Research Article

# Development and Validation of UV Spectroscopic Method for the Simultaneous Estimation of Bilastine and Montelukast in Combined Dosage Form

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**ABSTRACT**

A simple, robust, precise, UV spectroscopic method has been developed for the simultaneous estimation of Bilastine and Montelukast sodium in bulk and tablet dosage forms. In this paper the estimation of those drugs was carried out by simultaneous equation method. This method is based on measurement of absorption at251 nm and272 nm i.e., λmax of Bilastine and Montelukast sodium respectively. The linearity observed for Bilastine is in the range of 10-50µg/ml and for Montelukast is in the range of 5-25µg/ml. The accuracy of methods was assessed by recovery studies and was found to be within the range of 99%-101% for both Bilastine and Montelukast. The developed methods were validated with respect to linearity, accuracy (recovery), and precision. The method can be employed for estimation of pharmaceutical formulations with no interference from any other excipients and diluents. The results were validated as per ICH guidelines.

**Keywords:** Bilastine, Montelukast sodium, ICH, Validation etc.

## INTRODUCTION

Histamine and cysteinyl leukotriene (CysLTs) are potent inflammatory mediators involved in both seasonal allergic rhino conjunctivitis (SARC) and asthma. A combination therapy against both these agents may provide additive benefit.[1] Bilastine is a novel new-generation antihistamine that is highly selective for the H1 histamine receptor, has a rapid onset and prolonged duration of action.1 It has a chemical formula of C28H37N3O3. Montelukast is a member of the leukotriene receptor antagonist (LTRA) category of drugs with molecular formula $C_{35}H_{36}ClNO_3S$ and is indicated for the prophylaxis and chronic treatment of asthma. The Recommended dose of Bilastine and Montelukast sodium is 20 mg and 10 mg respectively.[1] Bilastine is chemically known as 2-[4-[2-[4-[1- (2-ethoxyethyl) benzimidazol-2-yl] piperidin-1- yl] ethyl] phenyl]-2-methylpropanoic acid.[2]. Bilastine is a novel new generation antihistamine that is highly selective for the H1 histamine receptor, has a rapid onset and prolonged duration of action. Histamine plays a major role in the allergic reaction and is released by mast cell degranulation. This histamine binds with H1 receptors, activates the receptors and causes allergic reactions. Bilastine binds with H1 receptor and prevents the activation of H1 receptor by histamine. Thus, it acts as an antagonist for histamine. Bilastine shows no cardiotoxic, sedative side effects and undergoes minimal or no first pass metabolism. It has less chance to undergo drug-drug interactions. Therefore, it is useful for treating patients suffering with renal/ hepatic dysfunction. Bilastine, a piperidine class antihistamine medication used for the treatment of allergic rhinitis and chronic urticaria.[3]

Montelukast is 1-[[[(1R)-1-[3-[(1E)-2-(7-Chloro-2-Quinolinyl) Ethynyl] Phenyl]-3-[2-(1-hydroxy-1-methyl-ethyl) Phenyl] Propyl] Thio] Methyl] cyclopropane acetic acid.[4] Montelukast Sodium is a selective, Potent and Orally Active Antagonist of the Cysteinyl, CysTL1, Leukotriene receptor used for the treatment of Asthma in children's and adults. Montelukast Sodium is a potent drug, selectively CystLT1 receptors antagonist. It is indicated for the prophylaxis and chronic treatment of asthma in adults and pediatric patients.[5,6]

*The synergistic combination of Bilastine plus Montelukast has a dual action and is an attractive treatment option in allergic rhinitis patients with hyperreactive airway disease such as asthma. Both classes of drugs are required for achieving better results*[7,8]

BIL and MONT is a new drug combination. Therefore, there are very few reports of HPLC and UV method development for this new combination. Therefore, this is an attempt to develop novel, simple, robust, accurate method for the determination of efficacy and safety of BIL and MONT combination. This method was fully validated according to International Conference on Harmonization (ICH) and ready for the application in routine analysis without interference of excipients.Published Papers on this drug combination and in combination with another drug by UV[9,10,11], HPLC[12,13,14,15].

