Correlation of *in Vitro* Release Rate and *in Vivo* Absorption Characteristics of Four Chlorpheniramine Maleate Extended-Release Formulations

Parviz Mojaverian,[1] Elaine Radwanski,[1] Chin-Chung Lin,[1] Phillip Cho,[2] Winston A. Vadino,[2] and Jack M. Rosen[2,3]

Received May 31, 1991; accepted October 22, 1991

An *in vitro/in vivo* correlation was established for four formulations of chlorpheniramine maleate (histamine, H_1-blocker) extended-release tablets exhibiting different *in vitro* release rate characteristics. *In vitro* release rate data were obtained for 12 individual tablets of each formulation using the USP Apparatus 2, paddle stirrer at 50 rpm in 1000 ml of distilled water at 37.0 ± 0.5°C. Inspection of the individual and mean release rate data indicated that the *in vitro* release rate of chlorpheniramine maleate was consistent with the intended design of the four extended-release formulations. The *in vivo* bioavailability and pharmacokinetics of these formulations were evaluated in 24 healthy subjects under fasting conditions. Wagner Nelson analyses of the *in vivo* data revealed extended release absorption profiles for all four formulations. Linear regression analyses of the mean percentage of dose absorbed versus the mean *in vitro* release resulted in a statistically significant correlation ($r^2 > 0.98$, $P < 0.001$) for each formulation. Qualitative rank-order correlations were observed among all combinations of *in vitro* and *in vivo* parameters. These data support a Level A correlation between the *in vitro* release rate profiles and the *in vivo* absorption for chlorpheniramine maleate determined under fasting conditions.

KEY WORDS: chlorpheniramine maleate; extended-release tablet; release rate; absorption profile; *in vitro/in vivo* correlation.

INTRODUCTION

An extended-release chlorpheniramine maleate product indicated for the temporary relief of hay fever symptoms, is under development by the Schering-Plough Corporation. Evaluation of product performance is strongly dependent on *in vitro* techniques and *in vivo* bioavailability. The results of *in vivo* bioavailability studies, often dictate formulation modification(s) to achieve the optimum performance characteristics of the marketed product. Therefore, it is essential that *in vitro* release rate methods have appropriate discriminating ability to enable their use in the screening of experimental formulations. Unless sufficient experimental data are available to demonstrate that the *in vitro* method reflects *in vivo* performance, release rate data carry very little importance in predicting overall product bioavailability which affects clinical efficacy and response. Release rate testing has been utilized primarily as a research method to assist in formulation development or as a quality control procedure to routinely determine the uniformity of production batches. The establishment of a meaningful correlation between the *in vitro* release and the *in vivo* bioavailability parameter(s) can then serve as a means of predicting bioavailability. This may reduce the need for costly additional clinical studies. Further, a valid *in vitro/in vivo* correlation facilitates the development of product specifications based on bioavailability data, the most meaningful criterion of quality assurance and predictable performance. Validation of the *in vitro* dissolution procedure as a predictor of *in vivo* performance is best achieved by comparing absorption characteristics with the release rate profiles of formulations specifically designed to show faster and slower release profiles than the intended marketed formulation. The *in vitro* procedure should be compendially acceptable to regulatory agencies and possess the ability to discriminate products with varying degrees of bioavailability. Many of the techniques that have been reported for establishing such correlations were of the simple-point type, where the percentage dissolved is correlated with a parameter of drug bioavailability (1), while others assumed *in vivo* and *in vitro* kinetics were constant for all the dosage forms tested (2–4). Some investigators utilized statistical moment methods (5–7), an *in vivo* dissolution rate intercept method (8), deconvolution methods (9), or, more recently, a computer simulation using the process termed "biorelevant dissolution" (10).

The purpose of this study was to establish an *in vitro/in vivo* correlation between an *in vivo* parameter (percentage of dose absorbed) and the *in vitro* release rate profiles for the four chlorpheniramine maleate extended-release formulations.

