ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Development and Validation of an RP-HPLC method for the estimation of Tolterodine in Raw materials and Tablet dosage forms

Vijaya Bhaskar S*[1], Seshagiri Rao JVLN [2], and Vijaya Sree [1]

[1]Department of Pharmaceutical Analysis, St. Johns Pharmacy College, Vijayanagar, Bangalore, Karnataka, India - 560104
[2]AU College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, Andhra Pradesh, India – 530003

ABSTRACT

A rapid, simple, reliable, accurate, and sensitive high performance liquid chromatography method for the determination of Tolterodine in raw materials and tablet dosage forms was developed and validated. Tolterodine was chromatogramed on Kromosil Symmetry C_{18} (150 X 4.6 mm; 5 µm) using a mobile phase consisting of phosphate buffer (pH 3.0) and acetonitrile in the ratio of 45:55 v/v. The flow rate was maintained at 1ml/min and eluents were detected with the retention time of 2.25 min. at 284 nm by using UV detector absorbance. The proposed method was validated by determining accuracy, precision, and linearity was observed in the range of 20 – 100µg/mL of the drug. The mean recovery of the drug was indicating high level of accuracy of the method. Due to its simplicity, accuracy and high precision of the proposed HPLC method was found to be appropriate for the estimation of Tolterodine in bulk and pharmaceutical dosage forms.

Keywords: Tolterodine, HPLC and Validation.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Tolterodine is chemically described as, (R)-2-[3-(di isopropyl amino)-1-phenyl propyl]-4-methyl phenol. Tolterodine is an antimuscarinic drug which is used to treat urinary incontinence. Urinary incontinence is characterized by uninhibited contractions causing an uncontrollable urge for urine. Tolterodine acts on M1, M2, M3, M4 and M5 subtypes of muscarinic receptors whereas older antimuscarinic agents used for overactive bladder acts only on M3 muscarinic receptor [1].

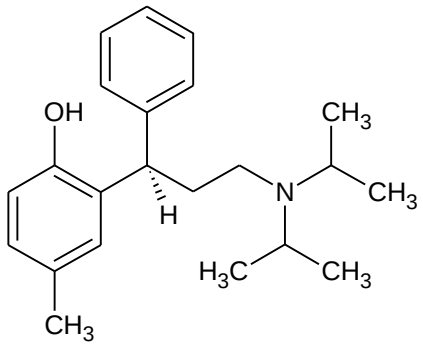


Figure 1: Chemical structure of Tolterodine.

The literature survey reveals that, Tolterodine was estimated by HPLC methods for stability indicating [2 & 3], pharmaceutical assays [4, 5& 6], enantiomers estimation by Chiral HPLC [7& 8] and simultaneous estimation method by HPLC [9] with different columns and mobile phases of various pH. There are some other methods like UPHPLC [10], HPTLC [11], spectrophotometric methods [11 -16] were also found in the literature. The present method describes the rapid, accurate and precise RPHPLC method for the estimation of Tolterodine in raw materials and tablet dosage forms by using different method.

MATERIALS AND METHODS

The reagents and chemicals used in this procedure are Acetonitrile, Potassium dihydrogen phosphate, ortho phosphoric acid and water of HPLC grade. A Waters 2695 model chromatograph equipped with a reverse phase Symmetry C_{18} column (100 x 4.6 mm; 5 µm) Kromosil was employed for the study.

Sample injection was performed with an automatic injector. Detection was done by a Waters 287 nm dual λ absorbance UV detector and the output signal was monitored and integrated using Waters empower software. Solubility of compound was enhanced by sonication on ultrasonic cleaner. A UV spectrum of tolterodine was taken on a UV 3000 UV visible – spectrophotometer (Labindia) in order to select the working wavelength for detection of drug. All the weights in the experiments were taken with a Sartorius electronic balance.

Preparation of Phosphate buffer of pH 3.0: Phosphate buffer was prepared by dissolving 7.0 gm of potassium dihydrogen phosphate in 1000 mL of HPLC water. The pH of the solution was adjusted to 3.0 with ortho phosphoric acid.

