Available online at www.sciencedirect.com
SCIENCE DIRECT®
Il Farmaco 60 (2005) 599–603
IL FARMACO
http://france.elsevier.com/direct/FARMAC/

Stability of aztreonam in AZACTAM

Marianna Zając [a,*], Anna Jelińska [a], Judyta Cielecka-Piontek [a], Irena Oszczapowicz [b]

[a] *Department of Pharmaceutical Chemistry, University of Medical Sciences in Poznań, Poznań, Poland*
[b] *Department of Modified Antibiotics, Institute of Biotechnology and Antibiotics, Warszawa, Poland*

Received 10 November 2004; revised and accepted 21 April 2005

Available online 01 June 2005

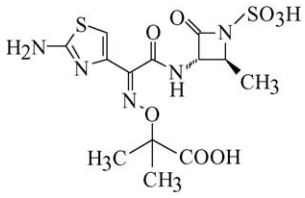
Abstract

The influence of temperature and relative humidity on the stability of aztreonam in AZACTAM was investigated. Changes of the concentration of aztreonam were followed using the HPLC method with UV detection. The first-order rate constants of the reversible reaction of isomerization *Z*-aztreonam ⇌ *E*-aztreonam and the parallel reaction *Z*-aztreonam → products were determined at RH = 76.4% and T = 313, 323, 333, 343 and 353 K, and at T = 343 K and RH = 50.9%, 60.5%, 66.5% and 76.4%. The thermodynamic parameters—energy, enthalpy and entropy of these reactions were calculated.

Keywords: Aztreonam; Stability in solid phase; Kinetic and Thermodynamic parameters

1. Introduction

Aztreonam is the first antibiotic from the monobactam family to have been therapeutically approved. It was introduced in 1987 in the form of injections in the USA and is now used in most European countries in the treatment of infections against Gram-negative bacteria.



Aztreonam is derived of 3-aminomonobactamic acid (3-AMA). The sulfonic acid substituent in the 1-position of the ring activates the beta-lactam moiety, while the aminothiazolyl oxime side chain in the 3-position and the methyl group in the 4-position ensures beta-lactamase stability [1,2].

In previous studies, the general and specific acid–base catalysis over a pH range from 3.50 to 10.50 at 35 °C [3], the decomposition of aztreonam in aqueous solution of Tris at a constant temperature (35 °C) and ionic strength (0.5 mol l^{-1}) in the presence of metal ions (Zn(II), Cd(II), Co(II), Cu(II), Ni(II) and Mn(II) [4], photodegradation of aztreonam under UV light [5], the stability of aztreonam in 5% dextrose and 0.9% sodium chloride injection [6] were investigated.

The purpose of this study was to evaluate the stability of aztreonam AZACTAM in an atmosphere of increased relative humidity.

2. Experimental

2.1. Material and reagents

Azactam—a sterile, nonpyrogenic, sodium-free, white powder containing approximately 780 mg arginine per g of aztreonam for intramuscular or intravenous use (E.R. Squibb and Sons Ltd.). Diprophylline suitability with FP VI.

Buffer salts used and other chemicals were commercial products of analytical grade.

2.2. Analytical procedure

The method used in the experiments is a modification of the procedure presented in USP 24.

The liquid chromatograph was equipped with an L-6000 pump, an LC-2UV detector (Shimadzu) and a Rheodyne 7120 (California, USA) with a 20 μl fixed-loop injector.

* Corresponding author. Tel.: +48 618 546 651; fax: +48 618 546 652.
E-mail address: mzajac@amp.edu.pl (M. Zając).

0014-827X/$ - see front matter © 2005 Elsevier SAS. All rights reserved.
doi:10.1016/j.farmac.2005.04.009

A Merck (Darmstadt, Germany) analytical column (LiChrospher RP-18, 5 μm particle size, 25 cm × 4 mm, ID) was used as the stationary phase. The mobile phase consisted of methanol and phosphate buffer (1:4) (6.8 g KH_2PO_4 dissolved in water to make 1000 ml, and adjusted with 1 mol l^{-1} H_3PO_4 to a pH of 3.0 ± 0.1). The flow rate was 1.0 ml min^{-1}. UV detection was set at 270 nm. All chromatographic operations were conducted under ambient conditions.

