International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 4 July-Aug 2023, pp: 653-666 www.ijprajournal.com ISSN: 2249-7781

Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Montelukast and Fexofenadine

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Submitted: 10-07-2023 Accepted: 20-07-2023

ABSTRACT
A New, Rapid, Accurate, Precise, reproducible reverse phase high performance liquid chromatography (RP-HPLC) method has been developed and validated for the simultaneous estimation of Montelukast Sodium and Fexofenadine Hydrochloride in Combined dosage form (tablet). The chromatographic separation was achieved on C18 Column (4.6mm×250mm) particle packing size is 5µm. as a Mobile phase Methanol : Water (0.1% Acetic Acid PH adjusted 3.5 with OPA) 35 : 65 at flow rate of 1 ml/min and UV detection wavelength used was 250 nm. The Linearity over the concentration range of 2-10µg/mL and 24-120µg/mL for Montelukast Sodium and Fexofenadine Hydrochloride. Correlation coefficient value was found to be (r^2= 0.999) for Montelukast Sodium and (r^2=0.999) for Fexofenadine Hydrochloride. The % recovery of the method was found to be within 98-102% Respectively. The Limit of detection and Limit of quantification were 0.02105 µg/mL and 0.0638 µg/mL for Montelukast Sodium and 0.24110 µg/mL and 0.73063 µg/mL for Fexofenadine Hydrochloride. The present successfully validated and developed method was perform as per the ICH guidelines. The proposed developed RP-HPLC method can be applied for identification and quantitative determination of Montelukast Sodium and Fexofenadine Hydrochloride in bulk drug and drug formulation.

KEYWORDS: - Validation, RP-HPLC, Montelukast Sodium, Fexofenadine Hydrochloride Simultaneous Estimation.

I. INTRODUCTION

Fexofenadine HCl (FEXO), chemically designated as (±)-4-[1-hydroxy-4-(4 hydroxydiphenylmethyl)-1- piperidinyl]-butyl]-∝,∝-dimethyl benzeneacetic acid hydrochloride 1 is a histamine H1 receptor antagonist used in patients with allergic rhinitis. It is freely soluble in methanol, ethanol and slightly soluble in water, chloroform and practically insoluble in haxene. The molecular weight is 538.13 and the emperical formula is C32H39NO4HCl

Montelukast Sodium (1-[[[(1R)-1-[3-[(1E)-2-(7-chloro-2-quinolinyl) ethenyl] phenyl]-3-[2-(1-hydroxy-1- methylethyl) phenyl] -propyl] thio] methyl] cyclopropaneacetic acid, monosodium salt is a white colored powder and it is freely soluble in ethanol, methanol, and water and practically insoluble in acetonitrile. Molecular weight of Montelukast Sodium is 608.2 g/mol and formula is C35H35ClNO3S.Na

It has been demonstrated in recent studies that the treatment of allergic rhinitis with concomitant administration of an anti-leukotriene and an antihistamine shows significantly better symptom relief compared with the modest improvement in rhinitis symptomatology with each of the treatments alone.

The review of literature revealed that several methods are available for the determination of montelukast sodium and fexofenadine hydrochloride individually. Reported method for estimation Fexofenadine hydrochloride in dosage form are spectrophotometry, spectrofluorometry, dissolution, RP-HPLC, and similarly for estimation Montelukast sodium in dosage form are spectrophotometry, spectrofluorometry, LC-MS[24-25] , RP-HPLC and HPTLC .

But, there is no any analytical method has been reported yet for combination of these drugs. There for the present research work aims to develop a simple, sensitive, accurate and reproducible method for simultaneous estimation of Montelukast sodium and fexofenadine hydrochloride in combined dosage form by HPTLC method.

