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Original Article

RAPID, HIGHLY EFFICIENT AND STABILITY INDICATING RP-UPLC METHOD FOR THE QUANTITATIVE DETERMINATION OF POTENTIAL IMPURITIES OF CARVEDILOL ACTIVE PHARMACEUTICAL INGREDIENT

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ABSTRACT

Objective: The main objective of the proposed study was to develop a sensitive, rapid and stability indicating reverse phase UV-UPLC method for the quantitative determination of potential impurities in carvedilol.

Methods: The chromatographic separations were achieved on waters Acquity UPLC BEH C_{18} column (100 mm length 2.1 mm ID with 1.7 µm particle size, Waters corporation, MA, USA). Mobile phase A consisted, 0.04% trifluroacetic acid in water and mobile phase B consisted as 0.04% trifluroacetic acid in acetonitrile with a gradient programme (T_{min} A:B) $T_0$90:10, $T_4$65:35, $T_7$40:60, T_{10}20:80, $T_{10.1}$ 90:10. The column temperature was maintained at 60 ˚C and the detection was carried out at 240 nm. The flow rate was set to 0.5 mL/min.

Results: Efficient chromatographic separation was achieved on UPLC BEH C_{18} stationary phase in gradient mode using simple mobile phase. In forced degradation study, major degradation of the drug substance was found to occur under oxidative stress conditions to form carvedilol hydroxylamine. The method was validated according to ICH guidelines with respect to specificity, precision, linearity and accuracy. Regression analysis showed the correlation coefficient value greater than 0.999 for carvedilol and its five impurities. Detection limit of impurities was in the range of 0.002–0.004% indicating the high sensitivity of the newly developed method. Accuracy of the method was established based on the recovery obtained between 96.7% and 108.1% for all impurities.

Conclusion: A new, rapid and highly efficient UPLC method was developed, which separates all impurities and degradation products of carvedilol. The method has been validated in order to ascertain the suitability and stability indicating power of the method.

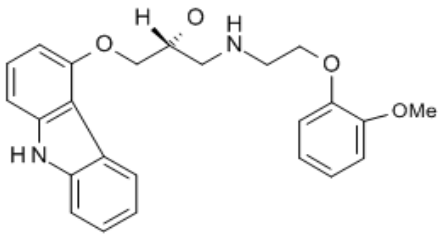
Keywords: Carvedilol, Impurities, RP-UPLC, Validation, Forced degradation.

INTRODUCTION

Carvedilol, (±)-1-(carbazol-4-yloxy)-3-((2-o-methoxyphenoxy) ethyl)amino)-2-propanol (Figure 1), is nonselective β-blocking agent with vasodilatation properties attributed mainly to its blocking activity at α_1-receptors. Carvedilol has much greater antioxidant activity than other commonly used blockers. It has been prescribed as an antihypertensive agent, an antiangina agent [1-4], and for treatment of congestive heart failure (CHF) [5].

Carvedilol is both a beta blocker (β1, β2) and an alpha blocker (α1), Norepinephrine stimulates the nerves that control the muscles of the heart by binding to the β1- and β2-adrenergic receptors. Carvedilol blocks the binding to those receptors [6] which both slows the heart rhythm and reduces the force of the heart's pumping. This lowers blood pressure thus reducing the workload of the heart, which is particularly beneficial in heart failure patients. Norepinephrine also binds to the α1-adrenergic receptors on blood vessels, causing them to constrict and raise blood pressure. Carvedilol blocks this binding to the α1-adrenergic receptors too [7], which also lower blood pressure. Carvedilol is a racemic compound and the stereoselectivity of the carvedilol enantiomers was established. The effects of the levorotatory S(-)-enantiomer are vasodilatation and beta blocking. The R(+)-enantiomer is a pure vasodilatation agent. HPLC methods for the determination of carvedilol related impurities were reported in USP, EP and BP [8-9]. Different analytical methods have been reported for the determination of carvedilol, its metabolites and enantiomers including liquid chromatography, liquid chromatography-mass spectrometry-mass spectrometry (HPLC/MS/MS), and electrophoresis [10-15].

