BIOSPECTRA
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TROMETHAMINE UNSPECIFIED DEGRADATION PRODUCTS VIA GC-FID

TABLE OF CONTENTS

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 µm 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

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 µL 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
System Suitability	
Diluent	≥1
LOQ	≥3
Impurity-level Standard	5
Samples	
Samples	≤6 (1 injection each)
Diluent	1
Impurity-level Standard (QC Check)	1
• Repeat the sample injection sequence if additional samples are to be analyzed • Samples may be substituted with diluent injections	

7.3. System Suitability Criteria

TABLE 3: SYSTEM SUITABILITY ACCEPTANCE CRITERIA

System Suitability Parameter	Acceptance Criteria
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:

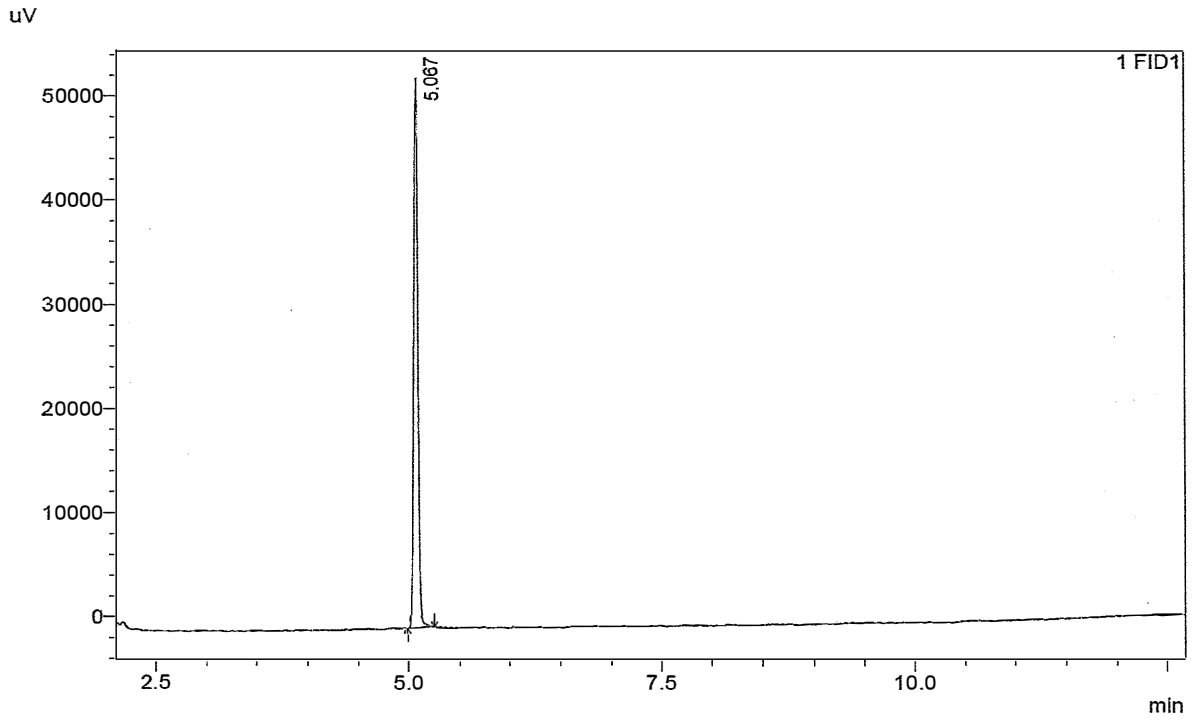

FIGURE 1. IMPURITY-LEVEL STANDARD SOLUTION (0.2 MG/ML TROMETHAMINE)

