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RP-HPLC Method Development and Validation for the Estimation of Loratadine in Bulk and Pharmaceutical Dosage form

Saroj Kumar Raul [1]*, B.V.V Ravi kumar[2], Ajaya Kumar Patnaik [3], Ram Prasad Padhy[4], Lagnajit Mohapatra[1], B. Bahugunachary[5]
[1]*M.R. College of Pharmacy, Phool-Baugh, Vizianagaram, A.P, India.*, [2]*Roland Institute of Pharmaceutical Sciences, Berhampur, Orissa, India.*
[3]*Departments of Chemistry, Ravenshaw University, Cuttack, Orissa, India.*, [4]*Joypore College of Pharmacy, Rondapali, Joypore, Orissa, India.*
[5]*Bright Labs, Kothapet, Dilshuk Nagar, Hyderabad.*

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ABSTRACT

A simple, selective, linear, precise and accurate RP-HPLC method is developed and validated for estimation of Loratadine in pharmaceutical dosage form. Isocratic elution at a flow rate of 1.2 mL min -1 was employed on Inertsil-Extend C_{18} column at ambient temperature. The mobile phase consisted of methanol: water 70:30 (v/v) and the detection was monitored at 220 nm. Linearity was observed in concentration range of 5-175 µg/mL. The retention time for Loratadine was 3.06 min. The method was validated as per the ICH guidelines. The proposed method can be successfully applied for the estimation of Loratadine in pharmaceutical dosage forms.

Key words: *Dosage forms, Estimation, Method development, Loratadine, RP-HPLC, Validation.*

INTRODUCTION

Loratadine (**Fig.1**) is second generation selective histamine H_1 antagonist that binds to the histamine H1 receptor. Chemically it is described as ethyl-4-(8-chloro-5, 6-dihydro-11H-benzo [5, 6] cyclohepta [1, 2-b] pyridine-11-ylidene)-1-piperidinecarboxylate.Loratadine competes with free histamine and exhibits specific, selective peripheral H_1 antagonistic activity. This block the action of endogenous histamine, which subsequently leads to temporary relief of the negative symptoms (eg. nasal congestion, watery eyes) brought on by histamine. Loratadine also appears to suppress the release of histamine and leukotrienes from animal mast cells, and the release of leukotrienes from human lung fragments. It is used for treatments of symptoms such as itching, runny nose, watery eyes, and sneezing from "hay fever" and other allergies [1].

Literature survey reveals that few HPLC and spectrophotometric methods [2-11] has been reported for the estimation of loratadine. The aim of the present study is to develop a simple, precise and accurate reversed-phase HPLC method for the estimation of loratadine in pharmaceutical dosage form as per ICH guidelines.

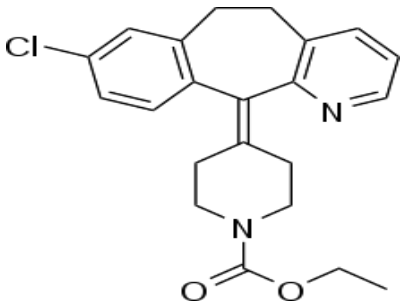

Fig. 1: Chemical structure of Loratadine

MATERIALS AND METHOD

Instrumental and analytical conditions:

The HPLC analysis was carried out on Young Lin Acme HPLC (9000) equipped with UV detector with auto Sampler and running on Autochro 3000 software. The column used is Inertsil-Extend C_{18} (250 × 4.6 mm, packed with 5 µm) and detection was performed at 220 nm. The injection volume of sample was 20 µL and the run time was 5 minutes. An isocratic mobile phase containing methanol and water at 70: 30 (v/v) was carried with the flow rate at 1.2mL min^{-1}. The mobile phase was filtered through 0.45µm membrane filter and degassed before use.

Reagents and chemicals:

Loratadine working standard was kindly gifted by Dr. Reddy's Laboratory, Hyderabad. Tablets were purchased from local pharmacy manufactured by Cipla (Loratin). Methanol (HPLC grade; Merck), water (HPLC grade; Milli-Q), were used in the study.

Preparation of mobile phase:

700mL of methanol and 300mL of water were transferred into a 1000 mL mobile phase bottle, mixed and sonicated up to 15 minutes to degas the mobile phase. Then it was filtered through 0.45 µm membrane filter under vacuum. The same mobile phase was used as diluent.

Preparation of standard solution:

10 mg of loratadine reference standard was weighed accurately and transferred into a 10mL volumetric flask containing 5 mL of methanol. Then it was sonicated for 10 minutes to dissolve the drug completely. The volume was adjusted with the mobile phase to get stock solution of 1000 µg/mL. 1 mL of this stock solution was transferred into a 10 mL volumetric flask and the volume was made up to the mark with mobile phase. This solution was filtered through 0.45 µm membrane filters, which give a solution of strength 100µg/mL.

