Bioavailability / Bioequivalence

- Selection of CROs
- Selection of a Reference Product
- Metrics (AUC, C_{max}/t_{max}, Shape of Profile)
- Acceptance Ranges (0.80 – 1.25 and beyond)
- Sample Size Planning (Literature References, Pilot Studies)
- Steps in bioanalytical Validation (Validation Plan, Pre-Study Validation, In-Study Validation)
- Study Designs
- **Protocol Issues**
- Evaluation of Studies
- Advanced Topics
- Avoiding Pitfalls

previous Lecture

next Lecture

Bioavailability / Bioequivalence

- **Protocol Issues**

Testing whether or not animals "kiss"

Bioavailability / Bioequivalence

- **Protocol Issues**
  - Whatever Procedure you have not stated *a-priori* in the Protocol *may* not be accepted by Regulatory Authorities!
    - Planning Phase
      - Sufficient number of blood samples (most important around t_{max}!) / urine collection periods.
      - Sampling long enough to cover ≥80 % of AUC_{∞}.
      - Wash-out periods long enough (≥3× $t_{½}$, recomm. ≥5× $t_{½}$).
      - Saturation phase long enough to reach Steady-State (≥5× $t_{½}$, recommended ≥7× $t_{½}$).
      - Pre-dose samples during saturation phase (compliance!)

Bioavailability / Bioequivalence

- **Protocol Issues**
  - …If you did not write it down, you did not do it… (inofficial G*x*P Guideline)
    - Standardization as far as possible; only as far as feasible.
      - Format of Study Protocol as close as possible to the format of ICH/GCP Study Reports.
      - Transfer of Study Medication from the Sponsor to the CRO.
      - Selection of subjects.
      - Recruitment (advertisements, database query).
      - Timing of Administration (time of day, day of week).
      - Posture during Administration and post-dose.
      - Nutrition, fluid intake, smoking during Hospitalization periods.

Bioavailability / Bioequivalence

- **Protocol Issues**
  - …If you did not write it down, you did not do it… (inofficial G*x*P Guideline)
    - Standardization as far as possible; only as far as feasible.
      - Rules of Conduct (pre-dose sleep, movies, sporting activities) during Hospitalization periods.
      - Rules of Conduct during Ambulatory periods.
      - Procedure for blood sampling / urine collection (*e.g.*, cooling prior to centrifugation, light protection).
      - Protection against sample-mix-up during plasma-separation (*e.g.*, Barcodes, Four-Eye-Principle).
      - Storage of samples (preferably together with QCs for bio-analytics).

Bioavailability / Bioequivalence

- **Protocol Issues**
  - …If you did not write it down, you did not do it… (inofficial G*x*P Guideline)
    - Standardization as far as possible; only as far as feasible.
      - Procedure to deliver unused Study Formulations from the CRO to the Sponsor.
      - Archiving of clinical sata (Screenings, CRFs).
      - Shipment of samples (preferably in two parts, datalogger).
      - Bioanalytical Protocol.
      - Results from valid runs only.
      - Storage of samples preferably at least 6 months after acceptance of Study Report.

Bioavailability / Bioequivalence

- **Protocol Issues**
  - …If you did not write it down, you did not do it… (inofficial G*x*P Guideline)
    - Standardization as far as possible; only as far as feasible.
      - → Bioanalytical Report including 20 % of Chromatograms.
      - → Documented transfer of analytical data for Biostatistics (paper, datafiles).
      - → Biostatistical Protocol (model, methods, handling of Outliers, data-input and storage, software).
      - → Evaluation according to Protocol.
      - → Biostatistical Report which allows re-calculation of the Study.

Bioavailability / Bioequivalence

- **Protocol Issues**
  - …If you did not write it down, you did not do it…

    (inofficial G*x*P Guideline)
    - Standardization as far as possible; only as far as feasible.
      - → Clinical Study Report according to ICH-Guideline.
      - → Archiving of data (at least 15 Years).
      - → Financial Issues.

Bioavailability / Bioequivalence

- **Protocol Issues**
  - If anything happens which would change the Conduct of the Study
    - Avoid ‘Protocol Deviations’, whenever possible
    - Protocol Amendment
      - if a different batch will be tested.
      - if Laboratory Normal Ranges change prior to start.
      - if the bioanalytical method changes.
      - Any change which may influence the safety of volunteers is rated **‘Substantial’** and must get a new Vote from the IEC.
      - Only minor changes (*e.g.*, typing Errors, the company shipping samples,…) is rated **‘Administrative’**. The IEC will only be notified.

Bioavailability / Bioequivalence

- **Protocol Issues**
  - If anything happens which would change the Conduct of the Study
    - If a ‘Protocol Deviation’ is unavoidable
      - Have an SOP for such a case (*i.e.*, describing a procedure which will authorize study personell to act *against* the Protocol).
      - Whenever possible ‘over-document’ in such a case (since questions may arise months/years later).

