Research Paper

Simultaneous Estimation of Montelukast Sodium and Levocetirizine HCl by RP-HPLC Method Development in Pharmaceutical Tablet Dosage Form

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The chromatographic analysis was performed by Hypersil BDS C18 ,250 $\times$ 4.6 mm, 5 μ particle size with mobile phase consisting of methanol and sodium hydrogen phosphate and orthophosphoric acid buffer (pH 7.0) in the ratio of 75:25 v/v, at a flow rate of 1.2 ml/min and eluents monitored at 230 nm. The method was validated for linearity, accuracy, precision, robustness and application for assay as per ICH guidelines. The retention times of montelukast and levocetirizine were 12.65 and 4.458 min, respectively. The calibration curves of peak area versus concentration, which was linear from 8-28μg/ml for montelukast and 4-14μg/ml for levocetirizine, had regression coefficient (r^2) greater than 0.998. The method had the requisite accuracy, precision, and robustness for simultaneous determination of montelukast and levocetirizine in tablets. The proposed method is simple, economical, accurate and precise, and could be successfully employed in routine quality control for the simultaneous analysis of montelukast and levocetirizine in tablets.

Key words : MON (Montelukast sodium), LEV (Levocetirizine hydrochloride), RP-HPLC (Reverse phase –High performance liquid chromatography) .ICH (International Conference on Harmonization), μ (Micron).

INTRODUCTION

Montelukast sodium, Chemically ; 2-[1-[[1-[3-[2- [(7-chloro-2- quinolyl)] vinyl] phenyl]-3- [2-(1-hydroxy-1-methyl-ethyl) phenyl]-propyl] sulfanyl methyl] cyclopropyl] acetic acid sodium salt[1].Lucasts reduce acute reactions to aspirin in sensitive patients, but have not been shown to be particularly effective for aspirin- sensitive asthma in the clinic. They inhibit exercise induced asthma and decrease both early and late responses to inhaled allergen. They relax the airways in mild asthma but are less effective than salbutamol. Their action is additive with B2-adrenoreceptor agonists. They also reduce sputum eosinophilia[2]. Levocetirizine hydrochloride, Chemically (R)- [2-[4-[(4-Chlorophenyl) phenylmethyl]-1-piperazinyl]ethoxy] acetic acid dihydrochloride[3].

Levocetirizine a selective long acting peripheral H1 receptor antagonist antihistamine. The increased polarity of Levocetirizine may decrease distribution of the drug into CNS, resulting in reduced potential for adverse CNS effects. As it

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does not dihydrochloride is the R-enatiomers of cetrizine HCl, a racemic compound with antihistaminic properties. It is an orally active and selective H_1-receptor antagonist. Histamines act on H_1 receptors, causing the symptoms commonly seen in allergic reaction. Levocetirizine inhibits these H_1 receptors[4-5]. For individual estimation of these drugs, several methods are available in the literature [6–11]. Some methods which are available in literature are for the simultaneous estimation of Montelukast and Levocetirizine [12–18].

The aim of this work is to develop an accurate, specific, repeatable, and validated method for simultaneous determination of Montelukast and Levocetirizine in both bulk and tablet formulations.

EXPERIMENTAL

Materials

Pure Montelukast (MON) and Levocetirizine (LEV) were used as working standards, gifted from Balaji drugs. Pontasahib (H.P). India. Tablets containing 10 mg of MON and 5 mg of LEV were purchased from market of Balsons pharma, India and used within their shelf life period. Methanol and water (HPLC-grade) were purchased from Merck, India. All other chemicals and reagents employed were of analytical grade, and purchased from Merck and Ranbaxy, India.

Instrumentation

A Shimadzu HPLC system consisting of a LC-2010 CHT binary gradient pump, an inbuilt auto sampler, a column oven and dual wavelength absorbance detector (DAD) was employed throughout the analysis. The data were acquired through the Empower-2 software. The column used was Hypersil BDS symmetry C18, 250×4.6 mm, 5μm. A Bandline sonerex sonicator was used for enhancing the dissolution of the compounds.

