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PHARMACY INFOPEDIA
ISSN: 2347-7881

Research Article

Development and Validation of HPTLC Method for Simultaneous Estimation of Sildenafil Citrate and Dapoxetine Hydrochloride in Combined Dosage Form

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ABSTRACT

A novel, precise, accurate and economic high-performance thin-layer chromatographic (HPTLC) method was developed, optimized and validated for simultaneous determination of Sildenafil Citrate and Dapoxetine Hydrochloride. The chromatographic separation was performed on precoated silica gel 60 GF_{254} plate with hexane: methanol: diethyl amine 9.2:1.6:1.2 (v/v/v) as mobile phase. The plate was developed to distance of 8.0 cm at ambient temperature. The developed plate was scanned and quantified at their single selected wavelength of 241 nm for Sildenafil Citrate and Dapoxetine Hydrochloride. Experimental conditions such as band size, chamber saturation time, migration time of solvent front, etc. were critically studied and the optimum condition were selected. The drugs were satisfactorily resolved with R_F 0.21 ± 0.02 for Sildenafil Citrate and 0.72 ± 0.02 for Dapoxetine Hydrochloride. The method was validated for linearity, accuracy, precision, and specificity. The calibration plot was linear between 2000–12000 ng per spot for Sildenafil Citrate and 1200–7200 ng per spot for Dapoxetine Hydrochloride. The limits of detection for Sildenafil Citrate and Dapoxetine Hydrochloride were 210 and 75ng per spot respectively and limit of quantification for Sildenafil Citrate and Dapoxetine Hydrochloride were 450 and 240ng per spot respectively. It is a user-friendly and important tool for analysis of combined fixed dosage forms. Methods were validated statistically and recovery studies were carried out. The method herein described can be employed for quality control and routine analysis of drugs inpharmaceutical formulations.

Keywords: Sildenafil Citrate, Dapoxetine Hydrochloride, HPTLC, Validation

INTRODUCTION

Sildenafil Citrate (SIL) is chemically *N*1-[[3-(6, 7-dihydro-1-methyl-7-oxo-3-propyl-1*H*pyrazolo [4, 3-*d*] pyrimidin-5-yl)-4-ethoxyphenyl] sulfonyl]-4-methylpiperazine citrate[1] **(Figure 1)** is a well-known phosphodiesterase type 5 (PDE5) inhibitor[2]. It is official in Indian Pharmacopoeia (IP) [3], United State Pharmacopoeia (USP). IP and USP describe liquid chromatography method for its estimation [4-5]. Literature survey reveals UV [6-8], HPLC [9-12], HPTLC [13-17] methods for estimation of SIL alone. Literature survey also reveals HPLC methods for determination of SIL with other drugs in combination. Dapoxetine Hydrochloride (DPX) is chemically (+)(*S*)-N,N-dimethyl-□-[2-(1-naphthalenyloxy)ethyl]-benzenemethanamine hydrochloride[18-20] **(Figure 2).** Dapoxetine Hydrochloride (DPX) is not official in any Pharmacopoeia. Literature survey reveals HPLC [21-22] methods for determination of DPX alone. The combination

How to cite this article: CA Prajapati, BS Patel, R Badmanaban; Development and Validation of HPTLC Method for Simultaneous Estimation of Sildenafil Citrate and Dapoxetine Hydrochloride in Combined Dosage Form; PharmaTutor; 2014; 2(10); 142-152

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ISSN: 2347-7881

of these two drugs is not official in any pharmacopoeia; hence no any official method is available for the simultaneous estimation of SIL and DPX in their combined synthetic mixture or dosage forms. Literature surveys reveal only simultaneous method UV and HPLC for SIL and DPX in synthetic mixture or combined dosage forms. The present communication describes simple, sensitive, rapid, accurate, precise and cost effective chromatographic method.

EXPERIMENTS

Apparatus

Chromatographic separation of drugs was performed on Merck TLC plates precoated with silica gel 60 F254 (20×20 cm, with 250µm layer thickness). The samples were applied onto the plates as a band with 6 mm width using Desaga 100 µl sample syringe (Hamilton, Switzerland) with an AS-30win sample applicator (Desaga, Switzerland). Linear ascending development was carried out in a twin trough glass chamber (200x100). Densitometric scanning was performed using Densitometer CD-60 TLC scanner (Proquant) in the range of 254- 366 nm and operated by proquant software (Desaga).