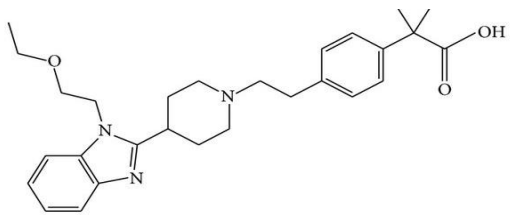



**Figure 1:** Structural formula of Bilastine

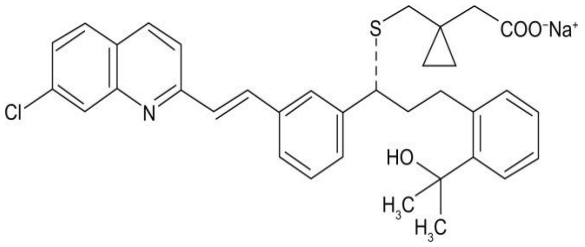



**Figure 2**: Structural formula of Montelukast Sodium

## MATERIALS AND METHODS

### Instruments

Shimadzu UV-1800 double beam spectrophotometer was used to record the spectra of sample and reference solutions using pair of quartz cells of 10 mm path length. All weighing was carried out on Sansui Vibra DJ-150S-S weighing balance. Sonicator of Fast Clean is used for the purpose of sonication, Filter papers of Sartorius Stedim Biotech of grade 292 are used for the filtration purpose.

### Chemicals

Bilastine (20 mg) and Montelukast (10 mg) pure drugs were obtained as a gift sample from Centaur Pharmaceuticals India. The combined formulation B-Latine-MK (20 mg/10 mg) of the two drugs purchased from Vikram Pharmacy Jalgaon. Analytical grade methanol purchased from Merck Chemicals Pvt. Ltd. Mumbai.

### Preparation of stock solution and selection of wavelength

#### *Bilastine stock solution*

An accurately weighed quantity of Bilastine (10 mg) was taken in 10 mL volumetric flask and dissolved in methanol (8 mL) with the help of ultrasonication for about 10 min. Then the volume was made up to the mark using methanol to get Bilastine standard stock solution (1 mg / mL).

#### *Bilastine working solution*

Bilastine standard stock solution (5 mL) was diluted to 50 mL using 80 % v/v methanol to get working standard solution (100 µg/mL).

#### *Montelukast stock solution*

An accurately weighed quantity of Montelukast (10 mg) was taken in 10 mL volumetric flask and dissolved in methanol (8 mL) with the help of ultrasonication for about 10 min. Then the volume was made up to the mark using methanol to get Montelukast standard stock solution (1 mg / mL).

#### *Montelukast working solution*

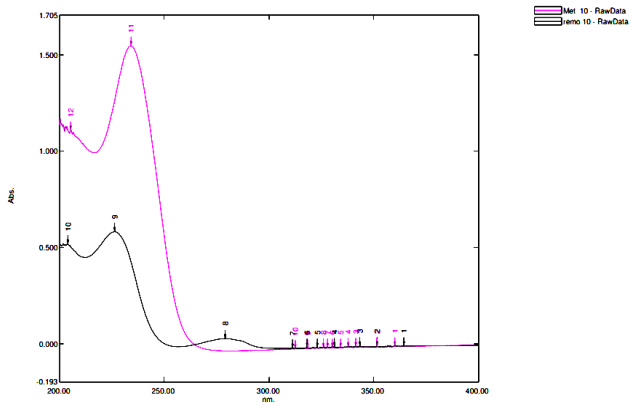
Montelukast standard stock solution (5 mL) was diluted to 50 mL using 80% v/v methanol to get working standard solution (100 µg / mL).

#### *Determination of λ Max of Individual Component*

An appropriate aliquot portion of Bilastine (0.4 mL) and Montelukast (0.2 mL) were transferred to two separate 10 ml volumetric flasks, the volume was made up to the mark using80 %v/v methanol to obtain Bilastine (40 µg/mL) and Montelukast (20 µg/mL). Drug solutions were scanned separately between 200 nm to 400 nm. Bilastine shows the $\lambda_{max}$ at 251 nm while Montelukast shows $\lambda_{max}$ at 272 nm.

