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Short communication

The stability of the amorphous form of cefuroxime axetil in solid state

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Abstract

The stability of the amorphous form of cefuroxime axetil was studied by means of the stress stability test. The degradation was evaluated using the HPLC method with UV detection (278 nm), as described in the monograph of Cefuroxime Axetil in European Pharmacopoeia. Liquid chromatography was performed with a H5 SAS Hypersil column (5 μm particle size, 250 mm × 4 mm), the mobile phase consisted of a mixture of 38 volumes of methanol and 62 volumes of a 23 g l^{-1} solution of ammonium dihydrogen phosphate, a flow rate of 1.2 ml min^{-1}, and the internal standard was a solution of acetanilide in a mixture (1:1) of acetonitrile and water at a concentration of 0.2 mg ml^{-1}. At an increased temperature at RH = 0%, the degradation of cefuroxime axetil (CFA) diastereoisomers is the reversible first-order reaction, while that occurring in humid air (RH > 25%) is the reversible first-order autocatalytic reaction with Δ^3-isomers and *E*-isomers of cefuroxime axetil and cefuroxime as the main products.

Keywords: Cefuroxime axetil—amorphous form; Stability in the solid state

1. Introduction

Cefuroxime axetil (1-acetoxyethyl ester of cefuroxime, CFA), widely used in therapy, is a prodrug of cefuroxime. This second generation-cephalosporin is active against a wide range of Gram-positive and Gram-negative organisms and it is resistant to most β-lactamases. Cefuroxime axetil has only weak antimicrobial activity but after oral absorption is rapidly hydrolised to cefuroxime and for this reason its antimicrobial spectrum corresponds to that of the parent cephalosporin [1–4].

CFA in position 3 has a carbamoyl group which gives it a considerable metabolic stability, and in position 7 it has a methoxyimino group which causes its high stability to β-lactamase attack and together with the furyl ring contributes to the antibacterial properties of the molecule by enhancing its activity against Gram-negative bacteria. The 1-acetoxyethyl ester group in position 4 of CFA ensures its lipophilicity and promotes the intestinal absorption of cefuroxime.

In a molecule of CFA there are three kinds of isomerism. The first is isomerism *R* and *S*, due to the presence of asymmetric carbon atom in the ester group, whereas isomerism Δ^2 and Δ^3 results from the presence of the double bond in the dihydrothiazine ring of the cephalosporin molecule. The third kind of isomerism *Z* and *E* (*syn* and *anti*) is connected with the presence of the double bond in methoxyimino group in position 7.

The assay of CFA according to European Pharmacopoeia is to be over 96.0% but the content of the inactive Δ^3-isomers, the most common impurity, should not exceed 1.5%.

For the preparation of pharmaceutical forms only the amorphous form is used. In comparison with the crystalline form that one has better physicochemical and biological properties, e.g. significantly higher solubility and bulk density as well as a higher absorption degree after oral administration [5,6].

The stability of the crystalline form of cefuroxime axetil in solid state and the kinetics of hydrolysis in aqueous solutions, in a pH range of 1–9, the kinetics of photoisomerisation in methanol–water solutions, and stability in human intestinal juice have been reported in the literature [7–10].

The aim of present study was to determine the effect of temperature at RH = 0% and 76.4%, and of humidity (RH 25–76.4%) at 363 K on the stability of the amorphous form of cefuroxime

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axetil in the solid state. The kinetic and thermodynamic parameters of degradation were also calculated.

2. Experimental

2.1. Materials and reagents

Cefuroxime axetil (CFAA/011/2003) made by Institute of Biotechnology and Antibiotics in Warsaw, Poland, Cefuroxime sodium salt, made by Bioton, Warsaw, Poland, Δ^3-isomers of cefuroxime axetil – LGC Prochem, 6 Annopol Str., 03-236 Warsaw, Poland, *E*-isomers of cefuroxime axetil was obtained by the Institute of Biotechnology and Antibiotics in Warsaw according to the monograph of Cefuroxime axetil in European Pharmacopoeia, acetanilide – Sigma–Aldrich, 19 Bastionowa Str., 61–663 Poznań, Poland. All other chemicals were of analytical or HPLC grade.

