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Research Article

Analytical method development and validation for estimation of sildenafil citrate from tablet dosage form by using RP-HPLC

Shrikrishna B. Baokar*[1], B.Shirke[2], V.Sivanand[3], G.K.Pratheesh[3]

[1]Department of Pharmaceutical Chemistry, SVPMS College of Pharmacy, Malegaon (Bk), Tal- Baramati, Dist-Pune, Maharashtra, 413115, India
[2] Analytical Department, Shalina Private Laboratories Limited, Jejuri, Dist-Pune, Maharashtra, India
[3] Department of Pharmaceutical Analysis, Ultra College of pharmacy, Madurai, Tamilnadu, India

ABSTRACT

This study Study was aimed at develop and validate RP- HPLC method for the assay of sildenafil citrate in tablets' formulation A chromatographic system comprising Partisil 10 ODS C18 (250 x 4.6 mm, 5µm) column, a mobile phase of Buffer solution (pH2.0): acetonitrile, a flow rate of 1.5 ml/min and a UV detector set at 228 nm has shown good chromatographic separation for sildenfil. The degree of linearity of the calibration curves, the percent recoveries of sildenafil and related substances, the limit of detection (LOD), and limit of quantitation (LOQ) for the HPLC method have been determined. The HPLC method under study was found to be specific, precise, accurate, reproducible indicating stability and robust

Keywords: Sildenafil citrate; Viagra; HPLC; validation.

1. INTRODUCTION

Sildenafil citrate is designated chemically as 1-[[3-(6, 7-dihydro-7-oxo-3-propyl-1H-pyrazolo [4, 3-d] pyrimidin-5-yl)-4-ethoxyphenyl] sulfonyl]-4-methylpiperazine and it is a popularly known as Viagra (Figure 1).

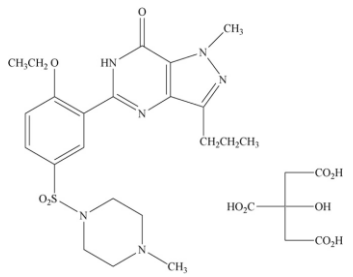

Figure 1: Structure of Sildenafil citrate

This is a novel oral agent for the treatment of penile erectile dysfunction (Boolell M *et al., 1996*). It is an active inhibitor of the type V-cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase on penile erectile activity and causes cGMP to accumulate corpus cavernosum (Boolell M *et al.,* 1996, Turko I *et al.,* 1999, Lowentritt B *et al.,* 1999, Brock G *et al.,* 2000). The structural formula is C22H30N6O4S. It is an ampholyte with pKa value 4 (pyridinium ion) and 8.8 (benzimidazole). Sildenafil citrate is twice more soluble in methanol than in water. Its solubility decreases with pH up to 9 when it starts to increase again. A few methods based on HPLC were reported for the determination of sildenafil citrate in biological and pharmaceutical products. A reverse-phase HPLC method using acetonitrile-phosphate buffer-water (28:4:68 v/v/v) with detection at 230 nm was utilized for the simultaneous determination for sildenafil and its metabolite (UK-103,320) using the automated sequential trace enrichment of dialysates(Cooper J *et al.,* 1997). Reverse-phase HPLC method using 70 mM potassium phosphate monobasic buffer of pH 3.0 containing 100 mM triethylamine: acetonitrile (7:3 v/v) as the mobile phase at 225 nm for the separation of sildenafil citrate formed due to oxidation (Martel A *et al.,* 1997). RP-HPLC method for the determination of sildenafil citrate by using Lichrospher C18 column with water-acetonitrile as the mobile phase and UV detection at 245 nm (Dinesh N *et al., 2002*). A reverse-phase HPLC method for the determination of its related substances in commercial formulations and tablets was reported (Draghmeh N *et al.,* 2001). The method was developed utilizing a monolithic silica column and an isocratic elution of acetonitrile: water and detection at 292 nm with a flow rate of 2.0 ml/m (Hassan Y *et al.,* 2009).

2. EXPERIMENTAL

2.1 Instrumentation

A High Performance Liquid Chromatography system (Agilent 1100) was used for the analysis, Mettler Tremedo as weighing balance and Bronson sonicator, Millipore Milli Q plus purification system, borosil glass apparatus were used for experimental process.

