BIOSPECTRA
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BioSpectra Revalidation Addendum Report

VALIDATION REPORT FOR THE MANUFACTURE OF:

TROMETHAMINE (TRIS), BIO EXCIPIENT OR BELOW GRADES

TO BE MANUFACTURED AS THE FOLLOWING CODES:

TROMETHAMINE (TRIS) TR32XX OR BELOW GRADES

TO BE MANUFACTURED AT:

BIOSPECTRA, INC., 1474 ROCKDALE LANE, STROUDSBURG PENNSYLVANIA, 18360

IN COMPLIANCE WITH THE STANDARDS OF:

THE JOINT IPEC – PQG GOOD MANUFACTURING PRACTICES
GUIDE FOR PHARMACEUTICAL EXCIPIENTS
ICH Q7 GUIDANCE

MANUFACTURED TO BE SUITABLE FOR USE AS:

PHARMACEUTICAL EXCIPIENT FOR DRUG MANUFACTURING PROCESSES

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1. INTRODUCTION

This Report is for the execution on *Tris Bio Excipient Revalidation Protocol Addendum 2020, Historic DCN: 20-003014, v. 1.0, Current DCN: BSI-PRL-0310.* The purpose of this Report is to summarize the execution of Revalidation Addendum Protocol. One batch of Tris, TR3200, was manufactured per change control SCC20-01, "Additional Tris RM Supply" which initiated this validation.

Manufactured Lot Number	Finished Good Lot Number
TR3200-561-0220-PV	TR7201-344-0220

The validation batch was manufactured using *Tris Batch Record Batch Number: TR3200-561-0220-PV, Historic DCN: 16-000619 v. 8.1, Current DCN: BSI-MPR-0009.* The revalidation addendum protocol describes the process as performed using Process Suite 1 of the BioSpectra, Stroudsburg, PA facility. This process suite is intended to manufacture excipients while complying with IPEC and ICH Q7 guidelines.

2. OBJECTIVE

The objective of this Report is to provide the results of BioSpectra Revalidation Addendum Protocol for the Manufacturer of Tromethamine (TRIS), Bio Excipient or Below Grades. The validation conforms to the requirements established by the FMEA, the Joint IPEC-PQG Good Manufacturing Practices Guide for Pharmaceutical Excipients, and ICH Q7 Guidelines.

3. SCOPE

This report applies to:

- The execution of BioSpectra Revalidation Addendum Protocol for the Manufacturer of Tromethamine (TRIS), Bio Excipient or Below Grades, *Historic DCN: 20-003014, v. 1.0, Current DCN: BSI-PRL-0310* which took place in Process Suite 1 of the BioSpectra Stroudsburg PA facility.
- The manufacturing process as described in the current Batch Record, *Tris Batch Record Batch Number: TR3200-561-0220-PV, Historic DCN: 16-000619 v. 8.1, Current DCN: BSI-MPR-0009,* under which one batch was validated. It is lot number TR3200-561-0220-PV. The product was Re-Assigned to Finished Good Lot Number TR7201-344-0220.
- All Manufacturing and Quality personnel that were involved in the creation, execution or documentation of the Protocol and Report.

The validation protocol validated the manufacturing process of Tromethamine (TRIS), Bio Excipient in Process Suite 1 located in BioSpectra's Stroudsburg PA facility.

All analysis listed in Table 4, Critical Quality Attributes (CQA), and Table 5, Required Analysis, of this report were performed during the execution of the validation protocol. Specifications and approval requirements for all raw materials (RM) and components have been created; therefore, the RM and components are not covered by this report except that only approved RM and components were used. This Report, Training Records, the Executed Protocol and the Degradation and Impurity Profile Protocol are filed in the TRIS Bio Excipient Revalidation Protocol Addendum 2020 Report Binder. The Degradation and Impurity Profile Protocol and Report, protocol Training Form, QC testing results, the Batch Record for the validation batch, and the executed protocol are attached electronically in MasterControl to this report.

