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NEW SIMPLE RP-HPLC METHOD FOR THE ESTIMATION OF SILDENAFIL CITRATE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT: A new simple, rapid, accurate, precise and sensitive RP-HPLC method was developed for the estimation of sildenafil citrate in bulk and pharmaceutical dosage form. The separation of sildenafil citrate was achieved on a Phenomenex C_{18} Column (150 X 4.6 mm i.d, 5µ particle size) with a mobile phase consisting of methanol and Buffer (50:50 % v/v). The flow rate was 1.5 ml/min and photodiode array detection at 290nm. The retention time of Sildenafil citrate was found to be 4.842 min. Calibration curve was found to be linear in the concentration range of 70 - 210 µg/ml and the correlation coefficient value was found to be (R^2) 0.9998. LOD and LOQ were 2.361ng/ml and 7.156ng/ml respectively. The RSD value of Precision and recovery studies were less than 2 %, indicated that the method was precise and accurate.

INTRODUCTION: Sildenafil citrate (SLDC), an oral therapy for erectile dysfunction, is the citrate salt of sildenafil, a selective inhibitor of cyclic guanosine monophosphate (cGMP) – specific phosphodiestrase type 5 (PDES). SLDC is designated chemically 1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1*H* pyrazolo[4,3-*d*] pyrimidin-5-yl)-4-ethoxyphenyl]sulfonyl]-4-methylpiperazine citrate **(Fig. 1)**.

SLDC is a white to off-white crystalline powder with a solubility of 3.5 mg/mL in water and a molecular weight of 666.7. SLDC is formulated as blue, flim-coated rounded, diamond shaped tablets equivalent to 25mg, 50mg and 100mg for oral administration [1].

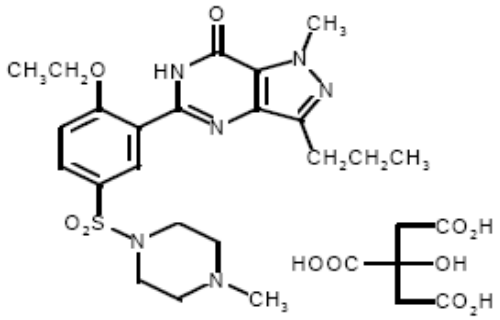

FIG. 1: SILDENAFIL CITRATE

Based on the literature survey, very few analytical methods like Voltammetry [2], HPLC [3, 4, 5, 6, 7, 8], LC-MS [9] and UV [10, 11] methods have been reported for the estimation of SLDC. But these methods are time consuming and expensive solvents are used for the estimation of SLDC. The present work describes a simple, rapid, precise, accurate and economical RP-HPLC method for the simultaneous estimation of SLDC in bulk and pharmaceutical dosage form.

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EXPERIMENTAL:

Drugs and chemicals: Pharmaceutical grade of SLDC was kindly supplied by Orchid Chemicals & Pharmaceuticals (P) LTD., (Chennai, India), HPLC grade methanol and water was used throughout the experiment. The commercial tablets (Viagra) used containing 50 mg of SLDC per tablet was manufactured by Orchid Chemicals & Pharmaceuticals (P) LTD., (Chennai, India).

Instrument: A Schimadzu HPLC system consist of LC-10AT-vp Solvent delivery system (pump), SPDM – 10AVP photodiode array detector, Rheodyne injector with 20µL loop volume, LC-Solution assisted for data collections and processing.

Preparation of ammonium dihdrogen phosphate buffer: 2.88gm of ammonium dihydrogen phosphate was accurately weighed and dissolved in 100ml water. Then the volume was adjusted up to 1000ml with water. pH of the resulting solution was adjusted to 3.5 with orthophosphoric acid.

Preparation of mobile phase: 50 % of the above buffer solution and 50 % of methanol were mixed thoroughly, degased for 20 min by ultrasonication. Then the mobile phase was filterred with 0.45µ membrane filter.

Preparation of Standard solution: 70mg of SLDC was accurately weighed and transferred in to a 100 volumetric flask separately and sufficient amount of methanol was added and sonicated for 10 minutes. Then the volume was diluted up to the mark with mobile phase. From this, 5ml of above stock solution was transferred to a 25 ml volumetric flask, diluted up to the mark with mobile phase.