Reagents and materials

Working standards of SIL and DPX were procured as gift sample from Emcure pharmaceutical, pune, India. Silica gel 60F_{254} TLC plate (20×20 cm, layer thickness 0.2 mm, Merck, Mumbai) was used as a stationary phase. All chemicals and reagent used were of analytical grade. Tablet containing SIL (100mg) and DPX (60mg) were procured from local pharmacy store (Olmesar-AV, Macleod Pharma).

Chromatographic conditions

Chromatography was performed on 10 cm × 10 cm precoated silica gel 60 F254 plate. Mixture of standard and sample solutions of SIL and DPX (5.0µl) were applied to the plates as 6-mm bands, 10 mm from X-coordinate and 10 mm from Y-coordinate by use of a DESAGA Sample applicator fitted with a 100-µL syringe. The constant application rate was 5s/µl and a nitrogen aspirator was used. Plates were developed with Hexane-Methanol-Diethyl amine (9.2:1.6:1.2 v/v/v) as mobile phase. Linear ascending development was performed in a 10 cm × 10 cm chromatography glass chamber previously equilibrated with mobile phase vapor for 20 min at room temperature (the optimum chamber-saturation conditions). The development distance was 80 mm (Approximately 12ml mobile phase was used for each development, which required 15 min). After development the plates were dried at room temperature. Densitometric scanning was performed with a DESAGA densitometer in reflectance mode at the wavelengths of 241 nm for SIL and for DPX. The slit height 0.40 mm and width 0.10 mm, and the scanning rate were 20 mm/s. The source of radiation used was the deuterium lamp. Evaluation was done by linear regression of peak area response against amount of drug. Proquant software was used for peak-area measurement and data processing.

Selection of detection wavelength

After chromatographic development bands were scanned over the range of 200-300 nm and the spectra were overlain. It was observed that both drugs showed considerable absorbance at close to 200 nm and 300nm. While selecting wavelength for measurement using multiple wavelengths the base line showed better at 241 nm .So, this was selected for quantitation as shown in **Figure 3** .

Standard Preparation

10 mg of SIL reference substance were accurately weighed and transferred to 10 ml volumetric flask.10 mg of DPX reference substance were accurately weighed and transferred to another 10 ml volumetric flask The powder was dissolved in 5 ml methanol

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ISSN: 2347-7881

with vigorous shaking and diluted up to 10 ml with same solvent to furnish concentration of 1000 µg ml^{-1} SIL and 1000 µgml^{-1} DPX. Take 4 ml from SIL volumetric flask and 2.4 ml from the DPX volumetric flask in 10ml volumetric flask diluted with methanol to 10 ml to furnish concentration of 400 µg ml^{-1} and 240 µg ml^{-1}. This solution was utilized to prepare the solution for linearity range**(fig. 4)**

Preparation of Tablet Formulation

Twenty tablets for SIL and DPX were weighed and ground to a fine powder separately 1:0.6 ratio of label claim quantity of powder equivalent to 100 mg SIL and 60 mg DPX were weighed, mixed and transferred to a 100 ml volumetric flask. The powder was sonicated to dissolve in 60 ml methanol and diluted up to mark with methanol. The solution was filtered through Whatman filter paper No. 41 then 4 ml of the filtered solution was diluted to 10ml with the same solvent to furnish a solution containing 400µg ml^{-1} and 240 µg ml^{-1} SIL and DPX respectively **(fig. 5)(Table-1)**

Method Validation[23-26],

The method was validated by establishing linearity, accuracy, inter day and intra day precision of measurement and repeatability of sample application, robustness and ruggedness. The limit of detection and limit of quantification were also determined.

Linearity and Range

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. Linearity was studied by analyzing six concentrations of the drug, and process was repeated for five times each. It was done over the concentration range of 2000-12000ng/spot for SIL and 1200-7200ng/spot for DPX.The calibration curves were constructed by plotting peak areas versus concentrations.

