RESEARCH ARTICLE

Simultaneous Estimation of Montelukast and Doxofylline in Bulk Drug and Tablet Dosage Form by UHPLC Method

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ABSTRACT

The study's primary goal is to create a novel ultra-high-performance liquid chromatography (UHPLC) technique that is exact, sensitive, and accurate. The primary goal is to calculate the dosages of montelukast and doxofylline in both pharmaceutical and bulk forms. A C18 (AGILENT) column was used to achieve the chromatographic separation of the drug and contaminants. The mobile phase consisted of 0.1% OPA and 57:43% v/v methanol (a pH of 4.2 with TEA). The detection was performed at 278 nm utilizing UV detection. According to the results, dinoxyline and montelukast were effectively eluted at retention durations of 3.523 and 4.918 minutes, respectively, with the flow rate adjusted at 1.0 mL/dinoxyline, with good resolution. The suggested method demonstrated linearity in the dosage ranging from 1 to 5 µg/mL of montelukast and 40 to 200 µg/mL of doxofylline. The range of recovery percentages for montelukast and doxofylline is 100.565 to 101.061%. The method's validation was carried out in compliance with the specifications of the International Symposium on Harmonisation, resulting in good precision, sensitivity, accuracy, linearity, specificity, and robustness. In conclusion, the developed method successfully separates and estimates doxofylline and montelukast. Its application in routine analysis of these compounds in pharmaceutical formulations is viable and reliable.

Keywords: Doxycycline, Montelukast, UHPLC, Simultaneous estimation.

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INTRODUCTION

Doxofylline, or doxophylline, is a pharmaceutical belonging to the xanthine derivative class. It is employed at asthma therapy, exerting antitussive and bronchodilator effects while functioning as a phosphodiesterase inhibitor. And bronchodilators, such as doxofylline, are utilized to alleviate asthma symptoms by clearing lung mucus and reducing airway inflammation. Doxofylline is specifically employed to address symptoms related to asthma and certain respiratory conditions, demonstrating efficacy in diminishing the urge to cough and facilitating increased airflow to the lungs. The chemical composition of doxofylline is described as a 1-(1,3-dimethylpurine-2,6-dione)-1,3-dimethyloxolan-2-ylmethyl. It is essential to use doxofylline under the supervision of a healthcare professional for optimal management of asthma symptoms.

Montelukast, categorized as a leukotriene receptor antagonist (LTRA), is employed to both maintain asthma treatment and alleviate symptoms related to seasonal allergies. It works as an oral CysLT1 antagonist, inhibiting the action of leukocyte D4 and its analogs (LTC4 and LTE4) on the lings and bronchial tubes' CysLT1 cysteinyl leukotriene receptor. This binding effect reduces bronchoconstriction brought on by leukotrienes, leading to diminished inflammation. This reduction in inflammation helps relieve airway narrowing resulting from swelling. Montelukast also induces relaxation in the bronchial tube walls. Not only does it alleviate asthma symptoms, but it also treats high temperature and allergic rhinitis. Chemically, montelukast is defined as [R–(E)]2-(ethenyl] phenyl] 2-(7-chloro-2 quinolinyl) 1– [1– [3– [23– [2-(ethyl-11-methyl acetic acid (methyl phenyl] propyl] thio] methyl] cyclopropane. It's important to note that montelukast and the previously mentioned doxofylline play distinct roles in managing asthma. Figure 1 illustrates the chemical structures of both doxofylline and montelukast.

To enhance patients on ongoing treatment for chronic obstructive pulmonary disease (COPD) with asthma, a combination of doxofylline and montelukast is frequently employed. This combination is commercially available, prompting the selection of these drugs for analytical studies. The literature has already documented various methods for separate valuation of these drugs.[1-12] Additionally, some current

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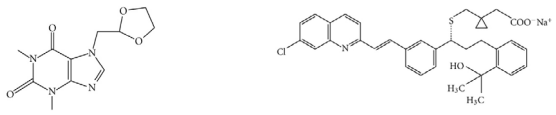

Doxofylline Montelukast

Figure 1: Chemical structures of both Doxofylline and Montelukast

approaches address the estimate of bambuterol and montelukast concurrently,[17-18] while others concentrate on the simultaneous estimation of montelukast and levocetirizine.[13–16] The current method for simultaneously estimating montelukast and doxofylline together in a formulation is easy to use, dependable, and reasonably priced.

