

**DEVELOPMENT AND VALIDATION OF A RP-HPLC METHOD FOR ESTIMATION OF LEVOCETIRIZINE AND MONTELUKAST IN PHARMACEUTICAL DOSAGE FORM**

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ABSTRACT

A simple, specific and accurate reverse phase high performance liquid chromatographic method was developed for the simultaneous determination levocetirizine and montelukast in pharmaceutical dosage form. The column used was Thermosil C₁₈ (150×4.6 mm, 3.5µm) in isocratic mode, with mobile phase containing phosphate buffer-acetonitrile (30:70) adjusted to pH 3.6 using ortho phosphoric acid was used. The flow rate was 1.0 mL/ min and effluents were monitored at 232 nm. The retention times of levocetirizine and montelukast were 2.213 min and 5.674 min, respectively. The linearity for levocetirizine and montelukast were in the range of 50-90 mg/mL and 100-140 mg/mL respectively. The recoveries of levocetirizine and montelukast were found to be 100.31% and 100.37%, respectively. The proposed method was validated and successfully applied to the estimation of levocetirizine and montelukast in combined tablet dosage forms.

Keywords: Validation, RP-HPLC, Levocetirizine, Montelukast

INTRODUCTION

Levocetirizine (LEV), 2-(2-{4-[(R)-(4-chlorophenyl)(phenyl) methyl] piperazin-1-yl} ethoxy) acetic acid is a second generation H₁ antihistamines marketed for the treatment of perennial and seasonal allergic rhinitis and chronic idiopathic urticaria. It is the most active enantiomer of cetirizine and has a favorable pharmacokinetic profile. Levocetirizine is rapidly and extensively absorbed, minimally metabolized and has a volume of distribution (V_d) which is lower than other compounds from the same. Montelukast sodium is chemically (R-(E))-1-(((1-(3-(2-(7-chloro-2-quinolinyl) ethenyl) phenyl)-3(2-(1-hydroxy-1-methylethyl) phenyl)propyl) thio)methyl) cyclopropaneacetic acid, monosodium salt.

It is primarily used for the treatment of asthma in children and adults. It is a potent selective inhibitor of leukotriene D₄ (LTD₄) at the cysteinyl leukotriene receptor cysLT₁.^[3-5] Literature review reveals that some analytical methods have been reported for

levocetirizine dihydrochloride^[6-9] and montelukast sodium^[10-12] individually as stability indicating and in biological fluids or in combination with other drugs in pharmaceutical dosage forms. . In the present study we have proposed validated simple RP-HPLC method for the simultaneous determination of levocetirizine and Montelukast in their combined tablet dosage form.

MATERIALS AND METHODS

A Waters HPLC system consisting of a Water 2695 binary gradient pump, an inbuilt auto sampler, a column oven and Water 2487 dual wavelength absorbance detector (DAD) was employed throughout the analysis. The data was acquired using Empower 2 software. The column used was Thermosil C₁₈ (150×4.6 mm, 3.5µm). A Bandline sonerex sonicator was used for enhancing dissolution of the compounds. A Digisum DI 707 digital pH meter was used for pH adjustment. Analytically pure LEV and MON were obtained as gift samples from M/s Blue Cross Ltd., (Mumbai, India) and M/s

Mercury Laboratories Ltd., (Vadodara, India), respectively. Acetonitrile, methanol, water (E. Merck, Mumbai, India) were of HPLC grade, while ortho-phosphoric acid and potassiumdihydrogen phosphate (S. D. Fine Chemicals, Mumbai, India) were of Analytical grade used for the preparation of mobile phase.

Preparation of mobile phase and stock solutions:

Potassium dihydrogen phosphate was weighed (7.0 g) and dissolved in 1000 ml of water. Finally the pH was adjusted to 3.6 with ortho phosphoric acid (0.1 M). The solution was sonicated for 10 minutes and filtered using Whatman filter paper (No.1) and used. LEV and MON were weighed (25 mg each) and transferred to two separate 25 ml volumetric flasks and dissolved in mobile phase, which gives 1000 µg/mL of LEV and MON. LEV and MON solutions were further diluted with mobile phase to obtain final concentration 100µg/mL each.

