ORIGINAL ARTICLE

Development of Analytical Method and its Validation in Bilayer Tablet Containing Fexofenadine Hydrochloride (Immediate Release Layer) and Montelukast Sodium (Sustained Release Layer)

Sourav Thakur[1#], Bhupendra Singh[2#], Manish Vyas[3*], Geetanjali Saini[4], Ravi Shankar Yadav[5], Gopal Khatik[6], Amit Chaudhary[7], Neha Sharma[8]

[1]Department of Quality Assurance, School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India, [2]Department of Quality Assurance, Abhilashi College of Pharmacy, Mandi, Himachal Pradesh, India, [3]Department of Ayurveda, School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India, [4]Department of Pharmaceutical Chemistry, Abhilashi College of Pharmacy, Mandi, Himachal Pradesh, India, [5]Department of Pharmacognosy, Abhilashi College of Pharmacy, Mandi, Himachal Pradesh, India, [6]Department of Pharmaceutical Chemistry, School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India, [7]Department of Pharmaceutics, School of Pharmacy, Abhilashi University, Mandi, Himachal Pradesh, India, [8]Department of Pharmacology, School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India

[#]First two authors share the equal contributions.

Abstract

Aim: The aim of the present study was to develop and validate the analytical method for bilayer tablets. **Materials and Methods:** Simultaneous estimation of fexofenadine hydrochloride (HCl) and montelukast sodium in bilayer tablets by ultraviolet spectrophotometric method. **Results and Discussion:** The absorption maxima of fexofenadine HCl and montelukast sodium were found to be 259 nm and 285 nm, respectively, using phosphate buffer pH 6.8. The method obeys Beer's law in the concentration range of 24–84 µg/ml and 2–14 µg/ml for fexofenadine HCl and montelukast sodium, respectively. Different analytical parameters such as limit of detection, limit of quantitation, accuracy, and precision were determined as per International Council for Harmonisation guidelines Q2 (R1). **Conclusion:** The accuracy of the method was found to be 99.71% and 99.13% for fexofenadine HCl and montelukast sodium, respectively. There is no interference shown by the excipients of the formulation in the method and the method can be used for routine quality control.

Key words: Limit of detection, limit of quantitation, ultraviolet spectroscopy

INTRODUCTION

Montelukast is a leukotriene receptor antagonist. It is used for the treatment of asthma and seasonal allergies. It is mainly orally administered. It acts by blocking leukotriene D4 on cysteinyl leukotriene receptor CysLT1 present in the bronchial tubes and lungs, which leads to a reduction in bronchoconstriction caused by leukotriene, thus reducing inflammation. It cannot be used for treating acute asthma.[1] It has no interaction with other antiallergic medications like theophylline due to its specificity. Singulair® is the marketed formulation of montelukast and is marketed in several countries including the United States by Merck & Co. It is available in the form of oral granules, chewable tablets, and oral tablets. Cipla markets montelukast under

Address for correspondence:
Manish Vyas, Department of Ayurveda, School of Pharmaceutical Sciences, Lovely Professional University, Phagwara - 144 411, Punjab, India.
Phone: +91-9780210783. E-mail: vymanish@gmail.com

Received: 14-01-2019
Revised: 08-03-2019
Accepted: 29-04-2019

the brand name Montair® in many countries including India.[2]

Fexofenadine hydrochloride (HCl) (Allegra) is antihistaminic in nature and is used for treating hay fever and other allergic symptoms. It was developed as an alternative to terfenadine. It does not pass through the blood–brain barrier readily, and hence, drowsiness is less as compared to the first-generation antihistamines.[3]

The bilayer tablets of fexofenadine HCl and montelukast sodium were prepared in house. The bilayer tablets of fexofenadine HCl and montelukast sodium were prepared in house to target the release of the drugs in intestine at pH 6.8. No method was reported for the simultaneous estimation of the fexofenadine and montelukast in the given medium. Hence, the method is best suited for the simultaneous estimation of both of the drugs in the given medium.[5]

MATERIALS AND METHODS

Instruments

The analytical method for the estimation was performed on ultraviolet spectrophotometer (Shimadzu-1800). Weighing of the other chemicals was done on an analytical balance.

Chemicals and reagents

The drug samples of fexofenadine HCl and montelukast sodium were obtained from Yarrow Chemicals Ltd., Mumbai. A marketed formulation Allegra-M tablet was procured from the market. All other chemicals were of analytical grades.

