IJPSR (2020), Volume 11, Issue 3 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 12 May 2019; received in revised form, 19 September 2019; accepted, 13 November 2019; published 01 March 2020

STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF BILASTINE AND ITS IMPURITIES BY UPLC METHOD

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Keywords:

Bilastine,
Stability indicating,
Validation, UPLC

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ABSTRACT: This study describes the development and validation of stability indicating UPLC method for Bilastine and its impurities, an anti-allergic drug. Bilastine was subjected to stress degradation under different conditions recommended by the International Conference on Harmonization to observe the degradation products. The successful separation of Bilastine from its synthetic impurities and degradation impurities formed under stress conditions were achieved on Acquity UPLC CSH Phenyl-hexyl (2.1 mm × 150 mm, 1.7 µ), and the gradient mode mobile phase consists of 0.05% TFA in water and 0.05% TFA in Acetonitrile. The mobile phase flow rate was 0.10 ml/min. The column temperature was maintained at 25 °C. The sample temperature was maintained at ambient and wavelength fixed at 275 nm UV-detection. It was found that the method of RPUPLC with UV-detection system for the analysis of Bilastine impurities determination and also applied in qualitative and quantitative analysis. The developed UPLC method was validated with respect to specificity, precision, linearity, ruggedness, and robustness. A validation study has been performed as per ICH guidelines.

INTRODUCTION: Bilastine is a novel no sedative H1-receptor antagonist, which may be used for the symptomatic treatment of chronic idiopathic urticarial and allergic rhinoconjunctivitis [1]. Pharmacological studies have shown that the drug is highly selective for the H1 receptor in both *in-vivo* and *in-vitro* studies, and with no apparent affinity for other receptors. The absorption of Bilastine is fast, linear and dose-proportional; it appears to be safe and well-tolerated at all doses levels in healthy population [2].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(3).1312-21

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(3).1312-21

Bilastine is a selective histamine H1 receptor antagonist (Ki = 64 nM). During allergic response mast cells undergo degranulation, which releases histamine and other substances. By binding to and preventing activation of the H1 receptor, Bilastine reduces the development of allergic symptoms due to the release of histamine from mast cells.

EXPERIMENTAL:

Reagents and Chemicals: Trifluoroacetic acid, Acetonitrile, Hydrochloric acid, Sodium hydroxide, Potassium dihydrogen phosphate, and Hydrogen peroxide was procured from Merck. Water (Milli-Q). Analytical grade chemicals were used as received.

Instrumentation: The analytical separations were carried out on the Waters UPLC system with PDA detector. The analytical column was waters (RP-UPLC) using an Acquity UPLC CSH Phenyl-hexyl

(2.1 mm × 150 mm, 1.7 µ), and the mobile phase consists of two Channels A and B. Channel-A 0.05% TFA in water and Channel-B: 0.05% TFA in Acetonitrile. The flow rate was 0.1 ml/min. The column temperature was maintained at 25 °C, and sample temperature was maintained at ambient and wavelength fixed at 275 nm UV-detection. The control of UPLC system and data collection was Empower 3 software.

