Validation of stability indicating high performance liquid chromatographic method for estimation of Desloratadine in tablet formulation

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Abstract A simple, sensitive and specific stability indicating high performance liquid chromatographic (HPLC) method for the estimation of Desloratadine was developed and validated. Desloratadine was separated and quantitated on Inertsil ODS-3 V column (250 mm length, 4.6 mm id, 5 μm particle size) using a mixture of methanol–phosphate buffer of pH 7.0 (70:30 v/v) as a mobile phase and at a flow rate of 1.0 mL/min. Quantification was achieved with an UV detector at 254 nm over the concentration range of 5–75 μg/mL. The applied HPLC method allowed the separation and quantification of Desloratadine with good linearity ($r^2 = 0.999$) in the studied concentration range. Limit of detection and limit of quantification were found to be 1.28 μg/mL and 3.89 μg/mL, respectively. The method was validated as per the International Conference on Harmonization (ICH) guidelines. Desloratadine stock solution was subjected to different stress conditions. The degraded product peaks were well resolved from the pure drug peak with significant difference in their retention time values. Stressed samples were assayed using developed HPLC method. Statistical analysis of the data showed that the method is precise, accurate, reproducible, and selective for the analysis of Desloratadine. The method was successfully applied to the estimation of Desloratadine in tablet dosage form.

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1. Introduction

Desloratadine (DLT) is a non sedative, long acting antihista-
mine with selective peripheral H1 receptor antagonistic activ-
ity. 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
the literature. Xu et al., reported a liquid chromatographic-
tandem mass spectrometric (LC/MS/MS) method for the
The present study describes the validation of a stability-indicating RP-HPLC method for the determination of DLT in the presence of its degradation products according to the ICH guidelines (ICH, 2005). The developed method is applied for the routine analysis of DLT in pharmaceutical tablet dosage form.

2. Experimental

2.1. Materials and methods

The pure DLT powder was obtained from Divi’s Laboratory Ltd., India. HPLC grade methanol, acetonitrile, water, triethylamine, tetrahydrofuran, and orthophosphoric acid were purchased from E. Merck (India) Ltd., Mumbai. Analytical grade sodium hydroxide, hydrochloric acid, ammonia, monobasic potassium phosphate, potassium dihydrogen phosphate, ammonium acetate, and glacial acetic acid were purchased from Spectrochem Pvt. Ltd. India.

2.2. Apparatus

The method was developed using a WATERS 2695 instrument equipped with a photo diode array detector 2996, isocratic pump system, auto injector, and Inertsil ODS-3V column (150×4.6 mm id, and 5 μm particle size).

2.3. Chromatographic condition

The Inertsil ODS-3V column was used at ambient temperature. The mobile phase consisted of methanol–phosphate buffer (70/30, v/v) with the final pH adjusted to 7.0 ± 0.02 and was pumped at a flow rate of 1 mL/min. The mobile phase was filtered through a Nylon 0.45 μm membrane filter and degassed before use. The elution was monitored at 254 nm, and the injection volume was 20 μL.

2.4. Preparation of Desloratadine standard stock solution

A 50 mg of standard DLT was accurately weighed and transferred to a 100 mL volumetric flask and dissolved in 75 ml of mobile phase. The flask was sonicated for 10 min. The flask was shaken and the volume was made up to the mark with the mobile phase to give a solution containing 500 μg/mL of DLT. The above solution was filtered through whatman filter paper (0.45 μ). From this stock solution the working standard solution was prepared by further diluting 5 ml of stock solution to 50 ml using the mobile phase.

2.5. Sample preparation for determination of Desloratadine in tablet dosage form

Twenty tablets were weighed and finely powdered. A mass equivalent to 50 mg DLT was weighed and transferred to a
100 mL volumetric flask, and the mobile phase (75 mL) was added. The solution was sonicated for 15 min, and the final volume was diluted to the mark with the mobile phase to obtain a solution of DLT (500 µg/mL). 5 ml aliquot of this solution was further diluted to 50 mL with the mobile phase to obtain a 50 µg/mL solution of DLT, which was used for determination of DLT. The solutions were filtered through a Nylon 0.45 mm membrane filter.

2.6. Method validation

Validation of the developed HPLC method was carried out as per the International Conference on Harmonization (ICH) guidelines Q2 (R1).

