BIOSPECTRA
100 Majestic Way, Bangor, PA 18013 / www.biospectra.us

BIS-TRIS
2023 VALIDATION LOTS
LONG TERM STABILITY REPORT

TABLE OF CONTENTS

1. OVERVIEW:

The purpose of this report is to analyze and conclude on the data obtained from the long-term stability study of Bis-Tris manufactured at BioSpectra's Bangor, PA facility. Testing intervals are designated by T_n, where n designates the number of months on stability. Testing is performed every three months for the first year, every six months for the second year, and annually for each subsequent year in order to maintain that the manufactured product remains stable under the specified conditions and for the specified interval of time. The analysis of the compiled data may also aid in a re-evaluation of the retest date for the finished good product.

This Long-Term Stability analysis will assess the stability of Bis-Tris validation lots BTRI-0123-00003-PV, BTRI-0123-00004-PV, BTRI-0123-00005-PV and BTRI-0123-00006-PV that completed eighteen (18) months of long-term stability in September 2024 and lot BTRI-0123-00045-PV that completed nine (9) months of long-term stability in August 2024. This study includes the following analyses: Absorbance (0.1M) @ 280 nm, Absorbance (0.1M) @ 340 nm, Appearance and Color, Assay (Dried Basis), Identification (IR), Loss on Drying, Melting Point, pH (1%) and pKa. Results from all analyses are summarized in Table 2 through 11. The data was analyzed utilizing a Shelf-Life Plot, which determines the point in time at which the slope would exceed the acceptance criteria. As long as the slope has a statistically significant difference from zero using a 95% confidence limit, an estimated time in months can be established at which the acceptance criteria will no longer be met, i.e. the Shelf Life. This allows BioSpectra to ensure that the product is stable over the time period in which it is part of the stability program. All quantitative data was analyzed using these methods. This study will be used to establish shelf life for all product codes of Bis-Tris. The following product codes are commercially available:

- BTRI-3250

2. REFERENCES:

2.1. BSI-SOP-0136, Stability Testing Program
2.2. BSI-SOP-0146, Stability Inventory
2.3. BSI-SOP-0289, Stability Indication Protocol
2.4. Current USP
2.5. ICH Q1E

3. SAMPLE DESIGNATION:

3.1. Samples placed on the stability program in 2023 consisted of five validation lots of Bis-Tris. Stability samples from these lots were put into P/P and Labline packaging configuration. The samples were packaged in accordance with the Stability Inventory SOP. Reference Table 1, below, for packaging configuration and description. The type of packaging utilized in this stability study was equivalent to the finished product packaging.

TABLE 1: PACKAGING DETAILS

Packaging Configuration	Packaging Description
Poly/Poly (P/P)	Samples are packaged into small poly bags and sealed with a zip tie. All individual samples are then placed into a poly drum.
Labline (HDPE Bottle)	Samples are packaged into a HDPE Lab Screw-Top Bottle.

4. STORAGE:

4.1. The Packaging and Storage requirements for Bis-Tris are to be in tightly closed container, and stored in a dry, well-ventilated area away from incompatible substances. For this study, the samples were stored in the Long-Term Stability Chamber H03SC01 at the Bangor, PA facility from April 2023 until the last sample time point in September 2024. Storage conditions have been continuously measured and recorded utilizing MadgeTech data loggers with regulated conditions for temperature (15-30°C) and humidity (monitor). For this period, the maximum temperature recorded was 26.20°C, the minimum temperature was 21.81°C, the average temperature was 25.41°C, and the average Mean Kinetic Temperature was 25.41°C. The maximum relative humidity recorded was 80.5%, the minimum relative humidity was 43.6%, and the average relative humidity was 61.2%. Maximum and minimum values that are outside limits for temperature and humidity are due to opening the door of the chamber as explained in Temperature and Humidity Monitoring Assessments for the chambers. Section 5 will include any excursions from these conditions that resulted in an investigation.

5. INVESTIGATIONS:

5.1. BDI24-13, Out of range humidity for the Real Time Stability Chamber H03SC01 caused by improper work order completion to prevent water leaking from the stability chamber. On 1/15/24 while conducting a maintenance walkthrough of the Bangor facility water was observed on the floor of room H03RM01. The issue was found to be a faulty pump and later repaired. There was no impact to the current list of materials in the stability chamber.

5.2. BDI24-126, Out of specification humidity and temperature for H03SC01 occurred on 8/15/24 with a humidity reading of 54.4% and a temperature of 21.81°C. It was discovered that a 20-amp fuse had blown. The fuse was replaced and the chamber went back into specification on 8/16/24 with a humidity reading of 62.3%. There is no impact on the stability samples as this excursion lasted less than 24 hours.

6. LOT EVALUATION:

TABLE 2: BTRI-0123-00003-PV P/P

Analysis	Specification	T_0	T_3	T_6	T_9	T_{12}	T_{18}
Absorbance (0.1M)	**0.04 a.u. max @ 280 nm**	0.0096	0.0091	0.0097	0.0105	0.0128	0.0101
	0.02 a.u. max @ 340 nm	0.0002	0.0003	0.0003	0.0004	0.0021	0.0040
Appearance and Color	**White Crystalline Powder**	White Crystalline Powder	White Crystalline Powder	White Crystalline Powder	White Crystalline Powder	White Crystalline Powder	White Crystalline Powder
Assay (Dried Basis)	**99.0-101.0%**	99.97%	99.87%	100.05%	100.11%	100.03%	100.21%
Identification (IR)	**Conforms to Reference Standard**	Conforms to Reference Standard	Conforms to Reference Standard	Conforms to Reference Standard	Conforms to Reference Standard	Conforms to Reference Standard	Conforms to Reference Standard
Loss on Drying	**≤1.0%**	<0.0144%	0.0277%	0.0160%	<0.0079%	0.0804%	0.1140%
Melting Point	**100-105°C**	103.5°C	103.4 °C	103.5 °C	104.2 °C	104.2 °C	104.2 °C
pH (1%)	**8.8 – 9.8**	9.33	9.48	9.45	9.49	9.28	9.46
pKa	**6.3 – 6.7**	6.564	6.653	6.579	6.562	6.603	6.562

