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Development, Validation of RP-HPLC Method and GC MS Analysis of Desloratadine HCL and It's Degradation Products

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ABSTRACT

Introduction: Efficacy and safety of drug therapy is directly related to the stability of active pharmaceutical ingredient (API) and drug product used. Forced degradation studies (also called stress testing) are performed for better understanding of API and drug product stability. Thus, stress testing is a prognostic research tool used to ascertain stability of drug molecule and provide support for developing stability indicating method.

Aim: The research aim is Development, Validation of RP-HPLC Method and GC MS Analysis of Desloratadine HCL and Its Degradation Products.

Objective: The objective of current study was to develop validated specific stability indicating reversed-phase liquid chromatographic method for the quantitative determination of Desloratadine HCl in bulk sample in the presence of degradation products.

Method: Desloratadine HCL was subjected to variable pH, oxidative, dry heat and photolytic stress condition as per ICH guideline for stability study. Stressed samples were further studied by validated RP-HPLC method and also studied by GC-MS to characterise degradation products (Fig 15).

Result: At oxidative stress, degradation products were generated and detected by GCMS. Slight degradation was observed in acidic and alkaline stress while no degradation was observed in other stress conditions. Separation of degradation products from pure drug was achieved on C18 column 5µ (4.6 X 250 mm) using the mobile phase consists a mixture of Orthophosphoric acid (0.1%V/V), Acetonitrile and Methanol (50:35:15 V/V/V). The detection was carried out at 242 nm. The proposed validated LC method was used to quantify the stressed test solutions in order to ascertain stability indicating potential of the method.

Conclusion: The established LC approach has been shown to be suitable for determining the quality of Desloratadine HCl from its dosage form and assessing its stability when required.

Key Words: Reversed-Phase Liquid Chromatographic Method, Validation, Stability study, Gas Chromatography and Mass Spectrometry (GCMS), Orthosphosphoric acid

INTRODUCTION

8-chloro-6, 11-dihydro-11-(4-piperdinylidene)-5*H*-benzo [5,6] cyclohepta[1,2-b]pyridine Desloratadine (DSLR) is a tricyclic antihistamine, an antagonist at histamine H1 receptors, and at all subtypes of the muscarinic acetylcholine receptor. It is originally used for the treatment of allergies and allergic rhinitis. [1,2]

It is reported that Desloratadine exhibit antihistaminic activity and anti-inflammatory activity.[1]Desloratadine impedes release of proinflammatory cytokines, such as the interleukin (IL) 6 and IL-8.

It has a long-lasting effect and in moderate and low doses, does not cause drowsiness as it does not readily enter the central nervous system.[3] It is demonstrated that Desloratadine has no adverse effects on the central nervous system,

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cardiovascular system, or gastrointestinal system, and no significant drug interactions.[1]

Spectrophotometric analytical methods have been reported for the determination of DSLR in Pharmaceutical formulation. [4,5,6] Several analytical methods including liquid chromatography with ultraviolet detection [7,8,9] or mass spectrometric detection [10], gas chromatography with nitrogen phosphorous detection [11] have also been reported for simultaneous estimation of DSLR and other drugs from their combined dosage form. Few methods have also been developed for estimation of DSLR in biological samples and applied pharmacokinetic studies. [12,13,14]

The structural characterisation of significant degradation products allows for the identification of the degradation process by which the degradation products are produced. This further helps with the quantitative determination of the drug, in the presence of its process related impurities and degradation products. All new drug substances and new dosage form must be evaluated with a stability-indicating assay method before being released, according to Current Good Manufacturing Practices.[15,16]To date, no stability-indicating HPLC assay method for the determination of Desloratadine is available in the literature. It was felt necessary to develop a stability-indicating liquid chromatography (LC) method for the determination of Desloratadine as bulk drug and as pharmaceutical dosage forms and to separate the drug from the degradation products under the International Conference on Harmonization (ICH) suggested conditions (hydrolysis, oxidations, photolysis and thermal stress).[17]

Therefore, the principal objective of this study was to develop a new, simple, economical, precise, and reproducible stability indicating RP-HPLC method with a wide linear range and good sensitivity for assay of Desloratadine in the bulk drug and in the pharmaceutical dosage form. [18,19]

MATERIAL AND METHODS

Chemicals

Desloratadine reference substance was received as a gift sample from ZIM laboratory. HPLC grades Acetonitrile, Methanol and Distilled water were used. All other reagents used in this study were of AR grade. Tablet DESLOR 5 Sun Pharmaceuticals Ltd. was used for the estimation.