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Volume 8, Issue 4 July-Aug 2023, pp: 653-666 www.ijprajournal.com ISSN: 2249-7781

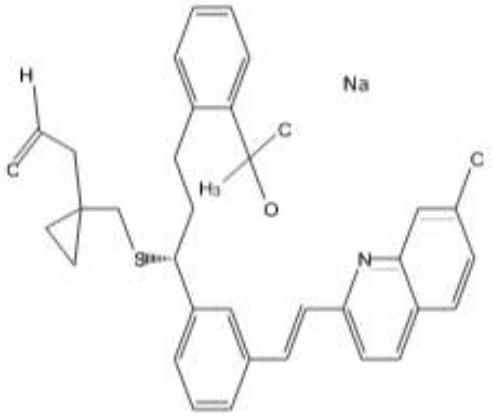

Fig. 1: Structural of Montelukast sodium

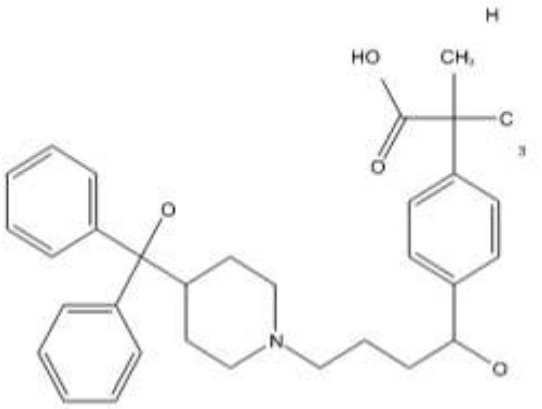

Fig. 2: Structural of fexofenadine HCl

II. MATERIALS AND METHODS :-

- **Selection and Procurement of Drug**

Table 1: Drug and Drug Supplier

Sr.No.	Name Of Drug	Grade	Drug Suppplier
1	Montelukast Sodium	API	Swapnaroop drug and pharmaceutical
2	Fexofenadine Hydrochloride	API	Swapnaroop drug and pharmaceutical

- **List of reagents & chemicals used**

Table 2: List of Reagents and Chemicals used

Sr.No	Name of chemical	Grade	Manufacturer
1	Orthophopsphoric acid (OPA)	HPLC	Avantor Performance material India Ltd. Thane, Maharashtra
2	Methanol	HPLC	Merck Specialities Pvt. Ltd. Shiv Sager Estate 'A' Worli, Mumbai
3	Water	HPLC	Merck Specialities Pvt. Ltd.Shiv Sager Estate 'A' Worli, Mumbai

- **Instruments and Equipment's**

Table No.3.: Instrument (HPLC) Details used during Method Development

Sr.No	Name of Instrument	Company Name
1	HPLC Instrument	Agilent Tech. Gradient System With Auto injector (Chemstation 10.1 software)
2	UV-Spectrophotometer	Analytical Technologies Limited
3	Column(C_8)	Agilent C_{18} (250mmX 4.6mm,5µm)
4	pH meter	VSI pH meter (VSI 1-B)
5	Balance	WENSAR™ High Resolution Balance.
6	Sonicator	Ultrasonic's electronic instrument

- **Chromatographic Conditions:**

Table No.4.: Chromatographic conditions (HPLC) details used during method Development

1.	HPLC	Agilent Tech. Gradient System With Auto injector

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2.	Software	Chemstation 10.1 software
3.	Column	(Agilent) C18 column (4.6mm x 250mm)
4.	Particle size packing	5 m
5.	Stationary phase	C-18 (Agilent)
6.	Mobile Phase	Methanol : Water (0.1% Acetic Acid PH adjusted 3.5 with OPA) 35 : 65
7.	Detection Wavelength	250 nm
8.	Flow rate	1 ml/min
9.	Temperature	25
10.	Sample size	20 l
11.	pH	3.5
12.	Run Time	15 min
13.	Filter paper	0.45 m

- **METHOD DEVELOPMENT OF HPLC:**

Table No.5: Selection of mobile Phase.

Sr.No.	Mobile Phase
1.	METHANOL+ Water OPA 0.1 % (90:10% v/v) 0.7ml 250 nm
2.	METHANOL+ Water OPA 0.1% (80:20 % v/v) 1ml 250 nm
3	METHANOL+ Water OPA 0.1% (70:30 % v/v) 1 ml 250 nm
4	METHANOL+Water OPA 0.1% (30:70 % v/v) 1 ml 250 nm
5	METHANOL+ Water OPA 0.1% (50:50 % v/v) 1ml 250 nm
6	ACETONITRILE+ Water OPA 0.05% (80:20 % v/v) 1.ml 250 nm sample in mobile phase
7	Methanol+ Water 0.1%ACETIC ACID (35:65 % v/v) 1 ml 250 nm

- **Selection of wavelength by UV-Visible Spectrophotometry:-**
- **Preparation of standard stock solution:-**
- **Fexofenadine standard stock solution : (Stock I)**

An accurately weighed quantity, 120 mg of Fexofenadine (FXD) was dissolved in methanol in a 50 ml volumetric flask and volume made up to 10.0 ml to produce a solution of 2400 ug/ml.

