ORIGINAL ARTICLE

HPLC STUDIES FOR ASSESSING THE STABILITY OF CARVEDILOL TABLETS

ANNE-MARIE CIOBANU[1#], ANCA LUCIA POP[1#]*, SIMONA CRIŞAN[2#], MARIANA PALI[2#], GEORGE TRAIAN ALEXANDRU BURCEA-DRAGOMIROIU[1#], DANIELA ELENA POPA[1#], DUMITRU LUPULIASA[1#], MARIA BÂRCĂ[1#]

[1] *"Carol Davila" University of Medicine and Pharmacy, Faculty of Pharmacy, 6 Traian Vuia Street, Bucharest, Romania*
[2] *"AC HELCOR" Pharmaceutical Company, R&D Centre, 6 Victor Babeş Street, Baia Mare, Romania*

**corresponding author: anca.pop@umfcd.ro*
[#] *All authors have equal contribution.*

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Abstract

Stability studies evaluate the effect of environmental factors on the quality of the drug product and they are developed to estimate the shelf life and the storage conditions. These studies should be designed following the guidelines issued by ICH, WHO and EMA, and they involve cost and time consumption, but also scientific expertise. All current guidelines recommend that the stability-indicating nature of the analytical procedure should be demonstrated by inclusion of forced degradation studies results in the validation of the procedures, together with chromatograms of the stressed samples. In the current study, two validated stability-indicating HPLC methods were used: one for the assay and one for the related substances. The stability of carvedilol tablets was demonstrated, both in accelerated (6 months) and long term conditions (36 months).

Rezumat

Studiile de stabilitate evaluează efectul factorilor înconjurători asupra calității produselor medicamentoase și se desfășoară pentru a estima perioada de valabilitate și condițiile de păstrare ale acestora. Aceste studii trebuie proiectate respectând cerințele ghidurilor ICH, OMS și EMA și necesită costuri considerabile prin necesarul de resurse financiare și de timp, dar și de personal cu expertiză științifică. Toate ghidurile în vigoare recomandă să se demonstreze natura indicatoare de stabilitate a metodei analitice prin includerea în datele de validare a rezultatelor studiilor de degradare forțată și prezentarea cromatogramelor probelor degradate. În prezentul studiu s-au utilizat două metode HPLC indicatoare de stabilitate, validate: una pentru dozarea substanței active și una pentru determinarea impurităților înrudite. A fost demonstrată stabilitatea comprimatelor cu carvedilol, atât în condiții accelerate (6 luni), cât și în condiții pe termen lung (36 luni).

Keywords: stability studies, stability testing, carvedilol, HPLC

Introduction

The stability of a pharmaceutical product represents the capability of a particular formulation in a specific closure system to remain within its physical, chemical, microbiological, toxicological, protective and informational specifications [3]. Stability testing is a complex process completed in order to ensure the preservation of the product quality, safety and efficacy throughout the shelf life and it is mandatory for the approval of any pharmaceutical product. Stability testing evaluates the effect of environmental factors on the quality of the drug product and it is developed to estimate the shelf life, the storage conditions and the labelling instructions. These studies should be designed following the guidelines issued by ICH, WHO and EMA and they are cost and time consuming, but also offer scientific expertise.

One of the essential analytical tools used in assessing drug product stability is the High Performance Liquid Chromatography (HPLC) [1, 4]. HPLC can separate, detect and quantify the active ingredient but also the impurities formed during manufacturing and storage of the drug product [8]. In order to determine the degradation pathways and degradation products formed during storage, forced degradation studies should be conducted [2]. These studies demonstrate the stability-indicating nature of the analytical procedure. All current guidelines recommend the inclusion of the results of forced degradation studies in the validation of the procedure, including chromatograms of the stressed samples. A stability-indicating method is "a validated quantitative analytical procedure that can detect changes in a quality attribute(s) of the drug substance and drug product during storage" [12]. A stability-indicating method accurately measures the active ingredients, without interference from process impurities, degradation products, excipients, or other potential impurities [9].

Carvedilol or (±)-1-(carbazol-4-yloxy)-3-((2-(o-methoxyphenoxy)ethyl)amino)-2-propanol is a non-selective beta-blocking agent used in the treatment of mild to moderate hypertension and *angina pectoris* [5, 7] or in congestive heart failure [6], alone or in combination with other drugs. Carvedilol is a racemic compound. Both enantiomers are active: the levo-rotatory S(-)-enantiomer effects are vaso-dilatation and beta blocking, while the R(+)-enantiomer effect is vasodilatation.