Chromatographic conditions: A reverse phase C18 column equilibrated with mobile phase phosphate buffer-acetonitrile (30:70) adjusted to pH 3.6 was used. Mobile phase flow rate was maintained at 1.0 mL/min and effluents were monitored at 232 nm. The sample was injected using a 20 µL fixed loop, and the total run time was 10 min. Appropriate aliquots of LEV and MON stock solutions were taken in different 10 ml volumetric flasks and diluted up to the mark with mobile phase to obtain final concentrations of 50, 60, 70, 80, 90 µg/mL of LEV and 100, 110, 120, 130, 140 µg/mL of MON. The solutions were injected using a 20 µl fixed loop system and chromatograms were recorded. Calibration curves were constructed by plotting average peak area versus concentrations and regression equations were computed for LEV and MON.

Determination of LEV and MON in their combined dosage forms:

The content of twenty tablets were taken and weighed. Powder equivalent to LEV 5mg and MON 10mg was accurately weighed and transferred to a 50 ml volumetric flask and 20 ml of mobile phase was added to the same and flask was sonicated for 5 min. The flask was shaken, and the volume was diluted to the mark with the same mixture. The above solution was filtered using Whatman filter paper No.1. Appropriate volume of the aliquot was transferred to a 50 ml volumetric flask and the volume was made up to the mark with mobile phase to obtain 60 µg/ mL of LEV and 120µg/mL of MON. The solution was sonicated for 10 min. The solution was injected at above chromatographic conditions and peak areas were

measured. The quantification was carried out by keeping these values to the straight line equation of calibration curve. The method was validated^[13] for accuracy, precision, specificity, detection limit, quantitation limit and robustness.

Accuracy: The accuracy of the method was determined by calculating recoveries of LEV and MON by method of standard additions. Known amount of LEV and MON were added to a pre quantified sample solution, and the amount of LEV and MON were estimated by measuring the peak areas and by fitting these values to the straight-line equation of calibration curve.

Precision: The intraday and inter day precision study of LEV and MON was carried out by estimating the corresponding responses 3 times on the same day and on 3 different days (first, second and fifth day). The results are reported in terms of relative standard deviation. The Repeatability studies were carried out by estimating response of 3 different concentrations of LEV and MON for triplicate and results are reported in terms of relative standard deviation (RSD).

Specificity: Commonly used excipients were spiked into a pre weighed quantity of drugs. The chromatogram was taken by appropriate dilutions and the quantities of drugs were determined.

Detection limit and quantitation limit: A calibration curve was prepared using concentrations in the range of 0.1-2 µg/ml for LEV and MON µg/ml for ASP (expected detection limit range). The standard deviation of y-intercepts of regression lines were determined and kept in following equation for the determination of detection limit and quantitation limit. Detection limit= $3.3\sigma /s$; quantitation limit= $10\sigma/s$; where σ is the standard deviation of y-intercepts of regression lines and s is the slope of the calibration curve.

Robustness: Robustness of the method was studied by changing the composition of organic phase by \pm % and the pH by ± 0.2 , and also by observing the stability of the drugs for 24 h at 35° temperature in the mobile phase.

RESULTS AND DISCUSSION

Optimization of mobile phase was performed based on resolution, asymmetric factor and peak area obtained for both LEV and MON. The mobile phase phosphate buffer-acetonitrile (30:70) adjusted to pH 3.6 using ortho phosphoric acid was found to be

satisfactory and gave two symmetric and well-resolved peaks for LEV and MON. The resolution between LEV and MON was found to be 6.2, which indicates good separation of both the compounds. The retention time for LEV and MON were 2.213 min and 5.674 min, respectively (Figure 1).

The asymmetric factors for LEV and MON were 1.26 and 1.32, respectively. The calibration curve for LEV was obtained by plotting the peak area of LEV versus the concentration of LEV over the range of 50-90 µg/mL, and it was found to be linear with $r^2 = 0.9992$. Similarly, the calibration curve for MON was obtained over the range of 100-140 µg/mL and was found to be linear with $r^2 = 0.9994$. The data of regression analysis of the calibration curves are shown in (Table-1).

