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RESEARCH PAPER

DEVELOPMENT AND VALIDATION OF STABILITY-INDICATING RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF MONTELUKAST SODIUM AND DOXOFYLLINE IN TABLET FORMULATION

Jitendra Prajapati[1], Manisha Jadav[2]*, Nayan Kshatri[3] and Lalit Lata Jha[4]

[1]Department of Quality Assurance, Parul Institute of Pharmacy; [2]Department of Quality Assurance, School of Pharmacy; [4]Department of Pharmaceutics, School of Pharmacy; Parul University, Limda, Waghodia-391760, Gujarat, India; [3]Cadila Pharmaceuticals Limited, Bhat-382210, Ahmedabad, Gujarat, India

**E-mail*: manisha.jadav121112@paruluniversity.ac.in
Tel.: +91 9510715628.

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Montelukast sodium and doxofylline exhibit bronchodilator, anti-inflammatory, and mucoregulator activities. A stability-indicating RP-HPLC method was developed and validated as per ICH guidelines for their simultaneous estimation. Quantitation was performed at 274 nm, with linearity observed in the concentration ranges of 100–300 µg/ml for montelukast sodium and 80–240 µg/ml for doxofylline, yielding correlation coefficients of 1.0 and 0.9990, respectively. Separation was achieved on a Hypersil BDS C18 (100 x 4.6 mm, 5 µm) column using a phosphate buffer (pH 3): acetonitrile mobile phase in a gradient program at 1 ml/min flow rate. Retention times were 3.27 min for montelukast sodium and 8.27 min for doxofylline. Forced degradation under hydrolytic, oxidative, and thermal stress confirmed method specificity. Percentage recoveries ranged from 99.22-99.77% for doxofylline and 99.83-99.90% for montelukast sodium. This precise, economical method is suitable for tablet dosage analysis.

Key words: Doxofylline, Forced degradation, Montelukast sodium, Tablet dosage form, RP-HPLC.

INTRODUCTION

Bronchial asthma is a persistent inflammatory ailment of the airways, characterized by reversible airflow obstruction, leading to symptoms like breathlessness, chest tightness, and wheezing. Derived from the Greek word "asthma," meaning "panting," asthma comprises various types, namely allergic asthma, exercise-induced asthma, cough-variant asthma, occupational asthma, and nocturnal asthma. This chronic inflammatory disease can be categorized as intrinsic or extrinsic, with triggers ranging from allergens and inflammatory mediators to viruses and other stimuli. Understanding the distinct types and triggers of asthma is crucial for effective management and treatment strategies [1].

Stability refers to a drug's ability to maintain its desired characteristics and quality over time. It's crucial for the safety and effectiveness of a drug. Stability testing examines how a drug changes under different conditions, like temperature and humidity. This testing includes various assessments to ensure a drug remains stable within its packaging and storage conditions. A stability indicating assay method (SIAM) is a precise test that detects any reduction in the active ingredient due to degradation [2]. FDA regulations emphasize that stability studies should use stability-indicating techniques that

precisely quantify the active substance without being influenced by degradation products or impurities. These techniques monitor alterations in the drug substance and product on the levels of chemistry, physics, and microbiology. Forced degradation studies play vital role in developing stability-indicating methods by demonstrating specificity, identifying degradation pathways, and revealing degradation products. This information helps improve drug formulation, manufacturing, and packaging processes to enhance the quality of the drug product [3,4].

A stability indicating assay method is created through stressing the active pharmaceutical ingredient (API) under conditions more severe than those used for accelerated stability testing. Stress testing, or forced degradation, reveals degradation pathways and products that might arise during storage, aiding formulation, manufacturing, and packaging development. Stressing the API in various forms (solution and solid-state) produces samples that simulate likely storage conditions, used to develop the SIAM. The SIAM's objective is to distinctly identify all resulting products, including the API and degradation products, without overlapping. Typically, 5-10% API degradation is aimed for, avoiding compound destruction or irrelevant degradation. The US Pharmacopoeia mandates using a stability-indicating assay for potency assessment during stability studies, with liquid chromatography commonly employed to separate and quantify target analytes [5-7].

