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STABILITY STUDY OF AMLODIPINE BESYLATE AND BISOPROLOL FUMARATE IN AQUEOUS SOLUTIONS

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Abstract: Valuable information concerning stability of compounds can be obtained by using different media (water, hydrochloric acid or sodium hydroxide) for dissolution of active pharmaceutical substances. Furthermore, additional knowledge is gained by performing experiments at different temperatures. This research paper deals with the stability of amlodipine besylate and bisoprolol fumarate in different media at different temperatures, whereby certain conclusions are drawn. For stability assessment, chemical kinetics approach was used, and constant rate (k), half-time ($t_{1/2}$) and activation energy (Ea) were used for prediction of compound stability degree. The stability of amlodipine besylate and bisoprolol fumarate were tested, both separately and in mixture, in water and in 0.01M HCl. All the investigated solutions were treated at two temperatures 25° and 70°C at the following time intervals: 0, 1 h, 24 h, 48 h and 72 h. Hydrophilic Interaction Liquid Chromatography – HILIC method, previously developed and validated, was used. On the basis of obtained results it was concluded that amlodipine-besylate was more stable in water than in acid medium, more stable in mixture rather than individually and more stable at lower temperatures.

This was confirmed by the obtained values of monitored parameters: amlodipine besylate Ea = 30.68 kJ mol^{-1}, k (25 °C) = 0.000333 mM h^{-1}, k (70 °C) = 0.00169 mM h^{-1}; amlodipine besylate in mixture Ea = 42,414 kJ mol^{-1}, k (25 °C) = $1.27 \cdot 10^{-4}$ mM h^{-1}, k (70 °C) = 0.0012 mM h^{-1}. Based on the obtained approximate Ea value for bisoprolol fumarate in acid (59 kJ mol^{-1}) and in water (56 kJ mol^{-1}), bisoprolol fumarate showed excellent stability against the media in which it was studied. On the other hand, the temperature had a significant effect on the stability of bisoprolol fumarate. These results provide the relevant information about the stability of the tested active substances, and may be of importance during the development of an appropriate pharmaceutical product. A bigger influence on the stability of bisoprolol fumarate had a temperature effect.

Keywords: amlodipine besylate, bisoprolol fumarate, stability in solutions, chemical kinetics.

1. INTRODUCTION

Water has a very wide application in pharmaceutical practice. Water is used as an excipient in final product, in production of active pharmaceutical ingredients, for cleaning/laundry equipment and containers [1] and finally water is an excellent solvent. Water's ability to dissolve a large number of substances lays in its physicochemical properties: small molecular volume (which allows easy penetration into the crystal lattice of substances), a powerful permanent dipole, ability to form hydrogen bonds and high dielectric constant [2]. Water is official by the European Pharmacopoeia of 2011. (Ph. Eur. 7.0), containing the following monographs for water: Purified Water – *Aqua purificata*, Highly Purified Water – *Aqua valde purificata*, Water for Injections – *Aqua ad iniectabile* [3] and water for dilution of concentrated solution for hemodialysis [2]. Potable water serves as a raw material for preparation of all these official water types. Potable water is usually subjected to distillation processes, ion exchange (exchange ions/demineralization), reverse osmosis and ultrafiltration. Water must have appropriate

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physicochemical values and microbiological parameters (conductivity, total organic carbon, microbial contamination, nitrates, heavy metals, aluminum, bacterial endotoxins) that correspond to Ph. Eur. 7.0 regulations. In addition, limits for acidity, alkalinity, chlorides, sulfates, ammonium, calcium, magnesium, oxidizable substances, residue after evaporation and bacterial contamination are specified as well [2]. Water also affects the stability of drugs. The chemical stability of a drug is of great importance, since it becomes less effective as it undergoes degradation. Also, drug decomposition may yield some side-effects with products that are harmful for the patient. In modern pharmaceutical analysis, stability studies of pharmacodynamic active substances are of great importance. Stability is quantitatively expressed as the expiration date of the drug, *i*.e. as the time during the drug stay suitable for use if kept under proper defined conditions. The importance of stability is highlighted by regulations prescribed by the European Medicines Agency (EMA) and International Conference of Harmonization (ICH) [4,5].

Chemical kinetics has a very wide application in the study of the stability of pharmaceutical preparations since stability depends on degradation rate of active substances [6]. Hydrolysis of the drug entity can be a major factor in the instability of solutions. So, in order to design stable products, we must first understand how our product degrades. Temperature is one of the primary factors affecting drug stability [7].

Temperature is an important parameter because most reactions proceed faster at elevated temperatures in comparison with lower temperatures. By performing forced degradation studies at elevated temperatures, also known as accelerated stability studies, one can predict the stability of compounds. In accelerated stability testing, a product is stressed at several high (warmer than ambient) temperatures and the amount of heat input required to cause product failure is determined [8]. This is done to subject the product to a condition that accelerates degradation. This information is then projected to predict the shelf life or used to compare the relative stability of alternative formulations. This usually provides an early indication of the product shelf life and thus shortening the development schedule [9,10].

