INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

DEVELOPMENT AND VALIDATION OF A REVERSED PHASE HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF LEVOCETIRIZINE AND MONTELUKAST SODIUM IN TABLET DOSAGE FORM

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ABSTRACT

A simple, accurate, rapid and precise isocratic reversed-phase high-performance liquid chromatographic method has been developed and validated for simultaneous determination of levocetirizine and montelukast sodium in tablets. The chromatographic separation was carried out on Atlantis C-18 analytical column (4.6×150 mm; 5µm) with a mixture of 10Mm acetonitrile:ammonium acetate (65:35 % v/v and pH 4.2 was adjusted with orthophosphoric acid) as a mobile phase; at a flow rate of 1.0 mL/min. UV detection was performed at 230 nm. The retention times were 3.03 and 6.28 min for levocetirizine and montelukast sodium respectively. Calibration plots were linear (r^2=0.999) over the concentration range of 25-75 µg/mL for levocetirizine and 50-150 µg/mL for montelukast sodium. The method was validated for accuracy, precision, specificity, linearity, and sensitivity. The proposed method was successfully used for quantitative analysis of tablets. No interference from any component of pharmaceutical dosage form was observed. Validation studies revealed that method is specific, rapid, reliable, and reproducible. The high recovery and low relative standard deviation confirm the suitability of the method for routine determination of levocetirizine and montelukast sodium in bulk and tablet dosage form.

Keywords: Levocetirizine, Montelukast Sodium, RP-HPLC, Tablets.

INTRODUCTION

Levocetirizine (LEVO) is a third-generation non-sedative antihistamine, developed from the second-generation antihistamine cetirizine (Fig. 1). Chemically it is 2-(2-(4[R]-[4-chlorophenyl) methylpiperazin-1-ethoxy)acetic acid. Levocetirizine works by blocking histamine receptors. Montelukast sodium (MONT) is a selective leukotriene receptor antagonist. Chemically it is 1-[({(*R*)-*m*-[(*E*)-2-(7-chloro-2-quinolyl)-vinyl]-α-[*o*-(1-hydroxy-1-methylethyl) phenethyl]-benzythio) methyl] cyclopropane acetate, monosodium salt (Fig. 2). It is used in the management of chronic asthma, allergic, rhinitis and as prophylaxis for exercise-induced asthma. It should not be used to treat an acute asthma attack [1].

Literature survey reveals that few spectroscopic methods[2-8], HPTLC methods[9-12], HPLC[13-24] and capillary electrophoresis[25] for determination of LEVO and MONT in single and combination with other drugs. Therefore, an attempt has been made to develop an accurate, rapid and reproducible reverse phase HPLC method for simultaneous determination of LEVO and MONT in tablet dosage form and validate it, in accordance with ICH[26] guidelines.

MATERIALS AND METHODS

Chemicals and reagents

Pharmaceutical grade of LEVO and MONT were kindly supplied as gift samples by Dr. Reddy's Laboratories Ltd., Hyderabad, India, certified to contain > 99% (w/w) on dried basis. Commercially available MONTAIR LC (Cipla) and LEVETA M (Alembic) tablets purchased from local market. Tablets claimed to contain 5 mg of LEVO: 10 mg of MONT have been utilized in the present work. All chemicals and reagents used were HPLC grade and purchased from Merck chemicals, India.

Chromatographic conditions

Separation was performed with Waters HPLC equipped with a pump-515, auto sampler- 2960 and UV detector-2998, operated at 254 nm. Empower software was applied for data collecting and processing. A systronics-361 pH meter was used for pH measurements. The separation was achieved on a Atlantis C-18 (4.6×150 mm, 5 µm) analytical column. The mobile phase consisted of acetonitrile: ammonium acetate buffer 65:35 (v/v) pH 4.2 was adjusted with orthophosphoric acid. The flow rate was 1.0 mL/min and UV detection was performed at 230 nm. The mobile phase was shaken on an ultrasonic bath for 30 min. the resulting transparent mobile phase was filtered through a 0.45 µ membrane filter (Millipore, Ireland). The injection volume was 20 µL and all the experiments were performed at ambient temperature.

