WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 2, 883-895. Research Article ISSN 2277– 7105

DEVELOPMENT AND VALIDATION OF A RP- HPLC METHOD FOR DETERMINATION OF FINASTERIDE IN PHARMACEUTICAL DOSAGE FORMS

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Article Received on
27 Nov. 2019,

Revised on 17 Dec. 2019,
Accepted on 07 Jan. 2020,

DOI: 10.20959/wjpr20202-16648

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ABSTRACT

To develop a simple, cheap, accurate, precise, linear and rapid Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method and validate as per ICH & USP guidelines for the quantitative estimation of Finasteride in pharmaceutical dosage forms. The separation was conducted by using mobile phase consisting of acetonitrile:water in the ratio (60:40). The wavelength was found at 245nm. Chromatographic determination was performed on Agilent 1220 Infinity LC with ezchrome software with variable wavelength detector. The separation was conducted at the flow rate of 1.10 ml/min using variable wavelength detector. The developed method resulted in finasteride eluting at 3.71min. The method was found to be linear over the concentration range 2-12µg/ml with coefficient regression R2 –0.9994. The precision is exemplified by relative standard deviation of 1.15 to 1.8%. Percentage Mean recovery was found to be in the range of 97 to 99%, during accuracy studies. The limit of detection (LOD) and limit of quantitiation (LOQ) was found to be 1.783ng/ml and 5.40 ng/ml respectively. A cheap, accurate, precise, linear and rapid RP-HPLC method was developed and validated for the quantitative estimation of finasteride tablets as per ICH guidelines and hence it can be used for the routine analysis in various pharmaceutical industries.

KEYWORDS: RP-HPLC, Finasteride, Method Validation.

INTRODUCTION

5a-reductase inhibitors were developed around 20 years ago. Finasteride was the very specific inhibitor of the human type 2 5a-reductase enzyme developed for clinical use. 5a-reductase inhibitors (5a-RIs), finasteride and dutasteride, have been approved for treatment of lower urinary tract symptoms, because of benign prostatic hyperplasia, with marked clinical efficacy. Finasteride is also permitted for treatment of hair loss (androgenetic alopecia). Although the adverse side effects of these agents are thought to be nominal, the extent of adverse effects on sexual function, Gynecomastia, depression, and quality of life remains ill-defined.

Finasteride is a competitive inhibitor for 5 alpha-reductase enzyme, being currently used as a pharmacological therapeutic approach of male androgenic alopecia and benign prostatic hyperplasia. Administration of Finasteride is able to induce behavioral changes to animals, while in humans depressive symptoms were relatively frequently reported. For this reason, it was suggested for Finasteride to be carefully administered to patients presenting a history or a high risk to developing depression.

In humans, primarily Type I 5á-reductase is found in the sebaceous glands of most regions of skin including scalp, and in liver, muscle, and brain with low levels also present in prostate that may boost in prostate cancer. Approximately one-third of circulating DHT is due to the type I 5á-reductase. The Type II 5á-reductase isozyme is found in prostate, seminal vesicle, epididymis, and hair follicles as well as in liver and is responsible for the remaining two-thirds of circulating DHT. Because of this profile of tissue specific expression and the specificity of finasteride inhibition in humans, few adverse reactions are observed in other organ systems. Finasteride has no affinity for the androgen receptor and exhibits no known androgenic, anti-androgenic, estrogenic, anti-estrogenic, or progesterone-like activity.

The rodent 5á-reductase isozymes also differ in the mechanism by which finasteride inhibits their enzymatic activity. Finasteride ay finasteride at steady-state mean peak concentrations of finasteride were 9.2 ng/mL (25 nmol/L). The volume of distribution of finasteride is 76 L/kg. Its plasma protein binding is 90%. The drug has been found to cross the blood–brain barrier, whereas levels in semen were found to be untraceable. Finasteride is widely metabolized in the liver, It has two major metabolites, which are the *tert*-butyl side chain mono-hydroxylated and mono-carboxylic acid metabolites. These metabolites show approximately 20% of the inhibitory activity of finasteride on 5α-reductase. Hence, the

metabolites of finasteride are not principally active. The drug has a terminal half-life of 5-6 hours in adult men (18–60 years of age) and a terminal half-life of 8 hours or more in elderly men (more than 70 years of age). It is eliminated as its metabolites 57% in the feces and 40% in the urine.

