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ANALYTICAL METHOD VALIDATION REPORT: RESIDUAL SOLVENTS BY HEADSPACE GC-FID: GLYCERIN

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1. PURPOSE:

1.1. The purpose of this Report is to:

1.1.1. Provide performance data demonstrating that the Residual Solvent Analysis by Head-Space GC-FID in Glycerin is adequately evaluated and validated.

1.1.2. Provide proof that the Residual Solvent Analysis by Head-Space GC-FID in Glycerin at the set specification meets all requirements for System Suitability, Accuracy, Precision, Linearity, Specificity, Range, Limit of Quantitation (LoQ), Intermediate Precision, and Solution Stability.

1.1.3. To ensure that the proper reagents and testing materials were used and the correct documentation was provided for evaluation.

2. SCOPE:

2.1. This Analytical Method Validation Report applies to Residual Solvents Analysis by Head-Space GC-FID in Glycerin.

2.2. The Residual Solvents Analysis by Head-Space GC-FID in Glycerin was validated as a Category II Quantitative Analysis.

2.3. Residual Solvents in Glycerin Specifications:

Table 1: Residual Solvents in Glycerin Specifications

Analyte	Specification
Methanol	3000ppm

3. RESPONSIBILITIES:

3.1. The Senior Product Life Cycle Manager or designee, is responsible for the control, implementation and maintenance of this report.

3.2. The Laboratory Analysts, and/or qualified designees, were responsible for performing the testing stated in the protocol and for performing the Validation.

3.3. The Laboratory Analysts, and/or qualified designees, performing the analysis, with help from the Senior Product Life Cycle Manager, if necessary, were responsible for completing the Analytical Method Validation Report using conclusions made from the results obtained from testing.

4. REFERENCES:

4.1. BSI-PRL-0348, Analytical Method Validation Protocol: Residuals Solvents by Head Space GC-FID

4.2. BSI-SOP-0098, Balance SOP

4.3. BSI-SOP-0126, Laboratory Notebooks

4.4. BSI-SOP-0134, Pipette SOP

4.5. BSI-SOP-0316, Shimadzu QP2010S GC SOP

4.6. BSI-SOP-0436, Analytical Methods Validation Master Plan

4.7. ICH Q3A

4.8. USP NF <621> Chromatography

4.9. USP NF <467> Residual Solvents

4.10. USP NF <1467> Residual Solvents – Verification of Compendial Procedures and Validation of Alternative Procedures

5. PRE-VALIDATION REQUIREMENTS:

5.1. Equipment

5.1.1. All equipment used in this Validation was in proper working order and with current calibrations if applicable. This is included in the Materials and Equipment portion of this Analytical Method Validation Report.

5.2. Personnel
5.2.1. All personnel executing this Validation were properly trained on the analysis technique.
5.3. Supplies:
5.3.1. Any supplies used in the Validation were clean and appropriate for the intended use. A list of supplies used is included in the Materials and Equipment section of the report and identified with the supplier and description.
5.4. Reagents:
5.4.1. All reagents were current, met required specifications, and were suitable for their intended use. A list of reagents used is included in the Analytical Method Validation Report. This includes: reagent name, lot number, manufacturer, date of opening, part number, and expiration date (if applicable).

5.5. Reference Standards:
5.5.1. Any standards used in this Validation are listed in the Materials and Equipment section of the Analytical Method Validation Report. The name of the reference standard, lot number, date of manufacture, date of opening, date of expiration, and part number must be provided in this document and recorded during Validation testing.

6. MATERIALS AND EQUIPMENT:

6.1. All materials and equipment utilized in this Validation are outlined in this section.
6.2. Equipment and Instrumentation
6.2.1. Analytical Balance
6.2.2. Micropipettes
6.2.3. GC-FID
6.2.3.1. Make: Shimadzu
6.2.3.2. Model: GC-2010 with Head-Space Autosampler
6.2.4. GC Column
6.2.4.1. Make: Phenomenex
6.2.4.2. Model: Zebron ZB-624 30m x 0.25mm x 1.40µm
6.2.4.3. Part Number: 7HG-G005-27

Table 2: Equipment and Instrumentation

Equipment	Part Number / Model	Manufacturer	Serial Number	Calibration Due Date
Analytical Balance	Secura 124-1S	Sartorius	29212172	4/30/25
1000µL - 10000µL Micropipette	Research Plus	Eppendorf	G54479H	3/20/25
500µL - 5000µL Micropipette	Research Plus	Eppendorf	H33986M	8/31/25
100µL - 1000µL Micropipette	Research Plus	Eppendorf	Q28940G	8/31/25
2µL - 20µL Micropipette	Research Plus	Eppendorf	R12216C	6/30/25
GC-FID	GC-2010, GCMS-QP2010S	Shimadzu	O20385050364	9/25
Head-Space Autosampler	HS-20	Shimadzu	O20715200319	9/25
GC Column: Zebron ZB-624 30m x 0.25mm x 1.40µL	7HG-G005-27	Phenomenex	1051537	Not Applicable

6.3. Reagents and Reference Standards:

6.3.1. **Glycerin:** Purchased Commercially.

6.3.2. **Methanol Certified Reference Standard:** Purchased Commercially.

6.3.3. **Purified Water:** In-House or Purchased Commercially.

