RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF FINASTERIDE AND TAMUSLOSIN IN TABLET FORMULATIONS

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ABSTRACT
The object of the present study was to develop a simple, precise simultaneous HPLC method for estimation of Tamsulosin (TAM) and Finasteride (FIN) in bulk and pharmaceutical dosage form. The method involves the use of easily available inexpensive laboratory solvents. The separation was achieved on spherisorb C-18 column with isocratic flow detected at 210nm. The mobile phase consisted of methanol: 0.03mM phosphate buffer pH 3.5 (70:30v/v) with flow rate of 1.0 mL/min. A linear response was observed over concentration range of 4-40μg/mL for tamsulosin and 10-80μg/mL for finasteride. Limit of detection and limit of quantitation for tamsulosin were 1.13μg/mL and 3.43μg/mL, and for finasteride were 1.37μg/mL and 4.21μg/mL, respectively. The recovery of tamsulosin and finasteride was found to be in the range of 98.80-100.24% and 99.33-100.33%, respectively. The low %RSD value indicated a good precision and stability of the analytical method. The analysis concluded that the method was selective for simultaneous estimation of tamsulosin and finasteride can be potentially used for the estimation of these drugs in combined dosage form.

Key words: HPLC, tamsulosin, finasteride, validation, pharmaceutical dosage form.

INTRODUCTION
During the drug development process, liquid chromatographic methods are used to determine the quality of the drug substance (active pharmaceutical ingredient) and drug product. RP-Liquid chromatography is the most accepted analytical technique in the pharmaceutical industry. Finasteride (Figure 1) is an antiandrogen which acts by inhibiting 5-alpha reductase, the enzyme that converts testosterone to dihydrotestosterone. In high dose, it is used to treat prostate cancer, while in low doses used in benign prostatic hyperplasia.

It is chemically known as N-(1,1-dimethylethyl)-3-oxo-(5alpha,17beta)-4-azandrost-1-ene-17-carboxamide. (Thimmaraju et al., 2011). Tamsulosin hydrochloride (Figure 2) is an antagonist of alpha1A adrenoceptors in the prostate and chemically described as (-)-(R)-5-[2-[[2-(o-ethoxyphenoxy) ethyl] amino] propyl] -2-methoxybenzenesulfonamide, mono-hydrochloride. It is used to reduce urinary obstruction and relieve the symptoms associated with symptomatic benign prostatic hyperplasia. Both drugs in combination used in the treatment of benign prostatic hyperplasia, enlarged prostate (Merck Index., 2006; Patel et al., 2010; Thimmaraju et al., 2011 and Basavaiah et al., 2007).

Figure 1. Chemical structure of tamsulosin.

Figure 2. Chemical structure of finasteride.
As per prior literature survey, it was found that there are few analytical methods are available for quantitation of finasteride alone as well as in biological fluid by various analytical technique such as polarographic analysis (Amer., 2005), HPLC in biological fluid (Constanzer et al., 2001; Carlucci et al., 1997), HPLC in formulation (Patel et al., 2010; Thimmeraju et al., 2011 and Syed et al., 2001) and LC-MS (Constanzer et al., 1994; Guo et al., 2007; Xiaohong et al., 2008 and Ptacek et al., 2000). There are few methods reported for tamsulosin estimation alone as well as in biological fluid are HPLC (Soeishi et al., 1990), LC-MS (Matsushima et al., 1997; Rahkonen et al., 2007; Din et al., 2002 and Ramakrishna et al., 2005) in pharmaceuticals formulation. However there are very few HPLC methods are reported for simultaneous estimation of TAM and FIN in combined pharmaceutical formulations (Patel et al., 2010; Thimmeraju et al., 2011). However, the limited number of HPLC methods that are available for regular routine analysis of TAM and FIN in pharmaceutical formulations (laboratory developed tablet dosage form) employ the use of high cost solvents that are found to be highly complex and are associated with increasing numbers of process variables, which makes them less acceptable for routine analysis. This paper reports a rapid and sensitive HPLC determination method with UV detection, useful for routine quality control for simultaneous estimation of both drugs in the combined dosage form. The method requires strong optimization process like mobile phase composition, pH of mobile phase, and flow rate. Therefore an attempt was made to develop and optimize new solvent system for simultaneous estimation of FIN and TAM in bulk as well as in tablet formulation. The developed method was validated in accordance with the ICH guidelines (ICH,Q2R1 2005).

**MATERIAL AND METHODS**  
**Chemicals and reagents**  
TAM and FIN were donation from Ranbaxy limited, India. Methanol (HPLC grade), disodium hydrogen phosphate and acetic acid glacial (AR Grade) were purchased from E-Merk Ltd. (Mumbai, India). Ultra-purified HPLC grade water was obtained from Milli-Q® system (Millipore, Milford, MA, USA) water purification unit. Mobile phase was filtered using 0.45µ nylon filters made by Millipore (USA) and was sonicated using sonicator.

