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Development of a Stability Indicating RP-HPLC Method for Determination of Rupatadine Fumarate in Tablet Dosage Form and its Validation.

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ABSTRACT

A simple, precise, cost effective stability indicating RP-HPLC method has been developed and validated for the determination of Rupatadine Fumarate in pharmaceutical tablet. The developed method gave discrete identification and determination of rupatadine in presence of degradant products under a variety of stress conditions. A Spherisorb CN, 250 x 4.6mm, 5µ (Water, Ireland) was used for chromatographic separation. The mobile phase comprised of a mixture of Phosphate buffer pH 4.4 and acetonitrile in the ratio 40:60 at a flow rate of 1.5 ml / min, which was filtered and degassed prior to use. The detection was performed at 242 nm using a PDA detector. Subjecting the drug solution to stress degradation proved that the drug was susceptible to acid-base hydrolysis, hydrogen peroxide thermal and UV degradation. The degradants were well separated from its active pharmaceutical ingredient. The method was also validated according to ICH guidelines. Hence, the developed method was found to be both specific and stability indicating.

Keywords: RP-HPLC, Stability indicating, ICH guidelines, Validation

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INTRODUCTION

Rupatadine Fumarate is a second generation, non-sedating, long acting histamine antagonist with selective peripheral H_1 receptor antagonist, used for the treatment of allergies. The drug is off white to pinkish crystalline powder and has a molecular formula of $C_{26}H_{26}ClN_3.C_4H_4O_4$, molecular weight 532.03 g / mol. It is soluble in methanol and ethanol, very slightly soluble in chloroform and insoluble in water [1]. The chemical structure of structure of Rupatadine Fumarate is shown in Figure 1.

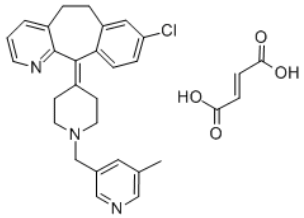

Figure 1: Chemical structure of Rupatadine Fumarate

Literature survey revealed some analytical methods for determination of rupatadine. Extractive spectroscopic method is reported for rupatadine in combination with loratadine [2]. There are reports on UV [3, 4], HPLC [5, 6] and HPTLC [7] methods for determination of rupatadine fumarate in bulk and pharmaceutical dosage form. An HPLC-MS method is reported for determination of Rupatadine in human plasma [8]. Literature survey presented the need for developing a stability indicating HPLC method in accordance with ICH guidelines [9], for determination of Rupatadine Fumarate in pharmaceutical dosage form. Some review articles have given an insight on performing stress degradation studies [10]. The method was validated in accordance with ICH Q2 (R1) [11].

EXPERIMENTAL

Materials

The API was obtained as a gift sample from Hetero healthcare Pvt. Ltd. Andheri East, Mumbai, Maharashtra. Rupatadine tablets (Rup AL 10 mg) were procured from Hetero healthcare Pvt. Ltd. Andheri East, Mumbai, Maharashtra. Solvents required for method development included Potassium di hydrogen orthophosphate, acetonitrile (HPLC grade), Ranbaxy, HPLC grade water was obtained from Milli Q purification system. All other chemicals were of analytical grade (Merck).

Instrumentation

Chromatographic separation was carried out on Shimadzu LC - 10ATvp, Japan and Agilent-HP1100 series with DAD detector and Chemstation software (Germany).

Chromatographic Conditions

A Spherisorb CN, 250 x 4.6mm, 5µ (Water, Ireland) was used for chromatographic separation. The mobile phase comprised of a mixture of Phosphate buffer pH 4.4 and acetonitrile in the ratio 40:60 at a flow rate of 1.5 ml / min, which was filtered and degassed prior to use. The detection was performed at 242 nm using a PDA detector and the injection volume was 50 µL.

