BIOSPECTRA
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TREHALOSE, DIHYDRATE LONG-TERM STABILITY REPORT: 2021 VALIDATION LOTS

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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 Trehalose, Dihydrate. 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 analysis will assess the stability of Trehalose validation lots TRED-0121-00002-PV, TRED-0121-00003-PV and TRED-0121-00004-PV that completed thirty-six (36) months of long-term stability in August 2024 and are scheduled to finish at sixty (60) months in August 2026. This study includes the following analyses: Appearance, Assay, Color and Clarity of Solution, Dextrins (Dextrins, Soluble Starch and Sulfite), Identification Test A (UATR), Impurities, pH at 25°C, Specific/Optical Rotation, and Water (by Karl-Fischer Titration). Bioburden and Endotoxin testing was added per BCC22-60, however this testing has not yet been completed through t=36 (Refer to BDI24-173 in section 5). Bioburden and endotoxin will be analyzed and included in the remainder of the stability study. Results from all applicable analyses are summarized in Tables 2 through 7. 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 for 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 Trehalose. The following product codes are commercially available.

- TRED-3250
- TRED-3251
- TRED-3252
- TRED-3253
- TRED-4250
- TRED-7251

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 initially placed on the stability program consisted of three lots of Trehalose. Stability samples from these lots were put into Poly/Poly (P/P) packaging configuration. The samples were packaged in accordance with the Stability Inventory SOP, BSI-SOP-0146. Reference Table 1 for packaging configuration and description. The type of packaging utilized in this stability study was based on BioSpectra packaging offered to the customer.

TABLE 1: PACKAGING DETAILS

Packaging Configuration	Packaging Description
Poly/Poly (P/P)	Samples are individually placed into small poly bags and are sealed with a zip tie. All samples are then placed into a poly pail and sealed.

4. STORAGE:

4.1. The Packaging and Storage requirements for Trehalose are to be in tightly closed container stored in a cool, well-ventilated area away from incompatible substances. For this long-term study, the samples were stored in the long-term stability chamber H03SC01 at the Bangor, PA facility from July 2021 (Lot TRED-0121-00002-PV started 07/28/21, and lots TRED-0121-00003-PV and TRED-0121-00004-PV started 08/02/21) until July 2024 when the T=36 (36-month) timepoint samples were pulled. The rest of the samples will remain in the long-term stability chamber until the study is completed in August 2026. Storage condition in the long-term stability chamber have been monitored continuously using MadgeTech data loggers with regulated conditions for temperature (25°C ±2) and relative humidity (60% ±5). For the time period of July 2021 until August 2024, the maximum temperature recorded was 26.31°C, the minimum temperature recorded was 22.63°C (Refer to BDI22-143), the average temperature recorded was 25.45°C, and the average kinetic temperature recorded was 25.45°C. The maximum relative humidity recorded was 80.5%, the minimum relative recorded was 31.1%, and the average relative humidity recorded was 61.2%. Maximum and minimum values that are outside the limits for temperature and humidity are due to opening the door of the chamber as explained in the Temperature and Humidity Monitoring Assessments. Section 5 will include any excursions from these conditions that resulted in an investigation.

5. INVESTIGATIONS:

5.1. **BDI22-61**: This discrepancy investigation covers missing MadgeTech logger data points for temperature and humidity in the Real Time Stability Chamber from 01/28/22 to 02/09/22. The logger was reset on 02/09/22 and functioned properly until the end of the collection period. The backup analog chart recorder on the Stability Chamber showed that there were no deviations in Temperature and Humidity during this time period. It was determined that there was no impact to the stability samples at this time.

5.2. **BDI22-138**: This discrepancy investigation covers out of specification (OOS) humidity readings in the Real Time Stability Chamber on 04/26/22 for approximately four hours. The low humidity readings were not accounted for by entry into the chamber, and the temperature during this time period was within specification. It was determined that the humidifier was not working properly due to a value that regulates the flow of humid air being left closed after chamber maintenance. The alarm for humidity deviations did alert staff and lead to the problem being addressed.

It was determined that due to the brief time period of lower humidity, there was no significant impact to the stability samples.

