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TRIS HYDROCHLORIDE 2022 LONG TERM STABILITY REPORT

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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) validation lot manufactured at the Rockdale 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 confirm 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 real-time stability report assesses the stability of Tris Hydrochloride lot THCL-0222-00215 that completed eighteen (18) months of real-time stability in March 2024. The study includes the following analyses: Absorbance (1M), Appearance and Color, Assay (Dried Basis), Identification (IR), Loss on Drying (105°C), Melting Range, and pH (0.5M). Results from all analyses are summarized in Table 2. 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, DCN: BSI-SOP-0136. The specifications for the stability indicating analyses are established in accordance with the Stability Indication Protocol, DCN: 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
- THCL-3257
- THCL-3259
- THCL-3260
- THCL-4220
- THCL-4221
- THCL-5201
- THCL-7201
- THCL-7204

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:

Samples placed on the Stability Testing Program consisted of one Tris Hydrochloride lot packaged into EziDock poly bags. These samples were packaged in accordance with the Stability Inventory SOP. Reference Table 1 below, for packaging configurations and descriptions. The type of packaging utilized in this stability study were based on BioSpectra final packaging.

TABLE 1: PACKAGING DETAILS

Packaging Configuration	Packaging Description
Poly/Poly (P/P) with EziDock Poly Bag	Samples are packaged into EziDock poly bags. All individual samples are then placed into a poly drum.

4. STORAGE:

Samples were placed on stability in BioSpectra's Rockdale Ln. 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 warehouse conditions; temperature (specification: 15-30°C), humidity (specification: monitor) and mean kinetic temperature (specifications: monitor). The samples were stored in the Rockdale warehouse from October 2022 through March 2024. The maximum temperature of the warehouse during this time was 29.75°C and the minimum temperature of the warehouse during this time was 11.73°C. The average mean kinetic temperature was 20.88°C. See Section 5 for the discrepancy investigations initiated for temperature excursions.

5. INVESTIGATIONS:

- 5.1. **SDI22-184** was initiated due to out of specification low temperature readings between 10/18/22 and 10/31/22. Two loggers recorded OOS low temperatures with the lowest reading of 13.40°C due to the AC unit being turned on instead of the heat. This had no impact on the Tris Hydrochloride stability samples as Tris Hydrochloride does not have a temperature requirement.
- 5.2. **SDI23-07** was initiated for MadgeTech STRD-4 data logger being damaged and unavailable to provide data for December 2022. This had no impact on the stability samples as all other loggers recorded temperatures within specification and Tris Hydrochloride does not have a temperature requirement.
- 5.3. **SDI23-76** was initiated due to an out of specification low temperature reading on 3/9/23. This was due to an empty propane tank which feeds the Stroudsburg warehouse heaters. This had no impact on the stability samples as the other six data loggers did not fall outside the specified temperature range and Tris Hydrochloride does not have a temperature requirement.
- 5.4. **SDI23-128** was initiated due to a data logger not able to download temperature and humidity data for 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.

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- 5.5. **SDI23-159** was initiated due to a Data logger to able to down load temperature and humidity data for the month of July 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 and Tris Hydrochloride does not have a temperature requirement.
- 5.6. **SDI24-17** was initiated for missing temperature and humidity data for the Stroudsburg warehouse on 1/17/24. This was due to the logger sustaining damages from a 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 and Tris Hydrochloride does not have a temperature requirement.

