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*Short communication*

SHORT COMMUNICATION

# Monitoring of the photochemical stability of carvedilol and its degradation products by the RP-HPLC method

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*Abstract:* A sensitive, selective, precise and stability-indicating, new high-performance liquid chromatographic method for the analysis of carvedilol both as a bulk drug and in formulations was developed and validated. As the method could effectively separate the drug from its degradation products, it can be employed as a stability-indicating one. The method was validated for linearity, selectivity, precision, robustness, LOD, LOQ and accuracy. The chromatographic separation was achieved on a Chromolit RP 8e, 100 × 4.6 mm, analytical column. The mobile phase consisted of a mixture of acetonitrile and water (45:55, V/V) (pH 2.5), pH adjusted with formic acid. The absorbance was monitored with a UV detector at 280 nm and the temperature of the analyses was 40 °C. The flow rate was 0.5 mL/min. The linearity ($r \geq 0.999$), reproducibility (0.68–1.27 %) and recovery (99.71–101.58) were found to be satisfactory. This method enables the simultaneous determination of carvedilol and its degradation products, as well as stability.

*Keywords:* carvedilol, 4-hydroxycarbazole, RP-HPLC, stability.

## INTRODUCTION

Carvedilol is a non-selective beta-receptor blocking agent and a vasodilatation drug with antioxidant activity.[1] It has been confirmed that carvedilol shows a far greater antioxidant activity than other commonly used beta-blockers.[2,3] Carvedilol is used in the treatment of mild to moderate hypertension and angina pectoris,[4,5] congestive heart failure (CHF)[6] and is often used in combination with other drugs. Chemically it is named (±)-1-(carbazol-4-yloxy)-3-((2-(*o*-methoxyphenoxy)ethyl)amino)-2-propanol. Carvedilol is a racemic compound.

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.

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Carvedilol is officially recognized in the European Pharmacopoeia in the pure form, but not in dosage forms. Different analytical methods have been developed for the determination of carvedilol, its metabolities and enantiomers including liquid chromatography,[7–12] liquid chromatography-mass spectrometry-mass spectrometry (HPLC/MS/MS),[13] and electrophoresis.[14–16] There are also some published reports of carvedilol determination by spectrofluorimetry[17] and differential pulse voltammetry.[18] However, there are neither published reports of the quantification assay of carvedilol in the presence of its impurities nor stability studies of this drug.

Therefore, the aim of this work was to determine the optimal conditions for simultaneous separation and determination of carvedilol and its potential impurities in pharmaceutical dosage forms, as well as to monitor the photochemical stability of carvedilol. A new stability-indicating RP-HPLC method was validated for the simultaneous determination of 4-hydroxycarbazole, impurity C and carvedilol. Impurity C, chemically named (2*RS*)-1-[benzyl-[2-(2-methoxyphenoxy)ethyl]amino]-3-(9*H*-carbazol-4-yloxy)propan-2-ol is a Pharmacopoeia and 4-hydroxycarbazole a non-Pharmacopoeia impurity.

