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CHROMATOGRAPHIC METHOD FOR DEVELOPMENT AND VALIDATION OF CINNARIZINE USING ICH GUIDELINES

Waghmare Sonali Arun*[1] and Phatangare Nitin Gangadhar[2]

S. N. D College of Pharmacy Babhulgaon, Tal.Yeola, Dist. Nashik, Maharashtra, India.

***Corresponding Author: Waghmare Sonali Arun**

S. N. D College of Pharmacy Babhulgaon, Tal.Yeola, Dist. Nashik, Maharashtra, India.

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ABSTRACT

In the present research work, a successful attempt was made for determination of Cinnarizine in Bulk form by High performance liquid chromatography. The method was developed by experimentation, based on literature survey. The simplicity, rapidity, reproducibility and economy of the proposed method completely fulfil the objective of this research work. The HPLC method was developed and validated for estimation of Cinnarizine. The mobile phase was consisting of Methanol: water (87:13) pH3. Detection was done at 255 nm. The method was found to be simple, linear, rapid, accurate, precise, reproducible and robust. The % RSD was found within limit. The result showed that proposed method was suitable for the accurate, precise and rapid determination of in Cinnarizine its bulk form. Before making experimentation with ion-exchange or ion-pair chromatography, ion suppression by pH controls and reverse phase chromatography should be tried for ion forming organic compounds. Ion-pair chromatography should be preferred to Ion Exchange chromatography.

KEYWORD*:* Cinnarizine, High performance liquid chromatography, Derivative Spectrophotometry, Q absorbance method, Normal Phase Chromatography.

I. INTRODUCTION

Analytical Chemistry is a measurement of science consisting of a set of powerful ideas and methods that are useful in all fields of science and medicine. It seeks ever improved means of measuring the chemical composition of natural and artificial materials. This branch of chemistry, which is both theoretical, and a practical science, is practiced in a large number of laboratories in many diverse ways while analytical method, is a specific application of a technique to solve an analytical problem. Methods of analysis are routinely developed, improved, validated, collaboratively studied and applied. The discipline of analytical chemistry consists of qualitative and quantitative analysis. Modern analytical methods are extremely sensitive and they require only small amount of sample material to provide precise and detailed information. The scope of drug analysis includes the analytical investigation of bulk-drug materials, the intermediates in their synthesis, products of drug research (potential pharmacons), drug formulations, impurities and degradation products of drugs, biological samples containing the drugs and their metabolites. Obtained data of drug analysis can contribute to the maximal efficacy and maximal safety of drug therapy and the maximal economy of the production of drugs. The efficacy, safety and economy of drug therapy are extremely important issues not only from the point of view of public health, but their financial, moreover political, aspects are also immense. As a consequence of this, pharmaceutical and biomedical analysis is among the most important branches of applied analytical chemistry. To fulfill the rapidly increasing demands as regards the number and the quality of analytical measurements, great efforts has to been made in the development of analytical method. Because of these reasons analytical methods are in widespread use. They are widely used in monitoring the use of drugs and medicines, product development and stability studies. Analytical methods are often classified as classical / traditional and instrumental method. This classification is largely historical with classical methods and it is replacing by highly sensitive instrumental methods.[1,2]

1.1. Instrumental methods[1,3]

Instrumental methods are used to investigate analytes using scientific instruments. Modern analytical chemistry is dominated by sophisticated instrumentation, the roots of analytical chemistry and some of the principles used in modern instruments are from traditional techniques many of which are still used today. These methods are extremely sensitive and they require only small amount of sample material to provide precise and detailed information. Because of these reasons analytical methods are in widespread use.

1.2. UV- Visible spectrophotometer[2,4,5]

Absorption spectroscopy is the measurement of the absorption of electromagnetic radiation from definite and narrow wavelength range by molecules, ions and atoms of chemical substance. Techniques most commonly employed in analytical field ultraviolet, visible, infrared and atomic absorption spectroscopy. The ultraviolet–visible spectroscopy is one of the most frequently employed techniques in pharmaceutical analysis. It involves the measurements of the amount of ultraviolet (190-380 nm) or visible (380-800 nm) radiation absorbed by a substance in solution. Instruments which measure the ratio or a function of the ratio, of the intensity of two beams of light in the ultraviolet-visible region are called ultraviolet-visible spectrophotometers. Absorption of light in both the ultraviolet and visible regions of the electromagnetic spectrum occurs when the energy of the light matches that required to induce in the molecule an electronic transition and its associated vibrational and rotational transitions. There are four types of transitions observed in UV-visible spectroscopy $\sigma \rightarrow \sigma^*$, $\pi \rightarrow \pi^*$, $n \rightarrow \pi^*$ and $n \rightarrow \sigma^*$.

1.3. Introduction to Derivative Spectrophotometry[2]

Derivative spectrophotometry, which consists in the differentiation of a normal spectrum, offers a useful means for improving the resolution of mixtures, because it enhances the detectability of minor spectral features. It involves the conversions of a normal spectrum to its first, second or higher derivative spectrum. In the context of derivative spectrophotometry, the normal absorption spectrum is referred to as the fundamental, zero order, or D0 spectrum whereas for first derivative D1 spectrum, second derivative D2 spectrum and so on. The first derivative spectrum is plot of rate of absorbance with wavelength against wavelength.

1.4. MODES OF CHROMATOGRAPHY[6]

Modes of chromatography are defined essentially according to the nature of the interactions between the solute and the stationary phase, which may arise from hydrogen bonding, Vander walls forces, electrostatic forces or hydrophobic forces are based on the size of the particles (e.g. Size exclusion chromatography)

Different modes of chromatography are as follows -

- Normal Phase Chromatography
- Reverse Phase Chromatography
- Reverse Phase – ion pair Chromatography
- Ion Chromatography
- Ion-Exchange Chromatography
- Affinity Chromatography

1.5. Adsorption Chromatography /Normal Phase Chromatography [7]

In normal phase chromatography, the stationary phase is a polar adsorbent and the mobile phase is generally a mixture of non-aqueous solvents.

The silica structure is saturated with silanol groups at the end. These OH groups are statistically disturbed over the whole of the surface. The silanol groups represent the active sites (very polar) in the stationary phase. This forms a weak type of bond with any molecule in the vicinity when any of the following interactions are present.

- Dipole-induced dipole,
- Dipole-dipole,
- Hydrogen bonding,
- π-Complex bonding

1.6. HPLC System[7,8]

The importance of Chromatography is increasing rapidly in pharmaceutical analysis. The exact differentiation selective identification and quantitative determination of structurally closely related compounds. Another important field of application of chromatographic methods is the purity testing of final products and intermediates (detection of decomposition products and by-products). As a consequence of the above points, chromatographic methods are occupying an ever-expanding position in the latest editions of the pharmacopoeias and other testing standards. The modern form of column chromatography has been called high performance, high pressure, and high-resolution and high-speed liquid chromatography. High-Performance Liquid Chromatography (HPLC) is a special branch of column chromatography in which the mobile phase is forced through the column at high speed. As a result the analysis time is reduced by 1-2 orders of magnitude relative to classical column chromatography and the use of much smaller particles of the adsorbent or support becomes possible increasing the column efficiency substantially.

II.MATERIALS AND INSTRUMENTS

2.1. Procurement of drug sample

Table No. 2.1.: Details of drug sample.

Name of Drug	Quantity	Drug Supplier
Cinnarizine	10 gm.	Chempro Pharma Pvt. Ltd, Mumbai

2.2. Reagents and chemicals

All the chemicals used are of HPLC and AR grade. Chemicals used are as follows

Table No. 2.2: Reagents and Chemicals.

Sr. No	REAGENTS	GRADE	MANUFACTURES
1	Methanol	HPLC	Merck specialities private limited, Mumbai
2	Water	HPLC	Merck specialities private limited, Mumbai
3	Acetonitrile	HPLC	Merck specialities private limited, Mumbai

III.RESULTS AND DISCUSSION

3.1. Identification of drug

3.1.1. Organoleptic properties of drug

Table No. 3.1.: Organoleptic properties of drug.

