Stability Indicating UV-Spectroscopic Method Development and Validation of Silodosin in Bulk and Capsule Dosage Form-A Green Analytical Approach

Soumya Stuti Patnaik[1], Dravidamani Meena[2], Sayyad Mustak [1], Arshiya Nazneen[3],Smriti Gohri[4], Tiru Kondur HimaBindu Kasyap[1], Ravi Kumar Vakkalagadda[1], Sandip Sen[1]*

1. *School of Pharmacy, Guru Nanak Institutions Technical Campus, Khanapur, Ibrahimpatnam, Telangana-501506, India*
2. *Department of Pharmaceutical Analysis, Seven Hills College of Pharmacy, Tirupati, Andhra Pradesh-517561, India*
3. *Srikrupa Institute of Pharmaceutical Sciences, Velikatta, Kondapakka, Siddipet, Telangana-502277,India*
4. *Department of chemistry, IIMT College of Medical Sciences, O-Pocket, Ganganagar, Meerut, Uttar Pradesh-250001, India.*

sandipsen2010@gmail.com *

Abstract

Silodosin is a medication for the symptomatic treatment of benign prostatic hyperplasia. It acts as an α -adrenoreceptor antagonist with high uroselectivity (selectivity for the prostate). It was approved by USFDA in August 2008. The present study was aimed to develop and validate stability indicating green UV-VIS spectrophotometric method for the estimation of Silodosin, in bulk and capsule dosage form. The UV-spectroscopic method was developed by using water and methanol (9:1). The absorption maxima (λ_{max}) of the drug was found to be 267nm. The methods were validated as per ICH guidelines. A linear response was observed in the range of 10-50µg/ml with a regression coefficient of 0.998. The average percentage assay was calculated and found to be 99.00-99.9 %. The percentage relative standard deviation was found to be less than 2%. The stress studies showed no significant change in absorption maxima of drugs, and no interfering peaks were found under alkaline, acidic, oxidative and thermal degradation conditions.

KEYWORDS: Silodosin, UV-Spectroscopic method, ICH-Guidelines, Method Validation

1. INTRODUCTION

Silodosin (Figure-1) is chemically designated as 1-(3-hydroxypropyl)-5-[(2*R*)-({2-[2-[2-(2, 2, 2-trifluoroethoxy) phenoxy] ethyl} amino) propyl] indoline-7-carboxamide[1] (Figure-1).It is a selective third-generation α1A adrenergic receptor antagonist[2]. Being a drug it was first approved by FDA in the year 2008. It selectively affects the prostate and urinary bladder as a therapeutic agent for the treatment of the signs and symptoms of benign prostatic hyperplasia. It causes smooth muscle relaxation by antagonizing the α1A adrenergic receptor in the lower urinary tract [3].

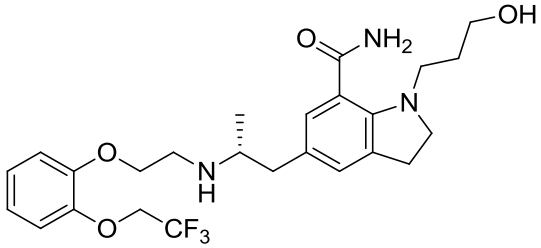

Figure-1: Structure of Silodosin

The literature survey reveals the development and validation of various analytical methods to determine the Sildosin in pharmaceutical or clinical samples. Jahan and Malipatil et al. in 2014 reported

the UV spectrophotometry method[4]. Similarly, the spectrofluorimetry method was developed by Bhamre and Rajput in 2014[5]. More sensitive analytical methods like high-performance liquid chromatography (HPLC) was reported by Vali et al. (2012)[6], Jahan and Malipatil (2014)[7]; Aneesh and Rajasekaran (2012)[8], high-performance thin-layer chromatography (2012) [9], ultra-high-performance liquid chromatography (UHPLC) by Shaik et al.[10]; Prasad et al[11] and liquid chromatography-tandem mass spectrometry (LC-MS/MS) by Zhao et al.[12] and electrochemical sensing methods by Er et al.[13].

