Eurasian Journal of Analytical Chemistry

Eurasian J Anal Chem 9(1): 49-57, 2014

Development and Validation of a Reversed-Phase HPLC Method for Simultaneous Estimation of Rupatadine Fumarate and Montelukast Sodium from Their Combined Dosage Forms

Tanneru Raja[1], A. Lakshmana Rao [*], M.V.L. Ramanakanth[1], D. Ramya[1] and S. Padmaja[1]

[*]V. V. Institute of Pharmaceutical Sciences, Gudlavalleru - 521 356, A.P., India
[1]Hindu College of Pharmacy, Guntur - 522 002, A.P., India

Abstract

A simple, accurate, precise and rapid reversed-phase high performance liquid chromatographic (RP-HPLC) method has been developed and subsequently validated for the simultaneous estimation of Rupatadine Fumarate and Montelukast Sodium from their combination drug product. The proposed method is based on the separation of the two drugs in reversed-phase mode using Symmetry C-8 analytical column (150 x 4.6 mm; 5 µ). The optimum mobile phase consisted of acetonitrile: phosphate buffer p^H 4.7 adjusted with o-phosphoric acid (60:40, v/v), mobile phase flow rate of 1.2 mL min-1 and UV detection was set at 254 nm. The retention times were 3.22 and 10.67 min. for Rupatadine Fumarate and Montelukast Sodium, respectively. The method was validated according to ICH guidelines. It was found to be accurate and reproducible. Linearity was obtained in the concentration range of 100-300 µg mL^{-1} for both Rupatadine Fumarate and Montelukast Sodium with correlation coefficients of 0.999 and 0.999 respectively. Mean percent recovery of triplicate samples at each level for both drugs were found in the range of 98.7% to 99.5% with RSD of less than 2.0%. The proposed method can be successfully applied in the quality control of bulk manufacturing and pharmaceutical dosage forms.

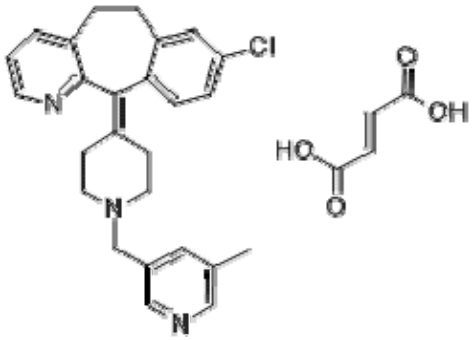
Keywords:

Rupatadine; Montelukast; HPLC; Validation

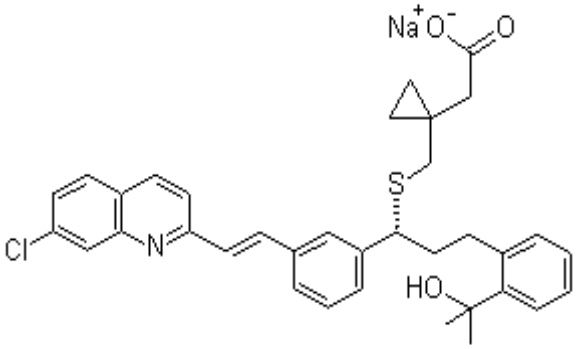
1. Introduction

Rupatadine Fumarate (RUP) (Fig. 1A) is a well-known non-sedating H_1 antihistaminic drug. Chemicaly it is 8-chloro-6,11-dihydro-11-(1-((5-methyl-3-pyridyl)methyl)-4-pyperidylidine)-5H-benzo-(5,6)-cyclohepta-(1,2-b) pyridine. It is potent, orally active and it was developed as a therapeutic agent for the treatment of seasonal allergic rhinitis and chronic idiopathic urticaria [1]. Montelukast Sodium (MTK) (Fig. 1B) is a specific cysteinyl leukotriene receptor antagonist belonging to a styryl quinolines series. Chemically it is 2-[1[1(R)-[3-[2(E)-(7-chloroquinololin-2-yl) vinyl] phenyl]-3[2-(1-hydroxy-1-methylethyl) phenyl]propylsulfanylmethyl] cyclopropyl]acetic acid sodium salt. It is developed as a therapeutic agent for the treatment of bronchial asthma [2].

