World Journal of Pharmaceutical Science and Research
www.wjpsronline.com
Research Article
ISSN: 2583-6579
SJIF Impact Factor: 5.111
Year - 2025
Volume: 4; Issue: 3
Page: 1249-1277

TO DEVELOP AND VALIDATE SUITABLE ANALYTICAL METHODS FOR THE QUANTIFICATION OF TOLTERODINE TARTRATE USING UV-VISIBLE SPECTROPHOTOMETRY AND REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (RP-HPLC)

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Article Received: 07 May 2025 // Article Revised: 27 May 2025 // Article Accepted: 19 June 2025

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DOI: https://doi.org/10.5281/zenodo.15774905

How to cite this Article: Kajal Madhukar Bhivsane, Pallavi Patharkar (2025). TO DEVELOP AND VALIDATE SUITABLE ANALYTICAL METHODS FOR THE QUANTIFICATION OF TOLTERODINE TARTRATE USING UV-VISIBLE SPECTROPHOTOMETRY AND REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (RP-HPLC). World Journal of Pharmaceutical Science and Research, 4(3), 1249-1277. https://doi.org/10.5281/zenodo.15774905

ABSTRACT

The aim of this research was to develop and validate a stability-indicating Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method for the estimation of Tolterodine Tartrate (TRT) in bulk and tablet dosage forms. Chromatographic separation was achieved using a C18 column (250 mm × 4.6 mm, 5 µm particle size) with a mobile phase consisting of Propylene glycol: Water (pH 4.5) (pH adjusted with Orthophosphoric acid): Methanol in the ratio of 50:40:10. The flow rate was maintained at 1.0 mL/min, and detection was carried out at 282 nm using a UV detector. The retention time of drug was found to be 5.793 for Tolterodine Tartrate (TRT). The method was validated according to ICH guidelines for linearity, accuracy, precision, specificity, limit of detection (LOD), limit of quantification (LOQ), and robustness. Linearity was observed in the concentration range of 10-50 µg/mL for TRT, with correlation coefficients (r^2) of 0.9993. The method demonstrated good accuracy, with recovery rates found in the range of 99.80% to 100.86 % for TRT. Precision, both intra-day and inter-day, showed relative standard deviation (RSD) values of less than 2%. The stability study involves the good results in all stability conditions. The marketed formulation of Tolterodine Tartrate was analysis using UV and RP-HPLC the percent assay by both methods was found to be 98.23% and 98.68% respectively. The developed RP-HPLC method is reliable, efficient, and suitable for the estimation of Tolterodine Tartrate in in bulk and tablet dosage forms. It can be successfully applied for routine quality control and stability testing of these agents.

KEYWORDS: Tolterodine Tartrate, Uv Spectroscopy, HPLC, Validation, Tablet Dosage form.

INTRODUCTION

1.1 Background of the Study

Pharmaceutical analysis plays a pivotal role in drug development, formulation, quality control, and regulatory compliance. The accurate quantification of active pharmaceutical ingredients (APIs) is essential to ensure the efficacy, safety, and quality of pharmaceutical products. Among the wide range of techniques available, UV-Visible spectrophotometry and high-performance liquid chromatography (HPLC), particularly Reverse Phase HPLC (RP-HPLC), are among the most widely used for routine analysis due to their accuracy, reproducibility, and sensitivity.

Tolterodine Tartrate is a competitive muscarinic receptor antagonist, primarily used for the treatment of overactive bladder (OAB) symptoms such as urinary frequency, urgency, and incontinence. Owing to its pharmacological importance and frequent use in clinical practice, the development of validated analytical methods for its quantification in pharmaceutical formulations is of critical importance.

1.2 Need for the Study

Analytical methods are crucial for the pharmaceutical industry to monitor and maintain drug quality throughout the product lifecycle. While several methods for Tolterodine Tartrate estimation have been reported, the continuous evolution of drug formulations, regulatory requirements, and demand for robust and reproducible techniques necessitate ongoing method development and validation.

UV-Visible spectrophotometry offers a simple and cost-effective approach for routine analysis, especially in developing countries, whereas RP-HPLC provides high sensitivity and specificity, suitable for complex matrices. The aim of this study is to develop, optimize, and validate reliable methods for the quantification of Tolterodine Tartrate using both UV-Visible and RP-HPLC techniques according to ICH guidelines.[1]

1.3 Tolterodine Tartrate

1.3.1 Chemical Profile

- **Chemical Name**: (R)-N,N-diisopropyl-3-(2-hydroxy-5-methylphenyl)-3-phenylpropanamine L-(+)-tartrate
- **Molecular Formula**: C22H31NO·C4H6O6
- **Molecular Weight**: 475.6 g/mol
- **Solubility:** Freely soluble in water and ethanol.

Tolterodine Tartrate is the tartrate salt form of tolterodine, which exists as a racemic mixture. It belongs to the class of antimuscarinics and exerts its pharmacological action by competitively inhibiting acetylcholine at muscarinic receptors in the bladder, leading to decreased detrusor muscle activity.[2]

1.3.2 Pharmacokinetics and Clinical Use

Tolterodine undergoes extensive first-pass metabolism in the liver primarily via CYP2D6 and CYP3A4 isoenzymes. It has a half-life of approximately 2–4 hours and is excreted mainly in the urine. Clinical studies have demonstrated its efficacy in reducing OAB symptoms with fewer adverse effects compared to older antimuscarinics.[3]

1.4 UV-Visible Spectrophotometry in Drug Analysis

UV-Visible spectrophotometry is based on the measurement of absorption of ultraviolet or visible light by a substance in solution. It is a widely used method in pharmaceutical analysis due to its simplicity, rapidity, and relatively low cost. The technique follows Beer-Lambert's Law, where the absorbance is directly proportional to the concentration of the absorbing species.

Advantages of UV-Visible Spectrophotometry

- Cost-effective and accessible
- Quick and non-destructive
- Suitable for routine quality control analysis
- Requires minimal sample preparation

Several studies have reported spectrophotometric methods for estimating Tolterodine Tartrate, typically in the range of 280–290 nm due to its aromatic moieties.[4]

1.5 High Performance Liquid Chromatography (HPLC)

HPLC is a powerful analytical technique widely used for qualitative and quantitative drug analysis. RP-HPLC, in particular, utilizes a non-polar stationary phase and a polar mobile phase, which makes it ideal for the separation of moderately polar compounds such as Tolterodine Tartrate.

Principles of RP-HPLC

In RP-HPLC, compounds are separated based on their hydrophobic interactions with the stationary phase. Parameters such as mobile phase composition, flow rate, detection wavelength, and column temperature can be optimized to enhance the resolution and sensitivity of the method.

Advantages of RP-HPLC

- High resolution and sensitivity
- Capable of separating complex mixtures
- Suitable for stability-indicating methods
- Reproducible and robust

1.6 Analytical Method Development and Validation

Analytical method development involves selecting an appropriate technique, optimizing parameters, and ensuring that the method meets the analytical requirements for specificity, precision, accuracy, and sensitivity.

ICH Guidelines (Q2(R1)) for Validation Parameters[1]

- **Specificity:** Ability to measure the analyte in the presence of components such as impurities, degradants, or matrix.
- **Linearity:** Ability to obtain test results that are directly proportional to the concentration.
- **Accuracy:** Closeness of the test results to the true value.
- **Precision:** Reproducibility of the results under the same conditions.
- **Limit of Detection (LOD)** and **Limit of Quantification (LOQ)**: Lowest concentration levels that can be reliably detected or quantified.

- **Robustness:** Capacity to remain unaffected by small changes in analytical parameters.

Proper method validation ensures the reliability of the test results and regulatory compliance for product approval.

1.9 Significance of the Study

- Provides validated, reproducible methods for routine drug analysis.
- Offers a comparative approach using both low-cost (UV) and advanced (HPLC) techniques.
- Supports pharmaceutical industries in ensuring drug quality and regulatory compliance.
- Can serve as a reference method in academic and industrial research.

1.10 Scope and Limitations

Scope

- Application of developed methods to tablet formulations.
- Use of standard validation parameters for ensuring accuracy and reliability.

Limitations

- The UV method may lack specificity in complex formulations.
- HPLC requires access to more sophisticated instrumentation and solvents.

MATERIALS AND INSTRUMENTS

A. Drug acquirement

Table: Medication test Providers.

