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THE STABILITY OF CEFPROZIL IN ORAL SUSPENSION CEFZIL

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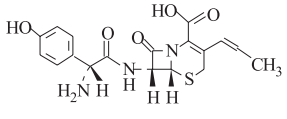
Abstract: The stability of cefprozil in oral suspension CEFZIL was studied by means of the stress stability test. Degradation was evaluated by using an HPLC method with UV detection (280 nm), as described in the monograph of Cefprozil for Oral Suspension, in USP 25. At increased temperature and relative air humidity the degradation of cefprozil in CEFZIL occurs as reversible-consecutive and parallel reactions. The reversible reaction of isomerization is approx. 10 times faster than the parallel degradation reaction of individual isomers. The first-order rate constants of the parallel reactions *Z*-cefprozil → product 1 and *E*-cefprozil → product 2 were determined at RH = 76.4% at T = 333, 338, 343, 348 and 353 K, and at T = 333 K and RH = 50.9, 66.5, 76.4 and 90.0%. The thermodynamic parameters E_a, $\Delta H^{\neq}$ and $\Delta S^{\neq}$ of these reactions were calculated.

Keywords: cefprozil, stability in solid state, kinetic and thermodynamic parameters

Cefprozil is a new, second generation, orally administered cephalosporin. It is active against a wide range of aerobic Gram-positive and Gram-negative bacteria, as well as certain anaerobic organisms. Cefprozil is hydrolyzed more slowly than other cephalosporins in the presence of penicillinase-producing *Staphylococcus aureus* isolates and in the presence of non-producers of penicillinase (1-7). In a molecule of cefprozil three kinds of isomerism are observed. The first is isomerism *Z* and *E* (*cis* and *trans*), due to the presence of an asymmetric carbon atom in the propenyl group at position 3 of the dihydrothiazine ring, whereas isomerism Δ^2 and Δ^3 results from the presence of double bond in the dihydrothiazine ring of a cephalosporin molecule. The third kind of isomerism is connected with the presence of 7-(*R*-amino-(4-hydroxyphenyl) acetylamino group (optical isomerism).



Cefprozil is applied in therapy as tablets containing 250 mg or 500 mg of cefprozil. The form of oral suspension dosage for pediatric purposes is also available as granules in multidose bottles. One 5 mL dosage of suspension contains 125 mg or 250 mg of cefprozil. Cefprozil in CEFZIL is a mixture of isomers, approximately 9:1, *Z* to *E* (8). The predominant *Z* form is much more active against Gram-negative organisms.

HPLC methods used in pharmaceutical analysis and pharmacokinetic studies of two isomers of cefprozil and simple, selective, spectrophotometric methods for quantitative determination of cefprozil in substance and in its pharmaceutical formulations have been developed (9, 10).

The aim of this study was to evaluate the stability of cefprozil in CEFZIL under stress storage conditions. The kinetic and thermodynamic parameters of degradation were also calculated.

EXPERIMENTAL

Material and reagents

Cefprozil for Oral Suspension – a dry mixture of cefprozil and one or more suitable buffers, flavors, preservatives, suspending agents, and sweeteners were products of Bristol-Myers Squibb. Salicylic acid (conforming to FP VI) was used as an internal standard. Other chemical substances and solvents were from Merck (Darmstadt, Germany) and were of analytical or high-performance liquid chromatographic grade.

Chromatographic conditions

Changes in the concentration of the two isomers (*Z* and *E*) of cefprozil were recorded using a HPLC method (an LC-6A pump, an SPO-6AV spec-

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trophotometric detector, a Chromatopac C-RGA, Shimadzu Co., Ltd., Kyoto, Japan). The method used in the experiments is a modification of the procedure presented in the USP 25 for Cefprozil for Oral Suspension. The only modification introduced was the internal standard, salicylic acid, used to determine cefprozil. Chromatography was carried out on a LiChrospher RP-18 column (Merck, Germany) (250 × 4 mm, 5 mm particle size). The mobile phase consisted of a mixture of acetonitrile and monobasic ammonium phosphate solution (10.35 g of $NH_4H_2PO_4$ was dissolved in water to make 900 mL, and adjusted, if necessary, with phosphoric acid to pH 4.4; 100 mL of acetonitrile was added and mixed). The flow rate was 1.0 mL/min. The liquid chromatograph was equipped with UV detector set at 280 nm. The injector was a Rheodyne 7120 with a loop of 50 µL. The internal standard was a solution of salicylic acid in a mixture (1:9 v/v) of acetonitrile and water at a concentration of 0.1 mg/mL. The study was performed at ambient temperature.

