VER
Vascular & Endovascular Review

Method Development and validation by Liquid chromatography Mass spectroscopy (LC-MS) for Bilastine by a stability indicating assay method

Kavya M C[1], Dr. P. Kumar Nallasivan[2]

[1]Research Scholar,
[2]Professor,
Faculty of Pharmacy, Karpagam Academy of Higher Education, Pollachi main road, Eachanari, Coimbatore, Tamilnadu, India.
pkns75@gmail.com

ABSTRACT

Bilastine, a newer second-generation antihistamine (SGAH) with minimal sedative effects, was recently introduced in the Indian market following approval from the Drugs Controller General of India. It has been prescribed for use in adults and adolescents aged 12 years and older across most countries since 2019. The recommended dosage is 20mg once daily for various treatment of allergic rhino-conjunctivitis and urticaria. Upon conducting a literature review, it was found that only a limited number of analytical methods—such as Ultraviolet spectroscopy, HPTLC, HPLC and fluorimetry — have been reported. Therefore, a novel, selective and sensitive Liquid approach was established for quantification of Bilastine in bulk and pharmaceutical dosage forms using Liquid Chromatography–Mass Spectrometry (LC-MS).

Analysis employed Agilent 1290 Infinity II LC system together with Agilent 6470 Triple Quadrupole Mass Spectrometer (or their equivalents). Chromatographic separation has been carried out on Poroshell Stable Bond (SB) C18 column (4.6×150mm, 2.7µm), using mobile phase composed of acetonitrile, water, methanol in a 40:30:30 ratio. Flow rate has been maintained at 0.5mL/min and detection has been carried out at 215nm with total run time of 6 minutes. The method has been confirmed as per International Council for Harmonisation (ICH) guidelines, encompassing parameters, namely specificity, accuracy, linearity, limit of detection (LOD), precision, limit of quantification (LOQ), robustness. The calibration curve showed linearity over a concentration range of 5–100µg/mL, with an LOD of 0.24µg/mL and an LOQ of 0.74µg/mL. All validation parameters met acceptable criteria, confirming the method's accuracy and precision.

KEYWORDS: LCMS, Bilastine, LOD, LOQ, ICH, Method development, Validation.

How to Cite: Kavya M C, P. Kumar Nallasivan, (2025) Method Development and validation by Liquid chromatography Mass spectroscopy (LC-MS) for Bilastine by a stability indicating assay method, Vascular and Endovascular Review, Vol.8, No.2, 329-334.

INTRODUCTION

The work aimed to develop and optimize simple LCMS technique for evaluation of bilastine in bulk and formulations. Many modern medicines, especially those with multiple active ingredients, can be efficiently analyzed using LC-MS. This technique stands out for its speed, precision, and ability to accurately identify specific compounds even in complex mixtures. It's also highly automated, which makes it ideal for routine testing in pharmaceutical labs.

One of biggest benefit of LC-MS is that it skips the need for lengthy extraction and isolation steps. Unlike older methods, it doesn't require buffer solutions, making the entire process simpler, cleaner, and faster.

Chronic spontaneous urticaria (CSU) is predominantly treated with second-generation H1-antihistamines (SGAHs). After receiving approval from the Drugs Controller General of India, Bilastine, a newer non-sedating SGAH, has been recently introduced in India [1]. For symptomatic treatment of allergic rhino-conjunctivitis and urticaria recommended dosage administered is 20mg/day. [2]. On literature survey, it was found that few U.V. spectroscopy, HPTLC, HPLC and fluorimetric methods are only available. [3] [4] [5]. Degradation studies are available for evaluation of Bilastine in tablet dosage form. In literature survey, there has been a lack of discussion on advanced chromatographic method development approaches specifically focused on pharmaceutical development. The goal of current work was to create rapid, simple, sensitive LC-MS technique for estimating bilastine in both bulk and tablet dosage forms with better retention time. By employing a more robust method that consistently produces reliable and high-quality data, technique has been confirmed in accordance with ICH guideline Q2 (R1).[6] Forced degradation study is a valuable tool for developing stability-indicating methods, providing insights into degradation pathways and products of drug substance. This assists to ensure specificity of stability indication methods while enhancing our understanding of active pharmaceutical ingredients and drug products.

