Quantification of the Effect of Excipients on Bioavailability by Means of Response Surfaces I: Amoxicillin in Fat Matrix

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Abstract □ A study was carried out to determine the effect of a fat excipient on the bioavailability of amoxicillin tablets. Three formulations with a fat excipient content of 10, 20, and 30%, respectively, were administered to 15 healthy volunteers according to a Latin-square design. The excretion curves were characterized with the help of two parameters, namely, the quantity of drug excreted between 0–2 and 0–12 hr postadministration, respectively. The effect of the fat excipient content was quantified with the use of polynomials whose corresponding orders were given by the ANOVA. In the case of both parameters, a quadratic response to the fat excipient content was found. At the same time, a dissolution study was carried out using a previously established method. Here, the parameters used to characterize the dissolution curves were the quantities of dissolved drug in 30 and 180 min, respectively. Again, a quadratic response to the fat excipient content was observed in the case of both parameters.

Keyphrases □ Amoxicillin—quantification of the effect of excipients on bioavailability by means of response surfaces, fat matrix □ Bioavailability—quantification of the effect of excipients by means of response surfaces, amoxicillin in fat matrix □ Excipients—quantification of the effect on bioavailability by means of response surfaces, amoxicillin in fat matrix

The quantification of the effect that certain excipients and technological procedures have on drug bioavailability is an interesting aspect of the field of pharmaceutical technology. Most of the investigations carried out with human subjects have used commercial formulations, that is, formulations whose composition and elaboration process are unknown (1–3). Therefore, only the bioequivalence or bioinequivalence of these formulations can be established. *In vitro* studies of drug release and drug dissolution, although more numerous, have failed to reflect the biopharmaceutical or clinical consequences of drug–technological factor interaction (4, 5).

BACKGROUND

Bioequivalence studies are usually based on the general linear model of ANOVA. This model, when applied to a three-way crossover design (formulation, subject, and period), takes the following form:

$$y_{ijk} = \mu + F_k + S_i + W_j + e_{ijk} \qquad \text{(Eq. 1)}$$

where y_{ijk} denotes the observation on the ith subject with the kth formulation on the jth period; μ represents the general mean; F_k, S_i, and W_j represent the effects produced by subject, formulation, and period, respectively; and e_{ijk} is the error associated with observation y_{ijk} and is assumed to have normal distribution with the mean zero and variance constant for all observations, σ^2. If this study were not confined only to establishing the bioequivalence or bioinequivalence between two formulations, but extended to include the quantification of the effects of excipients and technological factors on bioavailability, then the term F_k would have to be subdivided to meet these new demands. For example, if the study were to englobe factors A and B, and if the effect of each of these factors and of their mutual interaction were desired, then the term F_k would have to be replaced by the term:

$$A_m + B_n + AB_{mn} \qquad \text{(Eq. 2)}$$

where A_m represents the effect of factor A at level m, B_n the effect of factor B at level n, and AB_{mn} the effect of their interaction. Thus, the ANOVA could indicate the way in which these factors modify bioavailability and help establish not only the optimum formulation but also the *in vivo–in vitro* correlations.

Table I—Percent Composition and Mean Weight [a] of Formulations A, B, and C

Formulation	Amoxicillin Trihydrate	Fat, %	Talc, %	Mean Weight
A	85%	10	5	514
B	75%	20	5	582
C	65%	30	5	672

[a] Mean weight in kilograms.

The object of this study, which comprises three separate studies, is to quantify the effects which two excipients (a synthetic fat and a silica colloid) have on the bioavailability of amoxicillin tablets. Quantification was obtained by means of response surfaces expressed as low-order polynomials, a technique used to quantify the effects of excipients on some physicochemical properties of different dosage forms (6, 7). At the same time, a study was carried out to determine the effect of the above excipients on the dissolution rate of amoxicillin in formulations tested *in vivo*. Finally, the correlation between both series of data was also studied.