[*] **Corresponding Author**
E-mail: dralrao@gmail.com
ISSN: 1306-3057



(A)



(B)

Fig. 1. Chemical structures of (A) Rupatadine Fumarate and (B) Montelukast Sodium

Literature survey reveals that few spectroscopic methods [3-9], chromatographic methods [10-16] and capillary electrophoresis [17] for determination of RUP and MTK in single and combination with other drugs. To the best of our knowledge hitherto there is no HPLC method reported for simultaneous determination of binary mixture containing RUP and MTK. Therefore, an attempt has been made to develop a simple, accurate, rapid and reproducible reverse phase HPLC method for simultaneous determination of RUP and MTK in tablet dosage form and validated in accordance with ICH guidelines [18].

2. Experimental

2.1 Materials and Reagents

Pharmaceutical grade of RUP and MTK were kindly supplied as gift samples by Dr.Reddy's Laboratories Ltd., Hyderabad, India, certified to contain > 99% (w/w) on dried basis. Commercially available Rupanex-M (Dr.Reddy's Laboratories Ltd, Hyderabad, India) tablets claimed to contain 10 mg of RUP and 10 mg of MTK was purchased from local market. All chemicals and reagents used were HPLC grade purchased from Merck Chemicals Ltd, Mumbai, India.

2.2 Chromatographic system and conditions

Separation was performed with Waters HPLC equipped with a pump-515, auto sampler- 2707 and UV detector-2998, operated at 254 nm. Empower software was applied for

data collecting and processing. A Systronics-361 pH meter was used for pH measurements. The separation was achieved on a C-8 (150 x 4.6 mm, 5 µ) analytical column. The mobile phase consisted of acetonitrile: phosphate buffer P^H 4.7 was adjusted with o-phosphoric acid in the ratio 60:40 (v/v). The flow rate was 1.2 mL min^{-1} and UV detection was performed at 254 nm. The mobile phase was shaken on an ultrasonic bath for 30 min. the resulting transparent mobile phase was filtered through a 0.45 µ membrane filter (Millipore, Ireland). The injection volume was 20 µL and all the experiments were performed at ambient temperature.

2.3 Standard and Test Solutions

2.3.1 Preparation of Standard Solution

Standard stock solutions were prepared by dissolving separately 10 mg of RUP and MTK in 10 mL diluents. The standard calibration solutions were prepared by appropriate dilution of the stock solution with mobile phase to reach a concentration range of 100-300 µg mL^{-1} for RUP and MTK. Triplicate 20 µl injections were made for each concentration and injected under the optimized conditions described above. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

2.3.2 Preparation of Test Solution

Twenty tablets were accurately weighed, their mean weight was determined and they were mixed and finally powdered. A portion equivalent to about 10 mg was accurately weighed and transferred into a 10 mL volumetric flask containing 7 mL of mobile phase, sonicated for 30 min and diluted to 10 mL with mobile phase. The resulting solution was centrifuged at 3000 rpm for 5 min. Supernatant was taken and after suitable dilution the sample solution was then filtered using 0.45 µ filter. A 20 µL volume of sample solution was injected into HPLC, six times. The peak areas for the drugs were measured at 254 nm and amounts of RUP and MTK were determined using the related linear regression equations.

2.4 Method validation

The developed method was validated according to ICH guidelines. The system suitability was evaluated by six replicate analysis of RUP and MTK mixture at concentration of 200 µg mL^{-1}. The acceptance criteria were % RSD of peak areas and retention time less than 2%, theoretical plates numbers (N) at least 5000 per each peak and tailing factors (T) less than 1% for RUP and MTK.

Standard calibration curves were prepared in the mobile phase with five concentrations ranging from 100-300 µg mL^{-1} for RUP and MTK triplicate into the HPLC system keeping the injection volume constant. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs. To study the reliability and suitability of the developed method, recovery experiments are carried out at three levels 80%, 100% and 120%. Known concentrations of commercial tablets were spiked with known amounts of RUP and MTK. At each level of the amount six determinations were performed and the results obtained were compared with expected results.

