Kinetics of Hydrolysis of Bisoprolol Hemifumarate in Aqueous Acidic Solutions

MATEUSZ SZALKA,[1] EDWARD ROKASZEWSKI,[1] KRZYSZTOF KACZMARSKI[2]

[1]*Product Development Department*, ICN Polfa Rzeszów S.A., 35-959, Rzeszów, Poland

[2]*Department of Chemical and Process Engineering*, Rzeszów University of Technology, 35-959, Rzeszów, Poland

Received 31 January 2012; revised 13 November 2012; 28 June 2013; 7 July 2013; accepted 7 July 2013

DOI 10.1002/kin.20809
Published online 3 August 2013 in Wiley Online Library (wileyonlinelibrary.com).

ABSTRACT: The kinetics of hydrolysis of bisoprolol hemifumarate in acidic conditions was studied using high-performance liquid chromatography. For this purpose, different hydrohalic acids and one weak carboxylic acid were used. The rate constants, the order of the reaction, and the activation parameters: enthalpy, entropy, and energy of activation were calculated. A proposition for the mechanism of degradation was provided. © 2013 Wiley Periodicals, Inc. Int J Chem Kinet 45: 744–754, 2013

INTRODUCTION

Bisoprolol hemifumarate is a very popular drug substance used in the treatment of many cardiovascular diseases such as hypertension, arrhythmia, and coronary failure. Bisoprolol belongs to the beta-blockers group, which inhibits the synthesis of catecholamines (i.e., adrenaline) reducing their concentration in a human organism. Beta-blockers are also commonly used as illegal doping agents; they reduce tremors and cardiac frequency, significantly improving the physical performance [1,2]. Chemically bisoprolol is a derivative of aminopropanol and is used as a racemic mixture for therapeutic purposes. The full formula is presented in Fig. 1.

The lipophilic character of this drug substance is mainly determined by its aromatic ring. The alkaline properties, on the other hand, depend on the alkanoamine chain. The bisoprolol base has a p*K*a of 9.5 [3], the lipophilicity measured by a partition coefficient puts bisoprolol somewhere between less lipophilic atenolol and more lipophilic propranolol [4], and this position is a key factor for the theoretically ideal pharmacokinetic properties of bisoprolol. Bisoprolol is stable at a pH above 5.0 also in strong alkaline conditions.

In spite of the degradation studies [5] carried out for this drug substance, there is no information on the kinetics of hydrolysis in acidic aqueous solutions as well as no proposition for a detailed mechanism of degradation. The scope of this study is to demonstrate an explanation for the mechanism of hydrolysis and calculation of the activation parameters, which may be important for preparation of stable drug product formulations containing bisoprolol hemifumarate and compatible excipients.

Krzek et al. [6] found a correlation between the polarity of beta-blockers and their rate of degradation. According to the studies carried out for atenolol, acebutolol, and propranolol, the stability of beta-blockers increases with the increasing lipophilicity. The most stable is propranolol, then acebutolol and atenolol.

Correspondence to: Mateusz Szalka; email: mateusz.szalka@valeant.com

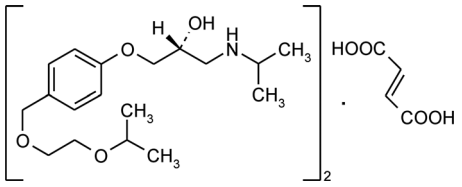

Figure 1 Structure of bisoprolol hemifumarate.

Table I Gradient Program for the HPLC Method

Time (min)	Mobile Phase A (%) (v/v)
0 → 12	57
12 → 21	57 → 30
21 → 22	30 → 57
22 → 30	57

The analyzed drugs significantly differ in log *P* values. According to Carda-Broch and Berthod [7] the log *P* for propranolol is 1.16, acebutolol −0.40, and atenolol −0.83, whereas the log *P* of bisoprolol is 0.04. The scope of this study was also to determine whether bisoprolol fits into this correlation.

EXPERIMENTAL

Instrumentation

A HPLC Waters 2659 separations module (maximum operating pressure 345 bar) with a photodiode array detector (PDA 2998) from Waters (Milford, MA) was used. An EC Nucleosil column, 100–5 C18 HD, 5 μm, 250 × 4.6 mm, was supplied by Macherey-Nagel (Düren, Germany).

Materials

Triethylamine (≥99.5%, HPLC grade) was supplied by Sigma Aldrich (Steinheim, Germany). Methanol of HPLC grade, orthophosphoric acid p.a. (85%), and anhydrous potassium dihydrogen phosphate p.a. were supplied by POCH S.A. (Gliwice, Poland).

Fumaric acid (>99%) and hydroiodic acid (57%) were supplied by Acros Organics (Geel, Belgium). Hydrochloric acid (35–38%), hydrobromic acid (48%), sodium chloride (p.a.), potassium bromide (p.a.), and potassium iodide (p.a.) were supplied by POCH S.A. (Gliwice, Poland). Bisoprolol hemifumarate, working standard, was synthesized at ICN Polfa Rzeszow S.A. (Rzeszów, Poland).

Analytical Method

Gradient elution high-performance liquid chromatography (HPLC) was applied for the analysis of reaction mixtures with the EC Nucleosil column, 100–5 C18 HD, 5 μm, 250 × 4.6 mm. The flow rate was set to 0.8 mL/min, the UV light absorbance at 225 nm wavelength, column temperature at 50°C, and sample injection volume at 10 μL. The time of a single analysis, 30 min, was determined by the gradient elution program presented in Table I. The mobile phase A was prepared by adding 1.36 g of potassium dihydrogen phosphate and 1 mL of triethylamine to 1 dm^3 of purified water; the pH of this solution was adjusted to 5.5 by diluted (8.5% v/v) phosphoric acid. The mobile phase B was HPLC-grade methanol.

Validation of the Analytical Method

The HPLC method was validated according to International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use guidelines; the results for all important parameters are presented below.

The limit of detection was set to 0.014 μg/mL and the limit of quantitation 0.048 μg/mL and linearity (peak areas vs. the concentration of bisoprolol) with the regression factor $r = 1.00$. Repeatability, measured as a deviation from peak areas, is characterized by relative standard deviation not more than 2.0%. Accuracy, measured by a recovery method, falls within the range from 95.0% to 105.0%. The analytical method is specific, accurate, precise, and linear.

