WWW.IJRPR.COM

**International Journal of Research Publication and Reviews**

Journal homepage: www.ijrpr.com ISSN 2582-7421

# Analytical Method Development and Validation for Simultaneous Estimation of *Montelukast* & *Bilastine* by RP-HPLC and Stability Studies

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**ABSTRACT**

A simple, Accurate, precise method was developed for the simultaneous estimation of the Bilastine and Montelukast in bulk and pharmaceutical dosage form. Chromatogram was run through Std Asce RP C18 150 x 4.6 mm, 5µm. Mobile phase containing Buffer Methanol: water taken in the ratio 70:30 was pumped through column at a flow rate of 1.0 ml/min. Buffer used in this method was 0.01N Na2hpo4 buffer. Temperature was maintained at 30°C. Optimized wavelength selected was 215.0 nm. Retention time of Bilastine and Montelukast were found to be 2.231 min and 2.865 min. %RSD of the Bilastine and Montelukast were and found to be 0.1% and 0.3% respectively. %Recovery was obtained as 100.07% and 99.81% for Bilastine and Montelukast respectively. LOD, LOQ values obtained from regression equations of Bilastine and Montelukast were 0.08, 0.24 and 0.01, 0.04 respectively. %Assay was obtained as 99.76% and 99.36% for Bilastine and Montelukast respectively. Regression equation of Montelukast is y = 80029x + 24896, y = 54895x + 363.57 of Bilastine. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

**Key Words:** Montelukast, Bilastine, RP-HPLC

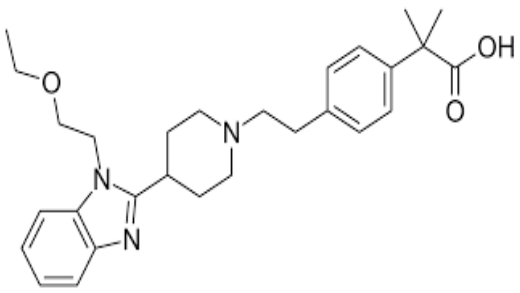
## INTRODUCTION:

The safety and efficacy of a medication are significantly influenced by its quality. The quality of analytical data is contingent upon the methodologies used to generate it. Consequently, it is imperative to develop robust and dependable analytical methods in order to formally certify medications and their formulations to regulatory bodies. In general, the quality and safety of a medicine are guaranteed by the effective monitoring and control of the assay and contaminants. The safety of the drug is determined by impurities, while its potency is determined by a test. The efficacy of pharmaceuticals in treating patients is dramatically impacted by their assay.

**Drug Profile Bilastine and Montelukast:**

**1. Drug: Bilastine**

Description: Bilastine is an innovative, next-generation antihistamine with a short half-life, lengthy duration of action, and good selectivity for the H1 histamine receptor.

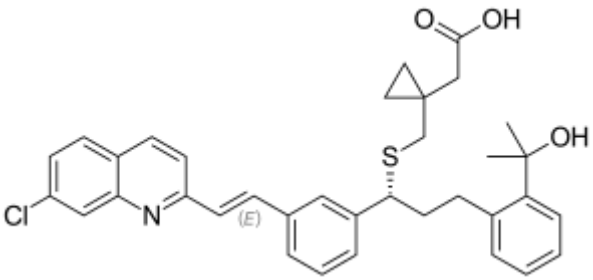



**Structure of Bilastine**

**MOA:** During an allergic reaction, mast cells degranulate, releasing many chemicals including histamine. By attaching to and blocking the activation of H1 receptors, bilastine reduces allergy symptoms caused by mast cell histamine production. Absorption: 1.13 hrs Tmax ½: 14.5 hrs

**2. Drug: Montelukast**

Description: Asthma treatment includes montelukast to counteract exercise-induced bronchoconstriction.



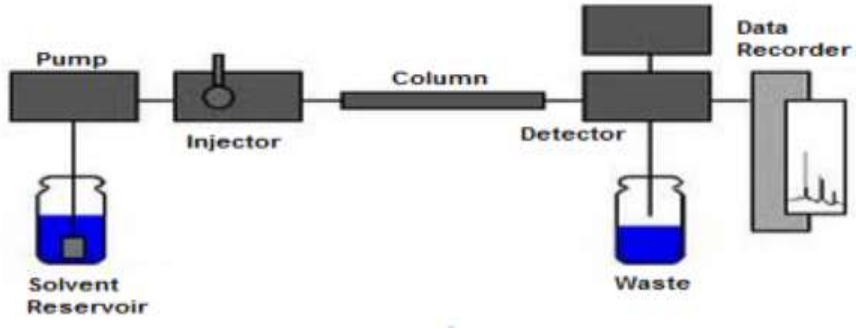

**Structure of Montelukast**

**MOA:** Mast cells and eosinophils produce cysteine leukotrienes (CysLT) such LTC4, LTD4, and LTE4,Absorption: 64% bioavailability,Metabolism: Analysis has established that montelukast undergoes significant metabolism, mostly mediated by the cytochrome P450 3A4, 2C8, and 2C9 isoenzymes.

