BIOSPECTRA
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ANALYTICAL METHOD VALIDATION REPORT: RESIDUAL SOLVENTS BY HEAD SPACE GC FID METHANOL IN HEPES

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1. PURPOSE:

1.1. The purpose of this Report is to:

1.1.1. Provide a comprehensive validation report that the method for methanol analysis in HEPES products follows USP <467> and <1467>.

1.1.2. Ensure that the quantification of residual solvents is adequately evaluated and validated as a Category II Quantitative analytical method.

1.1.3. Provide capability data of the analytical method and a finished testing procedure based on data acquired during validation intended for routine use.

1.1.4. Prove that the procedure for determining the amount of residual solvents in samples via GC-FID meets all requirements for quantitative method as stated below.

TABLE 1: VALIDATION PARAMETERS AND ACCEPTANCE CRITERIA

Parameters	Procedure	Acceptance Criteria
Specificity	Obtained GC chromatograms of the following to demonstrate that the peaks of interest are resolved from each other and there is no interference among peaks, identified each peak retention time. Methanol – 3,000 ppm Limit	NLT 1.5 Resolution between peaks of interest
Accuracy	Performed accuracy experiments by spiking specified residual solvents into an appropriate sample matrix over a minimum of five (5) concentration levels encompassing 50% to 150% of the analyte specification. Calculated the average recovery at each concentration level.	80 to 120% Recovery at Each Concentration
Linearity and Range	Prepared a minimum of five concentration levels of residual solvents encompassing the minimum range from the reporting level to at least 120% above the specification of the analyte. Analyzed at the limit, at least 120% above, and at least 80% below in triplicate.	Correlation coefficient of determination: (r^2) NLT 0.90
Precision	Prepared 6 replicate samples of residual solvent mix at the 100% limit for each solvent using the same lot of material. Calculated %RSD.	NMT 20% RSD
Method/Intermediate Precision	Performed "Precision" section of the protocol and calculated the %RSD of both sets of data at the 100% limit.	NMT 20% RSD
Detection Quantification (LOQ)	Reported the analyte level that gives a minimum signal-to-noise ratio of 10:1 (USP).	NLT Specified Limit
Solution Stability	Analyzed aged (previously prepared) standard and spiked sample solutions against a freshly prepared standard solution.	Report % recovery of aged sample and standard solution. NMT 20% variation.

2. SCOPE:

2.1. This Analytical Method Validation Report applies to the Quantification of Specified Aqueous Soluble Solvents via headspace GC-FID determination.

2.2. This method validation is a Category II quantitative analytical method validation.

2.3. The method applies to the analysis of residual solvents in water and in 10% w/v solutions of HEPES.

2.4. This validation was performed and validated in compliance with USP <1467> Quantitative validation of alternative procedures. This includes specificity, linearity and range, limit of quantitation, accuracy, repeatability (precision), intermediate precision, and solution stability.

3. RESPONSIBILITIES:

3.1. The Laboratory Technology Manager, or other qualified designee, is responsible for the control, training, implementation, and maintenance of this report.
3.2. The Analytical Chemistry Specialists, or other qualified personnel, are responsible for performing the testing stated in the protocol.
3.3. The Laboratory Technology Manager and Analytical Chemistry Specialists are responsible for completing the Method Validation Report using conclusions made from the results obtained.

4. REFERENCES:

4.1. BSI-PRL-0353, Analytical Method Validation Protocol: Residual Solvents USP 1467:HEPES-Methanol
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/MS SOP
4.6. BSI-SOP-0436, Analytical Methods Validation Master Plan
4.7. ICH Q3A
4.8. USP NF <467>, Organic Impurities in Drug Substances and Drug Products
4.9. USP NF <621>, Chromatography
4.10. USP NF <1467>, Residual Solvents – Verification of Compendial Procedures and Validation of Alternative Procedures

5. 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.
5.2. Personnel
5.2.1. All personnel executing this validation are trained in accordance with the Analytical Methods Validation Master Plan, BSI-SOP-0436. All personnel executing this report are trained on GC analysis or are considered Subject Matter Experts.
5.3. Supplies:
5.3.1. All supplies in this analytical method validation were appropriate for the intended use.
5.4. Reagents:
5.4.1. All reagents were current and met required specifications and were suitable for the intended use.
5.5. Reference Standards:
5.5.1. Any standards required in this validation report are listed in the Materials and Equipment section of the Analytical Method Validation Report.

