Research Article

Validated RP-HPLC Method for Simultaneous Estimation of Sildenafil Citrate and Dapoxetine HCl in Tablet Dosage Form

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ABSTRACT
The simple, reliable and reproducible HPLC methods were developed for the analysis of Sildednafil citrate and Dapoxetine HCl (API). The column used was Phenomex Luna 5µ C_{18} (12) 100A,(250 × 4.6m.m × i.d, 5µ).The mobile phase used was Ammonium acetate buffer (10mM,pH 4.3) Acetonitrile isocratic run at the flow rate of 1.7mL/min with UV (PDA) detector at 239nm at room temperature. Extraction of Sildenafil citrate and Dapoxetine HCl from tablet was carried out using methanol. Linearity was observed in the range from 2 to 20µg/ml for Sildenafil citrate with a correlation coefficient (R2) 0.99. The values of linearity range and correlation coefficient (R2) 1.2 to 12µg/ml and 0.99 respectively for Dapoxetine HCl . Parameters of validation prove the precision, accuracy, robustness, and specificity of the method and it's applicability for the Assay of Sildenafil citrate and Dapoxetine HCl. The method is suitable for routine analysis of the drug.

Keywords: Sildenafil citrate, Dapoxetine HCl, RP-HPLC analytical method.

INTRODUCTION

Sildenafil citrate (SIL) was patented in1996 and launched in May 1998 as first oral drug approved by Food and Drug Administration (FDA) to treat erectile dysfunction (ED) in the United States. It is also effective for treatment of pulmonary arterial hypertension (PAH).

Sildenafil citrate 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 Dalton. Molecular formula is $C_{22}H_{30}N_6O_4S$. .Chemically, designated as 1-[[3-(6, 7-dihydro-1-methyl-7-oxo-3-propyl-1*H* pyrazolo [4, 3-*d*] pyrimidin-5-yl)-4ethoxyphenyl] sulfonyl]-4-methylpiperazine citrate. Its structural formula is given below in the Fig.1.

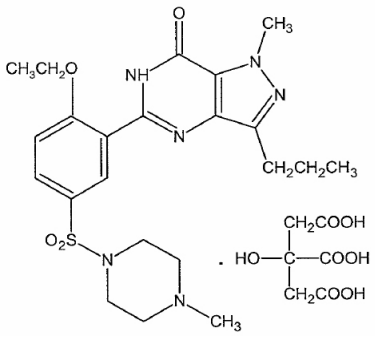


Fig.1: Sildenafil Citrate

The parasympathetic nerves are stimulated when man arouses sexually, leading to penile erection as result of release nitric oxide (NO) which works by activation of the enzyme guanylate cyclase responsible for converting guanosine triphosphate (GTP) to 3'5' cyclic guanosine monophosphate (cGMP)[1].

The cGMP is a potent vasodilator vital erection of the penis. Sildenafil citrate selectively inhibits the enzyme PDE-5A (phosphodiesterase-5A) that hydrolyzes cGMP. Thus it increases level of cGMP by preventing it from breaking down. Consequently smooth muscle relaxation leads to vasodilation and increased inflow of blood into the spongy tissue of the penis causing an erection by fascinating the signaling actions of nitric oxide (NO) in penile smooth muscle. The most common side effects of Sildenafil citrate are headache, facial flushing, and upset stomach. Less commonly cyanopsia (bluish vision), blurred vision, or sensitivity to light may briefly occur[2].

Dapoxetine HCl(DAP) is designated chemically as (S)-N, N-dimethyl-3-(naphthalen-1-yloxy)-1 phenylpropan-1-amine with an empirical formula of $C_{21}H_{23}NO$ and molecular weight of 305.413g. This drug is mainly useful in erectile dysfunction as selective serotonin reuptake inhibitor (SSRI)[3]. SSRI's are a class of compounds typically used as antidepressants in the treatment of depression, anxiety disorders, and some personality disorders. They can also sometimes be effective and used in treating premature ejaculation problems, impotence and some cases of insomnia. The drug's mechanism of

action is thought to be related to inhibition of neuronal reuptake of serotonin and subsequent potentiation of serotonin activity and increase the ejaculation time[4]. Its structural formula is given below in the Fig.2.

