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QUANTITATIVE ANALYSIS OF SILODOSIN IN CAPSULES USING UV SPECTROPHOTOMETRY AND RP-HPLC METHODS: APPLICATION TO DISSOLUTION TESTING

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ABSTRACT

Assay of Silodosin in capsule formulation was done by two ultraviolet spectrophotometric and one isocratic RP-HPLC method. The wavelength of maximum absorption for Silodosin in water was found at 268nm (method-I).The area under the absorption curve was recorded between 263nm and 273nm for each solution (method-II). A reversed phase column C_{18} (250 x 4.6 mm, 5µm particle size), mobile phase consisting of phosphate buffer pH 3.2: Acetonitrile (60:40v/v) with a flow rate 1.0 mL/min. was used. The effluents of the column were monitored through a variable wavelength UV detector at 230nm (method-III). The proposed methods obeys linearity in the range of 10-60 µg/mL and 4-20 µg/mL for method-I, II and method-III respectively with correlation coefficient values above 0.999. The % RSD was found to be less than 2.0.The mean recoveries were found in the range of 97.50-99.75%. Validation of the developed methods was performed in terms of accuracy, precision, linearity, etc. The proposed HPLC method has been applied to dissolution testing of the formulation.

Key words: Silodosin, Reversed Phase HPLC, Validation, Dissolution testing.

INTRODUCTION

Silodosin[1] is used for the symptomatic treatment of benign prostatic hyperplasia. It acts on α1-adrenoceptor antagonist with high uroselectivity. Chemically it is 1-(3-hydroxypropyl)-5-[(2R)-({2-[2-[2-(2, 2, 2-trifluoroethoxy) phenoxy] ethyl} aminopropyl] indoline-7-carboxamide. Literature survey reveals that there was a spectrophotometric[2]method and few HPLC[3-4] methods and a liquid chromatography-mass spectrometry[5] method were reported for the determination of Silodosin in bulk, pharmaceutical preparations and in biological fluids. However most of the available methods have limitations such as long run times, low sensitivity, uneconomical and have poor symmetry. Keeping in view of these we have decided to develop a simple, accurate, precise and reliable spectrophotometric and RP-HPLC method for the estimation of Silodosin in pharmaceutical dosage forms and application of the proposed methods for release studies.

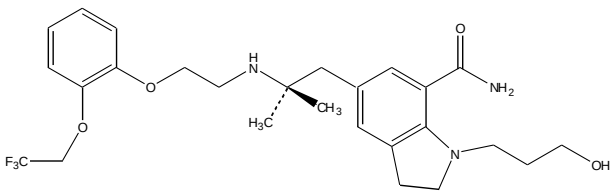


Fig.1.Chemical structure of Silodosin

MATERIALS AND METHODS

Silodosin pure material was gifted by Hetero Pharmaceutical Industries pvt. Ltd., Hyderabad. Silodosin is available as capsule formulation under different brand names which include silodal (manufactured by MSN laboratories and marketed by Ranabaxy) containing 8.0 mg of Silodosin and were procured from the local pharmacy. All chemicals and solvents were of analytical reagent grade. Acetonitrile and water (HPLC grade) was obtained from Merck Pvt. Ltd., Mumbai. Quantitative HPLC was performed on a high pressure gradient high performance liquid chromatograph (Shimadzu HPLC, Class VP series) with two pumps, manual injector with loop volume of 20 µL (Rheodyne), programmable variable wavelength UV detector and a reversed phase column (Phenomenex C_{18}, 250 mm length, 4.6 mm internal diameter and particle size 5 µm). The output signal was monitored and integrated using "Spincotech" software.

A Systronics Double beam UV- visible spectrophotometer 2203 with 1 cm matched quartz cells was used for all spectral and absorbance measurements and solutions were prepared in double distilled water. In-vitro dissolution studies were performed for the capsule dosage form using USP dissolution apparatus I(basket type, lab India dissolution apparatus)

Preparation of standard drug solutions for Method-I and Method-II:

100 mg of Silodosin pure drug was accurately weighed, transferred into a 100 mL volumetric flask containing 30 mL of double distilled water and sonicated for about 10 minutes. The volume was made

up to the mark with double distilled water to get the stock solution (1mg/mL). This solution was further diluted with the same to get the working standard solution.