6. MATERIALS AND EQUIPMENT:

TABLE 2: EQUIPMENT

Item ID	Manufacturer	Model	Serial Number	Due Date
Analytical Balance	Sartorius	MSE-224S	24801744	10/2020
Analytical Balance	Sartorius	Practum-612-1S	0031950175	10/2020
Eppendorf Pipette: 0.5-5 mL	Eppendorf	Research Plus	L21310F	01/31/21
Eppendorf Pipette: 1-10 mL	Eppendorf	Research Plus	M22725F	01/31/21
Eppendorf Pipette: 30-300 µL	Eppendorf	Research Plus	P53563H	11/30/20
Eppendorf Pipette: 100-1000 µL	Eppendorf	Research Plus	M27701G	12/31/20
Eppendorf Pipette: 1-10 mL	Eppendorf	Research Plus	G54479H	11/30/20
GC FID Detector	Shimadzu	GC-2010	020385050364	08/2020
GC Headspace Autosampler	Shimadzu	HS-20	SW0207152-00319	08/2020

6.1. Supplies:

TABLE 3: SUPPLIES

Item ID	Manufacturer	Model	Part Number	Serial Number
20 mL Verex Headspace Vial	Phenomenex	23 x 75 mm	AR0-3270-13	Not Applicable
Verex Seal Vial Cap	Phenomenex	20 mm diameter, PTFE/Silicone	AR0-5250-13	Not Applicable
150 mL Beakers	VWR	Not Applicable	Not Applicable	Not Applicable
Volumetric Flasks, Class A	VWR/Pyrex	Various sizes	Not Applicable	Not Applicable
Vespel Graphite Ferrule 1/16" x 0.4mm	Phenomenex	Cat. No. AGO-4708	Not Applicable	Not Applicable
0.4/0.5mm ID, Metal Encapsulated Vespel Graphite Ferrule	Phenomenex	Cat. No. AG0-8881	Not Applicable	Not Applicable
GC Column: 30 m Capillary Column USP Phase G43	Phenomenex Zebron	Not Applicable	7HG-G005-27	1051537

6.2. Reagents

TABLE 4: REAGENTS

Reagent	Supplier	Part Number	Lot Number	Expiration Due
HEPES	BioSpectra, Inc	Not Applicable	HE3200-027-0620	Not Applicable
HEPES	BioSpectra, Inc	BP310	186633	09/30/23
Purified Water	BioSpectra, Inc.	Not Applicable	D10DI01-072820; D10DI01-072920	07/28/20; 07/29/20

6.3. Reference Standards:

6.3.1. Methanol reference standard was purchased as single use ampules and were opened at time of use. Ethanol came from a bulk lot.

TABLE 5: REFERENCE STANDARDS					
Reference STD	Purity	Supplier	Part Number	Lot Number	Expiration Date
Methanol	99.8%	Sigma Aldrich	PHR1372	LRAB7353	12/31/22
Ethanol (SDA 3C)	95.0%	Tilley	Not Applicable	BLD200521-3C	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 min
7.1.8. Pressurization Time: 0.50 min
7.1.9. Pressure Equilibration Time: 0.50 min
7.1.10. Load Time: 1.00 min
7.1.11. Load Equilibration Time: 0.50 min
7.1.12. Injection Time: 1.00 min
7.1.13. Needle Flush Time: 1.00 min
7.1.14. GC Cycle Time: 7.00 min
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/min
7.2.6. Column Flow: 2.32 mL/min
7.2.7. Linear Velocity: 47.6 cm/sec
7.2.8. Purge Flow: 2.0 mL/min
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 6: OVEN TEMPERATURE PROGRAM		
Rate (^{O}C per Min)	**Temperature (°C)**	**Hold Time (min)**
-	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 min
- 7.3.16. CRG(INJ): OFF
- 7.3.17. APC1: 75.0 kPa

