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Short communication

Stability indicating RP-LC determination of sildenafil citrate (Viagra) in pure form and in pharmaceutical samples

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Abstract

A simple, precise, sensitive and stability-indicating reverse-phase high performance liquid chromatographic (RP-HPLC) method has been developed for the quantitation of sildenafil citrate (SC) in pure form and its pharmaceutical formulations. Method employs water and acetonitrile (48:52 v/v) as mobile phase with flow rate of 1 ml min^{-1}, LiChrospher C18-5 μm (25 × 0.46 cm) column and UV detection set at 245 nm. The internal standard method using piroxicam (PX) as the internal standard is used. The linear dynamic range of SC was found to be 0.05–7.5 μg ml^{-1}. The proposed method is successfully employed for the determination of SC in the tablets. The excipients present in the formulations do not interfere with the assay procedure. Analytical parameters were calculated and full statistical evaluation is included.

Keywords: RP-HPLC; Sildenafil citrate; Piroxicam; Internal standard; Pharmaceutical formulations

1. Introduction

Sildenafil citrate (SC) or citrate salt of sildenafil is an anti-impotent drug, a selective inhibitor of cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type 5 (PDE5). SC is chemically designated as 1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazole [4,3-d] pyrimidin-5-yl)-4-ethoxyphenyl]sulfonyl]-4-methylpiperazine citrate. The activity of sildenafil as an efficacious, orally active agent for the treatment of male erectile dysfunction has been reported by many authors [1–3]. No official (pharmacopoeial) method has been found for the assay of SC formulations. However, Cooper et al. [4] developed an assay procedure for the simultaneous estimation of sildenafil and its metabolite in plasma using automated sequential trace enrichment of dialysates and high performance liquid chromatography. Indravadan et al. [5] described a reverse-phase high performance liquid chromatographic (HPLC) method for the determination of SC in plasma, and Segall et al. [6] reported RP-HPLC method for the determination of SC in the

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presence of its oxidative-induced degradation products. Mohammed [7] reported a stability-indicating HPLC method for the determination of SC in pharmaceutical preparations and its application to kinetic studies. Miao et al. [8] reported an RP-HPLC method for the determination of SC and its related compound in tablets. Altokka et al. [9] described a Flow Injection Analysis (FIA) method for the determination of SC using UV detection. Daraghhmeh et al. [10] reported HPLC method for the determination of SC and related substances in the commercial products and tablet dosage form. However, all the reported HPLC methods are critical of the pH of the mobile phase used.

Present communication, which describes the RP-HPLC assay procedure exclusively for SC in pure form and in formulations, is simple, precise and sensitive. Present method uses simple mobile phase without the need of buffer, involves no complex procedure to prepare sample solutions [5,8], and offers better sensitivity than some of the reported methods [5,7,8]. Also, present assay procedure employs internal standard method with piroxicam (PX) as internal standard. It is known that an internal standard, if properly chosen and used, can compensate for several types of both random and systematic errors. The internal standard should provide a signal that is similar to the analyte signal in most ways but sufficiently different so that the two signals are readily distinguishable by the instrument. It is interesting to note that PX itself, an important anti-inflammatory drug, can effectively be used as internal standard for the analysis of SC.

2. Experimental

2.1. Instrumentation and reagents

A Merck-Hitachi 7000 series low pressure gradient HPLC system equipped with an L7100 low pressure quaternary gradient pump, L7612 solvent degasser, a Rheodyne model 7125 injection valve with a fixed loop of 20 μl and Merck-Hitachi L7400 multiwavelength UV–Vis detector was used. The analyte peaks were resolved on a LiChrospher C18-5 μm (25 × 0.46 cm) column. A PC-based Winchrom chromatographic software condition was used for data integration.

SC and PX were obtained as gift samples from Sun pharmaceuticals (Mumbai, India). Acetonitrile and water used for mobile phase were HPLC grade obtained from E. Merck, India Ltd, Chennai. Triply distilled water was employed for all other purposes.