The concept of accelerated stability testing is based upon the Arrhenius equation, as shown in equation 1.

$$k = Ae^{-Ea/RT} \quad (1)$$

k – rate constant
Ea – activation energy, J mol^{-1}
A – the frequency factor, s^{-1}
R – universal gas constant, 8.314 J mol^{-1} K^{-1}

Ea is used as a measure of temperature dependence of the rate constant. Ea values of about 40–130 kJ mol^{-1} are generally observed in the degradation of drug substances. The term Ea is a measure of how sensitive the degradation rate of a drug is to temperature changes. Estimation of rate constant for drug degradation is an important step in predicting the stability of pharmaceuticals. Knowing how such rate constant changes with temperature in a quantitative way may allow predicting the stability at other temperatures.

The aim of this paper is to investigate the stability of amlodipine besylate (AB) and bisoprolol fumarate (BF) individually and in mixtures influenced by different media: water (H2O) and hydrochloric acid (HCl), and at different temperatures (25 °C and 70 °C). As mentioned above, kinetics parameters give very valuable information concerning stability. In order to estimate the stability of AB and BF systems, the following kinetics parameters were monitored: rate constant (k), half-time reactions (t1/2) and the activation energy (Ea). The tested analytes (AB and BF) are official in Ph. Eur. 7.0 which provides a method for determination of both compounds and their related substances – Reverse Phase High Performance Liquid Chromatography method (RP–HPLC), column C18 (250 x 4.6 mm, 5 μm particle size), with isocratic elution method for AB and a gradient elution method for BF [3]. Chemical structure of analyzed compounds is shown in Figure 1.

After reviewing literature it was shown that AB and BF were determined in pharmaceutical dosage forms mostly using the RP–HPLC on a C18 column [11,12]. Also, the combination of HPLC method and spectrophotometric method was used to determine BF in combination with metoprolol and hydrochlorothiazide from biological materials using HPLC and spectrophotometric methods [13−15] as well as to determine AB from tablets and biological material in combination with lisinopril, losartan, hydrochlorothiazide, metoprolol, olmesartan or other antihypertensive drugs [16−21]. L. Kristoffersen et al. described a method for simultaneous determination of BF and other beta-blockers from biological material by high pressure liquid chromatography in combination with mass spectrometry [22]. There are numerous papers about determination of BF and other beta-blockers, either alone or in mixture with ranitidine, omeprazole, citalopram or other drugs by using Hydrophilic Interaction Liquid Chromatography (HILIC) method [23−26].

Concerning literature, it was concluded that there were no stability studies of AB and BF degradation performed by HILIC method. Also, there is no

literature data concerning chemical degradation kinetics on these two analytes (both individually and in mixtures) with different medium and different temperatures. This research paper will provide very valuable information regarding stability of these two very important active substances and may be of great help during development of appropriate pharmaceutical products.

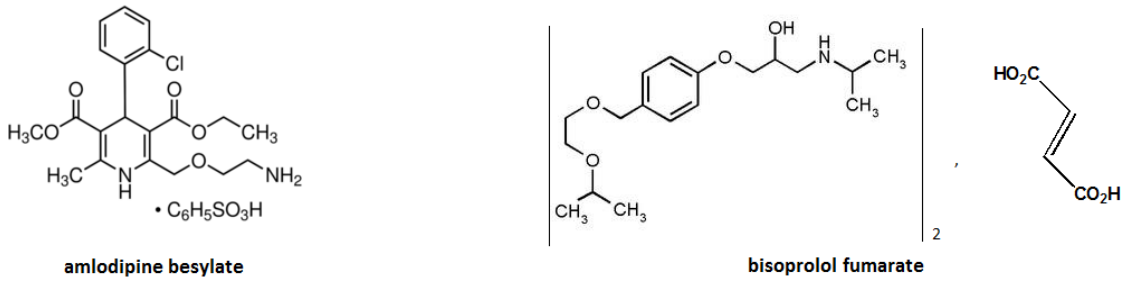

Figure 1. Chemical structure of analyzed compounds

2. METHODS AND MATERIALS

Chromatographic system. The experiments were performed on chromatographic system *Agilent Technologies HP 1200*, which consists of the HP 1200 pump, HP 1200 UV/VIS detector and *ChemStation Software, Origin and Windows XP* for collection and data analysis

Other equipment and accessories. pH meter Cyberscan pH 11 (Eutech, Malaysia), a magnetic stirrer (Falco, Italy), a system for filtering water (Whatman, Germany), ultrasonic bath (Bandelin, Sonorex digitec, Germany), Incubators I (Instrumentaria ST-05, Croatia), Incubator II (Binder, Germany). Method: Hydrophilic Interaction Liquid Chromatography (HILIC), previously developed and validated

Reagents. Acetonitrile (Fisher Scientific, England), Acetic acid (Lachner, Czech Republic), All reagents were of the HPLC grade.

HPLC water quality obtained by system Barnstead, distilled water obtained by system Barnstead and ammonium acetate (Lachner, Czech Republic)

Standards. Bisoprolol fumarate and Amlodipine besylate (working standards).

