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A novel spectrophotometric three point detection method for estimation of montelukast sodium and bilastine individually as well as simultaneously in bulk drug, aqueous samples and formulations

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Montelukast sodium and bilastine have been approved in fixed dose combination of 10 mg and 20 mg, respectively, by CDSCO for treatment of allergic rhinitis in adults. Quantitative estimation of drugs is of vital importance during various stages of drug development. Simultaneous estimation of such drugs is a concern as these interact with each other during estimation. Bilastine was not found to interact montelukast sodium at its absorption maximum i.e. 344 nm whereas montelukast sodium was found to interact bilastine throughout UV range. So, montelukast sodium has been simply evaluated at 344 nm as single standard while developing a novel method for estimation of bilastine simultaneously nullifying the interaction of montelukast sodium. The calibration graphs for montelukast sodium and bilastine are linear over concentration ranges of 6-40 µg/mL and 4-40 µg/mL, respectively. As per ICH Q2 (R1) guidelines, both the curves have been found to be specific, precise, accurate, robust and rugged as indicated by the results of validation parameters within specified limits. Also, the method neither requires any complex equipment nor specific software. The calibration curves, thus formed, are useful to evaluate the respective drug alone and in the mixtures/ samples of these two drugs in combination.

Keywords: Bilastine, Montelukast Sodium, Quantitative estimation of drug, Simultaneous estimation, Spectrophotometric method, Three point detection

Introduction

Montelukast sodium and Bilastine have been approved in fixed dose combination of 10 mg and 20 mg respectively by CDSCO for treatment of allergic rhinitis in adults[1]. Montelukast is available as a sodium salt of 2-[1-[(R)-[-2(E)-(7-chloroquinolin-2-yl) vinyl] phenyl] propyl-sulfonylmethyl] cyclopropyl. Montelukast sodium should be taken at a dose of 10 mg once daily[2]. It is used for the prophylaxis and chronic treatment of asthma in adults and paediatric patients who are 12 months of age and older[3].

Bilastine is 2-[4-[1-(2-ethoxyethyl)-1H-1,3-benzo diazol-2-yl]piperidin-1-yl]phenyl-2-methylpropanoic acid[4]. It is an innovative new generation antihistamine with a rapid onset and extended duration of action that is highly selective for the H1 histamine receptor[4]. Bilastine should be taken at a dose of 20 mg once daily. It is used to treat the symptoms of allergic urticaria and rhino conjunctivitis in adults and children older than 12 years old[5].

Various HPLC, UPLC and UV methods have already been reported for bilastine alone or in combination with other drugs. In this sequence, several chromatographic techniques have been accessible for assessing montelukast sodium both on its own and in conjunction with other medications. Ranjan *et.al*. in 2013 developed RP-HPLC method for estimation of montelukast sodium in plasma using ammonium acetate buffer and acetonitrile in 20:80 ratio[6]. Katta *et al*. validated an UPLC method for determination of bilastine and its impurities using trifluoroacetic acid and acetonitrile as solvent system[7]. Andhale *et al*. developed a RP-HPLC method for their simultaneous estimation using Acetonitrile: Phosphate buffer pH 6.8 as solvent system[4].

Several UV spectrophotometric methods have also been found to be established by different researchers for concurrent estimations of the two drugs. Prajapati *et al.*, developed a UV spectrophotometric method in 0.1 N hydrochloric acid and 0.1 N sodium hydroxide as solvents[8]. Similarly, Vyas *et al.*[9], Padhiyar *et al.*[3], Kothari *et al.*[10], developed and validated HPLC and UV spectrophotometric methods in methanol. Chandra *et al*., developed a HPLC method in acetonitrile solvent[11]. A review of these

methods indicates that none of these methods have been developed in water or other aqueous buffer media which is necessary to evaluate the drugs in the aqueous dissolution samples. Further, aqueous media are eco-friendly in nature. Similar are the cases with the methods developed by Mohan Raj *et al*.[12], Detroja *et al*.[13], Peethala *et al*.[14], Vallakeerthi *et al*.[15], Vijayalakshmi *et al*.[16], Roshdy *et al*.[17], Prajapati *et al*.[18], where authors used different organic solvents alone or in combinations.

