

SIMULTANEOUS ESTIMATION OF LEVOCETIRIZINE DIHYDROCHLORIDE AND MONTELUKAST SODIUM BY RP-HPLC METHOD

Sushma Somkuwar¹, A.K. Pathak^{1*}

Department of Pharmacy, Barkatullah University, Bhopal, M.P., [India] 462026

Abstract

A reverse phase high performance liquid chromatography method has been developed for the simultaneous estimation of Levocetirizine hydrochloride and Montelukast sodium in tablet formulation. The determination was carried out on a SUPELCOSILTM, LC-8 (15cm X 4.6 mm, 5 μ m) column using a mobile phase of 0.02M potassium dihydrogen phosphate buffer solution: methanol (40:60 v/v, pH 5.0). The flow rate was 1.0 ml/min with detection at 218 nm. Levocetirizine dihydrochloride and montelukast sodium showed a linear response in the concentration range of 5-20 μ g/ml and 10-40 μ g/ml, respectively. The results of analysis have been validated statistically and by recovery studies. Developed method was found to be simple, accurate, precise and selective for simultaneous estimation of Levocetirizine dihydrochloride and Montelukast sodium in tablet dosage forms.

Key Words: *Levocetirizine dihydrochloride, Montelukast sodium, RP-HPLC, Simultaneous estimation.*

Introduction

Levocetirizine dihydrochloride is chemically (RS)-2-{4-[(R)-p-chloro- α -phenylbenzyl]-1-piperazinyl} ethoxyacetic acid dihydrochloride. Its molecular formula is $C_{21}H_{25}Cl_2N_2O_3 \cdot 2HCl$. The recommended dose of Levocetirizine is 5mg per day. Levocetirizine (as levocetirizine 2HCl) is a third generation non-sedative antihistamine, acts by blocking histamine receptors. It is used in the treatment of several allergic reactions, viz., allergic rhinitis, idiopathic urticaria, hay fever etc. HPLC method of analysis for Levocetirizine Hydrochloride in bulk as well as from tablet is available. Montelukast is a specific cysteinyl leukotriene receptor antagonist belongs to a styryl quinolines series with the chemical name 2-[1-[1(R)-[3-[2(E)-(7-chloroquinolin-2-yl)vinyl]phenyl]-3[2-(1-hydroxy-1-methylethyl)phenyl]propyl sulfanyl methyl] cyclo-propyl] acetic acid. Its molecular formula is $C_{35}H_{36}ClNO_3S$. [1-3] The recommended dose of Montelukast is 10mg per day. It is developed as a therapeutic agent for the treatment of bronchial asthma and exercise induced bronchospasm. [4-5]

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 multicomponent dosage forms. [6-11] The quantitative analysis of such multicomponent formulations is very important. HPLC has become a useful instrument for drug analysis since it is the instrument of choice in conducting quantitative estimation. The instrument computes accurate results within minimal time. The method validation which ensures that the selective method will give reproducible and reliable results adequate for intended purpose. Thus, the objective of this work was to develop an accurate, specific, repeatable and validated HPLC method for simultaneous determination of Levocetirizine dihydrochloride and Montelukast Sodium in tablet dosage form.

Material And Methods

Methanol used was of HPLC grade of Rankem India and Milli Q water was used for the preparation of the mobile phase. All other reagents like KH_2PO_4 , KOH, H_3PO_4 used were of AR/GR grade. Reference standards of Levocetirizine dihydrochloride and Montelukast sodium were procured from Torque Pharmaceutical Pvt. Ltd, Baddi. and Ranbaxy Laboratories Ltd., Dewas.

