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Reversed Phase High Pressure Liquid Chromatography Technique fo Validation of Rupatadine Fumarate from Active Pharmaceutical Ingredient

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ABSTRACT

A simple, rapid and accurate high performance liquid chromatography method is described for determination of rupatadine fumarate from its active pharmaceutical ingredients. The separation of drug was achieved on Inertsil ODS-3 C18 (250 X 4.6 mm) 5µ column. The mobile phase was a mixture of acetonitrile and methanol (80:20 v/v) : buffer of pH 4.5 [70:30 % v/v]. The buffer of pH 4.5 was prepared from 0.01 M ammonium acetate and pH was adjusted with dilute acetic acid. The detection was carried out at wavelength 250 nm. The mixture of water: acetonitrile (30:70 % v/v) was used as a diluent. The method was validated for system suitability, linearity, accuracy, precision, robustness, stability of sample solution. The method has been successfully used to analyze rupatadine fumarate from pharmaceutical formulation.

Keywords: Rupatadine fumarate, Ammonium acetate, Acetic acid, Acetonitrile, Reversed phase HPLC, Methanol.

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INTRODUCTION

In this communication the present work proposes a new reversed phase HPLC method for assay of rupatadine fumarate from its active pharmaceutical ingredients. Its chemical name is 8-chloro-6,11- dihydro-11- [1-[(5-methyl- 3-pyridinyl)methyl]- 4-piperidinylidene]- 5H-benzo [5,6] cyclohepta [1,2-b]pyridine fumarate. Rupatadine fumarate is a second generation of antihistamine and platelet activity factor (PAF) antagonist used to treat allergies. It was discovered and developed by J.Urich y Cia, S. A.[1] Rupatadine possesses anti-allergic properties such as the inhibition of the de-granulation of mast cells induced by immunological and non-immunological stimuli and inhibition of the release cytokines, particularly of the TNF in human mast cell.[2] Literature survey reveals the Spectrophotometric [3-5] titration [6,7] , HPLC[8-13] methods have been reported for the estimation of rupatadine fumarate. A new, simple, rapid and reliable HPLC method is developed for the determination of rupatadine fumarate. This method can be used for the routine analysis. In the proposed method optimization and validation of this method are reported.

MATERIALS AND METHODS

Chemical and reagents

Reference standard of rupatadine fumarate was obtained from reputed firm with certificate of analysis. Ammonium acetate, acetic acid were used of analytical grade and the HPLC grade water was used from Merck. Standard and sample solutions were prepared in diluent such as water: acetonitrile (30:70% v/v).

Instrumentation

The HPLC system, Water Alliance (2695) HPLC system equipped with separation module and DAD detector (2996), was used. The chromatogram was recorded and peak quantified by mean of PC based Empower software.

A SHIMADZU analytical balance (0.01 mg) was used.

Preparation of standard solution

Standard solution

A 25 mg of standard rupatadine fumarate was weighted accurately and transferred in 25 ml volumetric flask. About 15 ml of diluent [water: acetonitrile (30:70 % v/v)] was added and sonicated for 5 minutes. The volume was adjusted up to the mark with diluent to give concentration as 1000 µg /ml. The working standard solution was prepared by diluting 5 ml of 1000 µg /ml solution to 25 ml with diluent to get concentration 200 µg /ml.

Sample preparation

Twenty tablets were weighed accurately and average weight of each tablet was determined. About 25 mg of rupatadine fumarate sample was weighted accurately and transferred in 25 ml volumetric flask. About 15 ml of diluent [(water: acetonitrile (30:70 % v/v)] was added and sonicated for 5 minutes. The volume was adjusted up to the mark with diluent to give concentration as 1000 µg /ml. The sample solution was prepared by diluting 5 ml of 1000 µg /ml solution to 25 ml with diluent to get concentration 200 µg /ml.

Chromatographic condition

Chromatographic separation was performed at 30°C temperature on a reverse phase Inertsil ODS-3 C18 (250 X 4.6 mm) 5µ column. The mobile phase was a mixture of acetonitrile and methanol (80:20 v/v) : buffer of pH 4.5 [70:30 % v/v]. The buffer of pH 4.5 was prepared from 0.01 M ammonium acetate and pH was adjusted with dilute acetic acid. The flow rate of the mobile phase was adjusted to 1.0 ml /min. The detection was carried out at wavelength 250 nm. (Figure. 1) The injection volume of the standard and sample solution was set at 20 µl.