Analytical method development and validation plays an important role in discovery, development and manufacture of pharmaceuticals. Validation is the process of providing documented evidence that the method does what it is intended to do. In other word the process of method validation ensures that the proposed analytical methodology is accurate, specific, reproducible and rugged for its intended use. Analytical techniques have different degrees of sophistication, sensitivity and selectivity, as well as, different cost and time requirements. First stage in the selection or development of method is to establish what is to be measured and how accurately it should measure. Effective method development ensures that laboratory resources are optimized, while methods meet the objectives required at each stage of drug development. Method validation, required by regulatory agencies at certain stages of the drug approval process, is defined as the "process of demonstrating that analytical procedures are suitable for their intended use.

MATERIALS AND METHODS

Chemicals and Reagents

Water, Methanol, Acetonitrile of Analytical and HPLC grade purchased from Finar Chemicals Pvt Ltd. Company (Gujarat India).

Instrument

HPLC analysis was performed on Agilent 1220 Infinity LC with EZchrome software with variable wavelength detector. With made of Agilent technologies, A manually operating Rheodyne injector with 20 µL sample loop was equipped with the HPLC system., Zobrax Eclipse XDBC18 column (4.6×150×5µm), Electronic weighing balance Sartorius Minebea co. Ltd Hot air oven made of NISCO Company (Biotechniques India), Sonicator made of the Ultrasonic's PCi Analytics sonicator.

METHODS

Selection of Wavelength Suitable wavelength for the HPLC analysis was determined by recording UV spectrum in the range of 200-400 nm for Finasteride. Suitable wavelength selected was 255 nm (Figure 2).

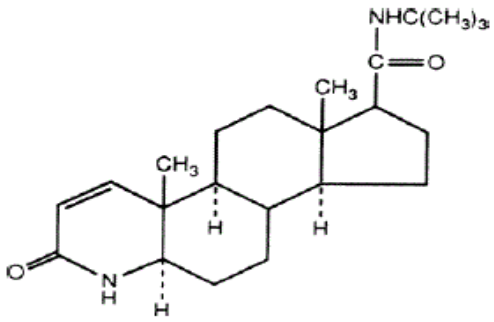

Figure 1: Chemical structure of Finasteride.

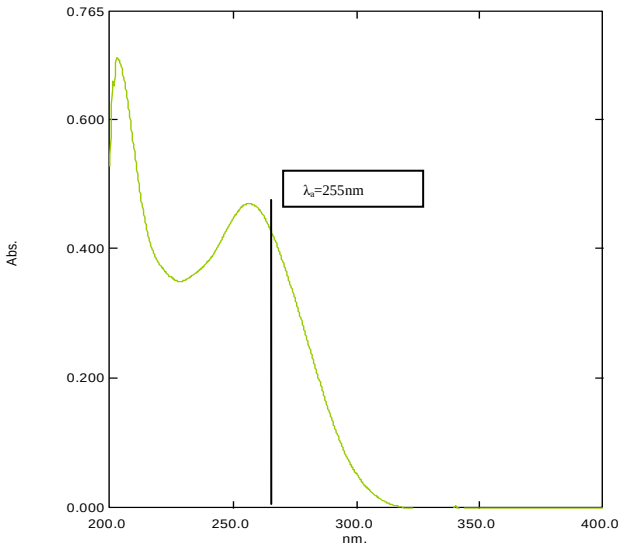

Figure 2: UV spectrum of Finasteride.

