Chinese Journal of Health Management
Volume 20 Issue 3, Year of Publication 2026, Page 467-476 | DoI-10.564220/1674-0815.2026.71

ISSN: 1674-0815

cjhmonline.com

DoI-10.564220/1674-0815

Chinese Journal of Health Management

Chinese Medical Association

Analytical Methods Development and Validation for Simultaneous Estimation of Tolterodine Tartrate and Mirabegron Acetate in Synthetic Mixture

Bansari J. Mehta[1], **Jitendra O. Bhangale**[2]
[1]Student, Smt. N. M. Padalia Pharmacy College, Navapura, Ahmedabad, Gujarat, India 382210.
[2]Professor and Principal, Smt. N. M. Padalia Pharmacy College, Ahmedabad, Gujarat, 382210, India.
Corresponding Author Email id: **jitu2586@gmail.com**

Article Information
Received: 06-12-2025
Revised: 12-01-2026
Accepted: 17-02-2026
Published: 25-03-2026

Keywords
Tolterodine tartrate, Mirabegron acetate, first-order derivative UV Spectrophotometric, RP-HPLC, Validation Parameters

ABSTRACT:
Tolterodine tartrate, a muscarinic receptor antagonist, and Mirabegron acetate, a β_3-adrenoceptor agonist, are widely used for the management of overactive bladder (OAB). Their combination offers enhanced therapeutic efficacy due to complementary mechanisms of action, particularly in patients inadequately controlled with monotherapy. This combination reported as an effective and safe choice for the management of OAB syndrome. However, despite the availability of analytical methods for individual drugs, no validated method has been reported for their simultaneous estimation. Therefore, this study aims to develop and validate simple, precise, and accurate first order derivative UV spectrophotometric and RP-HPLC methods for the simultaneous estimation of Tolterodine tartrate and Mirabegron acetate in synthetic mixture in accordance with ICH Q2 (R2) guideline. For the UV spectrophotometric method, Methanol was used as the solvent for analysis. The first order derivative method was selected using 221 nm and 283 nm wavelengths for measurement of Tolterodine tartrate and Mirabegron acetate, respectively. The zero-crossing point (ZCP) of Tolterodine tartrate was found to be 283 nm and 221 nm for Mirabegron acetate. Chromatographic separation was carried out under isocratic conditions using a C_{18} column. The optimized mobile phase consisted of Phosphate Buffer (pH 3.0 adjusted with ortho phosphoric acid): Acetonitrile (65:35 % v/v) to achieve well-resolved and symmetrical peaks of both analytes. The flow rate was maintained at 1.0 ml/min, and detection was performed at 278 nm wavelength. The linearity of Tolterodine tartrate and Mirabegron acetate for both methods were established in the range of 2-10 µg/ml and 25-125 µg/ml, respectively. Retention time for Tolterodine tartrate and Mirabegron acetate was 3.8 min and 6.2 min, indicating efficient separation. The developed methods were validated for parameters including specificity, linearity, range, precision, accuracy, limit of detection (LOD), limit of quantitation (LOQ), assay, system suitability. All validation results were found to be within acceptable limits. Both techniques were suitable for routine quality control analysis.

1. INTRODUCTION:

Overactive bladder (OAB) is a prevalent clinical condition characterized by urinary urgency, increased frequency, nocturia, and urge urinary incontinence, significantly impairing patients' quality of life. It results from involuntary contractions of the detrusor muscle and may occur independently or in combination with stress urinary incontinence as mixed urinary incontinence. Epidemiological data indicate that OAB affects approximately 11% of the population, with a considerable proportion of patients experiencing incontinence, thereby representing a significant healthcare burden [1]. Tolterodine tartrate, an antimuscarinic agent, reduces bladder overactivity by inhibiting muscarinic receptors and suppressing involuntary detrusor contractions [2]. In contrast, Mirabegron acetate, a β_3-adrenoceptor agonist, promotes bladder relaxation and increases storage capacity during the filling phase. Both drugs are widely used as effective oral therapies for the management of OAB [3]. Recent clinical studies have demonstrated that combination therapy of tolterodine tartrate and mirabegron acetate provides enhanced therapeutic efficacy compared to monotherapy. This synergistic effect arises from their complementary mechanisms of action, where tolterodine decreases bladder contractions while mirabegron facilitates detrusor relaxation. As a result, the combination improves urinary urgency and frequency, reduces urge incontinence episodes, enhances bladder capacity, and offers better symptom control with improved patient compliance and tolerability [4-7]. A comprehensive literature survey reveals those various analytical techniques, including UV spectrophotometry [8-12], RP-HPLC [13-17], Stability indicating RP-HPLC method [18, 19], bioanalytical method for estimation of tolterodine tartrate in human plasma by using RP-HPLC [20], HPTLC with fluorometric detection [21], and LC-MS/MS [22], Stability indicating UV Spectroscopy[23], have been reported for the individual and with other drugs. However, an extensive literature survey indicates that, no validated method has been reported for the simultaneous estimation of Tolterodine tartrate and Mirabegron acetate in a combined synthetic mixture, highlighting the need for the present study.

Recent studies demonstrate that combined administration of these drugs produces synergistic therapeutic effects, enhancing overall treatment efficacy. However, the lack of a validated analytical method for their simultaneous estimation necessitates the development of a precise, accurate, and reliable analytical methods on the basis of the present study. Therefore, the present study aimed to develop and validate a sensitive, precise, and robust RP-HPLC and first order derivative UV spectrophotometric methods for the simultaneous estimation of Tolterodine tartrate and Mirabegron acetate in a synthetic mixture, in accordance with ICH Q2 (R2) guideline [24].

