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Validated Stability Indicating Method For The Determination Of Tamsulosin In Pharmaceutical Dosage Form By Rp-Hplc.

P. Geetha Swarupa[1], Govind Chowdary[2], K. R. S. Prasad[1], K. Suresh Babu [3]*, and J. V. Shanmukha Kumar[1]
[1]*Department of Chemistry, Koneru Lakshmaiah Education Foundation, Vaddeswaram, Andhra Pradesh, India.*
[2]*Department of Chemistry, Vignan Institute of Technology and Sciences, Deshmuki, Nalgonda, Telangana, India.*
[3]*Department of Chemistry, Marri Laxman Reddy Institute of Technology and Management, Hyderabad, Telangana, India.*

Abstract

Tamsulosin in its pharmaceutical dosage has determined by a new validated stability- indicating method using RP-HPLC. Kromasil (250 x 4.6 mm, 5µm) column and mobile phase comprising of Buffer (0.1% Ortho-phosphoric acid)-Acetonitrile (50:50, v/v) were used to achieve the chromatographic separation at a wavelength of 270 nm with 1.0 ml/minute flow rate. The suggested method was linear with correlation coefficient (R^2= 0.9997) and regression equation y = 45801x + 2700.8. The limit of detection and limit of quantification were 0.010 and 0.031 respectively. The %RSD of precision, accuracy was < 2. Tamsulosin was subjected to stress conditions including acid, alkali, thermal, photolysis and oxidation degradation. Degradation of Tamsulosin was observed more in acidic medium. The proposed method was accurate, linear, precise, robust as per ICH guidelines.

Key words: *Tamsulosin, Stability- indicating method, RP-HPLC, Degradation, Stress studies.*

INTRODUCTION

Tamsulosin, an alpha-adrenoceptor blocker and a sulfamoylphenethylamine-derivative with highly selective for the alpha-adrenoceptors of the prostate [1]. Tamsulosin is used to treat indications of benign prostatic hyperplasia (BPH) [2]. Chemically it is known as 5-[2-[2-(2-Ethoxyphenoxy) ethyl amino] propyl]-2-methoxy-benzenesulfonamide **Fig. 1**.

As it is an antagonist at alpha-1A and alpha-1B-adrenoceptors and approximately 70% of the alpha1-receptors in human prostate are of the alpha-1A subtype[3] in the prostate the mechanism action involved in it is that these receptors block and causes relaxation of smooth muscles in the bladder neck and prostate, thus the urinary outflow resistance in men [4] decreases.

All analytical methods described in literature for the determination of Tamsulosin involve simultaneous estimation of Tamsulosin and other drugs by spectrophotometric method, UV spectroscopy method, RP-HPLC method, LC-ESI-MS/MS method. Determination of the enantiomers of Tamsulosin hydrochloride and its synthetic intermediates by chiral liquid chromatography, stability-indicating HPTLC method [5-22] in bulk and pharmaceutical dosage forms.

In the current work, we developed a simple, precise, accurate, selective, robust and stability -indicating liquid chromatographic method for the quantification of Tamsulosin in pharmaceutical dosage form in the presence of its degradation products as an alternative method. The developed stability-indicating method is validated as per ICH guidelines [23].

MATERIALS AND METHODS

Materials

Tamsulosin was obtained from SJS pharma lab and tablets (Label claim: 0.4 mg of Tamsulosin) was produced from the market. Acetonitrile, Ortho-phosphoric acid was obtained from Merck Specialities

Private Ltd. The other chemicals used in this process all are of HPLC grade. Milli Q water was used throughout the experiment.

Methods

A HPLC system Alliance waters 2695 HPLC equipped with 2996 PDA detector was used. Analysis was carried out using chromatographic column Kromasil 250mm x 4.6 mm, 5µ and the mobile phase consisting mixture of Acetonitrile and 0.1% ortho-phosphoric acid in the ratio (50:50, v/v). The mobile phase was injected into the HPLC system with a flow rate of 1.0 ml/min, before that it was degassed and filtered. The sample 10 µl was inserted in to the HPLC system and was detected at wavelength 270 nm.