Table 3: Reagents and Reference Standards

Reagent	Part Number	Lot Number	Manufacturer	CAS Number	Expiration Date	Date Opened
Glycerin	G2289-1L	SHBR2594	Sigma Aldrich	56-81-5	10/31/27	3/12/25
Purified Water	IQ 7005	F9SA14284H	Millipore Sigma	7732-18-5	Not Applicable	Not Applicable
Methanol Certified Reference Standard (Purity: 99.7%)	PHR1372-3x1.5mL	LRAD8537	Sigma Aldrich	67-56-1	8/31/28	3/12/25
						3/17/25

6.4. Supplies:

6.4.1. 20mL Headspace Vials and Caps

6.4.2. Beakers

6.4.3. Class A Volumetric Flasks

6.4.4. Metal Encapsulated Vespel Graphite Ferrule

6.4.5. Micropipette Tips

6.4.6. Transfer Pipettes

6.4.7. Vespel Graphite Ferrule

Table 4: Supplies

Supply	Manufacturer	Part Number
20mL Headspace Vials	Phenomenex	AR0-3270-13
20mL Headspace Vial Caps	Phenomenex	AR0-5250-13
150mL Beaker	Pyrex	1003
Volumetric Flasks, Class A	Pyrex	5640
Volumetric Flasks, Class A	VWR	Not Applicable
Transfer Pipettes	Samco Scientific	Not Applicable

7. METHOD PARAMETERS:

7.1. HS-20

7.1.1. Oven Temp: 80.0°C
7.1.2. Sample Line Temp.: 150.0°C
7.1.3. Transfer Line Temp: 155.0°C
7.1.4. Shaking Level: 1
7.1.5. Injection Count: 1
7.1.6. Pressurizing Gas: 176.2 kPa
7.1.7. Equilibrating Time: 15.00 minutes
7.1.8. Pressurization Time: 0.50 minutes
7.1.9. Pressure Equilibration Time: 0.50 minutes
7.1.10. Load Time: 1.00 minute
7.1.11. Load Equilibration Time: 0.50 minutes
7.1.12. Injection Time: 1.00 minute
7.1.13. Needle Flush Time: 1.00 minute
7.1.14. GC Cycle Time: 7.00 minutes
7.1.15. Check System Ready: Off
7.1.16. Extended System Ready Check: Off
7.1.17. Check GC Ready: Off
7.1.18. Extended GC Ready Check: Off
7.1.19. Needle Check: Yes
7.1.20. Action on Leak Check Error: Stop
7.1.21. Action with No Vial in Tray: Stop

7.2. GC-2010

7.2.1. Column Oven Temperature: 80.0°C
7.2.2. Injection Mode: Split
7.2.3. Flow Control Mode: Linear Velocity
7.2.4. Pressure: 175.2 kPa
7.2.5. Total Flow: 50.7 mL/minute
7.2.6. Column Flow: 2.32 mL/minute
7.2.7. Linear Velocity: 47.6 cm/second
7.2.8. Purge Flow: 2.0 mL/minute
7.2.9. Split Ratio: 20
7.2.10. High Pressure Injection: OFF
7.2.11. Carrier Gas Saver: OFF
7.2.12. Splitter Hold: OFF
7.2.13. Oven Temp Program

Table 5: Oven Temperature Program

Rate (°C/Minute)	Temperature (°C)	Hold Time (Minutes)
--	80.0	6.00

7.3. Ready Checks

7.3.1. Column Oven: YES
7.3.2. HS: NO
7.3.3. FID: YES
7.3.4. HS Carrier: YES
7.3.5. HS Purge: YES
7.3.6. APC1: YES
7.3.7. FID Makeup: YES

7.3.8. FID1 H2: YES
7.3.9. FID1 Air: YES
7.3.10. External Wait: NO
7.3.11. Auto Flame On: Yes
7.3.12. Auto flame Off: Yes
7.3.13. Reignite: Yes
7.3.14. Auto Zero After Ready: Yes
7.3.15. Equilibrium Time: 3.0 minutes
7.3.16. CRG(INJ): OFF
7.3.17. APC1: 75.0kPa

8. SAMPLE PREPARATION:

8.1. Pre-Requisite Solutions:

8.1.1. 10000ppm Methanol Stock Solution:
8.1.1.1. Prepared a 10000mg/L (ppm) solution of methanol in purified water by weighing 1.0g of standard directly into a 100mL volumetric flask, dissolving in Purified Water, filling to volume with Purified Water, and mixing thoroughly.
8.1.1.2. Calculate actual concentration based off Certificate of Analysis (CoA) purity.

8.2. Calibration Standards and Spike Diluent Preparation:

8.2.1. **NOTE:** Addition of solutions or reagents to head space vial may be done in any order.
8.2.2. Blank (0% Level):
8.2.2.1. Purified water or equivalent.
8.2.2.2. Pipette 10mL of standard into a 20mL headspace vial, crimp to seal, and mix thoroughly.
8.2.3. Calibration Level 1 (50% Level):
8.2.3.1. In a 100.0mL volumetric flask, add 1.50mL of *10000ppm Methanol Stock Solution*, dissolve in Purified Water, fill to volume with Purified Water, and mix well.
8.2.3.2. Pipette 10mL of standard into a 20mL headspace vial, crimp to seal, and mix thoroughly.
8.2.4. Calibration Level 2 (80% Level):
8.2.4.1. In a 100.0mL volumetric flask, add 2.40mL of *10000ppm Methanol Stock Solution*, dissolve in Purified Water, fill to volume with Purified Water, and mix well.
8.2.4.2. Pipette 10mL of standard into a 20mL headspace vial, crimp to seal, and mix thoroughly.
8.2.5. Calibration Level 3 (100% Level):
8.2.5.1. In a 100.0mL volumetric flask, add 3.00mL of *10000ppm Methanol Stock Solution*, dissolve in Purified Water, fill to volume with Purified Water, and mix well.
8.2.5.2. Pipette 10mL of standard into a 20mL headspace vial, crimp to seal, and mix thoroughly.
8.2.5.3. Prepare in duplicate, once for Calibration Curve and once for Solution Stability at the end of the run sequence.
8.2.6. Calibration Level 4 (120% Level):
8.2.6.1. In a 100.0mL volumetric flask, add 3.60mL of *10000ppm Methanol Stock Solution*, dissolve in Purified Water, fill to volume with Purified Water, and mix well.
8.2.6.2. Pipette 10mL of standard into a 20mL headspace vial, crimp to seal, and mix thoroughly.

8.2.7. Calibration Level 5 (150% Level):
- 8.2.7.1. In a 100.0mL volumetric flask, add 4.50mL of *10000ppm Methanol Stock Solution*, dissolve in Purified Water, fill to volume with Purified Water, and mix well.
- 8.2.7.2. Pipette 10mL of standard into a 20mL headspace vial, crimp to seal, and mix thoroughly.