## MATERIALS AND METHODS

**Materials:**

- Bilastine and Montelukast pure drugs (API) received from spectrum labs. Combination Bilastine and Montelukast Tablets received from local market. Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem
- **INSTRUMENTATION OF Rp-HPLC**




**Instruments:**

- Electronics Balance-Denver
- $p^H$ meter -BVK enterprises, India
- Ultrasonicator-BVK enterprises
- WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software.
- UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of Bilastine and Montelukast solutions.

**Methods:**

**Diluent:** Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50

**Preparation of buffer:**

**0.1%OPA Buffer**: 1ml of ortho phosphoric acid was diluted to 1000ml with HPLC grade water.

**Buffer: 0.01N Sodium hydrogen phosphate**

Accurately weighed 1.42gm of Sodium hydrogen phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then added 1ml of triethylamine then PH adjusted to 4.0 with dil. Orthophosphoric acid solution

High quality or grade 1 materials are used for the purpose of this method spectrum labs provided us with the API gift sample required

Other Chemicals and Solvents used are:

| | |
|---|---|
| Water | 0.1 - 2 N HCl |
| MeCN | $H_2O_2$ |
| Trimethyl Amine | NaOH |
| $KH_2PO_4$ | OPA |

**Sample Processing**

**Diluent:** $H_20$ and MeCN taken in a 50:50 ratio.

| | |
|---|---|
| **Std stock sol prep** | Dispense 10 mg of BLTE and 5 mg of MKST in 10 mL of vf, fill to the neck, sonicate for 10 to 15 minutes, and then fill to the mark with diluent. And designate as stocl sol<br>(100µg/ml of MKST and 200µg/ml BLTE). |
| **sample stock sol** | On average, 10 tablets were weighed and then put to 50 ml of vf. 25 ml of diluent was then added and the mixture was sonicated for couple of minutes. then filled with dil to the indicator mark.. (100µg/ml of MKST and 200µg/ml BLTE). |
| **100% solution or working sample/working std** | 1ml of the stock sol was added in 10ml vf and makeup with diluent. (10µg/ml of MKST and 20µg/ml BLTE). |

**Validation:**

**System suitability parameters:**

The system suitability parameters were determined by preparing standard solutions of Montelukast (10ppm) and Bilastine (20ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

The % RSD for the area of six standard injections results should not be more than 2%.

**Specificity:**

Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

**Precision:**

**std working sols (100% sol)** From stock sol 1 ml of the sol was added to a 10 ml vf and makeup with diluent. (50µg/ml BLTE, 25µg/ml MKST)

**Linearity:**

From stock sol following volumes are pipetted out in different vol flasks.

| Level | ml | Level | ml |
|---|---|---|---|
| 25 % | 0.25 ml from Pipette | 100 % | 1.0 ml from Pipette |
| 50 % | 0.5 ml from Pipette | 125 % | 1.25 ml from Pipette |
| 75 % | 0.75 ml from Pipette | 150 % | 1.5 ml from Pipette |

**Accuracy**:

| | |
|---|---|
| 50% sol | 0.5 ml of sample stock and 1 ml stock added to 10ml and makeup witl dil |
| 100% sol | 1 ml of sample stock and 1 ml stock added to 10ml and makeup witl dil |
| 150% sol | 1.5 ml of sample stock and 1 ml stock added to 10ml and makeup witl dil |

All levels must have a recovery rate between 98% and 102% to be eligible.

LOD and LOQ prep: 0.31 ml of stocl sol was added in 2 different 10ml vf and filled with dil

| | |
|---|---|
| LO-D | From above sol 0.1 ml taken in 10ml vf and makeup wil dil |
| LO-Q | 0.3 ml in 10ml vf and makeup with dil |