MATERIALS AND METHODS

Method Development

Various mobile phases, consisting of combinations of acetonitrile, methanol, water, buffers in different proportions, were experimented with. The mobile phase that was ultimately chosen was methanol:0.1% OPA (a pH of 4.2 with TEA) in a ratio of 57:43% v/v. This choice yielded the right peak characteristics and suitable resolution for doxofylline and montelukast. Arrangement suitability studies were conducted, and for standard solutions, peak asymmetry, the number of theoretical plates, and resolution were ascertained (Table 1). The obtained values confirmed the system's suitability for analyzing these drugs with combination. A representative chromatography of the standard solution is depicted in Figure 1. The chromatography separation was carried out using an AGILENT (1100) with a 20 µL sample injection loop and a UV-visible absorbance detector. The mobile phase had a methanol and 0.1% of orthophosphoric acid mixture with a 1.0 mL/min flow rate and a detection wavelength of 278 nm. Before using, a 0.2 m membranes filter was used to filter and degas the mobile phase. The analysis was conducted at ambient temperature using an injection volume of 20 µL.

Standard Solution Preparation

About 20 mg of the medication doxofylline and 5 mg of the working dose of montelukast Weigh the volumetric flask to 100 mL, then dilute it with 100 mL of mobile phase. In a 10 mL volumetric flask, pipette out 0.2 mL of the resultant solution, then dilute with mobile phase to make 100 mL.

Table 1: System suitability test

Parameter	*Method*
Column	id 4.6 x100 mm length
Particle size	2.5 µm
Stationary-Phase	$C1_8$(AGILENT)
Mobile-Phase	Methanol: 0.1 % OPA 57: 43
Detection of wavelength	278 nm
Flow rate	1.0 mL/min
Room Temperature	33
Size of sample	20 µL

Calibration Curve

The linearity of the system was measured by serially diluting the stock solutions to create a concentration range from 40 to 200 µg/mL for doxofylline and 1 to 5 µg/mL for montelukast. The calibration curves were obtained by plotting the peak area versus concentration for doxofylline (DOX) and montelukast (MONT), as illustrated in Figures 2 and 3. The decline calculations for the calibration curves are as follows: for doxofylline, ($y = 45.361x + 236.25$) with ($R^2 = 0.9993$), and for montelukast, ($y = 165.26x + 17.34$) with ($R^2 = 0.9991$).

Preparation of Sample Solutions

The method is effectively working for the analysis of a commercial sample. About 20 tablets were assessed, and their average weight is determined to be 488 mg. Next, the tablet was ground into a uniform size, and 244 mg of that size was dissolved in 100 mL of methanol. This solution underwent 15 minutes of sonication and 5 minutes of cyclomixing to ensure the extraction of the drug into the solution. A Millipore syringe filter (0.42 µ) was then used to filter the resulting solution. Following the specified protocol, the cleared solution was subsequently injected into the high-performance liquid chromatography (HPLC) system twice. The proposed method exhibited specificity, with no observable interference resulting from typical excipients in tablets such as lactose. The assay was calculated using the line regression equation for each drug. The percentages of single drugs in the formulation were determined and detailed in Table 2. The analysis results indicate a close alignment between the quantities of drugs found and the labeled claims of the formulations. This alignment attests to the analytical method's precision and consistency employed, confirming its suitability for evaluating the composition of the studied formulations.

Method Development

In adherence with the ICH guidelines, the validation process of the method encompassed assessing key parameters, including limits on quantitation, detection, linearity, accuracy, precision, and resilience. These evaluations collectively ensure the method's suitability for its planned application, confirming its ability to generate precise, accurate, and reliable results across a specified range and under varying experimental conditions. The chromatogram of Doxofylline and Montelukast shown in figure 4.

Linearity and range

The range for doxofylline was 40 to 200 g/mL, while for montelukast was 1 to 5 g/mL. The formulation's linearity was