So, an accurate, simple and precise UV spectrophotometric method has been established in water to estimate bilastine and montelukast sodium individually as well as simultaneously in bulk drugs, physical mixtures, formulations and aqueous samples. The established method was validated agreeing to ICH Q2 (R1) guidelines[19]. The developed method is different from the existing methods from the literature provided in the introduction section and has novelty to publish in such a way that while the existing literatures evaluate the these drug/s in non-aqueous solvent system, the developed method evaluate the drugs in aqueous solvent systems. The development of the method in the aqueous solvent systems is necessary because it will be required to evaluate the aqueous samples of drug release studies those used to be evaluated by HPLC or other separation methods as per the literature survey those are ultimately costly methods.

Experimental Section

Apparatus and chemicals

UV–visible spectrophotometer Shimadzu Double beam (UV-1800, Japan) was utilized with matching 1 cm quartz cells. The Sonicator (Branson Model 3510 Ultrasonic Cleaner, UK) as well as Analytical Balance (Sartorius CPA225D, Italy) were both used. Bilastine and montelukast sodium pure drugs were procured from Akums Pharmaceuticals Limited, Haridwar, Uttrakhand, India. Other chemicals used were of analytical grade.

Preparation of calibration curves for bilastine and montelukast sodium

Bilastine and montelukast sodium have been approved in fixed dose combination of 20 mg and 10 mg, respectively, by CDSCO for treatment of allergic rhinitis in adults[1]. So, their respective stock solutions as well as mixed standard solutions were prepared in the same ratio of concentrations. 10 mg of each drug were accurately weighed and dissolved separately in acetonitrile: methanol (7:3) in 10 mL capacity volumetric flasks to get 1000 µg/mL stock solutions A and B, respectively, for bilastine and montelukast sodium. Further, a 100 µg/mL working stock solution of bilastine (C) and a 50 µg/mL working stock solution of montelukast sodium (D) were prepared from stock solutions A and B, respectively, in 100 mL volumetric flasks by suitable dilutions using the same solvent system.

The prepared stock solution C and D were then suitably diluted with water to get 10 mL each of 24 µg/mL bilastine, 12 µg/mL montelukast sodium, and a mixed standard having 24 µg/mL bilastine and 12 µg/mL montelukast sodium. All the prepared solutions were then scanned between 400 nm-200 nm using a UV-visible spectrophotometer to get the wavelengths of maximum absorbance and other wavelengths of study as well as concentration ranges for both the drugs.

Working pure as well as mixed standard solutions of both the drugs were then prepared from these stock solutions C and D by appropriate dilutions with water in 10 mL capacity volumetric flasks so as to get different concentrations of montelukast sodium and bilastine as depicted in Table 1 to prepare calibration curves for both the drugs. Alternate pure and mix working standard solutions were prepared such that the linearity of the curves can explain the nullification of the interaction by presence of another drug. The calibration curve of montelukast sodium was then prepared at one of its wavelength of maximum absorbance ($\lambda_{max}M$) i.e. 344 nm as a single standard as it was not found to be interacted by bilastine at this wavelength.

The calibration curve of bilastine was prepared by application of the method as given by Prasad *et.al*[20-22] for evaluation of the drug in presence of turbidity and dissolved or undissolved impurities at three wavelengths i.e. at its wavelength of maximum absorbance ($\lambda_{max}B$) 281.5 nm and two other wavelengths equidistant on either side of $\lambda_{max}B$ i.e. λB1 of 279.5 nm ($\lambda_{max}B$ - 2) and λB2 of 283.5 nm ($\lambda_{max}B$ + 2) respectively plotting corrected absorbance according to the Eq. 1 i.e., the difference between absorbance at $\lambda_{max}B$ and average of absorbance at λB1 and λB2 against concentration.

$$\text{Corrected Absorbance} = \left[A(\lambda\text{maxB}) - \left(\frac{A(\lambda B1)+A(\lambda B2)}{2}\right)\right] \quad \ldots (1)$$

Table 1 — Standard solutions for montelukast sodium (MS), bilastine (BIL) and their blends