4. RESPONSIBILITIES

It was the responsibility of the Validation Team to ensure proper training was conducted in order to carry out the requirements of the Revalidation Addendum Protocol. The training for the Revalidation Addendum Protocol was described in each section of the Revalidation Addendum Protocol. All training records pertaining to the Revalidation Addendum Protocol are available via the Document Control department. It was the responsibility of qualified Manufacturing personnel to perform the manufacturing operations in accordance with the procedures described in the Batch Record and associated Section of the Revalidation Addendum Protocol. It was the responsibility of the Quality Control Laboratory to ensure the performance of all testing required by the Revalidation Addendum Protocol. All results were recorded in the appropriate laboratory documentation and transferred to spreadsheets to be provided to the Validation Supervisor or qualified designee for development of this report. All deviations or discrepancies were documented, reviewed, investigated, and submitted to the Validation Team, as listed in the approval section of the Revalidation Addendum Protocol, for review, investigation and closure.

5. CHANGES SINCE LAST VALIDATION

SCC20-01, Additional Tris RM Supply. Currently Tris and Tris HCl are manufactured with Raw Material from Angus (US) that is manufactured in the Sterlington, LA site. Angus is now capable of providing Tris from another manufacturing location, in Germany. This additional supply of Tris Raw Material from Angus was tested and assessed to ensure the material meets supplier approval expectations.

6. EXECUTIVE SUMMARY

The validation protocol contains executable forms which document the manufacturing, sampling and sample testing of the validation batch. Each of the forms were completed during the execution of the protocol. The forms are reviewed by the Quality Department to ensure the Validation Protocol was executed as written.

7. DESCRIPTION OF THE PROCESS

Reference Tris Bio Excipient Revalidation Protocol Addendum 2020, *Historic DCN: 20-003014, v. 1.0, Current DCN: BSI-PRL-0310* section 5 for a complete Description of the Process.

8. MANUFACTURING OBSERVATIONS

All Instruments used during the manufacture of the validation were in calibration, the instruments and calibration dates are listed in the following table. Manufacturing of TR3200-561-0220-PV began on 2/18/20 and ended on 2/22/20.

TABLE 1: MANUFACTURING INSTRUMENTATION CALIBRATION

Description	ID	Calibration Date	Due Date
Production Scale	TRFS02	10/11/19	4/20
IR Thermometer	616754	11/7/19	5/7/20
IR Thermometer	614538	8/5/19	8/5/20
Thermohygrometer	15-077-963	1/27/20	1/27/22
Digital Wall Clock	181173338	8/22/19	8/21
Digital Wall Clock	160190717	8/22/19	8/21

TABLE 2: CRITICAL PROCESS PARAMETERS (CPP)

PROCESS STEP	CPP	COMMENT
Batch Charge	Raw Material Charge	Raw material will be charged to the ML to achieve the most optimum yield quantities. Acceptable charge is 3300 ± 150 kg for finished goods lots. Wet crystal feed for Tris manufacturing the raw material charge can be varied.
Heating	Temperature	A temperature of 70 to 82°C is maintained to keep product in solution.
Purification	Recirculation	The solution is recirculated through a 3-micron Cuno filter until the solution is clear. A minimum of ½ hour is required to allow the carbon to affect purification. There is no maximum time, but to maintain production flow we will use 3-hours as a maximum during validation. Recirculation time starts when the filter aids are added.
	Filtration	It is then filtered through the 3-micron Cuno filter, 0.3 µM nominal Zeta filter and 400 mesh basket filter into the cooling tank.
Crystallize	Temperature	A final temperature of 18 to 20°C is achieved to ensure the yield is attained.
Drying	Hot Dryer Temperature	45 to 70°C for the inlet air temperature for the hot dryer
	Cold Dryer Temperature	Less than 40°C for the inlet air for the cold dryer

TABLE 3: VALIDATION BATCH MANUFACTURING SUMMARY

	Tris Lot Number:	**TR3200-561-0220-PV**
CPP	**Specification**	**Result**
Raw Material Charge	3300 ± 50 kg	3,334.7kg
Heating Temperature	Target: 75°C Hold at 74 to 76 °C	Temperature: 75.0°C
Recirculation Time	2 Hours	Start Time: 0201 End Time: 0401 Total Time: 2 hours
Cool Temp	Target: 18°C Hold at 18 to 20 °C	Temperature: 18.0°C

9. ANALYSIS

TABLE 4: CRITICAL QUALITY ATTRIBUTES (CQA)

Table 4 lists the CQA's required in the Revalidation Addendum Protocol.