8. SAMPLE PREPARATION:

8.1. Pre-Requisite Solutions:
- 8.1.1. Residual Solvent Stock Solutions:
  - 8.1.1.1. Prepared individual 10,000 mg/L (ppm) solutions of each residual solvent in purified water by weighing approximately 0.50 g of standard directly into a 50 mL volumetric flask. Mixed thoroughly. Calculated actual concentrations based off CoA/purity.

8.2. Calibration Standards and Spike Diluent Preparation:

NOTE: Addition of solutions or reagents to head space vial may be done in any order.
- 8.2.1. 0 ppb (Blank)
  - 8.2.1.1. Purified water or equivalent.
- 8.2.2. Calibration Level 1 (50% Level)
  - 8.2.2.1. In a 100.0 mL volumetric flask added the following:
    - 8.2.2.1.1. 1.50 mL of 10,000 ppm Methanol Stock Solution
  - 8.2.2.2. Diluted to 100.0 mL with water.
  - 8.2.2.3. Mixed thoroughly.
- 8.2.3. Calibration Level 2 (80% Level)
  - 8.2.3.1. In a 100.0 mL volumetric flask added the following:
    - 8.2.3.1.1. 2.40 mL of 10,000 ppm Methanol Stock Solution
  - 8.2.3.2. Diluted to 100.0 mL with water.
  - 8.2.3.3. Mixed thoroughly.
- 8.2.4. Calibration Level 3 (100% Level)
  - 8.2.4.1. In a 100.0 mL volumetric flask added the following:
    - 8.2.4.1.1. 3.00 mL of 10,000 ppm Methanol Stock Solution
  - 8.2.4.2. Mixed thoroughly.
  - 8.2.4.3. Aliquoted 2 vials of this solution, one for stability and one for calibration.
- 8.2.5. Calibration Level 4 (120% Level)
  - 8.2.5.1. In a 100.0 mL volumetric flask added the following:
    - 8.2.5.1.1. 3.60 mL of 10,000 ppm Methanol Stock Solution
  - 8.2.5.2. Diluted to 100.0 mL with water.
  - 8.2.5.3. Mixed thoroughly.
- 8.2.6. Calibration Level 5 (150% Level)

8.2.6.1. In a 100.0 mL volumetric flask added the following:
8.2.6.1.1. 4.50 mL of 10,000 ppm Methanol Stock Solution
8.2.6.2. Diluted to 100.0 mL with water.
8.2.6.3. Mixed thoroughly.
8.2.7. Linearity (300% Level)
8.2.7.1. In a 100.0 mL volumetric flask added the following:
8.2.7.1.1. 9.00 mL of 10,000 ppm Methanol Stock Solution
8.2.7.2. Diluted to 100.0 mL with water.
8.2.7.3. Mixed thoroughly.

8.3. Specificity Solutions
8.3.1. Specificity Solution 1- Blank
8.3.1.1. Pipetted 10 mL of purified water into a 20 mL head space vial.
8.3.1.2. Crimped to seal, mixed thoroughly.
8.3.2. Specificity Solution 2- Methanol
8.3.2.1. Pipetted 10 mL of purified water into a 20 mL headspace vial.
8.3.2.2. Added 0.1 mL of 10,000 ppm Methanol Stock Solution to head space vial.
8.3.2.3. Crimped to seal, mixed thoroughly.
8.3.3. Specificity Solution 3- Ethanol/2-Propanol/Methanol
8.3.3.1. Pipetted 10 mL of purified water into a 20 mL headspace vial.
8.3.3.2. Added 0.1 mL of 10,000 ppm Ethanol Stock Solution to head space vial.
8.3.3.3. Added 0.1 mL of 10,000 ppm Methanol Stock Solution to head space vial.
8.3.3.4. Crimped to seal, mixed thoroughly.
8.3.4. Specificity Solution 4- Sample Screen
8.3.4.1. Weighed and added 1.0 g of sample into a 20 mL head space vial.
8.3.4.2. Added 10 mL of purified water to head space vial.
8.3.4.3. Dissolved.
8.3.4.4. Crimped to seal, mixed thoroughly.

