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Stability Indicating RP-HPLC Method for Simultaneous Determination of Sildenafil and Duloxetine in Pharmaceutical Dosage Form

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ABSTRACT

A simple, fast and precise reverse phase, isocratic HPLC method was developed for the separation and quantification of Sildenafil and Duloxetine in pharmaceutical dosage form. The quantification was carried out using Symmetry C18-ODS 4.6X150mm, 3µm enhanced polar selectivity column and mobile phase comprised of potassium dihydrogen phosphate buffer and acetonitrile and water in proportion of ratio 30:65:5 and degassed under ultra-sonication. The flow rate was 0.6mL/min and the effluent was monitored at 244nm. The retention time of Sildenafil and Duloxetine were 4.3 and 3.4 respectively. The method was validated in terms of linearity, precision, accuracy, and specificity, limit of detection and limit of quantization. Linearity of Sildenafil and Duloxetine were in the range of 100 to 300µg/mL and 30 to 90µg/mL respectively. The percentage recoveries of both the drugs were 98.7% and 99.8% for Sildenafil and Duloxetine respectively from the tablet formulation.The method was found to be precise, accurate and specific during the study. The proposed method enables rapid quantification and simultaneous analysis of both drugs from commercial formulations without any excipients interference. The method can be used for routine analysis of marketed products of Sildenafil and Duloxetine in combined tablet formulation

Keywords: Sildenafil, Duloxetine, RP-HPLC, Validation, stability studies

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INTRODUCTION

Sildenafil citrate is designated chemically as 1-[[3-(6,7-Dihydro -1-methyl- 7-oxo-3-propyl -1H-pyrazolo [4,3-d] pyrimidin-5-yl) -4-ethoxyphenyl]sulphonyl]-4-methyl piperazine citrate (Fig.1) is a compound of the pyrazolo-pyrimidinyl-methyl piperazine class, and is used to treat male erectile dysfunction. Sildenafil citrate, sold as Viagra, Revatio and under various other trade names, is a drug used to treat erectile dysfunction and pulmonary arterial hypertension (PAH). It acts by inhibiting cGMP-specific phospho diesterase type5, an enzyme that promotes degradation of cGMP, which regulates blood flow in the penis. It relaxes the arterial wall, leading to decreased pulmonary arterial resistance and pressure. Various analytical methods have been reported for the assay of Sildenafil in pure form as well as in pharmaceutical formulations. They include dissolution methods[1], estimation in biological fluids[2-4], stability indicating hplc method[5], spectrophotometric methods[6], HPLC[7], Simultaneous estimation with Dapoxetine[8-9] and Tadalafil[10] and TLC[11] methods.

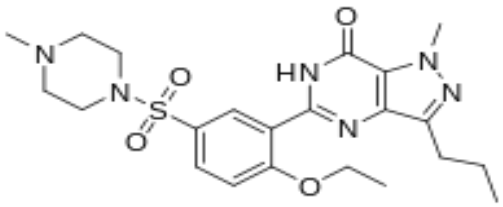


Figure1:Sildenafil

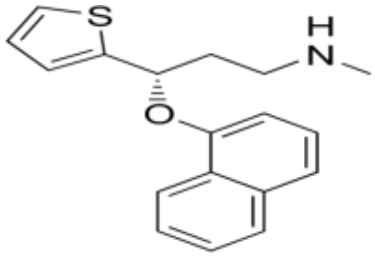


Figure 2: Duloxetine

Duloxetine hydrochloride is chemically as, N-methyl-3-(1-napthyloxy)-3-(thiophen-2-yl)-propan-1-amine. It belongs to the class narcoleptics. Duloxetine hydrochloride is a newer selective serotonin and nor epinephrine reuptake inhibitor (SSNRI) used for major depressive disorders. It has been approved by the US FDA for the treatment of major depressive disorder and for the diabetic peripheral neuropathic pain. Duloxetine is effective for major depressive disorder and generalized anxiety disorder (GAD). It can also relieve the symptoms of painful peripheral neuropathy, particularly diabetic neuropathy and it is used to control the symptoms of fibromyalgia. Literature survey reveals that the drug can be estimated by HPLC [12-14] and stability indicating HPLC method [15-17].