**Degradation studies:**

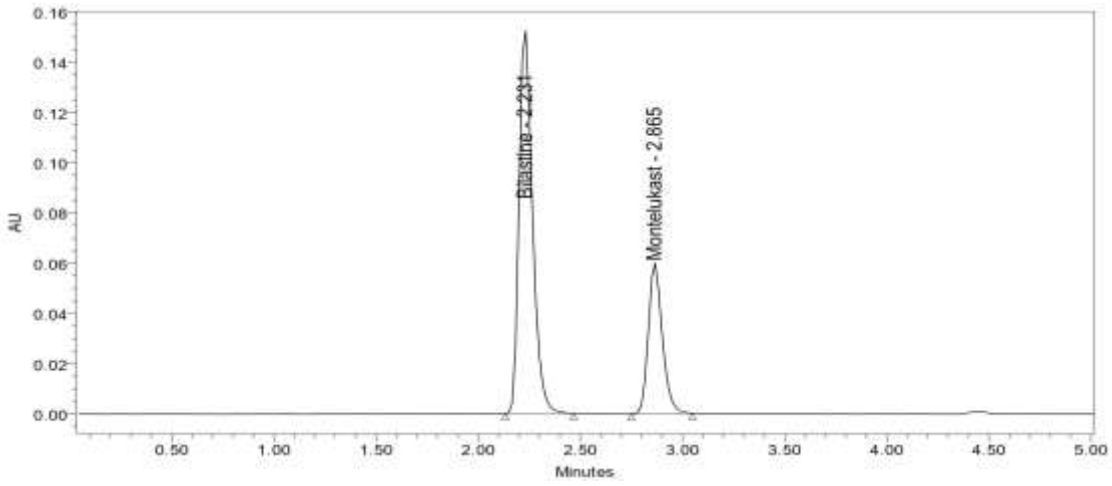
| method | prep |
|---|---|
| Oxidation study | After adding 1ml of a stock solution to 10ml of a 20% volume fraction of H2O2 and allowing it to sit in an oven at 60°C for 30 minutes, a chromatogram was produced by injecting a 10 µg/ml & 20 µg/m solution at 10 µl into HPLC. |
| Thermal Study | The stock solution was allowed to undergo thermal deterioration in an oven set at 105oC for 6 hours. Subsequently, a chromatogram was prepared by injecting a 10 µg/ml & 20 µg/m solution at 10 µl into HPLC. |
| UV study | The stock underwent degradation by exposure to UV radiation in the laboratory for a duration of 7 days. Upon injecting a 10 µg/ml & 20 µg/m solution at a volume of 10 µl into HPLC, a chromatogram was developed. |
| Neutral study | After refluxing the stock for 6 hours at 60 degrees Celsius, a chromatogram was prepared by injecting a 10 µg/ml & 20 µg/m solution at 10 µl into HPLC. |

## RESULTS AND DISCUSSION

Optimized wavelength selected was 224nm.

**Optimized Chromatogram**

| | |
|---|---|
| **Movable phase** | Methanol: Water (70:30 v/v) |
| **Column used** | Asce C18 (4.6mm x 150mm, 5µm) |
| **Stream rate** | 1 ml/min |
| **λ max** | 215 |
| **C Temp** | 24°C |
| **Inj** | 10 µL |
| **Range** | 5 min |
| **Observation** | Bilastine and Montelukast peak has good r2, tailing, plate count. |




**Structure of optimized Chromotogram**

**Remark:** With good resolution, Bilastine and Montelukast were eluted at 2.231 and 2.865 minutes, respectively. The plate count and tailing factor were highly satisfactory, leading to the optimization and validation of this approach.

**Validation:**

**System Suitability:**

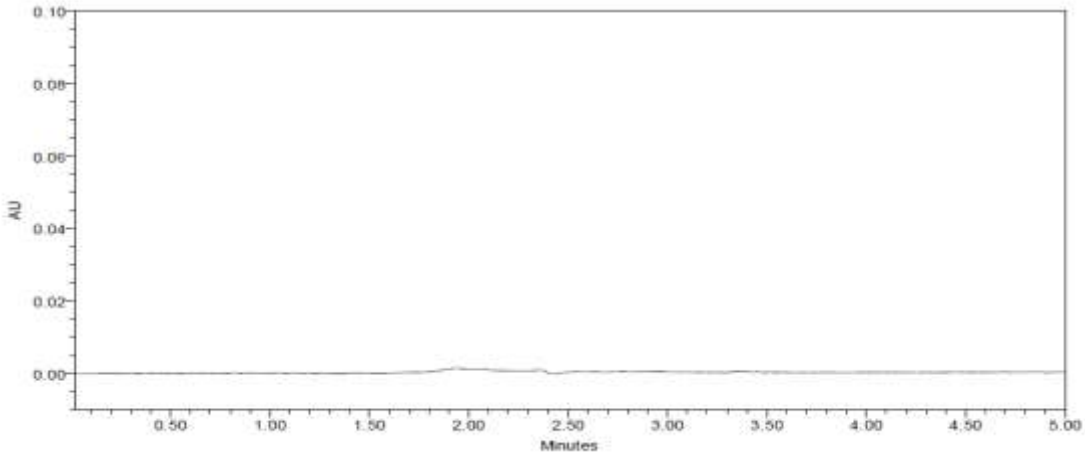
| S no | Bilastine | | | Montelukast | | | |
|---|---|---|---|---|---|---|---|
| Inj | RT (min) | USP Plate Count | Tailing | RT (min) | USP Plate Count | Tailing | RS |
| 1 | 2.219 | 5818 | 1.25 | 2.794 | 8117 | 1.19 | 4.7 |
| 2 | 2.219 | 5542 | 1.27 | 2.796 | 8161 | 1.19 | 4.6 |
| 3 | 2.221 | 5761 | 1.24 | 2.800 | 8250 | 1.21 | 4.6 |
| 4 | 2.222 | 5490 | 1.28 | 2.801 | 8650 | 1.22 | 4.6 |
| 5 | 2.223 | 5457 | 1.25 | 2.801 | 8183 | 1.21 | 4.6 |
| 6 | 2.223 | 5475 | 1.24 | 2.809 | 8347 | 1.19 | 4.8 |

