Acta Poloniae Pharmaceutica – Drug Research, Vol. 66 No. 5 pp. 563–569, 2009 ISSN 0001-6837
Polish Pharmaceutical Society

STABILITY OF THE CRYSTALLINE FORM OF CEFACLOR MONOHYDRATE AND ITS PHARMACEUTICAL PREPARATIONS

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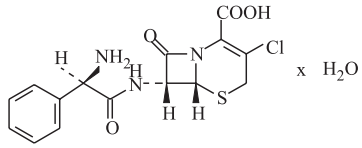
Abstract: The influence of temperature and relative air humidity on the stability of cefaclor monohydrate in crystalline form and in its pharmaceutical preparations (oral suspension and slow release tablets) was investigated. The process of degradation was studied by using high-performance liquid chromatography with ultraviolet (UV) detection, as described in the monograph of cefaclor in European Pharmacopoeia. The degradation of cefaclor monohydrate in substance, in oral suspension and tablets at relative air humidity RH > 50% is a first-order autocatalytic reaction relative to substrate concentration, while at 0% RH the degradation of cefaclor in substance is a first-order reaction relative to substrate concentration. The kinetic and thermodynamic parameters of degradation were calculated.

Keywords: cefaclor monohydrate, stability in solid phase, thermodynamic parameters, tablets, oral suspension

Cefaclor monohydrate is an oral, a semisynthetic second-generation cephalosporin antibiotic. This antibiotic is active against a wide range of Gram-positive and Gram-negative organisms, such as *Staphylococcus aureus*, *Streptococcus pneumonia*, *Haemophilus influenza*, *E. coli*, and many others and it is resistant to most β-lactamases (1-3).



Cefaclor is administered as capsules containing 250 or 500 mg of cefaclor monohydrate and as slow cefaclor tablets containing 375, 500 or 750 mg of cefaclor monohydrate. The form of oral suspension dosage for pediatric purposes is available as granules or powder in multidose and sachets. The constitution gives a suspension containing 125, 250 or 375 mg of cefaclor monohydrate.

Chromatographic methods (HPTLC, HPLC) (4-7) and spectrophotometric methods (UV, IR and ^{1}H-NMR) (5, 8-10) for the determination of cefaclor have been reported in the literature. The stability of cefaclor in aqueous solutions in a pH range of 1-12 and its stability in body fluids have also been described (11). In studies of the solid state of cefaclor many products of its degradation have been isolated and identified and the mechanisms of degradation relative to storage conditions have been established (8-10). The aim of the present study was to determine the effect of temperature at 76.4% RH and relative air humidity on the stability of cefaclor monohydrate in substance and in oral suspension and on the stability of cefaclor in slow release tablets under stress storage conditions.

EXPERIMENTAL

Materials and reagents

The crystalline form of cefaclor monohydrate was obtained from the Institute of Biotechnology and Antibiotics in Warsaw. The oral suspension CECLOR and tablets CECLOR MR were products of Polfa Kutno S.A. in Kutno. Salicylamide (conforming to FP VII) was used as an internal standard. All other chemical substances and solvents were the products of Sigma and were of analytical or high-performance liquid chromatographic grade.

Equipment

The chromatographic apparatus consisted of an LC-6A isocratic pump, a Rheodyne 7120 injector

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with a 50 μL loop, and an SPD-6AV UV-Vis detector set at 265 nm (all Shimadzu products). Separations was performed on a LiChrospher 100 RP-18 column (250 × 4 mm, 5 mm particle size; E. Merck).

Chromatographic conditions

The method applied was a modification of the procedure presented in the European Pharmacopoeia VI for cefaclor. In this study an internal standard (salicylamide) was used to determine cefaclor in the crystalline form and in its pharmaceutical preparations.

The mobile phase consisted of a mixture of 30 volumes of methanol and 70 volumes of a solution containing 1.0 g of sodium pentasulfonate and 10 mL of triethylamine adjusted to pH 2.5 with phosphoric acid(V) (1.42 kg/L)). The flow rate was 1.2 mL/min. The internal standard was a solution of salicylamide (1.0 mg/mL) in a mixture (3:7) of methanol and water. All chromatographic procedures were conducted at ambient temperature.