- **REMAINING TESTING INTERVAL PULL DATES**
  - T = 24; Scheduled for March 30, 2025
  - T = 36; Scheduled for March 30, 2026
  - T = 48; Scheduled for March 30, 2027
  - T = 60; Scheduled for March 30, 2028

TABLE 3: BTRI-0123-00003-PV LABLINE

Analysis	Specification	T_0	T_3	T_6	T_9	T_{12}	T_{18}
Absorbance (0.1M)	**0.04 a.u. max @ 280 nm**	0.0096	0.0086	0.0096	0.0096	0.0128	0.0089
	0.02 a.u. max @ 340 nm	0.0002	0.0002	<0.0030	0.0003	0.0021	0.0031
Appearance and Color	**White Crystalline Powder**	White Crystalline Powder	White Crystalline Powder	White Crystalline Powder	White Crystalline Powder	White Crystalline Powder	White Crystalline Powder
Assay (Dried Basis)	**99.0-101.0%**	99.97%	100.05%	100.09%	100.04%	100.08%	100.11%
Identification (IR)	**Conforms to Reference Standard**	Conforms to Reference Standard	Conforms to Reference Standard	Conforms to Reference Standard	Conforms to Reference Standard	Conforms to Reference Standard	Conforms to Reference Standard
Loss on Drying	**≤1.0%**	<0.0144%	0.0484%	0.0732%	0.0195%	0.0342%	0.0579%
Melting Point	**100-105°C**	103.5 °C	103.3°C	103.5°C	104.3°C	104.2°C	104.1 °C
pH (1%)	**8.8 – 9.8**	9.33	9.51	9.55	9.50	8.95	9.49
pKa	**6.3 – 6.7**	6.564	6.634	6.577	6.563	6.593	6.562

- **REMAINING TESTING INTERVAL PULL DATES**
  - T = 24; Scheduled for March 30, 2025
  - T = 36; Scheduled for March 30, 2026
  - T = 48; Scheduled for March 30, 2027
  - T = 60; Scheduled for March 30, 2028

TABLE 4: BTRI-0123-00004-PV P/P

Analysis	Specification	T_0	T_3	T_6	T_9	T_{12}	T_{18}
Absorbance (0.1M)	**0.04 a.u. max @ 280 nm**	0.0100	0.0093	0.0100	0.0120	0.0141	0.0110
	0.02 a.u. max @ 340 nm	0.0003	0.0011	0.0010	0.0013	0.0029	0.0041
Appearance and Color	**White Crystalline Powder**	White Crystalline Powder	White Crystalline Powder	White Crystalline Powder	White Crystalline Powder	White Crystalline Powder	White Crystalline Powder
Assay (Dried Basis)	**99.0-101.0%**	99.90%	100.02%	100.10%	100.39%	100.18%	99.93%
Identification (IR)	**Conforms to Reference Standard**	Conforms to Reference Standard	Conforms to Reference Standard	Conforms to Reference Standard	Conforms to Reference Standard	Conforms to Reference Standard	Conforms to Reference Standard
Loss on Drying	**≤1.0%**	<0.0193%	0.0547%	0.0433%	0.0230%	0.0610%	0.1427%
Melting Point	**100-105°C**	103.5°C	103.3°C	103.5°C	104.2°C	104.2 °C	104.1 °C
pH (1%)	**8.8 – 9.8**	9.08	9.39	9.53	9.44	9.45	9.47
pKa	**6.3 – 6.7**	6.547	6.627	6.582	6.565	6.603	6.565

- **REMAINING TESTING INTERVAL PULL DATES**
  - T = 24; Scheduled for March 30, 2025
  - T = 36; Scheduled for March 30, 2026
  - T = 48; Scheduled for March 30, 2027
  - T = 60; Scheduled for March 30, 2028

TABLE 5: BTRI-0123-00004-PV LABLINE

Analysis	Specification	T_0	T_3	T_6	T_9	T_{12}	T_{18}
Absorbance (0.1M)	**0.04 a.u. max @ 280 nm**	0.0100	0.0095	0.0101	0.0142	0.0142	0.0101
	0.02 a.u. max @ 340 nm	0.0003	0.0010	0.0008	0.0018	0.0026	0.0039
Appearance and Color	**White Crystalline Powder**	White Crystalline Powder	White Crystalline Powder	White Crystalline Powder	White Crystalline Powder	White Crystalline Powder	White Crystalline Powder
Assay (Dried Basis)	**99.0-101.0%**	99.90%	100.15%	100.16%	100.27%	100.07%	99.93%
Identification (IR)	**Conforms to Reference Standard**	Conforms to Reference Standard	Conforms to Reference Standard	Conforms to Reference Standard	Conforms to Reference Standard	Conforms to Reference Standard	Conforms to Reference Standard
Loss on Drying	**≤1.0%**	<0.0193%	0.0418%	0.0404%	0.0295%	<0.0076%	0.0850%
Melting Range	**100-105°C**	103.5°C	103.4°C	103.4°C	104.1°C	104.2°C	104.2 °C
pH (1%)	**8.8 – 9.8**	9.08	9.52	9.50	9.42	9.37	9.47
pKa	**6.3 – 6.7**	6.547	6.633	6.581	6.570	6.602	6.567

