Simultaneous Estimation of Montelukast Sodium and Fexofenadine HCL in Pharmaceutical Formulation by RP-LC-PDA

Anirudha R .Chabukswar*,Vishnu P. Choudhari, Swati C Jagdale, Shialesh N. Sharma, Nilesh A. Bari, Bharat D Pagare.

*Maharashtra Institute of Pharmacy, MIT Campus, Paud Road, Kothrud, Pune, 411038, MS, India; E-Mail: shaileshsharma321@gmail.com.

ABSTRACT:

High resolution RP-HPLC method has been developed for the simultaneous estimation of Montelukast Sodium and Fexofenadine HCL in pharmaceutical products. Separation was achieved by using a Kromasil C_{18}, 250 × 4.6 mm, 5 µm column with a the column temperature is 50°C, and detector wavelength is 241 nm gradient mobile phase composed of sol-A: Water, sol-B: Acetonitrile and Methanol(50:50) was used at the flow rate 1ml/min, The retention time of Montelukast sodium &, Fexofenadine HCL 3.306 & 2.214mins, respectively. The method is validated and shown to be linear. The correlation coefficients for Montelukast Sodium and Fexofenadine HCL are 0.999 and 0.999, respectively. The recovery values for Montelukast and Fexofenadine HCL ranged from 99.12–99.24% and 99.09–99.59%, respectively. The relative standard deviation for six replicates is always less than 2%. The developed method has wide applicable for the quantification of Montelukast and Fexofenadine in pharmaceutical dosage forms.

KEYWORDS: Montelukast and Fexofenadine HCL, Isocratic, RP-HPLC-PDA

INTRODUCTION

Montelukast(MON), is a specific cysteinyl leukotriene receptor antagonist belonging 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] propylsulfanylmethyl] cyclo-propyl] acetic acid sodium salt. It is developed as a therapeutic agent for the treatment of bronchial asthma by Merck and Co.(1) (Fig 1), A few UV Spectrophotometric and HPLC methods have been reported individually or in combination with other drugs for estimation of Montelukast.[2-11] Fexofenadine HCL (FEXO) is Fexofenadine α, α-dimethyl-4-[1- hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl] butyl] benzeneneacetic acid, is the most important terfenadine metabolite, prevents allergic inflammation[12]. It is nonsedating and does not decrease performance even in extremely high doses (Fig 2)[13]. There are various reports based on the evaluation of Fexofenadine by HPLC method in different dosage forms individually and with or in combination with other drugs.[14-17] To our knowledge there is no HPLC method reported for the combination, availability of an HPLC method with high sensitivity and selectivity will be very useful for the estimation of MON and FEXO in combined pharmaceutical dosage forms. Therefore the aim of the study was to develop a sensitive, precise, accurate and specific HPLC method for the determination of MON and FEXO simultaneously in formulation. The present work describes a simple reverse phase LC method for the determination of MON and FEXO in tablets. During present study efforts were directed towards use of mobile phase without salt to increase column life. The method was validated according to ICH guidelines

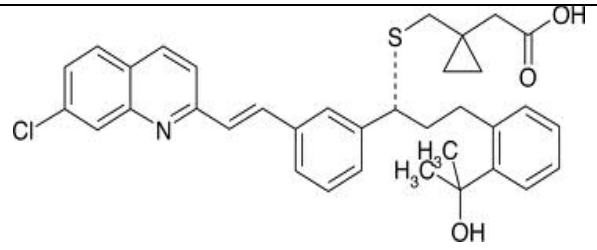

Fig.1structure of montelukast

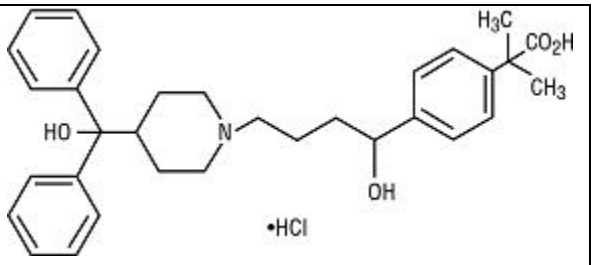

Fig.2 structure of Fexofenadine HCL

MATERIALS AND METHODS

Chemicals:
MON (purity, 99.92%) and FEXO (purity, 99.93%) were obtained from Cipla Pvt. Ltd. Mumbai, Methanol (HPLC grade) was purchased from E. Merck (India) Ltd, Worli, Mumbai, India. Double distilled water was used throughout the experiment. Tablets were purchased from Indian market, containing MON 10 mg and FEXO 120 mg each per tablet. (Tablet MONTAIR-FX^{TM}, Lot: T0027, Cipla Pvt. Ltd. (Mumbai).

