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DEVELOPMENT AND VALIDATION OF A RAPID RP-HPLC METHOD FOR THE ESTIMATION OF LORATADINE IN DRUG SUBSTANCE AND ITS 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 containing a mixture of dibasic potassium phosphate buffer (pH-7.2), methanol and Acetonitrile in the ratio of 7:6:6% v/v. Zodiac C-8 column (150×4.6mm, 5µm) was used. The analysis was performed with run time of 30 minutes at a flow rate of 1.0ml/min. The LRD was monitored at 254nm with UV detection and LRD was eluted at 22.0 min. The method was linear (r2 =0.999) at concentration ranging from 25.0 to 150µg/ml, precise (intra-day relative standard deviation [RSD] and inter-day RSD values < 1.0%), accurate (mean recovery = 99.5%), specific and robust. Detection and quantification limits were 20.0µg/ml and 61.0µg/ml, 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.*

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General 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:

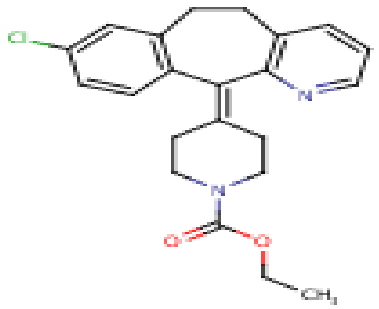

Figure: 1.10 Structure of Loratadine

Loratadine is a white to off white powder. It is practically soluble in water and slightly soluble in methanol and ethanol. A few analytical methods published that describe the quantification of Loratadine in plasma by liquid chromatography [1-3] and UV [4] detection. In the present investigation the authors propose a simple, sensitive and reproducible method for the determination of Loratadine.

The target of this study is to develop a new, simple and fast analytical method by RP-HPLC to quantify Loratadine in bulk and its capsule dosage forms. This validation study is carried out as per ICH guidelines Analytical methods are essential to characterize drug substances and drug products composition during all stages of pharmaceutical development. For routine analytical purpose it is always necessary to establish methods capable of analyzing huge number of samples in a short time period with high accuracy and precision.

Hence, there is a need for the development and validation of newer, sensitive, rapid, accurate and reproducible analytical methods for the estimation of Loratadine in bulk and pharmaceutical dosage form.

The literature survey reveals that there are several published procedures that describe the quantization of Loratadine in bulk and pharmaceutical formulation. A Reverse Phase HPLC method for the determination of Loratadine using a Zodiac C-8 column and a mixture of phosphate buffer, acetonitrile& methanol (7:6:6 v/v) as the mobile phase was reported.

OBJECTIVES OF THE STUDY:

It is an objective of the present study to make an attempt for the development of newer, sensitive and accurate methods for the estimation of Loratadine in bulk and pharmaceutical dosage form. According to the objective of study the plan of work comprising following:

Development of newer, sensitive and accurate methods for the quantitative estimation of Loratadine in pure bulk form.

Development of newer assay methods for Loratadine capsules.

Validate the newly developed analytical methods as per the ICH guidelines[5-7].

MATERIALS AND METHODS:

Materials:

Standards and chemicals:

All the chemicals and reagents for the development of new analytical methods to estimate Loratadine will be of analytical grade. Dibasic potassium phosphate, Acetonitrile, Methanol, Hydrochloric acid, Ortho Phosphoric acid and as well as water, A.R. grade were purchased from Fisher scientific, Mumbai, India. All other chemicals used were of HPLC grade or A.R. grade.

Preparation of mobile phase: Prepare a mixture of 0.01M dibasic potassium phosphate, methanol and Acetonitrile in the ratio of (7:6:6v/v). Adjust the PH 7.20 with ortho phosphoric acid solution. Filter through 0.45µ membrane filter and degas.

0.01M Dibasic potassium phosphate: Weigh accurately about 1.74g of anhydrous dibasic potassium phosphate transfer in to a1000 ml volumetric flask, dissolve and dilute to volume with water, mix well.

0.6M Dibasic potassium phosphate: Weigh accurately about 105 g of anhydrous dibasic potassium phosphate transfer in to a1000 ml volumetric flask, dissolve and dilute to volume with water, mix well. Filter through 0.45µ membrane filter.

