International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Original Article **CODEN: IJPNL6**

DEVELOPMENT OF A LIQUID CHROMATOGRAPHIC METHOD FOR PHARMACOKINETIC STUDIES OF TOLTERODINE TARTRATE ORODISPERSIBLE TABLETS IN RATS

Y Padmavathi*, Sumedha Kapre, K Latha, Renuka Menda, Rohini Kagithala

Department of Pharmaceutical Analysis and Quality Assurance, G. Pulla Reddy College of Pharmacy, Osmania University, Mehdipatnam, Hyderabad. Telangana, India.

***Corresponding author e-mail:** ypadmavathi@gprcp.ac.in

Received on: 18-05-2016; Revised on: 24-06-2016; Accepted on: 20-08-2016

ABSTRACT

A selective and high throughput reverse phase liquid chromatographic method was developed for studying pharmacokinetics of tolterodine tartrate (TT) orodispersible tablets (ODT) in rats. Quantification is achieved by the peak-area ratio method with reference to the internal standard. Metaxalone was used as internal standard. After liquid- liquid extraction the analyte and the internal standard were chromatographed on Enable 18H (5µ, 250×4.6mm) C18 column. Mobile phase used consists of phosphate buffer pH 3 and acetonitrile (55:45 %v/v). The elute was monitored with the UV–VIS detector at 280 nm with a flow rate of 0.8 mL/min. The peaks of the drug and IS were obtained at retention times of 6.74 min and 10.5 min respectively. The method was validated according to USFDA guidelines. The absolute recovery of analyte was consistent and reproducible. The method was successfully applied to study the pharmacokinetics of tolterodine tartrate orodispersible tablets in rats.

KEYWORDS: Tolterodine tartrate, Orodispersible tablets, Validation, USFDA

INTRODUCTION

Diabetes is a long-term condition often referred by doctors as diabetes mellitus, describes a group of metabolic diseases in which the person has high blood glucose, either because insulin production is inadequate or because the body's cells do not respond properly to insulin or both. Patients with high blood glucose will typically experience polyuria (frequent urination). Most common problem faced by diabetic (type 1, type 2, gestational diabetes) patients is urinary incontinence. Hence the treatment regimen for diabetis includes anti muscarinic drugs to subside the side effect called polyuria.

Tolterodine tartrate (TT) is a competitive, specific, muscarinic receptor antagonist with selectivity for the urinary bladder. Anti- muscarinic drugs block the action of a chemical messenger acetylcholine which send signals to brain that trigger abnormal bladder contractions associated with overactive bladder resulting in decrease in the frequency of incontinence episodes, the number of voids per day, and the number and severity of urgency episodes. It is rapidly and completely absorbed from the gastro intestinal tract and peak plasma concentration reaches at 0.5–2.5 hours for oral dispersible tablets. After oral administration, tolterodine is metabolized by cytochrome P-450 in the liver, resulting in the formation of 5-hydroxymethyl tolterodine (5-HMT), the major pharmacologically active metabolite, which exhibits an anti-muscarinic activity similar to that of tolterodine, contributes significantly to the therapeutic effect. [1-4]

Literature review revealed that several spectrophotometric, HPLC and LC-MS/MS methods have been reported so far for determination of tolterodine tartrate alone and its combination with other drugs.[5-17] There are very few methods

available for estimation of tolterodine tartrate in biological samples.[18,19] This paper describes the development and validation of a liquid chromatography method using UV detector for the pharmacokinetic studies of tolterodine tartrate oral dispersible tablets in rats. The structure of tolterodine tartrate is shown in figure 1.

MATERIALS AND METHODS

Chemicals and Reagents: Tolterodine tartrate and metaxalone were the gift samples from Micro labs, Bangalore and Aurobindo pharmaceuticals, Hyderabad respectively. HPLC grade acetonitrile, water and potassium di-hydrogen ortho phosphate were procured from S.D. fine chem. ltd (Mumbai, India). All other chemicals and solvents used were of analytical grade. The mobile phase and all the solutions were filtered through a 0.22µm cellulose acetate filters (Sartorius) prior to their use.