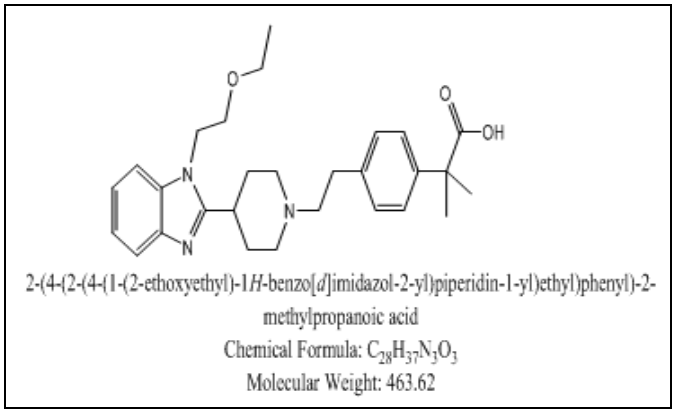

FIG. 1: CHEMICAL STRUCTURAL OF BILASTINE

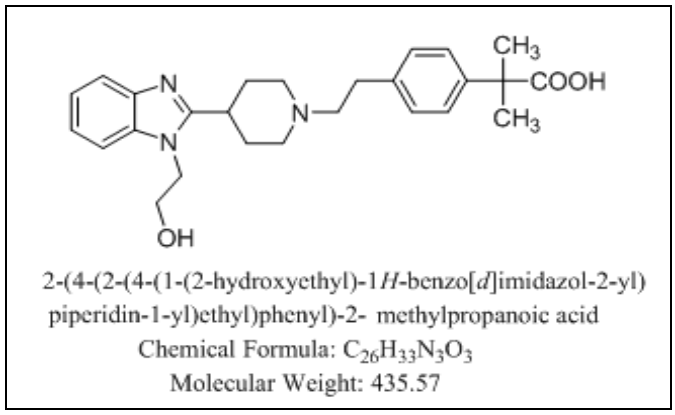

FIG. 2: CHEMICAL STRUCTURAL OF BILASTINE IMPURITY-A

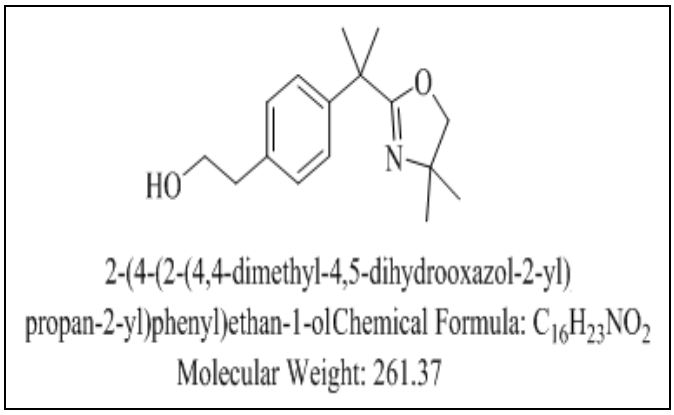

FIG. 3: CHEMICAL STRUCTURAL OF BILASTINE IMPURITY-B

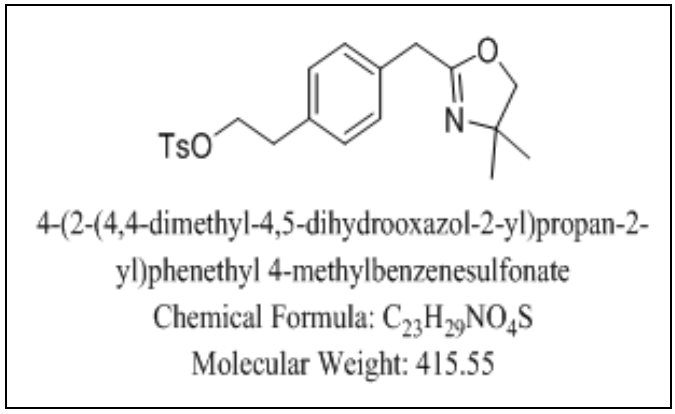

FIG. 4: CHEMICAL STRUCTURAL OF BILASTINE IMPURITY-C

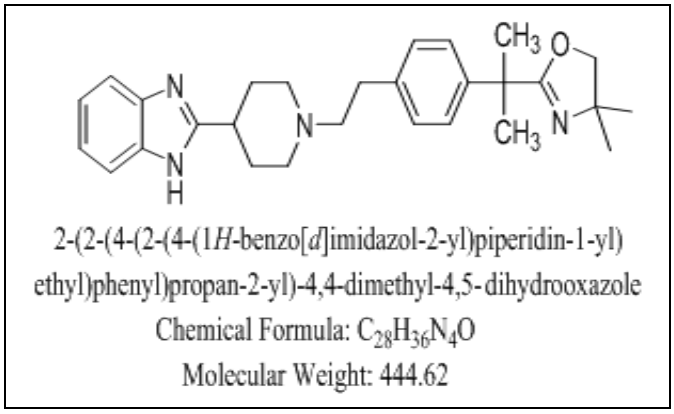

FIG. 5: CHEMICAL STRUCTURAL OF BILASTINE IMPURITY-D

Preparation Standard Solution and Sample Solution:

Standard Solution Preparation: A working standard stock solution of Bilastine was prepared by dissolving standard (equivalent to 0.5 mg/mL) 50 mg of Bilastine into 100 ml volumetric flask, to this added 50 ml of diluent and sonicated for 10 minutes at a temperature not exceeding 20 °C. Allowed the solution to attain room temperature and then diluted to the volume with diluent to have a solution with a concentration of 500 µg/ml.

Sample Preparation: Weighed 20 tablets and determined the average weight of the tablets and crush them to a fine powder by using mortar and pestle. Transfer crushed powder equivalent to 50 mg of Bilastine into 100 ml volumetric flask and added 50 ml of diluent and sonicated in ultrasonic bath for 20 min with intermediate shaking at a temperature, not more than 20 °C. Allowed the flask to attain room temperature and diluted to the volume with diluent. Filter the solution through 0.45 µm nylon membrane filter by discarding 4 ml of filtrate and injected the same solution (0.5 mg/ml).

Impurity Spiked Standard Preparation: Weighed accurately each impurity stock then spiked each stock to Bilastine standard to get the desired concentration of impurity-A, B, C and D as an Impurity standard.

DISCUSSION:

Method Optimization Parameters: An understanding of the nature of API (Solubility, Functionality, Acidity, or Basicity), the synthetic process, related impurities, the possible degradation pathways, and their degradation products are needed for successful method development in reverse-phase UPLC. In addition, successful method development should result a robust, simple, accurate, linear, precise and time-efficient method that is capable of being utilized in manufacturing setting.

Selection of Wavelength: The sensitivity of the UPLC method depends upon the selection of detection wavelengths. An ideal wavelength is one that gives good response for related substances and the drugs to be detected. The wavelength for measurement was selected as 255 nm from the absorption spectrum.

Selection of Stationary Phase: Proper selection of the stationary phase depends upon the nature of the sample and chemical profile. The drug selected for the present study was mixed polar compound and could be separated either by non-polar or mid polar stationary reverse phase chromatography. From literature survey, it was found that different C_{18}columns could be appropriately used for the separation of related substances for Bilastine.

Selection of Mobile Phase: Different mobile phase and stationary phases were employed to develop a suitable LC method for the quantitative determination of impurities in Bilastine. A number of column chemistries supplied by different manufacturers and different mobile phase compositions were tried to get good peak shapes and selectivity for the impurities present in Bilastine.

RESULTS: Poor peak shape and resolution was observed when BEH C18 RP Shield (100 mm × 2.1 mm, 1.7 µ) and gradient mobile phase programmed of Mobile Phase: A 0.1% OPA in water and Mobile Phase: B 0.1% OPA in Acetonitrile. There was no proper resolution of impurities and analyte peak and efficiency of the peak is also not achieved, and peak interferences are present.