Optimized chromatographic conditions

The chromatography elution was carried out in the isocratic mode using a mobile phase consisting of methanol and sodium hydrogen phosphate buffer (pH 7.0 adjusted with ortho phosphoric acid) in a ratio of 75:25 v/v. The analysis performed at ambient temperature using a flow rate of 1.2 ml/min with a run time of 4 min. The eluent was monitored using DAD at a wavelength of 230 nm. The mobile phase was filtered through whatmann filter paper No.41 prior to use.

Preparation of stock and standard solutions

A stock solution of MON and LEV(500 μg/ml) were prepared by taking accurately weighed 50 mg of MON and LEV as reference standard in 100 ml volumetric flask containing 50 ml of methanol and

then the volume was made up to the mark with methanol . The stock solution is protected from light using aluminum foil. Aliquots of the standard stock solution of MON and LEV were transferred , using A-grade bulb pipette into 10 ml volumetric flasks and solutions were made up to the mark with the mobile phase to give the final concentrations of 8-28μg/ml and 4-14 μg/ml of MON and LEV respectively.

Estimation of Montelukast and Levocetirizine from tablets

To determine the content of MON and LEV in tablets (Label claim: 10 mg and 5 mg), 20 tablets were taken and the powder equivalent to the weight of one tablet was accurately weighed and transferred to 50 ml volumetric flask and was dissolved in

25 ml of methanol and contents were weighed. An aliquot of volume was made up to the mark with methanol. The flask was sonicated for 20 minutes to affect complete dissolution. The solution filtered through a 0.45 μm millipore filter. A suitable aliquot of the filtered solution was transferred into a 100 ml volumetric flask and made up to the volume with the mobile phase to yield the concentration of 20μg/ml for MON and 10μg/ml for LEV. The experiments were performed six times under the optimized chromatographic conditions described prior. The peak areas were measured at 230 nm and concentration in the sample was determined by comparing the area of sample with that of the standard.
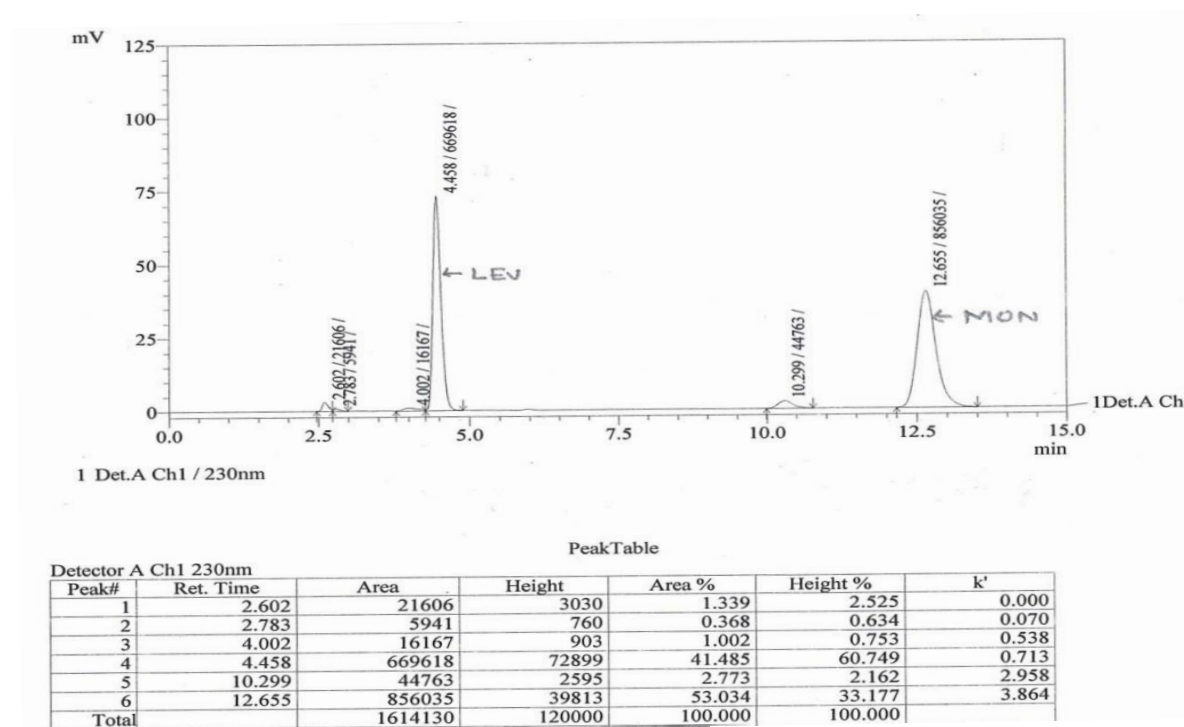

