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Development and Validation of Tolterodine by RP-HPLC Method in Bulk Drug and Pharmaceutical Dosage Forms

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Abstract: A new, simple, specific, sensitive, rapid, accurate and precise RP-HPLC method was developed for the estimation of Tolterodine in bulk and pharmaceutical formulations. Tolterodine was chromatographed on a hypersil C18 column (250x4.6mm I.D., particle size 5 µm) in a mobile phase consisting of Acetonitrile and 10 mM Ammonium acetate in the ratio 80:20 v/v. The mobile phase was pumped at a flow rate of 1.0 ml/min with detection at 283 nm. The detector response was linear in the concentration of 20-100 µg/ml. The intra and inter day variation was found to be less than 2%. The mean recovery of the drug from the solution was 99.39%. The proposed method is simple, fast, accurate, precise and reproducible hence, it can be applied for routine quality control analysis of Tolterodine in bulk and pharmaceutical formulations.
Keywords: RP-HPLC, Tolterodine, precision, accuracy.

Introduction

Tolterodine is an antimuscarinic drug that is used to treat urinary incontinence. Tolterodine acts on M1, M2, M3, M4 and M5 subtypes of muscarinic receptors whereas modern antimuscarinic treatments for overactive bladder only act on M3 receptors making them more selective. Tolterodine is chemically 2-[(1,S)-3-(di-isopropylamino)-1-phenylpropyl]-4-methylphenol. The structural formula is shown Figure 1. The molecular formula of Tolterodine is $C_{22}H_{31}NO$. The molecular mass of Tolterodine is 325.488 g/mol.[1] It is soluble in water, methanol, slightly soluble in ethanol and partially insoluble in toluene.[2] Literature survey reveals that, only few spectophotometric methods[3,4] and few analytical methods[5-7] have been reported for the quantitative estimation of Tolterodine in bulk drug and pharmaceutical formulation. Hence an attempt has been made to develop new HPLC methods for its estimation in bulk and pharmaceutical formulation with good precision, accuracy, linearity and reproducibility.

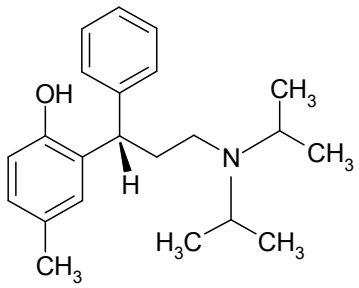

Figure 1. Chemical structure of Tolterodine

Materials and Methods

HPLC system is used for this study, the specifications are given below. An isocratic high pressure liquid chromatography (shimadzu HPLC winchrome - ES Series) with one LC-10 AT VP pumps, with UV/VIS detector SPD-10A VP, CTS-10 AS VP column oven (shimadzu), and a hypersil C-18 Column 250 mm x 4.6 mm i.d. particle size 5 µm) was used. The HPLC system was equipped with the software class Winchrome (Shimadzu).

Reagents and Chemicals

All the chemicals used were of HPLC grade and A.R. grade. Distilled water was used for making the solutions. The commercially available Tolterodine tablets were procured from the local market.

Chromatographic Conditions

The content of the mobile phase was Acetonitrile and 10 mM Ammonium acetate in the ratio of 80:20 % v/v. The mobile phase was filtered through 0.45 µm membrane filter and sonicated for 15 min. The flow rate of the mobile phase was maintained at 1.0 ml/min. The column temperature was set ambient and the detection was carried out by UV-detector wavelength at 283 nm. The run time was set at 10 min and the volume of the injection loop was 20 µL. Prior to injection of the drug solution, the column was equilibrated for atleast 30 min with the mobile phase flowing through the system.

Procedure

Stock solution of Tolterodine was prepared by dissolving 100 mg of Tolterodine in 100 ml standard volumetric flask containing approximately 50 ml of mobile phase and the solution was sonicated for 20 min and then the volume was made upto the mark with mobile phase to obtain a concentration of 1000 µg/ml. Subsequent dilutions of this solution were made with mobile phase to obtain the concentration range of 20-100 µg/ml. The standard solutions prepared as above were injected into the 20 µL loop and the chromatogram was recorded and shown in Figure 2.