* Corresponding Author
Email: krishnabaokar@gmail.com
Contact: +91-9960225455
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2.2 Chemicals and Reagents

Sildenafil Citrate was obtained from Shalina Laboratories, Jejuri (Pune) as a gift sample, Sildenafil citrate working standard, used from PPIM (Pharmaceutical Product of India Management) which is having % Purity - 99.57% for the estimation of sildenafil citrate in bulk and commercial formulations of sildenafil brand (Kifaru-50 Shalina Laboratories, Jejuri, Pune), 20 tablets were obtained from retail pharmacies. Each tablet was labeled contain 50 mg of sildenafil citrate. HPLC grade Methanol, Acetonitrile, Triethylamine, Phosphoric Acid - procured from Merck, India. Highly pure water was prepared by using Millipore Milli Q plus purification system.

2.3 Selection of Wavelength:

The wavelength for the analysis of Sildenafil Citrate (20ppm) was selected from the UV spectrum shown in fig.

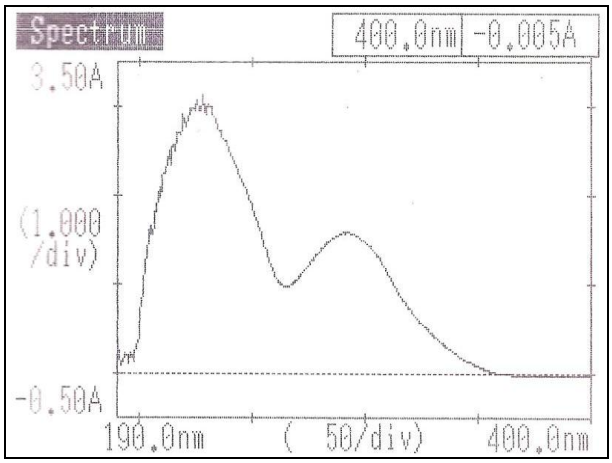


Figure 2: Wavelength Determination

A wavelength of 228 nm was selected for the analysis as it is having the maximum absorbance at this particular wavelength.

2.4 Chromatographic Conditions

As the drugs are polar in nature, a RP-HPLC method was preferred. The developed method for the estimation of Sildenafil Citrate required adequate resolution of the drug peaks in the chromatogram. Several solvent system were tried to obtain optimum resolution. Finally a peak of Sildenafil Citrate was well resolved with the solvent system of phosphate buffer (0.01% Triethanolamine in distilled water): Acetonitrile in the ratio 50:50, pH 2 adjusted with Phosphoric acid. The flow rate was tried with 1.5 ml/min, 1.7 ml/min and 2.0 ml/min. The peak shapes of drugs were showing fronting and tailing with 1.7 and 2.0 ml respectively. Finally, 1.5 ml/minute was selected for the analysis.

2.5 Preparation of Sildenafil Citrate Standard Solution:

Accurately 142.4mg of sildenafil citrate (142.4mg of Sildenafil Citrate is equivalent to 100mg of Sildenafil) was weighed and 100 ml methanol was added. 1.0 ml from this solution was taken and diluted to 100ml with methanol (10ppm solution) and chromatogram was recorded.

This developed chromatogram showed in the figure 3.

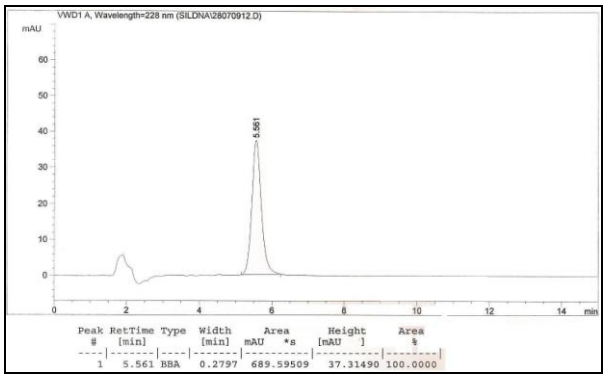


Figure 3: Method Development chromatograph

3. QUANTITATIVE DETERMINATION OF THE DRUGS USING THE

DEVELOPED METHOD

3.1 Standard Solution:

Accurately 50 mg Sildenafil Citrate was weighed and it was diluted with 100 ml methanol. From the resulting solution 1ml was taken and made up to 10 ml to give 50 µg/ml concentration of sildenafil citrate and it was used for chromatographic analysis. The developed chromatograph of standard solution is shown in figure 4.