0.05N Hydrochloric acid: Transfer 500 ml of water in to a 1000ml volumetric flask, to this add 83ml of hydrochloric acid dilute to volume with water and mix well. Transfer 50 ml of this solution in to a 1000 ml volumetric flask, dilute to volume with water and mix well.

Preparation of diluent: Measure accurately 400 ml of 0.05N Hydrochloric acid and 80 ml of 0.6M Dibasic potassium phosphate transfer in to a 1000 ml volumetric flask, dilute to volume with the mixture of methanol and Acetonitrile in the ratio of 1:1 and mix well. Filter through 0.45µ membrane filter.

Methods:

Instrumentation:

Quantitative HPLC was performed on a High performance liquid chromatography equipped with 2695pump and 2996 photodiode array detector was used. The output of signal was monitored and integrated using Agilent EZ Chrome Elite software.
Analytical methods for the estimation of Loratadine will developed with high sensitivity, accuracy, precision and economy. These methods will be validated as per the ICH guidelines. Following are the aforethought methods for the estimation of Loratadine in bulk and pharmaceutical dosage form:

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 [8-9]. Zodiac C-8 column (150×4.6mm, 5µm) was used with a mobile phase containing a mixture of dibasic potassium phosphate buffer (pH-7.2),methanol and Acetonitrile in the ratio of 7:6:6% v/v. The analysis was performed with run time of 30 minutes at a flow rate of 1.0ml/min. The LRD was monitored at 254nm with UV detection and LRD was eluted at 22.0min.

Chromatographic conditions:

The mobile phase used in this study was a mixture of dibasic potassium phosphate buffer (pH-7.2), methanol and Acetonitrile in the ratio of 7:6:6% v/v. Stationary phase was Zodiac C8 reverse phase column (150×4.6mm, 5µm) dimensions at ambient temperature. The contents of the mobile phase were filtered before use through a 0.45µ membrane. The mobile phase was pumped from the solvent reservoirs to the column at a flow rate of 1.0ml/min for 30min. The elute was monitored at 254nm using UV-detector. The retention time of the drug was found to be 22.50min.

Preparation of standard drug solution:

About 40.0mg of Loratadine was weighed accurately and transferred into a 100mL volumetric flask, dissolved and dilute diluent. The solution was sonicated for 15min and then the volume made up with a further quantity of the diluent to give 0.4mg/mL. 15uL of the solution was injected each time into the column for five times the corresponding chromatograms were obtained. From these chromatograms, the retention times and the areas under the peaks of the drug were noted.

Preparation of sample solution:

For the preparation of sample solutions, four capsules were weighed, powder was collected and mixed. A quantity equivalent to 40 mg of LRD was transferred into 100mL flask, to this 25mL of methanol, was added and sonicated for 10 min for dispersion of drug from its excipients. Then about 50mL of diluent was added and sonicated for 30minute to get extraction of the drug and finally diluted to 100mL volume to get 0.4mg/mL solution. The solutions were filtered through 0.45µ membrane filter before injection and 15µl solution was injected in duplicate injection in to the chromatographic system.

Method validation:

The objective of method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines Q2A and Q2B. Recommend validation characteristics depend on the type of analytical procedure. Method validation characteristics were tested in accordance with ICH guidelines. Method specificity was verified by comparing the chromatograms of sample of pharmaceutical preparation, standard solution and blank. Method precision, Recovery in the range of 50% to 150% of label claim of the drug using the blend, Linearity was tested in the range 25.0–150µg/ml. Intra and inter-day instrumental system Precision as well as repeatability and intermediate method precision were obtained using six replicates per day. Limits of detection and quantification were provided for LRD. Calculation was made by means of RSQ (Residual Square of regression).

RESULTS AND DISCUSSION

HPLC method development and optimization:

The chromatographic method was optimized by changing various parameters, such as the mobile phase composition, pH of the buffer used in the mobile phase. Retention time and separation of peak of LRD were dependent on pH of the buffer and the percentage of acetonitrile. Different mobile phases were tried, but satisfactory separation and good symmetrical peak were obtained with the mobile phases consisting of monobasic sodium phosphate monohydrate buffer, methanol and acetonitrile (pH-7.20) in the ratio of 7:6:6% v/v. A typical chromatogram obtained by using the aforementioned

mobile phase and 15µl of the injected assay preparation is illustrated in **Figure: 1.11.**