Sildenafil Citrate (Viagra) and Duloxetine, as one of the antidepressants are now combined to form Malegra DXT to cure Premature Ejaculation (PE). The most important thing that Malegra DXT does is to delay orgasm. This combination acts as a powerful medicine overall for a complete and healthy sexual intercourse. A single dose would prove satisfactory in achieving the

desired sexual results. Malegra DXT is a medication in a tablet form that does not cure only erectile dysfunction in men but also aids premature ejaculation.

Literature survey reveals that these drugs can be estimated separately and combination with other drugs. To till date, there have been no published reports about stability indicating HPLC method for the simultaneous estimation of Duloxetine and Sildenafil in pharmaceutical dosage forms. The objective of the present work is to develop and validate new analytical method for simultaneous determination of Sildenafil and Duloxetine in tablet dosage form in presence of their degradation products. The proposed method was validated as per the International Conference on Harmonization (ICH) method validation guidelines. This communication forms the first report of simple, sensitive and reproducible method for the simultaneous estimation of Sildenafil and Duloxetine from combined dosage form. This proposed method can be successfully employed for quality control during manufacture and for assessment of the stability of both drugs in bulk samples and combined dosage forms.

MATERIAL AND METHODS

Materials, Reagents and Chemicals

Sildenafil and Duloxetine were obtained as gift samples from Aurobindo Pharma Ltd, Hyderabad. Sildenafil and Duloxetine combined dosage form tablets were purchased from local market. HPLC grade acetonitrile and methanol, analytical grade potassium dihydrogen phosphate were obtained from Qualigens Fine Chemicals Ltd, Mumbai. Hydrochloric acid, sodium hydroxide, hydrogen peroxide of analytical grade were obtained from Merck Chemicals Ltd, Mumbai.

Chromatographic Conditions

Waters Alliance HPLC, integrated with Auto Sampler and UV detector was used. The output of signal was monitored and integrated using waters Empower 2 software. Symmetry C18-ODS 4.6 x150mm, 3µm particle size enhanced polar selectivity column was used as stationery phase. Mobile phase comprised of potassium di hydrogen phosphate buffer (1.36grams of KH_2PO_4 transferred into a 1000mL volumetric flask, add 500mL water, dissolve, sonic ate for five minutes, make volume up to the mark with water, acetonitrile and water in proportion of ratio 30:65:5 The mobile phase was mixed, filtered through 0.45µ membrane filter and degassed under ultra sonication. The mobile phase was used as diluents. Injection volume was 10µL and flow rate was 0.6mL/min and run time was 8 min. The column was maintained at ambient temperature and the eluent was monitored at 244nm.

Preparation of Standard Solution

Accurately weigh and transfer 10mg of Sildenafil and 10mg of Duloxetine working standard into a 10mL clean dry volumetric flask add about 7mL of mobile phase and sonicate to dissolve it completely, cool the solution to room temperature and dilute up to volume with mobile phase and used as standard stock solution. Pipette 2mL of Sildenafil and 0.6ml of Duloxetine standard stock solution into a 10mL volumetric flask and dilute up to volume with mobile phase and used as working standard solution.

Preparation of Sample Solution

Weigh and finely powder not fewer than 20 tablets. Accurately weigh and transfer a quantity of powder sample equivalent to 533.2mg of Sildenafil and Duloxetine into a 100mL clean dry volumetric flask add about 70mL of diluent and sonicate to dissolve it completely, cool the solution to room temperature and dilute to volume with diluent. Filter of the above sample solution through 0.45µmembrane filter. Pipette (60µg/ml of Duloxetine and 200µg/ml of Sildenafil) of the above filtered sample solution into a 10mL volumetric flask and dilute to volume with diluent. 10µL of the sample solution was injected in to the HPLC system.

VALIDATION OF THE PROPOSED METHOD

Specificity

A study conducted to establish specificity of the proposed method involved injecting diluent and placebo using the chromatographic conditions defined for the proposed method. The blank chromatogram showed no interference peaks at the retention time of Sildenafil and Duloxetine respectively. This indicates that diluent solution used in sample preparation do not interfere in the estimation of Sildenafil and Duloxetine. Similarly the placebo sample chromatogram showed no interference peaks at the retention time of Sildenafil and Duloxetine respectively. Additional peaks were observed in the channel may be due to excipients present in the formulations. These peaks however did not interfere with the standard peak indicating that the placebo used in sample preparation do not interfere in estimation of Sildenafil and Duloxetine in combination tablet, which demonstrates the specificity of the proposed method.

