ARC Journal of Pharmaceutical Sciences (AJPS)
Volume 3, Issue 1, 2017, PP 7-18
ISSN 2455-1538
DOI: http://dx.doi.org/10.20431/2455-1538.0301004
www.arcjournals.org

Analytical Method Development and Validation for Simultaneous Estimation of Doxofylline and Montelukast Sodium in Bulk and Pharmaceutical Dosage Form

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Abstract: *A rapid, precise and accurate reverse phase high performance liquid chromatographic method have been developed for the validated of* DOXOFYLLINE AND MONTELUKAS SODIUM*, in its original form as well as in pharmaceutical dosage form. Chromatography conditionswas carried out on a Phenomenex HYPERSIL ODS, RP-18,250×4.6mm ID, column using a mixture of Ammonium acetate: Methanol (60:40) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 219nm. The retention time of the* DOXOFYLLINE AND MONTE LUKAS SODIUM *was 2.34, 4.81 ±0.02min respectively. The method produce linearity responses in the range of 1.5-3.5mg/ml of* DOXOFYLLINE *and 60-140mg/ml of* MONTELUKAS SODIUM. *The limit of detection of Montelukast & Doxofylline 0.021 µg/ml & area 2.61 and 1.54 µg/ml & area 104.45 respectively. The limit of quantitation of Montelukast was found to be 0.06µg/ml & area 7.91 whereas for Doxofylline was 4.68 µg/ml & area 316.51. The percentage of recovery of Montelukast and Doxofylline was found to be 99.96% and 100.85% respectively. The method is useful for the quality and quality control of bulk and pharmaceutical formulations.*

Keywords: *DOXOFYLLINE, MONTELUKAST SODIUM, RP-HPLC, validation.*

1. INTRODUCTION

Montelukast is a leukotriene receptor antagonist (LTRA) with iupac name 2-[1- ({[(1R)-1- {3-[(E)- 2-(7- chloroquinolin-2- yl) ethenyl] phenyl}-3-[2-(2- hydroxypropan-2 yl) phenyl] propyl] sulfanyl} methyl) cyclopropyl]acetic. For the maintenance treatment ofasthma and to relieve symptoms of seasonal allergies. Ritonavir is a Antiretroviral drug 1,3-thiazol- 5-ylmethyl N-[(2S,3S,5S)-3-hydroxy-5-[(2S)-3- methyl-2 {[methyl({[2-(propan-2-yl)-1,3-tiazole4yl]methyl})carbamoyl]amino} butanamido]-1,6- diphenylhexanyl] carbamate. It is a xanthine derivative drug used in the treatment of asthma. (also known as doxophylline). It has anti tussive and bronchodilator effects, and acts as a phosphodiesterase inhibitor. Literature survey revealed that very few methods have been reported for the analysis Montelukast and doxofylline combinational dosage forms which include UV spectroscopy, Reverse Phase High performance Liquid Chromatography, Densitometric method, HPTLC methods

2. EXPERIMENTAL

Reagents and Chemicals

Montelukast API and doxofyllin API were obtained as gift sample from Chandra labs. Methanol, Sodium dihydrogen ortho phosphate Water, Potassium Dihydrogen ortho Phosphate, Dipotassium hydrogen ortho phosphate, Ammonium acetate, Tetra Hydro Furan, is used of HPLC grade and pur-chased

Instrumentation

Chromatographic separation was performed on a Shimadzu (LC 20 AT VP) HPLC with auto sampler and PDA Detector.variablewavelength programmable UV/VIS detector, HYPERSIL,ODS (C18 250x 4.6 ID) 5µm with10µl fixed loop.

Chromatographic Conditions

HYPERSIL, ODS (C18 250x4.6 ID) 5µm were the column used for separation. Mobile phase Containing a mixture of Ammonium acetate: Methanol in 1000 ml of water in the ratio (60:40) v/v was delivered at a flow rate of 1.0 ml/min with detection at 219nm. The mobile phase is filtered through a 0.45 nylon filter and sonicated for 20 min.

Method Development

Ammonium acetate, methanol and water in different proportions were tried and finally Ammonium acetate, methanol = 60:40 v/v was selected appropriate mobile phase which gave good resolution, retention time and acceptable system suitability parameters.

