STATISTICAL ANALYSIS OF PHARMACOKINETIC DATA--- BIOEQUIVALENCE STUDY

By

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ABSTRACT

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Bioequivalence (BE) studies are widely carried out in the pharmaceutical industry. The assessment of BE adopted by the Food and Drugs Administration (FDA) is a moment-based criterion evaluating log-transformed pharmacokinetic responses such as Area Under the Curve (AUC), Maximum Concentration ($C_{\max}$), which are usually estimated from drug plasma time profiles. Average BE (ABE) is based solely on the comparison of population averages but not on the variances, while Population BE (PBE) and individual BE (IBE) approaches include comparisons of both averages and variances. The objective of this thesis is to review the standard approaches to statistical analyses of pharmacokinetic data. It also covers estimation of AUC, $C_{\max}$ and other pharmacokinetic (PK) parameters as introduced in a Non-Compartmental Analysis (NCA) approach and Compartmental Models Analysis approach. Widely cited data sets from the published literature are used to illustrate these two approaches. They show the benefits of parameter estimation and subsequent statistical inference with an appropriate compartmental model, even though the model fitting could be a little complicated.

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CHAPTER 1 INTRODUCTION

1.1 Pharmacokinetics (PK)

PK is the study of the movement of drugs in the body, involving the processes of absorption, distribution, metabolism, and excretion (ADME), including the rate and extent of each of these processes. The science of PK concerns on how the body converts an active drug molecule into metabolites, the time course of drugs in the body, and what the body does to the drug. PK data are often derived from blood (serum or plasma) samples in small to medium-size datasets of individuals over time.

From a PK profile, pharmacokinetic parameters can be estimated such as Area Under the Curve (AUC), Maximum Concentration ($C_{\max}$), Time to Maximum Concentration ($T_{\max}$), Half Life ($T_{1/2}$), Bioavailability, Clearance, Volume of Distribution, etc. These PK parameters are very useful in optimization of the dosage form and dose interval.

1.2 Area Under the Curve (AUC)

AUC has units of concentration×time (e.g., $mg \times hr / L$), is a measure of the total systemic exposure of a drug integrated over time. AUC is usually estimated from concentration-time data. There are two major approaches to estimation of AUC: one is Non-Compartmental Analysis (NCA), which calculates the AUC following the trapezoidal rule by adding up the area under the curve between consecutive time points, the requirement of the Food and Drugs Administration (FDA)[1][2] for AUC estimation; the other is compartmental modeling analysis, which will be discussed in chapter 3.

1.3 Peak Concentration (C_{max})

C_{max} refers to the maximum (or peak) serum concentration that a drug achieves in a specified compartment or test area of the body after the drug has been administrated and prior to the administration of a second dose, i.e., in brief, C_{max} is the maximum concentration observed.

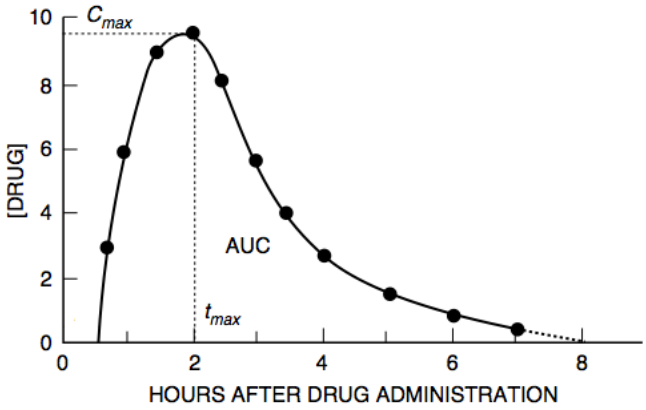

Figure 1 Schema of plasma concentration-vs.-time curve.

In bioequivalence studies, the key pharmacokinetic parameters are log-transformed AUC and C_{max}.[3] Figure 1 shows the schema of AUC, C_{max} and T_{max} in the plasma concentration -vs.-time curve after a single oral drug dose, cited from Atkinson AJ, et al. 2007[4] with a little modification. T_{max} is Time to Peak Concentration, the term used in pharmacokinetics to describe the time at which the C_{max} is observed.

1.4 Bioequivalence (BE)

BE studies are widely carried out in the pharmaceutical industry. The US Food and Drugs Administration -Code of Federal Regulations (FDA-CFR) definition[5] of BE is that the absence

of a significant difference in the rate and extent to which the active ingredient in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study. Although it is seen sometimes that measures used in BE study are pharmacological or clinical end-points, the most sensitive measures used in BE studies[6] are drug concentrations in the blood. From subject-level time and concentration data, subject-level AUC and $C_{\max}$ can be estimated, which form the BE outcome data for statistical analyses. If the drugs' plasma concentration curves are superimposable, then these drugs are considered as bioequivalent in extent and rate of absorption.

The FDA guidelines for BE studies recommend a minimum of 12 samples collected over time following drug administration with an additional sample prior to dosing.[2] These investigations on BE are best made through randomized clinical trials, and BE of two drugs is assessed by analysis of logarithmic transformed AUC and $C_{\max}$ typically obtained from a crossover design.

1.5 Crossover Design

Crossover Design is probably the most commonly used statistical design for comparing bioequivalence between two formulations of a drug. We shall refer to a two-sequence, two-period, crossover design as the standard 2×2 crossover design, also called AB|BA design.

Table 1 The standard 2×2 crossover design

Crossover Designs for Two Formulations	Period 1	Period 2
Sequence AB = 1	A	B
Sequence BA = 2	B	A

A standard 2×2 crossover study will generate paired outcomes (Y_1, Y_2) in two sequences of subjects: (a) In sequence AB subjects receive drug A in period 1 and are crossed over to drug B

in period 2; (b) In sequence BA subjects receive drug B in period 1 and are crossed over to drug A in period 2, as shown in Table 1. The dosing periods are separated by a washout period of sufficient length for the drug received in the first period to be completely metabolized or excreted from the body. Now, let's discuss here treatment effect, period effect and carry-over effect.

1.5.1 Treatment Effect. The objective of a cross-over trial is to focus attention on within-patient treatment differences, the difference between different measurements in the same subject, also called within-subject difference. The difference between these measurements removes any component that is related to the differences between the subjects, which is called 'subject-effect', from the comparison.

1.5.2 Period Effect. The within-subject difference could also be thought of as a comparison between the two treatment periods, which is the reason why usually one group of subjects received the treatments in the order AB and the other group received the treatments in the order BA.

1.5.3 Carryover Effect. A carryover effect is defined as effect of the treatment from the previous time period on the response at the current time period. The presence of carryover is an empirical matter.[7] It depends on the design, the setting, the treatment, and the response. The washout periods are usually included in the design, to allow the active effects of a treatment given in one period to be washed out of the body before each subject begins the next period of treatment. The disadvantage of the 2×2 crossover trial is that several important effects, such as

carryover effect and interaction effect are aliased with each other. Therefore, the carryover effect cannot be removed just by randomization alone in 2×2 crossover design. Washout period has to be clarified, usually 5 Half-life time ($T_{1/2}$) is necessary.

The objective of crossover design is to estimate the treatment effect, the expected difference in mean of logarithm transformed AUC and $C_{\max}$ of drug A versus drug B obtained from each subject. In each sequence, Treatment A and Treatment B produce a difference measure, provided that the period effect can be assumed to be constant, the information from both sequences can be combined to obtain the estimate of expected difference. Since the difference measure is within one subject, the difference removes any 'subject-effect' from the comparison.

1.6 Statistical Model

Consider a statistical model without considering carryover effect used by Byron Jones and Michael G. Kenward.[7] Let Y_{ijk} =response in *k*-th patient, in *j*-th period for *i*-th treatment. There is implied 'nesting' of treatment in period.

The model is:

$$Y_{ijk} = \mu + \tau_i + \pi_j + \alpha_k + \varepsilon_{ijk} \qquad (1)$$

where

μ is effect of an overall mean;

τ_i is effect of *i*-th treatment effect, *i*=1, 2, ..., *t*;

π_j is effect of the *j*-th period effect, *k*=1, 2, ..., *p*;

α_k is random effect associated with the *k*-th subject.

