# DEVELOPMENT AND VALIDATION OF ANTI ALLERGIC DRUGS : RP-HPLC

**Varun.Dasari [*], Perli.Krantikumar, S.K.Godasu, Pothula.Raju, Gopinadh.Vuyyala**
**Sri Indu Institute of Pharmacy, Sheriguda (V), Ibrahimpatnam (M), Hyderabad.**

[*]**Correspondence to Author:**
**Dr.VARUN DASARI**
Professor & Principal
Department of Pharmaceutics
Sri Indu Institute of Pharmacy
Hyderabad 501510
**Mail.id:drdvarun@gmail.com**

**ABSTRACT:**

A straightforward and specific LC technique is depicted for the assurance of Bilastine and Montelukast in tablet measurements structures. Chromatographic partition was accomplished on a Waters AcquityC18(50mm x2.1 mm ID) 1.8µm utilizing portable stage comprising of a combination of 55 volumes of blended Phosphate Support pH 3.5: Acetonitrile (75:25) %v/v with location of 265nm. Linearity was seen in the reach 20-60 µg/ml for Bilastine (r2 =0.9995) and 10-30 µg/ml for Montelukast (r2 =0.9997) for how much medications assessed by the proposed techniques was in great concurrence with the name guarantee. From the above trial results and boundaries, it was reasoned that, this recently evolved strategy for the synchronous assessment Bilastine and montelukast was viewed as basic, exact, precise and high goal and more limited maintenance time makes this technique more adequate and savvy and it very well may be actually applied for routine examination in research establishments, quality control office in implied in enterprises, supported testing labs concentrates on in not so distant future.
**Keywords:** Bilastine, Montelukast, RP-HPLC, Simultaneous estimation.

## INTRODUCTION:

Bilastine is an original new-age allergy med that is profoundly specific for the H1 receptor, has a quick beginning and drawn out term of activity. Bilastine is a specific receptor H1 receptor bad guy (Ki = 64Nm).[1] During unfavorably susceptible reaction pole cells go through degranulation which discharges receptor and other subastances. By restricting to and forestalling actuation of the H1 receptor, bilastine lessens the advancement of hypersensitive side effects because of the arrival of receptor from pole cells.[2] IUPAC Name of Bilastine is 2-[4-(2-{4-[1-(2-ethoxyethyl)- 1H-1,3-benzodiazol-2-yl] piperidin-1-yl} ethyl) phenyl]-2-methylpropanoic corrosive. Substance Recipe of Bilastine is C28H37N3O3. Atomic Load of Bilastine is 463.622 g·mol−1. Bilastine is dissolvable in the natural dissolvable chloroform at a convergence of roughly 30 mg/ml.

Montelukast is a leukotriene receptor bad guy utilized as a feature of an asthma treatment routine, to forestall practice instigated bronchoconstriction, and to treat occasional hypersensitive rhinitis.[3] Cysteinyl leukotrienes (CysLT) like LTC4, LTD4, and LTE4, among others, are eicosanoids delivered by various cells like pole cells and eosinophils. When such CysLT tie to relating CysLT receptors like CysLT type-1 receptors situated on respiratory aviation route smooth muscle cells, aviation route macrophages, and on different favorable to provocative cells like eosinophils and some particular myeloid foundational microorganisms exercises that work with the pathophysiology of asthma and hypersensitive rhinitis are stimulated.[4] IUPAC Name is 2-[1-({[(1R)- 1-{3-[(E)- 2-(7-chloroquinolin-2-yl) ethenyl] phenyl}-3-[2-(2-hydroxypropan-2-yl) phenyl] propyl] sulfanyl}

methyl) cyclopropyl] acidic corrosive. Synthetic Equation is C35H36ClNO3S. Sub-atomic Weight is 586.183 g·mol−1. Montelukast sodium is a hygroscopic, optically dynamic, white to grayish powder. Montelukast sodium is unreservedly solvent in ethanol, methanol, and water and basically insoluble in acetonitrile.