Sr. No.	Organoleptic Property	Cinnarizine
1	**Colour**	White powder
2	**Odor**	Odorless

3.1.2 Melting point of drug

Table No.3.2: Melting point of drug.

Sr. No.	Name of drug	M.P. (˚C)
1	Cinnarizine	117-120 ^{o} C

3.1.3 Solubility Study

Table No. 3.3.: Solubility Study of Cinnarizine.

Sr. No	Solvents	Solubility
1	Water	Freely soluble
2	Methanol	Freely soluble
3	DMSO	Soluble

3.2. FTIR spectrum of Cinnarizine

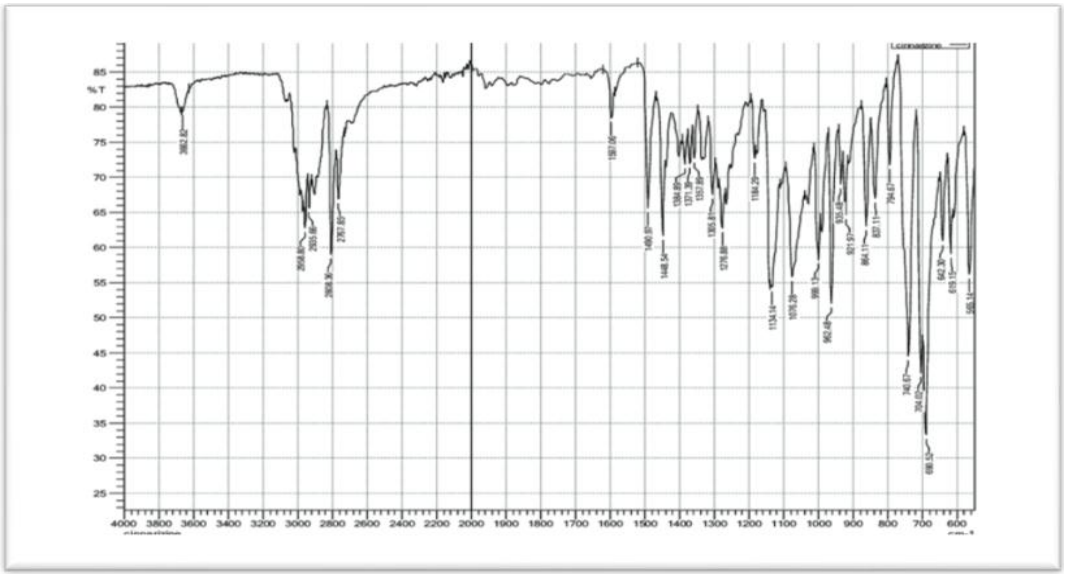

Fig.No.3.1: IR Spectrum of Cinnarizine.

Table No. 3.4: Interpretation of FTIR Spectrum of Cinnarizine.

Sr.No.	Functional group	Standard range (cm^{-1})	Observed range (cm^{-1})
1	C−H, Aromatic Stretching	3067-3025 $cm^{-1,}$	3066
2	C-H Alkane Stretching	2962-2853cm^{-1}	2808
3	Characteristic Peaks of Mon substituted benzene	2000-1667cm^{-1}	1800
4	C=C Aromatic	Near 1600cm^{-1}	1595
5	Piperazine ring N,N disubstituted	140-1500cm^{-1}	1492

3.3. Development of HPLC method for Cinnarizine

High performance liquid chromatographic method was developed and validated for determination of Cinnarizine in bulk form. Mobile phase consists of Methanol: Water (87:13) pH3. Chromatogram obtained was shows the maximum wavelength where the drug shows maximum response was 255 nm and is shown in Fig.3.2

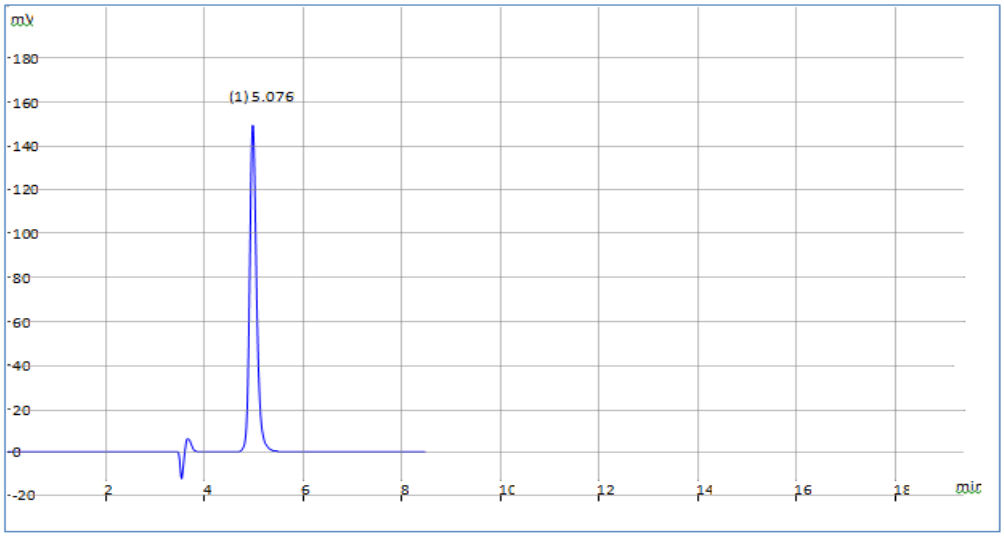

Fig. no.3.2: Typical chromatogram of Cinnarizine.

3.3.1. Linearity

Table No.3.5: Data of calibration curve of Cinnarizine by HPLC method.

Sr. No.	Conc. (µg/ml)	Area
1	10	614839 ±1680
2	20	1339880 ±2642
3	30	2032564 ±16880
4	40	2665713 ±5621
5	50	3251263 ±7513

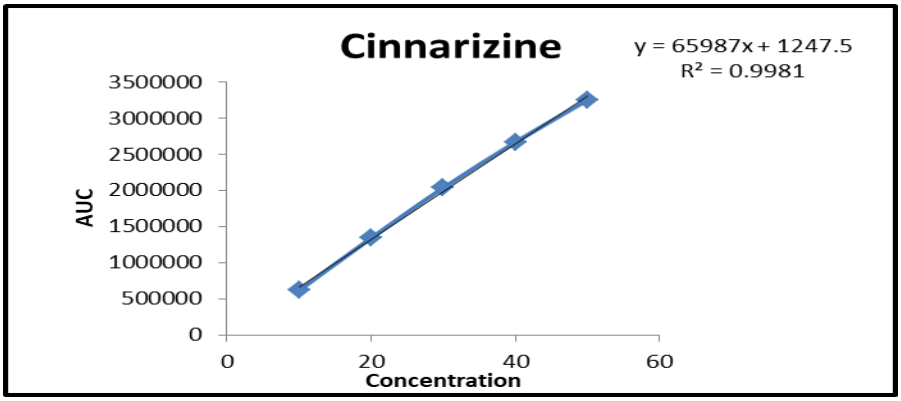

Fig. No.3.3: Calibration curve for Cinnarizine.

3.3.2. Optical characteristics

Table No. 3.6: Optical characteristics for Cinnarizine.

