Available Online through
www.ijpbs.com (or) www.ijpbsonline.com

IJPBS |Volume 2| Issue 4 |OCT-DEC |2012|332-337

Research Article
Pharmaceutical Sciences

DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR THE ESTIMATION OF TOLTERODINE TARTRATE IN TABLETS

N. RAMATHILAGAM[1], M.MEERADEVI[2] P.SOLAIRAJ[2], S.C. RAJESH[2]*

[1]Department of Pharmaceutical Analysis, Smt Sarojini Ramulamma College of Pharmacy, Mahabubnagar, Andhrapradesh

[2]Sankaralingam Bhuvaneshwari College of Pharmacy, Anaikuttam, Sivakasi-626130, Tamilnadu, India.

Corresponding Author Email: rtthilagam@gmail.com

ABSTRACT

A simple, specific, robust, accurate and precise isocratic HPLC method has been developed and subsequently validated for simultaneous determination of Tolterodine in pharmaceutical dosage forms. Kromosil C18 (250X4.6) mm 5µ with flow rate of 1ml/ min by using HPLCJASCO PU-1580 and UV/VIS JASCO UV-1570 at 281 nm. The separation was carried out using a mobile phase consisting of Acetonitrile: Methanol: Ammonium acetate buffer (pH 3.0) in the ratio of 30:30:40 respectively. The retention time for Tolterodine was found to be 4.99 min. The correlation coefficient was found to be 0.9991. The mean percentage recovery was found to be 102.65. The % estimation of the drugs was found near to 100 % representing the accuracy of the method. The proposed method was also validated and applied for the analysis of drugs in tablet formulation.

KEYWORDS

HPLC, Tolterodine, method development, validation.

INTRODUCTION

Tolterodine tartrate is chemically (R)-2-[3-[bis(1-methylethyl)-amino]1-phenylpropyl]-4-methylphenol [R-(R,R)]- 2,3 di hydroxy butanedioate, is muscarinic receptor antagonist[1-2]. Literature survey revealed HPLC reported for the determination of stability indicating studies and enantiomer impurities, Spectrophotometric method reported for the determination of coupling reaction and mass Spectrophotometric method determination of tolterodine tartrate in human plasma were reported for individual determinations of Tolterodine, and in their combination of Tolterodine and Tamsulosin but there is no work in HPLC method for the estimation of tolterodine individual dosage form. The proposed method presented here is simple, fast, accurate and precise and can be used for the determination in tablet dosage forms [3-8]. The method was validated as per ICH guidelines [9-17].

INSTRUMENT

Instrument used in present study was JASCO. HPLC. The pump used was JASCO PU-1580 pump. The samples were applied Kromosil C18 (250X4.6 mm) 5µ column with Rhedyne injector. The sample was performed using UV/VIS JASCO UV-1570 detector with flow rate 1ml/min and operated by JASCO LC –NeT II/ADC interface. The Shimadzu electronic balance (0.001gm sensitivity) was used for weighing purpose.

MATERIALS

Tolterodine raw material was supplied by micro labs. Hosur. TOLTER (Zydus) was taken for study which contains Tolterodine-4mg. Methanol (Merck Ltd., Mumbai, India) and HPLC grade acetonitrile (Molychem, mumbai), ammonium acetate, glacial acetic acid (Finar chemicals, Ahmadabad and HPLC grade water.

Preparation of mobile phase

Acetonitrile, methanol and ammonium acetate buffer (pH 3.0) in the ratio of 30:30:40 were

mixed, sonicated for 10 minutes and filtered through the membrane filter of micron 0.45µ.

Preparation of standard solution

10 mg of Tolterodine were dissolved in methanol by sonication and makeup to 10 mL. The working standard solution concentration was prepared 20µg/mL.

Preparation of sample solution

A quantity of tablet powder equivalent to 0.159 g was accurately weighed and transferred to 100 mL volumetric flask, dissolved in few mL of methanol, sonicated for 15 min and made up to the volume by mobile phase acetonitrile, methanol and ammonium acetate buffer (pH 3.0) in the ratio of 30: 30:40. This will give the concentration of 20 µg of Tolterodine per mL solution of tablet sample.

METHOD DEVELOPMENT

Selection of wavelength

Stock solutions of 10mg/mL were prepared for each drug in methanol and further diluted to get the concentration of 10µg/mL for Tolterodine was prepared with methanol. The wavelength was selected by scanning the above standard drug between 200 to 400 nm. The scanned results showed that reasonably maximum absorbance was recorded at 281 nm; therefore 281 nm was selected as the detection wavelength for the HPLC investigation. [**Figure1**].

