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Evaluation of stability of cefuroxime axetil in solid state

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Abstract

The effect of temperature and relative atmospheric humidity on the stability of the crystalline form of cefuroxime axetil (CFA) in solid state was investigated. CFA is a mixture of diastereomers A and B. Changes in the concentration of the two diastereomers (A and B) of CFA were recorded by means of HPLC with UV detection. The degradation of diastereomers of CFA occurring at 0% relative humidity (RH) of the ambient air is a reversible first order reaction, while that occurring in humid air (RH > 50%) is an autocatalytic first order reaction relative to substrate concentration. Although it has been found, that diastereomer B is the more stable isomer, humidity has a stronger effect on this very diastereomer.

Keywords: Cefuroxime axetil; Stability in the solid phase; Kinetic and thermodynamic parameters

1. Introduction

Cefuroxime axetil (CFA) an ester prodrug of cefuroxime, exists as a mixture of diastereomers A and B [1]. After oral administration cefuroxime axetil is rapidly hydrolised by non-specific esterases to give high concentrations of free acid in blood and in body tissues.

Previous studies of the stability of CFA dealt with the kinetics of hydrolysis in aqueous solutions, over a pH range from 1 to 9 as well as with the kinetics of photoisomerisation in methanol–water solutions, effected by UV light (254 nm) [2,3]. Concentration changes of substrates and products of hydrolysis and photoisomerisation were recorded by means of reversed phase HPLC under isocratic conditions. In aqueous solutions-maximal stability was assessed over a range of pH from 3.5 to 4.5. The diastereomer A was always more reactive than the B diastereomer, the largest difference between these two diastereomers was found at pH 1. Hydrolysis of CFA was also studied in blood in vitro and in vivo [4,5].

The aim of the study was to evaluate the stability of CFA in solid state and to estimate the effect of temperature and relative air humidity

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on the degradation of diastereomers A and B of CFA.

2. Experimental

2.1. Chemicals and reagents

1-Acetoxyethyl ester of cefuroxime–CFA was obtained from Institute of Biotechnology and Antibiotics. Other chemical substances and reagents were products of the Sigma.

2.2. Chromatographic conditions

The chromatographic separation and quantitative determination were performed on a high-performance liquid chromatograph equipped with a pump model L-6000 (Hitachi), and an LC-2UV detector was used for detection. A Merck analytical column (LiChrosorb RP-18; 7 μm particale size, 250×4 mm) was used as the stationary phase. The injector was the Rheodyne 7120 with a noose of 20 μl. The mobile phase consisted of a methanol-solution of sodium dihydrogen phosphate 0.2 mol l^{-1} (50:50 v/v). The flow rate was 0.8 ml min^{-1}. UV detection was carried out at 278 nm.

2.3. Calibration curves

The calibration curve for CFA was taken in a mixture of acetonitryl and water (1:1 v/v), for solutions of concentrations varying from 0.82×10^{-4} to 7.38×10^{-4} g ml^{-1}. The internal standard was a solution of acetanilide in a mixture of acetonitryl and water (1:1 v/v) at a concentration of 2 mg ml^{-1}. To 1.0 ml of the so obtained solution of CFA 1.0 ml of a solution of internal standard was added and the so obtained solution was analysed by means of HPLC. A total of 20 μl of the samples to be analysed were fed to the HPLC-column and the emerging signals-recorded and analysed:

- the diastereomer A of CFA-emerged with a retention time of approx. 6.53 min
- the diastereomer B of CFA-emerged with a retention time of approx. 7.33 min
- the internal standard (acetanilide)-emerged with a retention time of approx. 6.03 min (Fig. 1)

The chromatograms were interpreted using the following equation: $h_i/h_s = f(c)$; where h_i is the value of the CFA signal (diastereomer A and B, respectively), and h_s represents the value of the internal standard signal.

