



# DEVELOPMENT AND VALIDATION OF UV-SPECTROPHOTOMETRY FOR SIMULTANEOUS DETERMINATION OF BILASTINE AND MONTELUKAST IN DRUG SUBSTANCES AND COMBINATION TABLETS

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## Abstract:

An accurate, specific, simple, fast, and precise UV spectrophotometric method has been developed and validated for the estimation of Montelukast (MONT) and Bilastine (BILA) in pharmaceutical formulation. The UV-spectrophotometric method includes Q- ratio method (Method I), Absorbance correction method (Method II), Simultaneous equation method (Method III), Area under curve method (Method IV), and first-order derivative method (Method V), For the development of the method I, wavelengths selected were 249nm isobestic point for MONT and BILA and 282nm for MONT and 344nm for absorbance correction method II in methanol. Developed method III wavelengths selected were 252nm and 282nm for estimation of MONT and BILA respectively while for method IV, Area under curve method (AUC) using two-wavelength in the range (279-283.8) nm and (336.6-352) nm in respectively and first-order derivative spectrum method V was obtained in methanol, wavelength selected were bilastine at 279nm zero crossing point (ZCP) for Montelukast and 282.2nm of montelukast ZCP for bilastine. These methods are linear for both drugs in the range of 10-60ug/mL for montelukast and 20-120ug/ml for bilastine in methanol. The % recovery of MONT and BILA was found near to 100%. Validation of the developed methods was carried out for their accuracy, precision, ruggedness, and robustness according to ICH

guidelines. The methods were found to be precise as % RSD less than 2%. The proposed method was successfully applied for the estimation of montelukast and bilastine in bulk and commercial tablet dosage form.

**Keywords:** Bilastine (BILA), Montelukast sodium (MONT), UV spectrophotometry, Q ratio method, Absorption Correction method, simultaneous equation method, Area under curve method, first-order derivative method.

## Introduction:

Bilastine (BILA) is the second generation of H<sub>1</sub> receptor antagonists that allude mainly to allergic rhinitis and chronic urticarial {1}. A white crystalline powder of Bilastine is a molecular weight of 463.622g/mol. It is sparingly soluble in methanol and freely soluble in HCL, NaOH, and Chloroform {2}. It is an antihistamine agent which blocks the action of histamine with the chemical name -2-[4-[2-[4-[1-(2-ethoxyethyl) benzimidazol-2-yl] piperidin-1-yl] ethyl] phenyl]-2-methylpropanoic acid {3} (fig.1). The highly recommend dose of Bilastine is well tolerated. It has no anticholinergic and cardiotoxic effects and does not penetrate the central nervous system but shows minor sedation. It has been shown that to improve health-related quality of life {4, 5}. Practically clinical observation shows that levocetirizine and bilastine have comparable efficacy mad more effective than desloratadine of efficacy. {6} The literature survey found to be very few methods for the determination of Bilastine alone and in combination with other drugs in pharmaceutical formulation, such as UV {7,8}, HPLC {9,12}, and UPLC {13}.

Montelukast sodium (MONT) belongs to a class of leukotriene receptor antagonists (LTRA), used for the treatment of asthma who have difficulties in breathing and seasonal allergy {14}. Montelukast is binding with the cysteinyl leukotriene receptor CysLT<sub>1</sub> site on the mast cell thus blocking the secretion of leukotriene D<sub>4</sub>. This blockage helps in relief from the caused by leukotrienes such as broncho-constriction, and obstruction in the airway {15}. It is a hygroscopic nature, optically active; the white to off-white powder of montelukast sodium is molecular weight 608.18, freely soluble in ethanol, methanol, and water, and practically insoluble in acetonitrile. Its chemical name is 2-[1-[1(R)-[3-[2(E)-(7-chloroquinolin-2-yl) vinyl] phenyl]-3[2-(1-hydroxy-1-methylethyl) phenyl]-propyl-sulfanylmethyl] cyclopropyl] acetic acid sodium salt {16} (fig.2). Literature survey revealed that several analytical methods have been reported in the determination of montelukast sodium alone and combination with other drugs in pharmaceutical preparations, spectrophotometry {17-22}, HPLC {23-36}, HPTLC {37}.

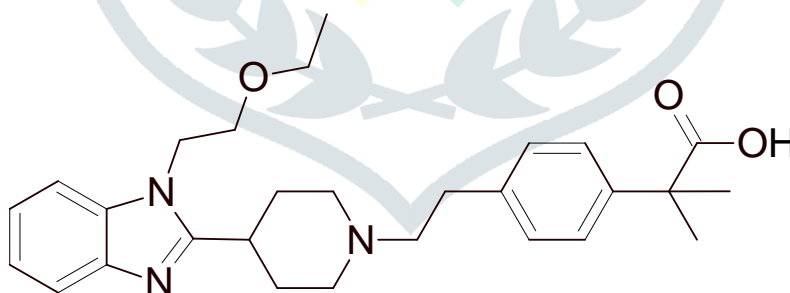


fig.1 chemical structure of bilastine

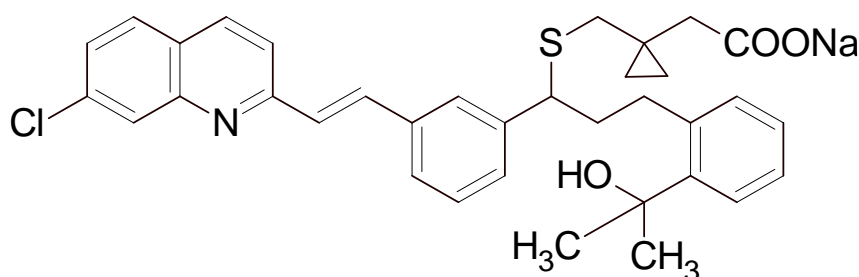


fig 2. chemical structure of montelukast sodium

## Experiment:

### Materials and Methods:

#### Instrument

A UV-Visible spectrophotometer, model V630 (Jassco) and Shimadzu1700 UV-Spectrophotometer having two matched cells with 1cm light path length Quartz cell cuvette used for all absorbance measurements over the range of 200-400nm. A digital Citizen analytical balance (contech) and ultrasonic sonicator were used in the study.

