Stability indicating HPLC method for simultaneous quantification of sildenafil citrate and dapoxetine hydrochloride in Pharmaceutical products

Kai Bin Liew[1,2] and Kok Khiang Peh[2]*

[1]Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, No 1, Jalan Menara Gading, UCSI Height, UCSI University, Cheras, Kuala Lumpur, Malaysia

[2]School of Pharmaceutical Sciences, University Sains Malaysia, Minden, Penang, Malaysia

Abstract: A stability-indicating HPLC-UV method for the simultaneous determination of sildenafil citrate and dapoxetine hydrochloride in solution and tablet was developed. The mobile phase was comprised of acetonitrile and 0.2M ammonium acetate buffer. The analyte was eluted at 3.392min and 7.255min for sildenafil citrate and dapoxetine HCl respectively using gradient system at a flow rate of 1.5mL/min. The theoretical plates count was>2000, tailing factor <1.30, capacity factor 3.19-7.58 and peak asymmetry factor <1.08.The method was linear from 5-180 and 1-40µg/mL with a correlation coefficient of 0.9999 and 0.9994 for sildenafil citrate and dapoxetine HCl respectively. The drug solution was stable at ambient room temperature (26˚C) for 48hours.Both drugs were found susceptible to oxidation and the drug content dropped slightly in acid and alkali condition but stable under UV light and heat. No interference from tablet excipients and degradation products was found.

Keywords: Dapoxetine HCl; degradation product; sildenafil citrate; simultaneous quantification; stability indicating HPLC method; stress degradation study.

INTRODUCTION

Off-label and unregistered drug use are reported worldwide as an increasing problem. Use of drug combination other than those approved by authority agencies poses a serious threat to the consumer due to the unknown safety and toxicity profile of combination drug effect. Countless new warnings are raised each year concerning the use of unregistered pharmaceutical products that are mis-leadingly advertised for the enhancement of male sexual performance. There are many unregistered pharmaceutical products sold in the illegal drug trade markets and combination product of sildenafil citrate and dapoxetine HCL is one of the example. The safety and toxicology profile of this combination has never been proven and the product has not been approved yet for the indication by any established drug control agency in the world. Consequently, the analytical method to simultaneous identification and quantification of these two drugs are of great interest.

Sildenafil citrate is an oral agent used for the treatment of erectile dysfunction and sold under trademark of Viagra. Its chemical formula is 1-[[3-(6, 7-dihydro-7-oxo-3-propyl-1Hpyrazolo [4, 3-d] pyrimidin-5-yl)-4-ethoxyphenyl] sulfonyl]-4-methylpiperazine (Morales *et al*, 1998). Sildenafil citrate belongs to a pharmacological group called type phosphodiesterase V (PDE 5) inhibitor which inhibits V-cyclic guanosine monophosphate (cGMP) specific phosphodiesterase on penile erectile activity. It is commonly prescribed as first line oral therapy for erectile dysfunction.

Dapoxetine Hydrochloride (Dapoxetine HCl) belongs to a pharmacological group called selective serotonin reuptake inhibitor (SSRI). It's chemical name is (1S)-N,N-dimethyl-3-naphthalen-1-yloxy-1-phenylpropan-1-amine hydrochloride (Giri *et al*., 2012). Dapoxetine HCl is used for the treatment of anxiety disorder and depression. It was found to delay the ejaculation in men during sexual relationship and patented for the indication of premature ejaculation (PE) more recently (Reddy *et al*., 2010).

Laumann *et al*. (1999) reported that at least 30% of PE men have concomitant erectile dysfunction. Tang and Khoo (2011) suggested that erectile dysfunction is associated with PE. Sildenafil is recommended as the first line oral therapy for erectile dysfunction whereas dapoxetineis approved for the treatment of premature ejaculation. In view of the comorbidity cases of erectile dysfunction and premature ejaculation, combination of sildenafil citrate and dapoxetine HCl might be a potential solution. As such, there is a need to develop a rapid, simple and reproducible analytical method to simultaneously quantify both drugs in a single run.

