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# Sustained-release etodolac bioavailability and dose proportionality: correlation between in vivo and in vitro performance

M. Dey, R. Enever, M. Marino *, J. Michelucci, D. Smith, R. Warner ** and R. Weierstall

*Wyeth-Ayerst Research Inc., Rouses Point, NY 12979 (U.S.A.)*

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## Summary

Ultradol is a new, non-steroidal, anti-inflammatory drug under clinical investigation. Sustained-release (SR) formulations, ranging from 200 to 600 mg in strength, have been developed to improve patients convenience and subsequent compliance, when compared with twice daily (b.i.d.) administration of immediate-release capsules. In vitro dissolution testing and clinical bioavailability studies have been completed for the SR formulations. A pilot bioavailability study was conducted in order to evaluate the relationship between in vitro dissolution and absorption. The computer program NONLIN was used to model both in vitro drug release and in vivo plasma concentration–time profiles. Based on the results of the pilot study and the kinetic modeling, optimum target in vitro release rates were identified. Dosage forms exhibiting these in vitro release profiles were evaluated in a bioavailability and dose proportionality study over the range of 200–600 mg daily doses. The SR formulations were bioequivalent to their respective immediate release doses and were dose-proportional. The in vivo performance was accurately predicted by in vitro dissolution data.

## Introduction

Etodolac (Ultradol) is a new, non-steroidal, anti-inflammatory drug presently marketed internationally and is the subject of a New Drug Application in the United States. Etodolac has been shown to be effective in the treatment of rheumatoid arthritis and osteoarthritis and in the relief of moderately severe postsurgical pain (Jacob et al. (1985); Giglo and Campbell (1986)). Development of sustained-release (SR) formulations of etodolac, ranging from 200 to 600 mg in strength, was undertaken to facilitate patient convenience and thus improve patient compliance. SR formulations should provide equivalent extent of bioavailability as immediate release formulations when measured by area under the plasma etodolac concentration-time curves (AUC).

This paper describes the use of plasma etodolac data to define a correlation between the rate of in vitro dissolution and bioavailability. Once established, this in vitro–in vivo correlation was used to set target in vitro release rates for two additional

* Present address: Squibb Pharmaceuticals, P.O. Box 4000, Princeton, NJ 08540, U.S.A.
** Present address: Mylan Pharmaceuticals, P.O. Box 4310, Morgantown, WV 26506, U.S.A.
*Correspondence:* M. Dey, Wyeth-Ayerst Research Inc., 64 Maple Street, Rouses Point, NY 12979, U.S.A.

strengths of the same formulation. The in vivo performance of these SR dosage forms was compared to equivalent daily doses of immediate release Ultradol capsules in a steady state bioavailability/dose proportionality study.

## Materials and Methods

*Dosage forms*

The etodolac reference solution and Ultradol immediate release capsules and SR tablets were manufactured by Ayerst Laboratories, Inc. Immediate release capsule formulations were used in the strengths of 100 and 200 mg. The SR tablet strengths were 200, 400 and 600 mg. The aqueous reference solution contained 25 mg of etodolac/ml.

*Subjects*

A total of 51 adult male volunteers participated in two different studies. All subjects were in good health and physical condition. Their ages ranged from 18 to 35 years (mean 27 years). Their weights ranged from 58 to 86 kg (mean 71 kg) and their heights from 163 to 188 cm (mean 177 cm). All subjects gave their written consent after the study objectives and procedures had been explained to them. No abnormalities were found on medical examination, in the results of laboratory tests, electrocardiograms or chest x-rays.

*Study design*

*Study I*

A pilot study was conducted as a 3-period, cross-over, bioavailability study in 14 subjects. Subjects were assigned, in a random manner, to one of 3 groups. The sequence of dosing for these groups was in accordance with a $3 \times 3$ latin square design. Each subject was administered single 400 mg oral doses of etodolac as experimental SR formulations, A or B, or the reference solution.

*Study II*

The bioavailability and dose proportionality of the chosen SR formulation from the first study were evaluated in a second study. This second study was conducted as a steady state, randomized, incomplete block in 37 volunteers. All 37 subjects received a 400 mg daily dose of Ultradol as 200 mg immediate release capsules b.i.d. and as a single 400 mg SR tablet. Eighteen of the 37 subjects received a 200 mg daily dose as 100 mg Ultradol capsules b.i.d. and as a single 200 mg SR tablet. The remaining 19 subjects received a 600 mg daily dose as 300 mg Ultradol capsules (100 plus 200 mg) b.i.d. and as a single 600 mg SR Ultradol tablet. All doses were administered for 3 days. Steady state conditions were confirmed by comparison of the plasma etodolac concentration in the pre-dose blood samples on days 1–3.