5.3. **BDI22-143**: This discrepancy investigation covers missing MadgeTech logger data points for temperature and humidity in the Real Time Stability Chamber from 11/20/21 to 12/03/21. It was determined that the missing data points were due to depletion of the batteries in the data loggers. When the batteries were replaced, the devices worked as intended. The backup analog chart recorder on the Stability Chamber showed that there were no deviation in the Temperature and Humidity during this time period. It was determined that there was no impact to the stability samples at this time.

5.4. **BDI23-42**: This discrepancy investigation covers the use of the wrong Maltotriose standard being used for assay and related substances for Trehalose by HPLC. The Maltotriose standard that was used was for qualitative use only, and this test method is a category II quantitative analysis. After performing the investigation and an impact assessment, it was determined that the samples analyzed show no peak corresponding to the retention time of Maltotriose, therefor the quantitation of Maltotriose was not required. The original results were considered acceptable and reported as the final values.

5.5. **BDI23-53**: This discrepancy investigation covers testing for Appearance testing not being completed before the due date. Sample paperwork was placed in the QC Office prematurely before all of the testing was complete, and the missed testing was not caught until after the two-week due date. Due to this being a qualitative test, it was determined that completing this testing two days past the due date had a low impact on the study at this time interval.

5.6. **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.

5.7. **BDI24-173**: This discrepancy covers the failure to test Endotoxin and Bioburden for Trehalose stability after approval of BCC22-60. The additional testing was to be completed on any new lots of Trehalose placed on the stability program and the next time intervals for any Trehalose lots already on the stability program. Upon approval of the updated Trehalose stability data card, new data cards were not printed for any lots already on the stability program, therefore the additional testing was not completed. The following lots/time points were included for the incomplete stability testing: TRED-0121-00002-PV t=36 P/P, TRED-0121-00003-PV t=36 P/P and TRED-0121-00004-PV t=36 P/P. There was no impact to the Trehalose lots on the stability program as all previous time points did not use Bioburden or Endotoxin results to determine shelf life.

6. LOT EVALUATION:

TABLE 2: RESULT OF REAL TIME STABILITY ANALYSES FOR TRED-0121-00002-PV P/P

Time Point	Analyses and Specifications							
	Appearance	**Color and Clarity of Solution**		**Dextrins, Soluble Starch & Sulfite**	**Identification (IR)**	**pH @ 25°C**	**Specific/ Optical Rotation**	**KF Water**
	White to Off White Crystalline Powder	A720: ≤0.050 a.u.	A420 -A720: ≤0.100 a.u.	Passes Test	Conforms to Standard	4.5-6.5	+197° to +201° @ 20°C	9.0 – 11.0%
T_0	White Crystalline Powder	0.001	0.021	Passes Test	Conforms to Standard	5.5 @ 23.2°C	+199°	9.8%
T_3	White Crystalline Powder	<0.003	0.018	Passes Test	Conforms to Standard	5.5 @ 24.7°C	+199°	10.4%
T_6	White to Off-White Crystalline Powder	<0.050	0.014	Passes Test	Conforms to Standard	5.6 @ 23.1°C	+199°	9.6%
T_9	White Crystalline Powder	<0.003	0.018	Passes Test	Conforms to Standard	5.6 @ 24.3°C	+199°	9.7%
T_{12}	White to Off-White Crystalline Powder	0.002	0.022	Passes Test	Conforms to Standard	5.5 @ 24.5°C	+200°	9.2%
T_{18}	White to Off-White Crystalline Powder	<0.003	0.021	Passes Test	Conforms to Standard	5.8 @ 23.2°C	+199°	9.5%
T_{24}	White to Off-White Crystalline Powder	<0.003	0.021	Passes Test	Conforms to Standard	5.6 @ 23.1°C	+199°	9.4%
T_{36}	White Crystalline Powder	0.0013	0.0222	Passes Test	Conforms to Standard	5.54 @ 25.3°C	+199.73°	9.71%

