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Research Article

Stability indicating RP-HPLC method development and validation of Sildenafil Citrate in pure form

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ABSTRACT

A simple , selective, precise, sensitive and stability indicating reverse phase high performance liquid chromatographic (RP-HPLC) method was developed and validated for the analysis of sildenafil citrate in pure form. Chromatographic separation achieved isocratically on Inertsil ODS-3V (4.6 x 250) mm, 5µ column utilizing a mobile phase of water: acetonitrile (40:60)%v/v with flow rate of 1ml/min with UV detection at 214 nm at ambient temperature. The retention time was 6.91 min. The method is accurate (100.32 ± 0.84), precise (100.4 ± 1.04) and linear (R^2=0.999) within the range of 20% - 150% of standard drug concentration. The limit of detection (LOD) and limit of quantification (LOQ) was found to be 0.0177µg/ml and 0.0535 µg/ml respectively. Stress studies were carried out as per recommendations of ICH guidelines. The method was proved to be robust with respect to change in flow rate, mobile phase composition and column oven temperature. Solution stability was found to be more than 8 hrs.

Keywords: Sildenafil citrate; HPLC method; Validation; Stability indicating.

INTRODUCTION

Sildenafil citrate (Fig. No. 1) 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.

The structural formulae is $C_{22}H_{30}N_6O_4S$. It is a novel oral agent for the treatment of penile erectile dysfunction. 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 (Yada D.,2010, Al-Omari M.,2000).

Literature reveals few RP-HPLC method for the determination of Sildenafil citrate (Reddy P.,2010, Yada D.,2010, Al-Omari M.,2000) using buffers. Rangappa K., 2002 used the internal standard method for its determination.

Present communication describe the stability indicating RP-HPLC method exclusively for Sildenafil citrate in pure form using simple mobile phase without need of buffer and involves no complex procedure to prepare the sample solutions.

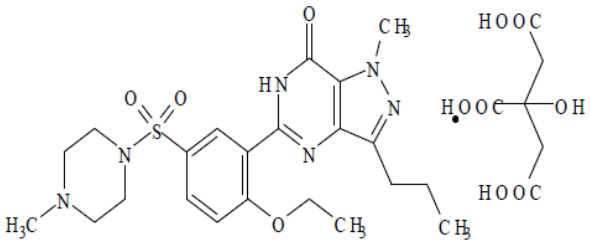

Figure 1: Structure of Sildenafil citrate

EXPERIMENTAL

Chemicals and Reagents

Sildenafil citrate was obtained as a gift sample from FDC Pvt. Ltd. India. Acetonitrile used was of HPLC grade and HPLC grade water was collected from Millipore –Q (0.22 µ).

Instrumentation and chromatographic conditions

Chromatographic separation was performed on a JASCO UV-2075 plus auto sampler chromatographic system equipped with Intelligent UV- Vis detector and data analyzed by using JASCO Chrompass version (1.8.6.1) software. Inertsil ODS-3V (4.6 x 250) mm, 5µ column was used for separation. Mobile phase consisting of a mixture of water: acetonitrile (40:60)%v/v was delivered at a flow rate of 1ml/min. The mobile phase was filtered through a 0.45 µ membrane filter and sonicated for 15min. Analysis was performed at ambient temperature. Detection was carried out at 214 nm. Chromatographic conditions were showed in (Table:1).

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Table 1: Chromatographic conditions

Chromatograph	JASCO HPLC system
Mobile phase	Water : Acetonitrile (40:60)%v/v
Column	Inertsil ODS-3V (4.6 x 250) mm, 5µ
Flow rate	1ml/min
Wavelength detection	214 nm
Injection volume	20µl
Temperature	Ambient
Retention time	6.91 min
Run time	10 min
Diluent	Mobile phase

Table 2: Method development conditions

Condition	Mobile phase	Ratio	Retention time	Tailing factor
1	Water Acetonitrile	20:80	4.51	1.05
2	Water Acetonitrile	50:50	10.4	1.04
3	Water Acetonitrile	40:60	6.91	1.16

Table 3: Stress conditions with % degradation

Stress condition	%LC	Average precision result	% Degradation
Acid degradation (0.1N HCL)	100.7	100.4	NIL
Acid degradation (0.5N HCL)	100.2	100.4	NIL
Acid degradation (2N HCL)	100.7	100.4	NIL
Base degradation (0.1N NaoH)	100.6	100.4	NIL
Base degradation (0.5N NaoH)	100.9	100.4	NIL
Base degradation (2N NaoH)	100.0	100.4	NIL
Peroxide degradation 2ml of 3% H_2O_2	78.4	100.4	21.6
Thermal degradation 105^0C 24 hrs	95.1	100.4	NIL
Photolytic degradation (Uv-364nm) 24 hrs	94.5	100.4	NIL

Table 4: Linearity of Sildenafil citrate

Concentration in %	Peak area[a]
Linearity-20%	480674
Linearity-50%	1215607
Linearity-80%	1982822
Linearity-90%	2175435
Linearity-100%	2555268
Linearity-110%	2666657
Linearity-120%	3051704
Linearity-150%	3855442

[a]average of two determination

Preparation of solutions

Test and standard solutions of Sildenafil citrate were prepared separately by dissolving in a diluent in a concentration to make 50µg/ml respectively.

