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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHIC METHOD FOR DETERMINATION OF DESLORATADINE IN TABLET DOSAGE FORM

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ABSTRACT
The present work describes development and validation of a new simple, accurate and precise stability-indicating high performance thin layer chromatographic (HPTLC) method for determination of Desloratadine as bulk drug and in tablet dosage form. As stability testing is key step in the development of new drug as well as formulation, stress degradation studies were carried out according to ICH guidelines. Desloratadine was found susceptible to all the analyzed stress conditions. HPTLC plates precoated with silica gel 60 F_{254} were used as the stationary phase and chromatographic separation was achieved by using Methanol: Benzene: Acetic acid (6: 4: 0.5, v/v/v) as mobile phase. Densitometric detection was carried out at 242 nm. The retention factor was found to be 0.55 ± 0.05. The developed method was validated with respect to linearity, accuracy, precision, limit of detection, limit of quantitation and robustness as per ICH guidelines. The developed method was found to be linear in the concentration range of 200-1000 ng band^{-1}. The LOD and LOQ for Desloratadine was found to be 31.47 ng band^{-1} and 95.37 ng band^{-1}, respectively. The developed method has been effectively applied for the drug estimation in tablet dosage form.

KEYWORDS: Desloratadine, Stability indicating method, HPTLC, Forced degradation studies.

INTRODUCTION
Desloratadine, chemically, 8-chloro-6, 11-dihydro 11-(4-piperdinylidene)-5H-benzo [5, 6] cyclohepta [1, 2-b] pyridine is a tricyclic H_1 inverse agonist that is used to treat allergies. It is used to treat allergic rhinitis, nasal congestion and chronic idiopathic urticarial.[1]

An extensive literature survey revealed that different analytical methods has been reported for quantitative analysis of Desloratadine. UV spectrophotometric methods for determination of Desloratadine in bulk and its tablet formulation has been reported.[2-5] High performance liquid chromatography (HPLC)[6-10] and High performance thin layer chromatography (HPTLC)[11-13] methods for the determination of Desloratadine either as single drug or in combination with other drugs in pharmaceutical formulations were also found in the literature. Quantification of desloratadine in human plasma by LC-ESI-MS/MS was also reported.[14]

To best of our information, no reports were found in the literature for determination of Desloratadine in pharmaceutical tablet dosage form by stability-indicating high performance thin layer chromatographic (HPTLC) method. High performance thin layer chromatography (HPTLC) is the most powerful analytical version of thin layer chromatography which is used for the analysis of pharmaceuticals to determine the purity of the drugs available from various sources by detecting the related impurities. The most adaptable technology available is HPTLC, which is renowned for its consistency, purity profile, assay values, and precision and accuracy of outcomes. The technique is simpler, provides more flexibility than HPLC and used as cost-effective quality-control tool for analysis of pharmaceuticals.[15] Hence the purpose of present work was to develop and validate a simple, sensitive, precise and accurate stability indicating HPTLC procedure for determination of Desloratadine as bulk drug and in tablet dosage form in accordance with International Conference on Harmonisation Guidelines.[16,17]

MATERIALS AND METHODS
Chemicals and reagents
Pharmaceutical grade working standard Desloratadine was obtained as a gift sample from Sun Pharmaceuticals Ltd. (Gujrat, India). The pharmaceutical tablet dosage

form Dazit 10 labelled to contain 10 mg (Sun Pharma Laboratories Ltd.) was procured from local pharmacy. All chemicals and reagents used for analysis were of analytical grade. Chemicals used viz. Methanol, Benzene, Acetic acid were purchased from Loba Chemie Pvt Ltd., India.

Instrumentation and chromatographic conditions

Chromatographic resolution of the drug 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 8 mm width using Camag 100 µL sample syringe (Hamilton, Switzerland). A constant application rate of 0.1 µL sec^{-1} was employed.

Linear ascending development was carried out in 10 ×10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) by using benzene: methanol: glacial acetic acid (6: 4 :0.5, v/v/v) as mobile phase. The saturation of mobile phase was done for 20 min in the chamber at room temperature. The length of chromatogram run was 75 mm. Densitometric scanning was performed on a CAMAG TLC scanner III at 242 nm for all developments operated by winCATS software version 1.4.2. Deuterium lamp emitting a continuous UV spectrum between 200 to 400 nm was used as radiation source.

Preparation of working standard stock solution

Accurately weighed 10 mg of Desloratadine transferred to 10 mL volumetric flask and dissolved in methanol to acquire solution of concentration 1000 ng μL^{-1} which was diluted further using methanol to get working standard solution of 100 ng μL^{-1}.

