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ANALYTICAL METHOD VALIDATION PROTOCOL: TROMETHAMINE DEGRADATION PRODUCTS VIA GC FID

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1. PURPOSE:

1.1. The purpose of this Report is to:

1.1.1. Ensure that the Tromethamine Assay and degradation products determination via GC-FID procedure is adequately evaluated and validated.

1.1.2. To provide a Validation Report to prove that the procedure for determining the amount of related substances in Tromethamine meets all requirements for quantitation as stated in the following acceptance criteria.

1.1.3. To provide evidence that the respective Analytical Method Validation Protocol was satisfied.

1.1.4. To report on any deviations from the protocol or specifications during execution of the validation study with appropriate justification and/or risk assessment.

1.1.5. 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: • Tromethamine • Method diluent • Individual authentic impurities and degradation products of the parent (if available) • For stability indicating test methods, also analyze solutions from stress testing of drug substance • Analyze solutions of unstressed drug substance and dark control sample from photostability for comparison.	The analyte is sufficiently separated from other impurities and from the drug substance, no peak interfering with the analyte peak. Retention / migration time and relative retention time of the analyte(s) are reported. Peak resolution for critical peak pairs is reported.
Accuracy at – Parent Level	Comparison of results with those obtained by another well-characterized technique	Difference of mean values ($n \geq 6$ samples): 98.0 – 102.0%: $\Delta \leq 1.0\%$ abs.
Linearity and range	Prepare solutions of the parent in duplicate, at a minimum of five levels (e.g., 80% to 120%) including 100% of the target concentration (e.g., 20 mg/mL). Calculate the regression line by the method of least squares (do not force through zero).	Correlation coefficient $R > 0.995$ Y-intercept bias: NMT 5.0%
Linearity and range – Low level impurity	Prepare a minimum of five concentration levels of Tromethamine encompassing a minimum range from the reporting level to at least 0.5%.	Correlation coefficient $R > 0.950$ Y-intercept bias: NMT 25.0%

Parameters	Procedure	Acceptance Criteria
Measurement Precision (Parent level)	At least 6 replicate measurements of a standard solution	srel ≤ 1.5% (n ≥ 6)
Intermediate Precision (Parent level)	At least 6 replicate measurements of a standard solution by a second analyst	srel ≤ 1.5% (n ≥ 12) If minimal approach is used, srel is calculated using the pooled individual data of the two runs. To pool the data, the following requirement has to be fulfilled: Difference of mean values: Δ ≤ 1.5% abs
Measurement Precision (Impurity level)	At least 6 replicate measurements of impurities at 0.1% level	0.05% ≤ Level < 0.15%: srel ≤ 20%
Intermediate Precision (Impuritylevel)	At least 6 replicate measurements of impurities at 0.1% level by a second analyst	Difference of mean values Δ: ≤ 30% rel
Detection limit (LOD)	Report the analyte level that gives a minimum signal-to-noise ratio of 3:1.	Report data
(LOQ)	Report the analyte level that gives a minimum signal-to-noise ratio of 10:1.	Signal-to-noise ratio: S/N ≥ 10 (minimum value to be reported, n ≥ 3) Replicate determinations (optional): Level ≥ 0.05%: srel: ≤ 15% (n ≥ 6)
Solution Stability	Re-assay aged standard and sample solutions against a freshly prepared standard solution	Parent peak in Standard solution: 98.0 - 102.0% Impurities in Sample solution: 0.05% ≤ Level < 0.15%: ≤ 30% srel
Robustness	Initial oven temperature, heating rate, column head pressure, injection time	The impact of parameter changes is assessed with regard to statistical significance and/or practical relevance.

2. SCOPE:

2.1. This Analytical Method Validation Protocol applies to the Tromethamine Assay and degradation products determination via GC-FID determination.
2.2. This method validation is a Category II quantitative analytical method validation.
2.3. 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 Systems is responsible for the control, implementation and maintenance of this protocol.
3.2. Analytical chemists or qualified designee is responsible for performing the testing stated in Section 8.0 of the report.
3.3. The Director of Laboratory Systems is responsible for completing the Method Validation Report using conclusions made from the results obtained from testing and supporting data.

