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Research Article

METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF SILDOSIN IN BULK AND PHARMACEUTICAL DOSAGE FORMS USING UV-VIS SPECTROPHOTOMETRY

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ABSTRACT

The present study describes a simple, accurate, precise and cost effective UV-VIS Spectrophotometric method for the estimation of Silodosin, a selective antagonist of alpha-1 adrenoreceptors used for reliving the symptoms of enlarged prostste, in bulk and pharmaceutical dosage form. The solvent used is methanol and the λ max or the absorption maxima of the drug was found to be 269nm. A linear response was observed in the range of 5-50µg/ml with regression coefficient of 0.994. The method was then validated for different parameters as per ICH guidelines. This method can be used for the determination of Silodosin in quality control of formulation without interference of the excipient

Keywords: Silodosin, ICH, UV-VIS Spectroscopy, Validation

INTRODUCTION

Silodosin is a medication for the symptomatic treatment of benign prostatic hyperplasia. It acts as an α_1-adrenoceptor antagonist with high uroselectivity (selectivity for the prostate). It was approved by USFDA in August 2008. The chemical name of silodosin is 1-(3-Hydroxypropyl)-5-[(2*R*)-2-({2-[2-(2,2,2-trifluoroethoxy)phenoxy]ethyl}amino) propyl]-2,3-dihydro-1*H*-indole-7-carboxamide[1,2]

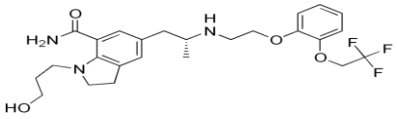

Fig 1: Structure of silodosin

MATERIALS AND METHODS

The solvent used was methanol. The instrument used was a double beam UV-VIS spectrophotometer (uv-1800, shimadzu).

METHOD DEVELOPMENT

Solubility Test: Solubility test for the drug silodosin was performed by various solvents. The solvents include Water, methanol, DMSO, methanol, ethanol and chloroform. However methanol was chosen as a solvent for developing the method.

Determination of λmax

Preparation of stock solution: Standard stock solution of silodosin prepared by dissolving 10mg of silodosin in methanol to produce a concentration of 1000µg/ml. 1ml of this stock solution was taken then diluted up to 10ml by using methanol to produce a concentration of 100µg/ml which is the standard stock solution.

From the above stock solution, 5ml was pipette out into a 10ml volumetric flask and the volume was made up to the mark with methanol to prepare a concentration of 50µg/ml. Then the sample was scanned in UV-VIS Spectrophotometer in the range 400-500nm using methanol as a blank and the wave length corresponding to maximum absorbance (λmax) was found to be 269nm (Fig 2).

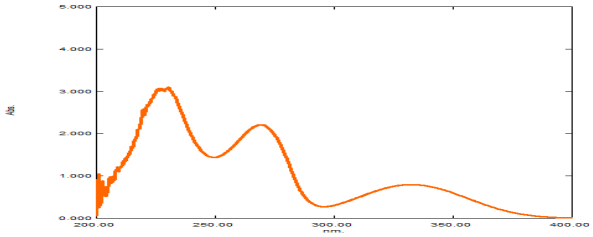

Fig 2: λmax of Silodosin showing at 269nm

Preparation of calibration curve

0.5ml, 1ml, 2ml, 3ml, 4ml, 5ml of 100ug/ml solution was diluted to 10ml using methanol to produce 5µg/ml, 10µg/ml, 50µg/ml, 30µg/ml, 40µg/ml, 50µg/ml solutions respectively. Then the construction of calibration curve was done by taking the above prepared solutions of different concentration ranging from 5-50µg/ml. Calibration curve was plotted by taking concentration on x-axis and absorbance on y-axis. The curve showed linearity in the concentration range of 5-50µg/ml, the correlation coefficient was found to be 0.994 (Fig 3).