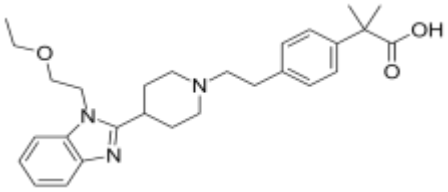


**Figure 1: Structure of Bilastine**

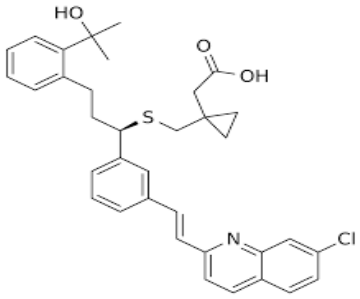


**Figure 2: Structure of Montelukast**

The writing review uncovered that There are not very many techniques detailed in that frame of mind for examination of Bilastine and Montelukast alone or in mix with different medications in the unadulterated structure and drugs definitions by RP-HPLC [6-10], RP-UPLC [11], UV [12]. Considering the requirement for a reasonable, savvy RP-HPLC technique for routine examination of Concurrent assessment of Bilastine and Montelukast in Tablet measurements structure, endeavors were made to foster straightforward, exact, precise and practical scientific strategy for the assessment of Bilastine and Montelukast. The proposed strategy will be approved according to ICH rules. The goal of the proposed work is to foster a new, basic, delicate, precise and practical scientific technique and approval for the Concurrent assessment of Bilastine and Montelukast in Tablet dose structure by utilizing RP-HPLC. To approve the created strategy as per ICH rules for the expected scientific application i.e., to apply the proposed technique for examination of the medication in its measurement structure. To apply the created technique for the concurrent assessment of Bilastine and Montelukast in Tablet measurement's structure.

## MATERIALS AND METHODS:

**Chemicals and Reagents:**: Bilastine and Montelukast were Gift tests got from Madras drugs, Chennai. NaH2PO4 was scientific grade provided by Finerchem restricted, Orthophosphoric corrosive (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

**Equipment and Chromatographic Conditions**: The chromatography was performed on a Waters 2695 HPLC framework, outfitted with an auto sampler, UV finder and Enable 2 programming. Investigation was done at 265 nm with segment Waters AcquityC18(50mm x2.1 mm ID) 1.8µm, aspects at 350C temperature. The streamlined portable stage comprises of Phosphate Support pH 3.5: Acetonitrile (75:25) %v/v Stream rate was kept up with at 0.5 ml/min and run time for 5 min.

**Preparation of solutions**
**Diluent:** Portable stage is utilized as Diluant.

**Preparation of stock standard solutions:**
Around 125 mg of BILASTINE and 100mg of Montelukast were weighed into a 100 mL volumetric flagon, to this 70mL of portable stage was added, sonicated and the volume was made up with the

versatile stage. Pipetted 5 mL of the unmistakable arrangement in to 50 mL volumetric carafe and make up volume with versatile stage.

**Preparation of Sample Solutions**

Pulverize more than 20tablets then gauge an amount of powder identical to 125mg of BILASTINE and 100mg of Montelukast in 100 mL volumetric cup and add70mL of versatile stage then sonicated it for 30min discontinuous shacking after 30min make up volume with portable stage. Pipetted 5 mL of the reasonable arrangement in to 50 mL volumetric carafe and make up volume with portable stage. Channel the arrangement through 0.45µm channel paper. The subsequent arrangement is utilized to record the chromatogram

**System:**

20µL of the norm, test are infused into the chromatographic framework and the regions for Bilastine and Montelukast tops are estimated and the %Assay are determined by utilizing the formulae.

**METHOD:**

The created chromatographic strategy was approved for framework appropriateness, linearity exactness, accuracy, roughness and vigor according to ICH rules.

