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ANALYTICAL METHOD VERIFICATION REPORT: SIEVERS M9 TOC ANALYSIS – TREHALOSE DIHYDRATE

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1. PURPOSE:

1.1. The purpose of this Method Verification Report is to:
- 1.1.1. Ensure that the Total Organic Carbon (TOC) procedure on the Sievers M9 TOC Analyzer is adequately evaluated and verified for Trehalose Dihydrate cleaning.
- 1.1.2. To summarize the results from the Trehalose Dihydrate Cleaning Total Organic Carbon (TOC) Analysis verification and demonstrate that the analytical method meets all requirements for:
  - 1.1.2.1. System Suitability
  - 1.1.2.2. Accuracy
  - 1.1.2.3. Precision
  - 1.1.2.4. Specificity
  - 1.1.2.5. Linearity
  - 1.1.2.6. Range
  - 1.1.2.7. Limit of Detection
  - 1.1.2.8. Limit of Quantitation
- 1.1.3. To summarize the results from the recovery of Trehalose Dihydrate rinse and swab samples from substrates of interest.

2. SCOPE:

2.1. This analytical method verification report applies to the Trehalose Dihydrate Cleaning Total Organic Carbon (TOC) Analysis for rinse and swab extraction samples using the Sievers M9 TOC Analyzer.

2.2. The Trehalose Dihydrate Cleaning Total Organic Carbon (TOC) Analysis was verified as a Category II Quantitative test.

2.3. **Reaction Chemistry:** The Sievers M9 TOC Analyzer is designed to measure the concentration of total organic carbon (TOC), total inorganic carbon (TIC), and total carbon (TC = TOC + TIC) in water samples. The Analyzer oxidizes organic compounds to form carbon dioxide (CO_2) using UV radiation and a chemical oxidizing agent (ammonium persulfate). CO_2 is measured using selective membrane-based conductometric detection.

3. RESPONSIBILITIES:

3.1. The Senior Manager of Product Life Cycle is responsible for the control, implementation, training and maintenance of this procedure.

3.2. Qualified personnel are responsible for data review and completing the Method Verification Report using conclusions made from the results obtained from testing.

4. REFERENCES:

4.1. BSI-PRL-0564, Sievers M9 TOC Verification Protocol
4.2. BSI-RPT-0915, Analytical Method Validation Report: TC Analysis – Trehalose Cleaning Detection Method
4.3. BSI-SOP-0098, Balance SOP
4.4. BSI-SOP-0126, Laboratory Notebooks
4.5. BSI-SOP-0133, Blue M Convection Oven Operation and Calibration SOP
4.6. BSI-SOP-0134, Pipette SOP
4.7. BSI-SOP-0244, VWR Gravity Convection Oven Operation and Calibration (Model Number 414005-106)
4.8. BSI-SOP-0293, Process Cleaning Validation Master Plan
4.9. BSI-SOP-0436, Analytical Methods Validation Master Plan
4.10. M9 Series Operation and Maintenance Manual
4.11. Sievers DataPro2 Software with DataGuard User Guide

5. MATERIALS AND EQUIPMENT:

5.1. All materials and equipment utilized in this verification are outlined in this section.

5.2. Equipment

TABLE 1: EQUIPMENT

Equipment	Model / Part Number	Manufacturer	Serial Number	Calibration Due Date	Date of Last Calibration
TOC Analyzer	M9	Sievers	22027246	2/25	2/29/24
Analytical Balance	MSE224S	Sartorius	24801744	10/31/24	4/12/24
Calibrated Micropipette	Research Plus	Eppendorf	I44388L	8/31/24	2/22/24
			H33986M	8/31/24	2/22/24
			M27701G	7/31/24	1/5/24
			N31016H	9/30/24	3/4/24

5.3. Standards and Reagents

TABLE 2: STANDARDS AND REAGENTS

Reagent / Standard	Lot ID	Manufacturer	CAS Number	Expiration Date
Trehalose Dihydrate	TRED-0123-00018	BioSpectra Inc.	6138-23-4	8/31/25
Purified Water	F9SA14284H	Millipore Sigma	7732-18-5	5/17/25
Tris Standard	723e	NIST	77-86-1	12/31/25
Acid Cartridge – 6M Phosphoric Acid	24044-ACID037	Suez	Not Applicable	2/13/25
Oxidizer Cartridge – 15% Ammonium Persulfate	24107-OXID104	Suez	Not Applicable	4/16/25