Montelukast Sodium is chemically known as 1-[[[(1R)-1-[3-[(1E)-2-(7-chloro-2-quinolinyl) ethenyl] phenyl]-3-[2-(1-hydroxy-1-methylethyl) phenyl]propyl]thio]methyl]cyclopropane acetic acid sodium salt and is a leukotriene receptor antagonist used for treating asthma and allergic rhinitis (**Figure 1**). It is a white to off-white crystalline powder with a melting point of 135–135.9°C and a molecular weight of 608.2 g/mole. Montelukast selectively targets the cysteinyl leukotriene CysLT1 receptor, blocking the effects of leukotrienes and mitigating asthma-related symptoms like airway edema and smooth muscle contraction. The drug is absorbed rapidly orally with a 64% bioavailability, primarily metabolized in the liver, and has a half-life of 3-4 h. Common side effects include stomach discomfort, tiredness, fever, and upper respiratory symptoms. Indications encompass asthma treatment and prophylaxis, exercise-induced bronchoconstriction prevention, and symptom relief for allergic rhinitis. Contraindications include hypersensitivity to the drug or its components. Doxofylline is a bronchodilator used to treat conditions like bronchial asthma, bronchospasm, chronic asthmatic bronchitis, and chronic obstructive pulmonary disease (COPD). It is white to off-white crystalline powder, chemically 7-(1,3-dioxolan-2-ylmethyl)-1,3-dimethylpurine-penta-dione, a molecular weight of 266.25 g/mol having a melting point of 144-146°C (**Figure 1**).

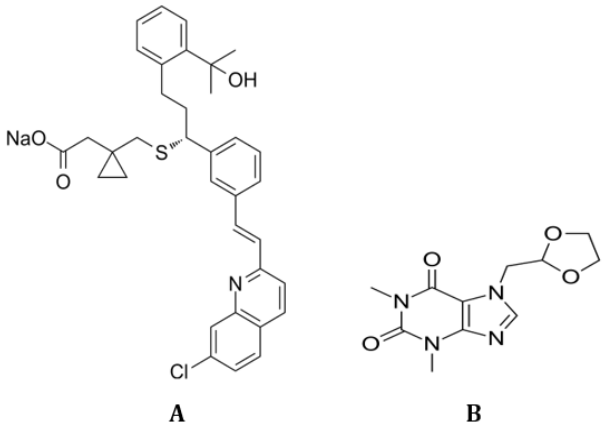

Fig. 1. Structures of Montelukast Sodium and Doxofylline

Doxofylline's mechanism of action involves inhibiting phosphodiesterase in bronchial smooth muscle, leading to relaxation and bronchodilation. It is metabolized in the liver, rapid and extensive oral absorption, distributes to various tissues and has a half-life of 7.42 h. Common side effects include headache, nausea, tachycardia, and the abdominal pain. Contra-indications include hypersensitivity to xanthine derivatives and acute myocardial infarction.

Montelukast sodium and Doxofylline are anti-asthmatic drug indicated for the treatment of bronchial asthma, chronic obstructive pulmonary disease and bronchitis. So, it was thought of interest to study the quality of Montelukast sodium and Doxofylline. Assay of drug is one of the critical parameters to ensure the quality of dosage form. Montelukast sodium and Doxofylline are official in pharmacopoeia.

The literature review revealed that no stability indicating chromatographic method is reported for determination of Montelukast sodium and Doxofylline. The aim of the present work is to develop and validate analytical method for estimation of Montelukast sodium and Doxofylline in tablet dosage form and to perform its stability indicating study according to ICH guidelines.

MATERIALS AND METHODS

Montelukast sodium API (MONTE) and Doxofylline API (DOXO) were provided by Cadila Pharmaceuticals Ltd, Ahmadabad, Gujarat, India. Acetonitrile and methanol (HPLC quality) were bought from Fischer Scientific India Pvt Ltd. in Mumbai, India. Orthophosphoric acid and sodium hydroxide obtained from Spectrochem. Hydrochloric acid LR and hydrogen Peroxide purchased from S.D. fine chemicals. Glacial acetic acid AR purchased from Qualigens fine chemicals. Sodium hydroxide purchased from Sisco Research.

Melting Point determination

Melting point of MONTE and DOXO were checked by open capillary method using paraffin bath assembly [8].

