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RP HPLC method for the determination of finasteride and tamsulosin in bulk and pharmaceutical formulations

Manish Kumar Thimmaraju*[1], Venkat Rao[2] and Srikanth Gurrala[3]

[1,2]Central Analytical Laboratory, Balaji Institute of Pharmaceutical Sciences, Narsampet, Warangal, Andhra Pradesh, India
[3]Gland Pharma Institute of Pharmaceutical Sciences, Medak, Andhra Pradesh, India

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ABSTRACT

A simple, sensitive, precise and specific reverse phase high performance liquid chromatographic method was developed and validated for the determination of Finasteride and 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 linearity range was found between 125-625µg/ml for Finasteride 10-50 µg/ml for Tamsulosin and internal standard (Bromhexine) 40µg/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 Finasteride and Tamsulosin in bulk and tablet dosage forms. LOD and LOQ for Finasteride and Tamsulosin were found to be 1.25, 4.166 and 0.495 and1.635.Accuracy (recoveries: finasteride-100.76% & Tamsulosin 99.06%) and reproducibility was found to be satisfactory.

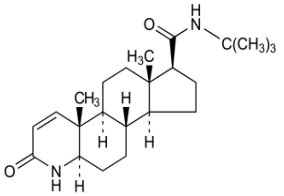
Keywords: Finasteride, Tamsulosin, RP-HPLC Method, Reverse phase chromatography, bromhexine Acetonitrile, Validation.

INTRODUCTION

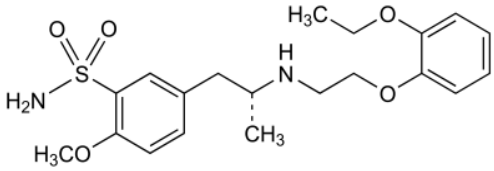
Finasteride , N- (1,1-dimethylethyl) –3-oxo-4-aza-5K-androst-1-ene- 172- carboxamide[1]. Finasteride, a type II 5 alpha reductase inhibitor, slowly reduces prostatic volume, Prostate growth and function is influenced by dihydrotestosterone.5 alpha-reductase enzyme converts

testosterone to dihydrotestosterone. Inhibition of 5 alpha reductase results in decreased level of dihydrotestosterone leading to reduction of prostate size. Finasteride has higher affinity for 5-R type II versus type I.Tamsulosin, 5- [(2R)-2[[2-(2-Ethoxy Phenoxy) ethyl] amino] Propyl}-2-methoxy benzene sulfonamide[2]. 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 finasteride and tamsulosin(Amer SM 2003[3], Amshumalli ,M.K et al., 2001[4] Constanzer ML et al., 1991[5] Carlucci G et al., 1997[6] ,Carlin JR et al.,1998,[7] , Higuchi S et al., 1997,[8] Soeishi Y et al., 1991[9]. The proposed method was found to be simple, precise, accurate and rapid for simultaneous determination of Finasteride and 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.

Hence, this method can be easily and conveniently adopted for routine analysis of Finsateride and Tamsulosin in combined dosage forms and can also be used for dissolution or similar studies.



(1)



(2)

Fig 1. Chemical structures of Finastride (1) and Tamsulosin (2)

Table-1 Optimized Chromatographic Conditions

Parameters	Method
Stationary phase (column)	(Hypersil odsc18 (250x4.6mm,packed with 5 micron)
Mobile phase	Acetonitrile:kh_2po_4, buffer (45:55)
Flow rate (ml/min)	1.6 ml
Run time (minutes)	15
Column temperature (°c)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	240
Internal standard	Bromhexine
Drugs Rt (min)	3.59 (FSD) and 6.051 (TMS)
Internal standard Rt (min)	10.7

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 (45:45) 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.**

MATERIALS AND METHODS

Finasteride, Tamsulosin, Water HPLC grade (triple distilled water), Acetonitrile HPLC grade (MERCK), Potassium Di hydrogen Phosphate (AR-Grade)**,** Methanol HPLC grade.