Preparation of the mobile phase: For the preparation of mobile phase, 450 mL of phosphate buffer was mixed with 550 mL of acetonitrile, degassed in an ultrasonic bath for 5 min, and filtered through a 0.45 µ membrane filter before use. The mobile phase was also used as a diluent for preparing the drug solutions. The flow rate of the mobile phase was maintained at 1.0 mL / min. The detection of the drug in the eluate was monitored at 284 nm.

Preparation of standard drug solutions: About 100mg of tolterodine was weighed accurately, transferred into a 100 mL volumetric flask and dissolved in 25 mL of the diluent. The solution was sonicated for 15 min and the volume made up to the mark with a further quantity of the diluent to get 1000 µg/mL of the drug. The working standard solution of tolterodine was prepared by diluting 0.1mL of the above stock solution and diluted to 20 mL in a volumetric flask to get a 20 µg/mL of the drug solution. Further dilutions ranging from 20 to 100 µg/mL of the drug were prepared from the stock solution in 10 mL volumetric flasks for plotting the calibration curve.

RESULTS AND DISCUSSION

A mixture of phosphate buffer (pH 3.0) and acetonitrile in the ratio of 45:55 v/v as the mobile phase on Kromosil Symmetry C_{18} (150 X 4.6 mm; 5µm) column resulted in a peak of the drug with good shape and resolution. A flow rate of 1.0 mL /min was found to be optimum in the range of 0.8 – 1.2 mL/min.

In the proposed method, the retention time of tolterodine was found to be 2.25 min. by using U.V. detector absorbance at 284 nm. Linearity was obeyed in the concentration range of 20-100 µg/mL of the drug. The regression equation of tolterodine concentration over its peak area was found to be Y=7500X+881.3 (r= 0.9982) where Y is the peak area and X is the concentration of tolterodine (µg/mL). The intra-day and inter-day drug variation by the proposed method showed an RSD of less than 2% (0.36% and 0.32%), indicating that the method is quite precise. The drug content in the tablets was quantified using the proposed method of analysis. The corresponding recovery of tolterodine was found to be 99.9%. This reveals that the method is quite accurate. The mean amount of tolterodine obtained in tablet dosage forms was 101.4%. The limit of detection and limit of quantitation were found to be 0.018 µg/mL and 0.036 µg/mL respectively indicating sensitivity of the method. The method tolerated minor variations in optimized chromatographic conditions indicating good robustness. The tailing factor (1.7), number of theoretical plates (2127.7) and HETP (7.050×10^{-5}) obtained were within the acceptable limits, which indicates efficient performance of the column. No interfering peaks were found in the chromatogram indicating that excipients used in tablet formulations did not interfere with the estimation of the drug by the proposed HPLC method.

