Research Article ISSN:2321-2624

International Journal of Medicine and Pharmaceutical Research
www.pharmaresearchlibrary.com/ijmpr

A Novel validated RP-HPLC method for the estimation of "Rupatadine" in its Bulk and Pharmaceutical Dosage forms

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Received: 23 September 2014, Accepted: 24 November 2014, Published Online: 10 December 2014

Abstract

A Novel Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method for the Estimation of "Rupatadine" has been developed. The developed method was found and proved to be a better one than the already existing ones. The mobile phase used was a combination of Methanol, Acetonitrile, Water in the ratio of 60:20:20 respectively. This mobile phase was used along with 3ml of Trifluoro acetic acid buffer (0.1%v/v). Specifications of the method include XTerra C_{18} Column, Flow rate of 0.5ml/min, Run time of 10mins, ambient temperature. Symmetric peak maxima was obtained and Retention time was found to be 2.085mins. The developed method can be considered as a very Economical method as the Retention time obtained is least when compared to the previously published methods for the Estimation of Rupatadine. Problems like Fronting, Tailing, Peak asymmetry were reduced due to the addition of Trifluoro acetic acid buffer (3ml), which is a Novel approach. The developed method was validated as per ICH Guidelines. R^2 was found to be 0.999. LOD and LOQ values were calculated and found to be 2.142 and 9.92 respectively. %Recovery was found to be 100.89% and %RSD was found to be 0.2. Standard Curves were linear over the concentration range of 15-75 µg/ml. Validation results serve as an authentic proof that the developed method is Reliable. Hence, this method is considered as a Novel, Reliable and Economic method. Thus, this method can be preferred for the routine analysis of Rupatadine in bulk and Pharmaceutical dosage forms.

Keywords: Rupatadine, RP-HPLC, Method development, Validation, ICH guidelines

Contents

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Manuscript ID: IJMPR2320

PAPER-QR CODE

1. Introduction

Aim

The main objective of this study is to develop an accurate and Economical method for the estimation of "Rupatadine" through RP-HPLC. Proving that the developed method is validated according to ICH guidelines and is better in its own way when compared to the previously existing methods. A thorough literature survey has been done and a goal was set and achieved accordingly."Rupatadine" is an anti Histaminic, anti Allergic agent. It's IUPAC name is 8-chloro-6,11-dihydro-11-[1-[(5-methyl-3-pyridyl)methyl]-4-piperidylidene]-5H-benzo[5,6] cyclohepta [1,2-b] pyridine.Chemical formula is $C_{26}H_{26}ClN_3$. Present study was performed by taking active pharmaceutical ingredient of Rupatadine and also "Rupanex" tablets (10mg).

Samples

Rupatadine API was kindly provided by Bright Labs, Kothapet, Hyd. A commercial tablet formulation "Rupanex" (10mg) from Dr. Reddy's Laboratories Ltd, Hyd, India was obtained from local market.

Reagents

Methanol, Acetonitrile, Water (Merck) were used which were HPLC grade. Trifluoro acetic acid buffer was prepared with a concentration of 0.1%v/v using HPLC grade water as diluent.

Apparatus

HPLC used was Waters 2695 Separation module with PDA detector (2996 model), XTerra C_{18} (150*4.6mm), 5µ column and automatic Injector. Software used was "Empower 2".

2. Experimental

Chromatographic Conditions

This Novel method was developed using XTerra C_{18} Column (150*4.6mm). Mobile phase used was Methanol : Acetonitrile : Water in the ratio of 60 : 20 : 20 along with 3ml of 0.1%v/v Trifluoro acetic acid buffer. Mobile phase was subjected to sonication and then used. Run time was set to 10mins, Flow rate was 0.5ml/min. Injection volume was 10µl. Column was equilibrated and then the analyte was injected to obtain Retention time.

