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ZORA Ž. STOILJKOVIĆ[1]
MILKA B. JADRANIN[2]
SVETLANA LJ. ĐURIĆ[1]
SLOBODAN D. PETROVIĆ[3]
MILKA L. AVRAMOV IVIĆ[4]
DUŠAN Ž. MIJIN[3]

[1]Zdravlje A.D., Actavis Company, Leskovac, Serbia
[2]University of Belgrade, IHTM - Center for Chemistry, Belgrade, Serbia
[3]University of Belgrade, Faculty of Technology and Metallurgy, Belgrade, Serbia
[4]University of Belgrade, IHTM - Center for Electrochemistry, Belgrade, Serbia

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INVESTIGATION OF FORCED AND TOTAL DEGRADATION PRODUCTS OF AMLODIPINE BESYLATE BY LIQUID CHROMATOGRAPHY AND LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY

Article Highlights

- Amlodipine besylate stock solutions were subjected to stressed conditions
- Acid and alkali hydrolysis, chemical oxidation and photodegradation were used
- Electrochemical degradation by cyclic voltammetry was also studied
- LCMS was used for the investigation of the degradation products
- Total degradation of amlodipine besylate was achieved in 5 mol/L NaOH at 80 °C for 6 h

Abstract

An isocratic, reversed-phase liquid chromatographic method was applied for the investigation of the degradation products of amlodipine besylate under stressed conditions in solution. Amlodipine besylate stock solutions were subjected to acid and alkali hydrolysis, chemical oxidation and photodegradation, as well as to the electrochemical degradation by cyclic voltammetry in 0.05 mol/L $NaHCO_3$ on gold electrode. The total degradation of amlodipine besylate was achieved in 5 mol/L NaOH at 80 °C for 6 h and the compound with molecular formula $C_{15}H_{16}NOCl$ was identified as a main degradation product. Under acidic (5 mol/L HCl at 80 °C for 6 h) stress conditions 75.2% of amlodipine besylate degradation was recorded. Oxidative degradation in a solution of 3% H_2O_2:methanol 80:20 at 80 °C for 6 h showed that amlodipine besylate degraded to 80.1%. After 14 days of expose in photostability chamber amlodipine besylate solution showed degradation of 32.2%. In electrochemical degradation, after 9 h of cyclization the beginning of amlodipine oxidation was shifted for 200 mV to more negative potentials, with the degradation of 66.5%. Mass spectrometry analysis confirmed the presence of dehydro amlodipine derivate with molecular formula $C_{20}H_{23}N_2O_5Cl$ in oxidative and acidic conditions, while in electrochemical degradation was detected in traces.

Keywords: amlodipine besylate, forced degradation, reversed phase liquid chromatography, liquid chromatography-mass spectrometry.

Amlodipine, *R,S*-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine is a calcium channel blocking agent with a long duration of action, widely used against hypertension and angina [1,2]. It was originally marketed in tablet form by Pfizer as a besylate salt under the trade name Norvasc [3] and generic amlodipine besylate tablets have been launched by different manufacturers in Europe over the last ten years [4]. Amlodipine besylate (Figure 1) belongs to the one of the most worldwide prescript cardiovascular drugs.

The identification and qualification of degradation products in pharmaceuticals is important since impurities may cause the undesirable effects on the patients and on the other hand may have influence on

Correspondence: D. Mijin, University of Belgrade, Faculty of Technology and Metallurgy, Belgrade, Serbia.
E-mail: kavur@tmf.bg.ac.rs
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quality, safety and efficacy of the drug product. The International Conference on Harmonization (ICH) has issued the Guidelines regarding qualification of impurities in drug substance and products as well as stability testing for registration of pharmaceutical for human use [5-7]. ICH Guidelines [8] also require the implementation of stress testing procedures for the identification of degradation products that are potentially occurring in drug substances, which can help to understand the possible degradation pathway for the drugs. This is important from the aspect of product formulation, the choice of packaging material and the drug product storage conditions.

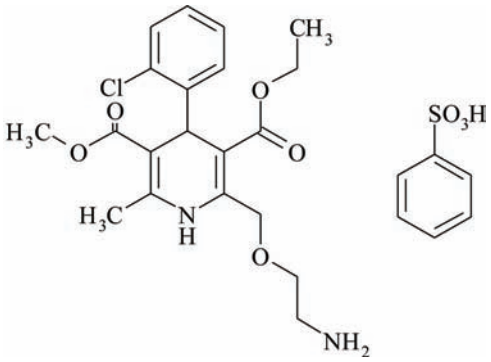


Figure 1. Structure of amlodipine besylate.

Amlodipine is photosensitive and undergoes an easy light-induced aromatization to pyridine derivative, as do other 1,4-dihydropyridine compounds [9,10]. The photostability and stability of amlodipine in liquid dosage forms have been also reported [11,12]. Regarding the stability and compatibility of amlodipine besylate in its solid formulations with different drug excipients, the influence of excipients and storage conditions on amlodipine besylate stability has been investigated. The major degradation product is amlodipine besylate glycosyl due to a well-known Maillard reaction between primary amines (amlodipine) and lactose as a water-soluble excipient [13]. The structural analysis of the Maillard reaction product was performed by LC-MS.

Photostability of amlodipine has also been monitored in several pharmaceutical inclusion systems characterized by plurimolecular aggregation of the drug and excipients with high molecular weight including cyclodextrins, liposomes and microspheres. Such matrixes prevent drug oxidation to the aromatic derivative through a number of chemical and physical barriers. These systems have shown a high degree of protection with a degradation rate always lower than in the usual solid drug formulations [14,15]. Thermal degradation of amlodipine base affords a mixture of three cyclic degradation products all resulting by intramolecular cyclisation reactions [16].

