Int J Med Res., 2015; 1(8): 411-418

IJMBR

International Journal of Medicobiological Research

(An International Peer Review Journal)

Journal homepage: *www.ijmedres.co.in*; ISSN: 0976-8971

Full Length Research Article

A Systematic Development of RP-HPLC Method for the Quantification of Montelukast Sodium in combination with an Antihistamine Drug

Vikas Wakchaure, *Dhananjay Mane*✍, Tukaram Sawant

[1]*Department of chemistry, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, Maharashtra-431004, India,*

✍**Corresponding author: *dvmane11@gmail.com***

Article information

Keywords:
RP-LC method,
Assay Method
Montelukast sodium
Fexofenadine Hydrochloride
Anti-histamine Drug

Received on : 01.11.2015
Revised on : 18.12.2015
Accepted on : 25.12.2015

ABSTRACT

A Simple, Selective, rugged, sensitive Isocratic RP-HPLC assay method has been developed for the quantitative determination of an antihistamine drug and Montelukast sodium as combination dosage. The developed method is applicable for the estimation and quantification of Assay. An Efficient chromatographic separation was achieved on a Phenyl column with simple mobile phase combination delivered in an isocratic mode and quantification was carried out using Ultraviolet detection at 220 nm wavelength. In the developed RP-HPLC method the known degradants are well separated and it was found to be selective for the assay analysis of drug contents in finished dosage form. Regression analysis shows r^2 value (correlation coefficient) greater than 0.999 for both the drugs. This method was capable of detecting both the drugs from 10% with respect to the test concentration of 240 µg/mL and 20 µg/mL of Antihistamine drug and Montelukast Sodium respectively for a 15 µL injection volume. The method has extraction capability from 10% 160% and consistent recoveries for Antihistamine drug and Montelukast Sodium (98.9% - 101.0%) and (98.7% - 101.0%) respectively.

1. INTRODUCTION

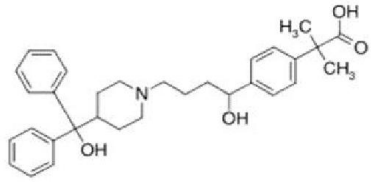
The Pharmaceutical analysis in medicinal field plays an important role in drug development, manufacture and its intended use. Many of drugs and drug formulations introduced for the treatment of critical disease and introduced into the market by pharmaceutical industries. Maximum drugs are given as the combination therapy drugs from all marketed drugs. So, it is necessary to identify category, possibilities of the drugs can arranged in such manner that medicament should have one with two active ingredients which reduces the complications and side effect due to placebo. To quantify the drug content from its dosages form Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) method was chosen. The developed method is sensitive, rugged, Simple and also reproducible in combinations.

A Novel drug in combination of Antihistamine drug with Montelukast Sodium has chosen for the development.Montelukast Sodium[1,2], 1-[(1R)-1-[3-[(1E)-2-(7-chloro-2-quinolinyl)ethenyl]phenyl]-3-[2- (1-hydroxy-1-methyl ethyl) phenyl] propyl] thio] methyl] cyclo propaneacetic acid (Figure. 1) is a white crystalline powder, soluble in water, alcohol, dimethyl formamide, and dimethyl sulfoxide. MLS is a selective leukotriene D_4 receptor antagonist that is used as an antiasthmatic. Fexofenadine Hydrochloride[3,4] (FFH) selected as antihistamine drug and combinations are available in the form of solid dosages. α,α-dimethyl -4- [1-hydroxy -4- [4-(hydroxyl diphenyl methyl)-1-piperidinyl] butyl]-benzeneacetic acid (Figure. 1), is a white crystalline powder, slightly soluble in water and soluble in alcohol. FFH is an anti-histamine H1 receptor antagonist. It is available in the ratio of (FFH:MLS) is 12:1.

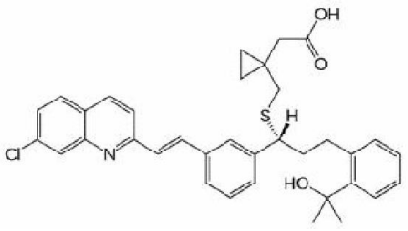
MLS and FFH combination tablet is available in the Indian market. According to literature survey, there are many analytical methods have been published and reported for estimation and determination of MLS by Reverse Phase High Performance Liquid Chromatography (RP-HPLC)[5-6] Photodegradation Kinetics [7],Spctrophometric methods,[8-9] Estimation by radioactive spectroscopy,[11] Voltametric determination [12] determination in Human plasma by HPLC coupled with ESI-MS/MS.[13-15] There are few analytical methods are available for the quantification of MLS and other possible formulations by HPLC.[10,16- 27] Estimationof FFH in

bulk drugs and finished dosages by RP-HPLC,[28-30] spectrophotometric determination,[31-33] dissolution method by HPLC.[34-35,57] Kinetic spectrometric estimation [36], HPLC with Fluorescence detection from human plasma [37], estimation in human serum and human plasma [38-40], HPLC/MS in human plasma [41]and Ion Chromatographic Method.[42] In combination with other formulations.[43,44] but there are very few method are available as a single method which was reported for simultaneous estimation of both the two drugs by RP-HPLC in combined dosage form.[21, 45-54]