Instruments: A gradient high performance liquid Chromatograph (SHIMADZU, Japan), HPLC pump LC-20AT, UV-visible detector SPD-20A and Enable-18H C18 column was used (250 x 4.6 mm, 5 µm). Data collection and integration were accomplished using LC Solutions, 1.25 version software. Other instruments used in the method development and validation, include vortex mixer (Model VX-200, Labnet International Inc., USA), sonicator (PCI Analytics 6.5 li200H), refrigerated centrifuge (Model C-24 BL, Remi, India) and deep freezer (Model BFS-345-S, Celfrost Innovations Pvt. Ltd., India). pH meter (Systronic digital pH meter 335) was used for measuring pH of all buffer systems.

Experimental Animals: Male albino Wistar rats (180-200 g) were obtained from National Institute of Nutrition, Hyderabad. The Institutional Animal Ethics Committee, G. Pulla Reddy College of Pharmacy, Hyderabad, India has approved the animal experimental protocol. (Registration No: 320/CPCSEA; Date 03/01/2001; IAEC Meeting No: 01/2014, Date: 10/2/2014; GPRCP/IAEC/10/14/ 2PAL/AE-1-RATS-M-15.)

Chromatographic Conditions: A reverse phase Enable-18H C18 column (250 x 4.6 mm, 5 µm) equipped with a guard column of same packing material was used for the study. The isocratic mobile phase consisted of an aqueous phase (10 mM phosphate buffer, pH 3.0) and acetonitrile (55:45 *v/v*). The HPLC system was stabilized for 1 h at 0.8 mL min^{-1} flow rate, through baseline monitoring prior to actual analysis. TT detection was monitored at a wavelength of 280 nm. An injection volume of 20 µL was optimized for final method. All analyses were performed at room temperature.

Method development

Preparation of standards and quality control samples: Standard stock solutions of tolterodine tartrate and metaxalone were prepared in acetonitrile (1mg/mL). Working standard solutions in the required concentration range were prepared by appropriate dilution of their stock solutions in the mobile phase. The calibration standards and quality control (QC) samples were prepared by spiking blank plasma with working standard solutions. Calibration standards were made at concentrations of 50, 100, 200, 400, 600, 800, 1000 and 1200 ng/mL and a working internal standard solution was prepared in 5 µg/mL. Quality control samples (QCs) were prepared at concentrations of 75 ng/mL, 500 ng/mL and 1100 ng/mL respectively. Aliquots of pooled plasma samples were taken in micro-centrifuge tubes and stored at −20 °C.

Selection of wavelength: Tolterodine tartrate solution was prepared in the mobile phase and was scanned in UV range from 200 to 400 nm. The wavelength at which maximum absorbance obtained was selected.

A liquid chromatographic method was developed by optimizing separation mode, mobile phase solvents - buffer and its pH, flow rate and type of column. Buffer and its strength play an important role in deciding the peak symmetries and separations. Different trials were performed using buffers and the best results were obtained using phosphate buffer (potassium dihydrogen ortho phosphate). Appropriate and desired peaks were obtained by using phosphate buffer of 0.05 M strength. Time taken for the elution of drug (retention time) is greatly influenced by the flow rate. Trials were performed to select an appropriate flow rate depending upon the elution of the drug from the column. Pooled plasma was used for spiking throughout the method development and validation studies.

Validation of analytical method: The method validation was performed as per the USFDA guidelines. [20] The method was validated for linearity, precision, accuracy, selectivity, recovery, and stability of analyte solutions.

Formulation Details: Oral dispersible tablets of 30mg weight containing 2 mg of tolterodine tartrate were prepared by direct compression technique in a tablet-punching machine using 4.7 mm round shape punch. [21]

Application of the method for pharmacokinetic study: Pharmacokinetic studies were carried out in male Wistar rats. The developed RP_HPLC method was used for analysis of rat plasma samples for determination of concentrations of tolterodine tartrate. The protocol was approved by the Institutional Ethical Committee at G. Pulla Reddy College of Pharmacy, Telangana, India. The experiments were conducted as per CPCSEA guidelines. The rats weighing 180–200 g were housed with free access to food and water, except for the final 12 h before experimentation. Rat dose was calculated according to their body weights from human adult dose. After a single oral administration of 40 µg of tolterodine tartrate (ODT), 0.5 mL of blood samples was collected from the retro orbital puncture at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5 and 6 h time points into disodium EDTA vials. The blood samples were immediately centrifuged (1000 rpm) for 10 min at cooling temperature. The supernatant plasma layer was separated and stored at -20^{0}C until analyzed. The plasma samples were analysed for tolterodine tartrate concentrations as described above. Different pharmacokinetic parameters - rate constant, biological half life, C_{max}, T_{max}, volume of distribution, AUC^t_0, AUC^∞_t were determined using WinNolin 6.3 software and results are tabulated in table 7. [22-24]

Extraction from plasma: Since the objective of this study was to develop a simple reliable method that would facilitate analysis of tolterodine tartrate in rat plasma in a large number of samples over a relatively short period of time and in a cost effective manner, an efficient extraction procedure was established. The literature search revealed, liquid – liquid extraction procedures for extraction of tolterodine tartrate from rat plasma.

