

Statistical Design and Analysis I

Helmut Schütz

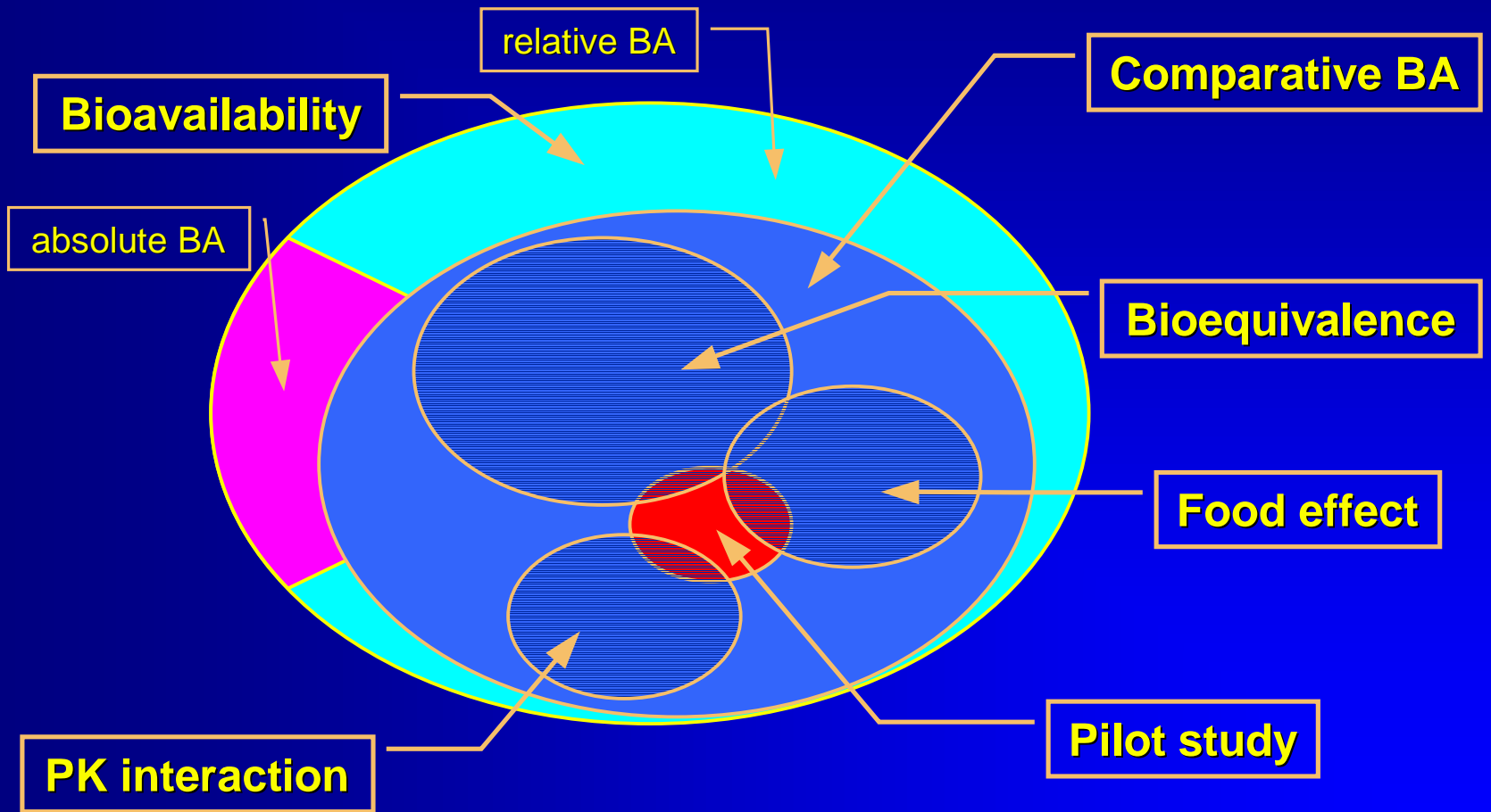
BEBAC

Consultancy Services for
Bioequivalence and Bioavailability Studies

1070 Vienna, Austria

helmut.schuetz@bebac.at

Terminology



Defining Study Objectives

- According to the EU NfG (3. Design and Conduct of Studies, paragraph 2):

‘A bioequivalence study is basically a comparative bioavailability study designed to establish equivalence between test and reference products.’

- Comparative BA,
- designed to demonstrate BE,
- reference = innovator’s product.

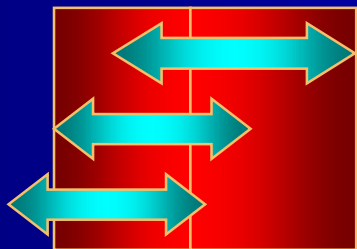
EMA Human Medicines Evaluation Unit / CPMP

Note for Guidance on the Investigation of Bioavailability and Bioequivalence (2001)

<http://www.emea.eu.int/pdfs/human/ewp/140198en.pdf#page=6>

Defining Study Objectives

- Comparative BA
 - true experiment; no bibliographic comp.
- Designed to demonstrate BE
 - variability,
 - deviation of test from reference,
 - drop-out rate,...
 - to be able (statistical power!) to demonstrate BE
- Reference = Innovator's product



#1: BE [90%–125%]

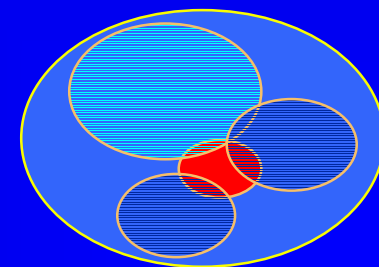
#2: BE [80%–110%]

#3: not BE [76%–103%]; (but 'BE' to #2)

Defining Study Objectives

- Definition of BE (EU NfG, Section 2.4)

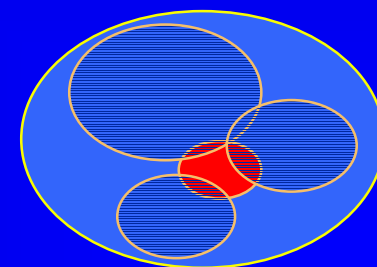
‘Two medicinal products are bioequivalent if they are pharmaceutically equivalent or pharmaceutical alternatives and if their bioavailabilities after administration in the same molar dose are similar to such degree that their effects, with respect to both efficacy and safety, will be essentially the same.’



Defining Study Objectives

- *In vivo* BE mandatory, if
 - Waiving (NfG Section 5.1.1) not possible
 - in MA of Generics
 - Manufacturing changes (EU Major variation type II(d)-(f) ~ FDA SUPAC Level 3)
 - Pharmacokinetic interaction studies,
 - Studies of fixed-combination products.

'[...] are similar to such degree that their effects, with respect to both efficacy and safety, will be essentially the same.'



Defining Study Objectives

- Statistical concept of BE also applicable to
 - Food effect studies,
 - Pharmacokinetic interaction studies,
 - Studies of fixed-combination products.

'[...] are similar to such degree that their effects, with respect to both efficacy and safety, will be essentially the same.'

EMA Human Medicines Evaluation Unit / CPMP

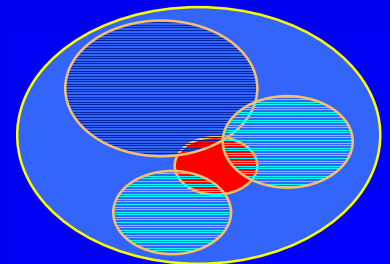
Modified Release Oral and Transdermal Dosage Forms: Section II (Quality)
CPMP/EWP/280/96 (1999)

EMA Human Medicines Evaluation Unit / CPMP

The Investigation of Drug Interactions
CPMP/EWP/560/95 (1997)

EMA

Fixed Combination Medicinal Products
CPMP/EWP/240/95 Rev. 1 (2008)



Defining Study Objectives

- Since *in vivo* BE relies on 'rich' PK data:
 - Sufficient number of blood samples (C_{\max} !) / urine collection periods
 - Sampling long enough to cover $\geq 80\%$ of AUC $_{\infty}$
 - Wash-out $\geq 3 \times t_{1/2}$ (recommended $\geq 5 \times t_{1/2}$)
 - Saturation phase long enough to reach steady-state: $\geq 5 \times t_{1/2}$ (recomm. $\geq 7 \times t_{1/2}$)
 - Pre-dose samples (carry-over, compliance)

EU Draft NfG (2008): for IR formulations no more sampling beyond 72 hours!