Preparation of standard stock solutions

Accurately weighed 10 mg each of fexofenadine HCl [Figure 1] and montelukast sodium [Figure 2] were separately dissolved in 1 ml methanol and the volume was made up to 10 ml with pH 6.8 phosphate buffer to obtain solutions of 1000 µg/ml each. These solutions were used as standard stock solutions for further analysis.

Preparation of pH 6.8 phosphate buffer

To a 1000 ml volumetric flask, 250 ml of freshly prepared 0.2 M monobasic potassium phosphate (KH_2PO_4) solution was transferred. To this, 112 ml of 0.2 M sodium hydroxide solution was added. The volume was made up to 1000 ml with distilled water. The pH was checked with the help of a pH meter. The pH was adjusted with orthophosphoric acid if the pH was more than 6.8, and with sodium hydroxide solution, if the pH was below 6.8.[6]

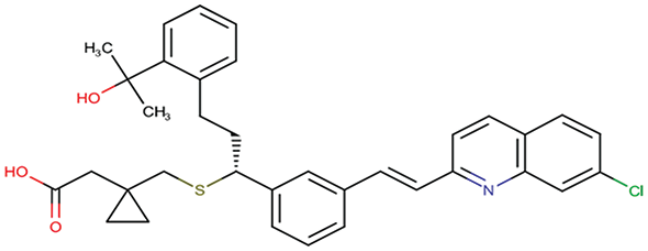

Figure 1: Chemical structure of montelukast[3]

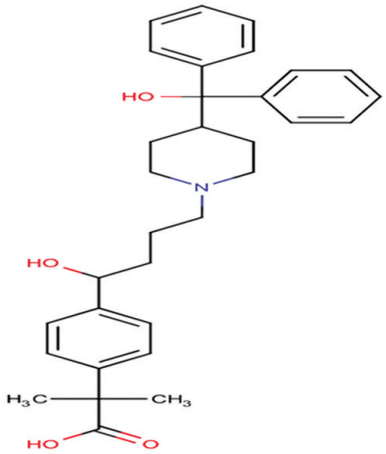

Figure 2: Chemical structure of fexofenadine[4]

Preparation of 0.2 M monobasic potassium phosphate solution

Accurately weighed monobasic potassium phosphate (27.22 g) was dissolved in a sufficient quantity of distilled water. The solution was then transferred into a 1000 ml volumetric flask. The volume was made up to 1000 ml with distilled water to get a 0.2 M monobasic potassium phosphate solution.

Preparation of 0.2 M sodium hydroxide solution

Accurately weighed sodium hydroxide pellets (8 g) were dissolved in a sufficient quantity of distilled water taken in a beaker. The solution was then transferred to a 1000 ml volumetric flask and volume was made up to 1000 ml with distilled water to obtain a solution of 0.2 M sodium hydroxide.

RESULTS AND DISCUSSION

Selection of analytical wavelength

From the standard stock solutions, appropriate dilutions were made to obtain final concentrations of 100 µg/ml fexofenadine HCl and 60 µg/ml montelukast sodium. The prepared solutions were then separately scanned in the wavelength range of 200–400 nm. Fexofenadine HCl showed maximum absorbance at 259 nm [Figure 3], while montelukast sodium

showed maximum absorbance at 285 nm [Figure 4]. These two wavelengths were selected for further analysis.

The method was developed and validated with respect to linearity, range, accuracy, precision, the limit of detection (LOD), and limit of quantitation (LOQ) as per International Council for Harmonisation (ICH) guidelines.[7,8]

Linearity and range

For each drug, appropriate aliquots were pipette out from standard stock solutions into a series of 10 ml volumetric flasks. The volume was made up to the mark with pH 6.8 phosphate buffer. The prepared aliquots for fexofenadine HCl (24–84 µg/ml) were scanned for absorbance at 259 nm [Figure 5]. The absorbance range was noted and determined if these aliquots followed Beer-Lambert's law or not. Similarly, aliquots for montelukast sodium (4–14 µg/ml) were prepared and scanned for absorbance at 285 nm [Figure 6]. The regression coefficients for fexofenadine HCl and montelukast sodium were determined.[9]

Determination of absorptivity at analytical wavelength

The absorbances obtained for each of the prepared aliquots were divided by their respective concentrations (g/100 ml) to obtain their absorptivity values [Tables 1 and 2].[10,11]

$$\text{Absorptivity} = \frac{\text{Absorbane}}{\text{Concentration}\left(\frac{\text{g}}{100\text{ ml}}\right)}$$