No compendial analytical procedures for testing carvedilol dosage forms are available in the European Pharmacopoeia, therefore any analytical procedure used for quality control of carvedilol tablets should be validated. Different analytical methods have been developed for the determination of carvedilol in tablets, however no long term or accelerated conditions stability studies of this drug were reported in the literature.

In this context, we investigated the stability of 6.25 mg carvedilol tablets, both in accelerated and long term conditions using two validated stability-indicating HPLC methods: one for the assay and one for the related substances.

Materials and Methods

Three batches of Carvedilol® 6.25 mg tablets packed in Al/PVC blisters kindly provided by AC Helcor (Romania) were undergone stability studies in the following conditions [10, 11]: long term (25°C ± 2°C, 60% R.U. ± 5%) for 36 months; accelerated conditions (40°C ± 2°C, 75% R.U. ± 5%) for 6 months.

The content of carvedilol in tablets and the related impurities were analysed by two stability-indicating validated HPLC methods at 0, 3, 6, 9, 12, 18, 24 and 36 months, for all three batches.

Carvedilol CRS and carvedilol for system suitability CRS (containing Impurity A and Impurity D) were purchased from Sigma-Aldrich Gmbh (Germany). Reagent-grade phosphoric acid, hydrochloric acid, formic acid, triethylamine and potassium dihydrogen phosphate were provided by Merck, Darmstadt, Germany. HPLC-grade acetonitrile and methanol were supplied by Merck, Darmstadt, Germany.

Instrumentation and chromatographic conditions: both chromatographic analyses were performed on a Jasco liquid chromatograph with UV detection at 240 nm.

Assay method

The stationary phased was a chromatographic column NUCLEOSIL 100 C8, 250 x 4.6 mm, 5 µm. The mobile phase was prepared by dissolving 0.52 g of sodium dodecyl sulphate in 75 mL of phosphate buffer pH 3.0 in a 1000 mL volumetric flask and sonicated. 720 mL of acetonitrile were added, and diluted with water to volume. Isocratic elution was performed at a flow rate of 1.3 mL/min and injection volume was 50 µL. All separations were carried out at a temperature of 40°C. Retention time for carvedilol was 25 minutes.

Sample solution preparation: 400 mg tablets powder (equivalent to 25 mg carvedilol) were weighed in a 100 mL volumetric flask. 10 mL water and 70 mL dilution mixture were added and then sonicated for 30 minutes. The volume was made up to the mark with mobile phase, mixed well and filtered (0.45 µm). 2.5 mL of this solution was diluted with methanol in a 50 mL volumetric flask (0.0125 mg/mL carvedilol).

Reference solution preparation: 25 mg carvedilol CRS were weighed in a 100 mL volumetric flask. 10 mL water and 70 mL dilution mixture were added and then sonicated for 30 minutes. The volume was made up to the mark with mobile phase, mixed well and filtered (0.45 µm). 2.5 mL of this solution was diluted with methanol in a 50 mL volumetric flask (0.0125 mg/mL carvedilol).

The carvedilol content was calculated using the formula:

$$Carvedilol\ content\ \left(\frac{mg}{tablet}\right) = \frac{A_S \times m_R \times \overline{M}_{20}}{A_R \times m_S}$$

where: A_S = carvedilol peak area of sample solution; m_R = weight of carvedilol CRS in reference solution (mg); $\overline{M}_{20}$ = mean tablet weight (mg); A_R = carvedilol peak area of reference solution; m_S = weight of carvedilol tablets in sample solution (mg).

Acceptance criteria: 95 - 105% of declared content (6.25 mg/tablet).

Related substances method

The stationary phase was a chromatographic column NUCLEOSIL 100 C8, 250 x 4.6 mm, 5 µm. The mobile phase was prepared by dissolving 1.77 g of potassium dihydrogen phosphate in 650 mL of water and the pH was adjusted with phosphoric acid to 2.0. 350 mL of acetonitrile were added and an isocratic elution was performed at a flow rate of 1 mL/min. The injection volume was 20 µL. All separations were carried out at a temperature of 55°C.

Sample solution preparation: 400 mg tablets powder (equivalent to 25 mg carvedilol) were weighed in a 25 mL volumetric flask. 15 mL mobile phase were added, then sonicated for 30 minutes and filtered (0.45 µm).

Reference solution (a) preparation: 1 mL of sample solution was diluted with mobile phase in a 100 mL volumetric flask. 1 mL of this solution was diluted with mobile phase in a 10 mL volumetric flask (carvedilol 0.1 % m/v).

