HELLENIC REPUBLIC
National and Kapodistrian University of Athens
EST. 1837

Bioequivalence studies

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Lesson plan

- **A1.** Introduction
- **A2.** Clinical (therapeutic) equivalence – Bioequivalence: General principles
- **A3.** Assessment of Bioequivalence
- **A4.** Why PK equivalence ensures therapeutic equivalence
- **A5.** Narrow Therapeutic Index (NTI) drugs
- **A6.** Highly variable drugs
- **A7.** Drugs interchangeability
- **A8.** Clinical design
- **A9.** Sample size estimation
- **A10.** Re-inventing the rate of absorption

A1.

Introduction

Bioequivalence (BE):

Bío- = life

&

Equivalence

Important discrimination

Equivalence

= two things are ***similar*** in terms of a property

Equality

2 = 2

3 = 3

or better:

2 mL = 2 mL

3 mg of Drug A = 3 mg of Drug A

When BE testing is used?

Bioequivalence testing is usually applied to assess the *in vivo* "equivalence" between **two** drug products of the **same** active moiety, namely:

- the test (**T**) (*or generic, or …*)

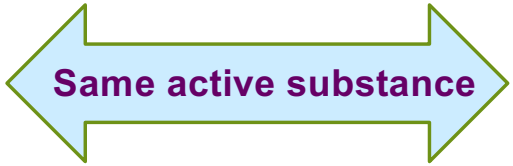
  and

- the innovator's (Reference, **R**) product

Bioequivalence testing

Product A

(Reference)



Product B

(Test)

e.g., generic

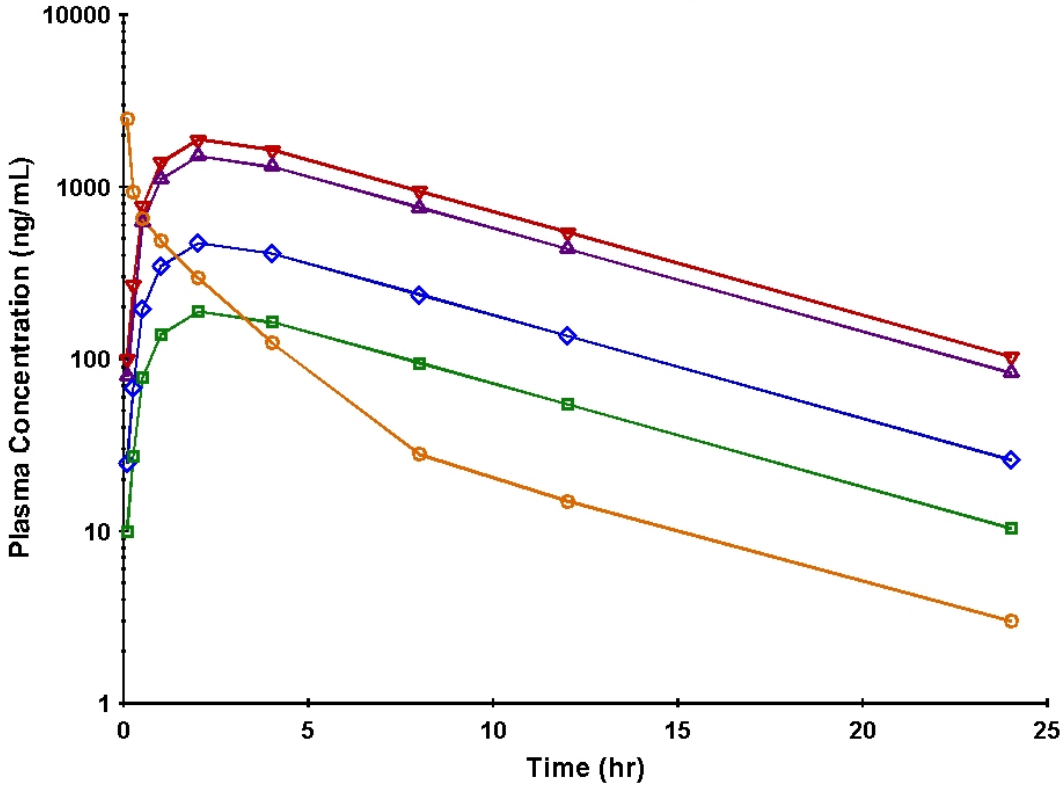
A2.

Clinical (therapeutic) equivalence –

Bioequivalence: General principles

Pharmacokinetic studies

➔ Pharmacokinetic measurements

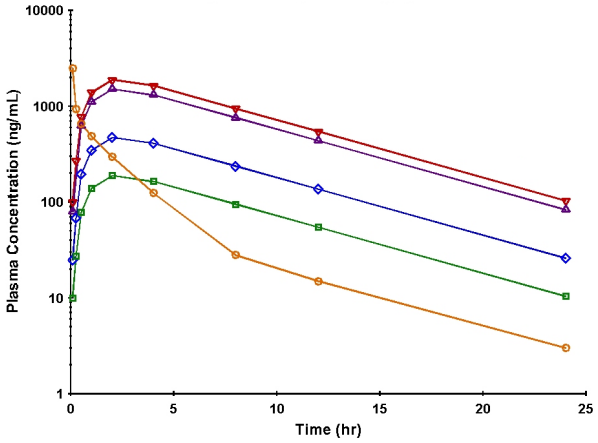


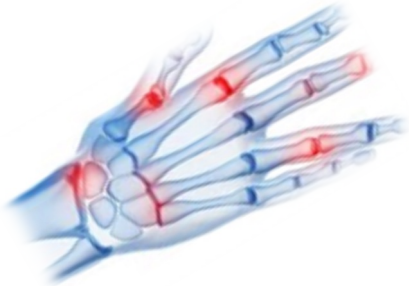
Bioequivalence studies = comparative **PK** studies

!! A rational question:

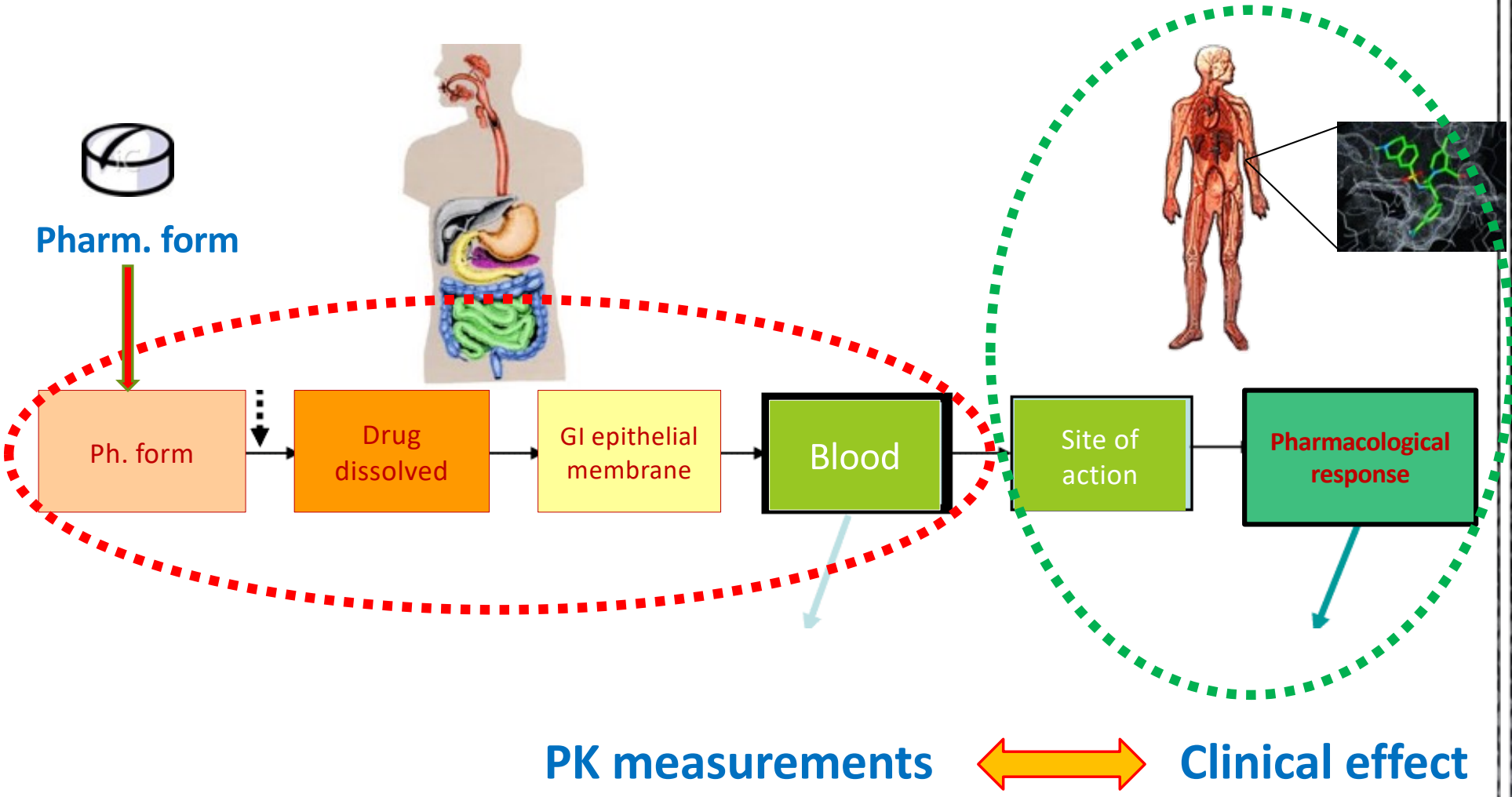
Why PK data are suitable for

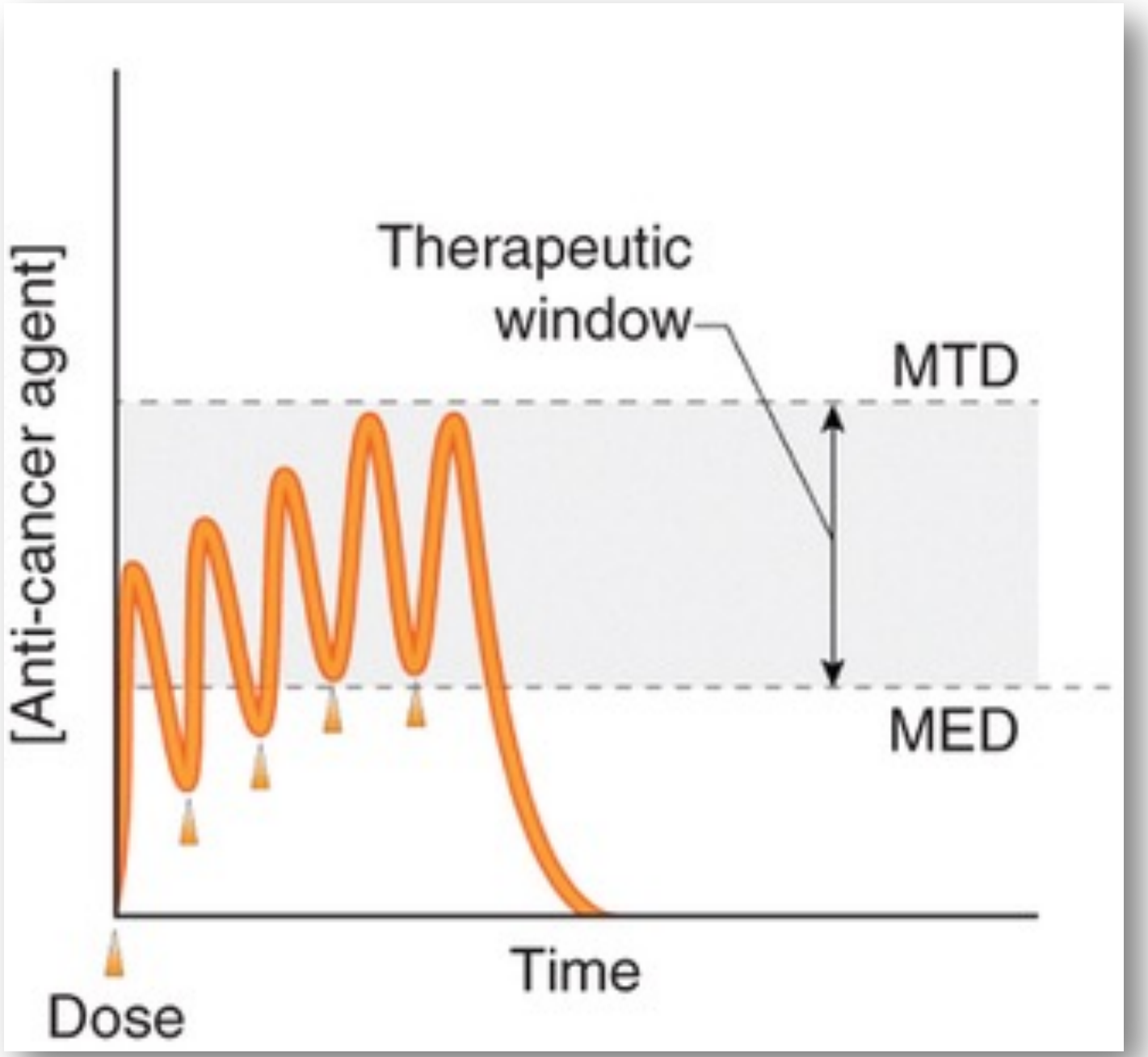
demonstrating 'Clinical Equivalence'?





Theoretical justification – Graphical illustration




Therapeutic
window
MTD
MED
[Anti-cancer agent]
Time
Dose

Thus:

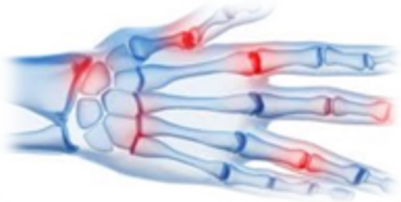
PK equivalence = Bioequivalence

Therapeutic equivalence

Same active substance

Product A

(Reference)



Product B

(Test)

«Ισοδυναμία»: ΟΧΙ χειρότερο & ΟΧΙ καλύτερο

Βιοϊσοδύναμα

Οχι βιοϊσοδύναμα

vs.

vs.

vs.

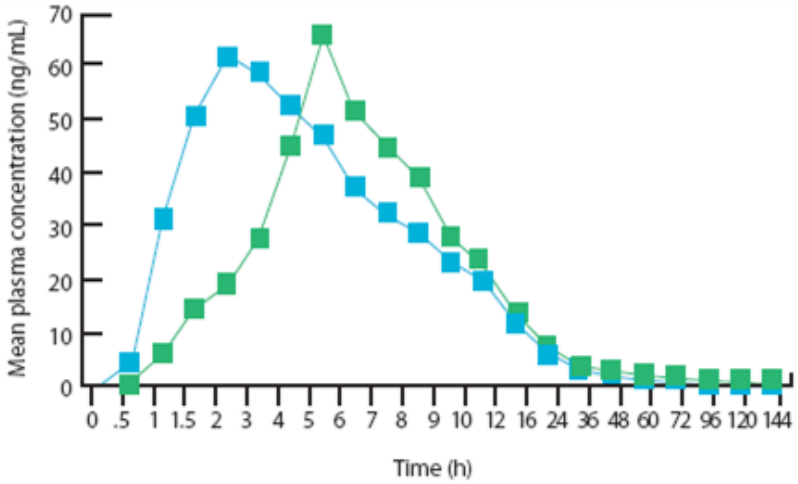
A3.

Assessment of Bioequivalence

The official EMA definition

Background

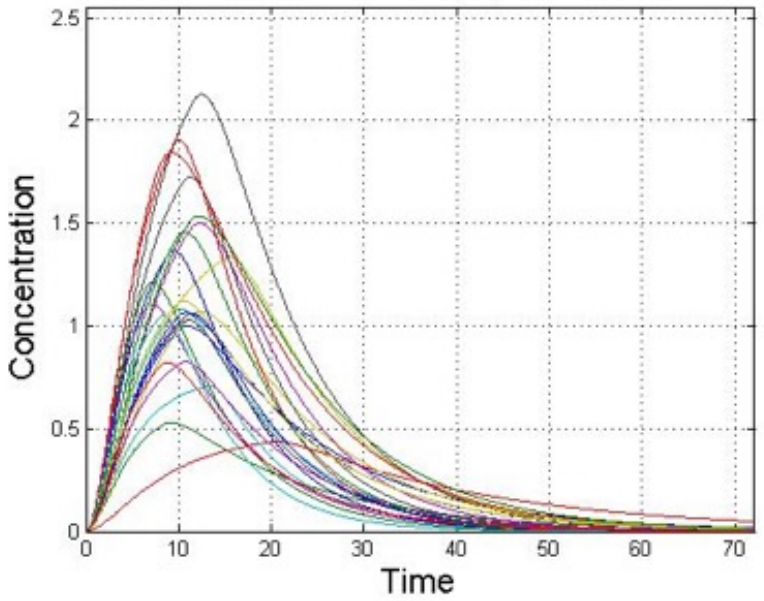
Two medicinal products containing the same active substance are considered bioequivalent if they are pharmaceutically equivalent or pharmaceutical alternatives and their bioavailabilities (rate and extent) after administration in the same molar dose lie within acceptable predefined limits. These limits are set to ensure comparable *in vivo* performance, i.e. similarity in terms of safety and efficacy.



The 1st step: Perform a bioequivalence study

Clinical studies in healthy volunteers …

➔ *Comparative PK analysis*



… how fast


European Medicines Agency

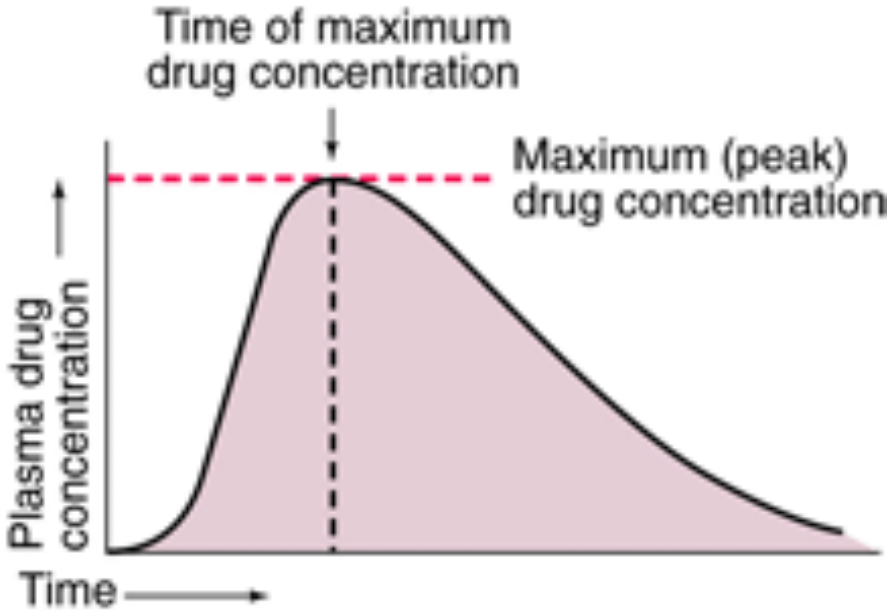
London, 20 January 2010
Doc. Ref.: CPMP/EWP/QWP/1401/98 Rev. 1/ Corr **

COMMITTEE FOR MEDICINAL PRODUCTS FOR HUMAN USE (CHMP)

GUIDELINE ON THE INVESTIGATION OF BIOEQUIVALENCE

Pharmacokinetic parameters

Actual time of sampling should be used in the estimation of the pharmacokinetic parameters. In studies to determine bioequivalence after a single dose, $AUC_{(0-t)}$, $AUC_{(0-\infty)}$, residual area, C_{max} and t_{max} should be determined. In studies with a sampling period of 72 h, and where the concentration at 72 h is quantifiable, $AUC_{(0-\infty)}$ and residual area do not need to be reported; it is sufficient to report AUC truncated at 72h, $AUC_{(0-72h)}$. Additional parameters that may be reported include the terminal rate constant, λ_z, and $t_{1/2}$.

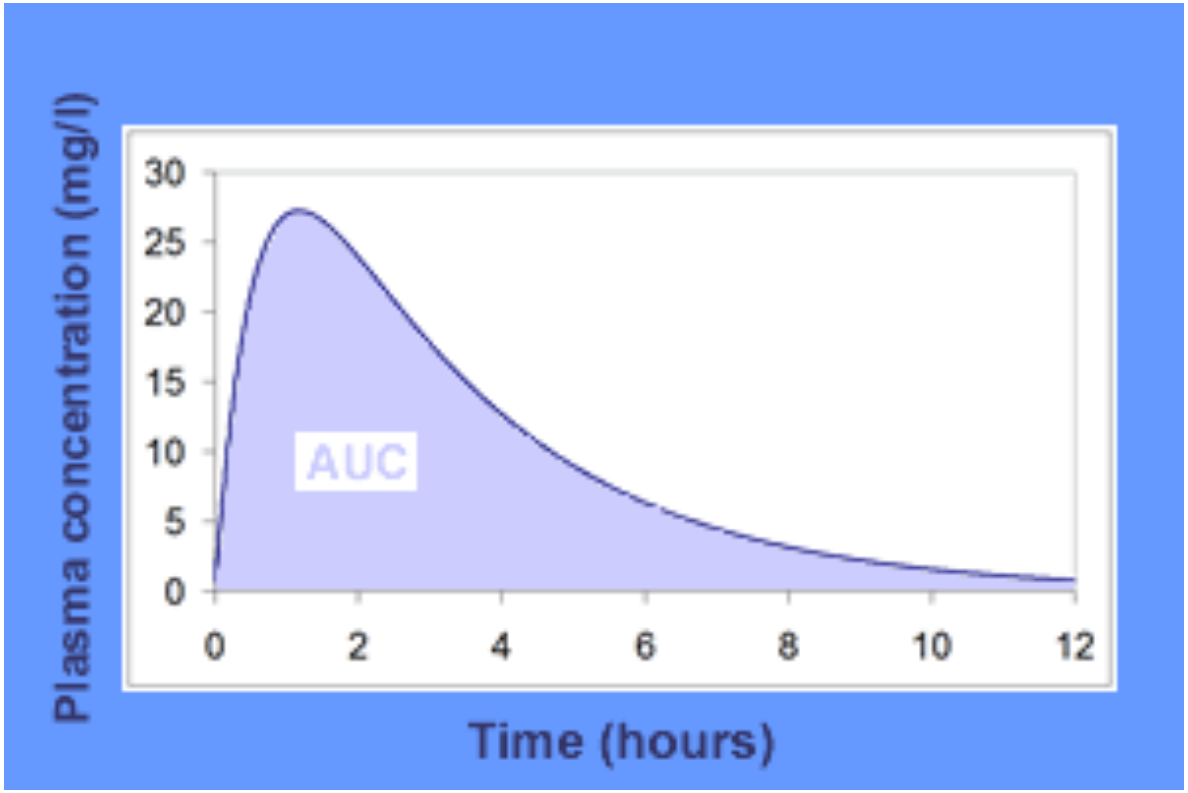


C_{max}: *Maximum plasma concentration*

… how much

Pharmacokinetic parameters

Actual time of sampling should be used in the estimation of the pharmacokinetic parameters. In studies to determine bioequivalence after a single dose, $AUC_{(0-t)}$, $AUC_{(0-\infty)}$, residual area, C_{max} and t_{max} should be determined. In studies with a sampling period of 72 h, and where the concentration at 72 h is quantifiable, $AUC_{(0-\infty)}$ and residual area do not need to be reported; it is sufficient to report AUC truncated at 72h, $AUC_{(0-72h)}$. Additional parameters that may be reported include the terminal rate constant, λ_z, and $t_{1/2}$.



