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Determination of Cinnarizine and Dimenhydrinate in Combined Tablet Dosage Form by Stability Indicating HPTLC Method

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Pooja Pawar, Padmanabh Deshpande*

All India Shri Shivaji Memorial Society's College of Pharmacy, Department of Pharmaceutical Quality Assurance, Kennedy Road, Near RTO, Pune-411001

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ABSTRACT

The present work describes the development and validation of new simple, accurate, precise and selective stability-indicating high-performance thin layer chromatographic (HPTLC) method for determination of cinnarizine and dimenhydrinate in combined tablet dosage form. The chromatographic separation was carried out using precoated silica gel $60F_{254}$ (10 × 10 cm) plates as the stationary phase and a mixture of toluene: ethyl acetate: methanol (7: 2.5: 0.5, v/v/v) as the mobile phase. Retention factors for cinnarizine and dimenhydrinate were found to be 0.72 ± 0.02 and 0.33 ± 0.02, respectively. The wavelength selected for detection was 266 nm. Drug samples were subjected to different stress conditions like hydrolysis, oxidation, photolysis and thermal degradation. The developed method has been validated for linearity, accuracy, precision, limit of detection and limit of quantification and robustness, as per ICH guidelines. Results were found to be linear in the concentration range of 250-1500 ng $band^{-1}$ for Cinnarizine and 500-3000 ng $band^{-1}$ for dimenhydrinate, respectively. The percentage of drug contents (Mean ± S.D.) obtained for cinnarizine and dimenhydrinate were 98.66±1.73 and 99.60±1.41, respectively. The developed method can be used for the simultaneous quantification of these drugs in combined tablet dosage form as well as for routine analysis in quality control laboratories.

1.0 INTRODUCTION

Cinnarizine, chemically, 1-benzhydryl-4-[(*E*)-3-phenylprop-2-enyl] piperazine is an antihistamine and calcium channel blocker used to treat cerebral apoplexy, post-trauma cerebral symptoms and cerebral arteriosclerosis [1]. Dimenhydrinate, 2-benzhydryloxy-N, N-dimethylethanolamine; 8-chloro-1, 3-dimethyl-7H-purine-2, 6-dione is used to treat nausea which occurs in motion sickness and also in the symptomatic treatment of vertigo [2].

An extensive literature review revealed that analytical methods such as UV spectrophotometry [3], High-Performance Liquid Chromatography (HPLC) [4-6] have been reported for determination of cinnarizine in pharmaceutical formulations either as single or in combination with other drugs. High-Performance Liquid Chromatography (HPLC) [7-8] methods for determination of dimenhydrinate either as a single drug or in combination with other drugs were found in the literature. Analytical methods such as UV spectrophotometry [9], HPLC [10-12] and HPTLC [13] were also available for simultaneous estimation of cinnarizine and dimenhydrinate in pharmaceutical formulations.

To best of our knowledge, no reports were available in the literature for simultaneous determination of Cinnarizine and Dimenhydrinate in the combined tablet dosage form by stability indicating HPTLC method. Based on this observation, we have developed a selective, accurate, precise and sensitive high-performance thin layer chromatography method for the simultaneous estimation of cinnarizine and dimenhydrinate in combined tablet dosage form in accordance with International Conference on Harmonisation Guidelines [14-15].

2.0 MATERIALS AND METHODS

2.1 Chemicals and reagents

Pharmaceutical grade working standard cinnarizine and dimenhydrinate was obtained as gift sample from Ajanta Pharma Ltd. (Aurangabad, India). The pharmaceutical dosage form used in this study was Vertigen tablets labeled to contain 20 mg of Cinnarizine and 40 mg of Dimenhydrinate were procured from the local market. Toluene, methanol and ethyl acetate (all AR grade) was purchased from Merck Specialties Pvt. Ltd. Mumbai, India.

2.2 Instrumentation and chromatographic conditions

Chromatographic separation of drugs was performed on Merck TLC plates precoated with silica gel 60 F_{254} (10 cm ×10 cm with 250 µm layer thickness) from E. MERCK, (Darmstadt, Germany) using a CAMAG Linomat V sample applicator (Switzerland). Samples were applied on the plate as a band with 5 mm width using Camag 100 µL sample syringe (Hamilton, Switzerland). Linear ascending development was carried out in 10 x 10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) by using toluene: ethyl acetate: methanol (7: 2.5: 0.5, v/v/v) as the mobile phase. The mobile phase was saturated in the chamber for 15 min.