Plasma sample preparation: 90 µL of plasma sample was spiked with 10µl internal standard solution (5 µg/mL) and 100 µL of acetonitrile was added. The mixture was vortexed for 1 minute. Centrifuged at 3000 rpm for 10 minutes at 4 ^{0}C. The organic layer was separated & filtered through a 0.2 µm cellulose acetate filter. The organic layer was evaporated on a vacuum evaporator at about 60 °C. The residue was reconstituted in 5 mL Acetonitrile and 20 µL was injected onto the HPLC column.

RESULTS AND DISCUSSION

Method development: Chromatographic analysis of the drug and IS was initiated under isocratic conditions with an aim to develop a simple separation process with a short run time. Separation was tried using various combinations of acetonitrile and phosphate buffer with varying contents of each component on two columns like C8 and C18, to get a good chromatographic separation with the desired response. Tolterodine tartrate being a polar drug, it can be easily separated by RP-HPLC. Therefore RP-HPLC was selected as the mode of separation. Generally longer columns provide better separation due to higher theoretical plate numbers. Therefore a C18 column with a particle size of 5µm and internal diameter of 4.6 mm was selected. For this method, 0.8 ml/min flow rate was optimized, where the retention time of tolterodine tartrate and internal standard metaxalone were 6.8 min and 10.5 min respectively.

TT and IS were well separated with the mobile phase consisting of phosphate buffer and acetonitrile (55:45, v/v), having pH around 3.0. The best resolution and sensitivity of the method was obtained at 280 nm with 0.8 mL/min flow rate of the mobile phase. The wavelength for UV detection of TT and IS was selected from the absorption spectrum taken in the mobile phase. The UV spectrum of TT is shown in figure 2.

For optimum detection and quantification of tolterodine tartraate with IS in rat plasma, it was necessary to adjust not only the chromatographic conditions, but also an efficient extraction method which gives consistent and reproducible recovery of analytes from plasma. The drug and internal standard were extracted from the plasma by liquid-liquid extraction method using acetonitrile.

Selectivity: Selectivity towards endogenous and exogenous plasma matrix components was assessed in rat plasma samples by analyzing blank and spiked samples at LLOQ level. It was performed in two sets, in the first set, plasma sample was extracted and directly injected for LC detection and in the second set, blank plasma spiked with LLOQ working solution of TT and IS were extracted and analyzed. The respective chromatograms are shown in figure 3 and 4. Chromatogram of a mixture of TT and IS revealed that they are well separated under LC conditions applied. Retention time was 6.74 min for TT and 10.5 min for IS. The absence of interference of plasma components around the zone of retention time of the TT and IS can be seen from the figures.

Limit of detection and limit of quantification: The limit of detection was 0.2 ng/mL at a signal-to-noise ratio of 3:1, and at the lower limit of quantification was 50 ng/mL with the precision and accuracy within 15%.

Linearity: The linearity of the method was determined by analysis of standard samples containing eight non-zero concentrations (50, 100, 200, 400, 600, 800, 1000 and 1200 ng/mL). The ratio of analyte to IS area versus analyte concentration was used for regression analysis. The lowest standard on the calibration curve was accepted as the limit of quantization (LOQ), if the analyte response was at least five times more than that of drug-free (blank) extracted plasma. The ratio of peak areas of tolterodine tartrate to IS at various concentrations of drug in plasma is shown in table 2. The eight – point calibration curve constructed was linear over the selected concentration range. (r^2 value 0.9994). Calibration curve is shown in figure 5.

