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SIMULTANEOUS DETERMINATION OF FINASTERIDE AND TAMSULOSIN IN COMBINED DOSAGE FORM BY USING RP-HPLC METHOD

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□ *A simple, accurate, economical, and reproducible reversed-phase high performance liquid chromatographic (RP-HPLC) method was developed and validated for the simultaneous determination of finasteride and tamsulosin in bulk and pharmaceutical formulations. The separation was achieved on a phenomenex C18 column (150 × 4.6 mm i.d, particle size of 5 μ) using a mixture of 0.1% triethylamine (pH adjusted to 7.01 ± 0.05 with 0.1% ortho phosphoric acid) and methanol in the ratio of 30:70% v/v as the mobile phase in an isocratic elution mode, at a flow rate of 0.7 mL/min. The detection was monitored at 220 nm. The retention times of finasteride and tamsulosin were found to be 5.8 ± 0.12 min and 2.9 ± 0.14 min, respectively. Excellent linearity range was found between 10–100 μg/mL for finasteride and 2–8 μg/mL for tamsulosin. The method was validated with respect to linearity, precision, accuracy, specificity, robustness, and ruggedness. The method was successfully applied for the simultaneous determination of finasteride and tamsulosin from the combined dosage form.*

Keywords finasteride, ICH, LOD, LOQ, RP-HPLC method, tamsulosin

INTRODUCTION

Finasteride (FIN), N- (1,1-dimethylethyl) -3-oxo-4-aza-5-androst-1-ene-17-carboxamide (Figure 1a)[1] is 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. The inhibition of 5-alpha reductase results in the decreased level of dihydrotestosterone leading to reduction of prostate size. Tamsulosin (TAM), 5-[(2 R)-2[[2-(2-ethoxy phenoxy) ethyl]

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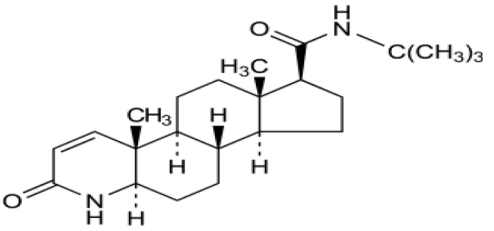
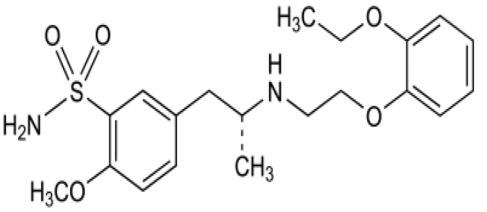


(a) finasteride



(b) tamsulosin

FIGURE 1 Structures of (a) finasteride and (b) tamsulosin.

amino]propyl}-2-methoxy benzene sulfonamide (Figure 1b)[2] is a selective alpha 1 adrenoceptor blocking agent. Smooth muscle tone is mediated by the sympathetic nervous stimulation of alpha 1 adrenoceptors, which are abundant in the prostate, prostatic capsule, prostatic urethra, and the 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 reduction in symptoms of benign prostatic hyperplasia (BPH).[3,4] TAM and FIN combination results in reduction of BPH progression to acute urinary retention.[5] Combination of TAM and FIN results in statistically significant benefits in quality of life scores, patient satisfaction, and urinary frequency.

A literature survey reveals that only a few analytical methods such as Visible,[6] UV,[7,8] and HPLC[9–13] methods were reported for FIN and TAM. Manish Kumar Thimmaraju et al.[14] executed the separation using a mobile phase of acetonitrile : KH_2PO_4 buffer (45:55) at a flow rate of 1.8 mL/min with a run time of 12 min. This enunciates the need for the development of a new method as flow rate of 1.8 mL/min directly increases the cost of analysis. Moreover, the use of 55% of buffer is not advisable because as the buffer percentage increases, there will be more chances of pressure fluctuations during the analysis. There also exists a scope of improvement in the matter of sensitivity as reported LOD and LOQ values seemed high.

