Method Development and Validation of new RP-HPLC Method for the Estimation of Bepotastine in Pharmaceutical Dosage Form

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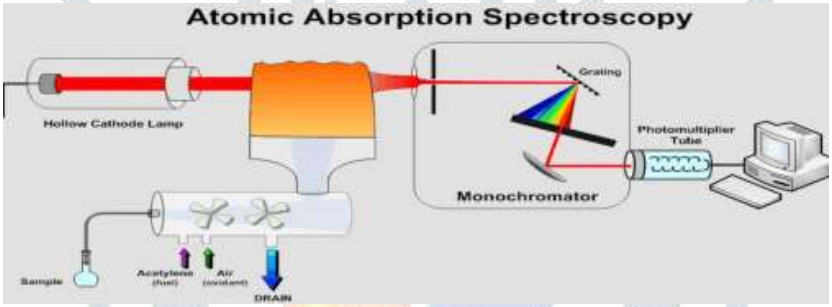
ABSTRACT
Validation is defined as "Establishing documented evidence which provide high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality characteristic. Performed a simple, specific and precise high performance thin layer chromatographic method and validated for estimation of Bepotastine in bulk drug and in ophthalmic solution. The chromatographic development was carried out on Inertsil ODS C18 (250×4.5 5µ) column using a mixture of Methanol: Acetonitrile (60:40)as mobile phase by Isocratic RP-HPLC technique. Bepotastine was found to shown appreciable absorbance at 254nm when determined spectrophotometrically and hence it was selected as the detection wavelength**.** System suitability was assessed by injecting 100µg/ml of Bepotastine standard concentration. The chromatograms confirm the presence of Bepotastine at 2.8 min without any interference. Number of theoretical plates was less than 2000 for the drug and tailing factor was less than Bepotastine standard. A Resolution of greater than 2 was observed. Concentration range of 10-250µg/ml for Bepotastine was found to be linear with correlation coefficient 0.999 for Bepotastine. Accuracy of the method was verified by performing recovery studies by standard addition method. The percent recovery was found to be 97.02% to 101.52% which indicates that the method was accurate. The limits of detection for Bepotastine was found to be 0.0249µg/ml and the limits of quantitation were found to be 0.0757µg/ml. Robustness was carried out by change in the flow rate (±0.1mL/min), and variation in wavelength (± 2 nm). Solution of 100µg/ml of Bepotastine concentration was prepared and injected for each varied operational condition and% R.S.D was found to be less than 2.The method was found to be specific for the combination of interest after verifying the chromatograms showing no interference of the excipients present.Hence, the method was well suitable for the estimation of the commercial formulations. Hence, the developed RP-HPLC method can be adopted for the routine analysis of Bepotastine in bulk and pharmaceutical dosage form in quality control laboratories.. Thus the proposed RPhplc method was found to provide a faster and cost effective quantitative control for routine analysis of Bepotastine hydrochloride as bulk drug and in ophthalmic solutions. Validation is a means to prove that an equipment or process actually performs as per design or requirement. This is achieved by measuring any attribute that is possible to quantify. The objective of the analytical procedure should be clearly understood since this will govern the validation characteristics, which need to be evaluated.Typical validation characteristics that should be considered are listed below. HPLC is a type of liquid chromatography that employs a liquid mobile phase and a very finely divided stationary phase. In order to obtain satisfactory flow rate liquid must be pressurized to a few thousands of pounds per square inch. The rate of distribution of drugs between stationary and mobile phase is controlled by diffusion process, if diffusion is minimized, a faster and effective separation can be achieved. The technique of high performance liquid chromatography is so called because of its improved performance when compared to classical column chromatography.

Key words: Bepotastine , RP-HPLC, Validation, chromatography, UV spectroscopy

INTRODUCTION

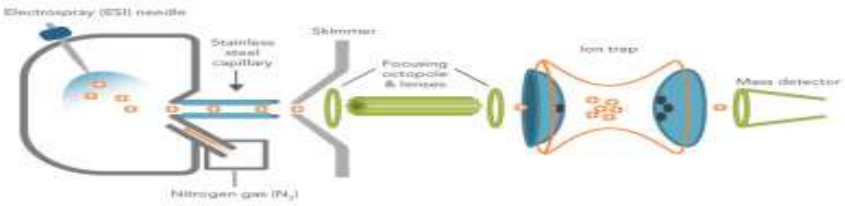
Bepotastine, belongs to category Histamine H1 Antagonist, non-Sedative. Its IUPAC name is 4-{4-[(4-chlorophenyl) (pyridin-2-yl)methoxy] piperidin-1yl}butanoicacid. The chemical formula is $C_{21}H_{25}ClN_2O_3$ Bepotastine is a non-sedating, selective antagonist of the histamine 1 (H1) receptor. Bepotastine was approved in Japan for use in the treatment [7.4] of allergic rhinitis and uriticaria/pruritis in July 2000 and January 2002, respectively, and is marketed by Tanabe Seiyaku Co., Ltd. under the brand name Talion. It is available in oral and opthalmic dosage forms in Japan. The opthalmic solution is FDA approved since Sept 8, 2009 and is under the brand name Bepreve. Because of a type 1 hypersensitivity reaction cascade that is triggered by antigen exposure, allergic conjunctivitis occurs. Allergen exposure is followed by conjunctival mast cell degranulation and histamine released as a result of the formation of complementary IgE cross-links on the conjunctiva. Due to the release of histamine, symptoms such as itching can be observed. Bepotastine works to relieve itchy eyes by three primary mechanisms of action. It is a non-sedating, selective antagonist of the histamine 1 (H1) receptor, a mast cell stabilizer, and it suppresses it suppresses the migration of eosinophils into inflamed tissues to prevent tissue damage and worsening of allergic inflammation of the conjunctiva. The main techniques employed for quantitative analysis are based upon a) Suitable chemical reaction based on either the amount of reagent needed to complete the reaction or the amount of reaction product obtained. Eg: Neutralization (Acid-Base reaction), Complex forming reaction, Precipitation reaction, Oxidation-reduction reaction.b) Appropriate electrical measurements which involve the measurement [14,3] of current, voltage or resistance in relation to the concentration of certain species in solution. Chromatography technique is a separation process employed for the separation of mixture of substances [10,11] and also for the identification of components, Eg: Gas chromatography, HPLC, HPTLC. e) X-ray Methods: When high speed electrons collide with a solid target, X-rays are produced. From the remittent X-ray emission, it is possible to identify certain emission peaks which are characteristics of elements contained in the target. The wavelength of the peaks can be related to the atomic numbers of the elements producing them. Spectrophotometer is an instrument for measuring the transmittance or absorbance of a sample as a function of the wavelength of electromagnetic radiation.



Spectrophotometer

Other optical components, such as lenses or mirrors, relay light through the instrument. A schematic representation of a UV/VIS spectrophotometer is shown below.Normal working range[12,27] for a spectrometer is 190 – 900 nm; working beyond 180nm requires special arrangements.