The present report studies the effect of a synthetic fat excipient at three equally spaced levels.

EXPERIMENTAL

Assayed Formulations and Analytical Method—Three formulations of amoxicillin trihydrate[1] tablets, containing 375 mg of anhydrous amoxicillin, were manufactured and studied. The percent composition of the three formulations, A, B, and C, is shown in Table I. The three formulations differed only in their synthetic fat[2] (mono-, di-, tripalmitate estearate of glycerin) content. In each case the hardness was 4 kg on the hardness tester scale[3].

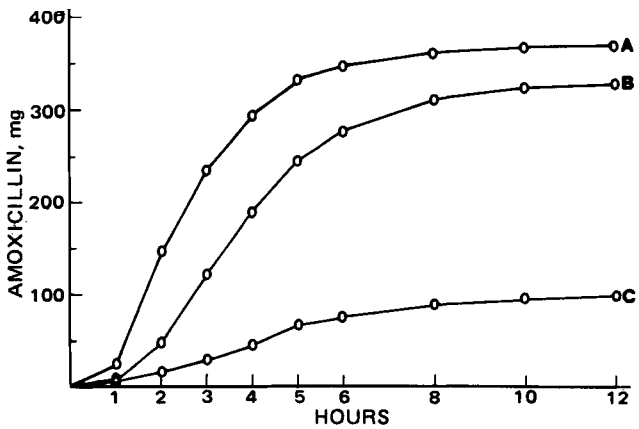

Figure 1—*Cumulative curves for the urinary excretion of unchanged amoxicillin.*

[1] Antibióticos S. A., lot A30H-106. Potency 859 μg/mg.
[2] Precirol, Gattefossé.
[3] Monsanto hardness tester.

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Table II—Mean Values and Variances [a] for the *In Vivo* Parameters Employed

Formulation	E_2	E_{12}
A	143.4 (4129.2)	369.8 (4248.7)
B	45.8 (1433.4)	329.8 (14349.2)
C	17.4 (136.9)	102.4 (2416.3)

[a] Variances in parentheses.

The *in vivo* and *in vitro* samples were assayed by means of a spectrophotometric method which was specific for intact drug (8).

Clinical Protocol—Urine analysis of the unchanged drug was carried out in 15 healthy volunteers of both sexes, whose ages ranged from 20 to 32. The subjects, tested for renal insufficiency, gave a negative response. A written consent was obtained from each subject. Subjects were divided at random into three equal groups. A Latin-square 3 × 3 design with five replicates was used, and the washing period between successive administrations was 5 days. Immediately before a standard breakfast, the fasted subjects were given two tablets equivalent to 750 mg of anhydrous amoxicillin. Urine samples were collected 1, 2, 3, 4, 5, 6, 8, 10, and 12 hr postadministration.

Pharmacokinetic Analysis—The effect of fat content on the quantity of drug absorbed and on the absorption rate were the two parameters used to characterize the urinary excretion curves. These parameters can be characterized by E_{12} the total quantity of drug, expressed in milligrams, excreted in the 12 hr following administration; and E_2 the quantity of drug, expressed in milligrams, excreted in the first 2 hr postadministration (during which time the maximum urinary excretion rate of the free drug was observed).

Characterization of the formulations by means of pharmacokinetic parameters was ruled out for two reasons. First, the absorption processes observed in Formulations B and C were too complex; second, to avoid the introduction of estimation error, which would have been confused with the experimental error and would have entailed the use of nonparametric tests and, consequently, the loss of information about the assay in question.