Ultra Performance Liquid Chromatography (UPLC) is a relatively new technique giving new possibilities in liquid chromatography, especially concerning the decrease of time and solvent consumption. The separation on UPLC is performed under very high pressures (up to 100MPa is possible in UPLC system), but it has no negative influence on the analytical column or other components of chromatographic system.



Molecular weight = 406.49, Molecular formula = $C_{24}H_{26}N_2O_4$

Fig. 1: Structure of Carvedilol.

Separation efficiency remains maintained or is even improved comparing to the conventional system using 5 µm particle packed analytical columns. As it is very well known from Van Deemter equations, the efficiency of the chromatographic process is proportional to particle size decrease. This model describing band broadening, which explains the relationship between the height equivalent of the theoretical plate (HETP) and linear velocity, one of the terms (path dependent term), is dependent on a diameter of particle packed into the analytical column [16].

Detailed literature study shown that there are no UPLC methods reported for the quantification of carvedilol impurities. In the

present study, we report the development and validation of a new UPLC method, enabling the determination of carvedilol potential impurities and other degradation products.

MATERIALS AND METHODS

Reagents and Chemicals

Samples of carvedilol and standards of Imp-1, Imp-2, Imp-3, Imp-4 and Imp-5 (Table 1) were received from Deepta laboratories, Mysore, India. HPLC grade methanol and acetonitrile were purchased from Rankem, Mumbai, India. Deionized water was prepared using a Milli-Q plus water purification system from Millipore (Bedford, MA, USA). AR grade Trifluoro acetic acid purchased from Qualigens Fine Chemicals, Mumbai, India. Analytical reagent grade potassium dihydrogen phosphate, ammonium acetate, sodium hydroxide, hydrochloric acid, hydrogen peroxide and ortho phosphoric acid were purchased from Merck India Limited (Mumbai, India).

Instruments

Samples were analysed on Agilent 1290 Infinity UPLC equipped with PDA detector (Agilent technologies, CA, USA). The MS studies were performed on Agilent 1290 Infinity UPLC coupled with single quadrupole 6140 MS and chemstation software (Agilent technologies, CA, USA) using multi mode source electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). The typical source conditions were; gas: nitrogen, drying gas flow: 10 L/min, nebuliser pressure: 30psig, drying gas temperature: 250 ˚C, Vaporiser temperature: 200 ˚C, Capillary voltage: 3500V, corono current: 4.0 µA, charging voltage: 2000 V. ^{1}H spectra were recorded using a Bruker 400 MHz spectrometer (Bruker, Fallanden, Switzerland) equipped with a 5 mm BBO probe and a z-gradient shim system. The ^{1}H spectra were recorded with 1s pulse repetition time using 30˚ flip angle. Samples were dissolved in dimethyl sulphoxide-d6. The ^{1}H chemical shift values were reported on the δ scale in ppm relative to DMSO-d_6 (2.50 ppm).

Chromatographic conditions

The chromatographic separations were achieved on waters Acquity UPLC BEH C_{18} column (100 mm length × 2.1 mm ID with 1.7 µm particle size, Waters corporation, MA, USA). Mobile phase A consisted, 0.04% trifluroacetic acid in water and Mobile phase B consisted as 0.04% trifluroacetic acid in acetonitrile with a gradient programme (T_{min}A:B) $T_0$90:10, $T_4$65:35, $T_7$40:60, T_{10}20:80, $T_{10.1}$ 90:10 with a post-run time of 2.5 min. The column temperature was maintained at 60 ˚C and the detection was carried out at 240 nm. The flow rate was set to 0.5 mL/min. The test concentration was about 100 µg/mL and the injection volume was 3 µL. A degassed mixture of mobile phase A and mobile phase B in the ratio of 70:30 (v/v) was used as diluent during the standard and test samples preparations.