PROCEDURE

Preparation of standard solution

2.5 mg of MONTELUKAST and 100 mg of DOXOFYLLINE was weighed in 100 ml of volumetric flask and dissolved in 10 ml of mobile phase and volume was made up to the mark with mobile phase. From above stock solution 2.5 µg/ml of MONTELUKAST and 100 µg/ml of DOXOFYLLINE were prepared by diluting 1ml to 10ml with mobile phase.

Procedure

Inject the samples to the RpHplc by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution and retention for performing valida tion parameters as per ICH guidelines

Linearity

Standard stock solutions of MONTELUKAST and DOXOFYLLINE (µ/ml) were prepared by dissolving 2.5 mg of MONTELUKAST and 100 mg of DOXOFYLLINE in 100 ml of mobile phase. After that filtered the solution using 0.45-micron syringe filter and sonicated for 5 min and diluted to 100ml with mobile.

Procedure for Analysis of Tablets

For Standard sample Standard stock solutions of MONTELUKAST and DOXOFYLLINE (micro gram/ml) were prepared by dissolving 2.5 mg of MONTELUKAST and 100 mg of DOXOFYLLINE insufficient mobile phase. After that the solution was filtered using 0.45-micron syringe filter and sonicated for 5min and diluted to 100 ml with mobile phase. Further dilutions were prepared in 5replicates of 2.5 µg/ml of MONTELUKAST and 100 µg/ml of DOXOFYLLINE were made by adding 1 ml of stock solution to 10 ml of mobile phase.

For Tablet sample20 tablets (each tablet contains 10 mg of MONTELUKAST and 400 mg of DOXOFYLLINE) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of MONTELUKAST and DOXOFYLLINE (µg/ml) were prepared by dissolving weight equivalent to 2.5 mg of MONTELUKAST and 100 mg of DOXOFYLLINE and dissolved in sufficient mobile phase. After that the solution was filtered using 0.45-micron syringe filter and sonicated for 5 min and diluted to 100ml with mobile phase.

3. METHOD VALIDATION

System Suitability

Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters like theoretical plates, resolution and asymmetric factor were evaluated.

Procedure

The standard solution was injected for five times and measured the area for all five injections in HP LC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Specificity

There is no interference of mobile phase, solvent and placebo with the analyte peak and also the peak purity of analyte peak which indicate that the method is specific for the analysis of analytes in their dosage form.

Standard Sample

Standard stock solutions of MONTELUKAST and DOXOFYLLINE (microgram/ml) were prepared by dissolving 2.5 mg of MONTELUKAST and 100 mg of DOXOFYLLINE dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5min

and dilute to 100 ml with mobile phase. Further dilutions are prepared in 5 replicates of 2.5 µg/ml of MONTELUKAST and 100 µg/ml of DOXOFYLLINE was made by adding 1 ml of stock solution to 10 ml of mobile phase.

Tablet Sample

20 tablets (each tablet contains 10 mg of MONTELUKAST and 400 mg of DOXOFYLLINE) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of MONTELUKAST and DOXOFYLLINE (µg/ml) were prepared by dissolving weight equivalent to 2.5 mg of MONTELUKAST and 100 mg of DOXOFYLLINE and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 100ml with mobile phase. Further dilutions are prepared in 5 replicates of 2.5 µg/ml of MONTELUKAST and 100 µg/ml of DOXOFYLLINE was made by adding 1 ml of stock solution to 10 ml of mobile phase.

Linearity

linearity responses in the range of1.5-3.5mg/ml of DOXOFYLLINE *and 60-140mg/ml of* MONTE LUKAS SODIUM . Linear regression data was given

Precision

The precision of the method was demonstrated by inter day and intraday studies. In the intraday studie s, solutions of standard and sample were repeated 3times in a day and percent relative standard deviati on (%RSD) was calculated. The intraday %RSD of Ritonavir and Atazanavir were foundto be 0.54 an d 0.8 respectively.In the interday variation studies, injections of standard and sample solutions were m ade on two days and % RSD was calculated. The interday % RSD for Ritonavir and Atazanavir were found to be 0.5 to 0.63 respectively. From the data obtained the developed RP-HPLC method was found to be precise.

Accuracy

Accuracy of the method was determined by Recovery studies. To the formulation (pre-analysed sample), the reference standards of the drugs were added at the level of 80%, 100%, 120%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in table. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analysed sample solution at three different levels 80%, 100%, 120%.