2.3. Conditions of the kinetic studies

For the experiments 10 mg samples of Azactam were 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 (RH ~ 76.4%) inserted in heat chambers set to the desired temperatures 313, 323, 333, 343 and 353 K. In order to examine the influence of humidity, samples were placed in desiccators containing saturated solutions of inorganic salts which ensured the desired relative humidity of the ambient air [8] (sodium bromide—RH = 50.9%, potassium iodide—RH = 60.5%, sodium nitrate—RH = 66.5% and sodium chloride—RH = 76.4%) inserted in heat chambers set to 343 K.

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 of methanol and water (1:4 v/v). The so obtained solutions were quantitatively transferred into measuring flaks and completed to a total volume of 10.0 ml the above-mentioned solvents. To 1.0 ml of the so obtained solution 1.0 ml of a solution of internal standard (diprophylline (0.4 mg ml^{-1}) in a mixture (1:4) of methanol and water) was added. Twenty microliters samples of the solutions were injected onto the column. The method was validated (selectivity, linearity, precision and limits of detection and quantitation) to assess its suitability for analyzing the stability of aztreonam in AZACTAM.

The values h/h_{IS} (h and h_{IS}—heights of aztreonam and internal standard peaks, respectively) were used to interpret the changes of concentration of *Z*-and *E*-aztreonam during the reaction.

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

$a \pm \Delta a$ and $b \pm \Delta b$ in the equation $y = ax + b$, standard deviation S_a, S_b, S_y and the coefficient of linear correlation r. The values $a \pm \Delta a$ and $b \pm \Delta b$ were obtained for $f = n - 2$ degrees of freedom with $\alpha = 0.05$.

3. Investigations and results

3.1. Validation of the HPLC method

3.1.1. Selectivity

The applied method was selective for *Z*-aztreonam (t_R = 4.20 min), *E*-aztreonam (t_R = 2.73 min), internal standard (diprophylline, t_R = 4.68 min) and the degradation product (t_R = 5.80 min) (Fig. 1).

Fig. 1. HPLC chromatogram of the Azactam after 7 h heating at 333 K and RH = 76.4%; 1 – *Z*-aztreonam, 2 – *E*-aztreonam, IS – internal standard (diprophylline), P – product.

3.1.2. Linearity

The linearity between h/h_{IS} (h and h_{IS}—heights of aztreonam and IS peaks) and the concentration of aztreonam in a mixture of methanol and water (1:4) ranging from 0.0702 to 0.9831 mg ml^{-1} was evaluated. The linear dependence $h/h_{IS} = f(c)$ was described by the equation $y = a \cdot c = (2.126 \pm 0.060) \cdot c$ (S.D. = 0.027, S_y = 0.0299, r = 0.9988 for n = 14 and α = 0.05).

3.1.3. Limits of detection and quantitation

The limits of detection (DL = 0.0464 mg ml^{-1}) and quantitation (QL = 0.141 mg ml^{-1}) were calculated from the formulas DL = 3.3 S_y/a and QL = 10 S_y/a.

3.1.4. Precision

The precision of the determination of aztreonam was estimated for three concentration of the standard solution of Azactam: 0.5, 1.0 and 1.5 mg ml^{-1} in a mixture (1:4) of methanol and water. The following results were obtained for the above concentration for $n = 8$ and $\alpha = 0.05$: x_1 = 0.5944, S.D. = 0.0039, R.S.D. = 0.66%, S_y = 0.014; x_2 = 1.2088, S.D. = 0.0015, R.S.D. = 0.13%, S_y = 0.013; x_3 = 1.7112, S.D. = 0.0012, R.S.D. = 0.07%, S_y = 0.0004.