Sr.No	Parameters	High performance liquid chromatography
1	λmax (nm)	255
2	Beer's law limit (µg/mL)	10-50
3	Regression equation[y]	y = 65987x + 1247.5
4	Slope[m]	65987
5	Intercept [c]	1247.5
6	Correlation coefficient [r^2]	0.9981
7	Limit of detection (LOD) (µg/mL)	0.084
8	Limit of quantitation (LOQ) (µg/mL)	0.254

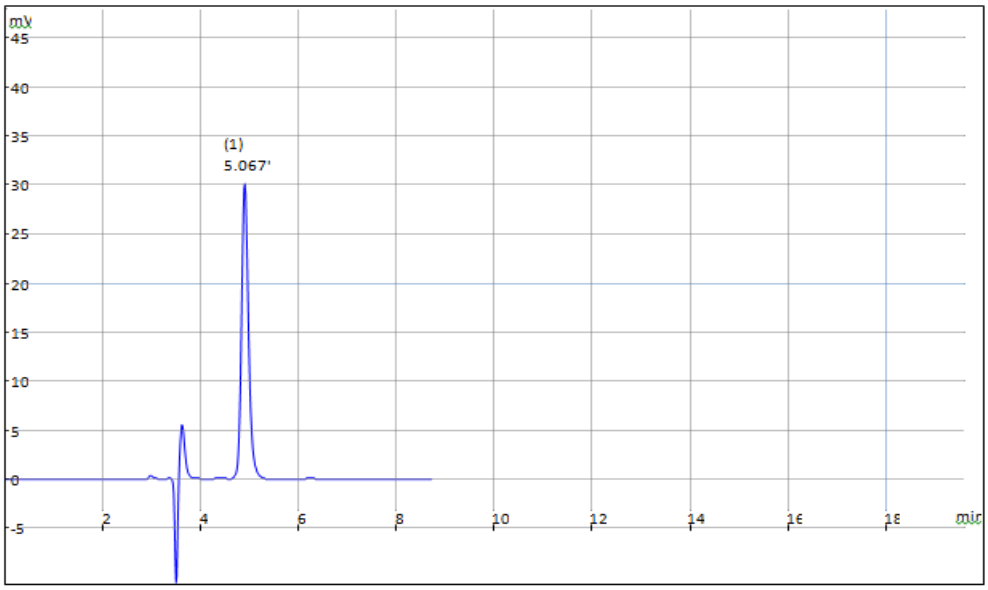

Fig. No. 3.4: Chromatogram of Linearity 10µg/ml Cinnarizine.

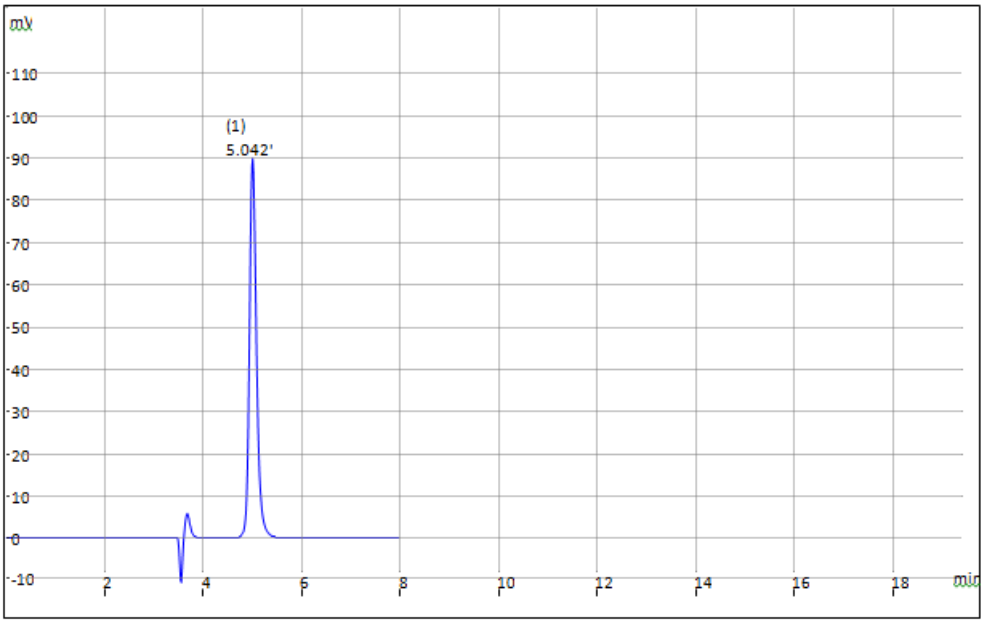

Fig. No. 3.5: Chromatogram of Linearity 20µg/ml Cinnarizine.

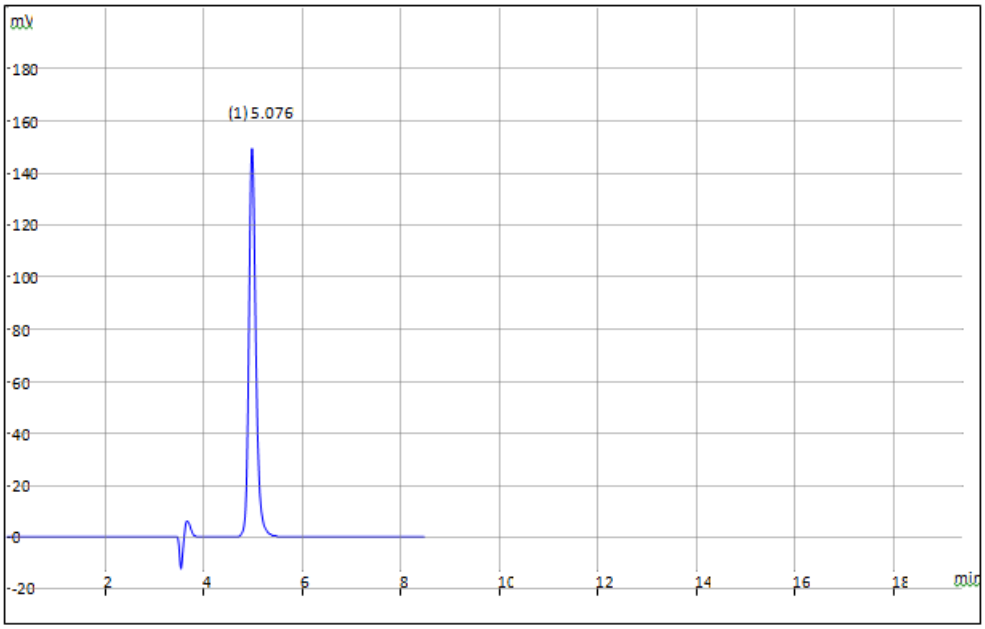

Fig. No. 3.6: Chromatogram of Linearity 30µg/ml Cinnarizine.

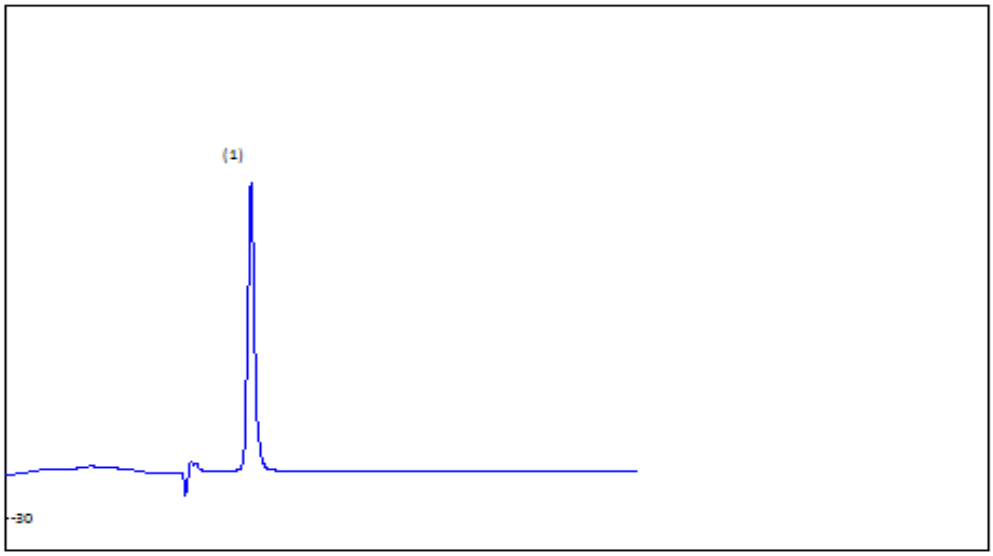

Fig. No. 3.7: Chromatogram of Linearity 40µg/ml Cinnarizine.

