Development and Validation of a Stability-indicating HPTLC Method for Analysis of Antiasthmatic Drugs

Patel Nilam[1], Patel Shirish[1], Patel Dhara[1]

[1]Department of Pharmaceutical Science, Faculty of Pharmacy, Hemchandracharya North Gujarat University, Gujarat, India

ABSTRACT

The objective of the present work was to develop a stability-indicating HPTLC method for Montelukast sodium (MTKT) and Levocetirizine dihydrochloride (LCTZ) in the presence of its degradation products generated from forced decomposition studies. Both drugs were subjected to acid, base, peroxide, and photo degradation. Successful separation of the drugs from the degradation products formed on aluminum-backed silica gel 60 F254 with Ethyl acetate: Methanol: Ammonia (7.0:1.4:0.7 v/v/v) as the mobile phase. Densitometric analysis of was performed at 231nm in concentration range 100-1200 ng/spot with range of recovery 99.91± 0.91% for MTKT and 50-600 ng/spot with range of recovery 99.28± 0.51 % for LCTZ by the HPTLC method. Statistical analysis proved the method to be repeatable, specific, and accurate for estimation of MTKT and LCTZ. It can be used as a stability indicating method due to its effective separation of the drugs from its degradation products.

KEYWORDS

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 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.

*Address for Correspondence:
Patel Nilam K.
Faculty of Pharmacy,
Department of Pharmaceutical Sciences,
Hemchandracharya North Gujarat University,
Patan-384265, Gujarat.
nilam_pharma@rediffmail.com

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], stereoselective 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[11-14] and LCMS[15-16] 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.[17-20] International Conference on Harmonization (ICH) guidelines requires stress testing to be carried out to elucidate the inherent

stability characteristics of the active drug substance. The aim of the present work was to develop an accurate, selective, precise, robust, and stability-indicating HPTLC method for the determination of MTKT and LCTZ in the presence of its degradation products and related impurities in tablets. The proposed method was validated according to ICH guidelines[21].
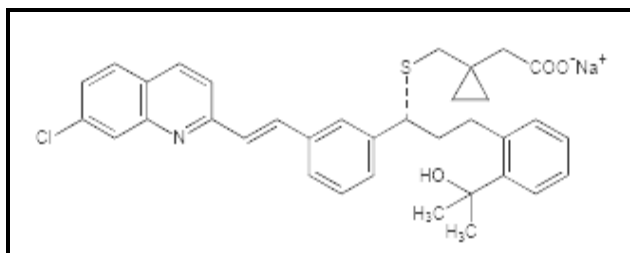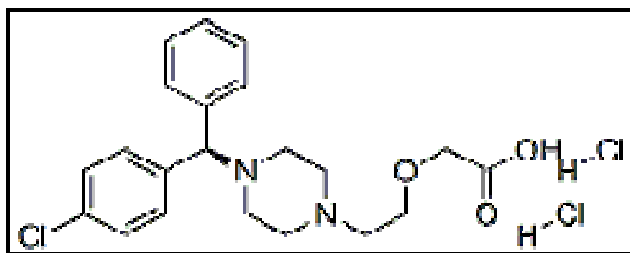

Figure: 1 Structure of Montelukast sodium

Figure: 2 Structure of Levocetirizine dihydrochloride

EXPERIMENTAL

INSTRUMENTS AND APPARATUS

A Camag HPTLC instrument consisted of Linomat V autosprayer, Scanner-III, flat bottom and twin trough developing chambers and viewing cabinet with dual wavelength UV lamps (Camag, Muttenz, Switzerland) were used. HPTLC plates used were of silica gel 60F-254, layer thickness 0.2 mm, 20X10 cm, aluminium backing (E. Merck, Darmstadt, Germany, supplied by Anchrom Technologists, Mumbai). Sartorius CP224S analytical balance (Gottingen, Germany) and ultrasonic cleaner (Frontline FS 4, Mumbai, India) were used during the research work.