After development, TLC plates were dried in a current of air with the help of a hair drier. Densitometric scanning was performed on CAMAG thin layer chromatography scanner III at 266 nm for all developments operated by winCATS software version 1.4.2. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 200 to 400 nm.

2.3 Preparation of standard stock solutions

Accurately weighed 10 mg of Cinnarizine was dissolved in 10 mL of methanol to get the solution having concentration 1000 µg mL^{-1} which was diluted further with methanol to acquire final working concentration 250 ng μL^{-1}. The standard solution for Dimenhydrinate was prepared by dissolving accurately weighed 20 mg in 10 mL of methanol to get the solution having concentration 2000 µg mL^{-1} from which 2.5 mL of solution was diluted with the same solvent to get solution having final concentration 500 ng μL^{-1}.

2.4 Selection of detection wavelength

After chromatographic development bands were scanned over the range of 200-400 nm. It was observed that both drugs showed considerable absorbance at 266 nm. So, 266 nm was selected as the wavelength for detection.

2.5 Analysis of marketed formulation

Twenty tablets were weighed accurately and finely powdered. A tablet powder equivalent to 10 mg Cinnarizine (20 mg of Dimenhydrinate) was weighed and transferred to a 10 mL volumetric flask having about 6 mL of methanol. The contents were sonicated for 15 min,

filtered and volume was made up to the mark with methanol. From the above solution, 2.5 mL of solution was diluted using the same solvent to achieve a final concentration of 500 ng μL^{-1} for Dimenhydrinate and 250 ng μL^{-1} for Cinnarizine. Two micrilitre volume of this solution was applied on the TLC plate to obtain a final sample concentration of 500 ng $band^{-1}$ for Cinnarizine and 1000 ng $band^{-1}$ for Dimenhydrinate. After chromatographic development peak areas of the bands were measured at 266 nm and the amount of each drug present in the sample was estimated from the respective calibration curve. The procedure was repeated six times for the analysis of the homogenous sample.

2.6 Stress degradation studies of bulk drugs

The stability studies were performed by subjecting the mixed standard solution of bulk drugs to the physical stress (hydrolysis, peroxide, heat, and light) and stability was accessed. The stress degradation studies were carried out at the initial drug concentration of 1000 µg mL^{-1} of Cinnarizine and 2000 µg mL^{-1} of Dimenhydrinate in methanol. The hydrolytic studies were carried out by mixing the drug solutions of Cinnarizine and Dimenhydrinate with 0.1 N HCl and 0.1 N NaOH and the resulting solutions were refluxed at 45ºC for 1 h 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 250 ng $band^{-1}$ and 500 ng $band^{-1}$ of Cinnarizine and Dimenhydrinate, respectively. Neutral hydrolysis study was performed by refluxing mixed standard solution of drugs with water and the resulting solution was refluxed at 45ºC for 1 h. The oxidative degradation was carried out in 6 % H_2O_2 and the sample was diluted with methanol to obtain a solution having concentration 250 ng $band^{-1}$ and 500 ng $band^{-1}$ of cinnarizine and dimenhydrinate, respectively. Thermal stress degradation was performed by keeping the solid drugs individually in the oven at 90ºC for a period of 6 h. Photolytic degradation studies were carried out by exposing both drugs individually to UV light up to 200-watt h square $meter^{-1}$ for 48 hrs. Thermal and photolytic samples were diluted with methanol to get the concentration of 250 ng $band^{-1}$and 500 ng $band^{-1}$ of Cinnarizine and Dimenhydrinate, respectively.

3.0 RESULTS AND DISCUSSION

3.1 Method optimization

The main aim in developing this stability indicating HPTLC method is to achieve the satisfactory resolution of drugs from each other and also from their degradation products.

Initially, many method trials were performed using different mobile phases in order to obtain better separation. Finally, the mobile phase comprising toluene: ethyl acetate: methanol (7: 2.5: 0.5, v/v/v) was selected as optimal for obtaining well defined and resolved peaks for the drugs. The densitometric evaluation was carried out at 266 nm. The retention factors were found to be 0.72±0.02 and 0.33±0.02 for Cinnarizine and Dimenhydrinate, respectively. Representative densitogram of a mixed standard solution of both drugs is shown in Figure 1.