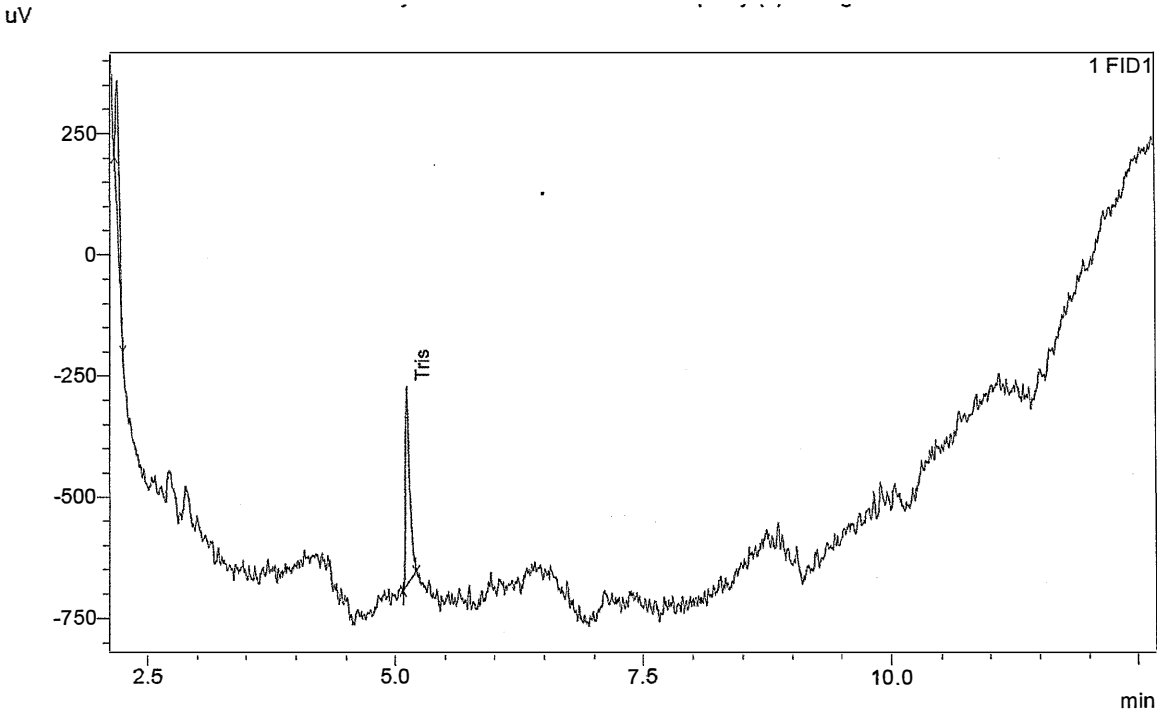

FIGURE 2. TRIS LOQ SOLUTION (0.006 MG/ML TROMETHAMINE)

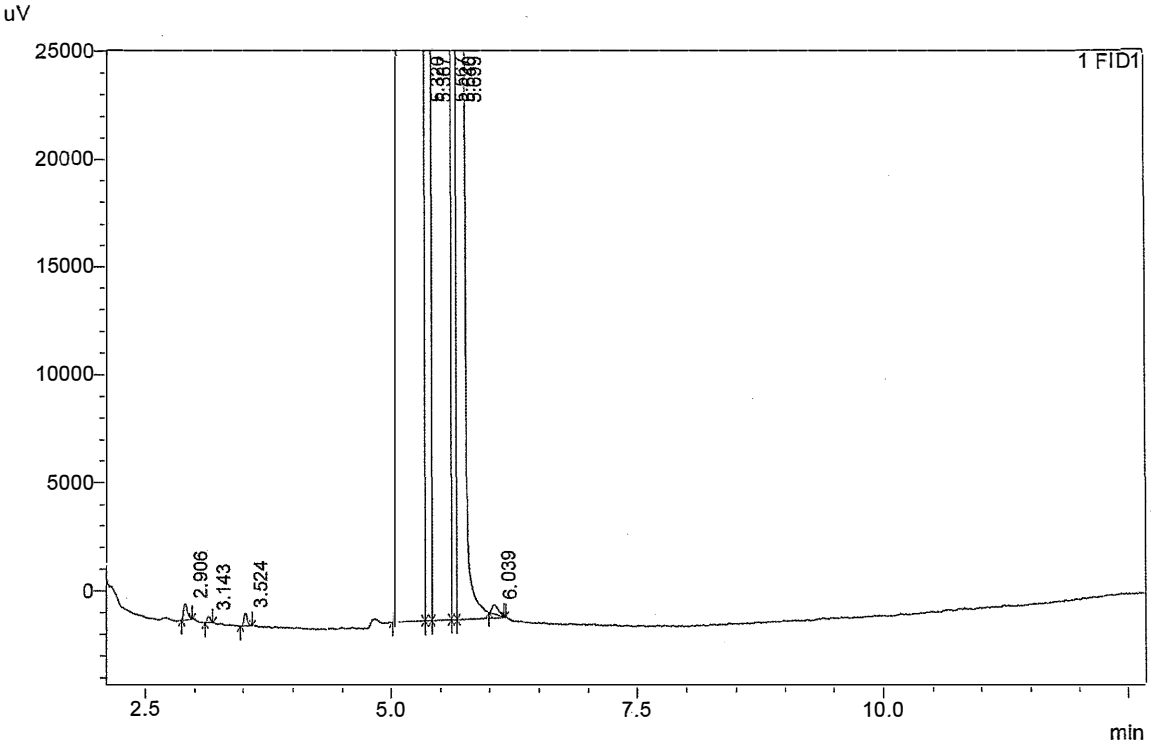

FIGURE 3. TRIS SAMPLE CHROMATOGRAM