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Analytical method development and validation of Tolterodine in pharmaceutical dosage forms by RP-HPLC

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Abstract

A new sensitive, simple, rapid and precise reversed phase high performance liquid chromatographic (HPLC) and a spectrophotometric method has been developed for estimation of tolterodine in tablet dosage form. Chromatography was performed by using the mobile phase, Potassium Phosphate pH 4.5 and acetonitrile mixed by a low pressure gradient program. Hypersil BDS C18 column was used. The wavelength detection was set at 205 nm. Linearity range for tolterodine was 10.0 – 60.0 µg/ mL. Linear calibration graphs values were found at 205 nm for tolterodine, The proposed method showed good linearity, precision and reproducibility and applied to the pharmaceutical dosage form containing the above mentioned drug without any interference by the excipients.

Keywords: Tolterodine • High performance liquid chromatography • Pharmaceutical dosage form

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INTRODUCTION

Tolterodine tartarate is chemically (R)-N,N-diisopropyl-3-(2-hydroxy-5-methylphenyl)-3-phenylpropanamine L-hydrogen tartrate. The molecular formula is $C_{26}H_{37}NO_7$ with a molecular weight of 473.58. Tolterodine is a competitive muscarinic receptor antagonist. Both urinary bladder contraction and salivation are mediated via cholinergic muscarinic receptors. After oral administration, tolterodine is metabolized in the liver, resulting in the formation of the 5-hydroxymethyl derivative, a major pharmacologically active metabolite. The 5-hydroxymethyl metabolite, which exhibits an antimuscarinic activity similar to that of tolterodine, contributes significantly to the therapeutic effect. Both tolterodine and the 5-hydroxymethyl metabolite exhibit a high specificity for muscarinic receptors, since either show negligible activity or affinity for other neurotransmitter receptors and other potential cellular targets, such as calcium channels. Tolterodine has a pronounced effect on bladder function. The main effects of tolterodine are an increase in residual urine, reflecting an incomplete emptying of the bladder, and a decrease in detrusor pressure, consistent with an antimuscarinic action on the lower urinary tract.

Both tolterodine and its active metabolite, 5-hydroxymethyltolterodine, act as competitive antagonists at muscarinic receptors. This result in inhibition of bladder contraction, decrease in detrusor pressure, and an incomplete emptying of the bladder in a study with ^{14}C-tolterodine solution in healthy volunteers who received a 5-mg oral dose, at least 77% of the radiolabeled dose was absorbed. Cmax and area under the concentration-time curve (AUC) determined after dosage of tolterodine immediate release are dose-proportional over the range of 1 to 4 mg. Based on the sum of unbound serum concentrations of tolterodine and the 5-hydroxymethyl metabolite ("active moiety"), the AUC of

tolterodine extended release 4 mg daily is equivalent to tolterodine immediate release 4 mg (2 mg bid). Cmax and Cmin levels of tolterodine extended release are about 75% and 150% of tolterodine immediate release, respectively. Maximum serum concentrations of tolterodine extended release are observed 2 to 6 hours after dose administration (1-9).

Literature survey shows that, a few analytical methods were reported for quantification of tolterodine by using capillary electrophorsis (10), HPLC (11-14), UPLC (15), GC-MS (16), LC-MS (17-22). Among all, quantification was done in pharmaceutical compounds (10-14), Biological (15-22).

The aim of the present study is to develop and validate simple, sensitive, rugged, reproducible, most economical method for quantification of tolterodine in pharmaceutical formulations(Tablet and pure drug) and same could be applied to quality control tests.

In this paper stability indicating RP-HPLC, their complete validation as per ICH guidelines [23-25] and comparison results with optimized experimental parameters has been described. The proposed methods were applied to pharmaceutical preparation, with satisfactory results.

