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Development and validation of HPTLC method for simultaneous estimation of montelukast sodium and fexofenadine hydrochloride in combined dosage form

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ABSTRACT

A simple, precise, specific and accurate high performance thin layer chromatographic method has been developed for the simultaneous determination of Fexofenadine hydrochloride (FEXO) and Montelukast sodium (MONT) in pharmaceutical dosage form. The separation was carried out on Merck HPTLC aluminum plates of silica gel G60 F254, (20 × 10 cm) with 250 µm thickness using ethyl acetate: methanol: ammonia (30%) (7: 3: 0.5, v/v/v/v) as mobile phase. HPTLC separation of the two drugs followed by densitometric measurement was carried out in the absorbance mode at 215 nm. The drugs were resolved satisfactorily with R_f values of 0.84 ± 0.01 and 0.24 ± 0.01 for MONT and FEXO, respectively. The linear regression analysis data for the calibration plots showed good linear relationship with R^2=0.9988 and 0.9995 for FEXO and MONT, respectively in the concentration range of 1800-9000 ng/spot for FEXO and 150-750 ng ng/spot for MONT. The method was validated for accuracy, precision, specificity and robustness. The limit of detection and quantitation were 100.6079 and 304.8726 ng/spot, respectively for FEXO and 40.0191 and 121.8456 ng/spot, respectively for MONT. The proposed developed HPTLC method can be applied for identification and quantitative determination of FEXO and MONT in bulk drug and drug formulation.

Keywords: fexofenadine hydrochloride; montelukast sodium; HPTLC; validation.

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 empiricalformula is $C_{32}H_{39}NO_4$•HCl[1-5]
- 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 $C_{35}H_{35}ClNO_3S.Na$[1-5]
- 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[6-9], spectrofluorometry[10-12], dissolution[13], RP-HPLC[14-19] , and similarly for estimation Montelukast sodium in dosage form are spectrophotometry[20-22], spectrofluorometry[23], LC-MS[24-25], RP-HPLC[26-30] and HPTLC[31-33].

- But, there is no any analytical method has been reported yet for combinaton 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.

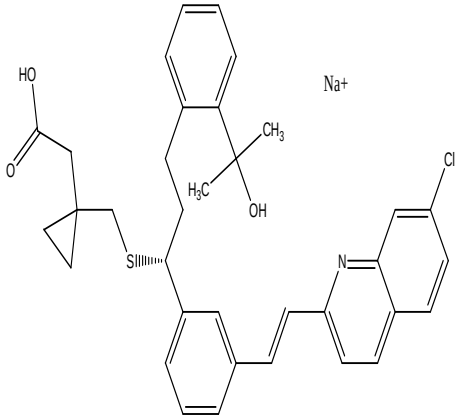

Fig. 1: Structural of Montelukast sodium

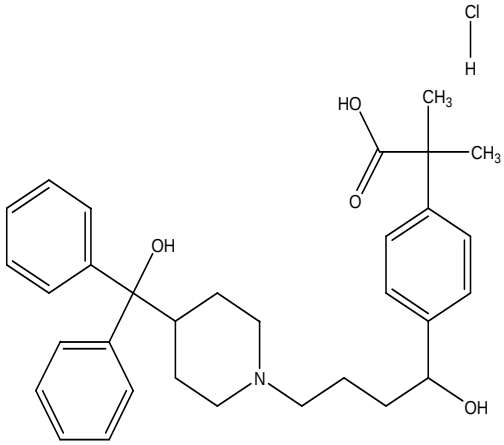

Fig. 2: Structural of fexofenadine HCl

MATERIALS AND METHODS

2.1. Materials:

Working standards of pharmaceutical grade FEXO (99.80 %, w/w) and MONT (99.9 %, w/w) were obtained as gift samples from Ami life science, Baroda and Zydus cadila, Ahmedabad. All chemicals and reagents of analytical grade were purchased from Merck Chemicals, Mumbai, India.

2.2. Selection of analytical wavelength:

Stock solutions of drugs were prepared in methanol separately. UV spectrum of 100µg/mL of individual drug was taken. Further, in situ HPTLC spectral overlain of FEXO and MONT was taken.

