The Dissolution and Bioavailability of Etodolac from Capsules Exposed to Conditions of High Relative Humidity and Temperatures

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Received August 19, 1992; accepted February 20, 1993

The dissolution and bioavailability of etodolac from capsules exposed to high relative humidity and temperature were compared to those from capsules stored at room temperature (RT). Dissolution of stressed and control capsules was evaluated using a USP basket apparatus at 100 rpm with 900 mL pH 7.5 phosphate buffer (0.05 *M*) at 37°C. The dissolution of etodolac from capsules exposed to stressed conditions was also evaluated with enzymes (pancreatin, 1%, w/v) added to the dissolution medium. The bioavailability of etodolac from capsules exposed to stressed conditions was compared in both dogs and humans to capsules stored at RT conditions. Capsules, 200 and 300 mg, exposed to stressed conditions failed the dissolution (without enzymes) specification [not less than 85% released (80% Q) in 30 min]. However, upon enzyme addition, all capsules met the specification. The rate and extent of absorption from these 200 and 300 mg etodolac capsules in dogs were equivalent to those from capsules stored at RT conditions that passed the dissolution specification. Similarly, the bioavailability of etodolac from 300 mg capsules that failed the dissolution specification upon exposure to stressed conditions was equivalent to that of control capsules in 24 adult male volunteers. Thus, an *in vitro* dissolution test with enzymes provides a better indication of stressed capsule performance *in vivo*.

KEY WORDS: dissolution; bioavailability; etodolac capsules; stressed conditions.

INTRODUCTION

Exposure of dosage forms to conditions of high temperature and relative humidity is an attempt to assess long-term stability of the dosage form in a relatively short period of time. While exaggerated (accelerated) conditions at temperatures greater than 30°C and humidities outside the range of 40–60% are not recommended by gelatin capsule shell manufacturers (1), storage conditions more stressful than these are routinely required by governmental agencies as evidence of the long term stability of the dosage form and the drug entity itself (2). The effect of high temperature and high relative humidity on gelatin is to render it partially insoluble in water due to polymerization (3,4). The extent of polymerization has been reported to be influenced by, among other things, the dye content in the gelatin (4,5). Very often a result of this polymerization on a capsule dosage form is a reduction in the *in vitro* dissolution rate in aqueous media without enzymes (5–9).

A concern is that this reduction in dissolution rate will manifest itself *in vivo* by a reduction in the rate and/or extent of absorption of the drugs administered to patients. However, *in vitro* dissolution media are extremely simplistic versions of the gastrointestinal milieu. The addition of enzymes to the dissolution media increases the dissolution rate of capsule dosage forms stored under these accelerated conditions (6,10). Since enzymes are a normal constituent of the GI tract and the digestive fluids, one would anticipate that the *in vivo* performance (bioavailability) would remain unaltered for formulations exposed to these accelerated conditions, assuming that the high temperature and high relative humidity did not alter the drug substance itself or cause a drug–excipient interaction.

Capsules (200 and 300 mg) of the nonsteroidal antiinflammatory/analgesic drug etodolac, when exposed to high temperature and humidity (40°C/75% RH), unpackaged or packaged in polyvinyl chloride (PVC) or PVC-ACLAR blisters, have been observed to fail a dissolution specification of 80% (Q) in 30 min. When packaged in high-density polyethylene (HDPE) bottles and exposed to the same 40°C/75% RH condition, these same capsules continue to conform to the dissolution specification. Studies were undertaken to (a) confirm that the stability of etodolac itself is not affected by these accelerated conditions, (b) examine the dissolution of stressed capsules, with and without enzymes, and (c) demonstrate that the *in vivo* bioavailability of etodolac capsules stored at 40°C/75% RH is not adversely affected.