ε_{ijk} is random error associated with the *k*-th subject who received the *i*-th treatment in the *j*-th period, *k*=1, 2, ..., $n_{i.}$

$\alpha_k \sim N\left(0, \sigma_\alpha^2\right)$, $\varepsilon_{ijk} \sim N(0, \alpha_\varepsilon^2)$, α_k and ε_{ijk} are independent.

$Var\left(Y_{ijk}\right) = Var\left(\alpha_k + \varepsilon_{ijk}\right) = \sigma_\alpha^2 + \alpha_\varepsilon^2 = \sigma^2$, and $Cov(Y_{ijk}, Y_{ij'k}) = \sigma_\alpha{}^2$ for all j ≠ j'.

In this random subject-effects model, σ_α^2 is the inter-subject variability and α_ε^2 is the within-subject variability.

In Table 2, there are only four sample means $\bar{y}_{11\cdot}, \bar{y}_{12\cdot}, \bar{y}_{21\cdot}$, and $\bar{y}_{22\cdot}$. $\bar{y}_{11\cdot}$ represents the mean of samples of treatment 1 and period 1, ..., $\bar{y}_{21\cdot}$ represents the mean of samples of treatment 2 and period 1; Table 3 lists the mean of treatment and period, period effect and treatment effect with regard to this 2×2 crossover design.

Table 2 Expected Cell Means for Model (1)

Sequence	Period	
	1	2
1	$\bar{y}_{11\cdot} = \mu + \tau_1 + \pi_1$	$\bar{y}_{22\cdot} = \mu + \tau_2 + \pi_2$
2	$\bar{y}_{21\cdot} = \mu + \tau_2 + \pi_1$	$\bar{y}_{12\cdot} = \mu + \tau_1 + \pi_2$

Table 3 Expected Means and Effects for Model (1)

Mean of Treatment A	$½(\bar{y}_{11\cdot} + \bar{y}_{12\cdot}) = \mu + \tau_1 + ½\pi_1 + ½\pi_2$
Mean of Treatment B	$½(\bar{y}_{21\cdot} + \bar{y}_{22\cdot}) = \mu + \tau_2 + ½\pi_1 + ½\pi_2$
Mean of Period 1	$½(\bar{y}_{11\cdot} + \bar{y}_{21\cdot}) = \mu + \pi_1 + ½\tau_1 + ½\tau_2$
Mean of Period 2	$½(\bar{y}_{12\cdot} + \bar{y}_{22\cdot}) = \mu + \pi_2 + ½\tau_1 + ½\tau_2$
Treatment (A-B) Effect	$\tau_1 - \tau_2$
Period (1-2) Effect	$\pi_1 - \pi_2$

It is known from empirical studies that after logarithmic transformation, AUC and C_{max} are normally distributed or may be assumed to be approximately normally distributed. The core modeling component of SAS proc GLIMMIX can be illustrated for both fixed and random subject-effects models.[7] Suppose there are variates including subject, period, direct treatment and response variables in the dataset, model fitting and inference for fixed subject-effect models follow conventional ordinary least squares (OLS) procedures and for random subject-effect models Restricted Maximum Likelihood (REML) analyzes are used.[7]

The benefit of crossover design is that each subject serves as their own control, and statistical efficiencies are gained with respect to power and precision. Although crossover design has great advantages, it also brings a potential disadvantage: (1) Carryover may be confounded with direct treatment effects. (2) There are at least 2 periods, patients may withdraw from the trial, or become "lost to follow-up".

Special consideration is needed while doing statistical analysis for the crossover design. The typical method of Null Hypothesis Significance Testing (NHST) is designed to assess the evidence against the null hypothesis. The null hypothesis is rejected if the observed p-value is less than the stated significance level α; if not rejected, the null hypothesis will be retained. However, equivalence is not concluded just because we do not reject null. Here, the Two One-Sided Tests (TOST) is applied to assessing bioequivalence.

1.7 Average Bioequivalence (ABE)

ABE is a conventional method for the BE study, which solely compares the population averages of a BE measure of interest but not the variances of the measures for the T and R products,

following the FDA 1992 guidance on Statistical Procedures for Bioequivalence Studies.[3][8]

TOST has been used to determine whether the ratio of the logarithm transformed averages of the measures for the test and reference products were comparable.[9]

The two null hypotheses of TOST: (1) the mean difference is larger than the upper value of the BE limit Δ; and (2) the mean difference is below the lower bound of the BE limit -Δ, versus the alternative hypothesis of the difference falls within the range of the BE limit.

$$H_{01}: \mu_T - \mu_R \geq \Delta,\ H_{02}: \mu_T - \mu_R \leq -\Delta \quad H_a: -\Delta < \mu_T - \mu_R < \Delta$$

where T is Test, and R is Reference.

BE is established at significance level of α if a t-interval of confidence $(1-2\alpha)\times 100\%$ is contained in the interval $(-\Delta, \Delta)$, which is called Westlake's Confidence Interval.[10] Therefore, to establish BE at significant level of $\alpha = 0.05$, a 90% confidence interval should fall within the BE limit $(-\Delta, \Delta)$. For PK measures after logarithmic transformation, $\Delta = \ln 1.25$, $-\Delta = -\ln 1.25$,[3] while for PK measures without logarithmic transformation, the BE limit is a little different.

For the case of no logarithmic transformation, let $D = \mu_T / \mu_R$ be the ratio of the averages of the measures for the Test and Reference products.

$$H_{01}: \mu_T / \mu_R \geq \varepsilon_U, H_{02}: \mu_T / \mu_R \leq \varepsilon_L \quad H_a: \varepsilon_L < \mu_T / \mu_R < \varepsilon_U$$

$\varepsilon_L = 4/5 = 0.8$, and $\varepsilon_U = 5/4 = 1.25$ on $D = \mu_T / \mu_R$ that define the region of equivalence.[3]

To establish BE at significant level of $\alpha = 0.05$, a 90% confidence interval should fall within the BE limit $(\varepsilon_L, \varepsilon_U)$.

ABE only compares the population averages of a BE measure, assesses no comparison between variances of the measure for T and R products, therefore, there are some limitations on ABE. FDA (2001)[3] recommends Population Bioequivalence (PBE) and Individual Bioequivalence (IBE), which include comparisons of both averages and variances of the measure. In Table 4, the evaluation criteria are listed for ABE, PBE and IBE.

Table 4 Bioequivalence Types and Evaluation Criteria

Bioequivalence/Parameters	Evaluation Criteria
ABE Population averages($\mu_T;\mu_R$)	$(\mu_T-\mu_R)^2 \le \theta_A^2$
PBE Population averages ($\mu_T;\mu_R$) Total variances ($\sigma_{TT}^2;\sigma_{TR}^2$)	$[(\mu_T-\mu_R)^2 + (\sigma_{TT}^2-\sigma_{TR}^2)]/\sigma_{TR}^2 \le \theta_P$ or $[(\mu_T-\mu_R)^2 + (\sigma_{TT}^2-\sigma_{TR}^2)]/\sigma_{T0}^2 \le \theta_P$
IBE Population averages ($\mu_T;\mu_R$) Intra-subject variances (σ_{WT}^2 , σ_{WR}^2) Subject-by-formulation interaction variance (σ_D^2)	$[(\mu_T-\mu_R)^2+\sigma_D^2+(\sigma_{WT}^2-\sigma_{WR}^2)]/\sigma_{WR}^2 \le \theta_I$ or $[(\mu_T-\mu_R)^2+\sigma_D^2+(\sigma_{WT}^2-\sigma_{WR}^2)]/\sigma_{W0}^2 \le \theta_I$

$\theta_A,\theta_P,\theta_I$: specified bounds by the FDA; σ_{T0}^2, σ_{W0}^2 :specified threshold value by the FDA.