**HPLC and chromatographic conditions**  
HPLC system (Waters) was used for the estimation drugs. The system was equipped by high pressure pump and a rheodyne injection valve with a 20µL loop used for sample injection. A Hypersil ODS C-18 column (250*4.6mm, i.d., 5µM particle size) was used in this research. The mobile phase was composed of methanol: 0.03mM phosphate buffer (pH 3.5), in the ratio of (70:30v/v) and flow rate was adjusted 1mL/min at room temperature (25±2°C). The mobile phase was prepared daily and degassed by ultrasonication before use.

**Preparation of standard drug solutions**  
The standard solutions of both drugs were prepared by dissolving 25mg of finasteride and 10mg of tamsulosin in 10mL of methanol in volumetric flask. The prepared solution was filtered and further diluted to make series concentration in linearity range of 4-40µg/mL for TAM and 10-80µg/mL for FIN.

**Assay of tablet**  
The analyses of in house developed formulations were performed by weighing 20 tablets. The tablets were powdered and appropriate portion of this powder, equivalent to 25mg of finasteride and 10mg of tamsulosin were weighed and transferred in a 10mL volumetric flask and further dissolved in methanol. This solution was sonicated for 10min to dissolve entire active from the tablet. Once the time had elapsed and the volumetric flask reached the environmental temperature (25°C). The solution was filtered through a 0.45pM filter to ensure the absence of particulate matter. The filtered solution was appropriately diluted further to get the desired concentration for the estimation.

**Method validation**  
The developed method was validated for parameters viz. linearity range, precision, accuracy, specificity, selectivity, limit of quantitation (LOQ), and limit of detection (LOD), robustness and solution stability.
**Linearity range:** The linearity of an analytical method is its ability of an analytical method to show a directly proportional relationship of a quantitative response to a specific concentration of an analyte within a given specified range of concentrations. Accurately measured aliquots of working standard solutions of TAM and FIN were diluted with methanol to obtain final concentrations of TAM (4-40µg/mL) and FIN (10-80µg/mL). Calibration curves were constructed by plotting peak areas vs concentrations, and regression equations were calculated.

**System suitability:** System suitability parameters were measured so as to verify the system performance. It was determined on six replicate injections of standard solutions. All important characteristics including capacity factor, peak resolution, and theoretical plate number were measured.

**Accuracy:** The accuracy was determined by standard additions method at three different levels, i.e. by multiple level recovery studies. The recovery studies were performed at 50%, 100%, 150% level from initial level. It was done by injection of solution (n=5) of known concentrations of drugs that had been prepared from stock solutions. Percent recovered were calculated for drug using regression equation.

**Precision:** The precision study was assessed by evaluating chromatographic responses of repeated injections (n=5) of known concentrations of both TAM and FIN over the concentration ranges studied. The intra-day precision refers to the use of analytical procedure within a laboratory over a short period of time using the same operator with same equipment. The inter-day precision involves estimation of variations in analysis when a method is used within a laboratory on different days, by same analysts. Repeatability is reported in terms of relative standard deviation (%RSD).

**Specificity and Selectivity:** It is used to check the proposed method of a combination tablet of TAM and FIN that contain commonly occurring excipient present formulations. The comparison of chromatogram of sample with chromatograms of the standard solution was done to check the variation in the retention time along with percentage recovery.

**Limit of Quantitation (LOQ) and Limit of Detection (LOD):** It was calculated by method which was based on the standard deviation (S.D) of the response and slope (S) of the calibration curve. LOD and LOQ were determined as follows.

\[
\text{LOD} = 3.3 \times \text{Standard deviation of y intercept} / \text{Slope of calibration curve}
\]

\[
\text{LOQ} = 10 \times \text{Standard deviation of y intercept} / \text{Slope of calibration curve}
\]

**Robustness:** The robustness of an analytical method refers to its capability to remain unaffected by small and deliberate variations in experimental condition. The conditions studied were flow rate (altered by ± 0.2mL/min), mobile phase composition (methanol ± 5%), and buffer pH (altered by ± 0.2).

**Stability:** The stability of drug solution was determined using the samples for short-term stability by keeping at room temperature for 12h and then analyzing. The long-term stability was determined by storing at 4°C for 30 days.

