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METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF MONTELUKAST AND ACEBROPHYLLINE IN BULK AND TABLET DOSAGE FORMS BY RP-HPLC

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ABSTRACT

A simple accurate, precise technique was developed for the simultaneous estimation of Montelukast and Acebrophylline in bulk and tablet dosage forms. Chromatogram was run through Hypersil-C8, BDS (150×4.6mm,5µ) columns. Mobile phase containing Acetonitrile and Phosphate buffer in the ratio of 60:40v/v was pumped through column at flow rate of 1ml/min. Temperature was kept in 30ºc.Optimized wavelength was set to be 280nm. Retention time for Montelukast and Acebrophylline was found to be 2.059 and 2.686. %RSD of Montelukast and Acebrophylline was found to be 1.06 and 0.63. %Recovery for Montelukast and Acebrophylline was obtained as 99.53 and 99.37.LOD and LOQ values were 0.008 & 0.63µg/ml for Montelukast, 0.32 & 0.98µg/ml for Acebrophylline respectively. Regression equation for Montelukast is y=62549x+985.3 and y=8492.5x+17955 for Acebrophylline respectively. This developed method was validated according to ICH guidelines and satisfactory results were obtained. So this method can be effectively applied in research institutions, QC departments in industries and approved testing laboratories.

INTRODUCTION

Montelukast is a leukotriene receptor antagonist that binds with the high affinity and selectivity to the cysteinyl leukotriene type-1 receptor located on respiratory airway smooth muscle cells, airway macrophages and on various pro inflammatory cells and inhibits broncho constriction. Acebrophylline is a mucolytic and inhibits intracellular phosphor diesterase associated with cAMP levels and facilitates bronchial muscles relaxation. Combination of both Montelukast and Acebrophylline were used to prevent symptoms of asthma and seasonal allergy symptoms. [1-4]

Materials and reagents: Montelukast and Acebrophylline (AR grade) were obtained from Spectrum laboratories. The HPLC grade Orthophosphoric acid, Acetonitrile and Phosphate buffer from Merck and HPLC grade distilled water from LobaChem.

Instrumentation: HPLC modelAlliance2695 Waters Company, detector Waters photodiode array 2996 with an automated sample injector. The output signal was monitored and integrated using Empower version 2.6 software by using columns like Discovery C8 BDS, Azilent C8 BDS, Hypersil C8 BDS columns were used.

Preparation of buffer:

Preparation of Phosphate buffer: Solution of 0.005M 1-octane sulfonic acid solution salt monohydrate (C8H17NaO3S.H2O,243g/mol) pH 6.7 was prepared in 400ml HPLC grade

water and the filtered through 0.45microns filter under vacuum filtration.

Preparation of mobile phase: Accurately measured 600ml of (60%) of HPLC acetonitrile and 400ml of phosphate buffer (40%) were mixed and degassed in a digital ultra sonicator for 25 minutes and then filtered through 0.45 microns filter under vacuum filtration.

Stock solution:

Preparation of standard stock solution: 10mg of Montelukast and 200mg of Acebrophylline API standards were accurately weighed and dissolve into 30ml of diluent in 50ml volumetric flak and allowed to sonicate for 20min.

Preparation of sample stock solution: By taking an average weight of 20 tablets, which were allow to crush in a motor by using pestle from which, accurately weighed 10mg equivalent weight of Montelukast and 200mg of Acebrophylline in 50ml volumetric flask and 30ml diluent was added and then allowed to sonicate for 25miuntes.

Preparation of working standard solution: From the stock solution 1ml was taken into a 10ml volumetric flask which is makeup with diluent up to the mark.

Preparation of sample working solution: From the above sample stock solution 1ml was transferred into 10ml volumetric flask which is diluted with diluent up to the mark. The final concentrations of Montelukast and Acebrophylline were found to be 20µg/ml and 400µg/ml respectively.

Procedure: The solutions (20µL) in duplicates were injected and the peak responses were measured. % assay were calculated for Montelukast and Acebrophylline.

RESULTS AND DISCUSSION:

Method validation: System suitability, linearity, accuracy, precision, repeatability, intermediate precision, limit of detection, limit of quantification, robustness and ruggedness of test method.