MATERIALS AND METHODS

Bioavailability Study of Etodolac in Dogs

Etodolac capsules, 200 mg (lot 7WFF) and 300 mg (lot 7WDQ) strengths, were obtained from the Clinical Supply Department, Wyeth-Ayerst Laboratories, Rouses Point, New York. The capsules were stored unpackaged and packaged in a controlled-temperature/humidity cabinet that was maintained at 40°C/75% RH. The unpackaged capsules were exposed directly to the 40°C/75% RH conditions in open petri dishes. The packaged capsules were contained within a blister card composed of 7.5 mil PVC/2 mil polyethylene/2 mil ACLAR with 1 mil aluminum foil backing. Both strengths of capsules were tested initially for dissolution, strength, degradation, and physical appearance. Samples (n = 12) of the unpackaged and PVC-ACLAR packaged capsules were periodically withdrawn over an 8- to 20-week period from the 40°C/75% RH storage cabinet and tested until the capsules failed USP Stage II criteria (11) for dissolution of 80% Q in 30 min. When the capsules met this criterion, strength, degradation, physical appearance, and bioavailability were evaluated. Etodolac capsules from the same batches were stored in HDPE bottles at 25°C (room temperature) and used as controls.

The bioavailability of etodolac from both packaged and

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0724-8741/93/0900-1295$07.00/0

unpackaged stressed capsules that failed dissolution was compared to control capsules from the same batch stored at room temperature. The bioavailability of both 200 and 300 mg etodolac capsules was tested using 12 beagle dogs, 6 (3 males and 3 females) assigned to each strength. For each strength, all dogs received a control capsule, a packaged stressed capsule, and an unpackaged stressed capsule with at least a 1-week washout between treatments. Blood samples were taken at 0 hr (predose) and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 9, 12, 15, and 24 hr after dosing. Plasma was immediately separated and frozen until analysis. Plasma etodolac levels were determined using the method of Cosyns *et al.* (12) modified for fluorescence detection.

Bioavailability Study of Etodolac in Humans

Etodolac 300 mg capsules (lot 1TJX) were stored unpackaged at 40°C/75% RH. Capsules (n = 12) were tested initially for dissolution, strength, degradation, and physical appearance. Samples were periodically withdrawn from storage and tested for dissolution until the mean dissolution was <50% released at 30 min and no individual capsule was >80% released in 30 min. When the capsules met these criteria, they were further tested for strength, degradation, and physical appearance. Etodolac capsules originating from the same batch were packaged in HDPE bottles and stored at 25°C to serve as controls.

To evaluate the effect of the accelerated conditions on the *in vivo* performance of etodolac from these capsules, a bioavailability study was conducted in 24 adult male volunteers. Each volunteer was randomized to two treatments. Each treatment consisted of a 300 mg etodolac capsule exposed to either unpackaged accelerated conditions or to room-temperature conditions in HDPE bottles. Each treatment was separated by a washout period of at least 1 week. Blood samples were taken at 0 hr (predose) and at 0.25, 0.5, 0.75, 1.0, 1.5, 2, 3, 4, 6, 8, 10, 12, 14, 16, 24, and 30 hr after dosing. Plasma was separated and frozen until analyzed. Plasma etodolac levels were determined by the HPLC method of Cosyns *et al.* (12).

The bioavailability of etodolac from capsules exposed to room temperature and accelerated conditions was compared using the maximum plasma etodolac concentration (C_{max}), time to maximum plasma concentration (t_{max}), and area under the plasma concentration time curve (AUC_{0-24} in dogs and AUC_{0-30} in humans). Statistical comparisons were made with an appropriate analysis of variance using the PROC GLM of SAS. For the dog study the MODEL statement used in the PROC GLM was MODEL = DOG TREATMENT. For the human study the MODEL statement was MODEL = SEQUENCE SUBJECT(SEQUENCE) TREATMENT PERIOD. The 90% confidence intervals (CI) were determined using the two, one-sided test (13).

Dissolution Methodology

Dissolution was performed using a USP basket apparatus in 900 mL of aqueous, pH 7.5 phosphate buffer (0.05 *M*) at 37°C. The basket rotation speed was 100 rpm. Pancreatin (1%, w/v) was added to the dissolution medium for studies conducted with enzymes. The dissolution of etodolac was monitored by UV detection at 278 nm.