DRUG PROFILE

Table-1. Drug profile of Bilastine

IUPAC Name	2-[4-[2-[4-[1-(2-ethoxyethyl)benzimidazol-2-yl]piperidin-1-yl]ethyl]phenyl]-2-methylpropanoic acid
Molecular Weight	463.6g/mol
Chemical Formula	$C_{28}H_{37}N_3O_3$
pka Value	(Strongest acidic) 4.04 (Strongest basic) 9.01
Tmax	1.13 h
Category	Second-generation antihistamine drug
Class	Benzimidazoles
Physical Form	White colour powder

Structure –

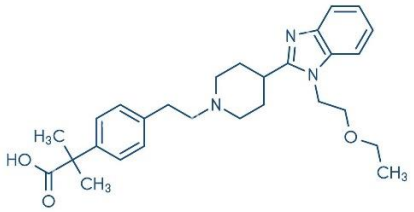

Figure 1. Structure of Bilastine

METHODS AND MATERIALS

2.1 Instrument

This work explains development and validation of Bilastine utilizing LC-MS procedure. Agilent 1290 Infinity II LC system has been employed to attain chromatographic separation, together with an Agilent 6470 Triple Quadrupole Mass Spectrometer has been provided with Electrospray Ionization (ESI) source.

Analysis has been carried out on a Poroshell SB C18 column (Dimensions: 4.6×150 mm, particle size: 2.7µm). Mobile phase consisted of mixture of acetonitrile (400 mL), distilled water (300 mL) and methanol (300 mL), delivered at flow rate of 0.5mL/min. Injection volume has been set at 10µL and column temperature regulated at 35°C to ensure optimal performance. Detection has been conducted at wavelength of 215nm, suitable for Bilastine's UV absorbance characteristics. The chromatogram obtained shown in Figure 4.

Retention time of Bilastine has been observed to be 3.6min which represented in Figure 6 and the run time was 06 min for each injection. Ionization Mode used in MS system was ESI. Various chromatographic conditions are explained in Table-2.

Table -2. Various chromatographic conditions used for the estimation of bilastine using LC-MS method

RAMETERS	**LUES**
tographic mode	tic
bile phase	N:Water: Methanol
ector	LC-MS
w rate	.5ml/min
ction volume	10µL
umn	oshell SB C18 4.6 x 150mm x 2.7µm
Column oven Temp	35°C
n time	6 mins
ention time	3.6 min

2.2 Chemicals and Reagent

Gift sample of Bilastine has been obtained from Synochem, a pharmaceutical company located in Uttarakhand. BL-Hist Tablets (Bilastine Tablets, 20mg) have been acquired from nearby pharmacy. Acetonitrile, Methanol, water have been utilised in HPLC grade and obtained from Merck Life Science Pvt. Ltd. in Mumbai, India.

1.3 Preparation of solutions for LC-MS method development of bilastine.

2.3.1 Preparation of mobile phase

Mobile phase has been generated by mixing Acetonitrile: Water: Methanol in ratio of [40:30:30]. The above mixture was sonicated before introducing into the LC-MS System.

2.3.2 Standard and sample preparation

Bilastine standard solution preparation: 0.05g of Bilastine has been precisely weighed and transferred to 50ml volumetric flask. Mobile phase solution has been utilized to dissolve Bilastine and volume has been filled up with same solution. (1000 µg/mL) Preparation of working standard solution: Working standard solutions of bilastine of 100µg/mL have been produced by pipetting out 1ml and diluting up to 10ml in SVF with mobile phase (Acetonitrile: Water: Methanol) [40:30:30] Sample solution preparation: Mobile phase has been utilised to dilute 0.05g of bilastine to 50ml in a volumetric flask. Drug is dissolved thoroughly by sonication and solution is filtered and diluted further by diluting 1 ml of it i 10ml volumetric flask. Mobile phase is later added to dilute solution to required volume.

2.4 Determination of Calibration curve.

Before injecting into LCMS system, both the standard and sample solutions have been carefully filtered using 0.45µm syringe filters to remove any particles that might interfere with the results. Each aliquot has been then injected into HPLC system utilizing a consistent volume of 10 µL. To understand how concentration affects the signal, a calibration curve was created by plotting known concentrations (varying from 5-100µg/mL) against corresponding peak areas. Using regression analysis, we calculated intercept, slope, correlation coefficient—key indicators of how well process performs.

