
- 7.14.1. Refer to Determination of Elemental Impurities by ICP-MS in Tris, DCN: 20-003602.

7.15. **ENZYME ACTIVITY Refer to Summary Sheet:**
- 7.15.1. RNase, DNase, and Protease as per SOPs.

7.16. **FORMALDEHYDE Refer to Summary Sheet:**
- 7.16.1. Refer to Analytical method: Quantification of Formaldehyde by GC-MS, DCN: 19-003090.

7.17. **HEAVY METALS as Pb Refer to Summary Sheet:**
- 7.17.1. Refer to 7.40: Trace metals for primary analysis.
- 7.17.2. EP/BP Test Sample preparation:
  - 7.17.2.1. Dissolve 2.0 g Tris in 20 mL of purified water, and add 1.2mL of concentrated hydrochloric acid to a 50-mL color-comparison tube.
- 7.17.3. 5 ppm limit specification – Test sample Preparation:
  - 7.17.3.1. Dissolve 4.0 g of Tris in 20 mL of purified water and neutralize with 2.4 mL of concentrated HCl to a 50-mL color-comparison tube.
- 7.17.4. Standard Lead Solution – On the day of use, dilute 10.0 mL of Lead Nitrate Stock Solution with purified water to 100.0 mL in a volumetric flask.
- 7.17.5. Standard Preparation – Into a 50 -mL color-comparison tube, pipette 2 mL of Standard Lead Solution prepared above, and dilute with purified water to 25 mL.
- 7.17.6. Test Preparation - In the 50-mL color comparison tube prepared above, dilute with purified water to 25 mL.
- 7.17.7. Monitor Preparation – Into a third 50 – mL color comparison tube, place 25 mL of a solution prepared as directed for Test Preparation and add 2.0 mL of Standard Lead Solution.
- 7.17.8. Procedure:

7.17.8.1. 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.
7.17.8.2. Dilute each tube with purified water to 40 mL and mix.
7.17.8.3. To all tubes 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 gently heated for about 20 seconds). Dilute with purified water to 50 mL, parafilm and mix by inversion.
7.17.8.4. Allow to stand for 2 minutes.
7.17.8.5. 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.

7.18. **HEAVY METALS (JPC 1997)** **Refer to Summary Sheet:**

7.18.1. Refer to 7.40 Trace metals for primary analysis.
7.18.2. Alternate Wet Method:
7.18.3. Standard Lead Solution (0.008mg/mL):
7.18.3.1. Immediately before use, dilute 8.0 mL of Lead Nitrate Stock Solution (~100ppm) to 100 mL with purified water in a volumetric flask.
7.18.4. Sample Preparation:
7.18.4.1. Accurately weigh 2.0g of sample and place in a quartz or porcelain crucible, cover loosely with a lid, and carbonize by gentle ignition.
7.18.4.2. After cooling, add 2 mL of Nitric Acid and 5 drops of sulfuric acid, heat cautiously until white fumes are no longer evolved, and incinerate by ignition between 500°C and 600°C.
7.18.4.3. Cool, add 2 mL of hydrochloric acid, evaporate to dryness on a water bath, moisten the residue with 3 drops of hydrochloric acid, add 10 mL of hot purified water, and warm for 2 minutes.
7.18.4.4. Then, add 1 drop of phenolphthalein TS, and ammonia TS dropwise until the solution develops a pale red color.
7.18.4.5. Add 2 mL of dilute acetic acid, and filter if necessary. Wash with 10 mL of purified water. Transfer the filtrate and washings to a Nessler tube and dilute to 50 mL with purified water.
7.18.5. Control Preparation:
7.18.5.1. Evaporate to dryness on a water bath, a mixture of 2 mL of nitric acid, 5 drops of sulfuric acid and 2 mL of hydrochloric acid. Moisten the residue with 3 drops of hydrochloric acid, add 10 mL of hot purified water, and warm for 2 minutes.
7.18.5.2. Then, add 1 drop of phenolphthalein TS, and ammonia TS dropwise until the solution develops a pale red color.
7.18.5.3. Add 2 mL of dilute acetic acid, and filter if necessary. Wash with 10 mL of purified water. Transfer the filtrate and washings to a Nessler tube, add 2.0 mL of Standard Lead Solution (0.008mg/mL Pb) and dilute to 50 mL with purified water.
7.18.6. Procedure:
7.18.6.1. To both the sample and the control, add 1 drop of Sodium Sulfide TS and mix thoroughly. Allow to stand for 5 minutes. Compare the colors of both solutions by viewing the tubes downward or transversely against a white background.

The test solution has no more color than the control solution to be reported as < 8ppm.

