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Validated RP-HPLC Method for the Estimation of Rupatadine fumarate in Bulk and Tablet Dosage Form

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ABSTRACT

A novel, simple and economic reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the estimation of Rupatadine fumarate in bulk and tablet dosage form with greater precision and accuracy. Separation was achieved on C18 column (250X4.6mm i.d.,5µm) in isocratic mode using Acetonitrile:Methanol:Water in the ratio of 40:50:10 (v/v/v) as mobile phase, pumped in to the column at flow rate of 1.0 mL min^{-1} and the detection of eluent from the column was carried out using variable wavelength UV detector at 244 nm. The total run time was 10 min and the column was maintained at ambient temperature. The retention time of Rupatadine fumarate was 7.350 min. The standard curves were linear over the concentration range of 10-60 µg mL^{-1} with R^2 9996 and the LOD and LOQ values for Rupatadine fumarate were 0.026 µg mL^{-1} and 0.056 µg mL^{-1} respectively. The percentage recovery was found to be 99.06 to 100.60, the % RSD of intraday and inter day precision was found to be 0.68 and 0.61, respectively. The percentage amount of a marketed tablet formulation of Rupatadine fumarate was found to be 99.08 %. The method was validated as per ICH guidelines. Validation studies demonstrated that the proposed RP-HPLC method is simple, specific, rapid, reliable and reproducible. Hence the proposed method can be applied for the routine quality control analysis of Rupatadine fumarate in bulk and tablet dosage forms.

Key words: Rupatadine fumarate, RP-HPLC, Method Development, Validation, ICH guideline

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INTRODUCTION

Rupatadine fumarate (RUPA) is a non-sedating H_1-antihistamine (second generation) and platelet-activating factor inhibitor. Chemically it 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. The structure of RUPA is shown in Fig.1.The drug is not official reported in pharmacopoeia. [1] .It is off white to pinkish crystalline powder that is soluble in soluble in methanol and ethanol, very slightly soluble in chloroform and insoluble in water. Rupatadine fumarate belongs to a class of medications called Antiallergic, Antihistaminic. It is potent and orally active that was developed as a therapeutic agent for the treatment of seasonal allergic rhinitis and chronic idiopathic urticaria.[1]

Literature survey reveals that two Spectrophotometric methods [2-3], six HPLC methods [4-9] have been reported for the estimation of Rupatadine fumarate in human serum and tablet formulation. The objective of the present work was to develop simple, rapid, accurate, specific and economic RP-HPLC method for the estimation of Rupatadine

fumarate in bulk and tablet. The method was further validated as per ICH guidelines [11] for the parameters like precision, accuracy, sensitivity, and linearity. The results of analysis were validated statistically and by recovery studies. These methods of estimation of Rupatadine fumarate were found to be simple, precise, accurate and economic.

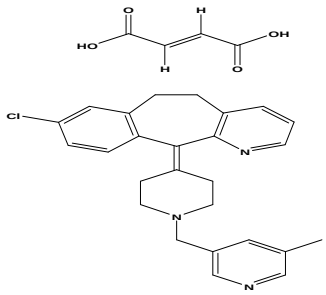

Fig 1: Structure of Rupatadine fumarate

MATERIALS AND METHODS

Samples

Rupatadine fumarate, 8-Chloro-6, 11-dihydro-11-[1-[(5-methyl-3-pyridinyl) methyl]-4-piperidinylidene]-5H-benzo [5, 6] cyclohepta [1, 2-b] pyridine fumarate, was kindly provided by Hetero Health Care Ltd. Mumbai, India. A commercial tablet formulation Rupanex from Dr. Reddy' s Laboratories Ltd, (Hyderabad, India) containing 10mg of RUPA was purchased from local market and used within their shelf life period.

Reagents

Methanol and acetonitrile were of HPLC grade, from Thomas Baker (India). Water used was bidistilled.

Apparatus

A Jasco HPLC system (Japan) composed of a PU-2080 plus pump equipped with a 7725i Rheodyne (CA, USA) injector, an UV-2075 plus UV–vis detector and a LC-Net II/ADC with inbuilt Borwin software.