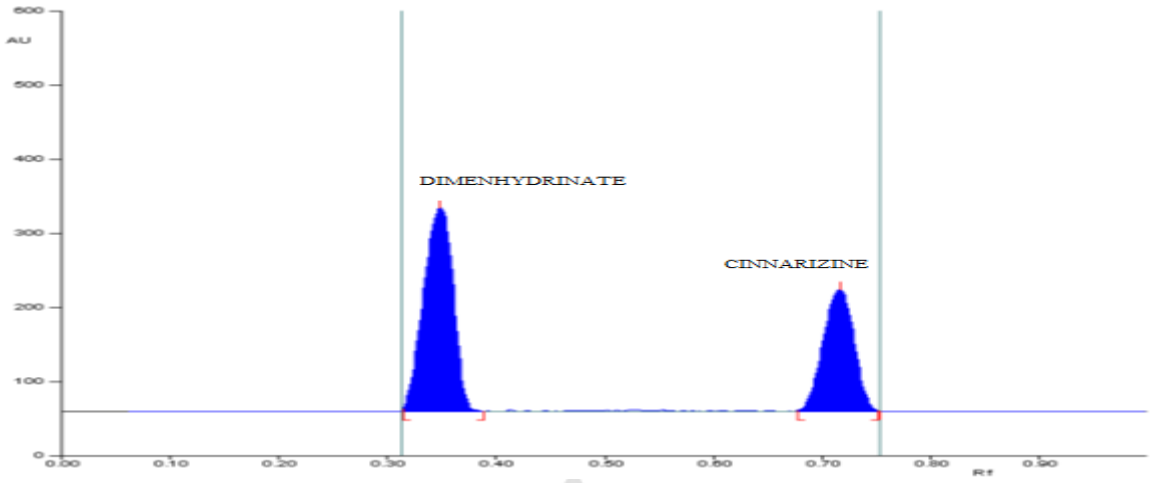


Fig. 1: Representative densitogram of mixed standard solution of Dimenhydrinate (1500 ng band^{-1}, Rf= 0.33±0.02) and Cinnarizine (750 ng band^{-1}, Rf= 0.72±0.02)

3.2 Forced degradation studies

The stress degradation results revealed the susceptibility of both the drugs to hydrolytic, oxidative, thermal stress conditions and stability under and photolytic stress conditions. Marked degradation in the densitograms was observed with the appearance of degradation products for cinnarizine and dimenhydrinate under acid and base hydrolysis while minor degradation was seen under neutral hydrolysis. Dimenhydrinate was found to undergo 13.48 % degradation after treatment with peroxide with a reduction in the peak area and without the appearance of the peak for degradation product while cinnarizine showed 11.36 % degradation with the appearance of the peak for degradation product at Rf 0.59. Figures 2 and 3 shows the densitograms of acid and alkali hydrolytic degradation, while Figures 4 and 5 show the densitograms of neutral and oxidative degradation, respectively. Peak purity results greater than 991 indicate that peaks for both drugs are homogeneous in all stress conditions tested. The unaffected assay of tablet formulation confirmed the stability indicating the power of the method. The findings of degradation studies are represented in Table 1.