Analysis of a standard mixture

The standard mixture was made in the ratio of 60:10 (fexofenadine HCl: montelukast sodium) from the working

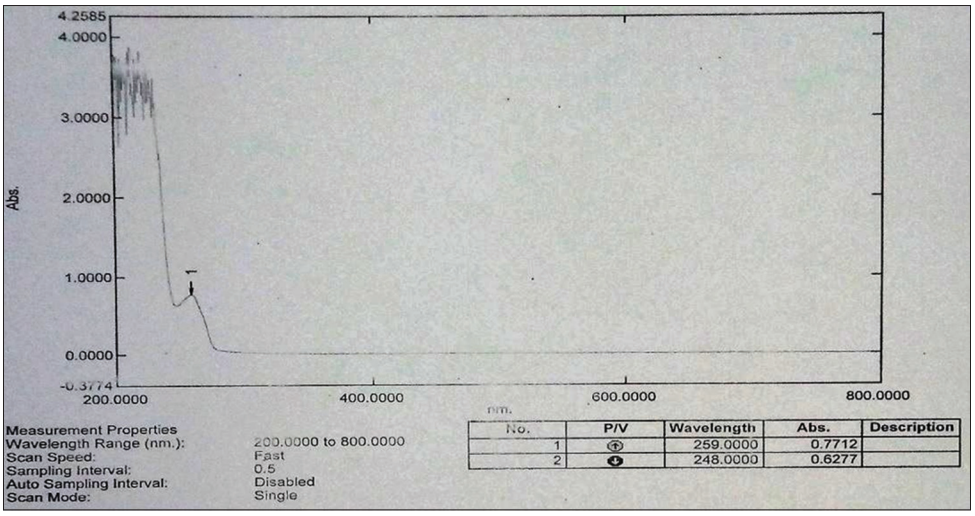

Figure 3: λ_{max} of fexofenadine hydrochloride at 259 nm

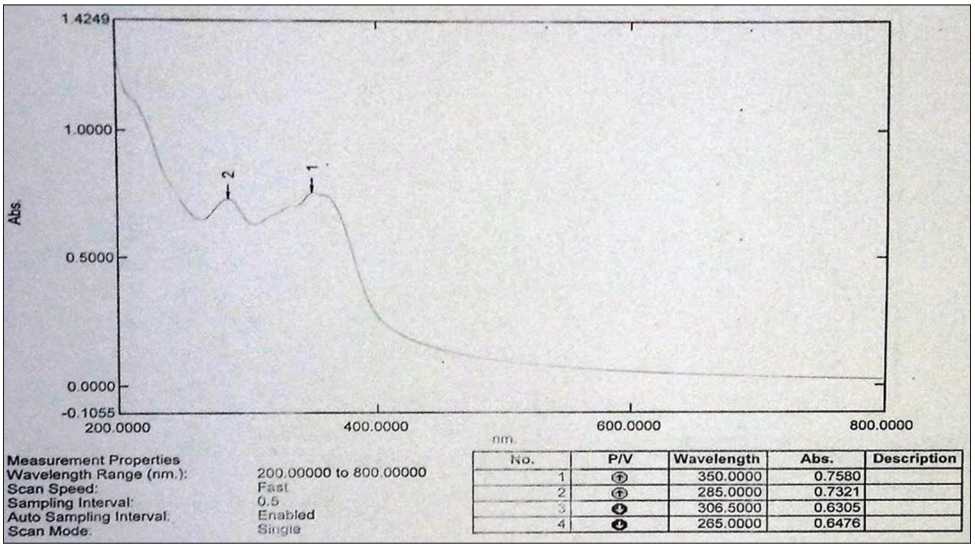

Figure 4: λ_{max} of montelukast sodium at 285 nm

stock solution. The standard mixture prepared was then scanned over the range of 200–400 nm. The absorbance was measured at 259 nm and 285 nm [Table 3]. The concentrations of each drug were calculated by putting the absorbance and absorptivity values in the following equations:

$$c_x = \frac{A_2 ay_1 - A_1 * ay_2}{ax_2 * ay_1 - ax_1 * ay_2}$$

$$c_Y = \frac{A_1 ax_2 - A_2 * ax_1}{ax_2 * ay_1 - ax_1 * ay_2}$$

Where,
C_x = Concentration of fexofenadine HCl (g/100 ml)
C_y = Concentration of montelukast sodium (g/100 ml)
A_1 = Absorbance of the mixture at 259 nm
A_2 = Absorbance of the mixture at 295 nm
ax_1 = Absorptivity of fexofenadine HCl at 259 nm
ax_2 = Absorptivity of fexofenadine HCl at 285 nm
ay_1 = Absorptivity of montelukast sodium at 259 nm
ay_2 = Absorptivity of Montelukast sodium at 285 nm.

