ISSN : 0974-7419 Volume 11 Issue 5

Trade Science Inc.

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 11(5) 2012 [180-187]

# RP-HPLC assay method for simultaneous estimation of tamsulosin hydrochloride and finasteride in pharmaceutical dosage form

**Pritam S.Jain[1*], Abdul R.Bakhshi[1], Sanjay B.Bari[2], Sanjay J.Surana[1], Amar J.Chaudhari[1]**

[1]Department of Pharmaceutical Chemistry, R.C.Patel Institute of Pharmaceutical Education and Research, Shirpur- 425405, Maharashtra, (INDIA)

[2]H.R.Patel Institute of Pharmaceutical Education and Research, Shirpur, Dist: Dhule (M.S.) 425 405 (INDIA)

E-mail: pritash79@yahoo.com; bakhshiabdul@rediffmail.com; barisb@rediffmail.com; sjsurana@rediffmail.com; chaudhariamar95@gmail.com

*Received: 19th January, 2012 ; Accepted: 13th February, 2012*

## ABSTRACT

A simple, specific, accurate and precise Reverse Phase High Performance Liquid Chromatographic method was developed for simultaneous estimation of tamsulosine hydrochloride and finasteride in tablet dosage form on RP $C_{18}$ BDS column (250mm×4.6 mm, 5µm) with a mobile phase consisting of methanol: water (70:30, v/v) (pH 3.7) adjusted with ortho phosphoric acid, with a flow rate of 1 ml/min, UV detection at 260 nm was used. The retention time for tamsulosine hydrochloride and finasteride were found to be as 2.68 and 7.33 min, respectively. Proposed method was validated for precision, accuracy, linearity, range, robustness, ruggedness and force degradation study. The calibration curve of tamsulosine hydrochloride and finasteride were linear over the range of 4-24 µg/ml ($r^2$=0.9997) and 20-120 ($r^2$=0.9998) µg/ml respectively. The method was found to be sensitive with limit of detection of tamsulosine hydrochloride and finasteride was determined 0.28 and 0.85 and limit of quantitation of was determined 1.03 and 3.22µg/ml, respectively. The method has been successively applied for the determination of tamsulosine hydrochloride and finasteride in tablets. There was no interference from the excipients commonly present in the tablets. Accuracy of the method was studied by the recovery studies at three different levels 80 %, 100 % and 120 %. The % recovery was found to be within the limits of the acceptance criteria with average recovery of 99.9%–100.54% for tamsulosine hydrochloride and 99.83%–100.92% for finasteride. The % RSD below 2.0 shows the high precision of proposed method.

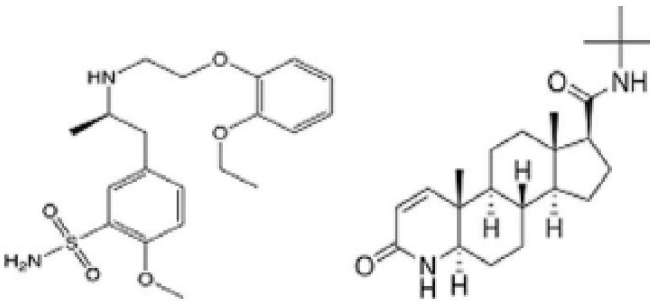


## KEYWORDS

Tamsulosine hydrochloride (TMS);
Finasteride (FIN);
RP-HPLC;
Method development;
Validation.

## INTRODUCTION

Tamsulosine hydrochloride (Figure 1) is chemically [(-)-(R)-5-[2-[[2-(*O*- ethoxyphenoxy)ethyl]amino] propyl]-2-methoxybenzenesulfonamide] and is official in Martindale – The Extra Pharmacopoeia and Merck Index[1, 2]. It 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



[a] [b]

**Figure 1 : Chemical structures of TMS [a] and FIN[b]**

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. Finasteride (Figure 1) is chemically *N*-(1,1-dimethylethyl)-3-oxo-(5α,17β)-4-azaandrost-1-ene-17-carboxamide and official in Merck Index[1, 2]. It is antagonist for the treatment of BPH and used as an anti-alopecia agent. It is a white crystalline powder freely soluble in methanol and chloroform administered orally.