Preparation of standard solution

Accurately weigh and transfer 50 mg of LEVO and 100 mg of MONT working standard into 100 mL volumetric flask, add about 30 mL of diluent and sonicate to dissolve it completely and make volume upto the mark with diluents (stock solution), from this stock solution pipette out 5 mL into 100 mL volumetric flask and dilute upto the mark with diluent. Mix well and filter through 0.45 µ filter.

Preparation of sample preparation

Twenty tablets were accurately weighed, their mean weight was determined and they were mixed and finally powdered. Transfer the sample equivalent to 50 mg of LEVO and 100 mg MONT in to a 100 mL volumetric flask. Add about 30 mL of diluents and sonicate to dissolve it completely and make volume upto the mark with diluents. Mix well and filter through 0.45 µ filter, from this stock solution pipette out 5 mL into 100 mL volumetric flask and dilute upto the mark with diluent. Mix well and filter through 0.45 µ filter.

Method validation

The developed method was validated according to ICH guidelines. The system suitability was evaluated by six replicate analysis of LEVO and MONT mixture at concentrations of 50 µg/mL and 100 µg/mL. The acceptance criteria are %RSD of peak areas not more than 2%, theoretical plates numbers (N) at least 3000 per each peak and tailing factors not more than 2.0 for LEVO and MONT.

Linearity

Standard calibration curves were plotted against the concentration ranging from 25-75 µg/mL for LEVO and 50-150 µg/mL for MONT. Different linearity levels was prepared and injected into the HPLC system keeping the injection volume constant.

Recovery

To study the reliability and suitability of developed method, recovery experiments were carried out at three levels 80%, 100% and 120%. Known concentration of commercial tablet was spiked with known amount of LEVO and MONT. At each level of amount six determinations were performed with expected results. The %RSD of individual measurements was also determined.

Precision

Precision of assay was determined by repeatability (intra-day) and intermediate precision (inter-day) for three consecutive days. Every sample was injected six times. The repeatability of sample application and measurements for peak area were expressed in terms of %RSD.

Specificity

All chromatograms were examined to determine whether compound of interest coeluted with each other or with any additional excipient peaks. Marketed formulation was analysed to determine the specificity of the optimized method in presence of common tablet excipients.

Limit of detection and limit of quantification

Limit of detection (LOD) and limit of quantification (LOQ) were estimated from signal-to-noise ratio. LOD and LOQ were calculated

using 3.3 σ/s and 10 σ/s formulae, respectively. Where, σ is the standard deviation of the peak areas and S is the slope of the corresponding calibration curve.

Robustness

To evaluate robustness of HPLC method a few parameters were deliberately varied. The parameters included variation of flow rate, buffer composition and pH of mobile phase.

RESULTS AND DISCUSSION

During the optimization of HPLC method, two columns symmetry C-18 and C-8 analytical column (4.6×250 mm; 5 µm) and (4.6×150 mm; 5 µm), two organic solvents (acetonitrile and methanol), two buffers (acetate and phosphate) at two different pH values (3 and 5) were tested. Initially methanol:acetate buffer, acetonitrile:acetate buffer, methanol:phosphate buffer, acetonitrile:phosphate buffer were tried in different ratios at pH 3-5. LEVO and MONT eluted with tried mobile phases. With acetonitrile:phosphate buffer two drugs eluted and run was 20 min, in order to decrease the run time, symmetry C-18 analytical column (4.6×150 mm; 5 µm) was selected, the mobile phase conditions were optimized so the peak area from the first eluting compound did not interfere with those from the solvent and excipients. Finally mobile phase consisting of mixture of acetonitrile:ammonium acetate buffer in ratio 65:35 (v/v) was selected as mobile phase to achieve maximum separation and sensitivity. Flow rates between 0.8 to 1.2 mL/min were studied. A flow rate of 1.0 mL/min gave an optimum signal to noise ratio with reasonable separation time using a C-18 analytical column (4.6×150 mm; 5 µm), the retention times for LEVO and MONT were observed to be 3.02 and 6.27 min respectively. Total run time was less than 13 min. The chromatogram at 230 nm showed a complete resolution at all peaks (Fig. 3). Validity of the analytical procedure as well as the resolution between different peaks of interest is ensured by the system suitability tests. All critical parameters tested meet the acceptance criteria on all days. As shown in chromatogram, two analytes are eluted by forming symmetrical peaks.

