

TRIS HYDROCHLORIDE
LONG TERM STABILITY REPORT: TH3200-085-0516

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1. OVERVIEW:

The purpose of this report is to analyze the data obtained from the Real-Time Stability of Tris Hydrochloride manufactured at BioSpectra's Stroudsburg, PA facility. Samples were placed on the Stability Testing Program in May of 2016 to fulfil the requirements of placing one lot of manufactured material per year on the Stability Testing Program. Testing intervals are designated by T_n , where n represents the number of months on stability. The long-term Real-Time Stability Program consists of testing every three months for the first year, every six months for the second year and annually for each subsequent year for a total of three years 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 be used to re-evaluate the retest period for future lots of manufactured material.

This Real-Time Stability report assesses the stability of one lot of Tris Hydrochloride. 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 and Shelf-Life Plot determinations have been created for quantitative analyses. Shelf-Life Plots determine 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 Predicted Shelf Life. This allows BioSpectra to ensure that the product will be stable over the time period in which it is part of the Stability Testing Program.

2. REFERENCES:

- 2.1. Current USP
- 2.2. ICH Q1
- 2.3. [Stability Testing Program](#)
- 2.4. [Stability Inventory](#)

3. SAMPLE DESIGNATION:

Samples placed on the Stability Testing Program consisted of one lot of Tris Hydrochloride. Stability samples from this batch were put into three different packaging configurations. 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 was based on BioSpectra final packaging.

TABLE 1: PACKAGING DETAILS

Packaging Configuration	Packaging Description
Poly/Fiber (P/F)	Samples are packaged into small poly bags and sealed with a ziptie. All individual samples are then placed into a fiber drum.
Tyvek/Poly (T/P)	Samples are packaged into small tyvek bags and sealed with a ziptie. All individual samples are then placed into a poly drum, along with a 5x8-unit desiccant.
Poly/Poly (P/P)	Samples are packaged into small poly bags and sealed with a ziptie. All individual samples are then placed into a poly drum.

4. STORAGE:

Samples were placed on stability in BioSpectra's Stroudsburg PA facility Stability Area, located in the quarantine area of the Warehouse. Although there are no storage requirements for Tris Hydrochloride, storage conditions were continuously monitored and recorded utilizing MadgeTech data loggers, with regulated conditions for temperature (15-30°C) and humidity (monitor). The maximum temperature of the warehouse during the stability study was 28.14°C and the minimum temperature of the warehouse was 12.63°C. See Section 5 for the discrepancy investigations initiated for temperature excursions.

5. INVESTIGATIONS:

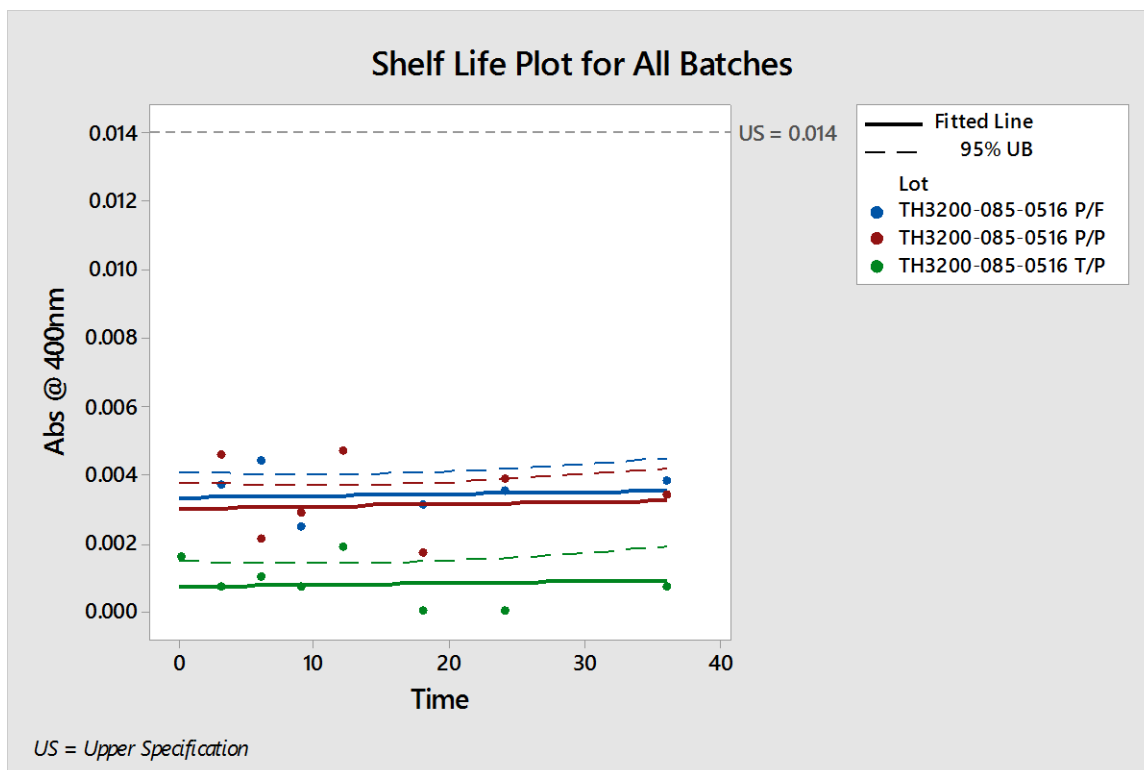
- 5.1. LI16-08 was initiated for OOS Absorbance at 400 nm for TH3200-085-0516 P/P and P/F T=3 samples. The investigation was found to have an assignable cause: analyst error. The re-tests of this material met the required specifications.
- 5.2. SDI16-57 was initiated for temperatures dropping below the minimum storage temperature of 15°C on 12/17/16. The temperature excursion had no effect on the Tris HCl stability samples, as the next time point was pulled and tested without issue.
- 5.3. SDI18-01 was initiated for temperatures dropping below the minimum storage temperature of 15°C on 1/7/18. The temperature excursion had no effect on the Tris HCl stability samples, as the next time point was pulled and tested without issue.