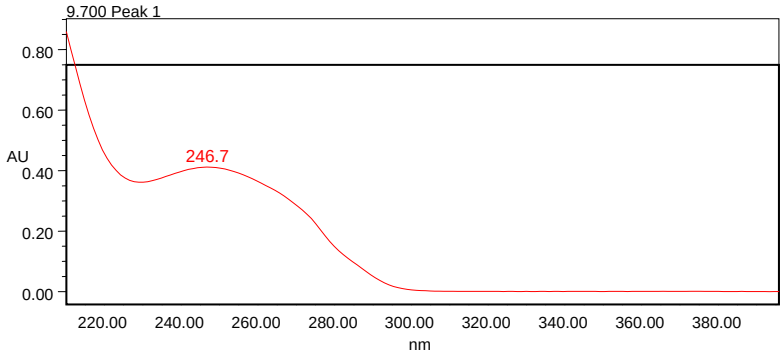

Figure 1: UV spectra of rupatadine fumarate

METHOD VALIDATION

System suitability

System suitability parameter is established to ensure that the validity of the analytical method is maintained whenever used. Typical variations are the stability of analytical solution, different equipment and different analyzer. In case of liquid chromatography typical variations are the pH of solution, the mobile phase composition, different columns, variation in temperature and flow rate.

Specificity

Specificity is the ability of the method to resolve the active ingredients. Hence blank (water: acetonitrile (30:70)), rupatadine fumarate were injected separately to prove specificity. The

typical chromatogram of the standard and sample assayed was given in figure 2 and 3 respectively.

Linearity

Under the experimental conditions described above, statistical evaluation of the data subjected to linearity are given in table 1. Linear calibration curve (Figure. 4) were obtained throughout the concentration range studied. Regression analysis was done on the peak area (y) v/s concentration (x). The statistical evaluation of the data subjected to regression analysis is tabulated in table 2.

Accuracy

The accuracy of method was determined by applying proposed method to synthetic mixture containing known amount of drug corresponding to 50 %, 100 % and 150 %. The accuracy was then calculated as the percentage of analyte recovered by the assay. The results of the recovery analysis are enclosed under table 3.

Precision

The method precision was established by carrying out the analysis of rupatadine fumarate. The assay was carried out of the drug using analytical method in six replicates. The value of relative standard deviation lies well with the limits (0.10 %). The results of the same are tabulated in the table 4.

Robustness

The robustness of the method was determined to check the reliability of an analysis with respect to deliberate variations in method parameters.

The typical variations are given as below:

Variation in the flow rate by $\pm$ 0.2 ml /min

Variation in wavelength by $\pm$ 5 nm

Variation in pH of buffer by $\pm$ 2 units

The results of the analysis of samples under the conditions of above variation indicated the nature of robustness of the method. Table 5(a, b and c)

Method application

Twenty tablets were weighed accurately and average weight of each tablet was determined. Powder equivalent to 25 mg of rupatadine fumarate sample was weighted accurately and transferred in 25 ml volumetric flask. About 15 ml diluent [water: acetonitrile (30:70)] was added and sonicated for 5 min to dissolve it. Further volume was made up to the mark with diluent to give 1000 µg /ml. Further 5 ml of this solution was diluted to 25 ml with diluent to give 200 µg /ml of rupatadine fumarate. From this solution 20 µl solution was injected under

specific conditions. The analyte peak was identified by comparison with that of respective standard. The percentage (%) assay results were expressed in table 4. It indicates the amount of rupatadine fumarate in the product meets the requirement.

RESULT AND CONCLUSION

The Parameters like theoretical plates (N), tailing factor, and relative standard deviation were determined. The results are shown in table 6 which indicates good performance of the system.

The reproducibility, repeatability and accuracy of the proposed method were found to be satisfactory which is evidenced by low values of standard deviation and percent relative standard deviation. The accuracy and reproducibility of the proposed method were confirmed by recovery experiments, performed by adding known amount of the drug to the pre-analyzed active pharmaceutical ingredient and reanalyzing the mixture by proposed method. In specificity no interference was observed from placebo with respect to rupatadine peak.