#### *Overlay spectra of Bilastine and Montelukast*

The overlay spectra of both drugs were recorded and two wavelengths 251 nm ($\lambda_{max}$ of Bilastine) and 272 nm ($\lambda_{max}$ of Montelukast) were selected for further study.




**Graph No. 1:** Overlay spectra of Bilastine and Montelukast

### Linearity study for Bilastine

An accurately measured aliquot portion of working standard solution of Bilastine was transferred to five separate 10 mL volumetric flasks. The volume was made up to the mark using 60 % v/v methanol to obtain concentrations of Bilastine (10µg/ml, 20µg/ml, 30µg/ml,40µg/ml,50 µg/ml). Absorbance of these solutions was measured at 251 nm, Calibration curve was plotted, absorbance Vs concentration.

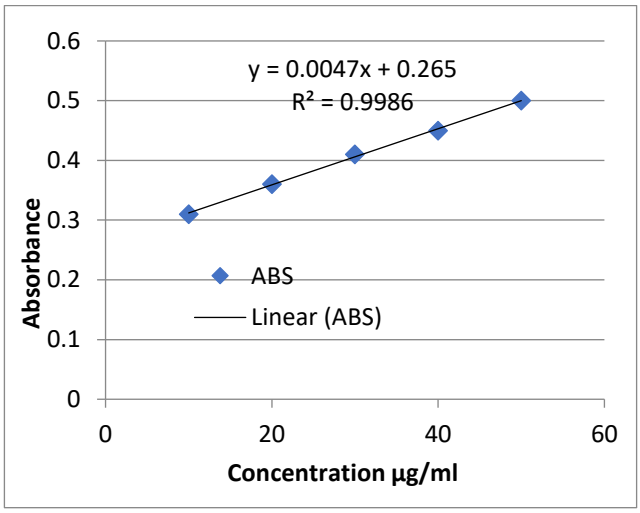
### Linearity study for Montelukast

Accurately measured aliquot portions of working standard solution of Montelukast were transferred to five separate 10 mL volumetric flasks. The volume was made up to the mark using 60% v/v methanol to obtain concentrations (5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml, 25µg/ml) Absorbance of these solutions was measured at 272 nm. Calibration curve was plotted, absorbance Vs concentration. The results are shown in the Table 1.

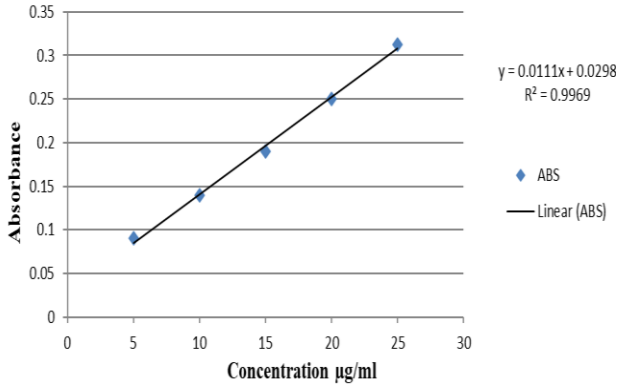
**Table 1:** Regression and Optical characteristics of BIL and MONT

| Parameters | Value for Bilastine | Value for Montelukast |
|---|---|---|
| Beer's law limit (µg/ml) | 5-80 µg/ml | 5-80 µg/ml |
| Regression Coefficient($R^2$) | 0.9986 | 0.9969 |
| Regression equation | y = 0.0047x + 0.2650 | y = 0.0111x + 0.0298 |
| Slope | 0.0047 | 0.0111 |
| Intercept | 0.2650 | 0.0298 |

The study of regression and optical characteristics of BIL and MONT are carried out in which Regression Coefficient ($R^2$) of BIL is 0.9986 and of MONT is 0.9969. The slope of BIL 0.0047 and slope of MONT is 0.0111 with Intercept of BIL 0.2650 and for MONT 0.0298.Therefore, Concentration Vs Absorbance are fairly linear between both co-ordinates by statistical manner and obey ICH guidelines.




