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Development and Validation of Analytical Method for Simultaneous Estimation of Theophylline and Montelukast in Pharmaceutical Dosage Form

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ABSTRACT:

A reversed-phase liquid chromatographic method has been developed and validated for estimation of Theophylline and Montelukast in Tabletdosage form. RP-HPLC method, Column used was C18(150 x 4.6 mm i.d.,5µm) with mobile phase containing 0.3 % Triflouro acetic acid in water pH 2.5: acetonitrile (20:80 %v/v). The flow rate (1.0 ml/min) and wavelength (230 nm). The retention time was found to be 4.201 mins and 6.124 mins of Theophylline and Montelukast respectively. Correlation co-efficient for Theophylline and Montelukast was found to be 0.999. Assay result of marketed formulation wasfound to be in 99.8 % and 98.6 % for Theophylline and Montelukastrespectively . The proposed method was validated with respect to linearity, accuracy, precision androbustness. Recovery was found in the range of 99.5 %– 101.7 %. Statistical Analysis proves that the developed methods weresuccessfully applied for the analysis of pharmaceutical formulations and can be used for routine analysis of drugs in QualityControl laboratories.

KEYWORDS Theophylline, Montelukast, HPLC, analytical method development, validation

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INTRODUCTION:

The IUPAC name of the Theophylline is 1,3-Dimethyl-3,7-dihydro-1H-purine-,6-dioneWithmolecular formula and molecular weight$C_7H_8N_4O_2$and 180.16402 g.mol^{-1}respectively.[1,2]

The molecular structure of the drug is given in Fig.1[3]

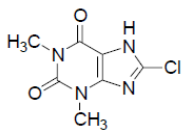

Figure 1 molecular structure of Theophylline

- Theophylline, also known as 1,3-dimethylxanthine, is a methyl xanthine drug used in therapy for respiratory diseases such as COPD and asthma under a variety of brand names.
- As a member of the xanthine family, it bears structural and pharmacological similarity to caffeine.[4]

The IUPAC name of the Montelukast is [R-(E)]-1-[[[1-[3-[2-(7-chloro-2-quinolinyl)ethenyl]phenyl]3-[2-(1-hydroxy-1-

methylethyl)phenyl]propyl]thio]methyl]cycl-opropaneacetic acid, monosodium salt with molecular formula and molecular weight $C_{35}H_{35}ClNNaO_3S$ and 608.17 $g.mol^{-1}$ respectively.[5,6]

The molecular structure of the drug is given in Fig.2[3]

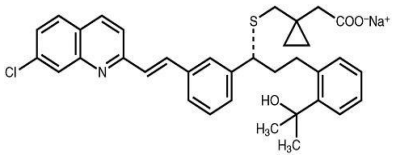

Figure 2 molecular structure of Montelukast

- Montelukast is a leukotriene receptor antagonist (LTRA) used for the maintenance treatment of asthma and to relieve symptoms of seasonal allergies.
- It is usually administered orally. Montelukast blocks the action of leukotriene D4 on the cysteinyl leukotriene receptor CysLT1 in the lungs and bronchial tubes by binding to it.
- This reduces the bronchoconstriction otherwise caused by the leukotriene, and results in less inflammation. Because of its method of operation, it is not useful for the treatment of acute asthma attacks.[7]

However no HPLCmethod has been reported till date for theestimation of Theophylline and Montelukast using the RP-HPLC method. The present paper describesthe analytical method development and validation of estimation of Theophylline and Montelukast inPharmaceutical dosage form using RP-HPLC. The proposed methodare optimized and validated as per ICH guidelines.[8]

Materials and methods

Materials:

a) Instruments

- Analytical Weighing Balance
- Sonicator
- FT-IR spectrophotometer
- HPLC system

b) Glasswares

- Beaker
- Conical flask
- Measuring cylinder
- Petri dish
- Pipette
- Volumetric flask

c) Chemicals

- Standard Montelukast sodium Gifted by Montage laboratories pvt. Ltd, Himmatnagar.
- Standard Theophylline Gifted by S Kant healthcare Ltd, Vapi.
- The commercial fixed dose combination Telekast-T Tablet was procured from local market. All solvents (HPLC grade) were obtained from Ranken Chemicals.

d) Method

- Chromatographic method

Methods

Working Standard preparation

- **Solution preparation of Montelukast : (10 µg/mL)**

10 mg of montelukast API was dissolved in 100 ml of diluent, fortther diluted 5 ml to 50 ml toget 10µg/ml concentration of montelukast in solution.

- **Solution preparation of Theophylline : (400 µg/mL)**

400 mg of Theophylline API was dissolved in 100 ml of diluent, further diluted 5 ml to 50 ml with diluent to get 400µg/ml concentration of Theophylline in solution.