S. No.	Volume of Stock C (mL)	Volume of Stock D (mL)	Volume of Water (mL)	Conc. of BIL (µg/mL)	Conc. of MS (µg/mL)	Conc. (µg/mL) of Mix Standard with	
						BIL	MS
1	0.4	0.4	9.2	-	-	4	2
2	0.6	-	9.4	6	-	-	-
3	-	0.6	9.4	-	3	-	-
4	0.8	0.8	8.4	-	-	8	4
5	1.2	-	8.8	12	-	-	-
6	-	1.2	8.8	-	6	-	-
7	1.6	1.6	6.8	-	-	16	8
8	2.0	-	8.0	20	-	-	-
9	-	2.0	8.0	-	10	-	-
10	2.4	2.4	5.2	-	-	24	12
11	2.8	-	7.2	28	-	-	-
12	-	2.8	7.2	-	14	-	-
13	3.2	3.2	3.6	-	-	32	16
14	3.4	-	6.6	34	-	-	-
15	-	3.4	6.6	-	17	-	-
16	3.6	3.6	2.8	-	-	36	18
17	3.8	-	6.2	38	-	-	-
18	-	3.8	6.2	-	19	-	-
19	4.0	4.0	2.0	-	-	40	20
20	-	6.0	4.0	-	30	-	-
21	-	8.0	2.0	-	40	-	-

Table 2 — Standard solutions of montelukast sodium (MS) and bilastine (BIL) for specificity studies

Solution	Concentration of MS solution (µg/mL)			Concentration of BIL solution (µg/mL)		
	MS		BIL	BIL		MS
	Without Excipients	With Excipients		Without Excipients	With Excipients	
Pure Standard	18.4	18.4	0	17.6	17.6	0
	23	23	0	22	22	0
	27.6	27.6	0	26.4	26.4	0
Mixed Standard	18.4	18.4	36.8	17.6	17.6	8.8
	23	23	46	22	22	11
	27.6	27.6	55.2	26.4	26.4	13.2

Validation of the prepared calibration curves

The prepared calibration curves were validated agreeing to ICH Q2 (R1) guidelines[19] on grounds of following parameters.

Specificity

Pure as well as mixed standard solutions of both the drugs in known concentrations were prepared separately in the given solvent system with and without common excipients as depicted in Table 2. UV spectra of all the solutions were obtained between 400 to 200 nm and were inspected for any variation in the wavelengths of study; and in absorbance/ corrected absorbance at the wavelengths of study.

Linearity

The linearity of the calibration curves prepared was determined using linear regression analysis. The linearity was also be proved by test for residuals[23].

Precision

Repeatability (n=6) was ascertained by analyzing diverse drug concentrations in mixed standards from independent stock solutions. Intraday as well as interday deviations in the analysis were got to measure intermediate precision of the suggested method. Standard solutions at different levels of 80%, 100% and 120% of midpoint of linearity range (in mixed standards) in 6 replicates were assessed

three times in a day for intraday variation. The alike procedure was trailed for three different days to study interday variation. The precision was determined as percent relative standard deviation (RSD).

Accuracy

Standard addition method was used to assess the accuracy of the developed method. The spiking of the known standard samples was done at three levels i.e. 80%, 100% and 120% of the midpoint of linearity range and the spiked drug content was determined in each sample using the developed method and the accuracy was determined by appropriate calculations.

Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) of the drugs by proposed method were determined by using calibration standards as per Eqs 2 and 3, respectively.

$$LOD = 3.3\left(\frac{SD_{Intercept}}{Slope}\right) \quad \ldots (2)$$

$$LOQ = 10\left(\frac{SD_{intercept}}{Slope}\right) \quad \ldots (3)$$

where "$SD_{intercept}$" is standard deviation of the intercept of regression line and "Slope" is the slope of the calibration curve.

Robustness

To determine the capacity of developed method to remain unaffected by small, but deliberate variations in method parameters and to indicate its reliability during normal usage, robustness study was performed. To check the robustness of the method, there were made minor changes in the solvent system and the absorbances were compared by single factor ANOVA.

Ruggedness

The notion of an analytical method to remain unaffected by varying environmental factors (Room Temperature, Humidity etc.) during normal usage is known as ruggedness of the method. To check the ruggedness of the method, there were made minor changes in the room temperature and the absorbances were compared by single factor ANOVA.

