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Advances in Bioresearch

ORIGINAL ARTICLE

A Validated Stability Indicating HPTLC Method for Estimation of Bilastine in Bulk and Tablet Dosage Form and Characterization of Impurities by LC-MS Method

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ABSTRACT

A sensitive, accurate and precise high-performance thin layer chromatographic method was developed for simultaneous estimation of Bilastine/BILAS in bulk and tablet dosage form. The successful separation was achieved on CAMAG HPTLC. The stationary phase and n-Hexane: Methanol: Iso Propyl Alcohol (1:5:4 v/v/v) as mobile phase. Chromatographic analysis was carried out in the reflectance/absorbance mode at 277 nm. The method was validated with respect to linearity, specificity, accuracy, precision, limit of detection and limit of quantitation and applied for analysis of drug in tablet dosage form. The Rf values were found to be 0.53 ± 0.02 for selected drug. The linear regression analysis this for the calibration plots showed a linear relationship in the concentration range 500-2500 ng/band with correlation coefficient 0.997. A validation study has been performed as per ICH guidelines. Functional groups was determined using Fourier transform Infrared spectroscopy (JASCO FTIR—4700). Potassium bromide (KBr) pellet was made and the sample was ground along with potassium bromide. For the characterization of degradation product using LC-MS/MS, For LC-MS study Inertsil C18 column (150 mm length x 2.1 mm , 3 µm particle size) and the mobile phase comprised of 0.1% formic acid and methanol (50:50 v/v) pumped at rate of 0.2 mL/min. was employed for chromatographic separation of Bilastine and its degradation products. The injection volume of drug was 10 µL and temperature of column was 40 °C.

KEYWORDS: *Bilastine, HPTLC, FT-IR, Mass spectrometry, Stress degradation.*

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INTRODUCTION

Bilastine/BILAS is highly non-sedating antihistamine indicated for the symptomatic treatment of allergic rhinoconjuctivities and urticaria[1]. Bilastine is novel ,second generation H1 receptor inhibitor chemically known as 2-[4-(2-{4-[1-(2-ethoxyethyl)-1H-1,3-benzodiazol-2-yl]piperidin-1-yl}ethyl)phenyl]-2-methylpropanoic acid[2] .It is highly selective for the H1 histamine receptor, has a rapid onset and prolonged duration of action. Molecular formula is C28H37N3O3 and molecular weight 463.61g/mol[2]. This plays important role in quality assurance of the product throughout its shelf life. The reason for selection of this method is because there is not much this available on HPTLC of Bilastine. Also drug was subjected to Fourier –Transform Infrared Spectroscopy for finding the functional groups present. Degradation product and the drug were characterized by using LC-MS/MS using Inertsil C18 column (150 mm length x 2.1 mm , 3 µm particle size) was employed for chromatographic separation of Bilastine and its degradation products. The injection volume of drug was 10 µL and temperature of column was 40 °C.
This method was adapted for the study of the selected drug because not much literature is available on this drug using HPTLC method, also this method includes stress degradation and further characterization of the impurities using LC-MS technique. Also the method incorporated is novel, accurate, precise and reproducible. This method is cost effective as compared to other reported methods and also it is less time consuming.

MATERIAL AND METHODS

HPTLC system-(Make: Camag, Model: CHF47150) Sample Applicator-CamagLinomat V Pressure required 3.5 bar, Syringe (Camag 100 µL), UV – Lamp (D2 & W), TLC Scanner (Camag TLC scanner III). WinCAT's software was used to obtain the this, FT-IR (Jasco FTIR-4700)

Bilastine drug was gifted by Symed Labs Ltd, Hyderabad, India. All other solvents and reagents were purchased from Merck Specialties Pvt. Ltd. Mumbai, India and were of analytical grade**.**

Chromatographic Condition

Chromatographic condition includes an Aluminium plates pre-coated with silica gel 60 F254 Merck as a stationary phase, Plate size of a 10 cm X 10 cm with a thickness of 200 µm maintained at room temperature (25 ± 5OC), with a mobile phase n-Hexane: Methanol: Iso propyl Alcohol 1:5:4% v/v/v. All the samples were applied and were detected at 277 nm.

Preparation of Standard stock solution

20 mg of BILAS was accurately weighed and 10 mL of methanol was added in a volumetric flask and sonicated for 10 minutes to remove the dissolve gas and the volume was made up by the methanol to 100ml (2000µg/ml).

Selection of Mobile Phase

Aliquot portions of standard stock solutions were applied on TLC plates in the form of band (band size: 6mm).After trying several combinations, the solvent system containing n-Hexane: Methanol: Iso- propyl Alcohol (1:5:4 v/v/v) was found to be giving good resolution.

Selection of Analytical Wavelength:

Standard stock solution was applied on TLC plate by CAMAG LINOMAT-V automatic sample applicator. The separated bands on the TLC plate were scanned between the wavelength ranges of 200-400 nm. The wavelength 277 nm was selected for Densitometric evaluation of separated bands.

Mass spectrometry conditions

The optimization of the instrument includes tuning of detector and electron spray ionization in a positive mode. 10 µg/mL of Bilastine in methanol was submitted to MS system in positive mode of ESI in the mass range of 100-540 Da. The instrument settings were applied as follows: de-clustering potential of 90 eV, entrance potential of 10 eV, cell exit potential of 10 eV and collision energy of 50 eV.