MATERIALS AND METHODS

Dosage Forms

Four formulations containing chlorpheniramine maleate were manufactured for use in this study. Each formulation was designed to exhibit different release rate properties from the proposed standard formulation.

The dosage forms used in this study were based on a matrix design using a mixture of cellulosic polymers. Drug release occurs by a combination of diffusion and erosion mechanisms. Varying the ratio of the cellulosic constituents will control the release rate of the drug from the dosage form. Once the designed standard formulation was identified based on release rate and *in vivo* bioavailability characteristics, additional formulations were prepared with release rates faster and slower than that of the reference standard.

[1] Drug Metabolism/Pharmacokinetics Department, Schering-Plough Research, Bloomfield and Kenilworth, New Jersey.

[2] Pharmaceutical Sciences Department, Schering-Plough Research, Bloomfield and Kenilworth, New Jersey.

[3] To whom correspondence should be addressed at Schering-Plough Research, PACRD, 2000 Galloping Hill Road, Kenilworth, New Jersey 07033.

0724-8741/92/0400-0450$06.50/0

In Vitro Dissolution Testing

Dissolution testing was performed using the USP Apparatus 2, paddle stirrer operating at 50 rpm. Based on solubility data, 1000 ml of water maintained at 37.0 ± 0.5°C was chosen as the dissolution medium. Multicomponent UV-VIS spectrophotometric analysis using a Hewlett Packard 8450 photodiode array spectrophotometer was used due to the interference of a dye blend in the tablet formulations. Approximately 10-ml aliquot samples were withdrawn from each dissolution vessel, filtered, and acidified with 0.1 *N* HCl to enhance and stabilize the absorbance spectra. The wavelength range for the multicomponent analysis was optimized after successful completion of drug linearity and recovery studies. Replicate multicomponent analyses of different concentrations of standard solutions yielded relative standard deviations of <0.4%.

In Vivo Studies

Study Design

Twenty-four healthy male subjects, with an age range of 19 to 39 years and average weights in accordance with current actuarial tables (±10%), were empaneled for this randomized, four-way crossover study. Each subject was determined to be in good health after medical history, physical examination, electrocardiogram, and laboratory tests. Twelve hours prior to the start of each treatment phase, the subjects were confined to the study area. A light snack was served on the night before drug administration after which an overnight fast was maintained. In the morning, each subject received one treatment with 180 ml of tap water from the four manufactured batches according to a computer generated random code. At least a 1-week drug-free washout period separated each treatment phase of this single-dose crossover study. Seven milliliters of blood was drawn immediately prior to drug administration (0 hr) and then again at specified times up to 120 hr after dosing. Chlorpheniramine plasma concentrations were determined by a previously validated, specific gas liquid chromatographic procedure with a limit of quantitation (LOQ) of 0.5 ng/ml (11).

Pharmacokinetic Analysis

Absorption profiles of chlorpheniramine maleate were evaluated for each subject using the Wagner Nelson function (12):

$$F(t) = C(t) + K * \mathrm{AUC}(t)$$

where K is the elimination rate constant calculated as the negative slope of the log-linear terminal phase of the plasma concentration–time curve. AUC(t) is the cumulative area under the plasma concentration–time curve from zero to time t, calculated by the linear trapezoidal rule. The Wagner Nelson function was expressed as a percentage of its asymptotic value, $K * \mathrm{AUC}(I)$, to yield the percentage of dose absorbed.

$$\%\ \text{dose absorbed} = \frac{C(t) + K * \mathrm{AUC}(t)}{K * \mathrm{AUC}(I)}$$

where AUC(I) is the area under the plasma concentration–time curve from time zero to infinity.

In Vitro/in Vivo Data Analysis

The method of Levy and Hollister (13,14) was utilized to determine the *in vitro/in vivo* correlation. This method which corrects for the *in vivo* lag time (T_{lag}), utilizes the Wagner Nelson method expressed in terms of the semilogarithmic percentage unabsorbed as a function of time. An intensity factor, I, and T_{lag} are defined as follows:

$$I = \frac{\text{Time required for 50\% absorption } \textit{in vivo}}{\text{Time required for 50\% release } \textit{in vitro}}$$

$$T_{lag} = \frac{t - \text{Lag Time}}{I}$$

where t is the original *in vitro* sampling interval.

