



## Concurrent Discriminative Emission Intensity Quantification of Fexofenadine hydrochloride and Montelukast Sodium

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### ABSTRACT

The synergistic effect of Fexofenadine Hydrochloride an anti-histamine agent and Montelukast Sodium in treating allergies by antagonizing histamine and leukotriene prompted their use as effective fixed dosage form combination. The current research scenario is about concurrent analysis of these drugs by spectrofluorimetric method with the wavelength of excitation and emission 261nm and 287nm for Fexofenadine Hydrochloride and 392nm 487nm for Montelukast Sodium. The Calibration curves were observed to be rectilinear over the concentration ranges 20-100 µg/mL for Fexofenadine Hydrochloride and 2-10 µg/mL for Montelukast Sodium with good correlation coefficient in the range of 0.997 and 0.999 respectively in phosphate buffer, pH 6.8. The LOD and LOQ were found to be 0.36 µg/mL and 2.53 µg/mL for Fexofenadine Hydrochloride and 0.73 µg/mL and 2.152 µg/mL for Montelukast Sodium respectively. The assay was found to be in range of 105% for fexofenadine hydrochloride and 110% for montelukast sodium solution and %RSD values for precision and accuracy studies were found to be less than 2. The results obtained for both drugs (fexofenadine and montelukast) for various parameters were validated according to ICH guidelines. The present method can be applied for quantification of both drugs concurrently in pharmaceutical dosage forms.

**Keywords:** Spectrofluorimetry, Fexofenadine Hydrochloride, Montelukast Sodium, Phosphate buffer.

### INTRODUCTION

Allergies were considered sixth most leading cause for chronic illness with millions over the world enduring mild symptoms like cough,

rashes, sneezing, hives, runny nose, scratchy throat, itchy eyes and in some it may even progress to severe manifestations like breathing problems, asthma attacks, blood pressure fluctuations and sometimes death.<sup>1-3</sup>



Allegra-M the highly effective dosage regimen to treat allergies is composed of Fexofenadine hydrochloride, the second generation antihistamine chemically known as "(±)-4-[1-hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]-butyl]- $\alpha,\alpha$ -dimethyl benzeneacetic acid hydrochloride" which functions by antagonizing H1 receptor and Monteleukast sodium which is non cysteinyl leukotriene receptor antagonist with the chemical name" [R-(E)]-1-[[[1-[3-[2-(7-chloro-2quinolinyl)ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]thio]methyl] cyclopropaneacetic acid, mono sodium salt.<sup>4-6</sup>

The tablet is composed of 120 mg of fexofenadine and 10 mg of monteleukast sodium. The 12:1 ratio of drugs in the dosage form imposes quite a challenge to the analytical chemist in developing a compatible method for the simultaneous estimation of two drugs. The dilution of the solution containing both drugs may affect the probability of detecting monteleukast sodium (10 mg) and high concentration of drugs drops down the sensitivity and specificity of the method. So the spectrofluorimetry was opted to develop simple, sensitive and highly selective method for simultaneous analysis of drugs from the dosage form.<sup>7</sup> The comparable sensitivity of spectrofluorimetric method is a good alternative to time consuming and expensive HPLC method.<sup>8</sup>

Literature study revealed that there are few spectrofluorimetric methods to determine these drugs individually but no simultaneous discriminative spectrofluorimetric analytical method was available for the given combination of drugs till date.<sup>5,9-17</sup> The present work intends to develop simple spectrofluorimetric method which has comparable sensitivity to HPLC, and is less expensive, less time consuming that can be utilised by any laboratories with limited infrastructure.

## MATERIALS AND METHODS

### Apparatus

All the spectrofluorimetric measurements were done by using Spectro-fluorimeter (Shimadzu-RF-5301PC-Japan) where xenon lamp with 1.0 cm quartz cells was equipped. The excitation and emission monochromators were fixed with 1.5mm slits. The pH values of buffer solutions were measured using Elico LI 120 instrument pH- meter.

### Reagents and Chemicals

All reagents and Chemicals are of analytical grade. Pharmaceutical grade of FEX certified to be 99.8% pure was obtained as gift samples from Gravity Pharma Hyderabad and MON was supplied by Neol Pharma, Shimla.

### Fluorimetric analysis of fexofenadine and montelukast

Fexofenadine and montelukast (10 mg) were weighed and transferred into 10 mL volumetric flask and dissolved in methanol. The flasks were shaken and volume was made up to the mark with methanol (1000  $\mu\text{g/mL}$ ).

Standard solution of both drugs (1000  $\mu\text{g/mL}$ ) were diluted appropriately with phosphate buffer, pH 6.8 solution, placed in the cuvette and analyzed using spectrofluorimeter. The excitation and emission wavelengths were identified. The excitation wavelength was fixed and solutions were scanned to get the emission spectrum.