Different analytical methods have been developed for the study of stressed degradation behavior of pharmaceutical active substances and amlodipine besylate including spectrophotometric method [17], HPTLC [18,19], HPLC [20-23], RP-HPLC [24-27], UPLC [28], CE [29] and LC-2D-NMR/LC-MS [30]. Forced degradation studies show that amlodipine is degrading slowly under thermal stress conditions [16], degrading faster under photo stress - more rapidly under 366 than under 254 nm [10] and even more under acidic, alkaline and oxidative stress conditions [21]. The aim of this work is investigation of forced degradation of amlodipine besylate under the different stressed conditions in solution in order to achieve the total degradation of amlodipine besylate.

EXPERIMENTAL

Apparatus

An Agilent 1100 series HPLC system equipped with a binary pump, 1200 Diode Array Detector, column thermostat, thermostatted autosampler and Spherisorb ODS1 column (250.0 mm×4.0 mm id, 5 μm particle size) was used. The effluents were monitored at 237 nm. Chemstation 32 software was used for the data acquisition. All the drugs and chemicals were weighed on a Sartorius AC 121S electronic balance (Sartorius Goettingen, Germany). A UV light cabinet Camag (Made in Switzerland) for forced degradation at 366 nm was used. Photodegradation processes were performed in a light cabinet type PSC 062 (Weiss Gallenkamp, UK) equipped with both the cool white fluorescent and near ultraviolet lamp, according to the ICH Guideline for photostability testing (option 2, ICH Q1B, 1996) [31]. In the present study, the power was maintained to 0.66 W/m^2 and 9.85 kLux during 14 days in order to achieve an overall illumination of not less than 1.2 million Lux h and 200 W h/m^2.

Materials and methods

Amlodipine besylate EP7 quality and amlodipine besylate CRS (chemical reference standard), amlodipine for peak identification, amlodipine impurity A, impurity B and impurity G were kindly provided from Zdravlje A.D., Actavis Company (Leskovac, Serbia). HPLC grade of methanol and acetonitrile were purchased from J.T. Baker. Ammonium acetate, hydrochloric acid, hydrogen peroxide and sodium hydrogen carbonate were purchased from Merck. Sodium hydroxide was purchased from J.T. Baker. All chemi-

cal used were of analytical grade. For preparing solution of ammonium acetate purified water was used.

LC Analysis

A Spherisorb ODS1 (octadecylsilyl silica gel) column (Waters, 250.0 mm×4.0 mm id, 5 μm particle size) was used, and the mobile phase consisted of 2.3 g/L ammonium acetate solution : methanol (30:70, *V*/*V*). The flow rate was maintained at 1.5 mL/min for related substances peak identification and 1.0 mL/min for forced degradation products. The mobile phase was passed through a 0.45 μm membrane filter (Sartorius Stedim Biotech GmbH, Goettingem, Germany) and degassed before use. The elution was monitored with diode array detector at 237 nm. The injection volume was 20 μl for related substances peak identification and 100 μl for degradation products. The test was carried out protected from light. All the chromatographic separations were carried out at controlled room temperature (20-25 °C).

LC-MS Analysis

An Agilent 1100 series HPLC system equipped with a degasser, autosampler, Waters Spherisorb ODS1 column (250.0 mm×4.0 mm id, 5 μm particle size) and DAD detector coupled with 6210 Time-of-Flight LC/MS system (Agilent Technologies) was used. An isocratic method (the same like was for LC) and the mobile phase consisted of 2.3 g/L of ammonium acetate solution:methanol (30:70, *V*/*V*) was used. The flow rate was maintained at 1.0 mL/min. The injection volume was 100 μL and the column temperature was maintained at 30 °C. The chromatograms were monitored at 237 nm. A personal computer system running MassHunter Workstation software was used for data acquisition and processing.

The positively charged molecular ions were obtained with electrospray ionization (ESI) at atmospheric pressure. The eluated compounds were mixed with nitrogen in the heated nebulizing interface, and polarity was tuned to positive with the following ESI parameters: capillary voltage 4.0 kV, gas temperature 350 °C, drying gas flow rate 12 L/min, nebulizer pressure 45 psig (310 Pa), and fragmentor voltage 140 V. Mass spectra were acquired over an *m*/*z* range of 100-2000.

Preparation of stock solution

Accurately weighed 10 mg of amlodipine besylate was transferred to a 10 mL volumetric flask and dissolved and diluted to the mark with methanol to obtain a stock solution of 1000 μg/mL. The freshly prepared stock solution was used.

Procedure for forced degradation study by HPLC

Preparation of sample solution

Alkaline degradation. Alkali degradation studies were carried out by using of 1 mL of stock solution and 9 mL of 0.1 mol/L NaOH. The solution was kept at room temperature for a period of 4 h, after which the solution was neutralized with 0.1 mol/L hydrochloric acid. The solution was diluted to 100 mL with mobile phase to prepare the solution of 10 μg/mL.

The second experiment was carried out by using of 1 mL of stock solution and 5 mL of 0.1 mol/L NaOH. The solution was kept at 80 °C for 6 h, after which the solution was neutralized with hydrochloric acid. The solution was diluted to 100 mL with mobile phase to prepare the solution of 10 μg/mL.

