

Development and Validation of a New RP-HPLC Method for the Simultaneous Quantitative Estimation of Sildenafil and Dapoxetine in API Form and Marketed Pharmaceutical Dosage Forms

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Sildenafil and Dapoxetine in API form and Marketed pharmaceutical dosage form by RP-HPLC. Chromatogram was run through Phenomenex Luna C18 (4.6mm×150mm, 5µm) Particle size Column and Mobile phase containing Methanol and Tri Ethyl Amine Buffer taken in the ratio of 35: 65% v/v was pumped through column at a flow rate of 1.0 ml/min. Temperature was maintained at 38°C. Optimized wavelength selected was 261 nm. Retention times of Sildenafil and Dapoxetine were found to be 2.256min and 5.427minutes respectively. The %RSD for the Repeatability and Intermediate Precision of the Sildenafil and Dapoxetine were found to be within limits. %Recovery was obtained was found to be within the limits for Sildenafil and Dapoxetine respectively. The LOD, LOQ values obtained from regression equations of Sildenafil and Dapoxetine were 2.63µg/ml and 3.84µg/ml & 7.92µg/ml and 11.54µg/ml respectively. Regression equation of Sildenafil and Dapoxetine was found to be $y = 10511x + 9597.2$ & $y = 6120.9x + 29119$ respectively. The Retention times was decreased and run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries. **Key Words:** Sildenafil and Dapoxetine, RP-HPLC, Method Development, Validation, Accuracy, Precision.

I. INTRODUCTION

Sildenafil, sold under the brand name Viagra among others, is a medication used to treat

erectile dysfunction and pulmonary arterial hypertension. It is also sometimes used off-label for the treatment of certain symptoms in secondary Raynaud's phenomenon¹. It is unclear if it is effective for treating sexual dysfunction in females. It can be taken orally (swallowed by mouth), intravenously (injection into a vein), or through the sublingual route (dissolved under the tongue). Onset when taken orally is typically within twenty minutes and lasts for about two hours. Sildenafil acts by blocking phosphodiesterase 5 (PDE5), an enzyme that promotes breakdown of cGMP, which regulates blood flow in the penis². It requires sexual arousal to work, and does not by itself cause or increase sexual arousal. It also results in dilation of the blood vessels in the lungs. The primary indication of sildenafil is treatment of erectile dysfunction (inability to sustain a satisfactory erection to complete sexual intercourse). Its use is now one of the standard treatments for erectile dysfunction, including for males with diabetes mellitus³. The IUPAC Name of Sildenafil is 5-[2-ethoxy-5-(4-methylpiperazin-1-yl)sulfonylphenyl]-1-methyl-3-propyl-6H-pyrazolo[4,3-d]pyrimidin-7-one. The Chemical Structure of Sildenafil is shown in following figure-1.

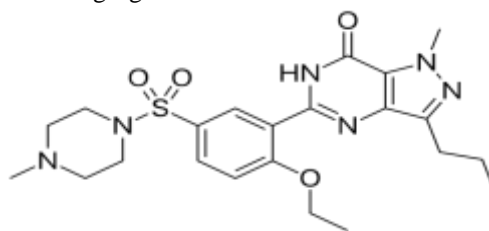


Fig-1: Chemical Structure of Sildenafil

Dapoxetine, sold under the brand name Priligy among others, is a selective serotonin reuptake inhibitor (SSRI) used for the treatment of premature ejaculation (PE) in men ages 18 to 64 years old. Dapoxetine works by inhibiting the serotonin transporter, increasing serotonin's action at the postsynaptic cleft, and as a consequence promoting ejaculatory delay⁴. As a member of the 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 antidepressant⁵. Dapoxetine is a selective serotonin reuptake inhibitor, for the treatment of premature ejaculation. In a phase II proof-of-concept study conducted by PPD, Dapoxetine demonstrated a statistically significant increase in ejaculatory latency when compared to placebo. Alza submitted a NDA to the FDA for Dapoxetine for the treatment of premature ejaculation in December 2004. In October 2005, the company received a FDA Non-Approvable letter from the FDA, at which time they planned to work with regulators to

address outstanding questions⁶. The IUPAC Name of Dapoxetine is (1S)-N,N-dimethyl-3-naphthalen-1-yloxy-1-phenylpropan-1-amine. The Chemical Structure of Dapoxetine is shown in following figure-2.