Preparation of mobile phase

3.4022 gram of potassium di hydrogen phosphate was dissolved in 500 ml of tripled distilled water, mixed thoroughly. The buffer and Acetonitrile were mixed in the ratio of 45:55.

Preparation of standard drug and internal standard solutions

Stock solution of the drug and internal standard were prepared by dissolving 125mg of Finsateride and 10mg of Tamsulosin in 100ml of methanol and 25 mg of internal standard (Bromhexine) was dissolved in 25ml of mobile phase. Daily working standard solutions of Finasteride & Tamsulosin 125-625µg/ml and 10-50µg/ml respectively, and internal standard 40µg/ml were prepared by suitable dilution of the stock solution with appropriate mobile phase.

Preparation of sample solution

For analysis of commercial formulations of tablets 20 tablets were weighed, powdered and weight equivalent to125mg and 10mg of Finasteride and Tamsulosin, which was transferred into 100 ml volumetric flask and dissolved in 100 ml methanol, filtered through a Whatmann filter paper. This solution was further diluted stepwise with mobile phase to get the concentration within the linearity range.

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 measurement was evaluated by analysing different concentrations of the standard solutions of Finasteride. and Tamsulosin. The Beer lamberts concentration was found to be between 125-625 µg/ml for Finasteride 10-50 µg/ml for Tamsulosin. Calibration curve was constructed by plotting average peak area against concentration and regression equation was computed. The results were shown in **Fig: 2&3** the slope, intercept and correlation coefficient values were found to be 1028.7, 170.81 and 0.9999 for Finasteride and 513.6, 44.781 and 0.998 for Tamsulosin.