Preparation of Standard Stock Solution

About 25mg of Rupatadine Fumarate working standard was accurately weighed and transferred to a 50 ml volumetric flask diluted up to the mark with mobile phase (Phosphate buffer pH 4.4 and acetonitrile in the ratio 40:60) was mixed. The resultant solution was sonicated and further diluted to obtain a working concentration.

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Sample Preparation

Around 20 tablets of Rupatadine Fumarate were weighed and powdered. About 25 mg of weight equivalent of powder was transferred to a 50 ml volumetric flask. About 30 ml of mobile phase was added and sonicated to disperse the tablet powder completely and diluted up to the mark with mobile phase. Further dilutions were made to obtain the working concentration of the solution.

Procedure

HPLC system was set as described under chromatographic conditions. Standard and sample solutions were prepared, injected and mean area counts for each sample was calculated. The drug solution was subjected to various stress conditions and further the method was also validated. The results are given below.

RESULTS AND DISCUSSION

Optimization of Chromatographic Conditions

During method development following attempts was made with respect to stationary phase, mobile phase and wavelength optimization.

Selection of Column

Two different columns were tried, as mentioned in Table 1.

The chromatogram is shown in Figure 2 and parameters are summarized in Table 2 and 3.

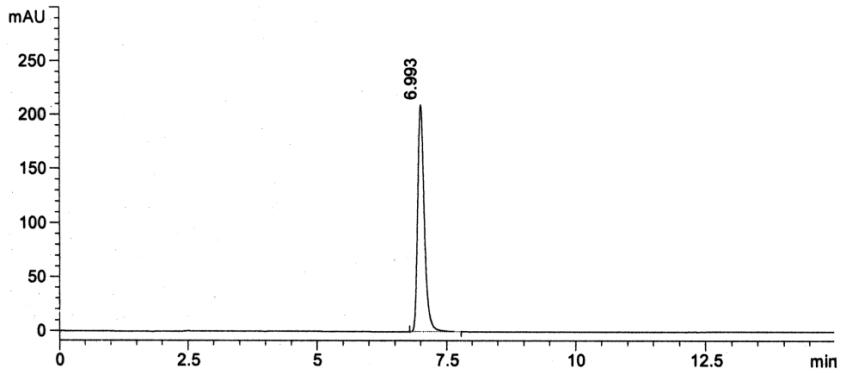

Figure 2: Separation on Spherisorb CN (Cyano), 250 x 4.6mm, 5µ (pH 4.4).

Table 1: Specifications of the columns used during method development

Column Type	Specifications
Spherisorb CN (Cyano)	250 x 4.6mm, 5µ (Water, Ireland)
Luna C_8 (Octylsilane)	250 x 4.6mm, 5µ (Phenomenax, USA

Table 2: Spherisorb CN (Cyano), 250 x 4.6mm, 5µ (pH 4.4)

Drug	R.T. Min.	Area	% Area	Tailing Factor	Theoretical Plates	Purity factor
Rupatadine Fumarate	6.99	1891.5	98.97	1.14	14,919	999.670

Table 3: Luna C_8 (Cyano), 250 x 4.6mm, 5µ (pH 4.4)

Drug	R.T., Min.	Area	% Area	Tailing Factor	Theoretical Plates	Purity factor
Rupatadine Fumarate	2.24	1877.6	98.74	1.18	7188	981.807

The instrument operating conditions and mobile phase composition was kept the same for both columns. Looking at the chromatogram and relevant data it was found that the spherisorb CN column gave good peak response and theoretical plates, affordable retention time with higher peak purity. Whereas, the Luna C_8 column did give good peak response with lower retention time but the number of theoretical plates and peak purity were low.

Selection of Mobile Phase

An additional mobile phase in the ratio of 0.02 M NaH_2PO_4 (pH 5.4 with dilute NaOH solution): Acetonitirile :: 80:20 at a flow rate of 1.2 ml/minute using Spherisorb CN column, 250 x 4.6mm, 5µ, Water (Ireland) at constant temperature of about 30°C at λ 305 nm was attempted. The result obtained is studied in the same manner as earlier. The resolution and theoretical plates for Rupatadine Fumarate was poor but peak purity of Rupatadine Fumarate was good. Table 4 shows the peak report on changing the mobile phase composition.