The third experiment was carried out by using of 1 mL of stock solution and 0.2 mL of 5 mol/L NaOH. The solution was kept at 80 °C for 6 h, after which the solution was neutralized with hydrochloric acid. The solution was diluted to 100 mL with mobile phase to prepare the solution of 10 μg/mL.

Degradation in 0.05 mol/L $NaHCO_3$. The solution of amlodipine besylate was prepared by dissolving 10 mg of the drug into 100 mL volumetric flask with 0.05 mol/L $NaHCO_3$. The freshly prepared drug solution was compared with the drug solution, which was kept at room temperature for 5 h.

The another test solution was prepared by dissolving 10 mg of the drug into a 100 mL volumetric flask with 0.05 mol/L $NaHCO_3$. The solution was kept at 80 °C for 5 h (final pH 8.87). The solution was diluted to 10 mL with mobile phase to prepare the solution of 10 μg/mL.

Oxidative degradation. For oxidative degradation studies, initially the solution containing 1 mL of stock solution and 9 mL 3% H_2O_2 was prepared. The solution was kept at room temperature for a period of 24 h, after which the solution was diluted to 100 mL with mobile phase to prepare the solution of 10 μg/mL.

Additionally, two samples for oxidative degradation were prepared. The studies were carried out by using of 1 mL of stock solution and 9 mL solution of 3% H_2O_2 : methanol = 80:20 (*V*/*V*). The solution was kept at 80 °C for a period of 2 h and then for 6 h. After that, the solution was diluted to 100 mL with mobile phase to prepare the solution of 10 μg/mL.

Photodegradation. The solution of amlodipine besylate was prepared by dissolving 14.3 mg of the drug into 100 mL volumetric flask with water.

5 mL of drug solution was exposed to the long wavelength (366 nm) UV light for 2 h and to photodegradation as per ICH guidelines for a period of 14 days (1.2 million Lux h and 200 W h/m^2).

Procedure for forced degradation study by LC-MS

In LC-MS procedure for investigation of amlodipine degradation products alkaline, acidic, oxidative and electrochemical degradation in 0.05 mol/L $NaHCO_3$ were included.

Stock solution. Amlodipine besylate at a concentration of 1 mg/mL in methanol was used in degradation study. The stock solution of amlodipine besylate was subjected to alkali, acidic and oxidative stress conditions.

Alkali degradation. The studies were carried out by using of 1 mL of stock solution and 0.2 mL of 5 mol/L NaOH. The solution was kept at 80 °C for 6 h, after which the solution was neutralized with hydrochloric acid. The solution was diluted to 100 mL with mobile phase to prepare the solution of 10 μg/mL.

Acidic degradation. The studies were carried out by using of 1 mL of stock solution and 0.2 mL of 5 mol/L HCl. The solution was kept at 80 °C for 6 h, after which the solution was neutralized with sodium hydroxide. The solution was diluted to 100 mL with mobile phase to prepare the solution of 10 μg/mL.

Oxidative degradation. The studies were carried out by using of 1 mL of stock solution and 9 mL solution of 3% H_2O_2:methanol = 80:20 (*V*/*V*). The solution was kept at 80 °C for 6 h. After that the solution was diluted to 100 mL with mobile phase to prepare the solution of 10 μg/mL.

Electrochemical degradation. Electrochemical degradation of amlodipine besylate (γ = 9.9 μg/mL) was carried out by cyclic voltammetry in 0.05 mol/L $NaHCO_3$ for 9 h on a gold electrode using equipment which has been described in detail [32].

RESULTS AND DISCUSSION

Amlodipine besylate is official in European Pharmacopoeia [33]. The objective of this study was to investigate the amlodipine besylate degradation products under the different stress conditions including electrochemical degradation.

In our preliminary experiment, the forced degradation study was carried out by subjecting amlodipine besylate to alkali hydrolysis, chemical oxidetion, photodegradation and degradation in 0.05 mol/L $NaHCO_3$ in order to achieve the complete degradation of amlodipine besylate. In addition, electrochemical degradation was also performed. Results are given in Figures 2 and 3, and Tables 1-4.

Alkali hydrolysis in 0.1 mol/L NaOH performed at room temperature for 4 h showed that amlodipine besylate was stable under the applied conditions. No changes in chromatogram of amlodipine besylate were found (Figure 2a). The chromatogram of alkali degraded sample in 0.1 mol/L NaOH at 80 °C for 6 h showed degradation product peaks at retention times (t_R) 3.015, 3.538, 4.899 and 5.154 min (Figure 2b). It was recorded that 42% of amlodipine besylate was degraded. When alkali hydrolysis of amlodipine besylate was performed in 5 mol/L NaOH at 80 °C for 6 h, complete degradation of amlodipine besylate was accomplished, with the main degradation product peak at retention time 20.878 min (Figure 2c). The degradation of the molecule is ascribed to the alkali hydrolysis of the acetyl groups of amlodipine [13].

Amlodipine besylate degradation was performed in 0.05 mol/L $NaHCO_3$ at room temperature for 5 h and a degradation of 4.2% in comparison to the freshly prepared solution of amlodipine besylate was recorded. When amlodipine besylate was degraded in 0.05 mol/L $NaHCO_3$ at 80 °C for 5 h, 30.7% of amlodipine besylate degradation was recorded. Chromatogram (Figure 2d) showed formation of compounds with retention times 4.802 and 5.023 min.

