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Research Article

HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF MONTELUKAST SODIUM AND LEVOCETIRIZINE DIHYDROCHLORIDE IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple and precise HPLC method was developed for estimation of Montelukast sodium (MTKT) and Levocetirizine dihydrochloride (LCTZ) in pure and pharmaceutical dosage form. HPLC separation was achieved with a Phenomenex (Torrance, CA) C_{18} column (250 mm × 4.6 mm id, 5 µm particle size) as stationary phase and methanol: Trichloroacetic acid: Acetonitril (90:5:5, v/v/v) as eluent, at a flow rate of 1.0 ml/min. UV detection was performed at 231 nm. The retention time of Levocetirizine dihydrochloride and Montelukast sodium were found to 2.4 and 3.5 min respectively. Results of the analysis were validated by recovery studies. The stability of Montelukast sodium was maintained by carrying out all the operations in amber colored glass wares with minimal exposure to light. The result of the studies showed that the proposed RP-HPLC method is simple, rapid, accurate & precise, which can be used for the routine determination of Montelukast sodium and Levocetirizine dihydrochloride in bulk and in its pharmaceutical dosage forms.

Keywords: Montelukast sodium, Levocetirizine dihydrochloride, HPLC, Assay, pharmaceutical dosage forms.

INTRODUCTION

Montelukast sodium 2-[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 1) is a fast acting and potent cysteinyl leukotriene receptor antagonist which is being used in the treatment of asthma[1]. The recommended dosing of MTKT is 10mg per day. Levocetirizine (Figure 2) 2-[2-[4-[(*R*)-(4-chlorophenyl)-phenyl methyl] piperazinyl-1-yl]ethoxy] acetic acid, the *R*-enantiomer of racemic cetirizine, is a selective, potent, H1-antihistamine compound indicated for the treatment of allergic rhinitis and chronic idiopathic urticaria[2].The LCTZ is official in IP-2007[3].The recommended dosing of LCTZ is 5mg per day.

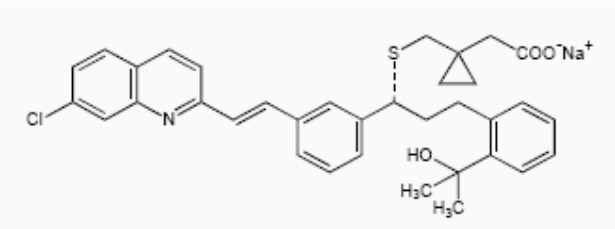

Fig. 1: Structure of Montelukast Sodium

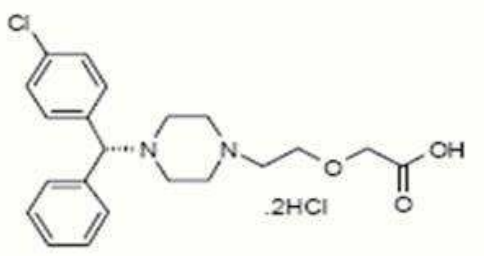

Fig. 2: Structure of Levocetirizine dihydrochloride

Only a few chromatographic methods have been reported for the determination of MTKT and LCTZ, in individual and in combination with other drugs in the open literature. Liquid chromatography with fluorescence detection[4–6], stereo selective HPLC for MTKT and its S-enantiomer[7], simultaneous HPLC and derivative spectroscopic method with loratadine[8], stability indicating HPLC method for MTKT in tablets and human plasma[9] reported for MTKT.Different spectrophotometric [10], HPLC[11-15] and LCMS[16,17] methods have been reported for the determination of cetirizine in pharmaceutical formulations and biological fluids.

During our literature survey, simple RP-HPLC,HPTLC and ratio derivative spectroscopy for determination of MTKT and LCTZ was found.[18-22].The reported simple RP-HPLC method used phosphate buffer (pH adjusted to 5.5 with dil. KOH): Acetonitrile (65:35, v/v) as a mobile phase. The goal of this study was to develop a method without using buffer in a mobile phase , has a less run time, and more sensitive compare to developed method for the analysis of Montelukast and Levocetirizine in formulations, using the most commonly employed C-18 column with UV detection and extremely low LOQ & LOD values.