Minutes

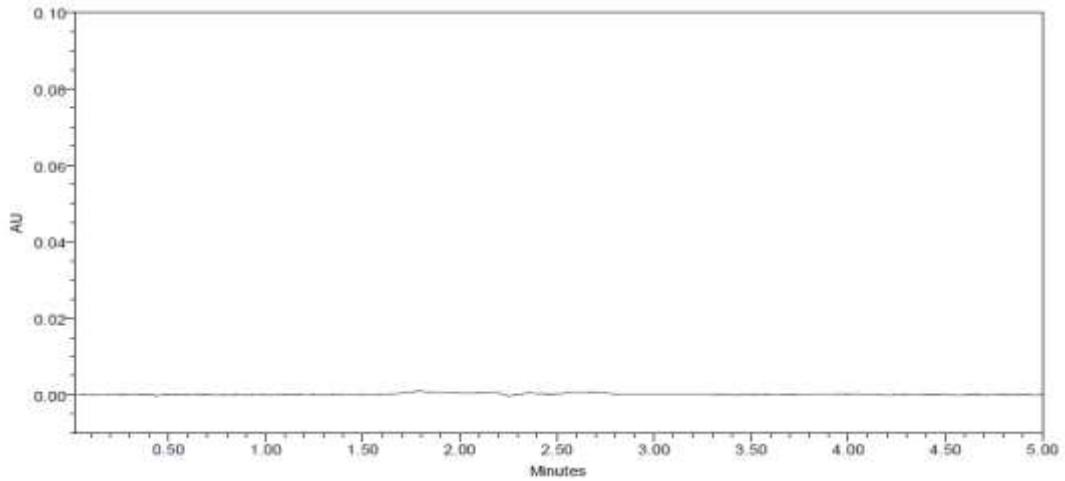
**Chromatogram of System Suitability**

**Remark:** The ICH criteria state that a plate count of more than 2000, a tailing factor of less than 2, and a resolution of more than 2 are required. Every system-appropriate parameter passed and remained within the range.

