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## A novel stability indicating RP-HPLC method for the simultaneous estimation of Sildenafil Citrate and Dapoxetine Hydrochloride in bulk and Pharmaceutical formulations

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### ABSTRACT

A novel reverse phase high performance liquid chromatographic (RP-HPLC) method has been developed and validated for simultaneous estimation of Sildenafil Citrate Dapoxetine Hydrochloride in pure and marketed formulations. Separation was carried out using column Hypersil BDS C<sub>18</sub> (100mm x 4.6mm x 5μ particle size) in isocratic mode using mobile phase composition of 0.1% orthophosphoric acid: Acetonitrile in the ratio of 50:50 and P<sup>H</sup> adjusted to 5.0±0.1 with sodium hydroxide of the flow rate was 1.0 ml/min and the effluent was monitored at 287nm. The retention time of Sildenafil Citrate was found to be 3.14 min and for Dapoxetine Hydrochloride 4 min. The method was linear from the concentration of 12.5 -62.5 μg/ml and 7.5-37.5 μg/ml for the estimation of Sildenafil Citrate and Dapoxetine Hydrochloride. The LOD and LOQ were found to be 0.824μg/ml and 2.499μg/ml for Sildenafil Citrate and of Dapoxetine Hydrochloride were found to be 0.90 μg/ml and 2.739 μg/ml. They undergo degradation in acids, alkalies, thermal and peroxides. The method was validated according to the guidelines of international conference on harmonization (ICH) and was successfully applied in the estimation and commercial formulations.

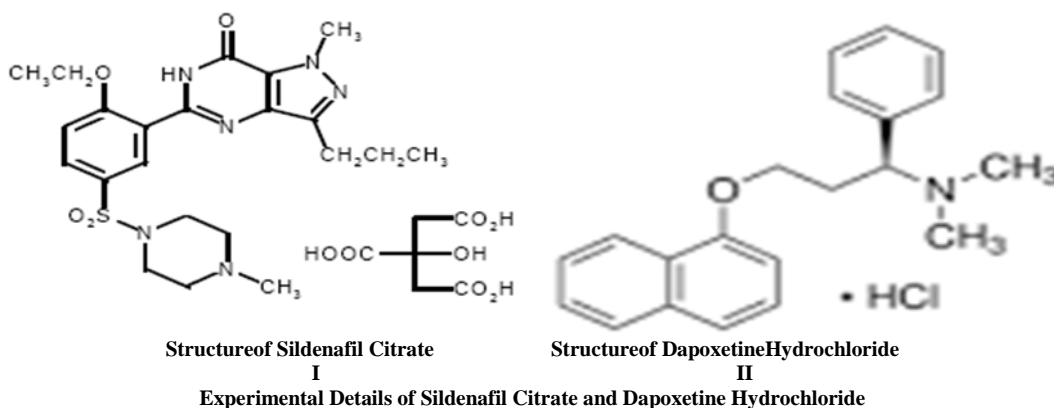
**Keywords:** Sildenafil Citrate, Dapoxetine Hydrochloride, RP-HPLC, method of validation and degradation studies.

### INTRODUCTION

Sildenafil Citrate (fig-1) is 2 - Hydroxy - 1,2,3 - propanetricarboxylate - 1-[[3-(6, 7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4, 3d] pyrimidin-5-yl)-4-ethoxyphenyl] sulfonyl] 4-methyl piperazine with molecular formula C<sub>22</sub>H<sub>30</sub>N<sub>6</sub>O<sub>4</sub>S•C<sub>6</sub>H<sub>8</sub>O<sub>7</sub> and molecular weight of 666.7g.mol<sup>-1</sup>. Sildenafil, sold as Viagra and other trade names, is a medication used to treat erectile dysfunction and pulmonary arterial hypertension (PAH). It acts by inhibiting cGMP-specific phosphodiesterase type 5 (PDE5), an enzyme that promotes degradation of cGMP, which regulates blood flow in the penis. The typical dosage of Sildenafil Citrate is 100 mg in men with erectile dysfunction [1,2,3].

