

HPTLC Method Validation for simultaneous determination of Tamsulosin Hydrochloride and Finasteride in Bulk and Pharmaceutical Dosage Form

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Summary

A new simple, precise, accurate and selective TLC-densitometry method has been developed for simultaneous determination of tamsulosin hydrochloride and finasteride in tablet dosage form. Chromatographic separation was performed on aluminum plate precoated with silica gel 60 F₂₅₄ using toluene: n-propanol: triethylamine (3.0:1.5:0.2 v/v) as mobile phase. Detection was carried out densitometrically at 260 nm. The RF value of tamsulosin hydrochloride and finasteride were 0.32 and 0.54, respectively. The reliability of the method was assessed by evaluation of linearity which was found to be 200 – 1200 ng/spot for tamsulosin hydrochloride and 1000 - 6000 ng/spot for finasteride. Accuracy of the method was assessed by percentage recovery and found to be 99.77 ± 0.71 % for tamsulosin hydrochloride and 99.75 ± 0.86 % for finasteride. The method can be used for routine analysis of tamsulosin hydrochloride and finasteride in tablet dosage form.

Keywords: Tamsulosin hydrochloride; Finasteride; TLC/densitometry; Validation

Introduction

Benign Prostate Hyperplasia (BPH) is a common condition in aging men. Chemically tamsulosin hydrochloride is [(-)-(R)-5-[2-[[2-(O-ethoxyphenoxy) ethyl]amino]propyl]-2-methoxybenzenesulfonamide] and is official in Martindale – The Extra Pharmacopoeia and Merck Index [1,2]. The chemical structures are shown in Figure 1, whereas chemically finasteride is N-(1,1-dimethylethyl)-3-oxo-(5 α ,17 β)-4-azaandrost-1-ene-17-carboxamide and official in Merck Index [1,2]. The chemical structures are shown in Figure 1.

Literature survey reveals various chiral separation techniques [3-6] along with impurities [7] for tamsulosin. More over LC [8, 9] coupled with MS-MS technique [10] is described for finasteride. Spectrophotometric method [11] and simultaneous estimation for tamsulosin and dutasteride is also given [12-13].

The purpose of this work is to establish and validate a simple accurate and reproducible procedure for quantitative TLC analysis of tamsulosin hydrochloride and finasteride in bulk and tablet dosage form as per ICH guidelines [14, 15].

Experimental

Chemicals and reagents

Tamsulosin hydrochloride and Finasteride was kindly gifted from

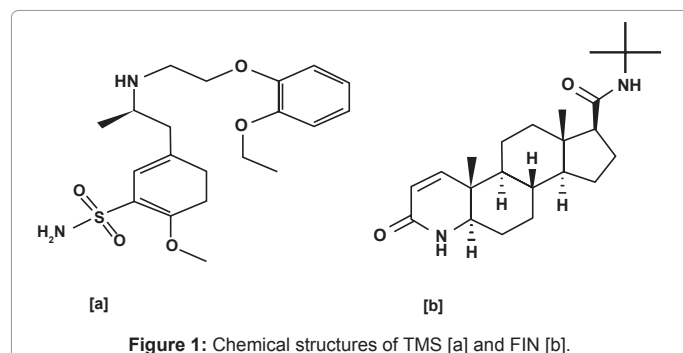
Sun Pharmaceuticals, Vapi (Gujarat), India. Urimax-F tablets containing 0.4 mg of tamsulosin hydrochloride and 5 mg of finasteride were obtained from commercial sources within their shelf life period. All the reagents and solvents used were of HPLC grade.

HPTLC instrumentation

The samples were spotted in the form of bands of width 6mm with a Camag 100 μ l sample (Hamilton, Bonaduz., Switzerland) syringe on precoated silica gel aluminium plate 60 F₂₅₄ (20 cm x 10 cm with 0.2 mm thickness), supplied by Anchrom technologists, (Mumbai) using a Camag Linomat applicator 5 (Switzerland). A constant application rate of 150 nl sec⁻¹ was employed and space between two band was 15 mm. The slit dimension was kept 6 mm x 0.45 mm. The mobile phase consisted of toluene: n-propanol: triethylamine (3.0:1.5:0.2 v/v). The optimized chamber saturation time for mobile phase was 20 min at room temp (25°C ± 2) and relative humidity 60% ± 5. The length of chromatogram run was approximately 80 mm. Subsequent to the development; TLC plates were dried in current of air with the help of an air dryer. Densitometric scanning was performed using Camag TLC scanner 3 in the absorbance mode at 260 nm and software used was winCATS 4.0.5. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum in the range of 190 - 400 nm.