Chromatographic conditions

The separation was performed on a 25 cm×4.6mm i.d. HiQ Sil-C18 HS column (Kya Tech, Japan). The flow rate was 1.0 mL min^{-1}. The injection volume was 20µl. The detection wavelength was set at 244 nm. The mobile phase consisted of Acetonitrile: Methanol: Water in the ratio of 40:50:10 (v/v/v). The run time was set at 10 min and column temperature was maintained at ambient. Prior to injection of analyte, the column was equilibrated for 30 min with mobile phase. The mobile phase was premixed, filtered through 0.45 µm membrane filter and degassed by sonication

3.0 Method Validation

3.1 Linearity

A stock solution of (1000 µg mL^{-1}) was prepared by dissolving 100 mg drug in 100 ml mobile phase then solutions of different concentrations (10–60 µg mL^{-1}) for construction of calibration plots were prepared from this stock solution. The mobile phase was filtered through a 0.45 µm membrane filter and delivered at 1.0 mL min^{-1} for column equilibration; the baseline was monitored continuously during this process. The detection wavelength was 244 nm. The prepared dilutions were injected in series, peak area was calculated for each dilution, and concentration was plotted against peak area.

3.2 Accuracy

Accuracy was determined by the standard addition method. Previously analyzed samples of RUPA (10 µg mL^{-1}) were spiked with 80, 100, and 120% extra RUPA standard and the mixtures were analyzed by the proposed method. The experiment was performed in triplicate. Recovery (%), RSD (%), and standard error (SE) were calculated for each concentration.

3.3 Precision

Precision was determined as both repeatability and intermediate precision, in accordance with ICH recommendations. Repeatability of sample injection was determined as intra-day variation and intermediate precision was determined by measurement of inter-day variation. For both intra-day and inter-day variation, solutions of RUPA at single concentrations was determined.

3.4 Reproducibility

The reproducibility of the method was checked by determining precision on a different column, analysis being performed by another analyst. For both intra-day and inter-day variation, solutions of RUPA at single concentrations (10μg mL^{-1}) were determined six times.

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

LOD and LOQ were determined by the standard deviation ($S_{y/x}$) method. LOD and LOQ were determined from the slope, S, of the calibration plot, $S_{y/x}$, by use of the formulae

$LOD = 3.3 \times S_{y/x}/S$ and

$LOQ = 10 \times S_{y/x}/S$.

3.6 Robustness

The robustness of the method was determined to assess the effect of small but deliberate variation of the chromatographic conditions on the determination of RUPA. Robustness was determined by changing the mobile phase flow rate to 0.9 and 1.1 mL $min^{-1}$and the concentration of methanol in the mobile phase to 48 and 52%.

3.7 Stability

The stability of the drug in solution during analysis was determined by repeated analysis of samples during the course of experimentation on the same day and also after storage of the drug solution for 48 hrs., under laboratory bench conditions (33 ± 1°C) and under refrigeration (8 ± 0.5°C).

3.8 Procedure for pharmaceutical formulation

For tablets, 20 units were weighed and finely powdered. An accurately weighed amount of the powder equivalent to 10 mg of Rupatadine fumarate was transferred into a 100 ml volumetric flask and sonicated for 15 min with 100 ml of mobile phase. The resulting suspension was filtered through 0.22μm membrane filter. A suitable aliquot of this filtrate was diluted with mobile phase in order to obtain a final concentration of 10 to 60 μg mL^{-1}. A 20 μl of the obtained solution was chromatographed.

RESULTS AND DISCUSSION

4.1 Method Development

The HPLC procedure was optimized with a view to developing a method. From several solvents and solvent mixtures investigated Acetonitrile: Methanol: Water in the ratio of 40:50:10 (v/v/v) was found to furnish sharp, well-defined peak with very good symmetry and low t_R (7.350 min) (Fig. 1). Various other mobile phases tried earlier either did not give well defined peak in a short time, therefore were not considered. The final selection on mobile phase composition and flow rate was made on the basis of peak shape (peak area, peak asymmetry & tailing factor), baseline drift, time required for analysis, and cost of solvent, and Acetonitrile:Methanol:Water in the ratio of 40:50:10 (v/v/v) was selected as the optimum mobile phase. Under these conditions the retention time was 7.35 ± 0.01 min.

