BJMHR
British Journal of Medical and Health Research
Journal home page : www.bjmhr.com

Analytical Method Development and Validation for Simultaneous Estimation of Sildenafil Citrate and Dapoxetine Hydrochloride In Pharmaceutical Dosage Form By RP-HPLC

Jitendra Patel[1], Tejas Thakar[1]*, Minakshi Dhoru[1], Sandip Dholakia[1], Madhubhai Patel[1]
1.Shankersinh Vaghela Bapu Institute of Pharmacy Vasan, Mansa Road, Gandhinagar.

ABSTRACT

Sildenafil Citrate (50mg) and Dapoxetine Hydrochloride (30mg) in combination used for the treatment of Premature ejaculation and Erectile dysfunction. Market survey and literature review revealed that there were so many methods available for single Sildenafil Citrate and Dapoxetine Hydrocloride and UV spectrophotometric method by absorbance correction method and simultaneous equation method (vierodt's method) are available in 2013 . So it was thought of intrest to develop and validate simple, accurate, sensitive and rapid new UV spectrophotometric and RP-HPLC method for simultaneous estimation of Sildenafil Citrate and Dapoxetine Hydrochloride in Pharmaceutical dosage form. In RP-HPLC method, chromatographic separation was achieved on Hypersil ODS C18 (250mm x 4.6 mm id, 5m particle size) column using Methanol:Water buffer at pH 3 (pH of buffer was adjusted to 3.0 with dilute 0.5% ortho-phoshporic acid) (95:05 v/v) as mobile phase. Detection was carried out at 225 nm.

Key words: Sildenafil Citrate, Dapoxetine Hydrochloride, RP-HPLC, Validation.

*Corresponding Author Email: jiturx@gmail.com
Received 27 July 2014, Accepted 13 August 2014

Please cite this article as: Patel *et al.*, Analytical Method Development and Validation for Simultaneous Estimation of Sildenafil Citrate and Dapoxetine Hydrochloride In Pharmaceutical Dosage Form By RP-HPLC. British Journal of Medical and Health Research 2014.

INTRODUCTION

Dapoxetine (Priligy) is a short-acting SSRI marketed for the treatment of premature ejaculation. Dapoxetine hydrochloride is one of the anti-depressants drugs called selective serotonin reuptake inhibitor (SSRI) that influence mood and behavior and is the only FDA approved drug to use in men for treatment of premature ejaculation. Premature ejaculation (PE), also commonly known as rapid or early ejaculation. It is defined by the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) Premature ejaculation (PE) is a common male sexual dysfunction1-3 whose patho-physiology is not completely understood and for which there are currently no approved pharmacological therapies. Premature ejaculation is also known as rapid ejaculation, rapid climax, premature climax, or early ejaculation.PE can be divided into two distinct entities: Acquired and Lifelong PE. Lifelong PE is a condition which has existed since the onset of sexual activity and is not reliant on either the conditions or the environment under which sexual activity is taking place. Acquired PE develops in an individual who has previously had normal ejaculatory control and can develop gradually or suddenly

As a member of selective serotonin reuptake inhibitor (SSRI) family, dapoxetine was initially created as an anti-depressant. 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 anti-depressant. Dapoxetine hydrochloride belongs to the class of drugs known as Selective Serotonin Reuptake Inhibitor (SSRI) and also acts as an anti-depressant. As the name suggest, it selectively inhibit the reuptake of serotonin in the neural cells. The inhibition of the serotonin preventing premature ejaculation in men. It is presumed that dapoxetine works by inhibiting serotonin transporter and subsequently increasing serotonin's action at pre-synaptic and post-synaptic receptors human ejaculation is regulated by various areas in the central nervous system (CNS).

Sildenafil citrate is prime treatment for erectile dysfunction. Sildenafil is a potent and selective inhibitor of cGMP-specific phosphodiesterase type 5 (PDE5), which is responsible for degradation of cGMP in the corpus cavernosum thus sildenafil protect the cyclic guanosine monophosphate (cGMP).

