The Relationship of Diastereomer Hydrolysis Kinetics to Shelf-Life Predictions for Cefuroxime Axetil

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Cefuroxime axetil, an ester prodrug of cefuroxime, is comprised of a 50:50 mixture of diastereomers A and B. The first-order hydrolysis kinetics of cefuroxime axetil were investigated as a function of pH, temperature, buffers, and ionic strength. Chromatographically identified hydrolysis products were cefuroxime, Δ^2-cefuroxime axetil, and α,β-sulfoxides. Buffer catalysis was observed in acetate and phosphate buffers. No significant kinetic effect was observed for ionic strength in the range μ = 0.1–1.0. The pH–rate profiles for hydrolysis of cefuroxime axetil isomeric mixture were obtained at 45, 35, and 25°C. The equation defining the cefuroxime axetil hydrolysis rate constant as a function of pH was $k_{obs} = k_H(a_H) + k_s + k_{OH}(K_W/a_H)$, exhibiting maximal stability in the pH range 3.5 to 5.5. The predicted profile at 5°C was in excellent agreement with experimental data in the pH range 3.6 to 5.5. In the pH range 1 to 9, the maximum difference observed for individual hydrolysis constants of isomers was 27%. Shelf-life estimates based on the hydrolysis rate constants for cefuroxime axetil as an isomeric mixture were shown to be equivalent to those based on individual hydrolysis rate constants for isomers A and B.

KEY WORDS: hydrolysis kinetics; cefuroxime axetil; shelf life; pH–rate profiles; isomeric mixture.

INTRODUCTION

The significance of isomeric mixtures has been neglected in drug stability studies. Cefuroxime axetil (Scheme I), an orally absorbed ester prodrug of the antibiotic cefuroxime (1–4), is a 50:50 mixture of diastereomers A and B resulting from esterification of cefuroxime with racemic 1-acetoxyethyl bromide. The HPLC assay for cefuroxime axetil involves summing the individually measured concentrations of A and B isomers in the mixture. Aqueous stability data on cefuroxime axetil were previously limited to a few studies in phosphate buffers (5).

The current research has shown that hydrolysis of the cefuroxime axetil isomeric mixture in solution during storage may be represented by

$$\text{isomer A} \xrightarrow{k_A} \text{cefuroxime}$$

$$\text{isomer B} \xrightarrow{k_B} \text{cefuroxime}$$

where the rates of loss are described by $-d[A]/dt = k_A[A]$ and $-d[B]/dt = k_B[B]$ and the total concentration of cefuroxime axetil as a function of time is $-d[A + B]/dt = k_A[A] + k_B[B]$. If $k_A = k_B$, then the total cefuroxime axetil concentration may be described by $-d[A + B]/dt = k_{obs}[A + B]$, where $k_{obs} = k_A = k_B$. However, if $k_A \neq k_B$ and k_{obs} is calculated from total concentration data, the resultant stability prediction may prove misleading. It was therefore of interest to evaluate the consequences of characterizing cefuroxime axetil stability based on its total isomeric concentration.

The specific objectives of this study were

(i) to determine the hydrolysis rate constants for the isomeric mixture (k_{obs}) and, individually, constants for isomer A (k_A) and isomer B (k_B) as a function of temperature, pH, buffers, and ionic strength; and

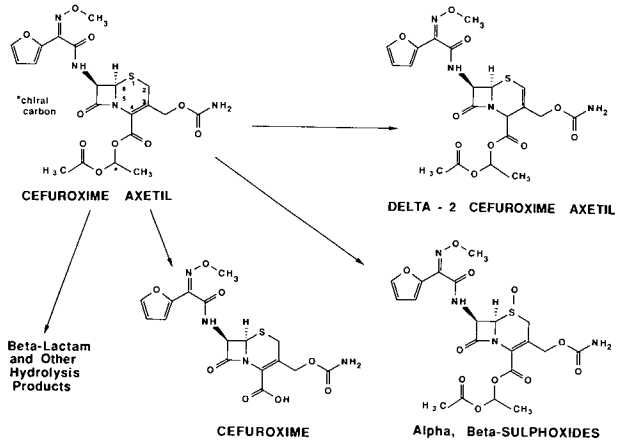
(ii) to predict stability based on the total isomeric mixture (k_{obs}) and to compare the results to those based on the individual components when $k_A \neq k_B$.