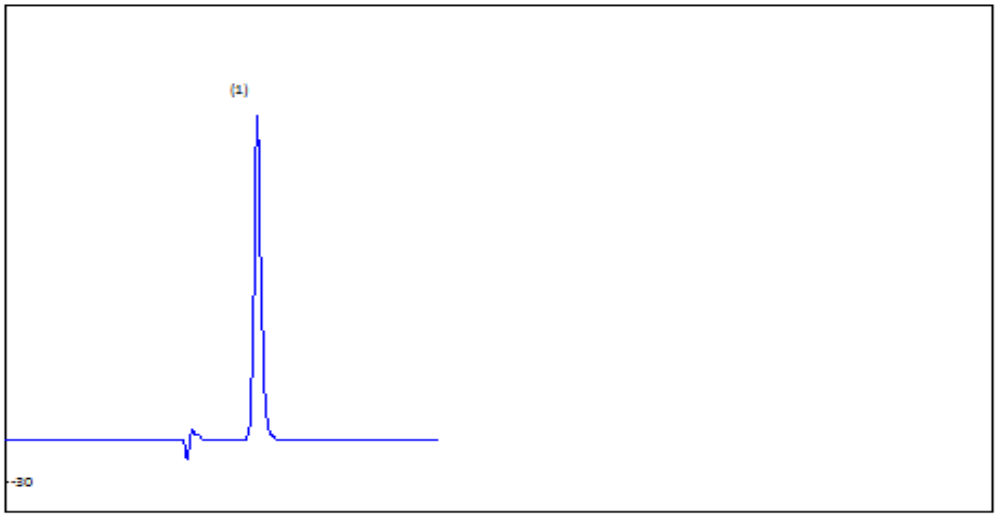

Fig. No. 3.8: Chromatogram of Linearity 50µg/ml Cinnarizine.

3.3.3. Accuracy

Table No.3.7: Data for recovery study of Cinnarizine by HPLC method

Level of addition	Standard added (µg/ml)	conc. (µg/ml)	Total conc. (µg/ml)	Area obtained*	Std Area	Drug recovered (µg/ml)	%Recovery
50%	10	20	30	2042365	2032564	30.14466	100.482199
	10	20	30	2051326		30.276921	100.923071
	10	20	30	2062345		30.439558	101.465194
100%	20	20	40	2671542	2665713	40.087466	100.218666
	20	20	40	2663215		39.962517	99.9062915
	20	20	40	2692654		40.40426	101.010649
150%	30	20	50	3245622	3251263	49.913249	99.8264982
	30	20	50	3225659		49.606245	99.2124907
	30	20	50	3265595		50.220407	100.440813

Table No.3.8: Statistical validation of Cinnarizine by HPLC method.

Level of addition	% Mean recovery*	SD	% RSD
50%	101	0.4924	0.487699
100%	100.4	0.5693	0.567125
150%	99.83	0.6142	0.615228

*****Average of three determination

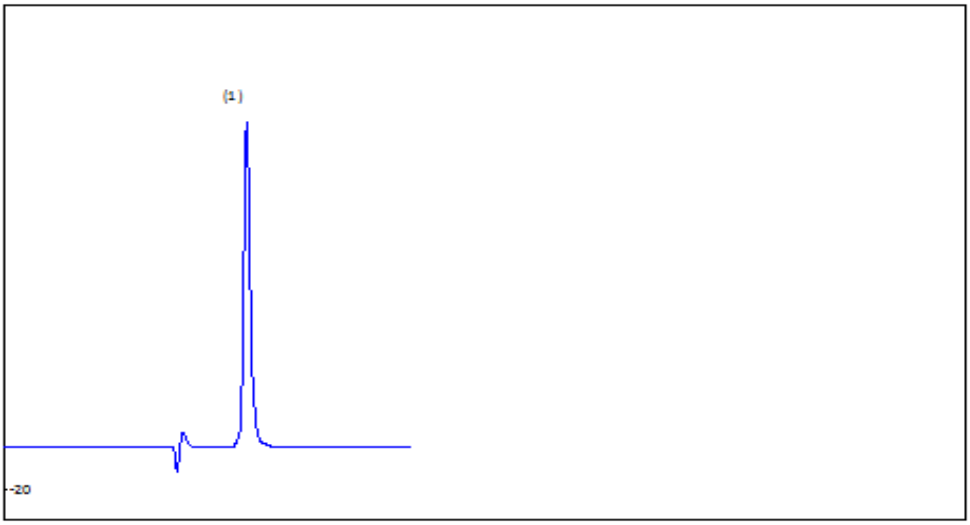

Fig. No. 3.9: A Chromatogram of % Recovery of 30µg/ml Cinnarizine.

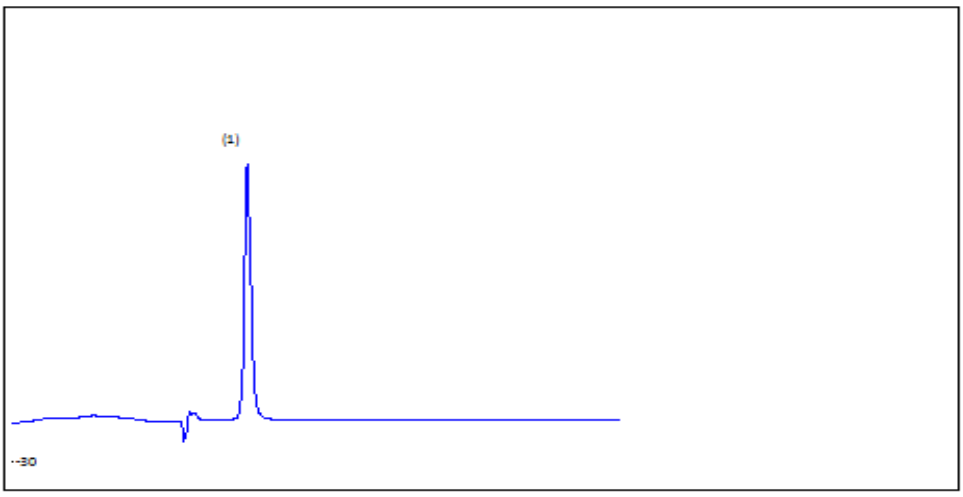

Fig. No. 3.10: A Chromatogram of % Recovery of 40µg/ml Cinnarizine.

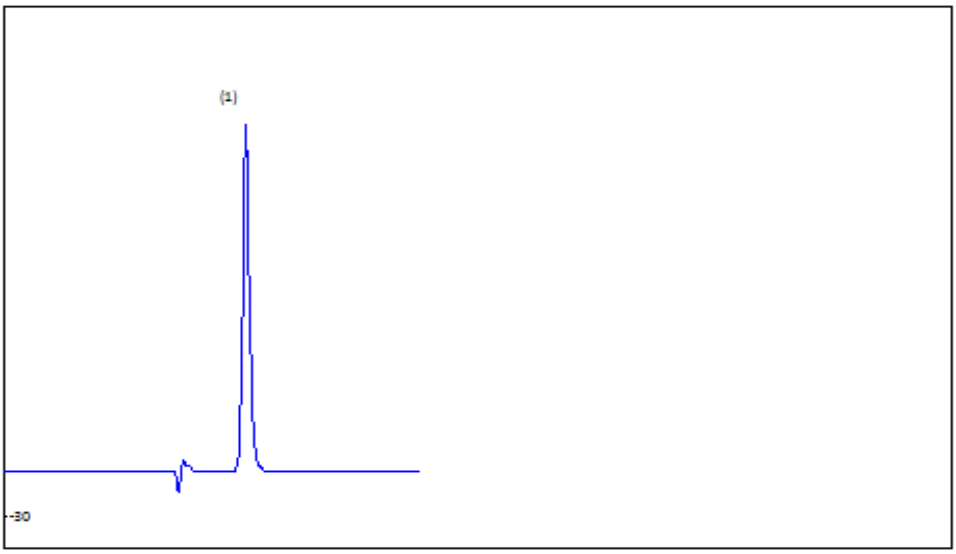

Fig. No. 3.11: A Chromatogram of % Recovery of 50µg/ml Cinnarizine.

