Short communication

Evaluation of stability of enalapril maleate in solid phase

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Abstract

The influence of temperature (from 383 to 348 K) and relative humidity (from 25.0 to 76.4%) on the stability of enalapril maleate in the solid phase was investigated. Changes in the concentration enalapril maleate were followed by a HPLC method with UV detection. The kinetic and thermodynamic parameters [E_a (kJ mol^{-1}) = 168.5 ± 27 for RH = 0% and 149.1 ± 48 for RH = 76.4%; $\Delta H^{\neq}$ (kJ mol^{-1}) = 166.1 ± 30 for RH = 0% and 146.6 ± 50 for RH = 76.4%; $\Delta S^{\neq}$ (J (K^{-1} mol^{-1})) = 120.3 ± 169 for RH = 0% and 82.1 ± 110 for RH = 76.4%] of the decomposition reaction were calculated.
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1. Introduction

Enalapril (ethyl enaprilate) is a pro-drug used in medicine to treat renin-dependent hypertension. The pro-drug does not manifest any direct biological activity. In the body—the pro-drug is hydrolysed by esterases to yield enaprilate—a competitive inhibitor of angiotensin converting enzyme (ACE) [1–7].

The following methods have been used for the estimation of enalapril maleate in substance: HPLC [8–10], spectroscopy VIS [11], potentiometric with enantioselective membrane electrode [12], and capillary electrophoretic [13]. In tablets: HPLC [14–18], spectroscopy VIS [19] and deriva-

tive UV spectroscopy [12,14,16,20] have been applied.

The available literature misses any kinetic data on the stability of enalapril maleate in solid state. However, it is know that in aqueous solution cyclisation and hydrolysis do occur [21]. In the literature there are data concerning the stability of enalapril maleate, but only of its tablet or suspension forms derived from short-term tests [22–26]. These data do not evaluate kinetic or thermodynamic parameters of these reactions.

My own observations indicating an essential influence of ambient temperature and humidity on the stability of this compound when in solid state have inclined me to undertake studies aiming at determination of kinetic and thermodynamic parameters of reactions occurring in a dry and humid atmosphere.

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The results of these studies will make it possible to evaluate the effect of humidity on the rate and mechanism of the reactions. Enalapril maleate in substance (in solid state) was chosen as the object to be investigated, because enalapril maleate is used predominantly in this form and thus we considered the knowledge of these parameters to be of valuable importance.

2. Experimental

2.1. Material and method

Enalapril maleate and cetirizine dichloride were derived from P.P.H.U. Biofarm Ltd. All the other reagents used were commercial preparations with a pro analysis grade of purity.

Liquid chromatography was performed with the aid of: a Shimadzu LC 6A-pump, a Cecil 1100 spectrophotometric detector, a computerised recorder and a Rheodyne sample injector (20 μl).

2.2. Experimental conditions

In order to determine the effect of humidity on the stability of enalapril maleate, vials containing samples of the preparation (0.0100 g) were placed in desiccators, and these in turn were kept in heat chambers set for the desired temperature. Changes in relative humidity of the environmental atmosphere have been achieved with saturated solutions of the respective inorganic salts: NaI (RH = 25.0%), NaBr (RH = 50.9%), KI (RH = 60.5%), NaNO$_3$ (RH = 66.5%) i NaCl (RH = 76.4%).

When determining the stability of the preparation in dry atmosphere, vials with the enalapril maleate were placed in sand baths that were located in heat chambers set to the desired temperatures.