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- **Montelukast standard stock solution : (Stock II)**

An accurately weighed quantity, 10 mg of Montelukast (MTL) was dissolved in methanol in 50 ml volumetric flask and volume made up to 50.0 ml to produce a solution of 200 ug/ml.

- **Preparation of Stock Standard Combination Solution :(Stock III) [FXD + MTL]**

Accurately weight and transfer 120 mg Fexofenadine and Montelukast 10 mg working standard into 50 ml volumetric flask as about diluent methanol completely and make volume up to the mark with the same solvent to get 2400 µg/ml standard for Fexofenadine and 200 µg/ml for Montelukast (stock solution) and 15 min sonicate to dissolve it and remove the unwanted gas, further an aliquots portion of Fexofenadine and Montelukast stock solution in ratio of 1:12 were mixed in volumetric flask in 50 ml and volume was adjusted up to mark with mobile phase from the resulting solution 0.1ml was transferred to 10 ml volumetric flask and the volume was made up to the mark with Methanol :Water (0.1% Acetic Acid prepared in (3.5 ml Methanol: 6.5ml Water (0.1% Acetic Acid) solvent.

- **HPLC used for chromatographic condition applies on the Preparation of standard solution:-**

- **Preparation of std. Fexofenadine solution: (Stock I)**

From the freshly prepared standard stock solution (2400 ug/ml), 0.1-0.5 ml stock solution was pipetted out in 50 ml of volumetric flask and volume was made up to 50 ml with mobile phase to get final concentration of 24-120 ug/ml.

- **Preparation of std. Montelukast solution: (Stock II)**

From the freshly prepared standard stock solution (200ug/ml), 0.1-0.5 ml stock solution was pipetted out in 50 ml of volumetric flask and volume was made up to 50 ml with mobile phase to get final concentration 2-10 ug/ml.

- **Preparation of std. Fexofenadine and Montelukast solution :(Stock III)**

From the freshly prepared standard stock solution (2400 ug/ml) Fexofenadine, and (200 ug/ml) Montelukast , 0.1-0.5 ml stock solution was pipetted out in 10 ml of volumetric flask and volume was made up to 10 ml with mobile phase to get final concentration 24-120 ug/ml for Fexofenadine and Montelukast 2-10 ug/ml respectively.

- **Selection of mobile phase:**

Each mobile phase was vacuum degassed and filtered through 0.45µ membrane filter. The mobile phase was allowed to equilibrate until OPA by baseline was obtained. The standard solution containing mixture of Fexofenadine and Montelukast was run with different individual solvents as well as combinations of solvents were tried to get a good separation and stable peak. From the various mobile phases tried, mobile phase containing Methanol and Water (0.1% Acetic Acid ph 3.5) was selected since it gave sharp, well resolved peaks with symmetry within the limits and significant reproducible retention time for Fexofenadine and Montelukast.

- **Studies of Calibration plot :-**
- **Optimization of Chromatographic condition:**

The following chromatographic conditions were established by trial and error and were kept constant throughout the analysis.

Column : C18 (250 mm× 4.6mm)
Particle size packing : 5µm
Detection wavelength : 250 nm
Flow rate : 1.1 ml/min
Temperature : Ambient
Sample size : 20 µl
Mobile phase : Methanol: Water (0.1% Acetic acid ph 3.5) (435: 65)

- **Calibration Experiment:**
- **RP-HPLC Method :**

a) Preparation of Calibration curve standard:

The above standard stock solution (2400:200µg/ml) of Fexofenadine and Montelukast was diluted with mobile phase to yield Five calibration curve (cc) standards with concentrations of 24,10,15,20 and 25 µg/ml of Fexofenadine and 5,10,15,20 and 25 µg/ml of Montelukast.