5.4. Supplies

TABLE 3: SUPPLIES

Supply	Manufacturer	Part Number
Low TOC Vials	Scientific Specialties	376740-TOC
Weight Boat / Paper	Cole Parmer	01017-05

6. PROCEDURE:

6.1. Start Up:

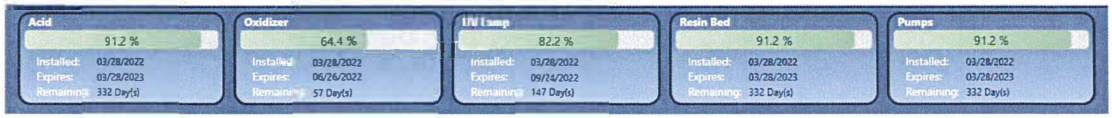
6.1.1. Check the consumables levels prior to performing analyses.

6.1.1.1. In the DataPro 2 software, select the Maintenance screen.

6.1.1.2. Select the Analyzer Panel.

6.1.1.3. Select the Consumables Tab.

6.1.1.4. Check the percentage of useful life remaining for each consumable: Acid, Oxidizer, UV lamp, Pumps, and Resin Bed. Reference the M9 Series Operation and Maintenance Manual if consumables need to be replaced.



6.1.2. DI Water Reservoir

6.1.2.1. Confirm that the DI water reservoir is filled to the proper level by observing the float disk in the DI reservoir. Reference the M9 Series Operation and Maintenance Manual, if needed.

6.2. Running a Protocol (Verification or Sample Analysis)

6.2.1. Check that the TOC and Conductivity Calibrations are current, prior to analyzing samples.

6.2.1.1. In the DataPro 2 software, select the Data Management screen.

6.2.1.2. Ensure that the most recent passing TOC and Conductivity Calibrations are current (annually calibrated).

6.2.2. Perform a syringe flush daily or if the Analyzer has been idle during the last eight hours.

6.2.2.1. In the DataPro 2 software, select the Maintenance Screen.

6.2.2.2. Select the Analyzer Panel.

6.2.2.3. Select the Analyzer Tab.

6.2.2.4. Insert a flush vial (filled with DI water) into position six (6) of the Autosampler Emergency rack.

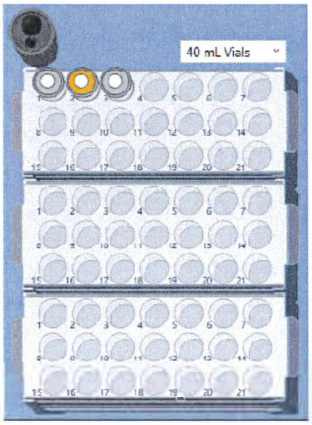
6.2.2.5. Change the number of flushes to three (3) minimum.

6.2.2.6. Click Start.

6.2.3. In the DataPro 2 software, select the Favorites screen.

6.2.4. Select the Protocol to run and click Load. The Home screen appears with the Setup tab active.

6.2.5. Load the sample vials into the Autosampler racks in the positions shown on the Sampling Rack graphic.



6.2.5.1. Samples may be added to the Protocol Table as needed by selecting the desired sample vial location on the Sampling Rack Graphic (the selected location will highlight orange) then selecting the "Apply Method to Vials (Insert)" icon on the Protocol Table window.

6.2.5.2. Samples may be removed from the Protocol Table as needed by selecting the sample row in the Protocol Table (the selected row(s) will highlight blue) then selecting the "Remove Selected Steps (Delete)" icon on the Protocol Table window.

6.2.5.3. Samples may be reordered in the Protocol Table as needed by typing the desired numeric order (starting with one (1)) in the "Step" column in the Protocol Table for each sample.