Percentage of dose released at T_{lag} and I for each formulation were determined from best-fit third-degree polynomial equations established for the *in vitro* release profiles.

RESULTS AND DISCUSSION

The objective of this study was to demonstrate a correlation between the percentage of dose released and the measured *in vivo* parameters for four chlorpheniramine maleate extended-release formulations.

In the present study mean pharmacokinetic and release rate data were used to determine all mathematical correlations to minimize intra and inter subject variability. Maximum drug absorption and serum concentrations were observed at approximately 8 hr after oral administration. *In vitro* release rate data up to the eighth hour were used in all linear regression analyses.

Mean cumulative *in vitro* release rate profiles for the four formulations are presented in Fig. 1. Inspection of the data indicated that the *in vitro* release rates of chlorpheniramine maleate are consistent with the intended formulation design and controlled by the amount of cellulosic polymer in each formulation.

The mean percentage dose absorbed-versus-time profiles for the various extended release formulations are shown in Fig. 2. The absorption rate over the 8-hr interval was similar for all extended release formulations. Mean plasma chlorpheniramine concentration–time profiles are illustrated in Fig. 3. Each formulation exhibited the anticipated extended-release profiles, differing only in the rate at which chlorpheniramine maleate was released and, thereby, available for absorption. Rank-order (qualitative) correlations were observed among these *in vivo* and *in vitro* parameters for each prototype formulation (Figs. 1–3). As the level of cellulosic polymer increased, the *in vitro* release rate decreased (Fig. 1), with correspondingly lower percentages ab-

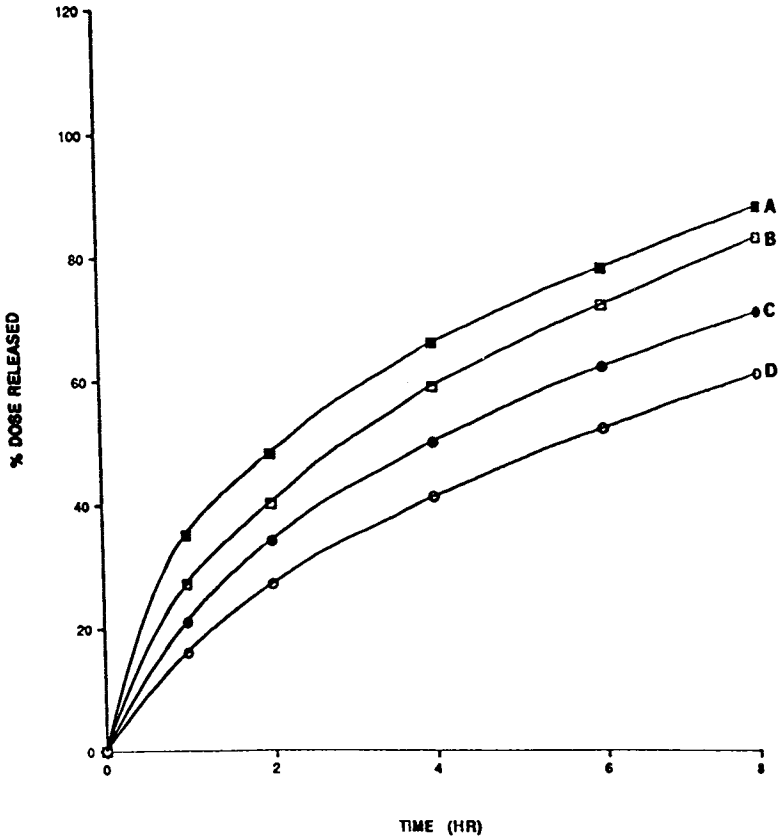

Fig. 1. Mean *in vitro* release profiles for four extended-release formulations of chlorpheniramine maleate over an 8-hr period.