Bioavailability / Bioequivalence

- Selection of CROs
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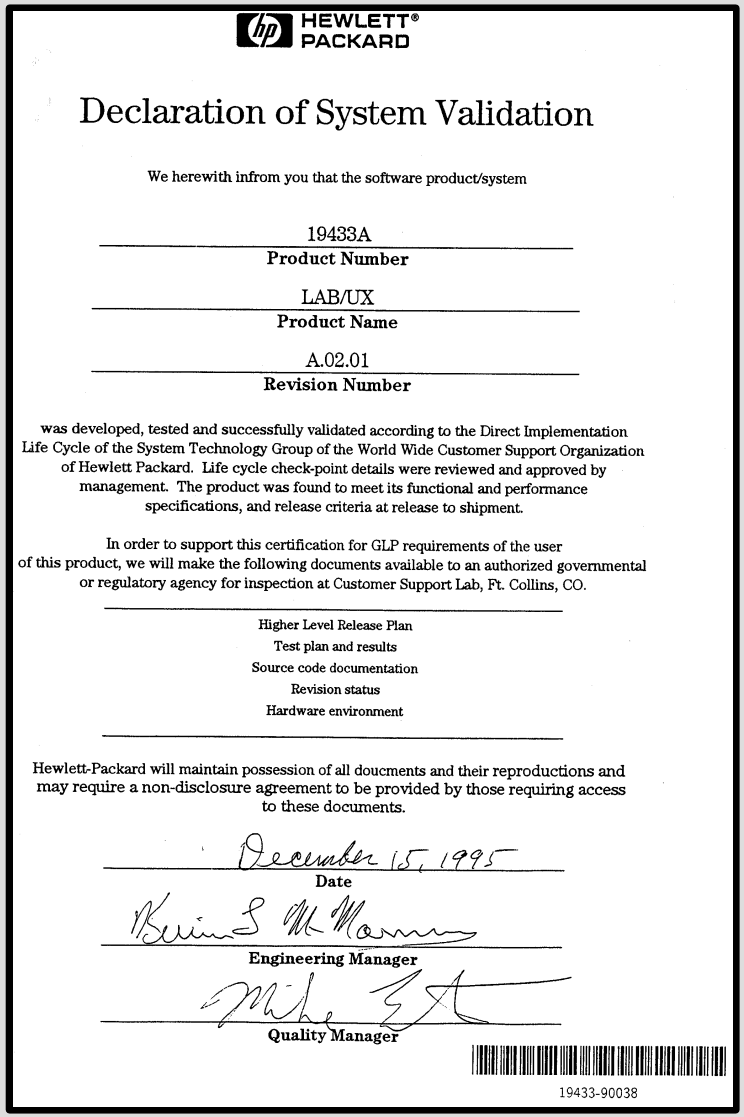
- Study Designs
- Protocol Issues
- **Evaluation of Studies**
- Advanced Topics
- Avoiding Pitfalls

Bioavailability / Bioequivalence

- **Evaluation of Studies**
  - Software
  - Parametric / Nonparametric
  - Outliers



HEWLETT® PACKARD

Declaration of System Validation

We herewith infrom you that the software product/system

19433A
Product Number

LAB/UX
Product Name

A.02.01
Revision Number

was developed, tested and successfully validated according to the Direct Implementation Life Cycle of the System Technology Group of the World Wide Customer Support Organization of Hewlett Packard. Life cycle check-point details were reviewed and approved by management. The product was found to meet its functional and performance specifications, and release criteria at release to shipment.

In order to support this certification for GLP requirements of the user of this product, we will make the following documents available to an authorized governmental or regulatory agency for inspection at Customer Support Lab, Ft. Collins, CO.

Higher Level Release Plan
Test plan and results
Source code documentation
Revision status
Hardware environment

Hewlett-Packard will maintain possession of all doucments and their reproductions and may require a non-disclosure agreement to be provided by those requiring access to these documents.

December 15, 1995
Date

Kevin S. McMann
Engineering Manager

Quality Manager

19433-90038

Bioavailability / Bioequivalence

- **Evaluation of Studies** (Software)
  - Types of Software
    - Commercial
      - Although Validation of software is mandatory according to ICH-GCP, rarely – if at all – current packages are validated.
      - Most ‘so-called’ validated software does not comply with current standards.
      - Try to get at least a statement of the Vendor about an applied SLC-Model (Software-Life-Cycle).
      - Have an Installation Plan.
      - Run Public-Domain datasets demonstrating ‘correct’ results.
      - Re-run datasets whenever you update the Operating System or install a new Version of the Package.
      - As a last resort you may claim the wide User-base.