Method Validation

Linearity

A stock solution of 1000µg/ml was prepared by using the premixed mobile phase as diluent. From this stock solution, different concentrations were prepared .i.e., 15, 30, 45, 60, 75µg/ml respectively. Detection wavelength should be 244nm. Column was equilibrated with flow rate of 0.5ml/min. Prepared dilutions were injected serially. Calibration curve was plotted by taking peak area and concentration of the prepared dilutions.

Accuracy

The standard solutions of accuracy 50%, 100% and 150%were injected into chromatographic system. Calculate the amount found and amount added for Rupatadine. Calculate the individual % recovery and mean % recovery values.

Acceptance criteria

- The % recovery for each level should be between 98.0 to 102.0%.

Precision

Repeatability

Preparation of stock solution

10mg Rupatadine working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask add about 7 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette out 0.45ml of the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

Procedure

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Acceptance criteria

The % RSD for the area of five standard injections results should not be more than 2.

Intermediate Precision / Ruggedness

To evaluate the intermediate precision (also known as ruggedness) of the method, precision was performed on different days by using different make column of same dimensions.

Preparation of stock solution

10mg Rupatadine working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask add about 7ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette out 0.45ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

Procedure: The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Acceptance criteria

The % RSD for the area of five sample injections results should not be more than 2%.

Assay

$$Assay\ \% = \frac{sample\ area}{Standard\ area} \times \frac{dilution\ of\ standard}{dilution\ of\ sample} \times \frac{P}{100} \times \frac{Avg.wt}{Lc} \times 100$$

Where,

Avg.wt = average weight of tablets

P= Percentage purity of working standard

LC= Label Claim of Rupatadine mg/ml

Specificity

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by injecting blank.

Robustness

As part of the robustness, deliberate change in the flow rate, mobile phase composition was made to evaluate the impact on the method. The flow rate was varied at 0.4ml/min to 0.6ml/min. Standard solution 45ppm of Rupatadine prepared and analysed using the varied flow rates along with method flow rate. The organic composition in the mobile phase was varied from 70% to 50% standard solution 45µg/ml of Rupatadine was prepared and analysed using the varied mobile phase composition along with the actual mobile phase composition in the method.

LOD

LOD's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Formula:

$$LOD = 3.3\ X\ \frac{\sigma}{S}$$

Where,

σ - Standard deviation (SD)

S - Slope

LOQ

$$LOQ = 10X\ \frac{\sigma}{s}$$

Where,

σ - Standard deviation

S - Slope

Procedure for Pharmaceutical formulation

20Tablets (Rupanex) were weighed and powdered. Accurately weighed amount of powder equivalent to 10mg was taken.

3. Results and Discussion

Method Development

A thorough study about Rupatadine drug and its estimation was done. Various trials were performed by using Methanol, Water, Acetonitrile individually and in combination of 2 or 3. By overcoming the obtained defects, the final mobile phase selected was Methanol : Acetonitrile : Water (60:20:20) along with 3ml of Trifluoro acetic acid buffer (1%v/v). Retention time obtained was 2.085mins. Optimized conditions are as follows:

Table 1: Optimized conditions

Parameter	**Condition**
Mobile phase	Methanol: Acetonitrile: Water (60:20:20) along with 3ml Trifluoroacetic acid buffer (1%v/v)
Flow rate	0.5ml/min
Column Temperature	Ambient
Column	XTerra C_{18} (150*4.6mm)
Injection volume	10µl
Run time	10mins
Retention time	2.085mins