7.19. **IDENTIFICATION TEST (A) (EP)** **Refer to Summary Sheet:**

7.19.1. Refer to 7.34 pH of 5%.

7.19.2. Solution must be strongly alkaline (pH >10).

7.20. **IDENTIFICATION TEST (D) (EP)** **Refer to Summary Sheet:**

7.20.1. Set up HPLC with the following parameters:

Parameter	Setting
Flow Type	Isocratic
Mobile Phase	25% Water / 75% Acetonitrile
Flow Rate	1.0 mL/min
Injection Volume	20 µL
Detector	UV 210nm
Column Temperature	30^0C
Run Time	9 minutes
Column Phase	Agilent ZORBAX phase or equivalent

7.20.2. Prepare mobile phase.

7.20.3. Prepare a 0.2% tris reference solution well dissolved in mobile phase, scale as required.

7.20.4. Prepare a 0.2% tris sample solution well dissolved in mobile phase, scale as required.

7.20.5. Analyze a blank (mobile phase), tris reference solution and tris sample solution.

7.20.6. The retention time of primary peak in the sample solution should correspond to the primary peak in the reference solution to pass test.

7.21. **IDENTIFICATION TEST (C) EP, (A) (USP)** **Refer to Summary Sheet:**

7.21.1. Follow Spectrum Two UATR SOP.

7.22. **IDENTIFICATION TEST (B) USP** **Refer to Summary Sheet:**

7.22.1. To 4.5mL of a saturated solution of Salicylaldehyde, add 0.5mL of glacial acetic acid and mix in a 50mL beaker.

7.22.2. Dissolve 1g of sample in 5mL of purified water.

7.22.3. Transfer 4.0mL of the sample solution to the above 50mL beaker and mix. A yellow color should be produced.

7.23. **IDENTIFICATION TEST (C) USP** **Refer to Summary Sheet:**

7.23.1. 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.

7.23.2. Dissolve 1g of sample in 5mL of purified water.

7.23.3. Transfer 0.5mL of the sample solution to the above 50mL beaker and mix. The color should change from light yellow to orange.

7.24. **IDENTIFICATION TEST (A) (JPC 1997)** **Refer to Summary Sheet:**

7.24.1. Prepare a 1:20 solution of sample by weighing 1 gram of sample and diluting with 20 mL of purified water.

7.24.2. To 5 mL of the sample solution, add 5 drops of cupric sulfate TS. A purple color must develop to report as Passes Test.

7.25. **IDENTIFICATION TEST (B) (JPC 1997)** **Refer to Summary Sheet:**

7.25.1. Prepare a 1:3 solution of sample by weighing 1 gram of sample and diluting with 3 mL of purified water.

7.25.2. Prepare sodium nitrite TS before use by weighing 5.00 grams of sodium nitrite on an analytical balance. Transfer to a 50 mL volumetric flask. Dissolve and Q.S. with purified water.

7.25.3. To 1mL of sample solution, add 3mL of dilute sulfuric acid. Cool in an ice bath and add sodium nitrite TS dropwise. The solution must effervesce and evolve colorless gas to report as Passes Test.

7.26. **INSOLUBLE MATTER** **Refer to Summary Sheet:**

7.26.1. Accurately weigh 20.0g of sample and transfer to a 600mL beaker.

7.26.2. Add 200mL of purified water and utilize a Teflon encapsulated magnetic stirring bar and electric stir plate to dissolve sample.

7.26.3. Heat to boiling and digest on a hot plate in a covered beaker for 1 hour.

7.26.4. 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.

7.26.5. Filter sample solution through conditioned filtering crucible and 6-15micron filter. Rinse thoroughly with at least 3 crucible volumes of hot purified water.

7.26.6. Dry the crucible at 105° ± 2°C for 1 hour.

7.26.7. Cool in ambient air for 15 minutes and reweigh.

7.26.8. Calculate the % Insoluble Matter as follows:

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

7.27. **IRON** **Refer to Summary Sheet:**

7.27.1. Refer to 7.40: Trace metals for primary analysis.

7.27.2. Alternate EP Wet method:

7.27.3. Test Solution:

7.27.3.1. Weigh 1.0g of sample and dilute to 10 mL with purified water in a graduated cylinder.

7.27.4. Standard Solution:

7.27.4.1. Iron standard solution (20 ppm Fe) R: Immediately before use, dilute with water R to 10 times its volume a solution containing ferric ammonium sulfate R equivalent to 0.863 g ferric ammonium sulfate heptahydrate and 25 mL of dilute sulfuric acid R in 500.0mL.

7.27.4.2. Iron standard solution (1 ppm Fe) R: Immediately before use, dilute Iron Standard Solution (20 ppm Fe) R to 20 times its volume with water R.