6.2.6. Type the lot number for each vial in the Lot # column.

6.2.7. Click the Run Protocol button to start the analyses.

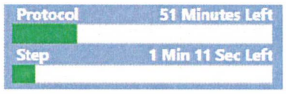
6.2.8. If the Analyzer has been idle during the last eight hours, and a reagent flush has not been performed, a message appears prompting a reagent flush.

6.2.8.1. Click Yes to display the Syringe Flush panel. Enter the number of flushes. (At least 3 flushes).

6.2.8.2. Insert a flush vial (filled with DI water) into position 6 of the Autosampler Emergency rack and click Start. When the flush completes, the Analyzer.

6.2.8.3. automatically begins sampling.

6.2.9. Progress bars indicate the time remaining for the Protocol and the current Step.



7. SOLUTION PREPARATION:

7.1. Control Solutions/ Standard Solutions

7.1.1. Five concentrations will be prepared from a *250 ppm Carbon Analyte stock solution.*

7.1.2. The 0, 0.125, 0.500, 1.000, 2.000 and 10.000 ppm concentrations will be prepared and analyzed in triplicate for assessment of accuracy, precision, linearity, range, and limit of quantitation and detection data.

7.1.2.1. *250 ppm Carbon analyte stock solution*

7.1.2.1.1. For Pure substances, determine the mass percentage of Carbon of the molecule of interest. Weigh (0.250 / % Carbon = g) of material of interest on the Analytical balance, transfer, dissolve, and dilute to 1000 mL with Purified water.

7.1.2.1.2. For substances of unknown carbon concentration; prepare a 100ppm stock and analyze to determine % Carbon content experimentally; use the % Carbon value in the equation above to prepare a 250ppm Carbon solution.

7.1.2.1.3. Experimentally Calculate % Carbon as:

$$\% \text{ Carbon} = [(\text{Result ppm C}) / (100\text{ppm})] * 100$$

7.1.2.2. 10.000 ppm (Concentration Level 5)

7.1.2.2.1. Pipette 1.200mL of the *250ppm Carbon stock solution,* record weight, and dilute to 30g with Purified water in a TOC vial.

7.1.2.3. 2.000 ppm (Concentration Level 4)

7.1.2.3.1. Pipette 0.240mL of the *250ppm Carbon stock solution,* record weight, and dilute to 30g with Purified water in a TOC vial.

7.1.2.4. 1.000 ppm (Concentration Level 3)
7.1.2.4.1. Pipette 0.120mL of the *250ppm Carbon stock solution,* record weight, and dilute to 30g with Purified water in a TOC vial.
7.1.2.5. 0.500 ppm (Concentration Level 2)
7.1.2.5.1. Pipette 0.060mL of the *250ppm Carbon stock solution,* record weight, and dilute to 30g with Purified water in a TOC vial.
7.1.2.6. 0.125 ppm (Concentration Level 1)
7.1.2.6.1. Pipette 0.015mL of the *250ppm Carbon stock solution,* record weight, and dilute to 30g with Purified water in a TOC vial.
7.1.2.7. 0 ppm (Blank)
7.1.2.7.1. Purified water in a TOC vial.

8. PERFORMANCE PARAMETERS:

8.1. **Calibration/System Suitability:**
8.1.1. Verify the calibration is current before use.
8.1.2. System Suitability: Analyze a 5.00ppm Tris Standard Solution and Reagent Water prior to analysis. Calculate Percent Recovery (%) as follows:

$$Percent\ Recovery\ (\%) = \frac{(Tris\ Standard\ (ppm) - Reagent\ Water\ Standard\ (ppm))}{5.00\ ppm} \times 100$$

8.1.3. Acceptance Criteria:
8.1.3.1. TOC and Conductivity Calibrations are current (calibrated annually).
8.1.3.2. Percent Recovery of 80% to 120%.