- **REMAINING TESTING INTERVAL PULL DATES**
  - T = 24; Scheduled for March 30, 2025
  - T = 36; Scheduled for March 30, 2026
  - T = 48; Scheduled for March 30, 2027
  - T = 60; Scheduled for March 30, 2028

TABLE 6: BTRI-0123-00005-PV P/P

Analysis	Specification	T_0	T_3	T_6	T_9	T_{12}	T_{18}
Absorbance (0.1M)	**0.04 a.u. max @ 280 nm**	0.0105	0.0092	0.0092	0.0126	0.0137	0.0092
	0.02 a.u. max @ 340 nm	0.0002	0.0003	0.0004	0.0018	0.0032	0.0033
Appearance and Color	**White Crystalline Powder**	White Crystalline Powder	White Crystalline Powder	White Crystalline Powder	White Crystalline Powder	White Crystalline Powder	White Crystalline Powder
Assay (Dried Basis)	**99.0-101.0%**	100.20%	99.86%	100.04%	100.18%	100.01%	99.91%
Identification (IR)	**Conforms to Reference Standard**	Conforms to Reference Standard	Conforms to Reference Standard	Conforms to Reference Standard	Conforms to Reference Standard	Conforms to Reference Standard	Conforms to Reference Standard
Loss on Drying	**≤1.0%**	0.0456%	0.0415%	0.0114%	0.0309%	0.0295%	<0.0061%
Melting Point	**100-105°C**	103.4°C	103.4°C	103.5°C	104.2°C	104.3°C	103.5 °C
pH (1%)	**8.8 – 9.8**	9.42	9.38	9.52	9.45	9.42	9.51
pKa	**6.3 – 6.7**	6.547	6.624	6.581	6.572	6.608	6.566

- **REMAINING TESTING INTERVAL PULL DATES**
  - T = 24; Scheduled for March 30, 2025
  - T = 36; Scheduled for March 30, 2026
  - T = 48; Scheduled for March 30, 2027
  - T = 60; Scheduled for March 30, 2028

TABLE 7: BTRI-0123-00005-PV LABLINE

Analysis	Specification	T_0	T_3	T_6	T_9	T12	T18
Absorbance (0.1M)	**0.04 a.u. max @ 280 nm**	0.0105	0.0096	0.0098	0.0117	0.0146	0.0099
	0.02 a.u. max @ 340 nm	0.0002	0.0003	0.0004	0.0014	0.0033	0.0035
Appearance and Color	**White Crystalline Powder**	White Crystalline Powder	White Crystalline Powder	White Crystalline Powder	White Crystalline Powder	White Crystalline Powder	White Crystalline Powder
Assay (Dried Basis)	**99.0-101.0%**	100.20%	99.82%	100.20%	100.04%	100.11%	100.03%
Identification (IR)	**Conforms to Reference Standard**	Conforms to Reference Standard	Conforms to Reference Standard	Conforms to Reference Standard	Conforms to Reference Standard	Conforms to Reference Standard	Conforms to Reference Standard
Loss on Drying	**≤1.0%**	0.0456%	0.0498%	0.0505%	0.0299%	0.0147%	0.0243%
Melting Point	**100-105°C**	103.4°C	103.4°C	103.6°C	104.2°C	104.4°C	103.9 °C
pH (1%)	**8.8 – 9.8**	9.42	9.49	9.48	9.46	9.45	9.54
pKa	**6.3 – 6.7**	6.547	6.634	6.581	6.570	6.601	6.564

- **REMAINING TESTING INTERVAL PULL DATES**
  - T = 24; Scheduled for March 30, 2025
  - T = 36; Scheduled for March 30, 2026
  - T = 48; Scheduled for March 30, 2027
  - T = 60; Scheduled for March 30, 2028

TABLE 8: BTRI-0123-00006-PV P/P

Analysis	Specification	T_0	T_3	T_6	T_9	T_{12}	T_{18}
Absorbance (0.1M)	0.04 a.u. max @ 280 nm	0.0090	0.0131	0.0105	0.0149	0.0110	0.0109
	0.02 a.u. max @ 340 nm	<0.0030	0.0014	0.0011	0.0035	0.0013	0.0025
Appearance and Color	White Crystalline Powder	White Crystalline Powder	White Crystalline Powder	White Crystalline Powder	White Crystalline Powder	White Crystalline Powder	White Crystalline Powder
Assay (Dried Basis)	99.0-101.0%	99.91%	99.54%	99.99%	100.27%	100.29%	99.80%
Identification (IR)	Conforms to Reference Standard	Conforms to Reference Standard	Conforms to Reference Standard	Conforms to Reference Standard	Conforms to Reference Standard	Conforms to Reference Standard	Conforms to Reference Standard
Loss on Drying	≤1.0%	0.0614%	0.0573%	0.0298%	0.0884%	0.0213%	0.0634%
Melting Point	100-105°C	103.6°C	103.4°C	103.5°C	104.2°C	104.3°C	104.2°C
pH (1%)	8.8 – 9.8	9.32	9.51	9.44	9.53	9.29	9.50
pKa	6.3 – 6.7	6.573	6.578	6.559	6.599	6.596	6.533

- **REMAINING TESTING INTERVAL PULL DATES**
  - T = 24; Scheduled for April 14, 2025
  - T = 36; Scheduled for April 14, 2026
  - T = 48; Scheduled for April 14, 2027
  - T = 60; Scheduled for April 14, 2028