RESULT AND DISCUSSION

Chromatography

All conditions were tested for method development (Table:2) only by using Inertsil ODS-3V (4.6 x 250) mm, 5µ column. Several mobile phase composition of Water: Acetonitrile were used for optimizing the chromatographic conditions. The parameter being focused was improvisation of retention time of Sildenafil citrate and column life by avoiding the complex buffer solutions. The selected method had shown no interference with the blank and the retention time of 6.910 min (Chromatogram: 1). The main advantage of this method is that it is simpler to carry out with regard to the preparation of samples and conditions used, compared to other methods and less time consuming. Method development conditions were showed in (Table:2).

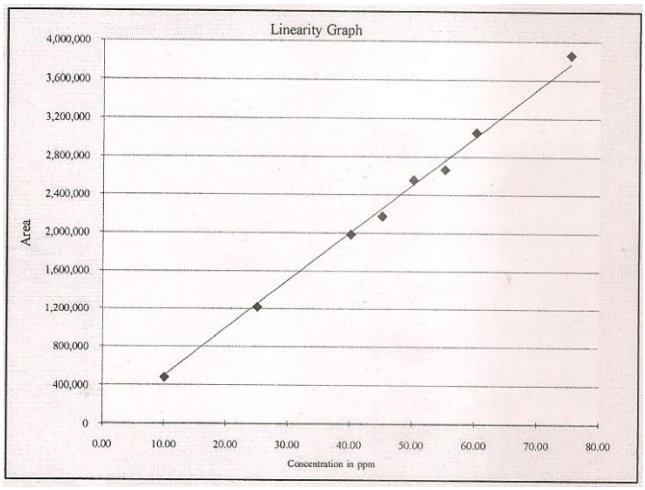

Figure 2: Linearity plot

Table 5: % recovery

% of API Added	Actual amount added in mg[b]	Peak area[c]	Amount recovered in mg[b]	% Recovery[b]
Accuracy-80%	39.93	2051707	39.99	100.16
Accuracy-100%	50.66	2595058	50.59	100.85
Accuracy-120%	60.14	3052405	59.50	99.92

[b] average of three weights

[c] average of six determination

Table 6: Precision and Ruggdness

Sample No	%Assay (Precision)	% Assay (Ruggdness)
Sample-1	100.0	99.6
Sample-2	101.6	99.4
Sample-3	101.1	99.2
Sample-4	100.7	99.2
Sample-5	100.1	99.1
Sample-6	98.6	99.0
Mean	100.4	99.25
SD	1.048	0.216
%RSD	1.045	0.002

Table 7: Robustness

Parameter	Flow rate		Mobile phase composition		Column oven temperature	
Changes in parameter	0.9 mi/min	1.1 ml/min	Low (Water: ACN) 38:62 % v/v	High (Water: ACN) 42:58 % v/v	20°C	30°C
%LC[d]	99.7	100.1	99.8	99.7	99.8	101.2
%RSD	0.290	1.026	0.116	1.047	0.657	0.457

[d] average of three determination

Validation of method

Specificity

The forced degradation of API was carried out as per ICH guidelines (ICH Q2B) in acid, base, oxidation, heat and photolysis. The acid, base and oxidation stress studies were carried out by refluxing API for 1hr at 70^0C with 1ml of HCL and NaOH (0.1N, 0.5N, 2N) and 1ml, 2ml of 3% hydrogen peroxide respectively. The thermal degradation was carried out by heating the drug powder at 105°C for about 24 hrs and the photo degradation was performed exposing the drug material to (Uv-364 nm) for 24 hrs. Except oxidation the drug was found to be stable under acid, base, heat and photolytic degradation. All the stress conditions with % degradation were showed in (Table: 3)