ISSN: 0975-8585

Figure 2: Chromatogram of Tolterodine

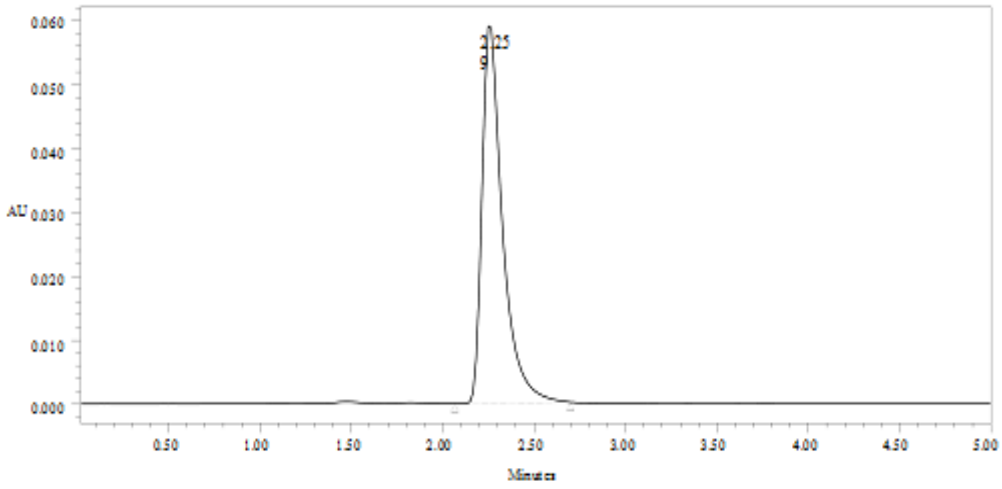


Figure 3: Calibration curve of Tolterodine

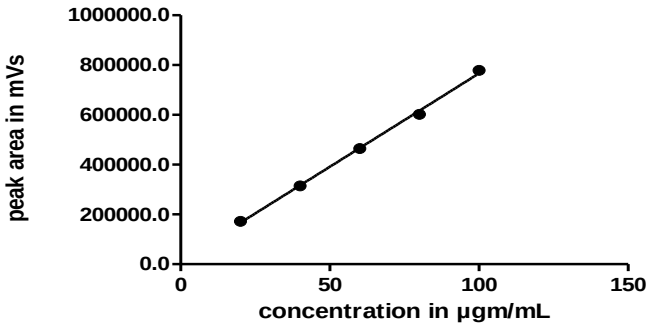
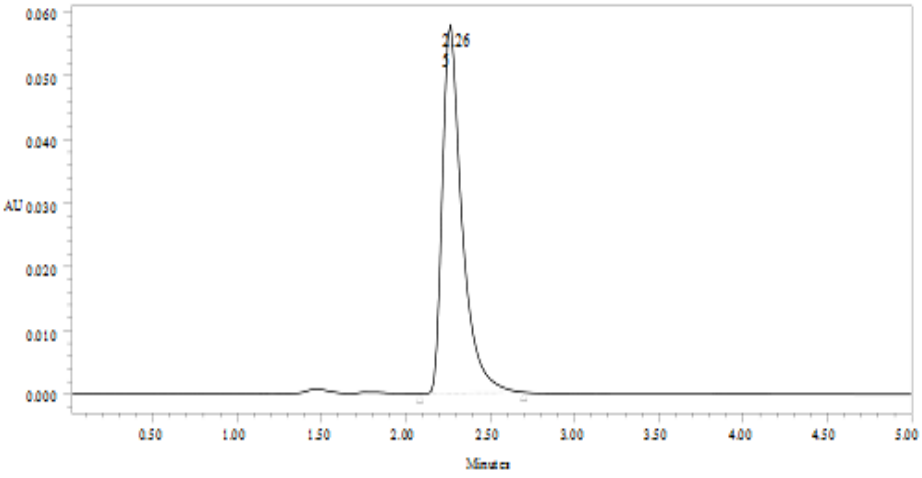


Figure 4: Chromatogram of Tolterodine formulation



ISSN: 0975-8585

Table 1: Optimized chromatographic conditions of the proposed method

S. No.	Parameter	Value
1.	Mobile phase	Phosphate buffer(pH 3.0)-acetonitrile (45:55v/v)
2.	Diluent	
3.	Stationary phase	Symmetry C_{18}, 150 X 4.6 mm; 5 µm
4.	Flow rate	1.0 ml/min
5.	Column temperature	25^0C
6.	Volume of injection	20 µL
7	Detection wavelength (λ_{max})	284 nm
8	Run time	5.00 min
9	Retention time	2.25 min

Table: 2 Linearity range of tolterodine.

S.No.	Concentration (µg/mL)	Area of the peak	Statistical analysis
1	20 µg/mL	172242	Slope=7500 Intercept= ±881.3 Correlation coefficient=0.9982
2	40 µg/mL	314507	
3	60 µg/mL	464742	
4	80 µg/mL	601968	
5	100 µg/mL	778476	

Table 3:Accuracy data of the proposed method

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	233753	5.0	5.02	100.5%	99.9%
100%	462955	10.0	9.95	99.5%	
150%	695705	15.0	14.9	99.7%	

Table 4: precesion of the method

Concentration Of Drug	Intraday precision			Inter day precision		
	Average peak area	S.D	%R.S.D	Average peak area	S.D	%R.S.D
60 µg/mL	463540	1672.4	0.36	461137	1496.0	0.32

