ACTA SCIENTIAE

**ISSN**: 2178-7727

# Analytical Method Validation For Bilastine And Montelukast Sodium Bilayered Film-Coated Tablets By Rp-Hplc Gradient Method

E. Leelavathy [1], S.M. Shaheedha [2], M. Shamshath Begum [3], R. Prakash[4]

[1,2,4] Crescent School of Pharmacy B S Abdur Rahman Crescent Institute of Science and Technology, Seethakathi Estate, GST Road, Vandalur Chennai, Tamil Nadu, India, 600048

[3] Department of Pharmaceutics, Faculty of Pharmacy, BIHER, SBMCH Campus, Chromepet, Chennai, Tamil Nadu, India, 600044.

Corresponding Email: shaheedhashabudeen@gmail.com 2

## ABSTRACT

The present study outlines the development and validation of a robust, precise, and accurate reverse-phase high-performance Liquid Chromatography (RP-HPLC) gradient method for the simultaneous estimation of Bilastine and Montelukast Sodium in bilayered film-coated tablet formulations. The chromatographic analysis was performed using a C18 column with a mobile phase comprising a gradient mixture of buffer and acetonitrile, monitored at an optimized wavelength. Method validation was carried out in accordance with International Council for Harmonisation (ICH) guidelines, covering parameters such as specificity, linearity, accuracy, precision, robustness, and system suitability. The developed method demonstrated excellent linearity over the concentration ranges of both drugs, with correlation coefficients ($r^2$) close to 0.999. Accuracy was confirmed through recovery studies, yielding results within acceptable limits (98–102%). Precision studies showed low %RSD values, indicating reproducibility. The method proved to be robust under slight variations in chromatographic conditions and was capable of distinctly separating the analytes without interference from excipients. These findings confirm that the developed RP-HPLC method is suitable for the routine quality control and analysis of Bilastine and Montelukast Sodium in bilayered tablet formulations.

**KEYWORDS:** RP-HPLC, Analytical Method Validation, Bilastine, Montelukast Sodium, Gradient Elution, Bilayered Tablets

## INTRODUCTION

Bilastine and Montelukast Sodium are widely prescribed medications used in the treatment of allergic conditions, particularly allergic rhinitis and asthma. Bilastine, a second-generation antihistamine, is known for its high selectivity for peripheral H1-receptors and minimal sedative effects [1]. Montelukast Sodium, a leukotriene receptor antagonist, is commonly employed for the management of asthma and allergic rhinitis by blocking the action of leukotrienes, which are responsible for allergic inflammation and bronchoconstriction [2]. Their combined administration offers a synergistic therapeutic effect and enhances patient compliance, especially in fixed-dose bilayered film-coated tablet formulations designed to release each drug in a controlled manner.

Pharmaceutical bilayer tablets offer numerous advantages over conventional dosage forms, including improved stability, controlled release of active pharmaceutical ingredients (APIs), and the ability to combine incompatible drugs in a single formulation [3]. However, the analysis of such multi-component dosage forms requires highly selective and sensitive analytical methods that can accurately quantify each API in the presence of excipients and degradation products. Among the available analytical techniques, Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) stands out due to its reliability, reproducibility, and capability to separate multiple compounds within a single run [4].

In the pharmaceutical industry, the validation of analytical methods is an essential requirement to ensure the accuracy, precision, specificity, and consistency of test results. Regulatory agencies such as the International

ISSN: 2178-7727

Council for Harmonisation (ICH) have outlined comprehensive guidelines for method validation, including parameters such as linearity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, robustness, and system suitability [5]. A properly validated analytical method is crucial for the routine quality control of pharmaceutical products and for ensuring that formulations meet the desired standards of safety and efficacy.

Despite the availability of methods for analyzing Bilastine and Montelukast Sodium individually, there is limited literature on a validated RP-HPLC gradient method capable of simultaneously estimating both drugs in a complex matrix such as a bilayered tablet. Additionally, gradient elution techniques are advantageous for separating components with differing polarities and retention behaviours, thereby enhancing method performance and resolution [6].

The primary objective of this study is to develop and validate an RP-HPLC gradient method for the simultaneous estimation of Bilastine and Montelukast Sodium in bilayered film-coated tablets. This involves optimizing chromatographic conditions and evaluating the method as per ICH Q2(R1) validation parameters to ensure its applicability for routine quality control and regulatory compliance.

