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UV – Spectrophotometric Method for Estimation of Sildenafil in Bulk and Tablet Dosage Form

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ABSTRACT

A simple, precise and accurate UV Spectrophotometric method has been developed and validated for estimation of Sildenafil in bulk and tablet dosage form. In this method Sildenafil shows λmax at 290nm using 0.1N HCl as a solvent and calibration graphs were plotted over the concentrations ranging from 5 to 30µg/ml of Sildenafil with correlation coefficient 0.999. The proposed method was validated as per ICH Q2 (R1) guidelines for precision, linearity, accuracy and recovery. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.113µg/ml and 0.343µg/ml respectively by simple UV spectroscopy. The proposed method was validated.

Keywords: Sildenafil, UV-Spectroscopy, Validation, 0.1N HCl.

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INTRODUCTION

Sildenafil is chemically 5-[2-ethoxy-5-(4-methylpiperazine-1-sulfonyl) phenyl]-1-methyl-3-propyl-1H,4H,7H-pyrazolo[4,3-d]pyrimidin-7-one. The molecular formula is $C_{22}H_{30}N_6O_4S$ and molecular weight is 474.6 g/mol. Sildenfail is a vasoactive agent used to treat erectile dysfunction and reduce symptoms in patients with pulmonary arterial hypertension (PAH). Sildenafil elevates levels of the second messenger, cGMP, by inhibiting its breakdown via phosphodiesterase type 5 (PDE5). PDE5 is found in particularly high concentrations in the corpus cavernosum, erectile tissue of the penis. It is also found in the retina and vascular endothelium. Increased cGMP results in vasodilation which facilitates generation and maintenance of an erection. The vasodilatory effects of sildenafil also help reduce symptoms of PAH[1].

In literature survey reveals various UV-spectroscopic and RP-HPLC methods[2-10]. In present study, simple, economical, accurate, reproducible analytical methods with better detection range for estimation of Sildenafil in its pure form and its pharmaceutical formulations were developed. The developed method was validated as per ICH guidelines[11].

MATERIALS AND METHODS:

The spectrophotometric measurements were carried out using a Shimadzu UV-1700 UV/Vis spectrophotometer with 1cm matched quartz cell and Shimadzu ELB 300 analytical balance, Sildenafil pure drug (99.95%) was obtained as a gift sample from Dr. Reddy's. All chemicals and reagents used were of analytical grade.

Preparation of Standard solution:

Standard drug of Sildenafil was proposed by dissolving 25mg pure Sildenafil in 0.1N HCl and transferred into 250ml volumetric flask to obtain 100µg/ml of stock solution. The standard solution of Sildenafil having concentration of 25µg/ml was scanned in UV range (200-400nm) in 1.0 cm cell against in solvent as blank and spectrum was obtained.

Determination of λmax:

25µg/ml of Sildenafil was prepared and scanned in UV range of 200-400nm and spectrum was obtained. The λmax was found to be at 290nm wavelength where absorbance was found maximum at this wavelength. Hence it is considered as absorbance maxima (λmax) shown in Figure-1.

Preparation of calibration curve:

Standard stock solution was suitably diluted with 0.1N HCl to obtain concentrations ranging from 5-30µg/ml. Absorbance of these solutions was measured at 290nm. Calibration curve was obtained by plotting graph between concentration and absorbance shown in Figure-2.

Preparation of test solution:

20 Tablets were weighed and its average weight was determined. An accurately weighed tablet powder equivalent to 25mg of Sildenafil transferred into 250ml volumetric flask dissolved in 0.1N HCl, sonicated for 10min and volume was made up to the mark. Solution was filtered using whattman filter paper (No.41) to obtain 100µg/ml stock solution.

VALIDATION:

Linearity:

The absorbance's were observed from 5 to 30µg/ml and were shown in Table-1. Linearity was obtained between 5 to 30µg/ml. Concentration graph was plotted for concentration and absorbance. The equation of calibration curve obtained was y = 0.035x - 0.003. The correlation coefficient (r) was 0.999 shown in Figure-2.

Accuracy:

To determine the accuracy of the method recovery was performed by standard addition method. To pre-analyzed sample known amount of standard Sildenafil was spiked in different concentrations. The recovery was performed at three levels 50%, 100% and 150% of standard Sildenafil. Solutions were analyzed and percentage recovery was calculated from calibration curve shown in Table-2

Precision:

Repeatability

Six concentrations of 20µg/ml were prepared and the absorbance's were read. The % RSD was calculated and shown in Table-3

Intraday and Interday Precision

The concentration of 20µg/ml, 40µg/ml and 60µg/ml of Udenafil (on label claim basis) was taken. The absorbance of the final solution was read after 0hr, 12hr and 24hr in 1.0 cm cell at selected wavelength. Similarly the absorbance of the same solutions was read on 1st, 2nd and 3rd day. All the solutions are prepared triplicate and analyzed.

