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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF FEXOFENADINE AND MONTELUKAST IN TABLETS DOSAGE FORM

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ABSTRACT

This research article describes simple, specific, accurate and precise Reverse Phase High Pressure Liquid Chromatographic method for the simultaneous determination of fexofenadine hydrochloride and Montelukast sodium in tablet dosage form. The chromatographic separation was achieved on 4.6 mm X 10 cm column containing packing L1 as stationary phase with a mobile phase comprising of water: methanol (35: 65) at a flow rate of 1.2mL/min, column temperature of 45^0C and UV detection at 254 nm. The retention time of Fexofenadine and Montelukast Sodium hydrochloride were 6.776 min and 3.776min respectively. The linearity were found to be in the range of 200-600 mg/mL and 200-600 mg/mL for Fexofenadine and Montelukast Sodium hydrochloride with correlation coefficient greater than 0.999. The proposed methods were validated as per ICH guidelines for the determination of drugs in tablet dosage form.

Keywords: Fexofenadine, Montelukast, RP-HPLC, Method Development

INTRODUCTION

Fexofenadine is a histamine H_1 receptor antagonist and has no sedative effects. It is the medication class of antihistamines. It is used to treat seasonal allergic rhinitis symptoms such as runny nose and sneezing. It works by preventing the release

of histamine a chemical in the body that causes allergic reactions **[1]**. It is a histamine H1 receptor antagonist and has no sedative effects. Its ability to reside in the zwitterionic state, which cannot cross the blood-brain barrier and does not sedate people, is the reason behind this **[2, 3]**. It is a second-generation antihistamine with a high degree of safety that can be added to current allergy medications **[4, 5]**.

It is a white to off-white powdery substance. It is freely soluble in methanol and ethanol, just moderately soluble in water and chloroform, and insoluble in hexane **[6]**. It is carboxylic acid metabolite of terfenadine, non-sedating selective histamine H1 receptor antagonist. Fexofenadine is chemically known as **(Figure 1)** (RS)-2-[4-[1-Hydroxy-4-[4-(hydroxyl-diphenyl-methyl)- 1-piperidyl]butyl]phenyl]-2-methyl-propanoic acid. It is the medication class of antihistamines. This drug contains an asymmetric carbon in its chemical structure and is administered clinically or is used as aglycoprotein probe as a racemic mixture of R- and S-enantiomers **[6]**.

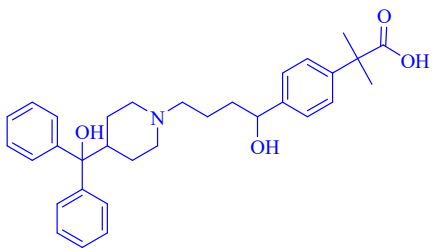

Figure 1: Structure of fexofenadine

Fexofenadine is a selective, second generation H1 receptor antagonist and non-sedative in nature, which have an additional impact on the inflammatory mediators. Montelukast is a highly selective type I receptor antagonist of leukotriene D4. The leukotrienes modifiers have bronchodilator and anti-inflammatory properties **[5–7]**. The literature review determined that using an antihistamine with Montelukast offers additional advantages. Montelukast and antihistamine combination therapy has boosting and complementary effects, successfully lowering symptoms. More efficient than antihistaminic alone at controlling the symptoms of allergic rhinitis is fexofenadine combined with Montelukast **[8, 9]**.

Montelukast sodium (MTKT) hygroscopic and optically active white to off white powder. It is freely soluble in methanol ethanol and water **(Figure 2)**. is chemically (S, E)-2-(1-((1-(3-(2-(7-chloroquinolin 2yl) vinyl) phenyl)-3- (2-(2-hydroxypropan-2-yl) phenyl) propylthio) methyl) cyclopropyl) acetic acid 3 is a leukotriene receptor antagonist used in the treatment of chronic asthma and allergic rhinitis **[4]**.