2.6.1. Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities and degradation products. Commonly used formulation excipients (hydroxypropylcellulose, mannitol, microcrystalline cellulose, polyethylene glycol 6000, and lactose monohydrate (Signet Ltd., Mumbai, India), and Methocel E5 Premium LV EP (Colcorcon Asia Pvt. Ltd., Goa, India) were spiked into a preweighed quantity of drugs. The chromatogram was taken by appropriate dilutions and the quantities of drugs were determined.

Specificity was also studied by performing the forced degradation study using acid and alkaline hydrolysis, chemical oxidation and dry heat degradation studies and interference of the degradation products were investigated. DLT was weighed (50 mg) and transferred to two separate 100 ml volumetric flasks, dissolved in a few ml of methanol and diluted up to the mark with methanol. This stock solution was used for forced degradation studies.

2.6.1.1. Alkali hydrolysis. To the separate 50 ml volumetric flask, 5 ml stock solutions of DLT were taken and 5 ml of 3 M NaOH was added to perform base hydrolysis. Flask was heated at 80°C for 2 h and allowed to cool to room temperature. Solution was neutralized with 3 M HCl and diluted up to the mark with the mobile phase.

2.6.1.2. Acid hydrolysis. To the separate 50 ml volumetric flask, 5 ml stock solutions of DLT were taken and 5 ml of 3 M HCl was added to perform acid hydrolysis. Flask was heated at 80°C for 2 h and allowed to cool to room temperature. Solution was neutralized with 3 M NaOH and diluted up to the mark with the mobile phase.

2.6.1.3. Oxidative stress degradation. To perform oxidative stress degradation, appropriate aliquots of stock solutions of DLT were taken in separate 50 ml volumetric flasks and 5 ml of 3% hydrogen peroxide was added. Flask was heated in a water bath at 80°C for 2 h and allowed to cool to room temperature and diluted up to the mark with the mobile phase.

2.6.1.4. Thermal (dry heat) degradation. Analytically pure sample of DLT was exposed in an oven at 80°C for 2 h. The solid was allowed to cool and 50 mg of DLT was weighed, transferred to separate 100 ml volumetric flasks and dissolved in 75 ml of methanol. Volume was made up to the mark with methanol. Solution was further diluted by the mobile phase taking appropriate aliquots.

2.6.1.5. Photochemical degradation. The photochemical stability of the drug was also studied by exposing the stock solution to an UV-chamber for 24 h. The resultant solutions were appropriately diluted with the mobile phase and analyzed.

All solutions were passed through a 0.45µm Whatman filter paper and injected in the liquid chromatographic system and chromatograms were recorded.

2.6.2. Calibration curve (linearity of the HPLC method)

Calibration curves were constructed by plotting peak area vs. concentrations of DLT, and the regression equations were calculated. The calibration curves were plotted over the six different concentration range 5–75 µg/mL of DLT. Aliquots of standard working solution were transferred to a series of 10 mL volumetric flasks and diluted to the mark with the mobile phase. Aliquots (20 µL) of each solutions were injected under the operating chromatographic condition described above (n = 6).

2.6.3. Precision

The intra-day and inter-day precision studies were carried out by estimating the corresponding responses three times on the same day and on three different days for three different concentrations of DLT (20, 30, 40 g/mL), and the results are reported in terms of relative standard deviation. The instrumental precision studies were carried out by estimating the response of three different concentrations of DLT (20, 30, 40 g/mL) six times and results are reported in terms of relative standard deviation.

2.6.4. Detection limit and quantitation limit

The limit of detection (LOD) is defined as the lowest concentration of an analyte that can reliably be differentiated from background levels. Limit of quantification (LOQ) of an individual analytical procedure is the lowest amount of analyte that can be quantitatively determined with suitable precision and accuracy. LOD and LOQ were calculated using the following equation as per ICH guidelines.

\[
LOD = 3.3 \times \sigma/S; \quad LOQ = 10 \times \sigma/S
\]

where \(\sigma\) is the standard deviation of y-intercepts of regression lines and S is the slope of the calibration curve.

2.6.5. Accuracy (% recovery)

The accuracy of the method was determined by calculating the recovery of DLT by the standard addition method. Known amounts of standard solution of DLT (0, 25, 50 and 75 µg/mL) were added to prequantified sample solutions of tablet dosage form. The amount of DLT was estimated by applying values of peak area to the regression equations of the calibration curve.

