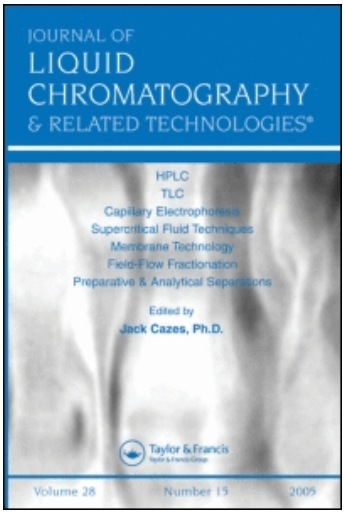

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Lanzanova, Fibele Analine , Argenta, Débora , Arend, Marcela Zart , Junior, Liberato Brum and Cardoso, Simone Gonçalves(2009) 'LC and LC-MS Evaluation of Stress Degradation Behavior of Carvedilol', Journal of Liquid Chromatography & Related Technologies, 32: 4, 526 — 543

To link to this Article: DOI: 10.1080/10826070802671481

URL: http://dx.doi.org/10.1080/10826070802671481

Journal of Liquid Chromatography & Related Technologies®, 32: 526–543, 2009

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826070802671481

LC and LC-MS Evaluation of Stress Degradation Behavior of Carvedilol

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Abstract: In the present study, forced and accelerated degradation studies of carvedilol (CAR) were carried out according to ICH guideline Q1A (R2). The drug was subjected to acid (1.0 N HCl), alkaline (1.0 N NaOH), and neutral hydrolytic conditions by refluxing at 90°C, as well as to oxidative (7.5% H_2O_2) decomposition, protected from light, at room temperature. Photolysis was carried out in solid state of the drug and in methanolic solution. The stress degradation samples were evaluated by LC and LC–MS. The kinetics of degradation were determined by the LC method, previously developed and validated for our group, that could separate the degradation products formed under various stress conditions.

An LC-MS/MS method was developed and validated and was found to be precise, accurate, specific, and selective. Tablets of CAR from three different batches (A,B,C) were subjected to climate chamber with 40±2°C and 75%±5 relative humidity for 6 months to evaluate the stability under accelerated conditions. The samples were assayed by LC-MS/MS and UV methods. The weight variation, hardness, disintegration time, friability, content, and dissolution test were also performed. The drug was relatively stable under acidic, neutral, and photolytic stress conditions, but showed instability under alkaline and oxidative conditions. The LC–MS m/z values of the two products obtained under oxidative

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conditions were 224.3 and 283.3 Daltons, respectively. The parameters of quality such as hardness, disintegration time, and dissolution were influenced by humidity and temperature for product B.

Keywords: Accelerated stability, Carvedilol, Forced degradation, LC-MS/MS method, Tablets

INTRODUCTION

Carvedilol (CAR, Figure 1) is an anti-hypertensive agent with non-selective α- and β1 adrenergic receptor blocking activities. It is available as tablet formulations and has been used for the treatment of hypertension, ischemic heart disease, and congestive heart failure.[1]

Stability testing provides evidence for the quality of the bulk drug and its final drug product when they are exposed to influence of environmental factors such as pH, temperature and humidity and includes long-term studies, where the product is stored at room temperature and humidity conditions, as well as accelerated studies where the product is stored under high heat and controlled humidity conditions. Forced degradation helps to determine the intrinsic stability of the molecule by establishing the degradation pathways.[2]

Stability studies are linked to the establishment and assurance of safety, quality, and efficacy of the drug product. In order to assess stability, the appropriate physical, chemical, biological, and microbiological testing must be performed. One of the evaluation criteria is the appearance of impurities and degradation products.[3]

The chemical and physical stability of CAR has been studied by some works in pharmaceutical formulations. Stability indicating ultraviolet spectroscopy methods were developed and validated for the determination of ezetimibe and CAR in pure form and their formulations.[4] A reversed-phase high-performance liquid chromatographic (RP-HPLC)

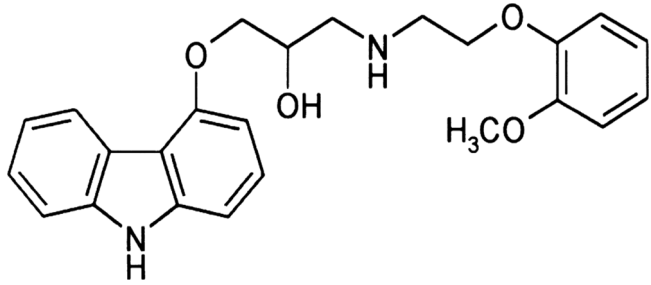

Figure 1. Chemical structure of carvedilol.