Bioavailability / Bioequivalence

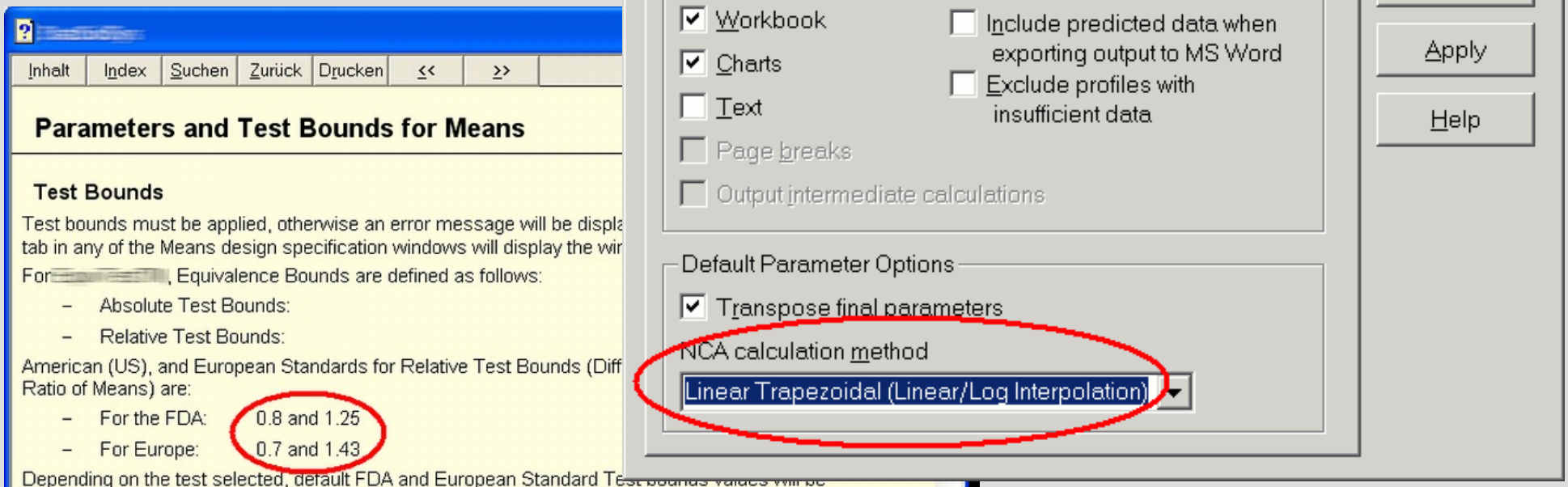
- **Evaluation of Studies** (Software)
  - Types of Software
    - Commercial
      - If you experience odd results, contact the Vendor's support and archive any correspondence (may be very helpful during a Regulatory Inspection).
      - If a Vendor offers a 'Validation Package', try to contact other users beforehand (*e.g.*, some Validation Packages cost more than the Software itself).

Bioavailability / Bioequivalence

- **Evaluation of Studies** (Software)
  - Types of Software
    - Commercial
      - Have SOPs describing the application for your evaluations – *not the Manual!*
      - The default-values of some programs may even lead to *'sub-optimal'* results…