2.3. Chromatographic analysis

The concentration changes of enalapril maleate occurring in the course of the experimental exposition of the studied samples were measured by means of a self elaborated HPLC separation method. The following parameters of the separa-

tion procedure were adopted: column: Hypersil MOS (5 μm), 250×4 mm ID (Merck), mobile phase: acetonitryl–solution A (50:50 v/v); preparation of the working solution A: take exactly 0.0680 g KH$_2$PO$_4$ and dissolve it in 450 ml water in a 500 ml volumetric flask, adjust the pH of this solution with 80% *ortho*-phosphoric acid to pH 2.2 and made up the volume with water to a total of 500 ml, mobile phase rate of flow 1.0 ml min^{-1}, internal standard: cetirizine dichloride (methanolic solution 0.2 mg ml^{-1}), detector UV: 215 nm.

For the elaboration and validation of the HPLC separation method, two kinds of reference substances were used: intact enalapril maleate and enalapril maleate incubated in dry air at 373 K, and another one incubated at 363 K, both in a stable with respect to humidity (RH = 76.4%) atmosphere were run through the procedure.

2.4. Sample preparation

In the test of 'enhanced ageing', vials containing the examined samples and kept under the experimental conditions were taken and their contents were dissolved in methanol next was added to make up for a total volume of 25.0 ml. To 1.0 ml of the sample solution to be investigated, 1.0 ml of a methanolic solution of cetirizine dichloride—internal standard (0.2 mg ml^{-1}) (solution A$_i$).

In parallel a 'comparative' solution of enalapril maleate in the methanol (concentration 0.4 mg ml^{-1}) was prepared (to be referred to as solution B). To 1.0 ml of solution B, 1.0 ml of a methanolic solution of cetirizine dichloride—internal standard (0.2 mg ml^{-1}) (solution B$_1$).

Samples (20 μl) of the respective solutions A$_i$ i B$_1$ were injected onto the column and further processed through the chromatographic procedure.

The percent loss of concentration of enalapril maleate (in solid state) subjected to the experimentally traced process of decomposition, was calculated from the following equation:

$$c \ (\%) = \frac{P_A \cdot c_B \cdot V}{P_B \cdot m}$$

where: $P_A - (P_A/P_{st})$ applies for the solution investigation; $P_B - (P_{B1}/P_{st})$ applies for the reference substance (P_{A_i}, magnitude of the enalapril maleate peak for solution A; P_{B_1}, magnitude of the enalapril maleate peak for solution B; P_{st}, magnitude of the internal standard peak); c_B, percent concentration of the comparative enalapril maleate sample in solution (0.04%); V, volume coefficient (25.0 ml); m, weighed amount of enalapril maleate (0.0100 g).

3. Results and discussion

3.1. Validation method HPLC

3.1.1. Selectivity

The elaborated method is selective towards the degradation products and internal standard. The following retention time for the substances, internal standard and product of deacay were obtained: approximately 4, 6 and 2, 3 min, respectively.

3.1.2. Linearity of the adopted HPLC method

Linearity of the adopted HPLC method was determined as the ratio of the peak height and concentration of enalapril maleate, over concentrations ranging from 0.0006 to 0.0048 mg ml^{-1}. The parameters of regression were the following $y = (323.4 \pm 5.7)x$; R.S.D. = 1.3; b, they were statistically nonsignificant; coefficient of linear correlation = 0.999. The values $a \pm \Delta a$ was computed for $f = n - 1$ degrees of freedom, with $\alpha = 0.05$.

3.1.3. Precision HPLC method

Parameters characterising the precision of the method were evaluated for ten individual samples of 0.4 mg ml^{-1} enalapril maleate, and the following results were obtained mean value $P_i/P_{is} = 0.9876$, standard deviation $s = 0.008$, variation coefficient = 0.81%.

3.1.4. Repeatability

The repeatability of method was tested by eight replicates and evaluated by the variation coefficient = 1.2%.

3.1.5. Robustness

The change of pH of the mobile phase within the range from 2.00 to 2.30, as well as the change of acetonitrile concentration in the mobile phase within the range from 48 to 53% yielded the best and desired, correct chromatographic separation of enalapril maleate from its decomposition product, as well as from eventual impurities.