IR spectral determination for identification of standard

In a hydraulic pellet press operating at a pressure of 7–10 tons, pellets of DOXO with KBr (Spectroscopic grade) and MONTE with KBr (Spectroscopic grade) were made and both the pellets were scanned from 400-4000 cm^{-1}, using an FT-IR instrument. The FTIR spectra of DOXO and MONTE were compared with reference spectra.

Chromatographic condition

The HPLC instrument, Dionex with Cromalion Software, model 2010C HT with UV Detector was used to carried out the analysis. Various optimizations were carried out during method development. Various trials were taken for selection of desired columns. Different columns like Cosmosil C8, Cosmosil C18, Inertsil BDS C18, Hypersil ODS C18, Develosil ODS C18, Dionex Acclaim C18 were tested in order to have better retention time and resolution. The column selected was Hypersil BDS C18 Column (100 mm × 4.6 mm, 5 µm.) The mobile phase consisted of buffer (1.36 g KH_2PO_4 dissolved in 1 lit. Water and adjusted to pH 3.0 with orthophosphoric acid) and acetonitrile. It was degassed, passed through a membrane filter (0.45 µm), and then injected 50:50 (*v/v*) from the solvent reservoir into the column. With a run time of 12 minutes, the detection wavelength was set at 274.0 nm and the mobile phase flow rate was maintained at 1.0 ml/min. The volume of injection loop was 20 µl. The column was equilibrated for at least 30 mins with the mobile phase running through the apparatus prior to the injection of the drug solution. The HPLC apparatus and column were kept at room temperature [9-11].

Standard stock solution preparation

By precisely weighing MONTE (50 mg) and DOXO (40 mg), dissolving in mobile phase, and diluting to the desired volume of 50 ml with mobile phase in the volumetric flask, a standard solution of MONTE (1000 µg/ml) and DOXO (800 µg/ml) was prepared.

Working standard solution preparation

5 ml of stock solution of MONTE and 5 ml of stock solution of DOXO was transferred into 25 ml volumetric flask and adjusted up to mark with mobile phase having to prepare 200 µg/ml and 160 µg/ml concentration. The first few drops of the filtrate were discarded after filtering the standard solution using 0.22 µm nylon membrane filter paper.

Sample solution preparation

Accurately measuring the weight of 20 tablets and their powdered contents, we then proceeded to transfer the quantity of powder equivalent to 10 mg of MONTE sodium and 800 mg of DOXO into a 50 ml volumetric flask. To this flask, we added 30 ml of the mobile phase and carefully filled it up to the mark, after which we subjected it to 15 mins of sonication to ensure complete dissolve the drugs. Following this step, the resulting solution underwent filtration using a 0.45 µm nylon membrane filter paper, and the initial few drops of the filtrate were discarded. This filtered solution was designated for use with MONTE. Subsequently, a 0.5 ml aliquot was extracted from the aforementioned solution and placed into a 50 ml volumetric flask, which was then filled to the mark. This second solution was designated for use with DOXO [12-15].

Preparation of placebo

A 50 ml volumetric flask containing 330 mg of placebo was filled with 30 ml of mobile phase, diluted to the appropriate level, and sonicated for 15 mins to completely dissolve the drugs.

Solution preparation for degradation study

Acid degradation

In a 50 ml volumetric flask, 10 ml of the stock solution was combined with 5 ml of 1N HCl. The flask was then gently heated on a water bath at 40°C for a duration of 30 mins. Afterward, the flask was allowed to cool to room temperature, and 5 ml of 1N NaOH was introduced to

neutralize the solution. The final volume was adjusted to the mark using diluent and thoroughly mixed. A blank sample was also prepared following the same procedure, excluding the addition of MONTE and DOXO.

Alkali degradation
5 ml of 1N NaOH was added to 10 ml of stock solution in 50 ml of volumetric flask, the flask was allowed to stand at RT for 30 mins and 5 ml of 1N HCl was added to neutralize the solution, final volume was made up to the mark with the diluent and mixed well. Similarly, blank was prepared without adding MONTE and DOXO.

Oxidation degradation
In a 50 ml volumetric flask, 10 ml of the stock solution was combined with 5 ml of a 10% H_2O_2 solution. The mixture was left to stand at room temperature for 30 minutes. Afterward, the final volume was adjusted to the mark using the diluent and thoroughly mixed. A similar blank sample was prepared, but without the addition of Montelukast sodium and DOXO.