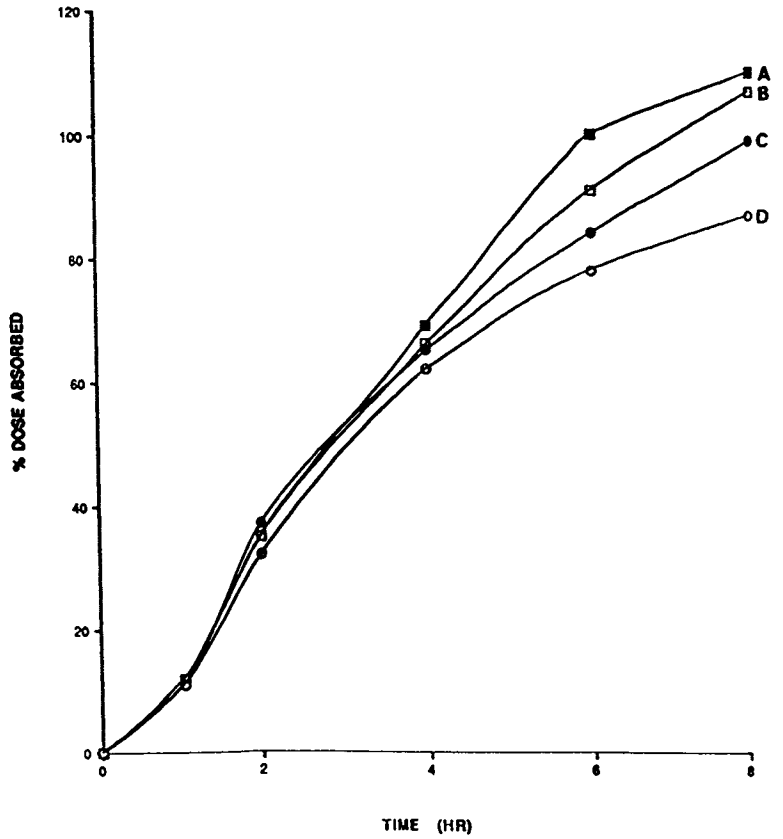

Fig. 2. Mean *in vivo* absorption profiles following oral administration of four chlorpheniramine maleate extended-release formulations over an 8-hr period. The percentage dose absorbed at each tested time point was calculated by the Wagner Nelson method.

