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UV-visible Spectrophotometric Estimation of Montelukast and Fexofenadine by Simultaneous Equation Method in Bulk & Combined Tablet dosage form

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Abstract

A simple, rapid, accurate and precise spectrophotometric method has been developed for simultaneous estimation of Montelukast Sodium and Fexofenadine HCl in Pure and tablet dosage form. Proposed method involves formation of 'simultaneous equations' at 259.60nm for Fexofenadine Hydrochloride and 283.00 nm for Montelukast Sodium using methanol as a solvent. The linearity was observed in the concentration range of 30-120 mg/ml for Fexofenadine HCl and 6-20 mg/ml for Montelukast Sodium. The correlation coefficient was found to be 0.9927 for fexofenadine HCl and 0.9985 for Montelukast Sodium. Thus the proposed method is reproducible which can be suitably applied for the estimation of FEXO &MONT in combined dosage forms. The results of analysis have been validated statistically and by recovery studies.

***Key-Words**:* Fexofenadine HCl (FEXO), Montelukast Sodium (MONT), Simultaneous equation method, spectrophotometry

Introduction

Fexofenadine hydrochloride (FEXO) (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 (MONT) (Fig. 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). The aim was present investigation to develop and validate spectroscopic methods (UV- Spectrophotometer) which are Accurate, Sensitive, Precise, and Economical method for simultaneous determination of Fexofenadine Hydrochloride and Montelukast Sodium in bulk drug and pharmaceutical tablet dosage form.

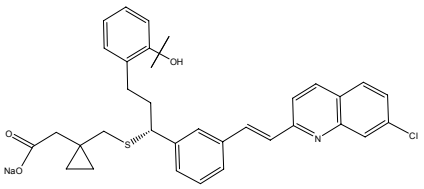

Fig. 1. Structure of Fexofenadine Hydrochloride

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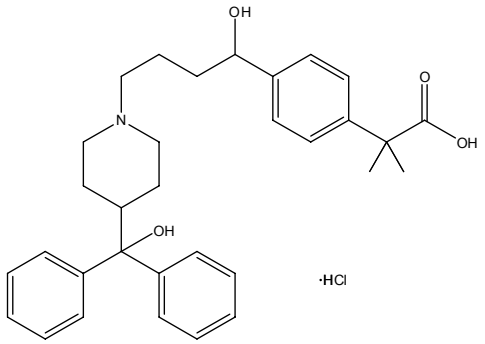

Fig. 2. Structure of Montelukast Sodium

Materials and Methods

Apparatus: A Shimadzu UV -2450 Double beam spectrophotometer has software UV-Probe 2.21 with path lenth 10 mm and variable Slit width was used for the absorbance measurements. All the solutions were freshly prepared using Methanol A.R. Grade.

Preparation of standard stock solutions: Accurately weighed Fexofenadine Hydrochloride and Montelukast Sodium were separately dissolved in sufficient quantity of methanol, then further diluted with methanol to give concentration of 1000µg/ml respectively for Fexofenadine Hydrochloride and Montelukast Sodium. These solutions were used as standard stock solution for the further analysis.

Selection of analytical wavelength: From the sample and standard stock solutions appropriate dilutions of both the drugs were made to obtain final concentration each containing 120 µg/ml of Fexofenadine Hydrochloride and 20 µg/ml of Montelukast Sodium. Solutions of both drugs were scanned in the wavelength range of 200 – 400 nm.

The wavelengths selected should be such that where each wavelength absorptivity difference between two components should be as large as possible. Fexofenadine Hydrochloride shows maximum absorption at wavelength (ëmax) 259.60nm and Montelukast Sodium shows

Reagents and Solutions	
Fexofenadine Hydrochloride	(Gaurav Enterprises, Indore, India),
Montelukast Sodium	(Melody Healthcare Pvt. Ltd., India),
Methanol AR grade	(S.D Fine chem. Ltd, India),
MONTEMAC- FXFexofenadine Hydrochloride, Montelukast Sodium	(Macleods Pharmaceuticals Ltd., India)

