SEPARATION AND DETERMINATION OF FEXOFENADINE HYDROCHLORIDE AND MONTELUKAST SODIUM IN TABLET DOSAGE FORM USING RP-HPLC

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ABSTRACT

A simple, precise and accurate reversed-phase high-performance liquid chromatographic method has been developed and validated for the simultaneous determination of Fexofenadine hydrochloride (FEX) and Montelukast sodium (MTKT) in their synthetic mixtures and combined tablet formulation. The chromatographic separation was achieved on a Thermo BDS HYPERSIL C_{18} column (250mm × 4.6 mm i.d., 5 µ particle size) at ambient temperature using simple isocratic mobile phase consisting of phosphate buffer (pH 6.0) and methanol (25: 75, v/v), pumped at a flow rate of 1.0 mL min^{-1}. UV detection was performed at 220 nm. The method was validated in the sample concentration ranges of 84–156 µg mL^{-1} for FEX and 7–13 µg mL^{-1} for MTKT, where it demonstrated good linearity with r = 0.9991 and 0.9995, respectively. The retention time (t_R) for FEX and MTKT were found to be 3.542 ± 0.01 and 7.142 ± 0.01 min, respectively. The validation of the proposed method was carried out for linearity, precision, robustness, limit of detection, limit of quantitation, specificity, accuracy and system suitability. The developed method can be used for routine quality control analysis of titled drugs in pharmaceutical dosage form.

Keywords: *Fexofenadine hydrochloride, Montelukast sodium, HPLC, Method development, Validation.*

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 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-

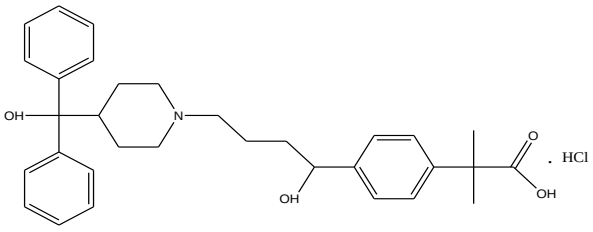
glycoprotein probe as a racemic mixture of *R*- and *S*-enantiomers [3, 4].

Montelukast sodium (MTKT) (Figure 1) 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 [6, 7].

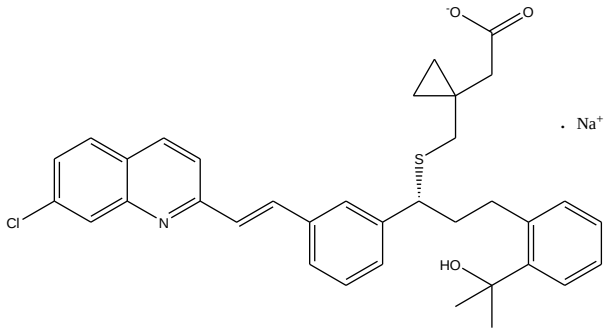
Literature survey reveals that Fexofenadine hydrochloride is estimated individually or in combination with other drugs by UV spectrophotometry [8, 9, 10], RP-HPLC [11, 12], biological fluid [13, 14, 15], LC/MS [16, 17], Stability indicating methods [18], HPTLC [19, 20] have been reported.

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

From the above literature survey it is very clear that no method has been reported for simultaneous determination of FEX and MTKT by HPLC. So, the present study is designed for the development and validation of simple, precise and accurate HPLC method for the simultaneous determination of FEX and MTKT in tablet formulation. The proposed method is validated as per ICH guidelines [34].



FEX



MTKT

Fig. 1. Structure of FEX and MTKT

EXPERIMENTAL

Materials

Working standards of pharmaceutical grade FEX and MTKT were obtained as a gift sample from Unichem Laboratories, Goa, India. It was used without further purification and certified to contain 99.6 % and 100.0 % (w/w) on dry weight basis for FEX and MTKT, respectively. 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. High purity deionized water was obtained from Millipore, Milli-Q (Bedford, MA, USA) water purification system.

