High performance liquid chromatographic method development for simultaneous analysis of doxofylline and montelukast sodium in a combined form

Abstract

Aim: Some literatures revealed that the high performance liquid chromatography (HPLC) method for single component or multicomponent analysis of montelukast sodium with other drugs. However, these methods deals with time consuming, so it is necessary to develop a cost-effective and less time consuming method for the estimation of doxofylline and montelukast sodium in combined pharmaceutical formulation. **Materials and Methods:** The separation was performed on an inertsil C8 (5 µm, 4.6 × 250 mm) column in isocratic mode with the mobile phase consisting a mixture of methanol and sodium phosphate buffer (75:25 v/v, pH 6.5 adjusted with orthophosphoric acid). The mobile phase was pumped at a flow rate of 1 mL min^{-1} and eluents were monitored at 230 nm. **Results:** The selected chromatographic conditions were found to separate doxofylline (retention time = 3.4 min) and montelukast sodium (retention time = 5.5 min) with a resolution of 5.47. The proposed HPLC method was validated with respect to linearity, accuracy, repeatability, specificity, robustness, and ruggedness as per International Conference on Harmonisation guidelines Q2(R1), November 2005 (Validation of Analytical Procedures: Text and Methodology). The percentage recoveries for doxofylline and montelukast sodium ranged from 98.1% to 101.7% and 98.2 to 101.9%, respectively, which indicated that the above method was enough accurate and precise. **Conclusions:** Hence, it was concluded that the developed method is suitable for routine analysis of these combination due to its less analysis time.

Key words: C8 column, less analysis time, percentage recovery, repeatability, ruggedness

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INTRODUCTION

Doxofylline is chemically designated as 7(1,3-dioxolone-2-yl-methyl)theophylline, presence of a dioxolane group in position 7 differentiates it from theophylline with lower side effects [Figure 1]. It is a new antibronchospastic drug recently introduced in therapy with pharmacological properties like theophylline, a potent adenosine receptor antagonist. Doxofylline does not affect gastric acid secretion, either *in vivo* or *in vitro*, unlike theophylline. It inhibits phosphodiesterase (PDE IV) which activates the consequent increase of cyclic AMP, which determines relaxation of the smooth musculature. It does not interfere with calcium influx into the cells or antagonize calcium channel blockers. Doxofylline appears to have decreased affinities toward adenosine A_1 and A_2 receptors, which may account for the better safety profile of the drug.[1,2] It is suitable for asthmatic patients with peptic ulcer disease.

Montelukast is a leukotriene receptor blocker, administered orally as a tablet in the dose of 5–10 mg per day. Chemically, it is represented as 2-[1-[(*R*)-[-2(*E*)-(7-chloroquinolin-2-yl)vinyl]phenyl] propyl-sulfanylmethyl]cyclopropyl]acetic acid sodium salt [Figure 2]. It is the only leukotriene modifier approved by the United States Food and Drug Administration (USFDA) for the use by children