CHEMICALS AND MATERIALS

Standard samples of MTKT and LCTZ were obtained as gifts from Zydus cadilla Healthcare, Ahmedabad, Gujarat, India. Combination tablet formulation containing MTKT equivalent to 10 mg and LCTZ 5 mg was procured from local pharmacy. Ethyl acetate, Methanol, Ammonia (S.D. Fine Chemicals, Mumbai, India) were used. All chemicals and reagents were of analytical reagent (AR) grade.

CHROMATOGRAPHIC CONDITIONS

Before analysis plates of silica gel 60F-254 HPTLC were cleaned by pre-development with methanol and activated at 110ºC for 5 min for solvent removal. Solutions of MTKT and LCTZ were applied to plates (10X10 cm) by means of a Linomat V automatic spotter equipped with a 100 µl syringe and operated with settings of band length, 6mm; distance between bands, 10 mm; distance from the plate edge, 10 mm; and distance from the bottom of the plate, 10 mm. The plate was developed in a twin trough chamber previously saturated for 30 min with the mobile phase, Ethyl acetate-Methanol-Ammonia 7:1.4:0.7 (v/v/v) to 8.5 cm. Densitometric scanning was performed using a Camag TLC scanner III in the reflectance-absorbance mode at 231 nm for all measurements and operated by the CATS software.

PREPARATION OF STANDARD SOLUTION

MTKT(1000 µg/ml) and LCTZ (500 µg/ml) stock were prepared by weighing accurately 10 mg MTKT and 5 mg of LCTZ powder into 2 separate 10 ml volumetric flasks; 5 ml methanol was added, shaken for a few minutes, and diluted to volume with methanol. From this solution, 1 ml of each was further diluted to 10 ml with methanol to obtain a mixed working standard solution of MTKT (100 ng/µl) and LCTZ (50 ng/µl).

METHOD VALIDATION

The HPTLC method was validated as per ICH guidelines.

Linearity

Calibration curves were plotted over the concentration range of 100-1200 ng/spot of MTKT and 50-600 ng/spot for LCTZ.

Accurately prepared mixed standard solutions of MTKT and LCTZ were applied to the plate. Each amount was applied and the plate was developed, using the previously described optimized mobile phase, and scanned. The calibration curves were constructed by plotting peak areas versus concentrations.

Accuracy (% Recovery)

The accuracy of the method was determined by calculating recoveries of MTKT and LCTZ by the standard addition method. Known amounts of standard solutions of MTKT and LCTZ were added at 80,100 and 120% levels to pre quantified sample solutions of MTKT and LCTZ. The amounts of MTKT and LCTZ were estimated by applying the obtained values to the regression equation of the calibration curve.

Method Precision (Repeatability)

Method precision for assay was established by determining the assay of six standard preparations under same conditions. Same sample solution 6 times on a plate with automatic spotter using the same syringe and by taking 6 scans of the sample spot for both MTKT (600ng/spot) and LCTZ (300 ng/spot) without changing the position of the plate. The repeatability was expressed in terms of relative standard deviation (RSD).

Intermediate Precision (Reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing mixed standard solution of MTKT and LCTZ at 3 different concentrations (100, 300 and 500 ng/spot for LCTZ; 200, 600 and 1000 ng/spot for MTKT) 3 times on the same day and on 3 different days. The results were reported in terms of relative standard deviation. (RSD).

Limit of detection and Limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the both the drugs were found by visual inspection.

Robustness

Robustness of the proposed method was estimated by changing different conditions like mobile phase composition ($\pm$ 0.2 ml for each component), mobile phase volume varied $\pm$ 3 %, scanning wavelength $\pm$ 1nm and R_f value and peak areas were measured after development of plate and % RSD was calculated. A concentration level of 300 ng per spot of LCTZ and 600 ng per spot of MTKT was employed.