2.2. Instrumental and chromatographic conditions

The method used in the experiments is a modification of the procedure presented in Ph. Eur. for Cefuroxime Axetil. The concentrations of CFA (diastereoisomers A and B) and the products of its degradation (Δ^3-isomers, *E*-isomers of cefuroxime axetil and cefuroxime) were determined using the HPLC method (a LC-6A pump, a SPO-6AV spectrophotometric detector, a Chromatopac C-RGA, Shimadzu Co., Ltd., Kyoto, Japan). The column HiCHROM H5 SAS Hypersil (25 cm × 4 mm, particle size 5 μm) was used as a stationary phase. The mobile phase consisted of a mixture of 38 volumes of methanol and 62 volumes of a 23 g l^{-1} solution of ammonium dihydrogen phosphate. The flow rate was 1.2 ml min^{-1}. The detection wavelength was 278 nm. The injector was a Rheodyne 7120 with a loop of 50 μl. The internal standard was a solution of acetanilide in a mixture (1:1) of acetonitrile and water at a concentration of 0.2 mg ml^{-1}. The study was performed at ambient temperature.

2.3. Selectivity

The selectivity of the HPLC method was examined for non-degraded, degraded samples and cefuroxime axetil, Δ^3-isomers, *E*-isomers of cefuroxime axetil and cefuroxime.

2.4. Linearity

Calibration curves for HPLC analysis were determined by linear regression. The linearity between P/P_{IS} (P and P_{IS}—areas of CFA and IS) and concentrations of CFA in a mixture of acetonitrile and water (1:1), ranging from 0.033 to 0.40 mg ml^{-1}, was evaluated. Linearity was also examined for three consecutive days in solutions of the same concentration prepared from the stock solution.

2.5. Precision

The precision of the method is expressed as the relative standard deviation (R.S.D.) of replicate measurements. In order to evaluate the repeatability (intra-day) of the method, eight samples of three different concentrations (low, $c = 0.133$ mg ml^{-1}; medium, $c = 0.267$ mg ml^{-1}; high, $c = 0.333$ mg ml^{-1}), were prepared and analyzed on the same day. The intermediate precision (inter-day) was studied by comparing the assays performed on two different days at a CFA concentration of 0.267 mg ml^{-1}.

2.6. Limits of detection and quantitation

The limits of detection (DL = 0.23 μg of CFA) and quantitation (QL = 0.70 μg of CFA) were calculated from the formulas $DL = 3.3S_y/a$ and $QL = 10S_y/a$, where S_y is the standard deviation of the blank signal and a is the slope of the corresponding calibration curve [11].

2.7. Kinetic measurements

The study of the stability of CFA in the solid state was performed using the stress degradation test (at 333–393 K, relative humidity 0% and 25–76.4%), which determines kinetic and thermodynamic relationships. Samples of CFA (10.00 mg) were accurately weighed into 5 ml vials. To evaluate the stability of CFA at relative humidity RH = 0%, the vials containing the studied substance were immersed in a sand bath that was placed in a heat chamber adjusted to 373 K, 378 K, 383 K, 388 K and 393 K. Samples tested for the influence of temperature in a humid environment were placed in desiccators containing sat-