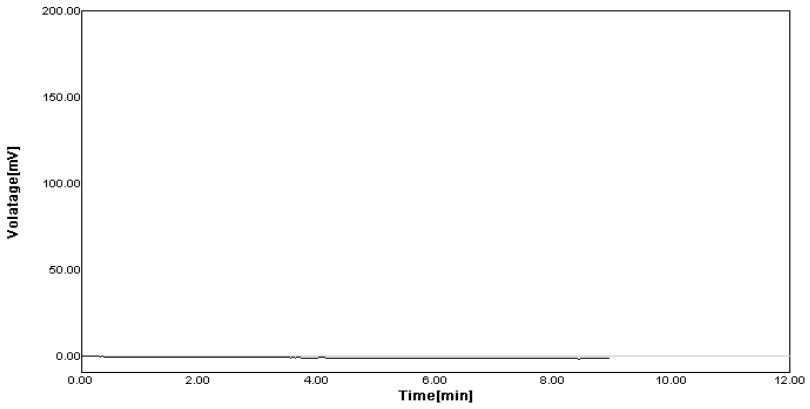

Figure 3: Typical Chromatogram of Blank solution.

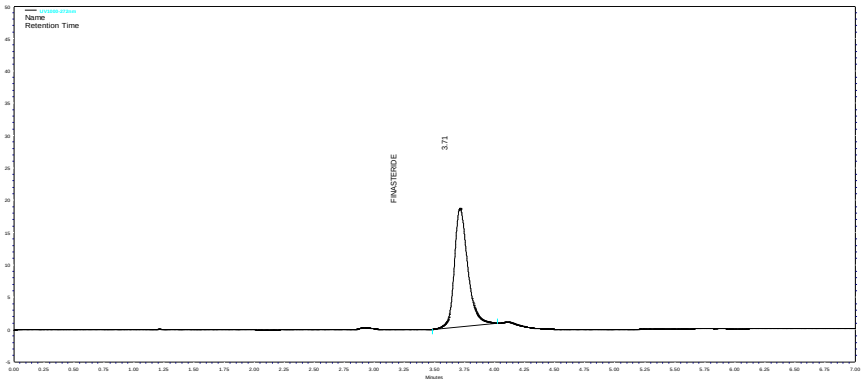

Figure 4: Typical chromatogram of the standard solution.

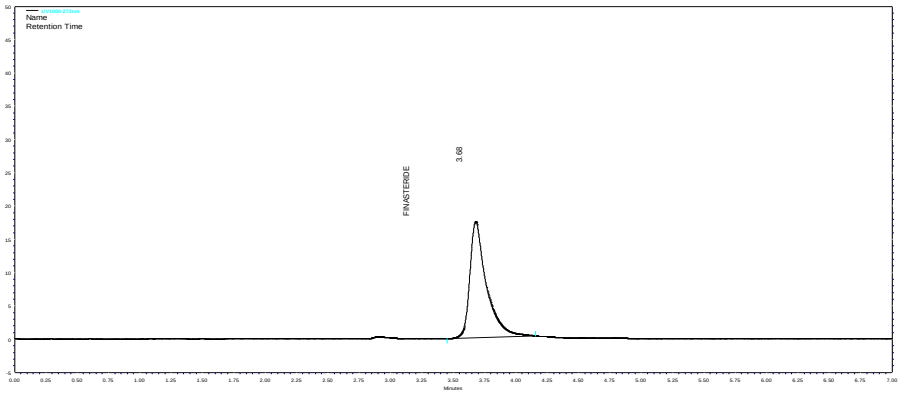

Figure 5: Typical chromatogram for the tablet formulation.

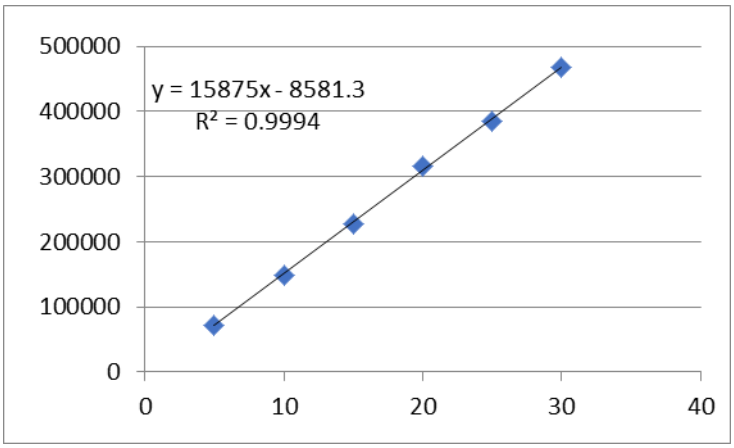

Figure 5: Calibration Curve.

