BIOSPECTRA
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STABILITY INDICATING REPORT: BIS-TRIS

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1. PURPOSE:

1.1. The purpose of this report is to document which Quality Control (QC) analyses are stability indicating through assessment of test results on chemically and physically stressed material, this information is used to build out stability testing criteria for the accelerated and long-term stability programs.

1.2. To determine which tests are stability indicating for Bis-Tris when the product is stressed in extreme conditions.

1.2.1. The first developmental lot of Bis-Tris were stressed in the following ways:

1.2.1.1. Thermal
1.2.1.2. Humidity / Hydrolytic
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1.2.1.4.1. Acid
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1.2.1.5. Oxidative

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1.2.3. The following tests were not performed after material manipulation:

1.2.3.1. Microbial Enumeration Tests (Bioburden)
1.2.3.2. Chloride
1.2.3.3. Endotoxin/ Bacterial Endotoxins
1.2.3.4. Enzymes
1.2.3.5. Water Content (KF) - (Represented as Loss on Drying)
1.2.3.6. Heavy Metals / Limit of Pb or Fe
1.2.3.7. Residue on Ignition

2. SCOPE:

2.1. This Report applies to the stability testing of BioSpectra manufactured Bis-Tris.

3. RESPONSIBILITIES:

3.1. The Associate Director of Product Life Cycle is responsible for the control, implementation, training and maintenance of this report.

3.2. The QC Analysts were responsible for performing the testing stated in this Report and recording all results in current laboratory documentation.

3.3. The stability manager is responsible for incorporating the findings of this report into the stability program for the applicable material.

4. REFERENCES:

4.1. BSI-PRL-0600, Stability Indicating Protocol: Bis-Tris
4.2. BSI-SOP-0090, Lambda 25 UV/Vis Operation and Calibration.
4.3. BSI-SOP-0098, Balance SOP.
4.4. BSI-SOP-0126, Laboratory Notebooks.
4.5. BSI-SOP-0140, Standardization of Titrants
4.6. BSI-SOP-0143, Metrohm Titrando 907 Auto-Titrator SOP
4.7. BSI-SOP-0244, VWR Gravity Convection Oven Operation and Calibration (Model Number 414005-106).
4.8. BSI-SOP-0254, Spectrum Two UATR SOP.
4.9. BSI-SOP-0255, XL200 pH/mV/Conductivity Meter SOP.
4.10. BSI-SOP-0256, MP50 Melting Range Operation and Calibration SOP.
4.11. BSI-SOP-0259, Fisher Scientific Isotemp Water Bath Operation Calibration SOP
4.12. BSI-SOP-0289, Stability Indication Report.
4.13. Current USP/ NF
4.14. ICH Q1A
4.15. Laboratory Notebook: BD6P13-14, 16, 19, 28-32, BR23P23-24

5. EQUIPMENT:

5.1. Refer to Laboratory Notebook Attachment BD6P13-14, 16, 19, 28-32, BR23P23-24.

6. PROCEDURE:

6.1. Stress Procedures:

6.1.1. Control:

6.1.1.1. Refer to Original testing results for Bis-Tris developmental lot BTRI-0122-00001. No stress applied to the material.

6.1.2. Thermal Stress:

6.1.2.1. Transferred 100.0922g of the crystal to a suitable tray and evenly spread to increase exposure area.

6.1.2.2. Heated sample between at 95°C for 22 hours to stress.

6.1.2.3. Froze the sample for 24 hours after heat exposure.

6.1.2.4. Allowed sample to reach room temperature before analysis.

6.1.3. Acid Stress:

6.1.3.1. Applied 2.5mL of hydrochloric acid per 100g of sample; mixed the acidic mixture thoroughly in a suitable container.

6.1.3.2. Dried the sample using a well-ventilated tray and used a mortar and pestle to homogenize after drying.

6.1.4. Basic Stress:

6.1.4.1. Applied 2.5mL of 50% sodium hydroxide per 100g of sample; mixed the basic mixture thoroughly in a suitable container.

6.1.4.2. Dried the sample using a well-ventilated tray and used a mortar and pestle to homogenize after drying.

6.1.5. Oxidative Stress:

6.1.5.1. Applied 2.5mL of 30% hydrogen peroxide per 100g of sample. Mix the mixture thoroughly and allow to react in an open container.