Preparation of standard solution

Exactly an amount of 4mg Tamsulosin working Standard was taken into a 10 ml clean dry volumetric flask. Initially 5 ml of diluent was added to the flask and sonicated for 30 minutes to get mix the solution properly and then made up to the mark of the flask with diluent. The stock solution prepared from the above 1 ml was pipetted out in to a 10 ml volumetric flask and made up to the mark with diluent. After preparing, solutions were filtered through a 0.45µ membrane filter prior to injection.

Preparation of sample solution

About 5 tablets were quantified and calculated the average weight of each tablet then the weight corresponding to 1 tablet was transferred into a 10 ml volumetric flask. Initially 7 ml of diluent was added and sonicated for 30 min and made up to the mark with the diluent. The contents of the volumetric flask were filtered. About 1 ml of filtered solution was pipetted out into a 10 ml volumetric flask and made up to the mark with diluent. 10 µl of these solutions were injected into the system and the peak area was recorded.

METHOD VALIDATION

The method was validated for the following parameters: Precision, accuracy, selectivity, robustness, limit of quantitation (LOQ), limit of detection (LOD) and system suitability.

System suitability

The working standard solutions of Tamsulosin were prepared as per test method and was injected six times into the HPLC system. The system suitability parameters like USP tailing, USP plate count and retention time were evaluated.

Linearity

Linearity test was performed by injecting different concentration levels of 10 µl of each in to the chromatographic system, peak area obtained from each chromatogram was noted. A graph is plotted between peak area (on y-axis) and concentration (on X-axis) to calculate the correlation coefficient.

Accuracy

The accuracy of Tamsulosin assay method was expressed from the percentage recoveries of calibration curve at three different concentration levels (50,100 and 150%), in triplicate manner.

Precision

The method precision (intra-day) of the assay method was estimated by calculating peak area ratios obtained from six repeated injections of Tamsulosin 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 estimated using two different analysts, different HPLC systems and different days in the same laboratory.

Limit of Detection and Limit of Quantification (LOD & LOQ)

The limit of detection and limit of quantification were established based on standard deviation of the response and the slope of the calibration curve. LOD & LOQ were calculated by using the following equations,

$$LOD = \frac{3.3 \times \text{standard deviation of the calibration curve}}{\text{Slope of the calibration curve}}$$

$$LOQ = \frac{10 \times \text{standard deviation of the calibration curve}}{\text{Slope of the calibration curve}}$$

Robustness

The robustness of the assay method was established by altering the HPLC conditions which include flow rate (+ 0.1 ml/min of actual flow rate), mobile phase composition (+ 5% of actual organic phase) and Temperature variation (+ 5o C of actual temperature). The standard and samples of Tamsulosin were injected by changing the conditions of chromatography at a concentration level of 10 µg/ml.

Forced degradation / stress studies

Forced degradation of Tamsulosin studies were performed to evaluate the stability indicating properties and specificity of the method.

Acid Degradation Studies:

Acid degradation studies were carried out by adding 1 ml of 2 N Hydrochloric acid to 1 ml of stock solution of TAMSULOSIN and was diluted to 40 µg/ml. The resultant solution 10 µl was injected into the system and the chromatogram obtained was used to assess the stability of sample.

Alkali Degradation Studies:

Alkali degradation studies were carried out by adding 1 ml of 2 N sodium hydroxide to 1 ml of stock solution of TAMSULOSIN and was diluted to 40 µg/ml. The resultant solution 10 µl was injected into the system and the chromatogram obtained was used to assess the stability of sample.

Dry Heat Degradation Studies:

To study the dry heat degradation the standard solution of TAMSULOSIN was placed in an oven at 105 °C for 1hour. The resultant solution was diluted to 40 µg/ml solution and10 µl were injected into the system and the chromatogram obtained was used to assess the stability of sample.

Photo Stability studies:

To assess the UV degradation of TAMSULOSIN, 400 µg/ml was prepared, and the beaker was exposed to UV chamber for 1hr or 200-Watt hours/m^2 in photo stability chamber. The resultant solution was diluted to 40 µg/ml solution and10 µl were injected into the system and the chromatogram obtained was used to assess the stability of sample.

Oxidation:

To assess the oxidative degradation 1 ml of 20% hydrogen peroxide (H2O2) was added to 1 ml of stock solution of TEMSULOSIN and were kept for 30 min at 600c. The resultant solution was diluted to 40 µg/ml solution and10 µl were injected into the system and the chromatogram obtained was used to assess the stability of sample.

