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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF MONTELUKAST SODIUM AND FEXOFENADINE HCL IN A PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, precise, specific and accurate reverse phase high performance liquid chromatography (RP-HPLC) method development and validation for the simultaneous estimation of Montelukast sodium and Fexofenadine HCl in pharmaceutical tablet dosage form. The different analytical performance parameters such as linearity, accuracy, precision, specificity, LOD and LOQ were determined according to ICH guidelines. RP-HPLC was conducted on Hypersil BDS C18 (250 mm length x 4.6 mm ID, 5µm) column. The mobile phase was consisting of mixed phosphate buffer pH-3 and Acetonitrile in the ratio (20:80% v/v) and the flow rate was 1ml/min. Montelukast sodium and Fexofenadine HCl were monitored using Shimadzu Prominence (Spinchrom ModuleLC-20AT) with auto sampling injector and UV detector. Linearity was observed in concentration ranges of 0.4-2.4 µg/ ml and 4.8-28.8 µg/ ml for Montelukast sodium and Fexofenadine HCl respectively. Regression equation of Montelukast sodium is y=42.98x+6.536, and of Fexofenadine HCl is Y = 5.859+11.58, Correlation coefficient was found to be 0.999, 0.999 for Montelukast sodium and Fexofenadine HCl respectively. The % recovery was found to be 99.74% for Montelukast sodium, 100.09% for Fexofenadine HCl. LOD value of Fexofenadine HCl and montelukast sodium was found to be 0.13, 0.026, respectively. LOQ value of Fexofenadine HCl and Montelukast sodium was found to be 0.397, 0.081 respectively.

Keywords: Montelukast sodium, Fexofenadine HCl, RP-HPLC, Linearity.

INTRODUCTION

Montelukast is a selective leukotriene receptor antagonist of the cysteinyl leukotriene CysLT $_1$ receptor. The cysteinyl leukotrienes (LTC $_4$, LTD $_4$, LTE $_4$) are products of arachidonic acid metabolism that are released from various cells, including mast cells and eosinophils. They bind to cysteinyl leukotriene receptors (CysLT) found in the human airway. Binding of cysteinyl leukotrienes to leukotriene receptors has been correlated with the pathophysiology of asthma, including airway edema, smooth muscle contraction, and altered cellular activity associated with the inflammatory process, factors that contribute to the signs and symptoms of asthma [1-4]. Fexofenadine is a second-generation selectively peripheral H1-blocker of the GI tract, large blood vessels, and bronchial smooth muscle. Blockage prevents the activation of the H1 receptors by histamine, preventing the symptoms associated with allergies from occurring [5-8]. Fexofenadine cannot cross the blood-brain barrier and therefore does not cause drowsiness. It also exhibits no anticholinergic, antidopaminergic, alpha adrenergic, or beta-adrenergic-receptor-blocking effects [9-12]

MATERIALS AND METHODS

Chemicals: Acetonitrile HPLC (Merck), Methanol HPLC (Merck), Potassium dihydrogen ortho

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phosphate AR (Merck), Di-Potassium hydrogen ortho phosphate AR (Merck), Ortho phosphoric acid AR (Merck), Water (Millipore)

Instrumentation and chromatographic conditions: Electronic balance Shimadzu linear over concentration range of 20- 120 µg/ml and 1060 µg/ml with correlation coefficients 0.998 and 0.999.HPLC Shimadzu Separation modulelc-20AT Prominence Liquid Chromatography. UV Detector, Chromatographic data Software: Spinchrom, Hypersil BDS 150×4.6mm. 5µ column. Vacuum filter pump, Mobile phase reservoir, Ultrasonicator pH meter

PREPARATION OF STANDARD AND SAMPLE SOLUTIONS

Preparation of Mobile Phase

The mobile phase was prepared by mixing mixed phosphate buffer (pH-3) and Acetonitrile in the ratio 20:80. The mobile phase is then filtered through 0.45µ filter paper and is sonicated using Ultra-Sonicator to remove the impurities and dissolved gases, as they may lead to unwanted peaks in the chromatogram.

