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DEVELOPMENT AND VALIDATION OF STABILITY-INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF THEOPHYLLINE AND MONTELUKAST SODIUM IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

The prime essence of this current study is to develop a simple, precise and accurate stability indicating RP-HPLC method for simultaneous estimation of Theophylline and Montelukast Sodium in bulk and pharmaceutical dosage form. The separation was achieved by using Hypersil BDS column, (150×4.6mm, particle size 5µ) with mobile phase containing 0.1% TFA: Acetonitrile: Methanol in the ratio 25:50:25 v/v/v at a flow rate of 0.7 mL/ min at detection of 250 nm. The developed method was validated according to ICH guidelines and was found to be linear, precise, accurate and robust. The linear response was observed in the range of 40-120 µg/mL for Theophylline and 0.5-5 µg/ mL for Montelukast Sodium. Method was precise with %RSD values of NMT 2 and was accurate with % recovery of 99.81% and 100.21% for Theophylline and Montelukast Sodium respectively. The method was robust enough to reproduce acceptable results under different method conditions. Upon performing

the forced degradation studies for pharmaceutical dosage form, the % assay of formulation for acid, alkali, oxidative and thermal degradation was performed and the amount of degradation achieved was within the acceptance limits *i.e.,* 5-20%. This method can be applied efficaciously for estimation of Theophylline and Montelukast Sodium quantitatively in the routine analysis and reliable for demonstrating and detecting any expected change or degradation in the drug product during stability studies.

Keywords: RP-HPLC, Theophylline, Motelukast Sodium, Validation, Forced degradation studies

INTRODUCTION

Theophylline, also known as 1, 3 - dimethylxanthine, is a methylxanthine drug used in therapy for respiratory diseases such as Chronic Obstructive Pulmonary Disease (COPD), asthma **[1-3]** and also for the treatment of conditions such as emphysema and chronic bronchitis **[4]**. Theophylline is a competitive nonselective phosphodiesterase inhibitor, which inhibits leukotriene synthesis, and reduces inflammation and innate immunity **[5-6].**

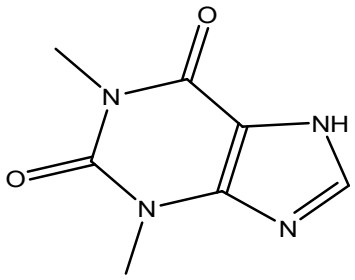


Figure 1: Chemical Structure of Theophylline

Montelukast Sodium (1-[[[(1R)-1-[3-[(1E)-2-(7-chloro-2-quinolinyl) ethenyl] phenyl]-3-[2-(1- hydroxy-1- methylethyl) phenyl] - propyl] thio] methyl] cyclopropane acetic acid **[7]**. Montelukast (sodium salt) is potent, selective CysLT1 receptor antagonist and is indicated for the prophylaxis and chronic treatment of asthma and to relieve symptoms of seasonal allergies in paediatrics and adults **[8-9]**.

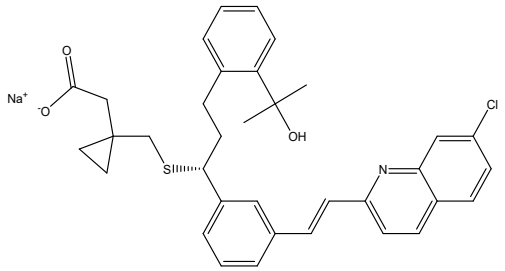


Figure 2: Chemical Structure of Montelukast Sodium

Literature review reveals that there were no stability indicating RP-HPLC methods developed for the simultaneous estimation of Theophylline and Montelukast Sodium **[10-15]**. The present work illustrates the development and validation of a Stability indicating simple, precise and accurate method for simultaneous estimation of Theophylline and Montelukast sodium by RP-HPLC in bulk and in Pharmaceutical dosage form.

