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ANALYTICAL METHOD VALIDATION REPORT: QUANTIFICATION OF FORMALDEHYDE BY DERIVATIZATION WITH PENTAFLUOROBENZYLHYDROXYL AMINE

TABLE OF CONTENTS

1. PURPOSE:

1.1. The purpose of this report is to:

1.1.1. Ensure that the Quantification of Formaldehyde by Derivatization with Pentafluorobenzylhydroxyl Amine (PFBHA) analytical method is adequately evaluated as a Category II Quantitative analytical method.

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

Parameters	Procedure	Acceptance Criteria
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 or mass fragment tables if identified.	Interference should not be present between peaks of interest and diluents or reagents used.
Accuracy	Perform accuracy experiments by spiking formaldehyde into an appropriate sample matrix over a minimum of three (3) concentration levels encompassing the reporting threshold to 120% of the analyte specification. Sample solutions for spiking are prepared in triplicate for each spike level (N = 9). Calculate the average recovery at each concentration level.	80 to 110% Recovery at Each Concentration
Linearity and Range	Prepare a minimum of five concentration levels of formaldehyde encompassing a minimum range from the reporting level to at least 120% above the specification of the analyte. Analyze at the limit, at least 120% above and at least 80% below in triplicate.	Correlation coefficient (R^2) $\geq$ 0.950
Method Precision	Use data from "Accuracy" to obtain %RSD of reported Formaldehyde content.	RSD $\leq$ 10.0%
Detection limit (LOD)	Report the analyte level that gives a minimum signal-to-noise ratio of 3:1 (USP)	Report
Detection Quantification (LOQ)	Report the analyte level that gives a minimum signal-to-noise ratio of 10:1 (USP)	Report
Intermediate Precision	NMT 10% RSD between identically prepared samples analyzed by two analysts, on two different calibration curves, on two different mass spectrometer tunes, and on two different days.	NMT 10% RSD
Solution Stability	Analyze aged (previously prepared) standard and spiked sample solutions against a freshly prepared standard solution	Report % recovery of aged sample and standard solution as well as storage conditions (Time, temperature, container)

2. SCOPE:

2.1. This Analytical Method Validation report applies to the Quantification of Formaldehyde via headspace GC-MS determination.
2.2. This method validation is a Category II quantitative analytical method validation.
2.3. The method applies to the analysis of formaldehyde (CAS 50-00-0) in water and in 10% w/v solutions of tris (tromethamine).

3. RESPONSIBILITIES:

3.1. The Executive Director, Quality Control is responsible for the control, implementation and maintenance of this report.
3.2. Qualified personnel are responsible for performing the testing stated in Section 9.0 of the report.
3.3. The Quality Control Manager was responsible for completing the Method Validation Report using conclusions made from the results obtained from the method validation protocol to assess the performance of the analysis.

4. REFERENCES:

4.1. BSI-PRL-0272, Analytical Method Validation Protocol: Quantification of Formaldehyde by GC-MS
4.2. BSI-SOP-0098, Balance SOP
4.3. BSI-SOP-0126, Laboratory Notebooks
4.4. BSI-SOP-0436, Analytical Methods Validation Master Plan
4.5. ICH Q2
4.6. ICH Q3A, B
4.7. ICH M7
4.8. Shimadzu QP2010S GC/MS SOP
4.9. USP NF <621>
4.10. Application Note: Perkin Elmer Detection and Quantification of Formaldehyde by Derivatization with Pentafluorobenzylhydroxyl Amine in Pharmaceutical Excipients by Static Headspace GC-MS, Padmaja Prabhu

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 performing this Validation must be properly trained in accordance with the Analytical Methods Validation Master Plan, DCN: 16-001438 v.3.0.
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 will be detailed in the analytical method validation report.

6.2. Equipment:

- 6.2.1. Analytical Balance
- 6.2.2. Automatic Pipette – 20 µL
- 6.2.3. Automatic Pipette – 1000 µL
- 6.2.4. Automatic Pipette – 5000 µL
- 6.2.5. Automatic Pipette – 10 mL
- 6.2.6. GC-MS
  - 6.2.6.1. Make: Shimadzu
  - 6.2.6.2. Model: GC-2010, GCMS-QP2010S
- 6.2.7. GC Column: Elite Series Capillary Column
  - 6.2.7.1. Make: Perkin Elmer Elite – 5 MS
  - 6.2.7.2. Part Number: N9316282
  - 6.2.7.3. Dimensions: 30 meter, 0.25 mm i.d., 0.25 µm film thickness
  - 6.2.7.4. Phase: 1,4-bis(dimethylsiloxy) phenylene dimethyl polysiloxane
- 6.2.8. Laboratory Notebook