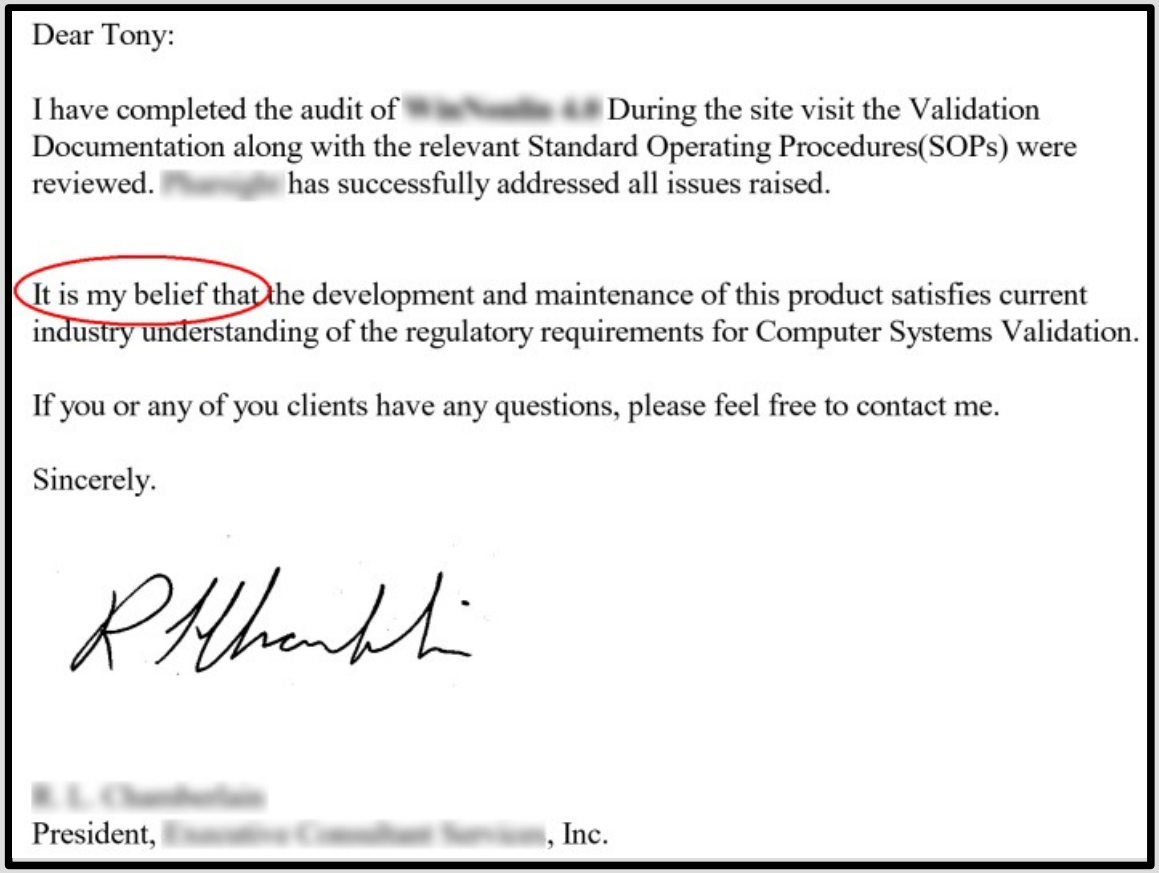
Bioavailability / Bioequivalence

- **Evaluation of Studies** (Software)
  - Types of Software
    - Commercial



Bioavailability / Bioequivalence

- **Evaluation of Studies** (Software)
  - Types of Software
    - Commercial
      - → Strong Beliefs
        'Validation Letter'



Dear Tony:

I have completed the audit of [redacted] During the site visit the Validation Documentation along with the relevant Standard Operating Procedures(SOPs) were reviewed. [redacted] has successfully addressed all issues raised.

It is my belief that the development and maintenance of this product satisfies current industry understanding of the regulatory requirements for Computer Systems Validation.

If you or any of you clients have any questions, please feel free to contact me.

Sincerely.

[redacted]
President, [redacted], Inc.

Bioavailability / Bioequivalence

- **Evaluation of Studies** (Software)
  - Types of Software
    - In-House
      - (Potentially) can be validated complying with ICH-GCP.
      - All points mentioned for commercial Software also apply.
      - It may be much easier to taylor such Software to your company's needs.
      - Is a necessity if modern methods*) simply are not implemented in commercial Packages.
      - Unfortunately Regulators often show a negative attitude towards In-House Software.

*) the Kolmogoroff-Smirnov-Test for Normality, which is outdated by the Shapiro-Wilk-Test since the mid-60ies of the last century was introduced to the BE-Module of the recent Version 5.0.1 of WinNonlin in 2005 (!)

Bioavailability / Bioequivalence

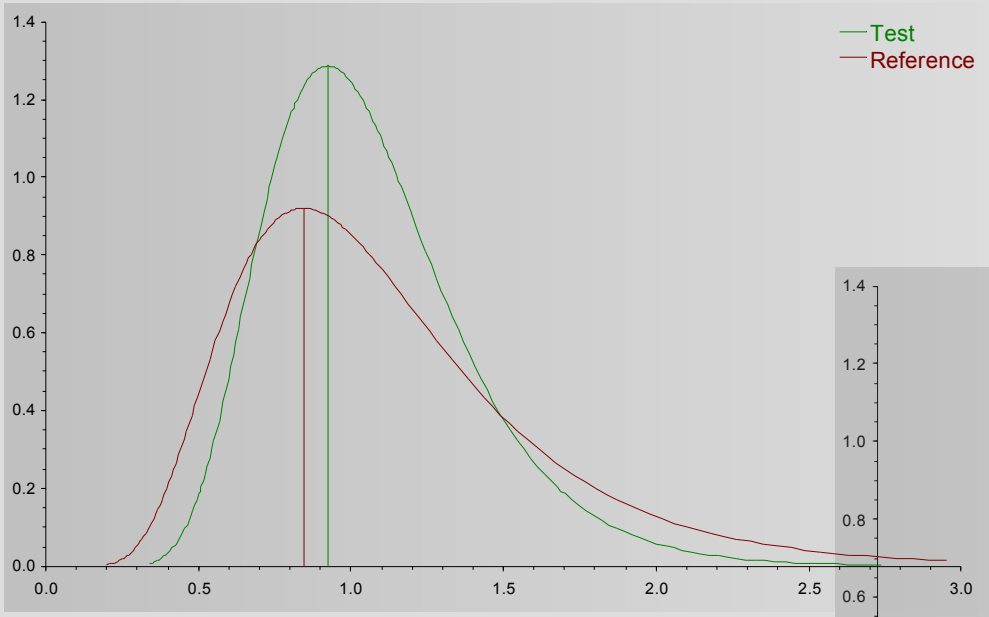
- **Evaluation of Studies**
  - Software
  - Parametric / Nonparametric
  - Outliers