Sr. No	Name of Drugs	Drug obtainment
1	Tolterodine tartrate	Tokyo Chemical Industry (India) Pvt. Ltd., Hyderabad

B. Reagents and synthetic substances utilized

Table: Regents and Synthetic Utilized.

Sr. No	Chemicals/ Reagents	Make	Grade
1	Water, Mili-Q	-	HPLC
2	Potassium Dihydrogen Phosphate	Dipa chemical Industry	EP
3	Ortho phosphoric acid	Dipa chemical Industry	EP
4	Propylene Glycol	Dipa chemical Industry	EP
5	Methanol	Advent	HPLC
6	Acetonitrile	Advent	HPLC

C. Instruments utilized

HPLC: Shimadzu LC 2010 System

Pump Reciprocating: Waters-510

Detector Waters : UV detector

Software: Empower

Column : Inertsil ODS 150mm x 4.6mm 3.5µ

Analytical Balance Digital: Analytical balance Essae

PH Meter: Digital pH meter

Sonicator: Ultrasonic Bath Sonicator

Filter: Nylon, PVDF, PTFE 0.45µm (Milipore)

UV Method Development[64]

1. Selection of Common Solvents

A solubility study was conducted to identify a suitable solvent system in which Tolterodine Tartrate is both completely soluble and chemically stable. Various solvents such as distilled water, ethanol, methanol, propylene glycol, acetonitrile, and their combinations were investigated. After comparative assessment, a binary solvent system comprising **Propylene Glycol (PG): Methanol in the ratio 10:90** was finalized. This solvent combination provided optimal solubility and stability, making it suitable for further spectral analysis and method development.

2. Preparation of Standard Stock Solution and Study of Beer-Lambert's Law

Preparation of Standard Stock Solution

An accurately weighed amount of Tolterodine Tartrate (10 mg) was dissolved in **PG: Methanol (10:90)** in a 10 mL volumetric flask. The solution was diluted to the mark with the same solvent to achieve a **stock solution concentration of 1000 µg/mL**. From this, **1 mL was withdrawn and diluted to 10 mL**, resulting in a working concentration of **100 µg/mL**.

Study of Beer-Lambert's Law

To investigate Beer-Lambert's Law applicability, aliquots of the stock solution were diluted with PG: Methanol (10:90) to prepare standard solutions in the concentration range of **5–25 µg/mL**. The absorbance of these solutions was measured at the wavelength of maximum absorbance (λmax). A calibration curve was plotted, and the regression equation was derived to evaluate linearity, confirming that Tolterodine Tartrate obeys Beer-Lambert's Law within the selected range.

3. Method Validation[65,66]

Method validation was performed in accordance with **ICH Q2(R1)** guidelines. The following parameters were evaluated:

A. Linearity

Linearity was assessed by preparing serial dilutions from the standard stock solution. Five concentrations were prepared and analyzed in 10 mL volumetric flasks. The calibration curve was constructed by plotting absorbance against concentration. Linearity was confirmed by evaluating the correlation coefficient (r^2), slope, and intercept.

B. Accuracy (Recovery Studies)

Accuracy was evaluated through recovery studies at **three concentration levels (80%, 100%, and 120%)** by spiking known quantities of Tolterodine Tartrate to pre-analyzed samples. The recovery percentage was calculated using the formula:

$$\% \text{ Recovery} = (SPS - SSP) \times 100\%$$

$$\text{Recovery} = \left(\frac{\text{SPS} - S}{SP}\right) \times 100$$

Where,

SPS = Amount found in the spiked sample

S = Amount found in the unspiked sample

SP = Amount of analyte added

C. Precision

Precision was expressed as the **% Relative Standard Deviation (%RSD)** of repeated measurements.

I. Intraday Precision: Samples of **10, 15, and 25 µg/mL** were analyzed three times on the same day at intervals (e.g., 11 AM, 1 PM, 3 PM). The %RSD of the readings was calculated.

II. Intraday Precision: The same concentrations were analyzed on **three consecutive days**, and %RSD values were determined to assess method reproducibility.

D. Robustness

Robustness was evaluated by deliberately varying the solvent system. Three solvent systems were tested:

- PG: Methanol (50:50)
- PG: Acetonitrile (50:50)
- PG: Methanol (90:10)

The absorbance of Tolterodine Tartrate was measured, and the **%RSD** was calculated to determine robustness against minor solvent changes.

E. Ruggedness

Ruggedness was assessed by performing analysis at three different temperatures:

- 25°C
- 37°C
- 60°C

The absorbance was recorded, and **%RSD** was computed to assess the method's resistance to external variations.

F. Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ were calculated using the formulae:

$$\text{LOD} = \frac{3.3 \times \sigma}{S}, \quad \text{LOQ} = \frac{10 \times \sigma}{S}$$

Where,

σ = Standard deviation of the y-intercept S = Slope of the calibration curve

These parameters reflect the method's sensitivity and the lowest detectable and quantifiable drug concentrations.

4. Analysis of Marketed Formulation by UV Spectrophotometry

The proposed UV method was applied for the estimation of Tolterodine Tartrate in a marketed formulation: **Roliten 2 mg tablets** (Sun Pharmaceutical Industries Ltd.), containing **2 mg of Tolterodine Tartrate**.

Sample Preparation

1. The tablet was finely powdered, and an amount equivalent to **2 mg of Tolterodine Tartrate** was transferred into a 10 mL volumetric flask.
2. The powder was dissolved using the previously selected solvent system **(PG: Methanol, 10:90)**.
3. The mixture was sonicated for 5–10 minutes and diluted to volume.
4. A stock solution of **200 µg/mL** was prepared.
5. From this, **1 mL was further diluted to 10 mL**, resulting in a final solution concentration of **20 µg/mL**.

Estimation and Assay Calculation

The absorbance of the prepared sample solution was measured and compared with that of the standard solution. The **percent assay** was calculated using the formula:

% Assay=(Absorbance of sampleAbsorbance of standard)×Label claim\%\ \text{Assay} = \left(\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}}\right) \times \text{Label claim}

HPLC Analysis[68,69]

1. Instrumentation

- **HPLC System**: Shimadzu LC-2010 series
- **Detector**: LC-UV-2075 UV/VIS
- **Software**: LC Solution
- **Column**: Intersil ODS-3V C18, 250 × 4.6 mm, 5 µm
- **Accessories**:
  - Elder digital balance
  - Prama ultrasonicator

2. HPLC Method Development

Mobile Phase Optimization

Multiple mobile phase combinations were evaluated. The optimized mobile phase selected:

- **Propylene Glycol: Water (pH 4.5 adjusted with Ortho-Phosphoric Acid): Methanol (50:40:10)**

Mobile Phase Preparation

- Filtered through **0.45 µm** membrane
- **Degassed** via ultrasonication for **10 minutes**

Chromatographic Conditions

- **Mode**: Isocratic
- **Flow Rate**: 1.0 mL/min
- **Detection Wavelength**: 282 nm
- **Column**: C18 (250 × 4.6 mm, 5 µm)

Sample Preparation

- **Standard Stock**: 10 mg TRT in 100 mL mobile phase → 100 µg/mL
- Filtered through **0.2 µ nylon membrane**

System Setup

- **Priming**: Manual air removal from tubing
- **Column Conditioning**:
  - HPLC-grade water (1 mL/min, 1 hr)
  - Methanol:Water (50:50) (30 min)
  - Acetonitrile (1 mL/min, 30 min)
- **Baseline Stabilization**: Detector ON for 1 hour before run
- **Sample Loading**: Auto-injector used

Method Validation[67,72]

Validation followed **ICH Q2 (R1)** guidelines.