Based on the above facts the present work was aimed to develop cost-effective, sensitive and reliable green analytical methods for the determination of biological and drug molecules. At this point, we focused on the cost of solvent, the accuracy of results and the wideness of study in respect of various samples within a short response time. Overall the present study has indicated stability-indicating accurate and efficient green analytical methods based on spectrophotometric for the determination of Silodosin in a pharmaceutical sample.

2.EXPERIMENTAL WORK:

The ELICO, Model: SL 244 double beam UV-VIS spectrophotometer using 1.00 cm quartz curettes Digital weighing balance (SHIMADZU AUX 220), P^H meter (INCO Lab) and ultra sonicator of (Citizen), Filtration membrane (0.22µ and 0.45µ) was used for analysis. Silodosin was obtained from Aurobindo Pharma Pvt. Ltd, Hyderabad, India. HPLC grade methanol, acetonitrile and triethylamine were purchased from S.D. fine chemicals.

2.1.UV- Spectroscopic Method Development:

2.1.1. Selection of solvent:

The solubility of Silodosin was determined in a variety of solvents as per Indian pharmacopeia standards. Solubility was carried out in polar to nonpolar solvents. From the solubility data methanol and water (10:90) were selected as a solvent for the analysis of Silodosin.

2.1.2. Preparation of solvent:

In a volumetric flask, a mixture of methanol & distilled water (v/v) was prepared in the ratio of 10:90 and then filter through a 0.45µm nylon membrane filter and degas before use.

2.1.3. Preparation standard stock solution of Silodosin:

Weigh 100 mg of Silodosin and transfer into a 100ml volumetric flask, dilute to volume with diluents and mixed well. Transfer 10.0 ml of the solution into a 100ml volumetric flask dilute to volume with diluents and mix well (concentration: about 100µg/ml of Silodosin). Transfer 3 ml of the (100µg/ml) Silodosin solution into a 10 ml volumetric flask dilute to volume with diluents and mix well (concentration: about 30µg/ml of Silodosin).

2.1.4. Preparation of working standard solution:

From the above stock solution pippetout 1,2,3,4 and 5 ml of solutions and diluted to 10 ml with diluents to get a concentration range of 10-50 µg/ml and used for further study.

2.1.5. Determination of suitable wavelength:

The working standard dilution of 30 µg/ml of the drug was scanned from 200-400nm, the instrument was scanned in spectrum mode and determine the absorbance. The study was carried out in triplicate.

2.1.6. Development of spectroscopic assay method:

Accurately weighed 20 capsules of Silodal-8 marketed by Sun pharma containing 8mg of silodosin as per label claim. A weight of 100 mg was accurately powdered was taken and dissolved in 70 mL of methanol: water (1:9) in 100ml of the standard flask and sonicate for 30 minutes and filter by membrane filter and made up the volume made up to mark. Transferred 10 ml from stock and diluted to

100 ml with solvent in 100ml volumetric flask to get concentration 100µg/ml. Again withdrawn 1, 2 and 3 ml of solution and diluted to 10 ml and used for the assay. Each solution was made in 3 sets and find out the absorbance of the above solution .finally determine the concentration by direct comparison method using the formula.

$$\frac{C1}{C2} = \frac{A1}{A2}$$

2.2. Validation Parameters:

The proposed method was extensively validated in terms of specificity, linearity, accuracy, precision, robustness, ruggedness, limits of detection (LOD) and quantification (LOQ) as per ICH guidelines[14]. For all the parameters percentage relative standard deviation was calculated.

***2.2.1. Preparation of test solutions for validation study of drug*s:**

Accurately weigh and transfer 100 mg of Silodosin working standard into 100 ml of clean dry volumetric flasks add about 70 ml of solvents and mobile phase used during method development and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). From the stock solution taken 1,2,3,4 and 5 ml of solution in a volumetric flask and further diluted to 10 ml to get 10-50 µg/ml concentration**.** Each sample was separately prepared in 6 sets for each parameter.