6.3. Reagents:

- 6.3.1. PFBHA
  - 6.3.1.1. Supplier: Acros
  - 6.3.1.2. Lot: A0400963
  - 6.3.1.3. Opened: 3/20/19
  - 6.3.1.4. Expires: 5/31/20
- 6.3.2. Tris base
  - 6.3.2.1. Supplier: Angus
  - 6.3.2.2. Lot: D609J7T031
  - 6.3.2.3. Opened: 11/5/19
  - 6.3.2.4. Expires: 7/29/22
- 6.3.3. Hydrochloric Acid
  - 6.3.3.1. Supplier: Fisher
  - 6.3.3.2. Lot: 0000125580
  - 6.3.3.3. Opened: 10/25/19
  - 6.3.3.4. Expires: 9/30/20
- 6.3.4. Cyclohexanone
  - 6.3.4.1. Supplier: Fisher Chemical
  - 6.3.4.2. Lot: 185310
  - 6.3.4.3. Opened: 7/8/19
  - 6.3.4.4. Expires: 9/30/23
- 6.3.5. Purified Water
  - 6.3.5.1. Supplier: BioSpectra Inc.
  - 6.3.5.2. Meets or Exceeds USP Purified Water specifications.

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

6.4.2. Funnels
6.4.2.1. Verex Seal, 20 mm diameter, PTFE/Silicone
6.4.2.2. Supplier: VWR
6.4.3. 150 mL Beakers
6.4.3.1. Supplier VWR
6.4.4. Volumetric Flasks, Class A, Various Sizes
6.4.4.1. Supplier: VWR
6.4.5. Vespel Graphite Ferrule
6.4.5.1. Manufacturer: Phenomenex
6.4.5.2. Catalog Number: AGO-4708

6.5. Reference Standards:
6.5.1. Formaldehyde (16% w/v in water)
6.5.1.1. Supplier: Thermo Scientific
6.5.1.2. Catalog Number: P128908
6.5.1.3. Lot: UE2770071, UJ2869271
6.5.1.4. Opened: Day of use, single use glass vial.
6.5.1.5. Expires:6/3/24, 11/27/24

7. METHOD PARAMETERS:

7.1. **HS-20**
7.1.1. Oven Temp: 60.0°C
7.1.2. Sample Line Temp.: 150.0°C
7.1.3. Transfer Line Temp: 160.0°C
7.1.4. Shaking Level: 3
7.1.5. Injection Count: 1
7.1.6. Pressurizing Gas: 75.0 kPa
7.1.7. Equilibrating Time: 20.00 min
7.1.8. Pressurization Time: 0.50 min
7.1.9. Pressure Equilibration Time: 0.20 min
7.1.10. Load Time: 0.20 min
7.1.11. Load Equilibration Time: 0.20 min
7.1.12. Injection Time: 0.20 min
7.1.13. Needle Flush Time: 5.00 min
7.1.14. GC Cycle Time: 40.00 min
7.1.15. Check System Ready: ON
7.1.16. Extended System Ready Check: 45 min
7.1.17. Check GC Ready: ON
7.1.18. Extended GC Ready Check: 10 min
7.1.19. Analysis Mode: Constant
7.1.20. Needle Check: Yes
7.1.21. Action on Leak Check Error: Continue
7.1.22. Action with No Vial in Tray: Skip

7.2. **GC-2010**
7.2.1. Column Oven Temperature: 50.0°C
7.2.2. Injection Mode: Split
7.2.3. Flow Control Mode: Linear Velocity
7.2.4. Pressure: 75.4 kPa
7.2.5. Total Flow: 8.8 mL/min
7.2.6. Column Flow: 1.30 mL/min
7.2.7. Linear Velocity: 41.4 cm/sec
7.2.8. Purge Flow: 1.0 mL/min

7.2.9. Split Ratio: 5
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 °C per Min	Temperature (°C)	Hold Time (min)
-	50.0	3.00
7.00	150.0	5.00
40.00	280.0	5.00

7.3. **Ready Checks**
7.3.1. Column Oven: YES
7.3.2. HS: YES
7.3.3. MS: YES
7.3.4. HS Carrier: YES
7.3.5. HS Purge: YES
7.3.6. APC1: YES
7.3.7. External Wait: NO
7.3.8. Equilibrium Time: 3.0 min
7.3.9. CRG(INJ): OFF
7.3.10. APC1: 75.0kPa

7.4. **Mass Spectrometer**
7.4.1. Group 1 Event 1
7.4.1.1. Start Time 1.00 min
7.4.1.2. End Time 30.00 min
7.4.1.3. ACQ Mode: Scan
7.4.1.4. Event Time: 0.20 sec
7.4.1.5. Scan Speed: 2000
7.4.1.6. Start m/z: 40.00
7.4.2. Group 1 Event 2
7.4.2.1. Start Time: 1.00 min
7.4.2.2. End Time: 30.00 min
7.4.2.3. ACQ Mode: SIM
7.4.2.4. Event Time: 0.20 sec
7.4.2.5. Ch1-m/z: 178.00
7.4.2.6. Ch2-m/z: 181.00
7.4.2.7. Ch3-m/z: 197.00