Preparation of sample solution:

Twenty tablets (Loratin) were weighed and their average weight was calculated. A quantity of the tablet powder equivalent to 50 mg of loratadine was transferred into a 50 ml volumetric flask containing 25ml of methanol and allowed to stand for 1hr with intermittent sonication to ensure complete solubility of the drug and then filtered through 0.45 µm membrane filter and the volume was adjusted up to the mark with mobile phase. Further 1 ml of the above stock solution was pipetteted into a 10 ml volumetric flask and the volume was adjusted up to the mark with mobile phase to give a concentration of 100µg/mL.

METHOD VALIDATION:

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision, accuracy,

***Corresponding author:**
Saroj Kumar Raul
Dept. of Pharmaceutical Chemistry, Maharajah's College of Pharmacy, Vizianagaram -2, A.P, India. Mobile-09392725971
**E-Mail: saroj.raul@rediffmail.com*

specificity, limit of detection, limit of quantification, robustness and system suitability.

Linearity:

From the standard stock solution, the various dilutions of loratadine in the concentration of 5, 10, 25, 50, 100, 150 and 175 µg/mL were prepared. Each of these solutions (20 µL) was injected six times in to the chromatographic system at the flow rate of 1.2mL min^{-1} and the peak areas and retention times were recorded. Calibration curve was obtained by plotting the peak area versus the applied concentrations of loratadine. The linearity range and corresponding graph are shown in **Table 1** and **Fig. 2**. A good linear relationship (r^2=0.999) was observed between the concentration range of 5-175 µg/mL.

Table No. 1: Linearity of loratadine

Concentration (µg/mL)	Average area
5	43173
10	82678
25	167458
50	336812
100	659419
150	977627
175	1145105

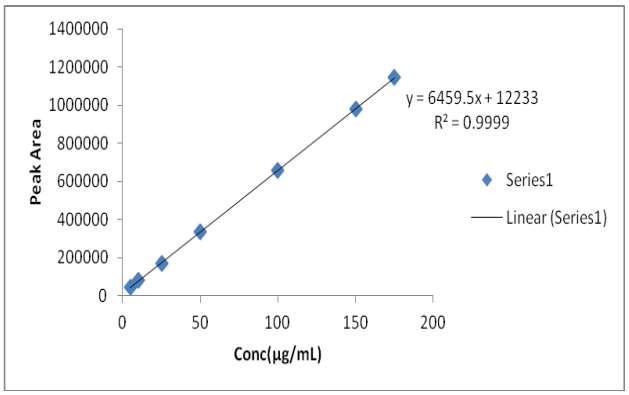

Fig. 2: Linearity curve of Loratadine

Precision:

Precision of an analytical method is defined as the agreement between replicate measurements of the sample. Precision of the method was checked by injecting replicate injections of 100 µg/mL of the solution for six times and the response was recorded. It is expressed as the % relative standard deviation of the replicate measurements and presented in **Table 2**.

Table No. 2: Precision of Loratadine

Injections	Area
1	658728
2	657826
3	658187
4	658301
5	657835
6	657863
Mean	658123.3
SD	357.9266
% RSD	0.05438

Accuracy:

To determine the accuracy of the proposed method recovery studies were carried out by adding different amounts (50%, 100%, and 150%) of the standard samples to the pre-analyzed formulation within the linearity range. At each level, samples were injected in triplicate and the recovery percentage was determined and presented in **Table 3**.

Table No. 3: Accuracy studies of Loratadine

%Conc	Amount added (mg)	Amount found (mg)	% Recovery	Mean Recovery
50%	5.0	4.93±1.6	98.6%	
100%	10.0	9.92±0.95	99.2 %	99.13 %
150%	15.0	14.94±0.84	99.6 %	

Specificity:

Spectral purities of loratadine chromatographic peaks were evaluated for the interference of the tablet excipients as per the methodology. In the work, a solution containing a mixture of the tablet excipients was prepared using the sample preparation procedure to evaluate possible interfering peaks and no interference peaks were observed.

Robustness:

To determine the robustness of the method, parameters like flow rate, composition of mobile phase, detection wavelength and sonication time, were varied from the optimized chromatographic conditions. This solution was injected thrice in each of chromatographic condition. Statistical analysis showed no significant difference between results obtained employing the analytical conditions established for the method and those obtained in the experiments in which variations of parameters were introduced. Thus the method showed to be robust which is shown in **Table 4**.

