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SIMULTANEOUS ESTIMATION OF METHOD DEVELOPMENT AND VALIDATION OF MONTELUKAST AND FEXOFENADINE BY USING REVERSE PHASE-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD

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ABSTRACT
A simple, specific, precise and accurate Stability indicating RP-HPLC method for simultaneous estimation of Montelukast and Fexofenadine in its pure and pharmaceutical dosage form has been developed and validated as per ICH Guidelines. The separation was achieved by Phenomenex Gemini ODS C18 (4.6mm×250mm) 5µm column and Acetonitrile: Methanol: Water (55:25:20% v/v) used as mobile phase, at a flow rate of 1 ml/min. Detection was carried out at 229 nm. Retention time of Montelukast and Fexofenadine were found to be 2.157 min and 3.631 min respectively. The method has been validated for linearity, accuracy, precision, robustness, LOD and LOQ. Linearity observed for Montelukast 10 – 30µg/ml and for Fexofenadine 6 - 14µg/ml. Developed method was found to be accurate, precise and simple, specific for simultaneous estimation of Montelukast and Fexofenadine in pure form and their Combined Pharmaceutical Dosage Form. The precision results are not more than 2%. The proposed method was successfully applied for the simultaneous estimation of both the drugs in commercial combined dosage form.

INTRODUCTION
Analytical chemistry[1] is the part of science engaged with isolating, distinguishing and deciding the general measures of the segments making up an example of issue. It is principally associated with the subjective recognizable proof or recognition of mixes and the quantitative estimation of the substances present in mass. HPLC[2] is a kind of fluid chromatography that utilizes a fluid portable stage and an in all respects finely partitioned stationary stage. So as to get palatable stream rate fluid must be pressurized to a couple of thousands of pounds for every square inch.

The rate of dispersion of medications among Stationary and versatile stage is constrained by dissemination process. In the event that dissemination is limited quicker and viable detachment can be accomplished. The systems of superior fluid chromatography are alleged in view of its improved exhibition when contrasted with old style section chromatography propels in segment chromatography into high speed, efficient, precise and profoundly settled technique for division.[3]

Montelukast[4],[5],[6] is chemically designed as (R,E)-2-(1-((1-(3-(2-(7-Chloroquinolin-2-yl)vinyl)phenyl)-3-(2-(2-hydroxypropan-2-yl)phenyl)propylthio)methyl)cyclopropyl)acetic acid, Montelukast is used as Anti-Asthmatic Agents[10] and Fexofenadine[7],[8],[9] is a chemically 2-(4-{1-hydroxy-4-[4-(hydroxyl diphenyl methyl) piperidin-1-yl] butyl} phenyl)-2-methyl propanoic acid and is used as Antihistamine drug.[11] Present work is intended to build up another, straightforward, quick, fast, precise, proficient and reproducible RP-HPLC strategy for the concurrent investigation of Montelukast and Fexofenadine. The created strategy will be approved by ICH rules.

MATERIALS AND METHODS
The working standards of Montelukast and Fexofenadine were procured from Sura Labs hyderabad,. Methanol and Water HPLC grade were obtained from LICHROSOLV (MERCK). Acetonitrile for HPLC obtained from Merck**.** HPLC (WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detector, pH meter (Lab

India), Weighing machine (Sartorius), glassware (Borosil), Digital ultra sonicator (Labman) was used.

Preparation of standard solution: Accurately weigh and transfer 10 mg of Montelukast and Fexofenadine working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 0.2ml of Montelukast and 0.1ml of Fexofenadine from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol. Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization: Initially the mobile phase tried was Methanol: Water and ACN: Water with varying proportions. Finally, the mobile phase was optimized to Acetonitrile and water in proportion 75:25 v/v respectively.

