

Method development and validation for the simultaneous determination of fexofenadine hydrochloride and montelukast sodium using rp-hplc

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Abstract—This research paper describes simple, specific, accurate and precise reverse phase high pressure liquid chromatographic method for the simultaneous determination of fexofenadine hydrochloride and Montelukast sodium in combined dosage form. The separation was carried out on agilent T.C – C₁₈ column(2)4.6×250m.m with 5 μm internal diameter using acetonitrile: TEA (P^H 6) (80:20v/v) as mobile phase at the flow rate of 1ml/min. RP-HPLC separation of the two drugs was carried out in the absorbance mode at 220 nm. The drugs were resolved satisfactorily with Rt values of 3.21 ± 0.01 and 6.66 ± 0.01 for FEX and MTKT, respectively. The linear regression analysis data for the calibration plots showed good linear relationship with r²=0.9996 and 0.9998 for FEX and MTKT, respectively in the concentration range of 12-144 μg/ml for FEX and 1-12μg/ml for MTKT. The method was validated for precision, robustness, specificity and accuracy. The limit of detection and quantitation were 1.41 and 4.29 μg/ml, respectively for FEX and 0.02 and 0.06 μg/ml, respectively for MTKT. The proposed developed RP-HPLC method can be applied for identification and quantitative determination of FEX and MTKT in bulk drug and drug formulation.

Keywords—fexofenadine hydrochloride; montelukast sodium; RP-HPLC; validation.

I. 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]. It is carboxylic acid metabolite of terfenadine, a non-sedating 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 [2]. 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] is a leukotriene receptor antagonist used in the treatment of chronic asthma and allergic rhinitis [3].

Literature survey reveals that Fexofenadine hydrochloride is estimated individually or in combination with other drugs by UV spectrophotometry [4,5,6], RP-HPLC [7,8,9], HPTLC [10, 11], in biological fluid by RP-HPLC [12,13,14], LC/MS [15], LC/MS/MS [16, 17] and Stability indicating HPLC and TLC method [18].

Similarly for Montelukast sodium, UV spectrophotometry [19,20], spectrofluorometry [21], RP-HPLC [22,23], HPTLC [24,25], plasma HPLC [26,27,28,29], LC/MS [30,31], and stability indicating HPLC methods [32,33] have been reported.

Above literature suggests that no method has been reported for simultaneous determination of FEX and MTKT by RP- HPLC. RP-HPLC method is sensitive, rapid and less time consuming. In RP- HPLC many samples are simultaneously used and solvent requirement is low. So, the present study is designed for the development and validation of simple, precise and accurate RP- HPLC method for the simultaneous determination of FEX and MTKT in tablet formulation. The proposed method is validated as per ICH guidelines [34].

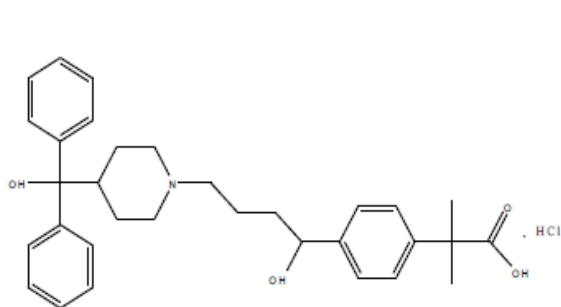


Figure 1. Structure of FEX

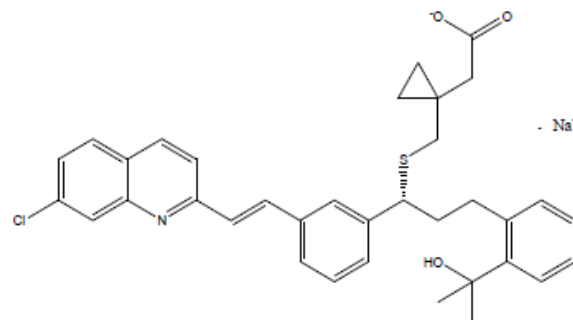


Figure 2. Structure of MTKT

II. 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, Hyderabad, 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 HPLC spectral overlay of FEX and MTKT was taken.

