Bilastine: stability-indicating a method using environmentally friendly by reversed-phase high-performance liquid chromatography (RP-HPLC)

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This study describes the development and validation of a new environmentally friendly analytical method for the determination of bilastine in coated tablets and the evaluation of its capacity to be stability-indicating as well. The ecofriendly analytical method was validated by specificity, linearity, accuracy, precision and robustness by reversed-phase high-performance liquid chromatography (RP-HPLC) according to International Conference on Harmonization guidelines (ICH) and Association of Official Analytical Chemists (AOAC). Isocratic LC separation was achieved on a RP18 column using a mobile phase of sodium dihydrogen phosphate aqueous buffer solution adjusted to pH (6.0 ± 0.1) with o-phosphoric acid (85% v/v) and triethylamine (0.3% v/v) and ethanol (EtOH) in the following proportions (60:40 v/v), at a flow rate of 1.0 mL·min^{-1} at temperature-controlled at 30 °C. The analytical method showed selectivity, good recovery and precision (intra- and inter-day), robustness, and linear over a range from 5.0 to 50 μg·mL^{-1}.

Introduction

Bilastine (Figure 1) 2-[4-(2-(4-(1-(2-ethoxyethyl)-1H-benzimidazol-2-yl) piperidin-1-yl) ethyl) phenyl]-2-methylpropionic acid, is a novel second-generation H1-antihistamine. It has been used for the symptomatic treatment of allergic rhinoconjunctivitis and urticarial without showing sedative or cardiotoxic effects (1,2).
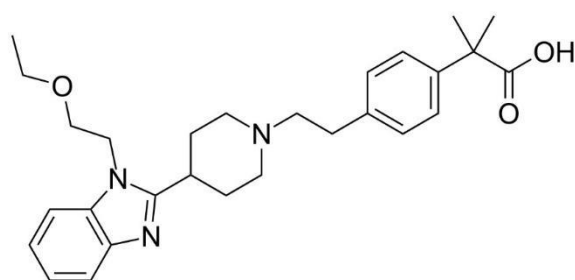

Figure 1 - Chemical structure of bilastine.

Bilastine was developed by FAES FARMA→ laboratories and it was approved for use in the European Union in 2010 and, since then, it has been introduced in clinical practice in more than 100 Countries of the European Union, Africa, Latin America, Asia and Canada adults over these 12 years (3). In Brazil, bilastine had approval for use in 2011 (4).

Although bilastine has been available in pharmaceutical market over a decade, there are no analytical methods described in official pharmacopeias and there are only few published papers reporting the determination in biological samples (5-7) and impurities determination by high performance liquid chromatography (HPLC) (8).

Green analytical chemistry (GAC) is a branch of green chemistry that targets for a minimal hazardous waste and to use friendly solvents to the environment (9). The main objective of green analytical chemistry is the development of new analytical greenest methods or making them greener than conventional methods, modifying an old one by incorporating procedures that aim the replacement of aggressive solvents for less hazardous chemicals (10). GAC was noticeable in the last twenty years due to its main effects on the atmosphere and the health of professional analysts (11).

The chromatographic methods often use solvents such as acetonitrile (ACN), methanol (MeOH) and other organic mobile phases polarity modifiers and mostly are toxic in nature and environmentally non-friendly (12). Since the last decade and further, ethanol (EtOH) has been reported to be used as a mobile phase modifier in the context of green separation approach for a variety of compounds in many HPLC methods (13,14).

EtOH is one of the greenest and environmentally compatible organic solvents, which makes it a particularly desirable solvent for green liquid chromatography (15) being an affordable reagent, economically viable and environmentally safe. Ecofriendly reverse phase high-performance liquid chromatography (RP-HPLC) methods using ethanol-based mobile phases have been widely reported for drug analysis in pharmaceutical formulations (16-21).

Chromatographic techniques have the potential to be ecofriendly through all steps of the analysis, from sample collection and preparation, to separation and final determination (22-25).

The present study aims to develop and validate a method of stability-indicating environmentally friendly using RP-HPLC. Safer, reliable, and greener for quantification of bilastine content in tablets using aqueous solution phases (buffer, TEA, orthophosphoric acid) and EtOH as the greener mobile phase component. To the best of our knowledge, this is the first report of bilastine determination using green approach principles.