MATERIALS AND METHODS

Chemicals. Cefuroxime axetil, cefuroxime, Δ^2-cefuroxime axetil, and α,β-sulfoxides were used as received from Glaxo Group Research, Ltd. (Greenford, Middlesex, England). All other chemicals and solvents were analytical or high-performance liquid chromatographic grade.

Kinetic Procedures. Reaction conditions and rate constants are given in Tables I and II. The pH values of the reaction solutions as well as those of the buffer standards used to calibrate the pH meter were measured at reaction temperatures. First-order hydrolysis kinetics were verified within the cefuroxime axetil concentration range of 1.0–10.0 $\times 10^{-4}$ *M* at pH 1.0 and 9.0 at 35°C.

Reactions were initiated by placing 0.5 ml of cefuroxime axetil stock solution into 20 ml of reaction solution equilibrated to the temperature studied in stoppered amber flasks. At selected times, approximately 0.8-ml samples were removed and cooled. From each sample, a 0.5-ml aliquot was taken and diluted 1:10 to a pH of 4 to 5 using hydrochloric acid or sodium hydroxide and refrigerated to quench the reaction. Reaction samples were quenched by dilution to this pH since cefuroxime axetil, an ester, was predicted and later observed to have a maximal stability at ~pH 5. No loss of cefuroxime axetil was observed after refrigeration for 24 hr. Samples were assayed on the same day of the experiment



Scheme I. Structures for chromatographically identified products in cefuroxime axetil hydrolysis.

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Table I. Experimental Conditions and Observed First-Order Rate Constants (k_{obs}) for Hydrolysis of (2.0–6.0) × 10^{-4} *M* Cefuroxime Axetil at 5, 25, 35, and 45°C, μ = 0.5

°C	pH[a]	Buffer concentration (*M*)			$10^3 \times k_{obs}$ (min^{-1})
		[HCOOH]	[HCOONa]	[NaCl]	
45.0	2.83	0.10	0.020	0.480	0.240
	± 0.04	0.08	0.016	0.484	0.241
		0.06	0.012	0.488	0.223
		0.04	0.008	0.492	0.201
		0.02	0.004	0.496	0.203
		[CH_3COOH]	[CH_3COONa]	[NaCl]	
5.0	3.60	0.10	0.010	0.490	0.00290
	± 0.05	0.08	0.008	0.492	0.00284
		0.06	0.006	0.494	0.00271
		0.04	0.004	0.496	0.00236
	4.57	0.060	0.060	0.440	0.00662
	± 0.01	0.048	0.048	0.452	0.00585
		0.036	0.036	0.464	0.00483
		0.024	0.024	0.476	0.00404
		0.012	0.012	0.488	0.00308
25.0	3.60	0.10	0.010	0.490	0.0298
	± 0.05	0.08	0.008	0.492	0.0287
		0.04	0.004	0.496	0.0235
		0.02	0.002	0.498	0.0212
	4.57	0.060	0.060	0.440	0.0778
	± 0.01	0.048	0.048	0.452	0.0713
		0.024	0.024	0.488	0.0455
		0.012	0.012	0.488	0.0307
35.0	3.60	0.10	0.010	0.490	0.106
	± 0.05	0.08	0.008	0.492	0.103
		0.06	0.006	0.494	0.0938
		0.04	0.004	0.496	0.0812
	4.57	0.060	0.060	0.44	0.270
	± 0.01	0.048	0.048	0.452	0.238
		0.036	0.036	0.464	0.186
		0.024	0.024	0.476	0.148
		0.012	0.012	0.488	0.0964
45.0	3.60	0.10	0.010	0.490	0.241
	± 0.05	0.08	0.008	0.492	0.212
		0.06	0.006	0.494	0.189
		0.02	0.002	0.498	0.148
	4.46	0.060	0.060	0.44	0.771
	± 0.01	0.048	0.048	0.452	0.641
		0.036	0.036	0.464	0.520
		0.024	0.024	0.488	0.418
		0.012	0.012	0.488	0.262
		[NaH_2PO_4]	[Na_2HPO_4]	[NaCl]	
5.0	5.51	0.10	0.0125	0.362	0.0211
	± 0.02	0.08	0.0100	0.390	0.0170
		0.06	0.0075	0.418	0.0135
25.0	7.41	0.0125	0.100	0.188	2.91
	± 0.05	0.010	0.080	0.250	2.34
		0.0075	0.060	0.312	1.82
		0.0050	0.040	0.438	1.25
		0.0025	0.020	0.438	0.768
35.0	5.51	0.100	0.0125	0.362	0.783
	± 0.02	0.080	0.0100	0.390	0.692
		0.060	0.0075	0.418	0.512
		0.040	0.0050	0.445	0.349
		0.020	0.0025	0.572	0.214

