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Research Article

Analytical Method Development and Validation for Roflumilast and Montelukast in Combine Dosage Form by RP-HPLC

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Abstract

The proposed HPLC method was found to be simple, specific, precise, accurate, rapid and economical for simultaneous estimation of Roflumilast and Montelukast in tablet dosage form. The developed method was validated in terms of accuracy, precision, linearity, robustness and ruggedness, and results will be validated statistically according to ICH guidelines. The Sample recoveries in all formulations were in good agreement with their respective label claims. From literature review and solubility analysis initial chromatographic conditions Mobile phase ortho phosphoric acid buffer: Methanol 25:75 were set (Buffer P^H 2.45 adjusted with Triethylamine), Inertsil C 18 (250×4.6mm, 5µ) Column, Flow rate 1.0 ml/min and temperature was ambient, eluent was scanned with PDA detector in system and it showed maximum absorbance at 254 nm. As the methanol content was increased Roflumilast and Montelukast got eluted with good peak symmetric properties. The retention times for Roflumilast and Montelukast was found to be 2.589 min and 3.711 min respectively. System suitability parameters were studied by injecting the standard five times and results were well under the acceptance criteria. Linearity study was carried out between 50% to 150 % levels, R^2 value was found to be as 0.999. By using above method assay of marketed formulation was carried out, 100.7% was present. Full length method was not performed, if it is done this method can be used for routine analysis of Roflumilast and Montelukast.

Keywords: *Inertsil C 18, Roflumilast and Montelukast, HPLC*

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1. Introduction

Roflumilast is a highly selective phosphodiesterase-4 (PDE4) inhibitor.[7] PDE4 is a major cyclic-3',5'-adenosinemonophosphate (cyclicAMP,cAMP) metabolizing enzyme [8] expressed on nearly all immune and pro-inflammatory cells, in addition to structural cells like those of the smooth muscle or epithelium.[7] The resultant increase in intracellular cAMP induced by roflumilast's inhibition of PDE4 is thought to mediate its disease-modifying effects, although its precise mechanism of action has yet to be elucidated**.**

Montelukast was first approved for clinical use by the US FDA in 1998 as Merck's brand name Singulair. The medication is a member of the leukotriene receptor antagonist (LTRA) category of drugs. Although capable of demonstrating effectiveness, the use of such LTRAs like montelukast is typically in addition to or complementary with the use of inhaled corticosteroids or other agents in asthma step therapy.1 Regardless, in 2008-2009, there were FDA-led investigations into the possibility of montelukast to elicit neuropsychiatric effects like agitation, hallucinations, suicidal behaviour, and others in individuals who used the medication.2 And although these kinds of effects are currently included in the official prescribing information for montelukast, the drug still sees extensive use worldwide via millions of prescriptions annually and has since become available as a generic and as a brand name product.

2. Materials and Methods

Instrumentation

The instrument used was HPLC waters 2690 separation module with photo diode array detector, Software-empower. The stationary phase used was Inertsil (250×4.6mm, 5µ) ODS C-18 RP-column Digital weighing balance-Model number BSA224SCW (Ascoset), Sonicator (Enertech)-SE60US, pH meter Model number AD102U.

Materials and reagents

Roflumilast and Montelukast were gift samples provided by Hetero Laboratories, Hyderabad, Ortho phosphoric acid, Potassium dihydrogen, Tri ethyl amine, Methanol and Water for HPLC were supplied by Merck India Ltd, Mumbai

Method development

Six trials were made by changing the mobile phase ratios and solvents Buffer: Methanol P^H 2.5 (30:70 v/v) Buffer: Methanol P^H 2.5 (30:70 v/v) Buffer: Methanol P^H 2.5 (60:40 v/v)Phosphate buffer: Methanol P^H 2.5(20:80v/v) Phosphate buffer: Methanol P^H 2.5 (55:45 v/v) Phosphate buffer: Methanol P^H 2.5 (25:75 v/v). Finally, the mobile phase was optimized to Methanol: Phosphate buffer P^H 2.5 (25:75 v/v).

