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Simultaneous Determination of Montelukast Sodium and Levocetirizine Dihydrochloride in Pharmaceutical Preparations by Ratio Derivative Spectroscopy.

V. Choudhari* ,A. Kale , S. Abnawe ,B.Kuchekar ,V.Gawli ,N. Patil

**Department of Pham. Analysis and Quality Assurance,
MAEER's Maharashtra Institute of Pharmacy, S.No. 124, MIT Campus, Paud Road, Kothrud, Pune-411038, MS, India.**

E-mail: viraj1404@rediffmail.com, Mb : +91 9421081141

Abstract: Simple, accurate, precise, and sensitive spectrophotometric method for simultaneous estimation of Montelukast (MON) and Levocetirizine (LEV) in combined tablet dosage form have been developed and validated. The ratio derivative spectroscopic method involves measurement of first derivative amplitude of ratio spectra at 250.4 nm for MON and 238.4 nm for LEV as two wavelengths for estimation. Beer's law is obeyed in the concentration range of 4-12 and 2-6 µg/mL for MON and LEV, respectively. LOD values for MON and LEV are found to be 0.09 µg/mL and 0.178 µg/mL, respectively. LOQ values for MON and LEV are found to be 0.277µg/mL and 0.591 µg/mL, respectively. The results of analysis have been validated statistically and recovery studies carried out in the range 80-120% to confirm the accuracy of the proposed method.

Key words: Montelukast, Levocetirizine, Ratio Derivative Spectroscopy.

Introduction

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.
Levocetirizine (as levocetirizine dihydrochloride) is chemically [2-[4-[(R)-(4-Chlorophenyl)phenylmethyl]-1piperazinyl]ethoxy]-acetic acid dihydrochloride a third-generation non-sedative antihistamine[1].LEV is official in European Pharmacopoeia and both are official in IP[2-3]. Literature survey reveals that there are UV and HPLC methods reported[5-15] for the estimation of MON in pharmaceutical formulations which include liquid chromatography coupled with mass spectrometry, HPLC, chiral liquid chromatography and capillary electrophoresis. The proposed methods are optimized and validated as per the International Conference on Harmonization (ICH) guidelines[4] Several methods[16-19] have been reported for the assay of LEV which include spectrophotometry, and liquid chromatography .The review of the literature revealed that there is no Spectrophotometric method available for this combination. Our efforts were to develop simple, rapid, accurate, reproducible and economical first order ratio derivative spectroscopic method for both the drugs in combined dosage forms. This paper describes first order ratio derivative spectrometry method for simultaneous estimation of MON and LEV in tablet formulations.

Materials and Methods

Drugs and Chemicals

Spectroscopic grade Methanol purchased from LOBA Chemie Pvt. Ltd., Mumbai was used throughout the study. Tablet used for analysis were MONTEK LC from two batches (Batch no AF60258 and AF 60350) manufactured by Atoz Life Sciences, Pondicherry, India having MON Sodium equivalent to MON 10mg and LEV 5 mg per tablet. Pure drug sample of MON % purity 98.5% was kindly supplied as a gift sample by Ranbaxy Laboratories Ltd., Dewas and pure drug sample of LEV % purity 99.8 was gifted by Mapro

Pharmaceuticals, Vapi, Gujrat. These samples were used without further purification.

Instruments

An UV-Visible double beam spectrophotometer (Varian Cary 100) with 10MM matched quartz cells were used for Spectrophotometric measurement. All weighing were done on electronic balance (Model Shimadzu AUW-220D).

Preparation of Standard Solution and Calibration Curve

Stock solution of each drug having concentration 1000µg/mL was prepared by dissolving MON and LEV separately in methanol. Aliquots of stock solutions were further diluted in methanol to get the solutions in the range of 4-12 µg/mL for MON and 2-6 µg/mL for LEV and were scanned in the wavelength range of 300–200 nm. Derivative amplitude of ratio spectra was obtained and was used for construction of calibration curve. The Beer's law was obeyed over the concentration range 4-12 µg/mL by MON and over the concentration range 2-6 µg/mL by LEV.