METHOD

The sample was applied Grace Vyadc C18 (250×4.6mm) 5µ column with Rheodyne injector in reverse saturation mode using acetonitrile, methanol and ammonium acetate buffer (pH 3.0) in the ratio of 30: 30:40 as mobile phase with flow rate of 1.0 mL/min. The sample was performed using UV/VIS JASCO UV-1570 detector with flow rate 1ml/min. The instrument computes accurate results within minimal time. The retention time was obtained 4.99 for Tolterodine respectively. The results of assay were reported in the **Table 1** and **Figure 2**.

VALIDATION OF THE METHOD

Accuracy

Accuracy was determined by tablet samples with different known concentrations of the drug (50%, 100% and 150%). Each concentration was injected in six times and the assay was performed as per the developed method. From this % recovery and the amount present or recovered were calculated. Results of recovery study are reported in **Table 2**.

Precision

Standard solution of tolterodine was prepared in same manner for the standard preparation. This solution containing 20 µg/mL of tolterodine. The repeatability was performed for six times. Results of precision was reported in **Table 3**.

Linearity

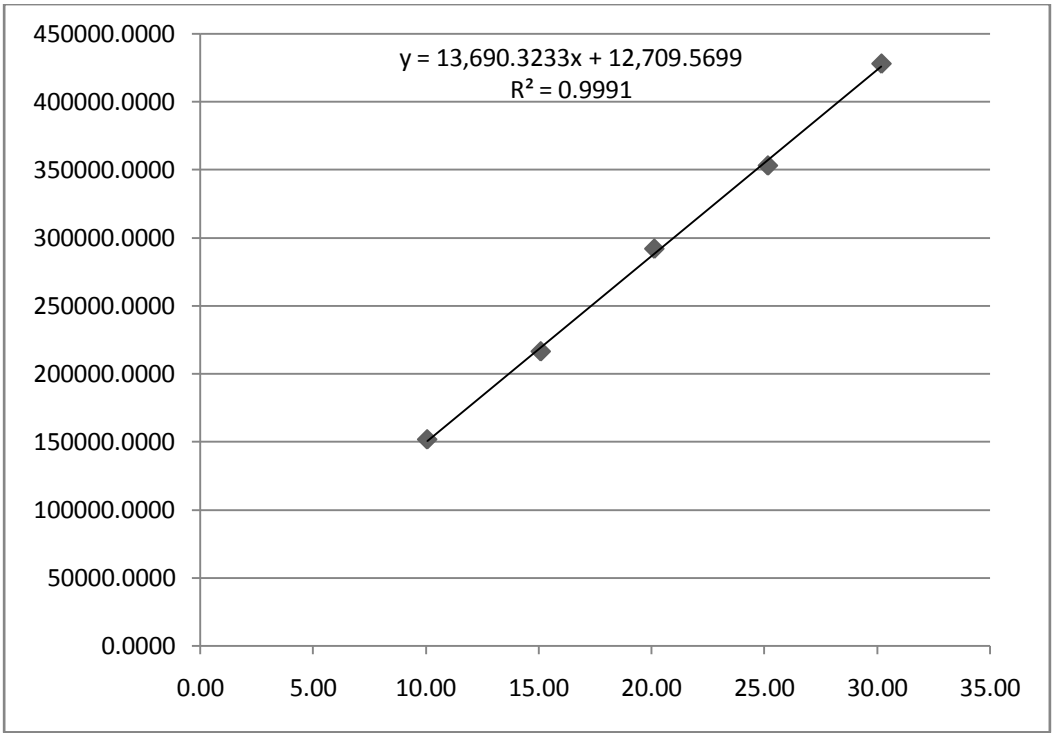
Linearity was determined in the range of 50-150 % (50, 75, 100, 125 and 150%) targeted concentration of assay procedure. five series of standard solutions containing 10, 15, 20, 25and 30 µg/mL of tolterodine was injected. Linearity of each- concentration and response ratio of each concentration was found. Linearity of each concentration is reported in **Table 4 and Graph 1**

Ruggedness

The above sample prepared solution and diluted to get the concentration of 20 µg of tolterodine per mL of tablet sample. From this 20 µL was injected through column separately by two different analysts in the same HPLC system and same column. The result was reported in a **Table 5**.

Robustness

The above prepared standard was determined by the variation of flow rate and variation of wavelength. The result was reported in a **Table 6**.