6.1.5.2. Once reaction (if any) ceased, transferred material to a well-ventilated tray, dried the sample and use a mortar and pestle to homogenize after drying.

6.1.6. Humidity/Hydrolytic Stress:

6.1.6.1. Transferred material to a suitable tray and evenly spread to increase exposure area.

6.1.6.2. Contacted Stability management to place the material, covered loosely, into the accelerated stability chamber and recorded conditions.

6.1.6.3. Allowed sample 42.5 hours exposure to accelerated conditions (40°C, 75.0% RH) before removing for testing.

6.1.7. Photolytic Stress:

Exposed sample to approximately 1.38 million lux hours of light.

7. ANALYTICAL DATA:

7.1. **ABSORBANCE (0.1M)** **(0.04a.u. Max at 280nm, 0.02 a.u. Max at 340nm):**

7.1.1. Procedure:

7.1.1.1. Prepare a 0.1M solution of the specified sample.

7.1.1.1.1. Accurately weigh 0.52g of sample.

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

7.1.1.1.3. Swirl to dissolve completely.

7.1.1.2. Refer to Lambda 25 UV/Vis Operation and Calibration to measure the Absorbance of thesample at the required wavelength.

7.1.2. Results:

Stability Indicating Results: 0.1M Absorbance Lot: BTRI-0122-00001			
Sample ID	280nm Result (a.u.)	340nm Result (a.u.)	Disposition
Control	0.0111	<0.003	Stability Indicating
Thermal Stress	0.0102	<0.003	
Humidity/Hydrolytic Stress	0.0099	<0.003	
Photolytic Stress	0.0096	<0.003	
Acidic Stress	0.0089	<0.003	
Basic Stress	0.0272	0.003	
Oxidative Stress	0.0689	0.020	

7.2. **APPEARANCE AND COLOR** **White Crystalline Powder:**

7.2.1. Procedure:

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

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

7.2.1.3. The sample should be white in color and characteristic of a crystalline powder. If the sample does not conform to these specifications, notify the QC Manager immediately.

7.2.2. Results:

Stability Indicating Results: Appearance and Color Lot: BTRI-0122-00001		
Sample ID	Result	Disposition
Control	White Crystalline Powder	Stability Indicating
Thermal Stress	White Crystalline Powder	
Humidity/Hydrolytic Stress	White Crystalline Powder	
Photolytic Stress	White Crystalline Powder	
Acidic Stress	Clumpy White Crystal	
Basic Stress	Clumpy White Crystalline Powder	
Oxidative Stress	Slightly Yellow Crystalline Powder	

7.3. **ASSAY and pKa** **99.0-101.0%, pKa 6.3-6.7:**

7.3.1. Procedure:

7.3.1.1. Standardize the Metrohm pH electrode as per Standardization of Titrants.

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

7.3.1.3. Accurately weigh 0.65g of Bis Tris sample.

7.3.1.3.1. 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 ~50mL).

7.3.2. Titrate with 0.1N HCl to a potentiometric endpoint using the Metrohm 907 Auto Titrator.

7.3.2.1. Each mL of 0.1N HCl is equivalent to 20.924mg of Bis Tris.

7.3.2.2. The pK_a should be reported on the assay printout from the Metrohm Auto-Titrator.

$$\%\ Bis\ Tris\ (As - Is) = \frac{mL\ of\ Titrant \times Normality\ of\ Titrant \times 20.924}{Sample\ Weight\ (g)}$$

$$\%\ Bis\ Tris\ (Dried\ Basis) = \frac{100 \times \%\ Bis\ Tris\ (As - Is)}{100 - \%\ LoD}$$

7.3.3. Results:

Stability Indicating Results: Assay and pKa Lot: BTRI-0122-00001			
Sample ID	Assay Result %	pKa	Disposition
Control	100.1	6.6	Stability Indicating
Thermal Stress	100.3	6.6	
Humidity/Hydrolytic Stress	100.1	6.6	
Photolytic Stress	100.2	6.6	
Acidic Stress	93.6	6.5	
Basic Stress	107.0	6.6	
Oxidative Stress	99.1	6.6	