Table 4: Peak report on Spherisorb CN (Cyano), 250 x 4.6mm, 5µ (pH 4.4), with 0.02 M NaH_2PO_4 (pH 5.4 with dilute NaOH solution): Acetonitirile :: 80:20

Drug	R.T Min	Area	% Area	Tailing Factor	Theoretical Plates	Purity factor
Rupatadine Fumarate	21.06	1744.0	97.80	2.49	10097	999.687

Selection of detection wavelength

Wavelength, 242 nm is selected because the drug, Rupatadine Fumarate produced maxima at that wavelength.

Identification

The retention time of the major peak in the chromatogram of sample preparation corresponds to that of Rupatadine Fumarate in standard preparation. Hence identity of drug is confirmed.

Forced Degradation Studies

Control Sample

Twenty tablets of Rupatadine Fumarate were weighed and powdered. About 25 mg of weight equivalent of powder was transferred to a 50 ml volumetric flask. About 30 ml of mobile phase was added and sonicated to disperse the tablet powder completely and diluted up to the mark with mobile phase. Further dilutions were made to obtain the working concentration of the solution. The control sample is the one to which no stress conditions were applied.

Acid degradation

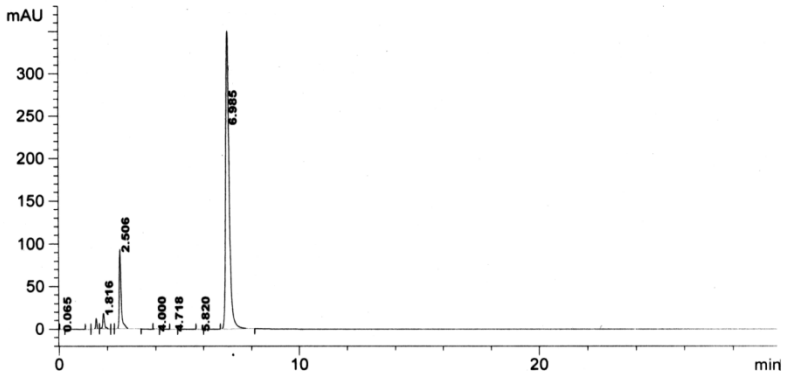

Figure 3: Chromatogram of acid degraded sample of Rupatadine Fumarate

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About 25 mg of weight equivalent of powder obtained from the triturated tablet was transferred to a 25 ml volumetric flask. To it, 25 ml of mobile phase and 5 ml of 1 N HCl was added and sonicated for 10 minutes. The sample was heated on a boiling water bath for 10 minutes, cooled to room temperature, diluted to volume with mobile phase and mixed well. The acidic forced degradation was performed in the dark in order to exclude the possible degradative effects of light. The solution was injected into the HPLC system. Figure 3 shows the chromatogram obtained.

Alkali degradation

About 25 mg of weight equivalent of powder obtained from the triturated tablet was transferred to a 25 ml volumetric flask. To it, 25 ml of mobile phase and 5ml of 1N NaOH was added and sonicated for 10 minutes. The contents were heated on a boiling water bath for 10 minutes, and cooled to room temperature, diluted to volume with mobile phase, mixed well. The alkaline forced degradation was performed in the dark in order to exclude the possible degradative effects of light. The solution was injected into the HPLC system. Figure 4 shows the chromatogram obtained on performing alkali degradation.

