

Research Article

Development and Validation of Stability-Indicating HPTLC Determination of Tamsulosin in Bulk and Pharmaceutical Dosage Form

S. B. Bari, A. R. Bakhshi, P. S. Jain, and S. J. Surana

R. C. Patel Institute of Pharmaceutical Education and Research (RCPIPER), Shirpur, District Dhule, Maharashtra 425 405, India

Correspondence should be addressed to P. S. Jain, pritash79@yahoo.com

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A simple, economic, selective, precise, and stability-indicating high-performance thin-layer chromatographic method for analysis of tamsulosin hydrochloride, both as a bulk drug and in formulations, was developed and validated according to ICH guidelines. The method employed HPTLC aluminium plates precoated with silica gel 60F-254 as the stationary phase while the solvent system consisted of toluene : methanol : triethylamine (3.5 : 1.2 : 0.2 v/v). The system was found to give compact spot for drug (R_f value of 0.52 ± 0.02). Densitometric analysis of tamsulosin was carried out in the absorbance mode at 280 nm. The linear regression analysis data for the calibration plots showed good linear relationship, $r^2 = 0.9982 \pm 0.0012$, with respect to peak area in the concentration range 400–2400 ng per spot. The mean value \pm SD of slope and intercept were 2.6553 ± 0.0173 and 777.7 ± 74.8 with respect to peak area. The method was validated for precision, recovery, and robustness. The limits of detection and quantitation were 20.49 and 62.10 ng per spot, respectively. Tamsulosin was subjected to hydrolysis, oxidation, and thermal degradation which indicate the drug is susceptible to hydrolysis, oxidation, and heat. Statistical analysis proves that the method is repeatable, selective, and accurate for the estimation of tamsulosin.

1. Introduction

Benign prostate hyperplasia (BPH) is a common condition in ageing 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]. Tamsulosin hydrochloride exists in two enantiomeric forms, but only R-isomer is the pharmaceutically active component. It is a new type of highly selective α -1-adrenergic receptor antagonist for treatment of BPH. Compared to other α -antagonists, tamsulosin hydrochloride has greater specificity for α -1 receptors in the human prostate and does not affect receptors on blood vessels. It is the most frequently prescribed medication for the treatment of lower urinary tract symptoms. It is a white to yellowish-white powder, slightly soluble in water, soluble in methanol, and chloroform-administered orally. Literature survey reveals the chiral separation by electrophoresis [3, 4], UV [5],

and HPLC methods [6–8], coupled with ESI-MS-MS are reported for the estimation of tamsulosin hydrochloride with its impurities in bulk and pharmaceutical formulations [9] as well as in biological fluids [10–17]. The present work deals with HPTLC stability for tamsulosin hydrochloride in bulk and pharmaceutical formulation which is economical and intended for better reproducibility of degraded products, and this work is genuine as it is not used by any other author.

2. Experimental

2.1. Materials. Tamsulosin hydrochloride was kindly gifted from Sun Pharmaceuticals, Vapi (Gujarat, India). All chemicals and reagents used were of analytical grade and purchased from Merck fine chemicals, Mumbai (Maharashtra, India).

2.2. HPTLC Instrumentation. The samples were spotted in the form of bands of width 6 mm with a Camag 100 microlitre sample syringe (Hamilton, Bonaduz, Switzerland)

using Camag Linomat 5 (Switzerland) sample applicator on precoated silica gel aluminium plate 60F-254 (10 cm × 10 cm with 0.2 mm thickness, E. Merck, Germany). A constant application rate of 200 nL/s was employed, and space between two bands was 8 mm. The slit dimension was kept 5 mm × 0.45 mm micro and 20-mm/s scanning speed was employed. The mobile phase consisted of toluene : methanol : triethylamine (3.5 : 1.2 : 0.2 v/v). Linear ascending development was carried out in twin trough glass chamber saturated with mobile phase. The optimized chamber saturation time for mobile phase was 20 min at room temperature. The length of chromatogram run was approximately 80 mm. Subsequent to the development, TLC plate was dried in a current of air with the help of an air dryer. Densitometric scanning was performed on Camag TLC scanner 3 in the absorbance mode at 280 nm. The source of radiation was deuterium lamp.