Defining Study Objectives

- PK metrics
 - Extent of bioavailability / Total exposure
 - single dose
 - AUC_t , AUC_∞ (plasma)
 - Ae_t , Ae_∞ (urine)
 - steady state
 - AUC_τ , AUC_{24h} , (plasma)
 - Ae_τ , Ae_{24h} , (urine)

Defining Study Objectives

● PK metrics

■ Rate of bioavailability / Peak exposure / Early exposure

■ single dose

➤ C_{\max} , (t_{\max} , partial AUC) (plasma)

➤ ΔAe_{\max} (urine)

■ steady state

➤ as above

➤ Fluctuation [$PTF = (C_{\max} - C_{\min}) / C_{av}$]

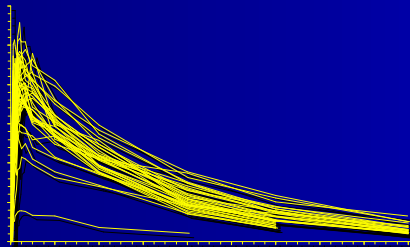
■ MR formulations

➤ MRT, HVD, $t_{75\%}$

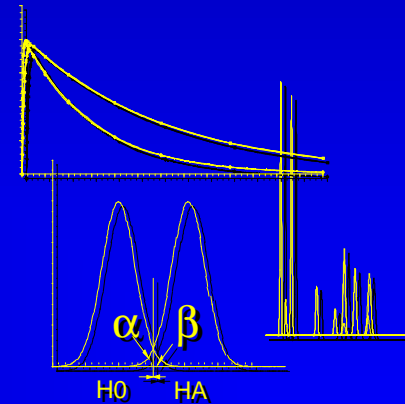
Assumptions: General



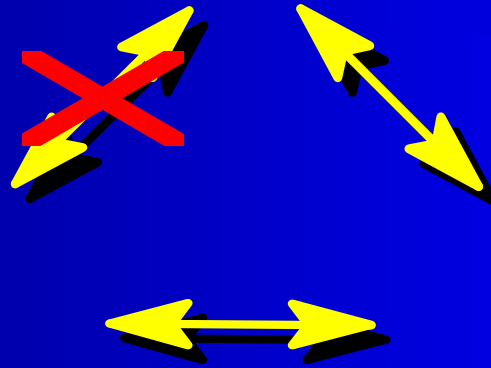
World *'Reality'*



Model *'Data'*



Theory *'Truth'*



Assumptions: Pharmacokinetics

$$\frac{F_1 \cdot AUC_1}{\cancel{D_1 \cdot CL_1}}, \frac{F_2 \cdot AUC_2}{\cancel{D_2 \cdot CL_2}}$$

$$F_{rel}(BA) = \frac{AUC_1}{AUC_2}$$

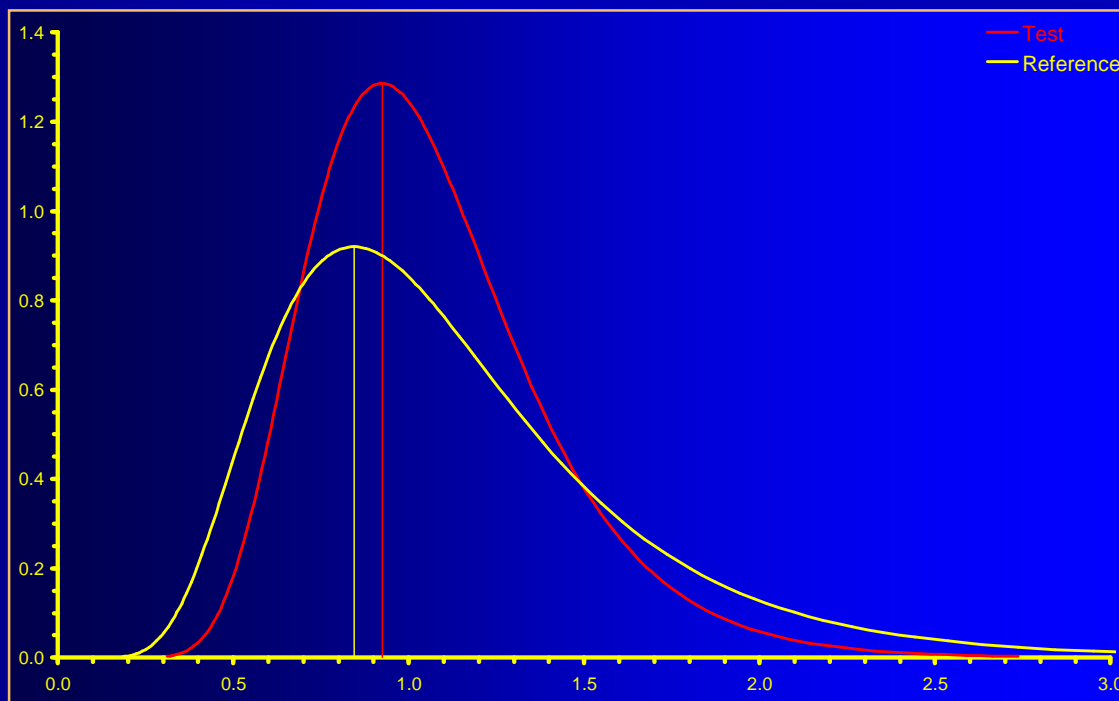
Assumption 1: $D_1 = D_2$ ($D_1/D_2 = 1^*$)

Assumption 2: $CL_1 = CL_2$

Assumptions: Statistics

Distribution

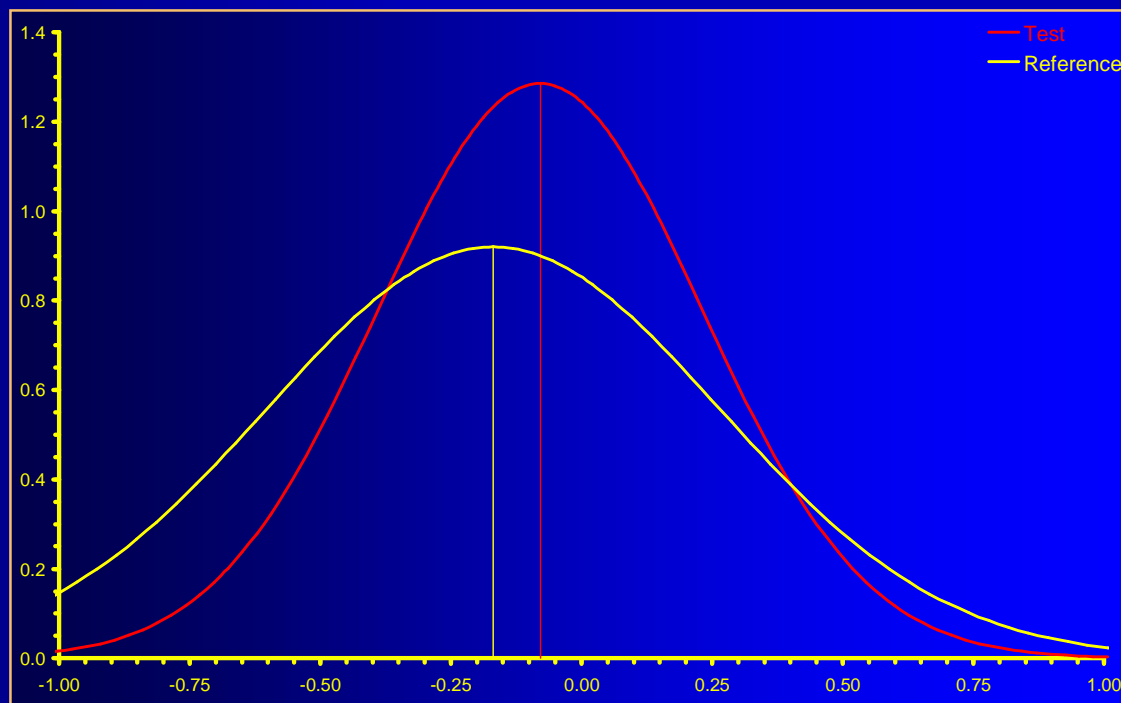
- IDD (Independent Identically Distribution)



Assumptions: Statistics

Multiplicative Model

- Log-Transformation (PK, Analytics)



Assumptions: Statistics

Multiplicative Model (X-over without carryover)

$$X_{ijk} = \mu \cdot \pi_k \cdot \Phi_l \cdot s_{ik} \cdot e_{ijk}$$

X_{ijk} : *ln*-transformed response of j -th subject ($j=1, \dots, n_i$) in i -th sequence ($i=1, 2$) and k -th period ($k=1, 2$), μ : global mean, μ_l : expected formulation means ($l=1, 2$: $\mu_1 = \mu_{test}$, $\mu_2 = \mu_{ref}$), π_k : fixed period effects, Φ_l : fixed formulation effects ($l=1, 2$: $\Phi_1 = \Phi_{test}$, $\Phi_2 = \Phi_{ref}$)

Assumptions: Statistics

Multiplicative Model (X-over without carryover)

$$X_{ijk} = \mu \cdot \pi_k \cdot \Phi_l \cdot s_{ik} \cdot e_{ijk}$$

s_{ik} : random subject effect, e_{ijk} : random error

Main Assumptions:

- All $\ln\{s_{ik}\}$ and $\ln\{e_{ijk}\}$ are independently and normally distributed about unity with variances σ_s^2 and σ_e^2 .
- All observations made on different subjects are independent.

