Research Article

Novel LC Method Development and Validation for Simultaneous Determination of Montelukast and Doxofylline in Bulk and Pharmaceutical Dosage Forms

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A novel rapid HPLC method was developed for simultaneous determination of montelukast and doxofylline in bulk and pharmaceutical dosage forms. Development of an analytical method for simultaneous estimation of drugs requires a lot of efforts and of course it is a challenging task. The method was developed by using C18 (150 mm × 4.6 mm, 5 μ m) column; mobile phase consisting of methanol and phosphate buffer at pH 4.5; the flow rate of 1.0 mL/min and ultraviolet detection at 280 nm. Both drugs were sufficiently resolved having retention time of 4.7 min and 1.9 min for montelukast and doxofylline, respectively. The method was validated as per ICH Guidelines for various parameters like precision, linearity, accuracy, ruggedness, and robustness. The validated method was applied to the commercially available pharmaceutical dosage form and obtained the desired result.

1. Introduction

In continuation to our earlier efforts [1, 2] to support our pharmaceutical fraternity, in this paper also we are trying to contribute with the analytical method of very important drugs required to cure most common disease, asthma.

Asthma is a chronic inflammatory disease that affects children and adults with symptoms such as a runny nose, nasal congestion, and sneezing. It causes inflammation of the airways in which the smaller passages of the lungs narrow. This causes wheezing, shortness of breath, mucus production, and coughing. With the present day's life style and due to environmental pollution, the population of asthma-infected people is drastically increasing. Hence, it occupies great medical importance. This can be controlled using anti-inflammatory drugs, bronchodilators, or leukotriene modifiers. Due to this reason, we have selected two important drugs, namely, montelukast and doxofylline for the study. Earlier, one method development for single drug, montelukast, using HPLC has been executed and submitted as a part of the author's dissertation work [3]. It has encouraged the authors

to extend this study with multidrug matrix. In the present study, two drugs montelukast and doxofylline combination has been selected, which are used in the medication and maintenance of asthma.

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 is a CysLT₁ antagonist and it blocks the action of leukotriene D₄ (and secondary ligands LTC₄ and LTE₄) 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. Decreasing inflammation reduces swelling that narrows airways. Montelukast also relaxes bronchial tube walls. Apart from being used to treat asthma, it is also used to treat symptoms of hay fever and allergic rhinitis. Montelukast sodium is described chemically as [R-(E)]-1-[[[1-[3-[2-(7-chloro-2 quinolinyl) ethenyl] phenyl]-3-[2-(1-hydroxy-1-methyl ethyl)phenyl]propyl]thio]methyl]cyclopropane acetic acid, monosodium salt.

FIGURE 1: Chemical structure of the drugs.

Doxofylline (also known as doxophylline) is a xanthine derivative drug used in the treatment of asthma. It has antitussive and bronchodilator effects and acts as a phosphodiesterase inhibitor. Bronchodilators are drugs used to relieve the symptoms of an asthma attack by clearing mucus from the lungs and reducing swollen airways. Doxofylline is used to treat the symptoms of asthma as well as certain other lung diseases and conditions. It is proven to alleviate symptoms by reducing the urge to cough and helping to increase the airflow to the lungs. Doxofylline is chemically described as 7-(1,3-dioxolan-2-ylmethyl)-1,3-dimethylpurine-2,6-dione. The chemical structures of montelukast and doxofylline are shown in the Figure 1.

For better maintenance therapy in patients suffering from asthma and chronic obstructive pulmonary disease (COPD), Montelukast and Doxofylline are used in combination. This drug combination is also available in the market. Hence, these drugs are selected for the analytical studies.

For individual estimation of these drugs, several methods are available in the literature [4–15]. Some methods which are available in literature are for the simultaneous estimation of montelukast and levocetirizine [16–19]. Some other methods available in the literature are for the simultaneous estimation of montelukast and bambuterol [20–22]. There is only one method [23] available in the literature for the simultaneous estimation of montelukast and doxofylline and that too has certain limitations. The present method of this paper is faster and user friendly than the method available in the literature for the simultaneous estimation of Montelukast and Doxofylline.

2. Experimental Section

2.1. Method Development. Selection of UV Maxima. A dilute solution of Montelukast showed the UV maxima at 286 nm and the absorbance has been found to be very poor due to less chromophoric nature of the compound. On the other hand, Doxofylline has exhibited the UV maxima at 273 nm, and 205 nm and the response was very good. To undertake the combined estimation of both drugs, it has been decided to select the UV maxima of 280 nm, which falls between the maxima of both the drugs and seems to be suitable.

Selection of Column. As C18 is the most widely used column, therefore, we decided to develop a method on a common column so that anybody can utilize this method for their application.

FIGURE 2: Temperature variation chromatograms.