Erectile dysfunction (ED) or impotence is sexual dysfunction characterized by the inability to develop or maintain an erection of the penis during sexual performance. A penile erection is the hydraulic effect of blood entering and being retained in sponge-like bodies within the penis. The process is often initiated as a result of sexual arousal, when signals are transmitted from the brain to nerves in the penis. Erectile dysfunction is indicated when an erection is difficult to produce. There are various circulatory causes, including alteration of the voltage-

gated potassium channel, as in arsenic poisoning from drinking water. The most important organic causes are cardiovascular disease and diabetes, neurological problems (for example, trauma from prostatectomy surgery), hormonal insufficiencies and drug side effects. After all, erections do start in the brain. Priapism is a persistent, usually painful, erection that lasts for more than four hours and occurs without sexual stimulation. Peyronie's disease is caused by scar tissue, called plaque, which forms along the length of the penis in the corpora cavernosa.

MATERIALS AND METHOD

Reagents used

- Sildenafil Citrate (provided by Emcure pharma Pvt. Ltd., Ahmedabad.)
- Dapoxetine Hydrochloride (Provided by Emcure pharma Pvt. Ltd., Ahmedabad.)
- HPLC grade Methanol (HPLC grade, Finar Chemicals Pvt. Ltd, Ahmedabad, India)
- HPLC grade Acetonitrile (Finar Chemicals Pvt. Ltd, Ahmedabad, India)
- Ammonium acetate buffer (AR grade, Oxford Laboratory)
- Ortho-phosphoric acid (Finar Chemicals Pvt. Ltd, Ahmedabad, India)
- Distilled Water

Preparation of main standard stock solution of Sildenafil Citrate

Accurately weighed quantity of Sildenafil Citrate 50 mg was transferred into 50 mL volumetric flask. Drug was dissolved in 40 mL of Methanol:Water (95:05 v/v), sonicated for 20 minutes and then diluted up to mark with Methanol:Water (95:05 v/v). This give main standard stock solution having strength of 1000 µg/mL.

Preparation of standard stock solution of Sildenafil Citrate

From main standard stock solution of (1000 µg/mL), 12.5 mL was pipetted out into 50 mL volumetric flask and volume was adjusted up to the mark with Methanol:Water (95:05 v/v) to get 250 µg/mL standard stock solution of Sildenafil Citrate.

Preparation of working standard solution of Sildenafil Citrate

From standard stock solution of Sildenafil Citrate (250 µg/mL), 1.0 mL, 1.5 mL, 2.0 mL, 2.5 mL and 3.0 mL were pipetted out into 10 mL volumetric flask and volume was adjusted to the mark with Methanol: Water (95:05 v/v) to get 25, 37.5, 50, 62.5, 75 µg/mL of working standard solution of Sildenafil Citrate.

Preparation of main standard stock solution of Dapoxetine HCl

Accurately weighed quantity of Dapoxetine HCl 50 mg was transferred into 50 mL volumetric flask. Drug was dissolved in 40 mL of Methanol: Water (95:05 v/v), sonicated for 20 minutes and then diluted up to mark with Methanol: Water (95:05 v/v). This give a main standard stock solution having strength of 1000 µg/mL.

Preparation of standard stock solution of Dapoxetine HCl

From main standard stock solution of Dapoxetine HCl (1000 µg/mL), 7.5 mL was pipetted out into 50 mL volumetric flask and volume was adjusted to the mark with Methanol:Water (95:05 v/v) to get 150 µg/mL standard stock solution of Dapoxetine HCl.

Preparation of working standard solution of Dapoxetine HCl

From standard stock solution of Dapoxetine HCl (150 µg/mL) 1.5 mL, 2.0 mL, 2.5 mL, 3.0 mL and 3.5 mL were pipetted out into 10 mL volumetric flask and volume was adjusted to the mark with Methanol:Water (95:05 v/v) to get 15, 22.5, 30, 37.5 and 45 µg/mL of working standard solution of Dapoxetine HCl.