Recovery for pharmaceutical formulations should be within the range of 100±5%. The % RSD of individual measurements was also determined. Precision of assay was determined by repeatability (intra-day) and intermediate precision (inter-day) for three consecutive days. Three different concentrations of RUP and MTK were analyzed in six independent series in the same day (intra-day precision) and three consecutive days (inter-day precision). Every sample was injected in triplicate. The repeatability of sample application and measurement of peak area for active compounds were expressed in terms of % RSD. All chromatograms were

examined to determine if compounds of interest co-eluted with each other or with any additional excipient peaks. Marketed formulations were analyzed to determine the specificity of the optimized method in the presence of common tablet excipients.

Limit of Detection (LOD) and Limit of Quantitation (LOQ) were estimated from the signal-to-noise ratio. LOD and LOQ were calculated using 3.3 σ/*s* and 10 σ/*s* formulae, respectively, where, σ is the standard deviation of the peak areas and *s* is the slope of the corresponding calibration curve. To evaluate robustness of HPLC method a few parameters were deliberately varied. The parameters included variation of flow rate, percentage of buffer in the mobile phase and pH of mobile phase.

3. Results and Discussion

During the optimization of HPLC method, two columns (Symmetry C-8, 250 × 4.6 mm, 5 µ and Symmetry C-8, 150 × 4.6 mm, 5 µ), two organic solvents (acetonitrile and methanol), two buffers (acetate and phosphate) at two different pH values (3 and 5) were tested. Initially methanol: acetate buffer, acetonitrile: acetate buffer, methanol: phosphate buffer, acetonitrile: phosphate buffer were tried in different ratios at pH 3 and 5. RUP and MTK eluted with tried mobile phases. Then, with acetonitrile: phosphate buffer all the two drugs eluted, but the analysis time was more than 15 min. In order to decrease the analysis time, column length was reduced from 250 to 150 mm. The mobile phase conditions were optimized so the peak from the first-eluting compound did not interfere with those from the solvent and excipients. Other criteria, time required for analysis, appropriate k range for eluted peaks, assay sensitivity, solvent noise were also considered. Finally a mobile phase consisting of mixture of acetonitrile: phosphate buffer pH 4.7 adjusted with o-phosphoric acid in ratio 60:40 (v/v), was selected as mobile phase to achieve maximum separation and sensitivity. Flow rates between 0.8 to 1.4 mL min^{-1} were studied. A flow rate of 1.2 mL min^{-1} gave an optimal signal to noise ratio with a reasonable separation time. Using a reversed phase C-8 column, the retention times for RUP and MTK were observed to be 3.22 and 10.67 min. respectively. Total analysis time was less than 13 min. The chromatogram at 254 nm showed a complete resolution of all peaks (Fig. 2).