**Specificity:**




**Chromatogram of blank**

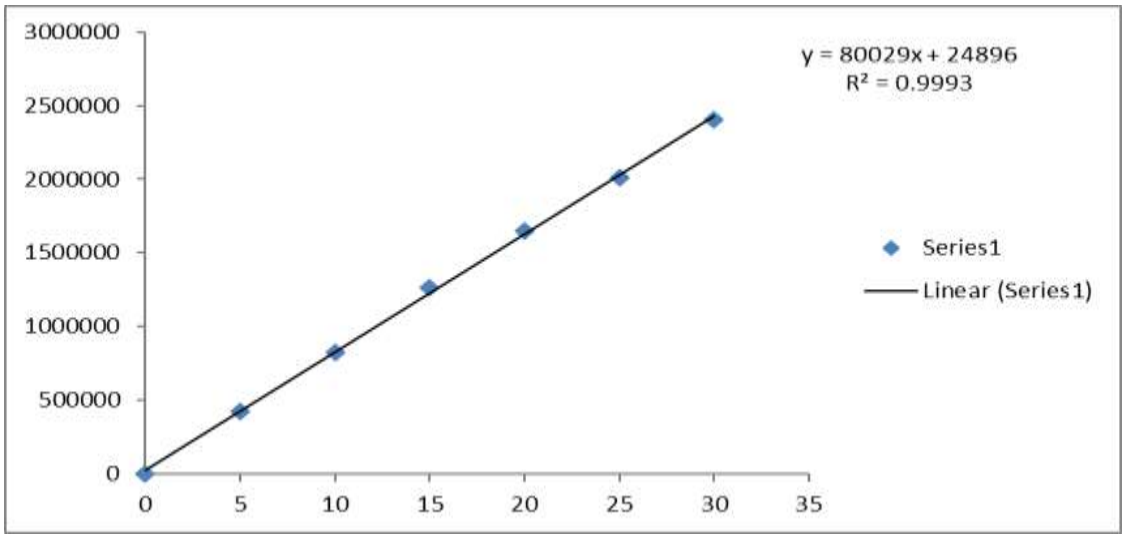



**Chromatogram of placebo**

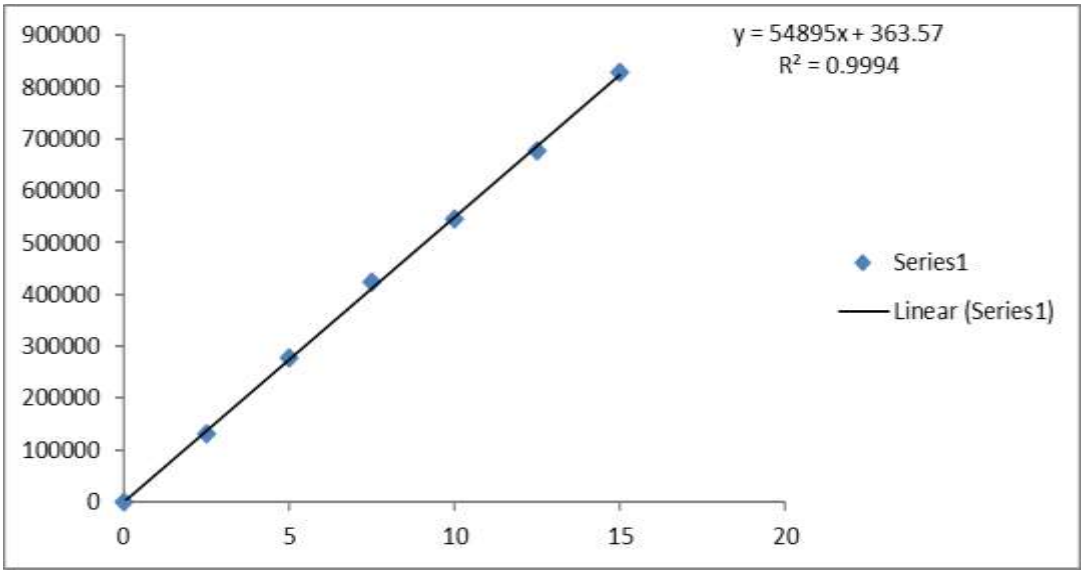
**Linearity:**

**Table 6.2 Linearity table for Bilastine and Montelukast and Pioglitazone.**

| Bilastine | | Montelukast | |
|---|---|---|---|
| Conc (µg/mL) | Peak area | Conc (µg/mL) | Peak area |
| 0 | 0 | 0 | 0 |
| 5 | 424274 | 2.5 | 131611 |
| 10 | 827338 | 5 | 277455 |
| 15 | 1261574 | 7.5 | 424775 |
| 20 | 1650367 | 10 | 545673 |
| 25 | 2011775 | 12.5 | 677432 |
| 30 | 2402025 | 15 | 827604 |




**Calibration data of Bilastine**




**Calibration data of Montelukast**

**Precision:**

**System Precision:**

**Table 6.3 System precision table of Bilastine and Montelukast**

| S. No | Area of Bilastine | Area of Montelukast |
|---|---|---|
| 1. | 1651717 | 546353 |
| 2. | 1649748 | 548458 |
| 3. | 1650174 | 544158 |
| 4. | 1655838 | 548523 |
| 5. | 1650423 | 548347 |
| 6. | 1650761 | 549023 |
| Mean | 1651444 | 547477 |
| S.D | 2253.0 | 1869.9 |
| %RSD | 0.1 | 0.3 |

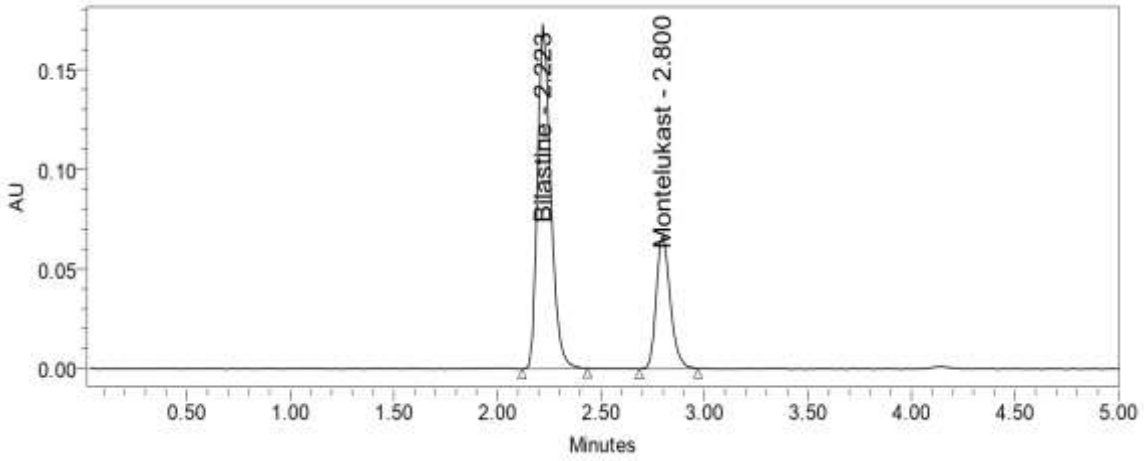



**Fig 6.18 System precision chromatogram**

**Discussion:**. Average area, standard deviation and % RSD were calculated for two drugs. % RSD obtained as 0.1% and 0.3% respectively for Bilastine and Montelukast. As the limit of Precision was less than "2" the system precision was passed in this method.