Chromatographic conditions

Agilent 1120 LC was used throughout analysis. The system consisted of binary solvent reservoir, manual injector (20 µl), Agilent C18 column, 5µ (4.6 X 250 mm) and VWD detector. EZ chrome Elite software was used for the data analysis and interpretation.

Preparation of Mobile phase:

Solvent A – Orthophosphoric acid (0.1%): An accurately measured 0.5 mL of OPA (%) transferred to 500 mL volumetric flask and diluted up to mark with HPLC grade distilled water. The flask was kept in ultra sonicator for 30 minutes. Then it was filtered through the 0.45µ membrane filter. Filtrate was again sonicated for 30 minutes.

Solvent B-Acetonitrile and Methanol also passes through the 0.45µ membrane filter and sonicated for 30 minutes separately. Then, accurately measured 350 mL methanol and 150 mL acetonitrile was taken in separate 500 mL beaker. Methanol was added to acetonitrile with continuous stirring. Thereafter, this mixture was sonicated for 30 minutes, passed through 0.45µ membrane filter and again sonicate for 30 minutes.

Solvent A and solvent B were transferred to the appropriate reservoir linked to channel A and channel B respectively. They were passed through the column in proportion (50:50 V/V) at flow rate 1 ml /minutes for sufficient time to saturate the stationary phase packed in column with mobile phase.

Linearity Study

Standard Stock Solution:

A standard stock solution (1000 ppm) was prepared by dissolving 100 mg of the DSLR HCL in 100 ml of methanol. Solution was sonicated for 30 minutes. An aliquot about 1 ml was pipette out from the above solution and diluted to 10 ml with methanol to make 100 ppm solution.

Working Standard Solution:

Appropriate aliquots were withdrawn from the stock solution and diluted up to10 mL with diluent to make standard solutions of concentrations 5, 10, 15, 20 and 25 ppm.

Calibration Curve:

Chromatogram was recorded for standard solutions with different concentration**(Fig.1)**. Graph of concentration (as x-value) versus area (as y-value) were plotted **(Fig. 2)**. The correlation coefficient, y-intercept and slope of the regression were calculated and mentioned below (**Table 1**).

Formula:

Concentration of test solutions were calculated by using slope and intercept equation obtained from the calibration curve of Desloratadine.

Slope and intercept equation: $y = m x \pm c$

Where, y - y axis value i.e. Absorbance of test solution

x - x axis value i.e. Concentration of test solution

m - slope of linear curve

c - y intercept at zero concentration

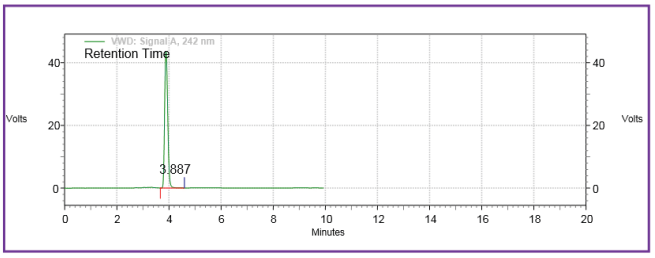


Figure 1: HPLC Chromatogram of Standard Desloratadine HCL.