In pharmaceutical drugs, Stability testing plays an integral part in drug product development in the manufacturing of pharmaceutical industry and market which has been consumed by human. The intended purpose of conducting stability testing study is to have an evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental conditions, such as temperature, humidity, and light; enabling recommendation of storage conditions, retests periods, and shelf lives to be established. The major two aspects of drug product that plays a major role in shelf-life determination are assay or purity of active ingredients, and degradants or impurities generated, during the stability study. The purity of drug product in stability test sample needs to be determined using stability-indicating method, as recommended by the International Conference on Harmonization (ICH) guidelines[55] and United States Pharmacopoeia (USP).[56] Even though stability-indicating assay methods have been reported for various drugs in drug products, maximum of them describe assay procedures for drug products containing one active drug substance. Very few stability-indicating methods are reported for assay of combination drug products containing two actives or more active drug substances. The main objective of this work was to develop an analytical procedure, which would prove that drug product which will be quantified using a stability-indicating method in combination drug product of MLS and FFH.There are few stability-indicating methods has been reported for determination of MLS and FFH. This manuscript describes the development and subsequent validation of a stability-indicating isocratic RP- HPLC method for simultaneous determination of MLS and FFH selectivity is proved in the presence of its known related compounds and unknown degradants. To establish the stability-indicating method, stress study (forced degradation) of drug substances and drug product was performed under different stress conditions (thermal, photolytic, UV exposure, acid and basic hydrolytic and oxidative) and stressed samples were analysed using the proposed method. The proposed method is having the capability to separate both the drugs from all the degradants which was found during stress study.



a) Fexofenadine Hydrochloride



b) Montelukast Sodium

Figure 1. Structures of Drugs

2. INSTRUMENTATION

2.1. High performance liquid chromatography

A waters alliance 2690 separation module with 998 photodiode array detectors used, the system was controlled and acquired data processed by the Chromeleon 7.0 version Software.

2.2. Chromatographic conditions

Chromatographic separation was achieved on 5µm Phenyl column, 250 x 4.6mm, (Phenomenax LUNA, USA) with a mobile phase containing 350 volumes of 0.04M of Sodium phosphate monobasic and 650 volumes of acetonitrile.Flow were maintained 2.0mL per minute, and column Temperature was maintained as 35ºC. Detection Wavelength were selected as 220nm and total run time as 8 minutes.

2.3. Standard and Sample Solution Preparation

i) Standard Solution Preparation

Weighed 120 mg of Fexofenadine Hydrochloride and 10 mg of Montelukast sodium standard and transferred it in to 100 ml volumetric flask, added 70 mL of diluent and dissolved and final volume made up to the mark with diluent. Pipetted out 10 mL of this solution and diluted to 50 mL with diluent.

ii) Test Solution Preparation

Weighed and taken 10 tablets and transferred it in to 500 mL volumetric flask, added 350mL of diluent added and then Sonicated 20 minutes with intermittent shaking. Final volume made up to the mark with diluent. Centrifuged the solution at 4000 RPM for 10 minutes. Further pipetted out 5mL of the supernatant solution and diluted to 50 mL with diluent.

2.4. Specificity and forced degradation studies

The specificityor stress study of a method is its suitability for analysis of a substance in the presence of known and unknown potential impurities. Stress testing of a drug substance can help to identify degradation products, using which one can establish degradation path ways and the inherent stability of the drug molecule. It can also be used to validate the stability indicating capacity of the analytical procedures used.

All stress studies were performed with 240 µg/mL of FFH and 20 µg/mL of MLS sample at 45°C. Acid hydrolysis was performed in 1N HCl for 45 Minutes. The study in basic solutionwas carried out in 1N NaOH for 30 minutes. Oxidation studies carried out in 3% $H_2O_2$for 30 Minutes.Photo

degradation carried out according to option 2 of Q1B in ICH guideline [28]The drug powder were exposed to heat for 2 days at 105°C.Samples withdrawn at the appropriate time analysis were performed by HPLC and peak purity was checked using PDA detector and results tabulated in table 1 and chromatograms of degradation study shown in figure 3.