## LITERATURE REVIEW

The accurate estimation of active pharmaceutical ingredients in combined dosage forms is a recurring challenge in analytical chemistry. Numerous studies have demonstrated the application of RP-HPLC for the estimation of single and multi-component drugs due to its high sensitivity, reproducibility, and selectivity. The literature reports various validated RP-HPLC methods for the quantification of Bilastine and Montelukast Sodium individually; however, simultaneous estimation using a gradient method in bilayered film-coated tablets remains limited.

In a study by Sharma et al., a validated HPLC method was developed for Montelukast Sodium in bulk and tablet dosage forms. The method was simple and effective for single drug analysis but was not extended to combination therapies [7]. Similarly, a validated UV-spectrophotometric method was proposed for Bilastine estimation, showing good linearity and accuracy, but with limitations in specificity in complex matrices like bilayered formulations [8].

RP-HPLC has become the method of choice for pharmaceutical analysis because of its precision in identifying and quantifying components in a mixture. Shetti et al. reported a method for simultaneous estimation of Montelukast Sodium and Levocetirizine Dihydrochloride in tablet dosage form using an isocratic RP-HPLC method. However, the fixed composition limited its application in formulations with different release profiles [9]. This emphasizes the need for gradient methods, especially in cases where the components have significantly different polarities and retention times.

Gradient elution methods, though more complex, offer improved resolution, especially in multi-component formulations. According to Kazakevich and Lobrutto, gradient techniques provide better separation for components that exhibit varying degrees of interaction with the stationary phase, making them highly effective for complex formulations like bilayered tablets [10].

ICH guidelines [5] have stressed the importance of method validation to establish the reliability and consistency of an analytical method. Parameters such as linearity, accuracy, precision, robustness, and specificity must be evaluated. Validation studies like the one conducted by Badr [11] describe the practical approach to assessing these parameters, particularly in pharmaceutical product development and quality assurance environments.

Additionally, the combination of Bilastine and Montelukast Sodium has been gaining pharmaceutical interest due to the complementary pharmacological effects and reduced dosing frequency, enhancing patient adherence [12]. However, few analytical methods cater to such fixed-dose combinations. Furthermore, bilayered tablets

ISSN: 2178-7727

pose unique challenges due to the presence of distinct layers, each possibly affecting the analytical outcome. Analytical methods for such formulations must be capable of separating and quantifying the APIs without cross-interference or matrix effects.

A recent study by Patil et al. involved the development of a stability-indicating RP-HPLC method for the simultaneous estimation of antihistamines with leukotriene antagonists, highlighting the growing need for robust methods in combined therapies [13]. Yet, a comprehensive method specific to Bilastine and Montelukast Sodium in a bilayered matrix using gradient elution and full ICH validation is still underexplored.

In conclusion, while multiple studies have addressed analytical method development for single or dual APIs, a validated gradient RP-HPLC method for Bilastine and Montelukast Sodium in bilayered tablets remains a research gap. This study addresses this gap by developing and validating such a method per ICH guidelines.

## MATERIALS AND METHODS

### 3.1 Chemicals and Reagents

- Bilastine and Montelukast Sodium working standards were obtained from a certified pharmaceutical manufacturer with ≥99% purity.
- Commercial bilayered film-coated tablets containing Bilastine (20 mg) and Montelukast Sodium (10 mg) were procured from the local market.
- Acetonitrile and methanol (HPLC grade) were purchased from Merck.
- Potassium dihydrogen phosphate, orthophosphoric acid, and triethylamine (analytical grade) were used for buffer preparation.
- HPLC-grade water was obtained by using a Milli-Q purification system.

### 3.2 Instrumentation and Chromatographic Conditions

The RP-HPLC system used for analysis was a Shimadzu LC-20AD system equipped with:

- Quaternary gradient pump
- UV detector
- Degasser
- C18 Column (250 mm × 4.6 mm, 5 µm)

**Table 1: Chromatographic conditions**

| Parameter | Condition |
|---|---|
| Column | Phenomenex C18, 250 mm × 4.6 mm, 5 µm |
| Mobile Phase | The gradient of Buffer: Acetonitrile (pH adjusted to 3.5 with OPA) |
| Flow Rate | 1.0 mL/min |
| Injection Volume | 20 µL |
| Column Temperature | Ambient (25 ± 2°C) |
| Detection Wavelength | 220 nm |
| Run Time | 10 min |