Recovery studies: The recovery of an analyte is the extraction efficiency of an analytical process, reported as a percentage of the known amount of an analyte carried through the sample extraction and processing steps of the method. It was performed at LQC, MQC and HQC levels. The extraction efficiency of TT was determined by comparing the peak areas obtained from extracted quality control samples with the peak area of aqueous working solution containing the same concentration of TT at three levels. The solvent that extracted the maximum amount of the drug from plasma sample, was selected for processing of the samples. The recovery of the drug was found to be 92.245$\pm$0.6%, 83.6$\pm$1.3% and 77.2$\pm$1.7% in acetonitrile, methanol and di-chloromethane, respectively at all three concentration levels, which confirm the extraction efficiency of the solvents. Among the three solvents, acetonitrile showed the maximum amount of the drug recovered, hence acetonitrile was selected as the solvent for liquid-liquid extraction. The extraction recoveries of tolterodine tartrate from plasma at three concentrations (75, 500, and 1100 ng /mL) are shown in table 3.

Precision: The precision of the measurements was determined by using three quality control samples and the results are reported in table 4. It was determined by calculating the percent coefficient of variation (% CV) for each level. The deviation at each concentration level from the nominal concentration was expected to be within ±15.0%, except at LLOQ, for which it should be within ±20.0%. The coefficient of variation (CV) of the intra - day assay of the drug ranged from 3.39% to 7.16% and for the inter-day assay was from 4.8% to 12.2%. Inter-day as well as intra-day replicates of TT resulted in a CV value less than 12.2%, which revealed that the precision degree of the proposed method is very high.

Stability: Experiments were performed to evaluate the stability of the analyte in stock solutions and in plasma samples under different conditions. Stock solution stability was performed by comparing the area response of stability sample of analyte and internal standard with the area response of the sample prepared from fresh stock solutions. Freeze–thaw stability, short-term stability and solution stability were performed at LQC and HQC level using five replicates at each level. The quality control standards containing 50 and 1200 ng/mL (n=5) of TT were subjected to the detection of stability of the drug in plasma. The initial assay of the samples was conducted. All the samples were analysed at standard chromatographic conditions to determine their peak areas. The results are presented in table 6. The results revealed that the final concentration of the drug in each quality control sample at stability conditions, i.e. freeze–thaw conditions, 24 h storage and one month storage was found to be similar with initial concentration. The CV value (n=5) of the final concentration of the drug after storing the samples in all the stability conditions was found to be less than 15%. There was no significant degradation observed since the deviations in concentration were within 15% of their nominal values.

Pharmacokinetic parameters: Mean plasma concentrations were calculated by using the developed liquid chromatographic method. Mean plasma concentration time profile following a single oral administration of tolterodine tartrate orodispersible tablets to rats is given in figure 6. The pharmacokinetic parameters like rate constant, biological half life, C_{max}, T_{max}, volume of distribution, AUC^{t}_{0}, AUC^{∞}_{t} were determined using winNolin 6.3 software and results are tabulated in table 7.

CONCLUSION

The developed liquid chromatographic method for estimation of tolterodine tartrate in rat plasma is selective and rugged, suitable for routine analysis of tolterodine tartrate in biological samples samples. This method has significant advantages in terms of simple, reproducible liquid extraction procedure and a chromatographic run time below 10 min. The extraction method gave consistent and reproducible recoveries for analytes and IS from plasma, with minimum matrix interference. The established LLOQ is sufficiently low to conduct a pharmacokinetic study of the test formulation of tolterodine tartrate oro dispersible tablets.

ACKNOWLEDGMENT

The authors are grateful to the management of G. Pulla Reddy College of pharmacy, for providing the necessary facilities to carry out the research work. Authors are thankful to Micro las, Bangalore and Aurobindo pharmaceuticals Hyderabad, India for providing the gift sample of the pure drug tolterodine tartrate.