Thermal degradation
Approximately 10 mg of MONTE and 800 mg of DOXO powder were placed in a petri dish and subjected to an 80°C oven for a duration of 30 mins. Following this, the powdered mixture was transferred into a 50 ml volumetric flask, and 30 ml of diluent was introduced. After 5 mins of sonication, the final volume was adjusted to the mark using the diluent.

Photo degradation
About 10 mg MONTE and 800 mg DOXO powder was kept in the petri dish and exposure to fluorescent light for 1.2 million lux h. Then, powder was transferred into a 50 ml volumetric flask, 30 ml diluent was added, sonicated for 5 mins and final volume was made up to the mark with diluent.

Calibration curve
Calibration curves were established across a broad concentration range, revealing a linear response within the intervals of 100-300 µg/ml for MONTE and 80-240 µg/ml for DOXO. To generate these curves, precise volumes of standard solutions of MONTE and DOXO (2.5, 3.75, 5.0, 6.25, and 7.5 ml) were transferred to a series of 25 ml volumetric flasks and then diluted to the mark using the mobile phase. Subsequently, 10 µl of each solution were injected under the chromatographic conditions specified earlier.
The calibration curves were constructed by plotting peak areas against the drug concentration, and regression equations were computed [16-20].

Method validation
The validity of the proposed method was assessed across various parameters, including linearity, range, repeatability, method precision, intermediate precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), robustness, ruggedness, and system suitability [21-25].

Linearity and range
The "range" denotes the span between the highest and lowest concentrations of the analyte in a sample, demonstrating that the analytical method maintains an appropriate level of precision, accuracy, and linearity. In this case, the linear response was confirmed to occur within the range of 100-300 µg/ml for MONTE and 80-240 µg/ml for DOXO.

Repeatability
To assess the repeatability of the method, standard solutions of MONTE and DOXO were injected repeatedly (n = 6) under identical chromatographic conditions. Measurements of peak area, retention time, and tailing factor were collected during these injections.

Intraday precision
Three sets of triplicates for different concentrations of MONTE (150 µg/ml, 200 µg/ml, and 250 µg/ml) and DOXO (120 µg/ml, 160 µg/ml, and 200 µg/ml) were simultaneously analyzed on a single day, and chromatographic data were collected at a wavelength of 274 nm. The relative standard deviation (%RSD) was subsequently computed based on these nine determinations.

Intermediate precision (Reproducibility)
Inter-day precision:
Over the course of three consecutive days, nine determinations were carried out for three different concentrations of MONTE (150 µg/ml, 200 µg/ml, and 250 µg/ml) and DOXO (120 µg/ml, 160 µg/ml, and 200 µg/ml). Chromatographic data were recorded at a wavelength of 274 nm, and the % RSD was subsequently calculated.

Different analysts:
Another analyst conducted nine determinations over the course of three consecutive days, involving three replicates for each of the MONTE concentrations (150 µg/ml, 200 µg/ml, and 250 µg/ml) and DOXO concentrations (120 µg/ml, 160 µg/ml, and 200 µg/ml). Chromatographic data were collected at 274 nm, and the %RSD was subsequently computed.

Accuracy (% Recovery)
To assess the method's accuracy, the recovery of MONTE and DOXO was determined using the Standard Addition method. In this approach, known quantities of standard solutions of MONTE (100 µg/ml, 150 µg/ml, and 200 µg/ml) and DOXO (80 µg/ml, 120 µg/ml, and 160 µg/ml) were introduced into a test solution containing MONTE (50 µg/ml) and DOXO (40 µg/ml). The concentrations of MONTE and DOXO were then determined by applying the obtained values to the regression equation derived from the calibration curve.

Limit of detection and limit of quantitation
The calculation of the limit of detection (LOD) was based on the analysis of five calibration curves employed to establish the method's linearity. The LOD can be determined using the formula: LOD = 3.3 × (SD/Slope), where SD represents the standard deviation of the Y-intercepts across the five calibration curves, and Slope corresponds to the mean slope of these curves. Similarly, the determination of the limit of quantification (LOQ) was carried out using the same set of five calibration curves used for assessing method linearity. The LOQ is calculated as follows: LOQ = 10 × (SD/Slope), where SD stands for the standard deviation of the Y-intercepts from five calibration curves, and slope denotes mean slope derived from curves.