Chromatographic conditions

From literature review and solubility analysis initial chromatographic conditions Mobile phase ortho phosphoric acid buffer: Methanol 25:75 were set (Buffer P^H 2.45 adjusted with Triethylamine), Inertsil C 18 (250×4.6mm, 5µ) Column, Flow rate 1.0 ml/min and temperature was ambient, eluent was scanned with PDA detector in system and it showed maximum absorbance at 254 nm.

3. Results and Discussion

System Suitability:

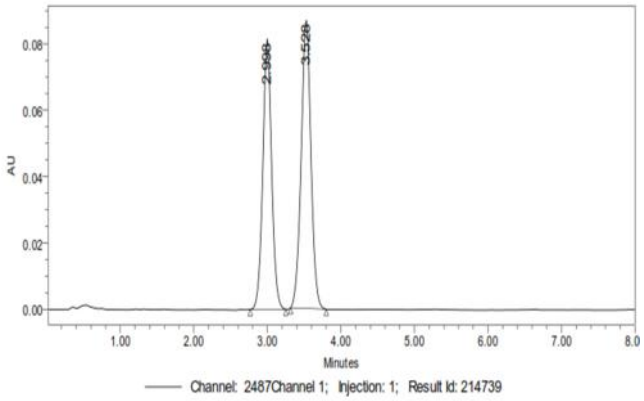

Figure 1 Chromatogram of sample system suitability

Report:

All the System suitability parameters were satisfied, thus the method passed the System suitability test.

Linearity:

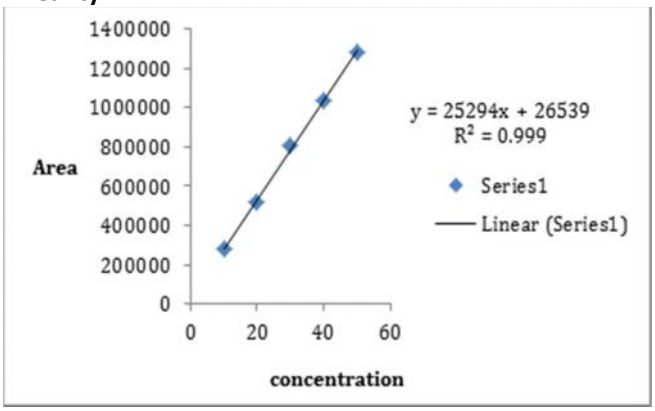

Figure 2 Linearity Graph of Montelukast

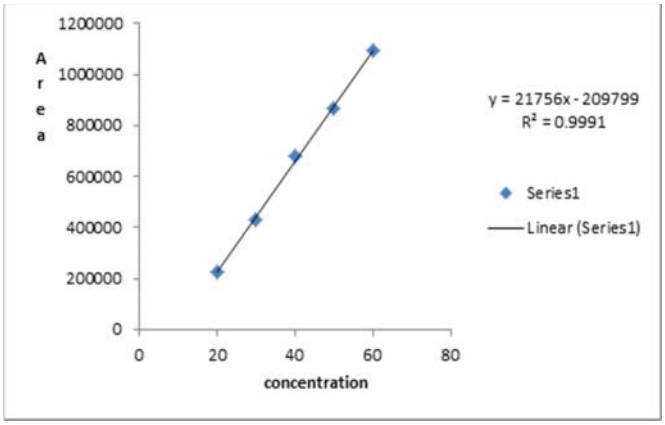

Figure 3 Linearity Graph of Roflumilast

Table 1 Preparation of Working standard solutions for Linearity

Sample ID	Roflumilast		Montelukast	
	Concentration (mcg/ml)	Area	Concentration (mcg/ml)	Area
20% of operating concentration	20	226418	10	277182
40% of operating concentration	30	432920	20	521695
60% of operating concentration	40*	677256	30*	808274
80% of operating concentration	50	869825	40	1033875
100% of operating concentration	60	1095759	50	1285804
Correlation Coefficient	0.999		0.999	

