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Development and Validation of HPTLC Method for Estimation of Desloratadine

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ABSTRACT

A new and rapid high-performance thin-layer chromatographic (HPTLC) method was developed and validated for quantitative determination of desloratadine. The HPTLC separation was achieved on an aluminum-backed layer of silica gel $60F_{254}$ using methanol : chloroform : toluene : ammonia (5.0+ 5.0 + 1.0 + 0.3 v/v/v/v) as mobile phase. Quantitation was achieved by densitometric analysis at 254 nm over the concentration range of 150–750 ng/mL. The method was found to give compact spot for the drug (R_f = 0.6 ± 0.01). The linear regression analysis data for the calibration plots showed good linear relationship with r^2 = 0.9997. The method was validated for precision, recovery, repeatability, and robustness as per the International Conference on Harmonization guidelines. The minimum detectable amount was found to be 21 ng/spot, whereas the limit of quantitation was found to be 65 ng/spot. Statistical analysis of the data showed that the method is precise, accurate, reproducible, sensitive and selective for the analysis of desloratadine. The method was successfully employed for the estimation of desloratadine as a bulk drug and in commercially available tablet formulation.

Keywords: desloratadine, HPTLC, method validation
Abbreviations: **DLT**, desloratadine; **HPLC**, high performance liquid chromatographic; **HPTLC**, high-performance thin layer chromatography; **ICH**, International Conference on Harmonization; **LC/MS/MS**, liquid chromatographic-tandem mass spectrometric

INTRODUCTION

Desloratadine (DLT) is a non sedative, long acting antihistamine with selective peripheral H_1 receptor antagonistic activity. DLT is slightly soluble in water, but highly soluble in methanol and propylene glycol. Chemically desloratadine is 8-chloro-6, 11-dihydro-11-(4-piperidinylidene)-5H-benzo [5,6] cycloheptane [1,2-b pyridine] (Schroeder *et al.* 2001).

No official method for the estimation of DLT is available in literature. Xu *et al.* (2007) reported liquid chromatographic-tandem mass spectrometric (LC/MS/MS) method for the simultaneous determination of DLT and its active metabolite 3-hydroxydesloratadine concentrations in human plasma for pharmacokinetics and bioequivalence studies. Liu *et al.* (2004) developed high performance liquid chromatographic (HPLC) method for the determination of DLT in dog plasma and was used for evaluating the bioequivalence of DLT fumarate tablets and DLT tablets in dogs. Ponnuru *et al*. (2012) developed LC-ESI-MS/MS method for quantitation of DLT in human plasma and applied for its pharmacokinetic study. Other methods reported for quantification of DLT in rat plasma with LC-MS (Chen *et al.* 2009), human plasma with LC-MS (Yang *et al.* 2003; Lu *et al.* 2005; Xu *et al.* 2007; Shen *et al*. 2007) LC-MS with nanospray ionization (Ramanathan *et al.* 2007), human plasma with HPLC (Shen *et al.* 2006; Ghosal *et al.* 2009) Wen *et al.* (2009) reported having developed a method by using liquid-liquid extraction with a concentration range of 0.1–20 ng/ml. Among all researchers, Yang *et al.* achieved the best results with high sensitivity at a linearity range of 25–10000 pg/ml with SPE extraction (Yang *et al.* 2003). Furthermore, the spectrofluorometric method (Method II) was reported to the *in-vitro* determination of the DLT in the presence of the parent drug loratadine in spiked human plasma and samples were prepared using solid phase extraction (El-Enany *et al.* 2007).

Spectrophotometric, spectrofluorometric and HPLC (Method I-IV) were developed and described by El-Enany *et al.* (2007) for the determination of DLT, in pharmaceutical preparations. Methods I and II are based on coupling DLT with 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole in borate buffer of pH 7.6 where a yellow colored reaction product was obtained and measured spectrophotometrically at 485 nm and spectrofluorometrically at 538 nm after excitation at 480 nm, respectively. Methods III and IV, on the other hand, involved derivatization of DLT with 2,4-dinitrofluorobenzene in borate buffer of pH 9.0 producing a yellow colored product that absorbs maximally at 375 nm. The same derivative was determined after separation adopting HPLC. El-Sherbiny *et al.* (2007) described HPLC procedure for analytical quality control of pharmaceutical preparations containing the loratadine and DLT was developed using a microemulsion as the eluent. Rao *et al.* (2010) separated DLT and its impurities in pharmaceutical dosage forms using ultra performance liquid chromatography (UPLC) method. Zheng *et al.* (2010) described gradient ion-pair chromatographic separation of DLT and related compounds in solid pharmaceutical formulation. Kubacak *et al.* (2005) reported quantitation of DLT in pharmaceutical formulation with electrophoresis. Razib *et al.* (2006) developed RP-HPLC assay method for estimation of DLT in marketed formulations. Sumarlik *et al.* (2005) reported TLC method for estimation of DLT in tablet formulation after extraction of samples with 96% ethanol.