TABLE 3: RESULT OF REAL TIME STABILITY ANALYSES FOR TRED-0121-00002-PV P/P CONTINUED

Time Point	Analyses and Specifications						
	Assay	**Maltotriose (Impurity B)**	**Total Impurities RRT <1.0**	**Total Impurities RRT >1.0**	**Glucose (Impurity A)**	**Other Impurities**	**Sum of Glucose, Maltotriose and Other Impurities**
	98.0-101.0%	≤0.5%	≤0.5%	≤0.5%	≤0.5%	≤0.2%	≤1.0%
T_0	99.6%	<0.5%	<0.5%	<0.5%	<0.5%	<0.2%	<1.0%
T_3	100.8%	<0.5%	<0.5%	<0.5%	<0.5%	<0.2%	<1.0%
T_6	99.5%	<0.5%	<0.5%	<0.5%	<0.5%	<0.2%	<1.0%
T_9	99.0%	<0.5%	<0.5%	<0.5%	<0.5%	<0.2%	<1.0%
T_{12}	99.4%	<0.5%	<0.5%	<0.5%	<0.5%	<0.2%	<1.0%
T_{18}	99.1%	<0.5%	<0.5%	<0.5%	<0.5%	<0.2%	<1.0%
T_{24}	99.5%	<0.5%	<0.5%	<0.5%	<0.5%	<0.2%	<1.0%
T_{36}	99.6%	<0.10%	0.12%	<0.01%	<0.10%	<0.01%	0.12%

FUTURE TESTING INTERVAL PULL DATES:

- T=48; Scheduled for July 28, 2025
- T=60; Scheduled for July 28, 2026

TABLE 4: RESULT OF REAL TIME STABILITY ANALYSES FOR TRED-0121-00003-PV P/P

Time Point	Analyses and Specifications							
	Appearance	**Color and Clarity of Solution**		**Dextrins, Soluble Starch & Sulfite**	**Identification (IR)**	**pH @ 25°C**	**Specific/ Optical Rotation**	**KF Water**
	White to Off White Crystalline Powder	A720: ≤0.050 a.u.	A420 -A720: ≤0.100 a.u.	Passes Test	Conforms to Standard	4.5-6.5	+197° to +201° @ 20°C	9.0 – 11.0%
T_0	White Crystalline Powder	0.002	0.014	Passes Test	Conforms to Standard	5.6 @ 23.4°C	+199°	9.5%
T_3	White to Off-White Crystalline Powder	<0.003	0.015	Passes Test	Conforms to Standard	5.5 @ 23.4°C	+199°	9.8%
T_6	White to Off-White Crystalline Powder	<0.003	0.017	Passes Test	Conforms to Standard	5.5 @ 23.0°C	+199°	9.8%
T_9	White to Off-White Crystalline Powder	<0.003	0.018	Passes Test	Conforms to Standard	5.6 @ 24.9°C	+199°	9.7%
T_{12}	White Crystalline Powder	<0.003	0.019	Passes Test	Conforms to Standard	5.6 @ 23.5°C	+199°	9.8%
T_{18}	White to Off-White Crystalline Powder	<0.003	0.019	Passes Test	Conforms to Standard	5.7 @ 24.5°C	+199°	9.4%
T_{24}	White Crystalline Powder	<0.003	0.019	Passes Test	Conforms to Standard	5.9 @ 27.0°C	+200°	9.7%
T_{36}	White Crystalline Powder	0.0023	0.0208	Passes Test	Conforms to Standard	5.47 @ 25.1°C	+201.00°	10.00%

TABLE 5: RESULT OF REAL TIME STABILITY ANALYSES FOR TRED-0121-00003-PV P/P CONTINUED

Time Point	Analyses and Specifications						
	Assay	**Maltotriose (Impurity B)**	**Total Impurities RRT <1.0**	**Total Impurities RRT >1.0**	**Glucose (Impurity A)**	**Other Impurities**	**Sum of Glucose, Maltotriose and Other Impurities**
	98.0-101.0%	≤0.5%	≤0.5%	≤0.5%	≤0.5%	≤0.2%	≤1.0%
T_0	100.3%	<0.5%	<0.5%	<0.5%	<0.5%	<0.2%	<1.0%
T_3	100.3%	<0.5%	<0.5%	<0.5%	<0.5%	<0.2%	<1.0%
T_6	100.8%	<0.5%	<0.5%	<0.5%	<0.5%	<0.2%	<1.0%
T_9	100.3%	<0.5%	<0.5%	<0.5%	<0.5%	<0.2%	<1.0%
T_{12}	99.3%	<0.5%	<0.5%	<0.5%	<0.5%	<0.2%	<1.0%
T_{18}	99.5%	<0.5%	0.2%	<0.5%	<0.5%	<0.2%	0.2%
T_{24}	99.3%	<0.5%	0.2%	<0.5%	<0.5%	<0.2%	0.2%
T_{36}	99.9%	<0.10%	0.17%	<0.01%	<0.10%	<0.01%	0.17%