3.3.4. Precision

Table No.3.9: Data for intraday precision of Cinnarizine by HPLC method.

Sr. No.	Conc. (µg/mL)	Area	Mean*	SD	%RSD
1	10	614959			
2	10	615785	614432	1679.693	0.273373
3	10	612552			
4	30	2015685			
5	30	2032564	2023969.33	16887.56	0.834378
6	30	2023659			
7	50	3256235			
8	50	3251263	3257551	7038.878	0.216079
9	50	3265155			

Fig. No. 3.12: A Chromatogram of Intraday Precision of 10µg/ml Cinnarizine.

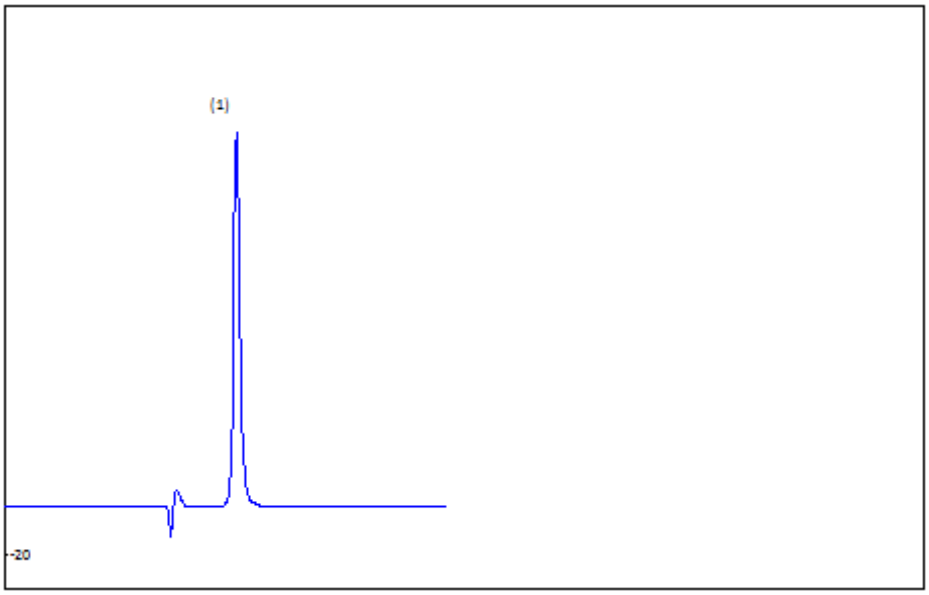

Fig. No. 3.13: A Chromatogram of Intraday Precision of 30µg/ml Cinnarizine.

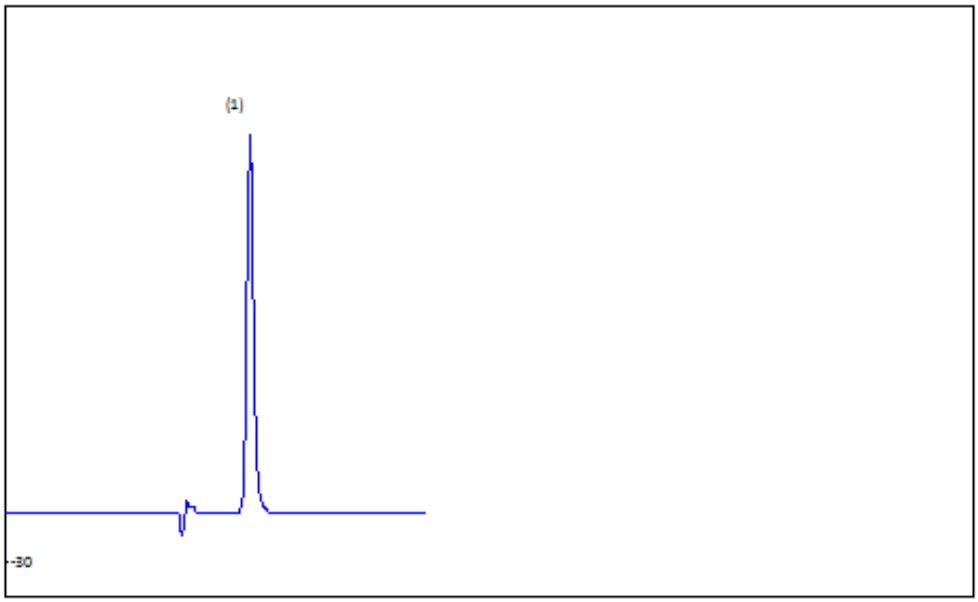

Fig. No. 3.14: A Chromatogram of Intraday Precision of 50µg/ml Cinnarizine.

Table No.3.10: Data for interday precision of Cinnarizine by HPLC method.

Sr. No.	Conc. (µg/mL)	Area	Mean*	SD	%RSD
1	10	613258	613279.667	3034.55801	0.49480819
2	10	610256			
3	10	616325			
4	30	2023526	2037444.67	16901.8362	0.8295605
5	30	2032556			
6	30	2056252			
7	50	3245622	3238386.33	23083.3062	0.71280273
8	50	3212552			
9	50	3256985			

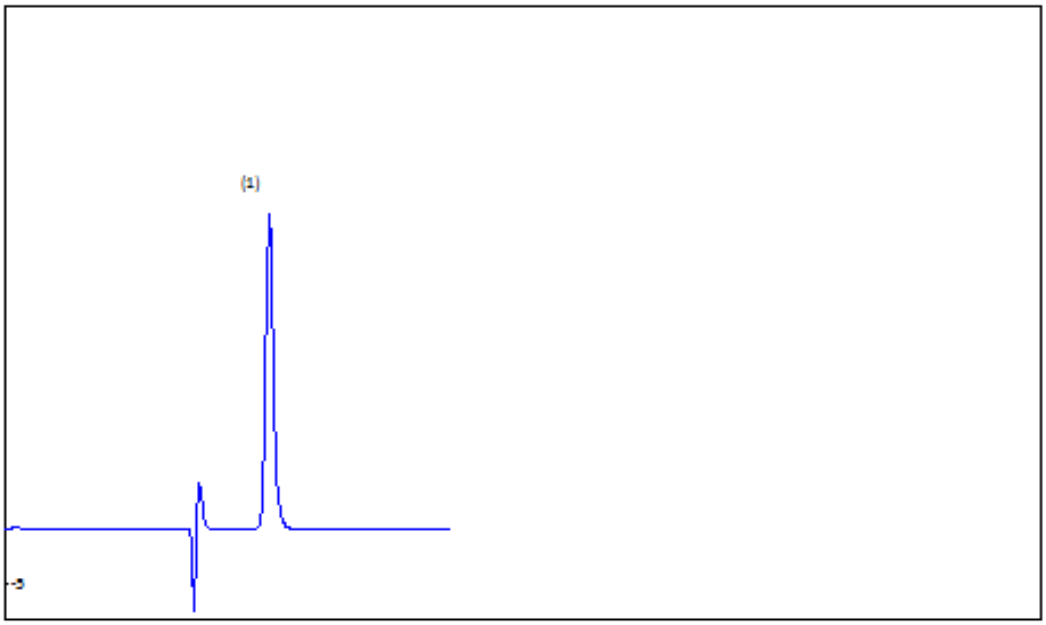

Fig. No. 3.15: A Chromatogram of Interday Precision of 10µg/ml Cinnarizine.