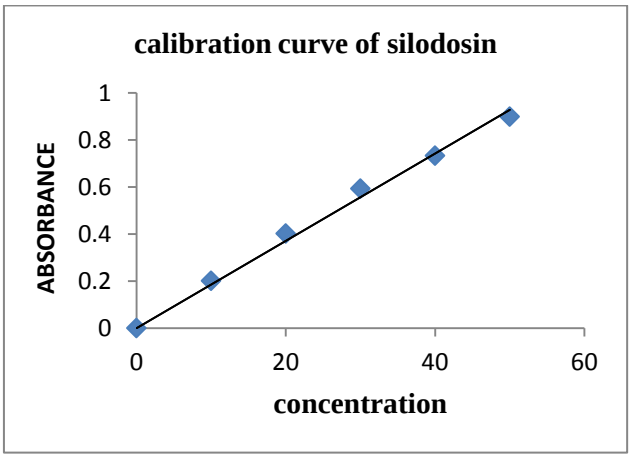

Fig 3: calibration curve of silodosin

METHOD VALIDATION

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce desired result or product meeting its predetermined specifications and quality characteristics [3].

The method was validated for different parameters like Linearity, Accuracy, Precision, Robustness, Ruggedness, Limit of quantification (LOQ), and Limit of Detection (LOD).

Linearity

The method was validated according to ICH Q2B guidelines for validation of analytical procedure in order to determine the linearity, sensitivity and accuracy of the analyte[4,5]. A calibration curve was generated with appropriate volumes of the working standard solutions for UV methods. The linearity found between the 10-50ug/ml. (Table1)

Table 1: Linearity of Silodosin

concentration	absorbance
10	0.201
20	0.402
30	0.593
40	0.733
50	0.899

Accuracy

The accuracy of the method was determined by preparing solutions of different concentrations that is 80%, 100%, 120% in which the amount of marketed formulation (SILODAL -100mg) was kept constant (10mg) and the amount of pure drug was varied that is 8mg, 10mg, 12mg for 80%, 100%, and 120% respectively. The solutions were prepared in triplicates and the accuracy was indicated by % recovery (Table 3).

Table 3: Accuracy Reading Of Silodosin

No. of preparations	Concentration (µg/ml)		% Recovery	Statistical Result		
	Formulation	Pure drug		Mean	SD	%RSD
80%	10	8	100.1			
80%	10	8	99.9	100.4	0.7	0.69
80%	10	8	101.2			
100%	10	10	101.2			
100%	10	10	99.9	100	1.15325	1.15
100%	10	10	98.9			
120%	10	12	100.9			
120%	10	12	99.9	100.9	1	0.99
120%	10	12	101.9			

Precision

Precision is the degree of repeatability of an analytical method under normal operational conditions. The precision of the assay was determined by repeatability (intraday) and intermediate precision (inter-day). The intraday precision study of different solutions of same concentrations were prepared and analyzed three times in a day i.e. morning, afternoon and evening, absorbance noted and result was reported as RSD % [4]. The inter-day precision was studied by comparing the assays on three different days and the results are documented as the standard deviation and RSD % (Table 4, 5&6).

Table 4: precision result showing repeatability of silodosin

Concentration (µg/ml)	Absorbance	Statistical Analysis
50	0.861	
50	0.862	
50	0.860	Mean =0.861
50	0.861	SD =0.001187
50	0.862	%RSD =0.14%
50	0.861	
50	0.862	
50	0.864	

Table 5: intra assay precision

Concentration (µg/ml)	Absorbance 1 (Morning)	Absorbance 2 (Afternoon)	Absorbance3 (Evening)	Average% RSD
50	0.861	0.861	0.861	
50	0.860	0.862	0.859	
50	0.861	0.860	0.861	
50	0.861	0.859	0.862	
50	0.863	0.862	0.861	
50	0.862	0.861	0.862	
50	0.860	0.864	0.861	
50	0.863	0.863	0.859	
50	0.863	0.864	0.864	
%RSD	0.14	0.50	0.18	0.16

Table 6: inter-assay precision

Concentration(µg/ml)	%RSD			Average % RSD
	Day 1	Day 2	Day 3	
50	0.13	0.15	0.18	0.16

Specificity

10mg of silodosin was spiked with 50% (5mg), 100% (10mg), 150% (15mg) of excipient mix (magnesium stearate) and the sample was analyzed for % recovery (Table 7).

