Validated stability-indicating RP-HPLC method for tamsulosine hydrochloride in pharmaceutical dosage form according to ICH guidelines: Application to stability studies

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ABSTRACT

A novel stability-indicating reversed phase high-performance liquid chromatographic assay method was developed and validated for quantitative determination of tamsulosine hydrochloride in bulk drugs and in pharmaceutical dosage form in the presence of degradation products. An isocratic, reversed phase HPLC method was developed to separate the drug from the degradation products, using an Ace5-C18 (250×4.6 mm, 5 μm) advance chromatography column, and 10 mmol L^{-1} methanol and water (70:30 v/v) as a mobile phase. The detection was carried out at a wavelength of 280 nm. The tamsulosine hydrochloride was subjected to stress conditions of hydrolysis (acid, base), oxidation, photolysis and thermal degradation. Degradation was observed for tamsulosine hydrochloride in base, in acid and in 30% H$_2$O$_2$. The drug was found to be stable in the other stress conditions attempted. The degradation products were well resolved from the main peak. The percentage recovery of tamsulosine hydrochloride was from (98.65 to 100.01%.) in the pharmaceutical dosage form. The developed method was validated with respect to linearity, accuracy (recovery), precision, system suitability, specificity and robustness. The forced degradation studies prove the stability indicating power of the method.

Keywords: Tamsulosine hydrochloride, HPLC, Validation, Stability, Degradation

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INTRODUCTION

Tamsulosine hydrochloride 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 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.

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

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estimation of tamsulosin hydrochloride with its impurities in bulk and pharmaceutical formulations as well as in biological fluids[3-12].

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 as bulk drug and pharmaceutical dosage form and separate the drugs from the degradation products under the International Conference on Harmonization (ICH) suggested conditions (hydrolysis, oxidations, photolysis and thermal stress) **[13-14]**. Therefore, the aim of the present study was to develop and validate a stability-indicating HPLC assay method for tamsulosine hydrochloride as bulk drug and in pharmaceutical dosage form as per ICH guidelines**[14]**.

MATERIALS AND METHODS

2.1 Material and reagents
Tamsulosine hydrochloride bulk drug (purity 99.8) and tablet tamsulosine hydrochloride (100 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.

2.2 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 232 nm.

2.3 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.

2.4 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 5 mg of tamsulosine hydrochloride was accurately weighed, transferred in a 50 mL volumetric flask, dissolved and diluted to 50 mL with the diluent A. From these stock solutions 2 mL of tamsulosine hydrochloride standard solution were transferred in a 10 mL volumetric flask and diluted with diluent B. This final solution contained 20 μg mL^{-1} of tamsulosine hydrochloride.

2.6 Sample solution (tablets)
Ten tablets of tamsulosine hydrochloride (100 mg) were finely ground using agate mortar and pestle. The ground material, which was equivalent to 10 mg of the active pharmaceutical ingredient, was extracted into diluent A in a 25 mL volumetric flask by vortex mixing followed by ultra sonication. Take 2 mL of it and dilute it to 40 mL with diluent B. The solution was filtered through a 0.45 μ nylon filter and an appropriate concentration of sample (20 μg mL^{-1} assay concentration) was prepared at the time of analysis.

2.7 Procedure for forced degradation study
Stability testing is an important part of the process of drug product development. The purpose of stability testing is to provide evidence of how the quality of a drug substance or drug product varies with time under a variety of environmental conditions, for example temperature, humidity, and light and enables recommendation of storage conditions, retest periods, and shelf life to be established. The two main aspects of drug product that play an important role in shelf-life determination are assay of the active drug and the degradation products generated during stability studies.

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2.8 Preparation of sample solution
2.8.1 Acidic degradation
5 mg drug was dissolved in the 5 mL of the diluent A. 50 mL of the 3 mol L^{-1} hydrochloric acid was added to it. The solution was kept for 1 h. 10 mL of solution was taken from it and neutralized with 3 mol L^{-1} sodium hydroxide. Then the solution was diluted with diluent B to prepare working solution of 20 μg mL^{-1} (pH of solution was 2.3).