#### Material

Montelukast and bilastine drugs were as gift samples. Methanol (AR grade) was purchased from Merck (India) Ltd., Mumbai, India. AR grade chemical and double distilled water were used during experimentation. Commercial pharmaceutical preparation (Bilacard – M tablet, Cadila pharmaceuticals, Ahmedabad) was procured from the local pharmacy shop, containing 10mg of Montelukast sodium and 20mg of Bilastine.

#### Preparation of standard stock solutions

Accurately weight quantities( 20mg)of MONT and (40mg) of BILA were dissolved in sufficient quantity of methanol in 100ml of amber-colored calibrated volumetric flasks individually &Diluted up to the mark with methanol to obtain standard solutions having a concentration of MONT (200 $\mu$ g/ml) and BILA (400 $\mu$ g/ml). The standard solutions were prepared in a dark place and it's protected from light.

#### Selection of wavelength

From the aloft working standard stock solution of MONT (200 $\mu$ g/mL) and BILA (400 $\mu$ g/mL) 5mL diluted were transferred into a 50ml volumetric flask and completed to mark with methanol to an obtained concentration of MONT (20 $\mu$ g/ml)and BILA (40 $\mu$ g/mL). The solution is protected from light. The working solutions of BILA and MONT were scanned in the UV- range of (200- 400 nm) against methanol as a blank. The overlain spectrum of drugs so recorded (Fig 3).The study of spectrum divulges that MONT shows a well-defined  $\lambda_{max}$  at 282nm and BILA shows a  $\lambda_{max}$  at 252nm; these two wavelengths were selected for the development of simultaneous equation method, 282nm  $\lambda_{max}$  for MONT, 249nm Isoabsorptive point for absorbance ratio method and 344nm for correction method. The area under the curve range of BILA at 279- 283.8nm and MONT at 336.6-352nm and the zero-crossing point for BILA at 279nm and zero-crossing point for MONT at 282.2nm for the first-order derivative method.

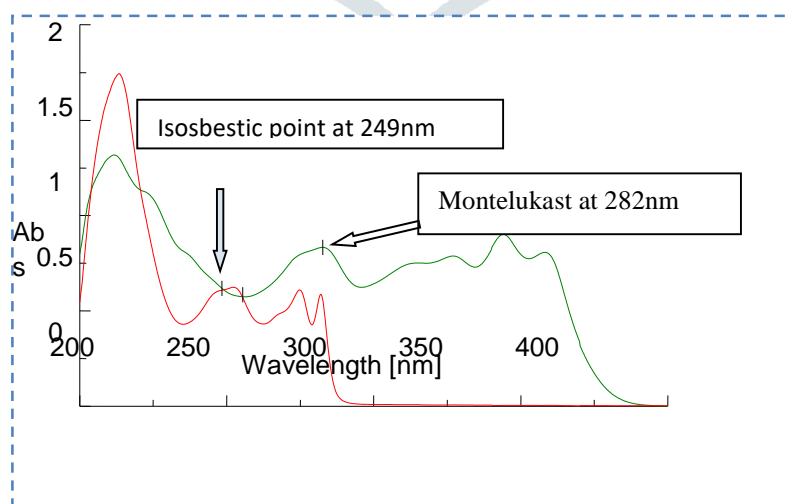


fig. 3 overlay spectra of mont and bila

## Preparation of calibration curve

The standard solution of MONT (200µg/ml), aliquots of 2.5, 5, 7.5, 10, 12.5 and 15mL were diluted into separate series of calibrated 50ml of a volumetric flask with the help of validated 5 mL pipette accurately and volume make up to the mark with methanol to this yielded solution of 10, 20, 30, 40, 50 and 60µg/ml of MONT protected from light. The absorbance was recorded range of 10-60µg/ml for MONT. The standard solution of BILA(400µg/ml), aliquots 2.5, 5, 7.5, 10, 12.5 and 15mL were diluted into a separate series of calibrated 50ml volumetric flask with the help of a validated 5ml pipette accurately and volume make up to the mark with methanol to the yielded solution of 20, 40, 60, 80, 100 and 120µg/ml of BILA. The absorbance was recorded range of 20-120µg/ml for BILA. The absorbance of each resulting solution was measured at 249nm, 252nm, 282nm and 344nm in a 1.0cm cell using a methanol blank. The graphs plotted Absorbance against concentration for MONT and BILA and their mixture respectively.

## Q- Ratio Analysis Method (METHOD I) (38-45)

The absorbance ratio method obeys Beer's law at all wavelengths; the ratio of absorbance at any single wavelength is a constant value independent of concentration or pathlength. At 249 nm, solutions of both drugs of the same concentration exhibit identical absorbance and consequently with zero difference. Isosbestic or iso-absorptive points are those wavelengths where both species absorb light equally. In Q - Absorbance ratio method uses a ratio of absorbance at two selected wavelengths, one which is an iso-absorptive point and the other being the  $\lambda_{\text{max}}$  of one of two components. From the overlay spectra of the two drugs, it was evident that MONT and BILA have an iso-absorptive point at 249 nm ( $\lambda_1$ ). The second wavelength used was 282nm at  $\lambda_{\text{max}}$  of MONT ( $\lambda_2$ ). BILA & MONT showed considerable absorbance at both wavelengths. (Fig. 3)

The concentration of two drugs of the mixture in 1:2 ratios at 249nm and 255nm can be calculated using the following equation 1 and 2. [38] (Fig 4, 5, 6, and 7).

