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# DEGRADATION AND IMPURITY PROFILE REPORT: BIS-TRIS

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## 1. PURPOSE AND SCOPE:

- 1.1. The impurity profiling of Bis-Tris was intended to identify and potentially quantify impurities found in Bis-Tris (CAS 6979-37-0) product manufactured and purified at BioSpectra.
  - 1.1.1. In the case where an impurity was found, a limit was set to the maximum allowable without measurable compromise to predetermined critical quality attributes or toxicity. In the case where a limit could not be set, a procedure was written and followed to identify if the possible impurity was present or not (i.e. an identity test, which is qualitative and not quantitative).
  - 1.1.2. The profiling results and data allowed BioSpectra to understand the purity and characteristics of Bis-Tris through all stages of manufacturing.
  - 1.1.3. The four stages of Bis-Tris that were tested were Raw Material, Mother Liquor, Wet Crystal, and Finished Good.
  - 1.1.4. Tables were generated to include all sample results in the Bis-Tris Degradation and Impurity Profile Report.
  - 1.1.5. The tests that were used to determine the presence of impurities and degradation products will be as follows:
    - 1.1.5.1. Absorbance
      - 1.1.5.1.1. All four stages.
    - 1.1.5.2. Appearance and Color
      - 1.1.5.2.1. Raw Material and Finished Good only.
    - 1.1.5.3. Assay
      - 1.1.5.3.1. All four stages.
    - 1.1.5.4. Bioburden
      - 1.1.5.4.1. Raw Material and Finished Good only.
    - 1.1.5.5. Elemental Impurities
      - 1.1.5.5.1. All four stages.
    - 1.1.5.6. Endotoxin
      - 1.1.5.6.1. Raw Material and Finished Good only.
    - 1.1.5.7. Identification (IR)
      - 1.1.5.7.1. All four stages.
      - 1.1.5.7.2. ML and WC Identification (IR) contains water contamination and is not representative of the finished product.
    - 1.1.5.8. Karl Fischer
      - 1.1.5.8.1. All four stages.
    - 1.1.5.9. Loss on Drying
      - 1.1.5.9.1. All four stages.
    - 1.1.5.10. Melting Range
      - 1.1.5.10.1. Raw Material and Finished Good only.
    - 1.1.5.11. Residual Solvents: Methanol
      - 1.1.5.11.1. Raw Material and Finished Good only.
    - 1.1.5.12. Residue on Ignition
      - 1.1.5.12.1. Raw Material and Finished Good only.
    - 1.1.5.13. Solubility 0.1M in Water
      - 1.1.5.13.1. All four stages.
- 1.2. All results were recorded in the appropriate laboratory documentation. The results were detailed and analyzed in this degradation and impurity profile report. This report includes all relevant data as well as references to the initial documented results. This report discusses any impurities found in the product and includes a specification for any limits on the impurities found when applicable.

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## 2. RESPONSIBILITIES:

- 2.1. The QC Analyst, or qualified designees, were responsible for performing the testing stated in the protocol and recording all results.
- 2.2. The Associate Director of Product Life Cycle, or designee, was responsible for completing the degradation and impurity testing report.
- 2.3. It was the responsibility of all personnel to read and understand the SDS and don the appropriate PPE for handling and disposing of chemicals in a safe manner.

## 3. REFERENCES:

- 3.1. BSI-ATM-0075, Analytical Method for the Determination of ICH Q3D Elemental Impurities (Class 1, 2A, 2B, 3 & 4) by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in Bis-Tris and Bis-Tris Hydrochloride
- 3.2. BSI-ATM-0095, Bis-Tris Testing Methods
- 3.3. BSI-PRL-0620, Degradation and Impurity Profile Protocol: Bis Tris
- 3.4. BSI-RPT-1301, Elemental Impurity Assessment: Bis-Tris E04 Validation 2023
- 3.5. BSI-SOP-0069, Preparation of Samples for Outside Testing
- 3.6. BSI-SOP-0090, Lambda 25 UV/Vis Operation and Calibration
- 3.7. BSI-SOP-0094, Muffle Furnace SOP and Calibration
- 3.8. BSI-SOP-0098, Balance SOP
- 3.9. BSI-SOP-0102, Degradation and Impurity Profiling SOP
- 3.10. BSI-SOP-0126, Laboratory Notebooks
- 3.11. BSI-SOP-0133, Blue M Convection Oven Operation and Calibration SOP
- 3.12. BSI-SOP-0134, Pipette SOP
- 3.13. BSI-SOP-0140, Standardization of Titrants
- 3.14. BSI-SOP-0143, Metrohm Titrand 907 Auto-Titrator SOP
- 3.15. BSI-SOP-0244, VWR Gravity Convection Oven Operation and Calibration
- 3.16. BSI-SOP-0254, Spectrum Two UATR SOP
- 3.17. BSI-SOP-0256, MP50 Melting Range Operation and Calibration SOP
- 3.18. BSI-SOP-0303, NexION 350X ICP-MS SOP
- 3.19. BSI-SOP-0345, Endosafe Nexgen-PTS Endotoxin Reader SOP
- 3.20. *ACS, Reagent Chemicals*, current edition

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4. **PROCEDURE:**

4.1. **ABSORBANCE** :

4.1.1. Refer to the Degradation and Impurity Profile Protocol: Bis Tris for testing methods and requirements. The results of the Absorbance testing are detailed in the table below.