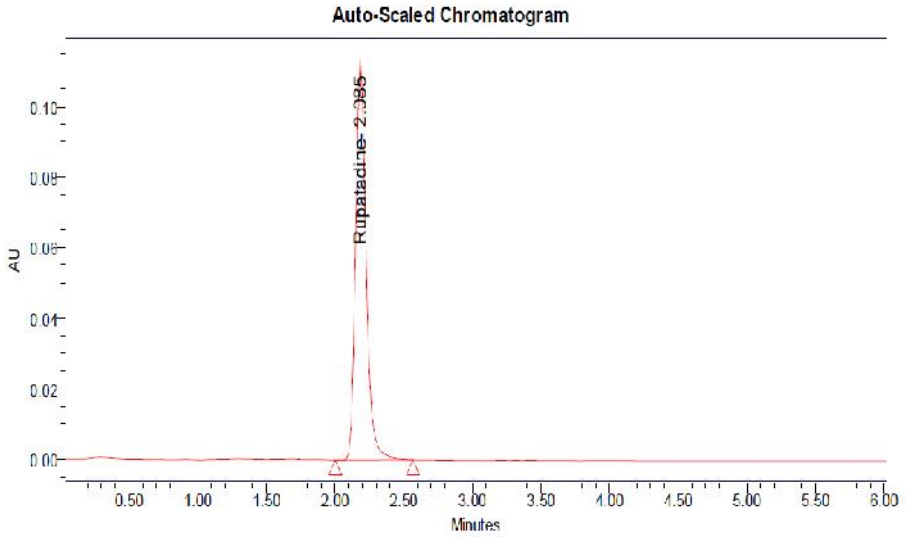


Figure 1: Optimized Chromatogram

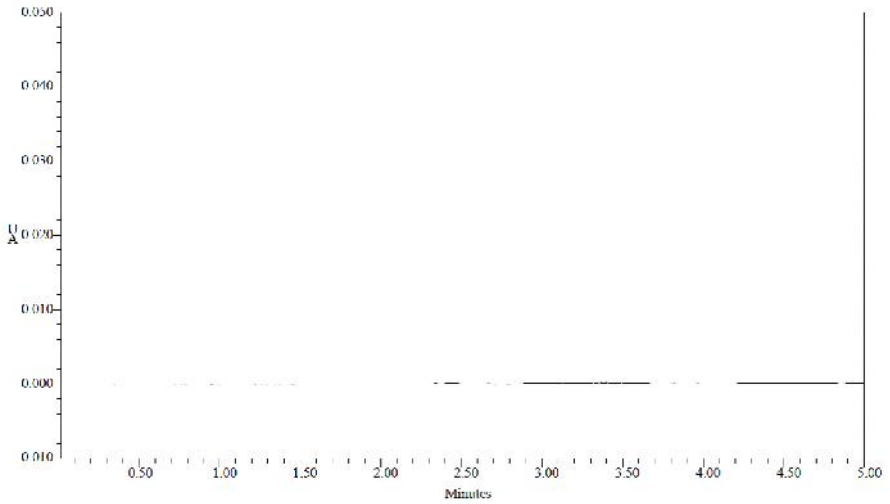
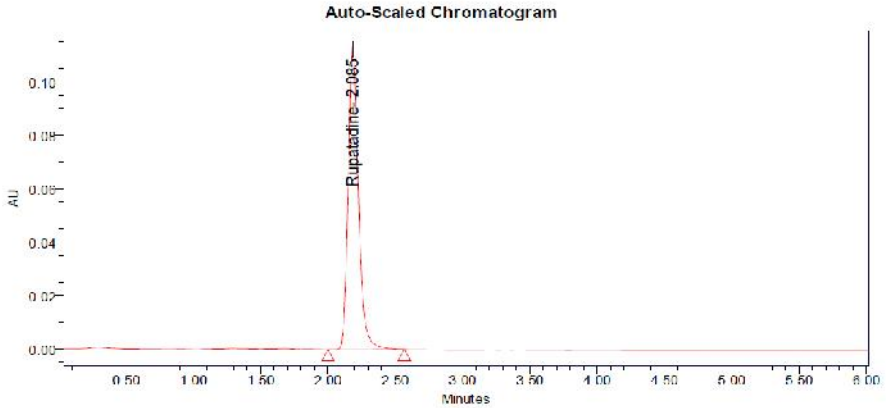


Figure 2: Chromatogram showing blank (mobile phase preparation)



Peak Results

	Name	RT	Area	Height	USP Resolution	USP Tailing	USP Plate Count
1	Rupatadine	2.085	604374	112446		1.22	3901

Figure 3: Chromatogram showing standard injection

Validation

Linearity

Calibration curve was plotted by taking Concentration on X-axis and Peak area on Y-axis. Various concentrations taken were 15, 30, 45, 60, 75µg/ml respectively. The curve obtained was linear. %RSD was calculated and was lower proving that the developed method is precise.Regression Coefficient was found to be 0.999. Low values of standard deviation, standard error, etc serve as a proof to show that the calibration plot did not deviate from linearity.

