RP-HPLC METHOD FOR THE ESTIMATION OF TAMSULOSIN IN BULK AND CAPSULES

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ABSTRACT

Objective: To develop a simple, sensitive and precise HPLC method for the quantitative estimation of tamsulosin in capsules.

Method: Chromatographic separation of the drug was achieved with an Hypersil BDS C_{18} column (250 mm x 4.6 mm I.D.; particle size 5 μm) analytical column using sodium percholate buffer (pH 3.0)-acetonitrile (60:40 *v/v*) as mobile phase. The instrumental settings are flow rate of 1.0 ml/min, column temperature at 40°C, detector wavelength of 225 nm and the run time was 10 min.

Results: The retention time of the drug was 6.52 min. Theoretical plates, plates per meter and height equivalent to theoretical plate for tamsulosin were 2760.03, 11040.12 and 9.058×10^{-5} respectively. The developed method shows linearity over a range of 2-10 μg/ml of tamsulosin with correlation coefficient of 0.9997. The relative standard deviation is less than 2%.

Conclusion: The proposed method was found to be suitable and accurate for quantitative determination of tamsulosin in capsules.

Keywords: RP-HPLC, Tamsulosin, Sodium percholate, Acetonitrile, Capsules.

INTRODUCTION

Tamsulosin [1-4] (TSN) is a sulfamoylphenethylamine-derivative alpha-adrenergic receptor blocker commonly used to treat signs and symptoms of benign prostatic hyperplasia. Tamsulosin is a selective, potent and competitive antagonist at alpha-1A and alpha-1B-adrenergic receptors in the prostate, prostatic capsule, prostatic urethra and bladder neck. Blockage of these receptors results in the relaxation of smooth muscles in the bladder neck and prostate, and consequently decreases urinary outflow resistance in men. Tamsulosin was first marketed in 1996 with trade name Flomax. Chemically, TSN is known as (R)-5-(2-[2-(2-Ethoxyphenoxy) ethylamino] propyl)-2-methoxybenzenesulphonamide hydrochloride (Fig. 1). Tamsulosin is a white crystalline powder with a molecular weight of 444.98 g/mol. It is sparingly soluble in water & methanol, slightly soluble in glacial acetic acid & ethanol, and practically insoluble in ether.
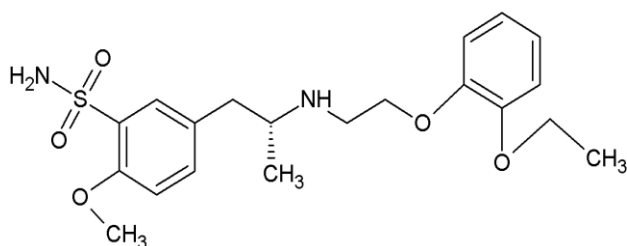

Fig. 1: Chemical Structure of Tamsulosin

For the determination of the TSN in bulk, pharmaceutical formulations and biological samples, various analytical techniques including HPLC[5-11], stability indicating HPLC[12], stability indicating HPTLC[13,14], LC-MS-MS[15-21], radioreceptor assay[22,23], potentiometry[24], voltametry[25], capillary electrophoresis[26], spectroflorimetry[27], UV[28] and visible spectrophotometry[29-31] are reported in the literature.

The main purpose of the present study was to establish a simple, sensitive, validated and low-cost RP-HPLC method for the determination of TSN in pure and capsules. The thorough literature survey has revealed that no RP-HPLC method has been reported for the estimation of TSN in bulk and capsules by using sodium perchlorate buffer and acetonitrile (60:40 *v/v*) as mobile phase. Hence, an attempt has been made to develop a new RP-HPLC method for its estimation with good accuracy, simplicity, precision and economy.

MATERIALS AND METHODS

Instrumentation

Quantitative HPLC was performed on a High Pressure Liquid Chromatograph (Shimadzu HPLC class VP series) with two LC-10 AT, VP pumps, variable wavelength programmable UV/Visible detector SPD-10A, VP, CTO-10AS VP column oven (Shimadzu), SCL-10A, VP system controller (Shimadzu). The HPLC system was equipped with the software "class VP series version 5.03 (Shimadzu)". Hypersil BDS C_{18} column [250 mm x 4.6 mm I.D.; particle size 5 μm] was used for the separation.

Chemicals

All chemicals were of HPLC grade quality. Acetonitrile, sodium perchlorate and orthophosphoric acid were from Qualigens, Mumbai, India.

Mobile phase

The mobile phase used for the analysis was prepared by mixing sodium perchlorate buffer (pH 3.0) and acetonitrile in the ratio, 60:40 *v/v*. The same mobile phase was also used as a diluent for the sample preparations. The buffer used in mobile phase contains 0.05 M sodium perchlorate monohydrate in double distilled water. The pH was adjusted 3.0 with orthophosphoric acid.

