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RP-UPLC METHOD DEVELOPMENT AND VALIDATION FOR QUANTITATIVE ESTIMATION OF LEVOCETIRIZINE IN LEVOCETIRIZINE DIHYDROCHLORIDE TABLETS

S. S. Shetgar [*1], D. Ramadevi [2], B. M Rao [3], K. Basavaiah [4]

Department of Inorganic and Analytical Chemistry [1, 4], Andhra University, Visakhapatnam -530003, Andhra Pradesh, India.

AU College of Pharmaceutical Science [2], Andhra University, Visakhapatnam - 530003, Andhra Pradesh, India

Vice President & Head ASAT & Corporate Quality Control [3], Dr. Reddy's Laboratories, Bachupally, Hyderabad - 500090, Telangana, India.

Keywords:

Levocetirizine, RP-UPLC, Rapid, Sensitive, Stability indicating

Correspondence to Author:
Mr. Sanjay S. Shetgar

Department of Inorganic and Analytical Chemistry, Andhra University, Visakhapatnam -530003, Andhra Pradesh, India.

E-mail: sanjayshetgar@yahoo.com

ABSTRACT: Current analytical research and experiments provide the development of a simple, sensitive, accurate, rapid, and stability indicative RP-UPLC method for estimation of Levocetirizine in Levocetirizine dihydrochloride tablets. This development was achieved using a Waters Acquity UPLC with BEH, C18 column of dimensions of 100 mm × 2.1 mm, 1.7 µm column at 0.3 mL/min flow rate and Acquity TUV detector at 230 nm. This method was validated based on the guidelines of the International Council on Harmonization (ICH). Linearity was demonstrated at 25% to 150% levels with R^2 value of 0.999. Precision and accuracy were in line with the ICH guidance with a mean recovery of 99.02%. The RP-UPLC method is sensitive with levels of Limit of detection (LOD) and Limit of Quantitation (LOQ) at 0.04µg/mL and 0.12µg/mL, respectively. Degradation studies in conditions of Oxidation, Acid, Base, Temperature, Water, UV light demonstrate there is no placebo as well as degradation impurities interfering with the Levocetirizine main peak. The simple, sensitive, accurate, rapid, and stability-indicating method makes it an efficient tool in routine quality control testing of the active pharmaceutical ingredient as well as in formulations.

INTRODUCTION: Levocetirizine comes under the class of antihistamine and is used to provide relief to patients who show symptoms such as sneezing, running nose, itching, and hives.

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On administration, the Levocetirizine Dihydrochloride selectively blocks histamine produced by the body when an allergic reaction occurs. Levocetirizine Dihydrochloride, **Fig. 1**, is levorotatory enantiomer of Cetirizine with IUPAC name 2- [2-[4-[(R)-(4-chlorophenyl) - phenyl (methyl)] piperazin-1-yl] ethoxy] acetic acid; dihydrochloride. Literature review for analytical estimations reveals that different analytical methods have been used for the determination of Levocetirizine. Most of these methods are for simultaneous estimations with other drug

combinations using the HPLC, HPTLC, and one RP-UPLC method [1-8]. In the literature review, came across one RP-UPLC method for simultaneous estimation of Levocetirizine and Montelukast sodium. However, it was developed for a liquid dosage form using a phenyl column with gradient elution. The objective of this study is to develop a simple, sensitive, accurate, stability-indicating and quick method for estimation of Levocetirizine in bulk as well as Levocetirizine tablets and validate it as per International council of harmonization (ICH) guidance so that it can be applied for routine analysis in the testing laboratories.

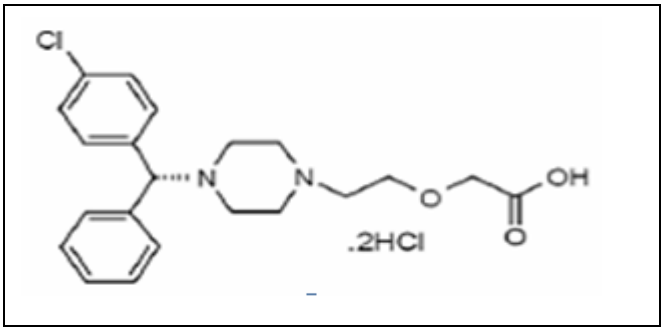

FIG. 1: STRUCTURE OF LEVOCETIRIZINE DIHYDROCHLORIDE

MATERIALS AND METHODS:

Materials: Levocetirizine dihydrochloride pure drug (API) and Levocetirizine Capsules (Xyzal) were obtained from Dr. Reddy's Laboratories. Other reagent and solvents like acetonitrile, methanol, potassium dihydrogen ortho phosphate buffer, ortho-phosphoric acid, glacial acetic acid was procured from Rankem and are of analytical reagent grade. Water for UPLC was obtained through the Milli-Q laboratory water generation system.