1 Det.A Ch1 / 230nm

PeakTable

Detector A Ch1 230nm

Peak#	Ret. Time	Area	Height	Area %	Height %	k'
1	2.602	21606	3030	1.339	2.525	0.000
2	2.783	5941	760	0.368	0.634	0.070
3	4.002	16167	903	1.002	0.753	0.538
4	4.458	669618	72899	41.485	60.749	0.713
5	10.299	44763	2595	2.773	2.162	2.958
6	12.655	856035	39813	53.034	33.177	3.864
Total		1614130	120000	100.000	100.000	

Figure 1: Chromatogram of MON and LEV

Method validation

Linearity: By appropriate aliquots of the standard MON and LEV solution with the mobile phase, six working solutions ranging between 8-28 μg/ml and 4-14 μg/ml were prepared. Each experiment was performed in triplicate according to optimized chromatographic conditions. The peak areas of the chromatograms were plotted against the concentration of MON and LEV to obtain the calibration curve.
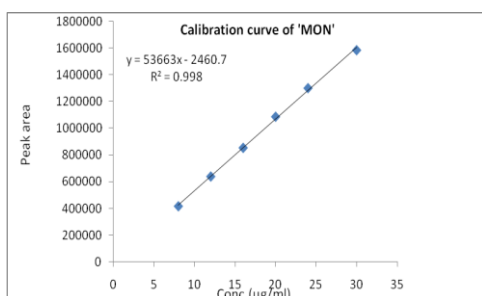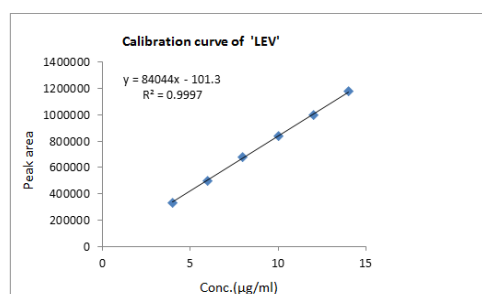

Figure 2: Linearity curve of MON

Figure 3: Linearity curve of LEV

Accuracy: Recovery studies by the standard addition method were performed with a view to justify the accuracy of the proposed method. Previously analyzed samples of MON and LEV to which known amounts of standard MON and LEV, corresponding to 80,100 and 120% of label claim were added. The accuracy

expressed as the percentage of analyte recovered by the proposed method.

Precision: Precision was determined as repeatability and intermediate precision, in accordance with ICH guidelines. The intra-day and inter-day precision were determined by analyzing the samples of MON and LEV at all concentration in linear range respectively. Determinations were performed with three replicates on the same day as well as on three consequent days.

Reproducibility: The reproducibility of the method was checked by determining precision on a same instrument, the analysis being performed by another person in the same laboratory. It was analyzing the samples of MON and LEV at different concentration in between 8-28 μg/ml and 4-14 μg/ml in triplicate respectively and calculates the amount of drug present in the sample.

Robustness: The robustness of the method was performed by deliberately changing the chromatographic conditions. The organic strength and buffer pH were varied by ±2% and 0.2 units, respectively.

