BIOSPECTRA
100 Majestic Way, Bangor, PA 18013 / www.biospectra.us

ANALYTICAL METHOD VALIDATION REPORT: ACETYLENE DETECTION BY HEAD SPACE GC FID 6N HCL IN 2-PROPANOL

TABLE OF CONTENTS

1. PURPOSE:

1.1. The purpose of this Report is to:

1.1.1. Provide a comprehensive validation report to validate acetylene detection in 6N HCl in 2-Propanol in compliance with ICH Q2.

1.1.2. Ensure that the detectability of acetylene is adequately evaluated and validated as a Category II Limit in comparison to previously approved materials.

1.1.3. To 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. To prove that the procedure for determining the amount acetylene in samples via GC-FID meets all requirements for a limit-based method as stated below.

Parameters	Procedure	Acceptance Criteria
System Suitability	Calibrate the GC-FID instrument with 5 levels of acetylene varying from 0-40ppm.	Correlation Coefficient (r^2) NLT 0.99
Specificity	Obtain GC chromatograms of the following to demonstrate that the peaks of interest are resolved from each other and there is no interference among peaks, identify each peak retention time. • Blank • Acetylene – 300ppm (0.03%) Limit • Sample (Unspiked)	NLT 1.5 Resolution between peaks of interest
Limit of Detection (LOD)	Report the analyte level that gives a minimum signal-to-noise ratio of 10:1 (USP).	NLT 50% of Specified Limit (150ppm)

2. SCOPE:

2.1. This Analytical Method Validation Report applies to the detection of acetylene via headspace GC-FID determination.
2.2. This method validation is a Category II Limit analytical method validation.
2.3. The method applies to the analysis of acetylene in water and in 9.4% v/v solutions of aqueous soluble articles.
2.4. This validation is intended to be performed and validated in compliance with ICH Q2 validation of analytical procedures. This includes system suitability, specificity, and detection limit.

3. RESPONSIBILITIES:

3.1. The Associate Director of Product Life Cycle is responsible for the control, implementation and maintenance of this report.
3.2. QC Analysts or Process Technology Chemists were responsible for performing the testing as stated in the Analytical Method Validation Protocol.
3.3. The Associate Director of Product Life Cycle was responsible for completing the Method Validation Report using conclusions made from the results obtained from this method validation report to assess the performance of the analysis.
3.4. Safety: Standard laboratory safety regulations apply. Before working with any chemical, read and understand the Safety Data Sheet (SDS).

4. REFERENCES:

4.1. BSI-PRL-0576, Analytical Method Validation Protocol: Acetylene Detection by Head Space GC FID
4.2. BSI-SOP-0092, Total Organic Carbon Water Analysis
4.3. BSI-SOP-0098, Balance SOP
4.4. BSI-SOP-0126, Laboratory Notebooks
4.5. BSI-SOP-0134, Pipette SOP
4.6. BSI-SOP-0316, Shimadzu QP2010S GC SOP
4.7. BSI-SOP-0436, Analytical Methods Validation Master Plan
4.8. ICH Q3A(R2)

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 were trained in accordance with the Analytical Methods Validation Master Plan.
5.3. Supplies:
5.3.1. All supplies used in this validation were appropriate for the intended use.
5.4. Reagents:
5.4.1. All reagents were current, met required specifications, and were suitable for their intended use.
5.5. Reference Standards:
5.5.1. All reference standards used in this method validation are listed in the Materials and Equipment section of this report.

