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Stability Indicating High Performance Thin Layer Chromatographic Method for Determination of Perphenazine in Tablet Dosage Form

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ABSTRACT

A simple, precise, sensitive and selective stability indicating high performance thin layer chromatographic (HPTLC) method has been developed and validated for the estimation of Perphenazine in tablet dosage form. The resolution of drug was achieved by use of pre-coated silica gel 60 GF_{254} plates as stationary phase and mixture of Toluene: Ethyl acetate: Methanol (5: 3: 2, v/v/v) as mobile phase. Densitometric evaluation of separated spot was performed at 258 nm. The retention factor was found to be 0.33 ± 0.07. As stability testing is major step in the development of new drug as well as formulation, stress degradation studies were carried out according to ICH guidelines. Perphenazine was found susceptible to all the analyzed stress conditions. The method was successfully validated according to ICH guidelines. The method was found to be linear in the concentration range of 200-1200 ng $band^{-1}$. The method was applied successfully for estimation of drug in tablet dosage form. The percentage drug content (Mean±S.D.) was found to be 99.78±1.55. The developed method can be used for the quantification of drug in tablet dosage form as well as for routine analysis in quality control laboratories.

INTRODUCTION

Perphenazine, chemically, 2-[4-[3-(2-chlorophenothiazin-10-yl)propyl]piperazin-1-yl] ethanol is Phenothiazine derivative used to treat the mental disorders and work by helping to restore the balance of certain natural substances (e.g., dopamine) in the brain [1]. Extensive literature survey with respect to quantitative estimation of Perphenazine revealed that analytical methods such as UV Spectrophotometry [2] and High Performance Liquid Chromatography [3-7] were reported for its determination in human plasma and/or pharmaceutical formulations either as single drug or in combination with other drugs. Chemometric method for the determination of Perphenazine in combination with other drugs was also found in the literature [8].

To best of our knowledge, no reports were available in the literature for determination of Perphenazine in tablet dosage form by stability indicating HPTLC method. Based on this information, we have developed a selective, precise, accurate and sensitive High Performance Thin Layer Chromatography method for the estimation of Perphenazine in the presence of its degradation products in accordance with ICH guidelines [9, 10].

MATERIALS AND METHODS

Chemicals and reagents

Analytically pure Perphenazine was obtained as gift sample from Emcure Pharmaceuticals Ltd., (Pune, India). Pharmaceutical tablet dosage form Trilafon labelled to contain 4 mg of Perphenazine was procured from local pharmacy. Toluene, methanol and ethyl acetate (all AR grade) were purchased from Merck specialties Pvt. Ltd. (Mumbai, India).

Instrumentation and Chromatographic conditions

CAMAG HPTLC system equipped with Camag Linomat V sample applicator, Hamilton syringe (100 µL), Camag TLC Scanner-3 with winCATS software version 1.4.2 and Camag twin- trough chamber (10×10 cm), Silica gel 60 F_{254} TLC plates (10×10 cm, layer thickness 0.2 mm, E. Merck, Germany) were used for the present study.

The chromatographic resolution of drug was accomplished by linear ascending development in 10 cm × 10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) using mixture of toluene: ethyl acetate: methanol (5: 3: 2, v/v/v) as mobile phase. The chamber was saturated with mobile phase vapor for 15 min. The development distance was 9 cm and the

development time approximately 15 min. The slit dimensions 5 mm × 0.45 mm and scanning speed of 20 mm sec^{-1} was employed. After chromatographic development, plates were dried and densitometric evaluation was done on CAMAG thin layer chromatography scanner III at 258 nm for all developments operated by winCATS software version 1.4.2.

Preparation of standard stock solution

Standard stock solution was prepared by dissolving 10 mg of the drug in 10 mL of methanol to get concentration of 1000 µg mL^{-1} which was diluted further with methanol to furnish final concentration of 100 ng μL^{-1}.

Selection of Detection Wavelength

Stock solution of the drug was prepared in 10 mL of methanol and was scanned over the range of 200-400 nm by using the UV Visible spectrophotometer. It was found that Perphenazine showed maximum absorbance at 258 nm and was selected as wavelength for the detection.