Validation of the HPLC method

The HPLC method was validated according to International Conference on Harmonisation Guidelines for Validation of Analytical Procedures (12).

Selectivity

The selectivity of the liquid chromatography method was examined for non-degraded and degraded samples. For the validation test the following substances were used: a comparative cefaclor monohydrate sample, a sample of cefaclor incubated in dry air at 373 K and a sample of cefaclor incubated at increased relative air humidity (76.4%), at 343 K.

Linearity

Calibration curves for HPLC analysis were determined by linear regression. The linearity between P/P_{IS} (P and P_{IS} – areas of cefaclor and internal standard) and the concentrations of cefaclor in a mixture of methanol and water (7:3), ranging from 0.2 to 0.6 mg/mL, was evaluated. To 1.0 mL of cefaclor solution 1.0 mL of internal standard solution was added and the so obtained solutions were analyzed. Fifty μL samples of these solutions were injected onto the column.

Precision

The precision of the method is expressed as the relative standard deviation (RSD) of replicate measurements. In order to evaluate the repeatability of the method (intra-day), eight samples of three different concentrations (low, c = 0.32 mg/mL; medium, c = 0.40 mg/mL; high, c = 0.48 mg/mL) were prepared and analyzed on the same day. The intermediate precision (intra-day) was studied by comparing the assays performed on two different days at a cefaclor concentration of 0.40 mg/mL.

Detection and quantitation limits

The LOD and LOQ parameters were determined from the regression equation, where: LOD = 3.3 S_y/a, LOQ = 10 S_y/a; S_y is a standard deviation and a is the slope of the calibration curve.

The conditions of kinetics studies

For the experiments 10 mg samples of cefaclor monohydrate and 40 mg samples of the oral suspension CEFACLOR (4.707 mg of cefaclor) were weighed into 5 mL vials. The samples of cefaclor monohydrate and the oral suspension tested for the influence of temperature in a humid environment were inserted in desiccators containing saturated solutions of sodium chloride 76.4% RH. The samples of cefaclor monohydrate were placed in heat chambers set to the desired temperatures: 333, 343,

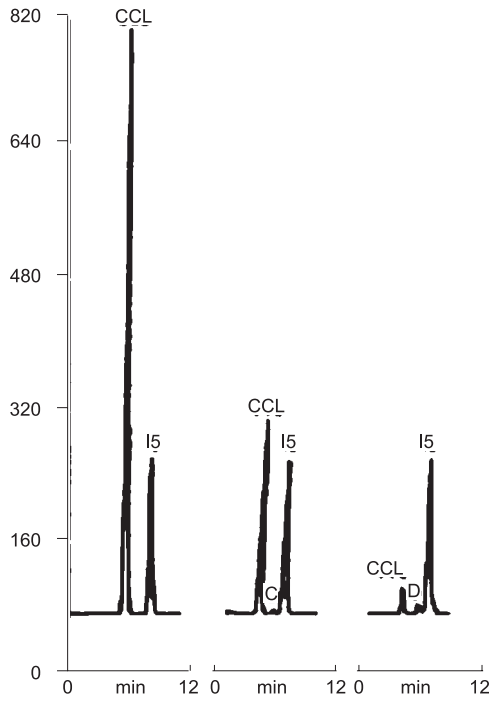

Figure 1. HPLC chromatograms of cefaclor (CCL), its degradation product (D) and internal standard (IS) after incubation at 363 K (RH = 76.4%): I at t = 0 h; II at t = 22 h; III at t = 30 h. Chromatographic conditions are described in the text.

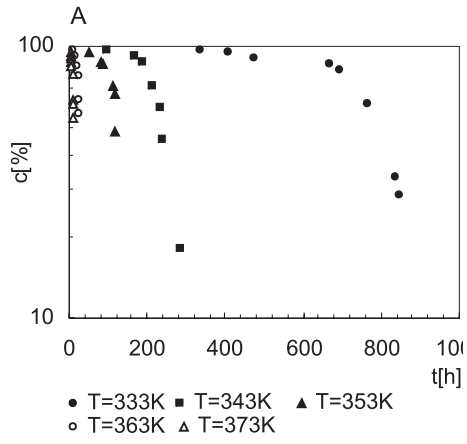

Figure 2. Semilogarythmic plots of ln c = f(t) for the degradation of cefaclor (A) and CECLOR (the oral suspension) (B) at different temperatures at RH = 76.4%.