Chromatographic conditions

The developed method uses a reverse phase C18 column, Inertsil ODS 3v (C8, 4.6×250mm, 5µm.), mobile phase consisting of a mixture of Acetonitrile: Water in the ratio (60 : 40). The mobile phase was set at a flow rate of 1.10 ml/min and the volume injected was 10 µl for every injection. The detection wavelength was set at 245 nm.

Selection of solvent

The ideal property of a solvent should be that the drug should be completely soluble in the solvent used. The drug should be stable in the solvent used and should be economical and volatile. After suitable literature survey, practical experience and taking above factors into consideration the suitable solvents selected of acetonitrile and water.

Selection of mobile phase

The samples were subjected to chromatography analysis using system mentioned above and several mobile phases were tried. Accordingly mobile phases comprising methanol, acetonitrile and water in different proportions were tried. Chromatograms were evaluated for parameters such as resolution, capacity factor, no of theoretical plates, and asymmetry factor. Mobile phase that gave a maximum resolution of drug from degradants product at a minimum retention time with good peak shape was chosen for further experiments.

SELECTION OF WAVELENGTH

The standard solution was prepared by dissolving 10 mg of Finasteride in 10 ml Diluent (1000µg/ml). Pipette out 1 ml in 10 ml volumetric flask and make up the volume with Diluent (100µg/ml). From 100µg/ml solution pipette out 1ml in 10ml volumetric flask and make up the volume with Diluent (10µg/ml). Sample was injected in HPLC system with Variable Wavelength Detector. The observed maximum area of drugs is at 245nm.

Preparation of standard stock solution

Weigh accurately 10 mg of Finasteride drug in 100 ml volumetric flask and dissolve in 100µ/ml.

Preparation of sample solution

20 Tablets (each tablet contains 10 mg of Finasteride) were weighed and taken into a mortar uniformly mixed. Test stock solutions of Finasteride (10µg/ml) and was prepared by dissolving weight equivalent to 10 mg of Finasteride and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 100ml with mobile phase. Further dilutions are prepared in 5 replicates of 10 µg/ml of Finasteride was made by adding 1 ml of stock solution to 10 ml of mobile phase.

Calibration Curve

Appropriate aliquots of standard stock solution (1000 µg/ml) was diluted to 100 mg/ml in 10ml volumetric flask and resultant solution was diluted up to the mark with mobile phase to obtain a final concentration of 2,4,6,8,10, & 12 µg/ml. These solutions were injected into chromatographic system and chromatograms were obtained and peak area ratio was determined for each concentration of drug solution. Calibration curve of Finasteride were constructed by plotting peak area ratio vs applied concentration of Finasteride and regression equation was computed. The sample solution was chromatographed and concentration of montelukast sodium in tablet samples was calculated using regression equation.

RESULTS AND DISCUSSION METHOD DEVELOPMENT

A Reverse phase HPLC method was developed keeping in mind the system suitability parameters i.e. tailing factor (T), the number of theoretical plates (N), run time and the cost effectiveness. The optimized method developed resulted in the elution of finasteride at 3.68 min. Figures 3 & 4 represent chromatograms of blank and standard solution (10µg/ml) respectively. The total run time is 6 minutes. System suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (Rt), number of theoretical plates (N) and peak Tailing factor (T) were evaluated for six replicate injections of the standard at working concentration. The results are given in Table 1.

Table 1: System suitability studies results.

Sr.No.	Parameters*	Finasteride
1.	Retention time (min)	3.71 min
2.	Number Of Theoretical plates (N)	6527
3.	Tailing factor (T)	1.019
4.	% RSD	0.7104
*Mean of six injections.		

Table 2: Calibration data for Finasteride.