There are a lot of publications on determination of sildenafil citrate (Reddy and Reddy, 2008; Nagaraju *et al*., 2003; Daraghmeh *et al*., 2001; Aboul-Enein and Hefnawy, 2003) or dapoxetine HCl (Giri *et al*., 2012; Hamilton and Cornpropst, 1993; Chandran and Kannan, 2012; Pandya

**Corresponding author:* e-mail: kkpehken@gmail.com

et al., 2012) by reversed phase high performance liquid chromatography (RP-HPLC) method. Up-to-date, there are only two publications on simultaneous quantification of sildenafil citrate and dapoxetine HCl using high performance liquid chromatography (Dhaduk *et al*., 2012; Langhneja *et al*., 2012). However, no stability indicating data was reported for both drugs and the standard calibration curves covered a narrow range of 2-14µg/mL for sildenafil citrate and 2.4-8.4µg/mL for dapoxetine HCl. Therefore, it was thought necessary to study the stability of Sildenafil citrate and Dapoxetine HCL under acidic, alkaline, oxidative, photolytic and heat conditions.

The aim of this study was to develop and validate a simple, rapid and reproducible stability-indicating reversed phase HPLC method to simultaneously quantifysildenafil citrate and dapoxetine HCl. Stress degradation study under acidic, alkaline, oxidative, photolytic and heat conditions were performed for the two drugs.

MATERIALS AND METHODS

Materials

Sildenafil citrate was obtained from Ind-Swift Laboratory Limited (India). Dapoxetine hydrochloride was obtained from Rakshit Drugs Pvt., Ltd. (India). Ammonium acetate, HPLC-grade acetonitrile, analytical grade hydrochloric acid, sodium hydroxide and analytical grade hydrogen peroxide solution were purchased from Merck (USA). Crospovidone XL-10 and povidone (PlasdoneK-29/32) were samples from ISP Technologies INC, (USA). Microcrystalline cellulose PH102 was obtained from FMC Corporation (USA). Mannitol was purchased from BASF (Malaysia). Starlac® was ordered from Meggle Group (Germany). Magnesium stearate was purchased from Peter Grevan (Holland). The materials were used as received.

Instrumentation

The HPLC system was comprised of a Shimadzu VP series (Japan) pump (LC-20AT vp) with solvent cabinet, a degasser (DGU-20A_3), a column oven (CTO-10S VP), an auto-injector (SIL-20A HT vp), UV/VIS detector (SPD-20A vp) and a computer software (LC-Solution VP).

Chromatographic condition

The separation was carried out using a Thermo Scientific Synchronize C-18 column (150x4.6mm ID, 5µm) (USA). The column temperature was set at 30˚C and detection wavelength of 240nm was used. Sample of 25µl was injected onto the column. The initial flow rate was set at 1.2mL/min.

Mobile phase optimization and elution mode

Different compositions of mobile phase were studied to determine the optimum mobile phase for good resolution and short elution time. Two elution methods were studied namely isocratic and gradient mode.

For isocratic elution, various compositions of mobile phase studied are shown in table 1. The 0.2M buffer solution was prepared by weighing 15.42 g of ammonium acetate and dissolving in 1L of distilled water. The buffer solution was mixed with acetonitrile accordingly. The pH was adjusted using glacial acetic acid. The mobile phase was filtered through 0.45µm nylon membrane filter (Whatman, UK) under vacuum using a filtration set and degassed using an ultrasonicator for 15 minutes.

On the other hand, for gradient elution, acetonitrile and 0.2 M buffer solution was run according to the time program presented in table 2.

Preparation of stock standard solution

Preparation of sildenafil citrate stock solution

20 mg of sildenafil citrate working standard was weighed and transferred into 25mLvolumetric flask.20 mL mobile phase comprising of acetonitrile and 0.2M ammonium acetate buffer solution (1:1, v/v) added to dissolve the drug. The volumetric flask was shaken using ultrasonicator for 5 min. The solution was diluted with mobile phase to volume. The stock standard solution had a concentration of 800µg/mL of sildenafil citrate.

