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RP-HPLC Method for the Determination of Tamsulosin in Bulk and Pharmaceutical Dosage Forms

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A sensitive, selective and precise high-performance liquid chromatographic method of analysis of tamsulosin both in a bulk drug and in formulations was developed and validated. The chromatographic separation was achieved on a Microbondapak C_{18} (250 cm × 4.6 mm, 5 µm) column using methanol:phosphate buffer (pH 7.8) (80:20 v/v) as a mobile phase. Solution concentrations were measured on a weight basis to avoid the use of an internal standard. The detection wavelength was 230 nm. This system was found to give the sharp peak for tamsulosin (RT at 3.42 ± 0.05 min). Tamsulosin was subjected to acid and alkali hydrolysis, oxidation, dry heat, wet heat and photo degradation. The drug underwent degradation under acidic and basic conditions and upon oxidation. The degraded products were well resolved from the pure drug with significant difference in their RT values. The response of the drug was found to be linear in the range of 5-250 µg/mL ($r^2 > 0.999$). The method was validated for the precision and recovery. The limit of detection and quantitation were 0.215 and 0.739 µg/mL, respectively. As the method could effectively separate the drug from its degradation products, it can be employed for analysis of stability samples.

Key Words: Tamsulosin, Stability indicating, Degradation, Validation and RP-HPLC.

INTRODUCTION

Tamsulosin, 5[(2R)-2-[2-(2-ethoxy phenoxy) ethyl amino] propyl]-2-methoxy benzene sulphonamide (Fig. 1) is an antagonist of α_{1A} adrenoceptors in the prostate[1]. Tamsulosin is not official in any pharmacopoeia. Although some pilot data on the HPLC[1] and LC-MS/MS[2,3] of tamsulosin have already been reported, but no simple and economic study on the drug has been performed. The scientific novelty of the present work is that the method used are simple, rapid, sensitive, less expensive and less time consuming compared with other published HPLC methods. This paper also deals with the assay of tamsulosin in presence of degradation products by HPLC. The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products[4], requires the stress testing to be carried out to elucidate the inherent stability characteristics of the active substances. Susceptibility to oxidation is one of the required tests. Also the hydrolytic and photolytic stability are required. An ideal stability indicating method is one that quantifies the drug and also resolves its degradation products. The aim of the present study is to develop an accurate, specific, reproducible and stability indicating method for the determination of tamsulosin in presence of its degradation products and for assessment of purity of bulk drug and stability of its dosage forms. Stability study of tamsulosin will provide the many informations which will help in formulation and storage and the characterization of the degraded compounds and may open a new scope of research on toxicity study of degraded components. The findings of toxicity study will help in scrupulous determination expiry, adverse effects *etc*. The proposed method was validated as per ICH guidelines[5,6].

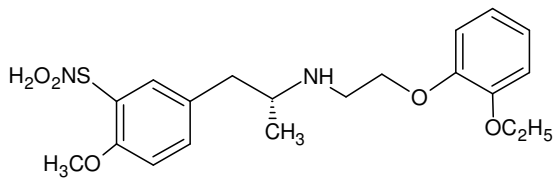

Fig.1. Chemical structure of tamsulosin

EXPERIMENTAL

Tamsulosin active pharmaceutical ingredient (API) was provided by Dr. Reddy's Laboratory Ltd., Hyderabad. It was used without further purification. All chemicals and reagents used were of HPLC grade and were purchased from Merck (India) Ltd., Mumbai.

An isocratic HPLC (Waters India, USA) with a single Waters 510 Pump, Waters 486 tunable absorbance detector

and RP-C_{18} column (Bondapak C_{18}, 250 mm × 4.6 mm, packed with 5 μm particle size) was used. The HPLC system was equipped with Millennium[32] software.

Chromatographic conditions: The mobile phase used was methanol and phosphate buffer (pH 7.8) in the ratio of 80:20 v/v. The mobile phase was filtered before use through a 0.45 μm membrane filter and degassed for 15 min. The components of the mobile phase were pumped from the solvent reservoir to the column at a flow rate of 1 mL/min that produced column backpressure 140-150 kg/cm^2. Ambient column temperature was maintained. The eluents were monitored at 230 nm. The volume of each injection was 20 μL.