method was developed for separation of CAR and its impurities from tablets and for monitoring of the photochemical stability of CAR and its degradation products.[5,6] A preparation of the amorphous form of CAR and the study of its physicochemical properties in comparison to pure drug was studied.[7] Stability of CAR in an oral liquid veterinary preparation was performed,[8] and for research and development of a new pharmaceutical formulation, an article was published.[9]

The aim of the present study was to evaluate and to compare the stability of carvedilol under stress conditions, as well as to evaluate the physical and chemical stability of tablets under controlled temperature and relative humidity, according to ICH guidelines.[2] The analyses were performed by liquid chromatography (LC),[10] liquid chromatography coupled with mass spectrometry methods (LC-MS/MS), and UV.[11]

EXPERIMENTAL

Chemicals and Reagents

Carvedilol chemical reference substance (purity, 99.91%) was purchased by DEG (São Paulo, Brazil). The purity of the reference substance was evaluated by NMR ^{13}C, NMR ^{1}H, and DSC (data not shown). Carvedilol tablets were obtained from commercial sources within their shelf life period and were identified as products A, B, and C:

Product A (Brazilian reference product, Coreg®)

Labeled to contain 25 mg of the drug and the following excipients: lactose, sacarose, magnesium stearate, povidone, crospovidone, and coloidal anhydrous silica.

Product B (generic product)

Labeled to contain 25 mg of the drug and the following excipients: lactose, microcrystalline cellulose, crospovidone, coloidal silicium dioxide, and magnesium stearate.

Product C

Labeled to contain 25 mg of the drug and the following excipients: lactose, microcrystalline cellulose, crospovidone, coloidal silicium dioxide, and magnesium stearate.

Analytical reagent grade sodium hydroxide (NaOH), hydrochloric acid (HCl), and hydrogen peroxide (H_2O_2) were purchased from Merck

(Darmstadt, Germany). LC grade acetonitrile, methanol, and formic, phosphoric, and acetic acids were purchased from Tedia (Fairfield, USA). Ultrapure water, obtained from a Labconco Water Purification Unit (Missouri, USA).

Forced Degradation Studies

Forced degradation studies were carried out under the conditions of hydrolysis (acid, alkali, and neutral), oxidation, and photolysis, as defined by ICH Q1A (R2)[2] and the approach suggested by Singh and Bakshi,[12] using CAR chemical reference substance at the concentration of 1 mg/mL solution (stock solution) for all degradation conditions. Acid and alkaline decomposition were performed by dissolving the drug in 1.0 N HCl and 1.0 N NaOH solutions, respectively. These solutions were refluxed at 90°C for 6 hours. The studies in neutral conditions were conducted similarly (reflux, 90°C, 6 hours) in water. Aliquots of hydrolytically degraded samples (one aliquot for each time) were neutralized, if necessary, diluted in mobile phase, and filtered through a 0.22 μm filter before performing CL analysis. The oxidative studies were carried out with 7.5% (v/v) hydrogen peroxide, at room temperature (25 ± 2°C) and protected from light, for a period of 15 hours. The photolytic studies were carried out by spreading the drug substance in solid state in a petri dish with a thickness of about 15 mm and in solution (dissolved in methanol) maintained in a quartz cell. These samples were covered with parafilm and exposed for 7 days in photostability chambers with an overall illumination of UVC-254 nm 30 W lamp (Philips, Holland). The distance between the lamp and the samples was 10 cm. The temperature in the chamber was controlled and always kept around 25°C.

Methods

UV

Analysis of the degraded tablets were carried out using the method previously developed and validated by Ieggli et al.,[11] following the conditions: A Shimadzu double-beam spectrophotometer (Shimadzu, Kyoto, Japan) model UV – 1601 PC, with a fixed slit width (2 nm) using 10 mm quartz cell. An amount of powder equivalent to 25 mg of CAR was placed in a 100 mL volumetric flask and about 5 mL of acetonitrile and 20 mL of ethanol were added. After shaking for 15 minutes, the volume was made up with ethanol and the solution was filtered through a quantitative paper filter (Schleicher & Schuell). Further dilution of the

filtrate was made with ethanol in order to give a final concentration of 4.0 μg/mL. The absorbances were measured at 244 nm.

