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Method development and forced degradation studies of carvedilol by RP-HPLC

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ABSTRACT

A simple, precise, rapid, accurate, selective and economic high performance liquid chromatographic (RP-HPLC) method has been developed for the estimation of Carvedilol in tablet dosage form. The method development can be achieved by Waters C_{18}, 5µm, 25cmx4.6mm i.d. column was used for the estimation of Carvedilol using Acetonitrile and Phosphate buffer and pH-6.00 can be adjusted with orthophosphoric acid as a mobile phase in the ratio of 60:40 v/v, at ambient temperature and the detector was set at wavelength 242nm.The method was validated for accuracy, precision, robustness and recovery studies. The recovery of the drug was found to be within the limits, relative standard deviation was found to be less than 2% for precession studies. The proposed method for quantitative estimation of Carvedilol was proved by method validation in accordance with the ICH guidelines. Carvedilol is subjected to stress conditions of acid, base, oxidation, and thermal and photolysis. Significant degradation is observed in acid and base stress conditions. The developed method can be successfully applied in the routine analysis of commercial pharmaceutical dosage forms. Degradation pathway for Carvedilol is established as per ICH recommendations by validated and stability-indicating reverse phase liquid chromatographic method.

Keywords: Carvedilol, RP-HPLC, Method development and Validation, ICH Guidelines.

INTRODUCTION

It is a non-selective β-blocker indicated in the treatment of mild to moderate congestive heart failure (CHF). It blocks beta-1 and beta-2 adrenergic receptors as well as the alpha-1 adrenergic receptors. Carvedilol is used in the management of congestive heart failure (CHF), commonly used as an adjunct to angiotensin-converting-enzyme inhibitor (ACE inhibitors) and diuretics[1][2][3]. It has been clinically shown to reduce mortality and hospitalizations in people with CHF. The mechanism behind its positive effect when used long-term in clinically stable CHF patients is not fully understood, but is thought to

contribute to remodeling of the heart, improving upon its structure and function. In addition, carvedilol is used in the treatment of hypertension and to reduce risk of mortality and hospitalizations in a subset of people following a heart attack. It can be used alone or with other anti-hypertensive agents.

Carvedilol is rapidly and extensively absorbed following oral administration, with an absolute bioavailability of approximately 25% to 35% due to a significant degree of first-pass metabolism.

Chemically it is 1-(9H-carbazol-4-yloxy)-3-{[2-(2-methoxyphenoxy) ethyl] amino} propan-2-ol and the molecular formula is $C_{24}H_{26}N_2O_4$ and the molecular weight is 406.4742 g/mol. The structure of Carvedilol is figure-1.

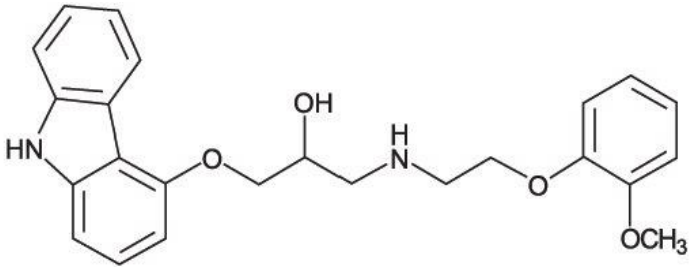

Fig-1: Chemical Structure of Carvedilol

The aim of the study is method development and validation of Carvedilol by RP-HPLC was not accurate showing deviations from system suitability parameters. It was found that the previous methods developed showed increased retention time and improper stability related values by using RP-HPLC method [4][5][6]. Hence the main objective of the present work is to develop a new accurate, precise method and validation of parameters including its stability related impurities. This new method was successfully developed and validated as per ICH guidelines; it can be utilized for the validation of Carvedilol in pharmaceutical dosage forms.

MATERIALS AND METHODS

Table-1: Instruments used

Sl. No.	Instruments/Equipments/Apparatus
1.	**WATERS HPLC** with Empower2 Software with Isocratic with UV-Visible Detector.
2.	**LABINDIA T-60** UV – Vis spectrophotometer
3.	Electronic Balance **(SHIMADZU ATY224)**
4.	Ultra Sonicator **(Wensar wuc-2L)**
5.	Thermal Oven
6.	**Waters ODS (C_{18}) RP Column, 250 mm x 4.6 mm,5µm**
7.	P^H Analyzer **(ELICO)**
8.	Triple Quartz Distillation Unit **(BOROSIL)**