2.5. Analytical Method Validation

As per ICH guidelines the method was validated in terms of following parameters.[55-56]

2.5.1. Precision

The precision determination of the impurities were checked by injecting six individual preparation of (240 µg/mL of FFH and 20 µg/mL of MLS) with 0.2% of all impurities and calculating % RSD of area for each compound. The ruggedness of the method also evaluated using different analyst, different instrument and different day in the same laboratory.

2.5.2. Linearity

Linearity solutions for the impurities were prepared by diluting the Linearity stock solution to the required concentration. The solution was prepared from 10% to 300% with respect to the Individual drug concentration in test solution. The calibration curve wasdrawn by plotting the area of response of impurities against the corresponding concentration. The slope, y-intercept, and regression coefficient of the calibration curve was calculated; as per table 2.

2.5.3. Accuracy

Accuracy determination of impurities was carried out in triplicate at 10%, 20%, 60%, 100 %, and 160 % of FFH and MLSconcentration 240 µg/mL and 20 µg/mL respectively. The % recovery for the impurities and % RSD was calculated as per table 3.

2.5.4. Robustness

The robustness of the method for the chromatographic conditions was purposely altered and the system suitability criteria were monitored and evaluated like, % RSD, retention time of both the actives, Number of theoretical plates, asymmetry of the peak. Flow rate was changed by ±0.2 units, pH of the buffer ±10%, column oven temperature were studied at ±5°C, and organic variation for mobile phase at ±10 %. In all the above condition components of the mobile phase were held constant.

2.5.5. Solution stability and mobile phase stability

A solution stability of FFH and MLS were carried out by a sample solution in a tightly capped volumetric flask at room temperature for 72 hours and refrigerator condition (2-8°C).Mobile phase stability carried out in room temperature about 72 Hours and found stable and no extraneous matter as present and system suitability criteria were met like, % RSD, retention time of both the actives, Number of theoretical plates, asymmetry of the peak.

3. RESULTS AND DISCUSSION

3.1. Optimization of chromatographic conditions

The method was developed to separate major degradation product for under various stress conditions. The main target of the chromatographic method is to get the separation for closely eluting impurities and degradants. The Sodium phosphate buffer selected. Since, the both the drug contents were quantified individually using monobasic phosphate buffer. The pH 2.5 and 4.5 was adjusted with diluted Ortho-phosphoric Acid. Then, pH was adjusted to 6.5 for dibasic phosphate buffer.

In all the above conditions the FFH Impurities were getting merged with MLS peak and so the pH slightly adjusted to 3.5.The FFH impurities were separated from FFH after addition of Sodium perchlorate. Then, the peak shape of the FFH was getting broad when we used 10mM phosphate buffer and Sodium perchlorate buffer. By increasing the buffer concentration the peak shape was observed symmetry. So, the finalised chromatographic conditions were optimised in phenyl column, 250mm, 4.6mm and 5 micron.
The chromatograms of optimised chromatographic condition are presented in figure 2.

Table 1. Stress testing (forced degradation data)

Type of Stress	Peak Purity		% Degradation
	FFH	MLS	
Acid Degradation	990	995	2.231
Base Degradation	990	994	3.602
Peroxide Degradation	990	999	9.021
Heat degradation	990	995	7.130
Sunlight Degradation	990	990	3.112
UV Degradation	990	990	3.235

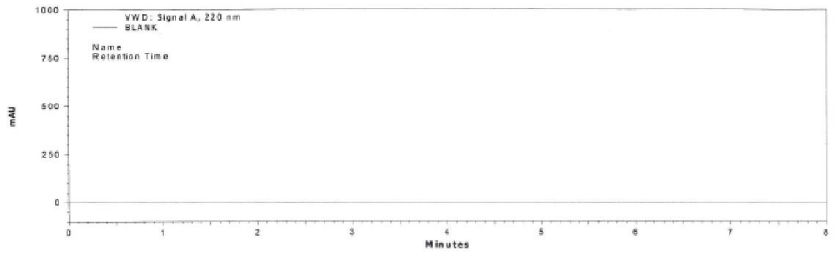
3.2. Discussion for analytical method validation

The diluent injected as a blank and no interference were observed in the retention time of FFH and MLS. The forced degradation study is carried out in hydrolysis (acid and base), Oxidation, thermal degradation and photolytic degradation has been performed and peak purity were found to be more than 0.999 as per the below table 1. There is no interference were observed in different stress condition and the % degradation were reported in table 1.