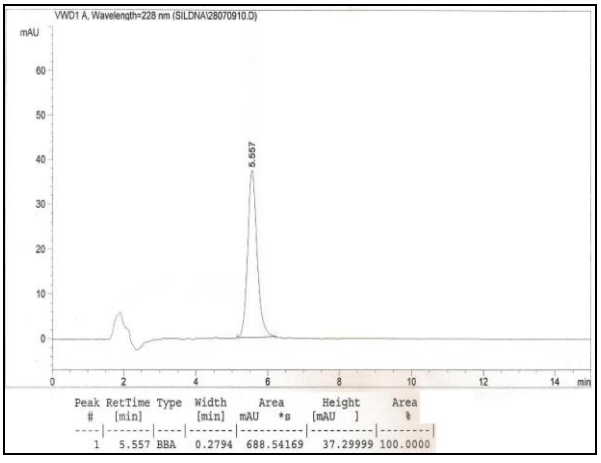


Figure 4: Quantitative Determination of Standard

3.2 Sample Solution

20 tablets were weighed and powdered. From this, powder equivalent to 50 mg of Sildenafil Citrate was taken and it was extracted with methanol and then the resulting solution is made up to 100 ml with Methanol. From the resulting solution 1 ml was taken and made up to 10 ml. It was used for chromatographic analysis. The developed chromatograph for sample solution is shown in figure 5.

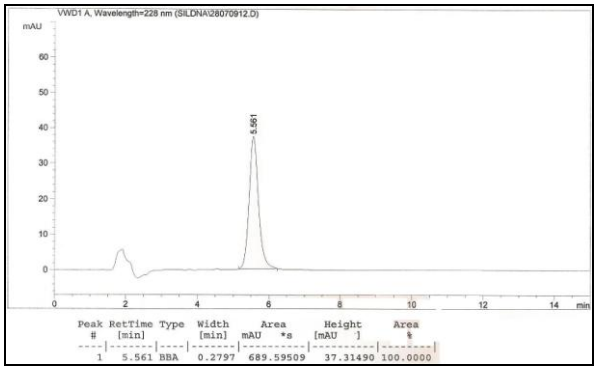


Figure 5: Quantitative Determination of Sample

Finally assay for this developed method summarized in Table 1.

bo containing all excipients except the drug and also the sample prepared from the same.

4.1.2 Standard Dilution

About 50 mg of Sildenafil Citrate was weighed in a 50 ml of Standard flask and it was dissolved with 10 ml of methanol. The volume was made up to 50 ml with Methanol.

4.1.3 Placebo Dilution

This placebo was prepared in similar manner that prepared for estimation with weighed amount of excipients which was found to be in 100 mg equivalent of Sildenafil Citrate tablet and then it was injected. Then mixtures of placebo with standard as well as separate standard solutions were also injected to specify no

Table 1: Quantitative Estimation

S.NO	Tablet sample	Label Claim in mg/tablet	Peak area		Amount found mg/tablet	Percent label claim %
			Test (Avg. Area of 3 readings)	Standard (Avg. Area of 3 readings)		
1	Sildenafil Citrate (Kifaru - 50)	50	689.5	700.5	49.21	98.42

Table 2: Specificity for Sildenafil Citrate

Sr. No.	Sample	Area Obtained	Percentage content of Drug % w/v
1	Placebo	0	0
2	Standard	689.59	98.42
3	Standard + Placebo	688.54	98.01

Table 3: Linearity

	10 PPM	20PPM	30 PPM	40 PPM	50 PPM
Sr. No.	PEAK AREA	PEAK AREA	PEAK AREA	PEAK AREA	PEAK AREA
1	492.26	974.89	1529.56	1963.00	2468.65
2	490.12	978.25	1535.56	1944.95	2450.54
MEAN	491.19	976.57	1532.56	1953.98	2459.6
SD	1.51	2.38	4.24	12.76	12.81
% RSD	0.31	0.24	0.28	0.65	0.52

4. VALIDATION

Validation of an analytical method is a process to establish that the performance characteristics of the developed method meet the requirement of the intended analytical application.

4.1 Specificity

The specificity of the method was evaluated by analyzing the sample solution added (known amount) with excipients at appropriate levels that the assay result is unaffected by the presence of extraneous materials.

