RESEARCH ARTICLE

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DEVELOPMENT AND VALIDATION OF HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY METHOD FOR SIMULTANEOUS ESTIMATION OF SILDENAFIL CITRATE AND DAPOXETINE HYDROCHLORIDE IN THEIR COMBINED TABLET DOSAGE FORM

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Abstract: The present manuscript describes new, simple, accurate, and precise high performance thin layer chromatography method for the simultaneous determination of Sildenafil Citrate and Dapoxetine Hydrochloride in combined tablet dosage form. Chromatographic separation of the drugs was performed on aluminium plates are coated with silica gel 60 F_{254} as the stationary phase and the solvent system consisted of Chloroform: Methanol: Acetonitrile : Glacial acetic acid (5:1:2.5:1.5 v/v). Densitometric evaluation of the separated zones was performed at 292 nm. The two drugs were satisfactorily resolved with R_f values 0.82 and 0.56 for Sildenafil Citrate and Dapoxetine Hydrochloride respectively. The linear regression data for the calibration plots showed good relationship with r^2 = 0.993 from 500-1500 ng/spot for Sildenafil Citrate and r^2 = 0.995 from 1000-3000 ng/spot for Dapoxetine Hydrochloride. The methods were validated for precision, accuracy, and recovery. The percentage recovery for Slidenafil Citrate was found to be 100.02% – 100.08% and 99.95% – 99.99%.Dapoxetine Hydrochloride. The limits of detection and quantification were 143.3625 and 434.4318 ng/spot per spot for Sildenafil Citrate and 315.4586 and 955.9351 ng/spot per spot Dapoxetine Hydrochloride, respectively.

Key words: Sildenafil Citrate, Dapoxetine Hydrochloride High Performance Thin Layer Chromatography

INTRODUCTION

Sildenafil Citrate (SIL) is Sildenafil citrate 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 is a compound of the pyrazolo-pyrimidinyl-methyl piperazine class, It is a selective inhibitor of cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type 5 inhibitor [1-4]. And used in For treatment of erectile dysfunction, To relieve symptoms of pulmonary arterial hypertension (PAH), For treatment of premature ejaculation[2].

It is official in British Pharmacopoeia (BP-99). Literature survey also reveals Spectrophotometric[5-6], HPLC[7-11], HPTLC[12] methods for determination of AMB with other drugs.

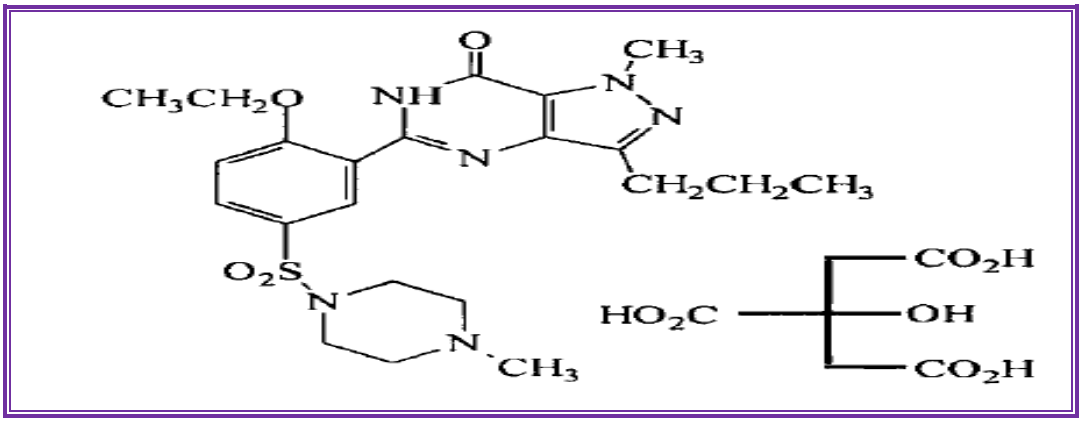

Figure 1 Structure of Sildenafil Citrate

Dapoxetine Hydrochloride (DAP) is chemically as (S)-N, Ndimethyl- 3-(naphthalen-1-yloxy)-1-phenylpropan-1-amine[13]. It is indicated for the treatment of Premature ejaculation and erectile dysfunction in men aged 18-64. Literature survey reveals, RP-HPLC[14, 1] methods for determination of DAP with other drugs.

