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CILAZAPRIL STABILITY IN THE PRESENCE OF HYDROCHLOROTHIAZIDE IN MODEL MIXTURES AND FIXED DOSE COMBINATION

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Abstract: The presented study aimed at the evaluation of hydrochlorothiazide influence on cilazapril stability in model mixture and fixed dose tablet formulation. The degradation of cilazapril in the presence of hydrochlorothiazide took place according to autocatalytic reaction kinetic mechanism, described mathematically by Prout-Tompkins equation. Hydrochlorothiazide coexistence with cilazapril in model mixture and fixed dose tablet without blister package accelerated cilazapril degradation in comparison with degradation of cilazapril substance. Values of reaction induction time shortened, while those of observed reaction rate constant increased. Increasing values of relative humidity and temperature have negative impact on cilazapril stability. Determined semi-logarithmic relationships: ln k = f(RH) and Arrhenius ln k = f(1/T) are linear and are cilazapril stability predictive. The blister (OPA/Alu/PVC//Alu) package of fixed dose tablets, constitutes absolute moisture protection and prevent cilazapril – hydrochlorothiazide interaction occurrence.

Keywords: cilazapril, hydrochlorothiazide, incompatibility, fixed dose combination

Uncontrolled hypertension, which is a clinical problem of great importance, can be treated by means of combination therapy. Combination of two or more antihypertensive drugs, in comparison to monotherapy, provides more effective blood pressure reduction, with limited side effects occurrence. One of possible preferred combinations in hypertension treatment constitutes angiotensin converting enzyme inhibitor (cilazapril) with thiazide diuretic (hydrochlorothiazide) (1, 2).

In many cases during the combination therapy, fixed dose pharmaceutical formulations are applied. They benefit in better compliance, while in one drug two pharmaceutically active ingredients are administered (3). Nevertheless, what is undoubtedly advantageous, from biological point of view can cause problems, while preparing stable pharmaceutical fixed dosage form. Coexistence of different pharmaceutically active ingredients may contribute to interactions or incompatibilities occurrence between them causing destabilization (4).

Cilazapril (Fig. 1) is (5*S*,8*S*)-5-{[(1*S*)-1-ethoxycarbonyl-3-phenyl-propyl]amino}-6-oxo-1,7-diazabicyclo[5.4.0]undecane-8-carboxylic acid

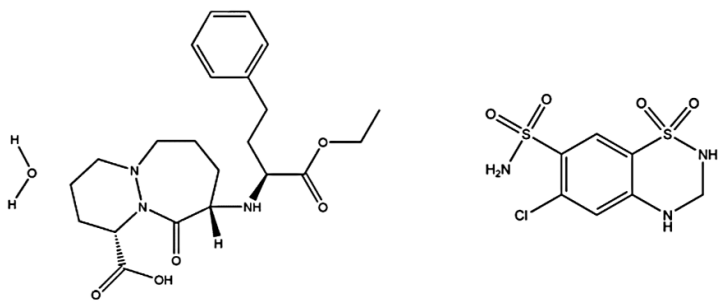

Figure 1. Chemical structures of A – cilazapril and B – hydrochlorothiazide

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monohydrate (5). Biologically its active form (cilazaprilat) prevents conversion of angiotensin I into angiotensin II leading to reduction in blood pressure values (6, 7).

Hydrochlorothiazide (Fig. 1) is 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide-1,1-dioxide (5). Its therapeutic action as a diuretic is owing to inhibiting sodium reabsorption in the renal tubules causing the increase in urinary excretion of water (8).

From chemical stability point of view it was reported that hydrochlorothiazide substance is stable (degradation was not observed) for five years, when stored at room temperature (9). On the other hand, cilazapril is unstable substance, what was proved in previous authors' studies. Cilazapril stability is negatively affected by environmental factors such as temperature and relative humidity and also excipients (hypromellose, lactose monohydrate and talc) (10, 11).

Taking into account the facts mentioned above, studying chemical influence of hydrochlorothiazide on cilazapril stability seems to be crucial, especially as no report about this issue is to be found in worldwide literature. Therefore, this study aimed at the evaluation of hydrochlorothiazide influence on cilazapril stability in model mixture and commercial fixed dose tablets.

EXPERIMENTAL

Materials

Cilazapril substance was kindly supplied by Biofarm (Poland) (batch number 1621816). Hydrochlorothiazide substance was purchased from Sigma-Aldrich (Germany). Pharmaceutical fixed dose tablet formulations of cilazapril 5 mg and hydrochlorothiazide 12.5 mg were purchased from Cefarm (serial number: E0072E2). Methanol and acetonitrile were HPLC grade (Merck, Germany). All other chemicals were of analytical reagent grade. Potassium phosphate monobasic, sodium bromide, sodium nitrate and sodium chloride were purchased from POCh (Poland). Oxymetazoline hydrochloride was purchased from Sigma-Aldrich (Germany). The distilled water was used throughout the study.