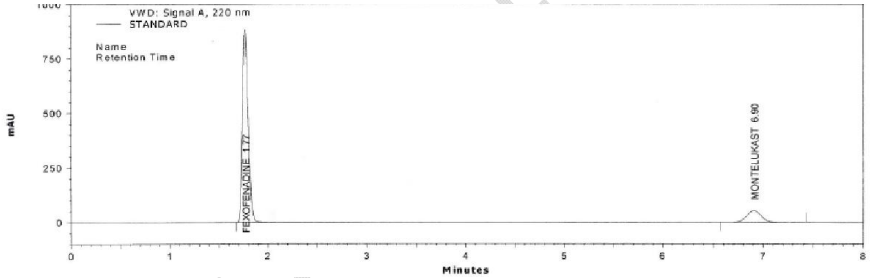
The precision study (system precision, repeatability, and ruggedness) were performed and found six replicate injections % RSD FFH and MLS were observed as1.2% and 1.1% respectively. The ruggedness study was performed with different instrument (Agilent 1260 series with multi wavelengthdetector with Chromeleon 7.0 version Software) and different day; the % difference was observed with Precision data of FFH and MLS are 2.5% and 2.2% respectively.

Figure. 1

i) Blank



ii) Standard Solution



iii) Test Solution

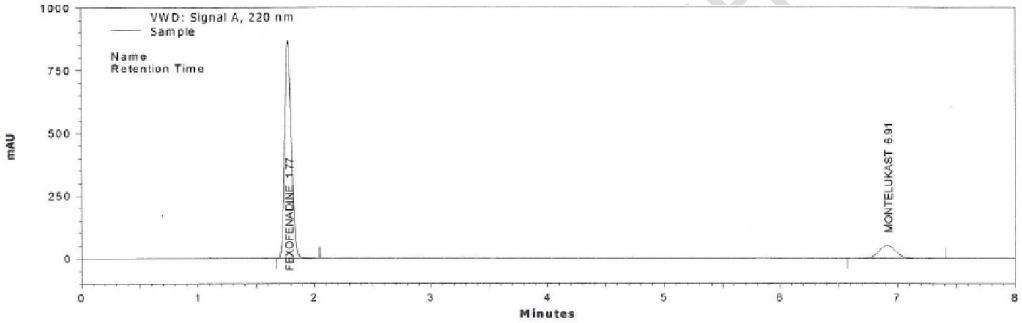
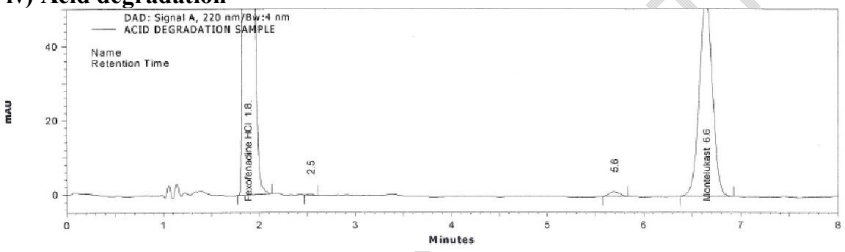
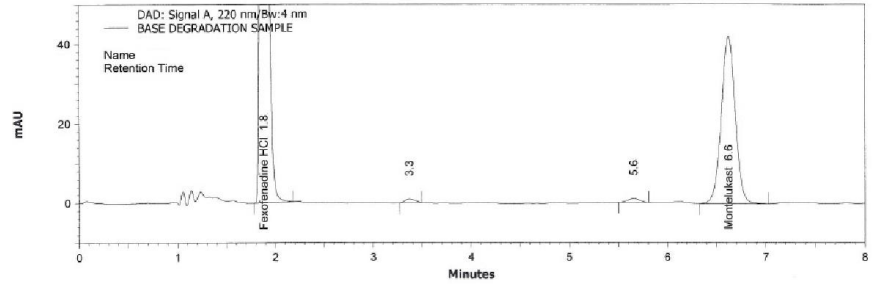