LC

Analysis of the degraded samples was carried out using the method previously validated by Ieggli,[10] following the conditions: a Shimadzu LC system (Shimadzu, Kyoto, Japan) equipped with an SCL-10A_{VP} system controller, LC-10 AD_{VP} pump, DGU-14A degasser, CTO-10A_{VP} column oven, SIL-10AD_{VP} autosampler, and an SPD-M10A_{VP} photodiode array (PDA) detector. The peak areas were integrated automatically by computer using a Shimadzu Class VP® V 6.12 software program. The LC analysis was carried out on a reversed phase Phenomenex Luna C_{18} column (250 mm × 4.6 mm I.D., with a particle size of 5 μm). A security guard holder (4.0 mm × 3.0 mm I.D.) was used to protect the analytical column. The Shimadzu LC system was operated isocratically at controlled-ambient temperature (25°C) using a mobile phase of phosphoric acid 0.1%, pH 3.0/acetonitrile (50:50, v/v), run at a flow rate of 1.0 mL/min using photodiode array (PDA) detector at 241 nm. The injection volume was 10 μL of solution containing 12.5 μg/mL for both standard and samples. The chromatographic separation was obtained within 10 minutes.

LC-MS/MS

The LC-MS/MS method was performed on a Waters Alliance LC system (Milford, MA, USA). The peak areas were integrated automatically by computer using a Masslynx (v 4.1) software program. The experiments were carried out on a reversed phase Synergi Fusion (Phenomenex, Netherlands) C_{18} column (50 mm × 4.6 mm ID, with a particle size of 4 μm and pore size of 100 Å). A security guard holder (4.0 mm × 3.0 mm ID) was used to protect the analytical column. The LC system was operated isocratically at controlled temperature (40°C) using a mobile phase of acetonitrile/acetic acid 0.1% (80:20, v/v). This was filtered through a 0.45 μm membrane filter (Millipore, Bedford, MA, USA) and run at a flow rate of 1.0 mL/min (split 1:5). The injection volume was 20 μL for both standard and samples. The triple quadrupole mass spectrometer (Waters, Milford, MA, USA), model Quattro micro, equipped with an ESI source using a crossflow counter electrode run in positive mode (ESI+), was set up in multiple reaction monitoring (MRM) mode, monitoring the transitions $406.7 > 100.3$ for CAR. For the optimization of mass spectrometer conditions, a mixed standard solution (1,000 ng/mL) containing CAR was directly introduced and the following parameters were selected: cone gas and desolvation gas

set at 50 and 400 L/h, respectively. Capillary voltage, extractor voltage, RF lens voltage, source temperature, and desolvation temperature were 3.90 kV, 2 V, 0.3 V, 120°C, and 400°C, respectively. The dwell time was set at 0.5 seconds; the collision gas pressure (argon) was 2.3×10^{-3} mbar. The cone voltage was 40 V and the collision energy was 30 eV. Data acquisition and analyses were performed using the software Masslynx (v 4.1) running under Windows XP on a workstation IBM PC.

Validation of the LC-MS/MS Method

The LC-MS/MS method was validated by the determination of the following parameters: specificity, linearity, range, precision, accuracy, limit of detection (LOD), limit of quantitation (LOQ), and robustness, following ICH guidelines.[13]

Specificity

The evaluation of specificity was performed by analyzing solutions of a placebo containing the same excipients as the commercial products in their usual concentrations. The samples were chromatographed to determine the extent to which mobile phase components and excipients could contribute to the interference with the analyte.

Linearity and Range

Linearity was determined by constructing three calibration curves. For the construction of each calibration curve, seven standard concentrations of CAR in the range of 10–1,500 ng/mL were prepared in acetonitrile/water (50:50, v/v). The peak area ratio of the drug against the respective standard concentrations was used for plotting the graph and the linearity evaluated by least squares regression analysis.

Precision

The precision of the method was determined by repeatability and intermediate precision. Repeatability was examined by six evaluations of the same concentration sample of CAR (1,000 ng/mL), using the product A, on the same day, under the same experimental conditions. The intermediate precision of the method was assessed by carrying out the analysis on two different days (inter-day) and also by another analyst performing the analysis in the same laboratory (between-analysts), using the products A and C.

Accuracy

The accuracy was evaluated applying the proposed method to the analysis of the in-house mixture of the tablet excipients with known amounts of the drug, corresponding to the concentrations of 80, 100, and 120%. The accuracy was calculated as the percentage of the drug recovered from the formulation matrix.

Limit of Quantitation and Limit of Detection

The limit of quantitation (LOQ), was taken as the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy, and the limit of detection (LOD), was taken as the lowest absolute concentration of analyte in a sample that can be detected but not necessarily quantified. These limits were calculated based on the ICH guideline.[13]

Robustness

The robustness of an analytical procedure refers to its ability to remain unaffected by small and deliberate variations in method parameters and provides an indication of its reliability for the routine analysis. The robustness was determined by analyzing the same sample (1,000 ng/mL) under a variety of conditions of the method parameters such as flow rate, column temperature, injection volume, and changing the mobile phase composition.