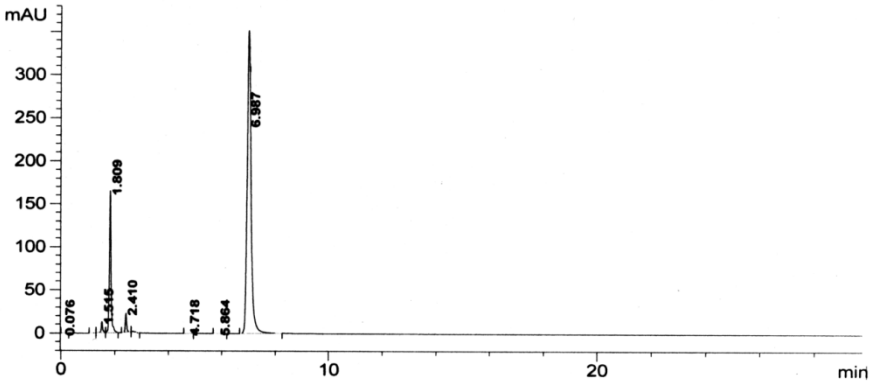

Figure 4: Chromatogram of alkali degraded sample of Rupatadine Fumarate

Peroxide degradation

About 25 mg of weight equivalent of powder obtained from the triturated tablet was transferred to a 25 ml volumetric flask containing 3.0 % H_2O_2. To it, 25 ml of mobile phase and 5 ml of 3 % H_2O_2 was added and sonicated for 10 minutes. The contents were heated on a boiling water bath for 10 minutes, and cooled to room temperature, diluted to volume with mobile phase, mixed well. The solution was injected into the HPLC system. Figure 5 shows the chromatogram.

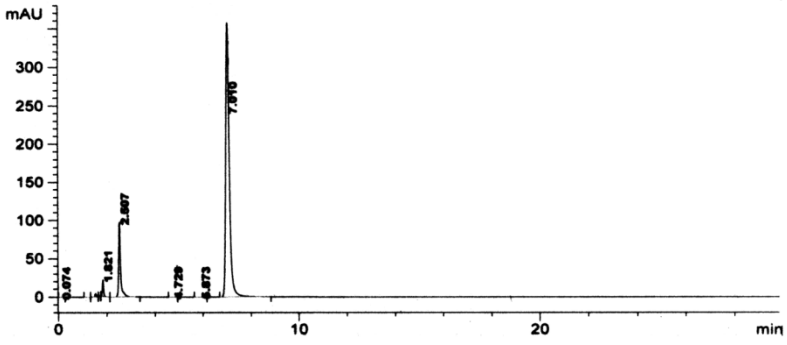

Figure 5: Chromatogram of peroxide degraded sample of Rupatadine Fumarate

UV degradation

About 25 mg of weight equivalent of powder obtained from triturated tablet (previously kept in UV light for 24 hours) was transferred to 50 ml volumetric flask. To it, 30 ml of mobile phase was added and

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sonicated for 10 minutes. The volume was made with mobile phase, mixed and was injected into the HPLC system. Figure 6 shows the chromatogram obtained.

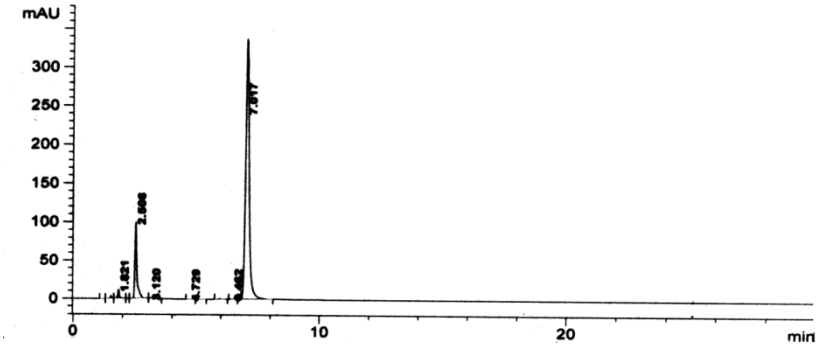

Figure 6: Chromatogram of UV degraded sample of Rupatadine Fumarate

Thermal degradation

About 25 mg of weight equivalent of powder obtained from triturated tablet was transferred to a 25 ml volumetric flask containing 30 ml of mobile phase and sonicated for 10 minutes. The contents were heated on a boiling water bath for 30 minutes, and cooled to room temperature, and volume was made up with mobile phase, mixed well. The solution was injected into the HPLC system. Figure 7 shows the chromatogram obtained.

