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Research Article

Development and Validation of RP-HPLC Method for the Estimation of Rupatadine in Bulk and Tablet Dosage Form

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Abstract

A simple, precise, sensitive, and rapid reversed phase high performance liquid chromatographic method for the analysis of Rupatadine in bulk and tablet dosage form with greater precision and accuracy has been developed and validated. The chromatographic separation was achieved by using Hypersil ODS, 150 × 4.6 mm, 5μm analytical column with a mobile phase consisting of 0.02 M phosphate buffer (pH 3.0, pH was adjusted with orthophosphoric acid), HPLC grade methanol and acetonitrile at the ratio of (45:30:25% v/v). The chromatographic condition was set at a flow rate of 0.5 ml/min, column oven temperature at 25ºC and detector wavelength of 242 nm using a photodiode array detector. Total run time was 13 min and retention time of Rupatadine was 7.94 min. The standard curves were linear over a concentration range of 20- 70 μl with an r2 value of 1.0. Theoretical plate for rupatadine was 7468 and tailing factor was 1.21. The proposed method was validated according to ICH guidelines for the parameters like linearity, accuracy, solution stability, system precision, specificity, robustness and it can be routinely used for routine quality control analysis of Rupatadine from Tablet dosage form.

Keywords: Rupatadine, RP-HPLC, method development, validation, tablet dosage form.

Introduction

Rupatadine fumarate is chemically 8-chloro-6, 11-dihydro-11-[1-[(5-methyl3-pyridinyl)methyl]-4-piperidinylidene]-5H-benzo[5,6]-cyclohepta[1,2b]pyridine fumarate (Figure 1).

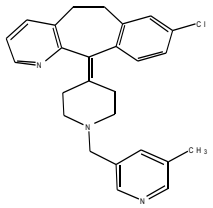

Figure 1: Chemical structure of rupatadine

It is a non-sedating H1-antihistamine (second generation) and Platelet Activating Factor (PAF) antagonist [1-4]. The drug is not officially reported in pharmacopoeia. It is off white to pinkish crystalline powder that is soluble in methanol and ethanol, very slightly soluble in chloroform and insoluble in water. Rupatadine fumarate belongs to a class of medications called antiallergic, antihistaminics. It is potent and orally active that was developed as a therapeutic agent for the treatment of seasonal allergic rhinitis and chronic idiopathic urticaria [5]. Rupatadine has a profile as an anti-allergic drug with potentially beneficial effects, such as the inhibition of mast cell degranulation, inhibition of neutrophil and eosinophil migration and inhibition of cytokine release [6-8].

Literature survey reveals that few analytical methods are available for estimation of rupatadine. But the existing methods are complex and time consuming; the proposed method was aimed to develop a simple, rapid, new, economical, precise and accurate method for the determination of rupatadine from its bulk and tablet dosage form.

Materials and Methods

Chemicals

Working standard of rupatadine with a potency of 99.58 % was collected from Shanghai Boyle Chemical Co. Ltd., China. The pharmaceutical formulation of rupatadine containing 10 mg rupatadine was procured from local market. HPLC grade Acetonitrile and Methanol was purchased from Merck, Germany, Potassium dihydrogen phosphate from Scharlau, Spain and analytical grade orthophosphoric acid were purchased from Sigma Aldrich, Germany. All other chemicals used were analytical or HPLC grade.

Chromatographic conditions

The analysis of drug was carried out on a Shimadzu LC-20 AT, prominence, equipped with an auto sampler (SIL-20AC HT, Shimadzu, Japan) and PDA detector (SPD- M20A, Japan) was used for the analysis. The data was recorded using LC-solution software. A Hypersil ODS, (150mm x 4.6mm, 5μm) column was used for the analysis. A powersonic 505 ultrasonic bath (Hwashin technology, Seoul, Korea) was used for degassing of the mobile phase.

In this HPLC method separation was carried out using a mobile phase consisting of 0.02M phosphate buffer with pH 3.0, (adjusted with orthophosphoric acid), Acetonitrile and Methanol in the ratio of 45: 25: 30% v/v. The mobile phase was mixed well before filtration, then sonicated and degassed before use. The column was maintained at a temperature of 25ºC with column oven (CTO-20AC) and the flow rate was 0.5 ml/min. Analysis was performed with injection volume of 10μl using PDA detection at 242 nm. The run time was set for 13 min. The optimized chromatographic condition is shown in Table 1.

Table 1. Optimized chromatographic conditions

Parameters	Conditions
Stationary phase (column)	Hypersil ODS, 150 × 4.6 mm, 5µm
Mobile Phase	0.02M Phosphate buffer : Acetonitrile : Methanol (45 : 25 : 30 v/v)
Flow rate (ml/min)	0.5
Runtime (min)	13
Column Temperature (ºC)	Ambient (25ºC)
Volume of Injection (µl)	10
Detection wavelength (nm)	242nm
Retention Time (min.)	7.94

Preparation of standard solution

About 32 mg of rupatadine fumarate WS equivalent to 25 mg of rupatadine was accurately weighed into a 100 ml volumetric flask and made up to the volume with mobile phase. The content of the volumetric flask were kept it in an ultrasonic bath for 20 min at 25ºC. Then standard solution was cooled to room temperature. 5 ml of this solution was diluted to 25 ml with diluting solution and mixed well. The final concentration was 50 μg /ml and this was used as stock solution.