Selection of analytical wavelength

Stock solutions of drugs were prepared in methanol separately. UV spectrum of 10 µg mL^{-1} of each individual drug was taken.

Instrumentation and chromatographic conditions

The HPLC system (Jasco corporation, Tokyo, Japan) consisted of a Pump (model Jasco PU- 2080 Plus) along with manual injector sampler programmed at 20 µl

capacity per injection was used. The detector consisted of UV/ VIS (model Jasco UV 2075). LC separations were performed on a Thermo BDS HYPERSIL C18 column (250×4.6 mm i.d., 5 µ particle size), Thermo Electron Corporation. Data was integrated using Jasco Borwin version 1.5, LC-Net II/ADC system. The mobile phase was consisted of Dipotassium hydrogen phosphate (0.02 M): Methanol (25: 75, v/v) and pH adjusted to 6.0 with ortho-phosphoric acid. The flow rate was set to 1.0 mL min^{-1} and UV detection was carried out at 220 nm at ambient temperature.

Standard solutions and calibration graphs

Stock standard solution containing FEX (1200 µg mL^{-1}) and MTKT (100 µg mL^{-1}) was prepared by dissolving 120 mg of FEX and 10 mg of MTKT in methanol in a 100 mL volumetric flask. This was further diluted with mobile phase to obtain working standard solutions in a concentration range of 84–156 µg mL^{-1} (i.e. 84, 96, 108, 120, 132, 144 and 156 µg mL^{-1}) for FEX and 7–13 µg mL^{-1} (7, 8, 9, 10, 11, 12 and 13 µg mL^{-1}) for MTKT. Constant volume of 20µL injections were made for each concentration six times and chromatographed under the above mentioned conditions. 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.

Sample preparation

To determine the content of FEX and MTKT simultaneously in tablet formulation Montair FX, 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 20 mL methanol, sonicated for 10 min. Then, diluted to 100 mL with mobile phase and sonicated for 20 min. with intermittent shaking. This solution was filtered through a 0.45 µm nylon syringe filter. 1 mL of the above solution was transferred to 10 mL volumetric flask and diluted to volume with mobile phase. The concentration achieved after the above dilution was 120 µg mL^{-1} of FEX and 10 µg mL^{-1} of MTKT. A constant 20 µL volume of sample solution was injected six times under the conditions described above. The peak areas were measured at 220 nm and their concentrations in the samples were determined using multilevel calibration curve developed on the same HPLC system under the same conditions using linear regression equation.

Method validation

The optimized HPLC method was validated with respect to the following Parameters. The validation was performed as per the ICH guidelines [34].

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 84, 120 and 156 µg mL^{-1} for FEX and 7, 10 and 13 µg mL^{-1} 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 120 µg mL^{-1} of FEX and 10 µg mL^{-1} of MTKT and the peak areas for real sample were expressed in terms of relative standard deviation (% R.S.D.).

Robustness

The robustness was studied by evaluating the effect of small but deliberate variations in the chromatographic conditions. The robustness of the method was studied by deliberately varying parameters like flow rate (± 0.1 mL min^{-1}), mobile phase composition (± 1 %) and pH of the buffer (± 0.1). Robustness of the method was done at three different concentrations 84, 120, and 156 µg mL^{-1} for FEX and 7, 10 and 13 µg mL^{-1} for MTKT.

Limit of detection (LOD) and limit of quantitation (LOQ)

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. LOD and LOQ of FEX and MTKT were determined by calibration curve method. LOD and LOQ were calculated by using following equations.

$$LOD = \frac{3.3 \times Sy.x}{S}; \quad LOQ = \frac{10.0 \times Sy.x}{S}$$

Where, Sy.x is Standard deviation of residuals from line; S is slope.