RESULTS AND DISCUSSION

The aim of the present work is to achieve the resolution between Tamsulosin and its degradation products. To develop a suitable LC method, different mobile phases and stationary phases were

employed. Various experiments were conducted using various columns to select best stationary and mobile phase that would give optimum resolution and better selectivity than the reported methods. The present proposed method was developed and validated using a stationary phase column Kromasil 250mm x 4.6 mm, 5µ and the mobile phase consisting mixture of 0.1% orthophosphoric acid and Acetonitrile in the ratio (50:50, v/v) at a flow rate 1 ml/min and a detection wavelength of 270nm. The injection volume is 10 µL and the retention time was 5 min.

System suitability parameters were evaluated, and the results are shown in the **Table 1**. And the standard chromatogram is shown in the **Fig. 2**.

Linearity was obtained in the concentration range of 10-60 ppm for Tamsulosin with correlation coefficient R2= 0.9997 and with regression equation y = 45801x + 2700.8. The calibration graphs are found to be linear which are shown in the **Fig. 3**.

LOQ was found to be 0.010 µg/ml and the **LOD** was found to be 0.031 µg/ml, results were shown in the **Table 2**.

The % RSD of **precision and robustness studies** were found to be < 2%, indicate the method was precise and robust. The results were shown in the **Table 3 & 4.**

Accuracy of the proposed was obtained from the percentage of recovery values ranged from 99.37 – 100.56. The average recoveries of three levels for Tamsulosin were 100.14 % and the results are shown in the **Table 5**.

To evaluate the stability of Tamsulosin, it was observed that the drug was decomposed under acid degradation and slightly under alkaline and peroxide degradation.
Degradation was not observed for Tamsulosin during stress conditions like thermal, water, UV. The percentage of the drug decomposed after the acid treatment (1 ml of 2N HCl) was found to be 4.16%, alkaline treatment (1 ml 2N NaOH) was found to be 3.40%, Peroxide degradation (1 ml 20% H2O2) was found to be 2.58%. The results were shown in the **Table 6**.
Typical chromatograms obtained for the assay of Tamsulosin stressed samples are shown in **Fig. 4-9.]**

CONCLUSION

It was concluded that the proposed RP-HPLC method developed for the determination of Tamsulosin was simple, accurate, sensitive, specific, precise and rapid. The method was proved to be superior than the reported methods. The mobile phase used was simple and economic. The method is found to be linear in the specified range, precise and robust. LOD and LOQ established by this method are lesser than the earlier reported methods. The study presented that the drug is stable for the thermal and photolytic degradation conditions whereas moderately degraded in acidic (4.16%), basic (3.40%) and in the oxidative (2.58%) conditions. Hence the proposed method stands validated and may be used for routine and stability sample analysis.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The authors stated that they have no conflict of interest.

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24.