8. METHOD PERFORMANCE REPORT:

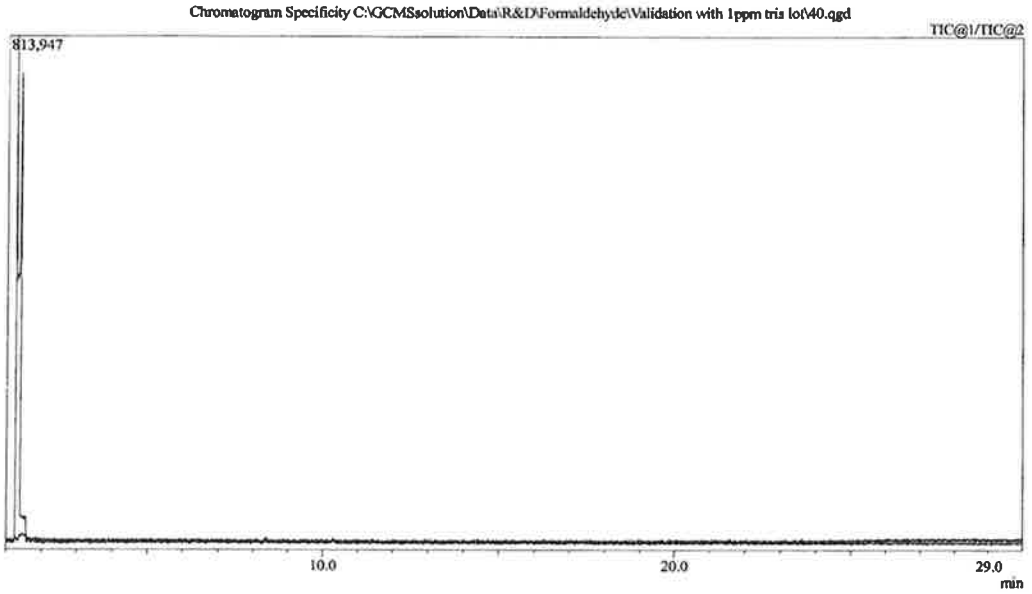
8.1. Evaluation of Performance Data:

8.1.1. Specificity:

8.1.1.1. GC Chromatograms were obtained of each analyte to ensure that there was no interference between peaks of interest.

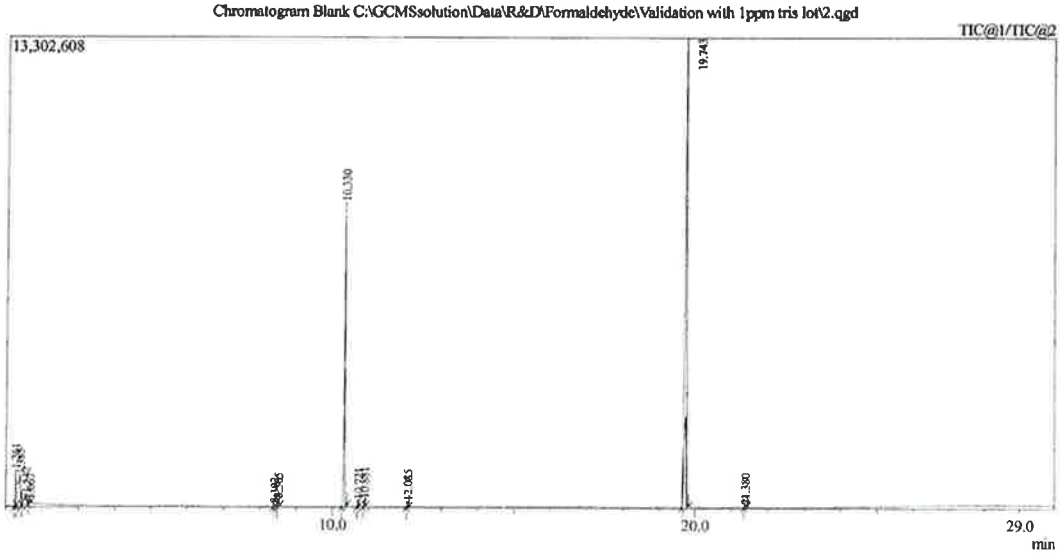
8.1.1.1.1. Specificity 1: Tris Sample Solution

