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Research Article

DEVELOPMENT OF ANALYTICAL METHOD AND ITS VALIDATION FOR SILDENAFIL CITRATE BY UV-SPECTROPHOTOMETRY

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ABSTRACT

Objective: The objective of this study is to develop a basic, delicate and precise UV-spectrophotometric method for Sildenafil citrate detection in the bulk drugs and the drug formulations and its validation. Method: The ideal conditions were established for the investigation or analysis of the drug. Results: The λ_{max} of 293.2nmwas found for the Sildenafil citrate. Within, 8 to 60µg/ml concentration range the method complied with Beer's Law and show exceptional sensitivity with linearity. Moreover, 1.012 and 3.036 were observed limit of detection and quantification, respectively. When the absorbance versus concentration graph was plotted on the calibration curves a linear relationship was observed with the coefficient correlation of 0.99. The observed regression coefficient (Y) of the calibration curves was found to be 0.0131*x*- 0.0191. The method was precise and accurate with the experiential value of 2.0325± 0.044. The stability of the test solution was up to 48 hours. Conclusion: The proposed analytical method is simple, economical and experimentally less time-consuming. Therefore, it will be appropriate for Sildenafil citrate analysis of pharmaceutical formulations in bulk.

Keywords: Sildenafil; Spectroscopy; Precise; Calibration

INTRODUCTION

Sildenafil citrate (SC) is pyrazolo-pyrimidinyl-methylpiperazine derivative. The IUPAC naming for sildenafil is "5-[2-ethoxy-5-(4-methylpiperazin-1-yl) sulfonylphenyl]-1-methyl-3-propyl-6 *H*-pyrazolo [4,3-d]pyrimidin-7-one". $C_{28}H_{38}N_6O_{11}S$ is the molecular formula (Fig. 1) and 661.700 is the molecular weight of SC. It is a crystalline, odorless, and ivory color powder having a salty or bitter taste. It melts at 186-190°C[1].The PKa and λmax (in 30% methanol water) are 8.7 and 290nm, respectively[2]. SC is a Phosphodiesterase's type 5 (PDE5) inhibitor specifically prescribed for management and treatment of male erectile dysfunction[3,4]. When it is orally administered, it absorbed promptly and shows the bioavailability of nearby 40%. The highest blood plasma concentration was attained by SC within few minutes to 2 hour (median1hour) when orally administered in the starved state[5], in which the action last up to 4 hours, but with lesser response as compared to that which is observed at 2 hours[6,7].

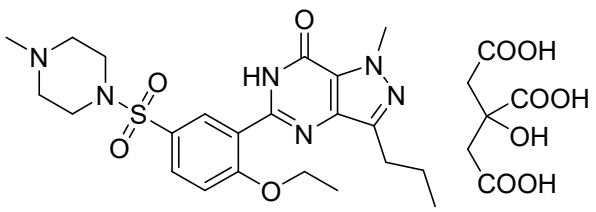

Figure 1: Sildenafil Citrate

Sildenafil citrate is primarily expelled as its metabolites in the feces and urine (80% and 13%of administered oral dose, respectively) irrespective of whether it is administered orally or intravenously[8]. Various techniques like chemometric[9], voltammetry[10,11], polarography[12,13]and measurement of mass by Electrospray Ionization (ESI) Technique in mass spectrometry[14], thin layer chromatography (TLC)[15-17], micellar electro-kinetic capillary chromatography[18,19], capillary zone electrophoresis[20], and high-performance liquid chromatography (HPLC)[21-25]gas chromatography (GC)[26,27]were well reported for analysis of SC.

MATERIALS AND METHODS

Reagents and chemicals

Sildenafil citrate of working standard was provided by Rachil Pharma, Hyderabad. Sildenafil citrate (Label claim: 50mg tablet) was manufactured by German Remedies Baddi, Solan (Himachal Pradesh), India. All other chemicals used in the analysis were AR grade.

