

## METHOD DEVELOPMENT & VALIDATION OF TOLTERODINE TARTRATE IN BULK AS WELL AS IN PHARMACEUTICAL FORMULATION BY USING RP-HPLC

S. ASHUTOSH KUMAR<sup>1\*</sup>, MANIDIPA DEBNATH<sup>2</sup>, J.V.L.N.SESHAGIRI RAO<sup>3</sup>

<sup>1,2</sup>A.K.R.G College of Pharmacy, Nallajerla, West Godavari, 534112, <sup>3</sup>Pharmaceutical Analysis, Yalamarty College of Pharmacy, Tarluwada Visakhapatnam, 530052, A.P. Email: ashu\_mpharm2007@rediffmail.com

Received: 16 Apr 2013, Revised and Accepted: 09 Jun 2013

### ABSTRACT

**Objective:** A simple and accurate Reverse Phase High Performance Liquid Chromatographic method was developed for the determination of Assay of Tolterodine Tablets 2mg. Tolterodine Tartrate the active pharmaceutical ingredient used in the treatment of urinary incontinence.

**Method:** A RP-HPLC method was developed for the method as well as for the validation. The process was achieved using a High performance liquid chromatography equipped with Auto Sampler and DAD or UV detector and the Column used for Method Development & Validation was Symmetry C18 (4.6 x 150mm, 5 µm, Make: Kromosil). The mobile phase was consists of Phosphate Buffer pH 3.0 and Acetonitrile HPLC grade. The binary gradient was made with a flow rate at 0.8 ml/min. The detection wavelength was selected 282 nm. The injection volume was 20 µL.

**Results:** The Accuracy was calculated and the % Recovery was found to be 98.1% to 100.2%. The method was found to be Linear over the range of 20 to 100µg per ml. The LOD for the drug was found to be 0.108µg/ml and LOQ for Tolterodine Tartrate was found to be 0.36µg/mL

**Conclusion:** The validated method yielded good results of precision, linearity, accuracy, and robustness. The proposed method was found to be suitable and accurate for the method development and validation and assay of compound Tolterodine Tartrate in active pharmaceutical ingredient. The developed method was validated to ensure the compliance in accordance with ICH guidelines and also well suitable for studies on HPLC.

**Keywords:** High performance liquid chromatography, Assay, Validation, Quantification, Tolterodine Tartrate, Detection.

### INTRODUCTION

Tolterodine Tartrate is described chemically as 2-(3-(diisopropylamino)-1-phenylpropyl)-4-methylphenol Tartrate (Fig.1). The Drug is listed in the Merck index. Tolterodine Tartrate is used for the treatment of urinary urge incontinence and other symptoms of overactive bladder [1]. Tolterodine Tartrate is a potent muscarinic receptor antagonist that is equipotent to oxybutynin in the bladder, but less potent in salivary glands, with the aim of improving tolerability (less dry mouth) in patients with overactive bladder [2-5]. A liquid chromatography-tandem mass spectrometry for Quantization of Tolterodine and its 5-hydroxy methyl metabolite in plasma samples was reported in the literature. Determination of Assay of Tolterodine Tartrate in Plasma, serum and urine by Capillary Gas chromatography coupled with Mass spectrometer was also reported in the literature. An enantio specific HPLC method for the determination of (S)-enantiomer impurities in (R)-Tolterodine Tartrate and Simultaneous Estimation of Tamsulosin HCL and Tolterodine Tartrate in pharmaceutical dosage form was also reported [6]. A HPLC method for determination of Tolterodine Tartrate in dosage forms was also available in literature for routine quality control samples [7]. A Validated Stability-Indicating HPLC Method for the determination of Related Components in Tolterodine Tartrate was also reported in the Literature [8].

The chemical structure of the Drug was represented in Fig. no.1

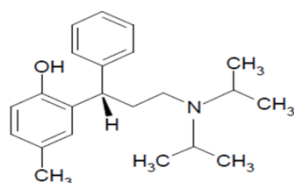


Fig. 1: It shows the Chemical Structure of Tolterodine

### MATERIAL AND METHOD

#### Chemicals and Reagents Used

Chemicals procured for the Method Development & as well as Validation were Water [HPLC Grade], Tolterodine [Working

Standard], Acetonitrile [HPLC Grade], Ortho phosphoric acid. All the chemicals were procured from STANDARD SOLUTIONS and Tolterodine Tablet 2mg was collected from the Local market manufacturer Ranbaxy, brand name ROLITEN.