TABLE 9: BTRI-0123-00006-PV LABLINE

Analysis	Specification	T_0	T_3	T_6	T_9	T_{12}	T_{18}
Absorbance (0.1M)	0.04 a.u. max @ 280 nm	0.0090	0.0104	0.0105	0.0121	0.0141	0.0104
	0.02 a.u. max @ 340 nm	<0.0030	0.0006	0.0010	0.0021	0.0042	0.0007
Appearance and Color	White Crystalline Powder	White Crystalline Powder	White Crystalline Powder	White Crystalline Powder	White Crystalline Powder	White Crystalline Powder	White Crystalline Powder
Assay (Dried Basis)	99.0-101.0%	99.91%	99.84%	100.26%	99.86%	100.04%	100.21%
Identification (IR)	Conforms to Reference Standard	Conforms to Reference Standard	Conforms to Reference Standard	Conforms to Reference Standard	Conforms to Reference Standard	Conforms to Reference Standard	Conforms to Reference Standard
Loss on Drying	≤1.0%	0.0614%	0.0111%	0.0379%	0.0607%	0.0220%	0.1136%
Melting Point	100-105°C	103.6°C	103.4°C	103.5°C	104.2°C	104.3°C	104.2°C
pH (1%)	8.8 – 9.8	9.32	9.57	9.46	9.52	9.41	9.44
pKa	6.3 – 6.7	6.573	6.593	6.580	6.599	6.600	6.536

- **REMAINING TESTING INTERVAL PULL DATES**
  - T = 24; Scheduled for April 14, 2025
  - T = 36; Scheduled for April 14, 2026
  - T = 48; Scheduled for April 14, 2027
  - T = 60; Scheduled for April 14, 2028

TABLE 10: BTRI-0123-00045-PV P/P

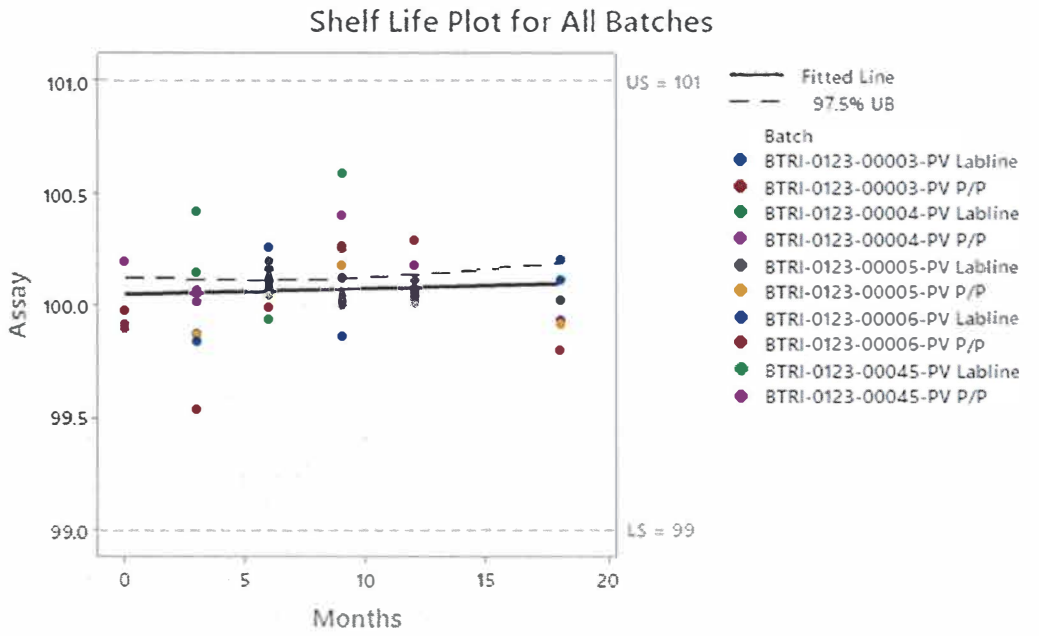
Analysis	Specification	T_0	T_3	T_6	T_9
Absorbance (0.1M)	**0.04 a.u. max @ 280 nm**	0.0089	0.0096	0.0108	0.0108
	0.02 a.u. max @ 340 nm	0.0002	0.0002	0.0005	0.0005
Appearance and Color	**White Crystalline Powder**	White Crystalline Powder	White Crystalline Powder	White Crystalline Powder	White Crystalline Powder
Assay (Dried Basis)	**99.0-101.0%**	100.20%	100.07%	100.13%	100.00%
Identification (IR)	**Conforms to Reference Standard**	Conforms to Reference Standard	Conforms to Reference Standard	Conforms to Reference Standard	Conforms to Reference Standard
Loss on Drying	**≤1.0%**	0.0739%	0.0761%	0.0388%	0.0263%
Melting Point	**100-105°C**	104.0°C	104.0°C	104.0°C	104.0°C
pH (1%)	**8.8 – 9.8**	9.43	9.43	9.49	9.34
pKa	**6.3 – 6.7**	6.607	6.619	6.579	6.558

- **REMAINING TESTING INTERVAL PULL DATES**
  - T = 12; Scheduled for November 20, 2024
  - T = 18; Scheduled for May 20, 2025
  - T = 24; Scheduled for November 20, 2025
  - T = 36; Scheduled for November 20, 2026
  - T = 48; Scheduled for November 20, 2027
  - T = 60; Scheduled for November 20, 2028