In Linearity correlation coefficient is found 0.999. [Figure. 4 and table 1,2]

Table 1: Statistical evaluation of the data subjected to linearity of rupatadine fumarate

Level	Conc. in ppm	Area	Mean Area
Level I 50%	100.24	3360309	3354956
		3349277	
		3355281	
Level II 80%	160.38	5319842	5321249
		5321323	
		5322582	
Level III 100%	200.48	6612461	6605267
		6600130	
		6603211	
Level IV 120%	240.576	7958853	7962960
		7960828	
		7969198	
Level V 150%	300.72	10122519	10058280
		10024309	
		10028011	

Table 2: Statistical evaluation of the data subjected to regression analysis

Parameters	Rupatadine fumarate
Correlation Coefficient (r)	0.9999
% Intercept (y)	-29120
Slope (m)	33368.23

The accuracy of rupatadine fumarate was found from 98.41% to 101.10% for 50%, 100% and 150%. [Table 3]

Table 3: Statistical evaluation of the data subjected to accuracy of rupatadine fumarate

Level	Test Name	Qty of Std.added (ppm)	Qty of test (ppm)	Total area obtained after adding	Total quantity obtained after adding (ppm)	Quantity recovered (ppm)	%Recovery
Level I (50%)	Test 1	99.56	10.07	3535224	108.03	97.96	98.39
	Test 2	99.56	10.08	3537656	108.11	98.02	98.46
	Test 3	99.56	10.07	3534474	108.01	97.94	98.37
						Mean 98.41	
Level II (100%)	Test 1	199.12	10.26	6797273	207.72	197.46	99.16
	Test 2	199.12	10.17	6791847	207.55	197.38	99.12
	Test 3	199.12	10.19	6794952	207.65	197.46	99.16
						Mean 99.15	
Level III (150%)	Test 1	298.68	10.23	10068072	307.67	297.44	99.58
	Test 2	298.68	10.85	10310287	315.07	304.22	101.86
	Test 3	298.68	11.05	10317658	315.30	304.25	101.86
						Mean 101.10	

The RSD of method precision is found to 0.21%. [Table 4]

The cumulative RSD of robustness by changing flow rate is 0.22%. [Table 5(a)]

The cumulative RSD of robustness by changing wavelength is 0.19%.[Table 5(b)]

The cumulative RSD of robustness by changing pH of buffer solution is 0.17%.[Table 5(c) tabulated in table 2.

Table 4: Statistical evaluation of the data subjected to method precision of rupatadine fumarate

Sr. No.	Sample name	Assay (%)
01	Test solution – 1	100.02
02	Test solution – 2	100.46
03	Test solution – 3	100.35
04	Test solution – 4	100.48
05	Test solution – 5	100.37
06	Test solution – 6	100.65
		100.39
Mean		0.20918
Standard Deviation		0.21
% RSD		

Table 5 (a): Statistical evaluation of the data subjected to Robustness of rupatadine fumarate by changing flow rate

Condition	Test Name	%Assay
Flow- 0.8 ml/min	Test 1	100.22
	Test 2	100.76
	Test 3	100.63
	Test 4	100.55
	Test 5	100.35
	Test 6	100.08

Flow- 1.0 ml/min	Test 1	100.02
	Test 2	100.46
	Test 3	100.35
	Test 4	100.48
	Test 5	100.37
	Test 6	100.65
Flow- 1.2 ml/min	Test 1	100.37
	Test 2	100.57
	Test 3	100.30
	Test 4	100.00
	Test 5	100.63
	Test 6	100.57
	Mean	100.41
	STD	0.223
	%RSD	0.22

Table 5 (b): Statistical evaluation of the data subjected to robustness of rupatadine fumarate by changing wavelength