2.6.6. Robustness

Robustness of the method was studied by changing the flow rate (±10%) composition of organic phase (±2%) and the pH (±0.2%) of mobile phase.
2.6.7. Solution stability
Stability of sample solutions was studied at ambient temperature for 24 h.

2.6.8. System suitability test
The system suitability test was carried out to evaluate the resolution and reproducibility of the system for the analysis to be performed, using five replicate injections of a reference solution containing 50 μg/mL of DLT. The parameters measured were peak area, retention time, theoretical plates, and tailing factor (peak symmetry).

3. Result and discussion

3.1. HPLC method development and optimization
Validation of a stability-indicating analytical method should demonstrate the capability of the method for the quantitation of the active pharmaceutical ingredient and the determination of possible degradation products without any interference. To obtain the best chromatographic conditions, the mobile phase was optimized to provide sufficient selectivity and sensitivity in a short separation time. Ammonium acetate buffer resulted in high sensitivity compared with phosphate buffer and phosphoric acid solution. The use of methanol resulted in better sensitivity and short analysis time, improving the peak symmetry (about 1.29). Columns from different sources were evaluated, and the Inertsil ODS-3 V analytical column was selected, as it provided the best chromatographic performance and acceptable peak characteristics, including tailing factor and the number of theoretical plates. Moreover, acceptable resolution of DLT and the degradation products was obtained, confirming the stability-indicating capability of the proposed method. For selection of the best wavelength of detection, a PDA detector was used.

A satisfactory separation with good peak symmetry and steady baseline was achieved with Inertsil ODS-3V column and methanol–phosphate buffer pH 7.0 (70 + 30, v/v) as the mobile phase at a flow rate of 1.0 mL/min. The quantitation of DLT was achieved at 254 nm. The optimized conditions of the HPLC method were validated for the analysis of DLT in tablet formulations and application for QC. Fig. 1 shows a typical chromatogram obtained by the proposed RP-HPLC method, demonstrating the resolution of the symmetrical peak corresponding to DLT. The retention time observed (5.2 min) allows a fast determination of the drug, which is suitable for QC laboratories.

3.2. Method validation
Forced degradations are performed to provide indications of the stability-indicating properties of an analytical method, particularly when there is no information available about the potential degradation products.

The results from the stress testing studies indicated that the method was highly specific for DLT. The drug was found to be unstable in basic medium. The degradation products were completely distinguishable from the parent compound. Alkali stress lead to 70% recovery with two unknown degradation peaks at 1.7 and 1.9 min, respectively, whereas a prominent peak of DLT was stable at 5.58 min (Fig. 2a). Peroxide stress lead to 84.5% recovery with one unknown degradation peak at 1.7 min, whereas a prominent peak of DLT was stable at 5.56 min (Fig. 2b). The force degradation studies in acid, thermal and UV degradation conditions of DLT resulted in a significant decrease of the peak area, 90.0%, 82.9%, and 88.1%, respectively, without any detectable degradation products (Fig. 2c–e). Table 1 outlines the results of degradation study of DLT at each stress condition.

Specificity is the ability to accurately and specifically measure the analyte of interest in the presence of other components that may be expected to be present in the sample matrix. It is a measure of the degree of interference from other active ingredients, excipients, impurities, and degradation products. Specificity in a method ensures that a peak response is due to a single component only. In the present study, the ability of the method to separate the drug from its degradation products and the non-interference of the excipients indicate the specificity of the method. Values of peak purity index were higher than 0.9999. These results indicated that the proposed method is specific and stability-indicating, and can be applied for stability studies and QC analysis of DLT in pharmaceutical products, with advantages when compared to the previously published methods.

The linearity of a method is defined as its ability to provide measurement results that are directly proportional to the con-
The linearity of the detector was obtained by diluting the analyte stock solution and measuring the associated responses, while the linearity of the analytical method was determined by making a series of concentrations of the analyte from independent sample preparations (weighing and spiking). The linearity data described in the present study demonstrate an acceptable linearity for DLT over the range of 80–120% of the target concentration. Linear correlation was obtained between peak areas and concentrations of DLT in the range of 5–75 μg/ml. The following regression equation was found by plotting the peak area (y) versus the DLT concentration (x) expressed in μg/mL: 
\[ y = 43562x + 25557 \]
The correlation coefficient \( r^2: 0.999 \) obtained for the regression line demonstrates the excellent relationship between peak area and concentration of DLT. Data of regression analysis are summarized in Table 2. Moreover, the relative SE of slope can be used as a parameter with respect to the precision of the regression, as a general acceptance criterion for the linearity performance of the analytical procedure. This parameter should be comparable to the RSD obtained in the evaluation of the precision. The result obtained for the RSD of the slope was 0.44%, which is lower than the mean value of 0.95% for the RSD of the precision.

