BIOSPECTRA
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DEGRADATION AND IMPURITY PROFILE REPORT: TRIS BIO EXCIPIENT CONTINUOUS 2022

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1. PURPOSE AND SCOPE:

1.1. The impurity profiling of Tris was intended to identify and potentially quantify impurities found in the Tris 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 allows BioSpectra to understand the purity and characteristics of Tris through all stages of manufacturing.

1.1.3. The three stages of Tris that were tested are Raw Material, Mother Liquor, and the finished product.

1.1.4. Tables were generated to include all sample results in this Tris Degradation and Impurity Profile Report.

1.1.5. The tests that were used to determine the presence of impurities and degradation products were as follows:

1.1.5.1. Absorbance

1.1.5.1.1. All three stages.

1.1.5.2. Assay

1.1.5.2.1. All three stages.

1.1.5.3. Chlorides

1.1.5.3.1. Raw Material and Finished Good Beginning Drum

1.1.5.4. Elemental Impurities with addition of Al, Ca, Fe, Mg, Mn, Zn

1.1.5.4.1. All three stages.

1.1.5.5. Identification (IR)

1.1.5.5.1. All three stages.

1.1.5.5.2. ML Identification (IR) contains water contamination and is not representative of the finished product.

1.1.5.6. Loss on Drying

1.1.5.6.1. Raw Material and Finished Good Beginning Drum

1.1.5.7. Melting Range

1.1.5.7.1. Raw Material and Finished Good Beginning Drum

1.1.5.8. pH of a 5% Solution

1.1.5.8.1. Raw Material and Finished Goods Beginning Drum

1.1.5.9. Related Substances: Organic Impurities

1.1.5.9.1. All three stages.

1.1.5.10. Residual Solvents: Methanol and Nitromethane

1.1.5.10.1. Raw Material and Finished Goods only.

1.2. All results were recorded in the appropriate laboratory documentation.

2. RESPONSIBILITIES:

2.1. The Executive Director of Quality Control was responsible for control, implementation, training,and maintenance of this procedure.

2.2. The QC Analysts were responsible for performing the testing stated in the protocol and recording all results.

2.3. The QC Systems Supervisor, or designee was responsible for completing the degradation and impurity testing report.

3. REFERENCES:

3.1. BSI-ATM-0007, Tris Testing Methods
3.2. BSI-ATM-0058, Analytical Method of Analysis: Trace Metal Impurities: Tris and THCl
3.3. BSI-ATM-0059, Determination of Elemental Impurities by ICP-MS in Tris
3.4. BSI-ATM-0062, Tris Related Substances Analysis Method via HPLC
3.5. BSI-PRL-0463, TRIS Continuous Stroudsburg S04, Process Validation Protocol
3.6. BSI-PRL-0512, Degradation and Impurity Profile Protocol: Tris Continuous 2022
3.7. BSI-RPT-1176, Elemental Impurity Assessment: Tris Continuous 2022 Process Validation
3.8. BSI-SOP-0090, Lambda 25 UV/VIS Operation and Calibration
3.9. BSI-SOP-0102, Degradation and Impurity Profiling SOP
3.10. BSI-SOP-0143, Metrohm Titrando 901 Auto-Titrator SOP
3.11. BSI-SOP-0254, Spectrum Two UATR SOP
3.12. BSI-SOP-0255, XL200 pH/mV/Conductivity Meter SOP
3.13. BSI-SOP-0256, MP50 Melting Range Operation and Calibration
3.14. BSI-SOP-0303, NexION 350X ICP-MS SOP

4. RESULTS:

4.1. ABSORBANCE

4.1.1. Refer to Degradation and Impurity Profile Protocol: Tris Continuous for testing methods. The results of Absorbance (1M Solution) are detailed in the table below. All results met requirements.

TABLE 1: ABSORBANCE

Lot Number	Stage of Material	Specification (a.u. max)			Result (a.u.)		
		260 nm	280 nm	400 nm	260 nm	280 nm	400 nm
PMAT-1221-00946-PD	Mother Liquor	0.1500 max.	2.0000 max.	2.2000 max.	0.1251	0.1048	0.0391
RMAT-0122-0079	Raw Material	0.20 max	0.20 max	Not Applicable	0.02	0.02	Not Applicable
RMAT-0721-0062					0.03	0.02	Not Applicable
RMAT-0122-0098					0.07	0.06	Not Applicable
TRIS-0222-00036-PV Drum 1	Finished Good	0.06 max	0.06 max	0.01 max	0.01	<0.06	<0.01

4.2. ASSAY (USP)

4.2.1. Refer to Degradation and Impurity Profile Protocol: Tris for testing methods. The results of Assay (USP) are detailed in the table below. All results met requirements.

TABLE 2: ASSAY (USP)

Lot Number	Stage of Material	Specification	Result
PMAT-1221-00946-PD	Mother Liquor	Monitor	39.79%
RMAT-0122-0079	Raw Material	99.0% min	99.5%
RMAT-0721-0062			99.9%
RMAT-0122-0098			99.6%
TRIS-0222-00036-PV Drum 1	Finished Good	99.0% - 101.0%	99.6%

4.3. **CHLORIDES** :

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

TABLE 3: CHLORIDES

Lot Number	Stage of Material	Specification	Result
RMAT-0122-0079	Raw Material	Monitor	<0.01%
RMAT-0721-0062			<0.01%
RMAT-0122-0098			<0.01%
TRIS-0222-00036-PV Drum 1	Finished Good	0.01% max	<0.01%

4.4. **ELEMENTAL IMPURITIES** :

4.4.1. Refer to Analytical Method Verification Protocol: Elemental Impurities via ICP-MS, DCN: BSI-ATM-0059 for testing methods. The results of Elemental Impurities are detailed in BSI-RPT-1070 Degradation and Impurity Profile Report: Tris 2022 – S04.