Validation of the HPLC method

The HPLC method was validated according to the guidelines of the International Conference on Harmonization (11).

Selectivity

The selectivity of the HPLC method was examined for non-degraded, degraded samples and internal standard (samples were stored at 343 K and 76.4% RH).

Linearity

Calibration curves for HPLC analysis were determined by linear regression. The linearity between P/P_{IS} (P and P_{IS} – areas of cefprozil and internal standard (W)) and concentration of cefprozil in a mixture (1:9) of acetonitrile and water, ranging from 6.51 µg/mL to 97.66 µg/mL, was evaluated. To 1.0 mL of cefprozil solution 1.0 mL of internal standard solution was added and the so obtained solutions were analyzed. 50 µL samples of these solutions were injected onto the column. Linearity was also examined for three consecutive days in solutions of the same concentration prepared from the stock solution.

Precision

The precision of the method is expressed as the relative standard deviation (RSD) of replicate measurements. In order to evaluate the repeatability (intra-day) of the method, eight samples of three different concentrations (low, c = 0.13 mg/mL; medium, c = 0.26 mg/mL; high, c = 0.39 mg/mL), were prepared and analyzed on the same days. The intermediate precision (inter-day) was studied by comparing the assays performed on two different days at cefprozil concentration of 0.26 mg/mL.

Limits of detection and quantitation

The limits of detection and quantitation were calculated from the formulas DL= 3.3 S_y/a and QL = 10 S_y/a, where S_y is the standard deviation and a is the slope of the corresponding calibration curve.

Kinetic measurements

The study of the stability of cefprozil in CEFZIL was performed using the stress degradation test (at 333–353 K, relative air humidity 50.9%–90.0%). Samples of preparation (25.00 mg, equivalent to 2.6 mg of cefprozil) were accurately weighed into 5 mL vials. Samples tested for the influence of temperature in a humid environment were placed in desiccators containing saturated solutions of sodium chloride (~76.4% RH) inserted in heat chambers set at 333, 338, 343, 348 and 353 K. To assess the effect of relative air humidity on the stability of cefprozil, the vials with CEFZIL were placed in desiccators containing saturated aqueous solutions of appropriate inorganic salts, which ensured the desired relative humidity of the ambient air (12) (sodium bromide, ~50.9% RH, sodium nitrate, ~66.5% RH, sodium chloride, ~76.4% RH, zinc sulfate, ~90.0% RH), and inserted in heat chambers set to 333 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 (1:9) 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. 50 µL samples of the solutions were injected onto the column.

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

RESULTS AND DISCUSSION

Changes in the concentration of cefprozil under the conditions of the study were evaluated using the HPLC method presented in USP 25 for Cefprozil for Oral Suspension. The HPLC method was validated with respect to selectivity, linearity, precision, detection limit and quantitation limits.

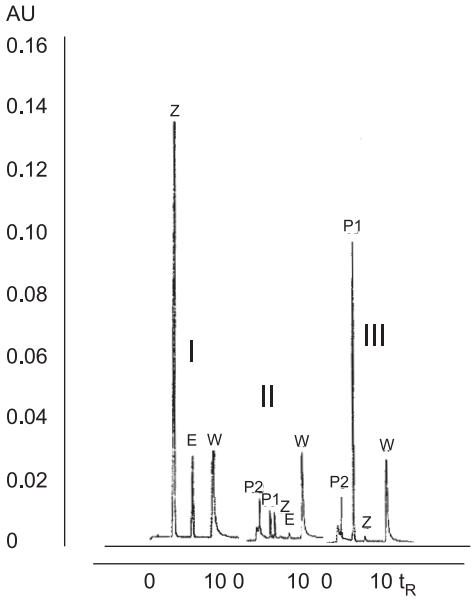


Figure 1. HPLC chromatograms of *Z*- and *E*-cefprozil, their degradation products (P_1 and P_2) and internal standard (W) after incubation at 343 K (76.4% RH): I at t = 0 h; II at t = 148 h; III at t = 456 h.