- **Sample Preparation for marketed formulation[9]**

Transferred 5 intact tablets in to 250ml of volumetric flask, added about 200ml of Diluent in to it,sonicated for 30 minutes with intermittent shaking, cooled to attain room temperature and made upto volume with Diluent. and filtered the solution with 0.45µ nylon filter. Further 5 ml of stock solutionpipette out in 100mL of volumetric flask and made up the volume with Diluent.

Theophylline: 400 ppm
Montelukast: 10 ppm

METHOD VALIDATION

Chromatographic conditions and System SuitabilityParameters:

Pumps: Mode of chromatography: Reversed PhaseChromatography
Mode of Elution: Isocratic
Flow Rate: 1.0 ml/min
Oven: Oven Temperature: 35° ± 2°C
Detector: Type: uv detector
Wavelength: 230 nm
Column: Waters symmetry C-18, 150X4.6 mm, 5µ
Sample Volume: 10 µl
Run time: 10 min
Mobile Phase: 0.3 % Triflouro acetic acid in water (pH2.5) :Acetonitrile(20:80 %v/v)

System Suitability Parameters:

Table 1: System Suitability Parameters

Sr. no.	System suitability parameter	Theophylline	Montelukast
1	Retention times (R_T)	4.1948	6.111
2	Theoretical plates (N)	15035	9997.1
3	Resolution (R_S)	-	7.8
4	Tailing factor (A_S)	1.0	1.1
5	% RSD	0.4	0.2

Linearity and Range (n=3):

The linearity response was determined by analyzing 5independent levels of calibration curve in the range of 200-600 µg/ml for Theophylline and 5-15 µg/ml for Montelukast.

The plot of peak area against concentration was plotted. Correlation coefficient and regression line equationswere calculated. Linearity range was established throughconsideration of required practical range and accordingto each drug concentration present in thepharmaceutical product, to give accurate, precise andlinear results**.**

Precision

Repeatability

Repeatability was determined by analyzing standardsolution of Theophylline having the concentration 400 µg/ml and Montlukast having the concentration 10 µg/ml. Scanned these solutions six times in a day. Theresults were reported in terms of % RSD (relativestandard deviation).

Intraday Precision

The intra-day precision of the proposed method wasdetermined by measuring the corresponding responses 3times on the same day for 3 different concentration of Theophylline for 200,400 and 600 µg/ml and Montelukast for 5,10 and 15 µg/ml.

The results werereported in terms of % RSD.

Interday Precision

The inter-day precision of the proposed method wasdetermined by measuring the corresponding responseson 3 different days over a period of 1 week for 3 different

concentration of Theophylline for 200,400 and 600 µg/ml and Montelukast for 5,10 and 15 µg/ml. Theresults were reported in terms of % RSD.

Accuracy (% Recovery)

The accuracy of the method was determined bycalculating recovery of Theophylline and Montelukast.by the Standardaddition method. Each solutionwas injected in triplicate and the percentage recoverywas calculated by measuring the peak areas and fittingthese values into the regression equation of therespective calibration curves.

Limit of detection and Limit of quantification

The limit of detection (LOD) and the limit ofquantification (LOQ) were calculated using the standarddeviation of y-intercept of calibration curve (σ) andaverage of slope (S) of the calibration curve.

$LOD = 3.3 \times \sigma / s$
$LOQ = 10 \times \sigma / s$

Robustness

The robustness was studied by analyzing the sample of Theophylline and Montelukast by deliberate variation in the methodparameters. The change in the response was noted.

Robustness of the method was studied by changingdifferent experimental conditions like temperature ofcolumn by ± 2°C, Flow rate by ±0.2 ml/min, Mobile phaseby ± 2 %.

RESULT

VALIDATION PARAMETER

Linearity and Range

Linear correlation was obtained between peak area andconcentration of Theophylline in the range of 200-400µg/ml and Montelukast in the range of 5-15 µg/ml. The linearity of the calibration curves wasvalidated by the value of correlation coefficients of theregression (r).

Table 2: Linearity data for Theophylline

% Linearity Level	Concentration (µg/ml)	Mean area	Correlation coefficient
50	200.1	3200224	
75	300.1	4802621	
100	400.1	6408087	0.9999
125	500.2	8007734	
150	600.2	9654005	

Table 3: Linearity data for Montelukast

% Linearity Level	Concentration (µg/ml)	Mean area	Correlation coefficient
50	5.0	4966959	
75	7.5	7452972	
100	10.0	9957146	0.9999
125	12.5	12442715	
150	15.0	14975626	

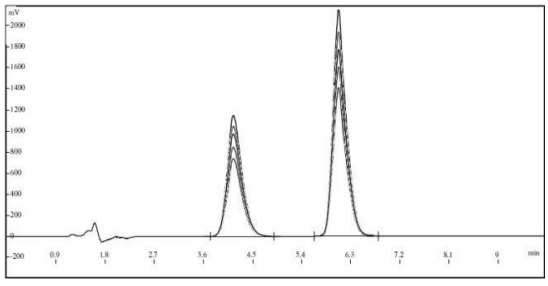

Figure 3: Overlay chromatogram of different concentration of Theophylline and Montelukast