Equipment Used: Waters -Acquity UPLC (Ultra performance Liquid Chromatograph system with Auto Injector and Tunable UV Detector. Software used is Empower 2, Sonicator (Ultrasonic), pH meter (Thermo scientific), Micro balance (Sartorius), Vacuum filter pump.

Method:

Chromatographic Conditions: The chromatographic system used was the Waters Acquity UPLC with BEH C18 column of dimension 100 mm × 2.1 mm, 1.7 µm.

The mobile phase comprised of 0.01N Potassium dihydrogen phosphate (KH_2PO_4) buffer and Acetonitrile in the ratio of 60:40 (pH 4.80). The flow rate was set at 0.3 mL/min with 2 µL injection volume and column oven temperature of 30 °C.

The diluent used was a mixture of 0.01N KH_2PO_4 buffer and Acetonitrile in the ratio 50:50. Detection was achieved with an Acquity Tunable UV detector at 230 nm. Instrument operation, data acquiring, and processing were done using Empower 2 software.

Mobile Phase and Solution Preparation: Preparation of 0.01N KH_2PO_4 Buffer (pH-4.8): 1.36 g of potassium dihydrogen phosphate was diluted to 1000 mL with Milli-Q grade water to get 0.01N KH_2PO_4 buffer.

Preparation of Mobile Phase: Mixture of 0.01N KH_2PO_4 Buffer (pH-4.8) and acetonitrile in the ratio of 60:40

Preparation of Diluent: Based upon the solubility of the drug, diluent was selected. 0.01N KH_2PO_4 Buffer and Acetonitrile were taken in the ratio of 60:40.

Preparation of Levocetirizine Standard stock solutions: Standard stock solution of 50 µg/mL was prepared by accurately weighing 2.5 mg of Levocetirizine Dihydrochloride in a 50 mL volumetric flask. About 30 mL of the diluent was added and sonicated for 10 min. The solution was made up to 50 mL with diluent.

Preparation of Levocetirizine Standard working solutions (100% Solution): A 5 µg/mL of Levocetirizine Dihydrochloride was prepared by diluting 1 mL of Levocetirizine Dihydrochloride stock solution to 10 mL with the diluent.

Preparation of Levocetirizine Sample Stock Solutions: 10 tablets were weighed and powdered. Then the average weight of each tablet was calculated (100.41 mg).

Weight equivalent to 1 tablet was transferred into a 100 mL volumetric flask, 50 mL of diluent was added and sonicated for 25 min, further, the volume was made up with diluent and filtered using 0.45 µ PVDF filters to get a solution of 50 µg/mL of Levocetirizine Dihydrochloride.

Preparation of Levocetirizine Sample working solutions (100% solution): 1 mL of filtered sample stock solution was transferred to 10 mL volumetric flask and made up with diluent to get a 5 µg/mL of Levocetirizine Dihydrochloride.

RESULTS AND DISCUSSION:

Optimization of Sample Preparation: The sample preparation needs to ensure complete extraction from the formulation mix. Tablet was finely powdered. Based on the solubility of the Levocetirizine, acetonitrile and potassium dihydrogen phosphate buffer combination were assessed and optimized for efficient extraction. The filter used was the standard 0.45µ PVDF filter which didn't demonstrate any retention of the drug on the filter.

Optimization of Chromatographic Conditions: The sensitivity of the UPLC method depends upon the selection of detection wavelength. An ideal wavelength is one that gives a good response for all impurities and analyte peaks to be detected.

The wavelength for measurement was selected as 230 nm from the absorption spectrum considering the UV maxima shown by Levocetirizine dihydrochloride in **Fig. 2**.

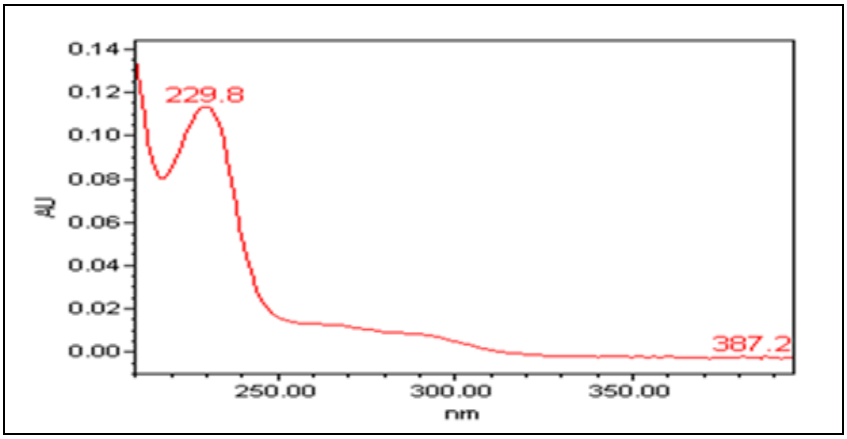

FIG. 2: UV SPECTRUM OF LEVOCETIRIZINE DIHYDROCHLORIDE

Various UPLC columns like X-Bridge, Hibar, HSS, BEH 100 mm lengths were evaluated. Mobile phase options of Water and Methanol mixture, 0.1% orthophosphoric acid and methanol mixtures, 0.1% orthophosphoric acid and acetonitrile mixtures and mobile phase combination of 0.01N potassium dihydrogen phosphate and acetonitrile mixture were evaluated. The use of water and methanol mixture was not satisfactory due to peak split and baseline disturbance. 0.1% orthophosphoric acid and methanol also did not give appropriate peak shapes.