Dapoxetine Hydrochloride (fig-II) is (S-(+)-N,N-Dimethyl-a-[2-(naphthalenyloxy) ethyl] benzene methanamine hydrochloride with molecular formula C<sub>21</sub>H<sub>23</sub>NO.HCl and molecular weight of 341.87g.mol<sup>-1</sup>. Dapoxetine, marketed as Priligy and Westoxetin 30 mg. It is the first compound developed specially for the treatment of premature ejaculation (PE) in men, works by inhibiting the serotonin transporter, increasing serotonin's action at the post synaptic cleft, and as a consequence promoting ejaculatory delay. As a member of selective serotonin reuptake inhibitor (SSRI) family, Dapoxetine was initially created as an antidepressant. However, unlike other SSRIs, Dapoxetine is absorbed and eliminated rapidly in the body. Its fast acting property makes it suitable for the treatment of PE but not as an antidepressant [4, 5,6].

Literature survey revealed that very few methods were reported for the simultaneous estimation of Sildenafil Citrate and Dapoxetine Hydrochloride by RP-HPLC method [7-12]. So an attempt has been made to develop an accurate precise and economically viable RP-HPLC method for the simultaneous estimation of combination of interest in the current research.



### Chemicals and reagents

HPLC grade acetonitrile and water purchased from E. Merck, Mumbai, India, and orthophosphoric acid and sodium hydroxide AR grade purchased from SD Fine Chem., Mumbai, India. The reference sample and branded formulation was supplied by Bio Leo Analytical labs, Hyderabad, India.

### Instruments and Columns

Waters HPLC model 2695 equipped with UV Visible detector using data handling system-waters alliance empower two software. Power Sonicator, model no: 405, Hwashin Technology, Korea. The column used in the development for determination is Hypersil BDS C<sub>18</sub> (100mm X 4.6 mm, 5 $\mu$ ).

### Selection of Chromatographic Method and Wave Length ( $\lambda_{max}$ )

Selection of chromatographic method in general is done taking into consideration of several parameters like the nature of the drugs, molecular weight and solubility. Since both the drugs selected are polar in nature. RP-HPLC was selected for initial chromatographic condition because of its simplicity and suitability. The Chromatographic conditions are given in the table-I.

### Chromatographic conditions:

Table : I

Method parameters	Optimisation conditions
Column	Hypersil BDS C <sub>18</sub> , 100 X 4.6 mm, 5 $\mu$ ,
Flow Rate	1.0 ml/min
Wave length	287 nm
Column temperature	30°C
Injection volume	20 $\mu$ L
Run time	10 minutes
Diluent	Mobile phase
Elution	Isocratic
Needle wash	Water: Acetonitrile 90:10 (v/v)

### Selection of Wave Length ( $\lambda_{max}$ )

The maximum absorbance of Sildenafil Citrate and Dapoxetine Hydrochloride was found to be 291nm and 311nm respectively. From the UV visible spectrophotometric results; Simultaneous estimation of two spectra shows maximum absorbance at 287nm (Fig-III).

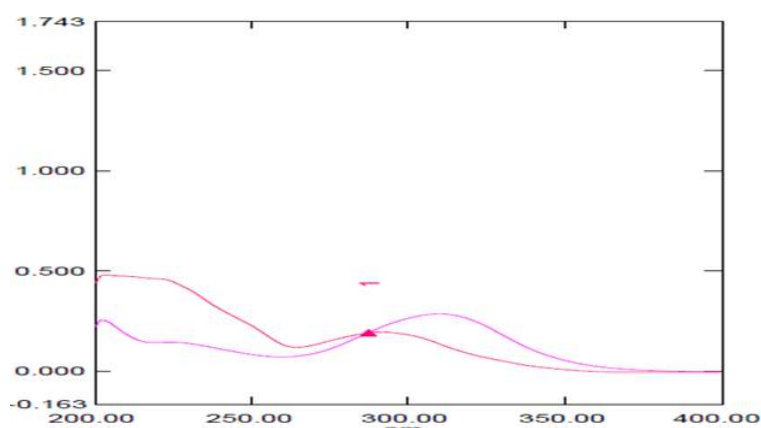


Fig-III Maximum absorbance spectrum of sildenafil citrate and Dapoxetine Hydrochloride

#### Preparation of mobile phase

Accurately measure the 0.1 % of orthophosphoric acid and dissolve in 1000 mL of water and P<sup>H</sup> is adjusted to  $5 \pm 0.1$  with 1M sodium hydroxide. The solution was filtered through 0.45 $\mu$  membrane filter and was degassed. A freshly prepared buffer solution: Acetonitrile in a ratio of (50:50 V/V) was filtered through 0.05 $\mu$  membrane filter and sonicated.