Table 5: Results of the robustness study (flow rate)

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	2023.9	1.7
2	1.0	2127.7	1.7
3	1.2	2018.1	1.5

Table 6: Results of the robustness study (mobile phase composition)

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2518.1	1.5
2	*Actual	2717.7	1.7
3	10% more	2211.3	1.7

* Actual Mobile phase composition is 55:45 of ACN: Buffer

Table 7: System suitability parameters of the proposed method

S.No.	Parameter	Result
1	Theoretical plates	2127.7
2	Tailing factor	1.7
3	HETP	7.050×10^{-5}
4	LOD (µg/mL)	0.018
5	LOQ (µg/mL)	0.036

Table 8: Recovery of tolterodine from formulations

S.No.	Formulation	Label claim (mg)	Amount found in mg (n=3)	% Recovery
1.	Tolter	2.0	2.008	100.4 %
2.	Flowchek	2.0	2.010	100.5 %

CONCLUSION

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the determination of tolterodine in pharmaceutical dosage forms. The values obtained for various validation parameters are within acceptance limits as per ICH guidelines[17]. Hence, this method can be easily and conveniently adopted for routine quality control analysis of in pure and its tolterodine pharmaceutical dosage forms.

ACKNOWLEDGEMENTS

The authors are thankful to Deputy Director and Principal of St.John's Pharmacy College for providing the facilities in carrying out the work.

REFERENCES

[1] http://www.drugbank.ca/drugs/DB01036.
[2] Radha Krishna S, Rao BM and Someswara Rao N. Rasayan J Chem 2009; 2(1): 144-150.
[3] Saxena Vinay, Zaheer Zahid and Farooqui Mazhar. Indian J Chem Tech 2006; 13(3): 242-246.
[4] Dwibhashyam VS, Keerthi P, Ratna JV and Nagappa AN. J Pharm Sci Tech 2009; 63(3): 234-239.

[5] Satish Kumar shetty and Arpan Shah. Int J Pharm Tech Res 2011; 3(2): 1083-1087.
[6] N Ramathilagam, M Meeradevi, P Solairaj, SC Rajesh. Int J Pharm Bio Sci 2012; 2(4): 332-337.
[7] Xia ZL, Chen Zh Y and Yao TW. Pharmazie 2007; 62(3): 170-173.
[8] Kumar YR, Ramulu G, Vevakanand VV, Vaidyanathan G, Srinivas K, Kumar MK, Mukkanti K, Reddy MS, Venkatraman S and Suryanarayana MV. J Pharm Biomed Anal 2004; 35(5): 1279-1285.
[9] Ravindra Kumar Nanda, Jagdish Gaikwad and Anand Prakash. J Pharm Res 2009; 2: 1786.
[10] Ramesh Yanamandra, Chandra Sekhar Vadla, Umamaheshwar Puppala, Blaram Patro, Yellajyosula LN. Murthy, Parimi Atchuta Ramaiah. Scientia Pharmaceutica 2012; 80: 101-114.
[11] Shaiba M, Maheswari R, Rahul Chakraborty, Sai Praveen P, Jagathi V. Res J Pharm Bio Chem Sci 2011; 2(1): 6-11.
[12] Shetty SK and Shah A. Int J Pharm Sci Res 2011; 2(6): 1456-1458.
[13] Shinde DB, Sangshetty JN, Rane VP, Chaudhari PD, Kolsure PK. Anal Chem Indian J 2007;5(1-6): 35-38.
[14] Nanda RK, Gaikwad J and Prakash A. Int J Pharm Tech Res 2009; 1(3): 420-423.
[15] Vanilatha S, Mare Theresa M, Prasanna N, ShantaKumari D, Harika B, Shirisha P, Archana M, Xavier, Gouri Bala Kumari K. Int J sci Inno Disco 2011; 1(2): 188-193.
[16] Syam Bab M, Viplava Prasad U, Kalyana Ramu B. Am J Pharm Tech Res 2012; 2 (4): 395-404.
[17] Validation of analytical procedures: Text and Methodology, ICH Hormonised Tripartite Guideline Q2 (R1), Commission of the European Communities (2005).