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Development and Validation Of RP-HPLC Method for Simulataneous Estimation Doxophyline and Montelukast Sodium in Tablet Dosage Form

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Abstract: A simple, rapid, accurate, precise and economical reverse phase high performance liquid chromato graphic method has been developed for simultaneous estimation of Doxophylline and Montelukast sodium. The separation of drugs was achieved on Inertsil Ph-3 column (250 mmx4.6 mm id, 5 µm particle size) with mobile phase of Ammonium acetate 0.05milimolar and Glacial acetic acid buffer (pH 3.5): Methanol (18:82 v/v) at flow rate of 1.2 ml/min. The detection was done at 274 nm and retention time was found to 4.4 mins and 8.4 mins for Doxophyline and Montelukast Sodium respectively. The run time for this method was 12 minutes. The validation was done as per ICH guideline. Doxophylline and Montelukast sodium showed a linear response in the concentration range of 15.99-47.98 µg/ml and 50.43-151.30 µg/ml respectively. The correlation co-efficient for Doxophylline and Montelukast sodium was found to be 0.9998 and 0.9999 respectively. The detection limit for Doxophylline and Montelukast was found to be 0.5 µg/ml and 0.2 µg/ml and quantitation limit 1.5µg/ml and 0.6 µg/ml respectively. The percentage recoveries obtained for Doxophylline and Montelukast sodium ranges from 101.4% to 100.3% and 100.0% to 101.5% respectively. This method can be successfully employed for routine analysis of both drugs in tablet dosage form.
Keywords: Doxophylline and Montelukast sodium, Validation.

Introduction:

Doxophylline (Figure 1) is chemically, 7-(1,3-dioxolan-2-ylmethyl)-1,3-dimethylpurine-2,6-dione has molecular formula $C_{11}H_{14}N_4O_4$ and molecular weight 266.26. It is new antibronchoplastic drug recently introduced in therapy, as a potent adenosine receptor antagonist. It is xanthine derivative drug used in the treatment of asthma.[1] It has antitussive and bronchodilator effects, and acts as a phosphodiesterase inhibitor.[2] Literature survey reveals that various bio-analytical methods are availabe for estimation of Doxophylline in human serum and blood, few spectrophotometric methods and HPLC methods for the quantitative estimation of Doxophylline in bulk and pharmaceutical formulations [4-8]. Montelukast sodium (Figure 2) is chemically(R,E)-2-(1-((1-(3-(2-(7-chlorquinolin-yl)vinyl)phenyl)propylthio)methyl)cyclopropyl) acetic acid sodium has molecular formula $C_{35}H_{35}ClNNaO_3S$ and molecular weight 608.17. Montelukast sodium is Leukotriene receptor and $CysLT_1$ antagonist which reduces bronchoconstriction caused by Leukotriene. It is used in treatment of asthma and it also relieve symptoms of seasonal allergies. Literature survey reveals that various bio-analytical methods are available for estimation of Montelukast sodium in human serum and blood and few spectrophotometric

methods and HPLC methods for the quantitative estimation of Montelukast sodium in bulk and pharmaceutical formulations [9-12]. There are various RP-HPLC methods are available for estimation of Montelukast in combination with othe drug like (Levocetrizine, Ambroxol, Bambuterol) [13-20]. There are three methods available in the literature for simultaneous estimation of Doxophylline and montelukast sodium [23-25].

Hence objective of this study was to develop selective, precise and reproducible RP-HPLC method for estimation of Doxophyline and Montelukast in tablet dosage form

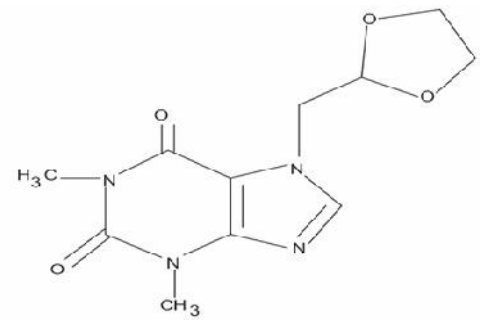

Figure 1: Structure of Doxophylline.