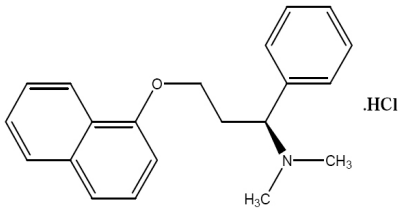


Fig.2 : Dapoxetine HCl

EXPERIMENTAL

Chemicals, Reagents and Solutions

- Sildenafil Citrate(99.5%) - Emcure pharmaceuticals, Pune.
- Dapoxetine HCl(99.5%) - Emcure pharmaceuticals, Pune.
- Ortho Phosphoric Acid (AR Grade) - Prowess Chemicals, Palakkad
- Acetonitrile **(** HPLC Grade) - S D Fine chemicals, Mumbai
- Methanol **(**HPLC Grade) - Prowess Chemicals, Palakkad
- Water **(**HPLC Grade) - Fischer Scientific
- Ammonium acetate(AR Grade) - Prowess Chemicals, Palakkad
- Laboratory glass wares - Borosilicate

Preparation of standard solutions

Primary solutions (1000µg/ml) of sildenafil citrate and dapoxetine HCl (600µg/ml) were prepared by accurately weighing 10mg sildenafil citrate and 6mg dapoxetine HCl and diluted to 10ml respectively by methanol. Stock solutions of sildenafil(200µg/ml) and dapoxetine (120µg/ml) were prepared by diluting 2ml primary solutions to 10ml respectively by methanol. A series of standard drug solutions in concentration range of 2-20µg/ml of sildenafil and 1.2-12µg/ml of dapoxetine were prepared by diluting appropriate volumes of standard stock solutions.

Optimization of Mobile Phase

The mobile phase containing mixture of ammonium acetate buffer (pH 4.3 adjusted with ortho phosphoric acid) and acetonitrile in the ratio 5:95 v/v was found to be most satisfactory as it gave good resolution and sharp peak.

Chromatographic Conditions

Chromatographic method : RP-HPLC
Selection of wavelength : 239nm
Stationary phase : Luna 5u, C18 column (250mm×4.6mm, 5µm)
Selection of mobile phase : Ammonium Acetate buffer(pH-4.3) : Acetonitrile
Solvent ratio : 05: 95
Flow rate : 1.7ml/minute
Temperature : room temperature