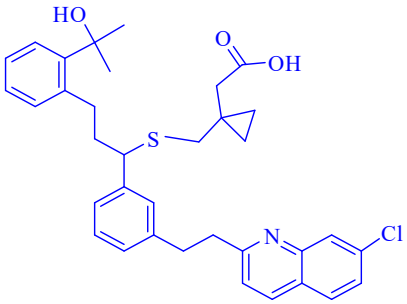

Figure 2: Structure of Montelukast

High-Performance Liquid Chromatography (HPLC)

High-performance liquid chromatography (HPLC) is fastest growing analytical technique for analysis of drugs. Its simplicity, high specificity and wide range of sensitivity makes it ideal for analysis of many drugs in both dosage forms and biological fluids. HPLC is a form of liquid chromatography to separate compounds that are dissolved in solution. HPLC is able to separate macromolecules and ionic species, labile natural products, a wide variety of other high molecular- weight poly functional groups and polymeric materials. Most of the drugs in multi component dosage forms can be analyzed by HPLC method because of the several advantages like rapidity, specificity, accuracy, precision and ease of automation in this method. HPLC method eliminates tedious extraction and isolation procedures **[17, 18]**.

Reverse Phase HPLC

The stationary phase generally is nonpolar (hydrophobic) in nature, while the mobile phase is a polar liquid, such as mixtures of water and methanol or acetonitrile. It works on the principle of hydrophobic interactions hence the more nonpolar the material is the longer it will be retained.

MATERIAL AND METHOD

The list of drugs, chemicals, and instruments used in this research are represented in below **Table 1, 2 and 3** respectively.

Table 1: Standards used

Sr. No.	NAME OF API	SUPPLIER
1	Fexofenadine	Pfizer Ltd
2	Montelukast	Curex Pharma Ltd

Table 2: Marketed formulation

FORMULATION	BRAND NAME	STRENGTH	NAME OF COMPANY	MFG. DATE	EXP. DATE
Fexofenadine+ Montelukast	Histakind-M	120mg Fexofenadine+10mg Montelukast	Mankind Pharma Ltd	OCT.2020	SEP. 2022

Table 3: Reagent use:

NAME OF CHEMICAL	GRADE	SUPPLIED BY
Methanol	HPLC Grade	Thermosil fine chem industry
Water	HPLC Grade	Thermosil fine chem industry

Table 4: Instrumentation for HPLC:

Sr. No.	INSTRUMENTS	SPECIFICATION
1	HPLC System	Shimadzu HPLC System
2	Pump	LC-20 AT Prominence Liquid Chromatography
3	Column	4.6 mm X 10 cm column containing packing L1
4	Detector	SPD-M20A PDA Detector
5	Data Processor	LC solution
6	Degasser	DGU-20 A5 Prominence Degasser

Method Development Parameters:

Selection of Suitable Solvent:

According to solubility tests and literature review FEX and MTKT were freely soluble in water, acetonitrile and methanol: water and acetonitrile mixture was selected as diluent for present research work.

Selection of Wavelength (for Detection)

By scanning the sample throughout the wavelength range of 200-400 nm against a blank of methanol, the UV- Spectrum of Fexofenadine and Montelukast were individually acquired.

After examination of the spectra, the wavelength of FEX and MTKT was found to be 257nm and 254nm which had use for this research work.

Preparation of mobile phase

Water 35% and 65 ml of methanol HPLC (70%) were mixed and degassed with the help of ultrasonic water bath for 5minutes and filtered through 0.45 µ filter under vacuum filtration.

Diluent:- Methanol

Preparation of standard solution (Mixed standard):

10 mg of Montelukast and 10mg of Fexofenadine working standards were accurately weighed and transferred into a 100 ml clean dry volumetric flask add about 60ml of diluent was added and sonicated to completely dissolve it and the volume was made up to the mark with the same solvent. (Stock solution) Further Fexofenadine 3.6ml &1.2ml of Montelukast of was pipetted from the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent **[19]**.

Preparation of sample solution

Weighed 10 tablets of HISTAKIND-M and powdered in glass mortar. The powder equivalent to the amount of active ingredient present in 10 tablets was transferred into a 100 ml clean dry volumetric flask, 60 ml of diluent was added to it and was dissolve completely and sonicated for about 30minutes by shaking at intervals of five minutes each and was diluted up to the mark with diluent and let it stand until the residue settles before taking an aliquot for further dilution (stock solution). 0.6ml of upper clear solution was transferred to a 10 ml volumetric flask and diluted with diluent up to the mark and the solution was filtered through 0.45 µm filter before injecting into HPLC system.