Method development: Method development was performed by changing various conditions like mobile phase, ratios or flow rates.

SYSTEM SUITABILITY: A standard solution of Montelukast and Acebrophylline working standards were prepared and were injected six times into the HPLC system. The system suitability parameters were evaluated from standard chromatograms by calculating %RSD (Relative Standard Deviation) from six replicate injections for Montelukast and Acebrophylline, retention times and peak areas.

LINEARITY: To establish the linearity of the method, serial dilutions were prepared to obtain the mixture of Montelukast and Acebrophylline ranging to 5ppm to 30ppm and 100ppm to 600ppm. The final solution was injected in duplicate manner. Calibration curve was plotted between mean peak area and concentration. The correlation coefficient and slope were determined from the calibration curve. Linearity chats of Montelukast and Acebrophylline were shown in figure No. 5 & 6. The correlation coefficient was found to be 0.9999 for both Montelukast and Acebrophylline. [5]

ACCURACY: Accuracy was conducted by drug assay was performed in triplicates as per test method with equivalent amount of Montelukast with Acebrophylline into each volumetric flask for each spike level to get concentration of Montelukast and Acebrophylline equivalent to 50%, 100% and 150% of the labeled amount as per the test method. The average % recovery of Montelukast with Acebrophylline was calculated.

PRECISION: Precision is measured by the multiple samples obtain from homogenous sample under prescribe conditions. It is a closeness degree of samples. As per ICH, precision is performed as repeatability, intermediate precision and reproducibility. At least six duplicates are to be in process and the %RSD was calculated and reported in table no. 6 & 7. [6]