Precision

Intra-day precision

Intra day precision was found by carrying out the analysis of standard drugs at three different concentrations in the linearity range of the drugs for three times on the same day. Each concentration was applied in triplicates and % RSD was calculated.

Inter- day precision

Inter day precision was found by carrying out the analysis of the standard drugs at three different concentrations in the linearity range of the drugs for three days and % RSD was calculated.

Repeatability

Repeatability of sample application

Repeatability of sample application was assumed by spotting 400 ng/ml of drug solution, 6 times on TLC plate followed by development of plate and recording the peak area for 6 spots and % RSD was calculated.

Repeatability of measurement

The repeatability of measurement of peak area was determined by spotting standard drug solution on TLC plate and developing the plate. The spot was scanned 6 times without changing the position of the peak and % RSD was calculated.

Accuracy

The accuracy of the method was determined by calculating recovery of SIL and DPX by the standard addition method. Known amounts of standard solutions of SIL were added to pre quantified sample solutions of DPX. Known amounts of standard solutions of DPX were added to pre quantified sample solutions of SIL. The amounts of SIL and DPX were estimated by applying obtained values to the regression equation of the calibration curve.

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Limit of Detection and Limit of Quantification (LOD &LOQ)

The sensitivity of measurements of atorvastatin and amlodipine by the use of the proposed method was estimated in terms of the Limit of Quantitation (LOQ) and Limit of Detection (LOD). These were calculated by the use equation LOD = 3.3 x N/B and LOQ = 10 x N/B, where 'N' is standard deviation of the peak areas of the drugs (n = 3), taken as a measure of noise, and 'B' is the slope of the corresponding calibration plot.

Standard and sample solution stability

Stability studies were also carried out by keeping the standard and sample solution prepared at room temperature for several hours and was spotted every time on a fresh plate. After development and scanning the plates were observed for change in peak areas and appearance of additional peaks. The RSD was calculated Additional peaks. The RSD was calculated.

Method Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness of the method was determined by small deliberate changes in mobile phase composition (± 2%), chamber saturation period (± 10%), and development distance (± 10%). The time from spotting to chromatography and from chromatography to scanning was varied from 10 min. When very small changes were made to the method conditions there were no marked changes in chromatographic behavior and content of the drug, % RSD was calculated.

Method Ruggedness

Ruggedness test was determined between two different analysts and instruments. The value of Percentage RSD was calculated.

RESULTS AND DISCUSSION

Linearity

Linearity and Range

The plate was developed, dried and scanned as represented 3D overlay chromatogram in **Figure 6&7.**The linear regression data showed a good linear relationship over a concentration range of 2000-12000 ng/spot for SIL **(fig.8)** and 1200-7200ng/spot for DPX**(fig.9)**.The slope, intercept and correlation coefficient values of SIL were found to be 0.255, 1039, and 0.9990 respectively and 0.343, 389 and 0.9994 respectively for DPX. The results are shown in **table 2**.

Precision

Precision was calculated as interday and intraday variations. The RSD (Relative Standard Deviation) was found to be not more than 2 % for both intraday and Interday precision **(Table 3).**

Repeatability

The repeatability showed excellent % RSD less than 0.6 after six applications **(Table 4).**

Accuracy

The percentage recovery for SIL was found to be 98.45 (at 50%), 98.65 (at 100%) and 99.03 (at 120%) with % RSD values ranging from 0.114 to 0.117 and 100.00 (at 50%), 98.93 (at 100%) and 98.32(at 120%) for DPX with % RSD values ranging from 0.100 to 0.124 **(Table 5**).

Method Precision (% Repeatability)

The RSD values for SIL and DPX were found to be 0.63% and 0.69% respectively. The RSD values were found to be <2 %, which indicates that the proposed method is repeatable.

LOD and LOQ

LOD values for SIL and DPX were found to be 210ng/spot and 75ng/spot, respectively and LOQ values for SIL and DPX were found to be 450ng/spot and 240ng/spot, respectively (Table 5). These data show that the proposed method

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is sensitive for the determination of SIL and DPX.