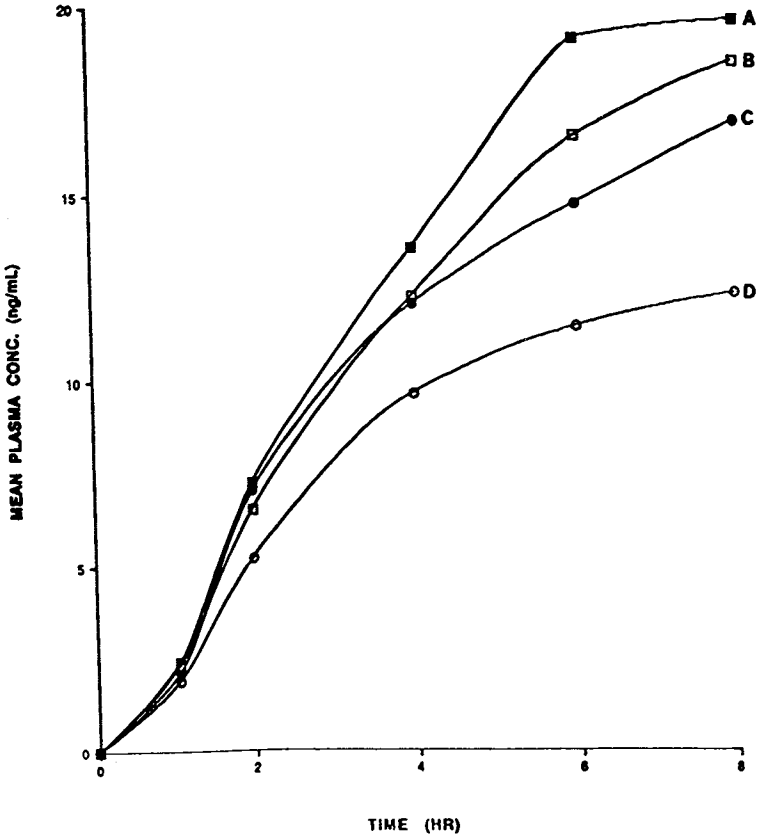

Fig. 3. Mean plasma concentration–time profiles for four chlorpheniramine maleate extended-release formulations following oral administration in 24 normal male subjects.

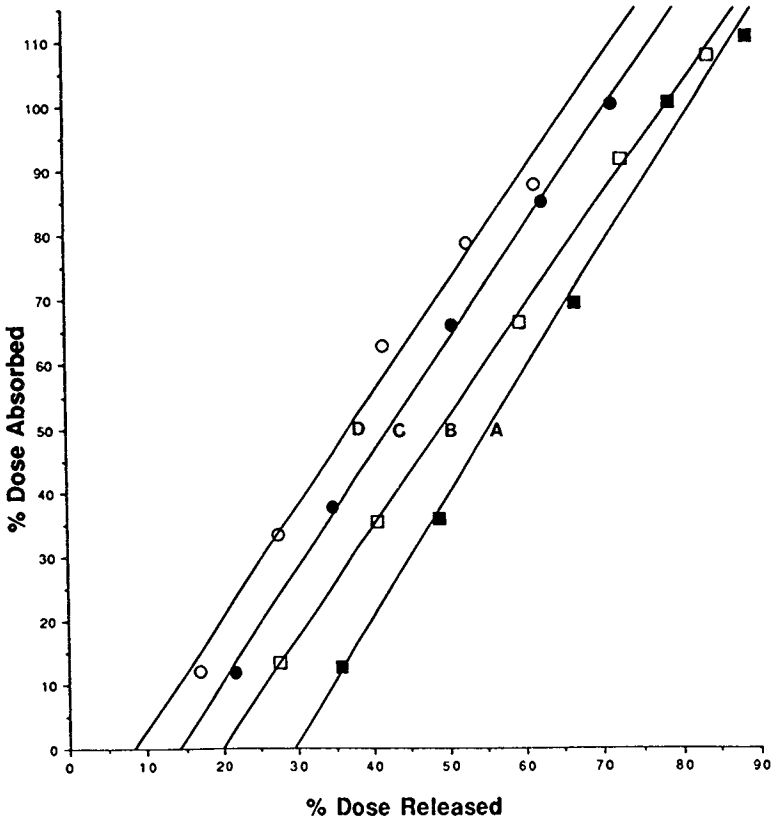

Fig. 4. Plots of mean percentage of dose absorbed versus mean percentage of dose released for the extended release formulations of chlorpheniramine maleate. The line of best fit is shown for each formulation. The corresponding regression equations are listed in Table I.

Table I. The *in Vivo/in Vitro* Regression Parameters for Four Chlorpheniramine Maleate Extended-Release Formulations Obtained by Linear Regression Analysis ($Y = aX + b$)

Formulation	Slope (a)	Intercept (b)	r^2	P value
A	1.93	−56.1	0.993	0.0003
B	1.71	−33.8	0.999	0.0001
C	1.75	−23.9	0.998	0.0001
D	1.73	−14.3	0.986	0.0007
Mean	1.78		0.994	
% CV	5.7		1.01	

Y = Mean % dose absorbed obtained from the Wagner Nelson analysis of the *in vivo* data in 24 normal subjects
X = Mean % dose released obtained from the *in vitro* release rate of extended-release tablets
a = Slope of the regression equation
b = The y intercept of the regression equation
r^2 = Coefficient of determination

sorbed and lower plasma concentrations (Figs. 2 and 3). Therefore, the rate at which chlorpheniramine maleate was released *in vitro* controlled the *in vivo* rate at which the drug appeared in the systemic circulation.