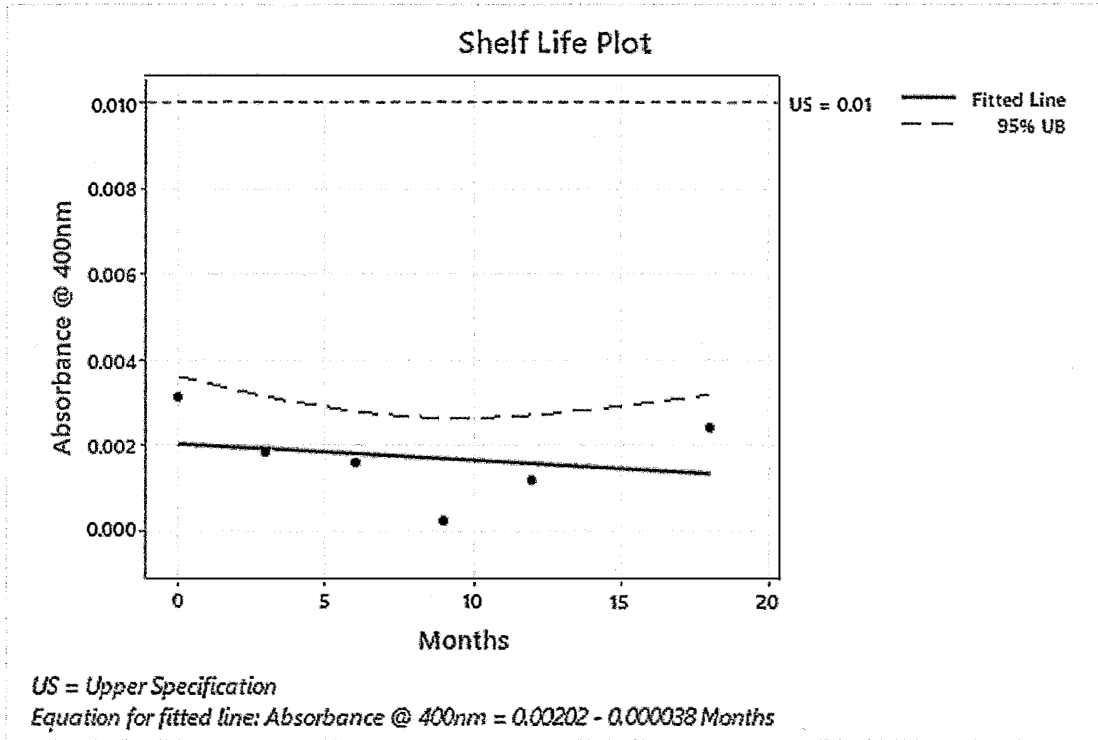
6. LOT EVALUATION:

TABLE 2: THCL-0222-00215 P/P WITH EZIDOCK POLY BAG

Analysis	Specification	T ₀	T ₃	T ₆	T ₉	T ₁₂	T ₁₈
Absorbance (1M)	0.01 a.u. max @ 400 nm	0.0031 a.u.	0.0018 a.u.	0.0016 a.u.	0.0002 a.u.	0.0012 a.u.	0.0024 a.u.
	0.06 a.u. max @ 280 nm	0.0197 a.u.	0.0101 a.u.	0.0106 a.u.	0.0081 a.u.	0.0092 a.u.	0.0120 a.u.
	0.06 a.u. max @ 260 nm	0.0221 a.u.	0.0120 a.u.	0.0124 a.u.	0.0093 a.u.	0.0108 a.u.	0.0140 a.u.
Appearance and Color	White/Crystals	White/Crystals	White/Crystals	White/Crystals	White/Crystals	White/Crystals	White/Crystals
Assay (Dried Basis)	99.0-103.0%	99.78%	99.69%	99.55%	99.61%	99.97%	100.21%
Identification (IR)	Passes Test	Passes Test	Passes Test	Passes Test	Passes Test	Passes Test	Passes Test
Loss on Drying	0.5% max.	0.4008%	0.2402%	0.1441%	0.0375%	0.0860%	0.0622%
Melting Range	147-153°C	151.0 – 152.0°C	150.6 – 151.8°C	150.6 – 151.6°C	151.5 – 152.5°C	151.7 – 152.7°C	151.7 – 152.3°C
pH (0.5M)	3.5 – 5.0	4.35	4.26	4.19	4.42	4.23	4.20