Standard and sample solution stability

Analyte should not decompose during development of the chromatogram and should be stable in solution as well as the solvent. The RSD was found below 2%. It was observed that the plates were stable up to 2 hours **(Table 6).**

Robustness

There were no significant changes in Rf and peak areas, which demonstrated that the developed HPTLC method is robust.

Ruggedness

The value of percentage RSD was below 2.0%, showed ruggedness of developed analytical method.

CONCLUSION

This study described a simple, selective and reliable HPTLC procedure for the simultaneous determination of Sildenafil Citrate and Dapoxetine Hydrochloride. Very few reports can be found for the analysis of this recently introduced pharmaceutical mixture. Reviewing the literature exposed that there were no reports for the application of a TLC-based method for the assay of this mixture. Obviously, the described HPTLC method offers selectivity advantage over the previously published spectrophotometric and HPLC method. Moreover, the proposed method does not require elaborate treatment or sophisticated experimental setup usually associated with HPLC methods of analysis. The HPTLC method uses a minimal volume of solvents and is suitable for use in quality control laboratories where economy and time are essential. Reliability was guaranteed by testing various validation parameters of the proposed method and successful application to laboratory prepared tablets without interference from commonly encountered dosage form additives. Finally, the proposed method was found accurate and precise; hence, it can be recommended for the routine analysis of the studied drugs either in bulk form or in their combined tablet dosage forms.

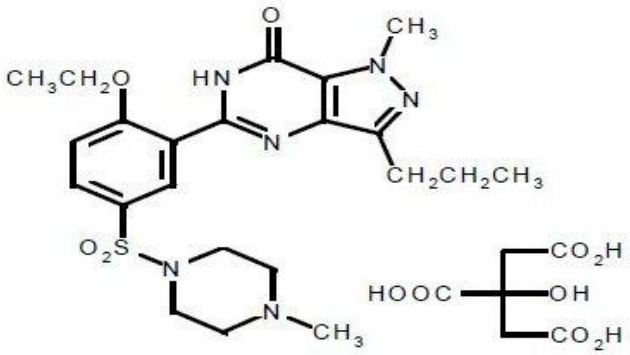

Fig. 1: Chemical structure of Sildenafil Citrate

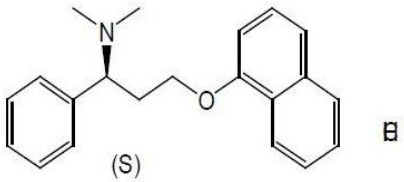

Fig. 2: Chemical structure of Dapoxetine Hydrochloride.

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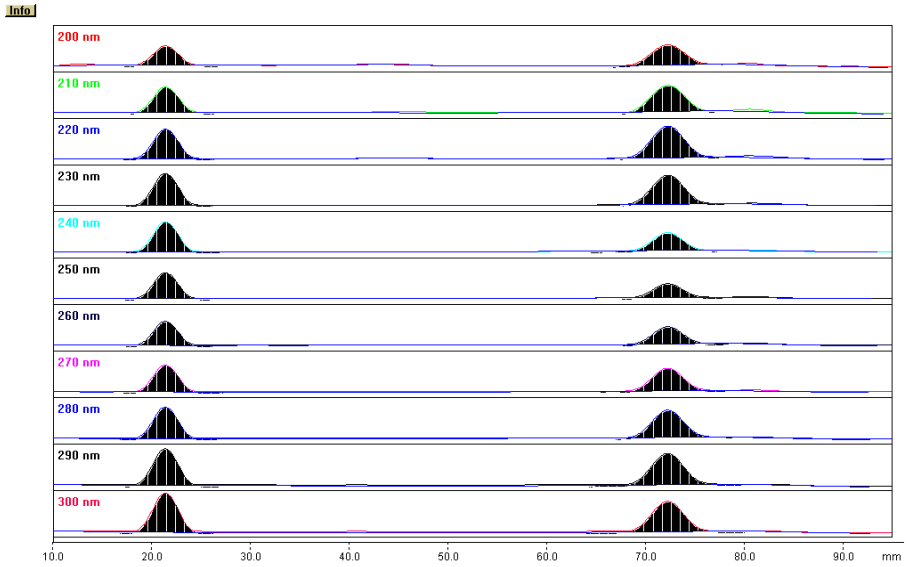