Robustness
To validate the robustness of the method, alterations were introduced in both the flow rate (±0.2 ml/min) and the pH of the mobile phase (±2 *v/v*). The %RSD for the measured area was then computed, with the criterion for acceptance being that it should not exceed 2% [26-29].

RESULTS AND DISCUSSION
A precise and accurate reversed-phase HPLC method was developed and validated for the quantification of MONTE and DOXO in tablet form. The chromatographic conditions were meticulously optimized to achieve effective compound separation. MONTE and DOXO were quantified utilizing a Hypersil BDS C18 (100 x 4.6 mm) 5 µm column, employing a mobile phase comprising phosphate buffer (pH 3) and Acetonitrile, flowing at 1 ml/min through a Gradient program, with detection at 274 nm. The retention times for MONTE and DOXO were determined to be 3.27 min and 8.27 min, respectively. The representative chromatogram of MONTE and DOXO is shown in **Figure 2**.

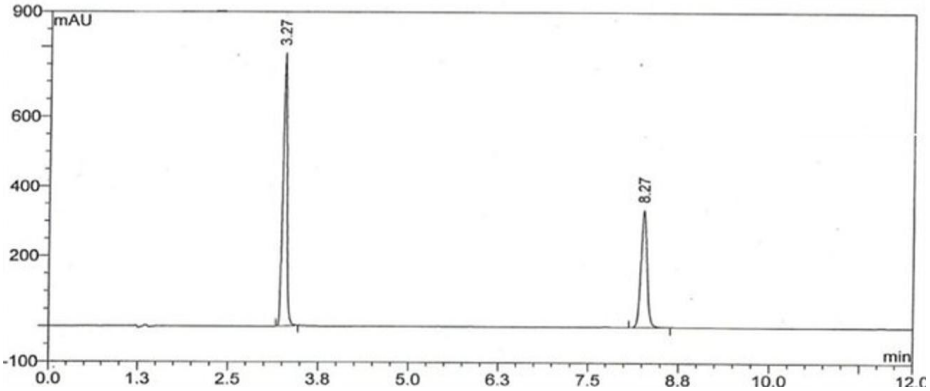

Fig. 2. Representative chromatogram of MONTE and DOXO

The linearity and range assessment, as shown in **Table 1**, established the range as 100–300 µg/ml for MONTE and 80–240 µg/ml for DOXO. The correlation coefficients were determined to be 1.0 for MONTE and 0.9990 for DOXO. The recovery percentages of MONTE and DOXO at different levels, as shown in **Table 2**, were within the ranges of 99.83–99.90% and 99.22–99.77%, respectively. Repeatability, intraday precision, and intermediate precision exhibited %RSD values of less than 2%. The robustness analysis, considering variations in flow rate and pH, yielded %RSD values below 2%, as shown in **Table 3**, confirming the

precision of the method and robustness. The assay results demonstrated % Assay values of 93.05% for MONTE and 96.89% for DOXO. The system suitability parameters, as summarized in **Table 4**, demonstrated compliance with the acceptance criteria, ensuring the reliability of the analytical method. An accuracy of the method, precision, and other validation parameters were systematically determined and statistically validated (**Table 5**).

Table 1. Regression analysis data for HPLC method for Montelukast sodium and Doxofylline

Parameter	Values (MONTE)	Values (DOXO)
λ_{max}	274 nm	274 nm
Range	100-300 µg/ml	80-240 µg/ml
Regression equation (y = mx + c)	y = 18278x + 11327	y = 17221x + 16510
Slope (m)	18278	17221
Intercept (c)	11327	16510
Correlation coefficient	1.00	0.9990

Table 2. Accuracy data for MONTE and DOXO

Drug	Level	Conc. (ppm)	Amount of sample taken (ppm)	Amount of std spiked (ppm)	Mean % recovery ± % RSD
MONTE	I	150	50	100	99.83 ± 0.77%
	II	200	50	150	99.44 ± 0.69%
	III	250	50	200	99.90 ± 0.38%
DOXO	I	120	40	80	99.22 ± 0.17%
	II	160	40	120	99.21 ± 0.37%
	III	200	40	160	99.77 ± 0.27%