2. MATERIALS AND METHODS:

2.1 Compounds and Components:

Tolterodine tartrate and Mirabegron acetate were bought from Zydus Lifesciences Pvt. Ltd. from Ahmedabad as gift samples. Finar Chemicals, Ahmadabad provided the HPLC-grade methanol, acetonitrile, and water that were employed. Ortho-phosphoric acid (75% AR grade) and potassium dihydrogen phosphate were procured from Astron Chemicals Ltd., India. All solutions were freshly prepared each day.

2.2 Scientific conditions with instrumentation:

The RP-HPLC process was successfully executed using the Clarify software, a Systronic RP-HPLC (LC-20-AD), a UV Detector SPD-20 A, and a Rheodyne injector equipped with a 20 µl loop. Reversal aspect strategies were employed during the technique's execution. Applying Phosphate Buffer (pH 3.0 adjusted with ortho phosphoric acid): Acetonitrile (65:35 % v/v) with a flow rate of 1 ml/min, both medicines had been isocratically eluted. The UV-Vis Detector's detecting wavelength has been configured at 225 nm. Every day, preparations containing mobile phases had been made and passed through 0.45 µm Millipore membrane filters and sonicate with Sonicator (Equitron, India) prior usage. Both a pH meter and a Kromstar C_{18} (250 × 4.6 mm, 5 µm) Column consisted utilised. At 25°C, the high-performance LC system was run at ambient temperature. Shimadzu UV Visible double beam spectrophotometer (Model 1900) along with UV probe 2.7 version software and 1.0 cm quartz cells were used for the UV Spectrophotometric technique. Balancing was carried out throughout on a Scale-Tec Analytical balance.

2.3 Preparation of stock solution:

An accurately weighed quantity of Tolterodine tartrate (4 mg) and Mirabegron acetate (50 mg) were separately transferred into two different 100 ml volumetric flasks. Each flask was then made up to the mark with the Methanol to prepare standard stock solutions, resulting in a final concentration of 100 µg/ml for both Tolterodine tartrate and Mirabegron acetate.

2.4 Preparation of sample solution:

Accurately 1 ml of the above mixture solution of Tolterodine tartrate (40 µg/ml) and Mirabegron acetate (500 µg/ml) was pipetted out into 10 ml volumetric flask and the volume was adjusted up to the mark with Methanol. Final concentration of Tolterodine tartrate was 4 µg/ml and Mirabegron acetate 50 µg/ml.

2.5 Preparation standard working solution:

The concentration ranges of **2-10 µg/mL for Tolterodine** and 25-125 **µg/mL for Mirabegron** were prepared from their respective stock solutions. Aliquots of **Tolterodine (0.5, 1.0, 1.5, 2.0, and 2.5 ml)** and **Mirabegron (0.5, 1.0, 1.5, 2.0, and 2.5 ml)** were pipetted into ten different 10 mL volumetric flasks and diluted to the mark with Methanol to obtain final concentrations of **2, 4, 6, 8, and 10 µg/ml for Tolterodine** and **25, 50, 75, 100, and 125 µg/ml for Mirabegron**, respectively. Under the optimized spectrophotometric conditions, the samples were analyzed using a 1 cm quartz cuvette in the UV spectrophotometer. Similarly, under the optimized RP-HPLC conditions, **20 µl of each standard working solution** was injected into the system for chromatographic analysis.

2.6 Preparation of 10% Orthophosphoric acid

A 10% orthophosphoric acid solution was prepared by accurately transferring 1.0 ml of concentrated orthophosphoric acid into a 10 ml volumetric flask containing a small quantity of HPLC-grade water. The solution was then diluted up to the mark with HPLC-grade water and mixed thoroughly to obtain the required concentration.

2.7 Preparation of 10mM Phosphate Buffer:

Accurately weighed 0.272 g of potassium dihydrogen phosphate (KH_2PO_4) was transferred into a suitable volumetric flask containing 200 ml of HPLC-grade water and allowed to dissolve completely. The solution was then filtered through a 0.45 µm membrane filter and sonicated for approximately 10 minutes to remove any dissolved gases. The pH of the buffer solution was adjusted to 4.0 using 10% orthophosphoric acid.

3. ANALYTICAL TECHNIQUES:

3.1 Method development:

3.1.1 Method I: UV Spectrophotometric Method:

A first-order derivative spectrophotometric method was used for the simultaneous estimation of Tolterodine Tartrate and Mirabegron Acetate in a synthetic mixture. Separate working standard solutions were scanned in the 200-400 nm range to obtain derivative spectra and determine suitable zero-crossing wavelengths for analysis. Standard stock solutions of both drugs were prepared in methanol at 100 µg/ml. Aliquots of 0.4 ml of Tolterodine Tartrate and 5 ml of Mirabegron Acetate were transferred into separate 10 ml volumetric flasks and diluted to volume with methanol to obtain concentrations of 4 µg/ml and 50 µg/ml, respectively.