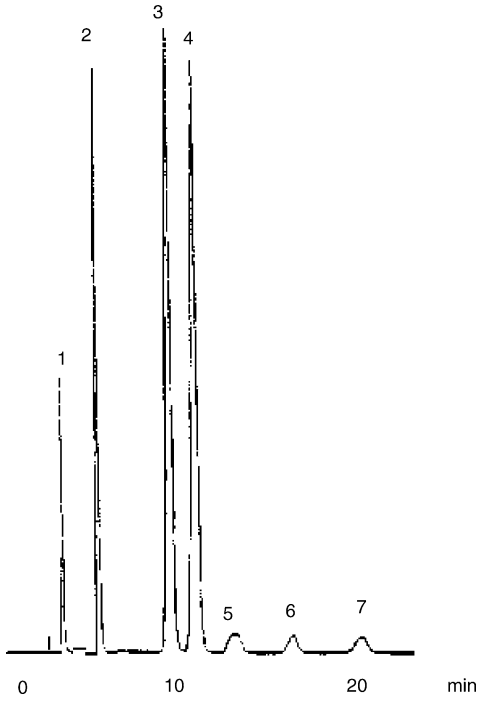

Fig. 1. HPLC chromatogram for cefuroxime axetil, products of its degradation and intenal standard: 1, cefuroxime; 2, internal standard; 3 and 4, cefuroxime axetil (diastereoisomers A and B); 5, Δ^3-isomers of cefuroxime axetil; 6 and 7, *E*-isomers of cefuroxime axetil. Chromatographic conditions are described in the text.

urated solutions of sodium chloride (RH ~76.4%) inserted in heat chambers set at 333 K, 343 K, 353 K, 363 K and 373 K. To assess the effect of relative humidity on the stability of CFA, the vials with CFA were placed in desiccators containing saturated aqueous solutions of appropriate inorganic salts which ensured the desired relative humidity of the ambient air (sodium iodide, RH ~25.0%, sodium bromide, RH ~50.9%, potassium iodide, RH ~60.5%, sodium nitrate, RH ~66.5%, sodium chloride, RH ~76.4%) and inserted in heat chambers set at 363 K. Each series comprised 10–15 samples. At definite time intervals, determined by the rate of degradation, the vials were removed, cooled to room temperature and the contents dissolved in a mixture (1:1) of acetonitrile and water. The so obtained solutions were quantitatively transferred into measuring flask, filled to 25.0 ml with the same mixture and filtered. To 1.0 ml of the sample taken 1.0 ml of internal standard solution was added. Fifty microliters samples of the solutions were injected onto the column.

Microsoft® Excel 2000 was used for the calculation of regression parameters.

3. Results and discussion

Concentration changes of CFA under the conditions of the study were assessed using the HPLC method. It was validated by selectivity, linearity, precision, detection limit and quantitation limit.

3.1. Validation of the HPLC method

3.1.1. Selectivity

The method is selective for CFA, Δ^3-isomers and *E*-isomers of cefuroxime axetil, cefuroxime and the internal standard, as shown in Fig. 1. In the chromatograms taken over a period of 0–25 min, the following peaks emerged:

- 1, corresponding to the cefuroxime, with a retention time ca. 2.8 min,
- 2, corresponding to the internal standard, with a retention time of ca. 5.0 min,
- 3 and 4, corresponding to the investigated substance (diastereoisomers A and B), with a retention times of ca. 10.2 min and 12.5 min, respectively,
- 5, corresponding to Δ^3-isomers of cefuroxime axetil, with a retention time of ca. 13.8 min and
- 6 and 7, corresponding to *E*-isomers of cefuroxime axetil, with a retention time of ca. 18.1 and 21.2 min, respectively (Fig. 1).

3.1.2. Linearity

The assays exhibited linearity between the response (y) (peak-area ratio of CFA over the internal standard) and the corresponding concentration of CFA (x), over the range 0.033–0.400 mg ml^{-1}. The equation for the calibration curve is $y = (5.62 \pm 0.11)x$; $r = 0.9996$; $n = 11$ (for the equation $y = ax + b$, the value b is insignificant).