Tablet formulation analysis

To estimate the content of Perphenazine in tablet dosage form, 20 tablets were weighed accurately and finely powdered. Powder quantity equivalent to 4 mg was weighed and transferred to a 10 mL volumetric flask containing approximately 6 mL of methanol. The contents were sonicated for 10 min, filtered and volume was made with the methanol. The resulting solution was filtered through Whatman filter paper No. 41 and 0.5 mL of filtrate was diluted further to 10 mL with methanol. Two micro-litre volume of this solution was applied to a TLC plate to provide final concentration of 400 ng $band^{-1}$. After chromatographic development the peak areas of the bands were measured at 258 nm and the quantity of drug present in sample was estimated from the respective calibration curve. Procedure was repeated six times for the analysis of homogenous sample.

Forced degradation study

Forced degradation studies were carried out to provide evidence on how quality of drug varies under the influence of a variety of environmental conditions like acidic, alkaline hydrolysis, oxidation, dry heat and photolytic degradation. The study was carried out at initial drug concentration of 1000 µg mL^{-1} in methanol. The acid and alkali hydrolytic studies were carried out by mixing the drug solution with 0.1 N HCl and 0.1 N NaOH and the resulting

solutions were kept at room temperature for 30 min separately to achieve degradation within the acceptable limit. The stressed samples of acid and alkali were neutralized with NaOH and HCl, respectively to furnish the final concentration of 1000 ng band^{-1}. The oxidative degradation was carried out by using the 3 % H_2O_2 solution. The thermal degradation study was performed by keeping drug sample in oven at 45ºC for a period of 7 d. The photolytic degradation studies were carried out by the exposure of drug sample to UV for period of 3 d.

RESULTS AND DISCUSSION:

Method optimization

The prime aim in developing the stability indicating HPTLC method is to achieve the satisfactory resolution of drug and its degradation products. Initially, many trials were conducted using different mobile phases in order to obtain better separation. The optimised mobile phase comprises of toluene: ethyl acetate: methanol (5: 3: 2, v/v/v) which shows well defined and resolved peak for the drug. Densitometric evaluation was carried out at 258 nm. The retention factor was found to be 0.33 ± 0.07. The representative densitogram of standard solution of drug is represented in Figure 1.

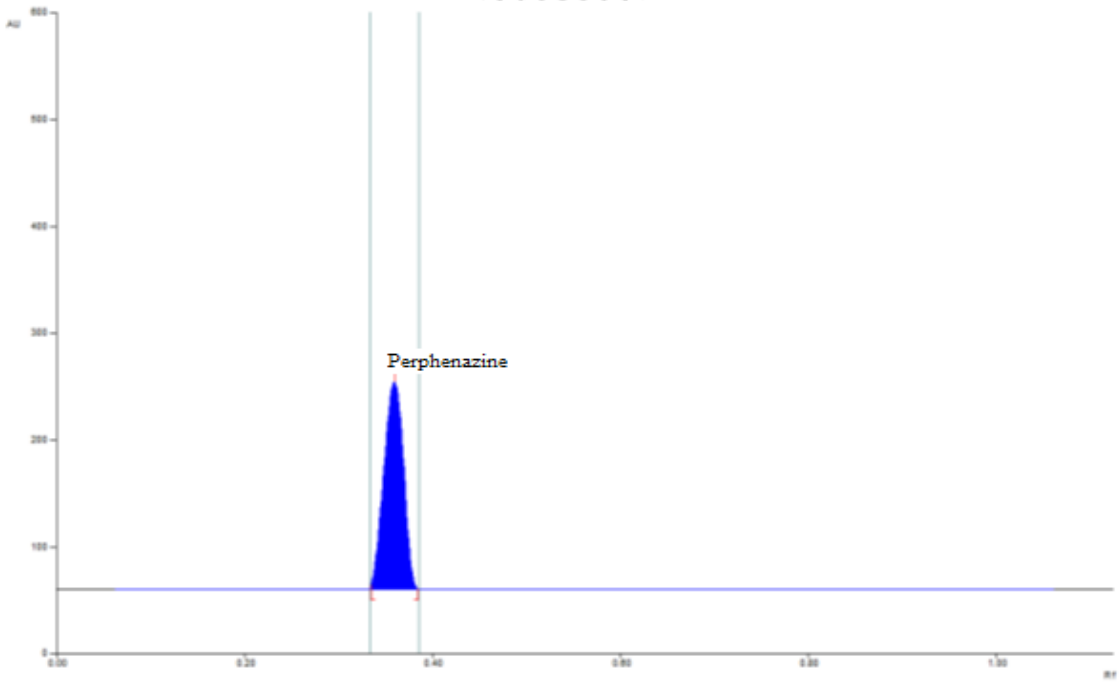

Fig. 1: Representative densitogram of standard solution of Perphenazine

(600 ng band^{-1}, Rf= 0.33±0.07)