Fig 3: Selection of wavelength for measurement

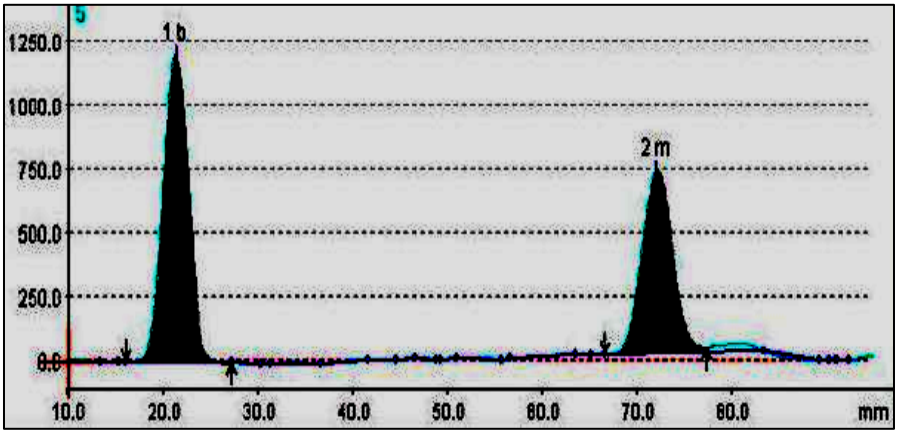

Fig: 4Chromatogram of SIL (10,000ng/band) and DPX (6000ng/band) standard with R_f of 0.21 and 0.72, respectively

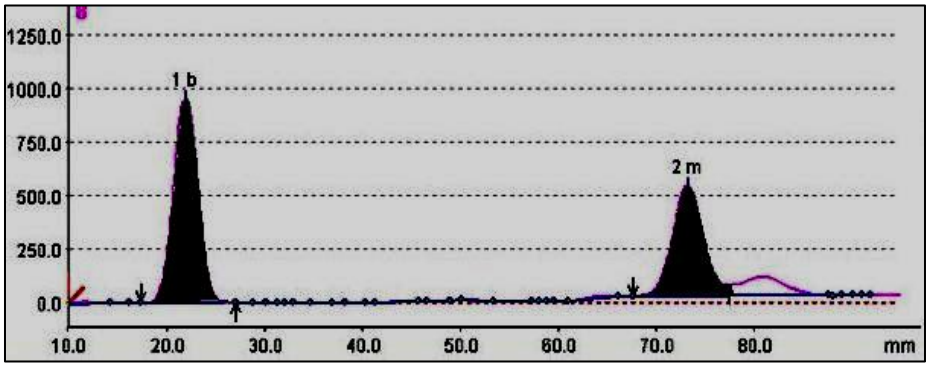

Fig. 5: Chromatogram of SIL (6000 ng/band) and DPX (3600ng/band) Sample solution with R_f of 0.21 and 0.72, respectively

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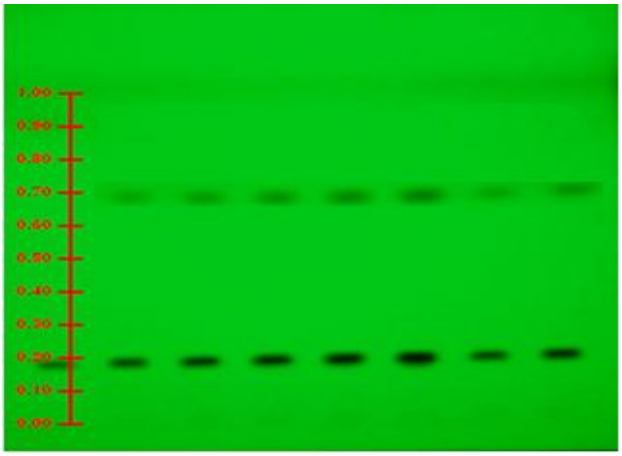