3.2. The kinetics of the degradation of aztreonam

3.2.1. Rate constants

The changes of the concentration of substrate and products (Fig. 2) show that the decomposition of aztreonam in

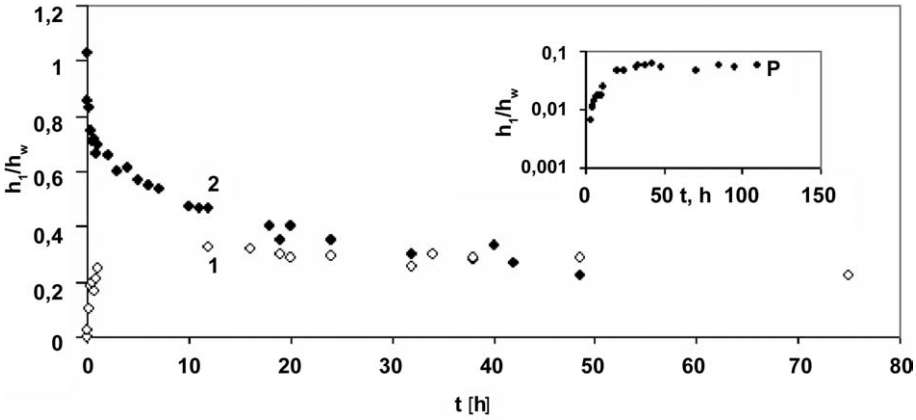


Fig. 2. Semilogarythmic plots of $h_i/h_{IS} = f(t)$ for *E*-aztreonam, *Z*-aztreonam and product (P) at 333 K and RH = 76.4%.

AZACTAM in an air of increased relative humidity is thought to be a consequence of the reaction illustrated in Scheme 1, where k_1 is the isomerization rate constant from *Z*-isomer to *E*-isomer, and k_{-1} is the reverse, and k_2 i k_3 are the degradation rate constants of *Z*- and *E*-isomers.

Similar mechanism of the degradation of aztreonam was observed in aqueous solution at pH 2.5 [7].

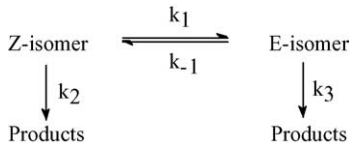
According to Scheme 1, the following kinetic equations can be obtained for *Z*- and *E*-isomers:

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

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

The first-order rate constants k_2 (Table 1) were computed for the values of *Z*-aztreonam concentration in the time range $t_e \rightarrow t_\infty$, in which the linear dependence $\ln c_i = f(t)$ was observed (Figs. 3 and 4).

The rate constants $k_s = k_1 + k_{-1}$ (Table 1) of the reversible reaction *Z*-aztreonam ⇌ *E*-aztreonam were determined from the remainder plots $\ln (c_i - c'_i) = f(t)$ (Figs. 5 and 6) in the time range $t_0 \rightarrow t_e$. The slopes of these plots $a = -k_s$. The values c'_i for time t_i were calculated by the extrapolation of the linear dependence $\ln c = f(t)$ in the time range $t_e \rightarrow t_\infty$. The value c'_0 for $t = 0$ denotes the concentration of *Z*-isomer in a state equilibrium.



Scheme 1.

The partial rate constants k_1 and k_{-1} (Table 1) of the reversible reaction were obtained from the following relationships $k_{-1} = k_s/(1 + K)$ and $k_1 = k_s - k_{-1}$.

The equilibrium constant (K) was calculated from the equation [8]:

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

The ratio *E*-aztreonam/*Z*-aztreonam was approximately 0.6 at 333 K and RH = 76.4%.