1.8 Population Bioequivalence (PBE)

PBE approach uses both the mean and the variance of log(AUC) and $\log(C_{max})$, to assess total variability of the measure in the population.[3][11]

For PBE, the parameter of interest is:

$$\Theta_{PBE}=\begin{cases}\dfrac{(\mu_T-\mu_R)^2+\sigma_{TT}^2-\sigma_{TR}^2}{\sigma_{TR}^2} & \text{when } {\sigma_{TR}}^2 > {\sigma_{T0}}^2 \text{ (Reference-scaled criterion)}\\ \dfrac{(\mu_T-\mu_R)^2+\sigma_{TT}^2-\sigma_{TR}^2}{\sigma_{T0}^2} & \text{when } {\sigma_{TR}}^2 \le {\sigma_{T0}}^2 \text{ (Constant-scaled criterion)}\end{cases}$$

In the equation, $\mu_T - \mu_R$ is still the mean difference between the test and reference. σ_{TT}^2 is the total variance of test, and σ_{TR}^2 is the total variance of reference. σ_{T0}^2 is the FDA specified threshold value, currently the values recommended by the FDA is $\sigma_{T0}^2 = 0.04$.[11] When the total variance of Reference σ_{TR}^2 is greater than the FDA specified threshold value σ_{T0}^2, Reference-scaled criterion is to be applied. Otherwise, Constant-scaled criterion is to be used. Currently FDA recommended value for θ_P is 1.7448,[11] if $\Theta_{PBE} < \theta_p$, and ABE is concluded, then PBE is also concluded.

1.9 Individual Bioequivalence (IBE)

IBE approach uses the means and variances of T and R, and the subject-by-formulation interaction to assess within-subject variability for the T and R products, as well as the subject-by-formulation interaction.

$$\Theta_{IBE} = \begin{cases} \dfrac{(\mu_T - \mu_R)^2 + \sigma_D^2 + \sigma_{WT}^2 - \sigma_{WR}^2}{\sigma_{WR}^2} & \text{when } \sigma_{WR}^2 > \sigma_{W0}^2 \text{ (Reference-scaled criterion)} \\ \dfrac{(\mu_T - \mu_R)^2 + \sigma_D^2 + \sigma_{WT}^2 - \sigma_{WR}^2}{\sigma_{W0}^2} & \text{when } \sigma_{WR}^2 \le \sigma_{W0}^2 \text{ (Constant-scaled criterion)} \end{cases}$$

Here, σ_{WT}^2 is within-subject variance for test drug, and σ_{WR}^2 is within- subject variance for reference drug. σ_{BT}^2 is between-subject variance for test drug, σ_{BR}^2 is between-subject variance for reference drug. $\sigma_D^2 = (\sigma_{BT} - \sigma_{BR})^2 + 2\,(1-\rho)\sigma_{BT}\sigma_{BR}$, assesses the subject-by-formulation interaction. ρ is the correlation coefficient between individual average test and reference formulation, they both contribute to the IBE determination.[3][11]

σ_{W0}^2 is specified threshold value, currently the FDA recommended $\sigma_{W0}^2 = 0.04$ .When the within variance of reference $\sigma_{WR}^2 > \sigma_{W0}^2$, Reference-scaled criterion is to be applied. Otherwise, Constant-scaled criterion is to be used. FDA recommended value for θ_I is 2.4948[11], if $\Theta_{IBE} < \theta_I$, and ABE is concluded, then IBE is also concluded.

The two-period two-treatment crossover design is the simplest prototype, which is not enough for PBE and IBE. When additional periods and/or treatments are considered, the possible configurations would increase. Some examples of a three-period two-treatment crossover design are (i) Sequences ABA and BAB; (ii) Sequences AAB, ABA and BAA; (iii) Sequences ABB and BAA.[3] Just as in the AB|BA design, we note that the treatment difference can be estimated in each period from any of these three designs. The benefit of having additional periods and/or treatments contributes to detecting if there is carryover effect in crossover design. Moreover, the IBE can be estimated with high order crossover design in addition to ABE and PBE.

CHAPTER 2 BIOEQUIVALENCE

A data set called "pkdata" from STATA documentation[12] is used here for our illustration. The data comprises two concentrations CONCA, CONCB in the same n=16 subjects assesses at 13 time points, including t=0. At baseline the concentration is zero. Eight patients are randomly assigned to sequence 1, which means they take the drug A in the period 1, and then take the drug B in the period 2; the other 8 patients are assigned to sequence 2, take the drug B in the period 1, and then take the drug A in the period 2. Assume the Drug B is the reference drug (R), and Drug A is the test drug (T). We will calculate AUC and $C_{\max}$ by Non Compartmental Analysis approach. Take the logarithmic transformation of AUC and $C_{\max}$ as the variables for the ABE study.

2.1 ABE

Consider the parameter AUC. Let $\mu_R = \mu_B$ be the reference group population mean, $\mu_T = \mu_A$ be the test group population mean. Let $\mu_D = \mu_T - \mu_R$ be the treatment difference, $-\Delta = -\ln 1.25$ be the lower bound, and $\Delta = \ln 1.25$ (=0.2231) be the upper bounds on $\mu_D = \mu_T - \mu_R$ that define the region of equivalence. ABE involves the calculation of a 90% CI for $\mu_D = \mu_T - \mu_R$, the difference in the means of log-transformed AUC. The ABE will be concluded based on the calculated 90% confidence limits falling within $-0.2231 \le \mu_T - \mu_R \le 0.2231$. First, the expected cell means for $log(AUC)$ in Table 5 are listed following notations used in Table 2.

Table 5 Expected Cell Means for $log(AUC)$

Sequence	Period	
	1	2
1	$\bar{y}_{11\cdot}=5.0069$	$\bar{y}_{22\cdot}=4.9270$
2	$\bar{y}_{21\cdot}=5.0077$	$\bar{y}_{12\cdot}=4.8740$

Similarly, the expected means and effects for $log(AUC)$ in Table 6 are listed following notations used in Table 3.

Table 6 Expected Means and Effects for $log(AUC)$

Mean of Treatment T	$½(\bar{y}_{11\cdot}+\bar{y}_{12\cdot})=4.9404$
Mean of Treatment R	$½(\bar{y}_{21\cdot}+\bar{y}_{22\cdot})=4.9673$
Mean of Period 1	$½(\bar{y}_{11\cdot}+\bar{y}_{21\cdot})=5.0073$
Mean of Period 2	$½(\bar{y}_{12\cdot}+\bar{y}_{22\cdot})=4.9005$
Treatment (T-R) Effect	-0.0269
Period (1-2) Effect	0.1067

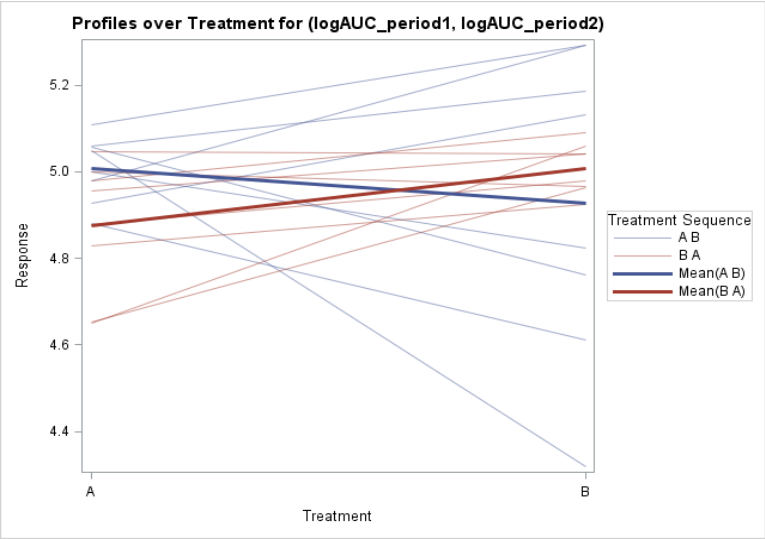

Figure 2 Profiles over treatment A and B for $log(AUC)$ in two periods.