2.2. Preparation of standard solutions

Standard stock solutions of 1 μg ml^{-1} of SC and PX were prepared using a mixture of water and acetonitrile (1:1 v/v) in separate volumetric flasks. From this standard stock solution, mixed standard (working standard) solutions were prepared by suitable dilution with the mobile phase to contain 0.01–8 μg ml^{-1} of SC and 8 μg ml^{-1} of PX as internal standard in different 10 ml volumetric flasks. Before being subjected to analysis, all the working standard solutions were filtered through 0.45 μ filter and degassed.

2.3. Preparation of sample solutions

Five tablets were weighed accurately and powdered well. The fine powder equivalent to 100 mg of SC was dissolved in 100 ml volumetric flask using water and acetonitrile (1:1 v/v). From this sample stock solution mixed solutions of working standard were prepared to contain 0.01–8 μg ml^{-1} of SC and 8 μg ml^{-1} of PX as internal standard in different 10 ml volumetric flasks. Before being subjected to analysis, all the working standard solutions were filtered through 0.45 μ filter and degassed.

Composition of the formulations studied is given below:

(i) Penegra: each tablet of SC contains 25 mg of sildenafil and red oxide of iron and titanium dioxide as the coloring agent.
(ii) Edegra: each tablet of SC contains 50 mg of sildenafil and indigo carmine as the coloring agent.
(iii) Silighra: each tablet of SC contains 50 mg of sildenafil and red oxide of iron and titanium dioxide as the coloring agent.

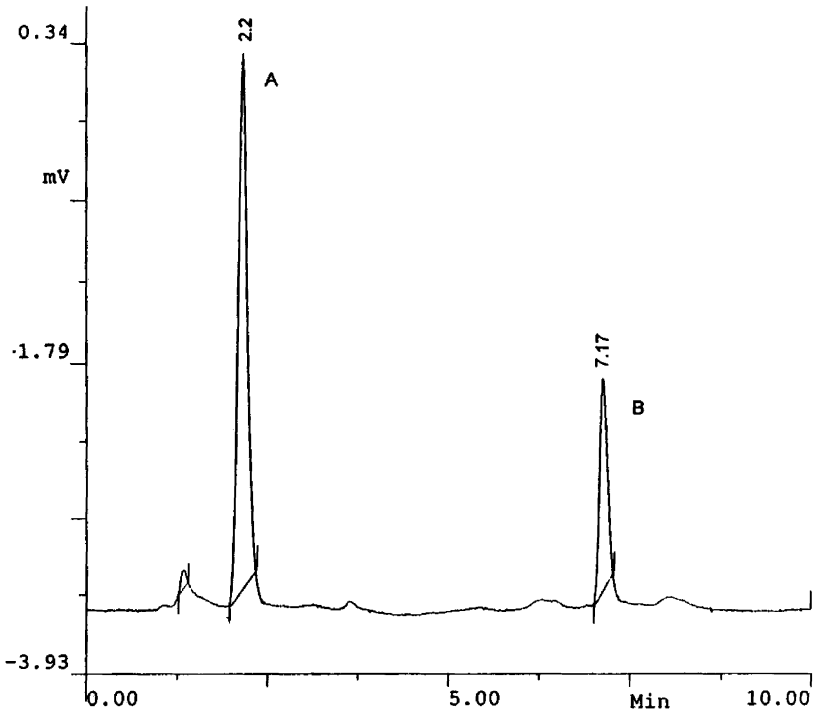

Fig. 1. Chromatogram showing the separation of internal standard PX (8 μg ml^{-1}, peak A) and SC (2 μg ml^{-1}, peak B).

2.4. Assay procedure

The chromatographic conditions followed for the assay of SC are given below:

mobile phase: water:acetonitrile (48:52 v/v)
column: LiChrospher C18-5 μm (25 × 0.46 cm)
flow rate: 1 ml min^{-1}
detection: UV set at 245 nm
injection volume: 20 μl
run time: 10 min.

With the above chromatographic conditions, 20 μl of the standard and sample solutions (12 different concentrations in the range containing 0.01–8 μg ml^{-1} of SC and 8 μg ml^{-1} of PX) were injected and the chromatograms were recorded. A typical chromatogram obtained by injecting a mixed standard solution containing 2 and 8 μg ml^{-1} of SC and PX, respectively, is shown in Fig. 1. The system suitability factors for this chromatogram are given in Table 1. The retention times for internal standard and SC were found to be 2.2 and 7.17 min, respectively. Concentration of the drug (pharmaceutical sample) was calculated by calculating the response factor of the standard solutions (peak area ratio of the standard peak and internal standard peak) and sample solutions.