#### Preparation of Stock solution

Weighed accurately about 50 mg of Sildenafil Citrate and 30 mg of Dapoxetine Hydrochloride and transferred into 100 mL volumetric flask, added 60 mL of diluent and the solution was sonicated to dissolve and dilute to the volume with mobile phase.

#### Standard preparation:

Transfer 10 mL of standard stock solution into 100 mL volumetric flask and dilute to volume with diluent.

#### Preparation of Sample solution

Weighed twenty tablets of Sildenafil Citrate and Dapoxetine Hydrochloride powdered uniformly in a mortar. An accurately weighed portion powder equivalent to 50 mg of sildenafil citrate and 30 mg of Dapoxetine Hydrochloride was transferred into 100 mL volumetric flask. The contents of the flask were sonicated for about 15 min for complete solubility of the drug and the volume was made up to 100 mL with mobile phase. Then the mixture was filtered through a 0.45 $\mu$  membrane filter. From the above solution 10 mL aliquot was taken into a separate 100 mL volumetric flask and diluted up to the volume with the mobile phase and mixed well.

#### Optimization of HPLC method

The HPLC method was optimized with an aim to develop an accurate and precise method for the estimation of Sildenafil Citrate and Dapoxetine Hydrochloride in pharmaceutical dosage forms. For the method optimization different mobile phases were tried but acceptable retention times, theoretical plates and good resolution observed with 0.1% orthophosphoric acid: Acetonitrile in the ratio of 50:50 using Hypersil BDS C18, 100 X 4.6 mm, 5 $\mu$ ,

#### Validated RP-HPLC Method for Sildenafil Citrate and Dapoxetine Hydrochloride.

Validation of the optimized method was performed according to the ICH guide lines [13-16]

#### Accuracy

For accuracy determination, three different concentrations were prepared separately i.e. 80%, 100%, and 120% of analyte and the chromatograms were recorded for the same. The results obtained for recovery were found to be within the limits. The results were given in the table-II

Table:II Results for accuracy

Accuracy	Amount added ( $\mu$ g/ml)		Amount recovered ( $\mu$ g/ml)		% recovered	
	SFC	DTH	SFC	DTH	SFC	DTH
80%	37.5	22.55	37.27	22.51	99.34	99.77
100%	50.10	30.20	49.90	29.96	99.58	99.12
120%	62.50	37.55	62.48	37.65	99.9	100.3
Overall mean of 3 levels % recovery						99.66

**Precision**

The precision of an analytical procedure express the closeness of agreement between a series of measurement obtained from multiple sampling of the same homogenous sample under the prescribed conditions. Precision of an analytical procedure is usually expressed in terms of variance, standard deviation, coefficient of variation of a series of measurement.

**System Precision**

System precision was determined by injecting six homogenous preparation solutions into HPLC System of a concentration 50µg/mL Sildenafil citrate and 30µg/mL Dapoxetine hydrochloride. The mean, standard deviation and % RSD for peak areas of Sildenafil citrate and Dapoxetine hydrochloride from standard solutions were calculated. The % RSD of both the drugs was found to be below 1. Hence the method is said to be Precise. The results were given in the table-III.

**Method Precision**

Method precision was determined by injecting six sample solutions of Single batch were analysed as per test method. The mean, standard deviation and % RSD for peak areas of Sildenafil citrate and Dapoxetine hydrochloride from sample solutions were calculated. The % RSD Sildenafil citrate and Dapoxetine hydrochloride was found to be below 1. Hence the method is said to be Precise. The results were given in the table-III