4. REFERENCES:

4.1. BSI-SOP-0098, Balance SOP
4.2. BSI-SOP-0126, Laboratory Notebooks
4.3. BSI-SOP-0134, Pipette SOP
4.4. BSI-SOP-0244, VWR Gravity Convection Operation and Calibration (Model Number 414005-106)
4.5. BSI-SOP-0436, Analytical Methods Validation Master Plan
4.6. ICH Q2A
4.7. ICH Q2B
4.8. ICH Q3A
4.9. Shimadzu QP2010S GC/MS SOP
4.10. USP NF <621>

5. VALIDATION REQUIREMENTS:

5.1. Equipment
5.1.1. All equipment used in this Validation must be in 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 laboratory analysts involved with the execution.
5.2.2. All supplies in this analytical method Validation must be appropriate for the intended use.
5.3. Reagents
5.3.1. All reagents must be current, meet required specifications, and be suitable for the intendeduse.

5.4. Reference Standards

5.4.1. 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. Micropipettes

6.2.3. Light source capable of producing 1.2 mil lux

6.2.4. GC-MS

6.2.4.1. Make: Shimadzu

6.2.4.2. Model: GC-2010, equipped with FID detector.

6.2.5. GC Column: 30m RTX-5 Amino column 0.53mm ID 1.00µm film thickness

6.2.5.1. Make: Restek

6.2.5.2. Part Number:12355

6.2.6. 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.

6.3.2. HPLC grade Methanol

6.3.3. 0.1N Hydrochloric Acid

6.3.4. 0.1N Sodium Hydroxide

6.3.5. 30% (v/v) Hydrogen Peroxide

6.4. Reference Standards

6.4.1. Tromethamine Certified Reference Material (NIST)

6.5. Supplies

6.5.1. Micropipette Tips

7. METHOD PARAMETERS:

7.1. GC-2010

7.1.1. Column Oven Temperature: 150.0°C
7.1.2. Injection Mode: Split
7.1.3. Injector temperature: 220.0°C
7.1.4. Detector temperature: 275.0°C
7.1.5. Flow Control Mode: Linear Velocity
7.1.6. Pressure: 25.0 kPa
7.1.7. Total Flow: 23.3 mL/min (Impurity Level) and 236.8 mL/min (Assay Level)
7.1.8. Column Flow: 3.05 mL/min
7.1.9. Linear Velocity: 29.2 cm/sec
7.1.10. Purge Flow: 5.0 mL/min
7.1.11. Split Ratio: 5 (Impurity level) and 75 (Assay level)
7.1.12. High Pressure Injection: OFF
7.1.13. Carrier Gas Saver: OFF
7.1.14. Splitter Hold: OFF
7.1.15. Oven Temp Program:

Rate ^{o}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

7.2. Ready Checks

7.2.1. Column Oven: YES
7.2.2. HS: NO
7.2.3. FID: YES
7.2.4. HS Carrier: NO
7.2.5. HS Purge: NO
7.2.6. APC1: YES
7.2.7. FID Makeup: YES
7.2.8. FID1 H2: YES
7.2.9. FID1 Air: YES
7.2.10. External Wait: NO
7.2.11. Auto Flame On: Yes
7.2.12. Auto flame Off: Yes
7.2.13. Reignite: Yes
7.2.14. Auto Zero After Ready: Yes
7.2.15. Equilibrium Time: 0.0 min

8. PROCEDURE:

8.1. Solution Preparation – System Suitability Solutions

8.1.1. Diluent (6% Water in Methanol)

8.1.1.1. Pipette 3 mL of water into a 50 mL volumetric flask, dilute to volume with methanol, and mix.

8.1.2. Assay Standard (20 mg/mL Tromethamine)

8.1.2.1. 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.

