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Development and validation of stability indicating method for the quantitative determination of carvedilol and its related impurities in pharmaceutical dosage forms using RP HPLC

L. Samba Siva Rao[1], P. Madhavan[2] and K. Vanitha Prakash[3]

[1]*Central Quality Control, Dr. Reddys Laboratories Ltd, IDA Bollaram, Hyderabad, Telangana, India*
[2]*CPS, Dr. Reddys Laboratories Ltd, Miyapur, Hyderabad, Telangana, India*
[3]*SSJ College of Pharmacy, Hyderabad, Telangana, India*

ABSTRACT

A simple, linear, rapid, precise and stability-indicating analytical method was developed for the estimation of related substances and degradants of Carvedilol API and tablets. The chromatographic separations were achieved using high performance liquid chromatograph (Inertsil ODS 3V, 150mm X 4.6mm, ID with 5 microns particles column) employing mixture of 0.1% Trifluoro acetic acid, methanol and acetonitrile (500:300:200v/v) as mobile phase in an isocratic elution mode with a 1.5 mL/min flow rate was chosen. Five impurities were eluted within fifteen minutes of run time. The column temperature was maintained at 55ºC and a detector wavelengths of 220nm (Amino impurity –E) & 240nm (Carvedilol & other impurities) was employed. Carvedilol was exposed to acid, base, thermal, photolytic, and oxidative stress conditions. The stressed samples were analyzed by the proposed method. The degradation of Carvedilol was observed under, oxidative hydrolysis, base hydrolysis, thermal and photolytic. The drug was found to be stable in all other stress conditions applied. Successful separation of the drug from organic impurities and degradation products formed under forced degradation was achieved. The developed HPLC method to determine the related substances and assay of Carvedilol can be used to evaluate the quality of regular production samples.It can be also used to test the stability samples of Carvedilol.

Key words: Reverse Phase Liquid Chromatography, Carvedilol, Validation, Stress conditions, Degradation Products.

INTRODUCTION

Carvedilol is a nonselective β-adrenergic blocking agent with α1-blocking activity. It is (±)-1-Carbazol-4-yloxy)-3-[[2-(o-methoxyphenoxy) ethyl]amino]-2-propanol. Carvedilol is used for treating high blood pressure and congestive heart failure. It is related to labetalol (Normodyne, Trandate).High blood pressure adds to the workload of the heart and arteries. If it continues for a long time, the heart and arteries may not function properly. This can damage the blood vessels of the brain, heart, and kidneys, resulting in a stroke, heart failure, or kidney failure. High blood pressure may also increase the risk of heart attacks. These problems may be less likely to occur if blood pressure is controlled.

Carvedilol is also used to prevent further worsening of congestive heart failure. It is also used to treat left ventricular dysfunction after a heart attack. Left ventricular dysfunction occurs when the left ventricle (the main pumping chamber of the heart) stiffens and enlarges and can cause the lungs to fill with blood.

Carvedilol belongs to a group of medicines called beta-adrenergic blocking agents, beta-blocking agents, or more commonly, beta-blockers. Beta-blockers work by affecting the response to some nerve impulses in certain parts of the body. As a result, they decrease the heart's need for blood and oxygen by reducing its workload. They also help the heart to beat more regularly. The FDA first approved carvedilol in 1995.

Literature survey reveals that there is no RP-HPLC methods reported for quantification of carvedilol in pharmaceutical formulation. However, a few methods have been used for quantification of carvedilol in biological fluids. High-performance liquid chromatography (HPLC) with fluorescence and mass spectrometer detectors, RP-HPLC and HPTLC with UV detector and gas chromatography (GC)-MS detector have been reported. These method require longer runtime and do not represent degradants studied in this papers. Therefore, the present study aims to develop and validate a simple, fast and economical RP-HPLC method for estimation of carvedilol in pharmaceutical dosage forms.

To the best of our knowledge, no method available in the literature can separate all five impurities (A,B,C,D &E) together using only a 15-minute run time. The method was validated as per ICH guidelines.

The HPLC system will significantly decrease the time and cost per sample in the analytical process while improving the quality of the results.

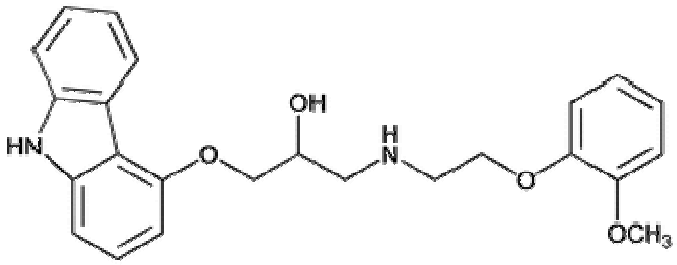
The chemical structures and names of Carvedilol and its impurities A,B,C,D &E depicted in Figure 1. Although five impurities are considered for method development and selectivity purposes, these impurities are not detected in the sample of Carvedilol.