Preparation of sample solution

About 20 tablets were weighed and crushed into fine powders. Powdered sample equivalent to 50 mg of rupatadine were taken into a 200 ml volumetric flask. About 3 ml of water was added to disperse the powders, and then drugs were dissolved in 100 ml of diluting solution and shaken at 250 rpm for 15 min by shaker. The volume was made up to the mark with diluting solution and sonicated for 20 minutes. It was then cooled to room temperature and the resultant solution was filtered through Whatman 41 filter paper. 5 ml of this solution was diluted to 25 ml with diluting solution. Final solution was filtered through 0.45μ membrane filter and the filtrate was used under the chromatographic condition. The amount of drug present in the sample was determined by comparison with the peak area of standard solution.

Method validation

The present method of analysis was validated according to the recommendations of ICH-1996 and USP-30 for the parameters like specificity, system suitability, accuracy, linearity, precision, robustness.

Specificity

Specificity of an analytical method is its ability to assess unequivocally the analyte in the presence of components that may be expected to be present. The specificity was done by injecting the excipients, diluting solution and standard solution of rupatadine to ensure that there is no interference of excipients and diluting solution in the chromatogram of rupatadine.

System suitability

System suitability was performed by injecting six replicates of standard solution at 100% of the test condition and two replicates of sample preparation at a 100% level to verify the accuracy and precision of the chromatographic system. This method was evaluated by analyzing the repeatability of retention time, tailing factor, theoretical plates of the column. System suitability data are given in Table 2.

Table 2. System suitability parameters of rupatadine

SI No	Parameters	Rupatadine
1	Tailor factor	1.21
2	Retention time	7.94
3	Theoretical plates	7468

Linearity and construction of calibration curve

Linearity of an analytical method is its ability to elicit test results directly proportional to the concentration of the analyte within a given range. The linearity of the chromatographic method was established by plotting a graph to concentration area of rupatadine standard and determining the correlation coefficient (r2). Linearity of rupatadine standard solution at a concentration level of 40%, 60%, 80%, 100%,120%,140% were injected into the HPLC system. The detector response was found to be linear form 40% to 140% of test concentration for rupatadine standard. The calibration plot of peak area against concentration was linear in the range investigated 20-70μg/ml. The linear regression equation was $y = 41521x + 4057$ and the regression coefficient (R2) was 1.0. The correlation coefficient was indicative of high significance. The low values of the standard deviation, the standard error of slope, and the intercept of the ordinate showed the calibration plot did not deviate from linearity. Linearity curve is shown in Figure 4 and data are shown in Table 3.

Table 3. Statistical data of calibration curves of rupatadine

Sl. no.	% test concentration	Concentration (μg/ml)	Average peak area
1	40	20	835126
2	60	30	1250372
3	80	40	1666809
4	100	50	2085412
5	120	60	2501639
6	140	70	2899845
	Regression co-efficient =		1.0

Accuracy

The accuracy of the method is the nearness of the result obtained to the true value. The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed tablet solution. Percent recovery was calculated by comparing the area before and after addition of the standard drug. The recovery was performed by adding rupatadine working standard at three concentration levels (50%, 100%, and 150%) and expressed as percent (%) recovered. Three samples were prepared for each recovery level. The percent recovery and % RSD at each level were calculated. Satisfactory recoveries ranging from 99.00 to 100.60% for rupatadine was obtained by the proposed method. The average recovery of three levels was 99.73%. Results are shown in Table 4.

Table 4. Results of accuracy experiment using recovery method

Level (%)	Amount of drug spiked (mg)	Found (mg)	Recovery (%) (n=3)
50	4.98	4.93	99.00
100	9.96	10.02	100.60
150	14.94	14.91	99.80
	Average Recovery		99.80
	SD		0.803
	% RSD		0.805

Stability of Analytical solution

The stability of analytical solutions was established by injecting the standard solution and sample solution at different time intervals up to 18 h (0, 4, 8, 12, 16, and 18 h) by keeping the auto sampler temperature at room temperature (25ºC). The response of standard solution and sample solution were measured and % differences of peak area were calculated. The values are presented in the Table 5.

Precision

The precision of an analytical method is the degree of agreement among individual test results where the method is applied repeatedly to multiple samplings. Precision of the assay was assessed with respect to repeatability, reproducibility and intermediate precision. Repeatability of sample injection was determined as intra-day variation and intermediate precision was determined by measurement of inter-day variation. Results of analysis for repeatability, intermediate precision, and reproducibility are given in the Table 6.

Robustness

Robustness was determined to assess the effect of small but deliberate variation of the chromatographic conditions on the determination of rupatadine. Percent assay of rupatadine, peak tailing, theoretical plates, % RSD were calculated. To study the effect of flow rate, it was changed to 0.1 units from 0.5ml/min to 0.4ml/min and 0.6ml/min. The effect of column temperature was studied at 23ºC and 27ºC instead of 25ºC. The effect of wavelength change was studied by changing the wavelength from 242 nm to 240 nm and 244 nm. Results are shown in the Table 7.