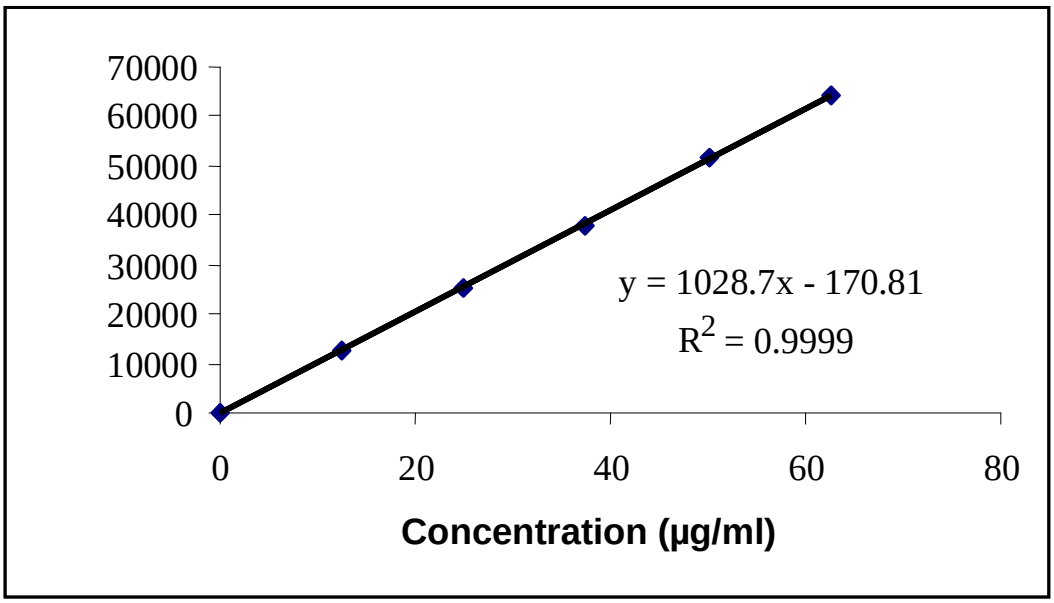


Fig.2 Linearity plot of Finasteride

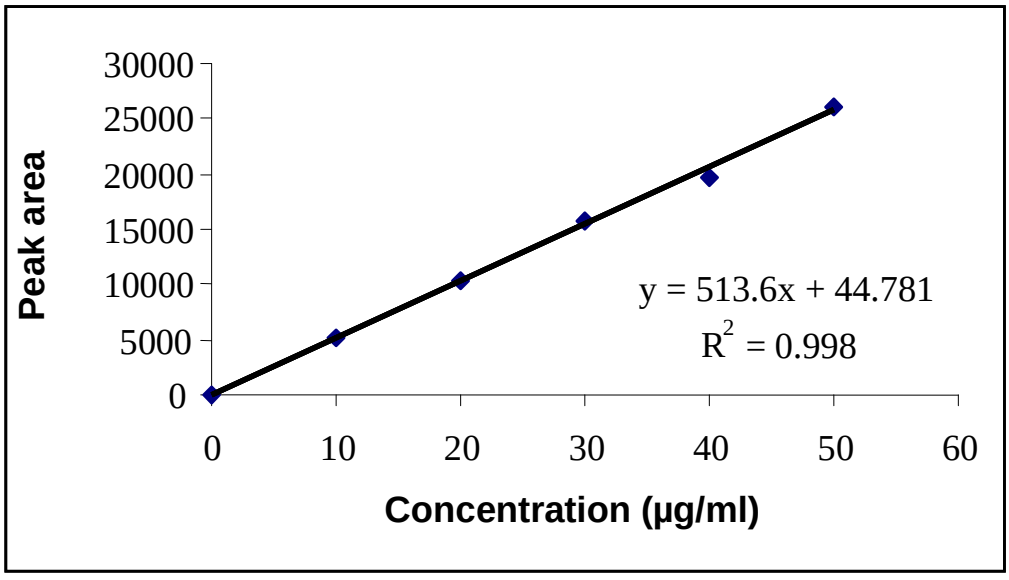


Fig.3 Linearity plot of Tamsulosin

Precision

The precision of each method was ascertained separately from the peak area ratios obtained by actual determination of eight replicates of a fixed amount of drug and internal standard. The intra-and inter-day variation in the peak areas ratio of the drug solution to that of internal standard was calculated in terms of percent RSD and the results are presented in the **Table 2**.

Table- 2 Intra – day and Inter – day Precision of Finasteride and Tamsulosin standard solutions

Drug	Theoretical concentration (µg /ml)	Intra-day concentration measured* (µg/ml)		Inter-day concentration measured* (µg /ml)	
		Mean (a)	RSD %	Mean (b)	RSD %
Finasteride	125	125.28	1.5663	12.04	1.7046
	250	250.168	0.6567	25.25	0.8172
	500	500.49	0.8268	50.21	0.336
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

**mean of three values*

Analysis of Finasteride and 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 in µg/ml on X-axis and peak area ratios on Y-axis) the results were shown **in table-3** Chromatogram was shown in **fig-4.**

Table-3 Assay of combined tablet dosage form

Drug	Sample No	Label claim (mg/tab)	Amount estimated (mg/tab)	% Label claim	% Deviation
Finasteride	1	5	4.92	98.4	(-) 1.6
	2	5	4.97	99.4	(-) 0.6
	3	5	4.82	96.4	(-) 3.6
	4	5	4.98	99.6	(-) 0.4
	5	5	4.93	98.6	(-) 1.4
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