The HPLC method was found selective for *Z*-cefprozil (t_R = 5.46 min), *E*-cefprozil (t_R = 8.37 min), internal standard (salicylic acid, t_R = 11.31 min) and the degradation products (P_1, t_R = 4.68 min and P_2, t_R = 2.59 min) (Figure 1).

The linearity of the method was obtained between the areas of the peaks and the concentration of cefprozil in the range 6.51 – 97.66 mg/mL. The linear dependence was described by the equation: $y = (5.62 \pm 0.11)\,x$; r = 0.9980; n = 15 (for the equation $y = ax + b$, the value b is insignificant; t_b = 0.998; $t_{k(13)}$ = 2.16).

The precision of the method was adequate because the RSD was less than 2% (0.40–1.60%). Intermediate precision was evaluated for 16 replicates and its variation coefficient was 1.96%.

Under the conditions of this study the detection limit was 5.96 mg/mL and the quantitation limit was 18.07 mg/mL.

Therefore, the procedure applied may be used to determine the stability of cefprozil in CEFZIL.

The kinetics of the degradation of cefprozil

Determination of rate constants

The degradation of cefprozil at increased temperature and relative air humidity occurs as reversible-consecutive and parallel reactions (Figure 2) and is a consequence of the reaction illustrated below.

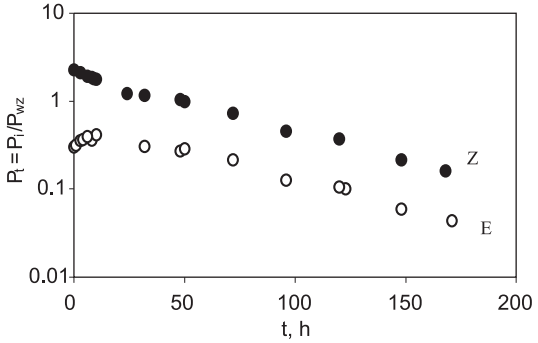


Figure 2. Semilogarithmic plots $P_t = f(t)$ for the degradation of *Z*- and *E*-cefprozil at 343 K (76.4%RH).

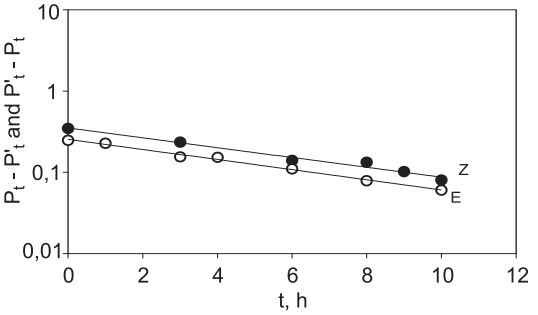


Figure 3. Semilogarithmic plots $(P_t - P'_t) = f(t)$ (*Z*-cefprozil) and $(P'_t - P_t) = f(t)$ (*E*-cefprozil) reaction of izomerization of *Z*- and *E*-cefprozil at 343 K (RH = 76.4%) at $t < t_e$.

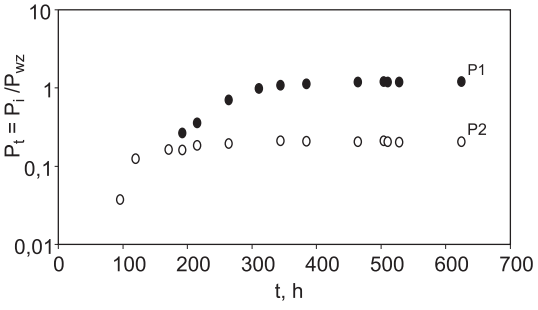
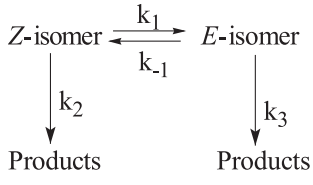


Figure 4. Semilogarithmic plots $P_t = f(t)$ of the reaction of formation of the degradation products of *Z*- and *E*-cefprozil (P_1 and P_2) at 343 K (76.4% RH).