6. LOT EVALUATION:

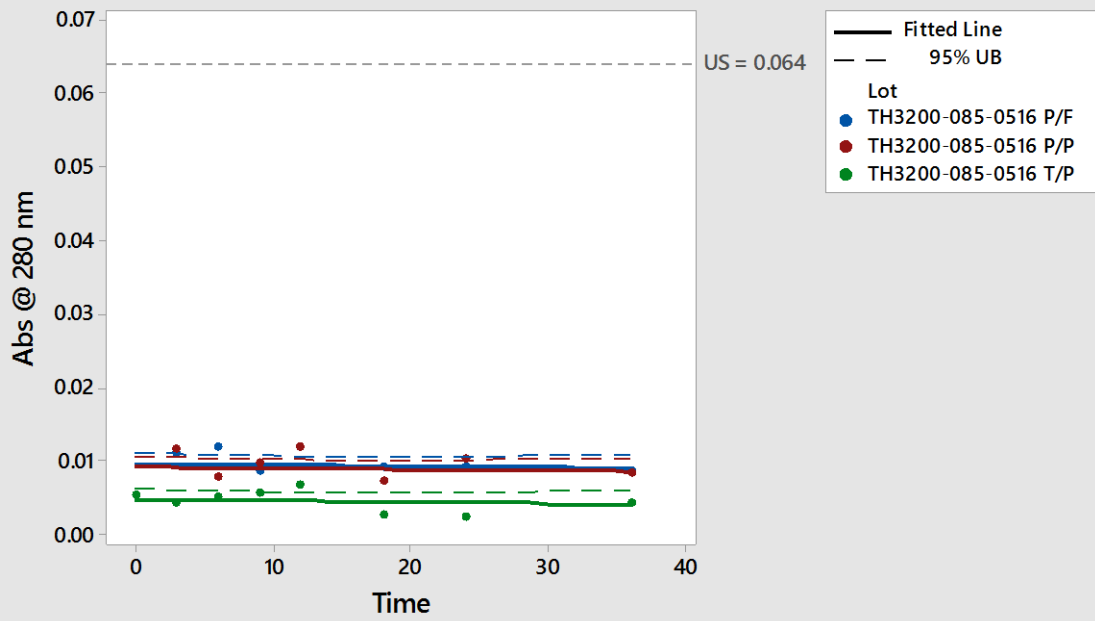
TABLE 2: ALL-INCLUSIVE STABILITY DATA

Tris Hydrochloride Stability Data											
Lot Number	Analysis	Specification	T ₀	T ₃	T ₆	T ₉	T ₁₂	T ₁₈	T ₂₄	T ₃₆	
TH3200-085-0516 (P/F)	Absorbance (1M)	0.01 a.u max @ 400 nm	0.0016	0.0037	0.0044	0.0025	0.0047	0.0031	0.0035	0.0038	
		0.06 a.u max @ 280 nm	0.0053	0.0110	0.0118	0.0087	0.0120	0.0092	0.0093	0.0085	
		0.06 a.u max @ 260 nm	0.0064	0.0130	0.0143	0.0105	0.0143	0.0112	0.0112	0.0100	
	Appearance and Color	White/Crystals	White/Crystals	White/Crystals	White/Crystals	White/Crystals	White/Crystals	White/Crystals	White/Crystals	White/Crystals	White/Crystals
	Assay	99.0 – 103.0%	99.40%	99.67%	99.66%	99.39%	99.63%	99.58%	99.86%	99.80%	
	Identification (IR)	Passes Test	Passes Test	Passes Test	Passes Test	Passes Test	Passes Test	Passes Test	Passes Test	Passes Test	Passes Test
	Loss on Drying (105°C)	0.5% max.	0.1257%	0.0520%	0.0162%	0.0783%	0.0474%	0.0343%	0.0474%	0.0595%	
	Melting Range	147 - 153°C	150.4-151.1°C	150.0-151.1°C	150.4-151.1°C	151.1-152.1°C	150.4-151.2°C	150.5-151.8°C	150.7-151.3°C	150.3-151.5°C	
pH (0.5M)	3.5 – 5.0	4.222 @ 22.72°C	4.208 @ 23.75°C	4.232 @ 22.48°C	4.211 @ 23.53°C	4.240 @ 22.54°C	4.197 @ 22.34°C	4.089 @ 23.35°C	4.201 @ 23.85°C		
TH3200-085-0516 (P/P)	Absorbance (1M)	0.01 a.u max @ 400 nm	0.0016	0.0046	0.0021	0.0029	0.0047	0.0017	0.0039	0.0034	
		0.06 a.u max @ 280 nm	0.0053	0.0115	0.0077	0.0098	0.0119	0.0072	0.0102	0.0083	
		0.06 a.u max @ 260 nm	0.0064	0.0136	0.0094	0.0120	0.0141	0.0090	0.0124	0.0100	
	Appearance and Color	White/Crystals	White/Crystals	White/Crystals	White/Crystals	White/Crystals	White/Crystals	White/Crystals	White/Crystals	White/Crystals	
	Assay	99.0 – 103.0%	99.40%	99.78%	99.57%	99.83%	100.05%	99.57%	99.88%	99.86%	
	Identification (IR)	Passes Test	Passes Test	Passes Test	Passes Test	Passes Test	Passes Test	Passes Test	Passes Test	Passes Test	
	Loss on Drying (105°C)	0.5% max.	0.1257%	0.0464%	0.0230%	0.0369%	0.0379%	0.0443%	0.0169%	0.0276%	
	Melting Range	147 - 153°C	150.4-151.1°C	149.5-150.8°C	150.5-151.1°C	151.1-151.9°C	150.6-151.7°C	150.3-151.8°C	150.6-151.3°C	150.5-151.6°C	
pH (0.5M)	3.5 – 5.0	4.222 @ 22.72°C	4.233 @ 22.73°C	4.238 @ 22.49°C	4.237 @ 22.80°C	4.239 @ 22.73°C	4.201 @ 22.24°C	4.083 @ 23.19°C	4.196 @ 24.16°C		
TH3200-085-0516 (T/P)	Absorbance (1M)	0.01 a.u max @ 400 nm	0.0016	0.0007	0.0010	0.0007	0.0019	0.0000	0.0000	0.0007	
		0.06 a.u max @ 280 nm	0.0053	0.0044	0.0052	0.0056	0.0067	0.0026	0.0023	0.0042	
		0.06 a.u max @ 260 nm	0.0064	0.0053	0.0065	0.0068	0.0082	0.0035	0.0036	0.0054	
	Appearance and Color	White/Crystals	White/Crystals	White/Crystals	White/Crystals	White/Crystals	White/Crystals	White/Crystals	White/Crystals	White/Crystals	
	Assay	99.0 – 103.0%	99.40%	99.80%	99.77%	99.59%	99.58%	99.55%	99.94%	99.91%	
	Identification (IR)	Passes Test	Passes Test	Passes Test	Passes Test	Passes Test	Passes Test	Passes Test	Passes Test	Passes Test	
	Loss on Drying (105°C)	0.5% max.	0.1257%	0.0331%	0.0214%	<0.0067%	0.0204%	0.0382%	0.0170%	0.0436%	
	Melting Range	147 - 153°C	150.4-151.1°C	149.9-151.1°C	150.6-151.6°C	150.9-152.1°C	150.6-152.1°C	150.3-151.8°C	150.6-151.3°C	150.0-151.3°C	
pH (0.5M)	3.5 – 5.0	4.222 @ 22.72°C	4.221 @ 23.65°C	4.248 @ 22.50°C	4.202 @ 23.10°C	4.237 @ 22.71°C	4.217 @ 22.31°C	4.104 @ 23.19°C	4.195 @ 24.07°C		