Selection of the Mobile Phase Composition. Various mobile phase compositions have been tried to establish the simultaneous estimation of both the drugs. We tried with the simple mixture of methanol and water, which was followed up with the combinations of various Buffers but out of these the combination of methanol and phosphate buffer has been found to be suitable. Buffer pH has also been optimized by performing the estimation at various pH, namely, 3.0, 4.5, and 7.5 and after evaluation of the peak shape and retention time, it has been concluded to adopt the buffer pH of 4.5.

Selection of Column Temperature. This study was quite interesting, and the determination of the combined drugs has been performed at three different temperatures, namely, 25°C, 40°C and 50°C. It has been found that the retention time of Doxofylline was unchanged (1.6 min) at all temperatures, whereas, the retention time of Montelukast has been found to be variable at different temperatures as 5.58 min at 25°C, 4.75 min at 40°C, and 4.22 min at 50°C. The representative graph is shown in Figure 2. Actually, 50°C is not a very suitable temperature for a routine analysis. So it was not selected for carrying out the analysis. We observed the difference of retention time of Montelukast between analysis at 40°C and 25°C to be almost 1 min. This was quite encouraging. Therefore, we decided to adopt 40°C of analysis temperature to speed up the analysis and elute both the peaks below 5 min, which is really demonstrating for the rapid estimation of this combination drug.

Optimised Method of Analysis and Other Chromatographic Conditions Are Below. The Chromatographic column, C18 (150 mm × 4.6 mm, 5µm) was used as a stationary phase.

FIGURE 3: Individual drugs.

FIGURE 4: Drug mixture.

FIGURE 5: Dosage form.

Mobile phase was prepared with Buffer and Methanol (10: 90). Buffer was prepared by dissolving 0.01 M Sodium Hydrogen orthophosphate in 1000 ML of water. The pH were adjusted to 4.5 with dilute OrthoPhosphoric acid. The diluents used for the analysis was prepared with Methanol and Water (80 : 20). Injection volume was 20 μ L. The pump flow rate was 1.0 mL/min. The column temperature was

maintained at 40°C. The eluent was detected at 280 nm. The run time was 20 min.

2.2. Materials, Reagents, and Chemicals. Montelukast and Doxofylline Standards were obtained from Startech Labs. Combination drug tablets used for the experiment, Telekast-D, was manufactured by Intas Pharmaceuticals. HPLC grade Methanol, Sodium Phosphate, and Orthophosphoric acid were obtained from Merck, Darmstadt, Germany.

2.3. Equipments. UV Visible spectrophotometer used was Shimadzu, model UV-2450. The HPLC instrument used was Shimadzu make, model-LC-2010 CHT. Class VP Software was used for data acquisition.

2.4. Preparation of Standard Solution. Mixed standard solution of 0.01 mg/mL of Montelukast and 0.4 mg/mL of Doxofylline (treat this as 100% for various experimental purposes) was prepared by taking 25 mg of Montelukast and dissolving in 25 mL diluents. 1 mL of this solution was mixed with 40 mg of Doxofylline in 100 mL volumetric flask diluted upto the mark with the diluent.

2.5. Preparation of Linearity Solutions. For Linearity 150%, 125%, 100%, 75%, and 50% solutions were prepared. 150% solution was prepared by dissolving 25 mL of Montelukast in 25 mL of diluent. 1.5 mL of this solution was added to 60 mg of Doxofylline and dissolved in 100 mL diluent. 20.83 mL of 150% solution was dissolved in 25 mL diluent for preparing 125% solution. 16.66 mL of 150% solution was dissolved in 25 mL diluent for preparing 100% solution. 12.5 mL of 150% solution was dissolved in 25 mL diluent for preparing 75% solution. 8.33 mL of 150% solution was dissolved in 25 mL diluent for preparing 50% solution.

2.6. Sample Preparation for Accuracy. Three different solutions were prepared for performing the accuracy studies. The first solution was prepared by dissolving 25 mg of Montelukast in 25 mL diluent. 1 mL of this solution was added to

TABLE 1: System suitability results.

Standard		Average	SD	%RSD
Montelukast	Retention time	4.79	0.002449	0.05
	Area	507292.8	4646.642788	0.91
	Resolution	12.414	0.074699398	0.6
	Theoretical plates	5660.126	88.82016032	1.56
	Asymmetry	0.948	0.017888544	1.88
Doxofylline	Retention time	1.97	0.000837	0.04
	Area	11536096.2	11488.81385	0.099
	Resolution	0	0	0
	Theoretical plates	1541.768	11.65102442	0.75
	Asymmetry	0.958	0.004472136	0.46

TABLE 2: Linearity results.