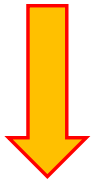
AUC: Area under the concentration – time curve

Classically:

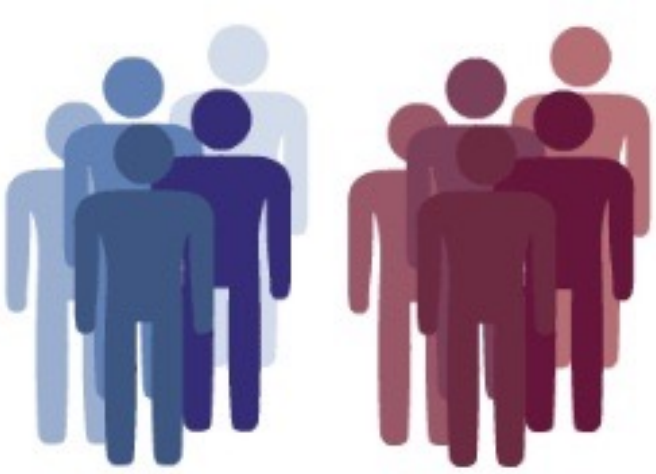
Average Bioequivalence (ABE)

Equivalence in the **averages** of the pharmacokinetic parameters

+/- 20%



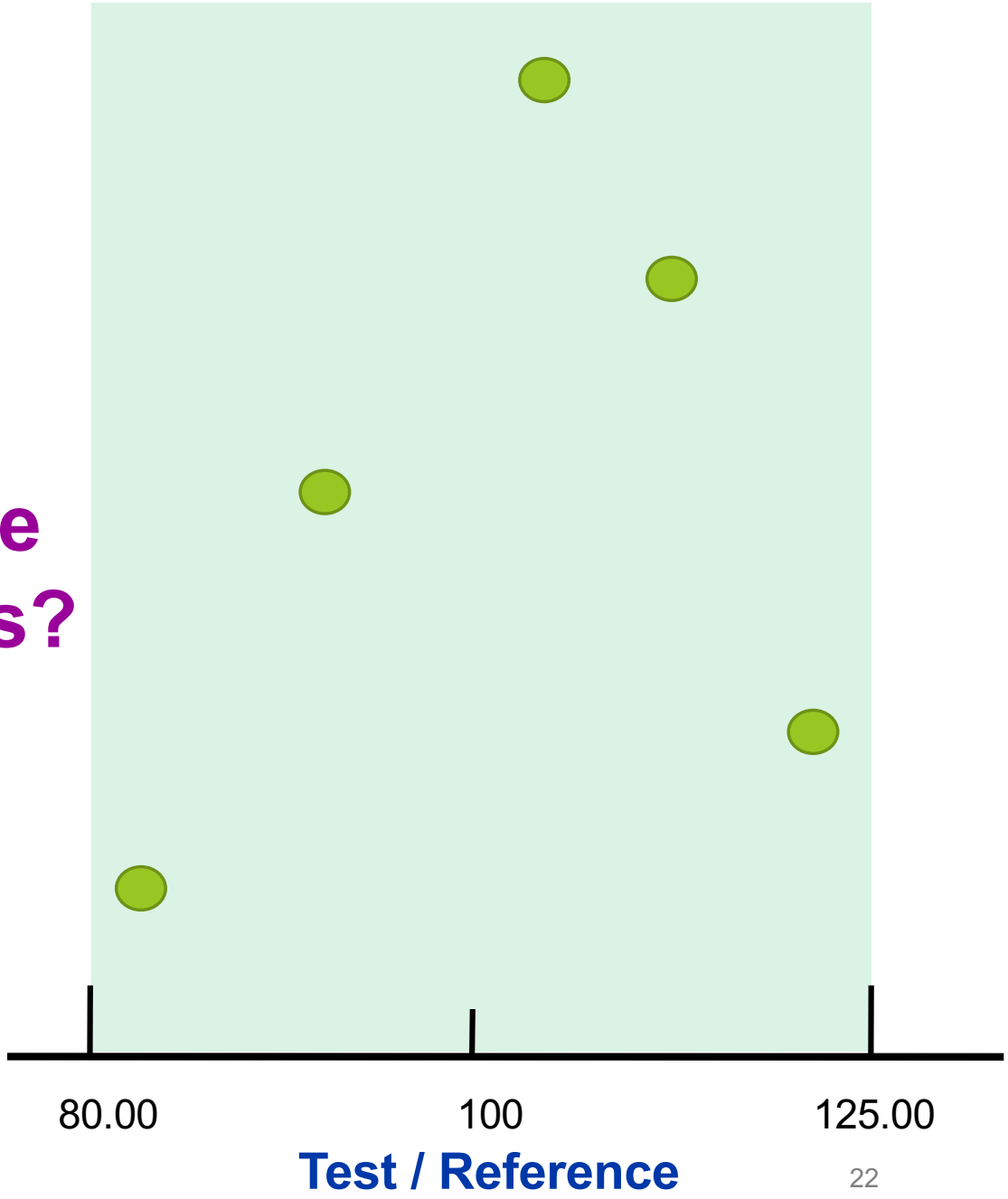
Rule: ***80 - 125%***

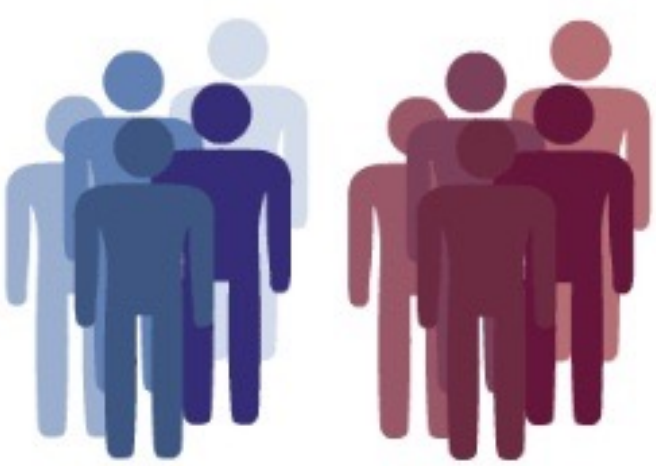


Comparison of the Mean PK estimates?

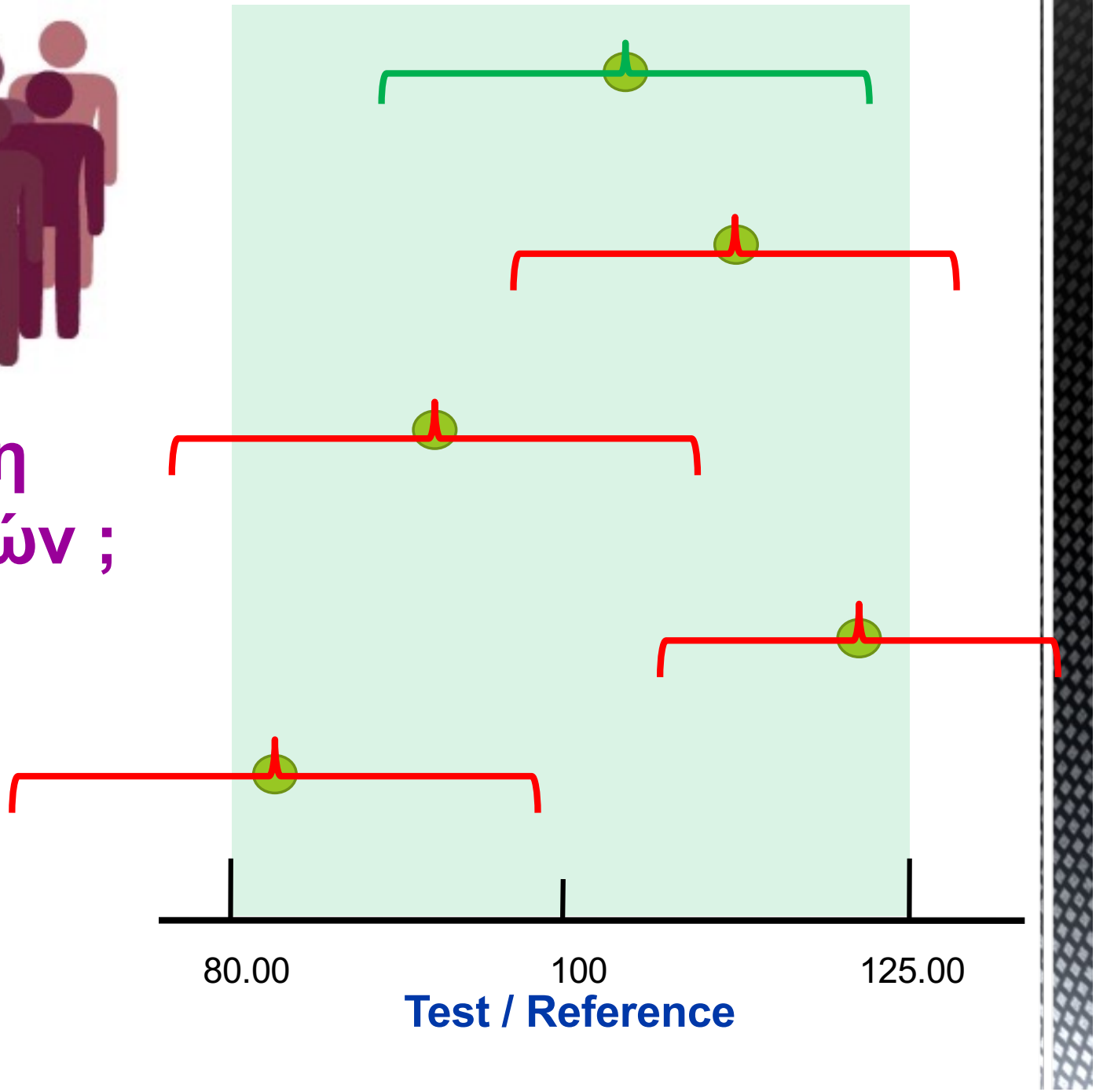
$$\frac{AUC_{Test}}{AUC_{Reference}}$$

Wrong !!





Σύγκριση μέσων τιμών ;



Statistical comparison

Basic steps

- ✓ *ln*-transformation: AUC_t, C_{max}
- ✓ General linear model (ANOVA)
  - ✓ Effects (…)
- ✓ Geometric Mean Ratio (GMR)
- ✓ 90% confidence interval (**90% CI**)
- ✓ Acceptance limits: **80.00% – 125.00%**

ln-transformation: AUC_t, C_{max}

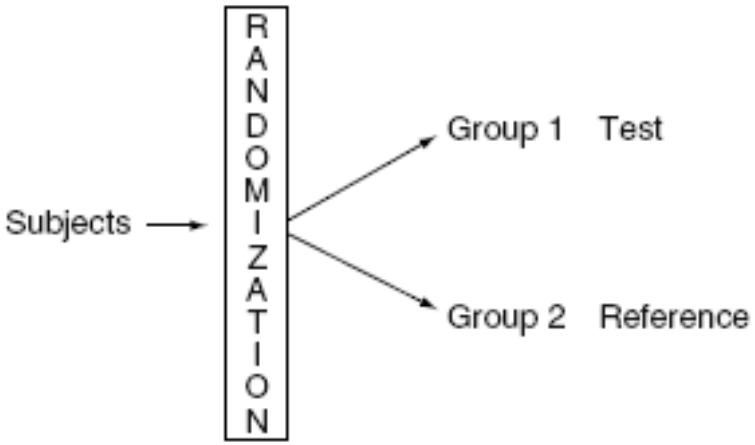
Logarithmic transformation

Cmax	AUCt
238.968	919.595
205.22	1049.641
188.84	1193.229
244.12	1253.65
296.62	1144.909
437.288	1535.726
328.892	1468.449
320.808	1542.425
124.296	678.515
201.68	800.88
144.364	664.454
68.408	478.592
140.828	616.558
191.116	668.816
223.392	934.644
197.924	1082.38825
135.752	563.33
119.164	544.126
134.212	706.52

ln(Cmax)	ln(AUC)
5.476	6.824
5.324	6.956
5.241	7.084
5.498	7.134
5.692	7.043
6.081	7.337
5.796	7.292
5.771	7.341
4.823	6.520
5.307	6.686
4.972	6.499
4.225	6.171
4.948	6.424
5.253	6.506
5.409	6.840
5.288	6.987
4.911	6.334
4.781	6.299
4.899	6.560

General linear model (ANOVA)

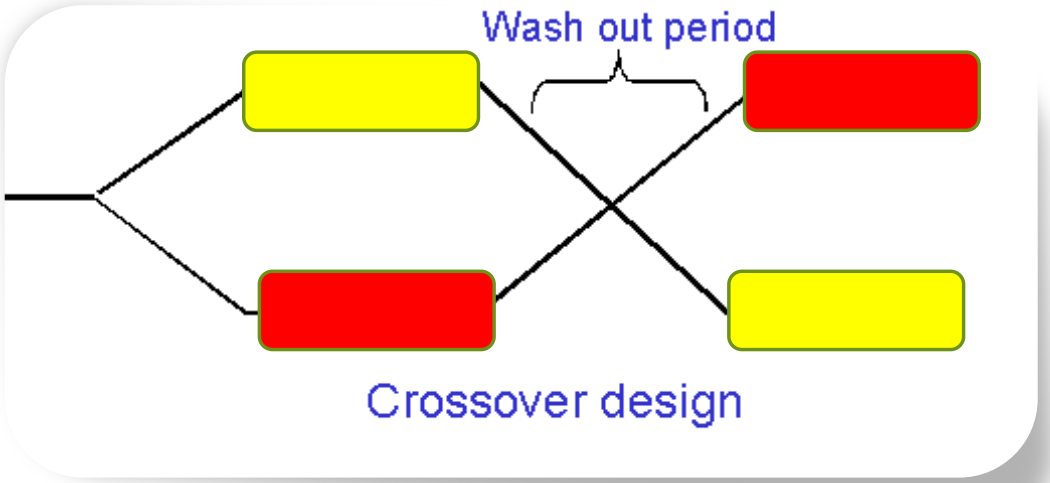
Parallel design



Effect:

Formulation (T, R)

Crossover design



Effects:

Formulation (T, R)
Period
Sequence
Subject (Sequence)

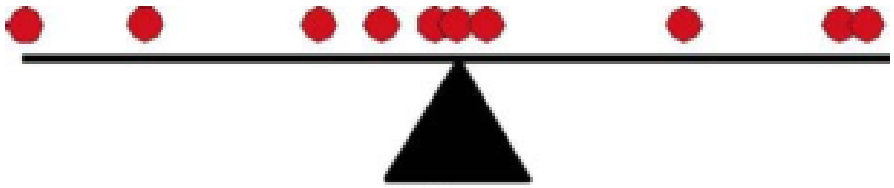
Statistical effects in model

- ✓Sequence effect
- ✓Subject (Sequence) effect
- ✓Formulation effect
- ✓Period effect
- ✓**Residual**

Analysis of variance (ANOVA) table for t-period, t-treatment crossover design

Sources of variation	*Degree of freedom (DF)*	*Sum of squares (SS)*	*Mean sum of squares (MS)*	*F Statistic*
Treatment	t^a-1	SST	MST	MST/MSE
Subject	n^b-1	SSS	MSS	MSS/MSE
Period	t-1	SSP	MSP	MSP/MSE
Error	(t-1)(n-2)	SSE	MSE	
Total	tn-1			

Geometric Mean Ratio (GMR)



In Statistics when we say **'Mean' (average)** we refer to:

$$\bar{X} = \frac{\sum_{i=1}^{n} x_i}{n}$$

But: **Geometric Mean** is: $= \exp\left[\frac{1}{n}\sum_{i=1}^{n} \ln a_i\right]$

Values	Arithmetic mean
10	30
20	
30	
40	
50	

Values	Ln(Values)	Sum of LN-values	Geometric Mean
10	2.303	16.300	26.052
20	2.996		
30	3.401		
40	3.689		
50	3.912		

Geometric Mean Ratio (GMR):

$$\mathbf{GMR} = \exp(m_T - m_R)$$

What 'type of variability' should be used for the construction of the 90% CIs?

Effects:

- ✓ Sequence (e.g., TR, RT)
- ✓ Period (e.g., I, II)
- ✓ Treatment (T or R)
- ✓ Subject(sequence)

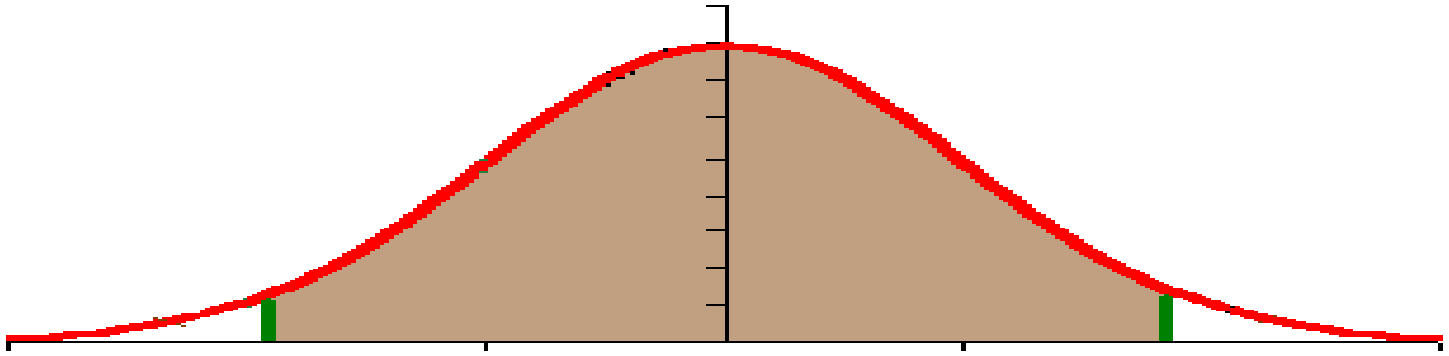
Residual error (or **MSE**) → ~ **WSV**

2x2

$$\left(m_T - m_R\right) \pm t_{0.05,N-2}\,\mathrm{MSE}\sqrt{\frac{1}{N_1}+\frac{1}{N_2}}$$

(2x2) clinical study

90% CI

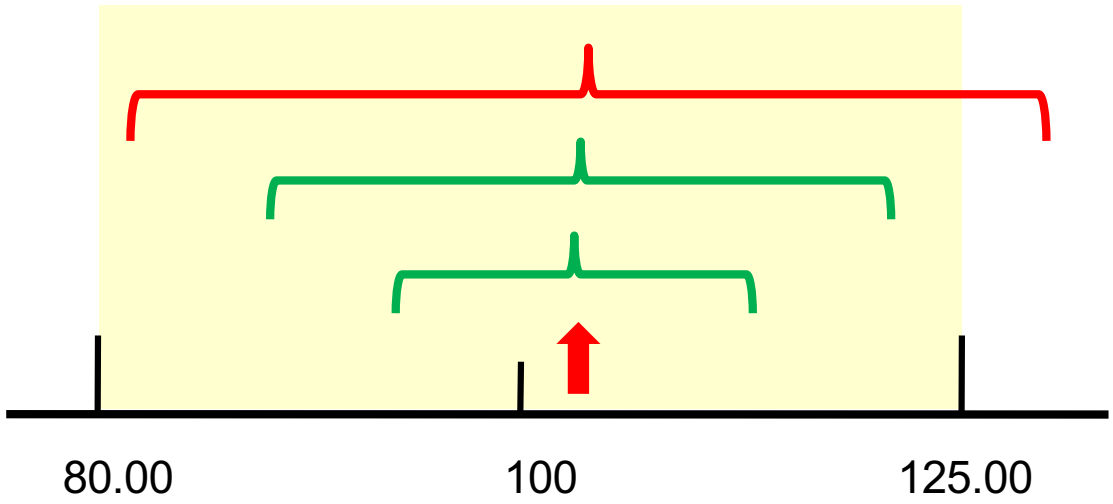


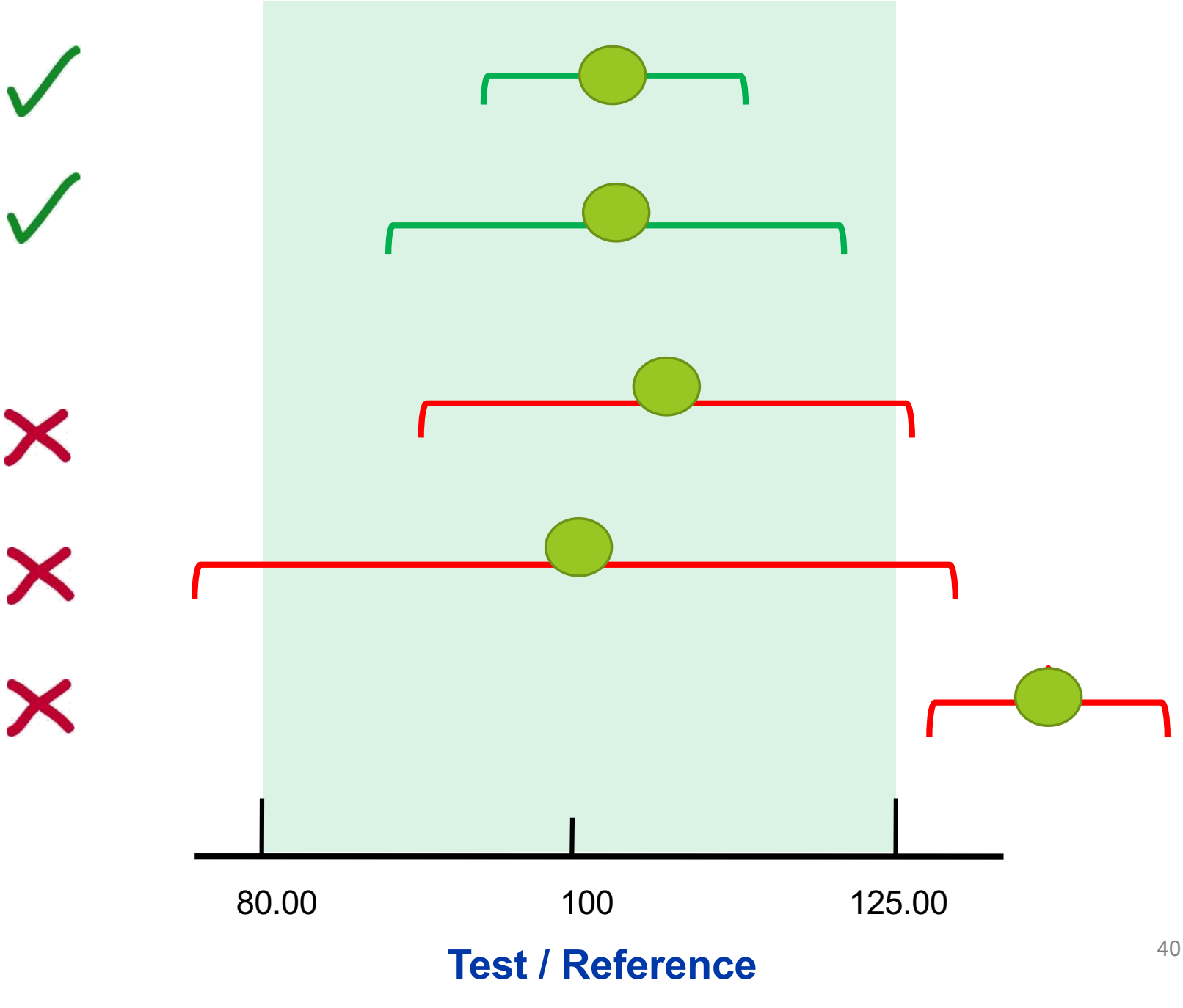
Acceptance limits:

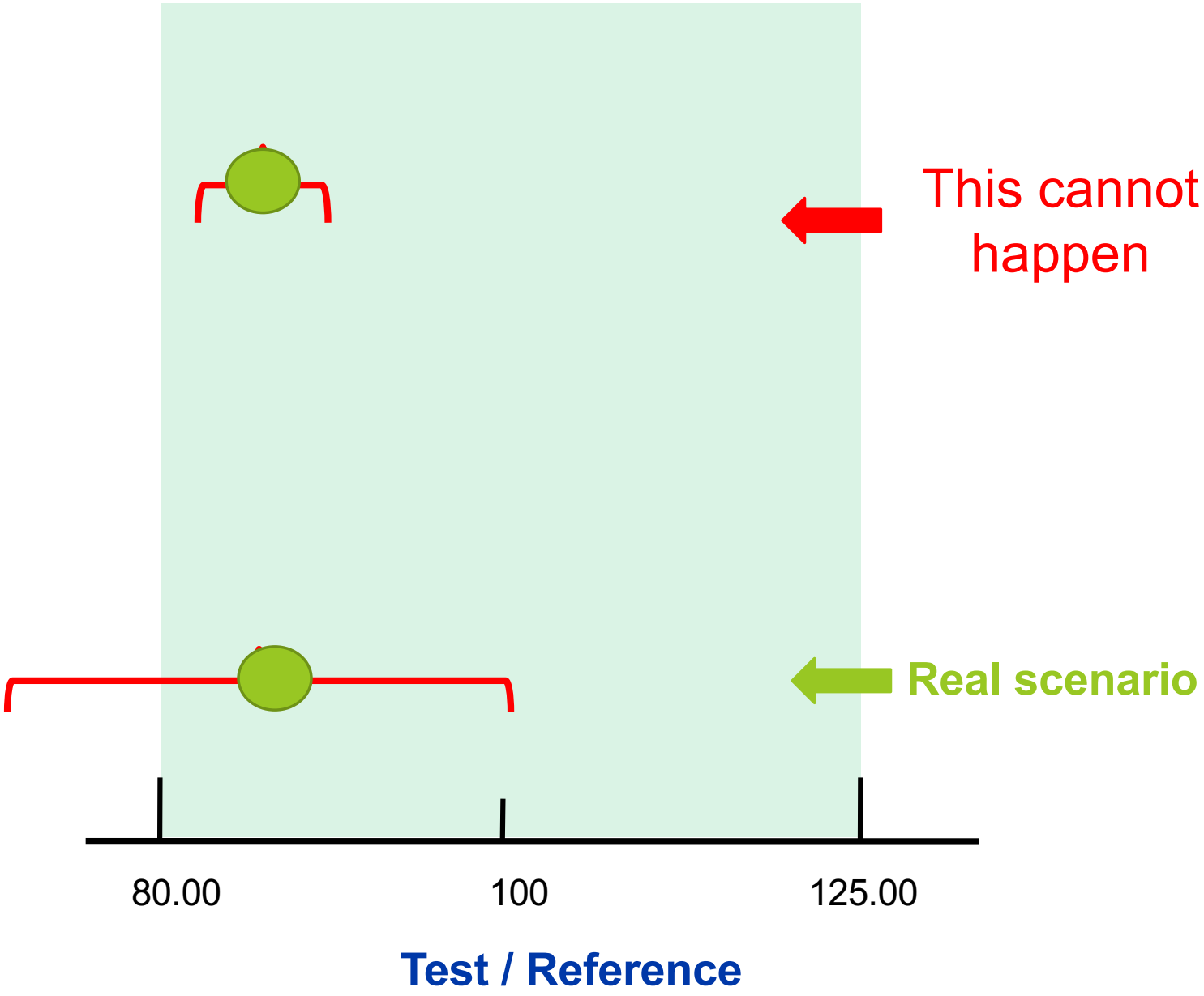
80.00 – 125.00%

90% Confidence Interval

Mean difference $\pm t_{\alpha, f(\mathrm{N})}$ **x** **Variability** **x** f (sample size)




80.00
100
125.00
Test / Reference


This cannot happen
Real scenario
80.00
100
125.00
Test / Reference

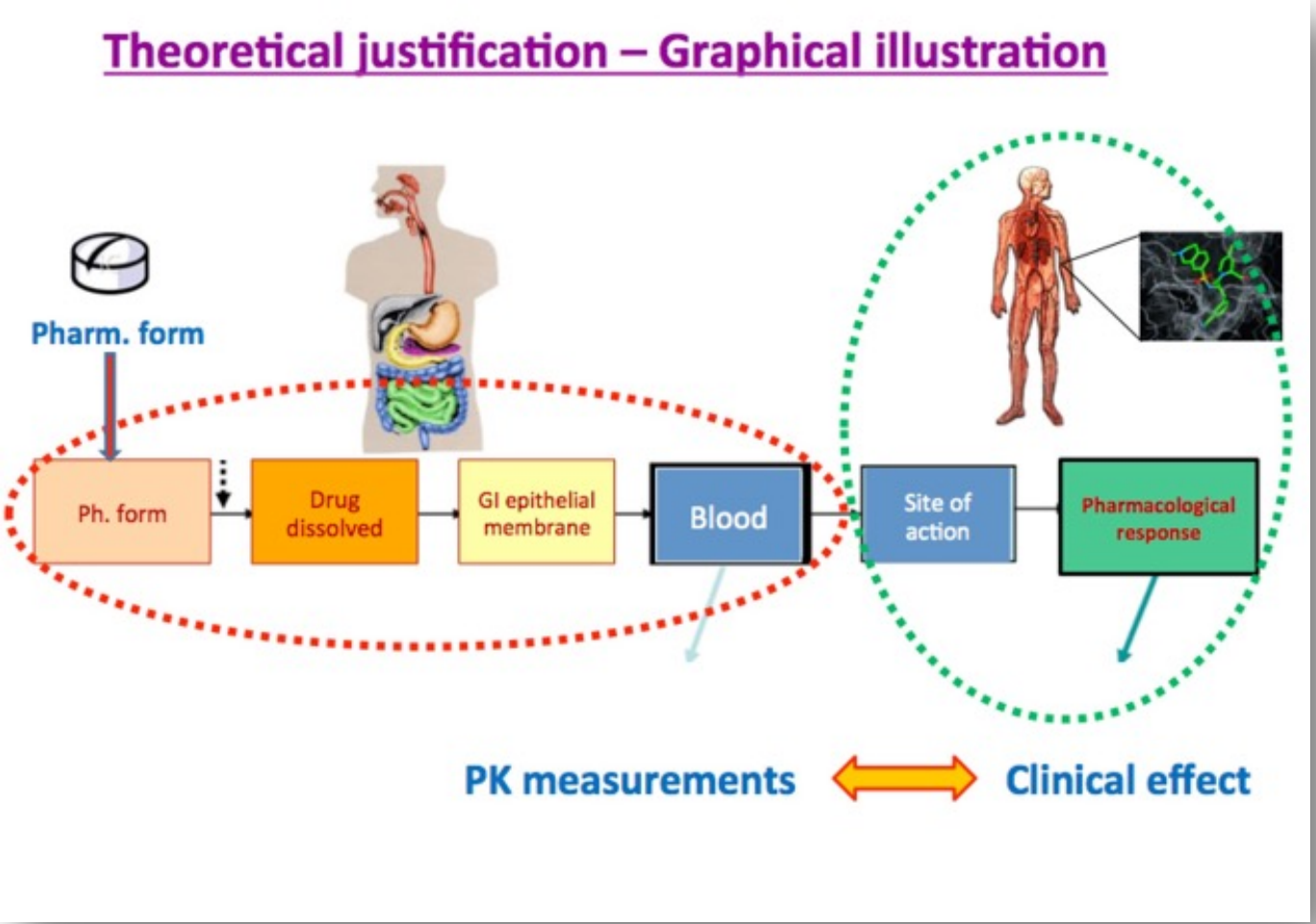
A4.

Why PK equivalence

ensures

therapeutic equivalence

… Remember !!


Theoretical justification – Graphical illustration
Pharm. form
Ph. form
Drug dissolved
GI epithelial membrane
Blood
Site of action
Pharmacological response
PK measurements
Clinical effect

Guidance for Industry

Bioavailability and Bioequivalence Studies for Orally Administered Drug Products — General Considerations

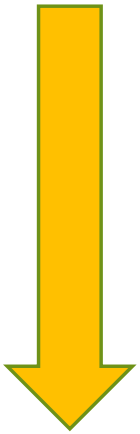
U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
March 2003
BP

Revision 1

A **Ranking** of available methods

(descending order of preference)

METHODS TO DOCUMENT BA AND BE



A. Pharmacokinetic Studies

B. Pharmacodynamic Studies

C. Comparative Clinical Studies

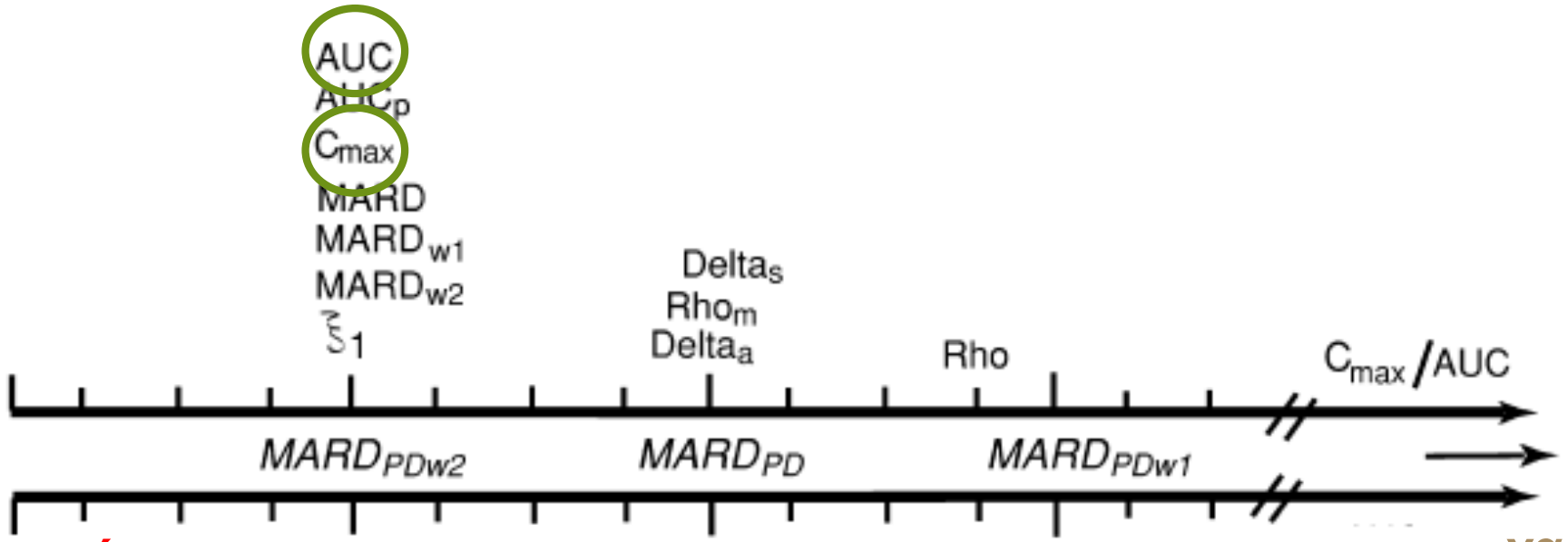
D. In Vitro Studies

Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmaceutical Sciences 19 (2003) 45–56
EUROPEAN JOURNAL OF PHARMACEUTICAL SCIENCES
www.elsevier.com/locate/ejps

Γ

Pharmacodynamic considerations in bioequivalence assessment: comparison of novel and existing metrics

Κατάταξη **Φαρμακοκινητικών** και **Φαρμακοδυναμικών** κριτηρίων



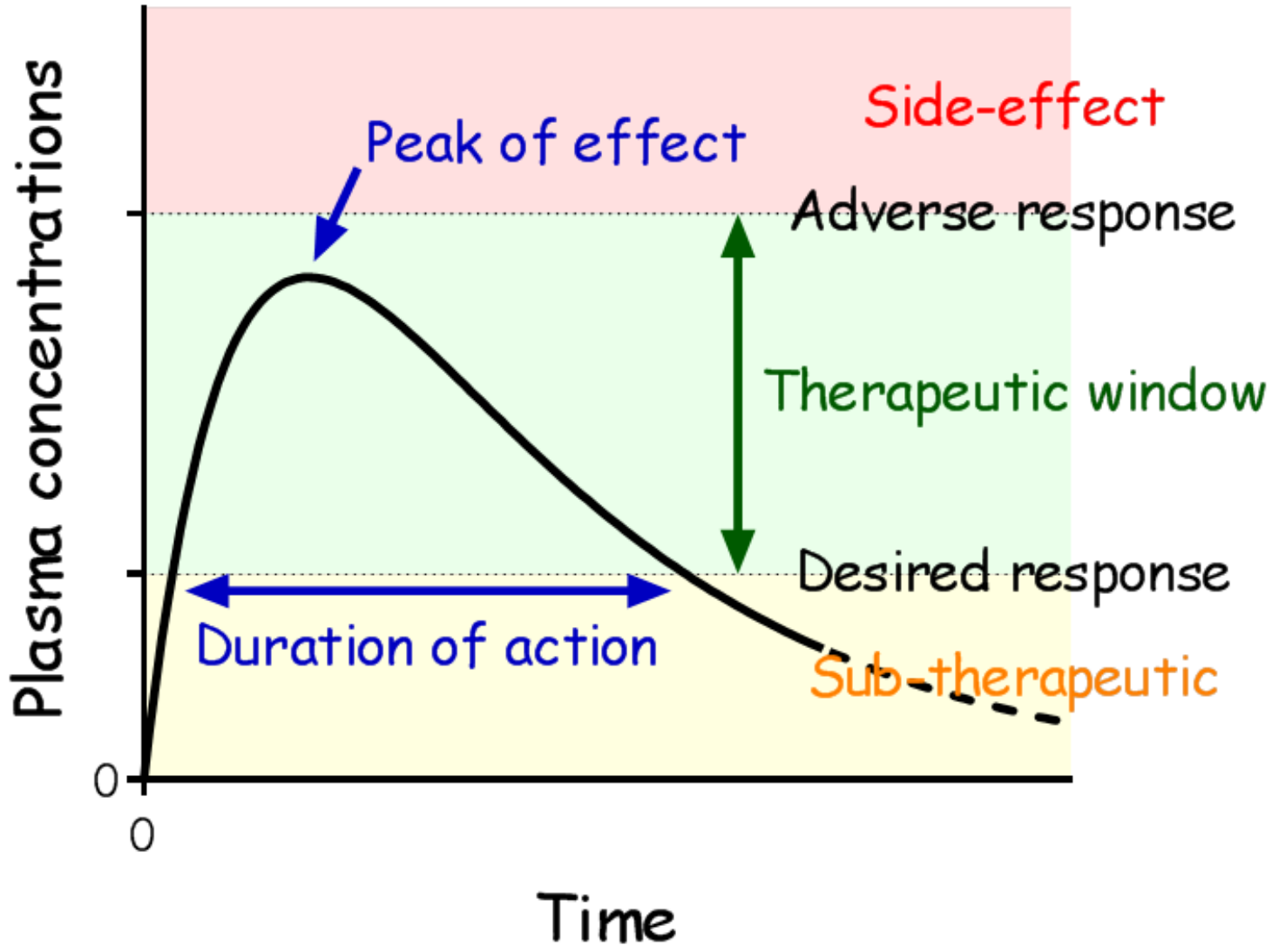
αυστηρό

χαλαρό

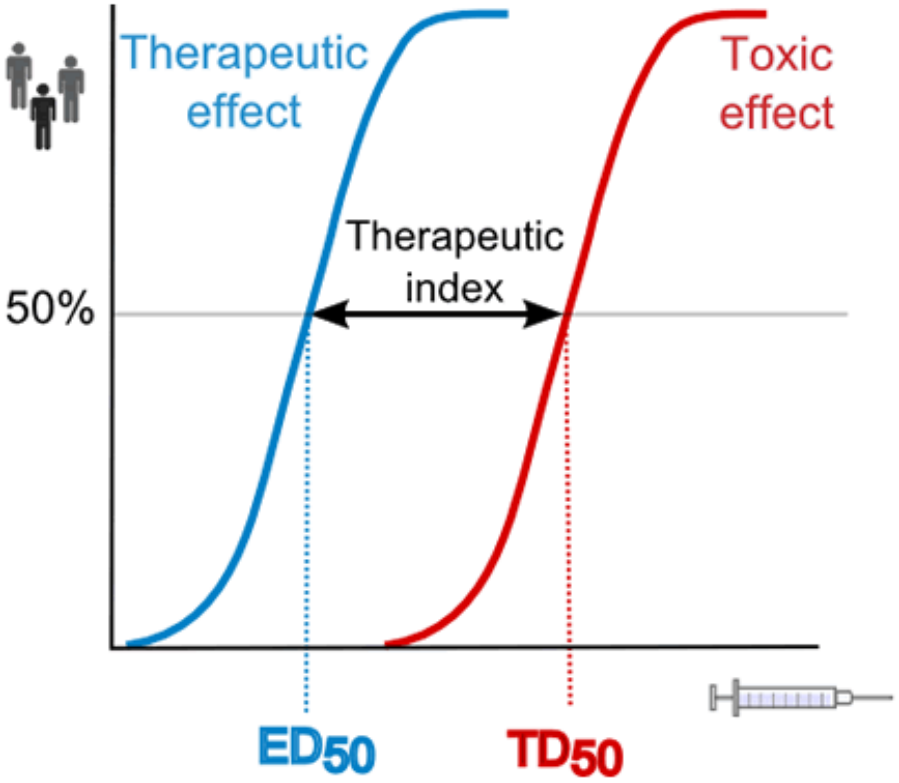
A5.

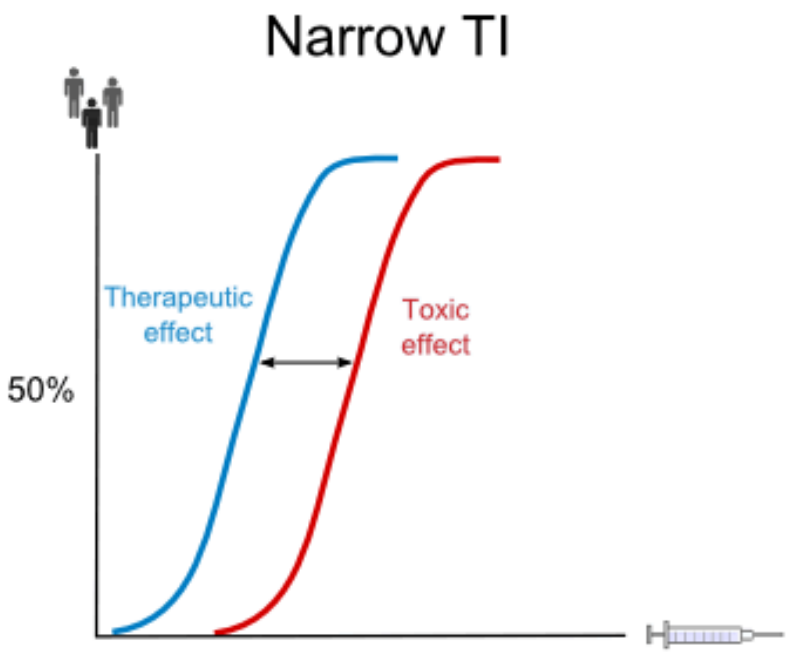
Narrow Therapeutic Index (NTI) drugs

Therapeutic window



Therapeutic Index





$$\text{Therapeutic Index} = \frac{\text{TD}_{50}}{\text{ED}_{50}}$$

The regulatory approach

European Medicines Agency

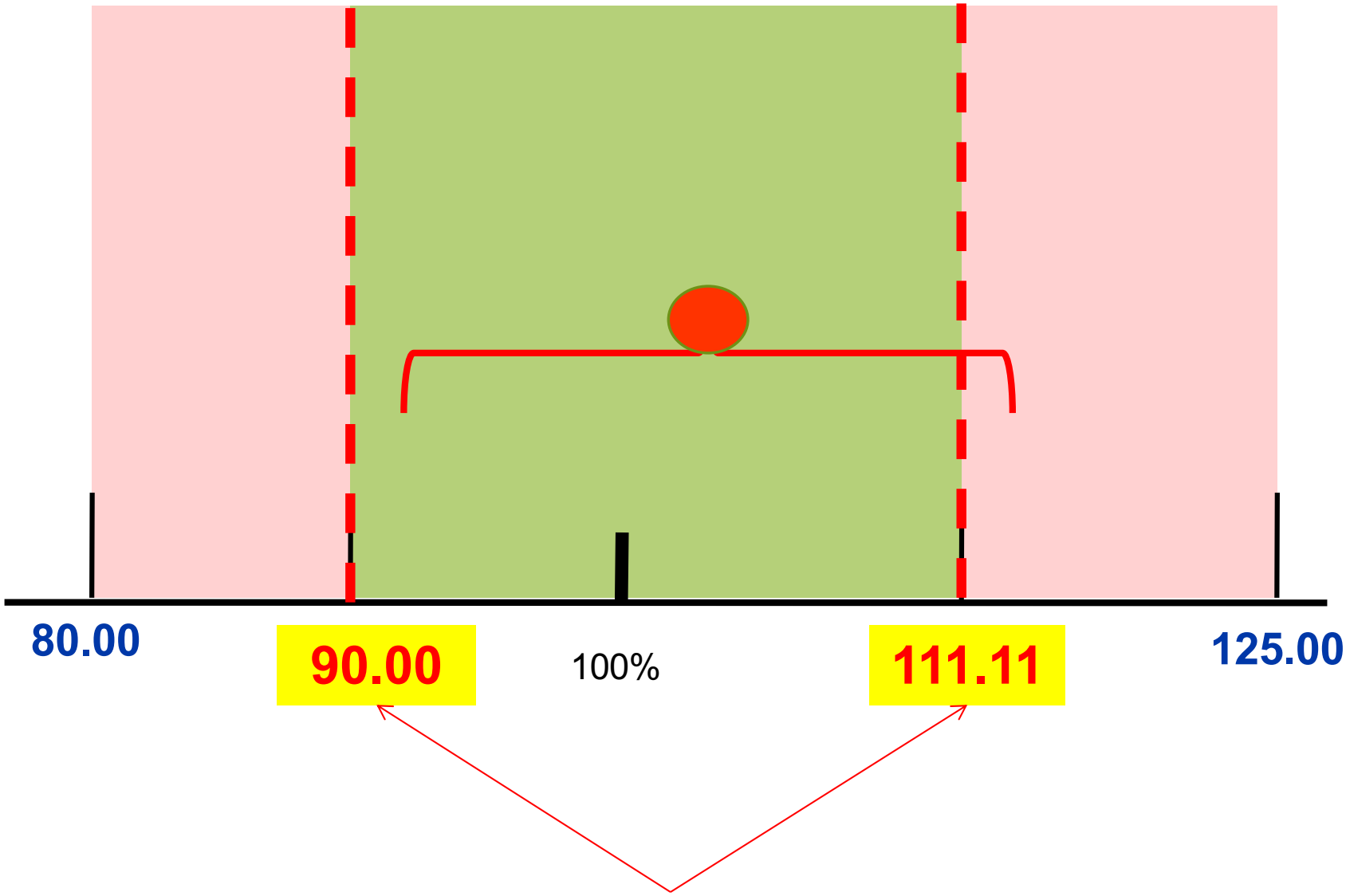
London, 20 January 2010
Doc. Ref.: CPMP/EWP/QWP/1401/98 Rev. 1/ Corr **