Standardized chromatographic condition:-

Table 5: Optimized chromatographic conditions

PARAMETERS	DESCRIPTION
Mode of operation	Binary
Diluents	Methanol
Column	4.6 mm X 10 cm column containing packing L1
Mobile phase	Methanol :Water (65:35)
Flow rate	1.2ml/min
Detection of Montelukast and Fexofenadine potassium	245nm
Temperature	45ºC
Injection Volume	20 µ
Run time	10 min
Detector	UV detector

METHOD VALIDATION

Accuracy

The accuracy of the method was determined by recovery studies using the standard addition

Method. Pre analyzed samples were spiked with standard drugs (FEX, MLK) **[12, 16]**.

The accuracy of analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness.

1ml (300µg/ml) binary mixture of standard drug solution was taken in three different flask label A, B and C. further add 0.5,1 and 1.5 ml of sample solution in same flask A,B and C. Spiked 50%,100% and 150% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 257nm and 254nm. The amount of FEX and MTKT was calculated at each level and %recoveries were computed.

Linearity and range:

The linearity of an analytical procedure is its ability (within given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample **[11, 14]**. The linearity for FEX and MTKT were assessed by preparing the solution in the range of 200-600µg/ml respectively. Take 2, 3, 4, 5, 6 ml of sample stock solution and transferred into 10ml volumetric flask and volume was made up to the mark with diluents. Linearity is calculated by the regression line by using a mathematical procedure of the results (that is, least mean squares) versus analyte concentration. Calibration curve was constructed by plotting peak area versus concentrations of analyte.

Precision

The standard solution was injected for multiple times and the areas for injections were measured in HPLC. The % RSD for the area of multiple replicate injections was calculated for system precision and 20 µL of sample solution was injected for multiple times and the peak area of the resulting

chromatogram was used for the calculation of standard deviation and relative standard deviation for method precision.Results should be expressed as relative standard deviation (RSD) or coefficient of variance **[15]**.

Intra and interday precision study of FEX and MTKT was carried out by estimating the corresponding responses multiple times on the same day and on same time is different days **[13]**.

Intra-day precision

Standard stock solution containing 200,400,600µg/ml of FEX and MTKT were analyzed multiple time on the same day and %R.S.D. was calculated.

Inter-day precision

Standard stock solution containing 200,400,600µg/ml of FEX and MTKT were analyzed three time on the different day and %R.S.D. was calculated.

Robustness

As the % RSD of retention time and asymmetry were within limits for variation in flow rate (± 0.1 ml). For this reason the allowable flow rate should be within 0.9 ml to 1.1 mL. The results of robustness for effect of variation in flow rate are tabulated in Table. Change following parameters, one by one and observe their effect on system suitability test and assay.

- Change mobile phase composition by ± 2 ml of organic solvent.
- Change flow rate ± 0.2 ml/min
- Change wavelength(nm) ±2nm

LOD and LOQ

The detection and quantification limits for the Montelukast and Fexofenadine were performed and calculated using S/N ratio method.The LOD was estimated from the set of 3 calibration curves used to determination linearity. The LOD may be calculated as,

LOD = 3.3 × (SD/Slope)

Where,

SD= Standard deviation of Y-intercepts of 3 calibration curves.

Slope = Mean slope of the 3 calibration curves.

The LOQ was estimated from the set of 3 calibration curves used to determine linearity.

The LOQ may be calculated as,

LOQ = 10 × (SD/Slope)

Where,

SD = Standard deviation of Y-intercepts of 3 calibration curves.

RESULTS AND DISCUSSION

Analytical Method Development

Several trials were made to get good peak resolution, acceptable plate count and tailing factor. Method was optimized for the simultaneous estimation of Montelukast and Fexofenadine in bulk and Pharmaceutical dosage form **(Figure 3)**.

Validation Analytical Method

Linearity

The linearity for FEX and MTKT were assessed by preparing the solution in the range of 200-600µg/ml respectively. Take 2, 3, 4, 5, 6 ml of sample stock solution and transferred into 10ml volumetric flask and volume was made up to the mark with diluents **(Table 7)**.

Accuracy

Table 9 and 10 displayed the recovery study (Accuracy) of FEX and MTKT. FEX and MTKT were used in a concentration of 50%, 100%, and 150%. For FEX, the % amount recovered were 98.41%, 99.96% and 98.42% respectively. For MTKT, the % amount recovered were 101.96%, 101.31%, and 100.90% respectively.