Linearity &Calibration Curve

Calibration curves were prepared by taking appropriate aliquots of Sildenafil and Duloxetine standard stock solutions in different 10 ml volumetric flask and diluted up to the mark with mobile phase to obtain final concentrations of 100, 150, 200, 250, 300 µg/ml of Sildenafil and 30, 45, 60, 75, 90 µg/ml of Duloxetine. These solutions (n=5) were injected through 10 µl loop system and chromatograms were obtained using 0.6 ml/min flow rate. The effluent was

monitored at 244 nm. Calibration curve was constructed by plotting average peak area against concentration and regression equation was computed.

Accuracy (Recovery Studies)

To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 50%, 100% and 150%. Known amounts of standard Sildenafil and Duloxetine were added to pre-analyzed samples and were subjected to the proposed HPLC method. The percentage of recoveries are found in the range of 98.7% and 99.0% for Sildenafil and Duloxetine respectively. From the data obtained, the proposed method found to be accurate.

Precision and Intermediate Precision

The precision of the method was demonstrated by inter day and intraday variation studies. In the intraday studies, five repeated injections of sample solutions were made in a day and the response factor of drug peaks and percentage of RSD were calculated. In the inter day variation studies, five repeated injections of sample solutions were made in different day with different make column of same dimensions. The repeatability of sample applications and measurement of peak area were expressed in terms of %RSD and found to be less than 2%.

Limit of Detection and Limit of Quantification

The Limit of Detection (LOD) and Limit of Quantification (LOQ) of the developed method were determined by injecting progressively low concentration of the standard solutions. The LOD is the smallest concentration of the analyte that gives a measurable response of signal to noise ratio of 3. The LOD for Sildenafil and Duloxetine were found to be 0.03 & 0.04 respectively. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified signal to noise ratio of 10. The LOQ was 0.1 & 0.15 of Sildenafil and Duloxetine respectively.

Robustness of Method

To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in the optimized method parameters were done. The effect of change in flow rate and mobile phase ratio on the retention time and tailing factor were studied. On evaluation of the results, it can be concluded that the variation in flow rate and changes in mobile phase composition affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate and mobile phase.

STABILITY STUDIES

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24hrs at room temperature. The results show that for

solutions, the retention time and peak area of Sildenafil and Duloxetine remained almost similar (% R.S.D. less than 2.0) and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 24hrs, which was sufficient to complete the whole analytical process. Further forced degradation studies were conducted indicating the stability of proposed method. The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the Sildenafil and Duloxetine using the proposed method.

Sample Preparation

Weigh and finely powder not fewer than 20 tablets. Accurately weigh and transfer a quantity of powder sample equivalent to 533.2 mg (100mg of Sildenafil and 30mg of Duloxetine) into a 100mL clean dry volumetric flask add about 70mL of diluent and sonicate to dissolve it completely, cool the solution to room temperature and dilute to volume with mobile phase. Filter the above sample solution through 0.45µ membrane filter. Pipette 2mL of Sildenafil and 2ml of Duloxetine (60µg/ml of Duloxetine and 200µg/ml of Sildenafil) the above filtered sample solution into a 10mL volumetric flask and dilute to volume with diluent. This sample solution is subjected to degradation with acid, base, and thermo, photolytic and peroxide.

Acid Degradation of Sample

To the 0.3ml sample stock solution (1000µg/ml), add 3mL of 0.1N acid (Hydrochloric acid), refluxed for 90minutes at 60°C, then cooled to room temperature, neutralize with 0.1N base (Sodium hydroxide) and dilute to volume 10ml with diluent. Filter the solution with 0.45 microns syringe filters and injected into HPLC system.

Base Degradation of Sample

To the 0.3ml sample stock solution (1000µg/ml), add 3mL of 0.1N base (Sodium hydroxide), refluxed for 60 minutes at 60°C, then cooled to room temperature, neutralize with 0.1N acid (Hydrochloric acid) and dilute to volume 10ml with mobile phase. Filter the above sample solution through 0.45µ membrane filter and injected into HPLC system.

Peroxide Degradation of Sample

To the 0.3ml sample stock solution (1000µg/ml) add 1mL of 30% peroxide, refluxed for 15 minutes at 60°C, then cooled to room temperature and dilute to volume with mobile phase. Filter the above sample solution through 0.45µ membrane filter and injected into HPLC system.