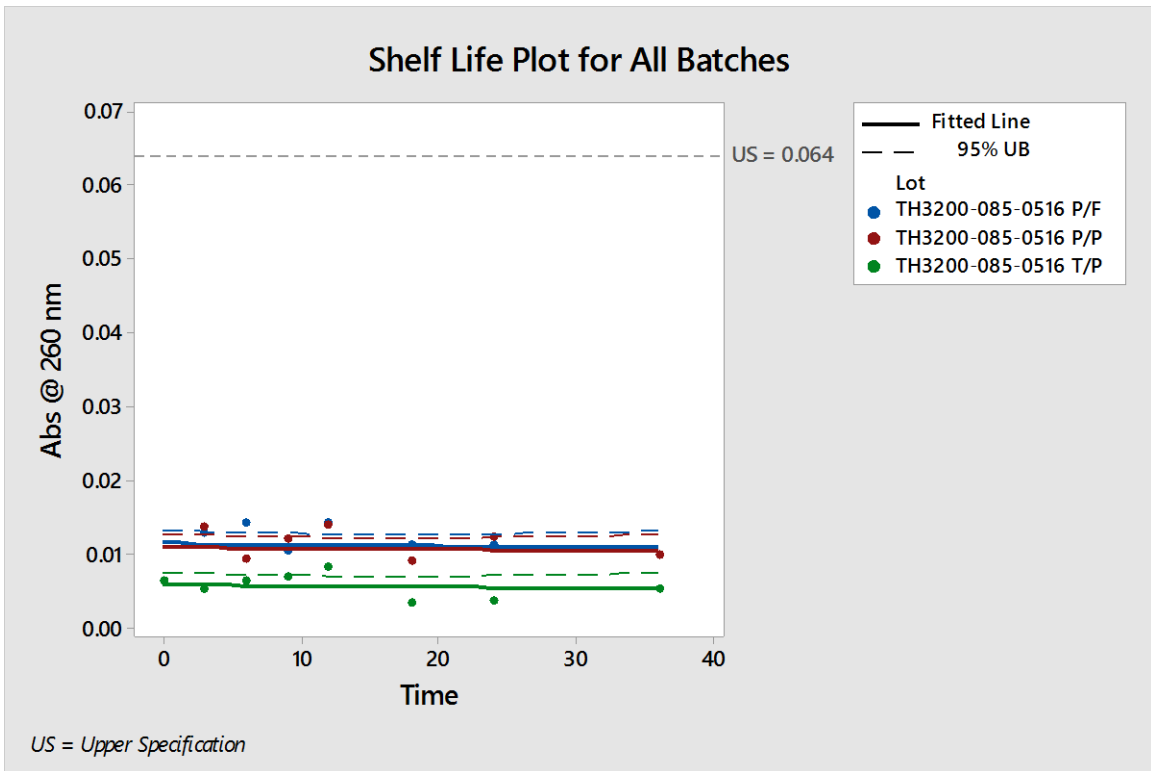
GRAPHS 1, 2 AND 3: SHELF LIFE PLOTS FOR ABSORBANCE @ 400 NM, 280 NM AND 260 NM



Shelf Life Plot for All Batches

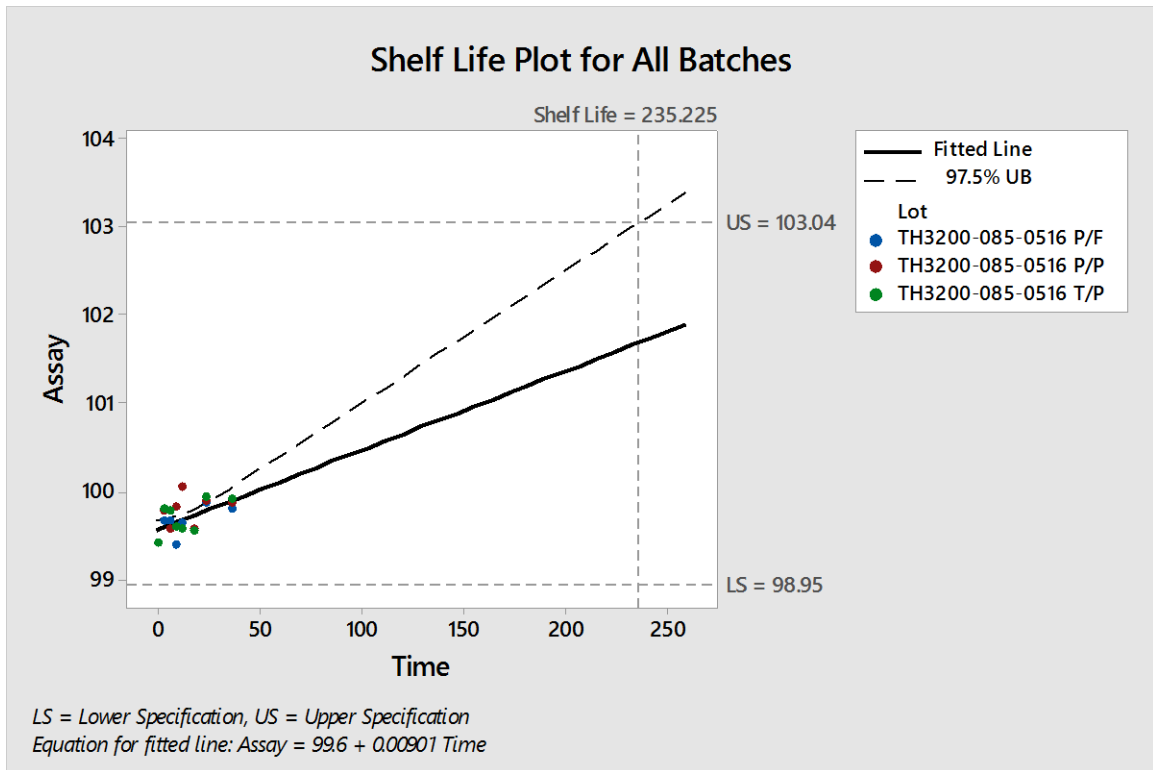


US = Upper Specification



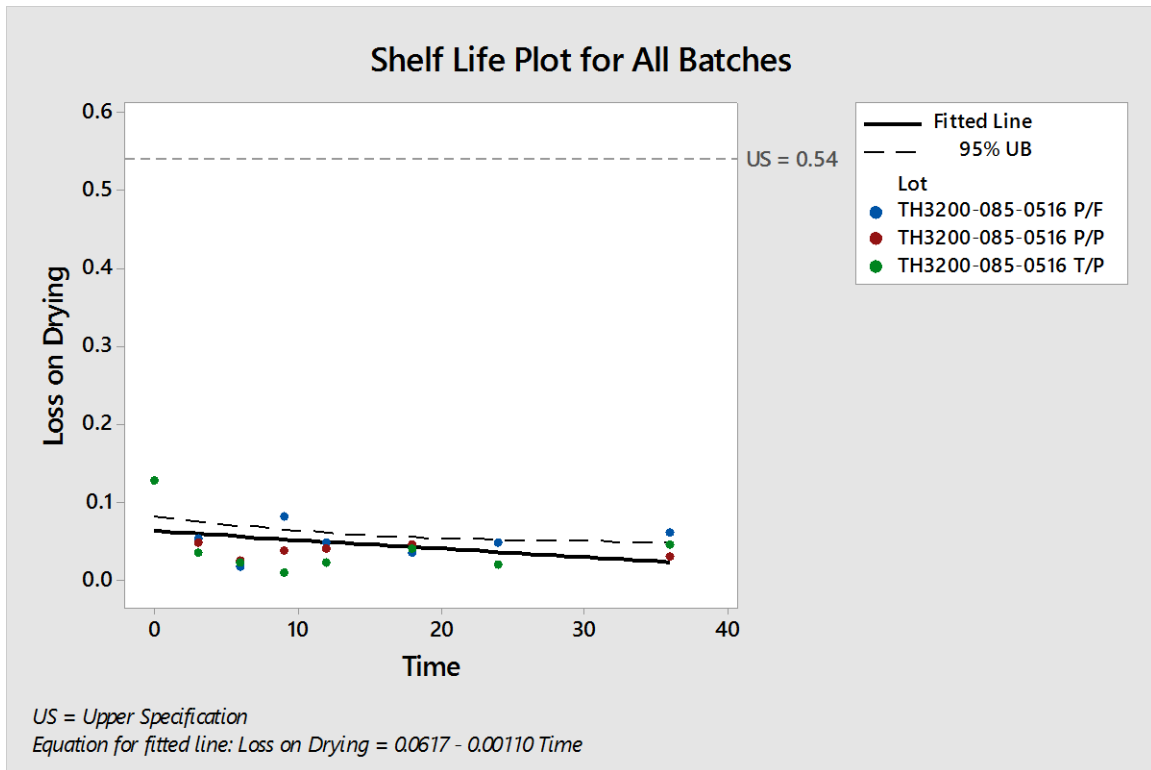
No shelf life is able to be determined for Absorbance, as the mean response slope is not significantly different from zero. There is no impact to the product or currently assigned expiration of this material.