2.4. Conditions of the kinetic studies

For the experiments, 10 mg samples of CFA were weighed into 5 ml vials. To assess the stability of CFA in dry air, the vials containing the studied substance were immersed in a sand bath that was placed in a heat chamber adjusted to temperatures of 373, 383, 388 and 393 K. Samples destined for the study of the impact of humidity were placed into exsicators inserted in heat chambers set to the desired temperatures: 333, 343, 353 or 363 K. The exsicators contained saturated solutions of inorganic salts, which safeguarded the desired relative humidity of the ambient air sodium bromide (RH = 50.9%), potassium iodide (RH = 60.5%), sodium nitrate (RH = 66.5%) and sodium chloride (RH = 76.4%) [6]. Each series comprised 10–15 samples.

After definite time intervals, determined by rate of degradation, the respective vials were taken out of the chamber, cooled to room temperature and the contents dissolved in a mixture of acetonitryl and water (1:1 v/v). The so obtained solution was quantitatively transferred into measuring flasks and made up to a total volume of 25.0 ml with the above mentioned solvent. To 1.0 ml of the so obtained solution (if necessary after filtration) 1.0 ml of a solution of internal standard was added. These samples were analysed according to the procedure described for calibration curves (Section 2.3).

3. Results and discussion

Concentration changes of CFA under the experimental conditions were assessed by means of

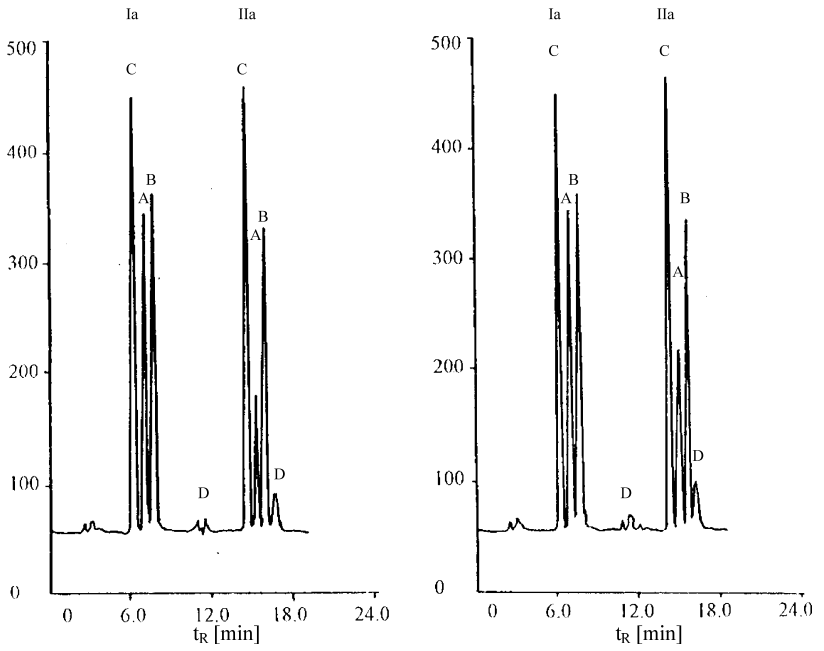

Fig. 1. HPLC chromatograms of the cefuroxime axetil (Ia, IIa) and of a mixture of a solid phase preparation of cefuroxime axetil and of its degradation products following incubation in an anhydrous, dry atmosphere at 393 K for 37 h (Ib) and at 363 K in a moist atmosphere (RH = 76.4%) for 28 h (IIb). (A, B) Diastereomers of cefuroxime axetil, (C) internal standard, (D) degradation products.

HPLC. The method applied was validated by the following parameters: selectivity, precision, linearity, detection limit and sensitivity. For the validation test, the following substances were used:

- a comparative CFA sample
- a sample of CFA heated to 393 K in dry air, and
- a sample of CFA heated to 363 K in a humid atmosphere of 76.4% RH.

3.1. Selectivity

The applied method is selective for the diastereomers A and B of CFA as well as for the internal standard (acetanilide) in the presence of degradation products of CFA, as shown by the chromatograms (Fig. 1).

3.2. Linearity

In the conditions studied, a linear dependence was obtained between the height of the peaks (diastereomers A and B) and CFA concentration over a range of 0.82×10^{-4}–7.38×10^{-4} g ml^{-1} ($y = (1627 \pm 33)x$, $r = 0.997$, $n = 9$ for diastereomer A and $y = (2330 \pm 46)x$, $r = 0.997$, $n = 9$ for diastereomer B).