#### Apparatus and Chromatographic Parameters

**Equipment:** High performance liquid chromatography equipped with Auto Sampler and DAD or UV detector.

**Column:** Symmetry C18 (4.6 x 150mm, 5 µm, Make: Kromosil)

**Flow rate:** 0.8mL per min

**Wavelength:** 282 nm

**Injection volume:** 20 µl

**Column oven :** Ambient

**Run time :** 5.0 min

#### Preparation of Phosphate buffer

The Buffer Solution was prepared by weighed accurately 7.0 grams of Potassium dihydrogen phosphate into a 1000ml beaker, dissolved and diluted to 1000ml with Water HPLC grade. The pH was adjusted to 3.0 by using Orthophosphoric acid.

#### Preparation of mobile phase

The Mobile Phase was prepared with a ratio of 50:50 of Buffer & Acetonitrile. The Buffer was taken 500mL and Acetonitrile was taken 500 mL and degassed in ultrasonic water bath for 5 minutes. Then it was filtered through 0.45 µ filter under vacuum filtration.

#### Diluent Preparation

The Mobile phase was used as diluent.

#### Preparation of the Tolterodine Standard & Sample Solution

##### Standard Solution Preparation

The Standard Stock Solution was prepared by weighed accurately and transferred 10mg of Tolterodine [Working standard] into a 10 mL volumetric flask added about 7 mL of Diluent and sonicated to dissolved it completely and the volume was made up to the mark

with the same solvent. Further from the above prepared Stock Solution pipette out 0.6 ml into a 10ml volumetric flask and diluted up to the mark with diluent. The resultant solution was mixed well and then it was filtered through 0.45µm filter.

#### Sample Solution Preparation

The Sample Stock Solution was prepared by weighed accurately 5 Tolterodine Tablets and calculated the average weight. The drug sample was accurately weighed equivalent to 10 mg and transferred into a 10 mL volumetric flask. About 7 mL of diluent was added and sonicated to dissolve it completely and then the volume was made up to the mark with the diluent. The resultant solution was mixed well and it was filtered through 0.45µm filter. Further from the above prepared Stock Solution pipette out 0.6 ml into a 10ml volumetric flask and diluted up to the mark with diluent. The resultant solution was mixed well and then it was filtered through 0.45µm filter.

#### The Standard & Sample Solutions Injected to the Column

About 20µL of standard and sample solutions were injecting to the Chromatographic system and the area for the Drug was measured and the % Assay was calculated by using suitable formulae.

#### System Suitability

The Tailing factor for the peak due to Tolterodine in Standard solution should not be more than 2.0. The Theoretical plates for the Tolterodine peak in Standard solution should not less than 2000.

#### Formula for calculating the Assay for the Drug Tolterodine

$$\text{Assay \%} = \frac{AT}{As} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{\text{Avg.Wt}}{\text{Label Claim}} \times 100$$

Where:

AT = Peak Area of Tolterodine obtained with test preparation

AS = Peak Area of Tolterodine obtained with standard preparation

WS = Weight of working standard taken in mg

WT = Weight of sample taken in mg

DS = Dilution of Standard solution

DT = Dilution of sample solution

P = Percentage purity of working standard

#### Results obtained for the System Suitability

- 1) The Tailing factor obtained from the standard injection was found to be 1.6
- 2) The Theoretical Plates obtained from the standard injection was found to be 2067.6

#### Calculated Assay Results for Tolterodine

$$\frac{634803}{634803} \times \frac{10}{10} \times \frac{0.6}{10} \times \frac{10}{82} \times \frac{10}{0.6} \times \frac{99.8}{100} \times \frac{82}{2} \times 100 = 100.2\%$$

#### Validation Development[9-10]

##### Precision

The precision of an analytical procedure was usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

##### Preparation of stock solution

The Stock Solution was prepared by weighed accurately and transferred 10 mg of Tolterodine working standard into a 10 mL volumetric flask. About 7 mL of Diluent was added and sonicated to dissolve it completely and the volume was made up to the mark with the same solvent.