2.3. Instrumentation and chromatographic conditions:

The HPTLC plates were prewashed with methanol and activated at 110 ^{o}C for 5 min prior to chromatography. The samples were spotted in the form of bands 6 mm width with a Camag 100 microlitre sample syringe (Hamilton, Bonaduz, Switzerland) on silica gel precoated HPTLC aluminum plate 60 F254, [(20 × 10 cm) with 250 µm thickness; E. Merck, Darmstadt, Germany, supplied by Anchrom Technologies, Mumbai] using a Camag Linomat 5 applicator (Switzerland). A constant application rate of 0.1µL/sec was used and the space between two bands was 7 mm. Linear ascending development was carried out in 20 cm × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The mobile phase was consisted of ethyl acetate: methanol: ammonia (30%) (7: 3: 0.5, v/v/v) and 20 mL was used per chromatography run. The optimized chamber saturation time with mobile phase was 30 min using saturation pads at room temperature (25 ^{o}C ± 2). The length of chromatogram run was 80 mm and run time was 20 min. Densitometric scanning was performed using a Camag TLC scanner III in the reflectance-absorbance mode and operated by winCATS software (V1.1.4, Camag). The slit dimension was kept at 5mm × 0.45 mm and the scanning speed was 10 mm/sec. The source of radiation used was a deuterium lamp emitting a continuous UV spectrum between 200 and 400 nm. All determinations were performed at ambient temperature with a detection wavelength of 215 nm. Concentrations of the compound chromatographed were determined from the intensity of the diffused light. Evaluation was by peak areas with linear regression.

2.4. Standard solutions and calibration graphs:

Mixed stock standard solution containing 1800 mg/mL of FEXO and 150 mg/mLof MONT was prepared in methanol by dissolving 180 mg of FEXO and 15 mg of MONT in 100 mL methanol. Mixed stock standard solution

was further diluted with methanol to obtain working standard solutions in a concentration range of 1800-9000 ng/spot for FEXO and 150-750 ng/spot for MONT. Each concentration was applied six times on the HPTLC plate. The plate was then developed using the previously described mobile phase. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs. Linear calibration curves were generated using least-squares linear-regression analysis.

2.5. Sample preparation:

Twenty Tablets were weighed and finely powdered. The powder equivalent to 900 mg (FEXO) of tablet formulation were accurately weighed and transferred to volumetric flask of 50 mL capacity. 15mL of methanol transferred to volumetric flask and sonicated it for 5 mins. The flask was shaken and volume was made up to the mark with methanol.

The above solution was carefully centrifuged at 4000 rpm for 15 min. It was filtered through vacuum filter using Whatman filter paper (No.41). The aliquot (1.0 mL) was transferred into 10 mL volumetric flask. and volume was made up to the mark with methanol to give a solution containing 150 µg/mL of MONT and 1800 µg/mL of FEXO. The plate was developed in the previously described chromatographic conditions. The peak area of the spots were measured at 215 nm for FEXO and MONT, respectively and the concentrations in the samples were determined using multilevel calibration developed on the same plate under the same conditions using linear regression equation.

2.6 VALIDATION PARAMETER OF THE DEVELOPED METHODS:-

2.6.1 Linearity

The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in sample within a given range. The range of analytical method is the interval between the upper and lower levels of analyte that have been demonstrated to be determined within a suitable level of precision, accuracy and linearity.

Standard mixture solution of MONT and FEXO having concentration of 150 to 750 ng/spot for MONT and 1800 to 9000 ng/spot for FEXO were spotted and developed as described in proposed method. Developed plates were subjected to densitometric measurements in absorbance mode at wavelength 215.0 nm using Camag TLC Scanner 3.