Preparation of mobile phase

A 10 mM phosphate buffer was prepared by dissolving 1.3609 g of potassium dihydrogen orthophosphate in 1000 mL of water. To this 1.5 mL of triethyl amine was added and pH was adjusted to 3.00 with orthophosphoric acid. Above prepared buffer and acetonitrile were mixed in the proportion of 60: 40 v/v. The mobile phase so prepared was filtered through 0.22 µm nylon membrane filter and degassed by sonication.

Preparation of standard drug solutions for Method-III (RP-HPLC):

About 100 mg of pure Silodosin was accurately weighed and dissolved in 50 mL of mobile phase in 100 mL volumetric flask to get 1 mg/mL stock solution. A series of standard solutions in the concentration range of 2, 4, 6, 8, 10 and 12 µg/mL were prepared by a suitable dilution of stock solution with the mobile phase.

Recommended procedure:
Method-1(Zero order)

Aliquots of standard drug (0.2 to 1.0 mL, 100µg/mL) solution in double distilled water were transferred into a series of 10 mL volumetric flasks and the solution was made up to 10mL with double distilled water. After setting the instrument for its spectral properties the solutions were scanned in the wavelength ranging from 200nm-400nm. The wavelength of maximum absorption for Silodosin was found at 268nm. Absorbance of the solutions was recorded at the selected wavelength. A Calibration curve was plotted by taking the absorbance on y-axis and concentration of standard solution of Silodosin on x-axis.

Twenty capsules of Silodosin were accurately weighed and powdered. A quantity of capsules powder equivalent to 50mg of Silodosin was accurately weighed and transferred into a 100 mL volumetric flask containing 50 mL of double distilled water. The solution was sonicated for extracting the drug for about 15minutes, filtered through a cotton wool and the filtrate was made up to volume with double distilled water. Working sample solution was prepared and the absorbance at 268nm was recorded. The amount of Silodosin in the dosage form was computed from its calibration plot.

Method-II (Area under curve):

Aliquots of standard drug (0.2 to 1.0 mL, 100µg/mL) solution in double distilled water were transferred into a series of 10 mL volumetric flasks and the solution was made up to 10mL with double distilled water. After setting the instrument for its spectral properties the solutions were scanned in the wavelength ranging from 200nm-400nm. The wavelength of maximum absorption for Silodosin was found at 268nm.The area under the

absorption curve was recorded between 263nm and 273nm for each solution. A Calibration curve was plotted by taking the area under the absorption curve on y-axis and concentration of Silodosin standard on x-axis.

Twenty capsules of Silodosin were accurately weighed and powdered. A quantity of capsules powder equivalent to 50mg of Silodosin was accurately weighed and transferred into a 100mL volumetric flask containing double distilled water. The solution was sonicated for extracting the drug for about 15minutes, filtered through a cotton wool and the filtrate was made up to volume with double distilled water. The solution was further diluted with double distilled water to get the strength of 100µg/mL solution. Working sample solutions were prepared the area under the absorption curve of the sample was recorded between 263nm and 273nm. The amount of Silodosin present in sample was computed from its calibration curve.

Method-III (RP-HPLC):

The HPLC system was stabilized for thirty min. by following the chromatographic conditions as described in Table 1 to get a stable base line. One blank followed by three replicates of a single standard solution of Silodosin was injected to check the system suitability of the method. The chromatographic run time of 12 min**.** was maintained for the elution of the drug from the column. The column effluents were monitored with UV detector at 230 nm.

Replicates of each standard solution (2, 4, 6, 8, 10 and 12 µg/mL) were injected into the chromatograph and the average peak areas were recorded. Calibration curve was plotted by taking concentration of standard Silodosin on X-axis and peak areas on Y-axis.

For the assay accurately the content of twenty Silodosin capsules was transferred into a cleaned and dry mortar and ground to a fine powder. From this capsule powder, equivalent to 50 mg of Silodosin was taken and transferred into a 100 mL volumetric flask. The drug was extracted with 50 mL of mobile phase and volume was made up to the mark with the same. The resulting solution was filtered through 0.22 µm nylon membrane filter and degassed by sonication. This solution was further suitably diluted for chromatography with mobile phase. Working sample solutions were prepared, injected and the area of each sample was recorded. The amount of drug present in sample was computed from its calibration graph.