In diagram, k_1 is the *Z* to *E* isomerization rate constant, k_{-1} is the reverse; k_2 and k_3 are the degradation

rate constants of *Z*- and *E*-isomers. The following kinetic equations can be used to calculate the degradation rate constants of *Z*- and *E*-isomers:

$$d[Z]/dt = -k_1[Z] + k_{-1}[E] - k_2[Z]$$

$$d[E]/dt = k_1[Z] + k_{-1}[E] - k_3[E]$$

The first-order rate constants k_2 and k_3 were calculated from the concentration of *Z*-cefprozil and *E*-cefprozil in the time range $t > t_e$ (Figure 2). Semilogarithmic plots $P_t = f(t)$ in the time range $t > t_e$ were linear and described by the equation: $\ln P_t = \ln P_0 - k_i t$ (i = 2 or 3).

The rate constants of the reversible reaction

Z-cefprozil ⇌ *E*-cefprozil

were calculated from the remainder plots $\ln (P_t - P'_t) = f(t)$ for *Z*-cefprozil and $\ln (P'_t - P_t) = f(t)$ for *E*–cefprozil in the time range $t_0 \rightarrow t_e$ (Figure 3). The slopes of these plots $a = -k_s$, which is the sum of rate constants k_1 and k_{-1} of the reactions, i.e. direct and reverse reactions. Partial reaction rate constants k_1 and k_{-1} were calculated using the equations $k_{-1} = k_s/(1+K)$ and $k_1 = k_s - k_{-1}$. Equilibrium constant K was calculated from the equation

$$K = k_1/k_{-1} = [E - \text{cefprozil}]_e/[Z\text{-cefprozil}]_e = (c_0 - c_e)/c.$$

For straight-line plots the parameters of the equation $y = a\,x + b$, $a \pm \Delta a$, $b \pm \Delta b$, *standard*

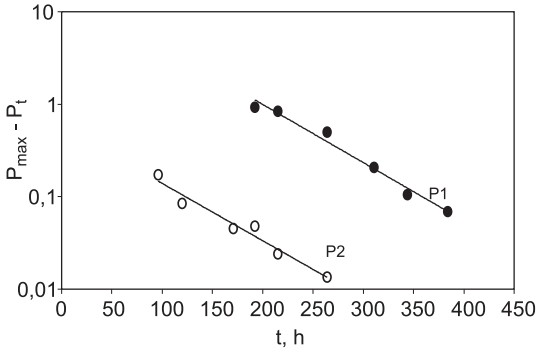


Figure 5. Semilogarithmic plots $(P_{max} - P_t) = f(t)$ for the formation of the degradation products of *Z*- and *E*-cefprozil (P_1 and P_2) at 343 K (76.4% RH).

Table 1. Kinetic and thermodynamic parameters for the reaction of the degradation of cefprozil in oral suspension CEFZIL at 76.4% RH.

Parameters	10^6 (k ± Δk), s^{-1}	Statistical evaluation $\ln k_i = f(1/T)$	Thermodynamic parameters
k_2			
333 K	0.794 ± 0.059	*a* = - 20747 ± 780	
338 K	1.74 ± 0.14	S_a = 245.1	E_a = 172.5 ± 6.5 (kJ/mol)
343 K	4.04 ± 0.37	*b* = 48.2 ± 2.3	$\Delta H^{\neq a}$ = 170.0 ± 8.9 (kJ/mol)
348 K	11.1 ± 1.3	S_b = 0.715	$\Delta S^{\neq a}$ = 155.6 ± 226.0 (J/K mol)
353 K	27.2 ± 1.9	r = -0.9994	
k_3			
333 K	0.794 ± 0.061	*a* = -20734 ± 654	
338 K	1.74 ± 0.13	S_a = 205.5	E_a = 172.4 ± 5.4 (kJ/mol)
343 K	4.04 ± 0.46	*b* = 48.1 ± 1.9	$\Delta H^{\neq a}$ = 169.9 ± 7.9 (kJ/mol)
348 K	11.2 ± 1.4	S_b = 0.600	$\Delta S^{\neq a}$ = 155.3 ± 229.0 (J/K mol)
353 K	27.0 ± 4.7	r = -0.9997	

E_a, activation energy; $\Delta H^{\neq}$, enthalpy; $\Delta S^{\neq}$, entropy; $E_a = -aR$ [J/mol]; $\Delta H^{\neq} = E_a - RT$ [J/mol];
$\Delta S^{\neq} = R\,[\ln A - \ln (k_B \cdot T/h)]$ [J/(K · mol)], where k_B stands for the Boltzmann constant ($1{,}3805 \cdot 10^{-23}$ J/K);
h, Planck constant ($6{,}6256 \cdot 10^{-24}$ J/s); *R*, universal gas constant (8,3144 /(K · mol));
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 effect of relative air humidity on the stability of cefprozil in oral suspension CEFZIL at 343 K.