3.3. Precision

Parameters characterising the precision of the method were evaluated for eight individual samples of 0.4 mg ml^{-1} CFA, and the following results were obtained mean value $h_i/h_s = 0.7326$, standard deviation $s = 0.005$, variation coefficient = 0.58% for diastereomer A and mean value $h_i/h_s = 0{,}7728$, standard deviation $s = 0.005$, variation coefficient = 0.59% for diastereomer B.

3.4. Detectability limit and sensitivity of the method

The concentration of CFA at which the height of the peak of the analysed substance is 10 times higher than the noise level was taken as the limit of detectability. In our experimental conditions this parameter was 0.82×10^{-4} g ml^{-1}.

The slopes of the curves plotted from the relationship $y = ax$ (see Section 3.2) for both diastereomers of CFA indicate a significant sensitivity of the applied method.

During the process of degradation of CFA in the solid phase, reaction products were formed exhibiting a yellow, light brown and dark brown coloration. The initial products of degradation were easily soluble in the acetonitryl–water solvent (1:1). In the further course of decomposition the ensuing products were no longer soluble in the above solvent mixture.

In samples incubated in humid conditions, the CFA peak heights (diastereomers A and B) decreased in the time interval $t_0 \rightarrow t_\infty$ from $h_i \rightarrow 0$ (h_i = CFA peak height) (Fig. 2). The degradation of CFA under humid conditions proceeded according to the model of an autocatalytic reaction. The rate constant of the first order reaction relative to the substrate concentration was calculated from the following equation:

$$\ln(1-\alpha)/\alpha = -\gamma \cdot t + c$$

where: $(1-\alpha)/\alpha = H_t/(H_0 - H_t)$. H_0 and H_t represent substrate concentrations at t_0 and t. $H_0 - H_t$ represent the product concentration at the time = t. c = a constant related to the induction time

$$H_i = h_i/h_s$$

The semilogarithmic plots of $H_t/(H_0 - H_t) = f(t)$ show a straight line relationship (Fig. 3) and their slopes constitute a measure of the reaction rate ($-\gamma = k_{obs}$).

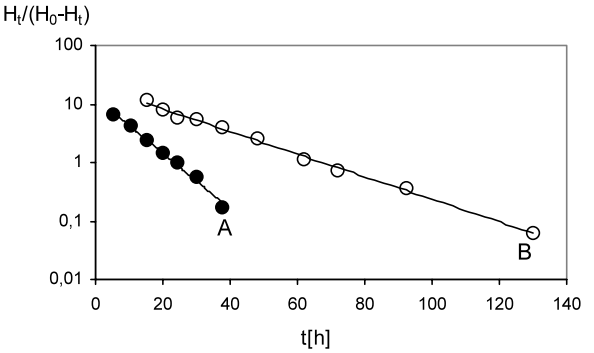

Fig. 3. Semilogarithmic plots of $H_t/(H_0 - H_t) = f(t)$ characterising the degradation of CFA following exposition to moist atmosphere of RH = 76.4% at 363 K ($H = h_i/h_s$).

The chromatographic peaks of analysed samples of CFA diastereomers incubated in dry air, over a period of time from $t_0 \rightarrow t_\infty$, showed a decline from $h_i \rightarrow h_\infty$ (Fig. 4). After some time, a steady state of the reaction was reached and the plots of $\ln(H_t - H_\infty) = f(t)$ presented straight lines (Fig. 5). The degradation of CFA in dry air thus occurs according to the mechanism of a reversible reaction:

$$[A] \underset{k_2}{\overset{k_1}{\rightleftarrows}} [B]$$

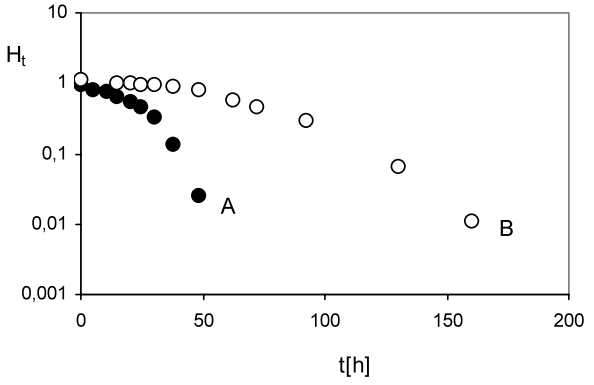

Fig. 2. Semilogarithmic plots of $H_t = f(t)$ characterising the degradation of CFA in a solid phase preparation in a moist atmosphere of RH = 76.4% at 363 K ($H = h_i/h_s$).

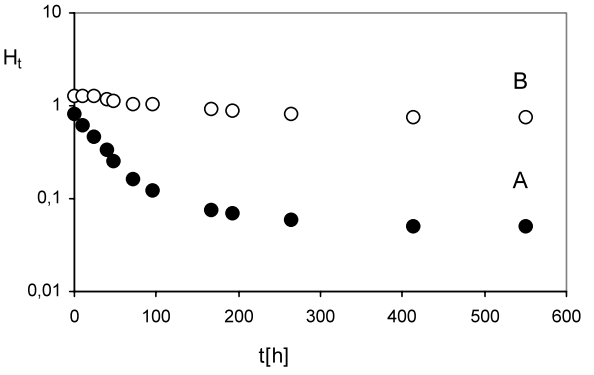

Fig. 4. Semilogarithmic plots of $H_t = f(t)$ characterising the degradation of CFA in a solid phase in an anhydrous, dry atmosphere at 383 K ($H = h_i/h_s$).

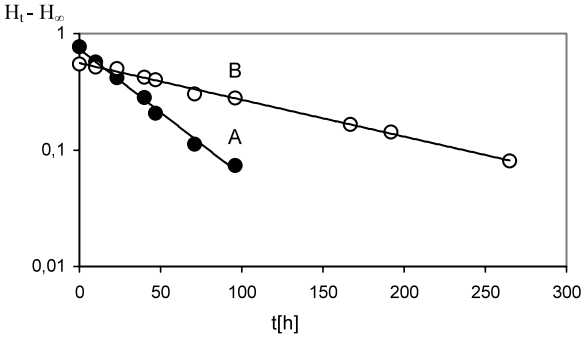

Fig. 5. Semilogarithmic plots of $(H_t - H_\infty) = f(t)$ characterising the degradation of CFA in a solid phase in an anhydrous, dry atmosphere at 383 K ($H = h_i/h_s$).

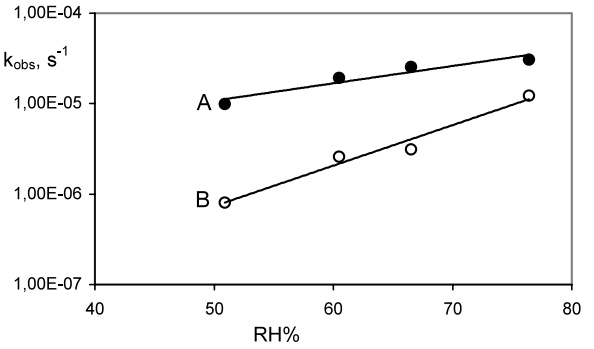

Fig. 6. Plots of the equation: $\ln k_i = f(\mathrm{RH}\%)$ characterising the reaction of CFA decay at 363 K.

where A stands for the substrate and B for the product of the reaction.

The summary rate constant of the first order reaction relative to the substrate concentration $k_{obs} = k_1 + k_2$ was calculated from the equation:

$$\ln(H_t - H_\infty) = \ln(H_0 - H_\infty) - k_{obs} \cdot t$$

The equilibrium constant K was calculated from the following equation:

$$K = k_1/k_2 = (H_0 - H_\infty)/H_\infty$$

The partial rate constants of the reaction of CFA decay occurring in dry air were computed from the following relationship: $k_2 = k_{obs}/(1+K)$; $k_1 = k_{obs} - k_2$.