Solutions. Basic solutions of amlodipine besylate and bisoprolol fumarate (c = 1 mg mL^{-1}) were prepared in acetonitrile. Working solutions of amlodipine besylate and bisoprolol fumarate (c = 100 mg ml^{-1}) were prepared by dissolving in water and 0.01M HCl.

Chromatographic conditions. Column – Luna 5 µ HILIC 200A (100 mm x 4.6 mm, 5 µm particle size), mobile phase is a mixture of acetonitrile–water solution of 10 mM ammonium acetate (pH 4.0 adjusted by concentrated acetic acid) at the ratio of 92:8 V/V. The flow rate was 1 mL min^{-1} and column temperature was 30 ^{o}C. UV detection was carried out at 230 nm and injection volume was 20 µL.

3. RESULTS AND DISCUSSION

Reference chromatogram of tested analytes AB and BF is presented in Figure 2. Reference chromatogram consists of two chromatographic peaks, one for BF (Area 2537.32 mAU min^{-1}, corresponding to the concentration of 0.1304 mM L^{-1}) and for AB (Area 2533.5 mAU min^{-1}, corresponding to the concentration of 0.1763 mM L^{-1}). By changing experimental conditions (solvent and temperature), we were able to monitor the process of degradation of AB and BF through the change in peaks surface which is proportional to concentration. We have monitored changes of peak area at the following time intervals: 0, 1, 24, 48 and 72 h. For simplicity and better presentation only results for two time intervals (1 h and 72 h) were presented. Well defined peaks were chosen.

a) Amlodipine besylate and Bisoprolol fumarate degradation – individually

Tables 1−3 below present parameters of degradation process of AB and BF in water and hydrochloric acid at 25° and 70 °C. Under every table there is an appropriate chromatogram that reflects the data presented in tables.

The data presented in Table 1 and Table 2 showed that the degradation process of AB is very much influenced by the temperature and the media. The degradation process that is conducted at elevated temperature (70 °C) and in acid media is more pronounced, leaving AB rather unstable under these experimental conditions (Figure 3 and Figure 4). BF, on the other hand, is quite stable in water despite higher temperature, since chromatogram (Figure 5) showed almost no change in peak surface. Degradation of BF is more affected by hydrochloric acid, as seen in Table 3 and Figure 6. Figure 6 also showed that as a result of BF degradation under acid influence, impurity peak appeared at 4.042 min retention time.

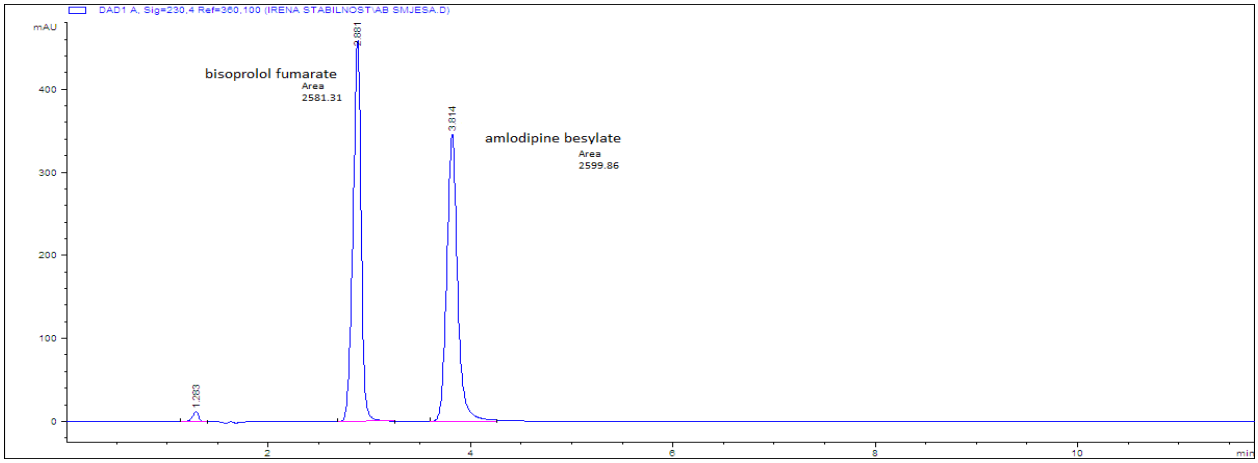

Figure 2. Chromatogram obtained under optimum chromatographic conditions

Table 1. Degradation of AB in the water at 25 °C and at 70 °C

Time for degradation	1 h	72 h
Temperature	25 °C	
Peak Area (mAU min^{-1})	2 442.41	2 270.02
Concentration (mM L^{-1})	0.17	0.158
Degradation (%)	3,59	10,4
Temperature	70 °C	
Peak Area (mAU min^{-1})	2 413.68	1 479.82
Concentration (mM L^{-1})	0.168	0.103
Degradation (%)	4,73	41,59

Table 2. Degradation of AB in the 0,01M HCl at 25 °C and at 70 °C

Time for degradation	1 h	72 h
Temperature	25 °C	
Peak Area (mAU min^{-1})	2 356.16	1 982.72
Concentration (mM L^{-1})	0.164	0.138
Degradation (%)	7	21.74
Temperature	70 °C	
Peak Area (mAU min^{-1})	2 413.67	732.7
Concentration (mM L^{-1})	0.168	0.051
Degradation (%)	4,73	71.08