3.2. Kinetic parameters

The assessed concentration changes of enalapril maleate that occurred during the incubation in an atmosphere with an increased humidity or in completely dry air (RH = 0%) substantiate the conclusion that the decomposition of the analysed substance is due to an autocatalytic reaction, characterised by a sigmoidal course of the relationship $c = f(t)$ (see Fig. 1) [27]. For the interpretation of relationship describing the process of decomposition of enalapril maleate in terms of an autocatalytic process, the equation of Prout–Tomkins [27] was used: $\ln c_t/(c_0 - c_t) = C - kt$, where c_0 stands for the percent concentration of enalapril maleate at zero time (t_0), c_t stands for the percent concentration of enalapril maleate at time t, C constitutes a parameter of the induction period, t stands for time, k stands for the velocity constant (s^{-1}).

Semilogarithmic plots of equation $c_t/(c_0 - c_t) = f(t)$ characterising the acceleration period show a straight-line relationship of this dependence with a negative slope that is equal to a negatively signed velocity constant. The velocity constants
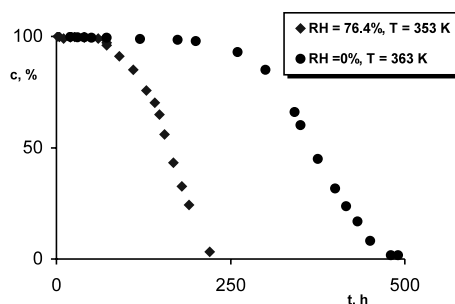

Fig. 1. Diagram presenting changes in concentration of enalapril maleate following exposition to a humid atmosphere (RH = 76.4%) and in dry atmosphere (RH = 0%).

Table 1
Regression parameters of $\ln c_t/(c_0 - c_t) = f(t)$ and kinetic parameters of enalapril maleate decomposition

Temperature (K)	Parameters	
	Regression of $\ln c_t/(c_0 - c_t) = f(t)$	Kinetic
Relative humidity (RH) 76.4%		
368	$n = 11$; $r = -0.995$; $a \pm \Delta a = -0.412 \pm 0.032$; $S_a = 0.014$; $b \pm \Delta b = 4.88 \pm 0.36$; $S_b = 0.16$	$k \pm \Delta k = (1.15 \pm 0.090) \times 10^{-4}$; $t_{0.1} = 6$ h; $t_{0.5} = 11$ h
363	$n = 13$; $r = -0.994$; $a \pm \Delta a = -0.121 \pm 0.0087$; $S_a = 0.0040$; $b \pm \Delta b = 4.15 \pm 0.19$; $S_b = 0.087$	$k \pm \Delta k = (3.35 \pm 0.24) \times 10^{-5}$; $t_{0.1} = 16$ h; $t_{0.5} = 35$ h
358	$n = 14$; $r = -0.996$; $a \pm \Delta a = -0.0926 \pm 0.0050$; $S_a = 0.0023$; $b \pm \Delta b = 5.62 \pm 0.32$; $S_b = 0.15$	$k \pm \Delta k = (2.57 \pm 0.14) \times 10^{-5}$; $t_{0.1} = 37$ h; $t_{0.5} = 61$ h
353	$n = 10$; $r = -0.993$; $a \pm \Delta a = -0.0371 \pm 0.0036$; $S_a = 0.0016$; $b \pm \Delta b = 5.98 \pm 0.52$; $S_b = 0.23$	$k \pm \Delta k = (1.03 \pm 0.10) \times 10^{-5}$; $t_{0.1} = 102$ h; $t_{0.5} = 161$ h
348	$n = 12$; $r = -0.998$; $a \pm \Delta a = -0.0207 \pm 0.00095$; $S_a = 0.00042$; $b \pm \Delta b = 5.81 \pm 0.25$; $S_b = 0.011$	$k \pm \Delta k = (0.576 \pm 0.027) \times 10^{-5}$; $t_{0.1} = 174$ h; $t_{0.5} = 280$ h
Dry atmosphere RH = 0%		
383	$n = 7$; $r = -0.995$; $a \pm \Delta a = -0.486 \pm 0.054$; $S_a = 0.021$; $b \pm \Delta b = 12.32 \pm 1.30$; $S_b = 0.50$	$k \pm \Delta k = (1.35 \pm 0.15) \times 10^{-4}$; $t_{0.1} = 21$ h; $t_{0.5} = 25$ h
373	$n = 15$; $r = -0.996$; $a \pm \Delta a = -0.0886 \pm 0.0050$; $S_a = 0.0023$; $b \pm \Delta b = 6.96 \pm 0.31$; $S_b = 0.14$	$k \pm \Delta k = (2.46 \pm 0.14) \times 10^{-5}$; $t_{0.1} = 54$ h; $t_{0.5} = 79$ h
368	$n = 11$; $r = -0.997$; $a \pm \Delta a = -0.0569 \pm 0.0034$; $S_a = 0.0015$; $b \pm \Delta b = 8.18 \pm 0.52$; $S_b = 0.23$	$k \pm \Delta k = (1.58 \pm 0.094) \times 10^{-5}$; $t_{0.1} = 105$ h; $t_{0.5} = 144$ h
363	$n = 11$; $r = -0.996$; $a \pm \Delta a = -0.0271 \pm 0.0022$; $S_a = 0.00094$; $b \pm \Delta b = 9.88 \pm 0.78$; $S_b = 0.33$	$k \pm \Delta k = (0.752 \pm 0.062) \times 10^{-5}$; $t_{0.1} = 284$ h; $t_{0.5} = 365$ h