The objective of this study with cross-over design is to focus attention on within-subject treatment differences. Figure 2 shows profiles over treatment for crossover designs. The subject-

profiles in Figure 2 are plotted for each sequence the change in each subject's response over the two treatment periods, which show no strong treatment effect or period effect.

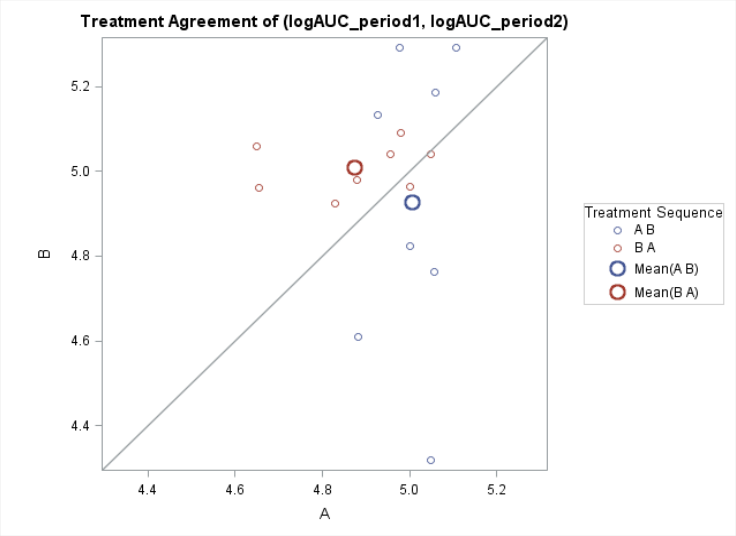

Figure 3 Treatment A vs. B Agreement of $log(AUC)$ in two periods.

The treatment agreement in Figure 3 is plotted for the response associated with the second treatment against the response associated with the first treatment. The figure indicates the strength of the treatment effect is small, and the treatment effect A-B is negative. The spread of points within sequence AB being wider indicates the bigger between-subject variability.

Table 7 TTEST output for $log(AUC)$

Treatment	Method	Mean	Lower Bound		90% CL Mean			Upper Bound	Assessment
Diff (1-2)	Pooled	-0.0269	-0.2231	<	-0.1462	0.0924	<	0.2231	Equivalent
Diff (1-2)	Satterthwaite	-0.0269	-0.2231	<	-0.1503	0.0965	<	0.2231	Equivalent

For crossover design, TOST option of PROC TTEST requests Schuirman's TOST equivalence test, with the option of specifying the equivalence bounds. After log - transformation of PK data, given the BE limit is (-0.2231, 0.2231), the assessment of BE is finally shown in Table 7. Exactly the same calculations can be carried out in PROC GLIMMIX with LSMEANS statement (1) compute least squares (LS) means of fixed effects (2) compute the 90% CI for LS-mean

difference, and (3) see if 90% CI falls in the stated BE limits (-Δ, Δ). The BE limits are (-0.2231, 0.2231) in ABE evaluation.

Table 8 GLIMMIX output for $log(AUC)$

Estimates							
Label	Estimate	Standard Error	DF	t Value	Pr> |t|	Lower	Upper
T-R	-0.02690	0.06774	14	-0.40	0.6973	-0.1462	0.09241

In Table 8, the PROC GLIMMIX output shows that the 90% CI (-0.1462, 0.09241) falls within the range (-0.2231, 0.2231), the ABE is concluded for $log(AUC)$ at significance level $\alpha = 0.05$

Table 9 Expected Cell Means for $log(C_{\max})$

Sequence	Period	
	1	2
1	$\bar{y}_{11\cdot} = 1.9763$	$\bar{y}_{22\cdot} = 2.5126$
2	$\bar{y}_{21\cdot} = 2.0101$	$\bar{y}_{12\cdot} = 2.4469$

Similarly, the expected cell means for $log(C_{\max})$ in Table 9 are listed following notations used in Table 2. $log(C_{\max})$ of Drug T and Drug R are estimated by PROC TTEST and PROC GLIMMIX procedures.

Table 10 Expected Means and Effects for $log(C_{\max})$

Mean of Treatment T	$½(\bar{y}_{11\cdot} + \bar{y}_{12\cdot}) = 2.2116$
Mean of Treatment R	$½(\bar{y}_{21\cdot} + \bar{y}_{22\cdot}) = 2.2614$
Mean of Period 1	$½(\bar{y}_{11\cdot} + \bar{y}_{21\cdot}) = 1.9932$
Mean of Period 2	$½(\bar{y}_{12\cdot} + \bar{y}_{22\cdot}) = 2.4798$
Treatment (T-R) Effect	-0.0498
Period (1-2) Effect	-0.4866

Similarly, the expected means and effects for $log(C_{\max})$ in Table 10 are listed following notations used in Table 3.

Figure 4 shows profiles over treatment for crossover designs. The subject-profiles in Figure 4 are plotted for each sequence the change in each subject's response over the two treatment periods, which show no strong treatment effect but maybe a period effect.

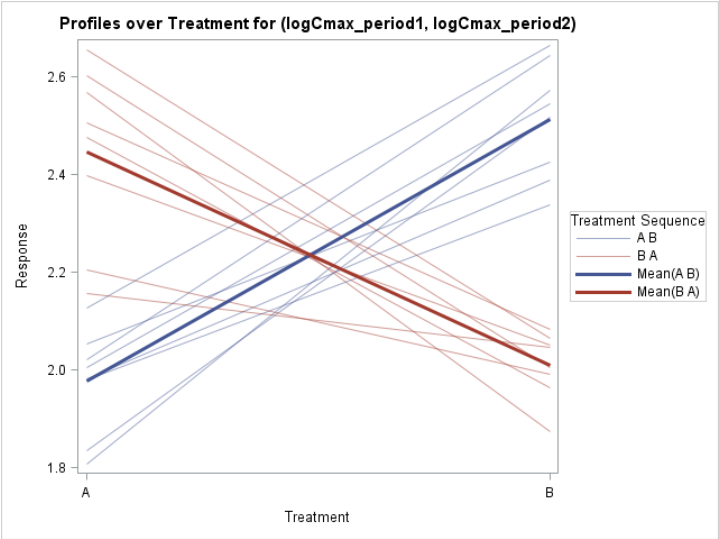

Figure 4 Profiles over treatment A and B for $log\left(C_{\max}\right)$ in two periods.

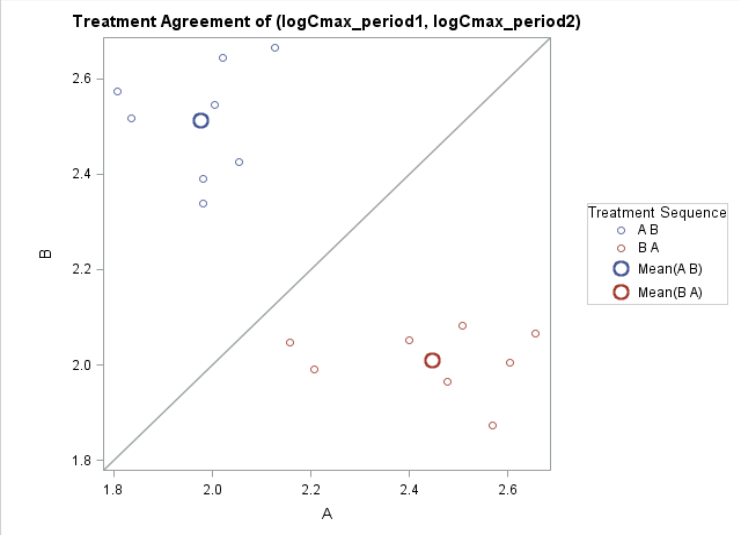

Figure 5 Treatment A vs. B Agreement of $log\left(C_{\max}\right)$ in two periods.

The treatment agreement in Figure 5 is plotted for the response associated with the second treatment against the response associated with the first treatment. The figure indicates the

strength of the treatment effect is small, and the treatment effect of A-B is negative. Substantial location differences between the two sequences indicate a strong period effect.