Reference solution (b) preparation: 5 mg carvedilol for system suitability CRS were weighed in a 50 mL volumetric flask. 35 mL mobile phase were added,

then mixed for dissolution. The volume was made up to the mark with mobile phase.
The impurities content was calculated using the following formulas:

$$Impurity\ content\ (\%) = \frac{A_I \times Cf \times 0.1}{A_R}$$

where: A_I = impurity peak area; Cf = correction factor (2.0 for Impurty A, 1.5 for Impurity D, 1 for unspecified impurities); A_R = carvedilol peak area of reference solution, 0.1 = reference solution concentration (%).
Total impurities content was calculated as sum of known and unknown impurities, using the following formula:

$$I_T = I_A + I_D + I_I$$

I_T = total impurities concentration (%); I_A = Impurity A concentration (%); I_D = Impurity D concentration (%); I_I = Unknown Impurity concentration (%).
Acceptance criteria: Impurity A < 0.2%, Impurity D < 0.15%, Unknown Impurity < 0.1%, total impurities < 0.5%.
There were disregarded peaks with area smaller or equal to 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05%).
The resolution factor between the impurities A and carvedilol peaks must be at least 3.5 in the chromatogram obtained with reference solution (b).

Results and Discussion

The validation of the HPLC assay method was performed for selectivity, linearity, LOD, LOQ, precision, accuracy and robustness [12].
The selectivity of the HPLC method was confirmed by injecting blank samples, placebo standard and sample solutions. No other peaks corresponding to the retention times of carvedilol (25 minutes) were noted, indicating that interfering substances were not present [8].
In order to establish the selectivity of the assay method regarding degradation products, force degradation studies were performed on placebo, references and samples using different conditions: acidic (0.1 M HCl, 2 h, 90 - 95ºC), alkaline (0.1 M NaOH, 2 h, 90 - 95ºC), thermic (1 h, 130ºC) and oxidative degradation (30% H_2O_2, 6 h, room temperature).

Table I

Results of force degradations studies – HPLC assay method

	Solution	Peak area (µV*min)	mg/mL	mg/tablets	Recovery (%)
Before degradation	Reference	3492196	0.0217	-	-
	Sample	3494892	-	6.38	-
Thermic degradation	Reference	3487485	0.0216	-	99.54
	Sample	1330283	-	2.42	37.93
Alkaline degradation	Reference	3412615	0.0212	-	97.70
	Sample	2571146	-	4.68	73.35
Acidic degradation	Reference	3378188	0.0205	-	94.47
	Sample	3222012	-	5.86	93.76
Oxidative degradation	Reference	3190060	0.0198	-	91.24
	Sample	2202775	-	4.00	62.69

Recovery (%) after forced degradation was calculated for reference and sample solutions and are listed in Table 1. Degradation of sample solution was observed in all tested conditions. The chromatograms for thermic degradation are presented in Figure 1. Supplementary picks are observed, as well as the decrease of the carvedilol peak.
The stability-indicating nature of the HPLC method was demonstrated.

The linearity of the relationship between the test results (peak areas, y) and the concentration (x) of diluted series of carvedilol standard solutions was examined in the range 50% - 150% relative to the working concentration. The solutions were injected three times.
The method was linear within the concentration range from 6.25 µg/mL to 18.75 µg/mL carvedilol (R^2 = 0.9973).