Preparation of dapoxetine HCl stock solution

40 mg of dapoxetine HCl working standard was weighed and transferred to a100mLvolumetric flask. 50mL of mobile phase comprising of acetonitrile and 0.2M ammonium acetate buffer solution (1:1, v/v) was then added to dissolve the drug. The volumetric flask was shaken using ultrasonicator for 5 min. The solution was diluted with mobile phase to make up the volume. The stock standard solution had a concentration of 400µg/mL of dapoxetine hydrochloride.

Preparation of working standard solution

Preparation of sildenafil citrate working standard solution

5mL of sildenafil citrate stock standard solution (800 µg/mL) was pipetted into 10-mL volumetric flask and diluted to final volume with mobile phase comprising of acetonitrile and 0.2M ammonium acetate buffer solution (1:1, v/v) and mixed well. This Working Standard Solution had a concentration of 400µg/mL of sildenafil citrate.

Preparation of dapoxetine HCl working standard solution

5mL of stock dapoxetine HCl standard solution (400µg/mL) was pipetted into 10-mL volumetric flasks and diluted to final volume with mobile phase comprising of acetonitrile and 0.2M ammonium acetate buffer solution (1:1, v/v) and mixed well. This Working Standard Solution had a concentration of 200µg/mL of dapoxetine HCl.

Preparation of standard drug solutions for standard calibration curve

Few concentrations of calibration standard containing combination of sildenafil citrate and dapoxetine HCl at ratios of 5/1, 10/2, 20/4, 40/8, 80/16, 120/32 and 180/40 µg/mL were prepared. The standard calibration curve was constructed using peak area versus known concentrations of sildenafil citrate and dapoxetine HCl. The linearity of sildenafil citrate and dapoxetine HCl was conducted using six set of the calibration standards.

Preparation of quality control (QC) standard solutions

Three concentrations of quality control samples containing combination of sildenafil citrate and dapoxetine HCl at 15/3µg/mL (low QC), 90/20µg/mL (medium QC) and 150/30µg/mL (high QC), were prepared and used in method validation.

System suitability study

The chromatographic parameters, such as, theoretical plates (N), tailing factor (T), peak asymmetry factor (As), capacity factor (k') and Resolution were calculated.

The number of theoretical plates (N) which is used to describe the quality of chromatographic column was determined from the following equation:

$$N = 16\left(\frac{t}{W}\right)^2$$

Where t = retention time of analyte and W=width of analyte peak.

The tailing factor (T) was determined from the following equation:

$$T = \frac{W_{0.05}}{2f}$$

Where $W_{0.05}$=Width of peak at 5% height and f = distance from the peak maximum to the leading edge of the peak. The value of not more than 2.0 is acceptable (Meyer, 1993; United States Pharmacopoeia, 2003).

Peak asymmetry factor (As) is the simplest way of measuring the degree of peak distortion (skew). The peak asymmetry was determined at 10% peak height. For a tailed peak, As>1. For a fronted peak, As<1. For a symmetric peak, As=1. The peak asymmetry factor was determined by the following equation.

$$As = \frac{a}{b}$$

Where b is the distance between the peak maximum point and the latest eluting porting of the curve, and a is the distance between the peak maximum point and the earliest eluting portion of the curve (Paul, 2000). The recommended acceptance criteria for asymmetry factor is between 0.9 to 1.1 (Snyder *et al*., 1997).

Capacity factor (k') is an indicator of efficiency of a column to retain sample molecule during an isocratic separation. Literature proposed the acceptable k' value ranges from 2-10 (Snyder *et al*., 1997). The capacity factor was determined by the following equation:

$$K' = \frac{T_R - T_o}{T_o}$$

Where TR is the analyte retention time and To is the non-retained substance retention time.

Resolution is calculated by the equation below:

$$RS = \frac{2(RT_a - RT_b)}{(W_a - W_b)}$$

Where RT_a and RT_b are the retention times of peak a and b and W_a and W_b are the widths at the baseline of the peaks.

