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New Validated RP-HPLC Method for Quantification of Rupatadine Fumarate Impurities in Solid Dosage Form Supported By Forced Degradation Studies

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ABSTRACT

A new stability indicating high performance liquid chromatography (HPLC) method has been developed and validated for the quantitative determination of potential impurity of rupatadine fumarate drug substance and drug product. A simple, specific, precise, robust and reproducible reverse high phase performance liquid chromatography (RP-HPLC) method was developed for the determination of potential impurity, desloratadine in Rupatadine fumarate drug substance and drug product. Chromatographic separation between impurity and Rupatadine achieved on the unisphere C_{18} (250 x 4.6 x 5µ) column. The mixture of aqueous buffer containing 0.3 M sodium acetate pH 4.4 ± 0.05 (adjusted with glacial acetic acid) and methanol in the ratio of 80:20 % (v/v) respectively. The mobile phase delivered at a flow rate of 1.0 ml/min and the detection was carried out at wavelength 245 nm. The chromatographic resolution between fumaric acid and desloratadine was found to be greater than 5. The response was determined and regression 'r' values (correlation coefficient) were obtained greater than 0.998 for related compound and Rupatadine fumarate. The LOQ values for desloratadine and Rupatadine fumarate were 0.25 µg/ml and 0.2 µg/ml respectively. The percentage recovery was found to be 98.0 % to 102.5 % for desloratadine. The drug was subjected to stress conditions as prescribed by ICH guideline. Degradation was fond to occur slightly under oxidative storage condition but drug was stable to acidic and basic hydrolysis, photolysis and thermal storage conditions. The developed method was validated as per ICH guideline.

Key words: Rupatadine Fumarate, RP-HPLC, Method Validation, Related Compound.

INTRODUCTION

Rupatadine fumarate (RUPA) is non sedating H, antihistamine (second generation) and platelet- activating factor inhibitor. Chemically it is 8-chloro-6, 11-dihydro-11-[1-[(5 methyl-3-pyridynyl) methyl]-4-piperidinylidene]-5H-benzo [5, 6] cyclopenta[1,2b]pyridine fumarate. The efficacy of RUPA as a treatment for allergic rhinitis (AR) and chronic idiopathic urticaria (CIU) has been investigated in adults and adolescents (aged over 12 years) in several controlled studies, showing a rapid onset of action and a good safety profile even in prolonged treatment period of a year.

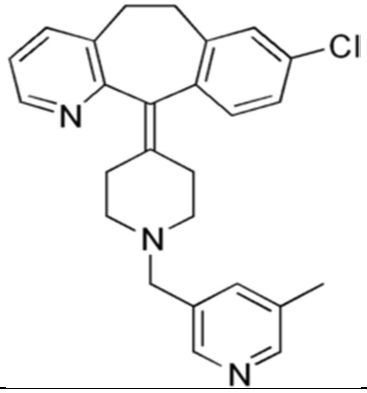
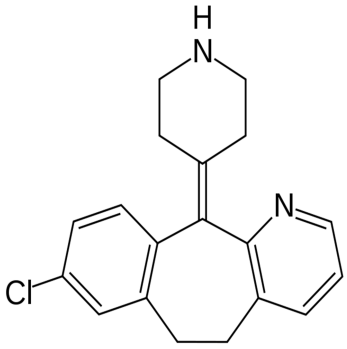
Rupatadine fumarate is not an official drug substance or drug product in the European Pharmacopeia, Indian Pharmacopeia (IP) and United State Pharmacopeia (USP).

Literature survey reveals that HPLC [1-3] spectrophotometric [4-9], and one titrimetric [10] method have been reported for the estimation of Rupatadine fumarate in human serum and tablet formulations. Very few methods have been appeared in the literature for the estimation of related compounds or impurities of rupatadine fumarate drug substance and drug products [11-13].The objective of the present was to develop simple, accurate, specific and economic RP-HPLC method for the estimation of related substances of Rupatadine fumarate in bulk drug and tablets. The developed method was successfully validated according to ICH guideline [14,15], for parameters like specificity, linearity, accuracy, precision and robustness. The results of analysis were validated statistically and by

recovery studies. In specificity study, desloratadine was found as a known degradation product, so desloratadine was selected for the validation parameters.