TABLE 4: ELEMENTAL IMPURITIES

Lot Number	Stage of Material	Specification	Result
PMAT-1221-00946-PD	Mother Liquor	Monitor	Refer to BSI-RPT-1176 for Elemental Impurities Profiling Report.
RMAT-0122-0079	Raw Material	Monitor	
RMAT-0721-0062			
RMAT-0122-0098			
TRIS-0222-00036-PV Drum 1	Finished Good	Report	

4.5. **IDENTITY (IR)** :

4.5.1. Refer to Degradation and Impurity Profile Protocol: Tris for testing methods. The results of Identity (IR) are detailed in the table below. All results met requirements.

TABLE 5: IDENTITY (IR)

Lot Number	Stage of Material	Specification	Result
PMAT-1221-00946-PD	Mother Liquor	Monitor	Passes Test (0.963194)
RMAT-0122-0079	Raw Material	Passes Test	Passes Test (0.993867)
RMAT-0721-0062			Passes Test (0.980635)
RMAT-0122-0098			Passes Test (0.992733)
TRIS-0222-00036-PV Drum 1	Finished Good	Passes Test	Passes Test (0.998315)

4.6. **LOSS ON DRYING** :

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

TABLE 6: LOSS ON DRYING

Lot Number	Stage of Material	Specification	Result
RMAT-0122-0079	Raw Material	Monitor	0.2200%
RMAT-0721-0062			0.1446%
RMAT-0122-0098			0.3004%
TRIS-0222-00036-PV Drum 1	Finished Good	0.5% max	0.3%

4.7. **MELTING RANGE** :

4.7.1. Refer to Degradation and Impurity Profile Protocol: Tris for testing methods. The results of Melting Range are detailed in the table below. All results met requirements.

TABLE 7: MELTING RANGE

Lot Number	Stage of Material	Specification	Result
RMAT-0122-0079	Raw Material	168 – 174°C	168 – 170°C
RMAT-0721-0062			170 – 171°C
RMAT-0122-0098			168 – 170°C
TRIS-0222-00036-PV Drum 1	Finished Good	168 – 172°C	171 – 172°C

4.8. **pH of a 5% or 1 in 20 SOLUTION** :

4.8.1. Refer to Degradation and Impurity Profile Protocol: Tris for testing methods. The results of pH of a 5% or 1 in 20 Solution are detailed in the table below. All results met requirements.

TABLE 8: PH OF A 5% OR 1 IN 20 SOLUTION

Lot Number	Stage of Material	Specification	Result
RMAT-0122-0079	Raw Material	Monitor	10.96 @ 23.1°C
RMAT-0721-0062			10.99 @ 23.1°C
RMAT-0122-0098			10.87 @ 23.1°C
TRIS-0222-00036-PV Drum 1	Finished Good	10.0 – 11.5	10.8 @ 25.0°C

4.9. **RELATED SUBSTANCES: Organic Impurities**

4.9.1. Refer to Degradation and Impurity Profile Protocol: Tris for testing methods. The results of Related Substances: Organic Impurities are detailed in the table below. All results met requirements.

4.9.1.1. As the Tris under analysis in this Degradation and Impurity Protocol and Report was Bio Excipient, Organic Impurities analysis was not required. Related substances results are reported as a limit test as per the requirements of Bio Excipient Tris.

TABLE 9: RELATED SUBSTANCES: ORGANIC IMPURITIES

Lot Number	Stage of Material	Specification	Result
			Related Substances Result:
PMAT-1221-00946-PD	Mother Liquor	Monitor	<0.03%
RMAT-0122-0079	Raw Material	Monitor	<0.03%
RMAT-0721-0062			<0.03%
RMAT-0122-0098			<0.03%
TRIS-0222-00036-PV Drum 1	Finished Good	0.1% max	<0.1%

4.10. **RESIDUAL SOLVENTS :**

4.10.1. Methanol and Nitromethane analysis were performed by Advanced Analytical Laboratories on the raw materials and individual drum sample.

TABLE 10: RESIDUAL SOLVENTS

Lot Number	Stage of Material	Specification		Result	
		Methanol	Nitromethane	Methanol	Nitromethane
RMAT-0122-0079	Raw Material	Report	Report	<150 ppm	<10 ppm
RMAT-0721-0062				<150 ppm	<10 ppm
RMAT-0122-0098				<150 ppm	<10 ppm
TRIS-0222-00036-PV Drum 1	Finished Good	≤ 300 ppm	≤ 15 ppm	<150 ppm	<10 ppm

5. CONCLUSION:

5.1. Water was identified as an intentionally introduced solvent due to the aqueous purification process, but was removed through drying and all finished material met moisture specifications.

5.2. Impurities were removed from Tris through the purification stage as shown in Absorbance analysis as the absorbances of the finished goods were less than that of the raw material.

5.3. In conclusion, all samples from all stages of the process met the required specifications as listed in the Degradation and Impurity Profile Protocol: Tris Continuous 2022.