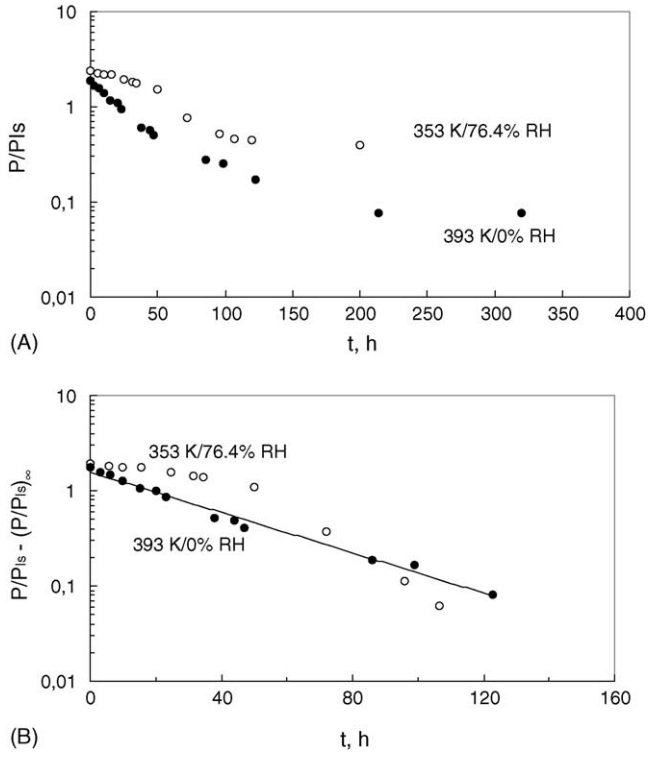

Fig. 2. Semilogarithmic plots $P/P_{IS} = f(t)$ (A) and $P/P_{IS} - (P/P_{IS})_\infty = f(t)$ (B) characterising the degradation of CFA in solid state at 353 K/76.4% RH and 393 K/0% RH.

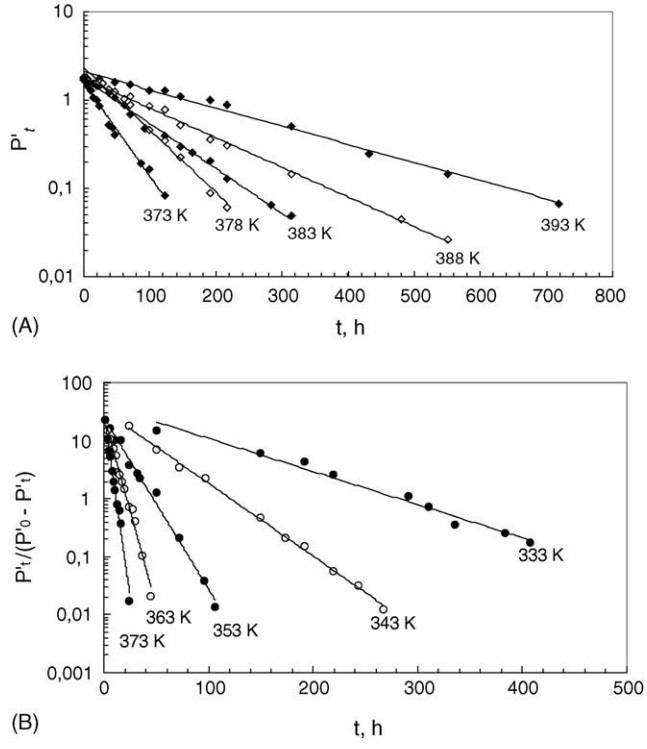

Fig. 3. Semilogarithmic plots $P'_t = f(t)$ (A) and $P'_t/(P'_0 - P'_t) = f(t)$ (B) for the degradation of cefuroxime axetil in solid state at an increased temperature at RH = 0% (A) and RH = 76.4% (B).