Apparatus

A double beam spectrophotometer systronics (2202-2203) was used for the detection of absorbance; Schimadzu (BL 220 H) weighing balance and pH meter (Systronics µ pH system 361) were used for experimental purpose.

Preparation of the Stock Solution

For the preparation of the stock solution, 100 mg of sildenafil citrate was accurately weighed in 100ml volumetric flask. Dissolved it in a small volume of HCl (0.1N) and then the volume is wake up with HCl (0.1N) up to 100ml.

Preparation of sample solution

Pipette out 10ml of the stock solution in another volumetric flask & then the volume was wake up with phosphate buffer saline (pH6.5). From the above standard solution, further aliquots were pipette into a 10 ml volumetric flask and then diluted with sufficient quantity of phosphate buffer saline (pH 6.5). A Blank solution was also prepared, similarly except for the addition of the drug. Measure the absorbance at λ_{max}293.2 nm.

Preparation of calibration curve

Different fractions were taken out from the standard stock solution and were diluted with phosphate buffer saline (pH 6.5) to give a series of concentration (8-40µg/ml). The $\lambda_{max}$of SC in the phosphate buffer saline (pH 6.5) was observed by UV spectrum, in the range of 200-400nm. It was found that the peak at 293.2nm showed maximum absorption which has been used for further measurements. Absorbance measurement was performed at 293.2nm against the phosphate buffer saline (pH 6.5) as blank along with 0.1NHCl as co-solvent. By absorbance versus concentration plot, the calibration curve was constructed.

Method Validation

The ICH guideline Q2 (R1) was considered for the authentication of the proposed method. Numerous validation parameters for method validation like Linearity and Range; Limit of Detection (LOD) and Limit of Quantification (LOQ); Accuracy and Precision; Ruggedness and Robustness were calculated.

Linearity and Range

Linearity is the ability to produce experimental results that were directly proportional to the analyst concentration in a specified range. Ten replicated analyses were performed separately to access linearity and Absorbance versus Concentration was plotted to obtain the graph of calibration. The least square regression method was employed for measurement of the linearity. The range of the proposed analytical method was given by the interval between the lower and the upper concentration levels of sildenafil citrate in standard solutions.

Accuracy

Accuracy is defined as the degree of how close the experimental results are, to the true value and the percentage of analyte recovered by performing an assay, from a known concentration of drug. The accuracy of the experiment was usually established by performing recovery studies. For which, 10 tablets were crushed in a pestle mortar. The powder (332mg) of the crushed tablet which is almost equivalent to the 50 mg SC was correctly weighed and transferred into a volumetric flask (50 ml) along with an appropriate amount of 0.1N HCl for dissolution, then the volume is wake up with 0.1N HCl to 50ml. Pipette out 10ml of the above solution into another volumetric flask of 100ml & the volume was made up of phosphate buffer saline (pH6.5). From the above standard solution, further aliquots of concentration 24 µg/ml were prepared in a volumetric flask of 10ml. Similarly, the Blank solution was also prepared without the addition of the drug. The absorbance was taken at λ_{max}293.2 nm.

Precision

Precision is defined as the degree of repeatability of the method under standard operating conditions. It was estimated for 3 concentration levels (15µg/ml) covering the entire linearity range by intermediate (inter-day) as well as repeatability (intra-day) studies and was reported as % RSD.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD is the minimum concentration of an analyte in a test sample that can be identified, but not quantified. The LOQ is the lowermost concentration of the standard curve that can be calculated with satisfactory accuracy and precision. The LOD and LOQ were estimated by considering the slope of the calibration curve and the standard deviation. The Following equations were used for the calculation;

$$LOD=3.3\sigma/S;\ LOQ=10\sigma/S$$

Where, σ denotes the standard deviation of the absorbance of the sample and, S denotes the slope of the related calibrations in the graph.

