REFERENCES

(1) M. Llabrés, J. L. Vila, and R. Martínez-Pacheco, *J. Pharm. Sci.*, **71**, 924 (1982).

(2) M. S. Barlett, *Biometrics*, **3**, 39 (1947).

(3) M. B. Brown and A. B. Forsythe, *Biometrics*, **30**, 719 (1974).

(4) W. G. Cochran and G. M. Cox, "Experimental Design," 2nd ed., Wiley, New York, N.Y., 1957, pp. 61–70.

(5) W. H. Barr, in "Dosage Forms Design and Bioavailability," J. Swarbrick, Ed., Lea & Febiger, Philadelphia, Pa., 1973, pp. 31–75.

Quantification of the Effect of Excipients on Bioavailability by Means of Response Surfaces III: *In Vivo–In Vitro* Correlations

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Abstract □ This study compares one of the previously studied formulations with commercial amoxicillin capsules. The results indicate that the percentage of the dose absorbed is similar in both formulations; nevertheless, the amoxicillin capsules present a higher absorption rate. The *in vivo–in vitro* correlations in terms of response surfaces, and the general correlation among all the formulations studied in the three articles of this series is discussed. The quantity of drug excreted in 2 hr and the quantity of drug dissolved in 30 min presents a correlation coefficient $r = 0.9458$ ($p < 0.01$) and the quantity of amoxicillin excreted in 12 hr and the quantity dissolved in 180 min presents a correlation coefficient $r = 0.9761$ ($p < 0.01$).

Keyphrases □ Amoxicillin—effect of excipients on bioavailability by means of response surfaces, *in vivo–in vitro* correlations □ Bioavailability—effect of excipients by means of response surfaces, amoxicillin, *in vivo–in vitro* correlations □ Excipients—effect on bioavailability by means of response surfaces, amoxicillin, *in vivo–in vitro* correlations

The comparison between Formulation E (1), previously studied, and a commercial amoxicillin capsule (Formulation S) was carried out. This study has a double purpose: first, to determine whether the absorption of the drug in Formulation E (which showed good absorption in previous studies) is equivalent to the absorption shown by the conventional formulations; second, to determine the degree of the individual variation for both types of formulations.

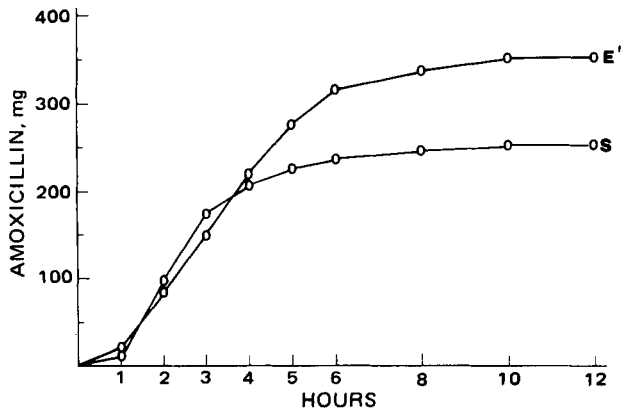

Figure 1—*Mean cumulative curves for urinary excretion of unchanged amoxicillin.*

EXPERIMENTAL

Assayed Formulations—A comparison is made between Formulation E, whose composition has been described (1) and commercial capsules[1] containing 500 mg of anhydrous amoxicillin. To differentiate the results of the present study from those obtained previously (1), Formulation E is termed E′ in the present report.

Clinical Protocol—Urinary excretion of unchanged drug was studied in 12 healthy volunteers of both sexes, whose ages ranged from 20 to 30 years and who showed no evidence of renal insufficiency. Subjects were divided at random into two equal groups of six. A Latin-square 2 × 2 design with 6 replicates was used, and the washing period was 5 days. The conditions of administration and sample times are the same as those described in the previous two reports (1, 2).

Pharmacokinetic Analysis—The parameters employed to characterize the excretion curves obtained are the same as those described previously (1, 2), E_2 and E_{12}.