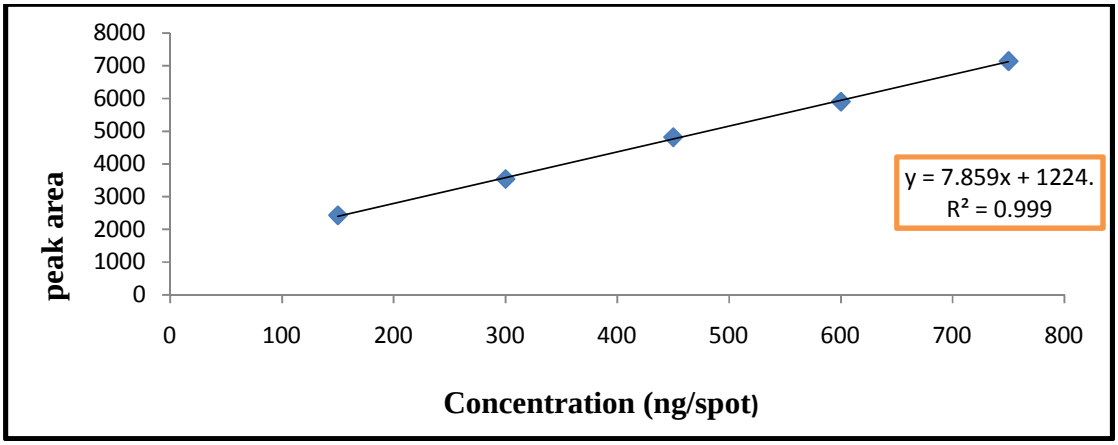

Figure 3: Calibration curve for Montelukast sodium

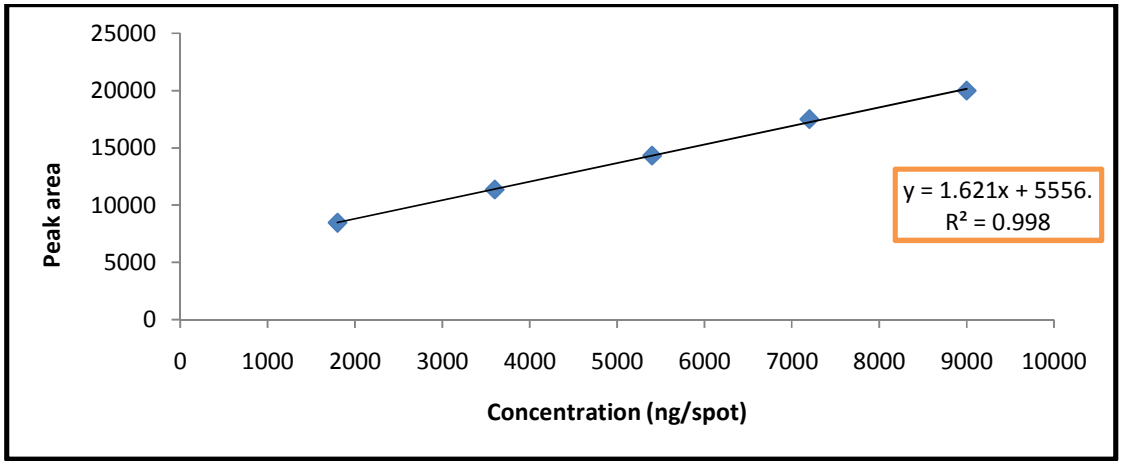

Figure.4: Calibration curve for Fexofenadine hydrochloride

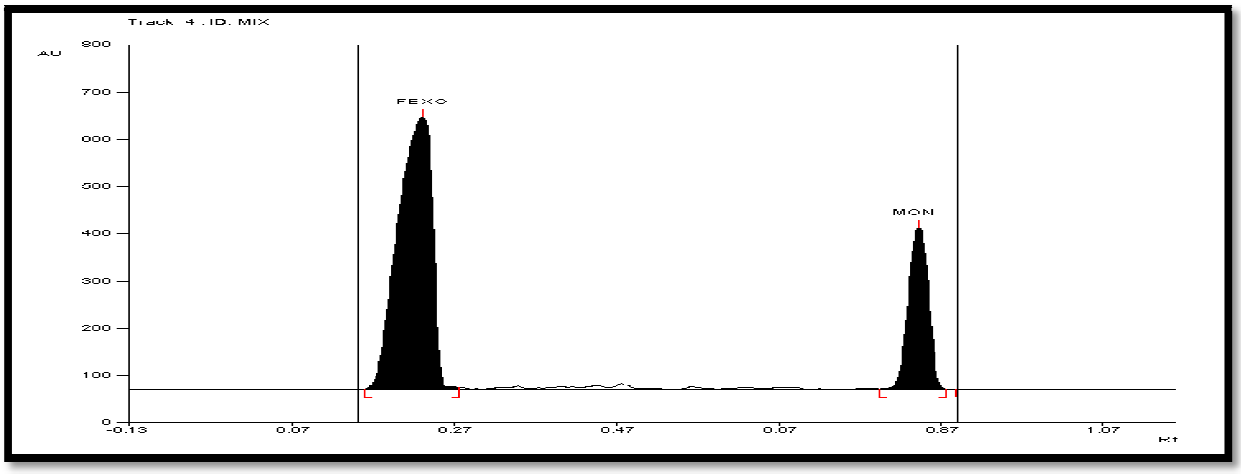

Figure 5: Chromatogram of Standard mixture Fexofenadine hydrochloride and Montelukast sodium in Ethyl acetate : Methanol : Ammonia (30%) (7: 3 : 0.5 v/v/v)

