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STABILITY INDICATING CHROMATOGRAPHIC METHOD FOR ESTIMATION OF BEPOTASTINE BESILATE

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Abstract :

A Simple, rapid, stability indicating HPTLC method has been developed for estimation of bepotastine besilate. HPTLC separation was carried out on Merck TLC aluminium sheets precoated with silica gel 60F_{254} using mobile phase as chloroform : methanol. Bepotastine besilate gave sharp peak at RF 0.53 ±0.03 at 225nm.calibration curve was linear in range 5-25ug/band for bepotastine besilate.

Stress degradation study was carried out according to ICH guidelines Q1A (R2) and the method was validated as per ICH guideline.

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INTRODUCTION:

Bepotastine is 2nd generation antihistamine. Its molecular formula is $C_{21}H_{25}ClN_2O_3$. Chemically it is benzenesulfonic acid;4-[4-[(S)-(4-chlorophenyl)-pyridin-2-ylmethoxy]piperidin-1-yl]butanoic acid as shown in fig.1.

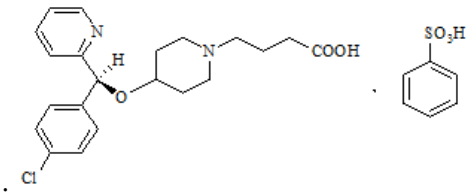

Fig.1.Structure Of Bepotastine Besilate

Molecular weight is 547.063 g/mol[1]. It is soluble in Acetonitrile and methanol. Bepotastine is not offical in IP/BP/USP. It was approved in Japan for use in the treatment of allergic rhinitis and urticaria/pruritus. It is available in ophthalmic solution and oral tablet . Its ophthalmic formulation has shown minimum systemic absorption,between 1 and 1.5% in healthy adults.It is a direct H_1 receptor antagonist that inhibits the release of histamine from mast cells. Literature survey revealed the estimation of Bepotastineby several techniques such as simultaneous estimation RP-HPLC techniques[2,3], HPLC method for Bepotastine in human plasma[4] and Study the duration of action of Bepotastine Besilate[5], Comparison study of Bepotastine Besilate[6,7].

MATERIAL AND METHOD:

Bepotastine was received as gift sample from Lupin Ltd, Aurangabad. All chemicals and reagents i.e Acetonitrile, Chloroform, Hydrochloric acid (HCL),and Hydrogen peroxied (H_2O_2), Sodium hydroxide (NaOH) were purchased from LOBA CHEMIE PVT. LTD., Mumbai. Methanol (AR grade) were purchased from S.D. Fine Chemical Laboratories, Mumbai .

Chromatographic Condition

The chromatographic separation was achived on aluminum plates precoated with silica gel $60F_{254}$ .In (10 cm × 10 cm with 250um layer thickness). Sample was applied on the plate as a band of 5 mm width using camag 100 ul sample syringe (Hamilton , Switzerland) with Linomat 5 applicator (camag Switzerland) . The mobile phase was composed of Chloroform : Methanol 5:5v/v .10 cm × 10 cm CAMAG twin trough glass chamber was used for linear ascending development of TLC plate under 15 min saturation condition and 10 ml mobile phase was used per run , migration distance was 80 mm. Densitometric scanning was performed using Camag TLC scanner 3 in the range of 400-200 nm, operated by win CATS software (version 1.4.3,Camag),slit dimensions were 3.00 × 0.45 mm and deuterium lamp was used as a radiation source.

Selection of analytical wavelength

From the standard stock solution (1000ug/ml)further dilution were done using ACN and scanned over the range of 200-400 nm, the λ_{max} was found to be 225nm, the spectrum is as shown in fig 2.

Preparation of standard stock solution

Standard stock solution of Bepotastine besilate was prepared by dissolving 100 mg of drug in 10 ml of ACN get concentration of 10,000ug/ml. It was diluted appropriately to obtain 100ug/ml solution. The resultant solution was applied on TLC plate.

Stress degradation studies [8,9]

- **Acid Hydrolysis**
  1 ml of stock solution (10,000 ug/ml) was mixed with 1 ml of 0.1N HCL and volume was made up to 10 ml with Acetonitrile. Solution was kept for 30min. A sample with 1N HCl was kept for 24hrs at RT.