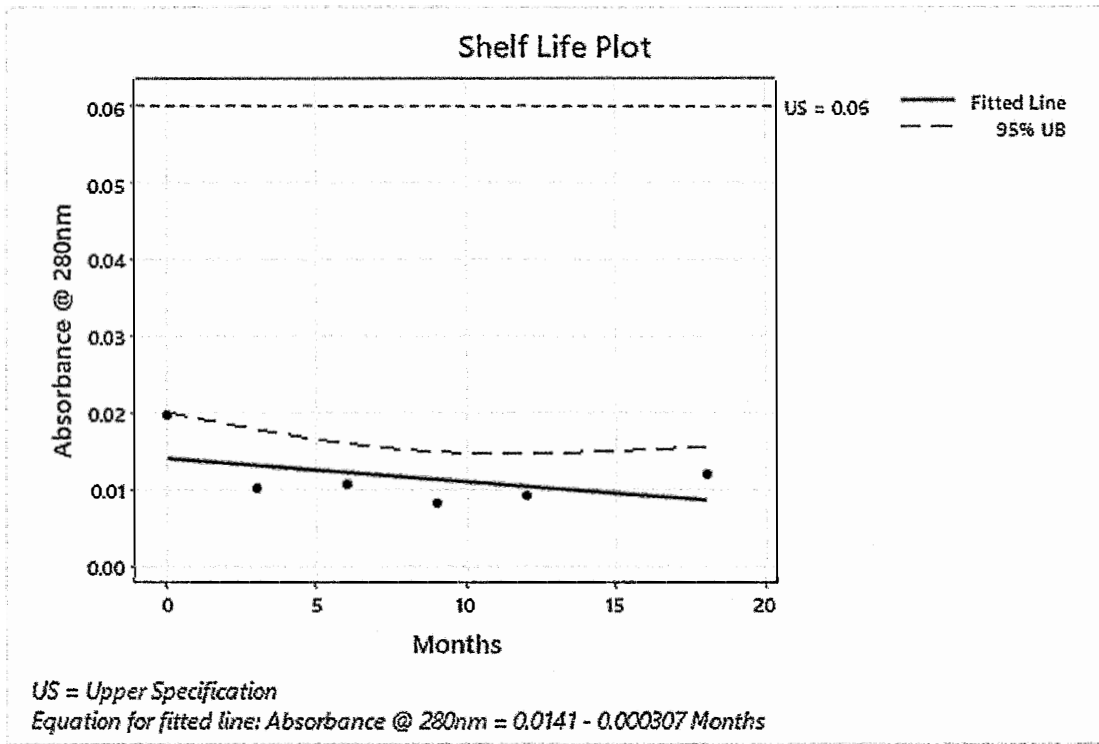
- REMAINING TESTING INTERVAL PULL DATES

- T=24: Scheduled to be pulled 10/14/24
- T=36: Scheduled to be pulled 10/14/25
- T=48: Scheduled to be pulled 10/14/26
- T=60: Scheduled to be pulled 10/14/27



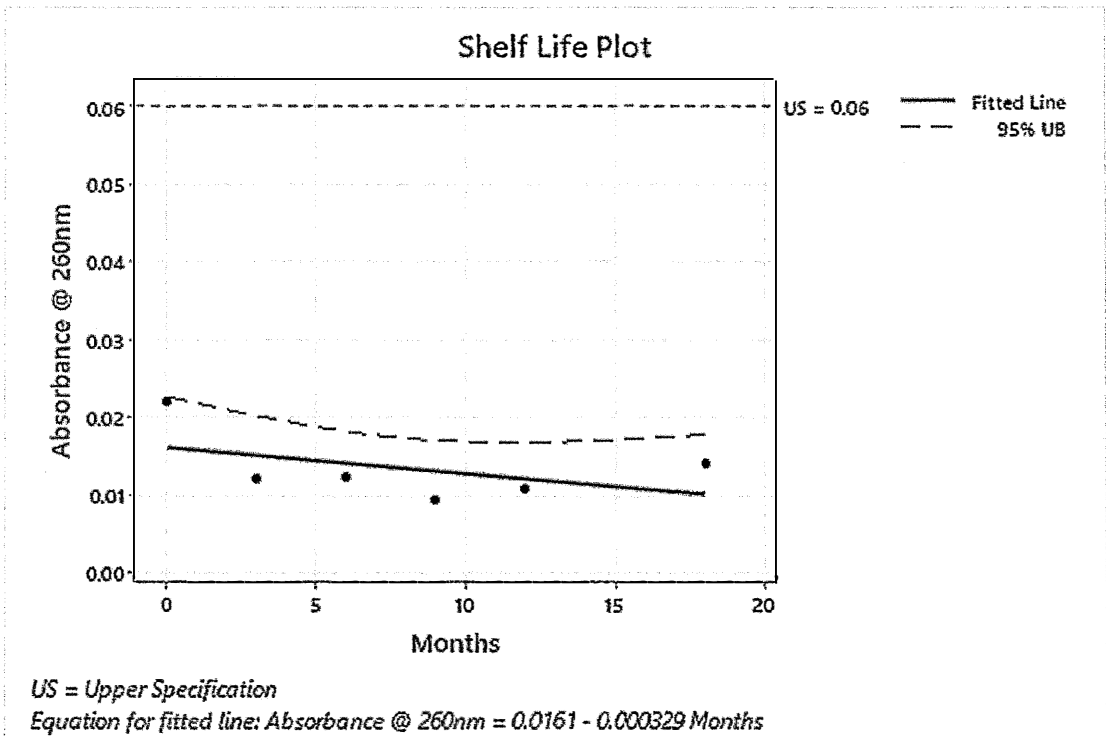
GRAPH 1: SHELF LIFE PLOT FOR ABSORBANCE (1M) @ 400 NM

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.