Preparation of standard stock solution of mixture of Sildenafil Citrate and Dapoxetine HCl

From main standard stock solution Sildenafil Citrate (1000 µg/mL) and main standard stock solution Dapoxetine HCl (1000 µg/mL), 5.0 mL and 3.0 mL were pipetteed out respectively in 50 mL volumetric flask and volume was adjusted to the mark with distilled water pH 3. Final concentration of mix working standard solution of Sildenafil Citrate and Dapoxetine HCl were 100 µg/mL and 60 µg/mL respectively.

Preparation of mix working standard solution of Sildenafil Citrate and Dapoxetine HCl.

From mix standard stock solution of Sildenafil Citrate (100 µg/mL) and Dapoxetine HCl (60 µg/mL), 2.5 mL, 5.0 mL, and 7.5 mL were pipetted out into 10 mL volumetric flask and 7.5 mL and 12.5 mL were pipette out into 20 mL volumetric flask and volume was adjusted to the mark with distilled water pH 3 to get 25, 37.5, 50, 62.5 and 75 µg/mL for Sildenafil Citrate and 15, 22.5, 30, 37.5 and 45 µg/mL for Dapoxetine HCl.

Preparation of sample solution

Twenty tablets were finely powdered and sample powder equivalent to 10 mg of Sildenafil Citrate and 06 mg of Dapoxetine HCl was weighed and transferred into a 100 mL of volumetric flask, dissolved and diluted up to mark with Methanol:Water (95:05 v/v). The solution was filtered through whatman filter paper no.42 (100 µg/mL of Sildenafil Citrate and 60 µg/mL of Dapoxetine HCl).

2.5 mL of above solution was transferred into a 10 mL volumetric flask and diluted up to the mark with distilled water (25 µg/mL of Sildenafil Citrate and 15 µg/mL of Dapoxetine HCl).This solution was used as working sample solution.

Preparation of placebo

All the ingredients except API (Magnesium stearate 5mg, sodium starch glycolate 1mg, aerosil 1mg, talcum 4mg) were accurately weighed and transferred into 100 mL volumetric

flask, dissolved and diluted up to mark with methanol. Then solution was filtered through membrane filter 0.22 µm to get clear solution. From this solution 25 mL was taken into 50 mL volumetric flask and diluted up to mark with water. Then from this solution 1 mL was transferred to 10 mL volumetric flask and diluted up to mark with water.

Preparation of mobile phase

Mobile phase was prepared by mixing 95 mL of HPLC grade methanol and 05 mL of HPLC grade water pH 3 (pH 3 was adjusted by dilute 0.5% ortho-phosphoric acid.

Selection of elution mode

Reverse phase chromatography was chosen because of its recommended use for ionic and moderate to polar compounds. It is not only simple, convenient but also better performing in terms of efficiency, stability and reproducibility. C-18 column was selected because it is least polar compared to C-4 and C-8 columns. C-18 column allows eluting polar compounds more quickly in comparison to non-polar compounds. In addition to this, PDA detector is used, which allows easy detection of the compounds in UV transparent organic solvents. A 250 x 4.6 mm column of 5µm particles packing was preferred as a starting point for method development. Isocratic mode was chosen due to simplicity in application and robustness with respect to longer column stability, shorter analysis time and reproducible Rt. This configuration provides a large number of theoretical plate's values for most separation.

Selection of wavelength

The sensitivity of RP-HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is the one that gives good response for the drugs that are to be detected. Chromatogram at 210.0, 225.0 and 280.0 nm was taken. In that at 225.0 nm, both drugs give good peak height and shape. So, 225.0 nm was selected for simultaneous estimation of Sildenafil Citrate and Dapoxetine HCl in pharmaceutical dosage form.

CHROMATOGRAPHIC CONDITIONS

- Stationary phase: C18 hypersil ODS (250 mm × 4.6 mm, 5 µm particle size)
- Mobile phase: Methanol:Water at pH 3.0 (pH of mobile phase was adjusted 3.0 with dilute Ortho-phosphoric acid) (95:05, v/v)
- Sample preparation: In Methanol
- Flow rate: 1.0 mL/min
- Column Temperature: 25±2ºC
- Injection volume: 20 µL
- Detection Wavelength: 225.0 nm
- Total run time: 06 minutes

METHOD VALIDATION

Chromatographic separation

Standard or sample solution was injected into column with 20 µL micro-syringe. The chromatogram was run for 06 minutes with mobile phase, Methanol:water at pH 3.0 (pH was adjusted 3.0 with 0.5% dilute ortho-phosphoric acid) (95:05, v/v), which was previously sonicated for 20 minutes for degassing and detection was carried out at 225.0 nm. The chromatogram was stopped after separation achieved completely. Data of peak like area, height, retention time, resolution etc was recorded using Clarity software.