Oxidative degradation (3% H_2O_2) of amlodipine besylate at room temperature for 24 h showed 5.3% amlodipine degradation with one degradation product peak at retention time 14.337 min. Oxidative degradation of amlodipine besylate in the solution of 3% H_2O_2:methanol 80:20 (*V*/*V*) at 80 °C for 2 h showed one degradation peak at retention times 14.335 min (Figure 3a) while oxidative degradation (3% H_2O_2-methanol 80:20) at 80 °C for 6 h showed three degradation products peak at retention times 7.582, 14.902 and 31.920 min (Figure 3b). Amlodipine besylate degradation was increased to 25.9 and 80.1%, respectively.

Chromatogram of amlodipine besylate photodegradation performed at 366 nm for 2 h showed three degradation products peaks at retention times 7.516, 10.259 and 14.395 min (Figure 3c) while chromatogram of amlodipine besylate photodegradation as per ICH guidelines for photostability testing for 14 days showed two degradation products peak at retention times 8.205 and 14.867 min (Figure 3d).

The degradation study thereby indicated that amlodipine besylate was found to be stable to alkali hydrolysis in 0.1 mol/L NaOH at room temperature for 4 h, while it was susceptible to degradation in 0.05 mol/L $NaHCO_3$, oxidative and photo stress conditions.

Amlodipine besylate in solution showed a decrease of 32.2% after 14 days of expose in a photostability chamber and 14.1% of decrease at 366 nm for 2 h.

According to the literature data [21] amlodipine decomposed to 25, 10 and 70% under acidic, neutral

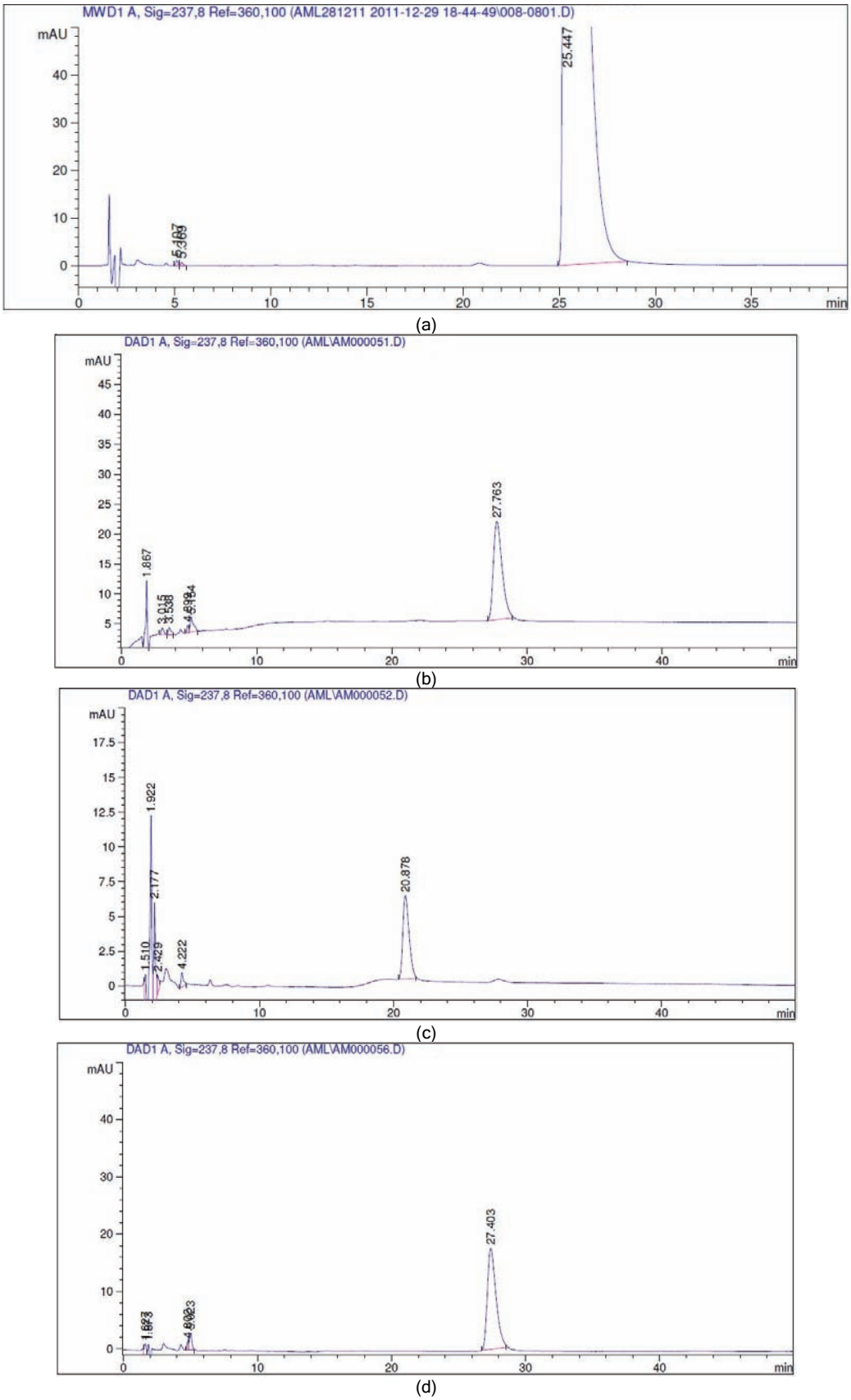
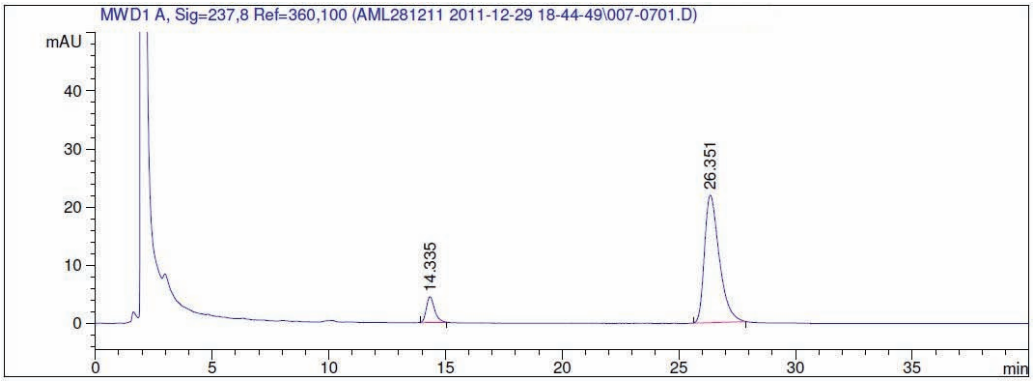
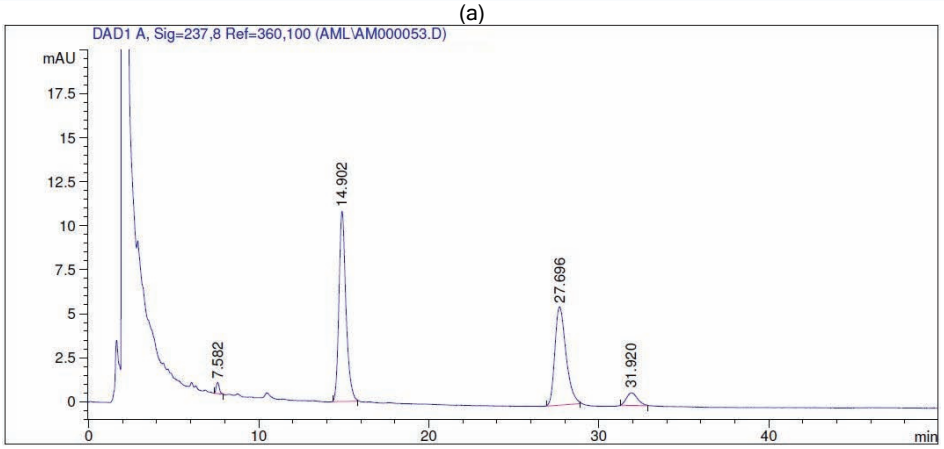