Table 3. Degradation of BF in the 0,01M HCl at 25 °C and at 70 °C

Time for degradation	1 h	72 h
Temperature	25 °C	
Peak Area (mAU min^{-1})	2 393.33	2 159.84
Concentration (mM L^{-1})	0.123	0.111
Degradation (%)	5,68	14.88
Temperature	70 °C	
Peak Area (mAU min^{-1})	2 303.11	175.12
Concentration (mM L^{-1})	0.118	0.009
Degradation (%)	9,23	93.1

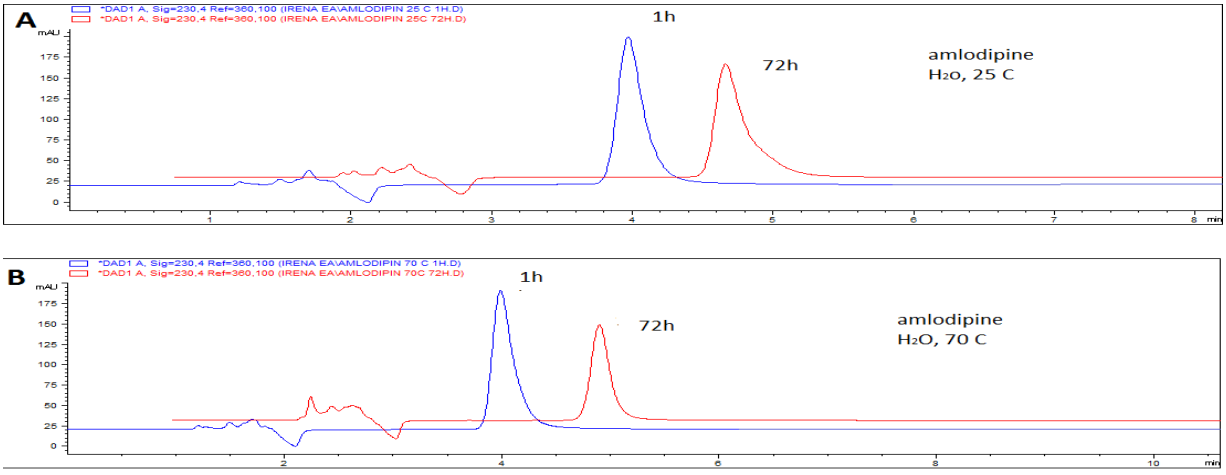

Figure 3. Degradation of AB in water at 25 °C and at 70 °C

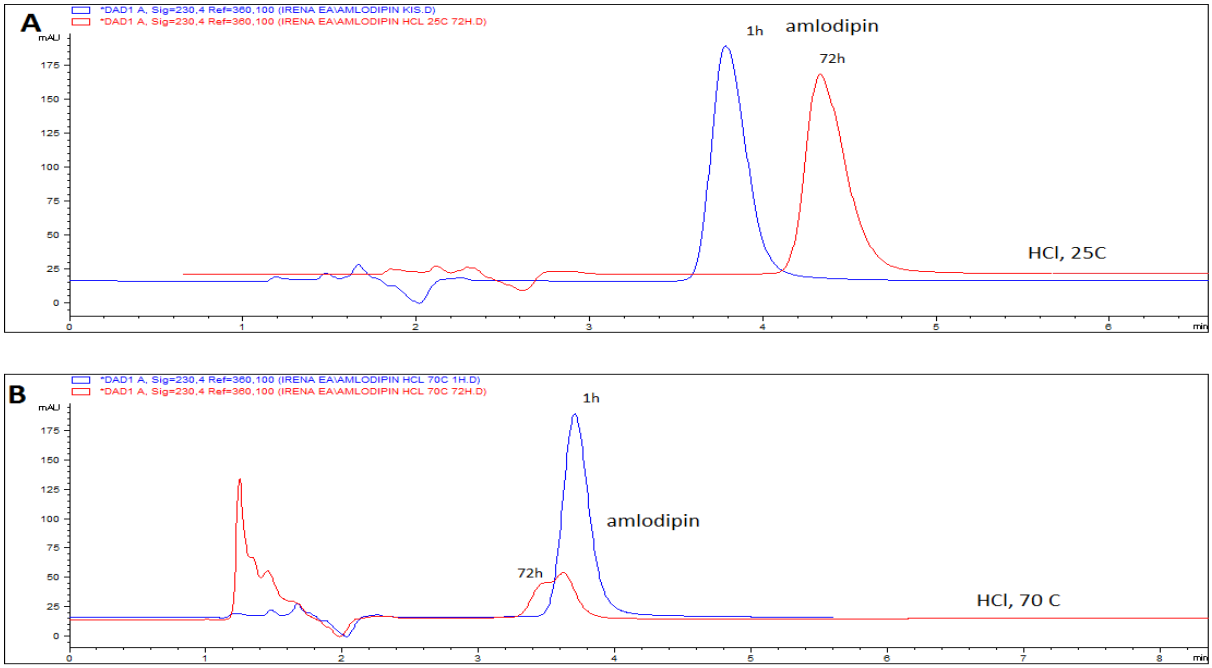

Figure 4. Degradation of AB in HCl at 25 °C and at 70 °C

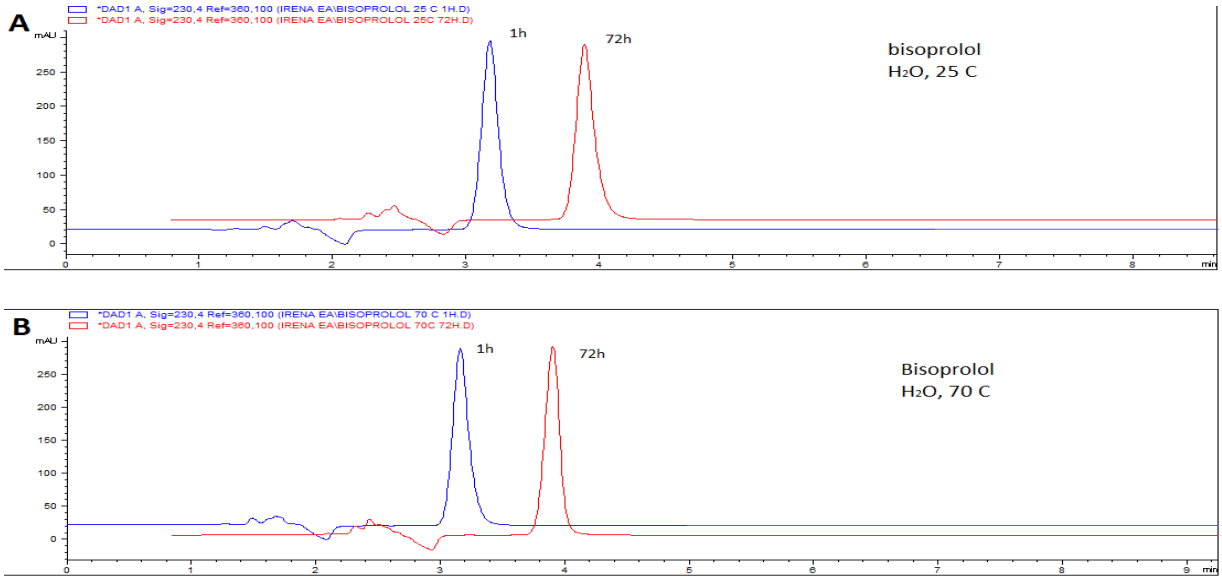

Figure 5. Degradation of BF in water at 25 °C and at 70 °C