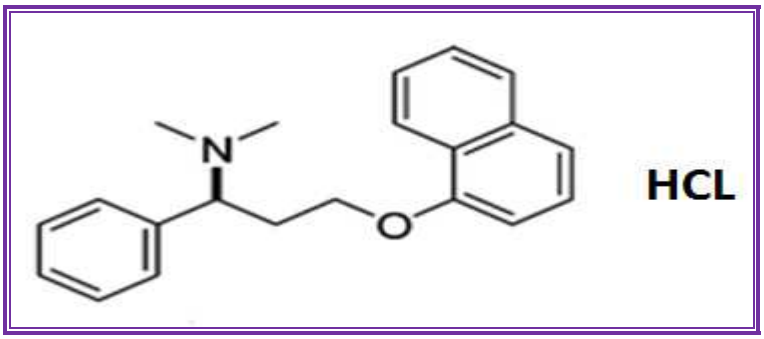

Figure 2 Structure of Dapoxetine Hydrochloride

The combined dosage forms of SIL and DAP are available in the market for the Treatment of Premature Ejaculation and Erectile dysfunction. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of SIL and DAP in their combined dosage forms. Literature survey does not reveal any simple HPTLC method for simultaneous estimation of SIL and DAP in combined dosage forms. The present communication describes simple, specific, rapid, accurate and precise chromatographic method based on High Performance Thin Layer Chromatographic method for simultaneous estimation of both drugs in their combined tablet dosage forms.

MATERIALS AND METHODS

Reagents and Materials

SIL and DAP bulk powder was kindly Purchased by Sunrise pharmaceutical Ltd, Ahmedabad Gujarat, India and Jai Radhe Sales, Ahmedabad, Gujarat, India respectively. The commercial fixed dose combination product SUPER P-FORCE (SIL – 100 mg, DAP – 60 mg) was procured from the local market which is manufactured by Sunrise Remedies Ltd.

All chemicals and reagents were of analytical grade and were purchased from Thermo fisher scientific Pvt. Ltd, Mumbai, India.

Instrumentation

CAMAG HPTLC instrument (Camag Muttenz, Switzerland) was used in this method. CAMAG HPTLC is equipped with CAMAG TLC scanner-3, Linnomate V Automatic sample applicator controlled by WIN CATS software (1.4.3 version). Aluminium packed silica Gel 60 F_{254} HPTLC plates (100 X 100 mm, layer thickness 0.2mm, E.MERCK). Linear ascending development was carried out in a 20 cm × 10 cm twin trough glass chamber (Camag Muttenz, Switzerland). The source of radiation used was deuterium lamp emitting a continuous UV spectrum between 190 to 400 nm.

Optimized chromatographic condition

Stationary phase:

Pre-coated silica gel 60 F_{254} Aluminium Plates (10x10cm)

Mobile phase:

Chloroform: Methanol: Acetonitrile: Glacial acetic acid (5:1:2.5:1.5 v/v)

Chamber saturation: 20 minutes

Development distance: 70mm

Development time: 15 minutes

Relative temperature: 25 ± 2˚C

Scanning Speed: 20 mm/sec

Detection wavelength: 292 nm

SIL R_f: 0.82

DAP R_f: 0.56

Preparation of standard stock solutions

The powder equivalent to 100 mg SIL and 60 mg DAP was accurately weighed and transferred to volumetric flask of 100 ml capacity. 50 ml methanol was transferred to volumetric flask and sonicated for 10 minutes. The flask was shaken and volume was made up to the mark with methanol. The above solution was filtered through what man filter paper (0.45µ). 2.5 ml of this aliquot was added to 25 ml volumetric flask and Volume was made up to the mark with Methanol to give a solution containing 1200 ng/µl SIL and 2000 ng/µl DAP. This solution was used for the estimation of DAP and SIL