System suitability test parameters

System suitability tests are used to verify that the resolution and repeatability of the system were adequate for the analysis intended. The parameters used in this test were the chromatographic peak resolution, theoretical plate number and tailing factor. The repeatability of these parameters was checked by injecting six times solution of Sildenafil Citrate and Dapoxetine HCl

Solution Stability

Standard and sample solutions were kept at 25^0C for 72 hours. Assay percentage at initial time period and at after every 24 hours were calculated. Assay percentage of initial time period was compared with these three time periods. The change in assay percentage was calculated. The % RSD of assay results should not be more than 2% for standard and sample.

Linearity and Range

The solution was prepared by pipetting out 1.5, 2.0, 2.5, 3.0 and 3.5 mL from standard stock solution of Sildenafil Citrate 250 µg/mL and 1.5, 2.0, 2.5, 3.0 and 3.5 mL from standard stock solution of Dapoxetine HCl 150 µg/mL into 10 mL volumetric flask and the volume was adjusted to mark with HPLC grade methanol:water (95:05 v/v). Mixed standard solution containing 25, 37.5, 50, 62.5 and 75 µg/mL of Sildenafil Citrate and 15, 22.5, 30, 37.5 and 45 µg/mL of Dapoxetine HCl. An aliquot of 20 µL of each solution was injected under operating chromatographic condition. Plot the calibration curve of Area versus respective concentration was plotted and found out correlation co-efficient and regression line equation for Sildenafil Citrate and Dapoxetine HCl. Each response was an average of five determinations.

Precision

Repeatability

Repeatability was determined by analyzing Sildenafil Citrate and Dapoxetine HCl test solution having the concentration 25 µg/mL of Sildenafil Citrate and 15 µg/mL of Dapoxetine HCl. The solution was measured six times.

% RSD was calculated for Sildenafil Citrate and Dapoxetine HCl.

Intraday precision

Intraday precision was determined by analysing Sildenafil Citrate and Dapoxetine HCl test solutions having concentration range 25, 50, and 75 µg/mL of Sildenafil Citrate and 15, 30, 45 µg/mL of Dapoxetine HCl. Each solution was measured in triplicate for three times in a day. % RSD was calculated for Sildenafil Citrate and Dapoxetine HCl.

Interday precision

Interday precision was determined by analysing Sildenafil Citrate and Dapoxetine HCl test solutions having concentration range 25, 50 and 75 µg/mL of Sildenafil Citrate and 15, 30 and 45 µg/mL of Dapoxetine HCl in different days. % RSD was calculated for Sildenafil Citrate and Dapoxetine HCl.

Accuracy

Accuracy was determined by calculating recovery of Sildenafil Citrate and Dapoxetine HCl by the standard addition method. Known amount of standard solution of 12.5, 25, 37.5. µg/mL of Sildenafil Citrate and 7.5, 15, 22.5 µg/mL of Dapoxetine HCl were added to pre-quantified working standard solution of 25 µg/mL of Sildenafil Citrate and 09 µg/mL of Dapoxetine HCl and get 37.5, 50.0, 62.5 µg/mL standard solution of Sildenafil Citrate and 22.5, 30, 37.5 µg/mL standard solution of Dapoxetine Hydrochloride. % RSD was calculated for Sildenafil Citrate and Dapoxetine HCl.

Limit of detection

The LOD is estimated by using standard deviation of Y-intercept and slop of calibration curve as per ICH guideline.

The LOD may be calculated as

$$\mathrm{LOD} = \frac{3.3 \times \mathrm{SD}}{\mathrm{slope}}$$

Where, SD = Standard deviation of response, Slope = Slope of the calibration curves

Limit of quantitation

The LOQ is estimated by using standard deviation of Y-intercept and slop of calibration curve as per ICH guideline.