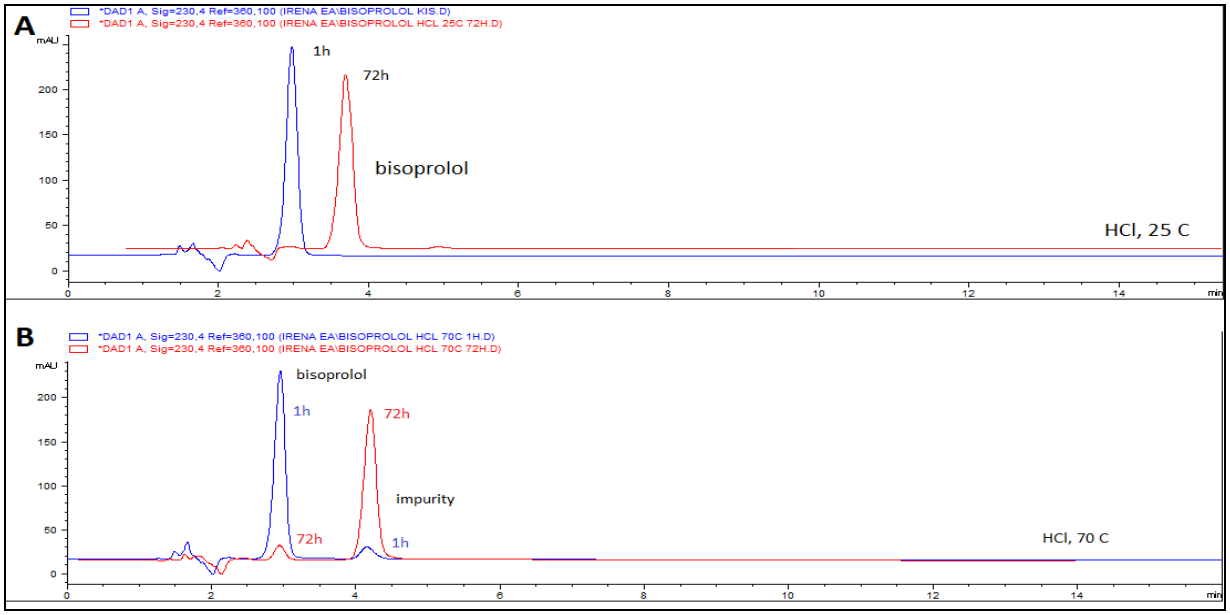

Figure 6. Degradation of BF in HCl at 25 °C and at 70 °C

b) Amlodipine besylate and Bisoprolol fumarate degradation – mixture

AB and BF were examined in a mixture, under the same conditions, in order to determine their mutual influence on stability. In Figure 7A it can be seen that in aqueous medium at 25 °C there was no significant change in size of the observed peaks. The peak area of BF remained unchanged, while AB in mixture showed greater stability, *i.e.* the area of its peak for 72 h was reduced by a total of 7 % (2 356.21 mAU min^{-1}, *i.*e. c = 0.164 mM L^{-1}).