Method

Cilazapril quantitative determination was conducted by means of validated, cilazapril stability indicating HPLC method (12). The Shimadzu HPLC system was equipped with: Shimadzu LC-6A liquid chromatograph pump with 7725 Rheodyne valve injector (20 µL fixed loop), Shimadzu SPD-6AV UV-VIS spectrophotometric detector and Shimadzu C-R6A Chromatopac integrator. The column: LiChroCART® 250-4 HPLC-Cradridge, LiChrospher® 100 RP-18 (5 µm) (Merck, Germany) served as the stationary phase. The mobile phase consisted of acetonitrile-methanol-phosphate buffer (pH 2.0) (60 : 10 : 30, v/v/v). The flow rate of mobile phase was 1.0 mL/min, column worked at ambient temperature and the injection volume was 20 µL. The detector wavelength was set at 212 nm.

Procedures

Cilazapril-hydrochlorothiazide model mixture

A model mixture in ratio 5 : 12.5 (w/w) of cilazapril substance with hydrochlorothiazide was prepared in mortar (45 min long micronization). The 5 : 12.5 (w/w) ratio was selected, while cilazapril and hydrochlorothiazide coexist in that proportion in fixed dose tablet formulation.

Samples for kinetic study were consisted of 20.00 mg accurately weighed model mixture powder placed in amber glass, uncapped vials. The samples were exposed to the conditions of kinetic study T = 343 K and various relative humidity levels RH: 50.9, 66.5 and 76.4%.

Tablets containing cilazapril and hydrochlorothiazide (fixed dose)

Each sample consisted of two fixed dose tablets, which were put in amber glass, uncapped vials. Simultaneously, under each isothermal kinetic condition, two series of samples were put: one containing tablets without blister and the second with tablets in blister package. The prepared samples were exposed to various temperatures T = 323-343 K and constant relative humidity RH = 76.4%.

Samples for HPLC analysis

Each 20 mg model mixture sample or fixed dose tablet was transferred quantitatively into 50 mL volumetric flask, afterwards methanol in volume of 25.0 mL was added. Prepared suspensions were shaken for about 15 min and filtered - quantitative filter (390, Munktell). Finally, 1.0 mL of clear supernatants were moved into vials and after mixing with 0.5 mL of oxymetazoline hydrochloride internal standard methanolic solution (0.2 mg/mL) and vortexing (0.5 min), obtained homogenous solutions were subjected to quantitative HPLC analysis.

Isothermal test conditions

Isothermal test protocol was applied in this study to shorten time necessary to conduct stability

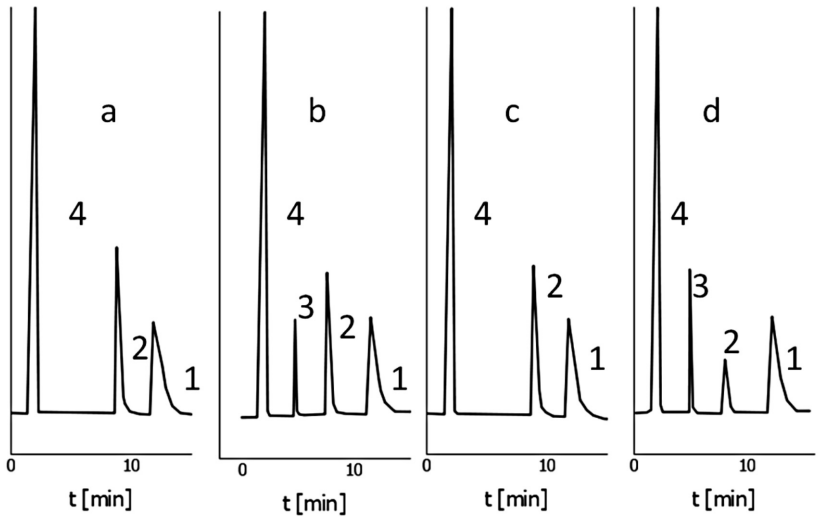

Figure 2. HPLC chromatograms of (a, b) cilazapril (2) – hydrochlorothiazide (4) model mixture and (c, d) cilazapril – hydrochlorothiazide fixed dose tablet before (a, c) and during (b, d) kinetic test, where (3) is a product of decomposition and (1) internal standard

analysis. Heat chambers (with the temperature control accuracy ± 1.0 K) guaranteed established isothermal test temperature. Proper relative humidity environments were obtained in closed desiccators that contain saturated solutions of inorganic salts (RH = 50.9% sodium bromide, RH = 66.5% sodium nitrate and RH = 76.4% sodium chloride). The equilibrate test conditions were established 24 h before the beginning of the study.