Dissolution Rate Studies—The apparatus and methodology used were both described in Part I (2).

Statistical Analysis—Cochran's test (3) confirmed the existence of heterogeneous variances for the treatments. Therefore, Scheffé's test modified by Brown and Forsythe (4) was employed (even though the logarithmic transformation of data stabilized the variances) in order to maintain homogeneity in the statistical treatment of data.

RESULTS AND DISCUSSION

A commercial formulation of 500 mg was chosen because the urinary excretion curves obtained for Formulation E in the previous study indicated the possibility of obtaining peaks similar to those obtained with 500-mg doses in conventional formulations. This approach, together with the comparison of the parameters E_2 and E_{12}, permits the possibility of determining whether or not there is a prolongation in the release–absorption process with respect to Formulation E′. This would explain the large individual variations observed in previous studies.

Figures 1 and 2 show the excretion and dissolution mean cumulative curves for both formulations. With respect to the E_2 parameter, the mean values and variances (in parentheses) obtained for Formulations E′ and S were 85.3 (2824.2) and 98.1 (2188.9), respectively. The corresponding figures for the E_{12} parameter were 354.4 (9003.6) and 253.6 (1895.9). The dissolution parameter D_{30} yielded mean values of 204.8 and 404.0 for Formulations E′ and S, respectively, whereas the D_{180} parameter yielded mean values of 634.0 and 471.5 for the respective formulations. The ANOVA results, using the method of Brown and Forsythe (4), show that both formulations are equivalent with regards to the E_2 parameter ($F_{1,22} = 0.392$) but differ significantly (0.01) with regards to the E_{12} parameter ($F_{1,22} = 11.190$). The quantity of intact drug excreted in urine was ~50% of the administered dose in the case of each formulation, which has been

[1] Clamoxil, lot 2L26, F. Bonet.

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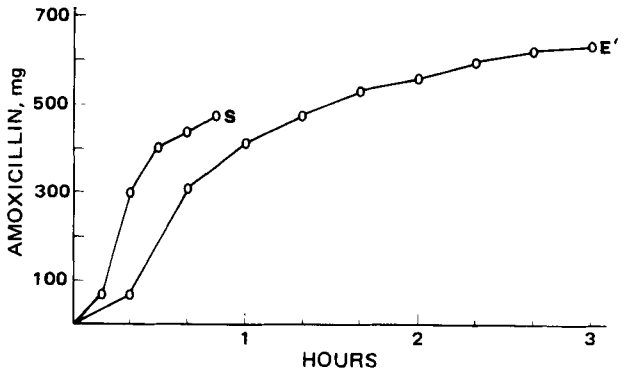

Figure 2—*Mean cumulative dissolution curves.*

discussed previously (5). The ratio between the observed values for the E_{12} parameter, in the case of each of the formulations, is the same as the ratio between doses, thus indicating equal absorption extent for both formulations. With respect to the E_2 parameter, statistically the ratio is equal to unity. This indicates a reduction in the absorption rate of Formulation E′ compared with Formulation S. Furthermore, there is a greater variation between subjects for Formulation E′, with respect to both parameters, which is why there are heterogeneous variances.

Response surfaces are not only useful for quantifying the effect of the different factors on bioavailability, but also serve as a basis for establishing the possible *in vivo–in vitro* correlations. Furthermore, this latter aspect is an important problem facing biopharmaceutics in the field of dosage form control. This importance is underlined by the numerous methods proposed for the study of such correlations (6, 7). Nevertheless, the fact that these methods are mainly empirical is not desirable. For this reason, some authors (6) point out that quantitative-type correlations, at least, "should probably be derived only when there is a theoretical reason for relating the variables."