Sr No.	Concentration (µg/ml)	Area
1.	5	7199
2.	10	148039
3.	15	226787
4.	20	315737
.5.	25	385449
6.	30	467382
Slope 1584x		

Table 3: Results of Accuracy studies for Finasteride.

Accuracy Recovery Level	% Recovery (AVG)	Statistical Analysis		
		Mean	SD	%RSD
80	101.59	102.18	1.1452	1.120
80	103.60			
80	101.45			
100	98.94	99.57	0.5485	0.5509
100	99.88			
100	99.90			
120	102.20	102.49	0.5254	0.5127
120	102.18			
120	103.10			

Table 4: Precision data for finasteride.

Sr. No.	Conc. (µg/ml)	Rt	Area	Interday			Rt	Area	Intra day		
				Mean	SD	%RSD			Mean	SD	%RSD
1.	7 ppm	3.70	92614	931110	641.0	0.69	3.68	93834	93137	610.6	0.66
		3.68	93834				3.68	92883			
		3.68	92883				3.68	92695			
2.	17ppm	3.69	284819	283935	2688.9	0.95	3.62	283062	281430	2062.3	0.73
		3.62	286070				3.62	282115			
		3.62	280915				3.62	272112			
3.	27ppm	3.68	421653	421752	770.3	0.18	3.66	421868	422370	1124.1	0.27
		3.66	421036				3.66	4236584			
		3.66	422567				3.66	421585			

Table 5: Robustness data for Finasteride.

		Change in wavelenth (244)		Change in wavelength (246)	
Sr.No.	Conc.	Area	Rt	Area	Rt
1	10	9123590	3.66	9259023	3.69
2	10	9124928	3.68	9189988	3.68
3	10	9125228	3.69	9225228	3.69
4	10	9252858	3.62	9252228	3.68
5	10	9258492	3.68	9356892	3.68
6	10	9123590	3.66	9356982	3.69
Mean		9168114	3.665	9273495	3.6733
SD		87850.9	0.02509	69084.8	0.01366
%RSD		0.74003	0.68458	0.7449	0.3719

Table 6: Change in flow Rate.

		Change in flow rate (0.9)		Change in flow rate (1.10)	
Sr.No.	**Conc.**	**Area**	**Rt**	**Area**	**Rt**
1	10	9123590	3.88	8852773	3.51
2	10	9124928	3.84	8824561	3.51
3	10	9125228	3.88	8656254	3.45
4	10	9252858	3.84	8756445	3.51
5	10	9258492	3.88	8656256	3.50
6	10	9123590	3.87	8753288	3.52
Mean		9168114	3.865	8783228	3.5
SD		87850.9	0.01974	117978.4	0.25298
%RSD		0.74007	0.5109	1.3434	0.72280

Tablet 7: Analysis data for % Assay.

Sr.No.	Conc	Rt	Area	% Assay
1.	10	3.68	9123466	
2.	10	3.66	9124928	
3.	10	3.64	9125228	
4.	10	3.66	9241446	
5.	10	3.68	9258492	99.94%
6.	10	3.68	9123590	
Mean		3.666	9166191	
SD		0.016329	65120.90	
%RSD		0.71043	0.71043	

METHOD VALIDATION

Validation of the analytical method is the process that establishes by laboratory studies in which the performance characteristics of the method meet the requirements for the intended analytical application. RPHPLC method developed was validated according to International Conference on Harmonization (ICH) and USP guidelines for validation of analytical procedures. The method was validated for the parameters like system suitability, specificity, linearity, accuracy, precision, ruggedness, limit of detection (LOD) and limit of quantitiation (LOQ).

System suitability parameters

The system suitability parameters were determined by preparing standard solutions of Finasteride and the solutions were injected six times and the parameters like % RSD, peak tailing, resolution and USP plate count were determined. The results are mentioned in Table 1. The standard chromatogram is shown in Fig. 4.

Specificity

Figures 3-5 for blank, standard drug solution and sample chromatogram reveal that the peaks obtained in the standard solution and sample solution at working concentrations are only because of the drugs as blank has no peak at the retention time of finasteride. Accordingly it can be concluded that, the method developed is said to be specific.