MATERIALS AND METHODS

Instrumentation:

HPLC 2030 C 3D Plus Shimadzu instrument equipped with PDA (Photo Diode Array) detector is utilised and data was analyzed by using LAB SOLUTIONS software.

Materials:

Analytically pure Theophylline and Montelukast Sodium were procured from Sigma Aldrich, Bangalore, India. Telekast - T® (Lupin Ltd.) was marketed pharmaceutical formulation containing 400mg of Theophylline and 10mg of Montelukast Sodium (drug sample) procured from local pharmacy. All the chemicals, reagents and solvents used were of analytical grade and HPLC grade water is used throughout the study.

Preparation of Standard Solution:

Weigh accurately 10mg of each Theophylline and Montelukast Sodium and transfer into 10ml volumetric flask individually. To each volumetric flask add 7mL of diluent (methanol: acetonitrile in the ratio of 50: 50 v/v) was added to dissolve and volume was made up to the mark with diluent (1000µg/mL).

Preparation of Sample solution:

20 tablets were weighed and crushed into a fine powder with mortar and pestle. Tablet powder equivalent to 400mg of theophylline and 10mg of Montelukast Sodium was accurately weighed and transferred into a 10ml volumetric flask, 7ml of diluent was added and volume is made up to the mark with diluent (solution 1) and then filtered with 0.45µ Millipore Nylon filter. From solution 1, 0.2ml was pipette out and transferred into a 10ml volumetric flask and volume is made up to the mark with diluent (solution 2). From solution 2, pipette out 1ml of solution and transfer into a 10ml volumetric flask and made up to the volume with HPLC water.

Method Development and Chromatographic Conditions:

Separation was achieved by using Hypersil BDScolumn, (150×4.6mm, particle size 5µ), mobile phase containing 0.1% TFA: Acetonitrile: Methanol in ratio 25:50:25 v/v/v was selected as optimized solvent for separation with a flow rate of 0.7mL/min and at detection wavelength of 250 nm with an injection volume of 10µL and run time was about 8 minutes.

METHOD VALIDATION: The method was validated according to ICH guidelines for evaluation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy etc., for the analyte.

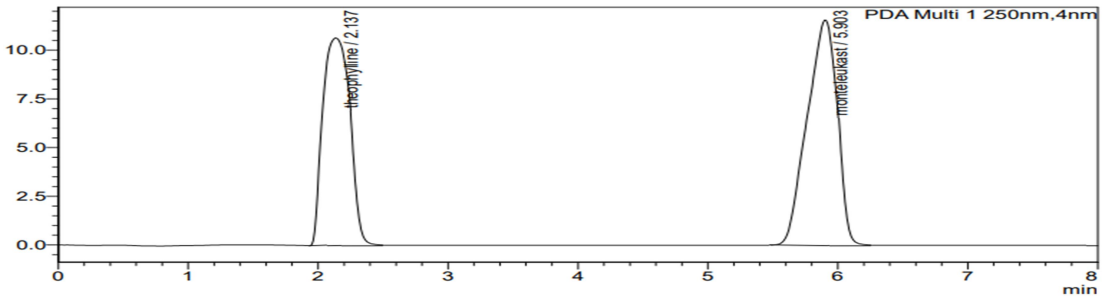


Figure 3: Optimized Chromatogram of Standard

RESULTS AND DISCUSSION

SYSTEM SUITABILITY: System suitability tests were performed by injecting standard solution containing Theophylline and Montelukast Sodium in the same concentration of optimized chromatogram and observed for the changes in the parameters such as retention time, number of theoretical plates, % RSD and tailing factor and were found to be within the acceptance criteria and data was represented in **Table 1**.

SPECIFICITY: Specificity is determined by comparing the chromatograms of blank, the standard and sample of Theophylline and Montelukast Sodium and observed for any interference with the analyte. Solutions of standard, sample and diluent were prepared as per the test method and injected into the chromatographic system and the chromatograms were recorded and there is no interaction of blank with the analyte of standard and sample.