Various methods as, determination of tamsulosine hydrochloride in pharmaceutical formulations by TLC-densitometry, determination of tamsulosine hydrochloride in human plasma by high-performance liquid chromatography, the chiral separation by electrophoresis and HPLC coupled with ESI-MS-MS are reported for the estimation of tamsulosine hydrochloride with its impurities in bulk and pharmaceutical formulations as well as in biological fluids[3-11] whereas five HPLC[12-16] methods with one LCMS[17] and UPLC[18] method was reported on finansteride determination in biological samples and in human plasma.

According to current good manufacturing practices, all drugs must be tested with a stability-indicating assay method before release. Till date, no stability-indicating HPLC assay method for the determination of tamsulosine hydrochloride is available in the literature. It was felt necessary to develop a stability indicating liquid chromatography (LC) method for the determination of tamsulosine hydrochloride and finasteride in combination as bulk drug and pharmaceutical dosage form. Therefore, the aim of the present study was to develop and validate a RP-HPLC assay method for tamsulosine hydrochloride as bulk drug and in pharmaceutical dosage form as per ICH guidelines[19].

## EXPERIMENTAL

### Material and reagents

Tamsulosine hydrochloride and finasteride bulk drug (purity 99.8) and tablet tamsulosine hydrochloride (0.4 mg) and finasteride (5 mg) were obtained from Sun Pharmaceuticals (Gujarat, India). Hydrochloric acid and sodium hydroxide pellets were obtained from Rankem Laboratories India. Methanol, o-phosphoric acid was obtained from Merck Specialities Private Ltd. Hydrogen peroxide is obtained from Fischer Scientific, India. All chemicals used are of HPLC grade. Milli-QWaterwas used throughout the experiment.

### Chromatographic conditions

The HPLC system used was a Shimadzu system equipped with a photodiode array detector. A chromatographic column of 250 mm length and internal diameter of 4.6 mm filled with octadecyl silane Ace5-C18 (Advance Chromatography Technology, USA) stationary phase with particle size 5 μm were used. The instrumental setting was at a flow rate of 1 mL $min^{-1}$. The injection volume was 20 μL. The detection wavelength was 260 nm.

### Mobile phase

The mobile phase consisted of methanol and water in the ratio (70:30 v/v). The pH 3.7 of mobile phase is adjusted with o-phosphoric acid in double distilled water. The mobile phase was premixed and filtered through a 0.45 μ nylon filter and degassed.

### Preparation of standard stock solutions

All solutions were prepared on a weight basis and solution concentrations were also measured on weight basis to avoid the use of an internal standard. Standard solution of tamsulosine hydrochloride was prepared by dissolving the drugs in the diluents and diluting them to the desired concentration. Diluent A was composed of methanol and diluent B was composed of water in the ratios of (70:30 v/v). Approximately

Full Paper

Standard stock solutions were prepared by dissolving 10 mg of TMS and 50 mg of FIN in 100 ml methanol that gives concentration of 100 μg/ml of TMS and 500 μg/ml of FIN.

**Linearity study**

From TMS stock solution aliquots of 0.4, 0.8, 1.2, 1.6, 2 and 2.4 ml were taken in 10 ml volumetric flasks and diluted up to the mark with methanol such that the final concentration of TMS in the range 4 – 24 μg/ml. From FIN stock solution aliquots of 0.4, 0.8, 1.2, 1.6, 2 and 2.4 ml were taken in 10 ml volumetric flasks and diluted up to the mark with methanol such that the final concentration of FIN in the range 20 – 120 μg/ml. Volume of 20 μL of each sample was injected with the help of Hamilton Syringe. All measurements were repeated three times for each concentration and calibration curve was constructed by plotting the peak area *vs* the drug concentration.

**Validation of proposed method**

The proposed method was validated as per ICH guidelines. The solutions of the drugs were prepared as per the earlier adopted procedure given in the experiment.

**Accuracy**

It was done by recovery study using standard addition method at 80%, 100% and 120 % level; known amount of standard TMS and FIN were added to preanalyzed sample (10 μg/ml of TMS; 40μg/ml of FIN) and subjected them to the proposed HPLC method.

**Precision**

Precision is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions.