Precision

The precision of the method was determined with the prepared aliquots. The precision of the method was verified by repeatability (intraday) and intermediate precision (interday) studies [Table 4].

Repeatability

Repeatability studies were performed by analysis of 60 µg/ml standard of fexofenadine HCl and 10 µg/ml of montelukast sodium. Method repeatability was achieved by repeating the same procedure 6 times on the same day for intraday precision. The repeatability of sample application and measurement was expressed in terms of relative standard deviation (% RSD) and standard error (S.E.) [Table 5].

Intermediate precision

The intermediate (interday) precision of the method was checked by performing the same procedure on different days under the same experimental conditions.

Table 1: Absorptivity values of fexofenadine hydrochloride

Concentration (µg/ml)	Absorbance at		Absorptivity at	
	259 nm	285 nm	259 nm	285 nm
36	0.081	0.048	22.50	13.33
48	0.109	0.055	22.71	11.46
60	0.125	0.058	20.83	9.67
72	0.150	0.061	20.83	8.47
84	0.178	0.066	21.19	7.86
			ax_1 = 21.61	ax_2 = 10.16

Table 2: Absorptivity values of montelukast sodium

Concentration (µg/ml)	Absorbance at		Absorptivity at	
	259 nm	285 nm	259 nm	285 nm
6	0.206	0.260	343.33	433.33
8	0.246	0.299	307.50	373.75
10	0.296	0.350	296.00	350.00
12	0.358	0.412	298.33	343.33
14	0.387	0.445	276.43	317.86
			ay_1=304.32	ay_2=363.66

Table 3: Data of standard mixture analysis

Drug	Concentration of drug taken (µg/ml)	Concentration of drug observed (µg/ml)±SD	% recovery	% RSD
Fexofenadine hydrochloride	60	60.15±0.4403	100.25	0.73
Montelukast sodium	10	9.81±0.0123	98.10	0.1253

Values represent mean±SD (*n*=6). SD: Standard deviation, RSD: Relative SD

Table 4: Results of intermediate precision of fexofenadine HCl and montelukast sodium

Drug	Concentration took (µg/ml)	Concentration observed (µg/ml)			Mean (µg/ml)	Standard deviation	% recovery	% RSD	Standard error
		Day 1	Day 2	Day 3					
Fexofenadine HCl	60.00	58.86±0.0153	60.27±0.0208	59.38±0.01	59.5	0.72	99.17	1.21	0.41
Montelukast sodium	10.00	9.84±0.0153	9.79±0.0153	9.78±0.0058	9.8	0.03	98.00	0.33	0.02

Values represent mean±SD (*n*=3). % RSD of fexofenadine HCl=1.53 (intraday) and 1.19 (interday). % RSD of montelukast sodium=0.56 (intraday) and 0.40 (interday). RSD: Relative standard deviation, HCl: Hydrochloride

LOD and LOQ

LOD is defined as the lowest amount of analyte that can be detected but may not get quantified. LOQ is the lowest amount of analyte that can be determined quantitatively with high accuracy and precision. To estimate the LOD and LOQ, individual standard deviations were determined of the sex replicates for 60 µg/ml of fexofenadine HCl and 10 µg/ml of montelukast sodium. The LOD and LOQ were then calculated by dividing the standard deviations of intercepts with slopes of their respective calibration curves [Table 6].

$$LOD = \frac{3.3 * \sigma}{S}$$

Where,
LOD = Limit of detection
σ = Standard deviation of the intercept
S = Slope of the calibration curve.

$$LOQ = \frac{10 * \sigma}{S}$$

Where,
LOQ = Limit of quantitation
σ = Standard deviation of the intercept
S = Slope of the calibration curve.

$$\sigma = \text{Standard error} * \sqrt{n}$$

Where,
n = number of observations
The S.E. was obtained from the regression analysis.

Accuracy and recovery

Accuracy of the method was validated by preparing three dilutions containing 80%, 100%, and 120% of the actual concentration. Triplicate determinations at each concentration level were executed and the obtained outcomes were compared with the expected outcomes.[11,12] Percentage recoveries were determined and found satisfactory as shown in Tables 7 and 8.

