Acta Poloniae Pharmaceutica – Drug Research, Vol. 60 No. 6 pp. 443–450, 2003

ISSN 0001-6837
Polish Pharmaceutical Society

THE STABILITY OF QUINAPRIL HYDROCHLORIDE – A MIXTURE OF AMORPHOUS AND CRYSTALLINE FORMS (QHCl–AC) – IN SOLID PHASE

BEATA STANISZ

Department of Pharmaceutical Chemistry, University of Medical Sciences,
6 Grunwaldzka Str., 60–780 Poznań, Poland

Abstract: The first–order rate constants and thermodynamic parameters (E_a [kJ · mole^{-1}] = 139.9 for RH=0% and 133.6 for RH=76.4%; $\Delta H^{\neq}$[kJ · mole^{-1}] = 137.4 for RH=0% and 131.1 for RH=76.4%; $\Delta S^{\neq}$ [J · (K^{-1} · mole^{-1})] = 35.4 for RH=0% and –207.8 for RH=76.4%) for the degradation of quinapril hydrochloride – a mixture of amorphous and crystalline forms (QHCl–AC) in solid state were calculated. The effect of humidity on the stability of QHCl–AC in the humidity range 25.0% to 76% at 363K is described by the equation $ln\ k_i = ax + b = (0.058 \pm 0.0086) \cdot RH\% - (14.19 \pm 0.50)$. Mechanism of degradation of QHCl–AC was investigated at 363 K (relative humidity 76.4%) and 373 K (relative humidity 0%).

Keywords: quinapril hydrochloride, stability in solid phase, HPLC, HPLC–MS

Quinapril hydrochloride is a pro–drug which after administration to the human body is hydrolysed to the active metabolite – quinaprilate. Quinaprilate (quinapril diacid), by acting on the enzyme angiotensin convertase (ACE) inhibits the transformation of angiotensin I into angiotensin II. This inhibition brings about peripheral vasodilatation and reduction of aldosterone secretion with the consequent depletion of sodium and concomitant increase of potassium levels (1–3).

So far conducted studies of quinapril hydrochloride were dealing only with the amorphous form of the drug (4–6). Because of the beneficial action of quinapril hydrochloride in arterial hypertension and the increasing interest in its application for the treatment of this disease (7–10), investigations aiming in more detail elucidation of the kinetics and mechanisms underlying the process of quinapril hydrochloride degradation seem to be well substantiated.

The aim of this work is to investigate the influence of temperature and humidity on the stability of QHCl–AC in solid phase and estimation of thermodynamic parameters.

In addition to that, the following parameters were included into the study protocol: evaluation of the mechanism of QHCl–AC decay (at an increased relative atmospheric moisture, RH =76.4% and in an anhydrous atmosphere, RH = 0%), kinetic interpretation of the formation or decomposition of degradation products under the experimental conditions. Another aim was to describe an adequate model of the reaction and evaluate the per cent participation of the two decay processes, that is: of cyclization and of hydrolysis in both, the moist and dry atmosphere. The investigations were performed by means of „the enhanced ageing test".

EXPERIMENTAL

Materials and reagents

Quinapril hydrochloride – a mixture of amorphous and crystalline state (QHCl–AC), quinaprilate – quinapril diacid (QAT), derivative quinapril – diketopiperazine (DKP) and lovastatine were derived from P.P.H.U. Biofarm Ltd. All other reagents used were commercial preparations with a pro analysis grade of purity.

HPLC method conditions

In this paper, a modified HPLC method was applied, used earlier for the study of stability of quinapril hydrochloride in tablets (11).

A Merck analytical column (Hypersil MOS, 5 μm particle size, 250 mm × 4 mm ID Merck) was used as the stationary phase. The mobile phase: acetonitrile – solution A (50:50 v/v); preparation of the working solution A: take exactly 0.0680 g of KH_2PO_4 and dissolve it in 450 ml of water in a 500 ml volumetric flask, adjust pH of this solution to 2.0 with 80% phosphoric(V) acid and made up the volume with water to a total of 500 ml, mobile phase flow rate 1.0 ml/min, internal standard: lovastatine (methanolic solution 0.16 mg/ml), detector UV: 215 nm.

HPLC – MS method conditions

Column: Hypersil MOS, 5 μm particle size, 250 mm × 4 mm ID Merck, mobile phase:

acetonitrile – water (90:10) with formic acid, flow rate: 0.5 ml/min.