2.5 Assay of bilastine in tablets

Using a mortar and pestle, 20 tablets had been thoroughly weighed and crushed into a fine powder. This resulted in precise measurement and transfer of amount of powder equivalent to 20mg of bilastine to 100mL volumetric flask. Drug has been dissolved completely by integerating approximately 50mL of ultrapure water and sonicating mixture for 5 minutes. Solution has been then topped up to 100 mL mark with ultrapure water.

From this stock solution, 1.00 mL has been measured and diluted to 10mL with water in volumetric flask, resulting in a final concentration of 20µg/mL. A 10µL volume of this sample has been injected into HPLC in triplicate. Samples were determined utilizing developed approach and the peak areas corresponding to bilastine have been recorded. These values have been later utilized for calculating amount of active ingredient available in tablets.

2.6. Analysis method conditions

This work discusses development and validation of Bilastine utilizing LC-MS process. Agilent 1290 Infinity II LC apparatus (or equivalent) was employed. MS System: Agilent 6470 Triple Quadrupole Mass Spectrometer.

Column has been used Poroshell SB C18 (4.6x150mmx2.7µm) with mobile phase comprised of acetonitrile (300ml), distilled water (300ml), methanol (400ml). The flow rate has been 0.5 ml/min, the injection volume has been 10µl and detection has been conducted at 215nm with column temperature of 35 ˚C. Retention time of bilastine has been observed to be 4.3min and run time for each injection has been 6 min. The molecular weight of bilastine is 463.4, Figure-2 shows the +ve ion detection of bilastine in LC-MS at 464.15 and figure 2 shows the –ve ion detection of bilastine in LC-MS at 462.17 The chromatogram obtained for bilastine is illustrtaed in Fig 5, with a maximum wavelength at 215nm.

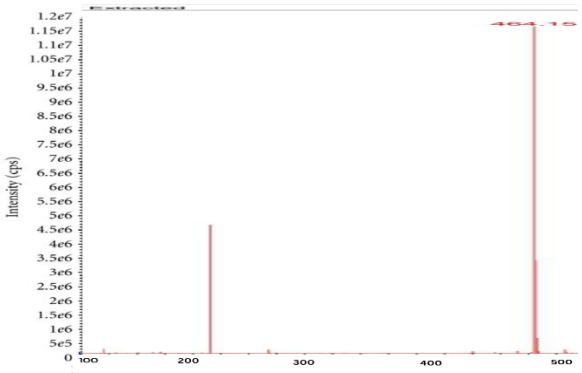

Figure-2. (+) ve Ion detection of bilastine at 464.15 in LC-MS system

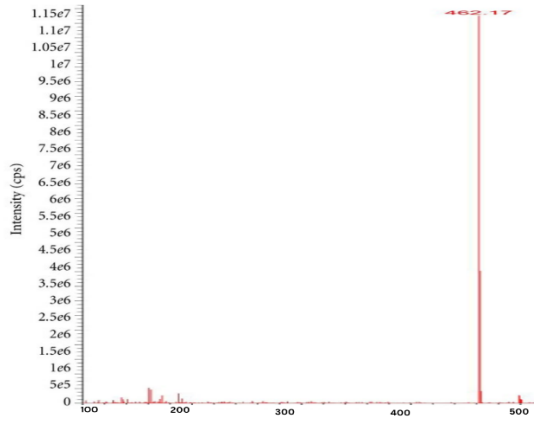

Figure-3. (-) ve Ion detection of bilastine at 462.17 in LC-MS system

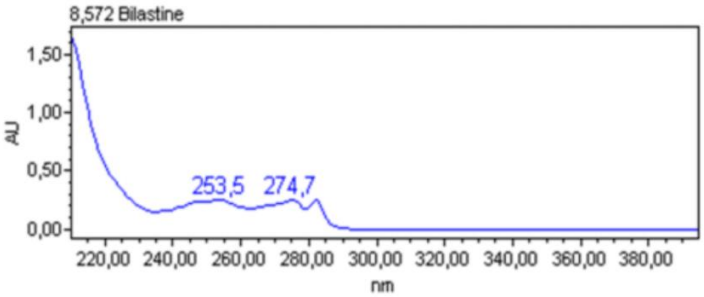

Figure 4. Chromatogram obtained for bilastine at maximum wavelength at 274.7 nm.

2.7 Validation of the developed method

To ensure reliability of established LC-MS technique for analyzing bilastine in bulk and tablet form, validation has been conducted as per guidelines set by ICH. Process was carefully evaluated for several key parameters—including accuracy, ruggedness, specificity, precision, linearity and range, system suitability and robustness. Validation of process performs consistently and accurately under various conditions, making it suitable for routine use.

