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Research Article

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Validated Stability-Indicating Isocratic RP-HPLC Method of Estimation of Montelukast Sodium and Fexofenadine Hydrochloride in Bulk and in Solid Dosage by Vieordt's Method

Rajeev Kumar P[1*] and Rekha Rajeev Kumar[2]

[1]Department of Pharmaceutics, School of Pharmacy, PRIST University, Chennai, Tamil Nadu, India
[2]Department of Pharmaceutical Analysis, School of Pharmacy, PRIST University, Chennai, Tamil Nadu, India

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ABSTRACT

A simple, speedy, precise, and accurate, stability-indicating reversed phase high performance liquid chromatographic method was developed and validated for simultaneous (Vieordt's method) determination of montelukast sodium (MTK) and fexofenadine hydrochloride (FXD). The sample injection volume was 20 µl and the quantification was attained by UV-VISIBLE detector at 248 nm. The chromatographic separation was achieved on X bridge C_{18}, 250 × 4.6 mm, 5 µm particle column, using an isocratic mobile phase comprising of acetonitrile: buffer (10 mM potassium dihydrogen phosphate solutions): methanol of pH 4.5 and in the ratio of 50:30:20 v/v/v at a flow rate of 1.5 ml/ min. The retention times for montelukast sodium and fexofenadine hydrochloride were found to be 3.62 min and 7.43 min, respectively. The drugs were exposed to thermal, photolytic, hydrolytic, and oxidative stress conditions, and the stressed samples were analyzed by the suggested method. Validation of the method was done as per International Conference on Harmonization (ICH) guidelines. Linearity was established for montelukast sodium and fexofenadine hydrochloride in the range of 0.020-0.100 mg/ml and 0.016-0.064 mg/ml, respectively. The limits of detection were 0.04 µg/ml and 0.07 µg/ml, respectively and the LOQ value 0.11 µg/ml and 0.023 µg/ml, for montelukast sodium and fexofenadine hydrochloride respectively. The method was found to be specific and stability-indicating as no interfering peaks of degradants and excipients were perceived. The proposed method is hence suitable for use in quality-control laboratories for quantitative analysis of both the drugs bulk and in combination, since it is simple and fast with good accuracy and precision.

Keywords: Montelukast sodium (MTK); Fexofenadine hydrochloride (FXD); Reversed-phase HPLC; Stability-indicating assay; Forced degradation studies; Method validation

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INTRODUCTION

Montelukast sodium (MTK), 1-[({(R)-m-[(E)-2-(7-chloro-2-quinolyl) vinyl]-α-[o-(1-hydroxyl-1-methylethyl) phenethyl] benzyl}thio) methyl] cyclopropaneacetate sodium[1,2] (Figure 1a). Montelukast (trade name Singulair) is a leukotriene receptor antagonist (LTRA) used for the maintenance treatment of asthma and to relieve symptoms of seasonal allergies. It is usually administered orally. Leukotriene receptor antagonist (LTRA) used for the maintenance treatment of asthma and to relieve symptoms of seasonal allergies [3]. Fexofenadine hydrochloride (FXD) [4-6] (Allegra, Telfast, Fastofen, Tilfur, Vifas, Telfexo, Allerfexo) is an antihistamine drug used in the cure of hay fever and similar allergy symptoms. It was developed as a successor of and substitute to terfenadine (brand names include Triludan and Seldane), an antihistamine with potentially serious contraindications. α, α-dimethyl-4-[1-hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl] butyl] benzeneneacetic acid, is the most important