GRAPH 4: SHELF LIFE PLOT FOR ASSAY



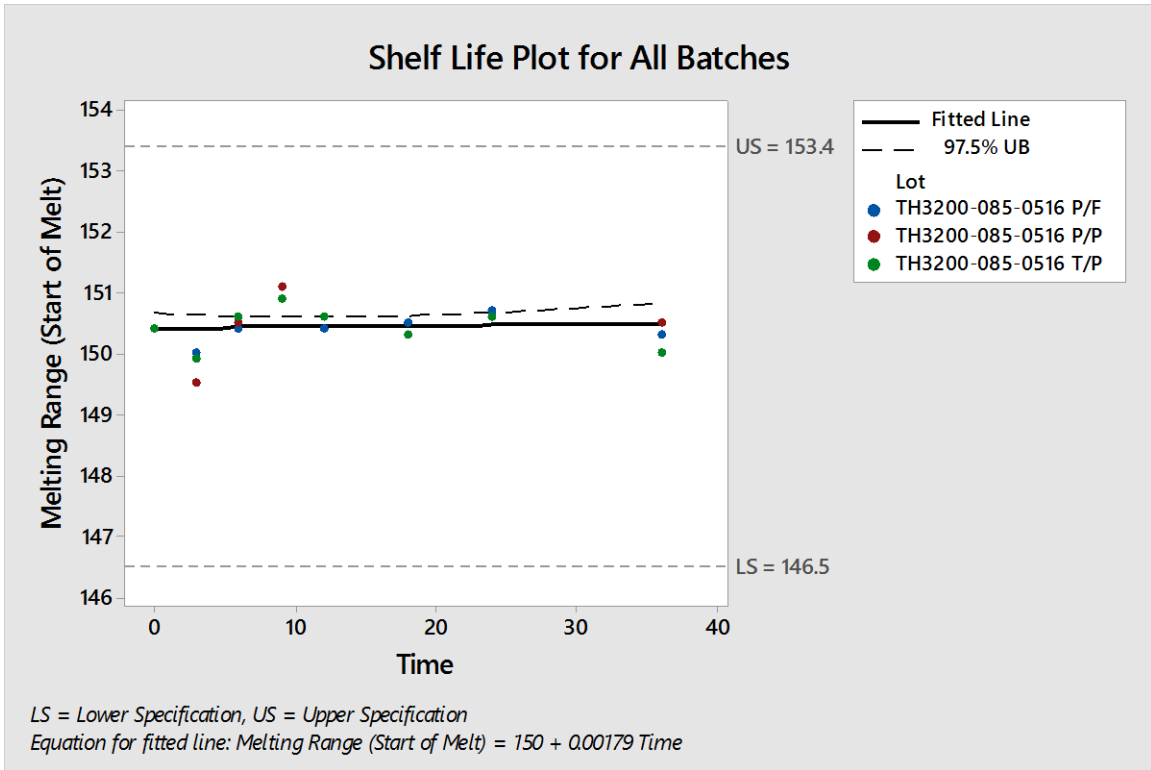
The predicted shelf life for Assay was determined to be 235.225 months. This is well beyond the thirty-six-month stability study, and shows no indication of issue to the product or the current shelf-life.

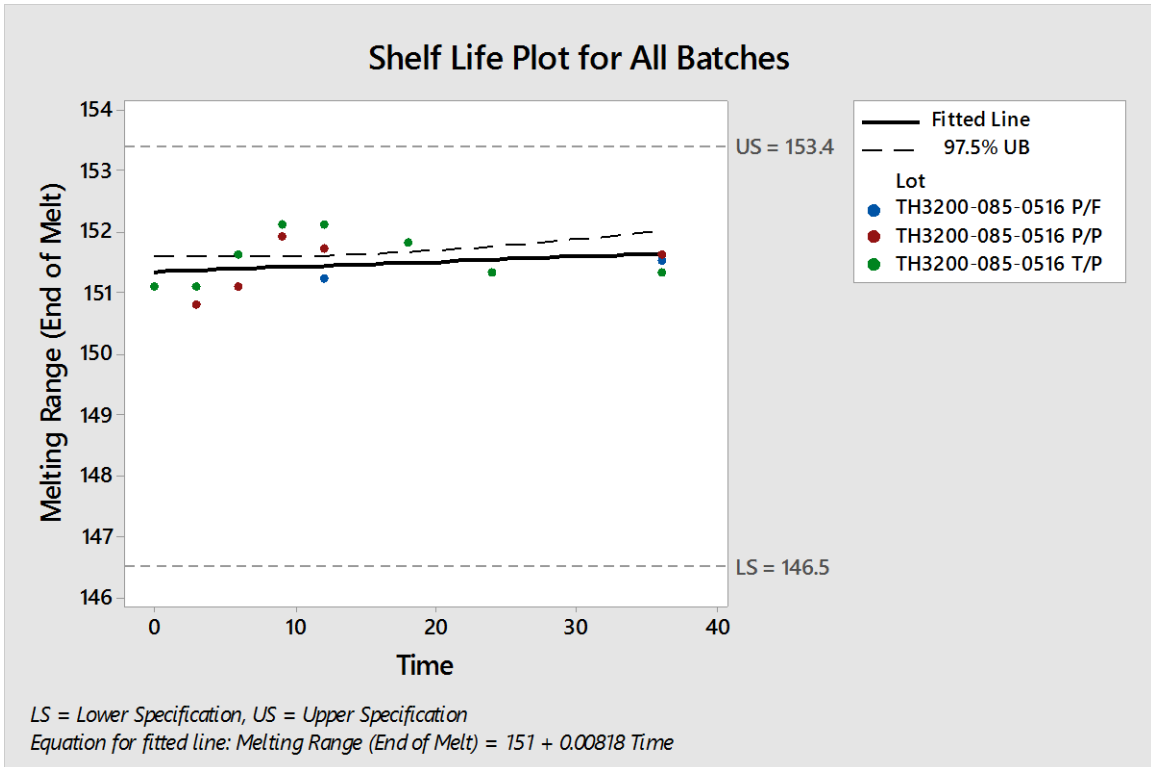
GRAPH 5: SHELF LIFE PLOT FOR LOSS ON DRYING



No shelf life is able to be determined for Loss on Drying, as the mean response slope is not significantly different from zero. There is no impact to the product or currently assigned expiration of this material.

