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Research Article

Development and Validation of Spectrophotometric Method for Simultaneous Determination of Sildenafil Citrate and Dapoxetine Hydrochloride in Their Combined Dosage Formulation

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ABSTRACT

A simple, accurate and precise spectrophotometric method has been developed for simultaneous estimation of Sildenafil Citrate and Dapoxetine Hydrochloride in combined dosage form. Simultaneous equation method is employed for simultaneous determination of Sildenafil Citrate and Dapoxetine Hydrochloride from combined dosage forms. In this method, the absorbance was measured at 291 nm for Sildenafil Citrate and 230nm for Dapoxetine Hydrochloride. Linearity was observed in range of 6-42µg/ml and 2-10µg/ml for Sildenafil Citrate and Dapoxetine Hydrochloride respectively. Recovery studies confirmed the accuracy of proposed method and results were validated as per ICH guidelines. The method can be used for routine quality control of pharmaceutical formulation containing Sildenafil Citrate and Dapoxetine Hydrochloride

Keywords: Sildenafil Citrate, Dapoxetine Hydrochloride, Simultaneous Estimation, UV Spectrophotometery

INTRODUCTION

Sildenafil Citrate (SIL; 1-[[3-(6, 7-dihydro-1-methyl-7-oxo-3-propyl-1Hpyrazolo [4, 3-d]pyrimidin-5-yl)-4-ethoxyphenyl]sulfonyl]-4-methylpiperazine citrate **[Figure 1]** is phosphodiesterase type 5 (PDE5) inhibitor and used in Sexual Dysfunction[1-3]. Dapoxetine Hydrochloride (DPX; (+)(S)-N,N-dimethyl-□-[2-(1-naphthalenyloxy)ethyl]-benzenemethanamine hydrochloride [**Figure 2**] is Serotonin Reuptake Inhibitor and used in premature ejaculation, epilepsy [4]. The combination of these drugs (100 mg SIL and 60 mg DPX) has been recently approved for the treatment of Sexual Dysfunction and premature ejaculation.

The literature reveals that several spectrometric and HPLC methods available for individual Sildenafil Citrate[5-8].A number of HPLC method are reported for DPX determination of DPX[9].no method has been reported for simultaneous estimation by U.V Spectrophotometric method in their combined tablet formulation.

MATERIALS AND METHODS

Apparatus

A shimadzu model 1700(Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained

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by UV-Probe system software (UV Probe version 2.31). An Electronic analytical balance (Acculab) and an ultrasonic bath were used in the study.

Reagents and Materials

Reference standard of SIL and DPX were obtained from Emcure Pharma. (Pune, India) as a gift sample, whereas their formulation obtained from local market. Analytical grade Methanol obtained from Finar Chemicals (Mumbai, India).

Preparation of standard stock solution

An accurately weighed quantity of SIL (50 mg) and DPX (50 mg) were transferred to a separate 0 ml volumetric flask and dissolved and diluted to the mark with methanol. Take 10 ml of above solution into 100 ml volumetric flask and dilute the mark with distill water to obtain standard solution having concentration of SIL (100 µg/ml) and DPX (100 µg/ml). This solution was used as working standard solution.

Method

In simultaneous equation method, six working standard solutions having concentration 6, 10, 14, 18, 22, 26, 30, 34, 38, 42 µg/ml for SIL and 2, 3, 4, 5, 6, 7, 8, 9, 10 µg/ml for DPX were prepared in Methanol and measured the absorbance at 291 nm (λ_{max} of SIL) and 230nm (λ_{max} of DPX), calculate absorptivity coefficients were calculated using calibration curve.

The concentration of two drugs in the mixture can be calculated using following equations

$$C_x = \frac{A_2 a_{y_1} - A_1 a_{y_2}}{a_{x_2} a_{y_1} - a_{x_1} a_{y_2}} \ldots\ldots\ldots\ldots (1)$$

$$C_y = \frac{A_1 a_{x_2} - A_2 a_{x_1}}{a_{x_2} a_{y_1} - a_{x_1} a_{y_2}} \ldots\ldots\ldots\ldots (2)$$

Where A_1, A_2 are absorbance of mixture at 291 nm (λ_1) and 230nm (λ_2) respectively, a_{x_1} and a_{x_2} are absorptivities of SIL at λ_1 and λ_2 respectively, a_{y_1} and a_{y_2} are absorptivities of DPX at λ_1 and λ_2 respectively, C_x and C_y are concentrations of SIL and DPX respectively.