Dissolution Rate Studies—Dissolution rate studies were carried out using the apparatus described previously (9). This apparatus was the flowthrough type without an accumulating reservoir. To calculate the quantity of dissolved drug at different times, the method proposed previously (10) was used. The following conditions were employed: angle agitation, 180; intensity of agitation, 6 oscillations/min; dissolution cell volume, 47.5 ml; flow rate, 1 ml/min. Enzyme-free juices, gastric and enteric, were the dissolution media employed. Three dissolution tests were carried out on each formulation using a tablet in each test, although the results refer to a 750-mg dose. The following parameters were employed to characterize the dissolution curves obtained: D_{180} was the quantity of drug, in milligrams, dissolved in 180 min, and D_{30} was the quantity of drug, in milligrams, dissolved in 30 min.

Statistical Analysis—Prior to the establishment of the ANOVA for the Latin-square design, the model was submitted to an additive test and to a variance homogeneity test in order to confirm its validity. Tukey's test (11) confirmed the additivity of the model. Nevertheless, Cochran's test (12) confirmed the existence of heterogeneous variances among the treatments for the two parameters under study. Therefore, the use of the three-way ANOVA in the Latin square lacked validity on account of power deficiency. To overcome this problem, and because the logarithmic transformation of data failed to stabilize the variances, the generalization of Scheffé's test proposed by Brown and Forsythe (13) was used. Although the latter test is a one-way ANOVA, in the presence of heterogeneous variances, it is a valid solution. According to Brown and Forsythe, the modification of Scheffé's probability is:

$$P_r\{(H - O)^2/(\sum Z_i^2 S_i^2/n_i) < (g - 1) F_{1-\alpha,g-1,f}\} \geq 1 - \alpha \quad \text{(Eq. 3)}$$

where $H = \Sigma Z_i \overline{X}_i$ is an estimation of $O = Z_i \mu_i$, f is defined by:

$$\frac{1}{f} = \sum \frac{C_i^2}{(n_i - 1)} \text{ and } C_i = \frac{(\sum Z_{ik}^2)\, S_i^2/n_i}{\sum_i \left(\sum_k Z_{ik}^2\right) S_i^2/n_i} \quad \text{(Eq. 4)}$$

where $\overline{X}_i$ is the mean of the treatments, S_i^2 is the variance, and g is the number of treatments.

For the *in vitro* parameters chosen, the corresponding one-way ANOVA was employed.

The study of response surfaces was achieved by subdividing the sum of squares for the term treatments of the ANOVA by means of a series of orthogonal polynomials which are defined by the number of factors studied as well as by the number of their levels (14). Therefore, such a study presupposes the use of an adequate experimental design. The subdivision in this study is achieved by means of the following polynomials (Z):

Treatments	Z_1	Z_2
T_1	−1	1
T_2	0	−2
T_3	1	1
Component	Linear	Quadratic

which subdivide the sum of squares for treatments in linear and quadratic responses. The levels of the factors studied must be equally spaced; if not, it would be impossible to carry out the orthogonal comparisons offered.

Once the type of response has been inferred, a polynomial function with the correspondent degree is adjusted to mean values by means of the least-square method.

RESULTS AND DISCUSSION

A quantitative study of bioavailability requires a grasp of two basic concepts. First, the concept of bioavailability includes the quantity of drug absorbed and the rate of absorption (15). Second, bioavailability is a random variable and, therefore, its interval (*i.e.*, a parameter which can measure interindividual variability) has to be estimated. The choice of the E_{12} parameter is clearcut, since it reflects the total quantity of drug absorbed, as the half-life of amoxicillin in ~1 hr. The E_2 parameter is mainly controlled by the rate of absorption, while depending to a certain degree on the total quantity of drug absorbed. There are two reasons for studying the variability of these parameters in the light of their respective variances: from a statistical point of view, variance gives a good idea of the interval; and variance homogeneity is a prequisite for the study of the ANOVA. Therefore, there is a combination of statistical and biopharmaceutical criteria.

