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Research Article

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Reversed phase high performance liquid chromatography method for determination of carvedilol hydrochloride from active pharmaceutical dosage form

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ABSTRACT

A simple, rapid and accurate high performance liquid chromatography method is described for determination of carvedilol hydrochloride from active pharmaceutical ingredients. The separation of drug was achieved on BDS hypersil C18 (150 x 4.6 mm i.d.) with 5 µ particle size column showed most favorable chromatographic pattern over the other columns. The mobile phase consisted of a mixture of buffer and acetonitrile (70:30 % v/v). The buffer was mixtures of 0.01 % tri-ethyl amine adjusted the pH 3.3 with ortho-phosphoric acid. The detection was carried out at wavelength 240 nm. The mixture of water and acetonitrile (50:50% v/v) was used as a diluent. The method was validated for system suitability, linearity, accuracy, precision, robustness, stability of sample solution. The method has been successfully used to analyze carvedilol hydrochloride from active pharmaceutical ingredients.

Keywords: Carvedilol hydrochloride, Acetonitrile, tri-ethyl amine

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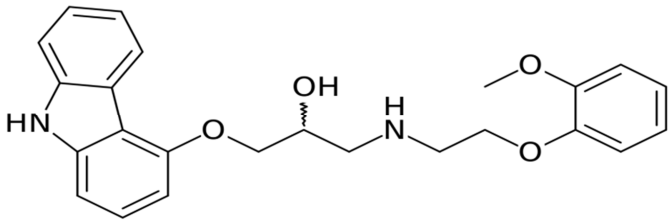
INTRODUCTION

Its chemical name is (±)-1-(isopropyl amino)-3-[*p*-(2-methoxyethyl)phenoxy]-2-propanol hydrochloride. Carvedilol hydrochloride is official in USP[1], EP[2] . Literature survey reveals the Spectrophotometric [3-8] HPLC [9-16],UPLC [17] methods for the estimation of carvedilol hydrochloride. Simple, rapid and reliable UV spectrophotometric methods are developed for the determination of carvedilol hydrochloride. These methods can be used for the routine analysis. In the proposed methods optimization and validation of this method are reported.

Structure of carvedilol



EXPERIMENTAL SECTION

Chemical and reagents

Reference standard of carvedilol hydrochloride was obtained from reputed firm with certificate of analysis. Tri-ethylamine, acetonitrile and ortho-phosphoric acid were used of analytical grade and the HPLC grade water was

used from Millipore. Standard and sample solutions were prepared in diluent [mixture of water and acetonitrile (50:50 % v/v)].

Instrumentation
The HPLC system used was MERCK Hitachi HPLC system equipped with auto sampler (D 7200 separation module) and UV detector (D- 7400). The chromatogram was recorded and peaks quantified by means of PC based EZChrom Elite software.

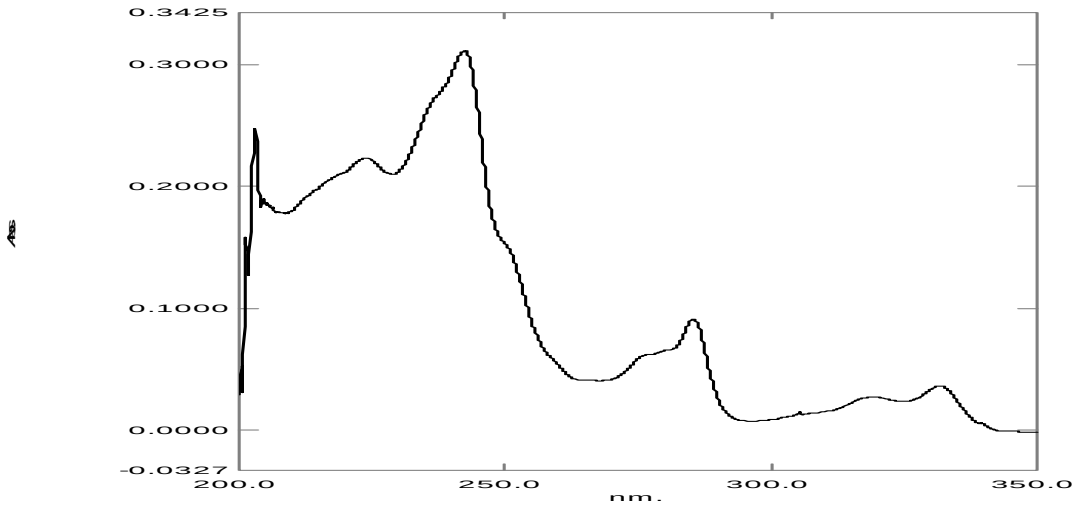
A SHIMADZU analytical balance (0.01 mg) was used.