terfenadine metabolite shown in Figure 1b. H_1-receptor antagonist recommended in patients with allergic rhinitis [7]. Fexofenadine hydrochloride, like other second and third-generation antihistamines, does not freely cross the blood-brain barrier, and so causes less drowsiness than first-generation histamine-receptor antagonists. It works by being an antagonist to the H_1 receptor. It has been termed as both second-generation and third-generation [3,8]. To the best of our knowledge no method is reported in literature for simultaneous determination of Montelukast sodium and Fexofenadine by high performance liquid chromatography (HPLC) using the specified chromatographic condition. The survey of literature showed few UV Spectrophotometric [9], HPLC [10-13], LC-ESI–MS/MS method [14] are available for the estimation of Montelukast sodium in pharmaceutical preparation and in biological fluids. Literature survey also showed that available for the estimation of UV spectrophotometric [15-17] for the estimation of Fexofenadine in pharmaceutical preparation. The objective of the present work was to develop and validate a simple, economic, rapid, precise, gradient and accurate stability-indicating method with good sensitivity for simultaneous determination of montelukast sodium and fexofenadine hydrochloride in accordance with ICH guidelines [18]. The proposed method was successfully applied to a solid dosage form of montelukast sodium and fexofenadine hydrochloride and analyzed in presence of commonly used tablet excipients. This method can also be employed for quality control during manufacture of drug product.

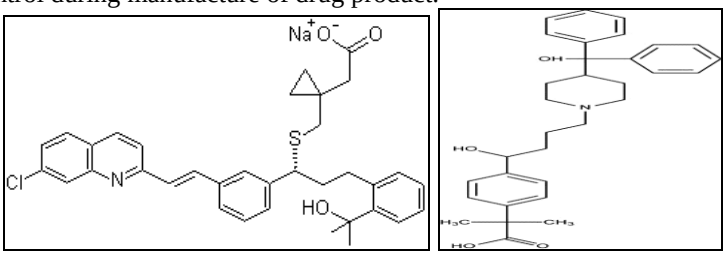

Figure 1: a) Structure of MTK; b) Structure of FXD

MATERIALS AND METHODS

Chemicals, Reagents, and Solutions

Montelukast sodium and fexofenadine were gift sample from Ami Life science, Baroda, Gujarat. HPLC grade acetonitrile, methanol and analytical grade potassium dihydrogen ortho phosphate, ortho phosphoric acid, triethylamine was obtained from S.D. Fine chemicals Ltd. (Mumbai, India). Hydrochloric acid, sodium hydroxide pellets and 3% v/v hydrogen peroxide solution were obtained from Ranbaxy Fine Chemicals, New Delhi (India). High purity water was prepared by Millipore Milli-Q plus purification system (Millipore, Bedford, USA).

HPLC Instrumentation and Chromatographic Conditions

The chromatographic system used to perform development and validation of this assay method was comprised of a Isocratic (LC 20AT) pump, UV-VISIBLE [SPD 20A] DETECTOR and a rheodyne manual injector with 20 µL loop, Column X bridge C_{18}, 250 × 4.6 mm, 5 µm particle connected to a multi-instrument data acquisition and data processing system. The flow rate of the mobile phase was 1.5 mL/min. The sample injection volume was 20 µL and the quantification was achieved by UV-VISIBLE (SPD 20A) detector at 248 nm. The isocratic program applied was carried out.

Mobile Phase Preparation

Mobile phase consisted of A, B and C. Mobile phase A and C was HPLC grade acetonitrile and methanol. Mobile phase B composed of buffer solution of 0.2 M potassium dihydrogen orthophosphate in 0.1 M phosphoric acid adjusted with triethylamine (0.5%) to pH 4.5. The above solution was filtered through 0.45 µm nylon filter. Finally the mobile phase A, B and C was mixed in the ratio of acetonitrile: buffer: methanol solutions of pH 4.5 and in the ratio of 50:30:20 v/v/v.

Standard Preparation

Accurately weighed and transferred 10 mg of montelukast and 120 mg of fexofenadine working standards into two separate 100 ml volumetric flask. Add 70 ml of mobile phase and sonicated for 10 minutes and diluted to volume with mobile phase. Further 1 ml of montelukast sodium and 1 ml of Fexofenadine standard stock solution was transferred into 100 ml volumetric flask and made up to the mark with mobile phase. The concentration obtained

was 10 µg/ml of montelukast sodium and 12 µg/ml of fexofenadine. The above solution was filtered through a 0.45 µ nylon membrane filtered.