Table I. Continued

°C	pH[a]	Buffer concentration (*M*)			$10^3 \times k_{obs}$ (min^{-1})
		[NaH_2PO_4]	[Na_2HPO_4]	[NaCl]	
	6.51	0.036	0.036	0.356	2.48
	± 0.05	0.024	0.024	0.404	1.81
		0.012	0.012	0.452	0.941
	7.43	0.0125	0.100	0.188	9.21
	± 0.05	0.0100	0.080	0.250	7.38
		0.0075	0.060	0.312	5.40
		0.0050	0.040	0.375	4.10
		0.0025	0.020	0.438	2.64
45.0	5.55	0.10	0.0125	0.362	2.170
	± 0.01	0.08	0.0100	0.390	1.820
		0.04	0.0050	0.445	0.907
		0.02	0.0025	0.472	0.585
	5.96	0.048	0.016	0.404	2.90
	± 0.01	0.036	0.012	0.428	2.29
		0.024	0.008	0.452	1.63
		0.012	0.004	0.476	0.979
45.0	6.54	0.060	0.060	0.260	9.96
	± 0.02	0.048	0.048	0.308	8.55
		0.036	0.036	0.356	6.34
		0.012	0.012	0.452	2.78
	7.44	0.0125	0.100	0.188	24.4
	± 0.06	0.010	0.080	0.250	19.8
		0.0075	0.060	0.312	15.5
		0.0050	0.040	0.375	12.2
		0.0025	0.020	0.438	7.29
		[$NaHCO_3$]	[Na_2CO_3]	[NaCl]	
25.0	9.00	0.16	0.020	0.280	34.0
	± 0.06	0.08	0.010	0.390	21.6
		0.04	0.005	0.445	17.1
35.0	8.96	0.16	0.020	0.280	84.9
	± 0.06	0.12	0.015	0.335	71.0
		0.08	0.010	0.390	60.6
		0.04	0.005	0.445	49.1
45.0	8.92	0.20	0.025	0.225	204.
	± 0.06	0.16	0.020	0.280	190.
		0.12	0.015	0.335	175.
		0.08	0.010	0.390	149.
		0.04	0.005	0.445	138.

[a] The initial and final pH values of reaction mixtures were constant.

except for those from reaction mixtures with long half-lives, where samples were stored at −70°C until time of assay. The content of cefuroxime axetil was not affected after 1 week at −70°C.

HPLC Analysis. The HPLC system for analysis of cefuroxime axetil consisted of a Model 600E Multisolvent Delivery System, a Model 490E variable wavelength detector, a Model 745 integrator, and a Model 712 automatic sample injection system (Waters Chromatography Division of Millipore, Milford, MA). Chromatographic separations (Fig. 1) with UV detection at 278 nm were achieved on a Hypersil SAS column (C1, 5 μm, 4.6-mm i.d. × 250 mm, Keystone Scientific Inc., State College, PA) using a mobile phase consisting of 62.5% aqueous 0.025 *M* ammonium acetate and 0.025 *M* acetic acid with methanol at 37.5% (v/v) and a flow rate of 1.5 ml/min. The capacity factors (k'), linear detection

