Research Article

Method Development and Validation for the Simultaneous Determination of Fexofenadine Hydrochloride and Montelukast Sodium in Drug Formulation Using Normal Phase High-Performance Thin-Layer Chromatography

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A simple, precise, specific, and accurate high-performance thin-layer chromatographic method has been developed for the simultaneous determination of fexofenadine hydrochloride (FEX) and montelukast sodium (MTKT) 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 toluene: ethyl acetate: methanol: ammonia (30%) (0.5: 7: 2: 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 220 nm. The drugs were resolved satisfactorily with \( R_f \) values of 0.21 ± 0.01 and 0.59 ± 0.01 for FEX and MTKT, respectively. The linear regression analysis data for the calibration plots showed good linear relationship with \( r^2 \) = 0.9996 and 0.9998 for FEX and MTKT, respectively, in the concentration range of 2400–10800 ng spot \(^{-1}\) for FEX and 200–900 ng spot \(^{-1}\) for MTKT. The method was validated for precision, robustness, specificity, and accuracy. The limits of detection and quantitation were 100 and 300 ng spot \(^{-1}\), respectively, for FEX and 50 and 100 ng spot \(^{-1}\), respectively, for MTKT. The proposed developed HPTLC method can be applied for identification and quantitative determination of FEX and MTKT in bulk drug and drug formulation.

1. Introduction

Fexofenadine hydrochloride (FEX) (Figure 1) (RS)-2-[4-[1-Hydroxy-4-[4-(hydroxy-diphenyl-methyl)-1-piperidyl]butyl]phenyl]-2-methyl-propanoic acid is used to relieve the allergy symptoms of seasonal allergic rhinitis (hay fever), including runny nose; sneezing; and red, itchy, or watery eyes; or itching of the nose, throat, or roof of the mouth in adults [1, 2]. It is carboxylic acid metabolite of terfenadine, a nonsedating selective histamine H1 receptor antagonist. This drug contains an asymmetric carbon in its chemical structure and is administered clinically or is used as a \( \beta \)-glycoprotein probe as a racemic mixture of \( R \)- and \( S \)-enantiomers [3, 4].

Montelukast sodium (MTKT) (Figure 2) is chemically (S, E)-2-((1-(3-(2-(7-chloroquinolin-2-yl)vinyl)phenyl)-3-(2-(2-hydroxypropan-2-yl)phenyl)propylthio)methyl)cyclopentyl)acetic acid [5] which is a leukotriene receptor antagonist used in the treatment of chronic asthma and allergic rhinitis [6, 7].

Literature survey reveals that fexofenadine hydrochloride is estimated individually or in combination with other drugs by UV spectrophotometry [8–10], RP-HPLC [11–13], HPTLC [14, 15], in biological fluid by RP-HPLC [16–18], LC/MS [19], LC/MS/MS [20, 21], and stability indicating method [22].

Similarly for montelukast sodium, UV spectrophotometry [23, 24], spectrofluorometry [25], RP-HPLC [26, 27],
HPTLC [26, 28], in biological fluid by HPLC [29–32], LC/MS [33, 34], and stability indicating HPLC methods [35, 36] have been reported. According to literature research no method has been reported for simultaneous determination of FEX and MTKT by HPTLC and HPLC. HPTLC method is cost effective, rapid, and less time consuming. In HPTLC many samples are simultaneously used and solvent requirement is low. The development and validation of simple, precise, and accurate HPTLC method for the simultaneous determination of FEX and MTKT in tablet formulation is described in the present study. The proposed method is validated as per ICH guidelines [37].

2. Experimental

2.1. Materials. Working standards of pharmaceutical grade FEX (99.60%, w/w) and MTKT (100.0%, w/w) were obtained as gift samples from Unichem Laboratories, Goa, India. Fixed dose combination tablets (MONTAIR FX, B. no. ACF1010, Cipla Ltd., MFG. 05/2011, EXP. 04/2013) containing 120 mg FEX and 10 mg MTKT were purchased from local pharmacy, Pune, India. 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 10 μg mL⁻¹ of individual drug was taken. Further, in situ HPTLC spectral overlain of FEX and MTKT was taken.