**Table: III Summary of validation parameters:**

S.no	Validation parameters	Sildenafil Citrate	Dapoxetine hydrochloride
1	Precision (n=6) RT(min) %RSD	0.0878	0.06
2	Method precision(n=6) RT(min) %RSD	0.0772	0.06
3	Linearity concentration range(µg/ml)	12.5-62.5	7.5-37.5
4	Ruggedness(day-1 and day-2) (n=6) %RSD	0.15	0.06
5	Robustness Change in Wavelength (±2nm). Column oven temperature (±5°C)	0.156	0.195
6	Specificity Interference from diluents and interference	No interference at the retention time of both drug peaks	
7	LOD(µg/ml)	0.824	0.904
8	LOQ(µg/ml)	2.499	2.739

**Linearity**

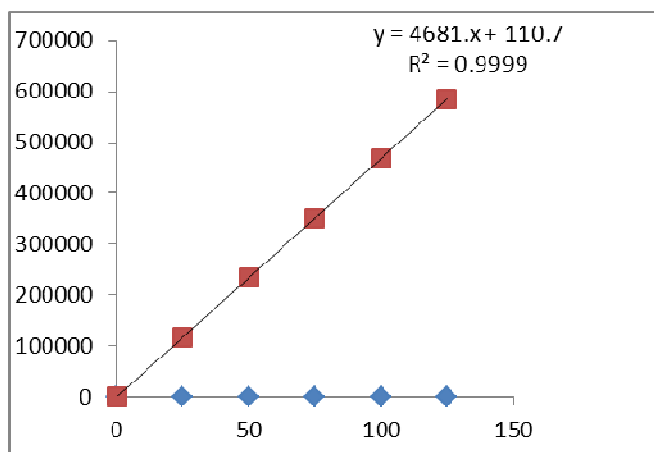
Linearity for Sildenafil citrate was determined in the range of 12.5µg/mL - 62.5µg/mL (Fig-IV) and Dapoxetine hydrochloride was determined in the range of 7.5µg/mL - 37.5µg/mL (Fig-V). A graph was plotted with concentration on X-axis and peak area on Y-axis and correlation coefficient was determined. The results were given in the table-IV

Concentration range (µg/mL) = 12.5-62.5

Correlation coefficient ( $r^2$ ) = 0.9999

Slope (m) = 4681

Intercept (b) = 110.7

**FigIV: Calibration curve for Sildenafil citrate**

Concentration range ( $\mu\text{g/mL}$ ) = 7.5- 37.5

Correlation coefficient ( $r^2$ ) = 0.9999

Slope ( $m$ ) = 24777

Intercept ( $b$ ) = -10960

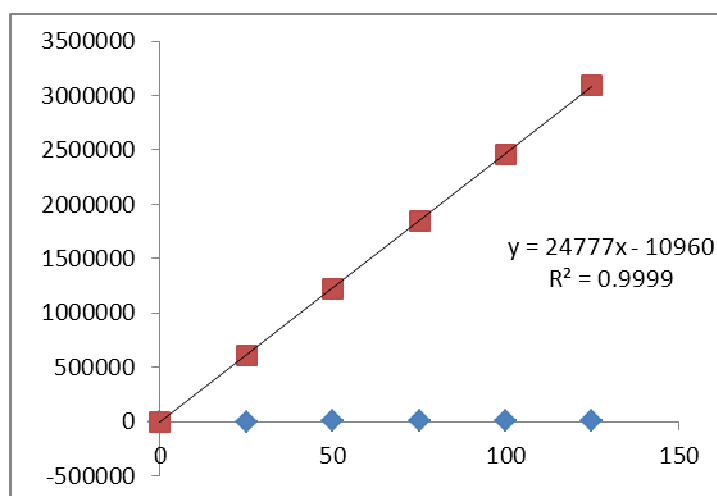


Fig-V: Calibration curve for Dapoxetine hydrochloride

Table no: IV Results for Linearity (n=6)

Parameters	SFC	DPH
Slope	4681	24777
Intercept	110.7	-10960
$R^2$	0.9999	0.9999
Regression equation	$Y=4681.x+110.7$	$Y=24777.x-10960$
Linearity range	12.5-62.5	7.5-37.5

### Ruggedness (Intermediate Precision)

The Ruggedness of the method has been verified by analysing the six samples of the same batch for method precision as per test method by different analyst using different instrument, different days. The analyst's prepared six samples of the same batch for two different days. Calculated % RSD for two different days by analyst in six samples for ruggedness results with the method precision reported in the table-III.