Figure 2. a) Liquid chromatogram of amlodipine besylate (AMB); chromatograms of alkali treated AMB at 80 °C for 6 h in b) 0.1, c) 5 mol/L NaOH and d) chromatogram of alkali treated (0.05 mol/L $NaHCO_3$) AMB at 80 °C for 5 h.



(a)



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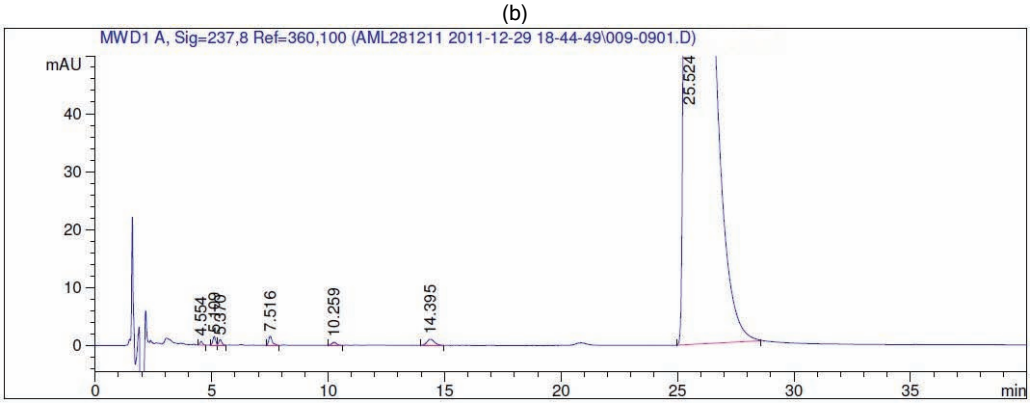
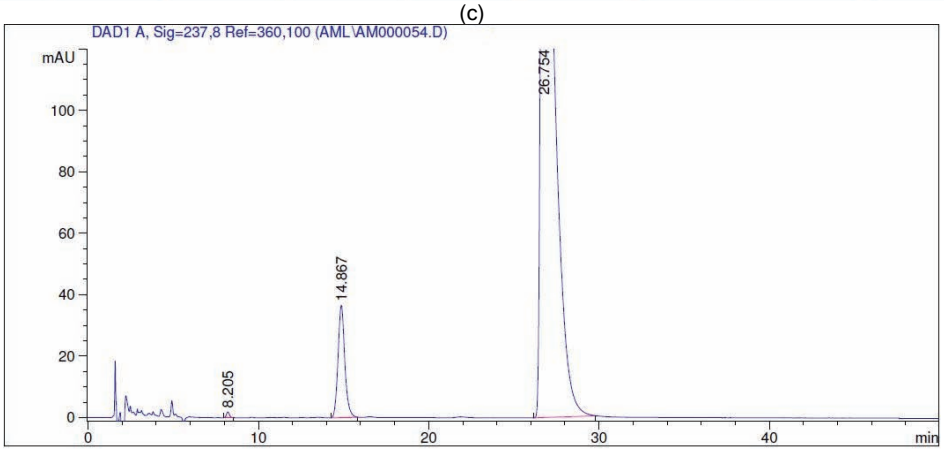


(c)



(d)

Figure 3. Chromatogram of oxidative degraded (3% H_2O_2:methanol = 80:20, V/V) AMB at 80 °C for a) 2 and b) 6 h; c) chromatogram showing photodegradation of AMB c) at 366 nm for 2 h and d) as per ICH guidelines for a period of 14 days.