Forced degradation studies

The stress degradation results revealed the susceptibility of the drug to hydrolytic, oxidative, and photolytic stress conditions. Noticeable degradation was observed for Perphenazine in acid and alkali hydrolytic condition with reduction in peak area and without appearance of degradation peaks. Significant degradation was observed for drug under oxidative stress with formation of degradation product at Rf 0.64. Marked degradation was seen in the densitogram along with peak for degradation product at Rf 0.17 under photolytic stress condition. Figures 2 and 3 represent the densitograms of acid and alkali hydrolytic degradation, while Figures 4 and 5 shows the densitograms of oxidative and photolytic degradation, respectively. The % degradation and % assay of active substance obtained after forced degradation is depicted in Table 1.

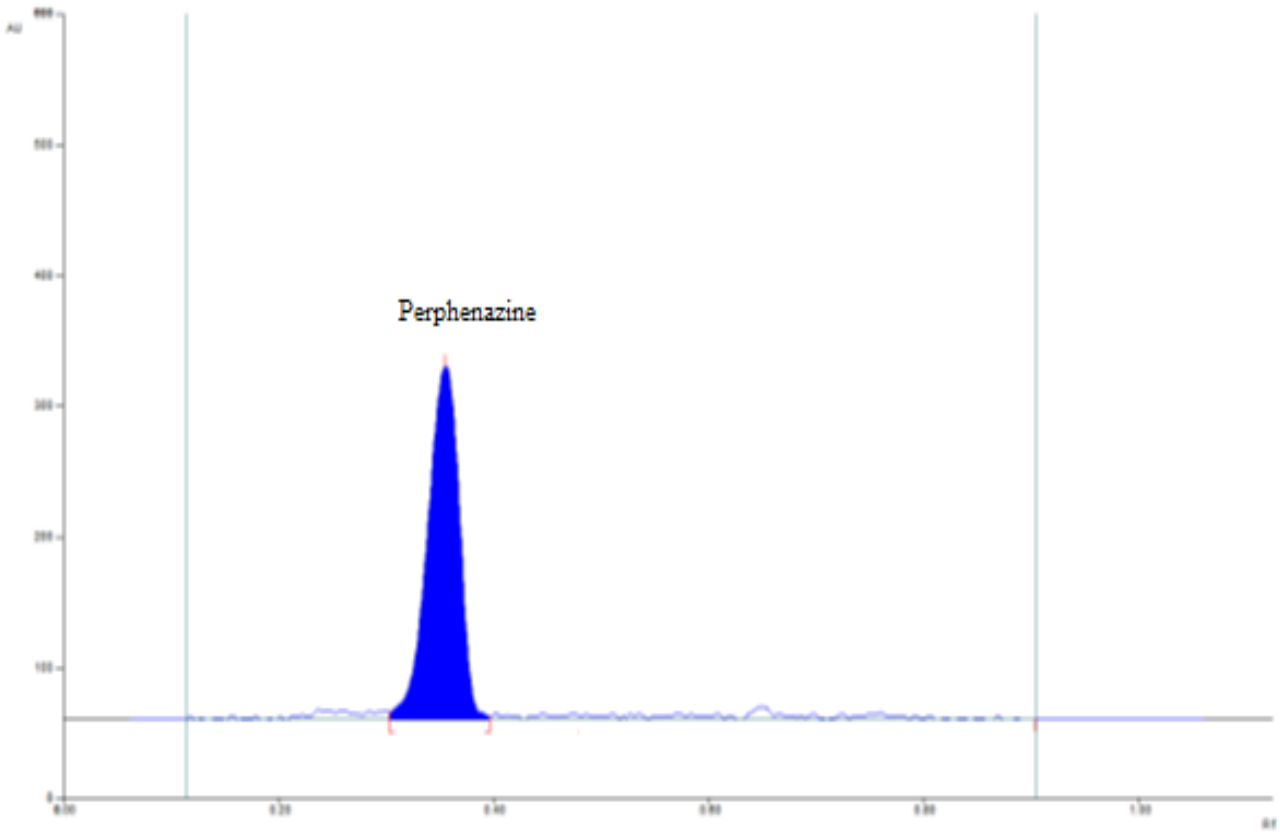