RESULT AND DISCUSSION

This article provides figures and this results from analysis by HPTLC system and mass spectrometry. Fig 2 reports the Spectra of Bilastine using Wincat's software and Fig.3 shows overly spectra of pure drug and tablet dosage form. Table 1 reports result of linearity studies and this of LOD and LOQ. Fig 4 describes the this of standard chromatogram of Bilastine. Table 2 describes the results of accuracy and other statistical values. Table 3 gives attributes related to the robustness results of Bilastine. Fig 5 shows the linearity plot of the drug. Fig 6 and fig 7 indicates the 2D and 3D spectra of the drug respectively. Table 4 gives the analysis of tablet formulation. Fig 8 shows acidic stress degradation indicating 5.52 %degradation. Fig 9 gives this on alkaline stress degradation that was found to be 3.28%. Fig 10 gives this on oxidative degradation which showed 11.08% degradation and fig 11 gives this on extended oxidative stress condition. Fig 12, fig 13 and fig 14 indicates this on thermal, photolytic and hydrolytic stress conditions respectively. Table 5 shows this of results of stress degradation conditions and table 6 indicates extended oxidation stress condition. Fig 15 gives this of mass spectrum of API and Fig 16 shows Mass spectrum of degradation product by hydrogen peroxide. Fig 17 reports probable fragmentation pattern of Bilastine. Table 7 reports this on LC-MS this of Bilastine and its degradation products and their major fragments. Table 8 reports this on Molecular formula of Degradant of Oxidative degradation. Fig 18 gives this on FT-IR spectra of Bilastine. Table 9 gives report on frequencies by FT-IR spectrum.