Table 1. Results of LC-MS analysis of amlodipine besylate in alkali degradation (5 mol/L NaOH) at 80 °C for 6 h; Identification for amlodipine was performed using standard. For other compounds, the proposed molecular formula was determined on the basis of precisely measured masses; (tr) - traces; compounds detected by mass spectrometer

No.	t_R / min	Measured molecular mass and measured mass of *m/z* ions	Molecular formula
1	1.976	348.0823; 349.0895, 100% (351.0851, 30%); 719.1593, 100% (721.1577, 30%) + + 406.1296; 407,1369, 100% (409.11351, 30%)	$C_{11}H_{21}O_{10}Cl$ or $C_{12}H_{17}N_4O_6Cl$ + + $C_{20}H_{23}N_2O_5Cl$ or $C_{25}H_{23}O_3Cl$
2	5.703	472.2546; 473.2619	$C_{21}H_{36}N_4O_8$ (tr)
3	7.461	414.2125; 415.2198 + 394.1149; 395.1222 + +378.1408; 379.1480	$C_{23}H_{30}N_2O_5$ (tr) + $C_{21}H_{18}N_2O_6$ (tr) + +$C_{18}H_{22}N_2O_7$ (tr)
4	8.835	278.1491; 279.1563; 301.1384; 317.1142	$C_{16}H_{22}O_4$ (tr)
5	15.425	246.2170; 247.2238; 264.2509; 269.2065	$C_{14}H_{30}O_3$ (tr)
6	17.372	290.2436; 291.2507; 308.2772; 313.2332	$C_{16}H_{34}O_4$ (tr)
7	19.418	261.0900; 262.0973, 100 % (264,0948, 30%)	$C_{15}H_{16}NOCl$
8	25.078	408.1435; 409.1504, 100% (411.1480, 30%); 431.1328, 100% (433.1313, 30%)	$C_{20}H_{25}N_2O_5Cl$ (amlodipine) (tr)

and alkaline stress conditions, respectively, and to 80% under oxidative stress conditions.

Beside HPLC analysis of amlodipine besylate degradation products under stress conditions, LC-MS study was performed. The LC-MS procedure included alkaline, acidic, oxidative and electrochemical degradation of amlodipine besylate. Electrochemical degradation of amlodipine besylate was performed in 0.05 mol/L $NaHCO_3$.

Table 1 shows results of LC-MS analysis of amlodipine besylate in alkali degradation (5 mol/L NaOH) at 80 °C for 6 h. Forced degradation under alkali conditions confirmed the complete degradation of amlodipine besylate, while the compound at retention time 19.41 min with molecular formula $C_{15}H_{16}NOCl$ was found as a main degradation product. Molecular mass, found value of *m/z* ions and proposed molecular formula of all potential amlodipine besylate degradation products are given in Tables 1-4.

LC-MS analysis of amlodipine besylate electrochemical degradation by cyclic voltammetry (0.05 mol/L $NaHCO_3$) for 9 h on a gold electrode is given in Table 2. The electrochemical experiment indicated degradation because after 9 h of cyclization the beginning of amlodipine oxidation was shifted for 200 mV to more negative potentials. Based on liquid chromatography results 66.5% of amlodipine besylate degradation was achieved. Three degradation products were found at (t_R) 7.521 min with the proposed molecular formula $C_{21}H_{18}N_2O_6$, $C_{23}H_{30}N_2O_5$ and $C_{18}H_{22}N_2O_7$.

The results of LC-MS analysis of amlodipine besylate in acid degradation (5 mol/L HCl) at 80 °C for 6 h are summarized in Table 3. Under acidic conditions, amlodipine besylate was degraded up to 75.2% (according to the LC results). It was found that the degradation product with molecular formula $C_{18}H_{35}NO$ at (t_R) 26.84 min is formed at significant level.

Table 2. Results of LC-MS analysis of amlodipine besylate electrochemical degradation by cyclic voltammetry (0.05 mol/L $NaHCO_3$) for 9 h on gold electrode; Identification for amlodipine was performed using standard. For other compounds, the proposed molecular formula was determined on the basis of precisely measured masses; (tr) - traces; compounds detected by mass spectrometer

No.	t_R / min	Measured molecular mass and measured mass of *m/z* ions	Molecular formula
1	7.521	394.1146; 395.1219 + 414.2132; 415.2196 + + 378.1408; 379.1481	$C_{21}H_{18}N_2O_6$ + $C_{23}H_{30}N_2O_5$ + + $C_{18}H_{22}N_2O_7$
2	8.893	278.1494; 279.1566; 301.1387; 317.1138	$C_{16}H_{22}O_4$ (tr)
3	14.511	406.1274; 407.1347, 100% (409.1333, 30%); 429.1172, 100% (431.1175, 30%)	$C_{20}H_{23}N_2O_5Cl$ (tr)
4	15.480	246.2175; 247.2242; 264.2514; 269.2071	$C_{14}H_{30}O_3$ (tr)
5	17.430	290.2441; 291.2511; 308.2778; 313.2337; 329.2108 + + 348.2971; 349.3044 + 335.3014; 336.3086	$C_{16}H_{34}O_4$ (tr) + $C_{18}H_{40}N_2O_4$ (tr) + + $C_{19}H_{37}N_5$ (tr)
6	19.858	334.2700; 335.2759; 352.3040; 357.2592	$C_{18}H_{38}O_5$ (tr)
7	25.078	408.1429; 409.1500, 100% (411.1477, 30%); 431.1323, 100% (433.1305, 30%); 447.1064, 100% (449.1081, 30%)	$C_{20}H_{25}N_2O_5Cl$ (amlodipine)