8.1.2.2. Sonicate, if necessary, to completely dissolve the Tromethamine.

8.1.2.3. Prepare in duplicate.

8.1.2.4. Label SS1 and SS2, respectively.

8.1.2.5. Retain SS2 FOR SOLUTON STABILITY.

8.1.3. Impurity-level Assay Standard (0.2 mg/mL Tromethamine)

8.1.3.1. Pipette 5 mL of the SS1 solution into a 50 mL volumetric flask, add 3 mL of water, dilute to volume with methanol, and mix well.

8.1.3.2. Pipette 5 mL of the solution prepared in Step 8.1.3.1. into a 50 mL volumetric flask, add 3 mL of water, dilute to volume with methanol, and mix well.

8.1.4. LOQ Solution (0.02 mg/mL Tromethamine)

8.1.4.1. Pipette 5 mL of the Impurity-level Assay Standard into a 50 mL volumetric flask, add 3 mL of water, dilute to volume with methanol and mix well.

8.1.4.2. Label flask: LOQ Solution

8.2. Solution Preparation – Stress Study

8.2.1. Acid Hydrolysis (20 mg/mL Tromethamine)

8.2.1.1. Transfer 200 mg of Tromethamine into a 10 mL volumetric flask, pipette 0.3 mL of 0.1N Hydrochloric Acid into the flask and stopper flask.

8.2.1.2. Place the solution in an oven set at 40°C for 5 days.

8.2.1.3. After 5 days, pipette 0.3 mL of 0.1N Sodium Hydroxide into the flask, dilute to volume with methanol and mix.

8.2.2. Basic Hydrolysis (20 mg/mL Tromethamine)

8.2.2.1. Transfer 200 mg of Tromethamine into a 10 mL volumetric flask, pipette 0.3 mL of 0.1N Sodium Hydroxide into the flask and stopper flask.

8.2.2.2. Place the solution in an oven set at 40°C for 5 days.

8.2.2.3. After 5 days, pipette 0.3 mL of 0.1N Hydrochloric acid into the flask, dilute to volume with methanol and mix.

8.2.3. Photolytic Sample (20 mg/mL Tromethamine)

8.2.3.1. Prepare in duplicate.

8.2.3.2. Transfer 200 mg of Tromethamine into a crystal dish, pipette 0.6 mL of water to the dish and dissolve.

8.2.3.3. Expose one (1) of the solutions to 1.2 million lux hours.

8.2.3.4. Keep the second solution (Control) in the dark until solution 1 has reached 1.2 million lux hours.

8.2.3.5. Carefully add 9.4 mL of methanol to the dish and mix. Transfer the solution into a 10 mL volumetric flask.

8.2.4. Thermal Sample (20 mg/mL Tromethamine)

8.2.4.1. Transfer 200 mg of Tromethamine to a 10 mL volumetric flask.

8.2.4.2. Store the sample in an oven set at 60°C for 5 days.

8.2.4.3. Pipette 0.6 mL of water, dilute to volume with methanol and mix.

8.2.5. Oxidative Sample (20 mg/mL Tromethamine)

8.2.5.1. Transfer 200 mg of Tromethamine into a 10 mL volumetric flask and pipette 0.5 mL of water and 0.1 mL of 30% Hydrogen Peroxide into the flask.

8.2.5.2. Allow the solution to sit for 2 days at room temperature.

8.2.5.3. Dilute to volume with methanol and mix.

8.2.6. Control Sample (20 mg/mL Tromethamine)

8.2.6.1. Transfer 200 mg of Tromethamine into a 10 mL volumetric flask, pipette 0.6 mL of water to the flask, dilute to volume with methanol and mix.

8.2.7. Hydrolysis Blank

8.2.7.1. Pipette 0.3 mL of 0.1N Hydrochloric Acid and 0.3 mL of 0.1N Sodium Hydroxide to a 10 mL volumetric flask, dilute to volume with methanol and mix.