The chemical structure and IUPAC name of rupatadine fumarate and desloratadine are presented in Figure 1.

Figure-1: Chemical structure and IUPAC name of rupatadine fumarate and desloratadine

Chemical Substance	IUPAC name	Chemical structure
1. Rupatadine fumarate	8-chloro-6, 11-dihydro-11-[1-[(5methyl-3-pyridynyl)methyl]-4-piperidinylidene]-5H-benzo[5,6] cyclopenta[1,2b]pyridine fumarate.	
2. Desloratadine	8-chloro-6,1 dihydro-11(4 piperidinylidine) -5-H- benzo [1,2-b]pyridine,	

MATERIALS AND METHODS

Materials

Reference standards of rupatadine fumarate and desloratadine and fumaric acid were obtained from reputed firm with certificate of analysis. Commercially available rupatadine tablets were purchased from market for this study. HPLC grade methanol and glacial acetic acid from Merck specialties chemicals was used for chromatographic separation. Sodium acetate was used of analytical regent grade from S.D. Fine chemicals. Water was de-ionized and further purified by means of a Milli –Q Elix water purification system, Millipore.

Instrumentation

The HPLC system used was Waters Alliance, equipped with auto sampler (2695 separation module) and photodiode array (PDA) detector (2996). The chromatogram was recorded and peaks quantified by means of PC based Empower 2 software.

Chromatographic conditions

Chromatographic separation was achieved on a reverse phase Unisphere C_{18} (250 x 4.6mm i.d. x 5µ) stain less steel column. A flow rate of 1.0 ml/min of HPLC analysis was conducted at ambient temperature. Mobile phase was consisted of buffer (2.5 gm of sodium acetate dissolved in 1000 ml of water, adjusted pH 4.4 ± 0.05 with glacial acetic acid) and methanol in the ratio of 20:80 %(V/V). The mobile phase was premixed, filtered through 0.45 µ membrane filtered and degassed by sonication. A mixture of water and methanol in the ratio of 20: 80 % (v/v) was used as diluent. The injection volume was 20 µl. The detector wavelength was set at 245 nm.

Preparation of solutions

Reference solution

Weighed accurately 25 mg of desloratadine and 5 mg of rupatadine and transferred into 50 ml volumetric flask. It was dissolved in about 25 ml of diluent with sonication and diluted to 50 ml using same diluent. 5 ml of this solution was further diluted to 50 ml using diluent. It was further diluted to get 5 µg/ml of desloratadine and 1µg/ml of rupatadine solution using same diluent.

Fumaric acid solution

Fumaric acid solution was prepared by transferring appropriate amount of fumaric acid into 50 ml volumetric flask and making volume with diluent to get 1000µg/ml of fumaric acid.

Sample solution

A powdered tablet equivalent to 50 mg rupatadine was weighed accurately. It was transferred to 50 ml volumetric flask. It was dissolved in small quantity of diluent by sonication for about 15 min and diluted to 50 ml volume using same diluent. This solution was filtered through Whatman filter paper no. 41. First few ml of the filtrate was discarded. The resulting solution was injected into HPLC system.

Method Development

Different column containing octyl and octadecyl silane stationary phase were tried for the separation and resolution. It was found that Unisphere C18 column offered more advantages over Inertsil and Hypersil BDS columns. Various mobile phase composition with different solvents and buffer composition were tried to achieve better separation between fumaric acid, desloratadine and rupatadine. The final selection of mobile phase composition and flow rate was made on the basis of peak shape (Peak area, peak asymmetry), baseline drift, time required and cost of solvents. Individual solution of fumaric acid, desloratadine and rupatadine were injected and retention time of all the three reference standards identified. desloratadine reference standard spiked in rupatadine sample solution injected in HPLC system and elution pattern of all the three (fumaric acid, desloratadine and rupatadine) and resolution parameters were studied as a function of pH of buffer of mobile phase. The pH effect showed that optimized conditions were reached at pH 4.4. Typical chromatograms of reference solution and sample solution spiked with desloratadine are given in Figure 2 and 3 respectively.