1. System Suitability

- Injected 20 µL of 10 µg/mL standard solution in **5 replicates**
- Parameters: **Retention Time, Peak Area, Tailing Factor, Theoretical Plates**

2. Specificity

- Ensured no interference at retention time of main peak in presence of excipients

3. Precision

- **System Precision**: Six replicates of 20 µg/mL solution
- **Method Precision**: Six test samples of known strength
- **Intra- & Inter-day Precision**:
  - Levels: 10, 20, 30 µg/mL
  - Intraday: every 2 hrs for 12 hrs
  - Interday: 3 different days

4. Accuracy (Recovery Study)

- **Standard Addition Method** at 80%, 100%, 120%
- % Recovery = A / (B + C) × 100

Where:

A = Total drug estimated

B = Amount of drug (mg)

C = Added pure drug (mg)

5. Linearity & Range

- Linear range: **10–50 µg/mL**
- Correlation coefficient and regression equation determined from calibration curve

6. Solution Stability

- Tested at 10 and 30 µg/mL
- Stored at **8 °C** and **room temperature** for 24 hrs

8. LOD & LOQ

- **LOD** = 3.3 × (σ/S)
- **LOQ** = 10 × (σ/S)
  - σ = Standard deviation of response
  - S = Slope of calibration curve

9. Robustness

- Flow rate changed to **0.8 mL/min and 1.2 mL/min**
- Compared assay and system suitability

10. Ruggedness

- Tested at 25°C, 37°C, and 60°C for 10, 20, 30 µg/mL solutions
- Evaluated % RSD

Analysis of Marketed Formulation by RP-HPLC

- **Tablet used**: Roliten 2 mg (Sun Pharma)
- **Sample Prep**:
  - Tablet powder equivalent to 2 mg TRT in 10 mL mobile phase
  - Sonicated 5–10 mins → Stock: 200 µg/mL
  - 1 mL diluted to 10 mL → 20 µg/mL

- **Analysis**
  - Injected into RP-HPLC
  - Compared area with standard
  - **% Assay Calculated**

RESULTS AND DISCUSSION

1. Organoleptic Properties

Table: Organoleptic Properties of Tolterodine Tartrate.

Tests	Specifications	Observation
Colour	White or almost white crystalline powder	White or almost white crystalline powder
Odour	Slight characteristic odor	Slight characteristic odor
Taste	Slightly bitter	Having a slight bitter taste
Solubility	Soluble in Water, Methanol, Ethanol, Propylene glycol	Soluble in Water, Methanol, Ethanol, Propylene glycol
Melting point	205.0 -209.0 °C	206.12 °C

1.1 FT-IR spectroscopy study

Identification of Tolterodine Tartrate was confirmed by FTIR Spectra. All peaks was found in Tolterodine Tartrate drug in figure 6.

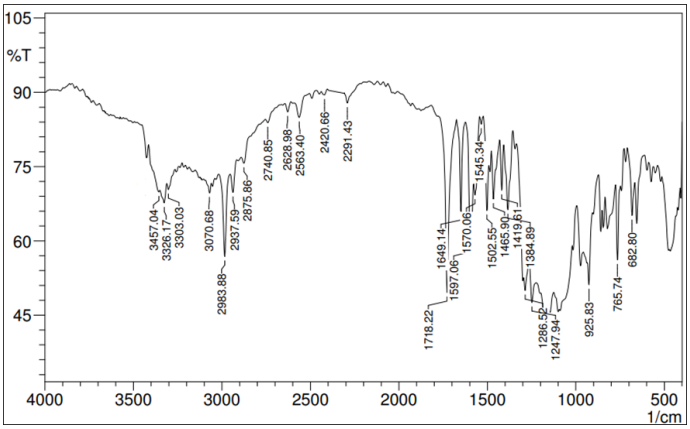

Fig: FTIR spectrum of Tolterodine Tartrate.

Table: Interpretation of IR spectrum of drug Tolterodine Tartrate.

Standard Frequency range	Observed Frequency	Function group present	Assignment
3200–3600	3457.04 cm−1,	O–H (alcohol/acid)	H-bonded OH group from tartrate
3000–3100	3070.68 cm−1,	Aromatic C–H stretch	Aromatic ring C–H
2850–2960	2875.86 cm−1, 2937.59 cm−1	C–H (alkyl stretch)	Aliphatic C–H stretch
1700–1725	1718.22 cm−1	COOH (from tartrate)	Sharp peak, carboxylic acid stretching
1450–1600	1465.90 cm−1	C=C (aromatic)	Aromatic ring vibrations
1250–1360	1286.52 cm−1	N–CH_3 (tertiary amine)	C–N stretching of dimethylamino group

Interpretation of Results

The FTIR spectrum of Tolterodine Tartrate reveals the presence of key functional groups that confirm its chemical structure. A broad absorption band around 3400 cm^{-1} corresponds to O–H stretching vibrations from the tartrate moiety, indicating hydrogen bonding. The sharp peak near 1718 cm^{-1} represents the C=O stretching of the carboxylic acid group. Aromatic C–H stretching vibrations are observed near 3050 cm^{-1}, while aliphatic C–H stretches appear around 2940 cm^{-1}, confirming both aromatic and aliphatic components. The C–N stretch of the tertiary amine is seen near 1286 cm^{-1}. These characteristic peaks collectively confirm the presence of alcohol, carboxylic acid, tertiary amine, aromatic, and ether groups in the Tolterodine Tartrate molecule.

2. DSC study of pure drug

Figures illustrates DSC profile of Tolterodine Tartrate. Tolterodine Tartrate showed a peak at 206.12^{0}C whereas, corresponding to the melting transition temperature and decomposition of drug. Such sharp endothermic peak signifies that Tolterodine Tartrate used was in pure crystalline state.

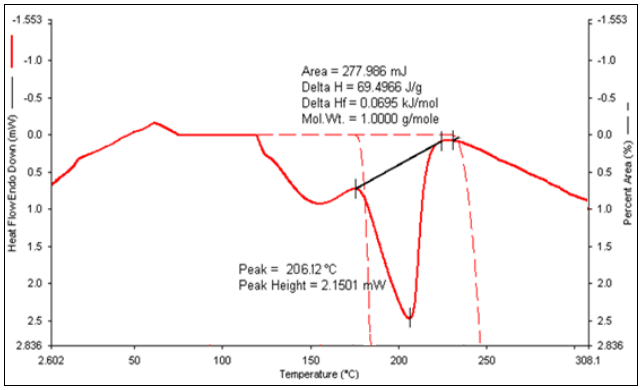

Fig. No. 10: DSC Thermogram of Tolterodine Tartrate.

The absence of exothermic peaks prior to melting indicates that drug, when considered individually, exhibit good thermal stability.

2. UV analysis

2.1 Determination of λmax

The determination of the maximum absorption wavelength (λmax) for Tolterodine Tartrate was conducted using a UV-visible spectrophotometer to identify the wavelength at which each drug exhibits maximum absorbance. Standard stock

solutions of both drugs were prepared in Propylene Glycol (PG): Methanol (10:90) and diluted to obtain a 10 µg/mL working solution. The solutions were scanned in the 200–400 nm range, and the λmax values were recorded.

Tolterodine Tartrate demonstrated maximum absorbance at approximately 282 nm. The selected λmax was further used for method development in RP-HPLC, ensuring optimal sensitivity and accuracy in drug quantification.

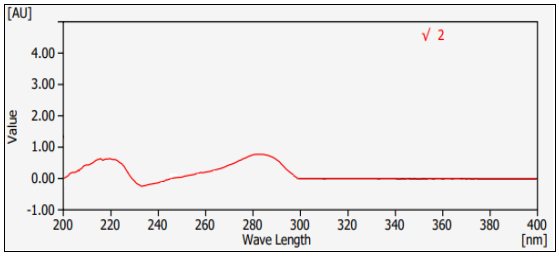

Fig: Maximum wavelength detection of Tolterodine Tartrate.

2.2 Development of standard curve for the Tolterodine Tartrate

Tolterodine Tartrate

The calibration curve of Tolterodine Tartrate was performed and graph plotted concentration vs. absorbance. The absorbance values of different concentration were noted. The regression equation was found to be y = 0.0494x-0.0213, with R^2 value of 0.9991. The graph was found to be linear.

Table: Concentration range and respective absorbance of Tolterodine Tartrate.

Sr. No.	Concentration (ppm)	Absorbance
1	5	0.259
2	10	0.518
3	15	0.782
4	20	1.001
5	25	1.253

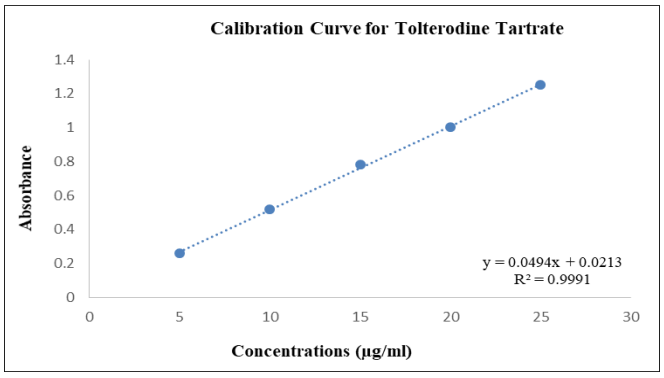

Fig. No.: Standard Curve for Tolterodine Tartrate.