Assay

20 tablets of Sildenafil were crushed into powder. An equivalent weight of 10mg was taken and dissolved in 10ml volumetric flask. 1ml was transferred into 10ml volumetric flask to get 100µg/ml. Again 1ml was taken from the above solution into 10ml volumetric flask to get the final concentration 10µg/ml. The observed value was compared with that of standard value (10µg/ml) without interference from the excipients used in the tablet dosage form. The assay & % purity was performed by taking two brands CAVERTA and REZUM with label claim 25mg and 50mg.

Ruggedness:

It was carried out by analyzing the sample by three different days and estimation of drug by proposed methods. Results of studies are shown in Table-8

RESULTS AND DISCUSSIONS:

Linearity

Beer's law was obeyed in concentrations ranging from 5 to 30µg/ml. The correlation coefficient values were above 0.999 which shows that absorbance was linear with concentration. The results were shown in Table-1 and Figure-1 and 2.

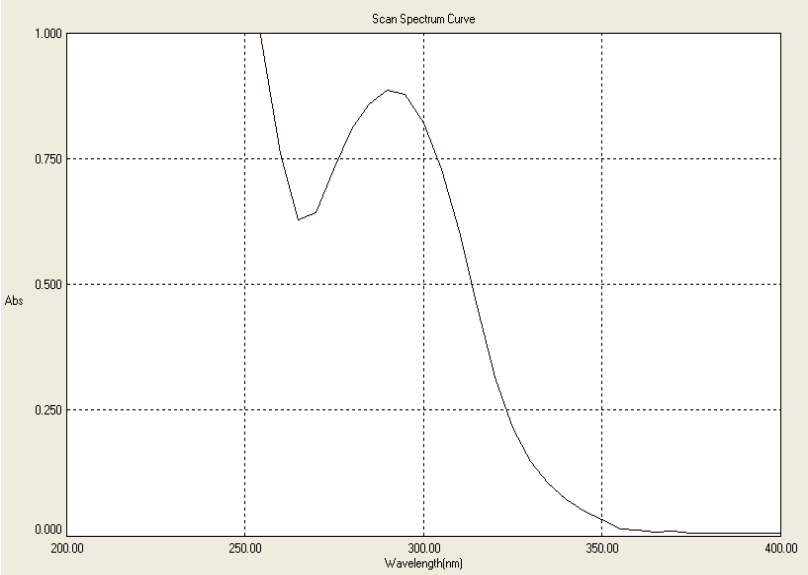

Figure 1: UV Spectrum of Sildenafil in 0.1N HCl at λmax = 290nm

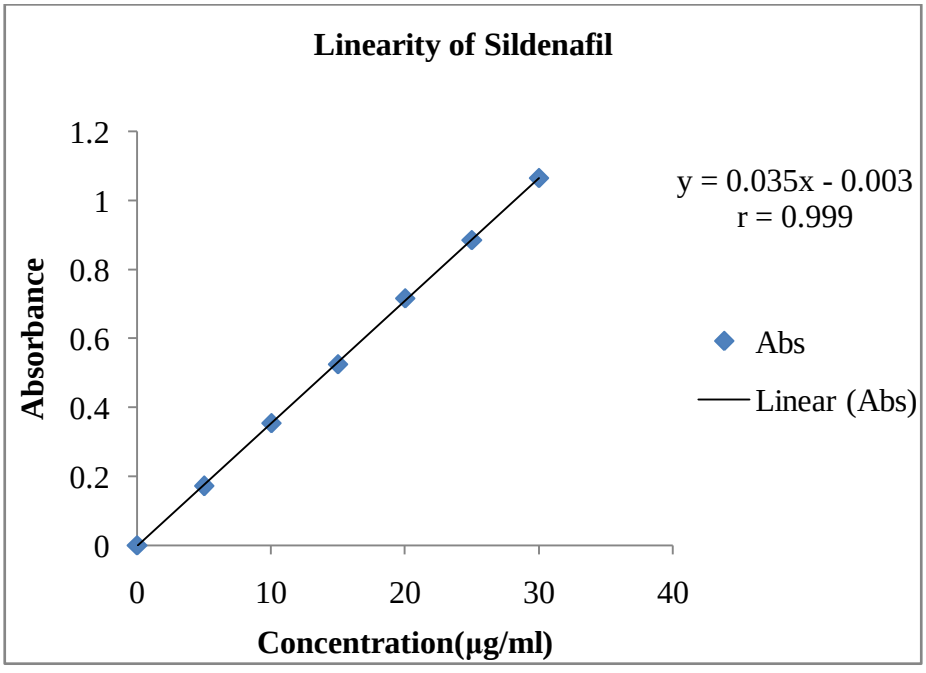

Figure 2: Calibration curve of Sildenafil in 0.1N HCl showing linearity relationship

Table 1: Calibration data for analysis of 0.1N HCl in 0.1N HCl at λmax = 290nm

Concentration (µg/ml)	Mean Absorbance (± SD)
5	0.172 (0.0006)
10	0.354 (0.0010)
15	0.531 (0.0015)
20	0.708 (0.0010)
25	0.885 (0.0006)
30	1.066 (0.0017)

Accuracy

Sildenafil standard was spiked with sample solution at 50%, 100% and 150%. The 10µg/ml sample solution was spiked with 5 µg/ml, 10 µg/ml and 15 µg/ml of standard solution. The recovery was found 99.72%w/v to 100.57%w/v. The recovery results were shown in Table-2.