8.1.1.1.1.1 No Peak Present when added alone in purified water.



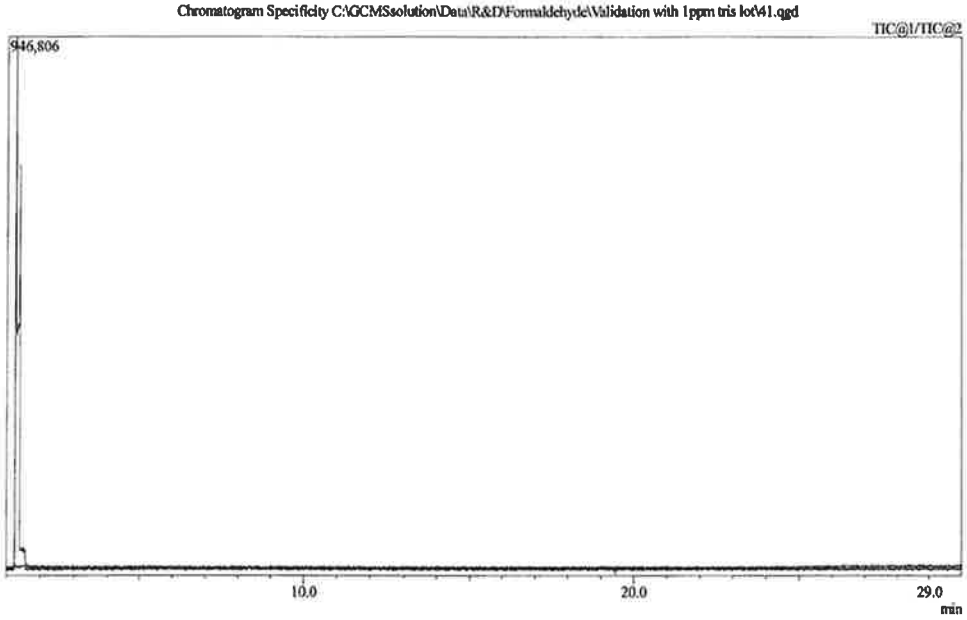
8.1.1.1.2. Specificity 2: Diluent(s) (Refer to Blank)

8.1.1.1.2.1 Relative retention of 1.9 to Cyclohexanone



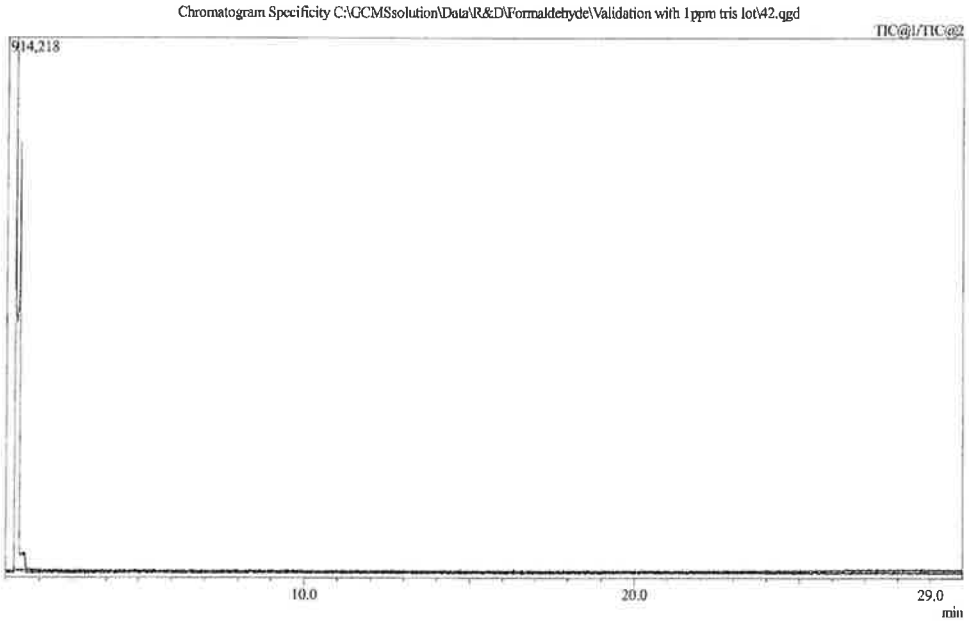
8.1.1.1.3. Specificity 3: Sodium Chloride

