# Validation and Application of a Modified RP-HPLC Method for the Quantification of Desloratadine in Pharmaceutical Dosage Forms

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**ABSTRACT:** The purpose of the study was to develop a simple, sensitive and rapid RP-HPLC method for the determination of desloratadine in marketed products. Chromatographic determination was performed in a reverse phase $C_{18}$ column (250 mm × 3.3 mm I.D. , 5µm particle size) using a mixture of acetonitrile : n-pentane sulphonic acid sodium salt monohydrate, adjusted to pH 3.0± 0.05 with phosphoric acid (60: 40 v/v) as mobile phase and delivered at a flow rate of 1 ml/min. The UV detection was set at 254 nm. The calibration range was from 2.0 to 40 µg/ml. The method was validated in term of linearity ($r^2$>0.98, RSD= 1.958%), precision (RSD=3.757 %) and accuracy (deviation<2.653%, RSD< 2.203%). The limit of quantification was 2 µg/ml and the limit of detection was 0.1 µg/ml. The linear ranges of desloratadine were 20.23 ± 0.368 µg/ml and 6.545 ± 0.0495 µg/ml in tablet (potency = 99.175 ± 0.718 %) and syrup (potency = 101.15 ± 1.838 %) respectively. The potency of desloratadine in marketed products was determined by this method with acceptable precision and reproducibility.

**Keywords**: Desloratadine, marketed products, RP-HPLC, development of a method

## INTRODUCTION

Desloratadine (descarboethoxyloratadine) is a non sedative, long acting antihistamine with selective peripheral $H_1$ receptor antagonistic activity.[1] Desloratadine 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]. It is available in different dosage forms: tablet and syrup. After oral administration, desloratadine selectively blocks peripheral histamine $H_1$ receptor, because the drug is excluded from entry to the central nervous system.[2] It has demonstrated antiallergic properties from in vitro studies. These include inhibiting the release of pro-inflammatory cytokines such as IL-4, IL-6, IL-8 and IL-13 from human mast cells/ basophilic as well as inhibition of the expression of the adhesion molecule P-selection on Endothelial cells.[3,4] Desloratadine concentration can be determined within 30 minutes of administration which is well absorbed with maximum concentration achieved after approximately 3 hours; the terminal phase half-life is approximately 27 hours. The degree of accumulation of desloratadine was consistent with its half-life and a once daily dosing frequency. The bioavailability was dose proportional over the range of 5 mg to 20 mg.[5]

In dosage forms, desloratadine has been determined using HPLC-MS and Hypersil CN column (150 mm × 5.0 mm I.D., 5 micron particle size) with a mixture of methanol, acetonitrile and phosphate buffer.[6] The reported techniques were tedious, insufficiently sensitive and required highly

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dedicated instrumentation. The present study describes a simple, reliable, sensitive and accurate method for the determination of desloratadine.

## MATERIALS AND METHODS

**Reagents.** Desloratadine (standard) was a kind gift from Eskayef Bangladesh Ltd. HPLC grade acetonitrile and methanol were obtained from Merck, Germany. Distilled deionized water was prepared by Aquatron deionizing water system. Phosphoric acid and n-pentane sulphonic acid sodium salt monohydrate were purchased from BDH (UK).

**Preparation of mobile phase and chromatographic condition.** RP-HPLC analysis was performed by isocratic elution with a flow rate of 1 ml/min at ambient temperature. The mobile phase was prepared with 0.005 M n-pentane sulphonic acid sodium salt monohydrate, adjusted to pH 3.0 ± 0.05 with phosphoric acid and acetonitrile (40: 60 v/v). The mobile phase was filtered through 0.22 µm filter tips and was degassed. The $\lambda_{max}$ for UV detection was set at 254 nm. AUFS (absorbance unit full scale) was kept at 0.002. The injection volume was 20 µl for all standards and samples. Before every injection every sample was filtered through 0.22 µm filter tips.

**Instrumentation.** An HPLC system (Shimadzu Corporation, Japan) consisting of L 7000 pump with online degasser equipped with 10 A system controller, UV-VIS detector (SPD-10 AV) and LC 10 AD pump was utilized. Data was acquired and computed by a C-R6A chromatopac. The analytic column used to achieve the chromatographic separation was a stainless steel symmetry $C_{18}$ reverse phase column (250 mm × 3.3 mm I.D., 5µm particle size) purchased from Ultrasphere, Beckman, USA.

**Preparation of the stock and the standard solutions.** 10 mg of desloratadine was accurately weighed and transferred to a 25 ml volumetric flask. 10 ml of mobile phase was added and sonicated to dissolve. The working standard solutions of various concentrations of 10, 15, 20, 25, 30, 35, 40, µg/ml were prepared by dilution of the stock solution with mobile phase.