Intraday Precision:

Table 11 displayed the study of intraday precision of FEX and MTKT. Both the drugs were used in a concentration of 200 µgm/ml, 400 µgm/ml, and 600µgm/ml were analyzed on the same day and % RSD was found to be 0.53, 0.25, 0.02 and Montelukast 0.14, 0.22, 0.15 respectively.

Interday Precision

Table 12 displayed the study of interday precision of FEX and MTKT. Both the drugs were used in a concentration of 200µgm/ml, 400µgm/ml, and 600µgm/ml were analyzed on different days and % RSD was found to be 0.15, 0.01, 0.04 and Montelukast 0.27, 0.14, 0.60 respectively.

Repeatability:

Repeatability or system suitability tests were carried out on standard solution of FEX and MTKT. Retention time of FEX and MTKT were found to be 6.447 and 6.711 respectively. The % RSD was 0.20 and 0.24 for FEX and MTKT respectively **(Table 13)**.

Robustness

Data for estimation of Fexofenadine and Montelukast

As the % RSD of retention time and asymmetry were within limits for variation in flow rate (± 0.1 ml). For this reason the allowable flow rate should be within 0.9 ml to 1.1 mL. The results of robustness for effect of variation in flow rate are tabulated in **Table 14**.

Limit of detection (LOD)

Limit of detection (LOD) of FEX and MTKT was found to be 0.192and 2.09279 respectively **(Table 15)**.

Limit of Quantitation (LOQ)

Limit of Quantitation (LOQ) of FEX and MTKT was found to be 0.192 and 2.0927 respectively **(Table 16)**.

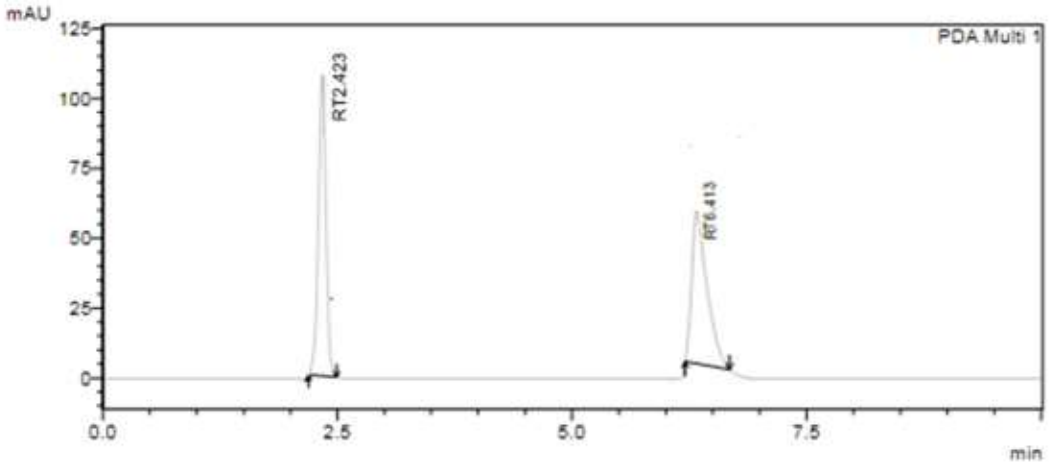

Figure 3: Optimized chromatographic condition

Table 7: Linearity data of Fexofenadine

Conc. µg/mL	RETENTION TIME	AREA I	AREA II	MEAN	SD	%RSD
200	6.499	14742.64	14744.65	14743.65	1.42	0.01
300	6.863	18678.6	18677.17	18677.88	1.01	0.01
400	6.629	22825.15	22830.13	22827.64	3.52	0.02
500	6.697	26819.11	26815.12	26817.12	2.82	0.01
600	7.014	3013.37	30517.38	30515.38	2.84	0.01