Table 11 TTEST output for $log(C_{max})$

Treatment	Method	Mean	Lower Bound		90% CL Mean			Upper Bound	Assessment
Diff (1-2)	Pooled	-0.0498	-0.2231	<	-0.1280	0.0285	<	0.2231	Equivalent
Diff (1-2)	Satterthwaite	-0.0498	-0.2231	<	-0.1285	0.0290	<	0.2231	Equivalent

For crossover design, TOST option of PROC TTEST requests Schuirman's TOST equivalence test, with the option of specifying the equivalence bounds. After logarithmic transformation of PK data, given the BE limit is (-0.2231, 0.2231), the assessment of bioequivalence is finally shown in Table 11.

Table 12 GLIMMIX output for $log(C_{max})$

Estimates							
Label	Estimate	Standard Error	DF	t Value	Pr> |t|	Lower	Upper
T-R	-0.04976	0.04379	14	-1.14	0.2749	-0.1269	0.02737

Similarly use PROC GLIMMIX with LSMEANS statement: (1) compute LS means of fixed effects, (2) compute the 90% CI for LS-mean difference, and (3) see if 90% CI falls in the stated BE limits (-Δ, Δ), here the BE limits are (-0.2231, 0.2231).

In Table 12, PROC GLIMMIX output shows that the 90% CI (-0.1269, 0.02737) falls within the range (-0.2231, 0.2231), the ABE is concluded for $log(C_{max})$ at significance level $\alpha = 0.05$.

Therefore, summarizing the two primary response variables for BE study, $log(AUC)$ and $log(C_{max})$, the ABE is concluded for T and R at the significance level $\alpha = 0.05$.

2.2 Sample Size Calculation

Sample size calculation for crossover design in bioavailability and bioequivalence study is an essential question, which establishes bioequivalence within meaningful limits in the case of logarithm transformed AUC and $C_{\max}$.[13] A minimum number of 12 evaluable subjects should be included in any BE study according to FDA guidelines.[3]

Based on Schuirmann's TOST procedure for interval hypothesis, use the data $\mu_D = \mu_T - \mu_R$ as the expected mean difference after logarithmic transformation. If the 100(1-2α)% CI $\left(\hat{\mu}_D - t_{\alpha,2n-2} \cdot \hat{\sigma}, \hat{\mu}_D + t_{\alpha,2n-2} \cdot \hat{\sigma}\right)$ of the mean μ_D is entirely within the BE limit (-ln1.25, ln1.25), then H_0 is rejected at significance level α and no drug-drug interaction is concluded; otherwise, H_0 fails to be rejected.

The type-I error α of the TOST procedure is often set as 5%.Total number of subjects should provide adequate power for BE demonstration, and the adequate power means at least 80% power to detect a 20% difference in products' BE. In practice the power usually is about 80% - 90%.

2.2.1 Formula for sample size. Let n = number of subjects required per sequence. α is the significant level, β is the type II error, CV = coefficient of variation, $CV = \sigma_e / \mu_R \cdot 100\%$, δ = the BE limit, ∇ is the expected difference compared to expected mean of Reference ,

$\nabla = \frac{\mu_T - \mu_R}{\mu_R} \cdot 100$; $\hat{\sigma}$ is the intra-subject standard deviation. Assuming a normal distribution of logarithm transformed PK data (AUC, $C_{\max}$), for $\nabla = 0$, n can be estimated[13][14] by

$$n \geq 2[t_{\alpha,2n-2} + t_{\beta/2,2n-2}]^2[\hat{\sigma}/\delta]^2$$

The approximate sample size calculation for the TOST tests for $\nabla > 0$, the equation is:

$$n \geq 2[t_{\alpha,2n-2} + t_{\beta,2n-2}]^2[\hat{\sigma}/(\delta - \nabla)]^2$$

The n on the right hand side is unknown (in the degrees of freedom). Start with an initial value n_0, the n_1 is calculated. The calculation is iterative until n is almost unchangeable.

As an illustration, consider the STATA dataset described at the beginning of this chapter.

Let α=0.05, β=0.2; For $log(AUC)$, $\delta = 0.2231$, $\hat{\sigma}$=0.1355, use the initial value $n_0 = 8$

$$n \geq 2\frac{[t_\alpha(2n-2) + t_{\beta/2}(2n-2)]^2}{\delta^2}\hat{\sigma}^2 \geq 2\frac{[1.761+1.345]^2}{0.2231^2}0.1355^2 \cong 8$$

So n per group = 8, and total n = 16.

The n per group =8 is also estimated from PROC POWER. The example is for ABE study. The number of subjects for PBE or IBE studies can be estimated by simulation according to the FDA guideline,[3] which will not be discussed here.

CHAPTER 3 AUC ESTIMATION

There are two representative examples of one compartmental pharmacokinetic models:[15][16]

(A) One compartmental model with i.v. administration; (B) One compartmental model with extravascular administration.

One compartmental model with i.v. dosing means administering a dose of drug over a very short time period, there is no absorption rate constant (k_a) considered. A one compartmental model with extravascular administration means absorption phase is involved in the whole process. As shown in Chapter 1, the $C_{\max}$ and $T_{\max}$ are simple measures for summarizing the absorption process. In one compartment model, assessment of $T_{\max}$ depends on the value of both elimination rate constant (k_e) and absorption rate constant (k_a).

$$T_{\max} = \frac{\log\left(k_a / k_e\right)}{k_a - k_e}.$$

Let us look into the concepts of k_e and k_a, and how they appear in a pharmacokinetic model.

3.1 Definition of PK Parameters

3.1.1 Elimination Rate Constant (k_e, units are h^{-1}) describes the rate of decrease in concentration per unit time, usually the time unit is hour. It is estimated from the log-linear terminal part of the concentration-time curve, $k_e = -slope$.

3.1.2 Absorption Rate Constant (k_a, units are h^{-1}) is the rate of absorption of a drug absorbed from its site of application according to assumption of first-order kinetics, which is for a drug

administered by a route (for example, oral) other than the intravenous. The first-order differential equation that governs the drug amount remained $X(t)$: $\frac{dX(t)}{dt} = -k_a X(t)$. $D = X(0)$ is the actual dose (mg) that is available to the body for kinetics, whereas the oral dose is given in mg/kg. Next we define the four most useful PK parameters characterizing the in vivo disposition of a drug.[15][16]

3.1.3 Half-life ($T_{1/2}$) is given by $T_{1/2} = \log(2)/\mathrm{k}_e$, that is, the time from $T_{\max}$ to reach one-half of the maximum concentration $C_{\max}$.

3.1.4 Bioavailability (F, has no unit) is described as the fraction of the extravascular dose of the administered drug that reaches the absorption depot. If the drug is injected intravenously, it is assumed that bioavailability F=100%. Bioavailability generally decreases when a medication is administered via other routes (such as orally), such as $F = \frac{AUC_{oral} \times Dose_{iv}}{AUC_{iv} \times Dose_{oral}}$, F is often measured by quantifying the "AUC".

3.1.5 Volume of Distribution (V, the units of volume, e.g., L) or apparent volume of distribution is a pharmacological, theoretical volume that the total amount of administered drug would have to occupy (if it were uniformly distributed), to provide the same concentration as it currently is in blood plasma. There are two quantities: the concentration $C(t)$ in plasma and the amount of drug $X(t)$ in tissue. It is assumed that $X(t)/C(t) = V$ is constant.

3.1.6 Clearance (CL , the units are volume per time, e.g., L/hr) is called the drug clearance rate, can be defined as the volume of plasma which is completely cleared of drug per unit time. CL is calculated using the dose administered divided by the subsequent measured AUC,

$CL = \frac{(Oral\ dose) \times F}{AUC} = \frac{X(0)}{AUC} = V \times k_e$, where F is the bioavailability. Unless we have information on F, the parameter CL/F is only identified, this is called the *apparent clearance*. With the drug plasma concentration-time profile, AUC can be estimated by NCA, and also by compartmental modeling analysis.

