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Simultaneous Estimation and Method Validation of Montelukast Sodium and Doxofylline in Solid Dosage form by RP-HPLC

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ABSTRACT

A reversed-phase high performance liquid chromatographic method has been developed and validated method for the simultaneous estimation of Doxofylline and Montelukast sodium in tablet dosage form. The method was carried out on a Inertsil C18 column with a mobile phase consisting of Acetonitrile: Methanol: Ammonium acetate buffer of pH 5.5 (10:70:20 % v/v) at a flow rate of 1.5 mL $min^{-1.}$ The Spectrophotometric detection was carried out at 274 & 347nm. The validation of the method was carried out and the results show that the proposed method is specific, accurate, precise and linear. The proposed method can be used for the simultaneous estimation of Montelukast sodium and Doxofylline in tablet dosage form by Rp-Hplc mehod.

Key words: RP-HPLC, Sustained dosage form, Montelukast sodium, Doxofylline.

1. INTRODUCTION

Montelukast is a potent, selective and orally active antagonist of the cysteinyl, CysTL1, leukotriene receptor used for the treatment of asthma in children and adults[1-3]. Montelukast, a leukotriene modifier, has clearly demonstrated the ability to ameliorate bronchoconstriction and indices of airway edema and abnormal mucus Production as observed in clinical trials[4].

Doxofylline (DX) is chemically7-(1,3-Dioxolan-2-ylmethenyl) Doxofylline. It used as a bronchodilator in asthma and chronic obstructive pulmonary disease[5,6].

Few methods have been reported in the literature for the estimation of Montelukast sodium[7-9] and Doxofylline[10,11] individually and in combination with other drugs. However, there is no method reported for the simultaneous estimation of Montelukast sodium and Doxofylline in combined dosage form by HPLC. So the aim of proposed work is to develop a simple, rapid, sensitive and accurate method for the simultaneous estimation of Montelukast sodium and Doxxyphilline, validate[12] the developed method.

2. MATERIALS AND METHODS

2.1. Reagents and chemicals

Ammonium acetate AR grade, Glacial acetic acid AR grade, Acetonitrile, Methanol and Water HPLC grade supplied by S.D fine chemicals. The standard drugs of Montelukast sodium Doxofylline were obtained Marcus Pharmaceuticals, Chennai and D Montus tab from Fourrts India Lab, Chennai.

2.2. Optimized Chromatographic Conditions

Stationary Phase: Inertsil C18 (4.6x250 mm, 5µm)

Injector : Rheodyne

Flow rate:	1.5ml
Operating temperature: temperature	Ambient
Selected wave length:	280 nm
Mobile phase ratio:	Acetonitrile: Methanol: Ammonium acetate buffer (10:70:20% v/v, pH 5.5)
Injection Volume:	20µl
Run Time:	10 min

2.3. PREPARATION OF SOLUTIONS

2.3.1. Preparation of buffer solution

3.85gm of Ammonium acetate was taken in a 1000ml volumetric flask, add 1ml of triethyl amine & add sufficient water to produce 1000ml, adjust the pH 5.5 with glacial acetic acid.

2.3.2. Preparation of mobile phase

Prepare the mobile phase with Acetonitrile: Methanol: Ammonium acetate buffer (10:70:20 % v/v pH 5.5)

2.3.3. Preparation of Montelukast standard solution

Accurately weighed quantity of 10mg Montelukast was transferred to a 100ml volumetric flask, dissolved in 25ml of mobile phase and the solution was made up the volume with mobile phase. From the above stock solution take 5ml was transferred to 100ml volumetric flask and make up the volume with mobile phase. The solution was filtered with 0.45µ filter and sonicated for 15min.

2.3.3. Preparation of Doxofylline standard solution

Accurately weighed quantity of 200mg Doxofylline was transferred to a 100ml volumetric flask, dissolved in 25ml of mobile phase, and the solution was made up the volume with mobile phase. The solution was filtered with 0.45µ filter and sonicated for 15min

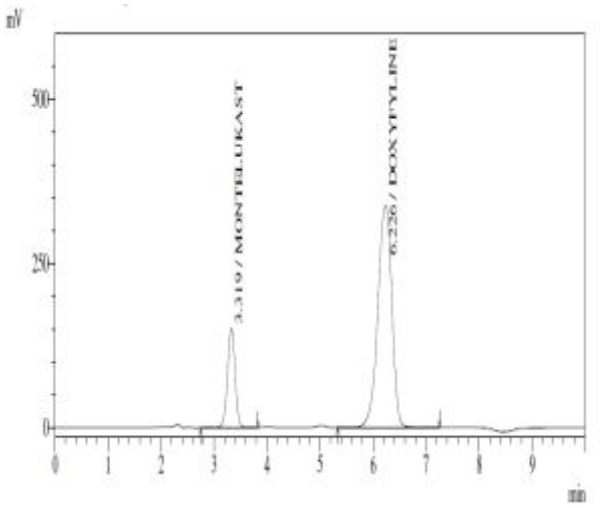
2.3.4. Preparation of sample solution

20 tablets are weighed and powdered 903.1mg of sample was transferred in to 100ml volumetric flask and add mobile phase to dissolve the sample. To make up the volume with mobile phase & filtered with 0.45µ filter paper. From the above stock solution take 5ml was transferred to 100ml volumetric flask and make up the volume with mobile phase. The solution was filtered with 0.45µ filter and sonicated for 15min.

