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KINETICS AND MECHANISM OF DEGRADATION OF CEFOZOPRAN HYDROCHLORIDE IN THE SOLID STATE

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Abstract: The process of degradation was studied by using an HPLC-DAD method. Two degradation products were identified with a hybrid ESI-Q-TOF mass spectrometer. The influence of temperature and relative air humidity (RH) on the stability of cefozopran hydrochloride was investigated. In the solid state the degradation of cefozopran hydrochloride was a first-order reaction depending on the substrate concentration. The kinetic and thermodynamic parameters of degradation were calculated.

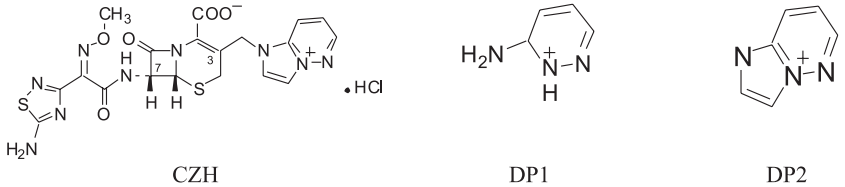
Keywords: cefozopran hydrochloride, HPLC, Q-TOF, stability, solid state

Cefozopran hydrochloride (CZH) (Fig. 1) is a parenteral, fourth-generation cephalosporin with broad spectrum activity against Gram-positive and Gram-negative organisms (1). Moreover, cefozopran has comparatively good activity against *Enterococci* and *Pseudomonas aeruginosa*, which are refractory to other cephalosporins (2). The main reason why CZH exhibits strong antimicrobial activity is assumed to be that it is stable against β-lactamase and that its action of inhibiting cell wall peptidoglycan cross-bridge formation is strong because it has a powerful affinity for penicillin binding proteins 1 and 2 of *Staphylococcus aureus* as well as for penicillin binding protein 3 of *Escherichia coli* and *P. aeruginosa* (3).

CZH has been clinically available for the treatment of various infections such as pneumonia, sepsis, urinary-tract and intra-abdominal infections in adult patients (2). CZH monotherapy is effective for the empirical treatment of pediatric cancer patients with febrile neutropenia (4-6). It is generally well-tolerated drug but fatal toxic epidermal necrolysis caused by CZH was observed (7). Probably, this adverse effect is caused by degradation products or impurities.

Chromatographic methods for the determination of CZH have proved the formation of many degradation products without their structural characterization (8-12).

The aim of this work was to investigate the kinetic of CZH degradation in the solid state and to identify degradation products.



CZH DP1 DP2

Figure 1. Chemical structures of CZH and its degradation products in solid state

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EXPERIMENTAL

Standards and reagents

CZH was obtained from CHEMOS GmbH, Regenstauf, Germany. It is a white or pale yellowish white, crystalline 98% pure powder soluble in water and conforms to the standards of Japanese Pharmacopoeia XV. All other chemicals and solvents were obtained from Merck KGaA (Germany) and were of analytical grade. High-quality pure water was prepared by using a Millipore Exil SA 67120 purification system (Millipore, Molsheim, France).

Kinetic analysis

For the kinetic study, the Dionex Ultimate 3000 analytical system consisted of a quaternary pump, an autosampler, a column oven and a diode array detector was used. As the stationary phase, a Lichrospher RP-18 column, 5 µm particle size, 250 mm × 4 mm (Merck, Darmstadt, Germany) was used. The mobile phase was composed of acetonitrile and 0.1% formic acid (8 : 92, v/v). The flow rate of the mobile phase was 1.0 mL/min and the injection volume was 10 µL. The wavelength of the DAD detector was set at 260 nm. Separation was performed at 30°C.

The stability tests were performed according to the International Conference on Harmonization Guidelines (13).