6. MATERIALS AND EQUIPMENT:

6.1. All materials and equipment used in this Validation are outlined in this section:
6.2. Equipment:
- 6.2.1. Automatic Pipette – 100µL - 1000 µL
  - 6.2.1.1. Manufacturer: Eppendorf
  - 6.2.1.2. S/N: R14419C
    - 6.2.1.2.1. Due: 2/28/23
  - 6.2.1.3. S/N: O39512B
    - 6.2.1.3.1. Due: 12/31/22
- 6.2.2. Automatic Pipette – 500µL - 5000µL
  - 6.2.2.1. Manufacturer: Eppendorf
  - 6.2.2.2. S/N: I45595H
    - 6.2.2.2.1. Due: 11/30/22
- 6.2.3. Automatic Pipette – 1mL - 10 mL
  - 6.2.3.1. Manufacturer: Eppendorf
  - 6.2.3.2. S/N: G54479H
    - 6.2.3.2.1. Due: 11/30/22
- 6.2.4. GC-FID
  - 6.2.4.1. Make: Shimadzu
  - 6.2.4.2. Model: GC-2010, equipped with FID detector.
  - 6.2.4.3. HS-20 S/N: 0207152
    - 6.2.4.3.1. Due: 9/30/23
  - 6.2.4.4. GC-FID S/N: 02038505
    - 6.2.4.4.1. Due: 9/30/23
- 6.2.5. GC Column: 30m Capillary Column USP Phase G43 or equivalent
  - 6.2.5.1. Make: Phenomenex Zebron
  - 6.2.5.2. Part Number:7HG-G005-27
  - 6.2.5.3. S/N: 1051537
- 6.2.6. TOC Analyzer
  - 6.2.6.1. Make: Shimadzu TOC-L
  - 6.2.6.2. S/N: H54325132005CS
    - 6.2.6.2.1. Due: 3/23
- 6.2.7. Laboratory Notebook
  - 6.2.7.1. Manufacturer: Thermo Fisher Scientific Inc.
  - 6.2.7.2. Cat. Number: 6300-1000

6.3. Reagents
- 6.3.1. Purified Water/Milli-Q Water
  - 6.3.1.1. Supplier: BioSpectra Inc.
  - 6.3.1.2. S/N: F9SA142884H
  - 6.3.1.3. Due: 6/23
  - 6.3.1.4. Meets or Exceeds USP Purified Water specification.
- 6.3.2. 10N NaOH
  - 6.3.2.1. Supplier: BioSpectra Inc.
  - 6.3.2.2. Lot: NAHY-0122-00054
  - 6.3.2.3. Exp: 10/31/24
- 6.3.3. 6N HCl in IPA
  - 6.3.3.1. Manufacturer: BioSpectra Inc.
  - 6.3.3.2. Lot ID: IHCL-0122-0013-PV (Middle)
- 6.3.4. 500ppb Carbon from Sucrose Standard
  - 6.3.4.1. Manufacturer: BioSpectra Inc. (In-House Solution)

6.3.4.2. Lot: BSP34P81
6.3.4.3. Exp.: 10/23
6.3.5. 500ppb Carbon from 1,4-benzoquinone Standard
6.3.5.1. Manufacturer: BioSpectra Inc. (In-House Solution)
6.3.5.2. Lot: BSP34P82
6.3.5.3. Exp.: 10/23
6.4. Supplies:
6.4.1. 20 mL Vial and Caps
6.4.1.1. Supplier: Phenomenex
6.4.1.2. Part Number: AR0-3270-13
6.4.1.2.1. Verex Headspace Vial, 23x75mm
6.4.1.3. Vial Cap Part Number AR0-5250-13
6.4.1.3.1. Verex Seal, 20 mm diameter, PTFE/Silicone
6.4.2. 150 mL Beakers
6.4.3. Volumetric Flasks, Class A, Various Sizes
6.4.4. Vespel Graphite Ferrule
6.4.4.1. Manufacturer: Phenomenex
6.4.4.2. Catalog Number: AGO-4708
6.4.5. Metal Encapsulated Vespel Graphite Ferrule
6.4.5.1. Manufacturer: Restek
6.4.5.2. Catalog Number: 24828
6.5. Reference Standards:
6.5.1. Acetylene
6.5.1.1. Industrial Grade; Compressed Cylinder 20lb

