International Journal for Pharmaceutical Research Scholars (IJPRS)

ISSN No: 2277-7873

RESEARCH ARTICLE V-1, I-4, 2012

Validated Stability Indicating RP-HPLC Method for the Determination of Silodosin in Pharmaceutical Dosage Form

Harischandran S*[1], Shankar Iyer R[2], Raju R[1], Shibi A[2], Sayana PS[2]

[1]*National College of Pharmacy, Department of Pharmaceutical Analysis, Calicut.*

[2]*New Udaya Pharmacy and Ayurvedic Laboratories, Kochi, Ernakulam.*

Manuscript No: IJPRS/V1/I4/00209, Received On: 05/12/2012, Accepted On: 13/12/2012

ABSTRACT

A simple, rapid and economic stability indicating high performance liquid chromatography method was developed for the determination of Silodosin in pharmaceutical dosage form. The chromatographic system comprised of a reverse phase Phenomenex C 18, 5µ Silica (250×4mm) column maintained at 25°C with mobile phase consisting of a mixture of methanol-water-acetonitrile-glacial acetic acid (60:27:10:3 % v/v) at pH 3.2 ± 0.1 with a flow rate of 1 ml/min, determined at 270 nm. The method was linear in the range of 10-100µg/ml. The results were validated according to ICH guidelines. The method could effectively separate the drug from its degradation products.

KEYWORDS

Silodosin, HPLC, Stability indicating, Validation, degradation.

INTRODUCTION

Silodosin is a highly selective third generation α_{1A}-adrenoceptor antagonist, chemically compound is - (-)-1-(3-hydroxypropyl)-5-[(2R)-2-({2-[2-(2,2,2trifluoroethoxy)phenoxy]ethyl}amino)propyl]-2,3-dihydro-1H-indole-7-carboxamide[1] - approved by FDA in 2008 for the treatment of the signs and symptoms of Benign Prostatic Hyperplasia (BPH). BPH is a common cause of lower urinary tract symptoms in men with an increasing prevalence with age, which results from proliferation of the stromal and epithelial cells of the prostate gland.[2,3] Alpha adrenergic antagonists are effective in relieving BPH symptoms. They relax smooth muscle in the prostate and bladder neck. An extensive literature survey revealed that there is a spectrophotometric method reported for the determination of Silodosin in pharmaceutical dosage form[4] and also a LC-MS method.[5] The present work is to develop a simple validated HPLC method with stability indicating for the quantification of Silodosin in dosage forms.

***Address for Correspondence:**
Harischandran S
National College of Pharmacy,
Manassery P.O,
Mukkom, Kozhikode - 673602
E-Mail Id: harischandran.s@gmail.com

EXPERIMENTAL

Chemicals and Reagents

Silodosin standard was kindly gifted by MSN Laboratories Ltd. Methanol, water and acetonitrile used were of HPLC grade and was procured from Merk chemicals. Capsules of Silodosin were purchased from local market.

Instruments and Apparatus

Thermo HPLC –P-680 system equipped with a surveyor pump and a manual injector of 20µL loop was used. Analysis was performed on a 250×4.60mm, 5µm Phenomenex C-18 column and detection was done with a PDA detector. The data acquisition was done on ChromQuest 5 software. All glass wares used for the preparation and dilution of standard and sample were of Class A.

© Copyright reserved by IJPRS

Chromatographic Conditions

Pre-filtered samples (20 µL) were injected into a Phenomenex C 18, 5µ Silica (250×4mm) Column maintained at 25°C. The mobile phase system consisted of methanol-water-acetonitrile-glacial acetic acid (60:27:10:3 % v/v) with pH 3.2 ± 0.1 and was run in isocratic mode at a flow rate was 1 ml/min through the column. The run time was 10 min/20µl injection and the elute was monitored at a wavelength of 270 nm. The temperature in the room was maintained at 25 ± 2°C.

