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Analytical method development and validation for the estimation of Cinnarizine by RP-HPLC in bulk and pharmaceutical dosage forms

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ABSTRACT

A simple, sensitive, accurate and precise RP-HPLC method was developed for the determination of Cinnarizine in bulk and pharmaceutical dosage forms. The method was developed by using ODS C18 column (250 × 4.6 mm, 5 µ) and the mobile phase composed of acetonitrile: buffer (0.1% *ortho*-phosphoric acid) in the ratio of 80:20v/v. The buffer pH was adjusted to 3. The retention time for Cinnarizine was found to be 4.427 min. Linearity range for Cinnarizine was found to be 10-60 µg/mL and the regression equation was found to be y = 130638x + 2529.6. % RSD for intra- and inter-day precision was found to be 0.52% and 0.29%. Average mean recovery was found to be 99.06%. LOD and LOQ values obtained for Cinnarizine were found to be 1.27 and 3.25 µg/mL respectively. The results are analyzed statistically and are found to be satisfactory. Hence this method can be successfully employed for analysis of Cinnarizine in tablet dosage form.

INTRODUCTION

Cinnarizine (Fig. 1) is a specific competitive H_1 receptor antagonist [1]. It inhibits contractions of vascular smooth muscles by blocking L-type and T-type voltage gated calcium channels preferably in the arterial smooth muscle. It is chemically 1-(diphenylmethyl)-4-(3-phenylprop-2-en-1-yl) piparazene [2,3]. Cinnarizine has also been implicated in binding to dopamine D_2 receptors, histamine H_1 receptors, and muscarinic acetylcholine receptors. Cinnarizine is used to control the vestibular symptoms of both peripheral and central origin and of labyrinth disorders including vertigo, dizziness, nystagmus, tinnitus, nausea and vomiting and prophylaxis of motion sickness. Cinnarizine also used for adjunct therapy for symptoms of peripheral arterial disease, prevention and treatment of kinesis.

A survey of literature found that several HPLC methods [4-12] were reported for estimation of Cinnarizine in combined dosage forms but limited methods were reported for individual estimation of Cinnarizine [13] by HPLC. However the reported methods required long run time, hence there is an attempt has been made to develop a simple, rapid and accurate RP-HPLC method for estimation of Cinnarizine in tablet dosage forms.

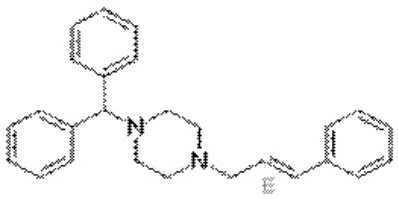

Fig. 1: Structure of Cinnarizine

MATERIALS AND METHODS

Instrument

Agilent 1260 infinity binary pump HPLC with open lab software was used for chromatographic studies.

Chemicals

Cinnarizine was purchased from Yarrow Chemicals, Mumbai, India. HPLC grade acetonitrile, ortho phosphoric acid, triethylamine were purchased from E. Merck (India) Ltd. Cinnarizine tablets were purchased from local market. Triple distilled water was used throughout experiment.

Preparation of Mobile phase

Buffer preparation

1 mL of *ortho*-phosphoric acid was transferred to 1000 mL volumetric flask and made upto volume with water. Adjusted the pH to 3.0 using triethylamine and the solution was filtered and sonicated for 5 min.

Acetonitrile: Buffer (0.1% *ortho*-Phosphoric acid) in the ratio of 80:20 v/v was used as mobile phase. The mobile phase was used as diluent.

Cinnarizine standard stock preparation

Accurately weighed and transferred about 50 mg of Cinnarizine working standard into a 50 mL clean dry volumetric flask, 20 mL of mobile phase was added, sonicate for 5 minutes, and diluted to volume with mobile phase.

Diluted standard

1 mL of the Cinnarizine standard stock solution was pippetted out and diluted to 10 mL with diluent.

Cinnarizine sample preparation

Twenty tablets were accurately weighed and ground to fine powder. An accurately weighed portion of powder sample equivalent to 50 mg of Cinnarizine was transferred to a 50 mL volumetric flask. The contents of the flask were sonicated for about 10 min for complete solubility of the drug and volume made up with further quantity of mobile phase. Further pipette 0.5 mL of the above stock solution into a 10 mL volumetric flask and the volume was made up to the mark with the mobile phase.