Dissolution testing of formulation by HPLC:

In-vitro dissolution studies were performed for the capsule dosage form using USP dissolution apparatus I(basket type, lab India dissolution apparatus), 100 rpm, thermostatically maintained at temperature 37 ± 0.5°C with a dissolution medium of 900mL of water for 45min. At predetermined time interval, samples of 5 mL were taken, filtered through 0.22µm membrane filter and drug content was

determined by HPLC method as described earlier.

RESULTS AND DISCUSSION

Chromatographic Conditions and System Suitability Parameters for assay of Silodosin capsules were given in Table no.1. The proposed method has good efficiency characteristics and system suitability. The tailing factor of Silodosin peak was 1.045. The number of theoretical plates of Silodosin peak was 315567.

Table.1.Chromatographic Conditions and System Suitability Parameters for Assay of Silodosin Capsules

Method Development Parameters and system suitability	
Stationary phase	Phenomenex C_{18} column (length: 250 mm, Internal diameter: 4.6 mm, Particle size: 5 µm)
Mobile phase	Buffer: ACN (60 : 40 v/v)
Flow rate (mL/min)	1.0
Column back pressure (kg/cm2)	118-125
Diluent	Mobile phase
Run time (min)	12min
Column temperature (oC)	Ambient
Volume of injection loop (µL)	20
Detection Wavelength (nm)	By UV at 230nm
Retention time (min.)	8.717
Theoretical plates (n)	15778
Plates per meter (N)	315567
Peak asymmetry	1.045

Specificity studies were conducted, in this method the diluents, mobile phase and the placebo solutions do not show any interference at the retention time corresponding to the peak of Silodosin.

The proposed methods obeys linearity 10-60 µg/mL and 4-20 µg/mL for method-I, II and method-III respectively. The correlation coefficient was found by the correlation and regression analysis and the coefficient of correlation was found to be 0.9994, 0.9991and 0.999 for method-I, method-II and method-III respectively. The optical characteristics and the regression analysis data concerning to the proposed methods is represented in Table no.2. The

representative spectra and chromatogram are shown in Fig. no.2 to Fig.no.4. The calibration plot for method-I, method-II and method-III are shown in Fig. no.5, Fig. no.6 and Fig. no.7 respectively.

Table.2.Optical characteristics, Regression data, Precision and Accuracy of the Proposed methods for Silodosin

Parameter	**Method-I** Zero order	**Method-II** Area under curve	**Method-III (RP-HPLC)**
λ_{max} (nm)	268	263-273	230
Beer's law limits (µg /mL)	10-60	10-60	4-20
Molar absorptivity (L. mole^{-1} cm^{-1})	5896.855	-----	---
Detection limits (µg / mL)	1.05156985	0.3602	0.549166
Sandell's sensitivity (µg /cm ^{2}/0.001 absorbance unit)	0.084034	-----	----
Optimum photometric range (µg / mL)	20-60	20-60	---
Regression equation (Y = a+ bc): Slope (b)	y = 0.0109x + 0.0036 0.0109	y = 0.0053x + 0.0014 0.0053	y = 5.298x +1.3512 15.298
Standard deviation of slope (S_b)	0.000116315	0.00001965	0.210209
Intercept (a)	0.0036	0.0014	1.3512
Standard deviation of intercept (S_a)	0.003521621	0.0005951	2.5457664
Correlation coefficient (r)	0.9994	0.9991	0.9992
% Relative standard deviation*	0.6215	0.6517	0.883587
%Range of Error (Confidence limits)* 0.05 level 0.01 level	0.65234851 1.023052973	0.6840 1.07275	0.91700079 1.43809692