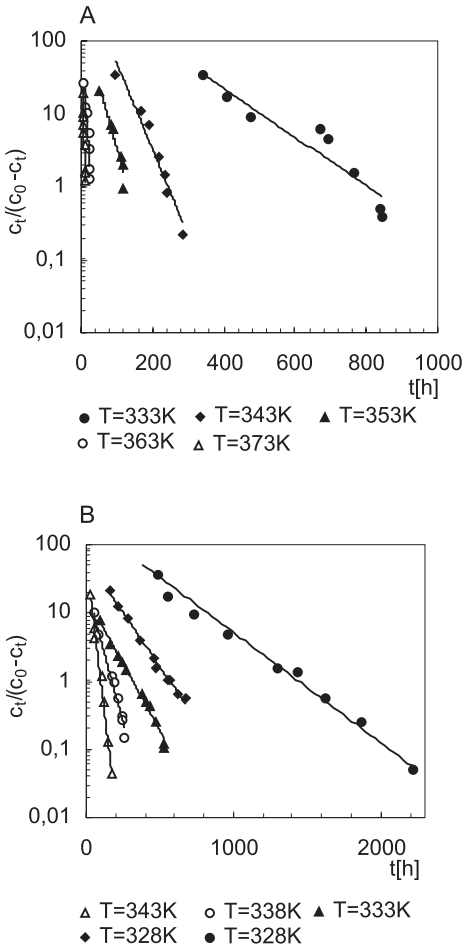

Figure 3. Semilogarythmic plots of ln $c_t/(c_0-c_t)$ = f(t) for the degradation of cefaclor (A) and CECLOR (the oral suspension) (B) at different temperatures at RH = 76.4%.

353, 363 and 373 K. The samples of the suspension were stored in heat chambers at 323, 328, 333, 338 and 343 K. In order to examine the influence of relative air humidity, the samples of cefaclor monohydrate and the oral suspension were placed in desiccators containing saturated solutions of inorganic salts which ensured the desired relative humidity of the ambient air (13) (sodium bromide – 50.9% RH, potassium iodide – 60.5% RH, sodium nitrate – 66.5% RH, sodium chloride – 76.4% RH and zinc sulfate – 90.0% RH), and inserted in a heat chambers set to 343 K.

To evaluate the stability of cefaclor monohydrate in dray air, the vials containing 10 mg of cefaclor monohydrate were immersed in sand bath placed in a heat chamber at 373 K.

Each batch to be studied comprised 10-15 samples. At specific time intervals, determined by the rate of degradation, the vials were removed, cooled to room temperature and the contents dissolved in a mixture (3:7) of methanol and water. The so obtained solutions were quantitatively transferred into measuring flasks and completed to a total volume of 25.0 mL with the same mixture of solvents. To 1.0 mL of the so obtained solution (after filtration) 1.0 mL of the internal standard solution was added. Fifty µL samples were injected onto the column. The concentration (%) of cefaclor relative to the comparative solution was calculated.

In order to determine the stability of cefaclor in slow release tablets, the tablets (CEFACLOR MR), they were stored in and without their commercial