Table II. First-Order Rate Constants (k_{obs}) for the Hydrolysis of (2.0–6.0) × 10^{-4} *M* Cefuroxime Axetil in HCl and in the Absence of Buffers in the pH range 2.83–9.00,[a] μ = 0.5

°C	HCl (*M*)	pH[b]	$10^3 \times k$ (min^{-1})
25.0	0.10	1.08	0.316
	0.075	1.19	0.235
	0.050	1.37	0.165
35.0	0.10	1.06	0.819
	0.075	1.17	0.643
	0.050	1.33	0.455
	0.025	1.63	0.248
	0.010	2.18	0.124
45.0	0.10	1.03	1.97
	0.075	1.14	1.59
	0.050	1.32	1.06
	0.025	1.61	0.608
	0.010	2.04	0.296
5.0		3.60	0.00290
		4.57	0.00222
		5.51	0.00208
25.0		3.60	0.0190
		4.51	0.0203
		7.41	0.205
		9.00	10.9
35.0		3.60	0.0667
		4.57	0.0551
		5.51	0.0657
		6.51	0.205
		7.43	0.820
		8.96	37.0
45.0		2.83	0.187
		3.60	0.123
		4.46	0.150
		5.55	0.146
		5.96	0.344
		6.54	0.964
		7.44	3.29
		8.92	120.0

[a] Based on intercept values for k_{obs} vs buffer concentration.
[b] The initial and final pH values of reaction mixtures were constant.

ranges, and coefficients of variation (%CV, six replicates) were as follows: cefuroxime axetil isomer A—7.2, (0.01–5.0) × 10^{-4} *M*, and 0.9%; and isomer B—6.2, (0.01–5.0) × 10^{-4} *M*, and 1.7%. Cefuroxime, α- and β-sulfoxides, and Δ^2-cefuroxime axetil were identified with *k'* values of 1.1, 3.4, 4.4, and 8.2, respectively, but were not quantified.

Mass balance was not achieved due to numerous unidentified degradation products. However, the specificity of the HPLC assay was demonstrated by chromatographing reaction mixtures (after ~2 half-lives) and comparing the UV spectrum of cefuroxime axetil to a reference standard. A diode array detector (LC 1090, Hewlett Packard, Palo Alto, CA) was utilized to obtain instantaneous UV spectra within the eluting peak. Peak purity was shown using the absorbance index technique (6). Further, complete disappearance of the sample peak was verified after appropriate reaction time and the hydrolysis of cefuroxime axetil showed first-order kinetics with no positive or negative deviation.

HPLC peak areas for isomers A and B were used to calculate hydrolysis rate constants k_A and k_B, respectively.

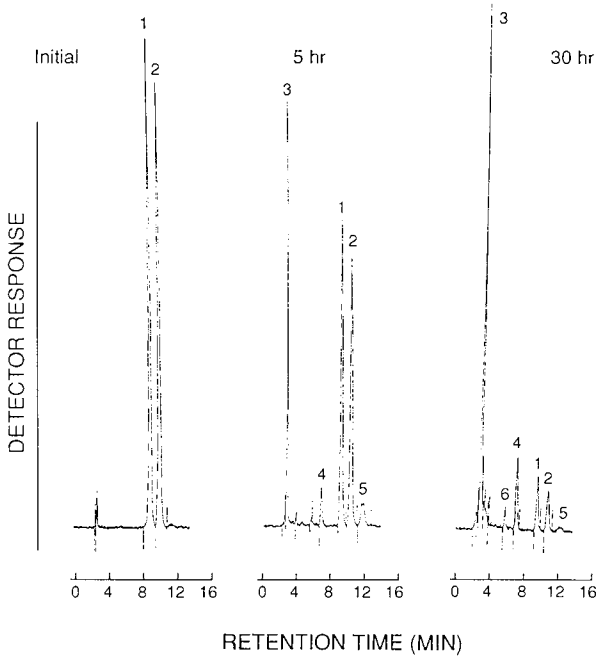

Fig. 1. HPLC chromatograms of cefuroxime axetil as a function of time at 25°C, pH 7.43; [phosphate] = 0.045 *M*, μ = 0.5. (1) Cefuroxime axetil isomer B; (2) isomer A; (3) cefuroxime; (4, 6) α,β-sulfoxides; (5) Δ^2-cefuroxime axetil.