Table-2: Recovery data of Sildenafil in 0.1N HCl

Ingredient	Amount of drug from formulation	Amount of standard added	Percentage added	Amount added	Amount found	% Recovery (Mean ± RSD)*
Sildenafil	10µg	5µg	50%	5	5.03	100.57 ± 0.57
Sildenafil	10µg	10µg	100%	10	9.99	99.91 ± 0.16
Sildenafil	10µg	15µg	150%	15	14.99	99.94 ± 0.22

*n=3 (Average of 3 determinations)

Precision

Repeatability was performed with six replicates of sample solution(10µg/ml). The %RSD was found to be 0.13. The intraday precision was carried out at 0hr, 12hr and 24hr with three different concentrations 10µg/ml, 20µg/ml and 30µg/ml. The results were discussed in Table-3, 4 and 5.

Table-3: Precision data of Udenafil in 0.1N HCl

S.No	Concentration(µg/ml)	Absorbance
1	25µg/ml	0.886
2	25µg/ml	0.885
3	25µg/ml	0.887
4	25µg/ml	0.884
5	25µg/ml	0.885
6	25µg/ml	0.884
Mean		0.885
Stddev		0.0012
%RSD		0.13

Table-4: Results of Intra-day Precision of Sildenafil in 0.1N HCl

Parameter	% Recovery Estimated (Mean + RSD)*		
	10µg/ml	20µg/ml	30µg/ml
At 0 hr	99.86 ± 0.16	99.76 ± 0.08	99.79 ± 0.2
At 12 hr	99.95 ± 0.28	99.81 ± 0.14	99.80 ± 0.24
At 24 hr	99.76 ± 0.43	100.04 ± 0.29	99.88 ± 0.33

*n=3 (Average of 3 determinations)

Table-5: Results of Inter-day Precision of Sildenafil in 0.1N HCl

Parameter	% Recovery Estimated (Mean + RSD)*		
	10µg/ml	20µg/ml	30µg/ml
Day-1	99.86 ± 0.33	99.95 ± 0.24	99.79 ± 0.11
Day-2	100.14 ± 0.43	100.24 ± 0.22	99.99 ± 0.16
Day-3	100.45 ± 0.19	100.11 ± 0.26	99.89 ± 0.24

*n=3 (Average of 3 determinations)

LOD and LOQ

The limit of detection was calculated by taking slope value in linearity graph and mean value in precision. The limit of quantification was calculated by multiplying 3.3 with LOD value. The LOD and LOQ were found to be 0.113µg/ml and 0.343µg/ml. The results were shown in Table-7.

Table-7: Lowest Limit of detection and Lowest Limit of quantification

LOD (µg/ml)	LOQ (µg/ml)
0.113	0.343

Ruggedness

Day to day analysis was performed in three days i.e., day-1, day-2 and day-3. The percentage purity with %RSD was calculated. The results were discussed in table-5. Analyst to analyst analysis was also performed with two analysts. The percentage purity with %RSD was calculated. The results were discussed in Table-8.

Table-8:Results of Ruggedness of Sildenafil in 0.1N HCl

Ruggedness	% RSD*
Analyst – 1	0.19
Analyst – 2	0.41

*n=3 (Average of 3 determinations)

Assay

Two brands of Sildenafil were taken CAVERTA and REZUM. The assay and percentage purity were 24.99 & 99.92±0.29 and 49.97 & 99.89±0.19. The results were shown in Table-6.

Table-6: Results of analysis of laboratory samples (Assay)

Sample	Label	Amount found	% Label claim
Sildenafil (Brand-1)	25mg	24.99	99.92 ± 0.29
Sildenafil (Brand-2)	50mg	49.97	99.89 ± 0.19

*n=3 (Average of 3 determinations)

Table-9: Validation Parameters

Parameters	Results
Beer's law limit (µg/ml)	5-30
Absorptivity (1mole^{-1}, cms^{-1})	1.6800 x 10^4
Sandell's sensitivity (µg/cm2/0.001)	0.0282
Correlation coefficient	0.999

Regression equation	Y = 0.035x - 0.003
Limit of detection	0.113
Limit of quantification	0.343
Precision % RSD	0.0012

CONCLUSION:

Attempt has been made to develop rapid, sensitive, economic, precise and accurate analytical method for Sildenafil in pure and pharmaceutical dosage form. The proposed method is based on UV Spectrophotometric absorption in UV region using 0.1N HCl as solvent. Maximum absorbance was found to be at 290nm). The optical characteristics such as Beer's law limit, correlation coefficient, slope, intercept, molar absorptivity, scandell's sensitivity were calculated and validated (Table-9). Hence the proposed method could be effectively adopted for routine quality control of Sildenafil in bulk and formulated tablet dosage form.

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