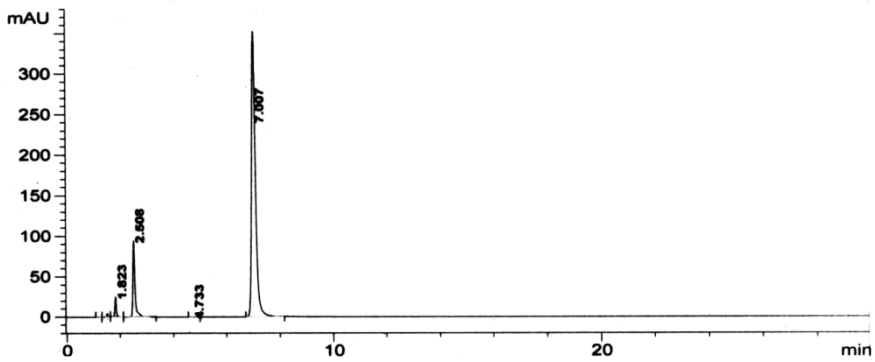

Figure 7: Thermal degraded sample of Rupatadine Fumarate

The control sample was evaluated relative to the standard concentration where as rest of the stressed condition samples (Sr.No.2 to 6) were evaluated relative to the control sample with respect to the % assay and % degradation. The percentage degradation results were calculated by area normalization method. The data obtained after performing degradation studies and studying its effects on determination of Rupatadine Fumarate and its degradants in pharmaceutical dosage form is given in Table 5.

Table 5: Stressed study data of Rupatadine Fumarate

Sr. No.	Stress Condition	% Assay	% Degradation	
			Single maximum	Total
1.	Control sample	98.02	Nil	Nil
2.	Acid	98.19	0.05	0.09
3.	Alkali	98.77	0.04	0.07
4.	H_2O_2	100.32	0.03	0.05
5.	UV	96.22	0.25	0.49
6.	Thermal	99.07	0.03	0.03

The chromatogram of the control sample did not show any additional peaks whereas, additional peaks at RT of 4.0 min, 4.72 min and 5.82 min were observed in the chromatogram obtained after subjecting the sample to acid degradation. The chromatogram of alkali degraded sample showed additional peaks at RT of 4.72 min and 5.86 min. The chromatogram of thermal degraded sample showed additional peaks at RT of

2.8 min, and 4.7 min. The chromatogram of hydrogen peroxide degraded sample showed additional peaks at RT of 4.73 min and 5.87 min. The chromatogram of UV degraded sample showed additional peaks at RT of 3.12 min, 4.74 min, 5.94 min and 6.47 min. Rest of the peaks, if any, were from its blank or placebo in each of these specified conditions. In each forced degradation samples where additional peaks were observed the response of the drug was changing from the initial control sample. In each of the stress conditions, the peak purity of Rupatadine Fumarate peak as determined by diode array detector was greater than 980.

Validation Studies

Linearity and range

The linearity of response for Rupatadine Fumarate assay method was determined by preparing and injecting solutions with concentrations of about 52.20 to 156.60 µg/ml of Rupatadine Fumarate using working standards. The results are shown graphically in Figure 8 and the mean area counts of linearity graph are given in Table 6.