8.1.1.1.3.1 No Peak Present when added alone in purified water.



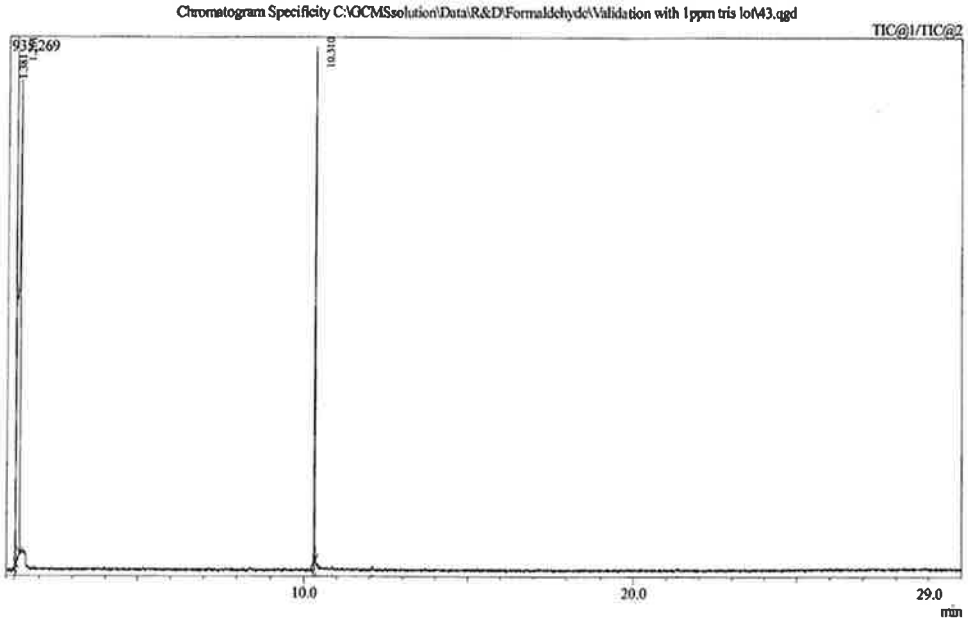
8.1.1.1.4. Specificity 4: Formaldehyde

8.1.1.1.4.1 No peak Present when added alone in purified water.



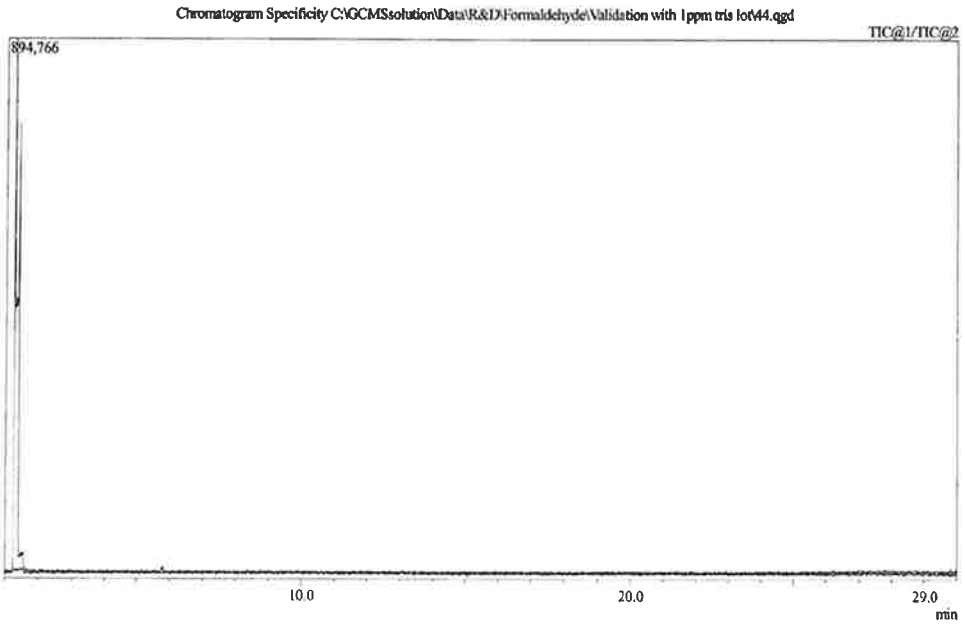
8.1.1.1.5. Specificity 5: PFBHA

8.1.1.1.5.1 No interfering derivative present.



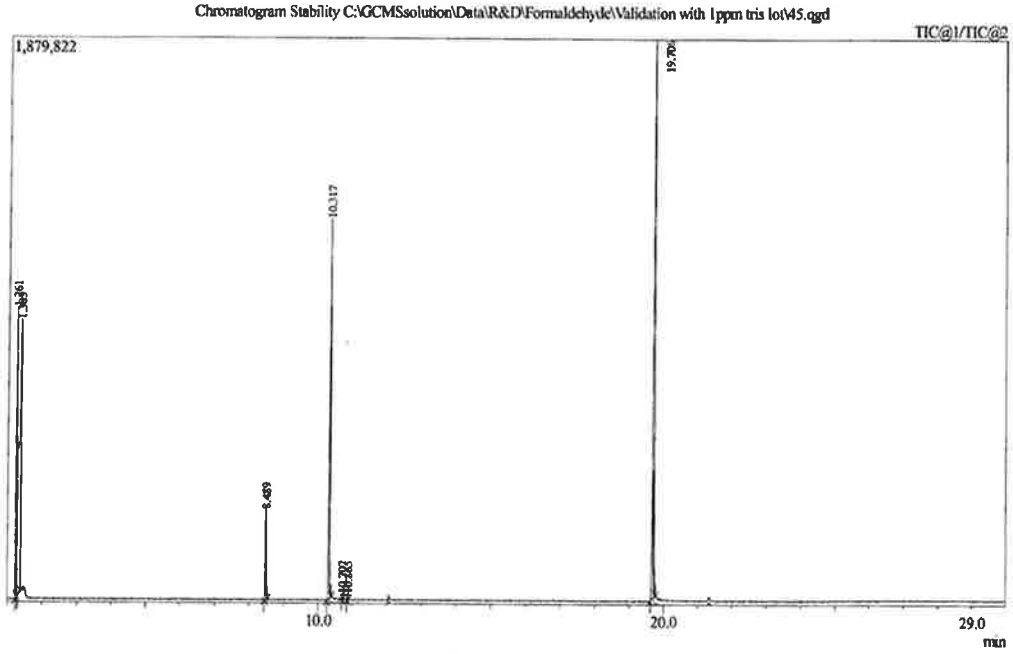
8.1.1.1.6. Specificity 6: Cyclohexanone

8.1.1.1.6.1 No peak present when added alone in purified water.