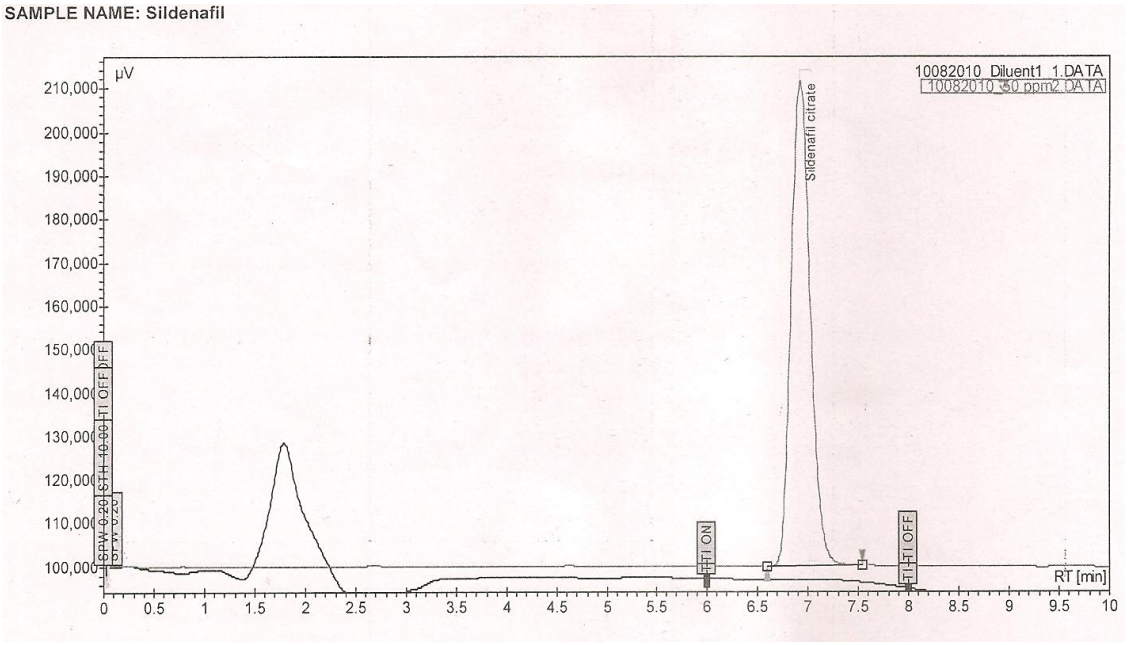

Figure 3: Chromatogram of sildenafil

Linearity, LOD & LOQ

The linearity solutions were prepared in mobile phase (diluent). Analyte solution has shown linear response for concentration levels ranging from 20% - 150% of the standard drug concentration (Table:4). The correlation co-efficient value was found to be 0.999. LOD and LOQ was calculated by STEYX method. The limit of detection (LOD) and limit of quantification (LOQ) was found to be 0.0177µg/ml and 0.0535µg/ml respectively. The linearity plot was shown in (Figure 2).

Accuracy: (% recovery)

The accuracy of the method was evaluated through standard addition method. In this known amount of standard Sildenafil citrate was added. This was done for 80%, 100% and 120% of the standard drug concentration (Table:5).

Precision

The method was found to be precise with six sample preparations for the quantification of Sildenafil citrate. The %RSD of Sildenafil citrate in six sample preparation was found to be less than 2.0% (Table:6).

Rugeddness

Rugeddness of the method was studied with system Shimadzu LC-2010 C HT auto sampler and analyst-II. The system deemed to be suitable as theoretical plates MT 2000, tailing factor and %RSD less than 2. The results of the robustness were showed in (Table:6)

Robustness

The robustness was investigated by varying the conditions with respect to change in flow rate, mobile phase composition and column oven temperature. The study was conducted at different flow rates of 0.9ml/min, and 1.1ml/min. The mobile phase composition was modified to high and low composition and column oven temperature was adjusted to 20°C and 30°C to study the effect of the mobile phase composition and column oven temperature respectively. Two injections of each of six sample preparation were injected. The method was found to be robust with respect to flow rate, mobile phase composition and column oven temperature without any changes in system suitability parameters such as tailing, theoretical plates and %RSD. (Table:7).

Solution stability

Solution stability of Sildenafil citrate in pure form was for the period of 8 hrs. The solution under study was compared with freshly prepared standard solution. It was found to be stable for period of more than 8 hours.

CONCLUSION

The method provides selective quantification of Sildenafil citrate without interference from blank affirming its stability- indicating nature. The proposed method is highly sensitive, reproducible, specific and rapid. The method was completely validated showing satisfactory data for all the method validation parameters tested. The developed method was robust in the separation and quantification of Sildenafil citrate. This method can be used for the routine analysis of production samples. The information presented herein could be very useful for quality monitoring of bulk samples and as well employed to check the quality during stability studies.

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