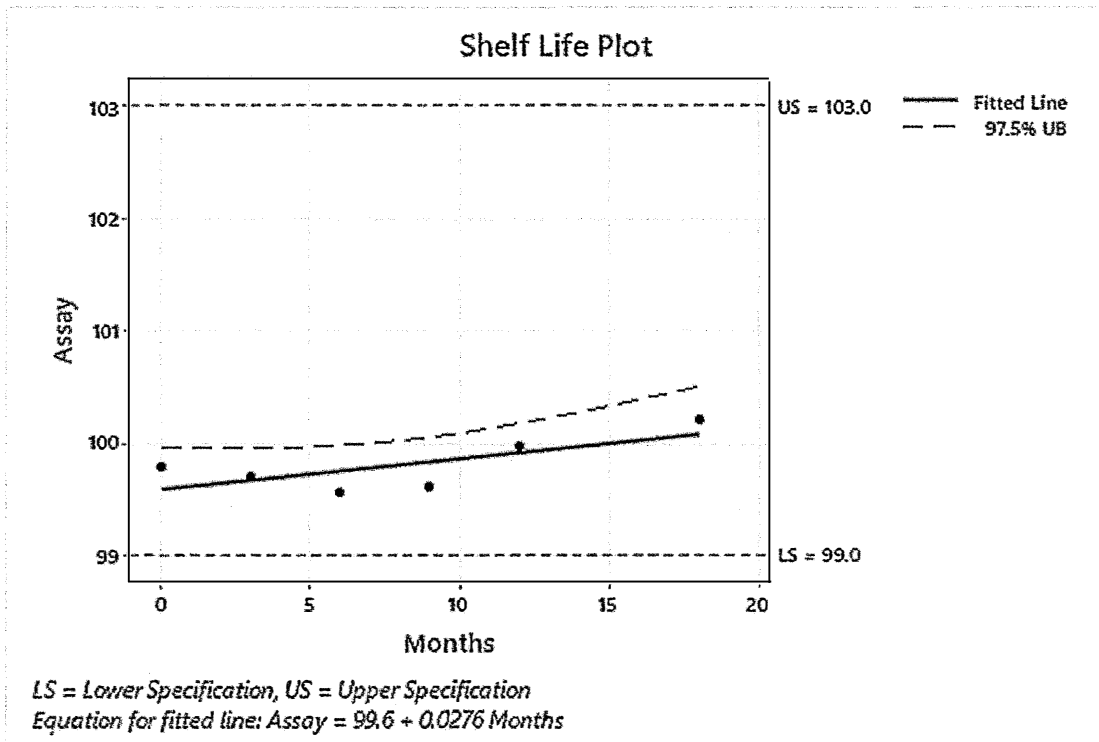
GRAPH 2: SHELF LIFE PLOT FOR ABSORBANCE (1M) @ 280 NM

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.



