Tmax: An Unconfounded Metric for Rate of Absorption in Single Dose Bioequivalence Studies

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Received August 24, 1995; accepted November 3, 1995

Purpose. While peak drug concentration (Cmax) is recognized to be contaminated by the *extent* of absorption, it has long served as the indicator of change in absorption *rate* in bioequivalence studies. This concentration measure per se is a measure of extreme drug exposure, not absorption *rate*. This paper redirects attention to Tmax as *the* absorption rate variable.

Methods. We show that the time to peak measure (Tmax), if obtained from equally spaced sampling times during the suspected absorption phase, defines a count process which encapsulates the rate of absorption. Furthermore such count data appear to follow the single parameter Poisson distribution which characterizes the rate of many a discrete process, and which therefore supplies the proper theoretical basis to compare two or more formulations for differences in the rate of absorption. This paper urges limiting the use of peak height measures based on Cmax to evaluate only for dose-dumping, a legitimate safety concern with any formulation. These principles and techniques are illustrated by a bioequivalence study in which two test suspensions are compared to a reference formulation.

Results. Appropriate statistical evaluation of absorption rate via Tmax supports bioequivalence, whereas the customary analysis with Cmax leads to rejection of bioequivalence. This suggests that the inappropriate use of Cmax as a surrogate metric for absorption rate contributes to the unpredictable and uncertain outcome in bioequivalence evaluation today.

KEY WORDS: bioequivalence; absorption rate; Tmax; discrete count variable; Poisson distribution.

INTRODUCTION

> There's no limit to how complicated things can get, on account of one thing always leading to another.
>
> —E. B. White.

A finding of bioequivalence (BE) serves as a surrogate for therapeutic identity (1). It is customary to evaluate bioequivalence in vivo in healthy subjects, by comparing both *rate* and *extent* of drug absorption of a test with a reference formulation. The area under the concentration time curve from time zero to time t (AUCt, where t is the last measurable time point) and similarly area under the curve from time zero to time infinity (AUC∞) are both recognized as uncontaminated measures of the extent of absorption.

The situation for rate is in a state of flux. Time to peak data, Tmax, are collected but with the typical irregular sampling schemes in vogue they are not easily amenable to proper statistical evaluation. In the interim, the continuous variable Cmax, the extreme observed concentration, has annexed the function of Tmax and performs as a substitute measure for the *rate* of absorption. Figure 1 shows clearly that rate or speed of absorption information resides in the discrete time, or x-axis measure, *not* the continuous concentration or y-axis one. The paper *questions* current illogical BE practice, i.e., analyze the continuous, y-axis, output, Cmax variable and *because* it has greater precision, *assume* it is telling us something intelligent about Tmax, which is a discrete, x-axis, input variable.

Such practice has non trivial consequences for inferences or study conclusions. Rate analysis by Cmax may reject bioequivalence while the more appropriate analysis by Tmax finds differently. We illustrate this with an example.

Cmax is recognized as a extent-contaminated measure of rate. For example, the ratio Cmax/AUC has been proposed, and is being evaluated, because it 'corrects for extent' (2). If the unit of measure for Cmax is (say) μg/ml, and the unit of measure for AUC∞ is (say) μg*hr/ml, the unit of measure for the ratio is hr^{-1}. However, approaching rate via a ratio of two wrong axis variables (Cmax/AUC) imparts to the outcome variable a spurious continuous form, and this too can have nontrivial consequences on study conclusions.

This paper redirects attention to Tmax as *the* absorption rate variable. Because absorption rate information resides therein we advocate 1) the routine use of equal spacing to collect samples, i.e., collect time data at a definite rate per hr during absorption and 2) simply analyze the corresponding count data (where count = Tmax times rate per hour). Such counts, multiples of the sampling times, are numeric integers. Feller (3) showed the ubiquitous single parameter Poisson distribution to be identified with numeric integer counts and process rates. The discrete Poisson distribution therefore provides a solid theoretical basis to compare two or more formulations for differences in the rate of absorption.

In this paper we urge that y-axis peak height measures based on Cmax evaluate only for dose-dumping, a legitimate extent safety concern with any formulation.

METHODS

Revised Sampling Needed During the Absorption Phase

The sampling times chosen to observe concentrations during a bioequivalence study attempt to balance conflicting objectives. First, an ethical imperative against unnecessary bloodletting translates into keeping sampling times to a practical minimum. Second, the need to sample densely enough throughout the suspected absorption phase so as not to miss the peak. Third, the desire to sample the time concentration curve for some three or more half lives beyond the peak to supply a good measure of the full extent of absorption.