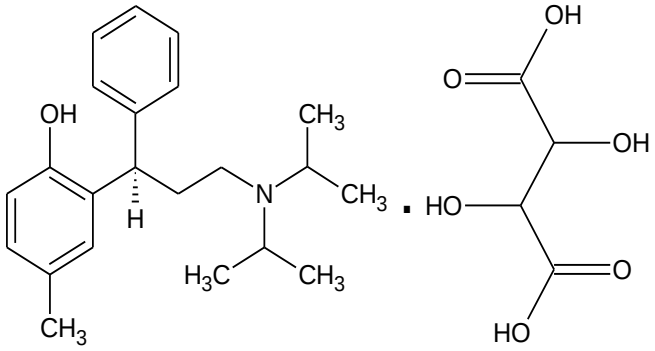

Fig. 1 Chemical structure of tolterodine tartrate

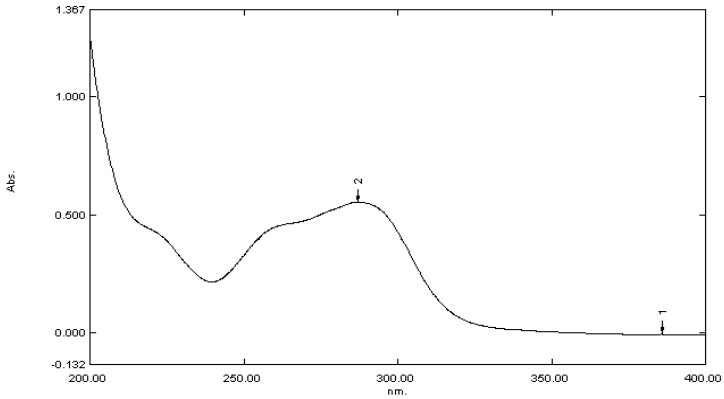

Fig.2. UV-spectrum of tolterodine tartrate

Table 1 Formula for preparation of tolterodine tartrate orodispersible tablets

S.no	Ingredients	Per tablet in mg
1	Magnesium stearate	0.5
2	Talc	0.5
3	Kyron	2
4	Tolterodine tartrate	2
5	Micro crystalline cellulose	Remaining

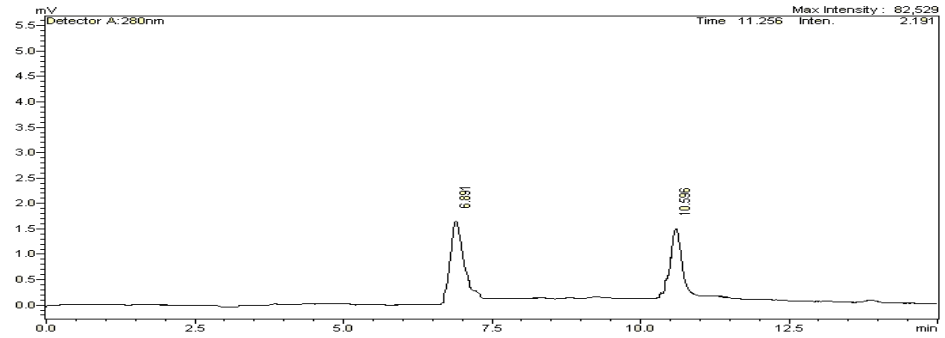

Fig.3. Chromatogram representing tolterodine tartarte and metaxalone in plasma

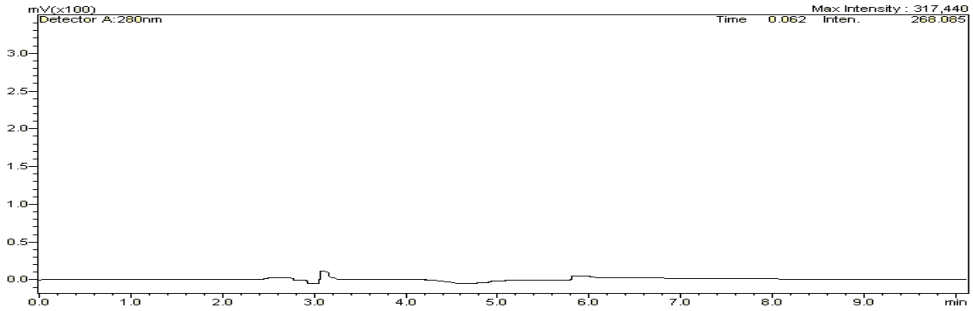

Fig. 4. Chromatogram of blank rat plasma

Table 2 Calibration data of tolterodine tartrate in rat plasma

Concentration (ng mL^{-1})	Mean peak area ratio*
50	0.29
100	0.32
200	0.36
400	0.45
600	0.54
800	0.63
1000	0.71
1200	0.79