S_a, S.D. slope regression of $\ln c_t/(c_0 - c_t) = f(t)$. S_b, S.D. value of b.

were calculated by means of the minimal square method, the obtained values are presented in Table 1.

The values $t_{0.1}$ and $t_{0.5}$ for the autocalalytic reaction (see Table 1) were calculated from the following equations: $t_{0.1} = (b - 2197)/k$ and $t_{0.5} = b/k$, where $t_{0.1}$ stands for the time needed for a 10% decay and $t_{0.5}$ stands for the time needed for a 50% decay, b stands for the value of the ordinate for $t = 0$ in dependence of $\ln c_t/(c_0 - c_t) = f(t)$, k is velocity constant of the reaction (s^{-1}).

3.3. Effect of humidity on the stability of enalapril maleate in solid state

The effect of humidity on the stability of enalapril maleate in solid phase, exposed to an environment with the following RH: 76.4, 66.5, 60.5, 50.9 and 25.0% and at 363 K ambient temperature is described by the following equation: $\ln k_i = a \text{RH}(\%) + b$, where k_i stands for the

reaction velocity constant (s^{-1}), RH stands for relative humidity (%) and b stands for regression coefficient (see Table 2).

Table 2
Effect of humidity on the stability of enalapril maleate at 363 K

Relative humidity %	$(k \pm \Delta k) \times 10^5$ s^{-1}	Parameters of the regression $\ln k_i = f(\text{RH}\%)$
76.4	3.35 ± 0.24	$a = 0.01960$; $b = -11.80$; $\Delta a = 0.00335$; $\Delta b = 0.196$; $S_a = 0.00105$; $S_b = 0.0615$; $r = 0.996$
66.5	2.89 ± 0.22	
60.5	2.33 ± 0.15	
50.9	2.04 ± 0.095	
25.0	1.24 ± 0.69	

S_a, S.D. slope regression of $\ln k_i = f(\text{RH}\%)$. S_b, S.D. value of b.

