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DEGRADATION AND IMPURITY PROFILE REPORT: TRIS – BIO FUISA AND BIO ACTIVE

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1. PURPOSE AND INTRODUCTION:

1.1. The impurity profiling of Tris Active Pharmaceutical Ingredient (API) was intended to identify and potentially quantify impurities found in the Tris API product manufactured and purified at the BioSpectra Bangor, PA facility.

1.1.1. The profiling results and data allows BioSpectra to monitor the purity and characteristics of Tris API through all stages of manufacturing.

1.1.2. The four stages of Tris API that were tested include Raw Material, Mother Liquor, Wet Crystal, and the finished product. There was at least one sample from each stage used for analysis, for each validation lot. A table was generated to summarize results.

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

1.1.3.1. Chlorides

1.1.3.1.1. Raw Material and Finished Goods only.

1.1.3.2. Formaldehyde

1.1.3.2.1. Raw Material, ML, WC and Finished Goods

1.1.3.3. Elemental Impurities

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1.1.3.4. Identification (IR)

1.1.3.4.1. Raw Material and Finished Goods only.

1.1.3.4.1.1. ML and WC Identification (IR) contains intentional water contamination and are not representative of the finished product.

1.1.3.5. Melting Range

1.1.3.5.1. Raw Material and Finished Goods only.

1.1.3.6. pH of a 5% Solution

1.1.3.6.1. Raw Material and Finished Goods only.

1.1.3.7. Related Substances: Organic Impurities

1.1.3.7.1. All four stages.

1.1.3.8. Residual Solvents: Methanol and Nitromethane

1.1.3.8.1. Finished Goods only.

1.1.3.9. Trace Metals: Aluminum, Calcium, Iron, Potassium, and Magnesium

1.1.3.9.1. Arsenic, Copper, Lead, and Nickel are Elemental Impurities but will also be analyzed for trace metal specifications.

1.1.3.9.2. All four stages.

1.1.4. Stability Indicating Analyses:

1.1.4.1. Absorbance

1.1.4.1.1. All four stages.

1.1.4.1.2. The sample preparation for Absorbance (40% Solution) was utilized for all samples.

1.1.4.2. Assay (USP)

1.1.4.2.1. All four stages.

1.1.4.3. Loss on Drying

1.1.4.3.1. Raw Material and Finished Goods only.

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

1.3. As the Tris Bio FUISA and Bio Active material produced at the BioSpectra Bangor, PA facility are manufactured in the same equipment with the same raw materials, this Report is to be considered supplemental to each product grade's validation.

2. RESPONSIBILITIES:

2.1. The QC department, or designee, is responsible for completing the Degradation and Impurity Report.

3. REFERENCES:

3.1. Degradation and Impurity Protocol: Tris Bio FUISA and Tris Bio Active DCN: 16-000077 v3.0

4. DATA PRESENTATION:

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

4.1.1. Refer to Degradation and Impurity Protocol: Tris Bio FUISA and Tris Bio Active for testing methods. The results of Absorbance (40% Solution) are detailed in the table below.

Lot Number	Stage of Material	Specification	Result @ 290 nm
D609JBI032	Raw Material	0.2 a.u. max @ 290 nm	0.0373 a.u.
D609JCB032			0.0414 a.u.
D609JBK032			0.0332 a.u.
TR1200-016-0220-PV WC Basket 1	Wet Crystal	Monitor @ 290 nm	0.0042 a.u.
TR1200-017-0220-PV WC Basket 2			0.0038 a.u.
TR1200-018-0220-PV WC Basket 4			0.0034 a.u.
TR1200-016-0220-PV ML	Mother Liquor	Monitor @ 290 nm	0.0400 a.u.
TR1200-017-0220-PV ML			0.0258 a.u.
TR1200-018-0220-PV ML			0.0230 a.u.
TR1200-016-0220-PV Drum 1	Finished Good	0.2 a.u. max @ 290 nm	0.0139 a.u.
TR1200-017-0220-PV Drum 2			0.0086 a.u.
TR1200-018-0220-PV Drum 4			0.0080 a.u.

4.2. **ASSAY :**

4.2.1. Refer to Degradation and Impurity Protocol: Tris Bio FUISA and Tris Bio Active for testing methods. The results of Assay are detailed in the table below.