Solution Stability

The stability of standard solutions was tested after 0, 6, 12, and 24 h of storage. The stability of the solutions was determined by comparing peak area percentage and peak purity at 300 ng per spot of LCTZ and 600 ng per spot of MTKT.

Specificity

The specificity of the method was ascertained by analyzing standard drugs and sample. The spots MTKT and LCTZ in samples were confirmed by comparing the R_f and spectra of the spots with that of standards.

ANALYSIS OF MTKT AND LCTZ IN TABLETS

To determine the content of MTKT and LCTZ in conventional tablets, 20 tablets were weighed, their mean weight determined and finely powdered. Powder equivalent to 10 mg of MTKT and 5 mg LCTZ were transferred into a 100 ml volumetric flask containing 20 ml methanol, sonicated for 30 min and diluted to the mark with methanol. 4 µl this solution was applied to the HPTLC plate at 200 ng per spot of LCTZ and 400 ng per spot of MTKT followed by development. Analysis was carried out in triplicate, peak areas were measured at 231 nm and sample concentrations calculated. The potential interference from excipients was also examined.

FORCED DEGRADATION OF MTKT AND LCTZ

Decomposition studies were performed in solutions containing drugs at a concentration of 0.6 mg/ml. Samples were withdrawn at suitable time intervals and subjected to HPTLC analysis. The drug was subjected under different stress conditions as follows:

(a)Acid Induced Degradation: 1 ml of the methanolic stock solution was diluted with each of 0.1 N HCl to 25 ml and was refluxed at 80ºC for 1 hr.

(b)Base Induced Degradation: 1 ml of the methanolic stock solution was diluted with each of 1.0 N NaOH to 25 ml and was refluxed at 80ºC for 10 hr.

(c)Oxidative Degradation: 1 ml of the methanolic stock solution was diluted with 3 % (v/v) of hydrogen peroxide to 25 ml. at room temperature for 18 hr

(d)Photostability: The photochemical stability of the drug was studied by exposing the drug to direct sunlight for 4 h kept on a terrace.

RESULTS AND DISCUSSION

Optimization of the chromatographic conditions

The TLC procedure was optimized with a view to develop a stability indicating assay method used for the quantification of the MTKT and LCTZ in pharmaceutical tablets. Both the pure drugs were spotted on the TLC plate and run in different solvent systems. Initially, neat solvents such as methanol, chloroform, and Ethyl acetate in different ratios were evaluated as mobile phase. Initially chloroform- methanol 4:1(v/v), chloroform: Ethyl acetate 4:1(v/v) were employed as mobile phase gave spot lacked of compactness and considerable less R_f value of MTKT. 0.45 R_f value MTKT was achieved with mobile phase Ethyl acetate: methanol 7:1.4(v/v) but less compact and diffused spot of LCTZ was observed. Sharp spot of LCTZ was obtained when Ammonia was added to this solvent system. Finally this mobile phase was optimized to achieve good peak symmetry and better separation of MTKT and LCTZ from degradation peaks obtained under different stressed conditions. The spots appeared to be more compact with a more symmetrical peak shape when TLC plates were pre-treated with methanol and activated at 110ºC for 5min. Well-defined standard spots along with its degradation products were obtained when the chamber saturation time was optimized to