Sample preparation for forced degradation studies

Stress study is a complementary part of stability testing wherein influence of environmental factors like pH, temperature, humidity, oxygen and light are evaluated on a drug substance and products. Stress testing of the drug substance was performed as per ICH guidelines Q1 (R2) and it can help to identify the likely degradation products, which can in turn help to establish the degradation pathways, the intrinsic stability of the molecule and specificity of the proposed method. Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities. Acid hydrolysis was performed in 0.01N, 0.1N, and 0.5N HCl at 70 ˚C for 2 days. The study in basic solution was carried out in 0.01N, 0.1N, 0.5N, 1N NaOH at 70 ˚C for 7 days. For study in neutral solution, the drug dissolved in water and was kept at 70 ˚C for 7 days. Oxidation studies were carried out at ambient temperature in 1%, 5%, 10% and 20% hydrogen peroxide for 3days. Samples were withdrawn at appropriate times and subjected to LC analysis after suitable dilution (100 µg/mL) to evaluate the suitability of the proposed method to separate carvedilol from its degradation products. The excess of acid or base in volumetric flasks were neutralized and made up to the volume with diluent. Corresponding blank solutions were prepared following the sample procedure without carvedilol sample. Thermal degradation was done at 105 ˚C for 72 h on the solid sample. Photodegradation studies were carried out according to option 2 of Q1B in ICH guidelines. Photolytic degradation was performed by keeping 250 mg of each sample in two separate losses on drying (LOD) bottles in a photo stability chamber model TP 0000090G (Thermo Lab equipments Pvt. Ltd., Mumbai, India). One bottle was covered with lid and then with aluminium foil (dark control) whereas another bottle (photolytic exposed sample) was covered with lid to get a minimum exposure of 1.2 million lux hours for light and 200 Wh/m2 for ultraviolet region. A 0.1 mg/mL samples were prepared for thermal degradation and photolytic degradation samples.

Preparation of stock solutions for method validation

A test preparation of 100 µg/mL of carvedilol API sample was prepared by dissolving in diluent. A stock solution of impurities was prepared by dissolving 5 mg each of Imp-1, Imp-2, Imp-3, Imp-4, Imp-5, and 5 mg of carvedilol in diluent and made up to 50 mL with diluent. Transferred 1 mL of each individual stock solution into a 100 mL volumetric flask and made up to volume with diluent. From this stock solution, standard solution of 0.10 µg/mL of each impurity and 0.10 µg/mL of carvedilol was prepared.

RESULTS AND DISCUSSION

Method development

The main objective of the UPLC method development was to achieve efficient separation of impurities, degradation products generated during stress studies and a short run time of the method. All impurities are listed in Table-1 and named based on their elution pattern. Carvedilol has a basic in nature due to the presence of an amino group with a p*K*a value of 7.97. However other commonly used β-blockers has p*K*a value around 9.5 and the difference is attributed primarily to the inductive effect of the β-O-atom, which lowers the basicity of the amino group. As a LC method development rule, one should work within ±1.5 pH unit of the pKa value of the analyte for good pH control of the mobile phase. This assures that the analytes are either 100% ionized or 100% non-ionized and should help control run-run reproducibility. On consideration of the above points, an acidic mobile phase was chosen as a part of initial method development screening of carvedilol and its potential impurities. Carvedilol is a hydrophobic drug, almost insoluble in aqueous media and freely soluble in organic solvents, for example methanol and acetonitrile.