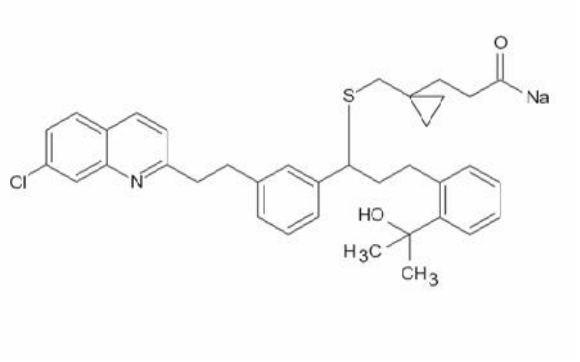

Figure 2: Structure of Montelukast sodium

Experimental

Materials and Methods

Reference standards of Doxophylline and Montelukast sodium were received from Startech labs. Marketed Preparation Dxoliin M® Tablets was obtained from Zydus Cadila Healthcare Ltd(Lable claim-Doxophylline-400 mg and Montelukast sodium-10 mg). Ammonium acetate, orthophosphoric acid, methanol, water and all regents were of HPLC grade and obtained from Merck (India) Ltd., Mumbai

Instrumentation and Chromatographic condition

The HPLC system (Shimadzu LC-2010CHT) consisted of quaternary pump system, software LC solution (Ver. 1.25, SP1) and auto injector programmed at 20µl capacity was used. The detector used was UV-Visible and operated at 274nm. The column used was Inertsil Ph-3 (250 mm 4.6 mm i.d-5 µ).The chromatographic separation was achieved on HPLC system using mobile phase Ammonium acetate buffer (pH-3.5 adjusted by glacial acetic acid) and Methanol in the ratio of 18:82 %v/v with flow rate 1.2 ml/min at 274 nm.

Experimental work

Preparation of standard solution

The standard stock solution of Doxophylline (32 ppm) (solution –A) and Montelukast sodium (100 ppm) (solution– B) was prepared by weighing Doxophylline (40 mg) and Montelukast sodium (52 mg) in 50 ml volumetric flask respectively. Then 25 ml of diluent was added and sonicated to dissolve. Volume was made up to the mark with diluents and mix.

Dilute 2.0 ml of standard preparation A and 5.0 ml of standard preparation B to 50.0 ml with diluents and mix.

Preparation of test solution (For Montelukast sodium)

Twenty tablets were weighed accurately and triturated to fine powder. The sample powder about 1519.98mg (Tablet powder Equivalent to 20 mg of Montelukast sodium) was transferred into 200 ml volumetric flask. Then

about 125.0 ml diluent was added and sonicated for 35 minutes with intermittent shaking. Then volume was made up to mark with diluent to make final standard concentration of Montelukast sodium (100 ppm). The test solution was filtered through 0.45µ (PVDF Millipore filter) and analyzed the filtrate (Stock Solution) by using HPLC system. The isocratic program was adopted analyze both component in single run by HPLC as shown **in** (Figure 3).

Buffer preparation

Accurately weighed 3.8 g ammonium acetate was dissolved in 1000 ml water and pH was adjusted to 3.5 with glacial acetic acid. The solution was filtered through 0.45 µm filter.

Preparation of test solution (For Doxophylline)

2.0 ml of above stock solution was diluted to 250 ml with diluents and mixed.

Method Validation

Validation was carried out with respect to various parameters, as required under ICH guideline Q2 (R1).

Linearity

To achieve linearity and range, stock solution containing Doxophylline (800 ppm) and Montelukast sodium (1040 ppm) were prepared. Doxophylline and Montelukast sodium Stock solutions were diluted to yield solutions in the concentration range of 15.99-47.98 µg/ml (50 % to 150%) and 50.43-151.30 µg/ml (50 % to 150 %) respectively.

Precision

Precision of the method was verified by method precision studies. The method precision was done by preparing six different sample preparations by one analyst. The results are presented in Table No.4.

Ruggedness

Ruggedness test was determined between different analyst, instrument and Column. The ruggedness of the method was done by sample application and measurement of peak areas were assessed by chromatography of six replicates of the concentration (100 µg/ml). The data obtained from ruggedness experiment are presented in Table 4.