Optimization of Column: The method was performed with various C18columns like Symmetry, X terra and ODS column. Phenomenex Gemini C18 (4.6×250mm) 5µ was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Optimized Chromatographic conditions

Instrument used	:	Waters Alliance 2695 HPLC with PDA Detector 996 model.
Temperature	:	36ºC
Column	:	Phenomenex Gemini ODS C18 (4.6mm×250mm) 5µm
Mobile phase	:	Acetonitrile: Methanol: Water (55:25:20% v/v)
Flow rate	:	1ml/min
Wavelength	:	229nm
Injection volume	:	10µl
Run time	:	6 minutes

METHOD VALIDATION

Preparation of mobile phase: Accurately measured 750ml of Acetonitrile (75%) of and 250ml of HPLC Water (25%) were mixed and degassed in a digital ultra sonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration. The Mobile phase was used as the Diluent.

System suitability: The standard solution was injected for five times and measured the area for all five injections in HPLC. The % RSD for the area of five replicate injections was found to be within the specified limits.

Drug Specificity study

Preparation of Sample Solution: Take average weight of Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Montelukast and Fexofenadine sample into a 10mL clean dry volumetric flask and add about 7mL of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Filter the sample solution by using injection filter which contains 0.45µ pore size. Further pipette out 0.2ml of Montelukast and 0.1ml of Fexofenadine from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent. Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

Preparation of drug solutions for linearity: Accurately weigh and transfer 10 mg of Montelukast and Fexofenadine working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I to V solutions: Pipette out 0.1ml (10ppm), 0.15ml (15ppm), 0.2 (20ppm), 0.25ml (25ppm), 0.3ml (30ppm) of the Montelukast and 0.06ml (6ppm), 0.08ml (8ppm), 0.1ml (10ppm), 0.12ml (12ppm), 0.14ml (14ppm) of the Fexofenadine from the above stock solutions in to a 10ml of five volumetric flask and dilute the solution, sonicate for 10minutes. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Precision

Repeatability: The standard solution was injected for five times and measured the area for all five injections in HPLC. The % RSD for the area of five replicate injections was found to be within the specified limits.

Intermediate precision: To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Accuracy: Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Montelukast and Fexofenadine and calculate the individual recovery and mean recovery values.

Robustness: The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.

Effect of Variation of flow conditions: The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

Effect of Variation of mobile phase organic composition: The sample was analyzed by variation of mobile phase i.e. Acetonitrile: Methanol and water was taken in the ratio and 50:30:20, 60:20:20 instead of 55:25:20 remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

RESULTS AND DISCUSSION

After repeating the same conditions got proper peaks, base line is good and we can go for repeatability. Therefore, separations of two peaks, base line, peak symmetry, resolution are proper.

Blank

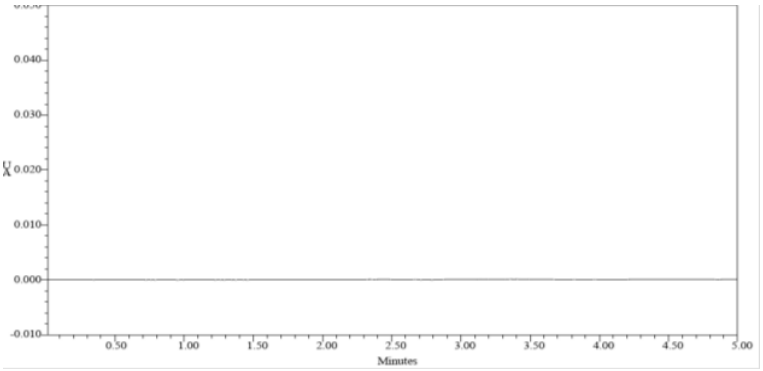

Fig. 1: Chromatogram showing blank (mobile phase preparation).

System suitability: system suitability results were shown in the table, values are within the acceptance criteria (% RSD NMT 2).

Table 1: Results of system suitability.