*Drug administration*

The first dose in each period, for both studies, was administered following a 10 h fast. Subsequent doses in the b.i.d. regimen were administered at 12 h after the initial dose. All doses were administered with 100 ml of water. Standard meals were served 2 h after the first dose and then again at 6 and 10 h, followed by a snack at 14 h.

*Blood sampling*

Blood samples were taken by venipuncture using 7 ml EDTA-treated, evacuated, blood sampling tubes. Samples were centrifuged in a refrigerated centrifuge (0–10 °C) for 15 min at 2500 rpm. The plasma was transferred to disposable, polypropylene tubes, capped, and frozen until analysis. All samples were submitted to the laboratory personnel without identification of the dose administered. The code was broken after analysis was complete.

*Dissolution testing*

Dissolution of the SR tablets was performed in an aqueous medium using USP apparatus. Quantitation of etodolac in the dissolution medium was performed by UV at an absorbance reading of 278 nm.

*Assay of etodolac in plasma*

Plasma etodolac concentrations were determined using two high-performance liquid chromatographic (HPLC) methods. Samples from the pilot study were analyzed by the HPLC/UV

method of Cosyns et al. (1983) with a limit of quantitation of 0.2 μg/ml. Samples from the second study were analyzed using both the method of Cosyns et al. (1983) and a more sensitive HPLC/fluorescence method with a limit of quantitation of 0.018 μg/ml.

Both methods use the same extraction procedures. Specifically, the plasma was acidified with 1 N HCl and extracted into 5 ml of 5 : 95 (V : V) isopentanol : hexane. Four ml of the hexane layer were removed and etodolac was back extracted into 1.0 ml of glycine buffer at pH 11.0. A 0.8 ml aliquot of the glycine buffer was acidified with 0.02 ml of 2.5 M phosphoric acid and 0.05 ml was injected onto a $C_{18}$ reverse phase HPLC column. The mobile phase in both cases was 32% (v/v) acetonitrile in 0.04 M potassium phosphate buffer. The UV method measured etodolac absorbance at 226 nm. The fluorescence method used an excitation wavelength of 280 nm and an emission wavelength of 350 nm.

For both methods, a matrix standard curve was run with each set of samples. For the fluorescence method, no internal standard was used. The mean recovery of the HPLC/fluorescence method was 103.1% (range: 100.0% at 0.035 μg/ml to 109.1% at 0.018 μg/ml) over an etodolac concentration range of 0.018 μg/ml to 1.11 μg/ml. The precision of the assay method as measured by the coefficient of variation (CV) ranged from 1.7% at 0.444 μg/ml to 5.8% at 0.018 μg/ml. The mean CV over the entire range (0.018 to 1.11 μg/ml) was 7.2%. Plasma spiked with etodolac at varying concentrations was analyzed along with the unknown samples each day to ensure acceptable performance of the method.

*Data analysis*

The relative rate of absorption of etodolac from each of the formulations administered in the bioavailability and dose proportionality study was assessed from the peak plasma etodolac level ($C_{max}$) and the time at which it occurred ($t_{max}$). The relative extent of bioavailability from these formulations was estimated by determining the area under the plasma etodolac concentration–time curve (AUC) over the 24 h dosing interval using the trapezoidal method. The statistical significance of differences in the data was assessed using an analysis of variance (ANOVA) appropriate for the study design. The computer program, SAS (Statistical Analytical Systems), was used to calculate the various parameters and perform the ANOVA.

The plasma etodolac concentration–time curves, following administration of the etodolac solution in the pilot study, were plotted log-linearly and feathered to define the pharmacokinetic model for etodolac and to obtain the initial microconstant estimates for each subject. These initial estimates were used in a subroutine for NONLIN (Metzler et al., 1974) to determine the least squares estimates of the microconstants. The initial estimate for sampling compartment volume ($V_1$) was determined from a previous study (Cayen et al., 1981). The fraction absorbed ($E$) was assumed to be 82% based on urinary excretion data following an oral dose of $^{14}$C-etodolac corrected for total $^{14}$C recovered (Cayen et al., 1981).