Table 7: Test for specificity showing no effect of excipients

Sample No	Excipient Conc. (%)	Silodosin Input (mg)	Silodosin recovered (mg)	Silodosin recovered (%)	Mean Recovered (%)	SD	%RSD
1	100	10	9.81	98.1			
2	50	10	10.05	100.5	100.1 %	1.877	1.87%
3	150	10	10.18	101.8			

Ruggedness

Analysis was carried out by different analyst in order to determine the ruggedness and the respective absorbance was noted and the result was indicated as% RSD (Table 8).

Table 8: Result showing Ruggedness

Analyst -1		
Conc.(µg/ml)	**Absorbance**	**Statistical analysis**
50	0.861	
50	0.861	Mean – 0.8606
50	0.862	SD – 0.00103
50	0.859	%RSD – 0.12%
50	0.860	
50	0.861	
Analyst -2		
Conc.(µg/ml)	**Absorbance**	**Statistical analysis**
50	0.861	
50	0.862	Mean – 0.860
50	0.861	SD – 0.0009
50	0.859	%RSD – 0.11%
50	0.861	
50	0.861	

Robustness

Analysis was carried out at two different temperatures ie, room temperature and 18°c to determine the robustness and the respective absorbance were noted and the result were indicated as% RSD (Table9).

Table 9: Result showing Robustness

Room temperature		
Conc.(µg/ml)	**Absorbance**	**Statistical analysis**
50	0.861	
50	0.863	Mean – 0.8616
50	0.862	SD – 0.00136

50	0.859	%RSD – 0.16%
50	0.862	
50	0.861	
Temperature 18°c		
50	0.861	
50	0.862	Mean -0.861
50	0.863	SD-0.0015
50	0.859	%RSD-0.17%
50	0.862	
50	0.861	

LOD and LOQ

The limit of detection (LOD) is defined as the lowest concentration of an analyte in the sample that can be detected. The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with an acceptable accuracy, precision and variability [4.5].

In this study, LOD and LOQ were based on the standard deviation of the response and the slope of the corresponding curve using the following equations-

$$LOD = 3\ s/m;\ LOQ = 10\ s/m$$

Where s, the noise of estimate, is the standard deviation of the absorbance of the sample and m is the slope of the related calibrations graphs.

The values of LOD and LOQ are found to be 0.5µg/ml and 1.55µg/ml respectively (Table 10).

DISCUSSION

A simple, rapid, sensitive and accurate analytical method for the quantitative determination of silodosin was developed according to ICH guidelines. Silodosin is a UV absorbing molecule with specific chromophores in the structure that absorb at a particular wavelength. The λmax of the drug for analysis was determined by scanning of the drug sample solutions in the entire UV region. It was found to be that maximum absorbance observed at the wavelength of 269nm. The developed method was found to be precise as the %RSD values for the intra-day and inter-day was found to be less than 2%. Recoveries of the drug were obtained at 98.1%-101.8%, indicating the method was accurate and specific. The LOD and LOQ were found to be in sub microgram level indicating the sensitivity of the method. The method was also found to be rugged and robust as indicated by the %RSD values which are less than 2%. The summary of validation parameters of proposed method is shown in table 10.

Table 10: summary of validation

Parameters	Values
Linearity Range	10-50
Precision(%)	0.16%
Accuracy(%)	98.9-101.9%
LOD(µg/ml)	0.5
LOQ(µg/ml)	1.55
Stability (h)	2

CONCLUSION

All the above factors lead to the conclusion that the proposed method is accurate, precise, simple, sensitive, robust and cost effective and can be applied successfully for the estimation of silodosin in bulk and pharmaceutical dosage formulations.

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