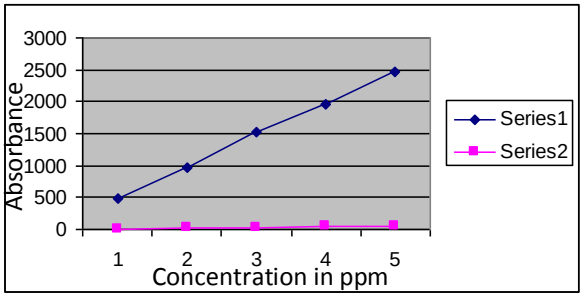
4.1.1 Placebo Method:

Specificity of the method was established by demonstrating that there is no interferences from the excipients. This was demonstrated by preparing the placeinterference of placebo with standard. Results for specificity are shown in Table 2.

4.2 Linearity

First the System suitability was checked with five replication of standard of 20ppm. Then the different concentrations of sample solution were injected from concentration range of 10 ppm to 50 ppm of the target analyte concentration including highest and lowest concentration i.e. 10ppm & 50ppm (Suddhasattya D *et al.,* 2010). All the dilutions were made with methanol and the solutions were injected and results for linearity shown in Table 3.

The correlation co-efficient and percentage curve fitting were calculated and is shown in fig.



Correlation of coefficient=0.998

Figure 6: Linearity curve of Sildenafil citrate

4.3 Accuracy

The accuracy of an analytical method is the closeness of the test results obtained by that method to the true value (Reddy P *et al.,* 2010). The accuracy of the analytical method was determined by applying the method to analyzed samples, to which known amounts of analyte have been added. The accuracy is calculated from the test results as the percentage of analyte recovered by the assay.

$$\text{Percentage Recovery} = \frac{\text{Mg found}}{\text{Mg added}} \times 100$$

Different concentrations of Sildenafil Citrate were prepared by taking 142.4mg of Sildenafil Citrate into 100 ml volumetric flasks and it was dissolved in methanol. This was considered as a 100% and the weight for 80% and 120% were calculated and these were 113.92mg and 170.88mg respectively. Result for accuracy shown in Table 4.

4.4 Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

4.4.1 System Precision

The first type of precision study is instrument precision or injection repeatability. Standard solution (20ppm) was injected in six replicate injections to check the Relative Standard Deviation (%RSD) for finding the precision of the system to be used for validation. Results for system precision are shown in Table 5.

Table 4: Accuracy

Sr. No.	Sample ID	Weight of Sample (mg)	Area	Amount Found in mg	% Recovery
1	80 %	113.92	555.09	113.77	99.86
		113.41	561.49	113.25	99.85
		113.65	562.78	113.49	99.85
2	100 %	142.42	688.54	142.23	99.86
		142.81	690.86	142.62	99.86
		142.66	689.59	142.47	99.86
3	120 %	170.86	829.33	170.63	99.86
		170.71	832.51	170.48	99.86
		170.93	832.00	170.70	99.86

Table 5: System Precision

Sr. No.	AREA OF 20 ppm
1	963.0
2	976.3
3	968.8
4	974.1
5	975.7
6	978.5
MEAN	972.5
SD	5.78
%RSD	0.59

4.4.2 Method Precision:

Method precision or intra-assay precision data are obtained by repeatedly analyzing, in one laboratory on one day, aliquots of homogeneous sample, each of which independently prepared according to method procedure. six times sample were prepared and each of those solution was injected. Mean of all of these values gives rise to the assay value obtained from method precision. Results for method precision are shown in Table 6.

Table 6: Method Precision

Sr. No.	PEAK AREA	PEAK AREA	PEAK AREA	PEAK AREA	PEAK AREA	PEAK AREA
1	965.01	995.63	972.21	962.43	1010.12	966.20
2	973.58	994.61	978.25	965.25	1008.99	974.05
MEAN	969.30	994.99	975.23	963.84	1009.56	970.13
SD	6.06	0.53	4.27	1.99	0.80	5.55
%RSD	0.63	0.05	0.44	0.21	0.08	0.57

Table 7: Ruggedness

Std.Batch.No.	SFC0280409		
Potency	98.00%		
Wt.Taken	101.1mg		
SET NO.	**Sample Name**	**AREA**	**ASSAY %**
1	Sildenafil Citrate-1	690.2	99.42
	Sildenafil Citrate-2	697.7	99.21
	Sildenafil Citrate-3	685.0	99.17
	Sildenafil Citrate-4	681.1	99.10
MEAN		688.5	99.22
SD		7.17	0.13
Std.Batch.No.	SFC0350509		
Potency	98.00%		
Wt.Taken	107.3mg		
SET NO.	**Sample Name**	**AREA**	**ASSAY %**
2	Sildenafil Citrate-1	954.3	99.47
	Sildenafil Citrate-2	987.3	99.27
	Sildenafil Citrate-3	1024.9	99.42
	Sildenafil Citrate-4	950.0	99.45
MEAN		979.12	99.40
SD		34.76	0.09