The apparent elimination half-life ($t_{1/2}\beta$) from plasma was calculated from the terminal log-linear portion of the plasma etodolac concentration-time curve ($\beta$ phase). The apparent volume of distribution during the $\beta$ phase ($V_D\beta$) was calculated by:

$$V_D\beta = Cl/\beta$$

Apparent oral clearance ($Cl$) was calculated for each subject as the product of the apparent volume of the central compartment ($V_1$) and the elimination rate constant from the central compartment ($k_{10}$).

*In vitro–in vivo correlations*

Correlations between in vitro dissolution and bioavailability were studied for each experimental SR candidate from Study I (formulations A and B). Dissolution data were plotted as percent released vs time. The first derivative of the dissolution curve was calculated over each time interval measured. These first derivatives were used as sequential zero order input functions to the pharmacokinetic model for etodolac to simulate plasma etodolac profiles. These simulations were then compared to the plasma profiles measured after dosing with either SR formulation A or B.

Based on the results of these simulation comparisons, target dissolution profiles were calculated for the higher (600 mg) and lower (200 mg) strength SR dosage forms. Subsequent comparison of the simulated profiles and the measured plasma etodolac levels from Study II were also made.

## Results

A 2-compartment, open model was found to adequately describe the pharmacokinetics of etodolac from a solution in man (Fig. 1). Absorption into and elimination from the central compartment (Compartment 1) were assumed to occur by first order processes ($k_a$ and $k_{10}$, respectively). The mean least squares estimates for the intercompartmental rate constants, $k_{12}$ and $k_{21}$, and other NONLIN fitted parameters for subjects receiving a 400 mg etodolac solution are presented in Table 1. The model parameters for the disposition of etodolac in subjects receiving a 200 mg capsule in the bioavailability/dose proportionality study (Study II) are compared to those determined for the solution from the pilot study (Study I) in Table 1. Similar values for $Cl$, $t_{1/2}\beta$, $V_1$, $V_D\beta$, $k_{21}$, $k_{12}$ and $k_{10}$ were determined in both studies. The apparent absorption rate constant of etodolac from the solution was approximately 10 times greater than from the immediate release capsules.

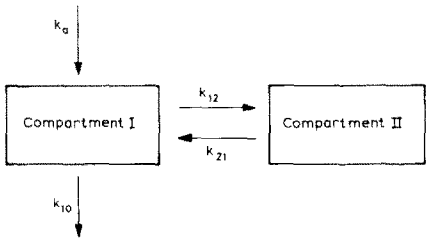


Fig. 1. Two compartment open model for etodolac disposition. Elimination rate constant, $k_{10}$, and intercompartmental rate constants, $k_{12}$ and $k_{21}$, are assumed to be first order. The apparent absorption rate constant, $k_a$, for the solution = 12.7 $h^{-1}$, for the capsule = 1.24 $h^{-1}$ and for the SR tablets = a series of zero order inputs.

The model parameter estimates were also determined when the model was constrained by a simultaneous fit of both the solution and capsule data (Table 1). The parameter estimates from the simultaneous fit indicate that the disposition of etodolac is consistent among different subject populations and is independent of the input.

The mean dissolution data from the SR formulations A and B, expressed as the mean percent released at each time point, are presented in Table 2. Rank order correlation between in vitro dissolution, measured as the rate of release of etodolac from the dosage form over each of the 4-h intervals, and relative bioavailability, measured as $AUC_{(SR)}/AUC_{(ref)}$, was observed. The release rate of etodolac from formulation A was 6–10% slower

TABLE 1

*Comparison of mean etodolac disposition constants and kinetic parameter estimates from a solution, an immediate release capsule and simultaneous fit of both the solution and capsule data*

| | Pilot Study ($n = 14$) (400 mg solution) | Bioavailability/dose proportionality study ($n = 37$) (200 mg capsule) | Simultaneous fit |
|---|---|---|---|
| $k_{10}$ ($h^{-1}$) | 0.406 (±0.128) | 0.391 (± 0.188) | 0.432 (±0.097) |
| $k_{12}$ ($h^{-1}$) | 0.446 (±0.293) | 0.696 (± 0.353) | 0.557 (±0.247) |
| $k_{21}$ ($h^{-1}$) | 0.347 (±0.239) | 0.276 (± 0.222) | 0.358 (±0.080) |
| $V_1$ ($l$) | 7.02 (±2.03) | 6.28 (± 2.73) | 6.62 (±1.47) |
| $k_a$ (solution) ($h^{-1}$) | 12.7 (±11.2) | | 4.02 (±1.65) |
| $k_a$ (capsule) ($h^{-1}$) | | 1.24 (± 0.711) | 1.39 (±0.37) |
| $t_{1/2}\beta$ (h) | 5.97 (±1.70) | 8.38 (± 3.84) | 6.75 (±1.52) |
| $Cl$ (ml/h × kg) | 36.3 (±7.36) | 38.5 (±13.6) | 42.2 (±4.96) |
| $V_{D\beta}$ (l/kg) | 0.31 (±0.10) | 0.465 (± 0.219) | 0.41 (±0.05) |

Values are mean (±S.D.)