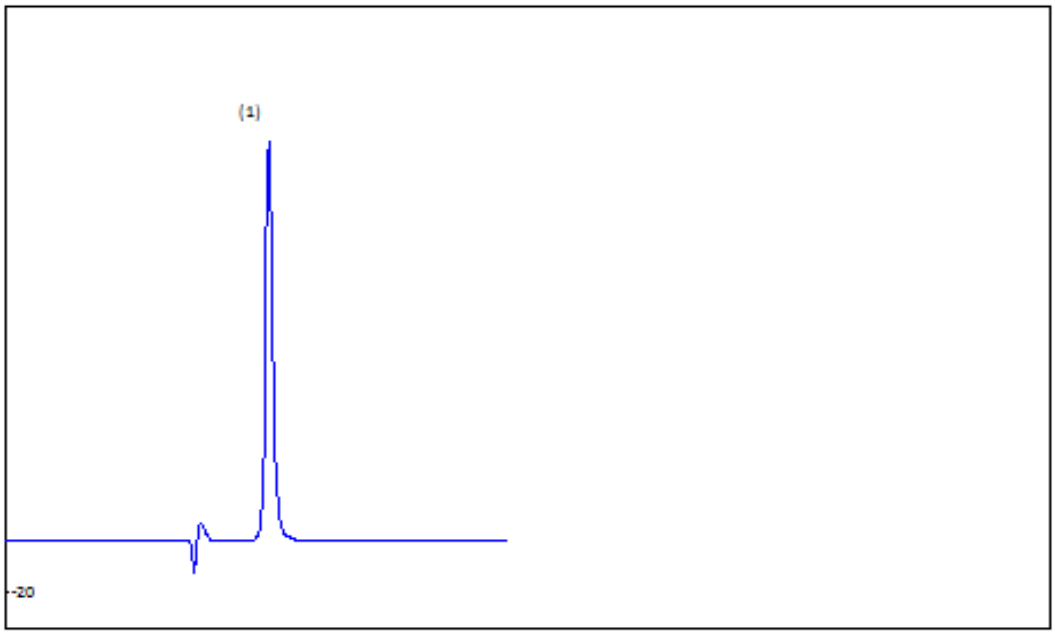

Fig. No. 3.16: A Chromatogram of Interday Precision of 30µg/ml Cinnarizine.

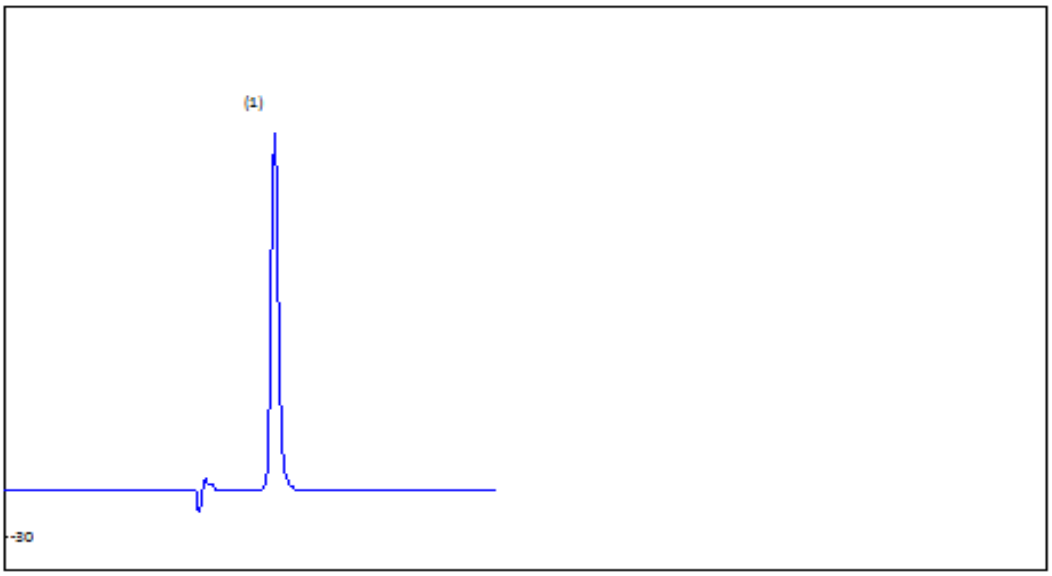

Fig. No. 3.17: A Chromatogram of Interday Precision of 50µg/ml Cinnarizine.

3.4. Limit of detection and limit of Quantitation

Table No. 3.11: Results of LOD and LOQ values of Cinnarizine.

Drugs	LOD (µg/ml)	LOQ (µg/ml)
Cinnarizine	0.084	0.254

3.4.1. Robustness

Robustness was studied by different deliberate variations in the chromatographic conditions. Results are shown in Table no. 49.

Table No.3.12: Data for Robustness study of Cinnarizine by HPLC method.

Sr.No	Parameter	Condition	Area	Mean*	SD	%RSD
1	Change in Flow rate (ml/min)	0.9	1333913	1362556	4329.28	0.31
2		1	1339880			
3		1.1	1337757			
1	Change in Wavelength (nm)	253	1340385	1363715	2175.33	0.15
2		255	1339880			
3		257	1341622			

*Average of three determination

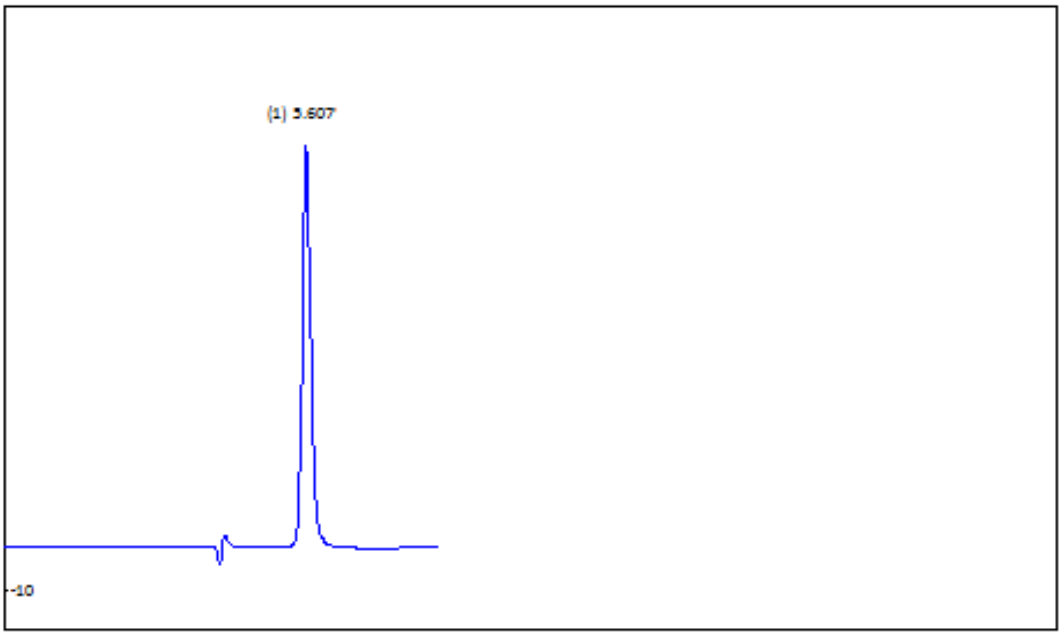

Fig. No. 3.18: Chromatogram of 20µg/ml Cinnarizine at flow rate 0.9 ml/min.