8.4. Linearity, Accuracy, and Precision Solution Preparation:
8.4.1. 50% Limit Level Residual Solvent Spike
8.4.1.1. Weighed 1.0 g of sample and added to 20 mL head space vial.
8.4.1.2. Added 10 mL of the 50% level spike diluent to the head space vial.
8.4.1.3. Dissolved.
8.4.1.4. Crimped to seal, mixed thoroughly.
8.4.1.5. Prepared in triplicate.
8.4.2. 80% Limit Level Residual Solvent Spike
8.4.2.1. Weighed 1.0 g of sample and added to 20 mL head space vial.
8.4.2.2. Added 10 mL of the 80% level spike diluent to the head space vial.
8.4.2.3. Dissolved.
8.4.2.4. Crimped to seal, mixed thoroughly.
8.4.2.5. Prepared in triplicate.
8.4.3. 100% Limit Level Residual Solvent Spike
8.4.3.1. Weighed 1.0 g of sample and added to 20 mL head space vial.
8.4.3.2. Added 10 mL of the 100% level spike diluent to the head space vial.
8.4.3.3. Dissolved.
8.4.3.4. Crimped to seal, mixed thoroughly.
8.4.3.5. Prepared 6 replicates.
8.4.4. 120% Limit Level Residual Solvent Spike
8.4.4.1. Weighed 1.0 g of sample and added to 20 mL head space vial.
8.4.4.2. Added 10 mL of the 120% level spike diluent to the head space vial.
8.4.4.3. Dissolved.
8.4.4.4. Crimped to seal, mixed thoroughly.

8.4.4.5. Prepared in triplicate.

8.4.5. 150% Limit Level Residual Solvent Spike

8.4.5.1. Weighed 1.0 g of sample and added to 20 mL head space vial.

8.4.5.2. Added 10 mL of the 150% level spike diluent to the head space vial.

8.4.5.3. Dissolved.

8.4.5.4. Crimped to seal, mixed thoroughly.

8.4.6. 300% Limit Level Residual Solvent Spike

8.4.6.1. Weighed 1.0 g of sample and added to 20 mL head space vial.

8.4.6.2. Added 10 mL of the 300% level spike diluent to the head space vial.

8.4.6.3. Dissolved.

8.4.6.4. Crimped to seal, mixed thoroughly.

9. PERFORMANCE PARAMETERS:

9.1. Calibration and System Suitability

9.1.1. Calibrated the GC-FID instrument using calibration levels 1, 2, 3, 4, and 5 along with a diluent blank (Standard 0 ppm) by pipetting 10 mL of each standard to a head space vial. Crimped to seal and mixed thoroughly. Prepared calibration standard 3 in duplicate, one for the calibration curve generation and once for solution stability analysis at the end of the run.

9.1.2. An r2 of NLT 0.95 was required for each solvent of interest.

9.2. Analyzed all samples prepared in Section 8 using the method parameters defined in Section 7.

9.3. Method Performance Data:

9.3.1. Specificity: Pass

9.3.1.1. Obtained GC chromatograms of the following to demonstrate that the peaks of interest are resolved from each other and there is no interference between peaks of interest; identify each peak retention time. Methanol is the only solvent of interest; however, ethanol was also analyzed to show methanol resolution from other residual solvent peaks. Resolution was to be NLT 1.5 between peaks.