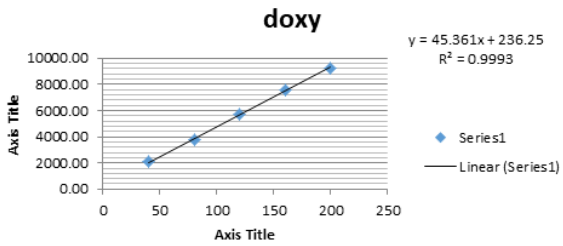

Figure 2: Calibration curve of doxofylline

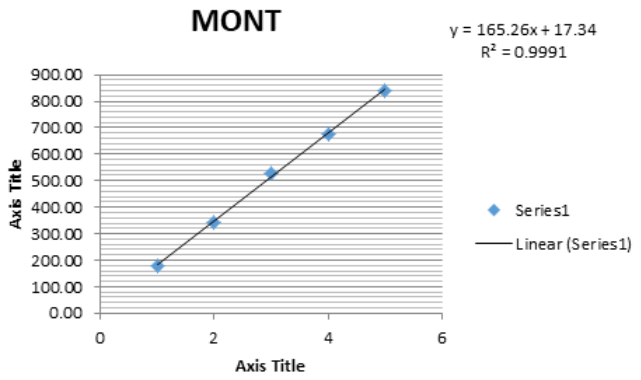

Figure 3: Calibration curve of montelukast

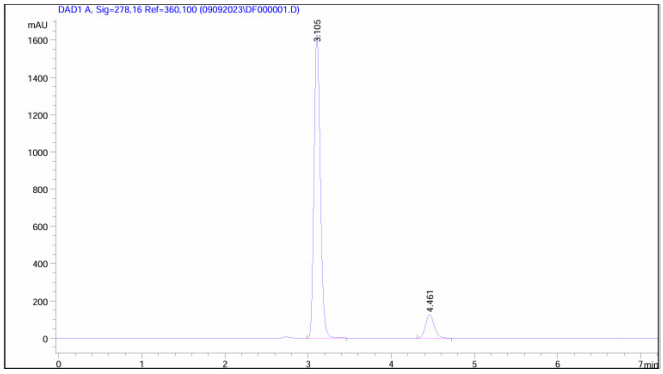

Figure 4: Chromatogram of doxofylline (3.105 minutes), montelukast 4.461 minutes, respectively

tested at five concentration levels. Montelukast's regression line equation was y = 165.26x + 17.34 R2 = 0.9991, while doxofylline was y = 45.361x + 236.25 R2 = 0.9993. The findings show a substantial association between the peak area and drug concentration within the advised concentration rang.

Accuracy and precision

Recovery tests at three different levels (80, 100, and 120%) were used to assess the correctness of the approach; Table 2 provides specifics on the computed recovered percentages. The recovery results confirm the method's correctness, which are within the range of 100 ± 2%. Studies on both intra-day and inter-day variance showed precision. Investigations conducted within a single day involved three consecutive injections of sample solutions. The percentage was used to evaluate precision. The relative standard deviation (%RSD) was computed. Inter-day variation tests, including three repeated standard and sample solutions injections over successive days yielded %RSD values (Table 3). The gathered data, which has a percentage RSD of less than 1.5%, shows how accurate the developed UHPLC process is. The limits of detection (LoD) and quantification (LoQ) were also established. To calculate LoD, use the formula LoD = (3.3 x standard deviation/Slope of the calibration curve), which represents the lowest analyte concentration generating a detectable reaction. Montelukast and doxofylline had LoD values of 0.0085 and 0.3825 g/mL, respectively. The formula for LoQ, which represents the lowest accurately measurable

Table 2: Recovery study of doxofylline (DOX) and montelukast (MONT)

Recovery level %	*%Mean Recovery**		*%R.S.D.*	
	DOX	*MONT*	*DOX*	*MONT*
80	100.46	98.60	0.06	0.33
100	100.12	102.64	0.01	0.01
120	101.30	99.52	0.06	0.16

Table 3: Intraday and interday precision study (Method precision)

Concentration (µg/mL)	*Doxofylline*		*Montelukast*	
	%RSD		*%RSD*	
	Intraday	*Interday*	*Intraday*	*Interday*
80	0.06	0.12	0.33	0.12
120	0.01	0.80	0.01	0.29
160	0.06	0.01	0.16	0.13

concentration, is LoQ = (10 x standard deviation/Slope of the calibration curve). The determined LoQ values for doxofylline and montelukast was 1.159 and 0.025 g/mL, respectively.

Robustness

Robustness has been assessed by deliberately introducing slight changes at the experimental procedures. In this method, intentional differences in pH, wavelength, and flow rate were implemented, and the resulting effects were observed. The method exhibited robustness, demonstrating its ability to withstand these deliberate alterations in wavelength, pH, and flow rate without significant impact on its performance.

RESULT AND DISCUSSION

The recommended method was found to be simple and demonstrated the concentration ranges of 1 to 5 g/mL for doxofylline and 40 to 200 g/mL for montelukast, which exhibit linearity, respectively. Accuracy and precision were confirmed through recovery studies, revealing %RSD values not exceeding 1.5%. For doxofylline and montelukast, the LoD and LoQ values are 0.3825 and 1.159 µg/mL, respectively, and 0.0085 and 0.025 µg/mL, accordingly, the method also showed sensitivity. The method's sensitivity and specificity are validated by these results taken together.

CONCLUSION

The suggested UHPLC technique for concurrent measurement of montelukast and doxofylline in mixed dose forms has shown excellent sensitivity, speed, simplicity, accuracy, and precision. Therefore, the current UHPLC method is deemed suitable for the routinely analyzed raw materials and compositions.