The areas for isomers A and B were combined to represent the total concentration of the isomeric mixture in calculating the hydrolysis rate constant k_{obs}. There was no evidence of isomer interconversion as the collected fractions of eluent containing pure A or B isomer did not give rise to the other isomer.

RESULTS AND DISCUSSION

Kinetics of Transformations. First-order loss was observed under all experimental conditions when assays were based on the individual areas under the isomer A or isomer B HPLC peaks or the sum of these areas, which represents the cefuroxime axetil isomer mixture. First-order rate constants were independent of the initial cefuroxime axetil concentration. Loss of cefuroxime axetil was described by $-d[C]/dt = kt$, which integrates to

$$\ln[C]_t = \ln[C]_o - kt \quad (1)$$

where $[C]_o$ is the initial concentration of isomer A, of isomer B, or of the sum of these cefuroxime axetil isomers; and $k = k_A$ for isomer A, $k = k_B$ for isomer B, and $k = k_{obs}$ for the observed loss of cefuroxime axetil isomeric mixture. The observed first-order rate constants for hydrolysis of cefuroxime axetil were obtained from plots of $\ln[C]_t$ versus time, which were linear for >80% loss of $[C]_o$. Figure 2 presents first-order plots for hydrolysis of individual isomers and of total cefuroxime axetil.

pH–Rate Profiles. The rate constants (k_{obs}) in the absence of buffer catalysis were obtained either from hydrochloric acid solutions or from intercepts of plots representing observed rate constant versus total buffer concentration

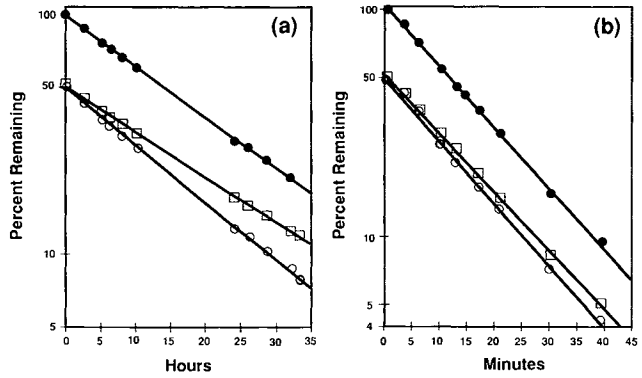

Fig. 2. Semilogarithmic plots of the percentage of (○) cefuroxime axetil isomer A, (□) isomer B, and (●) isomers A + B remaining as a function of time at 35°C, μ = 0.5: (a) 0.1 *N* HCl, pH 1.06; (b) 0.090 *M* carbonate buffer, pH 8.96.

(Fig. 3). The apparent first-order rate constants in the absence of buffer catalysis (Table II) were used to construct a pH–rate profile for cefuroxime axetil hydrolysis (Fig. 4). First-order rate constants for the hydrolysis of the cefuroxime axetil isomer mixture were described by

$$k_{obs} = k_H(a_H) + k_s + k_{OH}(K_w/a_H) \quad (2)$$

where a_H is the hydrogen ion activity, K_w is the dissociation constant for water at the temperature of the study (7), k_s is the first-order constant for spontaneous solvolysis of cefuroxime axetil, k_H is the catalytic constant for hydrogen ion, and k_{OH} is the catalytic constant for hydroxide ion. The catalytic constant values which produced the best fit (solid curves in Fig. 4) for the experimental data are listed in Table III.

Individual first-order hydrolysis rate constants, k_A and k_B, were determined using Eq. (1) under sufficient conditions in Tables I and II to conclude that reactivity differences were minimal. In the pH range 1 to 9, isomer A was less stable than isomer B, however, the maximum difference (27%) was observed at pH 1.06 (Fig. 2).