*Corresponding Author :

Prof. A. K. Pathak

Head of Department, Department of Pharmacy, Barkatullah University,

Bhopal, M.P. [India] Pin- 462026, Ph. (+91) 0755-2491846, 4297802 Fax- 0755-2491857 Email- anupampathak@yahoo.com

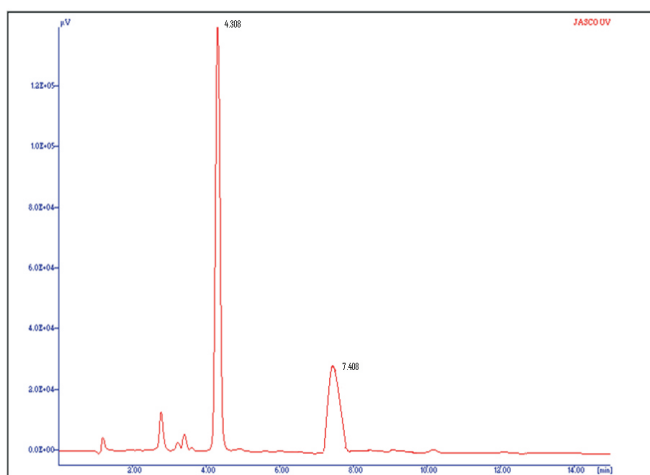
2.1 Chromatographic Conditions:

Chromatographic separation was performed on a JASCO HPLC 2000 liquid chromatographic system equipped with a Jasco PU-2080 plus solvent delivery system (pump), UV-2075 plus UV detector, Rheodyne 7725i injector with 50 μ l loop volume. Borwin ver. 1.50 data station was used for data collecting and processing. A SUPELCOSILTM LC-8 column (15cm x 4.6mm i.d. 5 μ) was used for the separation. The mobile phase was methanol: buffer (pH-5.0) (60:40, v/v) at a flow rate of 1.0 ml/min with detection at 218 nm. Under these conditions the retention times were typically 4.308 min for levocetirizine dihydrochloride and 7.408 min for montelukast sodium.

2.2 Preparation of standard solutions:

Standard stock solutions of 5 mg/ml levocetirizine dihydrochloride, and 10 mg/ml montelukast sodium were prepared using a mixture of methanol: buffer (60:40, v/v). From the standard stock solution, standard solution was prepared to get the solutions of concentration 10 μ g/ml of levocetirizine dihydrochloride, and 20 μ g/ml of montelukast sodium. A typical chromatogram obtained from standard solution is shown in Fig. 1.

Fig.1. Typical Chromatogram of Standard Solution



2.3 Preparation of Sample Solution:

20 tablets were weighed accurately and crushed, tablet powder equivalent to 5 mg of levocetirizine dihydrochloride and 10 mg montelukast sodium was taken in a 100 ml volumetric flask and diluted with mixture of methanol and buffer (60: 40 v/v) and filtered. The filtrate was made up to 100 ml with mobile phase and further dilutions were made to get a concentration of 10 μ g/ml of levocetirizine dihydrochloride and 20 μ g/ml of montelukast sodium. The resulted solution was used for the estimation.