A detector converts a light signal into an electrical signal. Ideally, it should give a linear response over a wide range with low noise and high sensitivity. Spectrophotometers normally contain either a photomultiplier tube detector or a photodiode detector. Photon Transducers: Convert [22,28] photon energy to electrical signal (current, voltage, etc.) Detectors based on photoelectric effect: Phototubes, Photomultiplier tubes, Phototube: Incident photon causes release of an electron Photocurrent Not best for low-light scenarios.



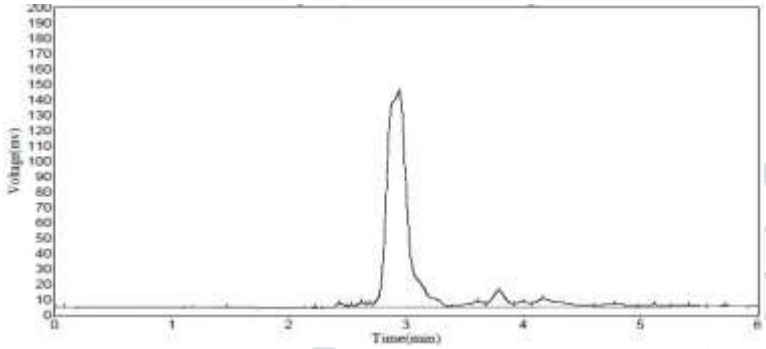
Detectors

MATERIALS AND METHODS

Method Development by Trial and Error method: Method development for the estimation of Bepotastine in dosage form was initiated based on the method development guidelines and literature [4,7] review. Several trials were conducted by varying the chromatographic parameters for optimization of method.

1. Chromatographic parameters of Trial 1

Mobile phase :	Methanol : Acetanitrile (20:80)
Column:	Inertsil ODS C18 (250x4.6x 5µ)
Detector wavelength:	254nm
Injection volume :	20 µL
Flow rate:	1.0ml/min
Theoretical plates:	1016

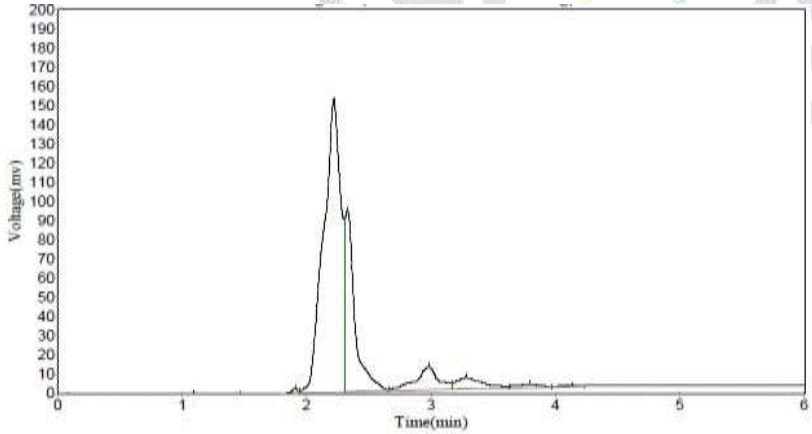


Chromatogram for Trial 1

Observation: Symmetry, Efficiency is not good and also peak tailing is observed.

2. Chromatographic parameters of Trial-2:

Mobile phase :	Methanol : Acetonitrile (40: 60)
Column:	Inertsil ODS C18 (150x4.6x5µ)
Detector wavelength:	254nm
Injection volume:	20 µL
Flow rate:	1.0 ml/min
Theoretical plates:	968



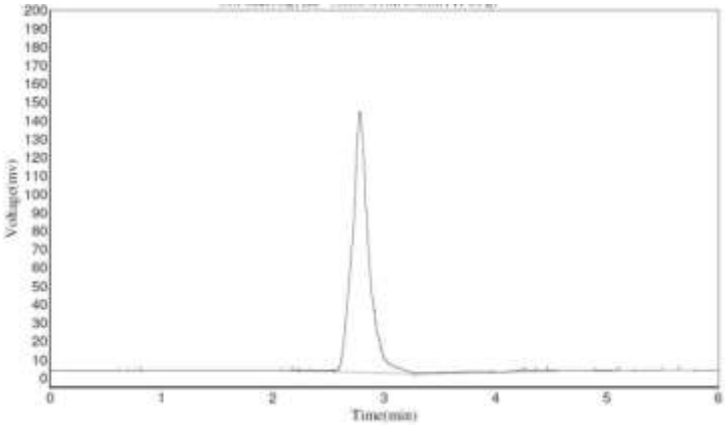
Chromatogram for Trial 2

Observation: Symmetry and Efficiency are not good and also peak tailing, broadening.

OPTIMIZED RP-HPLC METHOD

Chromatographic parameters

Mobile phase	:	Methanol : Acetonitrile (60:40)
Column	:	Inertsil ODS C_{18} (250x4.6x5µ)
Detector	:	254nm
Flow rate	:	1.2ml/min
Injection volume	:	20 µL



Chromatogram for optimized method

Optimized Chromatographic Parameters

Parameter	Condition
Mobile phase	Methanol:acetonitrile
Pump mode	Isocratic
Diluents	Mobile phase
Column	Inertsil ODS C_{18} (250x4.6x5µ)
Column temperature	Room temperature
wavelength	254
Injection volume	20µl
Flow rate	1.2ml/min
Run time	6min
Retention time	2.8min

PREPARATION OF SAMPLE SOLUTION:

1) For Rp-Hplc method:

Preparation of Standard stock solution: It was prepared by taking 50mg Bepotastine in 50ml volumetric flask[2,9] and make up the volume with mobile phase. If necessary sonicate the solution for 15 min.

Preparation of working standards: The working standard solutions were prepared by accurately transferring the (0.1, 0.5, 1.0, 1.5, 2.0, 2.5 ml) aliquots of the standard stock solution[11,13] in a series of 10 ml volumetric flasks. The volume was made upto mark with mobile phase to obtain concentrations of 10µg/ml-250 µg/ml.

Preparation of sample solution: Ten tablets were accurately weighed and finely powdered. A portion of the powder equivalent to about 10mg of Bepotastine was weighed accurately and transferred into100ml volumetric flask and mixed thoroughly for 20minutes for complete dissolution of Bepotastine with mobile phase and then the sample solution was filtered and diluted to 100ml with mobile phase to get concentration of 100µg/ml and used for analysis.

2) For UV method:

Preparation of Standard stock solution: A stock solution was prepared by taking 100mg Bepotastine in 100ml volumetric flask and make up the volume with methanol.

Preparation of working standards:The working standard solutions were prepared by accurately transferring the (0.1, 0.2, 0.3, 0.4, 0.5, 0.6 ml) aliquots of the standard stock solution[3,6] in a series of 10 ml volumetric flasks. The volume was made upto mark with methanol to obtain concentrations of 10µg/ml-60µg/ml.