2.3. Method Validation

2.3.1. Calibration Curve of Tamsulosin Hydrochloride. A stock solution of tamsulosin hydrochloride (1000 µg/mL) was prepared in methanol. From stock solution, a solutions of 400 µg/mL was prepared. The solution was then spotted in three replicate on TLC plate to obtain concentration of 400, 800, 1200, 1600, 2000, and 2400 ng per spot of tamsulosin hydrochloride, respectively. The data of peak area versus drug concentration were treated by linear least square regression.

2.3.2. Precision. Repeatability of sample application and measurement of peak area were carried out using six replicates of the same spot (800 ng per spot of tamsulosin hydrochloride). The intraday and interday variation for the determination of tamsulosin hydrochloride was carried out at three different concentration levels of 800, 1200, and 1600 ng per spot.

2.3.3. Robustness of the Method. By introducing small changes in the mobile phase composition, the effects on the results were examined. Mobile phases having different composition of toluene : methanol : triethylamine (3.5 : 1.2 : 0.2 and 3 : 1.2 : 0.2 v/v) were tried and chromatograms were run. The amount of mobile phase, temperature, and relative humidity was varied in the range of ±5%. The plates were prewashed by methanol and activated at 60 ± 5°C for 2, 5, and 7 min prior to chromatography. Time from spotting to chromatography and from chromatography to scanning was varied from 0, 20, 40, and 60 min. Robustness of the method was done at three different concentration levels: 800, 1200, and 1600 ng per spot.

2.3.4. Limit of Detection and Limit of Quantification. In order to determine detection and quantification limit, tamsulosin hydrochloride concentrations in the lower part of the linear range of the calibration curve were used. Tamsulosin hydrochloride solution of 200 µg/mL was prepared and 400, 500, 600, 700, and 800 ng/µL was applied in triplicate. The amount of tamsulosin hydrochloride by spot versus

TABLE 1: Summary of validation parameters.

Parameter	Data
Linearity range (ng per spot)	400–2400
Correlation coefficient	0.9982
Limit of detection (ng per spot)	20.49
Limit of quantitation (ng per spot)	62.10
Recovery ($n = 6$)	100.74
Precision (% R.S.D.)	
Repeatability of application ($n = 6$)	1.43
Repeatability of measurement ($n = 6$)	
Interday ($n = 6$)	1.45
Intraday ($n = 6$)	0.87
Robustness	1.05
Specificity	specific

average response (peak area) was graphed, and the equation for this was determined. The standard deviations (SD) of responses were calculated. The average of standard deviations was calculated (A.S.D.). Detection limit was calculated by $(3.3 \times \text{A.S.D.})/b$, and quantification limit was calculated by $(10 \times \text{A.S.D.})/b$, where “ b ” corresponds to the slope obtained in the linearity study of method.

2.3.5. Specificity. The specificity of the method was ascertained by analyzing standard drug and sample. The spot for tamsulosin hydrochloride in sample was confirmed by comparing the R_f values and spectra of the spot with that of standard. The peak purity of tamsulosin hydrochloride was assessed by comparing the spectra at three different levels, that is, peak start (S), peak apex (M), and peak end (E) positions of the spot.

2.3.6. Recovery Studies. The analyzed samples were spiked with extra 80, 100, and 120% of the standard tamsulosin hydrochloride, and the mixture was analyzed by the proposed method. At each level of the amount, six determinations were performed. This was done to check the recovery of the drug at different levels in the formulations (Table 4).

2.4. Analysis of Tamsulosin Hydrochloride in Prepared Formulation. To determine the concentration of tamsulosin hydrochloride in tablets (labeled claim: 0.4 mg per tablet), the contents of 25 tablets were weighed, their mean weight determined, and they were finely powdered. The powder equivalent to 10 mg of tamsulosin hydrochloride was weighed. The drug from the powder was extracted with methanol. To ensure complete extraction of the drug, it was sonicated for 30 min, and the volume was made up to 10.0 mL. The resulting solution was centrifuged at 3000 rpm for 5 min, and supernatant was analyzed for drug content. The 4 mL was taken and volume made up to 10.0 mL by methanol, to make a 400-µg/mL solution. The above solution (800 ng per spot) was applied on TLC plate followed by development and scanning as described in Section 2.2.