The zero-order spectra were recorded and converted to first-derivative spectra. Upon overlaying, Tolterodine tartrate showed a zero-crossing point at 283 nm, while Mirabegron acetate showed a zero-crossing point at 221 nm. Quantification was performed at 221 nm for Tolterodine tartrate (ZCP of Mirabegron acetate) and at 283 nm for Mirabegron acetate (ZCP of Tolterodine tartrate). The zero-order and first-order overlay spectra are presented in Figure 1 (a) and (b), respectively.

3.1.2 Method II: Reverse Phase High Performance Liquid Chromatography:

The isocratic analysis was carried out using Reverse phase chromatographic technique because of its recommended use for ionic and moderate to non-polar compounds using a mobile phase comprised Phosphate Buffer (pH 3.0 adjusted with ortho phosphoric acid): Acetonitrile (65:35 %v/v) at a flow rate of 1 ml/min found better separation of both the drug peaks. Prior to usage, the solvents were filtered through a 0.45 µ filter and sonicated for 10 min. The stationary phase was a Kromstar C_{18} (250 mm × 4.6 mm, 5 µm), and the eluent was observed by a U.V Detector at 278 nm showed in Figure 1.

3.2 Method Validation:

The developed method was validated with respect to specificity, linearity, range, accuracy, precision, limit of detection and limit of quantification, robustness, system suitability tests in accordance with the ICH Q2 (R2) guideline [24].

3.2.1 Specificity:

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically, these might include impurities, degradation products, and excipients. For Tolterodine and Mirabegron, specificity was demonstrated by comparing the chromatogram of the mobile phase and the test preparation solution, which confirmed that there was no interference from the mobile phase or excipient peaks with the peaks of Tolterodine tartrate and Mirabegron acetate.

3.2.2 Linearity & Range (n=6):
The linearity of Tolterodine tartrate and Mirabegron acetate was found to be in the range of 2-10 µg/ml and 25-125 µg/ml, respectively. Plot the calibration curve of Peak area Vs Concentration (µg/ml). Linearity of both the drugs was checked in term of slope, intercept and correlation coefficient.

3.2.3 Precision:
The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: Intermediate (Intraday) precision, Reproducibility (Interday precision), Repeatability.

3.2.3.1 Intraday Precision (n=3):
Solutions containing 2,4,6 µg/ml of Tolterodine tartrate and 25, 50, 70 µg/ml of Mirabegron acetate were analyzed three times on the same day and % R. S. D. was calculated.

***3.2.3.2 Interday Precision (n=3)*:**
Solutions containing 2, 4, 6 µg/ml of Tolterodine tartrate and 25, 50, 75 µg/ml of Mirabegron acetate were analyzed on three different successive days and % R. S. D, was calculated.

***3.2.3.3 Repeatability (n=6)*:**
Solutions containing 4 µg/ml of Tolterodine tartrate and 50 µg/ml of Mirabegron acetate were analyzed for six times and %R.S.D. was calculated. %R.S.D. was not more than 2%.

3.2.4 Limit of Detection (LOD):
Limit of detection can be calculated using following equation as per ICH Q2 (R2) guideline

$$\text{LOD} = 3.3 * \frac{\sigma}{S}$$

Where, SD = Standard deviation
Slope = the mean slope of the calibration curve

3.2.5 Limit of Quantification (LOQ):
Limit of quantification can be calculated using following equation

$$\text{LOQ} = 10 * \frac{\sigma}{S}$$

where, σ = Standard deviation
S = Mean slope of the corresponding calibration curve.

3.2.6 Accuracy:
The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Accuracy of the developed method was confirmed by doing recovery study as per ICH guideline at three different concentration levels 50%, 100%, 150% and the values were measured for Tolterodine tartrate (4 µg/ml) and Mirabegron acetate (50 µg/ml). This performance was done in triplicate.

3.2.7 Robustness:
Robustness is the ability of an analytical method to remain reliable and consistent when small, deliberate changes are made to method parameters. It indicates how well the method can tolerate variations without affecting the results. In liquid chromatography, this is assessed by examining factors such as changes in mobile phase pH or composition, using different columns from various batches or suppliers, and fluctuations in temperature or flow rate. Evaluating these factors ensures that the method performs consistently under routine conditions and produces dependable results.

3.2.8 Assay:
A synthetic mixture of Tolterodine tartrate (4 mg) and Mirabegron acetate (50 mg) was prepared with excipients including microcrystalline cellulose (8 mg), lactose (15 mg), magnesium stearate (12 mg), talc (7 mg), and starch (4 mg). The mixture was transferred to a 100 ml volumetric flask, sonicated, and diluted with methanol. After filtration, the solution yielded concentrations of 40 µg/ml (Tolterodine tartrate) and 500 µg/ml (Mirabegron acetate). Further dilution of 1 ml to 10 ml with mobile phase produced final concentrations of 4 µg/ml and 50 µg/ml, respectively. This solution was used for UV analysis (200-400 nm) using methanol as blank, and also analyzed by RP-HPLC.

3.2.9 System suitability test:
System suitability tests are a crucial part of liquid chromatography, designed to confirm that the system performs reliably and consistently for the analysis being conducted. These tests assess whether the system provides adequate resolution between peaks and reproducible results. Important parameters evaluated during system suitability testing include resolution, column efficiency, tailing factor, and the number of theoretical plates, all of which ensure the accuracy and reliability of the chromatographic analysis.

