Bioequivalence

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The bioequivalence of two formulations of the same drug may be determined by evaluating the similarity of their respective plasma concentration curves. The similarity of two plasma concentration functions can be measured by an index called the *bioequivalence index*. This paper shows how such an index may be defined and calculated.

KEY WORDS: bioequivalence; bioavailability; plasma concentration function; AUC.

DEFINITIONS OF BIOEQUIVALENCE

The generally accepted definition of *bioequivalence*, as reported by the Federal Register (1), is as follows:

> Bioequivalent means . . . drug products whose rate and extent of absorption do not show a significant difference when administered at the same dose under similar conditions.

This definition is based on the assumption that (2)

> two formulations that do not differ very much in the *rate* at and the *extent* to which they make the active ingredient available in the circulating blood will not differ much in their therapeutic efficacy.

The corresponding definition of *bioavailability* accepted by the FDA (1) is the following:

> "Bioavailability" means the rate and extent to which the active drug ingredient or therapeutic moiety is absorbed from a drug product and becomes available at the site of drug action.

MEASURES OF BIOEQUIVALENCE

In common practice, a test formulation and a reference formulation are said to be bioequivalent if it can be determined with a certain level of confidence, for instance, 95%, that an index Θ falls within a specified range, called the *acceptable interval* for bioequivalence. For instance, calling AUC and AUC′ the area under the curve of the plasma concentration function of the reference formulation and of the test formulation, respectively, the index Θ may be the ratio of the area under the curve of the two formulations, and it may be required that

$$0.80 \leqslant \frac{\text{AUC}'}{\text{AUC}} \leqslant 1.25$$

with the predetermined level of confidence. Alternatively, calling c_{max} and c'_{max} the maximum value of the plasma concentration function of the reference formulation and of the test formulation, respectively, it may be required that

$$-0.20 \leqslant \frac{c'_{max} - c_{max}}{c_{max}} \leqslant +0.20$$

with the same level of confidence (3).

It is obvious that AUC or c_{max} by themselves or taken together are not sufficient indicators of bioequivalence. In fact, two formulations with different absorption rates may show exactly the same AUC and exactly the same c_{max}, as shown in Appendix I.

The time of maximum concentration, t_{max}, may depend more on the absorption rate than AUC and c_{max}, but only when the rate of absorption is smaller than the rate of elimination. If those two rates are close, the value of t_{max} is ill determined, and if the rate of absorption is larger, t_{max} depends more on the rate of elimination.

A NEW INDEX OF BIOEQUIVALENCE

The estimation of bioavailability of a formulation requires the separate measurement of its rate of absorption and of its extent of absorption; but to determine that a new formulation is bioequivalent to an old one, it is certainly not necessary to estimate its rate and its extent of absorption. Both the letter and the spirit of the FDA definition of bioequivalence could be satisfied by showing that the active ingredient of the two different formulations is available in the plasma at the same time and in the same amount. In other words, it should be sufficient to show that the two formulations have plasma concentration functions sufficiently similar.

As a measure of the dissimilarity of the plasma concentration function $c_r(t)$ of a reference formulation from the plasma concentration function $c_x(t)$ of a test formulation, we can assume the index ξ_i defined by the formula

$$\xi_i = \left(\frac{\int_0^\infty |c_r(t) - c_x(t)|^i dt}{\int_0^\infty |c_r(t) + c_x(t)|^i dt} \right)^{1/i} \qquad (1)$$

where i is any positive integer.

The dimensionless number ξ_i can be called the *bioequivalence index;* it is always

$$0 \leqslant \xi_i \leqslant 1$$

This index is zero only when the two plasma concentration curves are indentical, i.e., when the two formulations are absolutely bioequivalent; it is one when one of the two curves is identically zero, i.e., when one of the two formulations is not absorbed at all.