MATERIALS AND METHODS

Active Pharmaceutical Ingredient (API) Tolterodine tartarate and working standards were kindly supplied by Bal Pharma Lt.d,(Banglore, India)

Matrix tablet of Tolterodine (4mg Tablet), manufactured by author (Vignan Pharmacy Colllege , Vadlamudi, Guntur, Andhrapradesh, 522213, India)

Chemicals and Reagents

Acetonitrile, Methanol (HPLC grade) obtained from J.T.Baker, Mumbai, Phosphoric acid(85%) (HPLC grade), Potassium Phosphate Monobasic, (ACS Grade), obtained from Merck, Mumbai. Milli Q water (HPLC Grade)

Apparatus and Equipment

A chromatographic system (Shimadzu Corporation, Japan) model Shimadzu VP, consisted of a system controller (CLASS-VP), on-line degasser (LC 2010C, Shimadzu), solvent delivery module (LC 2010C, Shimadzu), auto injector (LC 2010C, Shimadzu), column oven (LC 2010, Shimadzu), UV-VIS detector (LC 2010C UV PHARMASPEC 1700), Shimadzu and CLAS-VP software version=SPI, low pressure gradient pump, auto injector (SIL-10AD VP, Shimadzu), column oven (CTO-10AS VP, Shimadzu) and PDA detector (PDA-SPD-M10A VP, Shimadzu Diode Array Detector) and Chem station (software). Spectrophotometric analysis was carried out on a Shimadzu 1700 double beam spectrophotometer with a fixed width. Other apparatus used included Photo stability chamber, Hot air oven: Proto-Tech oven, Analytical balance: AX205, METTLER TOLEDO, pH Meter: Thermo Orion, model 420, Sonicator: Oscar Ultra Sonics OU-72(SPL).

Chromatographic Conditions

Mobile Phase Preparation

Solution A: Dissolve about 2.72g of Potassium dihydrogen phosphate into 1000ml of water. Add 2 ml of triethylamine and mix. Adjust pH 4.5±0.05 with diluted ortho phosphoric acid (5 ml of ortho phosphoric acid is dissolved in 50ml of water) mix and filter.

Solution B: Acetonitrile: Methanol (70:30) % v/v

The buffer and acetonitrile were degassed before for 15 min in an ultrasonic bath before use. The analysis was actually started after passage of mobile phase to stabilize the gradient program and reach equilibrium.

All solvents were filtered through 0.45µm PVDF Millipore filter and degassed in an ultrasonic bath before use.

Sample solvent Preparation: Mix of Solution A: Solution B: (60:40) % v/v

The chromatography was carried out on Hypersil BDS C18 ([250mm x 4.0mm] 5µ) analytical column at 1.8 ml min^{-1} flow rate of mobile phase with gradient programmer **(Table.I).** The injection volume was 10.0µL, Column oven temperature was at 30ºC. Detection at 205 nm, and chromatographic run time of 10.0 min was used. Prior to

injection of the drug solution, the column was equilibrated for at least 10 min with the initial time gradient mobile phase conditions flowing through the system.

Table.I.Gradient programme of the mobile phase

Mobile Phase Gradient Program				
Time(min)	**A%**	**B%**	**Flow rate(ml/min)**	**Gradient Program**
0.01	60	40	1.8	Initial
3.00	40	60	1.8	Linear
6.00	25	75	1.8	Linear
6.50	60	40	1.8	Linear
10.00	60	40	1.8	Isocretic

Preparation of Solutions

Preparation of Standard Solution

The Standard stock solution of Tolterodine tartrate (TTT) was prepared by dissolving 40 mg in 100 mL standard volumetric flask containing approximately 70 mL of sample solvent (diluent) and the solution was sonicated for 2 min, and the volume was made up to the mark with diluents. Further 5 ml of standard stock diluted to 50 ml of solvent to obtain the concentration of 40 µg/ mL.