Table 3. Robustness data

Sr. No.	Drug	Flow rate	Area	Mean	SD	% RSD
1	MONTE	0.9	3663138	3636422	23144.72	0.63
2		1.0	3623672			
3		1.1	3622456			
1	DOXO	0.9	2944100	2930076	12160.35	0.41
2		1.0	2923672			
3		1.1	2922456			
		pH				
1	MONTE	2.8	3562567	3569877	51337.36	1.43
2		3.0	3624478			
3		3.2	3522587			
1	DOXO	2.8	2845927	2865538	51814.4	1.80
2		3.0	2924295			
3		3.2	2826391			

Table 4. System suitability parameters

Parameter	Results of MONTE	Results of DOXO
Retention time (min)	8.26	3.27
Tailing factor (T)	1.06	1.12
Theoretical plates (N)	44488	14848
Resolution (R)	1.163	-

Table 5. Summary of validation parameters HPLC method for Montelukast sodium and Doxofylline

Parameters		Result (MONTE)	Result (DOXO)
Linearity		100-300 µg/ml	80-240 µg/ml
Repeatability	Intraday precision	1.63 %	1.20 %
	Interday precision	1.3 %	1.4 %
Accuracy	50%	99.83 ± 0.77 %	99.22 ± 0.17 %
	100%	99.44 ± 0.69 %	99.21 ± 0.37 %
	150 %	99.90 ± 0.38 %	99.77 ± 0.27 %
Assay		99.46 ± 1.25 %	99.54 ± 1.09 %
Limit of Detection (LOD)		0.517 µg/ml	0.628 µg/ml
Limit of Quantitation (LOQ)		1.56 µg/ml	1.90 µg/ml

Upon degradation assessment, in acidic conditions, MONTE and DOXO exhibited degradation percentages of 12.08% and 1.14%, respectively, as illustrated in **Figure 3**. Under alkaline conditions, MONTE and DOXO showed degradation percentages of 20.9% and 4.37%, respectively, as depicted in **Figure 4**. Oxidative conditions resulted in degradation of 20.34% for MONTE and 2.14% for DOXO, as shown in **Figure 5**. Finally, thermal conditions resulted in degradation of 23.28% for MONTE and 6.6% for DOXO, as illustrated in **Figure 6**. To evaluate stability, forced degradation experiments were conducted using 1% solutions of HCl, NaOH, H_2O_2, and thermal stress at 60°C. Interference from impurity peaks was assessed, revealing a 10-20% degradation for both MONTE and DOXO under the specified conditions using the developed HPLC method.

