BIOSPECTRA
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TRIS TESTING METHODS

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1. PURPOSE:

1.1. To provide the Laboratory personnel with a procedure for analyzing Tris.

2. SCOPE:

2.1. Applies to examination of Tris Raw Materials, In Process, Stability, and Finished Goods in the 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 Laboratory Manager is responsible for the control, training, maintenance and implementation of this procedure.
3.2. The Laboratory Analysts are responsible for compliance with the terms of this procedure. This includes notifying Quality Assurance and Laboratory Managers, or designees, if any analyses fail to meet their respective specifications.
3.3. The laboratory analysts are responsible for referring to the appropriate batch record and summary sheet for specifications for analyses of in-process and finished goods samples.

4. REFERENCES:

4.1. BSI-ATM-0050, Analytical Method: Quantification of Formaldehyde by Derivatization with Pentafluorobenzylhydroxyl Amine by GC MS
4.2. BSI-ATM-0058, Analytical Method of Analysis: Determination of Trace Metal Impurities by ICP-MS in Tris and Tris Hydrochloride
4.3. BSI-ATM-0059, Analytical Method of Analysis: Determination of ICH Q3D Elemental Impurities by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in Tris API
4.4. BSI-ATM-0062, Tris Identity and Related Substances via HPLC
4.5. BSI-ATM-0089, Analytical Method of Analysis: Trace Metals in Finished Goods Products by ICP-OES
4.6. BSI-ATM-0111, Assay of Tromethamine via GC-FID
4.7. BSI-ATM-0112, Tromethamine Unspecified Degradation Products via GC-FID
4.8. BSI-ATM-0131, Analytical Method for the Determination of Trace Metals in BioTech Products
4.9. BSI-MEM-1248, Tris Compendia Equivalency Summary
4.10. BSI-SOP-0090, Lambda 25 UV/Vis Operation and Calibration
4.11. BSI-SOP-0091, Portable Turbidimeter SOP and Calibration
4.12. BSI-SOP-0094, Muffle Furnace SOP and Calibration
4.13. BSI-SOP-0095, DNase (Endonuclease) Assay
4.14. BSI-SOP-0096, RNase (Ribonuclease) Assay
4.15. BSI-SOP-0098, Balance SOP
4.16. BSI-SOP-0126, Laboratory Notebooks
4.17. BSI-SOP-0133, Blue M Convection Oven Operation and Calibration SOP
4.18. BSI-SOP-0138, DNase (Exonuclease) Assay
4.19. BSI-SOP-0139, Protease Assay
4.20. BSI-SOP-0140, Standardization of Titrants

4.21. BSI-SOP-0143, Metrohm Titrando 907 Auto-Titrator SOP
4.22. BSI-SOP-0144, Metrohm 914 pH Conductometer Operation and Calibration
4.23. BSI-SOP-0242, Portable Turbidimeter Operation and Calibration
4.24. BSI-SOP-0254, Spectrum Two UATR SOP
4.25. BSI-SOP-0255, XL200 pH/mV/Conductivity Meter SOP
4.26. BSI-SOP-0256, MP50 Melting Range Operation, Verification and Calibration SOP
4.27. BSI-SOP-0303, NexION 350X ICP-MS SOP
4.28. BSI-SOP-0316, Shimadzu QP2010S GC/MS SOP
4.29. BSI-SOP-0422, Empower 3 General Procedure
4.30. BSI-SOP-0430, Tris Organic Impurities via UPLC
4.31. BSI-SOP-0573, MP90 Melting Range Operation, Verification, and Calibration SOP
4.32. BSI-SOP-0668, OPI-180 OD Handheld Colorimeter SOP
4.33. ACQUITY UPLC Quaternary Solvent Manager PLUS Series USP <621> Chromatography
4.34. ACQUITY UPLC TUV Detector Operator's Overview and Maintenance Guide
4.35. ACS, Reagent Chemicals, current edition
4.36. Current ChP
4.37. Current EP
4.38. Current USP
4.39. JPC edition

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. MP90 Melting Range Apparatus
5.8. Muffle Furnace
5.9. OPI-180 OD Handheld Colorimeter
5.10. Perkin Elmer NexION 350X ICP-MS
5.11. Perkin Elmer Avio 500 ICP-OES
5.12. Perkin Elmer Spectrum Two UATR
5.13. Perkin Elmer Flexar HPLC
5.14. XL200 pH/Conductivity Meter or equivalent
5.15. Waters H-Class HPLC/ UPLC or equivalent
5.16. Shimadzu QP2010 GC-MS with GC-FID Detector

6. REAGENTS:

6.1. **0.1N HCl:** Purchased Commercially.
6.2. **0.1N Silver Nitrate:** Purchased Commercially.
6.3. **1N Acetic Acid**: Dilute 60.0 mL of glacial acetic acid with water to make 1000 mL.
6.4. **200g/L Citric Acid Solution R**: Weigh 20 g of citric acid and dilute to 100 mL with Water R.
6.5. **2-Propanol R**: Purchased Commercially.