The analysis was repeated in triplicate. The possibility of excipient interferences in the analysis was studied.

2.5. Forced Degradation of Tamsulosin Hydrochloride

2.5.1. Hydrogen Peroxide-Induced Degradation. The 10 mg of tamsulosin hydrochloride was separately dissolved in methanolic solution of hydrogen peroxide (30.0%, v/v). The solution was kept for “16 h” at room temperature in the dark in order to exclude the possible degradative effect of light. The 4.0 mL of the above solution was taken and diluted up to 10.0 mL with methanol. The resultant solution was applied on TLC plate in triplicate (3.0 μ L each, i.e., 1200 ng per spot). The chromatograms were run as described in Section 2.2.

2.5.2. Dry Heat Degradation Product. The powdered drug was stored at 60°C for “2 h” under dry heat condition showed significant degradation. The degraded products were resolved from the standard. In all degradation studies, the average peak areas of tamsulosin hydrochloride after application was 1200 ng per spot of three replicates.

2.5.3. Acid and Alkaline Hydrolysis. Tamsulosin hydrochloride (10 mg) was separately dissolved in 10 mL methanolic solution of 2 M HCl and 2 M NaOH. This solution was kept for “6 h” at room temperature in the dark in order to exclude the possible degradative effect of light. Further, it was refluxed for “1 h” at 55°C. The 4 mL of the above solution was taken and neutralized, then diluted up to 10 mL with methanol. The resultant solution was applied on TLC plates in triplicates (3 μ L each, i.e., 1200 ng per spot). The chromatograms were run as described in Section 2.2.

2.5.4. Light Heat Degradation Products. Tamsulosin hydrochloride (10 mg) was dissolved in 10 mL of methanol. The solution was kept in the sun light for “24 h”. 4 mL of the above solution were taken and diluted up to 10 mL with methanol (Figure 6). The resultant solution was applied on TLC plate in triplicate (3 μ L each, i.e., 1200 ng per spot). The chromatograms were run as described in Section 2.2.

3. Results and Discussion

3.1. Development of Optimum Mobile Phase. TLC procedure was optimized with a view to developing a stability-indicating assay method. Initially, toluene : 1-propanol (3 : 2 v/v) was tried with good resolution with an R_f value of 0.3 for tamsulosin hydrochloride, but typical peak nature was missing; moreover, it was felt necessary to increase the R_f for better resolution of impurities. Finally, the mobile phase consisting of toluene : methanol : triethylamine (3.5 : 1.2 : 0.2 v/v) gave a sharp and well-defined peak at an R_f value of 0.52 (Figure 1). Well-defined spots were obtained when the chamber was saturated with the mobile phase for 20 min at room temperature.

3.2. Calibration Curves. The linear regression data for the calibration curves ($n = 3$), as shown in Table 1, showed

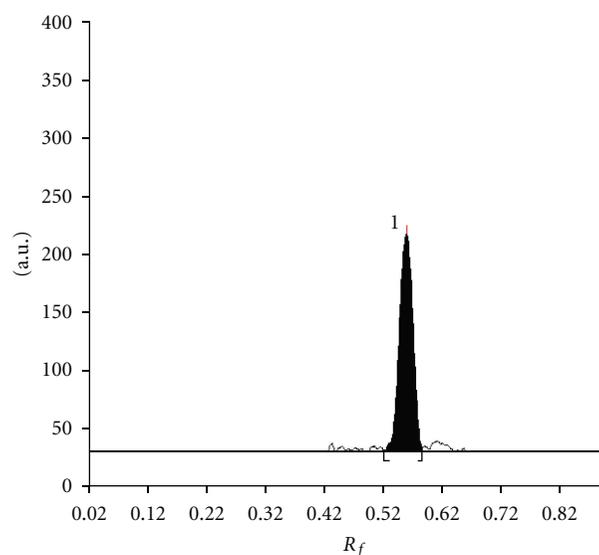


FIGURE 1: A typical HPTLC densitogram of tamsulosin hydrochloride ($R_f = 0.52$) in toluene : methanol : triethylamine (3.5 : 1.2 : 0.2, v/v) at 280 nm.