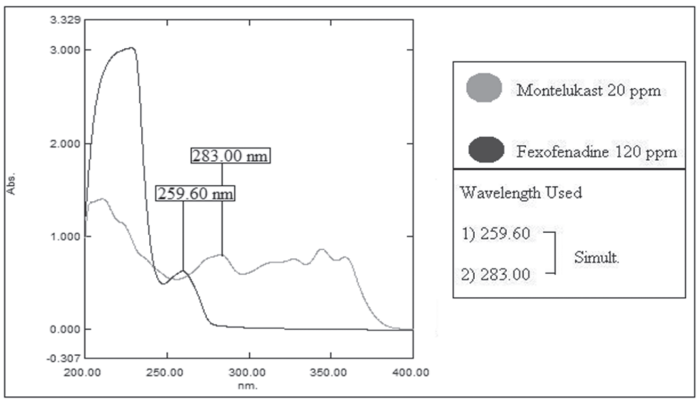

Fig. 3. Overlay spectra of Fexofenadine and Montelukast

Vol. 11 (4) 382-388 October 2017, ISSN 0973-8916 (Print), 2230-7303 (Online)

maximum absorption at wavelength (ëmax) 283.00 nm. Hence, the range for AUC method for Fexofenadine Hydrochloride was 259.60 ± 10 and for Montelukast Sodium was 283.00 ± 10 (Fig. 3).

Result and Discussion

Method validation for Simultaneous Linearity study : For each drug appropriate aliquots were pipette out from standard stock solutions into a series of 10 ml volumetric flasks. The volume was made up to the mark with methanol to get a set of solutions. The Absorbance of each of these solutions were measured at the selected wavelength i.e. 259.60nm and 283.00 nm for Fexofenadine and Montelukast respectively and plotted against concentration (Fig. 4-5 and Table-1 and 2). The concentration range over which the drugs obeyed Lambert-Beer's law. The range was found to be 30-120 mg/ml for Fexofenadine and 6-20 mg/ml for Montelukast.

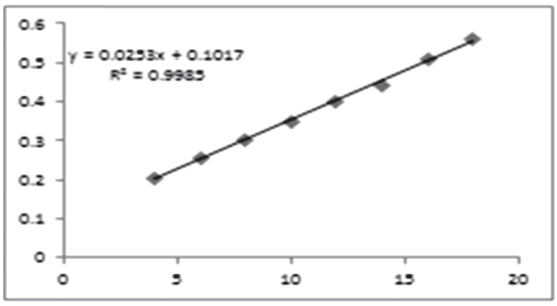

Figure 4: Calibration Curve of Montelukast Sodium

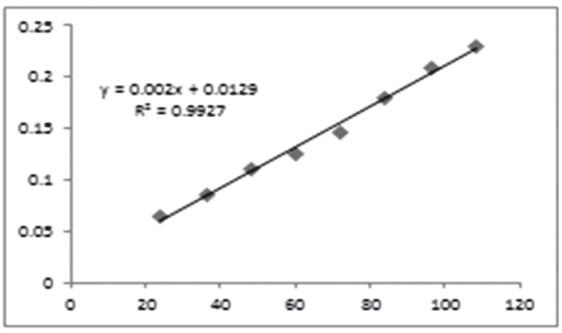

Fig 5. Calibration Curve of Fexofenadine HCl

Determination of absorptivity at analytical wavelength : For each drug appropriate aliquots were pipette out from standard stock solution and a series of dilutions of different concentrations were made for Fexofenadine and Montelukast in the concentration range 30-120 mg/ml for Fexofenadine and 6-20 mg/ml for Montelukast. Absorbances were then divided by concentration gm/L to get absorptivities values. These values are noted in table no.1 and 2.

Precision

Repeatability of method was established by analyzing various replicate standards of Fexofenadine and Montelukast. All the solutions were analyzed thrice, in order to record any intra-day and inter-day variation in the result. % RSD calculated from 3 replicate readings of absorbance values at each concentration confirm the precision of the method. The results of precision are given in Tables 3 and 4.

Analysis of Standard mixture

Standard Mix was made in the ratio of 120:20(FEXO:MONT) from the working stock solution of Fexofenadine and Montelukast (1000 µg/ml), 1.2 ml Fexofenadine and 0.2 ml of Montelukast were taken in 100 ml volumetric flask to Prepare 120 mg/ml of Fexofenadine and 10 mg/ml of Montelukast (Table 5). The standard mixtures prepared were then scanned over the range of UV i.e. 200 to 400nm.The absorbances were measured at selected wavelengths i.e. 259.60 and 283.00 nm. The concentrations of Fexofenadine and Montelukast were calculated by putting the absorbance value in equation.