Selection of detection wavelength

After chromatographic development bands were scanned over the range of 200-400 nm. It was observed that drug showed considerable absorbance at 242 nm. So, 242 nm was selected as the wavelength for detection.

Analysis of tablet dosage form

Commercial brand of tablets Dazit 10 containing 10 mg of drug was used to estimate the amount of Desloratadine in existing tablet formulation. For this, 20 tablets were weighed accurately and powdered. Powder quantity equivalent to 10 mg of was weighed and transferred to the 10 mL volumetric flask and 5 mL methanol was added and sonicated for 10 min. The solution was filtered using Whatman filter paper No. 41, and the volume was made up to the mark with methanol. The resulting solution was diluted further with methanol to get final concentration 100 ng μL^{-1}. Four micro-liter 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 242 nm and the amount of drug present in sample was estimated from the respective calibration curve. Procedure was repeated six times for the analysis of homogenous sample.

Stress degradation studies

Stress degradation studies were carried out to confirm the stability by exposing the bulk drug to different physical stress conditions recommended by ICH. The study was carried out at concentration of 1000 ng μL^{-1}. The acid and base hydrolytic studies were performed by treating stock drug solution separately with 0.1 N HCl and 0.1 N NaOH at room temperature for 1 h. The acid and alkali stressed samples were neutralized with NaOH and HCl, respectively to furnish the final concentration of 800 ng $band^{-1}$. Standard drug solution was treated with 3% H_2O_2 at room temperature for 45 min to perform the oxidative degradation and was diluted with methanol to obtain 800ng $band^{-1}$ solution. Thermal stress degradation was performed by keeping drug in oven at 70ºC for period of 1 h. The solid drug powder was exposed UV light up to 200-watt hour square $meter^{-1}$ to check photolytic degradation.

RESULTS AND DISCUSSION

Method optimization

The TLC procedure was optimized with a view to develop a stability indicating method which would be proficient to give the satisfactory resolution between Desloratadine its degradation products. Varied solvent systems comprising different ratios of benzene, chloroform, toluene, methanol, ethyl acetate, acetic acid were examined (data not shown) to achieve better separation and to resolve spot of Desloratadine from its impurities and other excipients present in formulation. Finally, the mobile phase comprising of benzene: methanol: glacial acetic acid (6: 4: 0.5, v/v/v) was chosen as optimum which gave acceptable resolution of drug with symmetrical peak shape. Densitometric detection was carried out at 242 nm. The retention factor (Rf) was found to be 0.55±0.05. Representative densitogram of standard solution of Desloratadine is represented in Figure 1.

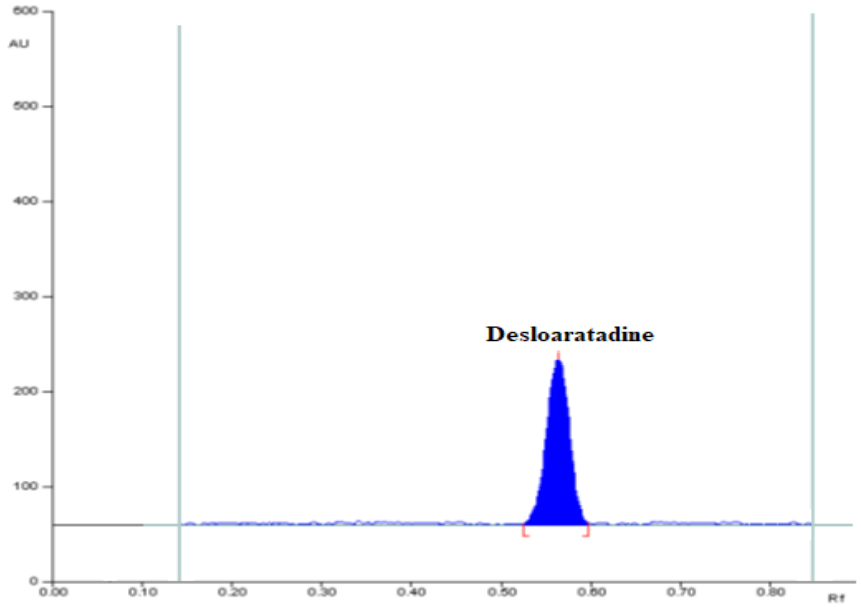

Figure 1: Representative densitogram of standard solution of Desloratadine. (800 ng band^{-1}, Rf= 0.55 ± 0.05)