**Table 2: Gradient Program**

| Time (min) | Buffer (%) | Acetonitrile (%) |
|---|---|---|
| 0 | 60 | 40 |
| 5 | 40 | 60 |
| 7 | 60 | 40 |
| 10 | 60 | 40 |

### 3.3 Preparation of Solutions

#### *3.3.1 Preparation of Standard Stock Solutions*

ISSN: 2178-7727

- Accurately weighed 20 mg of Bilastine and 10 mg of Montelukast Sodium were transferred into 100 mL volumetric flasks, dissolved in methanol, and volume was made up with the same.
- Further dilutions were made to obtain concentrations ranging from 10–60 µg/mL for Bilastine and 5–30 µg/mL for Montelukast Sodium.

### *3.3.2 Preparation of Sample Solution*

- Twenty tablets were weighed and powdered. An amount equivalent to 20 mg Bilastine and 10 mg Montelukast Sodium was transferred to a 100 mL flask.
- The mixture was dissolved in methanol with sonication for 20 minutes and filtered through a 0.45 µm membrane filter. The volume was made up with methanol.

## 3.4 Method Validation Parameters (as per ICH Q2(R1))

The method was validated for the following parameters:

### *3.4.1 System Suitability*

System suitability was assessed by injecting six replicates of the standard solution. The parameters evaluated included theoretical plates, tailing factor, resolution, and retention time.

**Table 3: Suitability of System**

| Parameter | Bilastine | Montelukast Sodium |
|---|---|---|
| Retention Time (min) | ~2.8 | ~5.4 |
| Theoretical Plates | >5000 | >4500 |
| Tailing Factor | <1.5 | <1.5 |
| Resolution | >2.0 | - |

### *3.4.2 Specificity*

Specificity was established by comparing chromatograms of blank, standard, and sample solutions. No interference was observed at the retention times of the analytes.

### *3.4.3 Linearity and Range*

Linearity was evaluated over the concentration range of:

- Bilastine: 10–60 µg/mL
- Montelukast Sodium: 5–30 µg/mL

**Table 4: Evaluation of Linearity over Different Concentration Range**

| Drug | Concentration Range (µg/mL) | Correlation Coefficient ($r^2$) |
|---|---|---|
| Bilastine | 10–60 | 0.9992 |
| Montelukast Sodium | 5–30 | 0.9987 |

### *3.4.4 Accuracy (Recovery Studies)*

Accuracy was assessed by the standard addition method at three concentration levels (80%, 100%, and 120%).

**Table 5: Accuracy**

| Level | % Recovery (Bilastine) | % Recovery (Montelukast) |
|---|---|---|
| 80% | 99.32 | 98.95 |
| 100% | 100.21 | 99.87 |
| 120% | 101.04 | 100.12 |

### *3.4.5 Precision*

- **Repeatability (Intra-day):** %RSD < 1.5%
- **Intermediate Precision (Inter-day):** %RSD < 2%

ISSN: 2178-7727

**Table 6: Precision**

| Drug | %RSD (Intra-day) | %RSD (Inter-day) |
|---|---|---|
| Bilastine | 1.04 | 1.29 |
| Montelukast Sodium | 1.12 | 1.38 |

**Table 7: LOD and LOQ**

| Drug | LOD (µg/mL) | LOQ (µg/mL) |
|---|---|---|
| Bilastine | 0.18 | 0.56 |
| Montelukast Sodium | 0.12 | 0.39 |

### *3.4.6 Robustness*

Minor variations in flow rate (±0.1 mL/min), mobile phase composition (±2%), and detection wavelength (±2 nm) did not significantly affect results, confirming method robustness.

## RESULTS AND DISCUSSION

### 4.1 Method Development

Several trials were conducted using different mobile phase compositions and gradient programs to optimize resolution, peak shape, and run time. The final optimized method using a gradient of phosphate buffer (pH 3.5) and acetonitrile achieved clear separation of Bilastine and Montelukast Sodium with well-resolved peaks at retention times of 2.8 min and 5.4 min, respectively.

### 4.2 System Suitability

System suitability testing ensures the performance of the chromatographic system before analysis. Parameters such as retention time, theoretical plates, resolution, and tailing factor were within acceptable limits (as per USP).