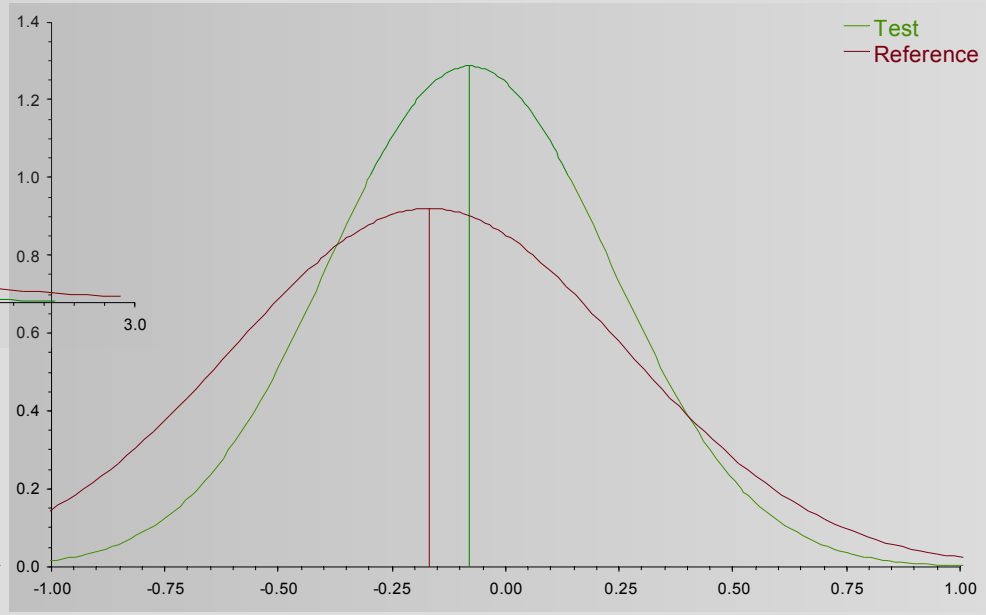
Bioavailability / Bioequivalence

- **Parametric / Nonparametric**
  - Parametric Evaluation (*e.g.*, Analysis of Variance – ANOVA, Generalized Linear Model – GLM)
    - Most powerful method for continuous data (*e.g.*, AUC, C_{max})
    - Assumption: Normal Distribution
      - unlikely for many biological parameters,
      - but may be resolved by suitable transformation (*e.g.*, taking logarithms),
      - independent identical distribution: common variance for both formulations – *true?*
    - Drawback: Very sensitive to Outliers

