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## TROMETHAMINE UNSPECIFIED DEGRADATION PRODUCTS VIA GC-FID

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**1. PURPOSE:**

- 1.1. To provide Analysts with a procedure for determining Tromethamine unspecified degradation products determination by GC with FID determination.

**2. SCOPE:**

- 2.1. This analytical method applies to the Tromethamine unspecified degradation products determination via GC-FID.
- 2.2. This Tromethamine unspecified degradation products method was validated as a Category II quantitative analytical method.
- 2.3. The unspecified degradation product specification is not more than 0.03% each.
- 2.4. The method applies to the Tromethamine raw materials, in- process materials, stability materials and finished goods material analysis.

**3. RESPONSIBILITIES:**

- 3.1. The Director of Laboratory Services is responsible for the control, training, implementation and maintenance of this procedure.
- 3.2. The analytical chemists, or qualified designees, are responsible for performing the testing in this procedure.
- 3.3. The analytical chemists performing this procedure, with help from the Laboratory Manager if necessary, are responsible for documenting the results obtained from testing.
- 3.4. Safety: Standard laboratory safety regulations apply. Before working with any chemical and understand the Safety Data Sheet (SDS).

**4. REFERENCES:**

- 4.1. BSI-PRL-0688, Analytical Method Validation Protocol: Tromethamine Assay and Degradation Products Via GC FID
- 4.2. BSI-RPT-1373, Analytical Method Validation Report: Tromethamine Unspecified Degradation Products Via GC-FID
- 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-0244, VWR Gravity Convection Operation and Calibration (Model Number 414005-106)
- 4.7. BSI-SOP-0436, Analytical Methods Validation Master Plan
- 4.8. Shimadzu QP2010S GC/MS SOP
- 4.9. USP NF <621>

**5. MATERIALS AND EQUIPMENT:**

**5.1. Equipment:**

- 5.1.1. Analytical Balance
- 5.1.2. Micropipettes
- 5.1.3. GC-MS
  - 5.1.3.1. Make: Shimadzu
  - 5.1.3.2. Model: GC-2010, equipped with FID detector.
- 5.1.4. GC Column: 30m RTX-5 Amino column 0.53 mm ID 1.00  $\mu$ m film thickness
  - 5.1.4.1. Make: Restek
  - 5.1.4.2. Part Number:12355
- 5.1.5. Laboratory Notebook

**5.2. Reagents:**

- 5.2.1. Purified Water/Milli-Q Water
  - 5.2.1.1. Supplier: BioSpectra Inc.
  - 5.2.1.2. Meets or Exceeds USP Purified Water specification.
- 5.2.2. Methanol, HPLC grade or equivalent

**5.3. Reference Standards:**

- 5.3.1. Tromethamine Certified Reference Material (NIST)
- 5.3.2. A secondary qualified reference is acceptable for use

**5.4. Supplies:**

- 5.4.1. Micropipette Tips
- 5.4.2. Class A volumetric flasks
- 5.4.3. Polypropylene transfer funnels or weighing boats

**6. METHOD PARAMETERS:****6.1. GC-2010**

- 6.1.1. Column Oven Temperature: 150.0°C
- 6.1.2. Injection Mode: Split
- 6.1.3. Injector temperature 220.0°C
- 6.1.4. Detector temperature 275.0°C
- 6.1.5. Flow Control Mode: Linear Velocity
- 6.1.6. Pressure: 25.0 kPa
- 6.1.7. Total Flow: 23.3 mL/min
- 6.1.8. Column Flow: 3.05 mL/min
- 6.1.9. Linear Velocity: 29.2 cm/sec
- 6.1.10. Purge Flow: 5.0 mL/min
- 6.1.11. Split Ratio: 5
- 6.1.12. High Pressure Injection: OFF
- 6.1.13. Carrier Gas Saver: OFF
- 6.1.14. Splitter Hold: OFF

**TABLE 1: OVEN TEMPERATURE PROGRAM**

Rate °C per Min	Temperature (°C)	Hold Time (min)
-	150.0	3.00
10.00	190.0	1.00
30.00	270.0	2.00
0.00	0.00	0.00