- **UV-Spectrophotometric Method-**

a) Selection of detection Wavelength:

Standard solutions were scanned in the range of 200-400nm, against 10 ml Methanol and volume make with water solvent system as reference Fexofenadine and Montelukast were showed absorbance maxima (lambda max) at 250 nm. If Two Fexofenadine and Montelukast sample Interact with this point is called Isobestic point Then detection of wavelength in Isobestic point in

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250 nm were selection wavelength is HPLC Method can be used.

b) Calibration standard drug and regression equation data:

From the standard stock solution of Fexofenadine and Montelukast, different concentration were prepared respectively in the range of 24-120 µg/ml for Fexofenadine (Figure No: 19) and 2-4 µg/ml for Montelukast and measured at 220 nm and 284 nm.

c) Calibration runs and regression analysis:

These calibration standard solutions were analyzed in three replicates using the under mentioned chromatographic conditions.

- Analytical column: Agilent C18 Column (250mm x 4.6mm, 5µm particle size).
- Injection volume : 20µl.
- Flow rate : 1. ml/min.
- Mobile phase: Methanol: Water ((0.1% Acetic Acid) (35:65 % V/V).
- Detection : 250 nm

- **Validation of method for analysis of Fexofenadine and Montelukast**

The developed method was validated as per ICH guidelines.

Linearity: Linearity of an analytical method is its ability to elicit test results that are directly or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range.

Determination: The linearity of the analytical method is determined by mathematical treatment of test results obtained by analysis of samples with analyte concentrations across the claimed range. Area is plotted graphically as a function of analyte concentration.

Acceptance Criteria: The plot should be linear passing through the origin. Correlation Coefficient should not be less than 0.999.

- **Preparation of standard stock solution for linearity:**

Average weight of tablet sample (equivalent to 120 mg of Fexofenadine and 10 mg of Montelukast) were weighed and transferred to 50 mL volumetric flask & diluent was added to make up the volume. Sonicated for 10 min with occasional swirling. 0.1 ml of this solution diluted up to 10 ml volumetric flask with diluents was added to make up the volume.

Table No.6: Table of linearity for RP -HPLC Method

Concentration (µg/mL)	
Fexofenadine	**Montelukast**
24	2
48	4
72	6
96	8
120	10

Accuracy (recovery):

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. Accuracy may often the expressed as percent recovery by the assay of known added amounts of analyte. The accuracy of an analytical method is determined by applying the method to analyzed samples, to which known amounts of analyte have been added. The accuracy is calculated from the test results as the percentage of analyte recovered by the assay.

Acceptance Criteria:

Mean recovery should be in the range of 98-102%. The Relative Standard Deviation should not be more than 2.0%.

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Preparation of standard stock solution:

120 mg of Fexofenadine and 10 mg of Montelukast working standards were weighed and transferred to 50 mL volumetric flask & diluent was added to make up the volume 0.1 ml of this solution diluted upto 10 ml with diluent.

Accuracy :

The accuracy was determined by Fexofenadine and Montelukast (equivalent to 120 mg of Fexofenadine and 10 mg of Montelukast (80 %, 100 % and 120 % of the label claimed, respectively) to quantity equivalent to average weight of marketed tablets. This powder mixture containing 120 mg of Fexofenadine and 10 mg of Montelukast were triturated and then subjected to chromatographic analysis using the described method. The resulting mixtures were analyzed in triplicates over three days. The % recovery of added drug was taken as a measure of accuracy.

Table No. 7: Table of Accuracy for Rp-HPLC Method

Sample	Amount Added (mg)	
	Fexofenadine	Montelukast
Accuracy 80%	19.2	1.6
Accuracy 100%	24	2
Accuracy 120%	28.8	2.4

- **Repeatability:**

Precision of the system was determined with the sample of RP-HPLC for. Two replicates of sample solution containing 96 µg/ml of Fexofenadine and 8 µg/ml Montelukast were injected and peak areas were measured and %RSD was calculated is was repeated for two times.

- **Precision:**

Precision of an analytical method is the degree of agreement among Individual test results when the procedure is applied repeatedly to multiple Samplings of a homogenous sample. Precision of an analytical method is usually expressed as standard deviation or relative standard deviation. Also, the results obtained were subjected to one way ANOVA and within-day mean square and between-day mean square was determined and compared using F-test.