8.2.8. Linearity (300% Level):
- 8.2.8.1. In a 100.0mL volumetric flask, add 9.00mL of *10000ppm Methanol Stock Solution*, dissolve in Purified Water, fill to volume with Purified Water, and mix well.

8.3. **Specificity Solutions:**

8.3.1. Specificity Solution 1- Blank:
- 8.3.1.1. Pipette 10mL of purified water into a 20mL headspace vial.
- 8.3.1.2. Crimped to seal, mixed thoroughly.

8.3.2. Specificity Solution 2- Methanol:
- 8.3.2.1. Pipette 10mL of purified water into a 20mL headspace vial.
- 8.3.2.2. Add 0.1mL of *10000ppm Methanol Stock Solution* to the headspace vial.
- 8.3.2.3. Crimp to seal, mix thoroughly.

8.3.3. Specificity Solution 3- Sample Screen:
- 8.3.3.1. Weigh and add 1.0g of sample to headspace vial.
- 8.3.3.2. Add 10mL of Purified Water to headspace vial.
- 8.3.3.3. Dissolve.
- 8.3.3.4. Crimped to seal, mix thoroughly.

8.4. **Accuracy, Precision, and Linearity Solution Preparation:**

8.4.1. 50% Level Residual Solvent Spike:
- 8.4.1.1. Weigh 1.0g of sample and add to a headspace vial.
- 8.4.1.2. Add 10mL of *Calibration Level 1 (50% Level)* solution to the headspace vial.
- 8.4.1.3. Dissolve
- 8.4.1.4. Crimp to seal, mix thoroughly.
- **8.4.1.5. Prepare in triplicate.**

8.4.2. 80% Level Residual Solvent Spike:
- 8.4.2.1. Weigh 1.0g of sample and add to a headspace vial.
- 8.4.2.2. Add 10mL of *Calibration Level 2 (80% Level)* solution to the headspace vial.
- 8.4.2.3. Dissolve.
- 8.4.2.4. Crimp to seal, mix thoroughly.
- **8.4.2.5. Prepare in triplicate.**

8.4.3. 100% Level Residual Solvent Spike:
- 8.4.3.1. Weigh 1.0g of sample and add to a headspace vial.
- 8.4.3.2. Add 10mL of *Calibration Level 3 (100% Level)* solution to the headspace vial.
- 8.4.3.3. Dissolve.
- 8.4.3.4. Crimp to seal, mix thoroughly.
- **8.4.3.5. Prepare six (6) replicates.**

8.4.4. 120% Level Residual Solvent Spike:
- 8.4.4.1. Weigh 1.0g of sample and add to a headspace vial.
- 8.4.4.2. Add 10mL of *Calibration Level 4 (120% Level)* solution to the headspace vial.
- 8.4.4.3. Dissolve.
- 8.4.4.4. Crimp to seal, mix thoroughly.
- **8.4.4.5. Prepare in triplicate.**

8.4.5. 150% Level Residual Solvent Spike:
- 8.4.5.1. Weigh 1.0g of sample and add to a headspace vial.
- 8.4.5.2. Add 10mL of the *Calibration Level 5 (150% Level)* solution to the headspace vial.
- 8.4.5.3. Dissolve.
- 8.4.5.4. Crimp to seal, mix thoroughly.
- **8.4.5.5. Single Preparation.**

8.4.6. 300% Level Residual Solvent Spike:
- 8.4.6.1. Weigh 1.0g of sample and add to a headspace vial.
- 8.4.6.2. Add 10mL of *Linearity (300% Level)* solution to the headspace vial.
- 8.4.6.3. Dissolve.
- 8.4.6.4. Crimp to seal, mix thoroughly.
- **8.4.6.5. Single Preparation.**

9. PERFOMANCE PARAMETERS:

9.1. **System Suitability:**

9.1.1. System Suitability was assessed by calibrating the GC-FID using the *Blank (0% Level)* and Calibration Levels 1 through 5 and determining the Calibration Coefficient (r^2).

9.1.2. Acceptance Criteria:
- 9.1.2.1. Calibration Coefficient (r^2): NLT 0.95.

9.2. **Accuracy:**

9.2.1. Accuracy was assessed across five (5) concentration levels.

9.2.2. Accuracy was assessed by comparing reported residual solvent result (ppm) with the prepared spike concentration (ppm) and calculating the Percent Recovery (%).

$$Percent\ Recovery\ (\%) = \frac{Residual\ Solvent\ Result\ (ppm)}{Residual\ Solvent\ Spike\ (ppm)} \times 100$$

9.2.3. Acceptance Criteria:
- 9.2.3.1. Percent Recovery (%): 80% - 120%.

9.3. **Precision:**

9.3.1. Precision was assessed over six (6) determinations at the 100% Concentration level.

9.3.2. Precision was assessed by calculating the Standard Deviation, Relative Standard Deviation (%RSD), and 95% Confidence Interval at each analysis level.

9.3.3. Acceptance Criteria:
- 9.3.3.1. Standard Deviation: Report
- 9.3.3.2. Relative Standard Deviation (%RSD): NMT 20%
- 9.3.3.3. 95% Confidence Interval: Report

9.4. **Linearity:**

9.4.1. Linearity was assessed across six (6) concentration levels.

9.4.2. Linearity was assessed by plotting and reporting the Slope, Y-Intercept, and Correlation Coefficient (r^2) of the Methanol Spike (ppm) versus the Average Methanol Area Count linear regression line.

9.4.3. Acceptance Criteria:
- 9.4.3.1. Slope: Report
- 9.4.3.2. Y-Intercept: Report
- 9.4.3.3. Correlation Coefficient (r^2): NLT 0.90

9.5. **Specificity:**

9.5.1. Specificity was assessed by obtaining chromatograms of *Specificity Solution 1 – Blank*, *Specificity Solution 2 – Methanol*, and *Specificity Solution 3 – Sample Screen* to demonstrate that the peaks of interest are resolved from one another and there is no interference between peaks of interest. Approximate retention times of peaks of interest were determined.