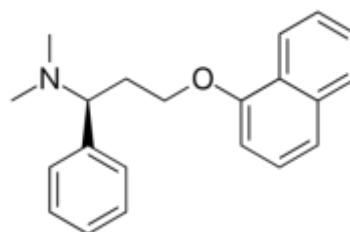


Fig-2: Chemical Structure of Dapoxetine

We have developed a new accurate and precise RP-HPLC method for the simultaneous determination of Sildenafil and Dapoxetine in a bulk and marketed pharmaceutical dosage forms. The developed method is validated as per ICH guidelines^{24,27}.

II. EXPERIMENTAL METHODS

Instruments Used:

Table-1: Instruments Used

S.No.	Instruments and Glass wares	Model
1	HPLC	WATERS Alliance 2695 separation module. 996 PDA detector, software: Empower 2
2	pH meter	LabIndia
3	Weighing machine	Sartorius
4	Digital Ultra Sonicator	Labman

Chemicals Used:

Table-2: Chemicals Used

S.No.	Chemical	Brand Names
1	Sildenafil	Cipla Ltd
2	Dapoxetine	Cipla Ltd
3	Water and Methanol for HPLC	LICHROSOLV (MERCK)
4	Acetonitrile for HPLC	Merck

HPLC Method Development:

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Sildenafil and Dapoxetine working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol⁷.

Further pipette 1ml of the above Sildenafil and 3ml of Dapoxetine stock solutions into a 10ml

volumetric flask and dilute up to the mark with Methanol.

Preparation of Sample Solution:

Take average weight of the Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Sildenafil and Dapoxetine sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent⁸⁻⁹.

Further pipette 1ml of Sildenafil and 3ml Dapoxetine above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure: Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines^{24,27}.

Preparation of Mobile Phase:

Accurately measured 350ml (35%) of Methanol, 650ml of Tri Ethyl Amine Buffer (65%) were mixed and degassed in digital ultra sonicator for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration¹⁰⁻¹¹.

Diluent Preparation:

The Mobile phase was used as the diluent.

Method Validation Parameters¹²⁻¹⁶

System Suitability

Accurately weigh and transfer 10 mg of Sildenafil and 10mg of Dapoxetine working sample into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 1ml of Sildenafil and 3ml of Dapoxetine from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:

The sample solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits¹⁷.

Specificity:

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Sildenafil and 10mg of Dapoxetine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 1ml of Sildenafil and 3ml of Dapoxetine from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of Sample Solution:

Take average weight of the Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Sildenafil and Dapoxetine sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 1ml of Sildenafil and 3ml Dapoxetine above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent¹⁸.

Procedure:

Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula¹⁹⁻²¹:

$$\% \text{ASSAY} = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Linearity and Range:

Accurately weigh and transfer 10 mg of Sildenafil and 10mg of Dapoxetine working sample into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Preparation of Level – I (60ppm of Sildenafil & 100ppm of Dapoxetine):

Pipette out 0.6ml of Sildenafil and 1ml of Dapoxetine stock solutions was taken in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (80ppm of Sildenafil & 200ppm of Dapoxetine):

Pipette out 0.8ml of Sildenafil and 2ml of Dapoxetine stock solutions was taken in a 10ml of volumetric flask dilute up to the mark with diluent²²⁻²⁵.

Preparation of Level – III (100ppm of Sildenafil & 300ppm of Dapoxetine):

Pipette out 1ml of Sildenafil and 3ml of Dapoxetine stock solutions was taken in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV (120ppm of Sildenafil & 400ppm of Dapoxetine):

Pipette out 1.2ml of Sildenafil and 4ml of Dapoxetine stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (140ppm of Sildenafil & 500ppm of Dapoxetine):

Pipette out 1.4ml of Sildenafil and 5ml of Dapoxetine stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Procedure:

Inject each level into the chromatographic system and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient²⁶⁻³⁰.

Precision

Repeatability

Preparation of Sildenafil and Dapoxetine Solution for Precision:

Accurately weigh and transfer 10 mg of Sildenafil and 10mg of Dapoxetine working sample into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 1ml of Sildenafil and 3ml of Dapoxetine from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

The sample solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits³¹⁻³⁵.