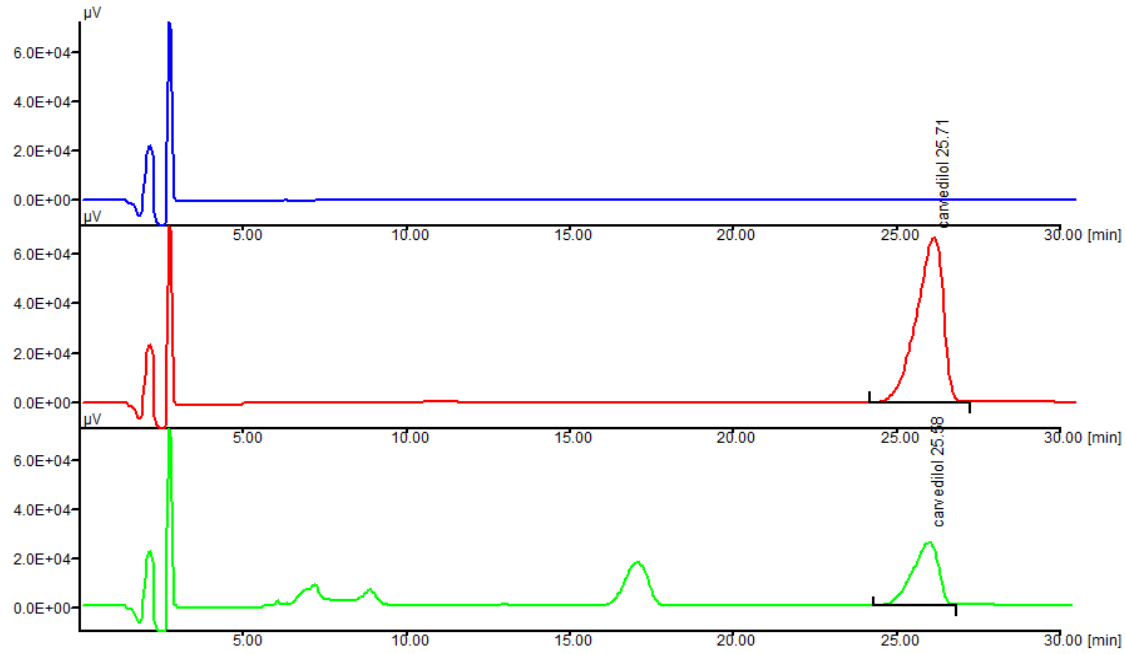

Figure 1.
The chromatograms of solutions after thermic degradation: placebo (blue), reference (red) and sample (green)

For the determination of the method repeatability, sample solution was prepared in six replicates and analysed according to the analytical procedure. Relative standard deviation (RSD %) of carvedilol assay results was calculated. With an RSD of 0.35% (RSD ≤ 2%) the method is repeatable.

For the determination of intermediate precision, sample solution was prepared in six replicates and analysed according to the analytical procedure by two different analysts, using the same sample, on different days. Relative standard deviation (RSD %) of carvedilol assay results was calculated. With an RSD of 0.31% (RSD ≤ 2%) the method is precise.

Accuracy was validated by analysing a synthetic mixture of drug product components (placebo), to which known amounts of carvedilol standard were added. Five solutions (concentration range 50 - 150% relative to the working concentration) were prepared and analysed. The average recovery was of 100.06%, RSD = 0.62%, the bias (%) proved to be ± 0.77 and the confidence interval (P = 95%) between 99.29 and 100.83.

The limit of detection (LOD), calculated to be three times the standard deviation of the noise ratio from the analysis of carvedilol, was 0.59266 µg/mL. The limit of quantification (LOQ), calculated to be ten times the standard deviation of the noise ratio from the analysis of carvedilol, was 1.9763 µg/mL.

For testing the robustness of the method, the influence of slightly modified chromatographic conditions was studied. The influences of the length of the chromatographic column (from 25 cm to 15 cm), the pH of the mobile phase (from 3.00 to 2.50), the mobile phase composition (increasing the content of acetonitrile with 10%), the column temperature (from 40°C to 45°C), the injection volume (from 50 µL to 20 µL), the flow rate of the mobile phase (from 1.2 mL/min to 1.3 mL/min and 1.4 mL/min) are within the set tolerance range (98% - 102% contained in carvedilol sample compared to the standard conditions of the method), so the method is considered robust.

The HPLC assay method was completely validated and it is suitable for the assay of carvedilol in tablets, both for release and shelf life specifications.

The validation of the HPLC related substances method was performed for selectivity, linearity, LOD, LOQ, precision, accuracy and robustness [12].

The selectivity of HPLC method was confirmed by injecting blank samples, placebo standard and sample solutions. No other peaks corresponding to the retention times of carvedilol (8 minutes) and its impurities (4.4 minutes for Impurity A and 40 minutes for Impurity D) were noted, indicating that interfering substances were not present.

In order to establish the selectivity of the assay method regarding unknown degradation products, force degradation studies were performed on placebo, references (a) and samples using different conditions: acid (0.1 M HCl, 2 h, 90 - 95ºC), base (0.1 M NaOH, 2 h, 90 - 95ºC), thermic (1 h, 130ºC) and oxidative degradation (30% H_2O_2, 6 h, room temperature).

Impurities content (%) after forced degradations was calculated for reference and sample solutions and they are listed in Table II.

Table II

Results of force degradations studies – related substances method

	Solution	Carvedilol Peak Area (µV*min)	Impurity A Peak Area (µV*min)	Unknown Impurities	Impurity A (%)	Unknown maximum impurity (%)	Total Impurities (%)
Before degradation	Reference	54780876	15061	3	< 0.05	< 0.05	< 0.5
	Sample	52261998	16684	4	< 0.05	< 0.05	< 0.5
Thermic degradation	Reference	55418151	11959	5	< 0.05	< 0.05	< 0.5
	Sample	38254437	6616546	9	6.82	16.57	25.1
Alkaline degradation	Reference	62697816	835	3	< 0.05	< 0.05	< 0.5
	Sample	62713843	17138	9	< 0.05	0.73	1.85
Acidic degradation	Reference	56337890	15565	3	< 0.05	< 0.05	< 0.5
	Sample	56026644	14454	8	< 0.05	< 0.05	< 0.5
Oxidative degradation	Reference	49362468	16281	9	< 0.05	0.32	0.52
	Sample	43895834	88929	10	0.60	5.98	8.21

The degradation of the sample solution was observed in all tested conditions. The chromatograms for oxidative degradation are presented in Figure 2. Supplementary picks are observed, as well as the decrease of the carvedilol peak and the increase of the peak of Impurity A.