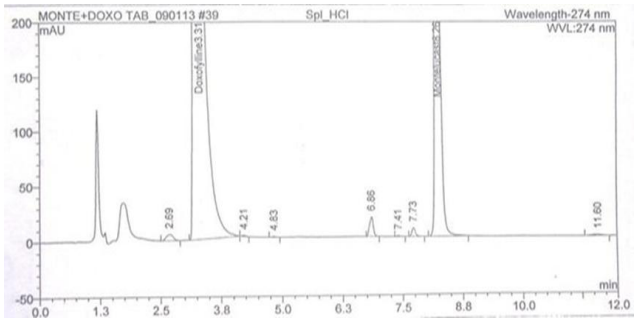

Fig. 3. Chromatogram of acid degradation

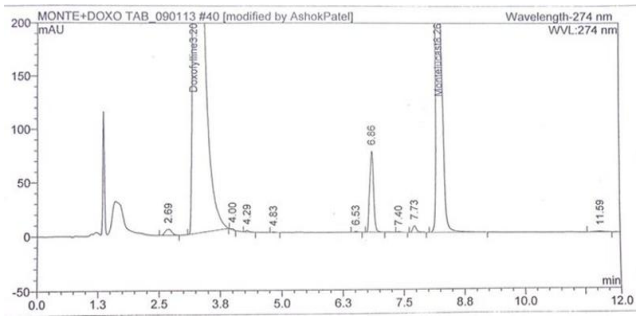

Fig. 4. Chromatogram of alkali degradation

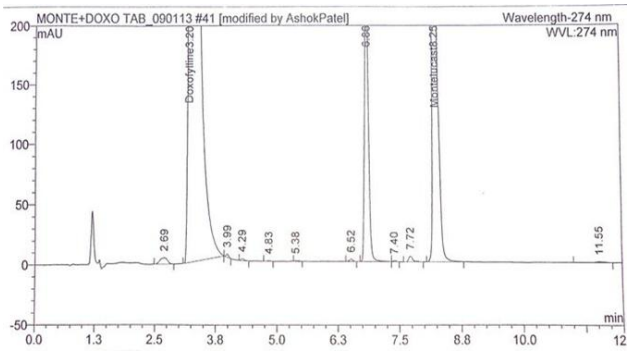

Fig. 5. Chromatogram oxidative degradation

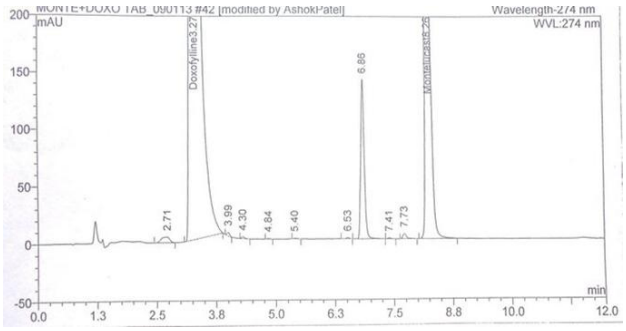

Fig. 6. Chromatogram of thermal degradation

CONCLUSION

In this study, we have developed a novel RP-HPLC method with stability-indicating properties and innovative solution for the simultaneous estimation of MONTE and DOXO in tablet formulations. An efficient and rapid RP-HPLC technique was devised and validated in accordance with the ICH Guideline. This method offers both sensitivity and precision in determining MONTE and DOXO within tablet formulations. Its accuracy and specificity were affirmed. This method's broad applicability for routine analysis of commercially available formulations was demonstrated. The developed method proves to be a high-throughput solution for quantifying MONTE and DOXO in tablet forms, showcasing exceptional precision, accuracy, and selectivity.