2.3. Instrumentation and chromatographic conditions:

2.3.1 Instruments and Apparatus

The chromatography was performed on a Agilent RP-HPLC instrument (LC-2010CHT) equipped with DAD detector and LC-solution software, C18 column (250×4.6 mm id, 5 µm particle size) was used as stationary phase. LC-GC (Axis)AGN 204 PO analytical balance and ultrasonic cleaner (1.5L 50H, PCI Mumbai) were used during the research work.

2.3.2 Reagents and materials

Standard samples of MTKT and FEX were obtained from Sun Pharmaceutical Pvt Ltd, (Vadodara, Gujarat). Combination tablet formulation containing Montelukast sodium equivalent to Montelukast 10 mg and fexofenadine hydrochloride 10 mg was procured from local pharmacy. Triple distilled water, methanol, acetonitrile, tri ethyl amine (loba chemicals) used were of HPLC grade.

2.4. Standard solutions and calibration graphs:

Accurately weighed MTKT (10 mg) and FEX(10 mg) standards were transferred to a 50 ml volumetric flask, dissolved in and diluted up to the mark with methanol to obtain a standard stock solution (200 mg/ml) of MTKT and FEX, each. From the above stock solution, an aliquot (2.5 ml) of the solution was transferred to 50 ml volumetric flask, and diluted up to the mark with mobile phase to obtain a working standard solution (10 µg/ml) of MTKT and FEX, each.

2.4.1 Preparation of Calibration Curve

Aliquots of 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 ml solutions of montelukast sodium and aliquots of 0.2, 0.4, 0.8, 1.2, 1.6, 2, 2.4 ml solutions of fexofenadine hydrochloride were used for mixed working standard solution (equivalent to 1, 2, 4, 6, 8, 10, 12 µg/ml and 12, 24, 48, 72, 96, 120, 144 µg/ml of MTKT and FEX each) were transferred in a series of 10 ml volumetric flasks, and the volume was made up to the mark with mobile phase. An aliquot (10 µl) of each solution was injected under the operating chromatographic conditions as described above and responses were recorded. Calibration curves were constructed by plotting the peak areas versus the concentrations, and the regression equations were calculated of fexofenadine hydrochloride and montelukast sodium respectively (fig 3 & fig 4). Each response was average of three determinations.