Materials and Methods

Bilastine reference substance (BRS) (99.0%) was purchased from TRC (Toronto, Ontario, Canada). Alektos® (Takeda, Brazil) was the pharmaceutical dosage chosen for the tests, consisting of tablets containing 20 mg of bilastine and purchased from a local drugstore. Ethanol HPLC grade was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The excipients contained in the dosage form (microcrystalline cellulose, sodium starch glycolate, magnesium stearate and colloidal silicon dioxide) were all pharmaceutical grades and were purchased from different suppliers. Phosphoric acid and triethylamine were purchased from Merck (Darmstadt, Germany). Purified water was prepared at the laboratory.

Instrumentation

HPLC equipment was a Shimadzu HPLC system (Shimadzu, Kyoto, Japan), consisting in a System Controller CBM-20A, a Solvent Delivery Unit LC-20AD, an On-line Degassing Unit DGU-20A, and a Photo-diode Array detector SPD-M20A. Integration of the peak areas was performed automatically by computer using a Shimadzu LC solution V1.24 SP1 software program. System was equipped with a Rheodyne® injector valve with a 20 µL loop volume of sample, was introduced into Auto-Sampler SIL, which consisted of a dual solvent delivery system. Photodegradation studies were carried out in a photostability UV chamber (1.0x0.17x0.17 m) with mirrors covering the inside walls and equipped with UV-C lamp (Ecolume ZW®, 254 nm, 30 W).

Preparation of bilastine sample solution

Twenty bilastine tablets were individually weighed, finely powdered and stored in amber flask. For the preparation of the stock solution, the equivalent of 5.0 mg of bilastine was transferred to a 25 mL volumetric flask and the volume was completed with a solution consisting of water and EtOH (1:1), the final solution was sonicated for 20 minutes, and volume was adjusted with the same solvent to yield a final concentration of 200.0 µg mL^{-1}. An aliquot of 2.0 mL of this solution was diluted in a 20 mL volumetric flask to yield a final concentration of 20.0 µg mL^{-1} using the same mixture of water and ethanol as solvent.

Preparation of BRS solution

To prepare this solution, 5.0 mg of bilastine reference standard was weighed, transferred to a 25 mL volumetric flask and added 25 mL of solution of water and EtOH (1:1), sonicated for 20 minutes, and the volume was completed up with the same solvent to yield a final concentration of 200.0 µg mL^{-1}. An aliquot of 2.0 mL of this solution was diluted in a 20 mL volumetric flask to yield a final concentration of 20.0 µg mL^{-1} using the same mixture of water and ethanol as solvent.

Chromatographic conditions

The chromatographic separation was performed on a Phenomenex® RP-18 column (150 x 4.6 mm, 5 µm). The elution was performed by isocratic system with a mobile phase containing pH 6.0 buffer solution and EtOH (60:40) at a flow-rate of 1.0 mL·min^{-1}. The buffer solution was composed by sodium dihydrogen phosphate 0,01 mol·L^{-1}, triethylamine (TEA) 0,3% (v/v) and the pH was adjusted with orthophosphoric acid 85% (v/v). Prior to the use, the mobile phase was filtered using 0.45 µm filter and sonicated for 15 minutes. The injection volume was 20 µL and the run time was set to 10 min. The column oven was set at 30 ℃ and the bilastine was determined by UV detection at 207 nm using photodiode-array.

Development and validation of the ecofriendly method

The validation of ecofriendly analytical method is essential for successful application in pharmaceutical analysis. The proposed method was validated according to International Conference on Harmonization (ICH) Q2R1 Guidelines and Official Methods of Analysis (26,27).

Selectivity

The selectivity of the method was evaluated for its ability to separate and properly identify the peaks of bilastine, excipients of formulation, impurities, and degradation products.