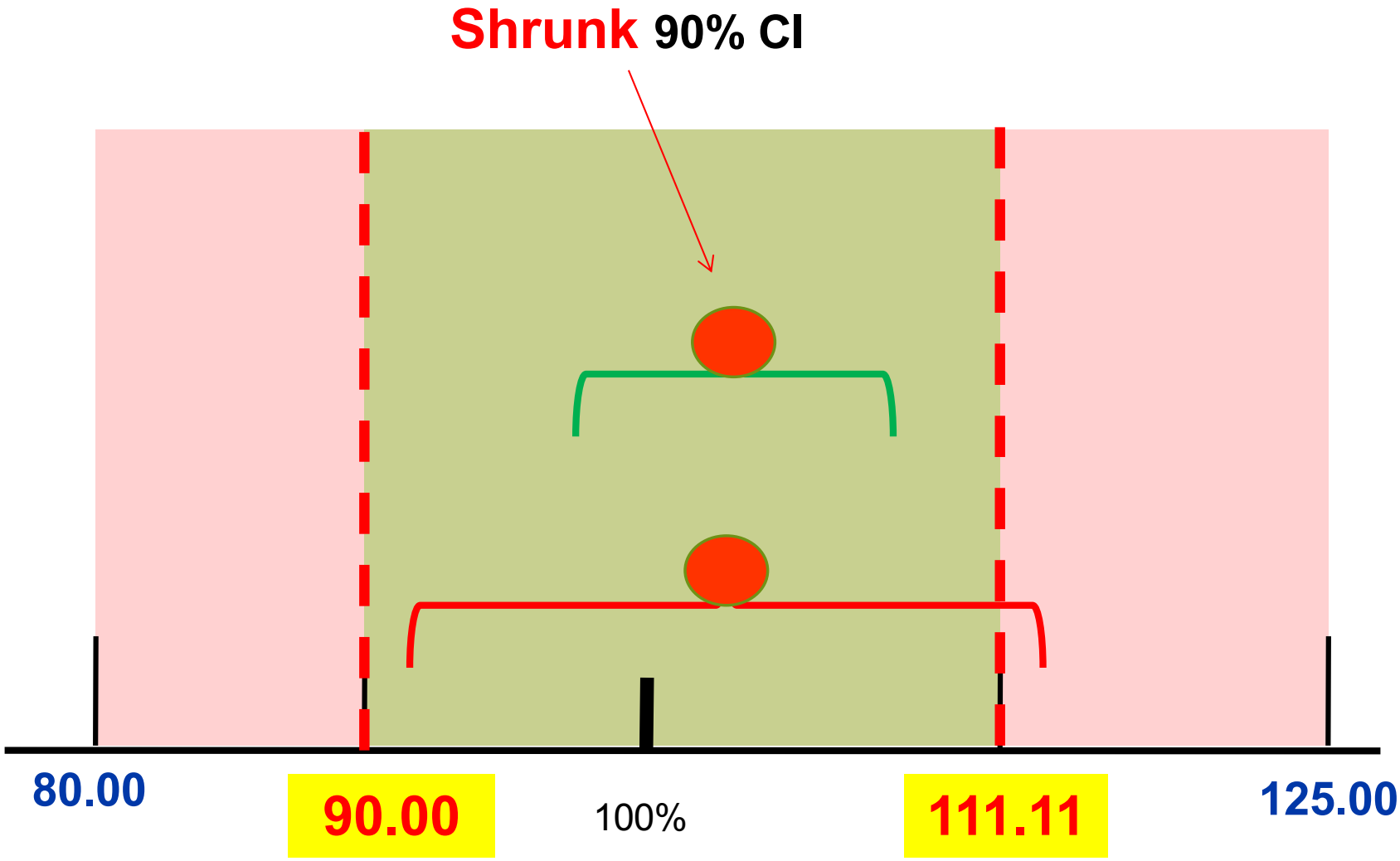
COMMITTEE FOR MEDICINAL PRODUCTS FOR HUMAN USE
(CHMP)

GUIDELINE ON THE INVESTIGATION OF BIOEQUIVALENCE

4.1.9 Narrow therapeutic index drugs

In specific cases of products with a narrow therapeutic index, the acceptance interval for AUC should be tightened to 90.00-111.11%. Where Cmax is of particular importance for safety, efficacy or drug level monitoring the 90.00-111.11% acceptance interval should also be applied for this parameter. It is not possible to define a set of criteria to categorise drugs as narrow therapeutic index drugs (NTIDs) and it must be decided case by case if an active substance is an NTID based on clinical considerations.

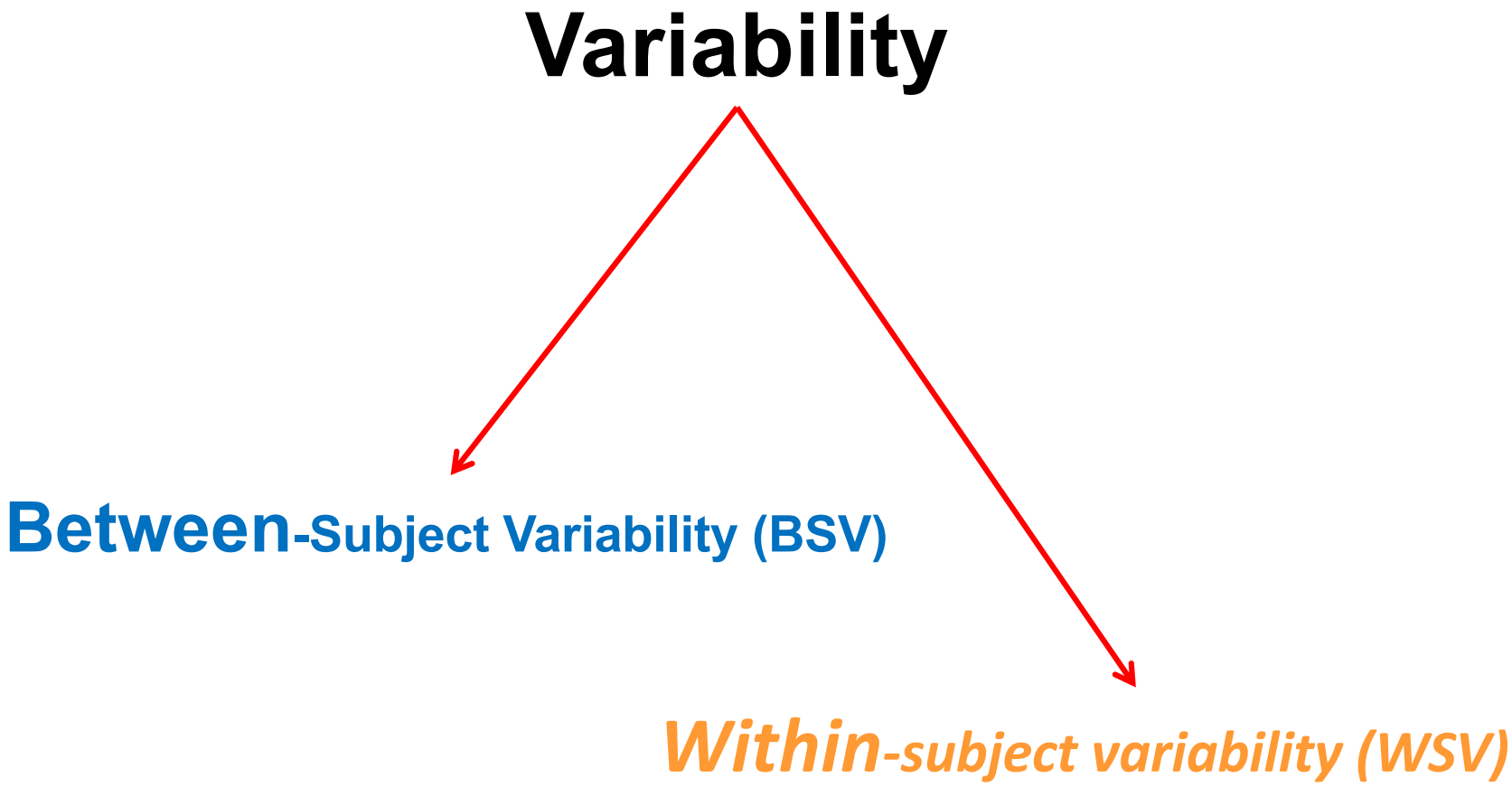

80.00
90.00
100%
111.11
125.00
tighter limits of acceptance


Shrunk 90% CI
80.00
90.00
100%
111.11
125.00

A6.

Highly variable drugs

A general classification



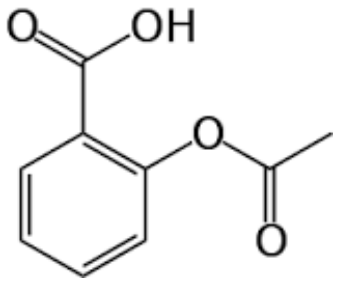
A regulatory definition …

Highly variable drug (or drug product):

= when the observed **coefficient of variation** (for a PK parameter)

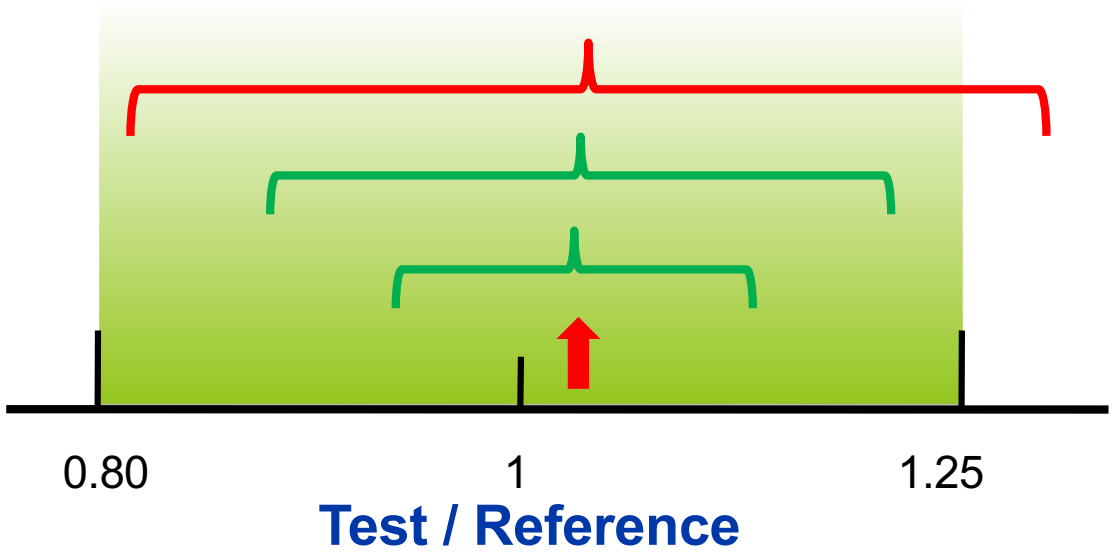
of the **within-subject** is **≥ 30%**,

regardless if it is due to the drug substance itself or comes from the product properties

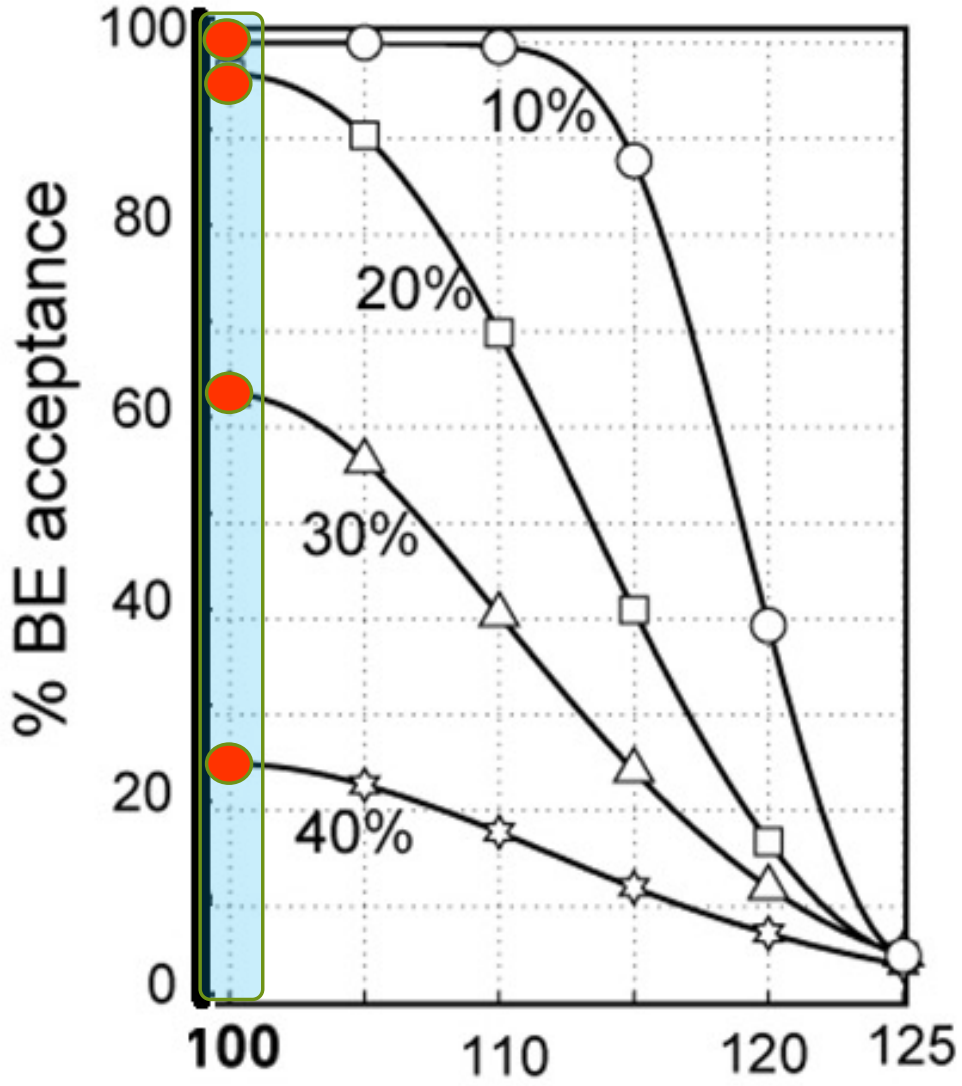


The impact of variability

Mean difference $\pm t_{\alpha,f(\mathrm{N})}$ x **Variability** x f (sample size)



Innovator's vs. Innovator's



- Same Lot
- Same blister
- **Different WSV**

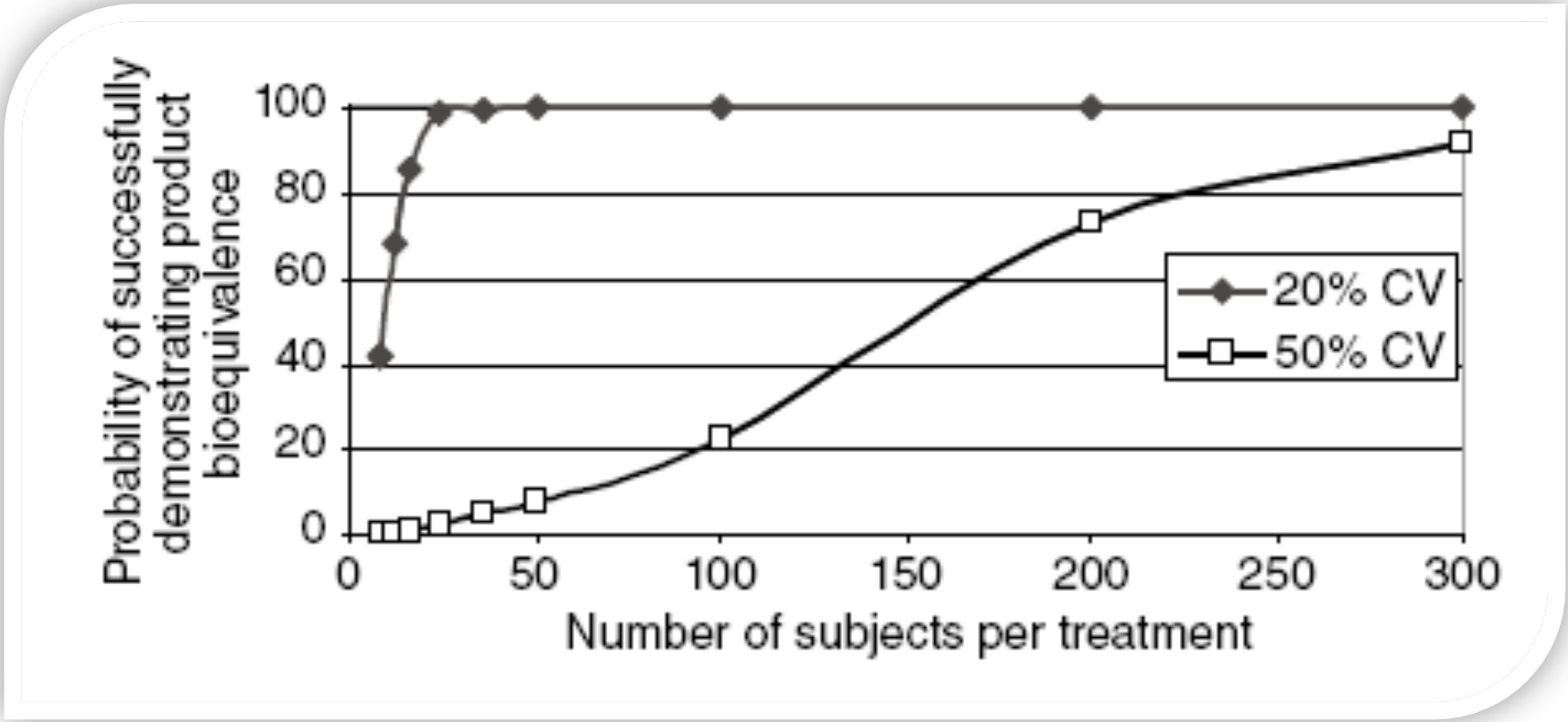
24 volunteers in a 2x2 design

Approaches to deal with high intrasubject variability

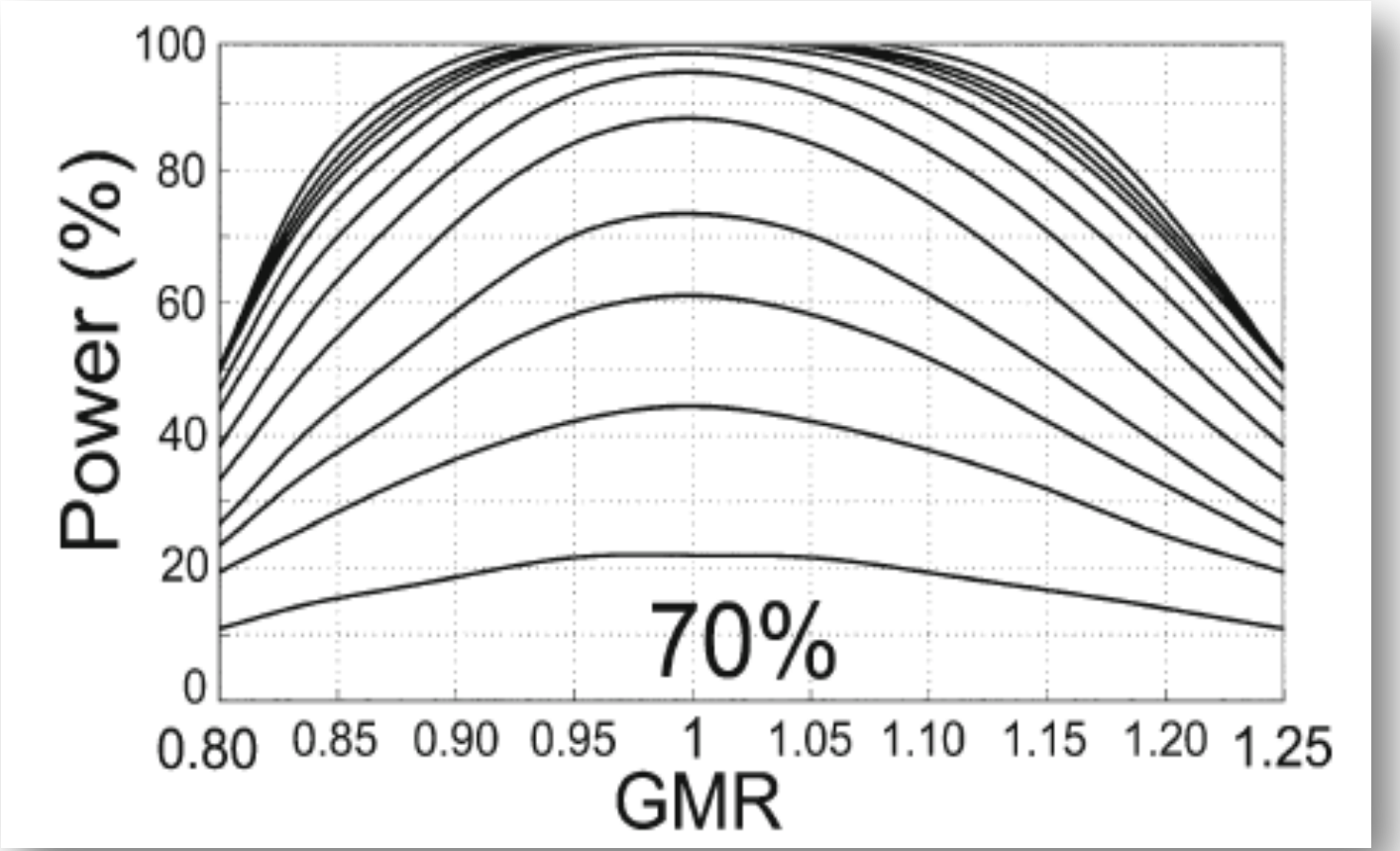
- ✓ **Increase** sample size
- ✓ **Steady-state** studies
- ✓ **Replicate** designs
- ✓ **Widening** the BE limits to prefixed constant values