At the temperature of 70 °C for 72 h, BF degraded only by 2.7 % (2 468.81 mAU min^{-1}, *i.*e. c = 0.126 mM L^{-1}), while the degradation of AB was 26.71 % (1 856.8 mAU min^{-1}, i.e. c = 0.129 mM L^{-1}) (Figure 7B). It can be concluded that the compounds are more stable in an aqueous medium in the mixture, rather than individually, even at high temperature. It can be assumed that AB and BF, when contained in a mixture, increase stability with each other in an aqueous medium, even at elevated temperature.

By observing the chromatogram of BF and AB in 0.01M HCl (Figure 8), it can be seen that both compounds are also more stable in the mixture rather than individually. Figure 8A shows the change of surface of tested analytes after 1 h, 24 h and 72 h.

For total 72 hours the degradation of AB is 10.4 % (2 270.02 mAU min^{-1}, *i.*e. c = 0.158 mM L^{-1}), and BF is 11,04 % (2 257.2 mAU min^{-1}, *i.*e. c = 0.116 mM L^{-1}**).**

Figure 8B shows the change at 70 °C the degradation of both compounds is significant, for AB, for 72 hours degradation it is 51.8 % (1 221.15 mAU min^{-1}, *i.e.* c = 0.085 mM L^{-1}), while for BF in 72 h the degradation is 79.29 % (525.48 mAU min^{-1}, *i.e.* c = 0.027 mM L^{-1}).

It is evident from Figure 8B that BF and AB are degraded in a high percentage. There are impurities at the retention time of 4,04 minutes which surface increases with time. This impurity was produced during the degradation of BF, as it can be seen in Figure 6B and Figure 8B.

Having reviewed stability and degradation of AB and BF systems from chromatograms under different experimental conditions, the obtained concentration in a proper time interval were further subjected to calculation in order to get certain kinetics parameters that could confirm our previously stated conclusions.

In Table 4 to Table 7 all the relevant kinetics parameters for stability study of AB and BF are summarized. The values for constant rate, half-time and activation energy for AB (Table 4 and Table 5) and BF (Table 6 and Table 7) degradation at 25 °C and 70 °C, both, in water and 0,01M hydrochloric acid are presented. From the obtained results it can be confirmed, once again, that the medium and temperature have a great impact on degradation of substances being analyzed. It should be mentioned that Ea for BF and AB alone in water is not presented in Tables 4 and 6 due to BF having constant rate equal to zero, while AB showed mixture kinetics.