table1: absorptivity values of bila & mont by q- ratio method

Drug	Absorptivity	
	$\lambda_1 = 249\text{nm}$	$\lambda_2 = 282\text{nm}$
MONT	$a_{X1} = 321.65$	$a_{X2} = 203.45$
BILA	$a_{Y1} = 156.2$	$a_{Y2} = 134.7$

$$C_X = \frac{Q_m - Q_y \times A_1}{Q_x - Q_y \times a_{X1}} \dots\dots\dots 1 \quad C_Y = \frac{Q_m - Q_x \times A_1}{Q_y - Q_x \times a_{Y1}} \dots\dots\dots 2$$

Where,  $Q_m$  is a ratio of absorbance  $A_1$  and  $A_2$  are absorbance of the mixture at 249nm and 255nm.  $Q_x$  is a ratio of absorptivity  $a_{X1}$  and  $a_{X2}$  at 249nm and 255nm.  $Q_y$  is a ratio of absorptivity  $a_{Y1}$  and  $a_{Y2}$  at 249nm and 255nm.  $C_X$  and  $C_Y$  is unknown concentrations of montelukast and bilastine respectively in a sample solution.

$$Q_x = a_{X2}/a_{X1}, Q_y = a_{Y2}/a_{Y1} \text{ and } Q_m = A_2/A_1$$

## Absorbance Correction method (method II)

The value of  $\lambda_{\text{max}}$  of MONT and BILA were determined by scanning the drug solution in the range 200-400nm and found to be at 249nm and 282nm, respectively. MONT and BILA also exhibit absorbance at 282nm, while BILA did not exhibit any interference at 344nm (Fig 3). To construct Beer's plot for MONT and BILA, the stock solution of 200µg/mL and 400µg/mL of both the drug was prepared in methanol. Also, Beer's plot was constructed for MONT and BILA of mixture solutions.

The concentration of two drugs in the mixture can be calculated by using equations 3 and 4 (Fig. 4, 7, and 8)

table2: absorptivity value of bila & mont by absorbance correction method

Drug	Absorptivity	
	$\lambda_1=282\text{nm}$	$\lambda_2=344\text{nm}$
BILA	$a_{X1}=162.67$	$a_{X2}=0$
MONT	$a_{Y1}=364.53$	$a_{Y2}=431.47$

$$C_{\text{MONT}} = \frac{A_2 a_{X1}}{a_{X1} a_{Y2}} \dots\dots\dots 3$$

$$C_{\text{BILA}} = \frac{A_2 a_{Y1} - A_1 a_{Y2}}{a_{X1} a_{Y2}} \dots\dots\dots 4$$

Where  $A_1$ = Absorbance of mixture solution at 282nm,  $A_2$ = Absorbance of mixture solution at 344nm,  $a_{X1}$ = absorptivity of BILA at 282nm,  $a_{X2}$ =absorptivity of BILA at 344nm,  $a_{Y1}$  = absorptivity of MONT at 282nm,  $a_{Y2}$  = absorptivity of MONT at 344nm,  $C_{\text{MONT}}$  = concentration of Montelukast,  $C_{\text{BILA}}$ = concentration of bilastine

Simultaneous Equation (Method III)

When samples contain two absorbing drugs that both absorbance at  $\lambda_{\text{max}}$  of the other. From the overlain spectra (Fig 3) two wavelengths, 252nm ( $\lambda_{\text{max}}$  of BILA) and 282nm ( $\lambda_{\text{max}}$  of MONT) were selected for the formulation of a simultaneous equation. The absorptivity was determined at both wavelengths selected for each drug. These values are an average of six estimations. The absorbance and absorptivity values were used for calculating the concentration of MONT and BILA by using the following equations 5 and 6 (Fig. 4, 7, 9, and 10)

table 3: absorptivity value of mont & bila by simultaneous equation method

Drug	Absorptivity	
	$\lambda_1=252\text{nm}$	$\lambda_2=282\text{nm}$
Bilastine	$a_{X1}=158.62$	$a_{X2}=162.67$
Montelukast	$a_{Y1}=296.35$	$a_{Y2}=364.53$

$$C_{\text{BILA}} = \frac{A_2 a_{Y1} - A_1 a_{Y2}}{a_{X2} a_{Y1} - a_{X1} a_{Y2}} \dots\dots\dots 5$$

$$C_{\text{MONT}} = \frac{A_1 a_{X2} - A_2 a_{X1}}{a_{X1} a_{Y2} - a_{X2} a_{Y1}} \dots\dots\dots 6$$

Where  $C_{\text{MONT}}$  and  $C_{\text{BILA}}$  are concentrated in Montelukast and Bilastine respectively in  $\mu\text{g}/100\text{mL}$ .  $A_1$  and  $A_2$  are the absorbances of the mixture at 252 nm and 282 nm respectively  $a_{X1}$ ,  $a_{X2}$ ,  $a_{Y1}$ , and  $a_{Y2}$  are the absorptivity of MONT and BILA at 252nm and 282nm.

Area under curve method (Method IV)

The absorption spectra from (200 to 400nm) of these solutions were recorded using methanol as blank. For simultaneous equation using determine the area under curve method (AUC).The area under the curve value for each component was recorded over the wavelength range of (279-283.8) nm ( $\lambda_1-\lambda_2$ ) and (336.6-352) nm ( $\lambda_3-\lambda_4$ ). Area wear integrated between two selected wavelengths for the analysis of both the drug which shows linearity response with increasing concentration (Fig. 11, 12).The calibration curves of MONT and BILA were prepared by using methanol in the concentration range of 10 – 60 $\mu\text{g}/\text{ml}$  and 20 -120 $\mu\text{g}/\text{ml}$  at their respective AUC range (Fig.13,14, and 15). The area absorptivity values were calculated at each wavelength range for the two components. By the simultaneous equations 7 and 8, calculate the concentration of analyst mixed standard and sample solution.