Table 1. Optimized Chromatographic Conditions

Parameters	Conditions
Stationary phase (column)	HiQ Sil-C18 HS
Mobile Phase	Acetonitrile:Methanol:Water in the ratio of 40:50:10 (v/v/v)
Flow rate (ml min^{-1})	1.0
Runtime (min)	10
Column Temperature(°C)	Ambient
Volume of Injection (μl)	20
Detection wavelength (nm)	244nm
Drug Retention Time(min.)	7.350

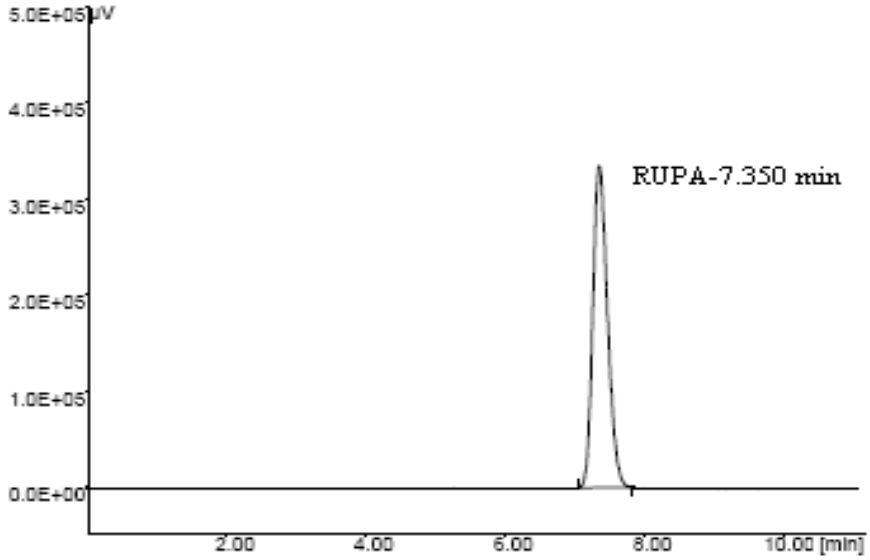

Figure 2. Typical chromatogram of RUPA in Acetonitrile: Methanol: Water (40:50:10)
t_R 7.350 min

5.0 Validation of the Method

5.1 Linearity

The calibration plot of peak area against concentration was linear in the range investigated (10–60 µg mL^{-1}). The low values of RSD and standard error show the method is precise. Statistical calculations were performed at the 5% level of significance. The linear regression data for the calibration plot are indicative of a good linear relationship between peak area and concentration over a wide range. The linear regression equation was y = 53294x + 359796 and the regression coefficient was 0.9996. 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. There were no significant differences between the slopes of standard curves constructed on different days.[11]

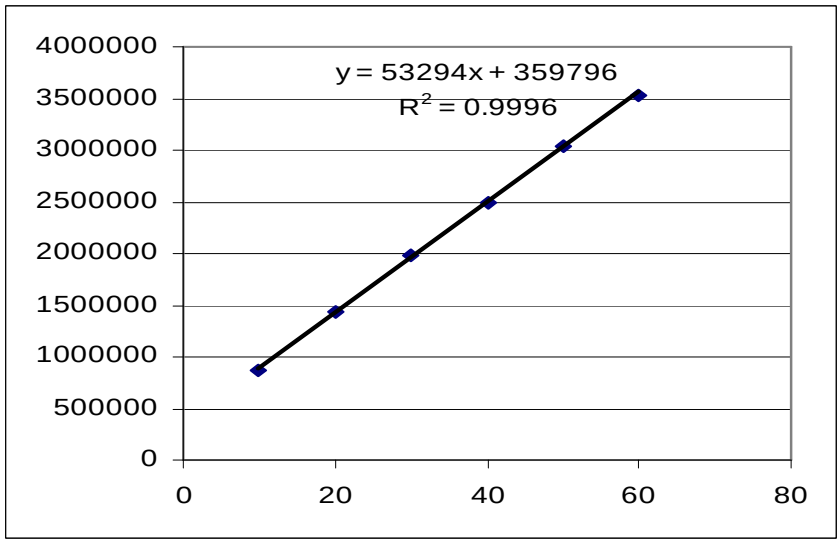

Figure 3: Calibration Curve of Rupatadine fumarate

Table 2. Statistical data of calibration curves of RUPA

Parameters	RUPA
Linearity (µg mL^{-1})	10 - 60
Regression equation	53294x + 359796
Correlation coefficient (R^2)	0.9996

Table 3. System Suitability Parameters

Parameters	Obtained Values
Theoretical plates (N)	5780
Tailing Factor	0.09
LOD (µg mL^{-1})	0.026
LOQ (µg mL^{-1})	0.056