Table 1: Observations of linearity study

Sr. No	Conc. of Desloratadine HCL (ppm)	Average Peak Area (for n = 3)
1	5	3029921
2	10	6059275
3	15	9057744
4	20	12117555
5	25	15126336
Linearity Range		5-25 PPM
Correlation coefficient (r^2)		0.999
Slope		605104
y-intercept		1349

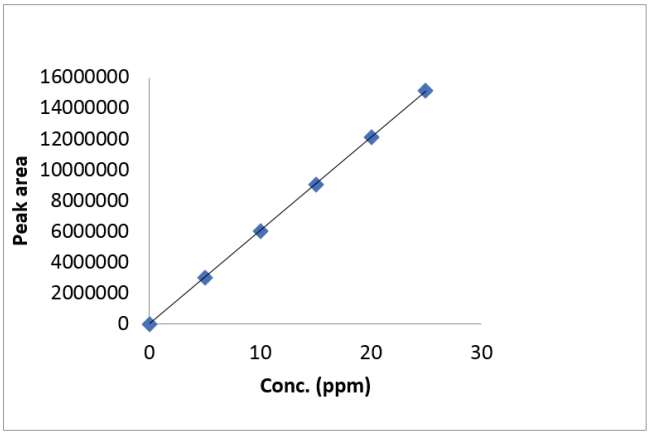


Figure 2: Calibration curve of DSLR HCl.

Assay:

An average weight of twenty tablets DESLOR (5 mg) was determined. Tablets were crushed to fine powder and uniformly mixed. An accurately weighed tablet powder equivalent to 10 mg DSLR was transferred to 100 ml volumetric flask. 30-40 ml methanol was added to flask and shaken for 5 minutes. Excipients were filtered off and filtrate was diluted to 100 ml with methanol. 1 ml of above solution was diluted to 10 ml with methanol.

Chromatogram of diluted test solution was recorded. Procedure was repeated for six times. The % content of drug was calculated by using following formula:

$$\% \text{ Content} = \frac{At}{As} \times \frac{Ds}{Dt} \times \frac{Ws}{Wt} \times \frac{Avg.\ wt.}{LC} \times 100$$

Where,

At = Peak area of sample solution
As = Peak area of standard solution
Ds = Dilution factor for standard
Dt = Dilution factor for sample
Ws = Weight of standard (mg)
Wt = Weight of sample (mg)
Avg. wt. = Average weight
LC = Label claim

Validation of HPLC Method

The proposed RP-HPLC method was validated as per the International Conference on Harmonization (ICH) guidelines Q2A (R1).[20,21]

Forced degradation study:

Standard Stock Solution: A standard stock solution (1000 ppm) was prepared by dissolving 100 mg of the DSLR HCL in 100 ml of methanol.

Acid Solution: One mL of stock solution was diluted up to 10 mL with 3 M HCl and refluxed at 80^{0}c for 8 hrs. 1 mL of the solution was taken in separate 10 mL volumetric flask and neutralized with 3 M NaOH. Final volume was adjusted to mark with diluent and analysed. [22,23]

Alkaline Solution: To separate 10 ml volumetric flask, 1 mL of stock solution taken and diluted in 3M NaOH to mark. Then solution was refluxed at 80^{0}c for 8 hrs. After the degradation time was achieved, 1 mL of the solution was neutralized with 3 M HCL and diluted to 10 mL with diluent. Final solution was used for the analysis. [22,23]

Oxidative Solution: Aliquot about 1 mL of stock solution and 4 mL was taken in 10 mL volumetric flask. This mixture diluted to mark with 0.3% H_2O_2 solution and heated at 80^{0}c for 8 hrs. After the degradation time was achieved, 1 mL of the solution was transferred to another 10 mL volumetric flask, diluted to mark and analysed. [22,23]

Thermal Degradation: Analytically pure sample of DLT was exposed in an oven at 110^{0}c for 12 hours and cooled to room temperature. Then, accurately weighed 100 mg sample was transferred to 100 ml volumetric flask and volume adjusted with methanol. Appropriate dilutions were done and final solution analysed. [22,23]

Photo stability Solution: A specific amount of compound was taken in a cleaned Petridish and exposed to radiations of wavelength 254 nm in UV chamber for 12 hrs. Accurately

weighed 100 mg sample was transferred to 100 ml volumetric flask and diluted with methanol. Solution was further diluted by diluent taking appropriate aliquot. [22, 24]

Fresh standard stock solution was prepared for the study of each stress condition. All solutions were passed through a 0.45l Whatman filter paper and injected in the liquid chromatographic system and chromatograms were recorded.

