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RP-HPLC method for the estimation of loratadine [LRD] in bulk drug and in tablet dosage forms

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ABSTRACT

A rapid and sensitive Reverse Phase High Performance Liquid Chromatographic [RP-HPLC] method was developed for the estimation of Loratadine [LRD] in pure and its capsule dosage forms. The method was validated as per International Conference on Harmonization [ICH] guidelines. The mobile phase used in this study is a mixture of (0.01M) Dibasic potassium phosphate, methanol and acetonitrile (7:6:6)% v/v. Stationary phase was Chemsil C8 reverse phase column (150×4.6mm, 5µm) dimensions at 30ºc temperature. The analysis was performed with run time of 30.0 minutes at a flow rate of 1.00ml/min. The LRD was monitored at 254nm with UV detection and LRD was eluted at 22.20min. The method was linear (r2 =0.999) at concentration ranging from 25 to 150µg/ml, precise (intra-day relative standard deviation [RSD] and inter-day RSD values < 1.0%), accurate (mean recovery = 99.5%), estimated from linearity by regression respectively. The results showed that the proposed method is suitable for the precise, accurate and rapid determination of LRD in bulk, its capsule dosage forms.

Keywords: Loratadine, RP-HPLC, Validation, Dosage form

INTRODUCTION

Loratadine is a derivative of azatadine and a second-generation histamine H1 receptor antagonist used in the treatment of allergic rhinitis and urticaria. Unlike most classical antihistamines (histamine H1 antagonists) it lacks central nervous system depressing effects such as drowsiness. IUPAC Name ethyl 4-{13-chloro-4-azatricyclo[9.4.0.0{3,8}]pentadeca-1(11),3,5,7,12,14-hexaen-2-ylidene}piperidine-1-carboxylate. Its molecular formula is $C_{22}H_{23}ClN_2O_2$ and its molecular weight is 382.883. The chemical structure is:

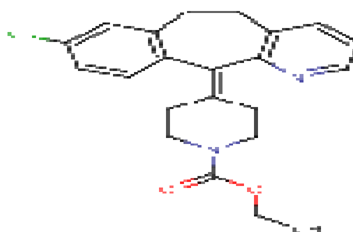


Figure: 1.01. Structure of Loratadine

Loratadine is a white to off white powder. It is practically soluble in water and slightly soluble in methanol and ethanol. It is official in USP[1], BP[2], IP[3]. It is non sedating peripheral histamine H1receptor antagonist. A literature survey reveals Spectrophotometric [4-5] and HPLC [6-13] methods

are reported for analysis of loratadine[LRD] in plasma and various biological fluids which suffer from undesirably long chromatographic run times and requirement for gradient analysis or use of an internal standard. The objective of this study were, therefore, to develop a simple, accurate, sensitive and validated RP-HPLC method for the quantification loratadine in bulk drug and in tablet dosage forms with good sensitivity. Method validation for the developed method was done according to ICH guidelines.

MATERIALS AND METHODS

Instrumental description:
High performance Liquid Chromatograph system equipped with pump, detector and injector [Agilent 1200 pumps with gradient mixer assembly]. Visible spectrophotometric detectors with a Data handling system EZ Chrome Elite software are used. A stainless steel column 150mm long, 4.6mm internal diameter filled with Octadecyl silane chemically bonded to porous silica particles of 5µm diameter. **Chemsil C8, 100X4.6, 5µm** is used.

Chromatographic conditions:
The mobile phase was delivered through the column at flow rate of 1.0mL/min. The column temperature was maintained at 30°C.The sample injection volume was 15µL.Agilent dual λ absorbance detector or equivalent was set at wavelength of 254nm. The details of chromatographic conditions were presented in **Table-1.01** for the determination of loratadine in bulk and pharmaceutical formulations.

Reagents:
Dibasic potassium phosphate : AR Grade
Acetonitrile : HPLC grade
Methanol : HPLC grade
Water : Milli-Q grade
Orthophosphoric Acid : Sd fine
Hydrochloric acid : Sigma Aldrich

Mobile phase Preparation:
Prepare a filtered and degassed mixture of 0.01M Dibasic potassium phosphate, methanol and acetonitrile (7:6:6). Adjust with 10% phosphoric acid solution to an apparent pH of 7.2. Filter through 0.45µ membrane filter.

0.01 M Dibasic potassium phosphate:
Transfer about 1.74 g of anhydrous dibasic potassium phosphate to a 1000 ml volumetric flask, dissolve in and dilute with water to volume and mix. Filter through 0.45µ membrane filter.

0.6 M Dibasic potassium phosphate:
Transfer 105 g of anhydrous dibasic potassium phosphate to a 1000 ml volumetric flask, dissolve in and dilute with water to volume and mix. Filter through 0.45µ membrane filter.

0.05 N Hydrochloric acid:
Transfer 500 ml of water to a 1000 ml volumetric flask, add 83 ml of hydrochloric acid, dilute with water to volume and mix. Transfer 50 ml of this solution into a 1000 ml volumetric flask, dilute with water to volume and mix. Filter through 0.45µ membrane filter.