Preparation of Sample Solution for drug substance

Ten tablets were weighed to determine the average tablet weight and powdered in a mortar. Powder equivalent to 20 mg of TTT was transferred into a 100mL volumetric flask. About 50mL of diluent was added and kept on a rotary shaker for 20 min to disperse the material completely, followed by sonication for 10 min, cooled to room temperature, made up to mark with diluent and mixed well. About 25mL of sample solution was centrifuged for 15 min at 2,500 rpm. The sample solution was filtered through a 0.45 µm Nylon-66 membrane syringe filter, and 10.0 mL of this solution was taken in a 50 mL volumetric flask and made up to volume with diluent.

$$\text{Drug substance Assay \% dried basis (mg/mL)} = \frac{A_{Spl} \times \text{mg. Std} \times 10 \times 100 \times 50 \times 100 \times \%P}{A_{Std} \times 100 \times 50 \times \text{mg. Spl} \times 5 \times 100\text{-}D \times 100}$$

Where,

A_{Spl} = Area of Tolterodine peak in test sample

A_{Std} = Area of Tolterodine peak in reference standard sample

Spl = Drug substance sample for assay

Spt = Drug substance standard for assay

%P = Potency of Tolterodine reference standard (as is basis)

D = Loss on drying of sample in %

Preparation of Sample Solution for drug Product

Ten tablets were weighed to determine the average tablet weight and powdered in a mortar. Powder equivalent to 20 mg of TTT was transferred into a 100 mL volumetric flask. About 50 mL of diluent was added and kept on a rotary shaker for 20 min to disperse the material completely, followed by sonication for 10 min, cooled to room temperature, made up to mark with diluent and mixed well. About 25 mL of sample solution was centrifuged for 15 min at 2,500 rpm. The sample solution was filtered through a 0.45 µm Nylon-66 membrane syringe filter, and 10.0 mL of this solution was taken in a 50 mL volumetric flask and made up to volume with diluent.

$$\text{Content of Tolterodine (Assay/Tablet) (mg/mL)} = \frac{A_{LT} \times W_L \times 10 \times 250 \times 50 \times P_L}{A_{LS} \times 50 \times 50 \times N \times 5 \times 100}$$

Where,

A_{LT} = Area of Tolterodine peak in test sample

A_{LS} = Area of Tolterodine peak in reference standard sample

W_L = Weight of Tolterodine reference standard

N = Number of tablets used for sample preparation

P_L = Potency of Tolterodine reference standard (as is basis)

$$\text{\% Labeled amount} = \frac{\text{Content of Tolterodine (mg/Tablet)} \times 100}{\text{Label claim (mg/Tablet)}}$$

Linearity and range

Stock solution TTT 200 µg /mLwas prepared by dissolving the appropriate amount in 100 mL of diluent and further diluted to the required concentrations with diluent. The solution was prepared at six concentration levels ranging from 25% to 150% of the target concentration 40 µg/ mL. Linearity of the method was studied by injecting six concentrations (10, 20, 30, 40, 50, 60 µg/ mL) of the drug prepared in the diluent in triplicate into the HPLC system keeping the injection volume constant. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs. The correlation coefficients, slopes and *Y*-intercepts of the calibration curve were determined.

Stress Studies

In order to prove the selectivity of the HPLC method, Tolterodine API, Placebo and its formulation were studied under various stressed conditions to perform forced degradation studies. Stress studies were carried out under the condition of acid/base hydrolysis, oxidation, thermal, humidity and photolytic, as mentioned in ICH guidelines. The stress conditions are mentioned in **Table.II.**

Table.II: Degradation Conditions

Condition	Detail
Thermal	80º C for 24 Hrs
U.V.	1.2 million lux (254 nm)for 48 Hrs
Acid	1 ml 1 N HCL and heated for 20 min at 60º C
Alkali	1 ml 1 N NaOH and heated for 20 min at 60º C
Oxidation	1 ml 0.3% H_2O_2
Humidity	40 ºC / 75% RH for more than 5 Days