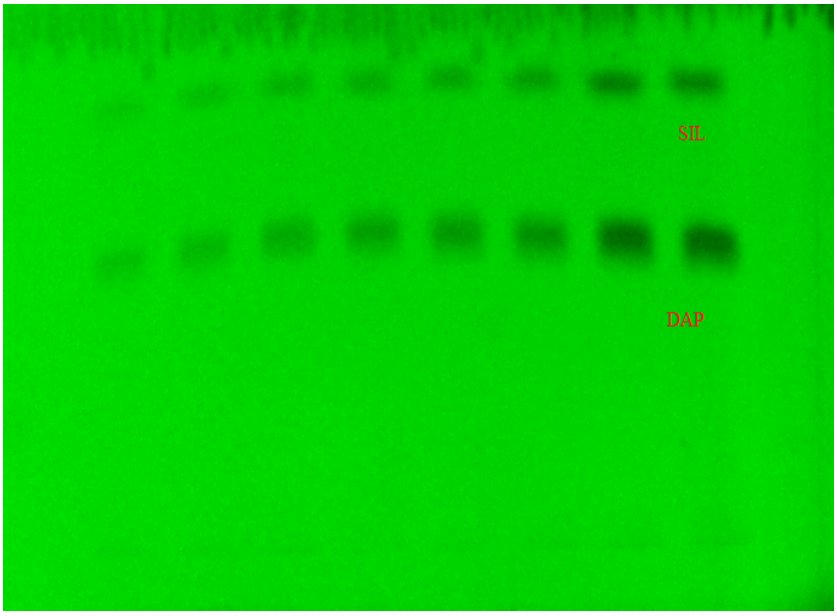

Figure 3 Photograph of developed HPTLC plate of SIL and DAP

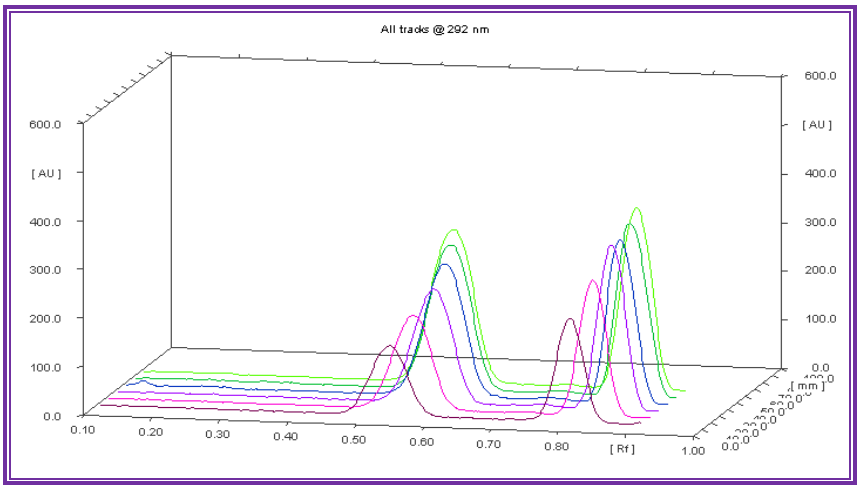

Figure 4 Overlain view of all tracks of SIL and DAP at 292 nm

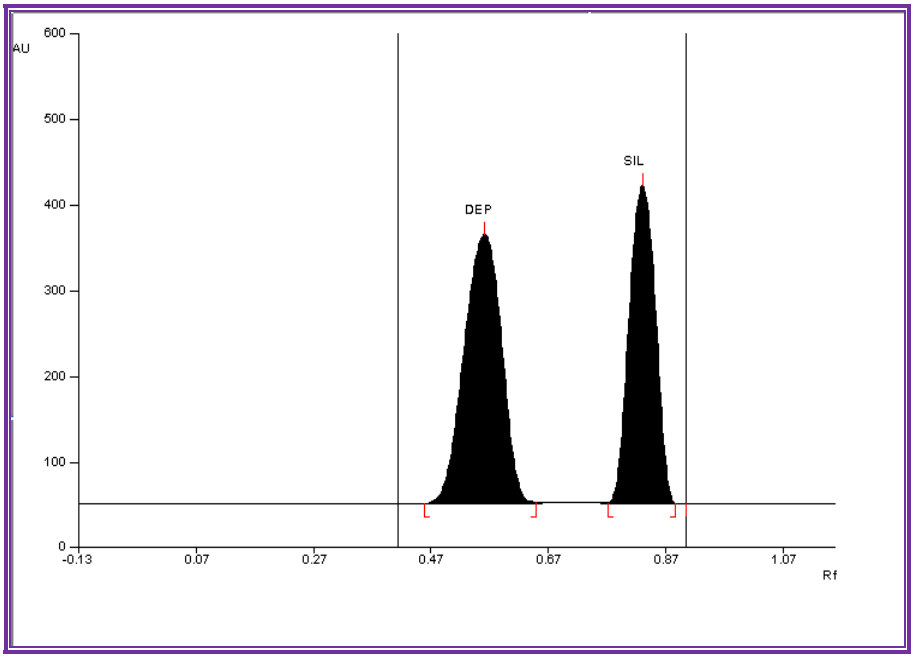

Figure 5 Densitogram of marketed formulation containing 1200 ng/spot SIL and 2000 ng/spot DAP

VALIDATION OF THE PROPOSED METHOD

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines[15].

Linearity and range

From the mixed standard stock solution 500-1500 ng/µl of SIL and 1000-3000 ng/µl of DAP, 1 to 5 µl solution spotted on HPTLC plate to obtain final concentration 1200ng/spot for SIL and 2000 ng/spot for DAP. Each concentration was applied six times to the HPTLC plate. The plate was then developed using the previously described mobile phase and the peak areas were plotted against the corresponding concentrations to obtain the calibration curves.

Precision

The precision of the method was verified by repeatability and intermediate precision studies.

a) Repeatability

Repeatability studies were performed by analysis of all concentrations (500, 750, 1000, 1250, and 1500 ng/spot for SIL and 1000, 1500, 2000, 2500, and 3000 ng/spot for (DAP) of the drug in six times on the same day.

b) Intermediate precision

The intermediate precision of the method was checked by intra day and inter day study. The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of SIL and DAP (500, 1000, 2000 ng/spot for SIL and 1000, 2000, 3000 ng/spot for DAP). The result was reported in terms of relative standard deviation (% RSD).

Specificity