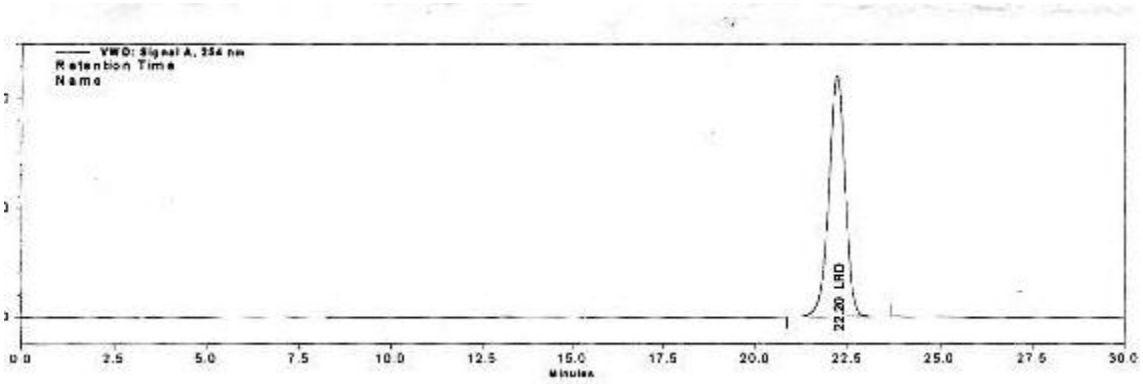

Figure 1.11: A typical chromatogram showing the peak of Loratadine

Method validation:

The analytical method was validated as per ICH guidelines with respect to parameters such as precision, accuracy, specificity, Linearity, range, limit of quantification [LOQ], limit of detection [LOD] and robustness.

Forced Degradation:

Control Sample: Weigh and finely powder not fewer than 20 Tablets. Accurately weigh and transfer equivalent to 40 mg of Loratadine into a 100 ml volumetric flask to this 25mL of methanol was added and sonicated for 10 min for dispersion of drug from its excipients. Then about 50mL of diluent was added, and sonicated for 30minute to get extraction of the drug and finally diluted to 100mL volume to get 0.4mg/mL solution. Filter the solution through 0.45 µm membrane Filter. Transfer 5.0 ml of the above solution into a 100 ml volumetric flask and dilute to volume with diluent. (Figure 1.12)

Acid Degradation Sample: Weigh and finely powder not fewer than 20 Tablets. Accurately weigh and transfer equivalent to 40 mg of Loratadine into a 100 ml volumetric flask to this 25ml of methanol was added and sonicated for 10 min for dispersion of drug from its excipients. Then about 50 ml of diluent was added. Then add 10ml of 5N acid, refluxed for 30min at 60°C, then cooled to room temperature, neutralize with 5N NaOH and dilute to volume with diluent and mix. Filter the solution through 0.45 µm membrane Filter. Transfer 5.0 ml of the above solution into a 100 ml volumetric flask and dilute to volume with diluent. (Figure 1.12)

Base Degradation Sample:

Weigh and finely powder not fewer than 20 Tablets. Accurately weigh and transfer equivalent to 40 mg of Loratadine into a 100 ml volumetric flask to this 25ml of methanol was added and sonicated for 10 min for dispersion of drug from its excipients. Then about 50 ml of diluent was added. Then add 10ml of 5N Base (NaOH), refluxed for 30min at 60°C, then cooled to room temperature, neutralize with 5N Acid

(HCl) and dilute to volume with diluent and mix. Filter the solution through 0.45 µm membrane Filter. Transfer 5.0 ml of the above solution into a 100 ml volumetric flask and dilute to volume with diluent. (Figure 1.12)

Peroxide Degradation Sample: Weigh and finely powder not fewer than 20 Tablets. Accurately weigh and transfer equivalent to 40 mg of Loratadine into a 100 ml volumetric flask to this 25ml of methanol was added and sonicated for 10 min for dispersion of drug from its excipients. Then about 50 ml of diluent was added. Then add 2ml of 30% Peroxide, refluxed for 30min at 60°C, then cooled to room temperature and dilute to volume with diluent and mix. Filter the solution through 0.45 µm membrane Filter. Transfer 5.0 ml of the above solution into a 100 ml volumetric flask and dilute to volume with diluent. (Figure 1.12)