The LOQ may be calculated as

$$\mathrm{LOQ} = \frac{10 \times \mathrm{SD}}{\mathrm{slope}}$$

Where, SD = Standard deviation of response, Slope = Slope of the calibration curves.

Robustness

The robustness study was performed to evaluate the influence of small but deliberate

variation in the chromatographic condition. The robustness was checked by changing small variation in parameters.

1) Mobile phase flow rate (±0.2mL/min)

2) pH (±0.2 units)

After each changes sample solution was injected and % assay with system suitability parameters were checked.

Analysis of prepared tablet formulation

Sample solution having strength 25 µg/mL of Sildenafil Citrate and 15 µg/mL of Dapoxetine Hydrochloride was used. The solution was measured six times.

% Recovery was calculated for Sildenafil Citrate and Dapoxetine Hydrochloride.

RESULTS AND DISCUSSION:

Results of system suitability test

Table:4.1 System suitability results for RP- HPLC method

Sr. No.	System suitability parameters	Observed Value		IP'2007 specification
		Sildenafil Citrate	Dapoxetine HCl	
1.	Number of theoretical plates (N)	4222.853	3411.598	>2000
2.	Tailing Factor (T_f)	1.215	1.178	< 2.0
3.	Resolution (R_S)	10.590		>2.0

Solution stability

Table: 4.2.1 Results of standard solution stability

Time	Assay ± S.D*		% RSD	
	Sildenafil Citrate	Dapoxetine HCl	Sildenafil Citrate	Dapoxetine HCl
Initial	99.96 ± 0.014	99.96 ± 0.011	0.0121	0.0137
After 24 hr	99.84 ± 0.101	99.86 ± 0.102	0.1003	0.1040
After 48 hr	99.41 ± 0.987	99.44 ± 0.987	0.9954	1.0003
After 72 hr	98.96 ± 1.027	98.91 ± 1.113	1.0334	1.1142

***Average of five determinations**

Table: 4.2.2 Results of sample solution stability

Time	Assay ± S.D*		% RSD	
	Sildenafil Citrate	Dapoxetine HCl	Sildenafil Citrate	Dapoxetine HCl
Initial	99.96 ± 0.014	99.94 ± 0.014	0.0171	0.0157
After 24 hr	99.44 ± 0.104	99.51 ± 0.132	0.1034	0.1331
After 48 hr	98.96 ± 0.987	98.99 ± 0.982	0.9983	0.9933
After 72 hr	98.84 ± 1.054	98.87 ± 1.104	1.0684	1.1208

***Average of five determinations**

Validation of RP-HPLC method

Linearity Range

Table:4.3.1.1 Linearity data of Sildenafil Citrate

Sr. No.	Conc.(µg/mL)	Mean Area ± S.D*	%RSD
1	25	60268.980 ± 127.530	0.236
2	37.5	152560.702 ± 289.931	0.190

3	50	255544.846 ± 319.330	0.124
4	62.5	346658.432 ± 337.302	0.097
5	75	457339.368 ± 375.120	0.082

Table:4.3.1.2 Linearity data of Dapoxetine HCl

Sr. No.	Conc.(µg/mL)	Mean Area ± S.D*	%RSD
1	15	31601.098 ± 154.100	0.487
2	22.5	59425.674 ± 211.441	0.355
3	30	89305.369 ± 269.36	0.301
4	37.5	119358.874 ± 294.03	0.246
5	45	145578.0146± 312.67	0.214