RESULTS

Etodolac Stability

Stability data for etodolac capsules are given in Table I. Although depressed dissolution rates are evident for both 200 and 300 mg strength capsules stored unpackaged or packaged in PVC-ACLAR blisters at 40°C/75% RH,

Table I. Stability Data for Etodolac 200 and 300 mg Capsules Stored Packaged and Unpackaged at 40°C/75% RH

Package	Storage condition	Strength (mg/cap)	Dissolution (ave % released)[a,b]	Degradation products (%)
		200 mg capsules used in dog biostudy		
—	Initial	199	101	ND[c]
Open petri dish	15 wk 40°C/75% RH	199	43	ND
PVC-ACLAR	20 wk 40°C/75% RH	NT[d]	50	ND
HDPE BTL[e]	20 wk 25°C	200	101	ND
		300 mg capsules used in dog biostudy		
—	Initial	292	100	ND
Open petri dish	8 wk 40°C/75% RH	296	29	0.04
PVC-ACLAR	20 wk 40°C/75% RH	NT	11	ND
HDPE BTL[e]	20 wk 25°C	299	100	ND
		300 mg capsules used in human biostudy		
HDPE BTL[e]	Initial	301	101	ND
Open petri dish	6 wk 40°C/75% RH	304	27	ND

[a] N = 6 capsules at initial, 12 capsules subsequently.
[b] Dissolution without enzymes.
[c] None detected.
[d] Not tested.
[e] Control.

Table II. Effects of Enzymes on Dissolution[e] of Etodolac 200 and 300 mg Capsules Stored Under Various Conditions

Storage conditions	Average percentage released at 30 min (range)			
	200 mg capsules		300 mg capsules	
	Without enzymes	With enzymes	Without enzymes	With enzymes
Control,[a] 25°C	101 (100–102)	NT[c]	100 (98–101)	NT
Unpackaged 8–15 weeks, 40°C/75% RH[a]	43 (21–98)	98 (97–100)	29 (4–48)	100 (96–103)
Packaged 20 weeks, 40°C/75% RH[a]	50 (8–98)	98 (96–100)	11 (3–26)	99 (98–101)
Control,[b] 25°C	NA[d]	NA	101 (99–105)	NT
Unpackaged 6 weeks, 40°C/75% RH[b]	NA	NA	27 (2–47)	100 (96–105)

[a] Capsules used in dog biostudy.
[b] Capsules used in human biostudy.
[c] Not tested.
[d] Not applicable.
[e] USP basket apparatus, 100 rpm, 900 mL, 37°C.

strength, degradation, and appearance showed little or no change. Control capsules stored at 25°C in HDPE bottles also showed no evidence of change in strength or presence of degradation products. Further, the dissolution of control capsules was unaffected.

Comparison of Dissolution with and Without Enzymes

The dissolution results of the 200 and 300 mg strength capsules used in both the dog and human biostudy, tested with and without enzymes are presented in Table II. Unpackaged capsules stored at 40°C/75% RH failed dissolution tests sooner (8–15 weeks) than did the PVC-ACLAR packaged capsules (20 weeks). The addition of enzymes (1% pancreatin) to the dissolution media resulted in conformance to the dissolution specification of 80% (Q) in 30 min.

Bioavailability of Stressed and Unstressed Capsules

Results of the bioavailability study in dogs are graphically shown in Figs. 1 and 2 for the 200 and 300 mg strength capsules, respectively. Values for the mean plasma etodolac AUC_{0-24}, C_{max}, and t_{max} values together with the statistical analyses are presented in Table III. The data indicate that both the unpackaged and the packaged etodolac capsules exposed to accelerated conditions were bioequivalent to capsules stored in HDPE bottles at room temperature. The ratios of mean parameters for AUC, C_{max}, and t_{max} for capsules exposed to accelerated conditions compared to control capsules are all within 80–120%. Additionally, none of the differences within capsule strengths were significantly different ($P > 0.05$). While some of the 90% CIs were outside the 80–120%, this most likely reflects the small number of animals used.