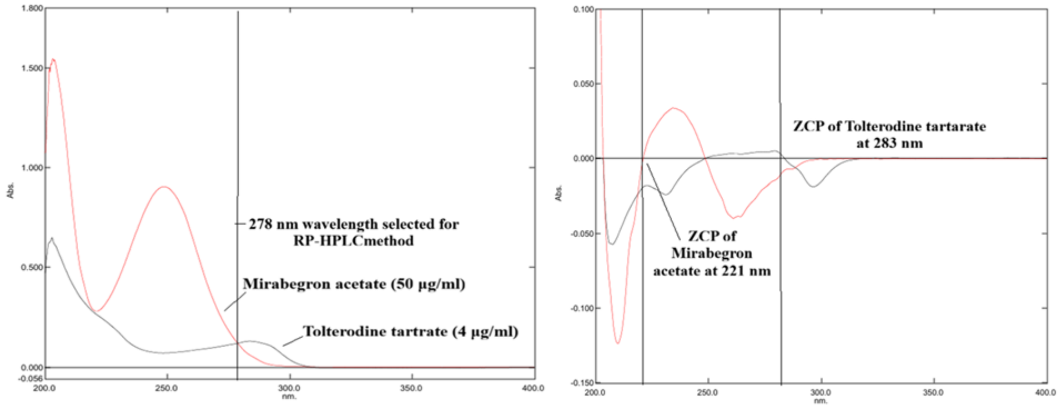
4. RESULTS AND DISCUSSION:

4.1 Method I: UV Method:
Among the different UV spectrophotometric approaches, the first-order derivative method provides significant advantages over conventional zero-order techniques for the simultaneous estimation of multiple components. It improves spectral resolution by reducing overlap and minimizing background interference, which enhances both selectivity and accuracy. In contrast to methods like simultaneous equation and absorbance ratio techniques, first-order derivative spectrophotometry enables measurement at zero-crossing wavelengths, allowing accurate determination of each component without interference from the other. Furthermore, this method offers greater sensitivity, improved baseline correction, and decreased matrix effects. Due to its simplicity, quick analysis, and effectiveness in resolving overlapping spectra, it is particularly suitable for routine analysis of combined pharmaceutical formulations.

4.1.1 Selection of wavelength for Tolterodine tartrate and Mirabegron acetate:
The remarkable absorbance of Tolterodine tartrate exhibited an absorption maximum at 221 nm, while Mirabegron acetate showed an absorption maximum at 283 nm shown in Figure 1 (b). The zero-order and First Order UV absorption spectra of Tolterodine tartrate (4 µg/ml) and Mirabegron acetate (50 µg/ml) in Methanol were showed in Figure 1 (a) and 1 (b), respectively.

4.1.2 First order derivative UV method development:
The overlapping absorption of Tolterodine tartrate and Mirabegron acetate in the 200-400 nm range is evident from the spectra, which makes direct quantification by conventional UV spectrophotometry difficult without compensating for spectral interference. The total absorbance of a mixture at a specific wavelength represents the sum of the individual absorbances of both drugs. When the absorption bands overlap, the concentration of each drug can be determined using their zero-order spectra [figure 1(a)]. To eliminate interference from overlapping components, the absorption spectra were converted into first-derivative spectra using $\Delta\lambda = 2$ nm and a scaling factor of 4. The amplitude values were measured at 221 nm (λ_1) for Tolterodine tartrate and at 283 nm (λ_2) for Mirabegron acetate, as shown in Figure 1 (b).

Chinese Journal of Health Management
Volume 20 Issue 3, Year of Publication 2026, Page 467-476 | DoI-10.564220/1674-0815.2026.71



1 (a) 1 (b)

Figure 1: Overlain UV Spectra of Tolterodine tartrate (4 µg/ml) and Mirabegron acetate (50 µg/ml) in Methanol (a) Zero Order (b) First order

4.2 Method II: RP-HPLC Method:

Pharmaceutical analysis frequently employs simultaneous estimation using RP-HPLC, which allows multiple compounds within a sample to be analyzed at the same time. Various reliable and effective methods have been developed to simultaneously estimate different components, such as drugs and their impurities, in pharmaceutical formulations. By selecting a suitable column, mobile phase, and detection system, RP-HPLC enables efficient separation and accurate quantification of target compounds. Overall, Reverse Phase High Performance Liquid Chromatography serves as a powerful analytical tool, providing precision and specificity for identifying chemical substances in synthetic mixtures.

Reverse phase chromatography was chosen because of its recommended use for ionic and moderate to non-polar compounds. Reverse phase chromatography is not only simple, convenient but also performs better in terms of efficiency, stability and reproducibility. C_{18} column was selected because it is least polar compare to C_4 and C_8 columns. C_{18} column allows eluting polar compounds more quickly compare to non-polar compounds. In addition to this UV detector is used which allows easy detection of the compounds in UV transparent organic solvents. Hence, C_{18} (250×4.6 mm) column of 5 µm particle packing was selected for separation of Tolterodine tartrate and Mirabegron acetate.

4.2.1 Selection of detection wavelength:

The sensitivity of RP-HPLC method that uses UV detection depends upon proper selection of detection wavelength. At 278 nm both drugs give good peak height and shape. So, 278 nm was selected for simultaneous estimation of Tolterodine tartrate and Mirabegron acetate in synthetic mixture. Overlay UV spectra of Tolterodine tartrate (4 µg/ml) and Mirabegron acetate (50 µg/ml) in Methanol has been shown in Figure 1 (a).