We advocate *equal* spacing of the sampling times from time zero (or other suitable initial time) until approximately two or three times the expected peak concentration time to improve the data and because this should have little impact on the total number of blood samples taken from a subject in the standard bioequivalence study. For example, a drug which has a Tmax of approximately half an hour in fasted subjects, and

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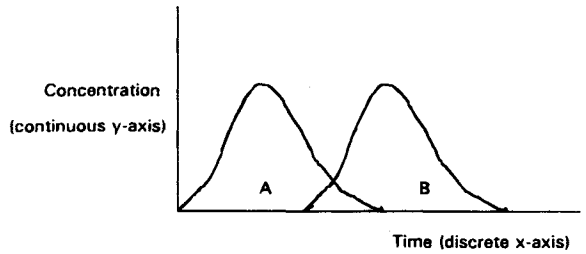

Fig. 1. Logical demonstration that information about the rate or speed of absorption resides in the discrete time or x-axis, *not* the continuous concentration or y-axis.

a similar short half-life, is easily densely sampled every fifteen minutes for the first two hours (nine samples) and with diminishing frequency thereafter through eight hours. An equal sampling interval through the absorption phase ensures that a subject's Tmax multiplied by the sampling frequency per hour is always a positive integer. These integer counts tell how long the absorption process takes to reach maximum concentration for each subject and they encapsulate the process rate.

Statistical Methodology for Counts

This section merely alerts the reader to a large and scientifically substantiated statistical methodology for analyzing discrete variables, including integer counts. For example, since the underlying distribution for a positive count is not the ubiquitous normal distribution many readers are likely familiar with, normal (Gaussian) theory methods should not be unthinkingly applied to counts. In a wide array of applications counts like those generated above have been found to be well-modeled by the pure random event Poisson distribution (3). The summary statistic for such counts across subjects for a formulation is supplied by the estimated distribution mean. Furthermore, comparison of the summary statistics from the counts for two different formulations determines whether formulations have the same rates of absorption. Modern computers and software make the proper analysis of counts very much more convenient than it used to be. Analysis of count data for subjects that arise from a crossover study may be analyzed by numerous procedures. Performing the computations for counts in particular within the context of a generalized linear model is a recent innovation comprehensively described in McCullagh and Nelder (4). SAS Institute (5) provides software that can perform the necessary computations. Cyrus Mehta and Nitin Patel (Cytel Software Corp., 6) have developed software to perform exact nonparametric inference for count data.

Reasons for Avoiding Ratios When We Can

Cmax, the extreme value of a concentration-time profile, is a single variable, whereas AUC is a composite variable similar to an average. Accordingly, AUC is better behaved and exhibits lower intrasubject variability than does Cmax. The ratio Cmax/AUC appears to cancel out the extent effect, but it retains unattractive aspects of *any* ratio. The variance of a ratio, X/Y is a relatively complex function of the variances of both X and Y and their covariance:

$$\mathrm{Var}(X/Y) \sim \mathrm{Var}(X)/Y^2 - 2 \times \mathrm{Cov}(X, Y)/Y^3 + X^2\mathrm{Var}(Y)/Y^4.$$

When Y is a constant, the two rightmost terms are zero and the ratio has variance proportional to Var(X). If X and Y do not correlate, the middle term becomes zero and the variance is proportional to $\mathrm{Var}(X) + X^2\mathrm{Var}(Y)/Y^2$. When X and Y do positively correlate, which we anticipate for Cmax and AUC, the result is likely to occur somewhere between these two extremes. In other words Cmax/AUC and Cmax have variance approximately the same order of magnitude, and furthermore there is the problem of spurious attribution of continuity to a discrete time variable. Because ratios obscure the correspondence between the attribute being measured, and the measurement chosen to do so, statisticians discourage forming ad hoc ratios of variables. The Cmax/AUC ratio exemplifies these disadvantages.

A Metric to Evaluate Dose-dumping

Whether Cmax is actually so extreme as to be potentially unsafe depends upon the other contributors to the concentration profile. The peak excursion statistic: Cex = Cmax- average of all other concentrations of the profile up to time t could signal whether formulation 'dumping' has occurred. This statistic, like AUC, is a composite measure statistic, but will behave like Cmax in applications. It too will exhibit substantial intrasubject variability. Cex, an always positive continuous random variable like Cmax, is log-normally distributed. It offers little that Cmax does not offer more directly. Both Cex and Cmax relate primarily to the question of extent while their connection to the question of rate is tenuous.