Quantitative *in vitro/in vivo* correlations were achieved between the two cumulative *in vitro* and *in vivo* parameters (Fig. 4). Linear correlation plots for percentage of dose absorbed and percentage of dose released are presented in Fig. 4. The corresponding regression parameters are summarized in Table I. These data indicate a statistically significant ($r^2 > 0.98$, $P < 0.001$) linear *in vitro/in vivo* relationship for all of the prototype formulations. The slopes of the regression lines were similar, with a relative standard deviation of 5.7% (Table I). The slopes reported for each formulation suggest that the chlorpheniramine maleate *in vivo* absorption rate was slower than the observed *in vitro* release rate (Table I). However, high correlation coefficients indicate that the *in vivo* absorption profiles of each formulation are correlated with the *in vitro* release profiles. The observed negative intercepts (Fig. 4) are due to the absorption lag time noted following oral administration of controlled-release dosage forms (15,16).

The *in vivo* lag time noted in Fig. 4 was corrected by using the Levy and Hollister method. The calculated lag times and intensity factors summarized in Table II indicate a rank order consistent with the observed *in vitro* release profiles. The percentage of dose absorbed at time t versus the percentage of dose released at T_{lag} are shown in Fig. 5. The regression analysis of these data revealed a significant linear correlation ($r^2 > 0.99$, $p < 0.0002$), with slope values approaching unity and passing through the origin (Fig. 5). The conditions for the present *in vitro* release rate procedure can predict *in vivo* performance.

Table II. Summary of Lag Times and Intensity Factors

Formulation	Lag time, T_{lag} (min)	Intensity factor (I)
A	30	1.04
B	42	0.90
C	45	0.70
D	55	0.58

CONCLUSIONS

For *in vivo* evaluation of oral extended-release products, one should consider both inter- and intrasubject variability in gastrointestinal (GI) motility, GI pH, site of drug absorption, and other pharmaceutical/physiological parameters (i.e., particle size, density, solubility, calorific values of meals, and gastric emptying) which affect the overall GI transit time (17–22). *In vitro* release testing is the other important element in the successful evaluation of an extended release product (23).

The significant correlations between the *in vitro* and the *in vivo* parameters reported here indicate that the *in vitro* release rate procedure is capable of discriminating between extended-release formulations having different *in vivo* bioavailabilities. *In vitro* testing by this procedure certainly represents a viable method with which to monitor production batches for quality assurance. The present *in vitro/in vivo* correlation method, which is consistent with Level A correlation guidelines described by the FDA/AAPS (Food and Drug Administration/American Association of Pharmaceutical Scientists) task force (24), provides the manufacturer with a valuable *in vitro* test that can be used to obtain useful information on the *in vivo* absorption behavior of such formulations.

ACKNOWLEDGMENTS

The contributions of the following Schering-Plough employees are gratefully acknowledged: Dr. Melton Affrime, who designed and supervised the clinical study; Dr. Arthur