Limit of Detection and Limit of Quantification

The Limit of detection and quantification were calculated using standard deviation of the resp onse and slope of calibration curve. The LOD for this method was found to be 0.021 µg/ml & area 2.61 for MONTELUKAST and 1.54 µg/ml & area 104.45 for DOXOFYLLINE. The LOQ for this method was found to be 0.06 µg/ml & area 7.91 for MONTELUKAST and 4.68 µg/ml & area 316.51 for DOXOFYLLINE

Robustness

Robustness of the method was checked by making slight changes in chromatographic conditions like mobile phase ratio, pH of buffer, flow rate. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed RP-HPLC method is robust.

RESULTS AND DISCUSSION

Chromatographic separation was achieved on a Hypersil C_{18} column. The optimum wave length for the determination of Montelukast and Doxofylline was selected at 219nmonthe basis of Isosbestic point. Various trials were performed with different mobile phases in differentratios, Ammonium acetate: Methanol (60:40) was selected as good peak symmetry and resolution between the peaks was observed. The Retentiontime of Montelukast and Doxofylline were found to be 2.34 and 4.81 respectively. The retention times for both the drugs were considerably less compared to the retention time obtained for the drugs in the other mobile phase.

The different analytical performance parameters such as linearity, precision, accuracy, and specificity, LOD, LOQ were determined according to International Conferenceon Harmonization ICHQ2B guidelines. The calibration curve for Montelukast was obtained by plotting peak area versus the concentration over the range of 1.5-3.5 µg/mL and for Doxofylline over the concentration range of 60-140 µg/mL. From linearity the correlation coefficient R^2 value was found to be 0.999 for Montelukast and .998 for Doxofylline. The proposed HPLC method was also validated for system suitability, system precision and method precision. The %RSD in the peak area of drug was found to be less than2%.The number of theoretical plates was found to be not less than2000, which indicates efficient performance of the column. The limit of detection of Montelukast & Doxofylline 0.021 µg/ml & area 2.61 and 1.54 µg/ml & area 104.45 respectively. The limit of quantitation of Montelukast was found to be 0.06 µg/ml & area 7.91whereas for Doxofylline was 4.68 µg/ml & area 316.51.The percentage of recovery of Montelukast and Doxofylline was found to be 99.96% and 100.85% respectively.