Specificity

The ability of an analytical method to unequivocally assess the analyte in the presence of other components. Specificity was assessed by a qualitative comparison between chromatograms obtained from sample, standard, blank and placebo solutions. Diluent was injected as a blank. Placebo interference study was conducted by injecting placebo solution prepared from the excipients most commonly used in pharmaceutical formulations including starch, lactose monohydrate, aerosil, hydroxypropylmethylcellulose, titanium dioxide and magnesium stearate. It was determined by the complete separation of FEX and MTKT with parameters like retention time (t_R), resolution (Rs) and tailing factor (T).

System suitability

The system suitability parameters with respect to theoretical plates (N), peak symmetry (*T*), selectivity (α), HETP (H) and resolution (Rs) between FEX and MTKT peaks were defined.

Accuracy

Accuracy of the method was carried out by applying the method to drug sample 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). At each level of the amount six determinations were performed and the results obtained were compared with expected results.

RESULTS AND DISCUSSION

Selection of analytical wavelength

UV spectrum of FEX and MTKT showed maximum absorbance at 220 nm and 344 nm, respectively. 220 nm was selected as a detection wavelength (Figure 2).

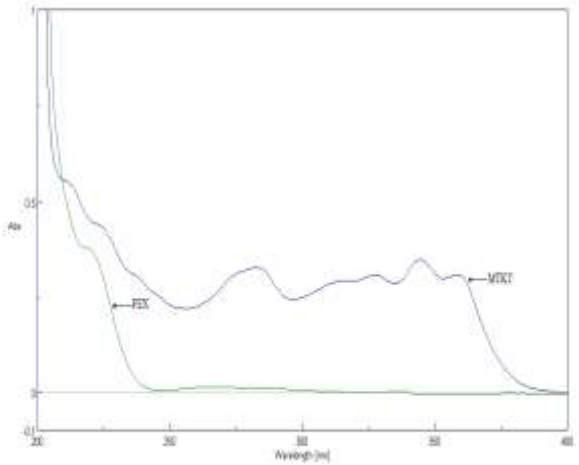


Fig. 2. UV spectrum overlay of FEX and MTKT

OPTIMIZATION OF PROCEDURES

The HPLC procedure was optimized with a view to develop a simultaneous assay method for FEX and MTKT. The stock standard solution was diluted with diluent to a concentration of 120 µg mL^{-1} for FEX and 10 µg mL^{-1} for MTKT. Then, the standard solution was injected into a Thermo BDS HYPERSIL C18 column (250×4.6 mm i.d., 5 µ particle size). Initially, an eluent mixture composed of phosphate buffer (pH 7.0): methanol; 25: 75 (v/v) solution was tried as mobile phase in which the FEX peak eluted along with the placebo peak [23]. To achieve the separation of placebo peak and FEX it was decided to decrease the pH of the mobile phase to 6.0 in order to increase the retention time of FEX as suggested from the pKa value of FEX. Complete separation of Placebo, FEX and MTKT obtained with the mobile phase composition phosphate buffer (pH 6.0): methanol; 25: 75 (v/v). Phosphate buffer 0.02 M (pH 6.0) was prepared by dissolving 3.48 g Dipotassium hydrogen phosphate in 1,000 mL millipore water and pH adjusted to 6.0 by addition of ortho-phosphoric acid. The flow rate was set to 1.0 mL min^{-1} and UV detection was carried out at 220 nm. The retention time (t_R) for FEX and MTKT were found to be 3.542 and 7.100 min, respectively (Figure 3). Acceptable retention time (t_R), plates, asymmetry and good resolution for FEX and MTKT were obtained.