Validation method

Selectivity: the applied method is selective towards the QHCl–AC, degradation products and lovastatinc (internal standard).

Linearity: the parameters of regression were the following: $y = (17.47 \pm 0.33) \cdot x$; b – was statistically insignificant, coefficient of linear correlation $r = 0.999$. The values of $\pm\Delta a$ were computed for $f = n-1$ degrees of freedom, with $a = 0.05$.

Precision: the precision of measurements of decomposition rates of QHCl–AC was established from the results of 8 repeated (under like conditions) determinations of velocity constant rates of degradation of QHCl–AC at 353 K, RH ~76%. The obtained results have shown that the adopted procedure for the determination of QHCl–AC is characterised by a good RSD (1.015%) value.

Condition of the kinetic studies

Weighed samples (0.0100 g) of the substance in open glass vials (5 ml) were placed in an automatically controlled head chamber at appropriate temperature. To investigate the effect of humidity, samples were placed in desiccators containing aqueous saturated solution of appropriate inorganic salts: sodium iodide for relative humidity RH ~25.0%, sodium bromide for RH ~50.9%, potassium iodide for RH ~60.5%, sodium nitrate for RH ~66.5% and sodium chloride for about 76.4% and placed into the head chamber (temperature 363 K).

Samples destined for investigation of the effect of temperature at a relative humidity of about 76.4% were placed in desiccators containing aqueous saturated solutions of sodium chloride and inserted in the head chamber adjusted to 338 K, 343 K, 348 K, 353 K and 363 K.

To assess the stability of QHCl–AC in dry air, the vials with the studied substance were immersed in a sand bath, in the head chamber adjusted to 353 K, 358 K, 363 K, 368 K and 373 K.

Samples destined for investigation of the mechanism of degradation QHCl–AC at a relative humidity of about 76.4% were placed a in desiccator containing aqueous saturated solution of sodium chloride and inserted in the head chamber adjusted to 363 K.

Samples destined for investigation of the mechanism of degradation QHCl–AC in dry air were immersed in a sand bath, in the head chamber adjusted to 373 K.

For the determination, samples were cooled at room temperature, dissolved in methanol, transferred into a volumetric flask and made up to 25 ml with the same solvent. For the analysis, 1.0 ml of this solution was mixed with 1.0 ml of the internal standard solution.

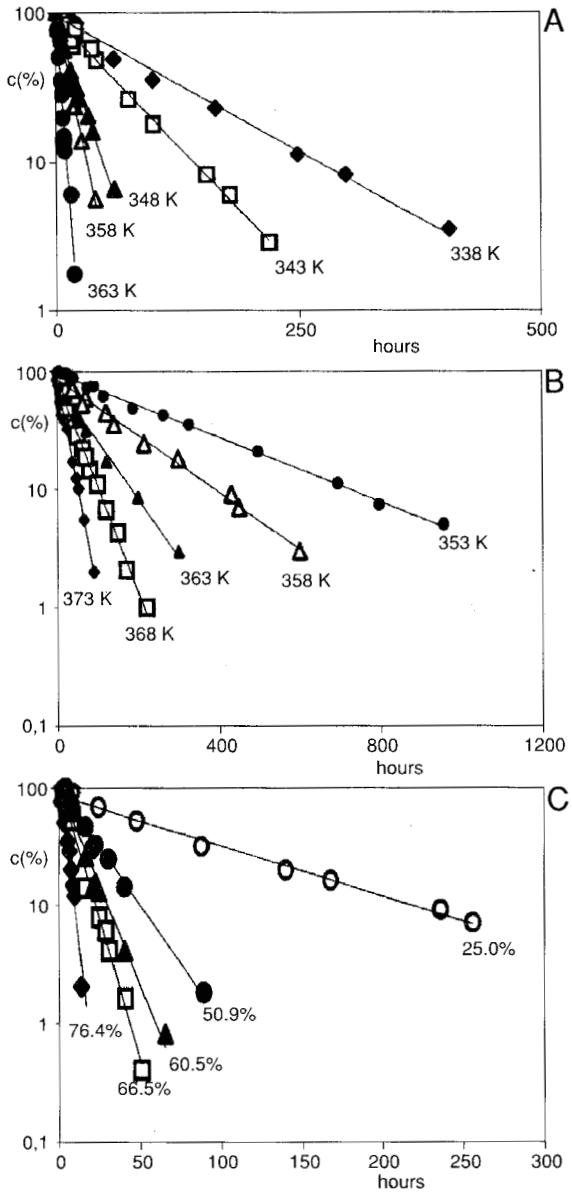


Figure 1. Semilogaritmic plots $c_i = f(t)$ for the degradation of QHCl – AC in solid phase
a) at relative humidity RH ~76% at different temperatures,
b) in dry air at different temperatures,
c) at different humidity, at 363 K