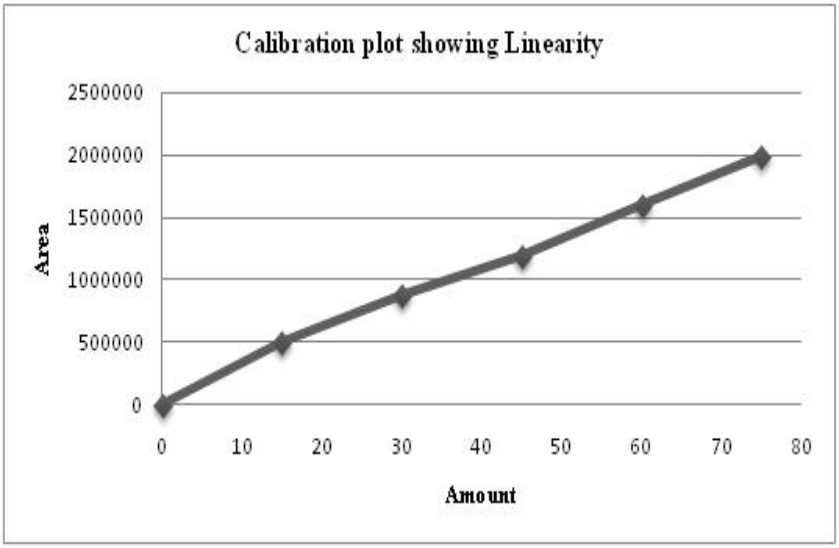


Figure 4: Calibration Plot showing Linearity

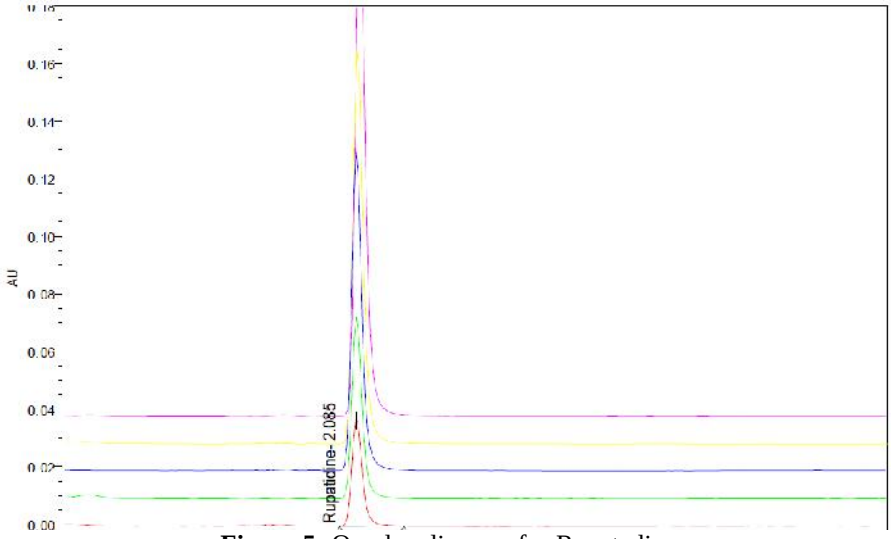


Figure 5: Overlay diagram for Rupatadine

Accuracy

Accuracy was determined by spiking 3 different concentrations .i.e., 50%, 100%, 150% and calculating the %Recovery and %RSD respectively.