Framework reasonableness boundaries: To assess framework appropriateness boundaries, for example, maintenance time, following variable and USP hypothetical plate count, the portable stage was permitted to move through the segment at a stream pace of 0.5 ml/min for 5 minutes to equilibrate the section at 35oC temperature. Chromatographic partition was accomplished by infusing a volume of 10 µL of standard into Waters AcquityC18(50mm x2.1 mm ID) 1.8µm, the portable period of piece Phosphate Support pH 3.5: Acetonitrile (75:25) %v/v was permitted to course through the section at a stream pace of 0.5 ml each moment. Maintenance time, following element and USP hypothetical plate count of the created technique are displayed in table 1,2.

**Assay of pharmaceutical formulation:** The proposed approved strategy was effectively applied to decide Bilastine and Montelukast in their tablet dose structure. The outcome acquired for Bilastine and Montelukast was equivalent with the comparing named sums and they were displayed in Table-3.

**Validation of Analytical method:**

**Linearity and Reach**: The linearity study was performed for the grouping of 62.5 to 188.5 µg/mL and 100 to 300 µg/ml. Each level was infused into chromatographic framework. The region of each level was utilized for computation of connection coefficient. Infuse each level into the chromatographic framework and measure the pinnacle region. Plot a diagram of pinnacle region versus focus (on X-hub fixation and on Y-pivot Pinnacle region) and compute the connection coefficient. The outcomes are displayed in table 4,5.

**Accuracy studies:** The not entirely set in stone by help of recuperation study. The recuperation technique completed at three level half, 100 percent, 150%. Infuse the standard arrangements into chromatographic framework. Work out the Sum found and Sum added for Bilastine and Montelukast and ascertain the singular recuperation and mean recuperation values. The outcomes are displayed in table 6,7.

**Precision Studies:** accuracy was caliculated from Coefficient of fluctuation for six reproduce infusions of the norm. The standard arrangement was infused for multiple times and estimated the region for each of the six Infusions in HPLC. The %RSD for the area of six repeat infusions was found. The resulte are displayed in table 8.

**Ruggedness:** To assess the halfway accuracy of the strategy, Accuracy was performed by various investigator. The standard arrangement was infused for multiple times and estimated the rate measure in HPLC. The %RSD for the area of five imitate infusions was found. The resulte are displayed in table 9.

**Robustness:** As a component of the Vigor, purposeful change in the Stream rate, Portable Stage sythesis, Temperature Variety was had to assess the effect on the strategy. The stream rate was changed at 0.4 ml/min to 0.6 ml/min. The outcomes are displayed in table 10.

**LOD and LOQ:** The responsiveness of RP not entirely set in stone from LOD and LOQ. Which were determined from the alignment bend involving the accompanying conditions according to ICH rules. The resulte are displayed in table 11.

LOD = 3.3σ/S and
LOQ = 10 σ/S, where
σ= Standard deviation of y intercept of regression line,
S = Slope of the calibration curve