Most of the methods reported were intended for the analysis of biological samples and require tedious procedures for sample pretreatment. The HPLC technique is excellent with respect to selectivity and sensitivity, but it cannot be used for routine analysis because of their speciality requirement and cost. Consequently, high-performance thin layer chromatography (HPTLC) based methods could be considered as a good alternative as they are being ex-

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plored as an important tool in routine drug analysis. Major advantage of HPTLC is its ability to analyze several samples simultaneously using a small quantity of mobile phase. This reduces time and cost of analysis. In addition, it minimizes exposure risks and significantly reduces disposal problems of toxic organic effluents, thereby reducing possibilities of environment pollution. HPTLC also facilitates repeated detection of chromatogram with same or different parameters. Furthermore, in case of HPTLC, there are no restrictions on the choice of solvents and mobile phases; drug and excipients can be dissolved in a suitable solvent that would evaporate during spotting on TLC plate leaving behind analyte as a thin band (Patel *et al.* 2010; Patel 2010a, 2010b; Patel *et al.* 2011a, 2011b). Therefore, for such methods, extraction procedure is not required always and could be developed for analyzing drug without any interference from excipients. The present study describes the development and validation of HPTLC method for routine estimation of DLT from bulk and pharmaceutical dosage forms such as tablets.

EXPERIMENTAL

Apparatus

The HPTLC system (Camag, Muttenz, Switzerland) consisted of Limomat V autosprayer connected to a nitrogen cylinder, a twin trough chamber (10 × 10 cm), a derivatization chamber, and a plate heater. Pre-coated silica gel 60 F_{254} HPTLC plates (10 × 10 cm, layer thickness 0.2 mm (E. Merck KGaA, Darmstadt, Germany) were used as stationary phase. HPTLC plates were prewashed twice with 10 mL of methanol and activated at 80°C for 5 min prior to sample application. Densitometric analysis was carried out using a TLC scanner III with winCATS software.

Reagents and materials

The pure DLT powder was obtained form Divi's Laboratory Ltd., (India) with 99.9% purity. Methanol, chloroform, toluene and ammonia were purchased from SDfine Chemicals (Ahmedabad, India). All other chemicals and solvents were of analytical reagent grade and used as received without further purification.

HPTLC method and chromatographic conditions

1. Sample application

The standard and formulation samples of DLT were spotted on pre-coated HPTLC plates in the form of narrow bands of lengths 6 mm, with 10 mm from the bottom and left margin and with 9 mm distance between two bands. Samples were applied under continuous drying stream of nitrogen gas at constant application rate of 150 nL/s.

2. Mobile phase and migration

Plates were developed using mobile phase consisting of methanol : chloroform : toluene : ammonia (5.0+ 5.0 + 1.0 + 0.3 v/v/v/v). Linear ascending development was carried out in 10 cm × 10 cm twin trough glass chamber equilibrated with mobile phase. The optimized chamber saturation time for mobile phase was 20 min at 25 ± 2°C. Ten milliliters of the mobile phase (5 mL in trough containing the plate and 5 mL in other trough) was used for each development and allowed to migrate a distance of 70 mm, which required 10 min. After development, the HPTLC plates were dried completely.

3. Densitometric analysis and quantitation procedure

Densitometric scanning was performed on Camag TLC scanner III in absorbance mode and operated by winCATS planar chromatography version 1.3.4 The source of radiation utilized was deuterium lamp. The spots were analyzed at a wavelength of 254 nm. The slit dimensions used in the analysis were length and width of 5 mm and 0.45 mm, respectively, with a scanning rate of 20 mm/s. These are selected as recommended by the CAMAG TLC Scanner III manual. It covers 70–90% of the application band length, which in the present case is 6 mm. The monochromator bandwidth was set at 20 nm. Concentrations of compound chromatographed were determined from the intensity of diffusely reflected light and evaluated as peak areas against concentrations using linear regression equation.