Thermal Degradation of Sample

To the 0.3ml sample stock solution (1000µg/ml), 3 ml of diluent was added in 10 ml of volumetric flask. Then, the volumetric flask was refluxed for 60 minutes and make up to 10ml with diluent. Filter the above sample solution through 0.45µ membrane filter and injected into HPLC system.

Photolytic Degradation of Sample

Sample of Sildenafil and Duloxetine tablet was exposed to near ultra violet lamp in photo stability chamber providing illumination for 1hr. Ten milligrams sample was dissolved in water and volume made up to 10 ml. From this solution dilutions were carried out to achieve the appropriate concentration. Filter the above sample solution through 0.45µ membrane filter and injected into HPLC system.

RESULT AND DISCUSSION

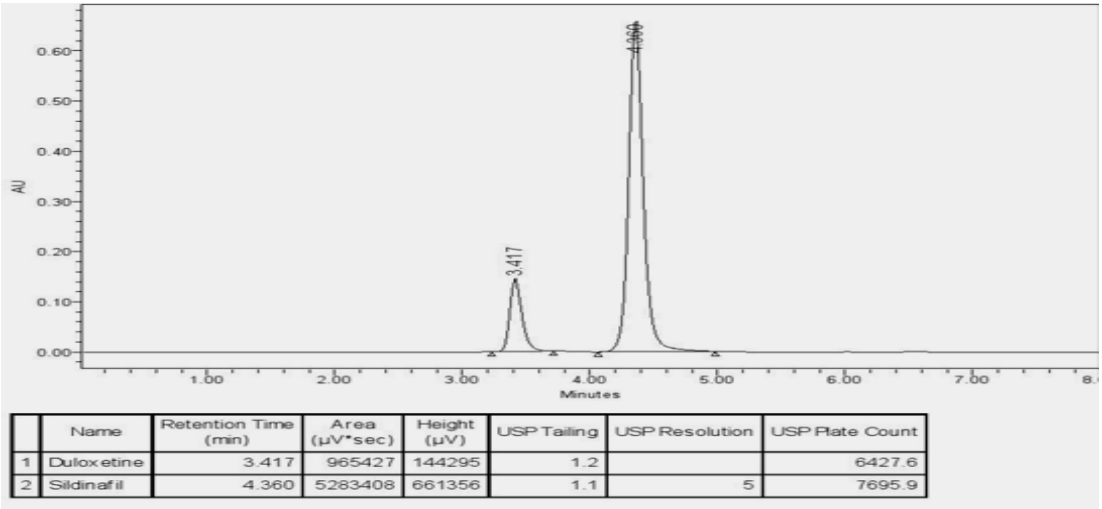
The mobile phase consisting of potassium dihydrogen phosphate buffer and acetonitrile and water in proportion of ratio 30:65:5 at 0.6ml/min flow rate which gave sharp, well-resolved peak with minimum tailing factor for Sildenafil and Duloxetine (Figure 3). The parameters like retention time, asymmetric factor, number of theoretical plates and tailing factors were evaluated for Sildenafil and Duloxetine. The results of system suitability parameters and chromatographic conditions are shown in Table 1. The calibration curve for Sildenafil and Duloxetine was found to be linear over the range of 100-300µg/ml, 30-90 µg/ml respectively and calibration curve **(**Figure 4, Figure 5) and results of linearity are shown in Table 2. The results of recovery studies are shown in Table 3. The proposed method was successfully applied to the determination of Sildenafil and Duloxetine in market formulation shown in Table 4. The LOD for Sildenafil and Duloxetine were found to be 0.03 & 0.04 respectively. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified signal to noise ratio of 10. The LOQ was 0.1 & 0.15 of Sildenafil and Duloxetine respectively as shown in Table 5. The %RSD of intraday and inter day precision study for Sildenafil and Duloxetine were found to be <2 as shown in Table 6. The results of robustness studies are shown in Table 7.

The forced degradation studies were also carried out as per *ICH* guidelines. There was complete separation of degradation peak and Sildenafil and Duloxetine peak, which demonstrate the specificity of assay method for estimation of Sildenafil and Duloxetine in the presence of its degradation products; it can be employed as a stability indicating one. This demonstrates that the developed Stability indicating HPLC method is simple, linear, accurate, sensitive and reproducible.

Percent degradation was calculated by comparing the areas of the degraded peaks in each degradation condition with the corresponding areas of the peaks of the drug under non degradation condition. The summary of degradation studies is given in Table 8 and the degradation chromatograms are shown in figures 6-10.