**Assay of the tablet and syrup products.** An average of ten tablets (Brand: Sedno 5 mg, of Square Pharmaceuticals Ltd., Bangladesh) were accurately weighed and made into fine powder in a mortar with pastel. Accurately weighed powder sample equivalent to average weight of each tablet (0.156 gm) was taken in a 25 ml volumetric flask. 10 ml of mobile phase was added and sonicated to mix uniformly. The final volume was made by mobile phase and filtered through 0.45µm filter. The solution was diluted 10 times and 20 µl (filtered through 0.22 µ filter tips) of this solution was directly injected into the HPLC injector port. The average content of the tablets was determined using the calibration curve. 1.5 g of desloratadine syrup (Desodin, Eskayef Bangladesh Ltd., 2.5 mg/ 5 ml) was accurately weighed and transferred into a 100 ml volumetric flask. 60 ml of mobile phase was added and sonicated to dissolve. The final volume was made by mobile phase. The preparation was filtered through 0.22 µ filter tips before injection.

**Development of method.** The mobile phase was chosen after several trials with acetonitrile and a buffer of n-pentane sulphonic acid sodium salt monohydrate in various proportions and at different pH values. A mobile phase consisting acetonitrile and a buffer (60: 40 v/v) was selected to achieve maximum separation and sensitivity. The buffer solution was prepared by dissolving 0.96 gm of n-pentane sulphonic acid sodium salt monohydrate in 900 ml deionized water. The pH of the buffer was adjusted to 3.0 ± 0.05 with diluted phosphoric acid. Then the solution was made to 1000 ml by adding deionized water and the solution was filtered through using 0.22µ nylon filter and degassed. Different flow rates in between 0.5 to 1.2 ml/min were studied. A flow rate of 1.0 ml/min gave an optimal signal to noise ratio with a reasonable separation time. Using a reverse phase $C_{18}$ column, the retention time was 7.46 ± 0.91 minutes. The maximum absorption of desloratadine was detected at 254 nm and this wavelength was chosen for the analysis. Different chromatograms of desloratadine in standard

solutions, tablets and syrups are presented in Figure 1.

**Preparation of standard curve for tablet.** Desloratadine, 10 mg (standard, supplied by Eskayef Bangladesh Ltd) was dissolved in 25 ml mobile phase. The concentration of the solution was 400 µg/ml. From this parent solution, solutions of various concentrations such as 40, 35, 30, 25, 20, 15 and 10 µg/ml were prepared using mobile phase (60: 40). 20 µl of each of the solutions was injected into the HPLC system and the run time was 15 minutes for each injection. Before injecting the drug solution, a blank was also injected. The Area Under the Curve (AUC) was plotted against concentration to get the standard curve.

**Preparation of standard curve for syrup.** 30 mg of desloratadine was weighed and transferred into a 100 ml volumetric flask. 60 ml of mobile phase was added and sonicated to dissolve. The volume was made 100 ml by mobile phase and mixed. Then it was filtered through 0.22µ filter tips. Solutions of various concentrations such as 2, 4, 6, 8 and 10 µg/ml were prepared using mobile phase. 20 µl of each of the solutions was injected into the injector port and the run time was 15 minutes for each injection. Before injecting the samples, blank was also injected. The Area Under the Curve (AUC) was plotted against concentration to get the standard curve.

## RESULTS AND DISCUSSION

**Linearity.** Table 1 presents the equation of the regression line, correlation coefficient ($r^2$), relative standard deviation (RSD %) values of the slopes. Excellent linearity was obtained for the compound between 10-40 µg/ml with $r^2$ values of 0.9820, 0.9984, 0.9990 and 0.9996.

**Precision.** The precision of the method (within-day variation of replicate determination) was checked by injecting desloratadine for 10 times. The precision of the method, expressed as the RSD % is given in Table 2.

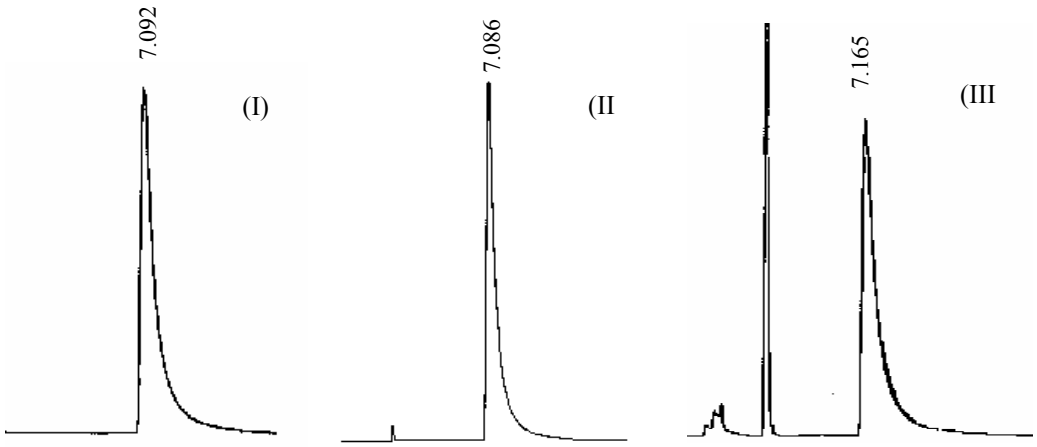


Figure 1. Different chromatograms of desloratadine in standard solution (I), tablet (II), syrup (III) with their retention time of 7.092, 7.086 and 7.165 minutes respectively.