***2.2.2. Linearity*:**

A linearity study was carried out at 10-50 µg/ml concentration for both methods**.** Each sample was prepared in 6 sets and measured percentage relative standard deviation (%RSD).

***2.2.3. Precision*:**

Precision is the degree of repeatability of an analytical method under normal operational conditions. The method precision study was carried out at 50 µg/ml respectively.

***2.2.4. Accuracy (Percentage recovery studies)*:**

To ensure the reliability (accuracy of method) recovery studies were carried out by mixing standard quality of standard drug with pre-analyzed sample formulation and contents were re-analyzed by the proposed method. To perform the recovery studies 3 dilutions were prepared using both standard drug and marketed formulation. The recovery study was carried out at 50,100 and 150 % levels for the spectroscopic method.

***2.2.5. Ruggedness*:**

Analysis was carried out by different analysts to determine the ruggedness study. The ruggedness study was carried out at the concentration of 50 µg/ml respectively.

***2.2.6. Robustness*:**

The robustness analysis was carried out at different conditions like change in temperatures i.e., room temperature and 40°C also by the change in flow rate. For the robustness study concentration of 20 µg/ml was selected.

***2.2.7. Detection Limit & Quantitation Limit*:**

In the present study, the LOD and LOQ were determined by the standard deviation of the response and the slope of the calibration curve using the formula 3.3σ/S and 10σ/S criteria, respectively; where σ is the standard deviation of y-intercepts of regression lines and s is the slope of the calibration curve.

2.3. Forced degradation and stability-indicating tests [15.16]:

Ten milligrams each of standard silodosin was dissolved to prepare the stock solution in a 25mL mixture of methanol: water (1:9, v/v) by sonication for 10 min. After the forced degradation process, each solution was filtered through a 0.20µm PTFE syringe filter before analysis.

***2.3.1. Acidic degradation*:**

In the presence of IS, 2.5 ml of 1.0 M HCl was added to 7.5 mL stock solution, and the mixture was kept at 55 °C for 1 h under reflux, cooled and neutralized with 1.0 M HCl to pH 7.0. Then, 6.25 ml of the solution was made up to 25 ml with ultrapure water. Finally, the solution was filtered through a 0.20µm PTFE syringe filter.

***2.3.2. Alkaline degradation*:**

In the presence of IS, 2.5 mL of 1.0 M NaOH was added to 7.5 mL stock solution, and the mixture was kept at 55 °C for 1 h under reflux, cooled and neutralized with 1.0 M NaOH to pH 7.0. Then, 6.25 ml of the solution was made up to 25 ml with ultrapure water. Finally, the solution was filtered through a 0.20 µm PTFE syringe filter.

***2.3.3. Oxidative degradation*:**

In the presence of IS, 2.5 mL of 5 % H_2O_2 was added to the 7.5 ml stock solution, and the mixture was kept at 55 °C for 1 h under reflux then cooled, and the volume of the mixture was made up to 25 ml with ultrapure water. Finally, the solution was filtered through a 0.20-µm PTFE syringe filter.

***2.3.4. Thermal degradation*:**

Ten milligrams of silodosin powders were kept at 80 °C for 24 h. After that, each powder was dissolved in a mixture of methanol: water (1:1, v/v) and acetonitrile: Triethylamine buffer pH4.6: Methanol (70:20:10% v/v). A mixture of these solutions was dissolved to get a required solution to make a required strength of 50 ppm SIL. The solution was filtered through a 0.20-µm PTFE syringe filter.