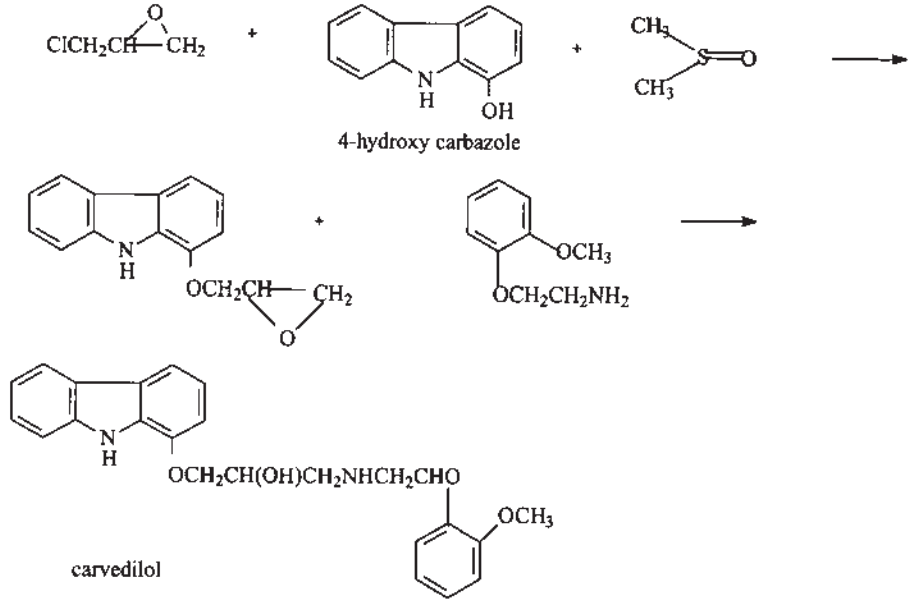


Fig. 1. Synthesis of carvedilol.

The chemical structures of carvedilol and its degradation product 4-hydroxycarbazole are given in Fig. 1. As can be seen, 4-hydroxycarbazole is used for the synthesis of carvedilol, hence it is questionable whether it is a synthesis impurity. This topic could be the subject of some future investigations.

## EXPERIMENTAL

*Chemicals and reagents*

A standard of carvedilol was supplied by NOSCH, Labs Private Limited, India. A standard of 4-hydroxycarbazole was obtained from Sigma-Aldrich (Gmbh, Germany). Reagent-grade acetic acid and formic acid were provided by Lachema (Brno, Czech Republic). HPLC-grade water, acetonitrile and methanol were supplied by Merck, Darmstadt, Germany. Impurity C was also supplied by Merck.

*Instrumentation and chromatographic conditions*

The chromatographic analyses were performed with a liquid chromatograph HP HPLC 1100, a Rheodyne 7725i injector valve with a 20 μL loop, and HP model G13141A UV-VIS variable wavelength detector.

The Analytiical column was Chromolit RP 8e, 100 × 4.6 mm. The mobile phase consisted of acetonitrile, water (45:55, V/V) (pH 2.5), pH adjusted with formic acid. Isocratic elution was performed at a flow rate of 0.5 mL/min. The UV-detection was performed at 280 nm. Injections were carried out using a 20 μL loop. All separations were carried out at a temperature of 40 °C.

*Preparation of samples and standard working solutions*

*Stock solutions*

The stock solution of 4-hydroxycarbazole had a concentration of 0.1 mg/mL; the stock solution of impurity C had a concentration of 0.2 mg/mL; the stock solution of carvedilol had a concentration of 0.1 mg/mL.

All the samples and standard working solutions were prepared in the mobile phase in the required concentrations and filtered through a 0.45 μm membrane filter.

*Calibration solutions*

A series of 5 standard solutions of 4-hydroxycarbazole were prepared by dilution of the stock solution to obtain a concentration range of 0.5–1.1 μg/mL. Calibration solutions for carvedilol were prepared by diluting the stock solution to obtain concentrations of 16–24 μg/mL. Calibration solutions for impurity C were prepared by diluting the stock solution to obtain concentrations of 0.96–1.44 μg/mL.

*Samples preparation*

Karvileks$^{R}$ tablets were used for the examinations. One tablet contains 12.5 mg active substance for carvedilol and other ingredients. The average mass of the tablets is 125 mg.

Sample solutions were prepared by first making stock solutions. Twenty tablets were weighed and finely powdered. Then the quantity of the powder containing 12.5 mg of carvedilol for assay (solution 1) and 50 mg for related substances (solution 2) were weighed in a 50 mL volumetric flask and about 30 mL of mobile phase was added to each one. Both flasks were sonicated in an ultrasonic bath for 30 min. The volume was made up to the mark with mobile phase, mixed well and filtered. For assay, 2.0 mL of solution 1 was diluted to 25.0 mL with mobile phase. The concentrations of carvedilol were 0.02 mg/mL for assay and 1 mg/mL for related substances.