Table-1: Chromatographic Conditions and system suitability parameters

Flow rate	0.6 ml/min
Wave length	244 nm
Injection volume	10µl
Temperature	25 ^{o}C
Runtime	8 min
column	Symmetry C18-ODS 4.6X150mm, 3µm particle size
Mobile phase	Potassium dihydrogen phosphate buffer, acetonitrile and water in proportion of ratio 30:65:5
Retention time	Sildenafil - 4.3 Duloxetine – 3.4
Theoretical plates	Sildenafil – 7695 Duloxetine – 6427
USP Tailing factor	Sildenafil – 1.1 Duloxetine – 1.2

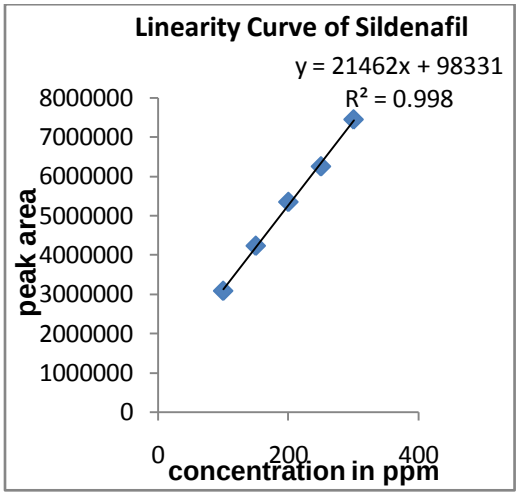


	Name	Retention Time (min)	Area (µV*sec)	Height (µV)	USP Tailing	USP Resolution	USP Plate Count
1	Duloxetine	3.417	965427	144295	1.2		6427.6
2	Sildinafil	4.360	5283408	661356	1.1	5	7695.9

Figure: 3 Chromatogram of Sildenafil and Duloxetine

Table 2: Linearity Results of Sildenafil& Linearity Results of Duloxetine

S.No	Concentration	Area	S.No.	Concentration	Area
1	100ppm	3090740	1	30ppm	567355
2	150ppm	4236091	2	45ppm	771224
3	200ppm	5350182	3	60ppm	989619
4	250ppm	6254060	4	75ppm	1147249
5	300ppm	7447171	5	90ppm	1367444
Correlation Coefficient		0.998	Correlation Coefficient		0.997



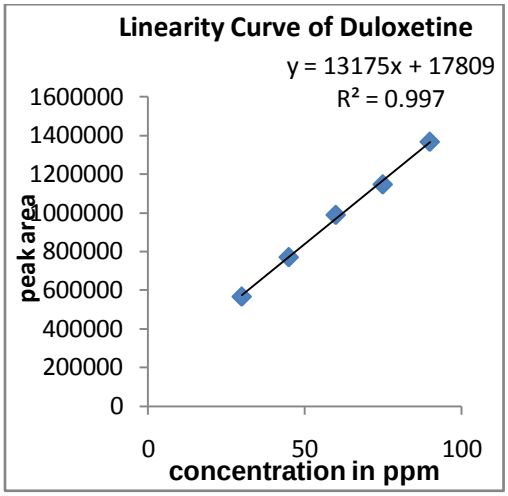


Figure: 4 and 5 calibration curves of Sildenafil and Duloxetine

Table 3: The Accuracy Results of Sildenafil & Duloxetine

Drug	%Conc. (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
Sildenafil	50%	2646318	4.97	4.87	98.0%	98.7%
	100%	5373366	10.0	9.89	98.9%	
	150%	8092466	15.0	14.9	99.3%	
Duloxetine	50%	483122	4.95	4.85	98.0%	99.0%
	100%	988952	10.0	9.93	99.3%	
	150%	1491976	15.0	14.9	99.8%	

Table 4: Results of Sildenafil and Duloxetine in Marketed Formulation

Marketed Formulation	Drug	Labeled Claim	% Amount Found	%RSD
	Sildenafil	100 mg	99.2	0.04
	Duloxetine	30 mg	99.4	0.2

