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STUDIES ON QUANTITATIVE ESTIMATION OF TOLTERODINE TARTRATE IN BULK AND PHARMACEUTICAL DOSAGE FORMS BY RP-HPLC

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ABSTRACT

The aim of present research work to develop precise accurate and reproducible RP-HPLC method for the quantitative estimation of Tolterodinetartrate in bulk and pharmaceutical dosage form. Chromatographic separation of Tolterodine tartrate by Phenomenex Luna C18 column 250x4.6mm 5µm, flow rate was 1.0 ml/min, mobile phase ratio was Methanol: 0.01m $NH_4H_2PO_4$ (80:20 %v/v), detection wavelength was 220 nm. Retention time of Tolterodine was 4.86 min. It was showing linear response in the range the 15-90 µg/ml and regression coefficient was found to be 0.999. Percentage mean recovery of Tolterodine was found to be 98.02%. The developed method can be successfully employed for the quality control analysis of Tolterodine tartrate in its pure form.

INTRODUCTION

Tolterodine Tartrate is the tartrate salt form of tolterodine, a benzhydryl compound and a muscarinic receptor antagonist possessing both anti-muscarinic and antispasmodic properties. Both tolterodine and its active metabolite, 5 hydroxy methyl tolterodine, competitively blocks muscarinic receptors, thereby inhibiting acetylcholine receptor binding. The 5-hydroxymethyl metabolite appears to contribute significantly to the therapeutic effects [2]. Literature survey reveals that few methods have been reported for the estimation of Tolterodine tartrate by using HPLC [3-8]. The present research works aims to develop and validate RP-HPLC method for the quantification of Tolterodine tartrate in bulk and pharmaceutical dosage form.

MATERIALS AND METHODS

Materials: Gift sample of Tolterodine tartrate working standard was procured from mylon laboratories, Banglore, Karnataka. The marketed formulation was purchased in local pharmacy. The other chemicals like HPLC grade methanol and water are made by merck labs, Mumbai. $NH_4H_2PO_4$

Was obtained from sd fine chemicals, Mumbai.

Instruments: The liquid chromatographic system was made up of shimadzu and proving software LC Solutions- 20 AD UFLC with UV-VIS detector, binary pump and septum injector valve with 20 µl fixed loop. Chromatographic system having Phenomenex Luna C_{18} ODS column (250 mm× 4.6 mm i.d. and 5 µm particle size).

Preparation of Mobile Phase: Preparation of mobile phase by using methanol and Phosphate Buffer in the ratio of 80:20 v/v. The prepared mobile phase was filtered through 0.45 um Nyoln 66 (N66) 47 mm membrane filter paper. After filtration it was ultra sonicated for 20 minute on ultra sonicator.

Preparation of stock solution of Tolterodine tartarate: API of Tolterodinetartarate (10mg) accurately weighed and transferred to 10 mL volumetric flask, dissolved in sufficient quantity of methanol and then diluted to the mark with mobile phase. The solution contains 1000ug/mL of Tolterodinetartarate. The solution was filtered through 0.45 um Nylon 66 (N66) 47 mm membrane filter paper and first few drops of filtrate were discarded.

Preparation of sample solution: Take 25 mg equivalent tablet powder of Tolterodinetartarate and dilute up to 25 mL with Mobile Phase. Sonicate it for 10 minutes. Filter the solution through Whatmann filter paper no. 41. This solution was used as sample solution.20 µL of the blank, standard and sample was injected in to the chromatographic system and areas for the Tolterodinetartarate the peaks were used for calculating the % assay.

Optimized Chromatographic Conditions:

The chromatographic method development for the Tolterodinetartate was optimized by several trails for various parameters as different column, flow rate and mobile phase; finally the following chromatographic method was selected for the separation and quantification of Tolterodinetartate in API and pharmaceutical dosage form by RP-HPLC method.

Method Validation

Specificity: Specificity of an analytical method indicates that the analytical method is its able to measure accurately and specifically the analyte of interest without any interference from blank. So here, the specificity was determined by the comparision of the chromatograms of Blank (mobile phase), Standard and sample solutions of Tolterodinetartarate.

Linearity: API of Tolterodinetartarate (10mg) accurately weighed and transferred to 10 mL volumetric flask, dissolved in with mobile phase. Solution of the drug at six different concentrations was analyzed and calibration curve was constructed by plotting mean response factor against the respective concentration. The method was evaluated by determination of the correlation coefficient and intercept value. Tolterodine tartrate follows linearity in the concentration range of 15- 90 µg/mL respectively.

Accuracy: The accuracy of the test method is demonstrated by % of recovery. Accuracy was performed in three different three concentration levels and injected three times (Like 50%, 100%, and 150%). The standard solutions of accuracy 50%, 100% and 150% were injected in to chromatographic system. Calculate the amount found and amount added for Tolterodinetartarate and calculate the individual % recovery and mean % recovery values.