GC-MS analysis

GC-MSanalysis of DSLR HCl stressed at acidic medium, alkaline medium and oxidative stress conditions was carried out to characterize degradation products. Separated degradation products and pure compound was achieved through gas chromatographic method. Separated compounds were characterized from their mass spectra.[18]

RESULTS

Optimization of Chromatographic Conditions

Several chromatographic runs were taken on C18 column 5µ (4.6 X 250 mm) with varying composition of mobile phase at flow rate 1 mL/min and detection wavelength 242 nm. As per the solubility of DSLR HCL, a mixture of orthophosphoric acid (OPA) (0.1%V/V) and methanol (50:50 V/V) was tried as a mobile phase and single peak obtained at retention time (RT) 4.2 minutes with insignificant plate count. Then, combination of orthophosphoric acid (0.1%V/V), Methanol and Acetonitrile (50:35:15 V/V) as a mobile phase gave a sharp single peak at RT 3.90 minutes with plate count above 5000. This chromatographic condition was finalised for HPLC method as it gave well resolved symmetric peak with reproducible retention time (Fig.1).

Assay

Proposed method was used for the estimation of DSLR from its tablet dosage form. Results of assay are summarized in table 2.

Table 2: Result of assay of DSLR HCL

Sr. No.	Wt. of standard in mg	Wt. of sample in mg	Peak area of standard	Peak area of sample	% DSLR content
1.	10	147.8	6023384	5980259	99.42
2.		148.2		6023426	99.86
3.		148.5		6053617	99.24
4		147.6		5971286	99.4
5		148.2		6014852	99.72
6		148.6		6029416	99.96
Statistical values for n=6				Mean	99.60
				SD	0.287
				%RSD	0.288

Method validation

The developed method was validated as per ICH guidelines Q2R1 for the various parameters shown in following table 3.

Specificity:

The specificity of the method for Assay was demonstrated by detecting Peak of STD, blank and sample solutions by developed HPLC method for rectification of specificity of method. Results of specificity study are shown in table 4.

Table 3: Validation Parameters

Sr. No	Parameters	For Assay
1	Specificity	√
2	Linearity	√
3	Accuracy	√
4	Precision	
4.1	System Precision	√
4.2	Method Precision	√
4.3	Intermediate Precision	√
5	Robustness	√
6	Solution Stability	√

Note: √ - Applicable

Figure 3: HPLC Chromatogram of blank solution.

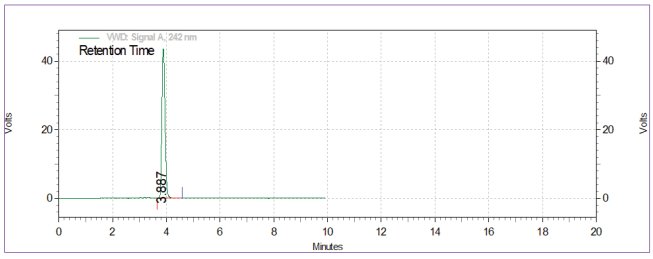


Figure 4: HPLC Chromatogram of sample solution.

Table 4: Results of Specificity study

Sr. No	Sample Name	RT (Min)	Tailing Factor	Plate Count	Peak Purity
1	Desloratadine HCL	3.907	1.192	5268	Pass
2	Blank	No peak is observed	NA	NA	Pass
3	Sample solution	3.907	1.183	5368	Pass

Precision

System precision

System precision was evaluated from five replicate injections of standard as per proposed method. The Peak area, average and % RSD were calculated and tabulated in Table 5.

Method Precision

The six sample solutions were prepared separately. Each sample solution was injected in HPLC system and analysed as per proposed procedure. The % assay, average and % RSD were calculated and tabulated in Table 5.

Intermediate precision

The Intermediate precision was determined by comparison of two independent analyses on different days. The data of the 1st day analysis was taken from the analysis of "Method precision". Results of Intermediate precision study were shown in table 5.

Linearity

The linearity of peak area response for DSLR HCL was determined from 50 % to 150 % level of test concentration. Accurately weighed quantity of tablet powder was diluted to obtain concentration in the range of 50-150 % of test concentration. Graph of concentration (as x-value) versus peak area (as y-value) was plotted as shown in fig 6 and observation in table 5.