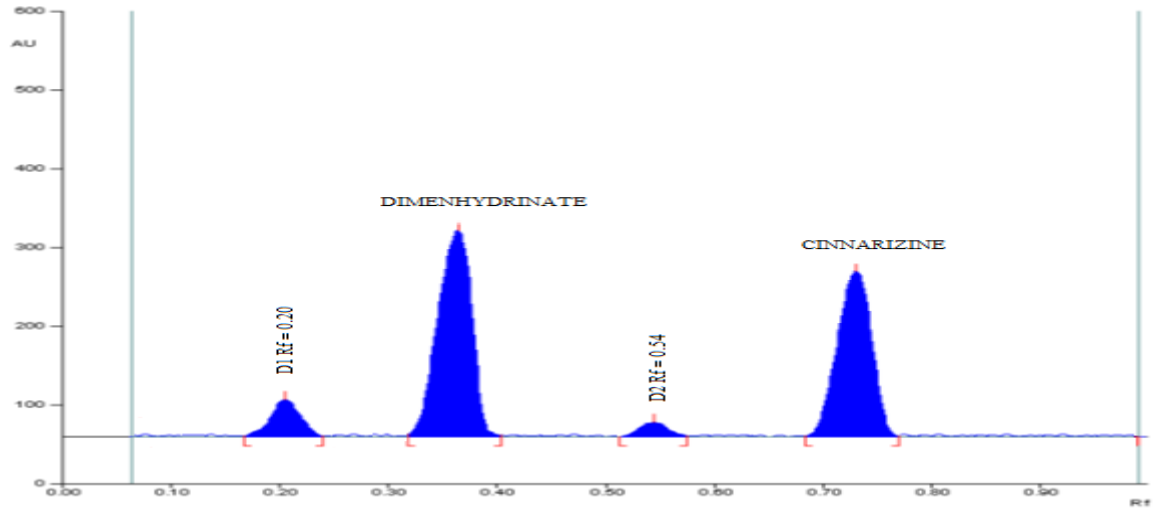


Fig. 2: Densitogram of a mixed standard solution of Dimenhydrinate (D1, Rf = 0.20) and Cinnarizine (D2, Rf = 0.54) obtained after acid degradation

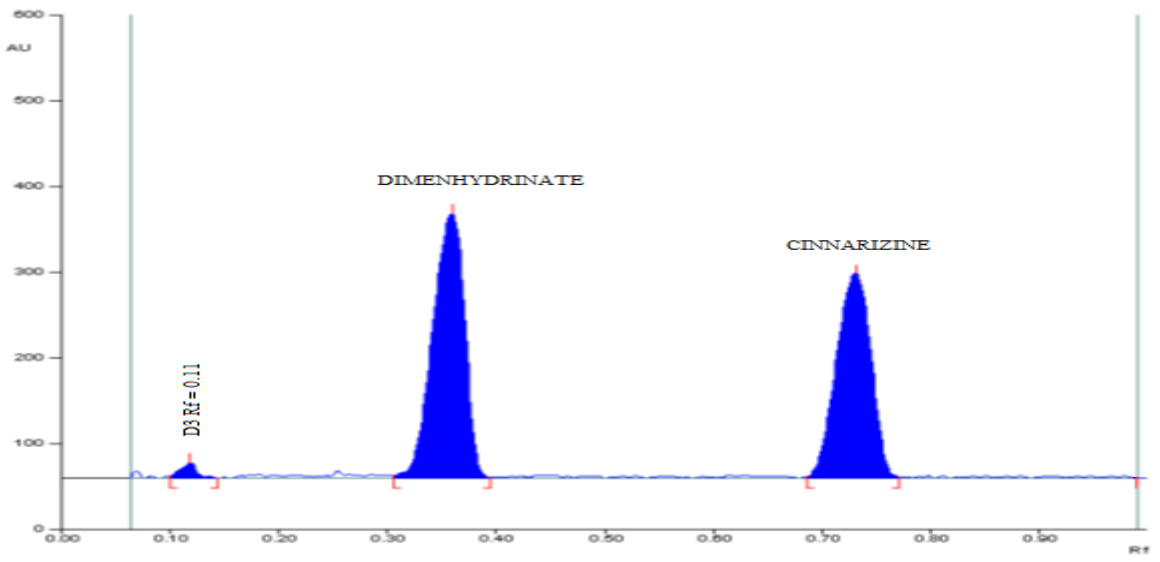


Fig. 3: Densitogram of a mixed standard solution of Dimenhydrinate (D3, Rf = 0.11) and Cinnarizine obtained after base hydrolysis