The objective of this work was to develop and validate an accurate, specific, precise, repeatable, HPLC method for determination of MTKT and LCTZ in combination dosage forms.

MATERIALS AND METHODS

Instruments and Apparatus

The chromatography was performed on a Shimadzu (Columbia, MD) RP-HPLC instrument (LC-2010CHT) equipped with PDA detector and LC-solution software, Phenomenex (Torrance, CA) C18 column (250×4.6 mm id, 5 µm particle size) was used as stationary phase. Sartorius CP224S analytical balance (Gottingen, Germany) and ultrasonic cleaner (Frontline FS 4, Mumbai, India) were used during the research work.

Reagents and materials

Standard samples of MTKT and LCTZ were obtained from Cedilla Healthcare Ltd, Ahmedabad. Triple distilled water, methanol, acetronitrile, Trichloroacetic acid (S. D. Fine Chemicals) used were of HPLC grade.

Preparation of Standard Solution

Accurately weighed MTKT (100 mg) and LCTZ (100 mg) standards were transferred to a 100 ml volumetric flask, dissolved in and diluted up to the mark with methanol to obtain a standard stock solution (1000 µg/ml) of MTKT and LCTZ, each. From the above stock solution, an aliquot (5 ml) of the solution was transferred to 50 ml volumetric flask, and diluted up to the mark with methanol to obtain a working standard solution (100µg/ml) of MTKT and LCTZ, each.

Preparation of Calibration Curve

Calibration curves were plotted over the concentration range of 0.5-30 µg/ml of MTKT and LCTZ. Accurately prepared standard solution of MTKT and LCTZ (0.05, 0.1, 0.5, 1, 1.5, 2, 2.5, and 3 ml) were transferred in 10ml volumetric flask and diluted with mark by using mobile phase. The calibration curves were constructed by plotting peak areas versus concentrations. An aliquot (10 µl) of each solution was injected under the operating chromatographic conditions as described above and responses were recorded. Calibration curves were constructed by plotting the peak areas versus the concentrations, and the regression equations were calculated. Each response was average of three determinations.

Preparation of Sample Solution

Twenty tablets were weighed and powdered. The quantity of the powder equivalent to 10 mg of MTKT and 5 mg of LCTZ was transferred to a 100 ml volumetric flask. The content was mixed with methanol (100 ml), sonicated for 20 min. to dissolve the drug as completely as possible. The solution was then filtering through a whatman filter paper no. 41. The volume was adjusted up to the mark with methanol**.** An aliquot (0.5ml) was transferred to a 10 ml volumetric flask and diluted up to the mark with mobile phase used for HPLC, to obtain sample solution of MTKT (5 µg/ml) and LCTZ (2.5 µg/ml).

RESULTS AND DISCUSSION

Method Development

Initially various mobile phases were tried in attempt to obtain the best separation and resolution between Montelukast and Levocetirizine. The mobile phase of Methanol: Trichloroacetic acid: Acetonitril (90:5:5, v/v/v) was found to be an appropriate mobile phase allowing the adequate separation of both the compounds using a Phenomenex (Torrance, CA) C_{18} column (250 mm × 4.6 mm id, 5 µm particle size), at a flow rate of 1.0ml/min. a typical chromatogram of separation of the two components is shown in figure 3. As the MTKT and LCTZ exhibit significant absorbance at wavelength 231nm, it was selected as detection wavelength for the simultaneous determination of MTKT and LCTZ in pharmaceutical dosage forms. The retention time of LCTZ and MTKT were found to be 2.4 and 3.5 respectively. The resolution, theoretical pate and tailing factors are prescribed in table.1.