**6.2. Ready Checks**

- 6.2.1. Column Oven: YES
- 6.2.2. HS: NO
- 6.2.3. FID: YES
- 6.2.4. HS Carrier: NO
- 6.2.5. HS Purge: NO
- 6.2.6. APC1: YES
- 6.2.7. FID Makeup: YES
- 6.2.8. FID1 H2: YES
- 6.2.9. FID1 Air: YES
- 6.2.10. External Wait: NO
- 6.2.11. Auto Flame On: Yes
- 6.2.12. Auto flame Off: Yes

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- 6.2.13. Reignite: Yes  
 6.2.14. Auto Zero After Ready: Yes  
 6.2.15. Equilibrium Time: 0.0 min

## 7. TESTING PROCEDURE:

### 7.1. Solution Preparation

- 7.1.1. Note: Solutions may be scaled as needed  
 7.1.2. Diluent (6% Water in Methanol)  
 7.1.2.1. Pipette 3 mL of water into a 50 mL volumetric flask, dilute to volume with methanol and mix.  
 7.1.3. Sample Solutions (20 mg/mL Tromethamine)  
 7.1.3.1. Accurately weigh 1.00 g of Tromethamine and transfer into a 50 mL volumetric flask, pipette in 3 mL of water, mix, dilute to volume with methanol and mix well. Sonicate if necessary to completely dissolve the Tromethamine.  
 7.1.3.2. Samples are to be prepared fresh each time for analysis.  
 7.1.4. Impurity-level Stock Standard Solution (20 mg/mL Tromethamine)  
 7.1.4.1. Accurately weigh 1.00 g of Tromethamine CRS and transfer into a 50 mL volumetric flask, pipette in 3 mL of water, mix, dilute to volume with methanol and mix well.  
 7.1.4.2. Sonicate if necessary to completely dissolve the Tromethamine.  
 7.1.4.3. Expiration: 7 days after preparation.  
 7.1.5. Impurity-level Standard Solution (0.2 mg/mL Tromethamine)  
 7.1.5.1. Pipette 0.5 mL of the Impurity-level Stock Standard into a 50 mL volumetric flask, add 3 mL of water, dilute to volume with methanol and mix well.  
 7.1.5.2. Expiration: 7 days after preparation  
 7.1.6. LOQ Solution (0.006 mg/mL Tromethamine)  
 7.1.6.1. Pipette 30  $\mu$ L of the Impurity-level Stock Standard into a 100 mL volumetric flask, add 6 mL of water, dilute to volume with methanol and mix well.  
 7.1.6.2. Label flask: LOQ Solution  
 7.1.6.3. Prepare fresh each time of analysis.

### 7.2. Injection Sequence

- 7.2.1. Inject samples with a split ratio of 5.

**TABLE 2: EXAMPLE INJECTION SEQUENCE**

Sample ID	Number of Injections
<b>System Suitability</b>	
Diluent	$\geq 1$
LOQ	$\geq 3$
Impurity-level Standard	5
<b>Samples</b>	
Samples	$\leq 6$ (1 injection each)
Diluent	1
Impurity-level Standard (QC Check)	1
<ul style="list-style-type: none"> <li>• Repeat the sample injection sequence if additional samples are to be analyzed</li> <li>• Samples may be substituted with diluent injections</li> </ul>	