Table 2: Accuracy Results

Spiked concentration	% Recovery	% RSD	Standard deviation
50%	100.2	0.2	1073.6
100%	98.8	0.3	3505.8
150%	96.5	2.0	36193.7

Precision

This includes intra-day and inter-day precision, carried out by using various concentrations in same day at different time intervals and in different days at same time interval. Determine Peak area and calculate % RSD to prove that the method is validated.

Table 3: Rupatadine Precision Results

S.No.	RT	Peak Area (µV*sec)	Height (µV)	USP Plate count	Tailing
1.	2.269	1187187	159416	2622.7	1.4
2.	2.264	1188125	161793	2758.1	1.5
3.	2.267	1189202	161854	2700.8	1.4
4.	2.270	1191196	159246	2619.9	1.5
5.	2.262	1192867	162665	2652.7	1.4
Mean		1189715.3		2670.8	1.4
S.D.		2308.1			
%RSD		0.2			

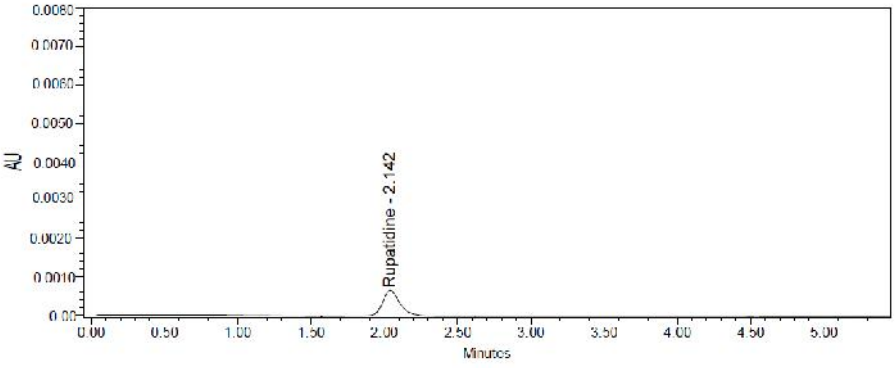
Intermediate Precision

Precision of the method was calculated by using different column by different analyst. Both intra day and inter day precision was determined. % RSD has no much variance. Hence, the developed method is said to be Reproducible.

Table 4: Intermediate Precision Results

S.No.	RT	Area (µV*sec)	Height (µV)	USP Plate count	Tailing
1	2.262	1192194	165295	2731.9	1.4
2	2.262	1192990	166061	2790.9	1.4
3	2.262	1193772	166385	2877.3	1.4
4	2.260	1194120	165951	2846.7	1.4
5	2.261	1197708	164956	2821.3	1.5
Mean		1194156.9		2813.6	1.4
Std.Dev		2119.9			
%RSD		0.2			

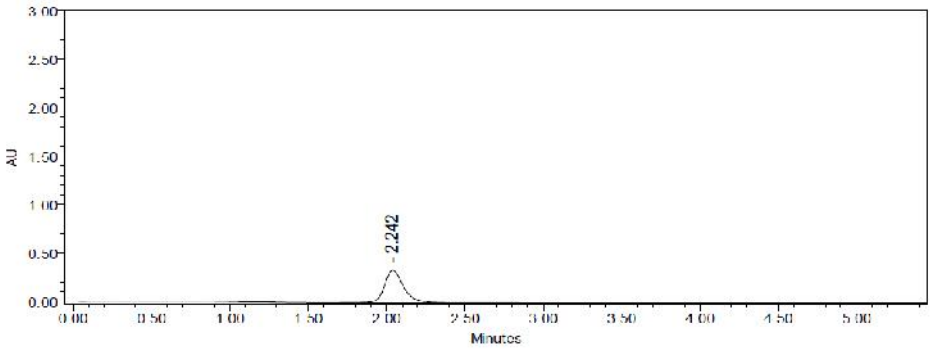
Limit of Detection (LOD) and Limit of Quantification (LOQ)