- **Base Hydrolysis**
  1 ml of stock solution was mixed with 1 ml of 5 N NaOH and volume was made up to 10 ml with ACN.Solution was kept for overnight at room temperature.

- **Neutral Hydrolysis**
  1 ml of stock solution was mixed with 1 ml of H_2O which is refluxed for 2hr and volume was made up to 10 ml with ACN. Solution was kept for overnight at RT and applied on TLC plate.

- **Oxidation Hydrolysis**
  1 ml of stock solution was mixed with 1 ml of 6% H_2O_2 and volume was made up to 10 ml with ACN. Solution was kept for overnight at RT and applied on TLC plate.

- **Degradation under Dry Heat**
  Effect of dry heat was studied by keeping drug in oven at 90ºC.Sample was withdrawn after 24 hr.Weighed 10 mg and dissolved in ACN to get solution of 1000ug/ml of Bepotastine besilate.

- **Photo-degradation studies[5]**
  Photolytic studies was carried out by exposure of drug in UV light upto 200 watt hrs/square meter and subsequently to cool fluoresent light to achieve an illumination of 1.2 million Lux hrs. sample was weighed , dissolved and diluted it with methanol upto 1000ug/ml of Bepotastine besilate.

Observation

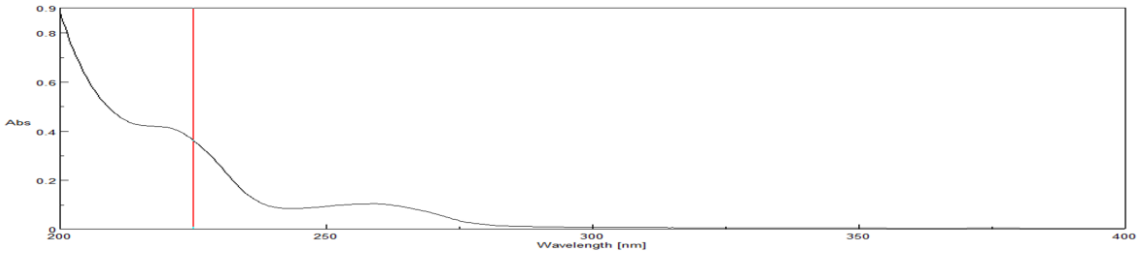

Fig.2. UV Spectrum of Bepotastine besilate (10ug/ml).

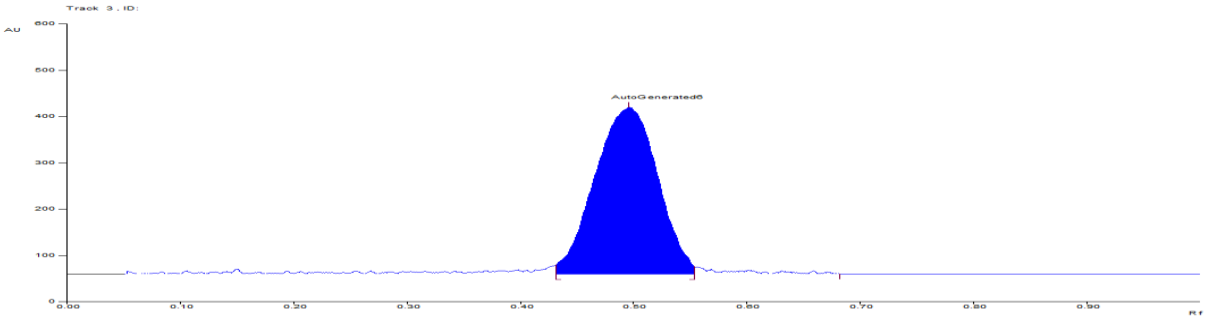

Fig.3.Densitogram of standard solution of Bepotastine besilate 10000 ng/band (Rf 0.50 ± 0.03)