Preparation of sample solution: Ten tablets were accurately weighed and finely powdered. A portion of the powder equivalent to about 100mg of Bepotastine was weighed[4.8] accurately and transferred into100ml volumetric flask and mixed thoroughly for 20minutes for complete dissolution of Bepotastine with methanol and then the sample solution was filtered and diluted to 100ml with methanol. And to get concentration of 1000µg/ml and used for analysis.

Validation of Method: The developed method was validated[10,14] according to the International Conference on Harmonization (ICH) guidelines for analytical method validation: Q2B

System Suitability.having optimized the efficiency of a chromatographic separation, the quality of the chromatography was monitored by applying the following system suitability tests:

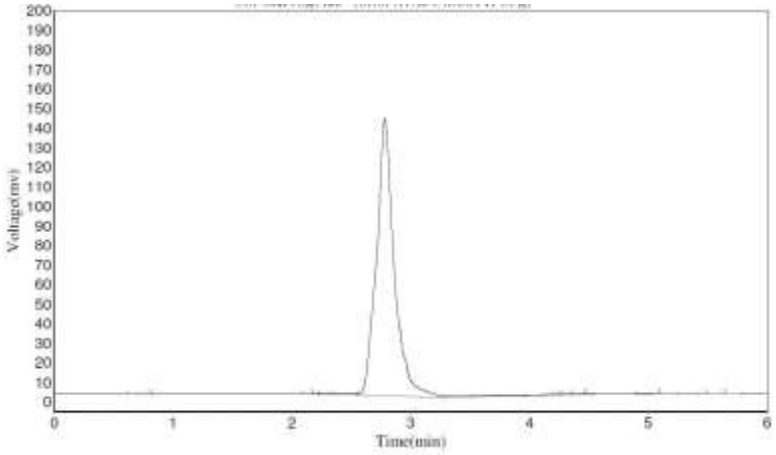
capacity factor, tailing factor and theoretical plates. The system suitability method acceptance[9,12] criteria set in each validation run were: capacity factor >2.0, tailing factor ≤2.0 and theoretical plates >2000. In all cases, the relative standard deviation (R.S.D) for the analytic peak area for two consecutive injections was < 2.0%. The system suitability test was performed using five replicate injections of standards before analysis of samples.

RESULTS AND DISCUSSION

1) SYSTEM SUITABILITY PARAMETERS

Tabular data of System suitability parameters:

Parameter	Bepotastine	Acceptance criteria
Peak assymmetric factor	1.195	NMT 2
Number of theoretical plates	5267	NLT 2000
Retention time(min)	2.857	-



Standard chromatogram of Bepotastine

2) LINEARITY FOR RP-HPLC METHOD

Standard curves were constructed using six standard concentrations in a range of 10-250µg/ml. The linearity of peak area responses versus concentrations was demonstrated by linear least square regression analysis.

Concentration(µg/ml)	Peak area(mv.sec)
10	417079
50	805358
100	1389879
150	1974988
200	2484887
250	3092886

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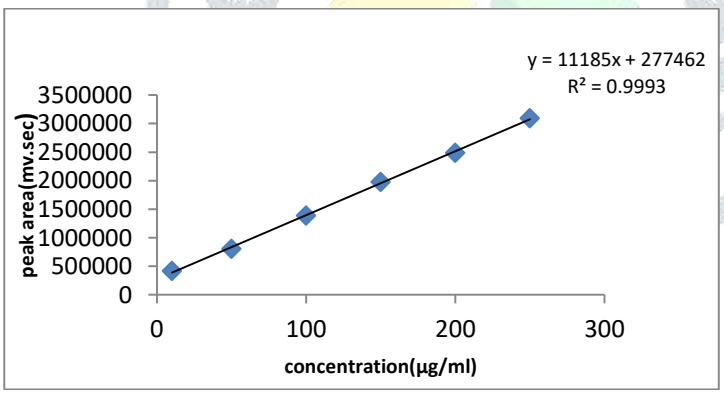
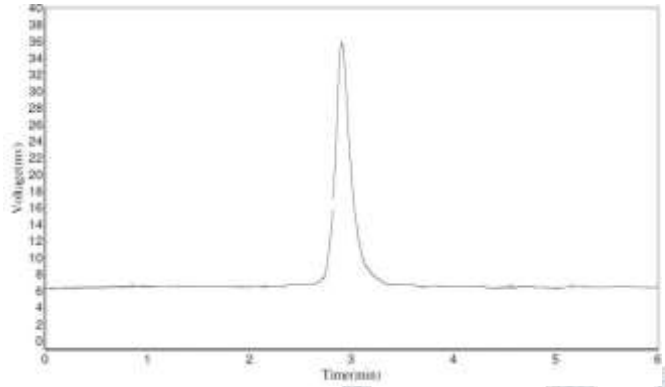
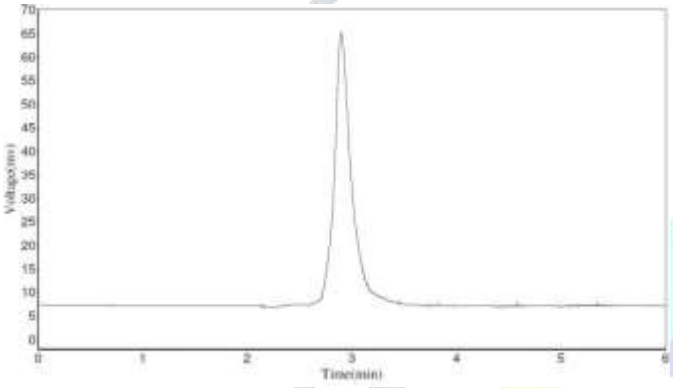


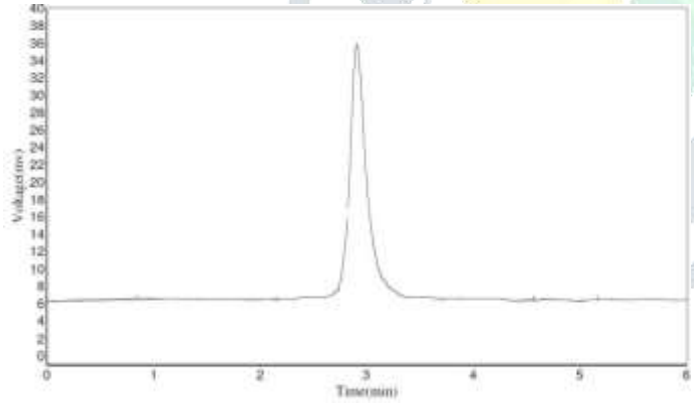
Linearity curve of Bepotastine by RP-HPLC method