4.2.2 RP-HPLC Method Development:

Liquid chromatography coupled with UV detection was used to develop a way for simultaneously measuring Azithromycin Dihydrate and Metronidazole Benzoate. Achieving acceptable peak symmetry and theoretical plates within a realistic time period was the aim. The chromatographic conditions were optimized by experimenting with various stationary and mobile phases. The mobile phase Phosphate Buffer (pH 3 adjusted with ortho phosphoric acid): Acetonitrile (65:35 % v/v) was selected because it was found to ideally resolve the peaks with retention time 3.8 min and 6.2 min for Tolterodine tartrate and Mirabegron acetate, respectively showed in figure 2 and table 1. Kromstar C_{18} (250×4.6 mm, 5 µm) column was used for separation of Tolterodine tartrate and Mirabegron acetate with flow rate of 1.0 ml/min at 278 nm.

Table 1: System suitability parameter

Parameters	Retention Time	Tailing Factor	Number of Theoretical plate	Resolution
Tolterodine tartrate	3.8 min	0.8	5964	2.5
Mirabegron acetate	6.2 min	0.7	9328	

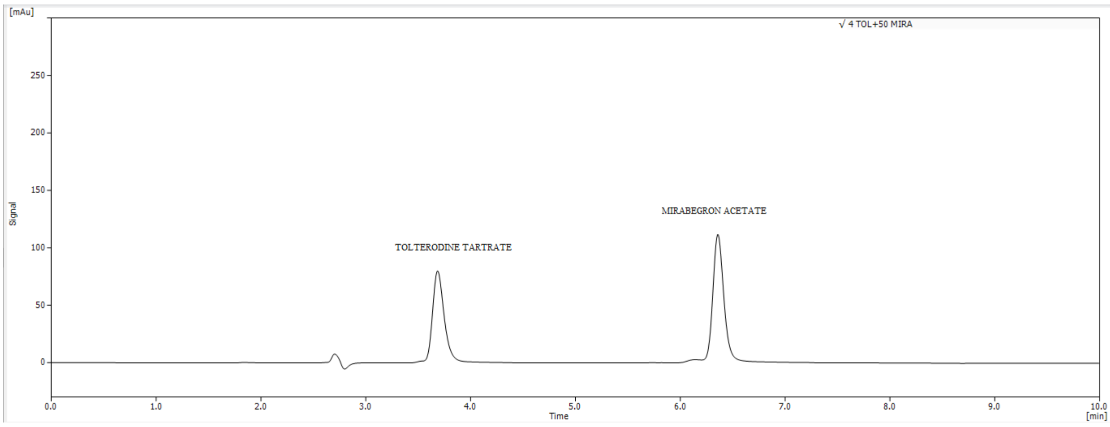


Figure 2: RP-HPLC Chromatogram of Tolterodine tartrate (4 µg/ml) and Mirabegron acetate (50 µg/ml) in 10 mM Phosphate buffer (pH 3.0 adjusted with Ortho phosphoric acid): Acetonitrile (65:35 %v/v) at 278 nm {Run time: 10 min, Flow rate: 1 ml/min}

4.3 Validation of the proposed methods:

4.3.1 Linearity and range:

For UV method, the mean absorbance was measured for linearity of Tolterodine tartrate (7.5-37.5 µg/ml) at 221 nm and Mirabegron acetate (20-100 µg/ml) at 283 nm showed in Figure 3 (a) and 3 (b), respectively. Linearity data of UV method showed in Table 2.

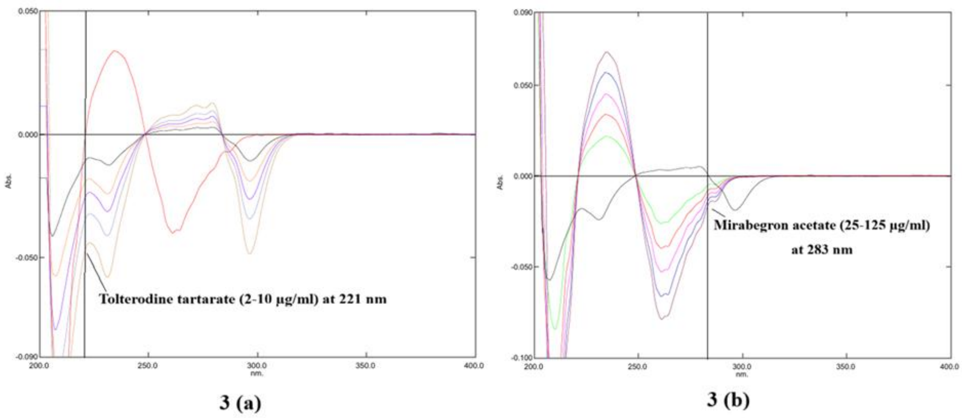


Figure 3: Overlain UV spectra of (a) Tolterodine (2-10 µg/ml) at 221 nm, (b) Mirabegron (25-125 µg/ml) at 283 nm

For the RP-HPLC method, the linearity of Tolterodine and Mirabegron was found to be 2-10 µg/ml and 25-125 µg/ml at 278 nm, respectively, which showed in Figure 4. Linearity data for the RP-HPLC method are shown in Table 2.