Thermal Degradation Sample: Powder collected from 20 tablets are exposed to heat at 105°C for about 5days. Then Weigh and finely powder not fewer than 20 Tablets. Accurately weigh and transfer equivalent to 40 mg of Loratadine into a 100 ml volumetric flask to this 25ml of methanol was added and sonicated for 10 min for dispersion of drug from its excipients. Then about 50mL of diluent was added and sonicated for 30minute to get extraction of the drug and finally diluted to 100mL volume to get 0.4mg/mL solution Then Filter the solution through 0.45 µm membrane Filter. Transfer 5.0 ml of the above solution into a 100 ml volumetric flask and dilute to volume with diluent. (Figure 1.12)

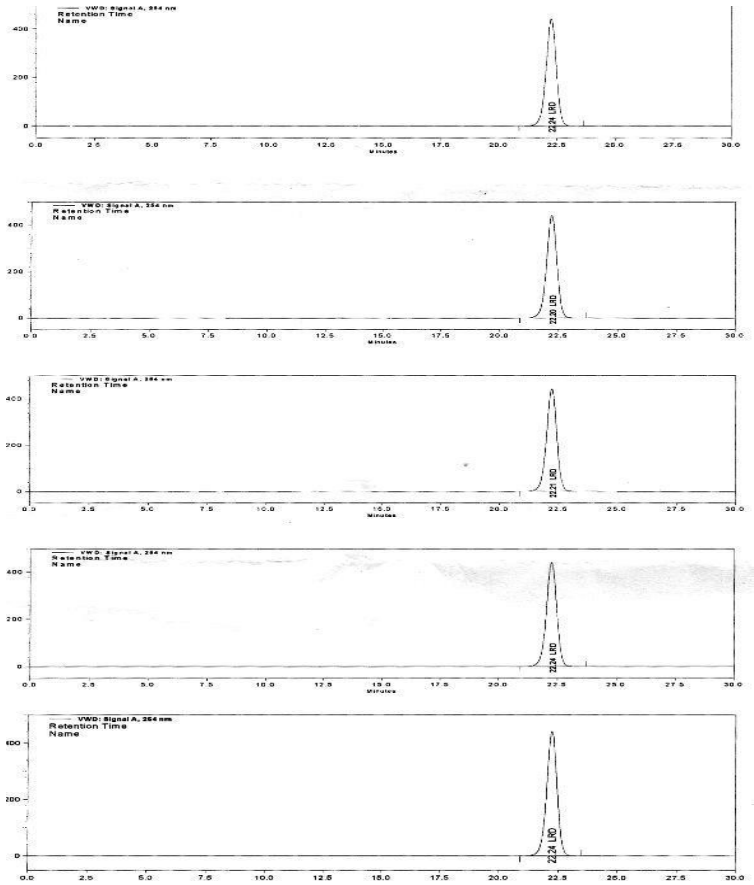

Figure 1.12: A typical HPLC Chromatogram showing the degradation profile of Loratadine by proposed method.

System suitability:

For system suitability, five replicates of standard solution were injected and studied the parameters like theoretical plates, tailing factor. The represented data was shown in **Table: 1.01.**

Table 1.01: System suitability parameters for LRD by proposed method

Name of the Compound	Theoretical plate	Tailing factor
Loratadine	3551	1.41

Specificity:

The HPLC chromatograms recorded for the placebo showed almost no peaks at the retention time of LRD. The peak for LRD is clearly separated from other excipients of the formulations. As there is no blank interference is not observed at the retention time of LRD. For Specificity Two replicates of standard solution were injected and studied the HPLC method presented in this specific for LRD.

Table 1.02: Specificity for LRD by proposed method

Loratadine	Inj-1	Inj-2	Avg	%RSD
RT	22.26	22.24	22.25	
Area	12694679	12615595	12655137	0.44

Precision:

In the study of the instrumental system precision where, a RSD of 0.21% was obtained for retention time and of 0.08% for the area obtained.

Table 1.03: System Precision for LRD by proposed method

Loratadine	Inj-1	Inj-2	Inj-3	Inj-4	Inj-5	Inj-6	Avg	%RSD
RT	22.27	22.28	22.28	22.28	22.28	22.29	22.28	0.21
Area	2535917	2531568	2535891	2537509	2533355	2535227	2534911	0.08

The method precision study for six sample preparations showed RSD of 0.13% was obtained for retention time and of 0.29% for the area obtained.