Kinetics of Degradation

The concentrations of the remaining CAR determined at the different time intervals of degradation studies were plotted in three different graphs, as follows: (a) concentration values against time (zero-order kinetics), (b) log of concentration versus time (first-order kinetics), and (c) reciprocal of concentration versus time (second-order kinetics). The determination coefficients (r) were obtained and the best fit observed. Each experiment was done in triplicate (analyzed by LC method) and average values were taken for the analysis.[14,15]

Accelerated Stability Samples

Samples of product A, B, and C were stored for six months under accelerated conditions of temperature and humidity ($40 \pm 2^{\circ}C/75\% \pm 5$ RH) in

climatic chambers (Mecalor, Brazil).[2,16] The samples were analyzed monthly by UV.[14] and LC-MS/MS. The weight variation, disintegration, hardness, and friability tests were performed according to Brazilian Pharmacopoeial,[17] and the content uniformity and dissolution test by methods previously developed and validated by the group.

RESULTS AND DISCUSSION

LC Studies on the Stressed Solutions

Preliminary stability studies of CAR under acidic, alkali, neutral, oxidative, and photolytic stress conditions were performed (Figure 2). No significant changes were observed after stress exposure of CAR in acid, neutral, and photolytic (both solid state and methanolic solutions) conditions. On the other hand, the drug degraded gradually in alkali condition with time after 6 hours of reflux at 90°C, forming an additional resolved peak at 10.67 minutes. CAR showed to be instable after exposure for 15 hours in 7.5% (v/v) hydrogen peroxide, at room temperature, with higher degradation. Two resolved peaks appeared at 6.22 and 9.31 minutes under this condition.

In the present work, the main observed degradation factor was the hydrogen peroxide. In the kinetics determination of the degradation, it was found that around 75% of CAR was degraded in 15 hours. The values of concentration, log of concentration, and reciprocal of concentration of the remaining drug versus time are shown in Table 1.

Through the evaluation of the correlation coefficients, it can be concluded that the degradation of CAR under the oxidative experimental conditions applied shows second-order kinetics. The calculated second-order degradation rate constant was $k = 0.0021\,\text{min}^{-1}$.

Validation of LC-MS/MS Method

To obtain the best chromatographic conditions, different columns and mobile phases consisting of acetonitrile/water or methanol/water were tested to provide sufficient selectivity and sensitivity in a short separation time. Modifiers such as ammonium acetate, and formic and acetic acids were tested. Acetic acid was selected because it was easily miscible with organic solvent and led to improved peak symmetry and ionization efficiency of CAR. The best signal was achieved using acetonitrile/acetic acid 0.1% (80:20, v/v) with a flow rate of 1.0 mL/min (split 1:5).

In the present study, electrospray (ESI) was used as the LC-MS/MS interface because the efficiency of ionization of CAR was