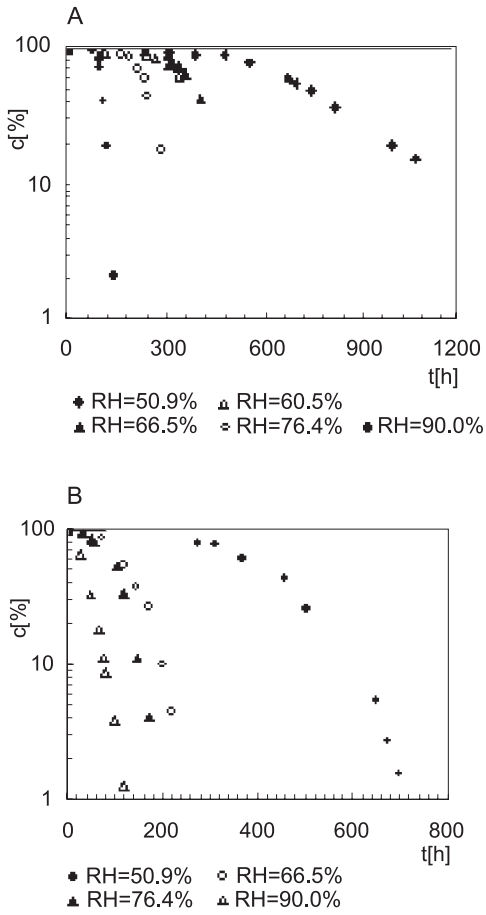

Figure 4. Semilogarithmic plots of ln c = f(t) for the degradation of cefaclor (A) and CECLOR (the oral suspension) (B) at 343K and various relative air humidity.

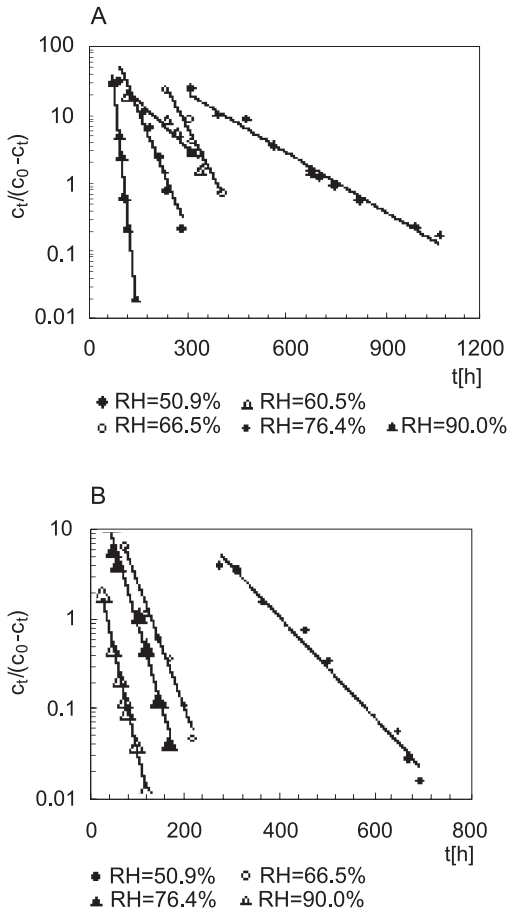

Figure 5. Semilogarithmic plots of ln $c_t/(c_0-c_t)$ = f(t) for the degradation of cefaclor (A) and CECLOR (the oral suspension) (B) at 343K and various relative air humidity.

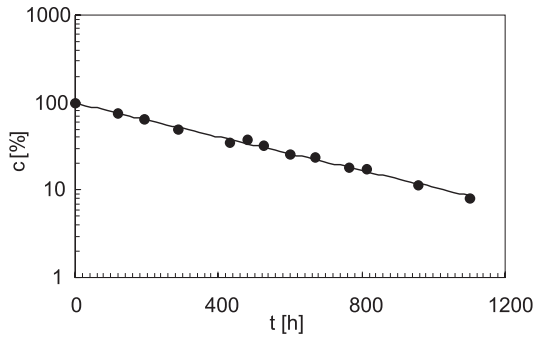

Figure 6. Semilogarithmic plot of ln c = f(t) for the degradation of cefaclor in solid phase at 373 K and RH = 0%.

package, at 76.4% RH. The tablets in the commercial package were stored at 333 K and those without it at 318 K. Depending on the rate of cefaclor degradation, the tablets were removed from the desiccators and triturated after cooling down to room temperature. Ten mg of the so obtained powder was weighed (6.848 mg of cefaclor) into 25.0 mL measuring flasks. The subsequent procedure was the same as for the cefaclor in substance and in oral suspension.