Intermediate Precision:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure:

Day 1:

The sample solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Day 2:

The sample solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Accuracy:

For Preparation of 50% Sample Stock solution:

Accurately weigh and transfer 10 mg of Sildenafil and 10mg of Dapoxetine working sample into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 0.5ml of Sildenafil and 1.5ml of Dapoxetine from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents³⁶⁻³⁸.

For Preparation of 100% Sample Stock solution:

Accurately weigh and transfer 10 mg of Sildenafil and 10mg of Dapoxetine working sample into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 1ml of Sildenafil and 3ml of Dapoxetine from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

For Preparation of 150% Sample Stock solution:

Accurately weigh and transfer 10 mg of Sildenafil and 10mg of Dapoxetine working Sample into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 1.5ml of Sildenafil and 4.5ml of Dapoxetine from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:

Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Sildenafil and Dapoxetine and calculate the individual recovery and mean recovery values³⁹⁻⁴².

Robustness:

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results. .

For Preparation of Standard solution:

Accurately weigh and transfer 10 mg of Sildenafil and 10mg of Dapoxetine working sample into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 1ml of Sildenafil and 3ml of Dapoxetine from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Effect of Variation of Flow Conditions:

The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded⁴³.

Effect of Variation of Mobile Phase Organic Composition:

The sample was analyzed by variation of mobile phase i.e. Methanol: Tri Ethyl Amine (35:65% v/v) was taken in the ratio and 40:60, 30:70 instead (35:65% v/v) remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

III. RESULTS AND DISCUSSION

Analytical Method Development:

Optimization of the HPLC method was carried out in experiments investigating mobile phase, flow rate, column, pH of the mobile phase, ratio of mobile phase, injection volume, and temperature of the column and solvents. The final isocratic chroma to graphic conditions shown in Table 1 are optimized for simultaneous analysis of Sildenafil and Dapoxetine. Test and standard samples were prepared in mobile phase as diluent which consist of a Methanol: Tri Ethyl Amine Buffer in ratio 35:65% v/v. The flow rate of 1.0ml/min, injection volume of 10µl, wave length of 261nm for Sildenafil and Dapoxetine is used at 38°C temperature conditions. Using this method, retention times of Sildenafil and Dapoxetine were 2.256min and 5.427min, respectively⁴⁴⁻⁴⁸. The Optimized Chromatographic Conditions were shown in following Table-3.

Table-3: Optimized Chromatographic Conditions:

Mobile phase	Methanol: Tri Ethyl Amine Buffer (35:65% v/v)
Column	Phenomenex Luna C18 (4.6mm×150mm, 5µm) Particle size
Flow rate	1.0 ml/min
Wavelength	261 nm
Column temp	38°C
Injection Volume	10 µl
Run time	10 minutes

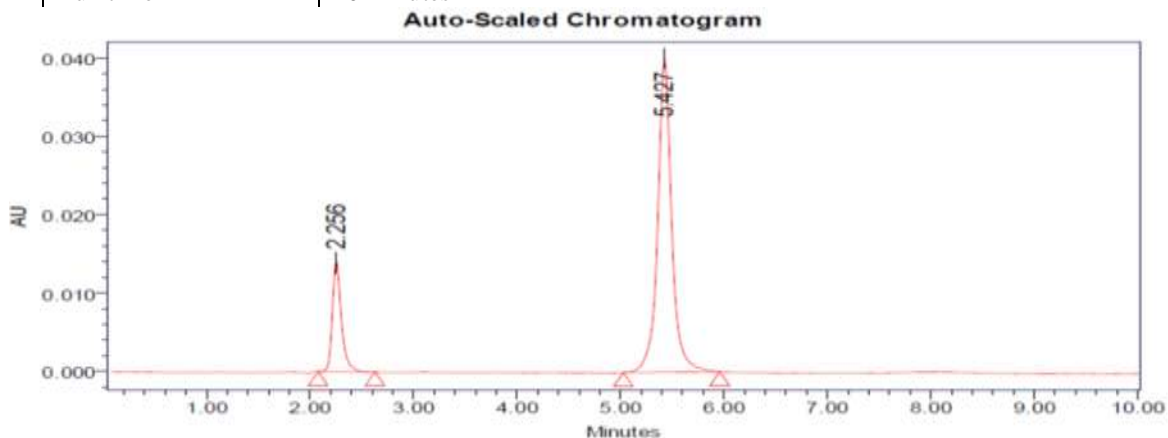


Fig-3: Optimized Chromatographic Condition

Validation of Analytical Method

The analytical technique was validated in accordance with ICH guidelines. Numerous factors, including limit of detection, accuracy, linearity, robustness, specificity, precision, and limit of quantification⁴⁹⁻⁵².