3. RESULT AND DISCUSSION

The detection wavelength of 274 &347nm was chosen in order to achieve a good sensitivity for quantitative determination of Montelukast sodium and Doxofylline in tablet dosage form. The mobile phase consisting of Acetonitrile: Methanol: Ammonium acetate buffer (10:70:20 % v/v, pH 5.5) offered a good separation at ambient temperature under these conditions using a flow rate of 1.5 mL min-1 and a run time of 10 min. Montelukast sodium elutes first and then Doxofylline shown in the chromatogram. Fig. 1which illustrates the separation of both active ingredients in this system. The method was adopted to analyze both components in a single run. The proposed method is simple and do not involve laborious time-consuming sample preparations.

Fig.1. Cromatogram of the sample solution



3.1. System suitability system precision

Montelukast sodium and Doxofylline standard solution as per test method were prepared to make five replicate injections were given and evaluated the system suitability and system precision parameters like theoretical plate number (N) and Peak asymmetry factor (As) were studied with the help of standard chromatograms. The results of system suitability and system precision were presented in Table 1.

3.2. Linearity and Range

The calibration graph was plotted with peak area in the Y-axis and concentration of standard solution in the X-axis. The degree of linearity was estimated by calculating the correlation coefficient. The plot is linear over the concentration range of 1 to 9 µg mL^{-1} and 160 to 240 µg mL^{-1} for Montelukast sodium and Doxofylline respectively. The results of linearity, limit of detection and limit of quantification were presented in Table 2.

3.3. Specificity

There was no interference from the sample solutions. It showed that developed method was specific for the analysis of Montelukast sodium and Doxofylline in solid dosage form.

3.4. Method precision

Precision of the method was demonstrated by repeatability studies. This was done by injection consecutively the sample solution 6 times and passing them through the assay procedure. The results showed that the method was precise. The results obtained were presented Table .3.

Table .1. System suitability Parameters

Compound (n=5)	Resolution	Tailing factor	Number of theoretical plate	Retention time	%RSD
Montelukast sodium	-	1.12	6554.6	7.54	0.51
Doxofylline	24.5	2.0	7088.6	15.32	0.34

Table .2. Linearity data for Montelukast sodium and Doxofylline

Compound (n=5)	Linearity Range	Correlation Coefficient	Slope (m)	Intercept
Montelukast sodium	1-9 µg/ml	0.9994	756.32	2.15
Doxofylline	160-240 µg/ml	0.9997	117.96	18.49

Table .3. Method precision results for Montelukast sodium and Doxofylline

Compound (n=6)	Concentration µg mL-1	Retention time (mean)	%assay (mean)	%RSD of assay
Montelukast sodium	1.5	3.31	99.71	0.56
Doxofylline	66.5	6.22	99.66	0.37

Table .4. The results of recovery studies for Montelukast sodium and Doxofylline

Compound	Spike level	Amount added (mg)	Amount Recovered (mg)	% Recovered
Montelukast sodium	80 %	8	7.9	98.75
	100 %	10.01	9.89	98.80
	120 %	11.9	11.8	99.15
Doxofylline	80 %	160.1	159.87	99.85
	100 %	199.9	200.1	100.1
	120 %	240.3	240.19	99.95

Table .5. Method robustness (%RSD) in normal and changed condition (n=5)

Compound	Condition	Change	%RSD
Montelukast sodium	Temperature	Normal	0.29
		-50C	0.48
		+50C	0.35
	pH	Normal	0.24
		-0.2 unit	0.60
		+0.2 unit	0.46
	Flow rate	Normal	0.31
		-2mL min-1	0.19
		+2mL min-1	0.25
	Mobile phase	Normal	0.28
		-2%	0.49
		+2%	0.65
Doxofylline	Temperature	Normal	0.22
		-50C	0.29
		+50C	0.49
	pH	Normal	0.36
		-0.2 unit	0.38
		+0.2 unit	0.50
	Flow rate	Normal	0.62
		-2mL min-1	0.58
		+2mL min-1	0.69
	Mobile phase	Normal	0.28
		-2%	0.48
		+2%	0.49

Table .6. Method Ruggedness

Analyst	Analyst-1, Instrument-1, Column-1	Analyst-2, Instrument-2, Column-2	%RSD
	% assay	% assay	
Montelukast Sodium	100.02	99.81	Analyst(1)-0.41 Analyst(2)-0.29
Doxofylline	99.62	99.08	Analyst(1)-0.35 Analyst(2)-0.49

3.5. Accuracy

To study reliability, suitability and accuracy of the method, adding a known quantity of the standard to the preanalyzed sample carried out recovery studies and recovery studies were done. The recovery was carried out at 80%, 100% and 120% level and the contents were determined from the respective chromatogram. From the results obtained we can conclude that the method was accurate. The results of recovery studies were presented in Table .4.

3.6. Robustness

For demonstrating the robustness of the developed method experimental conditions were purposely altered and evaluated. The method must be robust enough to withstand such slight changes and allow routine analysis of the sample.

3.7. Method Ruggedness

Defined by the USP as the degree of reproducibility of results obtained under a variety of conditions, such as different laboratories, analysts, instruments, environmental conditions, operators and materials. Ruggedness is a measure of reproducibility of test results under normal, expected operational conditions from laboratory to laboratory and from analyst to analyst. The results were presented in the Table 6.

3.8. Standard and sample solution stability

Standard and sample solution stability was evaluated at ambient temperature for 24 h. The percentage relative standard deviation was found below 2.0. It showed that the standard and sample solution was stable up to 24 h at ambient temperature.

4. Conclusion

From the above experimental data results and parameters it was concluded that the developed RP-HPLC method has the standard and sample preparation requires less time, no tedious extraction procedure was involved in the analytical process, suitable for the analysis of raw materials and run time required for recording chromatograms were less than 10 min.

Hence, the developed chromatographic method for Montelukast sodium and Doxofylline was found to be simple, precise, accurate and cost effective and it can be effectively applied for routine analysis in drug research, quality control department in industries and approved testing laboratories and etc.

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