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UV SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS DETERMINATION OF FEXOFENADINE HYDROCHLORIDE AND MONTELUKAST SODIUM IN TABLETS

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ABSTRACT

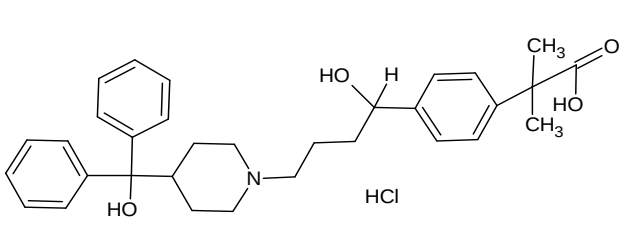
The present study describes the development and validation of a simple simultaneous equation method by UV spectrophotometry for estimation of Fexofenadine hydrochloride and Montelukast sodium in bulk and tablets. The maximum absorbance was measured at 259 nm and 344.5 nm for Fexofenadine hydrochloride and Montelukast sodium respectively in 0.1N NaOH. The calibration curves showed a linear relationship between absorbance and concentration in the range of 50-180 µg/ml for Fexofenadine hydrochloride and 1-35 µg/mL for Montelukast sodium with correlation coefficient of 0.998. The method was validated as per ICH guidelines and the outcome of the statistical analysis proved that the method was precise as the relative standard deviation was less than 2.0% for the assay calculated in intraday and interday precision. The mean percentage recoveries of Fexofenadine hydrochloride and Montelukast sodium calculated by standard addition were found to be 101.43 - 100.54% and 99.97-100.2% indicating accuracy of the method. The developed method was also checked in multicomponent mode and the assay values obtained were within the limits. The acceptable results of validation for the present study indicate the suitability of the method for routine quality control analysis of the combined drugs in tablets.

KEYWORDS: Fexofenadine hydrochloride, Montelukast sodium, Simultaneous equation, Validation, ICH guidelines

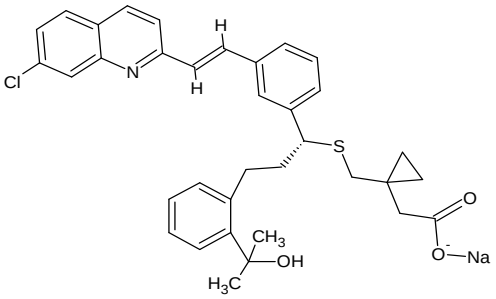
INTRODUCTION

Fexofenadine hydrochloride (FEX) is chemically 2-[4-[1-hydroxy-4-[4-[hydroxy (diphenyl) methyl] piperidin-1-yl] butyl] phenyl]-2-methylpropanoic acid; hydrochloride (Fig.1a), having molecular formula $C_{32}H_{39}ClNO_4$ and molecular weight 538.125 g/mol. It is white to off-white crystalline powder. It is freely soluble in methanol and ethanol, slightly soluble in chloroform and water and insoluble in hexane.[1] FEX is an antihistamine drug used in the treatment of hay fever and similar allergy symptoms which competes with free histamine for binding at H1-receptors in the GI tract, large blood vessels, and bronchial smooth muscle. It blocks the action of endogenous histamine, which subsequently leads to temporary relief of the negative symptoms (e.g. nasal congestion, watery eyes) brought on by histamine.[2]

Montelukast sodium (MKT) is chemically 2-[1-({[(1R)-1-{3-[(E)-2-(7-chloroquinolin-2-l) ethenyl]phenyl}-3-[2-(2-hydroxypropan 2yl) phenyl] propyl] sulfanyl} methyl) cyclopropyl] acetic acid; sodium (Fig.1b), having molecular formula $C_{35}H_{35}ClNO_3S$, and molecular weight 608.169g/mol. Montelukast is hygroscopic and optically active white to off white powder. It is freely soluble in methanol, ethanol and water.[1] MKT is a leukotriene receptor antagonist (LTRA) used for the maintenance treatment of asthma and to relieve symptoms of seasonal allergies. Montelukast inhibits the actions of leukotriene D_4 (LTD_4) at the cysteinyl leukotriene receptor ($CysLT_1$), preventing airway edema, smooth muscle contraction, and enhanced secretion of thick, viscous mucus.[3]



(a)



(b)

Figure 1: Chemical structure of
(a) Fexofenadine hydrochloride, (b) Montelukast sodium.