Patel and Patel[15] reported the separation of said combination using methanol:ammonium acetate:triethylamine, pH 9.2 (79.9:20:0.1 *v*/*v*), flow

rate of 1.0 mL/min with a run time of 8 min. This method can be considered for further modifications as exposure of alkaline pH 9.2 to a silanol based C18 column is not recommended because silanol groups tend to adsorb organic bases leading to the development of back pressure during the re-conditioning step.

The objective of this study was to develop and validate a simple, precise, economical, and reproducible RP-HPLC method for the simultaneous determination of FIN and TAM in bulk and pharmaceutical preparations.

EXPERIMENTAL

Apparatus

A SHIMADZU (Japan) HPLC instrument (LC-20AD) equipped with a UV-Visible detector, a rheodyne injector with a 20-µL loop, a phenomenex C18 column (150 mm × 4.6 mm i.d, 5 μ particle size), and LC-Solution software were used. Other instruments included were a SHIMADZU electronic balance, a BL-220H (SHIMADZU corp., Japan), a fast, clean ultrasonic cleaner, and a value 1 stage vacuum pump (Model VE115).

Materials

FIN and TAM pure powder were gift samples supplied from Intas Pharmaceutical Limited, India. Methanol (HPLC grade) and acetonitrile (HPLC grade) were purchased from Merck Ltd, India; triethylamine (AR grade) and ortho phosphoric acid (AR grade) were purchased from S.D. Fine Chem. Ltd, India. Water for HPLC was prepared by triple glass distillation and filtered through a 0.45-µ membrane filter (Gelman Laboratory, India).

Pharmaceutical Formulation

Formulation, Urimax F (5 mg FIN + 0.4 mg TAM per capsule), manufactured by Cipla Pharmaceutical Ltd, India, was purchased from the Apollo Pharmacy in Hyderabad, India.

Method Development and Optimization of Chromatographic Conditions

Chromatographic separation was performed on Shimadzu HPLC with Phenomenox C18 column (150 × 4.6 mm i.d, particle size of 5 μ) and constant flow pump. A wavelength of 220 nm was selected for the study.

Initially different combinations of water:acetonitrile, water:methanol, and water:acetonitrile: methanol were tried, but none of the combinations resulted in a proper resolution of the peaks. Then, different buffers were tried for the separation; those with 0.1% triethylamine buffer were found to be ideal for the work. Then, different pHs of the buffer were tried, out of which triethylamine adjusted to pH 7.01 with ortho phosphoric acid and produced good peaks. Then, the ratio of mobile phase was determined by varying the proportion of triethylamine and methanol. Finally, the mixture of 0.1% triethylamine buffer adjusted to pH 7.01 with ortho phosphoric acid and methanol (30:70% *v*/*v*) was selected for the simultaneous determination of both drugs. The retention times of TAM and FIN were found to be 2.9 and 5.8 min, respectively. Analysis was performed at ambient temperature. A chromatogram of standard drugs is shown in Figure 2. Optimization trials are listed in Table 1.

Preparation of Mobile Phase

The selected mobile was prepared by dissolving 0.1 mL of triethyamine in 100 mL of tripled distilled water, mixed thoroughly, and the pH of the solution was adjusted to 7.01 ± 0.05 with 0.1% ortho phosphoric acid. The buffer and methanol were mixed in the ratio of 30:70% *v*/*v*.

Preparation of Standard FIN and TAM Solutions

Each of FIN and TAM 10 mg were dissolved in 100 mL of mobile phase to obtain standard stock solutions of 100 µg/mL. Further working standard solutions of FIN and TAM, 10–100 µg/mL and 2–8 µg/mL, respectively,