In further trail made using BEH C18 (100 mm × 2.1 mm, 1.7 µ) Channel-A 0.05% TFA in water and Channel-B: 0.05% TFA in Methanol. There was no proper peak shape, baseline disturbance, and peak interferences are present. In one more trail made using Acquity UPLC CSH Phenyl-hexyl (2.1 mm × 150 mm, 1.7µ) Channel-A 0.05% TFA in water and Channel-B: 0.05% TFA in Acetonitrile. The resolution of both drug and impurities was achieved. These chromatographic conditions were selected for validation studies.

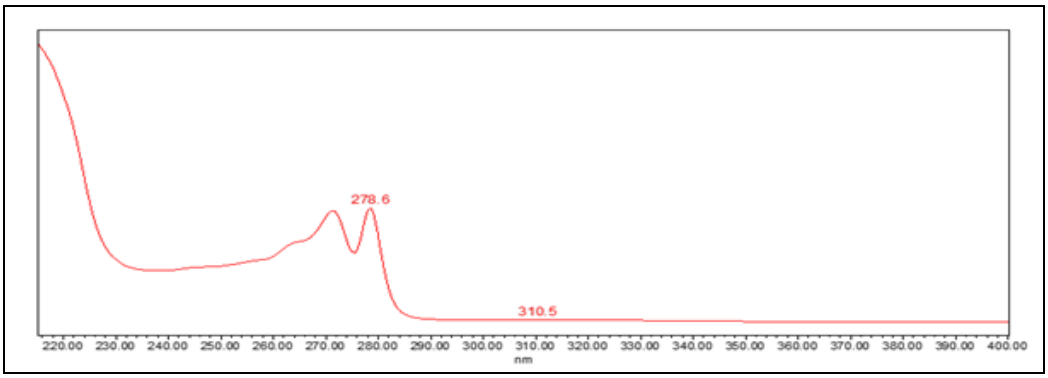

FIG. 6: PDA SPECTRUM OF BILASTINE

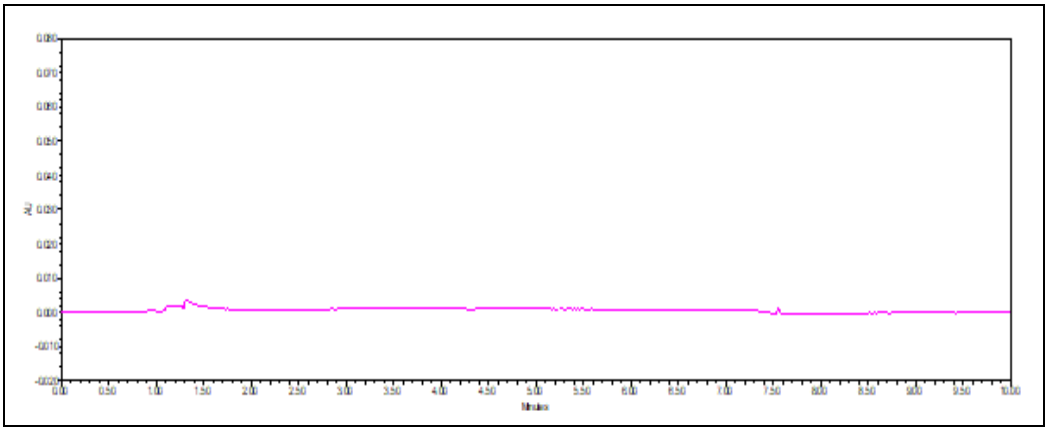

FIG. 7: TYPICAL CHROMATOGRAM OF BLANK

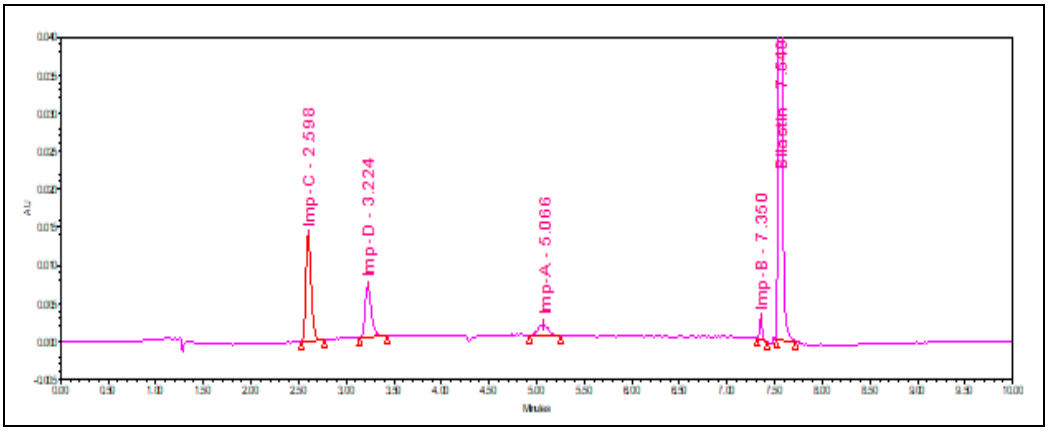

FIG. 8: TYPICAL CHROMATOGRAM SPIKED SAMPLE

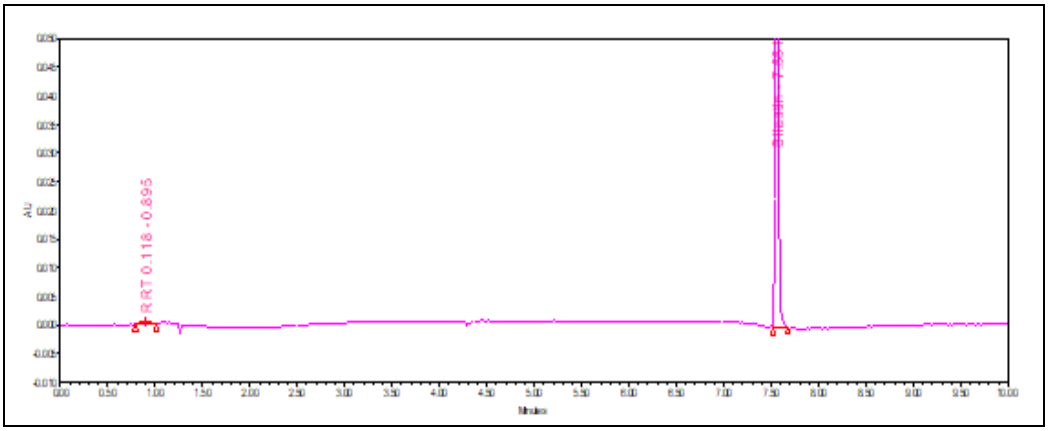

FIG. 9: TYPICAL CHROMATOGRAM OF AS SUCH SAMPLE

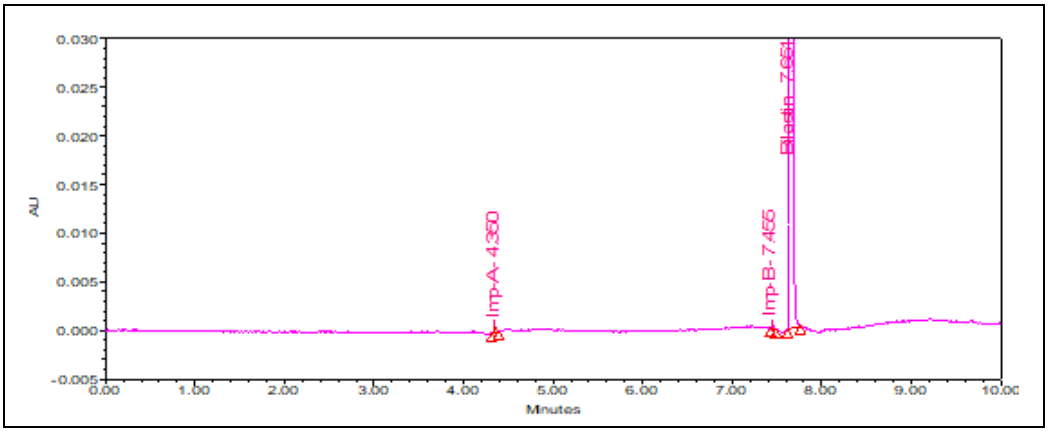

FIG. 10: TYPICAL CHROMATOGRAM OF ACID DEGRADATION SAMPLE

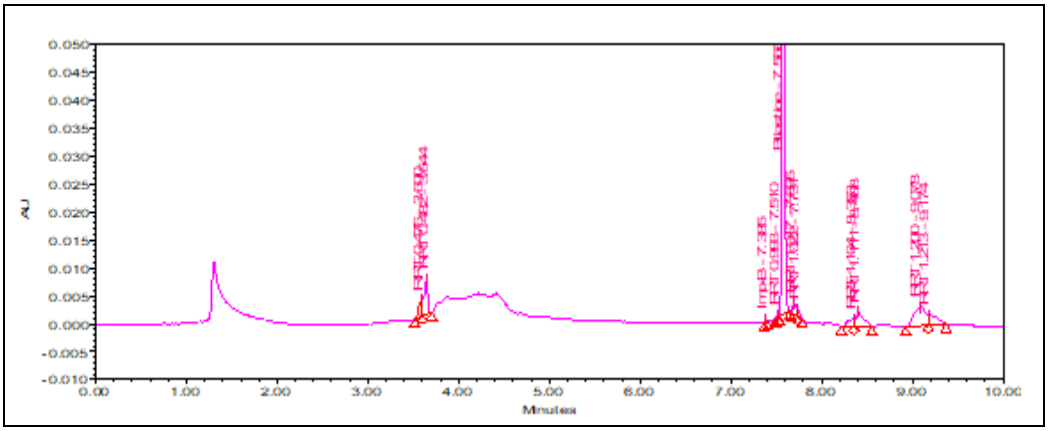

FIG. 11: TYPICAL CHROMATOGRAM OF ALKALI DEGRADATION SAMPLE

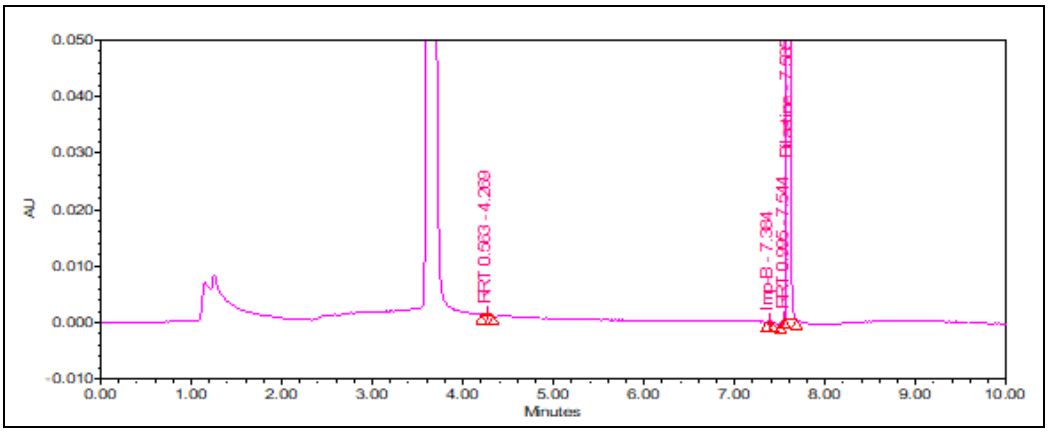