Preparation of standard drug solutions:

Stock solution of TSN (1 mg/ml) was prepared by dissolving 100 mg of TSN in 50 ml of mobile phase in a 100 ml volumetric flask and then made up to the mark with the same solvent. Working standard solutions of TSN (2, 4, 6, 8, 10 μg/ml) were prepared by suitable dilution of the stock solution with mobile phase.

Preparation of sample solution

The contents of capsule dosage form of urimax (Cipla Ltd., Mumbai, India), for TSN (100 mg), were weighed and then transferred to a 100 ml beaker containing about 50 ml mobile phase, and dissolved with the aid of sonication (10 minutes). The solution was filtered through 0.45μm membrane. The filtrate was transferred into a 100 ml volumetric flask and completed to the mark with mobile phase and mixed well. This solution was further appropriately diluted with mobile phase to obtain a concentration of 5 and 10 μg/ml.

Chromatographic conditions

The mobile phase was a mixture of sodium perchlorate buffer (pH 3.0) and acetonitrile (60:40 *v/v*). The contents of the mobile phase

were filtered before use through 0.45 μm membrane filter, degassed with a helium sparge for 15 min and pumped from the solvent reservoir to the column at a flow rate of 1 ml/min. The column temperature was maintained at 40±1°C. The injection volume of sample was 20 μl. The analyte was monitored at a wavelength of 225 nm.

Preparation of calibration curve

Prior to injection of the drug, the mobile phase was pumped for about 30 min to saturate the column thereby to get the base line corrected. Then, 20 μl of each working standard solution was injected automatically into the column in triplicate and the peaks were determined at 225 nm. The peak areas of TSN were plotted against the corresponding nominal concentration to obtain calibration curve. The concentration of the drug was calculated from the calibration curve or the regression equation.

Analysis of TSN in capsules

For this purpose, 20 μl of the sample solution, prepared as described under "Preparation of sample solution", was injected into HPLC system in triplicate. The chromatograms were recorded. The area under the peak was calculated. The TSN content in the capsules was calculated using the calibration curve or regression equation.

RESULTS AND DISCUSSION

HPLC Parameters fixation

In order to ascertain and establish optimum conditions for good resolution, rapid, accurate quantitative separation and estimation of TSN, the experimental conditions such as detection wavelength, mobile phase composition, flow rate of mobile phase, pH of mobile phase, type of column, column temperature were optimized by varying one variable at a time and fixing all other variables constant. The results are incorporated in the Table 1. Under the optimized chromatographic conditions the retention time of TSN was 6.50 min.

Specificity

Specificity of the proposed method is evaluated by comparing the chromatograms of blank sample (mobile phase) and tablet extract with chromatogram of pure drug sample. By comparing the chromatograms, it was observed that none of the peaks appears at the same retention time of TSN (Fig. 2). This established the specificity of the method.

Table 1: Optimized Chromatographic conditions

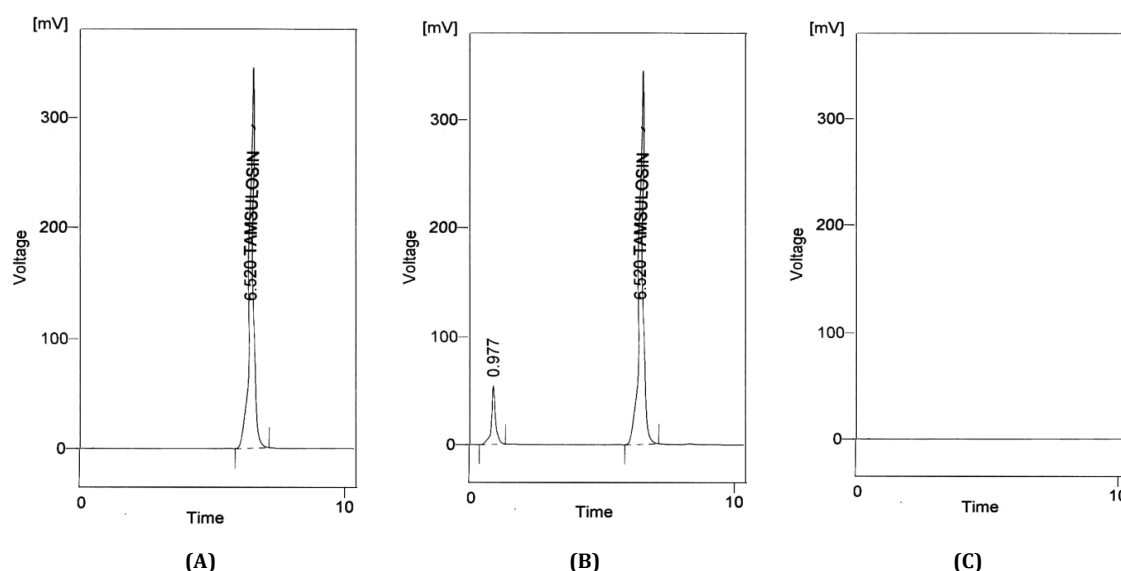| Parameter	Value
Column	C_{18} Hypersil BDS
Mobile Phase	Sodium perchlorate buffer (pH 3.0) : acetonitrile (60:40 *v/v*)
Flow Rate (ml /min)	1.0
Column Temperature (°C)	40
Detection Wavelength (nm)	225
Retention Time (min)	6.50

