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DEVELOPMENT AND VALIDATIONOF OF STABILITY INDICATING HPLC METHOD FOR DETERMINATION OF TOLTERODINE TARTRATE.

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ABSTRACT

A simple and rapid stability indicating HPLC method for tolterodine tartrate was developed and validated. The stationary phase used was Thermo C_{18} (250 x 4.6 mm) 5 µ with a mobile phase consisting of mixture of acetonitrile and water pH 3.0 in the ratio of 70:30 v/v at a flow rate of 1.0 ml/min. Detection was carried out at 220nm. The retention time observed was 7.1 ±0.1 min. The column was maintained at ambient temperature and 20µl of solutions were injected. The eluted compound was detected by using PDA detector. The linear regression analysis data for calibration plot show good relationship with coefficient of regression value, r^2=0.99 in the concentration range 5-25 µg/ml. The stress degradation studies were performed as per ICH guidelines. Tolterodine tartrate degraded with acid/base hydrolysis, thermal, oxidation and photolytic stress. The method was validated as per ICH Q2A(R1) guideline with respect to linearity, accuracy, precision, specificity and robustness. This method can be used for monitoring the stability of tolterodine tartrate. There are very few stability indicating method is present for determination of tolterodine tartrate.

KEYWORD: Tolterodine tartrate, stability indicating, HPLC, validation, ICH guideline.

INTRODUCTION

Tolterodine tartrate, is chemically 2-[(1R)-3-[bis(propan-2-yl)amino]-1-phenylpropyl]-4-methylphenol.[1] Tolterodine tartrate is a potent and competitive muscarinic receptor antagonist used for the treatment of urinary incontinence and other overactive bladder symptoms, such as urgency and high micturition frequency. The drug also increases functional bladder volume. The drug blocks muscarinic receptors, which can be found on the muscle cell of the bladder wall. Stimulation of these receptors causes the bladder to contract and empty. When these receptors are blocked, the muscle of the bladder wall contracts less. Tolterodine tartrate acts on M1, M2, M3, M4 and M5 subtypes of muscarinic receptors whereas modern anti muscarinic treatments for overactive bladder only act on M3 receptors making them more selective.[2] Some analytical methods which include UV-visible spectrophotometric, HPTLC and HPLC have been reported in the literature for the determination of Tolterodine tartrate in pharmaceutical preparations.[3-8] The method was validated as per ICH Q2A(R1) guideline with respect to linearity, accuracy, precision, specificity and robustness.[9-10]

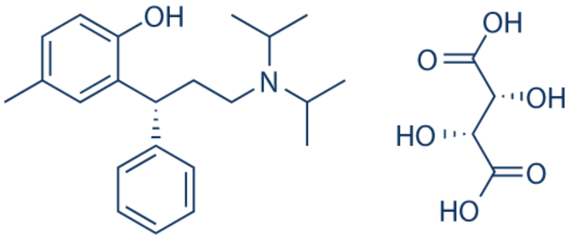

Figure1: chemical structures of tolterodine tartrate.

MATERIAL AND METHOD

Instruments

Quantitative HPLC was performed using isocratic high performance liquid chromatography (Jasco HPLC system) with a LC-PU 2080 Plus pump, manual injector with loop volume of 20µL (Rheodyne), programmable MD 2010 PDA detector and Thermo C18 (250 x 4.6 mm i.d, 5 µm particle size). The HPLC system was equipped with Borwin-PDA software (version1.5). An electronic balance (Shimadzu AY-120), UV-Visible (make JASCO, model V-550) spectrophotometer were used in this study.

Chemicals and reagents Analytically pure sample of Tolterodine tartrate was kindly supplied by Alkem laboratories Mumbai. Acetonitrile (HPLC grade) was purchased from

Merck specialties Pvt. Ltd.(Mumbai, India). Strong Ammonia and formic acid was purchase from Loba chemical. Elga Lab (PURELAB UHQ-II) water purification system.

Detection wavelength

From the standard stock solution further dilutions were done using acetonitrile and scanned over the range of 200–400 nm.