Test Preparation

Twenty tablets were weighed and the average weight of tablet was determined. The tablets were powdered and accurately weighed quantity of montelukast sodium equivalent to 10 mg of montelukast and 120 mg of fexofenadine in a 100 ml volumetric flask in mobile phase. This solution is then sonicated for 10 minutes and diluted to volume with mobile phase. Further 1 ml of this stock solution is taken in a 100 ml volumetric flask and made up to the mark with mobile phase (this 10 µg/ml of montelukast sodium and 12 µg/ml of fexofenadine sample solution. The sample was filtered through 0.45 µm nylon syringe filter.

RESULTS AND DISCUSSION

The conditions tested for the method development indicates that all the system suitability parameters according to ICH guidelines was achieved by using X bridge C_{18}, (250 × 4.6 mm, 5 µm particle) column using mobile phase composition consisting of a mixture of acetonitrile : buffer (0.2 M potassium dihydrogen orthophosphate in 0.1 M phosphoric acid adjusted with triethylamine (0.5%) to pH 4.5) : methanol (50:30:20, v/v) using a isocratic program with a flow rate of 1.5 ml/min throughout isocratic program with a detection wavelength of 248 nm for both the compounds with a injection volume of 20 µl. The peak at R_t3.62 min and 7.43 min for Montelukast sodium and Fexofenadine was observed in the chromatogram of the drug samples extracted from the tablet shown in Figure 2.

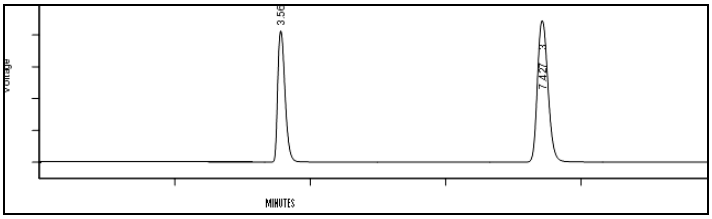

Figure 2: Chromatogram of test preparation

To validate RP-HPLC method, a series of tests were made using the most promising conditions. A calibration curve was made and concentration examined within the detection range from 0.020-0.100 mg/ml for Montelukast sodium and 0.016-0.064 mg/ml for fexofenadine. The correlation coefficient was found to be 0.9991 and 0.9998 for Montelukast sodium and fexofenadine respectively. The assay values are shown in (Table 1). The percent recovery of Montelukast sodium and fexofenadine were determined at determined at three different concentration levels like 80, 100 and 120%. The mean recovery for Montelukast sodium was 98.36-100.15% and 99.68-100.8% for fexofenadine. Data obtain from precision experiments are carried out for intraday and interday precision study for both Montelukast sodium and fexofenadine. The RSD values for intra day precision study and interday precision study was <2.0% for Montelukast sodium and fexofenadine. The result indicating that the method was accurate and confirms good precision. The results are presented under (Tables 2 and 3). The method was demonstrated to be robust over an acceptable working range of its HPLC operational conditions. The system suitability results within the acceptable limits and selectivity of individual substances were also not affected when subjected deliberately for varied chromatographic conditions. The result of the study confirms the robustness of the method. The result of robustness study of the developed assay method was established in (Tables 4 and 5). The stability of sample was checked by forced degradation in different stress conditions condition like acidic, alkali, thermal, oxidative degradation, photolytic and humidity conditions and % of degradation was calculated. The peak purity of the analyte was passed in all conditions (purity angle should be less than the threshold value). The following values in (Tables 2-6) indicate that any other impurity is not merging with the main peak (Figures 3-8). It was determined that the test preparation solution was found stable up to 48 hr at 2-5˚C and room temperature, as during this time the result was not decrease below the minimum percentage.