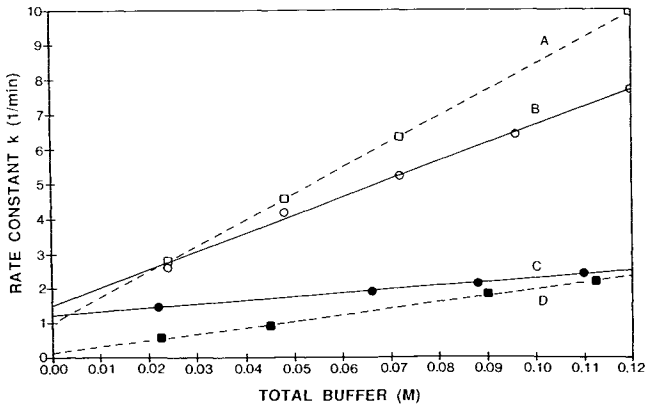

Fig. 3. Catalytic effect of buffer on the observed rate constants (k_{obs}) for the hydrolysis of cefuroxime axetil at 45°C, μ = 0.5, in phosphate buffers ($k \times 10^3$) at (A) pH 6.54 and (D) pH 5.55 and in acetate buffers ($k \times 10^4$) at (B) pH 4.46 and (C) pH 3.60. The intercepts represent the apparent rate constant in the absence of buffer.

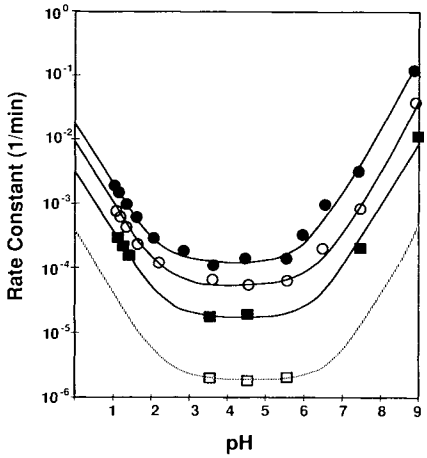

Fig. 4. pH–rate profiles for cefuroxime axetil hydrolysis constants (k_{obs}) at (□) 5°C, (■) 25°C, (○) 35°C, and (●) 45°C, μ = 0.5. The symbols represent experimental points. The solid lines are curves of best fit based on Eq. (2). The dashed curve represents the profile at 5°C predicted from the parameter values in Table III at 25, 35, and 45°C.

Temperature Dependence. Based on the Arrhenius relationship

$$\ln k = \ln A - E_a/RT \quad (3)$$

where R = 1.987 kcal/mol, T is the absolute temperature, and linear plots of ln k versus $1/T$ were used to determine the activation energies (E_a) and preexponential constants (A) for the first- and second-order catalytic constants (Fig. 5). Results are listed in Table III.

From Table III the catalytic constants k_H, k_s, and k_{OH} can be calculated as a function of temperature based on Eq. (3). The extrapolated values for k_H, k_s, and k_{OH} from Fig. 5a were used to predict the cefuroxime axetil hydrolysis rate constant as a function of pH at 5°C. The predicted values are in good agreement with those experimentally observed (Fig. 4).

Buffer Catalysis. At constant pH and in the presence of excess buffer, the rate constant, k_{obs}, for loss of cefuroxime axetil increased with increasing total buffer as shown in Fig. 3. The observed first-order rate constants were defined by

$$k_{obs} = k_{GA}[GA] + k_{GB}[GB] + k_i \quad (4)$$

where [GA] and [GB] are the concentrations of the acidic and basic buffer components, k_{GA} and k_{GB} are the catalytic

Table III. Summary of Catalytic Constants and Energetics for Hydrolysis of Cefuroxime Axetil (k_{obs})

	$k_H \times 10^2$ (M^{-1} min^{-1})	$k_s \times 10^4$ (min^{-1})	$k_{OH} \times 10^{-3}$ (M^{-1} min^{-1})
Temperature (°C)			
25.0	0.345	0.187	0.816
35.0	0.845	0.584	1.63
45.0	1.96	1.30	3.46
E_a (kcal/mol)	16.3	18.4	13.5
A (M^{-1} min^{-1})	3.58×10^9	6.54×10^8	7.16×10^{12}