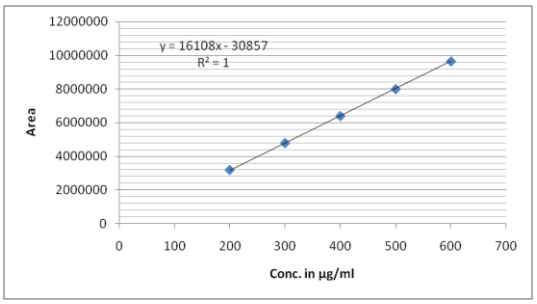

Figure 4: Calibration curve of Theophylline

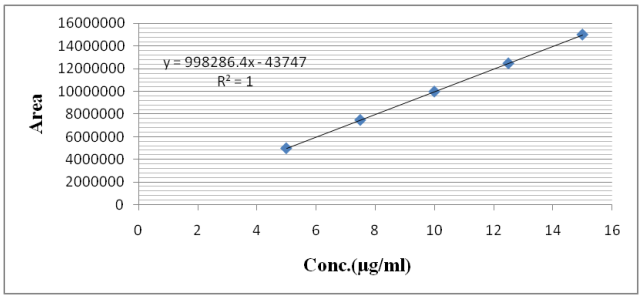

Figure 5: Calibration curve of Montelukast

Precision

Repeatability

The data for repeatability for Theophylline is shown inTable-4. The % RSD for Repeatability data was found to be 0.25%.

Table 4: Repeatability of Theophylline

Sr. no	Area	Mean	% RSD
1	6338050		
2	6331878		
3	6306195	6324139.5	0.25
4	6345016		
5	6313876		
6	6309822		

The data for repeatability for Montelukast is shown in Table-5. The % RSD for Repeatability data was found to be 0.17%.

Table 5: Repeatability of Montelukast

Sr. no	Area	Mean ± SD	% RSD
1	9818058		
2	9799919		
3	9775760	9789289.33±16944.6	0.17
4	9774194		
5	9780295		
6	9787510		

Intraday precision

The data for intraday precision for Theophylline is shownin Table-6. The % RSD for intraday precision was found to be 0.23%.

Table 6: Intraday precision for Theophylline

Sr. No.	Concentration (µg/ml)	Mean Area ± SD	% RSD
1	200.0	3184294±7334.6	0.23
2	400.0	6370284±13238.7	0.21
3	600.0	9597196±25354.4	0.26
Mean			0.23

The data for intraday precision for Montelukast is shownin Table-7. The % RSD for intraday precision was found to be 0.26%.

Table 7: Intraday precision for Montelukast

Sr. No.	Concentration (µg/ml)	Mean Area ± SD	% RSD
1	5.0	4937634±5731.0	0.12
2	10.0	9898388±15125.1	0.15
3	15.0	14887530±76370.9	0.51
Mean			0.26

Interday precision

The data for interday precision for Theophylline is shownin Table-8. The % RSD for intraday precision was found to be 0.82%.

Table 8: Interday precision for Theophylline

Sr. No.	Concentration (µg/ml)	Mean Area ± SD	% RSD
1	200	3167371±24499.4	0.77
2	400	6336430±48720.1	0.77
3	600	9546276±89868.9	0.94
		Mean	0.82

The data for intraday precision for Montelukast is shownin Table-9. The % RSD for intraday precision was found to be 0.70%.

Table 9: Interday precision for Montelukast

Sr. No.	Concentration (µg/ml)	Mean Area ± SD	% RSD
1	5.0	4911627±36956.1	0.75
2	10.0	9846155±52310.2	0.53
3	15.0	14809062±124741.3	0.84
Mean			0.70

Accuracy

Accuracy of the method was confirmed by recovery studyfrom marketed formulation at three level of standardaddition. Percentage recovery for Theophylline and Montelukast was found

to be 99.5 – 100.8 %.