For $i = 1$ the bioequivalence index ξ_1 is proportional to the sum of the areas in the (c, t) plane enclosed by the two curves of equation $c = c_r(t)$ and $c = c_x(t)$, all areas considered positive. By increasing the value of i in ξ_i, more weight will be given to the magnitude of the change in concentration from one formulation to the other than to the duration of that change. The choice of i implies the choice of a model that

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Table I. Bioequivalence Index ξ_1[a]

	k_a'															
f'	0.30	0.29	0.28	0.27	0.26	0.25	0.24	0.23	0.22	0.21	0.20	0.19	0.18	0.17	0.16	0.15
1.25	0.157	0.151	0.144	0.138	0.132	0.126	0.120	0.115	0.112	0.111	0.111	0.111	0.111	0.111	0.111	0.113
1.20	0.145	0.138	0.131	0.124	0.117	0.110	0.103	0.097	0.093	0.091	0.091	0.091	0.091	0.091	0.092	0.099
1.15	0.134	0.127	0.119	0.111	0.103	0.095	0.087	0.080	0.073	0.070	0.070	0.070	0.070	0.070	0.076	0.089
1.10	0.124	0.116	0.108	0.100	0.090	0.081	0.072	0.063	0.054	0.049	0.048	0.048	0.048	0.054	0.067	0.083
1.05	0.116	0.107	0.099	0.090	0.080	0.070	0.060	0.049	0.038	0.028	0.024	0.024	0.032	0.046	0.063	0.082
1.00	0.110	0.101	0.093	0.083	0.073	0.062	0.052	0.040	0.027	0.014	0.000	0.015	0.031	0.048	0.067	0.087
0.95	0.107	0.098	0.089	0.080	0.071	0.061	0.050	0.039	0.030	0.026	0.026	0.030	0.042	0.057	0.075	0.094
0.90	0.108	0.100	0.092	0.083	0.075	0.067	0.059	0.054	0.053	0.053	0.053	0.054	0.061	0.074	0.089	0.107
0.85	0.114	0.107	0.101	0.094	0.088	0.084	0.081	0.081	0.081	0.081	0.081	0.081	0.085	0.095	0.108	0.123
0.80	0.127	0.122	0.117	0.114	0.112	0.111	0.111	0.111	0.111	0.111	0.111	0.111	0.113	0.119	0.130	0.143
0.75	0.148	0.145	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.144	0.148	0.156	0.167

[a] Reference formulation: $f = 1$, $k_a = 0.2$, $k_e = 0.25$. Test formulation: $k_e' = 0.25$.

considers the consequences of any discrepancy between the value of $c_r(t)$ and the value of $c_x(t)$.

Appendix II shows the theoretical values of the indices ξ_1 and ξ_2 for some typical plasma concentration functions. It is clear that when the two formulations are not very dissimilar, ξ_1 and ξ_2 are quite close. In the rest of this paper I use only the bioequivalence index $\xi = \xi_2$.

COMPUTATION OF THE INDEX OF BIOEQUIVALENCE

Once the index of bioequivalence has been chosen in terms of the functions $c_r(t)$ and $c_x(t)$, the problem remains of its estimation from a finite number of samples of the two functions at the times $t_1, t_2, \ldots, t_n$, namely, from the two finite sequences,

$$\begin{cases} c_r(t_1), c_r(t_2), \ldots, c_r(t_n) \\ c_x(t_1), c_x(t_2), \ldots, c_x(t_n) \end{cases} \tag{2}$$

One method consists in fitting the data in the sequences (2) to a suitable model, for instance, a sum of exponentials, then using the two fitted functions $c_r(t)$ and $c_x(t)$ to compute the index of bioequivalence with definition (1). This method of course is model dependent, that is, the result depends upon the functions chosen to fit the data.