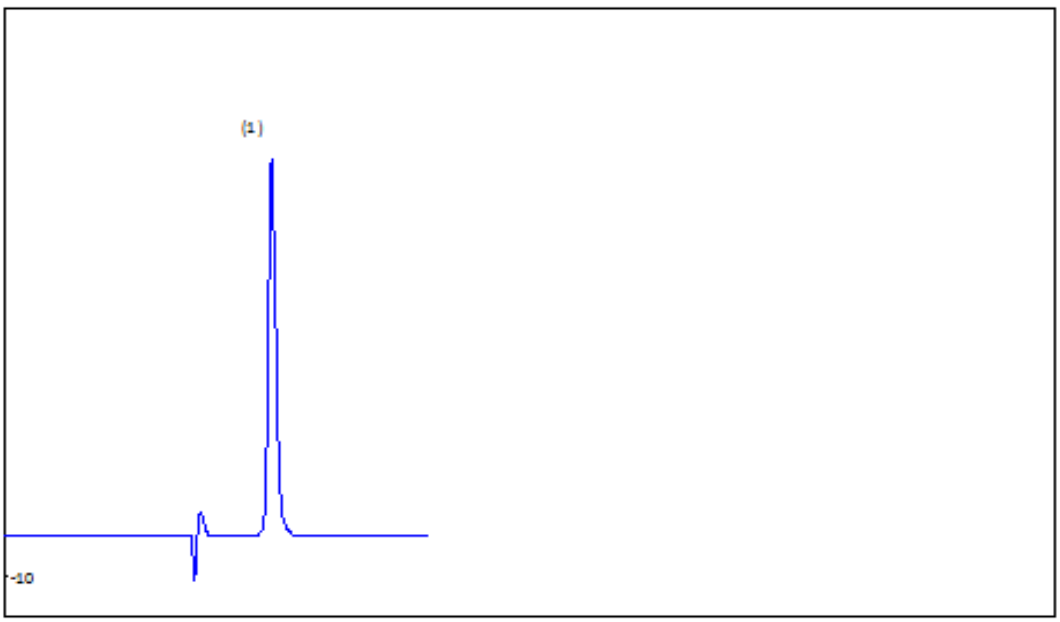

Fig. No. 3.19: Chromatogram of 20µg/ml Cinnarizine at flow rate 1.0 ml/min.

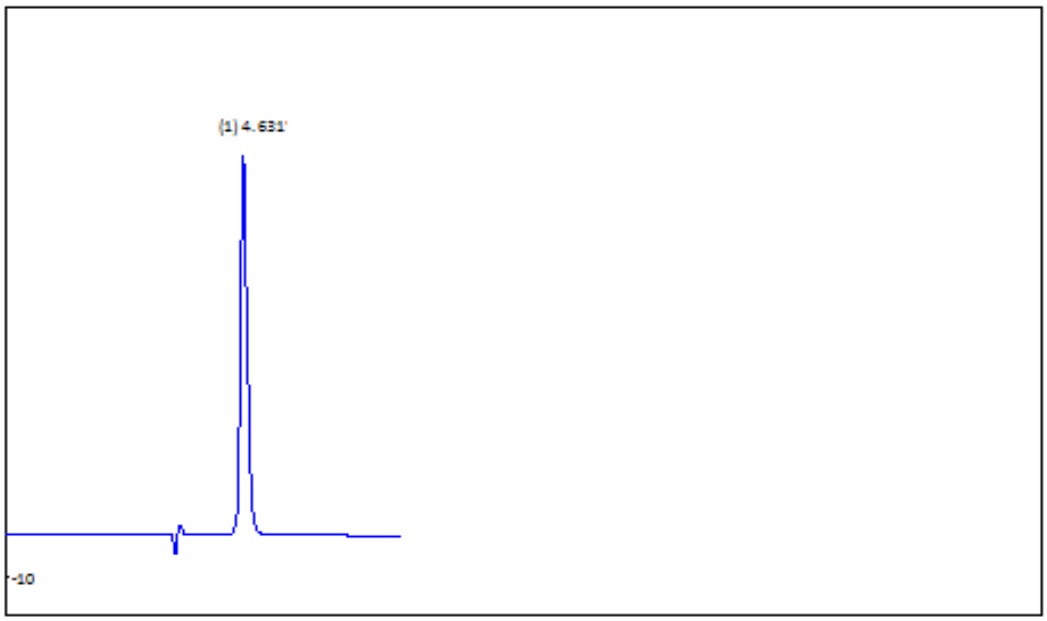

Fig. No. 3.20: Chromatogram of 20µg/ml Cinnarizine at flow rate 1.1 ml/min.

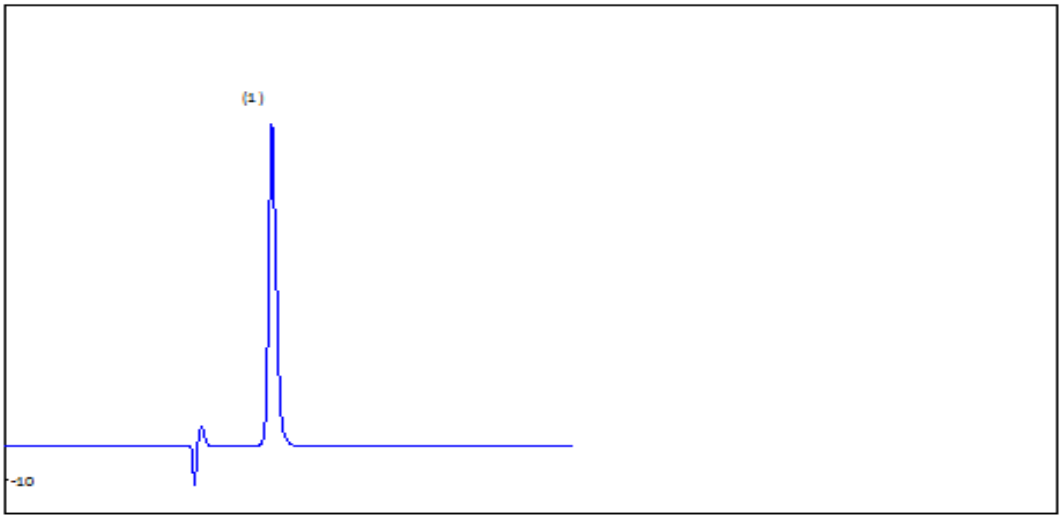

Fig. No. 3.21: Chromatogram of 20µg/ml Cinnarizine at Wavelength 253nm.

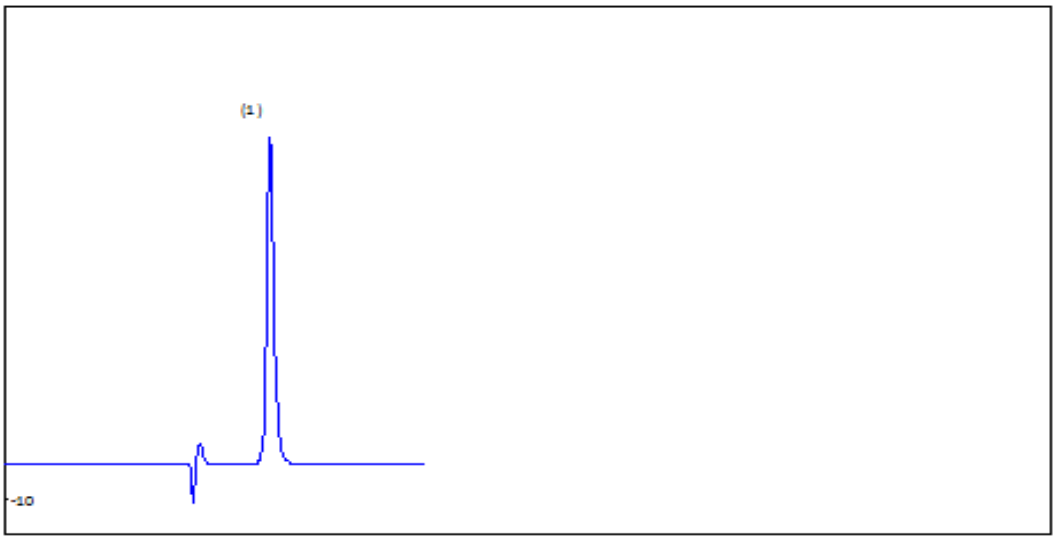

Fig. No. 3.22: Chromatogram of 20µg/ml Cinnarizine at Wavelength 255nm.