Table 10: Recovery Data of Theophylline

Accuracy Level %	Set no.	Area	Amount added (mg)	Amount recovery(mg)	%Recovery	Mean	%RSD
80 %	1	2535339	1600.00	1595.65	99.7	100.5	0.7
	2	2584708	1610.00	1626.72	101.0		
	3	2549722	1592.00	1604.70	100.8		
100%	1	3241476	2010.00	2040.07	101.5	100.5	0.9
	2	3172560	2000.00	1996.70	99.8		
	3	3167923	1990.00	1993.78	100.2		
120%	1	3877189	2410.00	2440.16	101.3	100.4	0.9
	2	3854889	2414.00	2426.13	100.5		
	3	3789423	2396.00	2384.93	99.5		

Table 11: Recovery Data of Montelukast

Accuracy Level %	Set no.	Area	Amount added (mg)	Amount recovery(mg)	%Recovery	Mean	%RSD
	1	3936	40.00	40.25	100.6		

80 %		328				10	1.1
	2	3936 490	39.75	40.25	101.3	0.3	
	3	3909 485	40.35	39.97	99.1		
	1	4896 676	50.00	50.07	100.1		
100%						10	0.6
	2	4889 166	50.25	49.99	99.5	0.1	
	3	4908 972	49.90	50.19	100.6		
	1	5965 343	60.00	61.00	101.7		
120%						10	0.1
	2	5920 818	60.40	60.54	100.2	0.5	
	3	5853 975	60.05	59.86	99.7		

Limit of detection and limit of quantification

The Limit of detection (LOD) and Limit of quantitation (LOQ) Theophylin and Montelukast as mention below table.

Table 12: Results of LOD and LOQ

Drug	Theophylline	Montelukast
LOD	0.521	0.0130
LOQ	1.581	0.0396

Robustness

The method is found to be robust as the results were notsignificantly affected by slight variation in composition ofmobile phase, Column temperature and flow rate of the mobile phase.

Table 13: Change the ratio of mobile phase

Standard repetitions (n=6)	**18 : 82**		**22 : 78**	
	Theophylline	Montelukast	Theophylline	Montelukast
Mean Area ± SD	6391100± 38978.9	9906135± 17035.9	6392535± 19029.9	9824652± 54439.1
% RSD	0.6	0.2	0.3	0.6

Table 14: Change the flow rate

Standard repetitions (n=6)	**0.8 ml/min**		**1.2 ml/min**	
	Theophylline	Montelukast	Theophylline	Montelukast
Mean Area ± SD	6985061± 28708.7	10879634 ±17845.6	5781165± 39693.3	8918004± 43199.3
% RSD	0.4	0.2	0.7	0.5

Table 15: Change the column temperature

Standard repetitions (n=6)	**40°C**		**30°C**	
	Theophylline	Montelukast	Theophylline	Montelukast
Mean Area ± SD	6372509± 24715.8	9906135± 17035.9	6371014± 21402.2	9832865± 24178.2
% RSD	0.4	0.2	0.3	0.2

Applicability of the method

The proposed RP-HPLC method was successfully appliedfor determination of Theophylline and Motelukast in tablet dosage form.The percentage was found to be satisfactory, which iscomparable with the corresponding label claim amount

Table 16: RP-HPLC method to Theophylline and Montelukast tablet formulation

Sr. no.	**Sample name**	**% Assay for Theophylline**	**% Assay for Montelukast**
1	Formulation	99.8%	99.6%

CONCLUSION

In Estimation of Theophylline and Montelukast in pharmaceutical dosage form,separation was achieved on Waters symmetry C-18, 150X4.6 mm, 5µ

at35ºCtemperature by using a mobile phase0.3 % Triflouro acetic acid in water (pH2.5) :Acetonitrile(20:80 %v/v) at a flow rate of 1.0 ml/min and UV detection was carried out at 230 nm. Data suggests that peak purity index ofthe drug was found to be greater than 0.999, so there isno co-elution of any degradation products with mainpeaks and the results obtained were found within the

acceptance criteria. Results of the validation forTheophylline and Montelukastof the above method were linear in therange of 200-600 µg/ml and 5-15µg/ml respectively . The % recovery was found to be 99.5%–101.7 %.The results of the precision studyindicate that the proposed method shown goodrepeatability with a % RSD of 0.3 % for Theophyllineand % RSD of 0.2 % for Montelukast. Similarly %RSD fromthe intraday precision data was found to be 0.23% for Theophylline and 0.26% for Montelukast and %RSD from the Interday precision data werefound to be 0.82% for Theophylline and 0.70% for Montelukast. Absolute differencebetween mean assay values of method precision and intermediate precision was found to be less than 2.0 %.Robustness is performed by making changes in flow rate, Mobile phase composition and Column temperature. The assayobtained after proposed changes compared with theassay obtained in normal conditions. According to the

acceptance criteria difference in the assay should not bemore than 2%. The results obtained are well within theacceptance criteria. The % assay results of 99.8 % for Theophylline and 98.6 % for Montelukast indicates that the proposed method wassuccessfully utilized for the estimation Theophylline and Montelukast in pharmaceutical dosage forms. Hence, the method can be termedas robust. Since the results are well within the limit ofacceptance criteria for all validation parameters,therefore the method can be considered as validated and

suitable for intended use. So, the proposed RP-HPLC assay method can be successfullyapplied for the estimation of Theophylline and Montelukast in pharmaceuticaldosage form.

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