8.2.8. Oxidative Blank

8.2.8.1. Pipette 0.1 mL of 30% Hydrogen Peroxide and 0.5 mL of water to a 10 mL volumetric flask, dilute to volume with methanol and mix.

8.3. **Solution Preparation – Assay Level Accuracy, Precision, and Linearity**

8.3.1. All solutions are to be thoroughly mixed after being prepared. Solutions may be scaled as needed.

8.3.2. 120% Tromethamine Calibration level (24 mg/mL Tromethamine)

8.3.2.1. Accurately weigh 1.20 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.

8.3.2.2. Prepare in triplicate.

8.3.3. 110% Tromethamine Calibration level (22 mg/mL Tromethamine)

8.3.3.1. Accurately weigh 1.10 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.

8.3.3.2. Prepare in triplicate.

8.3.4. 100% Tromethamine Calibration level (20 mg/mL Tromethamine)

8.3.4.1. 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.

8.3.4.2. Prepare six (6) 100% Tromethamine Calibration Level Solutions.

8.3.5. 90% Tromethamine Calibration level (18 mg/mL Tromethamine)

8.3.5.1. Accurately weigh 0.90 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.

8.3.5.2. Prepare in triplicate.

8.3.6. 80% Tromethamine Calibration level (16 mg/mL Tromethamine)

8.3.6.1. Accurately weigh 0.80 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.

8.3.6.2. Prepare in triplicate.

8.4. **Solution Preparation – Impurity-Level Precision and Linearity**

8.4.1. All solutions are to be thoroughly mixed after being prepared. Solutions may be scaled as needed.

8.4.2. 0.20% Tromethamine Calibration level (0.04 mg/mL Tromethamine)

8.4.2.1. Accurately weigh 50 mg of Tromethamine and transfer into a 50 mL volumetric flask, pipette in 3 mL of water, mix, dilute to volume with methanol and mix.

8.4.2.2. Pipette 2.0 mL of the solution from Step 8.4.2.1. into a 50 mL volumetric flask, add 3 ml of water, dilute to volume with methanol and mix.

8.4.2.3. Prepare in triplicate.

8.4.3. 0.15% Tromethamine Calibration level (0.03 mg/mL Tromethamine)

8.4.3.1. Accurately weigh 50 mg of Tromethamine and transfer into a 50 mL volumetric flask, pipette in 3 mL of water, mix, dilute to volume with methanol and mix (solution from Step 8.4.2.1 may be used).

8.4.3.2. Pipette 1.5 mL of the solution from Step 8.4.3.1. into a 50 mL volumetric flask, add 3 ml of water, dilute to volume with methanol and mix.

8.4.3.3. Prepare in triplicate.

8.4.4. 0.10% Tromethamine Calibration level (0.02 mg/mL Tromethamine)

8.4.4.1. Accurately weigh 50 mg of Tromethamine and transfer into a 50 mL volumetric flask, pipette in 3 mL of water, mix, dilute to volume with methanol and mix (solution from Step 8.4.2.1 may be used).

8.4.4.2. Pipette 1.0 mL of the solution from Step 8.4.4.1. into a 50 mL volumetric flask, add 3 ml of water, dilute to volume with methanol and mix.

8.4.4.3. Prepare six (6) 0.10% Tromethamine Calibration Level Solutions.

8.4.5. 0.05% Tromethamine Calibration level (0.01 mg/mL Tromethamine)

8.4.5.1. Accurately weigh 50 mg of Tromethamine and transfer into a 50 mL volumetric flask, pipette in 3 mL of water, mix, dilute to volume with methanol and mix (solution from Step 8.4.2.1 may be used).

8.4.5.2. Pipette 0.5 mL of the solution from Step 8.4.5.1. to a 50 mL volumetric flask, add 3 ml of water, dilute to volume with methanol and mix.