6.6. **50 0ppm Chloride Stock Solution**: Weigh 0.0824 g of NaCl and dilute to 100 mL with water R.

6.7. **5 g/L Potassium Permanganate in a 10 g/L Solution of Sodium Carbonate R**: Dissolve 1.0 g of Potassium Permanganate and 2.0 g of Sodium Carbonate in water R to make a total volume of 200 mL.

6.8. **6N Ammonium Hydroxide**: Prepare by diluting 400 mL of Ammonia Water, Stronger with water to make 1000 mL.

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. **Bromocresol Purple TS:** Dissolve 250 mg of bromocresol purple in 20 mL of 0.05N Sodium Hydroxide in a 250 mL volumetric flask. Dilute to volume with purified water.

6.14. **Ceric Ammonium Nitrate in 2N Nitric Acid:** Dissolve 40 g of Ceric Ammonium Nitrate in 2.0N Nitric Acid to make a total volume of 100 mL.

6.15. **Citric Acid Solution R,** (20% w/v or 200 g/L): Weigh 20 g of citric acid and dilute to 100 mL with water R.

6.16. **Cupric Sulfate TS:** Dissolve 12.5 g of Cupric Sulfate in purified water to make 100 mL.

6.17. **Dilute Acetic Acid:** Dilute 6 g of acetic acid (100) with water to make 100 mL (1 mol/L).

6.18. **Dilute Ammonia R1**: Dilute 41 g of concentrated ammonia R to 100 mL with water R.

6.19. **Dilute Nitric Acid R**: Dilute 20 g of Nitric Acid to 100 mL with Water R.

6.20. **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 100 mL (10%).

6.21. **Dilute Sulfuric Acid (EP)**: Add 5.5 mL of sulfuric acid R to 60 mL of water R, allow to cool and dilute to 100 mL with the same solvent.

6.22. **Glacial Acetic Acid**: Purchased Commercially.

6.23. **Glycerin:** Purchased Commercially.

6.24. **Glycerin Base TS:** To 200 g of glycerol, add purified water to bring the total weight to 235 g. Add 140 mL of 1N Sodium Hydroxide and 50 mL of purified water.

6.25. **Hydrochloric Acid, concentrated:** Purchased Commercially.

6.26. **Hydrochloric Acid (0.02N):** Slowly add 20 mL of 0.1N Hydrochloric Acid to 80 mL of purified water to make a total volume of 100 mL.

6.27. **Lead Nitrate Stock Solution (USP/EP/JPC)**: Weigh exactly 159.8 mg of Lead (II) nitrate and dissolve in 10 mL of dilute nitric acid and add water to make exactly 1000 mL. Prepare and store this solution using glass containers, free from soluble lead salts.

6.28. **LAL Reagent Water**: Purchased Commercially.

6.29. **Methanol R:** Purchased Commercially.

6.30. **Nitric Acid, Concentrated:** Purchased Commercially.

6.31. **pH 3.5 Acetate Buffer**: Dissolve 62.5 g of ammonium acetate in 62.5 mL of purified water, and add 47.0 mL 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 250 mL.

6.32. **Phenolphthalein TS**: Dissolve 1 g of phenolphthalein in 100 mL of ethanol (95).

6.33. **Reference Solution B_9**: Prepare immediately before use. Transfer 1.0 mL of Standard Solution B to 99.0 mL of 1% HCl.

6.34. **Salicylaldehyde:** Purchased Commercially.
6.35. **Silver Nitrate Solution R2**: See 0.1N $AgNO_3$.
6.36. **Sodium Nitrite:** Purchased Commercially.
6.37. **Solution S** (may be scaled as needed): Weigh 2.5 g of sample and dissolve in purified water. Dilute to a total volume of 50 mL with purified water.
6.38. **Sulfuric Acid, concentrated**: Purchased Commercially
6.39. **Thioacetamide TS**: Dissolve 4.0 g of thioacetamide in 100 mL of purified water.
6.40. **Thioglycolic Acid R**: Purchased Commercially.
6.41. **Tris IR Reference Standard:** Prepare a vial at 105°C for 30 minutes. Allow to cool in desiccator and weigh a maximum 10.0 g 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.
6.42. **Trometamol R/ Tromethamine CRS**: Purchased Commercially. Secondary reference standards may be used.