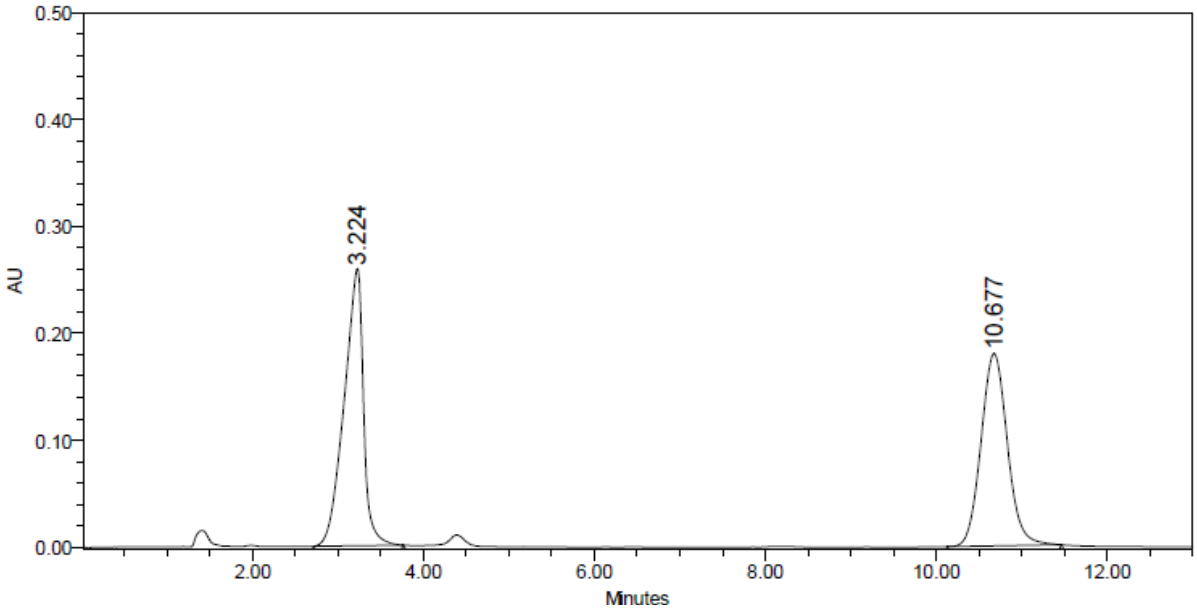


Fig. 2. Representative chromatogram of standard solutions of RUP and MTK

3.1 System suitability

The efficiency of the column was expected by the number of theoretical plates and the tailing factor. The system suitability data for RUP and MTK are shown in Table 1.

Table 1. Data indicating System Suitability Parameters

Parameters	RUP	MTK
Retention time (min)	3.22	10.67
Theoretical Plates	5850	5 450
Asymmetry	1.07	1.25
Resolution	2.3	5
Capacity Factor	0.46	0.49
Tailing Factor	0.8	1.2

3.2 Linearity and Range

Excellent linearity was obtained for all the two drugs in the range of 100-300 µg mL^{-1} for RUP and MTK. The correlation coefficient (r^2) were found to be greater than 0.999 (n=6) in all instances. The results of calibration studies are summarized in Table 2.

Table 2. Data indicating linearity of the proposed method

Parameters	RUP	MTK
Linearity range	100-300 µg mL^{-1}	100-300 µg mL^{-1}
Slope	27399	25827
Correlation coefficient	0.999	0.999

3.3 Recovery

The proposed method afforded high recoveries for RUP and MTK tablets. Results obtained from recovery studies presented in Table 3, indicate that this assay procedure can be used for routine quality control analysis of binary mixture in tablets.

Table 3. Result of accuracy data of RUP and MTK

Label claim (mg/tablet)	Amount added (%)	Total amount added (µg)	Amount recovered (µg)	Recovery±SD*, %	% RSD
RUP (10)	80	18	17.87	99.2±0.25	0.42
	100	20	19.88	99.4±0.39	0.50
	120	22	21.90	99.5±0.12	0.23
MTK (10)	80	18	17.89	99.3±0.68	0.32
	100	20	19.75	98.7±0.55	0.58
	120	22	21.88	99.4±0.26	0.36

*n=6, SD: Standard Deviation, % RSD: Relative Standard Deviation

3.4 Precision

Precision of the analytical method was found to be reliable based on % RSD (<2%) corresponding to the peak areas and retention times. As can be seen in Table 4, the % RSD values were less than 2, for intra-day and inter-day precision. Hence, the method was found to be precise for all the two drugs.

Table 4. Result of intra-day precision and inter-day precision for simultaneous determination of RUP and MTK

Drug	Concentration (µg mL^{-1})	Intra-day		Inter-day	
		Mean peak area	% RSD	Mean peak area	% RSD
RUP	150	2763264	0.03	2694320	0.029
	200	4138047	0.02	4137089	0.019
	250	5534869	0.50	5744876	0.51
MTK	150	2692930	1.81	2854965	1.91
	200	3898198	0.02	3954864	0.02
	250	5082546	0.19	5489625	0.20

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

LOD and LOQ were found to be 0.12, 0.40 µg mL^{-1} for RUP and 0.22, 0.80 µg mL^{-1} for MTK.

3.6 Robustness

In all deliberately varied conditions, the SD of retention times of RUP and MTK were found to be well within the acceptable limit. The tailing factor for all the two peaks were found to be <1.5 (Table 5).