The samples were put under stress conditions to provide a stability-indication and to investigate the selectivity properties of the method. The solution stock of bilastine was submitted to hydrolytic, thermal, oxidation and photolytic conditions. The peak purity test was performed by a photodiode array detector (PDA).

a) Acid Hydrolysis: This test was performed maintaining 1.0 mL of stock solution of bilastine (200 µg·mL^{-1}) in 1.0 mL of 1.0 mol·L^{-1} HCl for 1 and 2 hours at room temperature (25 °C) and protected from the light. Subsequently, the solution was neutralized by 1.0 mol·L^{-1}

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NaOH and diluted with a solution of water and EtOH (1:1) to a final concentration of 20.0 µg·mL⁻¹. The same procedure described above was performed in a water bath at 70 ºC for 1 and 2h.

b) Alkaline Hydrolysis: This test was performed maintaining 1.0 mL of stock solution of bilastine (200 µg·mL⁻¹) in 1.0 mL of 1.0 mol·L⁻¹ NaOH for 1 and 2 hours at room temperature (25 °C) and protected from the light. Subsequently, the solution was neutralized by adding 1.0 mol L⁻¹ HCl and diluting with a solution of water and EtOH (1:1) to a final concentration of 20.0 µg·mL⁻¹. The same procedure described above was performed in a water bath at 70 ºC for 1 and 2h.

c) Thermal degradation: This condition test was performed by adding 1.0 mL of stock solution of bilastine (200 µg·mL⁻¹) and maintained by 1 and 2 hours in a water bath and protected from the light at 70ºC and at room temperature (25°C). After that, the solution was diluted with a solution of water and EtOH (1:1) to a final concentration of 20.0 µg·mL⁻¹.

d) Oxidative degradation: This condition test was performed maintaining 1.0 mL of stock solution of bilastine (200 µg·mL⁻¹) in 1.0 mL of H_2O_2 30% for 1 and 2 hours and stored at room temperature (25°C) and protected from the light. The same procedure described above was performed in a water bath at 70 ºC. Then, the solution was diluted using a solution of water and EtOH (1:1) to a final concentration of 20.0 µg·mL⁻¹.

e) Photolytic degradation: This condition test was performed by adding 1.0 mL of stock solution of bilastine (200 µg·mL⁻¹) in Plastibrand® disposable cuvettes and subjecting them to UVC radiation for 4, 8 and 16 hours in the photostability chamber. Thereafter, all the solutions were diluted with a solution of water and EtOH (1:1) to a final concentration of 20.0 µg·mL⁻¹.

Linearity

The linearity of the method was determined by preparing five different concentrations of BRS in the concentration range of 5-50 µg·mL⁻¹. The peak areas of the chromatograms were plotted against the respective concentrations of the drug to obtain the analytical curves. The calculation of the linear regression was employed by using the method of least squares and the curves were validated using analysis of variance.

Precision

The precision of the method was evaluated using the parameters of repeatability (intra-day) and intermediate precision (inter-day) in two different days. The results were expressed as relative standard deviation (RSD) of the analytical results from of the results of the quantification of six bilastine sample solutions prepared at 20.0 µg·mL⁻¹. The results were statistically evaluated in terms of % RSD.

Accuracy

The accuracy was determined by percentage recovery by adding aliquots of 0.2, 0.5 and 1.0 mL of the solution standard to sample solution of 20.0 µg·mL⁻¹, corresponding 20, 50, and 100% of the nominal concentration. The procedure was performed in triplicate (n=9, n=3/concentration level).

Robustness

The investigated factors for robustness evaluation were flow rate (± 0.1 mL·min⁻¹), pH of buffer solution (± 0.1), proportion of the solvents of mobile phase (± 0.2 %,), and oven temperature (± 5 °C). In addition, others evaluated parameters were retention time, peak area, number of theoretical plates and asymmetry factor. These parameters were assessed throughout the validation procedure of the green method.

System suitability

The system suitability of the method was determined based on following chromatographic parameters: number of theoretical plates, resolution and tailing factor.

Results and Discussion

Evaluation of the proposed analytical procedures

According to the paper published by Welch *et al*. (28) a greener analytical method can be obtained by modifying the mobile phase polarity and EtOH was chosen and elected as the most appropriate solvent to replace acetonitrile (ACN) and methanol (MeOH). The US Environmental Protection Agency of toxic chemicals listed ACN as a hazardous solvent in January 2019**.** In replacement of both solvents listed above, the EtOH is a suitable choice due to be a biodegradable and cheapest solvent with lower volatility and toxicity characteristics than MeOH and ACN (29).

