BIOSPECTRA

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TRIS HYDROCHLORIDE 2023
LONG TERM STABILITY REPORT

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TABLE OF CONTENTS

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1. OVERVIEW:

The purpose of this report is to analyze and conclude on the data obtained from the long-term stability study of the Tris Hydrochloride (THCl) 2023 lot manufactured at the Stroudsburg, PA facility. Testing intervals are designated by T_n, where n = 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 report assesses the stability of one lot of Tris Hydrochloride THCL-0223-00034 that completed twenty-four (24) months of long-term stability in February 2025. This study includes the analyses listed in Table 1 below. Results from all analyses are summarized in Tables 4 and 5.

TABLE 1: STABILITY SPECIFICATION

Analysis	Specification
Absorbance 1M	≤0.06 a.u. @ 260 nm
	≤0.06 a.u. @ 280 nm
	≤0.01 a.u. @ 400 nm
Appearance and Color	White/Crystals
Assay (Dried)	≥99.0%
Identity (IR)	Passes Test
Loss on Drying @105°C	≤0.5%
Melting Range	147 – 153°C
pH (0.5M)	3.5 – 5.0
Water by Karl Fischer[1]	Monitor (Target is ≤0.5%)

[1]Added as a stability requirement in August 2024 and will only be reported for t=24 and subsequent time points.

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.

The stability program is designed to analyze the stability indicating analyses established for a product in accordance with the Stability Testing Program, BSI-SOP-0136. The specifications for the stability indicating analyses are established in accordance with the Stability Indication Protocol, BSI-SOP-0289, when a new product is manufactured. The study is used to trend the data to determine if there is any significant change over the course of the study to establish the shelf life of the product. This study will be used to establish shelf life for all product codes of Tris Hydrochloride. The following product codes are commercially available:

- THCL-3203
- THCL-3220
- THCL-3221
- THCL-3250
- THCL-3253
- THCL-3254

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- THCL-3257
- THCL-3259
- THCL-3260
- THCL-4220
- THCL-4221
- THCL-4251
- THCL-5201
- THCL-7201
- THCL-7204
- THCL-7205

2. REFERENCES:

2.1. BSI-LST-0149, Tris Hydrochloride Stability Data Card
2.2. BSI-SOP-0136, Stability Testing Program
2.3. BSI-SOP-0146, Stability Inventory
2.4. BSI-SOP-0289, Stability Indication Protocol
2.5. Current USP
2.6. ICH Q1E

3. SAMPLE DESIGNATION:

Samples placed on the Stability Testing Program consisted of one lot of Tris Hydrochloride packaged into P/F and Labline packaging configurations. These samples were packaged in accordance with the Stability Inventory SOP. Reference Table 2 below, for packaging configurations and descriptions. The types of packaging utilized in this stability study were based on BioSpectra final packaging.

TABLE 2: PACKAGING DETAILS

Packaging Configuration	Packaging Description
Poly/Fiber (P/F)	Samples are individually placed into small polyethylene bags and are sealed with a zip tie. All individual bags are then placed into a fiber drum with a 4-unit desiccant.
Labline (HDPE Bottle)	Samples are packaged into a HDPE Lab Screw-Top Bottle.

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4. STORAGE:

Samples were placed on stability in BioSpectra's Stroudsburg, PA facility stability area, located in the warehouse. Although there are no storage requirements for Tris Hydrochloride, storage conditions were continuously monitored and recorded utilizing MadgeTech data loggers for temperature (specification 15-30°C), humidity (specifications: monitor) and mean kinetic temperature (specifications: ≤25°C). The samples were stored in the Stroudsburg Warehouse from February 2023 through February 2025 and will continue until the end of the study in February 2028. The storage conditions for the time period of this study are detailed in Table 3. See Section 5 for the discrepancy investigations initiated for temperature excursions.

TABLE 3: STORAGE CONDITIONS

Condition	Specification	Value
Minimum Temperature	15-30°C	14.13°C
Maximum Temperature		29.75°C
Average Temperature		20.93°C
Mean Kinetic Temperature	≤25°C	20.99°C
Minimum Humidity	Monitor	4.2%
Maximum Humidity		82.5%
Average Humidity		36.0%

5. INVESTIGATIONS:

5.1. **SDI23-76** was initiated due to an out of specification low temperature reading. This was due to an empty propane tank. This had no impact on the stability samples as the other six data loggers did not fall outside the specified temperature range of 15 – 30°C during the missing time.