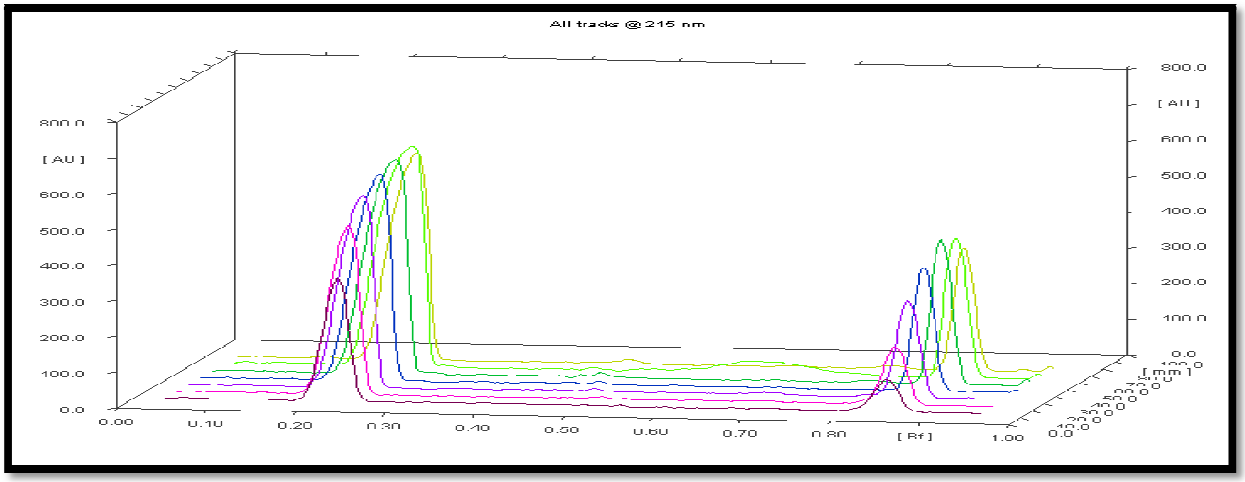

Figure 6: Three Dimension Chromatogram of calibration curve.

2.6.2 Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples. It provides an indication of random error results and was expressed as coefficient of variation(CV).

Intra and inter day precision

Intraday and interday precision was determined in terms of % RSD. Intraday precision was determined by analyzing in combined solution their respective calibration range for five times in the same day. Interday precision was determined by analyzing MONT and FEXO in for five days.

⇒**Procedure for Intraday Precision:** From combined standard solution of MONT and FEXO 150+1800ng/spot, 450+5400 ng/spot, 750+9000 ng/spot were spotted 5 times on same day and developed as described in proposed method. Developed plates were subjected to densitometric measurements in absorbance mode at wavelength 215.0 nm using Camag TLC Scanner 3 and % RSD was calculated.

⇒ **Procedure for Interday Precision:** From combined standard solution of MONT and FEXO 150+1800ng/spot, 450+5400 ng/spot, 750+9000 ng/spot were spotted 5 times on different days and developed as described in proposed method. Developed plates were subjected to densitometric measurements in absorbance mode at wavelength 215.0 nm using Camag TLC Scanner 3 and % RSD was calculated.

2.6.3 Accuracy

Accuracy may often be expressed as percentage recovery. It was determined by calculating the recovery of MONT and FEXO by application of the analytical method to mixtures of the drug product contents to which known amount of analyte have been added within the range ofthe method.

Tablet formulation solution:-

Weigh accurately 900 mg equivalent weight of tablet powder and dissolved in 50 mL of methanol to get concentrations 1500 µg/mL of MONT and 1800 µg/mL of FEXO. from this solution 1 mL was transferred into a series of 10 ml volumetric flask i.e. 80%, 100%, 120%.

Standard stock solution:-

Accurately weigh 37.5mg of MONT and 450 mg of FEXO standard were transferred to 50 mL of volumetric flask. The volume was adjusted to the mark with methanol. Thus final solution of mixture of MONT and FEXO obtained contain 750+9000 µg/mL. from this solution 1.6 mL, 2.0 mL, 2.4 mL were transferred into a series of above 10 ml volumetric flask. Samples were spotted and developed as described in proposed method. Developed plates were subjected to densitometric measurements in absorbance mode at wavelength 215.0 nm using Camag TLC Scanner 3.The mean % recovery from of peak areas calculated.

2.6.4 Specificity

Specificity of the method was determined by means of complete separation of pure drugs in the presence of other excipients normally present in the formulation. The specificity of the method was ascertained by peak purity profiling studies. Peak purity of MONT and FEXO was assessed by comparing their respective spectrum at peak start (S), peak apex (M) and peak end (E) position of the spots.The peak purity was determined on winCATS software using statistical equation.