- **Result of Intraday and Inter day Precision studies on RP-HPLC method for Fexofenadine and Montelukast**
- **Intra-day precision:**

Sample solutions containing 120 mg of Fexofenadine and 10 mg of Montelukast three different concentration (24µg/ml, 72µg/ml, 120µg/ml Fexofenadine and (2 µg/ml, 6µg/ml, 10 µg/ml) Montelukast. Fexofenadine and Montelukast were analyzed three times on the same day and %R.S.D was calculated.

- **Inter-day precision:**

Sample solutions containing 120 mg of Fexofenadine and 10 mg of Montelukast three different concentration (24µg/ml, 72µg/ml, 120 µg/ml Fexofenadine and (µg/ml, 10µg/ml, 15µg/ml) Montelukast. Fexofenadine and Montelukast were analyzed three times on the different day and %R.S.D was calculated.

- **Acceptance criteria:**

The Relative Standard Deviation should not be more than 2% for test.

- **Preparation of standard stock solution:**

120 mg of Fexofenadine and 10 mg Montelukast working standards were weighed and transferred to 50 mL volumetric flask & diluent was added to make up the volume. 0.1 ml of this solution diluted up to 10 ml with diluent.

- **Robustness:**

The mobile phase composition was changed in (±1 ml/ min-1) proportion (Fig No:44,45) of Methanol: Water (0.1% ph Acetic

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Acid) the mobile phase composition and the flow rate was (±1ml/ min-1) and the change in detection wavelength (±1 ml/ min-1) and the effect of the results were examined. it was performed using 120 µg/ml and 10 µg/ml solution of Fexofenadine and Montelukast in duplicate.

- **Detection Limit**

Based on the S.D. of the response and the slope of calibration curve, the detection limit (DL) was calculated as , DL = 3.3σ/S
Where,
σ = the S.D. of the y-intercepts of regression lines.
S = the slope of the calibration curve.
The slope S may be estimated from the calibration curve and S.D. was used should be calculated from the y-intercepts of regression line in calibration curve.

- **Quantitation Limit**

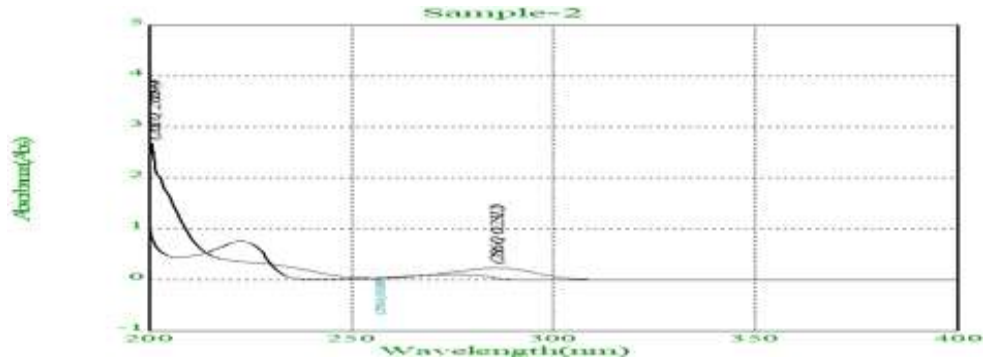
Based on the S.D. of the response and the slope of calibration curve, the quantitation limit (QL) was calculated as, QL = 10σ/S
Where,
σ = the S.D. of the y-intercepts of regression lines.
S = the slope of the calibration curve.
The slope S may be estimated from the calibration curve and S.D. was used should be calculated from the y-intercepts of regression line in calibration curve.

III. RESULT AND DISSCUSSION

As per the USP-XXVI system suitability tests were carried out on freshly prepared standard stock solution of Montelukast and Fexofenadine. These parameters signify good sensitivity, more ruggedness and robustness of the method.

Selection of wavelength

The standard solution of and Montelukast and Fexofenadine were separately scanned at different concentration in the range of 200-400 nm and the λmax was determined. The overlain spectrum of both the drugs was also run as shown in Figure. Hence the complete method was proceding with the wavelength 250 nm. **Iso-absorptive point of Fexofenadine and Montelukast**



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.