Global Harmonization?

Transformations (e.g. [...], logarithm) should be specified in the protocol and a rationale provided [...]. The general principles guiding the use of transformations to ensure that the assumptions underlying the statistical methods are met are to be found in standard texts [...]. **In the choice of statistical methods due attention should be paid to the statistical distribution [...].** **When making this choice (for example between parametric and non-parametric methods)** it is important to bear in mind the need to provide statistical estimates of the size of treatment effects together with confidence intervals [...].

ICH Topic E 9

Statistical Principles for Clinical Trials (1998)

Global Harmonization?

No analysis is complete until the assumptions that have been made in the modeling have been checked. Among the assumptions are that the repeated measurements on each subject are independent, normally distributed random variables with equal variances. Perhaps the most important advantage of formally fitting a linear model is that diagnostic information on the validity of the assumed model can be obtained. These assumptions can be most easily checked by analyzing the residuals.

Jones B and MG Kenward

Design and Analysis of Cross-Over Trials

Chapman & Hall, Boca Raton (2nd ed 2003)

Nonparametrics

The limited sample size in a typical BE study precludes a reliable determination of the distribution of the data set. Sponsors and/or applicants **are not encouraged to test for normality of error distribution** after log-transformation [...].

FDA, Center for Drug Evaluation and Research (CDER)

Guidance for Industry: Statistical Approaches to Establishing Bioequivalence (2001)

But: acceptable in

Turkey (MOH, November 2005)

Saudia Arabia (SFDA, May 2005)

Nonparametrics

5. In which cases may a non-parametric statistical model be used?

The NfG states under 3.6.1–Statistical analysis: “*AUC and C_{max} should be analysed using ANOVA after log transformation.*”

The reasons for this request are the following:

- a) the AUC and C_{max} values as biological parameters are usually not normally distributed;
- b) a multiplicative model may be plausible;
- c) after log transformation the distribution may allow a parametric analysis.

Comments:

a) – true b) – true c) – maybe, but may also terribly fail

EMA/CHMP/EWP/40326/2006

Questions & Answers on the BA and BE Guideline (2006)

Nonparametrics

5. In which cases may a non-parametric statistical model be used?

However, the true distribution in a pharmacokinetic data set usually cannot be characterised due to the small sample size, so it is not recommended to have the analysis strategy depend on a pre-test for normality. Parametric testing using ANOVA on log-transformed data should be the rule. Results from non-parametric statistical methods or other statistical approaches are nevertheless welcome as sensitivity analyses. Such analyses can provide reassurance that conclusions from the experiment are robust against violations of the assumptions underlying the analysis strategy.

Comment: It is well known that the efficiency of e.g., the Wilcoxon-Mann-Whitney test for normal distributed data is $3/\pi \approx 95.5\%$; for *not normal distributed data* the efficiency is $>100\%$!

Nonparametrics

4.1.8 Evaluation / Statistical analysis

The pharmacokinetic parameters under consideration should be analysed using ANOVA (or equivalent parametric method). The data should be transformed prior to analysis using a logarithmic transformation. A confidence interval for the difference between formulations on the log-transformed scale is obtained from the ANOVA model. This confidence interval is then back-transformed to obtain the desired confidence interval for the ratio on the original scale. A non-parametric analysis is not acceptable.

EMA/CPMP/EWP/QWP/1401/98 Rev. 1

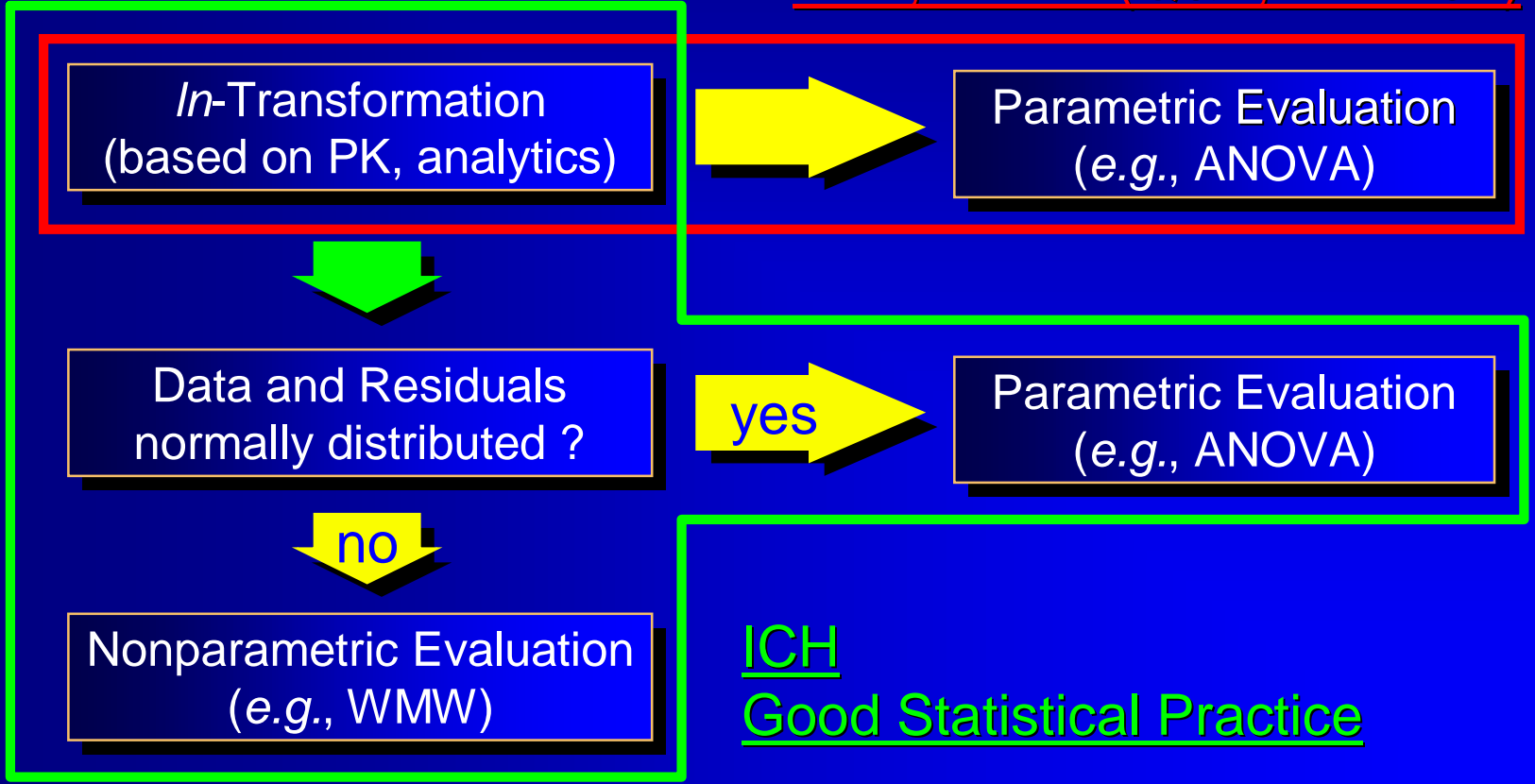
Draft Guideline on the Investigation of Bioequivalence (2008)

'Also interesting that they now say they will not accept non-parametric analyses. That seems a step backwards.'

(Walter Hauck, personal communication, Oct 2008)

Global Harmonization?

FDA, EMEA (Q&A, BE Draft)



ICH
Good Statistical Practice

Global Harmonization?