Table-2: Chemicals Reagents and Standards used

		Specifications		
S.No.	**Name**	**Purity**	**Grade**	**Manufacturer/Supplier**
1.	Doubled distilled water	99.9.%	HPLC	Sd fine-Chem ltd; Mumbai
2.	Methanol	99.9%	HPLC	Loba Chem; Mumbai.
3.	Acetonitrile	99.9%	HPLC	Loba Chem; Mumbai.
4.	Potassium dihydrogen orthophosphate	99.9	L.R.	Sd fine-Chem ltd; Mumbai
5.	Orthophosphoric acid	99.99	L.R	Sd fine-Chem ltd; Mumbai

RESULTS AND DISCUSSION

Method development

HPLC instrumentation & conditions

The HPLC system employed was **WATERS** with Empower2 Software with Isocratic with UV-Visible Detector.

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent.(After optimization of all conditions) for UV analysis. It scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Carvedilol, so that the same wave number can be utilized in HPLC UV detector for estimating the Carvedilol [7][8]. While scanning the Carvedilol solution we observed the maxima at 242 nm. The UV spectrum has been recorded on LABINDIA make UV – Vis spectrophotometer model T-60. The scanned UV spectrum is attached in the following page,

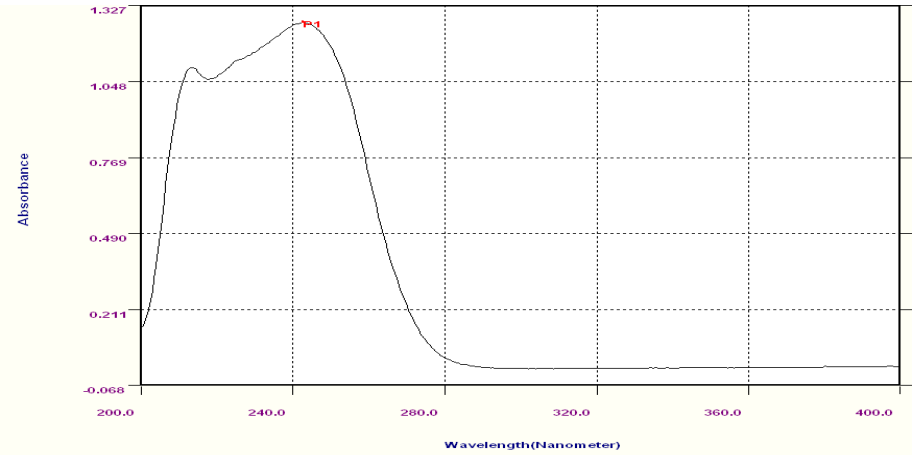

Fig-2: Uv Spectrum for Carvedilol

Sample & standard preparation for the analysis

25 mg of Carvedilol standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase.

Further dilution was done by transferring 0.4 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.

Optimization of chromatographic conditions

The chromatographic conditions were optimized by different means. (Using different column, different mobile phase, different flow rate, different detection wavelength & different diluents for sample preparation etc.

Table-3: Different trials used for method development

Column Used	Mobile Phase	Flow Rate	Wave length	Observation	Result
Waters C_{18}, 5µm, 25cmx4.6mm i.d.	Methanol : Water = 80 : 20	1.0 ml/min	242 nm	Low response	Method rejected
Waters C_{18}, 5µm, 25cmx4.6mm i.d.	ACN : Water = 40 :60	0.5 ml/min	242 nm	Broken peak	Method rejected
Waters C_{18}, 5µm, 25cmx4.6mm i.d.	ACN: water = 50 : 50	0.8 ml/min	242 nm	Tailing peak	Method rejected
Waters C_{18}, 5µm, 25cmx4.6mm i.d.	ACN: phosphate buffer = 50 : 50	1.2 ml/ min	242 nm	Broad Peak	Method rejected
Waters C_{18}, 5µm, 25cmx4.6mm i.d.	ACN : phosphate buffer = 60:40 (pH-6.0)	1.0 ml/min	242 nm	Nice peak	Method Accepted