A second method consists in substituting definition (1) with an equivalent definition valid for discrete sequences; for instance,

$$\xi^* = \left(\frac{\sum_{j=1}^{n} w_j [c_r(t_j) - c_x(t_j)]^2}{\sum_{j=1}^{n} w_j [c_r(t_j) + c_x(t_j)]^2} \right)^{1/2}$$

where w_j is an appropriate coefficient representing the weight that the sampling time t_j has in the determination of the whole functions $c_r(t)$ and $c_x(t)$. This method is not model-dependent in the ordinary sense, i.e., its result is a value that does not depend upon the choice of a particular function for fitting the data, but it is strongly contingent on the choice of the sampling times $t_1, t_2, \ldots, t_n$ and on the weights w_j. Of course if by time t_n most of the drug administered is known to have been eliminated, any extrapolation from t_n to $t = \infty$ would not add any significant information about the bioequivalence of the two formulations.

As a matter of fact, the first method is not as model-dependent as it may seem at first. If it is deemed satisfactory to consider the behavior of the functions $c_r(t)$ or $c_x(t)$ in the finite interval of time from t_1, to t_n, when most of the drug administered is known to have been eliminated, then any regular curve that fits the data in the interval t_1, t_n may be a good representation of the real curve $c_r(t)$ or $c_x(t)$, due to the smoothing effect of the integrals in definition (1).

Appendix III shows how to compute the index ξ using a linear spline approximation of functions $c_r(t)$ and $c_x(t)$.

CONCLUSION

From Tables I and II it is clear that, for two formulations with the same absorption and elimination rates, ξ_i is a linear function of the relative variation of f, the fraction absorbed; this was to be expected, due to the linear dependence of $c(t)$ on f. This is not the case for any variation of the rate of absorption, everything else being equal; this also was to be expected, due to the nonlinear dependence of $c(t)$ on the rate of absorption, at least in the model chosen for the construction of these Tables I and II. Furthermore, in both tables, ξ_i is not symmetrical around either column $k_a' = 0.20$ or row $f' = 1.00$, as a consequence of the strong nonlinearity of the processes involved. What ξ_i is measuring, after all, is neither the rate of absorption nor the fraction absorbed, but the consequences of the differences of those parameters in the two formulations.

Small shifts in lag times, i.e., delays before the absorption begins, ordinarily do not cause any change in AUC but may cause a considerable change in ξ_i; when these lag times are not considered important, their effect on ξ_i can be eliminated by introducing a time shift τ in $c_x(t)$, then determining the value of τ that minimizes ξ_i.[3]

Due to the statistical nature of the plasma concentration functions, we must expect variations in the concentration curves of the same formulation in different patients, and even of the same formulation in the same patient. Since bioequivalence tests are carried out in order to decide whether two formulations are sufficiently alike, a decision

[3] I am grateful to Dr. Ronald S. Siegel for this suggestion.

Table II. Bioequivalence Index ξ_2[a]

	k_a'															
f'	0.30	0.29	0.28	0.27	0.26	0.25	0.24	0.23	0.22	0.21	0.20	0.19	0.18	0.17	0.16	0.15
1.25	0.184	0.177	0.170	0.163	0.156	0.148	0.141	0.133	0.126	0.118	0.111	0.105	0.099	0.095	0.094	0.095
1.20	0.166	0.159	0.152	0.145	0.137	0.130	0.122	0.114	0.106	0.098	0.091	0.085	0.080	0.077	0.077	0.082
1.15	0.149	0.142	0.134	0.126	0.119	0.110	0.102	0.094	0.085	0.077	0.070	0.065	0.060	0.059	0.063	0.071
1.10	0.132	0.124	0.117	0.108	0.100	0.091	0.082	0.073	0.064	0.056	0.048	0.044	0.040	0.044	0.053	0.066
1.05	0.116	0.108	0.100	0.091	0.082	0.072	0.063	0.053	0.043	0.033	0.024	0.025	0.026	0.037	0.051	0.068
1.00	0.102	0.093	0.085	0.076	0.067	0.051	0.047	0.036	0.025	0.013	0.000	0.014	0.028	0.044	0.060	0.078
0.95	0.091	0.083	0.075	0.066	0.058	0.049	0.040	0.032	0.025	0.022	0.026	0.035	0.047	0.061	0.077	0.094
0.90	0.088	0.081	0.074	0.067	0.060	0.054	0.049	0.047	0.045	0.049	0.053	0.062	0.072	0.084	0.099	0.115
0.85	0.093	0.088	0.083	0.078	0.074	0.072	0.070	0.070	0.071	0.076	0.081	0.090	0.099	0.111	0.124	0.139
0.80	0.106	0.103	0.100	0.098	0.096	0.096	0.096	0.098	0.101	0.106	0.111	0.120	0.128	0.139	0.152	0.166
0.75	0.127	0.126	0.124	0.124	0.123	0.124	0.126	0.128	0.132	0.137	0.143	0.151	0.159	0.170	0.182	0.195

