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ANALYTICAL METHOD VALIDATION PROTOCOL: ACETYLENE DETECTION BY HEAD SPACE GC FID

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1. PURPOSE:

1.1. The purpose of this Protocol is to:

1.1.1. Provide a comprehensive validation protocol to validate acetylene analysis for products in compliance with ICH Q2.

1.1.2. Ensure that the detectability of acetylene is adequately evaluated and validated as a Category II Limit based test at a level of 300ppm based on a dilution of 1g/10mL in vitro sample preparation.

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 generate data to be detailed in a method validation report to prove that the procedure for determining the amount acetylenes in samples via GC-FID meets all requirements for quantitative method as stated below.

Parameters	Procedure	Acceptance Criteria
System Suitability	Calibrate the GC-FID instrument with 5 levels of acetylene varying from 10-50ppm.	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 Protocol 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 10% w/v solutions of aqueous soluble articles.
2.4. This validation is intended to be performed and validated 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 protocol.
3.2. QC Analysts, or Process Technology Chemists are responsible for performing the testing stated in Section 9.0 of the protocol.
3.3. The Associate Director of Product Life Cycle is responsible for completing the Method Validation Report using conclusions made from the results obtained from this method validation protocol to assess the performance of the analysis. Each material or product of interest will have an individual validation report summarizing method performance. The protocol may be executed routinely for qualification of new materials.
3.4. Safety: Standard laboratory safety regulations apply. Before working with any chemical, readand understand the Safety Data Sheet (SDS).

4. REFERENCES:

4.1. BSI-SOP-0098, Balance SOP
4.2. BSI-SOP-0126, Laboratory Notebooks
4.3. BSI-SOP-0316, Shimadzu QP2010S GC SOP
4.4. BSI-SOP-0436, Analytical Methods Validation Master Plan
4.5. ICH Q3A(R2)

5. VALIDATION REQUIREMENTS:

5.1. Equipment
5.1.1. All equipment used in this Validation must be 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, DCN: BSI-SOP-0436. All personnel executing this protocol are trained on GC analysis or are considered Subject Matter Experts. The protocol will be assigned as mark as read training to QC analysts involved with the execution.
5.3. Supplies:
5.3.1. All supplies in this analytical method Validation must be appropriate for the intended use.
5.4. Reagents:
5.4.1. All reagents must be current, meet required specifications and be suitable for the intended use.

5.5. Reference Standards:
5.5.1. Any standards required in this validation protocol are to be listed in the Materials and Equipment section of the Analytical Method Validation Report. The name of the reference standard, lot number, manufacture, date of opening, date of expiration, and part number will be documented.

6. MATERIALS AND EQUIPMENT:

6.1. All expected materials and equipment utilized in this Validation protocol are outlined in this section. This is a list of the materials and equipment required to satisfy the needs of the protocol. Expected materials and equipment are listed below. Any items not defined below willbe detailed in the analytical method validation report.

6.2. Equipment:

- 6.2.1. Analytical Balance
- 6.2.2. Automatic Pipette – 1000 µL
- 6.2.3. Automatic Pipette – 5000 µL
- 6.2.4. Automatic Pipette – 10 mL
- 6.2.5. GC-MS
  - 6.2.5.1. Make: Shimadzu
  - 6.2.5.2. Model: GC-2010, equipped with FID detector.
- 6.2.6. GC Column: 30m Capillary Column USP Phase G43 or equivalent
  - 6.2.6.1. Make: Phenomenex Zebron
  - 6.2.6.2. Part Number:7HG-G005-27
- 6.2.7. Laboratory Notebook

6.3. Reagents

- 6.3.1. Purified Water/MilliQ Water
  - 6.3.1.1. Supplier: BioSpectra Inc.
  - 6.3.1.2. Meets or Exceeds USP Purified Water specification.

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

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. 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.

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

ppm Acetylene Stock = (Stock Solution ppm C – Purified water ppm C) / 0.9225

8.1.1.3. 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:

$$(C_2)(V_2) = (C_1)(V_1)$$

(50ppm Acetylene Standard)(100mL) = (____ ppm Acetylene Stock)(V_1 mL)

8.2. Calibration Standards and Spike Diluent Preparation:

8.2.1. 0 ppm (Blank)

8.2.1.1. 10mL of Purified water or equivalent.