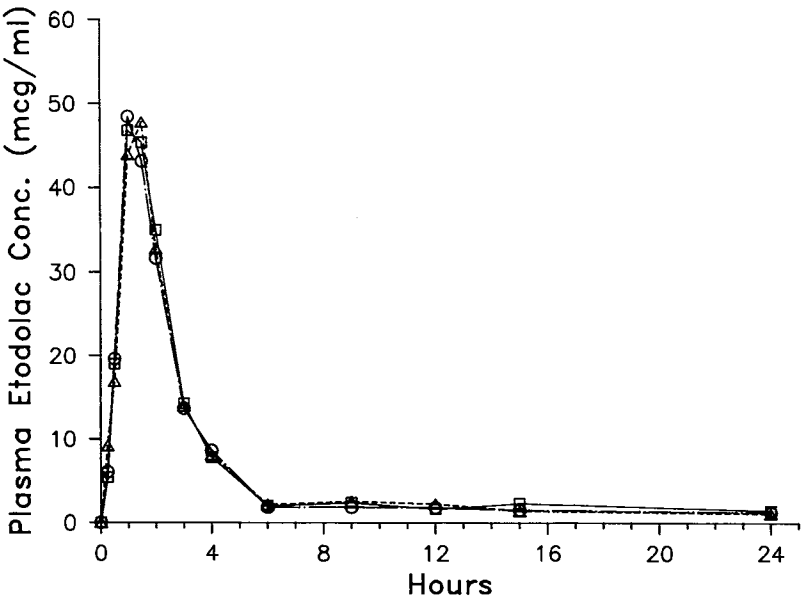

Fig. 1. Effect of capsule storage conditions on the bioavailability of etodolac in dogs from 200 mg capsules: (□) capsules stored at 40°C/75% RH unpackaged, failed dissolution; (△) capsules stored at 40°C/75% RH in PVC-ACLAR blisters, failed dissolution; (○) capsule stored at RT in HDPE bottles, passed dissolution.

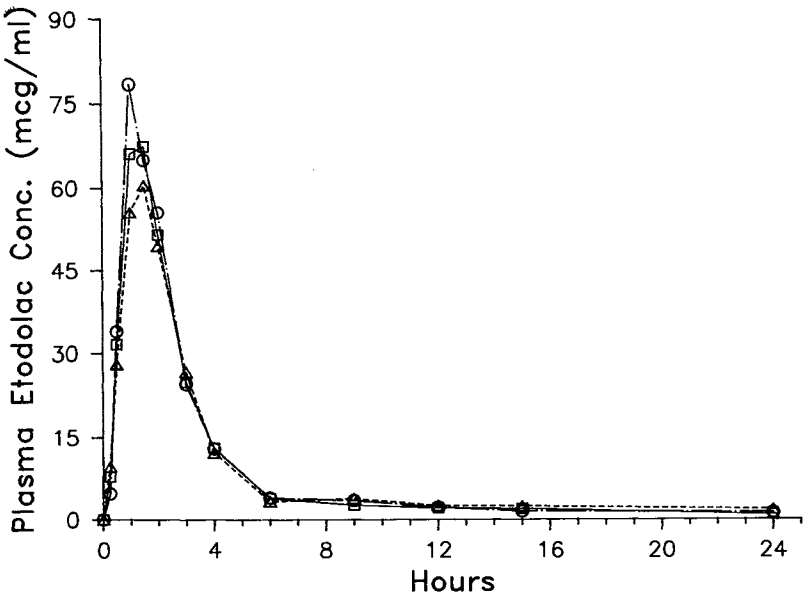

Fig. 2. Effect of capsule storage conditions on the bioavailability of etodolac in dogs from 300 mg capsules: (□) capsules stored at 40°C/75% RH unpackaged, failed dissolution; (△) capsules stored at 40°C/75% RH in PVC-ACLAR blisters, failed dissolution; (○) capsules stored at RT in HDPE bottles, passed dissolution.

Plasma etodolac levels from the human bioavailability study testing the 300 mg capsules are graphically presented in Fig. 3. The mean values for AUC_{0-30}, C_{max}, and t_{max} along with the statistical analyses are presented in Table IV. The results indicate that the capsules exposed to accelerated conditions were bioequivalent to capsules stored at room temperature. Mean values for AUC, C_{max}, and t_{max} are all within ±20% of the mean for the control capsules with no statistical difference between storage conditions ($P > 0.05$).

DISCUSSION

Data from stability testing of dosage forms stored under accelerated conditions, including formulations in hard gelatin capsules, are generally required for approval of new drug applications on the basis that short-term exposure of formulations at accelerated conditions are predictive of long-term exposure at room temperature conditions. The effects of high temperature and humidity on hard gelatin capsules has been well-known for some time. However, such testing continues to be required. The data from this study demonstrate that enzymes in the dissolution media may be used to indicate that the problem is with the capsule shell and thereby avoid the time and cost of conducting bioequivalence studies. Tests for product quality such as strength, degradation, and any other specifications must still be conducted to ensure that the accelerated conditions do not affect the formulated drug substance.