8.2. **Accuracy:**
8.2.1. Accuracy will be assessed over a minimum of 9 determinations over 3 concentration levels. Accuracy will be reported as the percent recovery. The data will be assessed by calculating the percent recovery for each concentration.

$$Percent\ Recovery\ (\%) = \frac{(Reported\ Value\ (ppm) - Blank\ Value\ (ppm))}{Theoretical\ Value\ (ppm)} \times 100$$

8.2.2. Acceptance criteria:
8.2.2.1. Samples with ≥1ppm Carbon should have a percent recovery of 80% to 120%.
8.2.2.2. Samples with <1ppm Carbon should have a percent recovery of 50% to 150%.

8.3. **Precision (NMT 20% RSD):**
8.3.1. The precision of the analytical procedure is determined by assaying a sufficient number of aliquots of a homogenous sample to be able to calculate statistically valid estimates of standard deviation or relative standard deviation (%RSD).

$$Standard\ Deviation\ (s) = \sqrt{\frac{\sum(X_i - \bar{X})^2}{n-1}}$$

$$\%RSD = \frac{Standard\ Deviation\ (ppm)}{Average\ (ppm)} \times 100$$

8.3.2. Acceptance Criteria:
8.3.2.1. Report Standard Deviation for each level.
8.3.2.2. A Relative Standard Deviation (%RSD) of NMT 20% at each level.

8.4. **Specificity:**

8.4.1. Specificity will be demonstrated by meeting requirements for accuracy and precision.

8.5. **Linearity:**

8.5.1. Linearity will be assessed across five (5) analysis levels. Plot the Average Response (ppm) vs. the Theoretical Spike Level (ppm), perform a linear regression, and report the Coefficient of Determination (r^2), Slope, and Y-Intercept.

8.5.2. Acceptance Criteria:

8.5.2.1. The Coefficient of Determination (r^2) should be NLT 0.99.

8.5.2.2. Report the Slope and Y-Intercept.

8.6. **Range:**

8.6.1. The range of the analytical method will be determined by the highest and lowest concentrations of analyte that produce suitable results for accuracy, precision, and linearity.

8.7. **Limit of Detection (LoD) / Limit of Quantitation (LoQ)**

8.7.1. The standard deviation will be determined for each of the following concentrations, Concentration Levels (ppm) plotted against the response (ppm), a linear regression performed, and the slope reported.

8.7.1.1. 1.000 ppm (Concentration Level 3)

8.7.1.2. 0.500 ppm (Concentration Level 2)

8.7.1.3. 0.125 ppm (Concentration Level 1)

8.7.1.4. 0 ppm (Blank)

8.7.2. The Limit of Detection (LoD) will be expressed as:

$$Limit\ of\ Detection\ (LoD) = \frac{3.3\sigma}{S}$$

8.7.2.1. Where:

8.7.2.1.1. S = Slope of the Calibration Curve.

8.7.2.1.2. σ = Average Std. deviation of the blank response.

8.7.3. The Limit of Quantitation (LoQ) will be expressed as:

$$Limit\ of\ Quantitation\ (LoQ) = \frac{10\sigma}{S}$$

8.7.3.1. Where:

8.7.3.1.1. S = Slope of the Calibration Curve

8.7.3.1.2. σ = Average Std. deviation of the blank response.