8.2.2. Calibration Level 1 (10ppm Acetylene)

8.2.2.1. Pipette 8.0mL of water to headspace vial.
8.2.2.2. Pipette 2.0mL of 50ppm Acetylene Standard Solution to a headspace vial.
8.2.2.3. Immediately cap and seal the vial.
8.2.2.4. Mix thoroughly.

8.2.3. Calibration Level 2 (20ppm Acetylene)
8.2.3.1. Pipette 6.0mL of water to headspace vial.
8.2.3.2. Pipette 4.0mL of 50ppm Acetylene Standard Solution to a headspace vial.
8.2.3.3. Immediately cap and seal the vial.
8.2.3.4. Mix thoroughly.
8.2.4. Calibration Level 3 (30ppm Acetylene)
8.2.4.1. Pipette 4.0mL of water to headspace vial.
8.2.4.2. Pipette 6.0mL of 50ppm Acetylene Standard Solution to a headspace vial.
8.2.4.3. Immediately cap and seal the vial.
8.2.4.4. Mix thoroughly.
8.2.5. Calibration Level 4 (40ppm Acetylene)
8.2.5.1. Pipette 2.0mL of water to headspace vial.
8.2.5.2. Pipette 8.0mL of 50ppm Acetylene Standard Solution to a headspace vial.
8.2.5.3. Immediately cap and seal the vial.
8.2.5.4. Mix thoroughly.
8.2.6. Calibration Level 5 (50ppm Acetylene)
8.2.6.1. Pipette 10.0mL of 50ppm Acetylene Standard Solution to a headspace vial.
8.2.6.2. Immediately cap and seal the vial.

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. Crimp to seal, mix thoroughly.
8.3.2. Specificity Solution 2- Acetylene
8.3.2.1. Refer to Calibration Level 3 (30ppm) Acetylene
8.3.3. Specificity Solution 3- Sample Screen
8.3.3.1. Weigh and add 1.0g of sample to head space vial.
8.3.3.2. Add 10 mL of purified water to headspace vial.
8.3.3.3. Dissolve.
8.3.3.4. Crimp to seal, mix thoroughly.

8.4. Limit of Detection Solution Preparations (Prepare in Triplicate):
8.4.1. 50% Limit Level Acetylene Spike (150ppm w/v in sample)
8.4.1.1. Weigh 1.0 g of sample and add to head space vial.
8.4.1.2. Add 7.0 mL of water to vial.
8.4.1.3. Add 3.0 mL of 50ppm Acetylene Standard Solution
8.4.1.4. Dissolve.
8.4.1.5. Crimp to seal, mix thoroughly.
8.4.2. 100% Limit Level Acetylene Spike (300ppm w/v in sample)
8.4.2.1. Weigh 1.0 g of sample and add to head space vial.
8.4.2.2. Add 4.0 mL of water to vial.
8.4.2.3. Add 6.0 mL of 50ppm Acetylene Standard Solution
8.4.2.4. Dissolve.
8.4.2.5. Crimp to seal, mix thoroughly.
8.4.3. 150% Limit Level Acetylene Spike (450ppm w/v in sample)
8.4.3.1. Weigh 1.0 g of sample and add to head space vial.
8.4.3.2. Add 1.0 mL of water to vial.
8.4.3.3. Add 9.0 mL of 50ppm Acetylene Standard Solution
8.4.3.4. Dissolve.
8.4.3.5. Crimp to seal, mix 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 5 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.

- Diluent
- Sample
- Acetylene

9.3.2. Limit of Detection (LOD), and Signal to Noise (SN): NLT 300ppm (0.03%)

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 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. DOCUMENTATION PROCEDURES:

10.1. Sample Preparation:

10.1.1. Record all related raw data including balance printouts if applicable in associated laboratory notebook.

10.1.2. Record lots numbers, associated calculations and any variance to solution preparations described in the preceding protocol with justification.

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

10.2. Instrument Run:

10.2.1. Print and initial and date sequence/batch file.

10.2.2. 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.

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

10.3. Report:

10.3.1. Upon satisfactory completion of the protocol, a method validation report will be written.

10.4. Standard Operating Procedure:

10.4.1. Upon satisfactory completion of this protocol, a standard operating procedure will be implemented into the appropriate test methods or as a stand-alone document to provide quality control personnel with an appropriate method for analysis for routine use.