9.5.2. Acceptance Criteria:

9.5.2.1. Peaks of interest are visually resolved from one another and there is no interference between peak of interest.

9.5.2.2. Resolution for peaks of interest: NLT 1.5.

9.5.2.3. Report approximate retention times of peaks of interest.

9.6. **Range:**

9.6.1. Range was assessed by showing an acceptable degree of Accuracy.

9.6.2. Acceptance Criteria:

9.6.2.1. A minimum range of 80% to 120% of the specification.

9.7. **Limit of Quantitation (LoQ):**

9.7.1. Limit of Quantitation (LoQ) was assessed by reporting the Signal-to-Noise ratio for the peaks of interest in the standard and spiked sample solution from at least three determinations.

9.7.2. Acceptance Criteria:

9.7.2.1. Signal-to-Noise Ratio: NLT 10.

9.7.2.2. Limit of Quantitation (LoQ): Not more than the specification.

9.8. **Intermediate Precision:**

9.8.1. Intermediate Precision was assessed by having a second analyst on a separate day perform System Suitability and an additional six (6) determinations of the *100% Level Residual Solvent Spike*. The Standard Deviation and Relative Standard Deviation (%RSD) will be calculated for individual and combined (Analyst I and II) results.

9.8.2. Acceptance Criteria:

9.8.2.1. Standard Deviation: Report

9.8.2.2. Relative Standard Deviation (%RSD): NMT 20%.

9.9. **Solution Stability:**

9.9.1. Solution Stability was assessed by analyzing a separately prepared *Calibration Level 3 (100% Level)* solution, analyzing it at the end of the sequence, and calculating the Percent Recovery (%) and Percent Variation in area counts.

9.9.2. Acceptance Criteria:

9.9.2.1. Percent Recovery (%): 80% to 120%

9.9.2.2. Percent Variation (%) in Area Counts: 80% to 120%

10. VALIDATION SUMMARY:

Table 6: Validation Summary

Performance Parameters	Acceptance Criteria	Results
System Suitability	• The Calibration Coefficient (r^2) must be NLT 0.95.	**Analyst I** • Calibration Coefficient (r^2): 0.9984 **Analyst II** • Calibration Coefficient (r^2): 0.9999
Accuracy	• Percent Recovery (%): 80% to 120%.	**50% Level** • Replicate 1: 106% • Replicate 2: 104% • Replicate 3: 102% **80% Level** • Replicate 1: 96% • Replicate 2: 100% • Replicate 3: 93% **100% Level** • Replicate 1: 95% • Replicate 2: 93% • Replicate 3: 97% • Replicate 4: 90% • Replicate 5: 94% • Replicate 6: 93% **120% Level** • Replicate 1: 97% • Replicate 2: 96% • Replicate 3: 95% **150% Level** • Replicate 1: 98%
Precision	• Standard Deviation: Report • Relative Standard Deviation (%RSD): NMT 20% • 95% Confidence Interval: Report	**100% Level** • Standard Deviation: 73.221ppm • %RSD: 3% • 95% Confidence Interval: 58.588ppm
Linearity	• Slope: Report • Y-Intercept: Report • Correlation Coefficient (r^2): NLT 0.90.	• Slope: 42.33 • Y-Intercept: 6 • Correlation Coefficient (r^2): 0.9989

Performance Parameters	Acceptance Criteria	Results
Specificity	• Peaks of interest are visually resolved from one another and there is no interference between peaks of interest. • Resolution for peaks of interest: NLT 1.5. • Retention Times: Report.	• Peaks of interest are visually resolved from one another and there is no interference between peaks of interest. **Resolution** • Not Applicable, Methanol is the only peak present. **Retention Times** • Methanol: 1.33 minutes
Range	• A minimum range of 80% to 120% of the specification.	• Range was established from 1500ppm to 4500ppm Methanol.
Limit of Quantitation (LoQ)	• Signal-to-Noise Ratio: NLT 10 • Limit of Quantitation (LoQ): NMT the specification.	**Signal-to-Noise Ratio** **50% Level** • Replicate 1: 1229 • Replicate 2: 1080 • Replicate 3: 1146 **Limit of Quantitation (LoQ)** • LoQ = 1515ppm Methanol
Intermediate Precision	• Standard Deviation: Report • %RSD: NMT 20%	**Individual (Analyst II)** • Standard Deviation: 26.622ppm • %RSD: 1% **Combined (Analyst I and II)** • Standard Deviation: 72.555ppm • %RSD: 3%
Solution Stability	• Percent Recovery (%): 80% to 120% • Percent Variation in Area Counts: 80% to 120%	**Analyst I** • Percent Recovery: 109% • Percent Variation: 111% **Analyst II** • Percent Recovery: 102% • Percent Variation: 103%

11. VALIDATION RESULTS:

11.1. System Suitability:

11.1.1. System Suitability was assessed by calibrating the GC-FID using the *Blank (0% Level)* and Calibration Levels 1 through 5 and determining the Calibration Coefficient (r^2).

11.1.2. Acceptance Criteria:

11.1.2.1. Calibration Coefficient (r^2): NLT 0.95.

11.1.3. Results:

11.1.3.1. All acceptance criteria were met for System Suitability on each day of analysis. Results are summarized in the "Analyst I and II System Suitability Results" Tables and Figures 1 and 2.