The retention time of Tolterodine was found to be 5.50 min. The calibration curve was constructed by plotting concentration against peak area ratio. The calibration curve is found to be linear and shown in Figure 3. The amount of Tolterodine present in sample was calculated through the standard calibration curve. The linearity experiment was carried out in triplicate to ascertain accuracy and precision of the method. The peak area ratios of the drug against concentration are found to be linear and the results are expressed in Table 1.

Table 1. Calibration data of the method

Concentration µg/ml	Peak area (n=3)
20	10267146
40	20281296
60	30358492
80	40846922
100	49931357

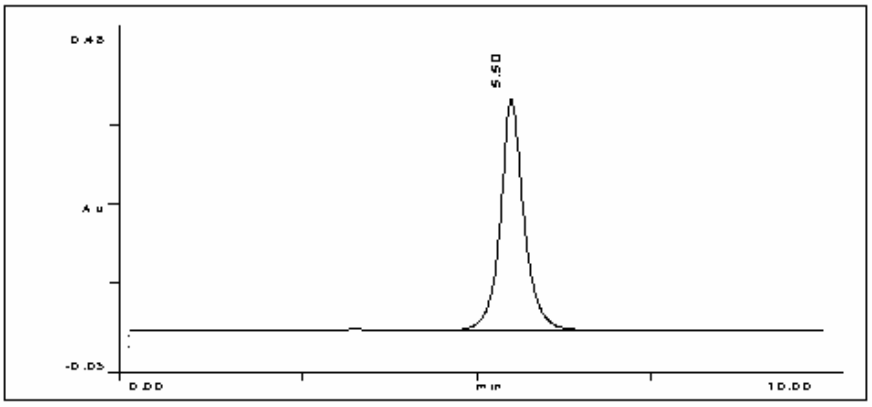

Figure 2. Typical chromatogram of Tolterodine

Table 2. Assay of Tolterodine

Formulation	Label claim (mg)	Amount found (mg)	% Amount found
Brand	2	1.989	99.45

Table 3. System suitability parameters

Parameter	Result
Linearity, µg/ml	20-100µg/ml
Correlation coefficient	0.9998
Retention time, min	5.50
Theoretical plates (N)	6125
Tailing factor	1.621
LOD, µg/ml	0.0457
LOQ, µg/ml	0.1384

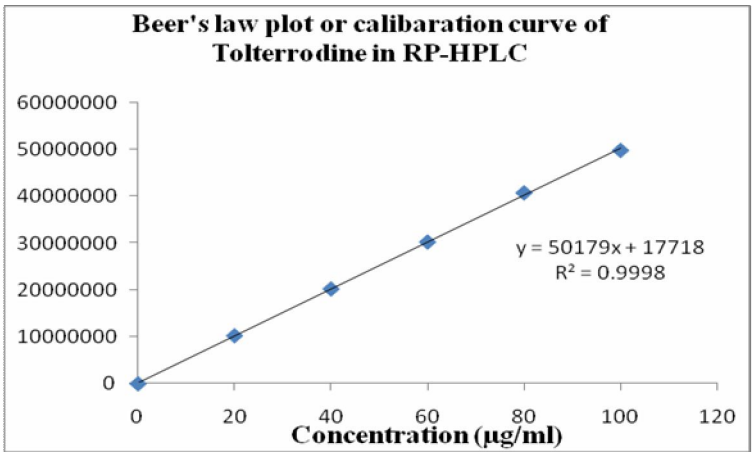

Figure 3. Calibration curve of Tolterodine

Assay

25 tablets each containing 2 mg of Tolterodine weighed accurately and powdered. A quantity equivalent to 50 mg of Tolterodine was weighed accurately and transferred to 50 ml volumetric flask containing approximately 30 ml of mobile phase. The contents were sonicated for 20 min and volume was made upto the mark with the mobile phase. The resulting solution was filtered through a membrane filter. The solution obtained was then diluted with the mobile phase so as to obtain a concentration of 50 µg/ml. Sample solution was injected under the same chromatographic conditions and the chromatogram was recorded in triplicate. The amount of Tolterodine present in tablet formulation was determined by comparing the peak area from the standard. The results are furnished in Table 2.