7. ANALYTICAL PROCEDURES:

7.1. ABSORBANCE (MOTHER LIQUOR):

7.1.1. **Note: ML Absorbance will be performed using 1cm cuvettes for all wavelengths.**
7.1.2. Prepare 10 mL of a 1:1 dilution with purified water of the submitted ML sample. Prepare by pipetting 5 mL of submitted ML into an LOD vial or small beaker. Add 5 mL of purified water to the same beaker/vial. Mix thoroughly.
7.1.3. Refer to Lambda 25 UV/Vis Operation and Calibration to measure the Absorbance of the sample.
7.1.4. Record results at specified wavelengths in the appropriate laboratory documentation and Batch Record.
7.1.4.1. Notify appropriate personnel if the results are within the action limits listed in the appropriate batch record.

7.2. ML ASSAY:

7.2.1. Perform a daily check or standardization of 0.1N HCl as per Standardization of Titrants.
7.2.2. Accurately weigh 0.5 g of ML sample.
7.2.3. Transfer accurately weighed sample to a suitable beaker.
7.2.4. Dissolve in 100 mL of water.
7.2.5. Add bromocresol purple TS.
7.2.6. Titrate with 0.1N HCl VS to a yellow endpoint.
7.2.7. Each mL of titrant is equivalent to 12.114 mg of Tris.
7.2.8. Calculate % Assay using the following equation:

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

7.3. ABSORBANCE (1M):

7.3.1. Prepare a 1M solution of the specified sample for the applicable wavelength measurements (250-300 nm range).
7.3.1.1. Accurately weigh 3.03 g of sample.

7.3.1.2. Transfer accurately weighed sample to a graduated cylinder and Q.S. to 25 mL with purified water.

7.3.1.3. Swirl to dissolve completely.

7.3.2. Prepare a 1M solution of the specified sample for the 400nm and 430nm measurement following steps 7.3.1.1-7.3.1.3, above.

7.3.2.1. **The 400 nm and 430 nm wavelength measurements require the use of 10 cm cuvettes.**

7.3.3. Refer to Lambda 25 UV/Vis Operation and Calibration to measure the Absorbance of the sample.

7.3.4. Analyze the following wavelengths on 2 different instrument methods:

7.3.4.1. One method will include 250 nm, 260 nm, 270, 280 nm, 290 nm, and 300 nm. Report results that are applicable to the testing required.

7.3.4.1.1. Requires the use of a 1cm Cuvette.

7.3.4.2. One method will include 400 nm and 430 nm. Report results that are applicable to the testing required.

7.3.4.2.1. Requires the use of a 10 cm Cuvette.

7.3.5. To report the 400 nm and 430 nm result perform the following calculation:

7.3.5.1. 400 nm Result = $\frac{Instrument\ Result\ a.u.}{10}$

7.3.5.2. 430 nm Result = $\frac{Instrument\ Result\ a.u.}{10}$

7.4. **ABSORBANCE (0.2M):**

7.4.1. Prepare a 0.2M solution of the specified sample for the applicable wavelength measurements (250-300 nm range).

7.4.1.1. Accurately weigh 0.6 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.

7.4.3. Analyze the sample on the method that includes the 250 nm, 260 nm, 270 nm, 280 nm, 290 nm and 300 nm.

7.4.3.1. Requires the use of a 1 cm Cuvette.

7.5. **ABSORBANCE (10% Solution):**

7.5.1. Prepare a 10% solution of the specified sample for the applicable wavelength measurements (250-300 nm range).

7.5.1.1. Accurately weigh 2.5 g of sample.

7.5.1.2. Transfer accurately weighed sample to a graduated cylinder and Q.S. to 25 mL with purified water.

7.5.1.3. Swirl to dissolve completely.

7.5.1.3.1. Sonicate if necessary to accelerate dissolution. Allow to cool to room temperature before analysis, if applicable.

7.5.2. Prepare a 10% solution of the specified sample for the 400 nm and 430 nm measurement following steps 7.4.1.1-7.4.1.3, above.

7.5.2.1. **The 400 nm and 430 nm wavelength measurements require the use of 10cm cuvettes.**

7.5.3. Refer to Lambda 25 UV/Vis Operation and Calibration to measure the Absorbance of the sample.

7.5.4. Analyze the following wavelengths on 2 different instrument methods:

7.5.4.1. One method will include 250 nm, 260 nm, 270, 280 nm, 290 nm, and 300 nm. Report results that are applicable to the testing required.

7.5.4.1.1. Requires the use of a 1 cm Cuvette.

7.5.4.2. One method will include 400 nm and 430 nm. Report results that are applicable to the testing required.

7.5.4.2.1. Requires the use of a 10 cm Cuvette.

7.5.5. To report the 400 nm and 430 nm result perform the following calculation:

7.5.5.1. 400 nm Result = $\frac{Instrument\ Result\ a.u.}{10}$

7.5.5.2. 430 nm Result = $\frac{Instrument\ Result\ a.u.}{10}$

7.6. **ABSORBANCE (40% Solution):**

7.6.1. Prepare a 40% solution of the specified sample for the applicable wavelength measurements (250-300 nm range).

7.6.1.1. Accurately weigh 10 g of sample.

7.6.1.2. Transfer accurately weighed sample to a graduated cylinder and Q.S. to 25 mL with purified water.

7.6.1.3. Swirl to dissolve completely.

7.6.1.3.1. Sonicate if necessary to accelerate dissolution. Allow to cool to room temperature before analysis, if applicable.