The stress degradation results demonstrated susceptibility of drug to hydrolytic, oxidative, thermal and photolytic stress conditions. The acid degraded sample of Desloratadine showed 12.46% degradation with decrease in peak area and without appearance of degradation products. Desloratadine after exposure to alkaline and oxidative stress condition showed significant degradation with product of degradation at Rf values 0.27 and 0.73 respectively. Desloratadine after exposure to heat and UV light also showed degradation without any degradation peaks but there was decrease in the area of drug as compared to initial area. Figures 2-4 represents the densitograms of acid, alkali and oxidative degradation. The findings of degradation studies along with % degradation and % of drug recovered are summarized in Table 1.

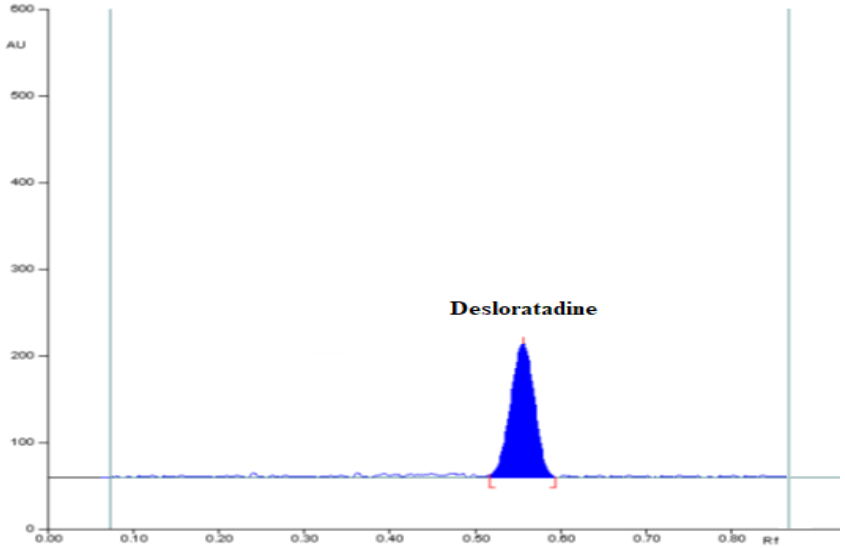

Figure 2: Densitogram after treatment with 0.1 N HCl at RT for 1 h.

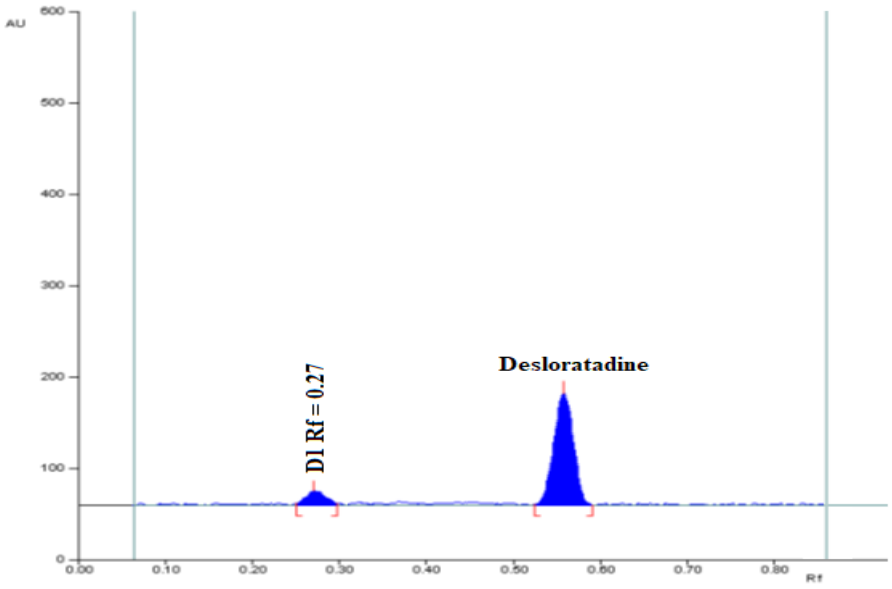

Figure 3: Densitogram obtained after treatment with 0.1 N NaOH with degradation product (D1, Rf = 0.27)