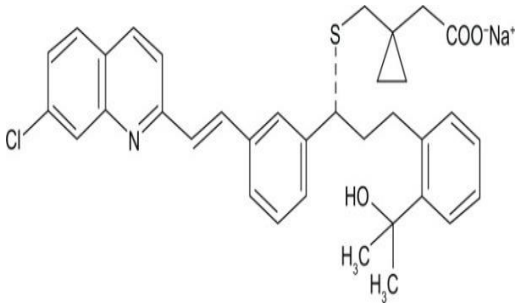

Fig-1: Structure of Montelukast

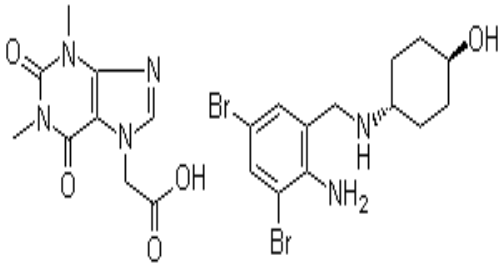

Fig-2 Structure of Acebrophylline

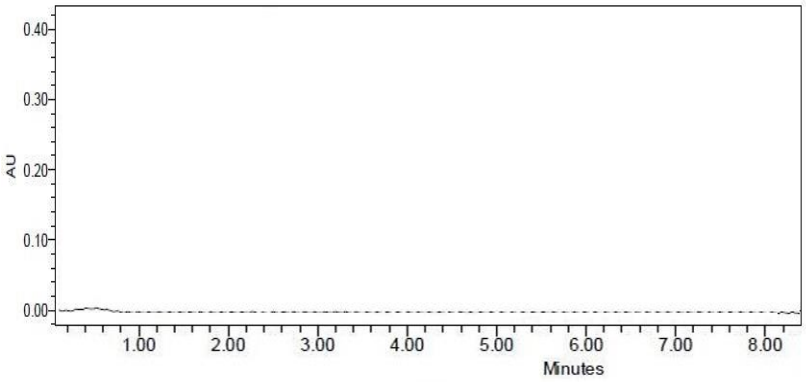

Fig-3 Chromatogram of blank

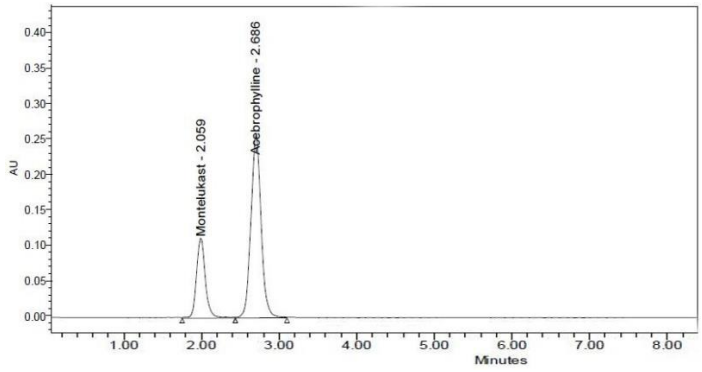

Fig-4 Chromatogram in optimized condition

Table-1 System suitability data of Montelukast

Injection	RT (min)	Peak area(AU)	USP Plate count	USP Tailing
1	2.044	1274217	2031	1.24
2	2.040	1282706	2064	1.20
3	2.042	1286826	2099	1.24
4	2.043	1283710	2157	1.22
5	2.042	1281615	2109	1.16
6	2.043	1289977	1972	1.19
Mean	2.042	1283175	2072	1.20
SD	0.001	5343.8	------	------
%RSD	0.07	0.4	------	------

Table-2 System suitability data of Acebrophylline

Injection	RT (min)	Peak area(AU)	USP Plate count	USP Tailing
1	2.688	3475141	2521	1.10
2	2.684	3471338	2552	1.09
3	2.688	3471542	2482	1.11
4	2.687	3473153	2393	1.07
5	2.688	3472831	2531	1.10
6	2.687	3483102	2637	1.08
Mean	2.687	4421	2519	1.09
SD	0.0015	0.13	------	------
%RSD	0.06		------	------

Table-3: Linearity results for Montelukast and Acebrophylline

S.No.	Concentration of Montelukast (µg/ml)	Peak area	Concentration of Acebrophylline (µg/ml)	Peak area
0	0	0	0	0
1	5	324281	100	857852
2	10	641248	200	1729794
3	15	941884	300	2573249
4	20	1284839	400	3463103
5	25	1570902	500	4246250
6	30	1873346	600	5089587

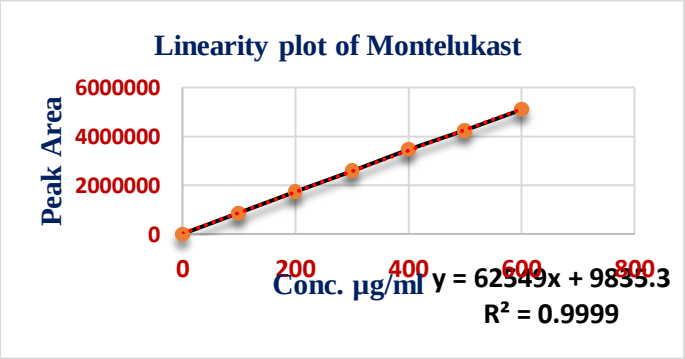

Fig-5 Linearity graph of Montelukast

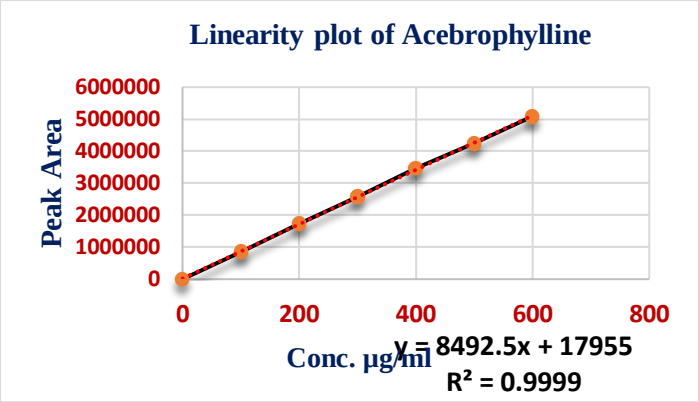

Fig-6 Linearity graph of Acebrophylline

Table-4 Accuracy data of Montelukast

% level	Amount spiked (µ/ml)	Amount recovered (µg/ml)	%Recovery	%Mean Recovery	%RSD
50%	50	9.81	98.16	99.62%	1.65%
	50	9.93	99.30		
	50	10.14	101.4		
100%	100	19.70	98.47	99.14%	0.70%
	100	19.82	99.10		
	100	19.97	99.85		
150%	150	29.98	99.94	99.84%	0.84%
	150	30.19	100.63		
	150	29.69	98.96		