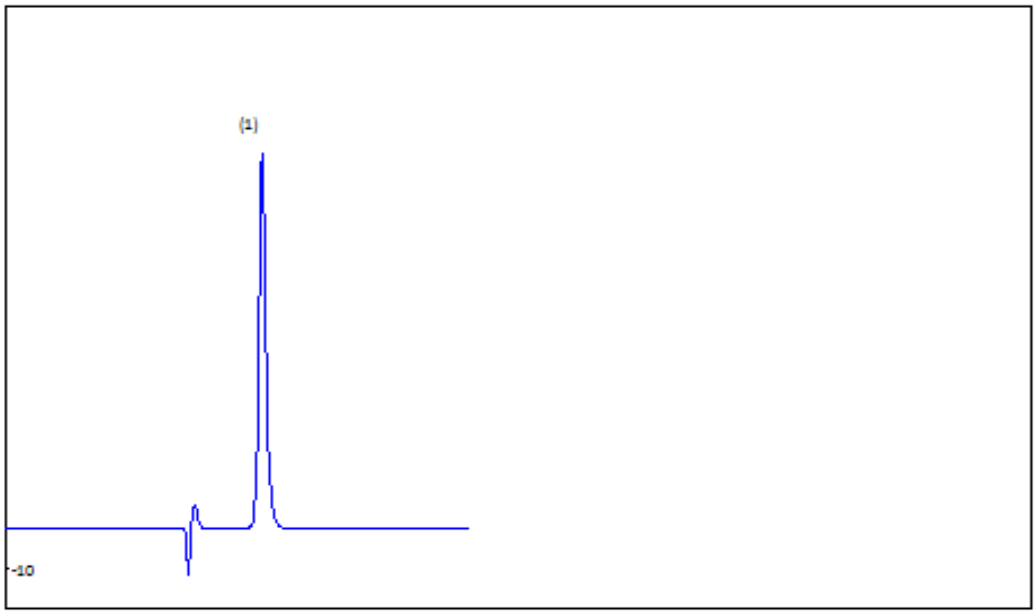

Fig. No. 3.23: Chromatogram of 20µg/ml Cinnarizine at Wavelength 257nm.

3.4.2. Ruggedness

Table No.3.13: Data for ruggedness study of Cinnarizine by HPLC method.

Sr. No	Analyst	Conc. (µg/ml)	Area	Mean area*	SD	% RSD
1	Analyst-I	30	2032565	2031067.33	6784.62927	0.33404256
			2023659			
			2036978			
2	Analyst-II	30	2032659	2036694	7426.92945	0.36465613
			2045265			
			2032158			

*****Average of three determination

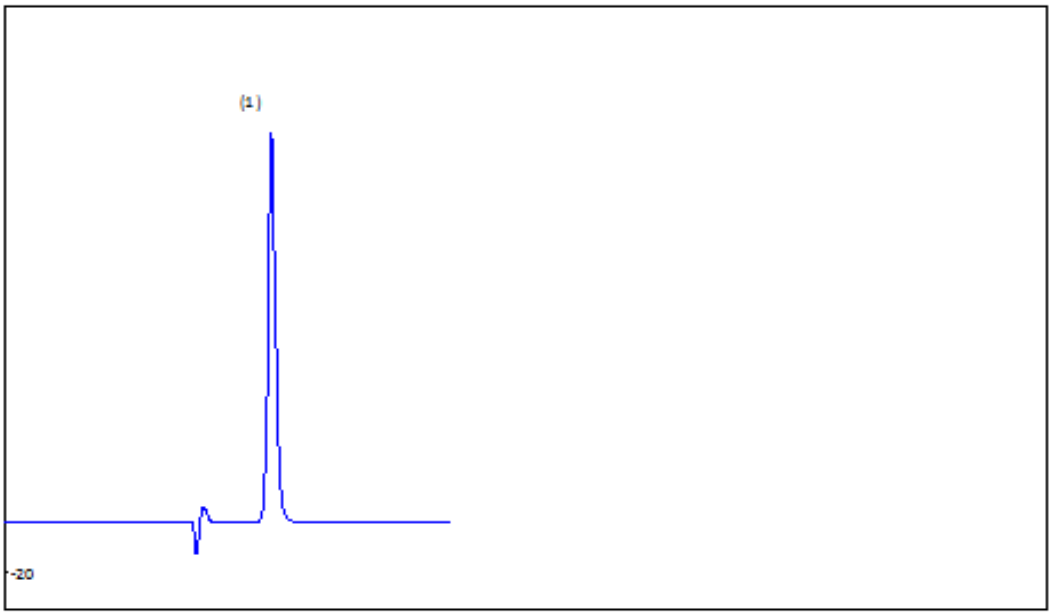

Fig. No. 3.24: Chromatogram of 30µg/ml Cinnarizine by Analyst –I.

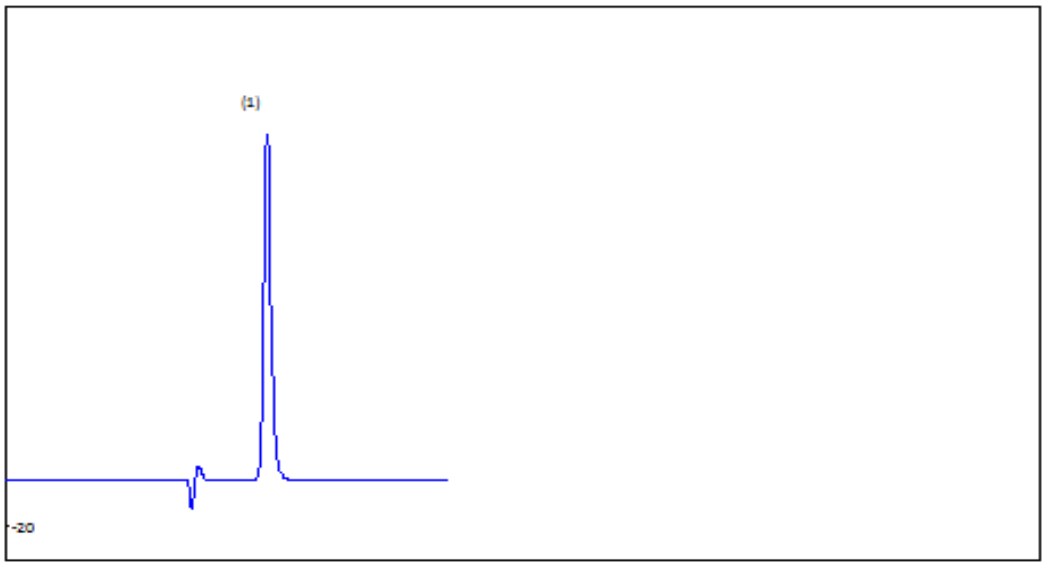

Fig. No. 3.25: Chromatogram of 30µg/ml Cinnarizine by Analyst –II.

3.4.3. Specificity

Table No.3.14: Data for specificity study of Cinnarizine by HPLC method.

Drug conc. (µg/ml)	Excipients (µg/ml)	Total conc. (µg/ml)	Area	Mean*	SD	%RSD
10	20	30	672869	673057.3333	519.7541085	0.07722286
10	20	30	673645			
10	20	30	672658			
20	20	40	1370589	1371722.333	3476.428963	0.25343533
20	20	40	1375624			
20	20	40	1368954			
30	20	50	1921659	1930366.667	9123.085242	0.47260893
30	20	50	1939855			
30	20	50	1929586			

3.4.4. System Suitability

System suitability parameters were measured to verify the system, method and column performance. Standard solution of Cinnarizine was injected into the system for five times and system suitability parameters were checked.

Table No.3.15: Data for System suitability study of Cinnarizine by HPLC Method.

Sr. No.	conc. (µg/ml)	Retention Time (min)	Theoretical plates	Asymmetry Factor
1	30	5.05	8487	1.25
2	30	5.02	8552	1.24
3	30	5.02	8462	1.25
4	30	5.01	8359	1.23
5	30	5.03	8252	1.24
6	30	5.1	8539	1.25
Mean		5.038	8441.83	1.243
SD		0.033115958	115.691688	0.00816497
%RSD		0.657280011	1.37045691	0.65669966

IV.CONCLUSION

The HPLC method was developed and validated for estimation of Cinnarizine. The mobile phase was consisting of Methanol: water (87:13) pH3. Detection was done at 255 nm. The method was found to be simple, linear, rapid, accurate, precise, reproducible and robust. The % RSD was found within limit. The result showed that proposed method was suitable for the

accurate, precise and rapid determination of in Cinnarizine its bulk form.

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