RESULTS

Method development

Trials were conducted by using different mobile phases in varying composition. By considering peak parameters the mobile phase composed of acetonitrile: buffer (0.1% *ortho*-phosphoric acid) in the ratio of 80:20 v/v was optimized. HPLC analysis of Cinnarizine was performed on ODS column (250 × 4.6 mm, 5µ). All samples were analysed with isocratic flow rate of 1 mL/min as well as column temperature of 25°C. UV 254 nm was used as suitable wavelength for detection of Cinnarizine. Injection volume was 20 µL and the total runtime was 5 minutes. Peak response was observed at 4.427 min and the optimized chromatogram was represented in Figure 3.

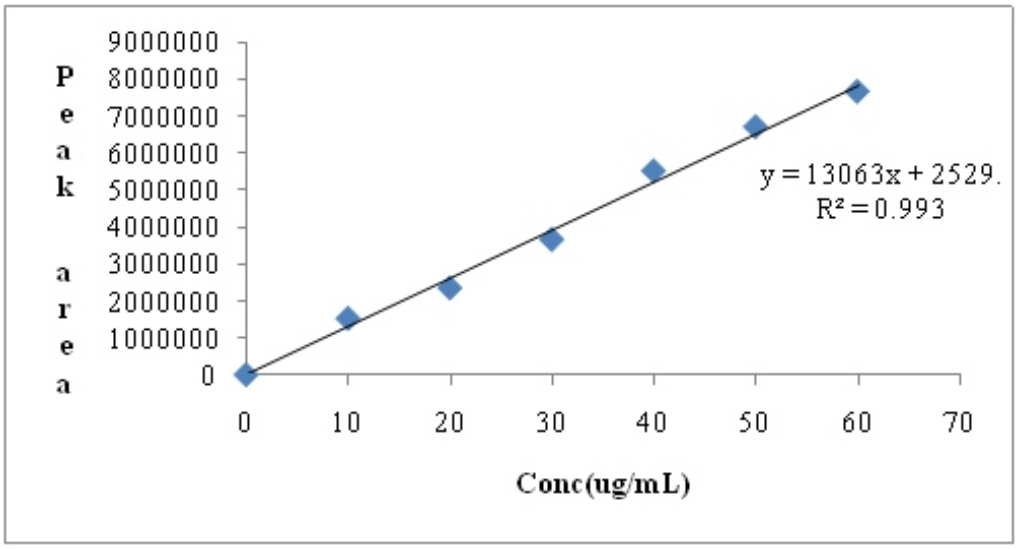

Fig. 2: Linearity curve of Cinnarizine

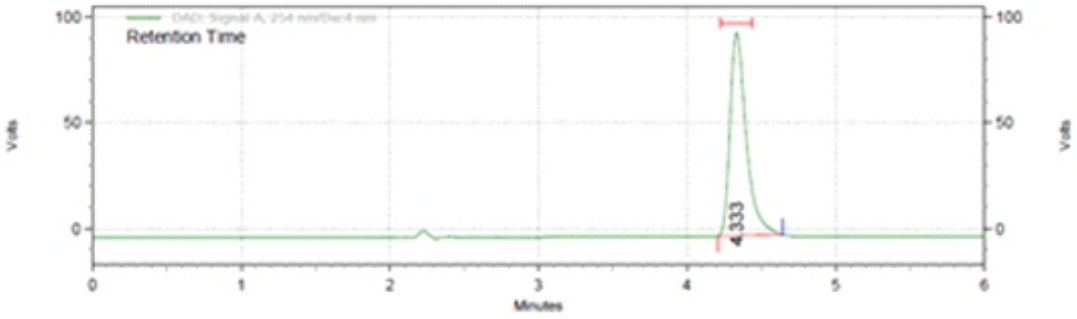

Fig. 3: Standard Chromatogram of Cinnarizine

Table 1: System suitability results for Cinnarizine

Injection	Retention time (min)	Peak area	Theoretical plates	Tailing factor (TF)
1	4.42	6745924	6896	1.52
2	4.39	6754382	6854	1.54
3	4.44	6752281	6910	1.55
4	4.43	6744723	6892	1.51
5	4.38	6743682	6908	1.55
6	4.45	6752657	6822	1.59
7	4.41	6754391	6882	1.6
8	4.46	6753285	6912	1.53
9	4.41	6747659	6890	1.53
10	4.37	6748589	6901	1.58
Mean	4.416	6749757	68867	1.55
SD	0.029889	4120.362	-	-
%RSD	0.67%	0.06%	-	-