7.6.2. Refer to Lambda 25 UV/Vis Operation and Calibration to measure the Absorbance of the sample.

7.6.3. Analyze with the following wavelength instrument method:

7.6.3.1. The method includes 250 nm, 260 nm, 270, 280 nm, 290 nm, and 300 nm. Report results that are applicable to the testing required.

7.6.3.1.1. Requires the use of a 1cm Cuvette.

7.6.4. To report 400nm and 430 nm measurements at this concentration prepare a separate 40% solution of the specified sample utilizing steps 7.5.1.1-7.5.1.3.1, above.

7.6.5. Refer to Lambda 25 UV/Vis Operation and Calibration to measure the Absorbance of the sample.

7.6.6. Analyze with the following wavelength instrument method:

7.6.6.1. The method that includes 400 nm and 430 nm. Report results that are applicable to the testing required.

7.6.6.1.1. Requires the use of a 10cm Cuvette.

7.6.7. To report the 400 nm and 430 nm result perform the following calculation:

7.6.7.1. 400 nm Result = $\frac{Instrument\ Result\ a.u.}{10}$

7.6.7.2. 430 nm Result = $\frac{Instrument\ Result\ a.u.}{10}$

7.7. **APHA COLOR, 20% SOLUTION:**

7.7.1. Sample Solution:

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

7.7.2. APHA 20 Standard Solution:

7.7.3. Pipette 2.0 mL of APHA no. 500 Pt-Co Standard into a 50-mL volumetric flask and mix thoroughly. Transfer to a 50 mL Nessler Color Comparison tube. 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.

7.8. **APPEARANCE AND COLOR:**

7.8.1. Place 25-50 g of the sample in a clean, dry glass beaker.

7.8.2. In an area with sufficient lighting, view the sample from all sides.

7.8.3. The sample should be white in color and characteristic of crystals.

7.8.4. 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.

7.8.5. If the sample does not conform to these specifications, notify Laboratory Management immediately.

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

7.9. **APPEARANCE OF SOLUTION (EP):**

7.9.1. Clear *(2.2.1.)* Turbidimetry

7.9.1.1. Rinse the sample bottle with Solution S twice.

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

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

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

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

7.9.1.6. Follow the appropriate SOP as follows:

7.9.1.6.1. Stroudsburg- Measure and record the turbidity of the sample according to Portable Turbidimeter Operation and Calibration SOP.

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

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

7.9.2. Colorless (*2.2.2. Method II*)

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

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

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

7.9.2.4. 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 oris not more intensely colored than reference solution B_9.

7.10. **ARSENIC:**

7.10.1. Refer to section 7.43 Trace Metals.

7.11. **ASSAY (USP/EP/ChP/JPC) dried basis:**

7.11.1. NOTE: This method will be followed to report as the result for all applicable compendia referenced in product specifications: USP, EP, ChP, and JPC.

7.11.2. Perform a daily check or standardization of the manual 0.1N HCl titrant as per Standardization of Titrants.

7.11.3. Crush a 1-3 g sample to a fine powder with a non-reactive mortar and pestle for 60-90 seconds.

7.11.4. Take special care to crush any larger crystals.

7.11.5. Dry the crushed material at room temperature (22° to 23°C) for 24 h in a desiccator over desiccant.

7.11.6. Accurately weigh 0.250 g of Tris previously dried as indicated above.

7.11.7. Transfer accurately weighed sample to a suitable clean, glass beaker. Dissolve in 100 mL of water.

7.11.8. Add bromocresol purple TS and titrate with 0.1N hydrochloric acid VS to a yellow endpoint.

7.11.9. Each mL of 0.1*N* HCl is equivalent to 12.114 mg of Tris:

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

7.12. **ASSAY (Ultrapure) dried basis:**

7.12.1. Perform a daily check or potentiometric standardization of 0.1N HCl as per Standardization of Titrants.

7.12.2. Crush a 1-3 g sample to a fine powder with a non-reactive mortar and pestle for 60-90 seconds.

7.12.3. Take special care to crush any larger crystals.

7.12.4. Dry the crushed material at room temperature (22° to 23°C) for 24h in a desiccator over desiccant.

7.12.5. Accurately weigh 0.5 g of Tris previously dried as indicated above.

7.12.6. Transfer accurately weighed sample to a suitable clean, glass beaker.

7.12.6.1. Ensure glass beaker has been previously rinsed with purified water and dried before transferring material to beaker.