2.8.2 Alkaline degradation
5 mg drug was dissolved in the 5 mL of the diluent A. 50 mL of the 0.5 mol L^{-1} sodium hydroxide was added to it. The solution was kept for 1 h. 10 mL of solution was taken from it and neutralized with 0.5 mol L^{-1} hydrochloric acid. Then the solution was diluted with diluent B to prepare working solution of 20 μg mL^{-1} (pH of solution was 14).

2.8.3 Oxidative degradation
5 mg drug was dissolved in 5 mL of diluent A and 50 mL of 30% H_2O_2 was added. The solution was kept for 4 h. Then the solution was diluted with diluent B to prepare working solution of 20 μg mL^{-1}.

2.8.4 Thermal degradation
10 mg drug was kept in the hot air oven for 48 h at 100^0C temperature. Then the working solution was prepared using diluent A and diluent B.

2.8.5 Photo degradation
10 mg of drug is exposed to the short wavelength (254 nm) and long wavelength (366 nm) UV light for 48 h. Then the working solution was prepared using diluent A and diluent B.

2.9 Specificity
Specificity is the ability of the method to assess unequivocally the analyte in the presence of components, which may be expected to be present. Typically, these might include degradation products, matrix etc.[13] The specificity of the developed HPLC method for tamsulosine hydrochloride was carried out in the presence of its degradation products. Stress studies were performed for tamsulosine hydrochloride bulk drug to provide an indication of the stability indicating property and specificity of the proposed method. Intentional degradation was attempted to stress conditions exposing it with acid (3 mol L^{-1} hydrochloric acid), alkali (0.5 mol L^{-1} NaOH), hydrogen peroxide (30%), heat (100^0C) and UV light (254 and 366 nm wavelength) to evaluate the ability of the proposed method to separate tamsulosine hydrochloride from its degradation products. For light and heat study, the study period was 48 h whereas for acid and base 1 h and for oxidation 4 h. Peak purity test for tamsulosine hydrochloride was by using PDA detector in stress samples.

RESULTS AND DISCUSSION

3.1 Optimization of chromatographic conditions
The primary target in developing this stability indicating HPLC method is to achieve the resolution between tamsulosine hydrochloride 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 and tamsulosine hydrochloride was achieved on Ace5 octadecyl silane C-18 stationary phase and 10 mmol L^{-1} methanol and water (90:10 v/v) as a mobile phase. The tailing factor obtained was less than two and retention time was about 3.3 min for the main peak and less than 4 min for the degradation products, which would reduce the total run time and ultimately increase the productivity thus reducing the cost of analysis per sample. Forced degradation study showed the method is highly specific and entire degradation products were well resolved from the main peak. The developed method was found to be specific and method was validated as per international guidelines.

3.2 Result of forced degradation experiments
Degradation was not observed for tamsulosine hydrochloride samples during stress conditions like heat, UV and light, except in base, acid and oxidation. Tamsulosine hydrochloride was degraded into acid (Figure 2), base (Figure 3) and oxidation (Figure 4) and forms polar impurities. In the acidic condition 7.54%, in the basic condition 4.88% after 1 h and in the oxidative condition 58.70% after 4 h, degradation was observed for tamsulosine hydrochloride. Peak purity results greater than 990 indicate that the tamsulosine hydrochloride peak is homogeneous in all stress

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conditions tested. The unaffected assay of tamsulosine hydrochloride in the tablets confirms the stability indicating power of the method (Table 1).

3.3 Determination of active ingredients in tablets
The contents of drug in tablets were determined by the proposed method using the calibration Curve.

Method Validation
4.1 Precision
Assay of method precision (intra-day precision) was evaluated by carrying out six independent assays of tamsulosine hydrochloride test samples against reference standard, the percentage of RSD of six assay values obtained was calculated. The intermediate precision (inter-day precision) of the method was also evaluated using two different analysts, different HPLC systems and different days in the same laboratory (Table 2).