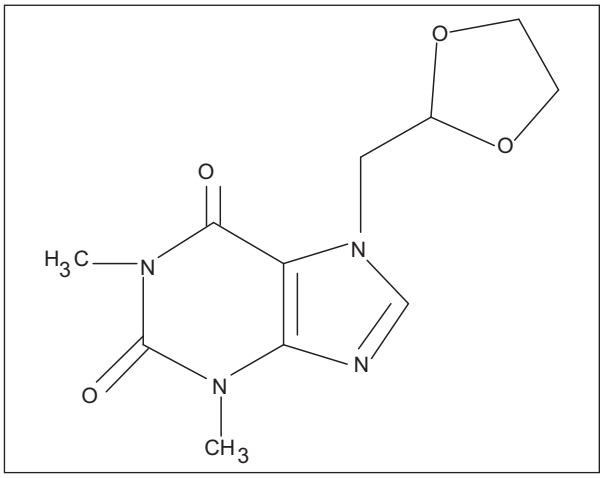

Figure 1: Chemical structure of doxofylline

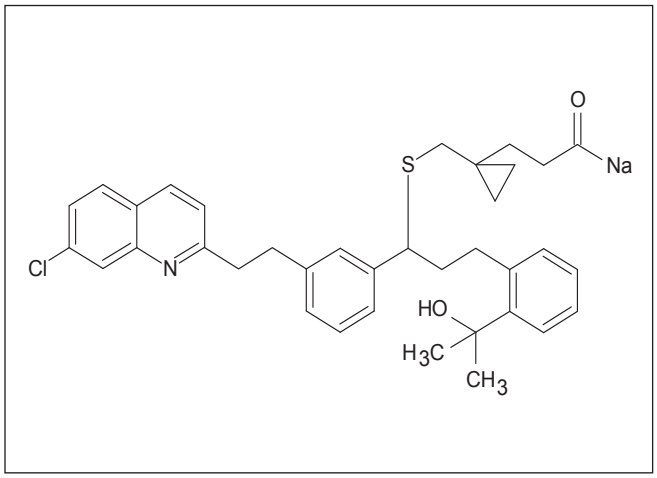

Figure 2: Chemical structure of montelukast sodium

from 2 to 12 years of age. Montelukast sodium primarily used for the treatment of asthma, it is a potent selective inhibitor of leukotriene D_4 (LTD_4) at the cysteine leukotriene receptor cysLT1. They induce bronco constriction, increase microvascular permeability, and are vasoconstrictor of coronary arteries. Their biological effects are transduced by a pair of G-protein coupled receptors. It binds to the human cysLT1 receptor.[3,4]

Giriraj *et al*. only estimated the content of montelukast sodium and doxofylline in a combined dosage form by reversed phase high performance liquid chromatography (RP-HPLC) using an Intersil C18 column and a 10:70:20 ratio of acetonitrile: methanol: ammonium acetate buffer, pH 5.5, as the mobile phase.[5] Few literatures revealed the development of a new HPLC method for determination of montelukast and its degradation product using a C18 column in solid dosage forms and in human plasma using LC-ESI-MS/MS.[6,7] Alsarra developed stability indicating a HPLC method for determination of montelukast in a tablet dosage form and in human plasma using a C18 column (5 μm, 3.9 × 150 mm) with UV detection at 345 nm.[8] Maliwal has taken the seminar on analytical method development, validation, and comparison of a first-order derivative spectroscopy method and stability indicating the HPLC method for the simultaneous estimation of doxofylline and montelukast in a pharmaceutical dosage form. Both the methods show enough robust and the same confidence limit for the same batch.[9] Some literatures revealed the new HPLC method for the determination of montelukast with other drugs such as bambrutal, loratidine, and cetrizine.[10-13]

MATERIALS AND METHODS

Instrumentation

A HPLC (Shimadzu prominence) method was developed using an Inertsil C8 coloum (5 μm, 4.6 × 250 mm) with a PDA detector. The sample volume of 20 μL was used throughout the analysis. Data were acquired and analyzed by LC software. The tablet "D-montus" with 650 mg of doxofylline and 10 mg of montelukast sodium was manufactured by Fourrts India, Chennai. All other reagents used were of HPLC grade.

Method development and optimization

Initially various mobile phases were tried in an attempt to obtain the best separation and resolution between doxofylline and montelukast sodium. The mobile phase consisted of methanol and 10 mM sodium phosphate buffer dibasic, pH 6.5, in the ratio of 75:25 was found to be an appropriate mobile phase allowing the adequate separation of both the compounds by using an Inertsil C8 (5 μm, 4.6 × 250 mm) column at a flow rate of 1 mL/min. A typical chromatogram of separation of the two components is shown in Figure 3.

As the doxofylline and montelukast sodium exhibit significant absorbance at wavelength 230 nm, it was selected as detection wavelength for the simultaneous determination of doxofylline and montelukast sodium in pharmaceutical dosage forms.

Standard solution preparation

An accurately weighed quantity of about 162 mg of the doxofylline working standard (WS) wastransferred into a 50 mL volumetric flask, then 20 mL of mobile phase was added to dissolve and 25 mg of montelukast sodium WS was weighed and transferred into the 50 mL

volumetric flask separately and made upto the volume with the mobile phase. Further, 5 mL of this montelukast sodium stock solution was transferred to the 50 mL volumetric flask containing doxofylline and diluted up to the mark with the mobile phase. The solution was mixed well and used for chromatographic injection.

Assay of formulation

Twenty tablets of the formulation were weighed and the average weight of one tablet was calculated. All 20 tablets were crushed and grounded to a fine powder. Powder equivalent to 165 mg of doxofylline (2.5 mg of montelukast sodium) was transferred into a 50 mL of volumetric flask and diluted up to the mark with the mobile phase and mixed well, then the solution was filtered through a 0.45 μm filter to obtain a clear filtrate. This solution was suitably diluted and used for analysis. After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, a fixed volume of 20 μL of the sample solution was loaded by an automatic sampler. The solution was injected, and chromatograms were recorded. The injections were repeated six times, and the peak area were recorded.