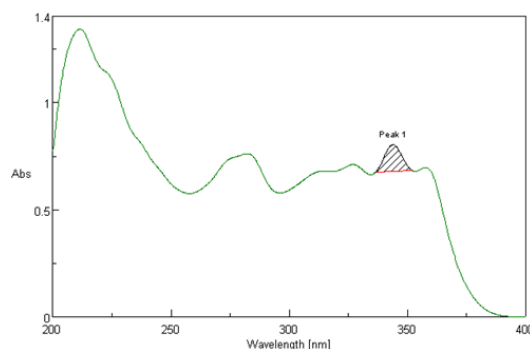


fig. 11 auc of mont at 336.6-352nm

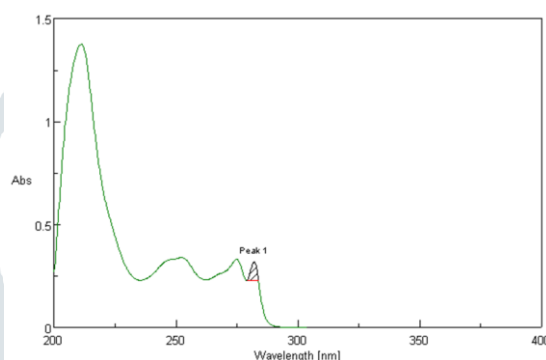


fig. 12 auc of bila at 279-283.8nm

$$C_x = \frac{A_2 a_{y1} - A_1 a_{y2}}{a_{x2} a_{y1} - a_{x1} a_{y2}} \dots\dots\dots 7$$

$$C_y = A_2 - a_{x2} \times C_x / a_{y2} \quad \text{or} \dots\dots\dots 8$$

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

Where  $a_{x1}$  (111.25) and  $a_{x2}$  (0) are absorptivities of BILA at (279-283.3nm) and (336.6-352nm) respectively.  $a_{y1}$  (9.55) and  $a_{y2}$  (502.2) are absorptivities of MONT at (279-283.3) and (336.6-352) respectively.  $A_1$  and  $A_2$  are the absorbance of the mixed standard at (279-283.3) and (336.352) respectively.  $C_{MONT}$  and  $C_{BILA}$  is the conc. of MONT & BILA, respectively in g/100ml.

### First-order derivative spectroscopy (Method V)

The absorption spectra were derivatized from first to fourth order. The First-order derivative spectrum was selected for the analysis of both drugs. The concentration of MONT and BILA were scanned in first-order derivative spectra range of 10-60 $\mu$ g/ml and 20-120 $\mu$ g/ml. The absorbance was measured at  $\lambda_{max}$  =288.4 nm,  $\lambda_{min}$ =270.0 nm & Zero crossing at 282.2nm for MONT and  $\lambda_{max}$  =277 nm,  $\lambda_{min}$ =280.8 nm & Zero crossing 279 nm for BILA (Fig 16, 17). At the zero-crossing point (ZCP) of MONT (282.2 nm), BILA showed the first-derivative absorbance, whereas, at the ZCP of BILA (279 nm), MONT showed the first-derivative absorbance respectively the amplitude difference was measured for each standard concentration, plotted against concentration, and a regression equation was calculated (Fig 18 and 19)

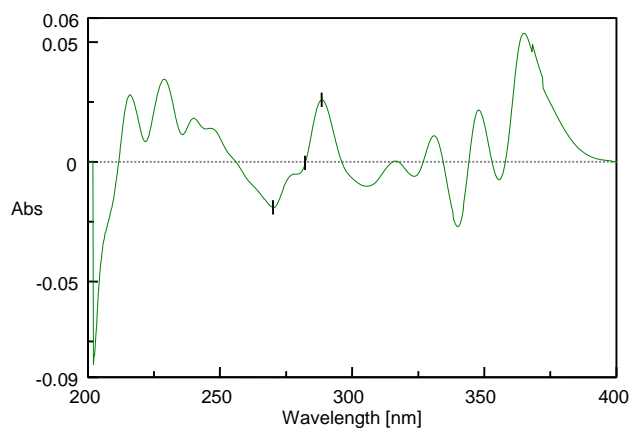


fig. 16: mont of first-order derivative at 282.2nm (zcp)

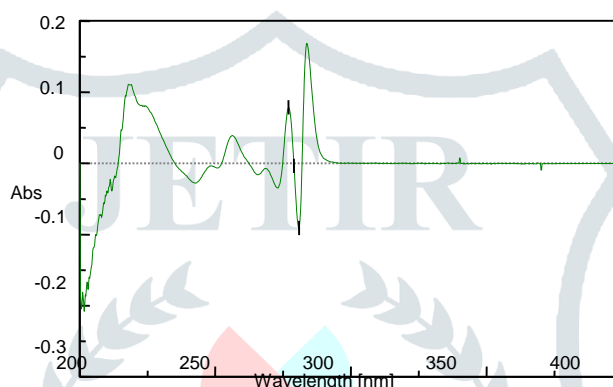


fig. 17: bila of first-order derivative at 279nm (zcp)

## Analysis of Market formulation

The combination of MONT and BILA (Labeled claim 10mg and 20mg) is available in the market formulation in various companies. Ten tablets of Bilacard-M were weighed and triturated in a mortar pestle and powder weighed equivalent to two tablets (0.6261g) was taken into a 100 ml volumetric flask. Add 50ml of methanol the solution was kept for sonication for 15 min, Filtered, through Whatmann filter paper no. 41. Filter the solution makeup to the volume 100ml with methanol, filtrate further 5ml of the above filtrate solution, diluted up to 50ml by using methanol to get a final concentration of 20 $\mu$ g/ml of MONT and 40 $\mu$ g/ml of BILA. These sample solutions were scanned in the range of 200-400nm. The absorbance of resulting solutions was measured at all selected methods wavelength in 1cm cell using methanol as blank. The relative concentration of two drugs in the sample was calculated using all equations (1-8) (Method I-V)

## Validation of the developed methods

The proposed method was validated according to the ICH Q2 (R1) guidelines (39) for validation of analytical procedure for a parameter like a linearity, accuracy, precision, robustness, and ruggedness for the analyte.