8.4.5.3. Prepare in triplicate.

8.4.6. 0.03% Tromethamine Calibration level (0.006 mg/mL Tromethamine)

8.4.6.1. Accurately weigh 50 mg of Tromethamine and transfer into a 50 mL volumetric flask, pipette in 3 mL of water, mix, dilute to volume with methanol and mix (solution from Step 8.4.2.1 may be used).

8.4.6.2. Pipette 0.3 mL of the solution from Step 8.4.6.1. into a 50 mL volumetric flask, add 3 ml of water, dilute to volume with methanol and mix.

8.4.6.3. Prepare in triplicate.

8.5. **Sample Preparation – Solution Stability**

8.5.1. Re-inject an Assay Standard (SS2) (Step 8.1.2.) and an Impurity-Level Standard (Step 8.1.3.) after 1 day, 3 days and 7 days.

8.5.2. Analyze the solutions against a freshly prepared Assay Standard (Step 8.1.2.).

8.5.3. Calculate the percent difference from the initial assay value.

8.6. **Injection Sequence**

8.6.1. Each sample will be injected once with a split ratio of 75 for the assay level and a second time with a split of 5 for the impurity level.

Sample ID	Number of Injections
System Suitability	
Diluent	≥1
LOQ	≥3
SS1	5
SS2 (Standard Check)	2
Diluent	1
Impurity-level Standard	5
Samples	
Samples	≤6 (1 injection each)
SS1 (QC Check)	1
Diluent	1
Impurity-level Assay Standard (QC Check)	1
• Repeat the sample injection sequence if additional samples are to be analyzed • Samples may be substituted with diluent injections	

8.7. **System Suitability Criteria**

System Suitability Parameter	Acceptance Criteria
The relative standard deviation of the Tromethamine peak from the first (5) injections of the SS1 solution.	NMT 1.0%
The average %Agreement between the first five (5) SS1 injections and each SS1 (QC Check).	99% to 101%
The relative standard deviation of the Tromethamine peak from the first (5) injections of the Impurity-level Assay Standard solution.	NMT 5.0%
The average %Agreement between the first five (5) Impurity-level Assay Standard injections and each Impurity-level Assay Standard (QC check)	96% to 104%
Average %Agreement between the first five (5) SS1 injections and the SS2 injections.	99% to 101%
The USP tailing factor of the Tromethamine peak from the first SS1 injection.	0.6 to 1.2
Signal to noise ratio for the LOQ injection.	NLT 10:1

9. CALCULATIONS:

9.1. Assay

9.1.1. Result = (r_u/Ar_s) (C_s/C_u) (100)

9.1.2. Where:

9.1.2.1. r_u= peak response of Tromethamine from the *Sample Solution*.

9.1.2.2. Ar_s= Average Peak response of Tromethamine from the *Standard Solution*.

9.1.2.3. C_s= Concentration of Tromethamine RS in the standard solution(mg/mL prepared * Purity of CRS).

9.1.2.4. C_u= concentration of Tromethamine in the *Sample Solution* (mg/mL).

9.2. Unspecified Impurities

9.2.1. Result = (r_u/Ar_s) (C_s/C_u) (100)

9.2.1.1. r_u= peak response of unspecified impurity from the *Sample Solution*.

9.2.1.2. Ar_s= Average Peak response of Tromethamine from the *Impurity-level Assay Standard*.

9.2.1.3. C_s= Concentration of Tromethamine RS in the *Impurity-level Assay Standard* (mg/mL prepared * Purity of CRS).

9.2.1.4. C_u= concentration of Tromethamine in the *Sample Solution* (mg/mL).

10. VALIDATION PROCEDURE:

10.1. System Suitability: Assay

10.1.1. Inject the Assay Standard (SS1), Assay Standard (SS2), and Assay Standard (SS1) (QC Checks) as per Section 8.6.

10.1.2. Acceptance Criteria:

10.1.2.1. Relative Standard Deviation of the tromethamine peak from the first five (5) injections of the SS1 solution: NMT 1%.