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: 100 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: 176.2 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

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: 176.2kPa

8. SAMPLE PREPARATION:

8.1. Pre-Requisite Solutions:

8.1.1. Acetylene Stock Solution:

8.1.1.1. Prepared a stock solution of acetylene in purified water by slowly gassing acetylene from a regulator under the surface of the purified water. Gassed 1-2 minutes, inverting the solution periodically to ensure homogenous dissolution. Mixed thoroughly and analyzed TOC of the gassed water (now the acetylene stock solution) and the water used to prepare the stock solution. Calculated actual concentration based off TOC analysis. Included calculations in notebook.

TOC Results		
System Suitability		
Reagent Water Area Count (R_W)	**Sucrose Standard Area Count (R_S)**	**1,4-Benzoquinone Standard Area Count (R_{SS})**
4.384	36.26	37.34
	Response Efficiency (%):	103.39%
Sample Analysis		
Sample ID	**Mean Area Count**	**TOC Result**
Purified Water Blank	5.017	74.52ppb
1:100 Acetylene Stock Standard Solution	372.7	5841ppb

8.1.1.2. Mixed thoroughly and calculated ppm Acetylene in the stock solution using the following equation:

$$ppm\ Acetylene\ Stock = \frac{Stock\ Solution\ ppm\ Carbon - Purified\ Water\ ppm\ Carbon}{0.9225}$$

$$ppm\ Acetylene\ Stock = \frac{584.100ppm - 0.07452ppm}{0.9225} = 633.09ppm$$

8.1.1.3. Prepared 100mL of 50ppm Acetylene standard by diluting an appropriate amount of the stock solution to 50ppm using the following equation by solving for V_1:

$$C_1V_1 = C_2V_2 \rightarrow V_1 = \frac{C_2V_2}{C_1}$$

$$V_1 = \frac{(50ppm\ Acetylene\ Standard)(100mL)}{633.09ppm\ Acetylene\ Stock} = 7.90mL\ Acetylene\ Stock$$

8.2. Calibration Standards and Spike Diluent Preparation:

8.2.1. 0 ppm Acetylene (Blank)

8.2.1.1. Pipetted 10mL of purified water to a headspace vial.

8.2.1.2. Pipetted 0.6mL of 10N NaOH to the vial.

8.2.1.3. Immediately capped and sealed the vial.

8.2.1.4. Mixed thoroughly.

8.2.2. Calibration Level 1 (9.43ppm Acetylene)

8.2.2.1. Pipetted 8.0mL of purified water to a headspace vial.

8.2.2.2. Pipetted 0.6mL of 10N NaOH to the vial.

8.2.2.3. Pipetted 2.0mL of 50ppm Acetylene Standard Solution to the vial.
8.2.2.4. Immediately capped and sealed the vial.
8.2.2.5. Mixed thoroughly.

8.2.3. Calibration Level 2 (18.87ppm Acetylene)
8.2.3.1. Pipetted 6.0mL of purified water to a headspace vial.
8.2.3.2. Pipetted 0.6mL of 10N NaOH to the vial.
8.2.3.3. Pipetted 4.0mL of 50ppm Acetylene Standard Solution to the vial.
8.2.3.4. Immediately capped and sealed the vial.
8.2.3.5. Mixed thoroughly.

8.2.4. Calibration Level 3 (28.30ppm Acetylene)
8.2.4.1. Pipetted 4.0mL of purified water to a headspace vial.
8.2.4.2. Pipetted 0.6mL of 10N NaOH to the vial.
8.2.4.3. Pipetted 6.0mL of 50ppm Acetylene Standard Solution to the vial.
8.2.4.4. Immediately capped and sealed the vial.
8.2.4.5. Mixed thoroughly.