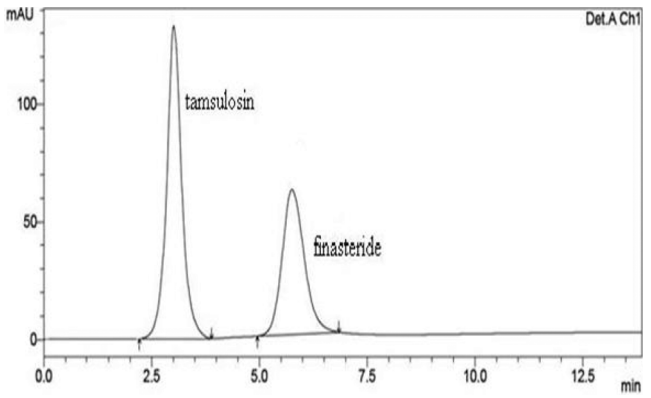


FIGURE 2 A typical chromatogram of standard tamsulosin (4 µg/ml) and finasteride (50 µg/mL) measured at 220 nm.

TABLE 1 Optimized Chromatographic Conditions

S. No	Mobile Phase Conditions	Observation
1	Water : Acetonitrile (10:90% *v*/*v*)	Less resolution
2	Water : Acetonitrile (20:80% *v*/*v*)	Broad peaks
3	Water : Acetonitrile : Methanol (20:75:5% *v*/*v*)	Fronting and Less resolution
4	Water : Methanol (30:70% *v*/*v*)	Fronting
5	0.1% Ortho phosphoric acid (pH adjusted to 4.01 with 0.1% Triethylamine): Methanol (30:70% *v*/*v*)	Broad peak shape and Tailing
6	0.1% Triethylamine (pH adjusted to 5.01 with 0.1% Ortho phosphoric acid): Methanol (30:70% *v*/*v*)	Broad peak shape and Tailing
7	0.1% Triethylamine (pH adjusted to 6.01 with 0.1% Ortho phosphoric acid): Methanol (30:70% *v*/*v*)	Broad peak shape and Tailing
8	0.1% Triethylamine (pH adjusted to 7.01 with 0.1% Ortho phosphoric acid): Methanol (30:70% *v*/*v*)	Symmetrical peaks
9	0.1% Triethylamine (pH adjusted to 7.01 with 0.1% Ortho phosphoric acid): Methanol (40:60% *v*/*v*)	Tailing
10	0.1% Triethylamine (pH 7.01): Methanol (30:70% *v*/*v*) with flow rate of 0.7 mL/min	Good resolution and sharp peaks

were prepared by suitable dilution of the stock solutions with the mobile phase.

Preparation of Sample Solution

For analysis of commercial formulations, 20 capsules were weighed and powdered. A weight equivalent to 10 mg and 0.8 mg of FIN and TAM, respectively, was taken and transferred into a 100-mL volumetric flask and dissolved with the mobile phase, filtered through a Whatman filter paper, and the solution was further diluted stepwise with a mobile phase to obtain the concentration within the linearity range.

Analysis of Formulation

The amount of drug present in the pharmaceutical formulation was calculated through the peak area by making use of the standard calibration curve (concentration in μg/mL on X-axis and peak area on Y-axis), the results are shown in Table 2.

TABLE 2 Analysis of Formulation

Drug Name	Amount Labeled (mg)	Amount Estimated (mg)	% Label Claim	% Deviation
Finasteride	5 mg	4.950	99.00	(−) 1.0
Tamsulosin	0.4 mg	0.397	99.25	(−) 0.75

RESULTS

Once the RP-HPLC method development was over, the method was validated in terms of parameters such as linearity, precision, LOD, LOQ, recovery, specificity, robustness, and ruggedness. The proposed RP-HPLC method was validated as per ICH (Q2B) guidelines.[16]

Linearity

The linearity measurement was evaluated by analyzing different concentrations of the standard Solutions of FIN and TAM. The Beer lamberts concentration was found to be between 10–100 μg/mL for FIN and 2–8 μg/mL for TAM. The calibration curve was constructed by plotting peak area against concentration, and the regression equation was computed. The slope, intercept, and correlation coefficient values are shown in Table 3.

Precision

The precision of the method was ascertained separately from the peak areas obtained by the actual determination of three replicates of three different concentrations taken within the linearity range for both the drugs. The intra- and inter-day variation in the peak areas of the drug solution was calculated in terms of percentage relative standard deviation [RSD (%)] and the results are presented in Table 4.