10.1.2.2. The average %Agreement between the first five (5) SS1 injections and each SS1 (QC Check): 99% to 101%.

10.1.2.3. Average %Agreement between the first five (5) SS1 injections and the SS2 injections: 99% to 101%.

10.1.2.4. The USP tailing factor of the Tromethamine peak from the first SS1 injection 0.6 – 1.2.

10.2. System Suitability: Degradation Products

10.2.1. Inject the Impurity-level Assay Standard, Impurity-level Assay Standard (QC Check), and the LOQ solution as per Section 8.6.

10.2.2. Acceptance Criteria:

10.2.2.1. Relative Standard Deviation of the tromethamine peak from the first five (5) injections of the Impurity-level Assay Standard solution: NMT 5.0%.

10.2.2.2. The average %Agreement between the first five (5) Impurity-level Assay Standard injections and each impurity level Assay Standard (QC Check): 96% to 104%.

10.2.2.3. The signal to noise ratio for the LOQ injections: NLT 10:1.

10.3. LOQ

10.3.1. Inject the LOQ solution as a sample as per Section 8.6 six (6) times.

10.3.2. Acceptance Criteria:

10.3.2.1. Signal to noise ratio is NLT 10:1.

10.3.2.2. The relative standard deviation of the tromethamine peak areas is NMT 20%.

10.3.2.3. The LOQ level is NMT 0.03%.

10.4. Linearity: Assay

10.4.1. Inject the 80%, 90%, 100%, 110%, and 120% Tromethamine Calibration Level Samples at least once (the average of the accuracy and precision samples may be utilized). Plot the peak area response against concentration and perform a linear regression by the method of least squares. Determine the Slope, Y-Intercept, Correlation Coefficient (r^2), and Y-Intercept Bias.

10.4.2. Acceptance Criteria:

10.4.2.1. Report the y-intercept, and slope of the linear regression.

10.4.2.2. Correlation coefficient (r^2): NLT 0.995.

10.4.2.3. Y-Intercept Bias: NMT 5.0%.

10.5. Linearity: Impurity-level Assay

10.5.1. Inject the 0.03%, 0.05%, 0.10%, 0.15%, and 0.20% Tromethamine Calibration level samples at least once (the average of the accuracy and precision samples may be utilized). Plot the peak area response against concentration and perform a linear regression by the method of least squares. Determine the Slope, Y-Intercept, Correlation Coefficient (r^2), and Y-Intercept Bias.

10.5.2. Acceptance Criteria:

10.5.2.1. Report the y-intercept, and slope of the linear regression.

10.5.2.2. Correlation coefficient (r^2): NLT 0.950.

10.5.2.3. Y-Intercept Bias: NMT 25.0%

10.6. Precision: Assay

10.6.1. Inject each of the six (6) preparations of the 100% Tromethamine Calibration Level samples.

10.6.2. Acceptance Criteria:

10.6.2.1. Relative Standard Deviation: $\leq 1.5\%$ ($n \geq 6$).

10.7. Precision: Impurity-level Assay

10.7.1. Inject each six (6) preparations of the 0.10% Tromethamine Calibration level samples.

10.7.2. Acceptance Criteria:

10.7.2.1. Relative Standard Deviation: $\leq 20\%$ ($n \geq 6$).

10.8. Accuracy: Assay Level

10.8.1. Inject each of the triplicate preparations of the 80%, 90%, 110%, and 120% Tromethamine Calibration Level Samples and inject each of the six (6) preparations of the 100% Tromethamine Calibration Level samples.

10.8.2. Acceptance Criteria:

10.8.2.1. Percent Recovery (%): All replicates are between 99% to 101%.

10.8.2.2. Comparison of results with those obtained by another well-characterized technique

10.8.2.3. Difference of mean values ($n \geq 6$ samples): 98.0 – 102.0%: $\Delta \leq 1.0\%$ abs.

10.9. Intermediate Precision: Assay

10.9.1. A different analyst or qualified designee will repeat the 100% Tromethamine Calibration Level portion of Section 10.6. (do not repeat the 80%, 90%, 110%, or 120% Tromethamine Calibration Level portions). This will be performed on a different day with separately prepared samples and standards (may be performed together with Intermediate Precision: Impurity-level Assay).