7.12.7. Dissolve in an appropriate amount of water and rinse all sides of the beaker to ensure no sample is lost (ensure that the sample dissolves, the electrode is covered, and/or the titration vessel will not overflow after titrant addition).

7.12.8. Titrate the solution with 0.1N HCl using a potentiometric endpoint determination.

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

7.13. **Assay (GC-FID):**

7.13.1. Refer to BSI-ATM-0111, Assay of Tromethamine via GC-FID for sample preparation and analysis.

7.14. **CHLORIDES:**

EP/ChP Method (0.01% max)

7.14.1. Sample Solution:

7.14.1.1. To 10 mL of Solution S add 2.5 mL of dilute nitric acid R in a test-tube.
7.14.1.2. Dilute to 15 mL with purified water.

7.14.2. Standard Solution:
7.14.2.1. Immediately before use, dilute 0.1 mL of 500 ppm Chloride Stock Solution to a total of 10 mL with purified water, in order to prepare chloride standard solution (5 ppm Cl) R.
7.14.2.2. Transfer to a test tube and add 5 mL of purified water.

7.14.3. Procedure:
7.14.3.1. To both the sample and standard solutions, add 1 mL of dilute nitric acid R and 1 mL of silver nitrate solution R2.
7.14.3.2. Compare the standard and samples solutions against a black background.
7.14.3.3. Allow to stand for 5 minutes, using a calibrated timer, protected from light.
7.14.3.4. Any opalescence in the test solution is not more intense than that in the standard.

USP Method (0.002% max)

7.14.4. Standard Preparation:
7.14.4.1. Pipette 0.057 mL of 0.02N HCl into a Nessler Color Comparison Tube and dilute to approximately 40 mL with purified water.

7.14.5. Sample Preparation:
7.14.5.1. Weigh 2.0 g of sample and dissolve in approximately 40 mL of purified water. If necessary, neutralize the solution with nitric acid to litmus.

7.14.6. Procedure:
7.14.6.1. Add to each solution, 1 mL of concentrated nitric acid and 1 mL 0.1N silver nitrate.
7.14.6.2. Q.S. to 50 mL with purified water. Cover with parafilm and mix by inversion.
7.14.6.3. 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.

7.15. **CLARITY AND COLOR OF SOLUTION (ChP/JPC 1997):**

7.15.1. Dissolve 1.0 g of sample in 10 mL of purified water. The solution must be clear and colorless.

7.16. **ENDOTOXIN:**

7.16.1. Sample Preparation using Endosafe Nexgen PTS Endotoxin Reader**:**
7.16.1.1. Accurately weigh 100 mg of sample into a sterile tube. Add 70 µL of concentrated HCl. Dilute to 10 mL with LAL reagent water, dissolve, and mix thoroughly.
7.16.1.2. Prepare a 1:1 dilution from the initial sample preparation and LAL reagent water for a final concentration of 0.0050 g/mL.
7.16.1.3. Analyze using a 0.5 - 0.005 EU/mL high sensitivity LAL cartridge.
7.16.1.4. Refer to Endosafe Nexgen PTS Endotoxin Reader SOP for instrument operation.

7.17. **ELEMENTAL IMPURITIES:**

7.17.1. Refer to Determination of Elemental Impurities by ICP-MS in Tris, DCN: BSI-ATM-0059.

7.18. **ENZYME ACTIVITY:**

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

7.19. **FORMALDEHYDE:**

7.19.1. Refer to Analytical method: Quantification of Formaldehyde by GC-MS, DCN: BSI-ATM-0050.

7.20. **HEAVY METALS as Pb:**

7.20.1. Refer to 7.43 Trace metals for primary analysis.

7.20.2. EP Test Sample preparation:

7.20.2.1. Dissolve 2.0 g Tris in 20 mL of purified water, and add 1.2 mL of concentrated hydrochloric acid to a 50-mL color-comparison tube.

7.20.3. 5 ppm limit specification – Test sample Preparation:

7.20.3.1. 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.

7.20.4. 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.

7.20.5. 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.

7.20.6. Test Preparation - In the 50-mL color comparison tube prepared above, dilute with purified water to 25 mL.

7.20.7. 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.

7.20.8. Procedure:

7.20.8.1. 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.

7.20.8.2. Dilute each tube with purified water to 40 mL and mix.

7.20.8.3. 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.

7.20.8.4. Allow to stand for 2 minutes.

7.20.8.5. 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.

7.21. **HEAVY METALS (ChP/JPC 1997):**

7.21.1. Refer to 7.43 Trace metals for primary analysis.

7.21.2. Alternate Wet Method:

7.21.3. Standard Lead Solution (0.008 mg/mL):

7.21.3.1. Immediately before use, dilute 8.0 mL of Lead Nitrate Stock Solution (~100 ppm) to 100 mL with purified water in a volumetric flask.