Preparation of Standard Solution

Standard stock solutions of Silodosin was prepared by dissolving 100mg of Silodosin in 100 ml volumetric flask with 50 ml methanol and sonicated for 10 min, the solution was then made up to 100 ml with methanol to get 1 mg/ ml solution. Working standards were prepared from this stock solution by diluting with mobile phase.

Preparation of Sample Solution

Twenty capsules (SILODAL) each containing 8 mg Silodosin were taken. A quantity of powder equivalent to 10 mg of Silodosin was weighed and transferred to a 10 ml standard flask and about 8 ml of methanol was added and sonicated for 10 minutes. Then the volume was made up with methanol. The solution was filtered through whatmann filter paper No. 1 and 1ml of the filtrate was diluted with mobile phase to get the concentration of 0.1 mg/ml of Silodosin. Before injection, both standard and sample were filtered through 0.2 µm syringe filter.

Method Validation

The method was validated as per ICH guidelines[6].

Linearity

Accurately measured standard solutions (100µg/ml) of Silodosin (1, 2, 5, 7, 10)ml were transferred to a series of 10 ml volumetric flask separately and diluted up to the mark with mobile phase to obtain the a concentration range of (10, 20, 50, 70, 100)µg/ml. The serial dilutions were injected. The calibration curve was constructed by plotting peak area against concentration of drugs. The slope, Y intercept and correlation coefficient were calculated.

Accuracy

The accuracy of the method was confirmed by studying the recovery at three different concentrations. Powdered drug sample of pre analyzed Silodosin 8 mg was taken containing approximately 8 mg of Silodosin was spiked with 2mL, 5mL and 10 mL stock solution of Silodosin to get concentrations of 25%, 62.5% and 125% of Silodosin. The % RSD was calculated.

Repeatability

Six determinations were carried out at 100% of the test concentration at three concentration levels.

Intermediate Precision

The precision of the method was determined by studying repeatability and reproducibility on 3 days. The area of drug peak and percentage relative standard deviation were calculated and presented.

Detection Limit

The limit of detection of the developed method was calculated from the standard deviation of the y-intercepts and slope of the calibration curves of Silodosin using the formulae as given below.

$$\text{Limits of Detection} = \frac{3.3 \times y - \text{intercept}}{\text{Slope of the calibration curve}}$$

Quantitation Limit

The limit of quantitation of the developed method was calculated from the standard deviation of the y-intercepts and slope of the calibration curves of Silodosin using the formulae as given below.

$$\text{Limits of Quantification} = \frac{10 \times y - \text{intercept}}{\text{Slope of the calibration curve}}$$

© Copyright reserved by IJPRS

Specificity

Silodosin standard solution was forced to degrade in presence of 10% hydrogen peroxide solution. To the solution containing degraded product of Silodosin, Silodosin standard solution was spiked. The separation of peak of standard from its degraded product was observed.

System Suitability

System suitability was determined by integrating the peaks of Silodosin standard solution of 0.1 mg/ml concentration.

Analysis of Silodosin in Marketed Formulation

For the analysis of commercial formulations, 20 capsules (SILODAL, SILOFAST and RAPILIF) each containing 8 mg Silodosin were taken separately. A quantity of powder equivalent to 10 mg of Silodosin was weighed and transferred to a 10 ml standard flask and about 8 ml of methanol was added andsonicated for 10 minutes. The volume was made up with methanol and filtered and the filtrate was further diluted with mobile phase to get the concentration of 0.1 mg/ml of Silodosin. Both the standard and samples were filtered through 0.2 µm syringe filter before injection. The amount of drug present in pharmaceutical formulation was calculated.

$$\text{Amount of Drug in sample} = \frac{\text{Peak area of test}}{\text{Peak area of Std}} \times \frac{\text{Conc.of Std}}{\text{Conc.of test}} \times \text{Avg.wt.}$$

Forced Degradation of Silodosin

To evaluate the stability indicating properties of the developed HPLC method, forced degradation studies were carried out in accordance to the ICH guidelines. The standard drugs were subjected to acid, base, oxidation, dry heat and photo-degradation studies. The forced degradation was performed in the dark to exclude the possible degradation effect of light.