TABLE 11: BTRI-0123-00045-PV LABLINE

Analysis	Specification	T_0	T_3	T_6	T_9
Absorbance (0.1M)	**0.04 a.u. max @ 280 nm**	0.0089	0.0095	0.0106	0.0108
	0.02 a.u. max @ 340 nm	0.0002	0.0001	0.0002	0.0005
Appearance and Color	**White Crystalline Powder**	White Crystalline Powder	White Crystalline Powder	White Crystalline Powder	White Crystalline Powder
Assay (Dried Basis)	**99.0-101.0%**	100.20%	100.42%	99.94%	100.59%
Identification (IR)	**Conforms to Reference Standard**	Conforms to Reference Standard	Conforms to Reference Standard	Conforms to Reference Standard	Conforms to Reference Standard
Loss on Drying	**≤1.0%**	0.0739%	0.1094%	<0.0071%	0.2179%
Melting Point	**100-105°C**	104.0°C	104.1°C	104.0°C	104.0°C
pH (1%)	**8.8 – 9.8**	9.43	9.45	9.41	9.58
pKa	**6.3 – 6.7**	6.607	6.599	6.588	6.592

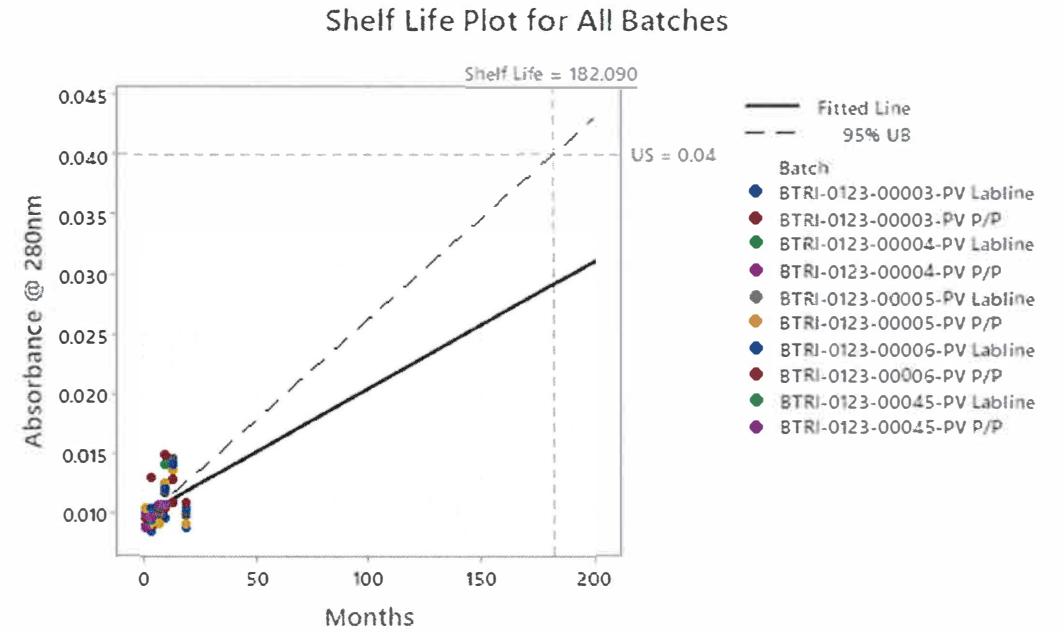
- **REMAINING TESTING INTERVAL PULL DATES**
  - T = 12; Scheduled for November 20, 2024
  - T = 18; Scheduled for May 20, 2025
  - T = 24; Scheduled for November 20, 2025
  - T = 36; Scheduled for November 20, 2026
  - T = 48; Scheduled for November 20, 2027
  - T = 60; Scheduled for November 20, 2028



LS = Lower Specification, US = Upper Specification
Equation for fitted line: Assay = 100 + 0.00264 Months