Table 7: Analyst I System Suitability Results

Calibration Level (%)	Methanol Spike (ppm)	Methanol Area Count	Slope	Y-Intercept	Calibration Coefficient (r^2)
0	0	0	476.370	-11156.4	0.9983818
50	151.49	59165			
80	242.39	108077			
100	302.99	132211			
120	363.59	161019			
150	454.49	205422			

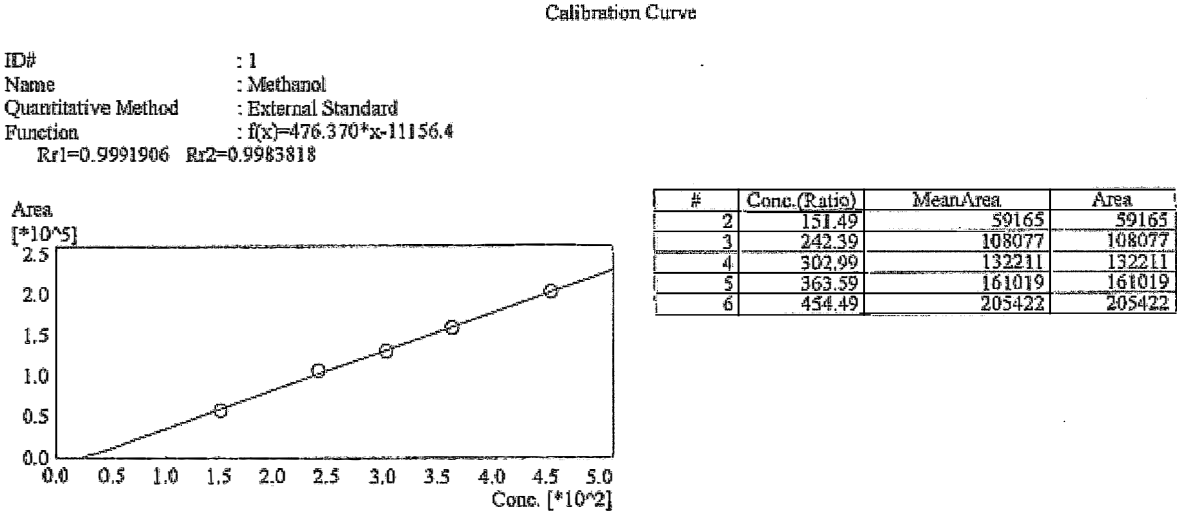

Figure 1: Analyst I Calibration Curve

Table 8: Analyst II System Suitability Results					
Calibration Level (%)	**Methanol Spike (ppm)**	**Methanol Area Count**	**Slope**	**Y-Intercept**	**Calibration Coefficient (r^2)**
0	0	0	28.9546	-2072.45	0.9999268
50	1543.66	42615			
80	2469.85	69797			
100	3087.31	86937			
120	3704.77	105035			
150	4630.97	132214			

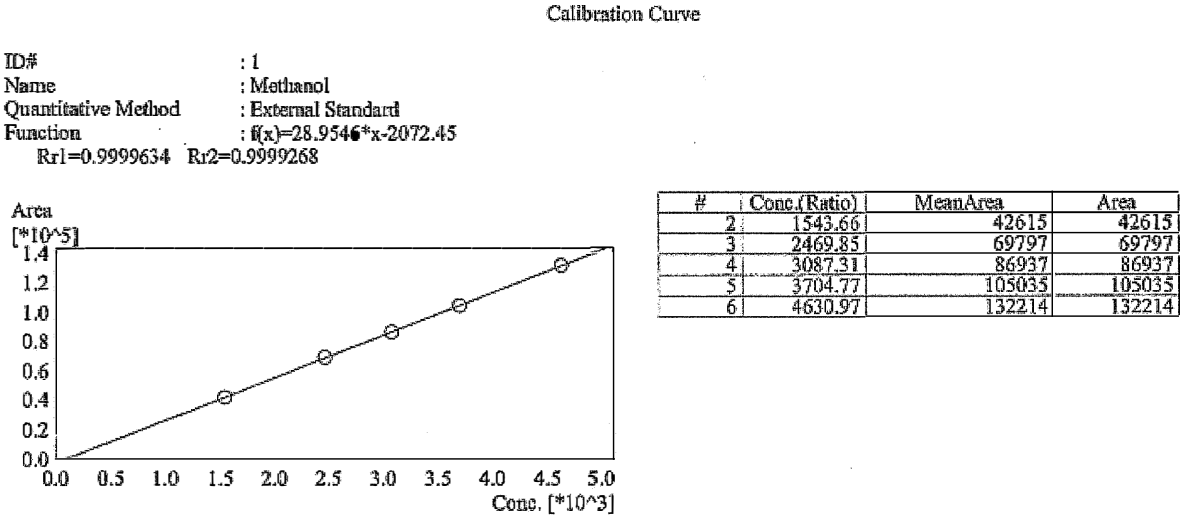

Figure 2: Analyst II Calibration Curve

11.2. **Accuracy:**

11.2.1. Accuracy was assessed across five (5) concentration levels.

11.2.2. Accuracy was assessed by comparing reported residual solvent result (ppm) with the prepared spike concentration (ppm) and calculating the Percent Recovery (%).

$$Percent\ Recovery\ (\%) = \frac{Residual\ Solvent\ Result\ (ppm)}{Residual\ Solvent\ Spike\ (ppm)} \times 100$$

11.2.3. Acceptance Criteria:

11.2.3.1. Percent Recovery (%): 80% - 120%.

11.2.4. Results:

11.2.4.1. All acceptance criteria were met for Accuracy. Results are summarized in the "Accuracy Results" table.

Table 9: Accuracy Results

Concentration Level (%)	Methanol Spike (ppm)	Replicate	Methanol Result (ppm)	Percent Recovery (%)
50	1514.94	1	1600.480	106
		2	1570.503	104
		3	1543.475	102
80	2423.91	1	2335.339	96
		2	2422.100	100
		3	2257.049	93
100	3029.88	1	2866.499	95
		2	2821.060	93
		3	2951.854	97
		4	2727.440	90
		5	2850.707	94
		6	2818.734	93
120	3635.86	1	3516.387	97
		2	3498.351	96
		3	3471.476	95
150	4544.82	1	4441.462	98

11.3. **Precision:**

11.3.1. Precision was assessed over six (6) determinations at the 100% Concentration level.

11.3.2. Precision was assessed by calculating the Standard Deviation, Relative Standard Deviation (%RSD), and 95% Confidence Interval at each analysis level.