9. RECOVERY STUDIES:

9.1. Recovery study previously performed. Refer to BSI-RPT-0915 for more information.

10. VERIFICATION SUMMARY:

TABLE 4: VERIFICATION SUMMARY

Performance Parameters	Acceptance Criteria	Results
Calibration / System Suitability	• TOC calibration is current • Percent Recovery of the 5ppm Tris Standard is 80% to 120%	• TOC Calibration = 2/29/24 • Percent Recovery = 102%
Accuracy	• Samples with ≥1ppm Carbon should have a percent recovery of 80% to 120%. • Samples with <1ppm Carbon should have a percent recovery of 50% to 150%.	**0.000ppm Carbon** • Replicate 1 = 0.0387ppm • Replicate 2 = 0.0374ppm • Replicate 3 = 0.0364ppm **0.125ppm Carbon** • Replicate 1 = 0.136ppm • Replicate 2 = 0.145ppm • Replicate 3 = 0.144ppm **0.500ppm Carbon** • Replicate 1 = 0.482ppm • Replicate 2 = 0.488ppm • Replicate 3 = 0.489ppm **1.000ppm Carbon** • Replicate 1 = 0.934ppm • Replicate 2 = 0.927ppm • Replicate 3 = 0.950ppm **2.000ppm Carbon** • Replicate 1 = 1.83ppm • Replicate 2 = 1.88ppm • Replicate 3 = 1.86ppm **10.000ppm Carbon** • Replicate 1 = 8.99ppm • Replicate 2 = 9.02ppm • Replicate 3 =9.08ppm
Precision	• Report the Standard Deviation for each concentration level. • The %RSD of the carbon concentration values is NMT 20% at each level.	**0.125ppm Carbon** • Standard Deviation = 0.005 • %RSD = 3.08 **0.500ppm Carbon** • Standard Deviation = 0.004 • %RSD = 0.78 **1.000ppm Carbon** • Standard Deviation = 0.012 • %RSD = 1.26 **2.000ppm Carbon** • Standard Deviation = 0.025 • %RSD = 1.36 **10.000ppm Carbon** • Standard Deviation = 0.046 • %RSD = 0.51

Performance Parameters	Acceptance Criteria	Results
Specificity	• All requirements are met for accuracy and precision.	• Accuracy and Precision requirements were met.
Linearity	• Report the slope and the Y-Intercept. • The Coefficient of Determination (r^2) should be NLT 0.99.	• Slope = 1.0078 • Y-Intercept = 0.0366 • Coefficient of Determination (r^2) = 1.0000
Range	• Report the lowest and highest concentrations of analyte that meet requirements for accuracy, precision, and linearity.	• 0.125ppm Carbon – 10.000ppm Carbon • 0.328ppm Trehalose Dihydrate – 26.247ppm Trehalose Dihydrate
Limit of Detection (LoD) / Limit of Quantitation (LoQ)	• Report the Limit of Detection (LoD). • Report the Limit of Quantitation (LoQ).	• Limit of Detection (LoD) = 0.004ppm Carbon (0.010ppm Trehalose Dihydrate) • Limit of Quantitation (LoQ) = 0.013ppm Carbon (0.034ppm Trehalose Dihydrate)

11. VERIFICATION RESULTS:

11.1. **System Suitability:**

11.1.1. System Suitability was assessed by analyzing a 5.00ppm Tris Standard Solution and Reagent Water prior to analysis and calculating the percent recovery (%). All acceptance criteria were met and are summarized in Tables 5 and 6.

11.1.2. Acceptance Criteria:

11.1.2.1. TOC Calibration is current (calibrated annually).

11.1.2.2. Percent Recovery of 80% to 120%.

TABLE 5: CALIBRATIONS

Calibration	Date and Time	Calibration Range
TOC	2/29/24 @ 1221	0-50ppm

TABLE 6: SYSTEM SUITABILITY RESULTS

Tris Standard Solution Concentration (ppm Carbon)	Tris Standard Solution Result (ppm Carbon)	Reagent Water Result (ppm Carbon)	Percent Recovery (80-120%)
4.9475	5.08	0.0190	102

11.2. **Accuracy:**

11.2.1. Accuracy was assessed with fifteen (15) determinations over five (5) concentration levels. The percent recovery was calculated by comparing the Reported Carbon Value (ppm) to the Theoretical Carbon Concentration (ppm). All acceptance criteria were met and are summarized in Table 7.

$$Percent\ Recovery\ (\%) = \frac{(Reported\ Value\ (ppm) - Blank\ Value\ (ppm))}{Theoretical\ Value\ (ppm)} \times 100$$

11.2.2. Acceptance criteria:

11.2.2.1. Samples with ≥1ppm Carbon should have a percent recovery of 80% to 120%.

11.2.2.2. Samples with <1ppm Carbon should have a percent recovery of 50% to 150%.

TABLE 7: ACCURACY RESULTS

Concentration Level (ppm)	Theoretical Value (ppm)	Determination	Result (ppm)	Percent Recovery (%)
0 ppm Carbon	0.000	1	0.0387	
		2	0.0374	
		3	0.0364	
0.125 ppm Carbon	0.105	1	0.136	94
		2	0.145	102
		3	0.144	101
0.500 ppm Carbon	0.445	1	0.482	100
		2	0.488	101
		3	0.489	101
1.000 ppm Carbon	0.899	1	0.934	100
		2	0.927	99
		3	0.950	102
2.000 ppm Carbon	1.800	1	1.83	100
		2	1.88	102
		3	1.86	101
10.000 ppm Carbon	8.924	1	8.99	100
		2	9.02	101
		3	9.08	101