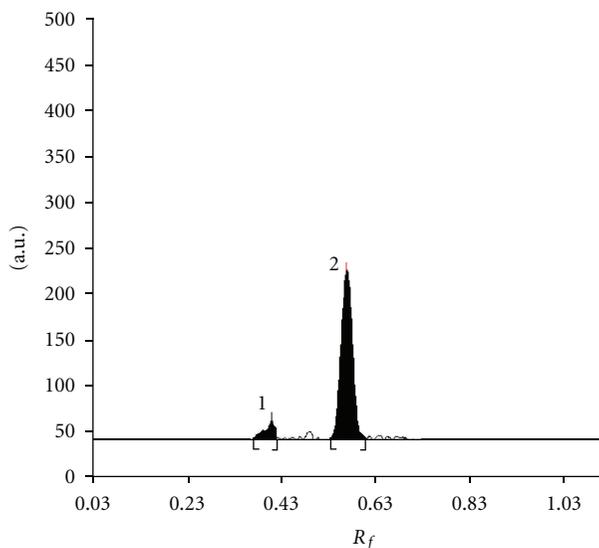


FIGURE 2: HPTLC densitogram of tamsulosin hydrochloride after treatment with 30% hydrogen peroxide for “16 h”.

a good linear relationship over the concentration range 400–2400 ng per spot with respect to peak area. No significant difference was observed in the slopes of standard curves (ANOVA, $P > .05$).

3.3. Validation of the Method

3.3.1. Precision. The repeatability of sample application and measurement of peak area were expressed in the terms of % R.S.D. and results are depicted in Table 2, which revealed intraday and interday variation of tamsulosin hydrochloride

TABLE 2: Intraday and interday precision of HPTLC method^a.

Amount (ng per spot)	Intraday precision				Interday precision			
	Mean area	SD	% R.S.D.	S.E. ^b	Mean area	SD	% R.S.D.	S.E. ^b
800	2974.6	15.00	0.50	25.08	800	43.43	1.50	8.661
1200	4015.5	32.01	0.80	23.81	1200	41.24	1.04	18.48
1600	5064.6	67.00	1.32	52.93	1600	91.68	1.82	38.68

^an = 6, ^bstandard error.

TABLE 3: Robustness of the method^a.

Parameter	SD ^b of peak area	% R.S.D. ^b
Mobile phase composition	31.35	1.05
Amount of mobile phase	34.83	1.14
Temperature	38.21	1.25
Relative humidity	36.26	1.19
Development distance	32.28	1.07
Time from spotting to chromatography	32.45	1.07
Time from chromatography to scanning	20.85	0.70

^an = 6, ^bAverage of one concentration: 800 ng per spot.

TABLE 4: Recovery studies^a.

Excess drug added to ^b the analyte (%)	Amount recovered (mg)	Recovery (%)	% R.S.D.	S.E.
0	809.23	101.15	0.40	4.99
80	1447.91	99.79	0.57	12.71
100	1613.94	100.59	0.70	17.21
120	1629.84	101.49	0.19	4.80

^an = 6, ^bmatrix containing 100 mg drug.

at three different concentration levels of 800, 1200, and 1600 ng per spot.

3.3.2. Robustness of the Method. The standard deviation of peak areas was calculated for each parameter, and the percentage of R.S.D. was found to be less than 2%. The low values of the percentage of R.S.D. values, shown in Table 3, indicated robustness of the method.

3.3.3. LOD and LOQ. The calibration curve in this study was plotted between the amount of analyte versus average response (peak area), and the regression equation was obtained ($Y = 3.284X + 416.0$) with a regression coefficient of 0.998. Detection limit and quantification limit were calculated by the method as described in Section 2.3.4 and were found to be 20.49 and 62.10 ng, respectively.

3.3.4. Specificity. The peak purity of tamsulosin hydrochloride was assessed by comparing the spectra at peak start, peak apex, and peak end positions of the spot which was obtained between standard and sample spectra of tamsulosin hydrochloride (Figure 7).