Preparation of Sample solution: 20 tablets were accurately weighed and powdered. Powder weight equivalent to 70 mg of SLDC was transferred to a 100ml volumetric flask, sufficient amount of methanol was added to dissolve by using sonicator.

Then, the volume was adjusted up to the mark with mobile phase and filtered with Whatmann filter paper. 5 ml of above filtered solution was diluted to 25ml with mobile phase. Resulting solution was filtered with 0.45µ membrane filter.

Optimized chromatographic conditions: The mobile phase consisted of methanol and Buffer (50:50 % v/v) and the flow rate was 1.5ml/min. Separation was achieved using a Phenomenex luna C_{18} (150mm X 4.6 mm i.d.) column with an average particle size of 5µ and the column was kept at ambient temperature. The column effluent was monitored at 290nm by PDA detection. SLDC was eluted at 4.842min. **(Fig. 2)**

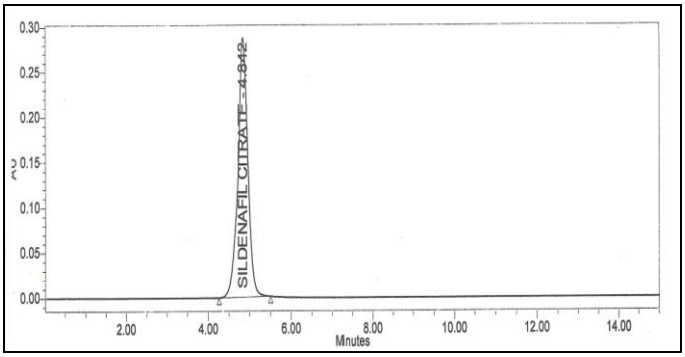

FIG. 2: CHROMATOGRAM OF SLDC

The developed method was validated, as per ICH guidelines [12].

RESULTS AND DISCUSSION:

Specificity: Specificity study was done by injecting the placebo and working standard solutions in to the HPLC, and the chromatogram of working standard solution was compared against placebo at 290nm. From the specificity study, no interference was observed from the excipients and solvents revealed that, the method is specific.

System suitability: System suitability was carried out by six replicate injections of working standard solution of SLDC, and % RSD of system suitability parameters like retention time, theoretical plates and asymmetric factors were calculated. The results were presented in **Table 1**.

TABLE 1: SYSTEM SUITABILITY VALUE FOR SLDC

Parameters		% RSD
Retention time	4.842	0.123
Theoretical plates	2366	0.326
Asymmetric factor	1.124	0.045

In system suitability studies the % RSD was found to be 0.123, 0.326, 0.045 for retention time, theoretical plates and asymmetric factor respectively, indicating that the proposed method is completely suitable with system.

Linearity: Appropriate volume from the stock solution was taken and diluted in the concentration range of 70,105, 140, 175 and 210 µg/ml for SLDC and the absorbance of each solution was measured at 290 nm. Calibration graph was plotted **(Fig. 3)** by concentration versus area.

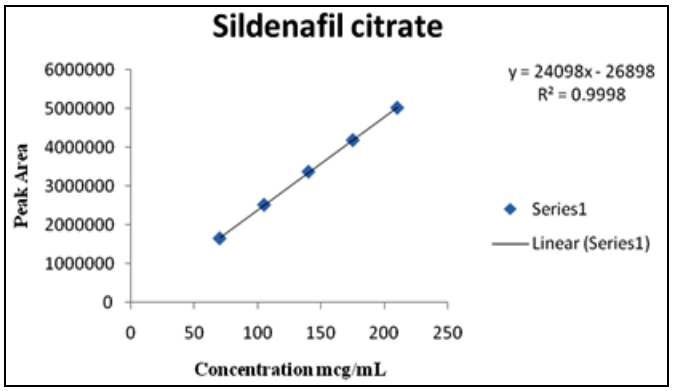

FIG. 3: CALIBRATION GRAPH FOR SLDC

SLDC was linear and obey's Beart's - Lambert's law in the concentration range of 70-210 µg/mL. The correlation coefficient (R^2) value was found to be 0.9998 for SLDC. Results exposed that good correlation existing between the concentration of the sample and their absorbances.

Accuracy: The accuracy of the method was studied by recovery experiments. The known amount of SLDC was added at 50 %, 75%, 100% 125% and 150% from the label claim to Placebo. The analysis was done by same method as per the assay procedure.