REFERENCES

1. Rang H, Dale M, Ritter J, Moore P. Pharmacology. Churchill Livingstone, Elsevier Science Ltd: Edinburgh, UK, 2007.
2. Parikh N, Patel N, Patel N. Stability indicating reverse phase HPLC method development and validation for estimation of chloramphenicol and flurbiprofen sodium in pharmaceutical dosage form. *Bull Pharm Res*. 2019; 9(1-3):160. doi:10.21276/bpr.2019.9.1
3. Singh S, Dahiya R. Stability-indicating RP-HPLC method for estimation of atorvastatin calcium in solid dosage form. *Bull Pharm Res*. 2014;4(1):9-13
4. Satoskar RS, Bhandarkar SD, Ainapure SS. Pharmacology and Pharmacotherapeutics. 16th edition, Popular Prakashan: Mumbai, India; 350-67.
5. Sharma BK. Instrumental Methods of Chemical Analysis. 23rd edition, Goel Publication House: New Delhi, India, 2002; 1-16.
6. Sethi PD. HPLC-Quantitative Analysis of Pharmaceutical Formulations. 1st edition, CBS Publishers and Distributors: New Delhi, India; 2010.
7. ICH, Impurities in New Drug Products. International Conference on Harmonization, IFPMA, Geneva, 1996.
8. Pallavi K, Babu S. Validated UV spectroscopic method for estimation of Montelukast sodium from bulk and tablet formulations. *Int J Adv Pharm Biol Chem*. 2012; 1(4):450-3.
9. Singh RM, Saini PK, Mathur SC, Singh GN, Lal B. Development and validation of a RP-HPLC method for estimation of montelukast sodium in bulk and in tablet dosage form. *Indian J Pharm Sci*. 2010;72(2):235-7. doi:10.4103/0250-474X.65023
10. Patil S, Pore YV, Kuchekar BS, Mane A, Khire VG. Determination of montelukast sodium and bambuterol hydrochloride in tablets using RP HPLC. *Ind J Pharm Sci*. 2009;71(1):58-61. doi:10.4103/0250-474X.51961
11. Ravisankar M, Uthirapathy S, Thangadurai A, Dhanapal K. Simultaneous estimation of fexofenadine hydrochloride and montelukast sodium in bulk drug and marketed formulation by RP-HPLC method. *Int Res J Pharm*. 2012;3:356-9.
12. Chabukswar AR, Choudhari VP, Jagdale SC, Sharma SN, Bari NA, Pagare BD. Simultaneous estimation of Montelukast Sodium and Fexofenadine HCL in pharmaceutical formulation by RP-LCPDA. *Int J Pharm Sci Res*. 2012;2(1):241-8.
13. Radhakrishna T, Narasaraju A, Ramakrishna MJ, Satyanarayana AJ. Simultaneous determination of montelukast and loratadine by HPLC and derivative spectrophotometric methods. *J Pharm Biomed Anal*. 2003;31(2):359-68. doi:10.1016/S0731-7085(02)00650-7
14. Abiram L. Simultaneous determination of montelukast sodium and bambuterol hydrochloride in tablet dosage form by ultraviolet spectrophotometry. *Int Res J Pharm Appl Sci*. 2011;1(1):1-8.
15. Pawar V, Pai S, Roa K. Development and validation of UV spectrophotometric method for simultaneous estimation of Montelukast Sodium and Bambuterol Hydrochloride in bulk and tablet dosage form. *Jordan J Pharm Sci*. 2008;1(2):152-8.
16. Pourghazi K, Khoshhesab ZM, Golpayeganizadeh A, Shapouri MR, Afrouzi H. Spectrophotometric determination of cetirizine and montelukast in prepared formulations. *Int J Pharm Pharm Sci*. 2011;3(2):128-30.
17. Patel NK, Pancholi SS. Spectrophotometric determination of montelukast sodium and levocetirizine dihydrochloride in tablet dosage form by AUC curve method. *Der Pharm Chem*. 2011;3(5):135-40.
18. Choudekar RL, Mahajan MP, Sawant SD. Validated RP-HPLC method for the estimation of rupatadine fumarate in bulk and tablet dosage form. *Der Pharm Chem*. 2012;4(3):1047-53.
19. Giriraj P, Shajan A. Simultaneous estimation and method validation of montelukast sodium and doxofylline in solid dosage form by RP-HPLC. *Int J Chem Pharm Sci*. 2011;2(1):79-89.
20. Patel RB, Parmar RR, Patel VM, Shah DA. Simultaneous estimation of montelukast sodium and theophylline in pharmaceutical dosage form by UV-spectrophotometric method. *Int J Inst Pharm Life Sci*. 2012;2(2):150-60.
21. Kumar JV, Ramachandran D, Saradhi SV. RP-HPLC method for the estimation of montelukast sodium in pharmaceutical dosage forms. *Curr Trends Biotech Pharm*. 2010;4(4):943-6.
22. Kamila MM, Mondal N, Ghosh LK. Development and validation of spectrophotometric method for estimation of anti-asthmatic drug doxofylline in bulk and pharmaceutical formulation. *Ind J Chem Tech*. 2007; 14:523-25.
23. Venkatesan S, Giriraj P, Myvizhi S, Kathiravan P, Rameshwar S. A simple HPLC method for quantitation of doxofylline in tablet dosage form. *Int J Pharm Chem Sci*. 2010;1(2):54-7.
24. Mittal A, Parmar S. Development and validation of rapid HPLC method for determination of doxofylline in bulk drug and pharmaceutical dosage forms. *J Anal Chem*. 2010;65(3):293-7. doi:10.1134/S1061934810030147
25. Gupta A, Rajkumar YV, Rawat S. Method development and alkali degradation study of doxofylline by RP-HPLC and LC-MS/MS, *Drug Invent Today*. 2014;33:1248-50.
26. Sivasubramanian L, Sarika V, Manikandan K, Lakshmi KS. RP-HPLC and HPTLC methods for determination of doxofylline in bulk and formulations. *J Pharm Res*. 2011; 4(3):643-4.
27. Xi Z. Determination of doxofylline in doxofylline injection by GC method. *Anhui Med Pharm J*. 2006;9.
28. Gannu R, Bandari SG, Sudke SG, Rao YM, Shankar BP. 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 Chromatogr*. 2007;19(19):149-60.
29. Rao RN, Naidu CG, Prasad KG, Santhakumar B, Saida S. Development and validation of a stability-indicating assay of doxofylline by RP-HPLC: ESI-MS/MS, ^{1}H and ^{13}C NMR spectroscopic characterization of degradation products and process-related impurities. *J Pharm Biomed Anal*. 2013;78:92-9. doi:10.1016/j.jpba.2013.02.004

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