8.2.5. Calibration Level 4 (37.74ppm Acetylene)
8.2.5.1. Pipetted 2.0mL of purified water to a headspace vial.
8.2.5.2. Pipetted 0.6mL of 10N NaOH to the vial.
8.2.5.3. Pipetted 8.0mL of 50ppm Acetylene Standard Solution to the vial.
8.2.5.4. Immediately capped and sealed the vial.
8.2.5.5. Mixed thoroughly.

8.3. Specificity Solutions

8.3.1. Specificity Solution 1- Blank
8.3.1.1. Pipetted 10mL of purified water into a headspace vial.
8.3.1.2. Pipetted 0.6mL of 10N NaOH to the vial.
8.3.1.3. Immediately capped and sealed the vial.
8.3.1.4. Mix thoroughly.

8.3.2. Specificity Solution 2- Acetylene
8.3.2.1. Refer to Calibration Level 3 (28.30ppm Acetylene).

8.3.3. Specificity Solution 3- Sample Screen
8.3.3.1. Added 9.0 mL of purified water to a headspace vial.
8.3.3.2. Pipetted 0.6mL of 10N NaOH to the vial.
8.3.3.3. Added 1.0mL of sample to the vial.
8.3.3.4. Immediately capped and sealed the vial.
8.3.3.5. Mixed thoroughly.

8.4. Limit of Detection Solution Preparations (Prepare in Triplicate):

8.4.1. 50% Limit Level Acetylene Spike (141.5ppm v/v in sample)
8.4.1.1. Added 6.0 mL of purified water to a headspace vial.
8.4.1.2. Pipetted 0.6mL of 10N NaOH to the vial.
8.4.1.3. Pipetted 1.0mL of sample to the vial.
8.4.1.4. Added 3.0 mL of 50ppm Acetylene Standard Solution to the vial.
8.4.1.5. Immediately capped and sealed the vial.
8.4.1.6. Mixed thoroughly.

8.4.2. 100% Limit Level Acetylene Spike (283.0ppm v/v in sample)
8.4.2.1. Added 3.0 mL of purified water to a headspace vial.
8.4.2.2. Pipetted 0.6mL of 10N NaOH to the vial.
8.4.2.3. Pipetted 1.0mL of sample to the vial.
8.4.2.4. Added 6.0 mL of 50ppm Acetylene Standard Solution to the vial.
8.4.2.5. Immediately capped and sealed the vial.

8.4.2.6. Mixed thoroughly.

8.4.3. 150% Limit Level Acetylene Spike (424.5ppm v/v in sample)

8.4.3.1. Pipetted 0.6mL of 10N NaOH to a headspace vial.

8.4.3.2. Pipetted 1.0mL of sample to the vial.

8.4.3.3. Added 9.0 mL of 50ppm Acetylene Standard Solution to the vial.

8.4.3.4. Immediately capped and sealed the vial.

8.4.3.5. Mixed thoroughly.

9. PERFORMANCE PARAMETERS:

9.1. Calibration and System Suitability

9.1.1. Calibrate the GC-FID instrument using calibration levels 1, 2, 3, 4, and a diluent blank (Standard 0 ppm).

9.1.2. An r^2 of NLT 0.99 is required for acetylene to pass system suitability.

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

9.3. Evaluating Performance Data:

9.3.1. Specificity:

9.3.1.1. Obtain 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. Due to the nature of analysis, not all constituents of analysis are expected to be seen.

- Blank
- Sample
- Acetylene

9.3.2. Limit of Detection (LOD), and Signal to Noise (S/N):

9.3.2.1. Report the mean signal to noise ratio for each solvent in the standard and spiked sample solution from at least three determinations; S/N is NLT 10.