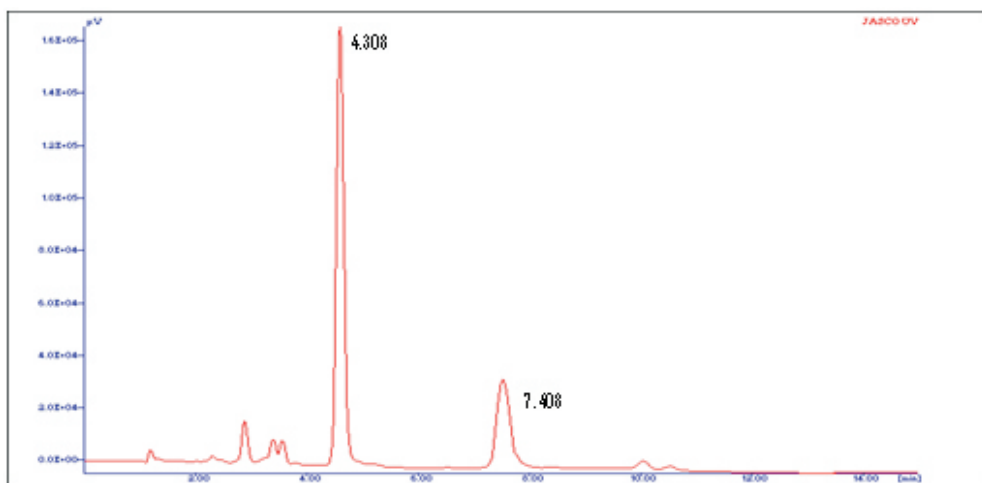
2.4 Assay Method

With the optimized chromatographic conditions, a steady baseline was recorded, the mixed standard solution was injected and the chromatogram was recorded. The retention time of levocetirizine dihydrochloride, and montelukast sodium was 4.308 ± 0.2 , and 7.408 ± 0.2 min respectively. This procedure was repeated for the sample solution obtained from the formulation.

The response factor (peak area ratio of standard peak and sample peak) of the standard solution and sample solution were calculated. Results are shown in Table 1 and the chromatogram recorded is shown in Fig.2.

Table 1 - Results of analysis of formulation

Drug	Labelled (mg/tab) amount present	Amount found (mg/tab)	% content assay
Levocetirizine dihydrochloride	5mg	4.96 mg	99.18%
Montelukast sodium	10mg	9.91 mg	99.08%

Fig. 2. Typical Chromatogram of Sample Solution

Method Validation

The proposed method was validated as per the International Conference on Harmonization (ICH) guidelines.

3.1 Accuracy:

To check the degree of accuracy of the method, recovery study was done in triplicate by standard addition method in each level at 80%, 100%, and 120% concentration. The percentage recovery and standard deviation of the percentage recovery were calculated and results are shown in Table 2.0

Table 2: Data for Recovery of Levocetirizine (Average of 3 readings)

Drugs	Level	Mean	S.D.	% RSD
Levocetirizine dihydrochloride	80%	99.06	0.355	0.358%
	100%	0.601	0.405	0.597%
	120%	100.68	101.45	0.399%
Montelukast sodium	80%	98.56	0.865	0.877%
	100%	99.47	0.525	0.527%
	120%	100.00	0.056	0.056%

3.2 Precision:

The precision of the method was determined by inter day and intraday (repeatability) variation studies. In the intraday studies, six repeated standard and sample solutions were injected and the response factor of drug peaks and percentage RSD were calculated. In the inter day variation studies, six repeated standard and sample solutions were injected for two consecutive days. Data for repeatability, intermediate precision, response factor of drugs peak and percentage RSD are reported in Table 3.

Table 3: Data for Repeatability and Intermediate precision (Day to Day analysis) of Levocetirizine and Montelukast (Average of 6 readings)

Levocetirizine dihydrochloride precision %R.S.D	Montelukast sodium precision %R.S.D	Levocetirizine dihydrochloride Intermediate precision	Montelukast sodium Intermediate precision
1.35%	1.19%	0.41%	0.29%

3.3 Linearity and Range:

The linearity of the method was ranging from 5.0 to 20.0 µg/ml for levocetirizine dihydrochloride and 10.0 to 40.0 µg/ml for montelukast sodium. The calibration curve was plotted between response factor and concentration of drugs. The slope and intercept value for calibration curve was $y = 29654x + 18298$ ($R^2=0.993$) for levocetirizine dihydrochloride and $y = 19748x + 55219$ ($R^2=0.997$) for montelukast sodium.

3.4 Limit of Detection and Limit of Quantification:

The Limit of Detection (LOD) and Limit of Quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD for levocetirizine dihydrochloride and montelukast sodium was found to be 2.493 µg/ml and 0.489 µg/ml respectively. The LOQ was 7.553 µg/ml and 1.482 µg/ml for levocetirizine dihydrochloride and montelukast sodium, respectively.

3.5 System suitability studies:

The column efficiency, resolution and peak asymmetry were calculated for the standard solutions and the results are presented in Table 4. The obtained values demonstrated the suitability of the system for the analysis of this drug combination.