Diluent:
Transfer 400 ml of 0.05 N Hydrochloric acid and 80 ml of 0.6 M Dibasic potassium phosphate to a1000 ml volumetric flask, dilute with a mixture of methanol and acetonitrile (1:1) to volume and mix. Filter through 0.45µ membrane filter.

Standard preparation:
Weigh accurately about 40 mg of Loratadine working standard in 100 ml volumetric flask, dissolve in and make upto the volume with diluent (0.4 mg/ml). Filter through 0.45µ membrane filter.

Sample preparation:
Weigh accurately about 40 mg of Loratadine sample in 100 ml volumetric flask, dissolve in and make upto the volume with diluent (0.4 mg/ml). Filter through 0.45µ membrane filter.

Estimation of Loratadine from commercial formulations by the proposed method:
Twenty tablets are weighed to get the average weight and pulverized. The sample powder, claimed to contain 40mg of active ingredient was transferred into 100mL volumetric flask and dilute to volume with water. This solution was further diluted stepwise with water, as under preparation of standard solutions to get different required. The area under the curve, the drug content per each tablet was calculated.

Table.1.01 Chromatographic conditions

Chromatographic Parameters	Peak Conditions
Mobile phase	0.01M Dibasic potassium phosphate, methanol and acetonitrile (7:6:6)% v/v. (adjusted to pH 7.20 by Orthophosphoric acid)
Column	Chemsil C_8 column (150mm x 4.6mm i.d., 5µm)
Flow rate	$1.0 mL.min^{-1}$
Detection	254nm
Injection volume	15 µL
Temperature	30 °C
Retention time	22.20 minutes
Run time	30 minutes

Procedure:
The composition and flow rate of the mobile phase was programmed from mother pump and the mobile phase buffer, acetonitrile and methanol (7:6:6) was passed through the 0.45µm membrane filter using millipore HPLC solvent filtration assembly, was delivered at 1.0mL/min for column stabilization. During this period, the base line was continuously monitored. The wavelength of detection was selected at 254nm. The prepared dilutions containing concentrations of Loratadine in the range 25.0 -150µg/mL were injected into the chromatograph. The stability of the solution of Loratadine during analysis was determined by repeated analysis of samples during the course of the experiment of the same day and also on different days after storing at laboratory bench conditions and in the refrigeration. Chromatogram parameters, retention time and asymmetry factor were standardized. A chromatogram indicating the separation of Loratadine is given in **Fig 1.01.**The retention time for Loratadine is 22.20min. The amount of the drug present in each pharmaceutical formulation was calculated through peak area ratio of component by making use of the standard calibration curve.

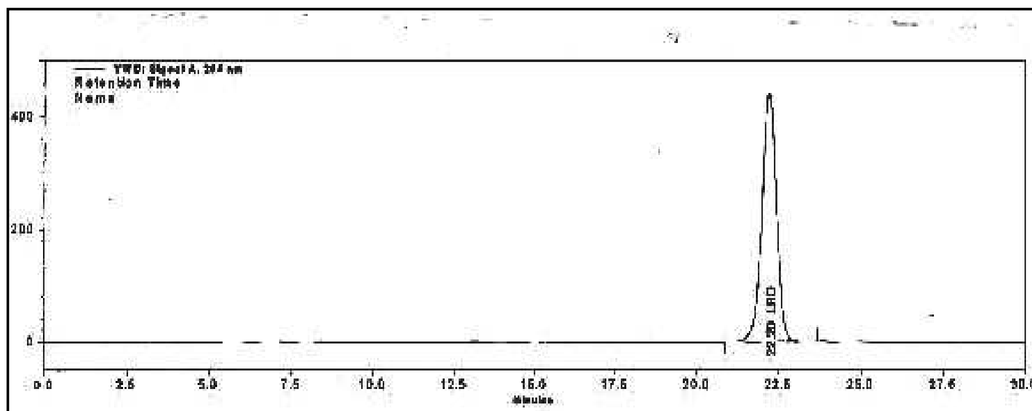
Method development:
Several tests were performed in order to get satisfactory separation-resolution of Loratadine in different mobile phases with various ratios of organic phase and buffers by using C_8 column. The ideal mobile phase used was 0.01M Dibasic potassium phosphate, methanol and acetonitrile (7:6:6) % v/v. Adjusted to pH 7.20 with Orthophosphoric acid to obtain satisfactory and good resolution. Increasing or decreasing pH of mobile phase by ± 0.2 does not show significant change in retention time of each analyte. The chromatographic conditions employed for the assay of LRD are reported in the **Table.1.01** and the typical chromatogram obtained was recorded respectively **Fig.1.01** The retention of Loratadine was 22.20minutes and was evaluated at a flow rate of $1.0 ml.min^{-1}$.

Method validation:
The method was validated following the parameters such as specificity, linearity, precision, accuracy, limits of detection and quantitation and robustness, following the ICH guidelines (ICH).

Specificity:
The specificity of the method was evaluated with regards to interference due to the presence of excipients in the pharmaceutical formulation. The placebo samples consisted of all the excipients without the active substance. Then, the specificity of the method was established by determining the peak purity of Loratadine in samples using a UV

detector, ranging between 190-400nm. To determine specificity with respect to sample compounds the responses of standard and sample solution were compared. No interferences were detected at the retention times of Loratadine in sample solution.