Graph 1: Linearity chart of standard Tolterodine

Table 1: Results of assay

Name of the drug	Amount present per tablet(mg)	% of assay
Tolterodine tartrate	4	102.5

Table 2: Results of recovery studies

Name of the drug	Amount taken (µg)	Amount found (µg)	% recovery	% of mean recovery
Tolterodine tartrate	2	2.1	102.53	102.50
	4	4.1	102.65	102.65
	6	6.1	101.78	101.78

Table 3: Results of precision study

Injection No.	Retention time	Peak area
1.	5.042	290530.000
2.	5.033	291429.000
3.	5.033	291491.863
4.	5.042	292408.791
5.	5.033	292771.250
6.	5.042	292623.500
Avg	**5.0375**	**291150.2877**
SD	**0.0049**	**538.1036**
%RSD	**0.10**	**0.18**

Table 4: Results of linearity studies of standard tolterodine

Concentration in µg/mL	Mean peak area
10	151773
15	216431
20	291875
25	352982
30	427836

Table 5: Ruggedness data of analyst – I and II

Analyst	Mean of peak area	%RSD	Recovery Quantity	% Recovery
Analyst-I	291150	0.21	4.106	102.65
Analyst-II	283982	0.04	4.114	102.86

Table 6: Robustness study

Parameters	Variations			
	Flow rate at 0.8ml\min	Flow rate at 1.2ml\min	Wavelength at 236 nm	Wavelength at 240 nm
Retention Time	6.233	4.417	5.308	5.317
Peak area	359498	328262	377228	399867

Figure 1: UV spectra of standard tolterodine

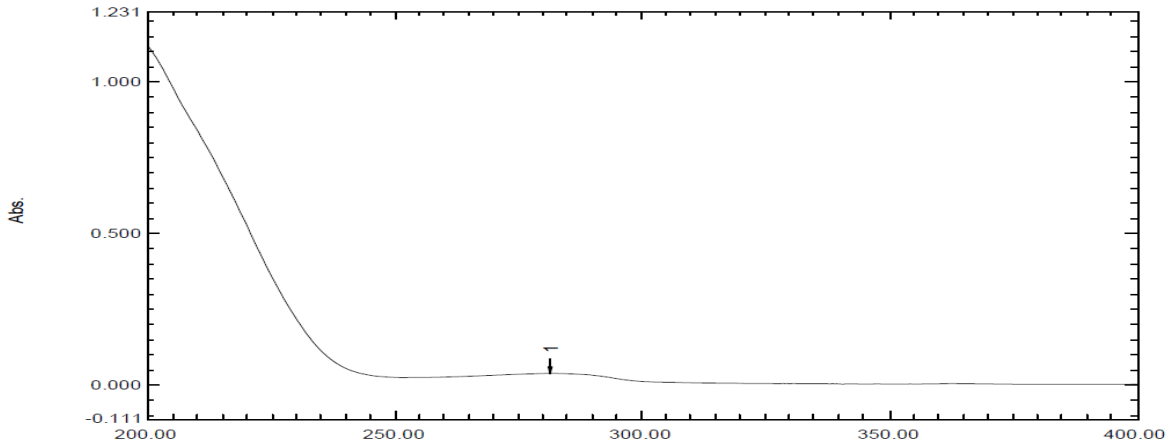
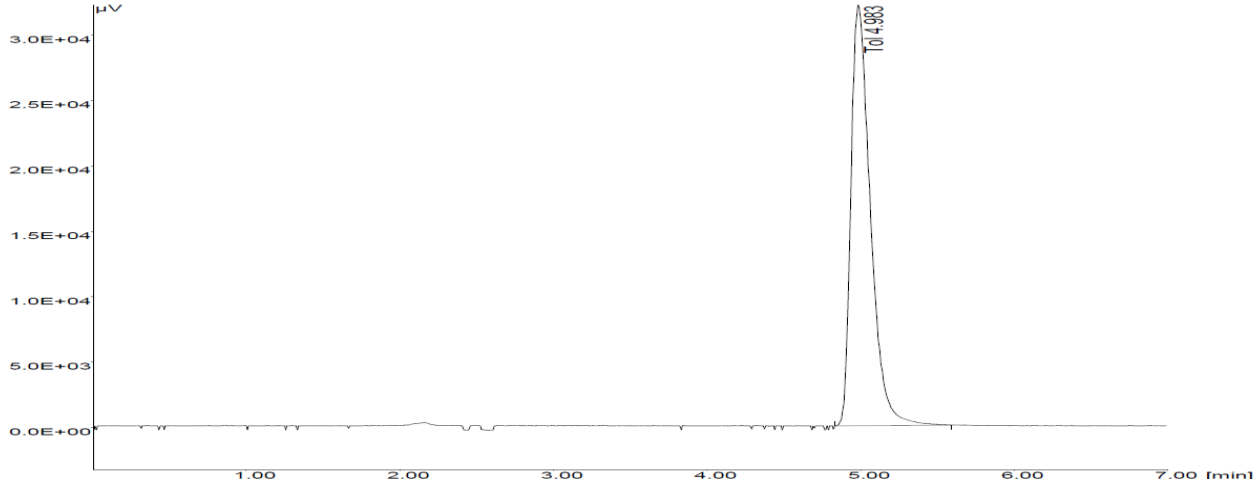


Figure 2: Assay chromatogram of Tolterodine



RESULT AND DISCUSSION

The present study was aimed to developing an accurate precise and linear HPLC method for analysis of tolterodine and in pharmaceutical dosage form as per CH guidelines. Tolterodine showed the linearity response over range 10, 15, 20, 25and 30µg/mL. The correlation coefficient for the drug was found to be 0.9991. The recovery studies of these drugs were found to be 102.65. The precision % RSD was found to be 0.18. The raggedness and robustness were studied with replicates standard solution of the drug and the result was found to be acceptance criteria.