Westlake (7) first suggested log transforms of Cmax and AUCt be analyzed, and a confidence interval method similar to that given by Schuirmann (8) could compare Cex or Cmax values between two formulations. The continuous data for subjects arising in a crossover study can be analyzed by generalized linear model procedures. A comprehensive approach to performing the computations is described in SAS Institute (9).

RESULTS

An Example

A single dose three way crossover study was conducted by Lilly Research Laboratories to assess bioequivalence of two test suspension formulations, A and B, against a reference formulation, C. Sixteen healthy male volunteers were randomized to receive antibiotic drug formulations in either one of three sequences: ABC, BCA or CAB, and began treatment; only fifteen subjects who completed the study contributed to the analysis reported. Formulation periods were separated by a 3 day washout period. Since food is known to delay the absorption of some antibiotics, blood samples were drawn from subjects after overnight fast followed by two additional hours of fasting at times 0 hrs, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, and 8.0 hrs after dosing in each period of formulation administration. Table 1 lists selected pharmacokinetic data for the study.

In accordance with FDA bioequivalence guidelines (1), analyses were applied to natural logarithmic transformations of three variables: Cmax, AUCt and AUC∞. Sequences were tested for significance. No significant differences between

Table 1. Selected Pharmacokinetic Data for a Bioequivalence Study

Subject	Sequence	Treatment	Tmax (hrs)	half-life (hrs)	Cmax (μg/ml)	AUCt (μg*hr/ml)	AUC∞ (μg*hr/ml)
1	CAB	A	0.75	0.582	10.7	12.8	12.9
2	ABC	A	0.50	0.533	16.0	13.4	13.7
3	BCA	A	0.50	0.567	12.1	10.1	10.4
4	CAB	A	0.75	0.523	15.7	13.8	14.2
5	CAB	A	0.50	0.528	10.6	11.4	11.6
6	BCA	A	0.50	0.594	13.5	12.3	12.8
7	ABC	A	0.75	0.644	14.0	14.4	14.7
8	ABC	A	0.50	0.463	15.2	12.9	13.1
9	BCA	A	0.50	0.527	12.5	9.4	9.6
10	BCA	A	0.75	0.573	13.9	15.8	16.0
11	CAB	A	0.50	0.538	19.1	14.1	14.4
12	ABC	A	0.25	0.566	13.2	12.5	12.8
13	CAB	A	0.25	0.467	12.8	11.5	11.7
14	ABC	A	0.50	0.579	14.1	15.2	15.4
15	BCA	A	0.50	0.524	16.2	18.1	18.2
16	ABC	A	0.50	0.827	6.3	12.1	12.4
1	CAB	B	0.25	0.561	21.6	14.9	15.2
2	ABC	B	0.75	0.554	11.7	14.7	14.8
3	BCA	B	1.25	0.486	7.3	10.8	11.2
4	CAB	B	0.50	0.481	17.8	15.4	15.7
5	CAB	B	0.50	0.525	10.7	10.8	11.1
6	BCA	B	0.75	0.527	14.4	16.3	16.4
7	ABC	B	0.75	0.542	11.7	13.2	13.3
8	ABC	B	0.25	0.488	17.4	13.1	13.2
9	BCA	B	0.75	0.433	8.7	8.4	8.5
10	BCA	B	0.50	0.629	8.6	15.9	16.3
11	CAB	B	1.75	0.460	7.8	13.6	13.7
12	ABC	B	0.50	0.506	12.6	12.5	12.9
13	CAB	B	0.50	0.473	16.2	12.4	12.6
14	ABC	B	1.25	0.849	7.1	14.1	14.6
15	BCA	B	0.50	0.486	16.2	16.8	17.2
16	ABC	B	0.75	0.413	12.7	12.3	12.5
1	CAB	C	0.75	0.491	10.6	11.9	12.2
2	ABC	C	0.25	0.480	19.4	14.7	14.9
3	BCA	C	0.50	0.586	9.1	11.9	12.1
4	CAB	C	1.00	0.526	15.9	12.8	12.9
5	CAB	C	0.50	0.545	15.2	12.2	12.5
6	BCA	C	0.50	0.488	16.7	14.5	14.7
7	ABC	C	0.50	0.779	14.3	14.6	15.0
8	ABC	C	0.75	0.445	12.7	10.8	11.0
9	BCA	C	0.75	0.464	12.1	10.3	10.5
10	BCA	C	0.50	0.628	9.9	13.4	13.6
11	CAB	C	0.25	0.477	17.3	14.9	15.1
12	ABC	C	0.50	0.494	19.6	14.3	14.6
13	CAB	C	0.25	0.570	19.9	11.6	11.8
14	ABC	C	0.50	0.604	11.6	15.6	15.9
15	BCA	C	0.50	0.520	19.8	16.0	16.3

sequences implied the basic validity assumption underlying the crossover design had not been violated. Single degree of freedom contrasts constructed from the sequence X period means served to compare both the new formulations against the reference. Confidence intervals (90%) were constructed for the difference in the means of both new formulations versus the reference formulation using the ln transformed data. The antilogarithms of each set of confidence limits supply 90% confidence limits for the ratio of test and reference product averages.