EXPERIMENTAL SECTION

2.1 Chemicals:

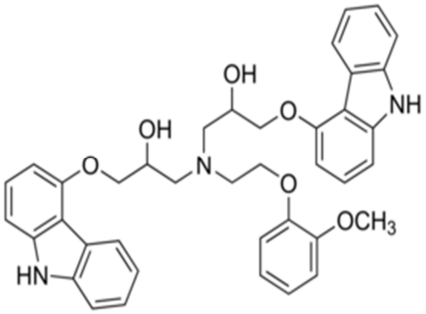
Samples of Carvedilol and its five impurities namely impurity A, B, C, D and impurity E (Fig.1) was received from Shodana Labs Pvt.Ltd, Hyderabad. Merck grade Methanol, Acetonitrile &Triflouro acetic acid was purchased from Merck, Darmstadt, and Germany. High purity water was prepared by using a Millipore Milli Q plus purification system.

Figure 1: Structure of Carvedilol and its impurities – A,B,C,D & E



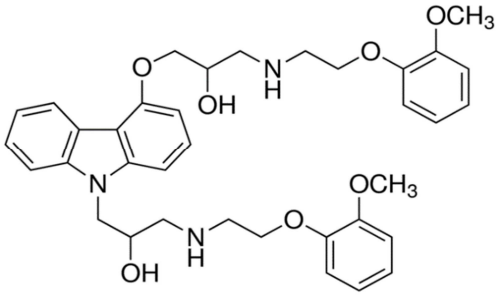
Molecular formula :$C_{24}H_{26}N_2O_4$
Molecular weight:406.47
Chemical Name**:** (2-Propanol, 1-(9H-carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy)ethyl]amino]-, (±)-; (±)-1-(Carbazol-4-yloxy)-3-[[2-(o-methoxyphenoxy)ethyl]amino]-2-propanol

Structure of Imp. A



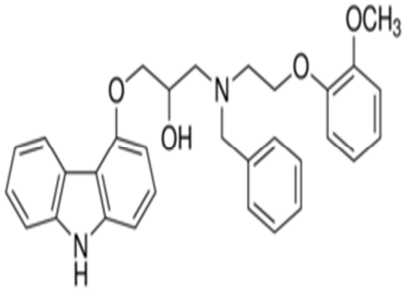
Molecular formula: $C_{36}H_{43}N_3O_7$
Molecular weight : 629.74
Chemical Name**:** 1-{{9-{2-Hydroxy-3-{[2-(2-methoxy- phenoxy)ethyl]amino}propyl propan-
}-9H-carbazol-4-yl}oxy}-3-{[2-(2-methoxy-phenoxy) ethyl] amino}-2-propanol.

Structure of Imp. B



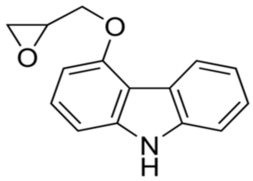
Molecular formula: $C_{39}H_{39}N_3O_6$
Molecular weight : 645.7
Chemical Name: 1,1′-{[2-(2-Methoxyphenoxy) ethyl] nitrilo}bis[3-(9H-carbazol-4-yloxy)-
2-ol.

Structure of Imp. C



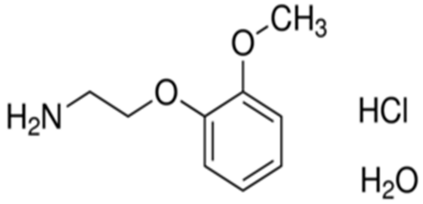
Molecular formula: $C_{31}H_{32}N_2O_4$
Molecular weight : 496.60
Chemical Name : (2RS)-1-{Benzyl[2-(2-meth-
oxyphenoxy)ethyl] amino}-3-(9H-carbazol-4-yloxy)-2-propanol

Structure of Imp. D



Molecular formula: $C_{15}H_{13}NO_2$
Molecular weight: 239.27
Chemical Name : 4-(2-Oxiranylmethoxy)-9H-carbazole

Structure of Imp. E



Molecular formula: $C_9H_{13}NO_2 \cdot HCl \cdot H_2O$
Molecular weight : 221.68
Chemical Name: 2-(2-Methoxyphenoxy)ethylamine hydrochloride monohydrate

2.2 Equipment:
The HPLC system used for the method development and validation consisted of gradient pumps from ShimadzuProminence, Ultra violet detector with dual nanometer from Shimadzu. Singapore, with autosampler and auto injector. The HPLC system was equipped with data acquisition and processing software "LC solutions software" Shimadzu. Singapore.

2.3 Preparation of Standard solutions:
A stock solution of Carvedilol was prepared by dissolving appropriate amount of substance in water and acetonitrile (1:1). Working solutions of 100µg /mL were prepared from the above stock solutions for the determination of related substance and assay. Stock solutions of impurities(mixture of Impurity A, B,C,D& E) at 0.1 mg/mL were also prepared in water and acetonitrile (1:1).