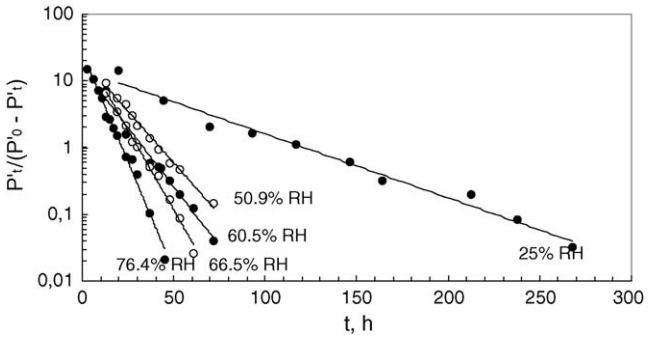

Fig. 4. Semilogarithmic plots $P'_t/(P'_0 - P'_t) = f(t)$ for the degradation of cefuroxime axetil in solid state at various humidities at 363 K.

3.1.3. Precision

Precision of the measurement was adequate, because the R.S.D. <2.0% (0.19–1.26%).

3.1.4. Limits of detection and quantitation

Under the conditions of this study the detection limits was 0.23 μg of CFA and the quantitation limit was 0.70 μg of CFA.

Therefore, the applied procedure may be used to determine the stability of CFA in the solid state.

3.2. The kinetics of the degradation of cefuroxime axetil

3.2.1. Determination of the rate constants

The degradation of cefuroxime axetil at an increased temperature and relative humidity is the reversible first-order autocatalytic reaction depending on the substrate concentration, whereas at an increased temperature and relative humidity RH = 0% it is the reversible first-order reaction depending on the substrate concentration.

From time t_0 to t_∞ the substrate values P/P_{IS} decreased to the value of $(P/P_{IS})_\infty$. After the calculation of $P/P_{IS} - (P/P_{IS})_\infty$ plots characteristic of the autocatalytic reaction (for RH > 25%) or the first-order reaction depending on the substrate concentration were obtained (Fig. 2).

To evaluate the rate constants of degradation the following rectilinear equations were used:

$$\ln (P')_t = \ln (P')_0 - k_{obs} t \quad (RH = 0\%)$$

$$\ln \frac{P'}{P'_0 - P'_t} = -\gamma t + c \quad (RH > 0\%)$$

where $P' = P/P_{IS} - (P/P_{IS})_\infty$, P'_0 and P'_t are the substrate concentrations after t_0 and t, $(P'_0 - P'_t)$—product concentration after time t.

Semilogarithmic plots $P'_t = f(t)$ (RH = 0%) and $P'_t/(P'_0 - P'_t) = f(t)$ (RH > 25%) (Figs. 3 and 4) are straight lines, and their slopes correspond to the rate constants of the reaction $(-k_{obs})$. For these straight-line plots the method of least squares was used to calculate the parameters of the equation $y = ax + b$, $a \pm \Delta a$, $b \pm \Delta b$, standard errors: S_a, S_b, S_y, and the correlation coefficient r. The values $\pm \Delta a, \pm \Delta b$ were calculated for $f = n - 2$ degrees of freedom and $\alpha = 0.05$.

The so obtained rate constant is the sum of rate constants k_1 and k_2, of the reactions, i.e. direct and back reaction. The partial reaction rate constants were calculated using the following equations:

$$k_2 = \frac{k_{obs}}{1 + K},$$

$$k_1 = k_{obs} - k_2$$

$$K = \frac{k_1}{k_2} = \frac{P_0 - P_\infty}{P_\infty}.$$

It was observed that the degradation of CFA diastereoisomers A and B at an increased temperature and relative humidity 0% and above 25% occurs at a similar rate. The values k_{obs} for each diastereoisomer and their sum calculated for the same conditions do not differ significantly (Table 1). In the aqueous solutions similar rates of decomposition of both CFA diastereoisomers were observed at pH 4.5–7 [7]. Thus in further calculations the rate constants of the sum of the CFA diastereoisomers were used.