DISCUSSION

Method Development

Development of a simple, rapid, rugged and reproducible HPLC method for the quantification of TTT required a number of trials to be carried out using different mobile phase compositions. As part of the method development, Suitable chromatographic conditions, Column, peak shape were finalized with different trails. Zorbax SB C18 (100 mm X 2.1 mm, 1.7 µm) column with Mobile phase combination (Potassium dihydrozen phosphate: Acetonitrile: Methanol: 40:60, 50:50, 60:40 v/v) with different gradient programs at a flow rate of 1.0 mL/ min were tried initially. Further with different column like Hypersil BDS C18 [250mm x 4.0mm] 5µ with Mobile phase combination (Potassium dihydrozen phosphate: Acetonitrile: Methanol: 40:60, 50:50, 60:40 v/v) with different gradient programs at a flow rate of 1.8 mL/ min were tried and finally Hypersil BDS C18 [250mm x 4.0mm] 5µ column with Mobile phase combination at gradient programme gave best chromatogram. For gradient program followed in chromatographic conditions. The typical retention time of TTT is 3.5 min in a total chromatographic run time of 10 min.(Fig.1) The resolution between TTT and the degradants generated after stress degradation were found to be good in the developed RP-HPLC assay method.

Method Validation

Complete validation parameters (linearity, precision, accuracy, specificity, solution stability, ruggedness and robustness) were performed, proving the HPLC method suitable for analysis as per International conference on Hormonization (ICH) guidelines. The HPLC method was found to be highly specific and linear. The peaks of the active ingredients, excepients and degradation products were separated completely with no overlaying. Thus the method was found to be highly specific and stability indicating for degradation products and formulation excipients.

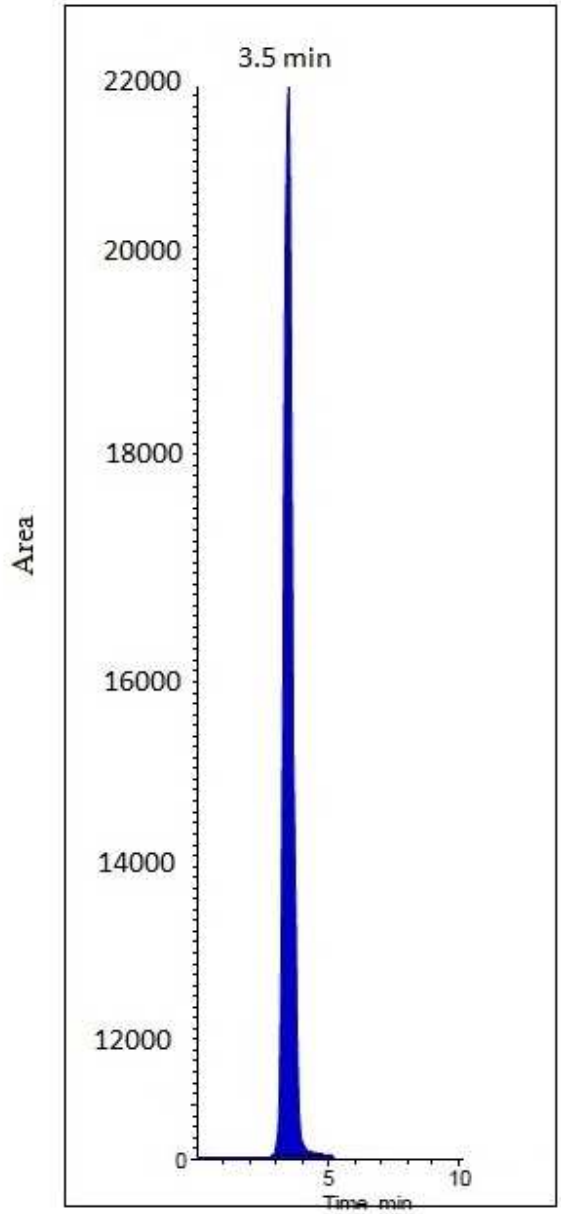

Fig. 1: Representative chromatogram of Tolterodine Tartrate (TTT) 40µg/ mL

RESULTS

System Suitability

System Suitability was daily performed during the entire validation. The system suitability and system precision results of HPLC method, are given in **Table III.** RSD in HPLC Method (n=5) = 0.1%.