11.3.3. Acceptance Criteria:

11.3.3.1. Standard Deviation: Report

11.3.3.2. Relative Standard Deviation (%RSD): NMT 20%

11.3.3.3. 95% Confidence Interval: Report

11.3.4. Results:

11.3.4.1. All acceptance criteria were met for Precision. Results are summarized in the "Precision Results" table.

Table 10: Precision Results

Concentration Level (%)	Methanol Spike (ppm)	Replicate	Methanol Result (ppm)	Standard Deviation (ppm)	%RSD	95% Confidence Interval (ppm)
100	3029.88	1	2866.499	73.221	3	58.588
		2	2821.060			
		3	2951.854			
		4	2727.440			
		5	2850.707			
		6	2818.734			

11.4. **Linearity:**

11.4.1. Linearity was assessed across six (6) concentration levels.

11.4.2. Linearity was assessed by plotting and reporting the Slope, Y-Intercept, and Correlation Coefficient (r^2) of the Methanol Spike (ppm) versus the Average Methanol Area Count linear regression line.

11.4.3. Acceptance Criteria:

11.4.3.1. Slope: Report

11.4.3.2. Y-Intercept: Report

11.4.3.3. Correlation Coefficient (r^2): NLT 0.90

11.4.4. Results:

11.4.4.1. All acceptance criteria were met for Linearity. Results are summarized in the "Linearity Results" table and Figure 3.

Table 11: Linearity Results

Concentration Level (%)	Methanol Spike (ppm)	Average Methanol Area Count	Slope	Y-Intercept	Correlation Coefficient (r^2)
0	0	0	42.33	6	0.9989
50	1514.94	63705			
80	2423.91	100227			
100	3029.88	124103			
120	3635.86	155354			
150	4544.82	200421			
300	9089.65	382270			

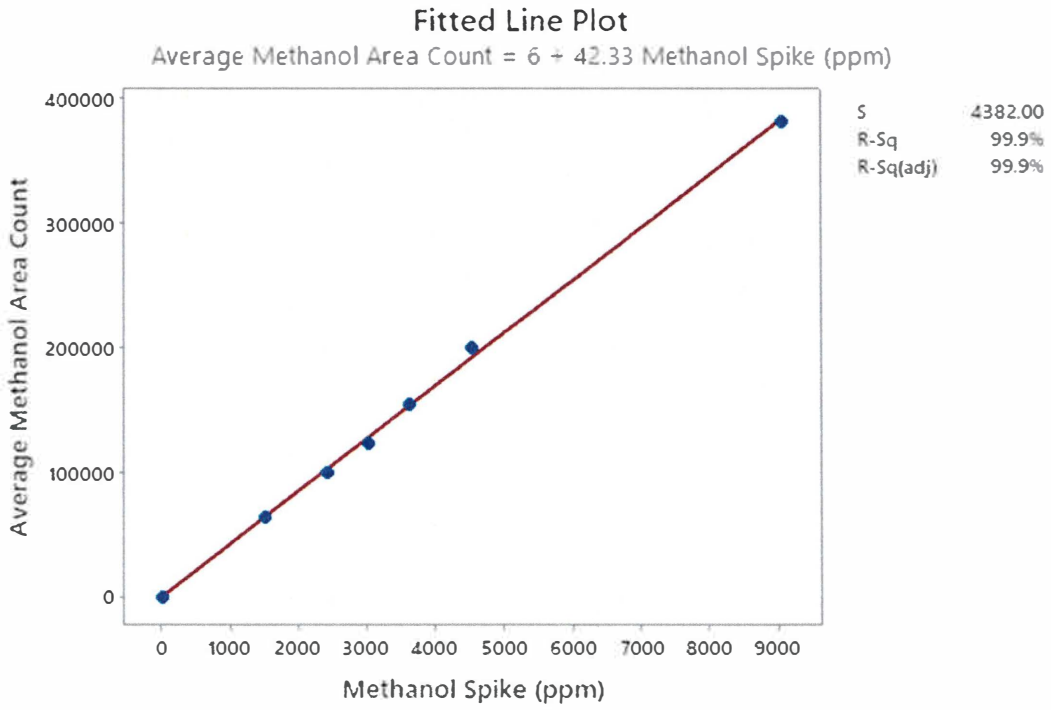

Figure 3: Glycerin Residual Solvents – Methanol Linearity plot of Methanol Spike (ppm) versus Average Methanol Area Count.

11.5. **Specificity:**

11.5.1. Specificity was assessed by obtaining chromatograms of *Specificity Solution 1 – Blank*, *Specificity Solution 2 – Methanol*, and *Specificity Solution 3 – Sample Screen* to demonstrate that the peaks of interest are resolved from one another and there is no interference between peaks of interest. Approximate retention times of peaks of interest were determined.

11.5.2. Acceptance Criteria:

11.5.2.1. Peaks of interest are visually resolved from one another and there is no interference between peak of interest.

11.5.2.2. Resolution for peaks of interest: NLT 1.5.

11.5.2.3. Report approximate retention times of peaks of interest.

11.5.3. Results:

11.5.3.1. All acceptance criteria were met for Specificity. Resolution values are listed as "Not Applicable" as the only peak present in any of the Specificity Chromatograms was Methanol. Results are summarized in the "Specificity Results" table and Figures 4 through 6.