Kinetic Testing

Kinetic measurements were studied in the aqueous acidic solutions for the initial concentration of bisoprolol 1.3×10^{-3} mol·dm^{-3} (1.0 mg/mL) if not stated otherwise. The temperatures for hydrohalic acids were 323.2, 333.2, and 343.2 K. The degradation of bisoprolol in fumaric acid occurred much more slowly, so the solutions were tested at higher temperatures, 353.2, 358.2, and 363.2 K. This temperature additionally guaranteed complete dissolution of fumaric acid in water. Three different hydrohalic acids were tested: hydrochloric, hydrobromic, and hydroiodic acids. Bisoprolol contains several ether linkages, and thus a protonation of various ether oxygen atoms followed by a nucleophilic attack may result in many different degradation products. The acid strength is also an important factor in the cleavage of ethers; hydroiodic and hydrobromic acids may be strong enough to cleave ether linkages that are often resistant to hydrochloric acid. After the assumed experimental time intervals, 2.0 mL

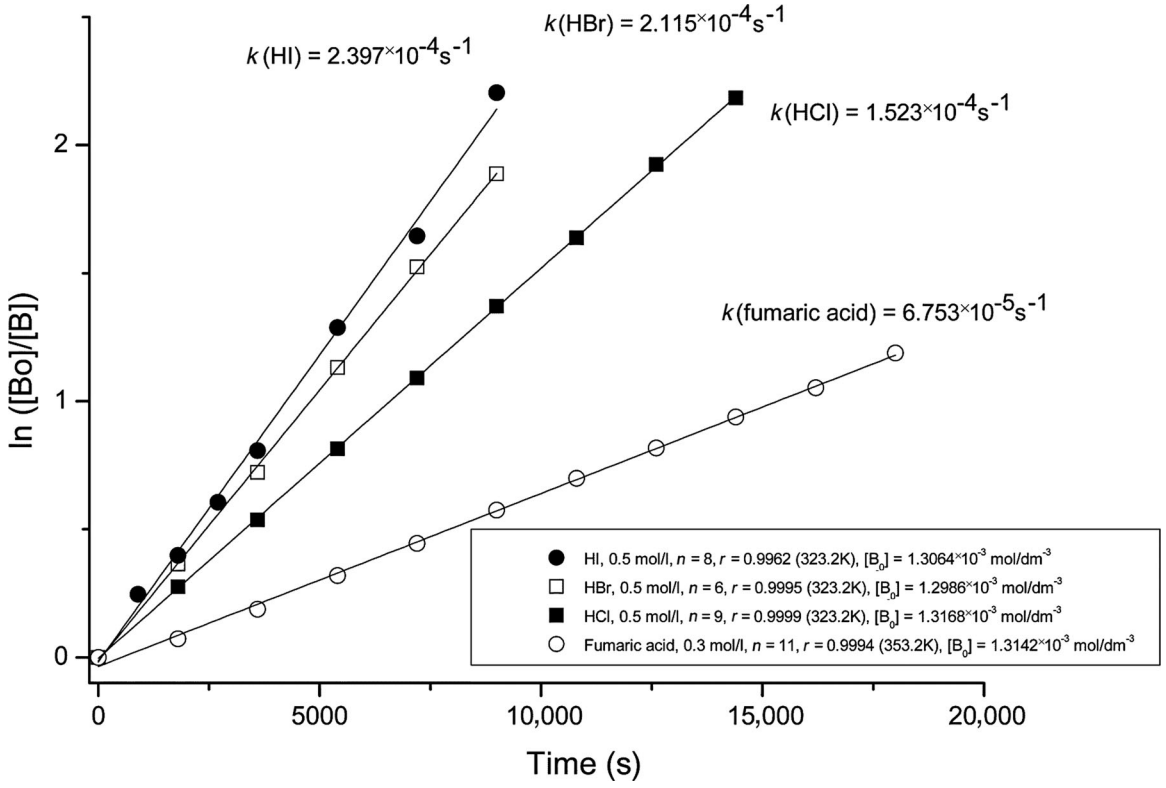

Figure 2 The pseudo–first-order hydrolysis reaction of bisoprolol hemifumarate.

of a reaction mixture was withdrawn from a reaction vessel and placed in the thermostated autosampler of the HPLC system. The autosampler temperature was set to 278.2 K to rapidly slow down the reaction; the samples were injected immediately after withdrawal. The peak areas of bisoprolol were measured for each determination and compared to the initial conditions. A temporary concentration of bisoprolol hemifumarate $b = [B]/[B]_0$ during the reaction was determined as a ratio of peak areas, $b = [B]/[B]_0 = [\text{Area}]_t/[\text{Area}]_0$, where $[\text{Area}]_0$ is the area at the beginning of the reaction, $[\text{Area}]_t$ is the peak area at the assumed time point, $[B]_0$ is the concentration of bisoprolol at the beginning of measurement, and [B] is the temporary concentration of bisoprolol at the assumed time point.

The total order, the rate of a reaction, the half-life time, and the activation parameters are presented in the next section. An explanation for the reaction mechanism is provided.

RESULTS AND DISCUSSION

In the aqueous solutions of hydrohalic acids, HX (HX = HCl, HBr, or HI), and in the solutions of fumaric acid, bisoprolol hydrolyzes according to the kinetics of pseudo–first-order reaction as presented in Fig. 2. The linear functions of $\ln([B_0]/[B])$ plotted against the reaction time are characterized by good regression coefficients, $r > 0.99$. The fastest reaction rate was obtained for hydroiodic, hydrobromic, and hydrochloric acids in a decreasing order. The slowest reaction was observed for a weak carboxylic acid. Surprisingly, only one degradation product arising due to the hydrolysis of the benzyl ether linkage was observed. The other ether connections remained unchanged, even when stronger acids (HBr and HI) were applied. As we can notice, the rate of hydrolysis of the benzyl ether linkage in bisoprolol directly depends on the acid strength and the type of a nucleophile. The initial concentrations of bisoprolol, the reaction temperatures, and obtained results are presented in Fig. 2. and Table II.