Preparation of Mixed phosphate buffer

Dissolve 2.950g Potassium dihydrogen ortho phosphate and 0.54g Di- Potassium hydrogen ortho phosphate in 1000ml water pH is adjusted 3.0 using dilute ortho phosphoric acid, from which 200ml is used.

Preparation of Standard Stock Solution

Accurately weighed and transfer 48mg of Fexofenadine HCl and 4mg Montelukast Sodium working standard into a 100ml clean dry volumetric flask and add mobile phase. The solution was sonicated for about 10mins and then made up to volume with mobile phase. Dilute 10ml of the above solution to 100ml with mobile phase. The prepared standard stock solution was wrapped with aluminum foil and stored in dark place for the further use.

Preparation of Sample stock solution

Accurately weighed and transfer a quantity of powder equivalent to 48mg of Fexofenadine HCl and 4mg Montelukast Sodium working sample into a 100ml clean dry volumetric flask containing mobile phase. The solution was sonicated for about 10mins and then made up to volume with mobile phase. Dilute 10ml of the above solution to 100ml with mobile phase. The prepared sample stock solution was wrapped with aluminum foil and stored in dark place for the further use.

Method validation

The HPLC method was validated in terms of precision, accuracy and linearity according to ICH guidelines. Assay method precision was determined using six-independent test solutions. The intermediate precision of the assay method was also evaluated. Assay method was evaluated with the recovery of the standards from excipients. Three different quantities (low, medium and high) of the authentic standards were added to pre analyzed tablet powder. The mixtures were extracted , and were analyzed using the developed HPLC method. Linearity test solutions were prepared. The LOD and LOQ for analytes were estimated by injecting a series of dilute solutions with known concentration. To determine the robustness of the method, the final experimental conditions were purposely altered and the results were examined. The flow rate was varied by (±) 0.1mL/min. Column temperature was varied by (±) 20°C and effect of column from different suppliers was studied. Measurement wavelength was varied by (±) 2nm.

RESULTS AND DISCUSSION

1. Optimization of the chromatographic conditions

The wavelength of maximum absorption of the drug10µg/ml of the drugs in methanol was scanned using UV-spectrophotometer in the range of 200nm-400nm against methanol as blank. The resulting chromatograms were show in the Fig3 and the absorption curve shows characteristic absorption at 240nm.

The optimization was done by changing the composition mobile phase ratio and flow rate. Different mobile phases tried for optimization of the method with the optimized chromatographic conditions, stock solutions of Montelukast sodium and Fexofenadine HCl were prepared in mobile phase (mixed phosphate buffer pH-3: Acetonitrile 20:80v/v) and 20µl of each solution was injected and recorded the chromatogram at 240nm.

Validation of the method

System Suitability

The system suitability of Montelukast Sodium and Fexofenadine HCl performed and data is tabulated were shown in Table1.

SPECIFICITY

The specificity study of Montelukast Sodium and Fexofenadine HCl performed and the resulting chromatograms were shown in Fig 4.

LINEARITY

The linearity was performed by stock solutions of Montelukast sodium and Fexofenadine HCl was prepared with mobile phase and mixture of Montelukast sodium concentration range 0.4-2.4 and concentration range 4.8-28.8 Fexofenadine HCl. Each solution was injected and recorded the chromatogram at 24nm. The calibration curve was found to be 0.999 for Montelukast sodium and 0.999 for Fexofenadine HCl respectively. The optical characteristics Fexofenadine HCl and Montelukast sodium are shown in Table 2.