Accuracy

The difference between theoretical added sample amount to the placebo and practically achieved sample amount from placebo (after HPLC analysis) is called accuracy of analytical method. Accuracy was determined at three different level 50%, 100% and 150% of the target concentration in triplicate. The results are presented in Table 5 and Table 6.

Solution stability

The standard and sample solutions were found stable up to 24 hours at room temperature. After 8, 24 hours the solutions were analyzed and results related to solution stability are summarized in Table 7.

Robustness

To evaluate Robustness of the method small but deliberate variation in the optimized RP-HPLC parameter. Small changes in the flow rate, pH, mobile phase ratio and column oven temperature were made to determine robustness of the method. Results are presented in Table 8.

LOD (Limit of Detection) and LOQ (Limit of Quantitation)

In order to estimate the limit of detection (LOD) and limit of quantitation (LOQ) values, the blank sample was injected six times and the peak area of this blank was calculated as noise level.

Result and Discussions

Solvent type, solvent strength, flow rate were varied to determine chromatographic conditions giving good separation. The mobile phase conditions were optimized so the peak from eluting compound did not interfere with those from solvents and exciepients. The final chromatographic conditions set for method were mobile phase of 0.05M Ammonium acetate buffer (pH-3.5 adjusted with glacial acetic acid): methanol (18 : 82 % v/v) with flow rate 1.2ml/min and temperature of 25 ˚c. The column used was Inertsil Ph-3 (250 mm 4.6 mm i.d-5 µ) and detection was carried out at 274 nm. System suitability parameter are shown in Table No.1.

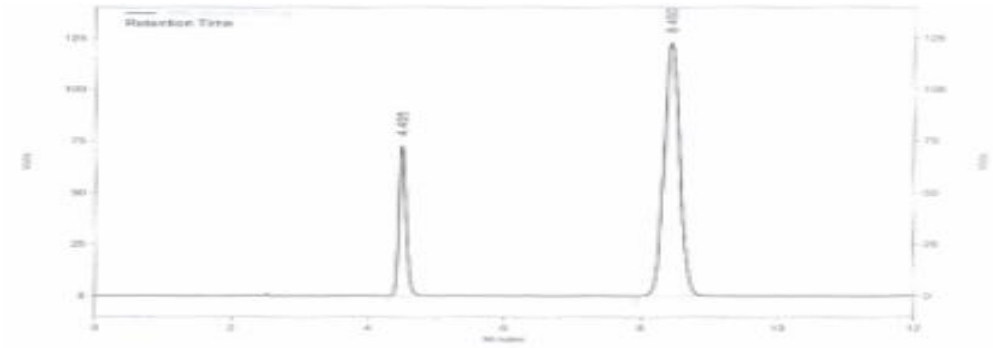

Figure 3: HPLC Chromatogram of Doxophylline and Montelukast sodium.

Table 1: System suitablity parameters found after RP HPLC Analysis.

Compound	Retention Time	Theoretical Plates	Asymmetry	Tailing Factor	Resolution	% RSD
Doxophylline	4.4 ± 0.0022	8929	1.10	1.11	0.00	0.2
Montelukast sodium	8.4 ± 0.0031	8574	1.00	1.04	5.48	0.1

Linearity:

The response of drug was linear in the concentration range 15.99-47.98 µg/ml (50 % to 150%) for Doxophyline and 50.43-151.30 µg/ml (50 % to 150 %) for Montelukast sodium. Calibration curve for both the drugs are shown in (Figure 4) and (Figure 5). The results of linearity are presented in Table No.2 and Table No.3.