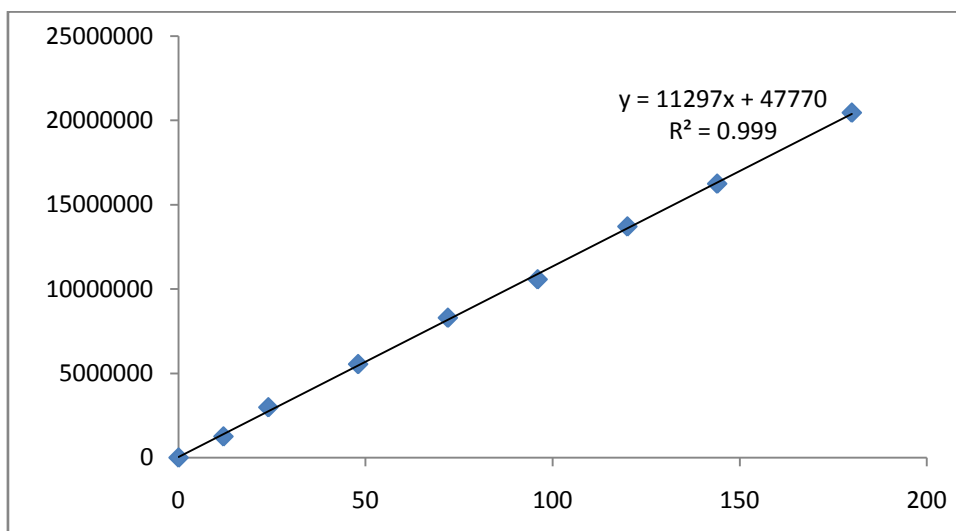


Fig: 3 calibration curve for fexofenadine hydrochloride

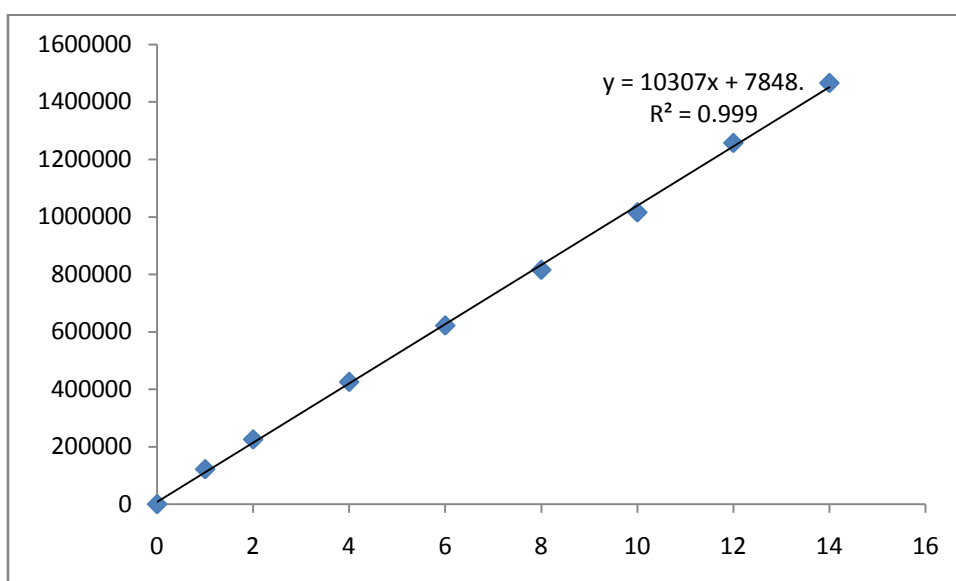


Fig: 4 calibration curve for montelukast sodium

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. MFG. 05/2011 EXP. 04/2013). 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 FEX was transferred in a 50 ml volumetric flask and methanol (30 ml) was added. The solution was sonicated for 15 min. The flask was allowed to stand for 5 min at room temperature and the volume was diluted up to the mark with methanol to obtain the sample stock solution of MTKT (10 $\mu\text{g/ml}$) and FEX (120 $\mu\text{g/ml}$). The solution was filtered through 0.45 μm -47mm membrane filter. An aliquot (2.5 ml) was transferred to a 50 ml volumetric flask and diluted up to the mark with mobile phase used for HPLC, to obtain working sample solution of MTKT (10 $\mu\text{g/ml}$) and FEX (120 $\mu\text{g/ml}$). An aliquot of the working test solution was transferred to a 10 ml volumetric flask and diluted up to the mark with mobile phase to obtain the sample solution of MTKT (6 $\mu\text{g/ml}$) and FEX (72 $\mu\text{g/ml}$). The peak areas were measured at 220 nm for FEX and MTKT, respectively.

III. METHOD VALIDATION

The optimized RP-HPLC method was validated with respect to the following Parameters as per the ICH guidelines [37].

3.1. Precision:

Precision of the method was determined with the standard and the test 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 24, 72, 108 µg/ml for FEX and 2, 6, 10 µg/ml for MTKT. Method repeatability was achieved by repeating the same procedure six times on the same day for intra-day 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 (% R.S.D.) and standard error (S.E.).

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 72 µg/ml for FEX and 6 µg/ml for MTKT and the peak areas for real sample were expressed in terms of relative standard deviation (% R.S.D.).