**Graph No.2:** Calibration curve of Bilastine at 251 nm




**Graph No.3:** Calibration curve of Montelukast at 272 nm

**Estimation of Laboratory mixture by proposed method**

***Method: Simultaneous Estimation Method***

If a drug sample contains two absorbing drugs (X and Y) each of which absorbs at the $\lambda_{max}$ of the other. Then, it may possible to estimate both drugs by this method. The scanning spectra of 40µg/ml solution of Bilastine and 20µg/ml solution of Montelukast show clear peaks at 251 nm and 272 nm respectively.

Amount of each drug was estimated using following equations,

$$C_x = \frac{A_2 \times ay_1 - A_1 \times ay_2}{ax_2\, ay_1 - ax_1\, ay_2}$$

$$Cy = \frac{A_1 \times ax_2 - A2 \times ax_1}{ax_2\, ay_1 - ax_1\, ay_2}$$

Where;

A1 and A2 are the absorbance of diluted mixture at $\lambda_1$ and $\lambda_2$

Cx and Cy are the concentration of X and Y respectively

ax1 and ax2 are absorptivities of X at $\lambda_1$ and $\lambda_2$ respectively

ay1 and ay2 are absorptivities of Y at $\lambda_1$ and $\lambda_2$ respectively.

The results are determined in the Table No. 2

**Table 2:** Results of Estimation of BIL and MONT in standard laboratory mixture

| Analyte | % Concentration estimated (Mean ± S.D) | % R.S.D |
|---|---|---|
| Bilastine | 99.7 ± 0.1277 | 0.1280 |
| Montelukast | 99.6 ± 0.1095 | 0.1098 |

The estimation of BIL and MONT in Standard Laboratory Mixture are carried out in which % concentration of BIL and MONT were found to be 99.7 and 99.6 respectively. Those values are fairly accurate by statistical manner and are as per ICH guidelines.

**Application of proposed method for Estimation of drugs in tablets**

Twenty 'B-Latine-MK' Tablets containing Bilastine (20 mg) and Montelukast (10 mg) were weighed and ground to fine powder. A quantity of sample equivalent to Bilastine (20 mg) and Montelukast (10 mg) was transferred into 100 mL volumetric flask containing methanol (60 mL), sonicated for 15 min and the volume was made up to the mark and filtered through Whatman filter paper (No. 45). This solution was (1 mL) transferred to 10 mL volumetric flaks, dissolved and volume was adjusted to the mark. The absorbances of the solutions were measured at 251 nm and 272 nm against blank. The concentrations of two drugs in sample were determined by using simultaneous equations. The results are shown in the Table No.3

**Table 3:** Results of Estimation of BIL and MONT in tablets dosage form.

| Analyte | Label claim (mg/tab) | % Label claim estimated (Mean±S.D) | % R.S.D |
|---|---|---|---|
| Bilastine | 20 | 99.16 ± 0.1140 | 0.9261 |
| Montelukast | 10 | 99.15 ± 0.167 | 0.9340 |

The results of Estimation of BIL and MONT in tablets dosage shows the % purity 99.16 & 99.15 with SD and RSD bellow 2 which is fairly accurate by statistical manner and are as per ICH guidelines.

**Validation of proposed method**

The proposed method was validated as per ICH guidelines.

**Accuracy (Recovery study)**

Accuracy of proposed method was ascertained on the basis of recovery study performed by standard addition method. A known amount of standard drug solutions was added to the tablet powder to make final concentrations in the range of 80%, 100% and 120% and re-analyzed it by the proposed method. The absorbance recorded and the % recoveries were calculated using formula,

**% Recovery = [A - B/ C] X 100**

Where,

A = Total amount of drug estimated

B = Amount of drug found on preanalysed basis

C = Amount of Pure drug added

The results are shown in the Table No.4

The results of Recovery study of BIL and MONT are found to be fairly accurate between 99.88 to 101.5% for BIL 99.75 to 100 % for MONT between various concentrations under observation by statistical way and are obey ICH guidelines.

**Precision**

Precision was determined as intra-day and inter-day variations. Intra-day precision was determined by analyzing Bilastine (10, 20, and 30 µg/mL) and Montelukast (5,10 and 15 µg/mL) for three times on the same day. Inter-day precision was determined by analyzing the same concentration of solutions for three different days over a period of week. The results are shown in the Table 5.

The Precision Study of BIL and MONT were carried out and Results are found to be fairly accurate by statistical manner and obeys ICH guidelines.