The similar dependence was observed in the case of *E*-isomer. The slope of the plot $\ln c = f(t)$ in the time range $t_e \rightarrow t_\infty$ equals $-k_3$ and the slope of the remainder plot $\ln (c'_i - c_i) = f(t)$ in the time range $t_0 \rightarrow t_e$ equals $-k_s$. The rate constant k_3 was smaller than k_2; at 333 K and RH = 76.4%, $k_2 = 5.73 \times 10^{-6}$ s^{-1} and $k_3 = 1.35 \times 10^{-6}$ s^{-1}.

3.2.2. The effect of temperature

The influence of temperature on the degradation of *Z*-aztreonam in AZACTAM is described by the Arrhenius relationship $\ln k_i = \ln A + a\,(1/T)$. The value $\ln A$ (A = frequency coefficient) and a (the slope of the plot $\ln k_i = f(1/T)$) were used to calculate energy (E_a), enthalpy ($\Delta H^{\neq}$) and entropy ($\Delta S^{\neq}$) of the reaction (Table 2) from appropriate equations [8].

Table 1
The first-order rate constants of the degradation of aztreonam in AZACTAM

Parameters	$(k_2 \pm \Delta k)$ (s^{-1})	$(k_s \pm \Delta k)$ (s^{-1})	k_1 (s^{-1})	K_{-1} (s^{-1})
T (K)	RH = 76.4%			
313	$(8.48 \pm 0.03) \times 10^{-7}$	$(9.58 \pm 0.01) \times 10^{-5}$	3.24×10^{-5}	6.34×10^{-5}
323	$(2.23 \pm 0.38) \times 10^{-6}$	$(2.39 \pm 0.29) \times 10^{-4}$	8.45×10^{-5}	1.55×10^{-4}
333	$(5.73 \pm 0.42) \times 10^{-6}$	$(6.34 \pm 1.06) \times 10^{-4}$	2.11×10^{-4}	4.24×10^{-4}
343	$(9.32 \pm 2.39) \times 10^{-6}$	$(1.62 \pm 0.26) \times 10^{-3}$	5.57×10^{-4}	1.07×10^{-3}
353	$(3.36 \pm 0.70) \times 10^{-5}$	$(2.69 \pm 1.23) \times 10^{-3}$	8.91×10^{-4}	1.81×10^{-3}
RH%	T = 343 K			
50.9	$(1.12 \pm 0.58) \times 10^{-6}$	$(2.20 \pm 0.85) \times 10^{-4}$	0.554×10^{-4}	1.65×10^{-4}
60.5	$(4.10 \pm 0.62) \times 10^{-6}$	$(3.64 \pm 0.15) \times 10^{-4}$	1.54×10^{-4}	2.10×10^{-4}
66.5	$(5.00 \pm 0.55) \times 10^{-6}$	$(5.25 \pm 0.12) \times 10^{-4}$	2.17×10^{-4}	3.12×10^{-4}
76.4	$(9.32 \pm 0.24) \times 10^{-6}$	$(16.2 \pm 0.26) \times 10^{-4}$	5.57×10^{-4}	10.7×10^{-4}

Table 2
The thermodynamic parameters of the degradation of aztreonam in AZACTAM

Parameters	k_2	k_s	k_1	k_{-1}
$\ln k_i = f(1/T)$	$a = -9770 \pm 1979$	$a = -9540 \pm 1659$	$a = -9460 \pm 1832$	$a = -9598 \pm 1596$
	$S_a = 622$	$S_a = 521$	$S_a = 576$	$S_a = 502$
	$b = 17.20 \pm 5.50$	$b = 21.23 \pm 4.36$	$b = 19.92 \pm 5.20$	$b = 21.00 \pm 4.43$
	$S_b = 1.87$	$S_b = 1.57$	$S_b = 1.73$	$S_b = 1.51$
	$r = -0.9939$	$r = -0.9945$	$r = -0.9944$	$r = -0.9959$
	$S_y = 0.1771$	$S_y = 0.1485$	$S_y = 0.1639$	$S_y = 0.1428$
E_a (kJ mol^{-1})	77.9 ± 11.0	79.4 ± 11.9	78.7 ± 13.3	79.8 ± 11.6
$\Delta H^{\neq}$ (kJ mol^{-1})	75.5 ± 13,55	76.9 ± 14.4	76.2 ± 15.8	77.3 ± 14.1
$\Delta S^{\neq}$ (J/(mol · K))	–112 ± 212	–68 ± 209	–79 ± 205	–70 ± 211