2.4 Chromatographic Conditions:
The Chromatographic separation was achieved on an Inertsil ODS 3V, 150mm X 4.6mm, ID with 5 microns particles column. 0.1% Trifluoro acetic acid, methanol and acetonitrile (500:300 :200v/v) used as a mobile phase. The mobile phase was filtered through nylon membrane (pore size 0.45 µm) and degassed by using vaccum pump and sonicate for 15 minutes prior to use. The flow rate of mobile phase was 1.5 mL/min. The column temperature was maintained at 55°C and wave length was monitored at 220 & 240 nm. The injection volume was 20µL. The standard and the test dilutions were prepared in water and acetonitrile (1:1).

2.5 Validation of the method:
2.5.1 Specificity:
Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities. The specificity of the developed HPLC method for Carvedilol was carried out in the presence of its impurities namely impurity A, B,C,D & E. Stress studies were performed for Carvedilol bulk drug to provide an indication of the stability indicating property and specificity of the proposed method. Intentional degradation was attempted to stress conditions of UV light (254nm), heat (70 °C), acid (0.5N HCl), base (0.5NNaOH),and Oxidation (3.0 % H_2O_2) to evaluate the ability of the proposed method to separate Carvedilol from its degradation products. For all degradation studies, period was 24 hours. Assay and related substance studies were carried out for stress samples against qualified Carvedilol reference standard. Assay was also calculated for Carvedilol samples by spikingall five impurities at the specification level (i.e., 0.1%).

2.5.2 Precision:
The precision of the assay method was evaluated by carrying out six independent assays of Carvedilol test samples against a qualified reference standard and calculate the %RSD of assay. The precision of the related substances method was checked by injecting six individual preparations of Carvedilol (0.1mg/ mL) spiked with 0.1 % of impurity A, B,C,D& E with respect to Carvedilol analyte concentration. %RSD of area for each impurity of A, B,C,D & E was calculated. The intermediate precision of the method was also evaluated using different analyst and different instrument in the same laboratory.

2.5.3 Limit of detection (LOD) and Limit of Quantification (LOQ):
The limit of detection and limit of quantification were determined at a signal to noise of 3:1 and10:1, respectively, by injecting a series of dilute solutions with known concentrations. Precision study was also carried out at the LOQ level by injecting five individual preparations of imp A, B,C,D& E and then calculated the %RSD of the peak area.

2.5.4 Linearity
Linearity test solutions for the assay method were prepared from Carvedilol stock solutions at five concentration levels from 50% to 250% of assay analyte concentration (50%, 100%, 150%, 200%, and 250%). The peak area verses concentration data was treated by least squares linear regression analysis.

Linearity test solutions for the related substance method were prepared by dilution of stock solution to the required concentrations. The solutions were prepared at five concentration levels from LOQ to 150% (100 µg/mL) of specification level of impurities namely - impurity A, B,C,D& E(LOQ 50% 75%, 100%, and 150%). Above test were carried out for 3consecutives days in the same concentration range for both assay and related substances method.The % RSD value for the Slope and Y-intercept of the calibration curve was calculated.

2.5.5 Accuracy:
The accuracy of the assay method was evaluated in triplicate at three concentration levels50%,100% and 150 % of test concentration (0.1 mg/mL).The percentage of recoveries were calculated. The accuracy study of impurities was carried out in triplicate at50%, 100%, & 150% of specification level (0.1%) to the Carvedilol analyte concentration (100µg /mL).The percentages of recoveries for impurities were calculated from the slope and Y- Intercept of the calibration curve.

2.5.6 Robustness:
To determine the robustness of the developed method, experimental conditions were deliberately altered and the resolution between Carvedilol and its impurity A, B,C,D& E was recorded. The effect of the methanol ratio in mobile phase preparation studied on resolution by varying by-5 to + 5 %, while other mobile phase components were held constant as stated in Chromatographic conditions. The column temperature was varied by -5 to + 5°C and flow rate of the mobile phase varied from – 0.1 to +0.1 mL/min.

2.5.7 Solution stability and Mobile phase stability:
The solution stability of Carvedilol in the assay method was carried out by leaving both the test solutions of sample and reference standard in tightly capped volumetric flasks at room temperature for 24 hrs. The same sample solutions were assayed for 6 hours interval up to the study period. The mobile phase stability was also carried out by assaying the freshly prepared sample solution against freshly prepared reference standard solution for 6 hours interval up to 48 hours. Mobile phase prepared was kept constant during the study period. The % RSD for the assay of Carvedilol was calculated during mobile phase and solution stability experiment. The solution stability of Carvedilol and its impurities in the related substance method was carried out by leaving spiked sample solution in tightly capped volumetric flasks at room temperature for 24 hours. Content of Impurity A, B,C,D& E were checked in the test solutions.