Optical characteristics

Fexofenadine hydrochloride and Montelukast sodium were estimated by simultaneous estimation method and their optical characteristics used for the same as represented in Table 9. The absorbance were recorded for the Fexofenadine hydrochloride and Montelukast sodium at 259 nm and 285 nm showing correlation coefficients 0.9954, and 0.995 respectively in the acceptance limits. %RSD for repeatabilities, intermediate precisions and accuracies were also found lower.

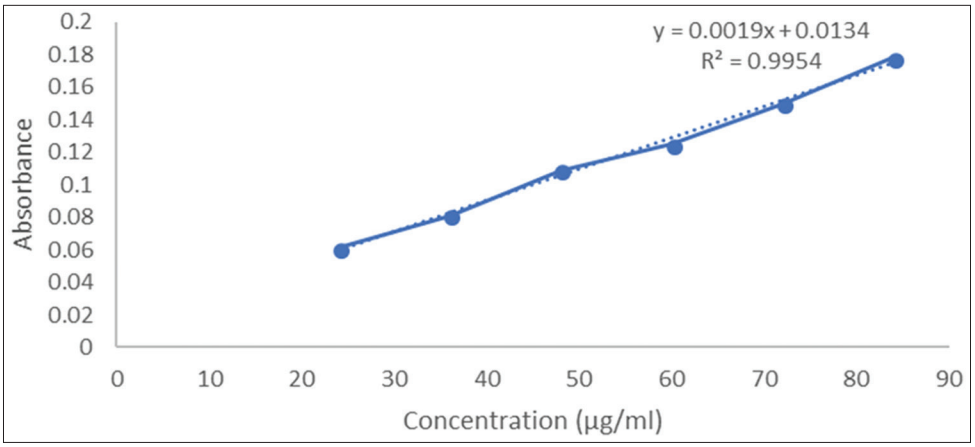

Figure 5: Linearity curve of fexofenadine hydrochloride

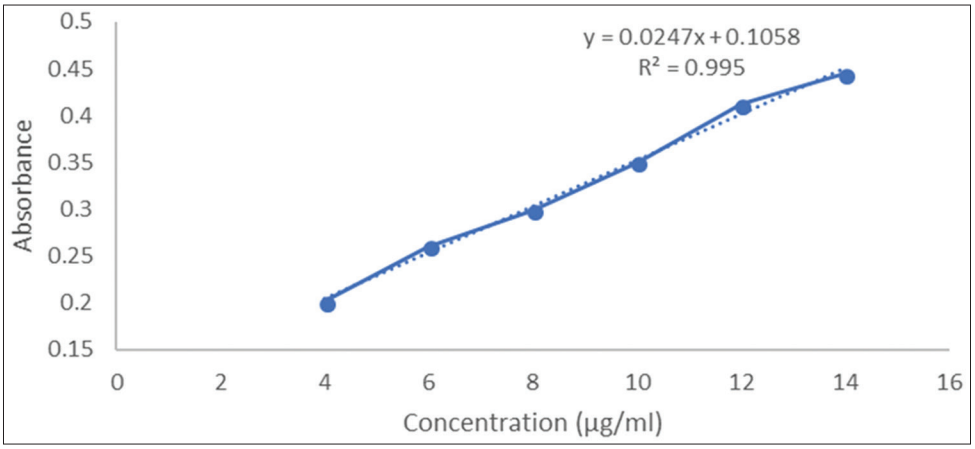

Figure 6: Linearity curve of montelukast sodium

Table 5: Results of repeatability of fexofenadine HCl and montelukast sodium

Drug	Concentration taken (µg/ml)	Concentration observed (µg/ml)	% recovery	% RSD	Standard error
Fexofenadine HCl	60.00	59.89±0.92	99.82	1.53	0.37
Montelukast sodium	10.00	9.82±0.05	98.2	0.56	0.02

Values represent mean±SD (*n*=6). RSD: Relative standard deviation, HCl: Hydrochloride

Table 6: Results of LOD and LOQ of fexofenadine HCl and montelukast sodium

Drug	Number of observations	Standard error	The standard deviation of the intercept	LOD (µg/ml)	LOQ (µg/ml)
Fexofenadine HCl	6	0.0038	0.0092	15.80	47.87
Montelukast sodium	6	0.0084	0.0207	2.77	8.38

LOD: Limit of detection, LOQ: Limit of quantitation, HCl: Hydrochloride

Table 7: Results of recovery studies of fexofenadine hydrochloride

Level of % recovery	Concentration taken (µg/ml)	Concentration observed (µg/ml)	% recovery	% RSD
Low (80)	48	47.86±0.4091	99.71	0.85
Middle (100)	60	59.89±1.0430	99.82	1.74
High (120)	72	72.31±0.8087	100.43	1.12