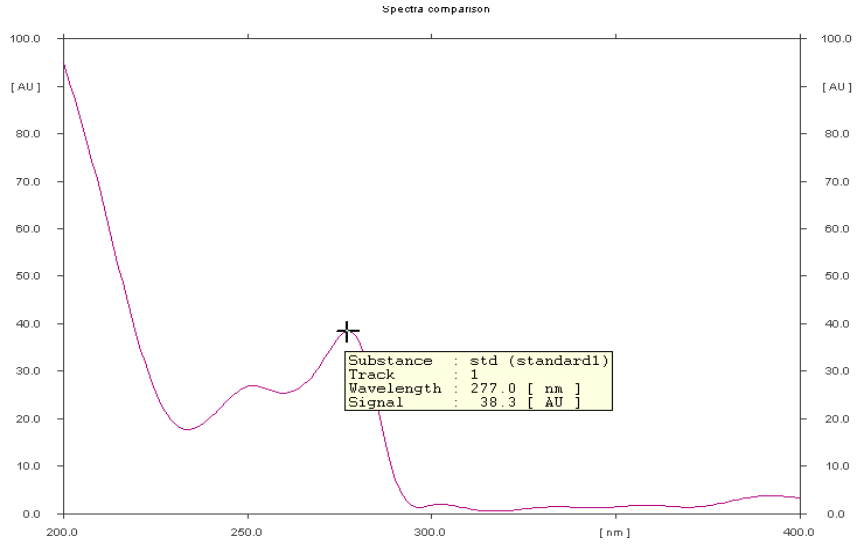

Fig .1:.Spectra of drug

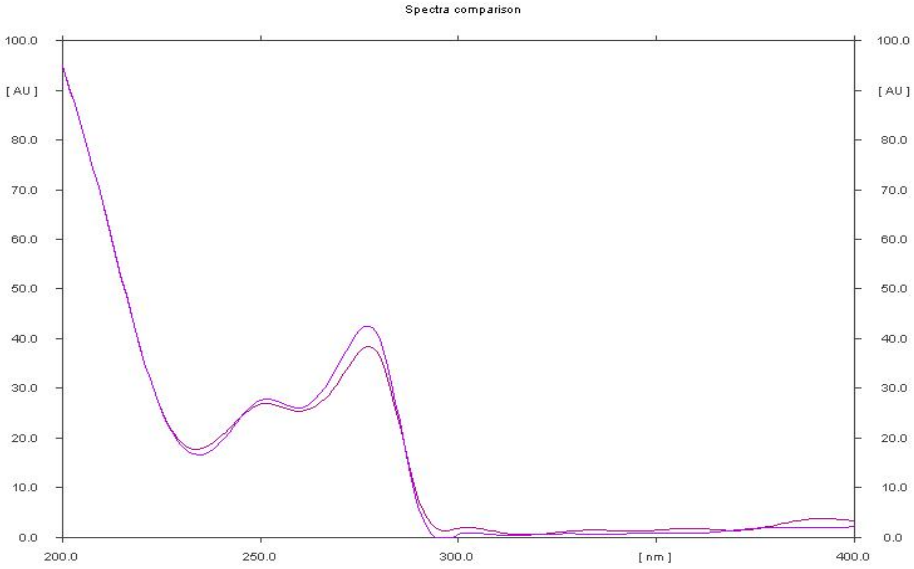

Fig .2: Overly spectra Standard + Sample

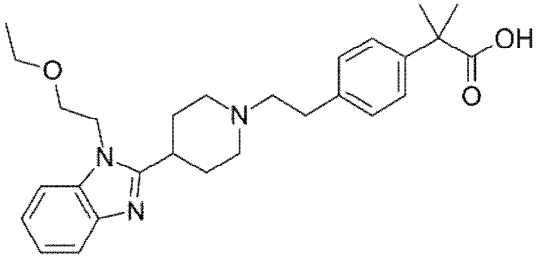

Fig .3: Structure of Bilastine

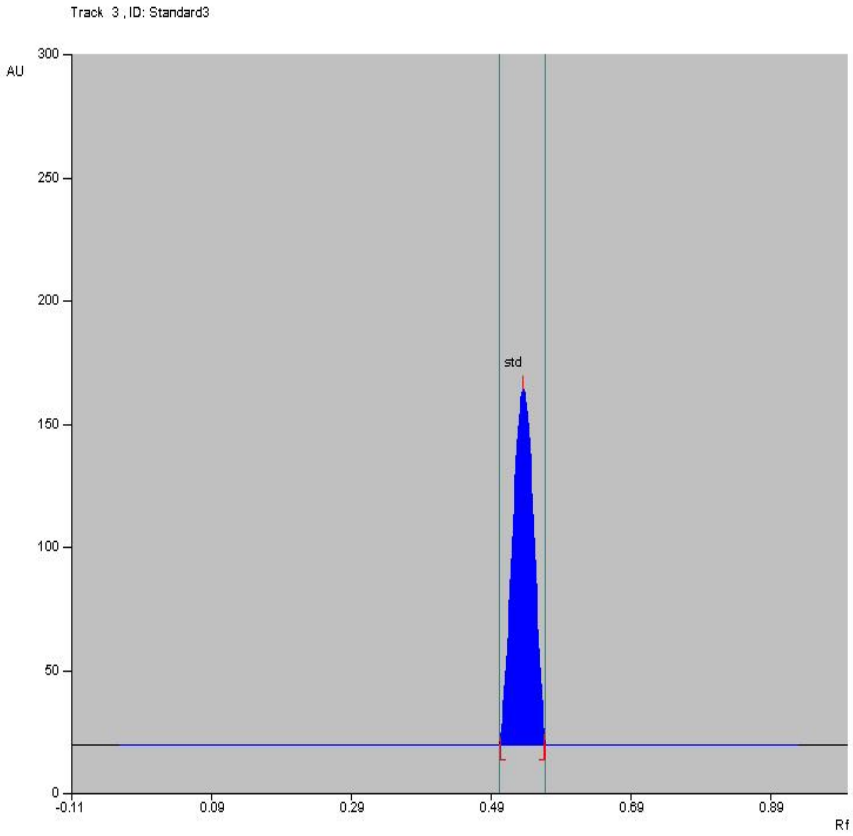

Fig .4: Standard chromatogram of Bilastine

Table no. :1 Linearity this, LOD and LOQ

Drug (Bilastine)	Concentration	Area	Rf
Linearity	50	1522.64	0.53
	100	2806.68	0.53
	150	3510.72	0.53
	200	4555.31	0.53
	250	5802.53	0.53
Linearity range	500-2500ng/band		
Regression equation	Y=555.839 + 2.568x		
Correlation coefficient	R2 = 0.9977		
Slope	2.568		
y- intercept	555.839		
LOD	1.53ng/band		
LOQ	6.43 ng/band		
Repeatability	**%Mean**	**SD**	**RSD**
	99.39	0.72	0.71
Intermediate Precision	**%Mean**	**SD**	**RSD**
	99.28	0.78	0.78