Selection of mobile phase

The standard solution of Tolterodine tartrate (10µg/ml) was injected into the HPLC system and run in different solvent systems. Different mobile phases like Methanol and water, methanol and acetate buffer, acetonitrile and phosphate buffer, methanol and phosphate buffer in varying proportion of mobile phase components, varying conditions of pH were tried in order to obtain the desired system suitability parameters for the Tolterodine tartrate

Optimized chromatographic conditions

The mobile phase consisted of acetonitrile and water (pH 3.0 adjusted by ammonia and formic acid) in the ratio of 70:30 v/v. It was then filtered through 0.45 µ membrane filter paper using vacuum filtration assembly and then sonicated using ultrasonic water bath for 15 min. The flow rate of mobile phase was maintained at 1 ml/min. The column and the HPLC systems were kept at ambient temperature.

Preparation of solutions

Preparation of standard stock solution

Prepared standard stock solution of Tolterodine tartrate in acetonitrile (100 µg/ml). Further dilution was made in Acetonitrile to get final solution of Tolterodine tartrate 5 to 25µg/ml.

Preparation of sample solution

5 Tablets were weighed and its average weight was determined. An accurately weighed tablet powder equivalent to 5mg of Tolterodine transferred into 10ml volumetric flask dissolved in acetonitrile, sonicated for 10min and volume was made up to the mark. Solution was filtered using whattman filter paper and diluted to obtain 10µg/ml stock solution.

Forced degradation studies

In order to determine whether the method is stability indicating, Forced degradation studies were carried under condition of acid, alkaline and neutral hydrolysis, oxidation, dry heat and photolysis as per ICH Q1A(R2) and Q1B guideline. The analysis was carried out by HPLC with a PDA detector. For each study, two samples were prepared: the blank subjected to stress in the same manner as the drug solution and working standard solution of Tolterodine tartrate subjected to degradation condition. Stress degradation conditions were optimized with respect to time of exposure and strength of reagent so as to obtain degradation in the range of 10 to 30%. Dry heat and photolytic degradation were carried out in solid state.

Acid hydrolysis

Acid induced degradation was performed by adding 1 ml of 1N Hydrochloric acid (HCl) to volumetric flask containing 1ml of Tolterodine tartrate standard solution (100µg/ml). The volume was made up to 10 ml with acetonitrile & kept for 24 hr in dark place. Final solution (10µg/ml) was injected.

Alkaline hydrolysis

Alkali induced forced degradation was performed by adding 1 ml of 1N sodium hydroxide (NaOH) to volumetric flask containing 1ml of Tolterodine tartrate standard solution (100µg/ml). The volume was made up to 10 ml with acetonitrile & kept for 24 hr in dark place.

Neutral Hydrolysis

Neutral hydrolysis was performed by adding 1ml of Tolterodine tartrate standard solution (100µg/ml) was mixed with 1ml of water in 10ml of volumetric flask and the volume was made upto the mark with mobile phase. Solution was kept for 24 hr dark place. Final solution (10µg/ml) was injected.

Oxidative Hydrolysis

Oxidative degradation was performed by adding 1ml of Hydrogen peroxide (H2O2, 30% v/v) to volumetric flask containing 1ml of Tolterodine tartrate standard solution (100µg/ml). The volume was made up to 10 ml with methanol & kept for 24 hr protected from light.

Degradation Under Dry Heat

Dry heat study was performed by keeping Tolterodine tartrate in oven at 60^0 C. A sample was withdrawn after 6 hours, weighed and dissolved in mobile phase to get solution of 10µg/ml of Tolterodine tartrate and then injected.

Photo-degradation studies

Photolytic degradation studies were carried out by exposure of drug to UV light up to 200 watt hours /square meter and subsequently to fluorescent light illumination not less than 1.2 million lux hours. Sample was weighed, dissolved in acetonitrile to get concentration of 1000 µg/ml. and further dilutions were made with acetonitrile to get final concentration (10 µg/ml).

RESULTS AND DISCUSSION

Selection of analytical wavelength

The standard solution was scanned over range of 200-400 nm to obtain a spectrum. The drug showed considerable absorbance at 220 nm.