Samples were withdrawn from desiccators and heat chambers after predetermined incubation time and subsequently analyzed.

RESULTS AND DISCUSSION

HPLC method for analysis

The HPLC method with UV detection (12) applied in this study was found to be suitable for the determination of cilazapril in the presence of hydrochlorothiazide under isothermal kinetic test conditions. The method selectivity towards cilazapril in the presence of hydrochlorothiazide, degradation product and components of fixed dose pharmaceutical formulation was proved. Chromatograms consisted of regular, sharp and well-separated peaks characterized by retention times: 2.3, 3.1 and 8.9 min, which matched: hydrochlorothiazide, product of decomposition and cilazapril, respectively. The internal standard used throughout the study was oxymetazoline hydrochloride, with retention time 12.6 min (Fig. 2).

Cilazapril degradation in the presence of hydrochlorothiazide in model mixture as well as in tablet dosage form progressed according to Prout-Tompkins mathematical degradation model, which indicates an autocatalytic reaction (13, 14). Kinetic parameters and half time of decomposition ($t_{0.5}$) were calculated on basis of Prout-Tompkins equation:

$$\frac{\ln c}{c_0 - c} = -k \cdot t + C \qquad (1)$$

where c_0 and c are cilazapril concentrations: initial and at time t of a kinetic study, respectively, k signifies the observed reaction rate constant and C – the constant related to reaction induction period.

The same kind of degradation (equal retention time) was observed, in pure cilazapril substance. In the authors' previous study, it was established by qualitative IR and HPLC-MS methods, that cilazaprilat is the degradation product (10). According to those findings, it could be stated, that also in the presence of hydrochlorothiazide cilazapril is decomposed by deestrification forming cilazaprilat. Such degradation has negative implications on drug bioavailability, while cilazaprilat, although biologically active, cannot be absorbed from gastrointestinal tract (15).

Cilazapril – hydrochlorothiazide model mixture

Model mixture of cilazapril and hydrochlorothiazide was in ratio 5 : 12.5 (w/w), because such proportions are administered during hypertension combined therapy (16).

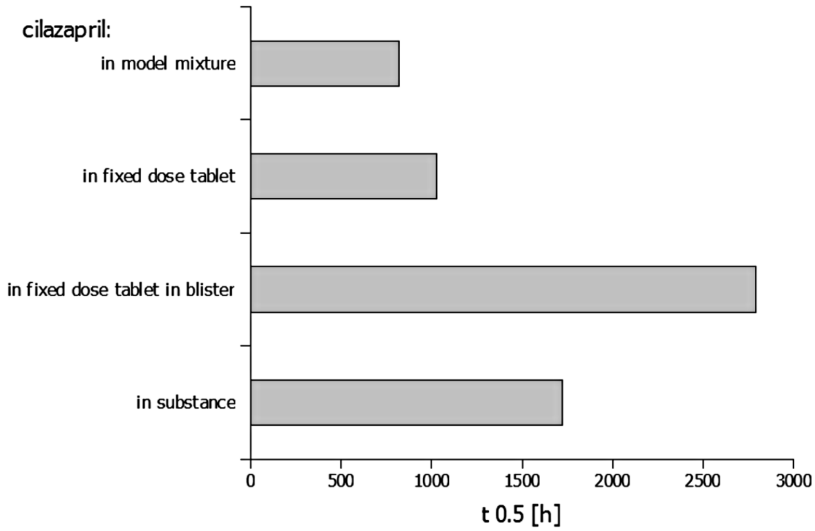

Figure 3. Comparison of half time of cilazapril decomposition in substance (10) and in the presence of hydrochlorothiazide in model mixture, tablet dosage form with and without blister package (T = 343 K, RH = 76.4 %)