1 Accuracy

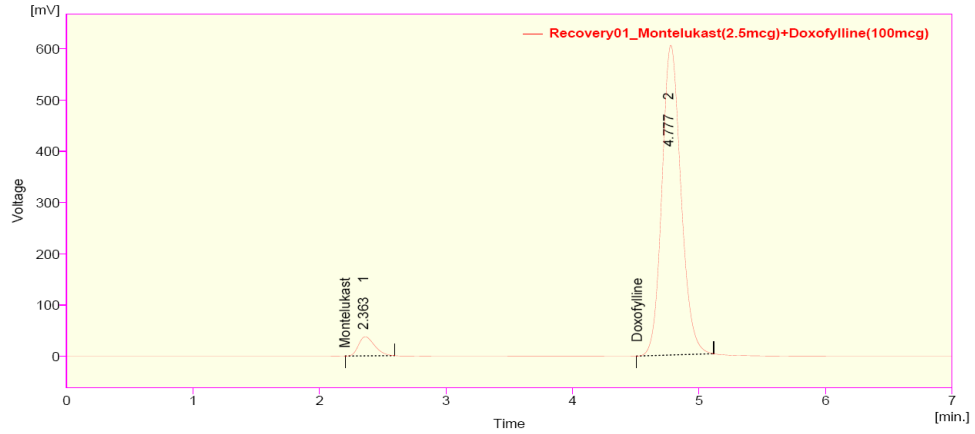

Fig 1.1. *Chromatogram of 80% recovery (injection 1)*

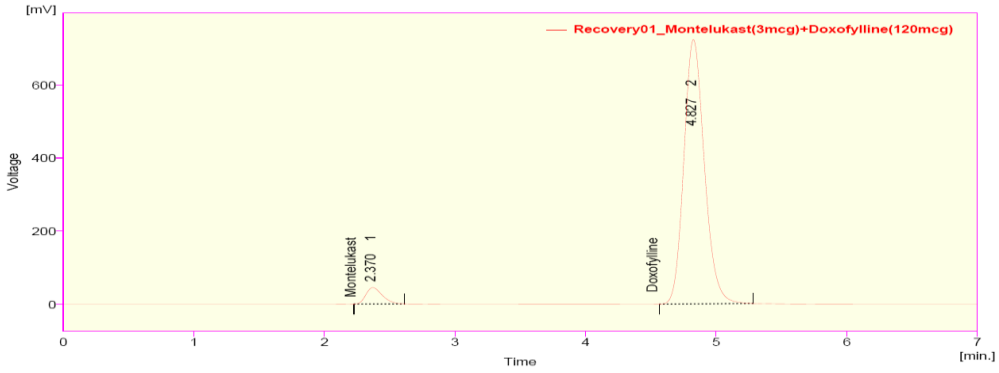

Fig 1.2. *Chromatogram of 100% recovery (injection 1)*

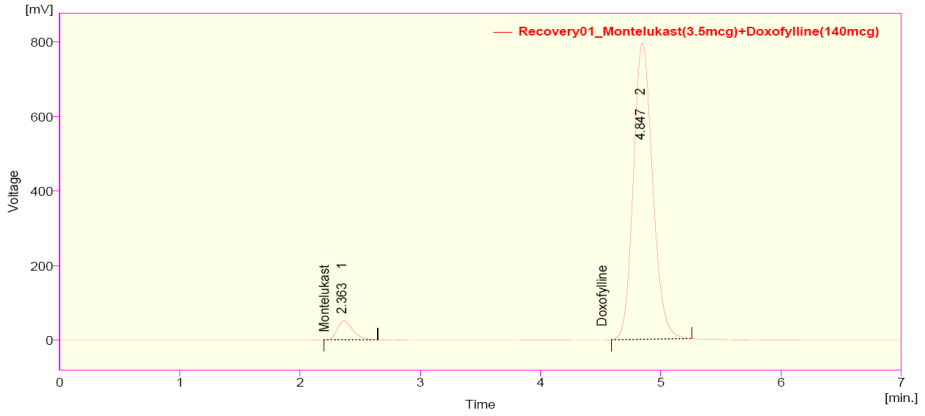

Fig 1.3. *Chromatogram of 120% recovery (injection 1)*

Table 1.1. *Recovery results for MONTELUKAST MALEATE*

Recovery level	**Accuracy MONTELUKAST MALEATE**					Average %Recovery
	Amount taken(mcg/ml)	Area	Average area	Amount recovered(mcg/ml)	%Recovery	
80%	2.5	295.482	299.503	2.46	98.50	99.96%
	2.5	301.897				
	2.5	301.131				
100%	3.0	393.495	382.759	3.02	100.52	
	3.0	370.824				
	3.0	383.957				
120%	3.5	453.643	453.048	3.54	101.01	
	3.5	453.573				
	3.5	451.928				

Table 1.2. *Recovery results for DOXOFYLLINE*

Recovery level	**Accuracy DOXOFYLLINE**					Average %Recovery
	Amount taken(mcg/ml)	Area	Average area	Amount recovered(mcg/ml)	%Recovery	
80%	100	6001.49	6007.368	99.00	99.00	100.85%
	100	6004.105				
	100	6016.51				
100%	120	7701.563	7746.327	121.42	101.18	
	120	7700.292				
	120	7837.127				
120%	140	8876.873	8911.900	142.72	101.77	
	140	8954.163				
	140	8904.665				