Table 1
Kinetics parameters for the reaction of the degradation of CFA in solid state at 373 K at RH = 76.4% and 0%

Kinetics parameters	Diastereoisomer A	Diastereoisomer B	Sum of diastereoisomers
RH = 76.4%			
$k \pm \Delta k$	$(8.12 \pm 0.81) \times 10^{-5}$	$(8.03 \pm 0.44) \times 10^{-5}$	$(8.09 \pm 0.66) \times 10^{-5}$
k_1 (s^{-1})	7.71×10^{-5}	6.42×10^{-5}	7.08×10^{-5}
k_2 (s^{-1})	4.14×10^{-6}	1.61×10^{-5}	1.01×10^{-5}
r	−0.993	−0.997	−0.994
n	10	11	11
RH = 0%			
$k \pm \Delta k$ (s^{-1})	$(1.31 \pm 0.09) \times 10^{-6}$	$(1.32 \pm 0.10) \times 10^{-6}$	$(1.32 \pm 0.09) \times 10^{-6}$
k_1 (s^{-1})	1.23×10^{-6}	1.20×10^{-6}	1.21×10^{-6}
k_2 (s^{-1})	0.830×10^{-7}	0.12×10^{-7}	1.00×10^{-7}
r	−0.995	−0.995	−0.994
n	13	11	13

Table 2
Kinetic and thermodynamic parameters for the reaction of the degradation of CFA in solid state at RH = 76.4% and 0%

T (K)	10^6 ($k \pm \Delta k$) (s^{-1})	Statistical evaluation $\ln k_i = f(1/T)$	Thermodynamic parameters
RH = 76.4%			
k_{obs}			
333	3.66 ± 0.47	$a = -9632 \pm 340$	
343	8.83 ± 1.14	$S_a = 107$	$E_a = (80.1 \pm 2.8)$ (kJ mol^{-1})
353	18.9 ± 1.3	$b = 16.4 \pm 1.0$	$\Delta H^{\neq a} = (77.6 \pm 5.3)$ (kJ mol^{-1})
363	41.8 ± 2.9	$S_b = 0.304$	$\Delta S^{\neq a} = (-108 \pm 237)$ (J K^{-1} mol^{-1})
373	80.9 ± 6.6	$r = -0.999$	
k_1			
333	2.57	$a = -10270 \pm 378$	
343	6.73	$S_a = 119$	$E_a = (85.4 \pm 3.1)$ (kJ mol^{-1})
353	15.3	$b = 18.0 \pm 1.1$	$\Delta H^{\neq a} = (82.9 \pm 5.6)$ (kJ mol^{-1})
363	34.2	$S_b = 0.338$	$\Delta S^{\neq a} = (-95.3 \pm 236)$ (J K^{-1} mol^{-1})
373	70.8	$r = -0.999$	
k_2			
333	1.08	$a = -7170 \pm 1365$	
343	2.10	$S_a = 429$	$E_a = (59.6 \pm 11.3)$ (kJ mol^{-1})
353	3.55	$b = 7.82 \pm 3.88$	$\Delta H^{\neq a} = (57.1 \pm 13.8)$ kJ mol^{-1}
363	7.62	$S_b = 1.22$	$\Delta S^{\neq a} = (-180 \pm 213)$ (J K^{-1} mol^{-1})
373	10.1	$r = -0.995$	
RH = 0%			
k_{obs}			
373	1.31 ± 0.09	$a = -11795 \pm 1323$	
378	2.15 ± 0.08	$S_a = 416$	$E_a = (98.1 \pm 11.0)$ kJ mol^{-1}
383	3.26 ± 0.15	$b = 18.1 \pm 3.5$	$\Delta H^{\neq a} = (95.6 \pm 13.5)$ kJ mol^{-1}
388	4.51 ± 0.37	$S_b = 1.09$	$\Delta S^{\neq a} = (-94.3 \pm 216)$ (J K^{-1} mol^{-1})
393	6.77 ± 0.52	$r = -0.998$	
k_1			
373	1.21	$a = -12051 \pm 1608$	
378	2.02	$S_a = 506$	$E_a = (100 \pm 13)$ (kJ mol^{-1})
383	3.14	$b = 18.7 \pm 4.2$	$\Delta H^{\neq a} = (97.7 \pm 15.9)$ (kJ mol^{-1})
388	4.29	$S_b = 1.32$	$\Delta S^{\neq a} = (-89.2 \pm 209)$ (J K^{-1} mol^{-1})
393	6.49	$r = -0.997$	
k_2			
373	0.100	$a = -7735 \pm 4879$	
378	0.123	$S_a = 1533$	$E_a = (64.3 \pm 40.6)$ (kJ mol^{-1})
383	0.122	$b = 4.53 \pm 12.74$	$\Delta H^{\neq a} = (61.8.7 \pm 43.0)$ (kJ mol^{-1})
388	0.219	$S_b = 4.01$	$\Delta S^{\neq a} = (-207 \pm 139)$ (J K^{-1} mol^{-1})
393	0.282	$r = -0.946$	