For the initial experiments, a BEH C_{18} column with 100 mm length × 2.1 mm ID column and 1.7 µm particle size was chosen. The oven temperature, detector wavelength and injection volume used were 25 ˚C, 240 nm and 5 µl were used. The optimal absorption wavelength for detection of the compounds was chosen especially with regard to absorption spectra of carvedilol impurities. Carvedilol and its impurities give higher detector response at 240 nm, therefore the final absorption wavelength for detection was chosen at 240 nm. UV spectra of carvedilol have been depicted in Figure 2. Many experiments were conducted to get a baseline resolution between carvedilol and impurities. The resolution between imp-1, imp-2 and carvedilol was poor when different UPLC columns viz; RP18, HSS and phenyl were used in different mobile phases containing phosphate, acetate and TFA along with acetonitrile, methanol, with pH ranging from 2 to 6. The optimum pH of the mobile phase selected was around in between 2.0 to 2.5 because at pH values higher than 3.0, a somewhat larger peak tailing and lack of resolution between impurities was resulted. And also at pH 2.5 for the mobile phase, carvedilol completely in ionized form and obtained a better peak shape. Use of C18 column with a 100 mm length × 2.1 mm ID column and 1.7 µm particle size, use of TFA as mobile phase modifier and acetonitrile–water mixture as mobile phase-B and column temperature at 60 ˚C was significant in achieving the desired resolution of carvedilol and its impurities. Studies on the effect of column temperature shown that a better baseline and low back pressure were observed at the higher temperature without affecting much resolution between impurities. After several trials for gradient profile, chromatographic conditions were finalized as described under section chromatographic conditions.

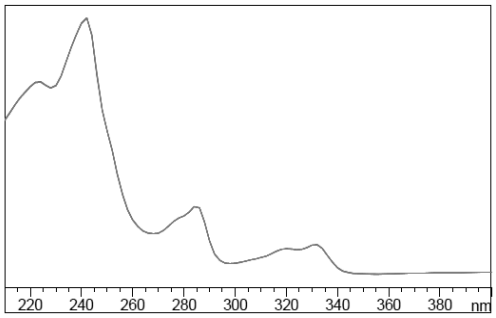


Fig. 2: UV absorption spectra of Carvedilol

Results of forced degradation

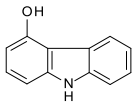
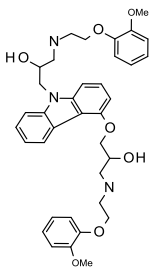
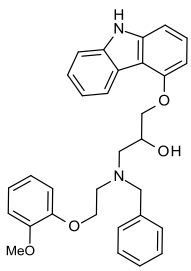
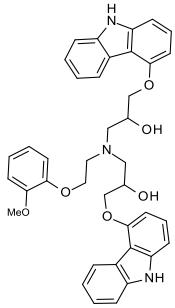
Carvedilol was found to be stable under stress conditions such as thermal, photolytic and basic hydrolysis conditions. According to a literature, a decomposition product as well as process related impurity 4-hydroxycarbozole was formed after carvedilol exposed to daylight almost 100 days. However, decomposition product was below 0.1% after keeping the sample such a long time. The newly developed method was able to separate this impurity from carvedilol and other known impurities. Significant degradation of the drug substance was observed under oxidative stress conditions leads to a major degradation product at RRT 0.93. LC/MS analysis was carried out to identify this degradation product of carvedilol using Agilent 6140 single quadrupole mass spectrometer. The degradation product shows the masses of 422 which is 16 higher masses than carvedilol mass 406. The impurity formed at RRT 0.93 was identified as shown in Table 1 and was formed due to the oxidation of secondary amine to hydroxyl amine after rearrangement of the N-oxide formation [17].

Acid hydrolysis stressed sample shown multiple peaks in the chromatogram. The LC/MS analysis has shown that mass spectra of the each peak having chlorine pattern indicating that the degradation products formed may be due to the side reaction between and carvedilol and hydrochloric acid.

Hence no further studies have been carried out to identify these degradation products. Chromatograms of forced degradation study have been depicted in Figure 4 and the summary report of forced degradation studies are depicted in Table 3.

Degradation studies and peak purity test results derived from PDA detector and LC/MS confirmed that the carvedilol peak was homogenous and pure in all the stress samples. The developed UPLC method was found to be specific in the presence of Imp-1, Imp-2, Imp-3, Imp-4, Imp-5 and their degradation products confirmed the stability indicating power of the newly developed method.