Instrumentation and chromatographic conditions:
The HPLC system consisted of a binary pump (model Waters 515 HPLC pump), auto sampler (model 717 plus Auto sampler), column heater, and PDA detector (Waters 2998). Data collection and analysis were performed using Empower- version 2 software. Separation was achieved on Kromasil C–18 (250 mm × 4.6 mm, 5.0 µ) columns maintained at 50^0C using column oven. Isocratic elution with Acetonitrile(ACN)/ methanol (MeOH)/water mobile phase(44:44:12) at the flow rate of 1 mL/min was carried out. The column was supported with waters kromasil C–18, (3.9×20mm, 5.0 µ) guard column. The detection was monitored at 241 nm and injection volume was 10 µL. The peak purity was checked with the photodiode array detector.

Preparation of Standard and Sample solutions and calibration graphs:
The stock solution of montelukast Sodium (MON) and fexofenadine HCL (FEXO) was prepared separately by dissolving accurately weighed 50 mg in 50 ml of methanol to obtain a final concentration of 1000.0 µg/ml. From this stock solution, standards within a 0.6-120 µg/ml and 0.05-10 µg/ml concentration range were prepared for FEXO and MON, respectively and were injected on to the column. A calibration curve was plotted as concentration of drugs versus peak area response. It was found to be linear for both the analytes. From the standard stock solution, a mixed standard solution was prepared containing 2µg/ml of MON and 24 µg/ml of FEXO and was used for system suitability study.

Method validation:
The HPLC method was validated in terms of precision, accuracy and linearity according to ICH guidelines. Assay method precision was determined using nine-independent test solutions. The intermediate precision of the assay method was also evaluated. Assay method was evaluated with the recovery of the standards from excipients. Three different quantities (low, medium and high) of the authentic standards were added to pre analyzed tablet powder. The mixtures were extracted as described in Section 2.3, and were analyzed using the developed HPLC method. Linearity test solutions were prepared as described in Section 2.3. The LOD and LOQ for analytes were estimated by injecting a series of dilute solutions with known concentration. To determine the robustness of the method, the final experimental conditions were purposely altered and the results were examined. The flow rate was varied by (±) 0.1 mL/min. Column temperature was varied by (±) 2^0C and effect of column from different suppliers was studied. Measurement wavelength was varied by (±) 1nm. The stability of the drug solution was determined using the samples for short-term stability by keeping at room temperature for 12 h and then analyzing. The long-term stability was determined by storing at 4^0C for 30 days.

RESULTS AND DISCUSSION

1.Optimization of the chromatographic conditions:

Mobile phase containing water and methanol was initially used. In this view water with methanol of different ratios was tried as a mobile phase at a different pH (3-7) and ratios were tried along with change in column temperatures. All the times peak shape was not proper and that for FEXO retention time was also too long (around 20 min). Mobile phase containing 25 % ACN was tried. In this mobile phase both drugs showed the development with reduced tailing. Two columns were used for performance investigations, including Kromasil C_{18} (5 micron 4.6 × 250 mm) and QuMONil C_8 (5 micron 4.6 × 250 mm), the first column was the most suitable one since it produced symmetrical peaks with high resolution. To improve the quality of peak and to elute MON earlier, 25% ACN was used in the MeOH(25%) and Water(50%), pH of which adjusted to around 3 – 4 was used, which showed reduced tailing and baseline disturbances when column was maintained at 45^o C. Now, mobile phase ACN: MeOH: Water (44: 44: 12 v/v) pH adjusted to 3 with OPA and column temperature 50^{o}C shown good resolution, peak shape and desired elution. Flow rate was set to 1 ml/min and UV detection was carried out at 241 nm. Chromatogram showed symmetrical peaks with good shapes; tailing factor for MON and FEXO was within range and the resolution of the standard drugs was satisfactory. Retention time of MON was 3.0727 min and that of FEXO was 4.106 min. Ultimately mobile phase consisting of ACN: MeOH: Water (44: 44: 12 v/v) pH 3 and 1 ml/min flow rate was selected for validation and short term stability studies. The mobile phase and sample solutions were filtered using 0.45 µ membrane filter and was degassed by ultrasonication for 15 min prior to use.