FIG. 12: TYPICAL CHROMATOGRAM OF OXIDATION DEGRADATION SAMPLE

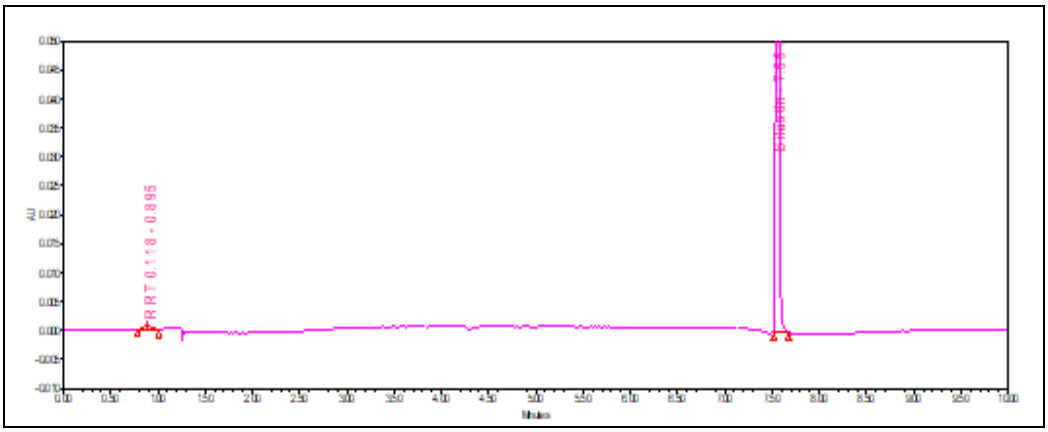

FIG. 13: TYPICAL CHROMATOGRAM OF THERMAL DEGRADATION SAMPLE

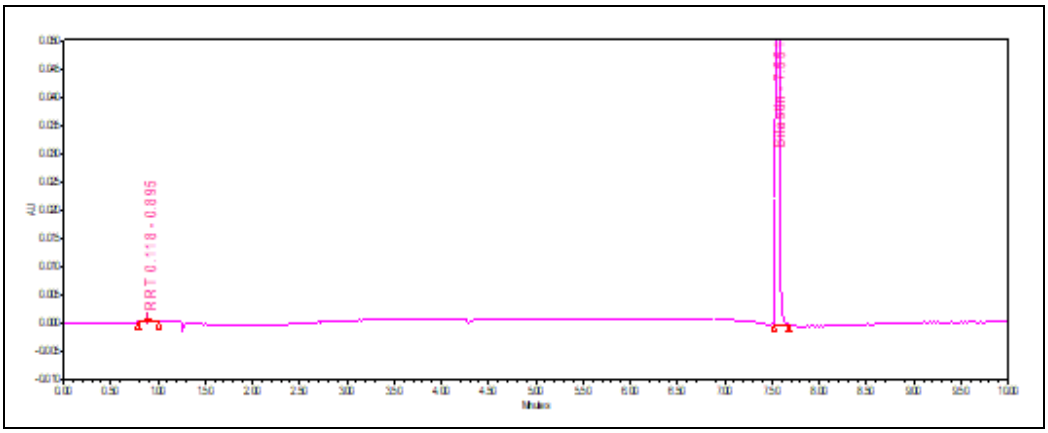

FIG. 14: TYPICAL CHROMATOGRAM OF PHOTOLYTIC DEGRADATION SAMPLE

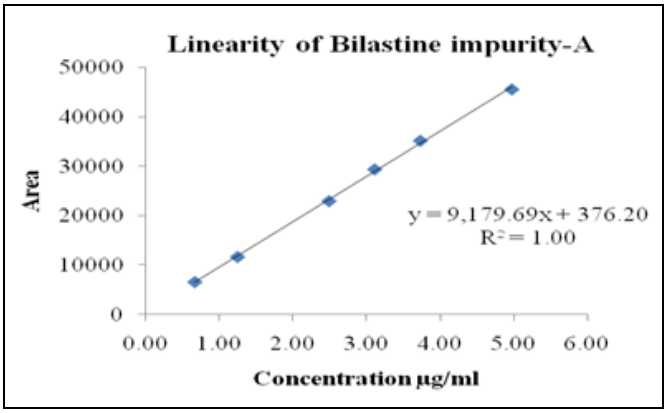

FIG. 15: LINEARITY OF DETECTOR RESPONSE FOR IMPURITY-A

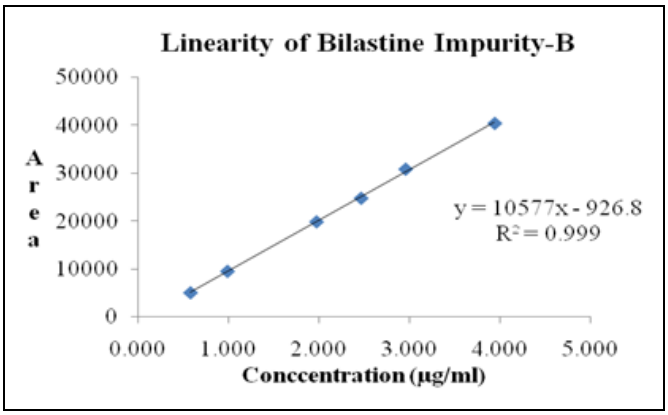

FIG. 16: LINEARITY OF DETECTOR RESPONSE FOR IMPURITY-B

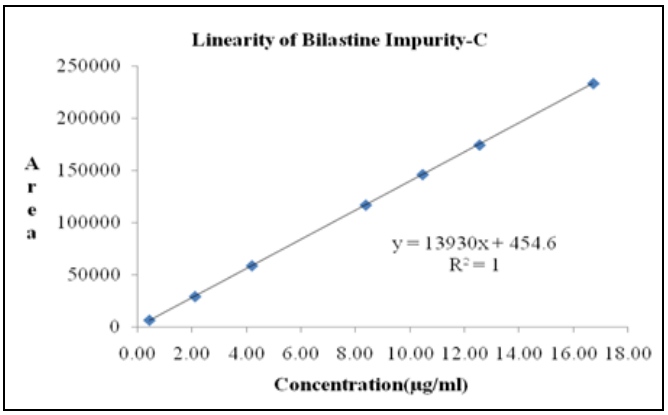

FIG. 17: LINEARITY OF DETECTOR RESPONSE FOR IMPURITY-C

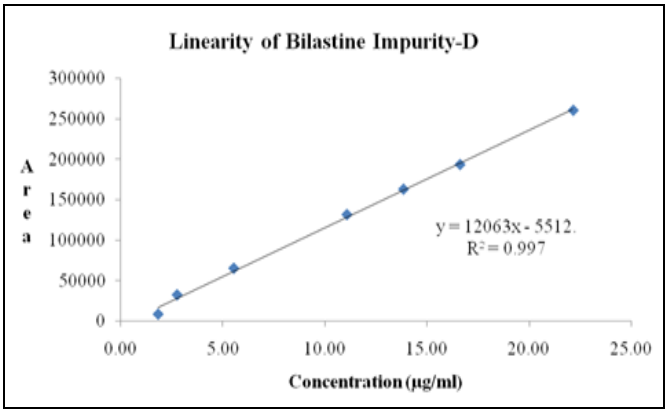

FIG. 18: LINEARITY OF DETECTOR RESPONSE FOR IMPURITY-D

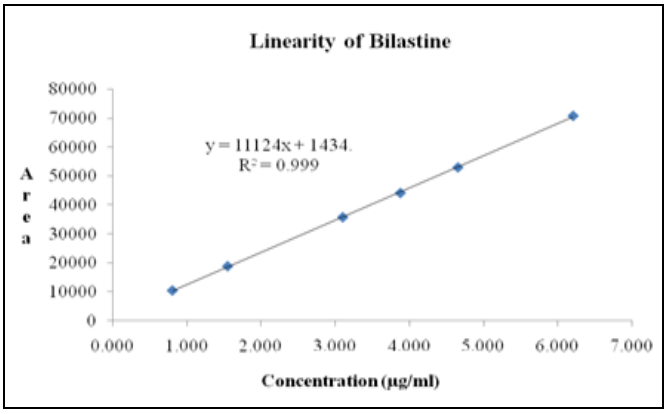

FIG. 19: LINEARITY OF DETECTOR RESPONSE FOR IMPURITY-E

Method Validation:

Specificity: Blank Interference: A study to establish the interference of blank was conducted. Diluent was injected as per the test method.

Spiked Sample Preparation: Weighed accurately each impurity stock then spiked each stock to Bilastine standard to get the desired concentration of Impurity-A, B, C, and D as an Impurity