7.27.4.3. Pipette 10.0 mL of Iron standard solution (1 ppm Fe) R into a graduated cylinder.

7.27.5. Procedure:
- 7.27.5.1. To both the test and standard solutions, add 2 mL of a 200 g/L citric acid solution R and 0.1 mL of thioglycolic acid R.
- 7.27.5.2. Mix, make alkaline with ammonia R and dilute to 20 mL with purified Water.
- 7.27.5.3. After 5 minutes, any pink color in the test solution is not more intense than that in the standard.

7.28. **LOSS ON DRYING** **Refer to Summary Sheet:**
- 7.28.1. Dry an LOD vial in the oven at 105 ± 2˚C for 30 minutes.
- 7.28.2. Cool for 15 minutes in a desiccator, weigh the LOD vial, and record results.
- 7.28.3. 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.
- 7.28.4. 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.
- 7.28.5. Place the LOD vial containing the sample into the oven and dry at 105°C ± 2°C for 3 hours.
- 7.28.6. Remove LOD vial from the oven and allow to cool in the desiccator for 15 minutes.
- 7.28.7. Reweigh the LOD vial and sample and retain the dried sample to perform the Assays.
- 7.28.8. Calculate the %LOD as follows:

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

7.29. **MICROBIAL CONTENT (TAMC/TYMC)** **Refer to Summary Sheet:**
- 7.29.1. Package no less than 35 grams of sample 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.

7.30. **MELTING RANGE/(Identification B EP)** **Refer to Summary Sheet:**
- 7.30.1. Refer to MP50 Melting Range Operation and Calibration SOP.

7.31. **SPECIFIED ORGANIC IMPURITIES** **Refer to Summary Sheet:**
- 7.31.1. The following impurities will be analyzed utilizing DCN:19-003129.
  - 7.31.1.1. 2-Nitroethanol
  - 7.31.1.2. 2-Nitropropane-1,3-Diol
  - 7.31.1.3. Tris(hydroxymethyl)nitromethane

7.32. **pH of a 0.1M SOLUTION @ 25 +/-2°C** **Refer to Summary Sheet:**
- 7.32.1. Accurately weigh 1.2g of sample. Transfer to a clean, dry 100mL graduated cylinder.
- 7.32.2. Q.S. to 100mL using purified water and dissolve.
- 7.32.3. Follow the appropriate SOP to measure and record the pH.

7.33. **pH of a 0.05M SOLUTION @ 25 +/-2°C** **Refer to Summary Sheet:**
- 7.33.1. Accurately weigh 0.6g of sample. Transfer to a clean, dry 100mL graduated cylinder.
- 7.33.2. Q.S. to 100mL using purified water and dissolve.

7.33.3. Follow the appropriate SOP to measure and record the pH.

7.34. **pH of a 5% or 1 in 20 SOLUTION @ 25 +/2°C Refer to Summary Sheet:**

7.34.1. Accurately weigh 5.0g of sample. Transfer to a suitable beaker.
7.34.2. Add 100mL of purified water and dissolve.
7.34.3. Follow the appropriate SOP to measure and record the pH.

7.35. **pH of a 1:100 SOLUTION @ 25 +/- 2°C Refer to Summary Sheet:**

7.35.1. Accurately weigh 1 grams of sample. Transfer to a suitable beaker.
7.35.2. Dissolve in 100 mL of purified water.
7.35.3. Follow the appropriate SOP to measure and record the pH.

7.36. **RELATED SUBSTANCES/UNSPECIFIED ORGANIC IMPURITIES Refer to Summary Sheet:**

7.36.1. Refer to DCN: 21-003960 for Primary Method via HPLC or DCN:19-003129 for secondary method via UPLC.
7.36.2. Alternate EP method for 1.0% specification
7.36.2.1. 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.
7.36.2.2. Test Solution (a):
7.36.2.2.1. Dissolve 0.20g in 1mL of purified water, with gentle heating, and dilute to 10mL with methanol R.
7.36.2.3. Test Solution (b):
7.36.2.3.1. Dilute 1mL of Test Solution (a) to 10mL with methanol R.
7.36.2.4. Reference Solution (a):
7.36.2.4.1. Dissolve 20mg of trometamol CRS in methanol R and dilute to 10mL with the same solvent.
7.36.2.5. Reference Solution (b):
7.36.2.5.1. Dilute 1mL of Test Solution (a) to 100mL with methanol R.
7.36.2.6. Procedure:
7.36.2.6.1. 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.
7.36.2.6.2. 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.
7.36.2.6.3. 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).