Table 3
Kinetic and thermodynamic parameters of enalapril maleate decay

T, K	$(k \pm \Delta k) \times 10^{-5}$ s^{-1}	Statistical evaluation ln $k_i = f(1/T)$	Thermodynamic parameters at the temperature 293 K
Dry atmosphere RH = 0%			
383	13.50 ± 1.49	$a = -20271.4 \pm 3362$; $S_a = 1543.1$; $b = 43.92 \pm 9.0$; $S_b = 4.15$; $r = -0.994$	$E_a = 168, 5 \pm 27$ (kJ mol^{-1}); $\Delta H^{\neq} = 166.1 \pm 30$ (kJ mol^{-1}); $\Delta S^{\neq} = 120.3 \pm 169$ (J (K^{-1} mol^{-1}))
373	2.46 ± 0.137		
368	1.58 ± 0.0940		
363	0.752 ± 0.0615		
Relative humidity (RH) 76.4%			
368	11.46 ± 0.896	$a = -17930.7 \pm 5797$; $S_a = 1821.9$; $b = 39.33 \pm 16.2$; $S_b = 5.08$; $r = -0.9849$	$E_a = 149.1 \pm 48$ (kJ mol^{-1}); $\Delta H^{\neq} = 146.6 \pm 50$ (kJ mol^{-1}); $\Delta S^{\neq} = 82.1 \pm 110$ (J (K^{-1} mol^{-1}))
363	3.35 ± 0.242		
358	2.57 ± 0.139		
353	1.03 ± 0.101		
348	0.576 ± 0.0266		

The semilogarithmic plot of the function expressed by the equation: $k_i = f(\text{RH\%})$ shows a straight line relationship of this association, and the slope of the curve expresses the effect of humidity on the stability of the substance under investigation (see Table 2)

3.4. Thermodynamic parameters of the enalapril maleate decay reaction

The relationship between the reaction velocity constants and environmental temperature (Table 3) is described by the Arrhenius equation: ln $k_i =$ ln $A - (E_a/R) \times 1/T$, where k_i stands for the reaction velocity constant (s^{-1}); A, frequency coefficient; $E_a/R = a$, vectorial coefficient of ln $k_i = f(1/T)$, R, universal gas constant (8.3144 J K^{-1} mol^{-1}; E_a, activation energy. The following magnitudes: activation energy (E_a), enthalpy ($\Delta H^{\neq}$) and entropy ($\Delta S^{\neq}$) were calculated.

4. Conclusions

The adopted HPLC method is a modification of the method presented by Xue-Zhi Qin and co-workers [18]. Changes introduced into this method pertained to both, the stationary phase and the mobile one (acetonitrile concentrations). These changes had a positive effect on the retention times of the substrate under study and its products of decomposition.

From the performed study it may be concluded, that both in dry and in humid atmosphere, the degradation of enalapril maleate is an autocatalytic reaction of first order kinetics with respect to substrate concentration. A diketopiperazine derivative seems to be the main degradation product of intermolecular cyclisation. This fact, as well as the insignificance of differences between reaction rate constants at 363 K obtained in a study of enhanced ageing in dry air ($k = (7.52 \pm 0.62) \times 10^{-6}$ s^{-1} and by the extrapolation of the relationship ln $k_i = f(\text{RH\%})$ to RH = 0% $k = (7.51 \pm 0.59) \times 10^{-6}$) indicate that humidity has no effect on the mechanism of the reaction degradating enalapril maleate.

This conclusion is further confirmed by the lack of significant differences between thermodynamic parameters found at 0 and 76.4% RH.

The obtained positive value of entropy confirms the monomolecular nature of the reaction.

The conducted study on the stability of enalapril maleate has shown that the investigated substance (in solid state) shows an appreciable stability at room temperature. The degradation constant of enalapril maleate at 298 K when extrapolated from the Arrhenius equation equal to $k = (4.78 \pm$

$0.16) \times 10^{-11}$ s^{-1} for RH $= 0\%$ and $k = (7.67 \pm 0.54) \times 10^{-10}$ s^{-1} for RH $= 76.4\%$ being.

Acknowledgements

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