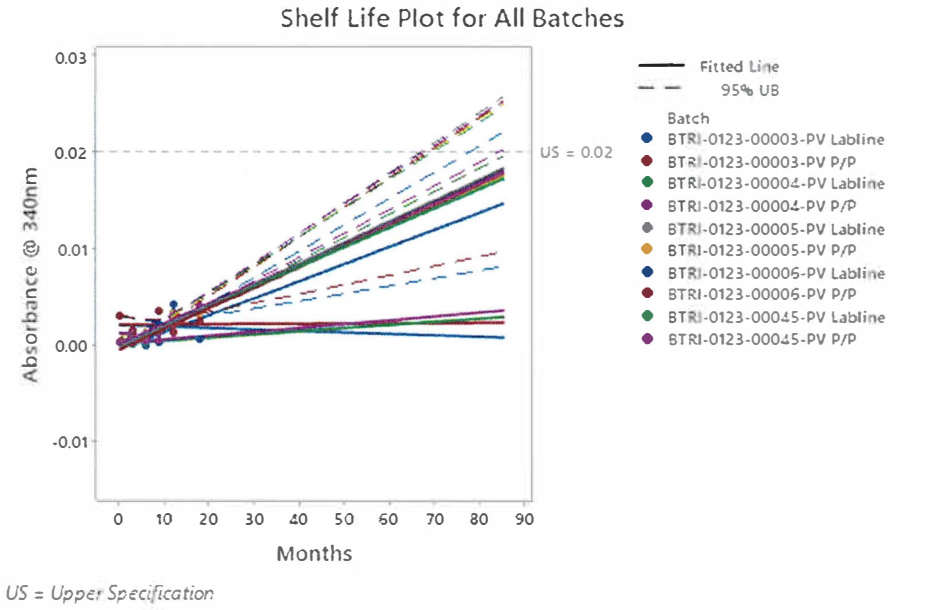
GRAPH 1: ASSAY

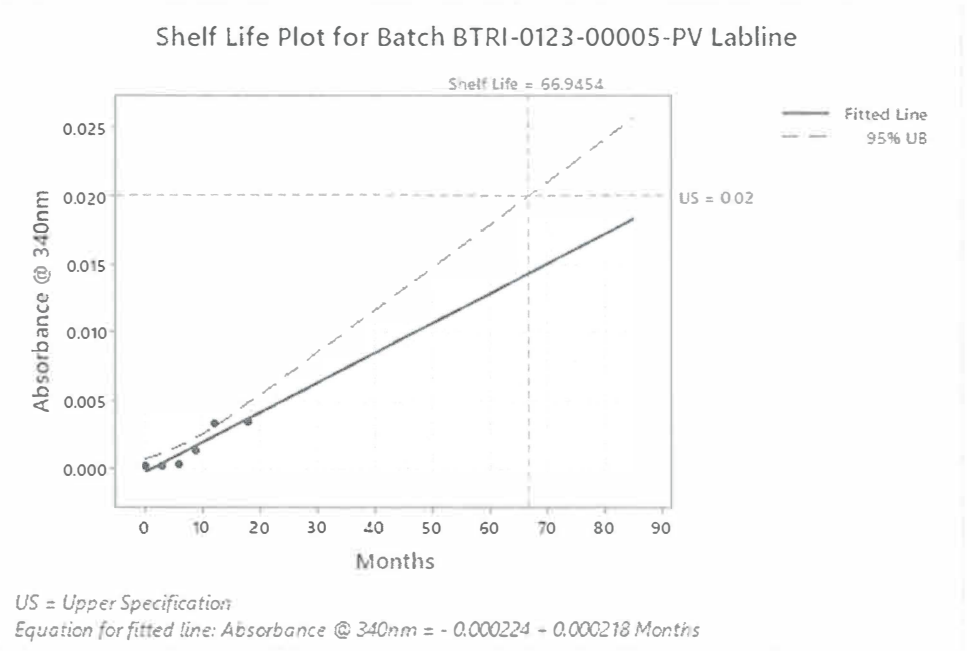
No Shelf-Life was able to be determined for Assay at the 18-month time point, as the mean response slope is not significantly different from zero using 95% confidence. There is no impact to the product or currently assigned retest period of this material.



GRAPH 2: ABSORBANCE @ 280 NM

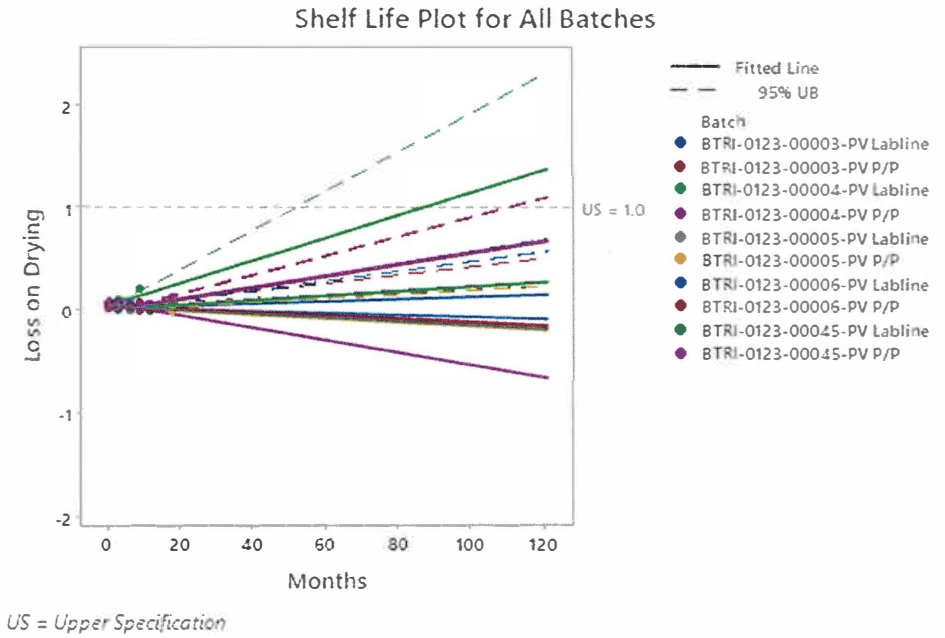
The predicted Shelf-Life for Absorbance @ 280 nm was determined to be 182.090 months as of the 18-month time interval. There is no impact to the product or currently assigned retest period of this material.