7.3. System Suitability Criteria

**TABLE 3: SYSTEM SUITABILITY ACCEPTANCE CRITERIA**

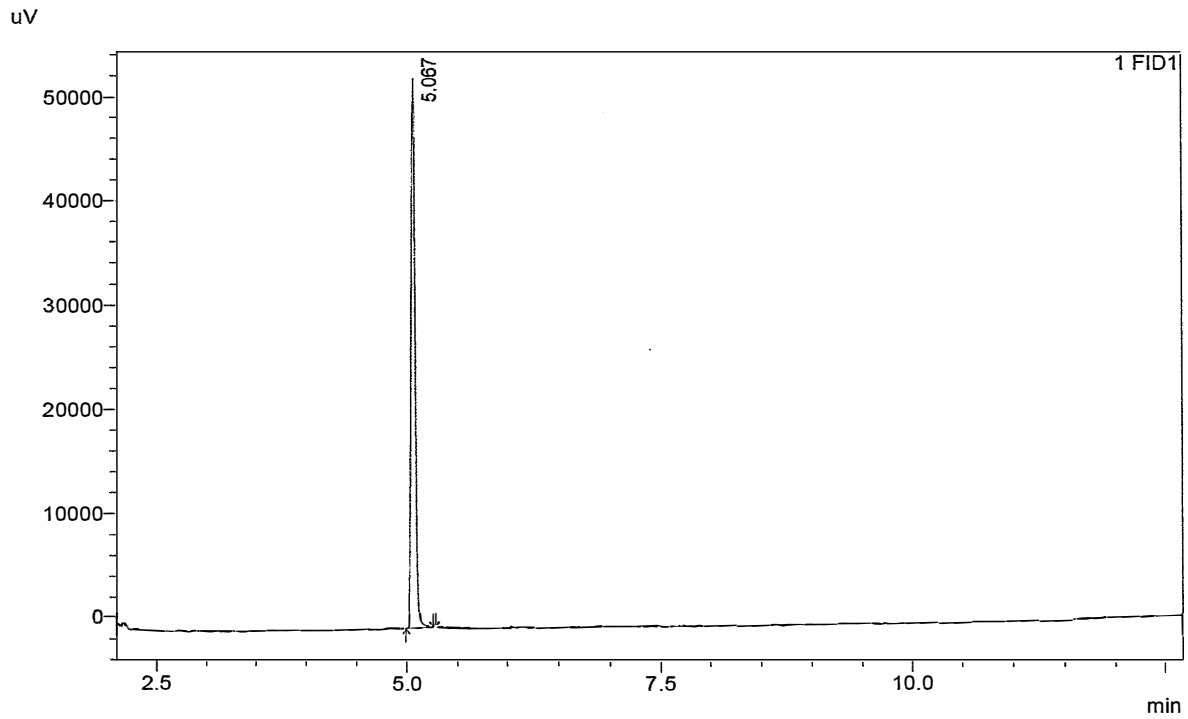
<b>System Suitability Parameter</b>	<b>Acceptance Criteria</b>
The relative standard deviation of the Tromethamine peak from the first (5) injections of the Impurity-level Standard solution.	NMT 20%
The average %Agreement between the first five (5) Impurity-level Standard injections and each Impurity-level Standard (QC check)	80% to 120%
Signal to noise ratio for the LOQ injection.	NLT 10:1