Table 2: Linearity results for Cinnarizine

S. No.	Concentration(µg/mL)	Peak area
1	0	0
2	10	1524864
3	20	2351570
4	30	3661857
5	40	5521805
6	50	6716467
7	60	7675020
Slope		125679
Intercept		85170.68
Regression Equation (y)		y = 130638x + 2529.6
Correlation Coefficient		0.9931

VALIDATION

System Suitability

Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters like theoretical plates, resolution and asymmetric factor were evaluated and the values are depicted in Table 1.

Linearity

Linearity was performed by preparing standard solution of Cinnarizine at different concentration levels i.e., 20-120 µg/mL. The absorbance was measured at 254 nm. Each measurement was carried out in triplicate. Linearity was proven by regression analysis by the least square method. The straight line in the calibration curve (Fig. 3) obeyed linearity in the concentration range of 20-120 µg/mL for Cinnarizine. The correlation coefficient, linearity results were presented in Table 2.

Precision

The precision of the method was confirmed by intra-day and inter-day analysis. The concentration used for the precision studies is 50 µg/mL and was assumed as 100%. To study the intra-day precision, the analysis of drugs was repeated for six times in the same day and for inter-day precision the analysis of drugs was

Fig. 3: Intra & inter-day results for Cinnarizine

S. No.	Intra-day Time (Hours)	Peak area	Inter-day (Days)	Peak area
1	0	6716589	1	6825523
2	3	6705421	2	6785259
3	6	6715948	3	6798562
4	9	6689985	4	6765892
5	12	6703245	5	6795826
6	15	6789251	6	6782357
Mean		6720073	Mean	6792237
SD		35264.43	SD	20006.05
%RSD		0.52%	%RSD	0.29%

Fig. 4: Accuracy results for Cinnarizine

Level	Standard conc. (µg/mL)	Conc. added (µg/mL)	Conc. found (µg/mL)	% Recovery	% Mean recovery
80%	10	40	39.22	98.05	98.5
80%	10	40	40.15	100.37	
80%	10	40	38.9	97.25	
100%	10	50	48.96	97.92	99.09
100%	10	50	49.5	99	
100%	10	50	50.18	100.36	
120%	10	60	59.82	99.7	99.61
120%	10	60	59.89	99.81	
120%	10	60	59.6	99.33	

Fig. 5: Robustness results of Cinnarazine

S.No.	Parameter	Optimised	Used	RT (min)	Peak area	%RSD
1	Flow rate (mL/min)	1	0.8	4.59	6719845	0.85
			1.0	4.48	6715483	0.75
			1.2	4.37	6714589	0.64
2	Wavelength (nm)	265	262	2.95	65148523	0.95
			265	2.97	6698985	0.35
			268	2.89	6624823	0.66

carried out for six days. Six replicate standard solution of Cinnarizine was measured with the same concentration and the %RSD was calculated. Results of intra-day inter-day precision were given in Table 3.

Accuracy

Accuracy was performed in triplicate as per test method with equivalent amount of Cinnarizine into each volumetric flask for each spike level to get the concentration equivalent to 80%, 100%, and 120% of the labeled amount as per the test method. The average % recovery of was calculated. The accuracy results were tabulated in Table 4.

Ruggedness

Ruggedness of the method was confirmed by the analysis of samples was done by different analysts. Samples of Cinnarizine at 100 µg/mL concentration were analyzed by different analysts. It was observed that there were no marked changes in absorbance, which demonstrated that the developed method was rugged in nature.

Robustness

To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like using different conditions like flow rate and wavelength. System suitability parameters were compared with that of method precision. The robustness results were furnished in Table 5.

Limit of detection and Limit of quantification (LOD & LOQ)

For this study six replicates of the analyte at lowest concentration were measured and quantified. The LOD and LOQ of Cinnarizine is given in Table 6.