4. Preparation of DLT standard stock solution

Stock solution was prepared by weighing DLT (10 mg). Weighed powder was accurately transferred to a volumetric flask of 100 mL and dissolved in and diluted to the mark with methanol to obtain a standard stock solution of DLT (100 µg/mL).

Method validation

Validation of the developed HPTLC method was carried out as per the International Conference on Harmonization (ICH) guidelines Q2 (R1) for specificity, sensitivity, accuracy, precision, repeatability, and robustness.

1. Specificity

The specificity of the developed method was established analyzing the sample solutions containing DLT in marketed tablets in relation to interferences from formulation ingredients. The spot for DLT in the sample was confirmed by comparing retention factor (R_f) values of the spot with that of the standard.

2. Sensitivity

Sensitivity of the method was determined with respect to limit of detection (LOD) and limit of quantification (LOQ). Noise was determined by scanning blank spot (methanol) six times. Series of concentrations of drug solutions (10–7500 ng/spot) were applied on plate and analyzed to determine LOD and LOQ. LOD was calculated as 3 times the noise level, and LOQ was calculated as 10 times the noise level. LOD and LOQ were experimentally verified by diluting the known concentrations of DLT until the average responses were approximately 3–10 times the standard deviation (SD) of the responses for six replicate determinations.

3. Linearity and calibration curves

Linearity of the method was evaluated by constructing calibration curves at six concentration levels. Calibration curves were plotted over a concentration range of 150–750 ng/spot. The calibration curves were developed by plotting peak area vs. concentrations (n = 6) with the help of the win-CATS software.

4. Accuracy

Accuracy of the method was evaluated by carrying the recovery study at three levels. Recovery experiments were performed by adding three different amounts of standard drug, i.e., 80, 100, and 120% of the drug, to the preanalyzed MME formulations, solution and conventional tablets, and the resultant was reanalyzed six times.

5. Precision

Precision was evaluated in terms of intra-day and inter-day precisions. Intra-day precision was determined by analyzing sample solutions of DLT from formulation at three levels covering low, medium, and higher concentrations of calibration curve for five times on the same day. Inter-day precision was determined by analyzing sample solutions of DLT at three levels covering low, medium, and higher concentrations over a period of seven days (n = 5). The peak areas obtained were used to calculate mean and % RSD (relative SD) values.

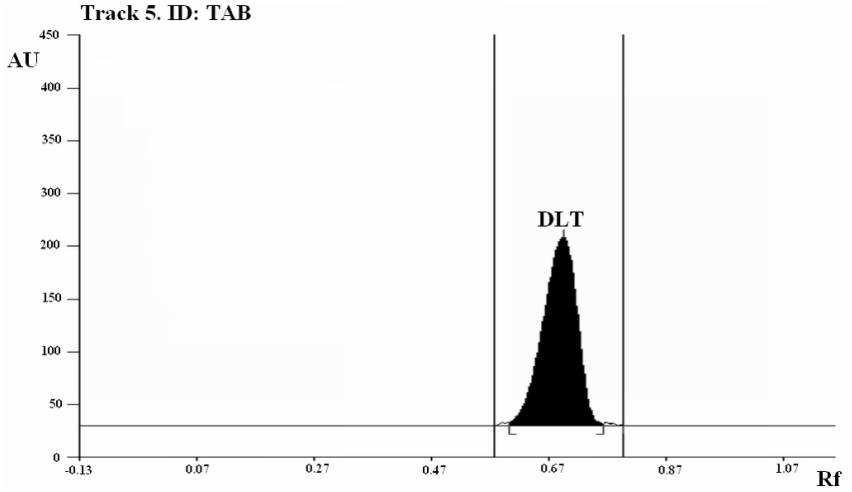

Fig. 1 Chromatogram of standard Desloratadine (500 ng/spot) using mobile phase methanol : chloroform : coluene : ammonia (5.0+ 5.0 + 1.0 + 0.3, v/v/v/v).