Height Equivalent to Theoretical Plate (HETP) is calculated by the equation below:

$$HETP = \frac{L}{N}$$

Where L is the length of column and N is the number of theoretical plate.

Precision and accuracy

Three quality control standard solutions containing combination of sildenafil citrate and dapoxetine HCl at concentrations of 15/3µg/mL, 90/20µg/mL and 150/30µg/mL as well as LOQ (5/1µg/mL) were prepared to determine the method precision and accuracy. For intra-day precision and accuracy, six sets of quality control standard solutions at four different concentrations were assayed on the same day. For inter-day precision and accuracy, six replicates of each quality control standard solution were injected over six consecutive days, with one standard curve on each day. The coefficient of variation (%CV) was determined to assess the precision of the assay. The coefficient of variation was derived from the following equation:

$$CV(\%) = \frac{S\tan darddeviation}{Meanvalue} \times 100\%$$

The accuracy was presented as the relative percentage error (% bias) of calculated concentration of the samples. % Bias was computed using the following equation:

$$\%Bias = \frac{(Calculatedconcentration - Cstd)}{Cstd} \times 100\%$$

Where C_{std}= the concentration of standard solution

Limit of quantification (LOQ)

The LOQ was the lowest point of concentration in the calibration curve. Acceptance criteria were precision (RSD 2%) and accuracy (% Bias 2%).

Stock solution stability

Stock solution stability was determined by keeping stock solutions of sildenafil citrate (800µg/ml) and dapoxetine

hydrochloride (400µg/ml) at room temperature (26˚C, 65% RH) and the samples were diluted to a concentration within the linear range and injected at 6 and 48 hours. The instrumental responses at 6 and 48 hours were compared with that of fresh samples at zero hour.

Robustness

Two parameters were evaluated for robustness namely flow rate and pH of mobile phase. Each factor selected was changed at three levels (-1, 0, +1) with respect to optimized parameters. Robustness of the method was done at the concentration level of high QC (150/30 µg/mL). The three selected flow rates were 1.4, 1.5 and 1.6mL/min whereas the selected pH values of buffer were 7.4, 7.5 and 7.6.

Preparation of tablet containing sildenafil citrate and dapoxetine HCl

100 tablets containing sildenafil citrate and dapoxetine HCl were prepared and used for stress degradation study. The tablets were prepared by wet granulation method. Crospovidone XL-10 was added intra-granularly (20%) and extra-granularly (80%). Sildenafil citrate, dapoxetine HCl, crospovidone XL-10, povidone, Starlac, mannitol and microcrystalline cellulose, were sieved through a mesh no. 60 of 0.4mm in diameter (Retsch, Germany). The powders were mixed for 5min (Kenwood, UK). Sufficient amount of distilled water was added to form a wet mass. The wet granules were dried in an oven (Carbolite, England) at 45°C for 2 hours. After drying, the dried granules were again passed through a mesh no. 35 of 0.25mm in diameter (Retsch, Germany). Crospovidone XL-10 was added extra-granularly to the granules and mixed. Magnesium stearate was added prior to compression into tablest. The tablet formulation is shown in table 3.

Application of HPLC method for determination of sildenafil citrate and dapoxetine HCL in tablet

Six (6) tablets were weighed and crushed using mortar and pestle separately. A portion of powder weight equivalent to the mean weight of six tablets (containing 70mg of sildenafil citrate and 35mg of dapoxetine HCl respectively) was taken and dissolved in a 100mL volumetric flask with mobile phase comprising of acetonitrile and 0.2M ammonium acetate buffer solution. The solution was subjected to sonication for 15 minutes. 1mL was drawn and diluted with mobile phase to 10mL in a volumetric flask to give a drug concentration of 70 µg/mL of sildenafil citrate and 35µg/mL of dapoxetine HCl. 25µL sample was injected into the HPLC system.