Table 1.04: Method Precision for LRD by proposed method

Loratadine	Inj-1	Inj-2	Inj-3	Inj-4	Inj-5	Inj-6	Avg	%RSD
RT	22.25	22.26	22.26	22.26	22.26	22.26	22.26	0.13
Area	2567559	2572075	2564177	2561582	2552521	2555495	2562235	0.29

For the intermediate precision, a study carried out by the same analyst working on different day. The results calculated as inter-day RSD corresponded to 1.0%. The same study was carried out for different analysts (n = 6 number of samples per analyst) obtaining a RSD of 0.42%. Both results together with the individual results are showing that the proposed analytical technique has a good intermediate precision.

Accuracy:

The accuracy of the method was determined on three concentration levels by recovery experiments. The recovery studies were carried out in triplicate preparations on composite blend collected from four capsules of Loratadine and analyzed as per the proposed method. The percentage recoveries with found in the range of 98.6 to 100.3 with an overall %RSD of 0.19%. From the data obtained which given in **Table: 1.05** the method was found to be accurate.

Table 1.05: Recovery studies for LRD by proposed methodLinearity:

% Level	Recovery Range	% RSD at each level	%RSD
50	99.5-100.3	0.19	0.19
100	98.9-99.3	0.19	
150	98.6-99.9	0.19	

Table: 1.06 Linearity of Response for LRD by proposed method

Level	Conc. (%)	Inj-1	Inj-2	Inj-3	Mean Area	%RSD
LOQ	0.003	30103	31742	28637	30161	5.39
LR-25%	0.025	615033	609319	622646	615666	1.09
LR-50%	0.05	1258166	1255701	1247526	1253798	0.44
LR-75%	0.075	1844806	1855948	1852979	1851244	0.31
LR-100%	0.1	2489787	2498117	2496042	2494649	0.17
LR-125%	0.125	3094254	3108682	3118425	3107120	0.39
LR-150%	0.15	3855585	3852934	3865774	3858098	0.18
Correlation coefficient					0.999	
% Y-intercept					-1.83	
Slope					25625824	
Intercept					-45671	

The standard curve was obtained in the concentration range of 25.0-150µg/ml. The linearity of this method was evaluated by linear regression analysis. Slope, intercept and correlation coefficient [r2] of standard curve were calculated and given in **Figure: 1.03** to demonstrate the linearity of the method.

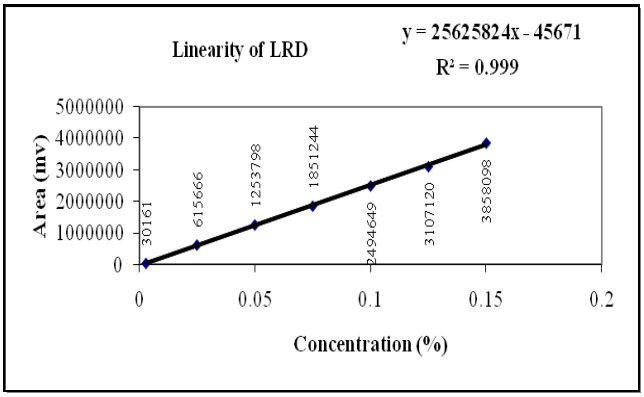

Figure: 1.03 Calibration curve for LRD

LOD and LOQ:

Limit of detection was found to be 20.0µg/ml and Limit of quantification was found to be 61.00µg/ml.

Table 1.07: Limit of detection for LRD by proposed method

Loratadine	Inj-1	Inj-2	Inj-3	Avg
RT	22.26	22.27	22.27	22.27
Area	7792	9160	7222	8058
S/N	2.99	4.05	3.47	3.5

Table 1.08: Limit of quantification for LRD by proposed method

Loratadine	Inj-1	Inj-2	Inj-3	Inj-4	Inj-5	Inj-6
RT	22.05	22.05	22.05	22.05	22.05	22.05
Area	30103	31742	28637	28077	27729	30658
S/N	11.34	11.01	10.58	11.02	10.06	11.11

CONCLUSION

We have developed a fast, simple and reliable analytical method for determination of LRD in pharmaceutical preparation using HPLC with UV detection. An analytical run takes about 30.0min. Separation of compounds is very fast, with good reproducibility and peak asymmetry. Validation of this method was accomplished, getting results meeting all requirements. The method is simple, reproducible, with a good accuracy and precision.

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