11.3. **Precision:**

11.3.1. Precision was assessed with triplicate determinations over five (5) concentration levels. The standard deviation and %RSD were determined at each concentration level. All acceptance criteria were met and are summarized in Table 8.

$$Standard\ Deviation\ (s) = \sqrt{\frac{\sum(X_i - \bar{X})^2}{n-1}}$$

$$\%RSD = \frac{Standard\ Deviation\ (ppm)}{Average\ (ppm)} \times 100$$

11.3.2. Acceptance Criteria:

11.3.2.1. Report Standard Deviation for each level.

11.3.2.2. A Relative Standard Deviation (%RSD) of NMT 20% at each level.

TABLE 8: PRECISION RESULTS

Concentration Level (ppm)	Determination	Result (ppm)	Standard Deviation (ppm)	%RSD
0.125 ppm Carbon	1	0.136	0.005	3.48
	2	0.145		
	3	0.144		
0.500 ppm Carbon	1	0.482	0.004	0.78
	2	0.488		
	3	0.489		
1.000 ppm Carbon	1	0.934	0.012	1.26
	2	0.927		
	3	0.950		
2.000 ppm Carbon	1	1.83	0.025	1.36
	2	1.88		
	3	1.86		
10.000 ppm Carbon	1	8.99	0.046	0.51
	2	9.02		
	3	9.08		

11.4. **Linearity**:

11.4.1. Linearity was assessed across five (5) analysis levels. The average response (ppm) was plotted against the Theoretical Spike Value (ppm) and analyzed via linear regression. Reported the Coefficient of Determination (r^2), Slope, and Y-Intercept. All acceptance criteria were met and are summarized in Table 9.

