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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

Suparna S. Tandulwadkar, Snehal J. More, Atul S. Rathore, Ajinkya R. Nikam, Lohidasan Sathiyanarayanan, and Kakasaheb R. Mahadik

Department of Pharmaceutical Chemistry, Poona College of Pharmacy, Bharati Vidyapeeth Deemed University, Erandwane, Pune, Maharashtra 411038, India

Correspondence should be addressed to Lohidasan Sathiyanarayanan, pharmsathiya@gmail.com

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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 F_{254}, (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 *P*-glycoprotein probe as a racemic mixture of *R*- and *S*-enantiomers [3, 4].

Montelukast sodium (MTKT) (Figure 2) is chemically (S, E)-2-(1-((1-(3-(2-(7-chloroquinolin-2-yl)vinyl)phenyl)-3-(2-(2-hydroxypropan-2-yl)phenyl)propylthio)methyl) cyclopropyl)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],

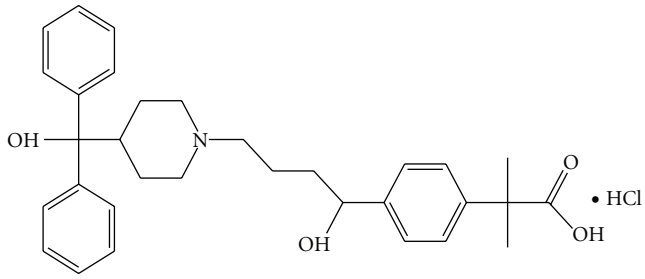

Figure 1: Structure of FEX.

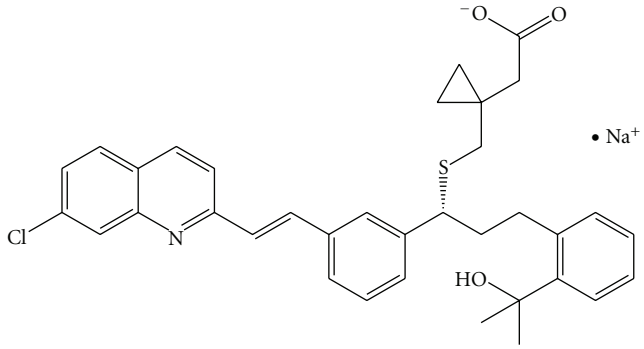

Figure 2: Structure of MTKT.

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.

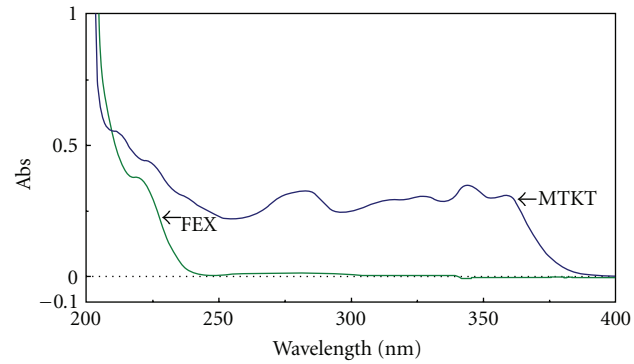

Figure 3: UV spectrum overlay of FEX and MTKT.

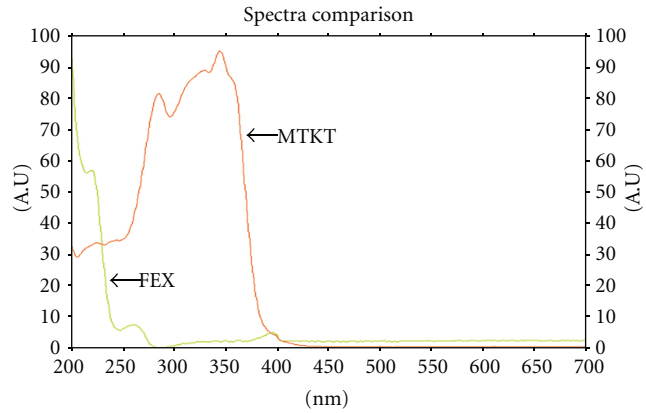

Figure 4: In situ HPTLC spectral overlain of FEX and MTKT.

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 F_{254}, ((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 slope[a]	1.620 to 1.682	6.796 to 7.003
Confidence limit of intercept[a]	798.1 to 1240	−77.73 to 45.49
r^2	0.9996	0.9998
Sy.x[b]	98.19	27.38
P value[c]	<0.0001	<0.0001

[a]95% confidence intervals.
[b]Standard deviation of residuals from line.
[c]P value is < 0.0001, considered extremely significant.