4.2 Accuracy (recovery test)
Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of the drugs in the placebo. The recovery was performed at three levels, 80, 100 and 120% of the label claim of the tablet (100 mg of tamsulosine hydrochloride). The recovery samples were prepared in the aforementioned procedure, and then 5 mL of tamsulosine hydrochloride solution were transferred into a 50 mL volumetric flask and diluted to volume with diluent B. Three samples were prepared for each recovery level. The solutions were then analyzed, and the percentage recoveries were calculated from the calibration curve. The recovery values for tamsulosine hydrochloride ranged from 98.65 to 100.01%. The average recoveries of three levels for tamsulosine hydrochloride were 99.5% (Table 3).

4.3 Linearity
The linearity of the response of the drug was verified at seven concentration levels, ranging from 10 to 150% of the targeted level (20 µg mL^{-1}). Concentration standard solutions containing 2-30 µg mL^{-1} of tamsulosine hydrochloride in each linearity level were prepared. Linearity solutions were injected in triplicate. The calibration graphs were obtained by plotting peak area versus the concentration data and were treated by least-squares linear regression analysis. The equation of the calibration curve for tamsulosine hydrochloride obtained $y = 28074 X + 4287$, the calibration graphs were found to be linear in the aforementioned concentrations. The coefficient of determination is 0.9993.

4.4 Limit of detection and limit of quantification (LOD and LOQ)
The limit of detection (LOD) and limit of quantification (LOQ) were determined by calibration curve method.[11] Specific calibration curve was constructed using samples containing the analytes in the range of LOD and LOQ. The LOD and LOQ for tamsulosine hydrochloride in LC were 0.24 and 0.73 µ g mL^{-1}, respectively. LOD and LOQ were calculated by using the following equations. LOD = 3.3Sa/b, LOQ = 10Sa/b. where Sa is the standard deviation of the calibration curve and b is the slope of the calibration curve. Precision at limit of quantification and limit of detection was checked by analyzing six test solutions prepared at LOQ and LOD levels and calculating the percentage RSD of area.

4.5 Robustness
To determine the robustness of the developed method experimental condition were purposely altered and the resolution between tamsulosine hydrochloride and acid degradation products were evaluated. The flow rate of the mobile phase was 1.0 mL min-1. To study the effect of flow rate on the resolution, it was changed by 0.2 units from 0.8 to 1.2 mL min-1. The effect of percent organic strength on resolution was studied by varying methanol from –10 to +10%. The resolution in the robustness study was not less than 3.5 in all conditions. The stability of the standard solutions and the sample solutions was tested at intervals of 24, 48 and 72 h. The stability of solutions was determined by comparing results of the assay of the freshly prepared standard solutions. The RSD for the assay results determined up to 72 h for tamsulosine hydrochloride was 0.53%. The assay values were within 1.5% after 72 h. The results indicate that the solutions were stable for 72 h at ambient temperature.

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Table 1. **Summary of forced degradation results**

Stress conditions	time / h	Assay of active	Degradation/(%) Substance	Peak purity[a]
Acid hydrolysis (3 mol L^{-1} HCl)	1	90.24	7.54	999
Base hydrolysis (0.5 mol L^{-1} NaOH)	1	88.69	4.88	999
Oxidation (30% H_2O_2)	4	40.08	58.70	999
Thermal (100^0C)	48	99.88	No degradation	999
Photo	48	99.65	No degradation	999

[a]*Peak purity values in the range of 990-1000 indicate the homogenous peak.*

Table 2. Result of precision of test method

Sample number	Assay of tamsulosine hydrochloride as % of labeled amount	
	Analyst 1(intra-day precision)	Analyst 2(inter-day precision)
1	99.97	99.75
2	99.87	99.40
3	99.98	99.23
4	99.56	99.31
5	99.45	99.43
6	99.68	99.13
Mean	99.75	99.45
RSD	0.12	0.101