Intra – day and Inter – day Precision

Intra – day precision were determined by analyzing, the three different concentrations 8 μg/ml, 12 μg/ml and 16 μg/ml of TMS, 40 μg/ml, 60 μg/ml and 80 μg/ml of FIN for three times in the same day. Day– to day variability were assessed using above mentioned three concentrations analyzed on three different days, over a period of one week.

**Repeatability**

It is measured by multiple injections of a homogenous sample of 8 g/ml of TMS and 40μg/ml of FIN that indicates the performance of the HPLC instrument under chromatographic conditions.

**Robustness**

To evaluate robustness few parameters were deliberately varied. The parameters include variation of flow rate, percentage of methanol using 8 μg/ml solution of TMS and 40 μg/ml of FIN.

**Sensitivity**

Sensitivity of the proposed method wer estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). LOD = 3.3 SD/S and LOQ = 10 SD/S, where SD is the residual standard deviation and S is the slope of the line.

**Specificity and selectivity**

The analytes should have no interference from other extraneous components and be well resolved from them. Specificity is a procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix, while selectivity is the procedure to detect qualitatively the analyte in presence of components that may be expected to be present in the sample matrix.

**Ruggedness**

From stock solutions, sample solutions of TMS (8 μg/ml) and FIN (40μg/ml) were prepared and analyzed by two different analysts using similar operational and environmental conditions. Peak area was measured for same concentration solutions, six times.

**System suitability test**

System suitability testing is essential for the assurance of the quality performance of the chromatographic system. Earlier prepared solutions for chromatographic conditions were tested for system suitability testing.

**Analysis of Pharmaceutical formulation**

To determine the contents of drugs in conventional tablets (Label claim 0.4 mg Tamsulosin hydrochloride and 5 mg of Finasteride per tablet); the twenty tablets

were weighed, their mean weight determined and they were finely powered. Powder equivalent to 4 mg TMS was transferred into a 100 ml volumetric flask containing 50 ml methanol. In that solution 6 mg of TMS bulk standard was added. The resulting solution was sonicated for 30 min and diluted to 100 ml with methanol. The solution was filtered, using 0.45 µm filter (Millifilter, Milford, MA). Excipients were separated by filtration. The solution was further diluted to get concentration 8µg/ml of TMS and 40 µg/ml of FIN, were subjected to proposed method and amount of TMS and FIN were determined.

## RESULTS AND DISCUSSION

### Optimization of chromatographic conditions

The primary target in developing this stability indicating HPLC method is to achieve the resolution between tamsulosine hydrochloride, finasteride and its degradation products. To achieve the separation of degradation products we used a stationary phase C-18 and combination of mobile phase 10 mmol $L^{-1}$ methanol with water. The separation of the degradation product, tamsulosine hydrochloride and finasteride was achieved on Ace5 octadecyl silane C-18 stationary phase and 10 mmol $L^{-1}$ methanol and water (70:30 v/v) as a mobile phase. The tailing factor obtained was less than two and retention time was about 2.68 and 7.33 min for TMS and FIN (Figure 2). The developed method was found to be specific and method was validated as per international guidelines.

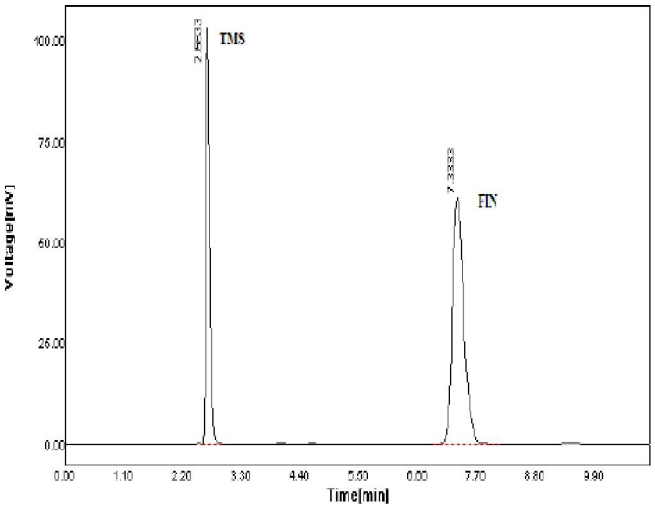



**Figure 2 : Chromatogram of standard Tamsulosine hydrochloride and Finasteride at 260 nm**

### Linearity study

Linearity was studied by preparing standard solutions at different concentration levels. The linearity range for tamsulosine hydrochloride and finasteride were found to be as 4-24 µg/ml and 20-120 µg/ml respectively (TABLE 1A & B). The regression equation for TMS and FIN were found to be as y= 24647 x + 7609.3 and y= 6550.9 x - 131.65 with correlation coefficient ($R^2$) 0.997and 0.998, respectively (Figure 3, 4).