Linearity

Linearity the method was tested from 80-120% of the targeted level of the assay concentration for analyte. Standard solutions contained 2-12 µg/mL of finasteride. Linearity solutions were injected in triplicate. The calibration graphs were obtained by plotting peak area against the concentration of the drugs. The equations of the calibration curves for the calibration graphs were found to be linear in the aforementioned concentrations with correlation coefficients 0.9994. The results are mentioned in Table 2 & calibration curve Fig.5.

Accuracy

Accuracy was determined by means of recovery experiments, by the determination of % mean recovery of sample at three different levels (80-120%). 20 blank tablets were powdered and mixed. This powder was then spiked with a quantity of finasteride. corresponding to 80%, 100% and 120% of the labeled claim. Each of these powder mixtures was analyzed in triplicate and the quantity of Finasteride was determined using calibration equation. Accuracy was reported as 989.57% of finasteride recovered. The results are mentioned in Table 3.

Precision

System precision

Six replicate injections of the standard solution at working showed % RSD (Relative Standard) less than 2 concerning the peak area for the drug, which indicates the acceptable reproducibility and thereby the precision of the system. System precision results are tabulated in Table 4.

Method precision

Method precision was determined by performing assay of sample under the tests of (i) repeatability (Intraday precision) and (ii) Intermediate precision/ Ruggedness/ Inter day precision) performed during 3 consecutive days by three different analysts, at working concentration.

Repeatability (Intraday precision)

Six consecutive injections of the sample from the same homogeneous mixture at working concentration showed % RSD less than 2 concerning % assay for the drug indicate that the method developed is method precise by the test of repeatability and hence can be understood that the method gives consistently. reproducible results shown in Table 4.

Intermediate Precision (Ruggedness / Inter day precision)

Six consecutive injections of the sample solution from the same homogeneous mixture at working concentration on three consecutive days by three different analysts, The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variation in the analytical procedure parameters.

Robustness

To evaluate HPLC method robustness a few parameters were deliberately varied. Change in wavelength ± 2nm, Change in flow rate ±0.2 ml/min. The results are mentioned in Table 5 & 6.

The % RSD less than 2 for % assay for the drug within and between days, which indicate the method developed is inter day precise / rugged.

Limit of Detection (LOD)

Limit of detection (LOD) of an analytical method may be defined as the concentration, which gives rise to an instrument signal that is significantly different from the blank. For spectroscopic techniques or other methods that rely upon a calibration curve for quantitative measurements, the IUPAC approach employs the standard deviation of the intercept (Sa), which may be related to LOD and the slope of the calibration curve, b. The limit of detection was found to be 1.783 µg/ml.

Limit of Quantification (LOQ)

The LOQ is the concentration that can be quantitate reliably with a specified level of accuracy and precision. The LOQ represent the concentration of analyte that would yield a signal-to-noise ratio of 10. The limit of quantification was found to be 5.40 µg/ml.

Analysis data for % Assay

The peak at 3.68 was observed for Finasteride in the chromatogram of the drug sample extracted from tablet. Experimental results of the amount of Finasteride in tablet, expressed

as percentage of label claim were in good agreement with the label claims thereby suggesting that here is no interference from any excipients, which are normally present in tablet. The drug content was found to be 99.94% and % RSD found to be is 0.71043 for Finasteride in tablet form.

CONCLUSION

A reverse phase HPLC isocratic method developed has been validated as per ICH & USP guidelines in terms of specificity, accuracy, precision, linearity, ruggedness, limit of detection and limit of quantitation, for the quantitative estimation of finasteride in pharmaceutical dosage form. A good linear relationship was observed for the drug between concentration ranges of 2-12µg/ml. The inter day and intraday precision results were good enough to say that the method developed is precise and reproducible. Accuracy studies revealed that mean recoveries were between 97 and 99%, an indicative of accurate method. Accordingly it can be concluded that the developed reverse phase isocratic HPLC method is accurate, precise, linear and rugged and therefore the method can be used for the routine analysis of finasteride in pharmaceutical dosage form.

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