At the constant initial concentration of bisoprolol hemifumarate (about 1.3×10^{-3} mol·dm^{-3}), the influence of temperature (333.2, 343.2, and 353.2 K) and the concentration of hydrohalic acid on reaction rates was determined. The concentrations of hydrohalic acids varied from 0.1 to 1.0 mol·dm^{-3}. As seen in Fig. 3, the effective rate constants of this reaction are the quadratic function of a concentration of hydrohalic acid according to Eq. (1). In our experiments, the concentration of a nucleophile $[X^-]$ was equal to the

Table II Kinetic Results for Hydrolysis of Bisoprolol in the Solution of Hydrochloric Acid

	Reaction Temperature 323.2 K				Reaction Temperature 333.2 K				Reaction Temperature 343.2 K			
[HCl] ($mol \cdot dm^{-3}$)	[B_0] ($mol \cdot dm^{-3}$)	(b_n)	$r(n)$	($k \pm \Delta k$) ($\times 10^{-4}\ s^{-1}$)	[B_0] ($mol \cdot dm^{-3}$)	(b_n)	$r(n)$	($k \pm \Delta k$) ($\times 10^{-4}\ s^{-1}$)	[B_0] ($mol \cdot dm^{-3}$)	(b_n)	$r(n)$	($k \pm \Delta k$) ($\times 10^{-4}\ s^{-1}$)
0.10	$1.2960 \cdot 10^{-3}$	0.72	0.9983(8)	0.176 ± 0.004	$1.2960 \cdot 10^{-3}$	0.65	0.9974(7)	0.61 ± 0.02	$1.2960 \cdot 10^{-3}$	0.49	0.9999(5)	1.95 ± 0.02
0.25	$1.3168 \cdot 10^{-3}$	0.32	0.9999(9)	0.597 ± 0.003	$1.3194 \cdot 10^{-3}$	0.22	0.9997(7)	2.07 ± 0.02	$1.3272 \cdot 10^{-3}$	0.10	0.9996(5)	6.3 ± 0.1
0.50	$1.3168 \cdot 10^{-3}$	0.11	0.9999(9)	1.522 ± 0.004	$1.3246 \cdot 10^{-3}$	0.05	0.9998(6)	5.43 ± 0.06	$1.3064 \cdot 10^{-3}$	0.06	0.9990(4)	15.9 ± 0.5
0.75	$1.3064 \cdot 10^{-3}$	0.09	0.9999(6)	2.62 ± 0.01	$1.3220 \cdot 10^{-3}$	0.08	0.9996(5)	9.1 ± 0.1	$1.3272 \cdot 10^{-3}$	0.06	0.9992(4)	28.3 ± 0.8
1.00	$1.3408 \cdot 10^{-3}$	0.02	0.9999(6)	4.49 ± 0.03	$1.3168 \cdot 10^{-3}$	0.01	0.9998(4)	14.4 ± 0.2	$1.3455 \cdot 10^{-3}$	0.02	0.9955(4)	43 ± 3
1.25	$1.2986 \cdot 10^{-3}$	0.09	0.9999(5)	6.73 ± 0.06	$1.3142 \cdot 10^{-3}$	0.01	0.9992(4)	21.6 ± 0.6	–	–	–	–
1.50	$1.3012 \cdot 10^{-3}$	0.08	0.9996(4)	9.5 ± 0.2	$1.3299 \cdot 10^{-3}$	0.01	0.9998(3)	30.6 ± 0.5	–	–	–	–
2.00	$1.3194 \cdot 10^{-3}$	0.01	0.9998(4)	17.1 ± 0.2	$1.3220 \cdot 10^{-3}$	0.05	0.9985(3)	51 ± 3	–	–	–	–

concentration of [H_3O^+] (excluding the experiments with added salts); thus Eq. (1) may be simplified to Eq. (2), where k_1 is a kinetic rate constant of hydrolysis according to the mechanism of the nucleophilic substitution type 1 (S_N1) and k_2 is a kinetic rate constant of hydrolysis according to the mechanism of the nucleophilic substitution type 2 (S_N2). As we can notice from Fig. 3, the k_1 rate constant slightly differs for all three hydrohalic acids; this is because the S_N1-type mechanism involves only a protonation of the benzyl ether oxygen atom and then a spontaneous cleavage of this linkage. There is no involvement of a nucleophile in the S_N1 mechanism; thus the reaction rates directly depend on the acid strength and its concentration. All hydrohalic acids are strong acids, which dissociate almost completely in the aqueous solutions; however, the level of dissociation may insensibly vary between them. The highest value of p*K*a has the strongest hydroiodic acid (p*K*a $= -10$) [8], then hydrobromic acid (p*K*a $= -9$) [8], and the lowest value has hydrochloric acid (p*K*a $= -7$) [9]. There are no important differences in the calculated dissociation of HI and HBr at the concentrations from 0.1 to 1.0 $mol \cdot dm^{-3}$, whereas the dissociation of HCl is slightly lower. The numbers of protonated molecules of bisoprolol are similar in the experiments with HI and HBr; thus k_1 rate constants of the S_N1 mechanism are almost the same. On the other hand, the protonation of bisoprolol is lower in hydrochloric acid solution, which explains the lower k_1 value.

The second mechanism of hydrolysis of bisoprolol is the nucleophilic substitution type 2 and occurs simultaneously. It is described by k_2 rate constants and involves a protonation of the benzyl ether oxygen atom and then the attack of a nucleophile (I^-, Br^-, or Cl^-). The rate of this reaction depends on the dissociation of acids but also on the type of nucleophile. As can be noticed from Fig. 3, the fastest reaction rate constants were obtained for HI, HBr, and HCl in a decreasing order. This confirms that the strength of the nucleophile decreases in the order I^-, Br^-, Cl^-.

The reaction rates determined for fumaric acid (p*K*a$_1$ = 3.03, p*K*a$_2$ = 4.54) [10] were much slower than for hydrohalic acids even at higher temperatures (353.2, 358.2, and 363.2 K). There are two explanations for this behavior. First, fumaric acid is a weak carboxylic acid with a low dissociation level; thus the low protonation of the benzyl oxygen atom in the bisoprolol molecule inhibits the S_N1 and S_N2 reactions. The second explanation is the lack of a strong nucleophile in the reaction mixture that additionally slows the nucleophilic substitution type 2. Water (reaction solvent) and the anion of fumaric acid may act as nucleophiles, but both are significantly weaker than the