RESULTS AND DISCUSSION

Changes in the concentration of cefaclor monohydrate under the conditions of the study were evaluated using the HPLC method presented in the European Pharmacopoeia VI for cefaclor and modified for this study. It was validated with respect to selectivity, precision and linearity. Detection and quantitation limits were also determined.

The HPLC method was found selective for the determination of cefaclor in the presence of its

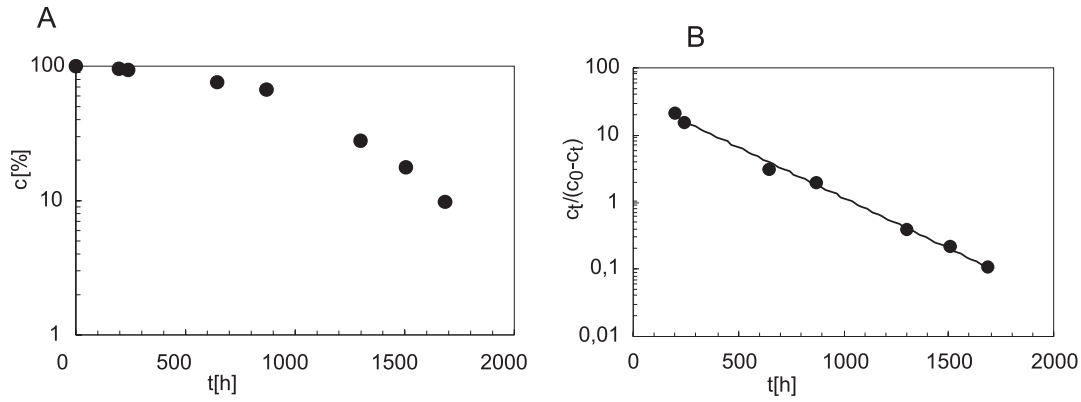

Figure 7. Semilogarithmic plots of ln c = f(t) (A) and ln $c_t/(c_0-c_t)$ = f(t) (B) for the degradation of CECLOR MR – slow tablets without their commercial pack at 333K and RH = 76.4%.

degradation products and the internal standard, as shown in Figure 1. Cefaclor formed a symmetrical peak, clearly separated form the peak of degradation products and that of the internal standard. In the chromatograms taken over a period of 0 to 12 min, the following peaks emerged: peak A, corresponding to cefaclor, with retention time of approx. 6.30 min; peak IS, corresponding to the internal standard, with a retention time of approx. 9.24 min and peak B, corresponding to the degradation product, with a retention time of approx. 8.08 min.

The linearity of the method was obtained between the areas of the peaks and the concentration of cefaclor in the range of 0.2 – 0.6 mg/mL. The equation for the calibration curve is y = (6.268 ± 0.180) × x; r = 0.9996; n = 11 (for the equation y = ax + b, the value b = 0.022 is insignificant; $t_b = b/S_b = 0.64$; $t_{k(9)} = 2.26$ for f = n – 2). The precision of the method was adequate, because the RSD was less than 2.0% (0.20 – 1.56%). Intermediate precision was evaluated for 16 replicates and its variation coefficient was 3.94%. Under the conditions of this

Table 1. Kinetic and thermodynamic parameters of the degradation of cefaclor monohydrate in the crystalline form and CECLOR (oral suspension) in solid state at constant relative air humidity (RH = 76.4%)

T (K)	$(k_i \pm \Delta k) \times 10^{-6}$ (s^{-1})	n	Statistical evaluation of ln k_i = f(1/T)	Thermodynamic parameters
CEFACLOR MONOHYDRATE				
333	2.164 ± 0.681	8	a = -12281 ± 3068	
343	7.541 ± 1.803	7	S_a = 1297	E_a = 102.1 ± 25.5 [kJ · mol^{-1}]
353	11.19 ± 3.44	6	b = 23.78 ± 10.22	$\Delta H^{\neq a}$ = 99.64 ± 27.99 [kJ · mol^{-1}]
363	41.32 ± 12.66	7	S_b = 3.683	$\Delta S^{\neq a}$ = -47.12 ± 159.94 [J · K^{-1} · mol^{-1}]
373	138.0 ± 18.2	9	r = -0.9837 S_y = 0.3321	
CECLOR (oral suspension)				
323	1.035 ± 0.133	10	a = -12771 ± 3076	
328	2.028 ± 0.164	10	S_a = 1300	E_a = 106.2 ± 25.6 [kJ · mol^{-1}]
333	2.534 ± 0.251	11	b = 25.62 ± 10.83	$\Delta H^{\neq a}$ = 103.7 ± 28.1 [kJ · mol^{-1}]
338	5.042 ± 0.683	8	S_b = 3.9003	$\Delta S^{\neq a}$ = -31.90 ± 154.87 [J · K^{-1} · mol^{-1}]
343	11.56 ± 1.30	7	r = -0.9848 S_y = 0.1853	