**Ruggedness**

Ruggedness of the proposed method was determined by analysis of aliquots from homogenous slot by two different analyst using same operational and environmental conditions. The results are shown in Table 6.

**Table 4:** Recovery study

| Drug in mixture solution (µg/ml) | | % Recovery ± S.D. | |
|---|---|---|---|
| **Bilastine** | **Montelukast** | **Bilastine** | **Montelukast** |
| 10 | 5 | 98.88 ± 1.817 | 99.75 ± 0.360 |
| 20 | 10 | 100 ± 1.855 | 99.90 ± 0.100 |
| 30 | 15 | 99.88 ± 1.301 | 98.91 ± 0.070 |

**Table 5:** Precision Study

| Drug | Conc. [µg/mL] | Intra-day Amount Found | | Inter-day Amount Found | |
|---|---|---|---|---|---|
| | | **Mean ±S.D [*n* = 5]** | **% R.S.D.** | **Mean ± S.D. [*n* =5]** | **% R.S.D.** |
| BIL | 10 | 9.94 ± 0.0630 | 0.6337 | 9.93± 0.0743 | 0.7484 |
| | 20 | 19.90 ± 0.1811 | 0.9100 | 19.89 ± 0.1799 | 0.9044 |
| | 30 | 29.97 ± 0.3762 | 0.9411 | 29.96 ± 0.3808 | 0.9529 |
| MONT | 5 | 4.91± 0.1606 | 0.8067 | 9.88 ± 0.07395 | 0.7999 |
| | 10 | 9.98± 0.3693 | 0.9237 | 19.85 ± 0.1192 | 0.9527 |
| | 15 | 19.97 ± 0.4904 | 0.6132 | 39.88 ± 0.3064 | 0.6627 |

**Table 6:** Ruggedness study

| | Bilastine 40 µg/ml | | Montelukast 20 µg/ml | |
|---|---|---|---|---|
| | **Amount found in µg/ml Mean ± S.D. (n=3)** | **% R.S.D** | **Amount found in µg/ml Mean ± S.D. (n=3)** | **% R.S.D** |
| Analyst I | 39.96 ± 0.2066 | 0.2584 | 19.84 ± 0.0953 | 0.9694 |
| Analyst II | 39.75 ± 0.4686 | 0.5876 | 19.95 ± 0.1670 | 1.6787 |
| Day I | 39.97 ± 0.2254 | 0.2819 | 19.01 ± 0.1081 | 1.080 |
| Day II | 39.81 ± 0.5412 | 0.6780 | 19.00 ± 0.1212 | 1.2124 |
| Instrument I | 39.85 ± 0.1184 | 0.1483 | 19.996 ± 0.1258 | 1.2587 |
| Instrument II | 39.86 ± 0.1228 | 0.1538 | 19.02 ± 0.09643 | 0.9624 |

The Ruggedness study of BIL and MONT are carried out and results are found to be fairly accurate by statistical manner and obeys ICH guidelines.

**LOD:** Limit of detection of Bilastine and Montelukast were found to be 2.2203 µg/ml and 1.2551 µg/ml respectively.

**LOQ:** Limit of Quantitation of Bilastine and Montelukast were found to be 6.72825µg/ml and 3.8035µg/ml respectively.

## RESULTS AND DISCUSSION

A simultaneous UV Spectrophotometric Estimation method was developed for Bilastine and Montelukast. The method employs 251 nm as λ1 and 272 nm as λ2 for formation of equations. Bilastine and Montelukast obeys Beer's law in the concentration range 10-50 µg/ml ($R^2$=0.9986) and 5-25 µg/ml ($R^2$=0.9969) respectively. The mean recovery for Bilastine and Montelukast was found to be 99.58 and 99.52 % respectively. The developed method were validated according to ICH guidelines and values of accuracy, precision and other statistical analysis were found to be in good accordance with the prescribed values.

## CONCLUSION

The proposed simultaneous UV Spectrophotometric Estimation method presented in this paper has advantages of simplicity, accuracy, precision and convenience for quantitation of Bilastine and Montelukast. The proposed method can be used for the quality control of Bilastine and Montelukast in typical laboratories.

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**Conflict of Interest:** The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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