**RESULTS AND DISCUSSION**

In this work a simple, sensitive and valid HPLC method has been developed for simultaneous estimation of FIN and TAM using RP-HPLC. There are various combination of mobile phases were initially attempted to elute simultaneously both components and to achieve sharp peaks. Most of the time FIN and TAM eluted with very small value of retention factor (k) associated with long retention time. The capacity factor (Kc) of both the drug was found to be optimum according to the studied parameters. The capacity factor obtained is within the accepted values, above 2 for the first peak and less than 10 for the last peak. During method development preference was given to methanol as choice of solvent. The various mobile phase compositions have been tried i.e. with methanol: water, methanol: formic acid 0.1%, methanol: sulphate buffer, however the result showed that both compounds FIN and TAM cannot be properly separated. The selected mobile phase composition consisted of methanol: 0.03mM phosphate buffer (pH - 3.5), in the ratio of 70:30v/v. Retention time of FIN and TAM was 3.52 and 8.03min (Figure 3), respectively.
Figure 3. Chromatograms of tamsulosin and finasteride reference sample.

Figure 4. Chromatograms of tamsulosin and finasteride test sample.

Figure 5. Linearity curve of Tamsulosin.

Figure 6. Linearity curve of Finasteride

**Linearity:** The linearity range for TAM and FIN were 4-40 μg/mL and 10-80 μg/mL, respectively (Figure 5 and 6). The correlation coefficient values were found to be greater than 0.99 for both the drugs. The relevant equations for these are $Y= 71217x + 12015$ and $Y= 66761x - 8554.9$ for TAM and FIN (Table I).

**System suitability:** The %RSD of peak area and retention time for both drugs was within 2% indicating that the method was suitable for analysis the drugs (Table II). The efficiencies of the column as expressed by number of theoretical plates for TAM and FIN were 6844±2% and 11577±1.96, respectively. The capacity factor and tailing factor for TAM and FIN were 2.1, 6.3, 0.44 and 0.82 respectively. Therefore the method complied with limits stipulated by USP for system suitability for the peaks.

**Accuracy (Recovery studies):** The recoveries of the drugs were determined by standard addition method. The result suggests that, the method can be considered accurate, as the %RSD of all the determinants were to be <2% which indicated that the method was accurate and also there was no interference of the excipients present in tablets (Table III).

**Precision:** In inter-day precision studies expressed by % RSD value of TAM and FIN were between 0.278-0.750 and 0.563-0.874, respectively. The result of intra-day precision study expressed as %RSD value were in range of 0.316-0.743 for TAM and 0.740-0.933 for FIN (Table 4). The % RSD results of inter-day precision and intra-day precision for both drugs were within 2.0% limit, which confirming good precision of developed analytical method.

**Specificity and selectivity:** Specificity and selectivity were studied for presence of interfering endogenous components. The results indicated that there were not much variations in retention time of pure drug and tablet formulation. The result showed that none of impurities were interfering in the assay. According to the chromatogram (Figure 4), the both peaks TAM, FIN were free from interference of formulation excipients, the solvent system and each other. This indicated the selected method was specific for simultaneously determination TAM and FIN.

**Limit of Detection and Limit of Quantification:** The LOD and LOQ of TAM were 1.13 and 3.43 μg/mL, respectively, while those of FIN were 1.37 and 4.21μg/mL.
%RSD of six replicate injections of TAM at LOD (1.13 μg/mL) and LOQ (3.43 μg/mL) were 1.85 and 1.07, respectively. Similarly, %RSD (relative standard deviation) of FIN at LOD (1.37 μg/mL) and LOQ (4.21 μg/mL) were 2.11 and 1.92. These values indicated that the method was very sensitive to quantify both drugs (Table I).

Robustness of method: In the robustness study, small deliberate variations in the optimized method parameters were done. The effect of change in mobile phase ratio, buffer concentration, and buffer composition on the peak shape and separation were studied. The method was found to be robust as small variations in the method parameters did not affect the peak shape and retention times of the drugs.

Table I. Analytical parameters of Tamsulosin and Finasteride

<table>
<thead>
<tr>
<th>Regression parameter</th>
<th>Tamsulosin</th>
<th>Finasteride</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range (μg/mL)</td>
<td>4-40</td>
<td>10-80</td>
</tr>
<tr>
<td>Slope</td>
<td>71217</td>
<td>66761</td>
</tr>
<tr>
<td>Intercept</td>
<td>12015</td>
<td>8554.9</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9989</td>
<td>0.9995</td>
</tr>
<tr>
<td>Retention time (min)*</td>
<td>3.52±0.007</td>
<td>8.03±0.003</td>
</tr>
<tr>
<td>LOD (μg/mL)*</td>
<td>1.13±0.021</td>
<td>1.37±0.029</td>
</tr>
<tr>
<td>LOQ (μg/mL)*</td>
<td>3.43±0.037</td>
<td>4.21±0.081</td>
</tr>
<tr>
<td>Recovery (μg)*</td>
<td>98.51±0.59</td>
<td>97.84±0.84</td>
</tr>
</tbody>
</table>