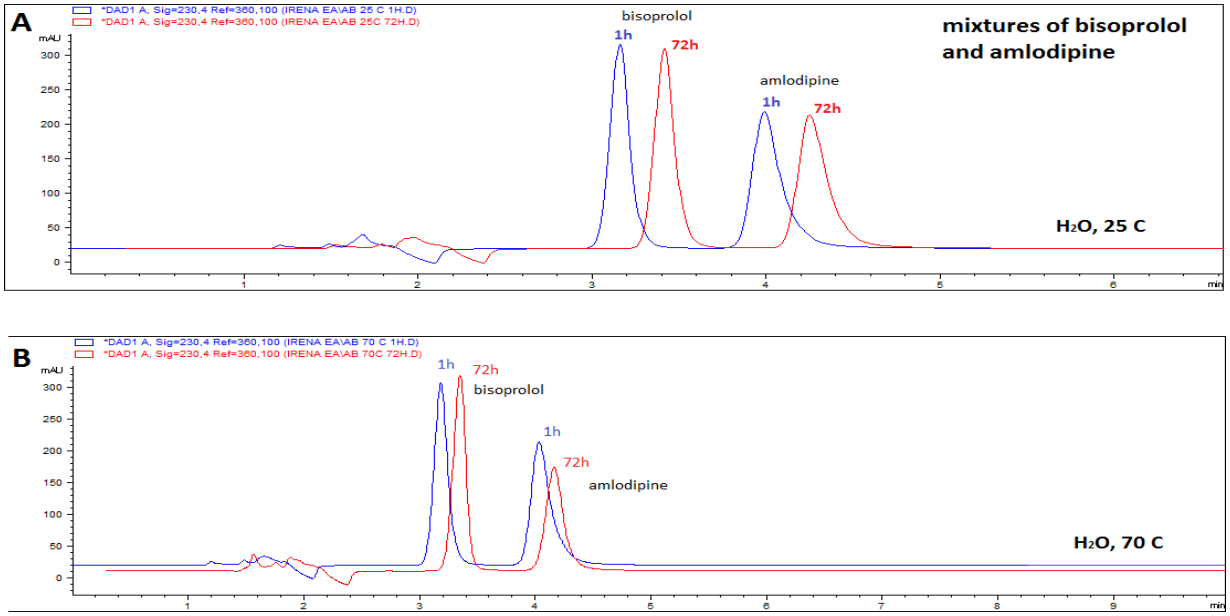

Figure 7. Degradation of mixtures BF and AB in water at 25 °C and 70 °C

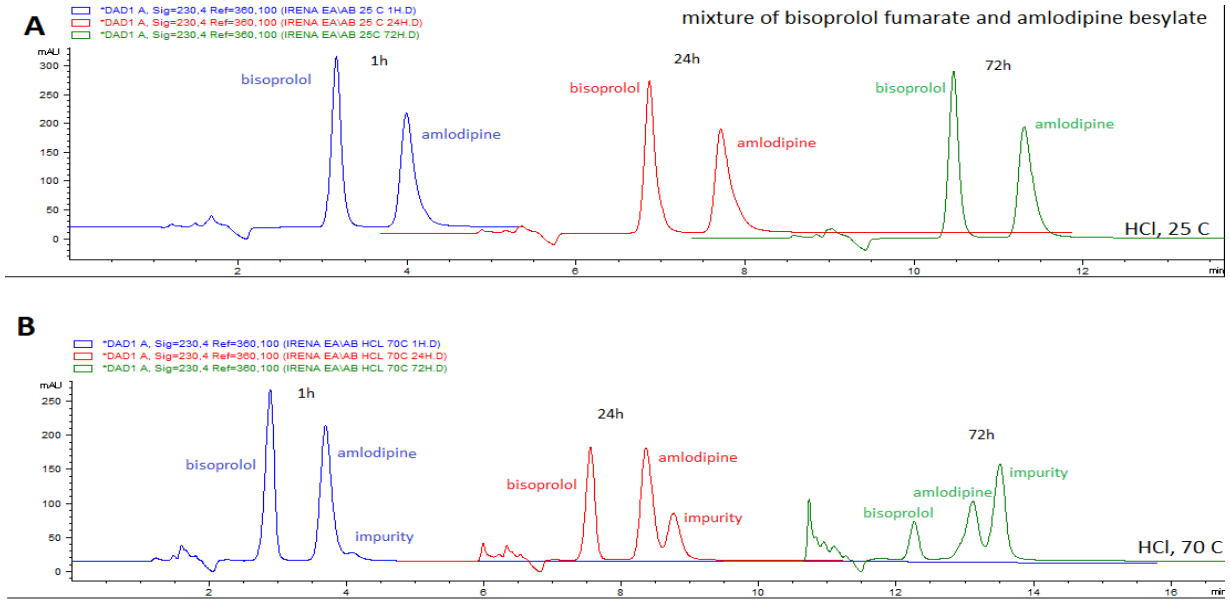

Figure 8. Degradation of mixtures BF and AB in 0,01 M HCl at 25 °C and 72 °C

Table 4. Kinetics parameter of AB in water

Parameter	25 °C	70 °C
k	$1{,}875 \cdot 10^{-4}$ mM h^{-1}	$5{,}163 \cdot 10^{-2}$ $mM^{-1}h^{-1}$
n	Zero order kinetics	Second order kinetics
$t_{1/2}$	460 h	117,76 h

Table 5. Kinetics parameter of AB in HCl

Parameter	25 °C	70 °C
k	$3{,}33 \cdot 10^{-4}$ mM h^{-1}	$1{,}69 \cdot 10^{-3}$ mM h^{-1}
n	Zero order kinetics	Zero order kinetics
$t_{1/2}$	246 h	50 h
Ea	30,68 kJ mol^{-1}	

Table 6. Kinetics parameter of BF in water

Parameter	25 °C	70 °C
k	0	$2{,}6 \cdot 10^{-3}$ mM^{-1} h^{-1}
n	-	Second order kinetics
$t_{1/2}$	0	2999 h

Table 7. Kinetics parameter of BF in HCl

Parameter	25 °C	70 °C
k	$1{,}634 \cdot 10^{-3}$ h^{-1}	$3{,}707 \cdot 10^{-2}$ h^{-1}
n	First order kinetics	First order kinetics
$t_{1/2}$	425 h	18 h
Ea	59,03 kJ mol^{-1}	

Table 8. Kinetics parameter of mixture of AB and BF in water

Parameter	AB		BF	
	25 °C	70 °C	25 °C	70 °C
k	$2{,}6\cdot10^{-3}$ mM^{-1} h^{-1}	$2{,}56\cdot10^{-2}$ mM^{-1} h^{-1}	0	$9\cdot10^{-4}$ mM^{-1} h^{-1}
n	Second order kinetics	Second order kinetics	-	Second order kinetics
$t_{1/2}$	2270,92 h	236,42 h	0	8812,11 h
Ea	43,218 kJ mol^{-1}		-	