30min at 25ºC temperature. Good resolution and considerable high R_f value for MTKT (0.49 ± 0.04) and for LCTZ (0.29 ± 0.02) were obtained for using optimized mobile phase Ethyl acetate: Methanol: Ammonia (7.0:1.4:0.7 v/v/v) with densitometric scanning at 231 nm (Figure 3).
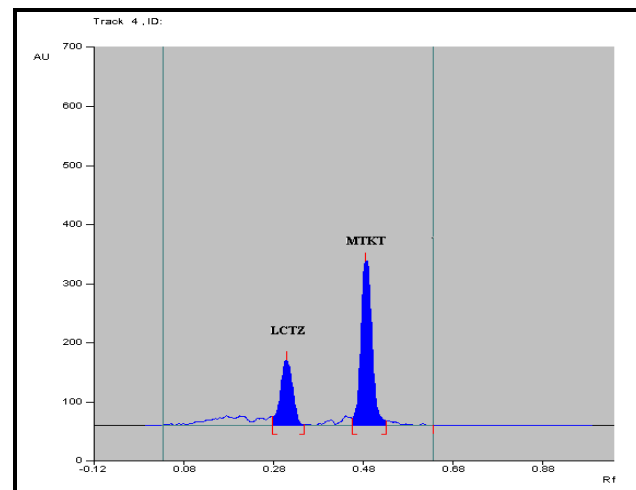

Figure: 3 A typical densitogram of standard Montelukast sodium and Levocetirizine dihydrochloride

Validation of the Proposed Methods

The developed method was validated, as described below, for various parameters like linearity and range, accuracy, precision, ruggedness, system suitability, specificity, LOQ, and LOD. Result of all validation parameters are summarized in Table 1.

Linearity and Range

The linearity curves were found to be linear over the concentration range 100-1200 ng/spot for MTKT and 50-600 ng/spot for LCTZ. A good linear relationship was observed over this range for MTKT (r^2 = 0.9950 ± 0.0051, slope =1.974 ± 0.2, intercept = 315.8± 50.3) and LCTZ (r^2 = 0.9980± 0.0051, slope = 9.686± 0.22, intercept = 302.2± 61.11).

Accuracy

Accuracy was performed by standard addition method. Mean recovery obtained for MTKT and LCTZ were 99.91 ± 0.91 % and 99.28 ± 0.51 %, respectively. The recoveries obtained by the HPTLC method for MTKT and LCTZ are shown in Table 2.

Table: 1 Summary of validation parameters of proposed HPTLC method

Parameters	LCTZ	MTKT
Range (ng/spot)	50-600	100-1200
Slope	9.686	1.974
Intercept	302.2	315.8
Correlation coefficient (r)	0.9980	0.9950
LOD (ng/spot)	20.0±0.51	40.0±0.63
LOQ (ng/spot)	50.0±1.05	100.0±1.12
%Recovery ± SD[a],(n=3)	99.28± 0.51	99.91±0.91
Repeatability of sample application (n=6)	0.42	0.34
Repeatability of sample measurement (n=6)	0.12	0.09
Interday precision (%RSD) (n = 3) at 3 range	0.29-1.64	0.60-1.39
Intraday precision (%RSD) (n = 3) at 3 range	0.23-0.35	0.22-0.50
% assay ± SD (n= 6)	98.10±1.32	99.19±0.10

[a]SD is Standard Deviation, [b]RSD is Relative Standard Deviation

Table: 2 Recovery data of MTKT and LCTZ

Drug	Amount present in formulation (µg/ml)	Amount Added (%)	% Recovery ± SD
MTKT	10	80	98.75+ 0.62
	10	100	98.83+ 0.28
	10	120	100.27+ 0.63
LCTZ	5	80	99.12+ 0.37
	5	100	101.16+ 1.10
	5	120	99.44+ 1.27

Precision

Repeatability of sample application and measurement of peak area was expressed as RSD and plates were six time scan without changing position of plate. The low values of RSD indicate that the proposed method is repeatable. The RSD value obtained for interday and intraday variation were 0.29-1.64 % and 0.23-0.35 % for MTKT and 0.60-1.39and 0.22-0.50% for LCTZ respectively, is low which indicates that proposed method is precise.

Specificity

The specificity of the method was ascertained by analyzing standard drugs and sample. The spots MTKT and LCTZ in samples were confirmed by comparing the R_f and spectra of the spots with that of standard. The peak purity of MTKT and LCTZ was assessed by comparing the spectra at three different levels, i.e., peak start (S), peak apex (M) and peak end (E) positions of the spot.