Results and Discussion

The overlay spectra of montelukast sodium (12 µg/mL), bilastine (24 µg/mL) and mixture thereof in the same concentration is presented in Fig. 1. It was observed that bilastine did not interact montelukast sodium between wavelengths of 300 nm to 400 nm in the UV region. So, the calibration curve of montelukast sodium (Fig. 2) was then prepared at one of its wavelengths of maximum absorbance ($\lambda_{max}M$) i.e. 344 nm as a single standard by plotting absorbance i.e. $A(\lambda_{max}M)$ against respective concentration.

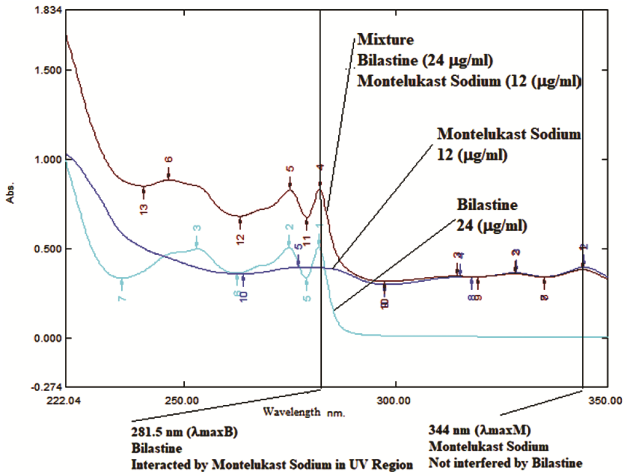

Fig. 1 — Overlay spectra of Bilastine (24 µg/ mL), Montelukast sodium (12 µg/ mL) and mixture thereof in the same concentration

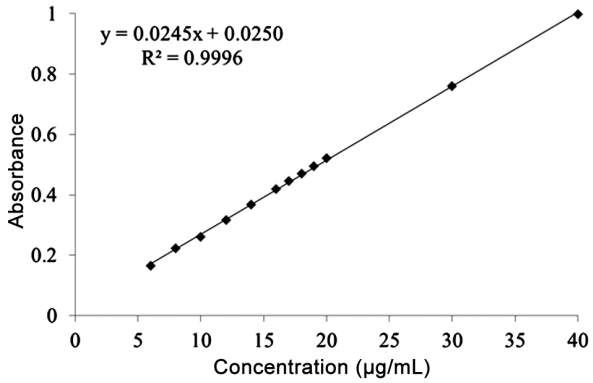

Fig. 2 — Calibration curve of Montelukast sodium in water

On the other hand, it was observed that montelukast sodium interacted bilastine throughout the UV scanning range i.e. 200 nm-400 nm. So, to nullify the interaction, the calibration curve of bilastine (Fig. 3) was prepared by plotting the corrected absorbance according to Eq. 1 against respective concentration.

The concept behind this is that the interaction of absorption of light by the two drugs, i.e. montelukast sodium and bilastine, in UV-visible

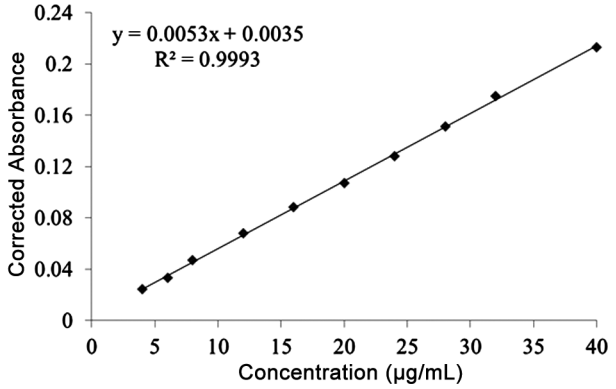

Fig. 3 — Calibration curve of Bilastine in water

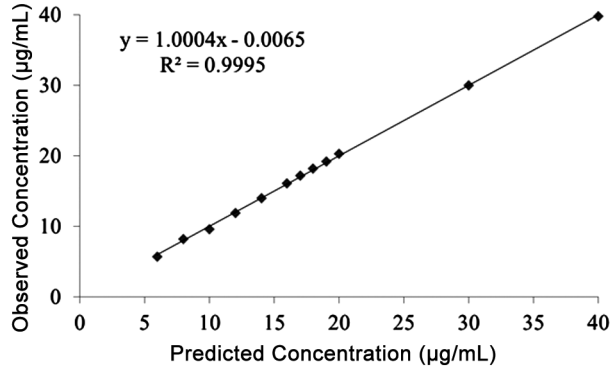

Fig. 4 — Linear curve of predicted concentration versus observed concentration for Montelukast sodium