RH (%)	$(k_i \pm \Delta k) \times 10^{-6}$ (s^{-1})	*n*	Statistical evaluation $\ln k_i = f(RH\%)$
k_2			
50.9	0.114 ± 0.036	7	
66.5	0.512 ± 0.097	9	$a = (11.1 \pm 6{,}8) \times 10^{-2}$
76.4	4.04 ± 0.37	9	*b* = -21.3 ± 4.8
90.0	8.06 ± 0.82	9	r = 0.9801
k_3			
50.9	0.108 ± 0.038	8	
66.5	0.512 ± 0.044	6	$a = (11.1 \pm 6{,}8) \times 10^{-2}$
76.4	4.04 ± 0.46	7	*b* = -21.3 ± 4.8
90.0	8.06 ± 0.76	7	r = 0.9801

errors: Sa, Sb, Sy, and correlation coefficient *r* were calculated. The values ± Δa and ± Δb were calculated for f = n – 2 degrees of freedom and a = 0.05. The ratio *E*-cefprozil/*Z*-cefprozil was approximately 0.18 at 343 K and 76.4% RH.

The rate constants of the formation of products P_1 and P_2 at 343 K (76.4% RH) were also calculated. The concentration of these products (expressed as P_t) changed from 0 to P_{max} (Figure 4). The remainder semilogarithmic plots ($P_{max} - P_t$) = f(t) of products P_1 and P_2 were linear (Figure 5) and their slopes were the rate constants of the formation of products P_1 or P_2. The values of the formation rate constants of products P_1 ($k = (4.02 \pm 0.68)10^{-6}$ s^{-1}) and P_2 ($k = (3.97 \pm 0.11)10^{-6}$ s^{-1}) are comparable with the degradation rate constants of *Z*-cefprozil ($k = (4.04 \pm 0.37)10^{-6}$ s^{-1}) and *E*- cefprozil ($k = (4.04 \pm 0.46)10^{-6}$ s^{-1}) under the same conditions. Under the other conditions only rate constants k_2 and k_3 were determined.

The effect of temperature

The values of reaction rate constants k_2 and k_3 were used to calculate the Arrhenius relationship in order to establish the influence of temperature on the rate of the reaction at 76.4% RH. Based on the parameters of the slope $\ln k_i = f(1/T)$, the energy of activation, enthalpy and entropy for 298 K of *Z*- and *E*-cefprozil were calculated (Table 1).

The effect of humidity

The influence of relative air humidity on the stability of cefprozil is expressed by the equations

$\ln k_2 = (11.1 \pm 6.8)10^{-2}$ (RH%) – (21.3 ± 4.8)

$\ln k_3 = (11.2 \pm 7.0)10^{-2}$ (RH%) – (21.4 ± 5.0)

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 cefprozil stability at 333 K, while the value $10^b = k_0$ represented cefprozil stability at 333K and at 0% RH (Table 2).

CONCLUSIONS

The degradation of cefprozil in CEFZIL occurs as reversible-consecutive and parallel reactions. The degradation of *Z*- and *E*-cefprozil yields two main products, P_1 and P_2. The reversible reaction of izomerization is approx. 10 times faster than the parallel reaction of degradation of individual isomers. The values of the formation rate constants of products P_1 and P_2 are comparable with the degradation rate constants of *Z*-cefprozil and *E*-cefprozil under the same conditions. At the time $t \rightarrow t_8$ the concentrations of products P_1 and P_2 reach maximal values, which indicates that they do not undergo further degradation. The thermodynamic parameters of the reactions *Z*-cefprozil ® product 1 and *E*-cefprozil ® product 2 and the influence of relative air humidity on these reactions do not differ significantly.

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