##### Preparation of 60 µg/ml solutions

Further from the above prepared Stock Solution pipette out 0.6 ml into a 10ml volumetric flask and diluted up to the mark with diluent.

The solution was mixed well and filtered through 0.45µm filter. The standard solution was injected for five times and the area was measured for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits. The data was represented in Table no.1

Table 1: It shows the results for Precision.

Injection	Area
Injection-1	667103
Injection-2	667059
Injection-3	669293
Injection-4	670508
Injection-5	671724
<b>Average</b>	669137
<b>Standard Deviation</b>	2064.7
<b>%RSD</b>	0.31

#### Acceptance Criteria

The % RSD for the area of five standard injections results should not be more than 2%.

#### Intermediate Precision (Ruggedness)

To evaluate the intermediate precision, precision was performed on different day by using different make column of same dimensions.

#### Preparation of stock solution

The Stock Solution was prepared by weighing accurately and transferred 10 mg of Tolterodine [Working standard] into a 10 mL volumetric flask added about 7 mL of Diluent and sonicated to dissolve it completely and the volume was made up to the mark with the same solvent.

#### Preparation of 60 µg/ml solution

Further from the above prepared stock Solution pipette out 0.6 ml into a 10ml volumetric flask and diluted up to the mark with the diluent. The resultant solution was mixed well and then it was filtered through 0.45µm filter. The standard solution was injected for five times and the area was measured for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits. The Data was represented in Table no. 2

Table 2: It shows the result for Intermediate Precision (Ruggedness).

Injection	Area
Injection-1	629965
Injection-2	630893
Injection-3	632215
Injection-4	633029
Injection-5	633771
<b>Average</b>	631975
<b>Standard Deviation</b>	1549.6
<b>%RSD</b>	0.25

#### Acceptance Criteria

The % RSD for the area of five standard injections results should not be more than 2%.

#### Accuracy

The accuracy of an analytical procedure expressed the closeness of agreement between the value which was accepted either as a conventional true value or an accepted reference value and the value found.

#### Preparation of stock solution

The Stock Solution was prepared by weighed accurately and transferred 10 mg of Tolterodine [Working standard] into a 10 mL volumetric flask. About 7 mL of Diluent was added and sonicated to dissolve it completely and the volume was made up to the mark with the same solvent.

### Preparation of 60 µg/ml solutions

Further from the above prepared Stock Solution 0.6 ml was pipette out into a 10ml volumetric flask and diluted up to the mark with diluent. The resultant solution was mixed well and it was filtered through 0.45µm filter. The following sample solutions were prepared from the above solution as follows:

#### For preparation of 50% solution (With respect to target Assay concentration)

The Stock Solution was prepared by weighed accurately and transferred 5.0 mg of Tolterodine API sample into a 10 mL volumetric flask and about 7 mL of Diluent was added and sonicated to dissolved it completely and the volume was made up to the mark with the same solvent. Further from the above Stock Solution 0.6ml was pipette out into a 10ml volumetric flask and diluted up to the mark with diluent. The resultant solution was mixed well and it was filtered through 0.45µm filter.

#### For preparation of 100% solution (With respect to target Assay concentration)

The Stock Solution was prepared by weighed accurately and transferred 10.0mg of Tolterodine API sample into a 10 mL

volumetric flask, about 7 mL of Diluent was added and sonicated to dissolved it completely and the volume was made up to the mark with the same solvent. Further from the above Stock Solution 0.6 ml was pipette out into a 10ml volumetric flask and diluted up to the mark with diluent. The resultant solution was mixed well and it was filtered through 0.45µm filter.

#### For preparation of 150% solution (With respect to target Assay concentration)

The Stock Solution was prepared by weighed accurately and transferred 15.0mg of Tolterodine API sample into a 10 mL volumetric flask, about 7 mL of Diluent was added and sonicated to dissolved it completely and the volume was made up to the mark with the same solvent. Further from the above Stock Solution 0.6 ml was pipette out into a 10ml volumetric flask and diluted up to the mark with diluent. The resultant solution was mixed well and it was filtered through 0.45µm filter.