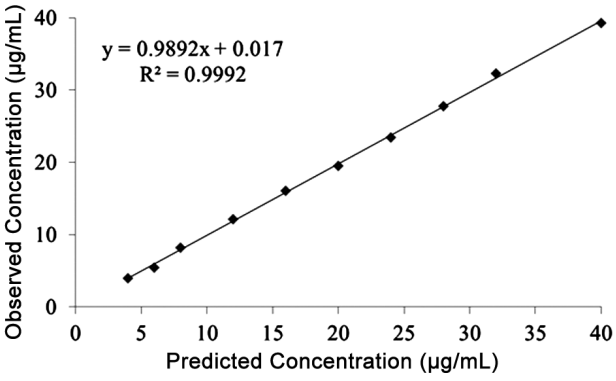

Fig. 5 — Linear curve of predicted concentration versus observed concentration for Bilastine

region (200 nm - 800 nm) is nullified according to Eq. 4, provided concentration of the pure standard of the respective drug and that of the mixed standard of the respective drug is same.

$$\left[A(\lambda maxB)^{P} - \left(\frac{A(\lambda B1)^{P} + A(\lambda B2)^{P}}{2}\right)\right] = \left[A(\lambda maxB)^{M} - \left(\frac{A(\lambda B1)^{M} + A(\lambda B2)^{M}}{2}\right)\right] \quad \ldots (4)$$

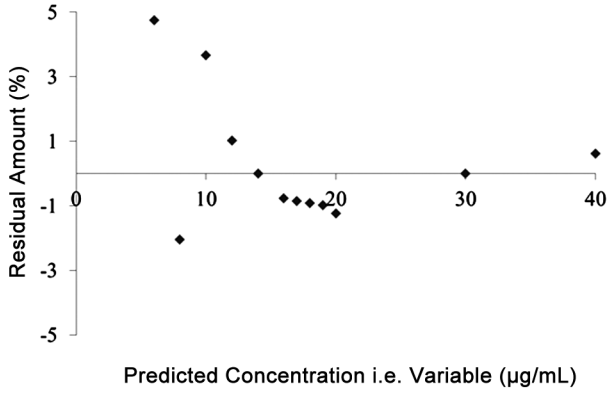

Fig. 6 — Random distribution curve of predicted concentration i.e. variable versus residual amount for Montelukast sodium

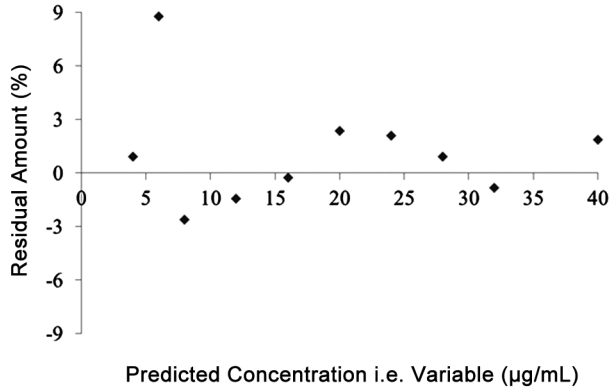

Fig. 7 — Random distribution curve of predicted concentration i.e. variable versus residual amount for Bilastine

where $\lambda_{max}B$ is wavelength of maximum absorbance of bilastine i.e., 281.5 nm; λB1 and λB2 are wavelengths of absorbance of bilastine equidistant on either side of its $\lambda_{max}B$ i.e. 279.5 nm ($\lambda_{max}B$ - 2) and 283.5 nm ($\lambda_{max}B$ + 2), respectively; superscript P indicates pure standard and superscript M indicates mixed standard.