Critical Quality Attribute	**Specification**
Appearance and Color	White / Crystals
Absorbance	0.01 a.u. max. @ 400 nm
	0.06 a.u. max. @ 280 nm
	0.06 a.u. max. @ 260 nm
Assay (Ultrapure)	99.9% minimum
Insoluble Matter	0.005% maximum
Loss on Drying	0.5% maximum
Melting Range	168 to 172 °C
pH 5% solution	10.0 to 11.5

TABLE 5: REQUIRED ANALYSIS

Required Analysis	Specification/Test Method
Mother Liquor (ML) Microbial Analysis	Monitor / USP <61> and <62> / MPL Laboratories
Wet Crystal (WC) Microbial Analysis	Monitor / USP <61> and <62> / MPL Laboratories
Raw Material (RM) Microbial Analysis	Monitor / USP <61> and <62> / MPL Laboratories
Mother Liquor (ML) Endotoxin Analysis	Monitor / USP <85> / BioSpectra *Historic DCN: 16-000367, Current DCN: BSI-SOP-0097*
Wet Crystal (WC) Endotoxin Analysis	Monitor / USP <85> / BioSpectra *Historic DCN: 16-000367, Current DCN: BSI-SOP-0097*
Raw Material (RM) Endotoxin Analysis	Monitor / USP <85> / BioSpectra *Historic DCN: 16-000367, Current DCN: BSI-SOP-0097*
Impurity Profile Testing	Report / BioSpectra *Historic DCN: 16-000454, Current DCN: BSI-PRL-0013*
In-Process (IP) Moisture Analysis (Dry)	≤ 0.24% / BioSpectra *Historic DCN: 16-000514, Current DCN: BSI-SOP-0141*
In-Process ML Absorbance (1:1)	Meets Specifications / BioSpectra *Historic DCN: 16-000491, Current DCN: BSI-SPC-0047*
In-Process WC Absorbance	Report / BioSpectra *Historic DCN: 16-000492, Current DCN: BSI-SPC-0048*
Finished Goods (FG) Analysis	Meets Specifications / BioSpectra DCN: *Historic DCN: 20-003014, v. 1.0, Current DCN: BSI-PRL-0310*
Microbial Analysis – Finished Goods	Monitor / USP <61> and <62> / MPL Laboratories
Endotoxin Analysis – Finished Goods	Monitor / USP <85> / BioSpectra *Historic DCN: 16-000367, Current DCN: BSI-SOP-0097*
Long-term Stability Analysis	Meets Specifications / BioSpectra *Historic DCN: 16-000505, Current DCN: BSI-SOP-0136*

10. QC TESTING

A single batch of Tris Bio Excipient Grade product was manufactured using RM from Angus which was manufactured in Germany. The manufacturing parameters in the current Batch Record were used for this validation. The batch met the acceptance parameters.

TABLE 6: QUALITY CONTROL BATCH ANALYSIS SUMMARY

This table summarizes Form 3: Quality Control Batch Analysis in the executed Revalidation Addendum Protocol.

	Tris Lot Number:	**TR3200-561-0220-PV**
Analysis	**Specification**	**Result**
ML Absorbance (1:1)	0.150 a.u. max. @ 400 nm	0.102 a.u.
	2.000 a.u. max. @ 280 nm	1.394 a.u.
	2.200 a.u. max. @ 260 nm	1.476 a.u.
ML Microbial Analysis	Report	< 10 CFU/g TAMC < 10 CFU/g TYMC
ML Endotoxin Analysis	Report	< 5.00 EU/mL
ML Impurity Profile	See Impurity Report	See Impurity Report
RM Approval Analysis	See RM Analytical Summary Sheet	See RM Analytical Summary Sheet
RM Microbial Analysis	Report	See the table below, RM Micro/Endo Results
RM Endotoxin Analysis	Report	See the table below, RM Micro/Endo Results
RM Impurity Profile	See Impurity Report	See Impurity Report
WC Absorbance Analysis	0.01 a.u. @ 400 nm max	<0.01 a.u.
	0.06 a.u. @ 280 nm max	0.01 a.u.
	0.06 a.u. @ 260 nm max	0.01 a.u.
WC Moisture Analysis	≤ 0.24%	0.13%
WC Microbial Analysis	Report	<10 CFU/g TAMC <10 CFU/g TYMC
WC Endotoxin Analysis	Report	< 1.2 EU/g
WC Impurity Profile	See Impurity Report	See Impurity Report
FG Microbial Analysis	Report	<10 CFU/g TAMC <10 CFU/g TYMC
FG Endotoxin Analysis	Report	<1.2 EU/g
FG Impurity Profile	See Impurity Report	See Impurity Report