3. RESULTS AND DISCUSSION

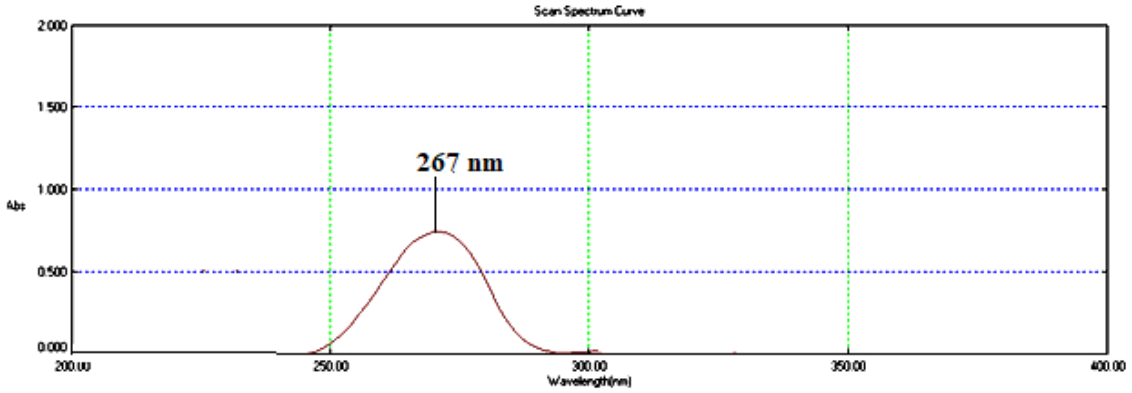

Figure-2: The λ max of Silodosin was found to be at 267 nm

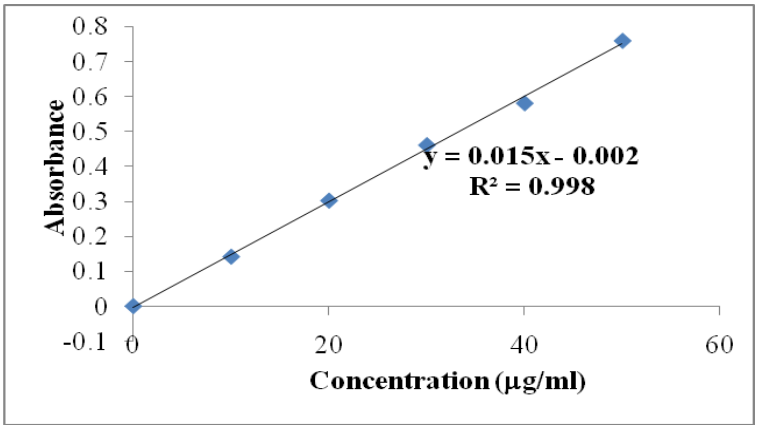

Figure-3: Calibration curve of Silodosin by spectroscopic method

Table-1: Assay studies for Silodosin capsule by spectroscopic method

S.No	Concentration of Standard	Absorbance of Standard	Absorbance of Sample	Conc. Found	% Purity	Average
1	10	0.1414	0.14	9.9	99	99.00%
2		0.1412	0.1401	9.92	99.2	
3		0.142	0.1407	9.9	99	
4	20	0.302	0.301	19.93	99.65	99.30%
5		0.3025	0.3001	19.84	99.2	
6		0.3029	0.3005	19.84	99.2	
7	30	0.4607	0.4436	28.8	96.2	96.30%
8		0.4618	0.4428	28.7	95.8	
9		0.4612	0.4481	29.14	97.15	

Table-2: Linearity values of Silodosin

Concentration (□g/ml)	Mean Absorbance	%RSD
0	0	0
10	0.1414	0.58
20	0.302	0.55
30	0.4607	1.47
40	0.5807	0.98
50	0.7594	1.37

Table-3: Precision result of silodosin

Concentration (µg/ml)	Absorbance
50	0.7594
50	0.7593
50	0.7593
50	0.7594
50	0.7593
50	0.7594
%RSD	**0.75**