### Linearity

Linearity was evaluated by making a standard solution in six different concentrations. The linearity study obeys Beer- Lambert's law concentration range was found to be 10-60  $\mu$ g/mL and 20-120 $\mu$ g/ml for MONT and BILA respectively. For each solution, the absorbance of MONT and BILA was measured at  $\lambda_1$  and  $\lambda_2$ . The calibration

curves of absorbance versus concentrations. Linear regression analysis was used to demonstrate the linearity of absorbance responses vs. concentrations. (Tablet 5)

### Accuracy<sup>o</sup>

To check the accuracy of the proposed method by a percentage of analyte recovered, recovery studies were carried out by assay from a known standard amount of MONT and BILA drug added at three different levels 50, 100, and 150% to the test solution of MONT (20 µg/mL) and BILA (40 µg/mL) as per ICH guidelines. At each level, the recovery research was carried out three times. The result of the recovery studies is reported in (Tables 6 and 7).

### Precision:

The precision of the proposed method was assessed as repeatability, inter-day precision, and inter-day precision. Repeatability was performed for six replicates of the sample solution. For intermediate Precision, Interday and Intraday precision were performed by the determined corresponding response of six replicates on the same and different days for test solution containing MONT (20 µg/mL) and BILA (40 µg/mL). The results of the study are presented in %RSD in (Tables 8 and 9).

### Ruggedness:

The Ruggedness of the proposed method is determined by the analysis of tablet sample solution by two different analysts and the result was recorded in (Table 10)

### Robustness:

The robustness of the method was evaluated by analysis of tablet sample solution small but deliberately varied spectrophotometric condition. A robustness study was carried out changing the methanol manufacture, instrumental variation, and changes in the scanning speed. The result of the robustness study was recorded in (Table 11)

## Results and Discussion:

The UV- spectrophotometric methods were developed for MONT and BILA which can be conveniently employed for routine analysis in pharmaceutical dosage form and will eliminate unnecessary tedious sample preparation. The suggested methods (Method I, II, and II) determine MONT and BILA shows absorptivity below (Table1, 2, and 3). All this method for the determination of MONT and BILA in the combined dosage form is simple, rapid, and accurate. Previously there are few methods of these two drug has been reported. The following developing methods (Method I-V) show the linearity of MONT and BILA at particular wavelengths by obeying Beer's law in the range 10-60ug/ml and 20-120ug/ml. The correlation coefficient, slope, and intercept obtained from each drug are shown in (Table5).The area under curve method (Method IV)solving by using a simultaneous equation, that measures the area of the sample solution at a selected two-wavelength range and calculates the concentration of the drug(Fig.13,14, 15).In the first-order derivative (Method V), linearity was obtained at 279nm and 282.2nm for the ZCP of BILA and ZCP of MONT respectively(Fig 18, 19).The commercial market formulation of tablets in combined dosage form used MONT (20mg) and BILA (40mg)performed all the methods (Method I-V) against mixed standards. The % Purity was found to be 99.96% and 99.93% of MONT and BILA respective drugs below (Table4).Tablet indicates that there is no interference from the formulation's excipients. It is simple and convenient to use for routine quality control analysis. As per the ICH guideline, the method validation parameters checked were linearity, accuracy, precision, robustness, and ruggedness. The recovery study result of MONT and BILA was close to 100% and the relative standard deviation (% RSD) was less than 2%. The results of the recovery study are shown in (Tables 6 and 7). The precision study was calculated as Interday and Intraday variation (%RSD) for both the drugs shown in (Tables 8 and 9). The ruggedness and robustness study calculated in (% RSD) shown in (Table 10, 11)

Table 4: market formulation % purity\*average of six determinations

Brand	Label claim mg/tab	% Purity
Bilacard-M tablet	Montelukast 10mg	99.96%
	Bilastine 20mg	99.93%

Parameter	Method I		Method II		Method III		Method IV		Method V	
	MONT	BILA	MON T	BILA	MONT	BILA	MON T	BILA	MON T	BILA
Linearity range(µg/ml)	10-60	20-120	10-60	20-120	10-60	20-120	10-60	20-120	10-60	20-120
Correlation coefficient	0.999	0.998	0.999	0.999	0.999	0.998	0.999	0.999	0.999	0.999
Slope	0.037	0.014	0.045	0.016	0.037	0.014	0.056	0.010	0.001	0.000
Intercept	0.012	0.049	0.05	0.016	0.012	0.030	0.094	0.017	0.012	0.000

Table 5: linearity studies and regression equations of the proposed methods

\*Average of six determinations, \*\*Method I= Q ratio method, Method II= Absorption correction method, Method III= Simultaneous equation method, Method IV= Area under curve method (AUC), Method IV= Firs order derivative method. \*\*\*MONT= Montelukast, BILA=Bilastine.

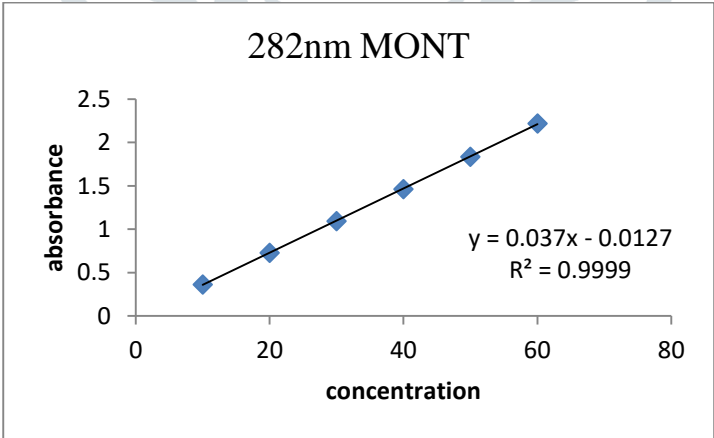


fig. 4 calibration curve of montelukast at 282nm by (method i, ii, iii)