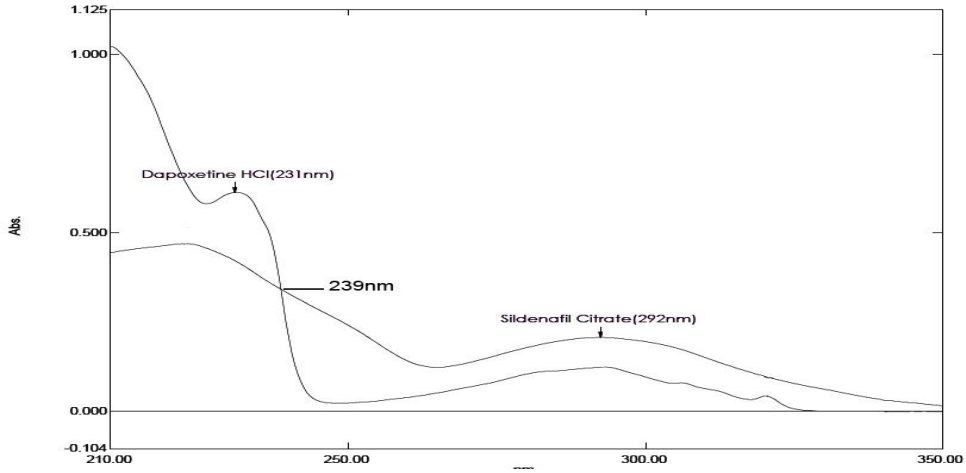
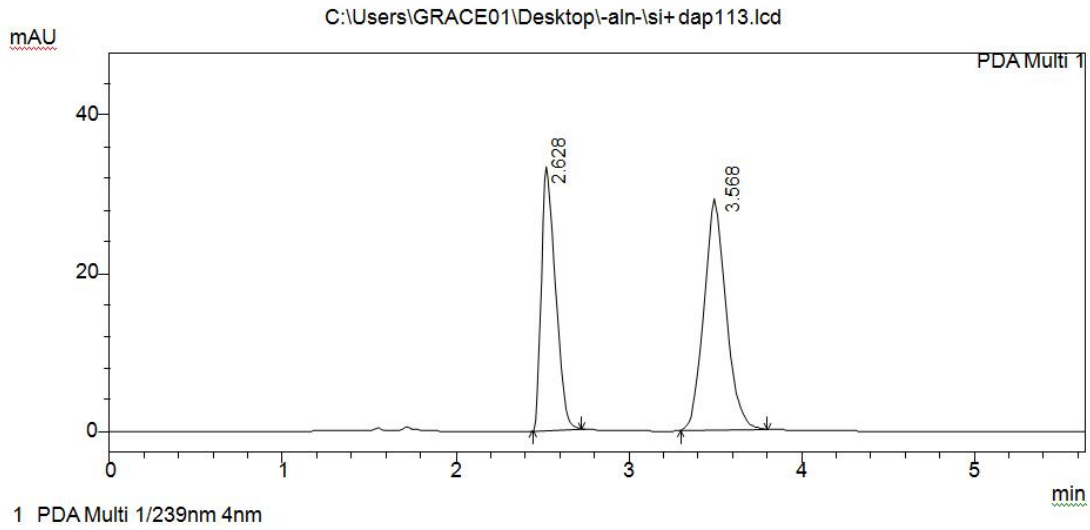


Fig. 3: Overlain spectra of Sildenafil Citrate and Dapoxetine HCl



PeakTable

PDA Ch1 239nm 4nm

Peak#	Ret. Time	Area	Theoretical Plate#	Tailing Factor (10%)	Resolution
1	2.628	146205	2992.381	1.375	0.000
2	3.568	157814	2735.261	1.078	4.352
Total		304019			

Fig. 3: HPLC Chromatogram of Sildenafil Citrate and Dapoxetine HCl

System Suitability Test (SST)

SST is commonly used to verify resolution, column efficiency, peak purity index and repeatability of chromatographic system to ensure its adequacy for a particular analysis. For SST chromatographic conditions were set as per the optimized parameters and mobile phase was allowed to equilibrate with stationary phase as was indicated by the study baseline. Five replicate injections of standard solution were made separately and the chromatograms were recorded (Table: 1).

Table 1: System Suitability Test

Drugs	Rs	N	Pi	T
Sildenafil Citrate		2992.381	1	1.375
Dapoxetine hydrochloride	4.352	2735.261	1	1.078

*mean of 5 observations

Estimation of Sildenafil Citrate and Dapoxetine HCl in Tablets by Proposed RP-HPLC Method

Twenty tablets were accurately weighed; average weight was determined and finely powdered. An accurately weighed quantity of tablet powder equivalent to 50mg of SIL and 30mg of DAP was transferred to 50ml volumetric flask and dissolved by sonication with sufficient quantity of methanol and then volume was made to the mark with methanol. The solution was then of filtered through Whatmann filter paper no. 41. The filtrate 2ml was taken in 10ml volumetric flask and volume made to the mark with methanol (Test stock solution). A series of different concentration range were prepared by diluting appropriate volumes of test stock solution in methanol.

Recording of chromatogram

A steady baseline was recorded with the fixed chromatographic conditions. Standard drugs solutions (2 to 20µg/ml of Sildenafil Citrate and 1.2 to 12µg/ml of Dapoxetine hydrochloride) were injected and chromatograms were recorded. Retention time of Sildenafil Citrate and Dapoxetine hydrochloride were found to be 2.6 and 3.5 minutes, respectively. This was followed by injection of sample solution obtained from tablet mixture (Table: 2).