Bioavailability / Bioequivalence

- **Parametric / Nonparametric**



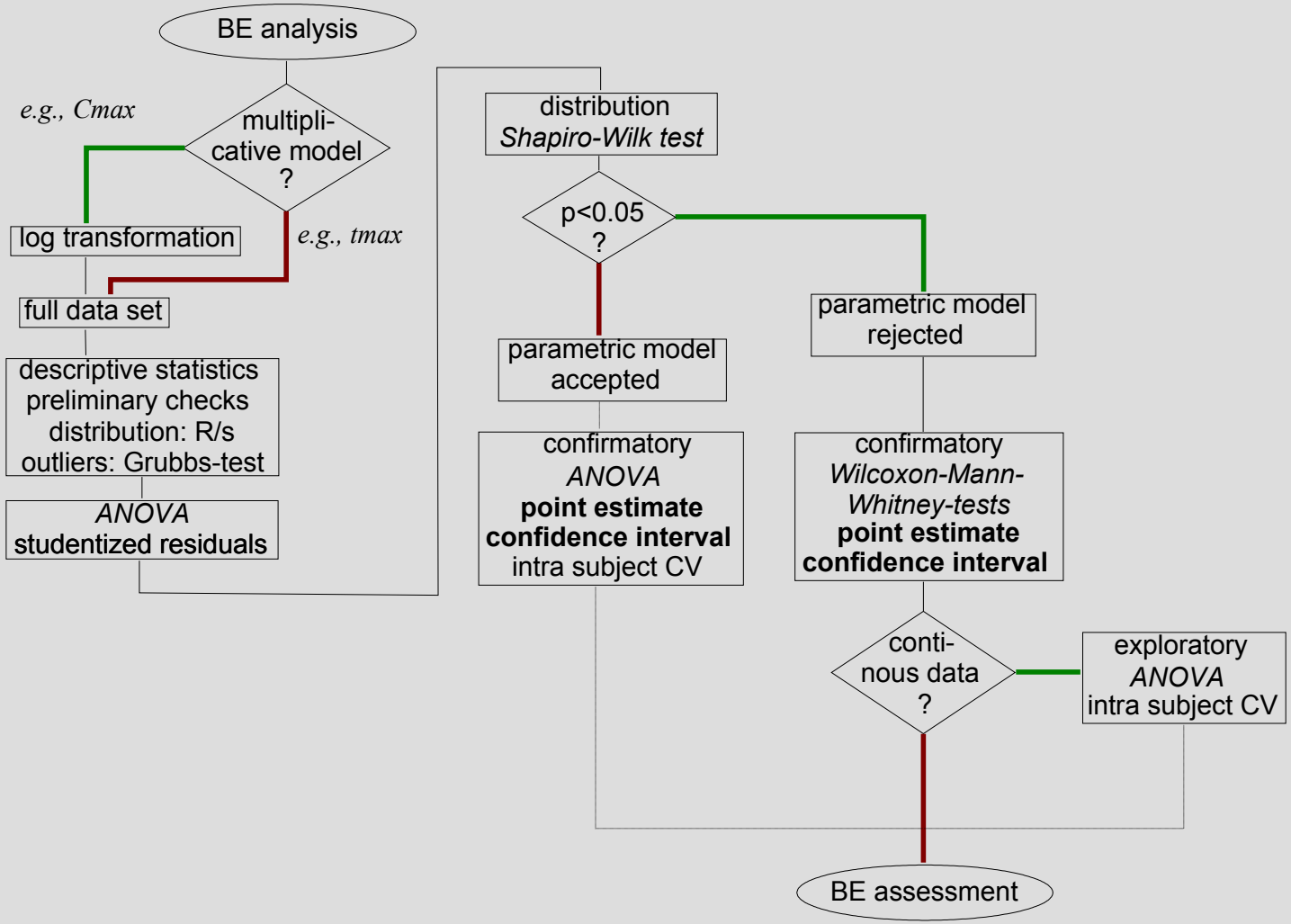


Bioavailability / Bioequivalence

- **Parametric / Nonparametric**
  - Nonparametric Evaluation (*e.g.*, Wilcoxon-Mann-Whitney)
    - Mandatory for discrete data (*e.g.*, t_{max})
    - Asymptotic power for continuous data 95.5 % ($3/\pi$)
    - Assumption: Continuous, Symmetrical Distribution Function
      - ➔ bivariate, continuous distribution function which is the same for both sequences – *true?*
    - not sensitive to Outliers
    - Drawback: Regulatory acceptance for PK parameters other then t_{max}?