Stress degradation studies

Drug solution containing combination of sildenafil citrate and dapoxetine HCL at 700µg/mL and 350µg/mL respectively was prepared by weighing 70mg of sildenafil citrate (equivalent to 50 mg of sildenafil) and 35mg of dapoxetine HCl powder (equivalent to 30mg of dapoxetine) and dissolving them in a 100mL volumetric flask. On the other hand, six (6) tablets were crushed with mortar and pestle separately. Powder with weight equivalent to the mean weight of six tablets (containing 70mg of sildenafil citrate and 35 mg of dapoxetine HCl respectively) was taken and dissolved in a 100mL volumetric flask. The mixture of 0.2M ammonium acetate buffer and ACN (50:50, v/v) was used as solvent.

For acid degradation study, 1mL of the sample solution was transferred into a 10mL volumetric flask. Two sets of flasks for each study were prepared. 3mL of 3M HCl was added into each of the flask. For the first set, 3mL of 3M NaOH was added immediately to neutralise the solution and adjusted to volume. It was served as zero hour sample. 25µL of the solution was injected into HPLC. Another set of flasks was left on the bench under room temperature (28˚C / 65% RH) and the same neutralization procedure was performed after three hours.

For alkali degradation study, 1mL of the sample solution was transferred into a 10mL volumetric flask. Two sets of flasks for each study were prepared. 3mL of 3M NaOH was added into each of the flask. For the first set, 3mL of 3M HCl was added immediately to neutralise the solution and the adjusted to volume. It was served as zero hour sample. 25µL of the solution was injected into HPLC. Another set of flasks was left on the bench under room temperature (28˚C / 65% RH) and the same neutralization procedure was performed after three hours.

For oxidative degradation with hydrogen peroxide, 1mL of the sample solution was transferred into a 10mL volumetric flask. Two sets of flasks for each study were prepared. 3mL of 35% H_2O_2 added into each of the flask. For the first set, the solution was adjusted to volume and 25µL of the solution was injected into HPLC immediately. It was served as zero hour sample. Another set of flasks was left on the bench under room temperature (28˚C/65% RH) and the same procedure was performed after three hours.

For heat degradation study, 1mL of the sample solution was transferred into a 10mL volumetric flask. Two sets of flasks for each were prepared. For the first set, the solution was adjusted to volume and 25µL of the solution was injected into HPLC immediately. It was served as zero hour sample. Another set of flasks was heated in water bath at 80˚C and the samples were injected after heating for 2 hours.

For UV light degradation, 1mL of the sample solution was transferred into a 10mL volumetric flask. Two sets of flasks for each study were prepared. For the first set, the solution was adjusted to volume and 25µL of the solution was injected into HPLC immediately. It was served as zero hour sample. Another set of flasks was stored in UV cabinet (254 nm) and the samples were injected after 24 hours.

Table 1: Various compositions of mobile phase used in isocratic elution

Acetonitrile: 0.2M Ammonium acetate buffer (v/v)	pH	Flow rate (mL/min)
30: 70	7.20	1.2
30: 70	6.00	1.2
30: 70	5.00	1.2
50: 50	7.40	1.2
70: 30	7.70	1.5
90: 10	7.90	1.5

Table 2: Various compositions of mobile phase and time programme for gradient system

Time Programme	Acetonitrile: 0.2M Ammonium acetate buffer (v/v)		
	Trial 1	Trial 2	Trial 3
0.00-4.49 min	50:50	50:50	50:50
4.50-7.99 min	60:40	75:25	90:10
8.00-8.01 min	50:50	50:50	50:50
8.02-9.00 min	50:50	50:50	50:50

RESULTS

Method development

Gradient elution system was used due to shorter retention time of interested compound. The composition of mobile phase of trial 3 was selected based on good separation and the shortest retention time of 3.39min for sildenafil citrate and 7.26min for dapoxetine HCl. A typical chromatogram of a combination of sildenafil citrate and dapoxetine HCl at 5µg/mL and 1µg/mL is shown in fig. 1.

