Bioavailability of Acetazolamide Tablets

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Abstract ☐ Plasma acetazolamide levels were measured by an enzymatic assay following single 250-mg oral tablet doses to 20 healthy volunteers; five different lots of acetazolamide tablets from a single manufacturer were used in a balanced incomplete block design. From the measured plasma levels, estimates of the bioavailability parameters (area under the plasma concentration *versus* time curve, time to peak plasma concentration, and peak plasma concentration) were obtained by least-squares digital computer fitting. No significant differences among the tablets were observed ($\alpha = 0.05$) for the analysis of variance of the area under the curve or time to peak parameters. Two tablets, however, provided statistically higher peak plasma concentrations than the other three. Thus, lot-to-lot bioinequivalence of acetazolamide tablets was observed. Some *in vitro* tests employed showed general trends for correlation with the *in vivo* data. However, considerable refinement of these techniques appears necessary for *in vitro* prediction of the observed lot-to-lot bioinequivalence.

Keyphrases ☐ Acetazolamide—bioavailability of various tablet lots compared in humans ☐ Bioavailability—acetazolamide, various tablet lots compared in humans ☐ Carbonic anhydrase inhibitors—acetazolamide, bioavailability of various tablet lots compared in humans

Acetazolamide is a potent carbonic anhydrase inhibitor and finds its primary use in the management of chronic simple and secondary glaucoma. The drug has been reported to be quantitatively absorbed from the human GI tract and to have a plasma half-life of about 100 min (1, 2). About 80% of the drug is excreted by tubular secretion of the anionic species, and 70–90% of the administered dose is recovered unchanged within 24 hr (3). The apparent volume of distribution in humans is about 20% of body weight (4).

Lehmann *et al.* (4) reported that acetazolamide was absorbed with an apparent half-life of approximately 0.5 hr and eliminated with an apparent half-life of 4.1 hr (range of 2.4–5.8 hr). Peak plasma levels were seen in 2 hr (range of 1.25–3 hr); however, the drug was taken soon after a meal. One subject showed poor absorption and had a flat plasma concentration–time curve with concentrations never exceeding 1.4 μg/ml after a 500-mg oral dose. Other investigators observed a lack of response in a patient given acetazolamide orally who responded to intramuscular therapy (5). Maren and Robinson (2) also reported variable acetazolamide absorption but only at high doses.

Variations in the bioavailability of acetazolamide dosage forms possibly may result from formulation factors or aging. Another sulfonamide-type diuretic, hydrochlorothiazide, has shown variable dissolution characteristics for tablet formulations (6, 7). Tannenbaum *et al.* (8) showed that product formulation could affect the bioavailability of hydrochlorothiazide dosage forms, although recently (9, 10) several groups showed that possible differences in dissolution may not be reflective of significant differences in the bioavailability of commercial products. These studies were based on urinary excretion data only, however.

Therefore, there may be variability in dissolution and/or

bioavailability with low solubility sulfonamides in tablet formulations. The possibility that such variations may occur with acetazolamide prompted the present study.

The objectives of this study were to establish a methodology for determining the biological equivalency of acetazolamide tablets in humans, to determine whether lot-to-lot variability in bioavailability occurs, to provide *in vitro* dissolution rate data capable of indicating potential lot-to-lot variability in acetazolamide tablets, and to correlate *in vitro* dissolution parameters with *in vivo* bioavailability data.

EXPERIMENTAL

Materials—Acetazolamide powder[1] was used to prepare standards. The 250-mg acetazolamide tablets[2] (designated A–E) used in the human studies were representative of tablets found in normal channels of distribution. All other chemicals were analytical reagent grade.

Disintegration and Dissolution Studies—Disintegration and dissolution measurements on the 250-mg acetazolamide tablets were carried out utilizing the USP XIX methods. The conditions were as follows: temperature, 37°; dissolution media, distilled water, simulated gastric fluid without pepsin, and pH 10 carbonate buffer; volume of dissolution medium, 900 or 1500 ml; and stirring speed, 50 or 100 rpm.