RESULTS AND DISCUSSION

Kinetic studies

In the presence of increased humidity and dry air, the concentration changes of QHCl–AC

occurred according to the first order reaction model $c_0 \rightarrow 0$. The plots $ln\ c_i = f(t)$ were linear (Figure 1a, 1b and 1c) and the observed rate constants were calculated by the least squares method according to the equation: $ln\ c_i = ln\ c_0 - k \cdot t$, where: c_i and c_0 represent concentration of QHCl–AC at time t and 0, respectively, k is the first–order rate constant.

The following statistical parameters of the respective equations were computed: $a \pm \Delta a$, $b \pm \Delta b$, S_a, S_b, S_y and the coefficient of linear correlation. The values of Δa and Δb were compouted for $f = n–2$ degrees of freedom, with $\alpha = 0.05$.

The determined reaction rate constants were employed for the calculation of the Arrhenius relationship:

$ln\ k_i = lnA - E_a/RT$, where k_i represent the respective reaction rate constants [s^{-1}], A = frequency coefficient, E_a = activation energy [J · mole^{-1}], R = universal gas constant (8.3144 J · K^{-1} · mole^{-1}), T = temperature [K].

For the relationship $ln\ k_i = f(1/T)$ straight line plots were obtained for both the humid and dry conditions of sample exposure (Table 1). From the parameters of the plot $ln\ k_i = f(1/T)$, the following thermodynamic parameters of the decomposition reaction of QHCl–AC in solid phase, pertaining to either of the conditions of sample incubation, i.e. in dry air and in an atmosphere of RH = 76.4%, were calculated: the activation energy (E_a), enthalpy ($\Delta H^{\neq}$) and entropy ($\Delta S^{\neq}$) for a temperature of 293 K (Table 1).

The effect of humidity on the stability of QHCl–AC at 363 K, in the humidity range from 25.0% to 76.4% is described by the equation: $ln\ k_i = ax + b$ = (0.058 ± 0.086) · RH% –(14.19 ± 0.50) (Table 2).

The slope (a = 0.058 ± 0.086) of the straight linear plot $ln\ k_i = f$ (RH%) characterises the effect of humidity on the stability of QHC–AC. The value b obtained by extrapolation of this dependence for

Table 1. The first–order rate constants and thermodynamic parameters for the decomposition of QHCl–AC in solid state

T(K)	10^6 k ± Δk, s^{-1}	–r	n	Statistical evalution ln k_i = f(1/T)	Thermodynamic parameters
Relative humidity, RH = 76.4%					
363	69.66 ± 0.37	0,9963	9	a ± Δa=–16071 ± 960	E_a = 133.62 ± 7.8
353	20.06 ± 8.32	0.9982	12	S_a = 301.9	[kJ · mole $^{-1}$];
348	10.76 ± 1.12	0.9985	11	b ± Δb = 4.46 ± 0.26	$\Delta H^{\neq}$= 133.14 ± 10.4
343	5.032 ± 0.38	0.9931	11	S_b = 0.866	[kJ · mole $^{-1}$];
338	2.722 ± 0.11	0.9965	11	r = –0.9995	$\Delta S^{\neq}$ = –207.8 ± 242 [J · (K^{-1} · $mole^{-1}$)]
Relative humidity, RH = 0%					
373	11.7 ± 0.75	0.9981	9	a ± Δa=–16822 ± 1973	E_a= 139.86 ± 16.4
368	5.76 ± 0.36	0.9965	11	S_a = 620.3	[kJ · $mole^{-1}$];
363	3.95 ± 0.38	0.9928	9	b ± Δb = 33.71 ± 5.43	$\Delta H^{\neq}$ = 137.4 ± 18.8
358	1.80 ± 0.085	0,9961	10	S_b = 1.70	[kJ · $mole^{-1}$];
353	0.869 ± 0.029	0.9986	12	r = –0.9979	$\Delta S^{\neq}$ = 35.36 ± 169 [J · (K^{-1} · mole $^{-1}$)]