System suitability tests: To ensure the validity of the analytical procedure, a system suitability test was established. Data from ten injections of 20μl of the working standard solution containing 20μg/ml for MON and 10μg/ml for LEV were used for

Table 1: Linearity data and their analytical performances for Montelukast sodium and Levocetirizine hydrochloride

Drugs	Conc. µg/ml	Peak area	Linear Range	Correlation co- efficient	Slope	Intercept
	8	416870	8-28µg/ml	0.998	53663	2460
MON	12	639668				
	16	854636				
	20	1088059				
	24	1302197				
	28	1586691				
	4	333692	4-14µg/ml	0.999	84044	101.3
	6	501423				
LEV	8	682458				
	10	839836				
	12	1001671				
	14	1178682				

the evaluation of the system suitability parameters like tailing factor, the number of theoretical plates and retention time

Table 2: System suitability parameters

Parameter	MON	LEV
Retention time (min.)	12.65	4.458
Resolution	20.30	1.091
No. of Theoretical plates	8283.25	5033.38
Tailing factor	1.225	1.39

Limit of detection and the limit of quantification:

Limit of detection (LOD) and limit of quantification (LOQ) was calculated, based on the ICH guidelines.

RESULTS AND DISCUSSIONS

A RP-HPLC method was proposed as a suitable method for the estimation of MON and LEV in the tablet dosage forms. The best chromatographic conditions were adequately selected. The selection of mobile phase and flow rate was made on the basis of peak shape, baseline drift, time required for analysis, and the mobile phase consisted of methanol and sodium hydrogen phosphate buffer (pH 7, adjusted pH with ortho phosphoric acid) in the ratio of 75:25 v/v at a flow rate of 1.2 ml/min and analyzed at 230 nm. The retention time observed (12.65 for MON and 4.458 for LEV) allows a rapid determination of these drugs. In Figure 1, a typical chromatogram obtained under these conditions is shown. The calibration plot of peak area against concentration was linear in the range of 8-28 µg/ml and 4-14µg/ml for MON and LEV respectively. The linear regression data for calibration

Table 3: Estimation of amount present in tablet dosage form

Brand name	Tablet Formulation	Label Claim per Tablet (mg)	% Label claim estimated (Mean) N=5	SD	%COV	% Drug estimated
CETCURE-MK	MON	10	10.06	0.698	0.7005	100.6
	LEV	**5**	5.02	1.397	1.3964	100.04

curves were indicative of a good linear relationship between peak area and concentration over a wide range (Table 1). The correlation coefficient was indicative of high significance. . The LOD and LOQ were found to be 0.0173 μg/ml and 0.0525 μg/ml, and 0.056 μg/ml and 0.188 μg/ml for MON and LEV respectively.

The accuracy was assessed from three replicates containing a concentration range of 80, 100 and 120%. The recovery of the method determined by spiking a previously analyzed test solution with standard MON and LEV solution, and the recovery values were found to be in the range of 99.16 - 100.33% and 99.4 - 99.73% respectively. The values of % recovery and %COV were indicated that the method is accurate.

The precision of the method was assessed in accordance with ICH guidelines. The low %COV (<2) values indicate that the method is precise. Reproducibility of the method was performed in the same laboratory on a same instrument which was performed by another analyst. The assay values and low %COV (<2) values

indicate that the method is reproducible.

The robustness was determined by analyzing the same sample under a variety of conditions. The factors consider being variations in the pH (0.2 units) and strength of methanol (±2%).The results and the experimental range of the selected variables, together with the optimized conditions. There were no significant changes in the chromatography pattern when the above modifications were made in the experimental conditions, showing that the method is robust. The system suitability tests were also carried out to evaluate the reproducibility of the system for the analysis to be performed. The results of system suitability tests are given in Table 2, showing that the parameters are within the suitable range.

The proposed method was applied to the analysis of marketed formulations and the results obtained are given in Table 3. The blank solution was prepared containing the components indicated in tablet dosage form except the active ingredient. No interference was observed from the tablet excipients. The MON and LEV content

was found to be 100.6% and 100.04% respectively.

CONCLUSION

The proposed RP-HPLC method is rapid, specific, accurate and precise for the quantification of MON and LEV from its tablet dosage form. The method has been found to be better than previously reported methods, because of its wide range of linearity, use of readily available mobile phase, lack of extraction procedures. All these factors make this method suitable for quantification of MON and LEV in tablet dosage forms. The method can be successfully used for routine analysis of MON and LEV in bulk drugs and pharmaceutical dosage forms without interference.

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