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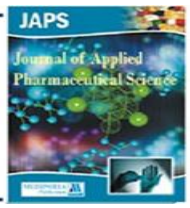

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RP HPLC Method for the determination of Tamsulosin in bulk and Pharmaceutical formulations

Manish Kumar Thimmaraju, Venkat Rao, Hemanth .K, P. Siddartha Kumar

Manish Kumar Thimmaraju, Venkat Rao, P. Siddartha Kumar
Central Analytical Laboratory, Balaji Institute of Pharmaceutical Sciences, Warangal, Andhra Pradesh, India

Hemanth .K
Shantha Institute of Pharmaceutical Sciences, Huzurabad, Andhra Pradesh, India

For Correspondence
Manish Kumar Thimmaraju
Email: manishcancer@gmail.com

ABSTRACT

A simple, sensitive, precise and specific reverse phase high performance liquid chromatographic method was developed and validated for the determination of Tamsulosin in bulk and tablet dosage forms. It was found that the excipient in the tablet dosage forms does not interfere in the quantification of active drug by proposed method. The HPLC separation was carried out by reverse phase chromatography on Shimadzu HPLC, 10-At detector with hypersil ODS C_{18} Column 250 X 4.6 mm (particle size of 5µ) and constant flow pump. Rheodyne injector with 20 µl loop with a mobile phase composed in the ratio acetonitrile: (0.05M) KH_2PO_4 buffer (45:55) at flow rate 1.8 ml /min. The detection was monitored at 240nm. The calibration curve for Tamsulosin was linear from 10-50µg/ml and internal standard (Bromhexine) 10µg/ml were prepared by suitable dilutions of the stock solution with appropriate mobile phase. The interday and intraday precision was found to be within limits. The proposed method has adequate sensitivity, reproducibility and specificity for the determination of Tamsulosin in bulk and its tablet dosage forms. LOD and LOQ for Tamsulosin were found to be 0.495 and 0.461.Accuracy (recoveries: 98.5-98.55%) and reproducibility were found to satisfactory.

Key words: Tamsulosin, RP-HPLC Method, Reverse phase chromatography, bromhexine Acetonitrile, Validation.

INTRODUCTION

Tamsulosin, 5- [(2R)-2[[2-(2-Ethoxy Phenoxy) ethyl] amino] Propyl} - 2-methoxy benzene sulfonamide. Tamsulosin is a selective alpha 1 adrenoceptor blocking agent. Smooth muscle tone is mediated by the sympathetic nervous stimulation of alpha1 adrenoceptors, which are abundant in the prostate, prostatic capsule, prostatic urethra, and bladder neck. Blockade of these adrenoceptors can cause smooth muscles in the bladder, neck and prostate to relax, resulting in an improvement in urine flow rate and a reduction in symptoms of BPH. According to the literature survey it was found that few analytical methods such as Visible, UV, polarographic analysis, HPLC other methods were reported for Tamsulosin, (Matsushima ., Takanuki .et al.,2004) (O'Neil , Smith , Heckelman , Budavari et al.,2001) (ICH Q2 R11995) (Nanda, Gaikwad and Prakashet al.,2009),(Chandorkar JG, Kotwal VB, Dhande et al.,2009,) (Macek , Klima and Ptacek et al.,2004) (Matsushima Takanuki , Kamimura et al.,2004), (Nilam .Gadhave Sanjay . Sawant et al.,2011)' (Rao , Talluri , Raju , Shinde , Rahkonen , Parssinen , Leppanen et al.,2008) The objective of the proposed methods to develop simple and accurate method for the determination of tamsulosin by UV spectrophotometric method in Pharmaceutical dosages forms.

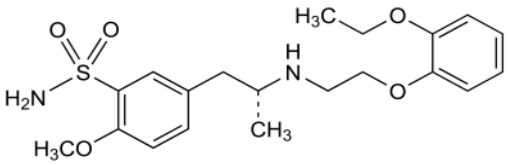

Fig-1 Tamsulosin.

Chromatographic conditions

Chromatographic separation was performed on Shimadzu HPLC, 10-At detector with Hypersil ODS C_{18} Column 250 X 4.6 mm (particle size of 5µ) and constant flow pump. Rheodyne injector with 20 µl loop. The composition of the mobile phase is in the ratio acetonitrile: (0.05M) KH_2PO_4 buffer (45:55) was delivered at flow rate 1.8 ml /min. The mobile phase was filtered through a 0.45 µ membrane filter and sonicated for 15min. Analysis was performed at ambient temperature.Bromohexine was used as internal standard. Optimized chromatographic conditions are listed in **Table -1.**

Table-1. Optimized Chromatographic Conditions.