2 Precision

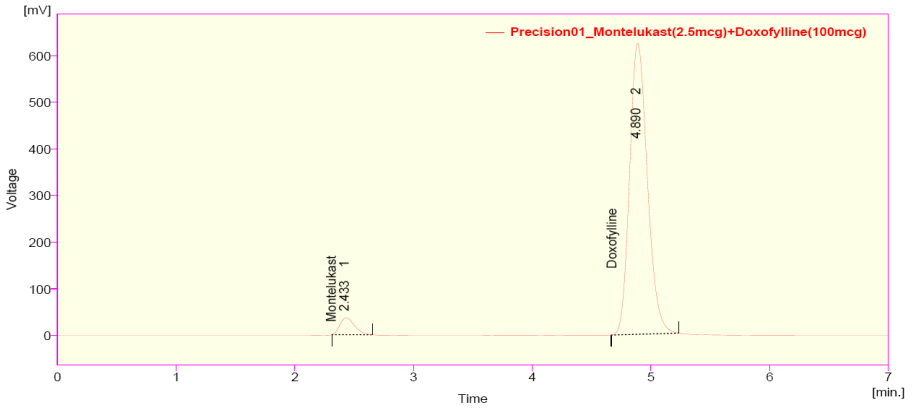

Fig 2.1. *Chromatogram of precision injection 1*

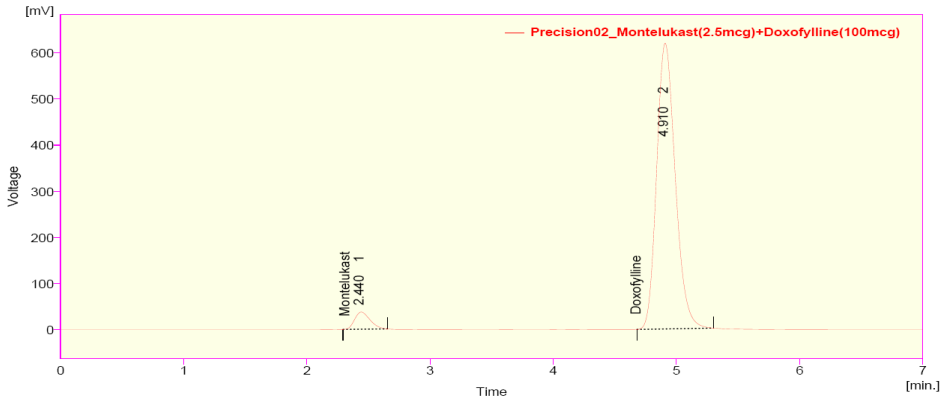

Fig 2.2. *Chromatogram of precision injection 2*

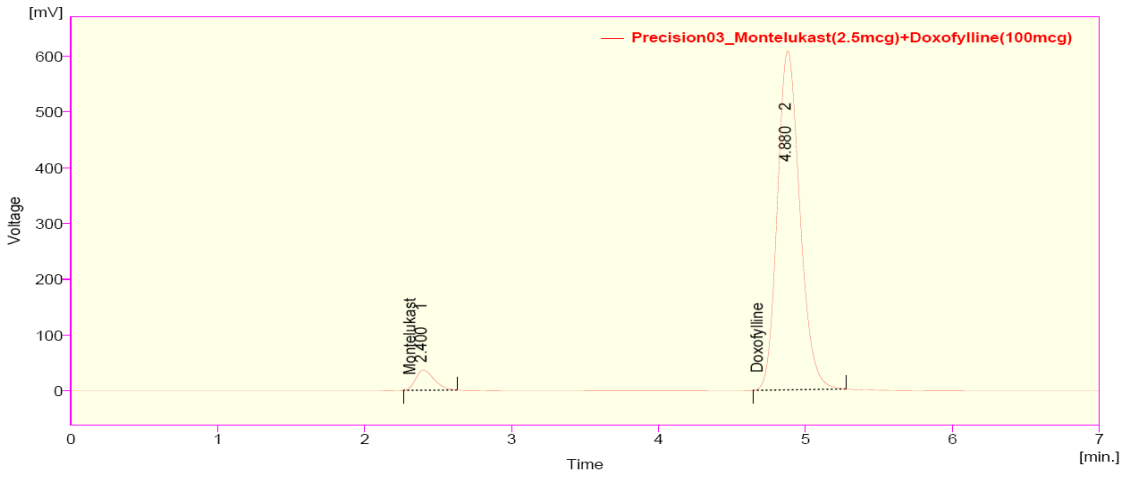

Fig 2.3. *Chromatogram of precision injection 3*

Table 2.1. *Results for Method precision of MONTELUKAST and DOXOFYLLINE*

MONTELUKAST MALEATE		
S.no.	Rt	Area
1	2.433	313.168
2	2.440	321.118
3	2.400	316.430
4	2.393	314.869
5	2.39	320.446
6	2.353	320.807
Avg	2.4015	317.806
St dev	0.0317	3.434
%RSD	1.32	1.08

DOXOFYLLINE		
S.no.	Rt	Area
1	4.890	6496.346
2	4.910	6510.812
3	4.880	6472.945
4	4.813	6438.985
5	4.863	6457.04
6	4.840	6394.355
Avg	4.866	6461.747
St dev	0.035	41.980
%RSD	0.72	0.65

Observation

Test results for DOXOFYLLINE and MONTELUKAST are showing that the %RSD of Assay results are within limits. The results were shown in table 8.2.7.

