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ANALYSIS

STABILITY OF CEFPIROME SULFATE IN AQUEOUS SOLUTIONS

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Abstract: The influence of pH on the stability of cefpirome sulfate was investigated in the pH range of 0.44 – 13.00. The degradation of cefpirome sulfate as a result of hydrolysis was a pseudo-first-order reaction. General acid-base hydrolysis of cefpirome sulfate was not observed. In the solutions of hydrochloric acid, sodium hydroxide, acetate, borate and phosphate buffer, $k_{obs} = k_{pH}$ because specific acid-base catalysis was observed. Specific acid-base catalysis of cefpirome sulfate consisted of the following reactions: hydrolysis of cefpirome sulfate catalyzed by hydrogen ions (k_{H+}), hydrolysis of dications (k_{1H_2O}) monocations ($k_{2\ H_2O}$), zwitter ions (k_{3H_2O}) and monoanions ($k_{4\ H_2O}$) of cefpirome sulfate under the influence of water. The total rate of the reaction was equal to the sum of partial reactions $k_{pH} = k_{H+} \times a_{H+} + k_{1H_2O} \times f_1 + k_{2H_2O} \times f_2 + k_{3H_2O} \times f_3 + k_{4\ H_2O} \times f_4$. Based on the dependence $k_{pH} = f(pH)$ it was found that cefpirome sulfate was the most stable in aqueous solutions in the pH range of 4-6.

Keywords: cefpirome sulfate, HPLC, stability in aqueous solutions

Cefpirome sulfate (CPS) (Fig. 1) is a new fourth-generation cephalosporin for parenteral administration. It is an oxime-type cephem with a 2-amino-thiazolylmethoxyimino group in the side chain at position 7, while at position 3 it is cyclopentapyridine. Those elements in structures are responsible for the high level of resistance to β-lactamases and the broad spectrum of antibacterial activity of CPS.

CPS is an active against Gram-positive bacteria, including *Staphylococcus aureus* and Gram-negative microorganisms such as *Pseudomonas aeruginosa* (1-3). It is clinically available for the treatment of various infections such as pneumonia and sepsis as well as urinary-tract and intra-abdominal infections in adult patients (4-7). The approved dosing regimen for intravenous CPS is 1–6 g daily in two or up to four divided doses (7). Fourth-generation cephalosporins have surprisingly few serious side effects, which makes them an attractive for use in the treatment of a wide variety of serious infections. The most common adverse symptoms are nonspecific circulatory disorders (chills, tachycardia, hypertension, nausea, dyspnea, cold perspiration, weak concentration, and dizziness). All adverse effects are of mild or moderate severity and do not last long. Spontaneous improvement leading to complete recovery can be observed. As most of the side effects of β-lactams are caused by the generation of degradation products, it is important to estimate their stability. Earlier studies have confirmed that cephalosporins are susceptible to degradation in aqueous solutions (8-14) and in the solid state (15-24). The degradation of CPS in the solid state in dry air and at increased relative air humidity was a first-order reaction (24). The kinetic mechanism of CPS degradation was not depending on storage conditions (24). CPS was reported to be stable in the pH range of 4-7, slightly unstable below pH 3 and to rapidly degrade at pH 9 and higher in aqueous solution (12). The degradation pathways both in aqueous solutions (12) and in solid state (24) were also described.

The aim of this work was to investigate the process of CPS degradation in the aqueous solutions in the pH range of 0.44–13.00 and determination the total rate of CPS degradation. The obtained results were compared with other fourth-generation cephalosporin cefoselis sulfate (CSS) (11).

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EXPERIMENTAL

Materials

CPS 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 Pharmacopeia XV. All other chemicals and solvents were obtained from Merck KGaA (Germany) and were of analytical grade. High-quality pure water was prepared using a Millipore Exil SA 67120 purification system (Millipore, Molsheim, France).

Instrumentation

Chromatographic separation and quantitative determination of CPS were performed by using the Dionex Ultimate 3000 analytical system consisted of a quaternary pump, an autosampler, a column oven and a diode array detector. As the stationary phase a Lichrospher RP-18 column, 5 μm particle size, 125 × 4 mm (Merck, Darmstadt, Germany) was used. The mobile phase composed of acetonitrile – 12 mM ammonium acetate (10 : 90, 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 270 nm. Separation was performed at 30°C (25).