7.21.4. Sample Preparation:

7.21.4.1. Accurately weigh 2.0 g of sample and place in a quartz or porcelain crucible, cover loosely with a lid, and carbonize by gentle ignition.

7.21.4.2. 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.

7.21.4.3. 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.

7.21.4.4. Then, add 1 drop of phenolphthalein TS, and ammonia TS dropwise until the solution develops a pale red color.

7.21.4.5. 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.

7.21.5. Control Preparation:

7.21.5.1. 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.

7.21.5.2. Then, add 1 drop of phenolphthalein TS, and ammonia TS dropwise until the solution develops a pale red color.

7.21.5.3. 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 (0.008 mg/mL Pb) and dilute to 50 mL with purified water.

7.21.6. Procedure:

7.21.6.1. 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 < 8 ppm.

7.22. **IDENTIFICATION TEST (EP-A):**

7.22.1. Refer to 7.37 pH of 5%.

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

7.23. **IDENTIFICATION TEST (EP-D), (ChP-2):**

7.23.1. Refer to DCN: BSI-ATM-0062 for Primary Method via HPLC.

7.24. **IDENTIFICATION TEST (USP-A), (ChP-3), (EP-C):**

7.24.1. Follow Spectrum Two UATR SOP.

7.25. **IDENTIFICATION TEST (USP-B), (ChP-1):**

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

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

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

7.26. **IDENTIFICATION TEST (USP-C):**

7.26.1. Prepare a 4 in 10 solution of Ceric Ammonium Nitrate in 2 N Nitric Acid. Transfer 0.5 mL of the resulting solution to a 50-mL beaker and add 3 mL of purified water.

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

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

7.27. **IDENTIFICATION TEST (A) (JPC 1997):**

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

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

7.28. **IDENTIFICATION TEST (B) (JPC 1997):**

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

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

7.28.3. To 1 mL of sample solution, add 3 mL 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.

7.29. **INSOLUBLE MATTER:**

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

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

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

7.29.4. Prepare a Gooch filtering crucible and 6-15-micron filter by drying at 105°C ± 2°C for 1 hour. Allow to cool in ambient air for 15 minutes and weigh.

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

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

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

7.29.8. Calculate the % Insoluble Matter as follows:

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

7.30. **IRON:**

7.30.1. Refer to 7.43 Trace metals for primary analysis.

7.30.2. Alternate EP Wet method:

7.30.3. Test Solution:

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

7.30.4. Standard Solution:

7.30.4.1. 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 500.0 mL.

7.30.4.2. 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.

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

7.30.5. Procedure:

7.30.5.1. 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.

7.30.5.2. Mix, make alkaline with ammonia R and dilute to 20 mL with purified Water.

7.30.5.3. After 5 minutes, any pink color in the test solution is not more intense than that in the standard.

7.31. **LOSS ON DRYING (USP/ChP/EP/JP):**

7.31.1. Dry an LOD vial in the oven at 105 ± 2˚C for 30 minutes.

7.31.2. Cool for 15 minutes in a desiccator, weigh the LOD vial, and record results.

7.31.3. If the substance to be tested is in the form of large crystals, reduce the particle size to about 2 mm by quickly crushing before weighing.

7.31.4. Transfer approximately 1-2 g 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.

7.31.5. Place the LOD vial containing the sample into the oven and dry at 105°C ± 2°C for 3 hours.

7.31.6. Remove LOD vial from the oven and allow to cool in the desiccator for 15 minutes.

7.31.7. Reweigh the LOD vial and sample and retain the dried sample to perform the Assays.

7.31.8. Calculate the %LOD as follows:

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

7.32. **MICROBIAL CONTENT (USP<61><62>):**

7.32.1. Package no less than 65 g 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 gram, *Staphylococcus aureus* Test for Absence per 1 gram.

7.32.2. Additional Product Codes may require Bile tolerant Gram-Negative Bacteria and *Candida albicans.* Bile tolerant Gram-Negative Bacteria Test for Absence per 1gram, *Candida albicans* Test for Absence per 1gram.

7.32.3. MPL Suitability Report Number to document ARF:

7.32.3.1. MPL Suitability Report #11M6239A is applicable to TAMC/TYMC, E.Coli, Salmonella, Pseudomonas aeruginosa, Staphylococcus aureus.

7.32.3.2. MPL Suitability Report #18P4320A is applicable to Bile tolerant Gram Negative and Candida albicans.

7.33. **MELTING RANGE/(Identification B EP), (USP/ChP/JP-C):**

7.33.1. Refer to BSI-SOP-0256, MP50 Melting Range Operation and Calibration SOP, or BSI-SOP-0573, MP90 Melting Range Operation, Verification, and Calibration SOP.