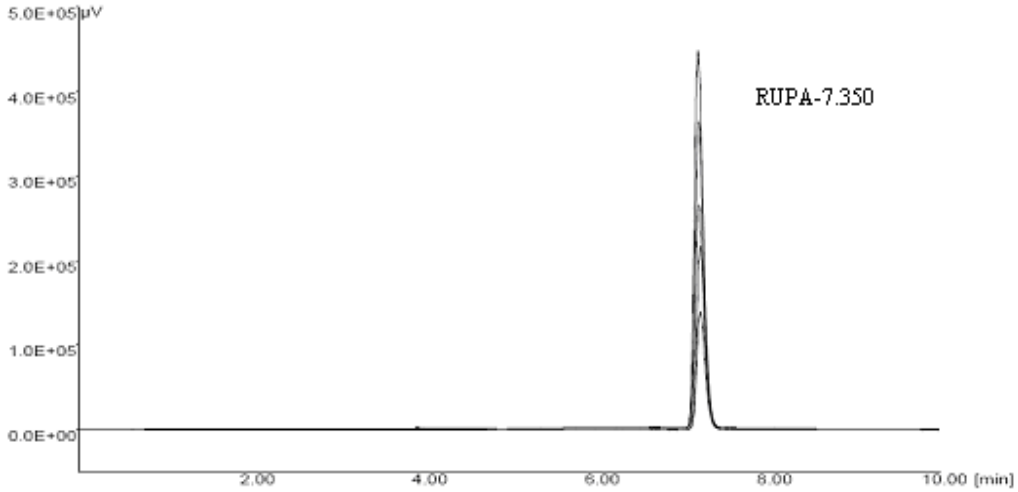

Figure 4. An overlain chromatogram of RUPA

5.2 Accuracy

The recovery of the method, determined by spiking a previously analyzed test solution with additional drug standard solution, was 99.06–100.60%. The values of recovery (%), RSD (%), indicate the method is accurate.[11]

Table 4. Result of Recovery Studies of Rupatadine fumarate

Level of Recovery	Amount Present in formulation (µg mL^{-1})	Amount of pure drug added (µg mL^{-1})	% Recovery*	R.S.D.	S.E.
80	10	8	99.06	1.49	0.027
100	10	10	100.48	0.95	0.038
120	10	12	100.60	1.68	0.035

** Indicates mean of three determination,, R.S.D. =Relative Standard Deviation, S.E. =Standard Error*

5.3 Precision

Intraday and inter-day precision were carried out for the various concentrations of the sample at different time intervals in the same day and at same time on different days. The concentration of the sample solution was determined as per the procedure given for the tablet formulation by determining peak area at selected analytical wavelength 244 nm. The variation of the results within the same day was analysed and statistically validated. [11]

Table 5. Results Analysis of Precision Studies

Concentration (µg mL^{-1})	Repeatability (intra day precision) *		Intermediate precision (inter day) *	
	% RSD	SE	% RSD	SE
10	0.68	0.58	0.61	0.85

** Indicates mean of six determinations, R.S.D. =relative Standard Deviation, S.E. =Standard Error*

5.4 Reproducibility

Reproducibility was checked by measuring the precision of the method on another column with analysis performed by another person. Both intra-day and inter-day precision were determined. There were no significant differences between RSD (%) values for intra-day and inter-day precision, which indicates the method, is reproducible. [11]

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

The LOD and LOQ of the method, determined by the standard deviation method, as described above, were 0.026 and 0.056 μg mL^{-1}, respectively, which indicated the method can be used for detection and quantification of RUPA over a very wide range of concentrations.[11]

5.6 Robustness

There was no significant change in the retention time of IND when the composition and flow rate of the mobile phase were changed. The low values of the RSD indicated the robustness of the method.[11]

Table 6. Robustness of the method

System suitability parameters	Normal condition	Change in condition	Change in % RSD
Flow Rate	1.0 mL min^{-1}	0.9 mL min^{-1} 1.1 mL min^{-1}	0.031 0.028
Mobile phase ratio (Acetonitrile: Methanol: water)	40:50:10	38:54:08 52:42:06	0.046 0.038

5.7 Analysis of RUPA from tablet formulation

The proposed method was applied to the determination of Rupatadine fumarate in tablets formulation (10 mg). The mean average (three replicates) was found to be 9.98 mg corresponding to a mean recovery of 99.08% with an R.S.D. of 0.025%. This result was in good agreement with the label value. It should be pointed out that the chromatogram of the solution of excipients is absolutely free of any peak indicating thus that no interference from the excipients is encountered.