LINEARITY: The linearity of Theophylline was demonstrated over the concentration range of 40-120 µg/mL and the linearity of Montelukast Sodium was demonstrated over the concentration range of 0.5-5 µg/mL. From the resultant data, parameters such as slope, intercept and regression equation were calculated and is supported by regression coefficient (R^2) of 0.999 and data was given in **Table 2**.

PRECISION: The precision of the method was determined by system precision and method precision using standard solutionby injecting the standard solution (80 µg/ml of Theophylline and 2 µg/ml of Montelukast Sodium) in six replicates and data was represented in **Table 3**.

ACCURACY: The accuracy of the method was based on the percentage recovery of the analyte that was added to weighed amounts at level 50%, 100% and 150% compared to the declared amounts by using tablet formulation (Telekast-T) and data was represented in **Table 4**.

LIMIT OF DETECTION (LOD) and LIMIT OF QUANTIFICATION (LOQ): The LOD of Theophylline and Montelukast Sodium were found to be 4µg/ml and 0.05 µg/ml respectively. The LOQ of Theophylline and Montelukast Sodium was

found to be 12µg/ml and 0.15 µg/ml respectively.

ROBUSTNESS: Robustness was performed with flow rate changed to 0.6mL/min and 0.8mL/min and mobile phase ratio is changed from 0.1% TFA: Acetonitrile: Methanol (25:50:25 v/v/v) to 30:45:25% v/v/v and 20:55:25%v/v/v and the data for robustness is tabulated in **Table 5**.

FORCED DEGRADATION STUDIES: The Theophylline and Montelukast Sodium pharmaceutical formulation (Telekast- T) were subjected to acidic (0.1N HCl), alkali (0.1N NaOH), peroxide (3% H_2O_2) conditions for 24 hours at room temperature and thermal degradation by placing in hot air oven at a temperature of 80ºC for 24 hours. After completion of the treatment, the solutions were left to return to room temperature and diluted with solvent to furnish sample solution containing 80 µg/ml of Theophylline and 2 µg/ml of Montelukast Sodium. The % assay of the drug obtained from the stressed sample was measured with PDA detector and compared with the chromatograms of untreated drugs in tablet solutionand data of degradation studies was given in **Table 6**.

Table 1: Data of System Suitability

Injection No	Theophylline		Montelukast Sodium	
	R_t (min)	Peak area	R_t (min)	Peak area
1	2.433	1402495	6.469	398777
2	2.438	1396049	6.530	393598
3	2.437	1390516	6.519	386967
4	2.435	1396950	6.503	394568
5	2.436	1406895	6.496	395687
6	2.435	1392091	6.489	396584
Mean		1397499	394364	
Standard Deviation		6223.463	4037.258	
% RSD		0.45	1.02	