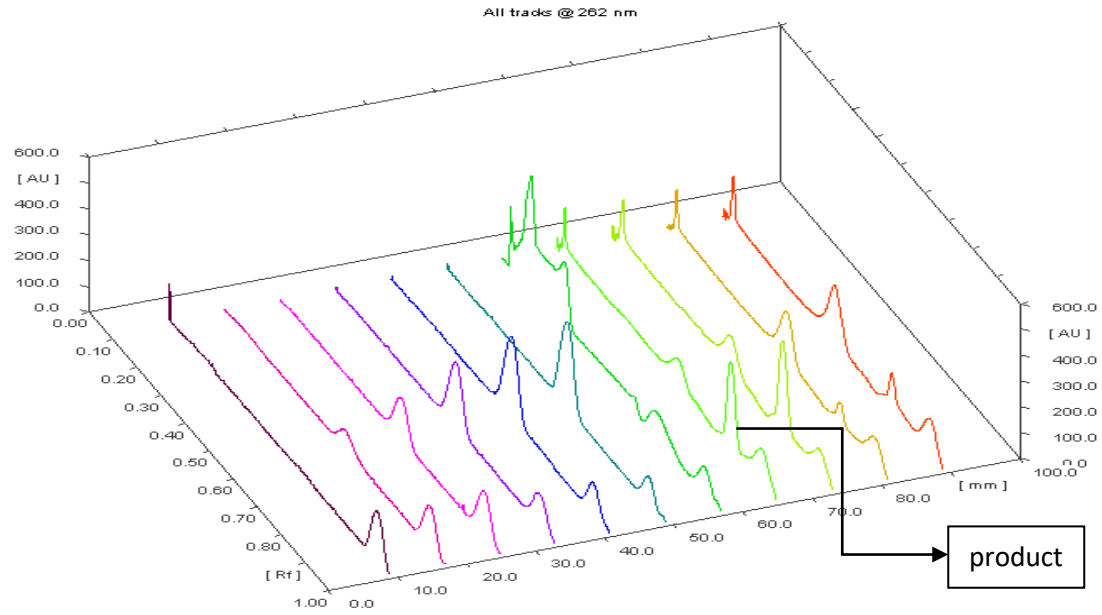

Fig.4.A. 3D Densitogram of acid hydrolysis

In above densitogram track first was blank and track 2 to 6 (5000-25000 ng/band) std linearity peak,track 8 to 11 acid treated sample. Degradation product was obtained at RF 0.78, upon keeping the solution at room temperature overnight.

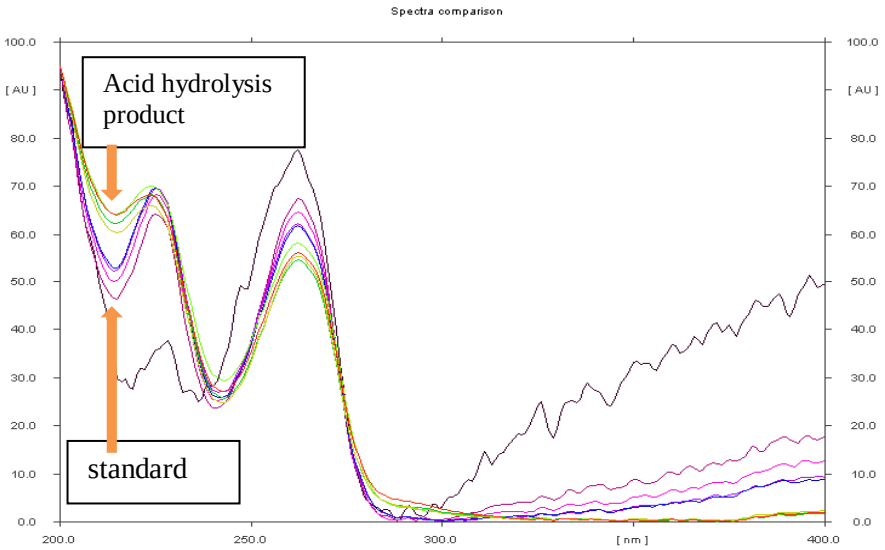

Fig.4.B. 3D spectral comparison of acid hydrolysis product and std

In Fig 4.B Spectrum of acid catalysed degradation product at RF 0.78 and std RF was obtained at 0.56 ± 0.03. Acid degradation was confirmed using HPLC method reverse phase column, Nucleosile C_{18}, was used. The column temperature was maintained at 45ºC. The mobile phase was ACN : MeOH : H_2O [0.1ml H_3PO_4 in100ml H_2O adjust the pH 3 with triethylamine] 70 : 20 : 10 v/v/v, flow rate 1ml/min and a detection wavelength was 225nm