Figures

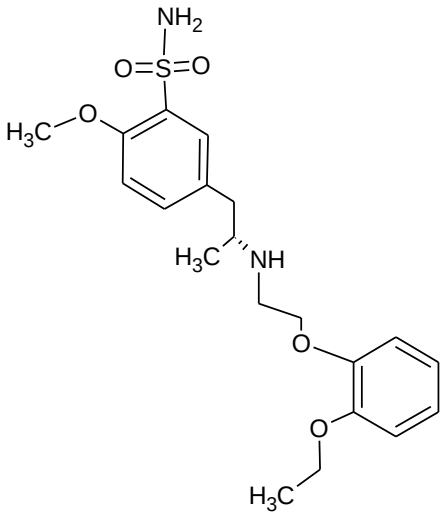

FIG.1: CHEMICAL STRUCTURE OF TAMSULOSIN

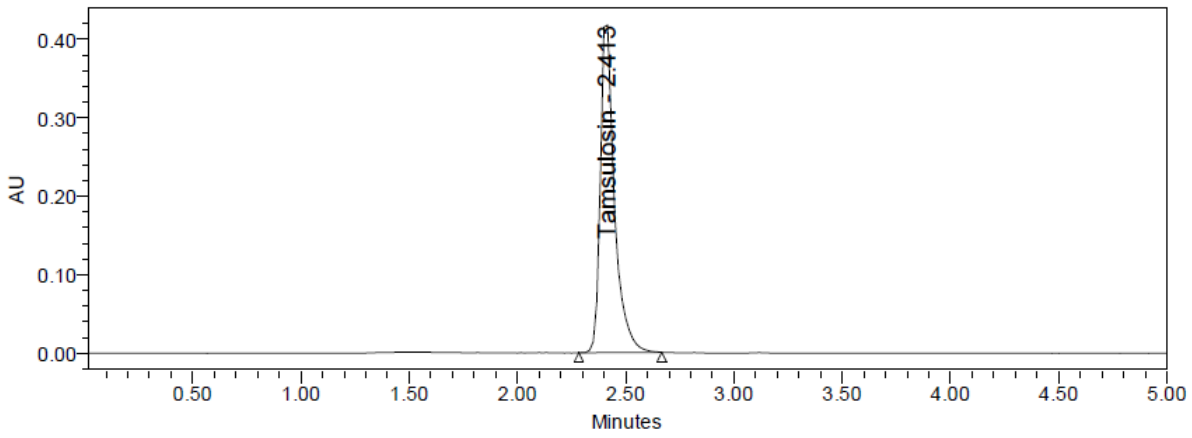

FIG. 2: STANDARD CHROMATOGRAM FOR TAMSULOSIN

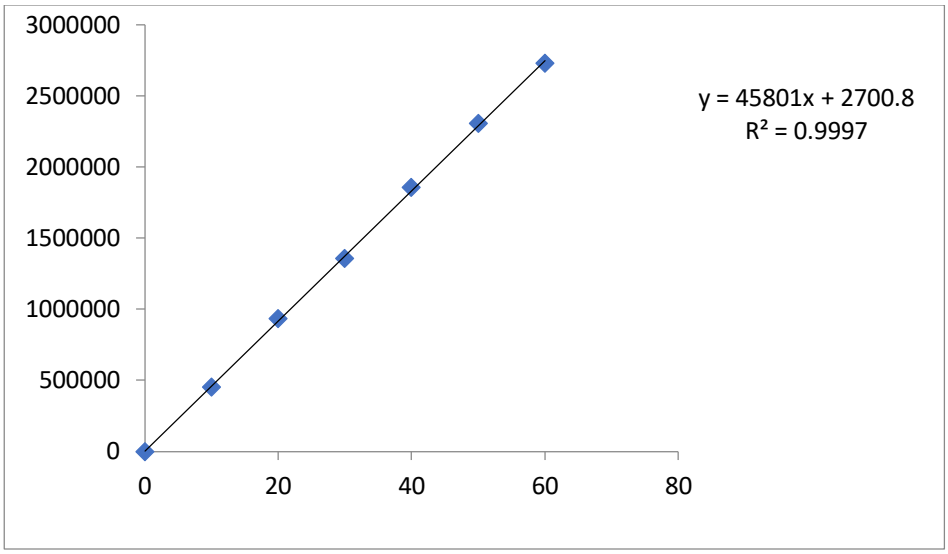

FIG. 3: LINEARITY GRAPH OF TAMSULOSIN

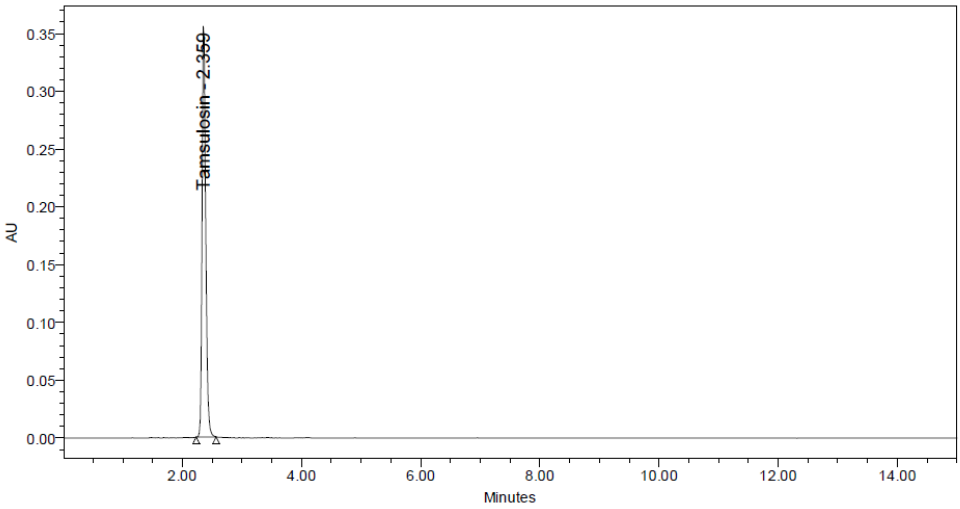

FIG. 4: TYPICAL CHROMATOGRAM OF TAMSULOSIN ON ACID DEGRADATION

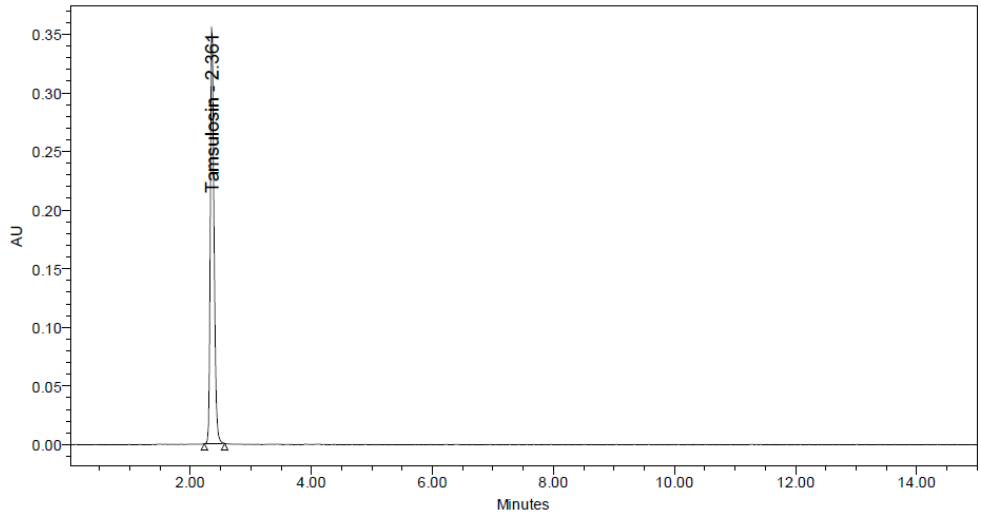

FIG. 5: TYPICAL CHROMATOGRAM OF TAMSULOSIN ON ALKALI DEGRADATION

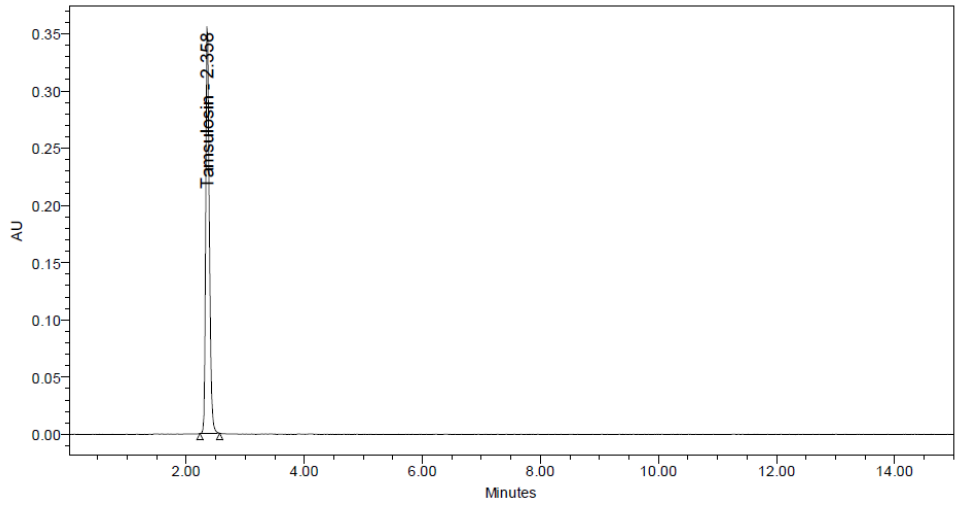

FIG. 6: TYPICAL CHROMATOGRAM OF TAMSULOSIN ON PEROXIDE DEGRADATION

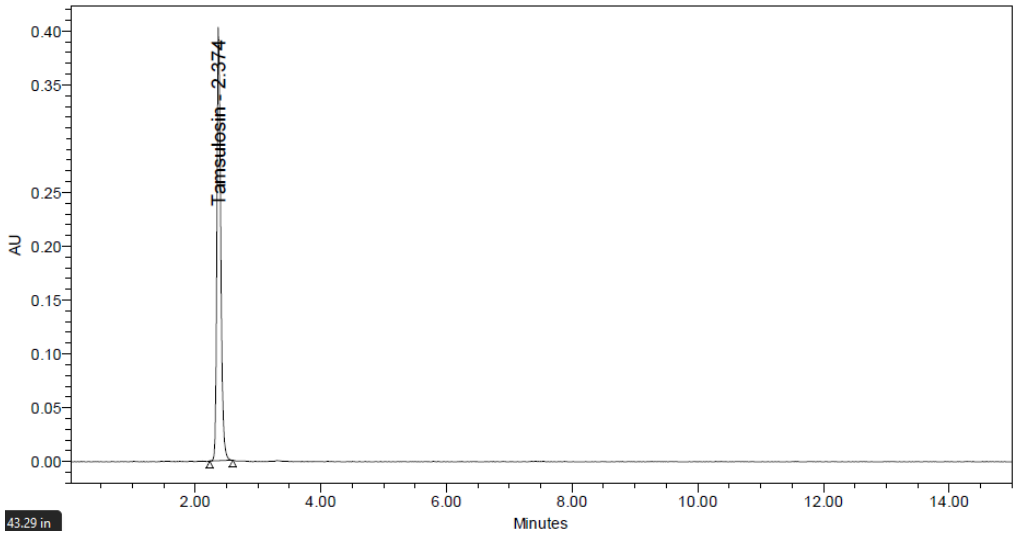

FIG. 7: TYPICAL CHROMATOGRAM OF TAMSULOSIN ON THERMAL DEGRADATION

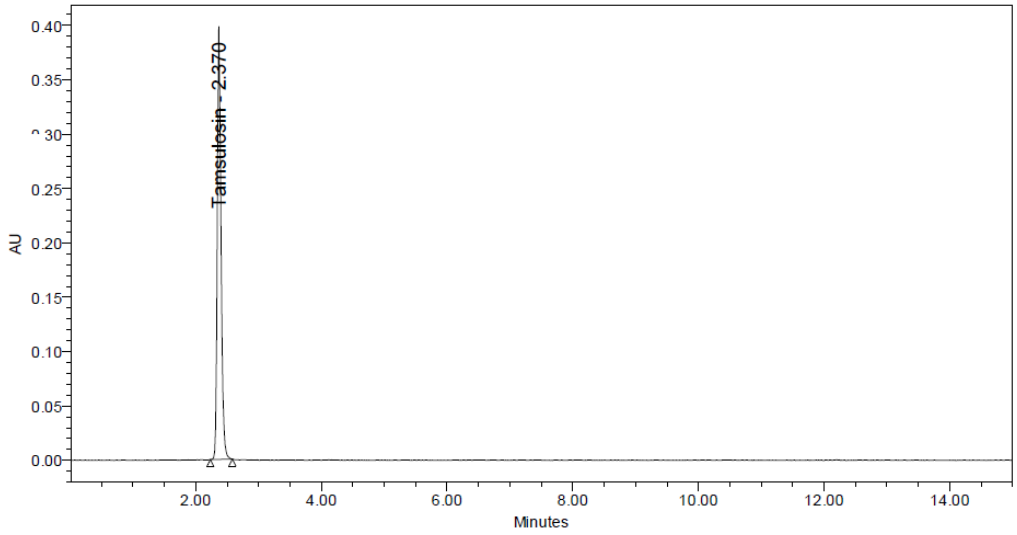