RESULTS AND DISCUSSION

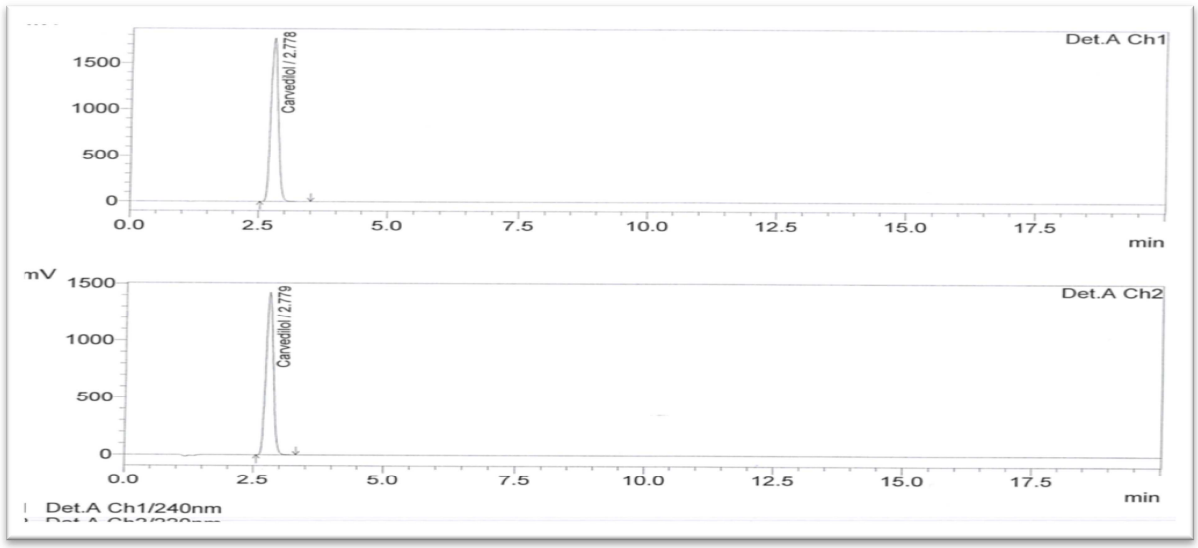
3.1 Optimization of Chromatographic conditions
The main objective of Chromatographic method is to separate Carvedilol from its impurities- A, B,C,D & E. Impurities were co-eluted using different stationary phases such as, Symmetry C18, 250mm X 4.6, 5µm,Zorabax C8, 150mm X 4.6, 5µm and X-Terra C18, 100mm X 4.6mm 5µm as well as different mobile phases. The Chromatographic separation was achieved on a Inertsil ODS 3V, 150mm X 4.6mm, ID with 5 microns particles column using a mixture of 0.1% Trifluoro acetic acid, methanol and acetonitrile (500:300 :200v/v) as mobile phase in an isocratic elution mode. The flow rate of the mobile phase was 1.5 mL / min, at 55 °C column temperature, the peak shape of the Carvedilol was found to be symmetrical. In optimized chromatographic conditions of Carvedilol, Imp –A,B,C,D& E were separated wither solution greater than 2, typical relative retention times were about .0.65, 4.54, 2.68,2.23 & 0.42respectively(Fig 2).The system suitability results are given in Table-1and developed HPLC method was found to specific for Carvedilol and its five impurities namely Imp –A,B,C,D &E(Fig .2).

Table-1:System suitability data

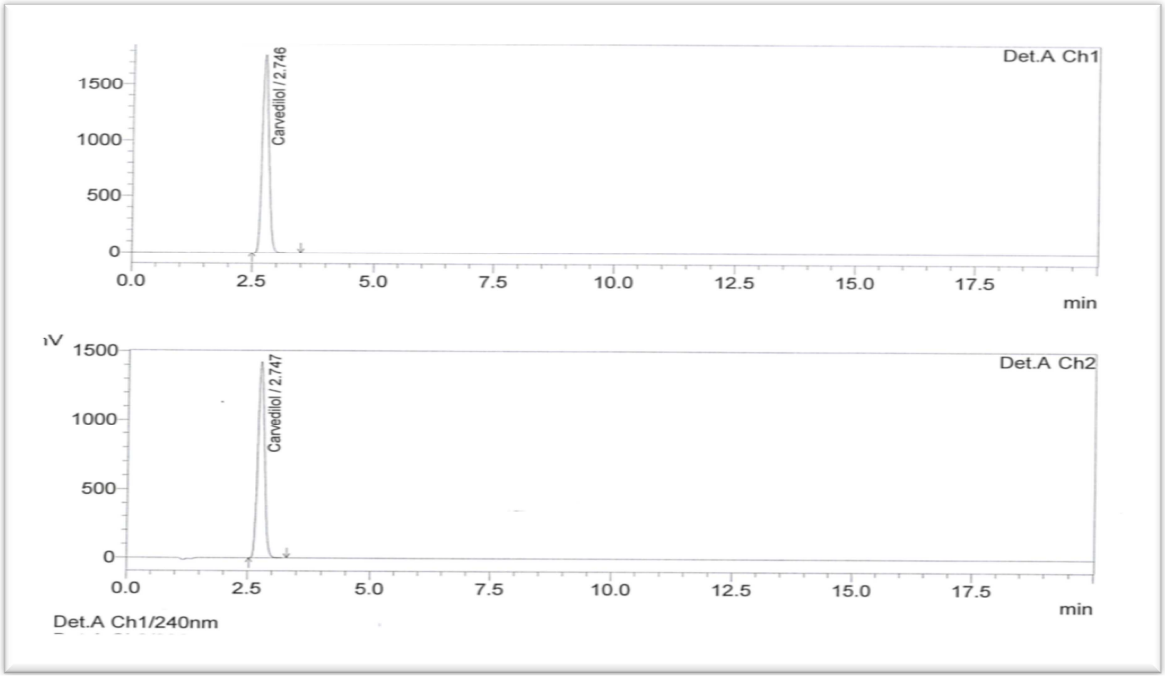
System suitability	Carvedilol	Imp. A	Imp. B	Imp.C	Imp. D	Imp.E
Rt	3.05	1.99	13.86	8.2	6.83	1.28
RRt	1	0.65	4.54	2.68	2.23	0.42
Rs	4.05	3.05	7.60	2.96	11.62	-
T	1.15	1.12	1.13	1.13	1.1	1.52
N	2500	2090	3468	3578	5200	2015