11.4.2. Acceptance Criteria:

11.4.2.1. The Coefficient of Determination (r^2) should be NLT 0.99.

11.4.2.2. Report the Slope and Y-Intercept.

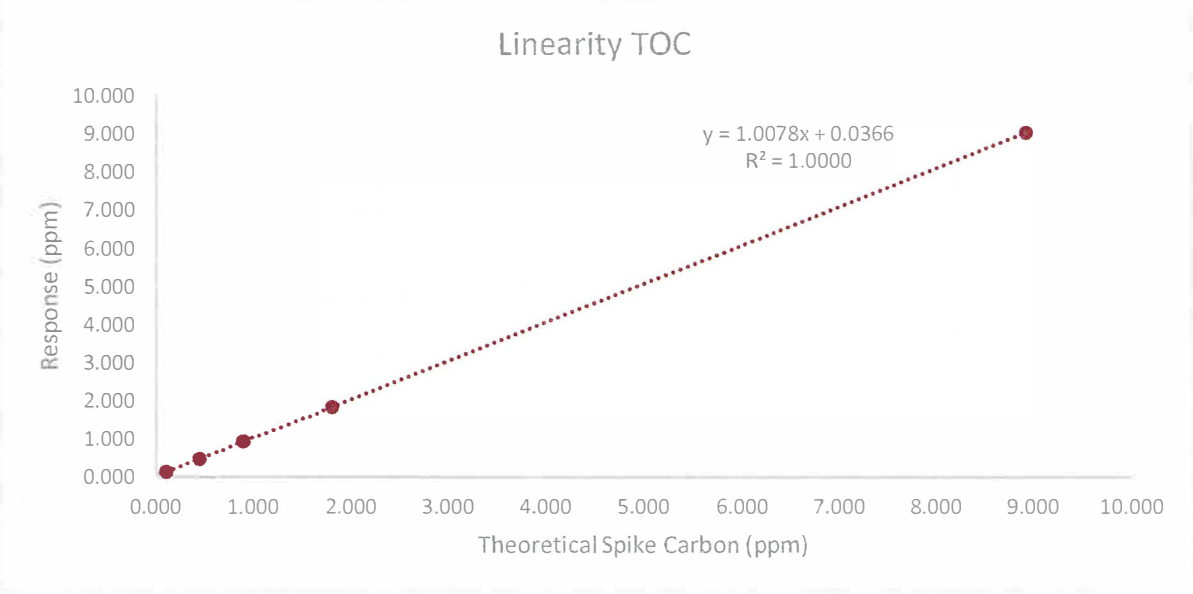


FIGURE 1: TREHALOSE DIHYDRATE CLEANING TOTAL ORGANIC CARBON (TOC) ANALYSIS LINEARITY – AVERAGE RESPONSE (PPM) VS. THEORETICAL SPIKED CARBON (PPM)

TABLE 9: LINEARITY RESULTS							
Concentration Level (ppm)	**Theoretical Spike Level (ppm)**	**Determination**	**Response (ppm)**	**Average Response (ppm)**	**Slope**	**Y-Intercept**	**r^2**
0 ppm Carbon	0.000	1	0.0387	0.0375	1.0078	0.0366	1.0000
		2	0.0374				
		3	0.0364				
0.125 ppm Carbon	0.106	1	0.136	0.142			
		2	0.145				
		3	0.144				
0.500 ppm Carbon	0.445	1	0.482	0.486			
		2	0.488				
		3	0.489				
1.000 ppm Carbon	0.898	1	0.934	0.937			
		2	0.927				
		3	0.950				
2.000 ppm Carbon	1.800	1	1.83	1.86			
		2	1.88				
		3	1.86				
10.000 ppm Carbon	8.924	1	8.99	9.03			
		2	9.02				
		3	9.08				

11.5. **Specificity**:

11.5.1. Specificity was demonstrated by meeting requirements for accuracy and precision. Refer to Sections 11.2 and 11.3 for disposition. All acceptance criteria were met and are summarized in Table 10.

TABLE 10: SPECIFICITY RESULTS	
Acceptance Criteria	**Result**
All requirements were met for Accuracy (refer to Section 11.2)	Pass
All requirements were met for Precision (refer to Section 11.3)	Pass

11.6. **Range:**

11.6.1. The range of the analytical method was determined by the highest and lowest concentrations of analyte that produce suitable results for accuracy, precision, and linearity.

Range of Analysis:

- **Carbon:**
  - **0.125ppm – 10.000ppm**
- **Trehalose Dihydrate:**
  - **0.328ppm – 26.247ppm**

11.7. **Limit of Detection (LoD) / Limit of Quantitation (LoQ):**

11.7.1. The Limit of Detection (LoD) and Limit of Quantitation (LoQ) were assessed across four (4) concentration levels, 0ppm through 1.000ppm. The concentration levels (ppm) were plotted against the average response and analyzed via linear regression. The Limit of Detection (LoD) and Limit of Quantitation (LoQ) were calculated as shown below and reported in Table 11.

11.7.2. The Limit of Detection (LoD) was expressed as:

$$Limit\ of\ Detection\ (LoD) = \frac{3.3\sigma}{S}$$

11.7.2.1. Where:

11.7.2.1.1. S = Slope of the Calibration Curve.

11.7.2.1.2. σ = Average Std. deviation of the blank response.

11.7.3. The Limit of Quantitation (LoQ) was expressed as:

$$Limit\ of\ Quantitation\ (LoQ) = \frac{10\sigma}{S}$$

11.7.3.1. Where:

11.7.3.1.1. S = Slope of the Calibration Curve

11.7.3.1.2. σ = Average Std. deviation of the blank response.