For monitoring the photochemical stability, carvedilol powder (1 g), distributed in a thin layer (<1 mm) on a small Petri dish, was exposed to continuous daylight for 100 days at room temperature (25 °C). The other sample was kept in a dark place to compare degradation with and without daylight exposition. The quantity of the powder containing 25 mg of carvedilol was dissolved in 25 ml of mobile phase and sonicated in an ultrasonic bath for 15 min (the concentration of the sample preparation were 1 mg/mL).

*Standard preparation*

The working standard solution for related substances contained carvedilol in a concentration of 1 mg/mL, 4-hydroxycarbazole in a concentration of 0.001 mg/mL and impurity C in a concentration of 0.002 mg/mL. The working standard solution for the test assay contained carvedilol in a concentration of 0.02 mg/mL.

## RESULTS AND DISCUSSION

### *Optimum conditions for the chromatographic procedure*

The combined effects of pH and mobile phase composition of the reverse-phase liquid chromatographic behavior of carvedilol and 4-hydroxycarbazole were studied. The effects of these factors were examined in the range of conditions where they provided acceptable retention, resolution, tailing factor and number of theoretical plates. pH between 2.1–2.7 has no significant influence on the results. The optimum pH was 2.5 because at pH values higher than 2.7, a somewhat larger peak tailing and inferior resolution resulted. In comparison with other commonly used β-blockers (p$K_a$ around 9.5), carvedilol has a p$K_a$ value of 7.97. The origin of the discrepancy in the p$K_a$ values between carvedilol and other β-blockers is attributed primarily to the inductive effect of the β-O-atom, which lowers the basicity of the amino group.[19] Different ratios of acetonitrile and water, between 30:70 to 70:30 were investigated and the composition of acetonitrile and water in mobile phase of 45:55 was found to be optimal, because higher concentrations of water disturbed the separation and resolution. If methanol was used instead of acetonitrile, the separation of carvedilol and the impurities was also with unsatisfactory resolution.

As is shown in Fig. 2, the retention times of carvedilol and its related substances were 3.4 min, 5.2 min, and 6.0 min, respectively. The separation of carvedilol from 4-hydroxycarbazole and impurity C is shown in Fig. 2.

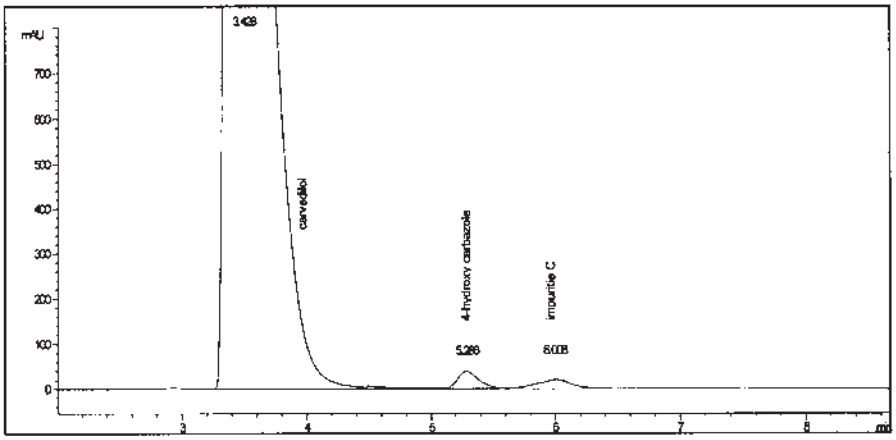


Fig. 2. Chromatogram showing the separation of carvedilol, 4-hydroxycarbazole and impurity C.