Preparation of Sample Solution and Formulation Analysis

Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 20 mg of LEV and MON Sodium equivalent to 40 mg of MON was transferred to 100 mL volumetric flask, 80 mL of methanol was added to the same flask, sonicated for 5 min and diluted to 100 mL with methanol and filtered through What man filter paper No. 41. Resulting solution was further diluted with methanol to obtain solution having concentration 8 µg/mL and 4 µg/mL of MON and LEV, respectively. The sample solution was scanned in the wavelength range of 300–200 nm. Derivative amplitude of ratio spectra was obtained and concentrations of the drugs were calculated by using calibration curve.

Theoretical Aspects

The absorption spectrum of mixture is divided by the absorption spectrum of standard solution of one of compound and first derivative of ratio spectrum is obtained, resulting spectra is independent of conc. of divisor. The conc. of active compounds are then determined from calibration graph obtained by measuring amplitude at points corresponding to minima or maxima. Using appropriate dilutions of standard stock solution, the two solutions were scanned separately. The ratio spectra of different MON standards at increasing concentrations were obtained by dividing each with the stored spectrum of the standard solution of LEV (4 µg/mL) as shown in Fig 2 (A) and the first derivative of these spectra traced, are illustrated in Fig 2 (B). Wavelength 250.4 nm was selected for the quantification of MON in MON + LEV mixture. The ratio and ratio derivative spectra of the solutions of LEV at different concentrations were obtained by dividing each with the stored standard spectrum of the MON (8 µg/mL) (Fig. 3 (A) and 3 (B) respectively). Wavelength 238.4 nm was selected for the quantification of LEV in MON + LEV mixture. Measured analytical signals at these wavelengths were proportional to the concentrations of the drugs. The amount of MON and LEV in tablets were calculated by using following equations

At 250.4 nm: $C_{MON} = d/d\lambda [A_{MON} / A_{LEV}] -$ Intercept (c) / Slope (m) ... (1)

At 238.4 nm: $C_{LEV} = d/d\lambda [A_{LEV} / A_{MON}] -$ Intercept (c) / Slope (m) ... (2)

Recovery Studies

The accuracy of the proposed method was checked by recovery studies, by addition of standard drug solution to preanalysed sample solution at three different concentration levels (80 %, 100 % and 120 %) within the range of linearity for both the drugs. The basic concentration level of sample solution selected for spiking of the drugs standard solution was 4 µg/mL of MON and 2 µg/mL of LEV.

Results and Discussion

Under experimental conditions described, calibration curve, assay of tablets, recovery studies and precision studies were performed. Using appropriate dilutions of standard stock solution, the two solutions were scanned separately. The zero order overlain spectra are shown in Fig 4. A critical evaluation of proposed method was performed by statistical analysis of data where slope, intercept, correlation coefficient is shown in Table 1. As per the ICH guidelines, the method validation parameters checked were linearity, accuracy and precision. Beer's law obeyed in the concentration range 4-12 µg/mL and 2-6 µg/mL with correlation coefficient of 0.999 and 0.997 for MON and LEV respectively. The proposed method was also evaluated by the assay of commercially available tablets containing MON and LEV(n = 6). The % assay was found to be 96.86 % for MON and 99.63 % for LEV as presented in Table 2. Results of recovery studies are shown in Table 2. For MON, the recovery study results ranged from 99.79% to 100.68% with % RSD values ranging from 0.394 % to 0.777 %. For LEV, the recovery results ranged from 99.44 % to 100.2 %, with % RSD values ranging from 0.425 % to 0.808 %. The accuracy and reproducibility is evident from the data as results are close to 100 % and standard deviation is low.

Conclusion

The validated Spectrophotometric method employed here proved to be simple, fast, accurate, and precise and sensitive thus can be used for routine analysis of Montelukast and Levocitrizine in combined tablet dosage form without prior separation.