Table-4: Accuracy study of Silodosin by standard addition method

Level	Total concentration	Absorbance	Amount Found	%Recovery	%RSD
50	30µg/ml	0.4036	26.28	87.6	0.6
		0.4028	26.22	87.4	
		0.4081	26.57	88.5	
100	40 µg/ml	0.5537	38.14	95.35	0.7
		0.5444	37.49	93.72	
		0.5679	39.11	97.79	
150	50 µg/ml	0.7082	46.62	93.25	0.9
		0.7092	46.69	93.38	
		0.7197	47.38	94.77	

Table-5: Ruggedness study of Silodosin

Concentration (µg/ml)	ANALYST-1	ANALYST-2
50	0.7592	0.7588
50	0.7593	0.7591
50	0.7595	0.7588
50	0.7596	0.759
50	0.7592	0.7591
50	0.7593	0.7587
%RSD	**0.80**	**0.60**

Table-6: Robustness result of silodosin

Concentration (µg/ml)	Absorbance	
	At room temperature	At 40 °C
20	0.3021	0.3022
20	0.3023	0.302
20	0.3021	0.3023
20	0.302	0.3022
20	0.3022	0.302
%RSD	**0.11**	**0.90**

Table-7: LOD and LOQ values for Silodosin

S.NO	PARAMETER	267 nm
1	LOD(µg/mL)	1.5(µg/ml)
2	LOQ(µg/mL)	3.3µg/ml

Table-8: Summary of Forced Degradation studies

Stressed Conditions	Heat temperature	Time	Percntage recovery study by spectrophometric method at 267nm	
			Drug recovered (%)	Drug decomposed (%)
Standard drug	Room temperature	-	100	-
Acid hydrolysis 0.1N HCl,	Room temperature	1hrs	86.87	13.13
Alkaline hydrolysis 0.1N NaOH,	Room temperature	1hrs	86.81	13.19
Oxidative degradation 10% H2O2	Room temperature	1hrs	82.84	17.16
Heat degradation	80°C	4 hrs	89.99	10.01

The absorbance maxima of silodosin were found to be 267 nm using water and methanol in 1:9 v/v (Figure 2). The method showed linearity in the concentration range of 10-50 µg/ml. The linearity curves for standard showing Correlation Coefficient (r^2) and linear regression equations $R^2 = 0.998$, y = 0.015x-0.002 respectively (Figure 3). The assay method was performed in capsule formulation purchased from the market. The average percentage assay was calculated and found to be 99.00 - 99.30 % in respect to 10-30 µg/ml (Table-1). The method was validated as per ICH guidelines. The precision study of the method was carried out by repeatability study. The % RSD was less than 0.75 (Table-3). The recovery studies were carried out at three different levels i.e. 50%, 100% and 150%. The result has shown a mean

percentage recovery of 87.5, 95.62 and 93.8 respectively (Table-4). The ruggedness study was carried out by different analysts the % RSD was found to be 0.8 and 0.6 shown in Table-5. The robustness study was carried out by a change in room temperature and at 40°C (Table-6). There were no recognized changes in results. The limit of detection was 1.5 µg/ml and quantification was found to be 3.8 µg/ml respectively (Table-7)**.**

After suitable spectroscopic method development Forced degradation studies were also performed to evaluate the stability and specificity of the proposed methods in different experimental conditions. The acidic, alkaline and oxidative degradation were studied by treating with 1.0 M HCl/1.0 M NaOH/ 5 % H2O2 solutions at 55 °C for 1 h, respectively. The thermal degradation was also studied by heating the SIL solution at 80 °C for 4 hrs. The whole degradation products were observed at 2.22-2.44 min respectively, in all proposed stress conditions (Fig. 5). The degradation study was carried out by a percentage recovery study (Table-8). The stress studies showed no significant change in retention times of drugs, and no interfering peaks were found within the retention time under alkaline, acidic, oxidative and thermal degradation conditions.

4. SUMMARY AND CONCLUSION

The results demonstrated that the method developed and validated in the current study is economical, accurate, precise and reproducible (relative standard deviation < 2%). Along with this the proposed method are well stable in different types of stressed conditions. The method can suitably be used for the routine analysis and quantitative determination of the bulk extracts and in the tablet dosage form.