7.34. **SPECIFIED ORGANIC IMPURITIES:**

7.34.1. The following impurities will be analyzed utilizing DCN: BSI-SOP-0430.

7.34.1.1. 2-Nitroethanol
7.34.1.2. 2-Nitropropane-1,3-Diol
7.34.1.3. Tris (hydroxymethyl)nitromethane

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

7.36. **pH of a 0.05M SOLUTION @ 25 +/-2°C:**
7.36.1. Accurately weigh 0.6 g of sample. Transfer to a clean, dry 100-mL graduated cylinder.
7.36.2. Q.S. to 100 mL using purified water and dissolve.
7.36.3. Follow the appropriate SOP to measure and record the pH.

7.37. **pH of a 5% or 1 in 20 SOLUTION @ 25 +/2°C (USP/EP-IDA/ChP):**
7.37.1. Accurately weigh 5.0 g of sample. Transfer to a suitable beaker.
7.37.2. Add 100 mL of purified water and dissolve.
7.37.3. Follow the appropriate SOP to measure and record the pH.

7.38. **pH of a 1:100 SOLUTION @ 25 +/- 2°C:**
7.38.1. Accurately weigh 1 g of sample. Transfer to a suitable beaker.
7.38.2. Dissolve in 100 mL of purified water.
7.38.3. Follow the appropriate SOP to measure and record the pH.

7.39. **RELATED SUBSTANCES (ChP/EP):**
7.39.1. Refer to DCN: BSI-ATM-0062 for Primary Method via HPLC.
7.39.2. Alternate EP method for 1.0% specification.
7.39.2.1. 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.
7.39.2.2. Test Solution (a):
7.39.2.2.1. Dissolve 0.20 g in 1 mL of purified water, with gentle heating, and dilute to 10 mL with methanol R.
7.39.2.3. Test Solution (b):
7.39.2.3.1. Dilute 1 mL of Test Solution (a) to 10 mL with methanol R.
7.39.2.4. Reference Solution (a):
7.39.2.4.1. Dissolve 20 mg of trometamol CRS in methanol R and dilute to 10 mL with the same solvent.
7.39.2.5. Reference Solution (b):
7.39.2.5.1. Dilute 1 mL of Test Solution (a) to 100 mL with methanol R.
7.39.2.6. Procedure:
7.39.2.6.1. Apply to the plate 10 µL of each solution. Develop over a path of 10 cm using a mixture of 10 volumes of dilute ammonia R1 and 90 volumes of 2-propanol R.
7.39.2.6.2. 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.

7.39.2.6.3. 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).

7.40. **RESIDUE ON IGNITION/SULFATED ASH (USP/ChP/EP/JPC):**

7.40.1. NOTE: The USP General Chapter will be followed to report as the result for all applicable compendia referenced in product specifications: USP, EP, ChP, and JPC.

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

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

7.40.4. Utilize forceps to insert and remove the crucible from the furnace.

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

7.40.6. For specification of 0.1% max:

7.40.6.1. Weigh 1.0 g sample in the previously ignited quartz crucible. Moisten the sample with 0.2 mL of sulfuric acid.

7.40.7. 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.

7.40.7.1. The rate of heating should be such that from ½ to 1 hour is required to volatilize the sample.

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

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

7.40.9. 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.

7.40.9.1. The rate of heating should be such that from ½ to 1 hour is required to volatilize the sample.

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

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

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

7.40.12. Calculate the %ROI as follows:

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

7.40.13. If the amount of the residue exceeds the limit specified, repeat the moistening with sulfuric acid using up to 1 mL, 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 0.0005 g or until the specification is met.

7.41. **RESIDUAL SOLVENTS:**

7.41.1. Prepare 10 g of sample to submit to the outside testing facility for Methanol and Nitromethane analysis.

7.41.1.1. Reference the following Method numbers on the testing Analysis Request Form:

7.41.1.1.1. For Methanol: MV-AATL-20-01

7.41.1.1.2. For Nitromethane: MV-AATL-20-02

7.42. **SOLUBILITY:**

7.42.1. Weigh 100 g of the sample.

7.42.2. Add 250 mL of purified water via graduated cylinder.

7.42.3. Gently heat and stir until all of the crystal is dissolved.

7.42.4. Solution should be clear and colorless.

7.42.4.1. If there is particulate matter present in the solution, filter using filter paper. If the particulates are removed, then the material is acceptable for manufacturing use only.