ACCURACY

The accuracy of the method was performed by recovery studies. To the 50 % of pre analysed formulation, a known quantity of Fexofenadine HCl and Montelukast sodium raw material solutions were added at different levels, injected the solutions. The chromatograms were recorded as shown in the Fig.5. The percentage recovery was found to be in the range between 98 – 102% for Fexofenadine HCL 100.09% and Montelukast sodium 99.733%. The % RSD was found to be 0.43, 0.462 and Fexofenadine HCl and Montelukast sodium. The values are given in the Table 3-5 the percentage recovery revealed that no interference produced due to excipients used in formulation. Therefore, the developed method was found to be accurate.

PRECISION

The precision of the method was confirmed by the system precision and method precision and chromatograms are shown in fig7 and Tab 6. The %RSD was found to be 0.69, 0.57 in system precision and 0.366, 0.524 in method precision for Fexofenadine HCl and Montelukast sodium respectively. It indicates that the method has good precision and data was shown in Table-6.

RUGGEDNESS

The intermediate precision (ruggedness) was performed to the Fexofenadine HCl and Montelukast sodium. The area was used for calculation %RSD and values were found to be 0.61, 0.40 for Fexofenadine HCl and 0.03, 0.34 for Montelukast sodium respectively .and data was shown in Table-7.

LOD AND LOQ

Limit of detection and Limit of Quantitation is calculated based on standard deviation and slope according to formula. The LOD of Fexofenadine HCl and Montelukast sodium was found to be 0.13 and 0.026 respectively. The LOQ of Fexofenadine HCl and Montelukast sodium was found to be 0.397 and 0.081 respectively and data was shown in Table-8.

ROBUSTNESS

Robustness was performed for wavelength variations 238nm, 242nm and flow rate variations 0.9ml, 1.1ml and the method is robust. All the above parameter with the ease of operation ensures that projected methods could be applied for the routine analysis of Montelukast Sodium and Fexofenadine HCl and pure tablet dosage form. The chromatograms shown in Fig 8-11.

Table 1. System Suitability of Fexofenadine HCl

S. No	Retention time (min)	Peak Area	Tailing Factor (T)	No. of Theoretical plates (N)
1	2.123	153.216	1.474	3325
2	2.127	154.225	1.526	3336
3	2.123	151.261	1.474	3325
4	2.127	152.826	1.526	3336
5	2.123	151.726	1.474	3325
Mean	2.1246	152.6508	1.4948	3329.4
S.D	0.002191	1.184805	0.028482	6.024948
%RSD	0.10	0.78	1.91	0.18

Table 2. Linearity for Montelukast sodium

S.No	Linearity level	Concentration (µg/ml)	Volume of stocksolution(ml)	Volume makeupto(ml)	Area
1	Linearity-1	0.4	1	10	24.287
2	Linearity-2	0.8	2	10	40.598
3	Linearity-3	1.2	3	10	57.900
4	Linearity-4	1.6	4	10	75.093
5	Linearity-5	2.0	5	10	92.186
6	Linearity-6	2.4	6	10	110.266

Table 3. Accuracy Spiking standard of Fexofenadine HCl and Montelukast Sodium

S. No	Injection No.	Retention time of Fexofenadine HCl	Area of Fexofenadine HCl	Retention time of Montelukast sodium	Area of Montelukast Sodium
1	Injection-1	2.123	153.782	5.627	93.286
2	Injection-2	2.127	152.924	5.623	92.816
3	Injection-3	2.123	153.056	5.607	93.964
	Average	2.124333	153.254	5.622333	93.35533

	S.D	0.002066	0.462	0.008287	0.577132
	%RSD	0.10	0.30	0.15	0.62

Table 4. Accuracy of Fexofenadine HCl

S. No	Weight of tablet powder taken	Amount of pure drug added	Sample peak area	Mean Area of spiking standard (24.0µg/ml)	Mean	Amount of total drug recovered (µg/ml)	% Recovery
1	2.4	19.2	137.904 138.733 138.733	153.254	138.456	21.68	100.41
2	2.4	24	169.886 168.317 168.668		168.957	26.46	100.25
3	2.4	28.8	198.661 197.942 198.866		198.489	31.08	99.58
			Mean				100.09
			SD				0.428993
			%RSD				0.43