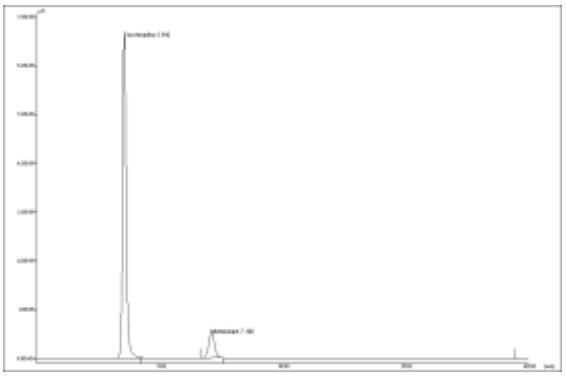


Fig. 3. Chromatogram of standard containing 120 µg mL^{-1} of FEX (t_R 3.542) and 10 µg mL^{-1} of MTKT (t_R 7.100)

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 84–156 µg mL^{-1}, and 7–13 µg mL^{-1}, respectively. The corresponding linear regression equation was y = 48390x – 15870 and y = 70640x + 57940, with square of correlation coefficient (r^2) of 0.9991 and 0.9995 for FEX and MTKT, respectively. Residual analysis was performed to ascertain linearity. The linearity of calibration graphs and adherence of the system to Beer's law was validated by high value of correlation coefficient. No significant difference was observed in the slopes of standard curves (Table 1). Residual

analysis was performed to ascertain linearity (Figure 4)

Tab. 1. Linear regression data for calibration curves (n=6)

Parameters	FEX	MTKT
Linearity range	84–156 µg mL^{-1}	7–13 µg mL^{-1}
Slope ± Standard error	48390 ± 663	70640 ± 715
Intercept ± Standard error	-15870 ± 8122	57940 ± 7292
Confidence limit of slope [a]	46680 to 50090	68810 to 72480
Confidence limit of intercept [a]	-367500 to 50120	39190 to 76690
r^2	0.9991	0.9996
Sy.x [b]	4214	3783

[a] 95% Confidence Intervals

[b] Standard deviation of residuals from line

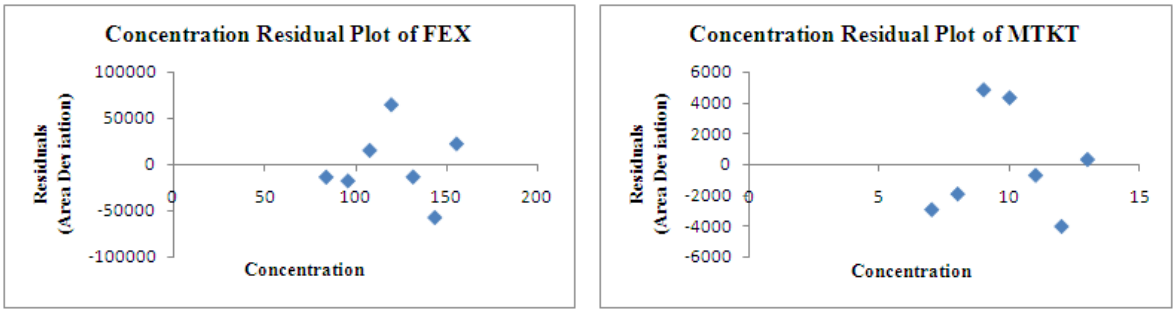


Fig. 4. Concentration versus Residual Plot of FEX and MTKT

Precision

The % R.S.D. values depicted in Table 2 shows that proposed method provides acceptable intra-day and inter-day variation of FEX and MTKT with respect to working standard.

Tab. 2. Intra-day and inter-day precision of FEX and MTKT (n=6)

Drugs	Conc. (µg mL^{-1})	Repeatability (intra-day)		Intermediate precision (inter-day)	
		Found conc. ± S.D.	%. R.S.D.	Found conc. ± S.D.	% R.S.D.
FEX	84	83.99 ± 0.06	0.07	84.00 ± 0.04	0.05
	120	120.13 ± 0.12	0.10	120.14 ± 0.20	0.17
	156	155.97 ± 0.86	0.55	155.84 ± 0.10	0.06
MTKT	7	7.05 ± 0.005	0.07	7.03 ± 0.008	0.11
	10	10.22 ± 0.086	0.84	10.25 ± 0.065	0.63
	13	12.69 ± 0.023	0.18	12.61 ± 0.073	0.58

The repeatability of real sample application and measurement of peak areas were expressed in terms of % R.S.D. and were found to be 0.14 and 0.72 for FEX and MTKT, respectively.