Filtered samples were withdrawn from the dissolution flasks at various times up to 1 hr, and the absorbance of the solution was determined at 265 nm. Where necessary to maintain the absorbance readings in the region from 1 to 2 units, the samples were quantitatively diluted.

Dissolution rate measurements also were obtained utilizing the rotating-filter–stationary basket apparatus (11).

Assay of Plasma Acetazolamide—Plasma acetazolamide levels were determined by a modified enzymatic method based on the inhibition of carbonic anhydrase activity. The method was originally developed by Maren (12) to evaluate the relative activity of compounds that inhibit carbonic anhydrase. Although little information on the sensitivity or reproducibility of the assay was available, the ease and rapidity of the technique indicated that it would be advantageous for the large number of samples generated in bioavailability studies. The Maren technique was modified to provide a rapid, accurate, and precise method for determining plasma acetazolamide; the procedure was described in detail previously (13).

Bioavailability Studies in Humans—*Selection of Subjects*—Normal, healthy, adult males, who were within 10% of their ideal body weight (14) and 21–40 years of age, were admitted to the study. Informed signed consent was obtained from each subject. One week prior to initiation of the study, the subjects were given a thorough physical examination including extensive laboratory tests. Subjects with test results outside normal limits were excluded, as were any individuals showing a history of any significant organ abnormality or disease.

Study Design—The basic experimental design employed to determine relative bioavailability of the five acetazolamide dosage forms was a balanced incomplete block design. The 20 subjects were randomly assigned to 10 different groups (two subjects per group), and each subject received three of the five possible treatments. The treatments were separated by a 1-week washout period, and the design balanced over weeks. The balanced incomplete block design utilized is shown in Table I.

Administration of Dosage Forms—The subjects were fasted overnight prior to and for 4 hr immediately after administration of a 250-mg

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Table I—Balanced Incomplete Block Design for Acetazolamide Bioavailability Study

Group	Subject	Week 1	Week 2	Week 3
I	1	A	B	C
	2	A	B	C
II	3	A	E	B
	4	A	E	B
III	5	B	C	D
	6	B	C	D
IV	7	B	D	E
	8	B	D	E
V	9	C	D	E
	10	C	D	E
VI	11	C	E	B
	12	C	E	B
VII	13	D	B	A
	14	D	B	A
VIII	15	D	C	A
	16	D	C	A
IX	17	E	A	C
	18	E	A	C
X	19	E	A	D
	20	E	A	D

[a] Treatments on different lots of 250-mg acetazolamide tablets.

acetazolamide tablet. The tablet was administered with 240 ml of water. Four hours postadministration, the subjects were given a meal and were allowed to eat and drink normally during the rest of the day.

The subjects were ambulatory throughout each 12-hr treatment day and were permitted to proceed with their normal daily routine inasmuch as was possible considering their availability for blood sampling. The subjects were not permitted to engage in any strenuous or athletic activities on the day of drug administration.

Blood Collection Schedule—The following blood samples were collected: 6-ml samples were drawn at 0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0, and 24.0 hr following drug administration.

The blood samples were drawn through an indwelling 19-gauge scalp vein needle into heparinized specimen tubes. The samples were centrifuged immediately, and the plasma was removed and frozen until assayed.

RESULTS AND DISCUSSION

Disintegration and Dissolution Studies—The results of the disintegration studies are shown in Table II. The data are generally internally consistent; *i.e.*, the rank order for disintegration is reasonably well maintained over the different disintegration media employed. Tablet E stands out significantly as the slowest disintegrating form tested.