Lot Number	Stage of Material	Specification	Result
D609JBI032	Raw Material	Monitor	99.9%
D609JCB032			99.7%
D609JBK032			99.8%
TR1200-016-0220-PV Drum 1	Finished Good	99.0 – 101.0%	99.43%
TR1200-017-0220-PV Drum 2			99.85%
TR1200-018-0220-PV Drum 4			99.98%

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4.2.2. As per section 4.2 of the Degradation and Impurity Protocol: Tris Bio FUISA and Bio Active, the Mother Liquor and Wet Crystal samples were also analyzed. These analyses were not required for completion of protocol. Specification is Monitor.

Lot Number	Stage of Material	Specification	Result
TR1200-016-0220-PV WC Basket 1	Wet Crystal	Monitor	97.95%
TR1200-017-0220-PV WC Basket 2			98.12%
TR1200-018-0220-PV WC Basket 4			98.49%
TR1200-016-0220-PV ML	Mother Liquor	Monitor	37.46%
TR1200-017-0220-PV ML			38.38%
TR1200-018-0220-PV ML			42.62%

4.3. **CHLORIDES :**

4.3.1. Refer to Degradation and Impurity Protocol: Tris Bio FUISA and Tris Bio Active for testing methods. The results of Chloride are detailed in the table below.

Lot Number	Stage of Material	Specification	Result
D609JBI032	Raw Material	0.01 % max	< 0.01%
D609JCB032			< 0.01%
D609JBK032			< 0.01%
TR1200-016-0220-PV Drum 1	Finished Good	0.01% max	< 0.01%
TR1200-017-0220-PV Drum 2			< 0.01%
TR1200-018-0220-PV Drum 4			< 0.01%

4.4. **ELEMENTAL IMPURITIES :**

4.4.1. Refer to Degradation and Impurity Protocol: Tris Bio FUISA and Tris Bio Active for testing methods. The results of Elemental Impurities are detailed in the table below.

Lot Number	Stage of Material	Specification	Result
D609JBI032	Raw Material	Monitor	Complies with USP <232> and <233>
D609JCB032			Complies with USP <232> and <233>
D609JBK032			Complies with USP <232> and <233>
TR1200-016-0220-PV WC Basket 1	Wet Crystal	Monitor	Complies with USP <232> and <233>
TR1200-017-0220-PV WC Basket 2			Complies with USP <232> and <233>
TR1200-018-0220-PV WC Basket 4			Complies with USP <232> and <233>
TR1200-016-0220-PV ML	Mother Liquor	Monitor	Complies with USP <232> and <233>
TR1200-017-0220-PV ML			Complies with USP <232> and <233>
TR1200-018-0220-PV ML			Complies with USP <232> and <233>
TR1200-016-0220-PV Drum 1	Finished Good	Complies with USP <232> and <233>	Complies with USP <232> and <233>
TR1200-017-0220-PV Drum 2			Complies with USP <232> and <233>
TR1200-018-0220-PV Drum 4			Complies with USP <232> and <233>

4.5. **FORMALDEHYDE :**

4.5.1. Refer to Degradation and Impurity Protocol: Tris Bio FUISA and Tris Bio Active for testing methods. The results of Formaldehyde are detailed in the table below.

Lot Number	Stage of Material	Specification	Result
D609JBI032	Raw Material	Monitor	10.2 ppm
D609JCB032			8.09 ppm
D609JBK032			8.8 ppm
TR1200-016-0220-PV WC Basket 1	Wet Crystal	Monitor	0.48 ppm
TR1200-017-0220-PV WC Basket 2			0.92 ppm
TR1200-018-0220-PV WC Basket 4			0.45 ppm
TR1200-016-0220-PV ML	Mother Liquor	Monitor	23.86 ppm
TR1200-017-0220-PV ML			11.21 ppm
TR1200-018-0220-PV ML			11.92 ppm
TR1200-016-0220-PV Drum 1	Finished Good	1 ppm max	0.77 ppm
TR1200-017-0220-PV Drum 2			0.66 ppm
TR1200-018-0220-PV Drum 4			0.53 ppm

4.6. **IDENTITY (IR) :**

4.6.1. Refer to Degradation and Impurity Protocol: Tris Bio FUISA and Tris Bio Active for testing methods. The results of Identity (IR) are detailed in the table below.