Drugs estimated in sample weight was calculated using formula

$$\% \text{ Estimation} = \frac{\text{As x Cstd x DF x Avg. wt. x 100}}{\text{AS x Wt. taken x Lc}}$$

As = Peak area of sample
AS = Peak area of standard
Cstd = Concentration of standard (µg/ml)
Avg .Wt. = Average weight of tablet (mg)

Table 2: Analysis of tablet mixture

Drug	Amont of tablet mixture (mg)		%Estimated	Assay(%) ± %RSD*
	Amount taken	Estimated		
Sildenafil Citrate	50	49.67	99.34	0.0378
Dapoxetine HCl	30	29.76	99.23	0.000035

*mean of six observations

VALIDATION OF RP-HPLC METHOD[4]

Linearity and range

Calibration graph was plotted using standard drug peak areas Vs. concentration of standard solutions (Table). Peak areas of these solutions were measured at 239 nm. The measured peak areas were plotted against concentrations. Linear regression data revealed an excellent linear relationship for both Sildenafil Citrate and Dapoxetine hydrochloride in the concentration range of 2-20µg/ml and 6-12µg/ml respectively(Table: 3-4), (Fig: 3-4).

Table 3: Calibration data of Sildenafil Citrate

S. No	Sildenafil Citrate	
	Concentration (µg/ml)	Peak Area
1	2	31097
2	4	60284
3	6	90260
4	8	118312
5	10	146128
6	12	178710
7	14	216675
8	16	246337
9	18	280397
10	20	313324

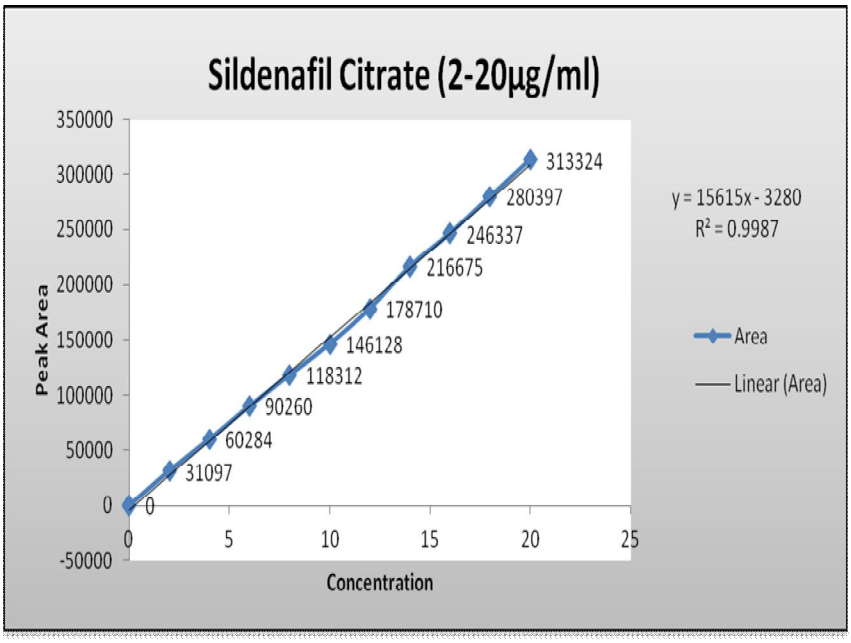


Fig 3: Calibration graph of Sildenafil Citrate (1.2-12µg/ml)

Table 4: Calibration data of Dapoxetine hydrochloride

Sl No	Dapoxetine hydrochloride	
	Concentration (µg/ml)	Peak Area
1	1.2	32323
2	2.4	65736
3	3.6	98200
4	4.8	123394
5	6	157749
6	7.2	198498
7	8.4	225484
8	9.6	256432
9	10.8	28210
10	12	325085