**TABLE 1A : Linearity study of TMS**

| Sr. No. | Concentration of TMS [µg/ml] | Mean peak area ± SD [n=5] | %RSD |
|---|---|---|---|
| 1 | 4 | 103245.4 ± 2024.31 | 1.96 |
| 2 | 8 | 210556.3 ± 3753.69 | 1.78 |
| 3 | 12 | 302203.2 ± 2961.57 | 0.98 |
| 4 | 16 | 400501.2 ± 3473.22 | 0.87 |
| 5 | 20 | 498634.6 ± 5446.75 | 1.09 |
| 6 | 24 | 600850.7 ± 7696.47 | 1.28 |

**TABLE 1B : Linearity study of FIN**

| Sr. No. | Concentration of FIN [µg/ml] | Mean peak area ± SD [n=5] | %RSD |
|---|---|---|---|
| 1 | 20 | 134601.7 ± 1452.07 | 1.08 |
| 2 | 40 | 260797.3 ± 4381.58 | 1.68 |
| 3 | 60 | 389445.7 ± 4739.63 | 1.22 |
| 4 | 80 | 524398.3 ± 5023.66 | 0.96 |
| 5 | 100 | 650343.8 ± 5068.11 | 0.78 |
| 6 | 120 | 791013.2 ± 9518.53 | 1.20 |

### Method Validation

### Accuracy

To check the degree of accuracy of the method, recovery studies were performed in triplet by standard addition method at 80%, 100% and 120% concentration levels. Known amounts of standard TMS and FIN were added to the pre-analyzed samples and were subjected to the proposed HPLC method. The % recov-