Lot Number	Stage of Material	Specification	Result
D609JBI032	Raw Material	Passes Test	Passes Test
D609JCB032			Passes Test
D609JBK032			Passes Test
TR1200-016-0220-PV Drum 1	Finished Good	Passes Test	Passes Test
TR1200-017-0220-PV Drum 2			Passes Test
TR1200-018-0220-PV Drum 4			Passes Test

4.7. **LOSS ON DRYING :**

4.7.1. Refer to Degradation and Impurity Protocol: Tris Bio FUISA and Tris Bio Active for testing methods. The results of Loss on Drying are detailed in the table below.

Lot Number	Stage of Material	Specification	Result
D609JBI032	Raw Material	Passes Test	0.0268%
D609JCB032			0.0548%
D609JBK032			0.0422%
TR1200-016-0220-PV Drum 1	Finished Good	Passes Test	0.0816%
TR1200-017-0220-PV Drum 2			0.0339%
TR1200-018-0220-PV Drum 4			0.2447%

4.8. **MELTING RANGE :**

4.8.1. Refer to Degradation and Impurity Protocol: Tris Bio FUISA and Tris Bio Active for testing method. The results of Melting Range are detailed in the table below.

Lot Number	Stage of Material	Specification	Result
D609JBI032	Raw Material	Monitor	170.5 – 172.0°C
D609JCB032			170.9 – 172.1°C
D609JBK032			170.8 – 171.9°C
TR1200-016-0220-PV Drum 1	Finished Good	168 – 172°C	170.9 – 172.0°C
TR1200-017-0220-PV Drum 2			171.1 – 172.2°C
TR1200-018-0220-PV Drum 4			171.3 – 172.2°C

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

4.9.1. Refer to Degradation and Impurity Protocol: Tris Bio FUISA and Tris Bio Active for testing method. The results of pH of a 5% solution are detailed in the table below.

Lot Number	Stage of Material	Specification	Result
D609JBI032	Raw Material	Monitor	10.88
D609JCB032			10.88
D609JBK032			10.91
TR1200-016-0220-PV Drum 1	Finished Good	10.0 – 11.5	10.72
TR1200-017-0220-PV Drum 2			10.75
TR1200-018-0220-PV Drum 4			11.06

4.10. **RELATED SUBSTANCES: Organic Impurities :**

4.10.1. Refer to Degradation and Impurity Protocol: Tris Bio FUISA and Tris Bio Active for testing methods. The results of Organic Impurities are detailed in the table below.

Lot Number	Stage of Material	Specification		Result	
		Formaldehyde	Unspecified	Formaldehyde	Unspecified
D609JBI032	Raw Material	Monitor		10.2 ppm	Not Detected
D609JCB032				8.09 ppm	Not Detected
D609JBK032				8.8 ppm	Not Detected
TR1200-016-0220-PV WC Basket 1	Wet Crystal	Monitor		0.48 ppm	Not Detected
TR1200-017-0220-PV WC Basket 2				0.92 ppm	Not Detected
TR1200-018-0220-PV WC Basket 4				0.45 ppm	Not Detected
TR1200-016-0220-PV ML	Mother Liquor	Monitor		23.86 ppm	Not Detected
TR1200-017-0220-PV ML				11.21 ppm	Not Detected
TR1200-018-0220-PV ML				11.92 ppm	Not Detected
TR1200-016-0220-PV Drum 1	Finished Good	NMT 1 ppm	NMT 1000 ppm	0.77 ppm	Not Detected
TR1200-017-0220-PV Drum 2				0.66 ppm	Not Detected
TR1200-018-0220-PV Drum 4				0.53 ppm	Not Detected

4.10.2. The following additional impurities were screened for in all four stages. Validation Testing Results verified that the following impurities met additional acceptance criteria. Results are detailed in the table below.

Organic Impurity Name	Specifications	Results
2-Nitropropane-1,3-diol	NMT 1ppm	NMT 1ppm
2-Nitroethanol	NMT 1ppm	NMT 1ppm
Tris(hydroxymethyl)nitromethane	NMT 1ppm	NMT 1ppm
Total Unspecified Impurities	NMT 0.03%	NMT 0.03%

4.11. **RESIDUAL SOLVENTS :**

4.11.1. Methanol and Nitromethane analysis was performed by an approved Service Provider on the individual drum samples. The Residual Solvents Results are detailed in the table below.