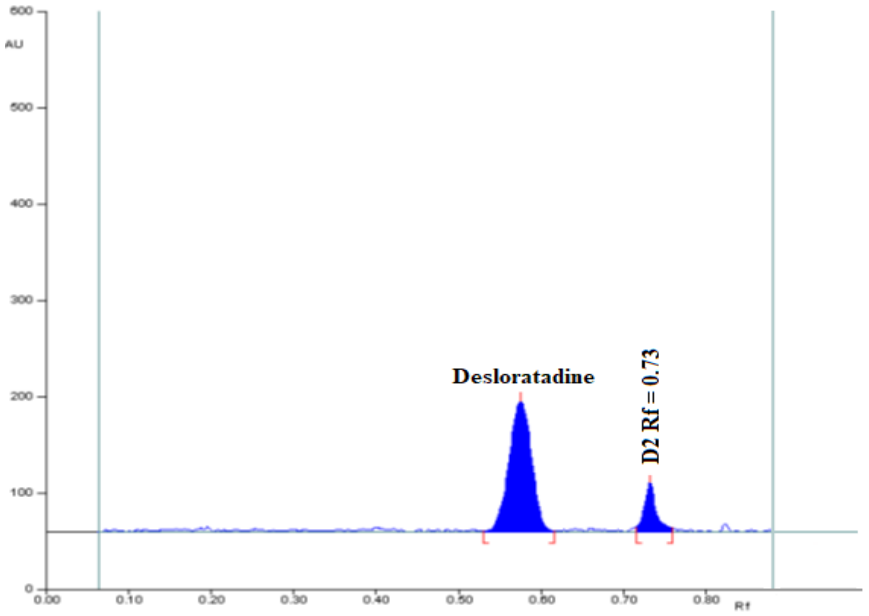

Figure 4: Densitogram after treatment with 3% H_2O_2 with degradation product. (D2, Rf = 0.73)

Table 1: Stress degradation studies.

Stress conditions	% Recovery	% Degradation
Acid hydrolysis (0.1 N HCl, Kept at RT for 1 h)	87.54	12.46
Base hydrolysis (0.1 N NaOH, Kept at RT for 1 h)	81.31	18.69
Oxidative degradation (3 % H_2O_2, Kept at RT for 45 min)	80.48	19.52
Thermal degradation (70^o C for 1 h)	90.39	09.61
Photolysis: UV light 200 watt h square meter^{-1}	83.32	16.68

Method Validation

The developed method was validated in terms of linearity, accuracy, intra-day and inter-day precision, limit of detection, limit of quantitation and robustness, in accordance with ICH Q2 (R1) guidelines.

Linearity

The linearity of proposed method was checked by spotting volumes 2, 4, 6, 8 and 10 µL of standard solution of Desloratadine (100 ng µL^{-1}) onto the TLC plates, developed and scanned under optimized chromatographic conditions. The method was found to

be linear in the concentration range 200-1000 ng band^{-1} with high correlation coefficient. The linear regression equation was found to be y = 2.1066x + 278.66 with correlation coefficient (R^2) value of 0.998. The calibration curve was obtained by plot of concentration vs peak area of drug is shown in Figure 5. A 3D densitogram obtained in the concentration range 200-1000 ng band^{-1} is shown in Figure 6.

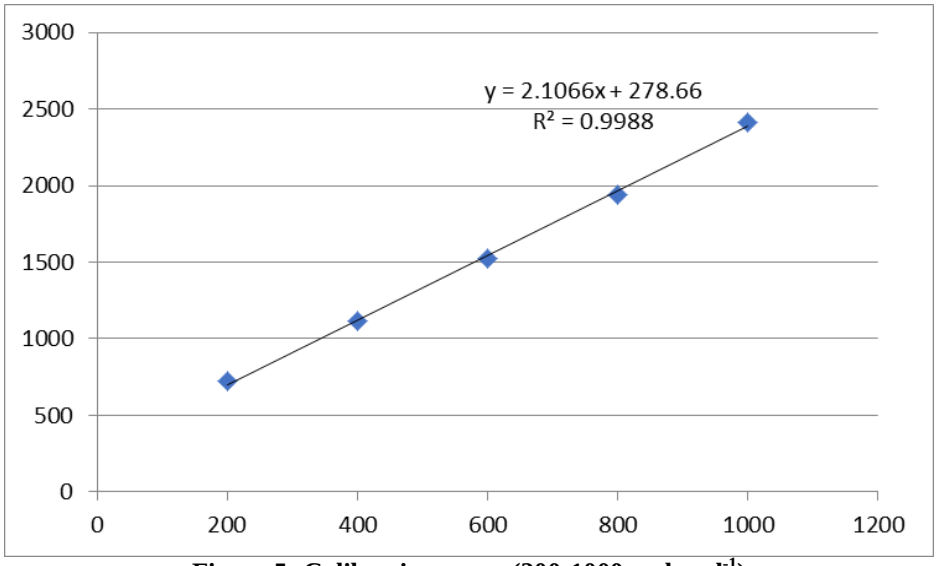

Figure 5: Calibration curve (200-1000 ng band^{-1})