Table 1
System suitability parameters for the HPLC assay of SC in the presence of internal standard PX

Parameters	PX	SC
Retention time (t_R) in min	2.2	7.17
Relative retention time (RRT) in min	–	4.21
Capacity factor (k)	0.66	4.27
Selectivity factor (α)	–	6.91
Resolution (R)	–	15.29
Number of theoretical plates (N^a)	827.58	13558.25
Height equivalent to theoretical plates (HETP) (h) in cm	0.030	0.0018

[a] Calculated as $N = 5.54\ (t_R/W_{0.5})^2$.

3. Results and discussion

3.1. Optimization of the variables

Optimum conditions, which are necessary for the quantitative analysis of the drug with maximum sensitivity, were established by a number of preliminary experiments. Optimum conditions were fixed by varying one parameter at a time by fixing other parameters constant and observing its effect on the response factor and also on the peak resolution. Effect of wavelength on the response factor and on the peak resolution was observed over the wavelength range 230–260 nm. 245 nm $\pm$ 2 unit was found to be optimal. Similarly, effect of composition of the mobile phase was studied by changing the composition of water and acetonitrile. The optimum ratio of water:acetonitrile was found to be 48:52 with $\pm$2 ml. Effect of flow rate was observed by varying the flow rate from 0.8 to 1.5 ml min^{-1}. Lower flow rates lead to increase in resolution time and high flow rates lead to considerable increase in the pressure. Therefore, 1 ml min^{-1} was found to be optimal for all measurements. Also, for internal standard, we tested different compounds and found that PX was the best. Under these optimum conditions we have observed good resolution between sample peak and internal stand peak with maximum sensitivity. Also it was observed that analyte concentration in solutions was stable over a period of 2 weeks and also found to be stable during the analysis.

3.2. Linearity and range

Linearity and range of the method were done by analyzing 12 different concentrations ($n = 5$) of the mixed standard solutions containing 0.01–8 μg ml^{-1} of SC and 8 μg ml^{-1} of PX under the chromatographic conditions mentioned above. The response factor of the standard solutions was calculated. The calibration curve was plotted using response factor versus concentration of the standard solutions. Calibration curve was found to be linear over the concentration range 0.05–7.5 μg ml^{-1}. The data were analyzed by linear regression least-squares fit method. The calibration graph shows negligible intercept and is described by the calibration equation $y = a + bx$, where y is the peak area, 'b' is the slope, 'a' is the intercept and 'x' is the concentration of the analyte. 'Relative standard deviation' (% $\pm$ RSD) values of 'b' and 'a' are found to be 0.84 and 0.68, respectively. To calculate the limit of quantitation (LOQ) and limit of detection (LOD) signal to noise ratio 10:1 and 3:1, respectively, were used. Linear regression least-squares fit data are given in Table 2.

Table 2
Linear regression least-squares fit data for the estimation of SC

Parameters	SC
Linear dynamic range (μg ml^{-1})	0.05–7.5
Regression equation (y[a])	
Slope (b)	0.1077
Intercept (a)	0.0067
Standard deviation of slope (Sb)	0.00127
Standard deviation of intercept (Sa)	0.0053
% $\pm$ RSD of 'b'	0.84
% $\pm$ RSD of 'a'	0.68
Correlation coefficient (R)	0.9995
LOQ (μg ml^{-1})	0.05
LOD (μg ml^{-1})	0.015

[a] $y = a + bx$, where x is the concentration in μg ml^{-1}.

3.3. Validation of the method

The validity of the method for the analysis of SC in its pure form and in its formulations was examined by analyzing various available formulations using the proposed procedure. To study the accuracy and to check the interference from the excipients used in the formulations, recovery studies were carried out by standard addition method. To the pre-analyzed formulation, known amounts of the analyte at three different concentration levels were added and assayed. The results obtained are given in Table 3. The recoveries were above 100% in most of the cases. The lower values of the RSD of the assay indicate that the method is precise and accurate. Also, the results depicted that the present method is useful for bulk drug analysis as well as commercial pharmaceutical formulations. Precision of the method was

demonstrated by repeatability studies. This was done by injecting consequently the standard solution 10 times and passing through the assay procedure. The lower value of RSD indicates that the method is precise.