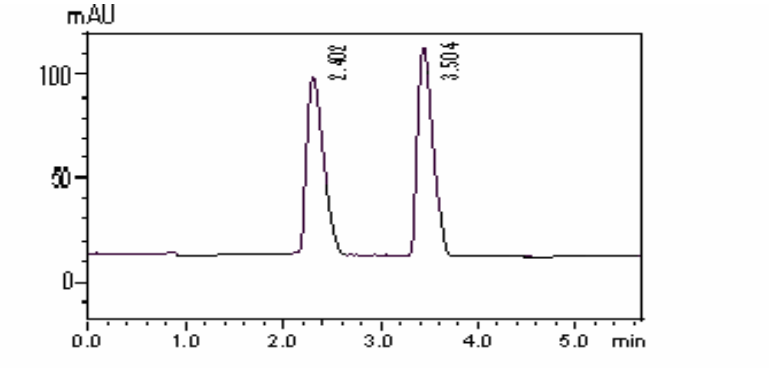

Fig. 3: HPLC spectra of Montelukast Sodium and Levocetirizine Dihydrochloride

Table 1: System Suitability Parameters

Parameters	MTKT ± RSD (n = 6)	LCTZ ± RSD (n = 6)
Retention time (min)	3.5± 0.29	2.4± 0.15
Tailing factor	1.25 ± 0.26	1.19 ± 0.20
Theoretical plates	4085 ± 0.35	3548 ± 0.39
Resolution	4.72 ± 0.19	

Validation of the Method

The retention time for LCTZ and MTKT was found to be 2.4 and 3.5 minutes respectively. For the evaluation of linearity, different concentrations of standard solution were prepared in the concentration range of 0.5-30 µg/mL for LCTZ and MTKT with correlation of 0.9965 for LCTZ and 0.9956 for MTKT. Accuracy of the method was ascertained by recovery study (n=3) (table 2). The concentration of the standard spiked to the sample was 50-150 % of the assay level. The method was found to be accurate with percent recoveries between 98.27% - 99.03% with standard deviation not more than 1.14 for LCTZ and recoveries between 98.12% - 99.04% with standard deviation not more than 0.71 for MTKT. There was good repeatability of proposed method with percentage RSD 0.373 for LCTZ and 0.305 for MTKT. The limit of detection (LOD) and limit of quantification (LOQ) of LCTZ were found to be 0.22µg/ml and 0.56µg/ml respectively. The limit of detection (LOD) and limit of quantification (LOQ) of MTKT were found to be 0.32µg/ml and 0.72µg/ml respectively. The result of specificity studies indicated no interference from excipients and mobile phase. The response was due to individual components only. All validation parameters were summarized in table 3.

Table 2: Recovery Studies

Drug	Tablet amount(µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml)*	Recovery	Coefficient of variation
LCTZ	1	0.5	0.491	98.27	0.667
	1	1	0.988	98.84	1.145
	1	1.5	1.485	99.03	0.836
MTKT	2	1	0.982	98.21	0.644
	2	2	1.980	99.04	0.711
	2	3	2.943	98.12	0.214

*Each value is the mean of three determinations.

Table 3: Summary of validation Parameters of Proposed HPLC method

Parameters	MTKT	LCTZ
Range (µg/ml)	0.5-30	0.5-30
Regression equation y=mx+c	Y = 580542x + 84143	Y = 502448x + 62869
Slope	580542	502448
Intercept	84143	62869
Correlation coefficient (r)	0.9956	0.9965
LOD (µg/ml)	0.32	0.22
LOQ (µg/ml)	0.72	0.56
%Recovery ± SD,(n=3)	98.46± 0.52	98.71±0.89
Repeatability (%RSD, n=6),	0.30	0.37
Interday precision (%RSD) (n = 3) at 3 range	0.53-1.02	0.59-1.12
Intraday precision (%RSD) (n = 3) at 3 range	0.32-0.50	0.45-0.51
% assay ± SD (n= 6)	98.80±0.40	99.16±0.89

[a]SD = Standard deviation, [b]RSD = Relative standard deviation

Analysis of LCTZ and MTKT in Formulation

The LCTZ and MTKT content were found to be 99.16±0.89 % and 98.806±0.407 respectively, of the label claim. The low value of % RSD indicated the method was suitable for routine analysis of the MTKT and LCTZ in pharmaceutical dosage forms. Result of assay was summarizing in table 4.

Table 4 : Assay Result

Dosage form	Drug	Label claim (mg)	Percent Label claim estimated*(Mean±SD)
Tablet	MTKT	10 mg	98.80± 0.407
	LCTZ	5 mg	99.163± 0.895

*Indicate average of six determination and SD denotes standard deviation

CONCLUSION

The proposed, the developed HPLC method is simple, liner, accurate, sensitive and reproducible. Thus, the developed method can be easily used for the routine quality control of bulk and tablet dosage from of Levocetirizine and Montelukast within a short analysis.

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