***Average of six determinations.**

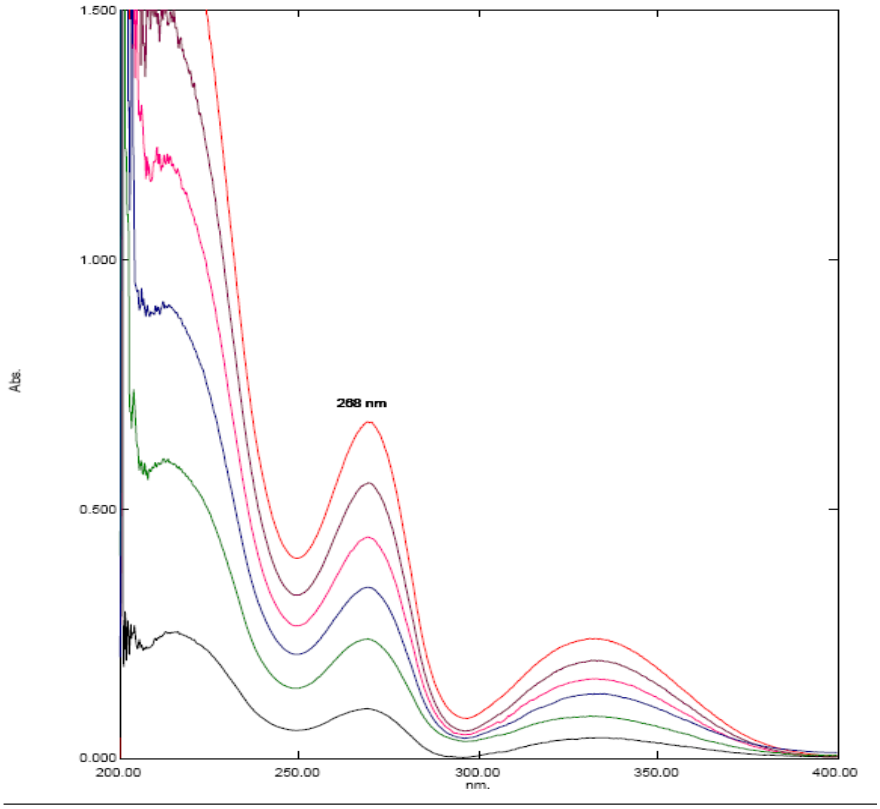
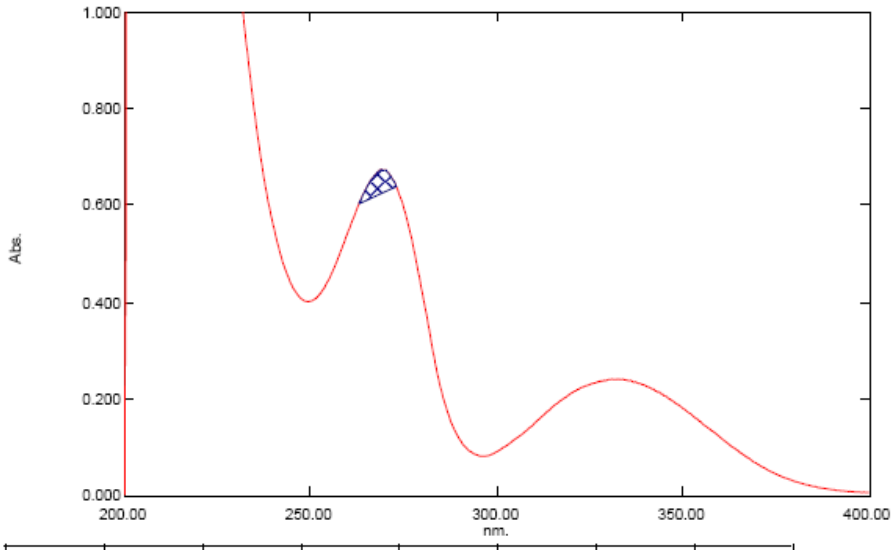


Fig.2.Overlay zero order Spectra of Silodosin in water : Concentration range 10-60µg/mL (from bottom to top)



Region	Color	Start	End	Divisor	Area	Result	Description
1		263.00	273.00	1.000	0.311	0.311	60 mcg
2							