3. Method Validation for Uv method development

3.1 Linearity

For the linearity of the Tolterodine Tartrate five point calibrations curve were plotted in a concentration range of 5-15 µg/ml. The equation was found to be y = 0.0494x-0.0213, with correlation coefficient of 0.9991. From the linearity study it was observed that the drug was found to be linear in the concentration range.

3.2 Accuracy

Accuracy of the proposed UV method for Tolterodine Tartrate was verified by conducting the recovery studies by using standard addition method. Standard drug concentration at three different percent levels was added to known amount of Tolterodine Tartrate. The percent recovery of added standards was calculated showed in the table. The results showed better % mean recovery for respective percent levels in table no 8. The % mean recovery values are closer to 100% showed high accuracy of the proposed UV analytical method.

Table: Evaluation data of Accuracy study of Tolterodine Tartrate.

	Tolterodine Tartrate				
Concentration (%)	**Origin level (µg/ml)**	**Amount added (µg /ml)**	**% Recovery**	**Mean % Recovery**	**% RSD**
80	5	4	98.46	99.613	1.163
80	5	5	99.61		
80	5	6	100.77		
100	15	12	99.87	100.298	0.390
100	15	15	100.38		
100	15	18	100.64		
120	25	20	99.68	99.950	0.332
120	25	25	100.32		
120	25	30	99.84		

3.3 Precision

Intra-day and inter-day precision study of drug were evaluated for the 5 µg/ml, 15 µg/ml and 20 µg/ml for Tolterodine Tartrate. Absorbance mean, percent assay and percent RSD were calculated for the intra-day as well as inter-day precision study showed in table no 9 & 10.

Table No.: Evaluation data for Intra-day and Inter-day study of Tolterodine Tartrate.

Intra-day	**Morning**			**Afternoon**			**Evening**		
Concentration Range (µg/ml)	**Mean**	**% Assay**	**% RSD**	**Mean**	**% Assay**	**% RSD**	**Mean**	**% Assay**	**% RSD**
5	0.258	99.742	0.804	0.256	101.04	0.981	0.250	101.465	1.284
15	0.781	100.213	0.195	0.775	101.29	1.909	0.782	99.872	0.461
25	1.252	99.973	0.121	1.251	99.973	0.122	1.251	100.213	0.201
Inter-day	**Day 1**			**Day 2**			**Day 3**		
Concentration Range (µg/ml)	**Mean**	**% Assay**	**% RSD**	**Mean**	**% Assay**	**% RSD**	**Mean**	**% Assay**	**% RSD**
5	0.257	98.832	1.029	0.258	101.419	1.564	0.253	100.395	1.425
15	0.785	99.872	0.127	0.784	100.340	0.482	0.785	100.00	0.382
25	1.252	100.514	0.092	1.232	101.920	1.702	1.247	100.749	0.970

3.4 Robustness

Robustness study was evaluated by using three different solvent. The method was found to be robust as indicated by the % RSD values which are less than 2%.

Table: Evaluation data for Robustness of Tolterodine Tartrate.

Tolterodine Tartrate			
Concentration (µg/ml)	**Solvents**	**Absorbance**	**% RSD**
20	Propylene glycol: Methanol (50:50)	1.001	0.208
20	propylene glycol: Acetonitrile (50:50)	0.998	0.358
20	Propylene Glycol: Methanol (90:10)	1.002	0.173

3.5 Ruggedness

Ruggedness study of drug was carried out at the three different temperature levels. From the results it was found that the method was rugged showing the % RSD value less than 2%.

Table: Evaluation data for Ruggedness of Tolterodine Tartrate.

Tolterodine Tartrate			
Concentration (µg/ml)	**Temperature (°C)**	**Absorbance**	**% RSD**
20	25	1.001	0.368
20	37	0.999	0.756
20	60	0.996	1.124

3.6 Limit of Detection (LOD) & Limit of Quantification (LOQ)

Form the results it was found that LOD & LOQ are in the sub-microgram level, which indicates the sensitivity of the method.

Table: Evaluation data for LOD & LOQ of Tolterodine Tartrate.

Tolterodine Tartrate	
LOD	0.211 µg/ml
LOQ	1.389 µg/ml

4 Analysis of Marketed Formulation by Uv visible spectrophotometer

The analysis of marketed formulations (Tolterodine Tartrate tablet IP 40 mg) (Glycinorm-40 Ipca Laboratories Ltd.) was carried out using Uv visible spectrophotometer. The percent assay test was carried out by forming suitable dilutions of marketed tablet. Make dilutions in such way that, the final concentration of Tolterodine Tartrate was in the range of standard dilutions.

Table: Analysis of marketed formulation.

	Standard (20µg/ml)	**Sample (marketed formulation) (20µg/ml)**
Absorbance	1.004	0.986
% assay	100	98.23

4 Method Development by Reverse Phase High Performance Liquid Chromatography

4.1 Optimization of Chromatographic Conditions and Method Development

In order to achieve the optimized chromatographic conditions to separate and quantify Tolterodine Tartrate, one or two parameters were modified at each trial and chromatograms were recorded with all specified chromatographic conditions. Various trials were carried out to finalize the optimized chromatographic conditions mentioned in the table no 14. Poor resolution, bad peak shapes, disturbances in base line were the few reasons of the rejections of the trials.

Table: Various Trials and Optimization of Chromatographic Conditions

Trial No	**HPLC System**	**Chromatographic Conditions**	**Observations**	**Remarks**
1	HPLC (Shimazdu LC 2010 with Uv detector)	**Mobile Phase-** Propylene glycol: Methanol (40:60) **Column -** Inertsil C18 (4.6 x 250mm, 5µm) **Flow rate-** 1 ml/min **Injection Volume-** 20µl **Pump mode-** Isocratic **Column temperature-** Ambient	Peak is not fully separated. peak shape was not acceptable	**Rejected**

		Wavelength- 282 nm		
2	HPLC (Shimazdu LC 2010 with Uv detector)	**Mobile Phase-** Propylene glycol: Acetonitrile (40:60) **Column -** Inertsil C18 (4.6 x 250mm, 5µm) **Flow rate-** 1 ml/min **Injection Volume-** 20µl **Pump mode-** Isocratic **Column temperature-** Ambient **Wavelength-** 282 nm	Peak was not fully separated but peak shape was good as compared to first trial	**Rejected**
3	HPLC (Shimazdu LC 2010 with Uv detector)	**Mobile Phase-** Propylene glycol: Water: Methanol (50:30:20) **Column -** Inertsil C18 (4.6 x 250 mm, 5µm) **Flow rate-** 1 ml/min **Injection Volume-** 20µl **Pump mode-** Isocratic **Column temperature-** Ambient **Wavelength-** 282 nm	Peaks were separated but the peak shapes was not good	**Rejected**
4	HPLC (Shimazdu LC 2010 with Uv detector)	**Mobile Phase-** Propylene glycol: Water: Methanol (50:40:10) **Column -** Inertsil C18 (4.6 x 250 mm, 5µm) **Flow rate-** 1 ml/min **Injection Volume-** 20µl **Pump mode-** Isocratic **Column temperature-** Ambient **Wavelength-** 282 nm	Peaks were separated but the first peak shape was not good	**Rejected**
5	HPLC (Shimazdu LC 2010 with Uv detector)	**Mobile Phase-** Propylene glycol: Water (pH 4.5) (pH adjusted with Orthophosphoric acid): Methanol (50:40:10) **Column -** Inertsil C18 (4.6 x 250 mm, 5µm) **Flow rate-** 1 ml/min **Injection Volume-** 20µl **Pump mode-** Isocratic **Column temperature-** Ambient **Wavelength-** 282 nm	Peaks shape were good, with good resolution and intensity at pH 4.5	**Accepted**

Blank Chromatogram

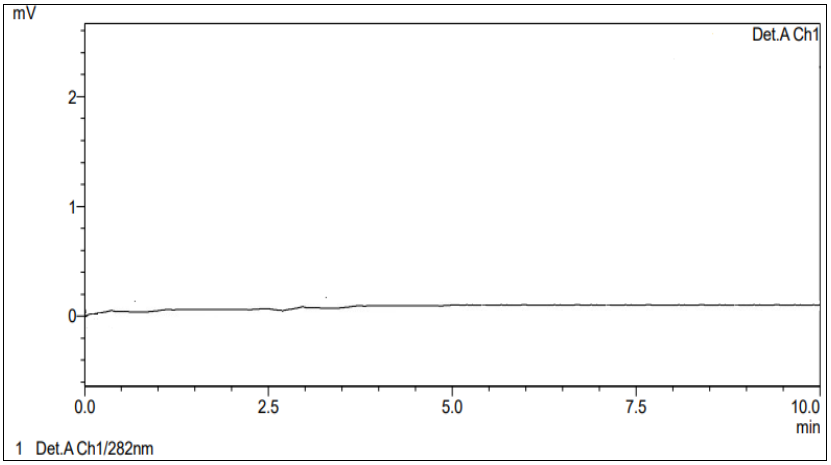

Fig.: Blank Chromatogram.