Table 2. The effect of humidity on the stability of QHCl–AC in solid state at 363 K

RH%	10^5 k ± Δk, s^{-1}	–r	n	Parameters of regression ln k_i = f(RH%)
25.0	0.315 ± 0.024	0.9951	11	a±Δa = 0.058 ± 0.0086
50.9	0.722 ± 0.072	0.9962	12	S_a = 0.00269
60.5	2.13 ± 0.16	0.9950	11	b±Δb = 14.19 ± 0.50
66.5	3.44 ± 0.33	0.9942	9	S_b = 0.1578
76.4	6.99 ± 0.37	0.9963	9	r = 0.9947

RH = 0% (b = k = 6.833 · $10^{-7}s^{-1}$) is lower than the value k at 363 K under dry air conditions (k = 9.953 · $10^{-6}s^{-1}$). Such results indicate differences in the mechanism of degradation of QHCl–AC occurring in the presence or absence of ambient humidity.

Mechanism of degradation of QHCl – AC in the presence or absence of ambient humidity

Chromatography was performed as described under Experimental.

The following signals on HPLC chromatograms were observed:

- the QHCl–AC signal at t_R = 6 min,
- three signals corresponding to the decomposition of QHCl–AC in the experimental conditions (RH = 76.4%, at 363 K): t_R = about 2.5 min, 3.5 min and 4.5 min,
- two signals corresponding to decomposition of QHCl–AC in a dry atmosphere (RH = 0%, at temp. 373 K): t_R = about 1.5 min and 4.5 min.

Identification of degradation products of QHCl–AC

HPLC–MS method

Mass spectra of the degradation products of QHCl–AC obtained by means of the ESI technique allowed to define their molecular masses (Figure 2):

- degradation products formed at RH = 76.4%, at 363 K; M = 410 (t_R about 14 min; ES^+, *m/z* = 411 and ES^-, *m/z* = 409), M = 420 (t_R about 9 min; ES^+, *m/z* = 421) and M = 392 (t_R about 6 min; ES^+, *m/z* = 393 and ES^-, *m/z* = 391),
- degradation products formed at RH = 0%, at 373 K; M = 420 (t_R about 9 min; ES^+, *m/z* = 421) and M = 452 (t_R about 6.5 min; ES^+, *m/z* = 353 and ES^-, *m/z* = 351).

The analysis of HPLC–MS spectra yielded the following conclusion: the product with M = 420 (ES^+, *m/z* = 421) is a derivative of QHCl–AC – diketopiperazine (DKP), whereas the product with a molecular mass M = 410 (ES^+, *m/z* = 411 and ES^-, *m/z* = 409) is diacid QHCl–AC, quinaprilate (QAT).

It has been assumed that the products with molecular masses M = 392 (ES^+, *m/z* = 393 and ES^-, *m/z* = 391) and M = 452 (ES^+, *m/z* = 353 and ES^-, *m/z* = 351) are formed in consecutive reactions.

To prove this assumption – changes in the P_i/P_{is} (P_i – area of peak QHCl–AC; P_{is} – area of peak internal standard) values for QHCl–AC and for its degradation products obtained in the HPLC procedure were analysed and the formation as well