TABLE NO.8:Chromatographic behavior of Fexofenadine and Montelukast mobile phase of various compositions.

Sr No.	Mobile Phase	Retention Time (min)		Remark
		FXD	MTL	
1.	METHANOL+ Water OPA 0.1 % (90:10 % v/v) 0.7ml 250 nm	3.02	3.28	Merge peak

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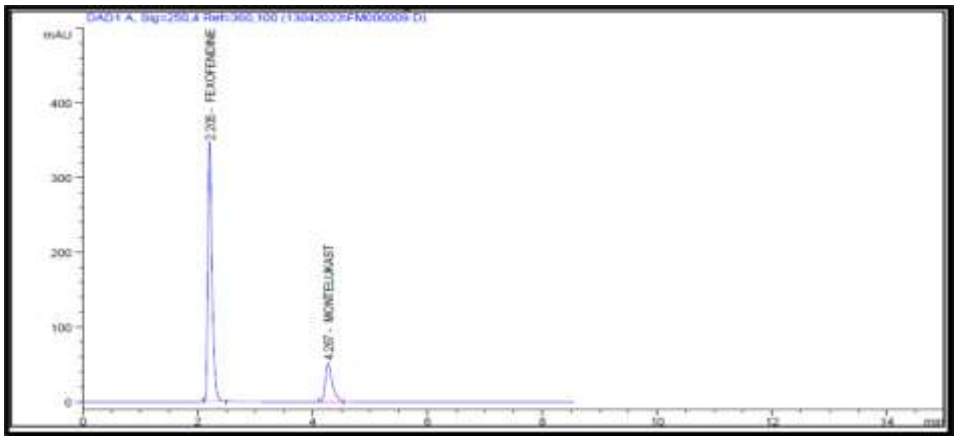
2	METHANOL+ Water OPA 0.1% (80:20 % v/v) 1ml 250 nm	3.54	4.23	Broad and merge peak
3	METHANOL+ Water OPA0.1% (70:30 % v/v) 1 ml 250 nm	4.74	6.74	Broad peak
4	METHANOL +Buffer OPA 0.1% (30:70 % v/v) 1 ml 250 nm	6.59	8.51	No Sharpe peak
5	ACETONITRILE +Water OPA 0.1% (50:50 % v/v) 1ml 250 nm	4.44	6.47	No peak
6	ACETONITRILE +Water OPA 0.05% (80:20 % v/v) 1.ml 250 nm	3.99	4.47	No sharp peak
7	**METHANOL+ Acetic Acid 0.1% (65:35 % v/v) 1 ml 250 nm**	**2.21**	**4.27**	**Resolve Peak and Sharp**



Chromatogram of standard Combination of Fexofenadine and Montelukast

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	2.205	1768.63770	5007	0.64	0.0000
2	4.267	437.82901	6488	0.59	12.45

In the standard mixture of Fexofenadine and Montelukast theoretical plates were found above 2000 i.e. for Fexofenadine 5007 and Montelukast 6488 at minimum RT 2.205 and 4.267 respectively.

METHOD VALIDATION

- **Linearity: -** Linearity of of Fexofenadine and Montelukast was observed in the range of 24-120µg/ml and 2-10µg/ml. Detection wavelength used was 250 nm.

The plot should be linear passing through the origin; Correlation Coefficient should not be less than 0.999.that concluded.

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Calibration graph of Montelukast and Fexofenadine for HPLC method

Regression Equation Data Y = mx+c	
Slope(m)	43.53 X
Intercept(c)	5.463
Correlation Coefficient	0.999

Regression Equation Data Y=mx+c	
Slope(m)	14.76x
Intercept(c)	10.64
Correlation Coefficient	0.999

Concentration µg/ml	**Area Montelukast**
2	95.8300
4	181.7900
6	271.5100
8	351.7200
10	438.0375

Concentration µg/ml	**Area Fexofenadine**
24	362.08
48	742.61
72	1064.11
96	1439.98
120	1768.61