Acid Induced Degradation

1 mL of the methanolic stock solution was diluted with 0.1 N HCl to 25 mL and was kept aside for 10 minutes. From the resulting solution 1 ml was diluted to 25ml with mobile phase and 20µL was injected.

Base Induced Degradation

1 mL of the methanolic stock solution was diluted with 0.1 N NaOH to 25 mL and was kept aside for 10 minutes. From the resulting solution 1 ml was diluted to 25ml with mobile phase. 20µL was injected.

Oxidative Degradation

1 mL of the methanolic stock solution was diluted with 10 % H2O2to 25 mL and was kept aside for 10 minutes. From the resulting solution 1 ml was diluted to 25ml with mobile phase. 20µL was injected.

Dry Heat Degradation

For dry heat degradation study, the standard powder drug was placed in an oven at 55ºC for 3 h. Appropriate dilution was prepared in mobile phase and then analyzed under the optimized chromatographic conditions.

Photo-degradation Study

For the photochemical stability of the drug, the standard powder drug was placed in direct sunlight for 4 h kept on a terrace.

RESULTS AND DISCUSSION

Optimization of Chromatographic Conditions

The RP-HPLC procedure was optimized with a view to develop a stability indicating assay method used for the quantification of the Silodosin in solid pharmaceutical dosage forms. The pure drug and the degraded products were mixed and several runs were done using different mobile phase solvent with varying ratios. The mobile phase with methanol, water, acetonitrile and glacial acetic acid in the ratio of 60:27:10:3 was selected with pH 3.2 which gave symmetric, well defined peak with good retention time of 2.2 min for Silodosin.

Validation of the Method

The calibration plot was linear over a concentration range of 10-100µg/ml. A good

© Copyright reserved by IJPRS

linear relationship was observed over this range (r^2 = 0.9994)

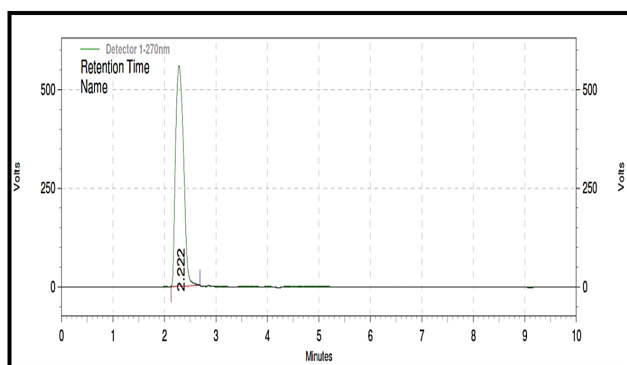

Figure 1: Chromatogram of Silodosin

Repeatability of injection was expressed as % RSD and was found to be 0.88486 for six replicate determinations at a single concentration. The low values of % RSD indicate that the proposed method is repeatable. The % RSD value indicates that the proposed method is repeatable.

The % RSD value obtained for reproducibility was between 0.291492 - 1.53448%, which indicates that the proposed method is precise.

The LOD and LOQ were found to be 0.0031628µg and 0.0105427µg respectively, and pointed towards adequate sensitivity.

Accuracy was determined on previously analyzed formulations after spiking with 25, 62.5 and 125 % of the standard drug. Mean recovery obtained is 100.29 %. [Table-1]

Table 1.Summary of Validation Parameters

Parameters	Values
Linearity	10-100 µg/ml
Correlation coefficient (r^2)	0.999
LOD	3 µg/ml
LOQ	10 µg/ml
Accuracy (%)	100.29 %
Precision (%RSD)	
Repeatability of sample injection (n=6)	0.88 %
Intermediate Precision	0.29 – 1.53 %
Specificity	Specific

The specificity of the method was demonstrated through forced degradation studies conducted on the sample using acid, alkaline, oxidative, thermal and photolytic degradations. The sample was exposed to these conditions and the main peak was studied for the peak purity, thus indicating that the method effectively separated the degradation products from the pure active ingredient. [Table-2]

Analysis of marketed capsule dosage form gave 95.37%, 94.79% and 95.30% for Silodal, Silofast and Rapilif respectively, per 8 mg capsules.