Five mg aliquots of CZH were weighed into glass vials. In order to test the influence of such environmental factors as temperature and humidity, the samples were placed in desiccators containing saturated solutions of inorganic salts: sodium bromide (RH ~50.9%), sodium nitrate (RH ~66.5%), sodium chloride (RH ~76.4%) and zinc sulfate (RH ~90.0%) that were in incubators (Wamed, Warszawa, Poland) set to the desired temperatures (333, 343, 353, and 363 K)

To evaluate the stability of CZH in dry air, the vials containing 5.0 mg of this substance were immersed in a sand bath placed in a heat chamber at 393 K.

Each batch to be studied comprised 8–12 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 (1 : 1, v/v) of acetonitrile and water. The solutions obtained in that way were quantitatively transferred into volumetric flasks and diluted to a total volume of 10.0 mL with the same mixture of solvents. After filtration, the 10 µL samples were injected onto the column.

LC-MS analysis

The LC-MS analysis was performed with the use of an Agilent Accurate-Mass Q-TOF LC/MS G6520B system with a DESI ion source and an Infinity 1290 ultra-high-pressure liquid chromatography system consisting of a G4220A binary pump, a G1330B FC/ALS thermostat, a G4226A autosampler, a G4212A DAD detector and a G1316C TCC module (Agilent Technologies, Santa Clara, USA). The chromatographic conditions were analogous to those described in the kinetic study. The MassHunter workstation software B.04.00 was used for the control of the system, data acquisition and qualitative analysis.

The Q-TOF detector was tuned in the positive (4 GHz) mode and the main parameters were optimized as follows: gas temp. 300°C, drying gas 10 L/min, nebulizer pressure 40 psig, capillary voltage 3500 V, fragmentor voltage 200 V, skimmer voltage 65 V, octopole 1 RF voltage 250 V. The data were acquired in the auto MS/MS mode with the mass range 50-1050 *m/z* and the acquisition rate 1.2 spectra/s (for MS and MS/MS data). The collision energy was calculated from the formula 2V (slope) × (*m/z*)/100 + 6 V (offset) and maximum 2 precursors per cycle were selected with an active exclusion mode after 1 spectrum for 0.2 min. To ensure accuracy in mass measurements, reference mass correction was used. Masses 121.0508 and 922.0097 were used as lock masses.

Calculations

The rates constants of a first-order reaction were calculated from:

$$\ln c = \ln c_0 - k_{obs} \times t \qquad \text{(equation 1)}$$

where c_0 and c are concentrations at time t = 0 and t, respectively, and k_{obs} is the observed rate constant of degradation (14).

Thermodynamic parameters (E_a, activation energy; $\Delta H^{\neq}$, enthalpy; $\Delta S^{\neq}$, entropy) were calculated from:

$$E_a = -a \times R \qquad \text{(equation 2)}$$

$$\Delta H^{\neq} = E_a - T \times R \qquad \text{(equation 3)}$$

$$\Delta S^{\neq} = R \times (\ln A - \ln(k_B \times T/h)) \qquad \text{(equation 4)}$$

where: k_B = Boltzmann's constant (1.3807×10^{-23} J × K^{-1}); h = Planck's constant (6.626×10^{-34} J × s); R = universal gas constant (8.314 $K^{-1} \times mol^{-1}$), T = temperature [K]; a = slope of the Arrhenius relationship; A = frequency coefficient where: (ln A = b) (14).

Statistical parameters of the respective equations were calculated using Microsoft Excel 2010.

RESULTS AND DISCUSSION

Changes in the concentration of CZH under stress study conditions were evaluated using the

Table 1. Q-TOF accurate mass elemental composition and MS/MS fragmentation of the analyzed substances.