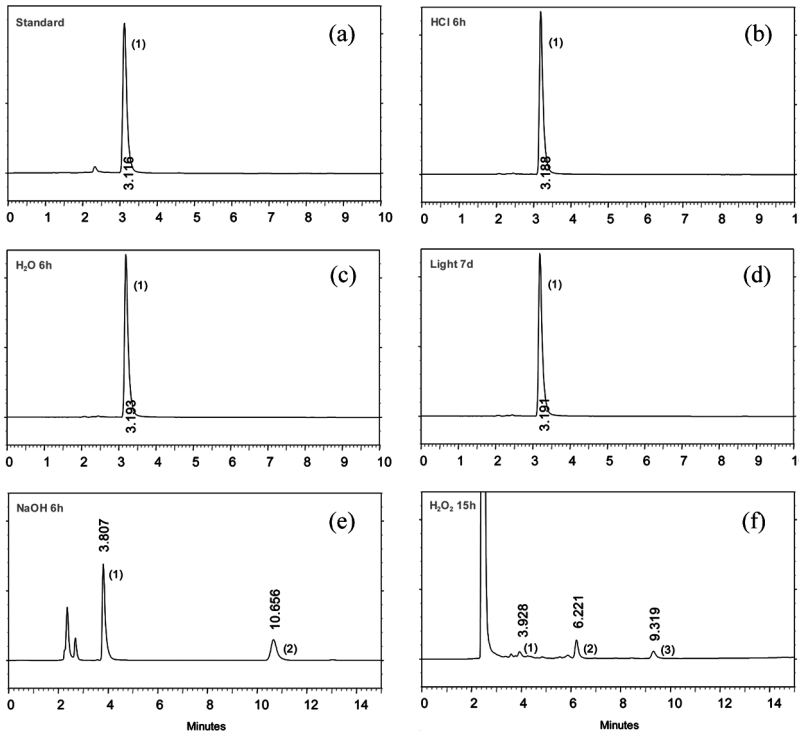

Figure 2. LC chromatograms of carvedilol. (a) Chemical reference substance solution. (b) After degradation in hydrochloric acid 1.0 N by refluxing at 90°C for 6 hours. (c) After degradation in water for 6 hours. (d) Methanolic solution of carvedilol after exposition to UV light for 7 days. (e) After degradation in sodium hydroxide 1.0 N by refluxing at 90°C for 6 hours. (f) After degradation in hydrogen peroxide 7.5% (v/v) at room temperature (25 ± 2°C), protected of light for 15 hours. Peak 1 – carvedilol, peak 2 and 3 – degradated forms.

higher than atmospheric pressure chemical ionization (APCI). The mass spectrometric response of the analyte was measured by using selected reaction monitoring in which the mass spectrometer is tuned to several sets of ions (multiple reaction monitoring, MRM). In this method, a set of precursor ion/product pairs was monitored. The protonated molecular ions $[M+H]^+$ of CAR observed on the full scan mass spectrum was m/z 406.7. Moreover, the collision energy in Q2 produced significant fragments for CAR (100.3, 193.9, and 221.8). The MS/MS transition $406.7 > 100.3$ was selected since the ion scan product with m/z 100.3 presented a higher abundance and stability for the CAR. The fragmentation patterns for CAR is are according to the literature.[18]

Table 1. Kinetics of degradation of oxidative solutions of carvedilol

Time (hours)	Concentration of carvedilol (μg/mL)	log concentration	1/Concentration
0	12.50	2.00	0.0100
1	11.74	1.97	0.0106
2	8.93	1.85	0.0140
3	8.32	1.82	0.0150
4	7.28	1.77	0.0172
5	6.37	1.71	0.0196
15	3.03	1.38	0.0413
r	0.8254	0.9611	0.9970

Values of correlation coefficients, *r*, for three reaction orders.

The coupling of LC with MS/MS detection in the MRM mode showed high specificity because only the ions derived from the analyte of interest (CAR) were monitored, and indicated that no interferences were detected from mobile phase and excipients of the formulation.

The linearity determined by three determinations of the concentrations in the range of 10–1,500 ng/mL gave the determination coefficient of 0.9955. The obtained calibration curve was $y = 369.11x + 12198$, where x is concentration and y is the peak area of the drug. These results indicated significant linearity of the calibration curve for the method.

The LOD and LOQ were obtained by using the mean of the slope (369.11 ± 0.56) and the standard deviation of the intercept (352.27) of the independent curves, determined by a linear regression line. The LOD and LOQ calculated were 2.86 and 9.54 ng/mL, respectively.

A typical chromatogram obtained by the proposed LC-MS/MS method, with the resolution of the symmetrical peak corresponding to CAR in pharmaceutical formulation, is shown in Figure 3. The low analysis time of 2.0 minutes allow a rapid determination of the drug, which is an important advantage for routine analyses.

The repeatability of the method was calculated as the RSD of six assays (of the product A) containing CAR in the same range of concentration (1,000 ng/mL) performed on the same day and under the same experimental conditions. The RSD value obtained was 0.58%. The intermediate precision was assessed by analyzing two samples of the pharmaceutical formulation (products A and C) on two different days (inter-day, Table 2); the RSD values obtained for the samples were 0.48 and 0.51, respectively. Between-analysts precision was determined by calculating the RSD for the analysis of two samples of the pharmaceutical formulation (product A and C) by two analysts; the values were found to be 0.41 and 0.51%, respectively (Table 3).

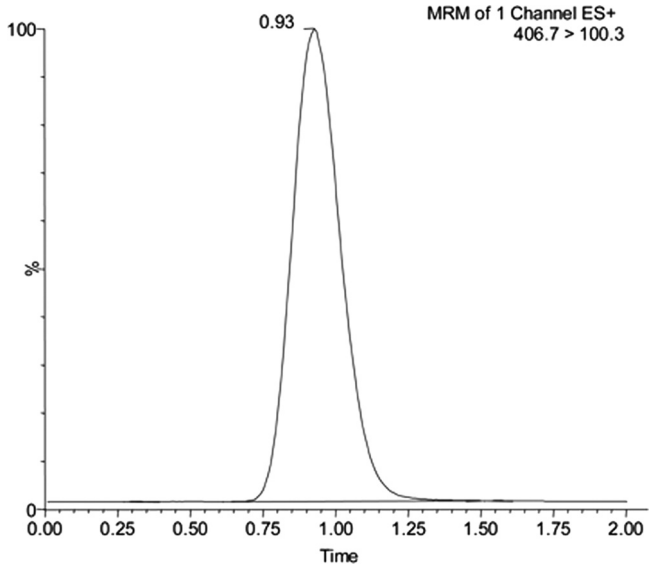

Figure 3. Representative LC-MS/MS chromatogram of carvedilol in tablets (1,000 ng/mL).