METHOD VALIDATION [10]

Linearity

The calibration curves were plotted over a concentration range of 6-42 µg/ml for SIL and 2-10 µg/ml DPX. Accurately measured standard stock solutions of each SIL (0.6ml, 1.0ml, 1.4ml, 1.8ml, 2.2ml, 2.6ml, 3.0ml, 3.4ml, 3.8ml, and 4.2ml) and DPX (0.2ml, 0.3ml, 0.4ml, 0.5ml, 0.6ml, 0.7ml, 0.8ml, 0.9ml, 1ml) were transferred to a series of 10 ml volumetric flask separately and diluted up to the mark with methanol. The absorbance of solution was then measured at 291 nm and 230nm. The calibration curves were constructed by plotting absorbance versus concentration and the regression equations were calculated.

Precision

Intraday Precision

Mixed solutions containing 6-42 µg/ml SIL and 2-10 µg/ml DPX was analyzed 3 times on the same day and % RSD was calculated.

Interday Precision

Mixed solutions containing 24-64 µg/ml SIL and 6-16 µg/ml DPX was analyzed on 3 different day and % RSD was calculated.

Accuracy

The accuracy of the method was determined by calculating recoveries of SIL and DPX in mixture by the standard addition method. Known amount of standard solutions of SIL (0, 2, 4 and 6 µg/mL) and DPX (0, 1.2, 2.4 and 3.6 µg/mL) were added to a pre-quantified sample solution of 10 µg/mL SIL + 6 µg/mL DPX mixture. The absorbance of SIL and DPX were recorded at λ_1 and λ_2. The percentage recovery was calculated by measuring the absorbance of both drug at their absorbance maxima and fitting these values into simultaneous equation. Each response was average of three determinations.

Limit of detection and Limit of quantitation

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The limit of detection (LOD) and the limit of quantitation (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where, σ = the standard deviation of the response and S = slope of the calibration curve.

Analysis of tablet dosage form

Take 10 tablets and weighed. Find out average weight. Take tablet powder equivalent to 50 mg of SIL and 30 mg of DPX was transferred in 100 ml volumetric flask, dissolved and diluted up to mark with methanol. The solution was sonicated for 15minutes. Filter the solution through Whatman filter paper no.42 and discard first few drops of filtrate. Pipette out 0.2 ml of the above solution in 10ml volumetric flask and diluted to mark with methanol. Absorbance of the resulting solution was measured at 291.0 nm and 230.0 nm against methanol, relative concentration of two drugs in the sample was calculated using above equation (1) and (2).

RESULT AND DISCUSSION

In simultaneous equation method, the primary requirement for developing a method for analysis is that the entire spectra should follow the Beer's law at all the wavelength, which was fulfilled in case of both these drugs. The two wavelengths were used for the analysis of the drugs were 291 nm (λ_{max} of SIL) and 230nm (λ_{max} of DPX) at which the calibration curves were prepared for both the drugs. The overlain UV absorption spectra of SIL (291 nm) and DPX (230nm) in methanol is shown in [**Figure 3]**. The validation parameters were studied at all the wavelengths for the proposed method. Accuracy was determined by calculating the recovery and the mean was determined [**Table 2]**. The method was successfully used to determine the amounts of SIL and DPX present in the tablet dosage forms. The results obtained were in good agreement with the corresponding labeled amount [**Table 3]**. Precision was calculated as repeatability and intra and inter day variations (% RSD) for both the drugs.

CONCLUSION

The developed simultaneous equation method is found to be simple, sensitive, accurate and precise and can be used for routine analysis of SIL and DPX. The developed method was validated as per ICH guidelines. Statistical analysis proved that the method is repeatable and selective for the analysis of SIL and DPX in their combined pharmaceutical formulations.