- Diluent – Acquired 07/28/20
  - No detectable peaks were observed.
- Sample - Acquired 07/28/20
  - No detectable peaks were observed.
- Methanol- Acquired 07/28/20
  - Methanol successfully identified at RT 1.388 min.
- Ethanol- Acquired 07/28/20
  - Ethanol was successfully identified at RT 1.579 min.
- 2-Propanol- Acquired 07/28/20
  - 2-Propanol was successfully identified at RT 1.738 min.
- Resolution Solution (Standard Mix) - Acquired 07/28/20
  - Resolution NLT 1.5
    - Methanol – Ethanol Resolution: 4.480
    - Ethanol – 2-Propanol Resolution: 3.599
- Example Chromatogram of Resolution

Chromatogram

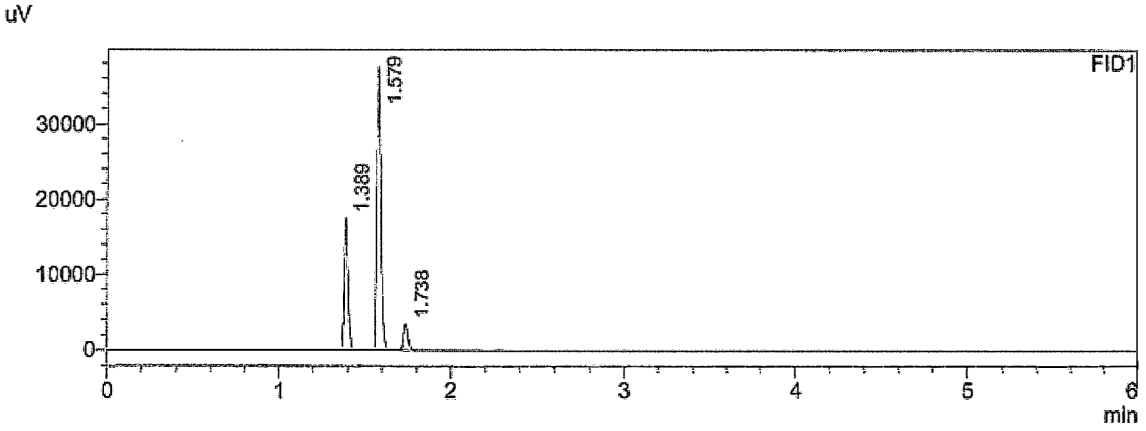

FIGURE 1: EXAMPLE CHROMATOGRAM OF RESOLUTION

9.3.2. Linearity: Pass

9.3.2.1. Using the data obtained from section 8.4, the average of each actual prepared spike concentration level vs. reported result is graphed below. Specification for the coefficient of determination, R2, is NLT 0.90.

9.3.2.2. Reported the correlation coefficient, y-intercept, and slope of the regression line.

9.3.2.3. Results:

9.3.2.3.1. Methanol: Pass

TABLE 7: METHANOL LINEARITY RESULTS

Methanol Spike (ppm w/w)	Average Result (ppm w/w)
1501.2	1690.204
2407.68	2365.292
3009.6	3031.678
3611.52	3498.394
4514.4	4274.859
9028.8	9016.902