n, number of experiments; $\Delta k = S_a\, t_{\alpha f}$; E_a, activation energy; $\Delta H^{\neq}$, enthalpy; $\Delta S^{\neq}$, entropy; $E_a = -a$ R (J mol^{-1}); $\Delta H^{\neq} = E_a - RT$ (J mol^{-1}); $\Delta S^{\neq} = R(\ln A - \ln(k_B T)/h)$, where: k_B stands for the Boltzmann constant (1.3807×10^{-23} J K^{-1}); h, Plancks constant (6.6256×10^{-24} 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.

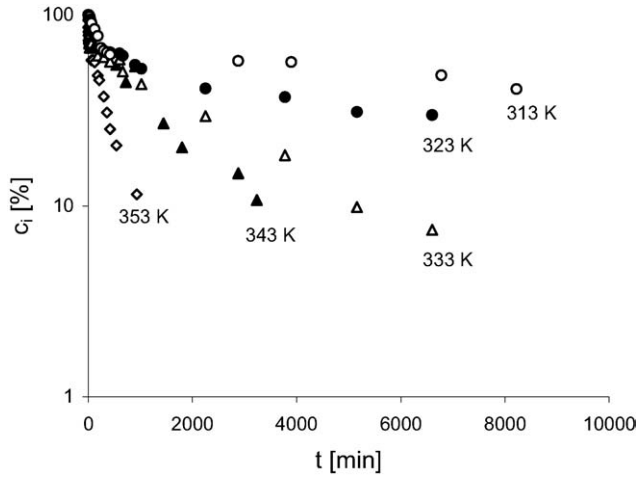


Fig. 3. Semilogarythmic plots of $c_i = f(t)$ of the degradation of the aztreonam in Azactam at different temperatures (RH = 76.4%).

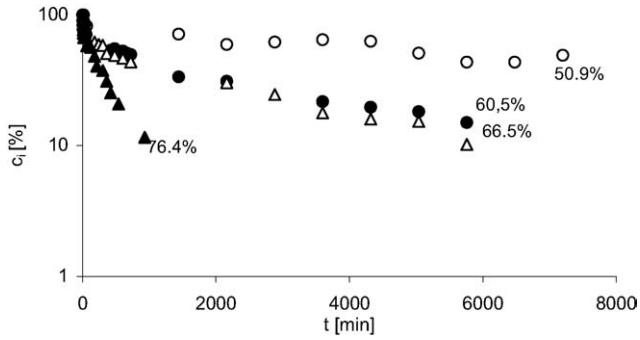


Fig. 4. Semilogarythmic plots of $c_i = f(t)$ of the degradation of the aztreonam in Azactam at various humidities at 358 K.

3.2.3. The effect of humidity

The effect of humidity (RH > 50%) on the stability of aztreonam in AZACTAM is described by the following equations:

$$\ln k_1 = (7.31 \pm 5.15)10^{-2} \cdot \mathrm{RH}\% - 14.3 \pm 1.0; \quad r = 0.9960$$

$$\ln k_{-1} = (8.87 \pm 1.56)10^{-2} \cdot \mathrm{RH}\% - 12.7 \pm 3.3; \quad r = 0.9414$$

$$\ln k_2 = (8.03 \pm 4.30)10^{-2} \cdot \mathrm{RH}\% - 17.6 \pm 2.8; \quad r = 0.9647$$