### Robustness

To evaluate the robustness, the following small deliberate variations are made in the method and analysed the sample in triplicate, Column oven temperature ( $\pm 5^\circ\text{C}$ ) Wavelength ( $\pm 2\text{nm}$ ). The system suitability was evaluated in each condition and compared the results with method precision results. The method is robust for change in wave length and column oven temperature. The % RSD reported in the table-III, IV

Table no: V Results for Robustness

Variations	Chromatographic parameters	
	Retention time (min)	Area ( $\mu\text{V}^2\text{Sec}$ )
<b>Standard</b>	3.143	4.033
Change in Column temperature at $\pm 50^\circ\text{C}$	3.155	4.054
	3.155	4.050
Change in wave length at $\pm 2\text{nm}$	3.152	4.047
	3.152	4.046
%RSD	0.156	0.195

### Specificity

Specificity shall be established by demonstrating that the procedure is unaffected by the presence of interference at the retention time of the Sildenafil citrate and Dapoxetine hydrochloride with respect to mobile phase, diluents, placebo and degradants. The specificity studies include deliberate degradation of the tablet sample by exposure to stress conditions, Specificity studies also include blank, placebo and sample solution (control sample), Sildenafil

citrate and Dapoxetine hydrochloride standard solution were injected into the HPLC system. There was no interference from the blank and placebo at the retention time of the peaks. Peak purity data reveals that Sildenafil citrate and Dapoxetine hydrochloride were homogeneous and there was no interference at the retention time of Sildenafil citrate and Dapoxetine hydrochloride peaks.

#### Degradation Studies:

Forced degradation or accelerated degradation is a process whereby the natural degradation rate of a product or material is increased by the application of additional stress. Forced degradation or stress test is undertaken to demonstrate specificity. When developing stability-indicating methods, particularly when little information is available about potential degradation products. These studies also provide information about the degradation pathways and degradation products that could form during storage. Forced degradation studies may help facilitate pharmaceutical development as well in areas such as formulation development manufacturing and packaging in which knowledge of chemical behaviour can be used improve a drug product.

Forced Degradation study was carried out by treating the sample under the following conditions. Weighed twenty tablets of Sildenafil Citrate and Dapoxetine Hydrochloride powdered uniformly in a mortar. An accurately weighed portion powder equivalent to 50 mg of Sildenafil Citrate and 30 mg of Dapoxetine Hydrochloride was transferred into 100 mL volumetric flask. The contents of the flask were sonicated for about 15 min for complete solubility of the drug and the volume was made up to 100 mL with mobile phase.

#### Acid degradation

5 mL of the above stock solution was transferred into 100 mL volumetric flask, and added 50 mL of diluent with intermittent shaking for 15 min. To this flask 5 mL of 0.1N HCl was added and sonicated for 30 minutes neutralized with 5 mL of 0.1N NaOH and diluted to volume with diluent and was analysed as per the test method for 12hrs.

#### Alkali degradation

5 mL of the above stock solution was transferred into 100 mL volumetric flask, and added 50 mL of diluent with intermittent shaking for 15 min. To this flask 5 mL of 0.1N NaOH was added and sonicated for 30 minutes neutralized with 5 mL of 0.1N HCl and diluted to volume with diluents and was analysed as per the test method for 12hrs.

#### Thermal degradation

The Drug substance was taken in Petri dish and exposed to a temperature of 105°C for 12hrs. Then the sample was taken and diluted with the diluent for further analysis. Treated sample was analysed as per the test method.

#### Peroxide degradation

5 mL of the above stock solution was transferred into 100 mL volumetric flask, and added 50 mL of diluent with intermittent shaking for 15 min. To this flask 5 mL of 30% hydrogen peroxide was added and sonicated for 30 minutes and diluted to volume with diluent and was analysed as per the test method. No peaks shall be eluted at the retention time of Sildenafil citrate and Dapoxetine hydrochloride.