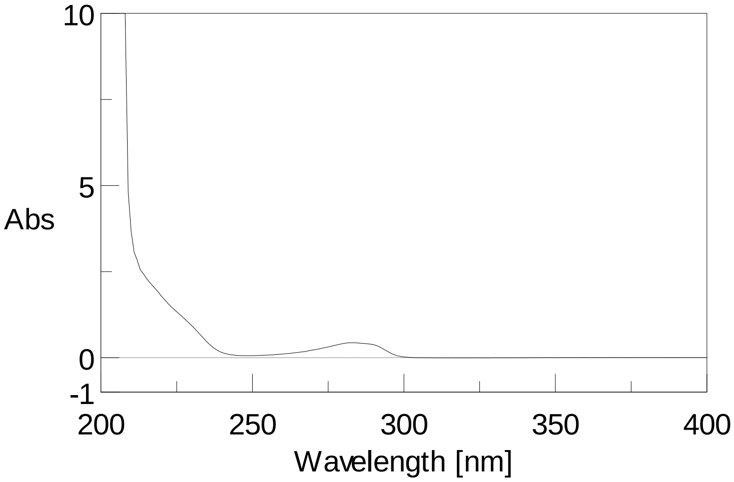

Figure 2: absorbance spectrum of tolterodie tartrate (10µg/ml)

Selection of mobile phase

After several trials, acetonitrile and water (100 ml water 0.2 ammonia pH 3.0 adjusts by formic acid) in the ratio of 70:30 was chosen as the mobile phase, which gave good resolution and acceptable peak parameters.

Chromatogram and system suitability parameter of drug

The column was equilibrated with the mobile phase (indicated by constant back pressure at desired flow rate). Working standard solution of drug (10µg/ml) was injected into the system. The retention time for the drug was found to be 7.1 ± 0.2 min. System suitability parameters of Tolterodine tartrate are summarized in

Table 1: System Suitability Parameter.

Name	RT(Min)	Conc. (µg/ml)	Area	Theoretical Plates	Asymmetry
TOLTERODIE TARTRATE	7.1	10	211289	2732	1.43

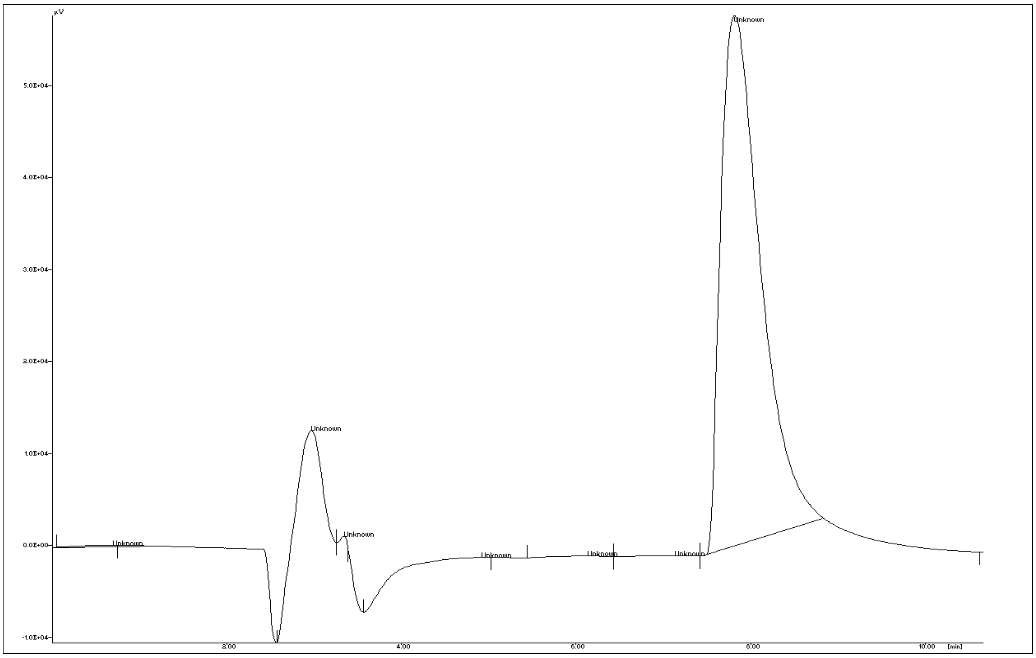

Figure 3: standard chromatogram of tolterodie tartrate (10µg/ml)

Stress degradation studies of bulk drug

At every injection of stress degraded sample to check presence of co-eluting other substance by peak purity studies. Stress degradation were performed as per ICH Q1A(R2) guideline[7]

Acid hydrolysis

Under acid hydrolysis, 72.12% Tolterodine tartrate was recovered with no peak of degradation.

Alkaline hydrolysis

After alkaline hydrolysis, 77.54% Tolterodine tartrate was recovered with no peak of degradation.