Table 3. Results of LC-MS analysis of amlodipine besylate in acid degradation (5 mol/L HCl) at 80 °C for 6 h ; Identification for amlodipine was performed using standard. For other compounds, the proposed molecular formula was determined on the basis of precisely measured masses; (tr) – traces; compounds detected by mass spectrometer

No.	t_R / min	Measured molecular mass and measured mass of *m/z* ions	Molecular formula
1	5.683	455.2278; 456.2397; 473.2616 + 351.1200; 352.1272, 100% (354.1125, 30%); 374.1095, 100% (376.1046, 30%); 390.0830, 100% (392.0822, 30%); 725.2321, 100% (727.2326, 30%);	$C_{21}H_{33}N_3O_8$ + + $C_{18}H_{22}NO_4Cl$ or $C_{13}H_{22}N_3O_6Cl$
2	6.648	391.1263; 392.1333, 100% (394.1311, 30%); 414.1159, 100% (416.1149, 30%); 430.0911, 100% (432.0893, 30%)	$C_{19}H_{22}N_3O_7Cl$ (tr)
3	7.070	389.0968; 390.1042, 100 % (392.1030, 30 %); 412.0866, 100 % (414.0851, 30 %); 428.0594, 100 % (430.0579, 30 %); 801.1903, 100 % (803.1884, 30 %)	$C_{15}H_{20}N_3O_7Cl$
4	7.452	414.2121; 415.2194 + 394.1143; 395.1216 + 378.1405; 379.1478	$C_{23}H_{30}N_2O_5$ + $C_{21}H_{18}N_2O_6$ or $C_{16}H_{18}N_4O_8$ + $C_{18}H_{22}N_2O_7$
5	8.824	278.1492; 279.1564; 301.1382; 317.1139	$C_{16}H_{22}O_4$
6	13.736	406.1274; 407.1346, 100% (409.1328, 30%); 429.1172, 100% (431.1153, 30%); 445.0905, 100% (447.0906, 30%); 835.2488, 100 % (837.2469, 30%) + 333.1110; 334.1184, 100% (336.1161, 30%); 356.1009, 100% (358.0981, 30%)	$C_{20}H_{23}N_2O_5Cl$ + + $C_{18}H_{20}NO_3Cl$ or $C_{13}H_{20}N_3O_5Cl$
7	15.367	246.2176; 247.2245; 264.2516; 269.2067	$C_{14}H_{30}O_3$ (tr)
8	17.310	290.2442; 291.2510; 308.2781; 313.2335; 329.2098	$C_{16}H_{34}O_4$ or $C_{17}H_{30}N_4$
9	19.397	261.0899; 262.0972, 100% (264.2516, 30%)	$C_{15}H_{16}NOCl$ (tr)
10	22.271	348.1227; 349.1300; 371.1115	$C_{19}H_{16}N_4O_3$ or $C_{18}H_{20}O_7$ or $C_{24}H_{16}N_2O$ (tr)
11	24.868	408.1429; 409.1499, 100% (411.1480, 30%); 431.1323, 100% (433.1306, 30%); 447.1064, 100% (449.1085, 30%)	$C_{20}H_{25}N_2O_5Cl$ (amlodipine)
12	26.840	281.2701; 282.2770; 299.3031; 304.2599; 563.5500	$C_{18}H_{35}NO$

LC-MS analysis of amlodipine besylate oxidative degradation (3% H_2O_2 : methanol 80:20) at 80 °C for 6 h is given in Table 4. Forced degradation of amlodipine besylate under oxidative conditions also showed the presence of compound $C_{18}H_{35}NO$, as like as in acid degradation, at (t_R) 26.94 min. Mass spectrometry technique confirmed the presence of compound with molecular formula $C_{20}H_{23}N_2O_5Cl$ corresponding to dehydro amlodipine derivate [33–35]. This compound is one of the forced degradation products in oxidative and acidic conditions, while in electrochemical degradation was found in traces.

To complete the MS analysis, LC-NMR preparative isolation of compounds obtained by forced deg-

Table 4. Results of LC-MS analysis of amlodipine besylate in oxidative degradation (3% H_2O_2:methanol, 80:20) at 80 °°C for 6 h; Identification for amlodipine was performed using standard. For other compounds, the proposed molecular formula was determined on the basis of precisely measured masses; (tr) – traces; compounds detected by mass spectrometer