The specificity of the method was determined by analyzing standard drug and test samples. The spot for SIL and DAP in the samples was confirmed by comparing the R_f and spectrum of the spot with that of a standard. The peak purity of SIL and DAP was determined by comparing the spectrum at three different regions of the spot i.e. peak start (S), peak apex (M) and peak end (E).

Accuracy

Accuracy of the method was carried out by applying the method to drug sample (SIL and DAP combination tablet) to which know amount of SIL and DAP standard powder corresponding to 80, 100 and 120% of label claim had been added (standard addition method), mixed and the powder was extracted and analyzed by running chromatogram in optimized mobile phase.

ANALYSIS OF SIL AND DAP IN COMBINED TABLET DOSAGE FORM

The powder equivalent to 100 mg SIL and 60 mg DAP was accurately weighed and transferred to volumetric flask of 100 ml capacity. 50 ml methanol was transferred to volumetric flask and sonicated for 10 minutes. The flask was shaken and volume was made up to the mark with methanol. The above solution was filtered through what man filter paper (0.45µ). 2.5 ml of this aliquot was added to 25 ml volumetric flask and Volume was made up to the mark with Methanol to give a solution containing 1200 ng/µl SIL and 2000 ng/µl DAP. This solution was used for the estimation of DAP and SIL.3.5 µl of the prepared sample was applied on pre-washed TLC plate, developed in the above mobile phase, dried in air and photo metrically analyzed by running chromatogram in optimized mobile phase. From the peak area obtained in the chromatogram, the amounts of both the drugs were calculated.