Fig.2:Chromatograms of (a) Carvedilol standard (b) Carvedilol sample (c)Carvedilol sample spiked with impurities

(a) Carvedilol Standard chromatogram



(b) Carvedilol sample chromatogram



(c) Carvedilol sample and spiked with impurities chromatogram

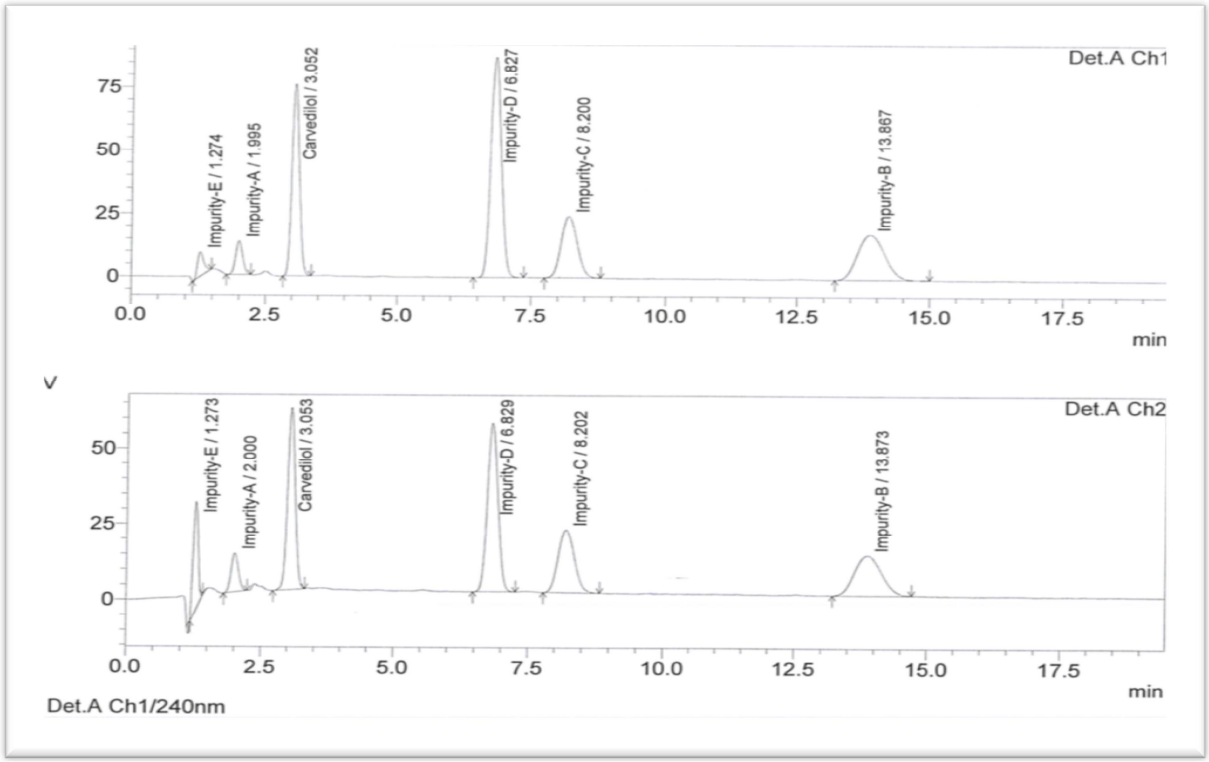
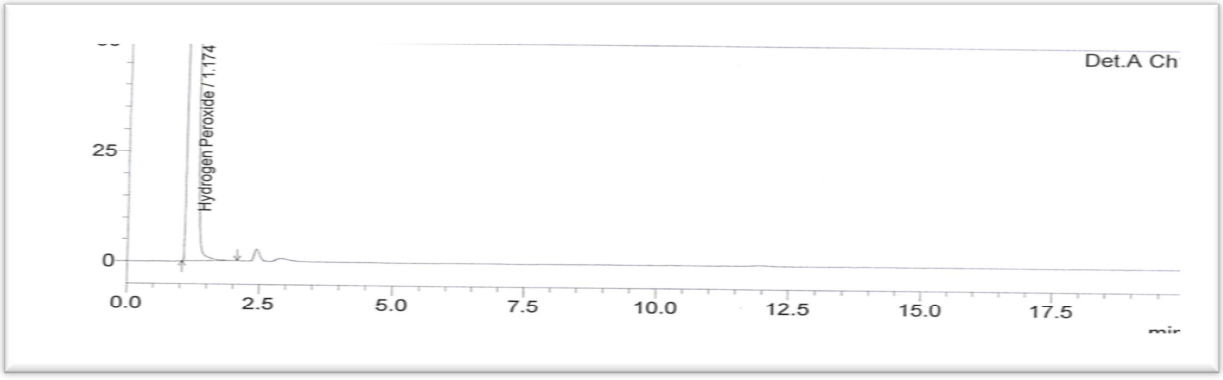
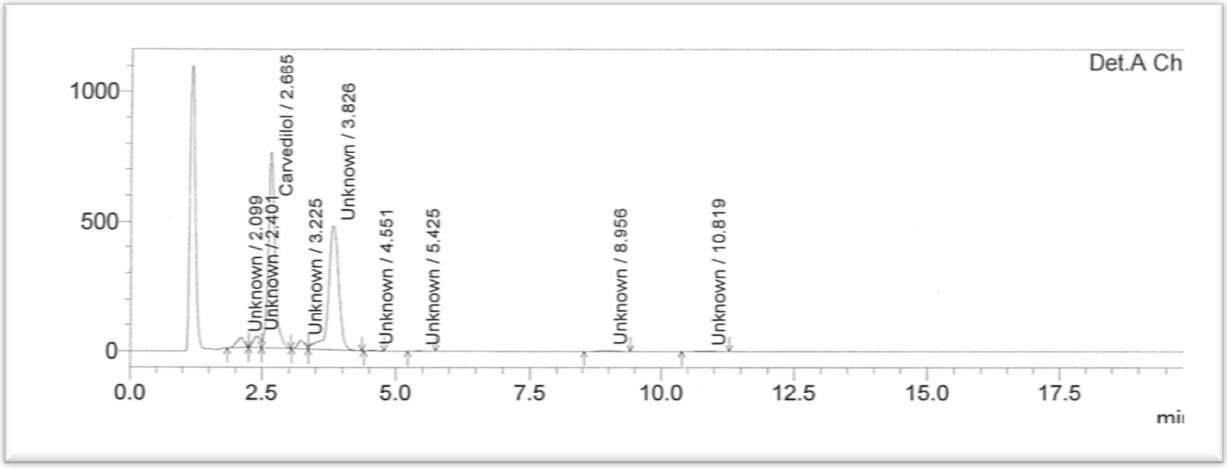