$\Delta k = S_a t_{\alpha f}$; 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}); $\Delta S^{\neq} = R(\ln A - \ln(k_B T)/h$ (J K^{-1} mol^{-1}), where: k_B stands for the Boltzmann constant (1.3807 10^{-23} J K^{-1}); h, Plancks constant (6.626 10^{-34} J s); R, universal gas constant (8.314 J K^{-1} mol^{-1}); T, temperature in K(t + 273 K); a, vectorial coefficient of the Arrhenius relationship and A, stands for the frequency coefficient.

[a] Calculated for 298 K.

3.2.2. The effect of temperature

The values of reaction rate constants k_{obs}, k_1 and k_2 were used to calculate the Arrhenius relationship in order to interpret the influence of the temperature on the reaction rate at RH = 76.4% and 0%. Taking into account the parameters of the slope $\ln k_i = f(1/T)$ obtained with the method of least squares, the thermodynamic parameters of the CFA decomposition reaction were calculated: energy of activation, enthalpy and entropy of activation for 298 K (Table 2). The differences in the thermodynamic parameters obtained for RH = 0% and 76.4% showed that relative humidity influences both the rate and mechanism of degradation. The energy of activation of the compound analysed at RH = 0% is higher at RH = 76.4%. The entropy of reaction

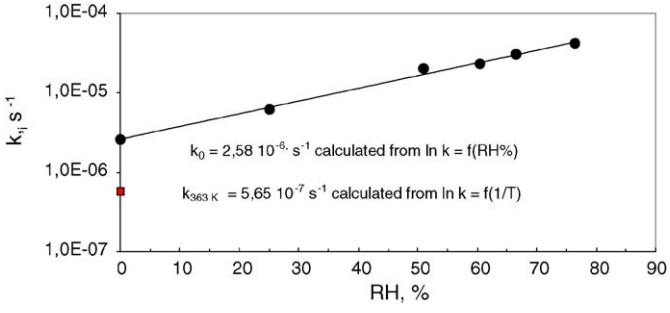

Fig. 5. A plot of $\ln k_i = f$(RH%) characterizing the degradation of cefuroxime axetil at 363 K.