Robustness

The influence of slightly changed parameters of the chromatographic conditions was tested according to ICH guidelines to demonstrate sufficient robustness of the method. The tests were carried out by injecting standard solution by changing detection wavelength (±2 nm) and flow rate of mobile phase (± 0.2 ml/minute). Results are as shown in table 5.

Accuracy

The accuracy was determined by standard addition method. A known but varying amount of Standard DSLR was spiked into pre-analysed Drug sample solution at 80%, 100% and 120% recovery levels of working concentration in triplicate. The spiked sample solution was injected in HPLC system and analysed by the proposed method. The percentage recoveries were calculated against respective levels and mentioned in Table 5.

Limit of detection (LD) & Limit of Quantification (LQ)

LD and LQ were mathematically determined through the calibration curve of predicated linearity. LD and LQ were calculated using the following equation as per ICH guidelines.

$LD = 3.3 \times \sigma/S;$

$LQ = 10 \times \sigma/S$

Where σ is the standard deviation of y-intercepts of regression lines and S is the slope of the calibration curve. Results are shown in table 10.

Forced degradation study

Various Conditions used for stability study of DSLR HCL. The forced degradation was carried out to achieve enough degradation. The following degradation behaviour of the drug was observed during the stress degradation studies. Summarised results of forced degradation study are shown in table 6.

Acidic condition

One degradation product (DP) was found to be generated in acidic solution. This DP I was separated from the DSLR peak by proposed HPLC method and the RT of DP I was found to be at 3.273 and DSLR peak at 3.970 minutes as observed in fig 5 A.

Alkaline condition

One degradation product found to be generated in alkaline solution. This DP was separated from peak of DSLR given HPLC method and the RT of DP II is found to be at 3.210 minutes. DSLR peak was obtained at 3.970 minute as observed in fig 5 B.

Oxidative condition

Two degradation products were found to be generated in H_2O_2 solution. These degradation products were resolved well by the developed HPLC method. Impurity was observed at RT DP III - 3.363, DP IV – 4.687 min and DSLR at 4.143 min as seen in fig 5 C.

Thermal degradation

Desloratadine HCL was found thermally stable. Impurities were not found at this stress condition as confirmed from fig 5 D.

Photo Stability condition

In photolytic solution of Desloratadine HCL was found stable. Impurities were not found at this stress condition and confirmed from fig 5 E.

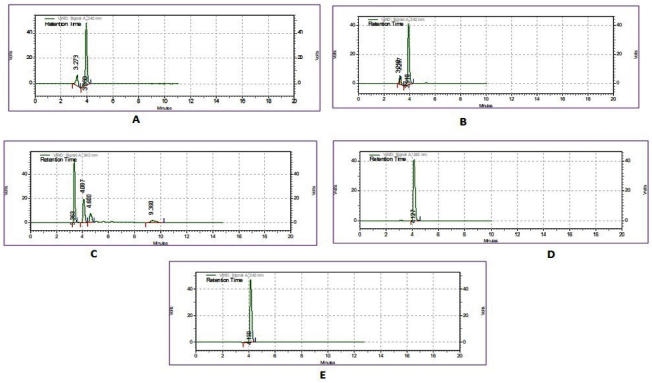


Figure 5: Chromatogram of Acidic(A), Alkaline (B), Oxidative (C), Thermal (D) and Photolytic (E).

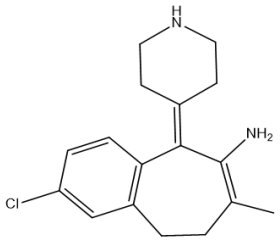
GC-MS analysis

Reference mass spectrum of Desloratadine HCl contains parent peak at m/z 310 and major fragments peak at m/z 294, 280, 266,245,230, 217,108 and 41 (Fig.6 A).

Acidic Hydrolytic Sample

One additional peak was observed in chromatogram at RT 17.135 referred as Degradation product –I (DP-I). A mass spectrum of DP-I contains parent peak at m/z 284. Major fragments were observed at m/z 241, 185,129 and 73(Fig 6 B).