System Suitability:

System suitability test is a fundamental part of liquid chromatography. It ensures that system is working correctly. The standard solution of Sildenafil and Dapoxetine was injected into the chromatographic system and recorded the chromatogram. System suitability parameters such as number of theoretical plates, retention time, and tailing factor were calculated⁵³⁻⁵⁴. The results were showing in table-4 and 5.

Table-4: Results of System Suitability for Sildenafil

S.No.	Name	Rt	Peak Area	Height	USP Plate Count	USP Tailing
1	Sildenafil	2.247	105698	18652	7592	1.08
2	Sildenafil	2.246	105874	18754	7584	1.09
3	Sildenafil	2.248	105698	18698	7562	1.08
4	Sildenafil	2.252	105465	18689	7549	1.08
5	Sildenafil	2.248	105236	18695	7591	1.09
Mean			105594.2			
Std. Dev			247.4049			
% RSD			0.234298			

Table-5: Results of System Suitability for Dapoxetine

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing	USP Resolution
1	Dapoxetine	5.452	1856985	63659	6359	1.05	5.86
2	Dapoxetine	5.484	1856754	63598	6384	1.04	5.85
3	Dapoxetine	5.491	1856985	63845	6395	1.05	5.86
4	Dapoxetine	5.482	1856574	63989	6345	1.04	5.86
5	Dapoxetine	5.491	1854735	63895	6395	1.05	5.85
Mean			1856407				
Std. Dev			950.2696				
% RSD			0.051189				

Specificity

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. Analytical method was tested for specificity to measure accurately quantities Sildenafil and Dapoxetine in marketed formulation⁵⁵⁻⁵⁷.

Conclusion: The % purity of Sildenafil and Dapoxetine in pharmaceutical dosage form (marketed formulation) was found to be 99.72%.

Linearity: Linearity of the method was performed by analyzing a standard solution of Sildenafil and Dapoxetine to obtain a solution in the concentration range is 60-140 µg/mL and 100-500 µg/mL for Sildenafil and Dapoxetinerespectively. The area of each level was calculated and graph of area versus concentration was plotted. The correlation coefficient was calculated in linearity plot⁵⁸.

$$\% \text{ASSAY} = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Table-6: Chromatographic Data for Linearity Study of Sildenafil:

Concentration µg/ml	Average Peak Area
60	648743
80	856982
100	1068542
120	1268984
140	1469853

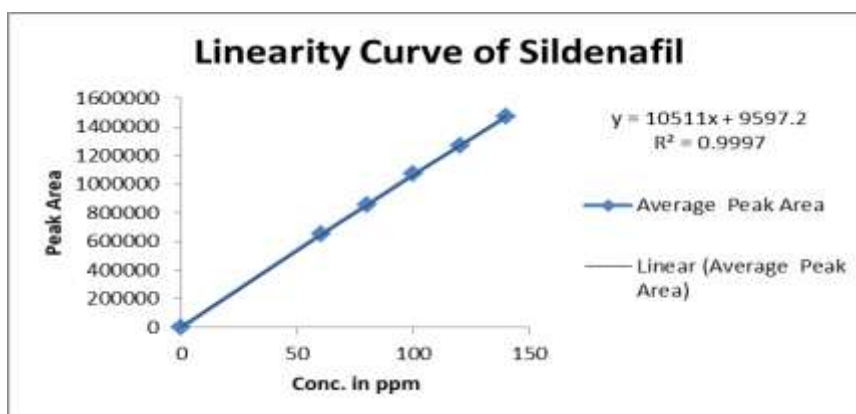


Fig-4: Calibration Curve for Sildenafil

Table-7: Chromatographic Data for Linearity Study of Dapoxetine

Concentration µg/ml	Average Peak Area
100	667564
200	1268547
300	1868598
400	2465487
500	3085864