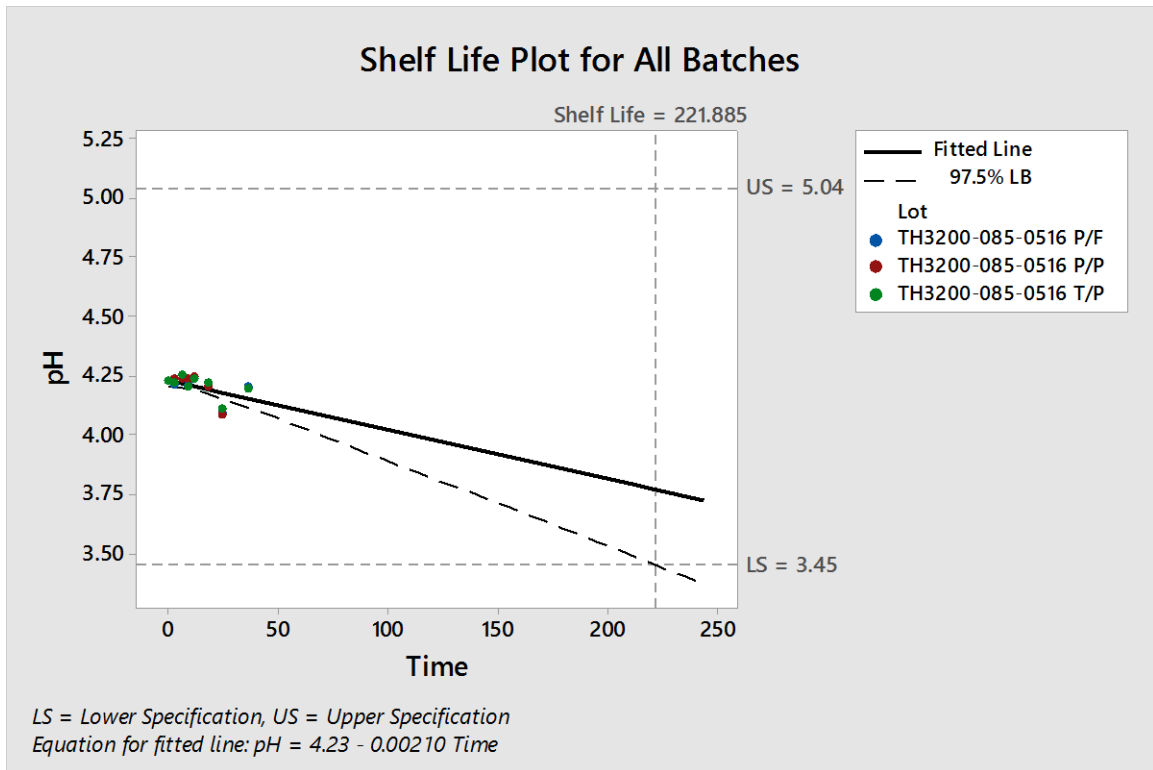
GRAPHS 6 AND 7: SHELF LIFE PLOTS FOR MELTING RANGE





No shelf life is able to be determined for Melting Range, as the mean response slopes for both the Start of Melt graph and End of Melt graph are not significantly different from zero. There is no impact to the product or currently assigned expiration of the lots.

GRAPH 8: SHELF LIFE PLOT FOR pH



The predicted shelf life for pH was determined to be 221.885 months. This is well beyond the thirty-six month stability study, and shows no indication of issue to the product or the current shelf-life.

7. CONCLUSION:

All data met the specifications set forth in the Stability Program. All known predicted Shelf Life estimates exceeded a Shelf Life of 36 months. The retest date will remain at two years. As the historical data and predicted Shelf Life plots indicate that the material is stable at 36 months, additional time may be given to extend the retest date to three years, if requested.

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.

8.1.1.1. 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.

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

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

2016 ANNUAL STABILITY PROGRAM ANALYSIS

TRIS HYDROCHLORIDE

Overview:

The purpose of this report is to analyze the data from the stability program set in place by BioSpectra. The Tris Hydrochloride (HCl) stability program was initiated in April of 2002. This program is continued on an annual basis in order to assure 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.

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 is significantly different from zero, 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 data can be found in the Tris Hydrochloride Stability Binder, Volume IV, the individual Analytical Summary Sheets for the analysis of the product, as well as an attachment to this report.

This real-time stability analysis assesses the stability of three Tris Hydrochloride lots that were initially put on stability in March of 2013, and came off stability in April of 2016. The study included the following analyses: Appearance and Color, Absorbance @ 400 nm, 280 nm, and 260 nm, Assay, Identification (IR), pH (0.5 M), and Melting Range. All Appearance and Color and Identification (IR) results met requirements. These results will not be analyzed as they are qualitative.

References:

ICH Q1A

Definitions:

CL: Control Limit, the average

UCL: Upper Control Limit, 3 sigma above the CL

LCL: Lower Control Limit, 3 sigma below the CL

OOL: Point(s) that fall outside the UCL or LCL

OOT: Out of Trend, this means that the material still meets control limits but was not in trend with the rest of the material.

OOS: Out of Specification, for the purpose of this stability analysis, OOS will mean that there is a point(s) that fall outside of the UCL or LCL.

Sample Designation:

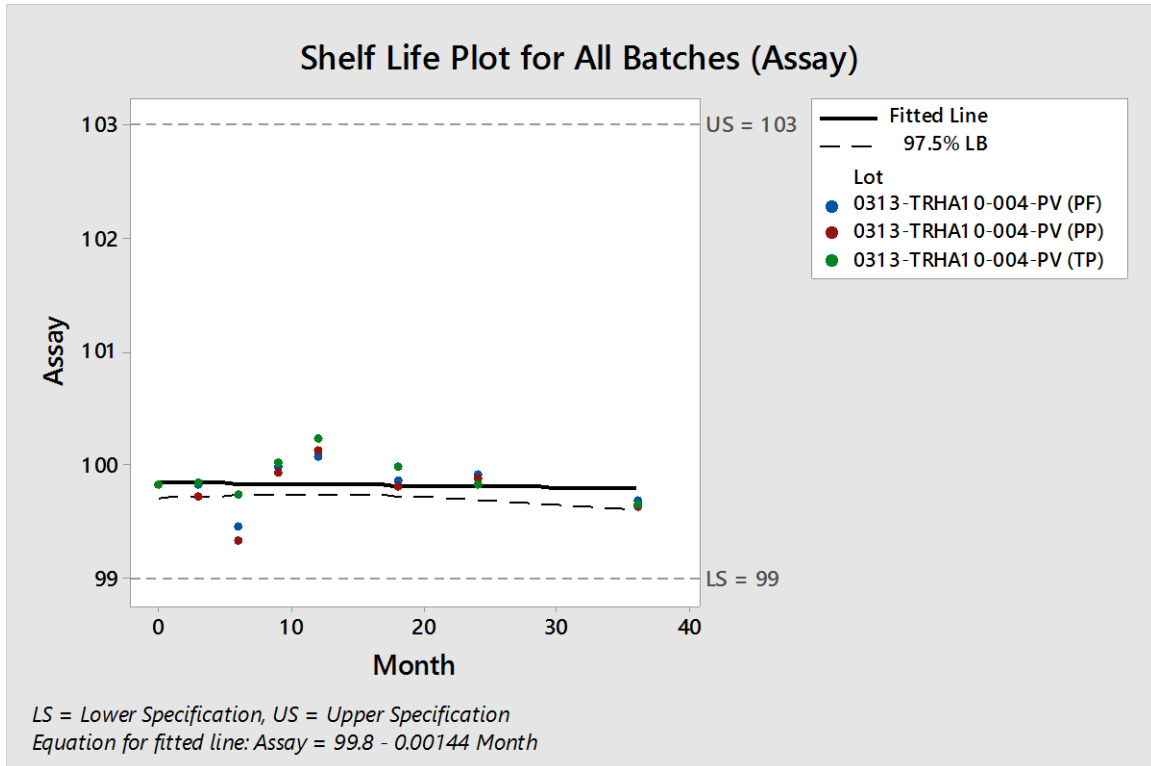
Samples initially placed on the stability program consisted of three Tris Hydrochloride Bio Excipient Validation Batches. Stability samples from each of the batches were put into three different types of packaging. The types of packaging are Poly/Poly (P/P), Tyvek/Poly (T/P), and Poly/Fiber (P/F). P/P samples were put into a poly bag that was goose-neck tied closed, which were then put in a larger poly bag that is goose-neck tied closed and placed into a poly drum. T/P samples were put into a poly drum lined with one Tyvek liner that was goose-neck tied closed. P/F samples were put into a poly bag and goose-neck tied closed, which were then put in a larger poly bag that is goose-neck tied closed and placed into a fiber drum. These batches were placed on stability in the Stroudsburg Stability Area, located in the quarantine area of the Warehouse. The type of packaging utilized in the stability samples were based on BioSpectra packaging.