standard. It was observed that known impurities were not co-eluting with each other and main analyte peak. Bilastine standard solution preparation and in spiked test preparation was calculated and found to be within the acceptable limit.

Forced Degradation Studies: Forced degradation studies were performed to establish the stability-indicating power of the method. In this study Bilastine raw material, finished product and placebo were subjected to acidic, basic, peroxide, thermal and photolytic stress studies on sample concentration of 0.5 mg/ml in the diluent.

Weigh and transfer 25 mg of Bilastine sample into 50 ml volumetric flask added 25 ml of diluent and sonicated for 20 min with intermediate shaking at temperature, not more than 20 °C and then added respective degradant (acid, alkali, oxidant) and performed the stress study. Samples were neutralized after degradation and then diluted to the volume with diluent and injected to verify the stability-indicating power of the analytical method.

TABLE 1: IMPURITY INTERFERENCE DATA

Preparation	RT	Peak response
Blank	NA	ND
Sample with spike impurities		
Impurity-A	5.06	11056
Impurity-B	7.35	5643
Impurity-C	2.59	54316
Impurity-D	3.22	29815
Bilastine	7.54	1973125

TABLE 2: STRESS CONDITION AND ITS RESULTS

S. no.	Stress condition	% Drug remained	% impurities
1	As such sample	100.0	0.02
2	1N HCl 60°C 2 h	99.5	0.42
3	1 N NaOH 60°C 2 h	95.0	5.50
4	3% H_2O_2 Bench top on 6 h	99.4	0.41
5	105 °C 48 h	99.7	0.13
6	Photolytic stability	99.9	0.05

Stress conditions under which the study was performed, the amount of Bilastine remains, % impurities generated and mass balance results were tabulated.

Precision:

System Precision: Standard solution was injected in six replicate injections to check the Relative Standard Deviation (% RSD) for finding the precision of the system to be used for validation.

TABLE 3: SYSTEM PRECISION DATA FOR BILASTINE

Injection	Area of standard
1	35498
2	34985
3	34159
4	35784
5	35412
6	35876
Avg.	35286
% RSD	1.80

Acceptance Criteria: RSD should not be more than 5.0%. The % RSD of peak area for Bilastine was found to be 0.54 which is below 5.0% indicates that the system gives a precise results.