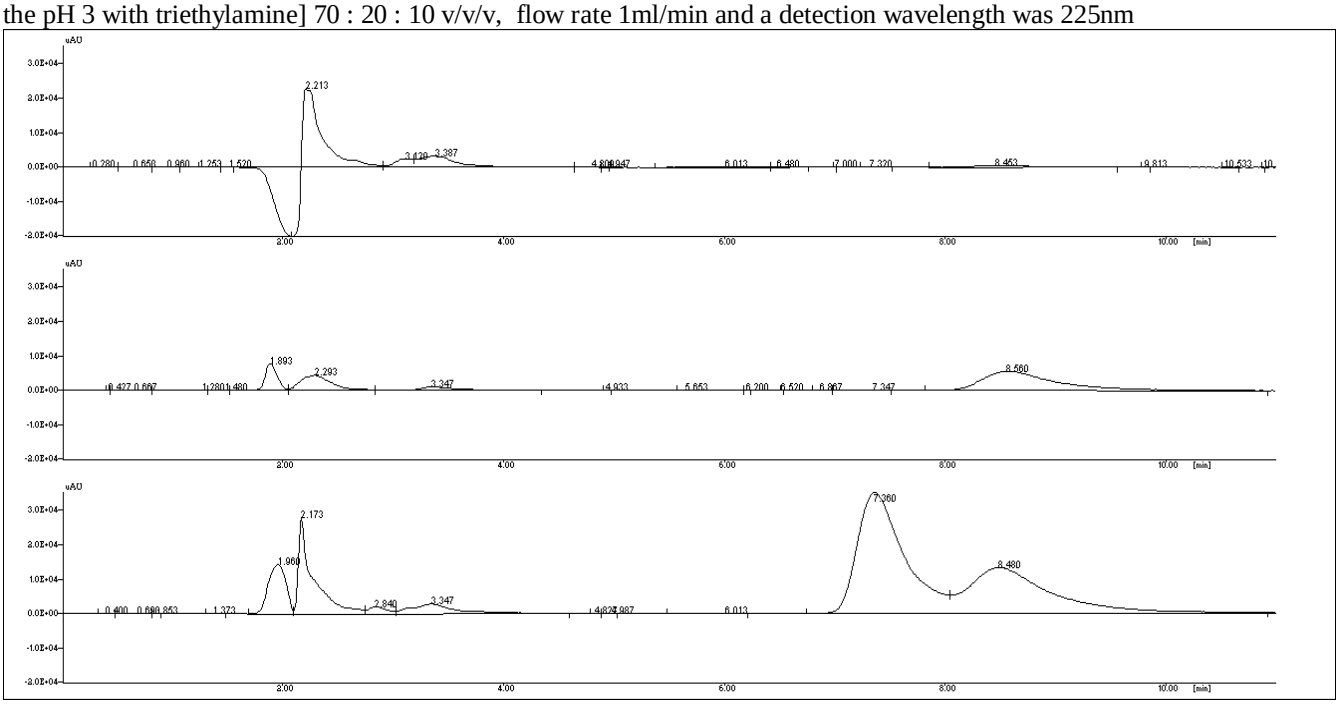

Fig.4 C: Typical chromatogram of a) blank, b) std (Bepo at Rt 8.560min), c)acid degradation sample (Product at Rt 7.360 min, Bepo at Rt 8.480)

- **Result of forced degradation studies**

The optimized stress degradation condition was obtained within ranged 10-30%. As shown in table