Fig. 2: (A) Chromatogram of pure TSN; (B) Chromatogram of TSN capsule; (C) Chromatogram of blank mobile phase

Performance calculations

To ascertain the system suitability for the proposed method, parameters such as relative retention, theoretical plates, HETP, and peak asymmetry have been calculated with the observed readings and the results are recorded in Table 2. The results indicate that the system is suitable for the assay of TSN.

Detection characteristics

To test whether the TSN has been linearly eluted from the column in the proposed method, without the usage of internal standard, different amounts of TSN (2, 4, 6, 8 and 10 μg/ml) were analyzed by the proposed method. The least square regression analysis was carried out for calculating the slope, intercept and correlation coefficient. The results are recorded in Table 2.

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ [32] of the proposed method were determined as signal-to-noise ratio 3 for LOD and 10 for LOQ by injecting a series of dilute solutions with known concentration (2 μg/ml). The calculated LOD and LOQ values were 0.594 and 1.98 μg/ml, respectively. These low values recommend the high sensitivity of the proposed method.

Precision

The precision of the proposed method was ascertained from the peak area of TSN obtained by the determination of eight replicates of fixed amount of TSN (10 µg/ml). The percent relative standard deviation was calculated and is presented in the Table 2.

Accuracy

To determine the accuracy of the proposed method, fixed amount of TSN (10 µg/ml) was analyzed by the proposed method. The percent ranges of errors (0.05 and 0.01 confidence limits) were calculated and are presented in the Table 2.

Table 2: Performance calculations, detection characteristics, precision and accuracy of the proposed method

Parameter	Value
Retention Time (Min)	6.52
Theoretical Plates (n)	2760.03
Plates per Meter (N)	11040.12
Height equivalent to theoretical plate (HETP) (mm)	9.058×10^{-5}
Peak asymmetry	0.979
Linearity range (µg/ml)	2-10
Regression equation (Y = a + bc)	
Slope (b)	1941
Intercept (a)	0.001
Regression Coefficient (r^2)	0.9997
Relative standard deviation* (%)	0.701
Percentage range of errors (Confidence limits)	
0.05 level	0.868
0.01 level	0.943

* Relative standard deviation for eight determinations

Interference studies

The effect of wide range of excipients and other additives usually present in the capsules of TSN in the determination under optimum conditions was investigated. The common excipients such as hydroxypropyl methylcellulose, lactose monohydrate, magnesium stearate, microcrystalline cellulose, polyethylene glycol 3000, and sodium starch glycolate have been added to the sample solution and injected. They have not disturbed the elution or quantification of TSN. In fact many have no absorption at 225 nm.

Table 3: Analysis of TSN in capsules

Amount of TSN (µg/ml)		% Recovery	% RSD
Taken	Found*		
5	4.98	99.60	0.859
10	10.02	100.20	0.647

* Average of five determinations

Analysis of capsules

To find out the suitability of the proposed method for the assay of capsules containing TSN was analyzed by the proposed method. The results are summarized in Table 3. The proposed method was more accurate with high recoveries indicating the absence of any interference from the excipients.

Table 4: Recovery studies

Amount of TSN (µg/ml)			% Recovery	% RSD
Taken	Added	Found*		
5	2.5	7.48	99.73	0.548
5	5	9.97	99.70	0.617

* Average of five determinations

Recovery studies

Recovery studies were conducted by analyzing each pharmaceutical formulation in the first instance for the active ingredient by the proposed method. Known amount of the pure drug was then added to each of the previously analyzed formulation, and the total amount of the drug was once again determined by the proposed method after bringing active ingredient concentration within the linearity

limits. The results are recorded in Table 4. The results were reproducible with low SD and RSD. No interference from the common excipients was observed and thereby establishes some degree of selectivity.

CONCLUSION

A new HPLC with UV detection method was proposed for the assay of TSN in bulk and capsules. The method is based on the use of a C18 column, with a suitable mobile phase, without the use of any internal standard. It can be seen from the results presented above, that the proposed method has good sensitivity. Statistical analysis of the results shows that the proposed procedures have good precision and accuracy. Results of analysis of pharmaceutical formulations reveal that the proposed method is suitable for their analysis with virtually no interference of the usual additives present in pharmaceutical formulations.

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