Neutral hydrolysis

After water treatment, 89.59% Tolterodine tartrate was recovered with no peak of degradation.

Oxidative Hydrolysis

Oxidative hydrolysis studies showed about 82.03% Tolterodine was recovered with no peak of Degradation

Degradation under dry heat

After the dry heat degradation, 85.28% Tolterodine was recovered with no peaks of degradation

Photo-degradation studies

After the photo degradation study under UV light 84.62% and Fluorescence light 93.13% Tolterodine was recovered with no peak of degradation.

Validation of Analytical Method

The method was validated as per ICH Q2 (R1) guidelines.[8]

Specificity

The specificity was carried out by determining the peak purity values which were found to be more than 998, indicating that there is non-interference of any impurities or degradation product at retention time of analytical peak. The study was performed by borwin software.

Linearity

Linearity was tested for the range of concentrations 10-50µg/ml. Each sample in five replicates was analysed and peak areas were recorded. The response factors are plotted against the corresponding concentrations of Tolterodine to obtain the calibration curve. Figure and represents the linearity and calibration curve for Tolterodine respectively.

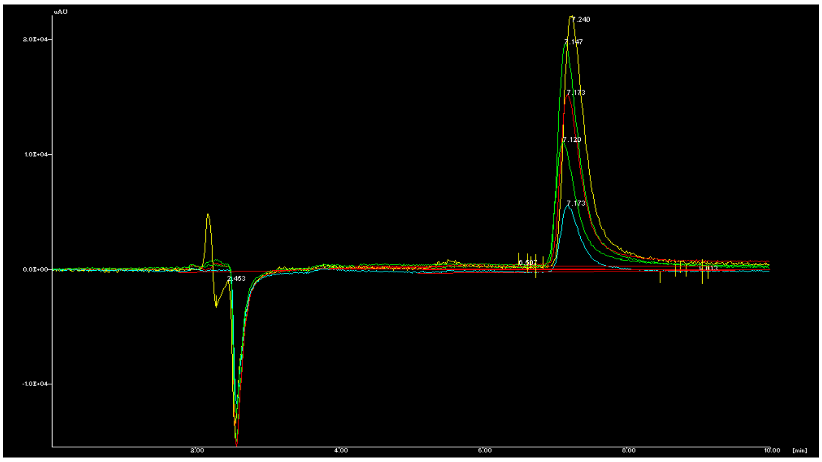

Figure 4: Chromatogram of linearity of Tolterodine (5-25µg/ml)

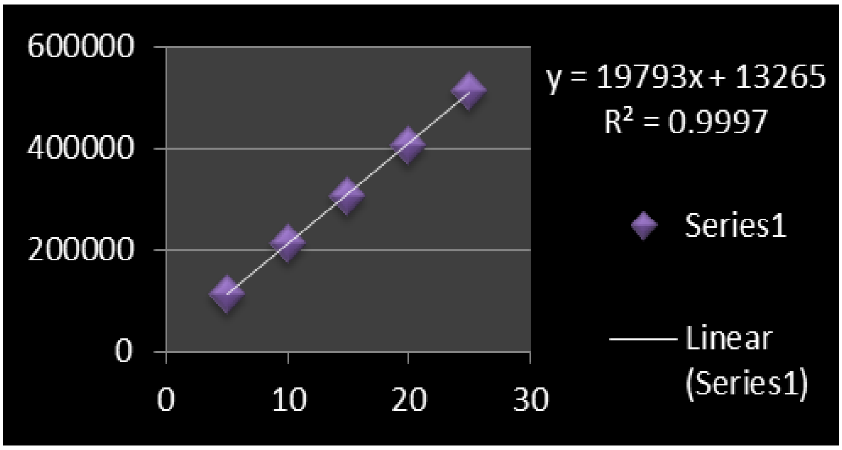

Figure 5: Calibration curve for Tolterodine.

Range

The linearity range of was found to be 5-25µg/ml.

Assay

Assay was carried out by dissolving sample equivalent to 5 mg of Tolterodine. It was repeated for six times. The sample solution was injected and area was recorded. Concentration and % purity was determined from linearity equation. The result found out to be 99.82%

Accuracy

The accuracy study was performed recovery studies were carried out by adding standard drug to marketed sample at three different levels 80%, 100% and 120 %.The accuracy study was performed for % recovery of Tolterodine tartrate. Concentration of sample chosen was 10 µg/ml of standard. These solutions were injected into HPLC system in triplicate to obtain the chromatogram. The drug concentrations were calculated by using linearity equation of Tolterodine. The results obtained are shown (Table 2)

Table 2: Recovery Studies of Tolterodine.