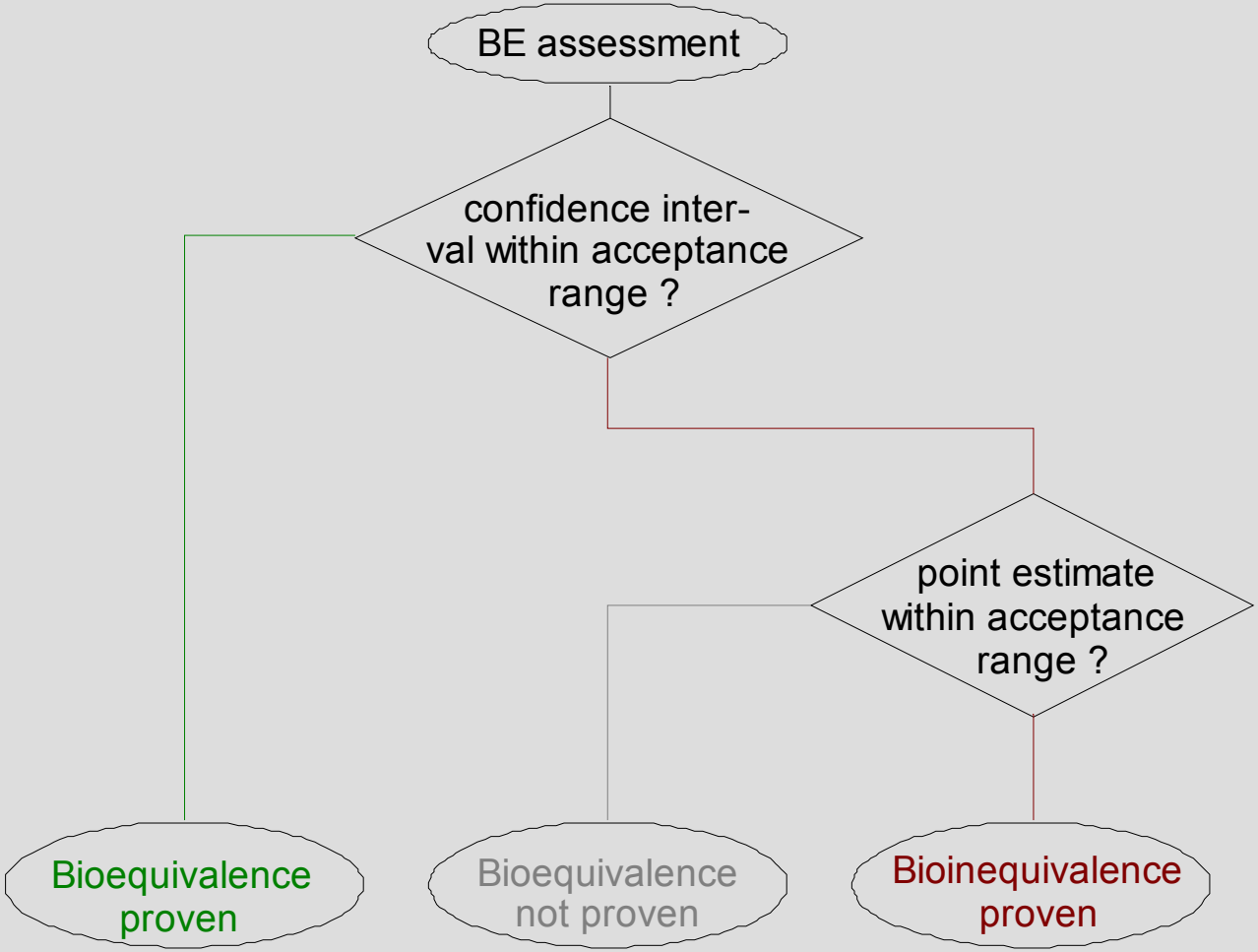
Bioavailability / Bioequivalence

- **Parametric / Nonparametric** (Decision Tree)



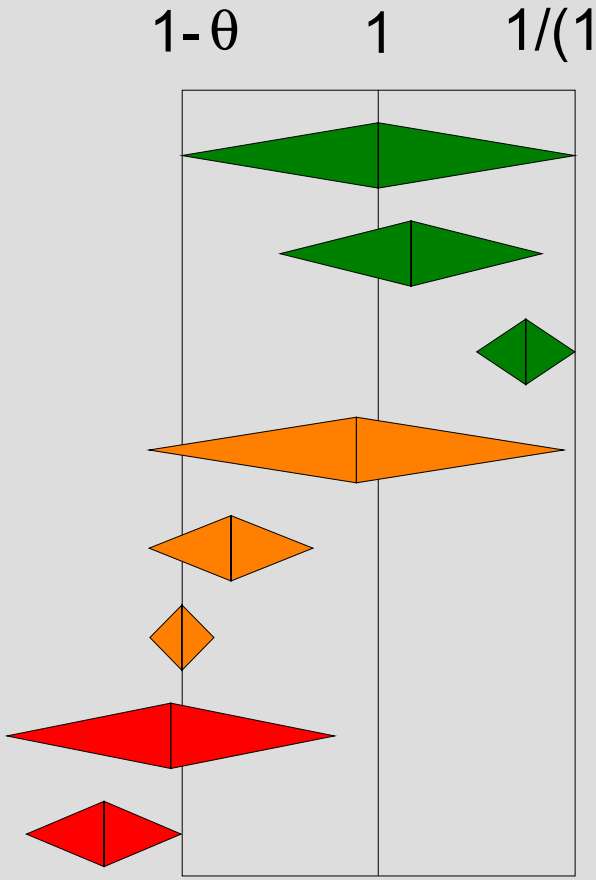
Bioavailability / Bioequivalence

- **Parametric / Nonparametric** (BE Assement)



Bioavailability / Bioequivalence

- **Parametric / Nonparametric** (BE Assement)



CI within both AI
bioequivalence =
lack of interaction proven

at least one CL ouside AI
PE inside AI
bioequivalence = lack of
interaction not proven

PE outside AL
bioinequivalence =
interaction proven

Bioavailability / Bioequivalence

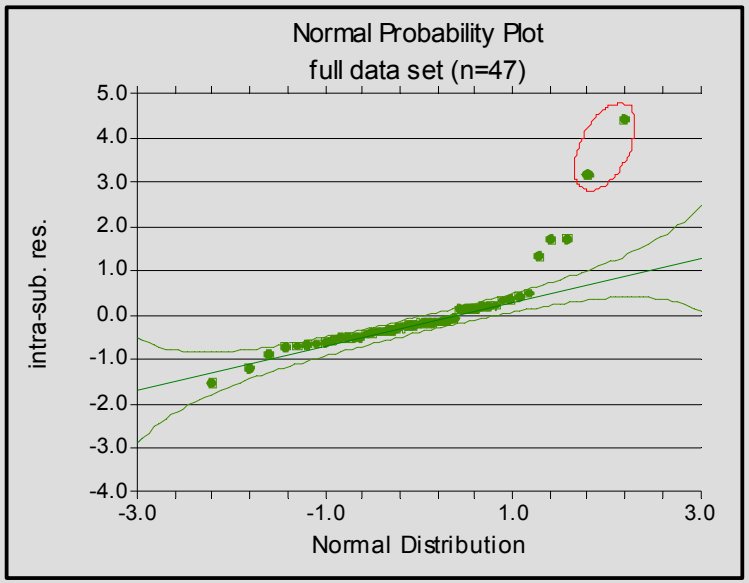
- **Outliers**
  - Parametric Methods are very sensitive to Outliers
    - A single Outlier may underpower a properly size study.
    - Exclusion of Outliers only possible if procedure stated in the Protocol, and reason can be justfied, *e.g.*,
      - Lacking compliance (subject did not take the medication),
      - Vomiting (up to $2 \times t_{max}$ for IR, at all times for MR),
      - Analytical problems (*e.g.*, interferences in chromatography);
      - not acceptable if only based on statistical grounds!
    - Remedy: Application of a valid statistical method!
    - Drawback: Regulatory acceptance?