Future Testing Interval Pull Dates:

- T=48; Scheduled for August 2, 2025
- T=60; Scheduled for August 2, 2026

TABLE 6: RESULT OF REAL TIME STABILITY ANALYSES FOR TRED-0121-00004-PV P/P

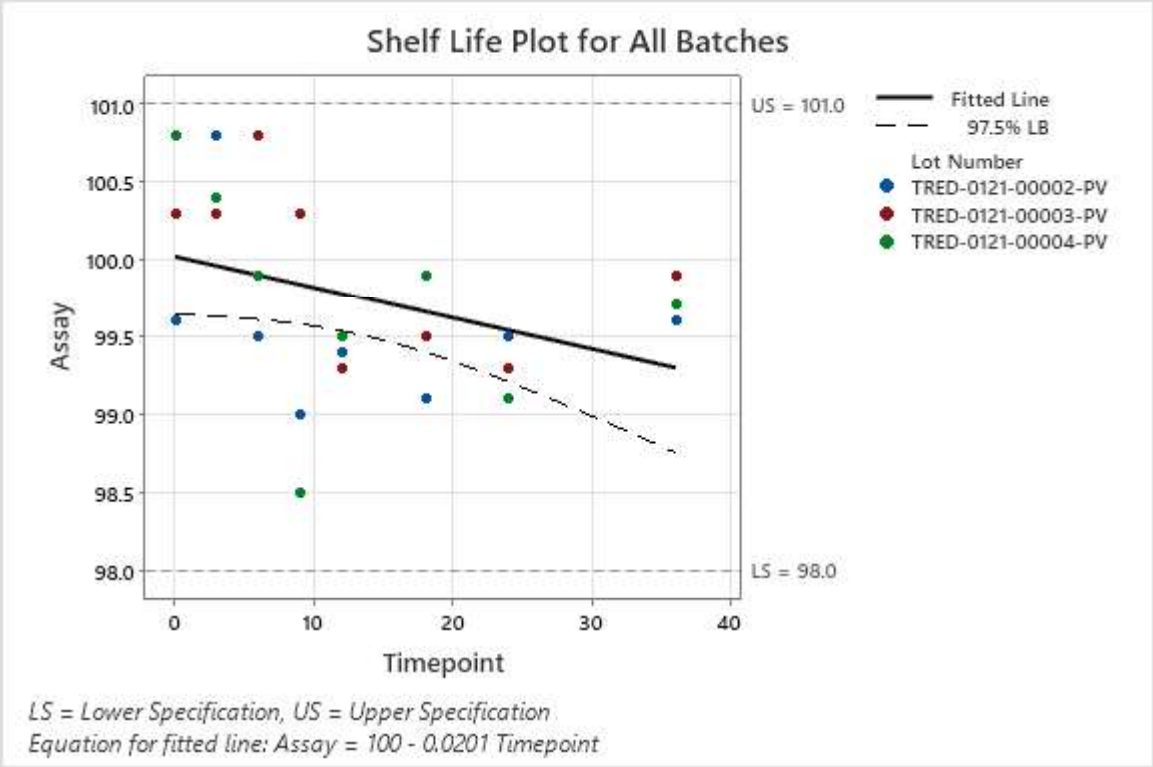
Time Point	Analyses and Specifications							
	Appearance	**Color and Clarity of Solution**		**Dextrins, Soluble Starch & Sulfite**	**Identification (IR)**	**pH @ 25°C**	**Specific/ Optical Rotation**	**KF Water**
	White to Off White Crystalline Powder	A720: ≤0.050 a.u.	A420 -A720: ≤0.100 a.u.	Passes Test	Conforms to Standard	4.5-6.5	+197° to +201° @ 20°C	9.0 – 11.0%
T_0	White Crystalline Powder	<0.003	0.014	Passes Test	Conforms to Standard	5.6 @ 23.0°C	+199°	9.4%
T_3	White to Off-White Crystalline Powder	<0.050	0.013	Passes Test	Conforms to Standard	5.4 @ 23.1°C	+199°	9.8%
T_6	White to Off-White Crystalline Powder	<0.003	0.016	Passes Test	Conforms to Standard	5.4 @ 23.1°C	+199°	9.8%
T_9	White to Off-White Crystalline Powder	<0.003	0.016	Passes Test	Conforms to Standard	5.6 @ 24.5°C	+199°	10.0%
T_{12}	White Crystalline Powder	<0.003	0.018	Passes Test	Conforms to Standard	5.6 @ 23.6°C	+199°	9.8%
T_{18}	White to Off-White Crystalline Powder	<0.003	0.017	Passes Test	Conforms to Standard	5.9 @ 23.2°C	+200°	9.8%
T_{24}	White Crystalline Powder	<0.003	0.018	Passes Test	Conforms to Standard	5.8 @ 25.9°C	+199°	9.5%
T_{36}	White Crystalline Powder	<0.003	0.0190	Passes Test	Conforms to Standard	5.62 @ 25.9°C	+200.40°	10.01%