7.4. **IDENTIFICATION TEST (UATR)** **Conforms to Reference Standard:**

7.4.1. Procedure:

7.4.1.1. Follow Spectrum Two UATR SOP, DCN: BSI-SOP-0254, for analysis.

7.4.1.1.1. Analyze the sample as-is.

7.4.2. Results:

Stability Indicating Results: Identification (IR) Lot: BTRI-0122-00001		
Sample ID	Result	Disposition
Control	Conforms to Reference Standard	Not Stability Indicating
Thermal Stress	Conforms to Reference Standard	
Humidity/Hydrolytic Stress	Conforms to Reference Standard	
Photolytic Stress	Conforms to Reference Standard	
Acidic Stress	Conforms to Reference Standard	
Basic Stress	Conforms to Reference Standard	
Oxidative Stress	Conforms to Reference Standard	

7.5. **LOSS ON DRYING** **NMT 1.0%:**

7.5.1. Procedure:

7.5.1.1. Note: Bis-Tris melting may occur during drying.

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

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

7.5.1.4. 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.

7.5.1.5. Transfer approximately 1- 2g of the sample to the LOD vial, and accurately weigh the vial and contents. By gentle, sidewise shaking, distribute the sample as evenly as possible in the LOD vial to a depth of about 5mm.

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

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

7.5.1.8. Reweigh the LOD vial and sample.

7.5.1.9. Calculate the %LOD as follows:

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

7.5.2. Results:

Stability Indicating Results: Loss on Drying (LOD) Lot: BTRI-0122-00001		
Sample ID	Result	Disposition
Control	<0.1%	Stability Indicating
Thermal Stress	<0.1%	
Humidity/Hydrolytic Stress	<0.1%	
Photolytic Stress	<0.1%	
Acidic Stress	1.0%	
Basic Stress	1.4%	
Oxidative Stress	2.0%	

7.6. **MELTING POINT** **100-105°C:**

7.6.1. Procedure:

7.6.1.1. Sample Preparation: As-Is

7.6.1.2. Refer to MP50 Melting Range Operation and Calibration SOP, DCN: BSI-SOP-0256, for analysis.

7.6.2. Results:

Stability Indicating Results: Melting Point Lot: BTRI-0122-00001		
Sample ID	Melting Point (°C)	Disposition
Control	105°C	Stability Indicating
Thermal Stress	106°C	
Humidity/Hydrolytic Stress	105°C	
Photolytic Stress	106°C	
Acidic Stress	104°C	
Basic Stress	104°C	
Oxidative Stress	104°C	

7.7. **pH of a 1% Solution @ 25 ±2°C** **8.8-9.8:**

7.7.1. Procedure:

7.7.1.1. Accurately weigh 1.0g of sample. Transfer to a suitable beaker.

7.7.1.2. Add 100mL of purified water and dissolve.

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

7.7.2. Results:

Stability Indicating Results: pH of a 1% Solution Lot: BTRI-0122-00001		
Sample ID	pH Result	Disposition
Control	9.5	Stability Indicating
Thermal Stress	9.5	
Humidity/Hydrolytic Stress	9.5	
Photolytic Stress	9.5	
Acidic Stress	7.8	
Basic Stress	11.4	
Oxidative Stress	8.8	

7.8. **SOLUBILITY 0.1M IN WATER** **Clear and Complete:**

7.8.1. Procedure:

7.8.1.1. Prepare a 0.1M solution of the specified sample.

7.8.1.1.1. Accurately weigh 2.09 grams of sample.

7.8.1.1.2. Transfer accurately weighed sample to a clean, dry glass beaker and dissolved sample in 100mL of purified water.

7.8.1.2. View sample from all angles under sufficient lighting, the solution should be clear and complete.

7.8.2. Results:

Stability Indicating Results: Solubility 0.1M (Aqueous) Lot: BTRI-0122-00001		
Sample ID	Solubility Result	Disposition
Control	Clear and Complete	Not Stability Indicating
Thermal Stress	Clear and Complete	
Humidity/Hydrolytic Stress	Clear and Complete	
Photolytic Stress	Clear and Complete	
Acidic Stress	Clear and Complete	
Basic Stress	Clear and Complete	
Oxidative Stress	Clear and Complete	

8. CONCLUSION:

8.1. The analyses recommended for the Bis-Tris Stability Testing Program are as follows:

8.1.1. Absorbance (0.1M)

8.1.2. Appearance and Color

8.1.3. Assay

8.1.4. Identification A (IR)

8.1.4.1. Note: Not found to be stability indicating, however at least one identification is required for stability.

8.1.5. Identification B (Melting Point/Range)

8.1.6. Loss on Drying

8.1.7. pH of a 1% Solution

8.1.8. pKa