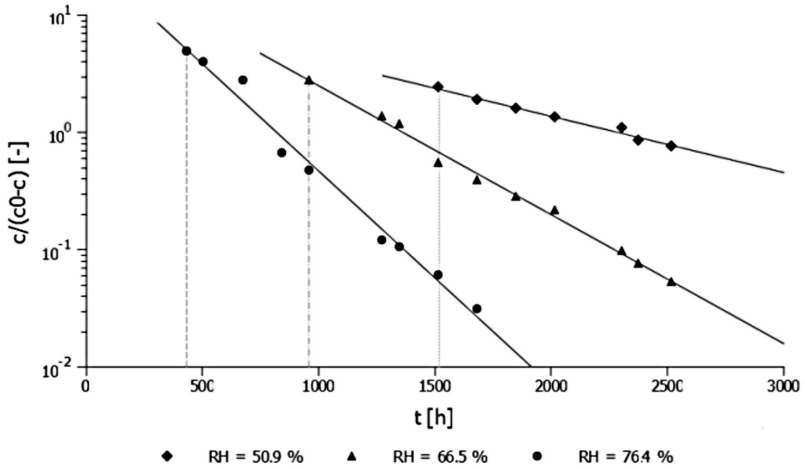

Figure 4. Semi-logarithmic plots of cilazapril decomposition in the presence of hydrochlorothiazide in the model mixture according to Prout-Tompkins relationship in isothermal kinetic study: T = 343 K, RH = 50.9, 66.5, 76.4 %. The points respond the experimental data, lines were calculated from Eq. 1 and dashed lines indicate the end of reaction induction time

The stability study results of cilazapril in substance and in the presence of hydrochlorothiazide comparison suggest that this drug combination in model mixture deteriorate the stability of cilazapril. Figure 3 shows differences in time of half decomposition, which in case of model mixture is lower than that of pure cilazapril substance. The consequence of those substances coexistence is cilazapril degradation acceleration, which is expressed in kinetic parameters values change: induction time and observed reaction rate constant. Those differences were statistically significant (Snedecor F-test, α = 0.05). Basing on above, it can be concluded that hydrochlorothiazide act as cilazapril degradation reaction accelerating agent (catalyst). The chemical reaction between those substances does not occur, while degradation product is the same as in case of cilazapril substance (10).

Table 1. The influence of relative humidity (RH) on cilazapril stability, while coexisting with hydrochlorothiazide in the model mixture (T = 343 K).

RH [%]	50.9	66.5	76.4
k [s^{-1}]	$3.06 \cdot 10^{-7} \pm 4.85 \cdot 10^{-8}$	$7.03 \cdot 10^{-7} \pm 4.87 \cdot 10^{-8}$	$1.17 \cdot 10^{-6} \pm 1.21 \cdot 10^{-7}$
$\ln k = a\ RH\% + b$			
a	0.0525 ± 0.0071	Sa	0.00056
b	-17.6690 ± 0.4671	Sb	0.0368
r	1.000		

Table 2. The influence of temperature on cilazapril stability in fixed dose combination tablet with hydrochlorothiazide with respect to the presence and lack of blister package (RH = 76.4%) with corresponding Arrhenius and thermodynamic parameters.

Tablet without blister				
T [K]	328	333	338	343
k [s^{-1}]	$7.09 \cdot 10^{-8}$	$1.89 \cdot 10^{-7}$	$6.74 \cdot 10^{-7}$	$1.16 \cdot 10^{-6}$
Δk [s^{-1}]	$4.29 \cdot 10^{-9}$	$2.40 \cdot 10^{-8}$	$1.31 \cdot 10^{-7}$	$1.97 \cdot 10^{-7}$
$\ln k = a \cdot 1/T + b$				
a	-21769.23 ± 9025.40	E_a [kJ/mol]	181.00 ± 75.04	
b	49.95 ± 26.91	ln$A$ [-]	49.95 ± 26.91	
Sa	2097.47	$\Delta H^{\neq}$ [kJ/mol]	178.52 ±77.52	
Sb	6.25	ΔS [J/(K mol)]	170.38 ± 21.16	
r	-0.0991			
Tablet in blister				
T [K]	328	333	338	343
k [s^{-1}]		$8.53 \cdot 10^{-8}$	$1.63 \cdot 10^{-7}$	$3.15 \cdot 10^{-7}$
Δk [s^{-1}] -*		$9.59 \cdot 10^{-9}$	$2.63 \cdot 10^{-8}$	$4.83 \cdot 10^{-8}$
$\ln k = a \cdot 1/T + b$				
a	-14903.02 ± 2324.71	E_a [kJ/mol]	123.91 ± 19.33	
b	28.47 ± 6.88	ln$A$ [-]	28.47 ± 6.88	
Sa	182.96	$\Delta H^{\neq}$ [kJ/mol]	121.43 ± 21.81	
Sb	0.54	ΔS [J/(K mol)]	-8.17 ± 187.72	
r	-1.000			

*only induction time was observed

Cilazapril degradation in the model mixture with hydrochlorothiazide was also influenced by relative humidity conditions. An increase of relative humidity under constant temperature caused changes in kinetic reaction parameters values: induction time shortened and observed reaction rate value rose (Fig. 4).