Fig.3: Stress study Chromatograms of Carvedilol a) peroxide blank b) peroxide degradation c) in base d) Photolytic degradation e) thermal f) acid degradation

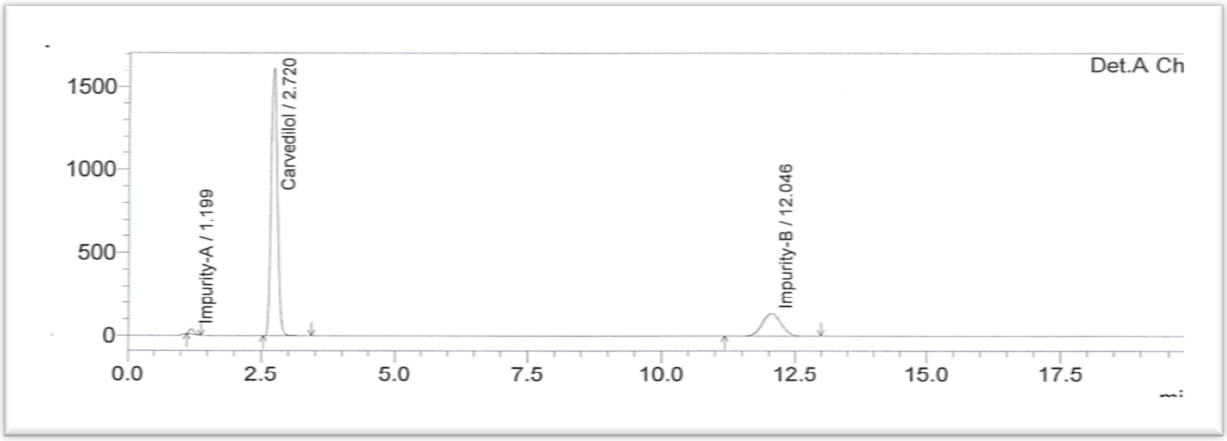
(a) Hydrogen peroxide blank chromatogram