Trial 1

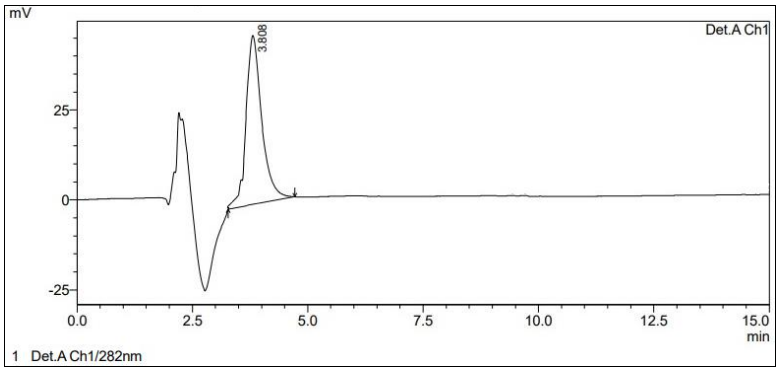

Fig.: HPLC Fingerprinting of Tolterodine Tartrate for trial. 1

Table: Evaluation parameter of trial 1.

Sr. No.	Name	Retention Time (min)	Area (µV*sec)	Height (µV)
1	Tolterodine Tartrate	3.808	268462	18935

Trial 2

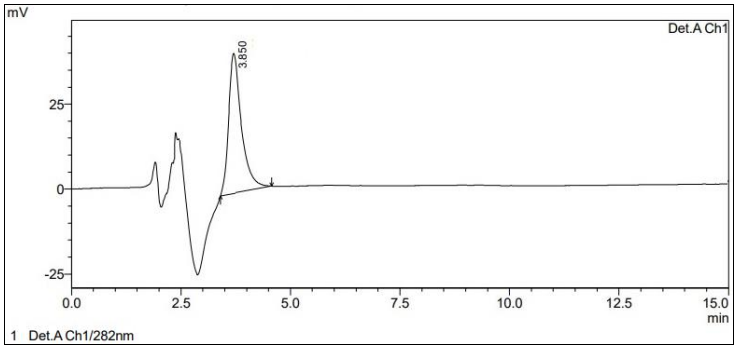

Fig.: HPLC Fingerprinting of Tolterodine Tartrate for trial 2.

Table: Evaluation parameter of trial 2.

Sr. No.	Name	Retention Time min)	Area (µV*sec)	Height (µV)
1	Tolterodine Tartrate	3.850	296854	23564

Trial 3

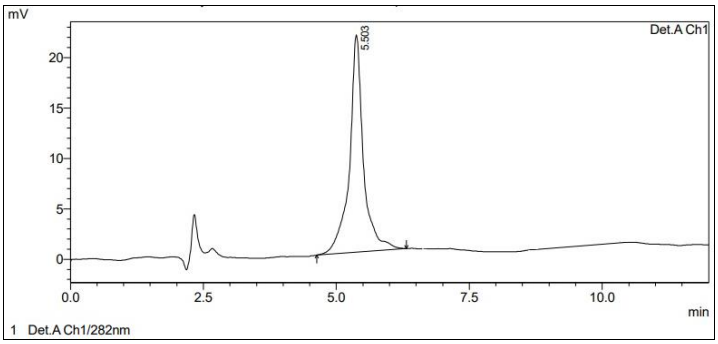

Fig.: HPLC Fingerprinting of Tolterodine Tartrate for trial 3.

Table: Evaluation parameter of trial 3.

Sr. No.	Name	Retention Time (min)	Area (µV*sec)	Height (µV)
1	Tolterodine Tartrate	5.503	372546	20463

Trial 4

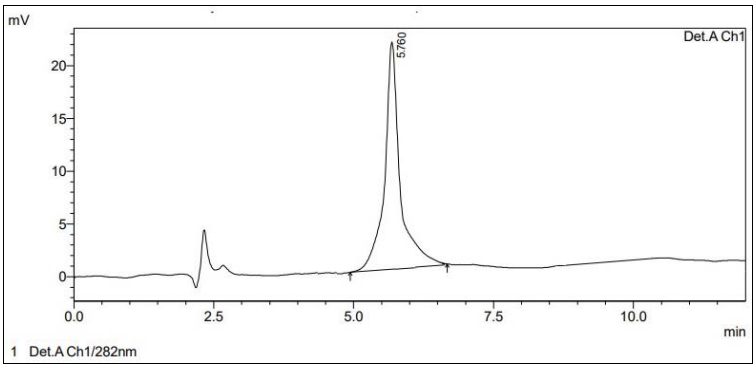

Fig.: HPLC Fingerprinting of Tolterodine Tartrate for trial 4.

Table: Evaluation parameter of trial 4.

Sr. No.	Name	Retention Time (min)	Area (µV*sec)	Height (µV)
1	Tolterodine Tartrate	5.760	387624	21357

Optimized trial

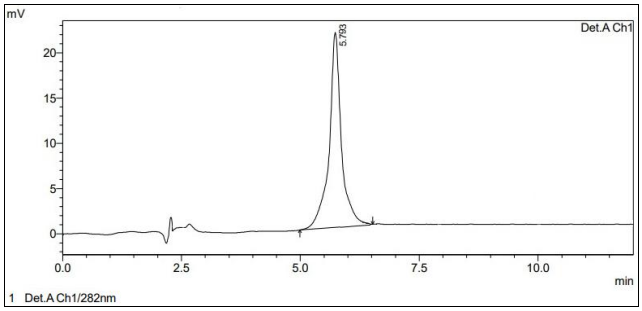

Fig.: Optimized HPLC Fingerprinting of Tolterodine Tartrate.

Table: Evaluation parameter of Optimized Tolterodine Tartrate trial.

Sr. No.	Name	Retention Time (min)	Area (µV*sec)	Height (µV)
1	Tolterodine Tartrate	5.793	391862	21486

4.2 Method Validation

The following parameters were considered for the analytical method validation of optimized method:

- System Suitability
- Specificity
- Linearity and Range
- Precision
  - ✓ System Precision
  - ✓ Method Precision
  - ✓ Inter-day Precision
  - ✓ Intraday Precision
- Ruggedness
- Accuracy (Recovery)
- Robustness
- Limit Of Detection(LOD)

- Limit Of Quantitation (LOQ)
- Solution Stability
- Application of method to the marketed dosage form

4.2.1 System Suitability

The HPLC method has been developed for the determination of the percentage assay of Tolterodine Tartrate in tablet dosage form. Parameters like Retention time, Peak area, tailing factor, and theoretical plates were found to be within acceptable limit.

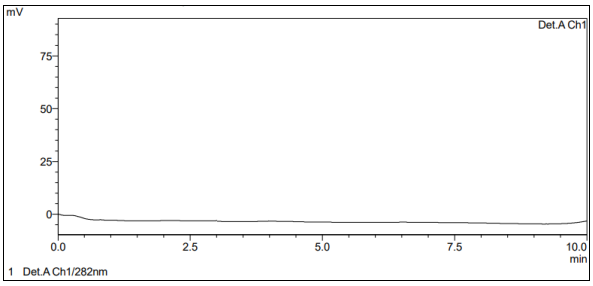

Fig.: Blank Chromatogram for System Suitability.

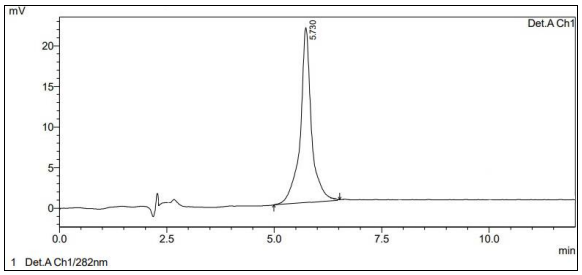

Fig.: System Suitability Chromatogram of Standard Tolterodine Tartrate 1.