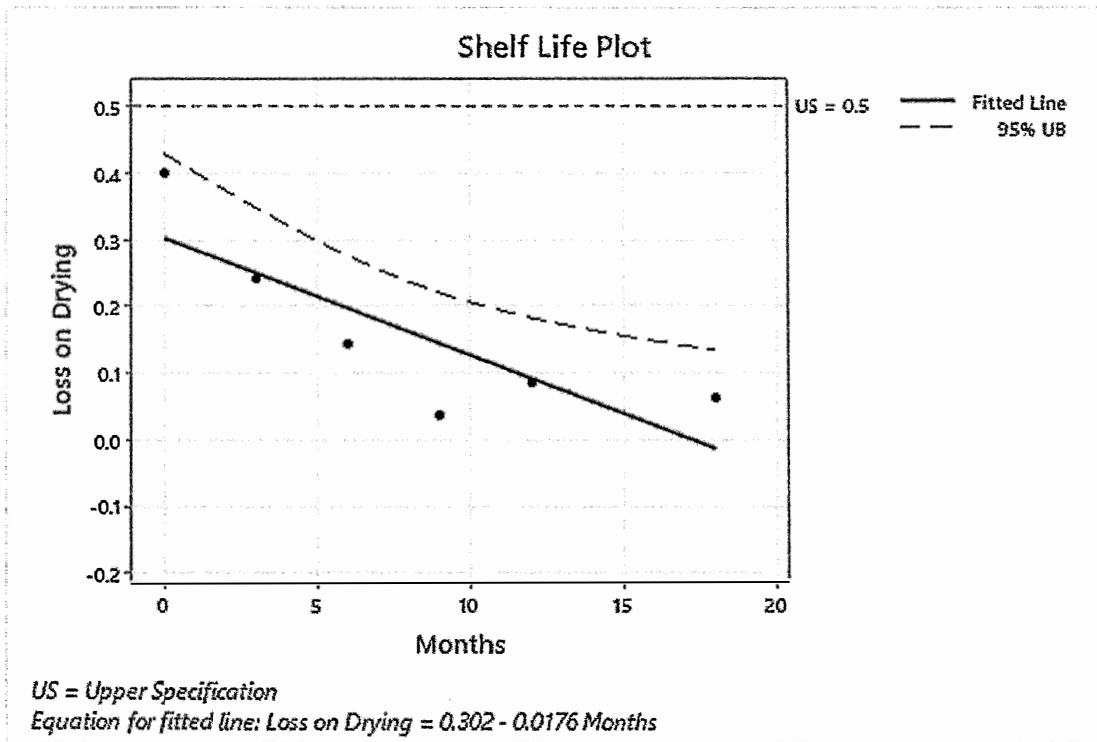
GRAPH 3: SHELF LIFE PLOT FOR ABSORBANCE (1M) @ 260 NM

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.