Kinetic studies

The degradation of CPS in aqueous solutions was studied at 353 K in hydrochloric acid (pH 0.44–1.39), phosphate (pH 2.22–3.38 and 6.16–7.95), acetate (pH 4.23–5.58) and borate (pH 8.32–9.84) buffers. The degradation of CPS in aqueous solutions of sodium hydroxide (pH 11.24–13.00) was studied at 298 K, 303 K, 308 K and 313 K and obtained results were extrapolated to 353 K. The pH values of the reaction solutions and those of the buffer used to calibrate the pH-meter were measured at reaction temperature. The ionic strength of solutions was adjusted to 0.5 M with a solution of sodium chloride (4 M). All solutions of CPS were protected from light. Degradation was initiated by dissolving an accurately weighed CPS (2.5 mg) in 12.5 mL of reaction solution heated to the required temperature. At specified time intervals, determined by the rate of degradation, samples of the reaction solutions (0.5 mL) were collected, neutralized if necessary and instantly cooled with a mixture of ice and water. Ten μL samples of the solutions were injected into the column.

RESULTS AND DISCUSSION

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

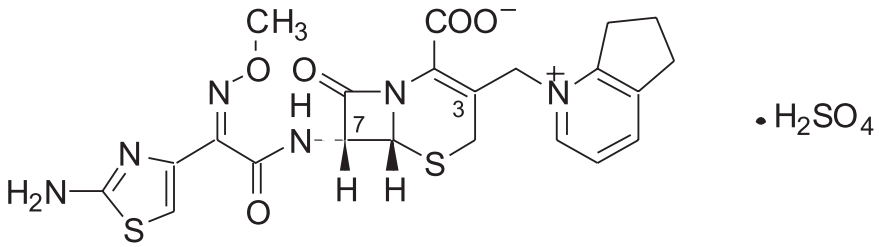

Figure 1. Chemical structure of cefpirome sulfate

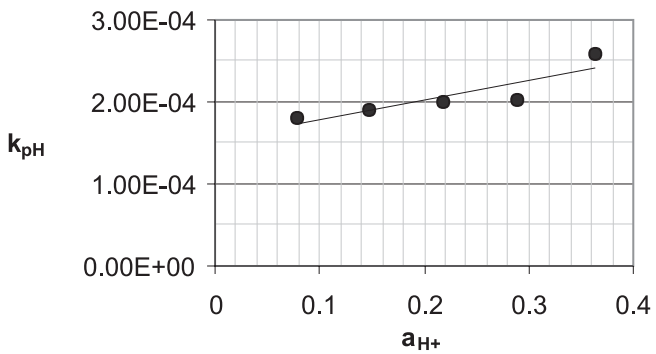

Figure 2. The plot $k_{pH} = f(a_{H+})$ for the degradation of CPS at 353 K

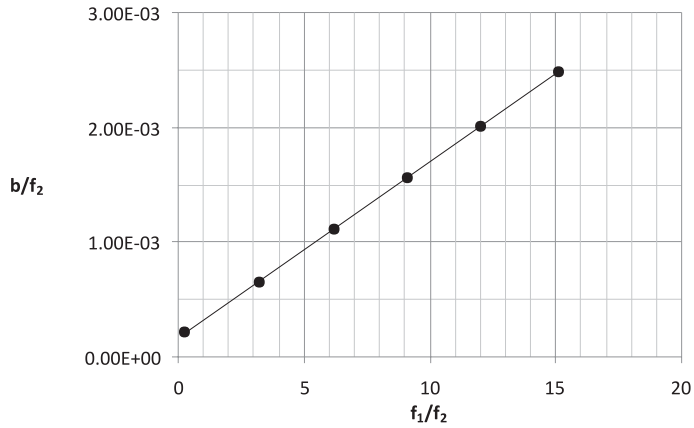

Figure 3. The plot $b/f_2 = f(f_1/f_2)$ for the degradation of CPS at 353 K

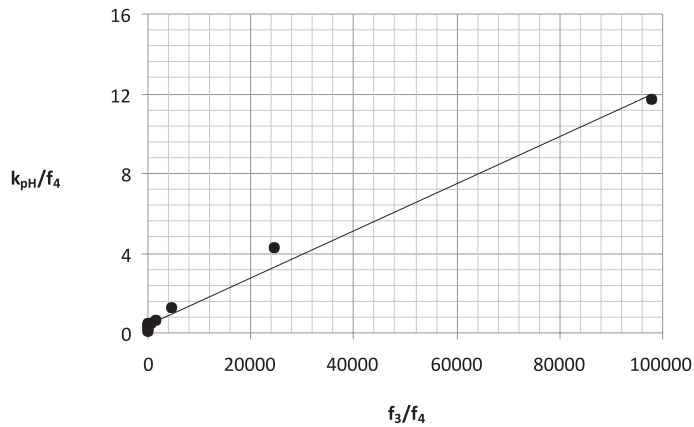

Figure 4. The plot $k_{pH}/f_4 = f(f_3/f_4)$ for the degradation of CPS at 353 K

HPLC method, which was linear in the range 7.2-300 mg/mL, precise (RSD 0.3-1.4%) and selective in the presence of CPS and its degradation products. The recovery of the method was in the range of RSD 98-104%. The LOD and LOQ were 2.4 and 7.2 mg/mL, respectively (25).

The observed rate constants of CPS were determined in the pH range of 0.44–13.00. The pH values of the reaction solutions in HCl were calculated from the equation pH = -log f_{HCl} [HCl]. The activity coefficients f_{HCl} were obtained from the literature or calculated by interpolation of literature data (26). The pH values of buffer were calculated by average value pH of buffer of different concentrations. The observed rate constants were described by the following equation of a pseudo first-order reaction:

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

where: c_t and c_0 are the time-dependent concentration and the initial concentration of CPS, at the time $t > 0$ and $t = 0$, respectively, k_{obs} is the observed rate constant of the pseudo first-order reaction of CPS degradation. The observed rate constants are equal to the slopes of the plots $\ln c_{CPS} = f(t)$, with a negative sign ($-k_{obs}$). The following statistical parameters of the equation $y = ax + b$ were calculated by using the least squares method: $a \pm \Delta a$, $b \pm \Delta b$, standard deviations Sa, Sb, Sy and the coefficient of linear correlation r. The values Δa and Δb were calculated for $f = n-2$ degrees of freedom and $\alpha = 0.05$. The number of measurements of c_t for each series ranged from 8 to 12.

Buffer catalysis

At constant pH, ionic strength and temperature, in the presence of excess acetate, borate and phos-

phate buffers, the observed rate constant of degradation of CPS did not depend on the total concentration of buffers. That indicates that components of those buffers did not catalyze the degradation of CPS. To verify that k_{obs} determined at different concentration buffer were statistically insignificant, the parallelism test was used. In the reaction solutions of HCl, buffers and NaOH general acid-base catalysis was not observed, therefore the values of $k_{obs} = k_{pH}$.

pH-rate profiles

The rate constants k_{pH} determined in hydrochloric acid, sodium hydroxide, phosphate, borate and acetate buffers were used to calculated the relationship log k_{pH} = f(pH). The semilogarithmic relationship k-pH indicates that in water solution (pH 0.44–13.00) the following reactions are possible:

- degradation of CPS catalyzed by hydrogen ions (k_{H^+}),
- degradation of dications (k_{1H_2O}), monocations (k_{2H_2O}), zwitter ions (k_{3H_2O}) and monoanions of CPS (k_{4H_2O}) under the influence of water.

The total rate of reaction is equal to the sum of partial reactions rates:

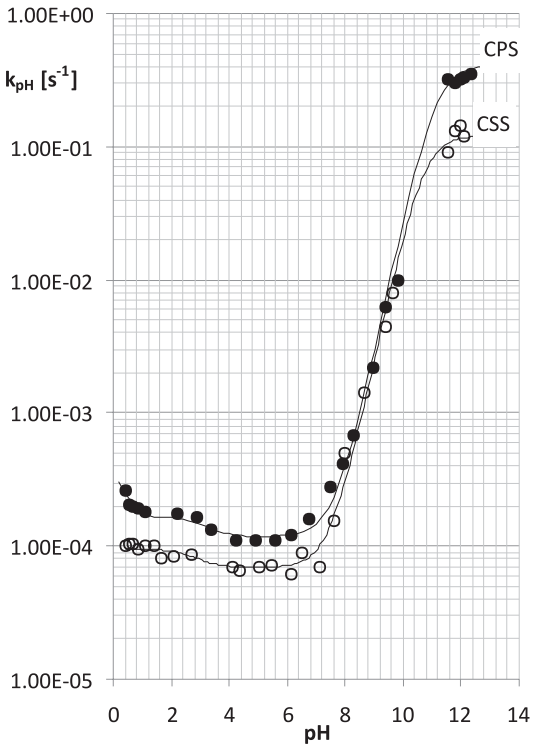

Figure 5. Log k_{pH} = f(pH) profile for the degradation of CPS and CSS (11) at 353 K. The points are determined experimentally and the lines were calculated from determined equation