  (e.g. 0.75-1.33, 0.70–1.43)
- ✓ **Individual** Bioequivalence
- ✓ **Scaled BE limits - Scaled Average Bioequivalence**

a) Sample size

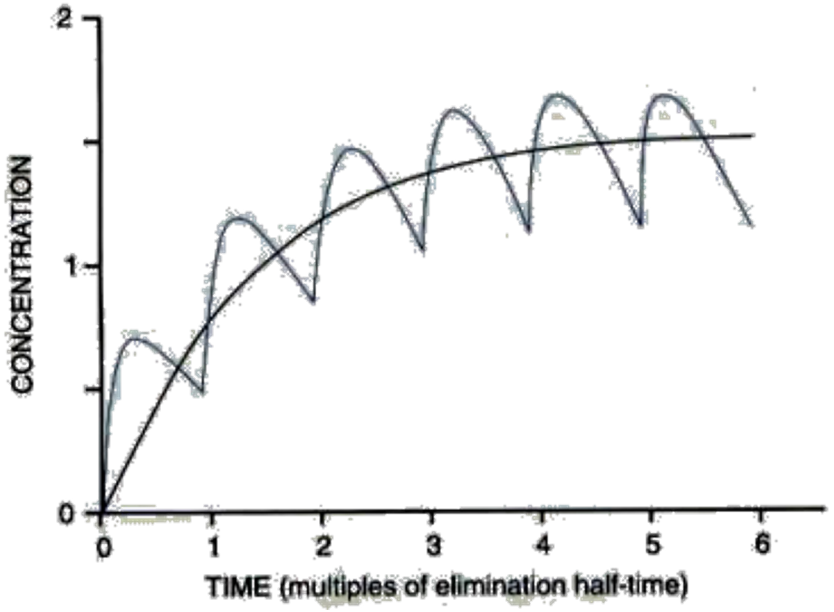


The effect of sample size



N

b. Steady state studies

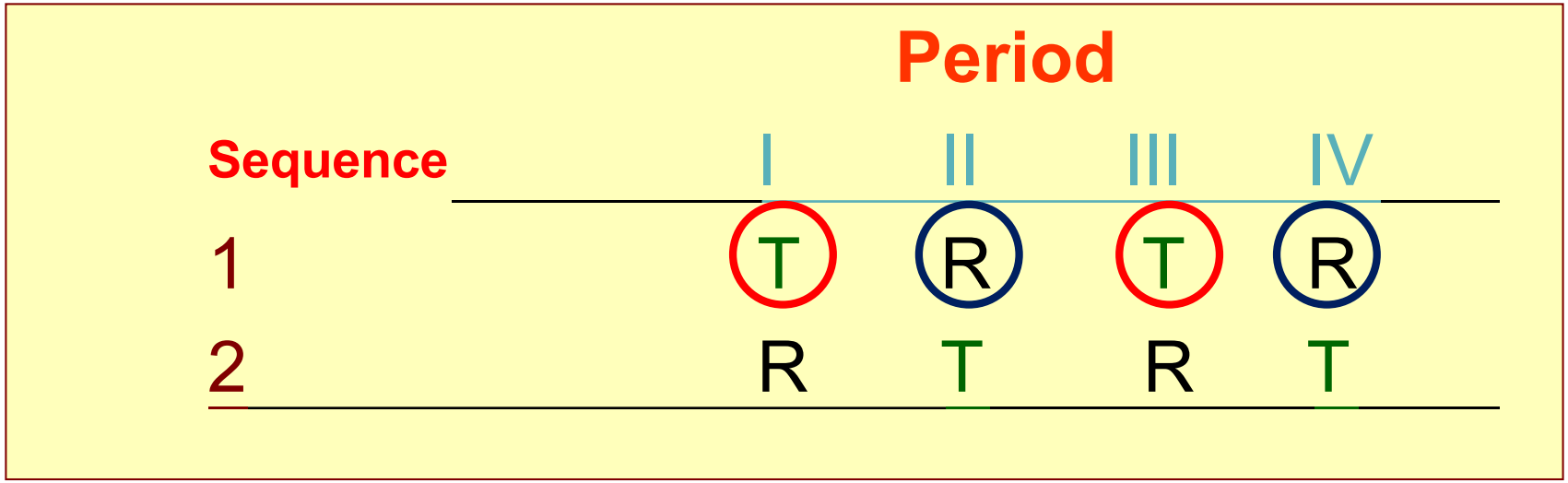


Multiple dosing

Variability of PK parameters is usually lower after multiple administration

- H. Blume, M. Elze, H. Potthast, and B. Schug. Practical strategies and design advantages in highly variable drug studies: multiple dose and replicate administration design. In H.H. Blume and K. Midha (eds.), *Bio-international '92: Bioavailability, Bioequivalence, and Pharmacokinetic Studies*. Medpharm, Stuttgart, 1995, pp. 117–122.
- Schug BS, Elze M, Blume HH. Bioequivalence of highly variable drugs and drug products: steady state studies. In: Midha KK, Nagai T, eds. *Bioavailability, Bioequivalence and Pharmacokinetic Studies.* Tokyo: Academic Societies Japan, 1996:101–6.

c) Replicate design - ABE



❖ H. Blume, M. Elze, H. Potthast, and B. Schug. Practical strategies and design advantages in highly variable drug studies: multiple dose and replicate administration design. In H.H. Blume and K. Midha (eds.), *Bio-international '92: Bioavailability, Bioequivalence, and Pharmaokinetic Studies*. Medpharm, Stuttgart, 1995, pp. 117–122.

❖ K.K. Midha, M. Rawson, J.W. Hubbard, E.D. Ormsby. Practical strategies and design advantages in highly variable drug studies: Replicate design. In H.H. Blume and K. Midha (eds.), *Bio-international '92: Bioavailability, Bioequivalence, and Pharmaokinetic Studies*. Medpharm, Stuttgart, 1995, pp. 117–122.

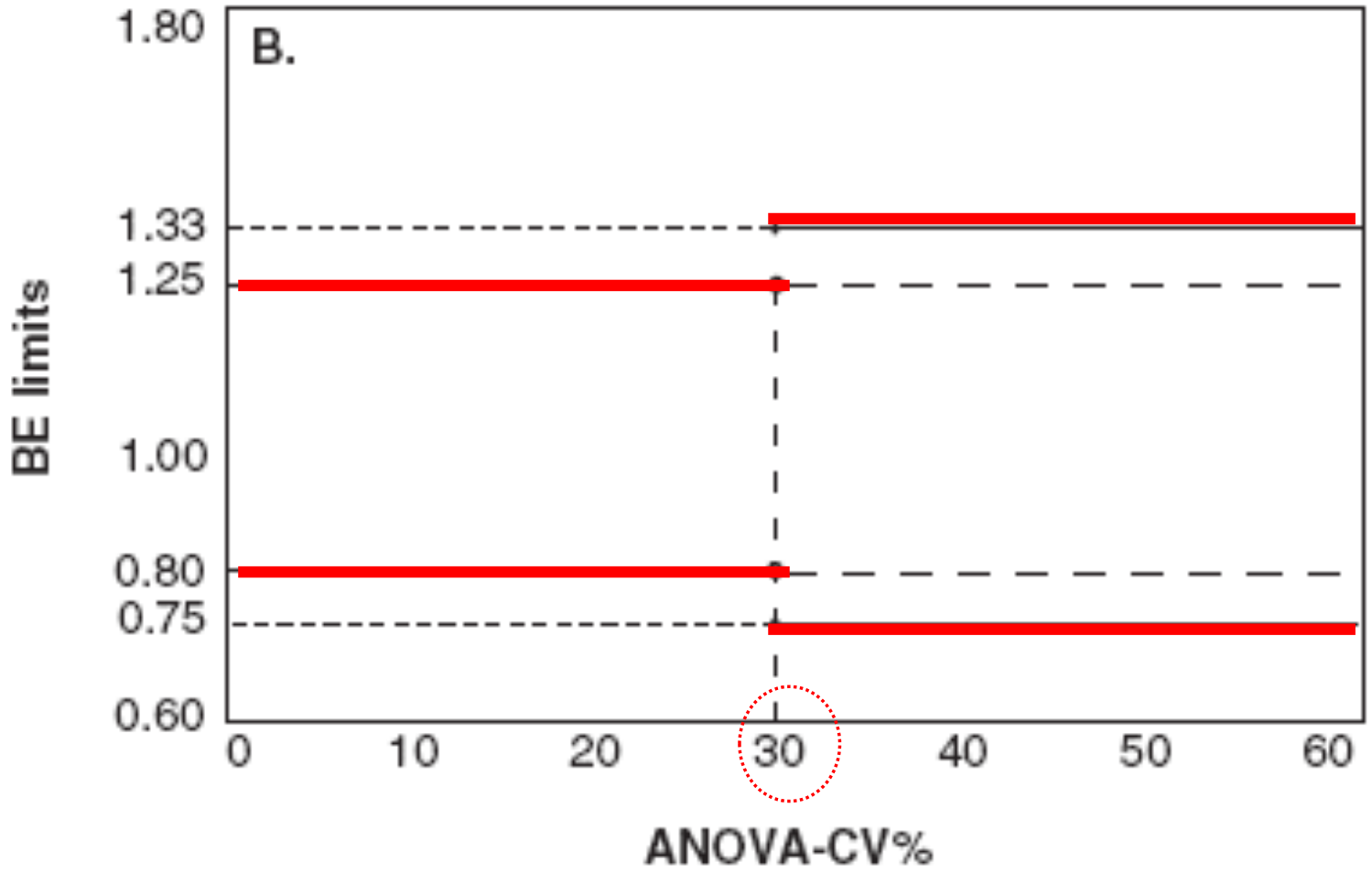
❖ V. Shah, A. Yacobi, W. Barr, L. Benet, D. Breimer, M. Dobrinska, L. Endrenyi, W. Fairweather, W. Gillespie, M. Gonzales, J. Hooper, A. Jackson, L. Lesko, K. Midha, P. Noonan, R. Patnaik, and R. Williams. Evaluation of orally administered highly variable drugs and drug formulations. *Pharm. Res*. 13:1590–1594 (1996).

d) Widening BE limits to pre-fixed values

❖ European Agency for the Evaluation of Medicinal Products. Note for Guidance on the Investigation of Bioavailability and Bioequivalence. Committee for Proprietary Medicinal Products (CPMP), London, 2001.

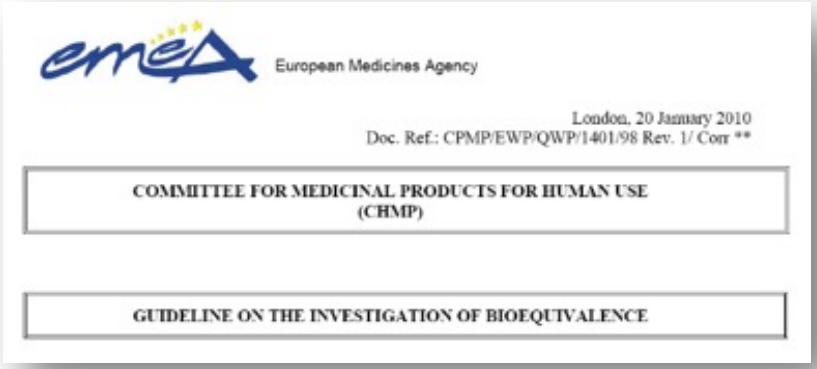
❖ H. Blume, I. McGilveray, and K. Midha. Report of consensus meeting: Bio-international'94, Conference on Bioavailability, Bioequivalence and Pharmacokinetics studies, Munich, Germany, 14-17 June 1994. *Eur. J. Pharm. Sci.* 3:113–124 (1995).

❖ L. Tothfalusi, L. Endrenyi, and K. Midha. Scaling or wider bioequivalence limits for highly variable drugs and for the special case of Cmax. *Int. J. Clin. Pharmacol. Ther.* 41:217–225 (2003).


B.
1.80
1.33
1.25
1.00
0.80
0.75
0.60
BE limits
0
10
20
30
40
50
60
ANOVA-CV%

e. Scaled BE limits –

Scaled Average Bioequivalence



European Medicines Agency

London, 20 January 2010
Doc. Ref.: CPMP/EWP/QWP/1401/98 Rev. 1/ Corr **

COMMITTEE FOR MEDICINAL PRODUCTS FOR HUMAN USE
(CHMP)

GUIDELINE ON THE INVESTIGATION OF BIOEQUIVALENCE

4.1.10 Highly variable drugs or drug products

Highly variable drug products (HVDP) are those whose intra-subject variability for a parameter is larger than 30%. If an applicant suspects that a drug product can be considered as highly variable in its rate and/or extent of absorption, a replicate cross-over design study can be carried out.

Those HVDP for which a wider difference in C_{max} is considered clinically irrelevant based on a sound clinical justification can be assessed with a widened acceptance range. If this is the case the acceptance criteria for C_{max} can be widened to a maximum of 69.84 – 143.19%. For the acceptance interval to be widened the bioequivalence study must be of a replicate design where it has been demonstrated that the within-subject variability for C_{max} of the reference compound in the study is >30%. The applicant should justify that the calculated intra-subject variability is a reliable estimate and that it is not the result of outliers. The request for widened interval must be prospectively specified in the protocol.

The extent of the widening is defined based upon the within-subject variability seen in the bioequivalence study using scaled-average-bioequivalence according to $[U, L] = \exp[\pm k \cdot s_{WR}]$, where U is the upper limit of the acceptance range, L is the lower limit of the acceptance range, k is the regulatory constant set to 0.760 and s_{WR} is the within-subject standard deviation of the log-transformed values of C_{max} of the reference product. The table below gives examples of how different levels of variability lead to different acceptance limits using this methodology.

Within-subject CV (%)*	Lower Limit	Upper Limit
30	80.00	125.00
35	77.23	129.48
40	74.62	134.02
45	72.15	138.59
≥50	69.84	143.19

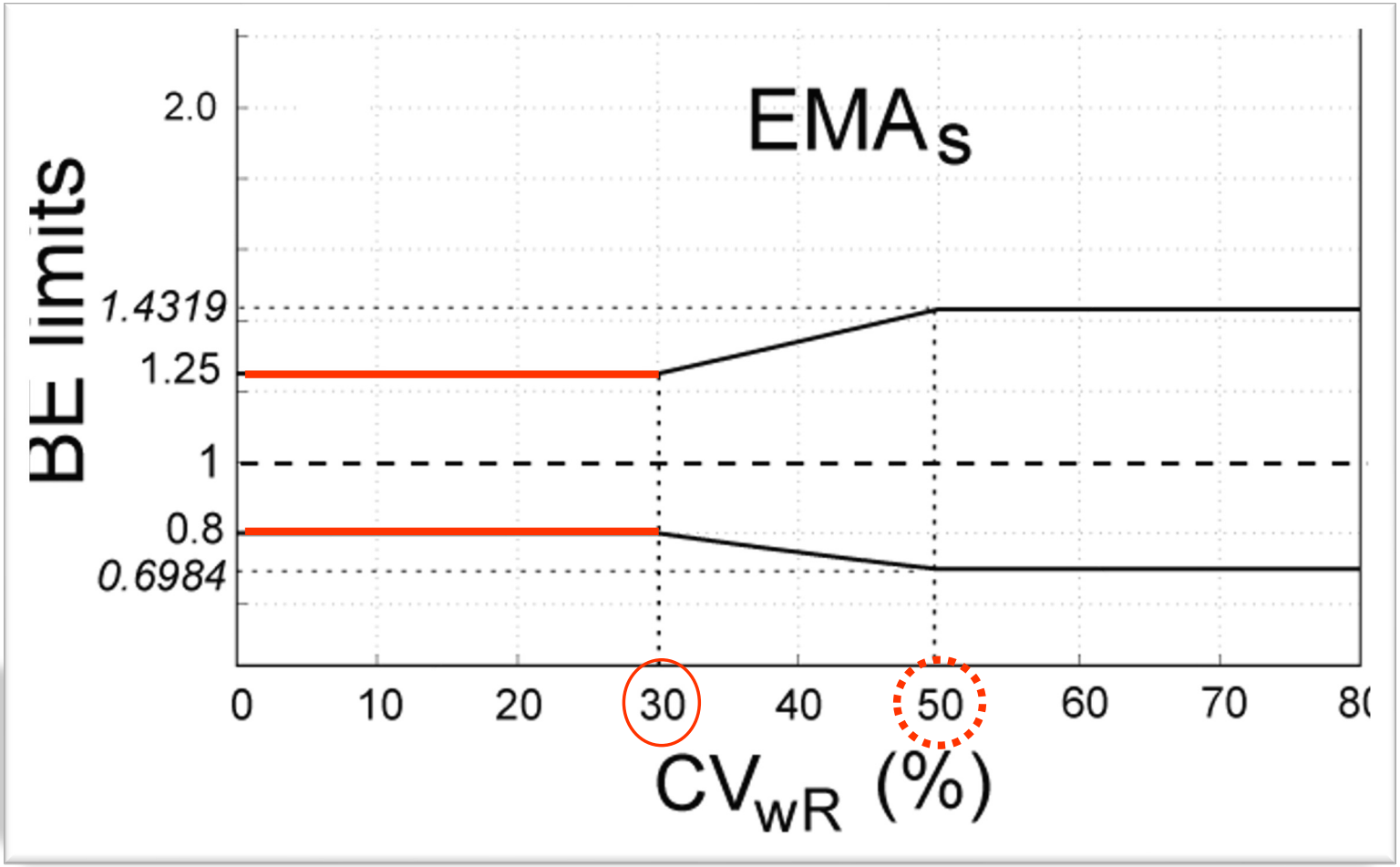
$$*\,CV(\%) = 100\sqrt{e^{s_{WR}^2} - 1}$$

The geometric mean ratio (GMR) should lie within the conventional acceptance range 80.00-125.00%.

The possibility to widen the acceptance criteria based on high intra-subject variability does not apply to AUC where the acceptance range should remain at 80.00 – 125.00% regardless of variability.

It is acceptable to apply either a 3-period or a 4-period crossover scheme in the replicate design study.

- **C_{max}**
- **GMR constraint: 0.80-1.25**
- CV_{WR} **> 0.30 ➔** scaling with **s_{WR}**
- Maximum CV_{WR}: **0.50 ➔ extreme limits: 69.84 - 143.19%**
- **3-** or **4-period** designs



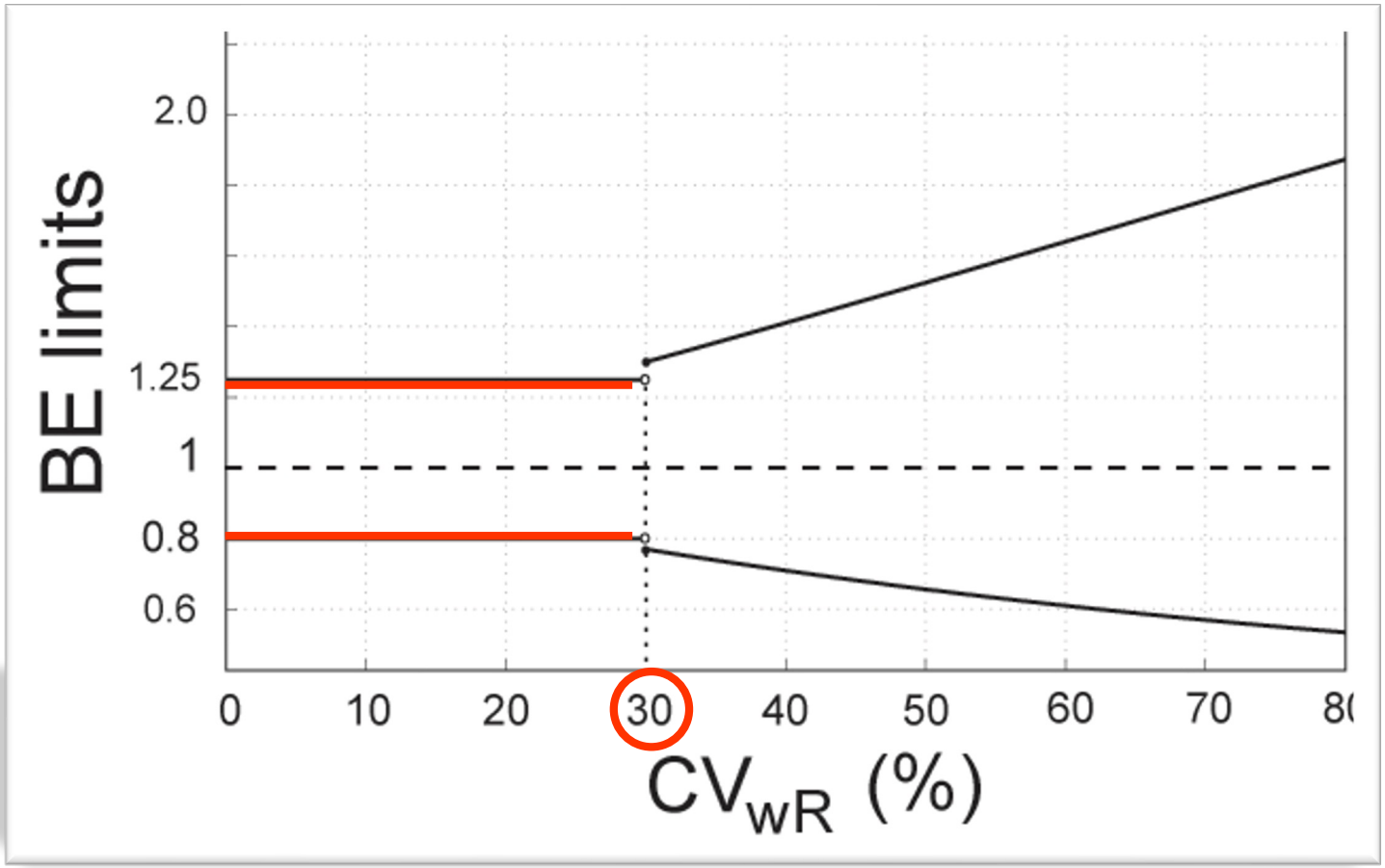
Upper/lower BE limit = $\exp(\pm k \cdot s_{WR})$

- *ln(1.25)*
- *CV_W=30%*

➔ ***k* = 0.760**

- **C_{max}, AUC**
- CV_{WR} **≥ 0.30** ➔ scaling with **s_{WR}**
- **GMR constraint**: **0.80-1.25**
- **3-** or **4-period** designs

- Davit B. Meeting of FDA Committee for Pharmaceutical Science, Rockville, MD. 2006, October 6.
- Haidar S. Meeting of FDA Committee for Pharmaceutical Science, Rockville, MD. 2006, October 6.
- Davit B. AAPS/FDA Workshop on BE, BCS, and Beyond, North Bethesda, MD. 2007, May 22.
- Haidar S. AAPS/FDA Workshop on BE, BCS, and Beyond, North Bethesda, MD. 2007, May 22.
- Haidar S, Davit B, Chen ML, et al., Pharm Res. 2008;15:237-41.
- Haidar S, Makhlouf F, Schuirmann D, et al., AAPS J. 2008;10:450-4.