5.2. **SDI23-128** was initiated due to a data logger not able to download temperature and humidity data for the month of June 2023. This was due to the logger being damaged during the movement of materials. This had no impact on the stability samples as the other six data loggers did not fall outside the specified temperature range of 15 – 30°C during the missing time.

5.3. **SDI23-159** was initiated due to a data logger to able to download temperature and humidity data for the month of July 2023. This was due to the logger having the battery dislodged during the movement of materials. This had no impact on the stability samples as the other six data loggers did not fall outside the specified temperature range of 15 – 30°C during the missing time.

5.4. **SDI24-17** was initiated for missing January Temperature and Humidity data for the Stroudsburg warehouse. This was due to the logger sustaining damages from the fall, rendering it unable to collect and/or communicate. This had no impact on the stability samples as the other loggers recorded temperatures within specification.

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6. LOT EVALUATION:

TABLE 4: THCL-0223-00034 P/F

Time Point	Analyses/Specifications									
	Absorbance (1M)			Appearance and Color	Assay (Dried)	Identity (IR)	Loss on Drying	Melting Range	pH	KF
	260 nm	280 nm	400 nm							
	≤0.06 a.u.	≤0.06 a.u.	≤0.01 a.u.	White/Crystals	≥99.0%	Passes Test	≤0.5%	147 – 153°C	3.5 – 5.0	Monitor
T_0	0.0148	0.0142	0.0017	White/Crystals	99.73%	Passes Test	0.2241%	151.0 – 151.8°C	4.18	
T_3	0.0077	0.0070	0.0007	White/Crystals	99.70%	Passes Test	<0.0038%	151.5 – 152.6°C	4.18	
T_6	0.0089	0.0078	0.0007	White/Crystals	99.82%	Passes Test	0.0548%	151.1 – 152.3°C	4.17	
T_9	0.0139	0.0121	0.0035	White/Crystals	99.67%	Passes Test	0.0309%	151.4 – 152.4°C	4.22	
T_{12}	0.0084	0.0070	0.0014	White/Crystals	100.09%	Passes Test	0.0384%	151.9 – 152.9°C	4.26	
T_{18}	0.0094	0.0082	0.0014	White/Crystals	100.16%	Passes Test	0.0619%	150.0 – 151.0°C	4.22	
T_{24}	0.0097	0.0085	0.0019	White/Crystals	99.79%	Passes Test	0.0365%	150.9 – 151.8°C	4.25	0.12%

- **REMAINING TESTING INTERVAL PULL DATES**
  - T = 36; Scheduled for February 2, 2026
  - T = 48; Scheduled for February 2, 2027
  - T = 60; Scheduled for February 2, 2028

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TABLE 5: THCL-0223-00034 LABLINE

Time Point	Analyses/Specifications									
	Absorbance (1M)			Appearance and Color	Assay (Dried)	Identity (IR)	Loss on Drying	Melting Range	pH	KF
	260 nm	280 nm	400 nm							
	≤0.06 a.u.	≤0.06 a.u.	≤0.01 a.u.	White/Crystals	≥99.0%	Passes Test	≤0.5%	147 – 153°C	3.5 – 5.0	Monitor
T_0	0.0148	0.0142	0.0017	White/Crystals	99.73%	Passes Test	0.2241%	151.0 – 151.8°C	4.18	
T_3	0.0066	0.0058	0.0003	White/Crystals	99.68%	Passes Test	0.0590%	151.5 – 152.6°C	4.18	
T_6	0.0066	0.0060	0.0003	White/Crystals	99.70%	Passes Test	0.1500%	151.1 – 152.3°C	4.20	
T_9	0.0134	0.0112	0.0035	White/Crystals	99.87%	Passes Test	0.2371%	151.3 – 152.3°C	4.23	
T_{12}	0.0070	0.0062	0.0014	White/Crystals	100.00%	Passes Test	0.2328%	151.8 – 152.8°C	4.27	
T_{18}	0.0070	0.0059	0.0007	White/Crystals	100.12%	Passes Test	0.2085%	149.8 – 150.6°C	4.22	
T_{24}	0.0076	0.0066	0.0012	White/Crystals	99.82%	Passes Test	0.1377%	150.9 – 151.9°C	4.27	0.24%