3.2 Non-Compartmental Analysis (NCA)

Based on the theory of statistical moments, the moments of a function are used in the analysis of pharmacokinetic data.[17] Suppose drug concentration $C(t)$ is a real-valued function defined on the interval [0, ∞); the zeroth moment of $C(t)$ is S_0 : $S_0 = \int_0^\infty C(t)dt = AUC$, "the area under the curve from time zero to infinity"; and the first moment of $C(t)$ is S_1 : $S_1 = \int_0^\infty t \cdot C(t)dt = AUMC$, "the area under the first moment curve", is the area under the curve of concentration-time versus time curve from time zero to infinity, $AUMC$ can be used to estimate some other PK parameters.

Non-compartmental Analysis estimate AUC using the trapezoidal rule without making any assumption concerning the number of compartments. Following the trapezoidal rule, concentration-time curve is considered as a series of trapezoids and the AUC estimate is the total area of all the trapezoids.

For non-compartment model, $AUC_{(0-t)}$ is AUC from 0 h to the last quantifiable concentration to be calculated; $AUC_{(0-\infty)} = AUC_{(0-t)} + AUC_{(t-\infty)}$, represents the total drug exposure over time. $AUC_{(0-\infty)}$ requires extrapolation of the elimination-phase curve beyond the last measurable plasma concentration. The extrapolation of AUC from *t* to infinity requires several assumptions: (1) At low concentrations, drug usually declines in mono exponential fashion; (2) The terminal elimination rate constant does not change over time or with different concentrations of circulating drug; (3) other processes such as absorption and distribution do not play a significant role in the terminal phase of the pharmacokinetic profile. These assumptions usually are valid in almost all PK applications. Therefore, $AUC_{(0-\infty)}$ can be calculated as

$AUC_{(0-\infty)} = AUC_{(0-t)} + C_{last} / k_e$, where C_{last} is the last observed quantifiable concentration and k_e is the terminal phase rate constant, $k_e = -slope$ (units: h^{-1}). When a regression line is fitted to terminal phase data points on log-scale, then elimination half-life $T_{1/2} = \log(2)/k_e$ can be estimated. With NCA, the observed $C_{\max}$ and $T_{\max}$ are obtained directly from the data without interpolation.

Non-compartmental analysis allows a simple estimation of AUC. It basically summarizes the concentration-time profile without modeling assumptions. However, non-compartmental methods are unable to visualize or predict plasma concentration- time profile for other dosing regimens. It assumes the kinetics to be linear and stationary (i.e., time-independent) for simple applications. In more sophisticated analyses of PK data, the one compartment model or multi-compartment analysis with nonlinear mixed effects models (NLMEM) are increasingly used in drug development.[18]

We will use the widely cited example of drug theophylline,[19][20][21] which has serum concentrations measured at 11 time points over a 25 hour period in 12 subjects to illustrate the NCA method and compare the results with that of a one compartment model.

3.3 One Compartment Model

Compartmental modeling in pharmacokinetics estimate the concentration- time curve using kinetic models that depend on the rate of drug distribution to the different parts of the body.[15]

In a one compartment model, the drug is considered to be distributed instantaneously into all parts of the body. The simplest case, if i.v. drug is received, it instantaneously equilibrates within the compartment and is eliminated at a constant rate k_e. For a concentration measure $C(t)$, first-order kinetics is assumed: $\frac{dC(t)}{dt} = -k_e C(t)$ where $k_e > 0$ is the elimination rate constant. We get $C(t) = C(0)\exp(-k_e t)$ where C(0) is the initial concentration. The elimination rate is h^{-1}, per hour. $C(0) = X(0)/V$ is the initial amount of dose in mg per unit volume in L, where V is the volume distribution.

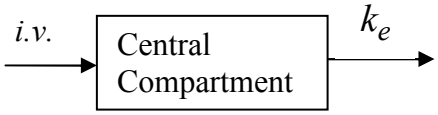

Figure 6 One compartmental model with i.v. administration.

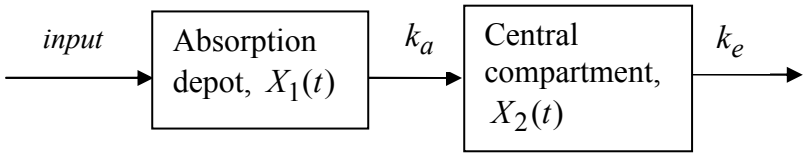

Figure 7 One compartmental model with extravascular administration.

For extravascular (such as oral) administration, the body receives the drug and is absorbed at constant rate k_a proportional to the amount of drug available for absorption. The drug instantaneously equilibrates within the compartment and is eliminated at a constant rate k_e. The first-order differential equations that govern the amounts $X_1(t)$: $\frac{dX_1(t)}{dt} = -k_a X_1(t)$, and $X_2(t)$: $\frac{dX_2(t)}{dt} = k_a X_1(t) - k_e X_2(t)$. The initial amounts are $X_1(0) = D$ and $X_2(0) = 0$; at time *t*, the drug amount in the absorption depot is: $X_1(t) = X_1(0)\exp(-k_a t)$, as shown in Figure 6; and the drug amount in the central compartment is $X_2(t) = \frac{k_a X_1(0)}{k_a - k_e}\left(\exp(-k_e t) - \exp(-k_a t)\right)$, as shown in Figure 7. Note that this equation makes sense only when $k_a > k_e$. The focus is on the equation of central compartment, which can be expressed as the concentration equation:

$$C(t) = \frac{k_a D}{V(k_a - k_e)}\left(\exp(-k_e t) - \exp(-k_a t)\right).$$

From the equations $k_e = CL/V$ and $X(0) = (Oral\,dose) \times F$, an operational expression of drug concentration in the central compartment at time *t* is

$$C(t) = \frac{k_a k_e (oral\,dose) \times F}{CL(k_a - k_e)}\left(\exp(-k_e t) - \exp(-k_a t)\right).$$

The unknown parameters are $k_a,\ k_e,\ CL$ that must be estimated from observed concentrations $\{C(t): t = 0,1,\ldots,m\}$ in individuals over a grid of time points.

3.3.1 AUC estimation. From the equation of the drug concentration, the AUC is defined as $AUC = \int_0^\infty C(t)dt$ and also denoted by $AUC_{(0-\infty)}$. From the formula for $C(t)$ we get

$$AUC = \frac{k_a k_e (oral\ dose) \times F}{CL(k_a - k_e)} \int_0^\infty \left(\exp(-k_e t) - \exp(-k_a t)\right) dt$$
$$= \frac{k_a k_e (oral\ dose) \times F}{CL(k_a - k_e)} \left(\frac{1}{k_e} - \frac{1}{k_a}\right) = \frac{(oral\ dose) \times F}{CL}.$$

Only if the underlying pharmacokinetic model is identified, can the parameters be accurately estimated, otherwise this method of estimation is not to be recommended.[22]

3.3.2 $T_{\max}$ and $C_{\max}$ estimation. Since $T_{\max}$ is the time to maximum concentration we obtain the maximum value of $C(t)$ by solving $\frac{dC(t)}{dt} = 0$ and showing that the unique solution is indeed the maximum value.

$$\frac{dC(t)}{dt} = \frac{k_a k_e (oral\ dose) \times F}{CL(k_a - k_e)} \left(-k_e \exp(-k_e t) + k_a \exp(-k_a t)\right).$$

The derivative is a continuous function; at t=0 it is positive; as $t \to +\infty$, the derivative approaches zero. When $k_a > k_e$, the solution $T_{\max}$ is given by $T_{\max} = \frac{\log\left(k_a / k_e\right)}{k_a - k_e}$.