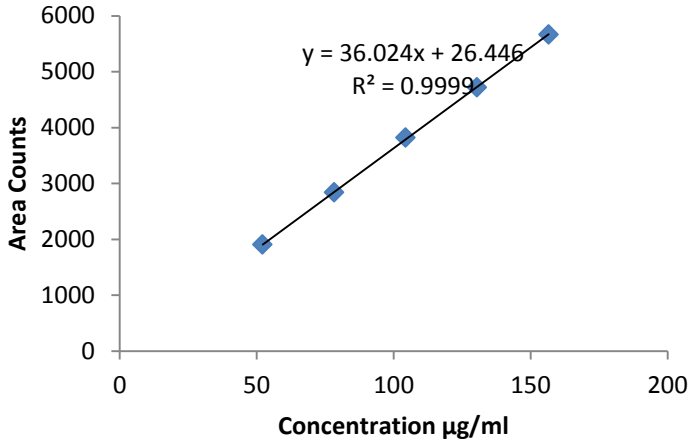

Figure 8: Linearity and Range of Rupatadine Fumarate

Table 6: Linearity and Range of Rupatadine Fumarate

Concentration (µg/ml)	Mean Area Counts
52.20	1903.085
78.30	2836.885
104.40	3815.855
130.50	4716.295
156.60	5664.47
Slope	36.02
Intercept	26.45
Correlation Coefficient (r)	0.9999

Specificity

Specificity of the method was established by demonstrating

No interference from blank

This was demonstrated by injecting a mobile phase as blank and then injecting a standard preparation as described under chromatographic condition. There was no peak at the retention time of Rupatadine Fumarate thereby indicating that there was no interference from the blank.

No interference from degradation products

This was demonstrated by carrying out forced degradation of the sample with 1 N HCl, 1 N NaOH, treating with 3.0% H_2O_2, heating on boiling water bath for 30 minutes and keeping under UV light for 24 hrs. The samples were prepared as per sample preparation for assay method and injected into the HPLC system having an HP 1100 series diode array detector. In each case, % peak purity of Rupatadine Fumarate peaks was determined to examine interference from degradation products.

Method Precision

Precision was determined through the estimate of the relative standard deviation (RSD) values. The precision studies were done by injecting the prepared standard solution at three different concentration levels in triplicate by injecting the standards at three different times on the same day. Results of this analysis are shown in Table 7.

Table 7: Method precision of Rupatadine Fumarate

Sample Preparation	% Assay Rupatadine Fumarate	% Deviation From Mean Assay value Rupatadine Fumarate
1	99.79	1.80
2	97.86	-0.16
3	98.04	0.02
4	97.26	-0.78
5	97.26	-0.77
6	97.92	-0.11
Mean	98.02	
± SD	0.93	
%RSD	0.95	

Accuracy

Accuracy (Recovery) study was performed by spiking 30%, 50% and 70% of Rupatadine Fumarate working standard to a pre-analyzed sample. The pre-analyzed sample was weighed in such a way that final concentration is half or 50% of the sample preparation before spiking. The percentage sum level of pre-analyzed sample and spiked amount of drug was 80%, 100% and 120% of simulated dosages nominal or target concentration of sample preparation. The accuracy of the analytical method was established in triplicate across its range according to the assay procedure. The results of accuracy are shown in Table 8.

Table 8: Accuracy of Rupatadine Fumarate

Sample Preparation	% Simulated Dosage Nominal	% Sum Level	% Amt. Recovered	% Recovery
Pre-analyzed sample				97.4733
1	80	80.46	81.18	100.9
2	80	79.98	81.19	101.52
3	80	80.24	81.09	100.57
1	100	100.9	101.52	100.61
2	100	101.08	101.5	100.41
3	100	101.26	101.6	100.34
1	120	121.24	121.85	100.5
2	120	121.04	121.7	100.54
3	120	120.96	121.66	100.58
Mean				100.66
±Standard Deviation				0.36
% Relative Standard Deviation				0.35

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Solution Stability

This was evaluated by injecting initially a freshly prepared standard and sample solutions and subsequently injecting the same at different time intervals. The peak response data of standard and sample is shown in Table 9.