EXPERIMENTAL

3.1 System suitability

System's suitability has been evaluated by injecting standard solution (10µl) under optimized chromatographic conditions. Factors, namely Chromatographic mode, Mobile phase, Detector, Flow Rate and Run time have been evaluated. According to values observed, system performs efficiently. Parameters and values of system suitability have been demonstrated in Table- 3 and Table-4.

3.2 Specificity

Blank, standard, sample solutions have been produced just as described earlier and each was carefully injected into LC-MS system. As the chromatograms were generated, retention times of all relevant peaks were carefully recorded. A summary of these findings is presented in Table-5.

Chromatogram showing two specific mass transition between 464.3 →145.1 and 464.3 →272. Figure-5 represents the fragmentation of parent ion (464.3 m/z) into 2 different products of (145.1 m/z) and (272 m/z)

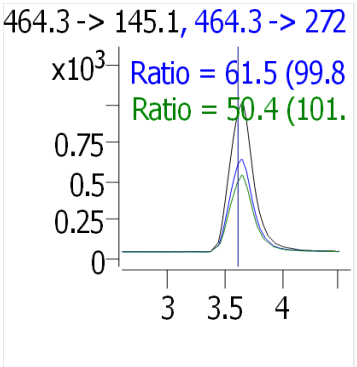

Figure 5. The LC-MS Chromatogram showing two specific mass transition between 464.3 →145.1 and 464.3 →272.

3.3 Linearity

For evaluating linearity of process, 5 levels of concentrations of 5 to 100µg/ml of standard solution bilastine were taken. The area under curve for each concentration is showed in Table-6 and the Linearity Curve obtained for bilastine at 215 nm is shown in Figure 6.

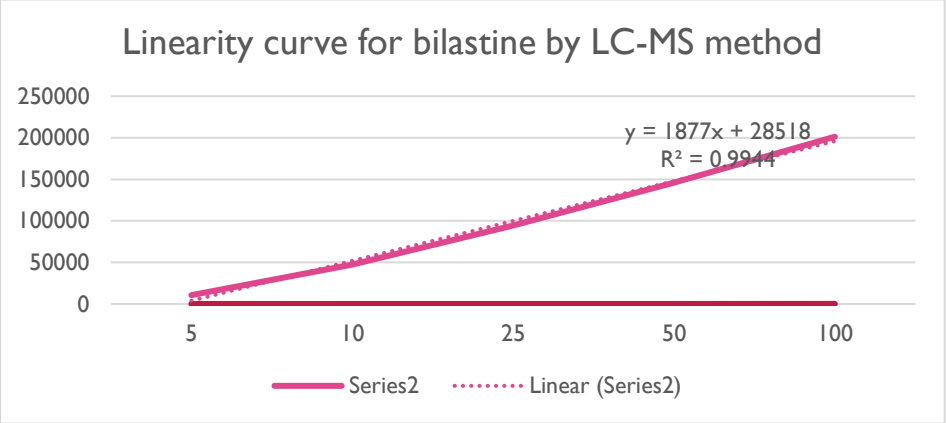

Figure-6. The Linearity Curve obtained for bilastine at 215 nm in various concentration between 5-100 µg/ml

3.4 Accuracy

The method's accuracy was evaluated through recovery investigations by determining percentage mean recovery of drug at 3 different levels: 80%, 100%, 120%. 3 determinations have been carried out at each level. Known quantity of standard pure drug has been integrated to pre-analyzed tablet powder and sample has been evaluated utilizing developed approach. The outcomes, presented in Table-5, showed that noted data have been within acceptable range, indicating good recovery values.

3.5 Precision:

Precision of analytical approach has been displayed by intra and inter day precision. In intraday precision, same sample was analyzed multiple times within single day while in inter day evaluated reproducibility of results when the same sample on different days. The accounts for variations was found to be in limit and shown in Table 6.

3.6 Robustness

Robustness of analytical approach has been performed through several parameters deliberately from optimized chromatographic conditions such as varying temperature and by addition of small amount of ammonium acetate in mobile phase. Findings have been depicted in Table-7.

3.7 Ruggedness

Ruggedness related to repeatability and reproducibility has been evaluated among numerous analysts. Value of STDEV has been observed to be lesser than 2.0. The value of %RSD found was illustrated in Table -8.