Observed reaction rates constant values changes while increasing relative humidity levels proceeds in semi-logarithmic manner:

$$\ln k = a \cdot RH + b \quad (2)$$

where a is a plot slope and b is an intercept. This relationship correlation is high, while coefficient of correlation (r) equals 1.000 (Table 1).

Cilazapril – hydrochlorothiazide fixed dose tablet

In fixed dose tablet, besides hydrochlorothiazide, also the excipients coexist with cilazapril. Each tablet component can affect cilazapril stability. As it is presented in Figure 3, half decomposition time of cilazapril in tablet with hydrochlorothiazide without blister package is slightly longer than that

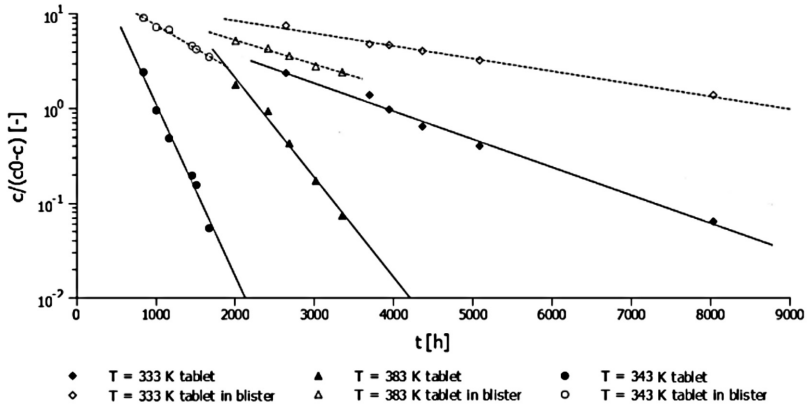

Figure 5. Semi-logarithmic plots of cilazapril decomposition in the presence of hydrochlorothiazide in fixed dose tablet (with and without blister package) according to Prout-Tompkins relationship in isothermal kinetic study: T = 333, 338, 343 K, RH = 76.4 %. The points respond the experimental data, but lines were calculated from Eq. 1

observed in the model mixture. This signifies that when mixed with excipients, interaction between cilazapril and hydrochlorothiazide is weaker. Nevertheless, cilazapril stability in fixed dose tablet formulation without blister is still lower than that of pure cilazapril substance. Kinetic parameters values change: induction time and observed reaction rate constant differ statistically (Snedecor F-test, α = 0.05). The greatest stability is assured by the fixed dose tablet package (blister: oriented polyamide (OPA) / aluminum (Alu) / polyvinyl chloride (PVC) // Alu). Since the well-quality OPA/Alu/PVC//Alu blister constitutes absolute protection against environmental relative humidity (17), it may be concluded that probably only in the presence of moisture interaction of cilazapril with hydrochlorothiazide occurs.

The increasing temperature (T = 323–343 K) under conditions of constant relative humidity RH = 76.4% was the factor that negatively affected cilazapril stability in fixed combination dosage form. Induction time value decreased and observed reaction rate increased. The blister package is of great importance for cilazapril stability, while significantly slows the degradation process (Fig. 5). Though, cilazapril – hydrochlorothiazide fixed dose tablets should be strictly stored in blister package under conditions of low surrounding temperature and humidity.

Observed reaction rate constants calculated from experimental data on basis of Eq. 1 depend on temperature in characteristic semi-logarithmic way according to Arrhenius relationship:

$$\ln k = a \cdot \frac{1}{T} + \ln A \qquad (3)$$

Activation energy was calculated from equation $E_a = - a \times R$, enthalpy $\Delta H = E_a - R \times T$, entropy $\Delta S^{\neq} = R\,(\ln A - \ln[k_B \times T/h])$, where R is universal gas constant, T stands for temperature (K), h - Planck constant, k_B - Boltzman constant and A is the frequency coefficient of Arrhenius equation. (Table 2).

CONCLUSIONS

Cilazapril stability is affected by the presence of hydrochlorothiazide in model mixture and tablet fixed dose form. The interaction occurs in form of the acceleration of cilazapril degradation reaction: shortening of the reaction induction time, as well as an increase of the observed reaction rate constant. It was also established that elevation of temperature and relative humidity values contributes to further cilazapril destabilization, though cilazapril-hydrochlorothiazide pharmaceutical formulations should be stored in dry and low temperature environmental conditions.

It was also established that OPA/Alu/PVC//Alu blister constitutes protection against cilazapril decomposition in the presence of hydrochlorothiazide. Therefore, it can be hypothesized that moisture in air is crucial factor for cilazapril – hydrochlorothiazide interaction.

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