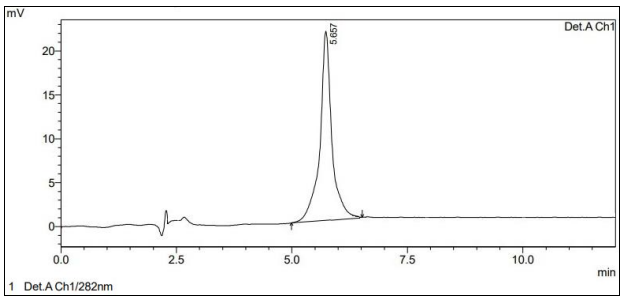

Fig.: System Suitability Chromatogram of Standard Tolterodine Tartrate 2.

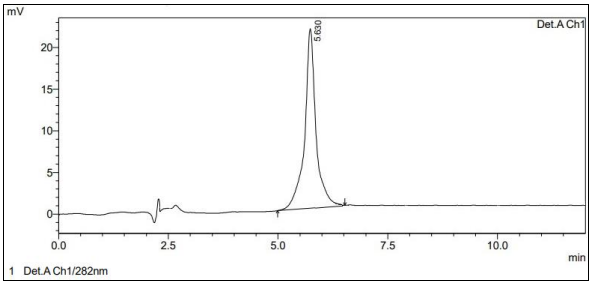

Fig.: System Suitability Chromatogram of Standard Tolterodine Tartrate 3.

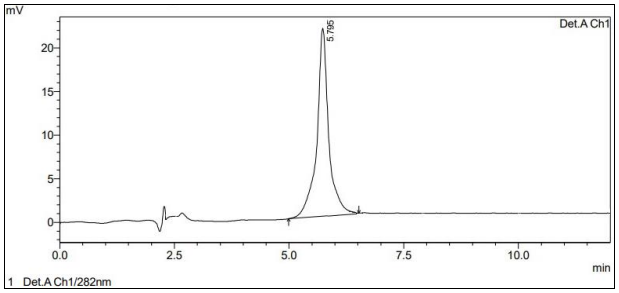

Fig.: System Suitability Chromatogram of Standard Tolterodine Tartrate 4.

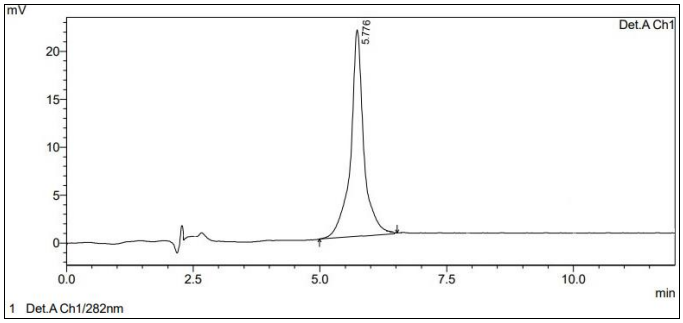

Fig.: System Suitability Chromatogram of Standard Tolterodine Tartrate 5.

Table: System Suitability Parameters of Tolterodine Tartrate.

Replicates		Retention time	Peak area	Tailing Factor	Theoretical Plates
1	TRT	5.730	391567	1.015	3592.494
2	TRT	5.657	392684	1.011	3592.685
3	TRT	5.630	393742	0.949	3827.273
4	TRT	5.795	392504	0.947	3794.905
5	TRT	5.776	392517	1.018	3670.583

4.2.2 Specificity

The absence of additional peaks in the chromatogram indicates non- interference of excipients. There was no interference from the blank at the retention time of analyte peak. The chromatograms of Blank and standard drug. The Retention time for Tolterodine Tartrate was found to be 5.765 min respectively.

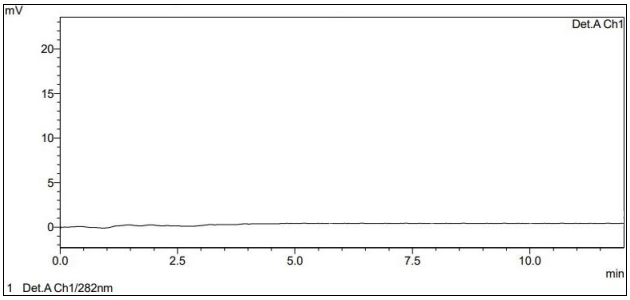

Fig.: Blank chromatogram for Specificity.

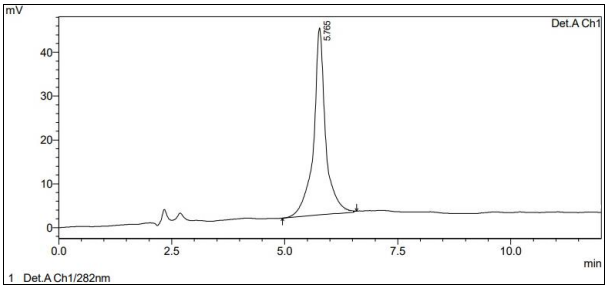

Fig.: Standard Chromatogram of Tolterodine Tartrate.

Table: Specificity Parameters for Tolterodine Tartrate.

Sr. No.	Name	Retention Time (min)	Area (µV*sec)	Tailing Factor	Theoretical Plate Count
1	TRT	5.765	794213	1.025	3586.314

4.2.3 Precision

a) System Precision

The system precision was performed by measuring the peak response for standard drugs solutions in six replicates. Peak areas, mean, standard deviation and % relative standard deviation (%RSD) for Tolterodine Tartrate was found to be 1.230%. The results were found well within the acceptable criteria. The chromatogram was showed in figure 24-29.

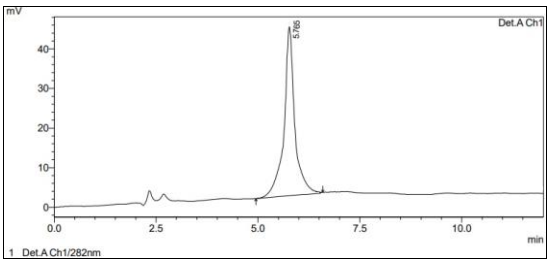

Fig.: Chromatogram of system precision 1.

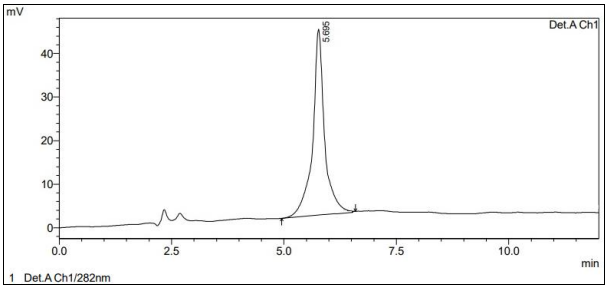

Fig.: Chromatogram of system precision 2.

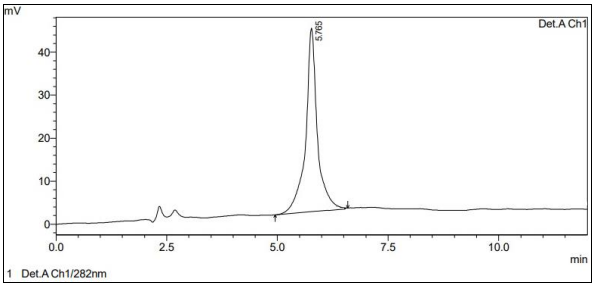

Fig.: Chromatogram of system precision 3.

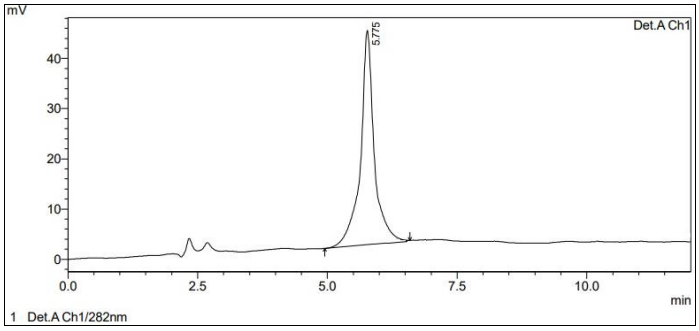

Fig.: Chromatogram of system precision 4.