Mass spectrum of degradation product (fig 14) revealed enough information about the structure. From m/z value of parent peak and fragment peak, degradation products were characterised as 2-chloro-7-methyl -5- (Piperidine-4-ylidene)-8, 9 dihydro-5H-Benzo [7] annulene-6-amine (DP-I). Predicted structures of degradation products were given as below

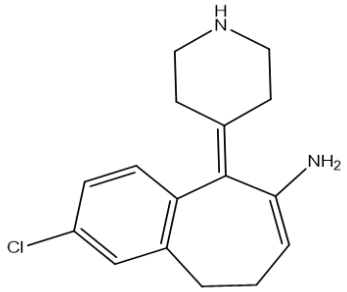


2-chloro-7-methyl-5-(piperidin-4-ylidene)-8,9-dihydro-5*H*-benzo[7]annulen-6-amine

Alkaline Hydrolytic Sample

One additional peak was observed in chromatogram at RT 5.823 referred as Degradation product –II. A mass spectrum of DP-II contains parent peak at m/z 264. Major fragments were observed at m/z 151, 137, 83, 69 and 55(Fig 6 C).

Mass spectrum of degradation product (fig 16) revealed enough information about the structure. From m/z value of parent peak and fragment peak, degradation products were characterised as 2-chloro-5- (Piperidine-4-ylidene)-8,9 dihydro-5H-Benzo [7] annulene-6-amine. (DP-II) Predicted structures of degradation products were given as below



2-chloro-5-(piperidin-4-ylidene)-8,9-dihydro-5*H*-benzo[7]annulen-6-amine

Oxidative Degradation Sample

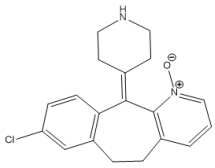
In GC-MS chromatogram, it was observed that standard Desloratadine eluted at RT 19.351 and its mass spectra. A mass spectrum of std. Compound contains parent peak at m/z 310 and major fragments peak at m/z 294, 280, 266,245,230, 217,108 and 41.

Additional peaks were observed in chromatogram at RT 16.389 and 17.961 referred as Degradation product –III (DP-III) and Degradation product-IV (DP-IV) peak respectively.

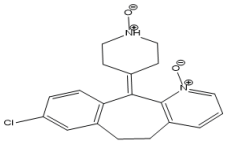
Mass spectrum of DP-III contains parent peak at m/z 327. Major fragments were observed at m/z 242, 215, 208,180,152 and 89. A mass spectrum of DP-IV contains parent peak at m/z 341. Major fragments were observed at m/z 265,207, 107, 87 and 74.

Mass spectrum of degradation products (Fig 6 D&E) revealed enough information about the structure. From m/z value of parent peak and fragment peak, degradation products were characterised as 8-chloro-11-(piperdine-4-ylidene)-6,11-dihydro-5H-benzo[5,6] cyclohepta[1,2-b] pyridine 1-oxide (DP-III) & 4-(8-chloro-1-oxido-5H-benzo[5,6]cycloheptal[1,2-b] pyridine-11(6H)-ylidene)piperidine1-Oxide (DP-IV) .

Predicted structures of degradation products were given as below



8-chloro-11-(piperidin-4-ylidene)-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine 1-oxide



4-(8-chloro-1-oxido-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11(6*H*)-ylidene)piperidine 1-oxide