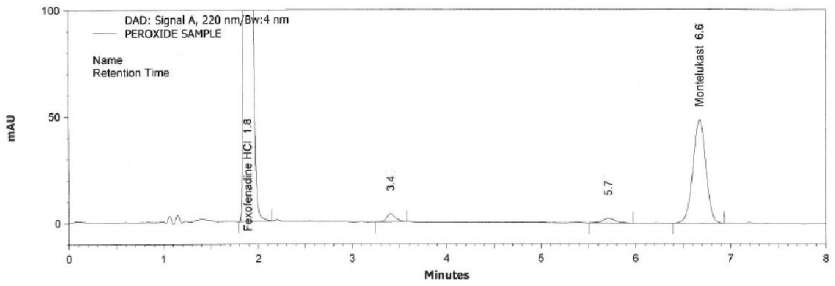
Figure. 2

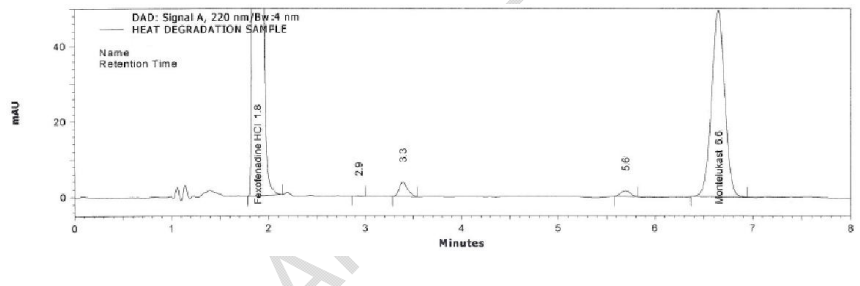
iv) Acid degradation



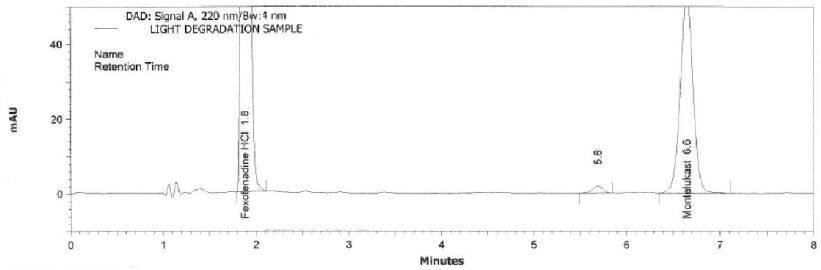
v) Base degradation with known impurities







viii) Sunlight Stress



ix) UV light Degradation

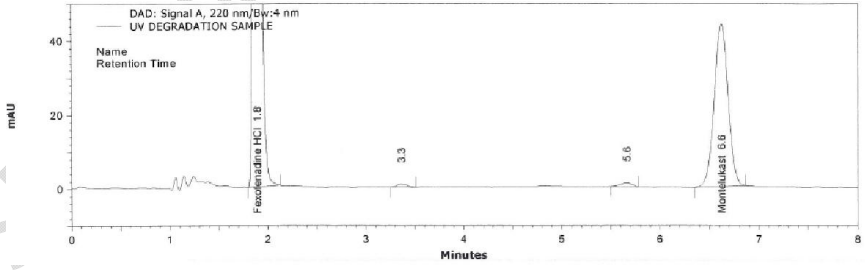


Table 2. Linearity

Concentration In µg/mL		Correlation Coefficient (r)		Coefficient of regression (m)		Constant of Regression (C)	
FFH	**MLS**	**FFH**	**MLS**	**FFH**	**MLS**	**FFH**	**MLS**
12.1	1.02						
24.3	2.04						
48.48	4.08						
121.21	10.2	1.000	1.000	4022.269	7204.077	10156.4	59.7
242.4	20.4						
363.6	30.6						

Table 3. Accuracy

% Level	% Recovery of FFH	% Recovery of MLS
10% Level	98.9	98.8
20% Level	99.4	98.7
60% Level	100.6	99.4
100% Level	101.0	100.7
160% Level	99.9	101.0

The linearity has been established in the range of 10% to 160% of both the drugs FFH and MLS respectively; the correlation coefficient was found to be more than 0.999. The Accuracy of FFH and MLS level, the % recovery was found as per table 3.The robustness study performed deliberate changes in chromatographic conditions as per listed analytical method validation, the system suitability parameters were found to be within the acceptance criteria.

4. CONCLUSION

The lower in % relative standard deviation for replicate preparations indicates that the method is precise. The value of % recovery is approximately in limit of 97% to 103%, which indicates that the method can be used for estimation of these two drugs in combined dosage forms without any interference due to the other components present in the formulations. There is no interference due to placebo mix, diluent, mobile phase, and degradation impurities. Hence, this study presents simple, accurate, precise and selective for the quantification of stability indication Assay method of two drugs in combined dosage form by RP-HPLC.

5. ACKNOWLEDGEMENTS

The authors wish thank to Wockhardt Pharmaceuticals Private Limited, Aurangabad, for providing infrastructure and facilities.