Robustness

The method was found to be robust, as small but deliberate changes in the method parameters have no detrimental effect on the method performance. The low value of relative standard deviation(less than 2%) was indicating that the method was robust.

Stability of standard and sample solutions

Stability of standard and sample solution of MTKT and LCTZ were evaluated at room temperature for 24 hr. The relative standard deviation was found below 2.0%. It showed that both standard and sample solution were stable up to 24 hr at room temperature.

LOD and LOQ

The LOD and LOQ were measured by using visual method. LOD and LOQ were 20.0 ± 0.51 and 50 ± 1.05 ng, respectively, for LCTZ and 40 ± 0.63 and 100 ± 1.12 ng, respectively, for MTKT pointed towards adequate sensitivity of the method.

Analysis of a Formulation

The proposed method was applied for the determination of MTKT and LCTZ in tablets. There was no interference from excipients commonly present in the tablets. The MTKT and LCTZ content were found to be 99.19 ± 0.1% and 98.10 ± 1.32%, 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.

Degradation Behavior of MTKT and LCTZ

Forced degradation study was carried out by subjecting the drug to acid and alkali hydrolysis, chemical oxidation and photolytic (sun light) conditions. The peaks of the degradation products were well resolved which are shown in Figure 4.The Rf of degradation products are shown in Table 3.
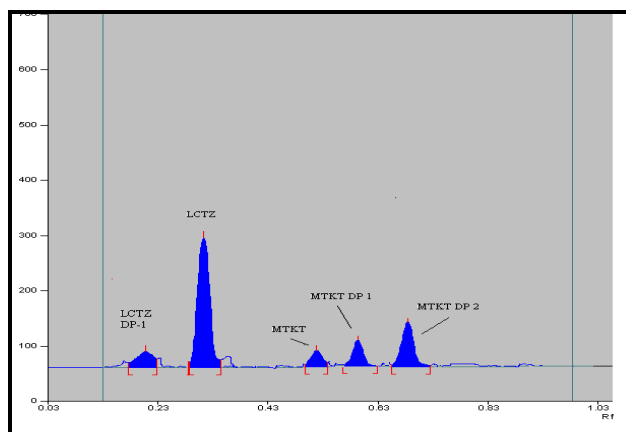

Figure: 4(a) Peaks obtained after acidic stress of Montelukast sodium and Levocetirizine dihydrochloride
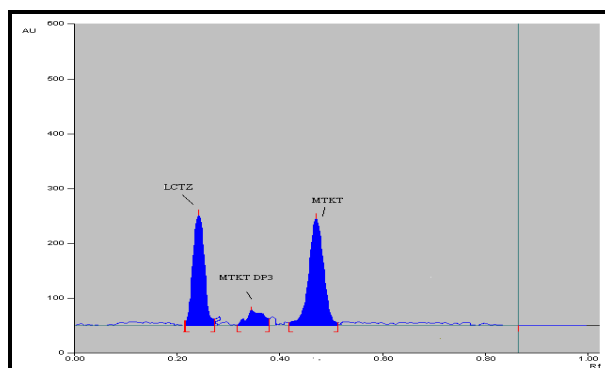

Figure: 4(b) Peaks obtained after basic stress of Montelukast sodium and Levocetirizine dihydrochloride
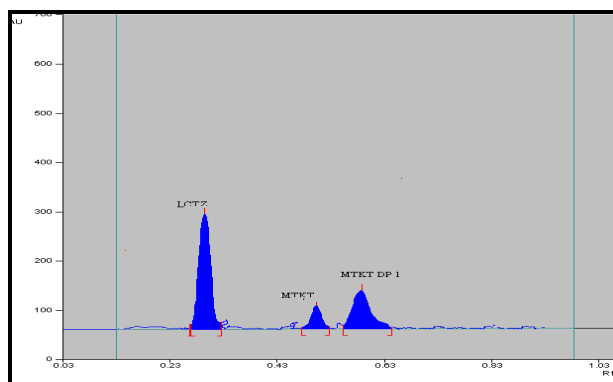