Validation procedure

The method was validated for the parameters such as system suitability, specificity, linearity and range, accuracy, precision, ruggedness, and robustness.[14] The system suitability was assessed by five replicate analysis of the drug at a concentration as per standard preparation. System suitability of the method was evaluated by analyzing the repeatability, peak symmetry (symmetry factor), theoretical plates of the column, resolution between the peaks, capacity factor, and relative retention.

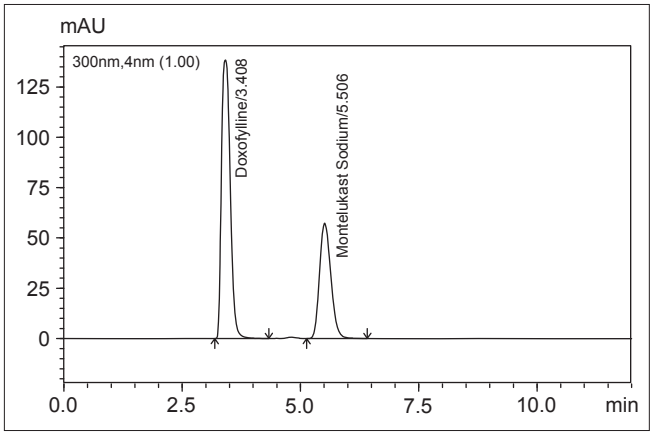

Figure 3: Chromatogram for doxofylline and montelukast sodium. Doxofylline and montelukast sodium peaks at retention time of 3.418 min and 5.506 min, respectively

Specificity was also determined in the presence of excipients used in formulation, and chromatogram was observed and compared with that of a standard peak. To evaluate the linearity of the method, serial dilutions were made from a standard stock solution in the working range with the diluent which contains a mixture of methanol and sodium phosphate buffer dibasic (75:25) and resolved on a C8 column.

To determine accuracy of the method in dosage formulation, a working standard of a drug was prepared. Samples for recovery studies were also prepared by spiking known amount of WS with placebo at three concentration levels (50%, 100%, and 150%) and analyzed.

The precision of the method was investigated with respect to repeatability. To determine intermediate precision, standard solutions of the drug at the 100% concentration level were analyzed three times within the same day (*intra-day* variation) and on three different days (*inter-day* variation).

Robustness studies were performed on method precision by making slight variations in flow rate, amount of the mobile phase and pH changes.

RESULTS AND DISCUSSION

The goal of this study was to develop a rapid, easy accurate, precise, reliable and least time consuming HPLC method for the analysis of doxofylline and montelukast sodium from the combined pharmaceutical formulation.

The newly developed method has been validated as per guidelines of the International Conference on the Harmonization of Technical requirements for the Registration of pharmaceutical for Human use [ICH 2005] and has recommended the accomplishment of specificity, linearity, precision, accuracy, ruggedness, and robustness of the method.

System suitability testing

Typical system suitability results were summarized in Table 1. All the values for the system suitability parameters were within limits. System suitability tests are an integral part of chromatographic methods and are used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed.

Range and linearity

The range of an analytical method is the interval between the upper and lower analytical concentration of a sample where the method has shown to demonstrate acceptable accuracy, precision, and linearity. The linearity of an analytical method is its ability to elicit test results that are directly proportional to the concentration of an analyte in the sample within a given range. The linearity of the method was observed in the expected concentrated range, demonstrating its suitability for analysis [Table 2]. The linearity curve of doxofylline and montelukast sodium was shown in Figures 4 and 5.

Accuracy

Accuracy of an analytical method is the closeness in agreement between the accepted true value or a reference value and the actual result obtained. Accuracy studies are usually evaluated by determining the recovery of a spiked sample of the analyte into the matrix of the sample to be analyzed. The results of accuracy studies are shown in Table 3. Recoveries of doxofylline and montelukast sodium were laid between 98% and 102%. This is evident that the method is accurate within the desired range.

WS is working standard of drug, % RSD is percentage relative standard deviation, $n = 3$ is three observation.