Recovery Level %	Amount taken (µg/ml)	Amount added (µg/ml)	Total concentration (µg/ml)	Area	% Recovery	% RSD
80	10	8	18	369921.7	100.1074	0.003
100	10	10	20	398311	97.2508	0.001
120	10	12	22	410404.7	100.2468	0.000

Precision

The precision study was performed the method was demonstrated by intra-day and inter-day studies. In the intra-day studies, 3 replicates of 3 standard solutions (10 15 and 20 µg/ml) were analyzed in a same day and percentage RSD was calculated (Table 3). For the inter-day variation studies, 9 replicates of 3 standard solutions (10, 15 and 20µg/ml) were analyzed on 3 consecutive days and percentage RSD was calculated (Table 4).

Table 3: Intra-Day Precision Study Of Tolterodine.

Concentration (µg/ml)	Area	Mean Area	SD	% RSD
10	210078	211135.3	0.462689	0.462829
	211679			
	211649			
15	303198	307582.7	1.7049	1.71983
	313122			
	306428			
20	400178	405312	1.31733	1.33014
	410604			
	405155			

Table 4: Inter-Day Precision of Tolterodine.

Concentration (µg/ml)	Area	Mean Area	SD	% RSD
10	203679	204902	1.20433	1.24388
	207649			
	203378			
15	310698	311949	2.10236	2.08976
	318722			
	306428			
20	414978	410896	1.4957	1.48904
	413604			
	404105			

Limit of Detection (LOD)

LOD is calculated from the formula

$$\mathrm{LOD} = \frac{3.3\ \sigma}{S}$$

Where,

σ = standard deviation of Y Interscept

S = slope of the calibration curve.

LOD of Tolterodine = 1.52 µg/ ml

Limit of Quantification (LOQ)

The Quantitation limit is expressed as: $LOQ = \frac{10\sigma}{S}$

LOQ of Tolterodine was found to be 4.63 µg/ ml

Robustness

Robustness of the method was determined by carrying out the analysis under conditions during which flow rate, concentration (strength), mobile phase ratio were altered and the effects on the peak area were noted

Table 5: Summary of Robustness Parameter.

SR NO	PARAMETER	AREA	MEAN	%RSD
1.	PLUS FLOW RATE (1.05)	216939	213305.33	
		213499		1.75
		209478		
2.	MINUS FLOW RATE (0.95)	203679	207375.33	
		207649		1.72
		210798		
3.	PLUS MOBILE PHASE COMPOSITION (68-32)	316398	314616	
		319722		1.96
		307728		
4.	MINUS MOBILE PHASE COMPOSITION (72 -28)	310478	310199.33	
		313622		1.15
		306498		
5.	PLUS WAVELENGTH 222	414999	410366	
		411464		1.28
		404635		
6.	MINUS WAVELENTH 224	401761	395376.66	
		393464		1.43
		390905		

Summary of validation study

Table 6: Summary of Validation Parameter.

Sr.No	Validation parameter	Tolterodine
1	Linearity Equation	y = 19793x + 13265
	(r2)	$R^2 = 0.9997$
	Range	5-25 µg/ ml
2	Precision (% RSD)	
	Interday	99.96
	Intraday	100.60
3	Accuracy	% recovery
	80	100.10
	100	97.25
	120	100.24

4	Limit of Detection	1.52 µg/ ml
5	Limit of Quantitation	4.63 µg/ ml
6	Specificity	Specific
7	Robustness	Robust

DISCUSSION

Krishna SR[6] et al, have reported A validated stability-indicating HPLC method for the determination of related substances and assay of tolterodine tartarate. In this work tolterodine expose to Acid (1 N HCl), Base (1NNaoH),oxidation (3.0 % H2O2) for 48 hours and thermal (60^{o} c) for 10 days conditions were used and present degradation was not mentioned.

CONCLUSION

The developed method is stability indicating, since the drug peak was found to be pure as Confirmed by peak purity profiling studies. The developed method is specific, accurate, precise, and robust and can be used for routine quality control as well as assessing the stability of Tolterodine

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