The chromatographic conditions were selected and finally settled after previous tests with distinct ratios of aqueous phase solution sodium dihydrogen phosphate buffer (water), and organic solvent EtOH. The water:EtOH (60:40 v/v) ratio had been selected for better chromatographic determination of the drug. Moreover, the ortho-phosphoric acid (85%) was added as an organic modifier to the aqueous phase to reach pH 6.0 for better peak symmetry. It was found that developed mobile phase at a 1.0 mL·min⁻¹ flow rate through the column showed good results without compromising peak parameters. The drug was eluted at 4.0 min., at the selected wavelength (207 nm).

Although EtOH showed satisfactory results, it was observed that it leads to high back pressure in conventional HPLC system due to its high viscosity (30). Thus, increasing the total pressure in the RP-HPLC system. In addition, it was also observed the lower the temperature (below 25 ºC) the higher the pressure.

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For the green analysis of bilastine were investigated in the development and validation of a HPLC method, a variety of mobile phases, components ratio, conditions, and its related compound in tablet pharmaceutical preparations in researched literature.

Selectivity

The method selectivity was performed to verify the interference of excipients, impurities and degradation products in the determination of the bilastine drug. The forced degradation studies were conducted to evaluate the stability-indicating capability and selectivity of the proposed eco-friendly method.

The selectivity analysis revealed that the (RP-HPLC) green method did not show any interference by the formulation excipients. since there were no other peaks at the same retention time of bilastine (Figure 2A). Photodiode array detection also supported the selectivity of the method and provided evidence of peak purity for bilastine. It is important to note that even though the several degradation product peaks can be observed, no one interference in the determination of bilastine was verified. It was observed that the bilastine peak presents appropriate resolution (Rs > 2) of the degradation products formed in stress conditions. The chromatographic peak purity tool was applied to verify the bilastine peak, showing that it was 100% pure in all cases. indicating the selectivity of the proposed green method. The results indicated that the eco-friendly method is indeed stability-indicating and that the drug can be evaluated both qualitatively and quantitatively in the presence of degradation products.

The obtained results in the forced degradation studies show that the drug is not susceptible to acid hydrolysis. basic hydrolysis and thermal conditions, but susceptible to oxidation and photodegradation. The results of forced degradation do not depend on the temperature, that is more pronounced in longer times and more drastic conditions. In the oxidative conditions (30% H_2O_2 for 2 h and heating) the drug degraded 62.07% In photolytic conditions bilastine degraded 37.30% in UVA light for 2 h, 72.35% in UVA light for 8h and 82.94% in UVA light for 16h.

Linearity

Linearity was calculated by constructing calibration curves (n=3) containing BRS. The parameter was assessed in the concentration ranges of 5-50 µg mL^{-1}. The validity of the assay was verified by means of ANOVA of the statistical data and according to it, showed that the method exhibited adequate linearity, significant linear regression (p < 0.05) and no significant linearity

deviation (p > 0.05). Besides that, Student's *t*-test showed that the experimental intercept in the regression equation is not significantly different from the theoretical zero value (p > 0.05). The linearity data was shown in Table 2 and (Figure 3).

Precision and Accuracy

Intra-day (*n* = 6) precision, performed by assaying the samples by the same analyst in three different days, showed the following results 100.20 ± 0.82; 100.22 ± 0.87 and 99.74±1.62 (mean ± RSD). The RSD for interday precision was 0.26% (*n* = 3). The low variability of the results indicated the precision of the method.

Accuracy was evaluated by the simultaneous determination of the analyte in solutions prepared by the standard addition method. The obtained results, 99.74±1.78; 99.22 ± 1.11 and 100.56 ± 0.98 (mean ± RSD %), revealed that the method enables accurate determination for each level, which indicated satisfactory accuracy.

System suitability testing and robustness

The obtained system suitability parameters were satisfactory according to the relevant USP-36 monograph Table 1. The system suitability of the method was checked based on chromatographic parameters including; retention time, tailing factor and number of theoretical plates. The optimum parameters were summarized in Table 1. The % RSD for the retention times of bilastine were found to be less than 2%. The theoretical number plate and tailing factor results were found to be satisfactory and within the limit.

The robustness of method was studied by deliberately modifying chromatographic conditions with a change in flow rate (0.8, 1.0 and 1.2 mL·min^{-1}) as well as pH of buffer solution (6.0 ± 0.1) and change in mobile phase composition (58:42, 60:40 and 62:38 v/v). Three different temperatures were studied: 25, 30 and 35 °C. but no significant enhancement of the peak shape was observed. This study was conducted to overcome peak broadening inducing by variation of organic solvent through increasing the temperature of the column.