## RESULTS AND DISCUSSION

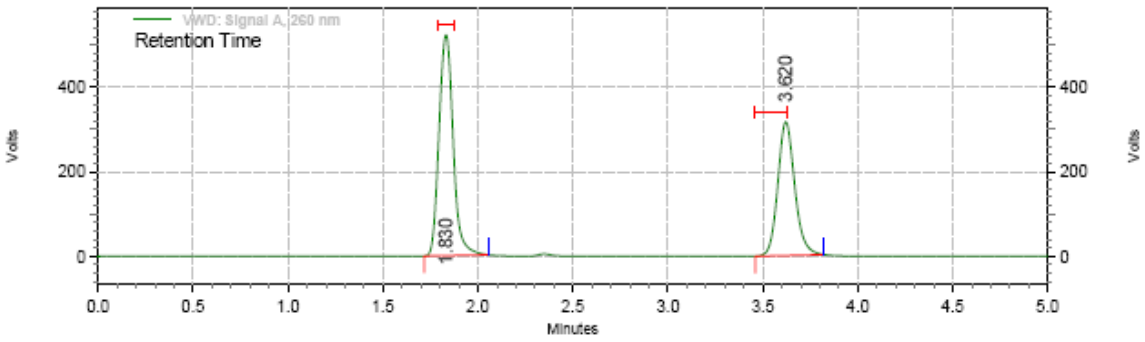


**Figure 3: Standard chromatogram**

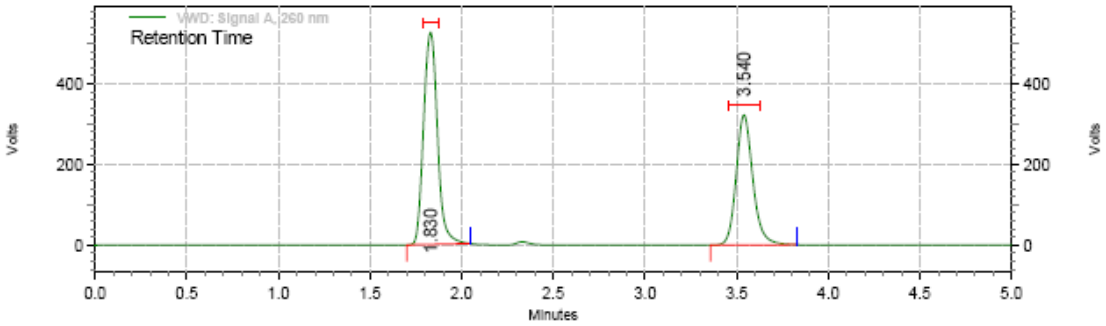


**Figure 4: Sample chromatogram**

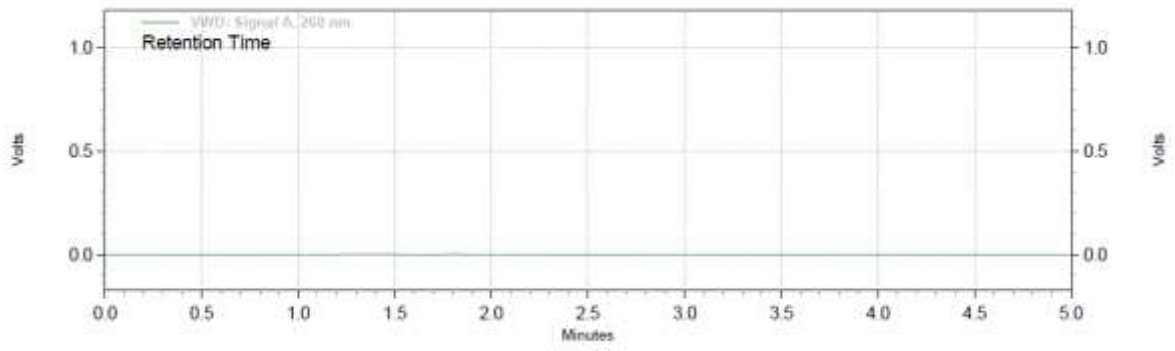


**Figure 5: Blank chromatogram**

**Table 1: System suitability parameters Bilastine**

| Injection | RT | Peak area | Theoretical plates (TP) | Tailing factor (TF) |
|---|---|---|---|---|
| 1 | 1.830 | 46340258 | 2945 | 1.21 |
| 2 | 1.827 | 46001582 | 2942 | 1.17 |
| 3 | 1.823 | 46033474 | 2949 | 1.18 |
| 4 | 1.830 | 46101716 | 2999 | 1.14 |
| 5 | 1.833 | 45923870 | 3041 | 1.15 |
| 6 | 1.827 | 45780149 | 3032 | 1.14 |
| Mean | 1.828 | 46030175 | - | - |
| SD | 0.003 | 187570 | - | - |
| %RSD | 0.2 | 0.4 | - | - |