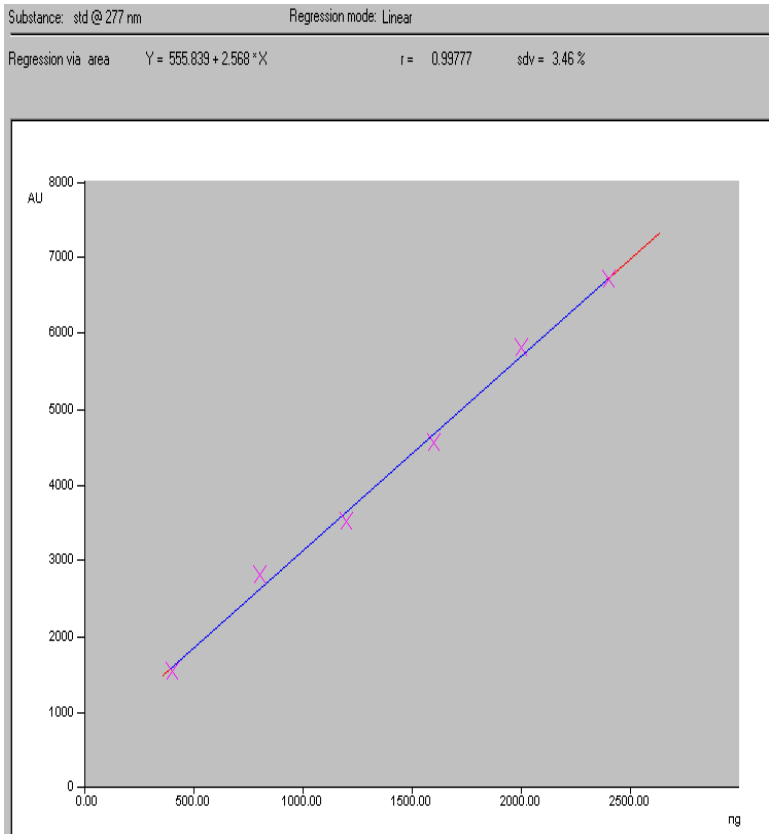

Fig.5: Linearity plot of Bilastine

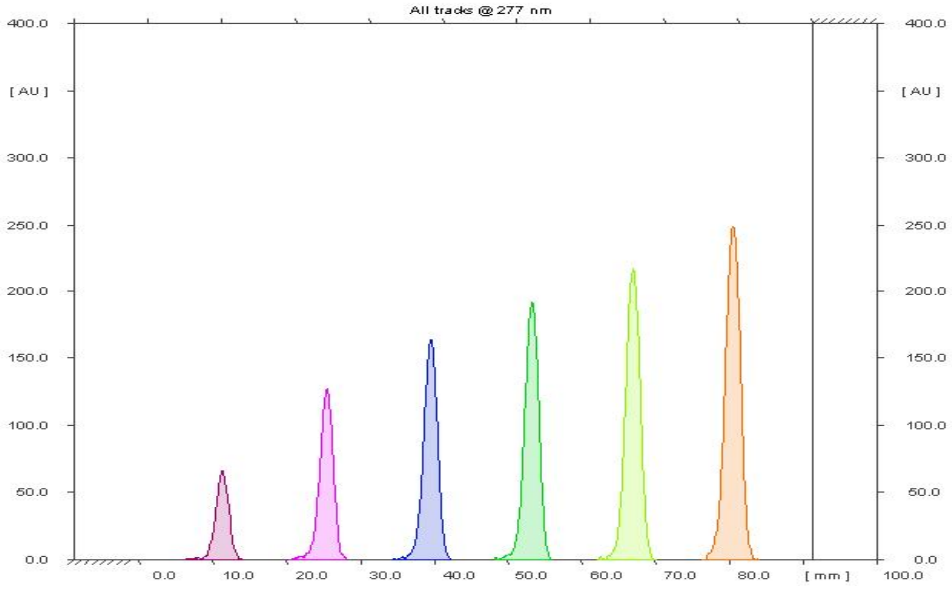

Fig.6: 2D Spectra of Bilastine using Wincat's software

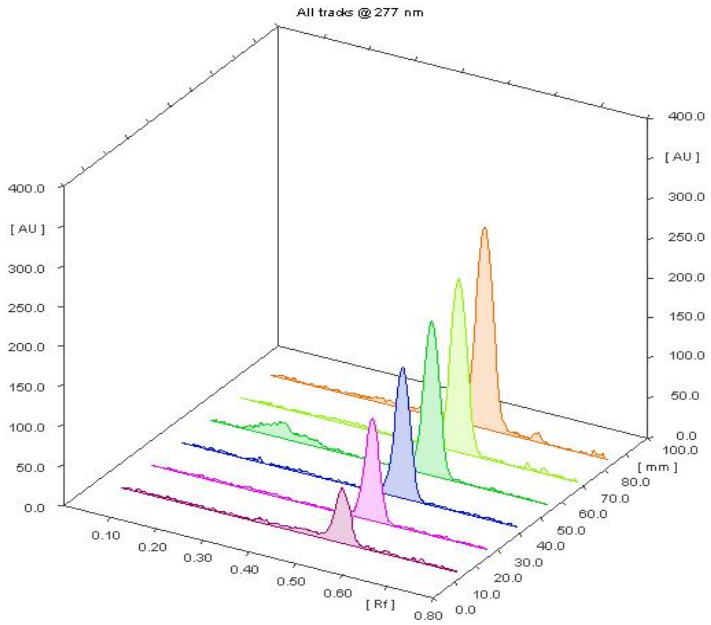

Fig.7:3D Spectra of Bilastine using Wincat's software

Table no. 2: Results of Accuracy and statistical values

Conc. Level (%)	Weight of tablet powder taken(mg)	Amount of drug added(mg)	Amount of drug recovered(mg)	% Recovery
80	160	16	16.24	101.5
	160	16	15.67	97.93
	160	16	15.70	98.12
100	160	20	20.24	101.2
	160	20	19.98	99.9
	160	**20**	**19.90**	**99.5**
120	**160**	**24**	**24.20**	**100.83**
	160	**24**	**23.97**	**99.87**
	160	**24**	**23.99**	**99.95**
Mean	**99.86**			
SD	**1.24**			
%RSD	**1.24**			

Table no. 3: Robustness results of Bilastine

Factor	Chromatographic Conditions		
Duration of chamber(±5 min)	Level	Peak Area	Rf values
5min	-5	3452.8	0.52
10min	0	3510.96	0.53
15min	+5	3646.73	0.55
	%RSD	0.55	
Amount of mobile phase(±1ml)			
09	-1	3446.68	0.44
10	0	3522.11	0.53
11	+1	3636.03	0.62
	%RSD	0.54	
Mobile phase composition±1ml			
2:4:4	±1ml	3477.74	0.52
1:6:3	0	3659.21	0.54
1:4:5	±1ml	3511.36	0.53
	%RSD	1.01	

Table no.4: Results of analysis of tablet formulation

Brand: Bilashine 20mg **Each film coated tablet contains 20 mg Bilastine**			
Sr. no.	Weight of tablet powder taken(mg)	Amount of pure drug estimated(mg)	% Label claim
1.	160	19.97	99.85
2.	160	20.2	101
3.	160	19.99	99.95
4.	160	20.3	101.5
5.	160	20.1	100.5
6.	160	20.2	101
Mean % Label claim	100.63%		
SD	0.650		
%RSD	0.64		