However, 0.01N potassium hydrogen phosphate acetonitrile combination gave good peak shape as well as quick peak elution. Different compositions of 0.01N potassium dihydrogen phosphate buffer and acetonitrile mixtures were evaluated at 50:50; 55:45; and 60:40 ratios to arrive at the optimum parameters. The final optimized condition was achieved using Waters Acquity BEH C18 column of dimensions 100 mm × 2.1 mm, 1.7 µm. with a flow rate of 0.3 mL/min with column oven temperature 30 °C and mobile phase consisting of 0.01N KH_2PO_4 buffer (pH 4.8) and Acetonitrile in the ratio of 60:40. Levocetirizine peak eluted well below 2 min, making it a quick method and hence the possibility to run multiple samples.

Method Validation: For the newly developed RP-UPLC method, validation was performed according to the ICH guideline for the analytical parameters such as system suitability test, specificity through degradation study of Levocetirizine tablets and placebo, linearity and range, precision, accuracy, the limit of detection (LOD), the limit of quantitation (LOQ) and robustness to demonstrate the adequacy of the method.

System Suitability Parameters: The system suitability parameters were determined by preparing standard solutions of Levocetirizine dihydrochloride (5 µg/mL) as six replicates and the parameters like Relative standard deviation (RSD), peak tailing and United States Pharmacopeia (USP) plate count were determined. The RSD was below

2% Retention time variation was observed in range 1.670-1.681 min with RSD of 0.2%. Plate count was in the range 5004 to 5093 with RSD of 0.7%. The tailing factor was in the range 1.56 - 1.57, with an RSD of 0.3%. Each of these meets the USP requirements and demonstrates good chromatographic practice. System suitability parameters such as RSD of six replicate dilute standard injections, Number of theoretical plates, and tailing factor for Levocetirizine are tabulated under **Table 1**.

TABLE 1: SYSTEM SUITABILITY PARAMETERS

S. no.	Levocetirizine Dihydrochloride		
Injection	**RT (minutes)**	**USP Plate Count**	**Tailing**
1	1.670	5004	1.57
2	1.674	5020	1.57
3	1.675	5016	1.56
4	1.676	5093	1.57
5	1.679	5007	1.57
6	1.681	5008	1.57
Avg.	1.676	5025	1.57
Std. Dev.	0.004	34.0	0.004
%RSD	0.2	0.7	0.3

Specificity: Specificity for the RP-UPLC method was demonstrated by no interference from the blank, placebo, and potential degradation impurities from the degradation study conducted in line with the ICH guidance. The chromatogram was reviewed for any interference from the blank, placebo, and potential degradation impurities. There were no interferences at the retention time of the levocetirizine peak. The % degradation is tabulated in **Table 2**.

TABLE 2: DEGRADATION DATA FOR LEVOCETIRIZINE TABLETS

S. no.	Degradation Condition	% Levocetirizine Undegraded	% Levocetirizine Degraded
1	Acid	94.06	5.94
2	Base	95.00	5.00
3	Oxidation	95.80	4.20
4	Thermal	97.01	2.99
5	UV	97.96	2.04
6	Water	98.96	1.04

Chromatograms for acid, base, oxidation, thermal, UV light, Water are shown in **Fig. 3, Fig. 4, Fig. 5, Fig. 6, Fig. 7, Fig. 8** respectively. There were no interferences at the retention time of the Levocetirizine peak. Chromatograms of Levocetirizine capsules placebo and Levocetirizine standard are shown in **Fig. 9** and **Fig. 10.** respectively.