The Standard and Sample solutions were injected to the chromatographic system for Accuracy 50%, 100% and 150%. The amount found was calculated and the amounts added for Tolterodine were calculated. The individual recovery and mean recovery values were also calculated. The Data was represented in Table no.3.

**Table 3: It shows the result for Accuracy**

% Concentration (at specific level)	Area	Amount Added (mg)	Amount recovery Found (mg)	% Recovery	Mean
50%	319003	5.0	5	100.2%	
100%	628847	10.0	9.87	98.7%	99.0%
150%	936919	15.0	14.7	98.1%	

### Acceptance Criteria

The % Recovery for each level should be between 98.0 to 102.0%.

#### Linearity

The linearity of the analytical procedure was its ability to obtain the test results which are directly proportional to the concentration (amount) of analyte in the sample.

#### Preparation of stock solution

The Stock Solution was prepared by accurately weighed and transferred 10mg of Tolterodine API sample into a 10 mL volumetric flask. About 7 mL of Diluent was added and sonicated to dissolve it completely and the volume was made up to the mark with the same solvent. The following solutions were prepared for the Linearity from the above prepared solutions.

**Preparation of Level - I (20µg/ml):** About 0.2ml of stock solution was taken into a 10 ml volumetric flask & diluted up to the mark with diluent

**Preparation of Level - I (40µg/ml):** About 0.4ml of stock solution was taken into a 10 ml volumetric flask & diluted up to the mark with diluent.

**Preparation of Level - II (60µg/ml):** About 0.6ml of stock solution was taken into a 10 ml volumetric flask & diluted up to the mark with diluent.

**Preparation of Level - III (80µg/ml):** About 0.8ml of stock solution was taken into a 10 ml volumetric flask & diluted up to the mark with diluent.

**Preparation of Level - IV (100µg/ml):** About 1.0ml of stock solution was taken into a 10 ml volumetric flask & diluted up to the mark with diluent.

The above prepared Levels were injected individually into the chromatographic system and the peak area was measured. A graph was plotted for peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and the correlation coefficient was calculated. The Data were represented in Table no. 4.

**Table 4: It shows the following results for Linearity**

S. No.	Linearity Level	Concentration (µg/ml)	Area
1	I	20 µg/ml	236168
2	II	40 µg/ml	429629
3	III	60 µg/ml	675098
4	IV	80 µg/ml	827109
5	V	100 µg/ml	1068003
Correlation Coefficient			0.99

### Acceptance Criteria

The Correlation coefficient should be not less than 0.999.

#### Limit of Detection

The detection limit of an individual analytical procedure was the lowest amount of analyte in a sample which can be detected but not necessarily quantities as an exact value.

#### Preparation of 60µg/ml solution

The Stock Solution was prepared by weighed accurately and transferred 10mg of Tolterodine [Working standard] into a 10 mL volumetric flask. About 7 mL of Diluent was added and sonicated to dissolve it completely and the volume was made up to the mark with the same solvent. Further from the above prepared Stock Solution 0.6 ml was pipette out into a 10ml volumetric flask and diluted up to the mark with diluent. The resultant solution was mixed well and then it was filtered through 0.45µm filter.

#### Preparation of 1.8% solutions At Specification level (0.108µg/ml solution)

The Solution was prepared by pipette out 1mL of 10µg/ml solution into a 10 ml of volumetric flask and diluted up to the mark with diluent. Further Pipette 1mL of 10µg/ml solution into a 10 ml of volumetric flask and diluted up to the mark with diluent. Further pipette 1.8mL of above diluted solution into a 10 ml of volumetric flask and diluted up to the mark with diluent.

**Calculation of S/N Ratio**

Average Baseline Noise obtained from Blank: 42 $\mu$ V

Signal Obtained from LOD solution (1.8% of target assay concentration): 122  $\mu$ V

$$S/N = 1223/42 = 2.90$$

**Acceptance Criteria**

The S/N Ratio value should be 3 for LOD solution.