2.3. Instrumentation and Chromatographic Conditions. The HPTLC plates were prewashed with methanol and activated at 110°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 V applicator (Switzerland). A constant application rate of 0.1 μLs⁻¹ was used and the space between two bands was 6 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 toluene: ethyl acetate: methanol: ammonia (30%) (0.5: 7: 2: 0.5, v/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°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 5 mm × 0.45 mm and the scanning speed was 10 mm s⁻¹. The source of radiation used was a deuterium lamp emitting a continuous UV spectrum between 200 and 400 nm. All determinations were performed
Table 1: Linear regression data for calibration curves (n = 6).

| Parameters                        | FEX                      | MTKT                      |
|-----------------------------------|--------------------------|---------------------------|
| Linearity range                   | 2400–10800 ng spot⁻¹     | 200–900 ng spot⁻¹         |
| Slope ± Standard error            | 1.651 ± 0.01             | 6.900 ± 0.04              |
| Intercept ± Standard error        | 1019 ± 90.27             | −16.12 ± 25.18            |
| Confidence limit of slope⁹        | 1.620 to 1.682           | 6.796 to 7.003            |
| Confidence limit of intercept⁹    | 798.1 to 1240            | −77.73 to 45.49           |
| r²                                | 0.9996                   | 0.9998                    |
| Sy.xb                             | 98.19                    | 27.38                     |
| P valuec                          | <0.0001                  | <0.0001                   |

*95% confidence intervals.

*Standard deviation of residuals from line.

*P value is < 0.0001, considered extremely significant.

2.4. Standard Solutions and Calibration Graphs. Mixed stock standard solution containing 12 mg mL⁻¹ of FEX and 1 mg mL⁻¹ of MTKT was prepared in methanol by dissolving 300 mg of FEX and 25 mg of MTKT in 25 mL methanol. Mixed stock standard solution was further diluted with methanol to obtain working standard solutions in a concentration range of 2400–10800 ng spot⁻¹ for FEX and 200–900 ng spot⁻¹ for MTKT. Each concentration was applied six times to 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. To determine the content of FEX and MTKT simultaneously in pharmaceutical dosage form MONTAIR FX (label claim: 120 mg FEX and 10 mg MTKT per tablet, B. no. ACF1010, Cipla Ltd.), twenty tablets were weighed and finely powdered. An accurate weight of the powder equivalent to 120 mg of FEX and 10 mg of MTKT was weighed. This was then transferred into a 100 mL volumetric flask containing 50 mL methanol, sonicated for 30 min, and made up to the mark with methanol. This solution was filtered through a 0.45 μm nylon syringe filter. The previous concentration achieved was 1200 ng μL⁻¹ of FEX and 100 ng μL⁻¹ of MTKT. 6 μL volume was spotted for six times to achieve a final concentration of 7200 ng spot⁻¹ for FEX and 600 ng spot⁻¹ for MTKT. The plate was developed in the previously described chromatographic conditions. The peak area of the spots was measured at 220 nm for FEX and MTKT, at ambient temperature with a detection wavelength of 220 nm. Concentrations of the compound chromatographed were determined from the intensity of the diffused light. Evaluation was by peak areas with linear regression.
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. Method Validation. The optimized HPTLC method was
validated with respect to the following parameters as per the
ICH guidelines [37].

2.6.1. Precision. Precision of the method was determined
with the standard and the real sample. The precision of the
method was verified by repeatability (intraday) and interme-
diate precision studies. Repeatability studies were performed
by analysis of three different concentrations of working stan-
dard of 2400, 7200, and 10800 ng spot$^{-1}$ for FEX and 200,
600, and 900 ng spot$^{-1}$ for MTKT. Method repeatability was
achieved by repeating the same procedure six times on the
same day for intraday precision. The intermediate (interday)
precision of the method was checked by performing same
procedure on different days under the same experimental
conditions. The repeatability of sample application and
measurement of peak area were expressed in terms of relative
standard deviation (% RSD) and standard error (SE).

An amount of the sample powder equivalent to the
label claim of FEX and MTKT was accurately weighed
and assayed. System repeatability was determined by six
replicate applications and measurement of sample solution at
a concentration of 7200 ng spot$^{-1}$ for FEX and 600 ng spot$^{-1}$
for MTKT and the peak areas for real sample were expressed
in terms of relative standard deviation (% RSD).

