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Original Article

Rp-HPLC Method for Simultaneous Estimation of Levocetirizine Dihydrochloride and Montelukast Sodium in tablets

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Abstract

A simple, selective and sensitive reverse phase high performance liquid chromatography (Rp-HPLC) method has been proposed for the simultaneous quantitative determination of levocetirizine dihydrochloride (LEV) and Montelukast sodium (MON) in pure form as well as in its pharmaceutical formulation. The chromatography was carried out on Waters C_{18} analytical column (15cm × 4.6 mm, 5µ) using a mobile phase of methanol: water (75:25 v/v). The flow rate was 1.0 ml/min with detection at 235 nm. The retention time of LEV and MON were found to 2.88 and 3.83 min respectively. The linearity for LEV and MON were in the range of 50-150 µg/mL and 100- 300 µg/mL respectively. The recoveries of LEV and MON were found to be 100.00% and 99.00%, respectively. The proposed method was validated and successfully applied to the estimation of LEV and MON in combined tablet dosage forms.

1. Introduction

Levocetirizine dihydrochloride (LEV), (2-[4-[(R)-(4-chlorophenyl) phenylmethyl]-1-piperazinyl]ethoxy]-acetic acid dihydrochloride) (Figure 1) is a third generation non sedative antihistamine, and is the active enantiomer of cetirizine dihydrochloride.[1]

LEV has the advantages of higher efficacy, less side effects, and longer duration over other antihistamines, and has begun to replace cetirizine in clinical therapy stepwise. It has been chemically proved that the half dosage form of LEV (2.5 mg) has comparable antihistaminic activity to normal amount (5.0 mg) of cetirizine in the treatment of allergic rhinitis and chronic idiopathic urticarial.[2]

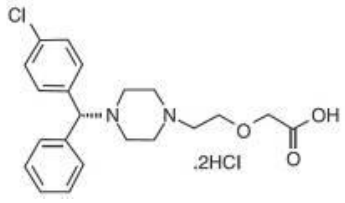

Figure 1: Chemical structure of Levocetirizine dihydrochloride

Montelukast is an oral selective leukotriene receptor antagonist that inhibits the cysteinyl leukotriene cysLT1 and has been shown to be effective in the treatment of chronic asthma and Chemically, it is2-[1-[(R)-[3-[2(E)-(7-chloroquinolin-2- yl)vinyl] phenyl]-3-[2-(1-hydroxy-1- methylethyl)phenyl]propyl -sulfanylmethyl] cyclopropyl] acetic acid sodium salt (Figure 2).[3]

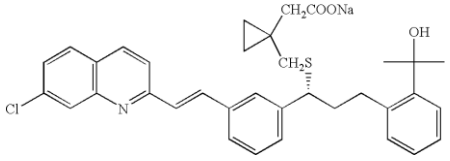

Figure 2: Chemical structure of Montelukast sodium

Literature reveals that various methods have been reported for analysis of Levocetirizine dihydrochloride and montelukast sodium in single component formulations but a less number of methods are available for the simultaneous estimation of these two drugs in multicomponant dosage forms.[4-9] Thus, the objective of this work was to develop an accurate, specific, repeatable and validated HPLC method according to ICH guidelines[10] for simultaneous determination of Levocetirizine dihydrochloride and Montelukast Sodium in tablet dosage form.

2. Materials and methods

2.1 HPLC–PDA instrumentation and chromatographic conditions

The HPLC system was an LC Waters (Waters, Milford, MA, USA) consisting of quaternary gradient system (600 Controller), in line degasser (Waters, model AF), photodiode array detector (Water, 2998 model) and auto sampler (Waters, model 717 plus). Data was processed using Empower Pro software (Waters, Milford, MA, USA). Chromatographic separation assay was performed with a Water's C-18 analytical column (150 mm × 4.6 mm inner diameter, 5 µm particle size, Waters, Dublin, Ireland) maintained at ambient temperature. The mobile

phase was pumped at a flow rate of 1.0 mL min−1. The detection wavelength was 235 nm.