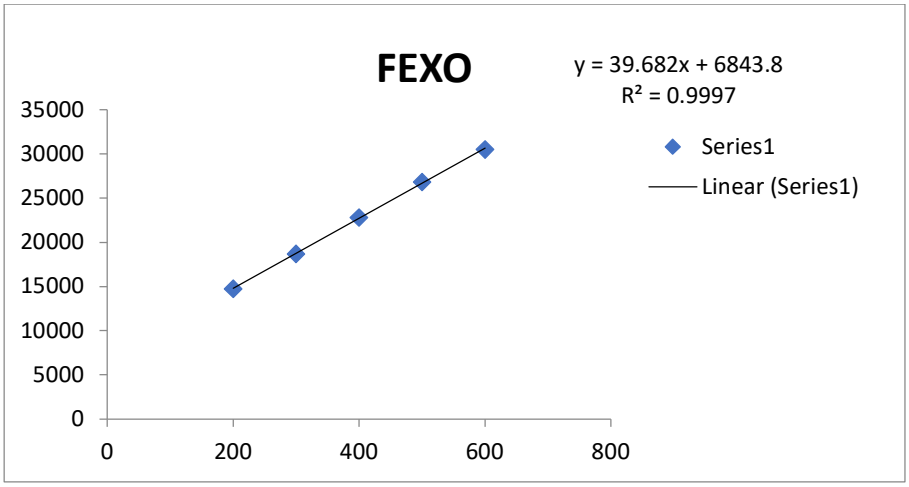

Figure 4: Calibration curve of fexofenadine

Table 8: Linearity of Montelukast

Conc. µg/mL	RETENTION TIME	AREA I	AREA II	MEAN	SD	%RSD
16	2.396	1037.65	1033.64	1035.65	2.84	027
24	2.433	1560.88	1981.14	1563.38	3.54	0.23
32	3.120	1981.14	1975.13	1378.14	4.25	0.21
40	2.420	2466.12	2468 .12	2467.12	1.41	0.06
48	2.276	2913.38	2917.38	2915.38	2.83	0.10

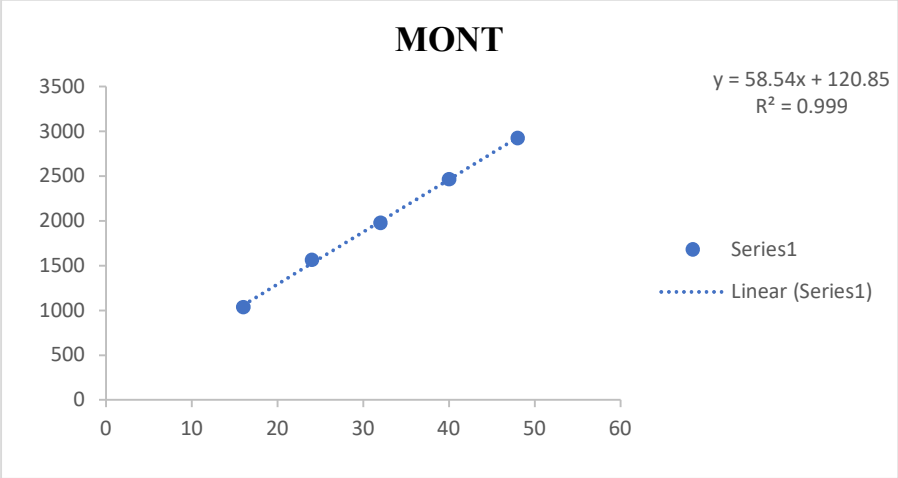

Figure 5: Calibration curve of Montelukast

Table 9: Recovery study of Fexofenadine (Accuracy)

SR. NO	Conc. µg/mL LEVEL %	RETENTION TIME	AREA	MEAN	AMOUNT ADDED	AMOUNT FOUND	% RECV
1	50	6.447	9699.64	9804.14	25	74.26	98.41
2	50	6.711	9908.64				
3	100	6.333	10701.64	10811.14	50	99.98	99.96
4	100	6.350	10920.64				
5	150	6.313	12709.640	11757.09	75	123.82	98.42
6	150	6.653	10804.540				

Table 10: Recovery study of Montelukast (Accuracy)

SR. NO	CONCENTRATION LEVEL %	RETENTION TIME	AREA	MEAN	AMOUNT ADDED	AMOUNT FOUND	% RECV
1	50	2.646	470.69	594.57	25	76.49	101.96
2	50	2.651	718.18				
3	100	2.652	592.25	590.285	50	100.66	101.31
4	100	2.647	588.32				
5	150	2.642	701.510	706.93	75	125.78	100.90
6	150	2.504	712.350				