The calibration curves (Fig. 2 and Fig. 3) have been found to be linear in concentration ranges of 6 µg/mL to 40 µg/mL and 4 µg/mL to 40 µg/mL for montelukast sodium and bilastine, respectively as indicated by their respective regression coefficients of 0.9998 and 0.9996.

The linearity has also been confirmed by the linearity of respective predicted versus observed concentration curves (Fig. 4 and Fig. 5) as well as by a random distribution curve of predicted concentration versus residual amount for both the drugs (Fig. 6 and Fig. 7). The parameters of standard curves have been presented in Table 3.

Table 3 — Regression parameters for calibration curves

Parameter	Value	
	Montelukast Sodium	Bilastine
Analytical wavelengths (nm)	λ_{max}M= 344	λ_{max}B= 281.5; λB1= 279.5; λB2= 283.5
Linearity range (µg/mL)	06-40	04-40
Regression equation	y = 0.0245x + 0.0250	y = 0.0053x + 0.0035
Correlation coefficient (r)	0.9998	0.9996
$SD_{Intercept}$ (n=6)	1.75×10^{-4}	3.50×10^{-4}

λ_{max}M and λ_{max}B = wavelength of maximum absorbance of Montelukast and Bilastine, respectively; λB1 and λB2 = wavelength equidistant on either side of λ_{max}B; $SD_{Intercept}$= standard deviation of intercept

Table 4 — Specificity studies for the developed analytical method

Conc. Taken (µg/mL)	Absorbance/ Corrected Absorbance*				Difference in Absorbance/ Corrected Absorbance**			
	Pure Standard		Mixed Standard		Pure Standard		Mixed Standard	
	Without Excipients (A_P)	With Excipients (A_{PE})	Without Excipients (A_M)	With Excipients (A_{ME})	$A_P - A_{PE}$	% ($A_P - A_{PE}$) w. r. t. A_P	$A_M - A_{ME}$	% ($A_M - A_{ME}$) w. r. t. A_M
				Montelukast Sodium[1]				
18.4	0.478± 0.0007	0.477± 0.0008	0.477± 0.0007	0.478± 0.0003	0.001	0.21	0.001	0.21
23	0.591± \0.0010	0.589± 0.0008	0.591± 0.0014	0.593± 0.0003	0.002	0.34	0.002	0.34
27.6	0.703± 0.0007	0.702± 0.0007	0.703± 0.0014	0.704± 0.0003	0.001	0.14	0.001	0.14
				Bilastine[2]				
17.6	0.0970± 0.0007	0.0965± 0.0008	0.0975± 0.0007	0.0970± 0.0003	0.0005	0.51	0.0005	0.51
22	0.1180± \0.0005	0.1175± 0.0004	0.1201± 0.0004	0.1197± 0.0003	0.0005	0.42	0.0004	0.33
26.4	0.1433± 0.0007	0.1426± 0.0007	0.1442± 0.0014	0.1435± 0.0003	0.0007	0.49	0.0007	0.49

*Mean ± SD of 6 replicate determinations; [1]Absorbance of the prepared solutions at 344 nm for montelukast sodium at 344 nm; [2]Corrected Absorbance as per equation 1 at λ_{max}B i.e. 281.5 nm two other wavelengths equidistant on either side of λ_{max}B i.e. 279.5 nm (λ_{max}B - 2) and 283.5 nm (λ_{max}B + 2)

The prepared calibration curves have been found to be specific as indicated by less than 0.49% difference in absorbance or corrected absorbance, whatever applicable, at different concentration levels of pure and mixed standard solutions for montelukast sodium (18.4, 23 and 27.6 µg/mL) and bilastine (17.6 µg/mL, 22 µg/mL and 26.4 µg/mL) respectively (Table 4).

The data for repeatability, intraday precision and interday precision have been presented in Tables 5-7. The low values of RSD i.e. always less than 2% (Tables 5-7) indicated that the developed method is repeatable and precise.

The accuracy was performed by recovery studies i.e. spiking method and the results have been presented in Table 8. The percent recovery of the added known amounts of the drug to a known concentration of the sample was found to be 100.04%±1.48%-101.53% ±0.10% and 99.44%±0.67%-101.32%±0.89% for pure samples of montelukast sodium and bilastine, respectively, and 100.28%±0.29%-100.65%±0.21% and 100.26%±1.34%-101.60%±0.94% for mixed samples of montelukast sodium and bilastine, respectively (Table 8).

