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DEVELOPMENT OF RP-HPLC METHOD FOR THE ANALYSIS OF LORATADINE AND ITS APPLICATION IN THE EVALUATION OF MARKETED PREPARATION

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Abstract

A simple, selective, rapid and precise reversed-phase high-performance liquid chromatographic method for analysis of Loratadine in bulk and tablet dosage form has been developed and validated. Chromatography was performed on a Waters C18, 5µm particle size, 25cmx4.6mm i.d. with ACN : Methanol = 85 : 15 v/v as mobile phase at a flow rate of 1.0 mL min−1. UV detection was performed at 212 nm. Total run time was 10 min. Loratadine were eluted with retention time of 5.776 minutes. The method was validated for accuracy, precision, linearity, specificity, and sensitivity in accordance with USP and ICH guidelines. Validation revealed the method is specific, rapid, accurate, precise, reliable, and reproducible. Calibration plots were linear over the concentration ranges 0-28µgmL−1 for Loratadine. Limit of detection were 0.06mg mL−1 and limit of quantification were 0.18mg mL−1 for Loratadine. The high recovery and low coefficients of variation confirm the suitability of the method for analysis of the drug in tablet dosage form. The validated method was successfully used for quantitative analysis of Loratin in Loratadine tablets.

Keywords: *Analytical Method Development, Estimation, Loratadine, Linearity, RP-HPLC.*

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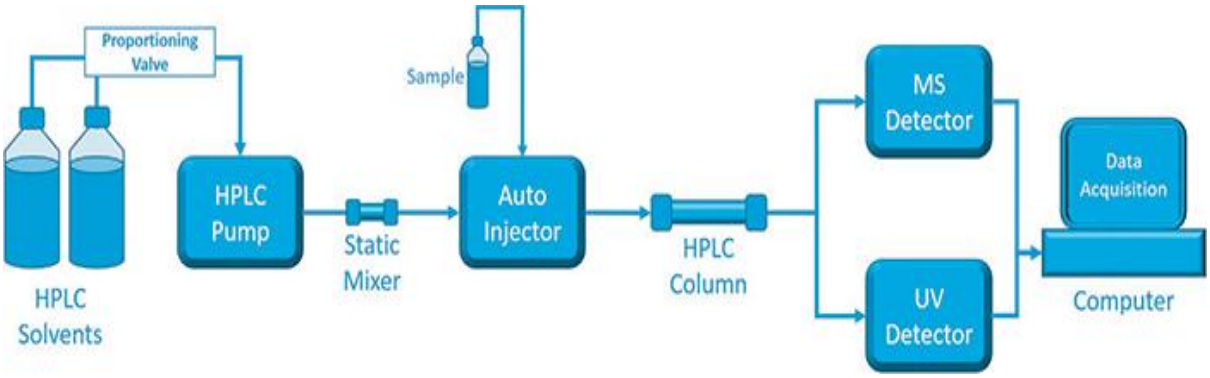
1. INTRODUCTION:

Chromatography technique developed substantially because of the work of Archer JohnPorter Martin and Richard Laurence Millington Synge during the 1940s and 1950s, for whichthey won the 1952 Nobel Prize in Chemistry. They established the principles and basic techniques of partition chromatography, and their work encouraged the rapid development of several chromatographic methods: paper chromatography, gas chromatography and what would become known as high-performance liquid chromatography. Since then, technology has advanced rapidly.

High-pressure liquid chromatography (HPLC)

Using this chromatography technique it is possible to perform structural, and functional analysis, and purification of many molecules within a short time, This technique yields perfect results in the separation, and identification of amino acids, carbohydrates, lipids, nucleic acids, proteins, steroids, and other biologically active molecules, In HPLC, mobile phase passes through columns under 10–400 atmospheric pressure, and with a high (0.1–5 cm//sec) flow rate. In this technique, use of small particles, and application of high pressure on the rate of solvent flow increases separation power, of HPLC and the analysis is completed within a short time.

Essential components of a HPLC device are solvent depot, high- pressure pump,commercially prepared column, detector, and recorder. Duration of separation is controlled with the aid of a computerized system, and material is accrued.



SOLVENT RESERVIOR: The contents of mobile phase are present in glass container. In HPLC the mobile phase or solvent is a mixture ofpolar and non-polar liquid components.

PUMP: The pump suctions the mobile phase from solvent reservoir & force it to column and then passes to detector. 42000 Kpa is the operating pressure of the pump.

SAMPLE INJECTOR: The injector can be a solitary infusion or a computerized infusion framework. The infusion of the fluid is givenwith o.1ml to 100ml of vol under high pressure (upto 4000psi).

COLUMNS: Columns are typically made of cleaned stainless steel, are somewhere around 50mm and 300mm long & have an inward distance across 2&5mm.

DETECTOR: The HPLC detector situated towards the end of the columndistinguishes the analytes as they elute from the chromatographic column. Regularly utilized detectors are UV spectroscopy & fluorescence etc..

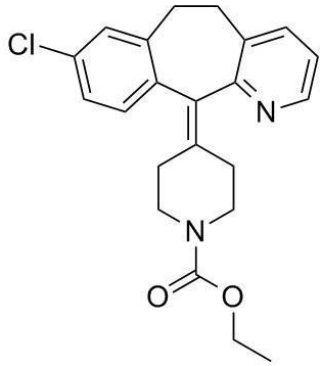
DATA COLLECTION DEVICE: Signals from the detector might begathered on graph & recorded.

DRUG PROFILE:

Name: Loratadine

Description: Loratadine is a second-generation antihistamine used to manage symptoms of allergic rhinitis.5 A lack of sedative and CNS adverse effects make loratadine, along with other second generation antihistamines, preferable over their 1st generation counterparts in many clinical situations.

Structure:



Categories: Anti-allergic Agents, Anti-pruritic Agent.

Weight: Average:382.883

Chemical Formula: $C_{22}H_{23}ClN_2O_2$

Mechanism of action: Histamine release is a key mediator in allergic rhinitis and urticaria.1278 As a

result, loratadine exerts it's effect by targeting H1 histamine receptors.