Amount of each drug was calculated using following formulae,

$$C_X = \frac{A_2\, a_{y1} - A_1\, a_{y2}}{a_{x2}\, a_{y1} - a_{x1}\, a_{y2}}$$

$$C_Y = \frac{A_1\, a_{x2} - A_2\, a_{x1}}{a_{x2}\, a_{y1} - a_{x1}\, a_{y2}}$$

Table 1. Linearity of Montelukast sodium

S. No.	Concentration (µg/ml)	Absorbance of Montelukast sodium at ëmax 259.60nm
1	2.4	0.065
2	3.6	0.086
3	4.8	0.111
4	6.0	0.127
5	7.2	0.148
6	8.4	0.18
7	9.6	0.211
8	10.8	0.231

Table 2: Linearity of fexofenadine

S. No.	Concentration (µg/ml)	Absorbance of fexofe nadine at ëmax 283nm
1	4	0.205
2	6	0.257
3	8	0.302
4	10	0.352
5	12	0.405
6	14	0.446
7	16	0.51
8	18	0.562

Where,

Cx and Cy: - Concentration of FEXO and MONT (gm/L) respectively.

A_1 and A_2: - Absorbances of mixture at 259.60 and 283.00 nm respectively.

ax_1 and ax_2 : - Absoptivity of FEXO at 259.60 and 283.00 nm respectively.

ay_1 and ay_2 : - Absoptivity of MONT 259.60 and 283.00 nm respectively.

Analysis of tablet formulation : Ten tablets were weighed accurately and powdered. Powder equivalent to 120 mg of Fexofenadine & 10 mg Montelukast was weighed and Spiking was done using 10 mg of Montelukast. Whole content was transferred to 100 ml volumetric flask, dissolved in 50 ml of methanol by shaking the flask for 20 min with the help of Sonicator and volume was made up to the mark with methanol. The solution was filtered through Whatmann paper no.41. An aliquot of working stock solution was made by diluting 0.1 ml of standard stock solution to 10ml of methanol to get concentration 120ìg/ml of Fexofenadine and 20ìg/ml of Montelukast. The results of tablet analysis are given in Table 6.

Recovery studies : Recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 80 %, 100 % and 120 %. In recovery study for Fexofenadine amount of standard drug solution added was 48 mg/ml, 60 mg/ml and 72 mg/ml in 80 %, 100 % and 120 % respectively. In recovery study for Montelukast amount of standard drug solution added was 8 mg/ml, 10 mg/ml and 12 mg/ml in 80 %, 100 % and 120 % respectively. The mixed sample solutions were analyzed to get the spectrum, the absorbance measured at 259.60 nm and 283.00 nm and the concentration of each drug was determined using the equations. At each levels of the amount, three determinations were performed. The results for recovery studies are given in Table 7.

Accuracy: The low values of S.D, %RSD, and 95% confidence interval indicate that method is precise. % recovery within limits indicates the non interference from the formulation excipients and confirms the accuracy and precision of the method.

UV spectrophotometric method : The method for the estimation of Fexofenadine and Montelukast was carried out by using a UV-Visible double beam spectropho-tometer (Shimadzu), model no. UV-2450.

Simultaneous Method for estimation of Fexofenadine and Montelukast : The method was found to be simple, accurate, reproducible and rapid, for routine analysis of the formulations.

Table 3. Results of method precision (Intra-day) for simultaneous equation method

Parameters	Standard Deviation		% R.S.D.		Standard Error	
	FEXO	MONT	FEXO	MONT	FEXO	MONT
Low	0.00107	0.006164	0.0122	0.0234	0.0006177	0.00355
Mid	0.00754	0.1177	0.0589	0.3278	0.004353	0.06795
High	0.005431	0.2416	.0293	0.5252	0.003135	0.1394

% R.S.D. = Relative Standard Deviation

Table 4: Results of method precision (Inter-day) for simultaneous equation method

Parameters	**Standard Deviation**		**% R.S.D.**		**Standard Error**	
	FEXO	MONT	FEXO	MONT	FEXO	MONT
Low	0.001326	0.0091	0.01535	0.03432	0.0007656	0.03408
Mid	0.0083267	0.006807	0.06699	0.018925	0.004807	0.003930
High	0.01124	0.00866	0.0615	0.0189	0.00648	0.005

% R.S.D. = Relative Standard Deviation

Table 5. Results of standard mixture analysis (no of determinations = 3)

Parameters	**Components**	
	Fexofenadine	**Montelukast**
Conc.of drug (µg/ml)	120	10
Drug content % ± SEM	101.26±0.3692	100.18±1.027
%RSD	0.6337	1.81135

RSD= relative standard deviation, SEM – Standard error of mean.