Trial-1

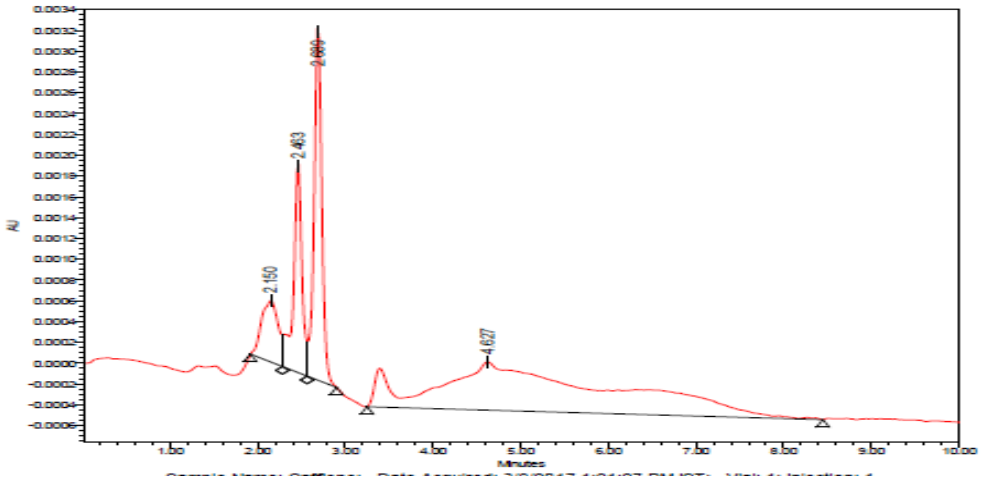

Fig-3: Chromatogram for Trial-1

Table-4: Results of Trial-1

Name	Rt	Peak Area	Tailing Factor	Plate Count
Carvedilol	2.689	102546	1.02	3125

Conclusion

Here More Impurity Peaks was observed, so method was rejected.

Trial-2

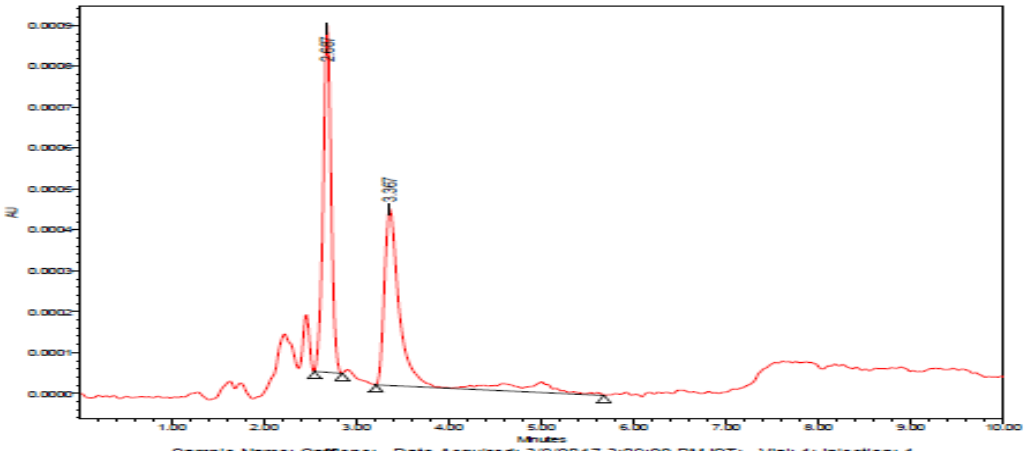

Fig-4: Chromatograms for Trial-2

Table-5: Results of Trial-2

Name	Rt	Peak Area	Tailing Factor	Plate Count
Carvedilol	2.687	125478	1.15	3251

Conclusion

Here along with the Peak tailing peaks was observed, so method was rejected.