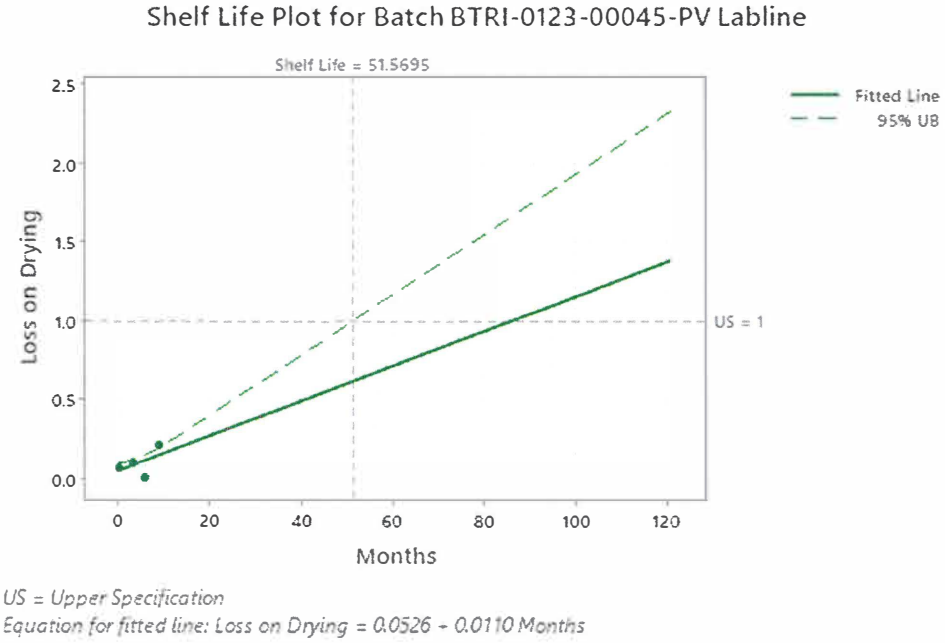




GRAPH 3: ABSORBANCE @ 340 NM

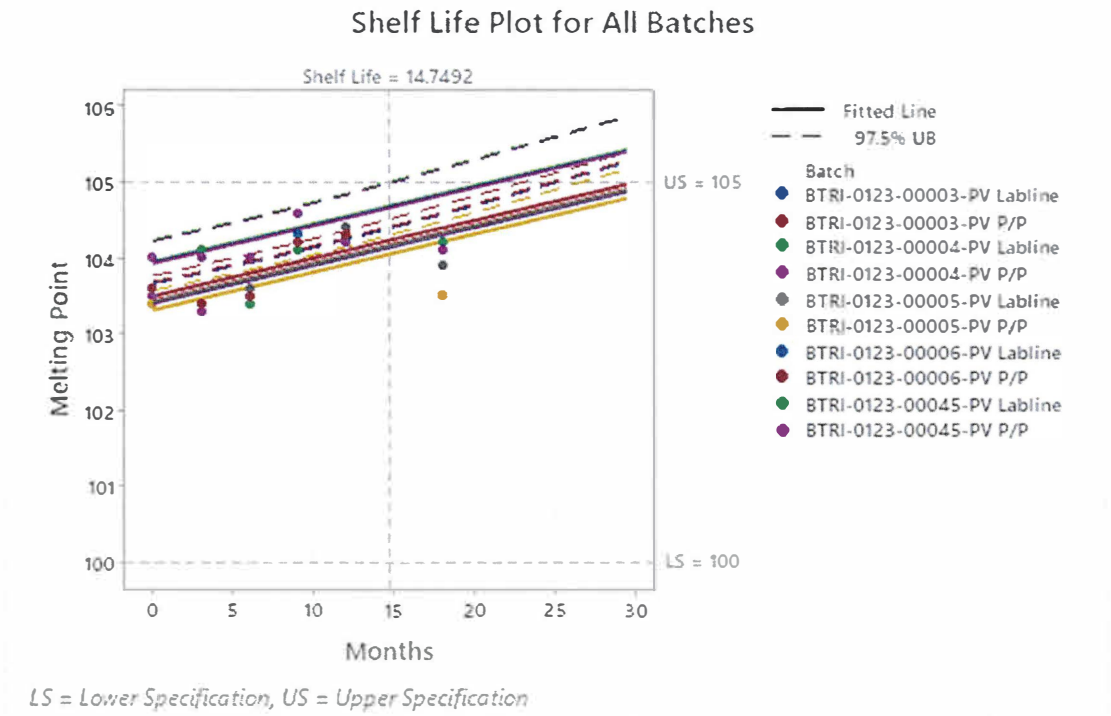
The predicted Shelf-Life for Absorbance @ 340 nm was determined to be 66.9454 months as of the 18-month time interval. There is no impact to the product or currently assigned retest period of this material.