Fig.3. Area under curve Spectrum of Silodosin in water : Concentration 60µg/mL

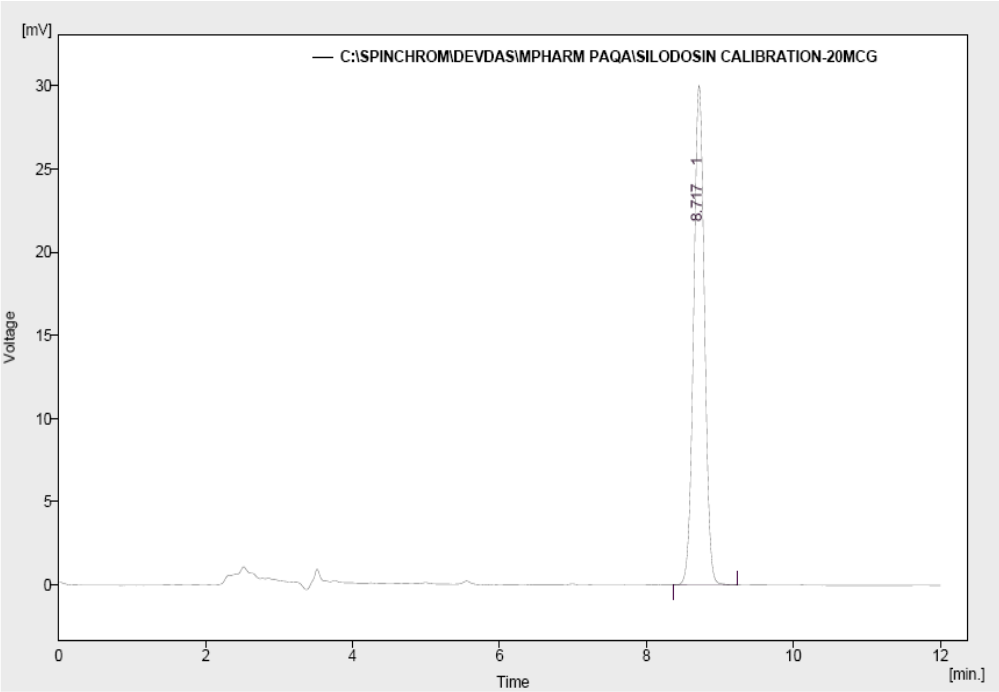


Fig.4.Representative Chromatogram of Silodosin standard (8 µg/mL)