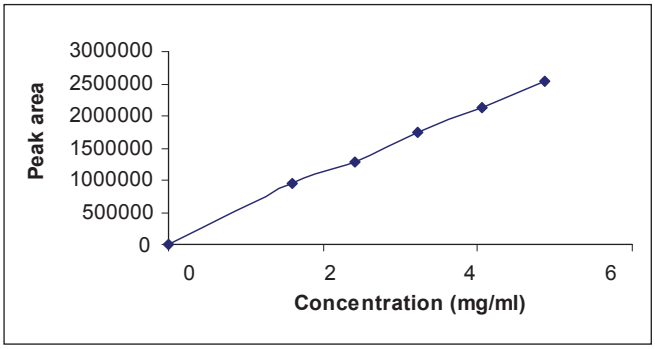

Figure 4: Doxofylline linearity curve. $Y = Mx + C$ = regression equation, R^2 = correlation co-efficient

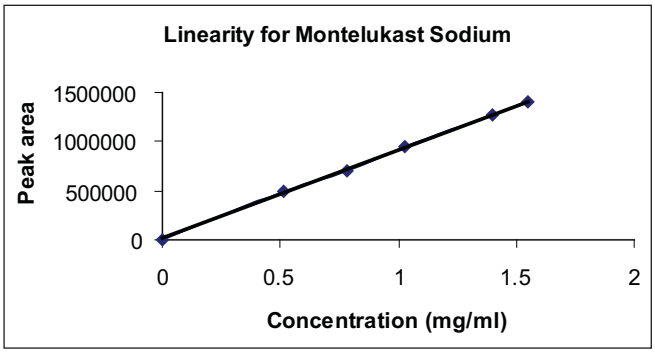

Figure 5: Montelukast sodium linearity curve. $Y = Mx + C$ = regression eqution, R^2 = correlation co-efficient

Precision

Precision is a measure of the ability of the method to generate reproducible results. The precision of a method is evaluated using three separate determinations for repeatability, intermediate precision, and reproducibility. The results of intra- and interday variations are shown in Table 4. The results obtained from intermediate precision also indicated a good method precision. All the data were within the acceptance criteria.

Ruggedness

The ruggedness of an analytical method is the degree of reproducibility of the test results obtained by the samples under a variety of conditions, such

Table 1: System suitability parameters

Parameters	Doxofylline	Montelukast sodium
Retention time (min)	3.418	5.506
Tailing factor (*T*)	1.27	1.21
Theoretical plate (*N*)	3339	3344
Resolution (*R*)	0.00	5.47

Table 2: Linearity data details for montelukast sodium and doxofylline

Doxofylline		Montelukast sodium	
Concentration (mg/mL)	Average peak area	Concentration (mg/mL)	Average peak area
1.6	945121	0.51	495937
2.42	1290784	0.78	700300
3.23	1736492	1.03	955010
4.05	2117767	1.40	1266364
4.87	2543130	1.55	1410963

mg/mL is milligram/milli litre, $n = 3$ is three observation

Table 3: Accuracy/recovery data for montelukast sodium and doxofylline—spiking method

Level of WS added	Doxofylline		Montelukast sodium	
	% Recovery	% RSD*	% Recovery	% RSD*
50%	100.8	0.32	98.2	0.62
	98.1		101.9	
	99.4		100.4	
100%	99.5	0.89	101.71	1.11
	101.7		100.2	
	100.3		99.0	
150%	100.3	1.41	99.5	1.57
	101.7		100.3	
	99.0		99.2	

Table 4: Intra- and inter-day precision study

Drug	Intra-day		Inter-day	
	% Content*	% RSD	% Content*	% RSD
Doxofylline	102.43	1.40	99.97	0.79
Montelukast sodium	101.10	1.39	101.94	0.88

% RSD is percentage relative standard deviation, $n = 5$ is five observations

Table 5: Ruggedness data for tablet analysis

Parameters	Doxofylline		Montelukast sodium	
	% Content*	% RSD	% Content*	% RSD
Analyst 1, Instrument 1, Column 1, Reagent 1	99.61	1.39	100.30	1.39
Analyst 2, Instrument 1, Column 1, Reagent 1	99.74	1.21	100.93	1.30
Analyst 1, Instrument 2, Column 1, Reagent 1	99.46	0.65	99.65	0.88
Analyst 1, Instrument 1, Column 2, Reagent 1	99.11	0.48	101.21	0.61
Analyst 1, Instrument 1, Column 1, Reagent 2	99.94	0.47	100.81	0.98