TABLE 7: RESULT OF REAL TIME STABILITY ANALYSES FOR TRED-0121-00004-PV P/P CONTINUED

Time Point	Analyses and Specifications						
	Assay	**Maltotriose (Impurity B)**	**Total Impurities RRT <1.0**	**Total Impurities RRT >1.0**	**Glucose (Impurity A)**	**Other Impurities**	**Sum of Glucose, Maltotriose and Other Impurities**
	98.0-101.0%	≤0.5%	≤0.5%	≤0.5%	≤0.5%	≤0.2%	≤1.0%
T_0	100.8%	<0.5%	<0.5%	<0.5%	<0.5%	<0.2%	<1.0%
T_3	100.4%	<0.5%	<0.5%	<0.5%	<0.5%	<0.2%	<1.0%
T_6	99.9%	<0.5%	<0.5%	<0.5%	<0.5%	<0.2%	<1.0%
T_9	98.5%	<0.5%	<0.5%	<0.5%	<0.5%	<0.2%	<1.0%
T_{12}	99.5%	<0.5%	<0.5%	<0.5%	<0.5%	<0.2%	<1.0%
T_{18}	99.9%	<0.5%	0.1%	<0.5%	<0.5%	<0.2%	0.1%
T_{24}	99.1%	<0.5%	0.2%	<0.5%	<0.5%	<0.2%	0.2%
T_{36}	99.7%	<0.10%	0.16%	<0.01%	<0.10%	<0.01%	0.16%