*Each mean value is the result of six analysis

Table II. System Suitability parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Tamsulosin</th>
<th>%RSD</th>
<th>Finasteride</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention time</td>
<td>3.52</td>
<td>0.241</td>
<td>8.02</td>
<td>0.322</td>
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<tr>
<td>Peak area</td>
<td>2989418</td>
<td>0.632</td>
<td>4234810</td>
<td>0.12</td>
</tr>
<tr>
<td>Theoretical plate</td>
<td>6844±2</td>
<td>0.069</td>
<td>11577±1.96</td>
<td>1.24</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>0.44±0.03</td>
<td>0.065</td>
<td>0.82±0.04</td>
<td>0.089</td>
</tr>
</tbody>
</table>

Table III. Recovery Table

<table>
<thead>
<tr>
<th>Theoretical content (µg/mL)</th>
<th>Excess drug</th>
<th>Recovery (µg/mL)</th>
<th>%Recovery</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tamsulosin</td>
<td>5</td>
<td>0</td>
<td>4.94</td>
<td>98.80</td>
</tr>
<tr>
<td>7.5</td>
<td>2.5</td>
<td>7.45</td>
<td>99.33</td>
<td>1.29</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
<td>9.90</td>
<td>99.00</td>
<td>0.91</td>
</tr>
<tr>
<td>12.5</td>
<td>7.5</td>
<td>12.53</td>
<td>100.24</td>
<td>1.02</td>
</tr>
<tr>
<td>Finasteride</td>
<td>4</td>
<td>0</td>
<td>3.98</td>
<td>99.50</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>5.96</td>
<td>99.33</td>
<td>0.67</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>7.99</td>
<td>99.87</td>
<td>0.89</td>
</tr>
<tr>
<td>12</td>
<td>8</td>
<td>12.04</td>
<td>100.33</td>
<td>1.71</td>
</tr>
</tbody>
</table>

Table IV. Statistical Evaluation of Precision of developed method

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (µg/mL)</th>
<th>n</th>
<th>Interday-precision</th>
<th>Intraday-precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean</td>
<td>RSD (%)</td>
</tr>
<tr>
<td>Tamsulosin</td>
<td>5</td>
<td>5</td>
<td>04.82</td>
<td>0.750</td>
</tr>
<tr>
<td>10</td>
<td>09.84</td>
<td>0.278</td>
<td>14.06</td>
<td>0.743</td>
</tr>
<tr>
<td>15</td>
<td>14.13</td>
<td>0.471</td>
<td>24.81</td>
<td>0.595</td>
</tr>
<tr>
<td>Finasteride</td>
<td>15</td>
<td>5</td>
<td>14.12</td>
<td>0.563</td>
</tr>
<tr>
<td>20</td>
<td>19.13</td>
<td>0.838</td>
<td>18.94</td>
<td>0.933</td>
</tr>
<tr>
<td>25</td>
<td>24.87</td>
<td>0.874</td>
<td>24.76</td>
<td>0.740</td>
</tr>
</tbody>
</table>

%RSD of six replicate injections of TAM at LOD (1.13 μg/mL) and LOQ (3.43 μg/mL) were 1.85 and 1.07, respectively. Similarly, %RSD (relative standard deviation) of FIN at LOD (1.37 μg/mL) and LOQ (4.21 μg/mL) were 2.11 and 1.92. These values indicated that the method was very sensitive to quantify both drugs (Table I).
The results showed that slight variations on the chromatographic conditions have negligible effect on the results showing that method was highly robust for its intended use.

**Stability:** Stability studies indicated that samples were stable when kept at bench top for 12 h (short-term), and refrigerated at 4°C for 30 days (long-term). Sample solution did not show any appreciable change in recovery value. The results of these stability studies are given in table VI, where the percent recoveries were within the acceptance range of 90–110%.

**Analysis of tablet:** Tamsulosin and Finasteride tablets were evaluated for the amount of TAM and FIN present in the formulation. Each sample was analyzed in triplicate after extracting the drug as mentioned in the sample preparation of the experimental section. The amount of drugs present was 98.51% and 97.85%, respectively for TAM and FIN. The reference chromatogram of standard pure drug (Figure 3) was matching with that of test sample chromatogram (drug extracted from the tablets) (Figure 4). The results are given in table I which shows high percentage recoveries and low RSD (%) values.

**CONCLUSION**

The proposed method was simple, sensitive and reproducible and hence can be used for simultaneous determination of TAM and FIN in bulk as well as in tablet formulations. The statistical analysis of the results has been carried out revealing high accuracy and good precision. The % RSD for all parameters was found to be low, which indicated that the method meet to the validity criteria. The assay results obtained by this method are in fair agreement. So, developed method can be used for routine quantitative simultaneous estimation of TAM and FIN in multicomponent pharmaceutical preparation.

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