TABLE 7: RAW MATERIAL TESTING RESULTS

Analysis		RM MICRO/ENDO RESULTS				
		SPECIFICATIONS	F110JATV12	F110JATV16	F100JAVV10	TR3200-559-0220
Microbial Analysis	TAMC	Report	<10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g
	TYMC	Report	<10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g
Endotoxin Analysis		Report	<1.2 EU/g	<1.2 EU/g	<1.2 EU/g	<1.2 EU/g

TABLE 8: QUALITY CONTROL CQA/FG TESTING

Tris Lot Number:			TR3200-561-0220-PV		
Analysis		Specifications	Composite	Basket 1	Basket 3
Absorbance (1M or 10%)	260 nm	0.06 a.u. max.	0.0077	0.0073	0.0081
	280 nm	0.06 a.u. max.	0.0064	0.0063	0.0067
	400 nm	0.01 a.u. max.	0.0000	0.0000	0.0000
Absorbance (40%)	280 nm	0.2 a.u. max.	0.0273	0.0252	0.0237
	290 nm	0.2 a.u. max.	0.0253	0.0244	0.0225
APHA Color, 20% Solution		20 APHA max	<20 APHA	<20 APHA	<20 APHA
Appearance and Color		White / Crystals	White/Crystals	White/Crystals	White/Crystals
Appearance of Solution		Passes Test	Passes Test	Passes Test	Passes Test
Assay (Ultrapure)		99.9% min.	100.53%	100.44%	100.38%
Assay (USP / EP/ JP)		99.8-100.1%	99.95%	99.90%	99.97%
Bioburden Microbial Analyses	TAMC	≤100 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g
	TYMC	≤100 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g
	Total Bioburden	≤ 500 CFU/g	<500 CFU/g	<500 CFU/g	<500 CFU/g
	Bile tolerant gram neg. bacteria	Absence in 1 g	Absent in 1 g	Absent in 1 g	Absent in 1 g
	Escherichia coli	Absence in 1 g	Absent in 1 g	Absent in 1 g	Absent in 1 g
	Pseudomonas aeruginosa	Absence in 1 g	Absent in 1 g	Absent in 1 g	Absent in 1 g
	Staphylococcus aureus	Absence in 1 g	Absent in 1 g	Absent in 1 g	Absent in 1 g
	Candida albicans	Absence in 1 g	Absent in 1 g	Absent in 1 g	Absent in 1 g
	Salmonella sp.	Absence in 10 g	Absent in 10 g	Absent in 10 g	Absent in 10 g
Chloride		0.01% max	<0.01%	<0.01%	<0.01%
Clarity and Color of Solution		Passes Test	Passes Test	Passes Test	Passes Test
Endotoxin		2.5 EU/g max.	<1.1 EU/g	<1.2 EU/g	<1.2 EU/g
Enzymes	DNase	None Detected	None Detected	None Detected	None Detected
	RNase	None Detected	None Detected	None Detected	None Detected
	Protease	None Detected	None Detected	None Detected	None Detected
Heavy Metals	USP	5 ppm max	<5 ppm	<5 ppm	<5 ppm
	JPC	8 ppm max	<8 ppm	<8 ppm	<8 ppm
USP Identifications	A (UATR)	Passes Test	Passes Test	Passes Test	Passes Test
	B	Passes Test	Passes Test	Passes Test	Passes Test
	C	Passes Test	Passes Test	Passes Test	Passes Test
EP Identifications	A	Passes Test	Passes Test	Passes Test	Passes Test