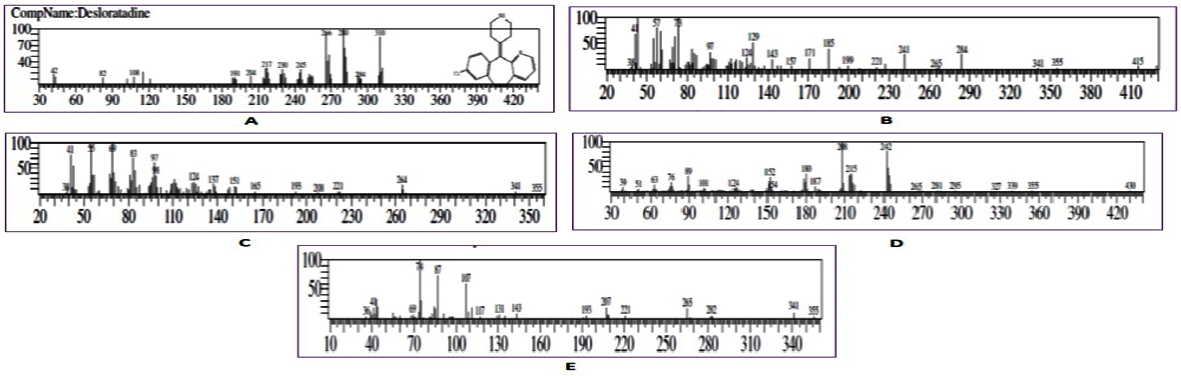


Figure 6: Mass spectra Pure Desloratadine (A), Acidic (B), Alkaline (C) & Oxidative (D & E) stressed sample.

Table 5: Summarised Results of validation study

Sr. No.	Parameters	Acceptance Criteria	Result
1	Specificity	No interference for Analyte peak	Specific
2	Linearity	R^2> 0.98	0.999
3	Precision		
3.1	System precision	% RSD <2.0	0.50
3.2	Method precision	% RSD <2.0	0.54
3.3	Intermediate precision	% RSD <2.0	0.56
4	Accuracy		
4.1	80 %	Average % Recovery should be in the range of 98% - 102 %	100.66
4.2	100 %		100.58
4.3	120 %		101.46
5	Robustness	System suitability parameters should comply	Robust
6	LOD		0.0049µg/mL
	LOQ		0.015 µg/mL

Table 6: Summarised results of forced degradation study

Stress condition			Peak name	RT min.	Area	USP Tailing	USP Plate count	% Content of DSLR	DSLR Peak purity
Strength	Time Hrs	Temp. °C							
3 M HCl	8	80	DP-I	3.273	1402881	1.451	3522	82.14	0.998
			DSLR	3.950	6451998	1.218	5420		
3 M NaOH	8	80	DP-II	3.210	903158	1.451	3146	87.17	1.000
			DSLR	3.910	6090056	1.207	4827		
0.3 % H_2O_2	8	80	DP- III	3.363	5843521	1.254	2561	49.85	0.995
			DSLR	4.087	3009332	1.26	3541		
			DP-IV	4.600	1319251	1.328	3054		
Photo Degradation 254 nm	24	RT	DSLR	4.130	6088539	1.182	4829	100.09	0.997
Thermal degradation	8	110	DSLR	4.129	6045791	1.256	5247	99.82	1.000

DISCUSSION

Desloratadine has been estimated using an RP-HPLC method from its tablet dosage form. According to ICH criteria, the proposed approach was validated. According to the results of the validation study, the developed HPLC technique is suitable for regular quantitative analysis of Desloratadine. The data from the forced deterioration research validated the stability of the proposed approach, demonstrating its potential. The developed HPLC technique was found to be capable of estimating Desloratadine and its degradation products simultaneously. The developed approach is specific and selective since degradation product peaks were successfully separated from the standard peak and from each other. In the chromatogram, no interference of any kind was seen. The method was proven to be reliable because results did not change much when method parameters were changed. The method was discovered to be linear and appropriate across a wide range of Desloratadine concentrations. According to the LOD and LOQ values, the method is highly sensitive. Because the mobile phase consisted of common solvent Orthophosphoric acid, methanol, and only a small amount of acetonitrile, the procedure was found to be inexpensive. When compared to buffer mobile phase, optimised mobile phase reduced the probability of column damage.