Table 12: Specificity Results

Solution ID	Analyte	Retention Time (minutes)	Resolution
Specificity Solution 1 – Blank	Methanol	Not Present	Not Applicable
Specificity Solution 2 – Methanol	Methanol	1.330	Not Applicable
Specificity Solution 3 – Sample Screen	Methanol	Not Present	Not Applicable

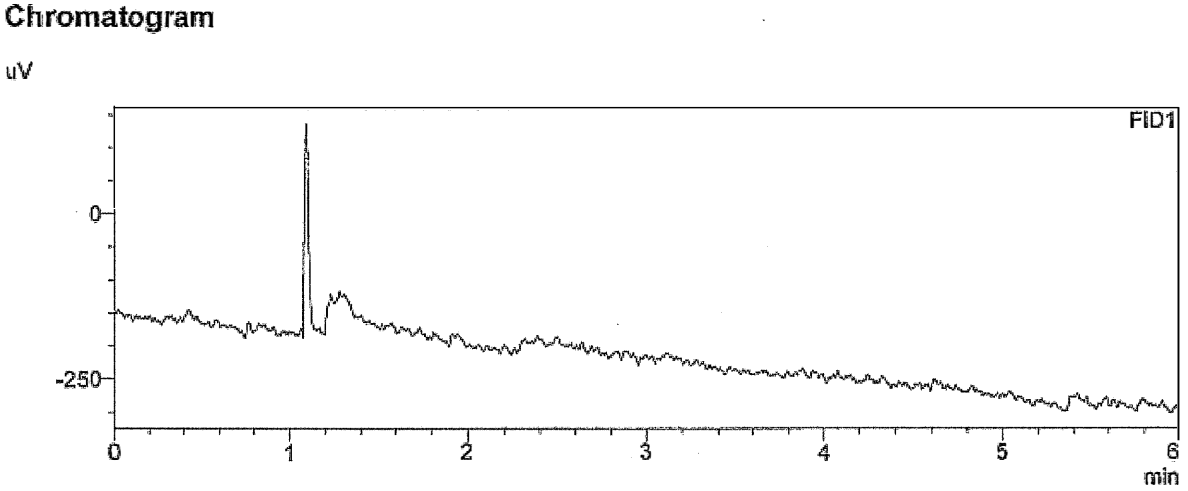

Figure 4: Specificity Solution 1 – Blank Chromatogram

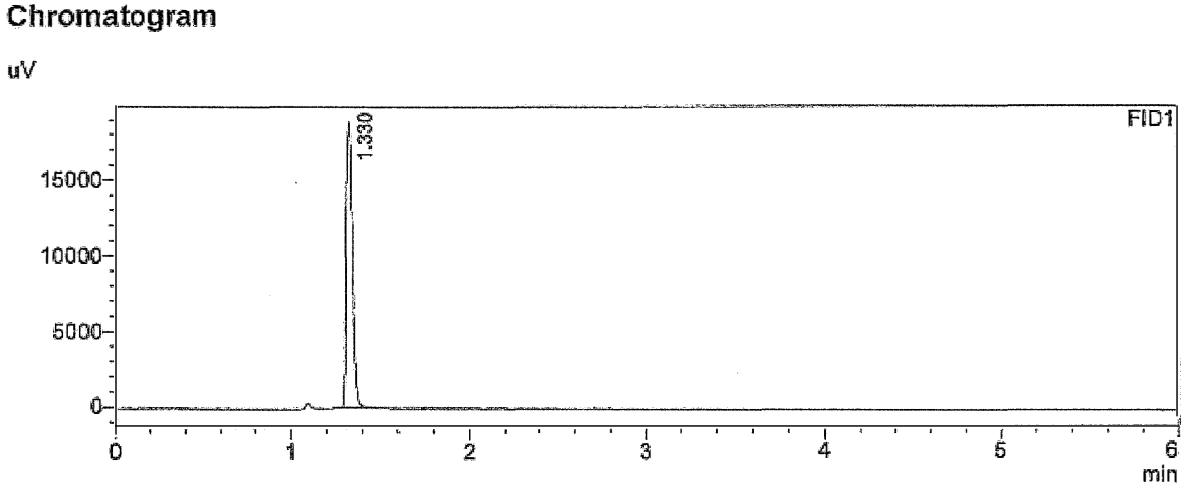

Figure 5: Specificity Solution 2 – Methanol Chromatogram

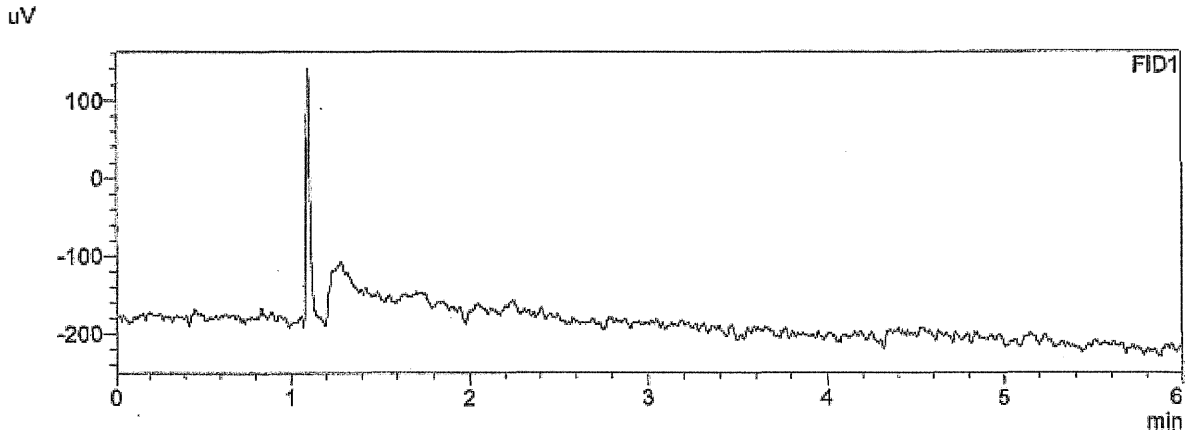

Figure 6: Specificity Solution 3 – Sample Screen

11.6. **Range:**

11.6.1. Range was assessed by showing an acceptable degree of Accuracy.

11.6.2. Acceptance Criteria:

11.6.2.1. A minimum range of 80% to 120% of the specification.

11.6.3. Results:

11.6.3.1. All acceptance criteria were met for Range. The range was determined to be from 50% to 150% of the specification.

Range of Analysis: 1500ppm Methanol – 4500ppm Methanol

11.7. **Limit of Quantitation (LoQ):**

11.7.1. Limit of Quantitation (LoQ) was assessed by reporting the Signal-to-Noise ratio for the peaks of interest in the standard and spiked sample solution from at least three determinations.