Table 5: LOD & LOQ results for Sildenafil & Duloxetine

Drug	LOD	LOQ
Sildenafil	0.03	0.1
Duloxetine	0.04	0.15

Table 6: The Precision Results for Sildenafil & Duloxetine

Injection	Interday		Intraday	
	Duloxetine area	Sildenafil area	Duloxetine area	Sildenafil area
Injection-1	960428	5294078	985648	5432312
Injection-2	961631	5292758	1010235	5442193
Injection-3	974165	5310288	994445	5454219
Injection-4	992792	5330655	990554	5459742
Injection-5	965259	5296927	994637	5485151
Average	970855	5304941	995104	5454723
STD	13391.5	15972.5	9215.9	20066.0
%RSD	1.38	0.30	0.93	0.37

Table 7: The Robustness Results for Sildenafil & Duloxetine

S.No	Flow rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
Duloxetine			
1	0.5	6125.3	1.1
2	0.6	6418.3	1.0
3	0.7	4855.5	1.1
Sildenafil			
1	0.5	7251.5	1.0
2	0.6	7640.3	1.0
3	7	6369.3	1.1
Change in organic phase composition in mobile phase			
Duloxetine			
1	10% less	6844.3	1.1
2	*Actual	6418.3	1.0
3	10% more	6451.9	1.1
Sildenafil			
1	10% less	7643.2	1.0
2	*Actual	7640.3	1.0
3	10% more	7266.3	1.1

***** Results for actual flow (0.6ml/min) have been considered from Assay standard.

***** Results for actual Mobile phase composition (65:30:5 Acetonitrile: Buffer: Water) have been considered from Accuracy standard.

Summary of Validation Parameters of Proposed RP HPLC

Parameters	Sildenafil	Duloxetine
Linearity range	100-300 ppm	30-90 ppm
Wavelength (nm)	244	244
Correlation Coefficient	0.998	0.997
Slope	21462	13175
Intercept	98331	17809
LOD (µg/ml)	0.03	0.04
LOQ (µg/ml)	0.1	0.15
% RSD		
Intraday precision	0.3	1.38
Interday precision	0.37	0.93