The robustness parameters were summarized in Table 1. The results for robustness were favorable for (% RSD < 2%) the developed eco-friendly method for the analysis of bilastine. However, chromatographic conditions such as pH 5.9 and flow rate of 1.2 ml/min are not comprised between the acceptable range of variation (98 to 102%) and should not be used.

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Table 1. System suitability parameters and robustness for proposed green method.

Parameter	Ret. time (min) Mean± RSD%	Tailing factor, T Mean± RSD%	Theor. plates, T Mean± RSD%	Mean±RSD(%) (20 µg mL^{-1})
Oven temperature (30°C ±5)				
25°C	3.888±0.15	1.024±0.44	2.374.844±0.73	99.80±0.22
30°C	3.697±0.18	1.055±0.48	2.914.246±1.80	99.88±0.38
35°C	3.567±0.14	1.049±0.53	2.919.736±1.09	99.43±1.99
Flow rate (1.0 mL min^{-1} ±0.2)				
0.8	5.134±0.17	1.081±0.23	4.662.849±0.63	98.72±018
1.0	4.149±1.37	1.187±1.28	4.067.715±1.78	98.09±0.72
1.2	3.268±0.43	1.020±0.83	2.155.638± 1.76	97.32±0.17
Ratio 60:40 (w:e v/v ±0.2)				
58:42	3.663±0.29	1.142±0.68	3.407.136±1.20	99.62±0.29
60:40	4.214±0.06	1.088±0.36	3.992.708±1.08	99.80±0.61
62:38	4.841±0.96	1.069±0.42	4.396.397±2.44	100.03±0.94
pH (6.0 ±0.1)				
5.9	4.085±1.15	1.140±0.42	3.005.075±0.016	97.48±0.50
6.0	4.189±1.06	1.126±0.75	3.051.090±1.00	98.72±0.51
6.1	4.341±3.08	1.111±0.55	3.112.428±0.87	101.06±0.25
Reference	For information	≤ 2	≥2000	102-98

n=3

Table 2. Linearity results of green analytical method.

Data	Method proposed
Range (µg mL^{-1})	5-50
Correlaction coeficiente. R	0.9998
Linear equation	44691x+4843.8
Linear regression (F-value) (critical F-value)	2826.81
Linear deviation	0.152