9.3.2.2. The 0% spike level sample response should be less than 50% spike level response.

9.3.2.3. The 50% spike sample response should be less than the 100% spike sample response.

9.3.2.4. The 100% spike level response should be less than the 150% spike sample response.

9.3.2.5. The 50% Spike Level requires a S/N of NLT 10.

10. PERFORMANCE RESULTS:

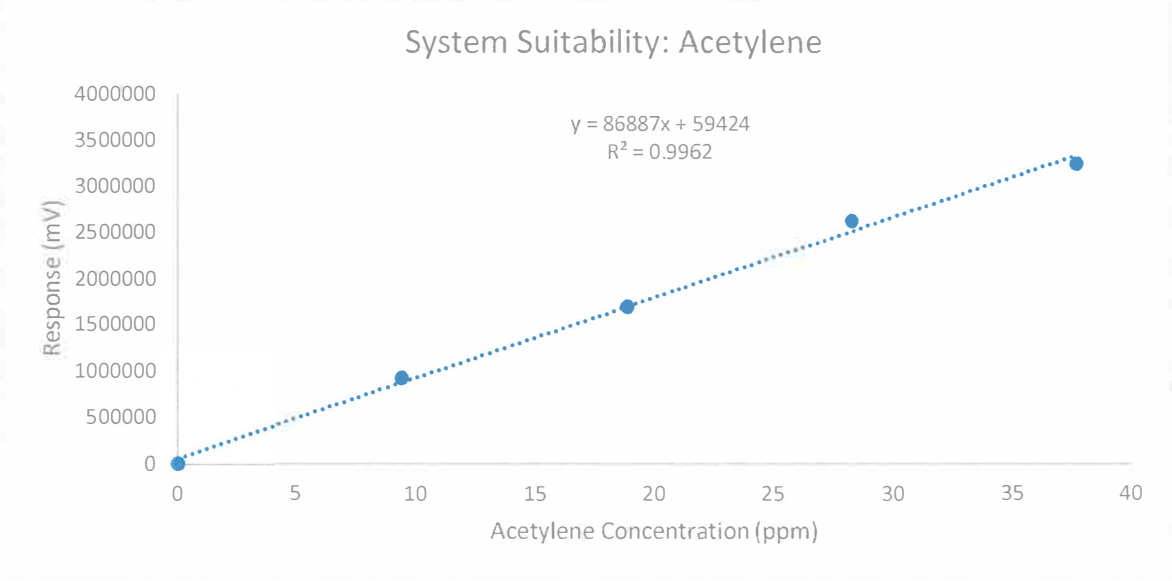
10.1. Analysis: Analyzed all samples prepared in Section 8 using the method parameters defined in Section 7.

10.2. **Calibration and System Suitability**

10.2.1. Calibrate the GC-FID instrument using calibration levels 1, 2, 3, 4 and a blank (0 ppm Acetylene) to verify the instrument has a linear and consistent response to acetylene at time of use.

10.2.2. An r^2 of NLT 0.99 is required for acetylene to pass system suitability.

Cal. Level	Standard	Acetylene Level (ppm)	Area Count	Result
1	0 (Blank)	0	4559	Pass
2	1	9.43	920570	
3	2	18.87	1689960	
4	3	28.30	2630308	
5	4	37.74	3248607	
		r^2 Value (NLT 0.99):	0.9962248	



10.3. **Specificity:**

10.3.1. Obtained GC chromatograms of the following to demonstrate that the peaks of interest are resolved from each other and there is no interference between peaksof interest; identify each peak retention time.