Fig. 6: TLC separation Plate

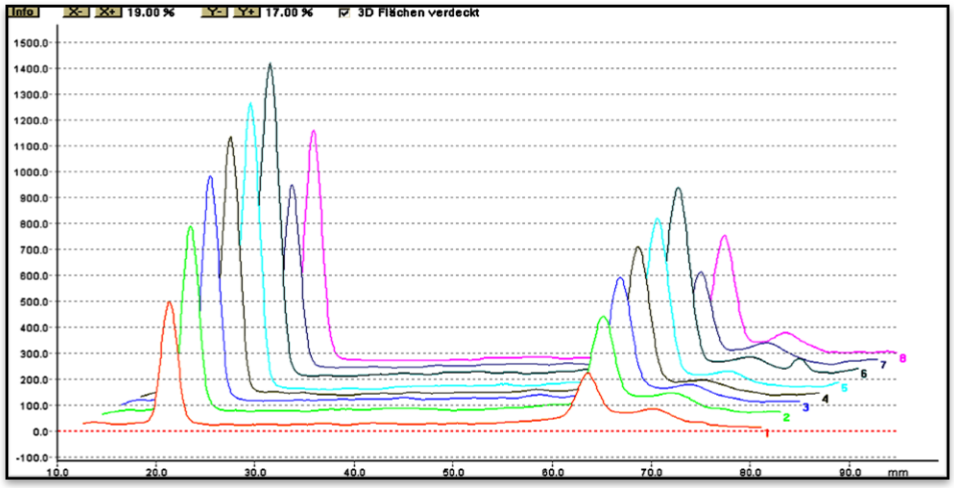

Fig. 7: 3D Overlay chromatogram of SIL and DPX

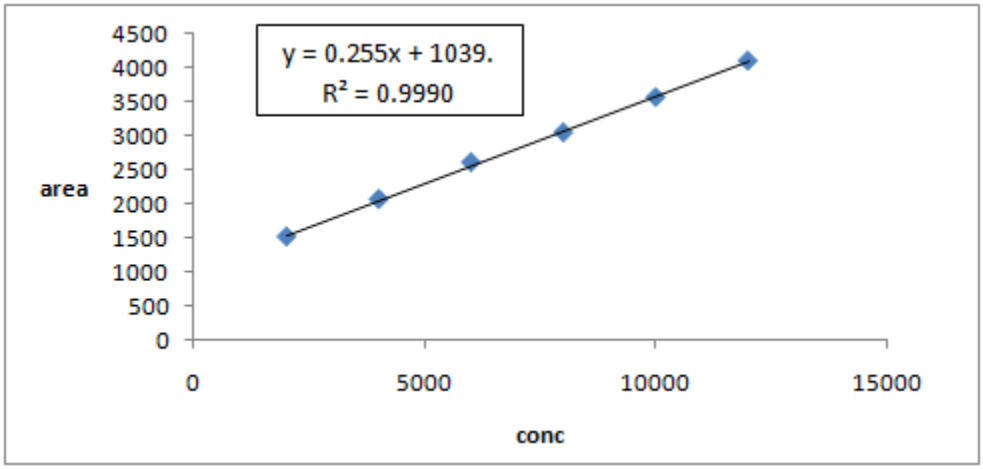

Fig.8:Calibration Curve of SIL at 241 nm

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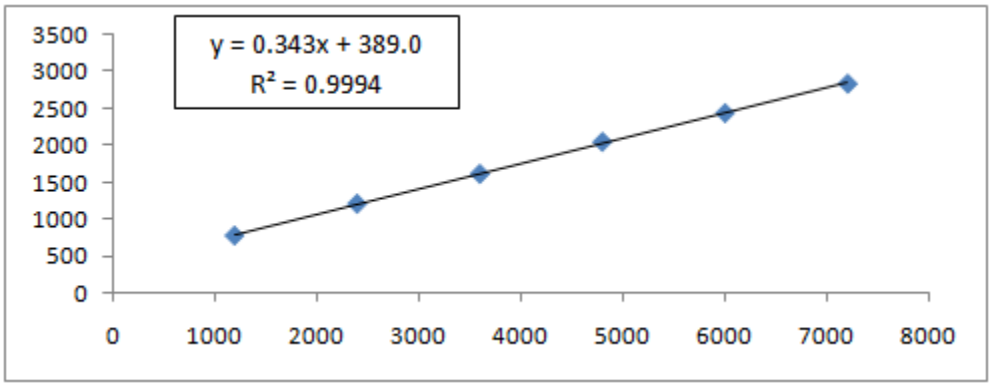