Fig. 2: Densitogram obtained after acid hydrolysis

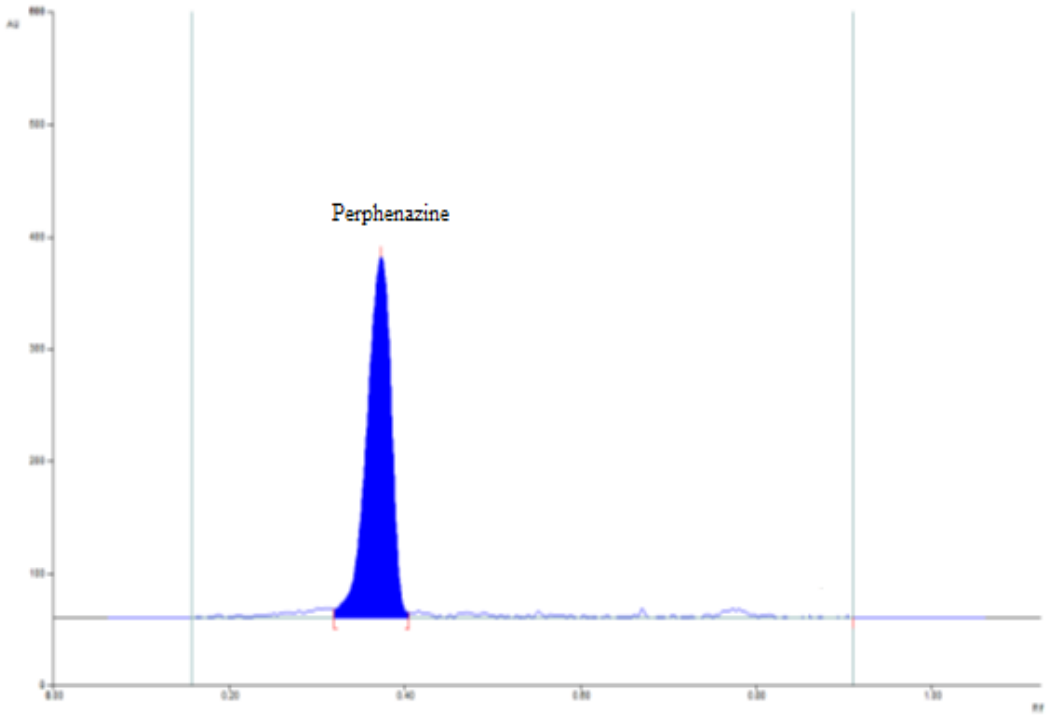

Fig. 3: Densitogram obtained after alkali hydrolysis

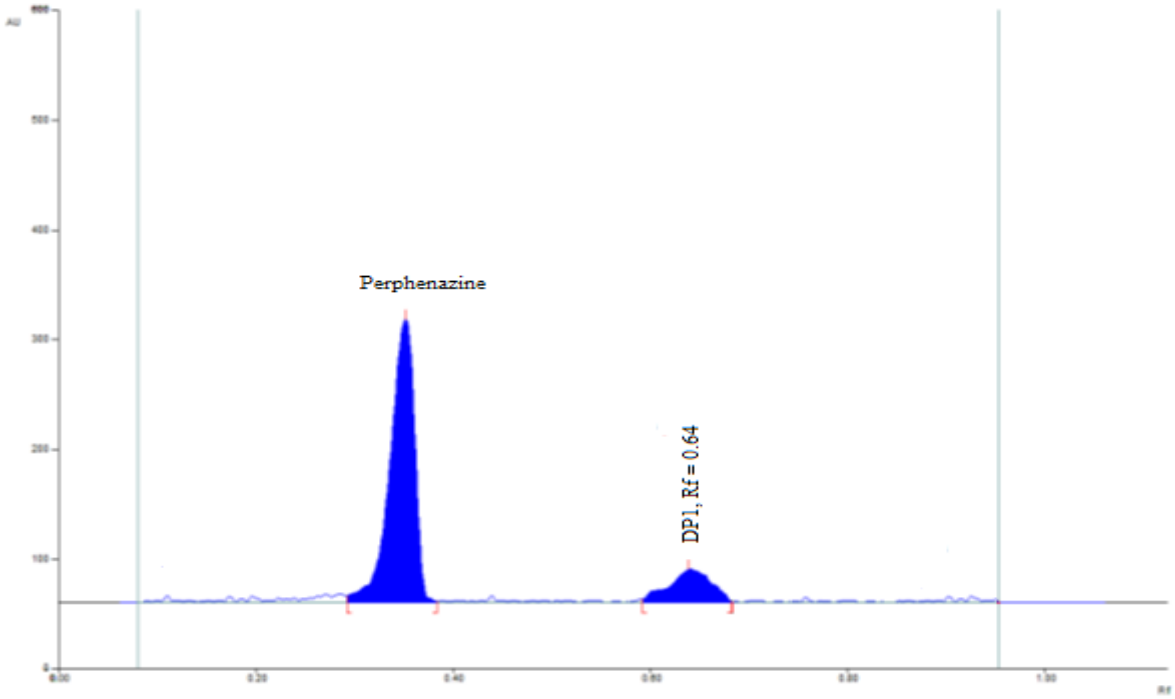

Fig. 4: Densitogram of Peroxide induced Perphenazine with degradation product

(DP1, Rf = 0.64)

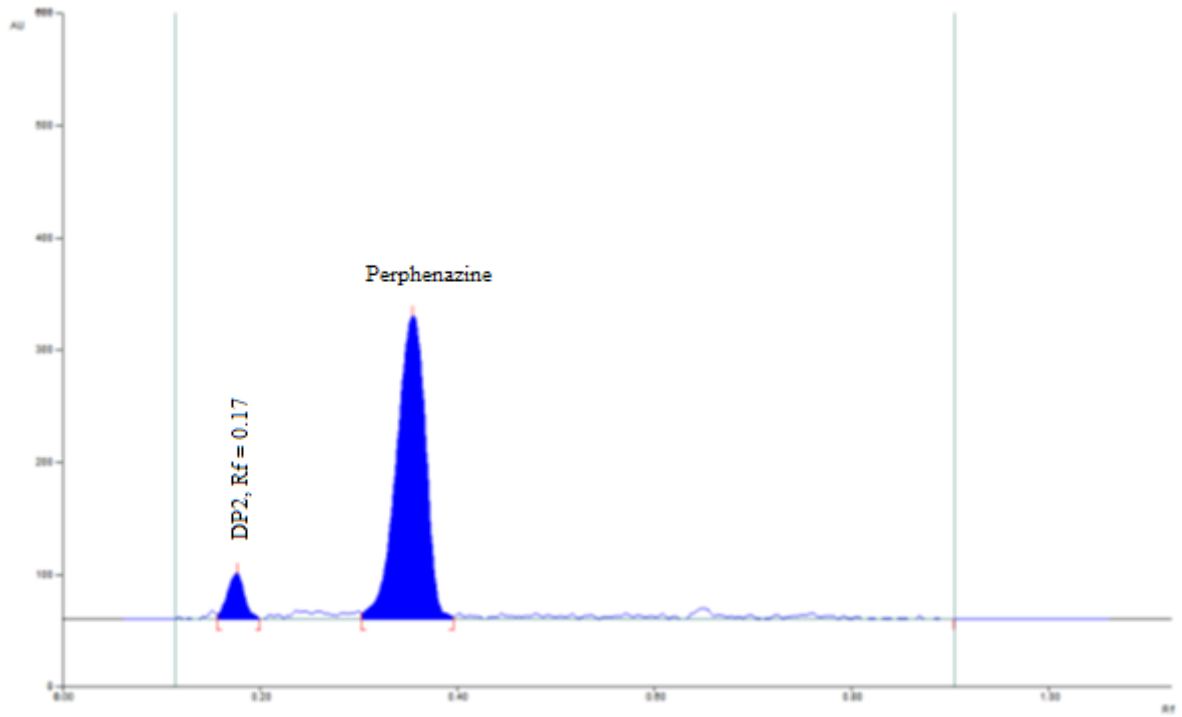

Fig. 5: Densitogram obtained after exposure to UV light with degradation peak

(DP2, Rf = 0.17)