8. CALCULATIONS:

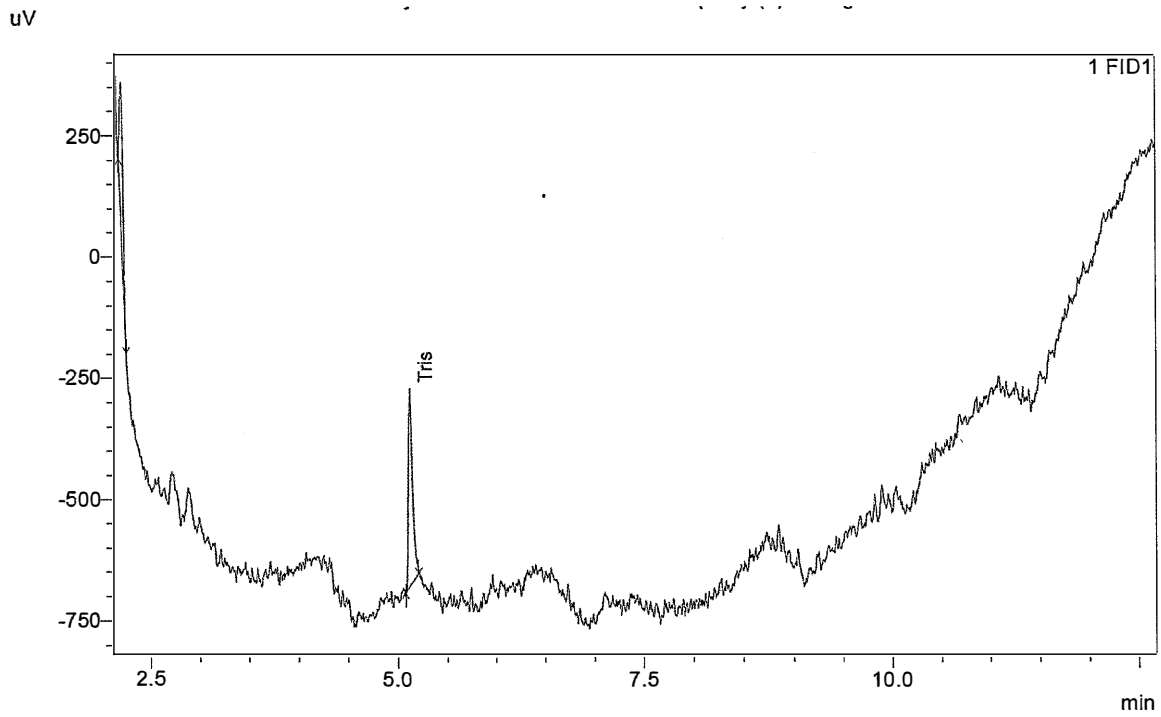
8.1. Unspecified Impurities

- 8.1.1. Report any peaks above the average peak area of the LOQ injections
- 8.1.2. Any peaks above the LOQ injections will result in the batch not meeting the specification limit of NMT 300 ppm.

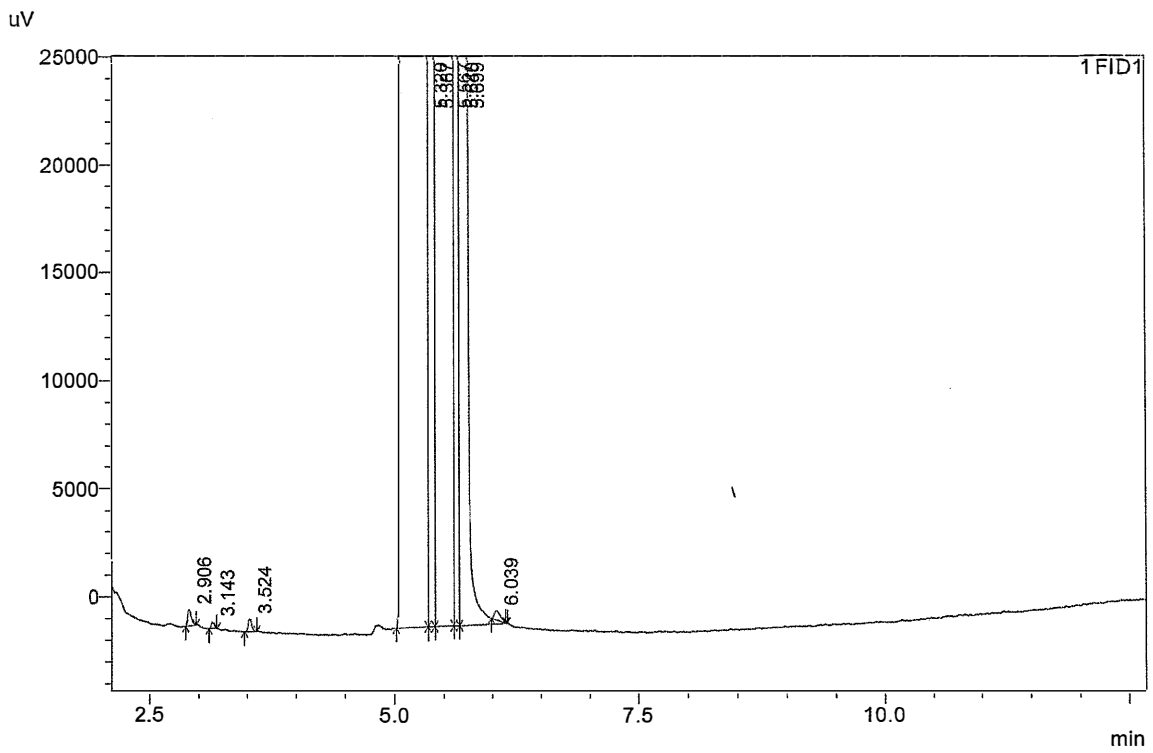
## 9. CHROMATOGRAMS:



**FIGURE 1. IMPURITY-LEVEL STANDARD SOLUTION (0.2 MG/ML TROMETHAMINE)**



**FIGURE 2. TRIS LOQ SOLUTION (0.006 MG/ML TROMETHAMINE)**



**FIGURE 3. TRIS SAMPLE CHROMATOGRAM**