The USP dissolution apparatus was utilized for most dissolution studies since it was readily available. The studies were run for 1 hr, and the times to achieve dissolution of different percentages of the acetazolamide present in a tablet were determined from plots of percent of dose dissolved *versus* time. The values obtained for the five tablets used in the human bioavailability studies are given in Table III. The dissolution data obtained using distilled water or simulated gastric fluid do not show any gross differences among the dosage forms under the conditions employed, although Tablets C and D dissolved faster than the other three products in gastric fluid.

In carbonate buffer, however, Tablet E shows markedly different dissolution characteristics (less than 20% in solution in 1 hr *versus* 20% in solution in generally less than 10 min for the other dosage forms). Thus, a nonphysiological dissolution medium detects differences in dissolution behavior of acetazolamide tablets not observable with more traditional

Table II—Disintegration Time (Seconds) of Different Lots of Acetazolamide Tablets by USP XIX Method

Tablet	Gastric Fluid[a]	Distilled Water[a]	Carbonate Buffer[b]
A	45.0 ± 5.0	82.3 ± 9.8	543 ± 240
B	37.7 ± 2.5	49.7 ± 8.4	374 ± 230
C	30.3 ± 3.1	24.0 ± 2.0	180 ± 13.4
D	65.0 ± 5.6	42.3 ± 3.2	388 ± 120
E	112.0 ± 15.2	124.0 ± 21.0	2169 ± 465

[a] Mean ± *SD*, n = 3. [b] Mean ± *SD*, n = 6.

**Table III—Dissolution Behavior of Different Lots of Acetazolamide Tablets Using the USP Apparatus**

Medium	Tablet	T_{10}	T_{20}	T_{25}	T_{30}	T_{40}	T_{50}	T_{75}
Water	A	12	25	32	39	52		
	B	13	27	34	42	58		
	C	11	33	51	>60	>60		
	D	9	25	34	45	>60		
	E	12	25	33	42	60		
Gastric fluid	A	11	24	31	37	55		
	B	12	25	33	40	58		
	C	12	23	28	32	42	54	
	D	8	20	25	32	44		
	E	10	23	32	41	60		
Carbonate buffer	A	6	12	14	17	23	30	44
	B	3	7	8	10	14	18	32
	C	2	3	4	5	9	15	46
	C	2	3	3	5	10	18	38
	D	3	4	5	6	9	12	30
	D	3	5	6	7	9	12	30
	E	26	>60					
	E	32	>60					

[a] Dissolution measurements were carried out under the following conditions: 900-ml dissolution volumes, 37°, 1-hr total time, and 50 rpm.

dissolution media.

The dissolution data obtained with the rotating-filter–stationary basket apparatus (Table IV) appear to show greater sensitivity to possible differences in the tablets. Although dissolution was relatively rapid under the conditions employed, greater percentage differences were observed between Tablets C and D and the others with this dissolution procedure than with the USP method.

Bioavailability Studies in Humans—The plasma samples obtained were assayed as outlined under *Experimental*. The plasma concentration *versus* time curves for each subject were analyzed according to a one-compartment open model with a lag time of the form shown in Scheme I:

$$\overset{\text{lag}}{\longrightarrow} G \overset{k_a}{\longrightarrow} B \overset{k_e}{\longrightarrow} U$$

Scheme I
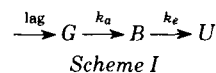
where G, B, and U represent the amounts of drug in the GI tract, total body, and the amount excreted, respectively; and k_a and k_e represent the overall first-order rate constants associated with the loss of drug from the GI tract and the body, respectively. Since measurements of drug concentrations were made in the central or total body compartment, the acetazolamide levels in this compartment as a function of time can be described by:

$$b = \frac{\gamma_a D_0 k_a}{(k_a - k_e) V_B} \{\exp[-k_e(t - L)] - \exp[-k_a(t - L)]\} \quad \text{(Eq. 1)}$$

where b represents the concentration of acetazolamide in the body compartment at any time, t; γ_a is the fraction of the dose, D_0, absorbed; V_B is the volume of distribution for the body compartment; and L is the time lag accounting for dosage form or physiological factors delaying drug absorption.