Table No. 4: Robustness studies of loratadine

Parameters	Adjusted to	Average Area	R_t	SD	% RSD
Flow rate	1.1 mL/min	717318	3.05	1573.89	0.22
	1.2 mL/min	658719.6	3.07	1043.44	0.16
	1.3ml/min	637882	3.26	1036.21	0.16
Mobile phase composition	Methanol: Water (75:25)	612302.8	2.98	1813.73	0.30
	Methanol: Water (70:30)	658492	3.11	1648.23	0.25
	Methanol: Water (65:35)	705755	3.21	1689.15	0.24

Ruggedness:

Inter day variations were performed by using six replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analyst on three different days over a period of one week. Ruggedness also expressed in terms of percentage relative standard deviation and statistical analysis showed no significant difference between results obtained employing different analyst.

Limit of detection (LOD) and Limit of quantification (LOQ):

According to the determined signal-to-noise ratio, the LOD and LOQ of loratadine obtained by the proposed method were 0.2µg/mL and 0.6µg/mL.

System Suitability

System suitability tests were carried out on freshly prepared standard stock solutions of loratadine and it was calculated by determining the standard deviation by injecting standards in six replicates at 6 minutes interval and the values were recorded and the system suitability parameters are shown in **Table 5**.

Table No. 5: System suitability of loratadine

Parameters	Results of the method
Average area	658666
Retention time(min)	3.076
Tailing Factor	1.08
Plate Count	3985.8
Plate per meter	15943.2
HETP	6.272×10^{-5}
Linearity range(µg/mL)	5-175
LOD(µg/mL)	0.2
LOQ(µg/mL)	0.6

Assay of Loratadine tablet:

Three different batches of Loratin were analyzed using the validated method. For the analysis, six replicates of each batch were assayed. Mean peak area of the drug was calculated and the drug content in the tablets was quantified and the results were presented in **Table 6**. The results shown were found to be in good agreement with the labeled amounts, which confirms the suitability of the method for the analysis of the drug in pharmaceutical dosage forms.

Table No. 6: Contents of loratadine in tablets

Sample tablet	Batch	Labelled Amount(mg)	Amount Found*(mg)±SD	%Amount Found
Loratin(10mg)	1	10	9.95±0.12	99.5
	2	10	9.97±0.41	99.7
	3	10	9.94±0.22	99.4

*Each value is an average of six replicate

RESULTS AND DISCUSSION

In this work a new sensitive, accurate and precise RP-HPLC method has been developed for the estimation of loratadine in bulk drug and pharmaceutical dosage form. A mixture of methanol and water in the ratio of 70:30 v/v was found to be most suitable for the separation of loratadine. The peak obtained was symmetrical and free from tailing. From the typical chromatogram the retention time was found to be 3.06 minute. A good linear relationship (r^2 = 0.999) was observed in the concentration range of 5-175 µg/mL. Low values of standard deviation are indicative of high precision of the method. The assay of loratadine tablet dosage form by the proposed method was found to be 99.5 percent. From the recovery studies it was found that about 99.13% of loratadine was recovered, which indicates high accuracy of the method. The absence of additional peaks in the chromatogram indicates non interference of the excipients in the tablet dosage form. Developed chromatographic method applied for the determination of loratadine in tablet formulation, given in **Table 7**. A typical chromatogram showing the separation of loratadine is shown in **Fig. 3**.

Table No. 7: Developed chromatographic conditions of loratadine

Parameters	Method
Stationary phase (column)	Inertsil Extend C_{18} (250 × 4.6 mm, packed with 5 µm)
Mobile Phase	70:30 (Methanol : Water)
Flow rate (ml/min)	1.2
Run time (minutes)	5.0
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	220
Drugs Rt (min)	3.06

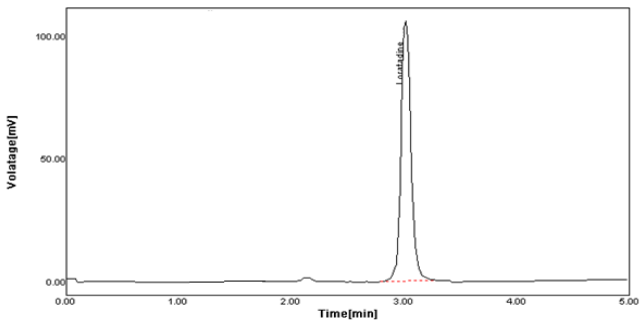

Fig. 3: Typical chromatogram of loratadine

CONCLUSION

A validated RP-HPLC method has been developed for the determination of loratadine in tablet dosage form. The study indicates that the developed method is simple, linear, precise, accurate, specific and reproducible. Thus the developed method can be successfully used for the routine analysis of bulk drug and dosage forms of loratadine within a short analysis time.

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