Table 5. Accuracy of Montelukast Sodium

S. No	Weight of tablet powder taken	Amount of pure drug added	Sample peak area	Mean Area of spiking standard(24.0 µg/ml)	Mean	Amount of total drug recovered (µg/ml)	% Recovery
1	0.2	1.6	83.914 83.893 83.743	93.35533	83.85	1.79	99.37
2	0.2	2.0	103.266 102.682 102.821		102.923	2.205	100.25
3	0.2	2.4	121.53 121.197 121.275		121.334	2.59	99.58
			Mean				99.733
			SD				0.459
			%RSD				0.462

Table 6. System precision of Fexofenadine HCl and Montelukast Sodium

S. No	Injection No.	Retention time of Fexofenadine HCl	Area of Fexofenadine HCl	Retention time of Montelukast Sodium	Area of Montelukast Sodium
1	Injection-1	2.123	152.904	5.627	92.93
2	Injection-2	2.127	153.703	5.623	92.094
3	Injection-3	2.123	151.152	5.607	92.185
4	Injection-4	2.127	153.262	5.62	93.402
5	Injection-5	2.123	151.474	5.627	92.185
6	Injection-6	2.123	152.726	5.63	92.303
	Average	2.124333	152.3368	5.622	92.5165
	SD	0.002066	1.0103	0.008287	0.528955
	%RSD	0..97	0.662	0.154	0.571

Table.7 Intermediate Precision (Ruggedness) of Montelukast Sodium

S. No	Injection. No	Retention time		Area	
		Analyst-1	Analyst-2	Analyst-1	Analyst-2
1	Injection-1	5.607	5.623	94.407	93.676
2	Injection-2	5.610	5.650	92.816	93.837
3	Injection-3	5.610	5.613	94.874	93.441
	Average	5.609	5.629	94.032	93.651
	SD	0.001732	0.01914	1.078945	0.199149
	%RSD	0.03	0.34	1.15	0.21