Estimation of Cinnarizine tablet dosage forms

Veritron tablets containing label claim Cinnarizine 25 mg was used for assay. The sample chromatogram was represented in Figure 4 and Table 7 shows the assay results of Cinnarizine in tablet dosage forms.

DISCUSSION

The present RP-HPLC method was developed for estimation of Cinnarizine by using acetonitrile:0.1% *O*-phosphoric acid buffer in the ratio 0f 80:20 v/v as mobile phase on ODS C18 column (250 × 4.6 mm, 5 µ). Cinnarizine peak was eluted at 4.427 min. The response of drug was found to be linear in the concentration range of 10-50 µg/mL. The regression equation was found to be Y=130638x+2529.6 The proposed RP- HPLC method was also validated for intra-day and inter-day precision. % RSD values for intra and inter-day precision was found to be 0.52 and 0.29 respectively. %Mean recovery was found to be in between 98.5-99.6%. The limit of detection and limit of quantitation was found to be 1.25 µg/mL and 3.85 µg/mL, which indicates the sensitivity of the method. The mixture of solvents used indicated well separation of two peaks with low tailing effects. The reliability and sensitivity of the validated method was

Table 6: LOD and LOQ of Cinnarazine

Parameter	Measured value (µg/mL)
Limit of detection(LOD)	1.27
Limit of quantification(LOQ)	3.85

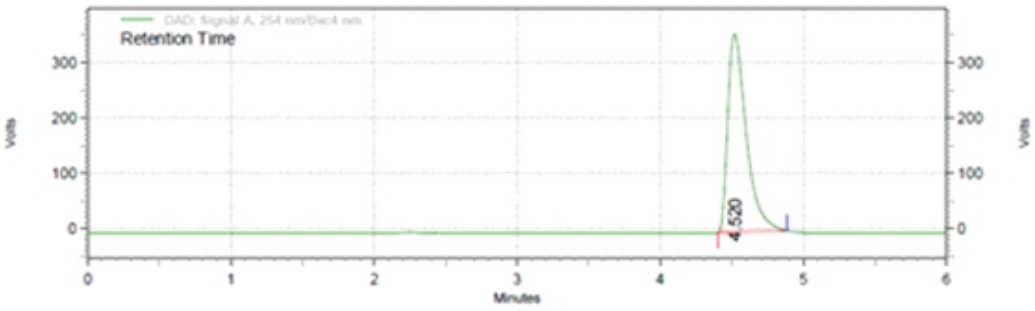

Fig. 4: Standard Chromatogram of Cinnarizine

Table 7: Assay results of Cinnarazine formulation

Formulation		Label claim	Amount found	%Assay
VERTIRON	Cinnarazine	25 mg	24.78 mg	99.12%

Table 8: Method Validation Summary

S. No.	Parameter	Observation
1	System suitability	The %RSD for retention time is 0.67% and for peak area is 0.06%
2	Precision	%RSD for Intra and Inter-day Precision 0.52 &0.29
3	Accuracy	%Mean recovery is between 98.5-99.6%
4	Linearity	Regression equation: Y=130638x+2529.6; R^2= 0.9936
5	LOD & LOQ	LOD: 1.25 µg/mL & LOQ: 3.85 µg/mL
6	Ruggedness	%RSD value is below 2%
7	Robustness	The % variation change in wavelength and flow rate is within limits

ensured with good linearity, accuracy and precision, within the ICH limits for method validation of analytical samples. The developed method can be easily applied for routine analysis of Cinnarizine in free of formulation dosage forms. The validation results were summarized in Table 8.

CONCLUSION

A new, reversed-phase HPLC method has been developed for analysis of Cinnarizine in commercial formulation. The developed RP-HPLC method was found to be simple, accurate, sensitive and precise proving reliability of the method. The method is very simple and involving no complicated sample preparations. The run time was relatively short, i.e. 4.427 min, which enables rapid quantitation of many samples in routine and quality control analysis of formulations. The optimized solvent system was used throughout the experimental work and no interference from any excipient was observed. The developed RP-HPLC method was validated as per the ICH guidelines. These results have shown that method could find practical application as a quality-control tool for analysis of Cinnarizine in pharmaceutical dosage forms in quality-control laboratories.

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