3.2. Robustness:

The robustness was studied by evaluating the effect of small but deliberate variations in the chromatographic conditions. The robustness was studied by analyzing the same samples of MTKT and FEX by deliberate variation in the method parameters. The change in the responses of MTKT and FEX were noted. Robustness of the method was studied by changing the extraction time of MTKT and FEX from tablet dosage forms by ± 2 min, composition of mobile phase by ± 2 % of organic solvent, flow rate by ± 0.2 ml/min and column oven temperature by ± 2^o C. The parameters used in system suitability test were asymmetry of the chromatographic peak, peak resolution, theoretical plates and capacity factor, as RSD of peak area for replicate injections. The robustness of the method was determined at three different concentration levels of 24, 72, 108 µg/ml for FEX and 2, 6, 10 µg/ml for MTKT.

3.3. 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. The limit of detection (LOD) and the limit of quantification (LOQ) of the MTKT and FEX, were calculated using the standard deviation of responses (N) and slopes (S) of respective calibration curves using signal-to-noise ratio.

$$\text{LOD} = 3.3 \times N/S$$

$$\text{LOQ} = 10 \times N/S$$

3.4. 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 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.

IV. RESULTS AND DISCUSSION

4.1. Selection of analytical wavelength:

UV spectrum of FEX and MTKT showed maximum absorbance at 220 nm and 344 nm, respectively (Figure5).

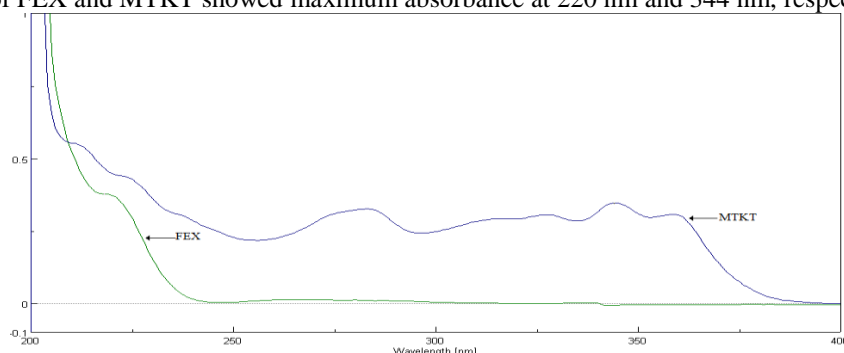


Figure 5. UV spectrum overlay of FEX and MTKT

4.2. 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 Acetonitrile :water(90:10 v/v) showing broad peak shape and complete separation was observed as the R_f of FEX was 0.40 and R_f of MTKT was 0.47 [26]. Several other ratios of the same mobile phase components were tested. Such ratios included acetonitrile: water (85:15), (80:20). However, since these mobile phases did not lead to the aimed result so, mobile phase was changed to acetonitrile:water(pH3) (80:20 v/v),(75:25v/v) which was showing void peak and incomplete separation of drugs was observed . However, since these mobile phases did not lead to the aimed result so, mobile phase was changed to acetonitrile : water(pH 4.1)(75:25v/v) which was showing incomplete separation of drugs .So the mobile phase changed to acetonitrile : TEA (pH 6) (85:15V/V),(82:18v/v) has showed good sharp peak but separation is not good. So mobile phase ratio has been changed to acetonitrile : TEA(pH 6) the result showed good separation with R_f of 0.20 and 0.60 for FEX and MTKT, respectively . Finally, a mobile phase with a combination of acetonitrile :TEA(pH 6)(80:20v/v) gave sharp, well-resolved peaks with R_t values of 3.21 ± 0.01 and 6.66 ± 0.01 for FEX and MTKT, respectively(fig 6). 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.