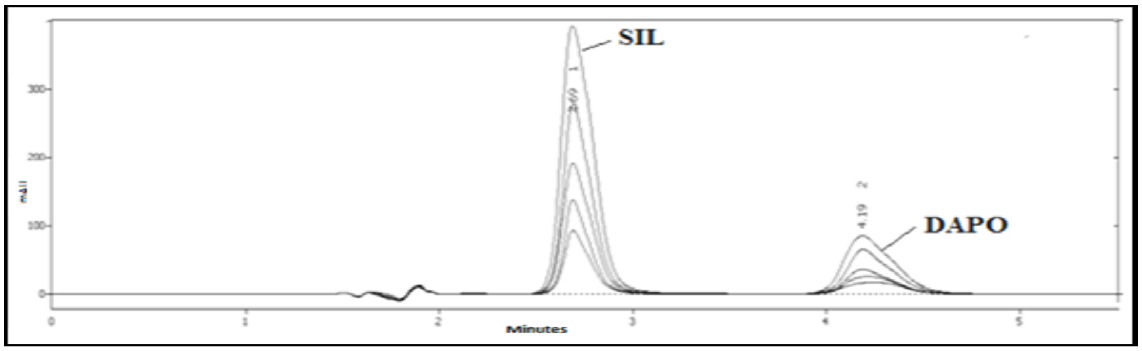

Figure 4.3.1.1 Overlain Chromatogram of for Sildenafil Citrate and Dapoxetine HCl

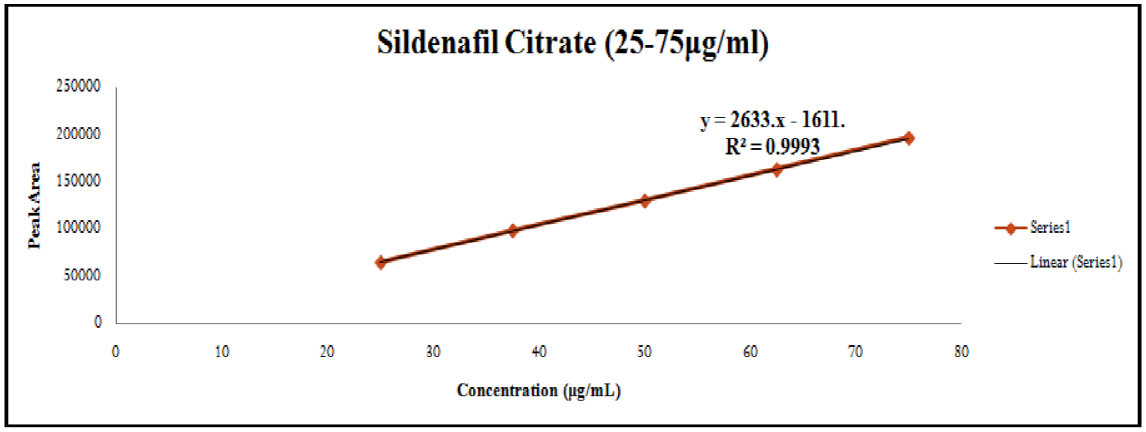

Figure: 4.3.1.2 Calibration curve of Sildenafil Citrate

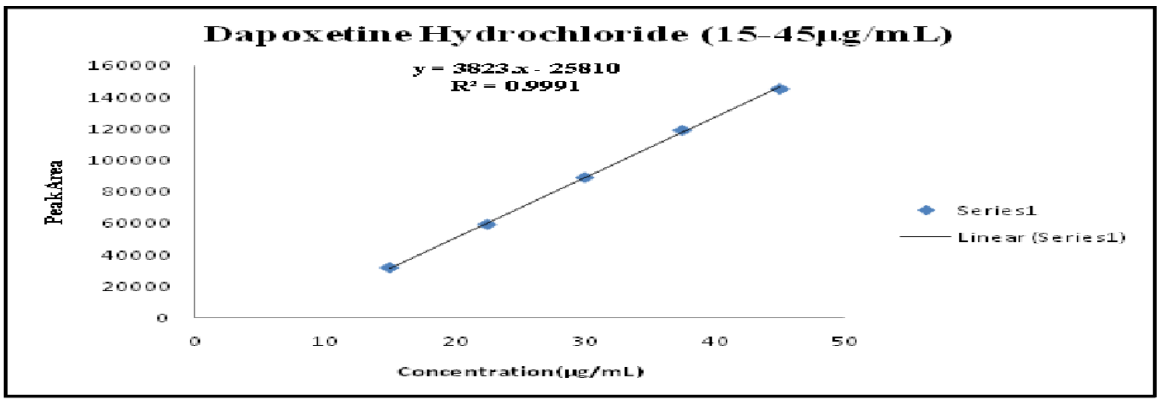

Figure: 4.3.1.3 Calibration curve of Dapoxetine HCl

Precision

Repeatability

Table: 4.3.2.1.1 Repeatability data for Sildenafil Citrate and Dapoxetine Hydrochloride