If y_i, $i = 1 \ldots, n$, denotes the concentration measured at time x_i, the model that is to be fit is:

$$E(y_i) = \frac{\alpha_1 \alpha_3}{(\alpha_1 - \alpha_2)} \{\exp[-\alpha_2(x_i - \alpha_4)] - \exp[-\alpha_1(x_i - \alpha_4)]\} \quad \text{(Eq. 2)}$$

Table IV—Dissolution Behavior of Different Lots of Acetazolamide Tablets Using the Rotating-Filter–Stationary Basket Apparatus

Tablet	T_{30} Average	T_{30} Range	T_{50} Average	T_{50} Range	T_{90} Average	T_{90} Range
A	4.1	4.5–3.6	5.9	5.1–6.9	9.00	7.8–10.9
B	1.9	1.6–2.3	2.7	2.3–2.8	5.7	5.2–6.1
C	1.1	1.0–1.2	1.48	1.4–1.6	3.0	2.5–4.0
D	1.6	1.3–2.0	2.16	1.7–2.9	3.96	3.4–4.4
E	4.0	3.1–4.7	5.25	4.0–6.9	8.03	6.7–9.6

[a] Experimental conditions: observations made on six tablets; medium = 1000 ml, pH 7.2 phosphate buffer; 37°; and 400 rpm.

Table V—Maximum Plasma Concentration Achieved (Micrograms per Milliliter) as a Function of Tablet Lot, Study Day, and Subject after Administration of 250-mg Acetazolamide Tablets

Tablet	Study Day 1		Study Day 2		Study Day 3	
	Subject	C_{max}	Subject	C_{max}	Subject	C_{max}
A	1	6.10	17	4.88	13	6.50
	2	8.11	18	10.13	14	Dropped
	3	6.02	19	5.71	15	8.76
	4	5.81	20	6.48	16	6.32
B	5	5.10	1	4.51	3	8.67
	6	12.11	2	10.31	4	13.10
	7	8.67	13	6.29	11	8.04
	8	10.52	14	Dropped	12	8.90
C	9	9.66	5	9.51	1	6.49
	10	8.40	6	17.94	2	13.46
	11	6.90	15	17.27	17	12.60
	12	12.86	16	11.43	18	12.99
D	13	9.57	7	15.63	5	9.43
	14	10.16	8	17.15	6	12.78
	15	14.12	9	11.25	19	8.07
	16	10.84	10	10.03	20	5.63
E	17	11.03	3	8.08	7	5.64
	18	7.79	4	11.12	8	9.23
	19	4.89	11	12.29	9	9.60
	20	9.01	12	4.91	10	9.20

where α_1 represents the rate constant k_a; α_2 represents k_e; α_3 represents the absorbed dose/apparent volume of distribution, $\gamma_a D_0/V_B$; and α_4 is the delay time, L.

The computational problem is to:

$$\text{minimize} \quad \sum_{i=1}^{n} [y_i - E(y_i)]^2$$
$$\alpha$$

i.e., to find the least-squares estimates of the parameters $\alpha = \alpha_1, \alpha_2, \alpha_3,$ and α_4. Gradient methods, which lead to iterative least-squares algorithms, are often unstable and fail to converge. For this reason, the Nelder–Mead (15) simplex algorithm, NELMIN, as coded by O'Neil (16), was employed to obtain the least-squares estimates. O'Neil's FORTRAN subroutine requires the FORTRAN function for the minimization problem described[3]. The initial estimates of the α values were determined graphically for each curve.

A FORTRAN program, CTCNM, utilizing the Nelder–Mead procedure, was then developed and run on a digital computer[4]. After obtaining the least-squares estimates, $\hat{\alpha}$, CTCNM computes estimates of time to maximum concentration, maximum concentration, and area under the concentration–time curve. Output also includes a table with the input data, fitted values, residuals, and the minimum sum of squares evaluated at $\hat{\alpha}$.