Table 1: Potential impurities of Carvedilol

S. No.	Structure	Mol. Wt.	IUPAC name	Code	Origin
1		183.21	4-hydroxy carbazole	IMP-1	Process
2		629.76	1-(4-(2-Hydroxy-3-(2-(2-methoxyphenoxy) ethylamino)propoxy)-9*H*-carbazol-9-yl) -3-(2-(2-methoxyphenoxy)ethylamino) propan-2-ol.	IMP-2	Process
3		496.61	1-(9*H*-Carbazol-4-yloxy)-3-(benzyl(2-(2-methoxyphenoxy)ethyl)amino)propan-2-ol	IMP-3	Process
4		645.76	3,3'-(2-(2-Methoxyphenoxy)ethylazanediyl) bis(1-(9*H*carbazol-4-yloxy)propan-2-ol).	IMP-4	Process

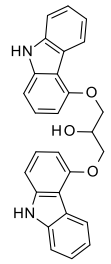
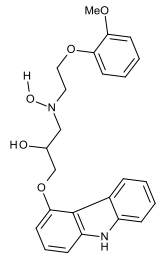
5		422.49	1,3-Bis(9H-carbazol-4-yloxy)-2-propanol	IMP-5	Process
6		422.49	*N*-[(2*RS*)-3-(9*H*-carbazol-4-yloxy)-2-hydroxy propyl]-*N*-[2-(2-methoxyphenoxy) ethyl] hydroxylamine.	-	Oxidative degradation impurity

Table 2: Method validation summary report

Parameter	Imp-1	Imp-2	Carvedilol	Imp-3	Imp-4	Imp-5
System suitability						
RT	4.30	4.50	4.86	6.26	6.64	7.30
RRT	0.88	0.93	1.00	1.29	1.37	1.50
Rs	-	3.42	6.28	23.79	7.76	11.66
N	57469	157825	79144	267067	300172	208317
T	1.05	1.06	1.08	1.29	1.06	1.10
Linearity						
r	0.9995	0.9973	0.9978	0.9994	0.9995	0.9992
Slope	74.88	20.11	32.144	29.232	38.52	55.093
Intercept	-0.1713	-0.0574	0.0198	-0.0397	0.0009	0.0164
Detection limit (%)	0.002	0.004	0.002	0.002	0.002	0.002
Quantitation limit (%)	0.006	0.01	0.006	0.006	0.006	0.006
Precision (QL)						
% RSD (n 6)	2.7	4.2	2.8	4.1	3.6	1.6
Repeatability (intra day)						
% RSD (n 6)	0.19	1.4	0.18	0.45	0.47	4.8
Intermediate precision (inter day)	0.54	1.4	0.24	0.74	0.91	5.0
% RSD (n 6)						
Accuracy at QL level (n 3)						
Amount added (%)	0.0060	0.0100	-	0.0060	0.0060	0.0060
Amount recovered (%)	0.0062	0.0101		0.0062	0.0064	0.0058
% Recovery	103.3	101.0		103.3	106.7	96.7
Accuracy at 80% level (n 3)						
Amount added (%)	0.08	0.08	-	0.08	0.08	0.08
Amount recovered (%)	0.0870	0.0823		0.0874	0.0840	0.0822
% Recovery	105.8	102.8		106.2	105.0	102.7
Accuracy at 100% level (n 3)		.				
Amount added (%)	0.10	0.10	-	0.10	0.10	0.10
Amount recovered (%)	0.1051	0.1039		0.1056	0.1046	0.1000
% Recovery	105.1	103.9		105.6	104.6	100.0
Accuracy at 120% level (n 3)						
Amount added (%)	0.12	0.12	-	0.12	0.12	0.12
Amount recovered (%)	0.1213	0.1218		0.1278	0.1308	0.1321
% Recovery	101.1	101.5		106.5	108.1	104.1

n, number of determinations; RT, retention time; RRT, relative retention time; Rs, USP resolution; N, number of theoretical plates; T, USP tailing factor; r, correlation coefficient.