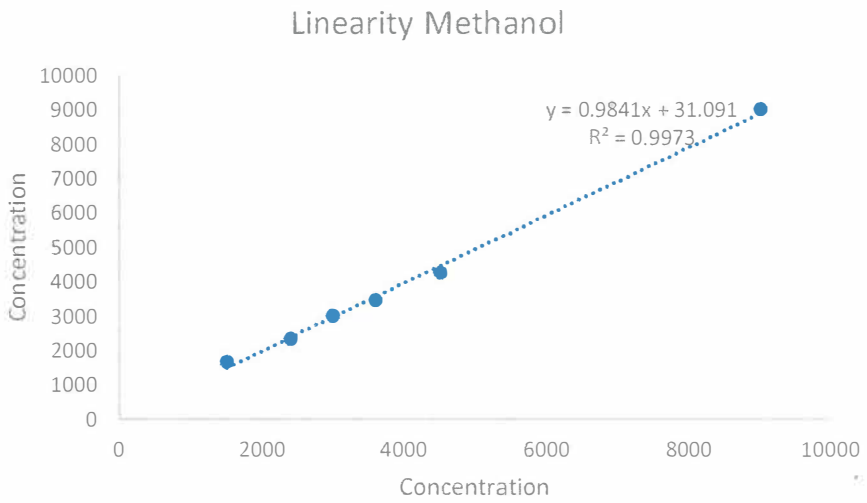

FIGURE 2: LINEARITY PLOT FOR METHANOL

9.3.3. Range:

9.3.3.1. Reported the concentration levels that meet requirements for accuracy and precision and are concurrently at least 80%-120% of the target specification.

9.3.3.1.1. Methanol: 1,500-3,600 ppm

9.3.4. Accuracy: Pass

9.3.4.1. Used the data obtained from analysis of solutions prepared in section 8.4 to assess accuracy at each concentration level.

9.3.4.2. Report data from a minimum of 9 determinations over a minimum of three concentration levels.

9.3.4.2.1. 0% Correction (1 Replicate)

9.3.4.2.2. 50% Level (3 Replicates for LOQ requirements)

9.3.4.2.3. 80% Level (3 Replicates)

9.3.4.2.4. 100% Level (6 Replicates)

9.3.4.2.5. 120% Level (3 Replicates)

9.3.4.3. Calculated and report % recovery as:

% Recovery = (Reported Concentration/Prepared Spiked Concentration)*100

9.3.4.4. Individual Solvent Accuracy Results

TABLE 8: METHANOL ACCURACY RESULTS				
% Limit	Methanol Prepared (ppm)	Methanol Reported (ppm)	Average % Recovery (80-120%)	Result
50%	1,501.2	1,675.033	113	Pass
50%	1,501.2	1,640.924		
50%	1,501.2	1,754.655		
80%	2,407.68	2,363.608	98	Pass
80%	2,407.68	2,309.069		
80%	2,407.68	2,423.198		
100%	3,009.6	3,178.931	101	Pass
100%	3,009.6	2,990.315		
100%	3,009.6	2,997.113		
100%	3,009.6	3,002.926		
100%	3,009.6	3,093.867		
100%	3,009.6	2,926.914		
120%	3,611.52	3,459.504	97	Pass
120%	3,611.52	3,489.077		
120%	3,611.52	3,546.602		

9.3.5. Precision: Pass

9.3.5.1. Reported the standard deviation and relative standard deviation (%RSD) at each replicate level. % RSD must not exceed 20% at the 100% level, n=6.

TABLE 9: METHANOL PRECISION RESULTS					
% Limit	Methanol Prepared (ppm)	Methanol Reported (ppm)	Standard Deviation	% RSD (NMT 20%)	Result
50%	1,501.2	1,675.033	58.364	3.5	Pass
50%	1,501.2	1,640.924			
50%	1,501.2	1,754.655			
80%	2,407.68	2,363.608	57.083	2.4	Pass
80%	2,407.68	2,309.069			
80%	2,407.68	2,423.198			
100%	3,009.6	3,178.931	89.731	3.0	Pass
100%	3,009.6	2,990.315			
100%	3,009.6	2,997.113			
100%	3,009.6	3,002.926			
100%	3,009.6	3,093.867			
100%	3,009.6	2,926.914			
120%	3,611.52	3,459.504	44.290	1.3	Pass
120%	3,611.52	3,489.077			
120%	3,611.52	3,546.602			

9.3.6. Limit of Quantification (LOQ), and Signal to Noise (SN): Report

9.3.6.1. Reported the mean signal to noise ratio for Methanol in the spiked sample solution from at least three determinations. Specification is NLT 10 for S/N.