Method Precision: The precision of the impurities and degradants method was determined by injecting six sample solutions spiked with impurities (Impurity-A, B and C) at the specification level. The samples were prepared as per the method and the result for precision study is tabulated in **Table 4.**

TABLE 4: RESULTS OF METHOD PRECISION

Sample name	Imp-A	Imp-C	Imp-D	Bilastine	Imp-B
Precision sample-1	0.3193	0.73440	0.9111	97.213	0.2979
Precision sample-2	0.3205	0.74560	0.9130	97.194	0.2937
Precision sample-3	0.3193	0.73440	0.9130	97.199	0.3011
Precision sample-4	0.3181	0.73520	0.9111	97.200	0.2958
Precision sample-5	0.3193	0.73520	0.9130	97.219	0.3053
Precision sample-6	0.3193	0.73680	0.9083	97.204	0.3042
Mean	0.32	0.74	0.91	97.2	0.30
%RSD	0.24	0.59	0.20	0.01	1.55

TABLE 5: RESULTS OF PRECISION AT LOQ LEVEL

S. no.	Imp-A	Imp-B	Imp-C	Imp-D	Bilastine
1	1824	5661	6291	7881	10873
2	1548	5671	6543	7732	10792
3	1710	5821	6409	7607	10531
4	1651	5657	6498	7593	10820
5	1669	6007	6512	8339	10565
6	1554	6434	6507	8110	10606
Mean	1659.3	5875.2	6460.0	7877.0	10697.8
% RSD	6.2	5.2	1.5	3.8	1.4

The method precession was performed with six replicate solutions of standard solutions prepared and the system suitability parameters found were within the acceptance criteria.

Precision at Limit of Quantitation: Inject the blank, precision at LOQ solutions six times into UPLC. Record the peak area of Impurity-A, Impurity-B, Impurity-C and Bilastine in each injection. Calculate the % RSD for the area of impurities and analyte peak. The % RSD for the area of impurities and analyte peak from six preparations should be not more than 10.0.

The Precision at Limit of quantitation parameter results met the acceptance criteria.

Limit of Detection (LOD) & Limit of Quantitation (LOQ): For the present developed UPLC method Limit of Detection was found to be 0.16 µg/ml for impurity-A, 0.16 µg/ml for impurity-B, 0.16 µg/ml for impurity-C and 0.17 µg/ml for Bilastine and Limit of Quantification was found to be 0.53 µg/ml for impurity-A, 0.52 µg/ml for impurity-B, 0.54 µg/ml for impurity-C and 0.55 µg/ml for Bilastine respectively. LOD and LOQ were determined based on signal to noise ratio.

The limit of the limit of quantitation and detection of quantitation values obtained for each impurity and Bilastine were within the acceptance criteria.

Linearity and Range: Bilastine and Impurities (impurities-A, B, C, and D) in the concentration levels from LOQ to 200% standard solution were injected into UPLC system. The linearity graph was plotted from LOQ to 200% of drug concentration. Report the linearity range as the range for determining the impurities.Results obtained are in tables **Table 7, Table 8, Table 9, Table 10** and **Table 11** and figures show the line of best fit for peak area versus concentration for each impurity.

TABLE 6: LOD FOR BILASTINE AND IMPURITIES

Impurity/ Compound name	Concentration in ppm	S/N
Impurity-C	0.12	5.16
Impurity-D	0.56	8.25
Impurity-B	0.18	5.7
Bilastine	0.25	7.0

TABLE 7: LOQ FOR BILASTINE AND IMPURITIES

Impurity/ Compound name	Concentration in ppm	S/N
Impurity-C	0.66	10.35
Impurity-D	0.41	40.52
Impurity-B	1.84	23.05
Bilastine	0.58	26.68

TABLE 8: LINEARITY OF DETECTOR RESPONSE IMPURITY-A

Levels (%)	Concentration (µg/ml)	Area response of Impurity-A
LOQ	0.66	6479
50	1.24	11568
100	2.49	22909
125	3.11	29347
150	3.73	35160
200	4.97	45579
Correlation Coefficient:		0.999
Slope :		9179
Intercept:		376.2
%Y-intercept:		1.64

TABLE 9: LINEARITY OF DETECTOR RESPONSE IMPURITY-B

Levels (%)	Concentration (µg/ml)	Area response of Impurity-B
LOQ	0.576	5070
50	0.984	9542
100	1.968	19878
125	2.459	24807
150	2.951	30873
200	3.935	40434
Correlation Coefficient:		0.999
Slope :		9179
Intercept:		376.2
%Y-intercept:		1.64

TABLE 10: LINEARITY OF DETECTOR RESPONSE IMPURITY-C

Levels (%)	Concentration (µg/ml)	Area response of Impurity-C
LOQ	0.41	6508
50	2.09	29187
100	4.18	58774
125	8.36	116907
150	10.45	146176
200	12.53	174538
Correlation Coefficient:		0.999
Slope :		9179
Intercept:		376.2
%Y-intercept:		1.64

TABLE 11: LINEARITY OF DETECTOR RESPONSE IMPURITY-D

Levels (%)	Concentration (µg/ml)	Area response of Impurity-C
LOQ	1.84	8126
50	2.77	32050
100	5.54	64863
125	11.08	131215
150	13.84	162631
200	16.61	193074
Correlation Coefficient:		0.999
Slope:		9179
Intercept:		376.2
% Y-intercept:		1.64

TABLE 12: LINEARITY OF DETECTOR RESPONSE BILASTINE

Levels (%)	Concentration (µg/ml)	Area response of Bilastine
LOQ	0.808	10519
50	1.551	18898
100	3.101	35811
125	3.877	44184
150	4.652	53004
200	6.203	70799
Correlation Coefficient:		0.999
Slope:		9179
Intercept:		376.2
% Y-intercept:		1.64

The method for the estimation of Impurity-A, B, C and Bilastine was found to be linear and the correlation coefficient was found to be 0.999, 1.000, 1.000, 1.000 and 1.000. The method was found to be linear in the range of LOQ to 200%.