TABLE 11: LIMIT OF DETECTION / LIMIT OF QUANTITATION RESULTS

Concentration Level (ppm)	Determination	Response (ppm)	Standard Deviation (σ)	Slope (S)	Limit of Detection (LoD)	Limit of Quantitation (LoQ)
0 ppm Carbon	1	0.0387	0.0012	0.9014	0.004	0.013
	2	0.0374				
	3	0.0364				
0.125 ppm Carbon	1	0.136				
	2	0.145				
	3	0.144				
0.500 ppm Carbon	1	0.482				
	2	0.488				
	3	0.489				
1.000 ppm Carbon	1	0.934				
	2	0.927				
	3	0.950				

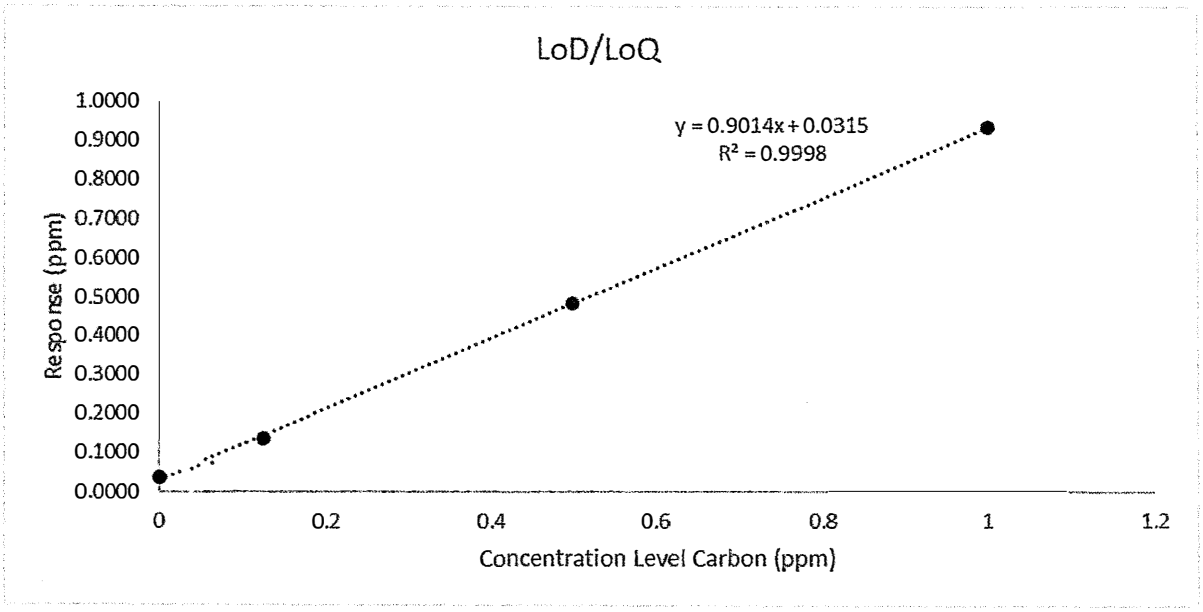


FIGURE 2: TREHALOSE DIHYDRATE CLEANING TOTAL ORGANIC CARBON (TOC) ANALYSIS LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTITATION (LOQ) – AVERAGE RESPONSE (PPM) VS. CONCENTRATION LEVEL CARBON (PPM)

12. CONCLUSION:

12.1. **Performance Summary:**

TABLE 12: PERFORMANCE SUMMARY	
Method Performance Indicator	**Result**
System Suitability	Pass
Accuracy	Pass
Precision	Pass
Specificity	Pass
Linearity	Pass
Range	0.125ppm – 10.000ppm Carbon Equivalent to: 0.328ppm – 26.247ppm Trehalose Dihydrate
Limit of Detection (LoD)	0.004ppm Carbon Equivalent to: 0.010ppm Trehalose Dihydrate
Limit of Quantitation (LoQ)	0.013ppm Carbon Equivalent to: 0.034ppm Trehalose Dihydrate
Recovery Study	Pass[1]

[1]Reference BSI-RPT-0915 for swab recovery data.

12.2. **Statement of Verification:** The method of analysis of Trehalose Dihydrate in rinse and swab samples is considered a verified method of analysis at all BioSpectra facilities with the Sievers M9 Total Organic Carbon (TOC) Analyzer and is approved for use.

12.3. Critical Changes, Discrepancies, or Failures

12.3.1. No critical changes, discrepancies, or failures are notable for this verification.