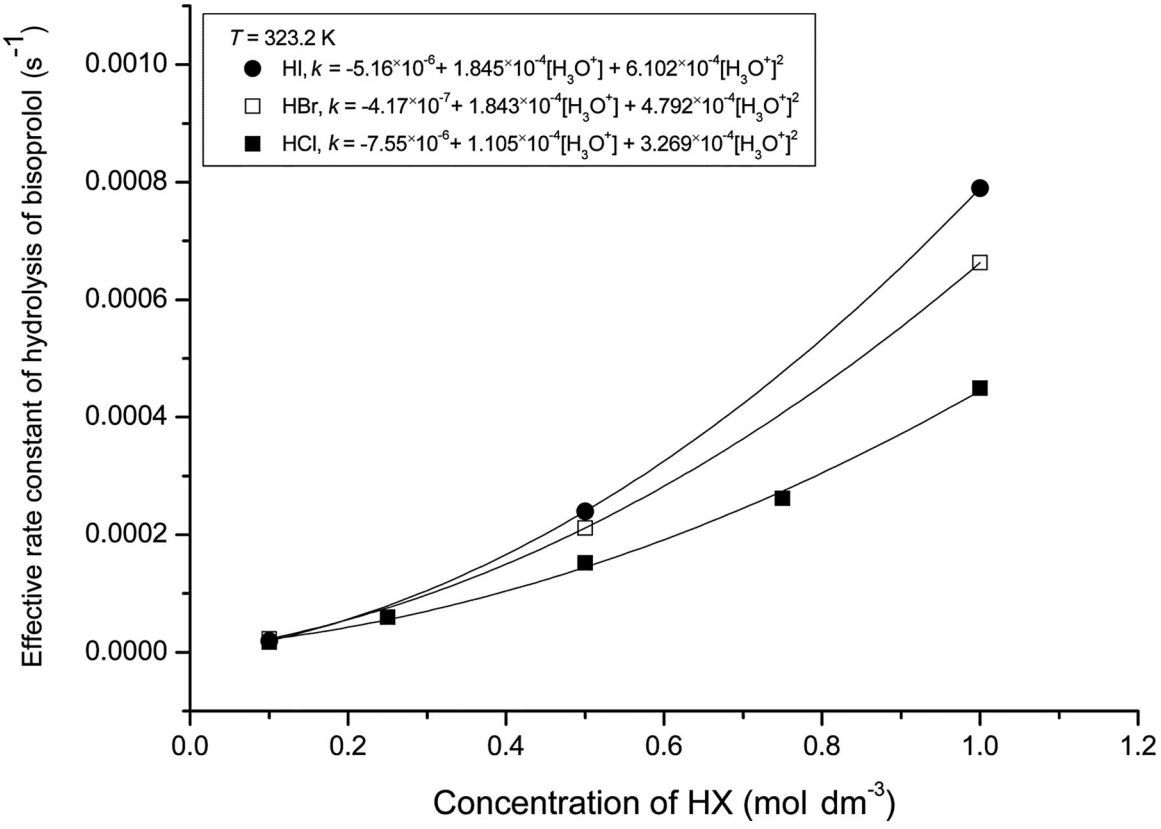

Figure 3 Impact of hydrohalic acid concentration on the effective rate constants of hydrolysis at 323.2 K.

halogen anions. The experiments carried out for fumaric acid will be omitted from further calculations and discussion due to low reaction rates.

$$k = k_0 + k_1\left[\mathrm{H_3O^+}\right] + k_2\left[\mathrm{H_3O^+}\right]\left[\mathrm{X^-}\right] \qquad (1)$$

$$k = k_0 + k_1\left[\mathrm{H_3O^+}\right] + k_2\left[\mathrm{H_3O^+}\right]^2 \qquad (2)$$

The obtained results are in agreement with the knowledge about hydrolysis of benzyl ethers, which may occur according to two important reaction mechanisms: nucleophilic substitution type 1 and type 2. Both possible mechanisms of the degradation are presented in Fig. 4.

The hydrolysis of bisoprolol molecule according to the S_N2 reaction mechanism is presented in Fig. 4(a). It begins with a protonation of the oxygen atom at the benzyl ether linkage and then the attack of a nucleophile on the benzyl carbon atom from the opposite side to the leaving group (isopropoxyethanol). The direct influence of a nucleophile was confirmed in the experiments carried out in 0.5 M HCl with the addition of various salts (0.1 M). The fastest reaction was observed in 0.1 M KI solution ($k_0 = 7.4785 \times 10^{-4}\ s^{-1}$), then in 0.1 M KBr ($k_0 = 7.2366 \times 10^{-4}\ s^{-1}$), and the slowest reaction occurred in 0.1 M KCl ($k_0 = 6.8626 \times 10^{-4}\ s^{-1}$). The ionic strength in all above-described experiments is constant; thus the rate of hydrolysis directly depends on the strength of the nucleophile ($I^- > Br^- > Cl^-$). Considering the reaction mechanism, we have assumed that an increase in the concentration of chloride ions (addition of KCl) enhances the probability of the S_N2 reaction. The addition of the same molar quantities of KBr or KI into the reaction vessel improves the nucleophilic character of anions; I^- and Br^- compete with Cl^- in the S_N2 reaction, increasing its rate. No reaction occurred in the experiments in which hydrohalic acids were substituted by appropriate salts. This proves that the isopropoxyethoxide anion is a poor leaving group (the lack of a stabilization of the negative charge); thus the S_N2 reaction requires a protonation of the benzyl oxygen atom to proceed. In all reactions with hydrohalic acids, only benzyl halides were observed as the degradation products.

The hydrolysis according to the S_N1 mechanism is described in Fig. 4(b); it also begins with a protonation of oxygen atom at the benzyl ether, but a nucleophile