Repeatability data for Sildenafil Citrate and Dapoxetine Hydrochloride

Drug	Conc. (µg/mL)	Peak Area Mean ± S.D*	%RSD
Sildenafil Citrate	25	45023.64 ± 664.10	0.57
Dapoxetine Hydrochloride	15	40934.41 ± 708.26	0.63

***Average of six determinantions**

Intraday precision

Table:4.3.2.2.1 Intraday precision data of Sildenafil Citrate and Dapoxetine HCl

DRUG	Concentration (µg/mL)(n=3)	Peak Area Mean ± S.D*	%RSD
Sildenafil Citrate	25	60438.408 ± 160.654	0.265
	50	255483.096 ± 372.761	0.145
	75	449561.760 ± 398.713	0.088
Dapoxetine HCl	15	31529.521 ± 118.459	0.375
	30	89494.509 ± 210.632	0.235
	45	145659.979 ± 268256	0.184

Average of three determinations

Interday precision

Table: 4.3.2.3.1 Inter day precision data for estimation of Sildeafil Citrate and Dapoxetine HCl

DRUG	Conc. (µg/mL)(n=3)	Peak Area Mean ± S.D*	%RSD
Sildenafil Citrate	25	60571.773±219.027	0.361
	50	255753.814±515.982	0.201
	75	145617.757±261.248	0.179
Dapoxetine HCl	15	31757.963±130.914	0.412
	30	89740.841±300.340	0.334
	45	145631.738±296.051	0.203

Average of three determination

ACCURACY

Table: 4.3.3.1 Recovery data for Sildenafil citrate and Dapoxetine HCl

Drug	Accuracy Level	Amt. of drug (µg/mL)	Amt. of drug added (µg/mL)	Amt. Recovered Mean (µg/mL)*	Mean %Recovery ± S.D*	Mean %RSD
Sildenafil Citrate	50%	25	12.5	12.460	99.68 ±0.138	0.139
	100%	25	25	24.403	99.59 ±0.115	0.116
	150%	25	37.5	37.33	99.56 ±0.087	0.088
Dapoxetine HCl	50%	15	7.5	7.473	99.64 ± 0.155	0.156
	100%	15	15	14.903	99.35 ± 0.101	0.102
	150%	15	22.5	22.343	99.30 ± 0.088	0.089

LOD and LOQ

Table: 4.3.4.1 LOD and LOQ data of Sildenafil Citrate and Dapoxetine HCl

Parameter	Sildenafil Citrate	Dapoxetine HCl
Mean slope	2633	3823
SD	103.01	234.05
LOD (µg/mL)	0.129	0.202
LOQ (µg/mL)	0.391	0.612

Robustness

The typical variations studied under flow rate and pH. The results are shown in the table 4.3.5.1, 4.3.5.2 Variation was within the acceptable range respect to peak asymmetry and theoretical plates, so the method was found to be robust.

Table: 4.3.5.1.1 Robustness data of Sildenafil Citrate.

Parameter		Peak Area		Tailing factor		Theoretical plate	
		Mean ± S.D. (n=3)	%RSD	Mean ± S.D. (n=3)	%RSD	Mean ± S.D. (n=3)	%RSD
Flow rate	0.8 mL/min	274468.646 ± 196.188	0.0714	1.215 ± **0.0005**	0.041	4224.572 **±3.0480**	0.072
	1.2 mL/min	274388.350 ± 169.137	0.0616	1.216 **±0.0005**	0.041	4216.547 ± **2.5178**	0.059
pH	2.8 mL/min	272556.528 ± 251.476	0.0922	1.215 ± **0.001**	0.082	4221.346 ± **11.8297**	0.280
	3.2 mL/min	274500.180 ± 197.970	0.0711	1.217 ± **0.001**	0.082	4184.267 ± **9.9987**	0.239