**Table 1. Linearity of the method.**

| Compound | $\lambda_{max}$ | Equation | $r^2$ | Slope (RSD %) |
|---|---|---|---|---|
| Desloratadine | 254 | **Y** = 24939 **X** + 4290.4 | 0.9820 | 1.958 |
| | 254 | **Y** = 25338 **X** + 2816.9 | 0.9984 | |
| | 254 | **Y** = 26027 **X** + 4443.6 | 0.9990 | |
| | 254 | **Y** = 25014 **X** + 4071.2 | 0.9996 | |

**X** = concentration (µg/ml); **Y** = Area
RSD% = (standard deviation / mean) x 100

**Table 2. The Precision of the developed method**

| Compound | $\lambda_{max}$ | Peak Area (Mean ± SD) (n =10) | RSD % |
|---|---|---|---|
| Desloratadine | 254 | 503490 ± 3015.5 | 3.757 |

**Reproducibility.** A standard working solution-containing desloratadine, producing final concentration of 10, 20 and 40 µg/ml was prepared. The prepared mixture of standard solution was injected 10 times as a test sample. From the respective area counts, the concentration of the desloratadine was calculated using the detector response (Table 3).

The potency was determined for tablets and syrup of marketed preparations (Table 4). The potencies were found 101.15% and 99.175% for tablets and syrup respectively.

**Table 3. The reproducibility of the method.**

| Compound | Standard concen-tration µg/ml | Measured concentration µg/ml, (n =10) (Mean ± SD) | RSD % | Deviation % |
|---|---|---|---|---|
| Desloratadine | 10 | 10.130 ± 0.223 | 2.203 | 1.287 |
| | 20 | 20.384 ± 0.329 | 1.612 | 1.888 |
| | 40 | 38.966 ± 0.462 | 1.187 | 2.653 |

Deviation (%) = (standard concentration - measured concentration) / standard concentration x 100.

**Table 4. Determination of drug content present in tablet and syrup (n = 10).**

| Dosage forms | AUC (Mean ± SD) | Equation | Concentration (µg/ml) | Amount (mg) per tablet or 5 ml syrup | Potency (%) |
|---|---|---|---|---|---|
| Tablet | 501464 ± 9176.832 | Y = 24939 X + 4290.4 | 20.23 ± 0.368 | 5.058 ± 0.092 | 101.15 ± 1.838 |
| Syrup | 162915.5 ± 1300.369 | Y = 25338 X+ 2816.9 | 6.545 ± 0.0495 | 2.48 ± 0.0141 | 99.175 ± 0.728 |

## CONCLUSION

The RP-HPLC method for the determination of desloratadine is validated in this study, has acceptable correlation coefficient, RSD (%) and deviation which makes it versatile and valuable in many applications, especially in pharmaceutical dosage form and drug concentration monitoring. The method can also be readily adapted for routine quality control analysis.

## REFERENCES


1. Schroeder, J.T., Schleimer, R.P., Lichtenstein, L.M. and Kreutner, W. 2001. Inhibition of cytokine generation and mediator release by human basophils treated with desloratadine. *Clinical & Experimental Allergy* **31**, 1369
2. Desloratadine data sheet, 2002. MIMS Bangladesh**. 4**, 119-121.
3. Mullol, J., Roca-Ferrer, J., Alobid, I., Pujols, L., Valero, A., Xaubet, A., Bernal-Sprekelsen, M. and Picado, C. 2006. Effect of desloratadine on epithelial cell granulocyte-macrophage colony-stimulating factor secretion and eosinophil survival. *Clinical & Experimental Allergy* **36**, 52-58.
4. Kowalski, M.L., Lewandowska, A., Wozniak, J., Makowska, J., Jankowski, A. and DuBuske, L. 2005. Inhibition of nasal polyp mast cell and eosinophil activation by desloratadine. *Clinical & Experimental Allergy* **60**, 80-85.
5. Gupta, S., Banfield, C., Affrime M, Marco, A., Cayen, M., Herron, J. and Padhi, D. 2002. Desloratadine demonstrates dose proportionality in healthy adults after single doses. *Clin Pharmacokinet*. **41**, Suppl 1:1-6.
6. Liu, L., Qi, M., Wang, P. and Li, H. 2004. High-performance liquid chromatographic method for the bioequivalence evaluation of desloratadine fumarate tablets in dogs. *J. Pharm. Biomed. Anal.* **10**, 34, 1013-1019.