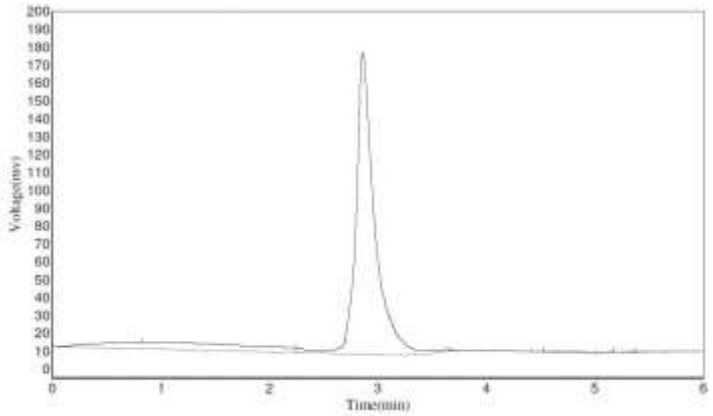
Chromatogram for 10µg/ml solution



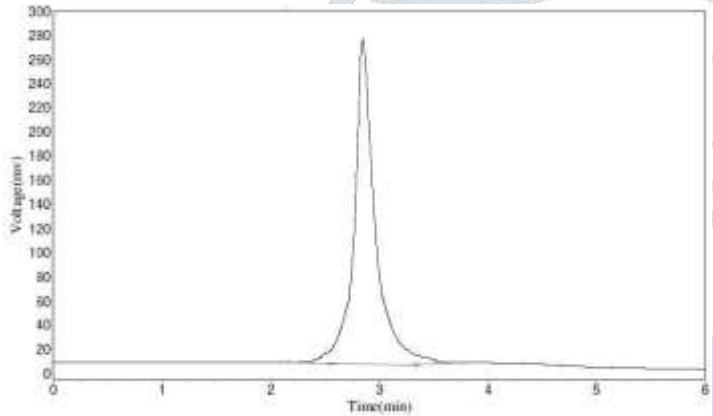
Chromatogram for 50µg/ml solution



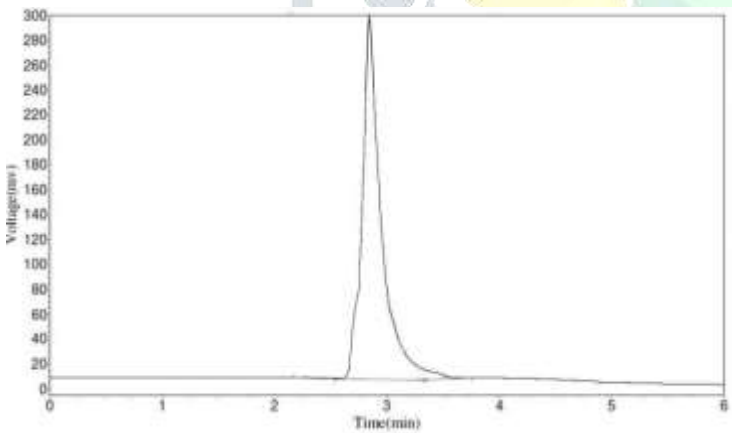
Chromatogram for 100µg/ml solution



Chromatogram for 150µg/ml solution



Chromatogram For 200µg/ml solution

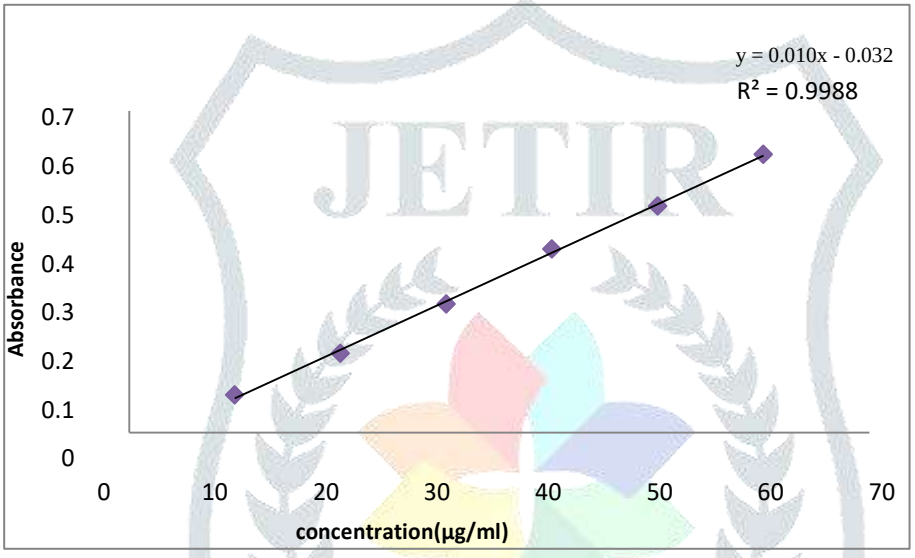


Chromatogram for 250µg/ml solution

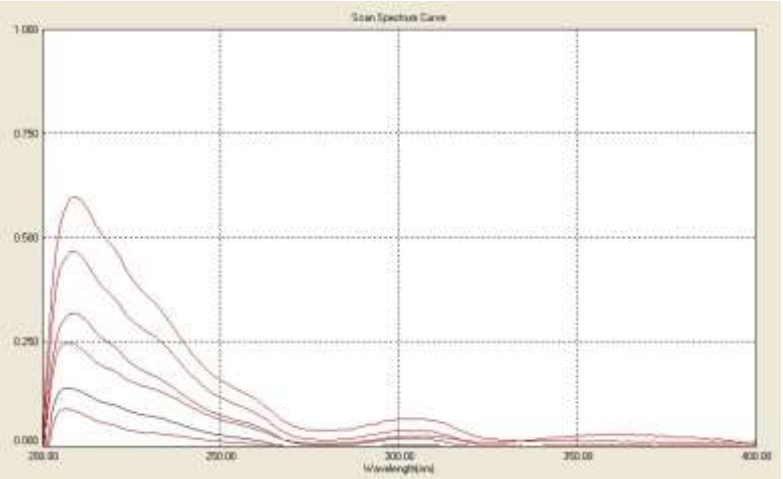
LINEARITY FOR UV METHOD

Linearity data for UV method

Concentration(µg/ml)_	Absorbance
10	0.081
20	0.172
30	0.279
40	0.399
50	0.492
60	0.605



Linearity curve of Bepotastine by Uv method



Linearity curves for 10,20,30,40,50,60µg/ml solutions

Analytical performance parameters of Bepotastine

PARAMETERS	**RP-HPLC METHOD**	**UV method**
Linearity range	10-250µg/ml	10-60µg/ml
Correlation coefficient($r^{2)}$	0.999	0.998
Slope(m)	27746	0.032
Intercept(C)	11185x	0.010x
Regression equation	Y=11185x+27746	Y=0.010x+0.032

3) ACCURACY

For Rp-Hplc Method : It was performed by standard addition method at 50%,100%and 150% level.