**Table 8: System Solubility**

| Parameter | Bilastine | Montelukast Sodium | Acceptance Criteria |
|---|---|---|---|
| Retention Time (min) | 2.8 | 5.4 | Consistent with standards |
| Theoretical Plates | 5587 | 4962 | >2000 |
| Tailing Factor | 1.19 | 1.24 | <2.0 |
| Resolution | - | 3.5 | >2.0 |

*Both analytes showed sharp, symmetric peaks with high efficiency and adequate resolution, confirming suitability for analysis.*

### 4.3 Specificity

The specificity was verified by injecting blank, standard, and sample solutions. No interference was observed at the retention times of Bilastine and Montelukast Sodium.

*The method is specific and can accurately estimate the APIs in the presence of excipients and other possible impurities.*

### 4.4 Linearity and Range

Calibration curves were constructed over a range of 10–60 µg/mL for Bilastine and 5–30 µg/mL for Montelukast Sodium. Excellent linearity was observed.

**Table 9: Linearity and Range**

| Drug | Concentration Range (µg/mL) | Equation | $r^2$ Value |
|---|---|---|---|
| Bilastine | 10–60 | y = 12543x + 2134 | 0.9992 |
| Montelukast Sodium | 5–30 | y = 13582x + 1043 | 0.9987 |

**ISSN**: 2178-7727

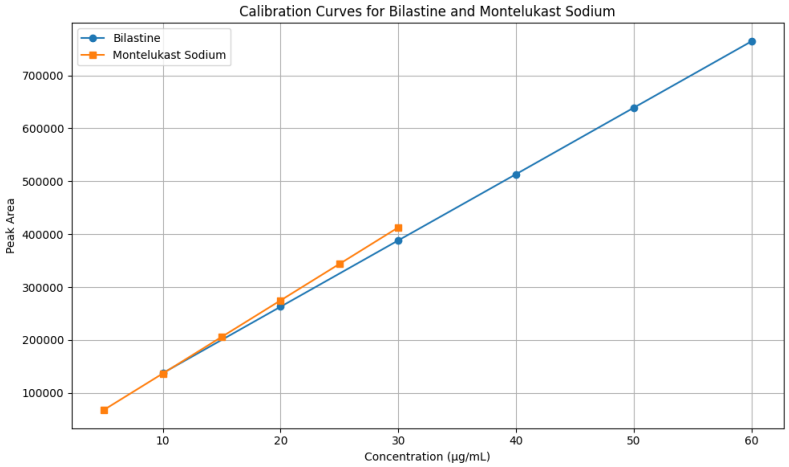


**Figure 1: Calibration Curve for Bilestine and Montelukast Sodium**

**Table 10: Accuracy (Recovery Studies)**

Recovery studies confirmed that the method could accurately quantify drugs within the matrix.

| Level | % Recovery (Bilastine) | % Recovery (Montelukast Sodium) |
|---|---|---|
| 80% | 99.32 | 98.95 |
| 100% | 100.21 | 99.87 |
| 120% | 101.04 | 100.12 |

*Recovery values within 98–102% confirm the method's accuracy and absence of matrix interference.*

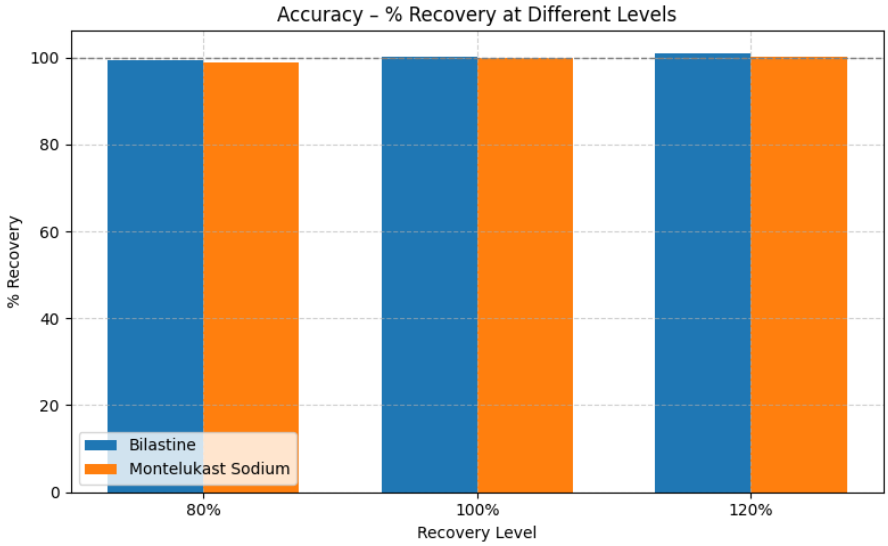


**Figure 2: Accuracy: % Recovery at Different Levels**

**Table 11: Precision**

Both intra-day and inter-day precision were evaluated by analyzing six replicates.