Sample preparation: A pure sample of tamsulosin procured from Dr. Reddy's Laboratory Ltd., Hyderabad, was used as reference standard in the study. About 100 mg of tamsulosin was weighed accurately and transferred into a 100 mL volumetric flask and dissolved in 50 mL of the mobile phase. Then the volume was made up with a further quantity of the mobile phase to get 1 mg/mL solution. Following this, the solution was sonicated for 15 min to degas it. Subsequent dilutions of this solution ranging from 5-250 μg/mL were made in 10 mL volumetric flasks by mobile phase. Twenty μL of this solution was injected each time into the stream of mobile phase at a flow rate of 1 mL/min. Each of the dilutions was injected 6 times into the column and the corresponding chromatograms were obtained.

Solutions containing 10, 90 and 200 μg/mL of tamsulosin were subjected to the proposed HPLC analysis to check the intra-day and inter-day variation of the method. The recovery studies were carried out by adding known amounts of tamsulosin to the preanalyzed samples and then analyzing them by the proposed HPLC method.

Method validation: Linear calibration plot for assay method was obtained over the calibration ranges tested, *i.e*., 5-250 μg/mL. The peak area *versus* concentration data was performed by least-squares linear regression analysis. The accuracy of the assay method was evaluated by injected 6 times at three concentration levels *i.e.*, 40, 50 and 60 μg/mL in bulk drug and formulation samples. The percentage recoveries were calculated from the slope and Y-intercept of the calibration curve obtained during linearity study of assay method. Repeatability of measurement of peak area was carried out using 6 replicates of the same sample (80 μg/mL of tamsulosin). The intra- and inter day variation for the determination of tamsulosin was carried out at three different concentration levels *i.e.*, 10, 90 and 200 μg/mL. The robustness was tested by introducing small changes in the mobile phase composition. Mobile phase having different compositions of methanol: phosphate buffer (80:20 ± 0.5 v/v) were tried and chromatogram was obtained. Also, the operating temperature, detection wavelength and relative humidity were varied in the range of ± 5 %. Robustness of the method was done at two different concentration levels of 70 and 150 μg/mL. In order to estimate the limit of detection (LOD) and lower limit of quantitation (LOQ), blank methanol:phosphate buffer (80:20 v/v) was sampled 6 times. The signal to noise ratio was determined. Limit of detection was considered at an S/N ratio of 3:1 and LOQ at an S/N ratio of 10:1. The specificity of the method was ascertained by analyzing standard drug and sample. The retention time (RT) of tamsulosin was confirmed by comparing the retention time with that of the standard Fig. 3(b). The use of placebo like calcium carbonate, starch and talc in different concentration was also studied by spiking the standard tamsulosin solution and no interference was observed in the chromatogram.

Analysis of marketed formulation: To determine the content of tamsulosin in conventional tablets (label claim: 0.4 mg/tablet), the tablets were powdered and the powder equivalent to 10 mg of tamsulosin was taken in a 100 mL volumetric flask and add approximately 80 mL of mobile phase. The content of the flask was then allowed to stand for 15 min with intermittent sonication to ensure complete miscibility of the drug and then filtered through 0.45 μm membrane filter. Final volume was made upto 100 mL with mobile phase to get a stock solution of 100 μg/mL. 2 mL of the resulting solution was transferred into a 10 mL of volumetric flask and diluted to the mark with mobile phase. A sample of 20 μL of this solution was directly injected. The average drug content (Table-1) of the formulations was quantified using the regression equation obtained for the pure sample.

TABLE-1
ASSAY OF TAMSULOSIN IN DOSAGE FORM

Brand name of the tablet	Labeled amount of drug (mg)	Mean (± SD) amount (mg) found by the proposed method (n = 6)	Mean % (± SD) labeled amount (n = 6)
Veltam	0.4	0.4051 ± 0.0051	101.3 ± 1.29
Dynapres	0.4	0.3996 ± 0.0064	99.68 ± 1.60

Accelerated degradation of tamsulosin: All degradations were done at a drug concentration of 150 μg/mL. For acid and alkali decomposition studies, initially the pure drug was mixed with approximately 20 mL of 0.1 N HCl and approximately 20 mL of 0.1 N NaOH separately. These mixtures were refluxed on a water bath for 4 h at 60 ºC. The forced degradation in acidic and basic media was performed in the dark in order to exclude the possible degradative effect of light. The resulting solution was neutralized by base and acid, respectively to avoid any interference of acid or base and suitably diluted with HPLC grade water to obtain solution of concentration of 150 μg/mL. 20 μL of resulting solution were injected into HPLC and the chromatograms were recorded. Tamsulosin was subjected to oxidative degradation by treating with 3 % v/v hydrogen peroxide solution for 24 h at ambient temperature. The powdered drug was stored in an oven at 60 ºC for 72 h to study dry heat degradation. Also the test solution was refluxed for 6 h on a water bath set at 60 ºC for wet heat degradation study. The photo chemical stability of the drug was also studied by exposing the drug to direct UV light (254 nm) for 24 h and the chromatogram were obtained. In all degradation studies the average peak area of tamsulosin of 6 replicates were obtained.