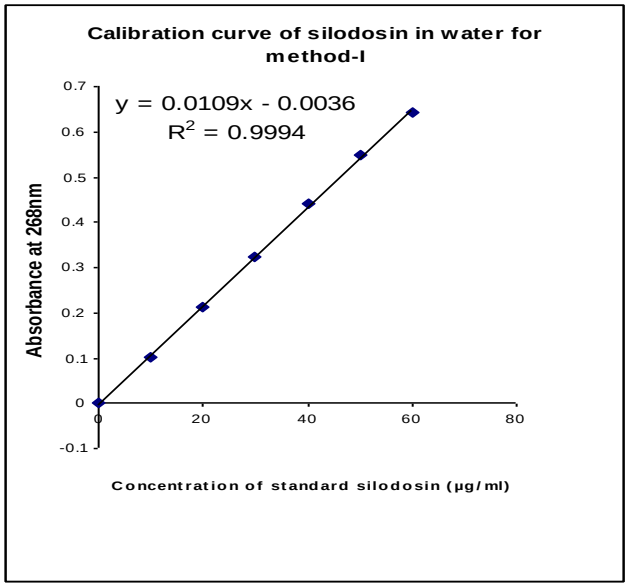


Fig.5.Calibration plot for method-I

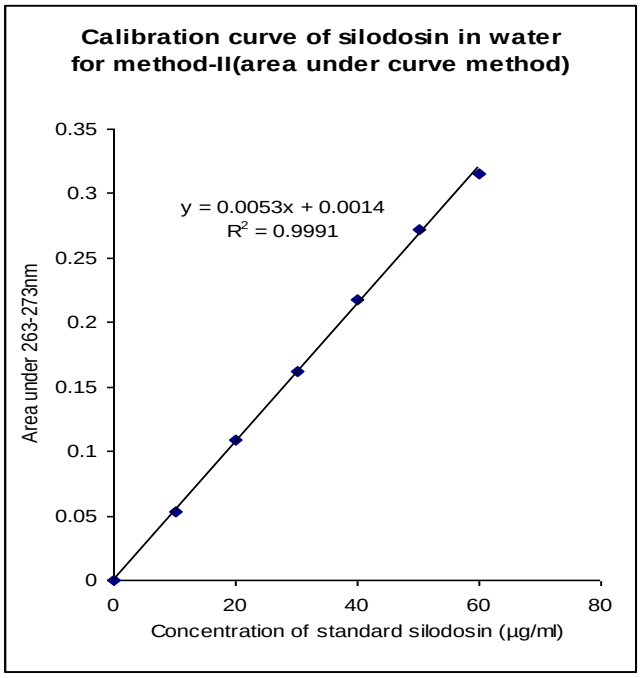


Fig.6.Calibration plot for method-II

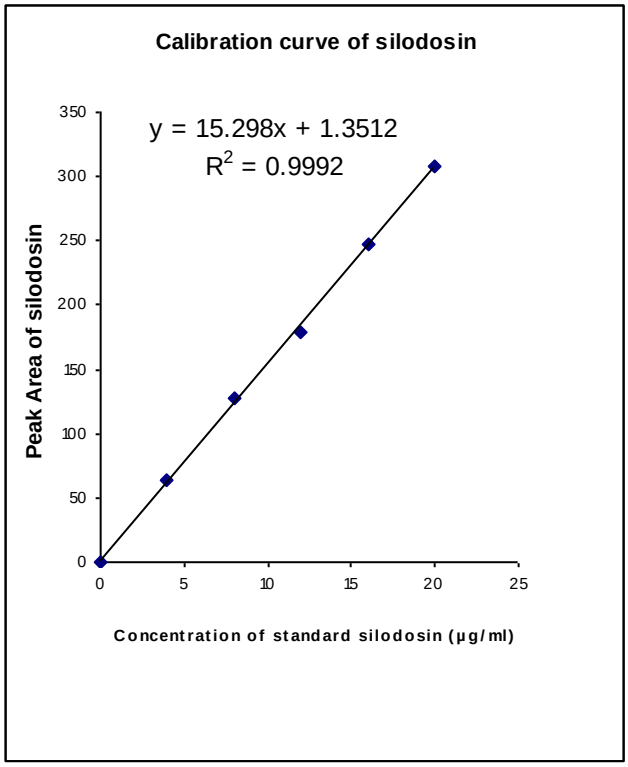


Fig.7.Calibration plot of Silodosin by HPLC

Accuracy was established by performing recovery studies at 3 levels in which known amount of analyte shall be added and recovery shall be carried out in three replicates of each concentration level. The % recovery at each level and % Mean Recovery were found to contain between 99.75%, 98.75and 97.50% for method-I, method-II and method-III respectively. The % RSD for the individual recoveries of each level and mean recovery were found to be less than 2.0 % for all cases. This recovery study indicates that the method has got good accuracy. The developed method was applied for the assay of *Silodal* capsule containing Silodosin and the assay results of Silodosin capsules of the sample were found to be with the limits. The mean assay results were very close to labeled amount of commercial Silodosin capsules. Assay and recovery study are tabulated in Table no.3. In-vitro dissolution was performed on silodal capsules containing Silodosin. The % cumulative drug release was found maximum within 30 min. The representative chromatograms of assay and dissolution sample are shown in Fig. no.8 and Fig.no.9. The dissolution profile curve of Silodosin is shown in Fig.no.10.