Although the conditions found in the GI tract are difficult to simulate, it is possible to study a series of factors which presumably affect the processes of release–dissolution of the drug in a biological medium, assuming that these processes are the limiting steps in absorption. The rate with which a drug is liberated from the dosage form is conditioned by two types of factors: the effect of the different excipients and technological factors and their possible interaction with the drug and the interactions between the dosage form and the GI environment. Some kind of physicochemical model is necessary so that the interaction between the technological factors of the dosage form and the physiological environment can be simulated *in vitro*. However, no such model is readily available. However, statistical models can be used, as is the case here, to see if the effects of the technological factors studied *in vitro* by means of certain parameters are comparable to the effects produced *in vivo*. Furthermore, this approach affords the possibility of obtaining the optimum conditions for *in vitro* testing.

Obtaining a correlation between the *in vivo* and *in vitro* response surfaces implies that the polynomials obtained from both series of data are related by means of any one operator; nevertheless, from a practical

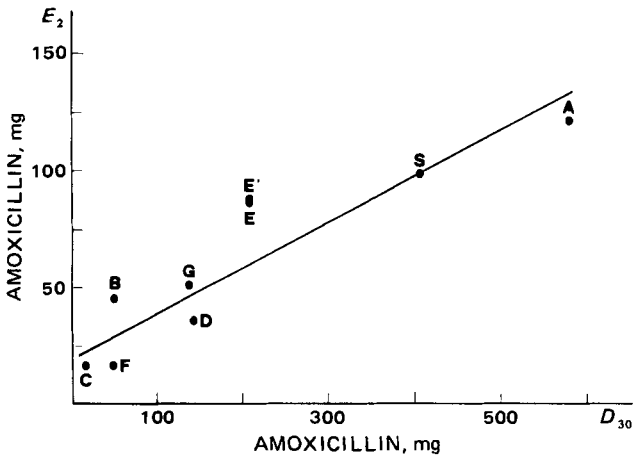

Figure 3—*Linear relationship between the amount of unchanged amoxicillin excreted in 2 hr and the amount dissolved in 30 min [y = 20.90 + 0.2169 x (r = 0.9458; F = 59.48; $\alpha < 0.01$)].*

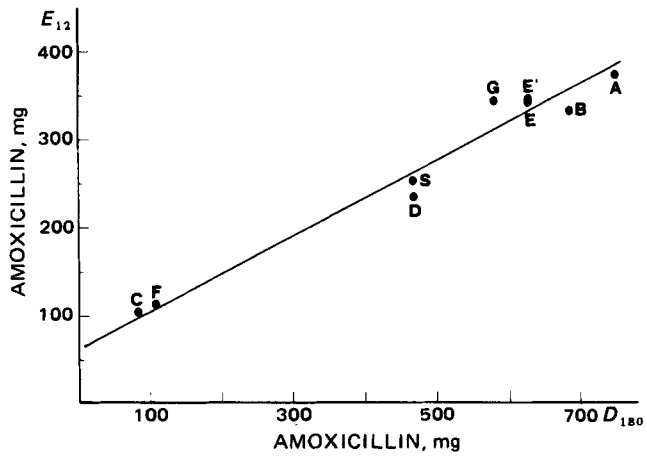

Figure 4—*Linear relationship between the amount of unchanged amoxicillin excreted in 12 hr and the amount dissolved in 180 min [y = 64.01 + 0.4278 x (r = 0.9761; F = 141.30; $\alpha < 0.01$)].*

point of view, it is desirable to have a linear relationship between both, that is, the polynomials obtained should have linear combination. Furthermore, the use of linear relations makes the predictions more plausible. Due to the nature of the experimental data, obtaining linear combinations, is not expected; nevertheless, the approximation to this situation can be tested by means of the linear regression of the polynomial coefficients obtained *in vivo versus* those obtained *in vitro*. If both polynomial functions are a linear combination, then a linear relationship with the ordinate at the origin, statistically equal to zero, will be obtained. This situation is analyzed by means of the ANOVA of the regression among coefficients, using as index the F value obtained for the regression term b_0. To establish precisely the coefficients of the polynomial function used *in vivo*, taking as a basis the *in vitro* counterparts, it is desirable to meet Wetz's criterion (8). According to Wetz, the minimum value of F necessary for equating prediction errors with experimental errors is four times the tabulated value. Another necessary condition is that the ordinate in the origin should be statistically equal to zero. It should also be noticed that the proportion constant for the polynomial coefficients is the same as one obtained by means of the regression between the *in vivo* and *in vitro* parameters.