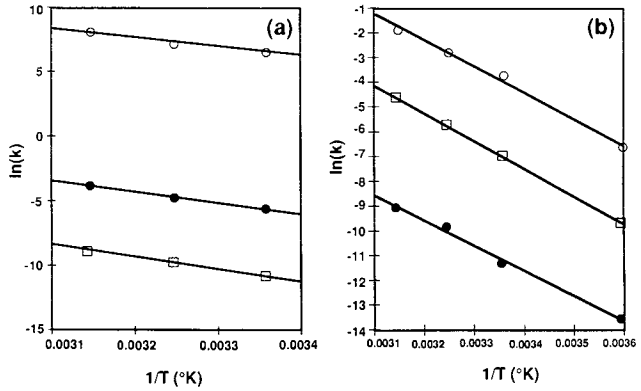

Fig. 5. Arrhenius plots of cefuroxime axetil hydrolysis (μ = 0.5) for (a) (●) k_H, (□) k_s, and (○) k_{OH} at 25, 35, and 45°C and for (b) (○) [HPO_4^{2-}] k_{GB}, (●) acetic acid k_{GA}, and (□) acetate k_{GB} at 5, 25, 35, and 45°C.

constants for the acidic and basic buffer components, and k_i is the rate constant in the absence of buffer. At a given pH, a plot of k vs [GA] was linear with an intercept, k_i, and slope = (k_{GA} + $k_{GB}[R]$), where R is the known ratio [GB]/[GA]. Thus having the slope and R values at two or more pH values allowed the calculation of both catalytic constants by simultaneous equations. The catalytic constants obtained in this way are listed in Table IV. Both the acetic acid and the acetate were catalytic. However, the catalysis was predominantly by acetate, which was about 50–80 times more efficient than the acidic component. For phosphate buffer, HPO_4^{2-} was catalytic but no significant catalysis by the acidic component ($H_2PO_4^-$) was found.

Stability Prediction. Rate constants for hydrolysis of the cefuroxime axetil isomer mixture in acetate or phosphate buffer were described by

$$k_{obs} = k_H a_H + k_s + k_{OH}K_W/a_H + k_{GA}[GA] + k_{GB}[GB] \quad (5)$$

where the constants k_H, k_s, k_{OH}, k_{GA}, and k_{GB} have been defined as a function of temperature. At a specified temperature, the catalytic constants in Eq. (5) can be calculated using the Arrhenius parameters listed in Table III for k_H, k_s, and k_{OH} and in Table IV for k_{GA} and k_{GB}. The ionic strength was varied from μ = 0.1 to 1.0 without significant alteration of the observed first-order rate constant. Over this range the observed rate constants (k_{obs}) were 0.00206 ± 6% min^{-1} in 0.1 *N* HCl and 0.000516 ± 5% in 0.072 *M* acetate buffer at pH 4.46. Thus, the rate constant for cefuroxime axetil hydrolysis can readily be predicted at a specified temperature, pH, and buffer concentration. Excellent agreement has been obtained for the observed and predicted stability in the optimal pH region 3.5–5.5 at 5°C.

Hydrolysis Pathways. Hydrolysis products were identified by comparing chromatograms of reference standards to those of reaction samples (Fig. 1). As illustrated in Scheme I, several hydrolysis routes for cefuroxime axetil were identified: rearrangement to Δ^2-cefuroxime axetil, conversion to cefuroxime, and minor detection of α,β-sulfoxides. Rearrangement of cephalosporin esters to Δ^2-isomers have been reported previously (8–11). In particular, the routes involving rearrangement to Δ^2-cefuroxime axetil and conversion to cefuroxime have been reported for cefuroxime axetil in solutions at pH 7.4. (5).