2.6.2. Robustness. The robustness was studied by evaluating
the effect of small but deliberate variations in the chro-
matographic 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, for example,
toluene: ethyl acetate: methanol: ammonia (30%) ((0.6: 7:
2: 0.5, v/v/v/v), (0.5: 7.1: 2: 0.5, v/v/v/v), (0.5: 7: 2.1: 0.5,
v/v/v/v), and (0.5: 7: 2.0: 0.6, v/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 2400,
7200, and 10800 ng spot$^{-1}$ for FEX and 200, 600, and 900 ng spot$^{-1}$
for MTKT.

2.6.3. Limit of Detection and Limit of Quantitation. The
detection limit of an individual analytical procedure is the
lowest amount of analyte in a sample that can be detected
but not necessarily quantitated as an exact value. The quanti-
tation limit of an individual analytical procedure is the lowest
amount of analyte in a sample that can be quantitatively
determined with suitable precision and accuracy. In order
to estimate the limit of detection (LOD) and limit of quantitation
(LOQ), the signal-to-noise ratio (S/N) of 3 and
10 was determined for six replicate determinations.

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.
Peak purity of FEX and MTKT was assessed by comparing
their respective spectra at peak start (S), peak apex (M), and
peak end (E) position of the spots.

2.6.5. Accuracy. Accuracy of the proposed method was
carried out by applying the method to pharmaceutical dosage
form (FEX and MTKT combination tablets) to which known
amounts of FEX and MTKT standard powder corresponding

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### Table 2: Intraday and interday precision of FEX and MTKT ($n = 6$).

| Drug | Conc. (ng spot$^{-1}$) | Repetability | Intermediate precision |
|------|------------------------|--------------|-----------------------|
|      |                        | Found conc. ± SD | % RSD | SE | Found conc. ± SD | % RSD | SE |
| FEX  | 2400                   | 2399.91 ± 12.41 | 0.52 | 5.08 | 2400.69 ± 17.75 | 0.74 | 7.24 |
|      | 7200                   | 7204.84 ± 15.53 | 0.22 | 6.36 | 7205.02 ± 8.72 | 0.12 | 3.57 |
|      | 10800                  | 10799.46 ± 10.73 | 0.09 | 4.39 | 10797.67 ± 27.95 | 0.26 | 11.41 |
| MTKT | 200                    | 200.50 ± 1.49 | 0.74 | 0.61 | 201.32 ± 0.96 | 0.47 | 0.39 |
|      | 600                    | 601.78 ± 1.17 | 0.19 | 0.47 | 600.56 ± 0.72 | 0.11 | 0.29 |
|      | 900                    | 900.65 ± 0.75 | 0.08 | 0.30 | 900.89 ± 0.55 | 0.06 | 0.22 |

### Table 3: Robustness testing of method ($n = 6$).

| Parameter                                      | FEX          | MTKT         |
|------------------------------------------------|--------------|--------------|
| SD of peak area$^a$                            | % RSD$^a$     |              |
| Mobile phase composition (±0.1 mL)             | 12.65        | 3.09         | 0.27 | 1.27 |
| Amount of mobile phase (±5%)                   | 15.71        | 2.40         | 0.33 | 0.98 |
| Time from spotting to chromatography (+10 min) | 12.36        | 2.55         | 0.24 | 1.04 |
| Time from chromatography to scanning (+10 min) | 8.75         | 2.87         | 0.18 | 1.17 |

$^a$ Average of three concentrations 2400, 7200, and 10800 ng spot$^{-1}$ for FEX and 200, 600, and 900 ng spot$^{-1}$ for MTKT, respectively.
3. Results and Discussion

3.1. Selection of Analytical Wavelength. UV spectrum of FEX and MTKT showed maximum absorbance at 220 nm and 344 nm, respectively (Figure 3). Further, in situ HPTLC spectral overlain of FEX and MTKT was taken and 220 nm was selected as scanning wavelength (Figure 4).