10.9.2. Acceptance Criteria:

10.9.2.1. Difference of mean values Δ: ≤ 1.5% rel

10.10. Intermediate Precision: Impurity-level Assay

10.10.1. A different analyst or qualified designee will repeat the 0.10% Tromethamine Calibration Level portion of Section 10.7. (do not repeat the 0.03%, 0.05%, 0.15%, or 0.20% Tromethamine Calibration portions). This will be performed on a different day with separately prepared samples and standards (may be performed together with Intermediate Precision: Assay).

10.10.2. Acceptance Criteria:

10.10.2.1. Difference of mean values Δ: 30% rel.

10.11. Range: Assay

10.11.1. The range of an analytical procedure is the interval between the upper and lower levels of analyte in the sample that have demonstrated suitable accuracy, precision, and linearity.

10.11.2. Acceptance Criteria:

10.11.2.1. Report the range of the analysis from the lowest level of analyte to the highest level of analyte that meets requirements for accuracy (section 10.8), precision (section 10.6), and linearity (section 10.4).

10.12. Range: Impurity-level Assay

10.12.1. The range of an analytical procedure is the interval between the upper and lower levels of analyte in the sample that have demonstrated suitable accuracy, precision, and linearity.

10.12.2. Acceptance Criteria:

10.12.2.1. Report the range of the analysis from the lowest level of analyte to the highest level of analyte that meets requirements for accuracy (section 10.8), precision (section 10.7), and linearity (section 10.5).

10.13. Specificity: Impurity-level Assay

10.13.1. Analyze acidic, basic, photolytic, thermal, and oxidative stress samples as well as a control, hydrolysis blank, oxidative blank, and diluent.

10.13.2. Acceptance criteria:

10.13.2.1. The analyte is sufficiently separated from other impurities and from the drug substance, no peak is interfering with the analyte peak. Retention / migration time and relative retention time of the analyte(s) are reported. Peak resolution for critical peak pairs is reported.

10.14. Solution Stability: Assay

10.14.1. Save and re-inject an Assay Standard (SS2) after 1 day, 3 days, and 7 days.

10.14.2. Acceptance criteria:

10.14.2.1. % Agreement between the first five (5) injections of a freshly prepared Assay Standard (SS1) and the aged Assay Standard (SS2) is 98.0 – 102.0%.

10.15. Solution Stability: Impurity-level Assay

10.15.1. Save and re-inject an Impurity-level Standard after 1 day, 3 days, and 7 days.

10.15.2. Acceptance Criteria:

10.15.2.1. Impurity-level: 0.03% ≤ Level < 0.15%: ≤ 30% rel.

10.16. Robustness: Assay

10.16.1. Prepare System Suitability Solutions as per Section 8.1. Evaluate each robustness condition in the table below:

	Low	Target	High
Initial Oven Temperature	145°C	150°C	155°C
Heating Rate	8°C/min	10°C/min	12°C/min
Column Head Pressure	22 Kpa	25 Kpa	28 Kpa

10.16.2. Acceptance criteria

10.16.2.1. All system suitability parameters are met.

11. DOCUMENTATION PROCEDURES:

11.1. A method validation report will be drafted after successful execution of this protocol summarizing method validation status and performance.

11.2. All data sheets and notebook pages, are to be signed and dated by the analyst executing the Protocol.

11.3. All equipment and instrumentation used in the execution of this protocol must be calibrated. Ensure that there is a certificate on file or appropriate standards are used if calibration is required.

11.4. Any critical changes made to the analytical method verification protocol must be noted in the Validation Report with supporting evidence for the change.