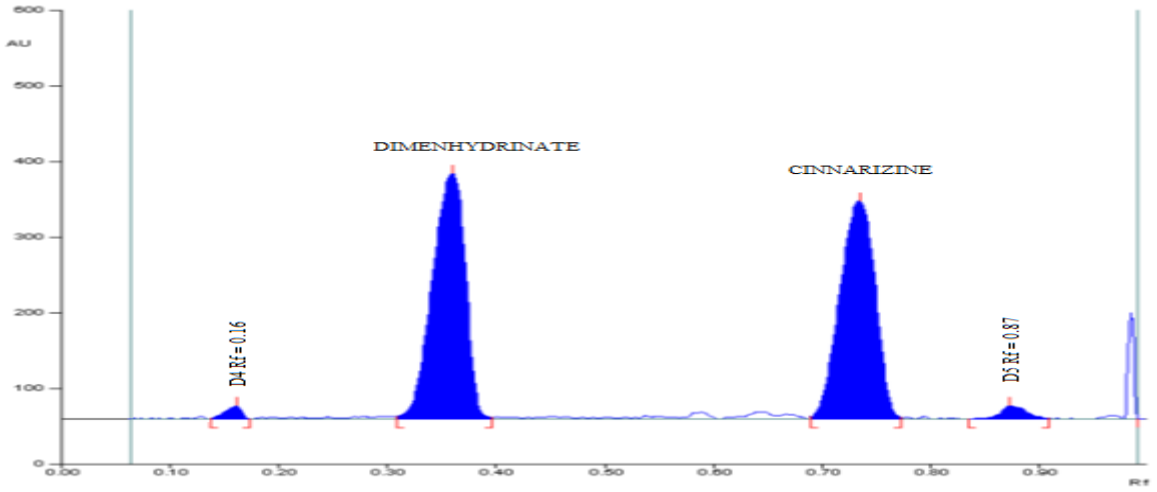


Fig. 4: Densitogram of Dimenhydrinate (D4, Rf = 0.16) and Cinnarizine (D5, Rf = 0.87) after neutral hydrolysis

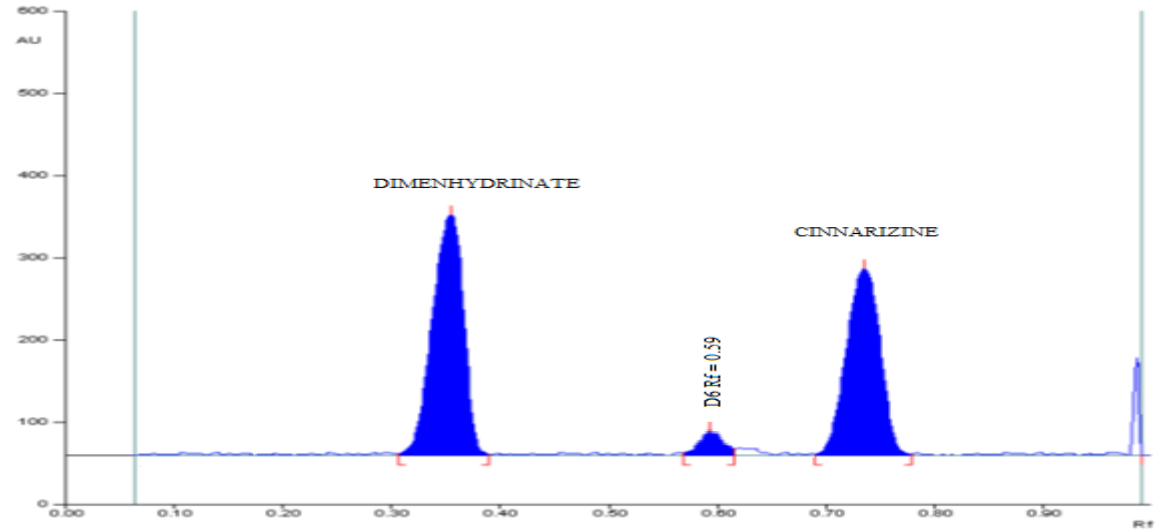


Fig. 5: Densitogram obtained Dimenhydrinate and Cinnarizine (D6, Rf = 0.59) after peroxide induced degradation