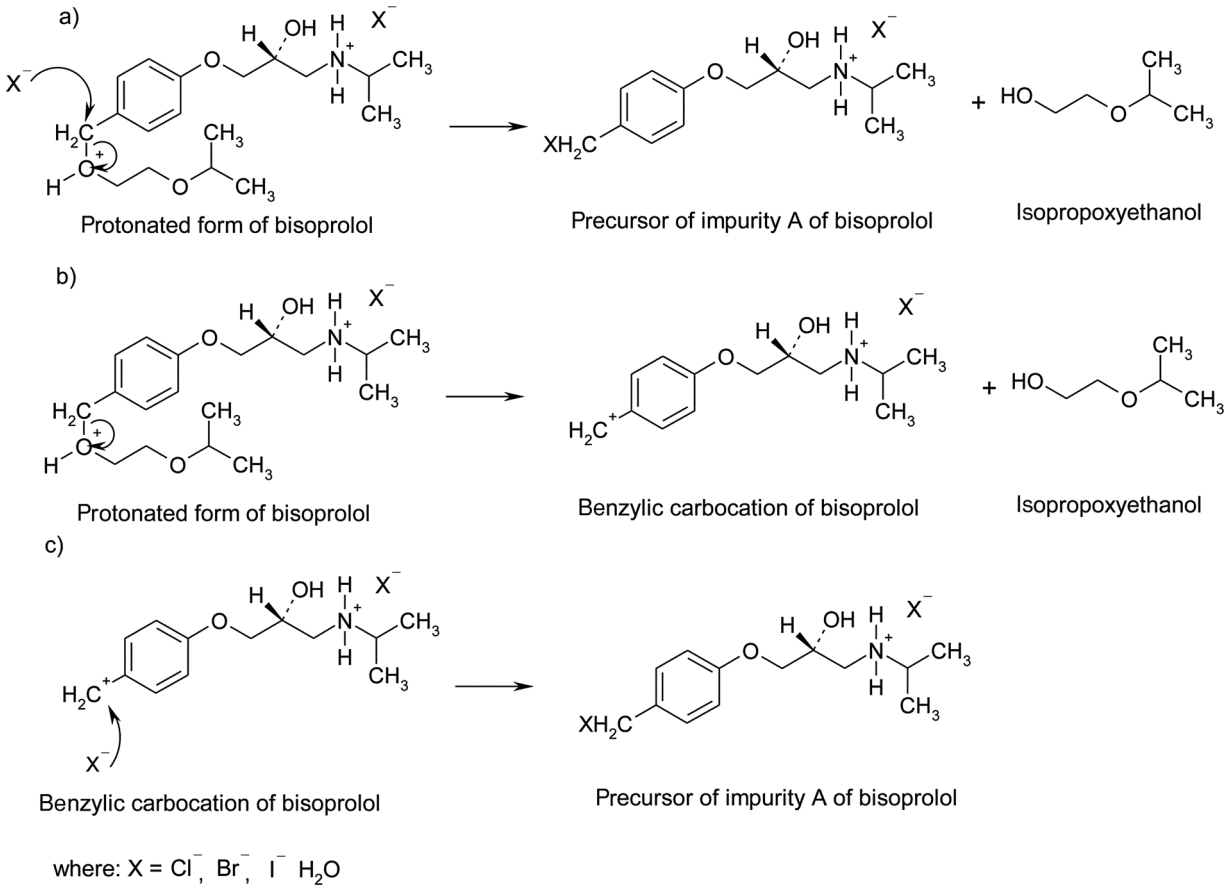

Figure 4 The mechanisms of degradation of bisoprolol in the aqueous acidic solutions. (a) Hydrolysis according to the nucleophilic substitution type 2 mechanism (S_N2), (b) hydrolysis according to the nucleophilic substitution type 1 mechanism (S_N1), and (c) termination of the S_N1 reaction mechanism.

is not involved in the further step. The cleavage of the benzyl ether linkage occurs spontaneously to form a benzylic carbocation and isopropoxyethanol as the leaving group. Benzyl carbocations are stabilized by the Π electron resonance from the aromatic ring and the solvation by polar protic solvents such as water.

Finally, Fig. 4(c) presents the termination of the S_N1 reaction mechanism, attack of a nucleophile on the carbocation, and formation of benzyl halides.

In addition, we have carried out a set of experiments for various initial concentrations of bisoprolol hemifumarate ranging from 1.3×10^{-3} to 2.6×10^{-1} mol·dm^{-3} in 1.0 mol·dm^{-3} solution of hydrochloric acid. These studies were performed to test the influence of the initial concentration of bisoprolol on the effective rate of hydrolysis. In all experiments, $\ln([B_o]/[B])$ was a linear function of time (with regression coefficients higher than 0.99), indicating the pseudo–first-order reaction in relation to bisoprolol (according to Eq. (5)). It may be noticed from Fig. 5 that the effective rate constants decrease with the increase of the concentration of bisoprolol hemifumarate. It was assumed that participation of the S_N1 and S_N2 mechanisms is different for various initial concentrations of bisoprolol; thus the effective rate constant k_0 is influenced by various participations of k_1 and k_2 rate constants. The results obtained for different initial concentrations of bisoprolol are presented in Fig. 5 and Table III.

$$-\frac{\mathrm{d}\left[\text{Bisoprolol}\right]}{\mathrm{d}t} = k_1\left[\text{Bisoprolol}\right]\left[\mathrm{H_3O^+}\right] + k_2\left[\text{Bisoprolol}\right]\left[\mathrm{H_3O^+}\right]^2 \quad (3)$$

$$-\frac{\mathrm{d}\left[\text{Bisoprolol}\right]}{\mathrm{d}t} = \left(k_1\left[\mathrm{H_3O^+}\right] + k_2\left[\mathrm{H_3O^+}\right]^2\right) \cdot \left[\text{Bisoprolol}\right] \quad (4)$$

$$-\frac{\mathrm{d}\left[\text{Bisoprolol}\right]}{\mathrm{d}t} = k_0\left[\text{Bisoprolol}\right],$$