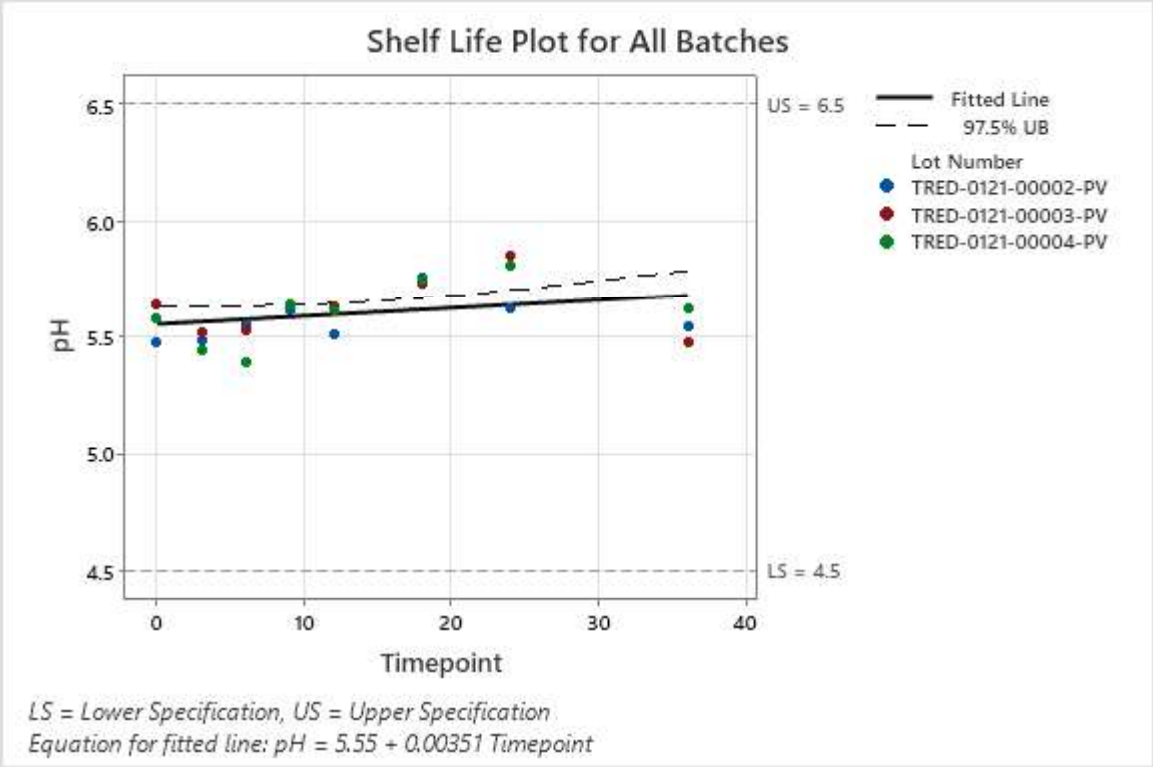
Future Testing Interval Pull Dates:

- T=48; Scheduled for August 2, 2025
- T=60; Scheduled for August 2, 2026



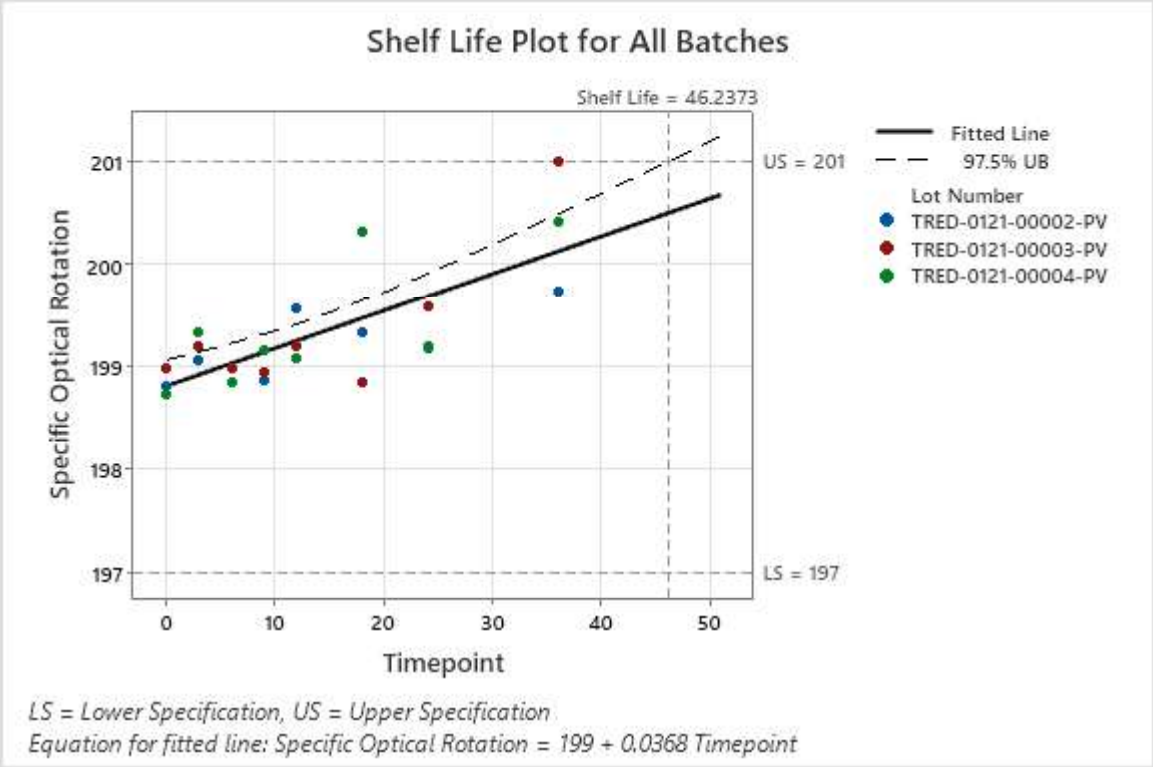
GRAPH 1: ASSAY

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.