Table7. Analysis of commercial formulation

Commercial formulation	Label claim (mg)	% Label claim estimated*	S.D.	%RSD
Tablet (Rupanex-M)	10	99.08	0.048	0.025

*SD= Standard deviation, RSD = Relative standard deviation, *Average of six determinations*

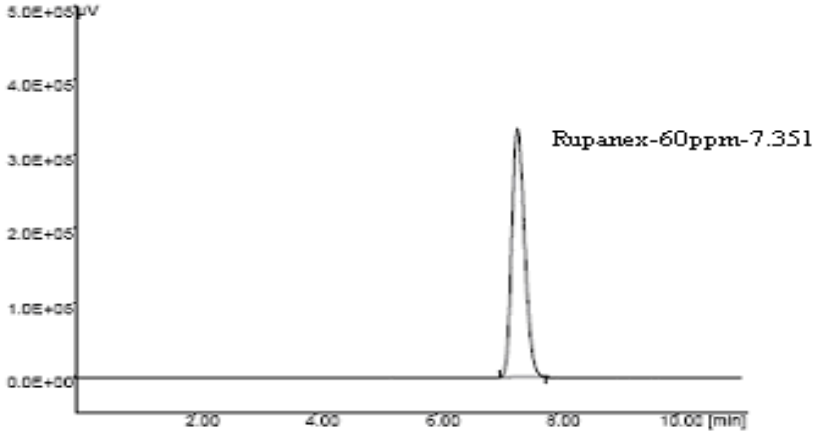

Figure 5. A chromatogram of RUPA formulation

CONCLUSION

A simple and rapid HPLC method has been developed for the determination of Indapamide. Statistical analysis of the results has been carried out revealing high accuracy and good precision. The method is reliable and convenient for routine control and stability assays of Indapamide in both raw material and tablets.

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REFERENCES

[1] J. Mullol, J. Bousquet , C Bachert, W.G. Canonica, Gimenez-Arnau A, M.L. Kowalski, *et al*. Rupatadine in allergic rhinitis and chronic urticaria. Allergy. **2008**; 63:5-28.
[2] P.G. Patel, V.M. Vaghela, S.G. Rathi, N.B. Rajgor, V.H. Bhaskar. Derivative spectrophotometry method for simultaneous estimation of rupatadine and montelukast in their combined dosage form. *Journal of Young Pharmacists*. **2009**; 1(4):354-8.
[3] A. Goyal, C.S. Sharma, G .Singh. Development of UV and visible spectrophotometric methods for estimation of Rupatadine fumarate from tablet formulation. *International journal of pharmaceutical research and development*. **2010** June 2(4).
[4] N. J. Malaviya*, R. K. Jat, R. Singh, K.G. Patel, RP-HPLC method development and its validation for assay of Rupatadine fumarate in Tablet dosage form *Inventi Rapid: Pharm Ana & Qual Assur*, Vol. 4 4/11/ **2012**
[5] WANG Hai-hong1, GE Ping2, ZHAO Wei3, HANG Tai-jun4, LI Tian-ao5, RP-HPLC determination of rupatadine fumarate and its related substances**,** *Chinese Journal of Pharmaceutical Analysis,* **2009**-05
[6] FU Chuan-shan, Determination of rupatadine fumarate by HPLC *China Tropical Medicine* **2009**,Vol. 9, No. 7 ,1352-1353
[7] L. Sergio, Dalmora, R. Daniele, Nogueira., Z Duilherme, Calegari., Ana C, Bergamo, e, Fernanda, Pavani, Stamm. Development and validation of a dissolution test with Reversed-phase liquid chromatography analysis for Rupatadine in tablet dosage forms. *Quim Nova*. **2010**;33(5):1150-4
[8] R. Daniele , Nogueira, Felipe B, Avila D, Clarice MB, Sergio D, L. Development and Validation of a Stability Indicating LC Method for the Determination of Rupatadine in Pharmaceutical Formulations. *Chromatographia*. **2007** 13 September 66:915-9.
[9] YAN Hui-juan, LI Qian, QIAO Jian, DENG Jun-gang, HUANG Hou-wu, LI Wei-yong, Determination of Plasma Concentration of Rupatadine Fumarate by HPLC-MS/MS **2008** 27 (10): 1174-1176
[10] A. H Beckett, J.B Stenlake,. Practical Pharmaceutical Chemistry. Fourth edition, Part 2, CBS Publishers and Distributors, New Delhi, **2002**, 275-278.
[11] ICH, Q2 (R1), Harmonized tripartite guideline, Validation of analytical procedures: text and Methodology International Conference on Harmonization.