Table 6. Results of commercial formulation analysis (no of determinations = 3) for simultaneous determination method

S. No.	**Labeled Claim(mg)**		**Amount Found (ig/ml)**		**% of Labeled Claim**	
	FEXO	MONT	FEXO	MONT	FEXO	MONT
1.	120	10	119.5	9.91	99.58	99.10
2.	120	10	119.8	9.95	99.83	99.5
3.	120	10	119.2	10.03	99.33	100.3
Mean					99.58	99.6
±Standard Deviation					0.25	0.06123
%RSD					0.00251	0.006144
±SEM					0.1443	0.3536

% R.S.D. - Relative Standard Deviation ; SEM - Standard error mean

The reproducibility, repeatability and accuracy of the method were found to be good which is evidenced by low values of standard deviation and percent relative standard deviation. The standard error at 95% confidence level 0.001357 & 0.001798 for Fexofenadine and Montelukast respectively shows the precision of the method. The percent recovery obtained indicates non-interference from the excipients used in the formulation. High Molar absorptivity 28047.002 & 2981.43 for Fexofenadine and Montelukast respectively and low Sandell's sensitivity 0.0173862 & 0.219023 for Fexofenadine and Montelukast respectively for the method reveals that the method is highly

Table 7. Recovery studies of Fexofenadine and Montelukast for Simultaneous estimation method (no of determinations = 3)

Level of % Recovery	**% Recovery***		**% RSD**		**±Standard Error**	
	FEXO	MONT	FEXO	MONT	FEXO	MONT
80	101.3	99.08	0.0002	0.0015	0.01200	0.0869515
100	101.4	102.56	0.0002	0.0062	0.01154	0.3622402
120	99.33	99.79	0.0005	0.0057	0.300231	0.3338337

% R.S.D. - Relative Standard Deviation

Table 8. Simultaneous Estimation of Fexofenadine HCl

S. No	Concentration (µg/ml)	259.60 nm	283.00 nm	Absorptivities	
				259.60 nm	283.00 nm
1.	36	0.086	0.05	23.89	13.89
2.	48	0.111	0.054	23.125	11.25
3.	60	0.127	0.057	21.16	9.5
4.	72	0.148	0.06	20.55	8.33
5.	84	0.18	0.065	21.42	7.73

$Ay_1 = 22.029$ $Ay_2 = 10.14$

Table 9. Simultaneous Estimation of Montelukast Sodium

S. No	Concentration (µg/ml)	283.00 nm	259.60 nm	Absorptivities	
				283.00 nm	259.60 nm
1.	6	0.284	0.257	473.5	428.33
2.	8	0.402	0.302	502.2	377.5
3.	10	0.48	0.352	480	352
4.	12	0.564	0.405	470	337.5
5.	14	0.636	0.446	454.2	318.5

$Ax_1 = 475.98$ $Ax_2 = 362.766$

sensitive. The LOD 5.94 & 2.023 for Fexofenadine and Montelukast respectively and LOQ 18 & 0.6678 for Fexofenadine and Montelukast respectively. Range found between 24-120 µg/ml for Fexofenadine and 6-20 µg/ml for Montelukast with correlation coefficient and respectively. Hence, this method can be successfully applied for the estimation of Fexofenadine and Montelukast in pharmaceutical formulation (Table 8 and 9).

Conclusion

The methods have wider linear range with good accuracy and precision. Hence, the data presented in the manuscript by spectrophotometry thus can be extended for routine analysis of Fexofenadine and Montelukast succinate in pharmaceutical industries, hospitals and research laboratories. Methods were validated as per regulatory guidelines. The results of recovery studies, LOD and LOQ for each of the methods were found to be satisfactory. Intra and inter-day variation of UV method was well within limit with % RSD < 2 %. Statistical analysis of all the method was done according to one-way ANOVA.

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