Robustness

The robustness is the ability to remain unaffected of an analytical method by small, but measurable disparities in validation parameters of the method and indicates its reliability throughout its normal usage. The robustness was estimated by calculating the impact of minor variations of the most significant variables like

- Change in acid normality (0.01N HCl) & (1N HCl)
- Change in acid type (0.1N H_2SO_4 instead of 0.1N HCl)

% RSD was determined for concentration for 15µg/ml at pH values mentioned above in triplicate.

Table 1: **Optical characteristic parameters for sildenafil citrate in phosphate buffer saline (pH 6.5)**

Parameters	Values
λ_{max}(nm)	293.2
Linearity range(µg/ml)	8-60
Correlation coefficient ($R^{2)}$	0.998
Intercept	0.0131
Slope	0.0191
LOD(µg/ml)	3.30
LOQ(µg/ml)	10.08
Sandell's sensitivity (µg/cm^2/0.001A.U)	0.6

Table 2: **Results of Accuracy of Sildenafil citrate**

Conc. Taken (mg)	Conc. Observed (mg)±SD	Standard deviation (σ)	Relative standard deviation (RSD)
50	49.52±0.004	0.004	0.965%

Each value is an average of three determinants.

Table 3: Results of Precision of Sildenafil citrate

Conc. Taken (µg/ml)	Absorbance	Conc. Observed (µg/ml)	Standard deviation (σ)	Relative standard deviation (RSD)	Actual concentration
15	0.328	16.48	0.09416	0.57%	15µg/ml
15	0.330	16.58			
15	0.310	16.35			
INTERMEDIATE PRECISION EVENING (DAY1)					
Conc. Taken (µg/ml)	**Absorbance**	**Conc. Observed (µg/ml)**	**Standard deviation (σ)**	**Relative standard deviation (RSD)**	**Relative standard deviation between morning and evening**
15	0.328	16.48	0.04714	0.29%	0.43%
15	0.330	16.58			
15	0.330	16.58			
INTERMEDIATE PRECISION MORNING INTER DAY (DAY II)					
Conc. Taken (µg/ml)	**Absorbance**	**Conc. Observed (µg/ml)**	**Actual concentration**	**Standard deviation (σ)**	**Relative standard deviation (RSD)**
15	0.328	16.48	15 µg/ml	0.16391	0.99%
15	0.330	16.58			
15	0.330	16.58			
INTERMEDIATE PRECISION MORNING (DAY III)					
Conc. Taken (µg/ml)	**Absorbance**	**Conc. Observed (µg/ml)**	**Standard deviation (σ)**	**Relative standard deviation (RSD)**	**Relative standard deviation for the day I, II, III**
15	0.331	16.64	0.04714	0.29%	0.43%
15	0.331	16.64			
15	0.332	16.69			

Each value is an average of three determinants.

Table 4: Results of Robustness of Sildenafil citrate

Conc. Taken (µg/ml)	Absorbance	Conc. Observed (µg/ml)	Actual amount present in µg/ml	Standard deviation (σ)	Relative standard deviation (RSD)
15	0.376	19	15 µg/ml	0.53475	2.96%
15	0.352	17.74			
15	0.370	18.68			
READINGS FOR ROBUSTNESS (1N HCL)					
Conc. Taken (µg/ml)	**Absorbance**	**Conc. Observed (µg/ml)**	**Actual amount present in µg/ml**	**Standard deviation (σ)**	**Relative standard deviation (RSD)**
15	0.289	14.44	15 µg/ml	0.43151	2.87%
15	0.301	15.07			
15	0.309	15.49			

Each value is average of three determinants.