Limit of detection and Limit of Quantitation:

LOD displays smallest concentration of analyte which could be confidently distinguished. It's the point where the analytical method says even if it's just barely above the baseline.

LOQ goes further, it's lowest concentration which can be assessed with acceptable precision, accuracy and consistency.
LOD and LOQ were calculated as,

$$LOD = 3.3\ \sigma / S$$
$$LOQ = 10\ \sigma / S$$

Where σ is standard deviation of lowest standard concentration and S is slope of standard curve. Found result is showed in Table 09.

RESULTS AND DISCUSSION

System suitability.

System's suitability has been assessed by injecting 10µl of standard solution under optimized chromatographic conditions. Parameters for Retention time (min) have been established including %RSD for injection precision. System's high performance is suggested by values that were attained. Table-3 displayed system suitability values.

Suitability Parameter	**Acceptance Criteria** % RSD	**Result** % RSD
Injection precision %RSD for Retention time (min)	NMT 2.0	1.61%

Injection precision for %RSD peak area (n = 6)	NMT 2.0	1.024%

Table 3 - The parameters of system suitability parameters.

4.2 Linearity

Linearity has been investigated through evaluating 5 standard solutions in range of 5-100µg/ml for Bilastine by LC-MS. Calibration curves with concentration versus area was plotted for each method. Linearity was measured by using calibration data, regression analysis as well as coefficient of correlation. Values obtained from linearity curve shown below in table -4.

Table 4. Values obtained from linearity curve for system suitability.

4.3 Accuracy

Method's accuracy was evaluated through recovery research by determining percentage mean recovery of drug at 3 various levels: 80%, 100%, 120%. 3 determinations have been carried out at each level. Known quantity of standard pure drug was integrated to pre-analyzed tablet powder or sample has been assessed utilizing developed technique. The outcomes presented in Table-5, showed that noted data were within acceptable range, indicating good recovery values.

Table 5 - Result for Accuracy with standard addition method.

4.4 Precision

The consistency of outcomes when same sample is analyzed many times within a single day (intraday) and also evaluated the determination in different days to determine in interday precision. Inter- and intra-day precision were assessed by injecting 10µl of standard solution under optimized chromatographic conditions. Observed data were within acceptable range and is shown in table -6.

Table 6- Results obtained for interday precision and intraday precision.

4.5 Robustness

Robustness of analytical process has been conducted through deliberately changing parameters from optimized chromatographic conditions such as varying temperature from 25-35°C. The another parameter involved in robustness was various concentration of mobile phase ie, 8mM and 10mM ammonium acetate in Water (Water: Methanol: ACN) in a 30:30:40 ratio. Observed outcomes are within limit and is depicted in Table- 7.

Table 7. Result for Robustness in different condition for flowrate and mobile phase.

4.6 Ruggedness.

Ruggedness was determined by different analysts. 10µl of standard solution has been injected under optimized chromatographic conditions for evaluating ruggedness. The value of STDEV was found to be lesser than 2, and is shown in Table 8.

Table 8. Result for Ruggedness obtained with different analysts

4.7 LOD and LOQ.

LOD and LOQ of drugs for both techniques have been calculated with standard deviation and slope. LOD and LOQ of each process were calculated and represented. LOD and LOQ have been calculated by utilising slope and standard deviation obtained for linearity curve. Results have been demonstrated in Table -9

Table 9. LOD and LOQ obtained for method validation of bilastine.

CONCLUSION

Simple, accurate, rapid, robust, precise LC-MS process has been established as well as confirmed for routine assessment of Bilastine in API or in tablet dosage form. Process has been suitable for determination of Bilastine. Chromatographic separation has been attained on Poroshell SB C18 (4.6x150mmx2.7µm) with mobile phase comprised of acetonitrile (400ml), distilled water (300ml), methanol (300ml). Flow rate has been 0.5ml/min, Injection volume has been 10µl and detection is at 215nm where column temperature has been 35 ˚C. Retention time of Bilastine has been observed to be 3.6min and run time for each injection has been 06 min.

Process has been established to fulfill ICH requirements and such validation comprised linearity, specificity, LOD, accuracy, LOQ, precision, robustness. Calibration curve has been linear over concentration range from 5-100µg/ml and lower LOD of 0.24µg/ml, LOQ of 0.74 µg/ml. Method's precision as well as accuracy were within the acceptable range. The methods that have been established are recommended for routine and quality control analysis. Value of standard deviation