- **REMAINING TESTING INTERVAL PULL DATES**
  - T = 36; Scheduled for February 2, 2026
  - T = 48; Scheduled for February 2, 2027
  - T = 60; Scheduled for February 2, 2028

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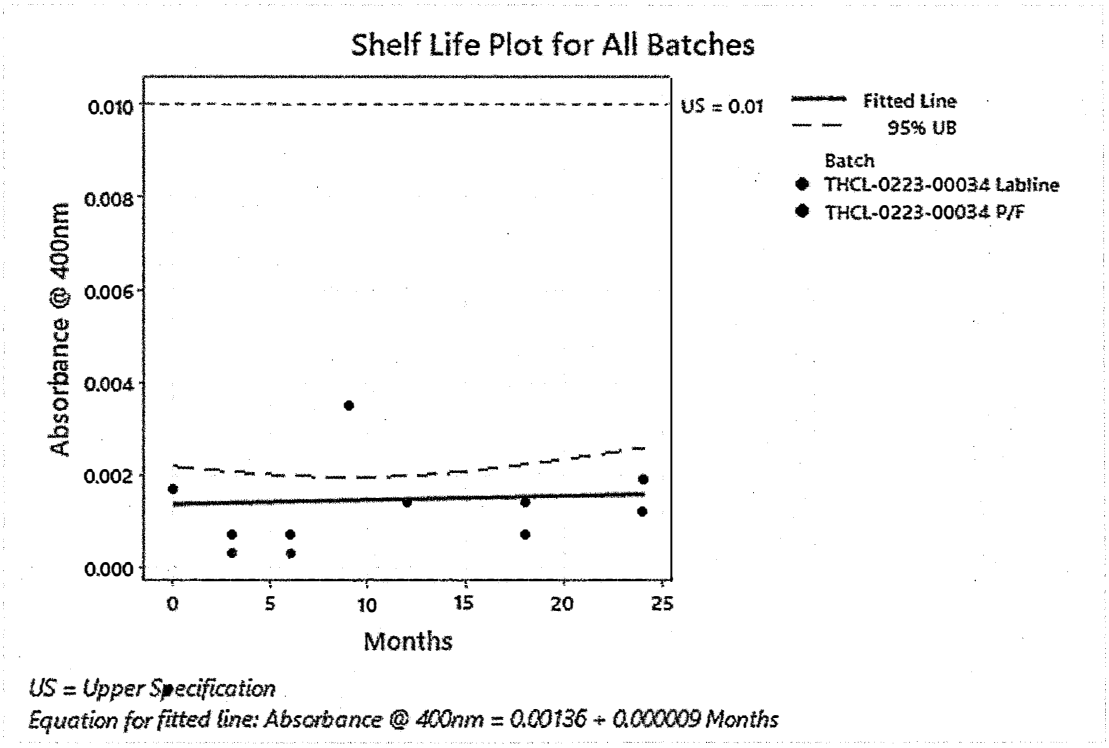

GRAPH 1: SHELF LIFE PLOTS FOR ABSORBANCE (1M) @ 400 NM

No Shelf-Life was able to be determined for Absorbance at 400 nm, 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.