Precision: The standard solution was injected for six times and measured the area for all five injections in hplc. It was performed in intra-day and inter day, Then calculate the % RSD of six replicate injections, it was found to be within the specified limits.

Robustness: As Part of the robustness, deliberate change in the flow rate, mobile phase composition was made to evaluate the impact on the method. The flow rate was varied at ± 10%. Standard solution 60 µg/mL of Tolterodinetartarate was prepared and analysed using the varied flow rates along with method flow rate. The Temperature was varied (± 5^{o}C)

Standard solution 40 µg/mL of Tolterodinetartarate was prepared and analysed using the varied flow rates along with method flow rate.

RESULTS AND DISCUSSION

Specificity: There is no interference of mobile phase, and placebo with the analyte peak and also the peak purity of analyte peak which indicate that the method is specific for the analysis of analytes in their dosage form. The results were shown in table 2.

Table 1: Chromatographic conditions

Column	Phenomenex Luna C18 column (250x4.6mm 5µm)
Mobile phase	Methanol: Phosphate buffer (80:20 v/v)
Detection wavelength	220 nm
Flow rate	1.0 ml/min
Injection volume	20µl
Column temperature	Ambient
Run time	8 min
Retention time	2.95 min
Elution mode	Isocratic

Table 2: Specificity Data

S.No	Peak Name	Observation	
1	Blank	Nil	
2	Placebo	Nil	
3	Standard	R_t: 2.95 min	λ max: 220 nm

System suitability: System suitability test was an integral part of method development and has been used to ensure adequate performance of the chromatographic system. The data was shown in table 3.

Table 3: Results of System Suitability

Parameter	Result	Acceptance Limit
Retention time (Rt)*	4.12 min	Less than 2
Resolution factor*	NA	--
Number of theoretical plates (N)*	3785	More than 2000
Tailing factor (T)*	1.35	Less than 2
* Number of injections: 6 replicates		

Linearity: Each aliquot was individually injected in to the chromatographic system and peak area was measured. Plot a graph of peak area versus concentration (on x – axis concentration and on y axis peak area) and the correlation was calculated. The results were given in table 4.

Table 4: Linearity

S. No	Concentration (µg/mL)	Peak Area
1	15	284852
2	30	562451
3	45	855471
4	60	1142548
5	75	1458752
6	90	1756248

Accuracy: Accuracy of the method was determined by Recovery studies. To the formulation (pre analyzed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%.The results were tabulated in table 5.

Table 5: Results of accuracy

Spiked Concentration (µg/ml)	Peak area	Amount added (µg/ml)	Amount Found (µg/ml)	Recovery	% Mean Recovery
50%	284852	15.12	14.98396	99.10024	99.40048
	284751		14.97864	99.0651	
	287542		15.12546	100.0361	
100%	562451	30.21	29.58639	97.93574	98.02489
	562451		29.58639	97.93574	
	563987		29.66718	98.20319	
150%	855471	45.14	45	99.68985	99.25581

Table 6: Intraday and Inter day precision results

S.No.	Intraday precision Area	Inter day precision Area
1	562451	568542
2	562152	564254
3	568745	568952
4	579854	584525
5	565421	559584
6	578542	557485
Mean	569527.5	567223.7
StdDev	7186.194	8813.686
%RSD	1.261782	1.553829

Table 7: Results of LOD & LOQ

S.No	Parameter	Slope	Standard Deviation	Value (µg/ml)
1	Limit of Detection	19682	7186	1.204
2	Limit of Quantification			3.651

Robustness: This method is robust for the analysis of tolterodine tartrate within the specified range of deviations in the experimental conditions. The results were shown in table 8(a) &8(b).

Table 8(a): Change of Flow rate (± 0.1mL)

S.No	Robust condition	0.9ml/min	1ml/min	1.1ml/min
1	Flow Rate	562451	558452	562451
2		563241	558542	562458
3		568452	568524	574521
4	Mean	564714.6667	561839.3	566476.7
5	Stddev	2662.301052	4726.916	5688.203
6	% RSD	0.471441811	0.841329	1.004137

Table 8(b): Change in Temperature (± 5°C)

S.No	Robust condition	30°C	35°C	40°C
1	Temperature	558562	568545	548562
2		568952	568524	562545
3		568542	557852	562548
4	Mean	565352	564973.7	557885
5	Stddev	4804.171798	5035.786	6592.357
6	% RSD	0.849766481	0.891331	1.181669