TABLE 2

*Mean dissolution of etodolac from Ultradol SR tablets (n = 12)*

| Time (h) | Formulation A % released | Formulation B % released |
|---|---|---|
| 0 | 0 | 0 |
| 4 | 26 | 32 |
| 8 | 45 | 53 |
| 12 | 58 | 68 |
| 14 | 64 | 73 |

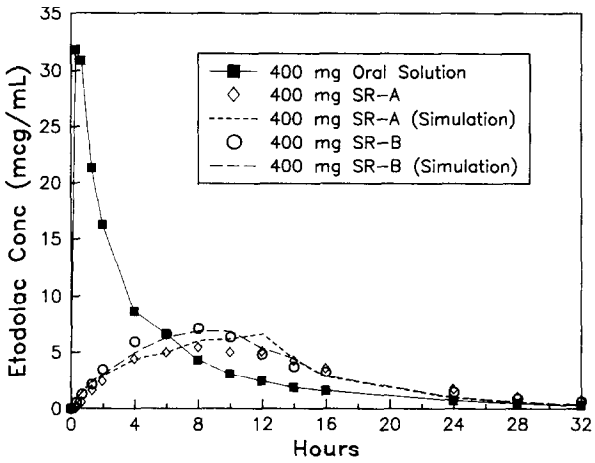


Fig. 2. Observed and simulated mean plasma etodolac concentrations from 400 mg Ultradol SR tablets and a 400 mg Ultradol solution. The observed concentrations were from Study I and the simulated concentrations were generated from in vitro dissolution data.

than from formulation B consistent with the difference in the relative bioavailabilities (78 and 84%, respectively).

Using the in vitro dissolution data and the 2 compartment model, plasma etodolac levels were simulated for SR formulations A and B. In Fig. 2, the simulated plasma concentrations are compared with those measured in the pilot study. From the results of the pilot study, SR formulation B was chosen as an acceptable sustained release formulation of etodolac based on its AUC being 84% of the reference solution (Table 3).

The mean results of the bioavailability/dose proportionality study (Study II) in which the 200, 400 and 600 mg SR formulations were compared to equivalent immediate release doses, are shown in Fig. 3. For each strength, the SR formulation showed equivalent bioavailability to its reference dose (Table 4). Etodolac $C_{max}$ and AUC values, whether from the SR formulation or the immediate release doses, were found to be linearly related to dose. When normalized to the 200 mg dose, no significant differences ($P \leq 0.05$) were observed between AUC or $C_{max}$ values (Table 4). Furthermore, for the immediate release capsules, the $t_{max}$ values were not significantly different. Similarly, no significant differences were observed for the $t_{max}$ values of the SR tablets.

Simulated plasma etodolac concentration–time curves using the dissolution data as input into the 2-compartment model for the 200, 400 and 600 mg SR formulations are compared to the mean results

TABLE 3

*Relative bioavailability of two 400 mg Ultradol SR formulations compared to a 400 mg solution*

| Parameter | Ultradol SR formulation A | Ultradol SR formulation B | Ultradol oral solution |
|---|---|---|---|
| $C_{max} \pm$ S.E.M. ($\mu$g/ml) | 7.73 ± 0.93 | 9.11 ± 1.25 | 36.8 ± 3.02 |
| % of reference | 20.9 | 24.7 | – |
| *P* | < 0.001 | < 0.001 | – |
| $t_{max} \pm$ S.E.M. (h) | 9.3 ± 1.1 | 7.0 ± 0.66 | 0.55 ± 0.08 |
| % of reference | 1597 | 1179 | – |
| *P* | < 0.001 | < 0.001 | – |
| AUC (0–∞) ± S.E.M. ($\mu$g × hr/ml) | 104 ± 7.6 | 111 ± 7.4 | 133 ± 7.7 |
| % of reference | 78.3 | 83.7 | – |
| *P* | 0.002 | 0.005 | – |