FIG. 8- TYPICAL CHROMATOGRAM OF TAMSULOSIN ON UV DEGRADATION

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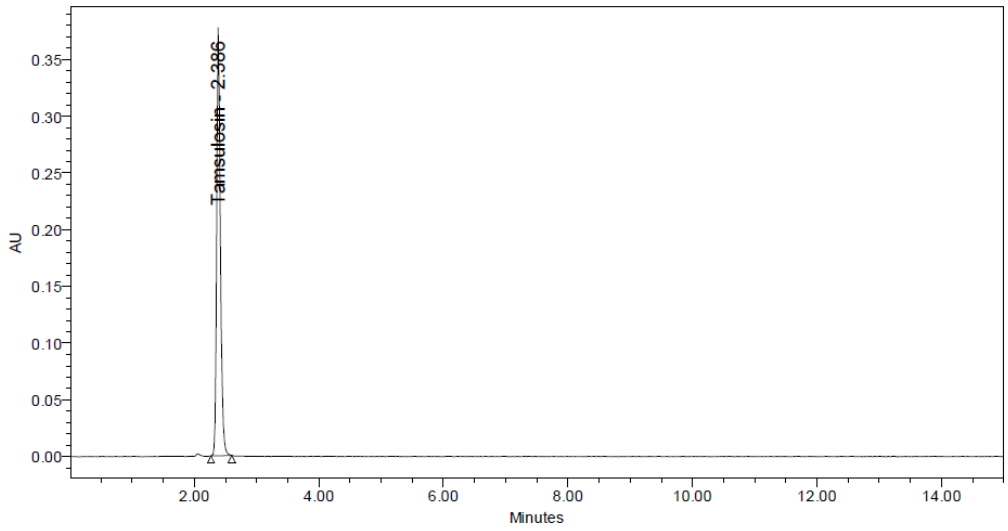

FIG. 9- TYPICAL CHROMATOGRAM OF TAMSULOSIN ON WATER DEGRADATION

Tables

TABLE 1: RESULTS FOR SYSTEM SUITABILITY FOR TAMSULOSIN

S. No	System suitability parameters	Tamsulosin values
1	USP tailing factor	1.44
2	USP plate count	7481
3	Mean Area	1897141
4	Retention time	2.4 min
5	% RSD	0.6

TABLE 2: LINEARITY, LOD & LOQ RESULTS FOR TAMSULOSIN

S. No	Parameters	Tamsulosin values
1	Regression Equation	Y=45801x+2700.8
2	Correlation Coefficient(R^2)	0.9997
3	Linearity range	10-60 ppm
4	LOD	0.010
5	LOQ	0.031

TABLE 3: RESULT OF PRECISION OF TEST METHOD FOR TAMSULOSIN

Sample number	Peak Area of Tamsulosin	
	Intra-day precision	Inter-day precision