Table. III: System suitability and System Precision

	Tolterodine
Retention time	3.51 ± 0.0211
Theoretical Plates (n)	11448
Asymmetry/Tailing (T)	1.00

Linearity

To achieve linearity and range for the method, stock solution of 200 µg/ mL was diluted to yield solutions in the range 10.0 to 60.0 µg/ mL for Tolterodine. The solutions were prepared in triplicate and analyzed by using 10 µL into HPLC. The linearity and range results are given in **Table. IV.** The calibration curve of is shown in Figure.2.

Table. IV: Characteristics of the Method from Standard calibration curve

	Tolterodine
Linearity Range	10.0-60.0µg/ ml
Correlation coefficient (r^2)	0.999
LOD	0.6µg/ mL
LOQ	10.0 µg/ mL

LOD=Limit of Detection, LOQ=Limit of Quantification

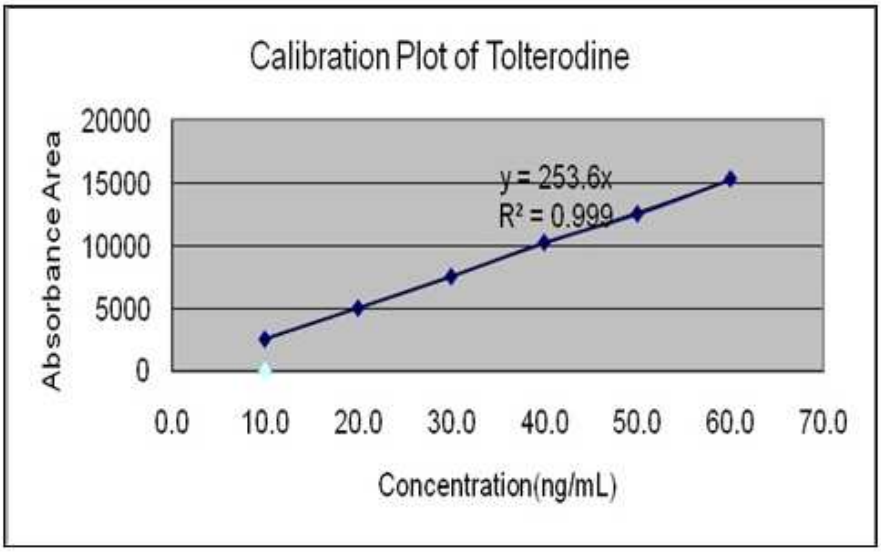

Fig. 2: Calibration Plot

Specificity

Specificity of developed method was established by determining peak purity of active component in standard preparation, test preparation and spiked sample preparation using PDA detector. There was no interference seen by excepients. 3 point peak purity of the drug Tolterodine was found to be greater than 0.999. Results of specificity of HPLC method are shown in **Table .V**.

Tab. V: Peak point Purity in HPLC method

Sets	3 point peak purity Tolterodine
Standard Solution	0.9998
Placebo spiked with API	0.9997
Test Solution	0.9992

Method Precision

The method precision (repeatability) was obtained by determining the assay by preparing six-sample preparation. The low value of standard deviation proved the method to be very precise. The method precision both Interday and Intraday results are shown in **Table VI.**

Table.VI: Method Precision

		Tolterodine
Intraday Precision		
	Assay (Mean ± SEM)	100.1
	% RSD of Assay	0.9%
Interday Precision		
	Assay (Mean ± SEM)	99.8
	% RSD of Assay	0.7%

Method Ruggedness

Ruggedness test was determined by two different analysts, instruments and columns in HPLC method. The results are shown in **Table. VII.**