The limit of detection (LOD) and limit of quantification (LOQ) of the drugs by proposed method have been found to be 0.047 µg/mL and 0.143 µg/mL respectively for montelukast sodium; and 0.109 µg/mL and 0.331 µg/mL, respectively, for bilastine as calculated by using Eqs (2) and (3).

As far as the test for robustness is concerned, the method has been found to pass the test for robustness as indicated by no significant difference ($p > 0.05$) in the quantitative evaluation of standard samples on changing the solvent system and changing the device. Similar results have been found on changing the room

Table 5 — Repeatability studies/ precision for the developed analytical method

Solution	Concentration (µg/mL)									
	Prepared Having		Found							
	MS	BIL	1	2	3	4	5	6	Mean*	RSD
					Montelukast Sodium					
Pure Standard	18.4	0	18.37	18.45	18.41	18.41	18.41	18.45	18.41±0.03	0.17
	23	0	22.98	23.10	23.10	23.10	23.10	22.94	23.05±0.08	0.32
	27.6	0	27.59	27.51	27.51	27.47	27.63	27.63	27.56±0.07	0.26
Mixed Standard	18.4	36.8	18.45	18.53	18.49	18.49	18.49	18.53	18.50±0.03	0.17
	23	46	23.06	23.18	23.18	23.18	23.22	23.02	23.14±0.08	0.35
	27.6	55.2	27.71	27.63	27.63	27.59	27.76	27.76	27.68±0.07	0.25
					Bilastine					
Pure Standard	0	17.6	17.64	17.26	17.64	17.45	17.45	17.45	17.48±0.14	0.81
	0	22	21.79	21.60	21.60	21.41	21.60	21.60	21.60±0.12	0.55
	0	26.4	26.51	26.32	26.32	26.51	26.51	26.51	26.45±0.10	0.37
Mixed Standard	8.8	17.6	17.45	17.26	17.64	17.45	17.45	17.45	17.45±0.12	0.68
	11	22	21.98	21.79	21.98	21.60	21.60	21.98	21.82±0.19	0.85
	13.2	26.4	26.13	26.32	26.32	26.13	26.13	26.32	26.23±0.10	0.39

*Mean ± SD of 6 replicate determinations

Table 6 — Intraday precision studies for the developed analytical method

Solution	Concentration (µg/mL)						
	Prepared Having		Found				
	MS	BIL	t1*	t2*	t3*	Mean**	RSD
				Montelukast Sodium			
Pure Standard	18.4	0	18.41±0.03	18.47±0.05	18.83±0.04	18.57±0.04	0.22
	23	0	23.05±0.08	23.22±0.06	23.37±0.05	23.21±0.06	0.27
	27.6	0	27.56±0.07	27.65±0.08	27.93±0.02	27.71±0.05	0.20
Mixed Standard	18.4	36.8	18.50±0.03	18.75±0.07	18.65±0.00	18.63±0.03	0.17
	23	46	23.14±0.08	23.41±0.08	23.41±0.08	23.32±0.08	0.35
	27.6	55.2	27.68±0.07	27.87±0.09	27.84±0.09	27.80±0.08	0.30
				Bilastine			
Pure Standard	0	17.6	17.48±0.14	17.26±0.12	17.08±0.17	17.27±0.14	0.83
	0	22	21.60±0.12	21.04±0.12	21.04±0.12	21.23±0.12	0.56
	0	26.4	26.45±0.10	26.45±0.10	26.45±0.10	26.45±0.10	0.37
Mixed Standard	8.8	17.6	17.45±0.12	17.42±0.14	17.92±0.10	17.60±0.12	0.69
	11	22	21.82±0.19	21.82±0.19	22.86±0.15	22.17±0.17	0.79
	13.2	26.4	26.23±0.10	25.84±0.16	26.79±0.16	26.29±0.14	0.53