Table 9: Solution stability of Rupatadine Fumarate

Time (Hrs)	Area Counts		% Deviation From Initial Area Counts	
	Standard	Sample	Standard	Sample
Initial	3810.44	3585.07	0.0	0.0
3	3819.08	3590.72	0.2	0.2
9	3835.64	3603.85	0.7	0.5
13	3848.89	3606.55	1.0	0.6
17	3844.38	3613.26	0.9	0.8
22	3850.44	3613.14	1.0	0.8

Ruggedness and Robustness

Method robustness and ruggedness was determined by analyzing same sample at normal operating conditions and also by changing some operating analytical conditions such as column make, mobile phase composition, pH, flow rate, instrument and analyst. The parameters and results are summarized below in Table 10.

Table 10: Robustness and Ruggedness of Rupatadine Fumarate

Chromatographic changes		
Factors	**Assay**	**% Deviation**
Column Make		
Spherisorb CN, 250x4.6mm, 5µ (Waters, Ireland)	98.02	0.0
Lichrosphere CN, 5µ, 250x4mm (Merck, Germany)	98.07	0.05
Mobile Phase Composition (Buffer:Acetonitrile)		
Buffer (pH 4.4) : Acetonitrile 30 : 70	98.02	0
Buffer (pH 4.4) : Acetonitrile 25 : 75	98.32	0.31
Flow Rate		
1.0 mL/min.	98.11	0.09
1.2 mL/min.	98.02	0
1.4 mL/min.	98.56	0.55
Pump		
Aglient 1100 series	98.02	0
Shimadzu 2010	97.97	-0.05
Detector		
Aglient 1100 series, DAD	98.02	0
Shimadzu 2010	98.14	0.12
Analyst		
Analyst 1	98.02	0
Analyst 2	98.45	0.44

CONCLUSION

From the above results with respect to variation in column and variation in mobile phase, Spherisorb CN (Cyano) column and the mobile phase as mentioned in chromatography condition was considered as the most suitable column for the validation and optimization, keeping in view the overall aim of a better resolution, faster analysis and better sensitivity. The stress study data indicates that the drug is susceptible to acid-base hydrolysis degradation, hydrogen peroxide degradation, thermal and UV degradation. The lower RT

of the degraded components indicates that they were more polar than the analyte itself whereas higher RT of the degraded components indicates that they were less polar than the analyte. The degradants are separated from its active pharmaceutical ingredient. Hence, the developed method was found to be both specific and stability indicating.

Validation studies results indicate that the response is linear over the range 52.20 to 156.60 µg/ml for Rupatadine Fumarate with coefficient of regression, R^2, value as 0.999.

Specificity studies show that a developed method was specific as there was no interference of the excipients and the degradation products.

The % relative standard deviation for precision studies for Rupatadine Fumarate was 0.95 %. This shows that precision of the method is satisfactory as % relative standard deviation (%RSD) is not more than 2.0%.

The recovery studies indicate that the individual recovery of Rupatadine Fumarate ranges from 100.4% to 101.5 % with mean recovery of 100.66 % and % relative standard deviation of 0.41%. The recovery of Rupatadine Fumarate by proposed method is satisfactory as % relative standard deviations is not more than 2% and mean recovery is between 100.4 % - 101.5 %.

The % deviation for solution stability studies of Rupatadine Fumarate standard and sample is 1.0 % and 0.8 % respectively for 22 hrs from initial response. This indicates that Rupatadine Fumarate is stable in analytical solution and samples need not be injected immediately after preparation as % deviation from initial area count is less than 2.0%.

The deliberate a forementioned changes in parameters for ruggedness and robustness alters the result of Rupatadine Fumarate to 0.03% to method precision study, which is not a significant change. The robustness and ruggedness of the method was established as the % deviation from mean assay value obtain from precision study is less than 2.0%.

Hence, it can be concluded that the developed method is simple, linear, specific, precise, accurate and rugged and can be employed in routine analysis of Rupatadine Fumarate tablets.

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