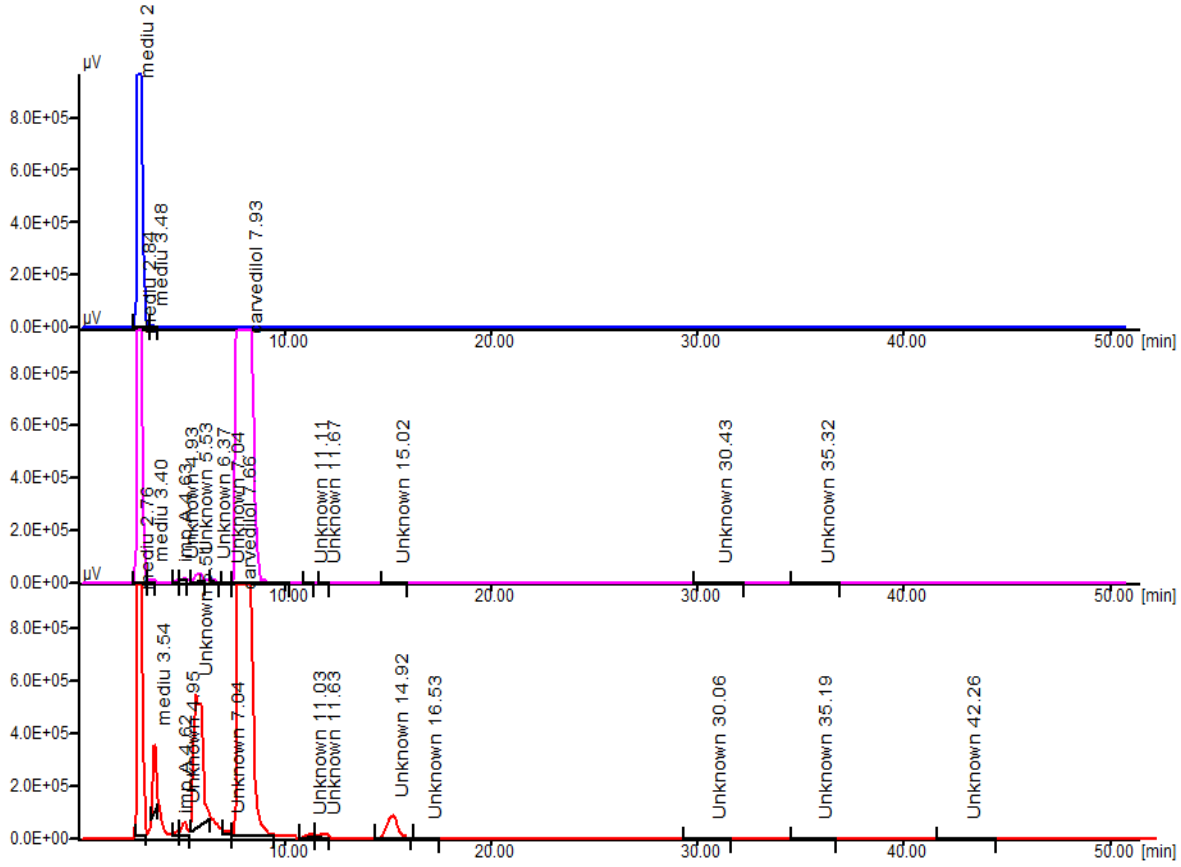

Figure 2.

The chromatograms of solutions after oxidative degradation: placebo (blue), reference (pink) and sample (red)

The stability-indicating nature of the HPLC method was demonstrated.

The linearity between peak areas (y) and the concentration (x) of diluted series of carvedilol standard solutions was examined in the range 50 - 150% relative to the working concentration. The solutions were injected in triplicate.

The method was linear within the concentration range from 0.5 µg/mL to 1.5 µg/mL carvedilol ($R^2 > 0.99$) and suitable for the determination of related substances in carvedilol tablets.