Figure-2: Typical Chromatogram of standard solution

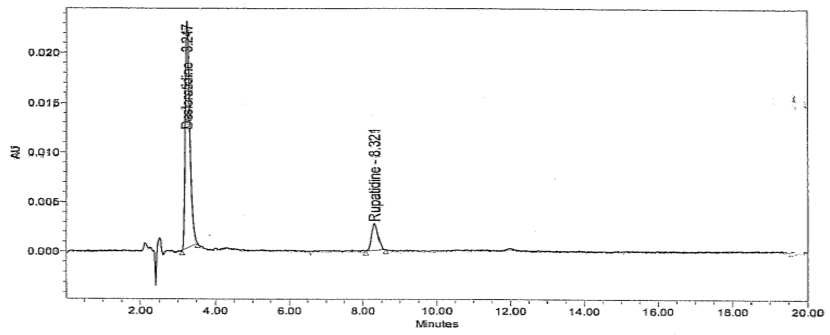
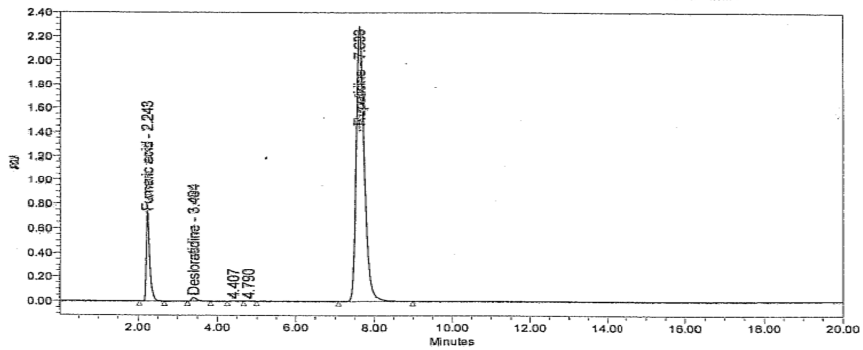


Figure- 3: Chromatogram of Sample solution spiked with desloratadine



The relative chromatographic figures of merit are reported in Table 1. The good chromatographic separation indicated that any of these drug substances could be used as internal standard for the assay of other drug.

Table 1: System performance parameter for fumaric acid rupatadine and desloratadine (n=6)

Substances	Retention time	Symmetry factor	Theoretical Plates	Resolution	% RSD
Fumaric acid	2.30	1.5	-	-	-
Desloratadine	3.25	1.6	3443	5.50	3.37
Rupatadine	8.30	1.35	10934	13.26	2.35

RESULTS AND DISCUSSION

Method Validation

System suitability

System performance parameters of developed method were determined by injecting reference solution in six replicates. Parameters such as numbers of theoretical plates, symmetry factor, resolution and relative standard deviation were determined. The results are summarized in Table -1, indicating good performance of the system.

Stress studies / Specificity

The specificity is the ability of the method to measure the analyte response in the presence of potent impurities. Retention time of individual compounds and diluent were injected, results are shown in Table-2. PDA detector was used to evaluate the homogeneity of the peak in the chromatogram. Chromatographic peak purity was determined for rupatadine fumarate spiked with known related substance as shown in Figure-3.