CONCLUSION

The proposed method gives good resolution of tolterodine within short analysis time (7 min). The validation parameters are validated and the results were complied with in the ICH guidelines. The method is very simple, specific, robust, accurate, rapid and precise for the determination of tolterodine and tablet dosage form. Therefore the method can be used in routine quality control analysis of the drug.

ACKNOWLEDGEMENT

The authors are thankful to Micro Lab. Pvt. ltd, Bangaloru for providing a gift samples, the authors are also thankful to Department of pharmaceutical analysis, Sankaralingam Bhuvaneshwari college of pharmacy, The Tamilnadu Dr. M.G.R University, sivakasi, Tamilnadu for encouragement.

REFERENCES

1) CIMS October-January (2011-2012) Tolterodine, page no: 358.
2) United States Pharmacopoeia-National Formulary, (24th) Asian Edition. USA: The United States Pharmacopoeia Convention Inc; (2000). p. 1428-1435.
3) Ravindra Kumar, Y., Ramulu, G., Vevakanand, V.V., Gopal Vaidyanathan, Keesari srinivas, Kishore Kumar, M., Mukkanti, K., Satyanarayana Reddy, M., Venkatraman, S. and Suryanarayana, M.V. (2004). A validated chiral HPLC method for the enantiomeric separation of tolterodine tartarate. Journal of Pharmaceutical and Biomedical Analysis 35 (5):1279-1285.
4) Beibei Zhang, Zunjian Zhang , Yuan Tian and Fengguo Xu. (2005). High performance liquid chromatography-electrospray ionization mass spectrometric determination of tolterodine tartrate in human plasma. Journal of Chromatography B 824(1-2): 92-98.
5) Saxena, Vinay Zaheer, Zahid Farooqui, Mazhar. (2006). Stability-indicating HPLC determination of tolterodine tartrate in pharmaceutical dosage form. Indian Journal of Chemical Technology 13(3):242-246.
6) Xia, Z. L., Chen, Zh. Y. and Yao, T. W. (2007). An enantiospecific HPLC method for the determination of (S)-enantiomer impurities in (R)-tolterodine tartarate. Pharmazie 62(3):170-173
7) Mohammed Ishaq, B., Vanitha Prakash, K., Manjula, B., Hari Kumar, C. and Usha Rani, G. (2010). New aurum coupling reaction for visible spectrophotometric determination of tolterodine tartrate in pharmaceutical preparations. International Journal of Chemical and Analytical Science 1(7): 165-167.
8) Mandava, V., Basavasewara Rao, Bhumireddy Chennakesava Reddy, Tumati Srinivas Rao, Jamini

Ranjan Mohanty, (2010).Determination of tamsulosine hydrochloride 0.2% and tolterodine tartrate 0.2% Combination Pellets by RP HPLC method. Research Journal of Pharmaceutical, Biolo gical and Chemical Sciences 1(1):136-140,

9) United States Pharmacopeia XXII (United States Pharmacopeia Convention, Rockville, MD, 1990)
10) Validation of Compedial Assay- Guidelines' Pharmacopieal Convention, Rockville, MD, 1985.
11) ICH guidelines for validation of analytical procedures: text and methodology, 1995, p.p1-15.
12) USP 25-NF 20 (United States Pharmacopieal convention, Rockville, MD, 2002), p.p 2256.
13) FDA, 'Analytical Procedures and Methods Validation: Chemistry, Manufacturing, Controls', Federal Register (Notices) 65 (1690, 52, 776, -52,777 (August 2000).
14) International Conference on Harmonization; Draft Guidance on Specifications: Test Procedures and Acceptance Criteria for New Drug Substance and Products: Chemical Substance, Federal Register (notices) 65, (251), 83041-83063 (2000).
15) Asian Guideline for Validation of Analytical Procedure Adopted from ICH guideline, Q2A27 Oct. 1994 and ICH Q2B, 6th Nov. 1994.
16) Guideline for Industry Text on Validation of Analytical Procedures, ICH Q2A, 1985, p.no.1-7.
17) Guidance for Industry Q2B Validation of Analytical Procedures: Methodology, 1996, P. no. 1-10.

***Corresponding Author:**
N. RAMATHILAGAM*
Department of Pharmaceutical Analysis,
Smt Sarojini Ramulamma College of Pharmacy,
Mahabubnagar, Andhrapradesh.
E.Mail: rtthilagam@gmail.com