Table 9. Kinetics parameter of mixture of AB and BF in HCl

Parameter	AB		BF	
	25 °C	70 °C	25 °C	70 °C
k	$1{,}272\cdot10^{-4}$ mM h^{-1}	$1{,}2\cdot10^{-3}$ mM h^{-1}	$1{,}13\cdot10^{-3}$ h^{-1}	$2{,}196\cdot10^{-2}$ h^{-1}
n	Zero order kinetics	Zero order kinetics	First order kinetics	First order kinetics
$t_{1/2}$	661 h	71 h	613 h	32 h
Ea	42,4146 kJ mol^{-1}		56,062 kJ mol^{-1}	

AB in HCl follows the zero order reaction, while BF follows the first order reaction at 25° and 70 °C (Table 5 and Table 7). This different type of reactions affects half-time of the substance. The concept of half-time is very important, since it plays a key role in determining how quickly a drug decreases after being absorbed. Generally, the half-life of zero order reaction decreases as the concentration decreases and it is dependent on the amount of initial concentration and constant rate, while the half-time of first order is practically independent of initial concentration. Half-time values reflect the statement concerning greater stability of AB and BF in mixture than individually. Temperature effect was quite as expected; higher temperatures gave higher values of constant rate, which confirms the hypothesis that degradation of AB and BF is faster. This trend was kept in AB and BF mixture too. The degradation of AB in the mixture happened faster than the degradation of BF in the mixture (Table 8 and Table 9). One should notice that BF was much more stable in HCl than AB since the value of Ea for BF (59.03 kJ mol^{-1}) (Table 7) had almost twice of Ea value for AB (30.68 kJ mol^{-1}) (Table 5). Furthermore, if we compare Ea results obtained for AB and BF, individually and in the mixture, such a great stability of BF was observed in each case, since activation energy of BF individually (59.03 kJ mol^{-1}) and in the mixture (56.06 kJ mol^{-1}) (Table 9) did not significantly change. Probably we could gain more information on BF stability in a more aggressive media.

4. CONCLUSION

We can conclude that the proposed HILIC method is applicable to the stability study of AB and BF, individually and in mixture. Upon analysing the data from the kinetics point of view we gained some valuable information and we can conclude as follows: the choice of solvents and elevated temperatures have impact on stability of AB and BF, more aggressive solvents and elevated temperatures contribute to a greater level of degradation, BF is more stable than AB, AB and BF in mixture stabilize each other making AB and BF more stable in mixture than alone which was confirmed by kinetics parameters.

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ИСПИТИВАЊЕ СТАБИЛНОСТИ АМЛОДИПИН-БЕСИЛАТА И БИСОПРОЛОЛ-ФУМАРАТА У ВОДЕНИМ РАСТВОРИМА

Сажетак: Коришћењем различитих медијума (вода, хлороводонична киселина или натријум-хидроксид) за растварање активних фармацеутских супстанци, може се добити доста корисних информација о стабилности испитиваних једињења. Поред тога, додатна знања стичу се извођењем експеримената на различитим температурама. У овом раду описано је испитивање стабилности амлодипин-бесилата и бисопролол-фумарата у различитим медијумима при различитим температурама и изведени су закључци о њиховој стабилности.

За проучавање стабилности примијењена је хемијска кинетика, а параметри који су коришћени за предвиђање степена стабилности испитиваних једињења су константа брзине реакције (к), полувријеме распада ($t_{1/2}$) и енергија активације (Ea). Стабилност амлодипин-бесилата и бисопролол-фумарата испитана је у води и 0,01 M HCl при чему је праћена њихова стабилност како појединачно тако и у смјеши. Сви испитивани узорци третирани су на двије различите температуре (25 ^{o}C и 70 ^{o}C) у одређеним временским интервалима (0 минута, 1 h, 24 h, 48 h и 72 h). Као метода за праћење процеса деградације коришћена је претходно развијена и валидирана течна хроматографија хидрофилних интеракција (енг. *Hydrophilic Interaction Liquid Chromatography* – HILIC). Добијени резултати су показали да је амлодипин-бесилат стабилнији у воденом него у киселом медијуму, затим да је стабилнији у смјеши него појединачно и да је стабилнији на нижим температурама. Ово је потврђено добијеним вриједностима праћених параметара: амлодипин-бесилат Ea = 30,68 кJ мол$^{-1}$, к (25 °C) = 0,000333 mM h^{-1}, к (70 °C) = 0,00169 mM h^{-1}; амлодипин-бесилат у смјеши Ea = 42,414 кJ мол$^{-1}$, к (25 °C) = 1,27 · 10^{-4} mM h^{-1}, к (70 °C) = 0,0012 mM h^{-1}. Бисопролол-фумарат је показао изванредну стабилност у односу на медијуме у којима је испитиван и добијена је приближна вриједност енергије активације за киселу (59 кJ мол$^{-1}$) и водену (56 кJ мол$^{-1}$) средину. С друге стране, температура је имала значајан утицај на стабилност бисопролол-фумарата. Добијени резултати дају релевантне податке о стабилности испитиваних активних супстанци и могу бити од значаја током развоја одговарајућег фармацеутског производа. Већи утицај на стабилност бисопролол-фумарата имао је температурни ефекат.

Кључне ријечи: амлодипин-бесилат, бисопролол-фумарат, стабилност у раствору, хемијска кинетика.