Tris Lot Number:			TR3200-561-0220-PV		
Analysis		**Specifications**	**Composite**	**Basket 1**	**Basket 3**
	B (Melting Range)	168-172°C	169.8-171.5°C	170.1-171.8°C	170.2-171.6°C
	C (UATR)	Passes Test	Passes Test	Passes Test	Passes Test
	D	Passes Test	Passes Test	Passes Test	Passes Test
JP Identifications	**A**	Passes Test	Passes Test	Passes Test	Passes Test
	B	Passes Test	Passes Test	Passes Test	Passes Test
Insoluble Matter		0.005% max	<0.0010%	<0.0010%	<0.0010%
Iron (Fe)		5 ppm max	<5 ppm	<5 ppm	<5 ppm
Loss on Drying		0.3% max	0.1177%	0.1455%	0.1774%
Melting Range		168-172°C	169.8-171.5°C	170.1-171.8°C	170.2-171.6°C
pH @ 25 ± 2°C	**0.1 M**	10.0 -11.0	10.478 @ 25.0°C	10.526 @ 24.4°C	10.524 @ 23.6°C
	5 % or 1 in 20	10.0-11.5	10.791 @ 23.9°C	10.808 @ 24.0°C	10.814 @ 23.6°C
Related Substances		0.1% max	<0.1%	<0.1%	<0.1%
Residue on Ignition		0.05% max	<0.0200%	<0.0200%	<0.0200%
Solubility (100g/250 mL)		Clear and colorless to Slightly Yellow Solution	Clear and colorless to Slightly Yellow Solution	Clear and colorless to Slightly Yellow Solution	Clear and colorless to Slightly Yellow Solution
Water (by Karl Fischer)		2.0% max	0.47%	0.18%	1.87%
Trace Metals	**Arsenic (As)**	1 ppm max	<1 ppm max	<1 ppm max	<1 ppm max
	Calcium (Ca)	1 ppm max	<1 ppm max	<1 ppm max	<1 ppm max
	Copper (Cu)	1 ppm max	<1 ppm max	<1 ppm max	<1 ppm max
	Iron (Fe)	1 ppm max	<1 ppm max	<1 ppm max	<1 ppm max
	Lead (Pb)	1 ppm max	<1 ppm max	<1 ppm max	<1 ppm max
	Magnesium (Mg)	1 ppm max	<1 ppm max	<1 ppm max	<1 ppm max
	Nickel (Ni)	15 ppm max	<15 ppm max	<15 ppm max	<15 ppm max
Quality Control Additional FG Testing					
Analysis		**Specifications**	**Composite**	**Basket 1**	**Basket 3**
Absorbance (1M or 10%)	**260 nm**	0.03 a.u. max.	0.0077	0.0073	0.0081
	280 nm	0.02 a.u. max.	0.0064	0.0063	0.0067
	400 nm	0.01 a.u. max.	0.0000	0.0000	0.0000
	430 nm	0.004 a.u. max	0.0000	0.0005	0.0007

		Tris Lot Number:	TR3200-561-0220-PV		
Analysis		**Specifications**	**Composite**	**Basket 1**	**Basket 3**
Absorbance (40%)	**400 nm**	0.02 a.u. max	0.0012	0.0003	0.0002
Assay		99.8 to 100.1%	99.95	99.90	99.97
pH (1:100)		10.3 to 10.7	10.464 @ 24.5°C	10.808 @ 24.0[1]°C	10.521 @ 23.4°C
pH (0.05 M)		10.3 to 10.5	10.327 @ 24.5°C	10.358 @ 24.5°C	10.309 @ 34.4°C