The bioavailability of etodolac from hard gelatin capsules was unaffected by exposure to accelerated storage con-

Table III. Mean Etodolac Bioavailability in Dogs from Stressed Capsules that Failed Dissolution Compared to Control Capsules

	200 mg capsule (n = 6)			300 mg capsule (n = 6)		
Parameter	Packaged	Unpackaged	Control	Packaged	Unpackaged	Control
AUC_{0-24} ± SD (μg × hr/mL)	138.9 ± 46.0	145.9 ± 25.8	135.7 ± 36.1	202.6 ± 50.2	202.8 ± 51.8	212.3 ± 53.9
Ratio (%)	102.4	107.5	—	95.4	95.5	—
90% CI of the ratio	83.9–120.8	89.0–125.9	—	76.2–114.7	76.2–114.8	—
P value	0.82	0.48	—	0.67	0.68	—
C_{max} ± SD (μg/mL)	52.4 ± 10	57.2 ± 9.0	53.1 ± 8.3	77.8 ± 17	78.5 ± 17	83.6 ± 13
Ratio (%)	98.6	107.7	—	93.0	93.9	—
90% CI of the ratio	90.1–107.1	99.2–116.2	—	79.1–107.0	80.0–107.9	—
P value	0.77	0.13	—	0.39	0.45	—
t_{max} ± SD (hr)	1.25 ± 0.42	1.33 ± 0.41	1.17 ± 0.26	1.25 ± 0.52	1.17 ± 0.41	1.17 ± 0.41
Ratio (%)	107.1	114.2	—	107.1	100.0	—
90% CI of the Ratio	77.6–136.6	84.7–143.7	—	72.7–141.5	65.6–134.4	—
P value	0.67	0.40	—	0.71	1.00	—

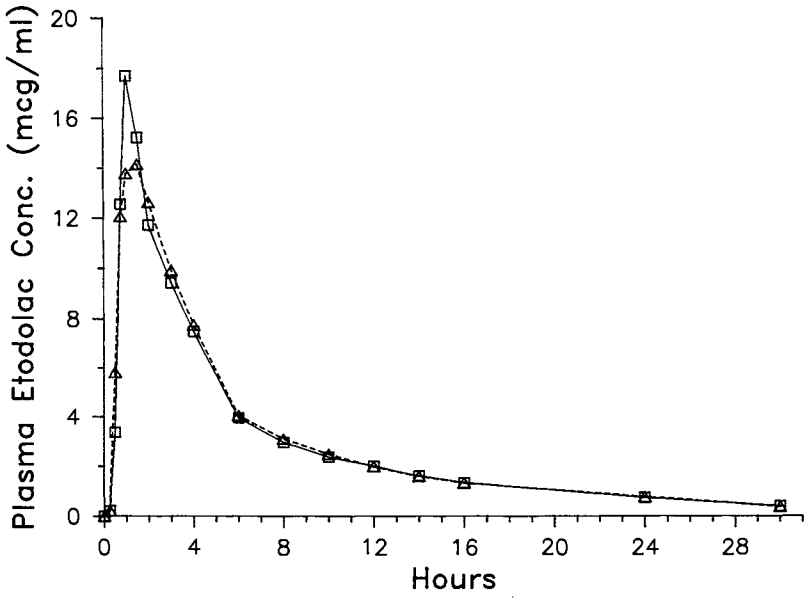

Fig. 3. Effect of capsule storage conditions on the bioavailability of etodolac in humans from 300 mg capsules: (□) capsules stored at 40°C/75% RH unpackaged, failed dissolution; (△) capsules stored at RT in HDPE bottles, passed dissolution.

ditions. Data from the dog study indicated no differences in the bioavailability of packaged or unpackaged capsules that failed dissolution compared to controls. The bioavailability of etodolac in humans from capsules stored under accelerated conditions was also equivalent to control capsules. Comparison of the results between the dog and the human studies suggests that the dog may be a useful model for studying the bioavailability of etodolac and perhaps capsule formulations of other drugs exposed to accelerated conditions. Previously, we have observed a similar phenomenon with capsules containing a combination of aspirin and codeine. In this instance, a dog (and human) biostudy also demonstrated that the *in vivo* performance of the capsules was not impaired. Clearly, the plasma etodolac data from both the dog and the human bioequivalence studies demonstrate that capsules stored under these stress conditions did not exhibit evidence of incomplete or delayed release.