Table 3: Summary report of forced degradation study

Stress condition	Time (h)	% Assay of active substance	% of degradation products	Mass balance* (%)
Acid Hydrolysis (0.5N HCl)	48	85.1	13.7	98.8
Basic Hydrolysis (1N NaOH)	168	99.6	-	99.6
Oxidation (20% H2O2)	72	75.6	22.7	98.3
Dry Heat (105 °C)	72	99.7	-	99.7
Wet Heat (70 °C)	168	99.2	-	99.2
UV (254 nm)	72	99.4	-	99.4

*It is the summation of assay of active substance and % of degradation products

Table 4: Mass and ^{1}H NMR chemical shift values

Name of impurity	Mass value (m/z) (M+H)	1H NMR chemical shift values, δ in ppm,
Carvedilol	407	8.2 (d, 1H, ArH), 7.45 (d, 1H, ArH), 7.3 (m, 2H, ArH), 7.1 (m, 2H, ArH), 6.8-7.0 (m, 4H, ArH), 6.67 (d, 1H, ArH), 4.1-4.3 (m, 5H, -O-CH2- and -CH), 3.71 (s, 3H, -CH3), 2.9-3.2 (m, 4H, -CH2).
IMP-1	184	8.15 (d, 1H, ArH), 7.4 (d, 1H, ArH), 7.35 (t, 2H, ArH), 7.15 (m, 2H, ArH), 6.9 (d, 1H, ArH), 6.55 (d, 1H, ArH).
IMP-2	630	8.26 (d, 1H, ArH), 7.61 (d, 1H, ArH), 7.33 (m, 2H, ArH), 7.23 (d, 1H, ArH), 7.15 (t, 1H, ArH), 6.82-6.98 (m, 8H, ArH), 6.72 (d, 1H, ArH), 3.91-4.50 (m, 10H, -CH2 and -CH), 3.70 (d, 6H, -CH3), 2.95 (m, 2H, -CH2), 2.95 (m, 2H, -CH2), 2.63 (m, 2H, -CH2).
IMP-3	497	8.19 (d, 1H, ArH), 7.4 (m, 3H, ArH), 7.27 (m, 5H, ArH), 7.06 (m, 2H, ArH), 6.89 (d, 1H, ArH), 6.82 (t, 1H, ArH), 6.69 (t, 1H, ArH), 6.61 (m, 2H, ArH), 3.95-4.24 (m, 5H, -O-CH2 and -CH), 3.82 (q, 2H, -CH2), 3.65 (s, 3H, -CH3), 2.75-2.93 (m, 4H, -CH2-).
IMP-4	646	8.24 (d, 2H, ArH), 7.41 (d, 2H, ArH), 7.28 (m, 2H, ArH), 7.21 (m, 2H, ArH), 7.06 (m, 4H, ArH), 6.85 (m, 2H, ArH), 6.57 (m, 4H, ArH), 3.9-4.2 (m, 8H, -O-CH2 and CH-), 3.59 (d, 3H, -CH3), 2.8-3.06 (m, 6H, -CH2).
IMP-5	423	8.25 (d, 2H, ArH), 7.45 (d, 2H, ArH),), 7.28 (m, 4H, ArH), 7.05 (m, 4H, ArH), 6.7 (d, 2H, ArH), 4.4-4.6 (m, 5H, -CH2- and CH.

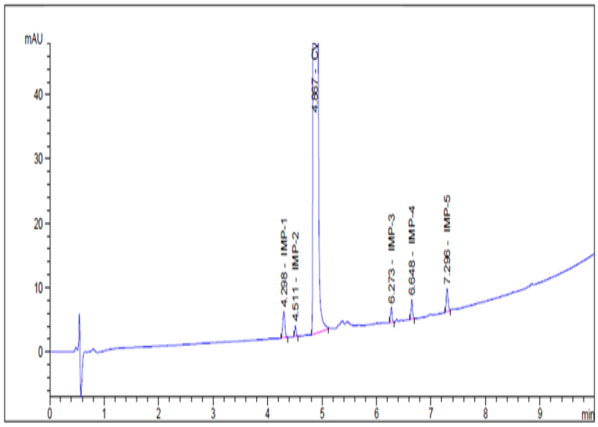


Fig. 3: Chromatogram of carvedilol spiked with impurities.