**Accuracy:**

**Table 6.6 Accuracy table of Bilastine and Montelukast**

| | **Bilastine** | | | **Montelukast** | | |
|---|---|---|---|---|---|---|
| % Level | Amount Spiked (µg/mL) | Amount recovered (µg/mL) | % Recovery | Amount Spiked (µg/mL) | Amount recovered (µg/mL) | % Recovery |
| 50% | 10 | 10.0 | 99.7 | 5 | 5.01 | 100.08 |
| | 10 | 9.9 | 99.5 | 5 | 4.99 | 99.89 |
| | 10 | 9.9 | 99.5 | 5 | 4.98 | 99.54 |
| 100% | 20 | 19.8 | 99.2 | 10 | 9.99 | 99.91 |
| | 20 | 19.9 | 99.4 | 10 | 9.93 | 99.31 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | 20 | 19.7 | 98.4 | 10 | 9.95 | 99.53 |
| 150% | 30 | 30.0 | 99.9 | 15 | 14.83 | 98.85 |
| | 30 | 30.0 | 100.1 | 15 | 15.01 | 100.05 |
| | 30 | 30.0 | 99.9 | 15 | 15.13 | 100.88 |
| Mean % recovery | 100.07 | | | 99.81 | | |

**Discussion:** Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 100.07% and 99.81% for Bilastine and Montelukast respectively.

**Sensitivity:**

**Table 6.8 Sensitivity table of Bilastine and Montelukast**

| Molecule | LOD | LOQ |
|---|---|---|
| Bilastine | 0.08 | 0.24 |
| Montelukast | 0.01 | 0.04 |