Loratadine binds to H1 histamine receptors found on the surface of epithelial cells, endothelial cells, eosinophils, neutrophils, airway cells, and vascular smooth muscle cells among others.7 H1 histamine receptors fall under the wider umbrella of G-protein coupled receptors, and exist in a state of equilibrium between the active and inactive forms.78 Histamine binding to the H1-receptor facilitates cross linking between transmembrane domains III and V, stabilizing the active form of the receptor.78 On the other hand, antihistamines bind to a different site on the H1 receptor favouring the inactive form.78 Hence, loratadine can more accurately be classified as an "inverse agonist" as opposed to a "histamine antagonist", and can prevent or reduce the severity of histamine mediated symptoms.

Method development and its validation for loratadine by rp-hplc

5.4.1. Selection of Wavelength

The standard stock solutions – 10 mg of Loratadine standard was transferred into 10 ml volumetric flask, dissolved & make up to volume with Methanol. Further dilution was done by transferring 1 ml of the above solution into a 10ml volumetric flask and make up to volume with methanol to get 10ppm concentration.

It scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Loratadine, so that the same wave number can be utilized in HPLC UV detector for estimating the Loratadine Further dilutions were made from the stock solution to get 0, 2, 4, 6, 8, 10 ppm was preparedfor calibration curve method.

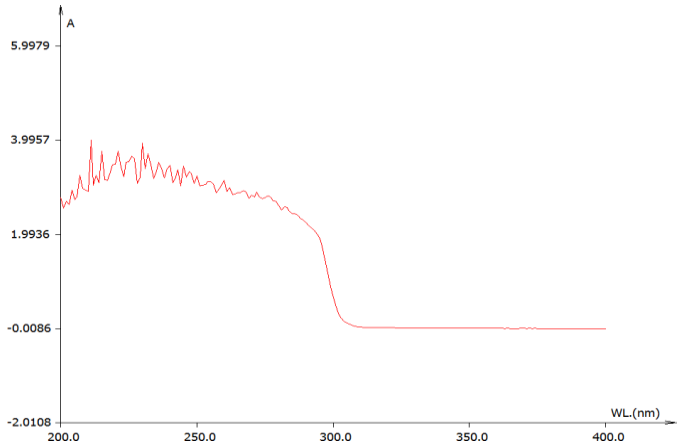


Figure-5.1: UV spectrum for Loratadine

Table-5.11: Summary of Optimized Chromatographic conditions

Mobile phase	ACN : Methanol = 85 : 15
Column	Symmetry ODS RP C18,5□m, 15mm x 4.6mm i.d.
Flow rate	1.0 ml/ min.
Wavelength	212nm
Sampling System	Automatic
Temp. of Auto sampler	Ambient
Volume of injection	10µl
Run time	10 mins
Mode of Separation	Isocratic

Preparation of mobile phase:
850ml of Acetonitrile and 150ml of Methanol were mixed well and degassed in ultrasonic water bath for 15 minutes. The solution was filtered through 0.45 µm filter under vacuum filtration.

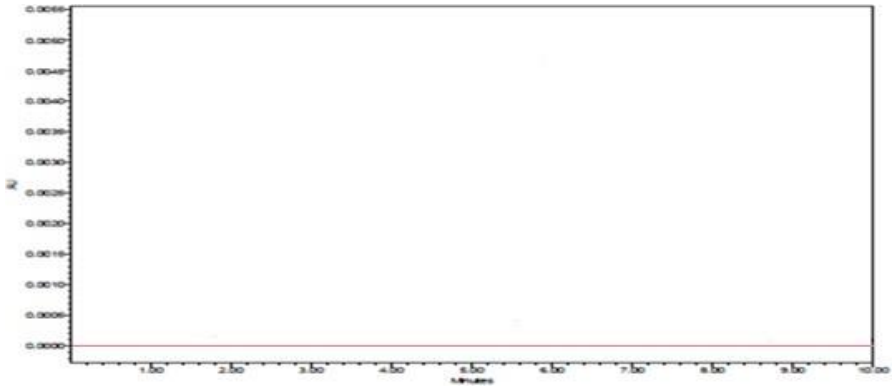
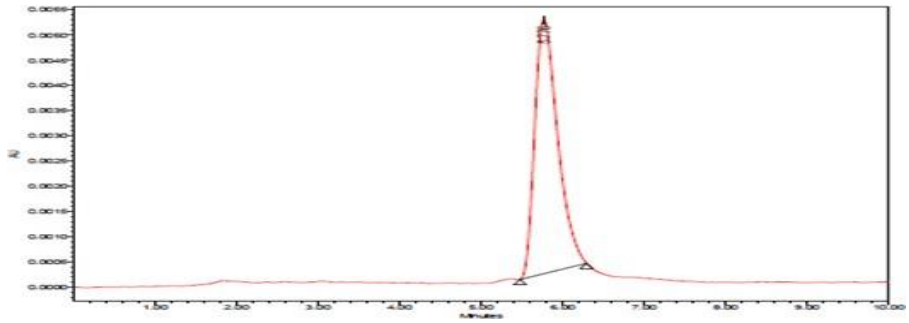


Fig-5.8: Chromatogram for Blank Solution



Fig–5.9: Chromatogram of Loratadine in Optimized Condition

RT	Peak Area	Theoretical Plates	Tailing Factor
5.776	218822	2682	1.09

METHOD VALIDATION

1. Accuracy:

Recovery study:

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of Loratadine were taken and added to the pre-analyzed formulation of concentration 20 g/ml. From that percentage recovery values were calculated. The results were shown in table-6.1.