Trial-3

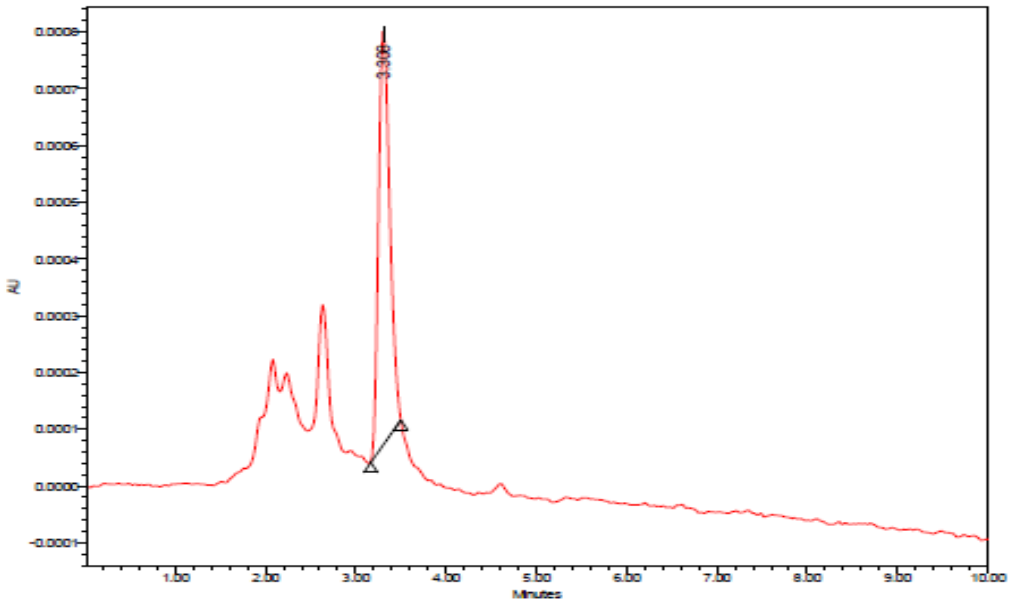

Fig-5: Chromatograms for Trial-3

Table-6: Results of Trial-3

Name	Rt	Peak Area	Tailing Factor	Plate Count
Carvedilol	3.308	135268	1.34	3125

Conclusion

Here also Peak was good but more tailing peaks were observed, so method was rejected.

Trial-4

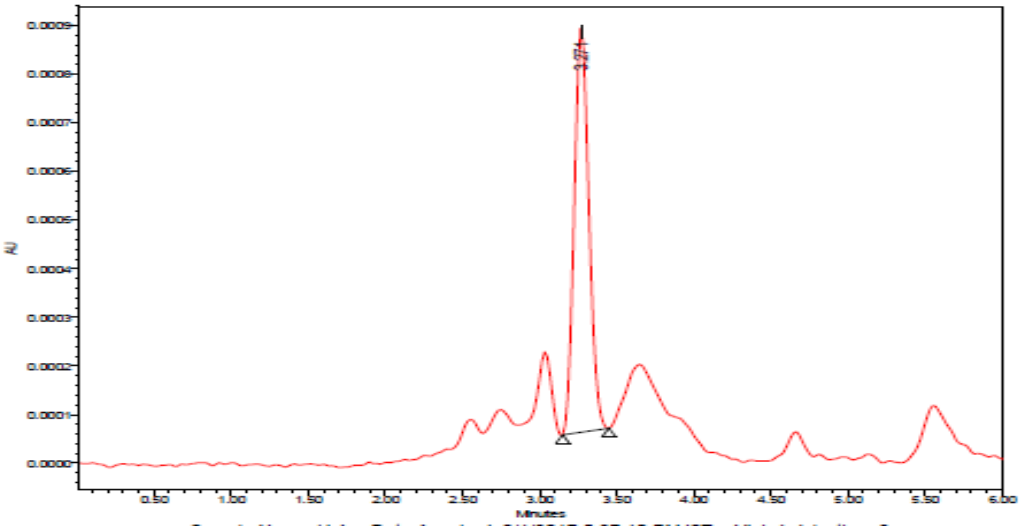

Fig-6: Chromatograms for Trial-4

Table-7: Results of Trial-4

Name	Rt	Peak Area	Tailing Factor	Plate Count
Carvedilol	3.271	10874	1.17	3541

Conclusion

Here Peak was good but tailing peaks were observed, so method was rejected.

Trial-5

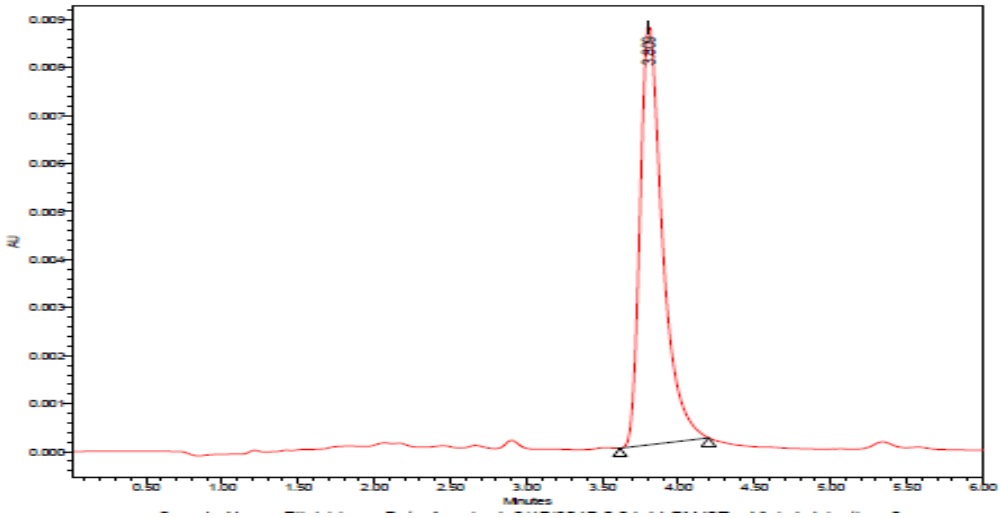

Fig-7: Chromatograms for Trial-5

Table-8: Results of Trial-5

Name	Rt	Peak Area	Tailing Factor	Plate Count
Carvedilol	3.809	104375	1.26	3134

Conclusion

Here Peak was good, so method was accepted.