### Standard solution composition

Standard stock solutions of 1000  $\mu\text{g/mL}$  for FEX and MON were prepared by dissolving 10 mg of each drug in 10 mL of Methanol. Serial dilutions were done from standard solution to prepare a working solution of concentration 20-100  $\mu\text{g/mL}$  & 2-10  $\mu\text{g/mL}$  for FEX and MON respectively using phosphate buffer, of pH 6.8 as solvent and fluorescence intensity quantified by spectrofluorimeter.

### Method validation

As per the guidelines of ICH (International Conference on Harmonisation) all the method parameters are validated<sup>18</sup>.

### Procedure for assay of tablet dosage form

20 Tablets of Marketed Formulation (Allegra-M), each containing 120 mg of FEX and 10 mg of MON were weighed in an analytical balance and powdered appropriately by crushing. The average weight of each tablet was recorded and 25 mg weight powder was transferred to 25 mL volumetric flask containing methanol. The solution was sonicated for 15 min, shaken vigorously and volume was brimmed up to the mark. The solution was further filtered and diluted with phosphate buffer of pH 6.8 to get concentrations of each drug with in linearity range for its quantification by spectrofluorimetry.

## RESULTS AND DISCUSSION

### Fluorescence intensity-effect of solvent

The estimation of Fexofenadine Hydrochloride and Montelukast Sodium has done in different solvents such as acetone, water, acetonitrile, DMSO, DMF, methanol and ethanol. The fluorescence was observed for both drugs only in methanol of all the solvents in the experiment so methanol was opted for the fluorescence measurement of both drugs (initial dilution).

### Fluorescence intensity-effect of pH

The effect of pH and different buffer systems on the fluorescence intensity was evaluated for both Fexofenadine Hydrochloride and Montelukast Sodium solutions. Fexofenadine Hydrochloride showed fluorescence with three buffers but the values were low for high concentration of Fexofenadine Hydrochloride. Fexofenadine Hydrochloride solution was found to be acidic because of carboxyl groups, so that supplementary analysis was performed with phosphate buffer of pH 6.8 and in case of Montelukast Sodium, there was no intensity observed up to pH 4.7. Beyond 4.7 the solution showed high intensity at pH 6.8. Therefore, further analysis was performed with phosphate buffer pH 6.8.

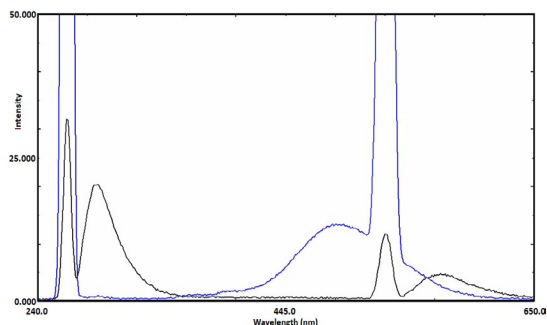
### Fluorescence spectra-wavelength selection

Both Fexofenadine Hydrochloride and Montelukast Sodium showed fluorescence in methanol. The fluorescence spectra of both drugs in phosphate buffer 6.8 were recorded. For Fexofenadine Hydrochloride, the excitation and emission wavelengths were 261 & 287nm and for Montelukast Sodium, the excitation and emission wavelengths were noted as 392 & 487nm.

### Fluorimetric analysis of Fexofenadine Hydrochloride and Montelukast Sodium

Fexofenadine Hydrochloride (100 µg/mL) and Montelukast Sodium (10 µg/mL) solutions were separately prepared in phosphate buffer pH 6.8. The different concentrations of fexofenadine and montelukast were added to match the ratio of drug concentration in marketed formulation (12:1). The solutions were scanned and overlaid to identify the emission maxima (Fig. 1), indicating that two peaks was observed. From the knowledge of drug solutions, a wavelength of ( $\lambda_{em}$ ) 287nm was identified for Fexofenadine Hydrochloride and ( $\lambda_{em}$ ) 487nm was designated for Montelukast Sodium. In spite of different drug concentration in solutions prepared,

the intensities of fluorescence remained the same in the graph plotted. Further a mixture of solution containing both drugs in the above concentration ratio was prepared and scanned. The result indicated similar intensity of signal for drugs both in the mixed solution or individual solutions. Therefore  $\lambda_{em}$  value was assigned for the Fexofenadine Hydrochloride and Montelukast Sodium as 287nm and 487nm.