- In almost all regulations two metrics are necessary to demonstrate BE, namely
 - extent (e.g., AUC_t , AUC_∞ , A_e), and
 - rate (e.g., C_{max} , PTF) of exposure.
- One exception: US-FDA (where AUC_∞ and AUC_t must demonstrate extent of BE)
 - Although stated in the GL, such a requirement is statistically flawed.
 - Multiplicity issues (what is the patient's risk?)
 - Impossible α -adjustment (interdependence)



There can be only one!

Basic Designs

- Single Dose / Multiple Dose
 - Cross-over
 - Standard 2×2
 - Higher Order Designs (for more than two treatments)
 - Latin Squares
 - Variance Balanced Designs (Williams' Designs)
 - Incomplete Block Designs
 - Replicate designs
 - Parallel Groups

Single Dose / Multiple Dose

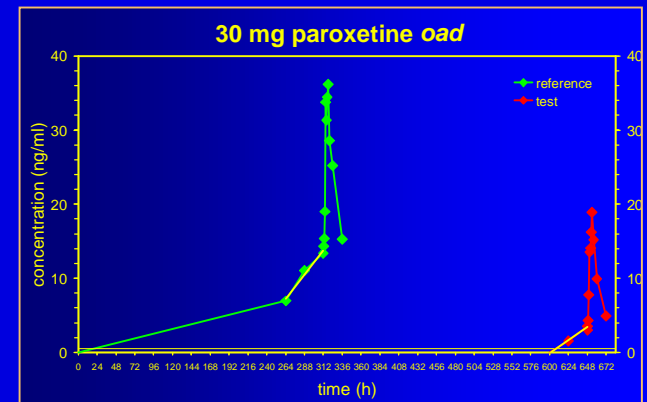
- Single Dose recommended in most GLs, but steady-state studies
 - may be required:
 - in the case of dose- or time-dependent pharmacokinetics
 - for some modified release products (additionally to single dose BE)
 - may be considered:
 - if problems of sensitivity preclude sufficiently precise plasma concentration measurements after SD administration. With current developments in bioanalytical methodology, you should have strong evidence of infeasibility if you claim the necessity of a MD study based on lacking methods.
Regulators are concerned with efficacy/safety issues – not with the budget of pharmaceutical companies!

Single Dose / Multiple Dose

- Steady-state studies

- **No Wash-out between Periods** (Switch-Over)!
- In order to fulfil the superposition principle of linear pharmacokinetics ($AUC_{\tau} = AUC_{\infty}$), you must demonstrate achievement of steady-state
 - Linear regression of pre-dose values in saturation phase

- slope (from at least the last three values) should not significantly ($p > 0.05$, two-sided) differ from zero,
- subjects not in steady-state at begin of the profile(s) should be excluded from the evaluation – **if stated in protocol!**



Single Dose / Multiple Dose

- Steady-state studies

- Demonstration of steady-state (cont'd)

- Multivariate method (simultaneous testing of all pre-dose values in all subjects)

- E.g., Hotellings T^2

- Benefit: additional statement possible when steady-state was reached

- Drawback: if significant result, no possibility to exclude particular subjects (rendering the entire study worthless).

- *t*-test of last two pre-dose values

- Pro: most easy to perform, relatively insensitive to outliers

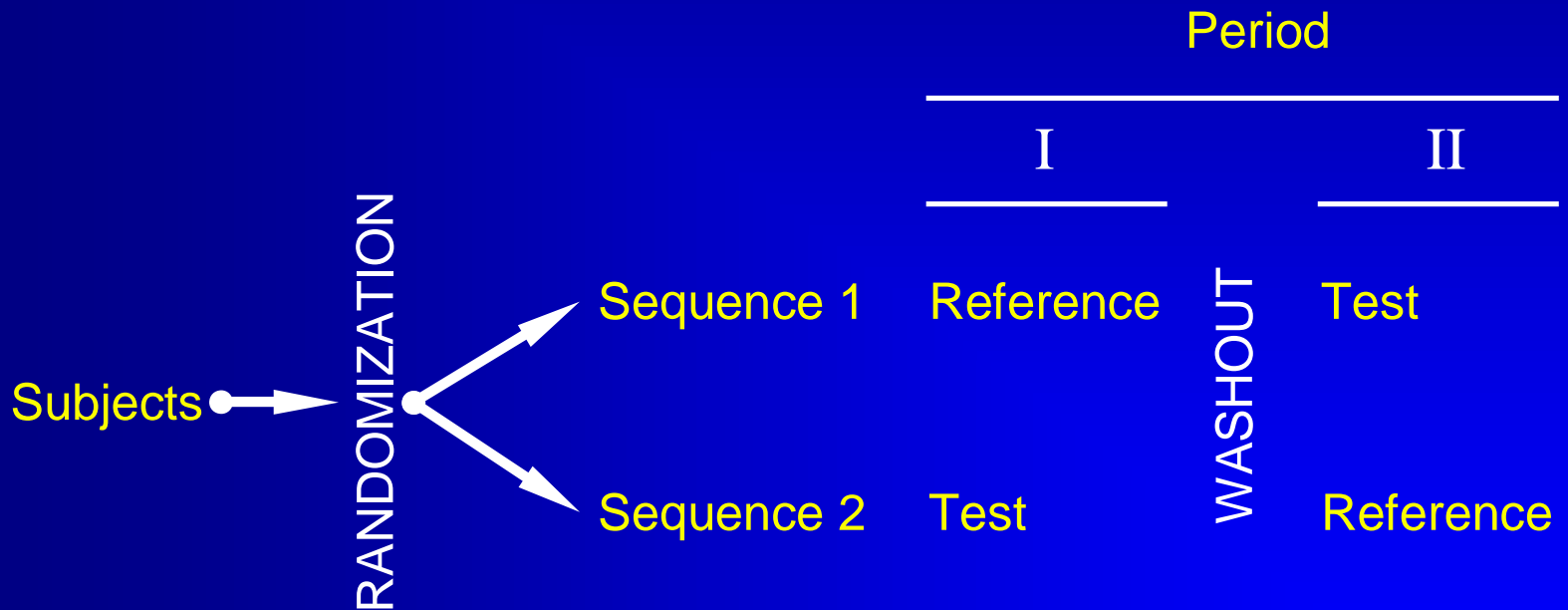
- Con: as above

Cross-over designs

- Standard $2 \times 2 \times 2$ (two-treatment two-sequence two-period) design
 - Each subject is randomly assigned to either sequence RT or sequence TR at two treatment periods
 - 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 circulation.
 - Smaller subject numbers compared to a parallel design, since the *within-subject* variability determines sample size (rather than *between-subject* variability).

Cross-over designs

- Standard 2x2x2 design



Assumptions: Cross-over

Multiplicative Model (X-over without carryover)

$$X_{ijk} = \mu \cdot \pi_k \cdot \Phi_l \cdot s_{ik} \cdot e_{ijk}$$

- All $\ln\{s_{ik}\}$ and $\ln\{e_{ijk}\}$ are independently and normally distributed about unity with variances σ_s^2 and σ_e^2 .
 - This assumption may not hold true for all formulations; if the reference formulation shows higher variability than the test formulation, a 'good' test will be penalized for the 'bad' reference.
- All observations made on different subjects are independent.
 - This assumption should not be a problem, unless you plan to include twins or triplets in your study...