**Limit of Quantification**

The Quantification limit of an individual analytical procedure was the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

**Preparation of 60 $\mu$ g/ml solution**

The Stock Solution was prepared by weighed accurately and transferred 10mg of Tolterodine Working standard into a 10 mL volumetric flask. About 7 mL of Diluent was added and sonicated to dissolve it completely and the volume was made up to the mark with the same solvent. Further from the above prepared Stock Solution 0.6 ml was pipette out into a 10ml volumetric flask and diluted up to the mark with diluent. The resultant solution was mixed well and then it was filtered through 0.45 $\mu$ m filter.

**Preparation of 6.0 %solution At Specification level (0.36 $\mu$ g/ml solution)**

The Solution was prepared by pipette out 1ml of solution into a 10 ml of volumetric flask and diluted up to the mark with diluent. Further 1ml of solution from prepared solution was transferred into a 10 ml of volumetric flask and diluted up to the mark with diluent. Finally 6.0mL from above diluted solution was pipette out into a 10 ml of volumetric flask and diluted up to the mark with diluent.

**Calculation of S/N Ratio**

Average Baseline Noise obtained from Blank: 42 $\mu$ V

Signal Obtained from LOQ solution (6.0% of target assay concentration): 418  $\mu$ V

$$S/N = 418/42 = 9.95$$

**Acceptance Criteria**

The S/N Ratio value should be 10 for LOQ solution.

**Robustness**

The robustness of an analytical procedure was a measured of its capacity to remain unaffected by small, but deliberate variations in method parameter and provides an indication of its reliability during normal usage.

**a) The flow rate was varied from 0.6 to 1.0 ml/min.**

The Standard solution 60  $\mu$ g/ml was prepared and analysed using the varied flow rates along with method flow rate. On evaluation of the above results, it was concluded that the variation in flow rate does not affected the method significantly. Hence it indicates that the method was robust even by change in the flow rate  $\pm$ 10%. The Data was represented in Table no. 5.

**Table 5: It shows the following results for the System Suitability with change in the flow rate.**

S. No.	Flow Rate (ml/ min)	System Suitability Results	
		USP Plate Count	USP Tailing
1.	0.6	2086.9	1.7
2.	0.8	2067.6	1.6
3.	1.0	2020.5	1.5

**b) The Organic composition in the Mobile phase was varied from 45% to 55%**

The Standard solution 60  $\mu$ g/ml was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method. On evaluation of the above results, it was concluded that the variation in 10% Organic composition in the mobile phase does not affected the method significantly. Hence it indicates that the method is robust even by change in the Mobile phase  $\pm$ 10%. The Data was represented in Table no. 6.

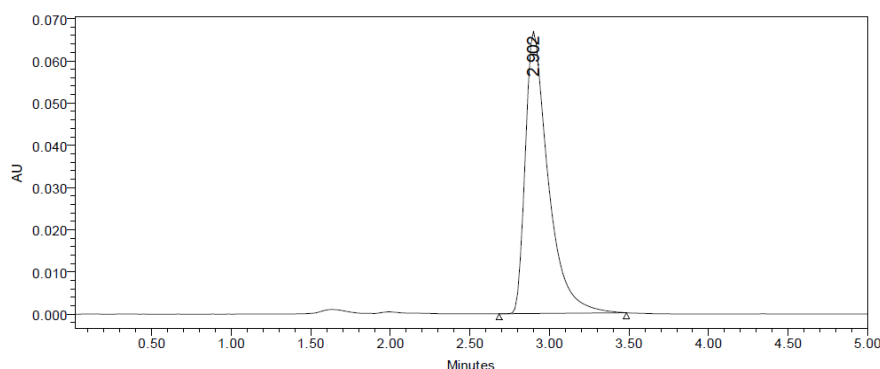
**Table 6: It shows the following results for System Suitability with change in Organic Composition.**

Sl. No.	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1.	10%	2443.9	1.6
2.	Actual	2067.6	1.6
3.	10% More	2166.3	1.7

**RESULT & DISCUSSION**

The present study was carried out to develop a sensitive, precise and accurate HPLC method for the analysis the drug in Bulk as well as in pharmaceutical formulations or dosage forms. In order to method

development under isocratic conditions, a mixtures of Phosphate Buffer whose pH was adjusted to 3 with Orthophosphoric acid and Acetonitrile HPLC grade were taken in different combinations and tested as mobile phase on a Symmetry C18 (4.6 x 150mm, 5  $\mu$ m, Make: Kromosil) column.