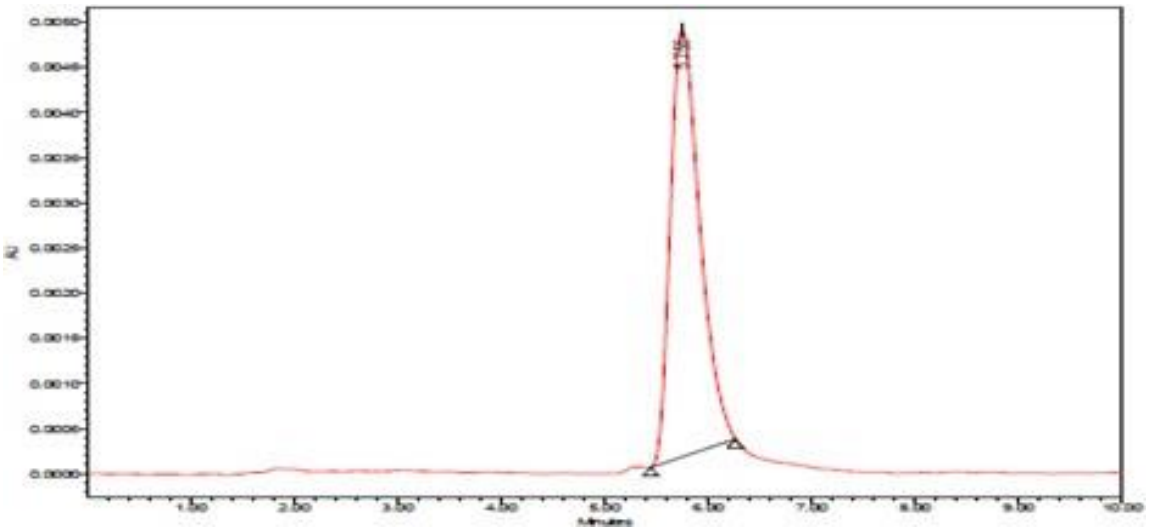


Fig-6.1: Chromatogram of 80% Accuracy-1Table-6.2: Readings of 80% Accuracy-1

RT	Peak Area	Tailing Factor	Theoretical Plates
5.755	180254	1.04	2815

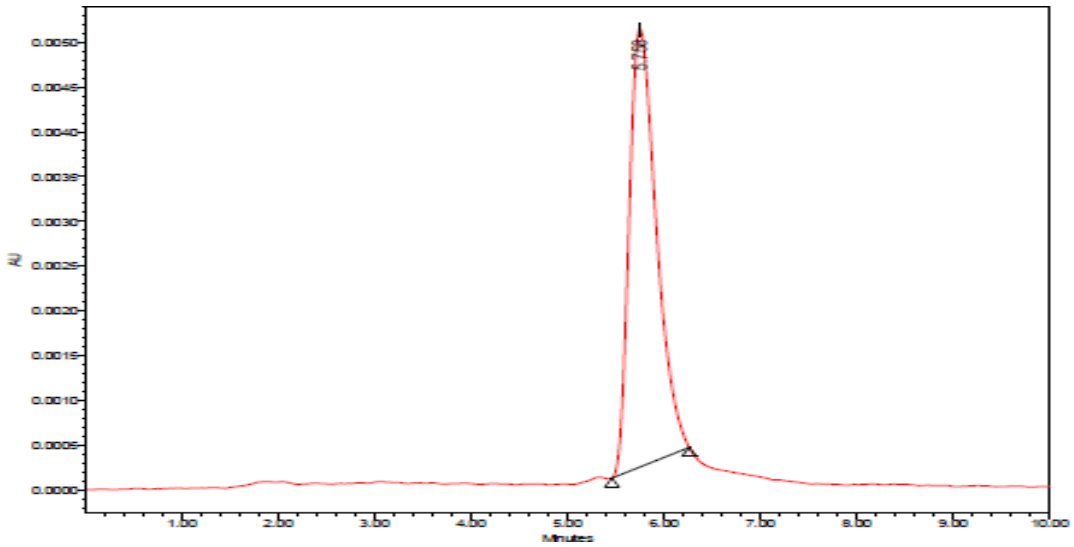


Fig.-6.2: Chromatogram of 80% Accuracy-2

Table-6.3: Readings of 80% Accuracy-2

RT	Peak Area	Tailing Factor	Theoretical Plates
5.758	181682	1.03	2913

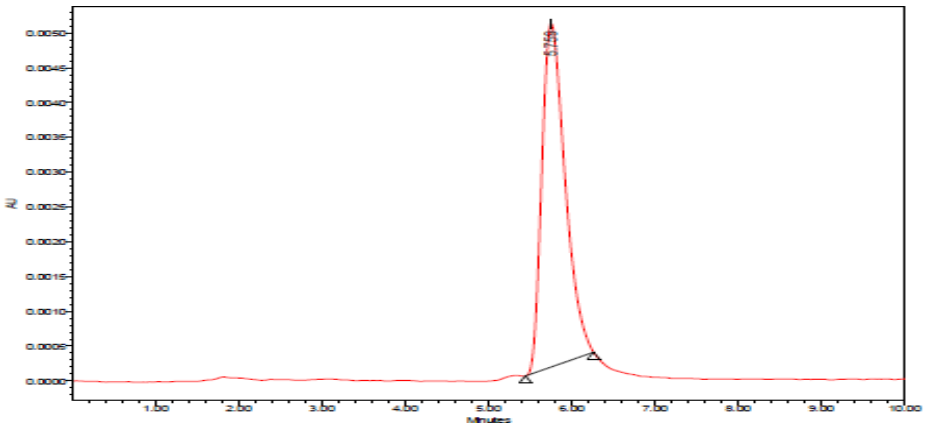


Fig.-6.3: Chromatogram of 80% Accuracy-3

Table-6.4: Readings of 80% Accuracy-3

RT	Peak Area	Tailing Factor	Theoretical Plates
3.538	180121	1.07	2687

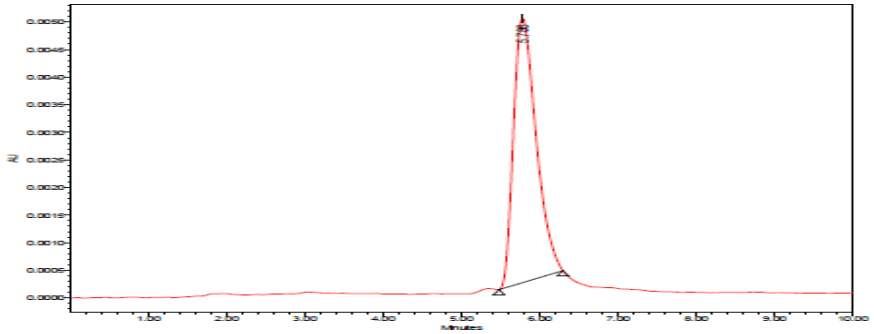


Fig.-6.4: Chromatogram of 100% Accuracy-1

Table-6.5: Readings of 100% Accuracy-1

RT	Peak Area	Tailing Factor	Theoretical Plates
5.786	226493	1.12	2852

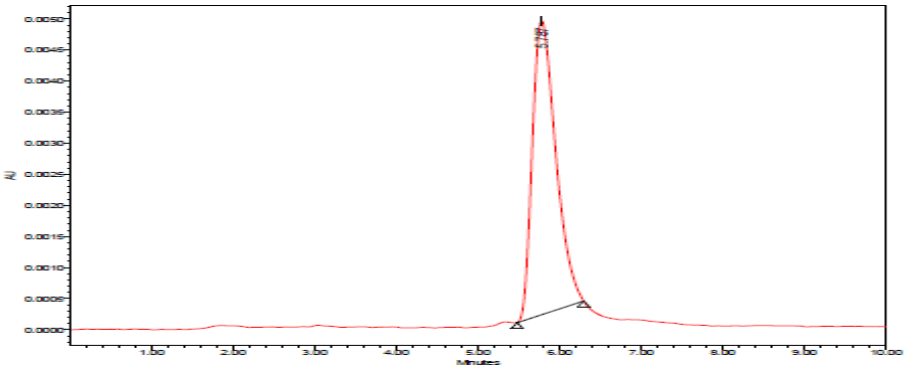


Fig.-6.5: Chromatogram of 100% Accuracy-2

Table-6.6: Readings of 100% Accuracy-2