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REFERENCES

1. Singh RM, Saini PK. Development and validation of a RP-HPLC method for estimation of montelukast sodium in bulk and in tablet dosage form. Indian Journal of Pharmaceutical Sciences. 2010; 72(2): 235–237. Available from: doi.org/10.4103/0250-474X.65023
2. Gannu R, Sudke SG. Development and validation of a stability-indicating RP-HPLC method for analysis of doxofylline in human serum. Application of the method to a pharmacokinetic study. Acta Chromatographica. 2007;19(19): 149–160.
3. Mittal A, Parmar S. Development and validation of rapid HPLC method for determination of doxofylline in bulk drug and pharmaceutical dosage forms. Journal of Analytical Chemistry. 2010; 65(3): 293–297. Available from: https://doi.org/10.1134/S1061934810030147
4. Gupta AS, Rawat M, Yadav JS. Method development and acid degradation study of doxofylline by RP-HPLC and LC-MS/MS. Asian Journal of Pharmaceutical Analysis. 2011; 1(1): 10–13. Available from: https://doi.org/10.5958/2231–5675
5. Saradhi SV, Sekaran CB, Reddy TV. Development and validation of novel analytical methods for estimation of doxofylline in bulk and dosage forms. European Journal of Chemistry. 2011; 2(3): 372–377. Available from: https://doi.org/10.5155/eurjchem.2.3.372-377.396
6. Venkatesan S, Myvizhi S. A simple HPLC method for quantitation of doxofylline in tablet dosage form. International Journal of Chemical and Pharmaceutical Sciences. 2010; 1(2): 54–57.
7. Alsarra IA. Development of a stability-indicating HPLC method for the determination of montelukast in tablets and human plasma and its applications to pharmacokinetic and stability studies. Saudi Pharmaceutical Journal. 2004; 12(4): 136–143.
8. Bharathi DV, Hotha KK, Naidu A. Quantification of montelukast, a selective cysteinyl leukotriene receptor (CysLT1) antagonist in human plasma by liquid chromatography-mass spectrometry: validation and its application to a human pharmacokinetic study. Biomedical Chromatography. 2009; 23(8): 804–810. Available from: https://doi.org/10.1002/bmc.1189 .
9. Patil SS, Bavaskar S, Mandrupkar SN. Development and statistical validation of spectrophatometry method for estimation of Montelukast in bulk and tablet dosage form. Journal of Pharmacy Research. 2009; 2(4): 714–716.
10. Challa BR, Awen BZ, Chandu BR. Method development and validation of montelukast in human plasma by HPLC coupled with ESI-MS/MS: application to a bioequivalence study. Scientia Pharmaceutica. 2010; 78(3): 411422. Available from: https://doi.org/10.3797/scipharm.1002-07
11. Rashmitha N, Raj TJ, Srinivas CH. A validated RP-HPLC method for the determination of impurities in montelukast sodium. EJournal of Chemistry. 2010; 7(2): 555563. Available from: https://doi.org/10.1155/2010/156593
12. Gupta A, Yadav V, Yadav JS. An analytical approach of doxofylline: a review. Asian Journal of Pharmaceutical Analysis. 2011; 1(4): 6770. Available from: DOI: https://doi.org/10.5958/2231-5675.2017.00002.3
13. Kumar BV, Mathur P, Rajesh N. Analytical method development and validation of Levocetirizine hydrochloride and Montelukast sodium in combined tablet dosage form by RP-HPLC. International Journal of Advances in Pharmaceutical Research. 2011; 2(7): 380–396.
14. Ashok kumar S, Raja MS, Perumal P. RP-HPLC method development and validation for simultaneous estimation of montelukast sodium and levocetirizine dihydrochloride. International Journal of Pharmaceutical Research. 2009; 1(4): 8–12.
15. Basu A, Basak K, Chakraborty M. Simultaneous RP-HPLC estimation of Levocetirizine Hydrochloride and Montelukast Sodium in tablet dosage form. International Journal of Pharm Tech Research. 2011; 3(1): 405–410.
16. Patil S, Pore YV, Kuchekar BS. Determination of montelukast sodium and bambuterol hydrochloride in tablets using RP HPLC. Indian Journal of Pharmaceutical Sciences. 2009; 71(1): 58–61. Available from: DOI: *https://doi.org/*10.4103/0250-474X.51961
17. Yanamandra R., Vadla C. S. Development and Validation of a Rapid RP-UPLC Method for the Simultaneous Estimation of Bambuterol Hydrochloride and Montelukast Sodium from Tablets. *Indian J. Pharm Sci.* 2012; 74(2): 116-121. Available from: DOI: *https://doi.org/*10.4103/0250-474X.103841
18. Patel DJ, Patel SA. Simultaneous determination of montelukast sodium and bambuterol hydrochloride in tablet dosage form by ultraviolet spectrophotometry (dual wavelength method). International Journal on Pharmaceutical and Biomedical Research. 2010; 1(3): 71–75.