To obtain $C_{\max}$:

$$C(T_{\max}) = \frac{k_a k_e (oral\ dose) \times F}{CL(k_a - k_e)} \left(\exp(-k_e T_{\max}) - \exp(-k_a T_{\max}) \right).$$

Although we defined $AUC = \int_0^\infty C(t)dt$, another quantity of interest is

$$AUC_{(0,T_{\max})} = \int_0^{T_{\max}} C(t)dt .$$

Using the formula $\int_0^{T_{\max}} \exp(-k_a t)dt = \frac{1 - \exp(-k_a T_{\max})}{k_a}$ and repeating the previous calculation gives

$$\begin{aligned} AUC_{(0,T_{\max})} &= \frac{k_a k_e (oral\ dose) \times F}{CL(k_a - k_e)} \int_0^{T_{\max}} \left(\exp(-k_e t) - \exp(-k_a t) \right) dt \\ &= \frac{k_a k_e (oral\ dose) \times F}{CL(k_a - k_e)} \left(\frac{1}{k_e} - \frac{1}{k_a} - \left\{ \frac{\exp(-k_e T_{\max})}{k_e} - \frac{\exp(-k_a T_{\max})}{k_a} \right\} \right) \\ &= \frac{(oral\ dose) \times F}{CL} - \frac{k_a k_e (oral\ dose) \times F}{CL(k_a - k_e)} \left\{ \frac{\exp(-k_e T_{\max})}{k_e} - \frac{\exp(-k_a T_{\max})}{k_a} \right\} \end{aligned}$$

The first term on the right hand side is $AUC_{(0,\infty)}$ that was calculated previously.

3.3.3 $T_{1/2}$ (half life) estimation. The half-life $T_{1/2}$ is the time from $T_{\max}$ to reach one-half of the maximum concentration $C_{\max}$. Initially at t=0 the concentration is zero. With the passage of time the concentration $C(t)$ increases to a peak $C_{\max}$ at time $T_{\max}$ and then $C(t)$ declines to zero asymptotically.

The relationship between elimination rate constant (k_e) and half-life ($T_{1/2}$) is:

$$k_e = \frac{\log 2}{t_{1/2}} .$$

The half-life ($T_{1/2}$) is determined by clearance (CL) and volume of distribution (V):

$$T_{1/2} = \frac{\log 2 \times V}{CL}.$$

An objective of PK studies is to obtain estimates of parameters from observations of concentrations $\{C(t) : t = 0,1,\ldots,m\}$ in individuals over a grid of time points. The parameterization may allow for some parameters to be individual-specific which makes them random effects instead of pure constants.

3.3.4 Application. Take the widely cited example of drug theophylline,[19][20][21] serum concentrations measured at 11 time points over a 25 hour period in 12 subjects. First the NCA method is used to estimate the PK parameters, comparing the results from the one compartment model. As described above, the one compartment model is used to estimate the PK parameters, and then estimates of AUC, $C_{\max}$ and $T_{\max}$ are derived from the formulas. Among the benefits of the one compartment model analysis is that the PK parameters are estimated together with their standard errors and 95% confidence intervals. In addition individual (subject-specific) prediction of drug concentration can be made.

3.3.5 Model for $C(t)$. Assume normal distribution for $C(t)$ given (k_a, k_e, CL). The parameters (k_a, CL) are subject-specific, i.e., random effects, but k_e is a fixed parameter. All parameters are transformed to their logged form: $log(k_a)$, $log(k_e)$ and $log(CL)$;and the random effects are jointly normal and independent of the error term in $C(t)$.

Therefore, formally the model is described by the equation (for one subject)

$$C(t) = \frac{k_a k_e (oral\ dose) \times F}{CL(k_a - k_e)}\left(\exp(-k_e t) - \exp(-k_a t)\right) + \varepsilon(t)$$

where

(i) the error term(s) $\varepsilon(t)$ are serially independent (within subject), normally distributed, mean zero and variance σ_ε^2 .

(ii) $log(CL) = \beta_1 + b_1, log\left(k_a\right) = \beta_2 + b_2, log\left(k_e\right) = \beta_3,$ with β_1, β_2, β_3 as fixed parameters, b_1, b_2 as subject-specific random effects — means 0, covariance matrix Σ (3-parameters $\sigma_1^2, \sigma_2^2, \sigma_{12}$).

(iii) (b_1, b_2) independent of the error term.

Across subjects independence is assumed. Hence we can construct a joint likelihood for the sample data $\{C_i(t): 0 \le t \le \tau, 1 \le i \le n\}$ for the *n*=12 subjects with 11 concentrations assessed at the same grid of time points from (0, 25hr).

Maximum likelihood estimation (MLE) provides estimates of all model parameters and their covariance matrix. There are 7 parameters: β_1, β_2, β_3, σ_1^2, σ_2^2, σ_{12}, σ_ε^2.The formulas for AUC, $C_{\max}$ and $T_{\max}$ for the compartment model now give their estimates. Because (k_a, CL) is individual-specific, we will get individual- specific estimates for the PK parameters. The additional big advantage of the compartment model is the calculation of standard errors of these estimates.

Table 13 shows the $AUC_{0-\inf}$ calculation by NCA approach, and AUC estimates by compartment model. It shows the values estimated by these two approaches are very similar except subject 1 and subject 10.

Table 13 Comparison of AUC by NCA and compartment model based estimates

		NCA	Compartment model based estimates			
Obs	subject	AUC_{0-inf}	AUC	Stderr AUC	Lower 95% CI	Upper 95% CI
1	1	270.004	144.816	7.00102	129.217	160.416
2	2	95.050	110.005	5.87935	96.905	123.105
3	3	107.599	111.415	5.95405	98.148	124.681
4	4	121.926	118.535	6.25209	104.604	132.465
5	5	146.878	137.689	6.62215	122.934	152.444
6	6	87.877	87.023	5.54399	74.670	99.376
7	7	115.931	106.554	6.28655	92.547	120.561
8	8	104.732	102.774	5.87002	89.695	115.853
9	9	96.641	95.818	5.34297	83.914	107.723
10	10	207.536	154.629	7.40864	138.122	171.137
11	11	85.472	96.932	5.54048	84.587	109.277
12	12	126.815	141.073	6.77517	125.977	156.169

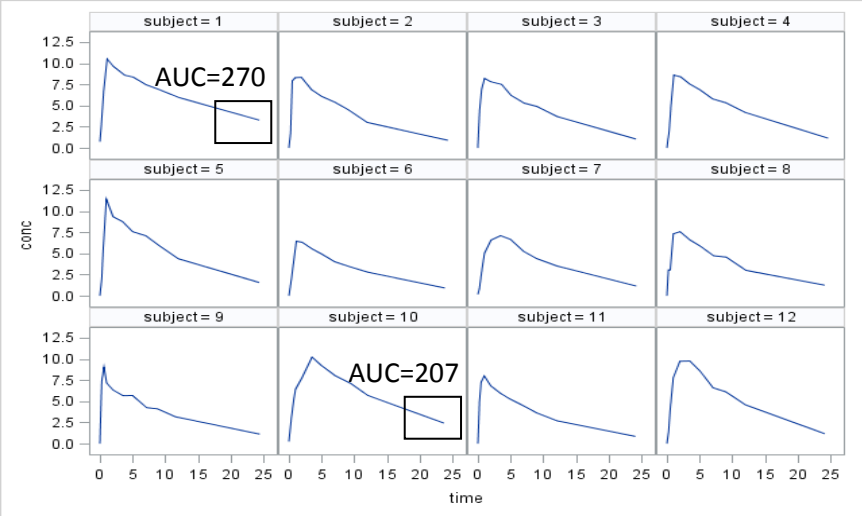

Figure 8 Individual concentration profiles by NCA.

Figure 8 shows individual concentration profiles by NCA, AUC of subject 1 and subject10by NCA are higher than AUC of most subjects. Higher C_{last} of these 2 subjects explains the higher AUC by NCA, and furthermore, explains the discrepancy of AUC by NCA and compartment model analysis.