Stress degradation studies

Acidic stress degradation

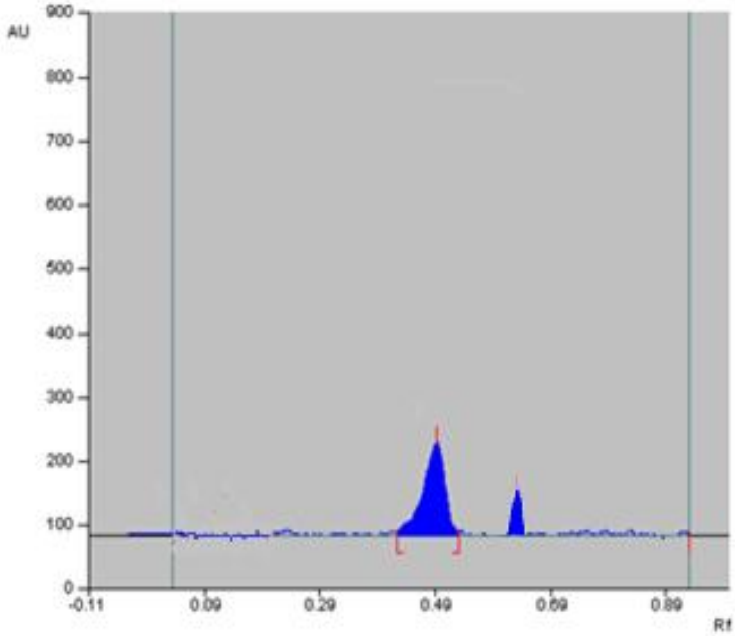

Fig .8: HPTLC densitogram of Bilastine in acidic condition

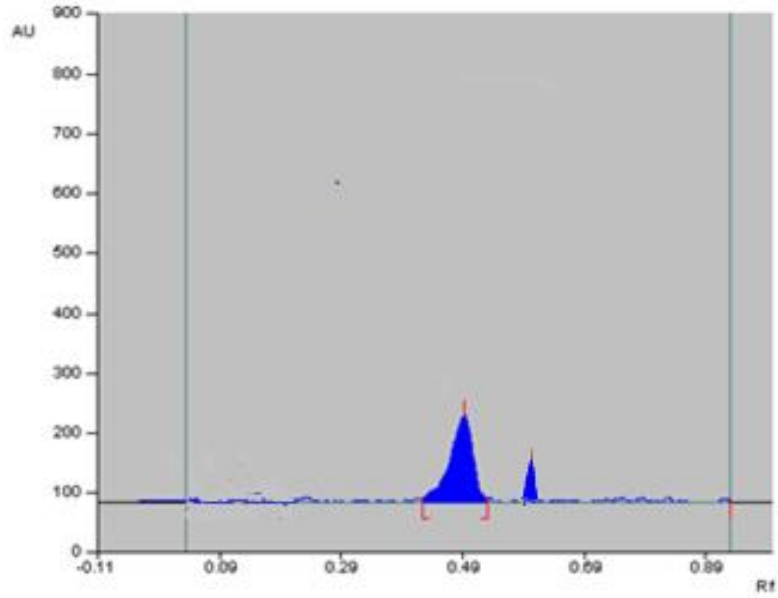

Fig. 11: HPTLC densitogram of Bilastine in alkaline condition

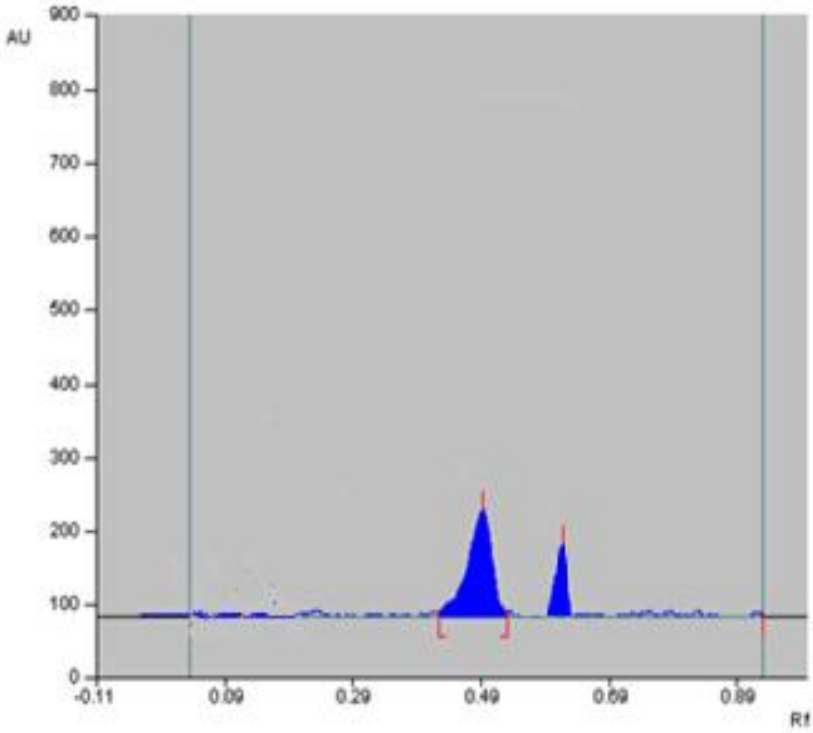

Fig.10: HPTLC densitogram of Bilastine in oxidative condition.

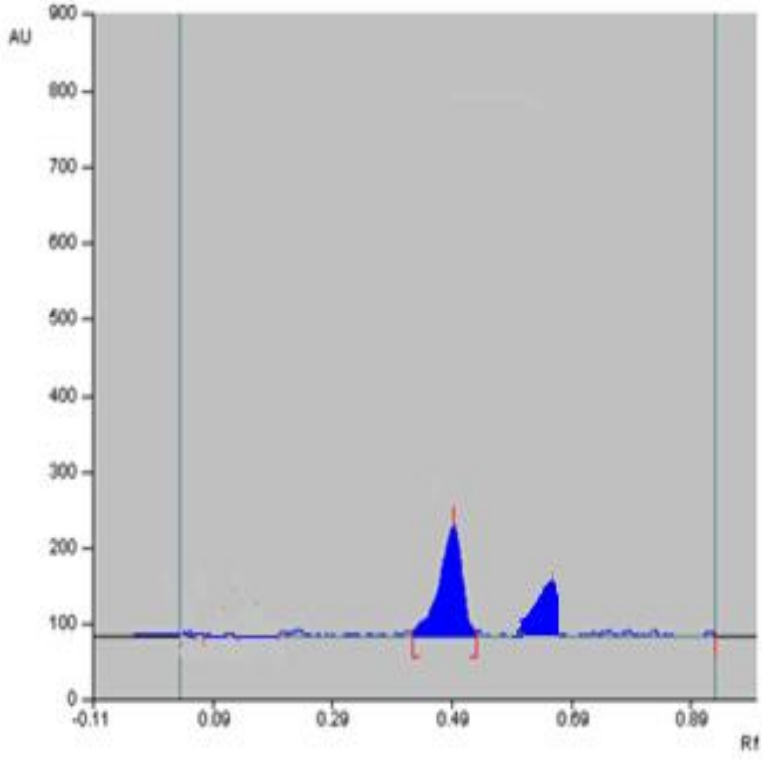

Fig .12: HPTLC densitogram of Bilastine in extended oxidative condition.