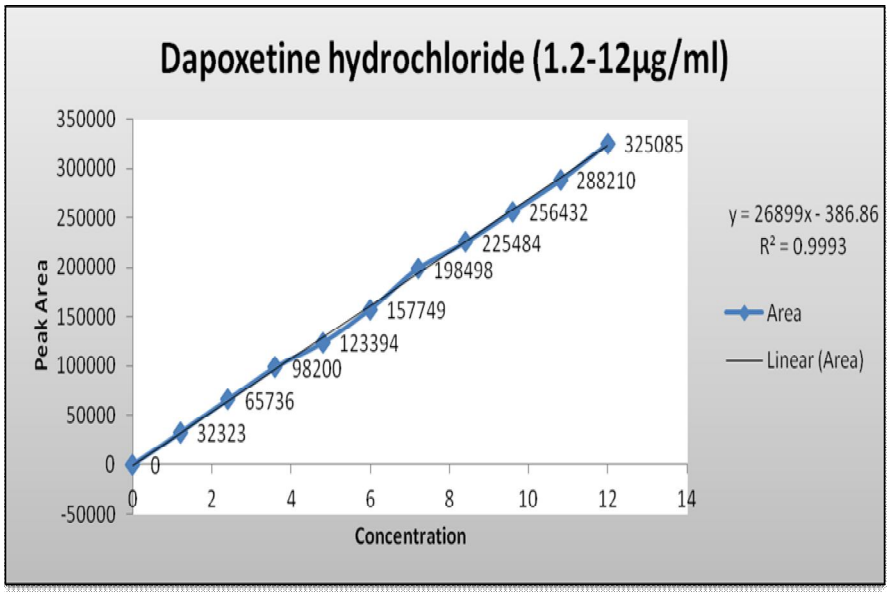


Fig 4: Calibration graph of Dapoxetine hydrochloride (1.2-12µg/ml)

Precision

Precision of method was determined by

a) Intraday precision

b) Inter day precision

c) Repeatability of injection

a) Intraday precision

Intraday precision was done by carrying out analysis of standard drugs solutions at a concentration in the linearity range (10µg/ml+6 µg/ml) for six times on the same day and %RSD was calculated (Table 5).

b) Inter day precision

Inter day precision was done by carrying out the analysis of standard drugs solutions at a concentration in the linearity range (10µg/ml+6 µg/ml) for three days and %RSD was calculated (Table 6).

c) Repeatability of injection

A standard solution of drugs (10µg/ml+6 µg/ml) was injected 6 times and its %RSD was calculated (Table 7).

Table 5: Intraday presicion

S. No	Peak area		%RSD*	
	Sildenafil Citrate (10µg/ml)	Dapoxetine HCl (6µg/ml)	Sildenafil Citrate (10µg/ml)	Dapoxetine HCl (6µg/ml)
1	146205	157814	0.00027	0.00009
2	146204	157935		
3	146173	157806		
4	146312	157874		
5	146301	157898		
6	146209	157891		

*mean of six observations

Table 6: Inter day presicion

Day	Concentration (µg/ml)		Peak area		%RSD*	
	Sildenafil Citrate	Dapoxetine HCL	Sildenafil Citrate	Dapoxetine HCl	Sildenafil Citrate	Dapoxetine HCl
1	10	6	146101	157698	0.0000065	0.0
2			146124	157580		
3			146104	157624		

*mean of three observations

Table 7: Repeatability of injection

Injection	Peak Area		%RSD*	
	Sildenafil Citrate (10µg/ml)	Dapoxetine hydrochloride (6µg/ml)	Sildenafil Citrate	Dapoxetine HCl
1	146099	157720	0.00000056	0.00026
2	146101	157698		
3	146120	157671		
4	146201	157693		
5	146195	157702		
6	146207	157731		

*mean of three observations

Accuracy

Recovery studies were done for determining accuracy parameter. It was done by mixing known quantity of standard drug with the analysed sample mixture and the contents were reanalyzed by the proposed method. Recovery studies carried out at 50 and 100% levels. The percentage recovery and its %RSD were calculated (Table 8).

Table 8: Recovery studies

Level	% Recovery		%RSD*	
	Sildenafil Citrate	Dapoxetine hydrochloride	Sildenafil Citrate	Dapoxetine HCl
50%	99.4	99.47	0.0266	0.000000056
100%	99.5	99.4	0.000056	0.0026

*mean of three observations

Robustness

In order to demonstrate the robustness of the method, the following optimized conditions were slightly varied.