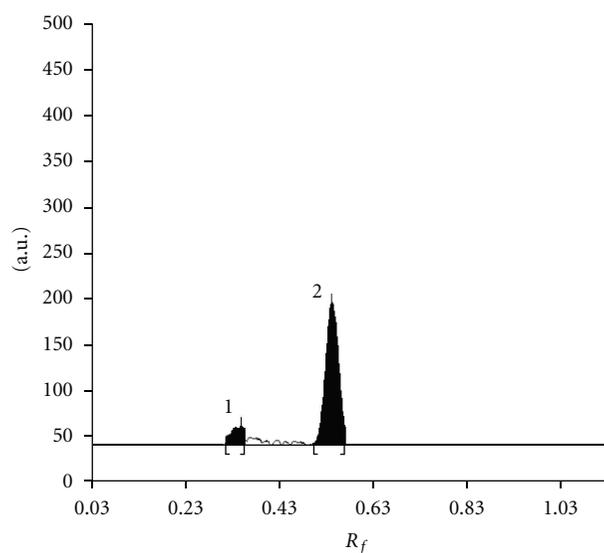


FIGURE 3: HPTLC densitogram of tamsulosin hydrochloride after photo degradation at “24 h” in sun light.

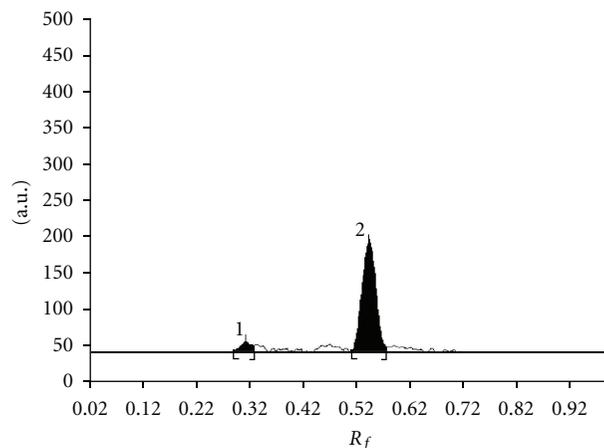


FIGURE 4: HPTLC densitogram of tamsulosin hydrochloride after HCl degradation for “8 h” at room temperature and “1 h” reflux.

3.3.5. Recovery Studies. The proposed method, when used for extraction and subsequent estimation of tamsulosin hydrochloride from pharmaceutical dosage forms after spiking with 80, 100, and 120% of additional drug, afforded recovery of 99.79%–101.59% as listed in Table 4. The data of summary of validation parameters are listed in Table 1.

TABLE 5: Forced degradation of tamsulosin hydrochloride.

Serial number	Sample exposure condition	Number of degradation products (R_f value)	Tamsulosin hydrochloride remained (ng/1200 ng) (\pm S.D, $n = 3$)	S.E.	Recovery (%)
1	2 N HCl "8 h" at room temp. reflux for "1 h"	1 (0.29)	1055.8 (7.90)	1.810	87.99
2	2 N NaOH "8 h" room temp. reflux for "1 h"	1 (0.26)	983.76 (5.01)	2.092	81.98
3	30% H ₂ O ₂ "16 h"	1 (0.34)	1020.66 (14.00)	2.034	85.06
4	Heat for "2 h" at 60°C	1 (0.31)	1028.72 (10.10)	2.244	85.73
5	Photo degradation kept for "24 h"	1 (0.28)	1069.81 (16.81)	0.900	89.15

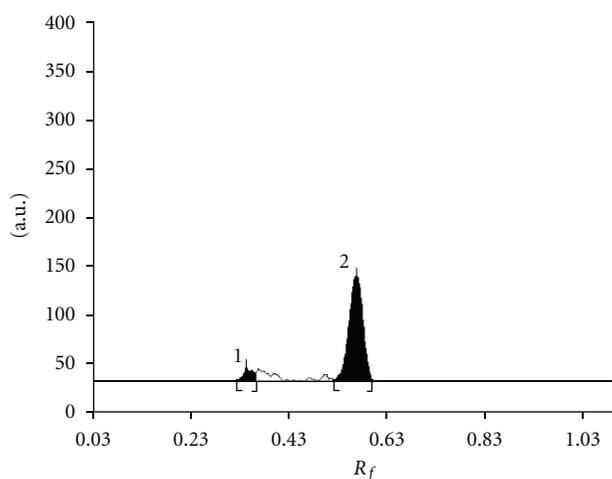


FIGURE 5: HPTLC densitogram of tamsulosin hydrochloride after NaOH degradation for "8 h" at room temperature and "1-hour" reflux.