7.43. **TRACE METALS:**

7.43.1. Available methods dependent on product code requirement:

7.43.1.1. Refer to Analytical Method of Analysis: Trace Metal Impurities: Tris and THCl, DCN: BSI-ATM-0058.

7.43.1.2. Refer to Analytical Method of Analysis: Trace Metals in Finished Goods Products by ICP-OES, DCN: BSI-ATM-0089.

7.43.1.3. Refer to Analytical Method for the Determination of Trace Metals in BioTech Products, DCN: BSI-ATM-0131.

7.44. **UNSPECIFIED IMPURITIES/TOTAL IMPURITIES:**

7.44.1. **Unspecified Impurities Analysis:**

7.44.1.1. Refer to BSI-ATM-0112 for sample preparation and analysis.

7.44.2. **Total Impurities Reporting Structure:**

7.44.2.1. Organic Impurity analysis will be performed on 3 different methods:

7.44.2.1.1. BSI-SOP-0430 will be used for the detection of the specified organic impurities listed in section 7.34.

7.44.2.1.2. BSI-ATM-0050 will be used for the detection of Formaldehyde, section 7.18.

7.44.2.1.3. BSI-ATM-0112 will be used for the detection of the Unspecified Impurities.

7.44.2.2. Total Impurities will be reported as ≤ 300 ppm for the final result as long as the Specified Impurities are less than or equal to the specification and Unspecified Impurities is less than or equal to the specification**.**

7.44.2.2.1. Limit of Detection for Unspecified Impurities method is 300 ppm. Any detection of any unspecified impurity would lead to a batch failure.

7.45. **WATER (by Karl Fischer Titration):**

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

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

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

7.45.4. Transfer the sample to the KF vessel by removing the rubber septum and adding the sample into the titration vessel.
7.45.4.1. Do not leave the rubber septum open for long periods of time as this will allow moisture to enter the titration vessel.
7.45.5. 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.
7.45.6. Check to make sure there is no residual sample stuck to the sides of the titration vessel.
7.45.6.1. If there is any sample stuck to the side, stop the stir bead from spinning before swirling the vessel to rinse the sides.
7.45.7. Once the method begins, check to ensure the sample is fully dissolved before the titration begins (i.e. before the stir command completes).
7.45.8. 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:

Table 1: Compendial Analyses

USP Compendia	ChP Compendia	EP Compendia	JPC 1997
Assay USP (dried basis)	Assay (dried basis)	Appearance of Solution	Assay (dried basis)
Chloride	Chlorides	Assay (dried basis)	Clarity and Color of Solution
Identification A (IR)	Clarity and Color of Solution	Chlorides	Heavy Metals (as Pb)
Identification B	Heavy Metals (as Pb)	Identification A (pH 5%)	Identification A
Identification C	Identification 1	Identification B (Melting Range)	Identification B
Loss on Drying	Identification 2	Identification C (IR)	Loss on Drying
Melting Range	Identification 3 (IR)	Identification D	Melting Range
pH (5%)	Loss on Drying	Loss on Drying	pH 1:100
Residue on Ignition	Melting Range	Related Substances	Sulfated Ash
	pH (5%)	Residue on Ignition	
	Related Substances		
	Residue on Ignition		

Table 2: One Method Utilized for Analysis

Analysis Name[1]
Assay (dried basis) (USP-NF), (EP), (ChP), (JPC 1997)
Chlorides (ChP), (EP)
Clarity and Color of Solution (ChP), (JPC 1997)
Heavy Metals (as Pb) (ChP), (JPC 1997)
Identification A (USP-NF), Identification 3 (ChP), Identification C (EP)
Identification B (USP-NF), Identification 1 (ChP)
Identification D (USP-NF), Identification 2 (ChP)
Loss on Drying (USP-NF), (ChP), (EP), (JPC 1997)
Melting Range or Temperature (USP-NF), (ChP), Identification B (EP), Melting Point (JPC 1997)
pH 5% (USP-NF), (ChP), Identification A (EP)
Residue on Ignition (USP-NF), (ChP), Residue on Ignition (JPC 1997), Sulfated Ash (EP)

[1]Refer to BSI-MEM-1248, Tris Compendia Equivalency Summary

8.1. In-House Validated Methods in Accordance with USP General Chapters

Table 3: <1225> Validation of Compendial Procedures

Analysis Name
Assay (Ultrapure)
Assay (GC-FID)
Elemental Impurities
Endotoxins
Formaldehyde
Heavy Metals /Trace Metals/Arsenic/Iron
Identification D for the EP monograph analysis
Organic Impurities: Specified
Organic Impurities: Unspecified
Related Substances for the EP monograph analysis
Water (by KF Titration)

Table 4: In House Methods for Product Quality Description

Analysis Name
Appearance and Color
Solubility

Table 5: Customer Requested Specifications

Analysis Name
Absorbance (1M)
Absorbance (0.2M)
Absorbance (10%)
Absorbance (40%)
APHA Color, 20% Solution
Assay (Ultra-Pure)
Enzyme Activity
Insoluble Matter
Microbial Content
pH (0.1M), pH (0.05M)

Table 6: Outside Laboratory Analysis

Analysis Name
Residual Solvents: Methanol and Nitromethane
Microbial Content