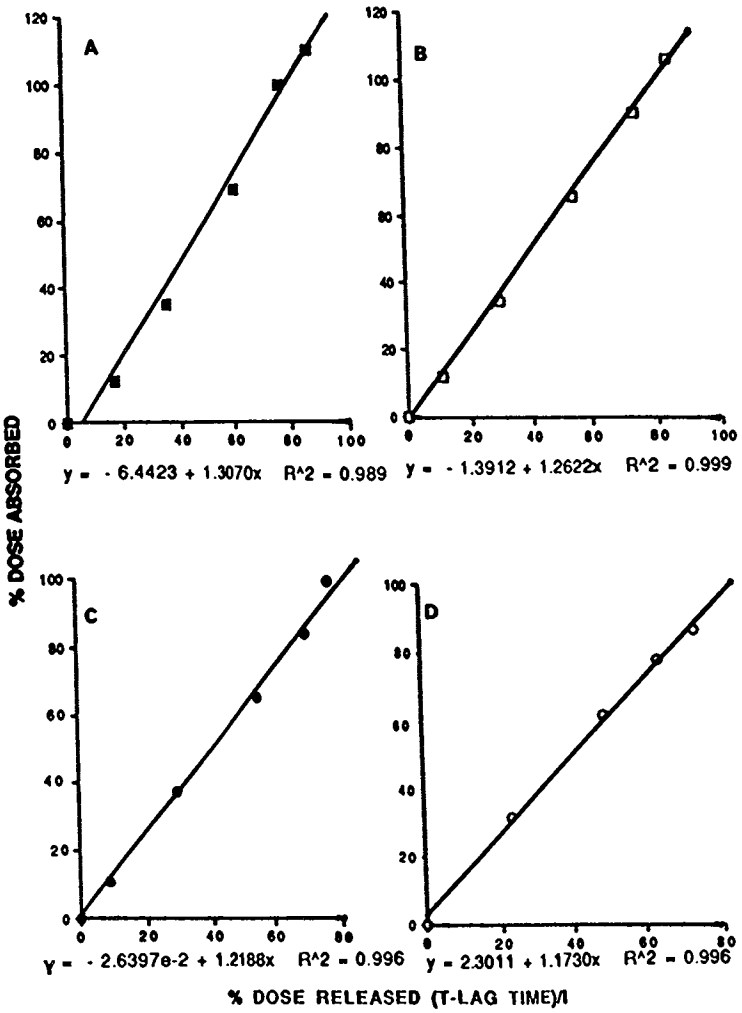

Fig. 5. Plots of mean percentage of dose absorbed versus mean percentage of dose released at T_{lag} for chlorpheniramine maleate extended-release formulations. The line of best fit is presented in each case.

de Silva, who did an editorial review of this paper; and Dr. Ladizlav Nemec, who provided computer expertise for the presentation of the mathematical correlations.

REFERENCES

1. V. Smolen. In-vitro drug release tests: A quantitative approach to their development for correlation to in-vivo availability. *Hosp. Pharm.* 4:14–18 (1969).
2. M. A. Gonzalez and A. L. Golub. Theo-Dur and Theo-Dur Sprinkle: Controlled release delivery systems for theophylline. *Drug Dev. Ind. Pharm.* 9:1379–1396 (1983).
3. J. M. Aiache, N. Pierre, E. Beyssac, V. K. Prasad, and J. P. Skelly. New results on an in-vitro model for the study of the influence of fatty meals on the bioavailability of Theophylline controlled-release formulations. *J. Pharm. Sci.* 78:261–263 (1989).
4. A. Yazigi and R. Sawchuk. In-vitro/in-vivo correlation and dissolution studies with oral theophylline dosage forms. *J. Pharm. Sci.* 74:161–164 (1985).
5. L. H. Block and U. V. Banakar. Further considerations in correlating in-vitro/in-vivo data employing mean-time concept based on statistical moments. *Drug Dev. Ind. Pharm.* 14:2143–2150 (1988).
6. P. J. McNamara, T. S. Foster, G. A. Digenis, R. G. Patel, W. A. Craig, P. G. Welling, R. S. Rapaka, V. K. Prasad, and V. P. Shah. Influence of tablet dissolution on furosemide bioavailability: A bioequivalence study. *Pharm. Res.* 4:150–153 (1987).
7. S. Riegelman and P. Collier. The application of statistical moment theory to the evaluation of in-vivo dissolution time and absorption time. *J. Pharmacokin. Biopharm.* 8:509–534 (1980).
8. D. Vaughn. A model independent method for estimating the in-vivo release rate constant of a drug from its oral formulations. *J. Pharm. Pharmacol.* 28:505–507 (1976).
9. N. Watari, N. Kaneniwa, and M. Hanano. Comparison if in-vivo dissolution of sulfa drugs calculated by deconvolution to absorption rates calculated by multicompartment model method. *Int. J. Pharm.* 6:155–166 (1980).
10. R. L. Williams, R. A. Upton, L. Ball, R. L. Braun, E. T. Lin, W. Liang-Gee, and L. J. Leeson. Development of a new controlled release formulation of chlorpheniramine maleate using in-vitro/in-vivo correlations. *J. Pharm. Sci.* 80:22–25 (1991).
11. H. Masumoto, Y. Tashiro, K. Matsumoto, A. Yoshida, and M. Hirayama. Simultaneous determination of codeine and chlorpheniramine in human plasma by capillary column gas chromatography. *J. Chromatogr.* 381:323–329 (1986).
12. J. G. Wagner and E. Nelson. Kinetic analysis of blood levels and urinary excretion in the absorption phase after single doses of drug. *J. Pharm. Sci.* 53:1392–1403 (1964).