10.3.1.1. Blank

10.3.1.1.1. Area count with acetylene < 0ppm Blank. No interference detected.

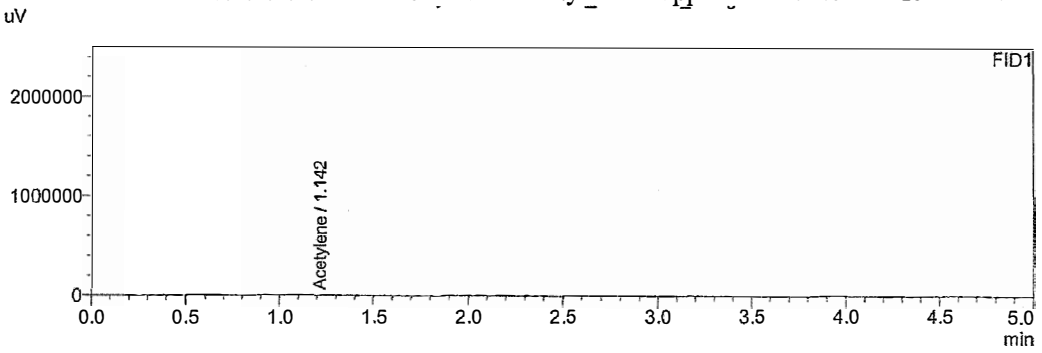


Figure 1: Blank Chromatogram

10.3.1.2. Sample

10.3.1.2.1. Retention Time Acetylene: 1.139 Min.

10.3.1.2.2. Retention Time 2-Propanol: 1.740 Min.

10.3.1.2.3. Resolution: >1.5

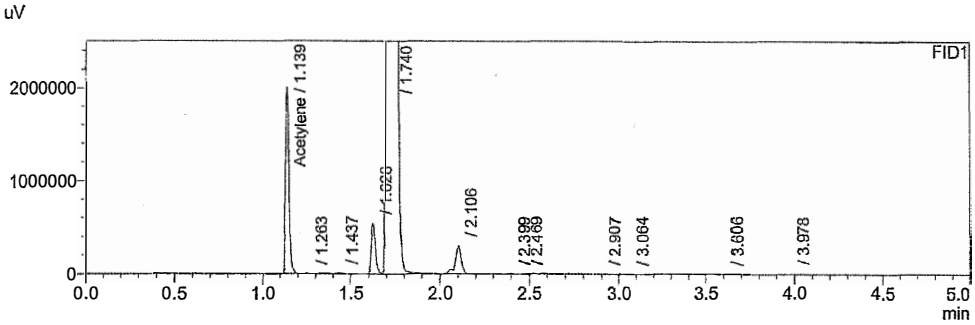


Figure 2: Sample and Acetylene Chromatogram

10.3.1.3. Acetylene

10.3.1.3.1. Area response of 2,630,308 detected, greater than blank response of 4,559.

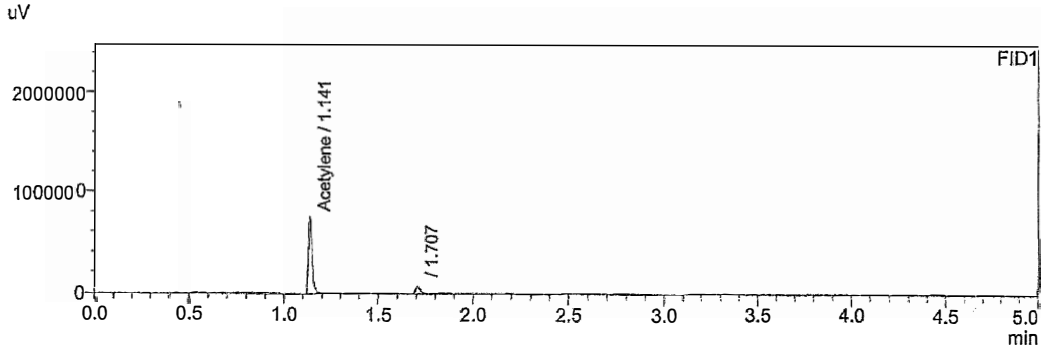


Figure 3: Acetylene Chromatogram

10.4. **Limit of Detection (LOD), and Signal to Noise (S/N):**

10.4.1. Report the mean signal to noise ratio for each solvent in the standard and spiked sample solution from at least three determinations; S/N is NLT 10.