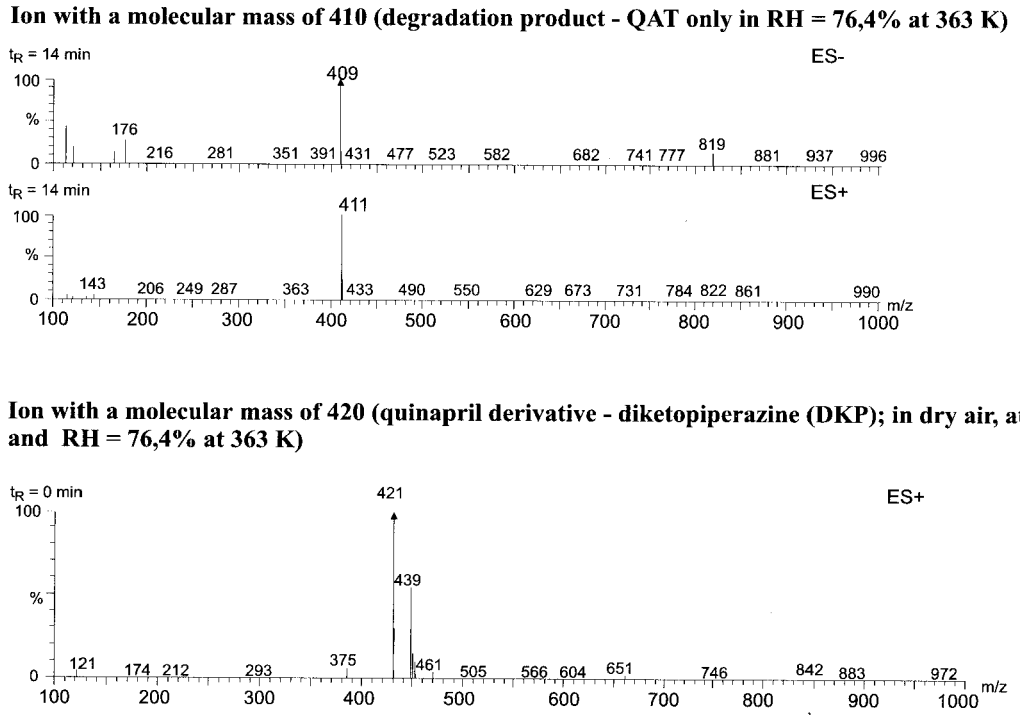


Figure 2. Mass spectra of the degradation products of QHCl–AC

as degradation velocity constants for each product were calculated.

Kinetic interpretation of the decomposition of QHCl–AC in dry air (RH = 0%) at 373 K

Velocity constants for both the formation and degradation of the individual compounds were determined by the least squares method according to the equations:

• degradation of QHCl–AC and derivative quinapril – diketopiperazine (DKP) (Figure 3a, Table 3)

$ln(P_i/P_{is})_{QHCl\ AC} = ln\ (P_0/P_{is})_{QHCl-AC} - k_1 \cdot t$

$ln(P_i/P_{is})_{DKP} = ln\ (P_0/P_{is})_{DKP} - k_2 \cdot t$

time interval $t = t_0 \rightarrow t_{max}$

• formation of the quinapril derivative – diketopiperazine (DKP) (Figure 3b, Table 3)

$ln(P_i' - P_t)_{DKP} = ln\ (P_0' - P_t)_{DKP} - k_1 \cdot t$

$P = P_i/P_{i.is.}$

P' – theoretical values, computed from the equation describing the degradation of DKP for a given time interval $t = t_0 \rightarrow t_{max}$

The velocity constants of QHCl–AC degradation and the formation of quinapril derivative – diketopiperazine (DKP) do not show any statistically significant differences (Table 3). These results indicate that the degradation of QHCl–AC in a dry, (anhydrous) atmosphere proceded in a reaction according to the following Scheme 1.

To prove that the reaction QHCl–AC → DKP is not accompanied by another reaction, we have calculated the value of the ordinate $(P_0')_{DKP}$ for t_0 using the following relationship: $ln\ (P_i/P_{is})_{DKP} = f(t)$, and taking into consideration results from the time intervals $t_{max} \rightarrow t_0$. Next, the theoretical value of $P_{DKP}/P_{is} = f(c_{DKP}, mole \cdot l^{-1})$ was calculated. The calculated value for time $t=0$ $c_{max,\ DKP}$ = 8.419 $\cdot 10^{-4}$ mole $\cdot l^{-1}$ and the value of initial concentration of QHCl–AC at zero time $c_{0,QHCl-AC}$ = 8.431 $\cdot 10^{-4}$ mole $\cdot l^{-1}$ are well comparable and thus indicate that the decomposition of QHCl–AC in a dry (anhydrous) atmosphere (RH = 0%, at 373 K) involves solely the cyclization reaction without involvement of any other process.