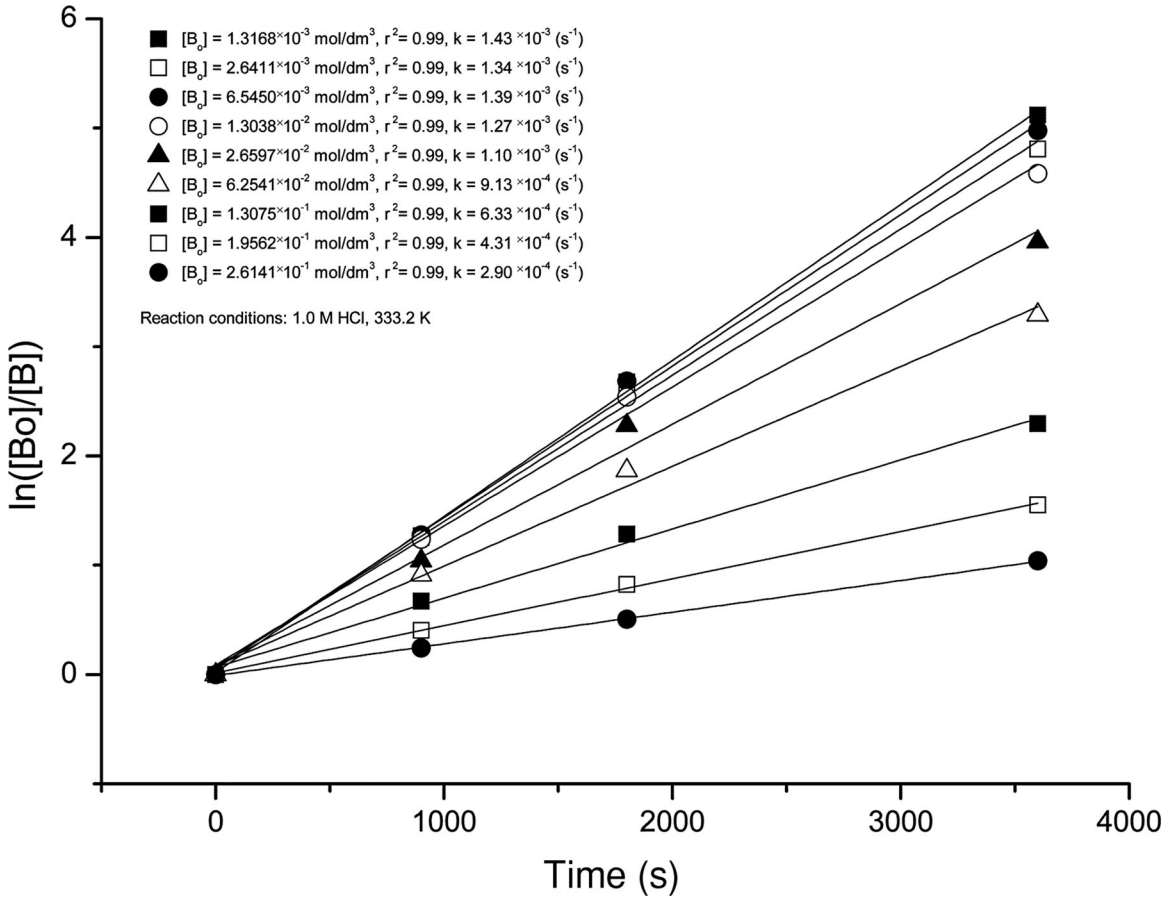

Figure 5 Determination of the reaction order in relation to bisoprolol.

Table III Influence of the Bisoprolol Hemifumarate Concentration on the Reaction Rate Constants (in 1 mol.dm^{-3} HCl Solution)

	Temperature 333.2 K		
[B_0] mol·dm^{-3}	b_n	r^2 (n)	($k \pm \Delta_I$) ($\times 10^{-3}$ s^{-1})
1.3168×10^{-3}	0.01	0.99 (4)	1.43 ± 0.02
2.6411×10^{-3}	0.01	0.99 (4)	1.34 ± 0.05
6.5450×10^{-3}	0.01	0.99 (4)	1.39 ± 0.04
1.3038×10^{-2}	0.01	0.99 (4)	1.27 ± 0.05
2.6597×10^{-2}	0.02	0.99 (4)	1.10 ± 0.07
6.2541×10^{-2}	0.04	0.99 (4)	0.91 ± 0.05
1.3075×10^{-1}	0.10	0.99 (4)	0.63 ± 0.03
1.9562×10^{-1}	0.21	0.99 (4)	0.43 ± 0.01
2.6141×10^{-1}	0.35	0.99 (4)	0.290 ± 0.004

$$\text{where } k_0 = k_1\left[H_3O^+\right] + k_2\left[H_3O^+\right]^2 \qquad (5)$$

The order of reaction in relation to a hydrohalic acid may be confirmed mathematically. The kinetic equation (6) may be further reduced to Eq. (7) by using high excess of hydrochloric acid. The experiments were carried out for eight different concentrations of hydrochloric acid ranging from 0.1 to 2.0 mol·dm^{-3} at 323.2 and 333.2 K and for the initial concentration of bisoprolol 1.3×10^{-3} mol·dm^{-3}. The order of a reaction in hydrohalic acid may be determined from Eq. (8) and Fig. 6. The graph of ln k plotted against ln [HX]

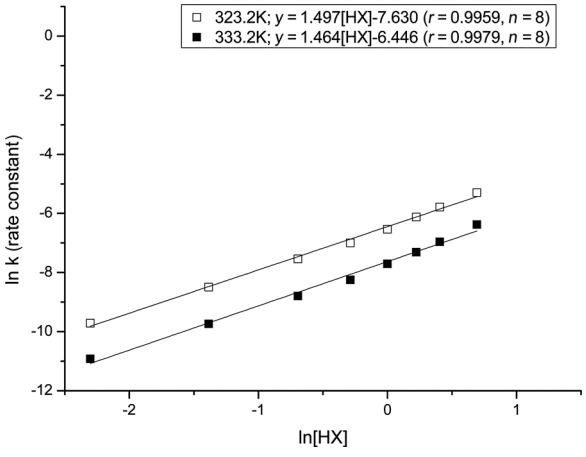

Figure 6 The order of a reaction in hydrohalic acid (isolation method).

has a slope z and an intercept $\ln k'$. The calculated order of the reaction (z) was approximately 1.5 (1.49 for 323.2 K and 1.46 for 333.2 K), indicating that there are two possible reaction mechanisms: the first involving only the hydronium ion and the second involving the hydronium ion and appropriate nucleophile. This is in conformance with our explanation of a hydrolysis by the nucleophilic substitution types 1 and 2. At this point, it is important to highlight that the overall reaction order in hydrohalic acid is an average value estimated for the reactions carried in 0.1–2.0 mol/dm^{-3} HCl solutions. At very low acid concentrations, the S_N1 mechanism may dominate, whereas at high acid concentrations the reaction will appear as second order in HX (the S_N2 mechanism). In fact, the data in Fig. 6 appear slightly curved, confirming that the reaction order in HX may change with the concentration of the catalyst:

$$v = k' \cdot \left[\text{Bisoprolol}\right] \cdot [\text{HX}]^z \tag{6}$$

$$v = k \cdot \left[\text{Bisoprolol}\right];\ \text{where } k = k' \cdot [\text{HX}]^z \tag{7}$$

$$\ln k = \ln k' + z \ln [\text{HX}] \tag{8}$$

We have mainly focused on the hydrolysis of bisoprolol hemifumarate; however, the kinetic studies carried out for the bisoprolol base (free amine) proved that there is no influence of fumaric acid on the reaction rate. The measurements were carried out at 333.2 K in 1.0 and 0.1 mol·dm^{-3} solutions of hydrochloric acid and 1.3×10^{-3} mol·dm^{-3} initial concentration of bisoprolol hemifumarate and bisoprolol base. The effective rate constant (k) obtained in 1.0 mol·dm^{-3} HCl solution was 1.4316×10^{-3} s^{-1} for bisoprolol hemifumarate and 1.4344×10^{-3} s^{-1} for the bisoprolol base. In 0.1 mol·dm^{-3} solution of hydrohalic acid, the rate constants for fumaric salt (6.0345×10^{-5} s^{-1}) and bisoprolol base (6.0088×10^{-5} s^{-1}) were almost the same. It was assumed that the dissociation of a weak carboxylic acid was inhibited in the presence of a strong hydrohalic acid and had no significant influence on the effective rate of hydrolysis.