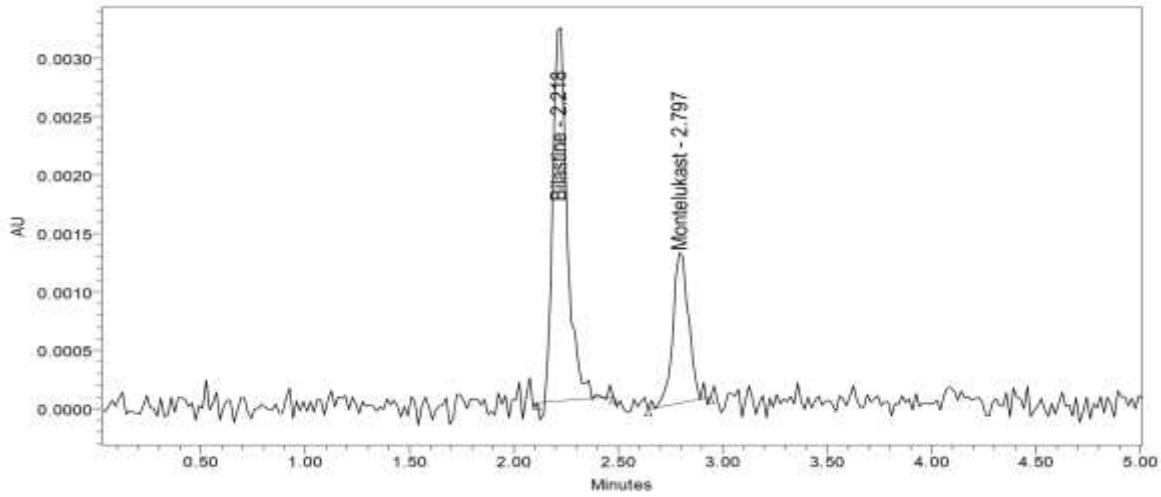



**Fig. No. 6.24 LOD Chromatogram of Standard**

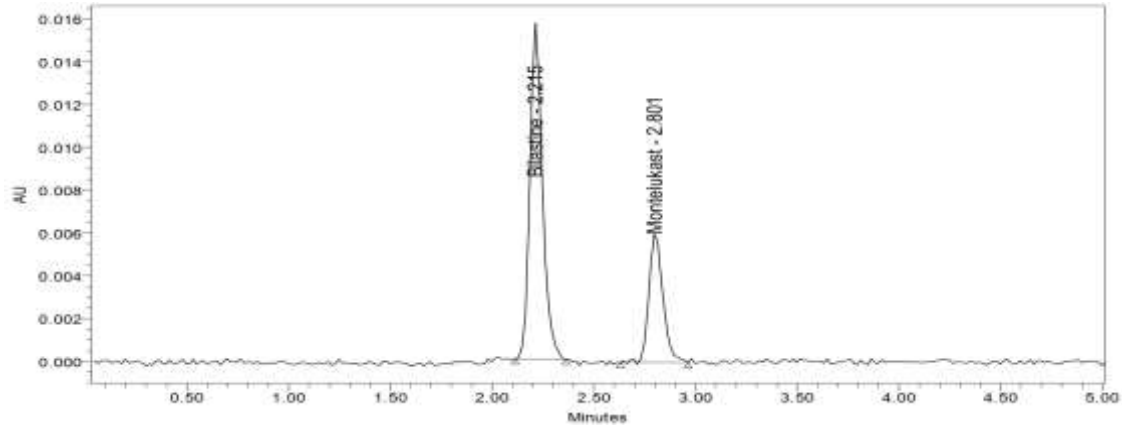



**Fig.No. 6.25 LOQ Chromatogram of of Standarard**

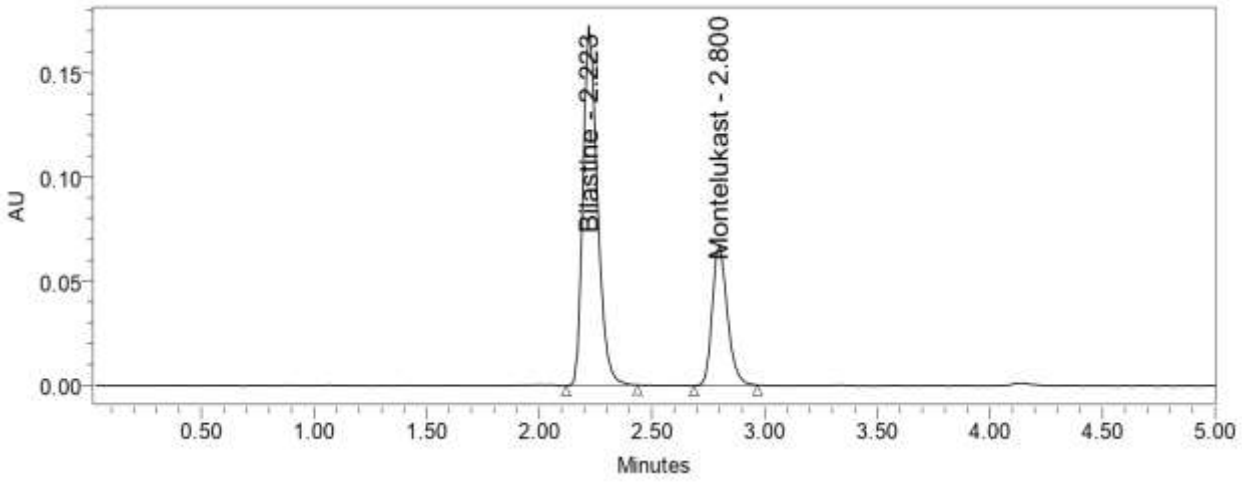
**Robustness:**

**Table 6.9 Robustness data for Bilastine and Montelukast.**

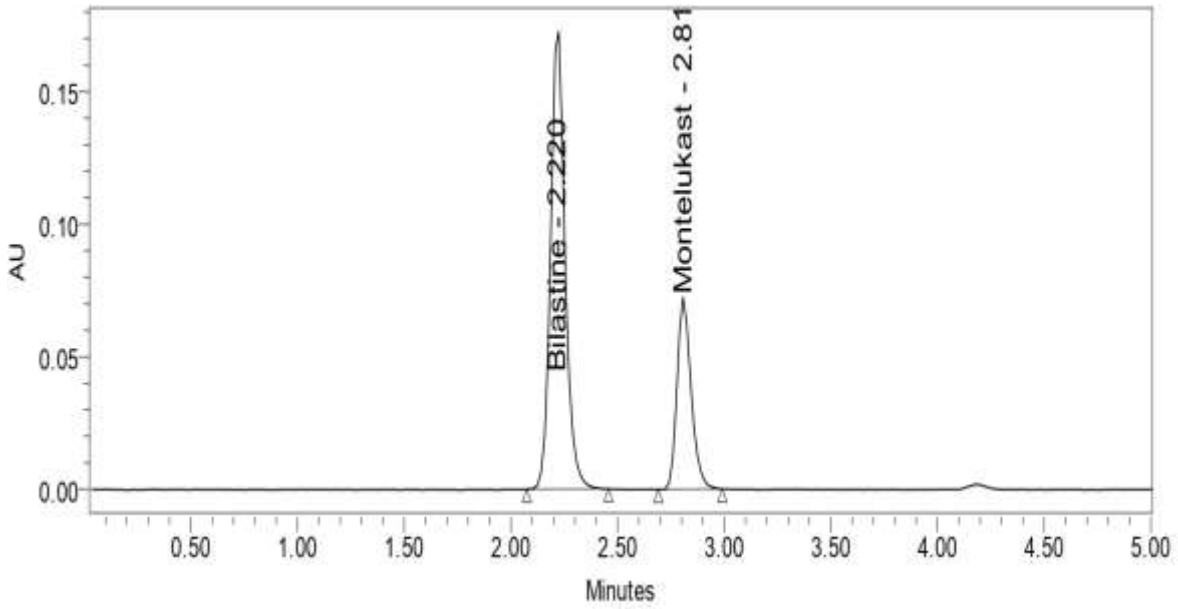
| S.no | Condition | %RSD of Bilastine | %RSD of Montelukast |
|---|---|---|---|
| 1 | Flow rate (-) 0.9ml/min | 0.3 | 0.2 |
| 2 | Flow rate (+) 1.1ml/min | 0.2 | 0.3 |
| 3 | Mobile phase (-) 65B:35A | 0.2 | 0.5 |
| 4 | Mobile phase (+) 75B:25A | 0.9 | 0.5 |
| 5 | Temperature (-) 27°C | 0.3 | 0.7 |
| 6 | Temperature (+) 33°C | 0.2 | 0.5 |