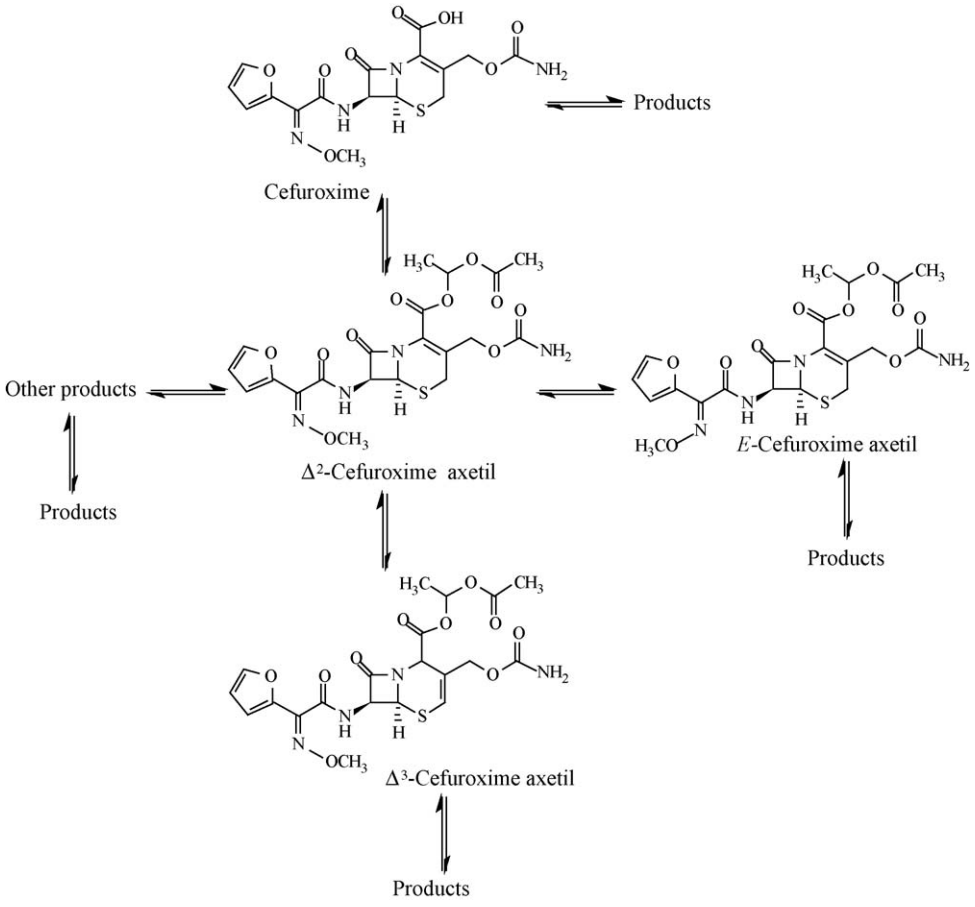

Fig. 6. The degradation of the amorphous form of cefuroxime axetil in solid state.

at RH = 0% and 76.4% is negative, which may suggest the bimolecular character of the reaction with a significant spatial limitation.

3.2.3. The effect of humidity

The influence of relative humidity on the stability of CFA is described by the equation $\ln k_i = a(\mathrm{RH}\%) + b$. The slope value a describes the influence of relative humidity on the stability of the compound, whereas the value $10^b = k_0$ when RH = 0%. The value of the rate constant of CFA degradation at 363 K and RH = 0% was calculated from the Arrhenius relationship. The extrapolated value $\ln k_{T=363\mathrm{K},\ \mathrm{RH}=0\%}$ was compared with the value k_0 of the degradation rate constant at 363 K and RH = 0% (Fig. 5). The value k_0 calculated from the equation $\ln k_i = f(\mathrm{RH}\%)$ was $2.58 \times 10^{-6}\ \mathrm{s}^{-1}$, whereas the value calculated from $\ln k_i = f(1/T)$ was $k_{363\mathrm{K}} = 5.65 \times 10^{-7}\ \mathrm{s}^{-1}$. This confirms the different mechanisms of the degradation of CFA at RH = 0% and >25%.

3.3. Conclusions

The differences in the kinetic mechanism of degradation of amorphous and crystalline forms of cefuroxime axetil are observed at an increased relative humidity. The degradation of amorphous cefuroxime axetil is the first-order autocatalytic reversible reaction, while in the case of the crystalline form of cefuroxime axetil degradation is the first-order autocatalytic reaction too but an irreversible one [10].

For the crystalline form of cefuroxime axetil degradation rates of diastereoisomers A and B are different. The degradation rate constants of amorphous CFA diastereoisomers A and B do not differ significantly.

The degradation of amorphous CFA yields three main products: Δ^3-isomers, *E*-isomers of cefuroxime axetil and cefuroxime regardless of relative humidity. All three products exept cefuroxime at RH = 0% underwent further decomposition in the consecutive reactions (Fig. 6).

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