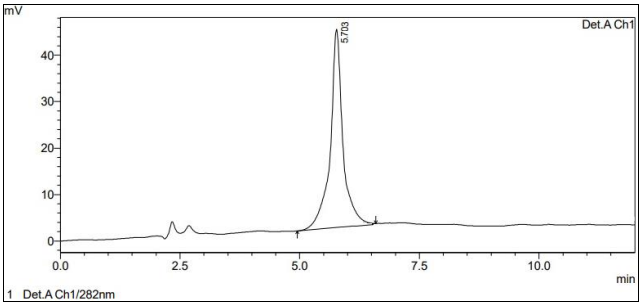

Fig.: Chromatogram of system precision 5.

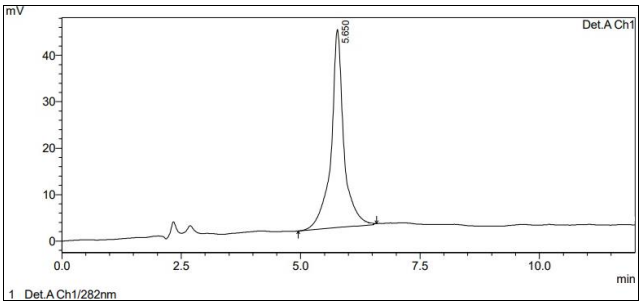

Fig.: Chromatogram of system precision 6.

Table: System Precision Data of Tolterodine Tartrate.

Sr. No.	Peak areas of TRT
1	794123
2	789547
3	794263
4	794052
5	794127
6	786542
Mean	**792109.00**
SD (±)	**3289.341**
RSD (%)	**0.415**
Acceptance Criteria	**% RSD Should not be more 2**

b) Method Precision

The method precision was performed by measuring the peak response for sample solutions in six replicates. The % assay for Tolterodine Tartrate in six samples was calculated. The results of % assay and % RSD are shown in table 23.

Table: Method Precision Data of Tolterodine Tartrate.

Sr. No.	% Assay of TRT (w/w)
1	100.52
2	101.34
3	99.37
4	100.28
5	99.64
6	100.54
Mean	**100.28**
SD (±)	**0.705**
RSD (%)	**0.705**
Acceptance Criteria	**% RSD Should not be more 2**

c) Intraday and Inter-day Precision

The % RSD in intraday precision for Tolterodine Tartrate (10, 20, 30 µg/ml) was found to be 0.718, 1.242, 0.285%. In inter-day precision % RSD for Tolterodine Tartrate (10, 20, 30 µg/ml) was found to be 00.926, 1.181, 1.126 %. Percent RSD in intraday and inter-day studies were found well within the acceptable limits. The results obtained are mentioned in the table no 24 & 25.

Table: Intraday Precision data of Tolterodine Tartrate

Tolterodine Tartrate					
Sr. no.	**Conc.**(µg/ml)	**Area**	**mean peak area**	**SD(±)**	**%RSD**
1	10	392746	391947	2041.352	**0.520**
		393468			
		389627			
2	20	794163	791359	4964.149	**0.627**
		785627			
		794286			
3	30	1252486	1252339	1424.725	**0.113**
		1250846			
		1253684			

Table: Inter-day precision data of Tolterodine Tartrate

Tolterodine Tartrate						
Sr. no.	**Day**	**Conc.** (µg/ml)	**Area**	**mean peak area**	**SD(±)**	**%RSD**
1	Day 1	10	392777	389929	2675.57	**0.686**
	Day 2		387468			
	Day 3		389542			
2	Day 1	20	788234	788840	1953.00	**0.247**
	Day 2		791025			
	Day 3		787263			
3	Day 1	30	1253864	1251839	2001.42	**0.159**
	Day 2		1251792			
	Day 3		1249862			

4.2.5 Accuracy (Recovery Study)

The accuracy of the assay method was evaluated by standard addition method in triplicate at 80%, 100% and 120% level of the labeled claim and the percentage recovery was calculated. The mean % recovery was found to be 100.19 % for Tolterodine Tartrate. The results of the recovery study are shown in the table 26.

Table: Recovery study for Tolterodine Tartrate.

Tolterodine Tartrate							
Level	**Set**	**Amount added(µg/ml)**	**Amount found(µg/ml)**	**%Recovery**	**Mean**	**SD**	**%RSD**
80%	1	10	9.915	100.86	100.23	0.553	**0.551**
	2	10	10.019	99.80			
	3	10	9.996	100.04			
100%	1	20	19.886	100.57	100.34	0.391	**0.390**
	2	20	20.024	99.88			
	3	20	19.892	100.54			
120%	1	30	29.983	100.05	100.02	0.079	**0.007**
	2	30	30.021	99.93			
	3	30	29.977	100.08			

4.2.6 Linearity and Range

Linearity for Tolterodine Tartrate was found to be in the range of 10-50 µg /ml with correlation coefficient value (r2) 0.9993 for Tolterodine Tartrate. The results were tabulated in table no 27 and graphically represented in figure 30-35.

Table: Linearity and Range for Tolterodine Tartrate.

10	392843
20	794156
30	1252578
40	1684623
50	2063462
Slope	**41860**
CC	**15230**

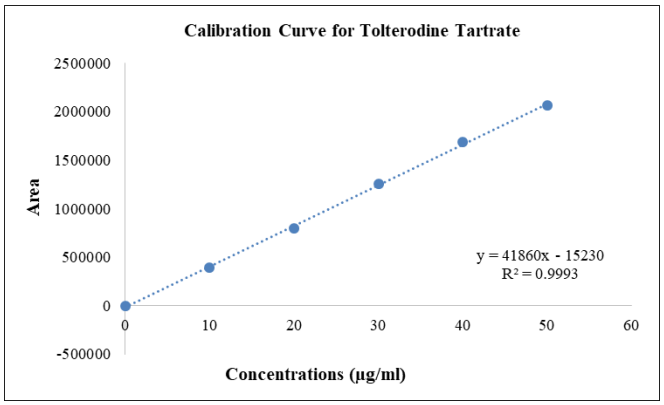

Fig.: Standard Curve for Tolterodine Tartrate.

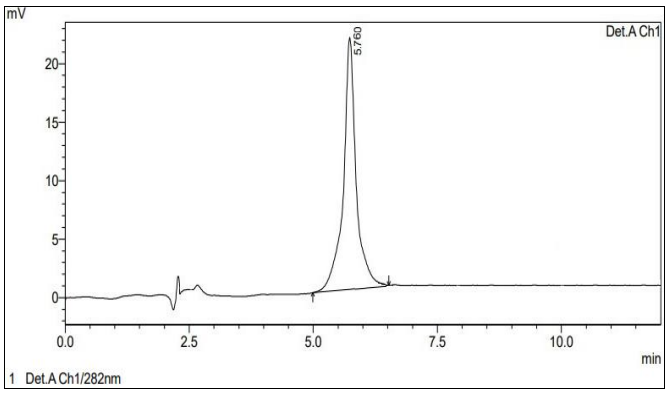

Fig: Standard Chromatogram for Linearity (TRT 10 µg /ml).

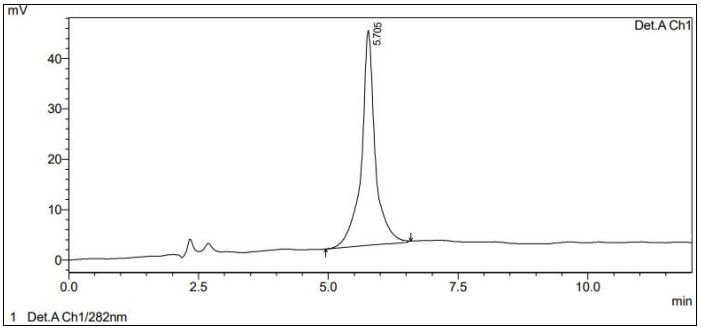

Fig.: Standard Chromatogram for Linearity (TRT 20 µg /ml).

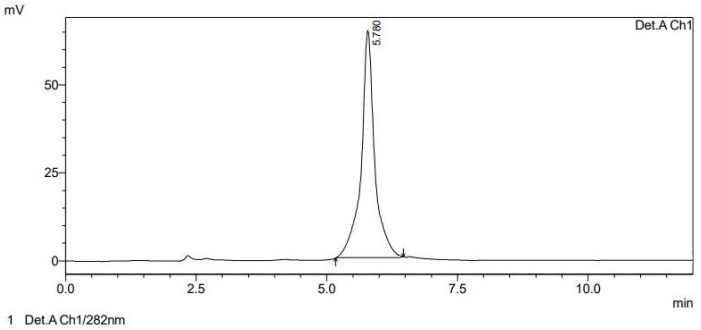

Fig.: Standard Chromatogram for Linearity (TRT 30 µg /ml).