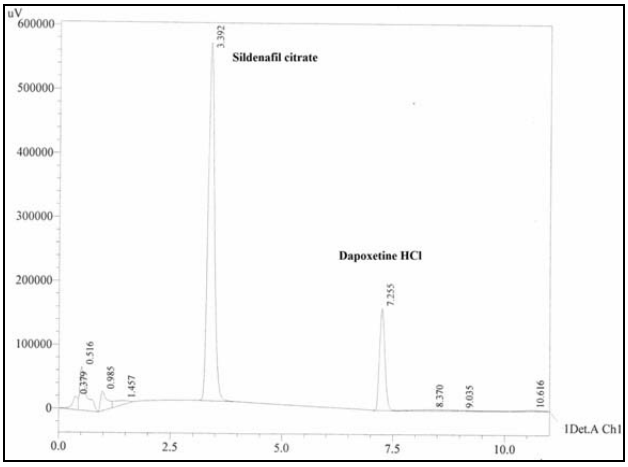

Fig. 1: Chromatogram of 5ug/mL of sildenafil citrate and 1ug/mL dapoxetine HCI solution (RT Sildenafil citrate = 3.392min and RT dapoxetine HCI=7.255 min)

System suitability

The results of theoretical plates (N), tailing factor (T), peak asymmetry factor (As), capacity factor (k'), resolution and Height Equivalent to Theoretical Plate (HETP) of the method at three QC concentrations and LOQ are shown in table 4 (a) for sildenafil citrate and table 4 (b) for dapoxetine HCl. The average theoretical plate was> 2000. The analyte peak tailing factor was < 2 and peak asymmetry factor was between 0.9-1.1. The capacity factor was in the ideal range of 2-10 (Snyder *et al*., 1997).

Table 3: Tablet formulation containing sildenafil citrate and dapoxetine HCl.

Ingredient	Amount per tablet (mg)
Sildenafil citrate	70
DapoxetineHCl	35
Crospovidone XL-10	5
Starlac	180
Mannitol	100
Povidone	5
Microcrystalline cellulose	100
Magnesium stearate	5

Linearity

The standard calibration curve exhibited an excellent linearity and a good correlation coefficient over the given range of 5-180µg/mL for sildenafil citrate and 1-40µg/mL for dapoxetine HCl. The mean linear regression equation from six calibration curves was y=36338.67 (±565.94) x-3057.50 (±18752.89) for sildenafil citrate and y=42687.00 (±523.86) x+9886.02 (±2217.86), (x=drug concentration, y=average peak area) with a correlation coefficient of 0.9999 (±0.0003) for sildenafil citrate and 0.9997 (± 0.0002) for dapoxetine HCl. The six standard calibration curves were injected over six days to test the reproducibility of the method. The results are presented in table 5.

Precision and accuracy

The results of precision and accuracy are shown in table 6 (a) for sildenafil citrate and table 6 (b) for dapoxetine HCl. For sildenafil citrate, precision and accuracy were tested at four concentrations namely LOQ, 15µg/mL,

Table 4(a): Results of system suitability tests for sildenafil citrate drug solution. Mean ± SD, N = 6.

Parameters	Sildenafil citrate concentration (µg/mL)			
	5.0	15.0	90.0	150.0
Theorectical plates	2697.79±159.28	2719.34±142.47	2686.08±121.73	2682.48±84.22
Tailing factor	1.21±0.03	1.21±0.01	1.23±0.01	1.24±0.01
Peak Asymmetry factor	1.07±0.03	1.05±0.04	1.06±0.03	1.07±0.02
Capacity factor	3.19±0.83	3.65±1.06	3.53±0.94	3.76±0.38
Resolution	12.21±0.08	13.46±0.10	11.84±0.05	12.78±0.12
HETP	55.77±3.34	55.29±3.01	55.94±2.52	55.96±1.78

Table 4(b): Results of system suitability tests for dapoxetineHCl solution. Mean ± SD, N = 6.