Table 2: Summary of Forced Degradation studies

Stress Condition	Rt of Silodosin (min)	Drug recovered (%)	Drug decomposed (%)
Standard drug	2.2	100	0
Acid hydrolysis 0.1N HCl, 10 min	2.2	15.12	84.88
Alkaline hydrolysis 0.1N NaOH, 10 min	2.2	12.35	87.64
Oxidative degradation 10% H2O2 10 min	2.2	17.43	82.59
Heat degradation 55C, 3 hours	2.2	100	0
Photolytic degradation Sunlight, 4 hours	2.2	100	0

System suitability of the method was determined by integrating the peaks of Silodosin. The number of theoretical plates was 3574, injection precision 0.3789 % and tailing

factor was 1.662, which indicate that the system used was suitable for the analysis.

Stability Studies

The drug was found to undergo acid, alkali and oxidative degradation very rapidly. Additional degradation peaks were found near to the peak of Silodosin. The peaks of Silodosin and its degradation products were clearly separated.

Heat degradation study of the drug revealed that the drug was heat stable under the prescribed conditions

In photo-degradation study, the color of the drug was changed from white to light brown, but no degradation was found under the prescribed conditions.

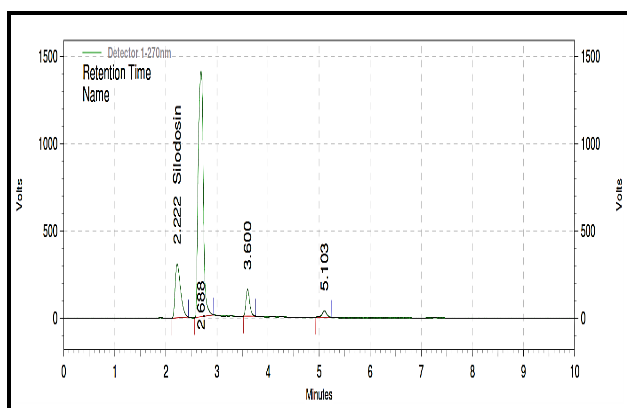

Figure 2: Chromatogram of Silodosin after Oxidative degradation with H_2O_2 10% v/v. The peak of Silodosin and its degradation products are separated

CONCLUSION

The proposed method was found to be simple precise, accurate, rapid and economic. Separation of Silodosin from its degradation products confirmed stability indicating properties of this method. Statistical analysis indicated that the method was reproducible and selective for the analysis of Silodosin in solid pharmaceutical dosage form without interference from excipients. Hence this method can be easily and conveniently adopted for routine analysis of Silodosin in its solid dosage form.

REFERENCES

1. Product Information: RAPAFLO(TM) oral capsules, silodosin oral capsules. Watson Pharma, Morristown, NJ, 2008.
2. Koda-Kimble MA. Applied Therapeutics: The Clinical Use Of Drugs. 9th Edition ed: Lippincott Williams & Wilkins; 2009.
3. Yoshida M. New clinical evidence of silodosin, an α1A selective adrenoceptor antagonist, in the treatment for lower urinary tract symptoms. International Journal of Urology, 2012, 1-11.
4. C R Sharma, " UV SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF SILODOSIN FROM ITS SOLID DOSAGE FORM ", Inventi Rapid: Pharm Analysis & Quality Assurance , Vol. 2012 , Article ID- " Inventi:ppaqa/310/12 ", 2012 [cited 2012 Nov 22] Available From http://www.inventi.in/Article/ppaqa/310/12.aspx
5. Cui Y. Determination of silodosin in human plasma by liquid chromatography–tandem mass spectrometry, Journal of Chromatography B. 2009, 877(29), 3724 - 3728.
6. “Validation of analytical methods: methodology,” in Proceedings of the 3rd International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH ’96) Guideline on Validation of Analytical Procedure-Methodology, Geneva, Switzerland, 1996.

© Copyright reserved by IJPRS