The combination therapy of Montelukast with antihistamine Fexofenadine provide enhancing and complimentary effects thereby reducing the symptoms effectively. Literature survey revealed several analytical methods for estimation of FEX and MKT individually using UV-Visible spectrophotometry[4-7] and RP-HPLC.[8-11] Few chromatographic methods including

RP-HPLC[12-18] and HPTLC[19] were also reported for the simultaneous estimation of FEX and MKT in dosage forms. Only a single UV spectrophotometric[20] method developed in methanol as solvent was reported for simultaneous estimation of both the drugs. Moreover methods were reported for estimation of FEX and MKT along with other drugs in combined formulation[21-22] The aim of the present study is to develop a simple, sensitive and economical validated UV spectrophotometric method for simultaneous analysis of FEX and MKT in tablets.

MATERIALS AND METHODS

Instruments

Absorbance measurements were made on shimadzu 1800 UV/Visible spectrophotometer with a pair of 10 mm matched quartz cells and a shimadzu digital balance was used for precise weighing of the samples.

Chemicals and reagents

Pure analytical samples of Fexofenadine hydrochloride (SVL Pharmaceuticals Pvt. Ltd., Hyderabad) and Montelukast sodium (Dr. Reddy's Laboratories, Hyderabad) were used in the present work. Commercial tablets of LUKOTAS FX (Intas Pharmaceuticals Ltd., India) containing 120 mg of FEX along with 10 mg of MKT were purchased from local pharmacy. All the solvents and chemicals used were of analytical reagent grade and distilled water was used throughout the study.

Preparation of standard stock solution (1000 µg/mL)

An accurately weighed quantity of 10.0 mg of pure FEX and 10.0 mg of pure MKT were transferred into two separate 10 mL volumetric flasks, dissolved in sufficient quantity of methanol, sonicated for 10 min and made up the volume with same solvent.

Preparation of working standard solution

From the above stock solution 5 mL of FEX was transferred to a 25 mL volumetric flask, 2.5 mL of MKT was transferred to a 25 mL volumetric flask and the volume was made up with 0.1N NaOH, to get concentration of 200 µg/mL (FEX) and 100 µg/mL (MKT) respectively.

Determination of Absorption maxima (λ_{max})

Solutions of 10 µg/mL FEX and MKT were prepared separately in 0.1N NaOH and scanned against blank in the range of 200-400 nm. Clear peaks were observed at 259 nm, 344.5 nm for FEX and MKT respectively and hence these wavelengths were chosen as the λ_{max}.

Simultaneous Equation method

Standard solutions of FEX and MKT in the concentration range of 50 -180 µg/mL and 1-35 µg/mL respectively were prepared in 0.1N NaOH and the absorbance of these solutions was measured at 259 nm and 344.5 nm respectively. The absorptivity values were calculated at the respective wavelengths for both the drugs. Two simultaneous equations[23] were formed as below using the A (1%, 1cm) values.

$$\mathbf{A_1 = 15.81bC_x + 710.0bC_y}$$

$$\mathbf{A_2 = 2.23bC_x + 374.42bC_y}$$

Where, C_x and C_y are the concentrations of FEX and MKT measured in gm/100 mL in sample solutions. A_1 and A_2 are the absorbances of mixture at selected wavelengths 259 nm (λ_1) and 344.5 nm (λ_2). 15.814, 2.23 are A (1% 1cm) values for FEX at λ_1 and λ_2 ; 710, 374.42 are A (1% 1cm) values for MKT at λ_1 and λ_2 respectively.