Table 3. Results of recovery tests of tamsulosine hydrochloride

Level of addition / (%)	Amount added / μg	Recovery /(%)	Average recovery / (%)
80	4.8	98.65	
100	6	100.01	99.5
120	7.2	99.84	
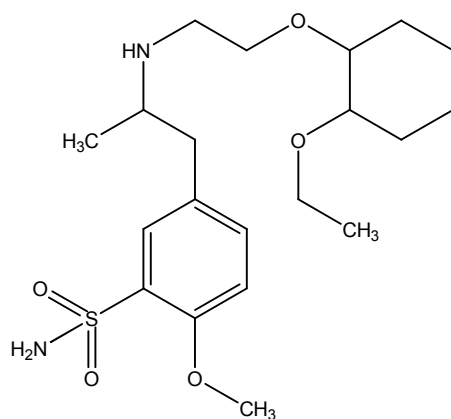
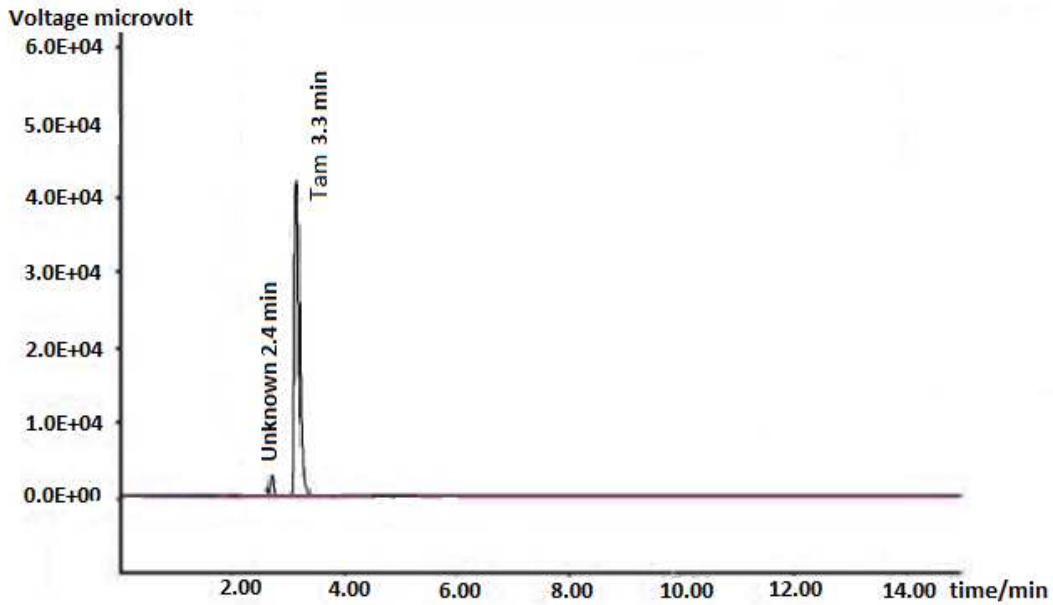**Fig 1. Chemical structure of Tamsulosine hydrochloride**

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Fig 2. Chromatogram of tamsulosine hydrochloride in acid degradation (acid Degraded product (2.4 min) and tamsulosine hydrochloride (3.3 min)). Concentration of tamsulosine hydrochloride injected was 20 μg mL^{-1}.
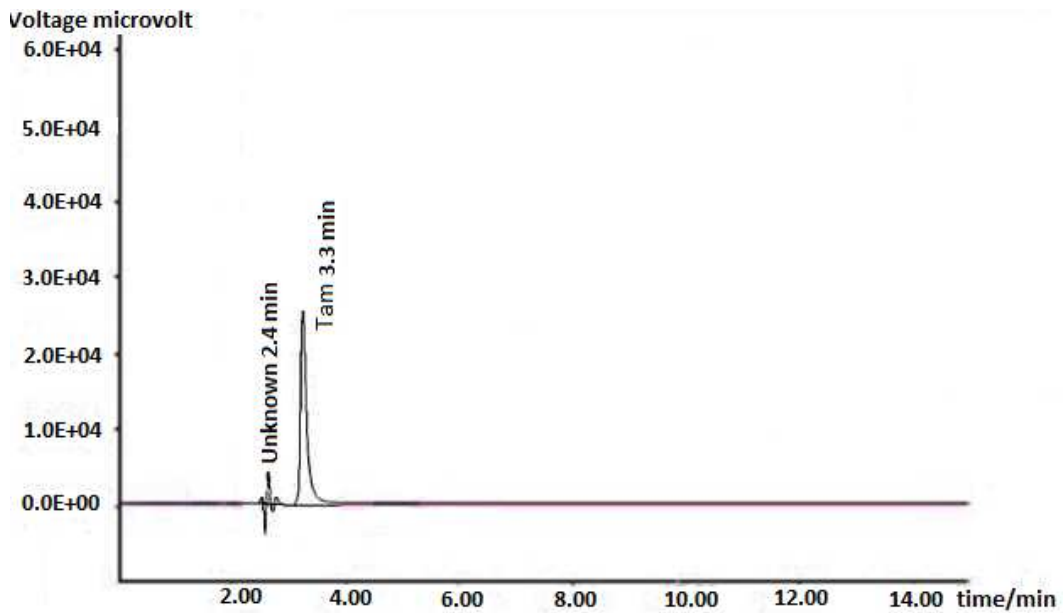