The value of testing the dissolution of hard gelatin encapsulated products after storage under accelerated conditions is questionable since the results are of little predictive value. If such tests continue to be required, the use of a dissolution medium containing enzymes would be capable of determining if any changes in dissolution were due to polymerization of gelatin. The addition of enzymes to the dissolution medium resulted in the acceptable dissolution of etodolac from capsules stored under accelerated conditions and correlated well with the results of the bioequivalency studies.

Table IV. Mean Etodolac Bioavailability in 24 Human Male Volunteers from 300 mg Capsules Exposed to Accelerated Storage Conditions Compared to Control Capsules

Parameter	Storage conditions	
	6 weeks at 40°C/75% RH	6 weeks at RT (Control)
AUC_{0-30} ± SD (μg × hr/mL)	86.2 ± 20	85.5 ± 24
Ratio (%)	100.9	—
90% CI of the ratio	95.1–106.6	—
P value	0.80	—
C_{max} ± SD (μg/mL)	22.7 ± 6.1	20.5 ± 7.8
Ratio (%)	110.7	—
90% CI of the ratio	95.6–125.8	—
P value	0.24	—
t_{max} ± SD (hr)	1.39 ± 0.9	1.67 ± 1.0
Ratio (%)	83.1	—
90% CI of the ratio	57.6–108.5	—
P value	0.27	—

ACKNOWLEDGMENTS

The authors are grateful to Ms. Nancy Barr, Mr. Michael Fournier, Ms. Diane Smith, Dr. Carole Ben-Maimon, and Dr. Karen Klamerus for conducting the stability and bioavailability studies.

REFERENCES

1. *Elanco Qualicaps, Technical Service Reference Manual* (1991).
2. *Guideline for Submitting Documentation for the Stability of Human Drugs and Biologics*, Center for Drugs and Biologics, Food and Drug Administration, 1987, pp. 11, 43.
3. E. M. Marks, D. Tourtellotte, and A. Andux. The phenomenon of gelatin insolubility. *Food Tech.* 22:1433–1436 (1968).
4. R. Clark and A. Courts. The chemical reactivity of gelatin. In A. Ward and A. Courts (eds.), *The Science and Technology of Gelatin*, Academic Press, New York, 1977, pp. 209–247.
5. K. S. Murthy, N. A. Enders, and M. B. Fawzi. Dissolution stability of hard-shell capsule products. I. The effect of exaggerated storage conditions. *Pharm. Tech.* 13(3):72–86 (1989).
6. K. S. Murthy, R. G. Reisch, Jr., and M. B. Fawzi. Dissolution stability of hard-shell capsule products. II. The effect of disso-

lution test conditions on in vitro drug release. *Pharm. Tech.* **13**(6):53–58 (1989).
7. S. A. H. Khalil, L. M. M. Ali, and M. M. A. Khalek. Effects of aging and relative humidity on drug release. I. Chloramphenical capsules. *Pharmazie* **29**:36–37 (1974).
8. P. York. The shelf life of some antibiotic preparations stored under tropical conditions. *Pharmazie* **32**:101–104 (1977).
9. M. Georgarakis, P. Hatzipantou, and J. E. Kountourelis. Effect of particle size, content in lubrication, mixing time and storage relative humidity on drug release from hard gelatin ampicillin capsules. *Drug Dev. Ind. Pharm.* **14**:915–923 (1988).
10. A. Ludwig and M. Van Ooteghem. Disintegration of hard gelatin capsules. *Pharm. Ind.* **43**:188–190 (1981).
11. *United States Pharmacopeia XXII*, Mack, 1989, Sect. 711, pp. 1578–1579.
12. L. Cosyns, M. Spain, and M. Kraml. Sensitive high performance liquid chromatographic method for the determination of etodolac in serum. *J. Pharm. Sci.* 72:275–277 (1983).
13. D. J. Schuirmann. A comparison of the two one-sided tests procedure and the power approach for assessing the equivalence of average bioavailability. *J. Pharmacokinet. Biopharm.* **15**:657–680 (1987).