50% Replicate (141.5ppm Spike)	Calculated (ppm)	S/N	Mean S/N (NLT 10)	Result
1	253.980	8,748.92	7,789.07	Pass
2	190.056	5,638.71		
3	254.530	8,979.59		

10.4.2. The 0% spike level sample response should be less than 50% spike level response.

50% Replicate (141.5ppm Spike)	Calculated (ppm)	50% Spike Level Response	0% Level Response	50% Spike Level Response Is Greater Than 0% Spike Level Response	Result
1	253.980	2,266,168	1,141,685	Yes	Pass
2	190.056	1,710,756		Yes	Pass
3	254.530	2,270,950		Yes	Pass

10.4.3. The 50% spike sample response should be less than the 100% spike sample response.

100% Replicate (283.0ppm Spike)	Calculated (ppm)	100% Spike Level Response	50% Level Response	100% Spike Level Response Is Greater Than 50% Spike Level Response	Result
1	336.105	2,979,727	2,082,625	Yes	Pass
2	292.284	2,598,983		Yes	Pass
3	296.868	2,638,811		Yes	Pass

10.4.4. The 100% spike level response should be less than the 150% spike sample response.

150% Replicate (424.5ppm Spike)	Calculated (ppm)	150% Spike Level Response	100% Level Response	150% Spike Level Response Is Greater Than 100% Spike Level Response	Result
1	379.981	3,360,948	2,739,174	Yes	Pass
2	352.001	3,117,845		Yes	Pass
3	361.607	3,201,302		Yes	Pass

10.4.5. The 50% Spike Level requires a S/N of NLT 10.

10.4.5.1. Result: 7,789.07; Pass

11. DOCUMENTATION PROCEDURES:

11.1. Sample Preparation:

11.1.1. Recorded all related raw data including balance printouts if applicable in associated laboratory notebook.

11.1.1.1. Refer to MV10P07-09

11.1.2. Recorded lots numbers, associated calculations and any variance to solution preparations described in the preceding report with justification.

11.1.2.1. Refer to MV10P08 for associated calculations.

11.1.3. Initial and date all applicable printouts and attachments as per laboratory notebooks SOP.

11.1.3.1. Refer to supporting documents for applicable printouts.

11.2. Instrument Run:

11.2.1. Data processing should be automated and performed within the method during data acquisition, any changes to the integration parameters should be justified with supporting evidence for the change and saved to the associated method file.

11.2.1.1. No Changes were made to the data processing parameters.

11.2.2. Specify the method file to be used during the method validation report and report base integration parameters.

11.2.2.1. Refer to method file: 6N HCl IPA Acetylene Screen and Quant.gcm

11.3. Critical Changes:

11.3.1. Dilution Volume adjustment.

11.3.1.1. In order to neutralize 6N HCl in IPA at a 1mL sample size, 0.6mL of 10N NaOH was added to all sample and standard vials in order to keep the dilution magnitude the same. The total volume adjustments were calculated and incorporated into the calibration curve. For example, the 10ppm Acetylene standard in 10mL was calculated to be 9.43ppm Acetylene.

11.3.2. Sample volume adjustment.

11.3.2.1. The initial protocol calls for 1.0g of sample into 10mL of purified water. Due to the volatile nature of the sample 1.0mL was added directly to the headspace vials via calibrated micropipette. Units for the final result are reported as acetylene; weight/volume expressed as ppm (mg/L). Volume expansion was corrected during spike recovery studies by reducing the amount of water added to each vial by 1.0mL to compensate for the sample addition. The change is not considered impactful as dilution factor is not utilized for result determination.

Example Calculation:

$$(10ppm\ Acetylene)(10mL) = (X\ ppm\ Acetylene)(10.6mL) = 9.43ppm\ Acetylene$$

12. CONCLUSION:

12.1. Based upon the studies performed in this validation, The GC-FID method used for analysis of acetylene in 6N HCl in 2-propanol is considered a validated method of analysis as a category II limit test at the BioSpectra Inc. Bangor PA quality control laboratory.