The regression obtained among the polynomial coefficients corresponding to the E_2 and D_{30} parameters, for Formulations A, B, and C is:

$$y = 1.1419 + 0.1995\,x \quad (r = 0.9999;\ F = 25{,}200.8) \qquad \text{(Eq. 1)}$$

in which the ordinate in origin is statistically equal to zero; the F value is superior to the quadruple of the value tabulated (645.6). Thus, both polynomials must be considered a linear combination within the experimental limits. From this the relationship between the E_2 and D_{30} parameters is as follows:

$$E_2 = 16.04 + 0.200\,D_{30} \quad (r = 0.9977;\ F = 226.5) \qquad \text{(Eq. 2)}$$

in which the y intercept is statistically equal to zero and the slope is almost equal to that obtained in the regression among polynomial coefficients. This coincidence confirms the existence of a linear combination between the response surfaces *in vivo* and *in vitro*.

Nevertheless, the regression obtained between the polynomial coefficients corresponding to E_{12} and D_{180} is:

$$y = -12.473 + 0.768\,x \quad (r = 0.9912;\ F = 56.47) \qquad \text{(Eq. 3)}$$

where the F value is smaller than the critical value tabulated for $\alpha = 0.05$. For this reason, the existence of a linear combination between sets of coefficients cannot be considered. Nevertheless, the relation between the parameters E_{12} and D_{180}:

$$E_{12} = 64.227 + 0.400\,D_{180} \quad (r = 0.990;\ F = 549.19) \qquad \text{(Eq. 4)}$$

presents an excellent linear correlation, even though there is no relationship between the *in vivo* and *in vitro* response curves; the relation, in fact, is exclusively empirical.

The existence of a linear combination between the polynomials obtained for E_2 and D_{30}, but not for E_{12} and D_{180}, suggests an alteration of the intensity factor (7), since the times corresponding to the *in vivo* and *in vitro* parameters chosen are multiples in both cases.

In the second study carried out on Formulations D, E, F, and G, it was pointed out that the two systems do not arrange the different formulations in the same way, and therefore, the existence of response surfaces proportional for the chosen parameters must be ruled out.

With regards to the general correlations, Figs. 3 and 4 show the regression of E_2 against D_{30} and E_{12} against D_{180}, respectively, for all the formulations studied. In spite of the large differences existing between the various formulations, a significant linear regression is obtained, especially in the case of E_{12} against D_{180}. Even the F value obtained for the regression term, b_0, of the ANOVA of the regression was higher than the quadruple of the critical value tabulated. The high F values indicate the capability of the apparatus employed for the *in vitro* studies.

A study of response surfaces is not necessary for establishing a routine control. Nevertheless, in biopharmaceutics, a knowledge of the technological factors which can interact with drugs, as well as of the physicochemical properties responsible for this interaction, is essential. A mechanistic model which would include all variables, both technologically and physiologically dependent, is not feasible, therefore, the use of response surfaces to quantify the effect produced by the factor studied is necessary. This quantification should be independent of therapeutic implications and should insist on establishing the necessary guidelines for obtaining an exact formulation of the dosage forms.

REFERENCES

(1) M. Llabrés, J. L. Vila, and R. Martínez-Pacheco, *J. Pharm. Sci.*, **71**, 924 (1982).

(2) M. Llabrés, J. L. Vila, and R. Martínez-Pacheco, *ibid.*, **71**, 927 (1982).

(3) W. G. Cochran, *Biometrics*, **3**, 22 (1947).

(4) M. B. Brown and A. B. Forsythe, *Biometrics*, **30**, 719 (1974).

(5) M. Barza and L. Weinstein, *Clin. Pharmacokinet.*, **1**, 297 (1976).