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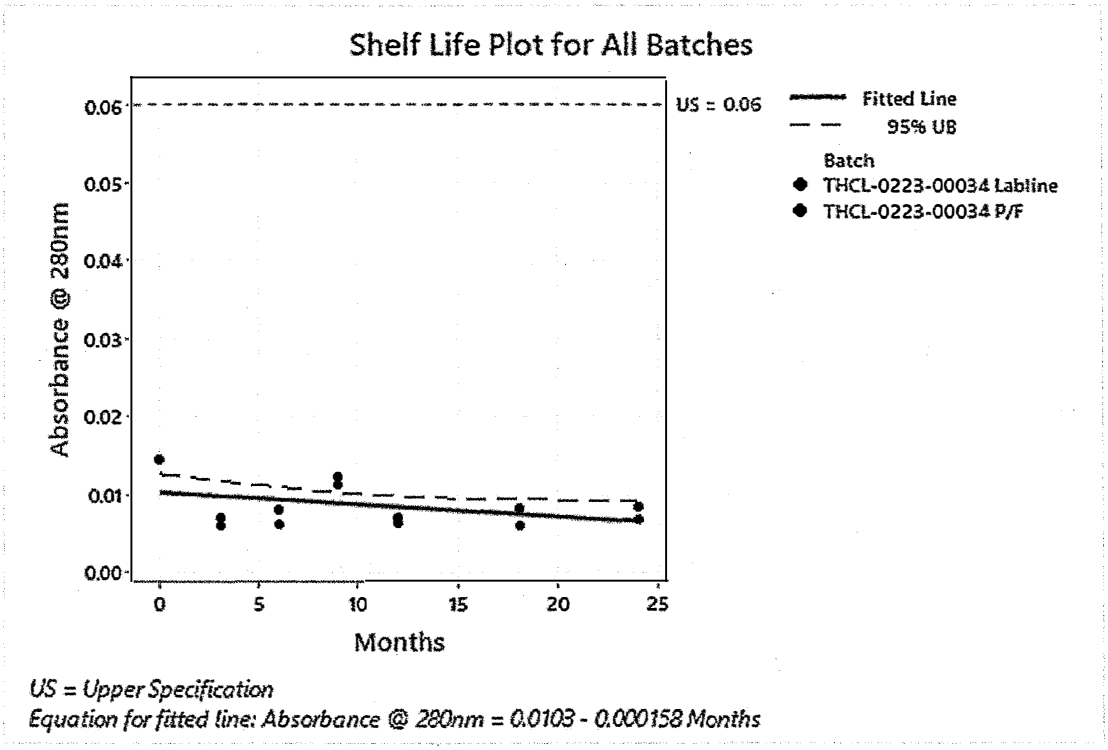

GRAPH 2: SHELF LIFE PLOTS FOR ABSORBANCE (1M) @ 280 NM

No Shelf-Life was able to be determined for Absorbance at 280 nm, 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.

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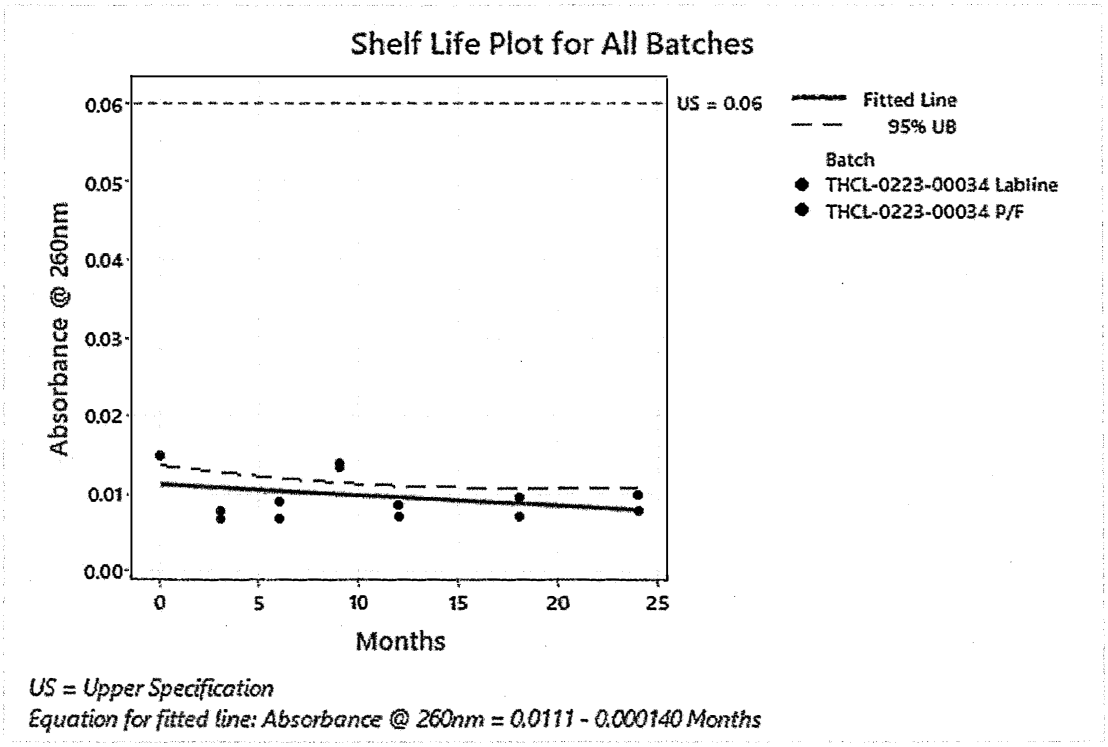

GRAPH 3: SHELF LIFE PLOTS FOR ABSORBANCE (1M) @ 260 NM

No Shelf-Life was able to be determined for Absorbance at 260 nm, 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.