Linearity was obtained for LEVO and MONT in the range of 25-75 µg/mL and 50-150 µg/mL. The correlation coefficient (r^2) was found to be greater than 0.999 in all instances. The results of calibration studies are summarized in Table 1. The proposed method afforded high recoveries for LEVO and MONT in tablet dosage form. Results obtained from recovery studies presented in Table 2. Indicate that this assay procedure can be used for routine quality control analysis of binary mixture in tablets. Precision of the analytical method was found to be reliable based on %RSD (<2%) corresponding to peak areas and retention times. As can be seen in Table 3 the %RSD values were less than 2 for intra-day and inter-day precision. Hence, the method was found to be precise for these two drugs.

The chromatograms were checked for appearance of any extra peaks under optimized conditions, showing no interference from common tablet excipients and impurities. Also the peak areas were compared with standard and percentage purity calculated was found to be within limits. LOD and LOQ were found to be 0.05 µg/mL and 0.17 µg/mL for LEVO, 0.10 µg/mL and 0.33 µg/mL for MONT. In all deliberately varied conditions, the %RSD for replicate injections of LEVO and MONT were found to be within the acceptable limit. The tailing factors for two peaks were found to be less than 1.5 and the results are shown in Table 4. The validate method was used in analysis of marketed tablet dosage form MONTAIR LC and LEVETA M) with a label claim 5 mg of LEVO and 10 mg of MONT tablet. The results for the drugs assay showed good agreement with label claims and the results are shown in Table 5.

Table 1: System suitability parameters of proposed method

S. No.	Parameters	LEVO	MONT
1	Linearity	25-75µg/ml	50-150 µg/ml
2	Theoretical plates	7451	10284
3	Asymmetric factor	1.09	1.21
4	Capacity factor	2.10	4.98
5	LOD	0.05 µg/mL	0.10 µg/mL
6	LOQ	0.17 µg/mL	0.33 µg/mL

Table 2: Accuracy data for proposed method[a]

Spiked level of drug (%)	Amount of drug added (µg/band)		%Mean recovery (n=6)		%RSD	
	LEVO	MONT	LEVO	MONT	LEVO	MONT
80	40	80	99.9	100.57	0.15	0.52
100	50	100	98.38	99.84	0.196	0.25
120	60	120	101.9	100.92	0.18	0.36

[a]n = 6

Table 3: Precesion data of proposed method[a]

Drug	Concentration (µg/mL)	Intra-day precision		Inter-day precesion	
		Mean peak area	%RSD	Mean peak area	%RSD
LEVO	25	972357	1.21	985674	1.32
	50	1976121	1.26	1995609	01.21
	75	2903486	1.41	2938957	0.5
MONT	50	2367187	1.6	2406781	1.4
	100	4673428	0.52	4657587	0.29
	150	6643621	0.52	6594732	0.46

[a]n = 6

Table 4: Robustness for flow rate variation of LEVO and MONT

Parameter	Flow rate variation-minus (0.8 mL/min)		Flow rate variation-plus (1.2 mL/min)	
	LEVO	MONT	LEVO	MONT
% RSD for five replication	0.2	0.4	0.3	0.6
Retention time	4.57	7.83	1.86	4.77
Theoretical Plates	5325	12674	8674	8731
Tailing Factor	1.02	1.34	1.05	1.23