Table:VI Results for degradation studies

Condition	Time	Retention time (min)		% of active drug present after degradation	
		SFC	DTH	SFC	DTH
Acid degradation	12	3.148	4.041	16.06	82.09
Alkaline degradation	12	3.149	4.040	16.02	82.76
Thermal degradation	12	3.147	4.040	16.28	79.12
Peroxide degradation	12	3.148	4.043	15.68	83.71

#### Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ of the developed method were determined by analysing progressively low concentration of the standard solutions using the developed methods. The results are given in the table-III

$$\text{LOD} = 3.3 \sigma / S \text{ and } \text{LOQ} = 10 \sigma / S$$

$\sigma$  = standard deviation of the response

S = slope of the calibration curve of the analyte.

#### Analysis of Marketed Formulations

Twenty tablets of sildenafil citrate and dapoxetine hydrochloride are weighed powdered uniformly in a mortar. An accurately weighed portion powder equivalent to 50 mg of sildenafil citrate and 30 mg of dapoxetine hydrochloride

was transferred into 100 mL volumetric flask. The contents of the flask were sonicated for about 15 min for complete solubility of the drug and the volume was made up to 100 mL with mobile phase. Then the mixture was filtered. From the above solution 10 mL aliquot was taken into a separate 100 mL volumetric flask and diluted up to the volume with the mobile phase and mixed well. Initially inject 20  $\mu$ L of blank solution, placebo solution, sample solution and standard solution, Disregard peaks due to blank and placebo if any. The results are given in the table-VII

Table no: VII Results for assay of marketed formulation

Drug	Quantity claim (mg/tablet)	*Quantity found (mg/tablet) $\pm$ SD	* % Assay found $\pm$ SD
Sildenafil Citrate	50	49 $\pm$ 0.261	98.80 $\pm$ 0.249
Dapoxetine Hydrochloride	30	29.76 $\pm$ 0.427	99.21 $\pm$ 0.483

\*Each value is a mean of three readings

### Recording of Chromatograms

The standard solutions stabilize the system until stable baseline is obtained. Initially inject the blank solution and placebo. The standard chromatograms were recorded by injected standard solutions and the peak areas of standard chromatograms were noted. Calibration graph was plotted using peak area Vs concentration. Then the sample solution was injected and the amount of Sildenafil citrate and Dapoxetine hydrochloride present in the formulation was calculated from the calibration curve. The amount of Sildenafil citrate and Dapoxetine hydrochloride present in per tablet Dapoxetine Hydrochloride and Sildenafil Citrate was found to be 79.16  $\pm$  0.688 mg. Total label claim for (P-FORE-30 mg of Dapoxetine Hydrochloride and 50 mg of Sildenafil citrate) formulation.

### RESULTS AND DISCUSSION

The goal of the study is to development of simple, rapid, sensitive, specific and accurate HPLC methods for the routine quantitative determination of samples. Hypersil C<sub>18</sub> BDS Column (100 mm x 4.6 mm; 5 $\mu$ ) was used as stationary phase. The mobile phase composition of 0.1% orthophosphoric acid: Acetonitrile in the ratio of 50:50 and P<sup>H</sup> adjusted to 5.0 $\pm$ 0.1 with sodium hydroxide were selected. A good linear relationship ( $r^2 = 0.9999$  &  $r^2 = 0.9999$ ) was observed in the range of 12.5  $\mu$ g/mL - 62.5  $\mu$ g/mL & 7.5  $\mu$ g/mL - 37.5  $\mu$ g/mL for sildenafil citrate and DapoxetineHCl. Recovery values obtained by the proposed method are accurate.