*Mean ± SD of 6 replicate determinations
** Mean ± SD of t1, t2 and t3

Table 7 — Interday precision studies for the developed analytical method

Solution	Concentration (µg/mL)						
	Prepared Having		Found				
	MS	BIL	d1*	d2*	d3*	Mean**	RSD
				Montelukast Sodium			
Pure Standard	18.4	0	18.41±0.03	18.21±0.03	18.71±0.03	18.50±0.03	0.19
	23	0	23.05±0.08	22.81±0.07	23.30±0.07	23.11±0.07	0.31
	27.6	0	27.56±0.07	27.27±0.05	27.84±0.07	27.60±0.06	0.22
Mixed Standard	18.4	36.8	18.50±0.03	18.70±0.03	18.66±0.03	18.66±0.03	0.17
	23	46	23.14±0.08	23.14±0.08	23.35±0.08	23.27±0.08	0.35
	27.6	55.2	27.68±0.07	27.97±0.07	27.93±0.07	27.90±0.07	0.27
				Bilastine			
Pure Standard	0	17.6	17.48±0.14	18.14±0.15	18.49±0.10	17.97±0.13	0.74
	0	22	21.60±0.12	22.30±0.15	22.42±0.19	21.98±0.16	0.71
	0	26.4	26.45±0.10	27.04±0.14	26.38±0.10	26.62±0.11	0.42
Mixed Standard	8.8	17.6	17.45±0.12	18.46±0.15	17.80±0.14	17.95±0.14	0.78
	11	22	21.82±0.19	22.42±0.15	22.77±0.22	22.46±0.18	0.81
	13.2	26.4	26.23±0.10	26.60±0.10	26.64±0.15	26.51±0.13	0.50

*Mean ± SD of 6 replicate determinations
** Mean ± SD of d1, d2 and d3

Table 8 — Accuracy studies for the developed analytical method

C_s (µg/ml)	C_a (µg/ml)	Pure Standard			Mixed Standard		
		C_t* (µg/ml)	%Recovery*#	RSD	C_t* (µg/ml)	%Recovery*#	RSD
			Montelukast Sodium				
	6.9	18.29±0.06	100.36±0.92	0.91	18.63±0.02	100.46±0.32	0.32
11.5	11.5	22.84±0.17	100.04±1.48	1.48	23.34±0.03	100.28±0.29	0.29
	16.1	27.71±0.02	101.53±0.10	0.10	27.90±0.03	100.65±0.21	0.21
			Bilastine				
	6.6	17.74±0.10	100.53±1.57	1.56	17.92±0.10	101.01±1.57	1.55
11	11	22.23±0.10	101.32±0.89	0.87	22.45±0.10	101.60±0.94	0.92
	15.4	26.42±0.10	99.44±0.67	0.67	26.70±0.21	100.26±1.34	1.34

C_s= Concentration of standard solution, C_a= Concentration of sample solution added and C_t= Total concentration found, #% Recovery= $[(C_t-C_s)/C_a]$x100, *Mean±SD of 6 replicate determinations

temperature and, hence, the prepared calibration curves have been found to pass the test for ruggedness.

Conclusion

Various authors developed assay methods for fixed dose combination formulation of montelukast sodium (10 mg) and bilastine (20 mg) to simultaneously estimate the two drugs avoiding the interaction by one drug to another in UV region. Montelukast sodium has been simply evaluated at 344 nm as single standard as bilastine was not found to interact the former at its absorption maximum i.e. 344 nm. To estimate bilastine which has been interacted by montelukast sodium throughout UV range, a novel method has been developed simultaneously nullifying the effect of montelukast sodium by plotting the corrected absorbance (as calculated by subtracting the average of absorbance at two wavelengths equidistant on either side of λmax form the absorbance at λ_{max}) against concentration.

The calibration curves for montelukast sodium and bilastine were found to be linear over concentration ranges of 6-40 µg/mL and 4-40 µg/mL, respectively. As per method validation guideline mentioned in ICH Q2 (R1), both the calibration curves were found to be specific, precise, accurate, robust and rugged as indicated by the results of validation parameters within specified limits.

The methods proposed were simple, accurate, precise, and neither require any complex equipment nor specific software. The calibration curves, thus formed, is useful to evaluate the respective drug i.e., montelukast and bilastine in mixture of the two drugs as well as in the samples having both the drugs in combination.

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Conflict of interest

The authors declare no conflict of interest.

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