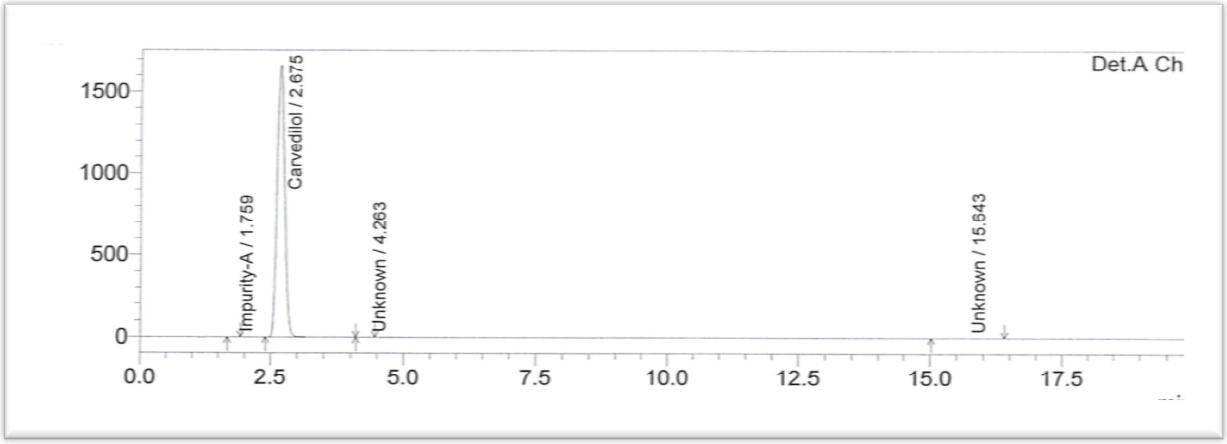
(b) Hydrogen peroxide degradation chromatogram



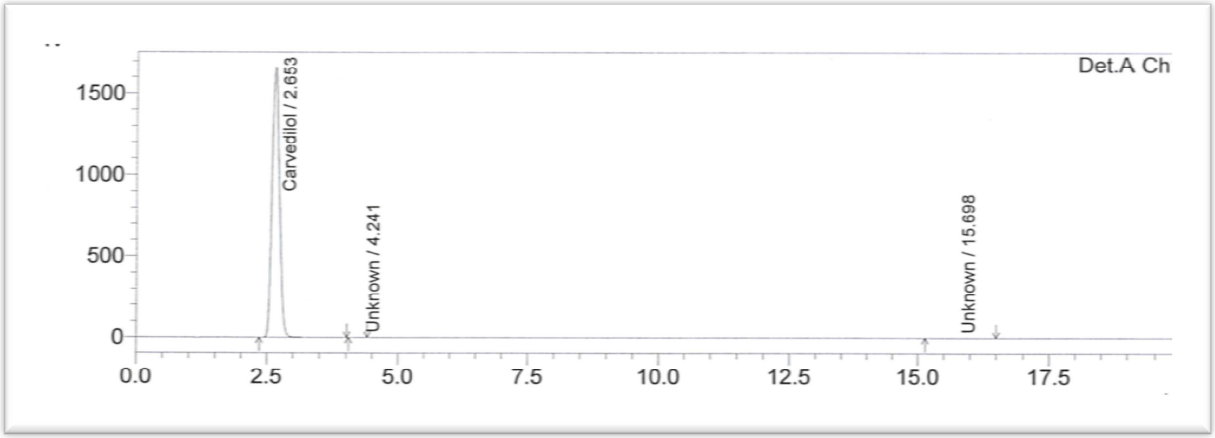
(c) Base degradation chromatogram



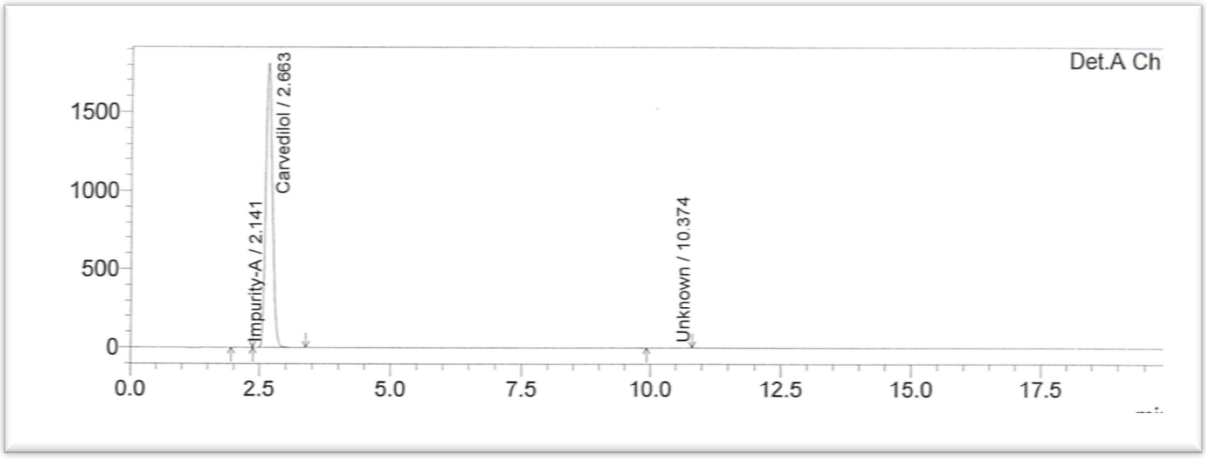
(d) Photolytic degradation chromatogram



(e) Thermal degradation chromatogram



(f) Acid degradation chromatogram



3.2 Results of forced degradation studies:

Degradation was not observed in Carvedilol sample when subjected to stress conditions like acid hydrolysis. Carvedilol was degraded to impurities A, B, C, D, E & unknown impurities under Oxidative hydrolysis, in base hydrolysis and heat and photolytic hydrolysis. **(Fig.3).**Peak purity test results confirmed that the Carvedilol peak is homogenous and pure in all the analyzed stress samples. The assay of Carvedilol is unaffected in the presence of impurities and its degradation products confirms the stability indicating power of the method. The summary of forced degradation studies is given in Table 2.