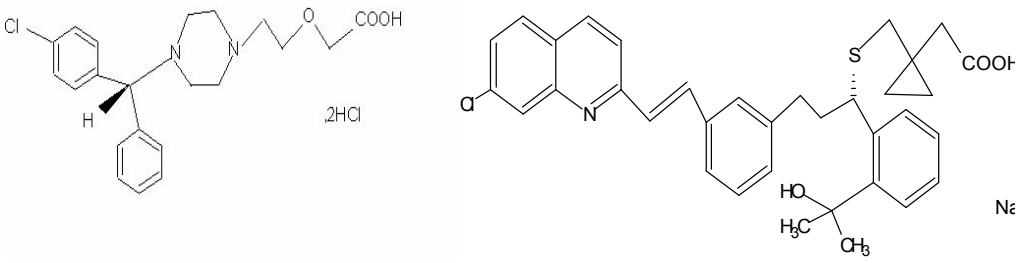
Table1. Optical Characteristics and Validation Data of MON and LEV

Parameters		**MON**	**LEV**
Working (nm)		250.4	238.4
Linearity		4-12 µg/mL	2-6 µg/mL
Molar absorptivity (l mole^{-1} cm^{-1})		726194.33	416042.09
Precision [a]	Inter-day (%RSD)	0.58	1.66
	Intra-day (%RSD)	1.15	0.83
LOD [a] ± SD		0.09 µg/mL	0.178
LOQ [a] ± SD		0.277 µg/mL	0.591µg/mL
Regression equation	Slope(m)	0.0123	0.0905
(Y=mx+c)	Intercept (c)	1.3902	0.1240
Regression coefficient(r^2)		0.999	0.997

[a] Denotes average of six estimations

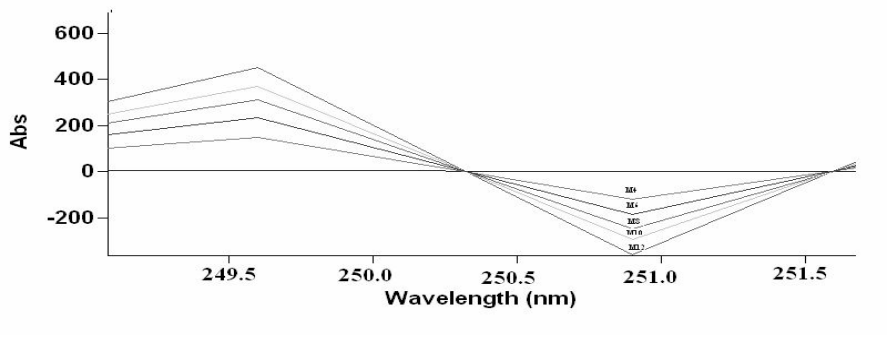
Table2. Statistical Validation Data of Tablet Formulation and recovery study

Drug	**Result of formulation analysis**			**Result of recovery study**		
	Amount present	**% of amount Found ± SD**	**%RSD (n=3)**	**Level recovery**	**% of recovery**	**%RSD (n=3)**
MON	10mg	96.86± 0.463	0.463	80	99.79	0.394
				100	100.68	0.617
				120	99.88	0.777
LEV	5 mg	99.63±0.234	0.234	80	99.44	0.425
				100	99.87	0.546
				120	100.2	0.808