The activation parameters were calculated using the Eyring equation [11]. Figure 7 is a graphical presentation of a linear form of the Eyring equation (9) for different hydrohalic acids at the concentration 1.0 mol·dm^{-3} at temperatures 323.2, 333.2, and 343.2 K. The enthalpy and entropy of activation were calculated from a plot of $\ln(k/T)$ versus $1/T$; this function is a straight line with a slope of $(-\Delta H^{\neq}/R)$ and an intercept $(\ln k_b/h + \Delta S^{\neq}/R)$. The enthalpy of activation decreases with an increase of the concentration of hydrohalic acid; this indicates larger participation

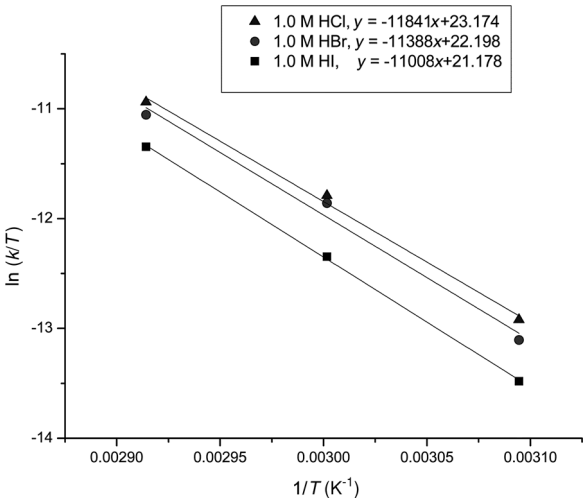

Figure 7 The linear form of the Eyring equation for three hydrohalic acids.

of the S_N2 mechanism in more concentrated solutions. This phenomenon was also confirmed by a decrease in the entropy of activation in the concentration range from 0.5 to 1.0 mol·dm^3 (compare with $\Delta H^{\neq}$ and $\Delta S^{\neq}$ values for the S_N1 and S_N2 reaction presented in Table VI). Summarizing the obtained results, we conclude that at low concentration of HX the S_N1 mechanism is favored, whereas at high acid concentration the S_N2 mechanism dominates. All obtained results are presented in Table IV.

$$\ln \frac{k}{T} = -\frac{\Delta H^{\neq}}{R} \cdot \frac{1}{T} + \ln \frac{k_b}{h} + \frac{\Delta S^{\neq}}{R} \tag{9}$$

In addition, we used available software to carry out the modeling to estimate the activation parameters for the two reaction pathways individually. The main model of the examined reaction was based on a quadratic function of Eq. (2) and the Arrhenius equation (10). The full description of our model is presented in Eq. (11), where A is a preexponential factor estimated empirically, E_a is the Arrhenius energy of activation, R is a gas constant, and T is a temperature. Equation (11) was solved numerically, simultaneously for all different experiments (all different initial conditions) at a given temperature. The model parameters were estimated by minimizing the sum of squares between experimental and theoretical values. For the nonlinear least-squares curve fitting, the Levenberg–Marquardt procedure modified by Fletcher [12] was used. The assessment of statistical significance of estimated values parameters at a fixed scan rate was based on the Student t test at the 0.05 significance level. The estimation was carried out for the data obtained for three hydrohalic acids at 323.2 K. Results are presented in Table V. Similar results were obtained at the

Table IV Enthalpy and Entropy of Activation for Different Hydrohalic Acids Calculated from a Linear Form of the Eyring Equation (9)

Hydrohalic Acid	Parameter	0.1 mol·dm^{-3}	0.5 mol·dm^{-3}	1.0 mol·dm^{-3}
HCl	$\Delta H^{\neq}$ (kJ/mol)	109.5	104.4	98.4
	$\Delta S^{\neq}$ (J/K·mol)	2.4	4.4	−4.9
HBr	$\Delta H^{\neq}$ (kJ/mol)	105.7	99.2	94.7
	$\Delta S^{\neq}$ (J/K·mol)	−7.8	−8.8	−13.0
HI	$\Delta H^{\neq}$ (kJ/mol)	109.4	106.2	91.5
	$\Delta S^{\neq}$ (J/K·mol)	2.2	13.5	−21.5

Table V Activation Parameters Estimated by a Reaction Model (11) for Three Different Hydrohalic Acids at 323.2 K

Hydrohalic Acid	Rate Constant of Reverse Reaction, k_0 (s^{-1})	Preexponential Factor for the S_N1 Mechanism, A_1	$k_1 = A_1 \cdot \exp(E_{a1}/RT)$ (s^{-1})	Preexponential Factor for the S_N2 Mechanism, A_2	$k_2 = A_2 \cdot \exp(E_{a2}/RT)$ (s^{-1})	E_a Calculated for the S_N1 Reaction Mechanism, E_{a1} (kJ/mol)	E_a Calculated for the S_N2 Reaction Mechanism, E_{a2} (kJ/mol)
HCl	-1.75×10^{-6}	1.155×10^{14}	1.630×10^{-4}	3.186×10^{11}	2.950×10^{-4}	110.4 ± 4.9	93.0 ± 5.1
HBr	-1.44×10^{-6}	4.649×10^{14}	1.822×10^{-4}	1.447×10^{11}	5.202×10^{-4}	107.7 ± 17.7	89.4 ± 11.2
HI	-1.66×10^{-6}	4.243×10^{14}	1.374×10^{-4}	2.873×10^{10}	6.566×10^{-4}	114.7 ± 19.3	84.4 ± 8.0

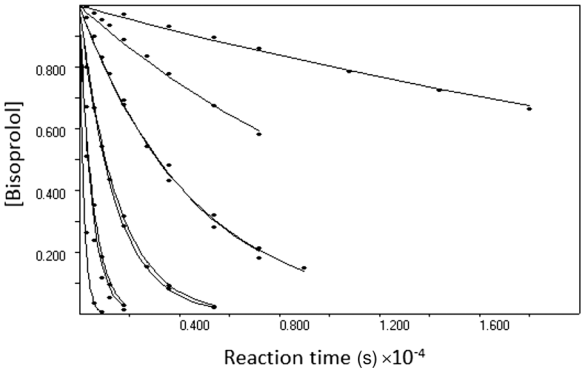

Figure 8 The exemplary graphical fit presenting estimation of results obtained for hydrobromic acid at 323.2 K.

temperatures 333.2 and 343.2 K. The exemplary graphical fit obtained after evaluation of the results for HBr at 0.1, 0.5, and 1.0 M at 323.2 K is presented in Fig. 8.

$$k = A\mathrm{e}^{-E_a/RT} \tag{10}$$

$$\frac{\mathrm{d[Bisoprolol]}}{\mathrm{d}t} = -\left(k_0 + A_1 \exp\left(\frac{E_{a1}}{RT}\right)\left[\mathrm{H_3O^+}\right] + A_2 \exp\left(\frac{E_{a2}}{RT}\right)\left[\mathrm{H_3O^+}\right]^2\right) \cdot \left[\mathrm{Bisoprolol}\right] \tag{11}$$