The absolute means obtained for accuracy were 100.3, 98.9, and 99.0%, from different solutions containing 800, 1,000 and 1,200 ng/mL, respectively, with a mean value of 99.40% and RSD of 0.79% as shown in Table 4.

In order to assess the robustness, different parameters were evaluated: flow rate, column temperature, injection volume, and changing the mobile phase composition. The results and the experimental range of the selected variables are given in Table 5, together with the optimized values. The obtained values demonstrated that the small variations do

Table 2. Inter-day precision data of LC-MS/MS for carvedilol in samples of pharmaceutical formulation

Sample	Day	Recovery[a] (%)	Mean[b]	RSD[c] (%)
A	1	98.8	99.2	0.48
	2	99.5		
C	1	98.6	98.9	0.51
	2	99.3		

[a]Mean of three replicates.
[b]Mean of two days.
[c]RSD = Relative standard deviation.

Table 3. Between-analysts precision data of LC-MS/MS for carvedilol in samples of pharmaceutical formulation

Sample	Analyst	Recovery[a] (%)	Mean[b]	RSD[c] (%)
A	1	98.0	97.8	0.41
	2	97.5		
C	1	98.7	98.3	0.51
	2	97.9		

[a]Mean of three replicates.
[b]Mean of two analysts.
[c]RSD = Relative standard deviation.

not affect the results, indicating that the method is reliable during routine analysis.

LC–MS Studies on Forced Decomposition Samples of Carvedilol

The full scan LC-MS spectra of CAR reference standard and oxidized forms after 15 hours of degradation with 7.5% (v/v) hydrogen peroxide are shown in Figure 4.

According to the work by Stojanovic et al.,[5,6] the method for monitoring the photochemical stability of carvedilol was proposed and when exposed to continuous daylight for 100 days at room temperature (25°C), carvedilol showed to be relatively photostable. The compound 4-hydroxycarbazole was detected in the exposed sample by LC analysis and 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 (2RS)-1-[benzyl[2-(2-methoxyphenoxy)ethyl]amino]-3-(9H-carbazol-4-yloxy)propan-2-ol, has a molecular mass

Table 4. Accuracy of LC-MS/MS for carvedilol in samples of pharmaceutical formulation

Nominal concentration (ng/mL)	Mean concentration found[a] (ng/mL)	RSD[b] (%)	Accuracy (%)
800	802.4	1.18	100.3
1000	988.6	0.47	98.9
1200	1188.5	0.94	99.0

[a]Mean of three replicates.
[b]RSD = Relative standard deviation.

Table 5. Results from of method robustness study

Conditions		Tablets % (mean ± sem)[a]	Tablets RSD (%)
Recommeded conditions[b]		99.6 ± 0.4	0.6
Mobile phase,	75:25	99.1 ± 0.2	0.8
acetonitrile:acetic acid 0.1%	85:15	101.3 ± 0.1	0.5
Column temperature (°C)	35	98.8 ± 0.3	0.3
	45	98.8 ± 0.2	0.8
Flow rate (mL/min)	0.8	98.1 ± 0.1	0.9
Split 1:5	1.2	97.7 ± 0.5	1.0
Injection volume (μL)	10	99.2 ± 0.4	0.4
	30	98.8 ± 0.7	0.8

[a]Sem is the standard error of the mean.

[b]The recommnended chromatographic conditions were acetonitrile:acetic acid 0.1% 80:20 (v/v) as mobile phase, Synergi Fusion C_{18} column, column temperature of 40°C, 1 mL/min flow rate (split 1:5) and 20 μL of injection volume.

of 496.60 and is denoted impurity C in the British Pharmacopoeia (BP). The 4-hydroxycarbazole with molecular mass of 183.21 is not mentioned in the BP and is used for the synthesis of CAR; hence, it can be supposed that it is a synthesis impurity.[19,20]

In this paper, it was detected that basic and oxidative factors are really damaging the CAR and the products of degradation observed were different from those. The obtained m/z values in positive ESI mode were compared to the molecular mass of the known degradation products and to the impurities listed in the drug monograph in the BP. The LC–MS m/z values of two oxidized forms have molecular masses of 224.3 and 283.3 Daltons, respectively (Figure 4). Based on the molecular mass, none of the products have structures similar to the impurities studied by Stojanovic et al.[5,6]