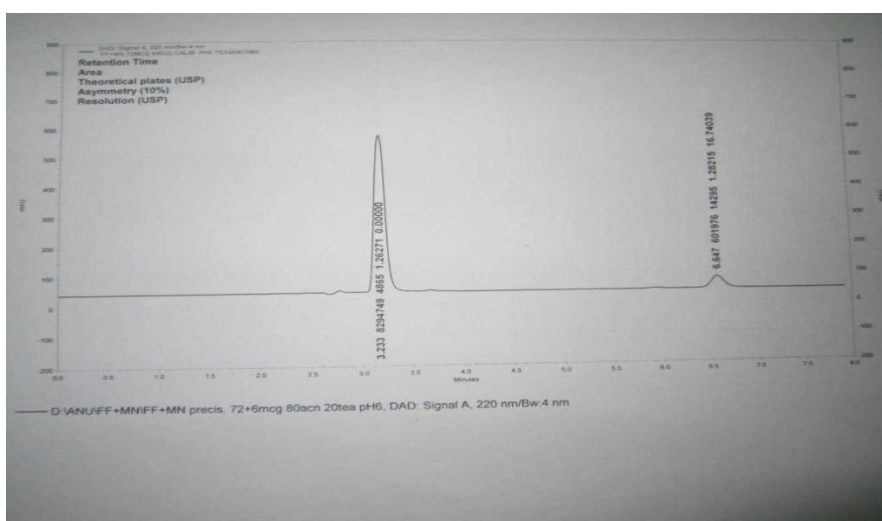


Fig: 6 High performance chromatogram of fexofenadine hydrochloride and motelukast sodium at 220 nm, with retention time of 3.22 and 6.64 min respectively

4.3. 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 12-144 $\mu\text{g/ml}$ and 1-14 $\mu\text{g/ml}$, respectively. The corresponding linear regression equation was $y = 111297X + 4770$ and $y = 10307X + 7849$ with square of correlation coefficient (r^2) of 0.9996 and 0.9998 for FEX and MTKT, respectively.

Table 1. Regression analysis data and summary of validation parameter for the proposed method

Parameters	FEX	MTKT
Linearity range	12-144 $\mu\text{g/ml}$	1-12 $\mu\text{g/ml}$
Slope	111297	10307
intercept	47770	7849
Correlation coefficient	0.9996	0.9998
LOD	1.41	0.02
LOQ	4.29	0.06
%Recovery(accuracy)	100.2 \pm 0.31	100.1 \pm 0.19
Repeatability(%RSD)	0.0026	0.0031
Precision		
Intra day	0.27	0.29
Inter day	0.37	0.21

RSD is a relative standard deviation, n is number of determinations, FEX is fexofenadine hydrochloride,MTKKT is montelukast sodium.

Table 2: System Suitability Test Parameters for Fex and Mtk for Proposed Method

parameters	FEX	MTK
Retention time(mins)	3.22	6.66
Asymmetry factor	1.26	1.28
Theoretical plates	4865	14295

4.4. Precision:

The % R.S.D. values depicted in TABLE 3 shows that proposed method provides acceptable intra-day and inter-day variation of FEX and MTKT with respect to working standard.

Table 3. Intra –day and inter d-day precision of fex and mtk (n=6)

Drug	Concentration(µg/ml)	Repeatability		Intermediate precision	
		Drug conc	Found conc	%RSD	Found conc.
FEX	24	23.99	0.52	42.69	0.74
	72	72.84	0.22	72.02	0.12
	108	107.46	0.09	107.67	0.26
MTK	2	2.15	0.74	2.32	0.47
	6	6.07	0.19	6.15	0.11
	10	9.65	0.08	9.89	0.06

The repeatability of real sample application and measurement of peak areas were expressed in terms of % R.S.D. and were found to be 0.61 and 0.26 for FEX and MTKT, respectively.

4.5. Robustness:

The standard deviation of the peak areas were calculated for each parameter and the % R.S.D. was found to be less than 2 %. The low values of the % R.S.D., as shown in TABLE 4 indicated the robustness of the method.