Assay of tablets

20 commercial tablets of FEX and MKT were triturated and powder equivalent to 30 mg of FEX and 2.5 mg equivalent of MKT was weighed and transferred to a 25 ml volumetric flask, dissolved in sufficient quantity of methanol, sonicated for 10 min and made up the volume to the mark with same solvent. The solution was filtered through Whatman filter paper. From this solution aliquots were taken in replicates, made up the volume to 10 mL with 0.1N NaOH and the absorbance of each sample solution was measured at 259 nm and 344.5 nm. The overlain absorption spectrum of FEX and MKT in pure and formulation is given in Fig. 2 and the assay values obtained are reported in Table 1.

Table 1: Results for simultaneous estimation of FEX and MKT in marketed formulation

BRAND	Label claim (mg/tablet)		Amount obtained* (mg/tablet)		Assay*(%w/w) ± SD	
	FEX	MKT	FEX	MKT	FEX	MKT
LUKOTAS FX	120	10	120.27	10.14	100.22 ± 0.40	101.4 ± 0.84

***Mean of six determinations*

Validation

The proposed method was validated according to ICH guidelines[24] for validation of analytical procedures in order to determine the linearity, precision and accuracy.

Linearity

Aliquots of the working standard solutions of FEX and MKT were suitably diluted in the concentration range of 50-180 µg/mL and 1-35 µg/mL respectively and analyzed as per the developed method. Calibration curves were plotted to verify the Beer's law and the results are reported in Table 2.

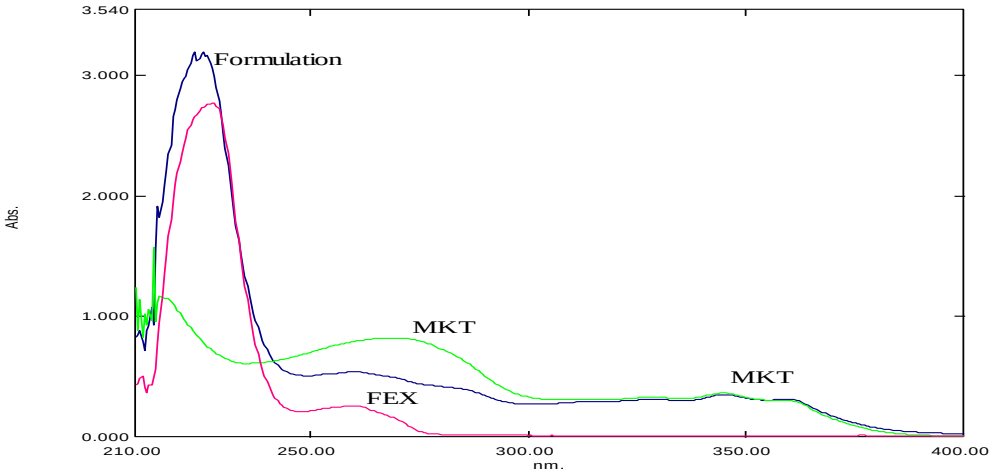


Figure 2: Overlain absorption spectrum of FEX and MKT in pure and formulation.

Precision

Intraday and interday precision studies were performed at three different concentrations of FEX and MKT formulation, each concentration prepared three times and analyzed. The assay was calculated for each preparation and the corresponding % RSD is reported in Table 3.

Table 3: Results for precision studies.

Conc. (µg/mL)		Intra day		Inter Day	
		*Assay (%) ± SD, RSD			
FEX	MKT	FEX	MKT	FEX	MKT
60	5	102.67 ± 1.01, 0.98	99.60 ± 1.85, 1.85	101.96 ± 0.67, 0.66	102.13 ± 0.67, 0.66
120	10	102.18 ± 0.28, 0.27	100.76 ± 0.89, 0.88	100.28 ± 0.41, 0.40	100.33 ± 0.85, 0.84
180	15	100.80 ± 0.33, 0.32	99.86 ± 1.74, 1.74	100.87 ± 0.76, 0.75	99.70 ± 1.73, 1.73

***Mean of three estimations.*

Accuracy

Accuracy of the proposed method was ascertained by standard addition method at 75%, 100%, and 125% wherein to a fixed concentration of the FEX and MKT tablet sample (60 µg/mL:5 µg/mL), varying concentrations of pure drug solutions were added and the absorbance was measured at the respective wavelengths. The percentage recovery at each level was analyzed using the simultaneous equations and the results are given in Table 4.