Table-5 Accuracy data of Acebrophylline

%level	Amount spiked (µg/ml)	Amount recovered (µg/ml)	%Recovery	%Mean recovery	%RSD
50%	50	198.80	98.43	99.39%	0.86%
	50	200.14	100.07		
	50	199.37	99.68		
100%	100	394.80	98.71	99.25%	0.64%
	100	396.42	99.10		
	100	399.81	99.95		
150%	150	594.84	99.14	99.47%	0.40%
	150	596.31	99.38		
	150	599.49	99.91		

Table-6 System precision (Repeatability) data for Montelukast and Acebrophylline

S.No.	Peak area	
	Montelukast	Acebrophylline
1	1274217	3475141
2	1282706	3471338
3	1286826	3471542
4	1283710	3473153
5	1281615	3472831
6	1283177	3483102

Mean	1283175	3474518
Std deviation	5343.8	4421.6
%RSD	0.4	0.1

Table-7 Intermediate precision data for Montelukast and Acebrophylline

S.No.	Peak area	
	Montelukast	Acebrophylline
1	1266254	3449951
2	1262389	3445251
3	1285323	3445111
4	1271812	3452630
5	1282137	3456716
6	1271850	3443451
Mean	1273294	3448852
Std deviation	8895.58	5166.40
%RSD	0.70	0.10

Table-8 LOD and LOQ values for Montelukast and Acebrophylline

Drug name	LOD(µg/ml)	LOQ(µg/ml)
Montelukast	0.008	0.03
Acebrophylline	0.32	0.98

Table-9 Robustness data for Montelukast and Acebrophylline

Montelukast		
Flow rate (ml/min)	Standard area	Tailing factor
0.8	1210409	1.26
1.0	1286826	1.24
1.2	1232819	1.27
Acebrophylline		
Flow rate (ml/min)	Standard area	Tailing factor
0.8	3271858	1.13
1.0	3471542	1.11
1.2	3306161	1.16

Table-10 Ruggedness (System to system variability) data of Montelukast

Montelukast					
S.No.	RT (min)	Peak area	Peak height	Peak plate count	Peak tailing
1	2.042	1286826	17914	2099	1.24
2	2.028	1311513	15963	2628	1.23
3	2.026	1286175	16029	2712	1.22
4	2.145	1260751	17248	2167	1.22
5	2.145	1275893	17829	2178	1.24
6	2.042	1284715	17657	2189	1.23
Mean		1284312			
SD		16588.23			
%RSD		1.29			

Table-11 Ruggedness (system to system variability) data of Acebrophylline)

Acebrophylline					
S.No.	RT (min)	Peak area	Peak height	Peak plate count	Peak tailing
1	2.684	3471542	38964	2482	1.11
2	2.554	3496394	39182	3431	1.19
3	2.557	3523088	38761	3743	1.14
4	2.790	3542185	38627	2479	1.00
5	2.787	3485490	39821	2395	1.21
6	2.684	3471542	39102	2482	1.11
Mean		3498373			
SD		28775.04			
%RSD		0.82			

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ): The LOD and LOQ were calculated by measuring the standard deviation of the response and slope. The results of LOD and LOQ were tabulated in table no.8.

ROBUSTNESS: The small deliberate changes in method like flow rate was made but there were no recognized change in the result. Standard solution prepared as per the test method was injected into HPLC system using flow rate 0.8ml/min, 1.0ml/min and 1.2ml/min. The system suitability parameters were evaluated and found to be within the limits for 0.8ml/min, 1.0ml/min and 1.2ml/min flow.

RUGGEDNESS:

System to system variability: Conducted on different HPLC systems, under similar conditions at different times. Six samples were prepared and each was analyzed as per test process. Comparison of both the results obtained on two different HPLC systems, showed that the assay test method is rugged for system to system variability.

CONCLUSION:

The developed RP-HPLC method for the analysis of Montelukast and Acebrophylline was found to be simple, sensitive, precise and accurate. The developed method was validated according to ICH guideline and satisfactory results were obtained. Hence, this method can be effectively applied for routine analysis in research institutions, quality control.

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