	Linearity range	Correlation coefficient
Montelukast	0.005–0.015 mg/mL	0.9941
Doxofylline	0.2–0.6 mg/mL	0.9935

40 mg of Doxofylline in 100 mL volumetric flask and made up the solution with 150% linearity solution. The second solution was prepared by dissolving 25mg of Montelukast in 25 ml diluent. 1mL of this solution was added to 40 mg of Doxofylline in 100 mL volumetric flask and make up the solution with 100% linearity solution. The third solution was prepared by dissolving 25 mg of Montelukast in 25 ml diluent. 1mL of this solution was added to 40mg of Doxofylline in 100 mL volumetric flask and make up the solution with 50% linearity solution.

2.7. Preparation of Sample Solution for Batch Analysis. The method was applied to the analysis of commercial sample, Telekast-D. The average weight of ten tablets was calculated and found to be 644.93 mg. The tablet was crushed to a homogeneous mixture and 59.43 mg of the tablet was dissolved in 100 mL of the mobile phase. The solution was sonicated for 5 minutes followed by cyclomixing for 5 minutes to extract the drug in solution. The resulting solution was filtered by using Millipore syringe filter (0.42 μ). The resulting clear solution was injected in HPLC in duplicate as per the developed method.

2.8. Analytical Method Validation

2.8.1. Specificity of the Method. Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities. This parameter was performed to know the Retention Time of each drug in a mixture and in the sample to understand if any chromatographic interference between the analytes or drug-excipient interference is present.

2.8.2. System Suitability. System suitability test is used to verify if the resolution and reproducibility of the chromatographic systems are adequate for the analysis to be done. The

tests are based on the fact that the equipment, electronics, samples to be analyzed constitute an integral system that can be evaluated as such. The limits for system suitability were set for theoretical plates, resolution, and asymmetry.

2.8.3. Linearity. Concentrations of the standard mixture, 50%, 75%, 100%, 125%, and 150% were prepared and injected, and chromatogram was recorded. A graph was plotted for the concentration of the corresponding drug versus area. The Correlation coefficient (r) for each drug was calculated.

2.8.4. Accuracy. To determine the accuracy in sample preparation, method of standard additions was made for measuring the recovery of the drugs. To the known standard solution concentrations of the drug (50%, 100%, and 150%) were added. Three different solutions were prepared as mentioned in Section 2.6. The accuracy was expressed as the percentage of the analyte recovery.

2.8.5. Robustness. To verify the robustness of the method, slight changes to the chromatographic conditions were made to check for any changes in the chromatogram. The analysis was done under variable flow rates, wave lengths, pH, and temperatures. The flow rate as per the developed method was 1 mL/min. This has been purposely changed to 0.8 mL/min and 1.2 mL/min, and the chromatogram was obtained. The wave length as per the developed method was 280nm. This has been purposely changed to 278 nm and 282nm. The pH in the developed method was 4.5. This was changed to 4.3 and 4.7, and the chromatogram was obtained. The column temperature in the developed method was 40°C. This was changed to 39.5°C and 40.5°C, and the chromatogram was obtained.

2.8.6. Ruggedness. To verify the ruggedness of the method, the analysis was done on different days and different chemists to check for any changes in the chromatogram. The percentage RSD for the retention time and area was calculated.

2.8.7. Performance of the Method on Commercial Samples. The method is said to be effective if it can be applied for the analysis of commercial tablets. For this purpose, performance

TABLE 3: Results for the accuracy of the method.

		Initial conc area	Sol 1 area	50% area	Sol 1-50% area	%Recovery
Sol 1	Montelukast	494506	785653	295518	291177	98.53
	Doxofylline	10344817	16526064	6302308	6181247	99.56
		Initial conc area	Sol 2 area	100% area	Sol 2-75% area	%Recovery
Sol 2	Montelukast	494506	988418	494506	493912	99.88
	Doxofylline	10344817	20787092	10344817	10442275	100.94
		Initial conc area	Sol 3 area	150% area	Sol 3-100% area	%Recovery
Sol 3	Montelukast	494506	1201875	712264	707369	99.31
	Doxofylline	10344817	25046850	14537835	14702033	101.13

FIGURE 6: Graphs showing linearity of the drugs.

test of the method has been conducted on the market sample Telekast-D, Batch no. 06TTD040 manufactured by Intas Pharmaceuticals.

3. Results and Discussion

The present work involves simultaneous estimation of Montelukast and Doxofylline using reverse phase HPLC. The current trend followed by the industries is developing a methodology which can save sophisticated instruments and chemist's valuable time by which the product analysis can be done very fast, thereby saving the time. This is the reason why people are more attracted towards UFLC (Ultra-Fast Liquid Chromatography) [24, 25] methods, though most of the pharmacopeia still have the HPLC methods only.

Keeping all these points in mind, the current method has been developed and it is very fast and encouraging. The developed method was validated with a holistic approach according to ICH guidelines and details of findings are expressed in what follows.