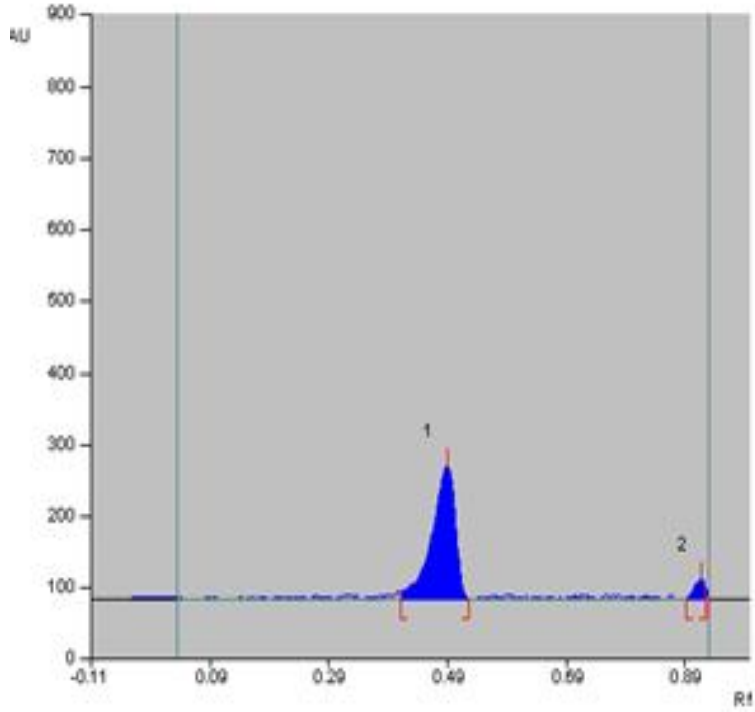

Fig.13: HPTLC densitogram of Bilastine in thermal condition.

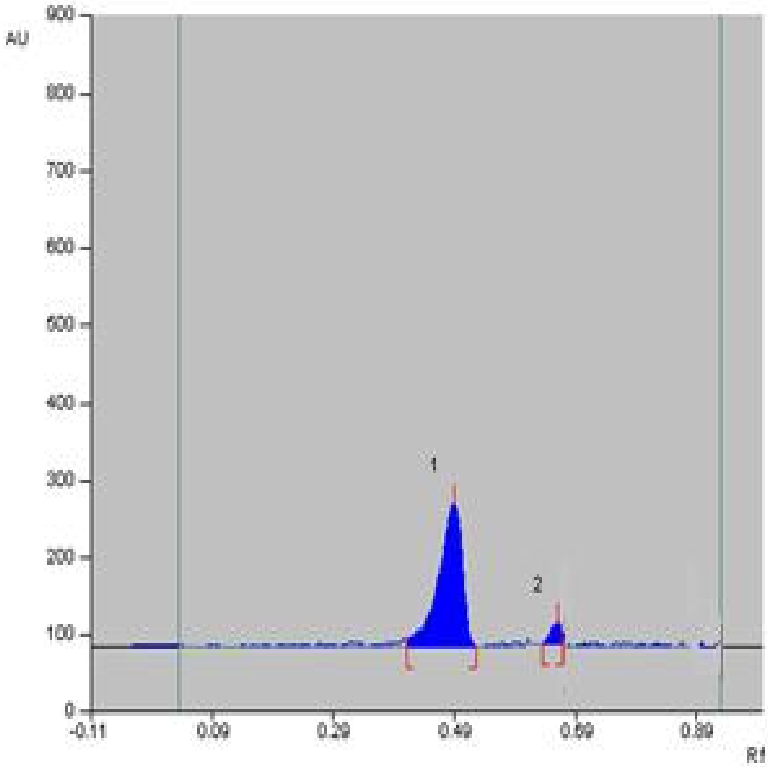

Fig.15: HPTLC densitogram of Bilastine in photolytic condition

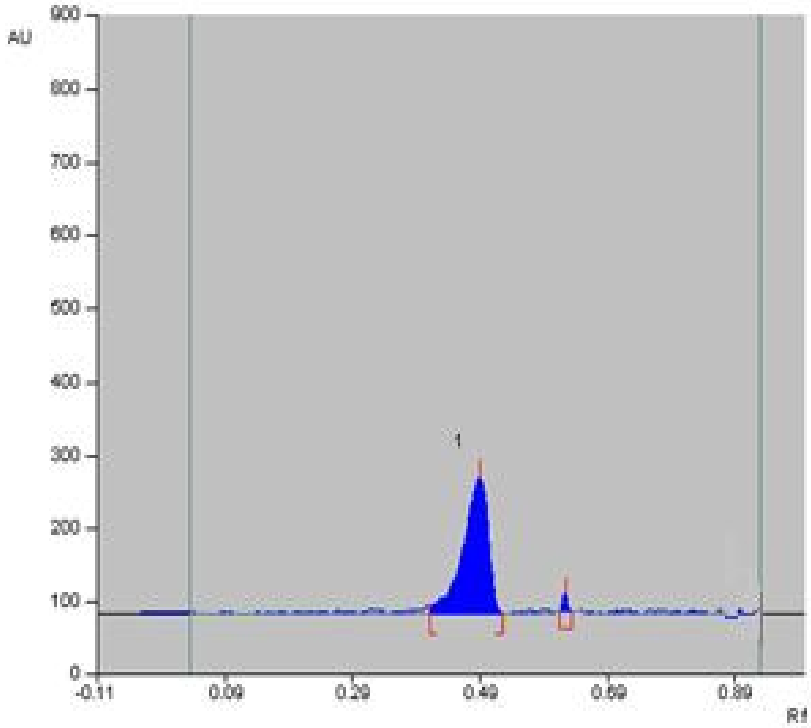

Fig.14: HPTLC densitogram of Bilastine in neutral condition.