Parameters	Method
Stationary phase (column)	Hypersil ODS C-18 250 x 4.6 mm, (packed with 5 micron)
Mobile Phase	Acetonitrile: KH_2PO_4 Buffer
Flow rate (ml/min)	1.8
Run time (minutes)	10
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	240
Internal standard	Bromhexine
Drug RT (min)	6.051
Internal standard RT (min)	10.7

METHODS AND MATERIALS

T.D.Water (Triple distilled water), Acetonitrile HPLC grade (MERCK), Potassium dihydrogen Phosphate, (AR- Grade), Methanol HPLC grade, Tamsulosin.

Preparation of standard drug and internal standard solutions

Stock solutions of the drug (pure) and internal standard were prepared by dissolving 10 mg of Tamsulosin in 100ml of Acetonitrile (HPLC Grade, MERCK) and 25 mg of internal standard (Bromhexine) in 25 ml of mobile phase separately in 25ml volumetric flasks. Daily working standard solutions of Tamsulosin were prepared between the range of 10-50µg/ml and internal standard (Bromhexine) 40µg/ml were prepared by suitable dilutions of the stock solution with appropriate mobile phase.

Preparation of sample solution

Twenty tablets were weighed to get the average weight and pulverized. The sample powder, equivalent to 10mg of active ingredient was extracted with acetonitrile sonicated for about 15 min and made to volume to get a stock solution of 1 mg/ml.This solution was filtered through a whatman filter paper. From this solution 0.1 to 0.5ml were taken and it was further diluted to 10ml with mobile phase as under preparation of standard solutions to get different concentrations required.

METHOD VALIDATION

Once the HPLC method development was over, the method was validated in terms of parameters like, precision, accuracy, linearity and range, LOD, LOQ, recovery studies, system suitability parameters etc. For all the parameters percentage relative standard deviation values were calculated. The proposed HPLC method was validated as per ICH guidelines.

Linearity and Range

The linearity of measurement was evaluated by analysing different concentrations of the standard solutions of the tamsulosin .The Beer lamberts concentration was found to be between 010-50 µg/ml. Calibration curve was constructed by plotting average peak area against concentration and regression equation was computed. The results were shown in **Fig: 2**.The slope, intercept and correlation coefficient values were found to be 5220, 8and 1.

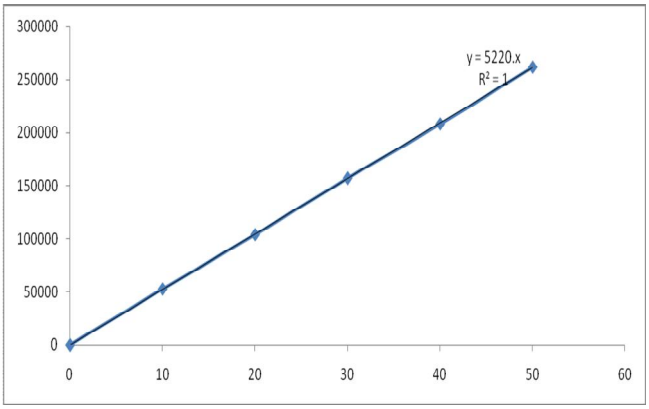

Fig-2 Linearity of Tamsulosin [Concentration (mcg/ml) (X-axis) **VS.** Peak Area (Y-axis)].

Table-3 Intra – Day and Inter – Day Precision **o**f Tamsulosin Standard.

Drug	Theoretical concentration (µg /ml)	Intra-day concentration measured (µg/ml)		Inter-day concentration measured (µg /ml)	
		Mean (a)	RSD %	Mean (b)	RSD %
Tamsulosin	10	10.166	1.818	10.24	1.399
	20	20.186	1.0885	20.21	0.7127
	30	30.246	0.8005	30.15	0.2375

Precision

Precision was evaluated by carrying out three independent sample preparation of a single lot of formulation. The sample solution was prepared in the same manner as described in the sample preparation. Percentage relative standard deviation (% RSD) was found to be less than 2% for within a day and day to day variations, which proves that that method is precise.
Results were shown in **Table 3**.

Analysis of Tamsulosin in its Formulations

The amount of drug present in each pharmaceutical formulation was calculated through peak area ratio of component

to that of internal standard by making use of the standard calibration curve (concentration µg/ml on X-axis and peak area ratios on Y-axis) the results were shown in **Table-4** The chromatogram was shown in **fig-3.**

Table 4 Analysis of Tamsulosin.