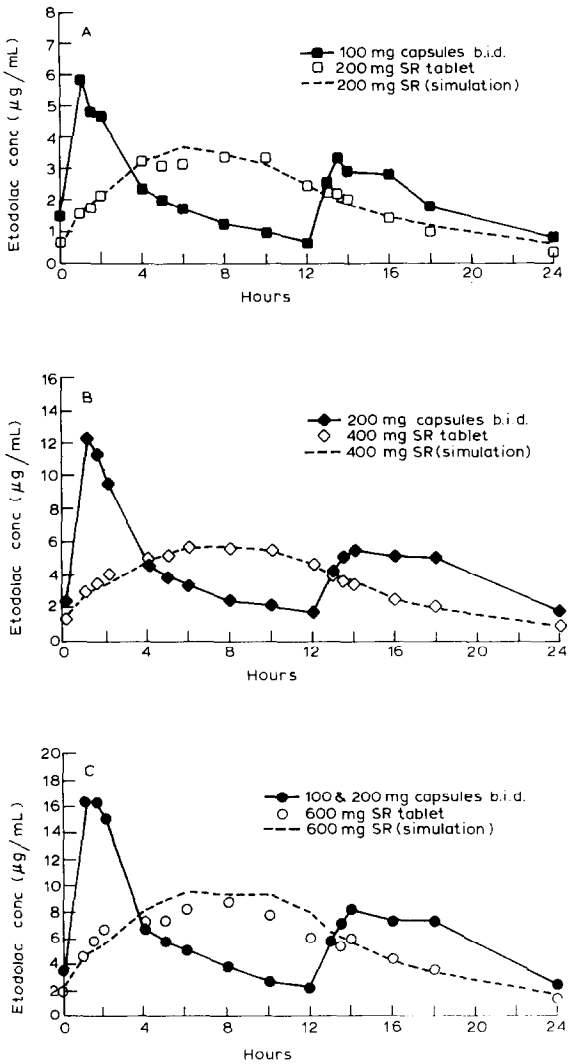


Fig. 3. Observed and simulated mean plasma etodolac concentrations from 200 (A), 400 (B) and 600 (C) mg Ultradol as either SR tablets or immediate release capsules. The observed concentrations were from Study II and the simulated concentrations were generated from in vitro dissolution data.

from Study II in Fig. 3. Again, good correlation was observed between the simulated and the observed levels in each case.

## Discussion

The etodolac disposition constants and kinetic parameters from Study I and II are remarkably close considering the two studies were carried out in different subjects. The slower apparent absorption rate from the capsule compared to the solution is consistent with the disintegration and dissolution time required for solid dosage forms compared to solutions. While there appears to be a relatively large difference in values of $k_{12}$ estimated from the solution and capsule, this difference may reflect poor parameter estimation as indicated by the relative standard deviations for both values. This is somewhat supported by a similar problem with the $k_{21}$ parameter estimates. The similarity in the parameter estimates determined by individual and simultaneous fits to the plasma data for the solution and capsule indicate that the input rates can be manipulated and provide very predictable plasma etodolac profiles.

Values for $t_{1/2}\beta$, $Cl$ and $V_D\beta$ are very similar to estimates previously reported by Cayen et al. (1981). In their work, the half-life was estimated to be 7 h compared to 6 and 8 h in the present studies and $Cl$ was estimated at 40.8 ml/h × kg compared to 36.3 and 38.5 ml/hr × kg reported here. If $Cl$ is calculated by non-compartmental methods i.e. $F \times$ dose/AUC, values of 34.7 and 42.8 ml/h × kg are obtained for the solution and capsule, respectively. The lower value for $Cl$ from the solution (34.7 ml/h × kg) may reflect more efficient systemic absorption than the value assumed here (0.82). If a value of 1.0 is assumed for $F$, as was done by Cayen et al. (1981), a $Cl$ value of 42.4 ml/h × kg is obtained. Their reported value for $V_D\beta$ was 0.41 l/kg where values in the present studies ranged from 0.31 to 0.46 l/kg.