3.2. Optimization of Mobile Phase. Optimization of mobile phase was done with a view to separate FEX and MTKT drugs. Since initially tested mobile phase, which was composed of toluene, ethyl acetate, methanol, and ammonia (30%) (2.5: 7: 2.5: 1, v/v/v/v) showing good peak shape but incomplete separation, was observed as the Rf of FEX was 0.40 and Rf of MTKT was 0.47 [26], Several other combinations of the same mobile phase components were tested. Such combinations included toluene, ethyl acetate, methanol, and ammonia (30%) ((2.5: 7: 3: 1), (2: 7: 2.5: 1), and (2: 7: 2.5: 0.5)). However, since these mobile phases did not lead to the aimed result so, mobile phase was changed to ethyl acetate, methanol, and ammonia (30%) (7: 1.5: 0.5 v/v/v/v) which was also the reported earlier [22]. The result showed good separation with Rf of 0.20 and 0.60 for FEX and MTKT, respectively, but MTKT peak showed significant tailing. Toluene (0.1, 0.3, 0.5 mL) was then added to mobile phase and in the subsequent run it was found that toluene was responsible for improving the peak shape of MTKT. Toluene, ethyl acetate, methanol, and ammonia (30%) (0.5: 7: 1.5: 0.5 v/v/v/v) showed desired peak shape of MTKT but Rf of FEX obtained was 0.18 which was below the desired range of Rf value (0.2–0.8). So, methanol was increased by 0.5 mL in the previous mobile phase which leads to the desired Rf value and good peak shape of the two drugs FEX and MTKT. Finally, a mobile phase with a combination of toluene, ethyl acetate, methanol, and ammonia (30%) (0.5: 7: 2: 0.5 v/v/v/v) gave compact, symmetrical, well-resolved spots with Rf values of 0.21 ± 0.01 and 0.59 ± 0.01 for FEX and MTKT, respectively. The development chamber was saturated for 30 min. The development was done for 80 mm on the plate and the development time was 20 min. After development, drying of the plates was done using air. Simultaneous detection of FEX and MTKT was performed at 220 nm since both compounds are well known to exhibit sufficient ultraviolet absorption at this wavelength.

3.3. Linearity. Linear relationships were observed by plotting drug concentration against peak areas for each compound. FEX and MTKT showed linear response in the concentration range of 2400–10800 ng spot−1 and 200–900 ng spot−1, respectively. The corresponding linear regression equation was y = 1.651x + 1019 and y = 6.8998x − 16.119 with square of correlation coefficient (r²) of 0.9996 and 0.9998 for FEX and MTKT, respectively. No significant difference was observed in the slopes of standard curves (Table 1). Residual analysis was performed to ascertain linearity (Figure 5).

3.4. Precision. The % RSD values depicted in Table 2 show that proposed method provides acceptable intraday and interday variation of FEX and MTKT with respect to working standard. The repeatability of real sample application and measurement of peak areas were expressed in terms of % RSD and were found to be 0.61 and 0.26 for FEX and MTKT, respectively.

3.5. Robustness. The standard deviation of the peak areas was calculated for each parameter and the % RSD was found to be less than 2%. The low values of the % RSD, as shown in Table 3, indicated the robustness of the method.

3.6. Limit of Detection and Limit of Quantitation. The signal/noise ratios 3:1 and 10:1 were considered as LOD and LOQ, respectively. The LOD and LOQ were found to be 100, 300 ng spot−1 and 50, 100 ng spot−1 for FEX and MTKT, respectively.

3.7. Specificity. The specificity was noticed by the complete separation of FEX and MTKT peaks. The peak purity of was assessed by comparing their respective spectra at the
peak start, apex, and peak-end positions of the spot, that is, \( r(S, M) = 0.9999 \) and \( r(M, E) = 0.9999 \) for FEX and \( r(S, M) = 0.9997 \) and \( r(M, E) = 0.9998 \) for MTKT.

3.8. Accuracy. As shown from the data in Table 4 satisfactory recovery percentage in the limit of 98–102% with small relative standard deviations (\% RSD) is obtained at various added concentrations. The results indicate that the method is highly accurate for simultaneous determination of FEX and MTKT.

3.9. Analysis of a Marketed Formulation. Using the proposed chromatographic method, assay of FEX and MTKT in their tablets (MONTAIR FX, label claim: 120 mg FEX and 10 mg MTKT per tablet, B. no. ACF1010, Cipla Ltd.) was carried out. The peaks at \( R_f \) 0.21 for FEX and 0.59 for MTKT were observed in the densitogram of the drug samples extracted from tablets. There was no interference from the excipients commonly present in the tablets (Figure 6). Satisfactory results were obtained for both drugs in a good agreement with the label claim. The drug content was found to be 99.82% ± 0.98 (\%RSD of 0.98) and 100.01% ± 1.06 (\% RSD of 1.07) for FEX and MTKT, respectively.

4. Conclusion

The developed HPTLC technique is precise, specific, robust, 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 or capsule formulation.

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