CONFLICT OF INTEREST

There is no conflict of interest.

ACKNOWLEDGMENT

The authors are also thankful to Srikrupa institute of Pharmaceutical sciences (Telangana, India) for providing the necessary facilities for research work

REFERENCES

1. *Sweetman SC, Martindale: the complete drug reference. 36th ed. London: The Pharmaceutical Press, 2009.*
2. *Goi Y, Tomiyama Y, Yokoyama A, Tatemichi S, Maruyama K, Kobayashi M and Yamaguchi O, Effect of silodosin, a selective α1A-adrenoceptor antagonist, on voiding behavior and bladder blood flow in a rat model of bladder outlet obstruction,* ***Eur J Pharmacol.*** *2015, 764, 489–496.*
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. *Jahan KK and Malipatil SM, Development and validation of new HPLC method for the quantitative estimation of silodosin in bulk and pharmaceutical formulation,* ***World J Pharm Pharm Sci.*** *2014a, 3, 960–969.*
5. *Bhamre P and Rajput SJ, Spectrofluorimetric method for the determination of silodosin in the bulk and pharmaceutical dosage form,* ***IAJPR****, 2014, 4, 5106–5110.*
6. *Vali SJ, Saladi SK, Sait SS and Garg LK, Development and validation of LC method for determination of the enantiomeric purity of silodosin in bulk drug substances,* ***Am J PharmTech Res****., 2012, 2, 750–758.*
7. *Jahan KK and Malipatil SM, Development and validation of new spectrophotometric methods for the quantitative estimation of silodosin in bulk drug and pharmaceutical formulations,* ***Int J Pharm Res Anal****., 2014, 4, 65–69.*
8. *Aneesh TP and Rajasekaran A, Development and validation of HPLC method for the estimation of silodosin in the bulk and pharmaceutical dosage form,* ***Int J Biol Pharm Res****., 2012, 3, 693–696.*
9. *Sayana PS, Iyer RS, Shibi A and Harischandran S, Development and validation of HPTLC method for quantification of silodosin in the bulk and pharmaceutical dosage form,* ***Pharma Innovation****, 2012, 1, 60–65.*
10. *Shaik JV, Saladi S and Sait SS. Development of stability-indicating UHPLC method for the quantitative determination of silodosin and its related substances****. J Chromatogr Sci. 2014****, 52, 646–653.*

11. *Prasad LAR, Rao JVLNS, Pamidi S, Prasad V and Hotha KK, New rapid UPLC method for the estimation of impurities in the capsule dosage form of silodosin,* ***Int J Anal Bioanal Chem,*** *2012, 2, 247–251.*
12. *Zhao X, Liu Y, Xu J, Zhang D, Zhou Y, Gu J and Cui Y, Determination of silodosin in human plasma by liquid chromatography-tandem mass spectrometry,* ***J Chromato graphy B.*** *2009, 877, 3724–3728.*
13. *Er E, Çelikkan H, Erk N and Aksu ML, A new generation electrochemical sensor based on graphene nanosheets/gold nanoparticles/Nafion nanocomposite for determination of silodosin,* ***Electrochim Acta****., 2015, 157, 252–257.*
14. *ICH Q2 (R1). International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceutical for Human Use. Validation of analytical procedures: text and methodology. Geneva, Switzerland; 2005.*
15. *Engin Er and Nevin Erk, An effective and sensitive stability-indicating chromatographic approach based on HPLC for silodosin assay,* ***Journal of Analytical Science and Technology****, 2016, 7, 1-8.*
16. *Harischandran S, Shankar Iyer R, Raju R, Shibi A and Sayana PS, Validated Stability Indicating RP-HPLC Method for the Determination of Silodosin in Pharmaceutical Dosage Form,* ***International Journal for Pharmaceutical Research Scholars****, 2012, 1, I-4.*