Recovery Studies

To determine the accuracy of proposed method recovery studies (50%, 100%, and 150%) were carried out by taking different amounts of bulk sample of FIN and TAM within the linearity range were taken and added to the pre-analyzed formulation. From that percentage recovery values were calculated and the results are presented in Table 5.

TABLE 3 Regression Analysis of the Calibration Curves

Parameters	Tamsulosin	Finasteride
Concentration range	2–8 μg/mL	10–100 μg/mL
Slope	45261	43059
Intercept	46378	39086
Correlation coefficient (r^2)	0.999	0.999

TABLE 4 Precision Studies

Drug	Concentration (μg/mL)	Intra-Day Concentration		Inter-Day Concentration	
		*Mean (μg/mL)	RSD (%)	*Mean (μg/mL)	RSD (%)
Finasteride	40	39.45	0.763	39.12	1.28
	50	48.90	1.250	48.50	1.36
	60	59.10	0.980	59.10	1.15
Tamsulosin	4	3.95	0.790	3.89	1.42
	5	4.93	0.860	4.87	1.30
	6	5.86	1.470	5.92	1.02

*mean of three observations.

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 produces a measurable response (signal-to-noise ratio of 3). The LOD values were found to be 0.2 μg/mL and 1.25 μg/mL for TAM and FIN, respectively (Figure 3 and Figure 4). The LOQ is the smallest concentration of the analyte that produces a response that can be accurately quantified (signal-to-noise ratio of 10). The LOQ values were found to be 7.5 μg/mL and 1.8 μg/mL for FIN and TAM, respectively.

Specificity

The commonly used excipients such as carboxy methyl cellulose (CMC), talc, starch, and magnesium stearate were mixed in appropriate proportions. They were spiked with the standard solution containing TAM and FIN. The specificity was assessed by the comparison of chromatogram of spiked solution with the pure drug solution. The method was found to be specific as there were no interferences observed with the analytes of interest.

TABLE 5 Recovery Studies

Drug	Level	Amount Added (μg/mL)	Amount Recovered (μg/mL)	% Recovery
Finasteride	50%	10	10.150	101.50
	100%	20	19.820	99.10
	150%	30	29.870	99.56
Tamsulosin	50%	1	1.012	101.20
	100%	2	1.980	99.00
	150%	3	3.020	100.66

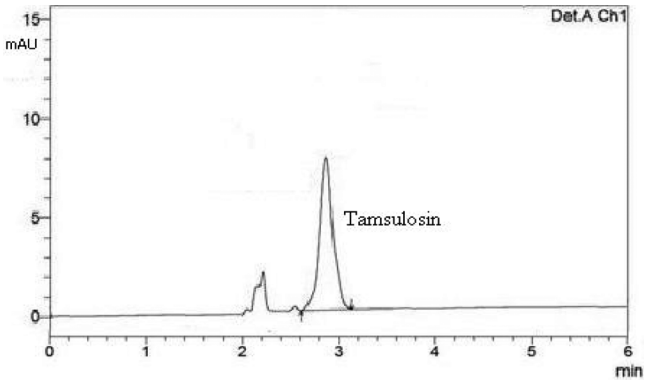


FIGURE 3 Chromatogram for limit of detection of tamsulosin.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate, variations in method parameters and provides an indication of its reliability during normal usage. The robustness of the method was investigated under a variety of conditions including changes of composition of buffer in the mobile phase and flow rate. The RSD (%) of TAM & FIN was calculated for each condition. Results are given in Table 6.