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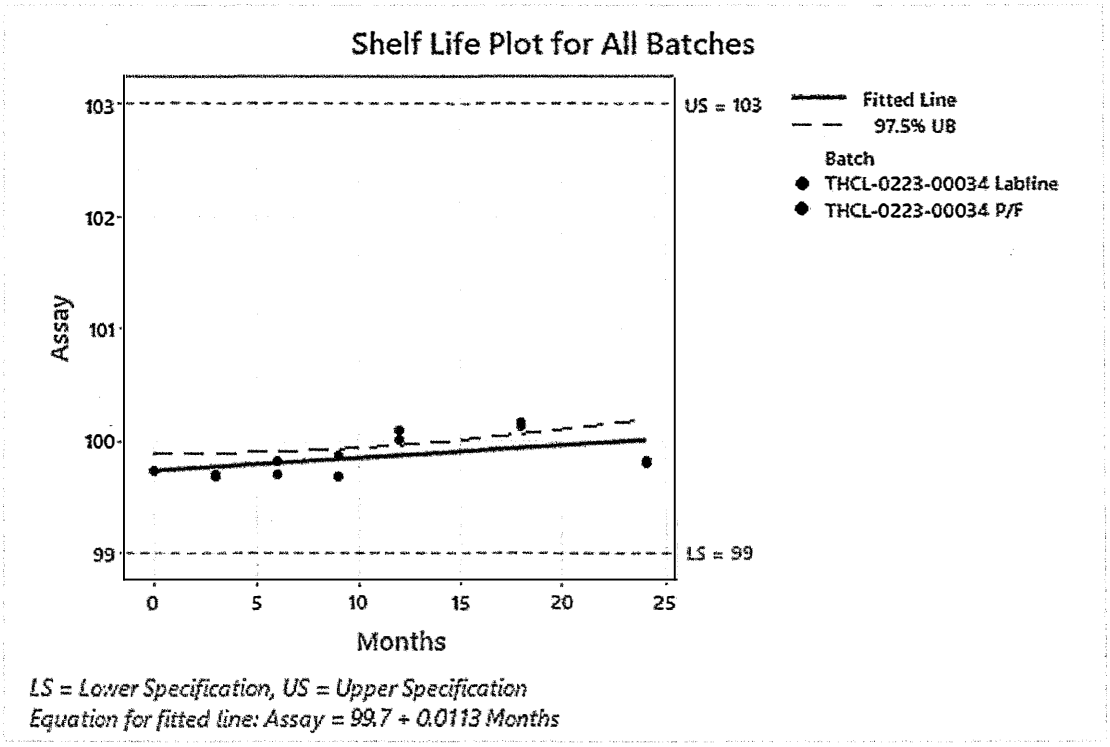

GRAPH 4: SHELF LIFE PLOT FOR ASSAY

No Shelf-Life was able to be determined for Assay, 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.

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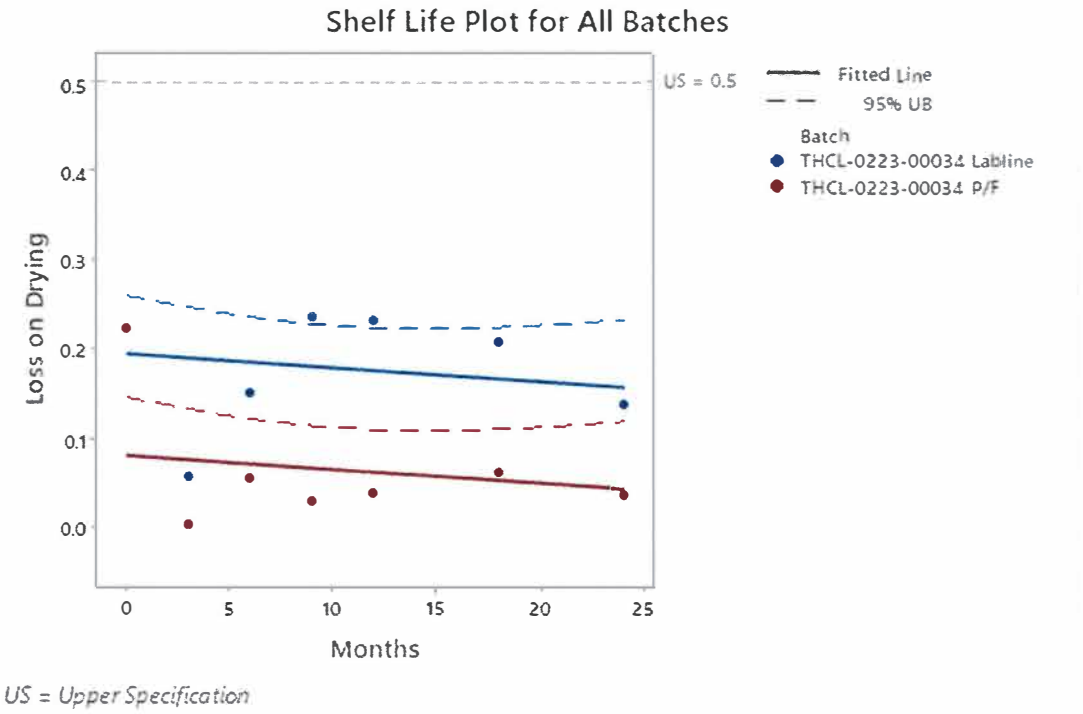