[a] Reference formulation: $f = 1$, $k_a = 0.2$, $k_e = 0.25$. Test formulation: $k_e' = 0.25$.

rule must be chosen based on the statistical properties of the index ξ. These properties can be determined only by simulating a sufficiently large number of concentration curves resulting from different rates and extents of absorption. The confidence level and the power of the test will then be determined exactly as is done with the classical indices of bioequivalence, such as AUC, t_m, and c_m.

APPENDIX I

Suppose that a fraction f of a drug is absorbed with a linear process at rate k_a and eliminated also with a linear process at rate k_e. If a unit dose is administered as a bolus, the plasma concentration function is

$$c(t) = \frac{1}{V_1} \cdot \frac{f \cdot k_a}{k_a - k_e} (e^{-k_e t} - e^{-k_a t})$$

and its AUC is

$$\int_0^\infty c(t)dt = \frac{1}{V_1} \cdot \frac{f \cdot k_a}{k_a - k_e} \left(\frac{1}{k_e} - \frac{1}{k_a}\right) = \frac{1}{V_1} \cdot \frac{f}{k_e}$$

and is proportional to the fraction absorbed and inversely proportional to the elimination rate but completely independent upon the absorption rate.

The plasma concentration function at time

$$t_{max} = \frac{\ln k_a - \ln k_e}{k_a - k_e}$$

reaches its maximum value,

$$c_{max} = \frac{f}{V_1} \left(\frac{k_e}{k_a}\right)^{k_e/(k_a - k_e)}$$

Again, the value c_{max} does not change from reference formulation to test formulation if the fraction absorbed changes in the same proportion as

$$\left(\frac{k_e}{k_a}\right)^{-[k_e/(k_a - k_e)]}$$

APPENDIX II

If a different formulation of the drug described in Appendix I has the same elimination rate but absorption rate k_a' and fraction absorbed f', we can compute its *bioequivalence index* ξ_i as

$$\xi_i = \left(\frac{\int_0^\infty |c_r(t) - c_x(t)|^i dt}{\int_0^\infty |c_r(t) + c_x(t)|^i dt}\right)^{1/i} = \left(\frac{\int_0^\infty \left|\frac{f \cdot k_a}{k_a - k_e}(e^{-k_e t} - e^{-k_a t}) - \frac{f' \cdot k_a'}{k_a' - k_e}(e^{-k_e t} - e^{-k_a' t})\right|^i dt}{\int_0^\infty \left|\frac{f \cdot k_a}{k_a - k_e}(e^{-k_e t} - e^{-k_a t}) + \frac{f' \cdot k_a'}{k_a' - k_e}(e^{-k_e t} - e^{-k_a' t})\right|^i dt}\right)^{1/i}$$

Tables I and II show some values of the bioequivalence indices ξ_1 and ξ_2 corresponding to some selected values of k_e, k_a, and f.