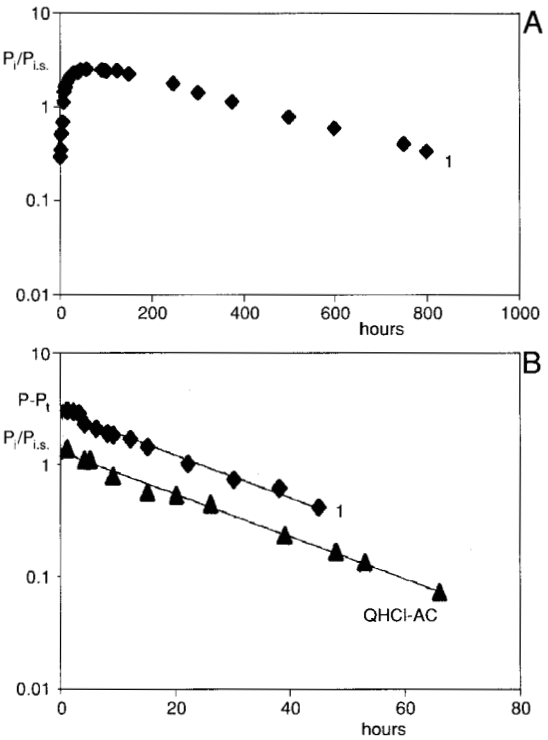


Figure 3. Semilogarithmic plots:
a) $P_i/P_{i.s.}$ = f(t) for derivative QHCl–AC DKP (1)
b) $P_i/P_{i.s.}$ = f(t) for degradation of QHCl–AC and $P_i' - P_t$ = f(t) for formation of derivative QHCl–AC DKP (1) in air dry at temp. 373 K.

Table 3. The apparent first–order rate constants for the degradation of QHCl–AC and the rate constants of formation and degradation of their products

Decomposition terms	Symbol	$k_1 \pm \Delta k_1$ [s^{-1}]	$k_2 \pm \Delta k_2$ [s^{-1}]	$k_3 \pm \Delta k_3$ [s^{-1}]
373K RH = 0%	QHCl [M = 438] DKP [M = 420]	$(6.99 \pm 0.72) \cdot 10^{-5}$ $(1.17 \pm 0.99) \cdot 10^{-5}$	$(7.69 \pm 0.41) \cdot 10^{-7}$	
363K RH = 76%	QHCl [M = 438] DKP [M = 420] QAT [M = 410]	$(6.97 \pm 0.32) \cdot 10^{-5}$ $(6.99 \pm 0.72) \cdot 10^{-5}$ $(6.94 \pm 1.01) \cdot 10^{-5}$	$(4.76 \pm 0.28) \cdot 10^{-7}$	$(1.78 \pm 0.20) \cdot 10^{-6}$

QHCl – Quinapril hydrochloride (mixture of amorphous and crystalline forms)
DKP – derivative QHCl–AC (diketopiperazine)
QAT – quinaprilate (diacid quniapril hydrochloride)

Kinetic interpretation of the decomposition of QHCl–AC in relative humidity RH = 76.4%, at 363 K

Velocity constants for both the formation and degradation of the individual compounds were determined by means of the method of smallest squares, for which the following equations were used:

• degradation of QHCl – AC, quinapril derivative – DKP and quinaprilate – QAT (Figure 4a, Table 3)

$$ln(P_t/P_{is})_{QHCl\text{-}AC} = ln\ (P_0/P_{is})_{QHCl\ AC} - k_1 \cdot t$$
$$ln(P_t/P_{is})_{DKP} = ln\ (P_0/P_{is})_{DKP} - k_2 \cdot t$$
$$ln(P_t/P_{is})_{QAT} = ln\ (P_0/P_{is})_{QAT} - k_3 \cdot t$$

• formation of quinapril derivative – DKP and quinaprilate – QAT (Figure 4b, Table 3)

$$ln(P_i' - P_t)_{DKP} = ln\ (P_0' - P_t)_{DKP} - k_1 \cdot t$$
$$ln(P_i' - P_t)_{QAT} = ln\ (P_0' - P_t)_{QAT} - k_1 \cdot t$$
$$P = P_t/P_{is}$$

P' – theoretical values, computed from the equation describing the degradation of DKP and QAT for a given time interval $t = t_0 \rightarrow t_{max}$

The velocity constants of QHCl–AC degradation and formation of quinapril derivative – DKP and quinaprilate – QAT do not show any statistically significant differences. These results indicate that the degradation of QHCl–AC in RH = 76% and a temperature of 363 K, proceeded in a parallel reaction according to the following Scheme 2.