6. Repeatability (system precision)

Repeatability of measurement of peak area was determined by analyzing different amount of DLT samples covering low, medium, and higher ranges of the calibration curve seven times without changing the position of plate. Repeatability of sample application was assessed by spotting DLT samples covering similar range of calibration curve seven times and analyzing them once.

7. Robustness

By introducing small changes in mobile phase composition, its volume, chamber saturation time, and slight change in the solvent migration distance, the effects on the results were examined. Robustness of the method was determined in triplicate at a concentration level of 400 ng/spot and the mean and % RSD of peak area was calculated.

Application of developed method

1. Analysis of DLT in formulations

Twenty tablets were weighed and finely powdered. Quantity equivalent to 10 mg of drug was weighed accurately and dissolved in 50 mL methanol. The solution was sonicated for 15 min and then filtered through Whatmann filter paper No. 41. The residue was washed thoroughly with methanol. The filtrate and washings were combined and diluted suitably with methanol to obtain a 100 µg/mL concentration of DLT. On HPTLC plates, 4 µL of these solutions were spotted and analyzed for DLT content using proposed method as described earlier. The possibility of interference from other components of the tablet formulation in the analysis was studied.

RESULTS AND DISCUSSION

To develop HPTLC method of analysis for DLT for routine analysis, selection of mobile phase was carried out on the basis of polarity. A solvent system that would give dense and compact spots with appropriate and significantly different R_f value for DLT was desired. Various solvent systems such as acetone-methanol, methanol–chloroform, methanol–toluene, toluene–ethyl acetate, hexane–ethyl acetate, hexane–acetone, Methanol-chloroform-toluene-ammonia, toluene–acetonitrile, and toluene–acetonitrile–glacial acetic acid were evaluated in different proportions. Among these, the solvent system comprising of methanol : chloroform : toluene : ammonia (5.0 + 5.0 + 1.0 + 0.3, v/v/v/v) gave good separation of DLT from its matrix with an R_f value of 0.6. It was observed that chamber saturation time and solvent migration distance are crucial in chromatographic separation as chamber saturation time of less than 15 min and solvent migration distances greater than 70 mm resulted diffusion of analyte spot. Therefore, methanol : chloroform : toluene : ammonia (5.0 + 5.0 + 1.0 + 0.3, v/v/v/v) solvent system with chamber saturation time of 20 min at 25°C and solvent migration distance of 70 mm was used as mobile phase. These chromatographic conditions produced a well-defined compact spot of DLT with optimum migration at $R_f = 0.6 \pm 0.01$ (**Fig. 1**). It also gave a good resolution of analyte from excipients used in formulation.

Table 1 Linear regression data for the calibration curves ($n = 6$).

Range (ng/spot)	$r^2 \pm$ SD	Slope ± SD	Intercept ± SD
150 - 750	0.9997 ± 0.001	14.084 ± 0.13	61239.27 ± 64.41

Under the experimental conditions employed, the lowest amount of drug that could be detected was found to be 21 ng/spot and the lowest amount of drug that could be quantified was found to be 65 ng/spot, with *RSD* <5%.

Specificity is the ability of an analytical method to assess unequivocally the analyte in the presence of sample matrix (Patel *et al.* 2011a, 2011b). DLT was separated from excipient with an R_f of 0.6 ± 0.01. There was no interfering peak at the R_f value of DLT from excipients present in commercial formulation, thereby confirming specificity of method.

Linearity of an analytical method is its ability, within a given range, to obtain test results that are directly, or through a mathematical transformation, proportional to concentration of analyte. Method was found to be linear in a concentration range of 150–750 ng/spot ($n = 6$), with respect to peak area. The regression data as shown in **Table 1** reveal a good linear relationship over the concentration range studied demonstrating its suitability for analysis. No significant difference was observed in the slopes of standard curves (ANOVA, $P > 0.05$).

Accuracy of an analytical method is the closeness of test results to true value (Patel *et al.* 2011a, 2011b). It was determined by the application of analytical procedure to recovery studies, where known amount of standard is spiked in preanalyzed samples solutions. Results of accuracy studies from excipient matrix are shown in **Table 2**; recovery values demonstrated the accuracy of the method in the desired range.