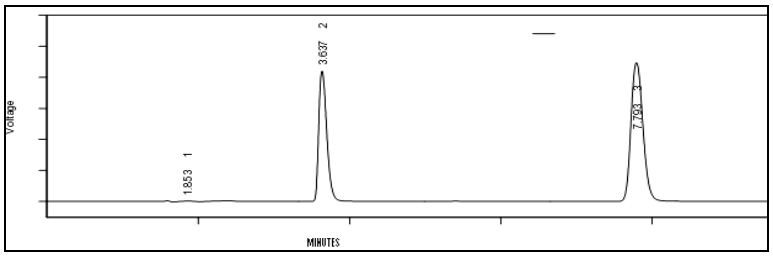

Figure 3: Chromatogram of acidic forced degradation study

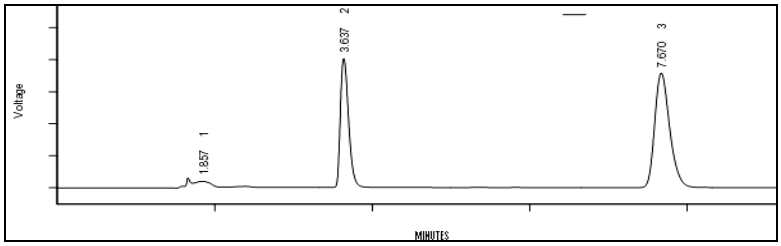

Figure 4: Chromatogram of alkali forced degradation study

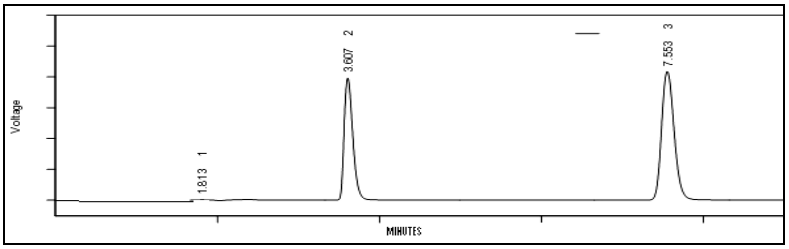

Figure 5: Chromatogram of oxidative forced degradation study

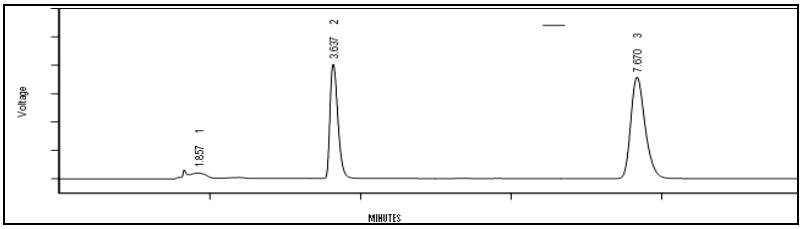

Figure 6: Chromatogram of thermal degradation study

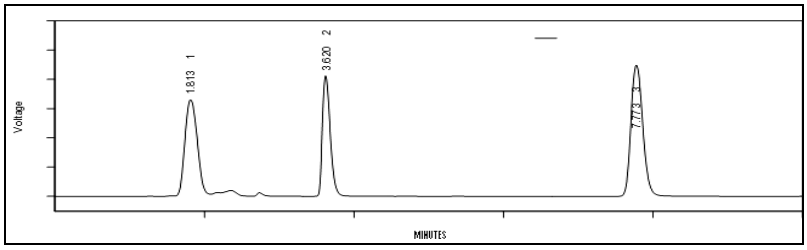

Figure 7: Chromatogram of UV-light degradation study

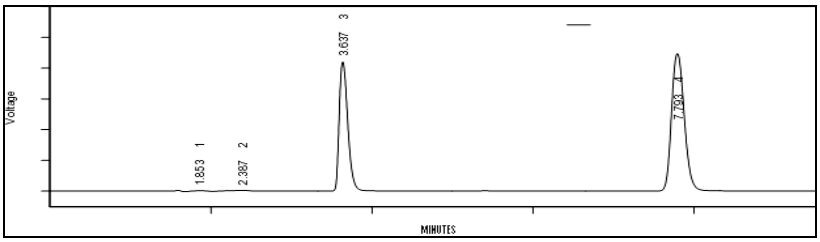

Figure 8: Chromatogram of humidity degradation study

Table 1: Assay of montelukast sodium and fexofenadine hydrochoride

Brand name	Compound	Amount found(mg)	%Assay
MONTAIR FX	Montelukast sodium	10.13	101.3
	Fexofenadine	119.89	99.9