		Tris Lot Number:	TR3200-561-0220-PV		
Analysis		**Specifications**	**Basket 5**	**Basket 7**	**Basket 9**
Absorbance (1M or 10%)	**260 nm**	0.06 a.u. max.	0.0067	0.0071	0.0074
	280 nm	0.06 a.u. max.	0.0056	0.0060	0.0059
	400 nm	0.01 a.u. max.	0.0000	0.0000	0.0000
Absorbance (40%)	**280 nm**	0.2 a.u. max	0.0217	0.0237	0.0211
	290 nm	0.2 a.u. max	0.0206	0.0225	0.0197
APHA Color, 20% Solution		20 APHA max	<20 APHA	<20 APHA	<20 APHA
Appearance and Color		White / Crystals	White/Crystals	White/Crystals	White/Crystals
Appearance of Solution		Passes Test	Passes Test	Passes Test	Passes Test
Assay (Ultrapure)		99.9% min.	100.48%	100.36%	100.29%
Assay (USP / EP/ JP)		99.8-100.1%	99.90%	99.81%	99.93%
Bioburden Microbial Analyses	**TAMC**	≤100 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g
	TYMC	≤100 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g
	Total Bioburden	≤ 500 CFU/g	<500 CFU/g	<500 CFU/g	<500 CFU/g
	Bile tolerant gram neg. bacteria	Absence in 1g	Absent in 1 g	Absent in 1 g	Absent in 1 g
	Escherichia coli	Absence in 1g	Absence in 1g	Absent in 1 g	Absent in 1 g
	Pseudomonas aeruginosa	Absence in 1g	Absent in 1 g	Absent in 1 g	Absent in 1 g
	Staphylococcus aureus	Absence in 1g	Absent in 1 g	Absent in 1 g	Absent in 1 g
	Candida albicans	Absence in 1g	Absent in 1 g	Absent in 1 g	Absent in 1 g
	Salmonella sp	Absence in 10g	Absent in 10 g	Absent in 10 g	Absent in 10 g
Chloride		0.01% max	<0.01%	<0.01%	<0.01%
Clarity and Color of Solution		Passes Test	Passes Test	Passes Test	Passes Test
Endotoxin		2.5 EU/g max.	<1.1 EU/g	<1.6 EU/g	<1.1 EU/g
Enzymes	**DNase**	None Detected	None Detected	None Detected	None Detected
	RNase	None Detected	None Detected	None Detected	None Detected
	Protease	None Detected	None Detected	None Detected	None Detected
Heavy Metals	**USP**	5 ppm max	<5 ppm	<5 ppm	<5 ppm
	JPC	8 ppm max	<8 ppm	<8 ppm	<8 ppm
	A (UATR)	Passes Test	Passes Test	Passes Test	Passes Test

[1] Value was from additional testing; lot selection would be required.

Tris Lot Number:			TR3200-561-0220-PV		
Analysis		**Specifications**	**Basket 5**	**Basket 7**	**Basket 9**
USP Identifications	**B**	Passes Test	Passes Test	Passes Test	Passes Test
	C	Passes Test	Passes Test	Passes Test	Passes Test
EP Identifications	**A**	Passes Test	Passes Test	Passes Test	Passes Test
	B (Melting Range)	168-172°C	169.9-171.5°C	170.2-171.5°C	170.0-171.6°C
	C (UATR)	Passes Test	Passes Test	Passes Test	Passes Test
	D	Passes Test	Passes Test	Passes Test	Passes Test
JP Identifications	**A**	Passes Test	Passes Test	Passes Test	Passes Test
	B	Passes Test	Passes Test	Passes Test	Passes Test
Insoluble Matter		0.005% max	<0.0010%	<0.0010%	<0.0010%
Iron (Fe)		5 ppm max	<5 ppm	<5 ppm	<5 ppm
Loss on Drying		0.5% max	0.1941%	0.3133%	0.3855%
Melting Range		168-172°C	169.9-171.5°C	170.2-171.5°C	170.0-171.6°C
pH @ 25 ± 2°C	**0.1 M**	10.0 -11.0	10.519 @ 24.5°C	10.519 @ 24.4°C	10.509 @ 24.2°C
	5 % or 1 in 20	10.0-11.5	10.805 @ 23.7°C	10.792 @ 23.8°C	10.801 @ 23.3°C
Related Substances		0.1% max	<0.1%	<0.1%	<0.1%
Residue on Ignition		0.05% max	<0.0200%	<0.0200%	<0.0200%
Solubility (100g/250 mL)		Clear and colorless to Slightly Yellow Solution	Clear and colorless to Slightly Yellow Solution	Clear and colorless to Slightly Yellow Solution	Clear and colorless to Slightly Yellow Solution
Water (by Karl Fischer)		2.0% max	0.77%	1.44%	1.04%
Trace Metals	**Arsenic (As)**	1 ppm max	<1 ppm max	<1 ppm max	<1 ppm max
	Calcium (Ca)	1 ppm max	<1 ppm max	<1 ppm max	<1 ppm max
	Copper (Cu)	1 ppm max	<1 ppm max	<1 ppm max	<1 ppm max
	Iron (Fe)	1 ppm max	<1 ppm max	<1 ppm max	<1 ppm max
	Lead (Pb)	1 ppm max	<1 ppm max	<1 ppm max	<1 ppm max
	Magnesium (Mg)	1 ppm max	<1 ppm max	<1 ppm max	<1 ppm max
	Nickel (Ni)	15 ppm max	<15 ppm max	<15 ppm max	<15 ppm max
Quality Control Additional FG Testing					
Analysis		**Specifications**	**Basket 5**	**Basket 7**	**Basket 9**
Absorbance (1M or 10%)	**260 nm**	0.03 a.u. max.	0.0067	0.0071	0.0074
	280 nm	0.02 a.u. max.	0.0056	0.0060	0.0059
	400 nm	0.01 a.u. max.	0.0000	0.0000	0.0000
	430 nm	0.004 a.u. max	0.0011	0.0001	0.0001
Absorbance (40%)	**400 nm**	0.02 a.u. max	0.0002	0.0008	0.0005
Assay		99.8 to 100.1%	99.90%	99.81%	99.93%
LOD		0.3%	0.1941%	0.3133%	0.3855[2]%