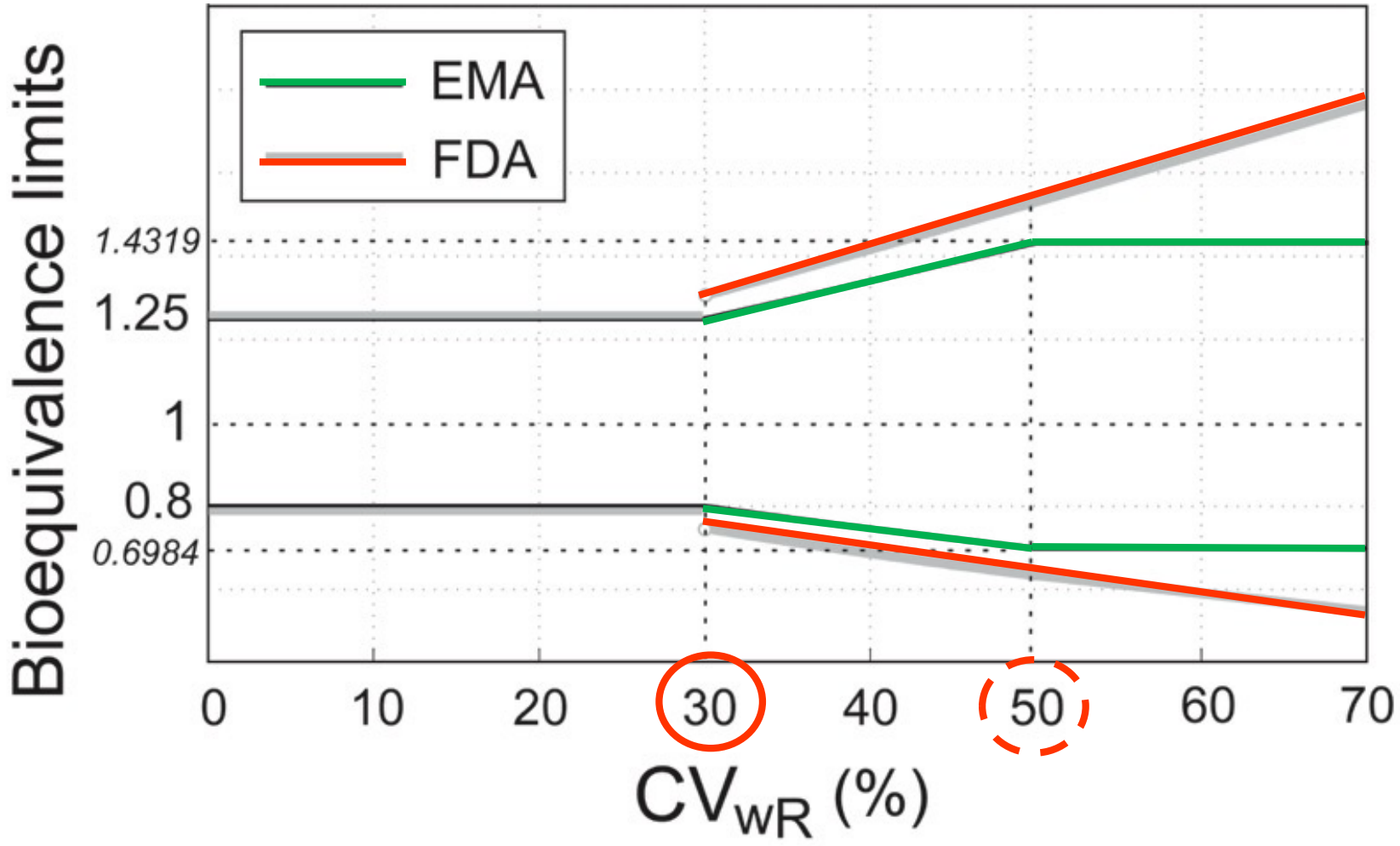


$$\text{Upper, Lower BE limits} = \exp\left(\pm \ln(1.25) \cdot \frac{\sigma_{WR}}{\sigma_{reg}}\right)$$

- s_{WR}
- s_{reg}: =0.25

➔ *k=0.892*

The scaled approaches of the **EMA** and the **FDA**

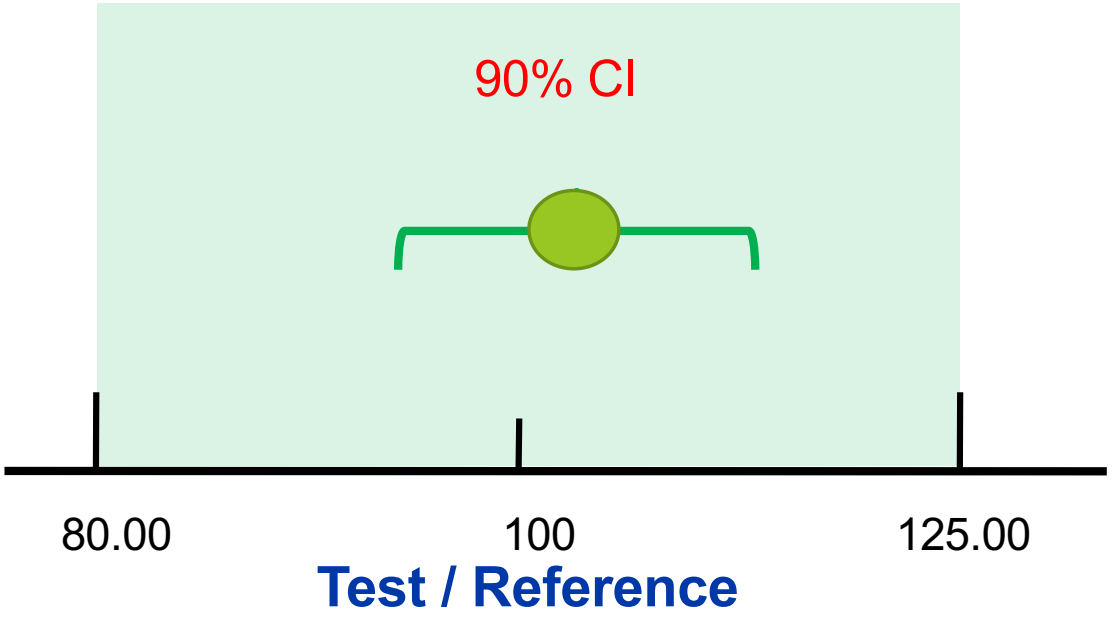


A7.

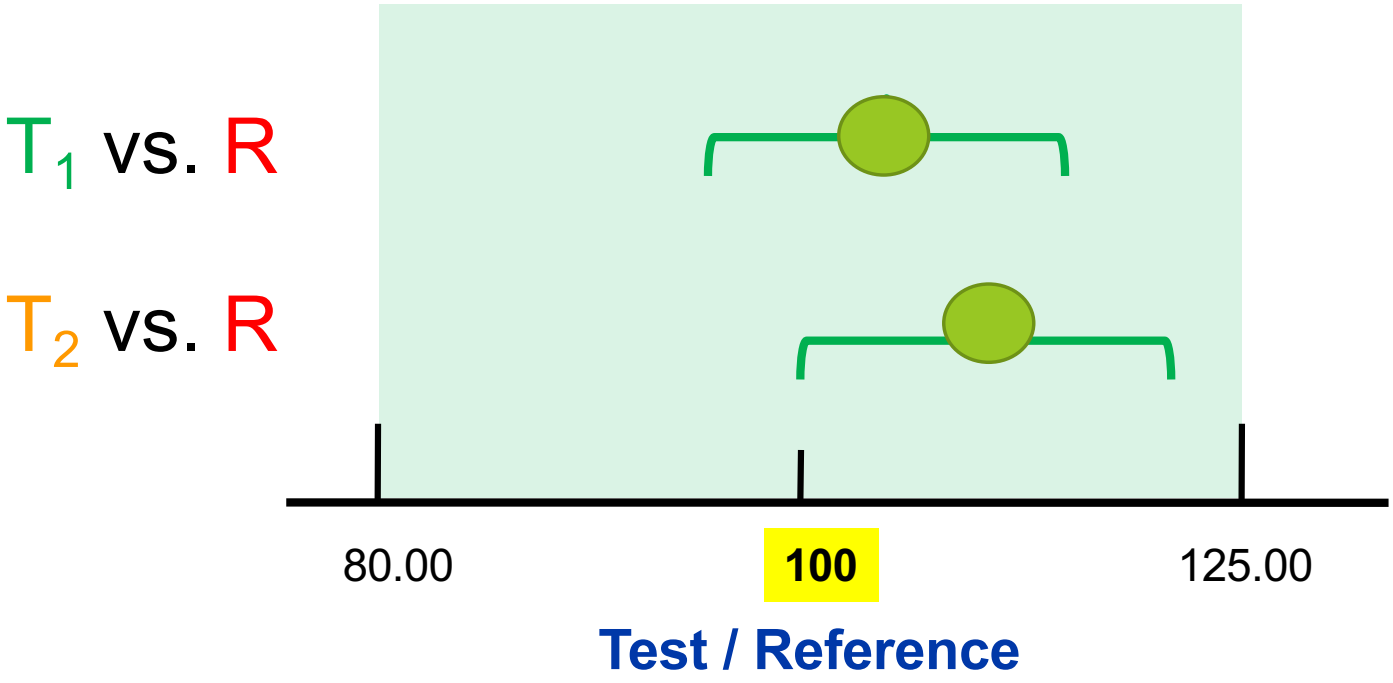
Drugs interchangeability

Classically, according to the existing BE assessment, we **believe that it is ensured**:

Reference → Test



... towards the same side

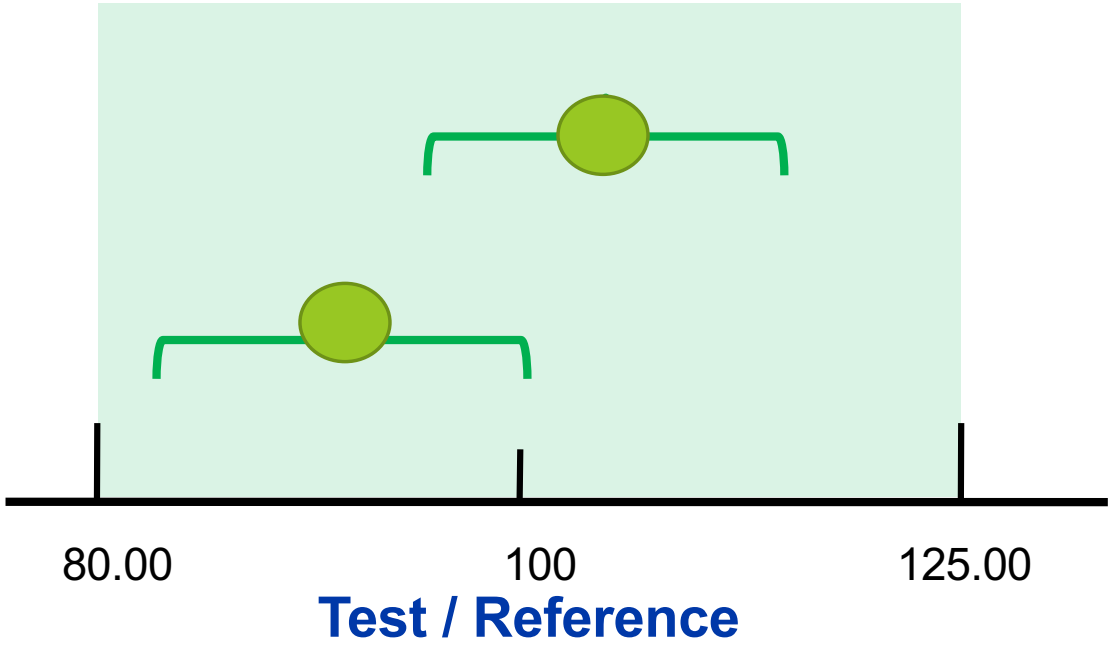


➔ Probably, more confident

… different sides

T_1 vs. R

T_2 vs. R



?

!! There is chance that BE between T_1 and T_2 is **not ascertained** … **even though** both of them were found to be bioequivalent with the R product.

A8.

Clinical design

Most appropriate

The **highest probability** to prove BE.

&

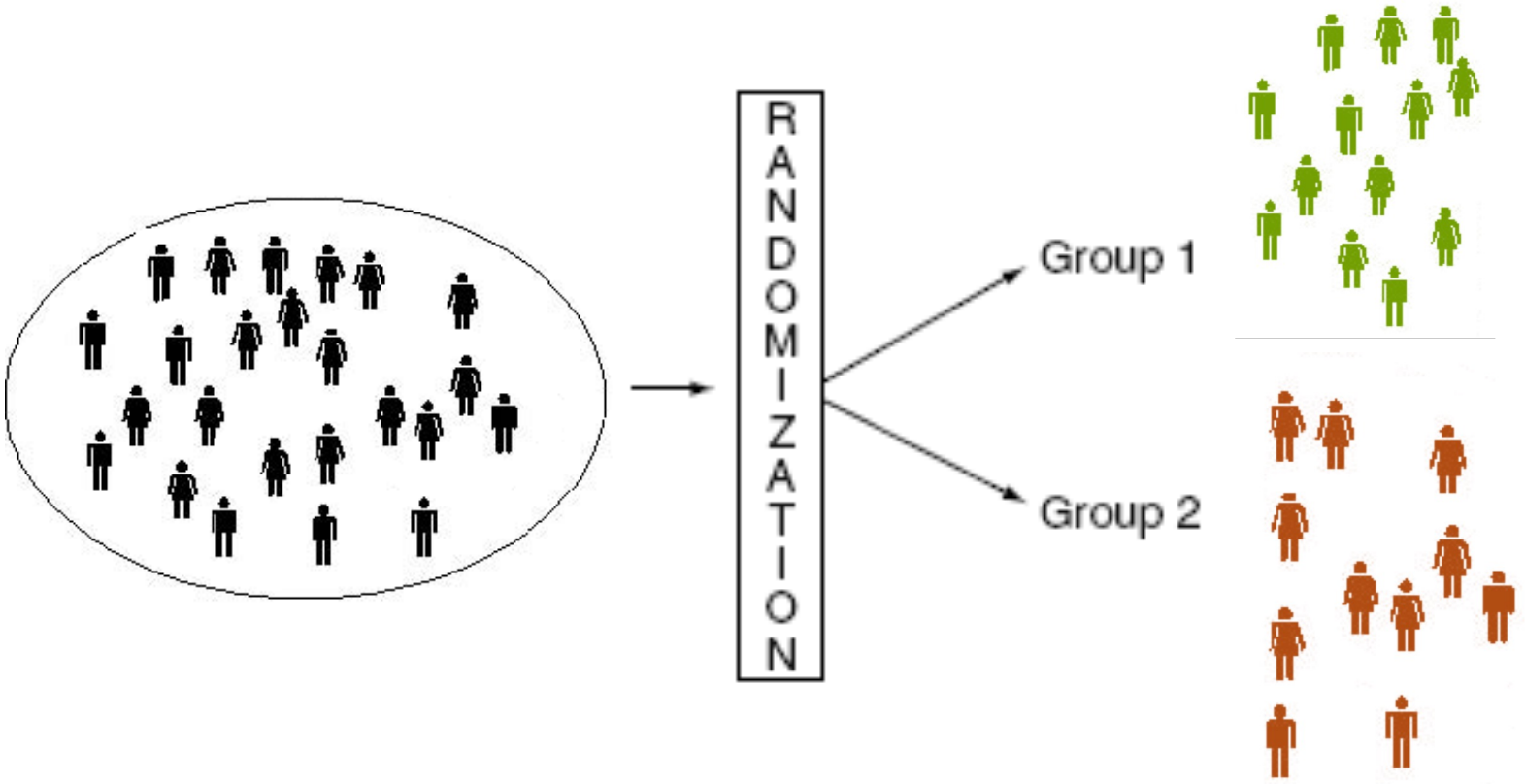
The **least human exposure** to drugs:

- sample size
- number of administrations per subject

&

The **lowest cost** for the sponsor.

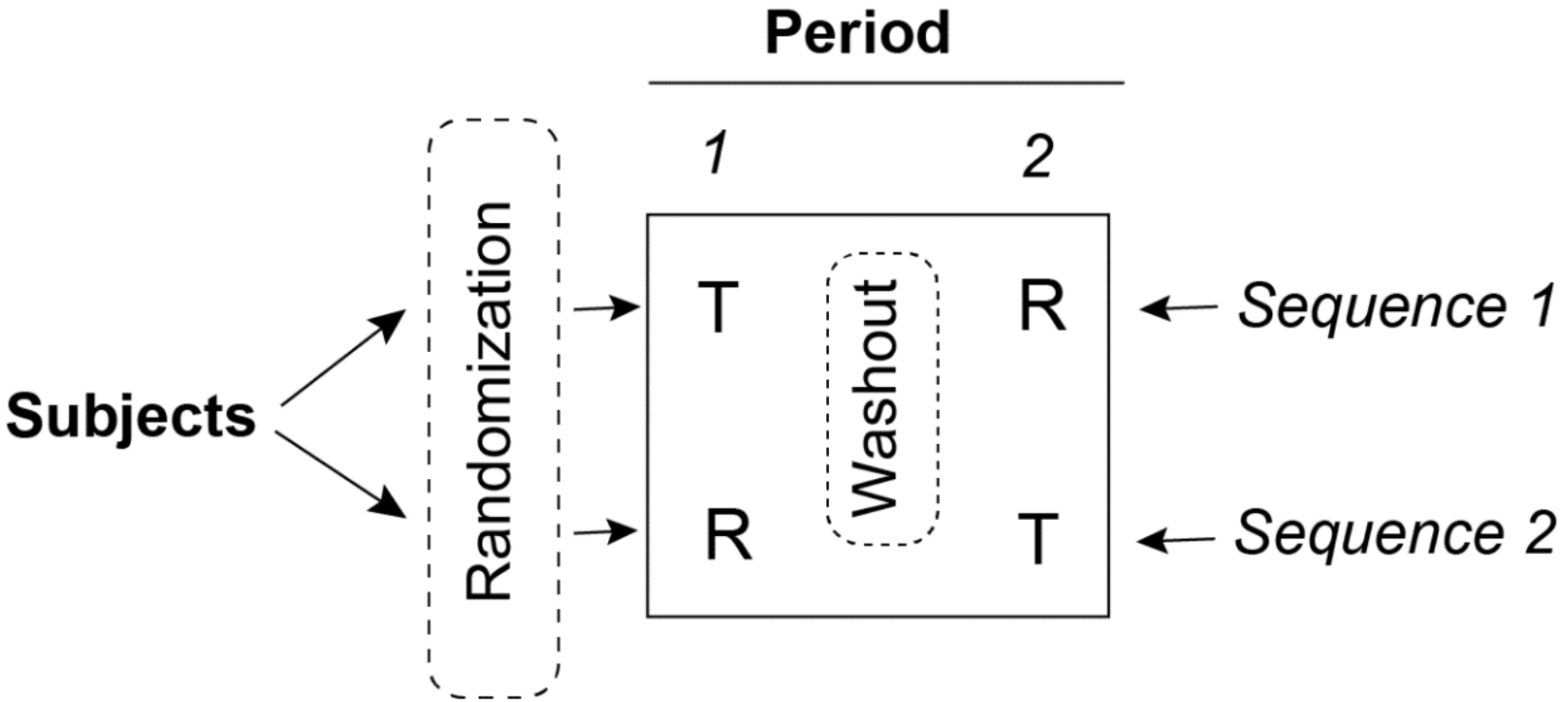
Parallel design



Currently: Long half-life drugs

2x2 Crossover design

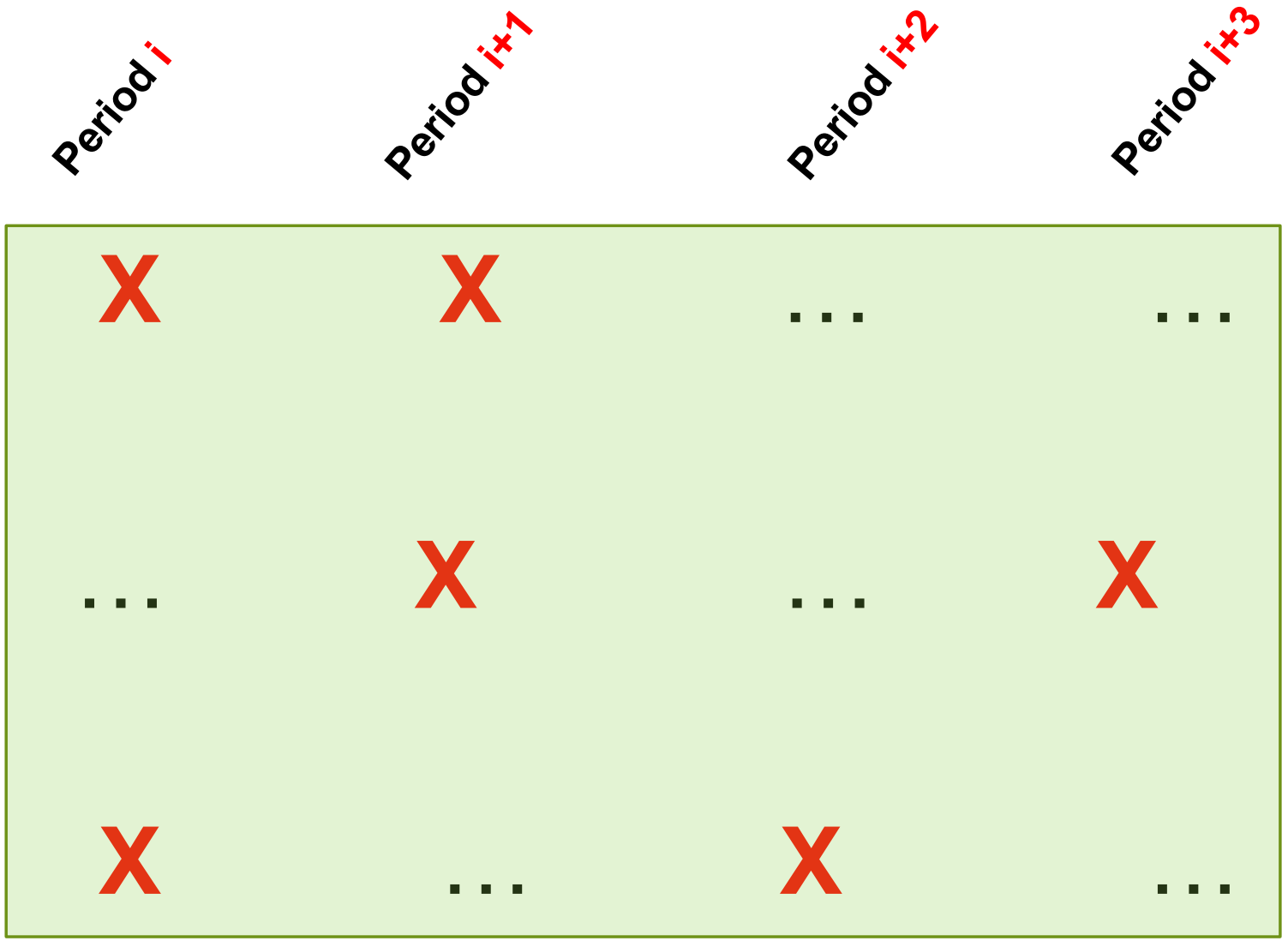
The Traditional design



Alternative designs:

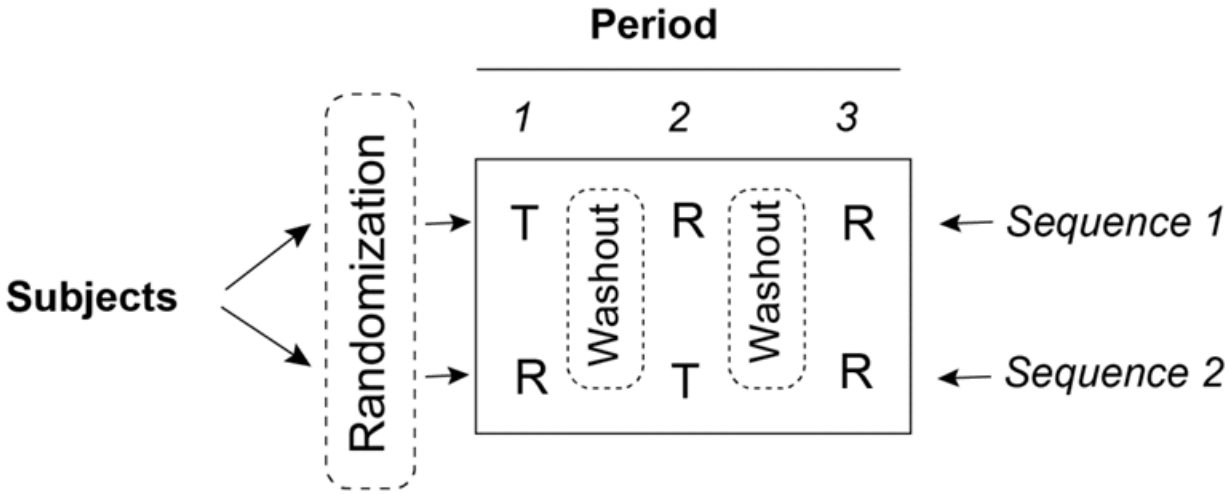
- Replicate
- Adaptive: Two-stage designs

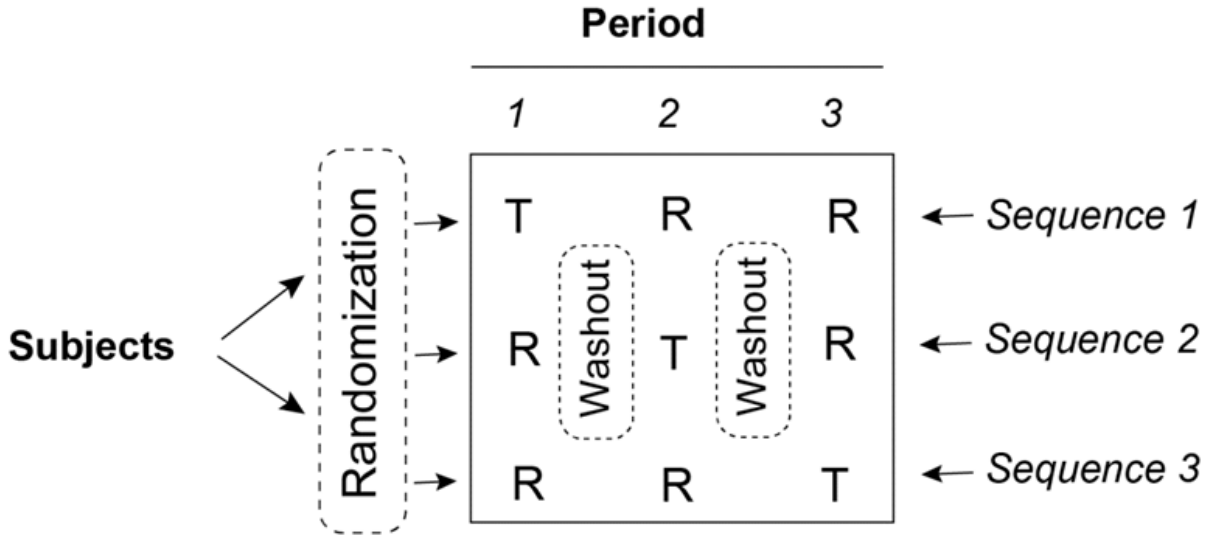
Replicate designs



3-period

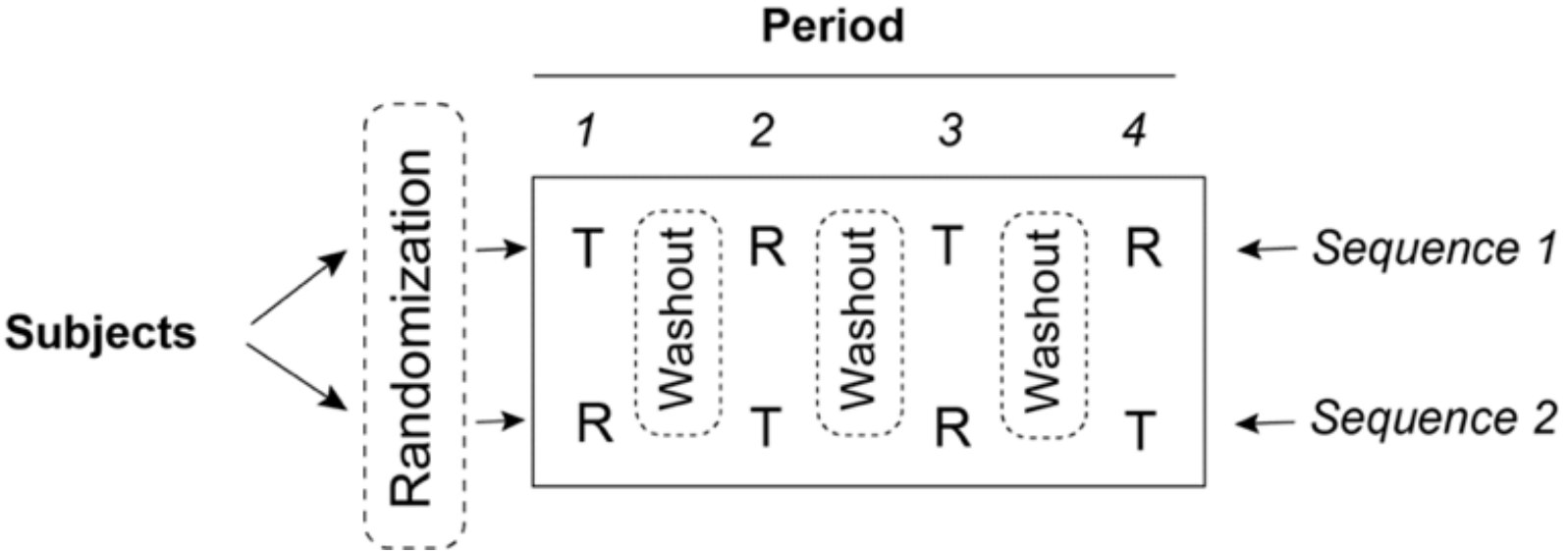
R → R


Period
1
2
3
Subjects
Randomization
T
R
R
Washout
Washout
R
T
R
Sequence 1
Sequence 2


Period
1
2
3
Subjects
Randomization
T
R
R
Washout
Washout
R
T
R
R
R
T
Sequence 1
Sequence 2
Sequence 3

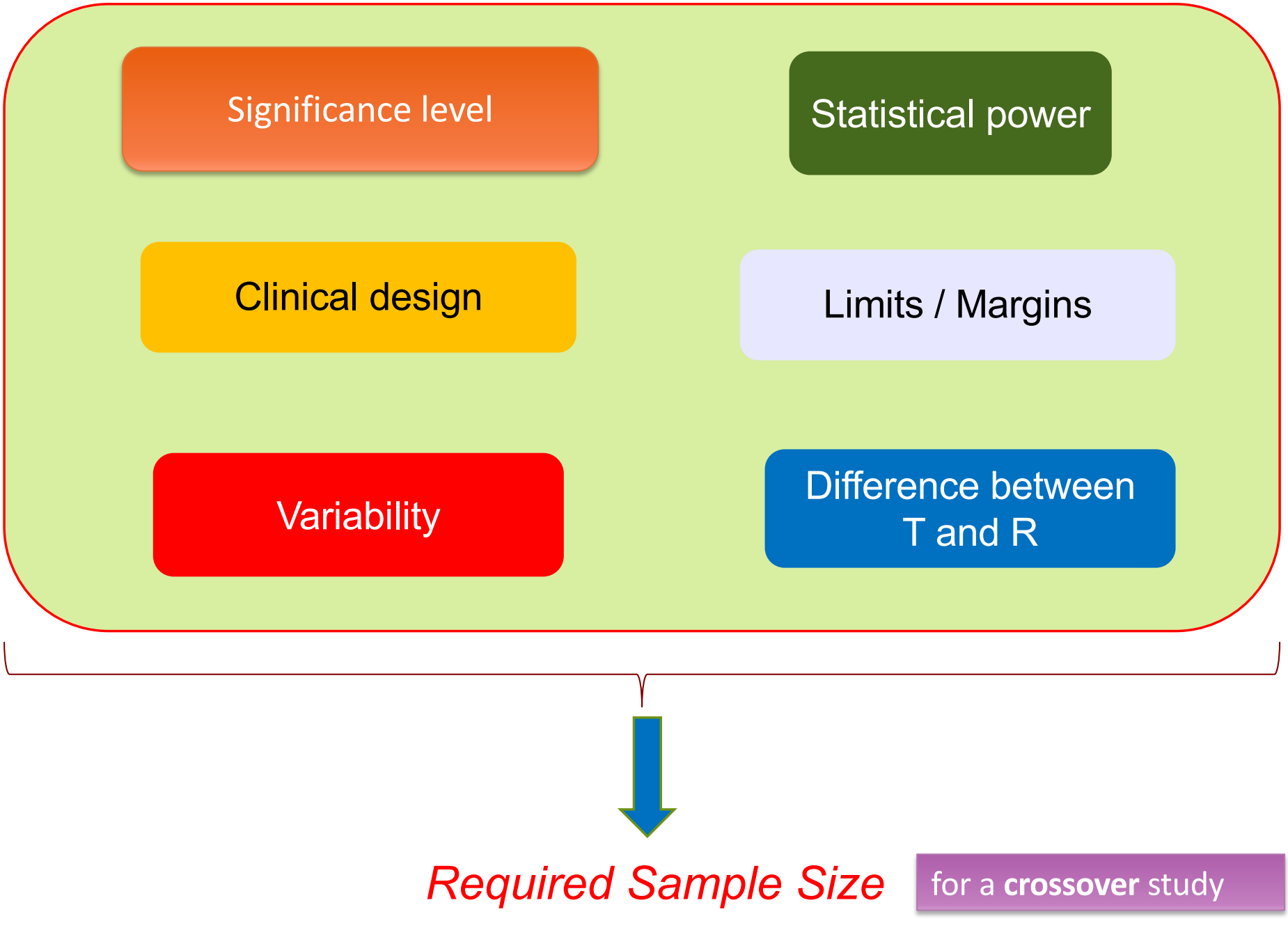
4-period

R → R
and
T → T



A9.

Sample size estimation


Significance level
Statistical power
Clinical design
Limits / Margins
Variability
Difference between T and R
Required Sample Size
for a **crossover** study

Tabular form
(EMA, US-FDA)

Table A1. Sample sizes for the requirements of EMA in 3-period studies

CV	80% POWER GMR 0.85	0.90	0.95
30%	194	53	27
35%	127	51	29
40%	90	44	29
45%	77	40	29
50%	75	40	30
55%	81	42	32
60%	88	46	36
65%	99	53	40
70%	109	58	45
75%	136	67	50
80%	144	72	54

Sample sizes for the requirements of FDA in 3-period studies

CV	80% POWER GMR 0.85	0.90	0.95	1.00	1.05
30%	145	45	24	21	24
35%	74	37	24	22	25
40%	60	33	24	22	24
45%	59	31	23	22	24
50%	66	30	24	22	23
55%	80	30	24	22	24
60%	88	31	24	23	24
65%	98	32	25	24	25
70%	106	35	26	25	26
75%	136	38	27	26	27
80%	144	40	29	27	29

Multiplicative model

$$n \geq [t(\alpha, 2n-2) + t(\beta/2, 2n-2)]^2 [CV/\ln 1.25]^2,$$

if $1 < \theta < 1.25$

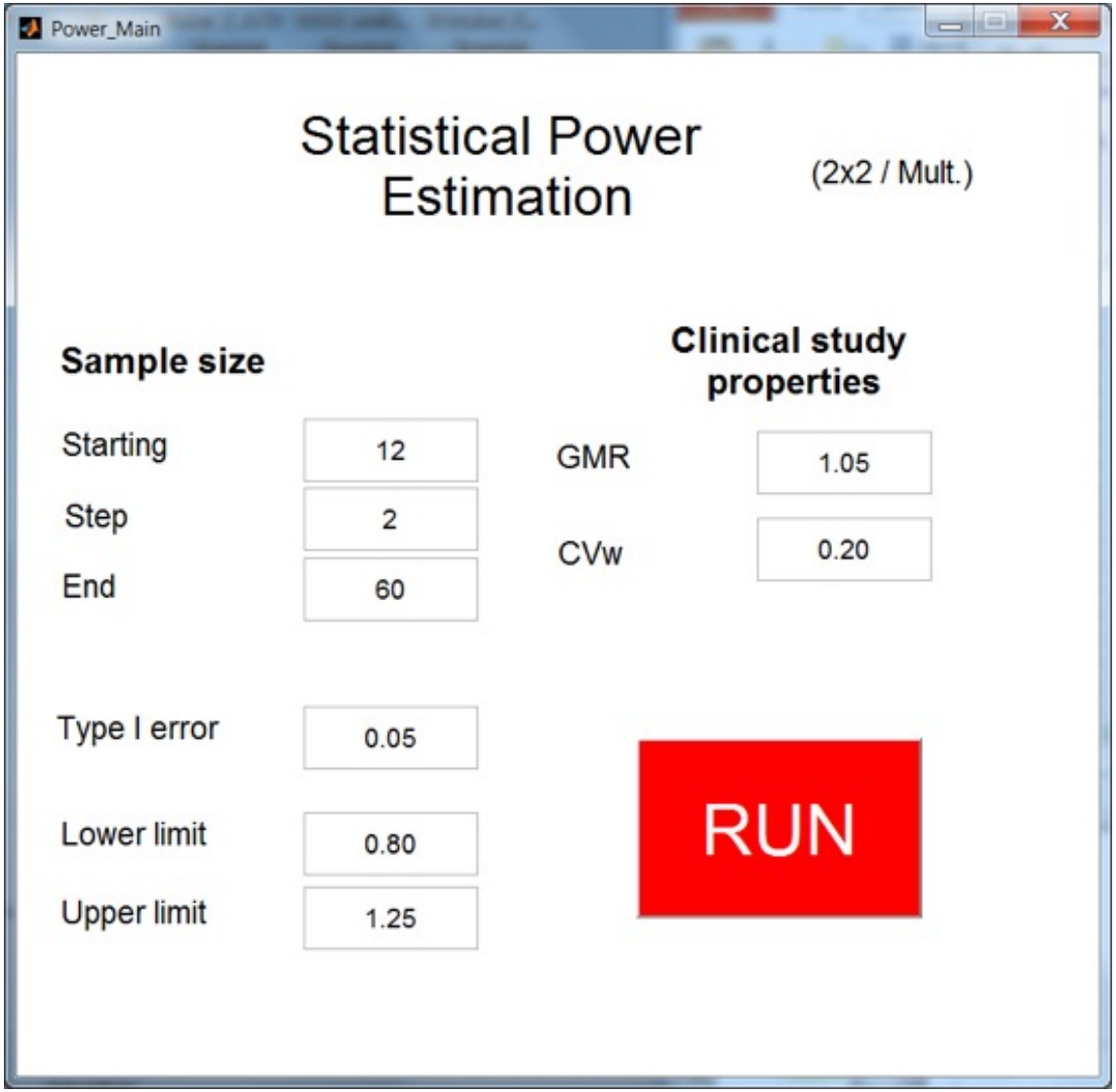
$$n \geq [t(\alpha, 2n-2) + t(\beta, 2n-2)]^2 [CV/(\ln 1.25 - \ln \theta)]^2$$

and if $0.8 < \theta < 1$

$$n \geq [t(\alpha, 2n-2) + t(\beta, 2n-2)]^2 [CV/(\ln 0.8 - \ln \theta)]^2$$

Diletti E, Hauschke D, Steinijans VW. Sample size determination for bioequivalence assessment by means of confidence intervals. *Int J Clin Pharmacol Ther Toxicol* 1991;29:1-8.

Hauschke D, Steinijans VW, Diletti E, Burke M. Sample size determination for bioequivalence assessment using a multiplicative model. *J Pharmacokinet Biopharm* 1992;20:557-61.


Power_Main
Statistical Power Estimation
(2x2 / Mult.)
Sample size
Starting
12
Step
2
End
60
Type I error
0.05
Lower limit
0.80
Upper limit
1.25
Clinical study properties
GMR
1.05
CVw
0.20
RUN

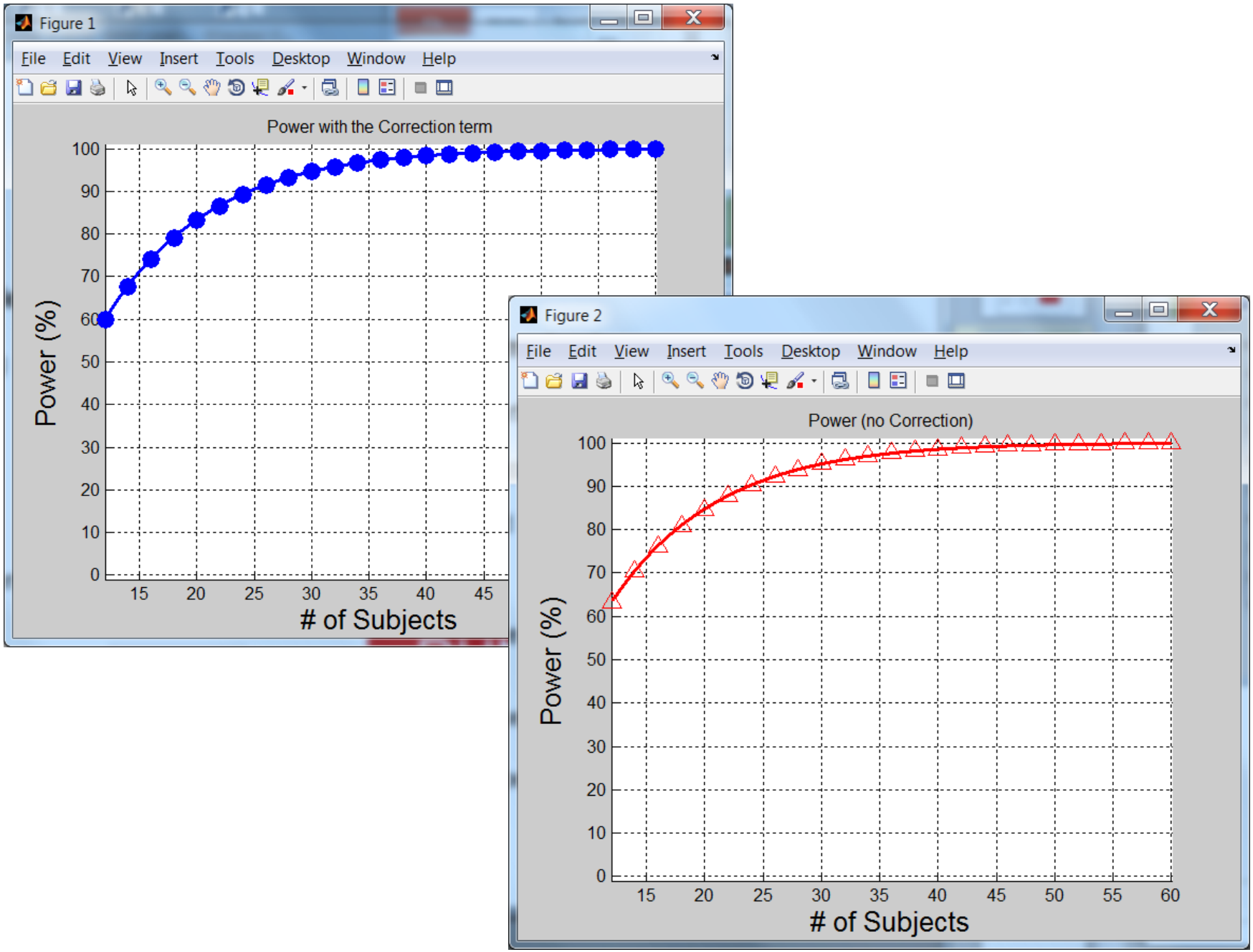
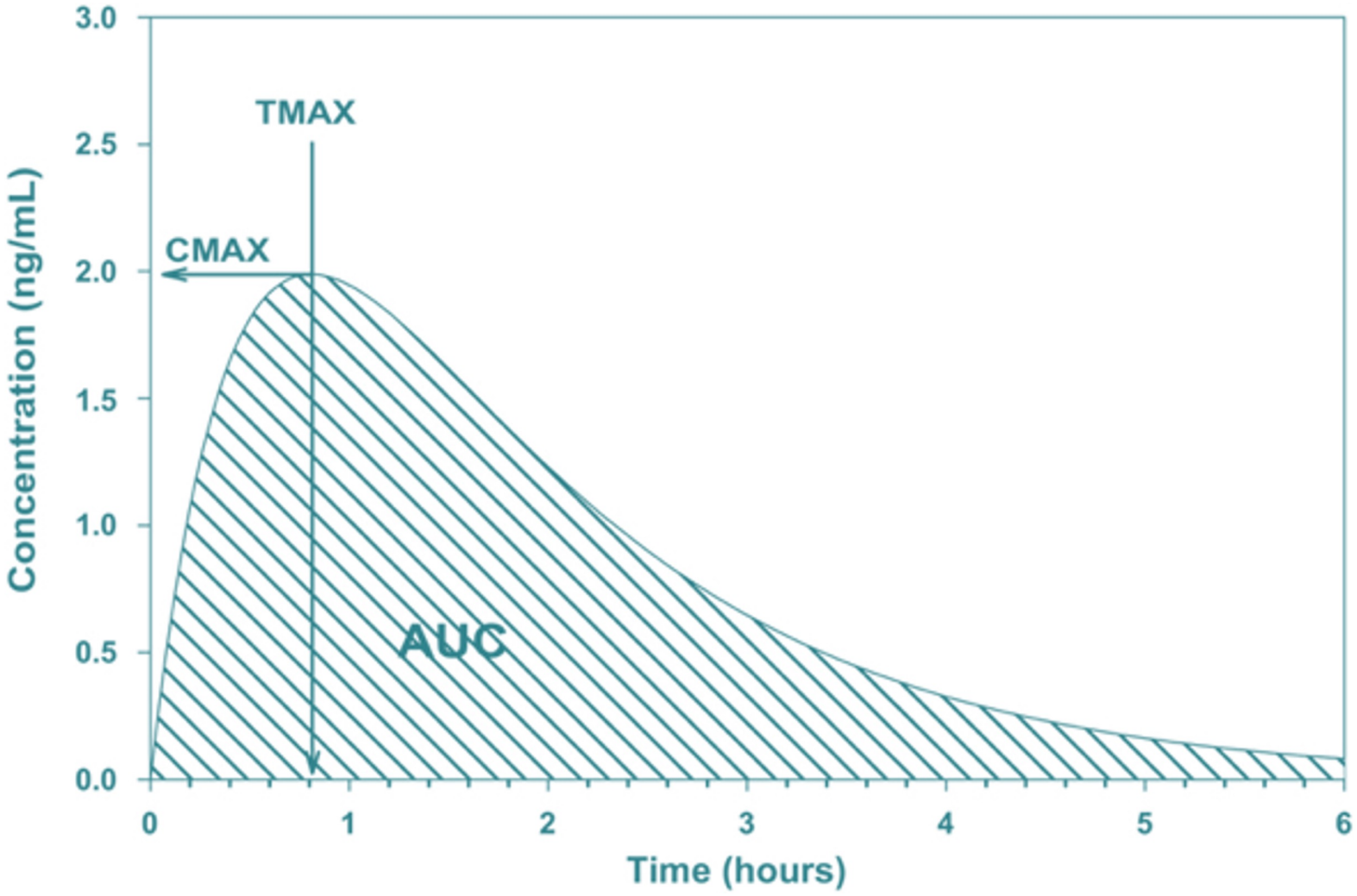


Figure 1
File Edit View Insert Tools Desktop Window Help
Power with the Correction term
Power (%)
# of Subjects
Figure 2
File Edit View Insert Tools Desktop Window Help
Power (no Correction)
Power (%)
# of Subjects

A10.