Lot Number	Stage of Material	Specification		Result	
		Methanol	Nitromethane	Methanol	Nitromethane
TR1200-016-0220-PV Drum 1	Finished Good	≤ 300 ppm	≤ 15ppm	≤ 300 ppm	≤ 15ppm
TR1200-017-0220-PV Drum 2				≤ 300 ppm	≤ 15ppm
TR1200-018-0220-PV Drum 4				≤ 300 ppm	≤ 15ppm

4.12. **TRACE ELEMENTS Al, As, Ca, Cu, Fe, K, Mg, Ni, and Pb:**

4.12.1. Refer to Degradation and Impurity Protocol: Tris Bio FUISA and Tris Bio Active for testing methods. The results of Trace Elements are detailed in the tables below.

Lot Number	Stage of Material	Specification (ppm max)					Result (ppm)				
		Al	As	Ca	Cu	Fe	Al	As	Ca	Cu	Fe
D609JBI032	Raw Material	Report					0.033	ND	4.655	0.022	0.150
D609JBK032							0.159	0.004	0.594	0.022	0.045
D609JBC032							0.199	ND	1.826	0.036	0.091
TR1200-016-0220-PV WC Basket 1	Wet Crystal	1					0.016	ND	1.190[2]	0.016	0.034
TR1200-017-0220-PV WC Basket 2							0.027	ND	0.715	0.044	0.030
TR1200-018-0220-PV WC Basket 4							ND	ND	0.266	0.012	ND
TR1200-016-0220-PV ML	Mother Liquor	Report					0.547	0.002	0.020	0.032	0.431
TR1200-017-0220-PV ML							0.508	ND	0.038	0.030	0.337
TR1200-018-0220-PV ML							0.440	0.003	ND	0.028	0.251
TR1200-016-0220-PV Drum 1	Finished Good	1					0.037	ND	0.446	0.006	0.020
TR1200-017-0220-PV Drum 2							0.054	ND	0.357	0.007	0.029
TR1200-018-0220-PV Drum 4							0.037	0.015	0.133	ND	0.062

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Lot Number	Stage of Material	Specification (ppm max)				Result (ppm)			
		K	Mg	Ni	Pb	K	Mg	Ni	Pb
D609JBI032	Raw Material	Report				0.186	0.318	1.159	0.009
D609JBK032						0.084	0.041	0.842	ND
D609JBC032						ND	0.117	0.950	0.001
TR1200-016-0220-PV WC Basket 1	Wet Crystal	1		0.4	0.5	0.307	0.092	0.340	ND
TR1200-017-0220-PV WC Basket 2						0.378	0.032	0.347	ND
TR1200-018-0220-PV WC Basket 4						0.078	ND	0.325	ND
TR1200-016-0220-PV ML	Mother Liquor	Report				0.370	0.062	0.726	ND
TR1200-017-0220-PV ML						0.092	0.007	0.813	ND
TR1200-018-0220-PV ML						0.283	ND	0.665	ND
TR1200-016-0220-PV Drum 1	Finished Good	1		0.4	0.5	0.185	0.020	0.185	ND
TR1200-017-0220-PV Drum 2						0.074	0.011	0.189	ND
TR1200-018-0220-PV Drum 4						0.078	ND	0.036	ND

[1]ND signifies Not Detected, or a value less than zero.
[2]Refer to BCL20-014.

5. CONCLUSION:

5.1. In conclusion, all sample from all stages of the process, met the required specification as dictated in the Degradation and Impurity Protocol: Tris Bio FUISA and Tris Bio Active. Nickel and formaldehyde were identified as objectionable impurities as per ICHQ3D and ICHM7 respectively. These impurities were monitored throughout processing from raw material to finished product to determine suitability of the purification. Levels of formaldehyde were reduced 93% through processing. Nickel was reduced by 86% to levels below the ICHQ3D control threshold for parenteral use assessed through option 1. Water was identified as an intentionally introduced solvent due to the aqueous purification process. Water content increased in 66% of the batches produced during validation. Water is not a toxic solvent and the resulting loss on drying content of NMT 1.0% meets USP requirements for tris. No limit adjustments or specification changes will occur as a result of the data acquired during protocol execution as the impurities met acceptance criteria set to the most stringent specifications of potential intended use.