For the interpretation of the straight curves plotted from $\ln H_t/(H_0 - H_t) = f(t)$ and $\ln(H_t - H_\infty) = f(t)$ the following statistical parameters of the respective equations were computed by means of the minimal square method: $y = ax + b$: $a \pm \Delta a$; $b \pm \Delta b$, standard errors: S_a, S_b, S_y, and the coefficient of linear correlation r. The values of $\pm\Delta a$ and $\pm\Delta b$ were computed 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: $\ln k_i = \ln A - E_a/RT$, where k_i represent the respective reaction rate constants [s^{-1}], A = frequency coefficient, E_a = activation energy [J mol^{-1}], R is universal gas constant (8.3144 J K^{-1} mol^{-1}), T is temperature (K).

For the relationship $\ln k_i = f(1/T)$ straight line plots were obtained for either of the diastereomers of CFA, for both the humid and dry conditions of sample exposure (Tables 1 and 2). Straight line plots of $\ln k_i = f(1/T)$ were also obtained for the partial rate constants (k_1 and k_2) of the reaction of CFA decay occurring in dry air (Table 2). From the parameters of the plot $\ln k_i = f(1/T)$ the following thermodynamic parameters of the reaction of degradation of CFA in solid state, pertaining to either conditions of sample incubation, i.e. in dry air and in an atmosphere of RH = 76.4%, were calculated: the activation energy (E_a), and enthalpy ($\Delta H^{\neq}$) and entropy ($\Delta S^{\neq}$) for the temperature 298 K (Tables 1 and 2). The results indicate that the B diastereomer of CFA is more stable than the A diastereomer in either of the studied conditions, i.e. in both the dry and humid atmospheres.

The effect of humidity on the stability of CFA is described by the equation:

$$\ln k_i = a(\mathrm{RH}\%) + b$$

The value of the slope a denotes the effect of humidity on the stability of CFA, while the value of $b = k_0$, characterises the stability of CFA at a relative humidity of RH = 0%. The plot of the equation $\ln k_i = f$ (RH%) is shown in Fig. 6. It documents the significantly greater impact of RH of ambient air on the degradation rate of the B diastereomer of CFA.

Table 1
Kinetic and thermodynamic parameters for the decomposition reaction of CFA in the solid phase in relative humidity RH = 76.4%

T (K)	$10^5(k \pm \Delta k)$ (s^{-1})	Statistical evaluation $\ln k_i = f(1/T)$	Thermodynamic parameters
Diastereomer A (k_A)			
333	0.25 ± 0.02	$a = -9799 \pm 1991$	$E_a = 81.5 \pm 16.5$ kJ mol^{-1}
343	0.62 ± 0.06	$S_a = 682.2$	$\Delta H^{\neq a} = 79.0 \pm 16.5$ kJ mol^{-1}
353	1.13 ± 0.10	$b = 16.5 \pm 5.7$; $S_b = 1.96$	$\Delta S^{\neq a} = -107.6 \pm 47.0$ J (K mol)$^{-1}$
363	3.05 ± 0.32	$r = -0.995$; $S_y = 0.126$	
Diastereomer B (k_B)			
333	0.054 ± 0.007	$a = -12\,457 \pm 1756$	$E_a = 81.5 \pm 16.5$ kJ mol^{-1}
343	0.15 ± 0.04	$S_a = 601.4$	$\Delta H^{\neq a} = 79.0 \pm 16.5$ kJ mol^{-1}
353	0.38 ± 0.03	$b = 22.9 \pm 5.1$; $S_b = 1.73$	$\Delta S^{\neq a} = -107.6 \pm 47.0$ J (K mol)$^{-1}$
363	1.23 ± 0.05	$r = -0.998$; $S_y = 0.111$	

[a] Calculated for the 298 K.