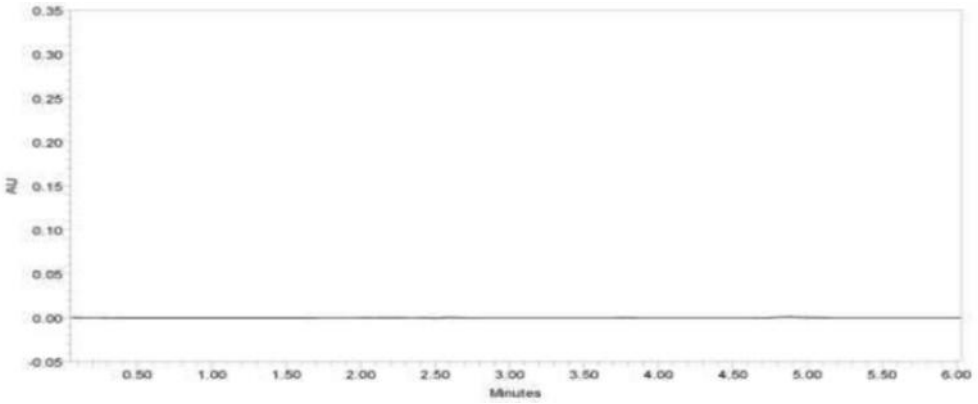

Figure 4 Chromatogram of Blank

Table 2 Precision Results for Roflumilast

	Peak name	**RT**	**Area**
1	Montelukast	3.557	819305
2	Montelukast	3.547	807157
3	Montelukast	3.544	804070
4	Montelukast	3.537	808474
5	Montelukast	3.534	804505
Mean			808702
Std.dev			6203.7
%RSD			0.77

Table 3 Precision Results for Montelukast

	Peak name	RT	Area
1	Roflumilast	3.019	691143
2	Roflumilast	3.011	685431
3	Roflumilast	3.004	683543
4	Roflumilast	2.997	683564
5	Roflumilast	2.994	683532
Mean			685443
Std.dev			3289
%RSD			0.4

Intermediate Precision: Intermediate precision of the analytical method was determined by performing method precision on another day by different analysts under same experimental condition. Assay of all six replicate sample preparations was determined and mean %assay value, standard deviation & %RSD was calculated.

Table 4 Intermediate Precision Results for Roflumilast

	Peak name	RT	Area
1	Montelukast	3.524	813507
2	Montelukast	3.533	817673
3	Montelukast	3.533	815189
4	Montelukast	3.517	815816
5	Montelukast	3.530	815356
Mean			815508
Std.dev			1492
%RSD			0.1

Table 5 Intermediate Precision Results for Montelukast

	Peak name	RT	Area
1	Roflumilast	3.001	673725
2	Roflumilast	3.009	672535
3	Roflumilast	3.010	676216
4	Roflumilast	2.997	679037
5	Roflumilast	3.007	677101
Mean			675723
Std.dev			2611
%RSD			0.3

Accuracy: Accuracy of the method was determined by recovery experiments. There are mainly 2types of recovery studies are there.

a) Standard addition method: To the formulation, the reference standard of the respective drug of known concentration was added, analyzed by HPLC and compared with the standard drug concentration.
b) Percentage method: For these assay method samples are prepared in three concentrations of 50%, 100%, and 150% respectively.

Acceptance criteria: The mean % recovery of the Roflumilast and Montelukast at each level should be not less than 95.0% and not more than 105.0%.

