

STABILITY INDICATING RP-HPLC METHOD FOR DETERMINATION OF TOLTERODINE IN SOLID DOSAGE FORM

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Received on : 14.07.2009

Revised : 15.09.09

Accepted : 17.09.09

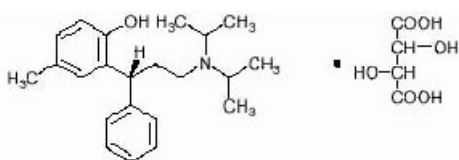
ABSTRACT

A simple, rapid, and accurate RP-HPLC method was developed for the determination of tolterodine in solid pharmaceutical dosage form. The method showed a linear response for concentration in the range of 1-15 µg/ml using acetonitrile:potassium dihydrogen phosphate (50:50, v/v) as the mobile phase with detection at 284 nm and a flow rate of 1 ml/min. The method was statistically validated for accuracy, precision, linearity, range, robustness, and stock solution stability. Recovery studies of the dosage form were also carried out and the mean recovery was found to be 98.35±1.01 %. Due to the simplicity, rapidity, and accuracy the method could be used for routine quality control analysis.

Keywords: Tolterodine; HPLC; Degradation studies.

INTRODUCTION

Tolterodine is a prescription drug used to treat overactive bladder (urinary incontinence). The chemical name of tolterodine is 2-[(1R)-3-[Bis(1-methylethyl)amino]-1-phenylpropyl]-4-methylphenol. The empirical formula of tolterodine tartarate is $C_{26}H_{37}NO_7$, and its molecular weight is 475.57. The structural formula of tolterodine tartarate is represented below¹



Tolterodine belongs to a family of drugs called antimuscarinics. Antimuscarinic drugs block muscarinic receptors, which activate certain circuits in the nervous system. There are several types of muscarinic receptors, including the M2 and M3 types. Tolterodine acts on the M2 and M3 subtypes of muscarinic receptors, while most antimuscarinic drugs only act on M3 receptors.² It is metabolized in the liver and the metabolites possess similar actions as the parent drug.³ The main effects of tolterodine are increase in residual urine, reflecting incomplete emptying of the bladder, and a decrease in detrusor pressure, consistent with an antimuscarinic action on the lower urinary tract. It is also used for the treatment of overactive bladder. Various methods are available for the analysis of tolterodine in literature like, chiral HPLC-UV⁴, GC-MS⁵, LC-MS⁶ etc. However, there are only few stability indicating HPLC methods reported in the literature for the estimation of tolterodine in bulk drug.

EXPERIMENTAL

Instrumentation

Quantitative analysis was performed using a High performance liquid chromatography with two LC-10AT VP pumps from Shimadzu Corp., Japan (Shimadzu Class LC-10A VP), with a PDA detector (SPD M-10AVP photo diode array detector, Shimadzu, Kyoto, Japan), supelco C₁₈ (250 X 4.6 mm, 5 µm) was the column used for the analysis. The HPLC system was equipped with class VP software, Version 5.03 (Shimadzu).

Reagents

Tolterodine was received as a gift sample from Dr. Reddy's Laboratories Ltd., Hyderabad, India. HPLC grade methanol, Potassium dihydrogen phosphate (Merck Chemicals, Mumbai) and Water HPLC grade (Milli-Q, Millipore, Mumbai) were used for the study. Commercially available tolterodine capsules, claimed to contain 2mg of the drug were procured from the local market.

HPLC Conditions

The experiment was performed using a (250 X 4.6 mm, 5 µm) supelco C₁₈ stainless steel column with acetonitrile:potassium phosphate buffer (10mM, pH 3.0±0.2) (50:50, v/v) as the mobile phase which was filtered through a nylon membrane (pore size 0.22 µm) and degassed before use. The analysis was performed at room temperature, with a flow rate of 1 ml/min, and the run time was 8.4 minutes. The injection volume for sample and standard was 20 µl. The column was equilibrated with mobile phase for at least 45 minutes prior to starting the analysis. The eluents were monitored at 284 nm and data acquired, stored and analysed using Class VP software, Version 5.03 (Shimadzu).

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A standard stock solution of tolterodine was prepared by dissolving equivalent to 10mg of tolterodine in a 10ml volumetric flask containing methanol (HPLC grade), sonicated for about 5 minutes and then made up to volume with methanol. Eight sets of the drug solution in the concentration range of 1-15 µg/ml were prepared and suitably diluted using the mobile phase. Each of these drug solutions (20µl) was injected six times into the column and the peak area and retention time was recorded.

Procedure for pharmaceutical formulation

Ten capsules were weighed and average weight was calculated. An amount equivalent to 10 mg of tolterodine was weighed and dissolved in methanol. Then the solution was sonicated for 10 min and filtered through 0.22µm filter. The filtrate was further diluted to get a concentration of 10 µg/ml and analysed by the same method by injecting 20 µl into the HPLC system.