Accuracy of level 50% in Rp-Hplc method:

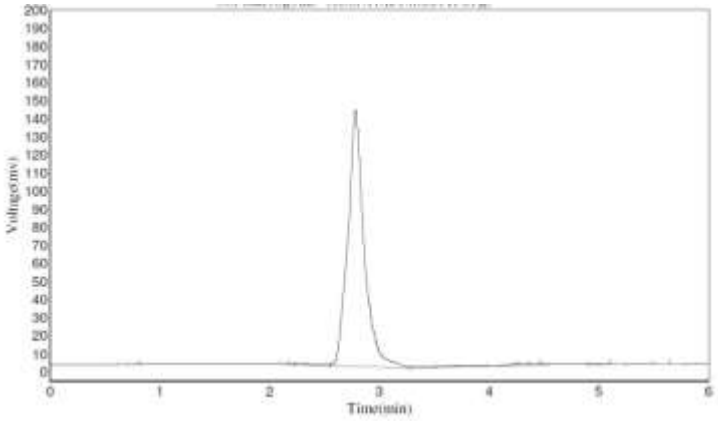
Level	STD	Level	Amount added	Total recovered	Recovered	%Recovery
50%	100	50	50	150	50	100
	100	50	50	150.057	50.057	100.114
	100	50	50	150.78	50.78	101.56
	100	50	50	150.2	50.2	100.4
	100	50	50	150.581	50.581	101.162
	100	50	50	149.05	49.05	98.1
					Mean	100.2226667
					SD	1.205765925
					%RSD	1.203087051

Accuracy of level 100% in RP-HPLC

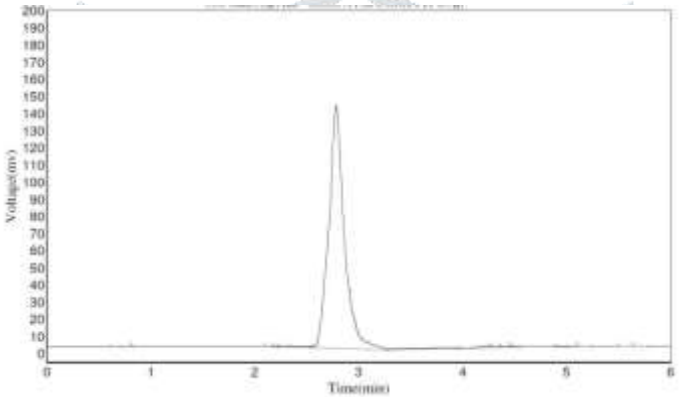
Level	STD	Level	Amount added	Total recovered	Recovered	%Recovery
100%	100	100	100	200.292	100.292	100.292
	100	100	100	200.602	100.602	100.602
	100	100	100	199.449	99.449	99.449
	100	100	100	199.527	99.527	99.527
	100	100	100	199.696	99.696	99.696
	100	100	100	198.892	99.892	99.892
					Mean	99.743
					SD	0.616244107
					%RSD	0.617831936

Accuracy of level 150% in Rp-Hplc method:

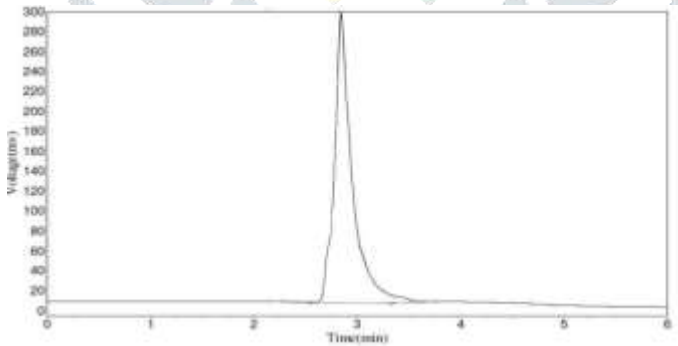
Level	STD	Level	Amount added	Total recovered	Recovered	%Recovery
150%	100	50	50	247.117	147.117	98.078
	100	150	150	247.271	147.271	98.18066667
	100	150	150	247.067	147.067	98.04466667
	100	150	150	245.541	145.541	97.02733333
	100	150	150	248.185	148.185	98.79006667
	100	150	150	248.118	148.118	98.74533333
					Mean	98.14434444
					SD	0.638985947
					%RSD	0.651067518



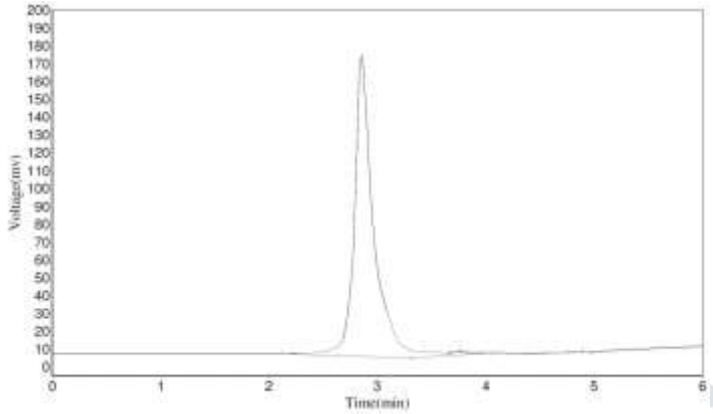
Chromatogram of Standard-1 of Bepotastine (150µg/ml)



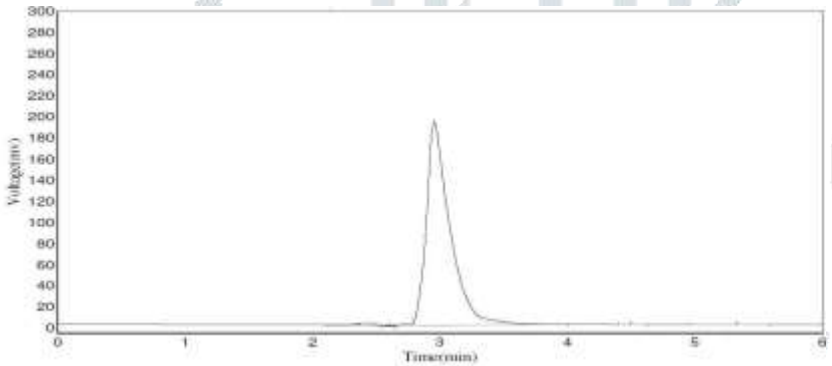
Chromatogram of Standard - 2 of Bepotastine (200µg/ml)



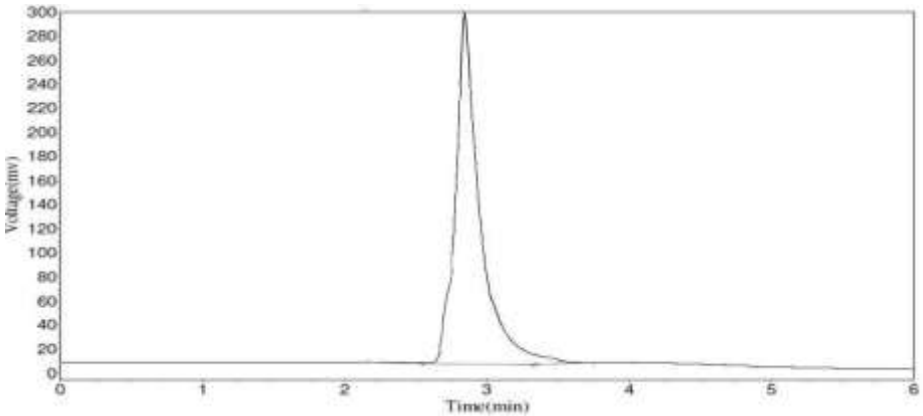
Chromatogram of Standard -3 of Bepotastine (200µg/ml)