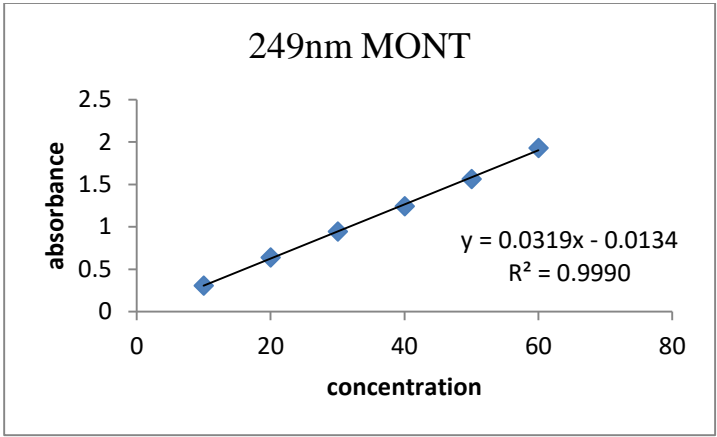


fig. 5 calibration curve of mont at 249nm by (method i)

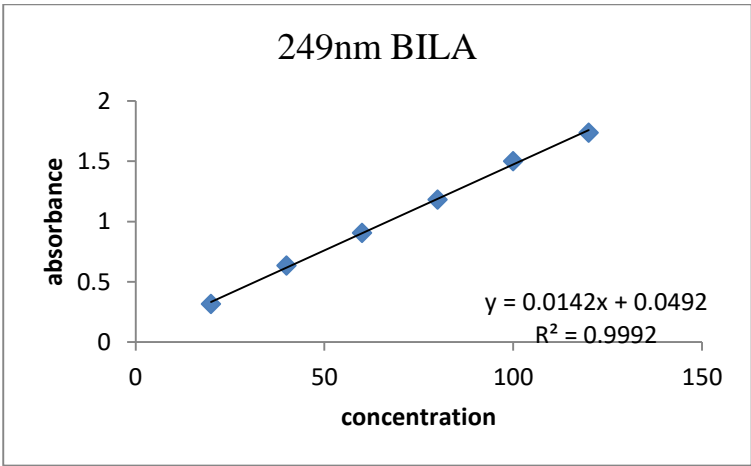


fig. 6 calibration curve of bila at 249nm by (method i)

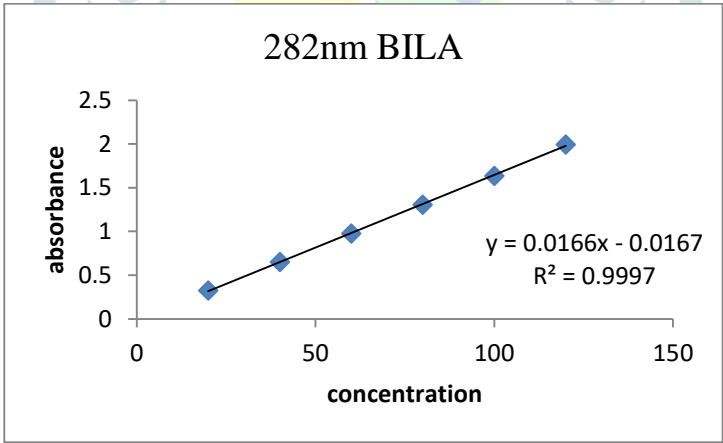


fig. 7 calibration curve of bilastine at 282nm by (method i, ii, iii)

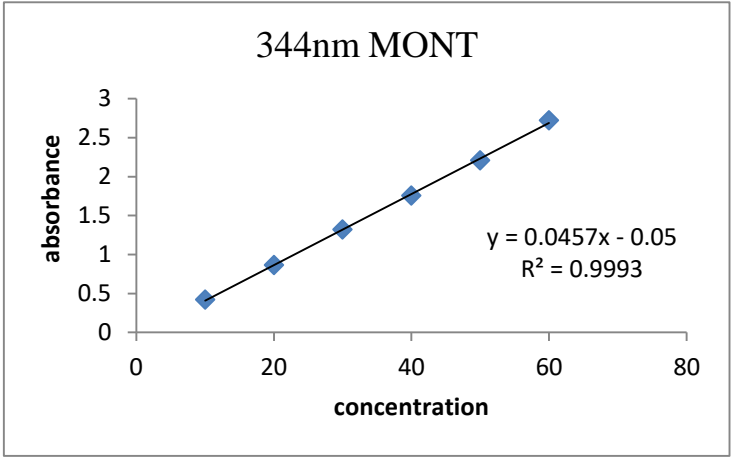


fig. 8 calibration curve of mont at 344nm by (method ii)

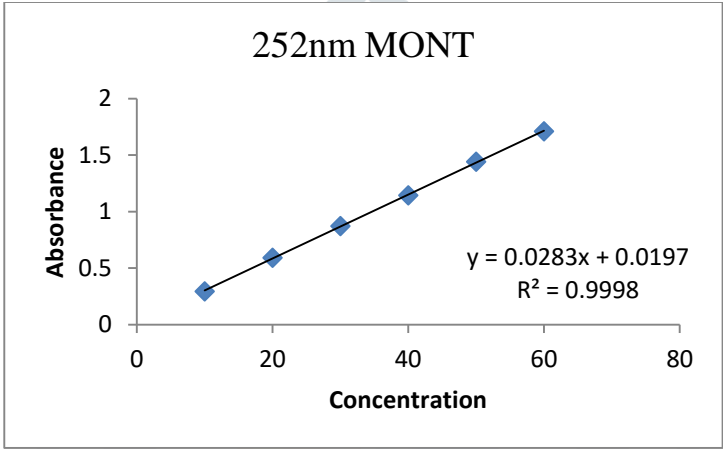


fig.9 calibration curve of mont at 252nm by (method iii)