For the determination of method repeatability, sample solution was prepared in six replicates and analysed according to the analytical procedure. Relative standard

deviation (RSD %) was calculated. With an RSD value of 0.26% (RSD ≤ 2%), the method is repeatable.
For the determination of the intermediate precision, sample solution was prepared in six replicates and analysed according to the analytical procedure by two different analysts, using the same sample, on different days. With an RSD value of 0.25 % (RSD ≤ 2%), the method is precise.
Accuracy was validated by analysing a synthetic mixture of drug product components (placebo), to which known amounts of carvedilol standard were added. Five solutions (concentration range 50% - 150% relative to the working concentration) were prepared and analysed. The experimentally obtained amounts of carvedilol were compared to the actual added amounts. The average recovery was of 100.71%, RSD = 0.21%, the bias (%) proved to be ± 0.27 and the confidence interval (P = 95%) between 100.44 - 100.98. LOD was 0.0145% and the LOQ was 0.0483%.
For testing the robustness of the method, the influence of slightly modified chromatographic conditions was studied. The influences of the type of the chromatographic column (from C8 cm to C18, Figure 3), the pH of the mobile phase (from 1.5 to 2.0 and 2.5), the mobile phase composition (increasing the content of acetonitrile with 10%), the column temperature (from 55°C to 45°C and 65°C), the injection volume (from 20 µL to 50 µL), the flow rate of the mobile phase (from 1 mL/min to 0.9 mL/min and 1.1 mL/min) are within the set tolerance range (resolution between carvedilol peak and Impurity A peak ≥ 3.5), so the method is considered robust.

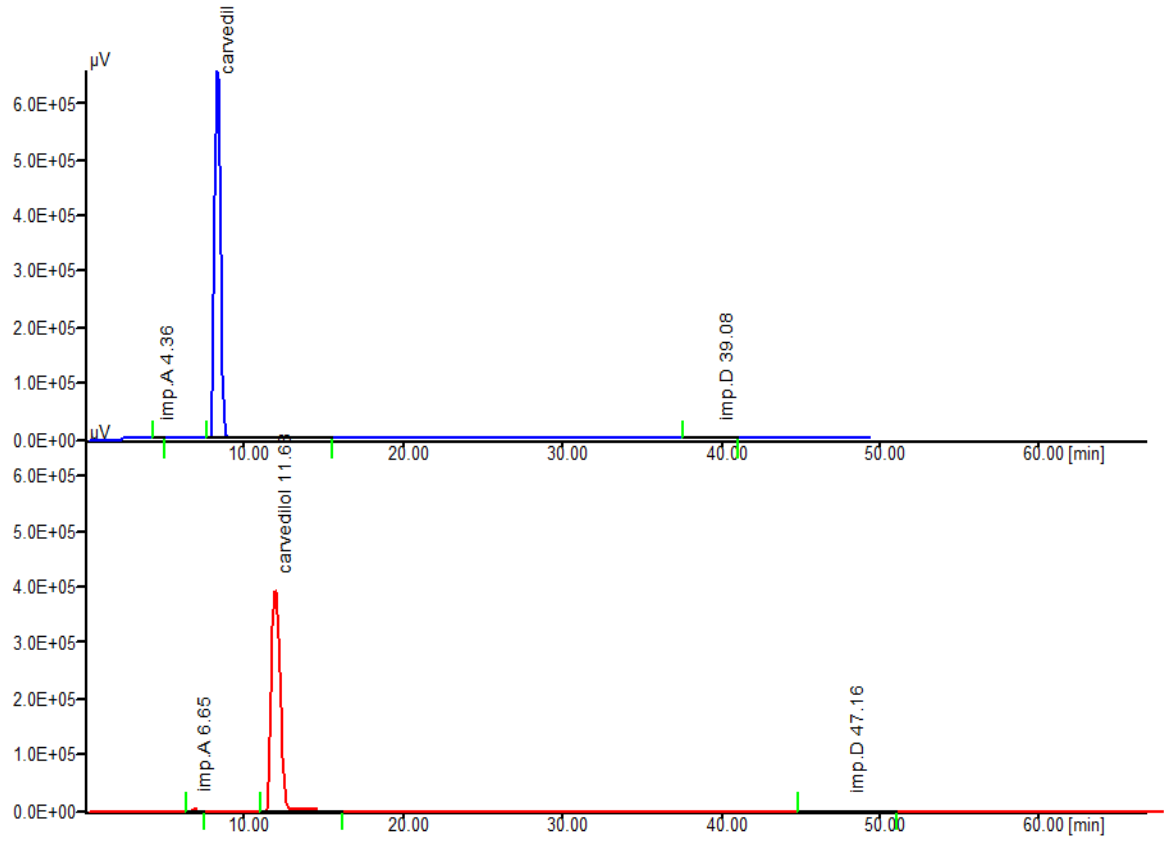

Figure 3.
The influence of chromatographic column type: C8 (blue), C18 (red)

The HPLC method was completely validated and it is suitable for the determination of relative substances in carvedilol tablets, both for release and shelf life specifications.
Stability studies
The stability testing was performed in order to investigate how the quality of the drug product changes with time under the influence of environmental factors. Accelerated and long term stability studies were conducted as described in materials and methods.
The two validated stability-indicating HPLC methods were used for monitoring the stability of carvedilol tablets by performing the carvedilol content and impurities content.
The results of stabilities studies are given in Table III for long term condition and in Table IV for accelerated condition.