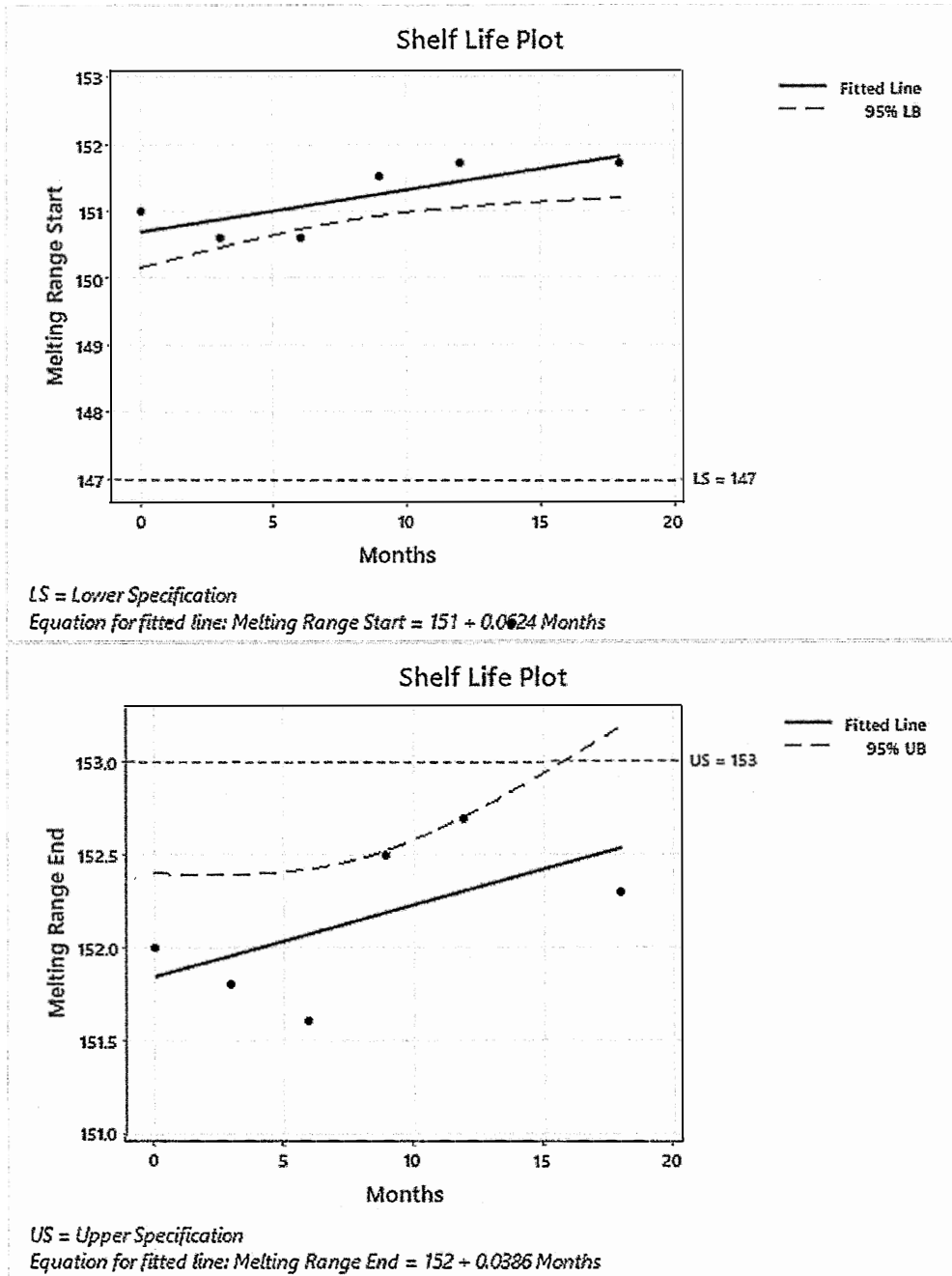
GRAPH 4: SHELF LIFE PLOT FOR ASSAY

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.



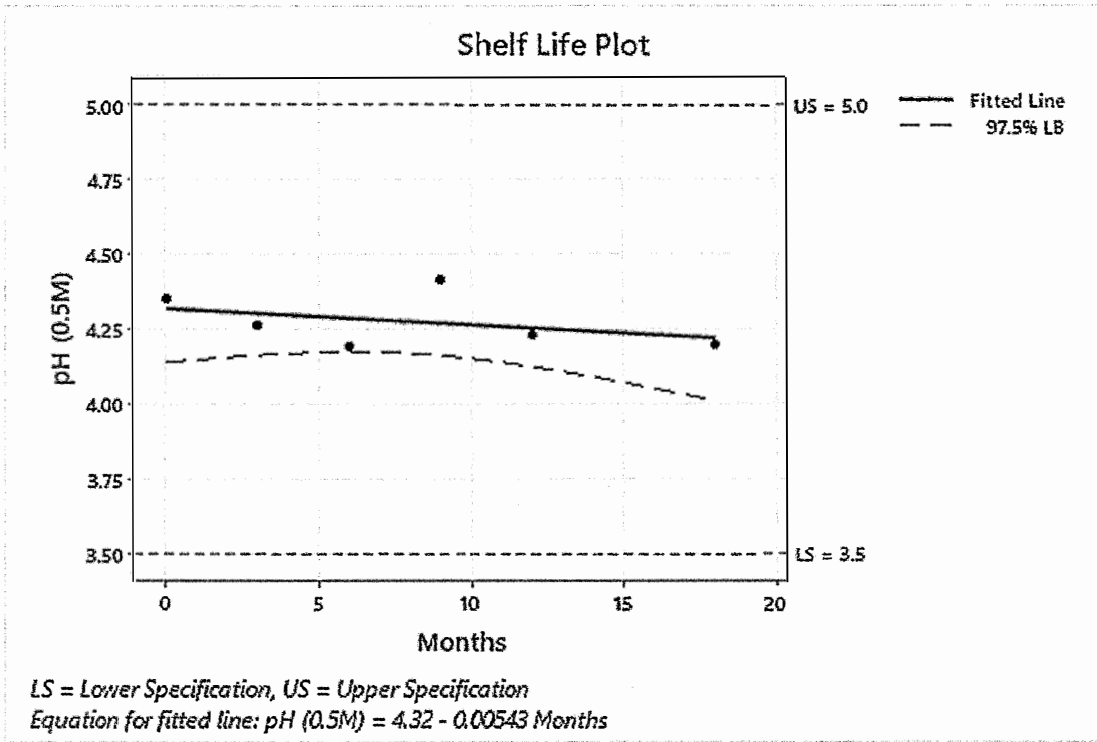
GRAPH 5: SHELF LIFE PLOT FOR LOSS ON DRYING

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.



GRAPHS 6 AND 7: SHELF LIFE PLOTS FOR MELTING RANGE

No shelf-life was able to be determined for Melting Point start or 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.



GRAPH 8: SHELF LIFE PLOT FOR PH

No shelf-life was able to be determined for pH (0.5M), 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:

In regards to the real time stability study for this 2022 batch of Tris Hydrochloride, 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 for long-term conditions (warehouse conditions of 15 – 30°C). The data obtained during this stability study along with the predicted shelf-life plots, supports the current retest date of 24 months for Tris Hydrochloride manufactured at BioSpectra's Stroudsburg, PA facility and packaged in Poly/Poly with the EziDock poly bag.

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.