GRAPH 5: SHELF LIFE PLOT FOR LOSS ON DRYING

No Shelf-Life was able to be determined for Loss on Drying, 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.

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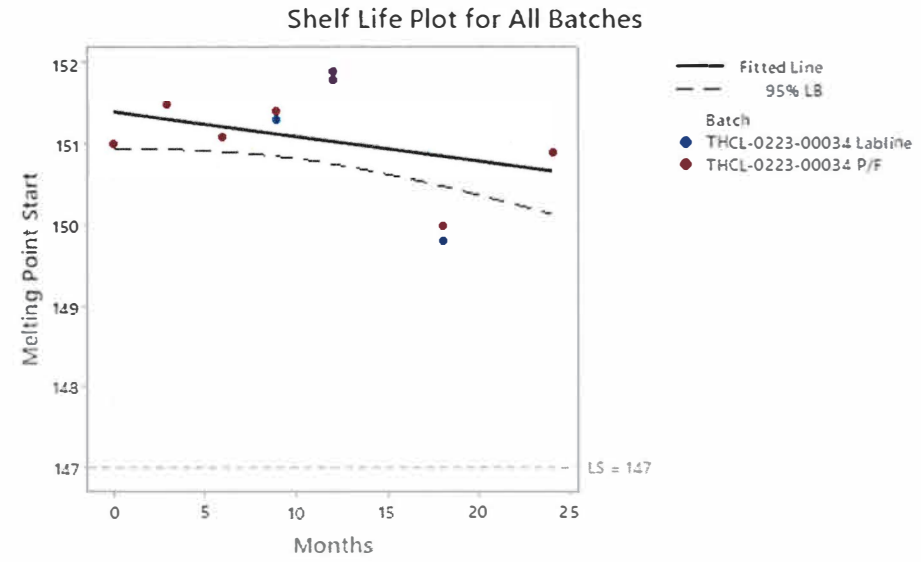
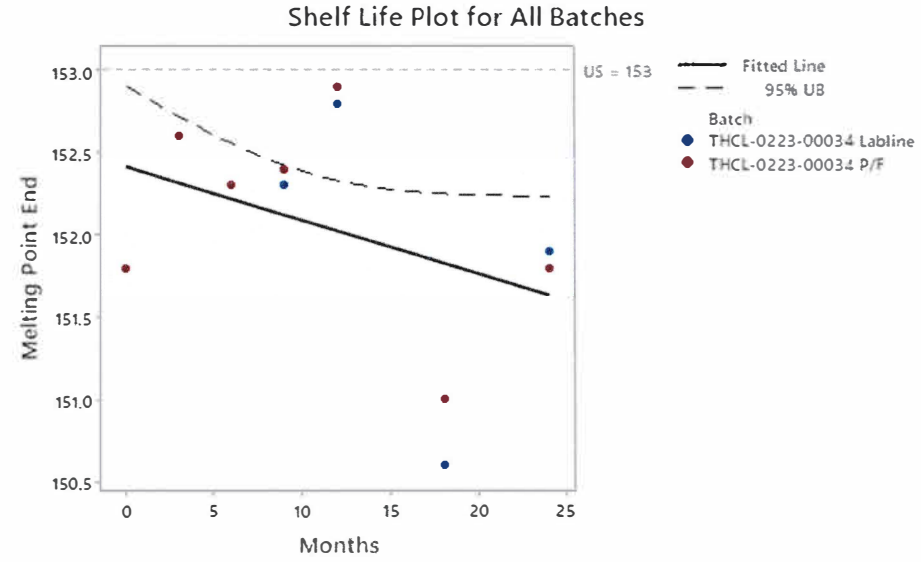