Table 1: Results of forced degradation studies

Sr. No.	Degradation Conditions	% Recovery	% Degradation
1	Acid/ 0.1N HCl Kept at RT for 30 min	87. 20	12.80
2	Alkali/ 0.1 N NaOH Kept at RT for 30 min	85.40	14.60
3	Oxidative 3 % H_2O_2 Kept at RT for 1 h	82.70	17.30
4	Dry heat/ 45^0C for 7 d	101.06	---
5	UV light	88.90	11.10

Validation of analytical method

The optimized analytical method was validated in terms of linearity, accuracy, intra-day and inter-day precision, limit of detection, limit of quantitation and robustness as per ICH guidelines.

Preparation of calibration curve

From the standard stock solution (1000 µg ml^{-1}), volumes 2, 4, 6, 8, 10 and 12 µL were spotted on TLC plate to obtain the concentration in the range 200-1200 ng $band^{-1}$. The plate

was developed and scanned under above established chromatographic conditions. Each standard in six replicates (n = 6) was analyzed and peak areas were recorded. The linearity was observed in the range of 200-1200 ng band^{-1} with linear regression equation y = 5.7481x + 394.06 and correlation coefficient 0.994. The calibration curve and 3 D spectrum obtained is shown in Figure 6 and 7.

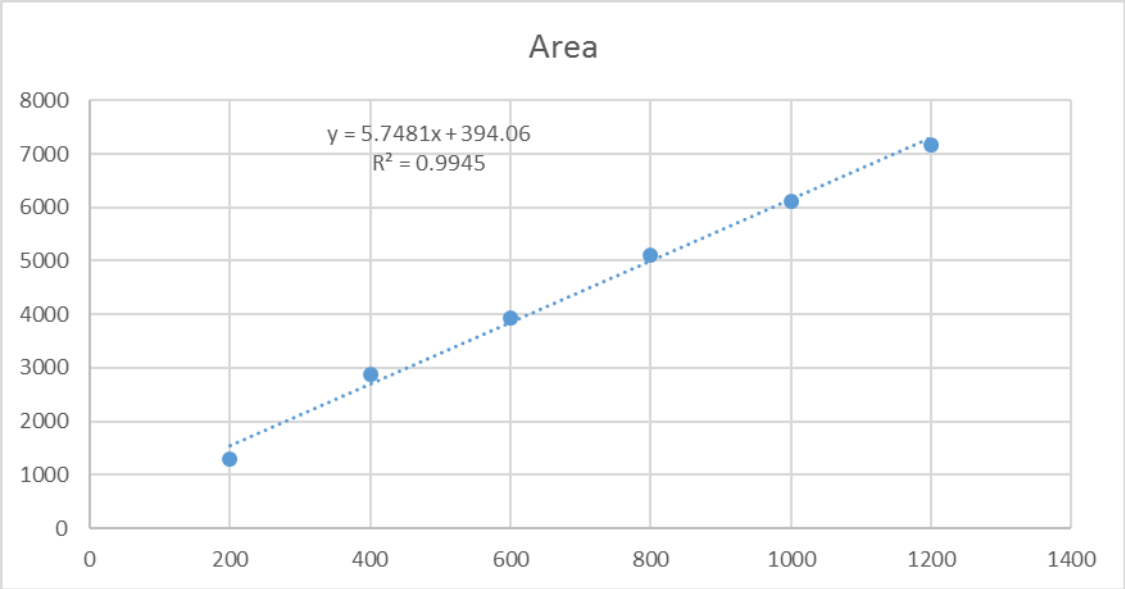

Fig. 6: Calibration curve

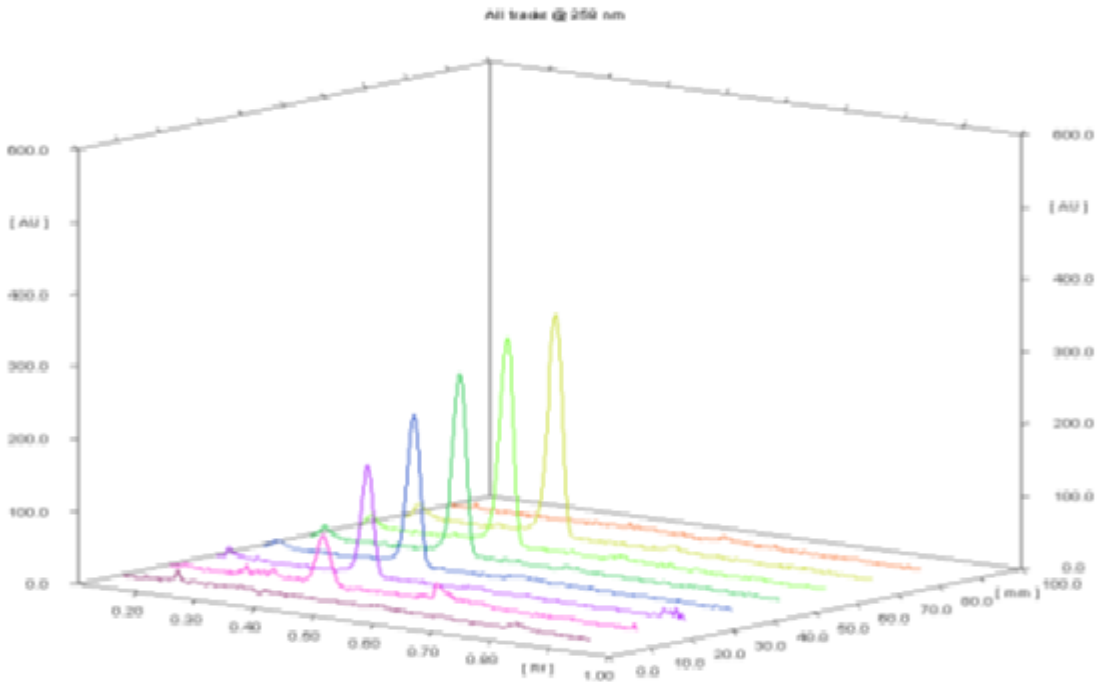

Fig. 7: 3D spectra of linearity in concentration range (200-1200 ng band^{-1})

Precision

The precision of the method was studied by intra-day and inter-day variation studies. In the intra-day studies, three replicates of three concentrations within concentration range were analysed on the same day and for the inter-day variation studies, three concentrations were analysed on three consecutive days and % RSD was calculated. The % RSD values obtained were well within the acceptable limit indicating the precision of developed method.

Table 2: Data of intra-day and inter-day variation studies

Concentration (ng band^{-1})	Intra-day precision			Inter-day precision		