Accuracy as recovery studies

The accuracy was determined by recovery studies. The known amount of standard drug was spiked to the pre analysed samples and the recovery of the drug was calculated. Accuracy was performed at three levels of 80 to 120% of standard concentration. A solution containing 10µg/ml of the sample which was extracted from the tolterodine capsule dosage form was spiked with 80-120% of the standard tolterodine solution (8, 10, 12µg/ml) and analyzed in same chromatographic condition. The percentage recoveries at three levels were found to be 99.33%, 100.36% and 98.36% for 80%, 100% and 120% respectively.

Precision

Repeatability is the results of the method operating over a short time interval (within a day) under the same conditions. The peak area of assay concentration of the drug (10µg/ml) was analysed on the same day six times. To assess the degree of reproducibility of the method, assay concentration (10µg/ml) was analyzed on different day. The assay procedure was repeated six times and the chromatogram was recorded and the % RSD was calculated.

Robustness

By introducing small but deliberate changes in the mobile phase pH (± 0.2), flow rate (± 0.1 ml/min) and the detection wavelength (± 5 nm) the robustness of the described method was studied. The robustness of the method was assessed for the assay concentration (10µg/ml).

Sensitivity

The sensitivity of the method was determined with respect to LOD and LOQ. In the present method, LOD and LOQ were calculated based on the standard deviation of the response and slope. $LOD = 3.3 \times SD/S$:

$LOQ = 10 \times SD/S$, where SD is the standard deviation of the blank response and S is the slope of the calibration curve.

Forced degradation studies

Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the method. Various stress conditions like alkaline, acidic, hydrogen peroxide, and thermal degradation were attempted. 10 mg of tolterodine was weighed accurately and transferred to 10ml volumetric flask and dissolved in 1M sodium hydroxide. Immediately after making up the volume, the solution was transferred to a round bottom flask and kept for refluxing at 80°C. Zero hour sample was taken and diluted with methanol. The samples were withdrawn at different time intervals (0, 1, 2, 3, 4, 6, 8, 12, 24hr) and final dilution was done with mobile phase and loaded into HPLC system. Similarly the degradation study was carried out in acidic (1N Hydrochloric acid) and oxidation (hydrogen peroxide 3%) and thermal degradation at 65°C for 15 days. Samples were collected at different time intervals and analyzed.

RESULTS AND DISCUSSIONS

An RP-HPLC with UV detection was developed for quantitative estimation of tolterodine in pharmaceutical dosage forms. The effect of pH, solvent strength, flow rate, temperature, wavelength, different buffers, and stationary phases were studied. In the developed method, the mobile phase consisted of 10mM potassium dihydrogen phosphate buffer (pH 3.0) and acetonitrile in the ratio of (50:50) with Supelco C₁₈ column (250mm X 4.6mm, 5µm) as stationary phase. The retention time of tolterodine was found to be 8.4 minutes (Fig.1).

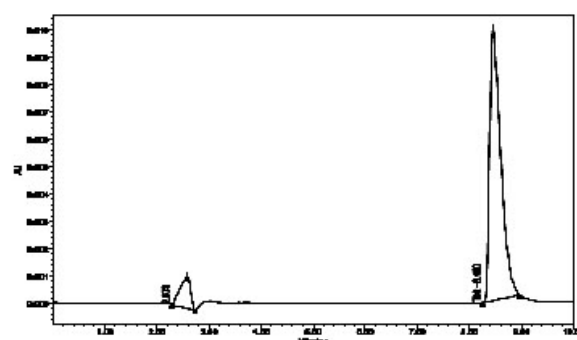


Fig. 1. Chromatogram of standard tolterodine, $R_t = 8.4$ minutes

The proposed HPLC method was linear over the range of 1-15 µg/ml, the coefficient of determination was found to be 0.999 ± 0.01 and % RSD for repeatability and intermediate precision was 0.95 and 1.57 respectively. The mean recovery was 98.35 ± 1.01 % and LOD and LOQ was found to be 0.1 and 1µg/ml respectively. Tolterodine subjected to various stress conditions like alkali hydrolysis, acidic hydrolysis, oxidative, and thermal degradation studies and

degradation products were analyzed. The results showed that tolterodine underwent alkali degradation and stable in other stress conditions. The developed method was able to separate the degradation products (Rt 6.18, 6.67 and 13.36 minutes) from the main analyte peak (Fig.2).

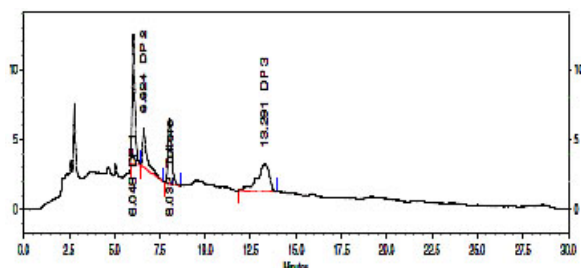


Fig. 2: Chromatogram of tolterodine in 48hrs 1N sodium hydroxide at 65° C

CONCLUSION

The statistical analysis proves that HPLC method is repeatable for the analysis of tolterodine in bulk drug and its solid dosage form. Since the forced degradation showed no interference and the degradation products were separated from tolterodine peak, the method is specific and stability-indicating. The developed method could be used in quality control of bulk drug and pharmaceutical dosage forms.

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