Table 2
Kinetic and thermodynamic parameters for the decomposition reaction of CFA in the solid phase in dry air RH = 0%

T (K)	$10^6(k \pm \Delta k)$ (s^{-1})	Statistical evaluation $\ln k_i = f(1/T)$	Thermodynamic parameters
Diastereomer A (k_A)			
k_A			
373	0.99 ± 0.10	$a = -16\,168 \pm 7523$	$E_a = 134.4 \pm 62.5$ kJ mol^{-1}
383	3.22 ± 0.35	$S_a = 1748.2$	$\Delta H^{\neq a} = 132 \pm 65$ kJ mol^{-1}
388	6.78 ± 0.73	$b = 29.6 \pm 19.6$; $S_b = 4.5$	$\Delta S^{\neq a} = 1.2 \pm 81.9$ J (K mol)$^{-1}$
393	8.06 ± 0.84	$r = -0.989$; $S_y = 0.176$	
k_{A1}			
373	0.83	$a = -17\,121 \pm 8134$	$E_a = 142.4 \pm 67.6$ kJ mol^{-1}
383	2.92	$S_a = 1890.3$	$\Delta H^{\neq a} = 139.9 \pm 70.1$ kJ mol^{-1}
388	6.41	$b = 31.9 \pm 21.2$; $S_b = 4.9$	$\Delta S^{\neq a} = 20.7 \pm 68.8$ J (K mol)$^{-1}$
393	7.65	$r = -0.988$; $S_y = 0.191$	
k_{A2}			
373	0.99	$a = -7251 \pm 3702$	$E_a = 60.3 \pm 30.8$ kJ mol^{-1}
383	3.22	$S_a = 860.3$	$\Delta H^{\neq a} = 57.8 \pm 33.3$ kJ mol^{-1}
388	6.88	$b = 3.8 \pm 9.6$; $S_b = 2.2$	$\Delta S^{\neq a} = -212 \pm 164.8$ J (K mol)$^{-1}$
393	8.06	$r = -0.986$; $S_y = 0.1$	
Diastereomer B			
k_B			
373	0.71 ± 0.06	$a = -9599 \pm 1712$	$E_a = 79.8 \pm 14.2$ kJ mol^{-1}
383	1.46 ± 0.05	$S_a = 397.8$	$\Delta H^{\neq a} = 77.3 \pm 16.7$ kJ mol^{-1}
388	2.02 ± 0.08	$b = 11.6 \pm 4.4$; $S_b = 1.0$	$\Delta S^{\neq a} = -148.5 \pm 207.9$ J (K mol)$^{-1}$
393	2.58 ± 0.30	$r = -0.998$; $S_y = 0.04$	
k_{B1}			
373	0.29	$a = -11\,708 \pm 8788$	$E_a = 97.3 \pm 73.1$ kJ mol^{-1}
383	0.53	$S_a = 2042.3$	$\Delta H^{\neq a} = 94.9 \pm 75.5$ kJ mol^{-1}
388	1.17	$b = 16.3 \pm 22.9$; $S_b = 5.3$	$\Delta S^{\neq a} = -109.5 \pm 54.7$ J (K mol)$^{-1}$
393	1.27	$r = -0.971$; $S_y = 0.21$	
k_{B2}			
373	0.42	$a = -8582 \pm 5569$	$E_a = 71.4 \pm 46.3$ kJ mol^{-1}
383	0.93	$S_a = 294.1$	$\Delta H^{\neq a} = 68.9 \pm 48.8$ kJ mol^{-1}
388	1.17	$b = 8.4 \pm 14.5$; $S_b = 3.4$	$\Delta S^{\neq a} = -175.2 \pm 124.4$ J (K mol)$^{-1}$
393	1.31	$r = -0.978$; $S_y = 0.13$	

[a] Calculated for the 298 K.

4. Conclusions

The kinetic mechanism of CFA decomposition depends on the storage conditions of the respective substance. In a dry ambient atmosphere the decomposition is the result of a reversible process and follows the kinetics of a pseudo-first order reaction. When stored in a humid environment ($RH > 50\%$), the degradation of CFA is of an autocatalytic nature.

Environmental humidity is a paramount factor determining the decomposition of CFA, especially at high temperatures.

The B diastereomer of CFA is more stable than the A one, both in a dry and in a humid ambient atmosphere.

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