**Fig. 2: It shows a model chromatograph of the Drug**

A binary mixture of Phosphate Buffer with pH 3 and Acetonitrile HPLC grade in 50:50 v/v proportion was proved to be the most suitable of all combinations since the chromatographic peaks were better defined and resolved and almost free from tailing. The retention time obtained for the drug Tolterodine was around 2.896 min. A model chromatogram was shown in Fig. no.2.

The Precision data was represented in Table no. 1. The accuracy of an analytical procedure expresses the closeness of agreement

between the value which was accepted either as a conventional true value or an accepted reference value and value found. The data found was in specified limits and represented in Table no.3. In order to test the linearity of the method, five dilutions of the working standard solutions of the drug in the range of 20 to 100µg per mL were prepared. The data was represented in Table no. 4. Each of the dilutions was injected into the column and the graph for the Linearity Curve was represented in Fig no. 3.

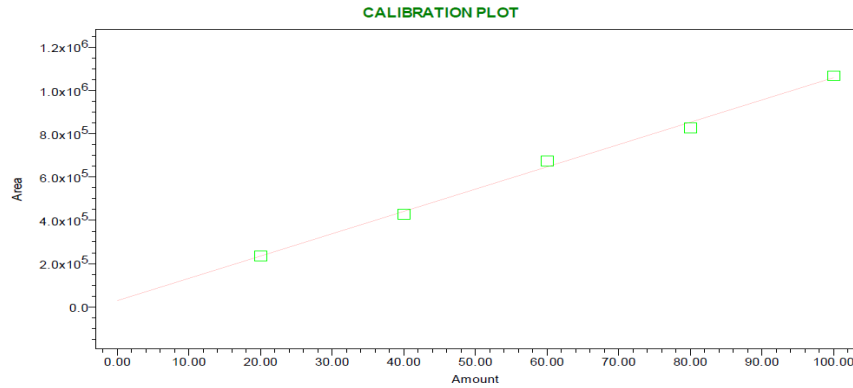


Fig. 3: It shows Linearity Curve for the Drug

The method was duly validated by evaluation of the required parameters. When Tolterodine was analyzed by the proposed method in the intra and inter-day (Ruggedness) variation results, a

low coefficient of variation was observed and it was represented in Table no. 2. It shows that the present HPLC method was highly precise and it was represented by Fig no. 4.

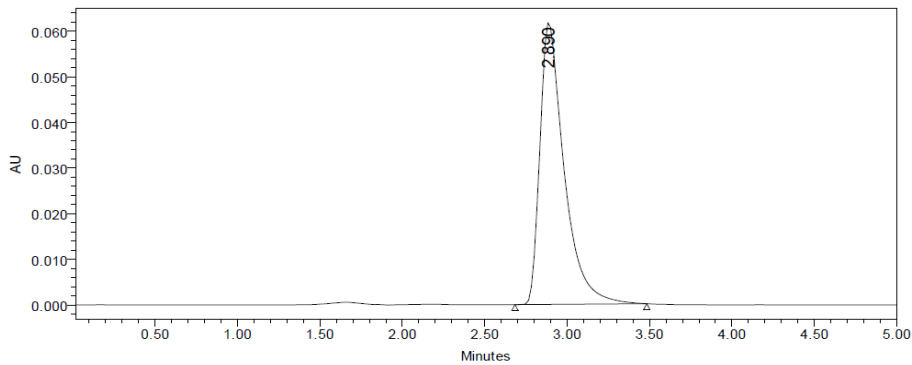


Fig. 4: It shows the Ruggedness of the Chromatograph

Robustness of the method was found out by testing the effect of small deliberate changes in the chromatographic conditions and the corresponding peak area. The factors selected for this purpose were flow rate and percentage composition variation in the mobile phase.