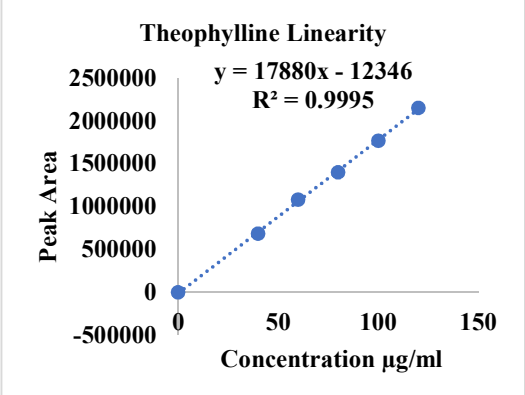


Figure 4: Linearity plot of Theophylline

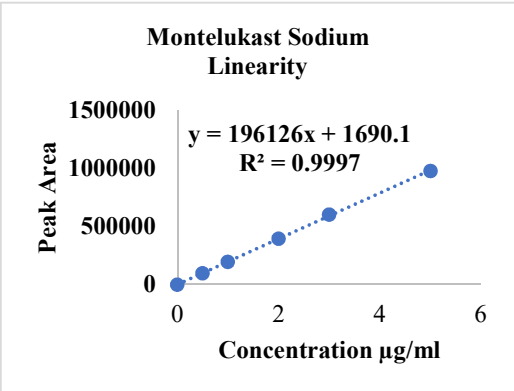


Figure5: Linearity plot of Montelukast Sodium

Table 2: Linearity data for Theophylline and Montelukast Sodium

Theophylline		Montelukast Sodium	
Concentration (µg/mL)	Peak Area	Concentration (µg/mL)	Peak Area
40	683023	0.5	98591
60	1077414	1.0	195182
80	1399175	2.0	394364
100	1767716	3.0	601546
120	2150551	5.0	975910
R^2 = 0.9995		R^2 = 0.9997	

Table 3: Data of System Precision and Method Precision

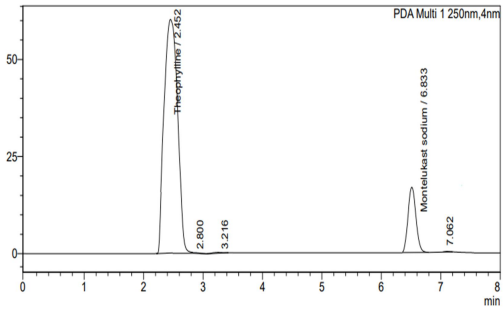
S. No	System Precision		Method Precision	
	Theophylline peak area	Montelukast Sodium peak area	Theophylline peak area	Montelukast Sodium peak area
1	1402445	398827	1402795	398562
2	1397002	393548	1394029	393786
3	1390566	387027	1390914	386203
4	1396901	394578	1397872	392376
5	1406845	395787	1407352	391286
6	1392041	396638	1394076	394274
Mean	1397633	394401	1397840	392748
SD	6167.061	4041.026	6191.802	4057.624
%RSD	0.44	1.02	0.42	1.03

Table 4: Accuracy data of Theophylline and Montelukast Sodium

%level	THEOPHYLLINE				MONTELUKAST SODIUM			
	Standard peak area	Sample peak area	% recovery	Mean % recovery	Standard peak area	Sample peak area	% recovery	Mean % recovery
50%	1397499	699895	99.85	99.81%	394364	199589	100.50	100.21%
	1397499	697895	99.38		394364	198546	99.79	
	1397499	698822	99.65		394364	199245	100.28	
100%	1397499	1400317	100.01		394364	397599	100.23	
	1397499	1403227	100.19		394364	399656	100.72	
	1397499	1393218	99.53		394364	395788	99.79	
150%	1397499	2112584	100.52		394364	596985	100.26	
	1397499	2096545	99.81		394364	594364	99.87	
	1397499	2088301	99.38		394364	598364	100.51	

Table 5: Data of Robustness of Theophylline and Montelukast Sodium

Parameter	THEOPHYLLINE			MONTELUKAST SODIUM		
	R_t (min)	Peak area	% RSD	R_t (min)	Peak area	% RSD
Change in Flow rate 0.9mL/min	1.902	1407564	0.44	5.010	398726	0.33
	1.914	1398687		5.017	400631	
Change in Flow rate 0.5mL/min	3.445	1398275	0.45	9.021	393345	0.25
	3.452	1407186		9.067	394778	
Change in Mobilephase ratio 20:55:25v/v/v	2.422	1395806	0.85	5.246	311001	0.42
	2.462	1379086		5.253	312889	
Change in Mobile phase ratio 30:40:25v/v/v	2.449	1439241	1.16	7.931	288568	0.84
	2.442	1415632		7.923	285145	