Table 2: Results of precision study

Set	Montelukast sodium (%Assay)		Fexofenadine (%Assay)	
	Intraday (n=6)	Interday (n=6)	Intraday (n=6)	Interday (n=6)
1	101.4	100.4	100.5	98.9
2	100.9	100.8	100.1	101.5
3	98.5	101.3	100.9	101.5
4	99.7	99.9	98.9	99.8
5	100.8	101.7	99.8	99.9
6	100.1	100.5	99.6	99.7
Mean	100.6	100.7	99.96	100.2
Standard deviation	0.513	0.219	1.1	0.423
% RSD	0.827	0.119	1.12	0.386

Table 3: Results of accuracy study

	Level (%)	Theoretical Concentration[a] (µg/ml)	Observed Concentration[a] (µg/ml)	% Recovery	% RSD
Montelukast sodium	80	41.52	41.35	99.59	0.49
	100	51.96	51.11	98.36	1.38
	120	62.28	62.9	100.15	0.32
Fexofenadine	80	482.6	481.1	100.08	1.23
	100	602.7	601.83	99.68	1.37
	120	723.2	729.2	100.8	1.68

[a]Each value corresponds to the mean of three determinations

Table 4: Evaluation data of robustness study of Montelukast sodium

Robust conditions	% Assay	System suitability parameters	
		Theoretical plates	Asymmetry
Flow 0.9 ml/min	101.2	5002	1.65
Flow 1.5 ml/min	100.8	5022	1.34
Buffer pH 5	99	5010	1.54
Buffer pH 5.5	99.4	5100	1.38
Buffer-ACN (60:40:10% v/v)	98.7	5018	1.73
Buffer-ACN (50:30:20% v/v)	99.5	5108	1.45
Column change	100	5090	1.39

Table 5: Evaluation data of robustness study of Fexofenadine

Robust conditions	% Assay	System suitability parameters	
		Theoretical plates	Asymmetry
Flow 0.9 ml/min	100.4	10156	1.65
Flow 1.5 ml/min	101.5	10256	1.44
Buffer pH 5	100.9	10100	1.5
Buffer pH 5.5	99.9	10313	1.33
Buffer-ACN (60:40:10% v/v)	99.7	10005	1.56
Buffer-ACN (50:30:20% v/v)	99.9	10181	1.45
Column change	101.1	10162	1.67

Table 6: Forced degradation studies of control sample (1 mg/mL) solution

Type of Degradation	No of unknown impurities	Montelukast sodium and fexofenadine peak area %	% Degradation
Control sample	Nil	99.63	_
Acid	Nil	99.92	_
Alkali	Nil	99.89	--
Peroxide	Nil	99.74	_
Thermal	Nil	99.96	_
Photolytic	Nil	99.97	_
Humidity	Nil	99.91	_

CONCLUSION

A simple, fast, accurate, and precise stability-indicating HPLC analytical method has been developed and validated for the routine analysis of montelukast sodium and fexofenadine hydrochloride in active pharmaceutical ingredient(API) and can be useful to pharmaceutical sold dosage forms. The results of stress testing was carried out according to the ICH guidelines reveal that the method is specific and stability-indicating. The developed method has the capability to isolate these drugs from their degradation products; common excipients used in pharmaceutical solid dosage forms, and hence can be applied to the analysis of routine quality control samples and samples obtained from stability studies.

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