$$k_{pH} = k_{H+} \times a_{H+} + k_{1H_2O} \times f_1 + k_{2H_2O} \times f_2 + k_{3H_2O} \times f_3 + k_{4H_2O} \times f_4$$

where f_1, f_2, f_3, f_4 – the fractions of the molecules of CPS. The values of $f_{1–4}$ were calculated after taking into account values pK_a of CPS that were about 1.62, 3.11 and 11.15 (27).

The catalytic rate constant (k_{H^+}) was calculated from the plot $k_{pH} = f(a_{H^+})$ taking into account k_{pH} values from pH 0.44 to 1.39, in which $f_1 + f_2 \rightarrow 1$. The plot $k_{pH} = f(a_{H^+})$ (Fig. 2) was linear with a positive slope $a = k_{H^+}$. The value of k_{pH} for $a_{H^+} = 0$ is equal $b = k_{1H_2O} \times f_1 + k_{2H_2O} \times f_2$ ($b/f_2 = k_{1H_2O} \times f_1/f_2 + k_{2H_2O}$). The values of k_{1H_2O} and k_{2H_2O} were calculated from the plot $b/f_2 = f(f_1/f_2)$ (Fig. 3). The plot $b/f_2 = f(f_1/f_2)$ is linear and the slope of this plot is equal k_{1H_2O} whereas k_{2H_2O} is equal to the value for b/f_2 for $f_1/f_2 = 0$. In the pH range 6.16–13.00 $f_3 + f_4 \rightarrow 1$, therefore $k_{pH} = k_{3H_2O} \times f_3 + k_{4\,H_2O} \times f_4$ $k_{pH}/f_4 = k_{3H_2O} \times f_3/f_4 + k_{4H_2O}$. The plot of $k_{pH}/f_4 = f(f_3/f_4)$ is linear (Fig. 4) and its slope is equal to k_{3H_2O} whereas value of k_{pH}/f_4 (b) for $f_3/f_4 = 0$ is equal to k_{4H_2O}. The consistency between theoretical profile calculated from equation 9 and the experimental results indicates that the equation 9 is correct (Fig. 5).

The influence of ionic strength

In hydrochloric acid and solutions of sodium hydroxide none influences of the ionic strength was observed. It confirms spontaneous hydrolysis of CPS under the influence of water and the hydrolysis of CPS molecules catalyzed by hydrogen ions.

CONCLUSION

In aqueous solutions, in the pH range 0.44–13.00, the degradation of CPS is a pseudo-first-order reaction. The hydrolysis of the dications, monocations, zwitter ions and monoanions of CPS are the partial reactions of hydrolysis. CPS, similarly to like other 4th generation cephalosporin - CSS (11), was the most stable at pH range from 4.0 to 6.5 and less stable above pH 11.24.

The analysis of the relationship log k_{pH} = f(pH) for the degradation of CPS indicate that CPS is more stable in acid than base pH values and has wide plateau region in acidic and neutral pH, which confirms influence of 3-non-acetoxy group at position 3 on the stability of cephalosporins (28). A comparison the above mentioned relationship for the degradation of CPS and CSS (11) indicates the following:

CSS is more stable in the pH range 0.44–8.00 and 10.00–13.00,

CSS and CPS demonstrate similar stability in pH range 8.00–13.00.

Stability of CPS and CSS is determined by spontaneous hydrolysis under influence of water depending on the substrate charge. For CPS in pH range 0.44–1.39 hydrolysis of CPS molecules catalyzed by hydrogen ions is observed.

The components of an acetate, borate and phosphate buffers do not catalyze the degradation of CPS while the acetate and phosphate buffers catalyze the degradation of CSS (11).

The stability of CPS in the presence of borate buffer is significantly lower than in phosphate or acetate buffers. Therefore, the components of borate buffer should not be used in pharmaceutical formulations of CPS as excipients.

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