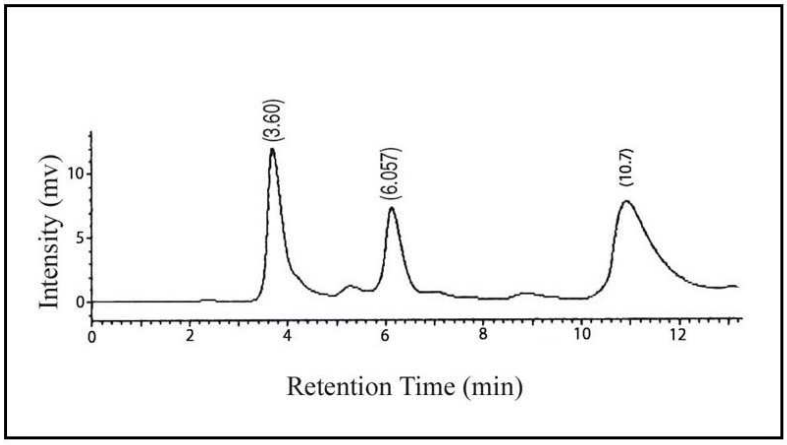


Fig-4 Finasteride and Tamsulosin in formulation with Internal Standard

Limit of Detection and Limit of Quantification

The limit of Detection (LOD) and limit of Quantification (LOQ) of the developed 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 Finasteride and Tamsulosin were found to be 1.25 &.095. 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 for Finasteride and Tamsulosin were found to be 4.166 &1.6335.

Recovery Studies

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

Table-4 Percentage Recovery

Drug	Amount Added (µg/ml)	Amount Recovered (µg/ml)	% Recovery
Finasteride	125 250 500	125.95 249.87 499.46	100.76 99.94 99.98
Tamsulosin	20 30 40	19.7 29.72 39.92	98.5 99.06 98.55

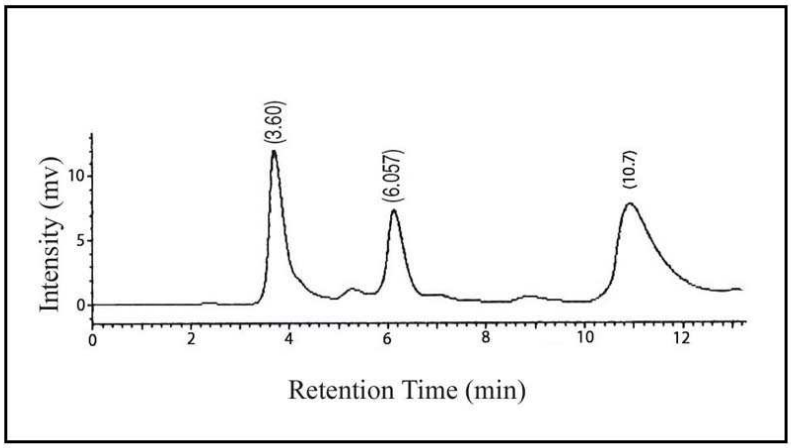


Fig- 4 Recovery of Finasteride and Tamsulosin with Internal Standard

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. (or) The USP (2000)[10] defines parameters that can be used to determine system suitability prior to analysis. 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 Finasteride and Tamsulosin combination in pharmaceutical formulations was validated or not. The results are recorded in **Table- 5**

Table -5

S.no.	Parameters	Obtained Values	
		Finasteride	Tamsulosin
1.	Theoretical plates (N)	2400	2300
2.	Resolution (R)	2.345	2.652
3.	Tailing factor (T)	1.221	1.097
4.	LOD (µg/ml)	1.25	0.495
5.	LOQ (µg/ml)	4.166	1.6335

RESULTS AND DISCUSSION

From the optical characteristics of the proposed method it was found that the drug obeys linearity within the concentration range of 125-625 µg/ml for Finasteride 10-50 µg/ml for Tamsulosin. From the results shown precision **table-2** 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 **table -4** it was found that the percent recovery values of pure drug from the preanalysed solutions of formulations were in between 98.12-100.76%, which indicates that the method is accurate, which reveals that commonly used excipients and additives present in the pharmaceutical formulations did not interfere in the proposed method. The system suitability parameters are within the specified limits for the proposed method.The proposed method was found to be simple, precise, accurate and rapid for simultaneous determination of Finasteride and 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 Finasteride and 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 Finasteride and Tamsulosin in pure form and its dosage forms and can also be used for dissolution or similar studies.

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