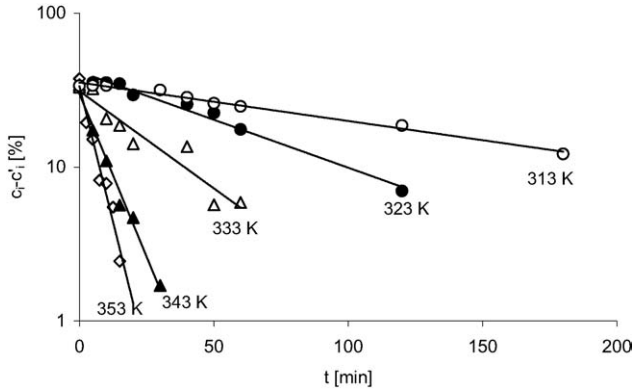


Fig. 5. Semilogarythmic plots of $c_i - c'_i = f(t)$ of the degradation of the aztreonam in Azactam at different temperatures (RH = 76.4%).

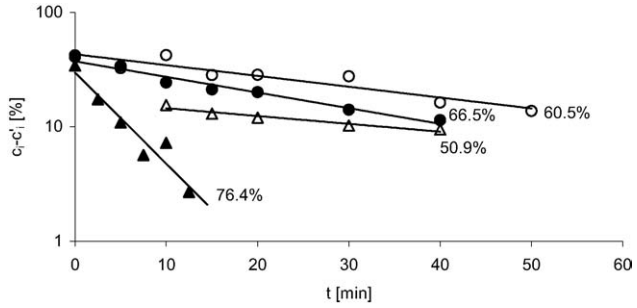


Fig. 6. Semilogarythmic plots of $c_i - c'_i = f(t)$ of the degradation of the aztreonam in Azactam at various humidities at 358 K.

4. Conclusions

The degradation of Aztreonam in AZACTAM in the air of increased relative humidity (50.9–76.4%) and temperature (313–353 K) is the effect of the parallel reaction (Scheme 1) as in the case of aqueous solutions at pH 2.5 [7]. However, the reversible reaction of isomerization *Z*-aztreonam ⇌ *E*-aztreonam is more important. The rate of this reaction is about 100 times greater than that of the parallel reaction *Z*-aztreonam → products, while the rate of the latter reaction is about four times greater than that of the subsequent reaction

E-aztreonam → products.

The thermodynamic parameters of each reaction (Table 2) as well as the effect of the relative air humidity in the range

50.9–76.4% on the rate of each reaction, do not show statistically significant differences.

Acknowledgements

This study was supported by a research grant from the University of Medical Sciences in Poznań, Poland (no. 501-3-0000225).

References

[1] Bristol-Myers Squibb Company, Princeton, NJ 08543, USA; Information of AZACTAM®.

[2] M. Zając, E. Pawełczyk, Pharmaceutical Chemistry, University of Medical Sciences in Poznań, 2000 (in Polish).

[3] R. Méndez, T. Alemany, J. Martin-Villacorta, Stability in aqueous solution of two monocyclic β-lactam antibiotics: aztreonam and nocardicin A, Chem. Pharm. Bull. (Tokyo) 40 (1992) 3222–3227.

[4] R. Méndez, T. Alemany, J. Martin-Villacorta, Catalysis of hydrolysis and aminolysis of non-classical β-lactam antibiotics by metal ions and metal chelates, Chem. Pharm. Bull. (Tokyo) 40 (1992) 3228.

[5] H. Fabre, H. Ibork, D.A. Lerner, Photodegradation kinetics under UV light of aztreonam solutions, J. Pharm. Biomed. Anal. 10 (1992) 645.

[6] L.A. Trissel, Q.A. Xu, J.F. Martinez, Compatibility and stability of aztreonam and vancomycin hydrochloride, Am. J. Health Syst. Pharm. 52 (1995) 2560.

[7] F. Bruchhausen, S. Ebel, A.W. Frahm, E. Hackenthal, Hagers Handbuch der Pharmazeutischen Praxis, Springer-Verlag, Berlin, 1993.

[8] E. Pawełczyk, T. Hermann, The Fundamentals of the Stability of Drugs, PZWL, Warszawa, 1982 (in Polish).