**Fig. 1. Fluorescence emission spectra of Fexofenadine Hydrochloride (100 µg/mL) and Montelukast Sodium (10 µg/mL) in phosphate buffer solution (pH 6.8)**

### Validation of Method

The evaluation of linearity was done by Least square regression method. All the values and responses for FEX were observed as linear with Pearson's correlation (R) coefficient noted as 0.997 in the concentration range of 20-100 µg/mL at wavelength 487nm. On the other hand, all the values and responses for MON were observed to be linear within a range 2-10 µg/mL concentration at wavelength 487nm with Pearson's correlation coefficient (R) of 0.999. The calibration curves for FEX and MON were shown in Fig. 2&3. The desirable conditions for the optimized method were shown in Table 1. The recovery percentages for FEX & MON are within the range of 98-99 and 97-99 respectively. The percentage relative standard deviation at every concentration level was <2 which indicates the method accuracy. The intra and inter day seems to be having no much different in terms of percentage relative standard deviation which announces the method reproducibility. The relatively least values of LOQ & LOD as tabulated in Table 1 expresses the method sensitivity. The spectrum obtained from the commercial formulation (Fexofenadine Hydrochloride and Montelukast sodium) was compared with spectra of the standard drug solutions. The spectrum of commercial formulation was similar and super-imposable on the individual solutions spectra and there was no interference observed from the excipients in the tablets. The scans were obtained by overlay method at the

analytical wavelengths (287 & 487nm) respectively for FEX and MON and were shown in Figure 4.

### Analysis of tablets

The method proposed was applicable for the ascertainment of the studied drugs in tablets. The results were satisfactory with less than two percent relative standard deviation for accuracy and precision. The assay of market formulations indicated % recovery to be 105% for Fexofenadine hydrochloride and 110% for Montelukast sodium by the proposed method. The results of the assay for both the drugs was presented in Table 2.

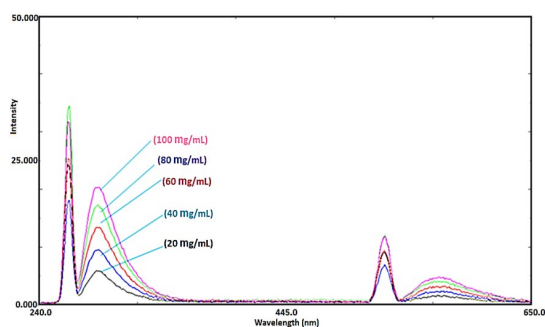


Fig. 2. Spectrofluorometric linearity range of Fexofenadine Hydrochloride (20-100 µg/mL) at emission wavelength 287 nm

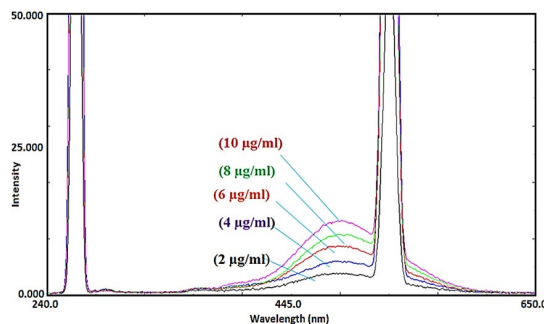


Fig. 3. Spectrofluorometric linearity range of Montelukast Sodium (2-10 µg/mL) at emission wavelength 487nm

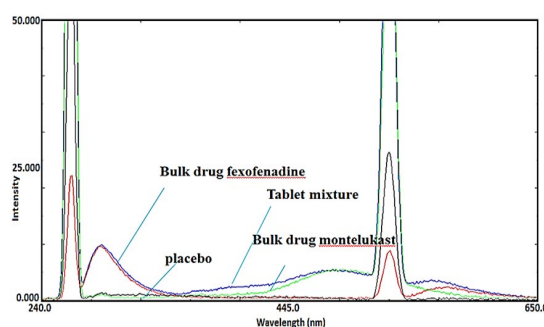


Fig. 4. Fluorescence spectra of placebo, bulk drug and tablet formulation

Table 1: Optimized system suitable spectrofluorimetric conditions

Parameter	Fexofenadine Hydrochloride	Montelukast Sodium
Excitation wavelength (nm)	261	392
Emission wavelength (nm)	287	487
Range (µg/mL)	20-100	2-10
LOD (µg/mL)	0.36	0.7
LOQ (µg/mL)	2.53	2.152
Correlation coefficient ( $r^2$ )	0.997	0.999
Slope (m)	0.2083	1.317
Intercept (c)	0.6439	0.0556
Regression equation	$Y=0.2083x+0.6439$	$Y=1.317x-0.0556$

Table 2: Assay data from analysis of tablet dosage form

Name of the drug	Formulation	Label claim (mg)	Amount found (mg)(AM ± SD) (n=3)	%RSD
Fexofenadine Hydrochloride	Allegra-M	120	126 ± 0.0051	0.05
Montelukast Sodium	Allegra-M	10	11 ± 0.006	0.11

### CONCLUSION

In the present workflow, a concurrent discriminative spectrofluorimetric method was developed for selective and specific quantification of Fexofenadine hydrochloride and Montelukast sodium in eco-friendly solvent phosphate buffer of pH 6.8. The scientific soundness of the method was confirmed by the validation results. This fluorimetric method can be

successfully applied in routine quality control studies of Fexofenadine Hydrochloride and Montelukast Sodium in bulk and tablet dosage form concurrently.

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