Full Paper

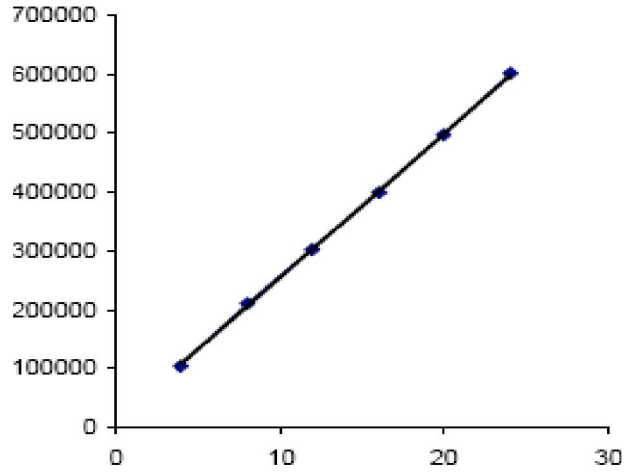



**Figure 3 : Calibration curve of Tamsulosine hydrochloride at 260 nm**

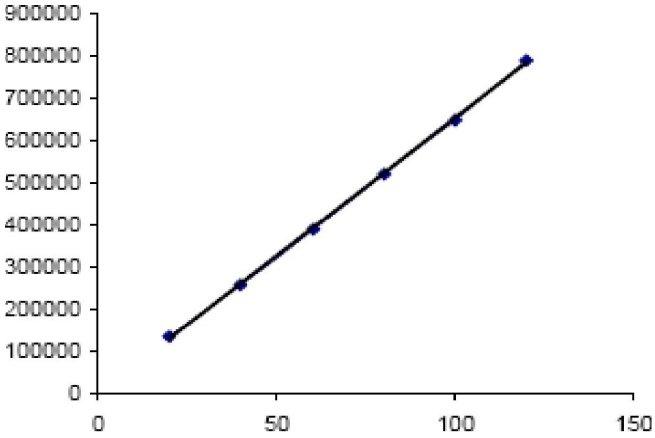



**Figure 4 : Calibration curve of Finasteride at 260 nm**

**TABLE 2A : Results of recovery studies of TMS**

| Drug | Initial amount [µg/ml] | Amount added [µg/ml] | Amount recovered ± S.D. [µg/ml, n = 3] | % Recovery | % RSD |
|---|---|---|---|---|---|
| TMS | 10 | 0 | 10.02 ± 0.11 | 100.20 | 1.19 |
| | 10 | 8 | 18.03 ± 0.12 | 100.31 | 1.48 |
| | 10 | 10 | 20.07 ± 0.47 | 100.54 | 0.95 |
| | 10 | 12 | 22.01 ± 0.13 | 99.90 | 1.11 |

**TABLE 2B : Results of recovery studies of FIN**

| Drug | Initial amount [µg/ml] | Amount added [µg/ml] | Amount recovered ± S.D. [µg/ml, n = 3] | % Recovery | % RSD |
|---|---|---|---|---|---|
| FIN | 40 | 0 | 40.37 ± 0.44 | 100.92 | 1.11 |
| | 40 | 32 | 31.95 ± 0.36 | 99.83 | 1.14 |
| | 40 | 40 | 80.33 ± 0.49 | 99.91 | 1.22 |
| | 40 | 48 | 47.99 ± 0.60 | 99.98 | 1.25 |

**TABLE 3A : Results of precision studies of TMS (Intra-day and inter-day)**

| Drug | Conc. [µg/ml] | Intra day Amount Found [µg/ml] | | Inter day Amount Found [µg/ml] | |
|---|---|---|---|---|---|
| | | Mean | % RSD [n= 3] | Mean | % RSD [n= 3] |
| TMS | 8 | 7.93 | 1.20 | 7.93 | 0.78 |
| | 12 | 12.22 | 1.89 | 11.91 | 0.63 |
| | 16 | 16.11 | 1.31 | 16.08 | 0.67 |

**TABLE 3B : Results of precision studies of FIN (Intra-day and inter-day)**

| Drug | Conc. [µg/ml] | Intra-day Amount found [µg/ml] | | Inter-day Amount found [µg/ml] | |
|---|---|---|---|---|---|
| | | Mean | % RSD [n= 3] | Mean | % RSD [n= 3] |
| FIN | 40 | 39.35 | 1.27 | 39.91 | 1.82 |
| | 60 | 60.72 | 0.56 | 59.02 | 1.15 |
| | 80 | 79.74 | 0.82 | 79.36 | 1.05 |

**TABLE 4A : Results of repeatability study of TMS**

| Sr. No. | Concentration [µg/ml] | Peak area |
|---|---|---|
| 1 | 8 | 201425.8 |
| 2 | 8 | 204875.2 |
| 3 | 8 | 204568.9 |
| 4 | 8 | 204523.6 |
| 5 | 8 | 202342.6 |
| 6 | 8 | 207536.2 |
| Mean ± SD | | 204212.9 ± 2143.61 |
| % RSD | | 1.04 |

**TABLE 4B : Results of repeatability study of FIN**

| Sr. No. | Concentration [µg/ml] | Peak area |
|---|---|---|
| 1 | 40 | 261328.5 |
| 2 | 40 | 257823.6 |
| 3 | 40 | 267124.9 |
| 4 | 40 | 259867.4 |
| 5 | 40 | 265143.5 |
| 6 | 40 | 264532.8 |
| Mean ± SD | | 262636.8 ± 3537.5 |
| % RSD | | 1.34 |

ery was found to be within the limits of the acceptance criteria with average recovery of 99.90– 100.50% for TMS and 99.83-100.92% for FIN. Results of recovery studies are shown in TABLE 2A & B.