SUMMARY OF OPTIMIZED CHROMATOGRAPHIC CONDITIONS

The Optimum conditions obtained from experiments can be summarized as below:

Table-9: Optimized Chromatographic Conditions

Mobile phase	Phosphate buffer (pH-6.0): Acetonitrile (40:60)
Column	Waters C_{18}, 5µm, 25cmx4.6mm i.d.
Wavelength	242nm
Flow rate	1.0 ml/ min.
Injection Volume	20 µl
Run time	06 min.
Column Temperature	Ambient

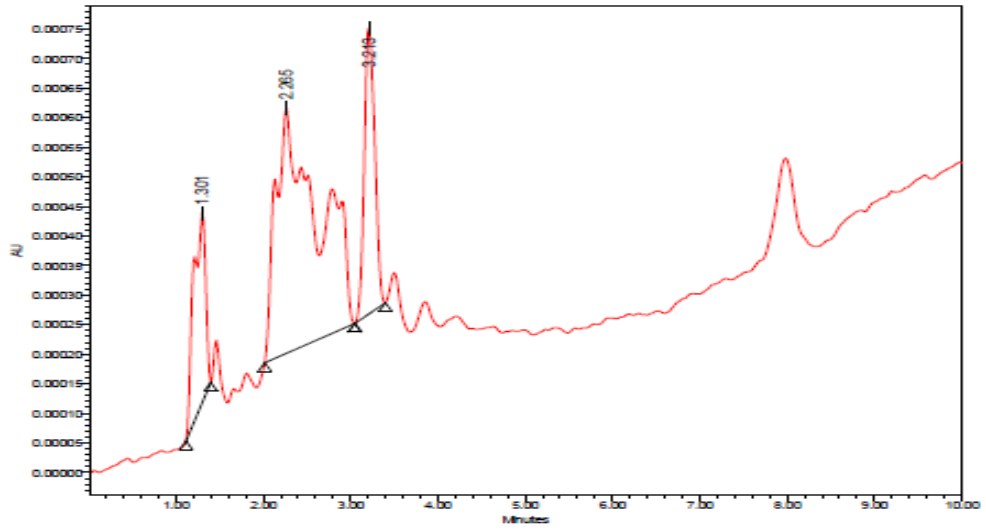

Fig-8: Chromatogram for Blank

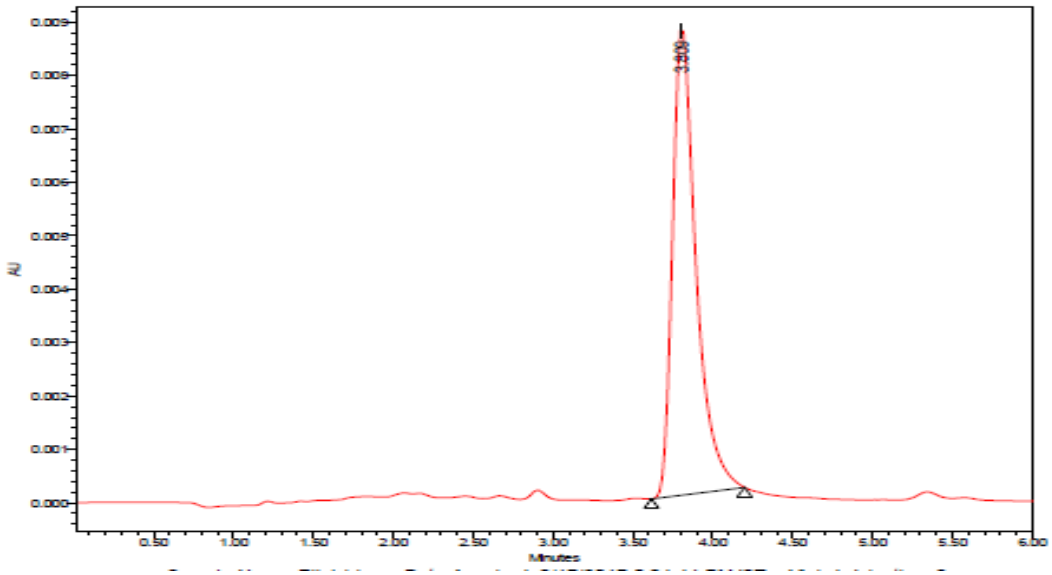

Fig-9: Optimised Chromatogram of Carvedilol

Table-10: Results of Optimized Chromatogram

Name	Rt	Peak Area	Tailing Factor	Plate Count
Carvedilol	3.809	104375	1.26	3134

FINAL RESULT & DISCUSSION

The selected and optimized mobile phase was mixture of Phosphate buffer (pH=6.00): Acetonitrile (40:60) and conditions optimized were: flow rate (1.0 ml/minute), wavelength (242 nm), Run time was 6.0 min. Here the peaks were separated and showed better resolution, theoretical plate count and symmetry. The proposed chromatographic conditions were found appropriate for the quantitative determination of the drug.

PREPARATION OF MOBILE PHASE