2. Validation of method

2.1 Specificity

The specificity of the HPLC method shows where complete separation of MON and FEXO was noticed in presence of tablet placebo (Fig 3A). In addition there was no any interference at the retention time of MON and FEXO in the chromatogram of tablet solution. In peak purity analysis with photo diode array detector, purity angle was always less than purity threshold for all the analytes. This shows that the peak of analytes was pure and excipients in the formulation did not interfere to the analytes.

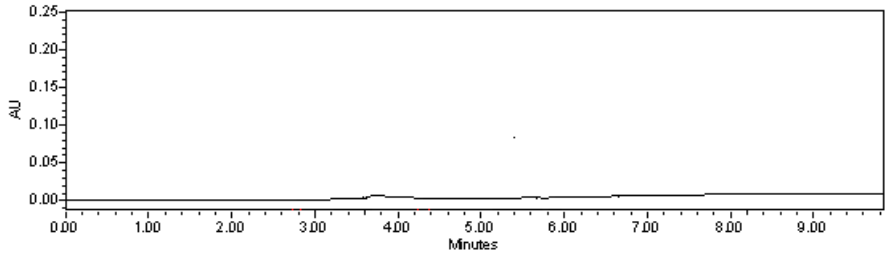

Fig no .3(A) Representative chromatograms obtained for the mobile phase

(B)

(C) for mobile phase and for formulation.

(D

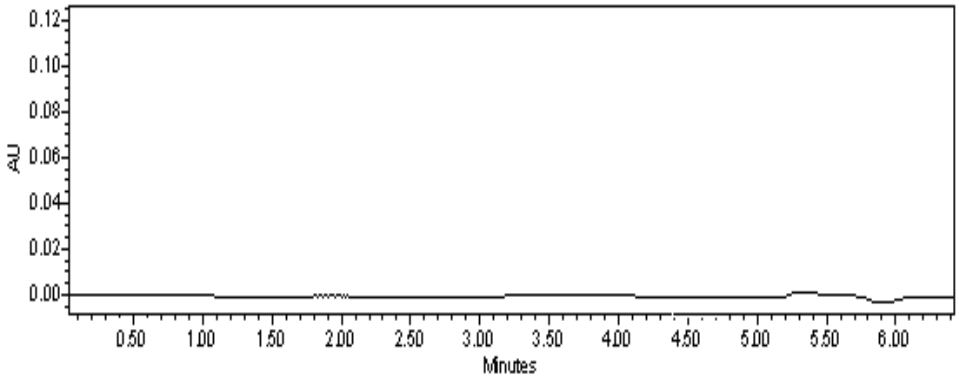

Fig no .3(B)- Representative chromatograms obtained for the placebo

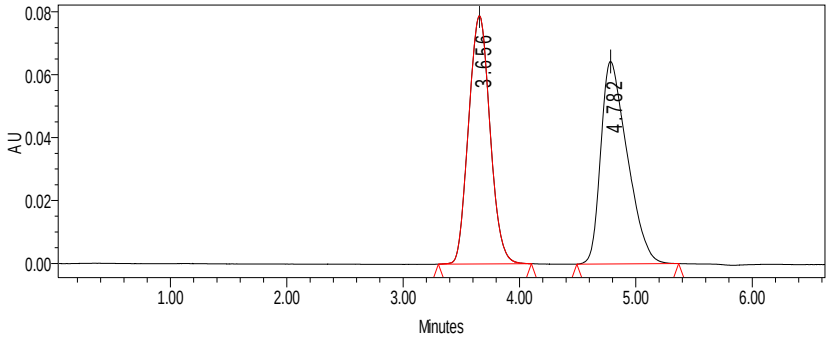

Fig no .3(C)- Representative chromatograms obtained for formulation

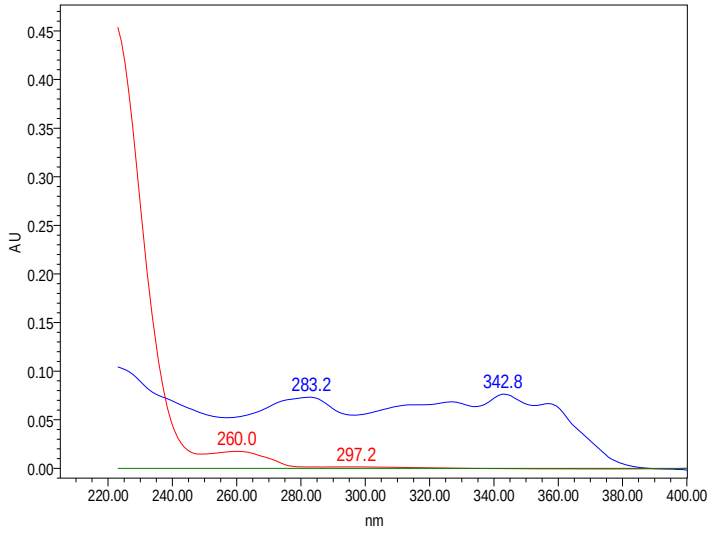

Fig no .3(D) Overlay uv spectra of MON& FEX

2.2 Precision and Accuracy

Precision:

The intra-day precision of the developed LC method was determined by preparing the tablet samples of the same batch in nine determinations with three concentrations and three replicate each on same day. The inter-day precision was also determined by assaying the tablets in triplicate per day for consecutive 3 days. The result obtained for Intra day and Inter day variations are shown for MON and FEXO in Table I& II respectively.

MON	**Measured concentration (µg/ml), % R.S.D**	
Conc. (µg/ml)	**Intra day**	**Inter day**
1	1.023,0.39	1.09,1.35
2	2.01, 0.75	2.22,0.75
6	6.33,0.45	6.12, 0.27

Table 1: Intraday and Inter day precision of MON (n=3)

FEXO	**Measured concentration (µg/ml), % R.S.D**	
Conc. (µg/ml)	**Intra day**	**Inter day**
12	12.19,0.26	12.41, 0.19
24	24.05, 0.44	24.12, 0.94
72	72.53,0.76	72.04,1.11

Table 2: Intraday and Inter day precision of FEXO (n=3)

Accuracy:

The accuracy of the method and recovery experiment was carried out. A known quantity of the pure drug was added to the placebo sample at the level of 25% to 150% of the test concentration. The recoveries of the drug product were determined and mean recoveries were in the range of 98.0-102.0 % which shows that there is no interference from excipients. Table-7 represents the recovery results. The results obtained are shown in Table III

SR NO	Active IngredientName	Spike level Average % Recovery						Average % Recovery
		25%	50%	75%	100%	125%	150%	
1	MONTELUKAST SODIUM	99.62	99.12	99.44	98.47	99.66	99.15	99.24%
2	FEXOFENADINE HCL	99.32	99.35	99.65	99.09	100.44	99.7	99.59

2.3 Linearity and Range

For the construction of calibration curves, seven calibration standard solutions were prepared over the concentration range. Linearity was determined for MON and FEXO in the range of 0.05-10µg/mL and 0.6-120 µg/mL. The correlation coefficient ('R^2') values were >0.9998(n = 6). Typically, the regression equations for the calibration curve was found to be Y = 386964X +65053 the correlation coefficient R^2 = 0.999 for MON(Fig 4) and Y=12727X+97637 and the correlation coefficient R^2 = 0.999 for FEXO(Fig 5).