Parameters	DapoxetineHCl concentration (µg/mL)			
	1.0	3.0	20.0	30.0
Theoretical plates	19260.69±161.70	18318.10±248.10	16972.57±202.94	16419.19±91.54
Tailing factor	1.14±0.01	1.19±0.02	1.28±0.01	1.30±0.01
Peak Asymmetry factor	1.05±0.03	1.03±0.04	1.05±0.04	1.06±0.02
Capacity factor	6.54±1.65	7.39±2.12	7.11±1.87	7.58±0.71
Resolution	16.45±0.10	16.16±0.06	15.80±0.05	15.64±0.03
HETP	7.79±0.07	8.19±0.11	8.84±0.12	9.23±0.24

Table 5: Results of six standard calibration curves.

No	Slope		Intercept		R^2	
	Sildenafil	Dapoxetine	Sildenafil	Dapoxetine	Sildenafil	Dapoxetine
1	35214	41861	34315	10020	0.9996	0.9993
2	36411	43191	-16223	7841.5	0.9990	0.9998
3	36501	42266	-13136	13770	0.9999	0.9995
4	36475	42714	-11214	7888.9	0.9998	0.9998
5	36750	43017	-7135	9084.7	0.9998	0.9998
6	36681	43073	-4952	10711	0.9999	0.9998
Mean	36338.67	42687.00	-3057.50	9886.02	0.9999	0.9997
SD	565.94	523.86	18752.89	2217.86	0.0003	0.0002

90µg/mL and 150µg/mL. Intra-day precision was in the range of 0.36-1.82% whereas intra-day accuracy was in the range of -1.00-1.52%. Inter-day precision and accuracy was in the range of 0.38-0.98% and 1.47-1.78% respectively. For dapoxetine HCl, precision and accuracy were tested at four concentrations namely LOQ, 3µg/mL, 20µg/mL and 30µg/mL. Intra-day precision was in the range of 0.79-1.32% whereas intra-day accuracy was in the range of 0.83- (-2.00)%. Inter-day precision and accuracy was in the range of 0.66-1.23% and -1.00-1.95% respectively. The results were within the ±2% range recommended by USP guideline. Hence, the method shows good precision and accuracy.

LOQ

The LOQ were 5µg/mL and 1µg/mL for sildenafil citrate and dapoxetine HCl respectively.

Stock solution stability

The percentage of sildenafil citrate remaining after six and forty eight hours kept under room temperature was 99.73% and 99.50% respectively, whereas for dapoxetine HCl were 99.70% and 99.32% respectively. The results suggest that the stock solution is stable at least for 48 hours under room temperature.

Robustness

The results of robustness are presented in table 7. The difference when the level of the parameters changed was not significant and acceptable (<2%). The results showed that the analytical system was robust.

Assay content

The validated HPLC method was applied to check the assay content of sildenafil citrate and dapoxetine HCl simultaneously in the tablet. The assay content calculated in this study was in a tight range within 98-102%.

Stress degradation studies

The results of acid, alkali, oxidation, heat and UV degradation are shown in table 8. The chromatograms of oxidative degradation study of tablets at three hour are presented in fig. 2. The method was able to separate both analytes and their degradation product peaks were eluted at 0.991min, 6.048min and 6.525min. Sildenafil citrate and dapoxetine HCl were found easily oxidised by

Table 6(a): Results of intra-day and inter-day precision and accuracy for sildenafil citrate. N=6.

Conc. (µg/mL)	Intra-day			Inter-day		
	Mean± SD (µg/mL)	Precision (% CV)	Accuracy (% Bias)	Mean ± SD (µg/mL)	Precision (% CV)	Accuracy (% Bias)
5.00	4.95±0.09	1.82	-1.00	5.08±0.05	0.98	1.60
15.00	15.21±0.13	0.85	1.40	15.22±0.08	0.53	1.47
90.00	91.37±0.50	0.55	1.52	91.56±0.45	0.49	1.73
150.00	152.16±0.55	0.36	1.44	152.67±0.58	0.38	1.78

Table 6(b): Results of intra-day and inter-day precision and accuracy for dapoxetine HCl. N=6.