3. Specificity

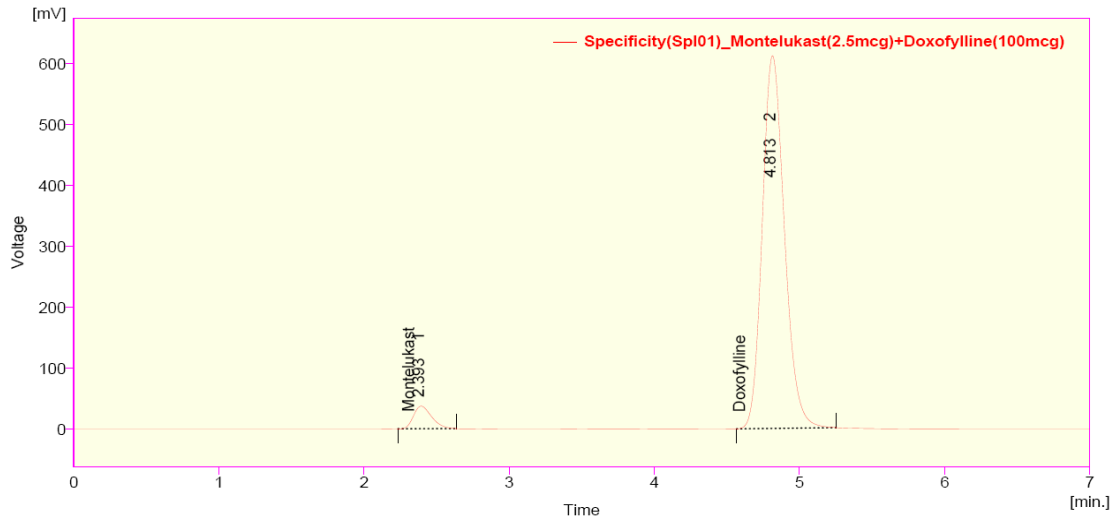

Fig 3.1. *Chromatogram for specificity of MONTELUKAST and DOXOFYLLINE sample*

Table 3.1. *Results for Specificity of MONTELUKAST and DOXOFYLLINE*

NAME	**Rt**	**AREA**	**Th.Plates**	**ASSYMETRY**	**RESOLUTION**
MONTELUKAST	2.393	321.015	2619	1.567	
DOXOFYLLINE	4.813	6474.853	4621	1.325	9.287

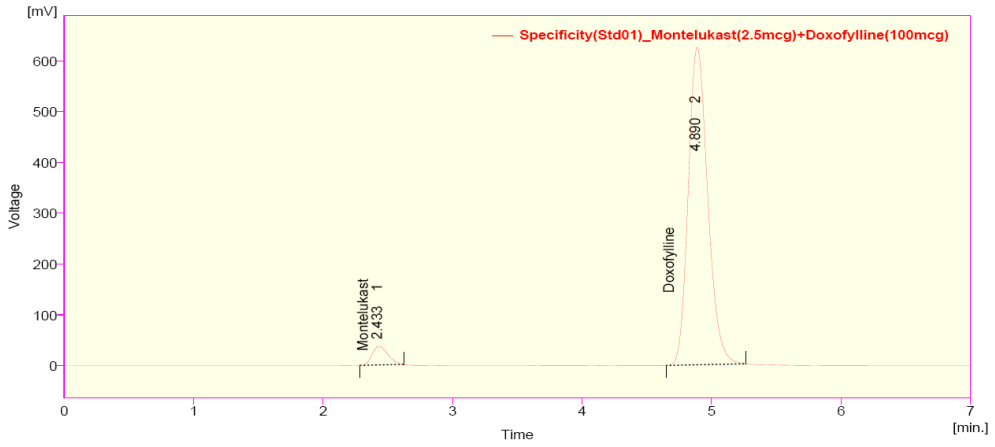

Fig 3.2. *Chromatogram for Specificity of MONTELUKAST and DOXOFYLLINE standard*

Table 3.2. *Results for Specificity of MONTELUKAST and DOXOFYLLINE*

NAME	Rt	AREA	Th.Plates	ASSYMETRY	RESOLUTION
MONTELUKAST	2.433	310.503	2756	1.467	
DOXOFYLLINE	4.890	6522.485	4966	1.300	9.637

Observation

It is observed from the above data, diluents or excipient peaks are not interfering with the MONTELUKAST and DOXOFYLLINE peaks.