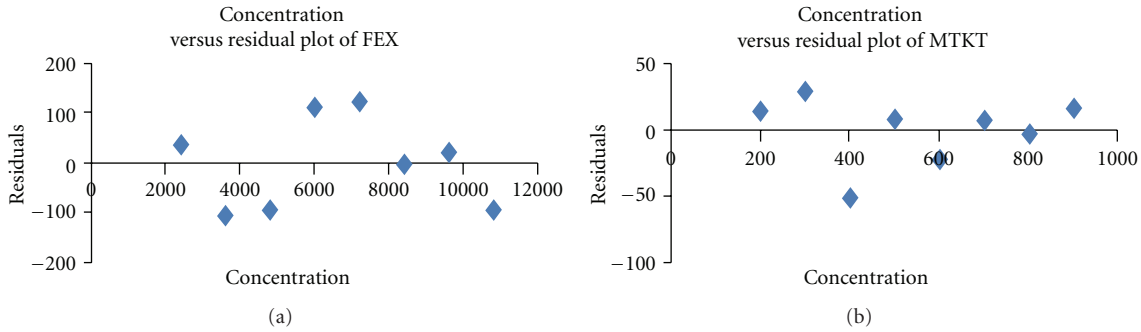

Figure 5: Concentration versus residual plot of FEX and MTKT.

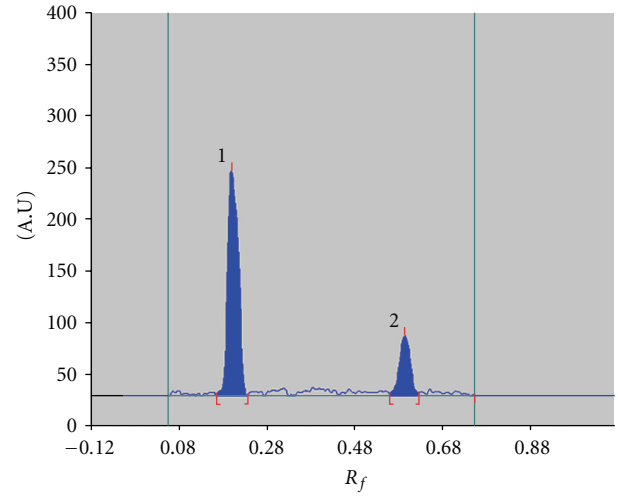

Figure 6: Densitogram of formulation containing 7200 ng spot^{-1} of FEX (R_f 0.21) and 600 ng spot^{-1} of MTKT (R_f 0.59).

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.

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 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. 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,

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

Drug	Conc. (ng spot^{-1})	Repatability			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	SD of peak area[a]		% RSD[a]	
	FEX	MTKT	FEX	MTKT
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.

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 intermediate precision studies. Repeatability studies were performed by analysis of three different concentrations of working standard 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 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, 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 quantitation 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

Table 4: Accuracy studies for the determination of (a) FEX and (b) MTKT ($n = 6$).

Excess drug added to the analyte (%)	Theoretical content (ng spot^{-1})	Measured conc. ± SD	Recovery (%)	%RSD	SE
(a) FEX					
80	4320	4318.25 ± 11.87	99.96	0.27	4.86
100	4800	4794.31 ± 9.47	99.88	0.20	3.88
120	5280	5269.76 ± 4.09	99.81	0.08	1.67
(b) MTKT					
80	320	320.24 ± 2.58	100.08	0.80	1.05
100	400	400.89 ± 1.63	100.22	0.40	0.66
120	480	479.57 ± 0.84	99.91	0.18	0.34

to 80, 100, and 120% of label claim had been added (standard addition method). The absolute recovery was calculated by comparing the peak areas obtained from standard solution of FEX and MTKT with the peak areas of samples of different concentration. Six determinations at each level of concentration were performed and the results obtained were compared with expected results.

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 R_f of FEX was 0.40 and R_f 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) which was also the reported earlier [22]. The result showed good separation with R_f 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 R_f of FEX obtained was 0.18 which was below the desired range of R_f value (0.2–0.8). So, methanol was increased by 0.5 mL in the previous mobile phase which leads to the desired R_f 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 R_f 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 (r^2) 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.

Acknowledgments

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