Chromatogram of Test- 1 of Bepotastine (150µg/ml)

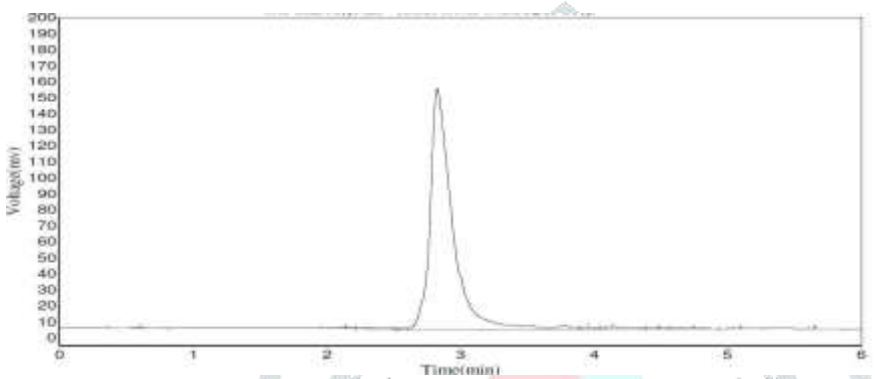


Chromatogram of Test- 2 of Bepotastine (200µg/ml)

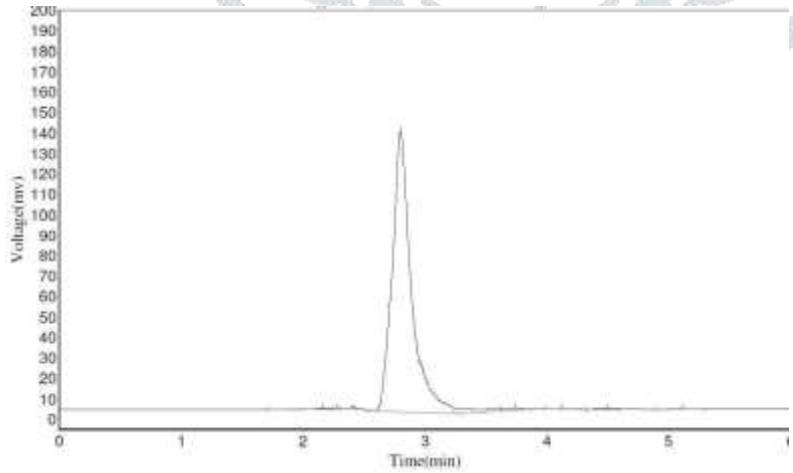


Chromatogram of Test- 3 of Bepotastine (250µg/ml)

4) SPECIFICITY



Chromatogram for Standard of Bepotastine

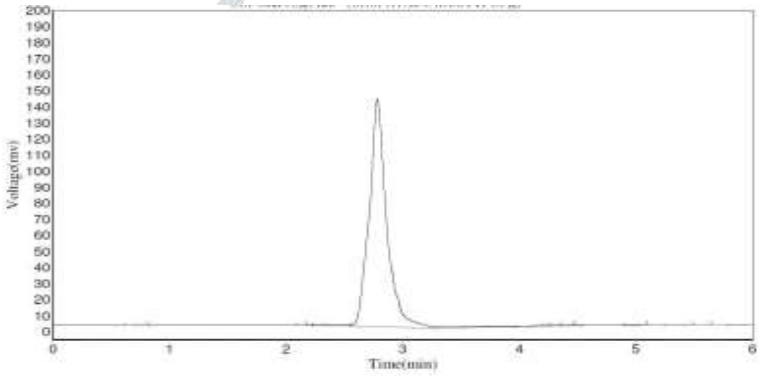


Chromatogram for Test of Bepotastine

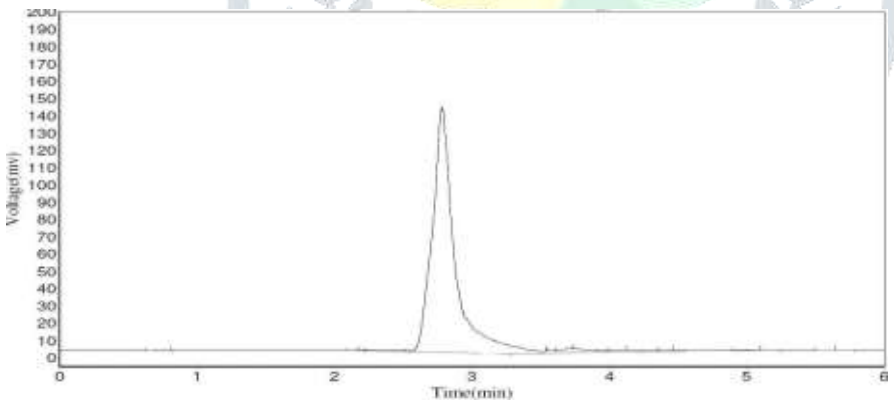
5) PRECISION

a) System Precision for RP-HPLC

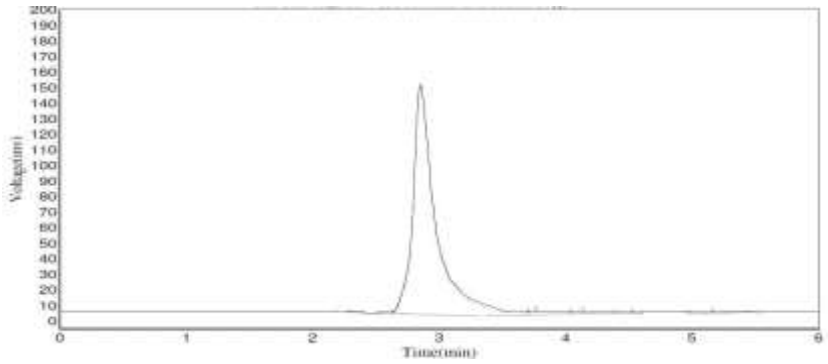
S.NO	R_t (min)	Peak area (mv.sec)
1	2.89	1389879
2	2.898	1390870
3	2.94	1381709
4	2.88	1371808
5	2.825	1387968
6	2.857	1375058
Mean	2.89	1382882
SD	0.047545968	8048.079647
% RSD	1.645189195	0.581978769



Chromatogram-1 of system Precision of Bepotastine (100µg/ml)



Chromatogram-2 of system Precision of Bepotastine (100µg/ml)