To show, to what extent the individual reactions participate in the decay of QHCl–AC, I have calculated the ordinate value $(P_0')_{DKP}$ for t_0 using the following relationship: $ln\ (P_t/P_{is})_{DKP} = f(t)$ or $(P_0')_{QAT}$ for t_0 $ln\ (P_t/P_{is})_{QAT}$ = f(t) and taking into consideration results from the time intervals $t_{max} \rightarrow t_0$. Next, the theoretical values of $P_{DKP}/P_{is} = f(c_{DKP}$, mole · l^{-1}) and $P_{QAT}/P_{is} = f(c_{QAT}$, mole · l^{-1}) were calculated which amount to $c_{max.\ QAT} = 5.019 \cdot 10^{-4}$ mole · l^{-1}, $c_{max.\ DKP} = 3.328 \cdot 10^{-4}$ mole · l^{-1} (initial concentration value of QHCl–AC at zero time $c_{0,QHCl\text{-}AC} = 8.431 \cdot 10^{-4}$ mole · l^{-1}). The obtained values indicate that the decomposition of QHCl–AC under conditions of increased relative moisture (RH = 76.4%) and temperature (363 K) involves hydrolysis of the ester bond and the cyclization reaction. The share of each of those processes is 59.53% and 39.47%, respectively.

CONCLUSIONS

1. In a dry atmosphere, the degradation of QHCl–AC conducts to quinapril derivative – dike-

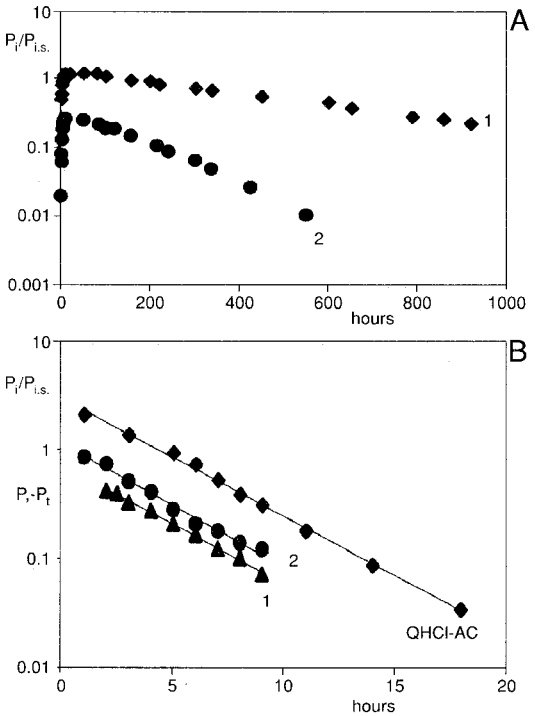
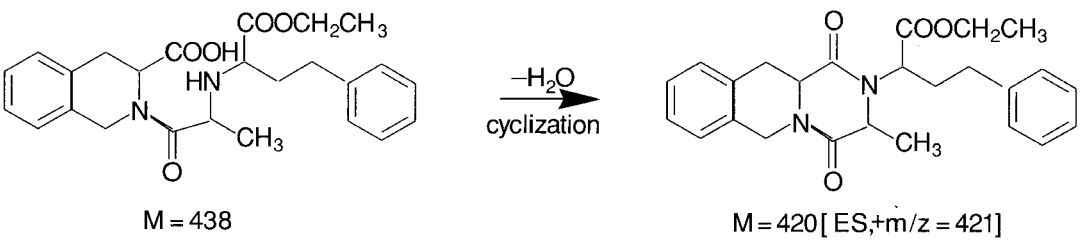
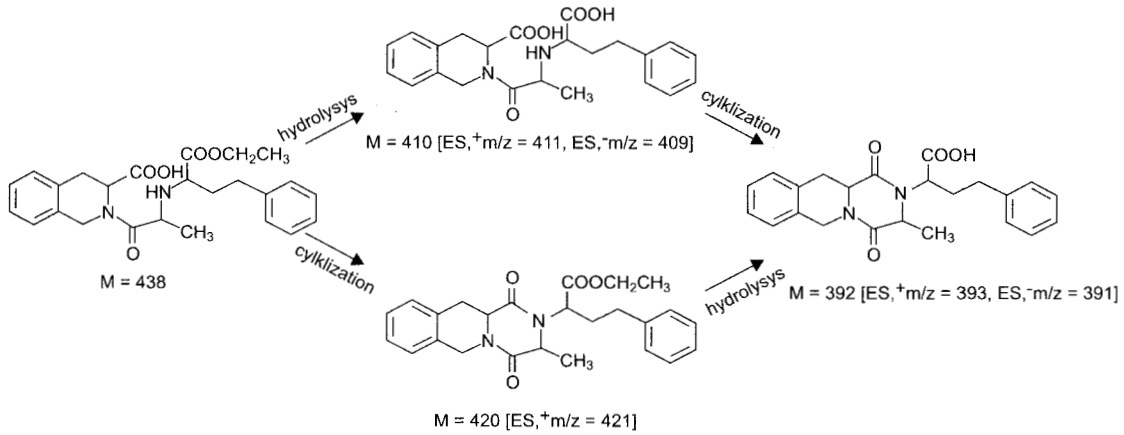