Table 1: Forced degradation studies of Cinnarizine and Dimenhydrinate

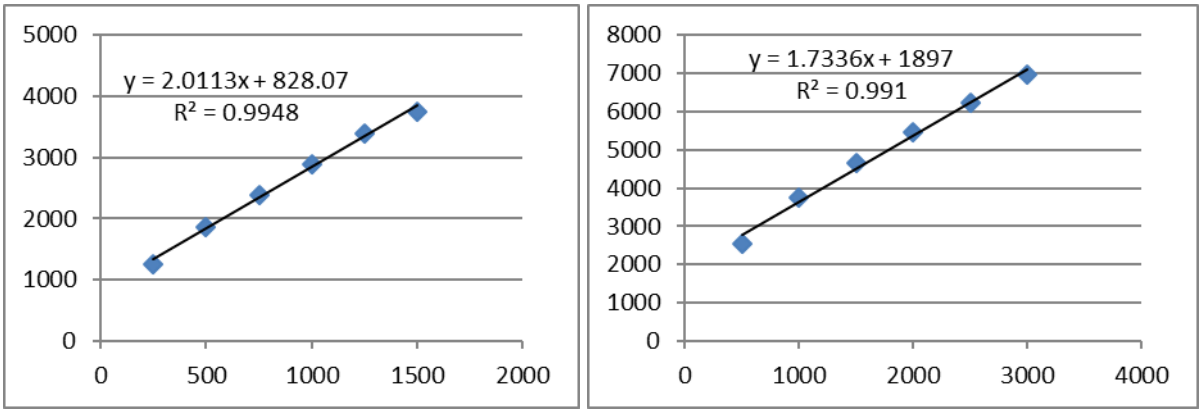
Stress conditions/ duration	Cinnarizine		Dimenhydrinate	
	% Assay	% of degradation	% Assay	% of degradation
Acidic / 0.1N HCl	89.78	10.22	81.88	18.12
Alkaline /0.1 N NaOH	90.88	9.12	87.21	12.79
Neutral/H_2O	90.33	09.67	91.94	08.06
Oxidative /6 % H_2O_2	88.64	11.36	86.52	13.48
Dry heat/ 90ºC/ 6 h	93.61	06.39	91.57	08.43
Photolysis	98.98	-----	99.18	-----

3.3 Method Validation

The optimized method was validated in accordance with ICH guidelines with respect to linearity, accuracy, intra-day and inter-day precision, limit of detection, limit of quantitation and robustness [14, 15].

3.3.1 Linearity

Volumes 1, 2, 3, 4, 5 and 6 µL of the standard stock solutions of Cinnarizine and Dimenhydrinate (250 ng $\mu L^{-1}$and 500 ng μL^{-1}) were applied by over spotting on TLC plate to obtain the concentration in the range 250-1500 ng $band^{-1}$ for Cinnarizine and 500-3000 ng $band^{-1}$ for Dimenhydrinate, respectively. Results were found to be linear in the concentration range indicated above. The linear regression equation and correlation coefficient were found to be $y = 2.0113x + 828.07$ and $R^2 = 0.994$ for Cinnarizine and $y = 1.7336x + 1897$ and $R^2 = 0.991$ for Dimenhydrinate, respectively. The calibration curves and 3 D spectra obtained for Cinnarizine and Dimenhydrinate are represented in Figure 6 and 7, respectively.