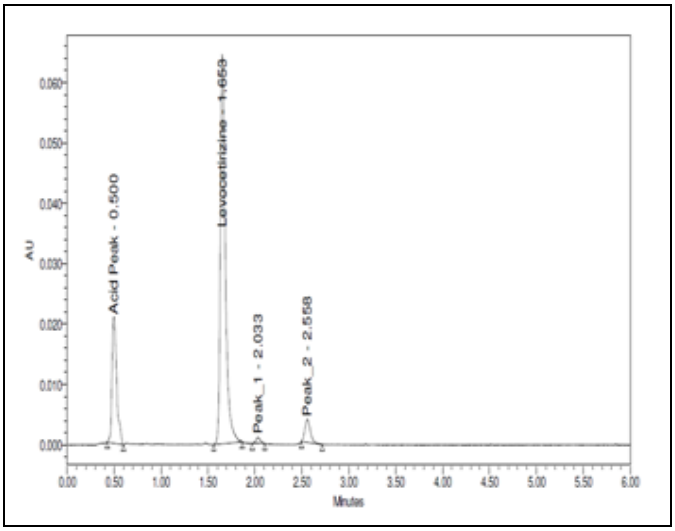

FIG. 3: ACID DEGRADATION

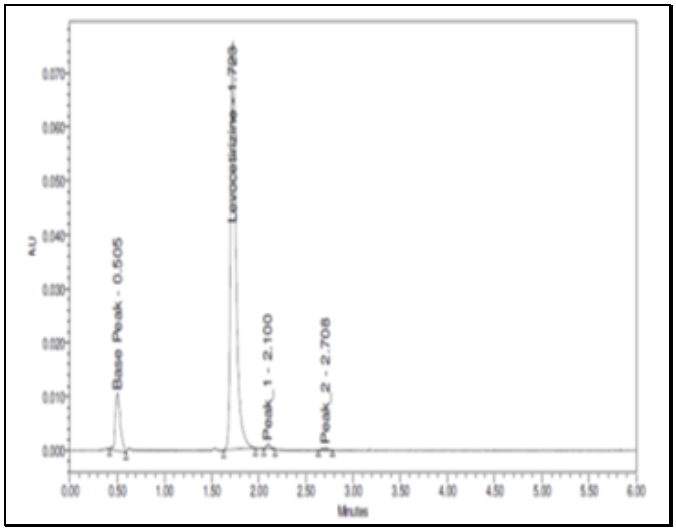

FIG. 4: BASE DEGRADATION

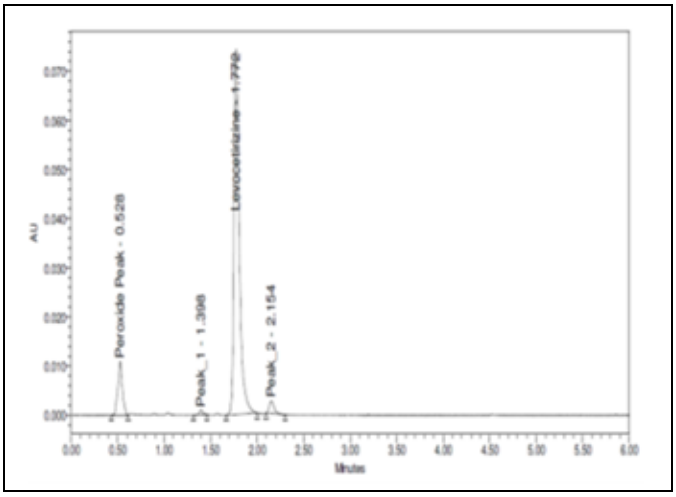

FIG. 5: OXIDATION DEGRADATION

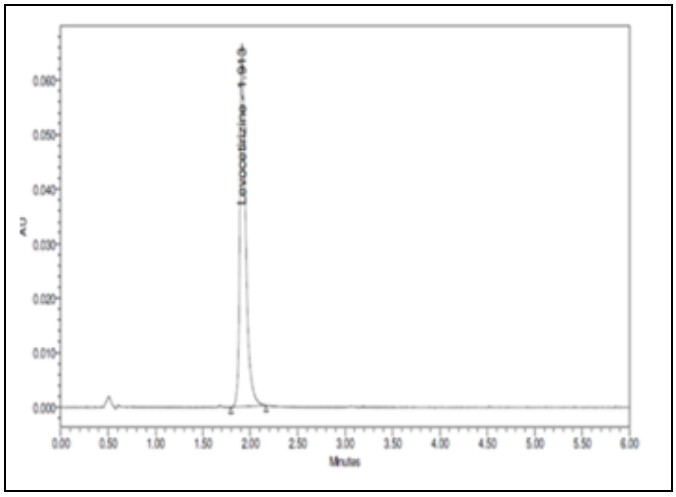

FIG. 6: THERMAL DEGRADATION

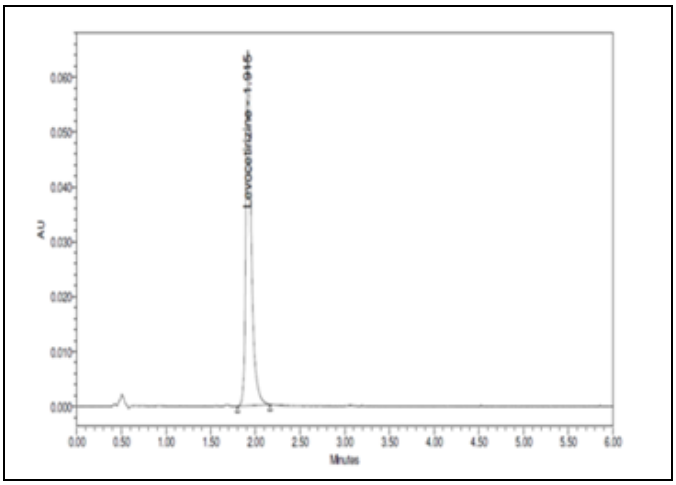

FIG. 7: UV DEGRADATION

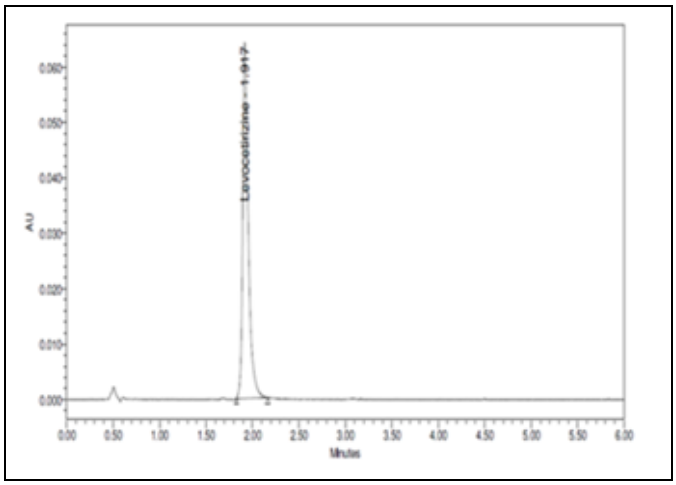

FIG. 8: WATER DEGRADATION

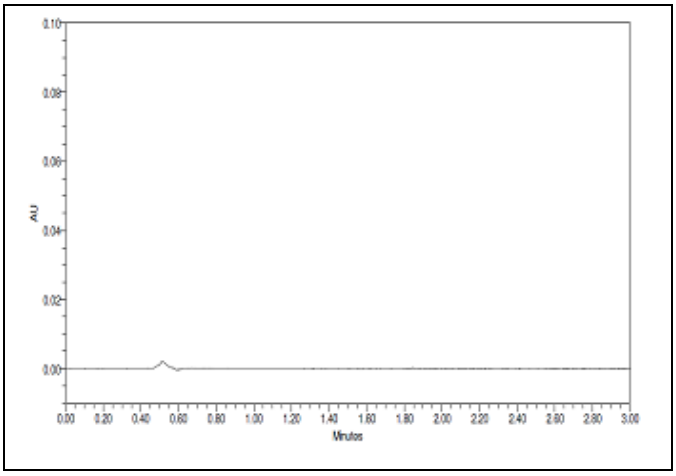

FIG. 9: FORMULATION PLACEBO

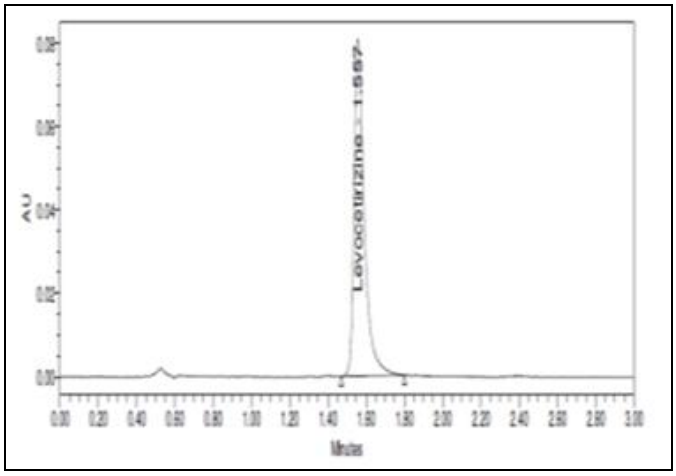

FIG. 10: LEVO STANDARD

Precision: The method precision of the RP-UPLC assay method as part of repeatability was checked by injecting six replicates of Levocetirizine sample solutions of concentration 5µg/mL. The % amount of Levocetirizine dihydrochloride found was calculated, and RSD was found to be 0.6%. Tabulated details are shown in **Table 3**.

Six sample solutions of concentration 5 µg/mL of Levocetirizine into the chromatograph in the optimized chromatographic conditions after 24 h of preparation. The % amount of Levocetirizine found was calculated, and RSD was found to be 0.3%. Tabulated details are shown in **Table 4**.