Table 2: Results of validation parameters for UV and RP-HPLC method

Sr. no	Validation parameters	UV Method		RP-HPLC Method	
		Tolterodine Tartrate	Mirabegron acetate	Tolterodine tartrate	Mirabegron acetate
1.	Detection wavelength (nm)	221 nm	283nm	278 nm	
2.	Linearity range	2-10	25-125	2-10	25-125
3.	Regression Equation (y=mx+c)	y = 0.0037x + 0.0026	y = 0.0001x + 0.0017	y = 71.283x – 51.506	y = 10.911x – 69.075
4.	Correlation Coefficient	0.9978	0.9952	0.9971	0.9997
5.	Intraday Precision (%RSD, n=3)	0.63-1.15	0.61-1.24	0.70-1.12	0.72-1.30
6.	Interday Pression (%RSD, n=3)	0.64-1.16	0.64-1.24	0.79-1.10	0.83-1.22
7.	Repeatability (%RSD, n=6)	0.92	1	0.77	1.15
8.	LOD (µg/ml)	0.10	1.82	0.06	0.88
9.	LOQ (µg/ml)	0.32	5.50	0.17	2.67

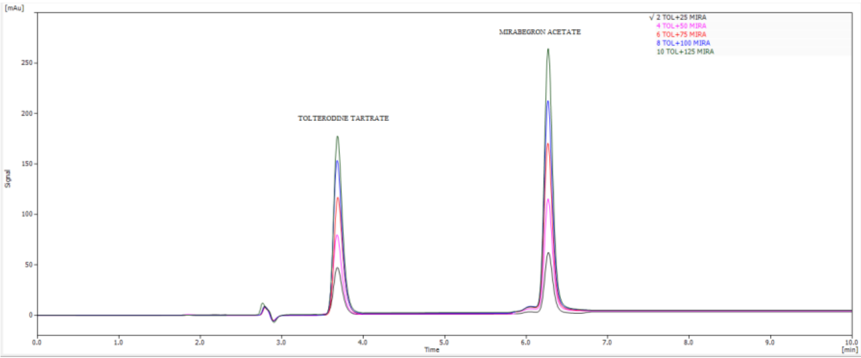


Figure 4: Overlain RP-HPLC chromatogram of Tolterodine tartrate (2-10 μg/ml) and Mirabegron acetate (25-125 μg/ml) at 278 nm {Run time: 10 min, Flow rate: 1 ml/min}

4.3.2 Precision:

Precision was assessed by intraday, interday, and repeatability studies. Tolterodine (2, 4, 6 μg/ml) and Mirabegron (25, 50, 75 μg/ml) were analyzed in triplicate on the same day and on three consecutive days. Repeatability was evaluated at 4 μg/ml for Tolterodine and 50 μg/ml for Mirabegron. The %RSD values for intraday, interday, and repeatability were all found to be less than 2%, indicating good precision of the method, as shown in Table 2.

4.3.3 LOD and LOQ:

In comparison, the RP-HPLC method exhibited lower LOD values of 0.10 μg/ml for Tolterodine and 1.82 μg/mL for Mirabegron, with LOQ values of 0.32 μg/ml for Tolterodine and 0.49 μg/ml for Mirabegron, respectively, indicating higher sensitivity of the HPLC method. The results of LOD and LOQ for both methods are shown in Table 2.

4.3.4 Accuracy:

The accuracy of the method was evaluated by recovery studies using the standard addition method. Known amounts of Loratadine and Famotidine were added to the pre-analysed sample at 50%, 100%, and 150% levels. The studies were performed in triplicate, and accuracy was expressed as % recovery. For the UV method, Tolterodine showed mean recoveries ranging from 99.33% to 99.80%, while Mirabegron exhibited recoveries between 99.92% and 99.96%. Similarly, the RP-HPLC method demonstrated mean recoveries of 99.50% to 99.90% for Tolterodine and 99.94% to 99.98% for Mirabegron. These results (Table 3) confirmed the high accuracy and reliability of both analytical methods.

Table 3: Recovery study data for UV and RP-HPLC Method

Name of Drug	% Level of recovery	Test Amount (μg/ml)	Amount of drug taken (μg/ml)	Total Std Amt (μg/ml)	Total amount Recovered (μg/ml)	% Mean Recovery ± SD(n=3)
UV Method						
Tolterodine tartrate	50	4	2	6	5.96	99.33±0.0305
	100	4	4	8	7.97	99.62±0.0800
	150	4	6	10	9.98	99.80±1.0100
Mirabegron acetate	50	50	25	75	74.94	99.92±0.9556
	100	50	50	100	99.95	99.95±1.0552
	150	50	75	125	124.95	99.96±1.5033
RP-HPLC Method						
Tolterodine tartarate	50	4	2	6	5.97	99.50±0.305
	100	4	4	8	7.98	99.75±0.800
	150	4	6	10	9.99	99.90±1.100
Mirabegron acetate	50	50	25	75	74.95	99.94±0.955
	100	50	50	100	99.97	99.97±1.035
	150	50	75	125	124.98	99.98±1.063

4.3.5 Assay:

From assay, final concentration of Tolterodine was 4 μg/ml and Mirabegron 50 μg/ml were run into UV and HPLC. Assay of the synthetic mixture by the UV method yielded assay values of 99.75 ± 0.825% for Tolterodine and 99.86 ± 0.978% for Mirabegron (n = 3). Similarly, the RP-HPLC method showed assay values of 99.80 ± 0.5682% and 99.98 ± 0.7624% for Tolterodine and Mirabegron, respectively, confirming the accuracy and precision of both methods. Its results are shown in Table 4.