% RSD is percentage relative standard deviation; *n = 5 is five observations: First column is the result for ruggedness using Analyst 1, Instrument 1, Column 1, Reagent 1; second column is the result for ruggedness using Analyst 2, Instrument 1, Column 1, Reagent 1; third column is the result for ruggedness using Analyst 1, Instrument 2, Column 1, Reagent 1; fourth column is the result for ruggedness using Analyst 1, Instrument 1, Column 2, Reagent 1; and fifth column is the result for ruggedness using Analyst 1, Instrument 1, Column 1, Reagent 2

Table 6: Robustness data for tablet analysis

Parameters		Doxofylline		Montelukast sodium	
		%RSD	% Content*	% RSD	% Content*
Flow rate (mL/min)	0.90	0.17	100.63	0.19	100.33
	1.1	0.09	101.55	0.11	100.87
Mobile phase ratio	87:13	0.07	101.92	0.16	98.67
	83:17	0.17	101.49	0.14	98.23
pH	6.3	0.41	101.78	0.42	98.71
	6.7	0.13	99.32	1.17	98.22

mL/min is millilitre/minute, % RSD is percentage relative standard deviation, the mobile phase ratio is methanol and sodium phosphate buffer (75:25 v/v, pH to 6.5 adjusted with orthophosphoric acid), n = 5 is five observations.

as different laboratories, different analysts, different instruments, different lots of reagents, and different days. The %RSD of below 2% indicated that the method was accurate with high precision [Table 5].

Robustness

Robustness is a measure of the performance of a method when small deliberate changes are made to the conditions of the method. The results of the robustness study are summarized in Table 6.

Solution stability

The working standard solution of montelukast sodium and doxofylline for analysis were kept in a bench top oven (at 25°C) and a refrigerator (at 5°C) and analyzed the solution at the time interval of 1, 2, 6, and 12 h. The chromatogram showed some additional peaks after 2 h in bench top conditions and after 12 h in refrigeration conditions. It was concluded that the solution was stable for 1 h at bench top conditions and 6 h at refrigeration conditions [Figure 6].

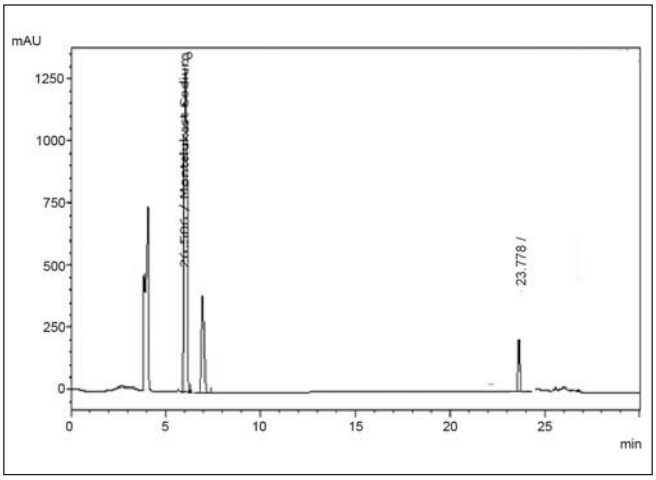

Figure 6: Solution stability for montelukast at refrigrator conditions after 12 h. Additional peaks obtained at 7.2 min and 23.7 min

CONCLUSIONS

It is a well known that the validation procedure is an integral part of the analytical method development. Therefore, the developed method was validated according to the ICH guidelines Q2 (R1). Based on the results, it can be concluded that there is no other co-eluting peak with the main peaks and that the method is specific for estimation of montelukast sodium and doxofylline. The proposed method has a linear response in the stated range and is accurate and precise. To our knowledge, the developed HPLC method is the first reported method for simultaneous determination of montelukast sodium and doxofylline from their combination drug product with very less retention time (3.408 min for doxofylline and 5.506 min for montelukast sodium) using a C8 column. Then, the stability study indicated that the standard stock solution was stable up to 6 h in the refrigrator. Therefore during the analysis, the standard and sample solutions should be kept in the refrigrator and used with in 6 h to get the better results. Taken together, these results clearly showed that this method can be used for routine analysis of montelukast sodium and doxofylline in their combined dosage form. The developed method can also be conveniently adopted for dissolution testing of tablets containing montelukast sodium and doxofylline.

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