RT	Peak Area	Tailing Factor	Theoretical Plates
5.787	223895	1.11	2715

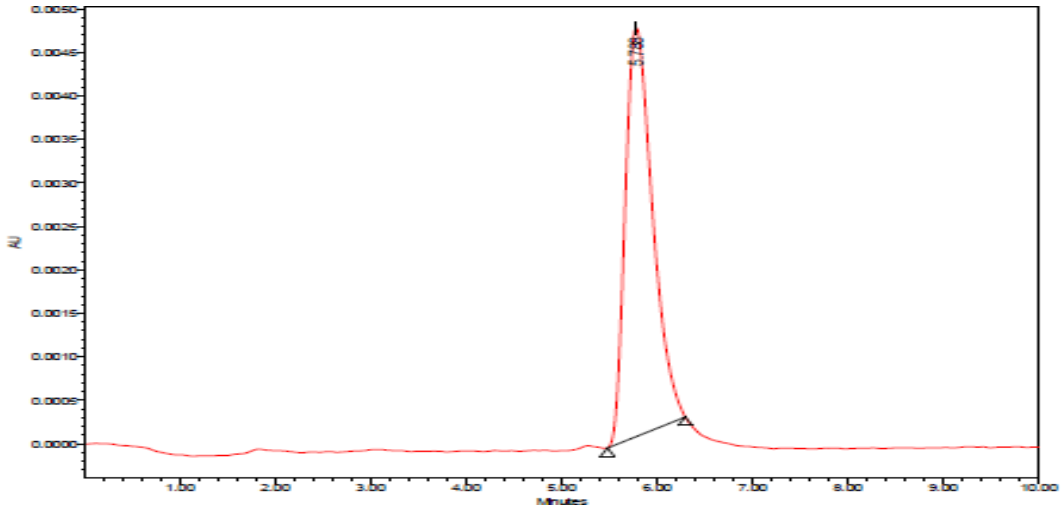


Fig.-6.6: Chromatogram of 100% Accuracy-3

Table-6.7: Readings of 100% Accuracy-3

RT	Peak Area	Tailing Factor	Theoretical Plates
5.788	224589	1.14	2906

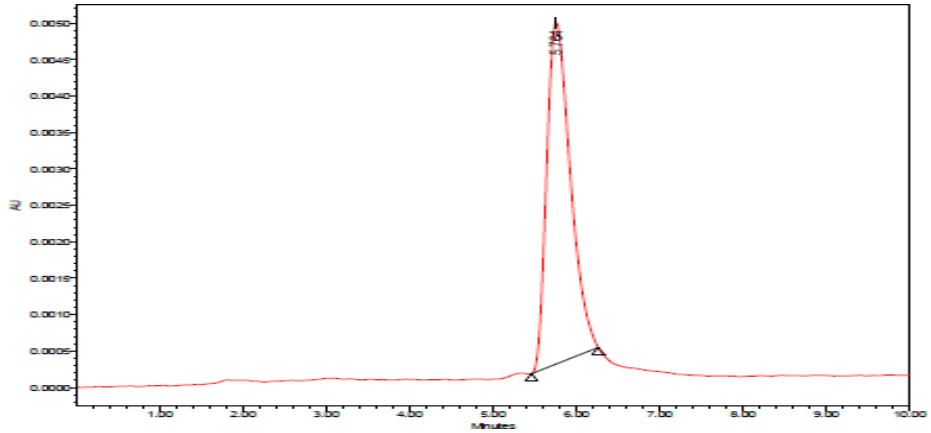


Fig.-6.7: Chromatogram of 120% Accuracy-1Table-6.8: Readings of 120% Accuracy-1

Fig.-6.: Reading of 120% Accuracy-

RT	Peak Area	Tailing Factor	Theoretical Plates
5.764	269542	1.13	2688

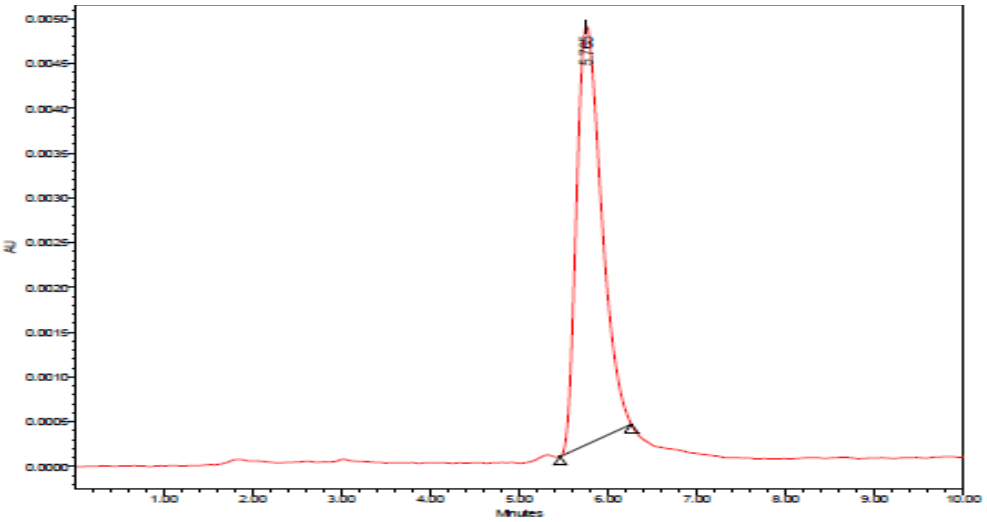


Fig.-6.8: Chromatogram of 120% Accuracy-

Table-6.9: Readings of 120% Accuracy-2

RT	Peak Area	Tailing Factor	Theoretical Plates
5.765	274878	1.12	2756

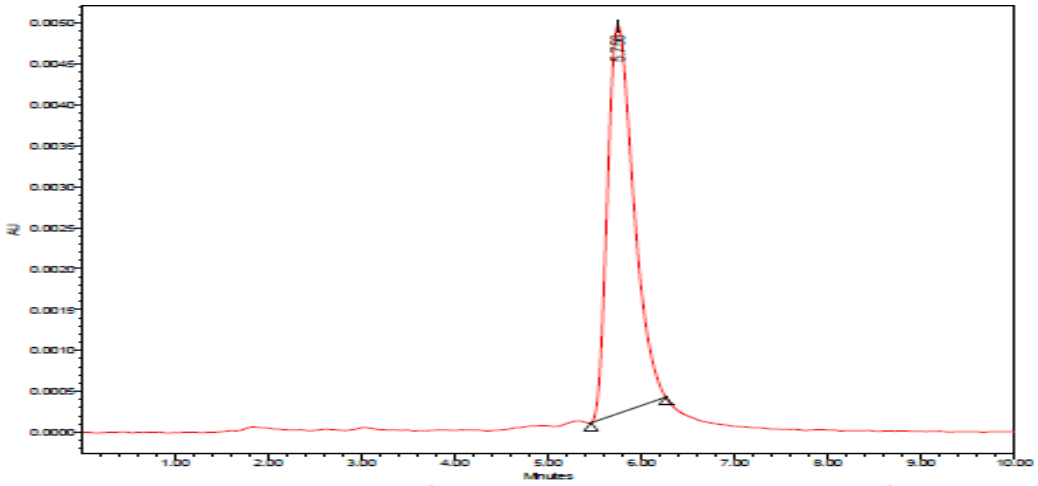


Fig.-6.9: Chromatogram of 120% Accuracy-3

Table-6.10: Readings of 120% Accuracy-3

RT	Peak Area	Tailing Factor	Theoretical Plates
5.756	396846	1.15	2817

Table-6.1: Accuracy Readings

Sample ID	Concentration (□g/ml)		Peak Area	% Recoveryof Pure drug	Statistical Analysis
	Amount Added	Amount Found			
S_1 : 80 %	16	15.92735	180254	99.54594	Mean= 99.78851667
S_2 : 80 %	16	16.05575	181682	100.3484	S.D. = 0.486310758
S_3 : 80 %	16	15.91539	180121	99.47121	% R.S.D.= 0.48734%
S_4 : 100 %	20	20.08479	226493	100.4239	Mean= 99.74928333
S_5 : 100 %	20	19.8512	223895	99.25598	S.D. = 0.604702551
S_6 : 100 %	20	19.91359	224589	99.56797	% R.S.D.= 0.60622%
S_7 : 120 %	24	23.9554	269542	99.81418	Mean= 100.93896
S_8 : 120 %	24	24.43517	274878	101.8132	S.D. = 1.022789303
S_9 : 120 %	24	24.28547	273213	101.1895	% R.S.D. = 1.01327%

2. Precision:

2.1. Repeatability

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug. Loratadine (API). The percent relative standard deviation was calculated for Loratadine are presented in the table-6.1