Table 5: Analysis of marketed formulations by proposed method

Brand name		Label claim (mg)	Amount Found (mg)*	% Label claim
Montair LC	LEVO	5	4.96	99.2
	MONT	10	10.24	102.4
Leveta M	LEVO	5	5.06	101.2
	MONT	10	9.98	99.8

*(n=6)

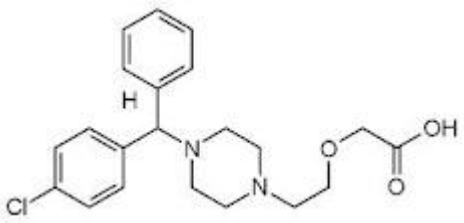

Fig. 1: Molecular structure of levocetirizine

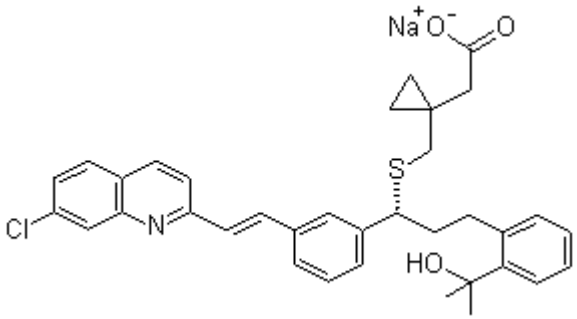

Fig. 2: Molecular structure of montelukast sodium

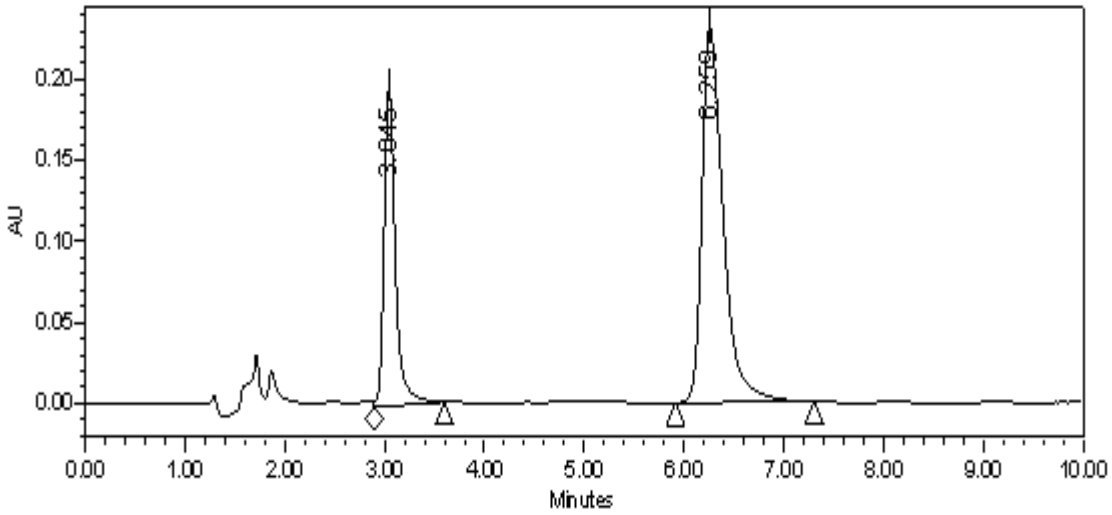

Fig. 3: Typical chromatogram of standard for LEVO and MONT

CONCLUSION

The developed HPLC method is simple, specific, accurate and precise for the simultaneous determination of LEVO and MONT in tablet dosage form. The developed method provides good resolution between LEVO and MONT. It was successfully validated in terms of system suitability, linearity, precision, accuracy, specificity, LOD, LOQ and robustness accordance with ICH guidelines. Thus the described method is suitable for routine analysis and quality control of pharmaceutical preparations containing these drugs either as or such in combinations.

ACKNOWLEDGEMENTS

The authors are thankful to Dr. Reddy's Laboratories Ltd, Hyderabad, for providing levocetirizine and montelukast sodium as gift samples for this work.

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