**Table 2: System suitability parameters Montelukast**

| Injection | Retention time | Peak area | Theoretical plates | Tailing factor | Resolution |
|---|---|---|---|---|---|
| 1 | 3.540 | 34134250 | 7587 | 1.14 | 13.25 |
| 2 | 3.580 | 33405725 | 7902 | 1.16 | 11.85 |
| 3 | 3.567 | 33639716 | 7664 | 1.18 | 11.72 |
| 4 | 3.580 | 33494709 | 7828 | 1.19 | 11.84 |

| | | | | | |
|---|---|---|---|---|---|
| 5 | 3.597 | 33681271 | 7886 | 1.19 | 11.95 |
| 6 | 3.573 | 33505619 | 7912 | 1.17 | 11.90 |
| Mean | 3.573 | 33643548 | - | - | - |
| SD | 0.019 | 260754 | - | - | - |
| %RSD | 3.540 | 34134250 | - | - | - |

**Table 3: Assay results for Bilastine and Montelukast**

| Drug | Label claim(mg) | Amount found(mg) | % Assay |
|---|---|---|---|
| BILASTINE | 125 | 124.1 | 98.8 |
| MONTELIKAST | 100 | 98.6 | 98.6 |

**Table 4: Linearity results for Bilastine**

| S.No | Concentration (µg/mL) | Area |
|---|---|---|
| 1 | 62.5 | 25055621 |
| 2 | 100 | 36806942 |
| 3 | 125 | 45311483 |
| 4 | 150 | 52619670 |
| 5 | 188.5 | 63933476 |

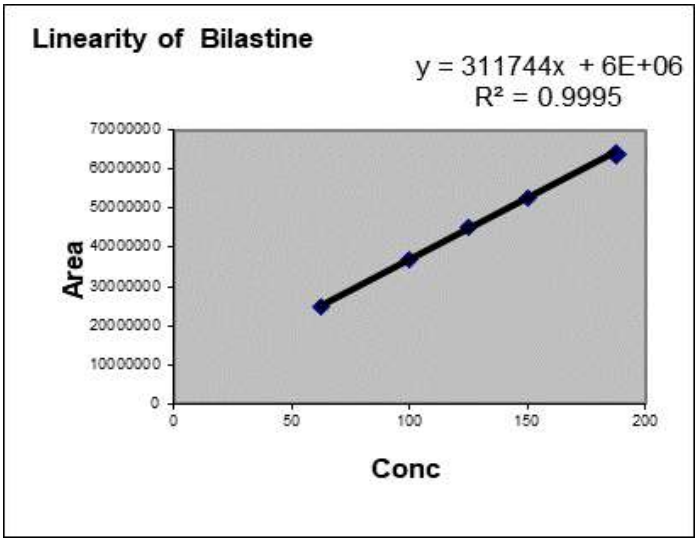


**Figure 6: Linearity graph for Bilastine**

**Table 5: Linearity results for Montelukast**

| S.No | Concentration (µg/mL) | Area |
|---|---|---|
| 1 | 100 | 16194209 |
| 2 | 160 | 26535716 |
| 3 | 200 | 33633340 |
| 4 | 240 | 39910697 |
| 5 | 300 | 49953204 |

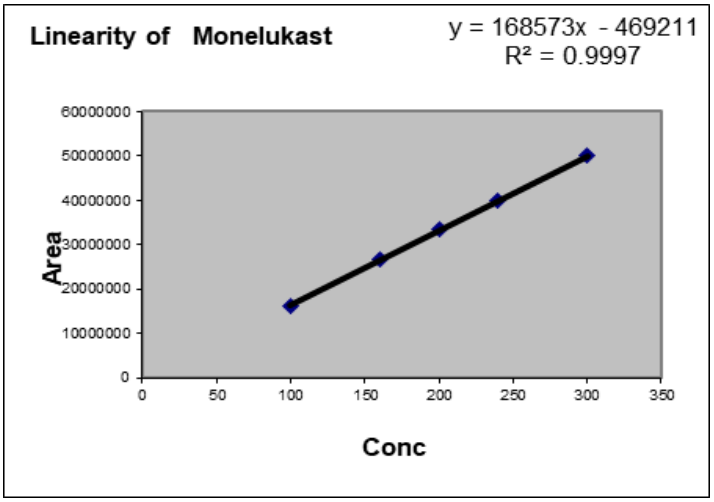


**Figure 7: Linearity graph for Montelukast**

**Table 6: Showing accuracy results for Bilastine**

| %Recovery | Amount present (µg/mL) | Amount found (µg/mL)* | Percent Recovery * | % Mean Recovery |
|---|---|---|---|---|
| 50% | 62.50 | **62.66** | 100.3 | 99.9 |
| 100% | 125.0 | **123.75** | 99.4 | |
| 150% | 188.5 | **188.17** | 100.4 | |