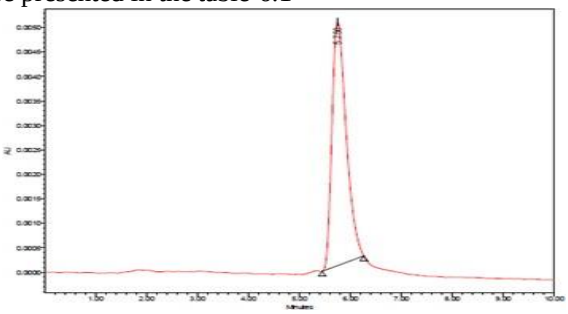


Fig.-6.10: Chromatogram of Repeatability-1

Table-6.12: Readings of Repeatability-1

RT	Peak Area	Tailing Factor	Theoretical Plates
5.756	216993	1.12	2965

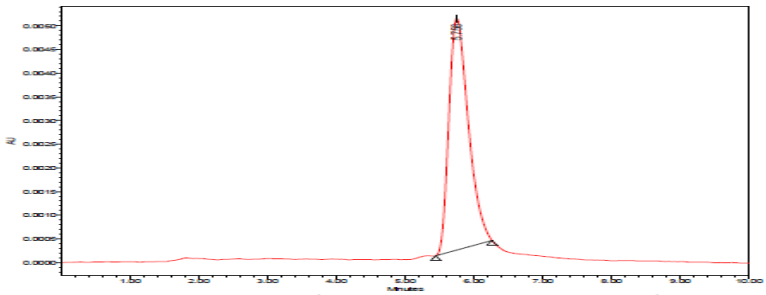


Fig.-6.11: Chromatogram of Repeatability-2

Table-6.13: Readings of Repeatability-2

RT	Peak Area	Tailing Factor	Theoretical Plates

5.756	216633	1.18	2926

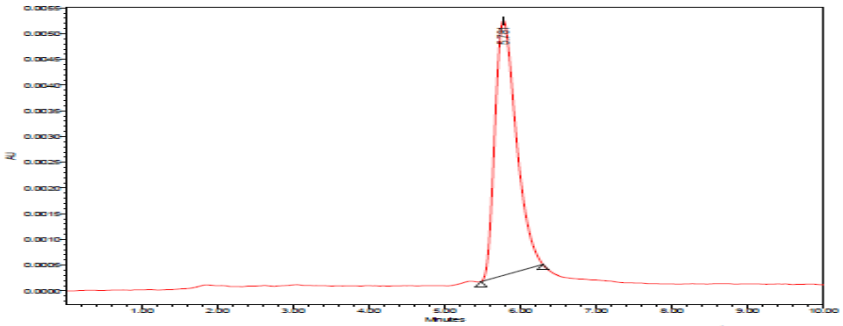


Fig.-6.12: Chromatogram of Repeatability-3

Table-6.14: Readings of Repeatability-3

RT	Peak Area	Tailing Factor	Theoretical Plates
5.781	217515	1.18	2621

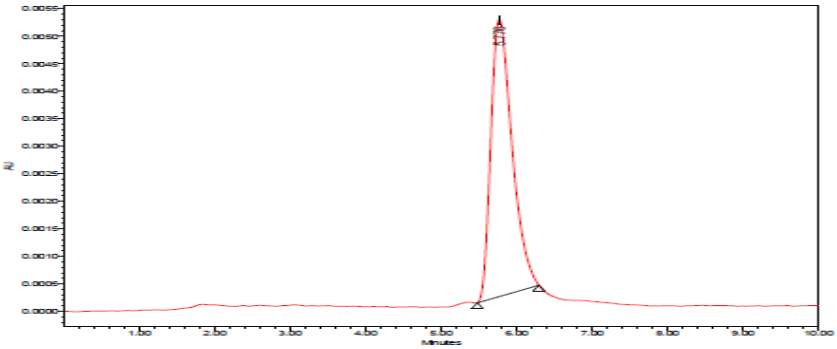


Fig.-6.13: Chromatogram of Repeatability-4

Table-6.15: Readings of Repeatability-4

RT	Peak Area	Tailing Factor	Theoretical Plates
5.776	218822	1.15	2764

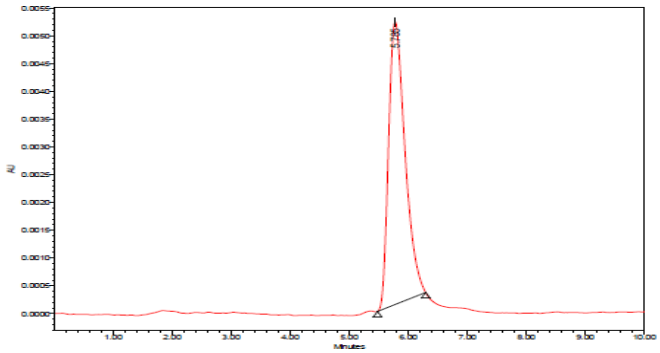


Fig.-6.14: Chromatogram of Repeatability-5

Table-6.16: Readings of Repeatability-5

RT	Peak Area	Tailing Factor	Theoretical Plates
5.785	218731	1.14	2565

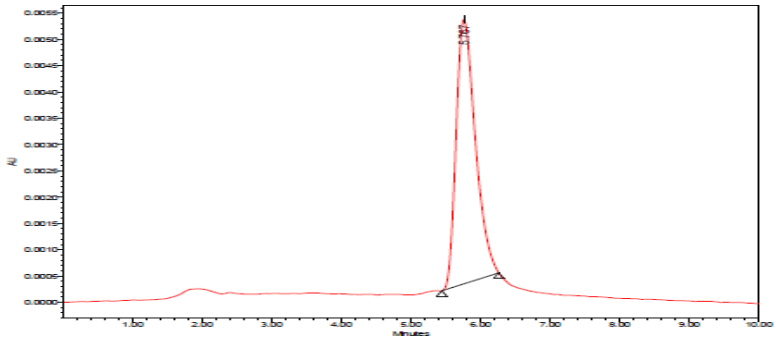


Fig.-6.15: Chromatogram of Repeatability-6

Table-6.17: Readings of Repeatability-6

RT	Peak Area	Tailing Factor	Theoretical Plates
5.767	217029	1.13	2922

Table-6.11: Repeatability Readings

HPLC Injection Replicates of Loratadine	Retention Time	Peak Area
Replicate – 1	5.756	216993
Replicate – 2	5.756	216633
Replicate – 3	5.781	217515
Replicate – 4	5.776	218822
Replicate – 5	5.785	218731
Replicate – 6	5.767	217029
Average	**5.770166667**	**217620.5**
Standard Deviation	**0.01251266**	**938.800032**
% RSD	**0.21685**	**0.4313**

1.1. Intermediate Precision:

The Intermediate Precision consists of two method: -

Intra Day: In Intra Day process, the 80%, 100% and 120% concentration are injected atdifferent intervals of time in same day.

Inter Day: In Inter Day process, the 80%, 100% and 120% concentration are injected atsame intervals of time in different days.

Table-6.18: Results of intra-assay & inter-assay

Conc. Of Loratadine(API) (µg/ml)	Observed Conc. Of Loratadine (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
16	16.23	0.34	16.07	0.76
20	20.48	0.57	20.18	0.82
24	24.13	0.52	24.19	0.68

2. Linearity & Range:

To evaluate the linearity, serial dilution of analyte was prepared from the stock solution was diluted with mobile phase to get a series of concentration ranging from 12-28µg/ml. The prepared solutions were filtered through Whatman filter paper (No.41). From these solutions, 20µl injections of each concentration were injected into the HPLC system and chromatographed under the optimized conditions. Calibration curve was constructed by plotting the mean peak area (Y-axis) against the concentration (X-axis).