## Precision

Precision was evaluated by carrying out six inde-

**TABLE 5A : Robustness evaluation of the HPLC method for TMS**

| Chromatographic conditions | $R_t$ | K' | T |
|---|---|---|---|
| **A: Mobile phase pH** | | | |
| 5 | 1.883 | 0.41 | 0.56 |
| 6 | 2.051 | 0.68 | 0.78 |
| 7 | 2.683 | 1.02 | 0.98 |
| Mean ± SD | 2.205 ± 0.40 | 0.703 ± 0.421 | 0.77± 0.210 |
| **B: Flow rate (ml/min.)** | | | |
| 0.90 | 8.2 | 0.42 | 1.89 |
| 1.0 | 6.99 | 0.98 | 1.02 |
| 1.1 | 4.55 | 1.34 | 0.49 |
| Mean ± SD | 6.580 ± 1.85 | 0.913 ± 0.46 | 1.133 ± 0.70 |
| **C: Percentage methanol in mobile phase (v/v)** | | | |
| 90 | 4.204 | 0.47 | 1.25 |
| 70 | 2.683 | 0.88 | 0.89 |
| 50 | 1.011 | 1.39 | 0.58 |
| Mean ± SD | 2.632 ± 1.59 | 0.913 ± 0.46 | 0.90 ± 0.33 |

**TABLE 5B : Robustness evaluation of the HPLC method for FIN**

| Chromatographic conditions | $R_t$ | K' | T |
|---|---|---|---|
| **A: Mobile phase pH** | | | |
| 5 | 6.562 | 0.62 | 0.41 |
| 6 | 6.780 | 0.73 | 0.69 |
| 7 | 7.333 | 0.94 | 1.08 |
| Mean ± SD | 6.891 ± 0.39 | 0.763 ± 0.162 | 0.726± 0.336 |
| **B: Flow rate (ml/min.)** | | | |
| 0.90 | 8.341 | 0.75 | 1.24 |
| 1.0 | 7.333 | 1.05 | 0.93 |
| 1.1 | 5.655 | 1.23 | 0.69 |
| Mean ± SD | 7.109 ± 1.356 | 1.010 ± 0.242 | 0.953 ± 0.275 |
| **C: Percentage methanol in mobile phase (v/v)** | | | |
| 90 | 10.233 | 0.57 | 1.78 |
| 70 | 7.333 | 1.12 | 0.97 |
| 50 | 4.324 | 2.02 | 0.42 |
| Mean ± SD | 7.296 ± 2.95 | 1.213 ± 0.73 | 1.11 ± 0.68 |

**TABLE 6A : Results of ruggedness study of TMS**

| Analyst | Amount found of TMS [%] | %RSD [n=3] |
|---|---|---|
| I | 99.40 | 0.76 |
| II | 99.58 | 0.60 |

**TABLE 6B : Results of ruggedness study of FIN**

| Analyst | Amount found of FIN [%] | %RSD [n=3] |
|---|---|---|
| I | 99.80 | 0.98 |
| II | 99.60 | 0.92 |