Table-2: Specificity Data

Compound Name	Retention time	RRT*	Purity angle**	Purity threshold
Desloratadine	3.25	0.39	1.60	1.75
Rupatadine	8.35	1.0	6.21	7.23

**RRT is calculated with respect to rupatadine peak.*

*** Purity angle and purity threshold of the related substance in the sample spiked with known related substances.*

In order to provide an indication of the stability- indicating properties of the proposed method, forced degradation studies were performed under various stress conditions [8]. Thus rupatadine fumarate sample, were stressed with 1.0 N HCl (heated on water bath at 80ºC for 60 min), 1.0 N NaOH solution (heated on water bath at 80ºC for 60 min) and 1 ml of 30% H_2O_2 (heated on water bath at 80ºC for 60 min). Thermal degradation at 105 ºC for 24 hrs and for photolytic degradation sample was exposed to UV degradation. After degradation treated sample neutralized with acid/base as required and analyzed as per the proposed method. Rupatadine was shown significant stability in all conditions except peroxide treatment. In peroxide stress degradation rupatadine showed about 12% degradation and the impurity peak were well resolved. The purity of the peaks obtained from stressed samples was verified using PDA detector. The obtained purity angle was less than the purity threshold for all stressed sample. An assay of samples was performed by comparison with reference standard, and the mass balance (% assay+% known impurity+% unknown impurities) for each of the stressed samples was calculated. The forced degradation data is presented in Table-3.

Table- 3: Forced Degradation data

Stress condition	Time	% assay active substance	Mass balance	Remark
Acid hydrolysis (1M HCl)	60 min	99.1%	99.8%	No major degradation
Base hydrolysis (1M NaOH)	60 min	99.3%	99.5%	No major degradation
Oxidation (30% H_2O_2)	60 min	87.4%	99.4%	One major degradation observed
Thermal (105 ºC)	24 hrs	99.2%	99.5%	No major degradation
Light (UV)	24 hrs	99.0%	99.4%	No major degradation

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ for desloratadine and rupatadine were estimated at a signal-to-noise (S/N) ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentrations. The precision was also carried out at the LOQ level by injecting six individual preparations of desloratadine in presence of Rupatadine and calculated the % RSD of the areas. The determined LOD and LOQ values are tabulated in Table-4.

Table- 4: Limit of detection (LOD) and limit of quantification (LOQ) data

Parameter	Desloratadine	Rupatadine
LOD	0.1 µg/ml	0.05 µg/ml
LOQ	0.25 µg/ml	0.2 µg/ml

Linearity

Linearity test solutions were prepared by diluting desloratadine and rupatadine standard stock solutions to the required level of concentration. The solutions were prepared as concentration levels from LOQ to 150% of the specification level.

The correlation coefficient, slopes and y-intercepts of the calibration curve were determined. The regression analysis data obtained is tabulated in Table-5.

Table 5: Linearity regression analysis data

Parameters	Desloratadine	Rupatadine
correlation coefficient (r)	0.999	0.998
Intercept (y)*	3759	3694
Slope (m)*	47504	32631

*For equation y = mx +c

Precision

The precision of the proposed method was performed by injecting six individuals test solutions preparation of (1000 µg/ml) rupatadine spiked with desloratadine impurity standard of specification level concentration. The % RSD of the area of desloratadine was calculated.

The intermediate precision of the method was evaluated using a different analyst and instrument located within same laboratory. These results from both the experiments were compared and confirmed the ruggedness of the method. The results obtained are tabulated in Table-6.

Table 6: Method precision and intermediate precision

Parameter	Desloratadine
Precision %RSD (n=6)	1.0 %
Intermediate precision %RSD (n=6)	0.95 %
Cumulative %RSD (n=12)	3.02 %

Accuracy

Accuracy of the proposed method was determined by applying the described method to powdered tablet containing known amount of desloratadine. For desloratadine, the recovery was calculated in six replicates (n=6) for each level of LOQ, 50%, 100% and 150%. The amount recovered was within 95 - 105% for each level. The % RSD of individual level of 50%, 100% and 150% was also within the limit of NMT 2.0 % and NMT 5.0 % for LOQ level. This indicates there is no interference due to excipients present in the pharmaceutical doses form. It was confirmed from results, the method is highly accurate, refer Table-7.