**Assay of Bilastine & Montelukast**

| | **Bilastine** | | | **Montelukast** | | |
|---|---|---|---|---|---|---|
| S.no | Std Area | Sample area | % Assay | Std Area | Sample area | % Assay |
| 1 | 1651717 | 1646588 | 99.51 | 546353 | 543324 | 99.04 |
| 2 | 1649748 | 1654192 | 99.97 | 548458 | 546648 | 99.65 |
| 3 | 1650174 | 1649693 | 99.69 | 544158 | 548763 | 100.03 |
| 4 | 1655838 | 1646533 | 99.50 | 548523 | 546348 | 99.59 |
| 5 | 1650423 | 1650946 | 99.77 | 548347 | 543283 | 99.04 |
| 6 | 1650761 | 1656357 | 100.10 | 549023 | 542007 | 98.80 |
| Avg | 1651444 | 1650718 | 99.76 | 547477 | 545062 | 99.36 |
| Stdev | 2253.0 | 3988.5 | 0.24 | 1869.9 | 2584.0 | 0.47 |
| %RSD | 0.1 | 0.2 | 0.2 | 0.3 | 0.5 | 0.47 |




**Chromatogram of Working Standard**




**Chromatogram of working Sample**

**Degradation Studies**

| **Type of degradation** | **Bilastine** | | | **montelukast** | | |
|---|---|---|---|---|---|---|
| | Area | % Rccovered | % Degraded | Area | % Recovered | % Degraded |
| Peroxide | 1548291 | 93.66 | 6.34 | 530555 | 96.81 | 3.19 |
| Thermal | 1626339 | 98.38 | 1.62 | 538262 | 98.22 | 1.78 |
| Uv | 1627309 | 98.44 | 1.56 | 542870 | 99.06 | 0.94 |
| Water | 1640524 | 99.24 | 0.76 | 550450 | 99.71 | 0.29 |

## Summary

| Parameters | | Bilastine | Montelukast |
|---|---|---|---|
| Linearity Range (µg/ml) | | 15-30µg/ml | 2.5-15µg/ml |
| $R^2$ | | 0.999 | 0.999 |
| (m) | | 80029 | 54895 |
| (c) | | 24896 | 363.57 |
| (Y=mx+c) | | y = 80029x + 24896 | y = 54895x + 363.57 |
| Assay (% mean assay) | | 99.76% | 99.36% |
| Specificity | | Specific | Specific |
| SP %RSD | | 0.1 | 0.3 |
| MP<br>%RSD | | 0.2 | 0.5 |
| %recovery | | 100.07% | 99.81% |
| LO-D | | 0.08 | 0.01 |
| LO-Q | | 0.24 | 0.04 |
| | 0.3 | 0.2 | 1.6 |

| | | | |
|---|---|---|---|
| Robustness | 0.2 | 0.3 | 0.2 |
| | 0.2 | 0.5 | 0.8 |
| | 0.9 | 0.5 | 0.3 |
| | 0.3 | 0.7 | 0.2 |
| | 0.2 | 0.5 | 0.2 |

## Conclusion:

Bilastine and Montelukast had 2.231 and 2.865-min retention times. Bilastine and Montelukast had 0.1 and 0.3. RSD, respectively. % Recovery was 100.07% with Bilastine and 99.36% for Montelukast. Bilastine and Montelukast regression models yielded LOD, LOQ values of 0.08, 0.24 and 0.01, 0.04. Bilastine regression equation is y = 80029x + 24896 and Montelukast y = 54895x + 363.57 1. In this method retention and run times were reduced.

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