| Drug | Intra-day (%RSD) | Inter-day (%RSD) | Acceptance Criteria |
|---|---|---|---|
| Bilastine | 1.04 | 1.29 | ≤ 2% |
| Montelukast Sodium | 1.12 | 1.38 | ≤ 2% |

*The low %RSD values confirm the repeatability and intermediate precision of the method.*

ISSN: 2178-7727

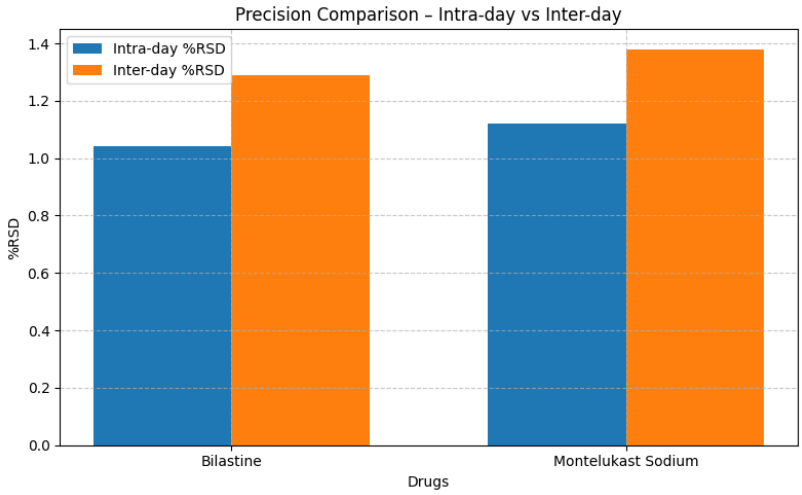


**Figure 3: Precision Comparison: Intra-day Vs. Inter-day**

**Table 12: LOD and LOQ**

Limits of detection and quantification were determined based on signal-to-noise ratios.

| Drug | LOD (µg/mL) | LOQ (µg/mL) |
|---|---|---|
| Bilastine | 0.18 | 0.56 |
| Montelukast Sodium | 0.12 | 0.39 |

*The method is sensitive enough to detect even trace quantities of the analytes.*

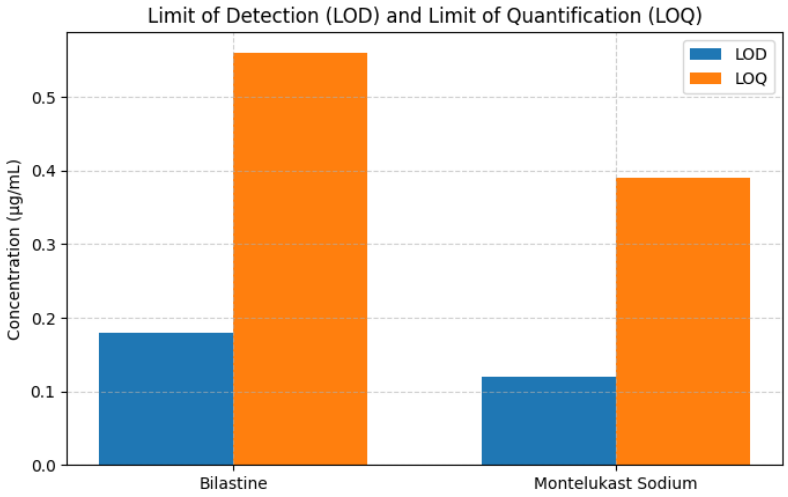


**Figure 4: LOD and LOQ**

### 4.8 Robustness

Method robustness was assessed by making deliberate changes to flow rate (±0.1 mL/min), wavelength (±2 nm), and mobile phase composition (±2%).

**Observations**: All variations resulted in %RSD < 2.0, and retention times and peak shapes remained consistent. '*The method is robust and reliable under minor experimental changes.'*

The developed RP-HPLC gradient method meets all required validation criteria as per ICH Q2(R1). It demonstrates excellent sensitivity, specificity, accuracy, precision, and robustness. The method is capable of separating Bilastine and Montelukast Sodium with high resolution in a bilayered matrix and is suitable for routine quality control in pharmaceutical industries. The use of gradient elution ensures enhanced separation efficiency, especially useful in multicomponent formulations with different physicochemical properties.