Mobile phase can be prepared by taking Phosphate Buffer (pH=6.00): Acetonitrile in the ratio of (40:60). Mobile phase was filtered through 0.45 µm membrane filter and degassed under ultrasonic [12] [13] [14] bath prior to use. The mobile phase was pumped through the column and maintained at a flow rate of 1.0 ml/min.

FORCED DEGRADATION STUDIES [15] [16]

Acid hydrolysis

Accurately weighed 25 mg. of pure drug was transferred to a clean & dry 25 ml volumetric flask [17] [18]. To which 0.1N Hydrochloric acid was added & make up to the mark & kept for 24 hrs. from that 1 ml was taken in to a 10 ml volumetric flask & make up to the mark with mobile phase, then injected into the HPLC system against a blank of HCl (after all optimized conditions).

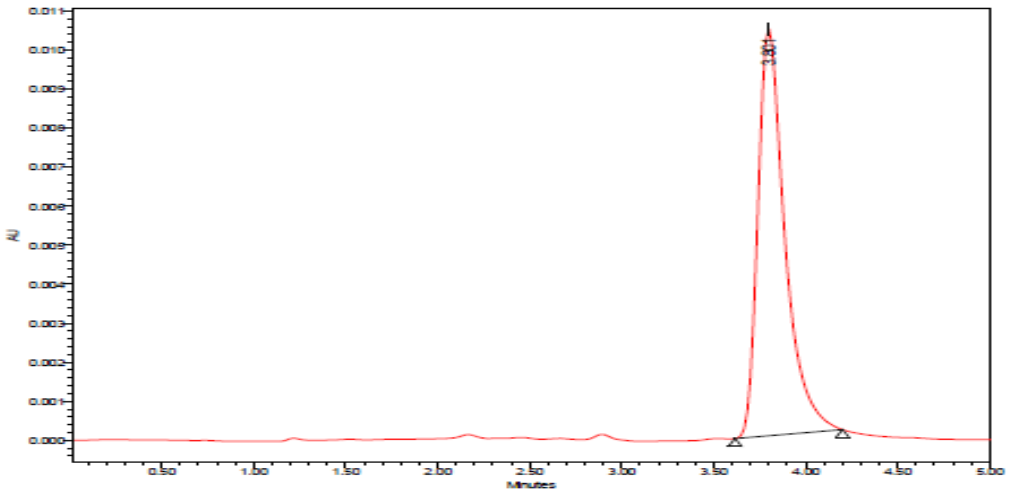

Fig-10: Chromatogram showing degradation for Carvedilol in 0.1 N HCl

Table-11: Results of Acid degradation

Name	Rt	Peak Area	Tailing Factor	Plate Count
Carvedilol	3.801	125250	1.29	3256

BASIC HYDROLYSIS

An accurately weighed 10 mg. of pure drug was transferred to a clean & dry 10 ml volumetric flask. To which 0.1N Sodium hydroxide was added & make up to the mark & kept for 24 hrs. From that 1 ml was taken in to a 10 ml volumetric flask & make up to the mark with mobile phase [19] [20], then injected into the HPLC system against a blank of NaOH (after all optimized conditions).