As per results of forced degradation study, it is concluded that Desloratadine degraded in acidic and alkaline solution. Desloratadine is gradually degraded at alkaline stress conditions. Percentage degradation is not significantly affected by strength of alkaline stressor. Desloratadine is extensively degraded at oxidative stress conditions. Around 50 % Desloratadine got degraded on treating with peroxide. Desloratadine is found more liable to oxidation. At oxidative stress condition, Strength of stressor i.e., conc. of H_2O_2 did not affect % degradation. Three degradation products were generated on oxidative degradation and one degradation product at each acidic and alkaline medium. Degradation products of Desloratadine HCl were identified as 2-chloro-7-methyl -5- (Piperidine-4-ylidene)-8, 9 dihydro-5H-Benzo[7] annulene-6-amine (DP-I), 2- chloro-5- (Piperidine-4-ylidene)-8,9 dihydro-5H-Benzo[7] annulene-6-amine (DP-II), 8-chloro-11-(piperdine-4-ylidene)-6,11-dihdro-5H benzo[5,6] cyclohepta[1,2-b]pyridine 1-oxide (DP-III) & 4-(8-chloro-1-oxido-5H-benzo[5,6]cycloheptal[1,2-b]pyridine-11(6H)-ylidene)piperidine1-Oxide (DPIV). Structures of degradation products reveal that pyridine and piperidine rings are liable to degradations at various stress conditions. According to structures of degradation products and pattern of fragmentations, it is concluded that unsubstituted pyridine and piperidine rings reduces stability of Desloratadine HCl. Among these, pyridine is highly sensitive to stress conditions. Stability of selected moiety can be improved substitution of rings with functional groups which less liable to degradations.

CONCLUSION

Simple, sensitive, rapid, robust, precise and accurate RP-HPLC stability indicating method has been developed and validated as per ICH Q1B. The results of stress testing are analysed in accordance with ICH principles, revealing that the procedure is both specific and stable. Application of this method for the analysis of DSLR HCL exposed to different stress conditions shows that degradation products did not interfere with the analytical determination. The proposed method was effectively utilised, and it is recommended for quantitative analysis of DSLR in pharmaceutical formulations for QC, when economy and speed are critical, and therapeutic efficacy is assured.

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All authors declared that there are no conflicts of interest.

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AUTHORS' CONTRIBUTION:

1. Ashish B Roge made significant contributions to the research by selecting a problem, conducting a literature review, conducting experimental work and writing a manuscript.
2. Sagar N Firke, assisted with the literature review, development of the RP-HPLC method, and force degradation study.
3. Shrinivas K Sarje helped with drug selection as well as laboratory work such as sample and reagent preparation.
4. Mahavir H Ghante assisted with data compilation, writing, and manuscript drafting.
5. Giridhar R Shendarkar has supervised the entire research project.
6. Nitin B Ghiware, has provided all the necessary facilities to carry out the research work.

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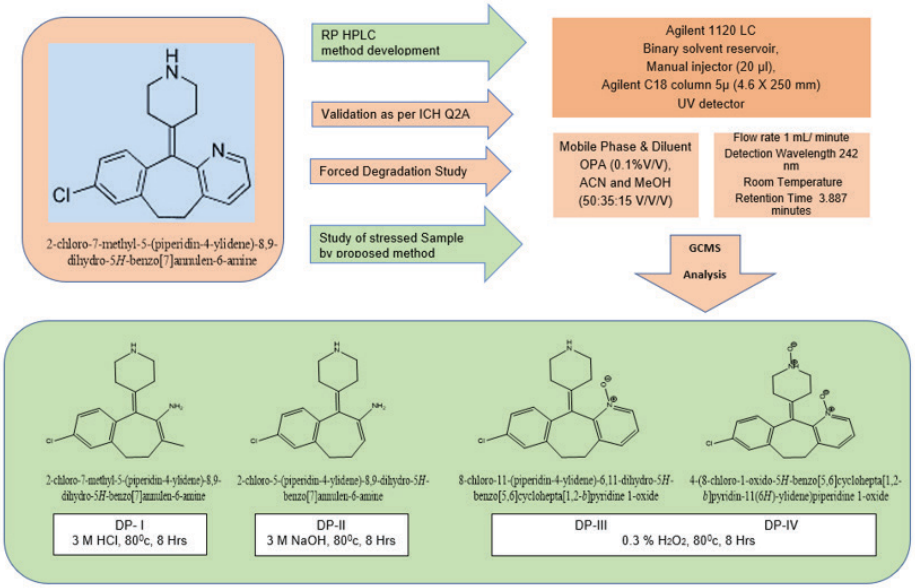

Figure 15: Graphical Abstract.