Drug	Peak Name	Injection 1	Injection 2	Injection 3	Injection 4	Injection 5	
Fexofenadine	RT	3.674	3.631	3.625	3.692	3.629	**Mean area:** 128649.6 **Std. Dev.:** 127.8761 **%RSD:** 0.099399
	Area (µV*sec)	128585	128698	128754	128457	128754	
	Height (µV)	85462	85745	85475	85687	85745	
	USP Plate count	9568	9578	9587	9568	9536	
	USP Tailing	1.8	1.9	1.8	1.9	1.8	
Montelukast	RT	2.152	2.157	2.141	2.133	2.166	**Mean area:** 62510 **Std. Dev.:** 139.6872 **%RSD:** 0.223464
	Area (µV*sec)	62356	62584	62365	62587	62658	
	Height (µV)	7568	7522	7586	7548	7542	
	USP Plate count	8569	8575	8536	8594	8514	
	USP Tailing	1.9	1.9	1.8	1.8	1.9	

Specificity: The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. Analytical method was tested for specificity to measure accurately Montelukast and Fexofenadine in drug product.

Linearity

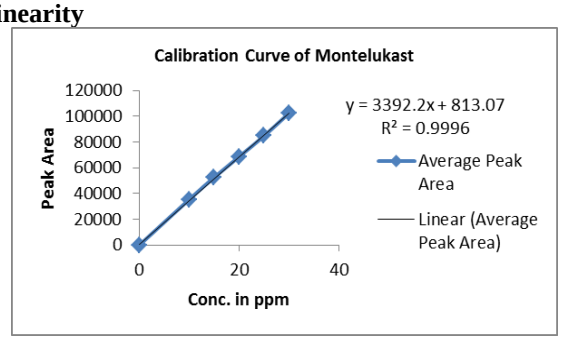

Fig. 2: Calibration Curve of Montelukast.

The plot of Concentration (x) versus the Average Peak Area (y) data of Montelukast is a straight line. Correlation Coefficient (r) is 0.99, and the intercept is 813.0. These values meet the validation criteria.

Table 2: Linearity study.

Drug	Concentration µg/ml	Average Peak Area
Fexofenadine	6	85987
	8	102587
	10	128569
	12	135847
	14	146859
Montelukast	10	35479
	15	52598
	20	68654
	25	84816
	30	102548

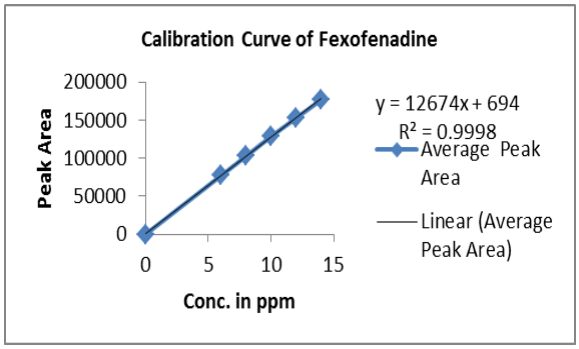

Fig. 3: Calibration Curve of Fexofenadine.

The plot of Concentration (x) versus the Average Peak Area (y) data of Fexofenadine is a straight line. Correlation Coefficient (r) is 0.99, and the intercept is 694. These values meet the validation criteria.

Precision: The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Repeatability: Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Table 3: Results of Repeatability.

Drug	Peak Name	Injection 1	Injection 2	Injection 3	Injection 4	Injection 5	
Fexofenadine	RT	3.603	3.608	3.600	3.696	3.629	**Mean area:** 128585 **Std. Dev.:** 102.2644 **%RSD:** 0.079531
	Area (µV*sec)	128568	128564	128475	128564	128754	
	Height (µV)	8568	8547	8598	8547	8564	
	USP Plate count	9542	9546	9578	9528	9575	
	USP Tailing	1.8	1.7	1.8	1.8	1.7	
Montelukast	RT	2.157	2.159	2.186	2.160	2.170	**Mean area:** 62356.6 **Std. Dev.:** 2.073644 **%RSD:** 0.003325
	Area (µV*sec)	62355	62354	62357	62358	62359	
	Height (µV)	7586	7584	7524	7534	7598	
	USP Plate count	8569	8542	8574	8534	8568	
	USP Tailing	1.9	1.9	1.9	1.8	1.8	

Table 4: Results of Intermediate Precision.