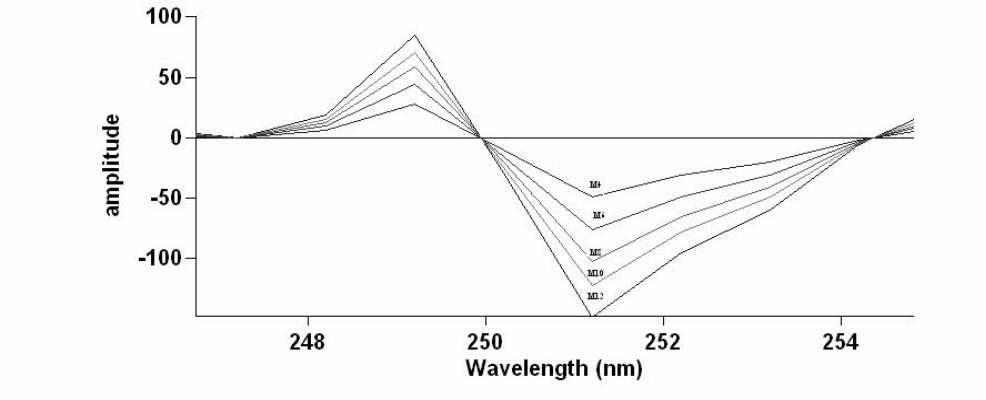


(a) **(b)**

Figure-1: Chemical structures of a) Montelukast and b) Levocetirizine

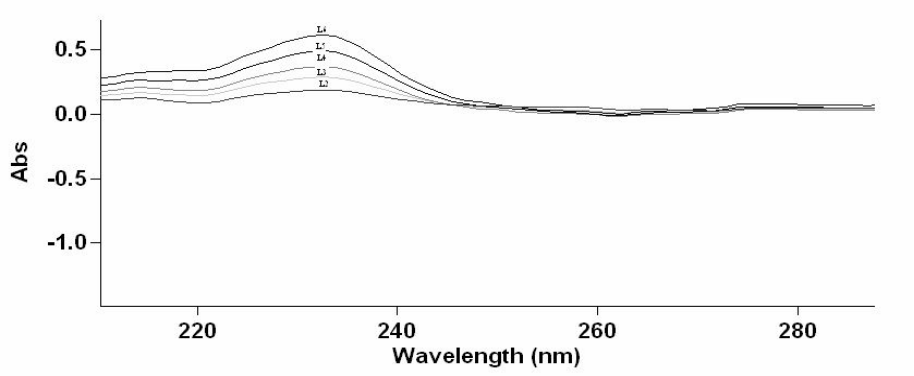


(A)

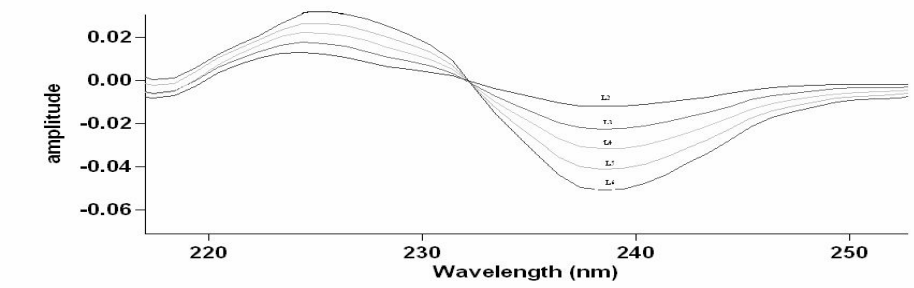


(B)

Figure-2. Ratio spectra (A) and first derivative of the ratio spectra (B) of M4, M6, M 8, M10 and M12 µg/mL solution of MON when 4 µg/mL solution of LEV is used as divisor. Where M=MON



(A)



(B)

Figure- 3. Ratio spectra (A) and first derivative of the ratio spectra (B) of L2, L3, L 4, L5and , L6µg/mL solution of LEV when 8 µg/mL solution of MON is used as divisor.
Where L=LEV

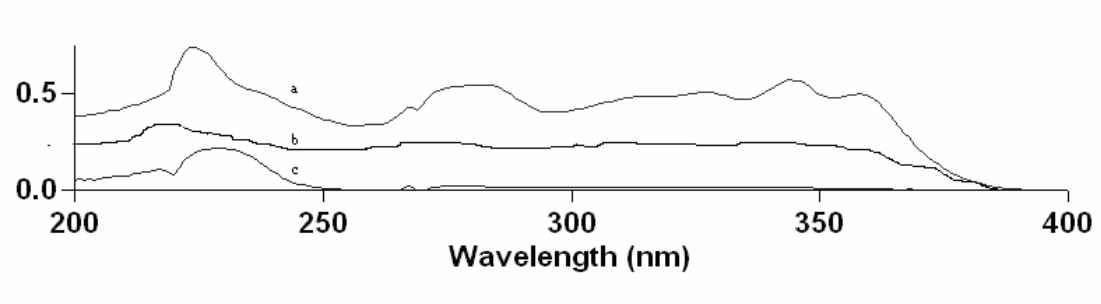


Figure- 4.Overlain zero order absorbance spectra of (a) MON (8 µg/mL) , (b) and their mixture (2 µg/mL LEV and 4 µg/mL MON) (c) LEV (4 µg/mL)