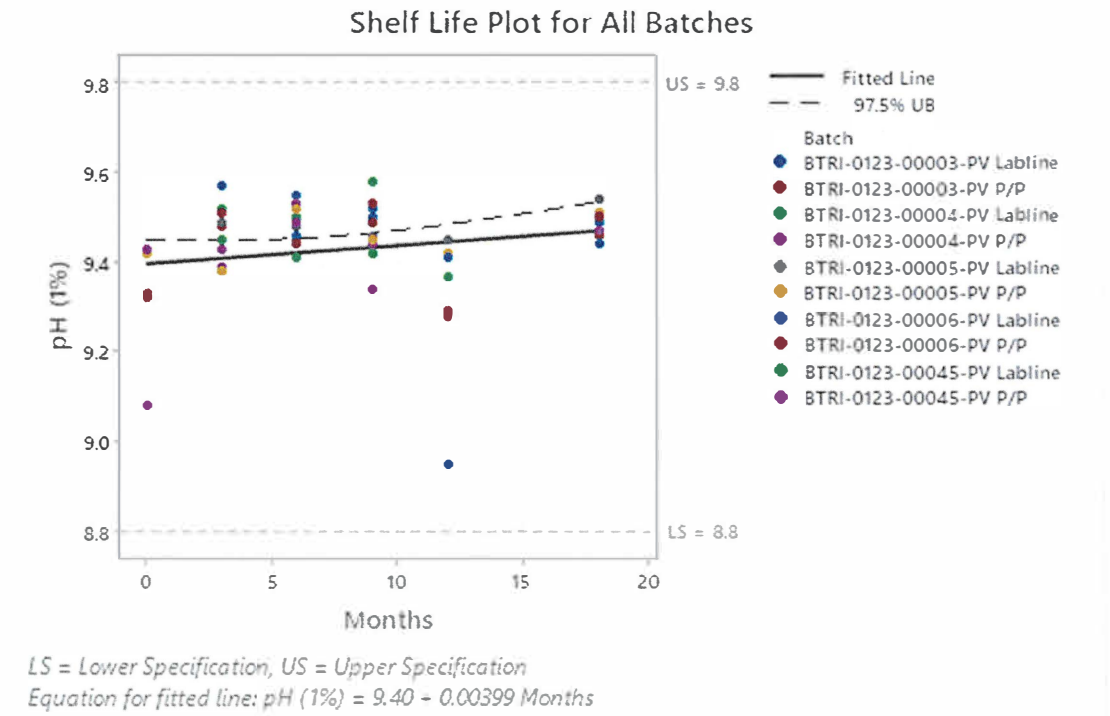
GRAPH 4: LOSS ON DRYING

The shortest predicted Shelf-Life for Loss on Drying was determined to be 51.5695 months as of the 9-month time interval. There is no impact to the product or currently assigned retest period of this material.



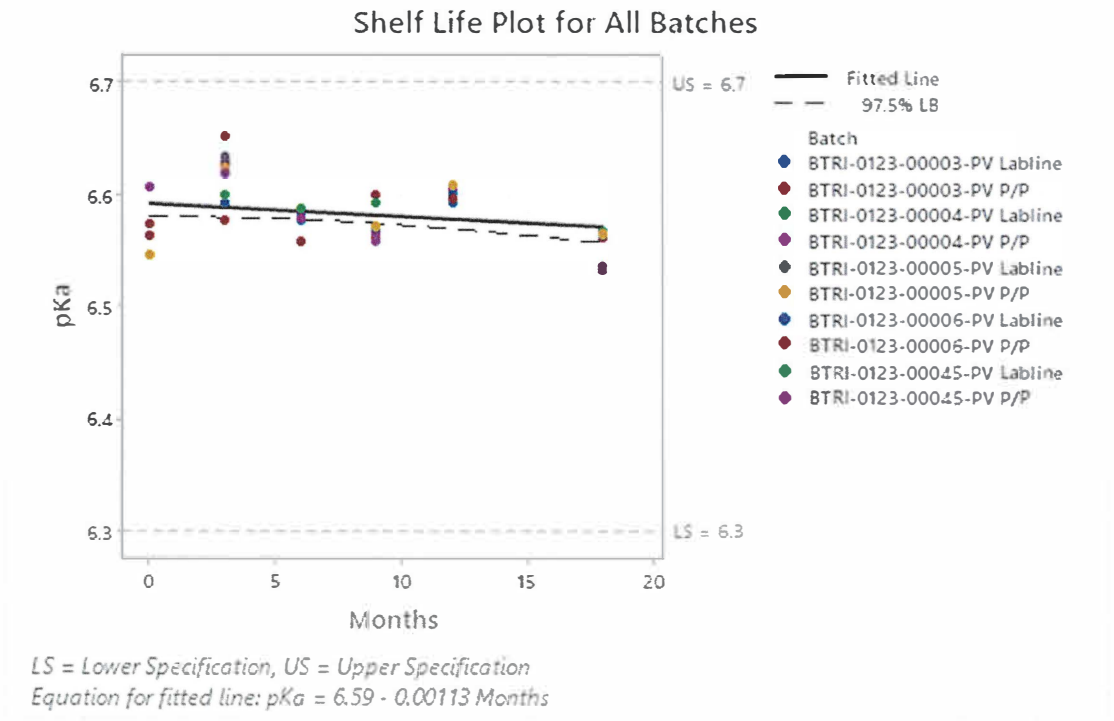
GRAPH 5: MELTING POINT

The predicted Shelf-Life for Melting Point was determined to be 14.7492 months as of the 18-month time interval. The shelf-life is defined as the time period in which you may be 95% confident that at least 50% of the response is within the required limits of specifications. All data regardless of the predicted model up to the current time point (9-month and 18-month) has met the required specification. There is no impact to the product or currently assigned retest period of this material.



GRAPH 6: PH (1%)

No Shelf-Life was able to be determined for pH (1%) at the 18-month time point, as the mean response slope is not significantly different from zero using 95% confidence. There is no impact to the product or currently assigned retest period of this material.



GRAPH 7: PKA

No Shelf-Life was able to be determined for pKa at the 18-month time point, as the mean response slope is not significantly different from zero using 95% confidence. There is no impact to the product or currently assigned retest period of this material.

7. CONCLUSION:

7.1. All data met the specifications set forth in the Stability Testing Program. In accordance with ICH Q1E, the retest date may be proposed for up to 2x, where x is the period covered by long-term stability data, but should be no more than 12 months beyond. Long-Term Stability Data displayed in this report, along with the predicted shelf-life plots, supports a retest date of 24 months for Bis-Tris manufactured at BioSpectra in the Bangor, PA facility.

8. STATEMENT OF COMMITMENT:

8.1. BioSpectra is responsible for the following regarding Stability Data in this report:

8.1.1. In the event that any stability analysis produces results found to be out of specification, the batch produced immediately before and after will be tested in full and analyzed in comparison with the batch in question.

8.1.2. This will serve to provide information to effectively ensure that the root cause of the investigation has not impacted the batch manufactured before or after the batch in question.

8.1.3. If a stability analysis is found to be out of specification, the batch will be withdrawn from the market through communication with the customer. Additionally, an investigation will be conducted to determine the possible withdrawal of the batches produced before and after the batch in question.

8.1.4. In the event that any out of specification results are confirmed, all authorized users of the material will be notified.