6. REFERENCES

[1] https://www.nlm.nih.gov/medlineplus/druginfo/meds/a600014.html
[2] http://www.drugs.com/cons/montelukast.html
[3] http://www.drugs.com/cdi/fexofenadine.html
[4] https://www.nlm.nih.gov/medlineplus/druginfo/meds/a697035.html
[5] Alsarra, I.A. (2004). 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 Pharma J.,*12:136-43.
[6] Raju, K.N., Swamy, T.G., and A.L. Rao. (2011). Development and validation of RP-HPLC method for the determination of montelukast sodium in bulk and in pharmaceutical formulation. *Int J Pharm ChemBiol Sci.,* 1:12-6.
[7] Roman, J., Breier, A.R., and M. Steppe. (2011). Stability indicating LC method to determination of sodium montelukast in pharmaceutical dosage form and its photodegradation kinetics. *J Chromatogr Sci.,* 49:540-6.
[8] Garg, L.K., Kumar, B.R., Sait, S.S., and T. Krishnamurthy. (2011). Determination of montelukast sodium in oral granules dosage forms by a simple and accurate UV spectrophotometric methods. *Int J Pharm Sci Rev Res.,* 7:69-72.
[9] Patel, N.K., and S.S. Pancholi. (2011). Spectrophotometric determination of montelukast sodium and levocetirizinedihydrochloride in tablet dosage form by AUC curve method. *Der Pharm Chem.,* 3:135-40.
[10] Radhakrishna, T., Narasaraju, A., Ramakrishna, M., A. Satyanarayana. (2003). Simultaneous determination of montelukast and loratadine by HPLC and derivative spectrophotometric methods. *J Pharm Biomed Anal.,* 31:359-68.
[11] Choudhari, V., Kale, A., Abnawe, S., Kuchekar, B., Gawli, V., and N. Patil. (2010). Simultaneous determination of montelukast sodium and levocetirizinedihydrochloride in pharmaceutical preparations by ratio derivative spectroscopy. *Int J Pharm Tech Res.,* 2:4-9.
[12] Alsarra, A., Al-Omar, M., Gadkariem, E.A., and F.Belal.(2005).Voltammetric determination of montelukast sodium in dosage forms and human plasma. *Farmaco.,* 60:563-7.
[13] Arayne, M.S., Sultana, N., and F. Hussain. (2009). Spectrophotometric method for quantitative determination of montelukast in bulk, pharmaceutical formulations and human serum. *J Anal Chem.,* 64:690-5.
[14] Challa, B.R., Awen, B.Z., Chandu, B.R., Khagga, M., and C.B. Kotthapalli.(2010). Method development and validation of montelukast in human plasma by]: Application to a bioequivalence study. *Sci Pharm.,* 78:411-22.
[15] Schoors, D.F., Smet, M.D., Reiss, T., Margolskee, D., Cheng, H., and P. Larson P. Single dose pharmacokinetics, safety and tolerability of MK-0476: A new leukotriene D4-receptor antagonist, in healthy volunteers. *Br J ClinPharmacol.,* 40:277-80.
[16] Kommana Bala Ram Kumar, Girija Sastry.V, G. Komala, PreethiPriyadarshini and P. Nares (2013). A Novel stability indicating Chromatographic method for the Simultaneous estimation of Levocetrizine and Montelukast in the combined dosage form by RP-HPLC. Int. J. Chem. Pharm. Sci., Vol 1(1):80-93.
[17] Patil, S., Pore, Y.V., Kuchekar, B.S., and V.G. Khire. (2009). Determination of montelukast sodium and bambuterol hydrochloride in tablets using RP HPLC. *Indian J Pharm Sci.,* 71:58-61.
[18] Eswarudu, M.M., Junapudi, S., and T.N. Chary.(2011). RP-HPLC method development and validation for simultaneous estimation of montelukast sodium and levocetirizine dihydrochloride in tablet dosage form. *Int J Pharm World Res.,* 2:145-6.
[19] Rathore, A.S., Sathiyanarayanan, L., and K.R. Mahadik. (2010). Development of validated HPLC and HPTLC methods for simultaneous determination of levocetirizine dihydrochloride and montelukast sodium in bulk drug and pharmaceutical dosage form. *Pharm Anal Acta.,*1:1-6.
[20] Rote, A., and V. Niphade. (2011). Determination of montelukast sodium and levocetirizine dihydrochloride in

combined tablet dosage form by HPTLC and first-derivative spectrophotometry. *J Liq Chromatogr Relat Technol.,* 34:155-67.

[21] HJ Vekaria, KS Muralikrishna and GF Patel (2011). Development and Validation of Spectrophotometric method for estimation of Fexofenadine HCL and Montelukast Sodium in Combined dosage form. Inventi Journals, Issue 4.

[22] Pourghazi, K., Khoshhesab, Z.M., Golpayeganizadeh, A., Shapouri, M.R., and H. Afrouzi. (2011). Spectrophotometric determination of cetirizine and montelukast in prepared formulations. *Int J Pharm Pharm Sci.,*3:128-30.