Table: 4.3.5.1.2 Robustness data of Dapoxetine Hydrochloride

Parameter		Peak Area		Tailing factor		Theoretical plate	
		Mean ± S.D. (n=3)	%RSD	Mean ± S.D. (n=3)	%RSD	Mean ± S.D. (n=3)	%RSD
Flow rate	0.8 mL/min	107489.210 ± 294.375	0.273	1.178 ± **0.0005**	0.042	3414.574 ± **3.3604**	0.098
	1.2 mL/min	117499.861 ± 239.550	0.203	1.1790 ± **0.0005**	0.042	3351.247 ± **3.1368**	0.093
pH	2.8 mL/min	106434.256 ± 278.361	0.261	1.178 ± **0.001**	0.084	3419.354 ± **9.0223**	0.263
	3.2 mL/min	107412.439 ± 155.169	0.144	1.180 ± **0.001**	0.084	3279.687 ± **9.1303**	0.278

Table: 4.3.5.1.3 Robustness data of Sildenafil Citrate and Dapoxetine Hydrochloride

Parameter		Retention time of Sildenafil Citrate (50 µg/mL)		Retention time of Dapoxetine Hydrochloride (30 µg/mL)	
		Mean ± S.D. (n=3)	%RSD	Mean ± S.D. (n=3)	%RSD
Flow rate	0.8 mL/min	2.91 ± 0.0132	0.455	4.65 ± 0.0149	0.320
	1.2 mL/min	2.36 ± 0.0189	0.799	4.26 ± 0.0033	0.336
pH	2.8	2.76 ± 0.0186	0.673	4.28 ± 0.0067	0.158
	3.2	2.71 ± 0.0085	0.315	3.54 ± 0.0060	0.169

4.3.6 Analysis of marketed formulation

Applicability of the proposed method was tested by analyzing the commercially available KUTUB. The results are shown in table 4.3.1

Table: 4.3.6.1 Analysis of marketed formulation

Formulation	Label claim (mg/tablet)		% Assay (Mean ± S.D*)	
	Sildenafil Citrate	Dapoxetine HCl	Sildenafil Citrate	Dapoxetine HCl
KUTUB	50	30	99.20 ± 0.259	99.46 ± 0.352

SUMMARY

Table: 5.1 Summary Data of validation parameters of HPLC for Sildenafil Citrate and Dapoxetine HCl.

Sr. no.	Parameters		Sildenafil Citrate	Dapoxetine HCl
1	Linearity range (µg/ml)		25-75	15-45
2	Regression equation		y = 2633.x –1611	y = 3823.x – 25810
3	Correlation coefficient		0.9993	0.9991
4	Precision			
4.1	Repetability(n=6) % RSD		0.57	0.63
4.2	Intraday (n=3) % RSD		0.265-0.088	0.184-0.375
4.3	Interday (n=3) % RSD		0.179-0.361	0.203-0.412
5	Accuracy or % Recovery		99.56-99.68	99.30-99.64
6	LOD		0.129	0.202
7	LOQ		0.391	0.612
8	Robustness (for 5% change in pH and Flow Rate)	Flow Rate 0.8 mL/min	0.041-0.455	0.042-0.320
		Flow Rate 0.8 mL/min	0.041-0.799	0.042-0.336
		pH 2.8	0.082-0.673	0.084-0.263
		pH 3.2	0.082-0.315	0.084-0.278
9	Solution stability		Stable up to 3 days	Stable up to 3 days
10	%Assay		99.20	99.46

CONCLUSION

A simple, economic, specific and robust RP-HPLC method has been developed and validated for the estimation of Sildenafil Citrate and Dapoxetine HCl in pharmaceutical dosage form. There was no interference from any excipients in the determination of drugs in pharmaceutical dosage form which indicates the method is specific. All method validation parameters lie within its acceptance criteria as per ICH Q2 (R1) guideline so we can conclude that method is specific, linear, accurate and precise. Hence, it can be successfully used for the routine analysis of Sildenafil Citrate and Dapoxetine HCl in pharmaceutical dosage forms.

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