Robustness

Each factor selected to examine were changed at three levels (−1, 0 and 1). One factor at the time was changed to estimate the effect. Thus, replicate injections (n = 6) of mixed standard solution at three concentration levels were performed under small changes of chromatographic parameters (factors). Results, presented in Table 3 indicate that the selected factors remained unaffected by small variations of these parameters.

Tab. 3. Robustness evaluation[a] of the method (n=6)

Factor	Level	Retention time (t_R)		Asymmetry (T)	
		FEX	MTKT	FEX	MTKT
A: Flow Rate (mL min^{-1})					
0.9	-1	3.721	7.250	1.29	1.07
1.0	0	3.542	7.126	1.29	1.07
1.1	+1	3.377	7.060	1.28	1.05
Mean ± S.D.		3.547 ± 0.17	7.145 ± 0.09	1.29 ± 0.01	1.06 ± 0.01
B: Percentage of methanol in the mobile phase (v/v)					
74	-1	3.561	7.215	1.31	1.07
75	0	3.542	7.126	1.29	1.07
76	+1	3.497	7.054	1.27	1.06
Mean ± S.D.		3.533 ± 0.03	7.132 ± 0.08	1.29 ± 0.02	1.07 ± 0.01
C: pH of the buffer					
5.90	-1	3.587	7.190	1.28	1.06
6.00	0	3.542	7.126	1.29	1.07
6.10	+1	3.511	7.087	1.25	1.07
Mean ± S.D.		3.547 ± 0.04	7.134 ± 0.05	1.27 ± 0.02	1.07 ± 0.01

[a] Average of three concentrations 84, 120, and 156 µg mL^{-1} for FEX and 7, 10 and 13 µg mL^{-1} for MTKT.

Limit of detection and limit of quantitation

The LOD and LOQ were found to be 0.29 and 0.87 µg mL^{-1}, respectively for FEX and 0.16 and 0.49 µg mL^{-1}, respectively for MTKT.

Specificity

There is no peak interference of blank and placebo at the retention time of FEX and MTKT which indicates that the method is specific for the analysis in their pharmaceutical dosage form. The specificity of the method is illustrated in

Figure 5 where complete separation of FEX and MTKT was noticed. The average retention time (t_R) ± S.D. for FEX and MTKT were found to be 3.542 ± 0.01 and 7.142 ± 0.01 min, respectively for six replicates. Tailing factor for peaks of FEX and MTKT was less than 2 ($T \leq 2$) and resolution was satisfactory (Rs≥2). The peaks obtained were sharp and have clear baseline separation.

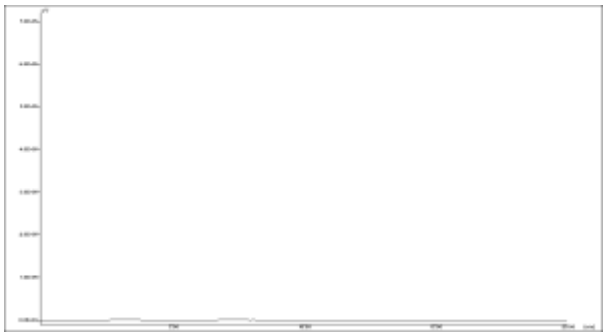


Fig. 5a.