RESULTS AND DISCUSSION

The results of validation studies on simultaneous estimation method developed for SIL and DAP in the current study involving as the mobile phase for HPTLC are given below. Chloroform: Methanol: Acetonitrile : Glacial acetic acid (5:1:2.5:1.5 v/v). The proposed method was found to be simple, specific, accurate, and precise for the routine simultaneous estimation of two drugs. The linearity range for SIL and DAP were found to be 500 – 1500 ng/spot and 1000-3000 ng/spot respectively. Regression analysis data and summary of all validation parameters is given in Table 1. Precision was calculated as repeatability (% RSD) and intra and inter day variation (% RSD) for both the drugs.

Accuracy was determined by calculating the recovery and the mean was determined. The LOD and LOQ were found to be 143.3625 and 434.4318 ng/spot respectively for SIL and 315.4586 and 955.9351 ng/spot respectively for DAP indicates sensitivity of the proposed method. The peak purity of SIL and DAP was assessed by comparing their respective spectra at the peak start, apex and peak end positions of the spot. The peak purity was found to be 0.999 and 0.992 for SIL and DAP respectively. The method was successfully used to determine the amounts of SIL and DAP present in tablets. The results obtained are in good agreement with the corresponding labelled amount. By observing the validation parameters, the method was found to be specific, accurate and precise. Hence the method can be employed for the routine analysis of these drugs in combinations.

CONCLUSION

Introducing HPTLC into pharmaceutical analysis represents a major step in terms of quality assurance. Today HPTLC is rapidly becoming a routine analytical technique due to its advantages of low operating costs, high sample throughput and the need for minimum sample preparation. The major advantage of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase-unlike HPLC - thus reducing the analysis time and cost per analysis.

The developed HPTLC technique is precise, specific and accurate. Statistical analysis proves that the method is suitable for the analysis of SIL and DAP in pharmaceutical formulation without any interference from the excipients. The common excipients and other additives are usually present in the tablet dosage form do not interfere in the analysis of SIL and

DAP in method, hence it can be conveniently adopted for routine quality control.

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Table 1

Regression analysis data and summary of validation parameters for the proposed method

Parameters	High Performance Thin Layer Chromatography method	
	SIL	DAP
Concentration Range (ng/spot)	500 – 1500	1000 – 3000
Slope (m)	4.24672	3.93872
Intercept (c)	4837.27	2003.047
Correlation Coefficient (r^2)	0.9935	0.9959
Accuracy (% recovery) (n = 3)	100.02 – 100.08 %	99.95 – 99.99%
Repeatability (%RSD) (n = 6)	0.40 %	0.63 %
Intraday (n = 3) (%RSD)	0.34 – 0.71 %	0.75 – 0.19 %
Interday(n = 3) (%RSD)	0.18 – 0.95 %	0.37 – 0.34 %
LOD (ng/spot)	143.36	315.45
LOQ (ng/spot)	434.43	955.93

Table 2

Recovery data of proposed method

Drug	Level	Amount taken (ng/spot)	Amount Recovered (ng/spot) (n=3)	% Recovery (n=3)
SIL	80 %	960	960.2	100.02±0.045
	100 %	1200	1200.9	100.08 ±0.035
	120 %	1440	1441.2	100.08 ±0.070
DAP	80 %	1600	1599.2	99.95 ± 0.029
	100 %	2000	1996.6	99.98 ± 0.080
	120 %	2400	2399.9	99.99 ± 0.051

Table 3

Analysis of SIL and DAP by proposed method

	Label claim (mg)		Amount taken (ng/spot)		Amount Recovered (ng/spot) (n=3)		% Label claim	
Tablet	SIL	DAP	SIL	DAP	SIL	DAP	SIL	DAP
SUPER P-FORCE	100	60	1200	2000	1198.49	2059.04	99.87 %	102.95 %

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