LS = Lower Specification
Equation for fitted line: Melting Point Start = 151 - 0.0301 Months



US = Upper Specification
Equation for fitted line: Melting Point End = 152 - 0.0324 Months

GRAPHS 6 AND 7: SHELF LIFE PLOTS FOR MELTING RANGE

No shelf-life was able to be determined for Melting Point Start, 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. No shelf-life was able to be determined for Melting Point End, 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.

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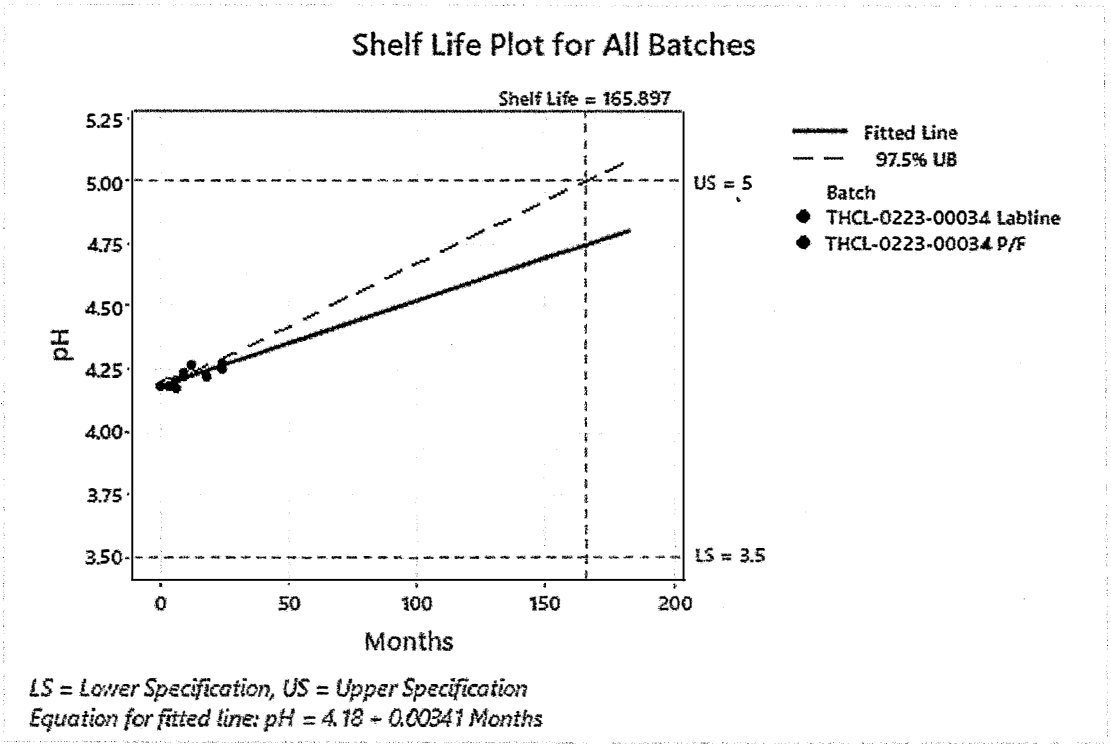

GRAPH 8: SHELF LIFE PLOT FOR PH (0.5M)

The predicted Shelf-Life for pH (0.5M) was determined to be 165.897 months as of the T=24-month time interval. There is no impact to the product or currently assigned retest period of this material.

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7. CONCLUSION:

In regards to the long-term stability study for Tris Hydrochloride, all data met the specifications set forth in the Stability Testing Program for the lot stored at the long-term condition. 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 for long-term conditions (warehouse conditions of 15 – 30°C). The Long-Term Stability Study data, along with the predicted shelf-life plots, supports a retest date of 24 months and an expiration date of 36 months upon request for a lot of Tris Hydrochloride manufactured at BioSpectra in the Stroudsburg, 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.

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