**Average of five determinations (n=5)*

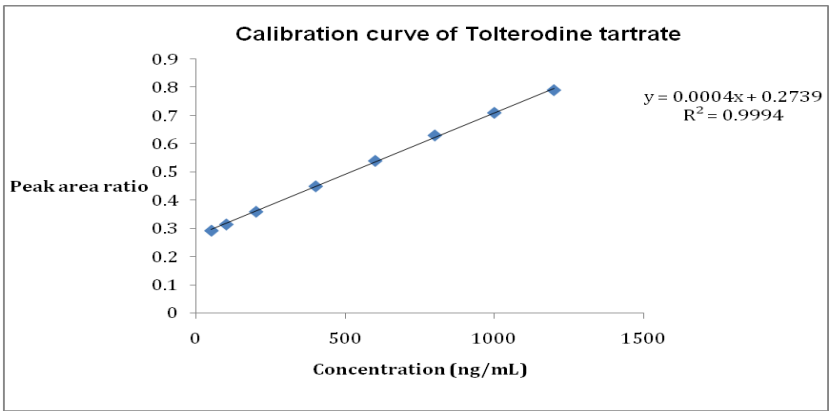

Fig.5 Calibration curve of tolterodine tartrate in plasma

Table 3 Recovery of tolterodine tartrate from rat plasma

Added concentration (ng/mL)	Mean detected concentration* (ng/mL)	Recovery (%)
75	70.4866	86.465
500	503.4	98.15
1100	1094.427	92.12

**Average of five determinations (n=5)*

Table 4 Precision of tolterodine tartrate in rat plasma

Spiked Concentration (ng mL^{-1})	Intra-day		Inter-day	
	Mean (ng mL^{-1})	RSD (%)	Mean (ng mL^{-1})	RSD (%)
50	42.874	7.16	22	12.2
75	68.6837	6.699	38.15	8.11
500	472.522	22.098	476.775	6.83
1100	1052.63	3.39	1035.25	4.8

Intra-day: n=5; Inter-day: n=3 days with 5 replicates per day; values were represented as ± SD mean

Table 5 Repeatability data

Accuracy level	Concentration (ng/mL)	Mean Peak area ratio*	Mean calculated concentration* (µg/mL)	Standard deviation	%CV
LOQ	50	0.291	42.874	3.0715	7.16
LQC	75	0.30137	68.6837	4.60115	6.699
MQC	500	0.463	472.522	22.098	5.3
HQC	1100	0.695	1052.63	35.787	3.39

**Average of five determinations (n=5)*

Table 6 Stability of tolterodine tartrate in rat plasma

Stability	Spiked concentration (ng mL^{-1})	Mean detected concentration (ng mL^{-1})	RSD (%)
Freeze-thaw[a]	50	38.376± 5.138	13.39
Freeze-thaw[a]	1200	1181.63± 140.6	11.9
Short term[b]	50	43.68± 4.004	9.16
Short term[b]	1200	1139.68± 102.73	9.01
Stock solution[c]	50	41.28± 1.682	4.07
Stock solution[c]	1200	1184.517± 44.764	3.78

[a] After three freeze (-20^{0}c) and thaw (room temperature) cycles; [b] After 24 hours of storing at room temperature; After 6 hours of storing at room temperature; Values are expressed as mean ± SD (n=5)

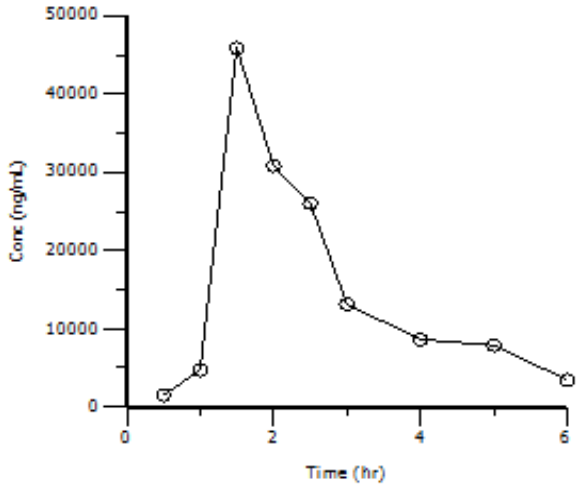

Fig.6 Mean plasma concentration–time profile of tolterodine tartrate in rats after oral administration of 0.24 mg kg^{-1}. Values were expressed as mean ± SD.