RESULTS AND DISCUSSION

The HPLC procedure was optimized with a view to develop a stability indicating assay method. Pure drug chromatogram was run in different mobile phase systems containing methanol and phosphate buffer in different ratios. The retention time and the tailing factor were calculated for each chromatogram.

Finally the mobile phase consisting of methanol:phosphate buffer (80:20 v/v) was selected which gave a sharp and symmetrical peak with minimum tailing. Calibration graph was found to be linear and in adherence to Beer's law over the concentration range of 5- 250 µg/mL (r^2 ± CV = 0.999, 0.813).

The repeatability of sample application and measurement of peak area were expressed in terms of percentage RSD and found to be 0.82. The percentage RSD for within and day to day analysis was found to be less than ± 2 % in all the cases (Table-2). The standard deviation and percentage RSD of peak area calculated for each parameter was less than ± 2 %, indicating robustness of the method. The LOD with a S/N ratio of 3:1 was found to be 0.215 µg/mL. The LOQ with S/N ratio of 10:1 was found to be 0.739 µg/mL. The peak purity of tamsulosin was assessed by comparing the chromatogram of standard and sample solutions. Good correlation (r^2 = 0.999) was obtained between standard and sample spectra of tamsulosin.

TABLE-2
SUMMARY OF VALIDATION PARAMETERS

Parameter	Data
Linearity range (µg/mL)	5-250
Linear regression equation	y = 12016x + 35602
Correlation coefficient	0.999
Limit of detection (µg/mL)	0.215
Limit of quantitation (µg/mL)	0.739
Recovery	99.90 ± 1.45
Precision (RSD %)	
Inter day*	0.29
Intra day*	0.97
Robustness	Robust
Specificity	Specific
Theoretical plate	5717
Tailing factor	1.12

*Each data represent the average of 6 reading.

A single peak at retention time 3.42 min (Fig. 2) was observed in the chromatogram of the drug samples extracted from the tablets. There was no interference from the excipient commonly present in tablets. The drug content was found to be 101.3 % with a SD of ± 1.29. It may therefore be inferred that degradation of tamsulosin had not occurred in the marketed formulations that were analyzed by this method (Table-1). The low SD value indicated the suitability of this method for routine analysis of tamsulosin in pharmaceutical dosage form. The proposed method when used for extraction and subsequent estimation of tamsulosin from pharmaceutical dosage form after spiking with additional drug provided recovery of 99.83-99.90 % (Table-3).

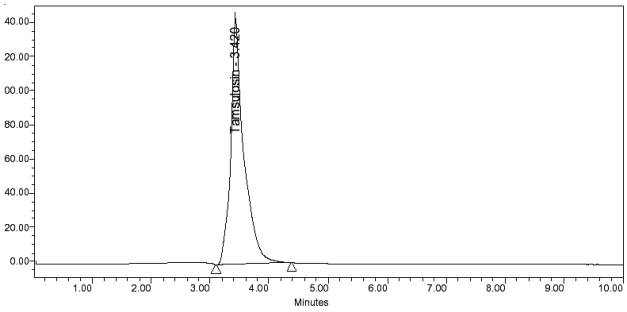

Fig. 2. Chromatogram of the tamsulosin extracted from the tablet

TABLE-3
RECOVERY STUDIES

Amount of drug added (µg) to solutions of formulation	Recovery from tablet formulation	
	Mean (± SD) amount (µg) found (n = 6)	Mean (± SD) % recovery (n = 6)
40	39.92 ± 0.81	99.89 ± 1.04
50	49.90 ± 0.81	99.83 ± 1.63
60	59.93 ± 0.87	99.90 ± 1.45