Accuracy: Recovery of Bilastine impurities in Bilastine was performed. The sample was taken and varying amounts of Bilastine impurities representing 50 to 150% of specification level were added to the flasks. The spiked samples were prepared as per the method and the results are tabulated in **Table 13.**

TABLE 13: ACCURACY STUDY OF BILASTINE

S. no.	Theoretical (%)	% Mean Recovery			
		Impurity-A	Impurity-B	Impurity-C	Impurity-D
1	50	104.1	102.95	97.23	96.40
2	100	110.0	104.18	91.88	92.24
3	150	98.0	106.79	95.60	95.21

SUMMARY: A simple, accurate and reproducible reverse phase UPLC method was developed for the estimation of Bilastine in bulk drugs and formulations. The optimized method consists of 0.05% TFA in water and 0.05%TFA in acetonitrilein gradient elution mode with a run time of 10 min and a flow rate of 0.1 ml/min. UV detection was carried out at a wavelength of 275 nm with an injection volume of 5.0 µL. Acquity UPLC CSH Phenyl-hexyl (2.1 mm × 15 mm, 1.7µ). The retention time of Bilastine was found to be 7.6 minutes. The developed method was validated as per ICH Q2A (R1) guideline. The proposed UPLC method was linear over the range of LOQ-200% level for impurities and analyte peak, the correlation coefficient was found to be 0.999 and 1.000 for all impurities and analyte peak. Recovery of the impurities and Bilastine was found to be within the limit 85-115%.

The relative standard deviation for system precision was found to be 0.54. Method precision was found to be below 2.0% impurities and analyte peak of Bilastine. Limit of Detection was found to be 0.16 µg/ml for impurity-A, 0.16 µg/ml for impurity-B, 0.16 µg/ml for impurity-C and 0.17 µg/ml for Bilastine and Limit of Quantification was found to be 0.53 µg/ml for impurity-A, 0.52 µg/ml for impurity-B, 0.54 µg/ml for impurity-C and 0.55 µg/ml for Bilastine respectively. The method developed was statistically validated in terms of selectivity, accuracy, linearity, precision and robustness. For Selectivity, the chromatograms were recorded for standard and sample solutions of Bilastine and its related substances. Selectivity studies reveal that the peak is well separated from each other.

CONCLUSION: The new UPLC method developed and validated for determination of related substances of Bilastine pharmaceutical dosage forms and assured the satisfactory precision and accuracy and also determining the lower concentration of impurities in its solid dosage form by RP-UPLC method. The method was found to be simple, accurate, linear, robust, rapid and they can be applied for routine analysis in laboratories and is suitable for the quality control of the raw materials, formulations and can be employed for bio-equivalence studies for the same formulation.

ACKNOWLEDGEMENT: The authors wish to thank Dr Reddy Laboratories for providing the analytical support to pursue this work.

CONFLICTS OF INTEREST: The authors declare no conflict of interest

REFERENCES:

1. Zuberbier T, Oanta A, Bogacka E, Medina I, Wesel F and Uhl P: Comparison of the efficacy and safety of Bilastine 20 mg *vs*. levocetirizine 5 mg for the treatment of chronic idiopathic urticaria: a multi-center, double-blind,

randomized, placebo-controlled study. Allergy 2010; 65(4): 516-28.
2. Scaglionte F: Safety profile of Bilastine: 2nd generation H1-antihistamines. Eur Rev Med Pharmacol Sci 2012; 16(14): 1999-05.
3. Berrueta LA, Fernandez-Armentia M, Bakkali A, Gonzalo A, Lucero ML and Orjales A: Matrix solid-phase dispersion technique for the determination of a new antiallergic drug, Bilastine, in rat faeces. J Chromatogr B 2001; 760: 185-90.
4. Lasseter KC, Sologuren A, La Noce A and Dilzer SC: Evaluation of the single-dose pharmacokinetics of Bilastine in subjects with various degrees of renal insufficiency. Clin Drug Investi 2013; 33: 665-73.
5. Terzic J, Popovic I, Stajic A, Tumpa A and Jancic-Stojanovic B: Application of analytical quality by design concept for bilastine and its degradation impurities determination by hydrophilic interaction liquid chromatographic method. J Pharm Biomed Anal 2016; 125: 385-93.
6. Silva ATD, Brabo GR, Marques ID, Bajerski L, Malesuik MD and Paim CS: UV Spectrophotometric method for quantitative determination of Bilastine using experimental design for robustness Drug Anal Res 2017; 01(2): 38-43
7. ICH. Harmonised Tripartide Guideline: Validation of Analytical Procedure: Text and Methodology Q2 (R1), 2005 17.pdf, acessado em Abril de 2017.
8. The United States Pharmacopeia, 38th ed., United States Pharmacopeial Convention: Rockville, 2015.
9. Heyden YV, Nijhuis A, Smeyers-Verbeke J, Vandeginste BGM and Massart DL: Guidance for robustness/ ruggedness tests in method validation. J Pharm Biomed Anal 2001; 24: 723-53.

How to cite this article:
Katta R, Murty NNVVSSN, Ramasrinivas and Rao GN: Stability indicating method development and validation for the determination of bilastine and its impurities by UPLC method. Int J Pharm Sci & Res 2020; 11(3): 1312-21. doi: 10.13040/IJPSR.0975-8232.11(3).1312-21.