Accelerated Sample Stability

In this study, two methods were employed to evaluate the CAR content after accelerated conditions (40 ± 2°C/75 ± 5% RH) for 6 months: the proposed LC-MS/MS method and the UV method.[14] No significant decomposition was observed for products A and C, while more than 10% decomposition occurred for product B (Table 6). According to a full scan LC-MS spectrum (Figure 5), additional peaks were not observed after exposition to temperature and humidity for 6 months. The values obtained

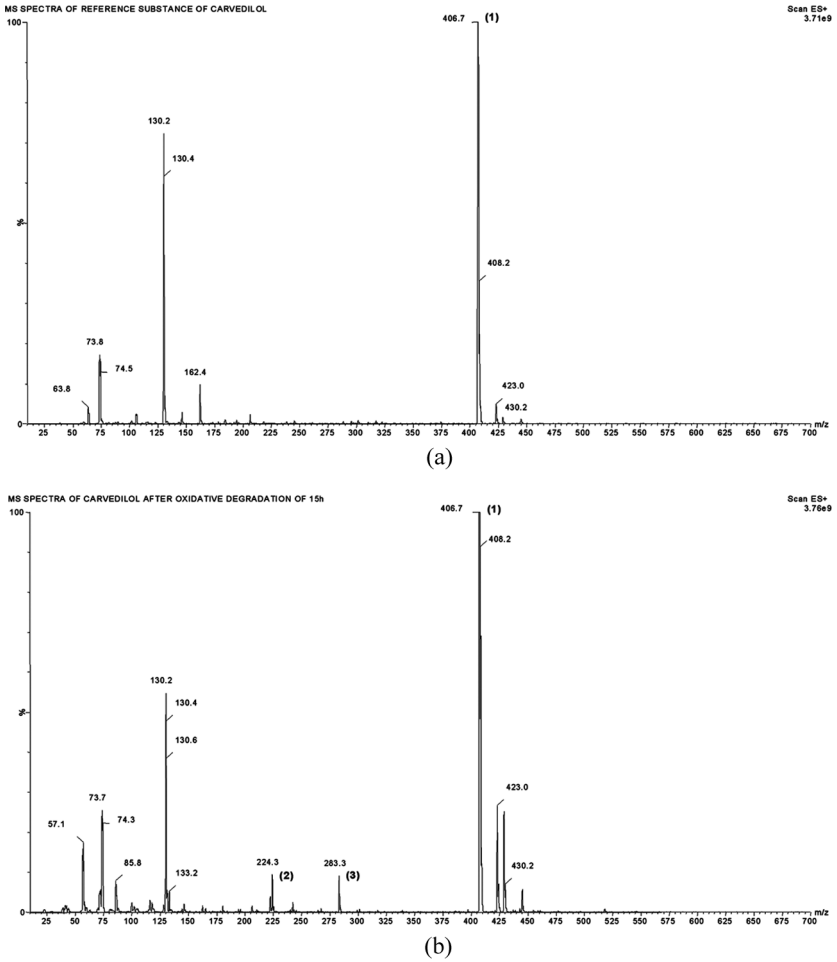

Figure 4. The full scan LC-MS spectra of carvedilol. (a) Chemical reference substance solution: peak 1 – carvedilol. (b) After degradation in hydrogen peroxide 7.5% (v/v) at room temperature ($25 \pm 2^{\circ}C$), protected from light for 15 hours.

from the two methods were compared statistically by the Student's *t*-test, showing non-significant difference ($P > 0.05$) between the experimental results. As shown in Table 7, the results of weight variation, disintegration, hardness, friability, content uniformity, and dissolution test of products A and C are in accordance with pharmacopoeial limits and specifications. However, the disintegration time and hardness value increased with time for product C, while the dissolution rate had a slight decrease for this product. For product B, changes in the disintegration time, hardness value, uniformity content, and dissolution rate were observed. According to

Table 6. Stability accelerated of product A, B and C for the assay, by UV and LC-MS/MS methods

	Assay (%)					
	LC-MS/MS[a]			UV[a]		
Month	A	B	C	A	B	C
0	99.1	98.1	99.9	98.6	100.5	99.7
1	100.2	99.5	99.3	99.1	98.5	101.0
2	98.9	95.4	98.2	96.8	98.0	98.9
3	98.8	99.5	100.0	97.9	98.7	99.7
4	99.7	99.0	99.5	96.9	96.6	98.9
5	97.7	88.1	100.3	95.7	87.4	99.0
6	99.4	86.3	98.5	95.1	82.9	96.0

[a]Mean of three determinations.