Structural Elucidation

Carvedilol and its all impurities (Imp-1, Imp-2, Imp-3, Imp-4, and Imp-5) were characterized with the help of MS and NMR spectroscopic techniques. The mass and ^{1}H NMR chemical shift values of these impurities are presented in Table 4.

Method validation

The newly developed method was validated for sensitivity, linearity, precision and accuracy, robustness and system suitability according to ICH guidelines [18]. Validation study was carried out for Imp-1, Imp-2, Imp-3, Imp-4 and Imp-5. The system suitability and selectivity were checked by injecting 100 µg/mL of carvedilol solution containing 0.1µg/mL of all impurities monitored throughout the validation. Method validation results are summarized in Table 2.

Limit of detection (LOD) and limit of quantitation (LOQ)

The limit of detection and limit of quantitation were determined for carvedilol and for each of the related substances as per ICH Q2R1 guideline. The LOD and LOQ for Imp-1, Imp-2, Imp-3, Imp-4 and Imp-5 and carvedilol were estimated at a signal-to-noise ratio of 3:1 and 10:1, respectively by injecting a series of diluted solutions with known concentration. The limit of detection and the limit of quantitation for imp-1, imp-3, imp-4, imp-5 and carvedilol were about 0.002% and 0.006% and for imp-2 was about 0.004% and 0.01% of analyte concentration i. e. 100 µg/mL respectively. The calculated LOQ concentrations of all the components were verified for precision by injecting six individual preparations of Imp-1, Imp-2, Imp-3, Imp-4, Imp-5, and carvedilol. The RSD of LOQ precision was in the range of 1.6–4.1 %.

Linearity and range

The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration of the analyte in the sample. A linearity test solution for related substance method was prepared by diluting the impurity stock solution to the required concentrations.

The solutions were prepared at six concentration levels from LOQ to 150% of the permitted maximum level of the impurity (i. e. LOQ, 0.02 µg/mL, 0.05 µg/mL 0.08 µg/mL, 0.10 µg/mL, 0.12 µg/mL, 0.15 µg/mL) was subjected to linear regression analysis with the least squares method. Calibration equation obtained from regression analysis was used to calculate the corresponding predicted responses.

The residuals and sum of the residual squares were calculated from the predicted responses. The correlation coefficient obtained was greater than 0.99 for all impurities. The result showed an excellent correlation between the peak and concentration of all impurities. The range of the method was from LOQ to 0.15 µg/mL of the analyte concentration (100 µg /mL).

Precision

Precision of the method was studied for method precision and intermediate precision. Method precision was checked by injecting six individual preparations of (100 µg/mL) carvedilol spiked with 0.1µg/mL of each impurity. In the intermediate precision study, the similar procedure of method precision was carried out by a different day. % RSD of areas of each impurity was within 5.0, confirming good precision at low level of the developed analytical method.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value, which is accepted either as a conventional true value or an accepted reference value and the value found. The accuracy of the method was evaluated in triplicate at LOQ, 80% level (0.08µg/mL), 100% level (0.1µg/mL) and 120% level (0.12µg/mL). The percentage recovery of all impurities in drug substance has been calculated. Chromatogram of carvedilol spiked with five impurities was depicted in Figure 3.

Robustness

To evaluate the robustness of the developed method, the chromatographic conditions were deliberately altered and the resolution between closely eluting peaks that is, Imp-1 and Imp-2 was evaluated. Close observation of analysis results of deliberately changed chromatographic conditions viz; flow rate (0.5±0.05 mL/min), Mobile phase composition (± 2% acetonitrile) and column temperature (60±5 ˚C) revealed that resolution between Imp-1 and Imp-2 was greater than 2.0 and no significant change in relative retention time for all impurities in spiked sample illustrating the robustness of the method.