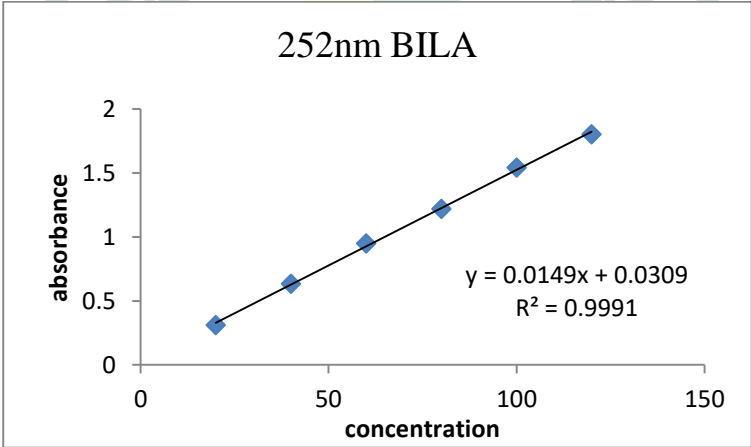


fig. 10 calibration curve of bila at 252nm by (method iii)

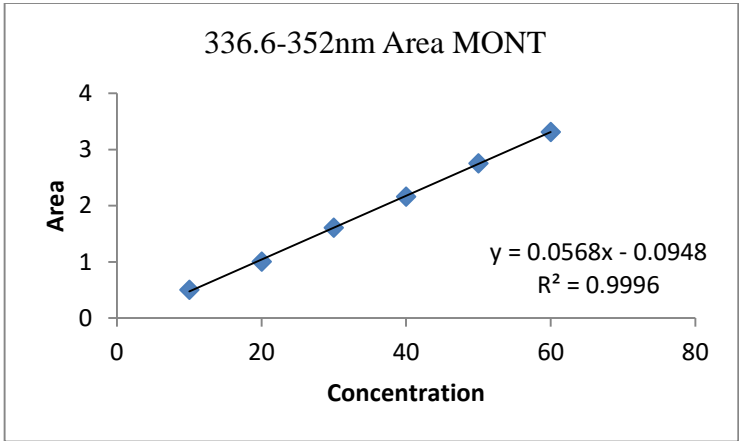


fig. 13calibration curve of auc mont at 336.6-352nm (method iv)

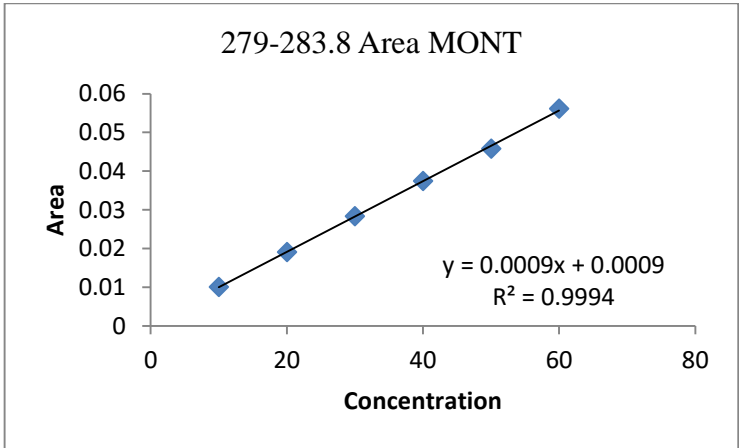


fig. 14 calibration curve of auc mont at 279-283.8nm (method iv)

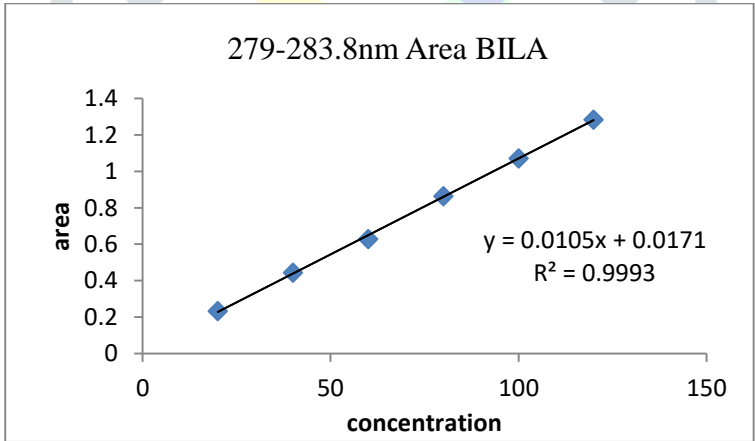


fig. 15 calibration curve of auc bila at 279-283.8nm (method iv)

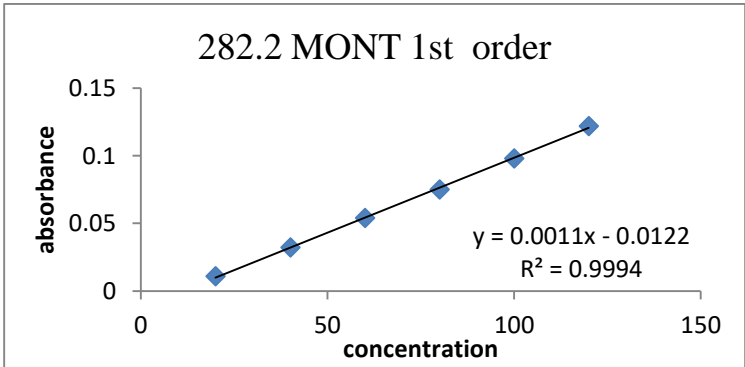


fig. 18 calibration curve of mont at 282.2nm for (method v)