Fig .9: Calibration Curve of DPX at 241 nm

Table 1 Analysis of Formulation (n = 6)

Sample No.	Label Claim		Amount Found		% Label Claim	
	SIL (mg)	DPX (mg)	SIL (mg)	DPX (mg)	SIL (%)	DPX (%)
1	50	30	49.84	30.03	99.68	100.11
2	50	30	49.94	29.79	99.87	99.29
3	50	30	49.03	29.65	98.05	98.84
4	50	30	49.25	29.65	98.49	98.82
5	50	30	49.06	29.41	98.12	98.03
6	50	30	49.46	29.70	98.92	99.01
Mean			49.43	29.71	98.84	99.02
S.D.			0.36	0.34	0.77	0.68

(n = no. of replicates, S.D. = standard deviation, RSD = relative standard deviation)

Table 2 Regression Analysis Data of SIL and DPX for proposed Method

Parameters	HPTLC method	
	SIL	DPX
Detection wavelength(nm)	241	241
Concentration range (ng/spot)	2000-12000 ng/spot	1200-7200ng/spot
Regression equation Y= mX + c	y = 0.255x + 1039	y = 0.343x + 389.0
Correlation coefficient	0.9990	0.9994
Slope	0.255	0.343
Intercept	1039	389
LOD(ng/spot)	210	75
LOQ(ng/spot)	450	240

Table 3Intra-day and Inter-day precision (n = 3)

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Drug	Conc. (ng/spot)	Intra-day precision		Inter-day precision	
		Mean ± SD	% RSD	Mean ± SD	% RSD
SIL	2000	1543±10.40	0.66	1567±15.64	0.99
	4000	2052±17.45	0.84	2008±23.21	1.16
	6000	2631±24.83	0.94	2613±30.64	1.17
DPX	1200	751±6.69	0.89	759±10.67	1.40
	2400	1251±12.57	1.00	1216±19.29	1.58
	3600	1631±20.04	1.02	1598±22.48	1.45

(n = no. of replicates, S.D. = standard deviation, RSD = relative standard deviation)

Table 4Repeatability of Sample Application (n = 6)

Sr. No	Peak Area			
	SIL (6000ng/spot)		DPX (3600ng/spot)	
	Std.		Sample	
1	2615	2746	1645	1650
2	2618	2778	1620	1655
3	2595	2730	1625	1670
4	2598	2735	1632	1630
5	2630	2770	1627	1660
6	2625	2756	1640	1630
Mean	2613.5	2752.5	1631.5	1649.1
SD	12.97	17.43	8.65	14.81
%RSD	0.49	0.63	0.53	0.89

(n = no. of replicates, S.D. = standard deviation, RSD = relative standard deviation)

Table 5 Recovery Data for the proposed Method (n = 3)

Drug	Level	Amount of sample taken (ng/spot)	Amount of standard spiked (%)	Mean% Recovery ± SD
SIL	I	4000	80 %	98.45± 0.66
	II	4000	100 %	98.65 ± 0.90
	III	4000	120 %	99.03 ± 0.80
DPX	I	2400	80 %	100.00± 1.87
	II	2400	100 %	98.93 ± 0.74
	III	2400	120 %	98.32 ± 0.99

Table 6 Recovery Data for the proposed Method(n = 3)

Conc. (ng/spot)	Time in (Hrs:Min)	Peak Area	
		SIL	DPX
6000	0:30	2616	1625
	1:00	2610	1620
	1:30	2622	1618
	2:00	2618	1622

ACKNOWLEDGEMENT

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ISSN: 2347-7881

The authors are thankful to Emcure Pharmaceuticals, Pune, India for providing gift sample of SIL and DPX for carry out the research work.

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