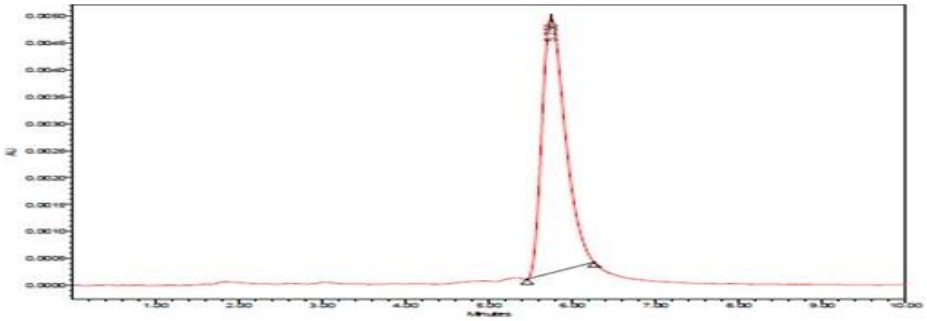


Fig-6.17: Chromatogram for Linearity (12ppm)

Table-6.20: Readings of Linearity-(12 ppm)

Drug Name	RT	Peak Area	Tailing Factor	Theoretical Plates
Loratadine	5.756	141302	1.02	2786

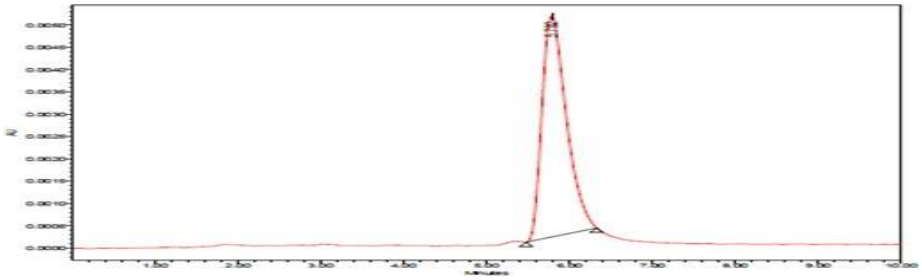


Fig-6.18: Chromatogram for Linearity (12ppm)

Table-6.21: Readings of Linearity-(16 ppm)

Drug Name	RT	Peak Area	Tailing Factor	Theoretical Plates
Loratadine	5.792	180283	1.05	2533

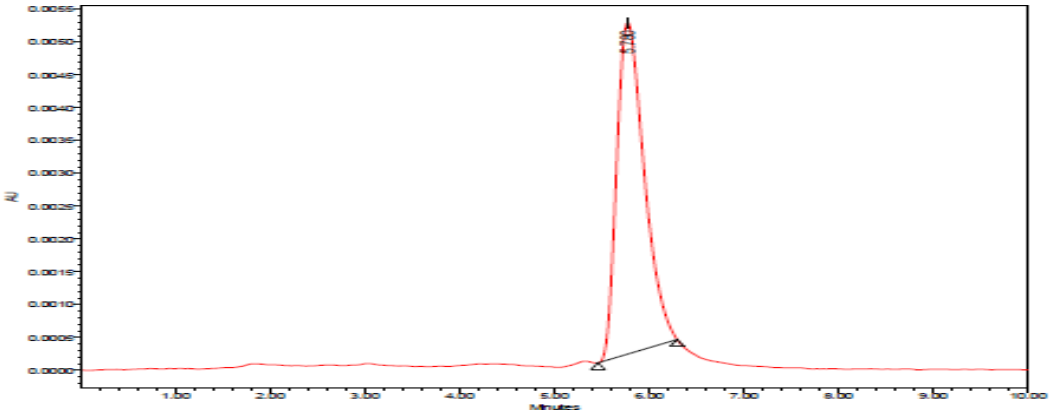


Fig-6.19: Chromatogram for Linearity (20 ppm)

Table-6.22: Readings of Linearity-(20 ppm)

Drug Name	RT	Peak Area	Tailing Factor	Theoretical Plates
Loratadine	5.780	226794	1.04	2915

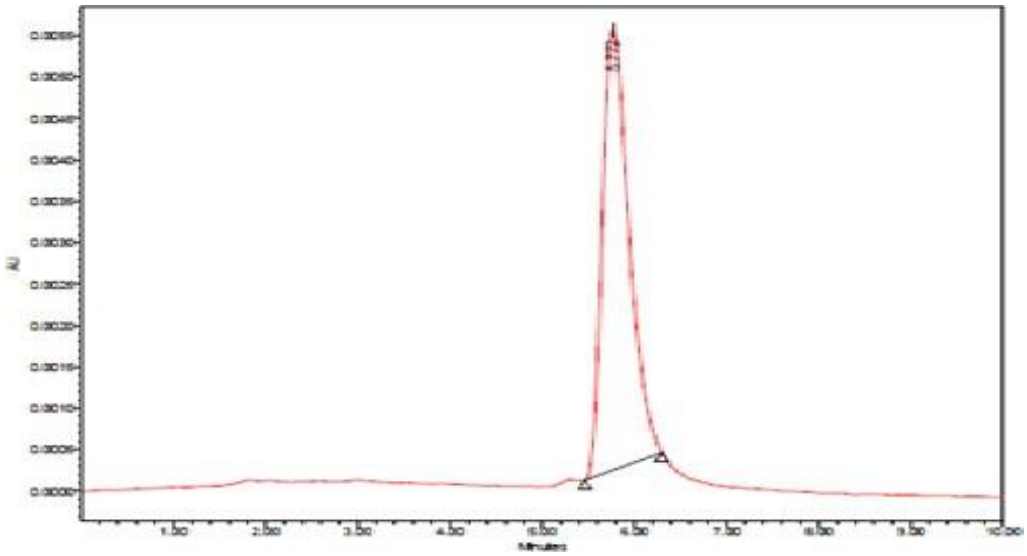


Fig-6.20: Chromatogram for Linearity (24 ppm)

Table-6.23: Readings of Linearity-(24 ppm)

Drug Name	RT	Peak Area	Tailing Factor	Theoretical Plates
Loratadine	5.772	272745	1.05	2897