The estimates of maximum concentration, time to maximum concentration, and the area under the concentration–time curve for each subject and tablet studies are given in Tables V–VII, respectively. Figure 1 shows the mean plasma concentration–time curves for each tablet and the computer fits of the data according to Eq. 2. Visual interpretation of the concentration–time curves shown should be made with the understanding that a complete crossover design was not employed.

Statistical Analysis of Data—The analysis of variance was utilized to determine whether the bioavailability of the five tablets studied was different. Each bioavailability parameter, area under the plasma concentration–time curve (AUC), time to peak concentration (t_{max}), and maximum concentration achieved (C_{max}), was treated as the response in a univariate analysis of variance. The statistical analysis was complicated by the loss of some data which occurred when one subject withdrew from the study. Since the design was no longer balanced, it was necessary to utilize regression methods to obtain the analysis of variance. The statistical model employed was:

$$Y_{ijk} = \mu + \beta_{ij} + \rho_j + \tau_k + \epsilon_{ijk} \qquad (\text{Eq. 3})$$

where μ is the overall mean; β represents subject effects; τ represents treatment effects; ρ represents a replication effect; $i = 1, 2, \ldots, 10$; $j = 1, 2$; and $k = 1, 2, \ldots, 5$. The ϵ's are assumed to behave as independent, random, normal variables with mean zero and constant variance. The parameters are subject to the usual constraints: $\sum_{i=1}^{10}\beta_{ij} = 0$ for $j = 1, 2$; $\sum_{k=1}^{5}\tau_k = 0$; and $\rho_1 = -\rho_2$. The model (Eq. 3) was expressed in matrix

[3] Available on request from the authors.
[4] CDC 6600-6400.

Table VI—Time to Maximum Plasma Concentration (Hours) as a Function of Tablet Lot, Study Day, and Subject after Administration of 250-mg Acetazolamide Tablets

Tablet	Study Day 1		Study Day 2		Study Day 3	
	Subject	t_{max}	Subject	t_{max}	Subject	t_{max}
A	1	6.53	17	4.87	13	2.38
	2	1.33	18	1.36	14	Dropped
	3	7.89	19	0.97	15	3.30
	4	5.83	20	4.54	16	6.77
B	5	4.84	1	4.28	3	2.71
	6	2.24	2	1.56	4	2.06
	7	1.71	13	5.70	11	2.18
	8	2.66	14	Dropped	12	2.71
C	9	3.83	5	5.28	1	5.25
	10	0.86	6	0.22	2	0.26
	11	5.29	15	1.55	17	0.67
	12	1.83	16	0.18	18	1.18
D	13	2.93	7	1.06	5	2.56
	14	0.20	8	0.67	6	0.70
	15	1.03	9	2.33	19	3.22
	16	3.60	10	2.52	20	2.58
E	17	0.65	3	7.74	7	4.19
	18	2.68	4	2.16	8	4.02
	19	0.68	11	0.35	9	1.86
	20	4.00	12	0.71	10	1.06

notation, and the computations were performed using the FIT command in Omnitab II (17). This program provides all of the results required in the subsequent analysis, including estimates of the parameters, the residual sum of squares, and estimates of the parameter variance–covariance matrix.

This approach permitted the construction of an analysis of variance table for the unbalanced incomplete block design data to determine whether bioavailability differences existed among the tablets studied. Analysis of the data in Table VII for the area under the plasma concentration–time curve (AUC) showed no significant differences among tablets at the 0.05 level. The ranked treatment means for the five tablets studied were (micrograms per milliliter $\times$ hours): A, 87.2; D, 88.1; E, 89.0; C, 91.9; and B, 103.1.