**ISSN**: 2178-7727

## CONCLUSION

The present study successfully developed and validated a robust and reliable RP-HPLC gradient method for the simultaneous estimation of Bilastine and Montelukast Sodium in bilayered film-coated tablets. The method was developed with careful optimization of chromatographic parameters, including mobile phase composition, gradient elution program, and detection wavelength, to achieve optimal peak resolution, symmetry, and reproducibility.

All validation parameters were evaluated in accordance with ICH Q2(R1) guidelines. The method showed excellent specificity with no interference from excipients or matrix components, and high linearity with correlation coefficients ($r^2$) greater than 0.998 for both drugs. The recovery results confirmed the accuracy of the method, with percent recoveries ranging from 98% to 102%. Precision studies (both intra-day and inter-day) produced %RSD values well below the acceptable threshold of 2%, indicating excellent repeatability. Furthermore, the method demonstrated strong robustness and sensitivity, as evidenced by low limits of detection and quantification.

The use of gradient elution in RP-HPLC proved particularly effective for separating the two analytes with differing chemical properties and retention behaviours. This method provides a significant improvement over conventional isocratic methods, particularly for the analysis of complex bilayered tablet formulations where excipient interactions and drug release mechanisms can affect quantification.

In conclusion, the validated RP-HPLC gradient method is well-suited for the routine quality control and quantitative analysis of Bilastine and Montelukast Sodium in bilayered film-coated tablets. It can be reliably adopted in pharmaceutical manufacturing and regulatory laboratories to ensure compliance with product specifications and therapeutic efficacy.

## REFERENCES

1. Kuna P, Bachert C, Nowacki Z, Van Cauwenberge P. Bilastine: a new H1-antihistamine for the treatment of allergic rhinitis and urticaria. Expert Opin Investig Drugs. 2009;18(3):313–22.
2. Virchow JC, Backer V, Kuna P, et al. Montelukast in the treatment of allergic rhinitis. Expert Opin Pharmacother. 2003;4(10):1801–9.
3. Shiyani B, Gattani S, Surana S. Formulation and evaluation of bilayer tablet of Metoclopramide hydrochloride and Ibuprofen. AAPS PharmSciTech. 2008;9(3):818–27.
4. Kazakevich Y, Lobrutto R. HPLC for Pharmaceutical Scientists. Hoboken, NJ: John Wiley & Sons; 2007.
5. International Council for Harmonisation (ICH). ICH Q2(R1): Validation of Analytical Procedures: Text and Methodology. Geneva: ICH; 2005.
6. Snyder LR, Kirkland JJ, Dolan JW. Introduction to Modern Liquid Chromatography. 3rd ed. Hoboken, NJ: John Wiley & Sons; 2011.
7. Sharma P, Gupta S, Rathore P. Development and validation of HPLC method for Montelukast Sodium in tablet dosage form. Int J Pharm Sci Res. 2013;4(5):1842–6.
8. Pawar SD, Dhamane H, Jadhav A. UV Spectrophotometric method for estimation of Bilastine in bulk and tablet dosage form. Int J Pharm Sci Rev Res. 2016;38(1):166–9.
9. Shetti NR, Reddy CV, Patil SM. Development and validation of RP-HPLC method for simultaneous estimation of Montelukast Sodium and Levocetirizine Dihydrochloride. Int J Pharm Pharm Sci. 2010;2(4):64–6.
10. Kazakevich Y, Lobrutto R. HPLC for Pharmaceutical Scientists. Hoboken, NJ: John Wiley & Sons; 2007.
11. Badr A. Validation of an analytical procedure: methodology and practical aspects. In: Quality Control of Herbal Medicines and Related Areas. IntechOpen; 2011.
12. Ridolo E, Montagni M, Bonzano L, Incorvaia C, Canonica GW. Bilastine: new insight into antihistamine treatment. Clin Mol Allergy. 2015;13(1):1–7.
13. Patil P, Chavan S, Deshmukh V. Development and validation of stability indicating RP-HPLC method for simultaneous estimation of Desloratadine and Montelukast Sodium in tablet dosage form. Der Pharmacia Lettre. 2012;4(2):531–6.

ACTA SCIENTIAE

**ISSN**: 2178-7727