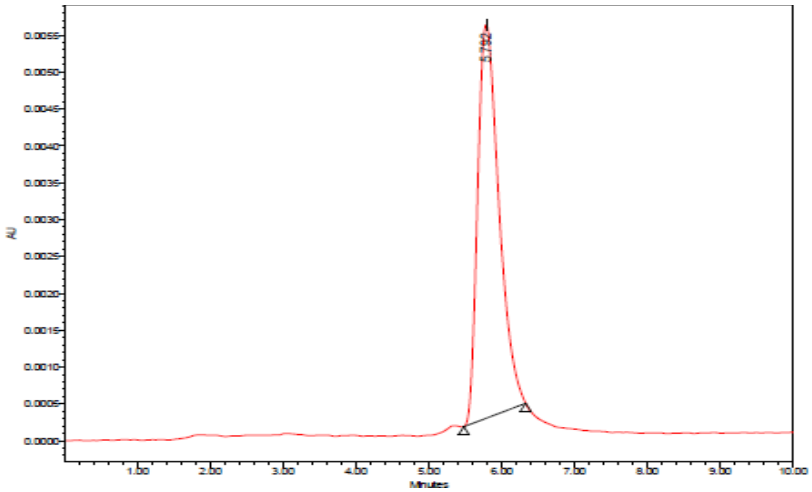


Fig-6.21: Chromatogram for Linearity (28ppm)

able-6.24: Readings of Linearity-(28ppm)

Drug Name	RT	Peak Area	Tailing Factor	Theoretical Plates
Loratadine	5.792	309734	1.07	2891

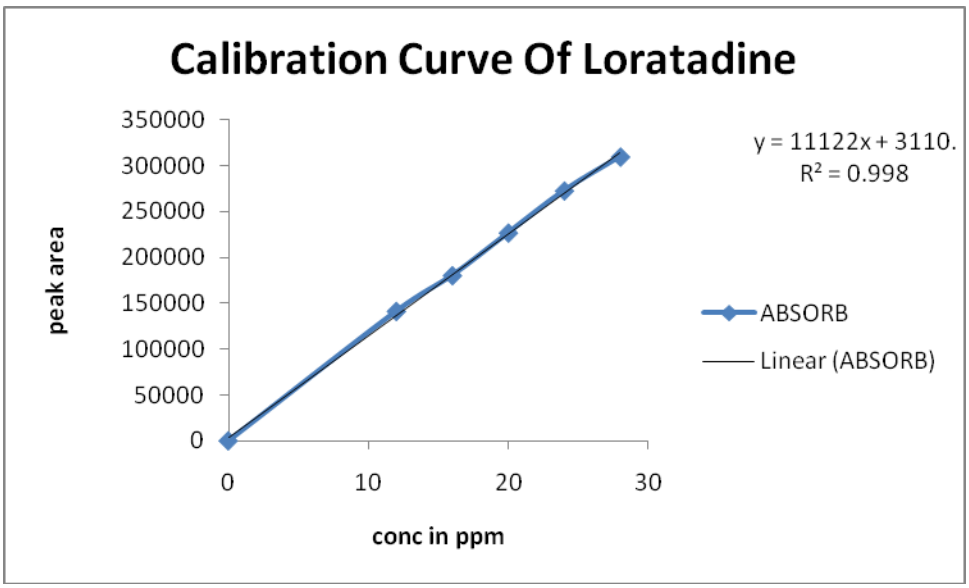


Fig-6.16: Calibration curve of Loratadine (API).

Table-6.19: Linearity Results

CONC.(µg/ml)	MEAN AUC (n=6)
0	0
12	141302
16	180283
20	226794
24	272745
28	309734

Observation: We observed that the calibration curve showed good linearity in the range of 12-28 µg/ml, for Loratadine (API) with correlation coefficient (R^2) of 0.998 (Fig-6.1). A typical calibration curve has the regression equation of y = 11122x + 3110 for Loratadine.

3. Method Robustness:

Influence of small changes in chromatographic conditions such as change in flow rate (0.1ml/min), Wavelength of detection (2nm) & organic phase content in mobile phase (5%)studied to determine the robustness of the method are also in favour of (Table-6.25, % RSD <2%) the developed RP-HPLC method for the analysis of Loratadine (API).

Table-6.25: Result of method Robustness test

Change in parameter	% RSD
Flow (1.1 ml/min)	0.26
Flow (0.9 ml/min)	0.22
More Organic	0.76
Less Organic	0.73
Wavelength of Detection (214 nm)	0.86
Wavelength of detection (210nm)	0.81

4. LOD & LOQ:

The Minimum concentration level at which the analyte can be reliable detected(LOD) & quantified (LOQ) were found to be 0.06 & 0.18µg/ml respectively.

5. System Suitability Parameter

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. Following system suitability test parameters were established. The data are shown in Table-6.26.

Table-6.26: Data of System Suitability Parameter

S.No.	Parameter	Limit	Result
1	Resolution	Rs □ 2	9.85
2	Asymmetry	T □ 2	Loratadine=0.19
3	Theoretical plate	N □ 2000	Loratadine=2947
4	Tailing Factor	T<2	Loratadine=1.25

Table-6.27: Recovery Data for estimation Loratin in Loratadine Tablets

Brand name of Loratadine	Labelled amountof Drug (mg)	Mean (□ SD) amount (mg) foundby the proposed method (n=6)	Assay % (□ SD)
Loratin tablets (10mg) (cipla Pharma)	10mg	9.9 (□ 0.472)	99 (□ 0.384)

RESULTS AND DISCUSSION:

- To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of Loratadine, different chromatographic conditions were applied & the resultsobserved are presented in previous chapters.
- Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution.
- In case of RP-HPLC various columns are available, but here Symmetry ODS RP C_{18}, 5□m, 15mm x 4.6mm i.d. column was preferred because using this column peak shape, resolution and absorbance were good.
- Mobile phase & diluent for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal (methanol, acetonitrile, water, 0.1N NaOH, 0.1NHCl).
- The drug was found to be soluble in Methanol, Ethanol, Acetone, DMSO, DMF, and Acetonitrile. soluble in Water. Using these solvents with appropriate composition newer methods can be developed and validated.
- Detection wavelength was selected after scanning the standard solution of drug over 200 to 400nm. From the U.V spectrum of Loratadine it is evident that most of the HPLC work can be accomplished in the wavelength range of 212 nm conveniently. Further, a flow rate of 1 ml/min & an injection volume of 20µl were found to be the best analysis.
- The result shows the developed method is yet another suitable method for assay which can help in the analysis of Loratadine in different formulations.

CONCLUSION:

A sensitive & selective RP-HPLC method has been developed & validated for the analysis ofLoratadine API. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility. The result shows the developed method is yet another suitable method for assay, purity whichcan help in the analysis of Loratadine in different formulations.

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