Table 4: Results for recovery studies.

Level of Recovery (%)	Drug in tablet (µg/mL)		Pure drug added (µg/mL)		*Drug recovered from tablet (µg/mL)		*Recovery (%) ± SD, RSD	
	FEX	MKT	FEX	MKT	FEX	MKT	FEX	MKT
75	60	5	45	3.75	60.33	5.01	100.54 ± 0.61, 0.61	100.2 ± 0.28, 0.27
100	60	5	60	5	60.46	5.05	100.77 ± 0.60, 0.59	100.0 ± 0.28, 0.28
125	60	5	75	6.25	60.86	4.99	101.43 ± 0.38, 0.39	99.97 ± 0.05, 0.05

*****_Mean of three estimations._

Multicomponent mode of analysis

The developed method was also applied in the multicomponent mode of analysis where in 6 mixed standard solutions of FEX and MKT in the ratio of 12:1, 10:1, 13:1, 8:0.5, 11:1, 13:2 (within the linearity range) were prepared in 0.1N NaOH and analyzed in replicates. All the solutions were scanned from 200 – 400 nm in multicomponent mode and the absorbance of each solution was measured at 259 nm (λ_{max} of FEX) and 344.5 nm (λ_{max} of MKT). Similarly sample solution from tablets was also suitably prepared and scanned in this mode. Inbuilt software automatically calculates the concentration of each drug in tablet sample solution and the results are reported in Table 5.

Table 5: Results for simultaneous estimation of FEX and MKT in marketed formulation by multicomponent mode.

BRAND	Label claim (mg/tablet)		Amount obtained* (mg/tablet)		Assay*(%w/w) ± SD	
	FEX	MKT	FEX	MKT	FEX	MKT
LUKOTAS FX	120	10	120.99	10.11	100.82 ± 0.21	101.1 ± 0.27

*****_Mean of three estimations_

RESULTS AND DISSCUSSION

Fexofenadine hydrochloride and Montelukast sodium exhibited maximum absorption at 259 nm and 344.5 nm. FEX and MKT obeyed Beer's law in the concentration range of 50-180 µg/mL and 1-35 µg/mL respectively. The % RSD (< 2.0) values obtained in the intraday precision study indicate the repeatability of the data and the interday precision results were also found to be satisfactory. The accuracy study executed by standard addition method has given acceptable results with an average percentage recovery of 100.75% w/w and 100.05% w/w for FEX and MKT respectively. The proposed method was also checked in the multicomponent mode and the results obtained were very precise and accurate with average assay of 100.82 ± 0.21 for FEX and 101.1 ± 0.27 for MKT as stated on the label claim. The spectral characteristics for the developed method such as Beer's law limits, regression analysis and Sandell's sensitivity are reported in Table 2.

Table 2: Spectral Characteristics, Precision and Accuracy of the developed method

Parameters	FEX	MKT
Range (µg mL^{-1})	50-180	1-35
$\lambda_{max,}$ (nm)	259	344.5
Slope (m)	0.001	0.039
Intercept (c)	0.027	-0.011
Correlation coefficient (r^2)	0.998	0.998
Repeatability (Assay (w/w %) ± SD, % RSD)	101.88 ± 0.54, 0.52	100.07 ± 1.49, 1.49
Intermediate precision (Assay (w/w %) ± SD, % RSD)	99.95 ± 0.63, 0.60	99.85 ± 1.63, 1.07
Accuracy (Recovery (%) ± SD)	100.91 ± 0.53	99.97 ± 0.41
Sandell's sensitivity (µg/cm^2/0.001)	0.66	0.027

CONCLUSION

A new, simple, sensitive and rapid UV spectrophotometric method was developed for the simultaneous analysis of Fexofenadine hydrochloride and Montelukast sodium in pharmaceutical formulations. The developed method is cost effective (as no organic solvent is majorly used) and yet precise and accurate within the established linearity as observed from the statistical results of validation. The present method can be very conveniently employed for the routine quality control analysis of FEX and MKT in tablets without any interference of excipents.

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Conflict of Interest: There are no conflicts of interest.

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