TABLE 3: METHOD PRECISION

S. no.	Levocetirizine Peak Area
1	289944
2	290568
3	292677
4	291748
5	288846
6	293358
AVG	291190
STD. DEV	1712.1
%RSD	0.6

TABLE 4: INTERMEDIATE PRECISION

S. no.	Levocetirizine Peak Area
1	276556
2	278194
3	276183
4	276019
5	276475
6	277167
AVG	276766
STD. DEV	803.3
%RSD	0.3

TABLE 5: LEVOCETIRIZINE LINEARITY DATA

Linearity Level (%)	Levocetirizine Concentration (µg\mL)	Levocetirizine Peak Response
0	0	0
25	1.25	75843
50	2.5	149315
75	3.75	217438
100	5	287840
125	6.25	364343
150	7.5	437256

Linearity and Range: To demonstrate the linearity and range of Levocetirizine Dihydrochloride RP-UPLC assay method, Levocetirizine standard solution of concentration 5 µg/mL was prepared. Using this stock, six standard solutions with concentrations at 25%, 50%, 75%, 100%, 125% and 150% corresponding to range 0 µg/mL 1.25 µg/mL, 2.5 µg/mL, 3.75 µg/mL, 5 µg/mL, 6.25 µg/mL and 7.5 µg/mL of Levocetirizine dihydrochloride were prepared and injected in the RP-UPLC system with the optimized chromatographic condition. Concentration versus peak area was plotted. The slope obtained was 57923, Y-Intercept was at 1652.2 and Correlation Co-efficient was found to be 0.9998, indicating it is linear across the range. Details of concentration and peak area are shown in **Table 5**. The linearity plot is shown in **Fig. 11.**

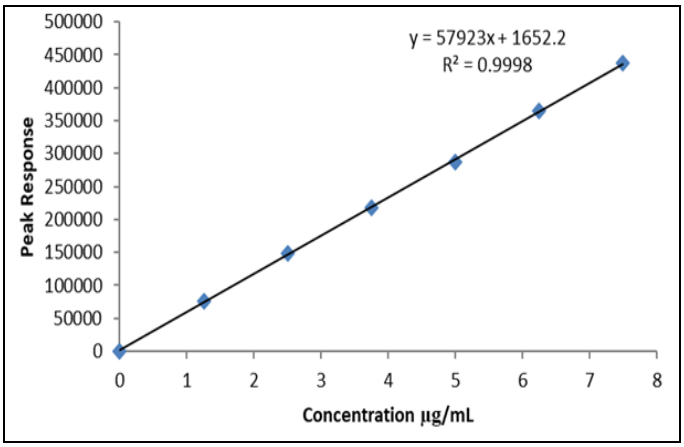

FIG. 11: LEVOCETIRIZINE LINEARITY PLOT

Accuracy: To demonstrate the accuracy of the Levocetirizine dihydrochloride RP-UPLC assay method Levocetirizine sample solution of concentration 5 µg/mL was prepared by taking one tablet equivalent of the powdered Levocetirizine tablet contents followed by sonication with the diluent and filtration. Known fixed quantities of Levocetirizine Standard stock solution were spiked onto Sample solution at levels of 50%, 100%, and 150%. Each of the sample solution spiked with the known concentration of the standard (50%, 100%, 150% of Levocetirizine dihydrochloride) was injected in triplicate in the optimized chromatographic system, and % recovery was in the range 99.46% to 101.58%, 98.13 - 99.31% and 100.20 - 101.11% at 50%, 100%, and 150% respectively. The average recovery was observed to be 99.89%. Detailed Levocetirizine Accuracy data is tabulated as **Table 6**.

TABLE 6: LEVOCETIRIZINE ACCURACY

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Average % Recovery
50%	2.5	2.49	99.46	99.89%
	2.5	2.54	101.58	
	2.5	2.49	99.74	
100%	5	4.91	98.13	
	5	4.97	99.31	
	5	4.95	99.09	
150%	7.5	7.53	100.37	
	7.5	7.52	100.20	
	7.5	7.58	101.11	

Limit of Detection (LOD) and Limit of Quantitation (LOQ): The LOD and LOQ were established by injecting dilute solutions of Levocetirizine dihydrochloride using the signal to noise ratio approach. The LOD and LOQ values were observed to be at 0.04 µg/mL and 0.12 µg/mL, respectively. Chromatograms for LOD and LOQ are shown in **Fig. 12** and **13**. respectively.