Comp. No.	Name	Retention time (min)	Measured mass (*m/z*)	Theoretical mass (*m/z*)	Mass error (ppm)	Molecular formula $[M+H^+]$	MS/MS fragmentation (*m/z*)	Fragmentation ions formulas
1	DP1	1.22	96.05512	96.05562	5.26	$C_4H_6N_3$	81.95996 68.05020 55.95761	$C_4H_6N_2$ C_4H_6N C_3H_3N
2	CZH	4.1	516.08805	516.08668	2.65	$C_{19}H_{18}N_9O_5S_2$	369.04325 325.05264 167.02687 120.05525	$C_{12}H_{13}N_6O_4S_2$ $C_{11}H_{13}N_6O_2S_2$ $C_7H_7N_2OS$ $C_6H_6N_3$
3	DP2	5.3	120.05543	120.05562	1.61	$C_6H_6N_3$	93.04294 79.02758	$C_5H_5N_2$ $C_4H_3N_2$

HPLC method and were linear in the range 20-300 mg/L, accurate (RSD 99.78-100.85 %), precise (RSD 0.12-0.47 %) and selective in the presence of CZH and its degradation products. The LOD and LOQ values were 6.72 and 20.51 mg/L, respectively. In the chromatograms of CZH developed over a period of 0 to 10 min, the following compounds were eluted: CZH with a retention time (t_R) of 4.1 min, degradation products (DP1) with t_R = 1.22 min and DP2 with t_R = 5.3 min.

The degradation of CZH in the solid state was a first-order reaction depending on the substrate concentration. The rate constants were determined from obtained results by using the least squares method for the equation:

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

where c_t and c_0 are the concentrations of CZH (y) at time t = 0 and t (x), respectively, and k_{obs} is the rate constant of the degradation reaction. 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 the linear correlation r ($y = c_t$, x = time). The values of error range of slope (Δa) and intercept (Δb) were obtained for f = n – 2 degrees of freedom, with $\alpha = 0.05$.

Other 4th generation cephalosporin: cefpirome sulfate (CPS) and cefepime dihydrochloride monohydrate (CPH) are degraded with the same kinetic mechanism as CZH (15, 16). Ceftriaxone disodium (CTD) and cefoselis sulfate (CSS), despite structural similarities to CZH, are degraded according to an autocatalytic reaction of the first order with respect to substrate concentration at increased RH and temperature (17, 18).

High-resolution LC-MS analysis allowed identification of the parent compound as well as degradation products of CZH in the solid state. As shown in Table 1, the molecular ions of CZH and its degradation products were found with very high accuracy (1.61–5.26 ppm), which permitted calculation of the chemical formulas for all the analyzed compounds. Additionally, the MS/MS fragmentation spectra that were obtained (main fragmentation ions are listed in Table 1) confirmed the proposed structures of all of the identified substances (Fig. 1).

Based on the kinetic mechanism of degradation identified as a first-order reaction depending on the substrate concentration, it may be assumed that products of CZH degradation (Fig. 1) do not have any catalytic effect on the degradation process in the solid phase.

The effect of RH on the stability of CZH at 363 K is presented in Table 2. As expected, RH turned

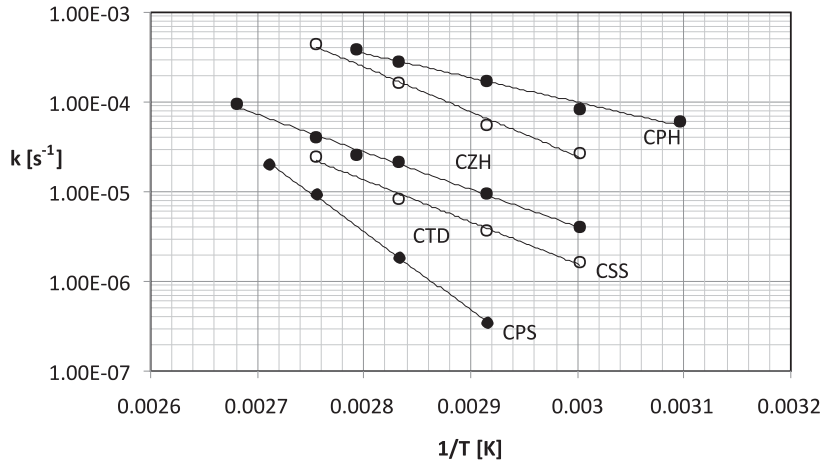

Figure 2. Semilogarithmic relationship k = f(1/T) for degradation of CZH, CPS (14), CPH (15), CTD (16) and CSS (17) at RH ~ 76.4%; CZH, CPS, CPH and CSS (4th generation cephalosporins), CTD (3rd generation cephalosporin)

Table 2.The effect of relative air humidity on the stability of CZH at 363 K.