Figure: 4(c) Peaks obtained after peroxide stress of Montelukast sodium and Levocetirizine dihydrochloride
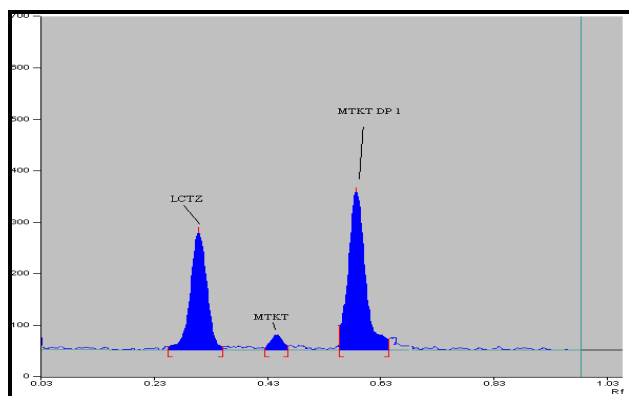
Figure: 4 (d) Peaks obtained after photolytic stress of Montelukast sodium and Levocetirizine dihydrochloride

degradation kinetics of Levocetirizine and Montelukast in biological fluids. Because the method separates the drug from its degradation products, it can be used as a stability-indicating method.

ACKNOWLEDGMENTS

Table: 3 Summary of degradation products of MTKT and LCTZ under different stress conditions

Stress condition/ duration/ state	Result for MTKT			Result for LCTZ
	Rf values of degradation products			Rf values of degradation products
	DP[c]-1	DP-2	DP-3	DP-1
Acidic/0.1NHCl/80°C/1hr/ solution	0.53	0.65	-	0.18
Alkaline/ 1N NaOH /80°C /10 hr/solution	-	-	0.36	Not Detected
Oxidative/3% H_2O_2 / RT[d]/ 18hr/solution	0.55	-	-	Not Detected
Sunlight/4hr/solution	0.56	-	-	Not Detected
[c]DP is Degradation Product, [d]RT is Room Temperature				

CONCLUSION

This HPTLC method is precise, specific, accurate, and stability indicating. Statistical analysis proved the method is repeatable and selective for the analysis of Levocetirizine and Montelukast as bulk drug and in pharmaceutical formulations. The method can be used to determine the purity of the drug obtained from different sources by detecting related impurities. It may be extended to determination of the

REFERENCES

1. Budavari S, editor, The Merck Index. 12th ed., Whitehouse Station, NJ: Merck & Co Inc, 1996. 1070.

2. Sweetman, S.C., Martindale, the Extra pharmacopoeia, 34th ed., London, Pharmaceutical press., 2004, 435.3, 11.

3. Indian Pharmacopoeia,Vol. II, Government of India, The controller of Publication, New

Delhi., 2007, 2, 1290.

4. Al-Rawithi S, Al-Gazlan S, Al-Ahmadi W, Alshowaier I, Yusuf A, Raines D, "Expedient liquid chromatographic method with fluorescence detection for Montelukast sodium in microsamples of plasma", J Chromatogram B Biomed Sci Appl., 2001, 754, 527–531.

5. Ochiai H, Uchiyama N, Takano T, Hara K, Kamei T. "Determination of Montelukast sodium in human plasma by column-switching high performance liquid chromatography with fluorescence detection", J Chromatogram B Biomed Appl., 1998,713, 409–414.

6. Chauhan B, Shubha Rani, Nivsarkar M, Padh H, "New liquid liquid extraction method for determination for Montelukast in small volume human plasma samples using HPLC with fluorescence detector", Indian J Pharm Sci., 2006, 68, 517–520.