Test and reference formulations are declared bioequivalent when the calculated limits are contained within the conventional bioequivalence range .80 to 1.25. Results of the bioequivalence evaluation are summarized in Table 2. The point estimate difference between B and C for the Cmax/AUC∞ ratio was −.18. The arithmetic 90% CI was −28.6% to −4.7% so this ratio variable here fails the bioequivalence interval (−20% to 20%) specification.

Since subjects *were* sampled at a constant rate of four per hour for two hours during the suspected absorption phase and all subjects peaked in concentration within two hours, the alternative procedure was easily implemented. Tmax could assume at most eight distinct values, and Tmax multiplied by four is

Table 2. 90% Confidence Limits on Separation of Means

Contrast	Variable	Ratio	Lower Limit	Upper Limit	Pass/Fail
A vs. C	Cmax	.95	.82	1.12	Pass
	AUCt	.99	.93	1.04	Pass
	AUC∞	.99	.94	1.04	Pass
B vs. C	Cmax	.82	.70	.97	Fail
	AUCt	1.01	.96	1.07	Pass
	AUC∞	1.01	.96	1.07	Pass

an integer, or count. Table 3 summarizes the answer to the question "How many quarters to reach Cmax?" for each subject and period on the study.

Analyses for these count data by stratified linear rank test (6) and within the crossover context by Poisson regression (5) are summarized in Table 4. Contrasts of sequence X period means similar to the ones used in the crossover analysis for continuous variables provided the two tests of the two Poisson regression mean parameter estimates given in Table 4. The Poisson regression deviance of 13.5053 is below its asymptotic chi-square value (24) for 24 degrees of freedom. The observed chi-square value corresponds to a p-value >.95 which usually is an indication the specified model fits the data very well. We have examined several examples and in all of them 'under dispersion' seems to be the norm. In general this should alert to the possibility of an incorrectly specified model (unlikely) or outliers in the data (much more likely). The phenomenon needs further investigation.

Table 5 shows how the Poisson regression computer printout leads easily to meaningful rate estimates. In the upper part of the table, with three treatment effects aliased with four sequence X period means, the program supplied estimates for the four sequence X period means (Table 5, column 3) need rectification into three treatment effects that sum to zero (column 4); treatment means are derived from these effects by adding each in turn to the overall mean (intercept). Column 5 shows count scale estimates obtained by applying the exponential transform to column 4 entries. Entries in parentheses are the square root of the entry above.

Haight (10) provides several methods for comparing Poisson means. Under the null hypothesis the three observed means (31.4, 43.4 and 31.4) are the same. The binomial probability law can evaluate them for significance. For example, to compare μ (B) and ω (C) we evaluate the appropriate confidence interval for $\theta = 0.5$ in 30 binomial trials, and observe whether $\mu/(\mu + \omega) = 0.58$ lies inside or outside the CI. If we use the software package (5) to evaluate a 60% CI for 15 successes in 30 trials we obtain .41–.59. Since .58 is barely contained within the range, B and C are significantly different at approximately the 20% level in yet another two sided test.

DISCUSSION

A B vs. C ratio estimate for Cmax below 1.0 implies the B formula has the *lower* extreme concentration value, so clearly dumping and attendant safety issues do not arise for B (Table 2). The example shows a suspected common occurrence in bioequivalence testing. The usurper variable Cmax declares that B and C are *not* bioequivalent in absorption *rate* under the standard approach, while the appropriate analysis (Table 5) applied to the logical estimator available for rate of absorption, i.e., Tmax, finds insufficient evidence to reject the null hypothesis. We should infer or conclude that B, while numerically lower in absorption rate by chance, has the same rate of absorption as C. Appropriate analysis therefore finds the two formulas, B and C, to be bioequivalent in both rate and extent!. Three outlier individuals here lead the two competing procedures to different conclusions.