Figure 4. Semilogarithmic plots:
a) $P_i/P_{i.s.}$ = f(t) for DKP (1), QAT (2)
b) $P_i/P_{i.s.}$ = f(t) for degradation of QHCl–AC and $P_i' - P_i$ = f(t) for formation of derivative QHCl–AC DKP (1) and QAT (2) at relative humidity (~76%) at 363 K.



Scheme 1. Degradation of QHCl–AC in dry air, at 373 K.



Scheme 2. Degradation of QHCl–AC under conditions of increased relative moisture (RH + 76.4%) at 363 K.

topiperazine (at temperatures ranging from 343 to 373 K) and proceeds according to the first order reaction in the case with QHCl in amorphous form only. The cyclization product – diketopiperazine undergoes further degradative processes.

2. Further studies have shown that in a dry hot atmosphere the cyclization reaction of QHCl–AC is not accompanied by any other parallel chemical process and the reaction proceeds with a 100% yield.

3. The activation energy of cyclization of QHCl in dry air, for a mixture of amorphous and crystalline forms does not show any significant differences compared with the respective activation energy for QHCl when in amorphous form solely (4).

4. In the presence of moisture, the decomposition of QHCl–AC proceeds with involvement of two parallel reactions – the hydrolysis, yielding quinaprilate and cyclization – yielding a DKP derivative. The yields of these reactions is as follows: 59.53% for the hydrolysis and 39.47% for the cyclization.

5. Quinaprilate – the product of hydrolysis also undergoes cyclization to yield a product with a molecular ion mass of M = 392, whereas the product of cyclization DKP may be hydrolyzed to yield a compound with a molecular ion mass M = 392 (Scheme 2).

Acknowledgements

I wish to thank the company P.P.H.U. BIO-FARM Ltd. for supplying quinapril hydrochloride pure substance.

REFERENCES

1. Zając M., Pawełczyk E.: in Chemia leków, Medical Academy, Poznań 2000.
2. Podlewski J.K., Chwalibogowska–Podlewska A.: Quinapril: in Leki Współczesnej Terapii (Ed. 14), p. 552, Wydawnictwo Fundacji Buchnera, Warszawa, 1999.
3. Pęczkowska M.: in Terapia i Leki 4–5, 97 (1997).
4. Guo Y., Byrn S.R., Zografi G.: J. Pharm. Sci 89, 128 (2000).
5. Guo Y., Byrn S.R., Zografi G.: Pharm. Res. 17, 930 (2000).
6. Li J., Guo Y., Zografi G.: J. Pharm. Sci 91, 229 (2002).
7. We G.C., Siroi M.G., Qu R., Liu P., Roulea J.L.: Cardiovasc. Drugs. Ther. 16, 29 (2002).
8. Barthelemy C., Eurin J., Lechat P., Masson F., Cortines M., Mougenot N., Soualmia H., Carayon A.: Peptides 23, 1161 (2002).
9. Ruiz O.M., Ruperez M., Lorenzo O., Esteban V., Blanco J., Mezzano S., Egido J.: Int. Suppl. 82, 12 (2002).
10. Sakata K., Pawlak R., Urano T., Takada A.: Pathophysiol. Haemost. Thromb. 32, 67 (2002).
11. Stanisz B.: Pharmazie 58, 249 (2003).

Received: 6.05.2003