Results and discussion

The present study was aimed at developing a simple precise and accurate HPLC method for the analysis of rupatadine in bulk drug and in pharmaceutical dosage form. The proposed method was specific because there was no interference of common excipients used in the tablet and impurity in the chromatogram of rupatadine (Figure 2 and Figure 3).

Figure 2. A typical chromatogram of rupatadine

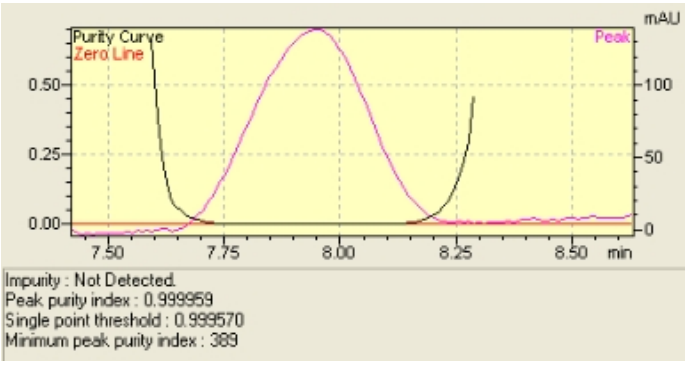

Figure 3: Purity curve of rupatadine

The method showed a good linear relationship of detector response and produces a regression coefficient R2 =1.0. The linearity of rupatadine at six different concentrations (20-70μg/ml) has shown in Table 3 and Figure 4.

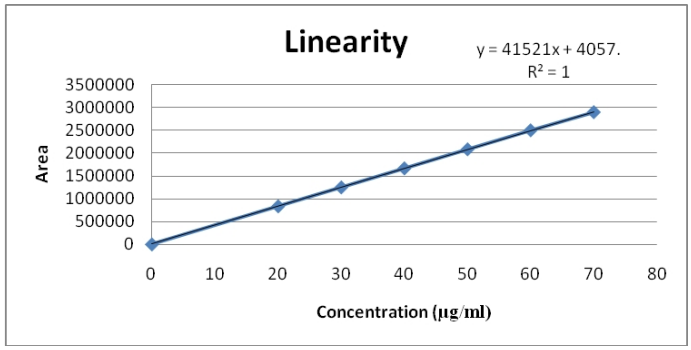

Figure 4: Calibration curve of Rupatadine

Results of accuracy were proven by the Table 4 and % RSD is 0.803, which is within the acceptable limit (less than 2.0). The % difference of peak area of Standard solution and Sample solution that were injected at periodic intervals were found to be within the specified limit (Table 5).

Table 5. Stability of standard and sample solution of rupatadine

Time Interval (h)	Standard		Sample	
	Standard peak area	% Difference	Sample peak area	% Difference
0	2083710	-	2080234	-
4	2084112	0.02	2081645	0.07
8	2083892	0.02	2081604	0.07
12	2083142	0.03	2080982	0.04
16	2082998	0.03	2080847	0.03
18	2082378	0.06	2079211	0.05

There were no significant differences between the % RSD values for intra-day and inter-day precision (Table 6), which indicates the method is reproducible. The deliberate changes in the method have not much affected the peak tailing, theoretical plates and the percent assay (Table 7). This indicates satisfactory robustness of the method. So, this method is applicable for determination of rupatadine from its bulk and tablet dosage form.

Table 6. Statistical analysis for repeatability, intermediate precision and reproducibility of rupatadine 10 mg tablet

Sample ID	Assay (% labeled amount)		
	Repeatability (Analyst 1)	Intermediate precision (Analyst 2)	Reproducibility (Analyst 3)
Sample-1	99.42	99.68	99.32
Sample-2	98.97	99.29	99.15
Sample-3	100.13	98.51	99.02
Sample-4	98.79	99.05	99.53
Sample-5	99.20	98.97	98.88
Sample-6	99.31	99.18	99.36
Average	99.30	99.11	99.21
SD	0.465	0.386	0.239
% RSD	0.469	0.390	0.241

Table 7. Results of robustness study

Sl. No.	Parameter	Variation	Assay % (n=3)
1.	Flow rate (± 10% of the set flow)	a) 0.4 ml/min b) 0.6 ml/min	a) 99.28 b) 98.93
2.	Wavelength (± 2.0 of set value)	a) 240 nm b) 244 nm	a) 99.01 b) 99.56
3.	Column oven temperature (± 2ºC of set temperature)	a) 23ºC b) 27ºC	a) 99.42 b) 99.20

Conclusion

The proposed RP-HPLC has been validated as per the recommendation of ICH guidelines. The method is accurate, precise, simple, less time consuming, cost effective and convenient for use. All the validation parameters of the analysis method showed satisfactory data with acceptable correlation co-efficient and lower % RSD. So the developed method can be used conveniently for analysis of quality control, stability and further studies.

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Declaration of Interest

The authors do not have any conflict of interest.

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