12.1.1. System Suitability: Pass

12.1.1.1. System suitability was demonstrated by a suitable r^2 value for the 5-point calibration curve, an r^2 Value of 0.99 or greater is required for analysis demonstrating linear response to acetylene over various concentration levels of interest. The r^2 value as tested was 0.9962248; meeting requirements.

12.1.2. Specificity: Pass

12.1.2.1. Specificity was shown as 2-propanol, any impurities, and acetylene elute at different times and have USP resolution >1.5 from one another.

12.1.3. Limit of Detection: 14.2ppm

12.1.3.1. Limit of Detection for acetylene was experimentally determined to be 50% of the 283ppm limit, or, 14.2ppm in vitro.

12.1.3.2. Lower limit of detection may be demonstrated by a S/N (Signal to noise) of NLT 10 for acetylene in a freshly prepared standard analyzed within a suitable GC sequence (After system suitability has passed).

13. ROUTINE TEST PROCEDURE:

13.1. Pre-Requisite Solutions:

13.1.1. Acetylene Stock Solution:

13.1.1.1. Prepare a stock solution of acetylene in purified water by slowly gassing acetylene from a regulator under the surface of the purified water. Gas 1-2 minutes, inverting the solution periodically to ensure homogenous dissolution. Mix thoroughly and analyze TOC of the gassed water (now the acetylene stock solution) and the water used to prepare the stock solution. Calculate actual concentration based off TOC analysis. Include calculations in notebook.

13.1.1.2. Mix thoroughly and calculate ppm Acetylene in the stock solution using the following equation:

$$ppm\ Acetylene\ Stock = \frac{Stock\ Solution\ ppm\ Carbon - Purified\ Water\ ppm\ Carbon}{0.9225}$$

13.1.1.3. Prepare 100mL of 50ppm Acetylene standard by diluting an appropriate amount of the stock solution to 50ppm using the following equation by solving for V_1:

$$C_1V_1 = C_2V_2 \rightarrow V_1 = \frac{C_2V_2}{C_1}$$

13.2. Calibration Standards, Limit Standard, and Sample Preparation Table:

Sample ID	Amount of 10N NaOH (mL)	Amount of Purified Water (mL)	Amount of 50ppm Acetylene Standard (mL)	Amount of Sample (mL)	Total Volume (mL)
Calibration Level 1 (0.00ppm)	0.60	10.0	0.00	0.00	10.6
Calibration Level 2 (9.43ppm)	0.60	8.00	2.00	0.00	10.6
Calibration Level 3 (18.87ppm)	0.60	6.00	4.00	0.00	10.6
Calibration Level 4 (28.30ppm)	0.60	4.00	6.00	0.00	10.6
Calibration Level 5 (37.74ppm)	0.60	2.00	8.00	0.00	10.6
Sensitivity Check Standard*	0.60	8.80	1.20	0.00	10.6
Sample Solution	0.60	9.00	0.00	1.00	10.6

*Corresponds to 60ppm w/v in 1:10.6mL diluted sample.

13.3. Calibration and System Suitability

13.3.1. Calibrate the GC-FID instrument using calibration levels 1, 2, 3, 4 and 5.

13.3.2. An r^2 of NLT 0.99 is required for acetylene to pass system suitability.

13.4. Analyze all samples prepared in Section 13.2 using the method parameters defined in Section 7.

13.5. Evaluating Testing Data:

13.5.1. System Suitability: r^2 value of NLT 0.99 is required for data report.

13.5.2. Sensitivity Check Standard: Prepare a *Sensitivity Check Standard* corresponding to the applicable limit of analysis and report S/N ratio. (60ppm w/v Example is illustrated in table 13.2); a S/N of NLT 10 is required.

13.5.3. Sample Solution: The corresponding acetylene response in the *sample solution* may not exceed the corresponding area response in the *Sensitivity Check Standard* solution.

13.6. Print and initial and date the sequence/batch file and transcribe results to applicable laboratory documentation.