4. Limit of Detection

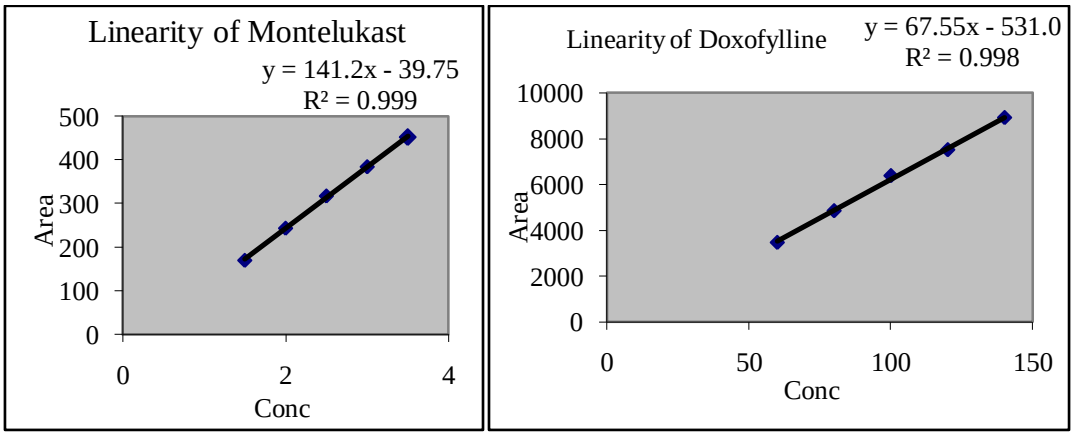

Fig 4.1. *Calibration graphs of DOXOFYLLINE & MONTELUKAST MALEATE*

Table 4.1. *Results for calibration graph*

	MONTELUKAST MALEATE		**DOXOFYLLINE**	
S.No.	Concentration µg/ml	Peak Area	Concentration µg/ml	Peak Area
1	1.5	169.476	60	3475.465
2	2	243.263	80	4854.281
3	2.5	317.33	100	6379.981
4	3	384.46	120	7506.113
5	3.5	451.928	140	8904.665
S.D.	0.8	112	31.62	2138
Slope	141.2		67.55	

Observation

The LOD for this method was found to be 0.021 µg/ml & area 2.61 for MONTELUKAST and 1.54 µg/ml & area 104.45 for DOXOFYLLINE

5. Limit of Quantification

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

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

Observation

The LOQ for this method was found to be 0.06 µg/ml & area 7.91 for MONTELUKAST and 4.68 µg/ml & area 316.51 for DOXOFYLLINE

6. Linearity and Range

Table 6.1. *Linearity Preparations*

Preparations	Volume from standard stock transferred in ml	Volume made up in ml (with mobile phase)	Concentration of solution (µg /ml)	
			MONTELUKAST	DOXOFYLLINE
Preparation 1	0.6	10	1.5	60
Preparation 2	0.8	10	2	80
Preparation 3	1.0	10	2.5	100
Preparation 4	1.2	10	3	120
Preparation 5	1.4	10	3.5	140

Table 6.2. *Linearity of MONTELUKAST*

S.no.	Conc.(µg/ml)	Area
1	1.5	169.476
2	2	243.263
3	2.5	317.33
4	3	384.46
5	3.5	451.928

Table 6.3. *Linearity of DOXOFYLLINE*

S.no.	Conc.(µg/ml)	Area
1	60	3475.465
2	80	4854.281
3	100	6379.981
4	120	7506.113
5	140	8904.665