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References

1. The Merck Index (2001) Merck research Laboratories. 13th edn, Maryndale J.O'Neil, Merck & Co., Inc., White house Station, NJ. USA.6281, 2030.
2. European Pharmacopoeia 6.0, v II, 1479.
3. Indian Pharmacopoeia, 2007, v II, 1290.
4. ICH Harmonised Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2 (R1) Nov 2005.
5. Liu L, Haiyung C, Jamie Z, Rogers J and Douglas J, Determination of Montelukast (MK-0476)and its S-enantiomer in human plasma by stereoselective high performance liquid chromatography with coloumn switching ,*J.of Pharmaceutical and Biomedical Analysis*, 1997, **63**,631.
6. Robert P, Pauline L, Wayne M. M and Elizabeth K, A rapid and sensitive method for the quantitation of montelukast in sheep plasma using liquid Chromatography/tandem mass spectrometry, *J. of Chromatography B*, 2007, **858**, 282–286.
7. Radhakrishna T, Narasaraju A, Ramakrishna M and Satyanarayana A, Simultaneous determination of montelukast and loratadine by HPLC and derivative spectrophotometric methods, *J. of Pharmaceutical and Biomedical Analysis*, 2003, **31**, 359_368.
8. Alsarra I, Al-Omar M, Gadkariem E.A and Belal F, Voltammetric determination of montelukast sodium in dosage forms and human plasma,*Il Farmaco*, 2005, **60**, 563–567.
9. Al-Rawithi S, Al-Gazlan S, Al-Ahmadi W, Alshowaier I. A, Yusuf A and Raines D. A, Expedient liquid chromatographic method with fluorescence detection for montelukast sodium in micro-samples of plasma, *J.of Chromatography B*, 2001, **754**,527–531.
10. Kitchen C. J, Wang A.Q, Musson D.G, Yang A.Y and Fisher A.L , A semi-automated 96-well protein precipitation method for the determination of montelukast in human plasma using high performance liquid chromatography/fluorescence detection ,*J.of Chromatography B,*1998, **713** ,409–414.
11. Al Omari M.M, Zoubi R.M, Hasan E.I, Khader T.Z and Badwan A.A, Effect of light and heat on the stability of montelukast in solution and in its solid state ,*J.of Pharmaceutical and Biomedical Analysis*, 2007, **45**, 465–471.
12. Ochiai H, Uchiyama N, Takano T, Harsa K and Kamei T, Determination of montelukast sodium in human plasma by columnswitching high-performance liquid chromatography withfluorescence detection, J.of Chromatography B,1998, **713**,409–414.
13. Yuliya S and Fiona R, Determination of montelukast sodium by capillary electrophoresis J.of separation science, 2008, **31**, 1137-43.
14. Sripalakit P, Kongthong B and Saraphanchotiwitthaya A, A simple bioanalytical assay for determination of montelukast in human plasma: application to a pharmacokinetic study. J.of chromatography B, Analytical technologies in the biomedical and life sciences, 2008, **869**, 38-44.
15. Chauhan B, Rani S, Nivsarkar M and Padh H, A new liquid-liquid extraction method for determination of montelukast in small volume human plasma samples using HPLC with

fluorescence detector ,Indian J.of Pharmaceutical Sciences, 2006, **68**, 517-520.

16. Smith G.A, Rawls C.M and Kunka R.L, An Automated Method for the Determination of Montelukast in Human Plasma Using Dual-Column HPLC Analysis and Peak Height Summation of the Parent Compound and Its Photodegradation Product ,Pharmaceutical Research, 2004, **21,** 1539-1544.
17. Arayne M.S ,Sultana N and Nawaz M, Simultaneous quantification of cefpirome and cetirizine or levocetirizine in pharmaceutical formulations and human plasma by RP-HPLC ,*J.of Analytical Chemistry*,2009,**63**,881-887.
18. Morita M.R, Berton D, Boldin R, Barros F.A.P, Meurer E.C, Amarante A.R, Campos D.R, Calafatti S.A, Pereira R, Abib E. Jr and Pedrazolli J. Jr, Determination of levocetirizine in human plasma by liquid chromatography–electrospray tandem mass spectrometry: Application to a bioequivalence study ,*J. of Chromatography. B*, Analytical technologies in the biomedical and life sciences, 2008, **862**,132.
19. Dhaneshwar S.R, Bhutale N. K, Mhaske D.V, Stability indicating HPLC method for the determination of levocetirizine dihydrochloride as bulk and in pharmaceutical dosage forms , *J.of Pharmacy and Pharmacology*, 2006, **58**, 99.

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