Table 4. Robustness testing of method (n=6)

Parameter	Peak area		%RSD	
	FEX	MTKT	FEX	MTKT
Flow rate(1.1 ml/min)	9415401	467033	0.0019	0.0023
Flow rate(0.9 ml/min)	11463892	521266	0.0025	0.0039
Wave length(218 nm)	10300605	520307	0.004	0.0051
Wave length(222nm)	9837740	373982	0.0058	0.007

4.6. 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 1.41,4.29 µg/ml and 0.02, 0.06µg/ml for FEX and MTKT, respectively.

4.7. Accuracy:

As shown from the data in TABLE 5 satisfactory recovery % in the limit of 98–102% with small relative standard deviations (% R.S.D.) obtained at various added concentrations. The results indicate that the method is highly accurate for simultaneous determination of FEX and MTKT.

Table 5. Accuracy studies for the determination of (a) fex and (b) mtk (n=6) Fex

Amount of drug formulation	Amount of pure drug added	%added	Peak area	Amount found	%recovery	%RSD
8	6	80	1399158	6.06	101	0.003
8	8	100	1598868	8.03	100.5	0.0045
8	10	120	1800524	10.08	100.7	0.0068

(a) MTKT

Amount of drug formulation	Amount of pure drug added	% added	Peak area	Amount found	%recovery	%RSD
96	72	80	18563621	71.9	100.5	0.0021
96	96	100	21132058	96.9	101.0	0.0036
96	120	120	23936827	121.9	101.6	0.0054

4.8. Analysis of a marketed pharmaceutical dosage form:

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. MFG. 05/2011 EXP. 04/2013) was carried out. Satisfactory results were obtained for both drugs in a good agreement with the label claim as shown in TABLE 6. The drug content was found to be $101.12\% \pm 0.98$ (%R.S.D. of 0.98) and $101.51\% \pm 1.06$ (%R.S.D. of 1.07) for FEX and MTKT, respectively.

Table 6. Assay results for tablets using the proposed method

formulation	Amount of drug taken		Amount of drug found		%amount found	
	FEX	MTKT	FEX	MTKT	FEX	MTKT
Tablets	96($\mu\text{g/ml}$)	8($\mu\text{g/ml}$)	97.1	8.12	101.1	101.5

V. CONCLUSION

The responses of sample solutions were measured at 220 nm for quantitation of FEX and MTKT by the proposed methods. The amount of FEX and MTKT present in the sample solutions were determined by fitting the responses into the regression equations of the calibration curve for FEX and MTKT, respectively. The mobile phase consisting of acetonitrile: TEA (tH6) (80:20v/v), at a flow rate of 1.0 ml/min was found to be satisfactory to obtain good peak symmetry, better reproducibility and repeatability for FEX and MTKT. Quantification was achieved with DAD detector at 220 nm based on peak area. The retention times were found to be 3.21 and 6.66 min for FEX and MTKT, respectively (Figure 1). Linear correlation was obtained between peak area and concentration for FEX and MTKT, each, in the range of 12-144 $\mu\text{g/ml}$ and 1-12 $\mu\text{g/ml}$ respectively (Table 1). The method was found to be specific as no significant change in the responses of FEX and MTKT was observed after 24 h. The percent mean recoveries obtained for MTKT and FEX were 100.2 ± 0.31 % RSD and 100.1 ± 0.19 % RSD (Table 1), which suggest accuracy of the method. The values of % RSD for intraday and interday variations were found to be 0.27 and 0.37 for FEX, and 0.29 and 0.21 for MTKT, respectively (Table 1). % RSD for repeatability was found to be 0.0026 and 0.0031 for FEX and MTKT, respectively. Low RSD values for precision suggest that the method is precise. The LOD and LOQ were found to be 1.41 and 4.29 $\mu\text{g/ml}$ for FEX, 0.02 and 0.6 $\mu\text{g/ml}$ for MTKT, respectively (Table 1), suggest sensitivity of the method. Results of system suitability testing are given in Table 2. The results obtained for FEX and MTKT were comparable with the corresponding labeled claim (Table 6).

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