No.	t_R / min	Measured molecular mass and measured mass of *m/z* ions	Molecular formula
1	4.275	444.2202; 445.2275	$C_{19}H_{32}N_4O_8$
2	5.706	472.2556; 473,2628	$C_{21}H_{36}N_4O_8$
3	7.464	414.2130; 415.2203 + 394.1154; 395.1227 + 378.1411; 379.1484	$C_{23}H_{30}N_2O_5$ + $C_{21}H_{18}N_2O_6$ or $C_{16}H_{18}N_4O_8$ + $C_{18}H_{22}N_2O_7$
6	8.843	278.1496; 279.1568; 301.1390; 317.1138	$C_{16}H_{22}O_4$ or $C_{17}H_{18}N_4$
7	13.564	406.1271; 407.1343, 100% (409.1328, 30%); 429.1172, 100% (431.1155, 30%); 445.0909, 100% (447.0932, 30%)	$C_{20}H_{23}N_2O_5Cl$
8	17.282	290.2437; 291.2507; 308.2777; 313.2328; 329.2084	$C_{16}H_{34}O_4$ or $C_{17}H_{30}N_4$
9	21.600	255.2543; 256.2615; 273.2879; 278.2434; 294.2168; 511.5183; 533.5006	$C_{16}H_{33}NO$
10	24.725	408.1431; 409.1496, 100% (411.1475, 30%); 431.1327, 100% (433.1301, 30%)	$C_{20}H_{25}N_2O_5Cl$ (amlodipine)
11	26.988	281.2701; 282.2770; 299.3031; 304.2599; 563.5500	$C_{18}H_{35}NO$

radation of amlodipine besylate should be carried out. This will be the subject of our further investigation.

CONCLUSION

The proposed study describes an isocratic, reversed-phase liquid chromatographic method for investigation of degradation products of amlodipine besylate under the stressed conditions in solution. Amlodipine-besylate stock solutions were subjected to acid and alkali hydrolysis, chemical oxidation and photo degradation as well as to the electrochemical degradation by cyclic voltammetry in 0.05 mol/L $NaHCO_3$ on gold electrode. The study showed the complete degradation of amlodipine besylate in 5 mol/L NaOH at 80 °C for 6 h, whereas moderately degraded in alkali 0.1 mol/L NaOH at 80 °C for 6 h (42.0%) and photo UV/Vis for 14 days (32.2%) conditions. Under the acid conditions, amlodipine besylate was degraded up to 75.2%. The drug was highly degraded in oxidative (80.1%) conditions. By cyclic voltammetry, 66.5% of amlodipine besylate degradation was achieved. After 9 h of cyclization the voltammogram shows that the beginning of amlodipine oxidation was shifted for 200 mV to more negative potentials. In addition, using of mass spectrometry technique we identified the presence of dehydro amlodipine derivate $C_{20}H_{23}N_2O_5Cl$ as one of the forced degradation products in oxidative and acidic degradation of amlodipine.

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ZORA Ž. STOILJKOVIĆ[1]
MILKA B. JADRANIN[2]
SVETLANA LJ. ĐURIĆ[1]
SLOBODAN D. PETROVIĆ[3]
MILKA L. AVRAMOV IVIĆ[4]
DUŠAN Ž. MIJIN[3]

[1]Zdravlje A.D., Actavis Company, Leskovac, Srbija
[2]Univerzitet u Beogradu, IHTM – Centar za hemiju, Beograd, Srbija
[3]Univerzitet u Beogradu, Tehnološko-metalurški fakultet, Beograd, Srbija
[4]Univerzitet u Beogradu, IHTM – Centar za elektrohemiju, Beograd, Srbija

NAUČNI RAD

ISPITIVANJE DEGRADACIONIH PROIZVODA AMLODIPIN-BEZILATA PRIMENOM TEČNE HROMATOGRAFIJE I TEČNE HROMATOGRAFIJE-MASENE SPEKTROMETRIJE U USLOVIMA FORSIRANE I POTPUNE DEGRADACIJE

Primenjena je izokratska RP-HPLC metoda za ispitivanje degradacionih proizvoda amlodipin-bezilata u uslovima forsirane degradacije. Osnovni rastvori amlodipin-bezilata su podvrgnuti kiseloj i alkalnoj hidrolizi, hemijskoj i fotodegradaciji kao i elektrohemijskoj degradaciji primenom ciklične voltametrije u rastvoru 0,05 mol/L $NaHCO_3$ na elektrodi od zlata. Potpuna degradacija amlodipin-bezilata je postignuta u 5 mol/L NaOH na 80 °C za 6 sati, a jedinjenje molekulske formule $C_{15}H_{16}NOCl$ je identifikovano kao glavni degradacioni proizvod. Pod uticajem kiseline (5 mol/L HCl) na 80 °C za 6 sati postignuto je 75,2% degradacije amlodipin-bezilata. U uslovima oksidativnog stresa u rastvoru 3% H_2O_2-metanol 80:20 na 80 °C tokom 6 sati pokazana je degradacija od 80,1%. Nakon 14 dana izlaganja rastvora amlodipin-bezilata fotodegradaciji u komori za fotostabilnost postignuta je degradacija od 32,2%. Kod elektrohemijske degradacije posle 9 sati ciklizacije početak oksidacije amlodipina se pomerio za 200 mV ka negativnijim potencijalima, sa degradacijom od 66,5%. Masenom spektrometrijom potvrđeno je prisustvo dehidro derivata amlodipina molekulske formule $C_{20}H_{23}N_2O_5Cl$ kao proizvoda forsirane degradacije u uslovima oksidacije i degradacije u kiseloj sredini. Pri elektrohemijskoj degradaciji amlodipina ovo jedinjenje nađeno je u tragovima.

Ključne reči: amlodipin-bezilat, forsirana degradacija, reverzno fazna tečna hromatografija, tečna hromatografija-masena spektrometrija.