Table 8. LOD and LOQ of Fexofenadine HCl and Montelukast Sodium

Parameter	Fexofenadine HCl	Montelukast Sodium
LOD	0.131	0.026
LOQ	0.3977	0.0814

Fig 1.Montelukast sodium

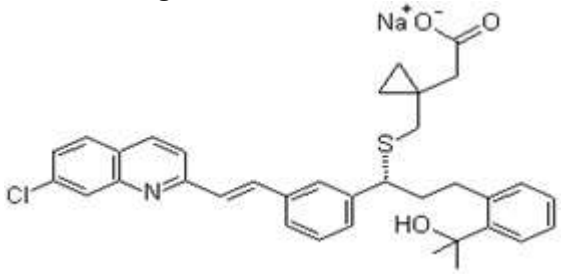


Fig 2 Fexofenadine HCl

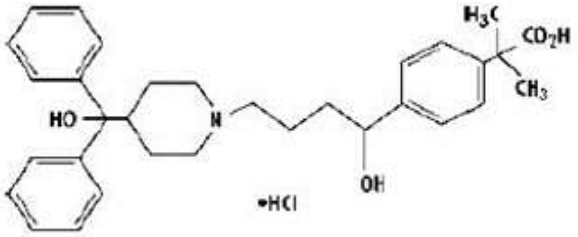


Fig 3. Spectrum of Fexofenadine HCl and Montelukast Sodium

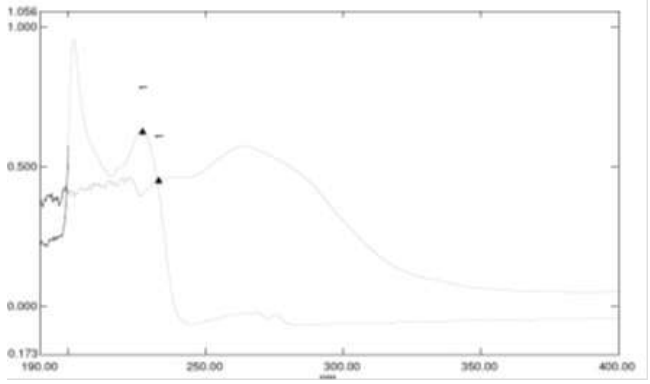


Fig 4. Chromatogram for Specificity-1

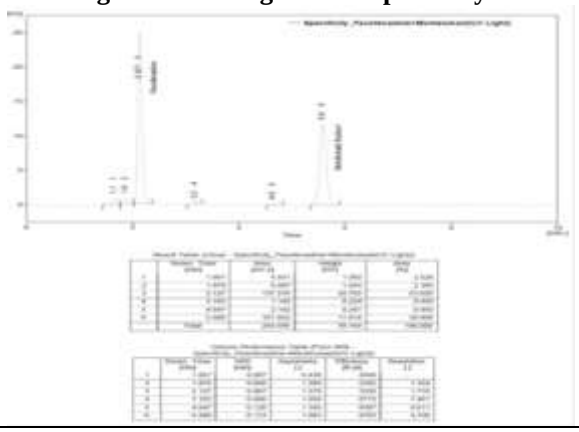


Fig 5. Chromatogram for Accuracy-1

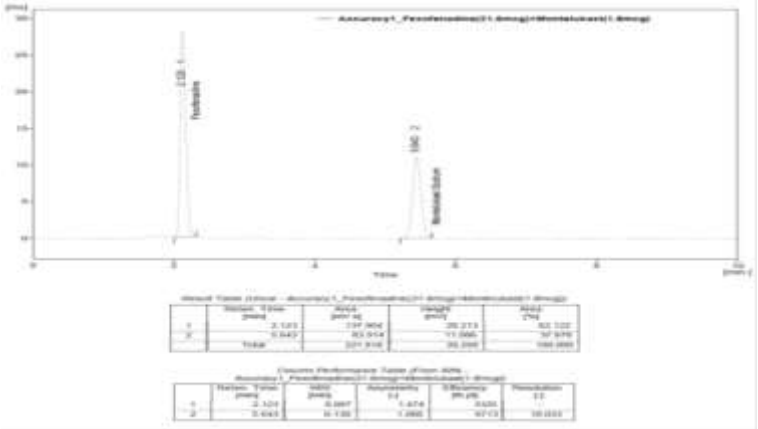


Fig 6. Chromatogram for Accuracy (Spiking standard)- 3

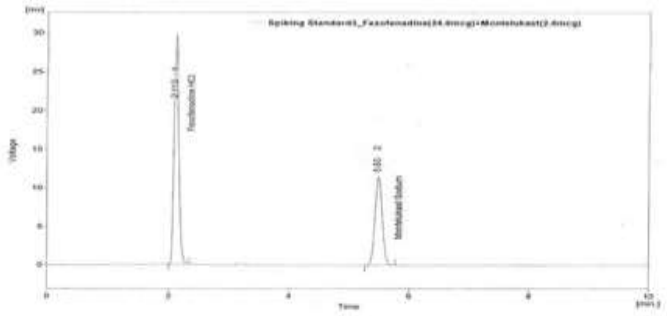


Fig 7. Chromatogram for System precision-

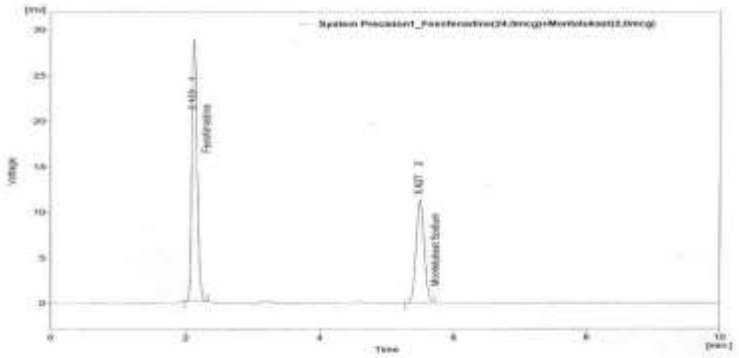