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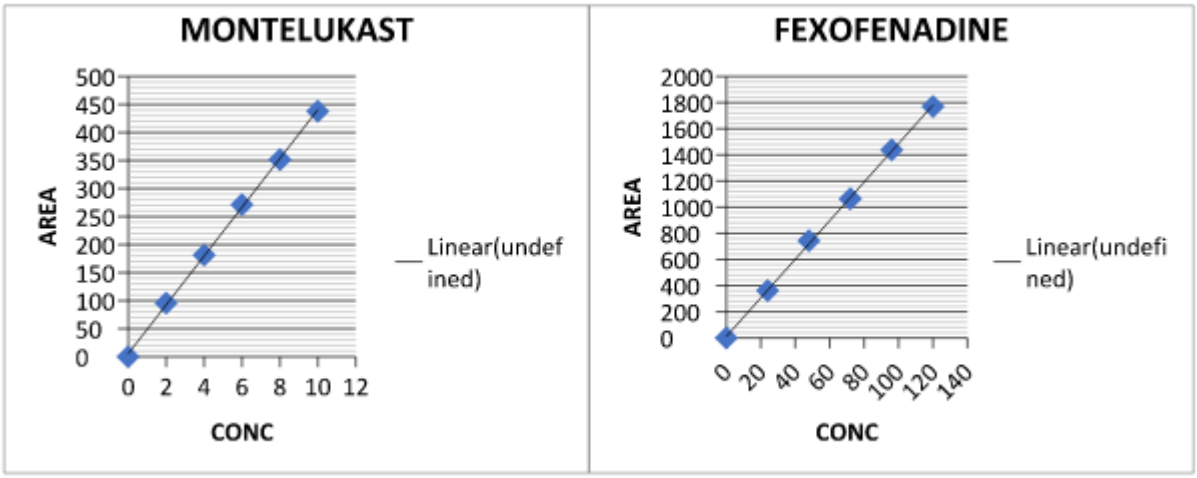

Linearity of Montelukast **Linearity of Fexofenadine**

- **ACCURACY -** Accuracy of RP-HPLC method is ascertained by recovery studies performed at different levels of concentrations (80%, 100% and 120%). The % recovery was found to be within 98-102%

TABLE NO.9: Result of Recovery data for Fexofenadine and Montelukast

METHOD	Drug	Level (%)	Amt. taken (µg/ml	Amt. Added (µg/ml	area Mean * ± S.D.	Amt. recovered Mean *±S.D.	%Recovery Mean *± S.D.
RP-HPLC Method	FXD	80%	24	19.2	43.32±0.076	19.32±0.076	100.64±0.40
		100%	24	24	47.99±0.078	23.99±0.078	99.96±0.33
		120%	2	28.8	52.75±0.081	28.75±0.081	99.84±0.28
	MTL	80%	2	1.6	3.61±0.008	1.61±0.008	100.54 ±0.51
		100%	2	2	4.00±0.016	2.00±0.016	100.06±0.79
		120%	2	2.4	4.39±0.016	2.39±0.016	99.59±0.67

***mean of each 3 reading for RP-HPLC method**

TABLE NO.10:Statistical Validation of Recovery Studies Fexofenadine and Montelukast

METHOD	Level of Recovery (%)	Drug	Mean % Recovery	Standard Deviation*	% RSD
Rp-HPLC Method	80%	FXD	100.64	0.40	0.39
		MTL	100.54	0.51	0.51
	100%	FXD	99.96	0.33	0.33
		MTL	100.06	0.79	0.79

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	120%	FXD	99.84	0.28	0.28
		MTL	99.59	0.67	0.68

***Denotes average of three determinations for RP-HPLC.**

- **REPETABLITY** - Repeatability studies on RP-HPLC method for Fexofenadine and Montelukast was found to be ,the %RSD was less than 2%, which shows high percentage amount found in between 98% to 102% indicates the analytical method that concluded.

TABLE NO.11&12: Repeatability studies on RP-HPLC for Fexofenadine and Montelukast

Sr.No.	Concentration of FXD (mg/ml)	Peak area	Amount found (mg)	% Amount found
1	96	1443.683	94.02	101.06
2	96	1441.572		
3	96	1445.626		
		Mean	1443.627	
		SD	2.02	
		%RSD	0.13	

Sr.No.	Concentration of MTL (mg/ml)	Peak area	Amount found (mg)	% Amount found
1	8	352.5645	7.98	100.40
2	8	352.8428		
3	8	355.6455		
		Mean	353.684	
		SD	1.70	
		%RSD	0.48	

PRECISION - Intraday and Inter day Precision studies on RP-HPLC for Fexofenadine and Montelukast which shows the high precision % amount in between 98% to 102% indicates to analytical method that concluded.