Fig.1.01. Typical HPLC chromatogram of standard solution of Loratadine [LRD]

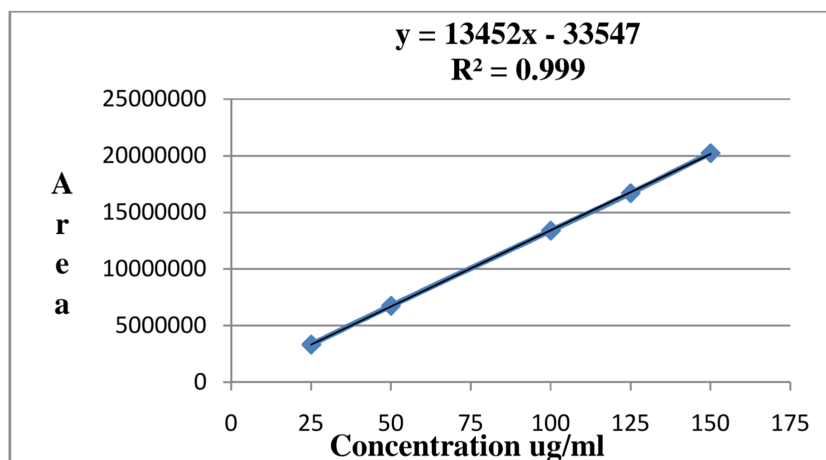


Linearity: The linearity for HPLC method was determined at six concentration levels ranging from 25.0-150.0µg.ml^{-1} for Loratadine**.** The calibration curve was constructed by plotting response factor against concentration of Loratadine which is represented in **Fig.1.02**. The slope and intercept value for calibration curve were **Y = 13452x – 33547 (R2 = 0.9990)** for Loratadine, where **Y** represents the ratio of peak area ratio of analyte to Loratadine and **X** represents analyte concentration. The results were satisfactory shown that significant correlation exists between response factor and concentration of drug. The results are shown in **Table.1.02.**

Table.1.02 Calibration of the RP HPLC for the estimation of Loratadine [LRD]

Concentration (µg)	Area
25.0	3319769
50.0	6746561
100.0	13381304
125.0	16691449
150.0	20229750
Regression equation :	Y = a X + b
Slope (a) :	13452
Intercept (b) :	33547
Correlation coefficient :	0.9990

Fig.1.02. Calibration Curve of Loratadine [LRD]



Precision: The precision of the proposed method was investigated by intra-day and inter-day determinations of Loratadine at three different concentrations of Loratadine (4, 8 and 12 µg/mL). The intra-day studies were performed in one day (for each level n=5) and inter-day studies in five days over a period of two weeks. The intra and inter-day precisions expressed as relative standard deviation values (RSD %) for Loratadine were found to be within 0.87-1.99 % and 0.99-2.03 %, respectively. The data proved good precision for the developed method.

Accuracy (Recovery): To further assess the accuracy of the method, recovery experiments were performed by applying the standard-addition technique. The recovery was assessed by determining the agreement between the measured standard concentration and added known concentration to the sample. The test was done by spiking the pre-analyzed tablet powder with pure Loratadine at three different levels [50, 100 and 150 % of the content present in the tablet powder (taken)] and the total was found by the proposed method. Each test was repeated three times. In all the cases, the recovery percentage values ranged between 99.53 and 99.89. Closeness of the results to 100% showed the fairly good accuracy of the method. The results are shown in **Table.1.03&1.04**

Table – 1.03: % Recovery for mixed Loratadine (LRD) solution

Con. of AC Solution	Area of Standard Solutions	Area of Spiked Solution	% recovery	Acceptance criteria
50%	6691062	6659982	99.53	
100%	13008401	12993311	99.88	98 to 102
150%	19791234	19571494	99.89	

Table-1.04: Assay and Recovery of Loratadine (LRD)

Pharmaceutical formulation	Labeled amount (mg)	Amount obtained by proposed method	% Recovery of proposed methods
Tablet	10	9.98	99.80 %

Stability: In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24hrs at room temperature. The results show that for solutions, the retention time and peak area of Loratadine remained almost similar (% R.S.D. less than 2.0) and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 24hrs, which was sufficient to complete the whole analytical process.

Ruggedness and Robustness: Ruggedness test was determined between two analysts, instruments, laboratory environment conditions and columns. Robustness of the method was determined by small deliberate changes in flow rate, mobile phase pH and mobile phase ratio. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was rugged and robust.

CONCLUSION

The reported RP-HPLC method developed by the author for the analysis of Loratadine [LRD] was proved to be simple, rapid and reproducible. The validation data indicate good precision, accuracy and reliability of the developed RP-HPLC method. The developed method offers several advantages in terms of simplicity in mobile phase, mode of elution, easy sample preparation steps and comparative short run time which makes the method specific and reliable for its intended use in routine analysis determination of Loratadine [LRD] in tablet dosage forms.

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