	Peak Name	RT	Area	Height	Injection	USP Plate Count	USP Tailing	Symmetry Factor
	Rupatidine	2.142	2809748	337063	1	1499	1.26	1.26

Figure 6: Chromatogram showing Limit of detection

The LOD was performed for Rupatadine was found to be 2.142



Peak Name	RT	Area	Height	Injection	USP Plate Count	USP Tailing	Symmetry Factor
Rupatidine	2.242	2809748	337063	1	1499	1.26	1.26

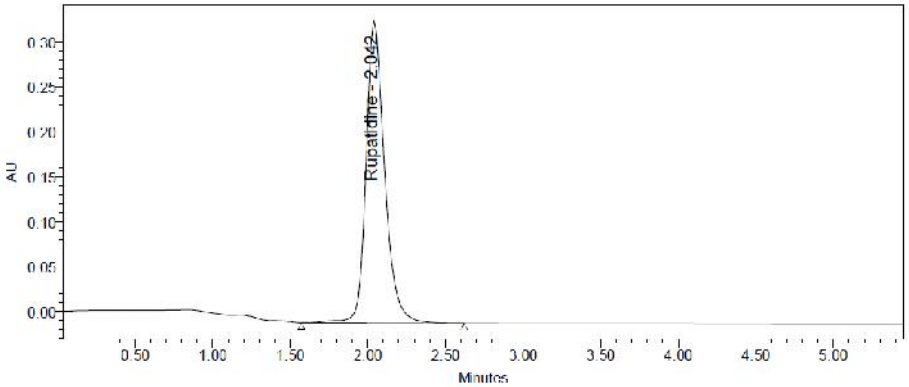
Figure 7: Showing results for Limit of Quantification

Table 5: Rupatadine results for Limit of Quantitation

Standard Deviation	Slope (S)	LOQ (µg)
371827.9	563365963	9.92

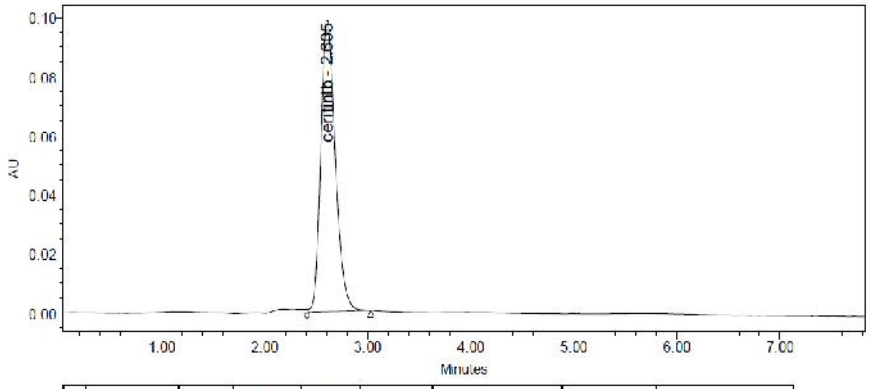
The LOQ was performed for Rupatadine was found to be 9.92.

Robustness: The method is said to be Robust, if the obtained %RSD values are low.



Peak Name	RT	Area	Height	Injection	USP Plate Count	USP Tailing	Symmetry Factor
Rupatidine	2.042	2609748	337063	1	2499	1.26	1.26