13. G. Levy and L. Hollister. Inter- and intrasubject variations in drug absorption kinetics. *J. Pharm. Sci.* **53**:1446–1452 (1964).
14. G. Levy and L. Hollister. Dissolution rate limited absorption in man: Factors influencing drug absorption from prolonged-release dosage forms. *J. Pharm. Sci.* **54**:1121–1125 (1965).
15. P. Mojaverian, M. L. Rocci, Jr., D. P. Conner, W. B. Abrams, and P. J. Vlasses. Effect of food on the oral absorption of enteric-coated aspirin: Correlation with gastric residence time. *Clin. Pharmacol. Ther.* **41**:11–17 (1987).
16. M. L. Rocci, Jr., P. Mojaverian, R. J. Davis, R. K. Ferguson, and P. H. Vlasses. Food-induced gastric retention and absorption of sustained-release procainamide. *Clin. Pharmacol. Ther.* **42**:45–49 (1987).
17. S. S. Davis. The design and evaluation of controlled release systems for the gastrointestinal tract. *J. Control. Release* **2**:27–38 (1985).
18. P. Mojaverian, K. Chan, A. Desai, and V. John. Gastrointestinal transit of a solid indigestible capsule as measured by radiotelemetry and dual gamma scintigraphy. *Pharm. Res.* **6**:719–724 (1989).
19. T. Itoh, T. Higuchi, C. R. Gardner, and L. Caldwell. Effect of particle size and food on gastric residence time of nondisintegrating solids in beagle dogs. *J. Pharm. Pharmacol.* **38**:801–806 (1986).
20. S. S. Davis, A. F. Stockwell, M. J. Taylor, J. G. Hardy, D. R. Whalley, C. G. Wilson, H. Bechgoard, and F. M. Christensen. The effect of density on the gastric emptying of single- and multiple-unit dosage forms. *Pharm. Res.* **3**:208–213 (1986).
21. P. Mojaverian, R. K. Ferguson, P. J. Vlasses, M. L. Rocci, Jr., A. Oren, J. A. Fix, L. J. Caldwell, and C. R. Gardner. Estimation of gastric emptying time of the Heidelberg capsule in humans: The effect of varying food composition. *Gastroenterology* **80**:392–397 (1985).
22. P. Mojaverian, P. H. Vlasses, P. E. Kellner, and M. L. Rocci. Influence of gender, posture, and age on gastric residence time of an indigestible solid. *Pharm. Res.* **5**:639–644 (1988).
23. Z. Hussein and M. Friedman. Release and absorption characteristics of novel theophylline sustained release formulations: in-vitro/in-vivo correlation. *Pharm. Res.* **7**:1167–1171 (1990).
24. J. P. Skelley, G. L. Amidon, W. J. Barr, L. Z. Benet, J. E. Carter, J. R. Robinson, V. P. Shah, and A. Yacobi. Report of the workshop on in-vitro and in-vivo testing and correlation for oral controlled/modified-release dosage forms. *J. Pharm. Sci.* **79**:849–854 (1990).