Table no.5: The results of the stress degradation studies of Bilastine

Sr.No	Stress test Conditions	Solvents	Temp.	Time	Peak area Std area= 3597.8		% Degradation
					Standard	Degradant	
1.	Acidic	0.1N HCl	RT	1hr	3298.248	223.641	5.52
2.	Alkaline	0.1 N NaOH	RT	1hr	3325.872	189.820	3.28
3	Oxidative	3%H2O2	RT	1hr	3178.520	378.908	11.08
4.	Thermal	-	60ºC	1hr	3438.510	98.840	2.12
5.	Photolytic	-	UV Light	24 hrs	3455.328	89.357	1.64
6.	Hydrolytic	Distilled Water	RT	1hr	3468.414	83.650	1.12

Table no. 6: Extended Oxidative Stress Degradation study

Sr.No	**Stress test Conditions**	**Solvents**	**Temp.**	**Time**	**% Degradation**
1.	Oxidative	6%H2O2	80ºC	6hrs	19.06

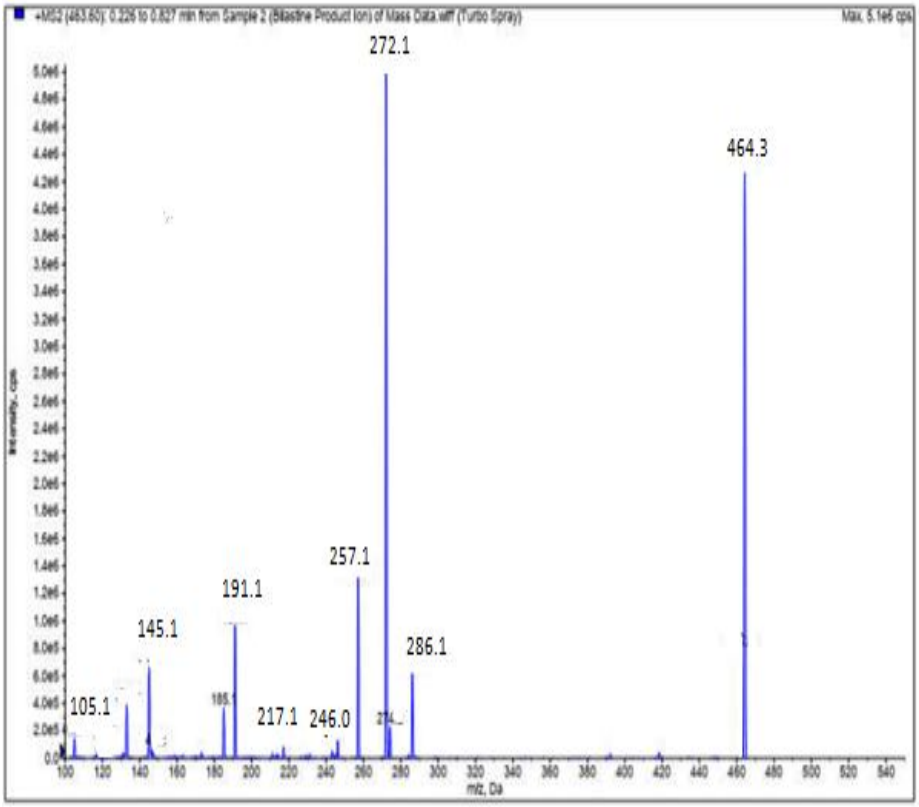

Fig.15: Mass spectrum of API

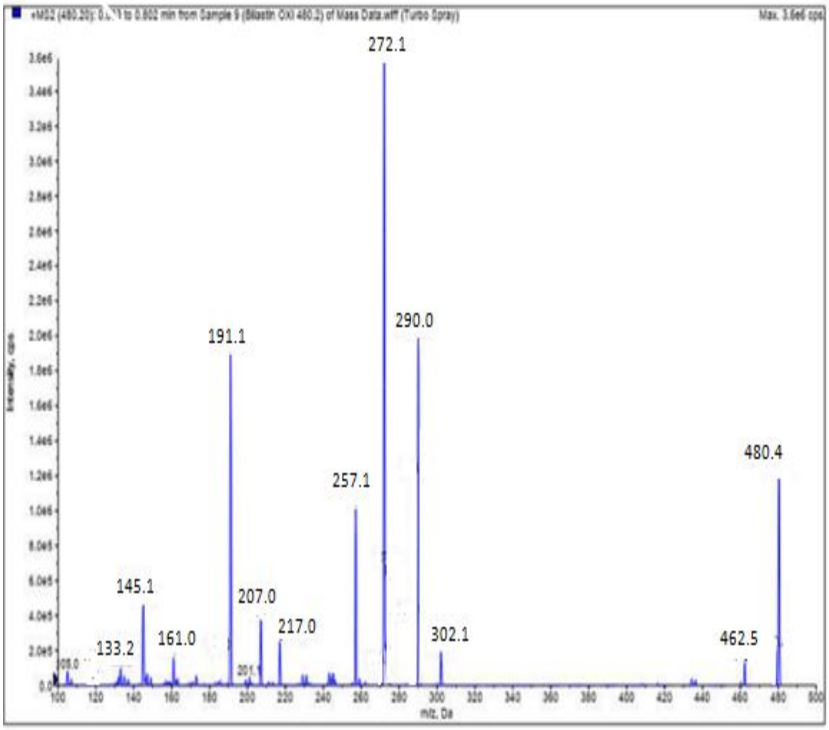

Fig .16: Mass spectrum of degradation product by hydrogen peroxide

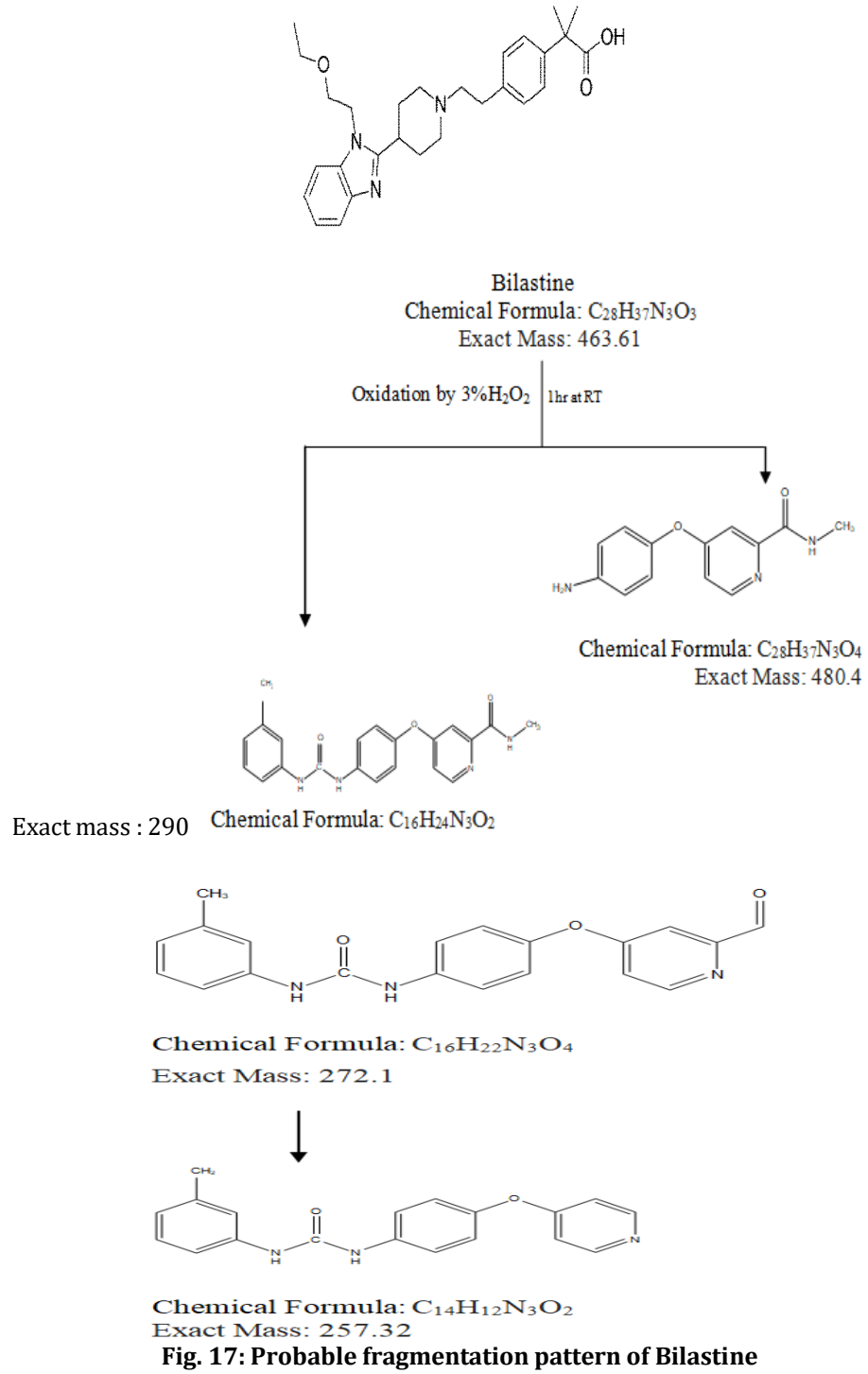

Fig. 17: Probable fragmentation pattern of Bilastine