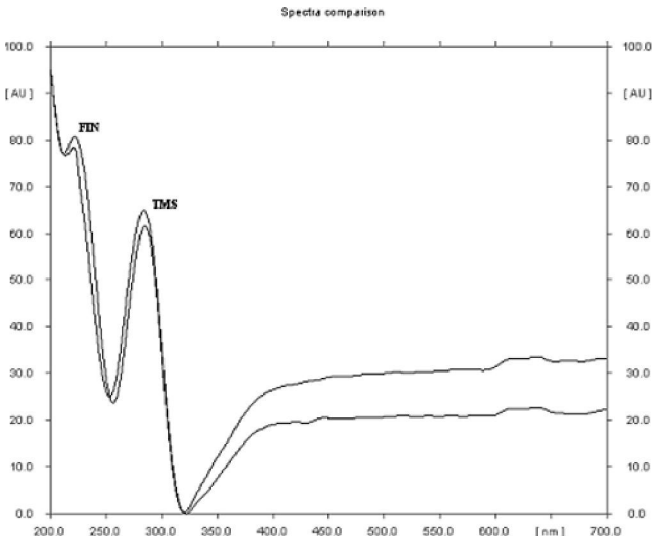



**Figure 5 : Overlain spectra of STADARD TMS and Fin at 260 nm**

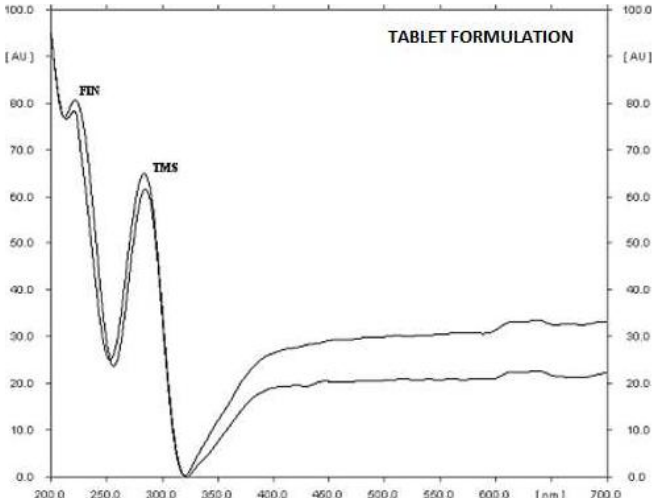



**Figure 6 : Overlain spectra of TMS and Fin in tablet formulation at 260 nm**

pendent sample preparations of a single sample by intra-day and inter-day precision. The sample preparation was carried out in same manner as described in

sample preparation. Percentage relative standard deviation (%RSD) was found to be less than 2% that proves method is precise shown in TABLE 3A & B.

### *Repeatability*

It is measured by multiple injections of a homogenous sample of 8 g/ml of TMS and 40µg/ml of FIN and the % R.S.D. was found to be less than 2 (TABLE 4A & B).

### Robustness of the method

To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in optimized method parameters were done. The effect of change in flow rate, pH retention time, and in mobile phase ratio were studied. The method was found to be unaffected by small changes like +/- 10% in flow rate, +/- 0.2 change in pH, shown in TABLE 5A &B.

### Sensitivity

LOQ and LOD can be determined based on visual evaluation, signal-to-noise approach, standard deviation of the response and slope. Limit of detection of TMS and FIN was determined 0.28and 1.06, respectively. Limit of quantitation of TMS and FIN was determined 0.85 and 3.22, respectively.

### Specificity and selectivity

The method is quite selective. There were no other interfering peak around the retention time of TMS and FIN; also the base line did not show any significant noise (Figure 5, 6).

### Ruggedness

Different analyst carried out precision studies in a similar manner carried out by first analyst. The % Assay was found to be 99.40-99.58%, and 99.60-99.80% of TMS and FIN, respectively. Percentage relative standard deviation (%RSD) was found to be less than 2% that proves method is rugged, shown in TABLE 6A & B.

### System suitability test

System suitability testing is essential for the assurance of the quality performance of the chromatographic system. The tailing factor, capacity factor, and theoretical plates for TMS and FIN were in the acceptance criteria as per the ICH guidelines (TABLE 7A & B).

**TABLE 7A : System suitability test for TMS**

| System suitability parameters | Proposed method |
|---|---|
| Retention time ($R_t$) | 2.833 |
| Capacity factor (K') | 0.96 |
| Theoretical plate (N) | 4128 |
| Tailing factor (T) | 1.04 |

**TABLE 7B : System suitability test for FIN**

| System suitability parameters | Proposed method |
|---|---|
| Retention time ($R_t$) | 7.33 |
| Capacity factor (K') | 0.99 |
| Theoretical plate (N) | 3655 |
| Tailing factor (T) | 1.03 |

**TABLE 8 : Analysis of tablet formulation**

**Brand name: Urimax-F**

**Batch no: DJ1505 Average wt: 0.114 gm**

| Drug | Label claim [mg] | Amount found [mg] | Amount found [%] |
|---|---|---|---|
| TMS | 0.4 | 0.3952 | 98.80 |
| | 0.4 | 0.4070 | 101.77 |
| | 0.4 | 0.3995 | 99.89 |
| | 0.4 | 0.3996 | 99.90 |
| | 0.4 | 0.3950 | 98.76 |
| | 0.4 | 0.4056 | 101.40 |
| | Mean ± SD | 0.4003 ± 0.005 | 100.08 ± 1.26 |
| | %RSD | 1.264 | 1.267 |
| **Drug** | **Label claim [mg]** | **Amount found [mg]** | **Amount found [%]** |
| FIN | 5.0 | 4.9575 | 99.15 |
| | 5.0 | 4.963 | 99.26 |
| | 5.0 | 4.9385 | 98.77 |
| | 5.0 | 5.0125 | 100.25 |
| | 5.0 | 4.918 | 98.36 |
| | 5.0 | 5.0895 | 101.79 |
| | Mean ± SD | 4.9798 ± 0.06 | 99.59 ± 1.24 |
| | %RSD | 1.25 | 1.25 |

### Analysis of Pharmaceutical formulation

The assay procedure was repeated for six times; the percentage content of TMS and FIN in the tablet formulation was determined as 98.76-101.77% and 98.36-101.79% respectively (TABLE 8).