Table 4: Data for System suitability (Average of 3 readings)

System suitability parameters	Components	
	Levo	Monte
Retention time(RT)min	4.46	7.34
Theoretical plates (N)	21118.67	6892.19
Tailing factor	1.66	1.061
Resolution	3.1	

Results And Discussion

Estimation of levocetirizine dihydrochloride and montelukast sodium 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 Fig. 1 and Fig. 2 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. The retention time of levocetirizine dihydrochloride and montelukast

sodium was found to be 3.308 ± 0.2 min and 7.408 ± 0.2 min respectively. The total time of analysis was less than 10 minutes. Results showed an excellent correlation between response factor and concentration of drugs within the concentration range.

Conclusion

The proposed RP-HPLC method for the simultaneous estimation of levocetirizine dihydrochloride and montelukast sodium in combined dosage forms is accurate, precise, linear, simple, rapid, and selective. It can, therefore, be conveniently adopted for estimation and routine quality control (QC) analysis of levocetirizine dihydrochloride and montelukast sodium.

Acknowledgement

Author is thankful to Department of Pharmacy, Barkatullah University, Bhopal (M.P.) for providing facilities to carry out the research and to Torque Pharmaceutical Pvt. Ltd, Baddi & Ranbaxy Laboratories Ltd., Dewas for providing the gift samples of drugs. Author is also thankful to Mr. Satish K. Sarankar and Mr. Anand Mundada (Department of Pharmacy, Barkatullah University, Bhopal) for their suggestions and help during the research.

References

1. Indian Pharmacopoeia. Government of India Ministry of Health and Family Welfare; Controller of Publications, vol II, 2007, 1290-1291
2. Maryadele JO, Neil Eds, The Merck Index, An Encyclopedia of Chemical, Drug's and Biologicals, Published by Merck Research Lab, Division of Merck and co. Inc., Whitehouse Station, NJ, 13th ed, 2001, 2022, 6258
3. Yadav B, Yadav S, Sensitive HPLC method for determination of related substance of Levocetirizine dihydrochloride in solid oral formulations. *J Pharm Res*, 3(12), 2010, 2817-2820
4. Gonikberg MW, Pharmacopeial Forum, United States Pharmacopeial Convection; USA, Vol-36(1), 2010, 1-3.
5. Moffat AC, Osselton DM, Clarke's Analysis of Drugs and Poisons. London: Pharmaceutical Press, 2004
6. Singh RM, Saini PK, Mathur SC, Singh GN, Lal B, Development and Validation of a RP-HPLC Method for Estimation of Montelukast Sodium in Bulk and in Tablet Dosage Form, *Indian J Pharm Sci*, 72(2), 2010, 235-237
7. Sharma S, Sharma MC, Kohli DV, Sharma AD, Development and Validation of TLC-Densitometry Method for Simultaneous Quantification of Montelukast Sodium and Levocetirizine Dihydrochloride Pharmaceutical Solid dosage form. *Der Pharmacia Lettre*, 2 (1), 2010, 489-94
8. Rathore AS, Sathinarayana L, Mahadik KR, Development of Validated HPLC and HPTLC Methods for Simultaneous Determination of Levocetirizine dihydrochloride and Montelukast sodium in Bulk Drug and Pharmaceutical Dosage Form. *Pharma Anal Acta, An Open Access Journal*, 1(1) 2010, 1-6
9. Sankar ASK, Baskar GN, Nagavalli D, Anandakumar K, Vetrichelvan T, Simultaneous Estimation of Montelukast Sodium and Levocetirizine Hydrochloride from Tablet Dosage Form. *Res J Phar and Tech*, 2(4), 2009, 443-445
10. Choudhari V, Kale A, Abnawe S, Gwale V, Simultaneous Determination of Montelukast Sodium and Levocetirizine Dihydrochloride in Pharmaceutical Preparations by Ratio Derivative Spectroscopy, *Inter J Pharma Tech Res*, 2(1), 2010, 4-9
11. Ashokkumar S, Senthil Raja M, Perumal P, RP-HPLC Method Development and Validation for Simultaneous Estimation of Montelukast Sodium and Levocetirizine Dihydrochloride, *Inter J Pharm Res*, 1 (4), 2009, 8-12