(6) J. G. Wagner, "Biopharmaceutics and Relevant Pharmacokinetics," 1st ed., Drug Intelligence Publications, Hamilton, Ill., 1971, pp. 121–147.

(7) J. Swarbrick, "Biopharmaceutics," 1st ed. Lea & Febiger. Philadelphia, Pa., 1970, pp. 265–296.

(8) J. W. Wetz in "Applied Regression Analysis," 1st ed., N. Draper and H. Smith, Eds., Wiley, New York, N.Y., 1966, p. 64.

Determination of Isoetharine in Plasma by Reversed-Phase Chromatography with Amperometric Detection

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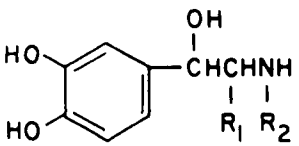
Received August 26, 1981, from the **Department of Drug Metabolism and Disposition* and the ‡*Department of Toxicology, Sterling-Winthrop Research Institute, Rensselaer, NY 12144.* Accepted for publication November 12, 1981.

Abstract □ A reversed-phase liquid chromatographic method for the determination of isoetharine in blood plasma, utilizing amperometric detection, is described. Plasma samples were extracted utilizing an ion-pair reagent, di-(2-ethylhexyl)phosphoric acid, to concentrate the catecholamine. Only minor differences were observed in the relative bioavailability of isoetharine hydrochloride and isoetharine mesylate after oral administration to rats. Observed plasma levels, at 1 hr after oral medication, were highly variable in dose-ranging studies at doses of 800–2500 mg/kg/day for 2 weeks.

Keyphrases □ Bioavailability—determination of isoetharine in plasma by reversed-phase chromatography with amperometric detection □ Reversed-phase chromatography—determination of isoetharine in plasma with amperometric detection □ Isoetharine—determination in plasma by reversed-phase chromatography with amperometric detection

Isoetharine (4-[1-hydroxy-2-[(1-methylethyl)-amino]-butyl]-1,2-benzenediol) (I) is a β-agonist which is widely



	R_1	R_2
I	CH_2CH_3	$-CH(CH_3)_2$
II	H	$-C(CH_3)_3$

used as a bronchodilator in inhalation therapy. Reversed-phase liquid chromatography with amperometric detection has been used extensively for the determination of endogenous and exogenous biogenic amines in various biological media (1). The most common methods for sample preparation include various modifications of the method of Anton and Sayre (2) in which the compound of interest is adsorbed to alumina through the catechol moiety. In addition, ion exchange resins (3) and boric acid gels (4) have been used to concentrate the analyte and separate it from interfering substances. In the present study, the catecholamine was extracted into an organic solvent through the formation of an ion-pair with di-(2-ethylhexyl)phosphoric acid (5).

The present report describes an analytical method developed for the determination of isoetharine in blood plasma, and the application of the method to the analysis of rat plasma in studies comparing the oral bioavailability of the hydrochloride and mesylate salts (the two marketed salt forms of isoetharine) and in dose-ranging toxicity studies.

EXPERIMENTAL

Reagents—Isoetharine[1] (hydrochloride and mesylate salts) (I), colterol[2] (mesylate salt, internal standard) (4-[2-[1,1-dimethylethyl)-amino]-1-hydroxyethyl]-1,2-benzenediol) (II), methanol[3], benzene[4], di-(2-ethylhexyl)phosphoric acid[5], were used as received. Water was deionized, distilled, and treated with high-intensity UV radiation[6]. All other chemicals were reagent grade or better and used without further purification.

A 1.5% (or 0.5%) solution of di-(2-ethylhexyl)phosphoric acid in ben-

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[2] Sterling-Winthrop Research Institute, Rensselaer, N.Y.
[3] OmniSolve, McB, Cincinnati, Oh.
[4] Nanograde, Mallinckrodt, St. Louis, Mo.
[5] Sigma Chemical Co., St. Louis, Mo.
[6] ORGANICpure, Sybron/Barnstead, Boston, Mass.

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