Fig 3.   Chromatogram of tamsulosine hydrochloride in base degradation (base degraded   product (2.4 min) and tamsulosine hydrochloride(3.3 min)).
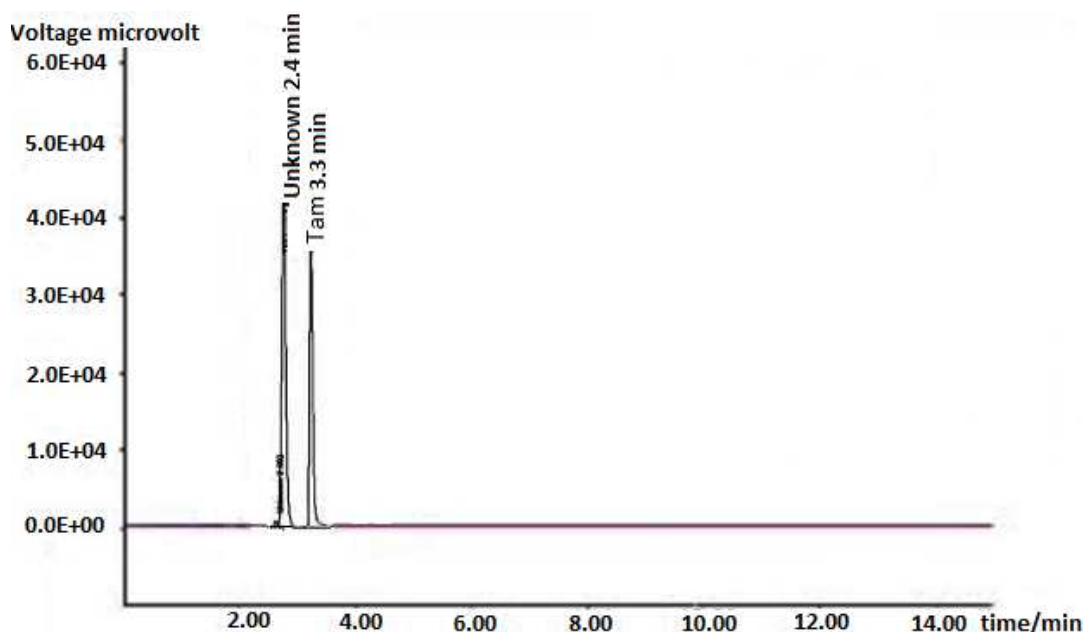
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Fig 4.   Chromatogram of tamsulosine hydrochloride in oxidative degradation (oxidative   degraded product (2.4 min) and tamsulosine hydrochloride (3.3 min)).

CONCLUSION

The developed method is stability indicating and can be used for assessing the stability of tamsulosine hydrochloride bulk drugs and pharmaceutical dosage form. The developed method is specific, selective, robust, rugged and precise. This method can be conveniently used for assessing stability assay of selected substances and dissolution of tablets containing tamsulosine hydrochloride in quality control laboratory. The study showed that the drug is stable for the thermal and photo degradation conditions where as moderately degraded in acid (7.54%) and base (4.88%) conditions but highly degraded in the oxidative (58.70%) conditions

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