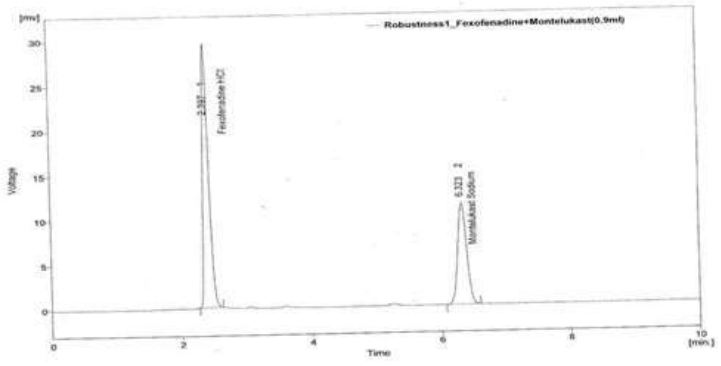


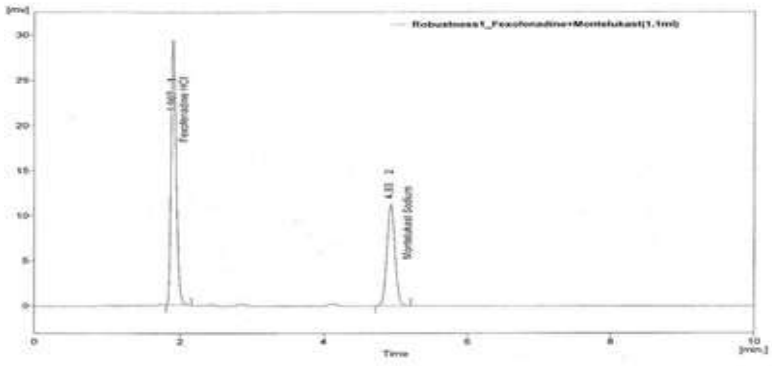
Fig 8. Chromatogram for Robustness-1 Flow rate 0.9 ml



	Retention Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.397	179.494	29.435	62.412
2	6.323	106.103	11.326	37.588
	Total	287.597	40.761	100.000

	Retention Time [min]	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Resolution [-]
1	2.397	0.097	1.524	3405	-
2	6.323	0.153	1.103	9422	18.485

Fig 9. Chromatogram for Robustness-2 Flow rate 1.1 ml



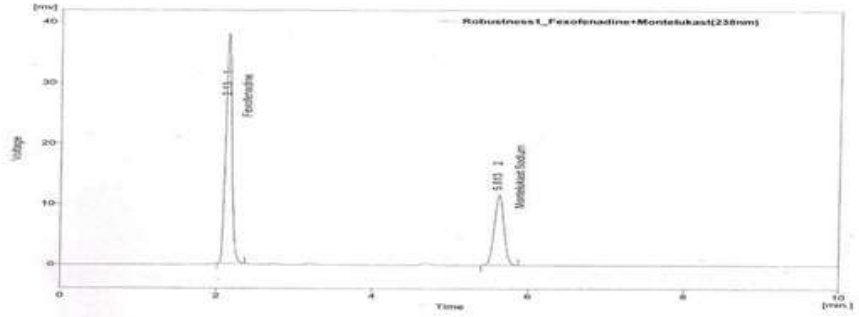
Result Table (Uncal - Robustness1_Fexofenadine+Montelukast(1.1ml))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	1.907	144.362	29.341	62.429
2	4.930	86.881	11.247	37.571
	Total	231.243	40.588	100.000