n, number of experiments; E_a, activation energy; $\Delta H^{\neq}$, enthalpy; $\Delta S^{\neq}$, entropy; $E_a = -aR$ [J · mol^{-1}]; $\Delta H^{\neq} = E_a - RT$ [J · mol^{-1}]; $\Delta S^{\neq} = R$ [ln $A - \ln (k_B \cdot T/h)$] [J · K^{-1} · mol^{-1}], where k_B stands for the Boltzmann constant (1,3805 · 10^{-23} J · K^{-1}); *h*, Plancks constant (6,6256 · 10^{-24} J · s^{-1}); R, universal gas constant (8,3144 J · K^{-1} · mol^{-1}); T, temperature In K (t + 273 K); a, vectorial coefficient of the Arrhenius relationship; *A*, stands for the frequency coefficient. [a]Calculated for 298 K.

Table 2.The influence of relative air humidity on the stability of cefaclor monohydrate in the crystalline form and CECLOR (oral suspension) in solid state at constant temperature (T = 343 K)

RH (%)	$(k_i \pm \Delta k) \times 10^{-6}$ (s^{-1})	n	Statistical evaluation of $\ln k_i = f(RH\%)$
CEFACLOR MONOHYDRATE			
50.9	1.867 ± 0.201	11	$a = (69.67 \pm 17.98) \times 10^{-3}$
60.5	2.978 ± 0.968	6	$S_a = 7.604 \times 10^{-3}$
66.5	5.690 ± 1.196	7	$b = 4.928 \times 10^{-8} \pm 3.532$
76.4	7.541 ± 1.803	7	$S_b = 0.5342$
90.0	30.42 ± 3.72	6	$r = 0.9826$ $S_y = 0.2281$
CECLOR (oral suspension)			
50.9	3.576 ± 0.436	8	$a = (36.39 \pm 18.16) \times 10^{-3}$
66.5	9.040 ± 1.097	6	$S_a = 7.68 \times 10^{-3}$
76.4	11.56 ± 1.30	7	$b = 6.549 \times 10^{-7} \pm 3.721$
90.0	14.97 ± 0.84	7	$S_b = 0.5563$ $r = 0.9583$ $S_y = 0.219$

study the detection limit was 0.02 mg/mL and the quantitation limit was 0.05 mg/mL. Therefore, the procedure was suitable for kinetic studies.

The kinetics of degradation of cefaclor

The degradation of cefaclor in substance and in oral suspension at an increased temperature and relative air humidity (RH > 50%) was a first-order an autocatalytic reaction depending on the substrate concentration (Fig. 2, 4), whereas at an increased temperature and relative air humidity 0% RH it is a first-order reaction depending on the substrate concentration (Fig. 6). At increased relative air humidity and at RH = 0%, at time $t_0 \rightarrow t_\mu$, the value $P/P_{IS} \rightarrow 0$. The rate constants of the reaction at increased relative air humidity were calculated from the equation:

$$\ln c_t/(c_o - c_t) = -k \times t + c$$

where c_o and c_t – substrate concentrations at t_0 and t; $c_o - c_t$ – product concentrations at time = t; c = a constant related to the induction time and k is the observed rate constant of degradation reaktion. The semilogarithmic plots $c_t/(c_o - c_t) = f(t)$ were straight lines and their slopes corresponded to the rate constants of the reaction ($-k_{obs}$) (Figures 3, 5).