Stability Prediction Using k_A *and* k_B. Table V lists some representative values selected for discussion of stability based on k_A and k_B in comparison to those based on k_{obs}. These selected pH values included regions wherein each of the three components in the pH–rate expression [Eq. (2)] predominated, i.e., pH 1.06 [$k_H(a_H)$ > 93%], pH 4.5–5.5 (k_s > 83%), and pH 8.92 [$k_{OH}(K_w/a_H)$ > 99%].

The shelf-life, T_{90}, for cefuroxime axetil was defined as the time to retain 90% of the initial concentration. Using the

Table IV. Catalytic Constants for Acidic (k_{GA}) and Basic (k_{GB}) Buffer Components ($10^3 \times M^{-1}$ min^{-1}) and Their Energetics in Phosphate and Acetate Buffers for Cefuroxime Axetil Hydrolysis (k_{obs})

	Phosphate	Acetate	
	k_{GB}	k_{GA}	k_{GB}
Temperature (°C)			
5.0	1.52	0.00149	0.0726
25.0	26.9	0.0133	0.987
35.0	68.5	0.0567	3.61
45.0	171.0	0.124	10.2
E_a (kcal/mol)	20.8	19.7	21.8
A (M^{-1} min^{-1})	3.92×10^{13}	5.35×10^{9}	1.07×10^{13}

Table V. Selected Rate Constants ($10^3 k$ min^{-1}) for Hydrolysis of Cefuroxime Axetil Isomer A (k_A) and Isomer B (k_B) and for the Observed Loss of the Cefuroxime Axetil Isomer Mixture (k_{obs})

pH (temp.)	Total buffer (M)[a]	k_A	k_B	k_{obs}
1.06[a]				
(35°C)	0.10	0.924	0.726	0.819
3.60[b]				
(35°C)	0.110	0.112	0.100	0.106
4.57[b]				
(5°C)	0.120	0.00714	0.00610	0.00662
	0.096	0.00634	0.00534	0.00585
	0.072	0.00539	0.00440	0.00484
	0.048	0.00442	0.00367	0.00404
	0.024	0.00340	0.00280	0.00308
5.51[b]				
(5°C)	0.1125	0.0222	0.0200	0.0211
	0.090	0.0180	0.0163	0.0170
	0.0675	0.0140	0.0130	0.0135
7.43[b]				
(35°C)	0.0900	7.41	7.30	7.38
	0.0450	4.87	4.04	4.10
	0.0225	2.84	2.66	2.64
8.92[b]				
(35°C)	0.090	62.9	57.7	60.6

[a] HCl, Table II.
[b] Buffers are described in Table I: acetate (pH 3.60 and 4.57), phosphate (pH 5.51 and 7.43), and carbonate (pH 8.92).

data for k_A and k_B and considering the isomers individually, the time required to decrease to 90% of the initial concentration of cefuroxime axetil was calculated and compared to the T_{90} for cefuroxime axetil based on the observed first-order rate constant for the isomeric mixture, $T_{90} = \ln 0.9/k_{obs}$. Using k_A and k_B, loss of the 50:50 mixture of isomers A and B was described by

$$\%(A + B)_t = (\%A)_o e^{-(kt)} + (\%B)_o e^{-(kt)} \quad (8)$$

where $(\%A)_o$ = initial percentage of isomer A = 50%, $(\%B)_o$ = initial percentage of isomer B = 50%, and $\%(A + B)_t$ represents the percentage remaining as a function of time. The time required to decrease to 90% was estimated by substituting the known values for k_A and k_B in Eq. (8) and simulating the percentage remaining as a function of time. The difference between the T_{90} calculated from the observed first-order rate constant k_{obs} and that estimated using individual constants in Eq. (8) was insignificant in all cases. For example, in the most extreme case, where k_A exceeded k_B by 27%, the T_{90} value was 129 min, whereas the estimate using Eq. (8) was 128 min. Further, the calculated time required to decrease to 10% remaining, T_{10}, was similar using both methods.

Hydrolysis of an isomeric mixture of a tertiary aliphatic chloride with a fourfold difference in isomeric reactivity has been described previously by biexponential loss (12). Cefuroxime axetil isomeric reactivity differences up to 27% did not affect the linearity of first-order plots for total concentration versus time.

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