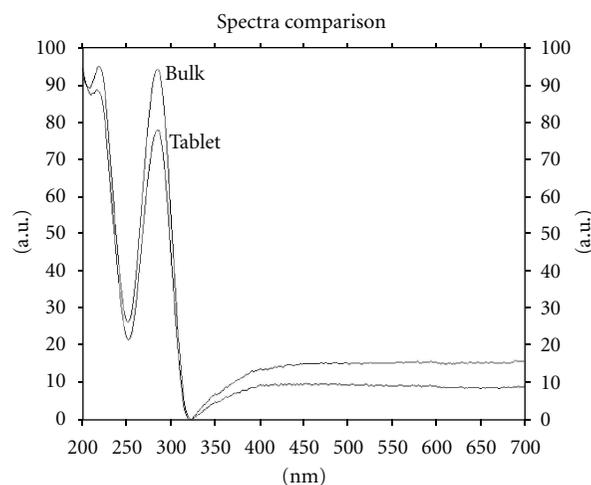


FIGURE 7: Absorption spectra of tamsulosin hydrochloride bulk and tablet formulation.

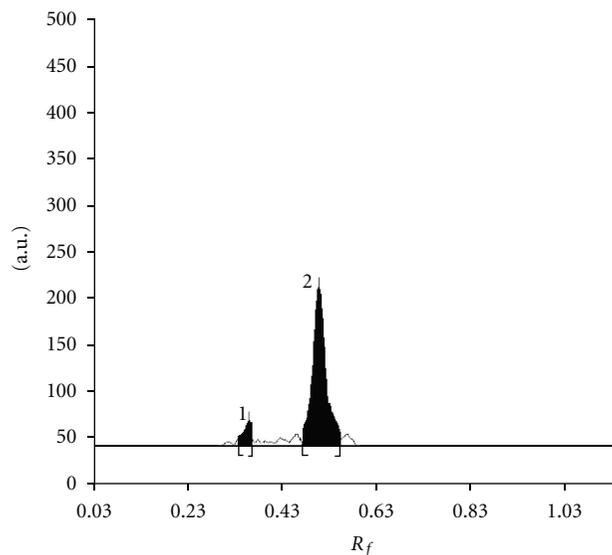


FIGURE 6: HPTLC densitogram of tamsulosin hydrochloride after "2-hour" dry heat degradation at 60°C.

3.4. Analysis of Prepared Formulation. A single spot of R_f 0.52 was observed in chromatogram of the tamsulosin hydrochloride samples extracted from tablet. There was no interference from the excipients commonly present in the tablets. The tamsulosin hydrochloride content was found to be 100.56% with an R.S.D. percentage of 0.68. It may, therefore, be inferred that degradation of tamsulosin hydrochloride had not occurred in the formulation that was analysed by this method. The low percentage of R.S.D. value indicated the suitability of this method for routine analysis of tamsulosin hydrochloride in pharmaceutical dosage forms.

3.5. Stability-Indicating Property. The chromatogram of samples degraded with acid, alkaline hydrolysis, hydrogen peroxide, and heat showed well-separated spots of pure tamsulosin hydrochloride as well as some additional peaks at different R_f values. The spots of degraded product were well resolved from the drug spot as shown in Figures 2, 3, 4, and 5. The conditions of degradation, number of degradation products with their R_f values, content of tamsulosin hydrochloride determined, and percentage recovery were calculated and listed in Table 5.

4. Conclusion

The developed HPTLC technique is precise, specific, accurate, and stability indicating. The developed method was validated based on ICH guidelines [18–20]. Statistical analysis proves that the method is repeatable and selective for the analysis of tamsulosin hydrochloride as a bulk drug and in pharmaceutical formulations.

The method can be used to determine the purity of the drug available from the various sources by detecting the related impurities. It may be extended to study the degradation kinetics of tamsulosin hydrochloride and for its estimation in plasma and other biological fluids. As the method separates the drug from its degradation products, it can be employed as a stability-indicating one.

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