8.1.1.1.7. Specificity 7: Formaldehyde Response

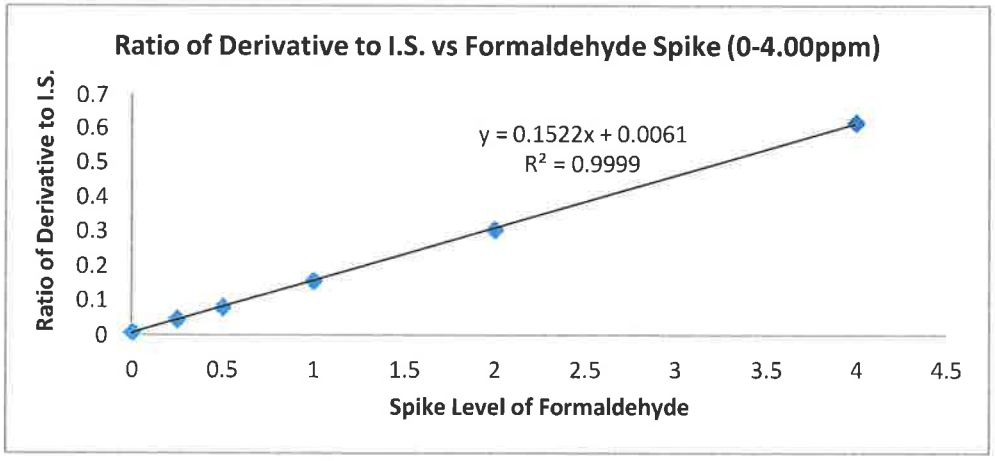
8.1.1.1.7.1 Clear formaldehyde response at 95ppb (0.95ppm calculated % w/w basis in 1g sample size) solution at retention time 8.489 min.

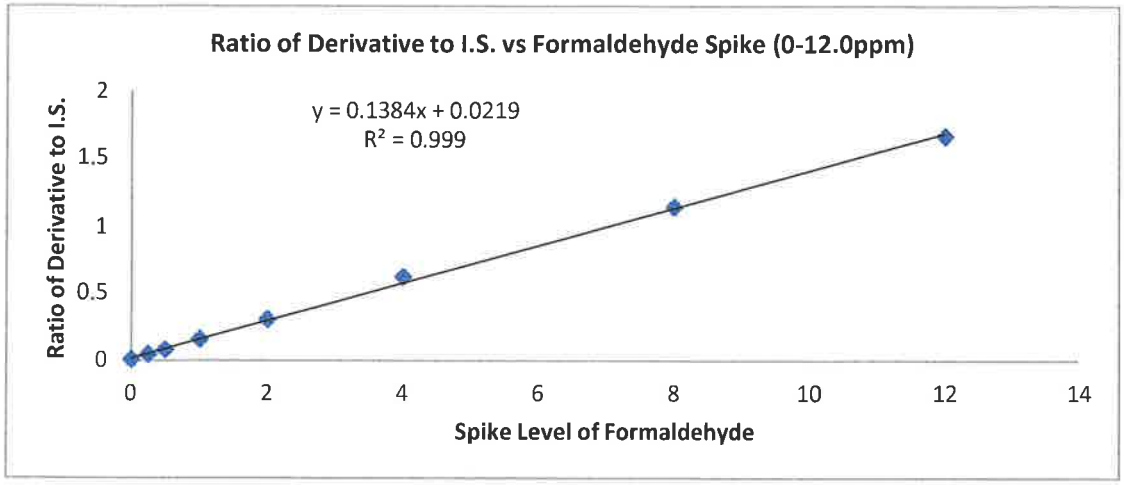


8.1.1.2. Conclusion: Specificity was achieved for all components of the analysis. Each volatile component had distinguished retention times and separation or did not appear in the chromatogram(s).