In retrospect some will claim that appropriate analysis of the rate data in this study merely confirms that 15 subjects cannot definitively answer the rate question. In our experience a sample in the vicinity of 30 is needed to begin to compare discrete variables with reasonable power. The insensitivity of absorption rate as measured in small bioequivalence studies is an issue beyond the scope of this paper. The bothersome evidence, illustrated by the study, is that appropriate statistical evaluation with Tmax supports bioequivalence in rate of absorption for the test suspension (B), whereas the customary analysis with illogical Cmax leads to rejection of bioequivalence.

The unpredictable and uncertain outcome arising from using Cmax as a metric for absorption rate has led to a search for surrogate metrics, and also to calls to modify the bioequivalence interval. We saw in the study example that the Cmax/AUC∞ ratio for B vs. C failed the bioequivalence interval specification when Cmax did. This was not surprising, it merely confirmed

Table 3. Frequency Tabulation of Counts for Three Formulations

Formulation	1	2	3	4	5	6	7	Total
A	2	9	4	0	0	0	0	15
B	2	6	4	0	2	0	1	15
C	3	8	3	1	0	0	0	15

Table 4. Two Analyses of Counts Formed from Tmax Observations

Test	Contrast	Inference form	Sided test	p-value
Stratified linear	A vs. C	Exact test	Two	1.0
rank test	B vs. C	Permutation	Two	0.18
Poisson	A vs. C	Asymptotic test	Two	1.0
regression	B vs. C	Likelihood ratio	Two	0.20

Table 5. Study Poisson Regression Absorption Parameter Estimates

Parameter		Program Estimate (ln scale)	Actual ln Estimate (sum terms)	exp(estimate) (count scale)	Estimate in minutes (= count × 15)
	Intercept	0.8473	0.8473	2.333	35.0
λ	A mean	0.2412	−0.1078	2.095	31.4
μ	B mean	.5776; .5513	0.2155	2.894	43.4
ω	C mean	0.2412	−0.1078	2.095	31.4
σ^2	deviance	0.5627		1.7554	26.3
σ	scale ($\sqrt{}$dev)	(0.7501)		(1.3249)	19.9

expected behavior for an ad hoc ratio. There are many proposals in the literature suggesting something be done about the Cmax interval. For example, Schultz and Steinijans (11) want to widen the bioequivalence range for Cmax from .8 − 1.25 to .7 − 1.43. Clearly a wider interval would help, but we contend a superior solution to the unpredictability problem lies in first using a refined sampling scheme so as to empower Tmax to function as *the* metric for absorption rate.

REFERENCES

1. Division of Bioequivalence Guidance Statement. *Statistical procedures for bioequivalence studies using a standard two-treatment crossover design.* Office of Generic Drugs, Food and Drug Administration, Rockville MD, 1992, pp. 1–13.
2. L. Endrenyi, S. Fritsch and W. Yan. C_{max}/AUC is a clearer measure than C_{max} for absorption rates in investigations of bioequivalence. *Int. J. Clin. Pharmac. Ther. Toxic.* **29**:394–399, 1991.
3. W. Feller. *An Introduction to Probability Theory and its Applications. 2nd edition.* John Wiley and Sons, New York, 1957, pp. 135–163.
4. P. McCullagh and J. A. Nelder. *Generalized Linear Models.* Chapman and Hall, London, 1989.
5. SAS Institute: *SAS/STAT™ Software. The Genmod Procedure, Release 6.09.* SAS Institute, Cary, NC, 1993, pp. 1–88.
6. Cytel Software Corp.: *StatXact. Statistical Software for Exact Nonparametric Inference,* Version 2. Cytel Software Corp., Cambridge, MA 02139, 1991, Chapter 4.
7. W. J. Westlake. Bioavailability and bioequivalence of pharmaceutical formulations. In: K. E. Peace (eds.), *Biopharmaceutical Statistics for Drug Development,* Marcel Dekker, New York, 1988, pp. 329–352.
8. D. J. Schuirmann. A comparison of the two one-sided tests procedure and the power approach for assessing the equivalence of average bioavailability. *J. Pharmacokin. Biopharm.* **15**:657–680, 1987.
9. SAS Institute: *SAS/STAT™ User's Guide, Release 6.3 Edition.* SAS Institute, Cary, NC, 1988, pp. 549–640.
10. F. A. Haight. *Handbook of the Poisson Distribution.* John Wiley and Sons, New York, 1967, pp. 98–99.
11. H.-U. Schultz and V. W. Steinijans. Striving for standards in bioequivalence assessment: a review. *Int. J. Clin. Pharmac. Ther. Toxic.* **30**:(Suppl. 1), S1-S6, 1992.