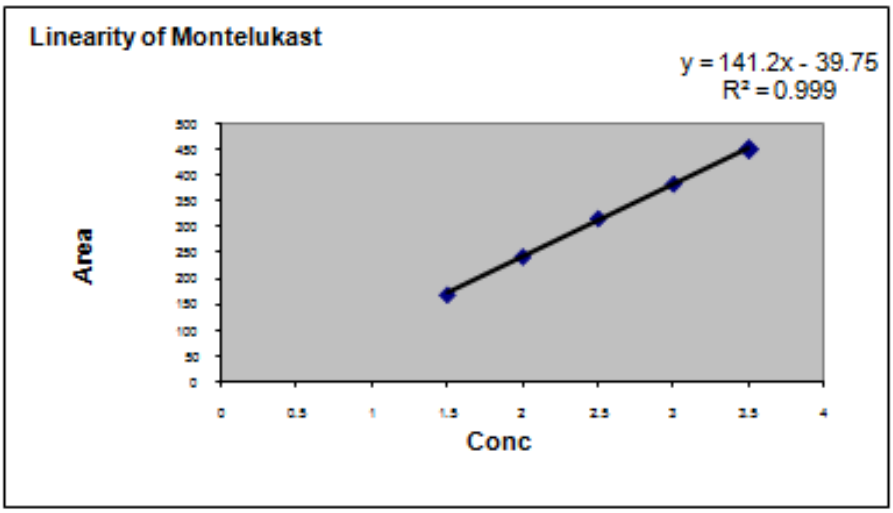

Fig 6.1. *Linearity graph of MONTELUKAST*

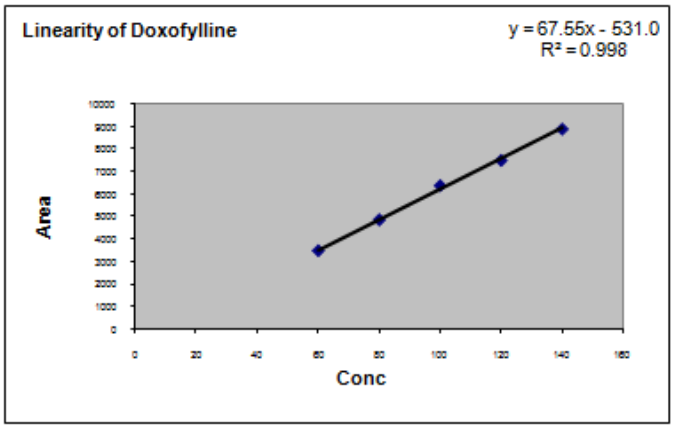

Fig 6.2. *Linearity graph of DOXOFYLLINE*

7 Robustness

Table 7.1. *Result of Robustness study*

Parameter	MONTELUKAST MALEATE		DOXOFYLLINE	
	Retention time(min)	**Tailing factor**	**Retention time(min)**	**Tailing factor**
Flow Rate				
0.8 ml/min	3.953	1.567	8.010	1.306
1.0 ml/min	2.100	1.500	4.017	1.239
Wavelength				
217nm	2.373	1.567	4.793	1.149
219 nm	2.347	1.581	4.837	1.150

8. System suitability

Table 8.1. *Results for system suitability of MONTELUKAST*

Injection	Retention time (min)	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	2.431	312.548	2630	1.500
2	2.401	312.324	2640	1.510
3	2.441	314.230	2627	1.503
4	2.403	317.879	2634	1.516
5	2.394	318.646	2634	1.503
6	2.391	318.242	2619	1.510
Mean	2.3915	315.806	-	-
SD	0.0417	3.644	-	-
%RSD	1.021	0.94	-	-

Table 8.2. *Results for system suitability of DOXOFYLLINE*

Injection	Retention time (min)	Peak area	Theoretical plates	Tailing factor	Resolution
1	4.870	6478.985	4207	1.183	9.126
2	4.860	6417.256	4270	1.178	9.142
3	4.890	6498.254	4211	1.175	9.175
4	4.803	6474.147	4295	1.172	9.163
5	4.833	6484.256	4215	1.170	9.128
6	4.820	6471.256	4219	1.171	9.176
Mean	4.851	6425.258	-	-	-
SD	0.038	40.210	-	-	-
%RSD	0.77	0.58	-	-	-

5. CONCLUSION

From the above experimental results and parameters it was concluded that, the developed method for the simultaneous estimation of MONTELUKAST&DOXOFYLLINE was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control

department in meant in industries, approved testing laboratories, bio-pharmaceutical and bio-equivalence studies and in clinical pharmacokinetic studies in near future.

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