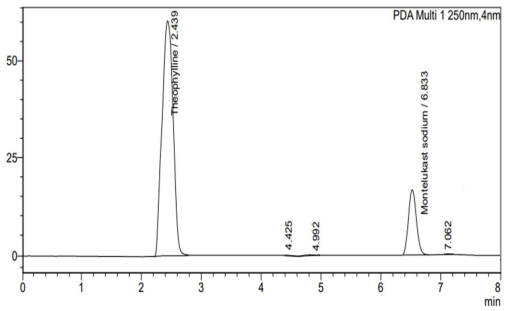
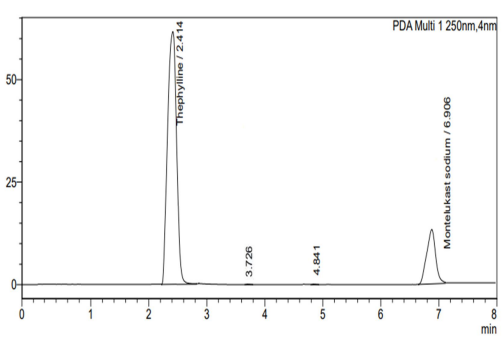


Figure 6: Acid degradation ChromatogramFigure 7: Alkali degradation Chromatogram



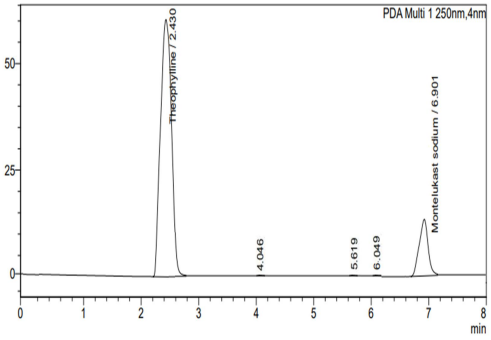


Figure 8: Oxidative degradationchromatogramFigure 9:Thermal degradationchromatogram

Table 6: Data of Degradation Studies

S. No	Degradative study	Degradation conditions	Peak area		% assay	
			Theophylline	Montelukast sodium	Theophylline	Montelukast sodium
1	Tablet assay	None	1397499	394364	100.23%	100.75%
2	Acid degradation	0.1N HCl for 24 hours	1295129	369656	92.51%	93.19%
3	Alkali degradatio	0.1N NaOH for 24 hours	1299129	359656	92.79%	90.67%
4	Oxidative degradation	3% H_2O_2 for 24 hours	1256129	349656	89.72%	88.15%
5	Thermal degradation	80^0 C for 24 hours	1322129	379656	94.44%	95.71%

DISCUSSION

In optimized chromatogram, Theophylline and Montelukast Sodium were eluted at 2.431 min and 6.463 min respectively. The method developed was validated according to ICH guidelines. Linearity was observedover the concentration range of40-120 µg/ml and 0.5 to 5 µg/ml with R^2 of 0.9995 and 0.9997 for Theophylline and Montelukast Sodium respectively and were within the R^2 of 0.999. Method was precise with %RSD values of NMT 2 which was within acceptance limits and was accurate with % recovery of 99.81% and 100.21% for Theophylline and Montelukast Sodium and was within the limits of 98% - 102%.Even with the changes in robustness parameters, separation was not affected and % RSDwas within the acceptable limits of NMT 2.From the Degradation studies, it was observed that Theophylline and Montelukast Sodium were stable towards acid, alkali, oxidative and thermal stress conditions and amount of degradation

achieved was within the acceptable limits of 5-20%.

CONCLUSION

A simple, precise and accurate stability indicating RP-HPLC method has been developed and validated for simultaneous estimation of Theophylline and Montelukast Sodium in its bulk and pharmaceutical dosage form.Based on the forced degradation studies carried out, the proposed stability indicating method is reliable for demonstrating and detecting any expected change or degradation in the drug product during stability studies and canbe used in stability studies for effective evaluations and also for the quantitative estimation of Theophylline and Montelukast Sodium in bulk and pharmaceutical dosage form in repetitive analysis.Based on literature, as there were no available stability indicating RP-HPLC method for the simultaneous estimation of Theophylline and Montelukast Sodium, the stability indicating method developed here wasnovel, robust andreproduce acceptable results under different method conditions.

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