Preparation of Standard preparation
Standard solution
A 10 mg of standard carvedilol hydrochloride was weighted accurately and transferred in 10 ml volumetric flask. About 5 ml of diluent was added and sonicated for 10 minutes. The volume was adjusted up to the mark with diluent to give concentration as 1000 µg /ml. The working standard solution was prepared by diluting 1 ml of 1000 µg /ml solution to 10 ml with diluent to get concentration 100 µg /ml.

Sample preparation
About 10 mg of carvedilol hydrochloride sample was weighted accurately and transferred in 10 ml volumetric flask. About 5 ml of diluent was added and sonicated for 10 minutes. The volume was adjusted up to the mark with diluent to give concentration as 1000 µg /ml. The sample solution was prepared by diluting 1 ml of 1000 µg/ml solution to 10 ml with diluent to get concentration 100 µg /ml.

Chromatographic condition
Chromatographic separation was performed at ambient temperature on a reverse phase BDS Hypersil C18 (150 x 4.6 mm i.d.) with 5 µ particle size column. The mobile phase was a mixture of buffer and acetonitrile (70:30 % v/v). The buffer was mixtures of 0.01 % tri-ethyl amine adjusted the pH 3.3 with ortho-phosphoric acid. The flow rate of the mobile phase was adjusted to 1 ml /min. The detection was carried out at wavelength 240 nm. (Fig. no.1) The injection volume of the standard and sample solution was set at 1.0 µl.

Figure 1: UV spectra of carvedilol hydrochloride



Method validation
System suitability
System performances of developed HPLC method were determined by injecting standard solutions. Parameter such as theoretical plates (N), symmetry, and area were determined. The results are shown in table 1 which indicates good performance of the system.

Table 1: System suitability parameters evaluated on standard solution of Carvedilol hydrochloride

Retention Time	Area	Area %	USP Plate Count	Symmetry
5.380	9739026	100	3881	1.53

Specificity

Specificity is the ability of the method to resolve the active ingredients. Hence blank, standard carvedilol hydrochloride was injected to prove specificity. The typical chromatogram of the standard and sample assayed are given in figure 2 and 3 respectively.

Figure 2: Typical chromatogram of carvedilol hydrochloride (standard)

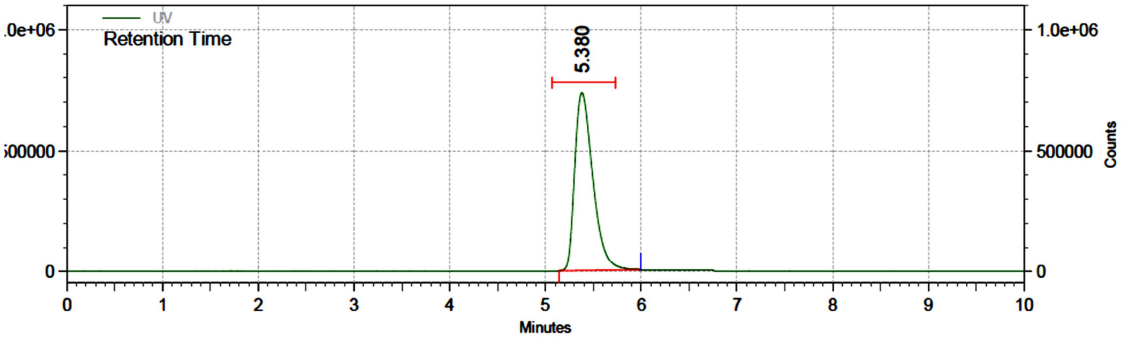
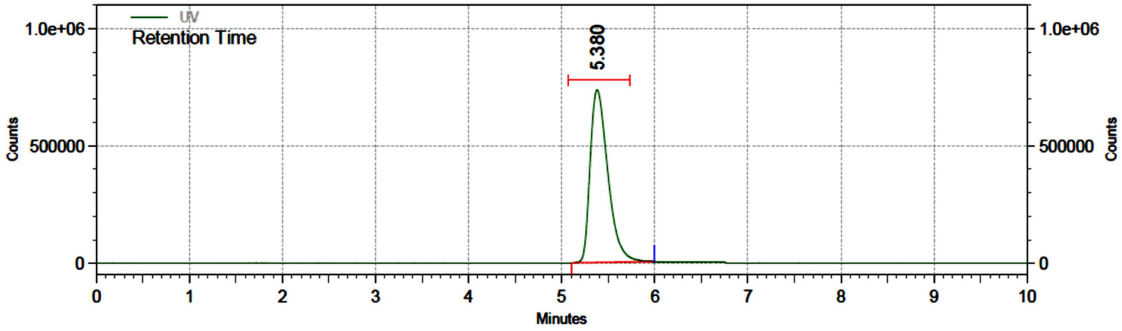