7.37. **RESIDUE ON IGNITION/SULFATED ASH Refer to Summary Sheet:**

7.37.1. NOTE: The USP General Chapter will be followed for USP, EP, and JPC 1997 testing, unless a dispute arises in which case the appropriate compendia chapter will be followed.
7.37.2. Turn on muffle furnace and allow it to stabilize at 600°C.Follow muffle furnace calibration procedure for operation of furnace.
7.37.3. Inspect a quartz crucible for cracks, chips and discoloration.

7.37.4. Utilize forceps to insert and remove the crucible from the furnace.
7.37.5. Ignite quartz crucible at 600 ± 50 °C for 30 minutes. Cool in a desiccator and weigh on an analytical balance.
7.37.6. For specification of 0.1% max:
7.37.6.1. Weigh 1.0 g sample in the previously ignited quartz crucible. Moisten the sample with 0.2 mL of sulfuric acid.
7.37.7. Volatilize the sample until the sample is thoroughly charred. Heat the sample slowly, so that the sample does not boil over and sample is not lost.
7.37.7.1. The rate of heating should be such that from ½ to 1 hour is required to volatilize the sample.
7.37.7.2. Continue to heat the sample until all the excess sulfuric acid has been volatilized.
7.37.8. Allow the sample to cool, and then moisten with 0.2mL of sulfuric acid.
7.37.9. 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.
7.37.9.1. The rate of heating should be such that from ½ to 1 hour is required to volatilize the sample.
7.37.9.2. Continue to heat the sample until all the excess sulfuric acid has been volatilized.
7.37.10. Ignite in the muffle furnace at 600 ± 50 °C for 15 minutes or until all carbon has been removed.
7.37.11. Cool in a desiccator for the same amount of time employed in the preparation of the crucible and weigh on an analytical balance.
7.37.12. Calculate the %ROI as follows:

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

7.37.13. 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.

7.38. **RESIDUAL SOLVENTS** **Refer to Summary Sheet:**
7.38.1. Prepare 10g of sample to submit to the outside testing facility for Methanol and Nitromethane analysis.

7.39. **SOLUBILITY** **Raw Material Only (Clear and Colorless):**
7.39.1. Weigh 100 grams of the sample.
7.39.2. Add 250 mL of purified water via graduated cylinder.
7.39.3. Gently heat and stir until all of the crystal is dissolved.
7.39.4. Solution should be clear and colorless.
7.39.4.1. If there is particulate matter present in the solution, filter using 1-5µm filter paper. If the particulates are removed, then the material is acceptable for manufacturing use only.

7.40. **TRACE METALS** **Refer to Summary Sheet:**

7.40.1. Refer to NexION 350X ICP MS SOP.

7.40.2. Refer to Analytical Method of Analysis: Trace Metal Impurities: Tris and THCl, DCN: 20-003601.

7.41. **WATER (by Karl Fischer Titration)** **Refer to Summary Sheet:**

7.41.1. Perform a standardization of the titrant (Composite 5) as per Standardization of Titrants.

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

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

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

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

7.41.5. 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.

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

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

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

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

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

8. COMPENDIAL DIFFERENTIATIONS

8.1. COMPENDIAL ANALYSES

USP Compendia	EP Compendia	JPC 1997
Identification B	Appearance of Solution	Clarity and Color of Solution
Identification C	Chlorides	Identification A
Chloride	Identification D	Identification B
		pH 1:100

8.2. Harmonized Methods

Analysis Name
Identification A (USP-NF), Identification C (EP)
Loss on Drying (USP-NF), (EP), (JPC 1997)
Melting Range or Temperature (USP-NF), Identification B (EP), Melting Point (JPC 1997)
pH (USP-NF), (EP), Identification A (EP)
Residue on Ignition (USP-NF), Residue on Ignition (JPC 1997), Sulfated Ash (EP)

8.3. In-House Validated Methods in accordance with USP General Chapters:

- <1225> Validation of Compendial Procedures

Analysis Name
Arsenic for JPC 1997 analysis
Assay (Dried Basis)
Elemental Impurities
Endotoxins
Formaldehyde
Heavy Metals /Trace Metals/Arsenic/Iron
Identification D for the EP monograph analysis
Organic Impurities
Related Substances for the EP monograph analysis
Water (by KF Titration)

8.4. In house Methods for Product Quality Description

Analysis Name
Appearance and Color
Solubility

8.5. Customer Requested Methods

Analysis Name
Absorbance (1M)
Absorbance 10%
Absorbance (40%)
APHA Color, 20% Solution
Assay (Ultra-Pure)
Enzyme Activity
Insoluble Matter
Microbial Content
pH (0.1M), pH (0.05M)

8.6. Outside Laboratory Analysis

Analysis Name
Residual Solvents: Methanol and Nitromethane
Microbial Content