The method was found to be robust enough that the peak area was not apparently affected by small variation in the chromatographic conditions. The Fig. no. 5, 6, 7 & 8 were represented the System Suitability.

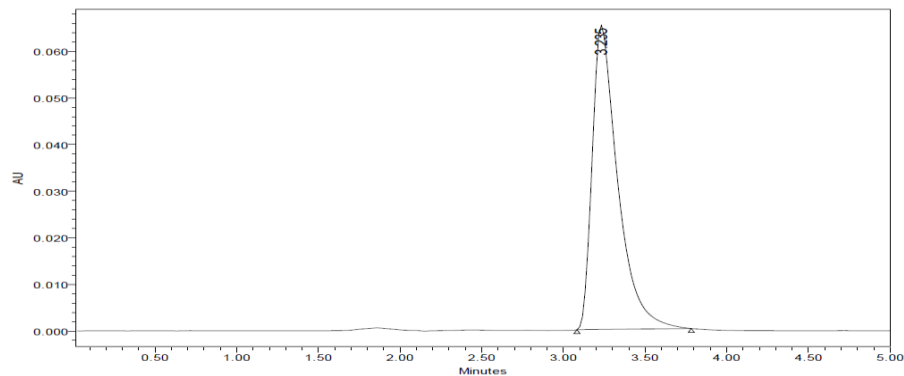


Fig. 5: It shows the System Suitability with decrease in the flow rate

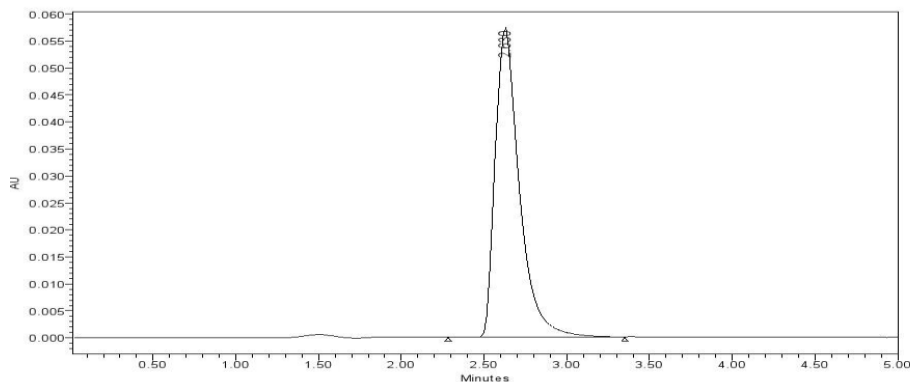


Fig. 6: It shows the System Suitability with increase in the flow rate

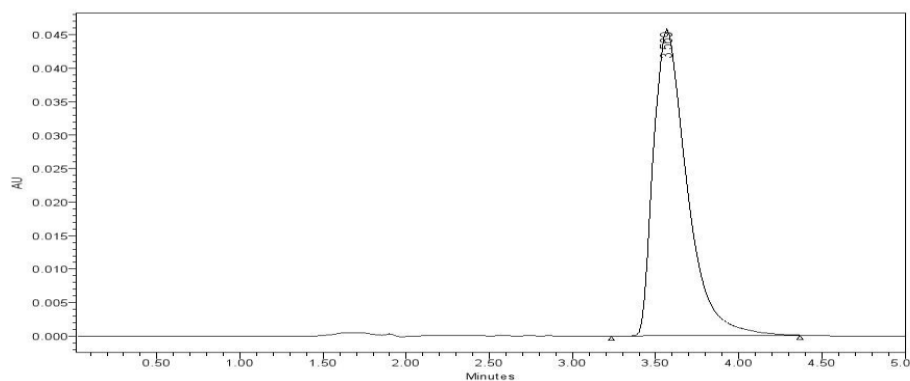


Fig. 7: It shows the System Suitability with decrease in the composition of the mobile phase.