Table III

Long term stability data

Sample	Initial	3 months	6 months	9 months	12 months	18 months	24 months	36 months
Assay (%)								
Batch P430510	97.60	96.56	97.68	96.40	100.64	99.36	98.24	99.44
Batch P440510	98.24	99.68	100.72	99.68	99.76	100.24	100.72	99.60
Batch P450510	97.44	97.01	99.25	98.29	100.03	99.41	99.17	98.08
Related substances								
Impurity A (%)								
Batch P430510	0.028	0.030	0.032	0.012	0.015	0.022	0.049	0.047
Batch P440510	0.050	0.050	0.043	0.049	0.036	0.018	0.023	0.049
Batch P450510	0.015	0.012	0.019	0.046	0.018	0.022	0.017	0.048
Impurity D (%)								
Batch P430510	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Batch P440510	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Batch P450510	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Unknown Impurity (%)								
Batch P430510	0.030	0.017	0.017	0.040	0.012	0.029	0.019	0.018
					0.010	0.016	0.016	0.033
						0.016		0.028
Batch P440510	0.018	0.020	0.0142	0.02	0.042	0.023	0.022	0.020
				0.045	0.029	0.031	0.011	0.045
						0.035	0.070	0.077
Batch P450510	0.017	0.040	0.044	0.028	0.017	0.050	0.024	0.019
							0.011	0.016
							0.027	0.020
Total Impurities (%)								
Batch P430510	0.058	0.047	0.049	0.052	0.037	0.083	0.084	0.126
Batch P440510	0.068	0.070	0.0572	0.114	0.107	0.107	0.126	0.191
Batch P450510	0.032	0.052	0.063	0.074	0.035	0.072	0.079	0.103

Table IV

Accelerated conditions stability data

	Sample	Initial	3 months	6 months
Assay (%)	Batch P430510	97.60	98.08	99.04
	Batch P440510	98.24	99.28	98.88
	Batch P450510	97.44	97.74	99.33
Related substances				
Impurity A (%)	Batch P430510	0.028	0.15	0.087
	Batch P440510	0.050	0.14	0.128
	Batch P450510	0.015	0.15	0.138
Impurity D (%)	Batch P430510	n.d.	n.d.	n.d.
	Batch P440510	n.d.	n.d.	n.d.
	Batch P450510	n.d.	n.d.	n.d.
Unknown Impurity (%)	Batch P430510	0.030	0.066	0.068
			0.046	0.016
			0.013	0.018
			0.024	0.076
				0.021
	Batch P440510	0.018	0.067	0.061
			0.047	0.021
			0.012	0.016
			0.016	0.079
			0.066	0.020
			0.022	
	Batch P450510	0.017	0.066	0.071
			0.045	0.021
			0.015	0.026
			0.013	0.090
			0.076	0.020
Total Impurities (%)	Batch P430510	0.058	0.299	0.286
	Batch P440510	0.068	0.370	0.325
	Batch P450510	0.032	0.365	0.366

Examples of sample chromatograms at the end of stability studies are shown in Figure 4 for long term conditions and in Figure 5 for accelerated conditions. No results were out of specifications, neither in the case of long-term stability studies nor in the case of accelerated stability studies. There were no observed trends for assay or for related substances, therefore, the product is considered stable over a shelf-life equal to the duration of the stability studies plus 12 months (4 years) in accordance with current quality guidelines [10, 11].