## CONCLUSION

The present study was conducted to develop and

validate a simple, sensitive and reproducible RP-HPLC method for quantitative determination of tamsulosine hydrochloride and finasteride. The developed chromatographic assay fulfilled all the requirements to be identified as simple, specific, selective and reliable method, including accuracy, linearity, recovery and precision data.

Furthermore, this simple and rapid RP-HPLC method can also be used successfully for the determination of tamsulosine hydrochloride and finasteride in pharmaceutical formulations without any interference from the excipients.

## ACKNOWLEDGMENTS

The authors are mostly thankful to Torrent research center for providing valuable drugs and to the principal, R C Patel Institute of Pharmaceutical Education and Research, Shirpur for providing necessary facilities to carry out the work.

## REFERENCES

**[1]** Martindale; 'The Complete Drug Reference', 29th Edition, Council of Royal Pharmaceutical Society; Great Britain, **(1989)**.

**[2]** S.Budavari; 'The Merck Index', 14th Edition, Merck and Co.Inc.; White House station, NJ, USA, **(1996)**.

**[3]** V.Maier, J.Horakova, J.Peter, E.Tesarov, P.Coufal, J.Sevik; J.Pharm.Biomed.Anal., **39**, 691 **(2005)**.

**[4]** Z.Zhang, G.Yang, G.Liang, H.Liu, Y.Chen; J.Pharm.Biomed.Anal., **34**, 689 **(2004)**.

**[5]** R.N.Rao, M.V.Talluri, A.N.Raju, D.D.Shinde, G.S.Ramanjaneyulu; J.Pharm.Biomed.Anal., **46**, 94 **(2008)**.

**[6]** M.Qi, P.Wang, R.Cong; Chromatogr., **59**, 251 **(2004)**.

**[7]** J.G.Chandorkar, V.B.Kotwal, N.S.Dhande, S.G.Gurav, V.V.Pande, P.V.Yadav; Pak.J.Pharm. Sci., **21**, 307 **(2008)**.

**[8]** L.Ding, L.Li, P.Ta, J.Yang, J.Zhang; J.Chromatogr. B., **75**, 767 **(2002)**.

**[9]** S.Yamada, C.Tanaka, M.Suzuki, T.Ohkura, R.Kimura, K.Kawab; J.Pharm.Biomed.Anal., **14**, 289 **(1996)**.

**[10]** K.Hanada, N.Tochikura, H.Ogata; Biol.Pharm. Bull., **30**, 1593 **(2007)**.

**[11]** P.Keski-Rahkonen, O.Parssinen, E.Leppanen, T.Mauriala, M.Lehtonen, S.Auriola; J.Pharm. Biomed.Anal., **17**, 606 **(2007)**.

**[12]** A.I.Segall, M.F.Vitale, V.L.Perez, M.L.Palacios, M.T.Pizzorno; J.Liq.Chromatogr.Rel.Tech., **25**, 3167 **(2002)**.

**[13]** J.Macek; J.Chromatogr.B., **764**, 207 **(2001)**.

**[14]** D.Hulya, C.Aysen, S.Serap; Anal.Chim.Acta., **557**, 252 **(2006)**.

**[15]** A.S.Akheel, K.Mungalimane, A.Amshumali; J.Pharm.Biomed.Anal., **25**, 1015 **(2001)**.

**[16]** K.Basavaiah, B.Somashekar; E.J.Chem., **4**, 109 **(2007)**.

**[17]** T.TsingHua; Acta.Pharm.Sin., **25**, 3167 **(2003)**.

**[18]** F.Szabolcs, F.Jeno, G.Katalin; J.Pharm.Biomed. Anal., **49**, 833 **(2009)**.

**[19]** ICH Guidelines Q2A. In: Text on Validation of Analytical Procedures, International Conference on Harmonization, Geneva, **(1994)**.