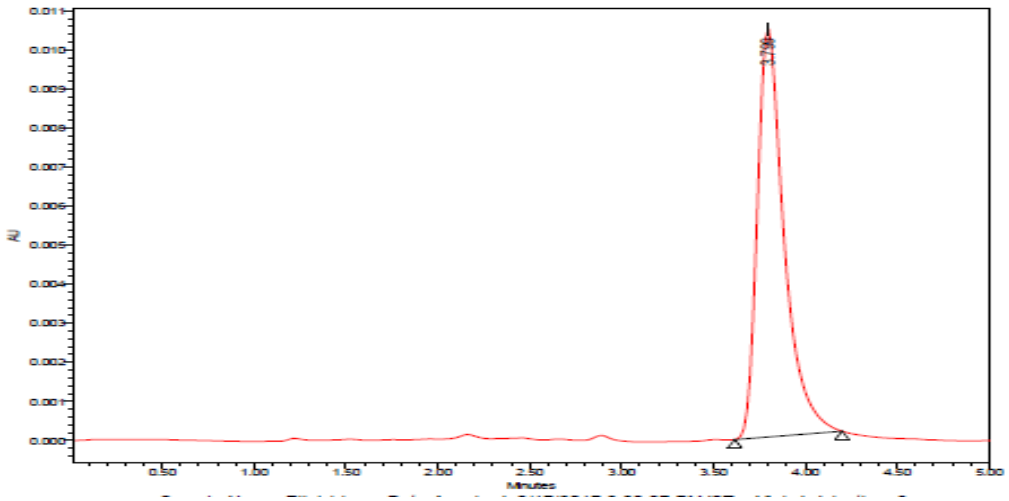

Fig-11: Chromatogram showing degradation related impurity in 0.1 N NaOH

Table-12: Results of Basic degradation

Name	Rt	Peak Area	Tailing Factor	Plate Count
Carvedilol	3.799	123162.5	1.45	3412

THERMAL DEGRADATION

An accurately weighed 10 mg. of pure drug was transferred to a clean & dry 100 ml volumetric flask, make up to the mark with mobile phase & was maintained at 50 ^{0}C. for 24 hrs[21] [22]. Then injected into the HPLC system against a blank of mobile phase (after all optimized conditions).

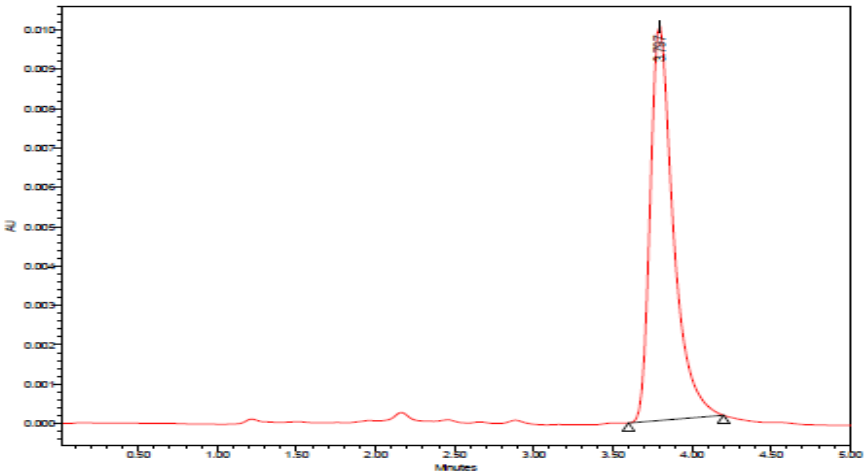

Fig-12: Chromatogram showing thermal degradation studies

Table-13: Results of Thermal degradation

Name	Rt	Peak Area	Tailing Factor	Plate Count
Carvedilol	3.797	117943.75	1.15	3256

PHOTOLYTIC DEGRADATION

Approximately 10 mg. of pure drug was taken in a clean & dry Petridis. It was kept in a UV cabinet at 254 nm wavelength for 24 hours without interruption. Accurately weighed 1 mg. of the UV exposed drug was transferred to a clean & dry 10 ml. volumetric flask [23] [24]. First the UV exposed drug was dissolved in methanol & make up to the mark.Then injected into the HPLC system against a blank of mobile phase (after all optimized conditions).