Table 7: Accuracy of desloratadine

Accuracy level (n=6)	% Recovery	% RSD
LOQ	98%	3.33
50%	102.5%	1.41
100%	98.2%	1.08
150%	98.3%	0.15

Robustness

Robustness of the method was studied by making small but deliberate changes in the optimized conditions. The effect of the deliberate changes were confirmed by the injecting reference solution and test solution and monitoring the resolution between fumaric acid and desloratadine, theoretical plates, symmetry factor and % RSD.

The different variations as given below:
Variation in flow rate by ± 0.2 ml/min
Variation in wavelength by ± 2nm
Variation in temperature by 5ºC

When one parameter changed rest all are other parameter were kept constant. The results are recorded in Table-8.

Table- 8: Robustness data.

Parameter	Resolution between fumaric acid and desloratadine	Theoretical plates		Symmetry factor		% RSD	
		Deslor.	Rupa.	Deslor.	Rupa.	Deslor.	Rupa.
Precision	5.6	3372	5717	1.7	1.2	0.79	2.81
Robustness (-wavelength)	5.1	4082	12156	1.65	1.20	4.38	2.72
Robustness (+wavelength)	5.2	4134	12455	1.67	1.18	4.0	4.63
Robustness (-flow rate)	5.9	1900	3491	1.8	1.23	0.55	4.9
Robustness (+flow rate)	4.15	1878	3221	1.59	1.33	0.96	2.64
Temp +5ºC	4.3	2020	4431	1.53	1.29	0.86	2.59

Des = Desloratadine , Rupa = Rupatadine

Method Application

The validated RP-HPLC method was applied to determination of related substances of rupatadine in drug substances and drug product. Twenty tablets powder blend was used. A portion equivalent to 50 mg of rupatadine fumarate was weighed accurately and dissolved in 30 ml of diluent. It was sonicated for 15 min and further diluted to 50 ml with diluent to get solution containing 1000 µg/ml of rupatadine.

CONCLUSION

A new, sensitive and stability indicating RP-HPLC method has been successfully developed for quantitative analysis of related substances in rupatadine fumarate drug substance and drug product. The proposed method has been successfully validated for specificity, linearity, precision, accuracy and ruggedness. The repeatability, reproducibility and accuracy of the proposed method were found to be satisfactory, which is evidenced by low value of relative standard deviation in comparison to previous methods. The accuracy of the proposed method was confirmed by recovery experiment, performed by adding known amount of impurity standard to the pre-analyzed formulation and reanalyzing the mixture by proposed method.

The previous method appeared in the literature for the assay of Rupatadine in drug substance and drug product by high performance liquid chromatography [1-3]. Few spectrophotometric methods are also available for the determination of Rupatadine [3-9]. One titration method is available for the assay of Rupatadine [10]. In previous methods for determination of related substances of rupatadine by HPLC method, X-tera column was used [8]. Such column is more expensive than the proposed Unisphere C18 column. In one of method ion pairing agent was used, where as in other method column oven temperature set at 50 ºC [8]. The proposed method involves use of methanol and acetate buffer which is volatile buffer. The use of volatile buffer in this method has a unique advantage over the other methods. The output from the HPLC system is directly applied to hyphenated mass spectrometry detector, which enables rapidly identification of impurities. The method enables high resolution of degradation products and impurities both from Rupatadine fumarate and from each other.

There is no use of acetonitrile and ion pairing agent, hence overall cost of analysis is less for proposed method. The proposed method has additional advantages over the existing methods and is more beneficial for analysis of such formulation than the previous methods.

Thus the proposed RP-HPLC method for the determination of related substances of rupatadine fumarate is precise, accurate, linear, robust, simple and rapid. Hence proposed RP-HPLC method is strongly recommended for the quality control of the raw material, formulations and dissolution studies. As the method is stability indicating in nature the same method can be used for the stability study also.

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