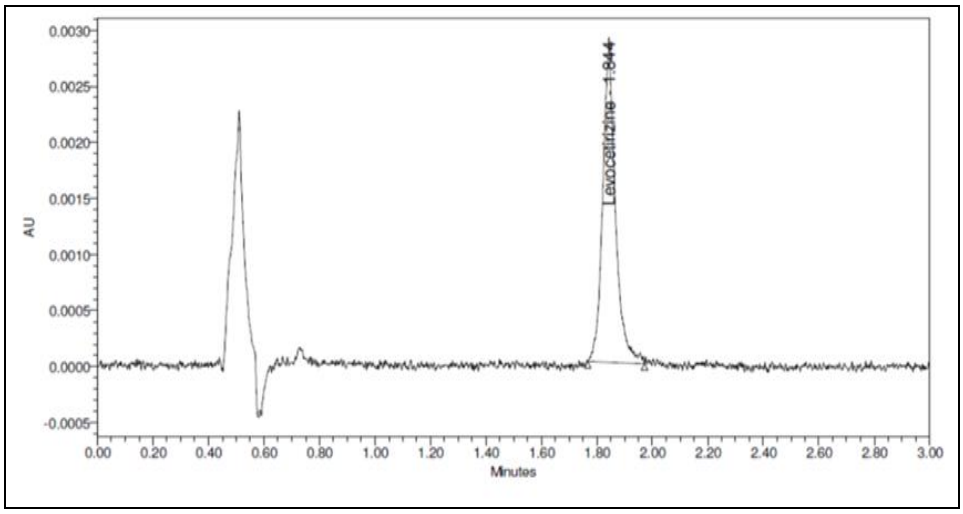

FIGURE 12: CHROMATOGRAM FOR LIMIT OF DETECTION

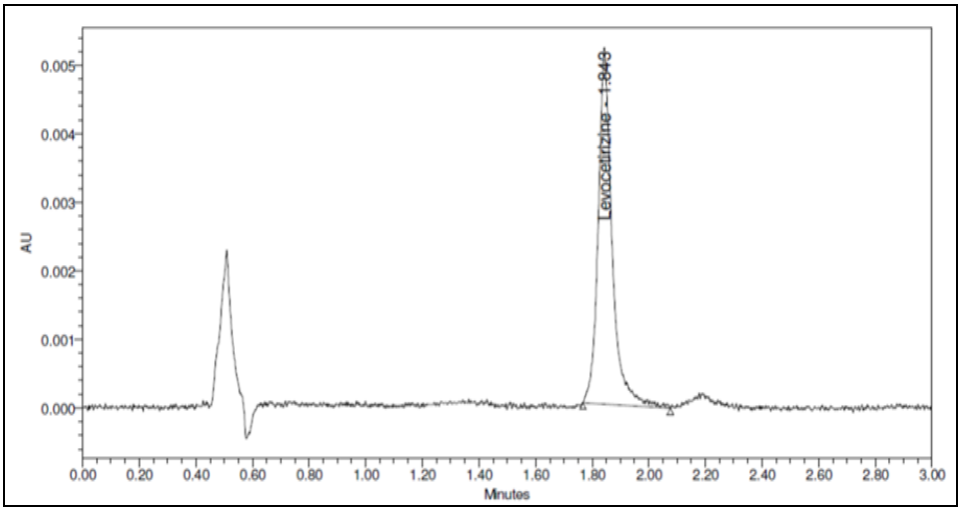

FIG. 13: CHROMATOGRAM FOR LIMIT OF QUANTITATION

Robustness: Robustness of the Levocetirizine dihydrochloride RP-UPLC Assay method was demonstrated small, deliberate changes in the method were made which could potentially occur in a laboratory environment by going on either side of the chromatographic parameters like Flow (+0.3 ml/min); Mobile phase composition (+2%); Temperature (+5 °C). Under each of these conditions, sample preparations were injected in duplicate into the optimized chromatographic conditions with the above changes. The system suitability parameters and amount of the Levocetirizine dihydrochloride content were calculated and found comparable. The RSD was in the range of 0.7% to 1.3% for content when assessed with the deliberate changes in the chromatographic parameters. Details are tabulated in **Table 7**.

TABLE 7: ROBUSTNESS - DELIBERATE CHANGES

Parameter	% RSD
Flow rate decrease (27ml/min)	1.0
Flow rate increase (0.33ml/min)	1.0
Mobile phase reduced ratio (65B:35A) *	0.8
Mobile phase increased ratio (55B:45A) *	1.0
Temperature decrease (25^0C)	1.3
Temperature increased (35^0C)	0.7

*Note: B-Buffer; A-Acetonitrile

Assay of Marketed Formulation: Assay of the Levocetirizine Dihydrochloride tablets batch was performed as per the validated RP-UPLC assay method. Standard and sample solutions were prepared at a concentration of 50 µg/mL of

Levocetirizine dihydrochloride and chromatographed. Six sample sets were prepared and assayed.

The results were observed to be 99.02 %, with RSD at 0.59%. The area count, content, and RSD are tabulated in **Table 8**.