11.7.2. Acceptance Criteria:

11.7.2.1. Signal-to-Noise Ratio: NLT 10.

11.7.2.2. Limit of Quantitation (LoQ): Not more than the specification.

11.7.3. Results:

11.7.3.1. All acceptance criteria were met for Limit of Quantitation (LoQ). The Limit of Quantitation (LoQ) was determined to be 1515ppm Methanol. Results are summarized in the "Limit of Quantitation (LoQ) Results" table.

Table 13: Limit of Quantitation (LoQ) Results – Calibration Standards

Concentration Level (%)	Methanol Spike (ppm)	Analyte	Replicate	Signal-to-Noise
0	0	Methanol	1	Not Applicable
50	151.49		1	1019.00
80	242.39		1	2080.72
100	302.99		1	2504.53
120	363.59		1	3077.74
150	454.49		1	4250.22

Table 14: Limit of Quantitation (LoQ) Results – Spiked Samples

Concentration Level (%)	Methanol Spike (ppm)	Analyte	Replicate	Signal-to-Noise
50	1514.94	Methanol	1	1228.57
			2	1080.30
			3	1146.30
80	2423.91		1	2124.05
			2	1964.73
			3	1767.87
100	3029.88		1	2751.07
			2	2558.92
			3	2545.84
			4	2143.78
			5	2468.08
			6	2773.05
120	3635.86		1	2942.68
			2	2984.80
			3	3149.80
150	4544.82		1	4004.81
300	9089.65		1	9202.21

Limit of Quantitation (LoQ): 1515ppm Methanol

11.8. **Intermediate Precision:**

11.8.1. Intermediate Precision was assessed by having a second analyst on a separate day perform System Suitability and an additional six (6) determinations of the *100% Level Residual Solvent Spike*. The Standard Deviation and Relative Standard Deviation (%RSD) will be calculated for individual and combined (Analyst I and II) results.

11.8.2. Acceptance Criteria:

11.8.2.1. Standard Deviation: Report

11.8.2.2. Relative Standard Deviation (%RSD): NMT 20%.

11.8.3. Results:

11.8.3.1. All acceptance criteria were met for Intermediate Precision. Results are summarized in the "Intermediate Precision Results" table.

Table 15: Intermediate Precision Results

Analyst	Concentration Level (%)	Methanol Spike (ppm)	Replicate	Methanol Result (ppm)	Standard Deviation (ppm)	%RSD
I	0	0	1	0		
	100	3029.88	1	2866.499	73.221	2.6
			2	2821.060		
			3	2951.854		
			4	2727.440		
			5	2850.707		
			6	2818.734		
II	0	0	1	0		
	100	3087.31	1	2962.774	26.622	0.9
			2	2936.678		
			3	2969.537		
			4	2922.048		
			5	2900.813		
			6	2919.493		
Combined (Analyst I and II):					72.555	2.5

11.9. **Solution Stability:**

11.9.1. Solution Stability was assessed by analyzing a separately prepared *Calibration Level 3 (100% Level)* solution, analyzing it at the end of the sequence, and calculating the Percent Recovery (%) and Percent Variation in area counts.

11.9.2. Acceptance Criteria:

11.9.2.1. Percent Recovery (%): 80% to 120%

11.9.2.2. Percent Variation (%) in Area Counts: 80% to 120%

11.9.3. Results:

11.9.3.1. All acceptance criteria were met for Solution Stability on each day of analysis. Results are summarized in the "Solution Stability Results" table.

Table 16: Solution Stability Results

Concentration Level (%)	Methanol Spike (ppm)	Initial Area Count	Final Area Count	Methanol Result (ppm)	Percent Recovery (%)	Percent Variation (%)
Analyst I						
100	3029.88	132211	146234	3303.952	109	111
Analyst II						
100	3087.31	86937	89184	3151.691	102	103

12. CONCLUSION

12.1. **Performance Summary:**

Table 17: Performance Summary	
Method Performance Indicator	**Result**
System Suitability	Pass
Accuracy	Pass
Precision	Pass
Linearity	Pass
Specificity	Pass
Range	1500ppm Methanol – 4500ppm Methanol
Limit of Quantitation (LoQ)	1515ppm Methanol
Intermediate Precision	Pass
Solution Stability	Pass

12.2. **Statement of Validation:** The Analytical Method for the determination of Residual Solvents by Headspace GC-FID in Glycerin is considered a validated method of analysis at the BioSpectra Inc. Majestic Facility.

12.3. **Excursions or Critical Changes to the Method Verification Protocol:**

12.3.1. **Critical Change – Residual Solvent Stock Solution -** The protocol referenced (DCN: BSI-PRL-0348) for this validation specifies to make a 1000ppm methanol solution to dilute down to the appropriate amounts. Due to the specification for methanol in glycerin being 3000ppm, a 10000ppm solution of methanol needed to be made. This was achieved by doubling the original weight of the methanol and reduce the original final volume of the solution by five times.

12.3.2. **Critical Change – Calibration Curve Generation -** The Lab Solutions software does not permit the usage of a blank or 'Zero' point on the calibration curve, as it uses that point to initialize the curve. As a result, the reported values for the concentration of methanol in glycerin samples should be calculated using the calibration curve generated in the Lab Solutions software from Standards levels 1 through 5.

12.3.3. **Critical Change – Solution Stability Acceptance Criteria –** The Analytical Method Validation Protocol states in Section 1 the acceptance criteria for Solution Stability as "Report % recovery of aged sample and standard solution. NMT 20% variation". Then, in Section 9, it states the acceptance criteria as "NMT 20% variation in associated area counts for LTBPS". Since these two sections list two different acceptance criteria, a percent recovery based on the methanol result (ppm) and a percent variation based on methanol area counts, the acceptance criteria has been updated to reflect these two requirements. Furthermore, the specification of NMT 20% has been restated as 80% to 120% which more directly aligns with the Percent Recovery and Percent Variation calculation results.