Storage Conditions:

Although there are no storage requirements for Tris Hydrochloride, storage conditions have been continuously monitored and recorded. The temperature and humidity was monitored continuously utilizing MadgeTech data loggers located in the Stroudsburg Warehouse with regulated conditions between 15-30°C.

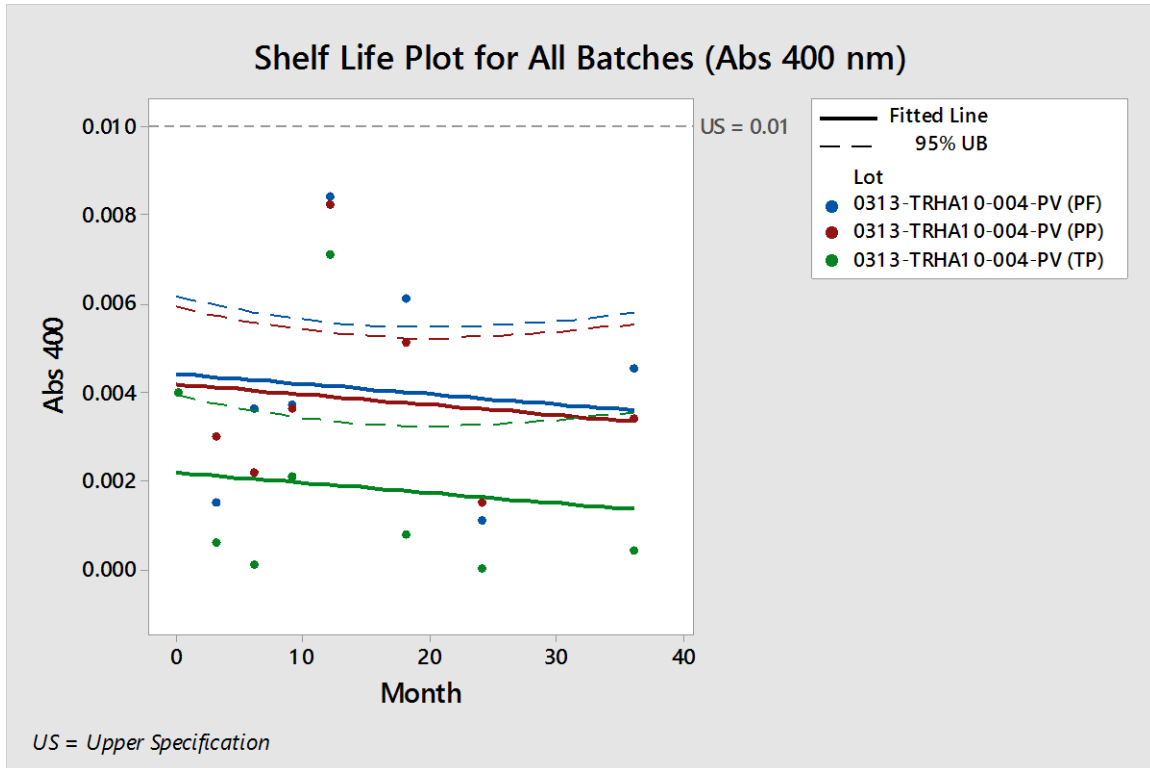
Lot Evaluation:

Graph 1: Shelf Life for All Batches (Assay)



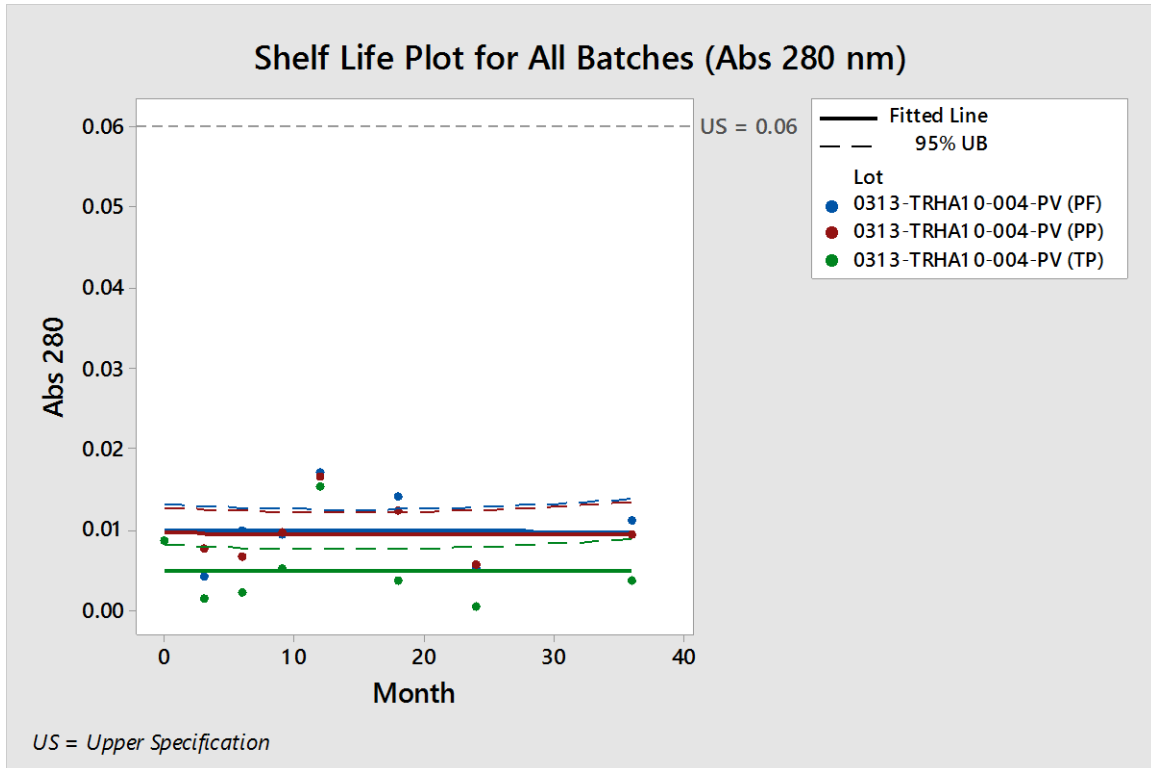
The estimated Shelf Life of Tris Hydrochloride for Assay Analysis cannot be predicted, as the mean response slope is not significantly different from zero. This is observed as there is little degradation of the product shown from this analysis.