The analysis of variance table for the AUC data showed:

source	df	SS	MS	F
treatments (adjusted)	4	1716.77	429.19	1.51
residual	34	9686.33	284.89	

critical value: $F_{0.05}(4,34) = 2.65$

Analysis of the data in Table VI for the time to achieve peak concentration (t_{max}) also showed no significant differences among the tablets tested. The ranked treatment means (hours) for the dosage forms studied were: D, 2.06; C, 2.38; E, 2.51; B, 2.52; and A, 3.96.

The analysis of variance table for the time to peak data showed:

source	df	SS	MS	F
treatments (adjusted)	4	19.71	4.93	1.74
residual	34	96.25	2.83	

critical value: $F_{0.05}(4,34) = 2.65$

Although no statistically significant differences could be noted, the mean data show that Tablet A took almost twice as long as Tablet D to reach peak plasma levels.

The peak plasma concentration data (C_{max}) presented in Table V show that statistically significant differences existed among the tablets tested. The ranked treatment means (micrograms per milliliter) for the five dosage forms were: A, 6.90; B, 8.55; E, 8.60; D, 11.28; and C, 11.44.

The analysis of variance table for the peak plasma concentration data showed:

source	df	SS	MS	F
treatments (adjusted)	4	143.21	35.80	6.21
residual	34	196.13	5.77	

critical value: $F_{0.05}(4,34) = 2.65$

To determine which of the tablets differed from one another, the standard deviation for the difference between two treatment means was calculated. The value calculated was that which yielded the largest value for the standard deviation from the variance–covariance matrix. Thus, the most conservative estimate available was used to test for differences among tablets.
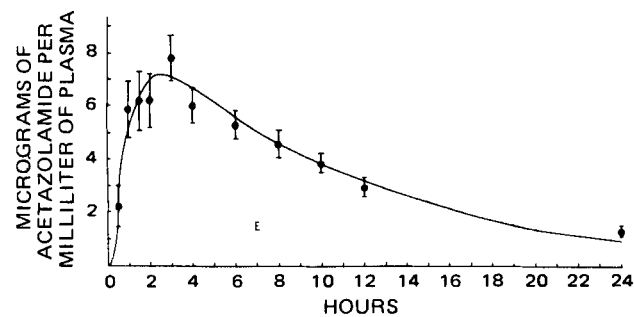
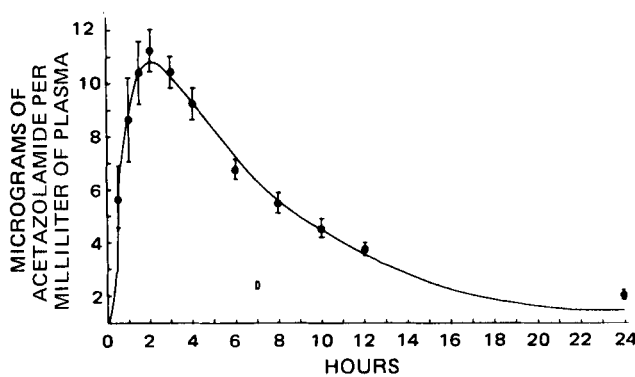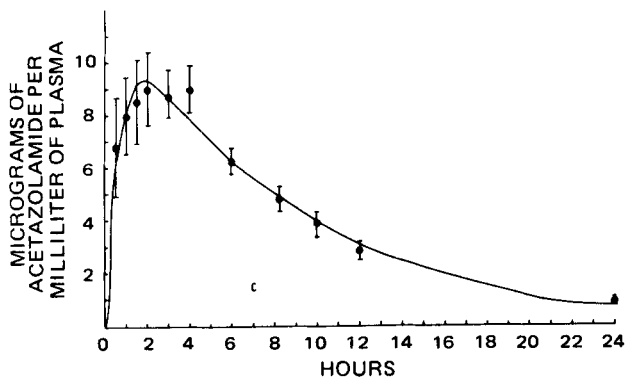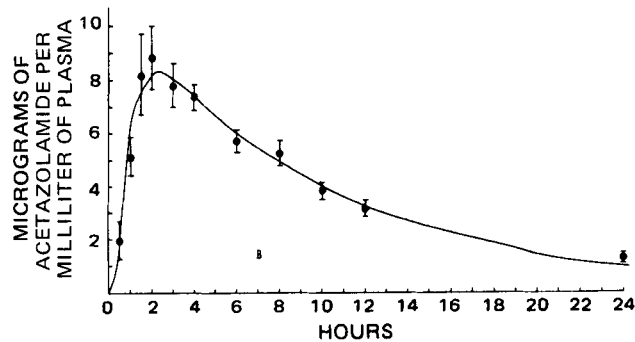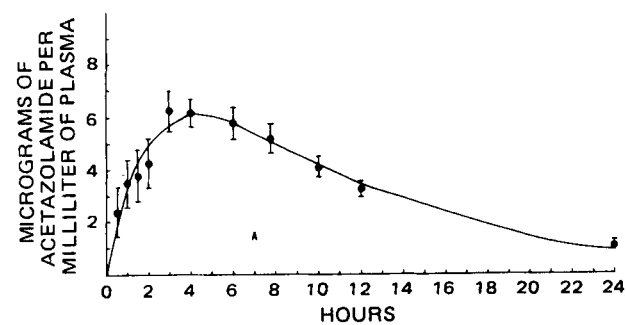Figure 1—*Plasma acetazolamide concentrations (micrograms per milliliter) as a function of time after oral administration of Tablets A–E. The curves are the computer fits of the data according to Eq. 2.*