Table III—Results of ANOVA for E_{12} [a]

i	H_i	$V(H)$	$g-1$	f	F	A	Results
1	−267.41	444.34	2	21	3.47	55.53	Significant linear response
2	−187.49	4270.80	2	21	3.47	172.16	Significant quadratic response
3	−39.96	1239.86	2	21	3.47	92.76	No significant contrast A *versus* B
4	−267.41	444.34	2	21	3.47	55.53	Significant contrast A *versus* C
5	−227.45	1117.71	2	21	3.47	88.07	Significant contrast B *versus* C

[a] $\alpha = 0.05$. The minimum value necessary for rejecting the null hypothesis is $A = \sqrt{(g-1)FV(H_i)}$ (Ref. 13).

Table IV—Results of ANOVA for E_2 [a]

i	H_i	$V(H)$	$g-1$	f	F	A	Results
1	−125.96	284.41	2	21	3.47	44.03	Significant linear response
2	69.26	666.67	2	21	3.47	68.02	Significant quadratic response
3	−97.61	370.85	2	21	3.47	50.73	Significant contrast A *versus* B
4	−125.96	284.41	2	21	3.47	44.03	Significant contrast A *versus* C
5	−28.35	104.69	2	21	3.47	26.95	Significant contrast B *versus* C

[a] $\alpha = 0.05$ (see Table III).

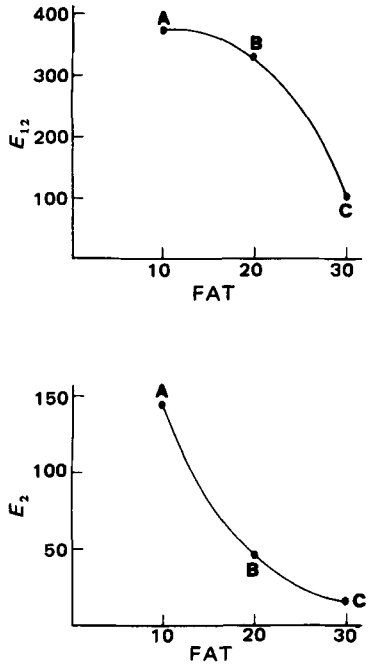

Figure 2—*Response curves for* E_{12} *and* E_2 *in function of fat content.*

Figure 1 shows the mean urine excretion accumulative curves of the three formulations studied. The mean values and the variances of the respective parameters are shown in Table II. The respective ANOVA results appear in Tables III and IV. The contrasts between pairs of formulations and the orthogonal contrasts used to study the influence of the excipient must be distinguished. From the contrasts between formulations, it can be inferred that A and B are bioequivalent using the mean value of the E_{12} parameter, the total quantity absorbed, but not so using the E_2 parameter, the rate of absorption. On the other hand, and in the case of both parameters, the variability of Formulation B is statistically greater than that of Formulation A. This means that Formulation B not only is absorbed more slowly but also its variability is much greater, two reasons for considering them to be bioinequivalent. Formulation C shows much lower values for both parameters.

With respect to the influence of the synthetic fat content on bioavailability, the ANOVA results imply a quadratic response for both parameters. Therefore, the following second-degree polynomials indicate a quantitative relationship between bioavailability and synthetic fat content in those formulations whose fat content lies within the limits of this study:

$$E_{12} = 222.320 + 24.138\,x - 0.938\,x^2 \qquad \text{(Eq. 5)}$$

$$E_2 = 310.280 - 20.150\,x + 0.346\,x^2 \qquad \text{(Eq. 6)}$$

in which x is the fat content expressed in percent. These polynomials are shown in Fig. 2. It can be seen that when the fat content is increased from 10 to 20%, there are no observable changes of importance, at first, in the mean quantity of drug absorbed; nevertheless, there is a marked decrease