The precision, evaluated as the repeatability of the method, was studied by calculating the RSD for six determinations of the 50 μg/mL sample of DLT performed on the same day and under the same experimental conditions. The obtained RSD value was 0.90%. The RSD values for repeatability study was found to be <1%, which indicates that the proposed method is repeatable. The intermediate precision was assessed by analyzing two samples of the pharmaceutical formulation on three different days (interday); the mean values obtained were 99.13% and 99.40% with RSDs of 0.64% and 0.83%, respec-

<table>
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<th>Stress condition</th>
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<th>Standard Desloratadine concentration (μg/ml)</th>
<th>Retention time (min)</th>
<th>% Area</th>
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Table 2 Regression analysis of calibration graphs for Desloratadine for the proposed HPLC.

<table>
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<th>Parameter</th>
<th>Desloratadine</th>
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<td>Intercept</td>
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<td>Standard deviation of the intercept</td>
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<tr>
<td>Correlation coefficient</td>
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The between-analysts precision was determined by calculating the mean values and the RSD for the analysis of two samples of the pharmaceutical formulation by three analysts; the values were found to be 99.46% and 99.97% with RSDs of 0.73% and 0.88%, respectively. The RSD values for intermediate precision was found to be <2%, which indicate that the proposed methods are reproducible.

The accuracy was assessed by the standard addition method for three replicate determinations of three different solutions containing 50, 100, and 150 μg/mL of DLT. The recoveries were obtained in a range of 98.15–99.50% for DLT using the proposed HPLC method. (Table 3) The high values indicate that the proposed HPLC method is accurate.

The LOD and LOQ were determined from slopes of linear regression curves. The limit of detection (LOD) and limit of quantification (LOQ) for DLT were found to be 1.28 and 3.89 μg/mL, respectively. (Table 3) LOD and LOQ data show that DLT can be accurately determined in the microgram quantity.

The results and the experimental range of the selected variables evaluated in the robustness assessment are given in Table 4, together with the optimized values. There were no significant changes in the chromatographic pattern when the modifications were made in the experimental conditions, thus showing the method to be robust. Solvent suitability study was carried out to establish the stability of the sample in an analytical solution (diluent) over a period of time during routine analysis. The stability of sample solutions was tested at intervals of 12 h, 24 h, and up to 48 h. The method was found to be rugged as there was no change in the area of DLT. Results of this solvent suitability study show a non-significant change (<2%) relative to freshly prepared samples. The RSD values calculated in the system suitability test for the parameters studied were within the acceptable range (RSD < 2.0%), as shown in Table 5, indicating that the system is suitable for the analysis intended.

3.3. Method application

The proposed RP-HPLC method was applied for the determination of DLT in tablet dosage forms, without prior separation of the excipients of the formulation. The results in Table 6 demonstrate the quality of the analyzed pharmaceutical samples and the applicability of the method for QC analysis.

4. Conclusions

This study is a typical example of the development of a stability-indicating assay following ICH guidelines. The results of stress testing are undertaken according to the ICH guidelines which reveal that the method is specific and stability-indicating. Based on the peak purity results obtained from the analysis of forced degradation samples using the described method, it can be concluded that there is no other coeluting peak with the main peaks, and the method is specific for the determination of DLT in the presence of degradation products.

A simple and rapid isocratic stability-indicating RP-HPLC method has been developed and validated for the determination of DLT in tablet dosage form. The results of the validation studies show that the RP-HPLC method is sensitive, accurate, specific, and stability-indicating. It possesses significant linearity (r² = 0.9999), precision with a mean RSD of 0.90%, high efficiency and resolution, and no interference from the excipients or degradation products, as was demonstrated. The proposed method was successfully applied and is suggested for the quantitative analysis of DLT in pharmaceuti-
aceutical formulations for QC, where economy and time are essential and to assure therapeutic efficacy.

Acknowledgements

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References