[2] Value was from additional testing; lot selection would be required.

Tris Lot Number:		TR3200-561-0220-PV		
Analysis	**Specifications**	**Basket 5**	**Basket 7**	**Basket 9**
pH (1:100)	10.3 to 10.7	10.501 @ 24.0°C	10.476 @ 24.0°C	10.464 @ 23.9°C
pH (0.05 M)	10.3 to 10.5	10.320 @ 24.2°C	10.255 @ 23.6°C	10.310 @ 24.2°C

Tris Lot Number:			TR3200-561-0220-PV		
Analysis		**Specifications**	**Basket 11**		
Absorbance (1M or 10%)	**260 nm**	0.06 a.u. max.	0.0069		
	280 nm	0.06 a.u. max.	0.0054		
	400 nm	0.01 a.u. max.	0.0000		
Absorbance (40%)	**280 nm**	0.2 a.u. max	0.0227		
	290 nm	0.2 a.u. max	0.0214		
APHA Color, 20% Solution		20 APHA max	<20 APHA		
Appearance and Color		White / Crystals	White/Crystals		
Appearance of Solution		Passes Test	Passes Test		
Assay (Ultrapure)		99.9% min.	100.35		
Assay (USP / EP/ JP)		99.8-100.1%	100.13		
Bioburden Microbial Analyses	**TAMC**	≤100 CFU/g	<10		
	TYMC	≤100 CFU/g	<10		
	Total Bioburden	≤ 500 CFU/g	<500		
	Bile tolerant gram neg. bacteria	Absence in 1g	Absence in 1g		
	Escherichia coli	Absence in 1g	Absence in 1g		
	Pseudomonas aeruginosa	Absence in 1g	Absence in 1g		
	Staphylococcus aureus	Absence in 1g	Absence in 1g		
	Candida albicans	Absence in 1g	Absence in 1g		
	Salmonella sp	Absence in 10g	Absence in 10g		
Chloride		0.01% max	<0.01		
Clarity and Color of Solution		Passes Test	Passes Test		
Endotoxin		2.5 EU/g max.	<1.2		
Enzymes	**DNase**	None Detected	None Detected		
	RNase	None Detected	None Detected		
	Protease	None Detected	None Detected		
Heavy Metals	**USP**	5 ppm max	<5 ppm		
	JPC	8 ppm max	<8 ppm		
USP Identifications	**A (UATR)**	Passes Test	Passes Test		
	B	Passes Test	Passes Test		
	C	Passes Test	Passes Test		
EP Identifications	**A**	Passes Test	Passes Test		
	B (Melting Range)	168-172°C	169.8 to 171.4		