TABLE 10: SIGNAL TO NOISE AND LOQ SUMMARY TABLE					
Solvent	Determination	Concentration (ppm)	Signal to Noise (S/N)	Average (S/N)	LOQ Result (ppm)
Methanol	1	1,500	1,376	1340	1500
	2		1,347		
	3		1,298		

9.3.7. Solution Stability: Pass

9.3.7.1. Analyzed a previously vialed and sealed 100% Standard Solution at the end of analysis run.

9.3.7.1.1. NMT 20% Variation in associated area counts.

9.3.7.1.2. %Change is calculated: % Change = (Final-Initial)/Initial x100

TABLE 11: SOLUTION STABILITY SUMMARY TABLE				
Solvent	Area Count Initial	Area Count Final	% Change	Result (NMT 20%)
Methanol	95,404	91,088	4.5	Pass

9.3.8. Intermediate Precision: Pass

9.3.8.1. On a different day, calibration, and with a different analyst (Analyst II) section 8.4.3. was repeated and analyzed. %RSD of results was not to exceed 20% when compared with data acquired from Analyst I for total of N=12 replicates.

TABLE 12: INTERMEDIATE PRECISION - METHANOL			
Analyst ID	Replicate	Reported Value (ppm)	% RSD (NMT 20%)
Analyst I	1	3,178.931	2.4
	2	2,990.315	
	3	2,997.113	
	4	3,002.926	
	5	3,093.867	
	6	2,926.914	
Analyst II	1	3,112.496	
	2	2,983.517	
	3	3,013.911	
	4	2,961.902	
	5	3,014.632	
	6	2,948.333	

10. STATEMENT OF VALIDATION:

10.1. The method used for analysis for residual solvents in HEPES for methanol is considered a validated method of analysis at the BioSpectra Bangor facility, based upon conclusions drawn from data obtained during the analyses performed in this validation.

10.1.1. Specificity was shown as methanol, ethanol, and 2-propanol eluted at different retention times and were adequately resolved in a specificity sample solution.

10.1.2. Linearity was shown for spiked samples over a 50% through 300% range of the target concentration., as evidenced by coefficients of determination (R2) of 0.9973.

10.1.3. Accuracy was shown for each of the spiked samples over a 50 – 120% range of the target concentration, based upon recoveries obtained within a 97-113% average for methanol. These results are within the 80-120% specification.

10.1.4. Precision was shown based upon the %RSD that is not more than 3% for six replicate injections for the residual solvent of interest at the 100% limit; this result falls within the NMT 20% acceptance criteria.

10.1.5. Intermediate precision, performed by a second analyst on a different day as the precision study, showed a %RSD of 2% for methanol in twelve total injections (six replicate injections each by Analyst 1 and Analyst 2), all results were within the NMT 20% acceptance criteria and meet requirements.

10.1.6. Solution stability showed 4.5% variation over the course of the validation, this result is within the NMT 20% specification and meets requirements.

10.1.7. The Limit of Quantitation (LOQ) for this method validation was established from 50%-120% for all analytes to be 1,500 ppm, which is less than the specification of methanol of 3,000 ppm.

11. CONCLUSION:

11.1. All precision, intermediate precision, specificity, and solution stability data are within acceptance criteria and the method is considered a validated method of residual solvent analysis for measurement of methanol in HEPES.

12. DEVIATIONS:

12.1. No significant changes were made to the validation protocol.

12.2. The 50% accuracy and linearity study were reperformed due to contamination in one of the triplicate preparations. The 50% spiked solutions were prepared by the second analyst and the results were combined with the remaining linearity and accuracy solutions. This was deemed acceptable since the accuracy met specification of 80-120% recovery and the coefficients of determination was greater than the acceptance criteria across preparations by two analysts.

12.3. The accuracy and linearity data from Analyst 1 were processed against the calibration curve from analyst 2 at the time of the execution of the protocol as the standard concentration was entered into the software incorrectly. For the purpose of data analysis for the report, the accuracy and linearity data from Analyst 1 was processed against the curve made from the standards used during the analysis in order to obtain the correct sample concentrations used for the spiked sample analysis. This was deemed acceptable as the calibration curve is constructed as a linear plot of concentration versus intensity and the sample concentrations are calculated from the linear trendline equation.