APPENDIX III

If we can approximate function $c(t)$ with linear splines, we put

$$c(t) = \frac{c_i - c_{i-1}}{t_i - t_{i-1}} t + \frac{t_i c_{i-1} - t_{i-1} c_i}{t_i - t_{i-1}}, \qquad i = 1, 2, \ldots n$$

where

$$c_i = c(t_i), \qquad i = 1, 2, \ldots, n$$

For the difference of the two functions $c_r(t)$ and $c_x(t)$ we have

$$c_r(t) - c_x(t) = \frac{\Delta c_i - \Delta c_{i-1}}{t_i - t_{i-1}} t + \frac{t_i \Delta c_{i-1} - t_{i-1} \Delta c_i}{t_i - t_{i-1}}, \quad i = 1, 2, \ldots, n$$

where

$$\Delta c_i = c_r(t_i) - c_x(t_i), \qquad i = 1, 2, \ldots, n$$

For their sum,

$$c_r(t) + c_x(t) = \frac{\Sigma c_i - \Sigma c_{i-1}}{t_i - t_{i-1}} t + \frac{t_i \Sigma c_{i-1} - t_{i-1} \Sigma c_i}{t_i - t_{i-1}}, \quad i = 1, 2, \ldots, n$$

where

$$\Sigma c_i = c_r(t_i) + c_x(t_i), \; i = 1, 2, \ldots, n$$

We compute now piecemeal the integral in the numerator of ξ_2,

$$\int_{t_{i-1}}^{t_i} [c_r(t) - c_x(t)]^2 dt = \int_{t_{i-1}}^{t_i} \left[\frac{\Delta c_i - \Delta c_{i-1}}{t_i - t_{i-1}} t + \frac{t_i \Delta c_{i-1} - t_{i-1} \Delta c_i}{t_i - t_{i-1}} \right]^2 dt = \frac{1}{3} [(\Delta c_{i-1})^2 + \Delta c_{i-1} \cdot \Delta c_i + (\Delta c_i)^2] \cdot (t_i - t_{i-1})$$

and the integral in the denominator,

$$\int_{t_{i-1}}^{t_i} [c_r(t) + c_x(t)]^2 dt = \int_{t_{i-1}}^{t_i} \left[\frac{\Sigma c_i - \Sigma c_{i-1}}{t_i - t_{i-1}} t + \frac{t_i \Sigma c_{i-1} - t_{i-1} \Sigma c_i}{t_i - t_{i-1}} \right]^2 dt = \frac{1}{3} [(\Sigma c_{i-1})^2 + \Sigma c_{i-1} \cdot \Sigma c_i + (\Sigma c_i)^2] \cdot (t_i - t_{i-1})$$

it follows that

Table III. Theoretical Values of ξ_2 Compared with the Values Computed Using a Linear Spline Approximation Truncated at Time $t = 40$[a]

Test formulation	ξ_2 (theoretical)	ξ_2 (computed)
$f' = 1.10, k_a' = 0.22$	0.064	0.063
$f' = 1.10, k_a' = 0.20$	0.048	0.048
$f' = 1.10, k_a' = 0.18$	0.040	0.041
$f' = 1.00, k_a' = 0.22$	0.025	0.024
$f' = 1.00, k_a' = 0.20$	0.000	0.000
$f' = 1.00, k_a' = 0.18$	0.028	0.027
$f' = 0.90, k_a' = 0.22$	0.045	0.046
$f' = 0.90, k_a' = 0.20$	0.053	0.053
$f' = 0.90, k_a' = 0.18$	0.072	0.070

[a] Reference formulation: $f = 1$, $k_a = 0.2$, $k_e = 0.25$. Test formulation: $k_e' = 0.25$.

$$\xi_2 \cong \left[\frac{\sum_{i=1}^{n} (\Delta c_{i-1})^2 + \Delta c_{i-1} \cdot \Delta c_i + (\Delta c_i)^2 \} \cdot (t_i - t_{i-1})}{\sum_{i=1}^{n} (\Sigma c_{i-1})^2 + \Sigma c_{i-1} \cdot \Sigma c_i + (\Sigma c_i)^2 \} \cdot (t_i - t_{i-1})} \right]^{1/2}$$

Table III compares some of the theoretical values of ξ_2 with the values computed with this formula, using 16 equally spaced sampling points up to time $t = 40$.

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