Table 14 Comparison of C_{max} by NCA and compartment model based estimates

		NCA	Compartment model based estimates			
Obs	subject	C_{max}	C_{max}	Stderr C_{max}	Lower 95% CI	Upper 95% CI
1	1	10.50	10.3420	0.33180	9.60273	11.0813
2	2	8.33	8.2205	0.32169	7.50370	8.9372
3	3	8.20	8.3918	0.32066	7.67732	9.1063
4	4	8.60	8.2855	0.31677	7.57966	8.9913
5	5	11.40	9.8981	0.32666	9.17026	10.6259
6	6	6.44	6.1226	0.30897	5.43414	6.8110
7	7	7.09	6.9453	0.31625	6.24068	7.6500
8	8	7.56	7.3470	0.31939	6.63534	8.0586
9	9	9.03	7.7379	0.31140	7.04408	8.4318
10	10	10.21	9.7244	0.31813	9.01554	10.4332
11	11	8.00	7.5903	0.32118	6.87468	8.3060
12	12	9.75	9.5540	0.32690	8.82562	10.2824

Table 15 Comparison of T_{max} by NCA and compartment model based estimates

		NCA	Compartment model based estimates			
Obs	subject	T_{max}	T_{max}	Stderr T_{max}	Lower 95% CI	Upper 95% CI
1	1	1.12	2.10624	0.18124	1.70241	2.51008
2	2	1.92	1.57581	0.16126	1.21650	1.93511
3	3	1.02	1.48354	0.17806	1.08680	1.88028
4	4	1.07	2.35712	0.21209	1.88455	2.82969
5	5	1.00	2.02911	0.15872	1.67545	2.38277
6	6	1.15	2.28098	0.27775	1.66212	2.89984
7	7	3.48	3.17439	0.32442	2.45154	3.89723
8	8	2.02	2.09445	0.22878	1.58470	2.60420
9	9	0.63	0.66861	0.13729	0.36270	0.97452
10	10	3.55	3.59319	0.26621	3.00003	4.18634
11	11	0.98	1.02902	0.15033	0.69407	1.36397
12	12	3.52	2.72684	0.20907	2.26101	3.19268

Similarly, the C_{max} and T_{max} estimates by NCA approach and compartment model are shown in Table 14 and 15. It indicates the values estimated by these two approaches are very similar. Our analysis shows the benefits of parameter estimation and subsequent statistical inference with an appropriate compartmental model, even though the model fitting could be a little complicated.

This pharmacokinetic model is well identified; the parameters can be accurately estimated. If it failed AUC estimation is recommended by NCA.[22] Failure to fit a compartment model to a given data set could be due to many factors. As seen the statistical model is highly non-linear, introduction of too many random effects can be problematic if the data set cannot support a complex structure. A good approach would start with a 'fixed' parameters model to obtain initial parameter values for building a more complex model. A few attempts might be needed before a stable model can be obtained.

Extension beyond a one compartment model is possible. Two-compartment models view the body as a central compartment that receives the drug with transfer from the central compartment to a peripheral blood compartment that absorbs the drug. Transfer in the opposite direction from peripheral to central is also possible. Elimination occurs from the central compartment.

CHAPTER 4 DISCUSSION

The objective of this thesis is to review the standard approaches to statistical analyses of pharmacokinetic (PK) data. It covers estimation of Area Under the Curve (AUC), Peak Concentration ($C_{\max}$) and other PK parameters and how a bioequivalence (BE) study can be conducted with crossover design. Parameters such as AUC and $C_{\max}$ are the key parameters in a PK study and used for bioavailability and bioequivalence. They are identified as population parameters and estimated from observed drug concentration-time profiles.

The assessment of AUC adopted by the Food and Drugs Administration (FDA) is the Non-Compartmental Analysis (NCA) approach that estimates AUC using the trapezoidal rule without making any assumption concerning the number of compartments. Following the trapezoidal rule, concentration-time curve is considered as a series of trapezoids and the AUC estimate is the total area of all the trapezoids. The other approach is based on compartmental models.

The one compartment model is used to estimate the PK parameters, such as Absorption Rate Constant (k_a), Elimination Rate Constant (k_e) and Clearance (*CL*). They can be estimated from observed concentrations $\{C(t): t = 0,1,\ldots,m\}$ in individuals over a grid of time points, and then estimates of AUC, $C_{\max}$ and $T_{\max}$ are derived from formulas. They show the benefits of parameter estimation and subsequent statistical inference with an appropriate compartmental model, even though the model fitting could be a little complicated. Among the benefits of the one compartment model analysis is that the PK parameters are estimated together with their

standard errors and 95% confidence intervals. In addition individual (subject-specific) prediction of drug concentration can be made. For Pharmacokinetic/Pharmacodynamic modeling, the compartmental pharmacokinetic models are widely used, providing continuous description of the drug concentration that can serve as the input of pharmacodynamic models.[23]

Fitting of compartmental models can be a complex and lengthy process. As seen the statistical model is highly non-linear, introduction of too many random effects can be problematic if the data set cannot support a complex structure. A good approach would start with a 'fixed' parameters model to obtain initial parameter values for building a more complex model. A few attempts might be needed before a stable model can be obtained. If it failed AUC estimation is recommended by NCA.[22]

The widely cited example of drug "theophylline" data[19][20][21] is used to illustrate these two approaches. Comparison of the PK parameters by NCA and one compartmental model shows the parameters estimated from these two methods are very close, the model is identified. Only when the underlying pharmacokinetic model parameters are identified, can AUC be accurately estimated, otherwise AUC estimation is recommended by the NCA.

BE studies are widely carried out in the pharmaceutical industry. For small molecule drug products, a bioavailability and bioequivalence study are required by FDA for approval of generic drug products, which contain the exact same active ingredient as the innovator drug. Biosimilars are large molecule biological drug products made via living systems. As generic forms of

biological products instead of the classical generic drugs, biosimilars are only similar to the reference product; with no exactly the same active ingredient as the innovator drug. The more stringent assessment include safety, purity, and potency, to show that a follow-on biologic is not clinically different from the reference biological product.[24]

Average bioequivalence (ABE) is based solely on the comparison of population averages but not on the variances, while population bioequivalence (PBE) and individual bioequivalence (IBE) approaches include comparisons of both averages and variances. For statistical analyses in a bioequivalence study, we used the "pkdata" example with AB|BA design to illustrate how the crossover design is applied and ABE is tested. SAS procedures PROC TTEST and PROC GLIMMIX are applied to the logarithm-transformed AUC and $C_{\max}$ to estimate ABE. Available from a public resource even though there are quality issues in this data, the "pkdata" example is the reasonable example of data with blood concentration time profiles, from which we can estimate AUC and $C_{\max}$, the two key parameters to compare in the BE study.

The deficit of the data for BE study includes: (1) For a AB/BA design, since there are only 4 combinations of periods and treatments, the period effect in this particular parameterization is aliased with the carryover effect.[25] Our results show that there is period effect when comparing $\log(C_{\max})$ for A and B. There is no information available if there is carryover effect, therefore, it is not clear if the period effect is a real period effect. The purpose of this thesis is to illustrate how a bioequivalence (BE) study can be conducted with crossover design, so we claim there is no carryover effects. (2) For the AB/BA design, it is not well-suited for comparison of the

within-unit variance σ_A^2 and σ_B^2 [25] in the statistical model, we have only have one common variance σ_ε^2 for treatment A and treatment B. Therefore, total variance of A and B cannot be calculated. The "pkdata" example cannot be used for Population BE and individual BE study. We will need a replicated crossover design.[3]

In recent years statisticians in the pharmaceutical industry have given attention to developing strategies for statistical analyses for *Biosimilars* and *Biobetters*. The FDA recently (April 2015) issued guidance on the scientific issues to be considered in demonstrating biosimilarity to a reference product.[26] The FDA's definition states: *Biosimilar* or *biosimilarity* means that "the biological product is highly similar to the reference product notwithstanding minor differences in clinically inactive components," and that "there are no clinically meaningful differences between the biological product and the reference product in terms of the safety, purity, and potency of the product."

Therefore, the role that crossover designs have in *bioequivalence* demonstration on a single endpoint or outcome measures must now be expanded in ways to assess multiple endpoints and measures. It will bring biostatisticians and methodologists in pharmacology together to craft the statistical designs and studies for clinical evaluations that can answer these questions.

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