Table 4: Analysis of synthetic mixture for UV and RP-HPLC Method

Name of Drug	Amount in synthetic mixture (μg/ml)	Mean Amount found (μg/ml)	% Assay ± SD (n=3)	%RSD
UV-Method				
Tolterodine tartrate	4	3.99	99.75 ± 0.825	0.82
Mirabegron acetate	50	49.98	99.96 ± 0.978	0.97
RP-HPLC Method				
Tolterodine tartrate	4	3.992	99.80±0.5682	0.56
Mirabegron acetate	50	49.99	99.98±0.7624	0.76

4.3.6 Robustness:

The robustness of the developed HPLC method was assessed by introducing small, intentional variations such as changes in analyst, slight modifications in flow rate, run time, and detection wavelength. The %RSD values were found to be within acceptable limits, confirming the method's robustness and reproducibility (Table 5).

Table 5: Robustness data

Condition	Variation	Tolterodine tartrate	Mirabegron acetate
		% Assay ± SD (n=3)	**% Assay ± SD (n=3)**
Flow rate (1 ml ± 0.2 ml/ min)	0.8 ml/min	99.42±3.5166	98.45±1.3730
	1.0 ml/min	99.65±5.2691	99.75±2.5545
	1.2 ml/min	99.76±7.4770	99.95±4.0286
Detection wavelength (278 nm ± 2 nm)	276	98.58±4.1268	99.65±2.9454
	278	99.92±4.4267	99.85±2.5055
	280	100.02±6.0256	99.99±5.3762
Mobile Phase (0.1% formic acid in Acetonitrile: PO_4 Buffer (20:80 ± 2 % v/v)	18:82	99.34±3.0784	99.47±4.1116
	20: 80	99.83±4.9421	99.65±1.0552
	22: 78	99.64±5.0143	100.05±4.1845

5. CONCLUSION:

The present study successfully demonstrated the development, optimization, and validation of first-order derivative UV spectrophotometric and RP-HPLC methods for the simultaneous estimation of Tolterodine tartrate and Mirabegron acetate in a synthetic mixture. The first-order derivative UV method effectively utilized zero-crossing wavelengths (221 nm and 283 nm) for selective, rapid, and accurate quantification of both drugs without spectral interference. The RP-HPLC method provided efficient chromatographic separation with well-resolved peaks at 278 nm with retention time of 3.8 min and 6.2 min for Tolterodine tartrate and Mirabegron acetate, respectively, along with good system suitability parameters. Both methods were validated as per ICH guidelines and exhibited excellent linearity, high accuracy (recoveries close to 100%), and precise results with low %RSD values for intraday, interday, and repeatability studies. The LOD and LOQ values confirmed adequate sensitivity, with RP-HPLC showing comparatively higher sensitivity than the UV method. No interference from excipients was observed, confirming the specificity of the methods. Overall, both analytical techniques were found to be simple, precise, accurate, economical, and reproducible. Hence, they are suitable for routine quality control analysis and simultaneous estimation of Tolterodine tartrate and Mirabegron acetate in synthetic mixture.

6. FUTURE PERSPECTIVES:

The first-order derivative UV method proves to be a rapid and cost-effective approach suitable for routine quality control analysis of Loratadine and Famotidine. Meanwhile, the RP-HPLC method, with its superior sensitivity and specificity, can be further applied to stability studies, impurity profiling, and the evaluation of finished pharmaceutical products containing these drugs. Future research may focus on applying these methods to commercially available formulations and further developing them for stability-indicating, bioanalytical, and pharmacokinetic investigations.

ACKNOWLEDGEMENT:

The author is thankful to Smt. N. M. Padalia Pharmacy College, Ahmedabad for facilities to carry out the research.

CONFLICT OF INTEREST:

The authors declare that there is no conflict of interest.

REFERENCES:

1. Leron E, Weintraub AY, Mastrolia SA, Schwarzman P, "Overactive Bladder Syndrome: Evaluation and Management," *Current Urology*, **2018**; *11(3),* 117-125. doi: 10.1159/000447205.
2. Nilvebrant L, Andersson KE, Gillberg PG, Stahl M, Sparf B. Tolterodine--a new bladder-selective antimuscarinic agent. Eur J Pharmacol. **1997**; *327(2-3)*: 195-207. doi: 10.1016/s0014-2999(97)89661-6.
3. Sharaf A and Hashim H, "Profile of Mirabegron in treatment of overactive bladder: place in therapy". Drug Des Devel Ther. **2017**, 11: 463-467. doi: 10.2147/DDDT.S101630.
4. Clinical Trial, The combination of Mirabegron and Tolterodine". Clinical Trial, gov ID. **2024**, 1-5. https://clinicaltrials.gov/study/NCT00689104.
5. Christopher R. Chapple, Francisco Cruz, Linda Cardozo, David Staskin, Sender Herschorn, Nurul Choudhury, Matthias Stoelzel, John Heesakkers, Emad Siddiqui, Safety and Efficacy of Mirabegron: Analysis of a Large Integrated Clinical Trial Database of Patients with Overactive Bladder Receiving Mirabegron, Antimuscarinics, or Placebo, European Urology, 77, 1, **2020**, 119-128, https://doi.org/10.1016/j.eururo.2019.09.024. (https://www.sciencedirect.com/science/article/pii/S0302283819307523)
6. Zhang Xiang, Mao Yinhui, Liu Yang, Sun Jilei, Sun Juntao, Pan Chenli, Wang Zhuo, Wei Zhitao, Yang Yong, "Mirabegron 50 mg once daily, long-term treatment maximizes benefit in middle-aged and older people with overactive bladder syndrome: a systematic review and meta-analysis of nine phase III, randomized, double-blind, parallel-design, placebo-controlled, multicenter, and multinational trials" *Frontiers in Surgery*, **2024**, 11, 1-10. doi: 10.3389/fsurg.2024.1372175.
7. Muhasina KM, Ghosh P, Nagappan K, Palaniswamy DS, Begum R, Islam MR, Tagde P, Shaikh NK, Farahim F and Mondal TK, "From Gut Microbiomes to Infectious Pathogens: Neurological Disease Game Changers." Molecular Neurobiology, **2025**, 62(1): 1184-1204. https://doi.org/10.1007/s12035-024-04323-0.
8. Siva SRG, Sarat PS, Ramachandran D, "UV-Spectrophotometric Assay Method for the Assay of Tolterodine in Pure and Formulations." *American Journal of Pharmacy and Health Research*, **2015**, *3(4),* 23-30.
9. Nanda RK, Gaikwad J and Prakash A. "Simultaneous Spectrophotometric Estimation of Tolterodine in Pharmaceutical Dosage form." *Research Journal of Pharmacy and Technology*, **2009**, *2(2),* 312-314.
10. Rao RN, Dr. D. Madhuri, Reddy SS, Rani K. TP. GK., "Development and Validation of derivative spectrophotometric method for estimation of mirabegron in bulk and tablet dosage form". *World Journal of Pharmaceutical Research*. **2017**, *6(14),* 760-767.
11. Shaikh FAV, Shaikh NK, Bhangale JO. "Development and Validation of UV-Spectrophotometric method for Simultaneous estimation of Colchicine and Rosuvastatin calcium in synthetic mixture." *J. Applied Bioanalysis*, **2025,** *11(s12),* 213-224. DOI: 10.53555/jab.v11si12.1676.
12. Shaikh NK, Bhangale JO. Analytical methods for detecting and quantifying Vildagliptin and Remogliflozin in bulk and combined dosage form and their potentiality, Journal of Molecular Science, **2025**, 35(3): 1100-1107. https://doi.org/10.004687/1000-9035.2025.147.
13. Rihana PSK, Srinivasa P, Chandrasekar KB, Challa BR, "Analytical method development and validation of Tolterodine in Pharmaceutical dosage forms by RP-HPLC." *Der Pharmacia Lettre*, **2014,** *6(3),* 246-254.
14. Sathish KS, Arpan S, "Development and Validation of Tolterodine by RP-HPLC Method in Bulk Drug and Pharmaceutical Dosage Forms." *international Journal of PharmTech Research*, **2011,** *3(2),* 1083-1087.
15. Konduru N and Gundla R, "Unique liquid chromatography technique for the determination of mirabegron in the presence of polymers; robustness by Design Expert in the light of Quality by Design." *Biomedical Chromatography*. **2022**, *36(10),* 5449.
16. Shaikh NK, Jat R, Bhangale JO. "Analysis of Vildagliptin and Nateglinide for simultaneous estimation using spectro-chromatographic methods", European Journal of Molecular and Clinical Medicine, **2020**, 7(8): 741-755. https://ejmcm.com/article_3194.html.
17. Suryawanshi R and Patil S, "RP-HPLC Method Development and Validation for the Estimation of Mirabegron in Bulk and Dosage Form." Journal of drug delivery Ther. **2020**, 10(1), 31-38.
18. Uday VH, Lahu B and Mrinalini CD, "Development and Validation of Stability Indicating HPLC Method for Determination of Tolterodine Tartrate." *World Journal of Pharmaceutical Research*, **2017,** *6(11),* 430-441.
19. Shaikh NK, Jat R, Bhangale JO. Development and validation of stability-indicating RP-HPLC and UV method for simultaneous quantitation of Repaglinide and Sitagliptin phosphate in combination, *American Journal of PharmTech Research*, **2020**, 10(6): 95-114. https://doi.org/10.46624/ajptr.2020.v10.i6.007.
20. Sinha AK, Manidipa D, Rao JVLN, Dasari GSS, "A new bioanalytical method development and validation for estimation of tolterodine tartrate in human plasma by using RP-HPLC." Indian Pharma. Drugs, **2015**, *52(7),* 23-28.
21. M Rizk, Zainab MM, Marwa MA, "Simultaneous ultra-sensitive analysis of tamsulosin hydrochloride and tolterodine tartrate binary mixture in their dosage form via high-performance thin-layer chromatography with fluorometric detection." *Journal of Planer Chromatography- modern TLC*, **2022,** *35(1),* 509-517.
22. Rihana PS, Puttagunnta SB, Chandrasekhar KB, Bahlul ZA, "A validated LC–MS/MS method for the determination of tolterodine and its metabolite in rat plasma and application to pharmacokinetic study." *Journal of Pharmaceutical Analysis*, **2013,** *3(6),* 489-499.
23. Kumar GV and M Kalyani, "stability indicating simultaneous estimation of vildagliptin and Mirabegron in bulk and pharmaceutical dosage form by using UV Spectroscopy." *World journal of pharmacy and pharmaceutical sciences.* **2017**, *6(5),* 912-925.
24. ICH, Q2 (R2) Validation of Analytical procedures: Text and Methodology International Conference on Harmonization, European Medicine Agency, **2022**, pp 1-33.