Table.1. : Summary of stress degradation of Bepotastine besilate

Stress degradation condition At 225nm	Bepotastine besilate		Peak purity at 225nm	
	Percent Recovery(%)	Percent Degradad(%)	r(s,m)	r(m,e)
Acid 1. (1N HCL,Overnight)	**26.3%**	**73.7%**	**0.9992**	**0.9983**
2. (0.1N HCL, 30 min.)	**76.93%**	**23.07%**	**0.9998**	**0.9993**
Base (5N NaOH, Overnight)	**83%**	**17%**	**0.9995**	**0.9998**
Oxidation(6%H_2O_2,Oernight)	**77%**	**23%**	**0.9993**	**0.9989**
Dry heat(90º 24hr)	**87.86%**	**12.14%**	**0.9998**	**0.9998**
Photo stability UV , 200 watt hrs/square meter	**More than100%**	-	**0.9988**	**0.9983**
Florescence , 1.2 million Lux.Hrs	**More than100%**	-	**0.9984**	**0.9986**
Neutral	**101.5%**		**0.9996**	**0.9998**

- **Validation of Analytical method[10]**

The validated method for various parameter according with ICH Q2 (R1) guidelines

Specificity

The specificity of the method was ascertained by peak purity profiling studies.The peak purity values were found to be more than 0.9900, indicating the non-interference of any other peak of degradation product or impurity.

Linearity

Linear relation between amount spotted vs peak area was obtained in the range of 5000-25000ng/band of Bepotastine besilate. The equation was found to be y=1024.1x+5610 for bepotastine besilate and also coefficient of correlation was found to be 0.9822.

Range

Bepotastine besilate = 5000-25000ng/band

Assay

Assay was performed on marketed formulation. Assay was determined by extrapolation of peak area from linearity equation which was found to be 106.25 % for Bepotastine besilate.

Accuracy

To check accuracy of the method, recovery studies were carried out by using marketed formulation to which standard was added at three different levels 80, 100, 120%. The drug concentration were calculated from respective linearity equation. The result of the recovery studies indicated that the method is accurate for estimation of drug in the blend. The result obtained is shown in.table 2.

Table 2: Recovery studies for Bepotastine besilate

Level (%)	% Recovery
80	101.68
100	100.97
120	101.06

Precision

The precision of method was demonstrated by intraday and interday variation studies. The result obtained for intraday and interday variation as shown in table

Table 3: Intra-day precision

Sr.no.	Amount (ng/band)	Area	Mean	SD	%RSD
1	10000	17065.8			
2	10000	17373.2			
3	10000	17251.0			
4	10000	17479.5	17386.45	224.47	1.291
5	10000	17734.5			
6	10000	17414.7			

Table 4: Inter-day precision

Sr.no.	Amount (ng/band)	Area	Mean	SD	%RSD
1	10000	17788.3			
2	10000	18017.4			
3	10000	17317.4			
4	10000	17467.9	17572.53	367.80	2.093
5	10000	17028.0			
6	10000	17816.2			

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD andLOQ were calculated as 0.78ng/band and 2.36 ng/band, respectively.

Robustness

Robustness of the method was deermined by carrying out the analysis under conditions during which chamber saturation time were altered, Time was also changed from spotting to development and development to scanning and the effects on the peak area was noted

Table.5. Summary of validation parameter

Sr.no	Parameter	Result
1	**Linearity**	y=1024.1x+5610 R^2 = 0.9822
2	**Rang**	5000-25000ng/band
3	**Precision**	**%RSD**
	Inter-day	2.093%
	Intra-day	1.291%
4	**Assay**	106.25**%**
5	**LOD**	0.78ng/band
6	**LOQ**	2.36ng/band
7	**Accuracy**	101.23
8	**Specificity**	Specific
9	**Robustness**	Robust

DISCUSSION:

The objective of work was to develop stability indicating HPTLC Method. In current work we have used Chloroform : Methanol 5:5v/v as mobile phase. So development method is simple and rapid. HPTLC has inherent advantage of high throughput.

CONCLUSION:

There are two research papers available in literature, for stress degradation of Bepotastine Besilate. The results mentioned in these papers do not match. So it was considered necessary to confirm the sensitivity of Bepotastine to different stress conditions. We have observed that Bepotastine is more sensitive to acid catalysed hydrolysis with a product of degradation getting well resolved. The developed method may be used for monitoring stability of Bepotastine Besilate

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