Table 11: Intraday Precision study of FEX and MTKT

SR. NO	Conc. mg/mL	FEXOFENADINE			MONTELUKAST		
		RETENTION TIME	AREA	%RSD	RETENTION TIME	AREA	% RSD
1	200	7.631	45852.85	0.53	2.396	1027.65	0.14
2	200	7.082	45752.77		2.409	1025.64	
3	400	6.847	8818.9043	0.25	2.399	1971.14	0.22
4	400	6.745	8820.5412		2.406	1965.12	
5	600	6.608	47852.85	0.02	2.419	2901.27	0.15
6	600	6.717	57852.85		2.437	2907.37	

*** Sum of %RSD of All the readings.**

Table 12: Interday Precision study of FEX and MTKT

SR.NO	Conc. mg/mL	FEXOFENADINE			MONTELUKAST		
		RETENTION TIME	AREA	%RSD	RETENTION TIME	AREA	%RSD
1	16	6.937	14752.65	0.15	2.628	1037.65	0.27
	16	7.781	14642.63		2.638	1033.64	
2	32	6.502	22907.15	0.01	2.622	1971.14	0.14
	32	7.679	22827.11		7.679	1975.13	
3	48	6.343	87052.85	0.04	2.684	2943.28	0.60

	48	6.302	87002.85		2.713	2918.28	

*** Sum of %RSD of All the readings.**

Table 13:- Repeatability study of FEX and MTKT

Sr. No.	Conc. µg/ml	FEXOFENADINE		MONTELUKAST	
		Retention time	Area	Retention time	Area
1	200	6.447	14849.646	2.646	1047.15
2	200	6.711	14892.64	2.651	1043.54
% RSD		0.20		0.24	

Table 14: Robustness data for estimation of FEX and MTKT

PARAMETERS	CONC. (µg/ML)	AMOUNT OF DETECTED (MEAN ±SD)	%RSD	AMOUNT OF DETECTED (MEAN ±SD)	%RSD
		FOR FEXOFENADINE		FOR MONTELUKAST	
Chromatogram of flow change 1ml	16+200	15958.91±15.04	0.09	1035.65±2.84	0.27
Chromatogram of flow change 1.3ml	16+200	14958.91±15.04	0.10	1458.90±15.04	0.10
Chromatogram of mobile phase change 34 + 66 ml	16+200	15398.±17.33	0.11	15398.0±17.33	0.11
Chromatogram of mobile phase change 36 + 64 ml	16+200	15393.09±7.64	0.05	15393.09±7.64	0.05
Chromatogram of comp change wavelength change 252 nm	16+200	6308.4±7.30	0.12	6308.4±7.30	0.12
Chromatogram of comp change wavelength change 256 nm	16+200	6954.02±12.58	0.18	6954.02±12.58	0.18

Table 15: Limit of Detection (LOD)

FEXOFENADINE	MONTELUKAST
Formula LOD = 3.3×avg S.D/Slope Avg.SD = 2.32 Slope = 39.682 LOD = 3.3x 2.32/39.682= 0.192	Formula LOD = 3.3×avg S.D/Slope Avg.SD =2.97 Slope = 4.6832 LOD = 3.3x2.97/4.6832 =2.09279

Table 16: Limit of Quantitation (LOQ)

FEXOFENADINE	MONTELUKAST
Formula LOQ = 10×avg S.D/Slope Avg.SD = 2.32 Slope = 39.682 LOD = 10x 2.32/39.682= 0.584	Formula LOQ = 10 ×avg S.D/Slope Avg.SD = 2.97 Slope = 4.6832 LOD = 10x2.97/4.6832=6.341

CONCLUSION

A simple RP-HPLC method was developed and validated successfully for simultaneous estimation of FEX and MTKT in tablet dosage form. The present study was validated as per the ICH guidelines and developed RP-HPLC technique is precise, linearity, repeatability and accurate method for analysis of FEX and MTKT in pharmaceutical preparations. The procedure can be readily used for selective analysis of drugs and repeatable results are obtained without interference from auxiliary substances. The method can be used for analysis of a few formulations on a single plate and is rapid and cost-effective for routine analysis of FEX and MTKT in tablet formulation.

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Conflict of Interest:

The authors have declared no conflict of interest.

Abbreviations: RP-HPLC**:** Reverse Phase High performance liquid chromatography; FXO: Fexofenadine; MKT: Montelukast sodium; ICH: International conference on harmonization; RSD: Relative standard deviation; SD: Standard deviation; LOD: Limit of detection; LOQ: Limit of Quantitation; Rf: Retention factor; API: Active pharmaceutical ingredient; UV: Ultraviolet.

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