TABLE 8: ASSAY OF THE MARKETED FORMULATION

Sample No.	Standard	Sample	%Assay
1	299137	289944	98.60
2	292776	290568	98.81
3.	294992	292677	99.53
4.	291234	291748	99.21
5.	291269	288846	98.22
6.	291505	293358	99.76
AVG.	293486	291190	99.02
STD. DEV	3118.2	1712.1	0.582
%RSD	1.1	0.6	0.59

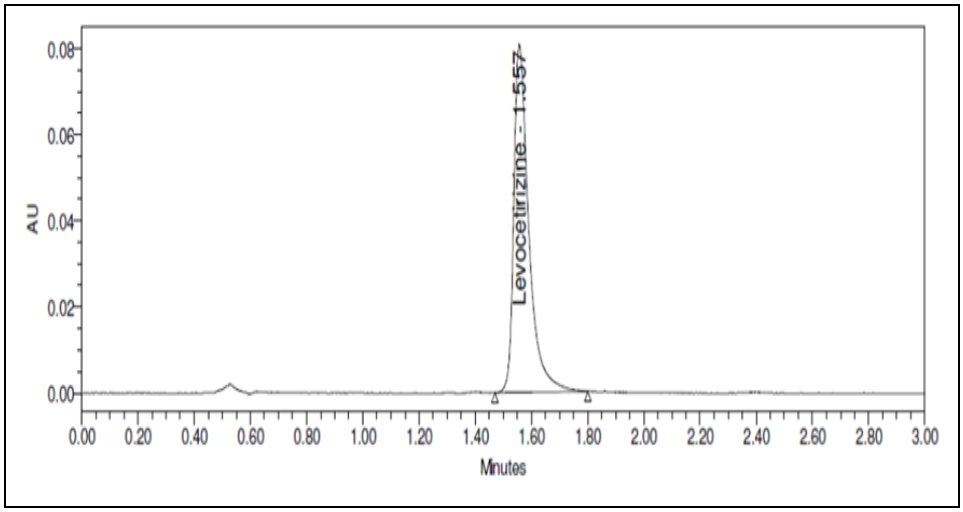

FIG. 14: CHROMATOGRAM OF LEVOCETIRIZINE STANDARD

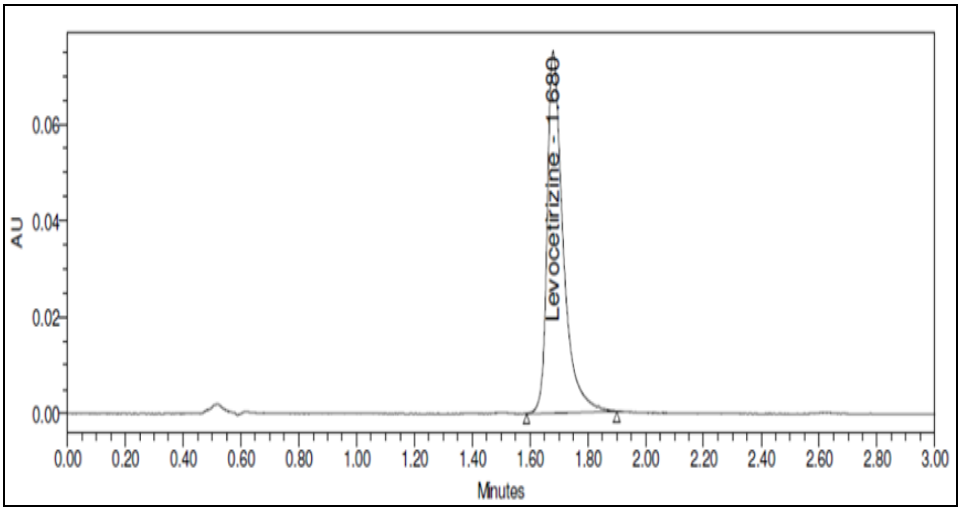

FIG. 15: CHROMATOGRAM OF THE LEVOCETIRIZINE SAMPLE

Chromatograms of typical standard and sample are shown in **Fig. 14** and **Fig. 15** respectively.

CONCLUSION: In conclusion, a simple, sensitive, accurate, and quick method was developed for the estimation of Levocetirizine Dihydrochloride in Levocetirizine Dihydrochloride Tablets by RP-UPLC technique. System suitability parameters were studied by injecting the standard six times, and results were well under the acceptance criteria. Linearity study was carried out between 25% to 150% levels, R^2 value was found to be > 0.999. Precision was found to be at an RSD

of 0.6% for repeatability and 0.3% for intermediate precision. LOD and LOQ were established at 0.04 µg/mL and 0.12 µg/mL, respectively. Recovery of Levocetirizine was found to be 99.89%. By using the validated RP-UPLC Assay method, the marketed formulation was tested, and the content of Levocetirizine dihydrochloride was observed to be in the range of 98.60 to 99.76%. Degradation studies of Levocetirizine conducted on lines on ICH demonstrated that the Levocetirizine peak purity thresholds were more than the purity angle. While the method is suitable for the estimation of Levocetirizine Dihydrochloride for routine and stability testing, it can also be used for estimation of Levocetirizine residues after cleaning of equipment and can be extended for impurity estimation in quality controls laboratory.

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CONFLICTS OF INTEREST: The authors have no conflict of interest to declare.

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