Solution stability and mobile phase stability

The solution stability of carvedilol and its related impurities was carried out by leaving both spiked and unspiked sample solutions in tightly capped UPLC vials at 25 ˚C for 48 h in an auto sampler. Content of each impurity was determined against freshly prepared standard solution. No significant changes were observed in the

content of any of the impurities. The solution stability and mobile phase stability experiment data confirms that the sample solutions and mobile phases used during related substance determination were stable for at least 48 hour.

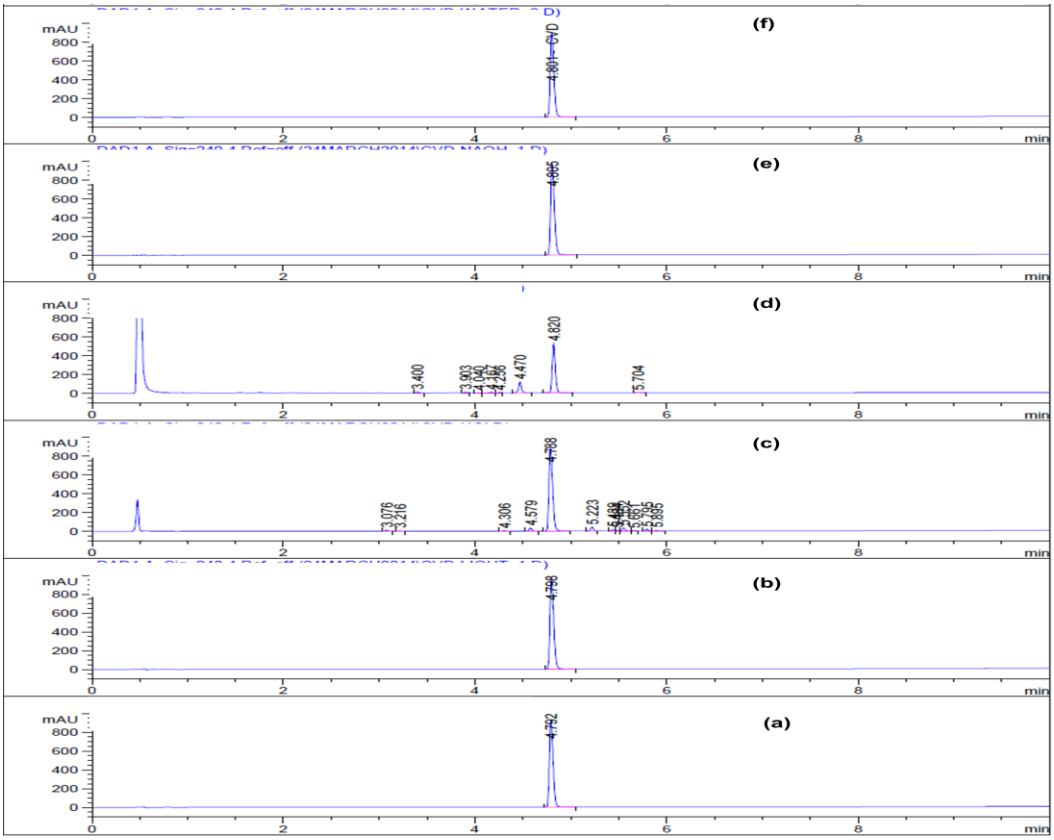


Fig. 4: (a) Typical chromatogram of carvedilol under stress conditions: (a) thermal degradation, (b) photolytic degradation, (c) acid hydrolysis,(d) oxidative degradation, (e) base hydrolysis and (f) in water

CONCLUSION

In this paper, a sensitive, selective, specific, accurate, validated and well-defined stability indicating UPLC method for the quantification of carvedilol process related impurities and degradation products were described. The major oxidative degradant was identified as carvedilol hydroxylamine. Detection limit for impurities was found to be as low as 0.002% and was found to have excellent resolution between impurities and carvedilol. This newly developed method has been validated as per regulatory requirements and can be used for routine and stability studies for the quantitative determination of potential impurities in carvedilol drug substance.

CONFLICT OF INTERESTS

Declared None

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