Tab.VII: Method Ruggedness

Day I: Analyst I, Instrument I & Column I	**Tolterodine**
Assay (Mean ± SEM)	101.5 ± 0.4242
% RSD	0.8%
Day II: Analyst II, Instrument II & Column II	
Assay (Mean ± SEM)	100.3 ± 0.2556
% RSD	0.56%

Recovery

To ensure the reliability and accuracy of the method, recovery studies were carried out in triplicate at 50%, 100% and 150% of target concentration. Results of accuracy study are within the range of 98% to 102% and RSD < 1%. Results are shown in **Table.VIII.**

Tab.VIII: Recovery Study

Levels	**Tolterodine** Mean % Recovery ± SEM, n=3	% RSD
50%	99.0±0.3551	0.8%
100%	98.9±0.2160	0.5%
150%	99.9±0.0849	0.2%

Method Robustness

Robustness of the method was determined by small deliberate changes in flow rate, buffer pH and column oven temperature. Even after these changes, almost same results were seen. The low values of relative standard deviation proved the method to be robust. The results are shown in **Table. IX**.

Tab. IX: Method Robustness

Changed Conditions	% Change	**Tolterodine** %RSD
	Normal	0.2%
Column Temp	+5º C	0.4%
	-5ºC	0.5%
Buffer pH	+0.2units	0.2%
	-0.2 units	0.6%
Flow Rate	+10%	0.5%
	-10%	0.7%

Solution Stability

Standard and sample solutions were evaluated at room temperature for 24 Hours. The solutions were analyzed after 2, 8, 18 and 24 hours the relative standard deviation was found to be below 2.0% in both the methods. The results are shown in **Table. X.**

Tab. X: Solution stability

Time	Tolterodine	
	% difference	
	Standard	Sample
2 Hours	0.0%	-0.8%
8 Hours	+0.5%	+0.3%
18 Hours	+0.5%	+0.3%
24 Hours	+1.0%	+0.4%

Assay

This validated method was successfully applied for the estimation of Tolterodine in tablet dosage form. The assay results were 101% for Tolterodine. Retention time approximately 3.5 minutes Tolterodine. The results of assay indicated that the method is specific for the analysis of Tolterodine without interference from the excipients used to prepare and formulate these tablets

Stability indicating nature of the Developed Method

Placebo (excepients), Tolterodine API, and tablets exposed to various stress conditions showed the peak purity as that of the normal condition. The degradation study clearly indicated that Tolterodine degrades in acid and alkali conditions the maximum. The degradation results clearly indicate that there is no merging of the impurity peaks with main analyte peaks of Tolterodine. The % assay of degraded samples summed up with % of observed impurities peaks (by area normalization) bringing close to 100%, suggested the HPLC method to be stability indicating. The degradation results are showed in **Table.XI.**

Table XI: Forced Degradation

Treated Parameters	Tolterodine		
	% Assay	*% Degradation*	3 point purity
As such	100%	--	0.9999
Humidity	99.1%	0%	0.9999
U.V.	98.5%	0%	0.9999
Oxidation	82.1%	15.4%	0.9990
Alkali	90.83%	8.2%	0.9993
Acid	83.1%	14.3%	0.9991
Thermal	94.53%	2.5%	0.9998

CONCLUSION

In this study, an accurate, rapid, simple, sensitive, reproducible and stability-indicating reversed-phase HPLC method with UV detection was described for the assay of Tolterodine Tartrate. The method was fully validated and applied successfully to quantify the drug in pharmaceutical dosage form, and API samples. A short chromatographic run time of 10 min allows the quantification of Tolterodine tartrate in bulk raw material, tablet dosage form in quality control laboratories, and is compatible with LC-MS technique where there is no need for traditional HPLC methods with complex mobile phase mixtures, long chromatographic run times and more solvent consumed methods. The developed RP-HPLC technique will eliminate significant time and cost per sample from analytical process while improving the quality of results. The proposed method is not hazardous to human health or to the environment and is more economic because a large number of samples can be analyzed in a short period of time.

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