In dry air, the plot $\ln c_t = f(t)$ was linear and the observed rate constant was calculated by the least squares method, according to the equation:

$$\ln c_t = \ln c_0 - k_{obs} \cdot t$$

For the interpretation of the straight line plotted from $\ln c_t/(c_o - c_t) = f(t)$ and $\ln c_t = f(t)$ the following statistical parameters of the equations were calculated by using the least squares method: $y = ax + b$, $a \pm \Delta a$, $b \pm \Delta b$, standard deviations S_a, S_b, S_y and the coefficient of linear correlation r. The values of $\pm \Delta a$ and $\pm \Delta b$ were obtained for $f = n - 2$ degrees of freedom, with a = 0.05.

The values of reaction rate constants k_{obs} were used to calculate the Arrhenius relationship in order to interpret the influence of the temperature on the reaction rate at 76.4% RH. The energy of activation and the thermodynamic parameters – enthalpy and entropy of activation for 298 K were calculated based on the parameters of the slope $\ln k_i = f(1/T)$ (Table 1).

The effect of humidity on the stability of cefaclor (Table 2) was described by the following equation:

$$\ln k_i = a \cdot (RH\%) + b$$

$\ln k_i = (69.7 \pm 24.2) \times 10^{-3} \times (RH\%) - 16.8 \pm 1.7$ (cefaclor in substance) and

$\ln k_i = (36.4 \pm 32.9) \times 10^{-3} \times (RH\%) - 14.2 \pm 2.4$ (cefaclor in oral suspension).

The semilogarithmic plots of $\ln k_i = f(RH\%)$ were straight lines, and the slopes of these curves reflected the effect of relative air humidity on cefaclor stability at 343 K, while the value $b = k_0$ represented cefaclor stability at 343 K and at 0% RH.

The rate constants of cefaclor degradation in substance and in oral suspension at 333 K and 76.4% RH were $(2.16 \pm 0.86)10^{-6}$ s^{-1} and $(2.53 \pm 0.25)10^{-6}$ s^{-1}, respectively.

The rate constants of cefaclor degradation at 373 K and 76.4% RH and 0% RH were (1.38 ±

$0.18)10^{-4}$ s^{-1} and $(6.16 \pm 0.35)10^{-7}$ s^{-1}, respectively, which demonstrates that increased relative air humidity determines cefaclor degradation.

The degradation of cefaclor in the slow release tablets was a first-order autocatalytic reaction depending on substrate concentration. The degradation of cefaclor in tablets without their commercial package at 318 K and 76.4% RH is shown in Figure 7A and the semilogarithmic straight-line relationship $c_t/(c_o - c_t) = f(t)$ is presented in Figure 7B ($k = 0.9621 \pm 0.0606)10^{-6}$ s^{-1}; $n = 7$; $r = -0.9969$). During incubation of tablets in their commercial package, in a humid environment 76.4%RH at 333 K, the content of cefaclor ranged from 93.2% to 117.9% of the declared value (117.9% after 296 h, 106.7% – 386 h, 95.6% – 478 h, 98.0% – 552 h, 90.2% – 695 h, 94.7% – 869 h and 93.2% after 1298 h), which indicates that the commercial package of CEFACLOR MR slow tablets provides satisfying protection from relative air humidity.

CONCLUSIONS

The degradation of cefaclor in substance and in its preparations – oral suspension and slow release tablets – is a first-order autocatalytic reaction depending on substrate concentration at increased temperature and relative air humidity. The degradation of cefaclor at increased temperature and 0% RH is a first-order reaction depending on the substrate concentration.

The study demonstrated that while the kinetic mechanism of the degradation of cefeclor depends on storage conditions, it does not depend on the excipients in pharmaceutical preparations of cefaclor. The difference between the influence of temperature on the stability of cefaclor in substance and in oral suspension was not statistically significant. However, the influence of relative air humidity on the stability of cefaclor in crystalline form, expressed as the rate of degradation, was approx. 2 times greater than in the case of oral suspension. The differences between the values of thermodynamic parameters of the degradation of cefaclor in substance and in oral suspension were not statistically significant. Ceclor MR slow release tablets stored in a commercial package receive satisfying protection from increased relative air humidity.

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Received: 18. 02. 2009