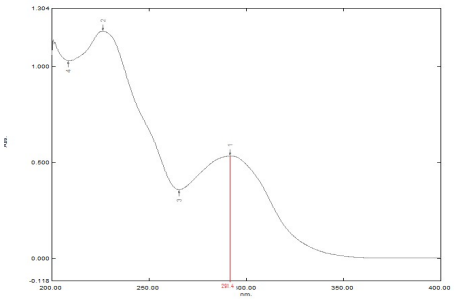

Figure 2: **UV absorption spectra of Sildenafil citrate in the phosphate Buffer saline (pH 6.5)**

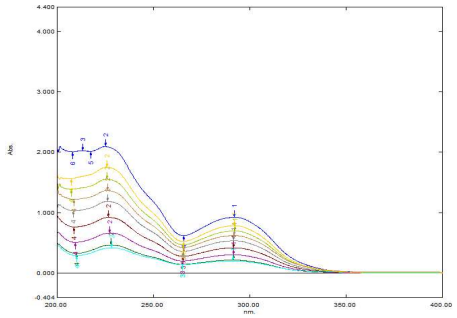

Figure 3: Overlay spectra of Sildenafil citrate in the phosphate buffer saline (pH 6.5)

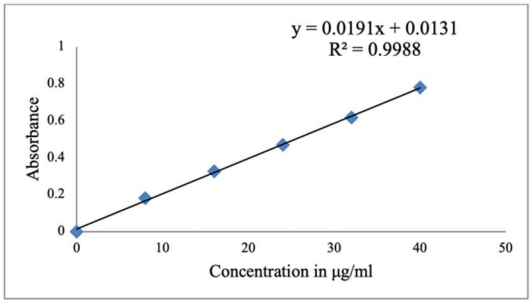

Figure 4: Calibration graph of sildenafil citrate in the phosphate buffer saline (pH 6.5)

RESULTS

Calibration Curve

The value of λ_{max} of Sildenafil citrate in the phosphate buffer saline (pH 6.5) was found to be 293.2nm (Fig. 2 and Fig. 3). The Absorbance range was found to be 0.189-0.780. The optical characteristic parameters are shown in Table 1.

Linearity and Range

The Range of Linearity was found to be 8-60µg/ml with a significantly high value of correlation coefficient, r2 = 0.998; the equation was represented as; **y = 0.0191x +0.0131** (Fig. 4)

Precision and Accuracy

The accuracy and precision have been summarized in Table 2 and Table 3 respectively. The Assay percentage was found to be 99.04%. The outcomes of precision (measurements of inter and intra-day) and accuracy exhibits excellent reproducibility with the percent relative standard deviation (%RSD) of less than 2.00%. Therefore, the results indicate that the method is highly precise and accurate.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ for sildenafil citrate were found to be 3.30µg/ml and 10.08µg/ml respectively.

Robustness

Data for robustness has been summarized and tabularized in Table 4.

DISCUSSION

This method validation was carried out with the objective to provide reliable and accurate method to estimate sildenafil citrate in bulk and in formulation. Validation of analytical method defines the documented procedure and provides the high degree of assurance of results meeting with predetermined specifications and quality attributes. The UV absorption spectrum of the sample (100µg/ml) was scanned, and major peak was obtained at 293.2nm as λ_{max} due to higher absorbance value. The calibration curve indicated the coefficient of correlation value of 0.998 which indicates linear relationship between concentration and absorbance. The results of accuracy and precision were found to be within limits in accordance with ICH Q2 (R1) guidelines.

Slight deviation was observed during robustness with change in acid composition and change in acid itself RSD slightly exceeded in accordance with, ICH Q2 (R1) guidelines. The sample was found to be stable and all other parameters were well within acceptance level i.e. LOD, LOQ and Sandell's sensitivity. The change in instrument, analyst and duration has not affected the method. The developed UV method was successful in accurate estimation of sildenafil citrate in formulation.

CONCLUSION

A UV-spectrophotometric method was proposed and validated. The proposed analytical method is simple, economical and experimentally less time-consuming. Therefore, it will be appropriate for Sildenafil citrate analysis of pharmaceutical formulations in bulk.

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