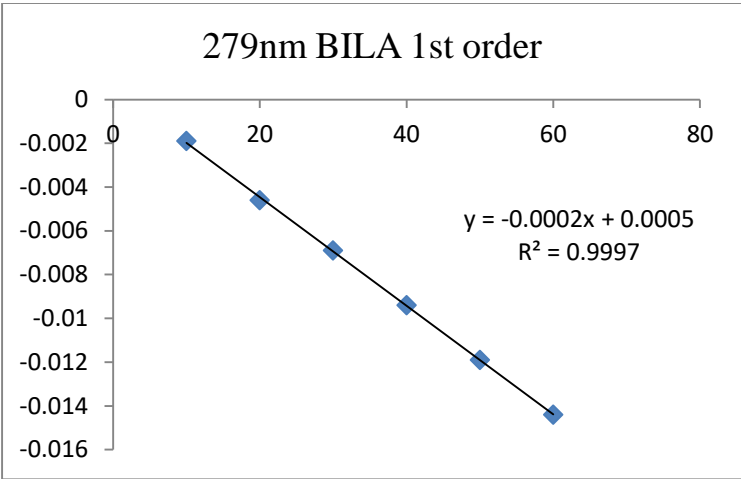


fig. 19 calibration curve of bila at 279nm for (method v)

table 6: recovery study (method i, ii, and iii)

sr. no	wt. of tablet powder taken (mg)	amt. of test solution (µg/ml)		amt of spickel (µg/ml) std. drug		% recovery of method i		% recovery of method ii		% recovery of arm	
		MON T	BILA	MONT	BILA	MONT	BILA	MONT	BILA	MONT	BILA
1	626.2	20.	40	10	20	100.6	97.45	98.00	98.3	98.7	98.2
2	626.2			20	40	97.35	100.3	97.7	98.05	100.35	99.72
3	626.1			30	60	99.13	99.03	98.03	97.91	97.76	99.36
				MEAN	99.02	98.92	97.91	97.08	98.93	99.09	
				SD	1.6253	1.4278	0.1824	0.1975	1.311	0.794	
				%RSD	1.6418	1.4432	0.1863	0.2014	1.325	0.801	

\***Method I**= Q ratio method, **Method II**= Absorption correction method, **Method III**= Simultaneous equation method, **Method IV**= Area under curve method (AUC), **Method IV**= First order derivative method. \*\***MONT**= Montelukast, **BILA**=Bilastine. \*\*\*% RSD= Relative standard deviation

table 7: recovery study (method iv, v)

Sr. No	Wt. of tablet powder taken (mg)	Amt. of test solution (µg/ml)		Amt of spickel (µg/ml) std. drug		% Recovery Method IV		% Recovery of Method V	
		MONT	BILA	MONT	BILA	MONT	BILA	MONT	BILA
1	626.2	20.	40	10	20	100.1	100.6	96.80	98.37
2	626.2			20	40	98.85	100.32	97.41	99.30
3	626.1			30	60	100.53	99.68	100.6	97.81
					MEAN	99.83	100.2	98.26	98.46
					SD	0.861	0.471	1.880	0.617
					%RSD	0.868	0.470	1.913	0.627

table 8: intra-day variation study

Intra-day	Hour	Drug	Concentration (µg/mL)	Method I %RSD	Method II %RSD	Method III %RSD	Method IV %RSD	Method V %RSD
	1hr	MONT	20 µg/mL	0.897	0.486	0.507	0.877	0.505
	2hr	BILA	40µg/mL	0.720	0.804	0.675	0.814	0.627
	4hr							

table 9: inter-day variation study

Inter-day	Day	Drug	Concentration (µg/mL)	Method I %RSD	Method II %RSD	Method III %RSD	Method IV %RSD	Method V %RSD
	1 day	MONT	20 µg/mL	1.295	1.628	1.216	1.693	1.377
	2 day	BILA	40µg/mL	1.542	1.527	1.130	1.507	1.285
	3 day							

table 10: ruggedness study

Analyst	Wt. of powder taken	% Label claim									
		Method I		Method II		Method III		Method IV		Method V	
		MONT	BILA	MONT	BILA	MONT	BILA	MONT	BILA	MONT	BILA
Analyst I	0.6261	98.69	99.75	99.41	98.36	100.52	99.48	101.06	100.38	99.37	98.88
Analyst II	0.6260	98.24	98.23	97.24	96.74	98.12	97.53	100.12	99.04	98.24	97.86
Mean		98.46	98.99	98.32	97.55	99.32	98.50	100.59	99.71	98.80	98.37
SD		0.318	1.074	1.513	1.145	1.697	1.378	0.664	0.947	0.799	0.721
%RSD		0.323	1.085	1.538	1.174	1.708	1.399	0.660	0.950	0.808	0.733

table 11: robustness study

Analyst		% Label Claim									
		Method I		Method II		Method III		Method IV		Method V	
		MONT	BILA	MONT	BILA	MONT	BILA	MONT	BILA	MONT	BILA
Chemical variation	Rankem	99.97	99.48	99.36	98.88	99.59	99.05	99.56	99.01	99.46	98.97
	Loba	100.04	99.54	99.61	99.13	99.63	99.07	99.40	98.92	99.54	99.00
%RSD		0.049	0.042	0.177	0.178	0.028	0.014	0.113	0.064	0.056	0.021
Changes speed variation	fast	99.30	98.84	99.32	98.85	99.43	98.94	99.68	99.15	98.97	98.49
	Medium	99.97	99.48	99.36	98.88	99.59	99.05	99.56	99.01	99.46	98.97
	slow	99.92	99.43	99.40	98.92	99.21	98.73	99.26	98.78	98.27	97.79
%RSD		0.374	0.358	0.040	0.035	0.191	0.164	0.217	0.188	0.604	0.602

Conclusion

There are few spectrophotometric methods are available in pharmaceutical formulation. From the experimental study, it can be concluded that the Q ratio method, Absorption correction method, simultaneous equation, Area under curve method, and first-order derivative method are developed for montelukast (MONT) and bilastine (BILA) in pharmaceutical formulation and combined dosage form. The proposed methods are simple, accurate, and fast for the determination of montelukast and bilastine in a combined dosage form. The method looks to be suitable for quality control in the pharmaceutical industry.

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