Table 7 Pharmacokinetic parameters of tolterodine tartrate oro dispersible tablets in Wistar Rat after oral administration of 0.24 mg Kg^{-1} body weight

Parameter	Result
Biological half life	1.71 hrs
C_{max}	45.79 µg/mL
T_{max}	1.5 hrs
V_d	6468.5 µL
AUC_0^t	0.082695 mg.hr/mL
$AUC_{t-\infty}$	0.091302 mg.hr/mL

C_{max}= peak serum concentration; T_{max}= time to reach peak concentration; AUC_{0-t}= area under the curve from zero to t; $AUC_{0-\alpha}$= area under the curve from zero to infinite; V_d= Volume of distribution;

REFERENCES

1. Indian pharmacopoeia, volume III, Indian pharmacopoeia commission, Ghaziabad, 2014: 2240-2241.
2. Drug bank of tolterodine tartrate, http://www.drugbank.ca/drugs/DB01036
3. Material Safety Data Sheet of tolterodine tartrate 1-9, Datasheets.scbt.com/sc-04708.pdf.
4. The Merck Index. 14th edition. USA: Merck Research Laboratories; 2006; 1637: 9528.
5. Vinay Saxena, Zahid Zaheer, Mazha rFarooqui. In J Ch Tech, 2006; 13: 242-246.
6. Ramathilagam N, Meeradevi M, Solairaj P, Rajesh SC. IJPBS, 2012; 2 (4): 332-337.
7. Murthy Dwibhashyam VSN, Keerthi P, VijayaRatna J, Nagappa AN. J Pharm Sci Technol, 2009; 63: 234-239.
8. Shetty SK, Shah A. IJPSR, 2011; Vol. 2(6): 1456-1458.
9. Ramesh Y, Chandra Sekhar V, Ramaiah PA. Sci pharm, 2012; 80 (1): 101-114.
10. Bala Kumar C, Lakshmi Narayanan B, Chandrasekar M, Malairajan M, Kumar EP. J Pharm Sci, 2014; 3 (3): 58-64.
11. Supriya M, Mhamunkar, Roshani Y, Vyavaharkar, Suvarna I Bhoir. IJPPS, 2012; 4(5): 320-322.

12. Ravindra Kumar Y, Ramulu G, Vevakanand VV, Gopal vaidyanathan, Keesari Srinivas, Kishore Kumar M, Mukkanti K, Satyanarayana Reddy M, Venkatraman S, Suryanarayana MV. J Pharm & BM, 2004; 35 (5): 1279-1285.
13. SyamBabu M, Viplava Prasad U, Kalyana Ramu B. AJPTR, 2012; 2 (4): 395-40.
14. El-Enany N, Walash MI, Belal F, Elmansi H. IJPSR, 2011; 2 (11): 2849-2855.
15. Kumar, Ashutosh S, Debnath, Manidipa, Sheshagiri Rao JV. J Res Chem, 2013; 6(7): 665-67.
16. Mohammed W. Nassar, Khalid A. Attia, Hamed M. Abou-Seada, Ahmed El-Olemy Allam. IJPSR, 2013; 4 (10): 3845-3849.
17. Munjed Ibrahim, Ghazi Bamagous, Ahmed Al-Sheikh, Safwan Fraihat. Erzurum. IACSIT press, 2014:45-49.
18. Rihna Parveen Shaik, Srinivasa Babu Puttagunta, Chandrasekhar Bannoth Kothapalli, Bahlu lZayed Sh.Awen, Challa BR. J Ph An, 2013; 3 (6): 489-499.
19. Beibi Zhang, Zunjian Zhang, Yuan Tian, FengguoXu. J Chr B, 2005; 824 (1-2): 92-98.
20. Bioanalytical Method Validation, Guidance for Industry, United States Food and Drug Administration, May 2001.
21. Leon Lachman. The theory and practice of Industrial pharmacy. 3rd edition, Varghese publishing house, USA.
22. Guideline on the need for non-clinical testing in juvenile animals for human pharmaceuticals for pediatric indications. European medicines agency pre-authorization. Evaluation of medicines for human use. London, 29 September 2005.
23. Shannon Reagan-Shaw, Minakshi Nihal, Nihal Ahmad. Dose translation from animals to human studies revisited. The FASEB Journal, 2002; 660 (22): 659-661.
24. Center for Drug Evaluation and Research. Estimating the safe starting dose in clinical trials for therapeutics in adult healthy volunteers, US. Food & Drug Administration, Rockville, Maryland, USA, 2002.