Table.3. Assay and recovery of Silodosin in SILODOL capsule dosage forms

Method	Pharmaceutical Formulation	Labelled Amount (mg)	Amount Found ± S.D	% Recovery by proposed methods** ± S.D
Method-I	Capsule	8mg	7.98	99.75±0.0014
Method-II	Capsule	8mg	7.9	98.75±0.0023
Method-III	Capsule	8mg	7.8	97.50±0.0068

***Average ± standard Deviation of six determinations, ** Average of six determinations.**

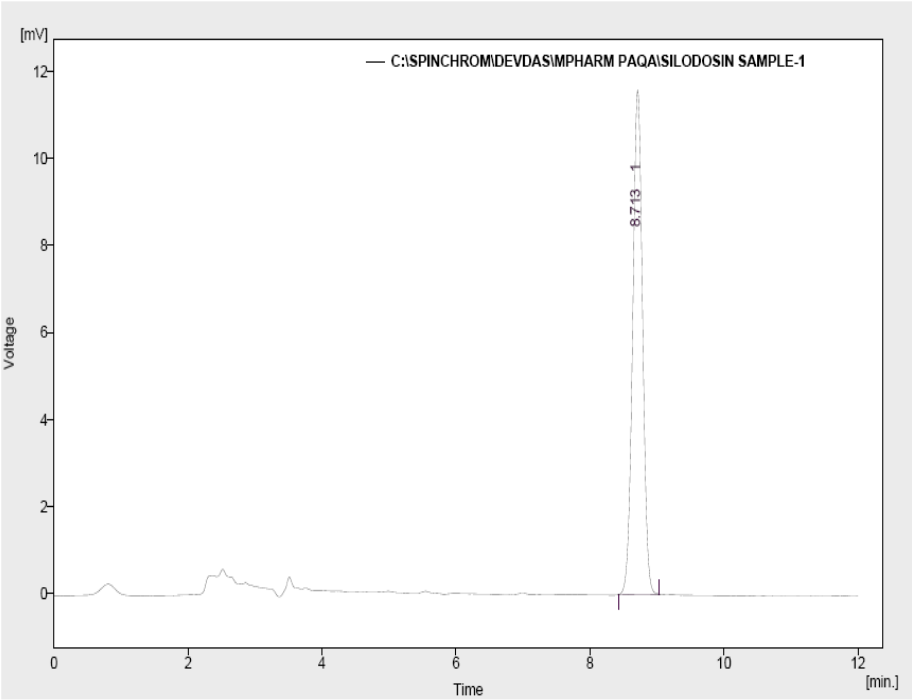


Fig.8.Representative Chromatogram of Silodosin Sample

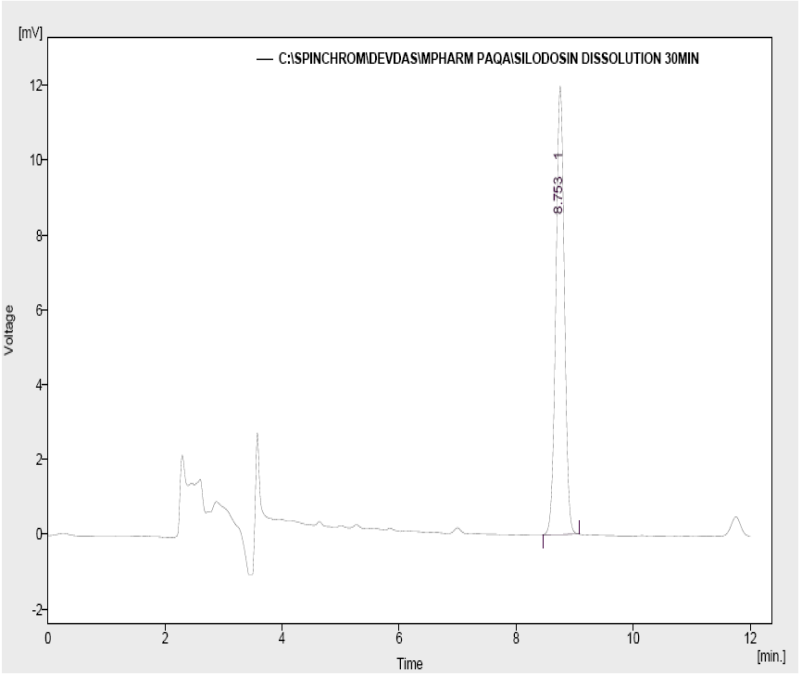


Fig.9.Representative Chromatogram of Silodosin dissolution Sample of 30min

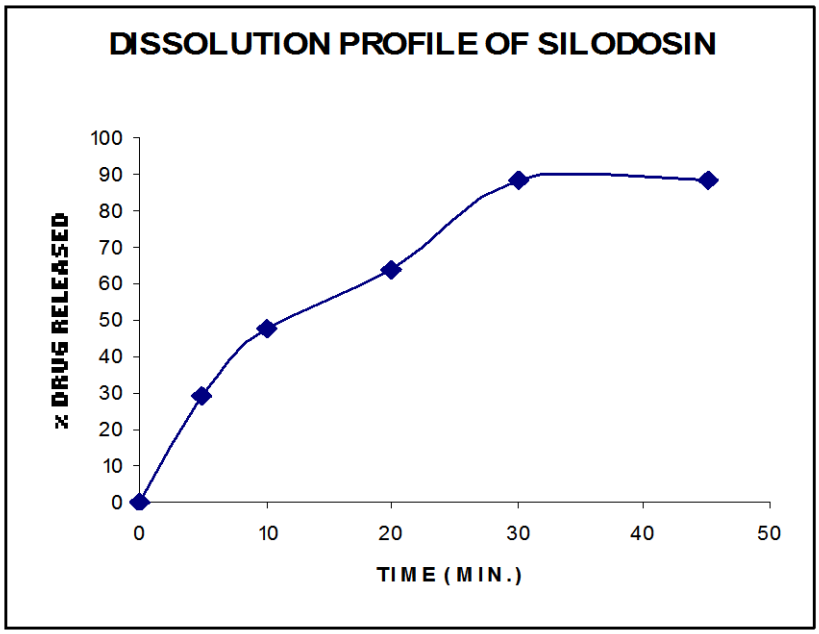


Fig.10.Dissolution profile curve of Silodosin capsules

Precision is carried out and the % RSD for the assay of Silodosin for six replicate samples was found to be 0.6215, 0.6517 and 0.883 for method-I, method-II and method-III respectively. Low % RSD values indicate the proposed methods have good precision. Changes in chromatographic conditions was studied in order to check whether there is any observable deliberate changes in the method and the method remains unaffected due to deliberate changes to the analytical method.

CONCLUSION

New spectrophotometric and RP-HPLC methods have been developed for the quantitative determination of Silodosin in bulk and capsule dosage form. Statistical analysis of the results shows that the proposed methods have good accuracy and precision. The methods are completely validated and show satisfactory results for all the method validation parameters tested and the methods were free from interferences of the other additives used in the formulation. As a