Preparation of standard solutions for linearity studies

An accurately weighed quantity of 10 mg TAM and 50 mg of FIN were transferred to two different 10 ml volumetric flasks, dissolved in methanol and volume was made up to mark with the same solvent to obtain concentration 1000 ng/ μ l of Tam and 5000 ng/ μ l of FIN respec-



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tively. From these solutions 2 ml stock solution of TAM and FIN was transferred to 10 ml volumetric flask and made up to mark. Aliquots of standard solutions 1, 2, 3, 4, 5 and 6 μ l of TMS and FIN were applied on TLC plate with the help of microlitre syringe, using Linomat 5 sample applicator to obtained the concentration of 200, 400, 600, 800, 1000 and 1200 ng per spot of TMS and 1000, 2000, 3000, 4000, 5000 and 6000 ng per spot of FIN respectively.

Method validation

Accurately weighed 10 mg of TMS and 50 mg of FIN were transferred to 10 ml volumetric flask, dissolved in methanol and volume was adjusted to mark. This mix standard solution was used for validation study.

Precision: Repeatability of measurement of peak area was determined by spotting 400 ng/spot of TMS and 2000 ng/spot of FIN. Precision of the method was assessed by intra-day and inter-day variations. Intra-day variations were assessed by spotting 400, 600, 800 ng/spot of TMS and 2000, 3000, 4000 ng/spot of TMS on TLC plate on three different times within the same day. Inter-day variations were performed by analyzing same concentrations described above for TMS and FIN in three different days over a period of week.

Specificity: Specificity of the method was ascertained by analyzing standard drug and sample. The mobile phase resolved both the drugs very efficiently, as shown in Figure 2. The spot for TMS and FIN was confirmed by comparing the RF and spectra of the spot with that of standard. A typical absorption overlain spectrum of TMS and FIN shown in Figure 3; wavelength 260 nm was selected for densitometric scanning. Peak purity of TMS and FIN was assessed by comparing the spectra of sample with that of standard at three different levels, i.e., peak start (S), peak apex (M) and peak end (E) positions.

Accuracy: The pre-analyzed samples were over spotted with extra 80, 100 and 120 % of the standard drug solution of TMS and FIN on TLC plate. The total concentrations of the drugs were determined. The experiment was conducted in triplicate. This was done to check for the recovery of the drug at different levels in formulation.

Robustness: Robustness of the method was performed by spotting 400 ng of TMS and 2000 ng of FIN on TLC plate by making small deliberate changes in chromatographic conditions. Mobile phases having different composition like toluene: n-propane: triethylamine (3.5: 1: 0.2 v/v/v) and toluene: n-propane: triethylamine (3.0: 1.5: 0.2 v/v/v) were tried and chromatograms were run. The development distance was varied from 7, 7.5 and 8 cm. The amount of mobile phase (4.7 and 9.4) was tried and chromatograms were run. Temperature and relative humidity was varied in the range of $\pm 5\%$. The plates were prewashed by methanol and activated at $60 \pm 5^\circ\text{C}$ for 2, 5 and 7 min prior to chromatography. Duration of saturation time of chamber was varied as 15, 20 and 25 min. Time from spotting to chromatography and time from chromatography to scanning was varied from 0, 20, 40 and 60 min. Robustness of the method was done at three different concentration levels.

Ruggedness: Ruggedness of the method was performed by spotting 400 ng of TMS and 2000 ng of FIN, respectively by two different analyst keeping same experimental and environmental conditions.