**TABLE 1: ABSORBANCE**

| Lot Number                      | Stage         | Specification |          | Result |        |
|---------------------------------|---------------|---------------|----------|--------|--------|
|                                 |               | 280 nm        | 340 nm   | 280 nm | 340 nm |
| BTRI-0123-00003-PV ML           | Mother Liquor | Monitor       | Monitor  | 0.0895 | 0.0302 |
| M155 0705                       | Raw Material  |               |          | 0.0117 | 0.0018 |
| BTRI-0123-00003 WC First Basket | Wet Crystal   |               |          | 0.0154 | 0.0034 |
| BTRI-0123-00003-PV Beginning    | Finished Good | 0.04 a.u.     | 0.02 a.u | 0.01   | <0.02  |

4.2. **APPEARANCE AND COLOR** :

4.2.1. Refer to the Degradation and Impurity Profile Protocol: Bis Tris for testing methods and requirements. The results of the Appearance and Color testing are detailed in the table below.

**TABLE 2: APPEARANCE AND COLOR**

| Lot Number                   | Stage         | Specification     | Result                   |
|------------------------------|---------------|-------------------|--------------------------|
| M155 0705                    | Raw Material  | White Crystalline | White Crystalline Powder |
| BTRI-0123-00003-PV Beginning | Finished Good | Powder            | White Crystalline Powder |

4.3. **ASSAY** :

4.3.1. Refer to the Degradation and Impurity Profile Protocol: Bis Tris for testing methods and requirements. The results of the Assay testing are detailed in the table below.

**TABLE 3: ASSAY**

| Lot Number                      | Stage         | Specification  | Result |
|---------------------------------|---------------|----------------|--------|
| BTRI-0123-00003-PV ML           | Mother Liquor | Monitor        | 47.29% |
| M155 0705                       | Raw Material  | 99.0% - 101.0% | 99.8%  |
| BTRI-0123-00003 WC First Basket | Wet Crystal   | Monitor        | 98.81% |
| BTRI-0123-00003-PV Beginning    | Finished Good | 99.0% - 101.0% | 100.2% |

4.4. **BIOBURDEN** :

4.4.1. Refer to the Degradation and Impurity Profile Protocol: Bis Tris for testing methods and requirements. The results of the Bioburden testing are detailed in the table below.

**TABLE 4: BIOBURDEN**

| Lot Number                   | Stage         | Specification |            | Result     |            |
|------------------------------|---------------|---------------|------------|------------|------------|
|                              |               | TAMC          | TYMC       | TAMC       | TYMC       |
| M155 0705                    | Raw Material  | Monitor       |            | <100 CFU/g | <100 CFU/g |
| BTRI-0123-00003-PV Beginning | Finished Good | ≤100 CFU/g    | ≤100 CFU/g | <100 CFU/g | <100 CFU/g |

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#### 4.5. ELEMENTAL IMPURITY :

4.5.1. Refer to the Degradation and Impurity Profile Protocol: Bis Tris for testing methods and requirements. The results of the Elemental Impurity testing are detailed in the table below.

**TABLE 5: ELEMENTAL IMPURITY**

| Lot Number                      | Stage         | Specification                   | Result  |
|---------------------------------|---------------|---------------------------------|---|
| BTRI-0123-00003-PV ML           | Mother Liquor | Monitor                         | Refer to<br>BSI-RPT-1301 for<br>Elemental Impurity<br>Assessment: Bis-Tris<br>E04 Validation 2023 |
| M155 0705                       | Raw Material  |                                 |   |
| BTRI-0123-00003 WC First Basket | Wet Crystal   |                                 |   |
| BTRI-0123-00003-PV Beginning    | Finished Good | Complies with<br>USP <232><233> |   |

#### 4.6. ENDOTOXIN :

4.6.1. Refer to the Degradation and Impurity Profile Protocol: Bis Tris for testing methods and requirement. The results of the Endotoxin testing are detailed in the table below.

**TABLE 6: ENDOTOXIN**

| Lot Number                   | Stage         | Specification | Result     |
|------------------------------|---------------|---------------|------------|
| M155 0705                    | Raw Material  | Monitor       | <10.0 EU/g |
| BTRI-0123-00003-PV Beginning | Finished Good | ≤50 EU/g      | <10 EU/g   |

#### 4.7. IDENTIFICATION TEST (IR) :

4.7.1. Refer to the Degradation and Impurity Profile Protocol: Bis Tris for testing method and requirements. The results of the Identification (IR) testing are detailed in the table below.