Table 5. Results of Robustness study

Factor[b]	Level	RUP		MTK	
		T_r[C]	T.F[d]	T_r[C]	T.F[d]
A: Flow rate (mL min^{-1})					
1.0	-1	3.28	1.1	10.59	0.8
1.2	0	3.22	0.8	10.67	0.6
1.3	1	3.32	1.4	10.71	1.1
Mean±SD (n=6)		3.27 ±0.05	1.1±0.05	10.69±0.02	0.83±0.035
B: % buffer in mobile phase (v/v)					
39	-1	3.17	1.2	10.74	0.94
40	0	3.22	0.78	10.67	0.9
41	1	3.28	1.3	10.70	0.86
Mean±SD (n=6)		3.273±0.055	1.09±0.016	10.70±0.03	0.9±0.8
C: pH of mobile phase					
4.6	-1	3.20	1.3	10.72	1.06
4.7	0	3.22	1.1	10.67	0.58
4.8	1	3.26	1.4	10.72	1.3
Mean±SD (n=6)		3.22±0.08	1.26±0.06	10.70±0.02	0.98±0.05

[a]Concentration used was 200 µg mL^{-1}. [b]Three factors were slightly changed at three different levels (-1,0,1), [c]Retention time, [d]Tailing factor

3.7 Analysis of Marketed Formulation

The validated method was used in the analysis of marketed conventional tablets Rupanex-M with a label claim: 10 mg of RUP and 10 mg of MTK per tablet. Representative

chromatogram is shown in Fig 3. The results for the drugs assay showed good agreement with the label claims (Table 6).

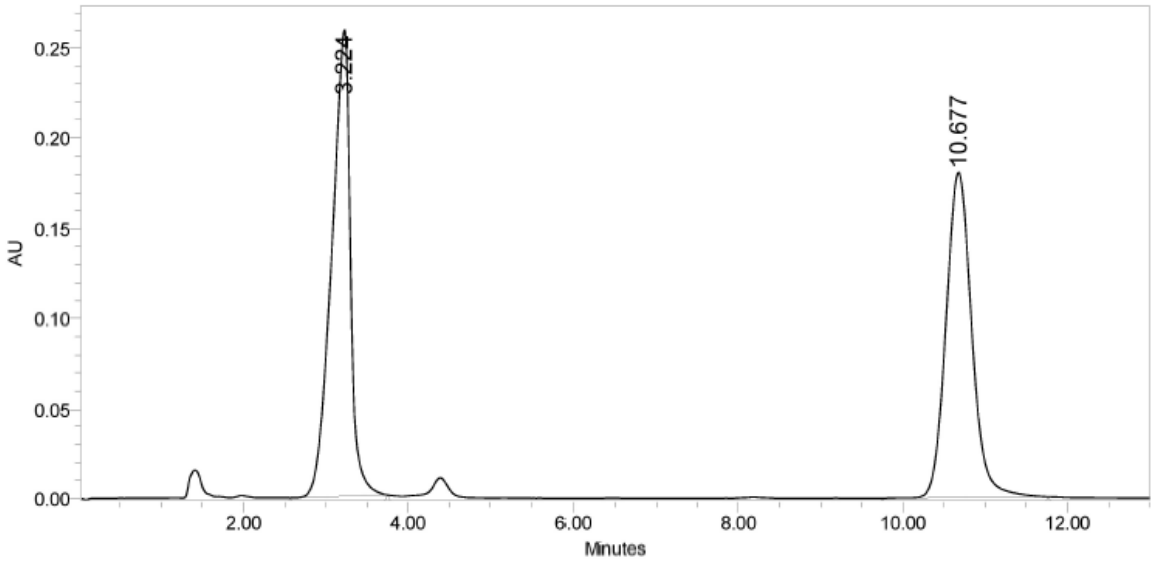


Fig. 3. Representative chromatogram obtained for marketed formulation for RUP and MTK

Table 6. Results of assay in commercial formulation

Compound	Label claim (mg)	Rupanex-M tablets	
		Amount found (mg)	% Drug content*
RUP	10	9.8	98
MTK	10	10.1	101

*n=6

4. Conclusion

The developed HPLC method is simple, specific, accurate and precise for the simultaneous determination of RUP and MTK from tablets. The developed method provides good resolution between RUP and MTK. It was successfully validated in terms of system suitability, linearity, precision, accuracy, specificity, LOD, LOQ and robustness in accordance with ICH guidelines. Thus, the described method is suitable for routine analysis and quality control of pharmaceutical preparations containing these drugs either as such or in combination.

5. Acknowledgements

The authors are thankful to Dr.Reddy's Laboratories Ltd, Hyderabad, for providing rupatadine fumarate and montelukast sodium as gift samples for this work, and also thankful to Hindu College of Pharmacy, Guntur, for providing required facilities for research work.