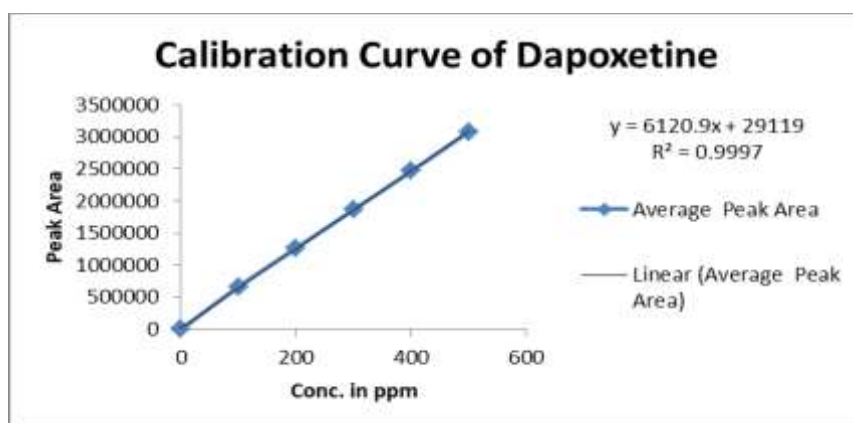


Fig-5: Calibration Graph for Dapoxetine

Precision: Precision of the method was determined by injecting five replicate of known concentration of Sildenafil 100 µg/mL and Dapoxetine 300

µg/mL have been analyzed by injecting into an HPLC column on the same day⁵⁹.

Table-8: Results of Repeatability for Sildenafil

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing
1	Sildenafil	2.269	105698	18569	7598	1.08
2	Sildenafil	2.255	105684	18547	7546	1.09
3	Sildenafil	2.252	105421	18594	7549	1.09
4	Sildenafil	2.267	105879	18574	7538	1.08
5	Sildenafil	2.260	105326	18563	7582	1.08
Mean			105601.6			
Std. Dev			224.5023			
% RSD			0.212594			

Table-9: Results of Method Precision for Dapoxetine

S. No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing	USP Resolution
1	Dapoxetine	5.274	1856985	63598	6359	1.05	5.86
2	Dapoxetine	5.266	1857458	63579	6357	1.04	5.85
3	Dapoxetine	5.265	1854795	63547	6358	1.04	5.86
4	Dapoxetine	5.278	1857469	63592	6357	1.05	5.86
5	Dapoxetine	5.305	1857685	63569	6345	1.04	5.85
Avg			1856878				
Std. Dev			1192.4				
% RSD			0.064215				

Intermediate Precision:

Day 1:

Table-10: Results of Intermediate Precision for Sildenafil

S. No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing
1	Sildenafil	2.248	115246	19685	7698	1.09
2	Sildenafil	2.245	116985	19654	7685	1.09
3	Sildenafil	2.242	115847	19675	7645	1.09
4	Sildenafil	2.239	116985	19682	7682	1.09
5	Sildenafil	2.243	115848	19654	7691	1.09
6	Sildenafil	2.246	116582	19647	7642	1.10
Mean			116248.8			
Std. Dev			710.3091			
% RSD			0.611025			

Table-11: Results of Intermediate Precision for Dapoxetine

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing	USP Resolution
1	Dapoxetine	5.284	1948592	64582	6459	1.05	5.96
2	Dapoxetine	5.293	1958245	64256	6475	1.06	5.95
3	Dapoxetine	5.306	1947584	64598	6498	1.05	5.96
4	Dapoxetine	5.319	1948675	64785	6472	1.06	5.95
5	Dapoxetine	5.346	1959854	64585	6493	1.05	5.96
6	Dapoxetine	5.352	1958246	64924	6438	1.06	5.96
Mean			1953533				
Std. Dev			5792.661				
% RSD			0.296522				

Day 2:

Table-12: Results of Intermediate Precision Day 2 for Sildenafil

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing
1	Sildenafil	2.255	102658	62584	6259	1.03
2	Sildenafil	2.260	102856	62359	6276	1.02
3	Sildenafil	2.242	102658	62451	6215	1.03
4	Sildenafil	2.245	102698	62584	6285	1.02
5	Sildenafil	2.260	102451	62758	6235	1.03
6	Sildenafil	2.255	102368	62154	6298	1.02
Mean			102614.8			
Std. Dev			176.9592			
% RSD			0.17245			

Table-13: Results of Intermediate Precision for Dapoxetine

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing	USP Resolution
1	Dapoxetine	5.266	1798952	62859	6265	1.03	5.42
2	Dapoxetine	5.265	1789854	62985	6289	1.02	5.43
3	Dapoxetine	5.306	1798659	62895	6279	1.03	5.42
4	Dapoxetine	5.293	1789898	62785	6285	1.02	5.43
5	Dapoxetine	5.265	1796856	62354	6249	1.03	5.42
6	Dapoxetine	5.266	1798568	62589	6245	1.02	5.43
Mean			1795465				
Std. Dev			4390.879				
% RSD			0.244554				

Accuracy: The accuracy of this method was determined by three different levels (50%, 100%, and 150%) by adding of unknown amount of

standard to sample at each level⁶⁰. Each sample was injected thrice. The obtained results are showed in following table-15 and 15.