(a) **(b)**

Fig. 6: Calibration curve for (a) Cinnarizine (b) Dimenhydrinate

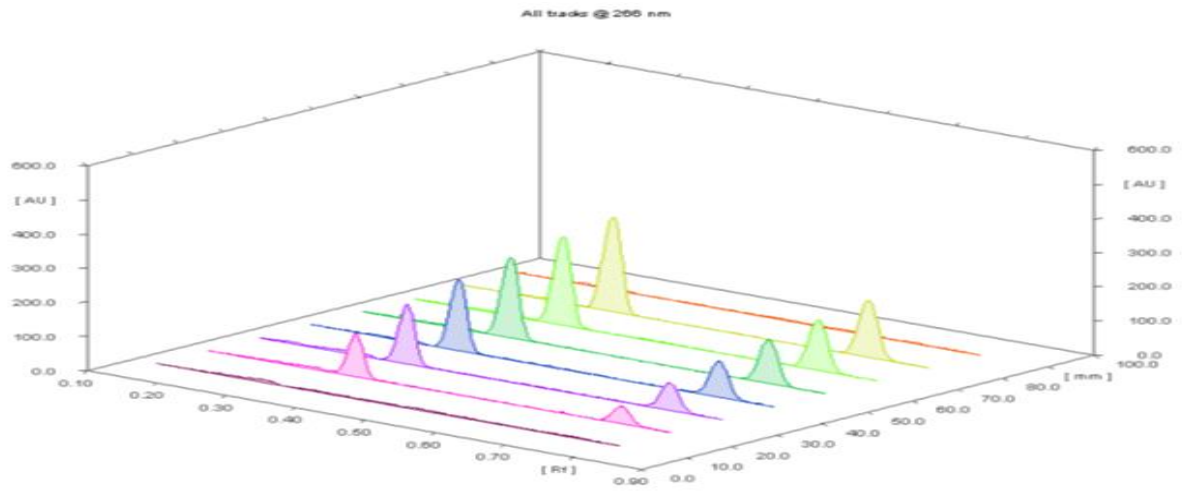


Fig. 7: 3 D spectra of linearity for Dimenhydrinate (500-3000 ng band^{-1}) and Cinnarizine (250-1500 ng band^{-1})

3.3.2 Precision

Set of three different concentrations in three replicates of mixed standard solutions of Cinnarizine and Dimenhydrinate were prepared. All the solutions were analyzed thrice on the same day and on three consecutive days in order to record intra-day and inter-day variations in the results. The % R.S.D. values for intra-day and inter-day precision were found to be in the range of 1.00-1.75 and 0.96-1.24 for Cinnarizine and 1.09-1.53 and 1.10-1.49 for Dimenhydrinate, respectively. The smaller values of % R.S.D. obtained indicate that the developed method is precise.

Table 2: Intra-day precision

Drug	Spotted concentration (ng band^{-1})	Mean area	Concentration found (ng band^{-1})	% R.S.D.*
Cinnarizine	750	2336	749.56	1.75
	1000	2835	997.98	1.00
	1250	3340	1248.90	1.10
Dimenhydrinate	1500	4484	1492.26	1.53
	2000	5390	2015.06	1.21
	2500	6252	2512.30	1.09

*Average of three determinations

Table 3: Inter-day precision

Drug	Spotted concentration (ng band^{-1})	Mean area	Concentration found (ng band^{-1})	% R.S.D.*
Cinnarizine	750	2350	756.52	1.24
	1000	2827	994.01	1.13
	1250	3324	1240.78	0.96
Dimenhydrinate	1500	4503	1503.22	1.49
	2000	5347	1989.88	1.10
	2500	6215	2490.96	1.10

*Average of three determinations

3.3.3 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 46.34 ng band^{-1} and 140.42 ng band^{-1} for Cinnarizine and 58.04 ng band^{-1} and 175.90 ng band^{-1} for Dimenhydrinate, respectively.

3.3.4 Accuracy

Recovery studies were carried out to check the accuracy of the method by standard addition method. It involved the addition of the standard drug to pre-analysed sample at three different levels 80, 100 and 120 %. The drug concentrations were calculated from respective linearity equation. The results of the recovery studies indicated that the method is accurate for the estimation of drugs in combined tablet dosage form.

Table 4: Recovery studies of Cinnarizine and Dimenhydrinate

Drug	Amount is taken (ng band^{-1})	Amount added (ng band^{-1})	Amount found (ng band^{-1})	% Recovery ± R.S.D.
Cinnarizine	500	400	901.05	100.11±1.43
	500	500	998.50	99.84±1.09
	500	600	1095.62	99.60±1.37
Dimenhydrinate	1000	800	1792.22	99.56±0.56
	1000	1000	1988.92	99.44±0.61
	1000	1200	2203.89	100.17±0.80

*Average of three determinations

3.3.5 Robustness

Robustness of the method was determined by making deliberate variations in method parameters. The parameters like mobile phase composition (± 2% methanol), wavelength (± 1 nm) were altered and the effect on the area of drugs was noted. The areas of peaks of interest remained unaffected by small changes of the operational parameters indicating that the method is robust.

4.0 CONCLUSION

A simple, precise, accurate and selective stability-indicating HPTLC method without interference from the excipients has been developed and validated for the simultaneous determination of Cinnarizine and Dimenhydrinate as bulk drugs and in the combined tablet dosage form. The developed method can be used for quantitative analysis of Cinnarizine and Dimenhydrinate in the pharmaceutical dosage form. The method was developed by using

easily available and economic solvents for analysis of drug hence can be considered as economic.

5.0 ACKNOWLEDGEMENT

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