Values represent mean±SD (*n*=3). RSD: Relative standard deviation

Table 8: Results of recovery studies of montelukast sodium

Level of % recovery	Concentration taken (µg/ml)	Concentration observed (µg/ml)	% recovery	% RSD
Low (80)	8	7.93±0.0139	99.13	0.17
Middle (100)	10	9.82±0.0563	98.20	0.57
High (120)	12	11.99±0.0385	99.92	0.32

Values represent mean±SD (*n*=3). RSD: Relative standard deviation

Table 9: Optical characteristics of simultaneous estimation method

Parameters	Fexofenadine hydrochloride	Montelukast sodium
Absorbance maxima (λ_{max})	259 nm	285 nm
Beer's law limit (µg/ml)	24-84	2-14
Correlation coefficient (R^2)	0.9954	0.995
Slope	0.0019	0.0247
Absorptivity	ax_1 = 21.61; ax_2 = 10.16	ay_1=304.32; ay_2=363.66
Repeatability	% RSD=1.53	% RSD=0.56
Intermediate precision	% RSD=1.21	% RSD=0.33
LOD	15.80	2.77
LOQ	47.87	8.38
Accuracy	% RSD (low)=0.85	% RSD (low)=0.17
	% RSD (middle)=1.74	% RSD (middle)=0.57
	% RSD (high)=1.12	% RSD (high)=0.32

LOD: Limit of detection, LOQ: Limit of quantitation, RSD: Relative standard deviation

CONCLUSION

The present analytical method for the simultaneous estimation of fexofenadine HCl and montelukast sodium in bilayer tablets was validated as per ICH Q2(R1) guidelines and the methods meet to the specific acceptance criteria. After studying all the parameters, it is concluded that the analytical method was specific, precise, accurate, and linear. The present analytical method can be used for the estimation of combined marketed formulations of fexofenadine HCl and montelukast.

ACKNOWLEDGMENT

The author is highly grateful to Dr. Monica Gulati, Senior Dean, Department of Pharmaceutical Sciences, Lovely Professional University, Phagwara, India, for providing necessary facilities to carry out the research.

REFERENCES

1. Jameson JL. Harrison's Principles of Internal Medicine. 14th ed. New Delhi: Tata Mc. Graw Ltd.; 1998.
2. Tripathi KD. Essentials of Medical Pharmacology. 6th ed. New Delhi: Published by Jaypee Brothers Medical Publisher; 2008.
3. Montelukast. Available from: https://www.drugbank.ca/drugs/DB00471. [Last accessed on 2019 May 09].
4. Fexofenadine. Available from: https://www.drugs.com/mtm/fexofenadine.html. [Last accessed on 2017 Dec 07].
5. Narang A, Nayak UY. Formulation design of bilayer dual-release tablet composition of fexofenadine HCl and montelukast sodium. Res J Pharm Technol 2016;9:1410-6.
6. Ministry of Health and Family Welfare. Indian Pharmacopoeia. Vol. 2. New Delhi: Controller of Publications, Ministry of Health and Family Welfare, Government of India. 2018. p. A48, A50, A54, A96, A99.
7. Validation of Analytical Procedures: Text and Methodology Q2 (R1). Geneva, Switzerland: International Conference on Harmonization; 2005. p. 11-2.
8. Beckett AH, Stenlake JB, editors. Practical Pharmaceutical Chemistry: Part II. 4th ed. United Kingdom: A and C Black; 1997. p. 275-336.
9. Raju KN, Swamy TG, Rao AL. Development and validation of RP HPLC method for the determination of montelukast sodium in bulk and in pharmaceutical formulation. Int J Pharm Chem Biol Sci 2011;1:12-6.
10. Willard-Hobart H, Lynne LM Jr., John AD. Instrumental Methods of Analysis. 5th ed. Von Nostrand: University of Michigan; 1974.
11. Chatwal GR, Anand S. Instrumental Methods of

Chemical Analysis. 5th ed. New Delhi: Himalaya Publishing House; 2002.

12. Bharat J, Bhupendra S. Method development and validation for simultaneous estimation of levosulpiride and rabeprazole sodium: A new analytical Q-absorbance ratio approach. Asian J Pharm Clin Res 2017;10:22-7.

Source of Support: Nil. **Conflict of Interest:** None declared.