Table 6 Accuracy Study of Roflumilast

Sample Id	Conc found (µg/ml)	Concn Obtained (µg/ml)	%Recovery	Mean recovery	Statistical Analysis
50%	5	5.01	100.2		
50%	5	4.96	99.2	99.73	%RSD= 0.505
50%	5	4.99	99.8		
100%	10	9.95	99.5		
100%	10	9.87	98.7	98.8	%RSD=0.66
100%	10	9.82	98.2		
150%	15	14.64	97.6		
150%	15	14.76	98.4	98.8	%RSD=1.45
150%	15	15.06	100.4		

Table 7 Accuracy Study of Montelukast

Conc (µg/ml)	Concn Obtained(µg/ml)	%Recovery of drug	Mean accuracy	%RSD
5	4.92	98.0		

5	4.96	99.2		
5	5.02	100.4	99.2	1.2
10	9.95	99.5		
10	9.94	99.4		
10	9.98	99.8	99.5	0.2
15	14.78	98.6		
15	14.94	99.6	99.0	0.530
15	14.83	98.8		

Limit of Detection and Limit Of Quantification: The Sensitivity of measurement of Roflumilast and Montelukast by use of the proposed method was estimated in terms of the Limit of Detection (LOD) and the Limit of Quantitation (LOQ). The LOD and LOQ were calculated by the use of the equations:

$$LOD = 3.3 \times \frac{\sigma}{S}$$

$$LOQ = 10 \times \frac{\sigma}{S}$$

Where, σ is the standard deviation of intercept of calibration plot and S is the average of the slope of the corresponding calibration plot. The LOD and LOQ values for Roflumilast and Montelukast were reported in the Table.

Table 8: LOD and LOQ Data of Roflumilast and Montelukast

Roflumilast Conc.(x) (µg/ml)	Peak Areas (y)	Statistical Analysis	Montelukast Conc.(x) (µg/ml)	Peak Areas (y)	Statistical Analysis
40	2004682	S = 39092 c = 618048	20	1184227	S = 39092 c =369381
40	2004587	LOD: 0.001µg/ml LOQ: 0.004µg/ml	20	1186425	LOD:0.005 µg/ml LOQ: 0.015µg/ml

Table 9: Robustness data for Roflumilast

Std. Replicate	Variation in flow rate		Variation in Mobile phase composition	
	Flow Rate 0.8ml/min	Flow Rate 1.2ml/min	Buffer: Methanol (40:60)	Buffer: Methanol (30:70)
Tailing factor	0.9	0.9	1.1	1.1
Theoretical plates	2690	2503	2707	2818

Table 10: Robustness data for Montelukast

Parameter	Variation in flow rate		Variation in Mobile phase composition	
Standard	Flow Rate 0.8ml/min	Flow Rate 1.2ml/min	Buffer: Methanol (40:60)	Buffer: Methanol (30:70)
Tailing factor	0.9	0.9	1.0	1.0
Theoretical plates	2716	2685	30018	3107

Report:

Roflumilast and Montelukast peaks in the chromatogram passed the system suitability criteria. %RSD of peak areas of Roflumilast and Montelukast was not more than 2.0% for variation in mobile phase composition. From the above data, it was concluded that the method was robust.

4. Conclusion

The proposed HPLC method was found to be simple, specific, precise, accurate, rapid and economical for simultaneous estimation of Roflumilast and Montelukast in tablet dosage form. The developed method was validated in terms of accuracy, precision, linearity, robustness and ruggedness, and results will be validated statistically according to ICH guidelines. The Sample recoveries in all formulations were in good agreement with their respective label claims. From literature review and solubility analysis initial chromatographic conditions Mobile phase ortho phosphoric acid buffer: Methanol 25:75 were set (Buffer P^H 2.45adjusted with Triethylamine), Inertsil C18 (250× 4.6mm,5µ) Column, Flow rate 1.0 ml/min and temperature was ambient, eluent was scanned with PDA detector in system and it showed maximum absorbance at 254 nm. As the methanol content was increased Roflumilast and

Montelukast got eluted with good peak symmetric properties. The retention times for Roflumilast and Montelukast was found to be 2.589 min and 3.711 min respectively. System suitability parameters were studied by injecting the standard five times and results were well under the acceptance criteria. Linearity study was carried out between 50% to150 % levels, R^2 value was found to be as 0.999. By using above method assay of marketed formulation was carried out, 100.7% was present. Full length method was not performed; if it is done this method can be used for routine analysis of Roflumilast and Montelukast.

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