Cross-over designs

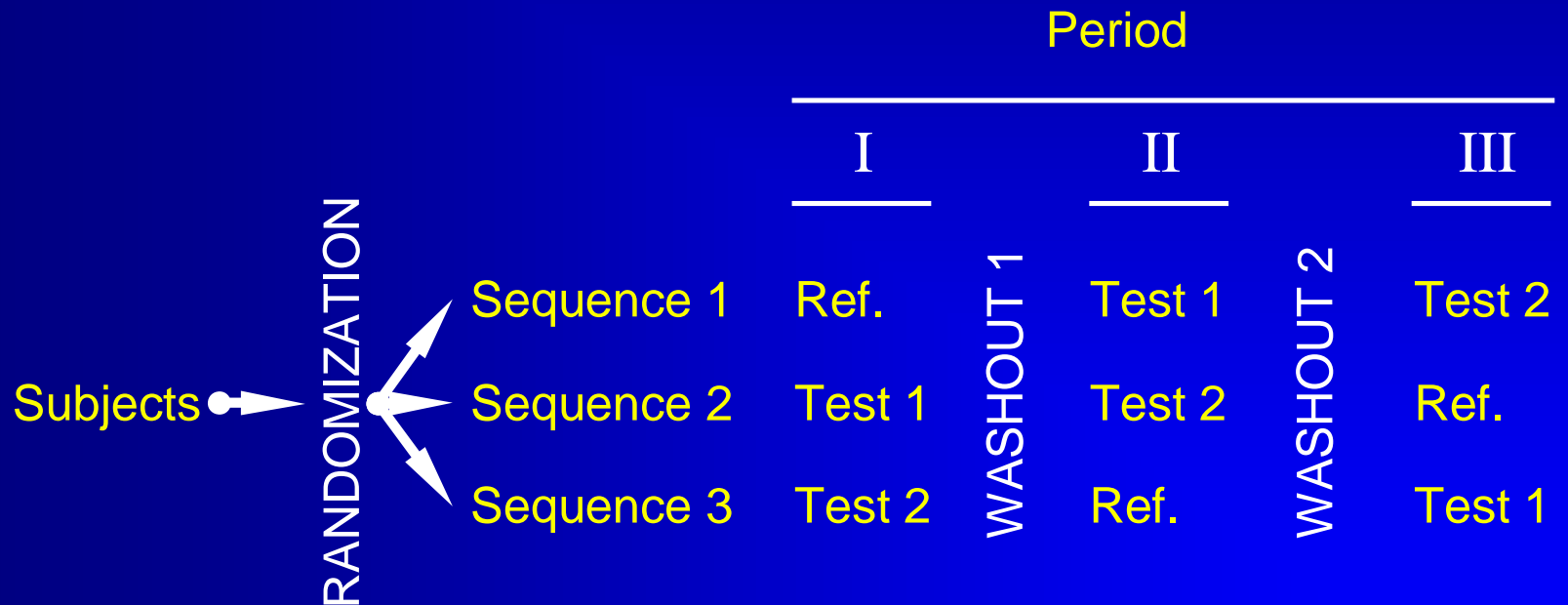
- Standard 2x2x2 design
 - Advantages
 - Globally applied standard protocol for BE
 - Straightforward statistical analysis
 - Disadvantages
 - Not suitable for drugs with long half life (→ parallel groups)
 - Not optimal for studies in patients with instable diseases (→ parallel groups)
 - Not optimal for HVDs (→ Replicate Designs)

Cross-over designs

- Higher Order Designs (for more than two treatments)
 - Latin Squares
Each subject is randomly assigned to sequences, where number of treatments = number of sequences = number of periods.

Cross-over designs

- 3x3x3 Latin Square design



Cross-over designs

● 3×3×3 Latin Square design

■ Advantages

- Allows to choose between two candidate test formulations or comparison of a test formulation with two references
- Easy to adapt
- Number of subjects in the study is a multiplicative of three
- Design for establishment of Dose Proportionality

■ Disadvantages

- Statistical analysis more complicated (especially in the case of drop-outs and a small sample size) – not available in some pieces of software
- Extracted pairwise comparisons are imbalanced
- May need measures against multiplicity (increasing the sample size)
- Not mentioned in any guideline

Cross-over designs

- Higher Order Designs (for more than two treatments)
 - Variance Balanced Designs (Williams' Designs)
 - For e.g., three formulations there are three possible pairwise differences among formulation means (*i.e.*, form. 1 vs. form. 2., form 2 vs. form. 3, and form. 1 vs. form. 3)
 - It is desirable to estimate these pairwise effects with the same degree of precision (there is a common variance for each pair)
 - Each formulation occurs only once with each subject
 - Each formulation occurs the same number of times in each period
 - The number of subjects who receive formulation i in some period followed by formulation j in the next period is the same for all $i \neq j$
 - Such a design for three formulations is the three-treatment six-sequence three-period Williams' Design

Cross-over designs

- Williams' Design for three treatments

Sequence	Period		
	I	II	III
1	R	T ₂	T ₁
2	T ₁	R	T ₂
3	T ₂	T ₁	R
4	T ₁	T ₂	R
5	T ₂	R	T ₁
6	R	T ₁	T ₂

Cross-over designs

- Williams' Design for four treatments

Sequence	Period			
	I	II	III	IV
1	R	T ₃	T ₁	T ₂
2	T ₁	R	T ₂	T ₃
3	T ₂	T ₁	T ₃	R
4	T ₃	T ₂	R	T ₁

Cross-over designs

● Williams' Designs

■ Advantages

- Allows to choose between two candidate test formulations or comparison of a test formulation with two references
- Design for establishment of Dose Proportionality
- Paired comparisons (e.g., for a nonparametric method) can be extracted, which are also balanced
- Mentioned in Brazil's (ANVISA) guideline

■ Disadvantages

- More sequences for an *odd* number of treatment needed than in a Latin Squares design (but equal for even number)
- Statistical analysis more complicated (especially in the case of drop-outs) – not available in some softwares
- May need measures against multiplicity (increasing the sample size)

Cross-over designs

- Extraction of 2x2 comparisons (T₁/R, T₂/R)

- Latin Squares

Seq.	P ₁	P ₂	P ₃
1	T ₁	T ₂	R
2	T ₂	R	T ₁
3	R	T ₁	T ₂

Seq.	P ₁ '	P ₂ '
1	T ₁	R
2	R	T ₁
3	R	T ₁

Seq.	P ₁ ''	P ₂ ''
1	T ₂	R
2	T ₂	R
3	R	T ₂

imbalanced

- Williams' design

Seq.	P ₁	P ₂	P ₃
1	T ₁	T ₂	R
2	T ₂	R	T ₁
3	R	T ₁	T ₂
4	T ₁	R	T ₂
5	T ₂	T ₁	R
6	R	T ₂	T ₁

Seq.	P ₁ '	P ₂ '
1	T ₁	R
2	R	T ₁
3	R	T ₁
4	T ₁	R
5	T ₁	R
6	R	T ₁

Seq.	P ₁ ''	P ₂ ''
1	T ₂	R
2	T ₂	R
3	R	T ₂
4	R	T ₂
5	T ₂	R
6	R	T ₂

balanced

Cross-over designs

- Higher Order Designs (cont'd)
 - Bonferroni-correction needed (sample size!)
 - *If more than one formulation will be marketed (for three simultaneous comparisons without correction patients' risk increases from 5 % to 14 %).*
 - *Sometimes requested by regulators in dose proportionality.*

k	$P_{\alpha=0.05}$	$P_{\alpha=0.10}$	$\alpha_{adj.}$	$P_{\alpha_{adj.}}$	$\alpha_{adj.}$	$P_{\alpha_{adj.}}$
1	5.00%	10.00%	0.0500	5.00%	0.100	10.00%
2	9.75%	19.00%	0.0250	4.94%	0.050	9.75%
3	14.26%	27.10%	0.0167	4.92%	0.033	6.67%
4	18.55%	34.39%	0.0125	4.91%	0.025	9.63%
5	22.62%	40.95%	0.0100	4.90%	0.020	9.61%
6	26.49%	46.86%	0.0083	4.90%	0.017	9.59%

Cross-over designs

- Replicate designs
 - Each subject is randomly assigned to sequences, where at least one of the treatments is administered at least twice.
 - Not only the global within-subject variability, but also the within-subject variability per treatment may be estimated.
 - Smaller subject numbers compared to a standard $2 \times 2 \times 2$ design – but outweighed by an increased number of periods.
 - Same overall number of individual treatments!
 - Mandatory in the EU if an extended acceptance range for C_{\max} (0.75–1.33) is aimed at (HVDP must be demonstrated in advance)

Cross-over designs

- Replicate designs

- Advantages

- Some experience from FDA's initiative on population BE (PBE) and individual BE (IBE)
 - Reference scaling average bioequivalence (RSABE)
 - Handling of outliers (subject-by-formulation interaction may be ruled out)

- Disadvantages

- Statistical analysis complicated (especially in the case of drop-outs and if RSABE is the target) – not available in standard software
 - Many publications, but still no agreement on methodology
 - Mentioned only in South African GL; will be adopted by FDA

Cross-over designs

- Replicate designs

- Examples

- Two-sequence three-period

T R T

R T R

Sample size to obtain the same power as a 2x2x2 study: 75%

- Two-sequence four-period

T R T R

R T R T

Sample size to obtain the same power as a 2x2x2 study: 50%

- and many others... (FDA for RSABE: TRR–RTR–RRT)
 - The statistical model is a little bit complicated – and dependent on the actual design

$$X_{ijkl} = \mu \cdot \pi_k \cdot \Phi_l \cdot s_{ij} \cdot e_{ijkl}$$

HVDs/HVDPs

- Highly Variable Drugs / Drug Products (intra-subject variability >30 %)

- ✓ USA Replicate Design recommended.