**TABLE 7: IDENTIFICATION (IR)**

| Lot Number                      | Stage         | Specification | Result                |
|---------------------------------|---------------|---------------|-----------------------|
| BTRI-0123-00003-PV ML           | Mother Liquor | Monitor       | Passes Test; 0.230041 |
| M155 0705                       | Raw Material  | Passes Test   | Passes Test; 0.997191 |
| BTRI-0123-00003 WC First Basket | Wet Crystal   | Monitor       | Passes Test; 0.982837 |
| BTRI-0123-00003-PV Beginning    | Finished Good | Passes Test   | Passes Test; 0.993222 |

#### 4.8. KARL FISCHER :

4.8.1. Refer to the Degradation and Impurity Profile Protocol: Bis Tris for testing methods and requirements. The results of the Karl Fischer testing are detailed in the table below.

**TABLE 8: KARL FISCHER**

| Lot Number                      | Stage         | Specification | Result |
|---------------------------------|---------------|---------------|--------|
| BTRI-0123-00003-PV ML           | Mother Liquor | Monitor       | 50.95% |
| M155 0705                       | Raw Material  |               | 0.10%  |
| BTRI-0123-00003 WC First Basket | Wet Crystal   |               | 1.30%  |
| BTRI-0123-00003-PV Beginning    | Finished Good | ≤1.0%         | 0.1%   |

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**4.9. LOSS ON DRYING** :

4.9.1. Refer to the Degradation and Impurity Profile Protocol: Bis Tris for testing methods and requirements. The results of the Loss on Drying analysis are detailed in the table below.

**TABLE 9: LOSS ON DRYING**

| Lot Number                      | Stage         | Specification | Result   |
|---------------------------------|---------------|---------------|----------|
| BTRI-0123-00003-PV ML           | Mother Liquor | Monitor       | 53.1754% |
| M155 0705                       | Raw Material  |               | 0.2901%  |
| BTRI-0123-00003 WC First Basket | Wet Crystal   |               | 1.4379%  |
| BTRI-0123-00003-PV Beginning    | Finished Good | ≤1.0%         | <1.0%    |

**4.10. MELTING POINT** :

4.10.1. Refer to the Degradation and Impurity Profile Protocol: Bis Tris for testing methods and requirements. The results of the Melting Point analysis are detailed in the table below.

**TABLE 10: MELTING POINT**

| Lot Number                   | Stage         | Specification | Result |
|------------------------------|---------------|---------------|--------|
| M155 0705                    | Raw Material  | 100°C - 105°C | 103°C  |
| BTRI-0123-00003-PV Beginning | Finished Good |               | 104°C  |

**4.11. RESIDUAL SOLVENTS** :

4.11.1. Refer to the Degradation and Impurity Profile Protocol: Bis Tris for testing methods and requirements. The results of the Residual Solvents analysis are detailed in the table below.

**TABLE 11: RESIDUAL SOLVENTS**

| Lot Number                   | Stage         | Specification        | Result   |
|------------------------------|---------------|----------------------|----------|
| M155 0705                    | Raw Material  | Monitor for Methanol | 1003 ppm |
| BTRI-0123-00003-PV Beginning | Finished Good |                      | <80 ppm  |

**4.12. RESIDUE ON IGNITION** :

4.12.1. Refer to the Degradation and Impurity Profile Protocol: Bis Tris for testing methods and requirements. The results of the Residue on Ignition analysis are detailed in the table below.

**TABLE 12: RESIDUE ON IGNITION**

| Lot Number                   | Stage         | Specification | Result   |
|------------------------------|---------------|---------------|----------|
| M155 0705                    | Raw Material  | Monitor       | <0.0200% |
| BTRI-0123-00003-PV Beginning | Finished Good | ≤0.1%         | <0.1%    |

**4.13. SOLUBILITY 0.1M IN WATER** :

4.13.1. Refer to the Degradation and Impurity Profile Protocol: Bis Tris for testing methods and requirements. The results of the Solubility analysis are detailed in the table below.

**TABLE 13: SOLUBILITY IN WATER**

| Lot Number                      | Stage         | Specification      | Result             |
|---------------------------------|---------------|--------------------|--------------------|
| BTRI-0123-00003-PV ML           | Mother Liquor | Monitor            | Clear and Complete |
| M155 0705                       | Raw Material  |                    | Clear and Complete |
| BTRI-0123-00003 WC First Basket | Wet Crystal   |                    | Clear and Complete |
| BTRI-0123-00003-PV Beginning    | Finished Good | Clear and Complete | Clear and Complete |

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## 5. CONCLUSION:

- 5.1. All samples met the specification for required analyses as dictated in the Degradation and Impurity Profile Protocol: Bis-Tris.
- 5.2. It can be concluded that there are no additional identifiable impurities present in the Bis Tris material at any stage of the process at this time.