Condition	Test Name	%Assay
Wavelength -(245 nm)	Test 1	100.00
	Test 2	100.44
	Test 3	100.36
	Test 4	100.32
	Test 5	100.50
	Test 6	100.58
Wavelength -(250nm)	Test 1	100.02
	Test 2	100.46
	Test 3	100.35
	Test 4	100.48
	Test 5	100.37
	Test 6	100.65
Wavelength -III (255nm)	Test 1	100.06
	Test 2	100.52
	Test 3	100.35
	Test 4	100.35
	Test 5	100.42
	Test 6	100.60
	Mean	100.38
	STD	0.188
	%RSD	0.19

Table 5 (c): Statistical evaluation of the data subjected to robustness of rupatadine fumarate by changing pH of buffer solution

Condition	Test Name	%Assay
pH - 4.3	Test 1	100.28
	Test 2	100.22
	Test 3	100.19
	Test 4	100.18
	Test 5	100.56
	Test 6	100.35

pH -4.5	Test 1	100.02
	Test 2	100.46
	Test 3	100.35
	Test 4	100.48
	Test 5	100.37
	Test 6	100.65
pH - 4.7	Test 1	100.38
	Test 2	100.37
	Test 3	100.13
	Test 4	100.54
	Test 5	100.12
	Test 6	100.18
	Mean	100.32
	STD	0.172
	%RSD	0.17

Table 6: System suitability parameters evaluated on standard solution of rupatadine fumarate

Retention Time	Area	Area %	USP Plate Count	USP Tailing
8.96 minutes	6600799	100.0	5584	1.34

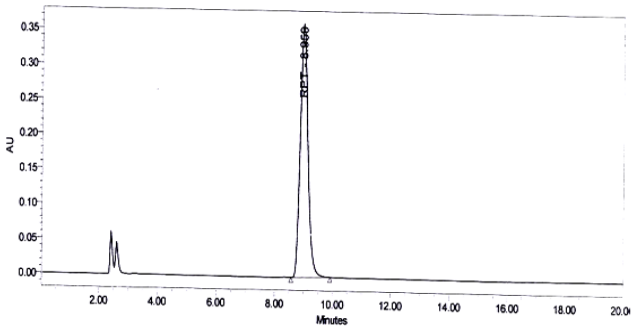

Figure 2: Typical chromatogram of rupatadine fumarate (standard)

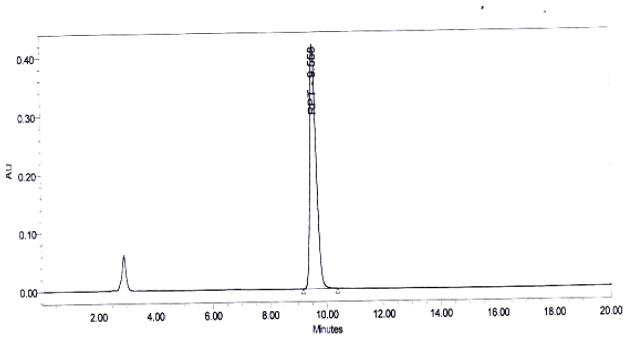

Figure 3: Typical chromatogram of rupatadine fumarate (sample)

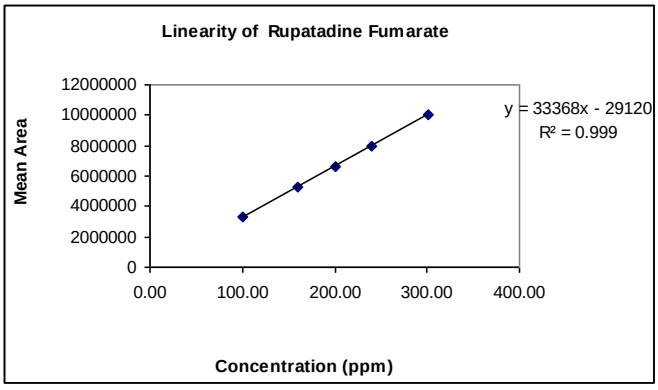

Figure 4: Linearity graph of rupatadine fumarate

Thus the proposed RP-HPLC method is used for the estimation of rupatadine fumarate from its

active pharmaceutical ingredient. It is more suitable, precise, accurate, linear, robust, simple and rapid method. Hence the proposed RP-HPLC method is strongly recommended for the quality control of the raw material, active pharmaceutical ingredient and pharmaceutical formulation.

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