- Reference Scaled Average Bioequivalence under discussion: minimum number of subjects (24 or 36), restriction on GMR (0.8–1.25)

- ± EU [...] under certain circumstances [...] alternative well-established designs could be considered such as [...] replicate designs for substances with highly variable disposition.

- Widening of acceptance range in a pivotal BE study (for C_{\max} only) after demonstration of reference HVDP (pilot replicate design).

- RSABE according to the Draft GL not acceptable!

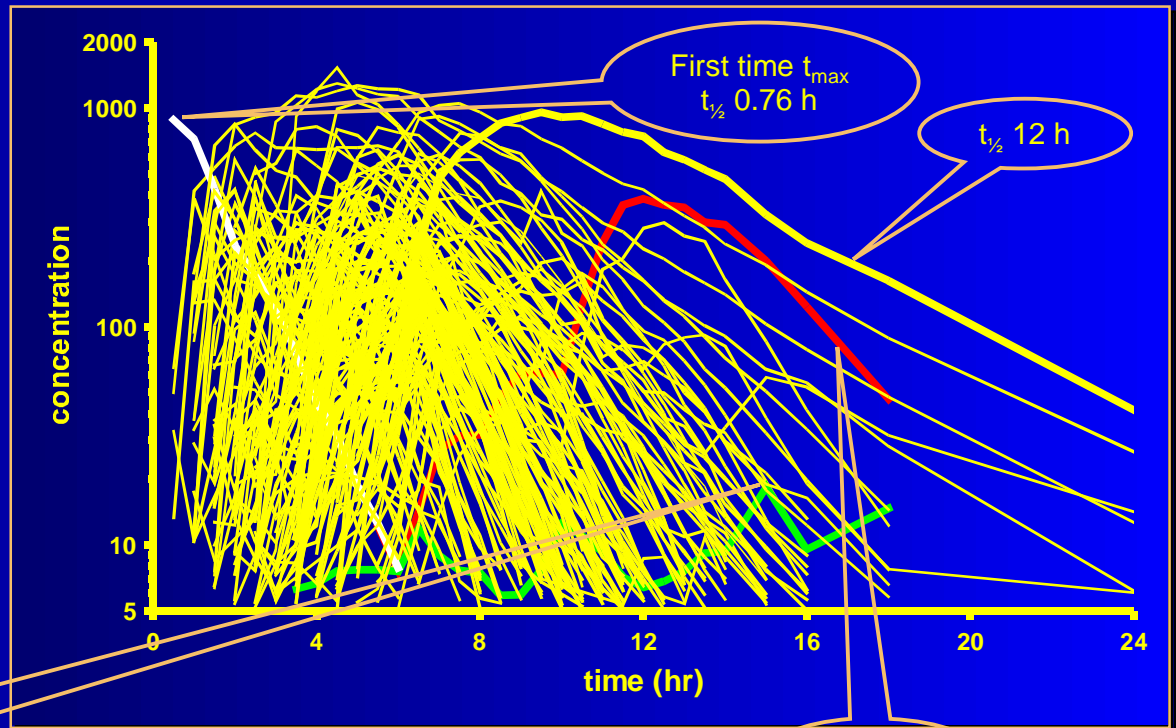
HVDs/HVDPs

- Does knowledge of the PK profile always help in demonstrating bioequivalence when a conventional BE study is unsuitable?
 - Omeprazole: Highly Variable Drug Product (HVDP), higher variability in fed state as compared to fasted state commonly observed, sensitive to low pH, breakdown of gastric resistant coating (especially of the reference product) not unusual, high variability in C_{\max}/t_{\max} due to variability in gastric emptying, ...

HVDs/HVDPs

- Attempt to deal with high variability in C_{max}

Powered to 90% according to CV from previous studies; 140 (!) subjects and to 80% for expected dropout rate. Sampling every 30 min up to 14 hours (7785 total).



t_{max} 15 h
 C_{max} 3.5xLLOQ

$t_{1/2}$ 3.15 h

HVDs/HVDPs

● Ways out?

- Replicate designs could be considered e.g. for substances with highly variable pharmacokinetic characteristics.
(EU BE Draft, Section 4.1.2)

- Nonparametric methods

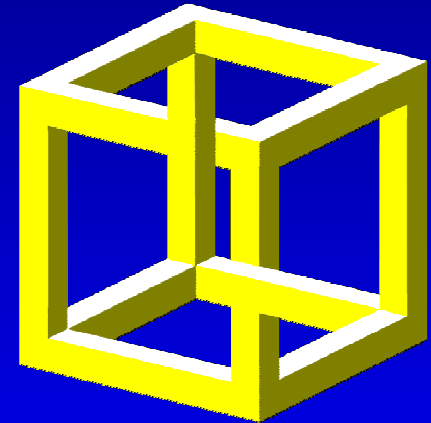
A non-parametric analysis is not acceptable.

(BE Draft, Section 4.1.8)

- Compartmental (Population PK) methods

The use of compartmental methods for the estimation of parameters is not acceptable.

(BE Draft, Section 4.1.5)



HVDPs

- All (!) ANDAs submitted to FDA/OGD 2003–2005 (1010 studies, 180 drugs)
 - 31% (57/180) highly variable ($CV \geq 30\%$)
 - of these HVDs/HVDPs,
 - 60% due to PK (e.g., first pass metabol.)
 - 20% formulation performance
 - 20% unclear

Davit BM, Conner DP, Fabian-Fritsch B, Haidar SH, Jiang X, Patel DT, Seo PR, Suh K, Thompson CL, and LX Yu

Highly variable drugs: observations from bioequivalence data submitted to the FDA for new generic drug applications

AAPS J 10(1): 148-56 (2008)

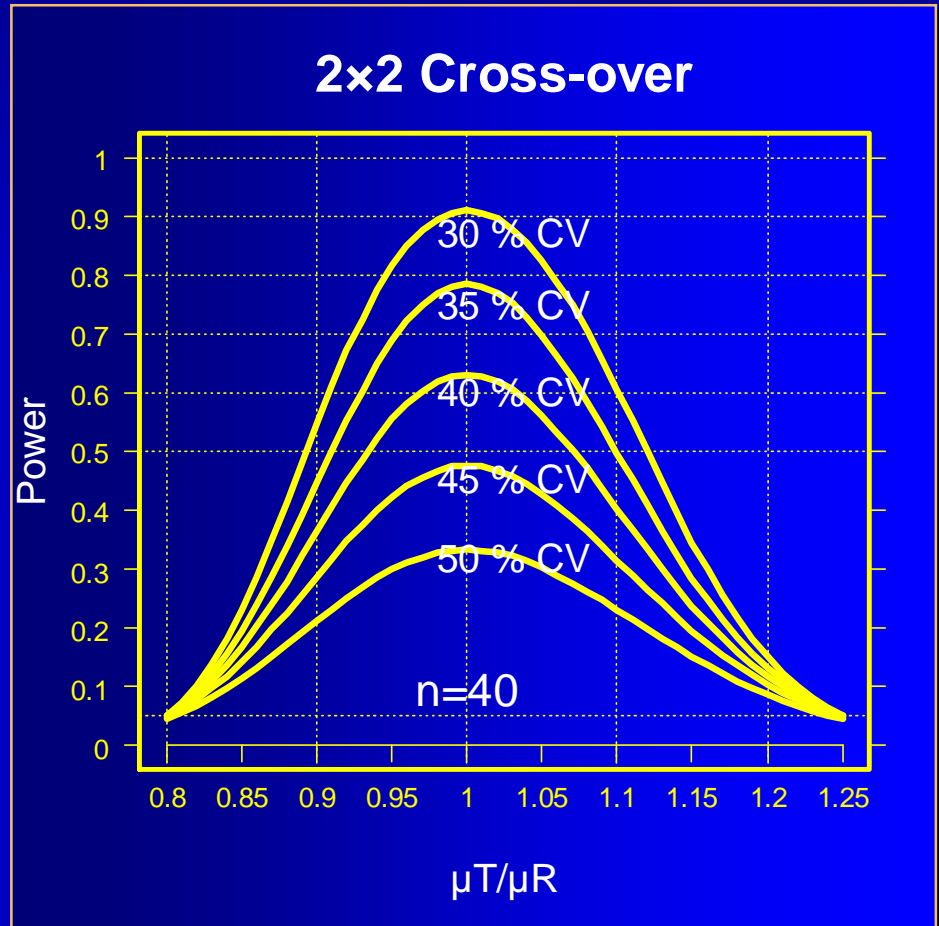
HVDPs

Power to show BE
with 40 subjects for
 $CV_{intra} = 30-50\%$

$\mu T/\mu R$ 0.95, CV_{intra} 30%
→ power 0.816

$\mu T/\mu R$ 1.00, CV_{intra} 45%
→ power 0.476 <
Roulette 0.486 (!)