Limit of detection (LOD) and limit of quantification (LOQ): In order to determine detection and quantification limit, concentrations in the lower part of the linear range of the calibration curve were used. Stock solutions of TMS (1000 $\mu\text{g}/\text{ml}$) and FIN (5000 $\mu\text{g}/\text{ml}$) were prepared separately and different concentrations 200, 240, 280, 320, 360 and 400 ng of TMS and 1000, 1200, 1400, 1600, 1800 and 2000 of FIN

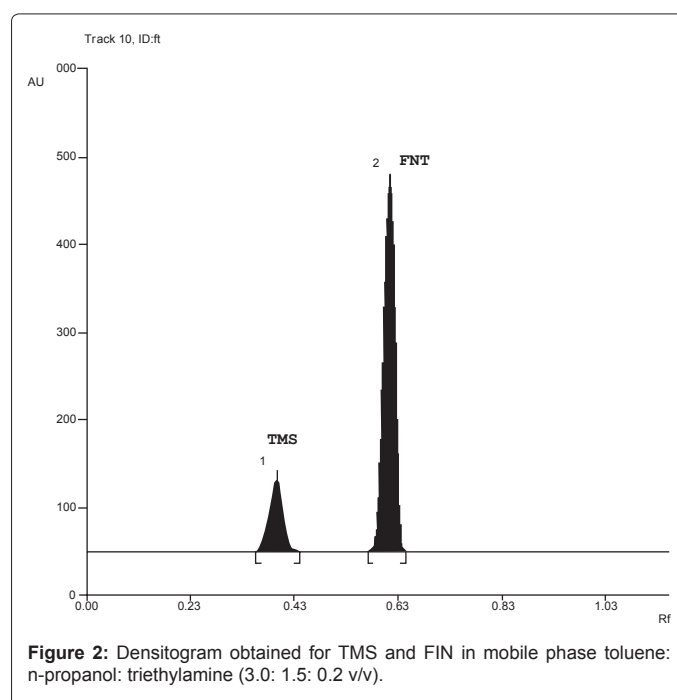


Figure 2: Densitogram obtained for TMS and FIN in mobile phase toluene: n-propanol: triethylamine (3.0: 1.5: 0.2 v/v).

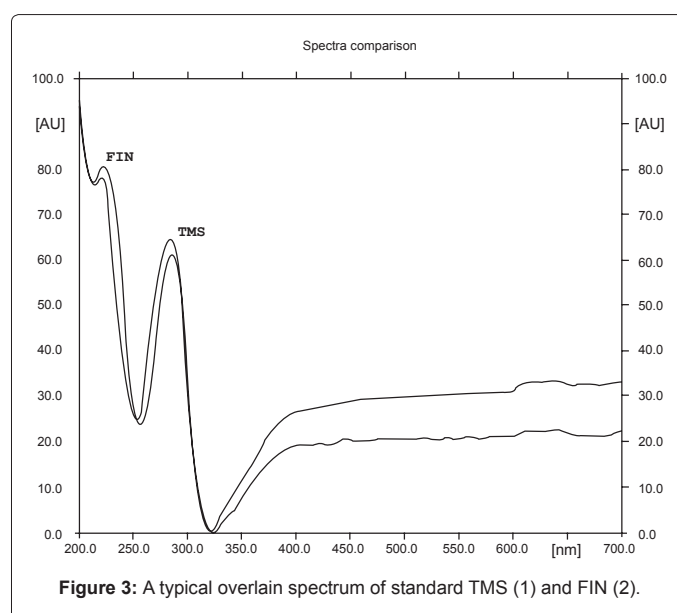


Figure 3: A typical overlain spectrum of standard TMS (1) and FIN (2).

were separately spotted on TLC plates in triplicate. The LOQ and LOD were calculated using equation $\text{LOD} = 3.3 \times \text{N}/\text{B}$ and $\text{LOQ} = 10 \times \text{N}/\text{B}$, where, N is standard deviation of the peak areas of the drugs ($n=3$), taken as a measure of noise, and B is the slope of the corresponding calibration curve.