Tris Lot Number:			TR3200-561-0220-PV		
Analysis		**Specifications**	**Basket 11**		
	C (UATR)	Passes Test	Passes Test		
	D	Passes Test	Passes Test		
Analysis		**Specifications**	**Basket 11**		
JP Identifications	**A**	Passes Test	Passes Test		
	B	Passes Test	Passes Test		
Insoluble Matter		0.005% max	<0.0010		
Iron (Fe)		5 ppm max	<5 ppm		
Loss on Drying		0.3% max	0.3855		
Melting Range		168-172°C	169.8 to 171.4		
pH @ 25 ± 2°C	**0.1 M**	10.0 -11.0	10.504 @ 24.0		
	5 % or 1 in 20	10.0-11.5	10.780 @ 23.8		
Related Substances		0.1% max	<0.1		
Residue on Ignition		0.05% max	<0.0200		
Solubility (100g/250 mL)		Clear and colorless to Slightly Yellow Solution	Clear and colorless to Slightly Yellow Solution		
Water (by Karl Fischer)		2.0% max	0.49		
Trace Metals	**Arsenic (As)**	1 ppm max	<1 ppm max		
	Calcium (Ca)	1 ppm max	<1 ppm max		
	Copper (Cu)	1 ppm max	<1 ppm max		
	Iron (Fe)	1 ppm max	<1 ppm max		
	Lead (Pb)	1 ppm max	<1 ppm max		
	Magnesium (Mg)	1 ppm max	<1 ppm max		
	Nickel (Ni)	15 ppm max	<15 ppm max		
Quality Control Additional FG Testing					
Analysis		**Specifications**	**Composite**		
Absorbance (1M or 10%)	**260 nm**	0.03 a.u. max.	0.0074		
	280 nm	0.02 a.u. max.	0.0059		
	400 nm	0.01 a.u. max.	0.0000		
	430 nm	0.004 a.u. max	0.0001		
Absorbance (40%)	**400 nm**	0.02 a.u. max	0.0005		
Assay		99.8 to 100.1%	100.13		
LOD		0.3%	0.2968		
pH (1:100)		10.3 to 10.7	10.464 @ 23.9		
pH (0.05 M)		10.3 to 10.5	10.272 @ 24.2		

TABLE 9: QUALITY CONTROL COMPOSITE TESTING SUMMARY

Table 9: Quality Control Composite Testing Summary, this data is from the Analytical Summary Sheet Tris Bio Contract Grade /TR7201, DCN: 16-001038 v.3.0 which is in the attached Batch Record.

Analysis		Specification	Test Result
Absorbance (1M)	260nm	0.06 a.u. max.	0.01
	280nm	0.06 a.u. max.	0.01
	400nm	0.01 a.u. max.	<0.01
Appearance & Color		White/Crystals	White/Crystals
Assay (Ultrapure)		99.9% min.	100.5%
Assay (USP)		99.0% - 101.0%	100.0%
Endotoxin		2.5 EU/g max.	<1.1EU/g
Enzymes	DNase	None Detected	None Detected
	RNase	None Detected	None Detected
	Protease	None Detected	None Detected
Heavy Metals		0.001% max.	<0.001%
Identity B		Passes Test	Passes Test
Identity C		Passes Test	Passes Test
Identity (IR)		Passes Test	Passes Test
Insoluble Matter		0.005% max	<0.001%
Loss on Drying		0.5% max.	0.1%
Melting Range		168-172°C	170-172°C
pH (0.1M)		10.0-11.0	10.5 @ 25.0°C
pH (5%)		10.0-11.5	10.8 @ 23.9°C
Residue on Ignition		0.1% max.	<0.1%
Trace Metals	Arsenic (As)	0.0001% max.	<0.0001%
	Calcium (Ca)	0.0001% max.	<0.0001%
	Copper (Cu)	0.0001% max.	<0.0001%
	Iron (Fe)	0.0001% max.	<0.0001%
	Lead (Pb)	0.0001% max.	<0.0001%
	Magnesium (Mg)	0.0001% max.	<0.0001%

11. INVESTIGATIONS

There were no QC Laboratory Investigations or Discrepancy Investigations performed regarding the execution of the Revalidation Addendum Protocol. There is one applicable Change Control, SCC20-18 *Analysis Result Reporting Equivalency.* SCC20-18 benefits the testing by providing precise results and by reducing waste and duplicate testing. Instrumental results will be used instead of Wet Chemistry testing.

12. CONCLUSION

BioSpectra has validated the Tris Bio Excipient manufacturing process using RM from an alternate location, (Germany). Tromethamine (Tris), Bio Excipient or Below Grade is validated to be compliant with key compliance grades up to and including the Bio Excipient Grade. The Tris Bio Excipient manufacturing process is an approved Bio Excipient manufacturing process performed in accordance with Joint IPEC – PQG Good Manufacturing Practice Guide for Pharmaceutical Excipients ICH Q7 Guidance. There were no QC Laboratory Investigations or Discrepancy Investigations performed during the execution of the Revalidation Addendum Protocol. All expectations of the validation have been met and approved on June 24, 2021. The results contained in this validation report deem Tris Bio Excipient manufactured, using this validated process, suitable for use as an Excipient for drug manufacturing processes. Additionally, the executed items are attached as supporting documentation to this validation report.