$\mu T/\mu R$ 0.95, CV_{intra} 45%
→ n=82 (power 0.807)



HVDPs (US/EU)

- Advisory Committee for Pharmaceutical Sciences (ACPS) to FDA (10/2006) on HVDs
- Follow-up paper in 2008 (likely to be implemented in next Guideline)
 - Replicate study design [TRR–RTR–RRT]
 - Reference Scaled Average Bioequivalence (RSABE)
 - Minimum sample size 24 subjects
 - Point estimate restricted to [0.80, 1.25]

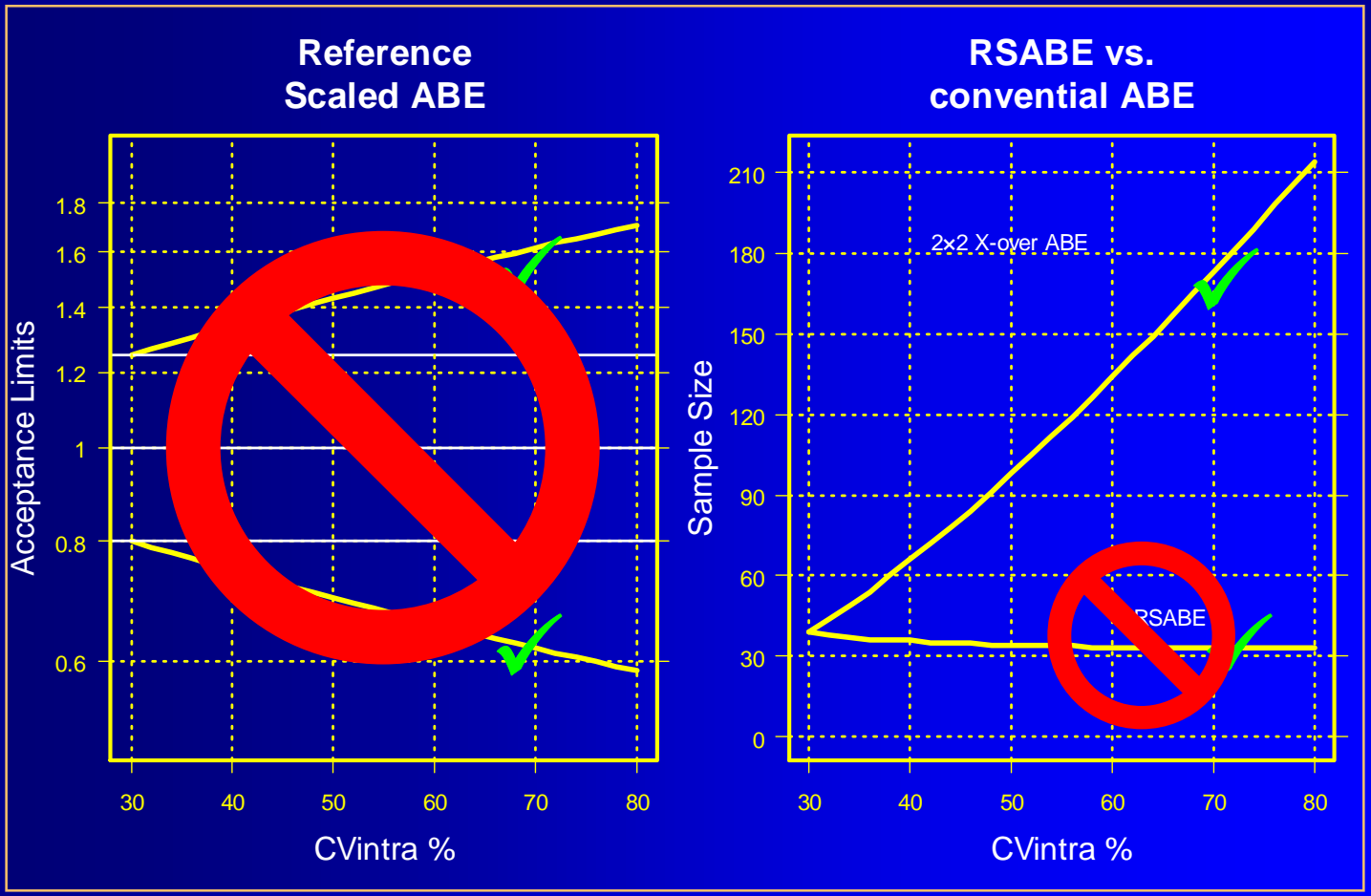
Haidar SH, Davit B, Chen M-L, Conner D, Lee LM, Li QH, Lionberger R, Makhlouf F, Patel D, Schuirmann DJ, and LX Yu

Bioequivalence Approaches for Highly Variable Drugs and Drug Products

Pharmaceutical Research 25/1, 237-241 (2008)

<http://www.springerlink.com/content/u503p62056413677/fulltext.pdf>

HVDPs (US/EU)



HVDs/HVDPs

- Is suggested EU-method of any good?
 - Replicate designs ... (BE Draft, Section 4.1.2) *without scaling*
 - **reduce** the number of subjects (to 75% for a 3-period design and to 50% for a 4-period design as compared to a conventional 2x2),
 - **but** keep the *theoretical* number of treatments constant:
 - The potential drop-out rate increases.
 - Practically *more* treatments must be administered in order to maintain the desired power!

HVDs/HVDPs

● Example

- AR [0.80, 1.25], CV_{intra} 49.5%, T/R 0.95%, power 80%, $n_{2 \times 2}$ 96
- expected dropout rate of 10% per washout
 - 2x2 study: 96+10=106 subjects, 212 treatments
 - 4x2 study: 48+16=64 subjects, 256 treatments
- Proposed FDA Scaling-Method:
AR [0.7006, 1.4273], PE [0.80, 1.25], n 34 (!)

Ethical?

HVDPs: $C_{ss,min}$

- EMEA Draft BE Guideline (2008)
 - Acceptance limits
 - [...] at steady state $AUC\tau$, $C_{max,ss}$, and $C_{min,ss}$ should be analysed using the same acceptance interval as stated above.
 - $C_{min,ss}$ was added probably after concerns for oxycodone, but this metric will be rather tough to meet for some drugs.
 - Since scaling is not allowed, sample sizes are expected to be very high (variability of $C_{ss,min} \gg C_{ss,max}$).

Early Exposure

- Partial AUCs for Rapid Onset Drugs
 - US-FDA 2003 (III.A.8.a.)
 - [...] that the partial area be truncated at the population median of T_{max} values for the reference formulation. We also recommend that at least two quantifiable samples be collected before the expected peak time to allow adequate estimation of the partial area.
 - Canada-TGD 2005
 - [...] $AUC_{Reftmax}$ for a test product is defined as the area under the curve to the time of the maximum concentration of the reference product, calculated for each study subject.

Early Exposure

- Partial AUCs for Rapid Onset Drugs (cont'd)
 - EU-EMA BE Draft 2008
 - When partial AUC is to be determined, frequent early sampling is recommended with preferably at least two quantifiable samples before expected t_{\max} . [...] partial AUCs can be used as a measure of early exposure. The partial area can in most cases be truncated at the population median of t_{\max} values for the reference formulation. However, an alternative time point for truncating the partial AUC can be used when clinically relevant. *The time point for truncating the partial AUC should be pre-specified and justified in the study protocol.*

Early Exposure (HVDP?)

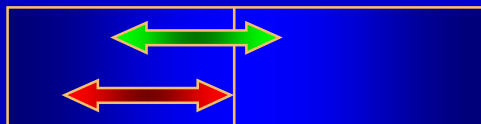
● Partial AUCs for Rapid Onset Drugs (cont'd)

Example	median $t_{\max\text{ref}}$	PE	nonparametric CI		BE	FDA	parametric CI		BE	TGD	BE
1	1.5 h	± 0.00 h	-0.25 h (85%)	+0.25 h (115%)	yes	90.1%	75.0%	110.1%	no (CV 26.4%)	85.7%	yes
2	1.5 h	+0.26 h	± 0.00 h (100%)	+0.50 h (130%)	no	66.1%	53.1%	82.0%	no (CV 29.7%)	62.4%	no

- Even for formulations with low intra-subject variability...
 - Example 1: AUC_t 13.3% C_{\max} 17.0%
 - Example 2: AUC_t 6.33% C_{\max} 9.43%
- ...it is unlikely to be able to demonstrate BE due to high variability of this metric. It is unclear how median $t_{\max\text{ref}}$ can be stated in the protocol (EMA) – the innovator's SmPC (=label) mostly states only the *arithmetic mean*.