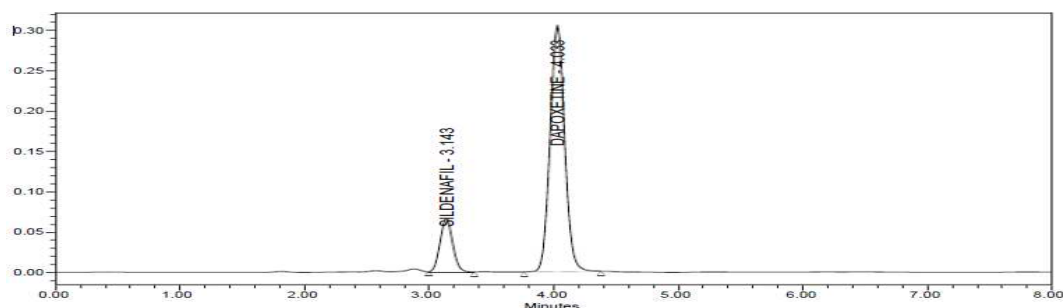


Fig VI: Standard chromatogram of sildenafil citrate and dapoxetine hydrochloride

The system precision was established by six replicate injections of the standard solutions containing analyte of interest. The value of relative standard deviation of Sildenafil citrate and Dapoxetine HCL was found to be 0.25 and 0.28 within the limit, indicating the injection repeatability of the method. The method precision was established by carrying out the analysis six times using the proposed method. The relative standard deviation of Sildenafil citrate and Dapoxetine HCL was found to be 0.57 and 0.12 within the limit, indicating the injection repeatability of the method. Six samples of the same batch were prepared on different days by the analysts. Calculated %RSD for two different days in six samples for ruggedness results with the method precision within the limits. The system suitability was evaluated in each condition and compared the results with method precision results. The method is robust for change in wave length and column oven temperature

The specificity studies include deliberate degradation of the tablet sample by exposure to stress conditions, Specificity studies also include blank, placebo solution, and sample solution (control sample), Sildenafil citrate and Dapoxetine hydrochloride standard solution were injected into the HPLC system. There was no interference from the blank and placebo at the retention time of the peaks. Peak purity data reveals that Sildenafil citrate and Dapoxetine hydrochloride were homogeneous and there was no interference at the retention time of Sildenafil citrate and Dapoxetine hydrochloride peaks. The method does not permit detection of degradation product for

sildenafil citrate and DapoxetineHCL. Although the drugs underwent degradation at different conditions the retention time was not altered (Table VI).The method is specific for the estimation of Sildenafil citrate and Dapoxetine Hydrochloride tablets. The sample solution was injected and the amount of Sildenafil citrate and Dapoxetine Hydrochloride present in the formulation was calculated from the calibration curve. The amount of Sildenafil citrate and Dapoxetine Hydrochloride present in per tablet of Dapoxetine Hydrochloride and Sildenafil citrate was found to be  $29.76 \pm 0.427$  mg and  $49 \pm 0.261$ mg .Total label claim for (P-FORE-30 mg of Dapoxetine Hydrochloride and 50mg of Sildenafil citrate) formulations (Fig VII)

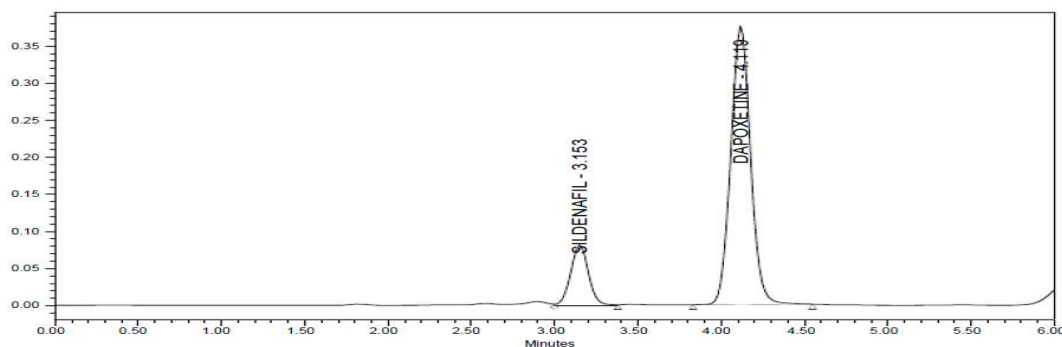


FIG VII: A typical chromatogram for assay of market formulation containing Sildenafil Citrate and Dapoxetine Hydrochloride

### CONCLUSION

The RP-HPLC method developed and validated allows a simple and fast quantitative simultaneous determination of Sildenafil Citrate and Dapoxetine Hydrochloride from its formulation. All the validation parameters were found to be within the limits according to the ICH [17,18]. The proposed method was found to be specific for the drugs of interest irrespective of the excipients present and the method was found to be simple, accurate, precise, rugged, and robust and can be involved in the routine analysis of the marketed formulation. Therefore this method can be employed in quality control to estimate the amount of Sildenafil Citrate and Dapoxetine Hydrochloride in pure and pharmaceutical dosage forms

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