n=3

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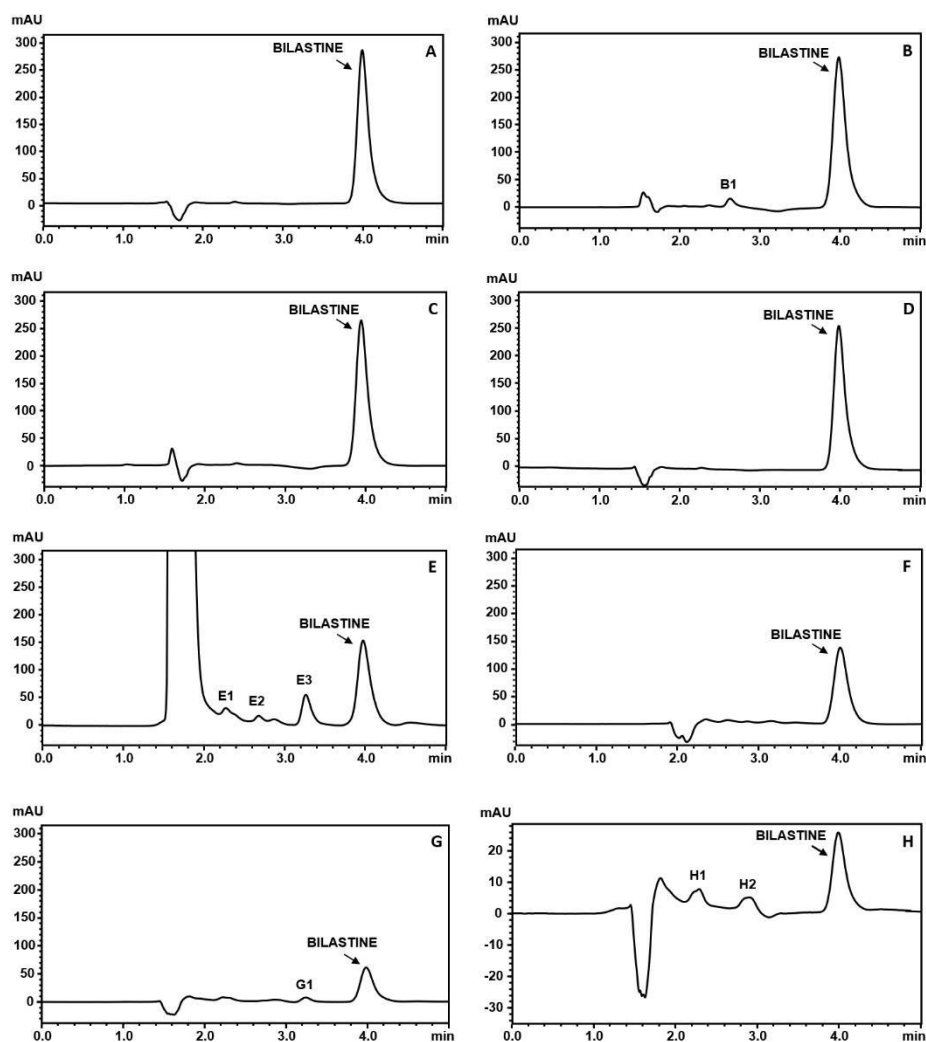
Figure 2. Chromatograms obtained from bilastine drug product (20.0 g mL^{-1}): A) Bilastine reference substance (BRS); B) chromatogram of bilastine acid-degraded and degradation product B1 acid hydrolysis (1.0 mol L^{-1} HCl for 2 h and heating); C) chromatogram of bilastine basic-degraded; under basic hydrolysis (1.0 mol L^{-1} NaOH for 2 h and heating); D) chromatogram of bilastine thermal-degraded under heating of 2hs; E) chromatogram of bilastine oxidative-degraded and degradation product, E1, E2 and E3 (30% H$_2$O$_2$ for 2 h and heating); F) chromatogram of bilastine photo-degraded, photodegradation (UVA 2 h.); G) chromatogram of bilastine photo-degraded and degradation product, G1, photodegradation (UVA 8 h.); H) chromatogram of bilastine photo-degraded and degradation product, H1,H2, photodegradation (UVA 16 h.) Chromatographic conditions: Phenomenex® RP-18 column (150 x 4.6 mm, 5 µm); buffer sodium dihydrogen phosphate 0,01M, Triethylamine (0,3% v/v); (pH adjusted to 6.0 with 85% (v/v) phosphoric acid) 1.0 mL^{-1} flow rate and buffer solution:EtOH (60:40, v/v); UV detection at 207 nm.
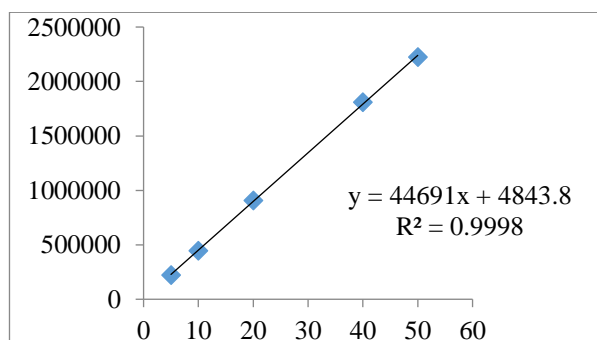
Figure 3. Bilastine reference substance calibration curve obtained by HPLC.

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Conclusions

An environmentally friendly RP-HPLC method was developed and statistically validated for the quantitative determination of bilastine in coated tablets. Validation experiments provided proof that the LC analytical method is linear, accurate, precise (repeatability and intermediate precision levels), specific, capable of separating the main drug from its degradation products, and robust. Due to these characteristics, the method has stability-indicating properties for bilastine in coated tablets and may be applicable to routine analysis. Furthermore, environmentally friendly methods have become of great interest to the field of pharmaceutical analysis to protect both the environment and the analyst's health.

Acknowledgments

Conflict of interest
The authors declare no conflicts of interest.

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