The chromatogram of the acid degraded sample for tamsulosin showed the peak at retention time 1.60 and 3.45 Fig. 3(c). The chromatogram of the base degraded sample showed the peaks at retention time 1.58 and 3.42 [Fig. 3(d)]. When the drug was exposed to oxidation conditions (3 % v/v hydrogen peroxide), a mild degradation was observed [Fig. 3(e)]. The areas of the degraded peaks were found to be lesser than the area of standard drug concentration 150 µg/mL indicating that tamsulosin underwent degradation under acidic, basic and oxidation conditions, as listed in Table-4. The peaks of degraded products were well resolved from the drug peak. When tamsulosin was subjected to dry heat degradation by stored in an oven at 60 °C for 72 h, wet heat degradation by refluxed for 6 h on a water bath set at 90 ºC, no degradation was observed. Test solution of tamsulosin prepared in mobile phase was subjected to photolytic degradation by exposing the same to UV light for 24 h; no degradation was observed.

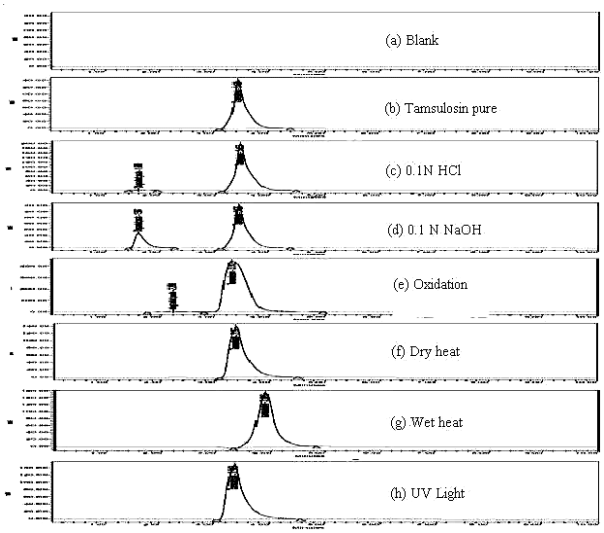

Fig. 3. HPLC chromatogram of tamsulosin under different stress conditions

Degradation studies there by indicate that the drug is susceptible to acid hydrolysis, basic hydrolysis and oxidation with maximum degradation was observed in basic hydrolysis.

Conclusion

The developed HPLC technique is precise, specific, accurate and stability indicating. Statistical analysis proved method was repeatable and selective for the analysis of tamsulosin as bulk drug and in pharmaceutical formulation without any interference from the excipient. Also the above result indicate the suitability of the method for acid, base, oxidative, dry heat, wet heat and photo degradation studies. As the method separates the drug from its degradation products, it can be employed for analysis of stability samples.

TABLE-4
DEGRADATION OF TAMSULOSIN

Stress conditions	Time (h)	Assay of active substance (%)	Assay of degraded product (%)	Mass balance (%)
Base hydrolysis (0.1 N NaOH)	4	65.49	33.56	99.05
Acid hydrolysis (0.1 N HCl)	4	93.75	4.64	98.21
Oxidation (3 % H_2O_2)	24	92.22	8.79	101.01
Dry heat (60 ºC)	72	99.8	–	99.8
Wet heat (60 ºC)	4	99.63	–	99.63
UV (254 nm)	24	99.25	–	99.25

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REFERENCES

1. B.M. Rao, M.K. Srinivasu, G. Sridhar, B.S.S. Reddy, T.V.S.K. Vittal, R.P. Kumar and K.B. Chandrasekhar, *Indian Drugs*, **43**, 39 (2006).
2. N.V.S. Ramakrishna, K.N. Vishwottam, S. Manoj, M. Koteshwara, S. Vishu and D.P. Verma, *Biomed. Chromatogr*., **19**, 709 (2005).
3 M. Qi, P. Wang and L. Liu, *J. Chromatogr. B*., **805**, 7 (2004).
4. Proceedings of International Conference on Harmonization of Technical requirements of Registration of Pharmaceuticals for Human Use, Stability Testing of New drug substances and products ICH-Q1A, Geneva (1993).
5. Proceedings of International Conference on Harmonization of Technical requirements of Registration of Pharmaceuticals for Human Use, Validation of analytical procedure ICH-Q2B Geneva (1996).
6. Proceeding of the International Convention on Quality for the Pharmaceutical Industry, ICH Guidance on Analytical Method Validation, Toronto, Canada (2002).