Validation of Proposed Method

Selectivity of the method was assessed on the basis of elution of Tolterodine using the above mentioned chromatographic conditions. This study was validated for linearity, precision, accuracy, limit of detection, limit of quantitation and robustness. The results are furnished in Table 3.

Linearity

The standard curve was obtained in the concentration range of 20-100 µg/mL. The linearity was evaluated by linear regression analysis using the least square method. It was found that correlation coefficient and regression analysis are within the limits.

Table 4. Precision of the proposed HPLC method

Concentration of Tolterodine (µg/ml)	Concentration of Tolterodine Found on			
	Intra-Day		Inter-Day	
	Mean (n=3)	C.V. (%)	Mean (n=3)	C.V. (%)
50	99.9367	0.0058	99.9433	0.0510

Table 5. Recovery studies of the proposed HPLC method

Sr No	List of % Recovery	Mean*	Standard Deviation*	Co-efficent of variation
1	80	99.2600	1.0452	1.0530
2	100	99.5367	0.1102	0.1106
3	120	99.3733	0.3761	0.3784

n=3

Precision

The precision was assesed in terms of intra-day and inter-day variation. The intra-day and inter-day variation in the peak area of drug solution was calculated in terms of coefficient of variation (C.V.). The results are furnished in Table 4.

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

The LOD and LOQ for Tolterodine were predicted basing on the parameters of standard error of estimate and slope, calculated from linearity of the response data of Tolterodine.

Robustness

The robustness was checked by changing the flow rate to 0.9 and 1.1 ml/min.

Accuracy

The accuracy of the HPLC method was assessed by adding known amount of standard drug solution to a pre-analyzed tablet formulation. The recovery studies were carried out in triplicate. The accuracy was expressed in terms of recovery at three levels 80%, 100% and 120%. The results are furnished in Table 5.

Results and Discussion

Optimization of the chromatographic conditions were carried out with various combinations of Acetonitrile and 10 mM Ammonium acetate and by observing the peak parameters, the run time of the method was set at 10 min, Tolterodine appeared on the typical chromatogram at 5.50 min, which indicates a good base line. When the same drug solution was injected 3 times, the retention time of the drug was same. Linearity range was observed in the concentration range of 20-100 µg/ml. The regression equation of Tolterodine concentration over its peak area ratio was found to be Y = 50179X + 17718 (r=0.9998) where Y is the peak area ratio and X is the concentration of Tolterodine (Fig. 3). The proposed HPLC method was also validated for intra-day and inter-day variation. The coefficient of variation in the peak area of the drug for 3 replicate injections was found to be less than 2%. The tailing factor was found to be 1.01, which indicates good shape of peak. The number of theoretical plates was found to be 6125, which indicates efficient performance of the column. The limit of detection and limit of quantitation was found to be 0.0457µg/ml and 0.1384 µg/ml which indicates the sensitivity of the method. The use of Acetonitrile and 10mM Ammonium acetate in the ratio of 80:20 v/v resulted in peak with good shape and resolution. The high percentage of recovery of Tolterodine ranging from 100.10-100.30 indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram indicating that excipients used in tablet formulation did not interfere with the estimation of the drug by proposed HPLC method.

Conclusion

The proposed HPLC method was found to be simple, rapid, sensitive, precise and accurate for the estimation of Tolterodine in pharmaceutical formulations. Hence, this method can easily and conveniently be adopted for routine quality control analysis of Tolterodine in bulk and pharmaceutical formulations.

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