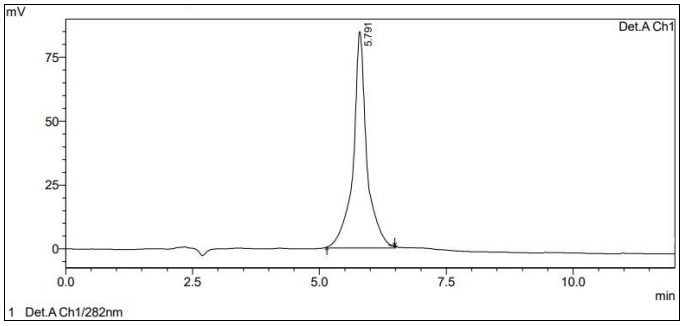

Fig.: Standard Chromatogram for Linearity (TRT 40 µg /ml).

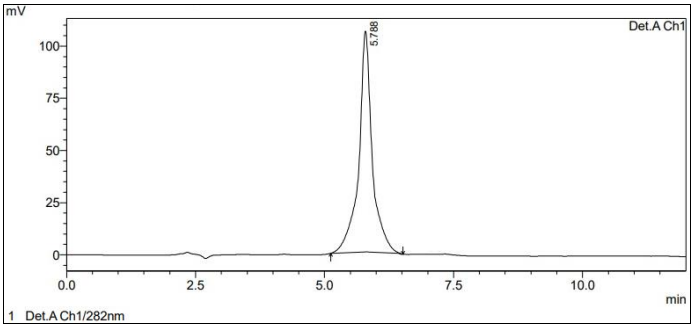

Fig.: Standard Chromatogram for Linearity (TRT 50 µg /ml).

4.2.7 Stability in Analytical Solution

No significant difference was found in the % Assay of both drugs before and after storing for 24 hrs in refrigerator and room temperature. This confirms the stability of the drugs in solutions. The percentage assay is tabulated in table 28.

Table: Solution Stability Data of Tolterodine Tartrate.

Time level	Refrigerator (25°C)	Room Condition (37°C)
Time in hrs	% Assay of TRT	
Initial	100.25 (±0.34)	99.12 (±0.032)
After 24 hrs	101.23 (±0.58)	100.09 (±0.046)

**Average of Six determination*

4.2.8 Limit of Detection (LOD) and Limit of Quantitation (LOQ)

For Tolterodine Tartrate LOD and LOQ were found to be 3.808 µg/ml and 1.154 µg/ml respectively. These values indicate that the method is suitable for the determination of the lower concentration and confirms that proposed method is sensitive for the determination.

4.2.9 Robustness

The robustness of an HPLC method is states about its capacity to remain unaffected by minor, deliberate alterations to its method parameters. This quality ensures the reliability of the method during routine usage. Percent (%) RSD at each condition was found less than 2. This indicates the robustness of the method. The results are tabulated in table no 29 & 30.

Table. Robustness data of Tolterodine Tartrate at flow rate 0.8 ml/min.

TRT				
Flow Rate (0.8ml/min)				
	Retention Time (min)	**Area**	**Theoretical Plate**	**Tailing factor**
	5.884	392762	3594.12	1.102
	5.846	392887	3498.02	1.113
	5.882	388624	3584.26	1.128
Avg	**5.870**	**391424**	**3558.80**	**1.114**
SD	**0.021**	**2425.97**	**52.871**	**0.013**
%RSD	**0.364**	**0.619**	**1.485**	**1.171**

Table: Robustness data of Tolterodine Tartrate at flow rate 1.2 ml/min.

TRT				
Flow Rate (1.2 ml/min)				
	Retention Time (min)	**Area**	**Theoretical Plate**	**Tailing factor**
	5.693	389674	3486.26	1.110
	5.564	392760	3562.22	1.103
	5.676	392864	3524.38	1.116
Avg.	**5.644**	**391766**	**3524.29**	**1.109**
SD	**0.070**	**1812.47**	**37.982**	**0.006**
%RSD	**1.241**	**0.462**	**1.077**	**0.586**

4.2.10 Ruggedness

The ruggedness parameter was determined by analyzing the different concentration at different temperature. The results were showed in table.

Table: Data of Ruggedness for Tolterodine Tartrate

TRT				
Change in Parameters	**Area of Standard**	**Mean**	**SD**	**%RSD**
25°C	392846	387338	5060.16	**1.306**
	382895			

	386274			
37°C	794153	791710	5173.28	**0.653**
	785768			
	795210			
60 °C	1251684	1246304	10074.70	**0.808**
	1252546			
	1234681			

6 Analysis of Marketed Formulation

The analysis of marketed formulation was carried out by using RP-HPLC. The percent assay parameter were analyzed and the results were shown below. The percent assay test was done by conducting the marketed tablets for suitable dilutions according to the standard concentrations.

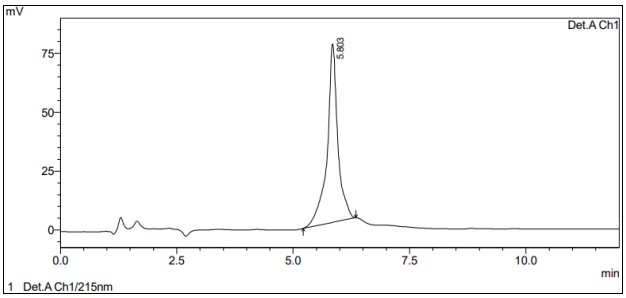

Fig.: Chromatogram of Marketed Formulation (Roliten 2mg Sun Pharmaceutical Industries Ltd.).

Table: Evaluation parameters of Marketed Formulation

Sr. No.	Name	Retention Time (min)	Area (µV*sec)	Tailing Factor	Theoretical Plate Count	% Assay
1	Marketed Formulation (Roliten 2mg Sun Pharmaceutical Industries Ltd.)	5.803 (TRT)	1662385 (TRT)	1.021 (TRT)	3487.53 (TRT)	98.68

The results of Marketed formulation (Tolterodine Tartrate tablet IP 40 mg) Roliten 2mg Sun Pharmaceutical Industries Ltd. was found to be satisfactory. The percent assay of Roliten 2mg tablet was found to be 98.68 % for Tolterodine Tartrate.

CONCLUSION

In the present work, developed simple, precise, accurate and low cost UV and RP-HPLC method. It is successfully applied for the simultaneous determination of Tolterodine Tartrate (TRT) in pharmaceutical preparations without the interferences of other constituent in the formulations. For identification of drug, multiple tests such as organoleptic characteristics, melting point, UV and FTIR spectroscopic analysis, solubility and DSC were performed.

UV method for TRT was developed by using the spectrum mode of analysis of Lasany LI-2702 Visible double beam spectrophotometer. Method was developed by using Propylene Glycol (PG): Methanol (90:10) as a solvent system. By scanning, the each solution was in the range of 200-400 nm. 282 nm was selected as a maximum wavelength for TRT. The method, obeys Beer's and Lambert law. Method was validated with the help of parameter as linearity, accuracy, precision (intraday and interday), LOD LOQ, ruggedness, robustness. HPLC method for analysis of Tolterodine Tartrate was developed by using HPLC system of Shimadzu LC 2010 series with C18 Intersil ODS-3V (250 x 4.6mm) column. Initially, various mobile phase compositions were tried, to get good optimum result. Mobile phase and flow rate selection was based on peak parameters. Chromatogram for drug was developed using triphasic mobile phase,

Propylene glycol: Water (pH 4.5) (pH adjusted with Orthophosphoric acid): Methanol in the ratio of 50:40:10 with flow rate 1 ml/min at 282 nm. The retention time of drug was found to be 5.473 for TRT. The calibration was linear in concentration range of 100-500 µg/ml for TRT. The low values of % R.S.D. indicate the method is precise and accurate. The mean recoveries were found in the range of 99.94% to 101.33 % for TRT.

The proposed method was validated in accordance with ICH parameters and the results of all Methods were very close to each other as well as to the label value of pharmaceutical tablet formulation. Therefore, there is no significant difference in the results achieved by the proposed method. Therefore it is suggested that the developed UV and RP-HPLC methods can be effectively applied for the routine analysis of TRT in different Dosage form.

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