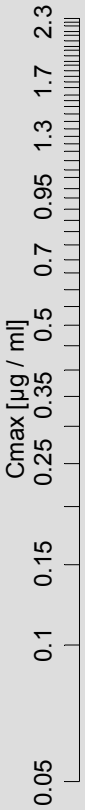
Bioavailability / Bioequivalence

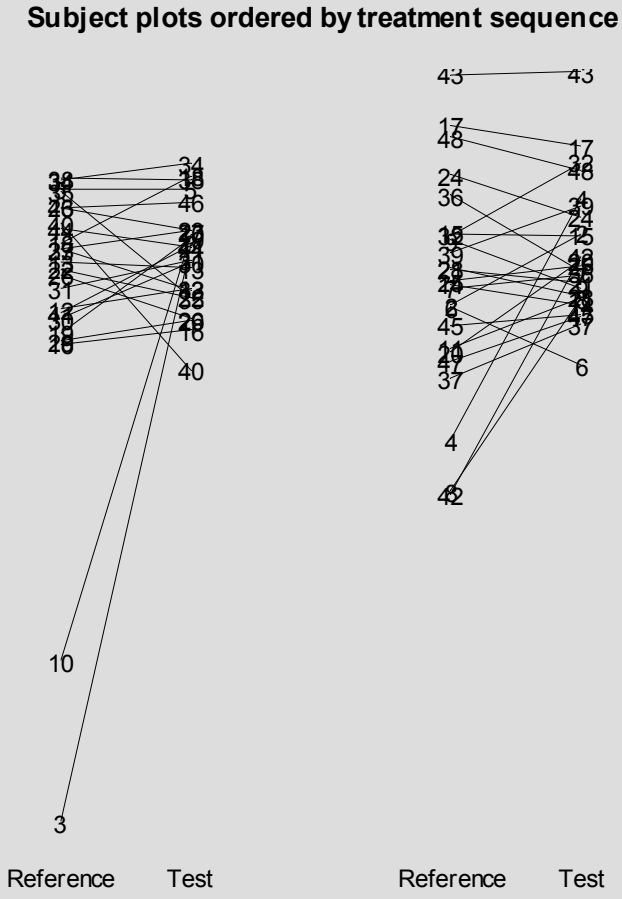
- **Outliers**
  - Parametric Methods are very sensitive to Outliers
    - Optional: stay with the parametric method, but
      - evaluation of both the Full Data Set, and the Reduced Data Set (Outlier/s exluded), and
      - discuss influence on the outcome of the study.

Bioavailability / Bioequivalence

- **Parametric / Nonparameric / Outliers**
  - Example: Lansoprazole







Bioavailability / Bioequivalence

- **Parametric / Nonparameric / Outliers**
  - Example: Lansoprazole

Test	Value	Probability	Decision (5%)
Shapiro-Wilk W	0.7339928	<0.000001	Reject normality
Anderson-Darling	3.98384	<0.000001	Reject normality
Martinez-Iglewicz	4.224289		Reject normality
Kolmogorov-Smirnov	0.2312414		Reject normality
D'Agostino Skewness	5.1629	<0.000001	Reject normality
D'Agostino Kurtosis	4.1551	0.000033	Reject normality
D'Agostino Omnibus	43.9204	<0.000001	Reject normality

Bioavailability / Bioequivalence

- **Parametric / Nonparameric / Outliers**
  - Example: Lansoprazole
  - Results (Nonparametric as Per Protocol, n=47)
    - AUC_{∞} 107.7 % [102.2 % – 116.1 %]
    - AUC_t 107.7 % [102.0 % – 116.4 %]
    - C_{max} 108.3 % [99.8 % – 118.8 %]
      - ➔ Deficiency Letter by Dutch Authority (MEB):
      - ➔ BE not assessed by ANOVA (although problems with the reference were known from previous studies with >50 subjects and decision tree was stated in the protocol),
      - ➔ CI for C_{max} calculated by ANOVA outside 0.80–1.25 (although extended range of 0.75–1.33 was clinically justified in the protocol),
      - ➔ Lacking justification and valid *explanation* of nonnormality (?)

Bioavailability / Bioequivalence

- **Parametric / Nonparameric / Outliers**
  - ANOVA (Reduced Data Set, n=45)
    - AUC_{∞} 108.8 % [101.8 % – 116.4 %]
    - AUC_{t} 108.9 % [101.8 % – 116.7 %]
    - C_{max} 108.6 % [99.1 % – 119.4 %]

      ➔ So what?