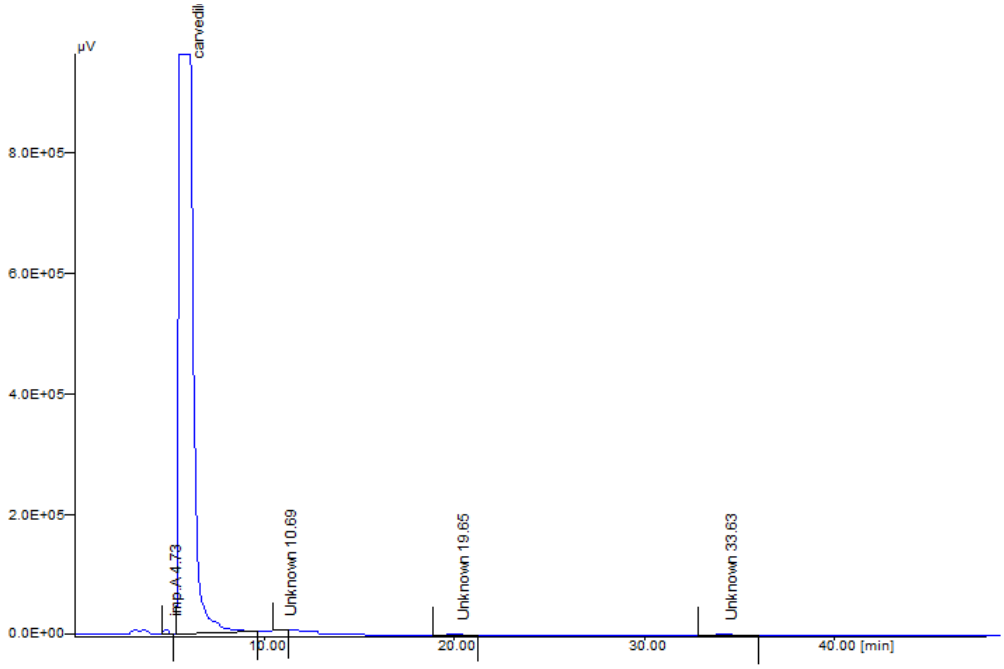

Figure 4.
Chromatogram of sample solution prepared from Batch P440510, after 36 month of storage in long term condition

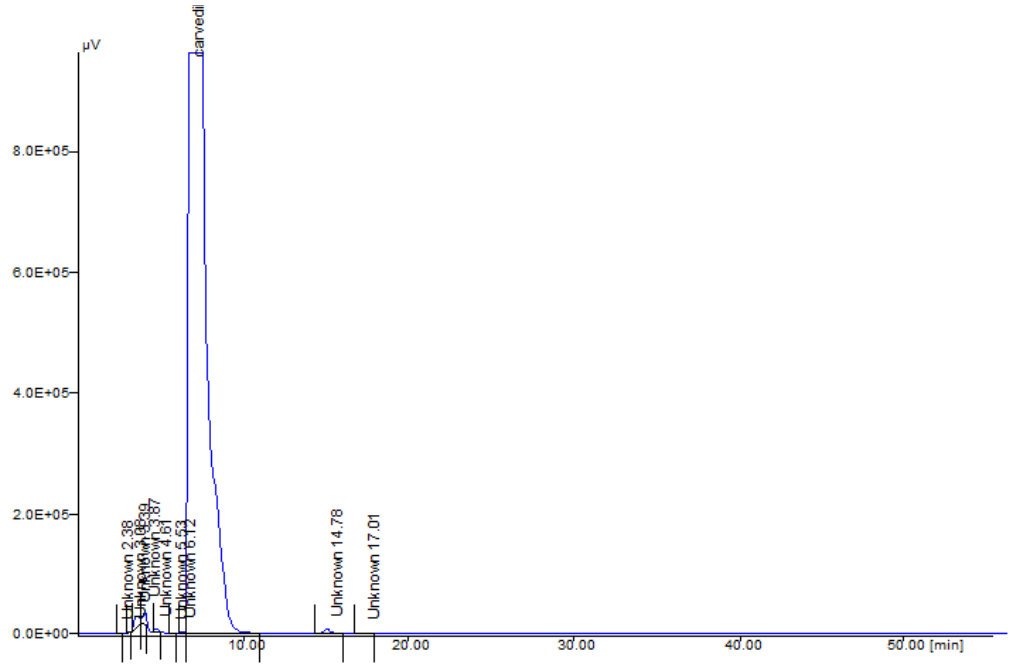

Figure 5.
Chromatogram of sample solution prepared from Batch P440510, after 6 month of storage in accelerated condition

Conclusions

Two key quality parameters were monitored through the stability studies of carvedilol tablets: assay and related substances, using two HPLC methods. In order to demonstrate the suitability of the analytical procedures for the intended purpose, the methods were validated and their stability-indicating nature were confirmed.

The results of the stability studies showed that 6.25 mg carvedilol tablets were stable. Since no trends were observed, regarding the assay and the related substances, the shelf-life can be calculated as equal to the duration of the stability studies plus 12 months (4 years) in accordance with current quality guidelines. However, results should be completed with more quality parameters (as tablet dissolution) monitored in stability studies in order to establish the shelf life and the storage condition of the drug product.

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