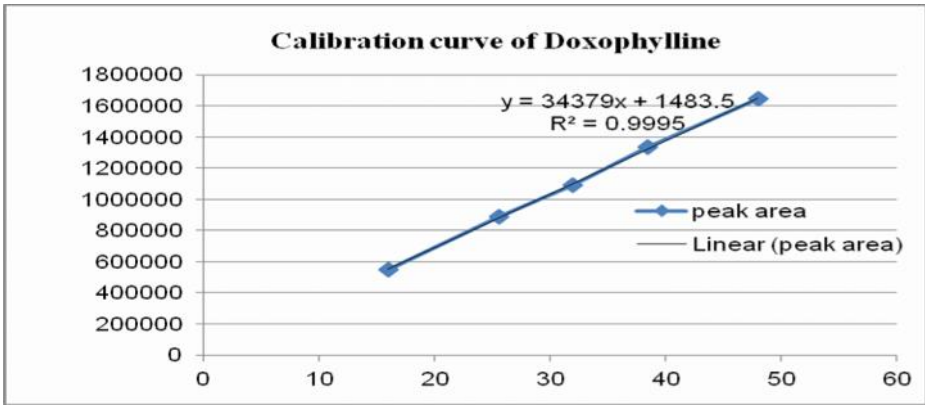

Figure 5: Calibration Curve of Doxophylline sodium

Table 2: Linearity data of Doxophylline

Linearity data for Doxophylline				
Linearity Level	**Doxophylline Stock solution taken in ml**	**Diluted to volume (mL) with diluent**	**Final conc (µg mL^{-1})**	**Area**
50%	1.0	50	15.9919	548314
80%	1.6	50	25.5870	888354
100%	2.0	50	31.9838	1089680
120%	2.4	50	38.3805	1333020
150%	3.0	50	47.9756	1645962

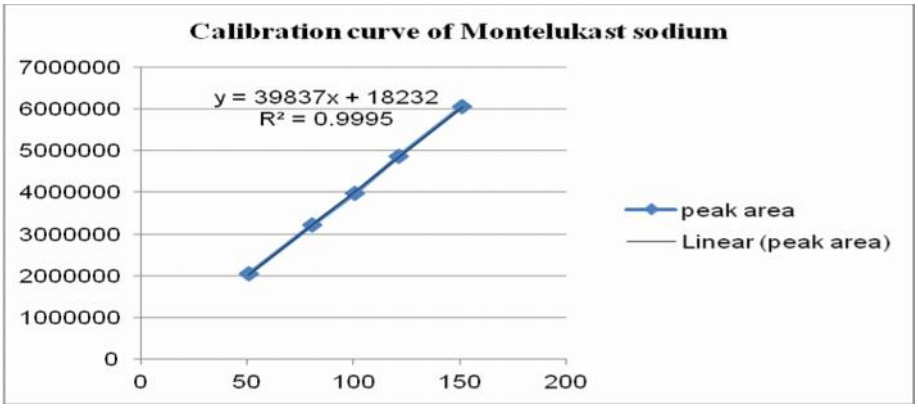

Figure 5: Calibration Curve of Montelukast sodium

Table 3: Linearity data for Montelukast sodium

Linearity data for Montelukast sodium				
Linearity Level	**Montelukast sodium Stock solution taken in ml**	**Diluted to volume (mL) with diluent**	**Final conc (µg mL^{-1})**	**Area**
50%	2.5	50	50.4328	2049898
80%	4.0	50	80.6925	3227557
100%	5.0	50	100.8656	3982419
120%	6.0	50	121.0387	4866767
150%	7.5	50	151.2984	6055267

Precision:

The result for Method Precision and Ruggedness are shown in table No.4. The method was found to be precise as the %RSD values for method precision and ruggedness were < 2 as recommended by ICH guideline Q2B.

Table 4: Results of Precision and Ruggedness of the method

Parameters	Doxophylline		Montelukast sodium	
	%Assay Mean*	% RSD	%Assay Mean*	% RSD
Method precision	99.4 %	0.9 %	116.8 %	0.3 %
Ruggedness	100.1 %	0.3 %	115.5 %	0.7 %

**n=6*

Accuracy:

The result for sccuracy are shown in table No.5 and 6. The %recoveries for Doxophylline and Mntelukast was found in the range of 100.8 to 101.2 and 99.3 to 100.2 respectively. The % RSD and % Recoveries for the individual measurment was calculated**.**

Table 5: Results of accuracy of Doxophylline

Accuracy data of Doxophylline					
Level	**Amount of drug added (mg)**	**Amount of drug recovered (mg)**	**Recovery (%)**	**Mean %**	**% RSD**
50%	400.30	404.85	101.4	101.2	0.8*****
	400.20	406.39	101.9		
	400.50	400.61	100.3		
100 %	800.40	798.16	100.0	100.8	0.7*****
	800.40	805.42	100.9		
	800.30	809.98	101.5		
150 %	1200.80	1220.05	101.9	101.1	1.4*****
	1200.50	1191.28	99.5		
	1200.50	1220.26	102.0		