1) ± 1 in ratio of methanol in mobile phase
2) ± 0.1 units in pH of buffer
3) ± 0.1 in flow rate

The response factors for these changed chromatographic parameters were almost same as that of the fixed chromatographic parameters and hence developed method is said to be robust (Table 9).

Table 9: Robustness

Chromatographic condition		Peak area	
		Sildenafil Citrate	Dapoxetine HCl
Mobile phase ratio	4:96	142124	154568
	6:94	142202	153580
pH of Water	4.2	146215	167264
	4.4	146368	166487
Flow rate	1.6	147895	166987
	1.8	147465	166745

*mean of six observations

Specificity

An investigation of specificity was conducted during the validation of identification tests, the determination of impurities and the assay. The procedures used to demonstrate specificity were depending on the intended objective of an analytical procedure.

In order to demonstrate the specifity of the method, a pinch of Lactose and Starch were added to the standard drugs mixture. The response factors for these changed chromatographic parameters were almost same as that of the fixed chromatographic parameters and hence developed method is said to be robust.

RESULTS AND DISCUSSIONS

Experimental results of the amount of Sildenafil citrate and Dapoxetine Hydrochloride in tablets, expressed as a percentage of label claims were in good agreement with the label claims thereby suggesting that there is no interference from any

of the excipients which are normally present. The drug content was found to be 99.34% for Sildenafil citrate and 99.23% for Dapoxetine Hydrochloride (Table: 2).

The Beer- Lambert's concentration range is lies between 2-20µg/mL for Sildenafil citrate and 1.2-12µg/mL for Dapoxetine HCl at 239nm with coefficient of correlation 0.9987 and 0.9993 respectively. The linearity graphs were shown in (Fig: 3-4). Drugs show good regression values at their respective wavelengths and the recovery study reveals that any small change in the drug concentration in the solution could be accurately determined by the proposed methods.

Precision was determined by studying the intermediate precision and the result indicates the precision under the same operating conditions over a short interval time and inter-assay precision. The standard deviation and % relative standard deviation are calculated for both drugs. Intermediate precision study expresses within laboratory variation in different days. In intra, inter-day precision and repeatability of injection study for both the drugs %RSD are not more than 1.0 indicating good intermediate precision (Table 5-7).

The validity and reliability of proposed methods are assessed by recovery studies. Sample recoveries for both the methods are in good agreement with their respective label claims, which suggests non-interference of formulation additives in estimation (Table 8).

An investigation of Robustness was conducted during the validation by suitably controlled analytical conditions. The response factors for these changed chromatographic parameters were almost same as that of the fixed chromatographic parameters and hence developed method is said to be robust (Table: 9).

An investigation of Specificity was conducted during the validation of identification tests. The response factors for these changed chromatographic parameters were almost same as that of the fixed chromatographic parameters indicating that both drugs having good specificity.

Table 10: Results of Validation parameters

Parameters	Values	
	Sildenafil Citrate	Dapoxetine HCl
Working λmax	239nm	239nm
Linearity (µg/mL)	2-20	1.2-12
Intercept	15615	26899
Slope	3280	386.86
Correlation coefficient	0.9987	0.9993
Intra-day (Precision) (%RSD)	0.00027	0.00009
Inter-day (Precision) (%RSD)	0.0000065	0.0
Repeatability of injection (%RSD)	0.00000056	0.00026
Resolution	0.0	4.352
Theoretical Plates	2992.381	2735.261
Tailing Factor	1.375	1.078
Peak Purity Index	1	1

CONCLUSION

Sildenafil Citrate and Dapoxetine HCl are available in combined pharmaceutical dosage form for the treatment of premature ejaculation. Here, one simple RP-HPLC method were developed and validated for their simultaneous analysis. The standard deviation and RSD for the methods are low, indicating high degree of precision of the methods. The RSD was found to be less than 2%. The % recovery was between 98-102% indicating high degree of accuracy of the proposed methods. The developed methods are simple, rapid, precise, and accurate and can be employed for the routine estimation of Sildenafil Citrate and Dapoxetine HCl.

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