6. References

1. Del Cuvillo A, Mullol J, Bastsa J, Davila I and Montoro J (2006) Comparative pharmacology of the H_1 antihistamines. J Investig Allergol Clin Immunol 16: 3-12.
2. Anick L, Claudine F and Guy M (2006) Montelukast regulates eosinophil protease activity through leukotriene independent mechanism. J Allergy clin Immunology 118 (1): 113-119.

3. Anju G, Sharma C S and Singh G (2010) Development of UV and Visible spectrophotometric methods for estimation of Rupatadine Fumarate from tablet formulation. Int J Pharm Dev 2(4): 10.
4. Rele R V, Desai P D and Sawant A (2010) Simple extractive spectrophotometric determination of Rupatadine Fumarate from pharmaceutical formulations. Int J Chem Sci 8(1): 22-28.
5. Patel P G, Vaghela V M, Rathi S G and Rajgor N B (2009) Derivative spectrophotometry method for simultaneous estimation of Rupatadine and Montelukast in their combined dosage form. J Young pharmacists 1(4): 354-358.
6. Patel D J, Patel S A, Patel S K (2010) Simultaneous determination of Montelukast Sodium and Bambuterol Hydrochloride in tablet dosage form by UV spectrophotometry (Dual wavelength method). Int J Pharm Biomed Res 1(3): 71-75.
7. Vishnu P, Anamika N, Satish A and Abhijit S (2011) Derivative and absortive factor spectrophotometric estimation of Montelukast Sodium and Levocetrizine Dihydrochloride from pharmaceutical formulation. Res J Pharm Tech 4(03): 386-389
8. Patel N K and Pancholi S (2011) Spectrophotometric determination of Montelukast Sodium and Levocetrizine Dihydrochloride in tablet dosage form by AUC curve method. Der Pharma chemica 3(5): 135-140.
9. Choudhari V, Kale A, Abnane S and Gawli V (2010) Simultaneous determination of Montelukast Sodium and Levocitrizine Dihydrochloride in pharmaceutical preparation by ratio derivative spectroscopy. Int J Pharm Tech 2(1): 04-09.
10. Yuan T, Jingjing Z, Jiabi L and Yun C (2008) High performance liquid chromatography – tandem mass spectrometric determination of Rupatadine in human plasma and its pharmacokinetics. J Pharm Biomed Anal 47(4-5): 899-906.
11. Smitha P, Pore Y V, Kuchekar B S and Khire V G (2009) Determination of Montelukast Sodium and Bambuterol Hydrochloride tablets using RP-HPLC. Ind J Pharm Sci 71(1): 58-61.
12. Alireza S, Afshin Z, Seyed M (2010) Rapid and sensitive determination of Montelukast in human plasma by HPLC method using monolithic column application to pharmacokinetic studies. J Bioequi Availab 2(06): 135-138.
13. Shaiba N, Devi K, Prashanthi R, Raghavi K and Sindhura M (2011) High performance thin layer chromatographic estimation of Rupatadine Fumarate. Res J Pharm Tech 4(3): 420-422.
14. Sane R T, Ajai M, Mandar M, Gunesh G (2004) HPTLC Determination of Montelukast Sodium in bulk drug and its pharmaceutical preparations. J Pharm Chem 17(1): 75-78.
15. Jun W, Zhanying H, Hua W, Guorong F (2009) Simultaneous determination of Rupatadine and its metabolite desloratadine in human plasma by a sensitive LC-MS/MS method Application to the pharmacokinetic study in healthy Chinese volunteers. J Pharm Biomed Anal 49(2): 347-353.
16. Daniele R N, Maximilianoda S S and Vitor T (2008) Determination of Rupatadine in pharmaceutical formulation by a validated stability – indicating method. J Sep Sci 31(17): 3098-3105.

17. Shakalisava Y and Ragan F (2008) Determination of Montelukast Sodium by capillary electrophoresis. J Sep Sci 31(17): 1137-1143.

18. ICH (1996) Proceedings of the International Conference on Harmonisation of Technical Requirement of Registration of Pharmaceutical for Human Use (ICH Harmonised Tripartite Guidelines). Validation of Analytical Procedures: Methodology, Q2R1, Geneva, Switzerland.