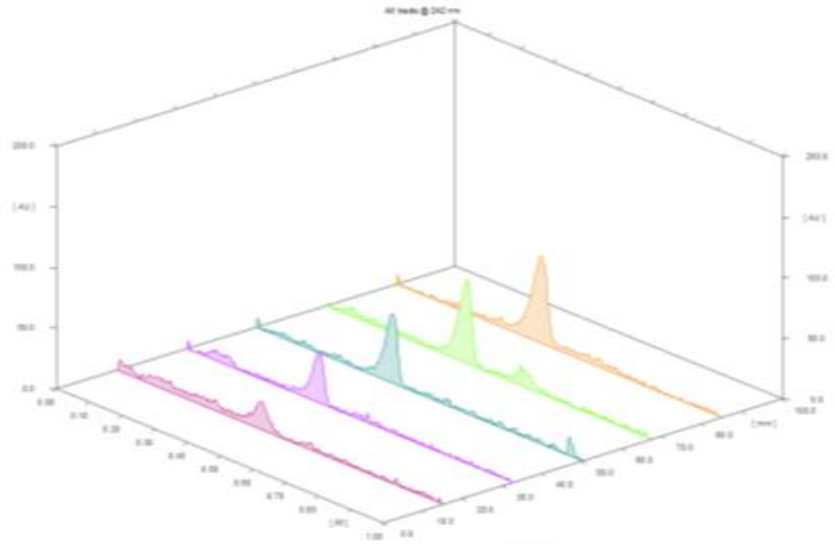

Figure 6: 3D densitogram in concentration range 200-1000 ng band^{-1}

Precision

The precision of the method was demonstrated by intraday and interday variation studies in which three replicates of three concentrations within the linearity range were analyzed on the same day and on three consecutive days, respectively and percentage R.S.D. was calculated. The % R.S.D. values obtained for intraday and interday variations were found to be 0.53 to 0.70 and 1.20 to 1.50, respectively. The lower values of % R.S.D. (< 2) indicated that method was found to be precise.

Table 2: Intraday precision studies.

Spotted concentration (ng band^{-1})	Average Area	Recovered concentration (ng band^{-1})	% R.S.D.*
400	1127	402.84	0.63
600	1551	603.75	0.70
800	1966	801.00	0.53

* Average of three determinations.

Table 3: Inter-day precision studies.

Spotted concentration (ng band^{-1})	Average Area	Recovered concentration (ng band^{-1})	% R.S.D.*
400	1120	399.50	1.39
600	1534	595.82	1.50
800	1945	792.76	1.20

* Average of three 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. The LOD and LOQ values were found to be 31.47 ng band^{-1} and 95.37 ng band^{-1}, respectively.

Accuracy

Accuracy of developed method was checked by performing recovery studies by standard addition method. It involved addition standard drug solution to pre-analyzed sample solution at three different levels 80, 100 and 120%. Basic concentration of sample chosen was 400 ng band^{-1} from tablet solution. The results of the recovery studies indicated accurateness of developed method for estimation of drug in tablet formulation.

Table 4: Recovery studies.

Drug	Concentration taken (ng band^{-1})	Concentration added (ng band^{-1})	Concentration found (ng band^{-1})	% Recovery±R.S.D.*
Desloratadine	400	320	717.63	99.67±0.89
	400	400	798.27	99.78±0.97
	400	480	882.73	100.31±0.72

*Average of three determinations.

Robustness

By introducing deliberate variation in the method parameters, the effects on the results were examined to check the robustness of the method. The parameters varied were mobile phase composition (± 1% methanol), wavelength (± 1 nm), saturation time (± 10 min) and the effect on the area of drug was noted. The areas of peaks of interest remained unaffected by small changes of the operational parameters which indicated robustness of the method.

CONCLUSION

Stability-indicating HPTLC-densitometric method without interference from degradation products and excipients has been developed and validated for the determination of Desloratadine as bulk drug and in tablet dosage form. The drug was found to be susceptible all analyzed stress conditions including heat and light. The developed method is simple, sensitive, precise, accurate, and reproducible. The developed method can be used for quantitative analysis of drug in pharmaceutical dosage form.

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