DRUG	Peak Name	Injection 1	Injection 2	Injection 3	Injection 4	Injection 5	
Fexofenadine	RT	3.611	3.623	3.684	3.697	3.684	**Mean area:** 130598.2 **Std. Dev.:** 193.8942 **%RSD:** 0.148466
	Area (µV*sec)	130254	130584	130658	130587	130659	
	Height (µV)	88952	88569	88745	88951	88254	
	USP Plate count	9785	9722	9763	9715	9725	
	USP Tailing	1.9	1.9	1.8	1.9	1.8	
Montelukast	RT	2.198	2.196	2.178	2.142	2.177	**Mean area:** 61702.17 **Std. Dev.:** 179.8915 **%RSD:** 0.291548
	Area (µV*sec)	61587	61593	61587	61985	61586	
	Height (µV)	7785	7798	7752	7746	7792	
	USP Plate count	8859	8854	8826	8849	8841	
	USP Tailing	1.9	1.9	1.8	1.9	1.8	

Accuracy: Accuracy at different concentrations (50%, 100%, and 150%) was prepared and the % recovery was calculated.

Table 5: The Accuracy results.

Drug	% Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
Montelukast	50%	34851.33	10.034	10	100.340	100.348%
	100%	68924.33	20.079	20	100.395	
	150%	102889	30.093	30	100.310	
Fexofenadine	50%	64596	5.041	5	100.820	100.492%
	100%	127586	10.011	10	100.110	
	150%	191854	15.082	15	100.546	

Limit of Detection: The detection limit of an individual analytical procedure is the lowest amount (1.1µg/ml of Montelukast and 2.3µg/ml of Fexofenadine) of analyte in a sample which can be detected but not necessarily quantities as an exact value.

Limit of Quantitation: The quantitation limit of an individual analytical procedure is the lowest amount (3.2µg/ml of Montelukast and 6.4µg/ml of Fexofenadine) of analyte in a sample which can be quantitatively determined.

Robustness: The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Montelukast and Fexofenadine Acid. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard and samples of Montelukast and Fexofenadine Acid were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor and plate count.

Table 6: Results for Robustness.

DRUG	Parameter used for sample analysis	Actual Flow rate of 1.0 mL/min	Less Flow rate of 0.9 mL/min	More Flow rate of 1.1 mL/min	Less organic phase	More organic phase
Fexofenadine	Peak Area	128568	134515	126854	124512	122564
	Retention Time	3.631	4.498	3.505	4.504	3.512
	Theoretical plates	9542	9254	9126	9245	4954
	Tailing factor	1.8	1.7	1.6	1.4	1.6
Montelukast	Peak Area	62354	65658	61245	60448	63698
	Retention Time	2.157	2.210	2.184	2.200	2.172
	Theoretical plates	8564	8154	8264	8415	8365
	Tailing factor	1.9	1.58	1.69	1.78	1.79

CONCLUSION

In the present examination, a straightforward, touchy, exact and precise RP-HPLC strategy was created for the quantitative estimation of Montelukast and Fexofenadine mass medication and pharmaceutical measurements structures. This technique was straightforward, since weakened examples are legitimately utilized with no starter substance derivatisation or filtration steps. Acetonitrile: Methanol: Water (55:25:20% v/v) was picked as the portable stage. The dissolvable framework utilized in this strategy was prudent. The %RSD esteems were inside 2 and the technique was observed to be exact. The outcomes communicated in Tables for RP-HPLC strategy was promising. The RP-HPLC technique is increasingly delicate, exact and exact contrasted with the Spectrophotometric strategies. This technique can be utilized for the standard assurance of Montelukast and Fexofenadine in mass medication and in Pharmaceutical dose structures.

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