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TABLE NO.13: Result of Intraday and Inter day Precision studies on RP-HPLC for Fexofenadine and Montelukast

METHOD	Drug	Conc^{n} (µg/ml)	Intraday Precision		Interday Precision	
			Mean± SD	%Amt Found	Mean± SD	%Amt Found
Rp - HPLC METHOD	FXD	24	23.80± 0.15	99.16	23.78±0.70	101.25
		72	71.20±1.18	98.89	71.22±1.11	98.92
		120	118.87±7.23	99.06	119.19±3.85	99.32
	MTL	2	2.04± 0.44	102.05	2.06±1.53	102.96
		6	6.10±2.06	101.62	6.08±0.46	101.33
		10	9.97±0.51	99.69	10.03±1.01	100.25

***Mean of each 3 reading for RP-HPLC method**

ROBUSTNESS - The changes were did flow rate (±1 ml/ min-1), PH of mobile phase composition (±1 ml/ min-1),and Wavelength (±1 ml/ min-1) .%RSD for peak area was calculated which should be less than 2%.the result shown in analytical method that concluded.

Table No.14 Result of Robustness Study of Fexofenadine

Parameters	Conc. (µg/ml)	Amount of detected (mean ±SD)	%RSD
Chromatogram of flow change 0.9 ml	120	1975.98±1.17	0.06
Chromatogram of flow change 1.1 ml	120	1609.10±1.63	0.10
Chromatogram of comp change 34 MEOH +66 WATER	120	1755.6±0.96	0.05
Chromatogram of comp change 36 Methanol+ 64 WATER	120	1786.93±2.01	0.11
Chromatogram of wavelength change -249 nm	120	1768.9±9.44	0.53
Chromatogram of wavelength change -251 nm	120	1786.02±2.46	0.14

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International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 4 July-Aug 2023, pp: 653-666 www.ijprajournal.com ISSN: 2249-7781

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Chromatogram of wavelength change -249 nm	10	1768.9±9.44	0.53
Chromatogram of wavelength change -251 nm	10	1786.02±2.46	0.14

Table No.15 Result of Robustness Study of Montelukast

LIMIT DETECTION

The LOD is the lowest limit that can be detected. Based on the S.D. deviation of the response and the slope The limit of detection (LOD) may be expressed as:

LOD = 3.3 (SD)/S

Where, SD = Standard deviation of Y intercept
S = Slope

Limit of detection = 3.3 X 1.08/14.76= 0.241109 (µg/mL)
Limit of Quantitation = 10 X 1.08 /14.76= 0.73063 (µg/mL)
The LOD and LOQ of Fexofenadine was found to be **0.24110** (µg/mL) and **0.73063** (µg/mL), analytical method that concluded.

LIMIT QUANTIFICATION

The LOQ is the lowest concentration that can be quantitatively measured. Based on the S.D. deviation of the response and the slope,
The quantitation limit (LOQ) may be expressed as:
LOQ = 10 (SD)/ S
Where, SD = Standard deviation Y intercept
S = Slope
Limit of Detection = 3.3X0.28/43.53=0.02105 (µg/mL)
Limit of Quantitation = 10 X0.28/ 43.53=0.06380 (µg/mL)
The LOD and LOQ of Montelukast was found to be **0.02105** (µg/mL) and **0.0638** (µg/mL) analytical method that concluded.

IV. CONCLUSION

Simple, rapid, accurate and precise RP-HPLC have been developed and validated for the routine analysis of Fexofenadine and Montelukast in API and formulation. This methods are suitable for the simultaneous determination of Fexofenadine and Montelukast in multi-component formulations without interference of each other. The developed methods are recommended for routine and quality control analysis of the investigated drugs in two component pharmaceutical preparations. The amount found from the proposed methods was in good agreement with the label claim of the formulation. Also the value of standard deviation and coefficient of variation calculated were satisfactorily low, indicating the suitability of the proposed methods for the routine estimation of tablet dosage forms.

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