Drug	Sample No	Label claim (mg/tab)	Amount estimated* (mg/tab)	% Label claim	% Deviation
Tamsulosin	1	0.4	0.392	98.0	(-)2
	2	0.4	0.394	98.5	(-)1.5
	3	0.4	0.390	97.5	(-)2.5
	4	0.4	0.391	97.75	(-)2.25
	5	0.4	0.389	97.25	(-)2.275

*Each value is average of five determinations ± standard deviation.

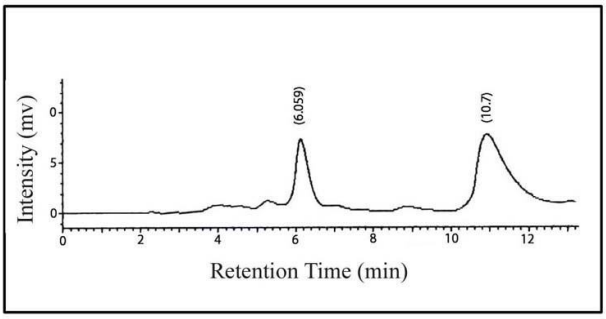

Fig-3 Assay of Tamsulosin.

Limit of Detection and Limit of Quantification

The limit of Detection (LOD) and limit of Quantification (LOQ) of the devel oped method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOD for Tamsulosin found to be 0.495 The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ was 0.461.It was concluded that the developed method is sensitive.

Recovery Studies

To determine the accuracy of proposed method recovery studies carried out by taking different amounts of bulk sample of Tamsulosin within the linearity range were taken and added to the pre-analysed formulation. From that percent recovery values were calculated. Results were given below in **Table-5.** Recovery chromatogram was shown in **fig-4**

System suitability parameters

System suitability parameters can be defined as tests to ensure that the method can generate results of acceptable accuracy and precision. The requirements for system suitability are usually developed after method development and validation have been completed. The USP (2000) defines parameters that can be used to determine system suitability prior to analysis.

Table-5 Percentage recovery.

Drug	Amount Added (µg/ml)	Amount Recovered (µg/ml)	% Recovery
Tamsulosin	20	19.7	98.5
	30	29.72	99.06
	40	39.92	98.55

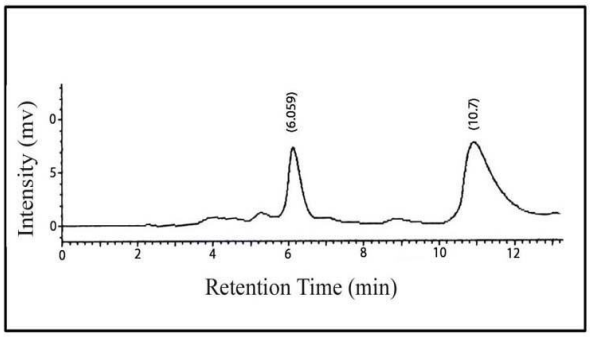

Fig-4 Recovery chromatogram.

The system suitability parameters like Theoretical plates, Resolution (R), Tailing factor (T), LOD (µg/ml), LOQ (µg/ml) were calculated and compared with standard values to ascertain whether the proposed RP-HPLC method for the estimation of Tamsulosin in pharmaceutical formulations was validated or not. The results are recorded in **Table-6.**

Table-6 System suitability parameters.

S.No	Parameters	Obtained Values
1.	Theoretical plates (N)	2300
2.	Resolution (R)	2.652
3.	Tailing factor (T)	1.097
4.	LOD (µg/ml)	0.495
5.	LOQ (µg/ml)	0.461

RESULTS AND DISCUSSION

From the optical characteristics of the proposed method it was found that the drug obeys linearity range within the concentration of 10-50µg/ml. From the results shown precision it was found that the percent RSD is less than 2%, which indicates that the method has good reproducibility. From the results shown in accuracy it was fond that the percent recovery values of pure drug from the preanalysed solutions of formulations were in between 98.5-98.55%, which indicates that the method is accurate. The system suitability parameters are within the specified limits and which refers the commonly used excipients and additives present in the pharmaceutical formulations did not interfere in the proposed method. The proposed method was found to be simple, precise, accurate and rapid for determination of Tamsulosin from pure and its dosage forms. The mobile phase is simple to prepare and economical. The sample recoveries in all formulations were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation.

CONCLUSION

A convenient and rapid RP- HPLC method has been developed for estimation of Tamsulosin in tablet dosage form. The assay provides a linear response across a wide range of concentrations. Low intra-day and interday % RSD coupled with excellent recoveries. Hence, this method can be easily and conveniently adopted for routine analysis of Tamsulosin in pure form and its dosage forms and can also be used for dissolution or similar studies.

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