Conc. (µg/mL)	Intra-day			Inter-day		
	Mean ± SD (µg/mL)	Precision (% CV)	Accuracy (% Bias)	Mean ± SD (µg/mL)	Precision (% CV)	Accuracy (% Bias)
1.00	0.98±0.01	1.02	-2.00	0.99±0.01	1.01	-1.00
3.00	3.04±0.04	1.32	1.33	3.04±0.02	0.66	1.33
20.00	20.34±0.16	0.79	1.70	20.39±0.25	1.23	1.95
30.00	30.25±0.24	0.79	0.83	30.44±0.25	0.82	1.47

Table 7: Results of robustness of analytical system, N=3.

Parameters		Sildenafil citrate	Dapoxetine HCl
		RSD (%)	RSD (%)
Flow rate (mL/min)	1.4	0.82	0.49
	1.5	0.63	1.32
	1.6	1.10	1.28
pH	7.4	0.67	0.95
	7.5	0.63	1.32
	7.6	1.18	1.67

Table 8: Results of stress degradation studies. Mean ± SD, N = 3.

Parameter		Percentage (%)			
		Assay of sildenafil citrate at zero hour	Assay of sildenafil citrate at three hour	Assay of Dapoxetine HCl at zero hour	Assay of Dapoxetine HCl at three hour
Acid hydrolysis	In drug solution	98.66±0.08	91.87±0.02	101.43±0.12	93.78±0.08
	In tablet formulation	99.87±1.87	88.34±0.57	100.23±0.43	90.88±0.26
Alkaili hydrolysis	In drug solution	99.26±0.05	97.72±0.10	100.79±0.04	99.10±0.02
	In tablet formulation	99.81±0.21	96.22±0.12	99.83±0.38	98.25±0.33
H_20_2 Oxidation	In drug solution	20.87±0.37	0.19±0.01	81.13±1.45	1.29±0.07
	In tablet formulation	25.36±0.79	0.54±0.09	78.34±1.55	0.28±0.63
Heat degradation	In drug solution	99.97±0.04	100.00±0.08	100.03±0.09	103.33±0.73
	In tablet formulation	99.16±0.84	100.45±0.25	100.72±0.16	99.38±0.08
UV degradation	In drug solution	100.00±0.12	100.10±0.05	100.07±0.19	100.37±0.20
	In tablet formulation	99.33±0.07	100.21±0.05	99.29±0.15	98.48±0.12

hydrogen peroxide even just after exposure for 5 minutes (from preparation to injection). The assay of both drugs dropped slightly in acid and alkali condition but was stable in UV light and heat.

DISCUSSION

A few types of column were tried to achieve good separation and resolution between sildenafil citrate and dapoxetine HCL. The columns were Thermo Scientific Synchronise C18 column (250x4.6 mm I.D., 5µm), Thermo Scientific Synchronise C18 column (150x4.6 mm I.D., 5 µm), Zorbax CN column (250x4.6mm I.D., 5 µm) and Zorbax Phenyl-Hexyl column (250 x 4.6 mm I.D., 5 µm). Thermo Scientific Synchronise C18 column (150 x 4.6 mm I.D., 5 µm) was chosen as the optimum column due to shorter retention time and better separation. The mobile phase composition and flow rate were varied to

achieve the optimum chromatographic conditions. Increase in acetonitrile content in mobile phase yielded shorter retention time of analyte peaks. However, the elution time of the analytes was too long for all the compositions of acetonitrile and 0.2 M ammonium acetate buffer studied in isocratic system, ranging from 15-25 min, which was unsuitable for analysis of large quantity of samples.

CONCLUSION

A simple, rapid and reproducible stability indicating HPLC method was successfully developed to simultaneously quantify sildenafil citrate and dapoxetine HCl in tablets. Both drugs were stable under heat and UV, dropped slightly in acidic and alkali condition, but very susceptible to hydrogen peroxide degradation.

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