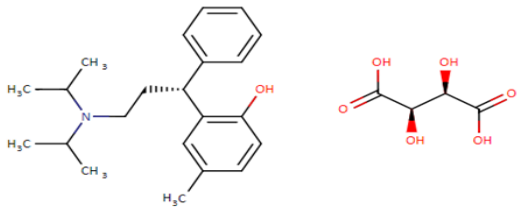

Fig 1. Chemical Structure of Tolterodine tartrate

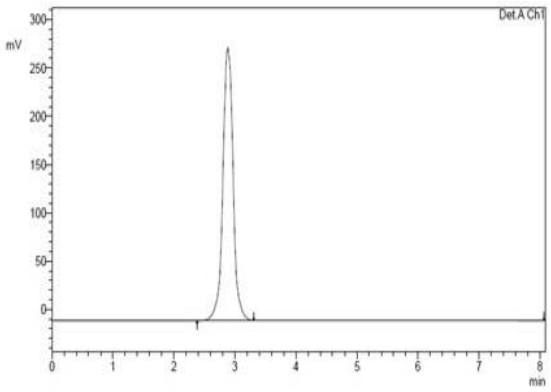

Fig 2: Optimized chromatogram of Tolterodine tartrate

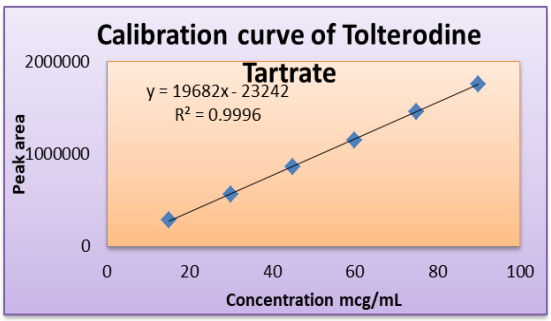

Fig 3: Calibration curve of Tolterodinetartate

DISCUSSION

A new method was established for estimation of Tolterodine tartrate by RP-HPLC method. The chromatographic conditions were successfully developed for

the separation of Tolterodine tartrate by using Phenomenex Luna C_{18} column 250x4.6mm 5µm, flow rate was 1.0 ml/min, mobile phase ratio was Methanol: 0.01m $NH_4H_2PO_4$ (80:20 %v/v), detection wavelength was 220 nm. The retention time was found to be 2.95 mins. The % purity of Tolterodine tartrate was found to be 100.77% respectively. The system suitability parameters for Tolterodine tartrate such as theoretical plates and tailing factor were found to be more than 2000 and less than 2 respectively. The linearity study for Tolterodinetartratewas found in concentration range of 15µg-90µg/ml and correlation coefficient (r^2) was found to be 0.9996, % recovery was found to be 98.02%, % RSD for repeatability was 1.261, % RSD for intermediate precision was 1.553 respectively. The precision study was precise, robust, and repeatable. LOD value was 1.204, and LOQ value was 3.651 respectively.

CONCLUSION

The developed method was validated as per ICH guidelines and was found to be within the prescribed limit. It concludes that the developed method was simple, selective, precise , accurate and robust. Hence the suggested RP-HPLC method can be used for routine analysis of Tolterodine tartrate in API and pharmaceutical dosage form.

REFERENCES

[1] Breaux and Jones, Understanding and implementing efficient analytical methods development and validation. J off pharmatech 2003; 5:110- 114.

[2] Indian pharmacopeia 2016, Vol 2.

[3] VinaySexana et al., Stability indication HPLC determination of tolterodinetartate in pharmaceutical dosage form. Ind J of chem tech 2006; 13: 242-246.

[4] SupriyaMhamunkar et al., RP-HPLC method development and validation for the simultaneous estimation of Tamsulosin HCL and Tolterodine Tartrate in pharmaceutical dosage form. IntJ of Pharm and PharmaSci 2012; 4 (5): 319-322.

[5] Lakshminarayanan B et al., Development and Validation of RP-HPLC Method for the Quantitative Estimation of Tolterodine Tartrate in Capsule Formulation. RGIJHS J PharmaSci 2013; 3(3): 132-135.

[6] S. Ashutosh Kumar et al., Method Development & Validation of Tolterodine Tartrate In Bulk As Well As In Pharmaceutical Formulation By Using RP-HPLC, Int J of Pharma and PharmaSci 2013; 5(3):175-180.

[7] Alok Paul et al., A Comparative Analysis of Detection of Tolterodine Tartrate with a HPLC-UV Method using Sodium-, Potassium-, and Ammonium Dihydrogen Phosphate Buffer in the Mobile Phase. Adv in Natural ApplSci 2012; 6(8): 205-208.

[8] Gowrisankar et al., UV spectrophotometric determination of tolterodinetartarate and cefepime. Asian J of Chem 2005; 17(3): 2028-2030.

[9] Ibrahim et al., Simple Spectrophotometric Methods for Determination of TolterodineTartrate in Pharmaceutical Forms. Int J of ChemTech Research 2015; 8(6): 665-669.

[10] ICH Q 2A, "Validation of analytical methods, definitions and terminology", ICH Harmonized tripartite guideline, (1999).

[11] Code Q2B, "Validation of analytical procedures; Methodology. ICH Harmonized tripartite guidelines. Geneva, Switzerland, 1996, PP 1-8.