[23] Patel, D.J., Patel, S.A., and S.K. Patel. (2010). Simultaneous determination of montelukast sodium and bambuterol hydrochloride in tablet dosage form by ultraviolet spectrophotometry (Dual wavelength method). *Int J Pharm Biol Res.,* 1:71-5.

[24] Patel, S.A., Patel, D.J., and N. J. Patel. (2011). Simultaneous spectrophotometric determination of montelukast sodium and bambuterol hydrochloride in tablets. *Int Res J Pharm.,* 2:154-8.

[25] Patel, S.A. (2011). Development and validation of spectrophotometric method for simultaneous determination of montelukast sodium and bambuterol hydrochloride in tablets. *Int J Pharm Biomed Res.,* 2:112-118.

[26] K. Chouhan, A. Gaur, V. Lawaniya, M.P. Singh, (2014) Method Development and its Validation for Simultaneous Estimation of Montelukast sodium and Olopatadine hydrochloride as API and in tablet dosage form by UV spectroscopy, International Journal of Pharmaceutical Research and Bio-science, Volume 3(2): 1009-1018.

[27] Singh, R.R., and M.V. Rathnam. (2012). A stability indicating RPHPLC method for the estimation of Montelukast Sodium and Fexofenadine hydrochloride in pharmaceutical preparations. *Int J Pharm Pharm Sci.,* 4:587-93.

[28] Sharaf, El-Din, M.K., Ibrahim, F., Eid, M.I., and M.E. Wahba. (2011). Validated stability indicating liquid chromatographic method for the determination of fexofenadine hydrochloride in presence of its degradation products. Application to tablets and content uniformity testing. *J Pharm Res.,* 4:2377-80.

[29] Radhakrishna, T., and O.G. Reddy. (2002). Simultaneous determination of fexofenadine and its related compounds by HPLC. *J Pharm Biomed Anal.,* 29:681-90.

[30] Maher, H.M., Sultan, M.A., and I.V. Olah. (2011). Development of validated stability-indicating chromatographic method for the determination of fexofenadine hydrochloride and its related impurities in pharmaceutical tablets. *Chem Cent J.,* 5:76-86.

[31] Kumar, K.S., Ravichandran, V., Mohan Maruga Raja, M.K, Thyagu, R., and A. Dharamsi. (2006). Spectrophotometric Determination of Fexofenadine hydrochloride. *Indian J Pharm Sci.,* 68:841-2.

[32] Polawar, P.V., Shivhare, U.D., Bhusari, K.P., and V.B. Mathur. (2008). Development and validation of spectrophotometric method of analysis for fexofenadine HCl. *Res J Pharm Tech.,*1:539-41.

[33] Narayana, B., and K. Veena. (2010). A new method for the spectrophotometric determination of fexofenadine hydrochloride. *Indian J Chem Tech.,*17:386-90.

[34] Oliveira, D.C., Weigch, A., and C.M. Rolim. (2007). Simple and reliable HPLC analysis of fexofenadine hydrochloride in tablets and its application to dissolution studies. *Pharmazie.,*62:96-100.

[35] Zafar, F., Shoaib, M.H., and R.I.Yousuf. (2011). Development of RP-HPLC method for fexofenadine determination in tablet formulations and development of dissolution method. *Pak J Pharm.,* 28:43-9.

[36] Patel, P.G., Vaghela, V.M., Rathi, S.G., Rajgor, N.B., and V.H. Bhaskar. (2009). Derivative spectrophotometry method for simultaneous estimation of rupatadine and montelukast in their combined dosage form. *J Young Pharm.,* 1:354-8.

[37] Uno, T., Yasui-Furukori, N., Takahata, T., Sugawara, K., and T. Tateishi. (2004). Liquid chromatographic determination of fexofenadine in human plasma with fluorescence detection. *J Pharm Biomed Anal.,* 35:937-42.

[38] Arayne, M.S., Sultana, N., Shehnaz, H., and A. Haider. (2011). RP-HPLC method for the quantitative determination of fexofenadine hydrochloride in coated tablets and human serum. *Med Chem Res.,*20:55-61.

[39] Miura, M., Uno, T., Tateishi, T., and T. Suzuki. (2007). Determination of fexofenadine enantiomers in human plasma with high-performance liquid chromatography. *J Pharm Biomed Anal.,* 43:741-5.

[40] Jain, D.K., Kumar, A.M., and D. Jain. Quantitative determination of fexofenadine in human plasma by UPLC. *Indian J Pharm Educ Res.,*44:295-300.

[41] Isleyen, E.A., Ozden, T., Ozilhan, S., and S. Toptan. (2007). Quantitative determination of fexofenadine in human plasma by HPLC-MS. *Chromatographia.,* 66:109-13.