**Table 7: Showing accuracy results for Montelukast**

| %Recovery | Amount present (µg/mL) | Amount found (µg/mL)* | Percent Recovery * | % Mean Recovery |
|---|---|---|---|---|
| 50% | 100 | 100.94 | 99.1 | 99.8 |
| 100% | 200 | 198.58 | 100.7 | |
| 150% | 300 | 300.95 | 99.7 | |

**Table 8: Precision results for Bilastine**

| Injection | BILASTINE | | MONTELUKAST | |
|---|---|---|---|---|
| | Area | %Assay | Area | %Assay |
| 1 | 45741313 | 98.8 | 33365477 | 98.9 |
| 2 | 45787695 | 98.9 | 33411360 | 99.1 |
| 3 | 46080749 | 99.6 | 33638194 | 99.7 |
| 4 | 45802928 | 99.0 | 33304993 | 98.7 |
| 5 | 45286836 | 98.8 | 32828676 | 98.3 |

| | | | | |
|---|---|---|---|---|
| 6 | 45786681 | 98.9 | 33039426 | 98.0 |
| Average | - | 98.8 | - | 98.6 |
| SD | - | 0.6 | - | 0.9 |
| %RSD | - | 0.6 | - | 0.9 |

**Table 9: Precision results for Montelukast**

| S.NO | Name | RT | Area | Height |
|---|---|---|---|---|
| 1 | Montelukast | 4.302 | 1401475 | 100274 |
| 2 | Montelukast | 4.305 | 1401345 | 100078 |
| 3 | Montelukast | 4.325 | 1402415 | 98425 |
| 4 | Montelukast | 4.315 | 1404775 | 98165 |
| 5 | Montelukast | 4.312 | 1408614 | 98154 |
| Mean | | | 1491354 | |
| Std.dev | | | 5882.5 | |
| %RSD | | | 0.38 | |

**Table 10: Ruggedness results of Bilastine and Montelukast**

| BILASTINE | **%Assay** | MONTELUKAST | **%Assay** |
|---|---|---|---|
| **Analyst 01** | 99.36 | **Analyst 01** | 100.15 |
| **Analyst 02** | 99.27 | **Analyst 02** | 100.38 |
| **% RSD** | 0.27 | **% RSD** | 0.57 |

**Robustness results**

**Table 11: Results for Robustness of BILASTINE and MONTELUKAST**

| Chromatographic changes | | Theoretical Plates | | Tailing factor | | Resolution |
|---|---|---|---|---|---|---|
| | | BILASTINE | MONTELUKAST | BILASTINE | MONTELUKAST | Between BILASTINE & MONTELUKAST |
| Flow rate (mL/min) | 0.4 | 3588 | 9450 | 1.1 | 1.2 | 4.5 |
| | 0.6 | 2455 | 7299 | 1.0 | 1.1 | 4.6 |
| Temperature(°C) | 25 | 3541 | 9524 | 1.1 | 1.2 | 4.4 |
| | 35 | 3652 | 9536 | 1.0 | 1.1 | 4.5 |

**Table 12: LOD, LOQ of Bilastine and Montelukast**

| Drug | LOD | LOQ |
|---|---|---|
| **Bilastine** | 0.16 | 0.995 |
| **Montelukast** | 0.55 | 1.635 |

## CONCLUSION:

The proposed HPLC technique was viewed as basic, exact, precise and touchy for the synchronous assessment of Bilastine and Montelukast in drug measurement structures. Thus, this technique can undoubtedly and helpfully take on for routine quality control examination of in unadulterated and its drug measurement structures.

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