8.1.2. Calibration Curves:

Formaldehyde (ppm) = (Ratio of Derivative-0.0061) / 0.1522

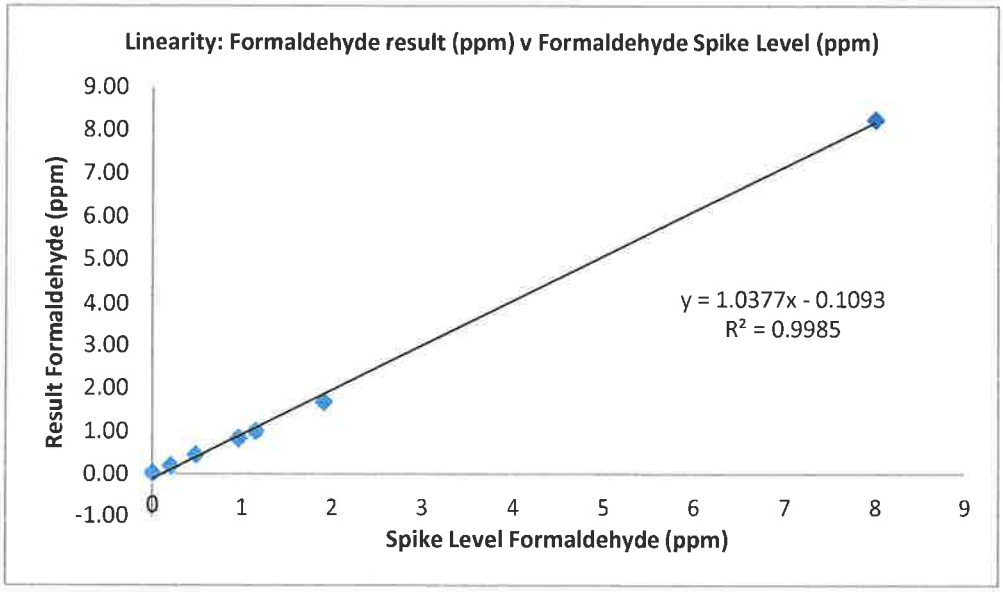




8.1.2.1. The figure above demonstrates a slight inhibition of the reaction between the PFBHA and formaldehyde at high concentrations of formaldehyde (>4.00ppm). The method shows consistent linearity up to 400ppb formaldehyde in solution, measuring equivalency of 4ppm formaldehyde per 1g of sample. The 0-4ppm calibration curve was used for sample calculations as the range is more appropriate for the limit of 0.95ppm max formaldehyde.

8.1.3. Linearity (NLT 0.95 R^2)

8.1.3.1. The data obtained from independent formaldehyde recovery analyses was used to graph the average of each formaldehyde level measured, n=7.



8.1.3.2. Correlation Coefficient: 1.0377

8.1.3.3. Y-Intercept: -0.1093 8.1.3.4. R2: 0.9985

8.1.3.4. Result: Pass

8.1.3.5. Conclusion: The analysis shows a clear and linear response for formaldehyde.

8.1.4. Accuracy (80-120% Recovery): Pass

8.1.4.1. Data:

Controlled Formaldehyde Spike Level (ppm)	Result Formaldehyde (ppm)[1]	% Recovery (80-120%)	Pass / Fail
0.20	0.18	90%	Pass
0.48 (50% Limit)	0.44	91%	Pass
0.95 (100% Limit)	0.83	87%	Pass
1.14 (120% Limit)	1.00	88%	Pass
1.90	1.67	88%	Pass
8.00	8.25	103%	Pass

[1]If data sets contained more than a single replicate the average of the data was used to calculated recovery data.

8.1.4.2. Calculated and reported % recovery as:% Recovery = (Reported Concentration/Theoretical Concentration)*100

8.1.4.3. Spike recoveries met requirements of 80-120% recovery from a range of 0.20ppm to 8.00ppm formaldehyde.

8.1.5. Precision (Repeatability) (NMT 10% RSD):

8.1.5.1. Result: Pass

8.1.5.2. Discussion: ICH Q2 defines precision or repeatability to be assessed at 6 determinations at 100% of the test concentration or a minimum of 9 determinations covering the specified range of the procedure. The data obtained and presented below meets ICH Q2 requirements for precision and therefore passes.

Controlled Formaldehyde Spike Level (ppm)	Result Formaldehyde (ppm)	% RSD (NMT 10%)	Result
0.48	0.45	3%	Pass
0.48	0.43		
0.48	0.42		
0.95	0.86	2.8%	Pass
0.95	0.83		
0.95	0.85		
0.95	0.81		
0.95	0.81		
0.95	0.81		
1.14	0.98	1%	Pass
1.14	1.00		
1.14	1.01		
8.00	8.14	2%	Pass
8.00	8.20		
8.00	8.41		

8.1.5.3. Accuracy and Precision were also performed at the 100% level with the proposed test sample introduced into the sample matrix, at n=6. The results are corrected for endogenous formaldehyde present in the testing sample. With tris introduced the method meets requirements for accuracy and precision.