The modeling was carried out for the results obtained at three temperatures, 323.2, 333.2, and 343.2 K, at three different concentrations of hydrohalic acids, 0.1, 0.5, 1.0 mol·dm^{-3}. The estimated k_1 and k_2 rate constants were then introduced to the Eyring equation to calculate the enthalpy and entropy of activation for the S_N1 and S_N2 reactions individually; results are presented in Table VI. The negative values of the entropy of activation for the S_N2 reaction indicate that the transition state is more ordered than molecules in a ground state. It stays in conformance with the mechanism of the S_N2 reaction where a nucleophile may attack on the protonated bisoprolol molecule only from the opposite side to the leaving group. On the other hand, positive values of the entropy of activation for the S_N1 mechanism confirm that the reactant in a ground state (a protonated form of bisoprolol) is more ordered than in a transition state.

Finally, a comparison of the half-lives of atenolol, acebutolol, and propranolol (1 M HCl, 333.2 K), which are, respectively, 0.84, 4.29, and 385 h, with the half-life of bisoprolol 0.13 h, indicates that the stability of beta-blockers does not depend on a lipophilicity as indicated by Krzek et al. The stability mainly depends on the reactivity of functional groups contained in the molecule.

CONCLUSIONS

In this article, we presented the results of the pseudo–first-order kinetics of hydrolysis of bisoprolol

Table VI Activation Parameters for Hydrolysis of Bisoprolol According to the S_N1 and S_N2 Mechanisms

Hydrohalic Acid	Parameter	Hydrolysis According to the S_N1 Mechanism	Hydrolysis According to the S_N2 Mechanism
HCl	$\Delta H^{\neq}$ (kJ/mol)	117.6	84.5
	$\Delta S^{\neq}$ (J/K·mol)	45.7	−51.7
HBr	$\Delta H^{\neq}$ (kJ/mol)	112.2	85.7
	$\Delta S^{\neq}$ (J/K·mol)	28.9	−42.9
HI	$\Delta H^{\neq}$ (kJ/mol)	120.9	80.4
	$\Delta S^{\neq}$ (J/K·mol)	54.4	−57.2

hemifumarate in the aqueous acidic solutions. The influence of three different hydrohalic acids and a weak carboxylic acid was tested. There are two possible reaction mechanisms of hydrolysis: the nucleophilic substitution types 1 and 2. As may be predicted, the S_N2 mechanism occurs more readily at high acid concentrations, whereas at low acid concentrations the S_N1 mechanism dominates. It was proved that the attack of the nucleophile in the S_N2 mechanism enhances separation of a leaving group and improves the reaction rate. The nucleophile entity is not engaged in the S_N1 mechanism; thus a cleavage of the ether linkage occurs spontaneously, separating the reactive benzyl carbocation from isopropoxyethanol. The S_N1 reaction requires higher energy of activation. The detailed explanation of both reaction mechanisms was provided. Equation (11) based on the Arrhenius equation presents a general model for the hydrolysis of bisoprolol, which was used to estimate the activation parameters by a specialized software.

Only benzyl halides were observed as the final products of a hydrolysis catalyzed by hydrohalic acids; these compounds may further react to form (*RS*)-1-(4-hydroxymethyl-phenoxy)-3-isopropylaminopropan-2-ol (impurity A of bisoprolol). Our studies confirm that impurity A may arise in drug product preparations due to the hydrolysis of the bisoprolol molecule. No other degradation products appeared; even hydrobromic or hydroiodic acids did not cleave the phenol ether linkage, which seems to be very stable in this molecule. Finally, the stability of beta-blockers depends not on the lipophilicity but on the reactivity of functional groups in the molecule.

NOMENCLATURE

A	Preexponential factor of Arrhenius equation
[B] = [Bisoprolol]	Concentration of bisoprolol, mol·dm^{-3}
$[B]_0 = [Bisoprolol]_0$	Initial concentration of bisoprolol, mol·dm^{-3}
b_n	Relative concentration of bisoprolol for the last point of determination
E_a	Energy of activation
E_{a1}	Energy of activation of the nucleophilic substitution type 1
E_{a2}	Energy of activation of the nucleophilic substitution type 2
h	Planck constant
k	Rate constant
k_1	Rate constant of the nucleophilic substitution type 1
k_2	Rate constant of the nucleophilic substitution type 2
k_b	Boltzmann constant
log P	Partition coefficient
n	Number of determinations
r	Regression coefficient
R	Gas constant
S_N1	Nucleophilic substitution type 1
S_N2	Nucleophilic substitution type 2
T	Temperature, K
v	Reaction rate
z	Order of reaction in relation to hydrohalic acid
$\Delta H^{\neq}$	Enthalpy of activation
$\Delta S^{\neq}$	Entropy of activation

BIBLIOGRAPHY

1. Van Baak, M. A. Sports Med 1988, 5, 209.
2. Cartoni, G. P.; Ciardi, M.; Giarrusso, A.; Rosati, F. J High Resolut Chromatogr 1988, 11, 528.
3. Cruickshank, J. M. Am Heart J 1980, 100, 160.
4. Bisoprolol, Cardioselective Beta-Blocker; Merck KGaA: Darmstadt, Germany, 2001.
5. Dulin, W. A. Drug Dev Ind Pharm 1995, 21, 393.
6. Krzek, J.; Kwiecień, A.; Żylewski, M. M. Pharm Dev Technol 2006, 11, 409.
7. Carda-Broch, S.; Berthod, A. J Chromatogr A 2003, 995, 55.

8. Brownstein, S.; Stillman, A. E. J Phys Chem 1959, 63, 2061.
9. Raamat, E.; Kaupmees, K.; Ovsjannikov, G.; Trummal, A.; Kütt, A.; Saame, J.; Koppel, I.; Kaljurand, I.; Lipping, L.; Rodima, T.; Pihl, V.; Koppel, I. A.; Leito, I. J Phys Org Chem 2013, 26, 162.
10. Dawson, R. M. C. Data for Biochemical Research; Clarendon Press: Oxford, UK, 1959.
11. Schwetlick, K. Kinetische Methoden zur Untersuchung von Reaktionsmechanismen; VEB Deutscher Verlag der Wissenschaften: Berlin, Germany, 1971; p. 88.
12. Fletcher, R. AERE-R6799, United Kingdom Atomic Energy Authority, Atomic Energy Research Establishment, Harwell: Berkshire, UK, 1971.