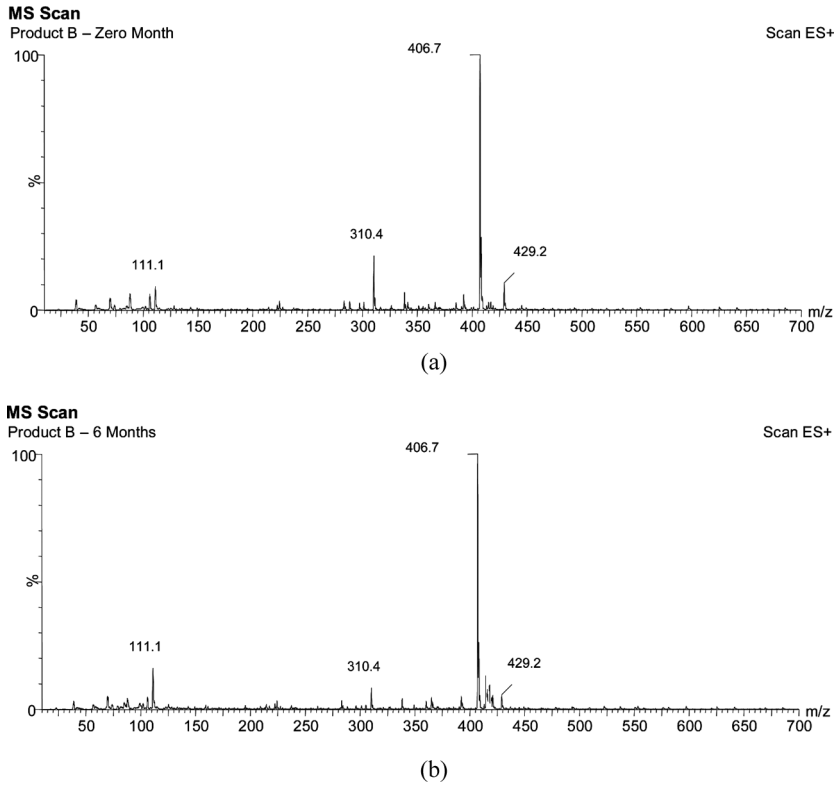

Figure 5. The full scan LC-MS spectrum of product B. (a) Before exposition to accelerated stability ($40 \pm 2°C/75\% \pm 5$ RH). (b) After accelerated stability for 6 months.

Table 7. Stability accelerated of product A, B, and C for all parameters, except to the assay

	0 month			6 months		
Parameters	A	B	C	A	B	C
Weight variation[a] (mg)	81.5	98.5	120.4	82.1	99.7	120.6
Disintegration[b] (min:sec)	2:50	1:00	0:44	3:00	10:0	3:05
Hardness[c] (N)	51.5	80.0	40.4	53.8	117.4	62.2
Friability[d] (%)	0.23	0.04	0.14	0.27	0.03	0.18
Content Uniformity (%) (n = 10)[e]	97.9–105.4	94.7–106.2	102.7–107.9	94.9–105.4	82.8–83.5	95.7–102.6
Dissolution (%) (n = 6)[f]	94.8	91.5	97.0	96.7	81.1	90.8

N = Newton.

Specifications: [a]Mean ± 7,5%, [b]maximum 30 minutes in water, [c]minimum 30 N, [d]maximum 1.5%, [e]variation: 85–115% and relative standard deviation ≤6%, [f]%dissolved: ≥85% in 60 minutes in buffer acetate pH 4.5.

ICH,[2] where significant changes occur at the accelerated condition, the shelf-life would depend of on the outcome of stability testing at the intermediate conditions, as well as the long-term condition.

CONCLUSION

The stability of the carvedilol was investigated using UV, LC, and LC-MS/MS procedures. These methods permit detection and quantitation of carvedilol in the presence of its degradation products. The kinetic studies indicate that carvedilol undergoes fast degradation in 7.5% (v/v) hydrogen peroxide solution. The rate of the oxidative route followed was second-order kinetics. The results of the validation studies show that the LC-MS/MS method is specific, accurate, and possesses significant linearity and precision characteristics without any interference from the excipients. Two degradation products, at 224.3 and 283.3 Daltons, were observed by LC-MS analyses of the degraded solution of carvedilol. Moreover, the values obtained from the UV and LC-MS/MS MS and methods in the study of accelerated stability of tablets containing CAR were compared statistically by the Student's *t*-test, showing non-significant difference ($P > 0.05$) between the experimental results.

ACKNOWLEDGMENTS

The authors thank FIPE (Fundo de Incentivo à Pesquisa) and FAPERGS (Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul) for the financial support.

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Received August 18, 2008
Accepted September 17, 2008
Manuscript 6392