Column Performance Table (From 50% - Robustness1_Fexofenadine+Montelukast(1.1ml))

	Reten. Time [min]	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Resolution [-]
1	1.907	0.082	1.444	3147	-
2	4.930	0.123	1.125	8452	17.499

Fig 10. Chromatogram for Robustness-3 (238 nm)



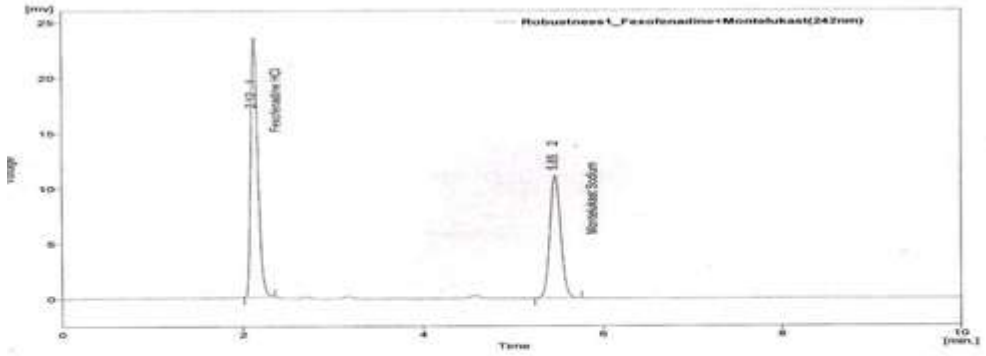
Result Table (Uncal - Robustness1_Fexofenadine+Montelukast(238nm))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.130	206.415	37.871	67.297
2	5.613	100.309	11.582	32.703
	Total	306.724	49.454	100.000

Column Performance Table (From 50% - Robustness1_Fexofenadine+Montelukast(238nm))

	Reten. Time [min]	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Resolution [-]
1	2.130	0.087	1.450	3340	-
2	5.613	0.140	1.111	8906	18.086

Fig 11. Chromatogram for Robustness-4 (242 nm)



Result Table (Uncal - Robustness1_Fexofenadine+Montelukast(242nm))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.120	126.014	23.462	57.730
2	5.650	93.731	11.101	42.270
	Total	221.745	34.564	100.000

Column Performance Table (From 50% - Robustness1_Fexofenadine+Montelukast(242nm))

	Reten. Time [min]	W05 [min]	Asymmetry [-]	Efficiency [th.pl]	Resolution [-]
1	2.120	0.087	1.526	3315	-
2	5.650	0.133	1.114	8801	17.885

CONCLUSION

A new method was established for simultaneous estimation Montelukast Sodium and Fexofenadine HCl. The chromatographic conditions were successfully developed for separation of Montelukast Sodium and Fexofenadine HCl by using Hypersil BDS C18 column (150x4.6mm, 5µ). The tablet dosage form (MONTOLIFE-FX) was selected for the analysis. The percentage purity was found to be 100.59%, 100.48% for Fexofenadine HCl and Montelukast Sodium, respectively. The precision of method was confirmed by repeatability of formulation for six times. The accuracy of the method was confirmed by recovery studies. The percentage recovery was found to be 100.09%, 99.733% for Fexofenadine HCl and Montelukast sodium respectively. The LOD values were found to be 0.13µg/ml and 0.026µg/ml for Fexofenadine HCl and Montelukast sodium respectively. LOQ values were found to be 0.397µg/ml and 0.081µg/ml for Fexofenadine HCl and Montelukast Sodium respectively. The analytical method is validated as per ICH guidelines. Simple, rapid and accurate and an isocratic RP – HPLC methods were developed for the estimation of Montelukast Sodium and Fexofenadine HCl in a pharmaceutical dosage form by RP – HPLC method.

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CONFLICT OF INTEREST

No interest

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