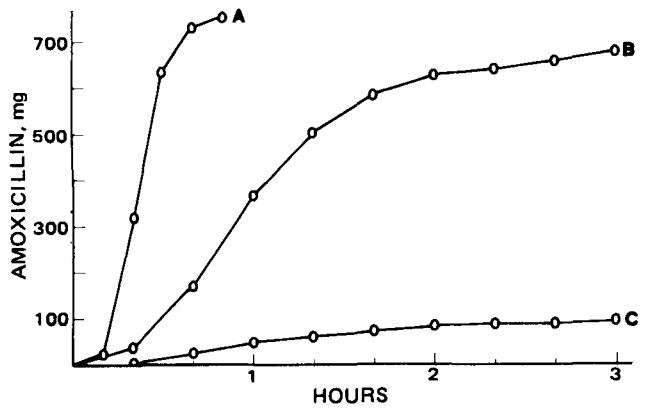

Figure 3—*Cumulative dissolution curves.*

Table V—Mean values of Three Dissolution Tests for the Chosen *In vitro* Parameters

Formulation	D_{30}	D_{180}
A	577.8	750.6
B	71.3	681.0
C	16.5	91.9

Table VI—Results of ANOVA for D_{180}

Source of Variation	Sum of Squares	Degrees of Freedom	F	α
Treatments	780,319.56	2	4013.14	<0.01
Linear	645,818.92	1	6642.83	<0.01
Quadratic	134,500.64	1	1383.46	<0.01
Residual	583.32	6		
Total	780,902.88	8		

Table VII—Results of ANOVA for D_{30}

Source of Variation	Sum of Squares	Degrees of Freedom	F	α
Treatments	632,495.56	2	176.25	<0.01
Linear	548,208.96	1	352.50	<0.01
Quadratic	84,286.60	1	46.97	<0.01
Residual	10,765.79	6		
Total	643,261.35	8		

in the rate of absorption. It can also be seen that when the fat content is increased from 20 to 30%, there are noticeable decreases in both E_2 and E_{12}. However, it can be seen that the variation coefficients obtained with the data listed in Table II increase when the fat content increases. In the case of the E_{12} parameter, these are 18, 36, and 48% for Formulations A, B, and C, respectively.

The use of these second-degree polynomials obtained from the mean values might lead to certain criticism. The ANOVA only indicates the existence of a statistically significant deviation in the linear relationship between the parameters and the synthetic fat content. This does not mean that the second-degree polynomial is the optimum function or that there is some physical–chemical basis for justifying its use. The fact is that it is the simplest linear function application to the statistical model used. With respect to the utility of these functions for determining the type of effect produced by the excipient, this depends on whether the function used can be said to be equivalent to the hypothetical function

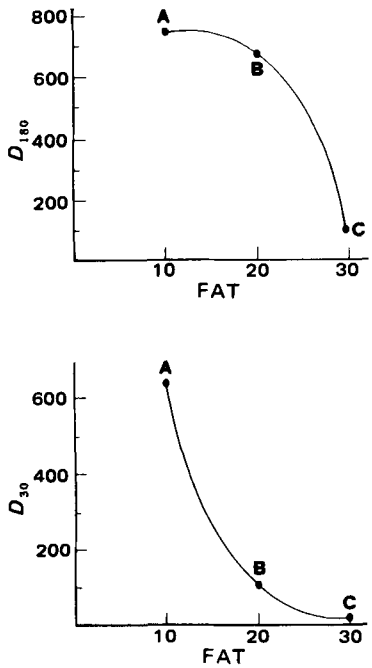

Figure 4—*Response curves for* D_{180} *and* D_{30} *in function of fat content.*

derived from those physical-chemical properties responsible for the modification involved and for the interaction between the dosage form and the physiological surroundings where drug release and drug absorption takes place. The best way to avoid any possible discrepancy between real behavior and that inferred from the ANOVA is to carefully choose the interval and the number of levels necessary for the study. The cost of the *in vivo* experiment necessarily limits the number of levels studied.