2.2 Chemical and reagents

Pure powder (>99.9% purity) of LEV and MON was supplied as a gift sample by Hetero drugs Pvt. Ltd., Hydrabad. The tablet Dosage form of LEV and MON was purchased from Anukar Pharmacy, Hyderabad. HPLC grade acetonitrile (ACN) were purchased from Scharlau (Sentmenat, Spain). HPLC grade water was obtained by distilling deionised water produced by a Milli-Q millipore water system (Milford, MA, USA). All the other reagents and materials were of analytical grade and supplied from commercial sources. The aqueous and organic components of the mobile phase were mixed and degassed under vacuum by the HPLC. The LC mobile phases were filtered through 0.2 µm cellulose acetate membrane filters (Sartorius Ste-dim Biotech S.A., Aubagne Cedex, France) with a solvent filtration apparatus.

2.3 Mobile phase

A mixture of methanol: water in the ratio of 75:25 %v/v was used as mobile phase. Mixed solvents were filtered through 0.2 µm cellulose acetate membrane filters (Sartorius Ste-dim Biotech S.A., Aubagne Cedex, France) with a solvent filtration apparatus, degassed and used as mobile phase. Same was used as diluents for the preparation of drug solutions.

2.4 Standard and sample solutions

Stock solution of LEV was prepared by dissolving 10 mg of LEV in a 10 ml volumetric flask, and the volume is made up with the diluent. Subsequent dilutions of this solution ranging from 50 to 150 µg/ ml were made with the diluent. 20 mg of MON was dissolved in 10 ml volumetric flask and the volume was made up with the diluents. Subsequent dilutions of this solution ranging from 100 to 350 µg/ ml were made with the diluent. Working solutions were prepared daily from the stock solutions. The containers used for storage were screw-capped tubes coated externally by aluminium foil.

2.5 Assay of pharmaceutical preparation:

To carry out the sample solution, 20 tablets were taken and weighed individually, obtaining afterwards the average weight of these tablets, finally they were ground. An appropriate portion of this powder, equivalent to 10 mg of LEV was weighed and placed in a 10ml volumetric flask, dissolving it in the mobile phase. This solution was sonicated for 10 min to dissolve and remove the entire active from the tablet. The solution was filtered if necessary. 5 ml of aliquot was taken and transferred to volumetric flask of 50 ml capacity and volume was made up to the mark with the diluent. This solution was used for the estimation of LEV and MON (100µg/ml).

3. Results and Discussion

Estimation of LEV and MON in tablet dosage forms by RP-HPLC method was carried out using optimized chromatographic conditions. The typical chromatogram of standard and sample solution is given in Figure 3 and Figure 4 respectively. The peak area ratio of standard and sample solutions was calculated. The results of analysis shows that the amounts of drugs were in good agreement with the label claim of the formulations.