Table-2: Summary of forced degradation

Stress conditions	Time (hours)	Assay of Active substance (%)	Total impurities (%)	Mass balance (Assay + impurities (%))
Normal	24	100	-	100
Acid hydrolysis	24	99.97	0.03	100
Base hydrolysis	24	79.10	20.9	100
Oxidation(3%H_2O_2)	24	46.90	53.1	100
Thermal at 70 ^oC	24	99.87	0.13	100
UV light	24	99.88	0.12	100

3.3 Precision

The % RSD of assay of Carvedilol during the assay method precision study was within 0.10% and the %RSD for the area of impurity–A, B, C, D & E in related substances method precision study was within 3.42 %, 2.12%, 2.76%, 0.67% & 3.0%. The % RSD of the assay results obtained in the intermediate precision study was within1.6%,

%RSD for the area of A, B, C, D & E were well within 3.51%, 2.25, 3.0, 0.95 & 3.18 conforming good precision of the method.

3.4 Limit of detection (LOD) and Limit of Quantification (LOQ):

The limit of detection of all impurities A,B,C,D &E were achieved1.59, 1.59, 1.51, 1.55 &1.41 µg/mL respectively for 20 µL injection volume. The limit of quantification of impurities namely A, B, C, D & E are 4.81, 4.81, 4.60, 4.66 & 4.27, µg/mL for 20µL injection volume. The precision at the LOQ concentrations for Imp -A, B, C, D & E were below 7.5%.

Table-3 : Limit of Detection and Limit of Quantification

Name	Imp. A	Imp. B	Imp.C	Imp.D	Imp.E
LOD	1.59	1.59	1.51	1.55	1.41
LOQ	4.81	4.81	4.6	4.66	4.27

3.5 Linearity:

The linearity calibration plot for the assay method was obtained over the calibration ranges tested, i.e. 50 to 250 µg/ mL and correlation coefficient obtained was greater than 0.999. Linearity was checked for assay method over same concentration range for 3 consecutives days. The %RSD value of the Slope and Y-Intercept of calibration curve were 1.4 and 2.5 respectively. The result shows that an excellent correlation existed between the peak area and concentration of the analysis. Linear calibration plot for the related substances method was obtained over the calibration ranges tested i.e. LOQ to 150 % for Imp - A, B, C, D & E. The correlation coefficient obtained greater than 0.999. Linearity was checked for the related substances method over the same concentration ranges for three consecutives days. The %RSD values of the Slope and Y-intercept of calibration curve were 3.42, 2.12, 2.76, 0.67, and 3.0 respectively. The above results shows that an excellent correlation existed between the peaks area and the concentrations of Imp - A, B, C, D & E.

Table-4: Linearity data

Name	Carvedilol	Imp.A	Imp.B	Imp.C	Imp.D	Imp.E
Linearity (n=3)						
Intercept	1328283.5	-38.12	819.94	-1608.43	-78.24	40.57
Slope	14556568.5	2483.73	24992.16	16642.54	33436.72	1793.52
r	0.99978	0.99921	0.99969	0.99963	0.99972	0.99972

n, number of determinations

3.6 Accuracy

The percentage recovery of Carvedilol in bulk drug samples was ranged from100 to 100.5% (Table-5).The percentage recoveries of all five impurities in Carvedilol samples varied from 98.5-100.8%.

Table-5: Accuracy data

Name	Carvedilol	Imp.A	Imp.B	Imp.C	Imp.D	Imp.E
Accuracy % Recovery	100-100.5	98.5-100.5	99.0-100.5	99.5-100.5	99.0-100.8	99.1-100.6

3.7 Robustness

In all the deliberate varied chromatographic conditions (flow rate, composition of organic solvent & column temperature) the resolution between critical pair, i.e. Carvedilol and imp A,B,C,D&E was greater than 2.0, illustrating the robustness of the method.

3.8 Solution stability and Mobile phase stability

The % RSD of assay of Carvedilol during solution stability experiments were within 0.1%. No significant changes were observed in the content of impurities namely imp – A,B,C,D &E during the solution stability and mobile phase stability experiments when performed using the related substance method. The solution stability and mobile phase stability experiment data confirms that the sample solution and mobile phase used during the assay and the related substance determination were stable for 48 hours.

CONCLUSION

The RP HPLC method developed for quantitative and related substance determination of Carvedilol is linear, accurate, precise, rugged, rapid and specific. The method was fully validated showing satisfactory data for all method validation parameters tested. The developed method is stability indicating and can be conveniently used by quality control department to determine the related substance and assay in regular Carvedilol production samples and also stability samples. In the RP HPLC technique all the impurities were separated within a short time.

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