The standard deviation between treatment means was 1.13. The critical value from the t distribution at the 0.05 level for 34 degrees of freedom is 1.69. Thus, if the difference between any two treatment means was greater than 1.91 $(SD \times t_{\alpha=0.05})$, the treatments (tablets) were considered to be statistically different. According to this method of analysis, the peak plasma concentrations from Tablets C and D were different from A, B, and E.

Table VII—Area under the Plasma Level–Time Curve (Micrograms per Milliliter × Hours) as a Function of Tablet Lot, Study Day, and Subject after Administration of 250-mg Acetazolamide Tablets

Tablet	Study Day 1		Study Day 2		Study Day 3	
	Subject	Area	Subject	Area	Subject	Area
A	1	91.06	17	52.89	13	128.29
	2	72.95	18	98.42	14	Dropped
	3	116.05	19	49.07	15	122.42
	4	84.22	20	70.38	16	102.92
B	5	167.65	1	131.28	3	137.77
	6	69.07	2	97.64	4	92.61
	7	57.79	13	114.95	11	69.44
	8	108.59	14	Dropped	12	85.83
C	9	91.14	5	120.26	1	79.69
	10	76.69	6	63.50	2	80.62
	11	86.20	15	142.16	17	61.45
	12	83.08	16	104.94	18	101.50
D	13	105.51	7	74.63	5	88.69
	14	131.44	8	89.80	6	51.85
	15	127.2	9	98.48	19	70.94
	16	88.61	10	68.14	20	70.41
E	17	76.16	3	148.05	7	55.16
	18	98.32	4	90.49	8	84.13
	19	45.21	11	67.50	9	98.40
	20	91.08	12	153.74	10	59.94

The rank order of the tablets on the basis of peak concentration attained was exactly the same as from the data on time to peak. This internal consistency from parameters that should be related lends support to the inference that differences exist among the tablets tested, at least in terms of the rate of availability.

In Vitro–In Vivo Correlations—The *in vivo* data indicate that while all of the tablets tested were equivalent in terms of the extent of absorption, C and D provided significantly higher peak plasma concentrations than A, B, and E. Analysis of the *in vitro* USP dissolution data showed that the same general trends held in gastric fluid and pH 10 carbonate buffer, although the observed differences did not appear to be of a sufficient magnitude to make the dissolution test discriminatory except for Tablet E in carbonate buffer. With water as the dissolution medium, there was no discrimination among tablets; in fact, C and D performed slightly more poorly than the others.