**n=3*

Table 6: Results of accuracy of Montelukast sodium

Accuracy data of Montelukast sodium					
Level	**Amount of drug added (mg)**	**Amount of drug Recovered (mg)**	**Recovery (%)**	**Mean %**	**% RSD**
50%	11.10	10.66	99.3	99.3	0.2*****
	11.10	10.68	99.4		
	11.40	10.94	99.1		
100 %	22.10	21.35	99.8	100.0	0.6*****
	22.20	21.65	100.7		
	22.50	21.67	99.5		
150 %	32.50	31.51	100.2	100.2	0.4*****
	32.40	31.56	100.6		
	32.70	31.58	99.80		

**n= 3*

Robustness:

The parameter chaged for robustness were Temperature, Mobile phase ratio, pH of mobile phase and flow rate. The triplicate (N=3) injections of standard drug were performed under standard condition with small but deliberate changes in the above mentioned parameter. Insignificant differences in the peak areas, asymmetry and resolution were observed and changes in % RSD was found to be within limit. The method was found to be robust.

Table 8: Doxophylline (Doxo) & Montelukast sodium (Mont)robustness Study.

Name of Drug	Temp. 34.5°C	Temp. 35.5°C	Flow rate 1.0 mL	Flow rate 1.4 mL	Mobile phase Ratio (16:8)	Mobile phase Ratio (20:80)	pH 3.3	pH 3.7	Mean %RSD
Doxo %RSD (N=3)	0.4	0.6	0.6	0.8	0.6	0.9	0.5	0.8	0.7
Mont %RSD (N=3)	0.2	0.3	0.3	0.7	0.2	0.5	0.3	0.5	0.4

Limit of detection and Limit of quantitation:

The LOD was calculated as three times the noise level while ten times the noise value gave the LOQ. The results of LOD and LOQ are mentioned in Table 9.

Solution stability:

Table 7: Standard & sample solution stability data Doxophylline and Montelukast sodium

Standard solution Stability					Sample solution stability			
Time (Hrs)	**Area**		**Difference (% RSD)**		**Area**		**Difference (% RSD)**	
	Doxo-phylline	**Monte-lukast-sodium**	**Doxo-phylline**	**Monte-lukast-sodium**	**Doxo-phylline**	**Monte-lukast-sodium**	**Doxo-phylline**	**Montel-ukast sodium**
0	596450	2151558	==	==	585151	2615791	==	==
8	605064	2171257	0.33	0.48	587782	2630981	0.34	0.51
24	607181	2187303	0.58	0.85	596401	2653903	0.57	1.1
31	611117	2223135	0.90	1.01	603322	2696726	0.88	1.23
% Mean RSD			0.60	0.78	% Mean RSD		0.59	0.94

Table 9: Summary of Validation Parameters of RP-HPLC Method for Simultaneous estimation of Doxophylline and Montelukast sodium

Parameter	Acceptance Criteria	Doxophylline	Montelukast sodium
Range of Linearity	Follows Beer Lambert's law	15.99-47.975 μg/ml	50.43-151.30 μg/ ml
Correlation Coefficient	Correlation coefficient r^2 > 0.999 or 0.995	0.9998	0.9999
LOD	S/N > 2 or 3	0.5 μg/ml	0.2 μg/ml
LOQ	S/N > 10	1.5 μg/ml	0.6 μg/ml
Precision	RSD < 2%	0.9%	0.3%
Ruggedness	RSD < 2%	0.3%	0.7%
Accuracy	Recovery 98- 102%	100.8% to 101.2%	99.3% to 100.2%
Specificity	No intereference of placebo	Complies	Complies
Solution Stability	> 12 hour	Stable for 24 hr %RSD = 0.46%	Stable for 24 hr %RSD =0.67%
Robustness	RSD NMT 2% in modified condition	Complies	Complies

Conclusion

Thus proposed method was found to be simple, rapid, accurate, selective and economical for simultaneous routine analysis of Doxophylline and Montelukast sodium in bulk and commercial dosage form. This method can also be used for determination of content uniformity and dissolution profiling of this product. It can be also applied for the industrial as well as academic purpose.

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