matter of fact results of the study indicate that the methods were found to be simple, reliable, accurate, sensitive, economical and reproducible and hence it can be concluded these methods can be employed for the routine quality control analysis of Silodosin in active pharmaceutical ingredient (API) and pharmaceutical capsule preparations.

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REFERENCES

1. Yoshida M, Homma Y, Kawabe K . Silodosin a novel selective α1A-adrenoceptor selective antagonist for the treatment of benign prostatic hyperplasia. Expert opin investing drugs; 16 (12): 1955-65.
2. Aneesh T.P, A.Rajasekaran. Method development and Validation for the Estimation of Sildosin in Bulk and Pharmaceutical Dosage forms Using UV-Vis Spectrophotometry. Asian Journal Of Pharmaceutical and Clinical Research 150-153 Vol 5, Issue 4, 2012.
3. Aneesh TP and Rajasekaran A. Development and validation of HPLC method for the estimation of silodosin in bulk and pharmaceutical dosage form;International Journal of Biological & Pharmaceutical Research. 2012; 3(5): 693-696.
4. V. Mohan Goud, A. Srinivasa Rao, S. Pragati Ranjan1, S. D. Shalinil, S. Sowmya and Bhagya Bhoga. Method Development and Validation of RP-HPLC Method for assay of Sildosin in Pharmaceutical Dosage Form; International Journal of Pharma Sciences Vol. 3, No. 2 (2013): 194-196.
5. Xia Zhao, Yuwang Liu, Junyu Xu, Dan Zhang, Ying Zhou, Jingkai Gu, Yimin Cui: Determination of Silodosin in human plasma by Liquid chromatography-tandem mass spectrometry; Journal of chromatography B, Analytical technologies in biomedical and life sciences. Vol 877, Issue 29, 2009; 3724-3728.