[42] Srinivas, L.D., Kumar, P.R., and B.S. Sastry. (2005). Ion association methods for the determination of fexofenadine in pharmaceutical preparations. *E-J Chem.,* 2:199-202.

[43] Karakus, S., and I. Kucukguzel. (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.,* 46:295-302.

[44] Saeed, A.M., Najma, S., Zeeshan, M.A., and S.F. Ahmed. (2010). Simultaneous determination of gliquidone, fexofenadine, buclizine, and levocetirizine in dosage formulation and human serum by RP-HPLC. *J ChromatogrSci.,* 48:382-5.

[45] Mona Pankhaniya, Parula Patel, JS Shah (2013), Stability-indicating HPLC method for simultaneous determination of montelukast and fexofenadine hydrochloride, Indian Journal of Pharmaceutical Sciences, Volume : 75 (3) 284-290.

[46] Hitesh Vekaria · VipulLimbasiya · Piyush Patel (2013), Development and validation of RP-HPLC method for simultaneous estimation of Montelukast Sodium and Fexofenadine hydrochloride in combined dosage form,Journal of Pharmacy Research; 6(1):134–139.

[47] M. KalyankarTukaram, R. Wale Risha and B. KakdeRajendra (2013), Development and Validation of RP-HPLC Method for Estimation of Montelukast Sodium and Fexofenadine Hydrochloride in Pharmaceutical Preparations, ChemSci Trans.,2(3), pp 889-899.

[48] Yakkala Lakshmi Manasa, Naidu Srinivasa Rao, MeghanaReddy.Regalla, (2013), Method Development and Validation for Simultaneous Estimation of Fexofenadine HCl and Montelukast Sodium by RP-HPLC in Pure and Combined Tablet Dosage form, Volume 2, Issue 6, 5948-5965.

[49] Ravisankar M, SubasiniUthirapathy, AnandaThangadurai, JambulingamMunusamy and KamalakananDhanpal (2012). Simultaneous Estimation of Fexofenadine Hydrochloride and Montelukast Sodium in bulk drug and marketed formulation by RP-HPLC method, International Research journal of Pharmacy, 3(4).

[50] Anuruddha R Chabukswar, Vishnu P. Choudhari, Shaliesh N. Sharma, Nilesh A. Bari and DhavalGhuge (2012). Ratio derivative Spectrophotometry method for Simultaneous estimation of Montelukast and Fexofenadine HCL in their combined dosage form. Asian J. Research Chem. 5(5).

[51] K. Prashanth Kumar, M. AkifulHaque, T. Pradeep Kumar, G. Nivedita, S. Hasan Amrohi, V.V.L.N. Prasad and Prakash V. Diwan (2012). Simultaneous determination of Fexofenadine HCL and Montelukast Sodium in Combined dosage form by using RPHPLC method. World J. Chem., 7(2):42-46.

[52] Rajeevkumar R. Singh and Manapragada V. Rathnam (2012). A Stability indicating RP-HPLC method for the estimation of Montelukast Sodium and Fexofenadine HCL in Pharmaceutical Prepareations. Int J Pharm Sci, Vol 4, Issue 2, 587-593.

[53] Mounikagodavarthi, K. Sujana and A. Pramila rani. Method development and Validation for Simultaneous determination of Fexofenadine HCL and Montelukast Sodium using RP-HPLC. IOSR Journal of Pharmacy. 2012, Vol 2 Issue 5, pp. 41-48.

[54] Eldin, A.B., Shalaby, A.A., and M. El-Tohamy. (2011). Development and validation of a HPLC method for the determination of montelukast and its degradation products in pharmaceutical formulation using an experimental design. *Acta Pharm Sci.,* 53:45-56.

[55] ICH harmonized tripartite guideline, stability testing of new drug substances and products. Q1A (R2) 2003 Feb;1–15.Rockville, MD: United States Pharmacopoeial Convention Inc; 2003. USP 33, The United States Pharmacopoeia, 26th Rev; p. 1151.Singh RM, Mathur, 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.,* (2010). 72:235–37.

[56] Stability testing of new drug substances and products. Q1A (R2) 2003 Feb; 1–15.Rockville, MD: United States Pharmacopoeial Convention Inc; 2003. USP 33, The United States Pharmacopoeia, 26th Rev; p. 1151.

[57] Kozan, I., Palabiyik, I.M., Karacan, E., and F. Onur. (2008). Spectrophotometric and high performance liquid chromatographic determination of fexofenadine hydrochloride in pharmaceutical formulations. *Turk J Pharm Sci.,* 5:175-89.