The detection limit for LEV and MON were 0.11 µg/mL and 0.04 µg/mL, respectively. The quantitation limit for LEV and MON were 0.36 µg/mL and 0.12 µg/mL, respectively, which suggest that a nano gram quantity of both the compounds can be estimated accurately. The validation parameters are summarized in (Table-1).

The recoveries of LEV and MON were found to be 100.31% and 100.37%, respectively. The system suitability test parameters are shown in (Table-1). The liquid chromatographic method was applied to the determination of LEV and MON in their combined dosage forms. The results for LEV and MON were comparable with the corresponding labeled amounts (Table-4).

CONCLUSION

Proposed study describes a new RP-HPLC method for the estimation of LEV and MON combination in mixture using simple mobile phase with low buffer concentration compared to the reported method. The method gives good resolution between both the compounds with a short analysis time (<10 min). The method was validated and found to be simple, sensitive, accurate and precise. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation. Therefore, the proposed method can be used for routine analysis of LEV and MON in their combined dosage form.

Table 1: Validation parameters and data for proposed method

Validation parameter	Results	
	LEV	MON
Linearity	50-90 µg/mL	100-140 µg/mL
Regression coefficient (r^2)	0.9992	0.9994
Limit of detection (µg/mL)	0.11	0.04
Limit of quantitation (µg/mL)	0.36	0.12
Accuracy (% recovery)*	100.31	100.37
Precision		
Repeatability of injection (%RSD)**	0.907	0.502
Intra-day precision (%RSD)*	0.666	0.687
Inter-day precision (%RSD)*	0.718	0.766
Reproducibility		
Intra-day precision (%RSD)*	0.809	0.533
Inter-day precision (%RSD)*	0.897	0.452
Assay value (%)	99.73	100.16
System suitability parameter		
Tailing factor	1.26	1.32
Number of theoretical plates	2676	5889
Resolution		6.2

* Replicates of three concentration levels (in three determinations); ** Ten repetitive injections of same homogeneous sample

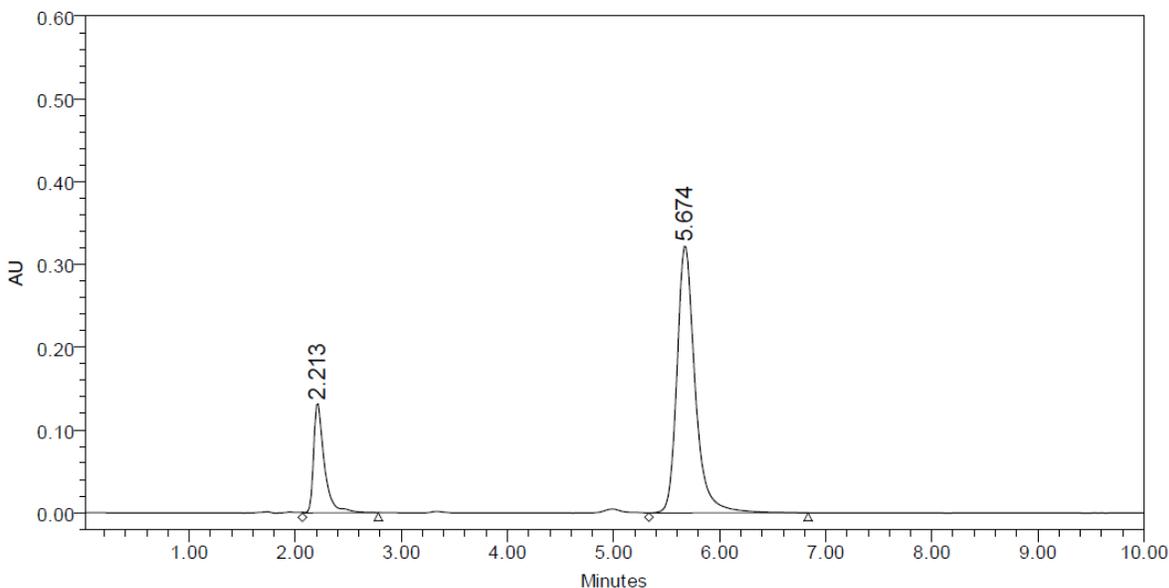


Figure 1: HPLC chromatogram of levocetirizine and montelukast in optimized chromatographic conditions

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