Ruggedness

The ruggedness of the method was assessed by comparison of the intra-day and inter-day assay results for FIN and TAM that has been performed by two analysts. The RSD (%) values for assays performed in

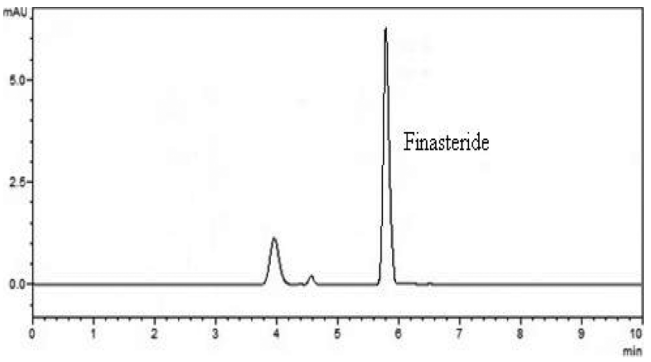


FIGURE 4 Chromatogram for limit of detection of finasteride.

TABLE 6 Robustness

Parameters	Tamsulosin		Finasteride	
Change in Flow rate (mL/min)	RT (min)*	RSD (%)	RT (min)*	RSD (%)
0.70 (original)	2.90	0.08	5.81	0.12
0.75	2.85	0.12	5.76	0.19
0.65	2.94	0.16	5.85	0.15
Change in Mobile phase composition (0.1% triethylamine (pH 7.01) : Methanol (% *v*/*v*)				
30:70 (original)	2.90	0.09	5.81	0.11
28:72	2.88	0.21	5.80	0.26
32:68	2.91	0.17	5.84	0.32

*Mean of three observations.

TABLE 7 System Suitability

Drug	Tamsulosin	RSD (%)*	Finasteride	RSD (%)*
Retention time	2.90	0.10	5.81	0.13
Tailing Factor	1.128	0.18	1.252	0.15
Theoretical plates	4186	0.24	5978	0.32

*Mean of three observations.

the different laboratories by two analysts were found to be less than 2, indicating the ruggedness of the method.

System Suitability

Six injections of 5 µg/mL (TAM) and 50 µg/mL (FIN) of the standard solution were given by increasing the injection volume and results are given in Table 7. The RSD (%) obtained for the retention time, tailing factor, and theoretical plates was found to be less than 2%, which indicated that the present method is suitable for the simultaneous analysis of TAM and FIN.

DISCUSSION

The mixture of 0.1% triethylamine buffer (pH 7.01 ± 0.05) and methanol (30:70% *v*/*v*) was employed for the simultaneous determination of both the drugs and supersedes the problems because of extreme pH conditions. The flow rate used was 0.7 mL/min, which directly reduces the cost of analysis and this parameter differentiated the developed method from the reported methods. The retention times of TAM and FIN were found to be 2.9 and 5.8 min, respectively. The developed method was validated as per ICH guidelines.[16] Calibration graphs were plotted using

standard peak areas versus the concentration of standard solutions. The slope, intercept, and correlation coefficient values were found to be 45261, 46378, and 0.999; and 43059, 39086, and 0.999 TAM and FIN, respectively. TAM was found to be linear in the range of 2 to 8 μg/mL and FIN in the range of 10 to 100 μg/mL.

The LOD of TAM and FIN were found to be 0.2 μg/mL and 1.25 μg/mL, respectively, indicating the sensitivity of the method compared to the reported methods. The LOQ of TAM and FIN were found to be 1.8 μg/mL and 7.5 μg/mL, respectively. Precision of the developed method was studied under intra-day and inter-day precision. Low RSD (%) values indicate that the method is precise. The recovery studies were carried out in three levels, that is, 50%, 100%, and 150% to ensure the reproducibility and reliability of the method by adding known amounts of standard drugs and analysis was carried out as per formulation procedure. The recovery values were found within the limits indicating that the method is accurate. System suitability parameters such as the number of theoretical plates (N) and tailing factor were studied and results indicated that the method is suitable for the simultaneous analysis of TAM and FIN.

CONCLUSION

A convenient and rapid RP- HPLC method has been developed for the estimation of FIN and TAM in combined dosage form. The assay provides a linear response across a wide range of concentrations. Low RSD (%) values of intra-day and inter-day precision and excellent recoveries indicate the repeatability and accuracy of the method. Hence, this method can be conveniently adopted for routine analysis of FIN and TAM in pure form and its dosage forms can also be used for dissolution or stability studies.

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