Relative air humidity (%)	10^{-1} (k ± Δk) [s^{-1}]	Statistical evaluation ln k = f(RH%)
50.9	(0.71 ± 0.16)	a = 0.06 ± 0.03
66.5	(2.33 ± 0.24)	S_a = 6.95 × 10
76.4	(4.52 ± 0.21)	b = -12.50 ± 2.16 S_b = 0.50
90.0	(7.46 ± 0.60)	r = 0.9871 S_y = 0.20

Table 3. Kinetic and thermodynamic parameters of degradation of CZH in solid state at RH ~ 76.4%.

T [K]	10^5 (k ± Δk) [s^{-1}]	Statistical evaluation ln k = f(1/T)	Thermodynamic parameters
333	(2.72 ± 0.19)	a = -11472.35 ± 3621.09	E_a = 95.385 ± 30.107 [kJ mol^{-1}]
343	(5.60 ± 0.53)	S_a = 841.52	$\Delta H^{\neq a}$ = 92.907 ± 30.107 [kJ mol^{-1}]
353	(16.08 ± 0.84)	b = 23.81 ± 10.42	$\Delta S^{\neq a}$ = – 46.93 ± 86.65 [JK^{-1} mol^{-1}]
363	(45.18 ± 2.09)	S_b = 2.42 r = 0.9947 S_y = 0.15	

[a] calculated for 298 K.

out to be key factor determining the degradation of CZH. The more rapid degradation of CZH at increased RH in comparison to CPS, CSS and CTD (Fig. 2, Table 4) resulted from the susceptibility of the C-3 and C-7 substituents to degradation under the influence of humidity. Therefore, the structure of those substituents is responsible for the pharmacological properties and stability of CZH in the solid state. In this study, the impact of temperature on CZH stability at increased RH was also investigated. The values of the 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 (Fig. 2). The energy of activation and the thermodynamic parameters, enthalpy and entropy for 298 K, were calculated from the parameters of the slope ln k_i = f(1/T) (Table 3). In the solid state at RH ~ 76.4% CPS had the highest activation energy (E_a) and CPH the lowest. Nevertheless, CSS was the most stable of all the compared cephalosporins, most likely due to a different kinetic mechanism of degradation. The stabil-

Table 4. Kinetic and thermodynamic parameters of degradation of CZH, CPS (15), CPH (16), CTD (17) and CSS (18) in solid state at constant relative air humidity (RH ~ 76.4%) and at T= 363 K

Cephem	E_a, [kJ mol^{-1}]	Half-life ($t_{0.5}$) [h]	Kinetic mechanism of degradation
CZH	95 ± 30	0.4	first-order reaction depending on the substrate concentration
CPS	167 ± 16 (15)	20.2 (15)	first-order reaction depending on the substrate concentration (15)
CPH	52 ± 12 (16)	0.4*	first-order reaction depending on the substrate concentration (16)
CTD	79 ± 9 (17)	23.1 (17)	autocatalytic reaction of the first order with respect to substrate concentration (17)
CSS	90 ± 26 (18)	34.5 (18)	autocatalytic reaction of the first order with respect to substrate concentration (18)

*calculated from extrapolated data

ity of CZH was very similar to that of CPH and was the lowest of the compared cephems.

CONCLUSIONS

The degradation of CZH in the solid state in dry air and at increased relative air humidity is a first-order reaction. The kinetic mechanism of CZH degradation does not depend on storage conditions. The degradation products of CZH do not have catalytic effect on the degradation process in the solid phase.

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