Figure 3: Typical chromatogram of carvedilol hydrochloride (sample)



Linearity

Under the experimental conditions described above, linear calibration curve were obtained throughout the concentration range studied. Regression analysis was done on the peak area (y) v/s concentration (x). The regression analysis data obtained is tabulated in table no. 2.

Table 2: Statistical evaluation of the data subjected to regression analysis

Parameters	Values
Correlation Coefficient (r)	0.9996
% Intercept (y)	184100
Slope (m)	227731

Accuracy

The accuracy method was determined by applying proposed method to synthetic mixture containing known amount of drug corresponding to 50 %, 100 % and 150 %. The accuracy was then calculated as the percentage of analyte recovered by the assay. The results of the recovery analysis are enclosed under table no.3.

Table 3: Statistical evaluation of the data subjected to accuracy of carvedilol hydrochloride

level	test	weight in mg	area	quantity added in µg /ml	quantity recovered in µg /ml	% recovery	mean recovery
80%	1	10.29	7948758	41.40	42.16	101.84	**101.99**
	2	10.46	7943056	41.40	42.13	101.76	
	3	10.38	7989232	41.40	42.38	102.36	
100%	1	10.25	9873960	51.75	52.37	101.20	**101.27**
	2	10.39	9867824	51.75	52.34	101.14	
	3	10.58	9899215	51.75	52.51	101.46	
120%	1	10.22	11761183	62.10	62.38	100.45	**100.50**
	2	10.37	11768690	62.10	62.42	100.52	
	3	10.35	11771061	62.10	62.43	100.54	
					Mean		**101.25**

Precision

The method precision was established by carrying out the analysis of carvedilol hydrochloride. The assay was carried out of the drug using analytical method in five replicates. The value of relative standard deviation lies well with the limits. The results of the same are tabulated in the table no. 4.

Table 4: Statistical evaluation of the data subjected to method precision of carvedilol hydrochloride

Test	wt of test	Area	% assay
Test -1	10.39	9739026	99.92
Test -2	10.33	9764657	99.60
Test -3	10.34	9803765	100.10
Test -4	10.32	9773785	99.60
Test -5	10.41	9735918	100.08
Test -6	10.39	9765910	100.19
	Mean Assay		**99.91**
	SD		**0.259**
	RSD		**0.259**

Robustness

The robustness of the method was determined to check the reliability of an analysis with respect to deliberate variations in method parameters.

The typical variations are given below:

Variation in the flow rate by $\pm$ 0.2 ml /min

Variation in mobile phase composition by $\pm$ 2 %

Variation in wavelength ± 5 nm

The results of the analysis of the samples under the conditions of the above variation indicated the nature of robustness of the method.

Method application

Twenty tablets were weighed accurately and average weight of each tablet was determined. Powder equivalent to 10 mg of carvedilol hydrochloride sample was weighted accurately and transferred in 10 ml volumetric flask. About 5 ml diluent was added and sonicated for 10 min to dissolve it. Further volume was made up to the mark with the diluent to give 1000 µg /ml. Further the 1 ml of this solution was diluted to 10 ml with diluent to give 100 µg /ml of carvedilol hydrochloride. From this solution 1.0 µl was injected specific conditions. The analyte peak was identified by comparison with that of respective standard. The (%) assay results were expressed in table no. 4. It indicates the amount of carvedilol hydrochloride in the product meets the requirement.

RESULTS AND CONCLUSION

The reproducibility, repeatability and accuracy of the proposed method were found to be satisfactory which is evidenced by low values of standard deviation and percent relative standard deviation. The accuracy and reproducibility of the proposed method was confirmed by recovery experiments, performed by adding known amount of the drug to the pre-analyzed active pharmaceutical ingredient and reanalyzing the mixture by proposed method. Thus the proposed RP-HPLC method is used for estimation of carvedilol hydrochloride from active pharmaceutical ingredient. It is more precise, accurate, linear, robust, simple and rapid method. Hence the proposed RP-HPLC method is strongly recommended for the quality control of the raw material, active pharmaceutical ingredient and pharmaceutical formulation.

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