The poor performance of Tablet E in the carbonate buffer dissolution test was probably due more to its disintegration characteristics than to any discrimination by the dissolution test. Tablet E had an extremely long disintegration time in carbonate buffer, which contributed to its very slow dissolution.

A better *in vitro* correlation with the *in vivo* data was obtained with the rotating-filter–stationary basket apparatus. A much greater percentage difference was observed between the tablets giving the highest blood levels (C and D) and the other three tablets than was seen with the USP apparatus. The rank correlation between the T_{90} for dissolution and the mean C_{max} values was excellent, indicating that this dissolution procedure may eventually provide a method for monitoring lot-to-lot variations in acetazolamide bioavailability. More extensive evaluation of the spin filter technique was limited by the number of tablets available.

While trends for a correlation between disintegration and/or dissolution test and *in vivo* bioavailability exist, the apparent *in vitro* test procedures would have to be refined considerably to increase their ability

to discriminate accurately among acetazolamide tablets. That considerable refinement would be necessary is accentuated by the fact that the *in vivo* data show statistical differences among tablets tested in only one bioequivalence parameter. Detection of this inequivalence by *in vitro* testing would require highly sensitive and statistically validated methodology.

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NOTES

Hydrolytic Degradation of 2,6-Dichlorobenzylthiopseudourea Hydrochloride

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Abstract □ Degradation of 2,6-dichlorobenzylthiopseudourea hydrochloride was followed in basic medium (pH 7.5) to isolate and characterize all possible degradation products. IR, Raman, and NMR spectroscopy, TLC, and elemental analysis were used to identify the products. Degradation of base-hydrolyzed 2,6-dichlorobenzylthiopseudourea hydrochloride produced 2,6-dichlorobenzylthiol and cyanamide and was followed by oxidation (air) to produce bis(2,6-dichlorobenzyl) disulfide, dimerization to give cyanoguanidine, and hydrolysis to yield urea. The kinetics of hydrolysis at 22.5° (pH 7.0 and 7.5) and at 37° (pH 7.0) revealed a pseudo-first-order reaction with respect to the substrate. Apparent first-order rate constants and energy of activation, entropy of activation, and half-life values were determined.

Keyphrases □ 2,6-Dichlorobenzylthiopseudourea—hydrolytic degradation in basic medium, products isolated and identified □ Degradation, hydrolytic—2,6-dichlorobenzylthiopseudourea in basic medium, products isolated and identified □ Hydrolysis—2,6-dichlorobenzylthiopseudourea in basic medium, products isolated and identified □ Thiopseudourea, substituted—hydrolytic degradation in basic medium, products isolated and identified

The novel thiopseudourea analogs exhibited gastric, antisecretory, spasmolytic, and antiulcerogenic activities in laboratory animals. 2,6-Dichlorobenzylthiopseudourea (I) was selected as a candidate for exhaustive pharmacological and chemical studies.

Thiopseudourea is chemically susceptible to hydrolytic degradation and scission of the sulfur–carbon bond in strongly basic solutions (1). Limited information is available on solution stability and on the degradation products resulting from base hydrolysis. Zuman and Fedoreňko (2) investigated the kinetic hydrolysis of several substituted thiopseudoureas at pH 11, using a polarographic method to detect thiols as degradation products. No reference was made of the formation of the disulfide as one major resultant product. The homogeneous base-catalyzed oxidation of *n*-butylthiol to form disulfide with molecular oxygen has been studied in aprotic (3) and aqueous (4) solutions.

The objectives of this investigation were to isolate all possible degradation products resulting from the base hydrolysis of I at pH 7.5 and to follow the hydrolysis kinetics of this entity in a neutral medium and a weakly basic buffered medium at pH 7.0 and 7.5 at 22.5° and at pH 7.0 at 37°. This study was also conducted to characterize the