Low Variability

- Drugs / Drug Products with $CV_{\text{intra}} < 10\%$
 - No specific statements in any guideline.
 - Problems may arise according to significant treatment effects in ANOVA (*i.e.*, although the 90% CI is within the acceptance range – 100% is not included) – even for the minimum sample size of 12.



- Denmark

- DKMA considers that the 90% CI for the ratio test versus reference should include 100% [...].
- Deviations may be accepted if they can be adequately justified not to have impact on either the overall therapeutic effect or safety profile of the product.

Danish Medicines Agency (DKMA)

Bioequivalence and labelling of medicinal products with regard to generic substitution (Jan 2006)

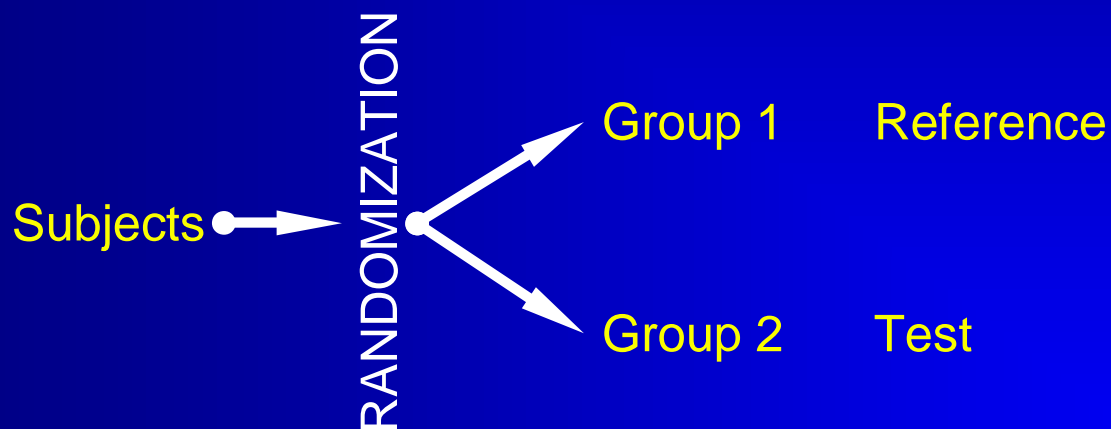
<http://www.dkma.dk/1024/visUKLSArtikel.asp?artikelID=6437>

Parallel Groups

- Two-group parallel design
 - Each subject receives one – and only one – treatment in a random fashion
 - Usually each group contains the same number of subjects.
 - Higher subject numbers compared to a cross-over design, since the *between-subject* variability determines sample size (rather than *within-subject* variability)

Parallel Groups

- Two-group parallel design



Parallel Groups

- Two-group parallel design

- Advantages

- Clinical part – *sometimes* – faster than X-over
- Straightforward statistical analysis
- Drugs with long half life
- Potentially toxic drugs or effect and/or AEs unacceptable in healthy subjects
- Studies in patients, where the condition of the disease irreversibly changes

- Disadvantages

- Lower statistical power than X-over (*rule of thumb*: sample size should at least be doubled)
- Phenotyping mandatory for drugs showing polymorphism

Parallel Groups

● Design Issues

■ EMEA NfG on BA/BE (2001)

■ 3.2.4 Genetic phenotyping

‘Phenotyping and/or genotyping of subjects should be considered for [...] all studies using parallel group design. If a drug is known to be subject to major genetic polymorphism, studies could be performed in panels of subjects of known phenotype or genotype for the polymorphism in question.’

■ Since the comparison is based on *inter-subject* effects

■ One study of the major phenotype/genotype

■ Two studies of the respective phenotype/genotype – only if requested!

Parallel Groups

● Evaluation

- FDA/CDER, Statistical Approaches to Establishing Bioequivalence (2001)
 - Section VI. B.1.d. Parallel Designs
 - ‘For parallel designs, the confidence interval for the difference of means in the log scale can be computed using the total between-subject variance. As in the analysis for replicated designs (section VI. B.1.b), equal variances should not be assumed.’
- The conventional t -test depends on the assumption that samples come from populations that have identical variances
 - ‘Naïve pooling’ of variances is relatively robust against unequal variances, but rather sensitive to inbalanced data
 - If assumptions are violated, the conventional t -test becomes liberal (*i.e.*, the CI is too tight; patient's risk >5%).

Sample Data Set

● 2x2x2 Cross-over Study

- 24 subjects (balanced:
TR=RT=12)
- Single dose
- Target parameter: AUC_{0-t}
- CV_{intra} 20.0%
- CV_{inter} 32.6%

<http://bebac.at/downloads/24sub.txt>
(CSV-format)

Trt	Rand	Sub	P ₁	P ₂
1	RT	1	44.1	39.1
1	RT	2	33.6	23.8
1	RT	3	45.5	40.8
2	TR	4	19.5	21.1
2	TR	5	67.2	51.5
2	TR	6	25.7	30.1
1	RT	7	35.3	26.7
1	RT	8	26.0	36.5
1	RT	9	38.2	57.8
2	TR	10	33.6	32.5
2	TR	11	25.1	36.8
2	TR	12	44.1	42.9
1	RT	13	25.6	20.1
1	RT	14	58.0	45.3
1	RT	15	47.2	51.8
2	TR	16	16.5	21.4
2	TR	17	47.3	39.4
2	TR	18	22.6	17.3
1	RT	19	17.5	30.1
1	RT	20	51.7	36.0
1	RT	21	24.5	18.2
2	TR	22	36.3	27.2
2	TR	23	29.4	39.6
2	TR	24	18.3	20.7

Parallel Groups: Example

- Evaluation (sample data set, period 1 only)
 - Original data set
 - **Balanced** (T 12, R 12)
 - **Equal variances** (s^2_R 0.1292, s^2_T 0.1796)
 - F-ratio test p 0.5947
 - Levene test p 0.5867
 - Modified data set
 - Values of subjects 4 – 6 multiplied by three
 - Subjects 22 – 24 removed
 - **Inbalanced** (T 9, R 12)
 - **Unequal variances** (s^2_R 0.1292, s^2_T 0.5639)
 - F-ratio test p 0.0272
 - Levene test p 0.1070

Parallel Groups: Example

- Evaluation (original data set)
 - Is your software able to give a correct answer?

Software / Method	equal variances	unequal variances
'manual' (Excel 2000)	63.51% – 110.19%	63.48% – 110.25%
R 2.8.1 (2008)	63.51% – 110.19%	63.49% – 110.22%
NCSS 2001 (2001)	63.51% – 110.19%	63.49% – 110.22%
STATISTICA 5.1H (1997)	63.51% – 110.19%	63.49% – 110.22%
WinNonlin 5.2.1 (2008)	63.51% – 110.20%	<i>not implemented!</i>
Kinetica 5.0.1 (2009)	63.51% – 110.19%	<i>not implemented!</i>
EquivTest/PK (2006)	63.51% – 110.18%	<i>not implemented!</i>

Parallel Groups: Example

● Evaluation (modified data set)

Software	equal variances	unequal variances
R 2.8.1 (2008)	81.21% – 190.41%	76.36% – 202.51%
NCSS 2001 (2001)	81.21% – 190.41%	76.36% – 202.51%

- Inflated α -risk in ‘conventional’ t -test (naïve pooling) is reflected in a tighter confidence interval.
- Preliminary testing for equality in variances is flawed*) and should be avoided (FDA).
- Approximations (e.g., Satterthwaite, Aspin-Welch, Howe, Milliken-Johnson) are currently not implemented in packages ‘specialized’ in bioequivalence testing (*WinNonlin*, *Kinetica*, *EquivTest/PK*)!

*) **Moser BK and GR Stevens**
Homogeneity of variance in the two-sample means test
 Amer Statist 46:19-21 (1992)

Thank You!
**Statistical Design
and Analysis I**
Open Questions?
(References in handouts of part II)

Helmut Schütz
BEBAC

Consultancy Services for
Bioequivalence and Bioavailability Studies
1070 Vienna, Austria
helmut.schuetz@bebac.at