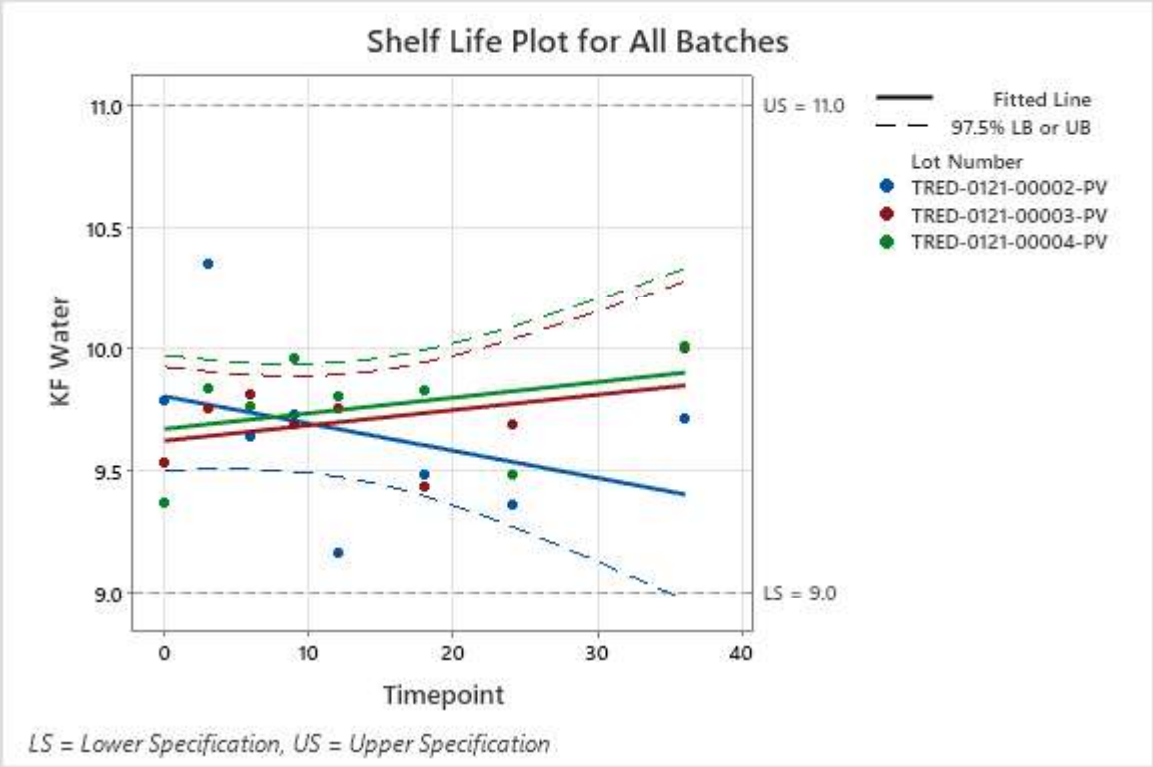
GRAPH 2: PH

No Shelf-Life was able to be determined for pH, 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: SPECIFIC OPTICAL ROTATION

The predicted Shelf-Life for Specific Optical Rotation was determined to be 46.2373 months at the T=36-month time interval. Results will continue to be monitored. All timepoint intervals did pass specification up to the T=36 sample. There is no impact to the product or currently assigned retest period of this material.



GRAPH 4: KF WATER

No Shelf-Life was able to be determined for KF Water, 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:

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 real time conditions. Long-term stability data up to 36 months displayed in this report along with the predicted shelf-life plots would support shelf life extension up to 46 months upon request. This stability report supports the current retest date of 24 months and expiration of 36 months for Trehalose manufactured at BioSpectra in the Bangor, PA facility, and will continued to be monitored.

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.