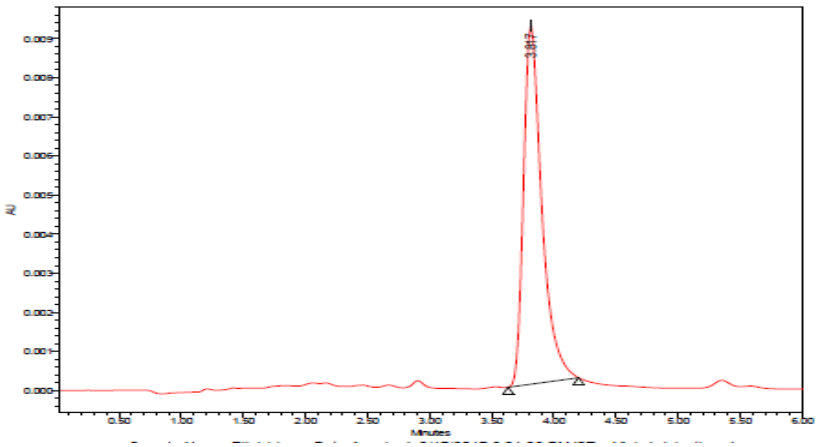

Fig-13: Chromatogram showing photolytic degradation

Table-14: Results of Photolytic degradation

Name	Rt	Peak Area	Tailing Factor	Plate Count
Carvedilol	3.817	109593.75	1.13	3652

OXIDATION WITH (3%) H_2O_2

Accurately weighed 10 mg of pure drug was taken in a clean & dry 100 ml. volumetric flask. 30 ml. of 3% H_2O_2 and a little methanol was added to it to make it soluble & then kept as such in dark for 24 hours [25] [26]. Final volume was made up to 100 ml. using water to prepare 100 ppm solution. The above sample was injected into the HPLC system.

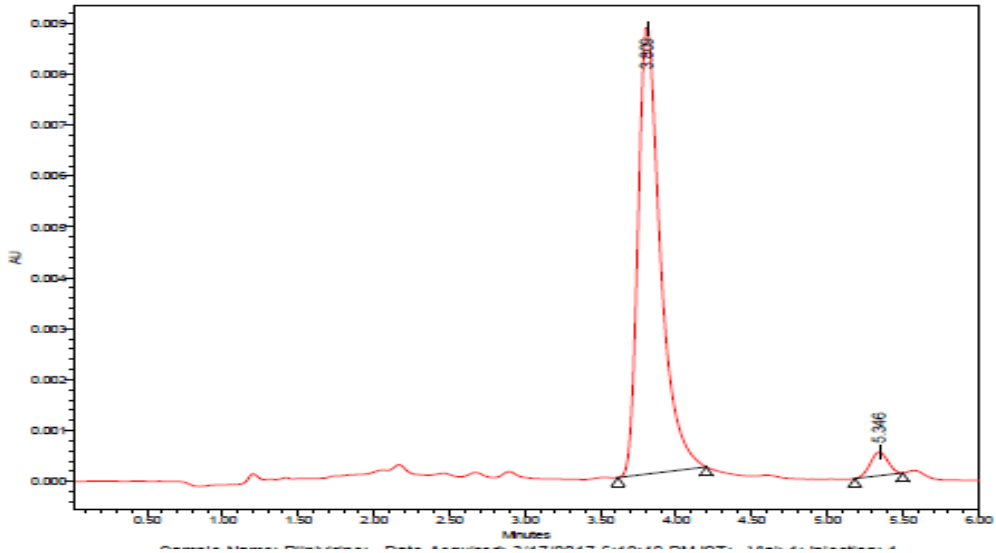

Fig-14: Chromatogram showing oxidative degradation

Table-15: Results of Oxidative degradation

Name	Rt	Peak Area	Tailing Factor	Plate Count
Carvedilol	3.809	106462.5	1.09	3657

Results of degradation Studies

The results of the stress studies indicated the **specificity** of the method that has been developed. Carvedilol was stable only in oxidation and photolytic stress conditions. The results of forced degradation studies are given in the following Table-16.

Table-16: Results of force degradation studies of Carvedilol API.

Stress condition	Time	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1M HCl)	24Hrs.	83.33	15.03	98.36
Basic Hydrolysis (0.1M NaOH)	24Hrs.	84.74	13.58	98.32
Thermal Degradation (50 ^{0}C)	24Hrs.	88.49	8.90	97.39
UV (254nm)	24Hrs.	95.23	4.30	99.53
3 % Hydrogen peroxide	24Hrs.	98.03	----------	99.03

CONCLUSION

The developed method, a new stability-indicating RPHPLC method has been developed for estimation of Carvedilol in tablet dosage form. The proposed method was validated and it was found to be simple, sensitive, precise, and robust and it can be used for the routine analysis of Carvedilol in tablet dosage form in the future.

The forced degradation studies were carried out in accordance with ICH guidelines and the results revealed suitability of the developed method is to study the stability of Carvedilol under various degradation conditions like acid, base, oxidative, thermal, UV and photolytic degradations.

Finally it was concluded that the method is simple, accurate, sensitive and has the ability to separate the drug from degradation products and excipients found in the dosage form.

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