Figure 1: The chemical structure of Sildenafil Citrate

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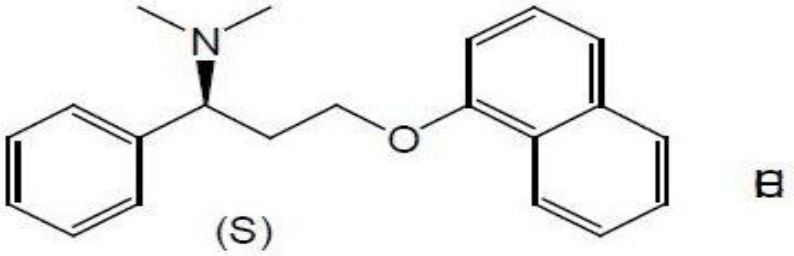

Figure 2: The chemical structure of Dapoxetine Hydrochloride

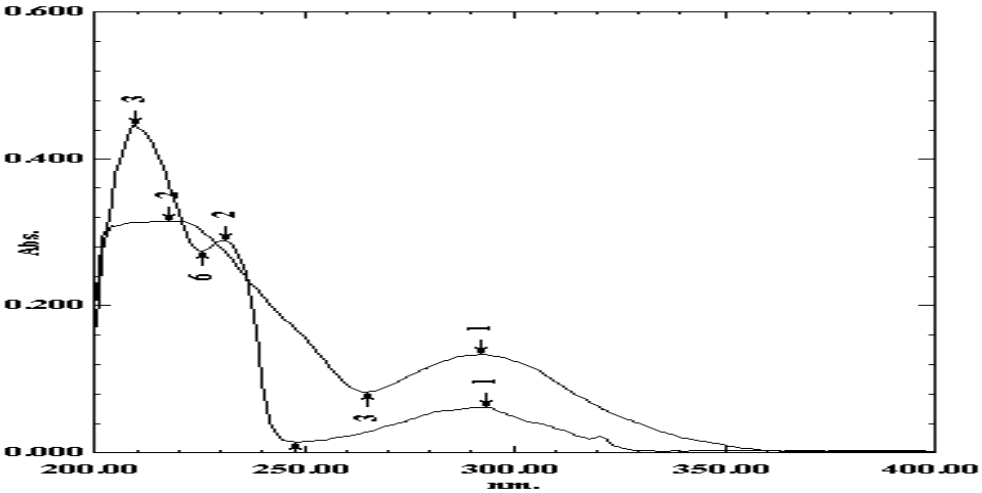

Figure 3: Overlain absorption spectra of SIL (291 nm) and DPX (230nm) in methanol.

Table 1: Regression Analysis Data and Summary of Validation Parameters for the Proposed Method

Sr. No	Parameters	SIL	DPX
1	Wavelength range (nm)	291	230
2	Beer's law limit (µg/ml)	6-42	2-10
3	Regression equation (y = mx + c)	y = 0.0196x + 0.0137	y = 0.089x + 0.022
4	Slope	0.0196	0.089
5	Intercept	0.0137	0.022
6	Correlation Coefficient (r^2)	0.9993	0.9995
7	System Precision (%R.S.D)[a] 1.Intraday Precision(n = 3) 2.Interday Precision(n = 3)	 0.53-0.89% 0.87-1.23%	 0.47-0.71% 1.20-1.59%
8	Accuracy (% recovery) (n = 3)	98.98-100.49%	99.11-100.44%
9	LOD[b](µg/ml)	0.70	0.28
10	LOQ[c] (µg/ml)	1.12	0.54
11	Assay (±S.D.)[d] (n = 3)	99.6 ± 0.84	100.3 ± 0.78

[a]RSD = Relative standard deviation. [b]LOD = Limit of detection. [c]LOQ = Limit of quantitation
[d]SD is Standard deviation and n is number of replicates

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Table 2: Recovery Data of Proposed Method

Drug	Level	Amount taken (µg/ml)	Amount added (µg/ml)	Amount added (%)	% Mean recovery (±S.D) (n = 3)
SIL	I	10	0	0	99.50 ± 1.7
	II	10	2	20	100.49 ± 1.74
	III	10	4	40	98.98 ± 1.18
	IV	10	6	60	100.35 ± 1.36
DPX	I	6	0	0	99.80 ± 1.56
	II	6	1.2	20	99.11 ± 0.43
	III	6	2.4	40	99.17 ± 0.86
	IV	6	3.6	60	100.44 ± 1.12

S.D is Standard deviation and n is number of replicates.

Table 3: Analysis of SIL and DPX by Proposed Method

Formulation	Labeled claim (mg)		Amount found (mg)		% Label claim (±S. D.) (n = 3)	
Kutub Tablet	SIL	DPX	SIL	DPX	SIL	DPX
	50	30	49.8	30.10	99.6 ± 0.84	100.3 ± 0.78

S.D. is Standard deviation and n is number of replicates.

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