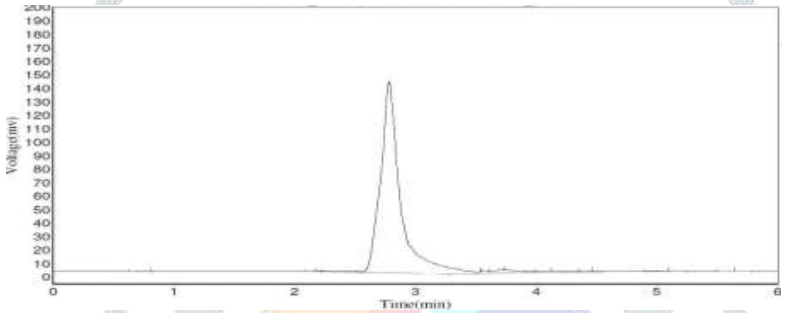


Chromatogram-3 of system Precision of Bepotastine (100µg/ml)

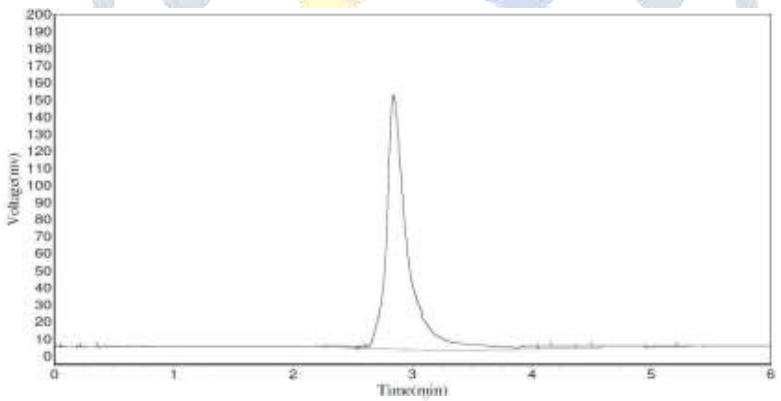
b) Method Precision for RP-HPLC:

Method Precision for Rp-Hplc method table:

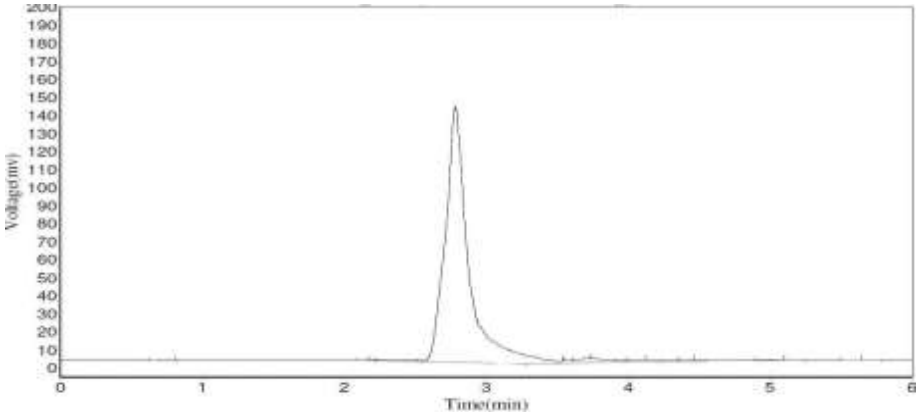
S.NO	Peak area (mv.sec)	% Labelled claim
1	1375623	100.5276882
2	1367299	101.1396922
3	1381142	100.1259827
4	1358812	101.7714003
5	1385642	99.80081435
6	1399221	98.83227882
Mean	1377956.5	100.3663094
SD	14191.07247	1.032398753
% RSD	1.029863604	1.028630782



Chromatogram-1 of Method Precision of Bepotastine (100µg/ml)



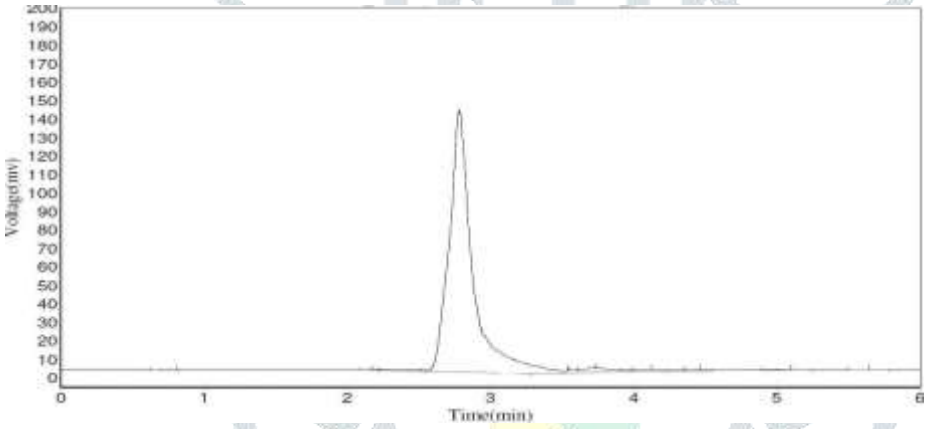
Chromatogram-2 of Method Precision of Bepotastine (100µg/ml)



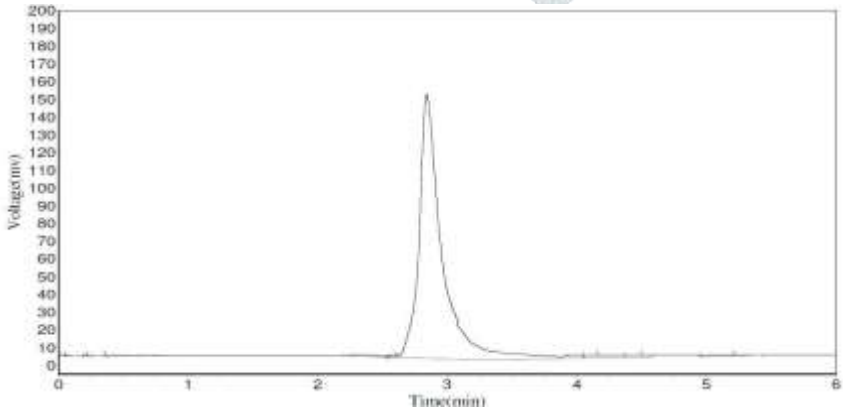
Chromatogram-3 of Method Precision of Bepotastine (100µg/ml)

C) Intermediate precision for RP-HPLC method

	ANALYST-1		ANALYST-2	
S.NO	**R_t(min)**	**Peak area (mv.sec)**	**R_t(min)**	**Peak area (mv.sec)**
1	2.892	1389879	2.884	1385965
2	2.901	1397256	2.875	1377715
3	2.871	1372689	2.912	1366121
4	2.869	1377661	2.819	1345688
5	2.877	1388821	2.9	1366127
6	2.9	1401127	2.833	1399910
Mean	2.885	1387905.5	2.8705	1373587.667
SD	0.014463748	10986.0312	0.037022966	18723.7953
% RSD	0.501343077	0.791554699	1.289774111	1.363130709



