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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.

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

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

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 BSI-RPT-1301 for Elemental Impurity Assessment: Bis-Tris E04 Validation 2023
M155 0705	Raw Material		
BTRI-0123-00003 WC First Basket	Wet Crystal		
BTRI-0123-00003-PV Beginning	Finished Good	Complies with 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%

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%

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

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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.