3.1. Specificity of the Method. The Retention times of the standard drugs individually were measured and it was found to be 4.78 min and 1.97 min for Montelukast and Doxofylline, respectively. The drugs were mixed and injected for taking the chromatogram. Both drugs were resolved sufficiently in the mixture. Retention time of both drugs in standard mix was found to be 4.78 min and 1.97 min for Montelukast and Doxofylline, respectively. This indicates that there is

no chromatographic interference between the analytes. The pharmaceutical dosage form (tablet) was then injected, and the chromatogram was obtained. The retention time of the drugs in the dosage form was found to be 4.78 min and 1.96 min for Montelukast and Doxofylline, respectively. There is no specific change in retention time of both drugs, which indicates that there is no drug-excipient interference. Respective HPLC chromatograms are represented in Figures 3, 4 and 5.

The resolution between Montelukast and Doxofylline was 12.36 which indicates a very good separation. The asymmetry factor for Montelukast and Doxofylline was 0.95 and 0.97, respectively. Therefore, this is a suitable method for the simultaneous estimation of Montelukast and Doxofylline in drug mixture and dosage forms.

3.2. System Suitability. Five injections of the standard mix were injected for this purpose. The retention time, areas, resolution, theoretical plates values, and peak Asymmetry were calculated for standard. Percentage RSD value was calculated. The Results obtained are given in following Table 1.

3.3. Linearity. The correlation coefficient (r) obtained was calculated and it was found to be greater than 0.99 for Montelukast and Doxofylline, which is well within the acceptance criteria. The results are shown in Table 2. The concentration was found to be proportional to the area and the response of the detector was determined to be linear over the range of

TABLE 4: Robustness results.

		Retention time %RSD	Area %RSD
Different flow rates			
0.8 mL/min	Montelukast	0.07	0.48
	Doxofylline	0.15	0.16
1.2 mL/min	Montelukast	0.12	0.31
	Doxofylline	0.24	0.39
Different pH values			
4.3	Montelukast	0.29	0.98
	Doxofylline	0.51	0.46
4.7	Montelukast	0.1	0.99
	Doxofylline	0.24	0.24
Different wave lengths			
278 nm	Montelukast	0.32	0.52
	Doxofylline	0.12	0.1
282 nm	Montelukast	0.29	0.76
	Doxofylline	0.38	0.49
Different temperatures			
39.5°C	Montelukast	0.09	0.34
	Doxofylline	0.19	0.15
40.5°C	Montelukast	0.03	0.15
	Doxofylline	0.06	0.21

TABLE 5: %RSD of the drugs on different days and different analysts.

	Retention Time %RSD	Area %RSD
Day 1 & analyst 1		
Montelukast	0.29	0.8
Doxofylline	0.12	0.34
Day 2 & analyst 2		
Montelukast	0.09	0.49
Doxofylline	0.11	0.41

TABLE 6: Estimation of the drugs in commercial samples.

Telekast-D	Label claim	Acquired data	%Recovery
Montelukast	10 mg/tab	10.03 mg/tab	100.30%
Doxofylline	400 mg/tab	399.48 mg/tab	99.87%

0.2 to 0.6 mg/mL for Montelukast and 0.5 to 1.5 mg/mL for Doxofylline as shown in the Figure 6.

3.4. Accuracy. The results indicate that the recoveries are well within the acceptance range of 80–120%; therefore, method is accurate and it can be used for the simultaneous estimation of Montelukast and Doxofylline. The percentage recovery of the results obtained are listed in Table 3.

3.5. Robustness. Due to deliberate change in the chromatographic conditions of the method, like flow rate, pH, wave lengths, and column temperature, excellent performance of the method was observed. This indicates that the method is Robust. The results obtained are shown in Table 4.

3.6. Ruggedness. Data acquired and %RSD of area and retention time were calculated for various trials and data tabulated in Table 5. Based on the data, it is evident that the method is rugged.

3.7. Performance of the Method on Commercial Formulations. As per the label claim, the commercial tablet, namely, Telekast-D contains 10 mg of Montelukast and 400 mg of Doxofylline. The quantification of both the drugs was done in the formulation and the results have been found to be in the range of 99.8 to 100.3%. This indicates that the method developed by us can be used for the simultaneous estimation of Montelukast and Doxofylline in any of the pharmaceutical dosage forms. The results obtained are as shown below in the Table 6.

4. Conclusion

The novel HPLC method developed is sensitive, unique, precise, user friendly, rapid, and reproducible for simultaneous estimation of Montelukast and Doxofylline in bulk mix and pharmaceutical dosages forms. The method was validated as per the ICH Guidelines. It is concluded that this method can be used by the industries and academic institutions for their combination drug estimation, which is fast as well as novel.

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