Significantly ($P < 0.05$) lower $C_{max}$ and later $t_{max}$ values were measured after the second daily administration of all doses (100, 200 and 100 plus 200 mg) of the immediate release capsules (Fig. 3). This supports the observation previously reported by Kraml et al. (1984) who noted that the plasma etodolac levels at 12 h after the first dose of a 100 mg b.i.d. administration were lower than 12 h after the second 100 mg dose. While it is tempting to suggest the etodolac pharmacokinetics may be subject to a diurnal effect, in both studies the fasting conditions were not maintained from the first to the second daily dose. The first daily dose was preceded by at least a 10 h fast while a meal

TABLE 4

*Relative steady state bioavailability and dose proportionality of Ultradol SR compared to equivalent daily dose of immediate-release Ultradol*

| Parameters | 100 mg b.i.d. | 200 mg SR | 200 mg b.i.d. | 400 mg SR | 300 mg b.i.d. | 600 mg SR |
|---|---|---|---|---|---|---|
| $C_{max}$ ± S.E.M. (μg/ml) | 7.34 ± 0.50 | 4.58 ± 0.46 | 14.5 ± 0.71 | 7.47 ± 0.36 | 20.8 ± 1.53 | 11.9 ± 1.08 |
| % of reference | – | 62.4 | – | 51.5 | – | 57.2 |
| *P* | – | < 0.001 | – | < 0.001 | – | < 0.001 |
| $C_{max}$ ratio | 1.00 ** | 1.00 * | 1.98 ** | 1.63 * | 2.84 ** | 2.59 * |
| AUC (0–24) ± S.E.M. (μg × h/ml) | 53.9 ± 4.1 | 51.5 ± 3.7 | 111 ± 5.5 | 95.1 ± 4.2 | 162 ± 11.7 | 146 ± 14.4 |
| % of reference | – | 95.6 | – | 85.4 | – | 90.0 |
| *P* | – | 0.607 | – | < 0.001 | – | 0.022 |
| AUC ratio | 1.00 ** | 1.00 * | 2.06 ** | 1.85 * | 3.01 ** | 2.83 * |
| $t_{max}$ ± S.E.M. (h) | 1.5 ± 0.2 | 7.2 ± 0.7 | 1.3 ± 0.1 | 7.9 ± 0.5 | 1.7 ± 0.3 | 7.8 ± 0.7 |
| % of reference | – | 480 | – | 608 | – | 459 |
| *P* | – | < 0.001 | – | < 0.001 | – | < 0.001 |
| $C_{max}/C_{min}$ ± S.E.M. | 7.82 ± 0.87 | 8.66 ± 1.11 | 8.16 ± 1.08 | 7.20 ± 0.93 | 7.47 ± 0.86 | 7.43 ± 1.02 |
| % of reference | – | 111 | – | 88.2 | – | 99.5 |
| *P* | – | 0.627 | – | 0.423 | – | 0.939 |

* Relative to 200 mg SR

** Relative to 100 mg b.i.d.

was served 2 h before and a snack 2 h after the second dose. Since food has been demonstrated to lower the rate of absorption of etodolac in dogs, the possibility that the lower $C_{max}$ after the second daily dose was due to a difference in the fasting status of the subjects cannot be ruled out.

Etodolac has been shown to be effective in relief from dental pain at single doses of 50 to 200 mg, from rheumatoid arthritis over the range of 100–600 mg/day and from osteoarthritis over the range of 100–600 mg/day (Pinalo, 1985). Kraml et al. (1984) reported the average plasma etodolac levels [AUC $(0-\tau)/\tau$] from 100, 200 and 300 mg b.i.d. doses of the immediate release capsules were 2.2, 4.6 and 6.8 μg/ml, respectively. Mean plasma etodolac levels from the 200, 400 and 600 mg Ultradol SR tablets, measured in the present study, were quite similar (2.1, 4.0 and 6.1 μg/ml, respectively) and are expected to provide equivalent therapeutic relief. A clinical study is ongoing to confirm the therapeutic equivalence of these SR formulations.

Development of SR dosage forms can be significantly aided by the utilization of computer models that are specific for the drug and the formulation. However, the disposition kinetics of the drug must be able to be characterized in order to define the basic model and to evaluate the effect of varying input rate on plasma levels. For Ultradol SR, when the input function was estimated from the in vitro rate of dissolution over discrete time intervals, a direct correlation between the dissolution profile and the relative bioavailability of the formulation was observed. Once established, the correlation between dissolution rate and bioavailability enabled accurate prediction of dosage form performance in vivo from in vitro dissolution profiles. Additional, such a correlation may be useful in establishing meaningful dissolution specifications and limits for sustained release dosage forms.

The present studies indicate that Ultradol SR is as equally bioavailable as the immediate release Ultradol capsules administered b.i.d. and is dose-proportional over the range of 200 to 600 mg. Further, the bioavailability of Ultradol SR could be accurately predicted from in vitro dissolution.

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*Statistical Analytical Systems,* SAS Institute, Cary, NC.