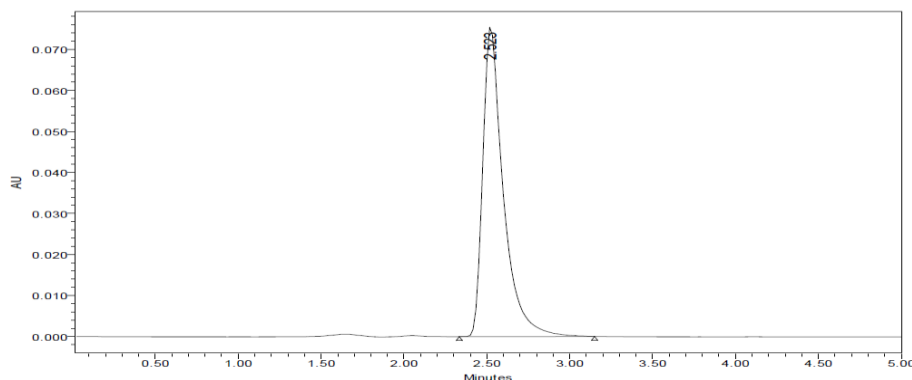


Fig. 8: It shows the System Suitability with increase in the composition of the mobile phase.

The system suitability parameters were within the limits as shown in Table 5 and 6. The Limit of detection and limit of quantification of the method were calculated based on standard deviation of the response and the slope (s) of the calibration curve at approximate levels of the limit of detection and limit of quantification. The LOD for the drug was found to be  $0.108\mu\text{g/ml}$  and LOQ for Tolterodine was found to be  $0.36\mu\text{g/mL}$ . The drug content formulations were quantified by using the proposed analytical method. The low coefficient of variation in the recovery data was indicated that the reproducibility of the method in dosage forms. It was concluded that the proposed HPLC method is sufficiently sensitive and reproducible for the analysis of Tolterodine in the Tablet formulation dosage forms within a short analysis time.

#### CONCLUSION

It was concluded that the proposed RP-HPLC method developed for the quantitative determination of Tolterodine in bulk as well as in its formulations was simple, selective, sensitive, accurate,

precise and rapid. The method was proved to be superior to most of the reported methods. The mobile phases were simple to prepare and economical. The sample recoveries in the formulation were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Hence the method can be easily adopt as an alternative method to report routine determination of Tolterodine depending upon the availability of chemicals and nature of other ingredients present in the sample. The method also finds use in clinical, biological and pharmacokinetic studies for the drug Tolterodine.

#### REFERENCE

1. <http://en.wikipedia.org/wiki/Tolterodine>
2. <http://www.rxlist.com/detrol-la-drug.htm>
3. National center of Biotechnology information. Available at: <http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0001025/> (access on April 2012).

4. ICH Harmonized Tripartite Guideline on validation of Analytical Procedures: Q 2 (R1) (2005).
5. United States Pharmacopeia Asian edition (2005).
6. Supriya M Mhamunkar, Roshani Y. Vyavaharkar & Suvarna I Bhoir; RP-HPLC Method Development and Validation for the Simultaneous Estimation of Tamsulosin HCL and Tolterodine Tartrate in pharmaceutical dosage form, International Journal of Pharmacy and Pharmaceutical Science. 2012; 4 Suppl 5:320-322.
7. Dwibhashyam V.S, Keerthi P, Ratna J. V, Nagappa A. N; Reverse-Phase High performance liquid chromatographic method for the determination of Tolterodine Tartrate in routine quality control samples. PDA J Pharm Sci Technol.2009; 63(3):234-239.
8. S Radha Krishna, B.M. Rao and N. Someswara Rao, A Validated Stability-Indicating HPLC Method for the Determination of Related Substances and Assay of Tolterodine Tartrate. Rasayan J. Chem.2009; 2(1):144-150.
9. Sethi, P.D., In; HPLC 'High Performance Liquid Chromatography', Quantitative Analysis of Pharmaceutical Formulations, 1st Edn; CBS Publishers and Distributors, New Delhi, 2001, 3-72,116-120.
10. Carolin Nimila, P. Balan, N. Chiranjeevi, Vinnakota.V. V. M. Kumar; Development and Validation of A Reverse Phase HPLC Method Of Simultaneous Estimation Of Tolperisone Hydrochloride And Paracetamol In Tablet Dosage Form, Vol 4, Suppl 5, 2012; 84-88.