2.6.5 Selectivity

Selectivity is the procedure to detect qualitatively the analyte in presence of components that may expected to be present in the sample matrix. commonly used excipients present in selected tablet formulation were spiked into a preweighed quantity of drugs. The absorbance was measured, and calculations determined the quantity of the drugs.

2.6.6 Limit of Detection (LOD)

The L.O.D. was estimated from the set of 5 calibration curves.

$LOD = 3.3 \times (S.D./Slope)$

Where,

S.D. = Standard deviation of the Y- intercepts of the 5 calibration curves.

Slope = Mean slope of the 5 calibration curves.

LOD of MONT and FEXO is described in table 1

2.6.7 Limit of Quantification (LOQ)
The L.O.Q. was estimated from the set of 5 calibration curves.

LOQ = 10 × (S.D./Slope)
Where,
S.D. = Standard deviation of the Y- intercepts of the 5 calibration curves.
Slope = the mean slope of the 5 calibration curves.

LOQ of MONT and FEXO is described in table 1

2.6.7 Robustness
The robustness was studied by evaluating the effect of small but deliberate variations in the chromatographic conditions. Following the introduction of small changes in the mobile phase composition (± 0.1 mL for each component), the effect on the results was examined. Mobile phases having different compositions, e.g.: ethyl acetate: methanol: ammonia (30%) (7: 3: 0.5, v/v/v), (7.1: 3: 0.5, v/v/v), (7: 3.1: 0.5, v/v/v), (7: 3.0: 0.6, v/v/v) were tried and chromatograms were run. The amount of mobile phase was varied over the range of ±5%. The time from spotting to chromatography and from chromatography to scanning was varied by +10 min. The robustness of the method was determined at three different concentration levels of 1800,5400, 9000 ng/spot for FEXO and 150, 450, 750 ng/spot for MONT.

RESULTS AND DISCUSSION

Under experimental conditions described, calibration curve, assay of tablets, recovery studies, precision studies, LODs & LOQs were performed. Using appropriate dilutions of standard stock solution, the two solutions were scanned separately. A critical evaluation of proposed method was performed by statistical analysis of data where slope, intercept, correlation coefficient were studied. Beer's law is obeyed in the concentration range 150-750 ng/spot for MONT and 1800-9000 ng/spot for FEXO and correlation coefficient of 0.9988 and 0.9995 for FEXO and MONT respectively. The proposed method was also evaluated by the assay of commercially available tablets containing FEXO and MONT (n = 6). The % assay was found to be 99.78 % for FEXO and 98.52 % for MONT. The accuracy and reproducibility is evident from the data as results are close to 100 % and standard deviation is low.

TABLE 1: Regression analysis data and summary of validation parameter for the proposed method

Sr. No.	Parameters	Results	
		Montelukast sodium	**Fexofenadine HCl**
1	Linearity Range	150-750 ng/spot	1800-9000 ng/spot
2	Regression equation	y = 7.8597x + 1224.8	y = 1.6219x + 5556.9
3	Correlation coefficient (R^2)	0.9995	0.9988
4	% Recovery ± SD	99.32 ± 0.7549	99.29 ± 0.1625
5	Precision (%RSD) Interday	0.8179	0.7880
	Intraday	0.3339	0.3989
6	LOD (ng/spot)	40.0191	100.6079
7	LOQ (ng/spot)	121.8456	304.8726
8	Robustness (%RSD)	0.4115	0.2551

Table 2: Analysis of marketed formulations

Tablet	Concentration of formulation (ng/spot)	Concentration Found (ng/spot)		% Mean Recovery	
	MONT : FEXO	* MONT	* FEXO	MONT	FEXO
Montair-FX	1800 + 150	147.78 ± 0.9160	1796.22 ± 0.7359	98.52	99.79

Table 3 : Specificity and Selectivity study

Parameter	MONT	FEXO
Specificity	99.06 %	99.32%
Peak purity	r (S, M) = 0.9999 r (M, E) = 0.9998	r (S, M) = 0.9997 r (M, E) = 0.9998
Selectivity	Selective	Selective

CONCLUSION

The developed HPTLC technique is precise, specific, robust and accurate method for analysis of FEXO and MONT in pharmaceutical preparations. The procedure can be readily used for selective analysis of drugs and repeatable results are obtained without interference from auxiliary substances. It can be successfully applied for simultaneous estimation of FEXO and MONT in tablet dosage forms without prior separation and any interference in quality control.

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