Re-inventing the “rate of absorption”

Rate of absorption



What is “**rate**”?

Rate: a quantity, amount, or degree of something measured per unit of something else

Rate = dx / dt

applied sciences https://doi.org/10.3390/app13010418 MDPI

Article

Machine Learning in Bioequivalence: Towards Identifying an Appropriate Measure of Absorption Rate

applied sciences https://doi.org/10.3390/app13042257 MDPI

Article

On the Interplay between Machine Learning, Population Pharmacokinetics, and Bioequivalence to Introduce Average Slope as a New Measure for Absorption Rate

pharmaceuticals https://doi.org/10.3390/ph16050725 MDPI

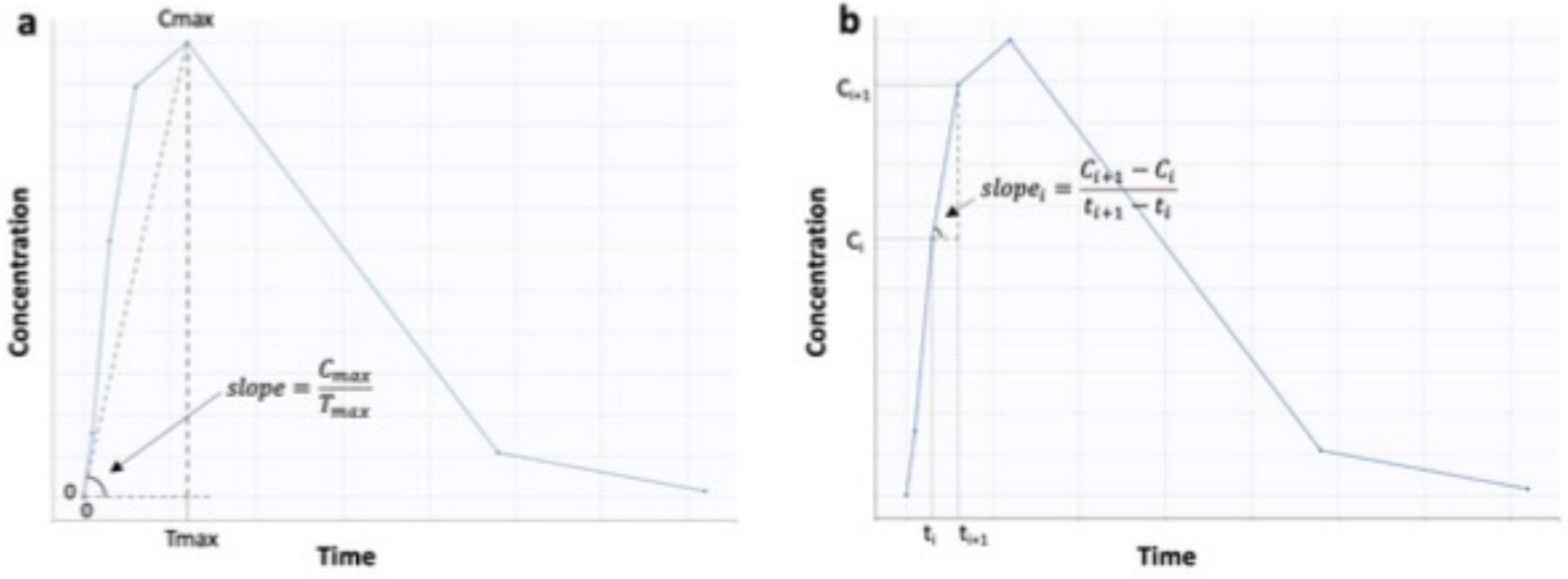
Article

An In Silico Approach toward the Appropriate Absorption Rate Metric in Bioequivalence

In Pharmacokinetics / Bioequivalence:

$$\text{Rate of absorption} = \frac{\text{Change in drug concentration}}{\text{Change in time}}$$

Average Slope (AS)



$$\text{slope}_i = \frac{C_{i+1} - C_i}{t_{i+1} - t_i} \quad \Rightarrow \quad AS = \frac{\sum_{i=1}^{n-1} \text{slope}_i}{n-1} = \frac{\sum_{i=1}^{n-1} \frac{C_{i+1} - C_i}{t_{i+1} - t_i}}{n-1}$$

More details:

$$\text{Sum of slopes} = \sum_{i=1}^{n-1} \frac{C_{i+1} - C_i}{t_{i+1} - t_i} = \sum_{i=1}^{n-1} \frac{C_{i+1} - C_i}{\Delta t_i}$$

In the special case where the sampling interval is constant ($\Delta t_i = \Delta t$):

$$\text{Sum of slopes} = \sum_{i=1}^{n-1} \frac{C_{i+1} - C_i}{\Delta t}$$

By eliminating the consecutive concentration values, we get:

$$\text{Sum of slopes} = \frac{C_n}{\Delta t}$$

$$AS = \frac{\text{Sum of slopes}}{\text{number of intervals}} = \frac{C_n}{n-1} = \frac{C_{max}}{\Delta t \times (n-1)}$$

The product $\Delta t \cdot (n-1)$ refers to Tmax, thus:

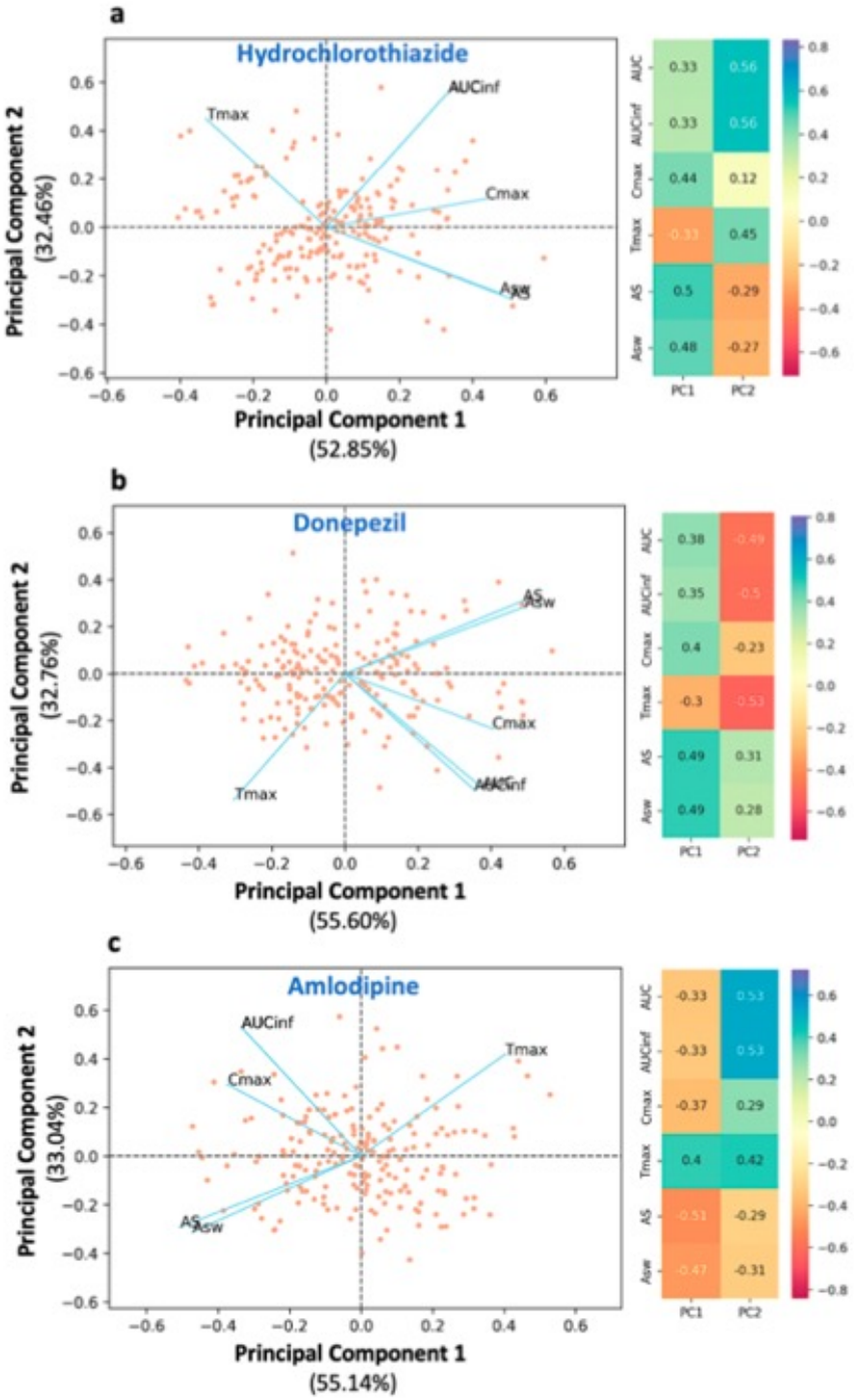
$$AS = \frac{C_{max}}{T_{max}}$$

A generalized version of AS: **Weighted AS**

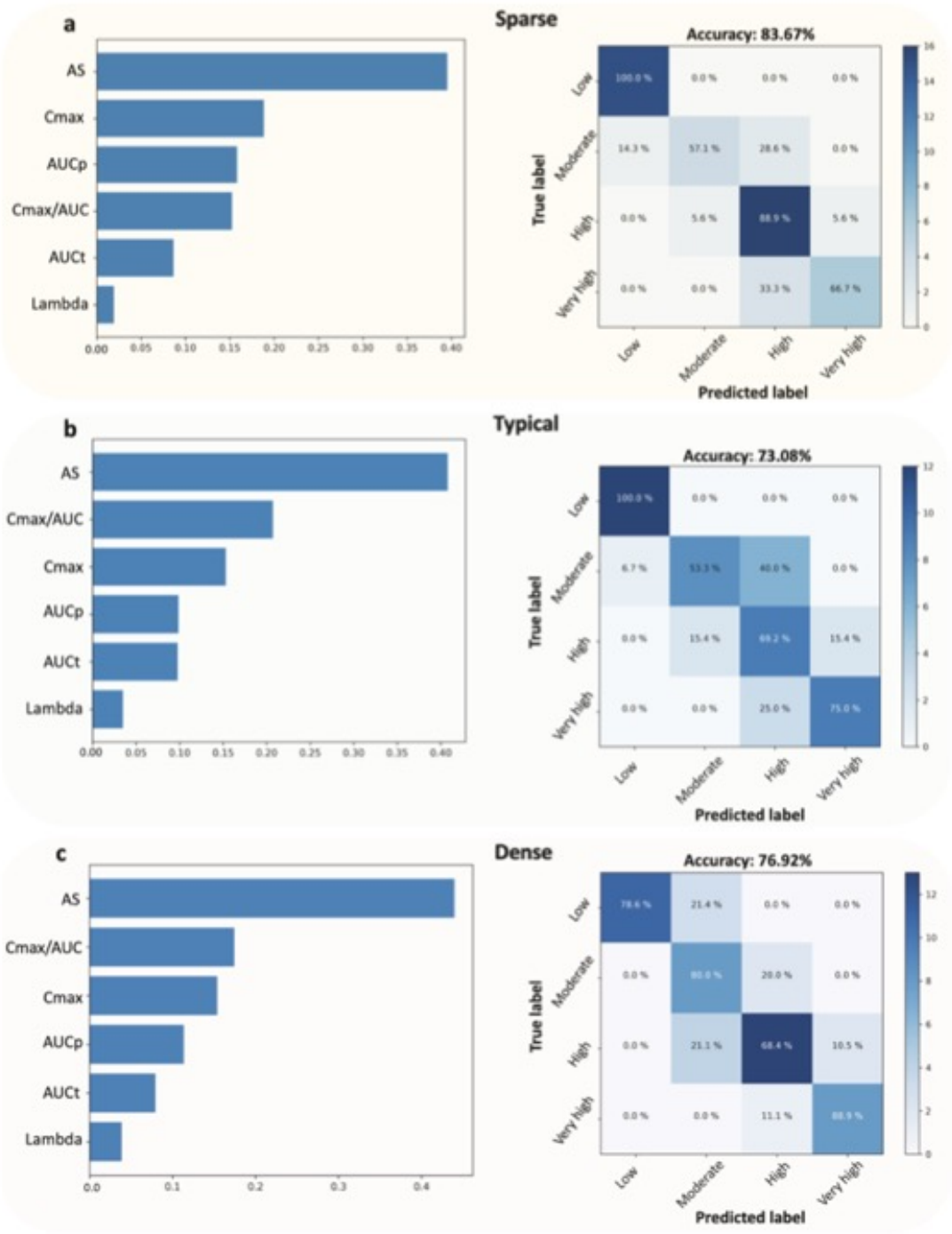
$$AS_w = \frac{\sum_{i=1}^{n-1}\left(\frac{Tmax-t_i}{Tmax} \times slope_i\right)}{n-1} = \frac{\sum_{i=1}^{n-1}\left(\frac{Tmax-t_i}{Tmax} \times \frac{C_{i+1}-C_i}{t_{i+1}-t_i}\right)}{n-1}$$

To place more emphasis on absorption kinetics

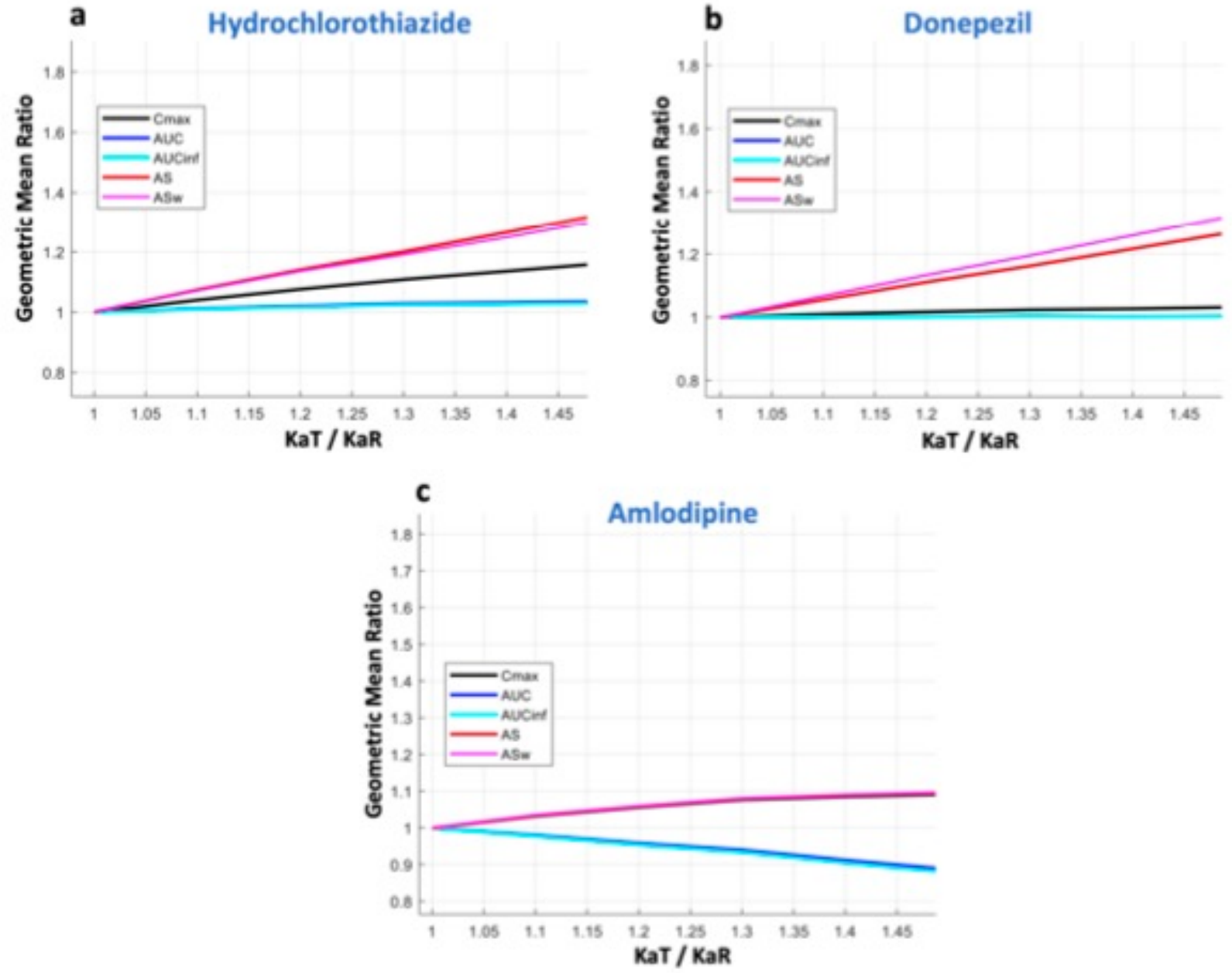
Principal Component Analysis (PCA)

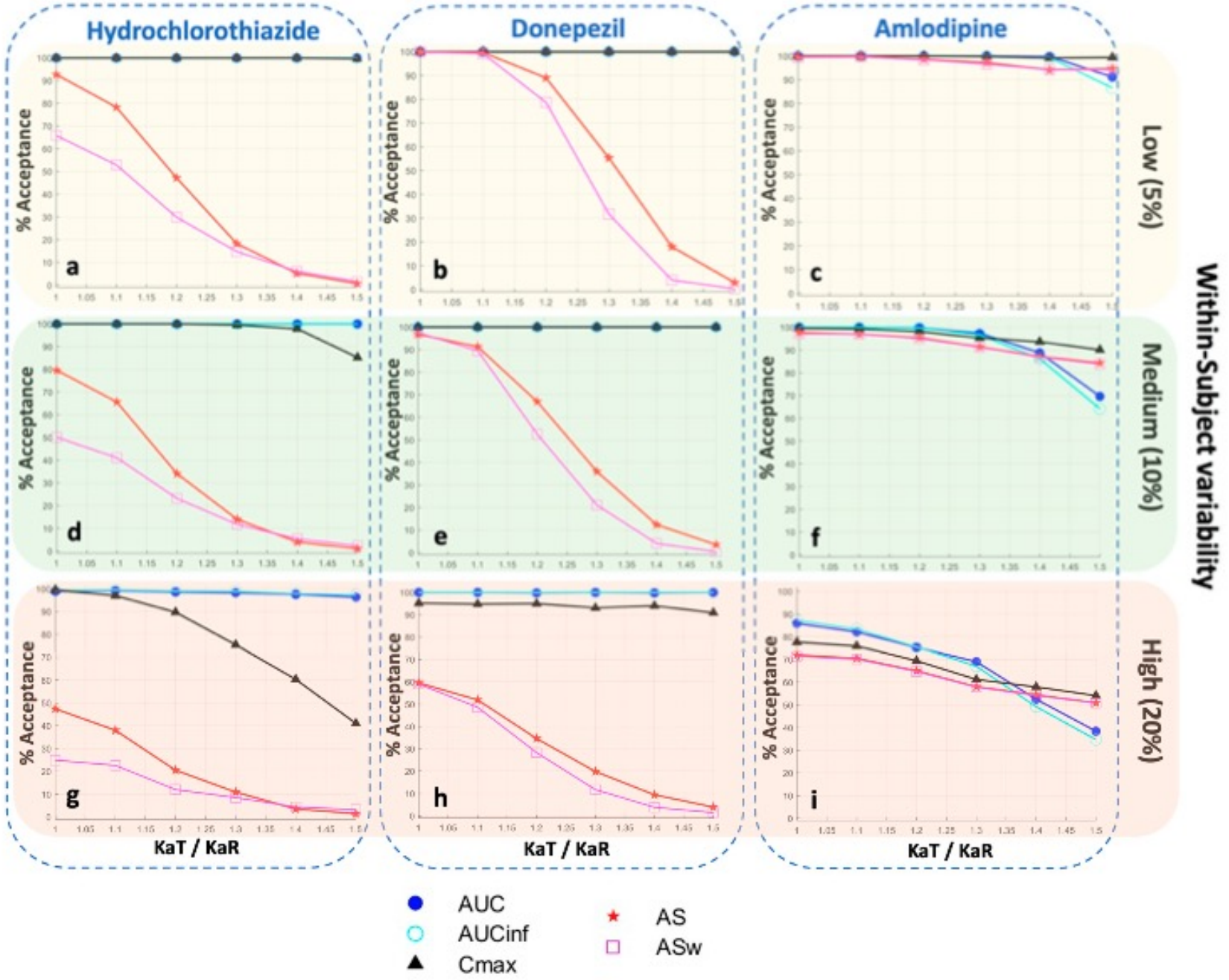


Random Forest (RF)



Sensitivity




Hydrochlorothiazide
Donepezil
Amlodipine
Low (5%)
Medium (10%)
High (20%)
Within-Subject variability
% Acceptance
KaT / KaR
a
b
c
d
e
f
g
h
i
AUC
AUCinf
Cmax
AS
ASw

Advantages:

(a) AS satisfies the fundamental theoretical reason for considering a parameter as a measure of absorption rate; namely, AS has **units of concentration/time** in contrast to all other measures proposed in the literature, which have meaningless units.

(b) The **machine learning** methods applied in this study showed that AS succeeds in **reflecting the "absorption rate"**, while Cmax and other existing metrics fail

(c) AS can be **estimated quite simply** using a **model-independent approach**, **without any assumptions**

(d) AS is a **generalization of Cmax/Tmax**, and therefore AS can be applied to either **equally or unequally spaced sampling schemes**

(e) Due to the calculation of AS, which relies on many data points, **estimation bias that might occur to Cmax/Tmax can be avoided** in the case of AS.

(f) The **weighted version** of AS (i.e., ASw) allows **more emphasis to be placed on early time points**, thus expressing more purely the absorption process.