1	1872101	1862947
2	1878063	1865780
3	1892087	1875558
4	1878249	1875949
5	1897295	1857616
6	1909573	1877087

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Avg	1887895	1869156
SD	14248.0	8162.7
% RSD	0.8	0.4

TABLE 4: RESULT OF ROBUSTNESS FOR TAMSULOSIN

Analytical conditions		Mean RT (min)	Mean Area	SD	RSD	Tailing factor	No. of Theoretical plates
Flow Rate (ml/min)	0.9	2.604	1953657	17101.4	0.9	1.33	6683
	1.0*	2.413	1897141	108332.2	0.6	1.44	7481
	1.1	2.308	1828523	12841.3	0.7	1.34	6117
Temperature (^{o} C)	20	2.404	1871381	10243.4	0.5	1.32	6727
	25*	2.413	1897141	108332.2	0.6	1.44	7481
	30	2.400	1854286	8302.3	0.4	1.29	6779
Mobile phase composition (v/v)	40:60	2.317	1854747	11560.2	0.6	1.24	6296
	50:50*	2.413	1897141	108332.2	0.6	1.44	7481
	60:40	2.623	2145864	15039.4	0.7	1.32	6666

*optimum conditions

TABLE 5: ACCURACY STUDY OF TAMSULOSIN

Sample no.	Spiked concentration (ppm)	*Measured concentration (ppm)	%* Recovery	%*RSD
1	50% (20 ppm)	19.99	100.56	0.64
2	100% (40 ppm)	40.38	100.50	
3	150% (60 ppm)	59.74	99.37	

*Mean of three replicates

TABLE 6: FORCED DEGRADATION STUDIES OF TAMSULOSIN

S.No.	Stress conditions	Retention Time	% Drug Recovered	% Drug decomposed
1	Standard drug	2.413	100	0
2	Acidic hydrolysis	2.359	95.84	4.16
3	Alkaline hydrolysis	2.361	96.60	3.40
4	Oxidative degradation	2.358	97.42	2.58
5	Photolytic degradation	2.370	99.58	0.42
6	Thermal degradation	2.374	99.28	0.72
7	Neutral degradation	2.370	99.82	0.18

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