3.4. Stability studies

Stability studies were carried out at laboratory temperature for a month to find potential stability problems of the drug in the formulations. Samples were analyzed at intervals of 0, 1, 2, 4, 8, 16 and 30 days by the recovery method mentioned above. The results obtained are given in Table 4. The percent RSD values between subsequent readings gave an indication of the stability of the drug in the formulations.

3.5. Ruggedness

Chromatographic parameters were not affected significantly with the slight changes in the chromatographic conditions like the composition of the mobile phase and flow rate (1 ml $min^{-1} \pm$ 0.1). Assay procedure described in the proposed method was repeated with different C18 columns and no significant change in chromatographic parameters was observed. Also, three different persons (analysts) carried out analyses and no considerable changes were noticed in the chromatographic parameters. All this shows that the proposed chromatographic procedure adopted in the method is rugged.

4. Conclusions

The results of the proposed HPLC method showed that the data are consistent with the label claim of the formulations. The calibration curve showed linear response over the range of concentration used in the assay procedure. The lower value of RSD for the reproducibility studies and recovery studies shows that the method is precise and accurate. Further, there is no interference of the excipients used in the formulations. The present RP-HPLC method uses simple mobile-phase water and acetonitrile without the need of

Table 3
Recovery study for spiked concentrations of drug to the preanalyzed dosage forms

Formulations	Label claim (mg per tablet)	Amount added (mg)	Recovery (% mean ± RSD)[a]	
			Proposed method	Reference method [7]
Penegra[b]	25	0	98.88 ± 0.48	98.20 ± 0.67
		5	100.73 ± 0.74	99.24 ± 0.74
		10	98.54 ± 0.20	98.12 ± 0.60
		15	101.07 ± 0.85	102.17 ± 1.14
Edegra[c]	50	0	101.12 ± 1.06	102.22 ± 1.53
		10	100.50 ± 0.29	99.18 ± 0.78
		20	100.68 ± 0.32	98.88 ± 1.02
		30	101.10 ± 0.82	101.76 ± 0.68
Silighra[d]	50	0	101.0 ± 0.72	101.56 ± 1.55
		10	99.36 ± 1.01	101.22 ± 0.28
		20	100.68 ± 0.52	101.18 ± 0.75
		30	100.75 ± 0.48	101.10 ± 0.88

[a] Average of four determinations.
[b] Marketed by Cadila Healthcare Ltd.
[c] Marketed by Sun pharmaceuticals industries Ltd.
[d] Marketed by Cipla Ltd.

Table 4
Stability study for the drug in different formulations

Formulations	Amount claimed (mg per tablet)	Time (days)	Amount found[a] (mg)	% Recovery	%±RSD
Penegra	25	0	24.72	98.88	0.48
		1	24.84	99.36	0.36
		2	24.90	99.60	0.30
		4	24.81	99.24	0.73
		8	24.78	99.12	0.63
		16	24.80	99.20	0.44
		30	24.76	99.04	0.72
Edegra	50	0	50.56	101.12	1.06
		1	50.21	100.42	0.86
		2	50.28	100.56	0.88
		4	50.12	100.24	0.64
		8	50.22	100.44	0.46
		16	50.28	100.56	0.38
		30	50.18	100.36	0.92
Silighra	50	0	50.50	101.0	0.76
		1	50.32	100.64	1.01
		2	50.28	100.56	0.82
		4	50.34	100.68	0.26
		8	50.30	100.60	0.74
		16	50.36	100.72	0.62
		30	50.28	100.56	0.85

[a] Average of four determinations.

buffer, and all other reported HPLC procedures are critical of the pH of the mobile phase used, involves no complex procedure to prepare sample solutions and offers better sensitivity than some of the reported methods. Thus, the developed HPLC method is simple, accurate, precise and rugged. Hence, this method can be adopted for the quality control of SC in bulk as well as in formulations.

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