Chromatogram of Analyst-1 for Bepotastine (100µg/ml)



Chromatogram of Analyst-2 for Bepotastine (100µg/ml)

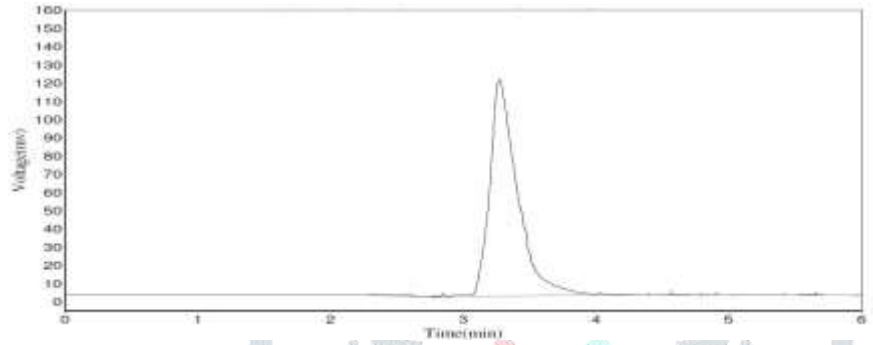
6) Limit of Detection & Limit of Quantification (LOD & LOQ):

METHOD	LOD	LOQ
RP-HPLC	0.024982	0.075702

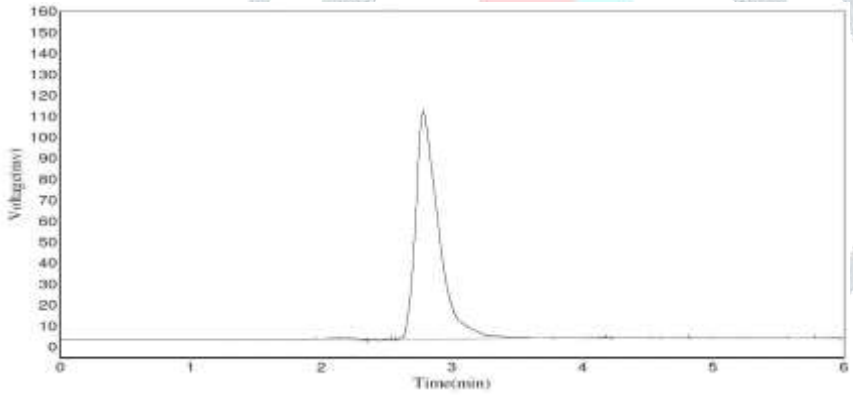
7) ROBUSTNESS

a) Effect of change in flow rate for Bepotastine in RP-HPLC:

Flow rate	Rt(min)	Peak area(mv.sec)	%RSD
STD(1.2ml/min)	2.8	1385965	0.791
1.1ml/min	3.2	1300145	0.642
1.3ml/min	2.7	1489911	1.201



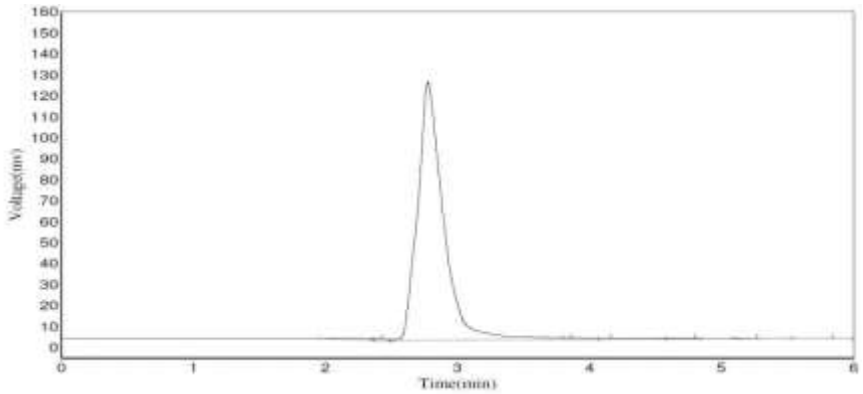
Chromatogram for flow rate of 1.1ml/min



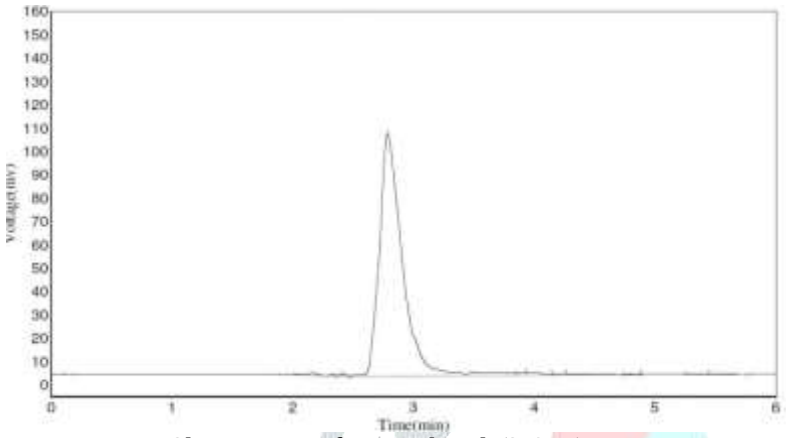
Chromatogram for flow rate of 1.3ml/min

b) Effect of Change in Wavelength for Bepotastine in Rp-Hplc method:

Wavelength(nm)	R_t(min)	Peak area(mv.sec)	%RSD
STD(254)	2.81	1385965	0.791
252	2.78	1328815	0.914
256	2.76	1215141	1.825



Chromatogram for Wavelength (252nm)



Chromatogram for Wavelength (256nm)

c) Effect of change in Wavelength for Bepotastine in UV method:

Wavelength	Asorbance						Mean	SD	%RSD
215	0.217	0.219	0.217	0.218	0.22	0.219	0.218333	0.001211	0.554684
219	0.215	0.216	0.218	0.219	0.217	0.218	0.217167	0.001472	0.677802

Summary of Validation Results

PARAMETERS	RP-HPLC METHOD
Linearity	10-250µg/ml
Correlation coefficient	0.999
Method precision(%RSD)	1.029
Accuracy	97.02%-101.56%
Range	Lowest level-10µg/ml Highest level-250µg/ml
LOD	0.0419
LOQ	0.1271

CONCLUSION:

In the present investigation simple, sensitive and economical new analytical method was developed for the Bepotastine by RP-HPLC method. The developed and validated RP-HPLC method was found to be more economical due to the short analytical run time of 6.0 minutes. The result of analysis of formulation and recovery studies obtained by HPLC method were statistically validated and high percentage of recovery studies suggest that the developed methods were free from interference of excipients used in formulation. The HPLC method was statistically validated in terms of accuracy, precision, linearity and reproducibility. Hence above methods can be employed in quality control laboratories to estimate the amount of Bepotastine in bulk and in commercial formulations.

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