The precision of an analytical method expresses the degree of scatter between a series of measurements obtained from multiple sampling of the same homogeneous sample under prescribed conditions. Intra-day precision refers to the use of analytical procedure within a laboratory over a short period of time using the same operator with the same equipment, whereas inter-day precision involves estimation of variations in analysis when a method is used within a laboratory on different days, by different analysts (Patel *et al.* 2010; Patel 2010a, 2010b; Patel *et al.* 2011a,

Table 2 Recovery studies (n = 6).

Formulation	Amount of drug analyzed (ng)	Amount of drug added (ng)	Theoretical concentration (ng)	Total amount of drug analyzed (ng)	% Recovery ± SD
Marketted	200	160	360	359.11	99.75278
Tablets	200	200	400	400.23	100.0575
	200	240	440	438.89	99.74773
				%Average recovery ± SD	99.85 ± 0.18

Table 3 Intra and inter-precision studies (n = 5).

Amount of drug spotted (ng)	Amount of drug detected (ng, mean ± SD)	% RSD
Intra-day (n = 5)		
100	99.83 ± 1.84	1.25
300	298.12 ± 1.79	0.74
600	600.05 ± 1.12	0.29
Inter-day (n = 5)		
100	100.03 ± 0.44	0.64
300	301.23 ± 1.05	0.48
600	595.89 ± 2.33	1.04

2011b). The results obtained are shown in **Table 3**. In all instances, % *RSD* values were less than 5% confirming the precision of the method.

Ten-microliter aliquots of samples containing 200, 400, and 600 ng of DLT were analyzed according to proposed method. In order to control scanner parameters, i.e., repeatability of measurement of peak area, one spot was analyzed without changing position of plate (n = 7). By spotting and analyzing the same amount several times (n = 7), precision of automatic spotting device was evaluated. %*RSD* was consistently less than 5% (**Table 4**), which was well below the instrumental specifications, ensuring repeatability of developed method as well as proper functioning of the HPTLC system.

The low values of % *RSD* (**Table 5**) obtained after introducing small deliberate changes in the developed HPTLC method confirmed the robustness of the method (Patel *et al.* 2011a, 2011b).

A single spot at R_f = 0.6 was observed in the chromatogram of DLT. No interference from the excipients present in the marketed tablet formulation was observed. Analysis of DLT tablets showed a drug content of 99.83%. The DLT content of the marketed formulations was found to be within the limits (±5% of the theoretical value) and is mentioned in **Table 6**. The low %*RSD* value indicated the suitability of this method for routine analysis of DLT in various formulations.

Table 6 Content of DLT in formulation.

Formulation	Actual concentration ng/spot	% DLT	% RSD
Tablets	500	99.83 ± 0.28	1.06

CONCLUSION

A new HPTLC method has been developed for the identification and quantification of DLT. Low cost of ingredients, faster speed, and satisfactory precision and accuracy are the main features of this method. Method was successfully validated as per ICH guidelines and statistical analysis proves that method is sensitive, specific, and repeatable. It can be conveniently employed for routine quality control analysis of DLT as bulk drug in marketed tablets.

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Table 4 Repeatability studies (n = 7).

Parameters	Amount of drug detected (ng, mean ± *SD*)		
Amount of olanzapine spotted (ng)	200	400	600
Measurement of peak area[a]	199.43 ± 1.23	398.56 ± 3.17	589.23 ± 9.05
%*RSD*	1.66	2.08	2.81
Sample application and derivatization technique[b]	200.18 ± 3.11	401.31 ± 6.05	595.98 ± 7.02
%*RSD*	2.39	3.49	4.79

[a] One spot is scanned eight times.
[b] Eight spots scanned once.

Table 5 Robustness of method (n = 3).

Parameters	Amount of DLT spotted (ng)	Amount of DLT detected (ng, mean ± SD)	%RSD
Mobile phase composition: 5.1:4.9:0.9:0.3	300	298.11 ± 4.21	2.17
Mobile phase composition: 4.9:5.1:1.1:0.3	300	299.23 ± 2.39	2.29
Mobile phase volume: 8 mL	300	301.14 ± 5.77	1.39
Mobile phase volume: 12 mL	300	298.32 ± 3.76	1.49
Chamber saturation time: 15 min	300	295.63 ± 3.86	2.57
Chamber saturation time: 25 min	300	297.49 ± 3.19	2.17
Solvent migration distance: 68 mm	300	302.57 ± 3.16	1.79
Solvent migration distance: 72 mm	300	303.14 ± 5.97	1.99

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