Table no.7: LC-MS this of Bilastine and its degradation products and their major fragments

Substance	Experimental mass	Molecular formula	Major fragments
Bilastine	463.3	C28H37N3O3+	302.1(C17H24O2N3+) 290.1(C16H24O2N3+), 288.1(C17H22O2N3+), 286.1(C17H20ON3+), 272.1((C16H22ON3+), 257.1(C15H19ON3+),
Oxidation degradation product of Bilastine	480.4	C28H37N3O4 +	

Table no. 8: Molecular formula of Degradant of Oxidative degradation

Degradant No.	Molecular formula	Comment
1.	C16H24N3O3	Loss of C12H14
2.	C13H17N3O3	Loss of C15H2O
3.	C16H22N3O2	Loss of C12H16O
4.	C14H12 N3O2	Loss of C14H24O

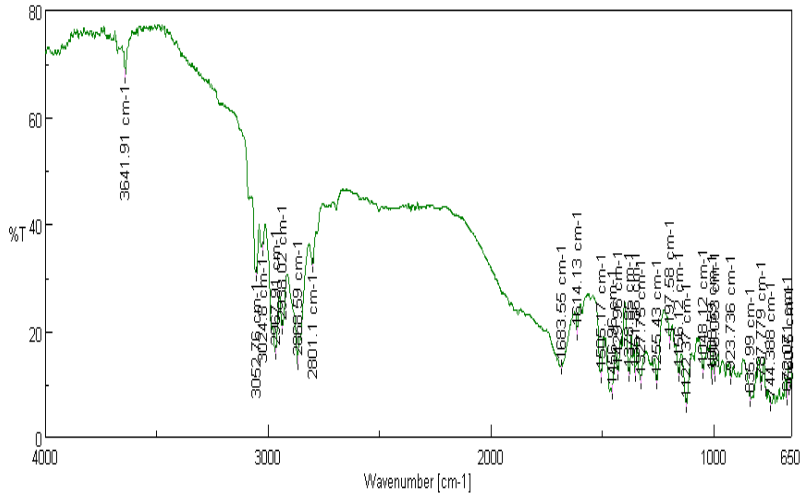

Fig.18. FT-IR Spectra of Bilastine

Table no.9: Results of FT-IR spectra

Observed frequency (cm-1)	Functional group
3641.91	O-H Stretch
2868	C-H Stretch
1225	C=O Stretch
1614.13	C=C ring Stretch
1378.85	N-H Stretch

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CONFLICT OF INTEREST

Author doesn't have any conflict of interest

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