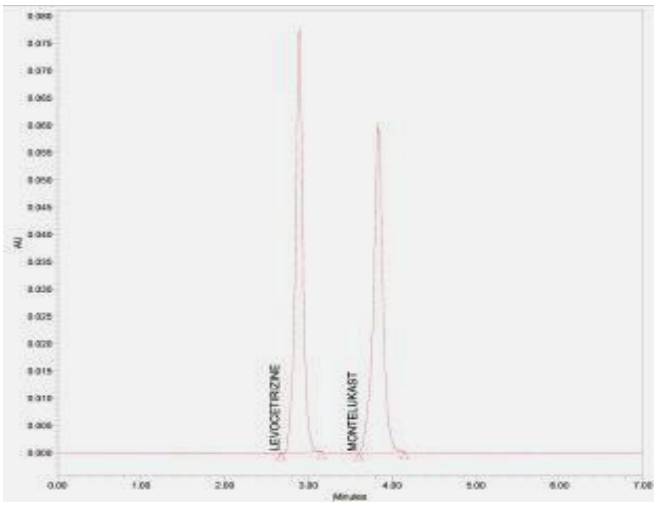

Figure 3: Typical chromatogram of standard sample of LEV and MON

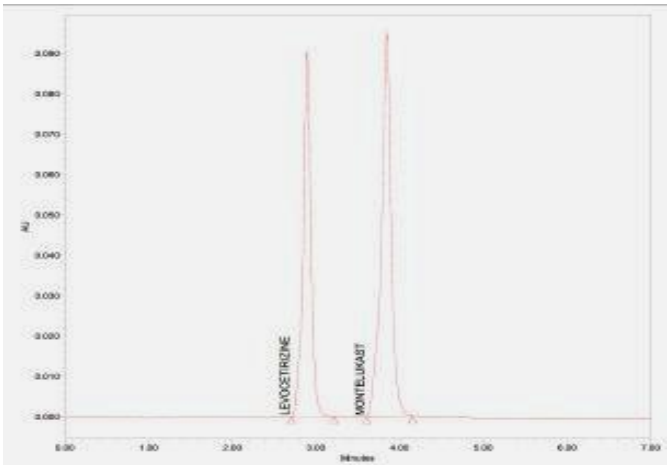

Figure 4: Typical chromatogram of tablet sample of LEV and MON

Optimization of mobile phase was performed based on resolution, asymmetric factor and peak area obtained for both LEV and MON. The mobile phase composed of a mixture of methanol: water in the ratio of 75: 25 v/v was found to be satisfactory and gave two symmetric and well-resolved peaks for LEV and MON. The summary of optimized chromatographic conditions was shown in table-1. The retention time of levocetirizine dihydrochloride and Montelukast sodium was found to be 2.88± 0.2 min and 3.83± 0.2 min respectively. The total time of analysis was less than 6 minutes. Results showed an excellent correlation between response factor and concentration of drugs within the concentration range.

Table 1: Optimized HPLC conditions for simultaneous estimation of Rosuvastatin and Ezetimibe

S. No	Parameter	Description/Value
1.	Stationary Phase	Water's C18 (250X4.6X5)
2	Mobile Phase	Methanol: water 75:25 v/v
3	Flow rate	1.0 ml/min
4	Detection Wavelength	235 nm
5	Detector	Photo diode array
6	Injection	autosampler -Waters, model 717 plus
7	Rt's	Levocetirizine: 2.88 min Montelukast: 3.83 min
8	Injection volume	10 µl
9	Column Temperature	40 °C
10	Run time	7 mins
11	Diluent	Mobile Phase

The calibration curve for LEV was obtained by plotting the peak area of LEV versus the concentration of LEV over the range of 50-150 µg/mL, and it was found to be linear with r2 = 0.9991. Similarly, the calibration curve for MON was obtained over the range of 100-300 µg/mL and was found to be linear with r2 = 0.9994. The data of regression analysis of the calibration curves are shown in (Table-2). The detection limit for LEV and MON were 0.4 ng/mL and 0. 1ng/mL, respectively. The quantitation limit for LEV and MON were 0.36ng/mL and 0.12ng/ml, respectively, which suggest that a nano gram quantity of both the compounds can be estimated accurately. The validation parameters are summarized in (Table-2).

The recoveries of LEV and MON were found to be 99.0 % and 100.00%, respectively. The system suitability test parameters are shown in (Table-2). The liquid chromatographic method was applied to the determination of LEV and MON in their combined dosage forms.

Table 2: Summary of validation Results

S. No.	Validation parameter	Results	
		Levocetirizine	Montelukast
1.	Linearity	50-150µg/ml	100-300 µg/ml
2.	Regression equation (y)	y = 95579x + 98597	y = 128494x + 127999
3.	Regression coefficient (r2)	R^2 = 0.9991	R^2 = 0.9994
4.	Limit of detection (µg/mL)	0.42 ng/mL	0. 16 ng/mL
5.	Limit of quantitation (µg/mL)	0.36 ng/mL	0.12 ng/ml
6.	Accuracy (Mean % recovery)*	99 %	100 %
7.	Precision (%RSD)		
	Intra-day precision (%RSD)*	0.19	0.18
	Inter-day precision (%RSD)*	0.41	0.24
8.	Mean Assay (% Purity)	100	99
9.	Tailing factor	0.968	0.920
10.	Number of theoretical plates	5697	6484
11.	Resolution	3.1	

** Replicates of three concentration levels (in three determinations); ** Ten repetitive injections of same homogeneous sample. All the values were expressed in mean± SD.*

4. Conclusion

Proposed study describes a new RP-HPLC method for the estimation of LEV and MON combination in mixture using simple mobile phase compared to the reported method. The method gives good resolution between both the compounds with a short analysis time (<7 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.

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