The dissolution accumulative curves obtained are shown in Fig. 3, while the mean values of the chosen parameters, D_{30} and D_{180}, are listed in Table V. A quadratic response for both parameters, with respect to the fat content, can be inferred from the one-way ANOVAs shown in Tables VI and VII. The polynomials, after least-squares adjustment, were:

$$D_{180} = 300.090 + 70.924\,x - 2.593\,x^2 \qquad \text{(Eq. 7)}$$

$$D_{30} = 1549.010 - 112.343\,x + 2.053\,x^2 \qquad \text{(Eq. 8)}$$

They are shown in Fig. 4. It is worth noting that this excipient exercises the same influence *in vitro* as it does *in vivo*.

Bioequivalence studies carried out on formulations whose composition is defined by an appropriate experimental design afford the opportunity of studying the effect of the different excipients on bioavailability; that is, the effect of these excipients on the quantity of drug absorbed and on the rate of absorption. The effect the excipients have on interindividual variability must also be considered. As has been pointed out previously (16, 17), it is not sufficient just to accept the null hypothesis for the parameters employed as an indication of bioequivalence between two formulations; the variability must also be similar.

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Quantification of the Effect of Excipients on Bioavailability by Means of Response Surfaces II: Amoxicillin in Fat–Silica Matrix

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Abstract □ This report studies the bioavailability of amoxicillin in different fat–silica matrixes. A urinary excretion study was carried out on four formulations containing fat and silica excipients. The formulations were administered to 24 healthy volunteers according to a Latin-square design. The following percent proportions of fat–silica were used: 15:3.75, 15:7.50, 30:3.75, and 30:7.50. The urinary excretion curves were characterized using the quantity of unchanged drug excreted between 0–2 and 0–12 hr postadministration, respectively as parameters. The ANOVA results showed that both excipients had an additive effect on the quantity of drug excreted between 0 and 2 hr, whereas the effect on the quantity of drug excreted between 0 and 12 hr was also one of interaction between both excipients. Quantification of the ANOVA results in terms of excipient content was conducted by means of the adequate linear functions. At the same time, a dissolution study was carried out using the quantity of drug dissolved in 30 and 180 min as parameters. The behavior was similar to that encountered for the *in vivo* parameters.

Keyphrases □ Amoxicillin—effect of excipients on bioavailability by means of response surfaces, fat–silica matrix □ Bioavailability—effect of excipients by means of response surfaces, amoxicillin in fat–silica matrix □ Excipients—effect on bioavailability by means of response surfaces, amoxicillin in fat–silica matrix

The aim of the present study is to determine the effect which the combination of two excipients, a synthetic fat[1] and a silica colloid[2], has on the bioavailability of amoxicillin tablets.

EXPERIMENTAL

Assayed Formulations—Four formulations of amoxicillin trihydrate[3] tablets were manufacturerd and studied. Formulations, D, E, F, and G, contained 375 mg of anhydrous amoxicillin, and their percent composition is shown in Table I. Hardness, in each case, was 5 kg on the hardness tester scale[4].

Clinical Protocol—The urinary excretion of unchanged drug was studied in 24 healthy volunteers whose ages ranged from 20 to 30 years and who showed no evidence of renal insufficiency. The subjects were randomly divided into four equal groups. A Latin-square 4 × 4 design with 6 replicates was used, and the washing period was 5 days. Immediately before a standard breakfast, fasted subjects were given two tablets equivalent to 750 mg of anhydrous amoxicillin. Urine samples were collected at 1, 2, 3, 4, 5, 6, 8, 10, and 12 hr postadministration.

Pharmacokinetic Analysis—Characterization of the urinary excretion curves was achieved by means of the parameters mentioned in the previous report (1).

Dissolution Rate Studies—The apparatus and methodology used were both described in the previous report (1).

Statistical Analysis—Heterogeneous variances for the treatments

[1] Precirol, Gattefossè.

[2] Aerosil, Degussa.

[3] Antibióticos S.A. lot A30H-106, potency 859 μg/mg.

[4] Monsanto hardness tester.

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