Accuracy and Precision at 100% Level with Tris n=6				
Controlled Formaldehyde Spike Level in Tris (ppm)	**Result Formaldehyde (ppm)**	**Recovered Formaldehyde (ppm)**	**% Recovery (80-120%)**	**% RSD (NMT 10%)**
0	0.60			5%
0	0.64			
0	0.58			
0.95	1.50	0.90	95%	4%
0.95	1.54	0.93	98%	
0.95	1.46	0.85	89%	
0.95	1.43	0.83	87%	
0.95	1.43	0.82	86%	
0.95	1.40	0.80	84%	

8.1.6. Intermediate Precision NMT 10% RSD: Pass

8.1.6.1. Intermediate precision was investigated by performing six replicates of the same sample spiked at the 100% of the limit of analysis by two different chemists, on two different days, with two different calibration curves and two different instrument tune parameters. The acceptance criteria was held to the same as instrumental precision, NMT 10% RSD. The analysis met requirements for intermediate precision which demonstrates that the analysis is robust as well as precise.

Intermediate Precision Data			
Analyst and Date	**Replicate ID**	**Result Formaldehyde (ppm)**	**% RSD (NMT 10%)**
Analyst I 12/19/19	1	1.50	9.9%
	2	1.54	
	3	1.46	
	4	1.43	
	5	1.43	
	6	1.40	
Analyst II 1/6/20	1	1.82	
	2	1.83	
	3	1.74	
	4	1.64	
	5	1.71	
	6	1.70	

8.1.7. Range: 0.04ppm-8.00ppm

8.1.7.1. Result: ICH Q2 Defines the range of impurity analyses from the detection limit to a measured degree beyond the intended specification and should commensurate with the level at which the impurity must be controlled. The range of the analysis is 0.04ppm-8.00ppm (mg/kg) formaldehyde with respect

to sample size. The level of range was determined to be from the limit of quantification level to 8.00ppm. Sample content higher than 8.00ppm formaldehyde should be appropriately diluted before use if higher than 8.00ppm.

8.1.8. Limit of Detection (LOD), Limit of Quantification (LOQ), and Signal to Noise (SN)

8.1.8.1. Reported the Signal to Noise of the response of the formaldehyde derivative in the blank samples by assessing the standard deviation of the response, n=6.

8.1.8.2. Data:

Blank ID	Derivative Area	Area I.S.	Ratio	Standard Deviation of Response (SN)
1	421293	43871073	0.009602979	0.0006
2	430556	47295692	0.009103493	
3	392628	48392525	0.008113402	
4	429667	50260008	0.008548884	
5	444660	52732297	0.008432403	
6	427887	54183324	0.007897024	

8.1.8.3. Limit of Detection: LOD is expressed as $3.3\sigma/S$: 0.01ppm

8.1.8.3.1. Where σ is the SN: 0.004

8.1.8.3.2. Where S is the slope of the calibration curve: 0.1522

8.1.8.4. Limit of Quantitation: LOQ is expressed as $10\sigma/S$: 0.04ppm

8.1.9. Solution Stability

8.1.9.1. Where σ is the SN: 0.004

8.1.9.2. Where S is the slope of the calibration curve: 0.1522

8.1.9.3. Assessed a 0.95ppm formaldehyde standard solution after at least 24 hours.

8.1.9.4. % Recovery was 81% for formaldehyde after 33 hours. Standard solutions and samples solutions should be freshly prepared for each analysis as the formaldehyde standard is not stabilized with chemical agents and over time may form polymers to paraformaldehyde (PFA).

9. CONCLUSION:

9.1. Modifications to Protocol:

9.1.1. Calibration:

9.1.1.1. The calibration curve was intended to be run from 0-8.00ppm, instead the curve was reduced to from 0-4.00ppm to better suit the intended range of analysis. Linearity was investigated to 12.0ppm and was found to still demonstrate linearity at higher than required levels. The maximum range of the analysis was not investigated further.

9.1.2. Acceptance Criteria:

9.1.2.1. % Recovery for accuracy was expanded from 90-110% to 80-120% to align with FDA recommendations.

9.1.3. Instrument Run:

9.1.3.1. The instrumental run and method did not undergo any modifications to the initial protocol.

9.2. **Standard Operating Procedure:**

9.2.1. A standard operating procedure was implemented for screening of water-soluble materials, including tris, utilizing this method. Refer to BSI-ATM-0050.

9.3. **Statement of Validation and Discussion**

9.3.1. The method of quantification of formaldehyde by derivatization with pentafluorobenzyl hydroxylamine is considered a validated method for use in quantifying formaldehyde in tris and water and is approved for official use at the BioSpectra Bangor PA facility.