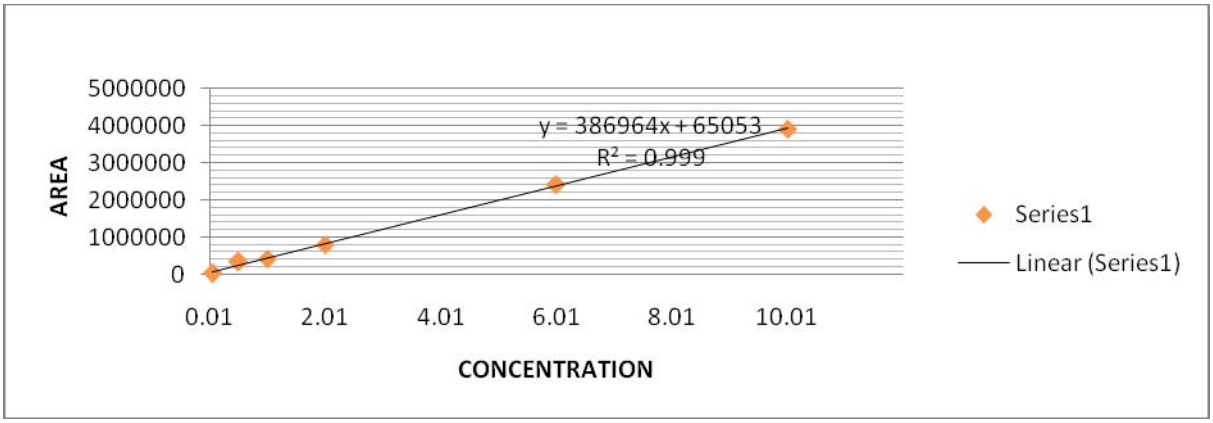

Fig no 4-Calibration curve for montelukast sodium

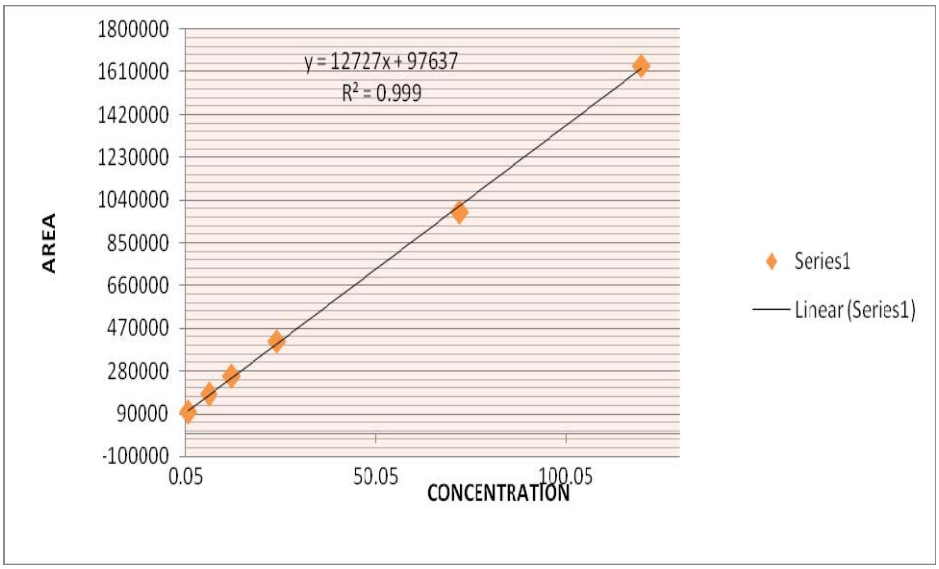

Fig no 5-Calibration curve for fexofenadine HCL

2.4 Sensitivity

LOD and LOQ for the procedure were performed on samples containing very low concentrations of analytes based on calibration curve method. Solutions of MON and FEXO were prepared in the range of 0.6-120µg/ml and injected in triplicate. Average peak area of three analyses was plotted against concentration. LOD and LOQ were calculated by using following equations.

LOD = (3.3 ×Syx)/b LOQ= (10.0 ×Syx)/b

Where Syx is residual variance due to regression; b is slope. The LOD and LOQ values were found to be 0.6, 0.094 µg/mL and 0.89, 0.028 µg/mL for MON and FEXO respectively.

2.5 Stability

Solution stability as described in method validation under experimental section was studied. Result of short-term, long-term, and the auto sampler stability of the MON and FEXO solutions were calculated form nominal concentrations and found concentration. Results of the stability studies were within the acceptable limit (98–102%).

2.7 Robustness

Robustness of the method was investigated under a variety of conditions including changes of flow rate, column oven temperature, column form different suppliers and wavelength of measurement. The mixed standard solution is injected in five replicates and sample solution of 100% concentration is prepared and injected in triplicate for every condition and % R.S.D. of assay was calculated for each condition. The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters has proven that the method is robust (Table IV)

Factor	Level	Mean % assay(n=3), % RSD of results	
		Montelukast	Fexofenadine HCL
Flow rate (mL/min)	1.05	100.04, 0.29	99.97, 0.06
	0.95	98.78, 0.24	100.16, 0.22
Column oven temperature (^{0}C)	50	100.41, 0.49	99.98, 0.24
	40	98.54, 0.58	100.05, 0.77
Separation Column	Column I[a]	100.6, 0.92	100.12, 1.29
	Column II[b]	99.9, 0.83	100.04, 0.94
Measurement wavelength (nm)	241.5	99.45, 0.53	100.15, 0.77
	238.5	99.8, 0.47	100.30, 0.28

CONCLUSIONS

A simple, specific, linear, precise, and accurate RP-HPLC-PDA method has been developed and validated for quantitative determination of Montelukast and Fexofenadine HCL in their binary mixture is carried out. All the parameters for the drugs met the criteria of ICH guidelines for method validation. The method is very simple and specific as all peaks are well separated and there is no interference by excipients peaks with total runtime of 8 min, which makes it especially suitable for routine quality control analysis work. The method can be used for individual analysis of the titled drugs or their binary combinations.