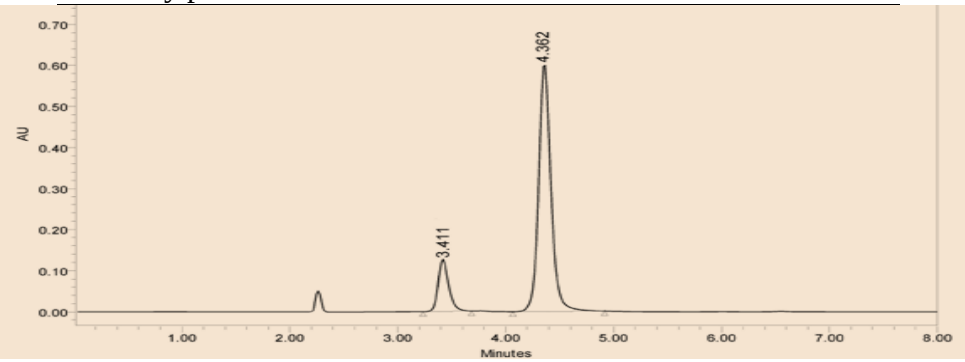


Figure 6: Acid Degradation chromatogram of Sildenafil & Duloxetine

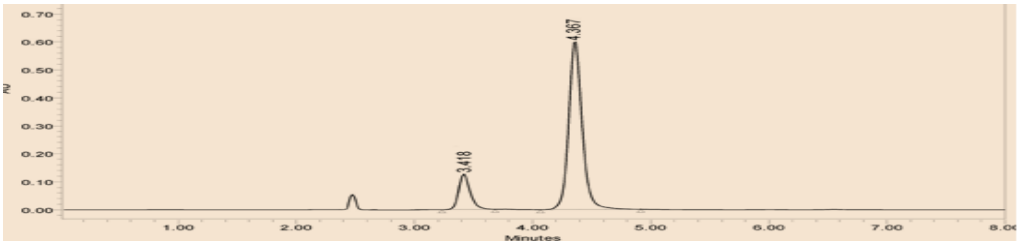


Figure 7: Base Degradation chromatogram of Sildenafil & Duloxetine

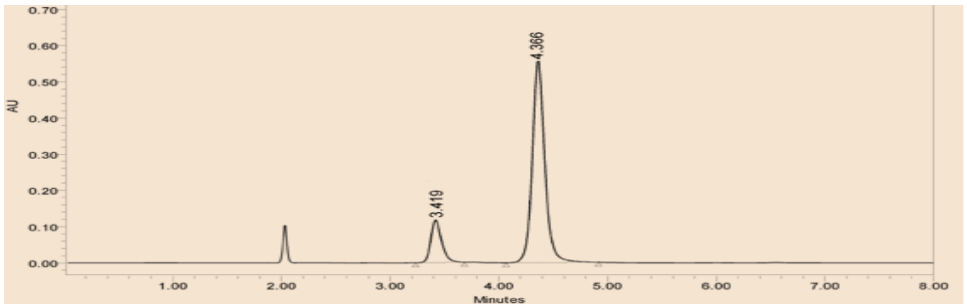


Figure 8: Peroxide Degradation chromatogram of Sildenafil & Duloxetine

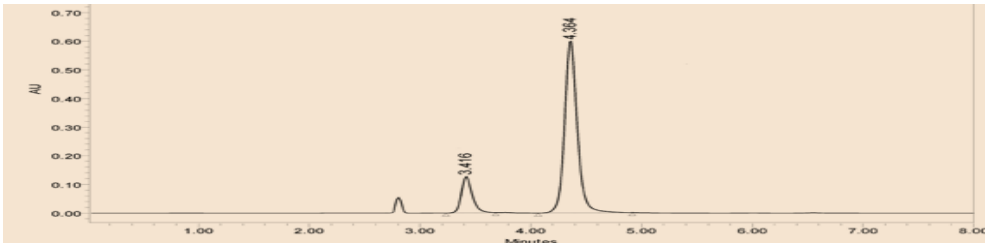


Figure 9: Thermo Degradation chromatogram of Sildenafil & Duloxetine

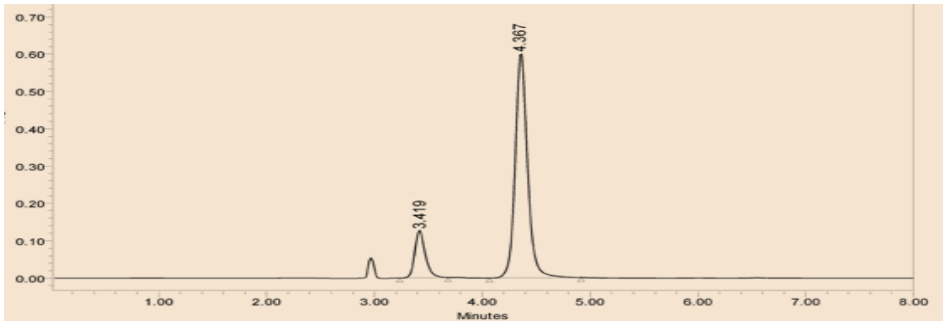


Figure 10: Photolytic Degradation chromatogram of Sildenafil & Duloxetine

From the above data of degradation profile it can be conclude that there is no interference found for Sildenafil and Duloxetine peak.

Table 8: The Degradation Results for Sildenafil (SIL) & Duloxetine (DUL)

Degradation parameter	Degradation Time	Results of Degradation							
		Peak area of degradated product		Peak area of standard		% of recovery		% of Degradation	
		SIL	DUL	SIL	DUL	SIL	DUL	SIL	DUL
Acid Degradation(0 .1N HCl)	90 min	4897195	894965	5265801	962328	93%	92.9%	7%	7.1%

Base Degradation (0.1NNaOH)	90 min	4844537	885342	5265801	962328	92%	92%	8%	8%
Peroxide Degradation (3% H_2O_2)	15 min	4475931	817979	5265801	962328	85%	85%	15%	15%
Thermal Degradation	60 min	4739221	866095	5265801	962328	90%	89.9%	10%	10.1%
Photolytic Degradation	60 min	4686563	856472	5265801	962328	89%	89%	11%	11%

CONCLUSION

Thus the proposed stability indicating RPHPLC method for the simultaneous determination of Sildenafil and Duloxetine in tablet dosage form was accurate, precise, linear, reliable, simple, economic and robust. The method has several advantages, including simple mobile phase, rapid analysis, simple sample preparation and improved selectivity as well as sensitivity. The developed method is rapid and completes a single run in relatively short time, i.e., eight minutes. The proposed method enables rapid quantification and simultaneous analysis of both drugs from commercial formulations without any excipients interference. The method can be used for routine analysis of marketed products of Sildenafil and Duloxetine in combined tablet formulation.

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