Table-14: The Accuracy Results for Sildenafil

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	539070	50	50.373	100.746%	100.36%
100%	1063578	100	100.274	100.274%	
150%	1587149	150	150.085	100.056%	

Table-15: The accuracy results for Dapoxetine

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	949127	150	150.328	100.218%	100.15%
100%	1867824	300	300.441	100.147%	
150%	2785321	450	450.359	100.079%	

Limit of Detection

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value⁶¹.

$$LOD = 3.3 \times \sigma / s$$

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Sildenafil: = 2.63 μ g/ml

Dapoxetine: = 3.84 μ g/ml

Limit of Quantitation

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determine⁶².

$$LOQ = 10 \times \sigma / S$$

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Sildenafil: = 7.92 μ g/ml

Dapoxetine: = 11.54 μ g/ml

Robustness: The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Sildenafil and Dapoxetine. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase $\pm 5\%$. The samples (marketed formulation) of Sildenafil and Dapoxetine were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count⁶³.

Stability Studies

The specificity of the method can be demonstrated by applying stress conditions using acid, alkaline, peroxide, thermal, UV, water degradations. The sample was exposed to these conditions the main peak of the drug was studied for peak purity that indicating the method effectively separated the degradation products from the pure active ingredient⁶⁴⁻⁶⁵.

Table-16: Results of Forced Degradation Studies of Sildenafil

S.No.	Stress Condition	Peak Area	% of Degraded Amount	% of Active Amount	Total % of Amount
1	Standard	105265	0	100%	100%
2	Acidic	101557.566	3.522	96.478	100%
3	Basic	104200.770	1.011	98.989	100%
4	Oxidative	102830.220	2.313	97.687	100%
5	Thermal	103990.240	1.211	98.789	100%
6	Photolytic	105154.471	0.105	99.895	100%

Table-17: Results of Forced Degradation Studies of Dapoxetine

S.No.	Stress Condition	Peak Area	% of Degraded Amount	% of Active Amount	Total % of Amount
1	Standard	1858475	0	100%	100%
2	Acidic	1795045.248	3.413	96.587	100%
3	Basic	1832214.748	1.251	98.749	100%
4	Oxidative	1800100.300	3.141	96.859	100%
5	Thermal	1829910.239	1.537	98.463	100%
6	Photolytic	1839276.953	1.033	98.967	100%

IV. SUMMARY AND CONCLUSION

In the present study the analytical method is developed for analysis of Sildenafil and Dapoxetine in bulk form and marketed pharmaceutical dosage form by using RP-HPLC. Analytical method was developed for analysis of Sildenafil and Dapoxetine in bulk form and marketed pharmaceutical dosage form by using

Phenomenex Luna C18 (4.6mm \times 150mm, 5 μ m) Particle size and of Methanol: Tri Ethyl Amine Buffer in the ratio of 35:65% v/v used as mobile phase at 1.0 mL/min. The UV detector wave length is 261 nm. The developed method is economically feasible than RP-HPLC method, reproducible, selective, precise, specific and accurate than existing methods. This method can be used as

alternative for HPLC methods. The advantages of the proposed method involve a simple procedure for sample preparation and relatively short time of analysis. Apart from this, it can be used for assays of Sildenafil and Dapoxetine in bulk forms or in pharmaceutical formulations. The proposed method was validated by testing its linearity, accuracy, precision, limits of detection, and limit of quantitation. The results of the analysis of pharmaceutical dosage forms by the proposed methods are highly reproducible, reliable, and are in good agreement with the label claims of the drug. The additives usually present in the pharmaceutical formulations of the assayed samples did not interfere with Sildenafil and Dapoxetine. It may be said that the proposed methods are precise, sensitive, and accurate, so that these can be used as standard Pharmacopeial methods for the determination of Sildenafil and Dapoxetine using the RP-HPLC systems with PDA detector.

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