Accuracy was determined by calculating the amount of drug recovered from each concentration. The results are presented in **Table 2**.

TABLE 2: RECOVERY RESULTS FOR SLDC

Concentration (%)	Added amount (mg)	Amount recovered (mg)	Amount recovered (%)
50	35.01	33.97	97.02
75	52.53	52.46	99.86
100	70.04	70.01	99.95
125	87.48	87.24	99.72
150	104.73	104.12	99.41

Results publicized that the proposed method was accurate because the amount of SLDC present in the sample solution at various levels is close to 100% of label claim.

Precision: Six individual sample preparations were prepared and the absorbances were measured at 0 hrs, 8 hrs, 16 hrs, Day-1, Day-2, Day-3, by Analyst-1and Analyt-2 in different instruments. The amount of SLDC present in the sample solutions was calculated and the values are presented in the **Table 3**.

TABLE 3: PRECISION RESULTS FOR SLDC

Parameters	Sampling time	SLDC		
		Amount present (mg)	Amount present (%)	RSD (%)
Intraday precision	0 hrs	49.65	99.30	0.028
	8th hrs	49.23	98.46	0.014
	16th hrs	49.14	98.28	0.066
Interday precision	I st Day	49.43	98.86	0.174
	2nd day	49.30	98.60	0.632
	3rd day	48.94	97.89	0.077
	Analyst -1	49.42	98.85	0.304
	Analyst -2	49.43	98.86	0.291
	Instrument -1	49.30	98.61	0.158
	Instrument -2	49.36	98.73	0.303

The low % RSD values indicated that the proposed method is precise.

Robustness: Robustness of the method was determined by changing the method procedure like wavelength, flow rate and mobile phase ratio. The results were presented in **table 4**.

TABLE 4: RESULTS OBSERVED BY CHANGING THE WAVELENGTH ± 1nm

Parameters		SLDC		
		Amount present (mg)	Amount present (%)	RSD %
Wavelength (nm)	288	48.69	97.38	0.417
	292	49.01	98.02	0.014
Flow rate	1.3	49.34	98.68	0.052
	1.7	49.32	98.64	0.296
Mobile phase	+ 2%	49.78	99.57	0.165
	-2%	49.58	99.16	0.435

The % RSD calculated from the robustness study was found to be less than 2 %.for SLDC, indicated that the method was robust.

LOD and LOQ: LOD and LOQ were determined by response versus slope method. The responses were taken from the area of 50% concentration of standard solution and the results were presented in the **Table 5**.

TABLE 5: LOD AND LOQ

Response of SLDC	
1644224	
1644212	
1644246	
SD	17.24
SLOPE	24098
LOD (ng/ml)	2.361ng/mL
LOQ (ng/ml)	7.156 ng/mL

From the results obtained the LOD and LOQ of SLDC were found to be 2.361ng/mL and 7.156 ng/mL respectively.

Stability: Sample solution was stored at room temperature for three days. Stability of the sample solution was determined by injecting the sample solution every day and the amount of SLDC present in the sample was calculated. The results are presented in the **table 6**.

TABLE 6: STABILITY DATA FOR SLDC

Day	SLDC	
	Amount present (mg)	Amount present (%)
1	49.62	99.24
2	49.56	99.12
3	48.84	97.68

Results shown that, the sample solution is stable for 3 days and do not shows any degradation of SLDC at room temperature.

Developed method was applied to the marketed dosage forms: Assay was performed according to above described procedure (section 2.6) for marketed dosage form (Viagra) and the amount of SLDC in each tablet was calculated. The results were incorporated in to **Table 7**.

TABLE 7: ASSAY RESULTS OF TABLETS DOSAGE FORMS

SLDC	
Amount present (mg)	Amount present (%)
49.62	99.24
49.44	98.88
49.56	99.12
48.90	97.80
49.35	98.70

CONCLUSION: Finally the developed method was applied successfully for marketed dosage form and the assay results were indicating that, the proposed method is successfully estimated the amount of the SLDC present in each tablet and was found to be 49.65mg indicating that this developed RP-HPLC method can be effectively used for the routine analysis of SLDC in bulk and pharmaceutical dosage forms without any interferences.

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