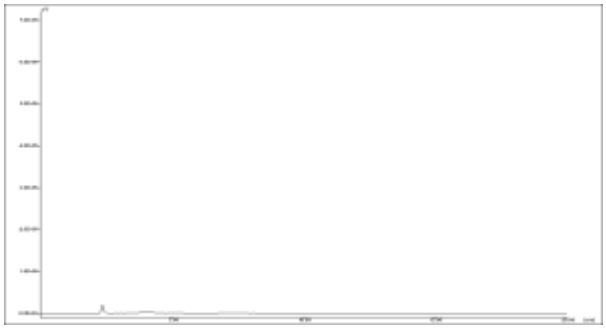


Fig. 5b.

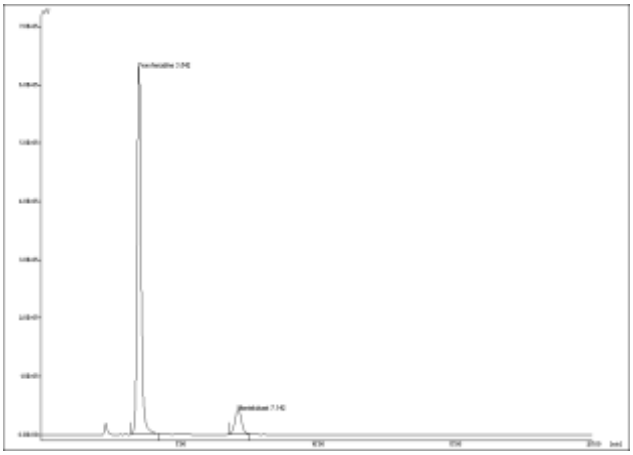


Fig. 5c: Chromatogram of a) Blank b) Placebo c) Sample containing 120 µg mL^{-1} of FEX (t_R 3.542) and 10 µg mL^{-1} of MTKT (t_R 7.142)

System suitability

System suitability parameters including theoretical plates, peak asymmetry(T), selectivity (α), HETP (H) and resolution (Rs) between FEX and MTKT peaks were calculated and summarized in Table 4.

Tab. 4. System suitability parameters for FEX and MTKT by the proposed HPLC method

Parameters	FEX	MTKT	Reference values
Theoretical plates (N)	4321.33	5318.09	N>2000
Peak asymmetry (T)	1.29	1.07	$T \leq 2$
Selectivity (α)[a]	---	5.036	α>1
HETP (H)[b]	0.058	0.047	---
Resolution (Rs)[a]	---	11.88	Rs≥2

[a] With respect to previous peak

[b] HETP (Height Equivalent to Theoretical Plate)

Accuracy

As shown from the data in Table 5, satisfactory recovery % with small relative standard deviations (% R.S.D.) were obtained at various added concentrations. The results indicate the method is highly accurate for simultaneous determination of the three drugs.

Tab. 5. Accuracy studies for the determination of (a) FEX (b) MTKT (n=6)

Excess drug added to the analyte (%)	Theoretical content (µg mL^{-1})	Measured conc. ± S.D.	Recovery (%)	%.R.S.D.
(a) FEX				
80	108	107.89 ± 0.61	99.90	0.57
100	120	119.85 ± 0.33	99.87	0.28
120	132	131.58 ± 0.42	99.68	0.32
(b) MTKT				
80	9	9.02 ± 0.06	100.22	0.67
100	10	10.01 ± 0.03	100.10	0.29
120	11	10.99 ± 0.06	99.91	0.55

Analysis of 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.) was carried out. Satisfactory results were obtained for both drugs in a good agreement with the label claims thereby suggesting that there is no interference from any of the excipients which are normally present in tablets. The drug content was found to be 99.91% ± 0.18 and 100.26% ± 0.36 for FEX and MTKT, respectively.

CONCLUSION

The developed HPLC technique is precise, specific, robust and accurate. Statistical analysis proves that the method is suitable for routine analysis of FEX and MTKT in tablet formulation.

ACKNOWLEDGEMENT

Authors thank Unichem Laboratories, Goa, India for providing gift sample of standards FEX and MTKT and Poona College of Pharmacy, Bharati Vidyapeeth Deemed University for providing facilities for carrying out this study.

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