Graph 2: Shelf Life for All Batches (Absorbance @ 400 nm)



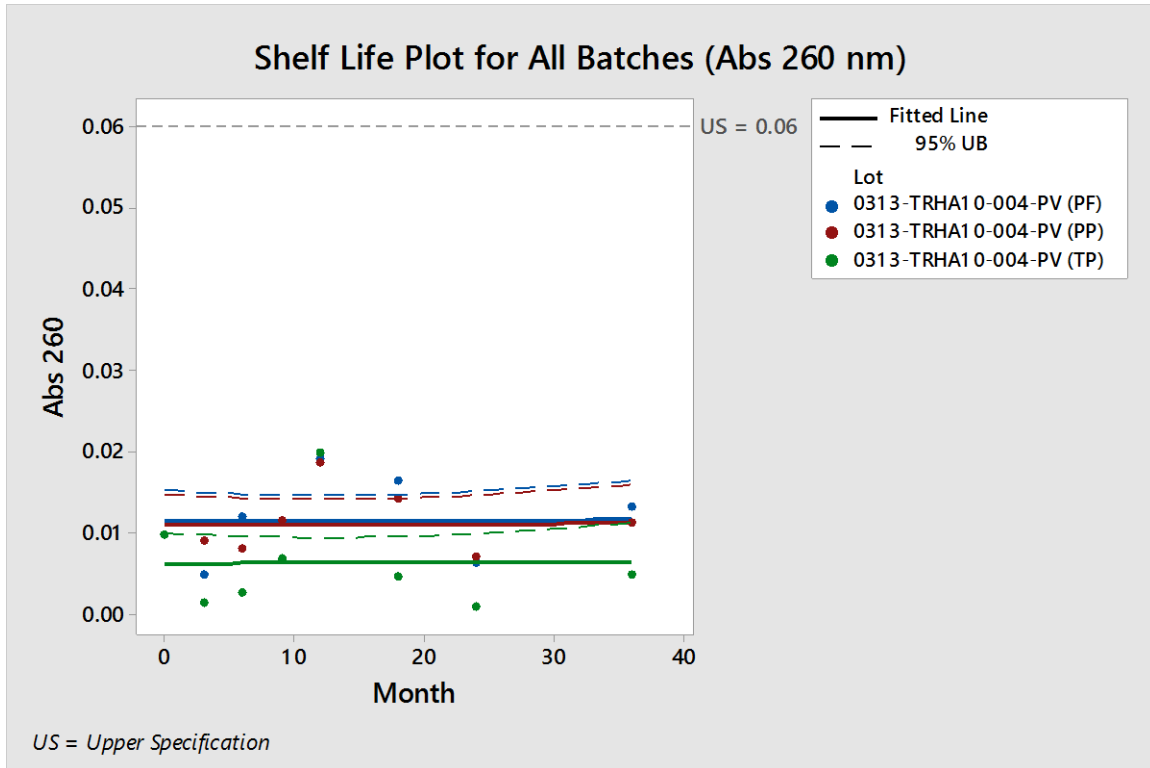
The estimated Shelf Life of Tris Hydrochloride for Absorbance Analysis at 400 nm cannot be predicted, as the mean response slope is not significantly different from zero. This is observed as there is little degradation of the product shown from this analysis.

Graph 3: Shelf Life for All Batches (Absorbance @ 280 nm)



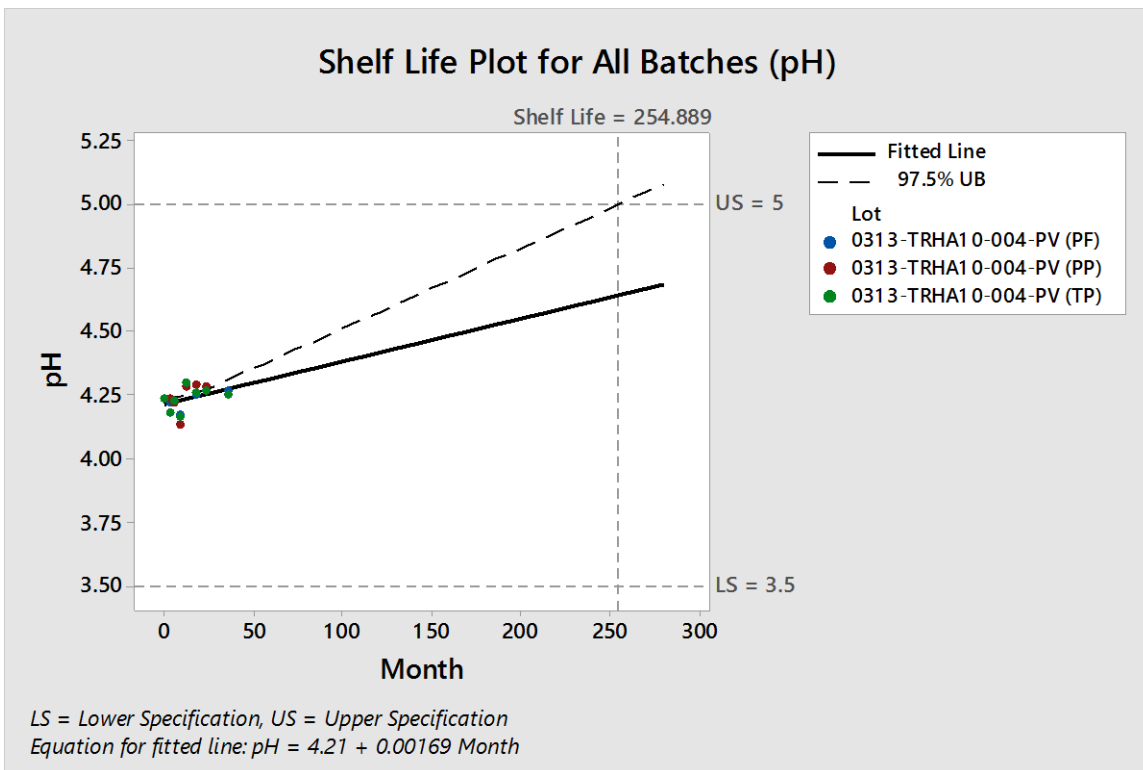
The estimated Shelf Life of Tris Hydrochloride for Absorbance Analysis at 280 nm cannot be predicted, as the mean response slope is not significantly different from zero. This is observed as there is little degradation of the product shown from this analysis.