Table 8: Ruggedness in comparison

SAMPLE	ASSAY-I	ASSAY-II
	SET-1	SET-II
1	99.42	99.47
2	99.21	99.27
3	99.17	99.42
4	99.1	99.45
MEAN	99.22	99.4
SD	0.14	0.09
% RSD	0.13	0.9
Set	I	II
Analyst	1	2
Instrument. No.	1100	1100
Date	11.11.2009	15.11.2009

4.5. Ruggedness

The ruggedness of an analytical method is degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions, such as different laboratories, different analysts, different instruments, different lots of reagents, different elapsed assay times, different assay temperatures, different days etc. To carryout ruggedness experiment, analysis was repeated with different column, different day, different analyst and different system. All results for ruggedness shown in two different sets and mentioned in Table.

4.6. Robustness

Robustness is the capacity of the method to remain unaffected by small deliberate variations in method parameters In the case of liquid chromatography, examples of typical variations are: Influence of variations in wavelength of detections (+/-5nm), Influence of variations in column temperature (+/-5), Influence of variations in mobile phase composition (+/- 2%), Influence of variations in flow rate (+/- 10%), Influence of varia-

Table 9: Robustness

Sr.No.	Parameters	Sildenafil Citrate	Acceptance Criteria
1	Wavelength +5nm	99.25%	99-101%
2	Wavelength -5nm	99.41%	99-101%
3	Column Temp. +5°C	99.24%	99-101%
4	Column Temp. -5°C	99.29%	99-101%
5	Mobile Phase 52:48	99.32%	99-101%
6	Mobile Phase 48:52	99.30%	99-101%
7	Flow Rate 1.3ml/min	99.24%	99-101%
8	Flow Rate 1.5ml/min	99.35%	99-101%
9	pH +0.2	99.39%	99-101%
10	pH -0.2	99.25%	99-101%

Table 10: System suitability

System Suitability Parameters	Sildenafil Citrate
Theoretical Plate number	6329.18,
Tailing	1.01

tions of pH in a mobile phase (+/- 0.2), All observed results are summarized Table 9.

5. SYSTEM SUITABILITY STUDIES

It is essential for the assurance of the quality performance of chromatographic system and six replicate injections should be given to the system. A solution of 20ppm solution of Sildenafil Citrate was prepared by diluting suitably with methanol and the same was injected and chromatograms were recorded. The observed No. of theoretical plates and tailing factor noted in Table.

6. RESULTS AND DISCUSSION

The result for the developed method, quantitative determination of the formulation using the developed method & validation of the developed method are shown in Table No.1 Specificity of the method was found out through non-interference of the placebo in identical conditions of assay. This confirms the specificity of the developed method. Linearity of the drug was obtained in the range of 10ppm to 50ppm of Sildenafil Citrate. The linearity coefficient and percentage curve fitting was found to be 0.9994 and 99.98% for Sildenafil Citrate. Accuracy of the method was determined through the recovery studies of the drugs. Recovery of the drugs was well within the acceptance limit (99% - 101%). Precision of the method was determined by analyzing the drug formulation by replicate injections and precision of the system was determined by mixed standard solutions. Percent RSD of the analyte was found to be within the limit of 2%, thus the developed method was found to provide high degree of precision and reproducibility. Ruggedness was determined by performing the assay with same condition on different days, by different analysts, different instrument and different column. The test results were found within limit 99 – 101%. The results were found to be reproducible, in spite of variations in conditions which could be normally expected from analysts to analysts. Robustness was determined by carrying out the assay during change in mobile phase ratio, wavelength, column temp, flow rate, and pH. Percent RSD was found to be within the limit NMT 2%. The values of RSD obtained with the change in mobile phase ratio makes it possible to carry out the method for sildenafil citrate with a small variation in mobile phase ratio. System suitability was determined by performing the assay with the same sample repeatedly. The number of theoretical plates was found to be 6329.18 for sildenafil citrate and the Tailing factor was found to be 1.01.

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