### *Quantitative determination*

The HPLC method was tested for selectivity, linearity, precision and accuracy. The response (peak area) was proportional to concentration over the tested ranges, *i.e.*, between 16–24 μg/mL for carvedilol, between 0.5–1.5 $\mu$g/mL for 4-hydroxycarbazole and between 0.96–1.44 $\mu$g/mL for impurity C. The parameters of calibration curve: slope (*a*), intercept (*b*), correlation coefficient (*R*) are given in the following regression equations:

$y = 70531x + 13.708$ for carvedilol $R^2 = 0.9992$

$y = 131040x + 4.230$ for 4-hydroxycarbazole $R^2 = 0.9995$

$y = 93442x - 5.424$ for impurity C $R^2 = 0.9993$

The selectivity of HPLC method was confirmed by injecting blank samples, placebos and standard solutions. No other peaks corresponding to the retention times of carvedilol and its impurities were noted, indicating that interfering substances were not present.

The precision of analytical system was investigated by using standard solutions. Six consecutive replicate injections of each sample gave relative standard deviations of 0.68 % for carvedilol, 1.02 % for impurity C and 0.87 % for 4-hydroxycarbazole.

The accuracy of the method was provided by the determination of 4-hydroxycarbazole and impurity C in the presence of carvedilol. A solution ($c$ = 1 mg/mL) containing carvedilol with no detectable amount of impurities was spiked with aliquots of the impurities at adequate concentrations. The limit of detection (LOD) was calculated to be three times the standard deviation of the noise ratio from the analysis of each compound.

The limit of quantification (LOQ) was measured as the lowest amount of analyte that can be reproducibly quantified above the baseline noise, for which duplicate injection resulted in a RSD ≤ 2 %. The validation parameters of the HPLC method are shown in Table I.

TABLE I. Validation parameters of the HPLC method

| Sample | RSD/% | Recovery/% | LOD/μg mL$^{-1}$ | LOQ/μg mL$^{-1}$ |
|---|---|---|---|---|
| Carvedilol | 0.68 | 101.58 | 0.007 | 0.026 |
| Impurity C | 1.02 | 100.43 | 0.042 | 0.061 |
| 4-Hydroxycarbazole | 0.87 | 99.71 | 0.036 | 0.048 |

*Stability study*

The purpose of the stability testing was to investigate how the quality of a drug product changes with time under the influence of environmental factors. Since daylight-induced degradation of the drug may have a negative impact on the quality, safety and effectiveness of pharmacotherapy, research focused on this point is definitely reasonable.

The RP-HPLC method for monitoring of the photochemical stability of carvedilol is presented here. Carvedilol was exposed to daylight. The presence of degradation products was observed. The percentage (%) of the degradation products were observed every 20 days for 100 days. The results are given in Table II.

TABLE II. Degradation products (%)

| Degradation products/time of exposure | 0 day | 20 day | 40 day | 60 day | 80 day | 100 day |
|---|---|---|---|---|---|---|
| 4-Hydroxycarbazole | 0.01 | 0.012 | 0.028 | 0.044 | 0.065 | 0.081 |
| Impurity C | – | – | – | – | – | – |
| Unknown impurity | – | 0.016 | 0.038 | 0.062 | 0.087 | 0.11 |

4-Hydroxycarbazole was detected in the exposed sample by RP-HPLC analysis. The impurity C– (2*RS*)-1-(2-(2-methoxyphenoxy)ethyl)amino)-3-(9*H*-carbazol-4-yloxy)propan-2-ol was not detected after this period under the described conditions. The presence of 4-hydroxycarbazole in the exposed sample of carvedilol was confirmed by agreement of $t_R$ value (HPLC) of the standard solution of 4-hydroxycarbazole and $t_R$ value of the solution of carvedilol after exposition to daylight. This agreement was also confirmed by application of an additional amount of standard solution (4-hydroxycarbazole) to the sample and a significant increase of the peak area was determined. Fig. 3 shows the chromatogram of a sample after 100 days of exposition to daylight. In addition, the presence of another unkown degradation product is visible on this chromatogram with a retention time of 7.68 min.