Graph 4: Shelf Life for All Batches (Absorbance @ 260 nm)



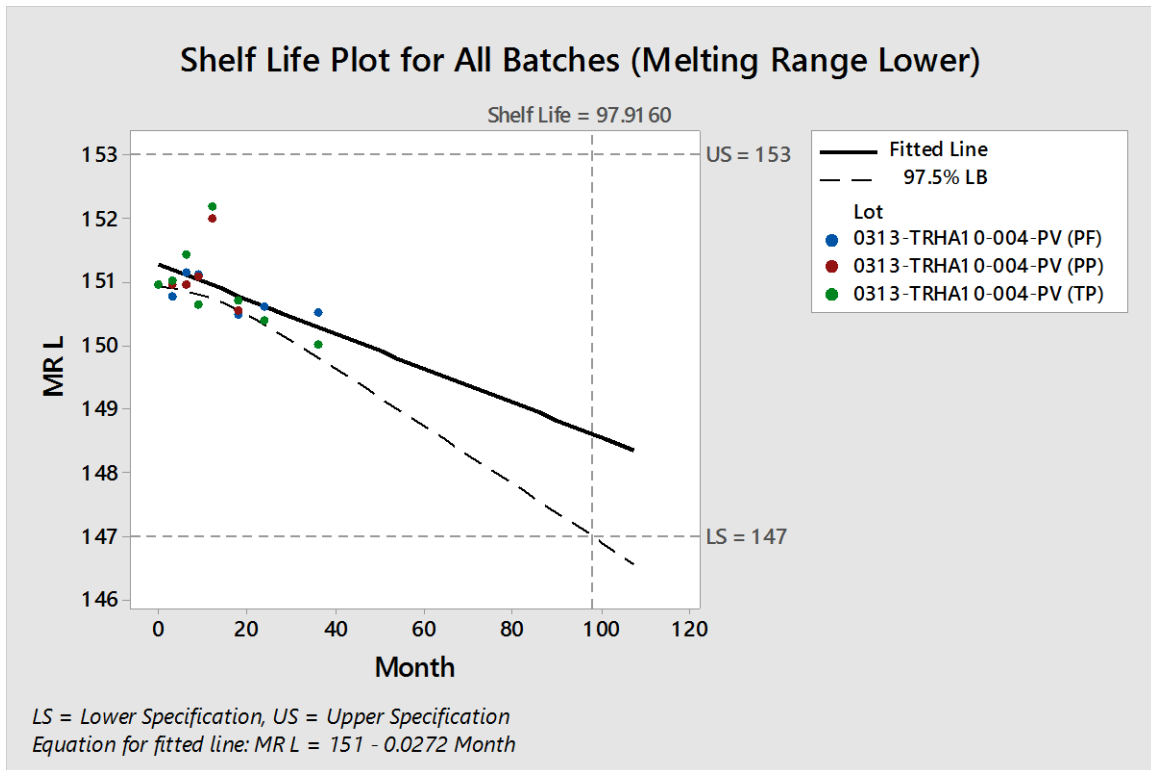
The estimated Shelf Life of Tris Hydrochloride for Absorbance Analysis at 260 nm cannot be predicted, as the mean response slope is not significantly different from zero. This is observed as there is little degradation of the product shown from this analysis.

Graph 5: Shelf Life for All Batches (pH 0.5 M)



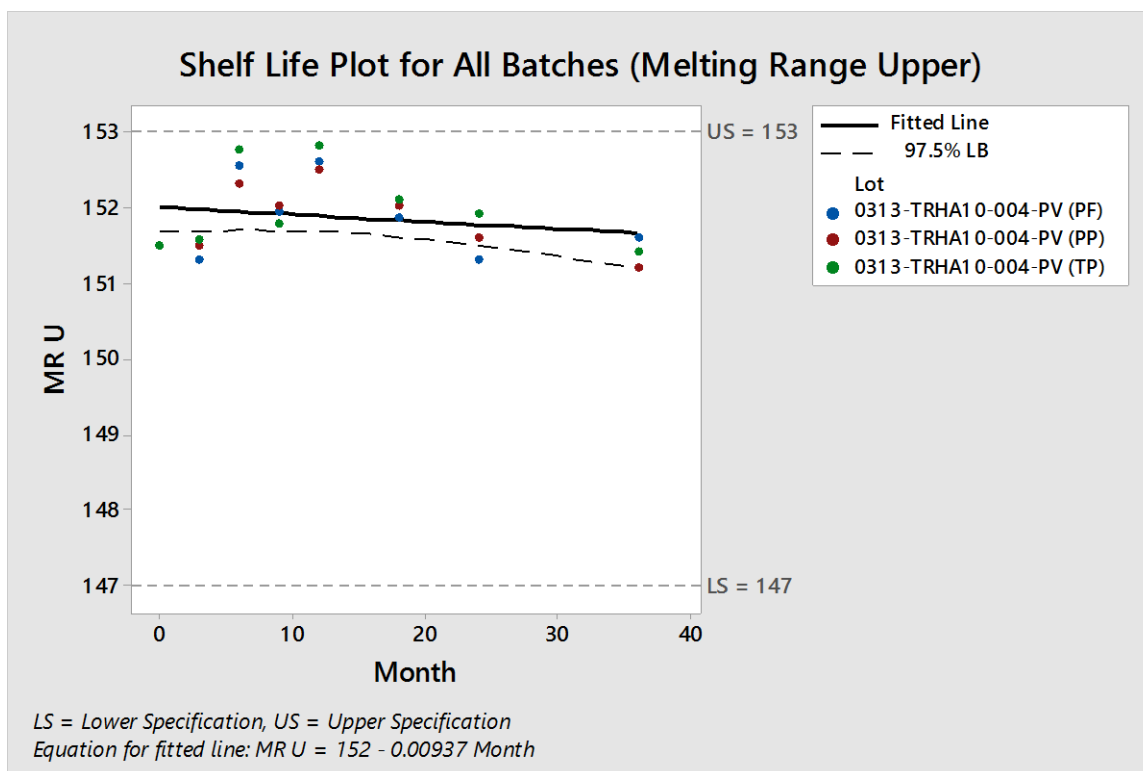
The estimated Shelf life of Tris Hydrochloride for pH Analysis (0.5 M) is predicted to be 254.89 months.

Graph 6: Shelf Life for All Batches (Melting Range Lower)



The estimated Shelf life of Tris Hydrochloride for Melting Range Analysis (Lower) is predicted to be 97.92 months.

Graph 7: Shelf Life for All Batches (Melting Range Upper)



The estimated Shelf Life of Tris Hydrochloride for Melting Range Analysis (Upper) cannot be predicted, as the mean response slope is not significantly different from zero. This is observed as there is little degradation of the product shown from this analysis.

Conclusion:

All lots of Tris Hydrochloride placed on stability in 2013, with the study concluding in 2016, were determined to be stable. All data met the specifications set forth in the Stability Program. All known predicted Shelf Life estimates exceeded a Shelf Life of 36 months. The retest date will remain at two years in accordance with the data assessment made above. Additional time after the two year may be given based on historical data and current data up to one year after a retest has been conducted.

Statement of Commitment

- BioSpectra is responsible for the following regarding Stability Data in this report:
 - All ongoing stability data points obtained from this program will be submitted to the DMF on an annual basis.

- 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.
- 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.
- If a stability analysis is found to be out of specification, the batch will be withdrawn from the market through communication with the Applicant and any additional customer. Additionally, an investigation will be conducted to determine the possible withdrawal of the batches produced before and after the batch in question.
- In the event that any out of specification results are confirmed, all authorized users of the material will be notified.