	Area	% Recovery	% RSD*	Area	% Recovery	% RSD*
600	3893	101.33	1.00	3897	101.50	1.06
	3847	100.10		3838	99.83	
	3821	99.33		3829	99.50	
800	4903	98.00	1.10	4991	99.87	1.06
	4983	99.75		4910	98.12	
	4994	100.02		5002	100.01	
1000	6109	99.40	1.03	7314	100.25	1.67
	6192	100.80		7461	102.41	
	6225	101.44		7232	99.08	

* Average of 3 determinations

Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. LOD and LOQ were found to be 19.71 ng band^{-1} and 58.10 ng band^{-1} respectively.

Accuracy

To check accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analysed sample solution at three different levels 80, 100 and 120%. Basic concentration of sample chosen was 400 ng band^{-1}. The % mean recovery was found to be 99.53±1.51 which indicated that the method is accurate for estimation of drug in tablet dosage form. The results obtained are presented in Table 3.

Table 3: Recovery studies

Drug	Concentration taken (ng band^{-1})	Concentration added (ng band^{-1})	Total concentration found (ng band^{-1})	% Recovery ± R.S.D.
Perphenazine	400	320	716.42	99.44±1.69
	400	400	797.84	99.66±1.53
	400	480	876.01	99.50±1.33

* Average of 3 determinations

Robustness

Robustness of the method was determined by making intentional variations in method parameters during which wavelength (± 1 nm), mobile phase composition (± 1 % methanol), were changed and the effect on the area was noted. The developed procedure was found to be robust as % RSD values were within limit (% RSD < 2).

CONCLUSION

Stability indicating HPTLC method for determination of Perphenazine as bulk drug and in tablet dosage form has been developed and validated. The developed method is simple, precise, accurate, and selective and can be used for quantitative analysis of Perphenazine in pharmaceutical dosage form as well as for routine analysis in quality control laboratories.

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