Figure 8: Chromatogram showing less flow rate 0.4ml/min

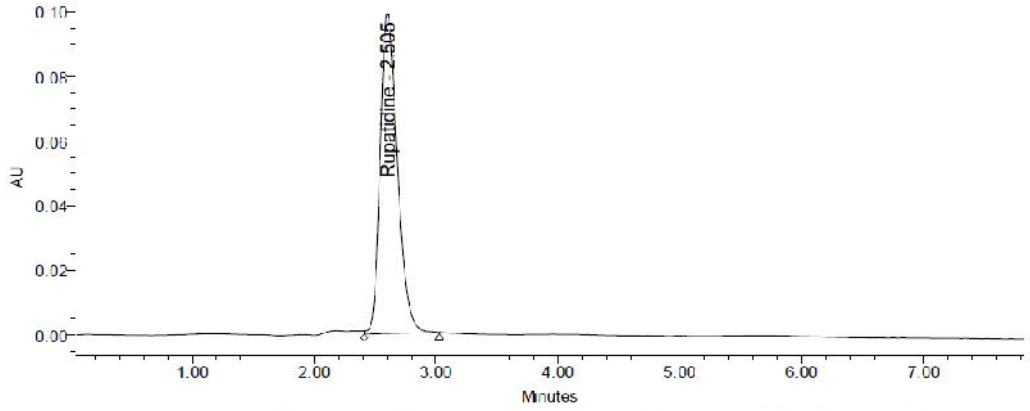


Peak Name	RT	Area	Height	Injection	USP Plate Count	USP Tailing	Symmetry Factor
Rupatidine	2.605	964188	99137	1	3659	1.29	1.29

Figure 9: Chromatogram showing more flow rate 0.6ml/min

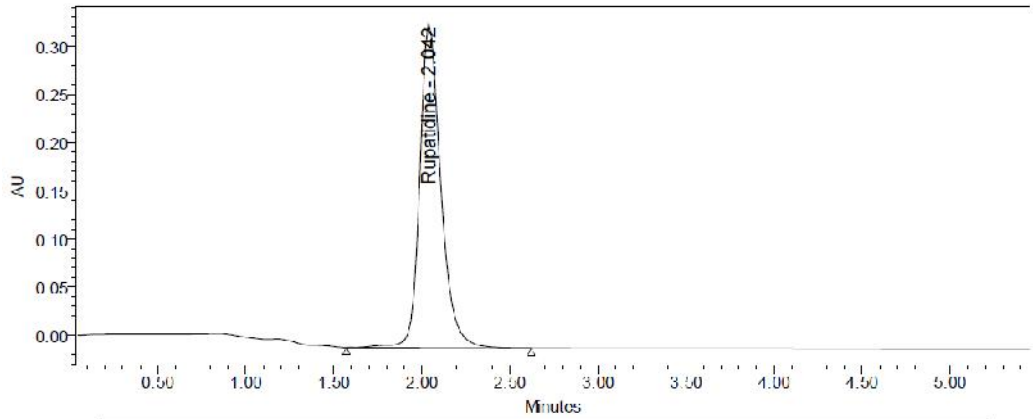
Table 6: Showing Robustness results for Rupatadine

S.No	Flow rate (ml/min)	Plate Count	Tailing
1.	0.4	2499	1.26
2.	0.5	3901	1.22
3.	0.6	3659	1.29



Peak Name	RT	Area	Height	Injection	USP Plate Count	USP Tailing	Symmetry Factor
Rupatidine	2.505	964188	99137	1	3659	1.29	1.29

Figure 10: Chromatogram showing more organic phase ratio



Peak Name	RT	Area	Height	Injection	USP Plate Count	USP Tailing	Symmetry Factor
Rupatidine	2.042	2809748	337063	1	2499	1.26	1.26

Figure 11: Chromatogram showing less organic phase ratio

Table 7: Showing system suitability results

S.No	% Change in organic phase	Plate Count	Tailing
1.	10% less	3659	1.29
2.	ACTUAL	3901	1.22
3.	10% more	2499	1.26

Analysis of Rupatadine Pharmaceutical formulation

The developed method was applies to Rupanex (10mg) tablets and mean recovery was found to be 100.2%

4. Conclusion

A Novel RP-HPLC method for the determination of Rupatadine has been developed, which was found to be Economical, Accurate and Reliable when compared to already existing ones. According to the statistical analysis performed, the developed method is said to be validated and can be preferred for regular analysis of Rupatadine and its formulation.

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