Application of proposed method to tablet formulation

Twenty tablets were weighed; average weight determined and crushed in to fine powder. An accurately weighed tablet powder equivalent to 4 mg of tamsulosin hydrochloride and 50 mg of finasteride was transferred into 25 ml volumetric flask containing 15 ml methanol, an accurately weighed quantity of standard tamsulosin hydrochloride (6mg) was added to obtain the proportion of 1:5 for TMS and Fin re-

Drugs	Conc. ng/ml	Intra-day		Inter-day	
		% Amount found*	% R.S. D.	% Amount found *	% R.S.D
TMS	400	101.19	1.70	101.34	1.68
	600	99.02	1.04	99.02	0.80
	800	101.76	1.10	101.42	0.65
FIN	2000	99.94	0.68	101.10	1.02
	3000	99.14	0.41	100.20	0.58
	4000	99.48	0.45	99.78	0.59

* mean of three estimations

Table 1: Intra-day and inter-day precision of HPTLC method.

Drugs	Label claim (mg/tablet)	Amount of standard drug added (%)	% Drug Recovered	% R.S.D.
TMS	0.4	0	100.02	1.43
		80	99.74	0.84
		100	99.32	1.28
		120	100.03	1.35
FIN	5	0	99.93	1.46
		80	99.72	0.88
		100	99.36	0.97
		120	99.99	0.60

* mean of three estimations at each level

Table 2: Results of recovery studies*.

Parameter	TMS	FIN
Linearity range (ng/spot)	200 – 1200	1000 – 6000
Correlation coefficient	0.9991	0.9987
Limit of detection (ng/spot)	17.47006	61.98041
Limit of quantitation (ng/spot)	52.93956	187.3935
% Recovery (n = 9)	99.77	99.75
Ruggedness (% R.S.D.)		
Analyst I (n = 3)	1.19	0.95
Analyst II (n = 3)	0.71	0.71
Precision (%R.S.D.)		
Repeatability of application (n = 6)	1.04	0.73
Intra-day (n = 3)	0.65 – 1.86	0.41 -0.68
Inter-day (n = 3)	1.04 – 1.70	0.58 – 1.02
Robustness	Robust	Robust
Specificity	Specific	Specific

Table 3: Summary of validation parameter.

spectively. The solution was sonicated for 10 min and volume was made up to mark with same solvent. The resulting solution was filtered using 0.41 µm filter (Millifilter, Milford, MA). The appropriate volume i.e. 5 µl containing 400 ng of TMS and 2000 ng of FIN was spotted on TLC plate.

Results and Discussion

HPTLC method development and validation

The TLC procedure was optimized with a view to develop method for simultaneous determination of TMS and FIN. The mobile phase toluene: n-propanol: triethylamine 3.0: 1.5: 0.2 (v/v) gave good resolution, sharp and symmetrical peak with R_f value of 0.35 for TMS and 0.60 for FIN. It was observed that prewashing of TLC plates with methanol (followed by drying and activation) and pre-saturation of TLC chamber with mobile phase for 20 min ensure good reproducibility and peak shape of both the drugs.

Validation

Linearity: The linear regression data for the calibration curves showed good linear relationship over the concentration range 200 – 1200 ng/spot for TMS and 1000 – 6000 ng/spot for FIN. Linear regres-

sion equation was found to be $Y = 1.0937 X + 32.884$ ($r^2 = 0.9991$) for tamsulosin hydrochloride and $Y = 0.4696 X + 274.89$ ($r^2 = 0.9987$) for finasteride.

Precision: The precision of the developed HPTLC method was expressed in terms of % relative standard deviation (% R.S.D.). The results depicted revealed high precision of the method is presented in Table I.

LOD and LOQ: Detection limit and quantification limit was calculated by the method as described in Specificity. The LOQ and LOD for TMS were 52.94 ng and 17.47 and for FIN, LOQ and LOD were found to be 187.39 ng and 61.98 ng. This indicates that adequate sensitivity of the method.

Accuracy: The proposed method when used for extraction and subsequent estimation of both the drug from pharmaceutical dosage forms after over spotting with 80, 100 and 120 % of additional drug; afforded recovery of 98 - 102 % as listed in Table II.

Robustness: The standard deviation of peak areas was calculated for each parameter and % R.S.D. was found to be less than 2 %.

Ruggedness of the method: Ruggedness of the method was performed by applying 400 ng and 2000 ng for TMS and FIN, respectively by two different analyst keeping same experimental and environmental conditions. The results summarized in Table III.

Conclusion

The developed HPTLC method is simple, precise, accurate and reproducible and can be used for simultaneous determination of TMS and FIN in tablets. The method was validated as per ICH guidelines.

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