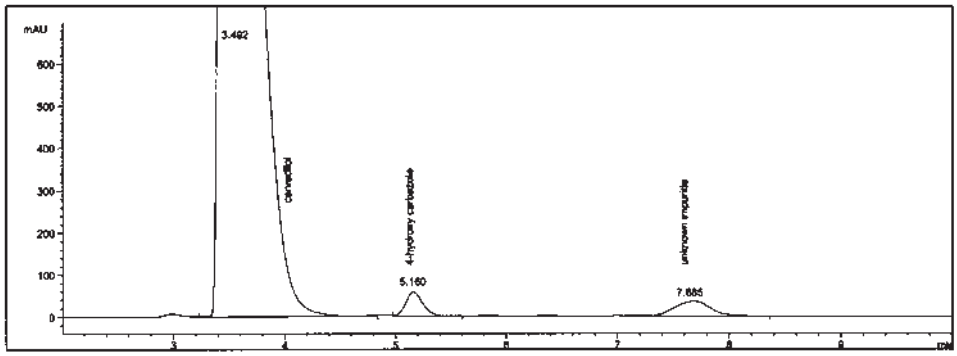


Fig. 3. Chromatogram of degraded carvedilol after a 100-day exposition to daylight.

## CONCLUSION

On the bases of the experimental results, the proposed method is suitable for the simultaneous qualitative and quantitative determination of carvedilol and related substances in pharmaceutical formulations, as well as for use for stability testing.

The method provides great sensitivity, adequate linearity and repeatability. It is also quicker and simpler for sample preparation than already offered procedures. The chromolit column guarantees better peak shape, better resolution and lower pressure during operation in comparison with previous methods.

It was shown that carvedilol is a relatively photostable compound. Carvedilol can be exposed to daylight to obtain its decomposition products. As the main decomposition product, 4-hydroxycarbazole was found by RP-HPLC. 4-Hydroxycarbazole was also determined in the sample of the raw material, hence it can be

supposed that it is a synthesis impurity. Impurity C was not found under the described conditions. The chromatogram of the exposed sample of carvedilol shows that some other impurity is formed. A further study is necessary for the identification of this decomposition product.

## ИЗВОД

## ПРАЋЕЊЕ ФОТОХЕМИЈСКЕ СТАБИЛНОСТИ КАРВЕДИЛОЛА И ЊЕГОВИХ ДЕГРАДАЦИОНИХ ПРОИЗВОДА RP-HPLC МЕТОДОМ

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Карведилол је неселективни бета блокатор са вазодилататорним и антиоксидативним својствима. Примењује се у терапији хипертензије, ангине пекторис и конгестивне срчане инсуфицијенције. Развијена је и валидирана нова HPLC метода за истовремену квалитативну и квантитативну анализу карведилола и његових нечистоћа као и за праћење фотохемијске стабилности овог једињења. Метода је валидирана на линеарност, селективност, прецизност, робустност, LOD, LOQ и тачност. Оптимални услови постигнути су коришћењем хроматографске колоне Chromolit RP 8e, 100 × 4,6 mm. Мобилну фазу представља смеша ацетонитрила и воде у односу 45:55 при pH 2,5. Анализа се одвија на температури од 40 °C при протоку 0,5 ml/min а таласна дужина UV детектора износи 280 nm. Линеарност ($r \geq 0{,}999$), репродуктивност (0,68–1,27 %), и рикавери (99,71–101,58) показују задовољавајуће вредности. Хроматографска анализа је показала да се као главни производ деградације карведилола јавља 4-хидроксикарбазол, док нечистоћа C није присутна под овим условима. Предложена RP-HPLC метода је брза, прецизна, тачна, осетљива, поуздана и применљива за квалитативну и квантитативну анализу Карвилекс$^R$ таблете, као и за праћење стабилности овог лека.

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