7. Liu L, Cheng H, Zhao JJ, Rogers JD, Determination of Montelukast (MK-0476) and S-enantiomer in human plasma by stereoselective high performance liquid chromatography with column switching, J Pharm Biomed Anal., 1997, 15, 631–638

8. Radhakrishna T, Narasaraju A, Ramakrishna M, Satyanarayana A, "Simultaneous determination of Montelukast and Loratadine by HPLC and derivative Spectrophotometric methods", J Pharm Biomed Anal., 2003, 31, 359–68.

9. Alsarra I, "Development of a stability-indicating HPLC method for the determination of Montelukast in tablets and human plasma and its applications to pharmacokinetic and stability studies", Saudi Pharm J., 2004, 12, 136–143.

10. A.F.M. El Walily, et al. "Spectrophotometric and high performance liquid chromatographic determination of cetirizine dihydrochloride in pharmaceutical tablets", J. Pharmaceut. Biomed. Anal.1998, 17, 435–442.

11. Sevgi K., et al., "Development and validation of a rapid RP-HPLC method for the determination of cetirizine or fexofenadine with pseudoephedrine in binary pharmaceutical dosage forms" J. Pharmaceut. Biomed. Anal., 2008, 46, 295-302

12. Sun Ok Choi., et al. "Stereoselective determination of cetirizine and studies on pharmacokinetics in rat plasma." Journal of Chromatography B., 2000, 744, 201–206.

13. Mee-Kyung Kima., et al. "Narrow-bore high performance liquid chromatographic method for the determination of cetirizine in human plasma using column switching" J.Pharmaceut.Biomed.Anal., 2005, 37, 603–609.

14. Jabera A.M.Y., et al. "Determination of cetirizine dihydrochloride, related impurities and preservatives in oral solution and tablet dosage forms using HPLC", J.Pharmaceut.Biomed.Anal., 2004, 36, 341–350.

15. Melanie M.T., et al, "Automated 96-well solid phase extraction and hydrophilic interaction liquid chromatography–tandem mass spectrometric method for the analysis of cetirizine in human plasma—with emphasis on method ruggedness",Journal of Chromatography B, 2005, 814, 105–111.

16. Mortia M.R, et al., "Determination of Levocetirizine in human plasma by liquid chromatography–electrospray tandem mass spectrometry: Application to a bioequivalence study", Journal of chromatography B, 2008, 862, 132-139.

17. Sharma Smita, Sharma M. C, Kohli D.V, Sharma A.D. "Development and Validation of TLC Densitometry Method for Simultaneous Quantification of Montelukast Sodium and Levocetirizine dihydrochloride Pharmaceutical Solid Dosage Form", Scholars Research Library, Der Pharmacia Lettre, 2010, 2(1), 489-494.

18. Choudhari V, Kale A, Abnawe S , Kuchekar B , Gawli V ,Patil N, "Simultaneous Deter-

-mination of Montelukast Sodium and Levocetirizine dihydrochloride in Pharmaceutical Preparations by Ratio Derivative Spectroscopy", IJPRIF, 2010, 2(1), 04-09.

19. Rathore Atul S, Sathiyanarayanan L and Mahadik K.R. "Development of Validated HPLC and HPTLC Methods for Simultaneous Determination of Levocetrizine dihydrochloride and Montelukast Sodium in Bulk Drug and Pharmaceutical Dosage Form" Pharmaceutica Analytica Acta, 1(1), 100-106.

20. Ashokkumar S, Raja Senthil M, Perumal P. "RPHPLC Method Development and Validation for Simultaneous Estimation of Montelukast sodium and Levocetirizine dihydrochloride", International Journal of Pharmaceutical Research, 2009, 1(4), 8-12.

21. "Topic Q2A Validation of Analytical Procedure: Methodology", International Conference on Harmonization (ICH), Geneva, Switzerland 1994.