REFERENCES

[1] Schoors DF, Smet MD, Reiss T, Margolskee D, Cheng H, Larson P, *et al*. ,Single dose pharmacokinetics, safety and tolerability of MK-0476: A new leukotriene D4-receptor antagonist, in healthy volunteers. ,Br J Clin Pharmacol 1995;40:277-80.

[2] Al-Rawithi S, AL-Gazlan U, Al-Ahmadi W, Alshowaier I, Yusuf A, Dale A. Raines expedient liquid chromatographic method with fluorescence detector for Montelukast sodium in micro samples of plasma. J Chromatogr 2001;7543:527-31.

[3] Chester J, Kitchen I, Amy Q, Wang I, Musson DG, Yang AY, *et al.* A semi-automated 96-well protein precipitation method for the determination of montelukast in human plasma using high performance liquid chromatography/fluorescence detection. J Pharm Biomed Anal 2003;31:647-54.

[4] Robert PA, Luka P, Mulletta WM, Elizabeth K. A rapid and sensitive method for the quantization of montelukast in sheep plasma using liquid chromatography/tandem mass spectrometry. J Chromatogr 2007;858:282-6.

[5] Chauhan B, Rani S, Nivsarkar M, 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 Pharm Sci 2006;68:517-20.

[6] Hong TH, Sharma R, Sustanto D, DI Maso M, Kwong E. The microwave assisted extraction (MAE) technique has been evaluated for the extraction of active pharmaceutical ingredients (API) from various solid dosage forms. J Chromatogr A 2007;1156:149-53.

[7] Hoang TH, Farkas R, Wells C, McClintock S, Maso MD. Application of pressurized liquid extraction technology to pharmaceutical solid dosage form analysis. J Chromatogr A 2002;968:277-61.

[8] Liu L, Cheng H, Zhao J, Roger D. Determination of Montelukast (MK0476) and its enantiomer separation in human plasma by stereo selective HPLC with column-switching. J Pharm Biomed Anal 1997;15:631-8.

[9] Ochiai H, Uchiyama N, Takano T, Hara KI, Kamei T. Determination of Montelukast sodium in human plasma by column-switching HPLC with fluorescence detector. J Chrom B Biomed Sci Appl 1998;713-2:409-14.

[10] 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.
[11] See MM. Thompson R, Grinberg N, Perpall HJ, Bicker G, Tway P. Chromatographic analysis of residual acetate in bulk drugs. J Liquid Chromatogr 1995;18:137-54.
[12] Gelfand, E.W., Cui, Z.H., Takeda, K., Kanehiro, A. and Joetham, A. (2002). Fexofenadine modulates T-cell function, preventing allergen-induced airwayinflammation and hyperresponsiveness. *J.Allergy Clin. Immunol.*, 110(1): 85-95.
[13] Sussman, G.L., Mason, J., Compton, D.,Stewart, J. and Ricard, N. (1999). The efficacy and safety of fexofenadine HCl and pseudoephedrine, alone and in combination, in seasonal allergic rhinitis *J. Allergy Clin. Immunol.*, 104(1): 100-106.
[14] 14) Karakus, S., Kucukguzel, I. and Kucukguzel, S.G. (2008). Development and validation of a rapid RP- HPLC method for the determination of cetirizine or fexofenadine with pseudoephedrine in binary pharmaceutical dosage forms. J. Pharm. Biomed. Anal., 46(2): 295-302.
[15] Hofmann, U., Seiler, M., Drescher, S. an Fromm, M.F. (2002). Determination of fexofenadine in human plasma and urine by liquid chromatography- mass spectrometry. J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci., 766(2): 227-233.
[16] Mikus, P., Valaskova, I. and Havranek, E. (2005). Determination of fexofenadine in tablets by capillary electrophoresis in free solution and in solution with cyclodextrins as analyte carriers. *Drug Dev. Ind. Pharm.*, 31(8): 795-801.
[17] Yamane, N., Tozuka, Z., Sugiyama, Y., Tanimoto, T., Yamazaki, A. and Kumagai, Y. (2007). Microdose clinical trial: Quantitative determination of fexofenadine in human plasma using liquid chromatography/electrospray ionization tandem mass spectrometry. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, 858(1-2): 118-128.
[18] Kullai Reddy Ulavapalli, J. Sriramulu, Useni Reddy Mallu and Varaprasad Bobbarala.(2011). Simultaneous determination of psuedoephedrine, fexofenadine and loratadine in pharmaceuticalproducts using high resolution RP-HPLC method. Journal of Pharmacy Research., 4(4),1219-1221