previous Lecture

next Lecture

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

Bioavailability / Bioequivalence

- **Sample Size Planning (Literature References, Pilot Studies)**
  - Suggested References
    - S.-C. Chow and J.-p. Liu;
      Design and Analysis of Bioavailability and Bioequivalence Studies.
      Marcel Dekker, New York (2nd ed. 2000)
    - S.-C. Chow, Shao, J. and H. Wang;
      Sample Size Calculations In Clinical Research.
      Marcel Dekker, New York (2003)

Bioavailability / Bioequivalence

- **Sample Size Planning (Literature References, Pilot Studies)**
  - The number of subjects required is determined by
    - the error variance associated with the primary characteristic to be studied as estimated from
      - → a pilot experiment,
      - → previous studies, or
      - → published data,
    - the significance level desired,
    - the expected deviation (Δ) from the reference product compatible with BE and,
    - the required power.

Bioavailability / Bioequivalence

- **Sample Size Planning (Literature References, Pilot Studies)**
  - Problems/Solutions
    - … the error variance associated with the ***primary characteristic*** to be studied …
      - ➔ Since BE must be shown both for AUC *and* C_{max}, and
      - ➔ if you plan your sample size only for the 'primary characteristic' (*e.g.*, AUC), in many cases you will fail for the secondary parameter (*e.g.*, C_{max}), which most likely shows higher variability – your study will be underpowered.
      - ➔ Based on the assumption, that CV is identical for test and reference (what if only the reference formulation has high variability, *e.g.*, ∗prazoles?).

Bioavailability / Bioequivalence

- **Sample Size Planning (Literature References, Pilot Studies)**
  - The number of subjects required is determined by
    - the error variance associated with the primary characteristic to be studied as estimated from
      - → a pilot experiment,
      - → previous studies, or
      - → published data,
    - the significance level desired,
    - the expected deviation (Δ) from the reference product compatible with BE and,
    - the required power.

Bioavailability / Bioequivalence

- **Sample Size Planning (Literature References, Pilot Studies)**
  - Problems/Solutions
    - … as estimated from
      - a ***pilot experiment***,
      - ***previous studies***, or
      - ***published data***,
    - The correct order should read:

      previous studies → pilot study → published data.
      - Only in the first case you ‘know’ all constraints resulting in variability.
      - Pilot studies are often too small to get *reliable* estimates of variability.
      - Advisable only if you have data from a couple of studies.

Bioavailability / Bioequivalence

- **Sample Size Planning (Literature References, Pilot Studies)**
  - The number of subjects required is determined by
    - the error variance associated with the primary characteristic to be studied as estimated from
      - → a pilot experiment,
      - → previous studies, or
      - → published data,
    - the significance level desired,
    - the expected deviation (Δ) from the reference product compatible with BE and,
    - the required power.

Bioavailability / Bioequivalence

- **Sample Size Planning (Literature References, Pilot Studies)**
  - Problems/Solutions
    - … the ***significance level desired***…
      - Throughout the NfG the significance level (α, error type I: patient's risk to be treated with an bioinequivalent drug) is fixed to 5 % (corresponding to a 90 % confidence interval).
      - Only in some very restrictive legislations (*e.g.*, Brazil's ANVISA), α must be tightened to 2.5 % for NTIDs (95 % confidence interval).
      - You may *desire* a lower significance level, but you will not get an approval anywhere!

Bioavailability / Bioequivalence

- **Sample Size Planning (Literature References, Pilot Studies)**
  - The number of subjects required is determined by
    - the error variance associated with the primary characteristic to be studied as estimated from
      - → a pilot experiment,
      - → previous studies, or
      - → published data,
    - the significance level desired,
    - the expected deviation (Δ) from the reference product compatible with BE and,
    - the required power.

Bioavailability / Bioequivalence

- **Sample Size Planning (Literature References, Pilot Studies)**
  - Problems/Solutions
    - … the ***expected deviation (Δ) from the reference***…
      - → Reliable estimate only from a previous full-sized study.
      - → If you are using data from a pilot study, allow for a safety margin.
      - → If no data are available, commonly a test/reference-ratio of 0.95 (Δ = 5 %) is used.
      - → If more than Δ = 10 % is expected, questions from the Ethics Committee are likely.

Bioavailability / Bioequivalence

- **Sample Size Planning (Literature References, Pilot Studies)**
  - The number of subjects required is determined by
    - the error variance associated with the primary characteristic to be studied as estimated from
      - → a pilot experiment,
      - → previous studies, or
      - → published data,
    - the significance level desired,
    - the expected deviation (Δ) from the reference product compatible with BE and,
    - the required power.

Bioavailability / Bioequivalence

- **Sample Size Planning (Literature References, Pilot Studies)**
  - Problems/Solutions
    - … the ***required power***.
      - Generally the power is set to at least 80 % (β, error type II: producers's risk to get no approval for a bioequivalent drug; power = 1 – β).
        Remember: 1 out of 5 studies will fail!
      - If you plan for power of less than 70 %, problems with the Ethics Committee are likely.
      - If you plan for power of more than 90 % (especially with *low* variability drugs), problems with the Regulator are likely ('forced bioequivalence').
      - Add subjects according to the expected drop-out rate.

Bioavailability / Bioequivalence

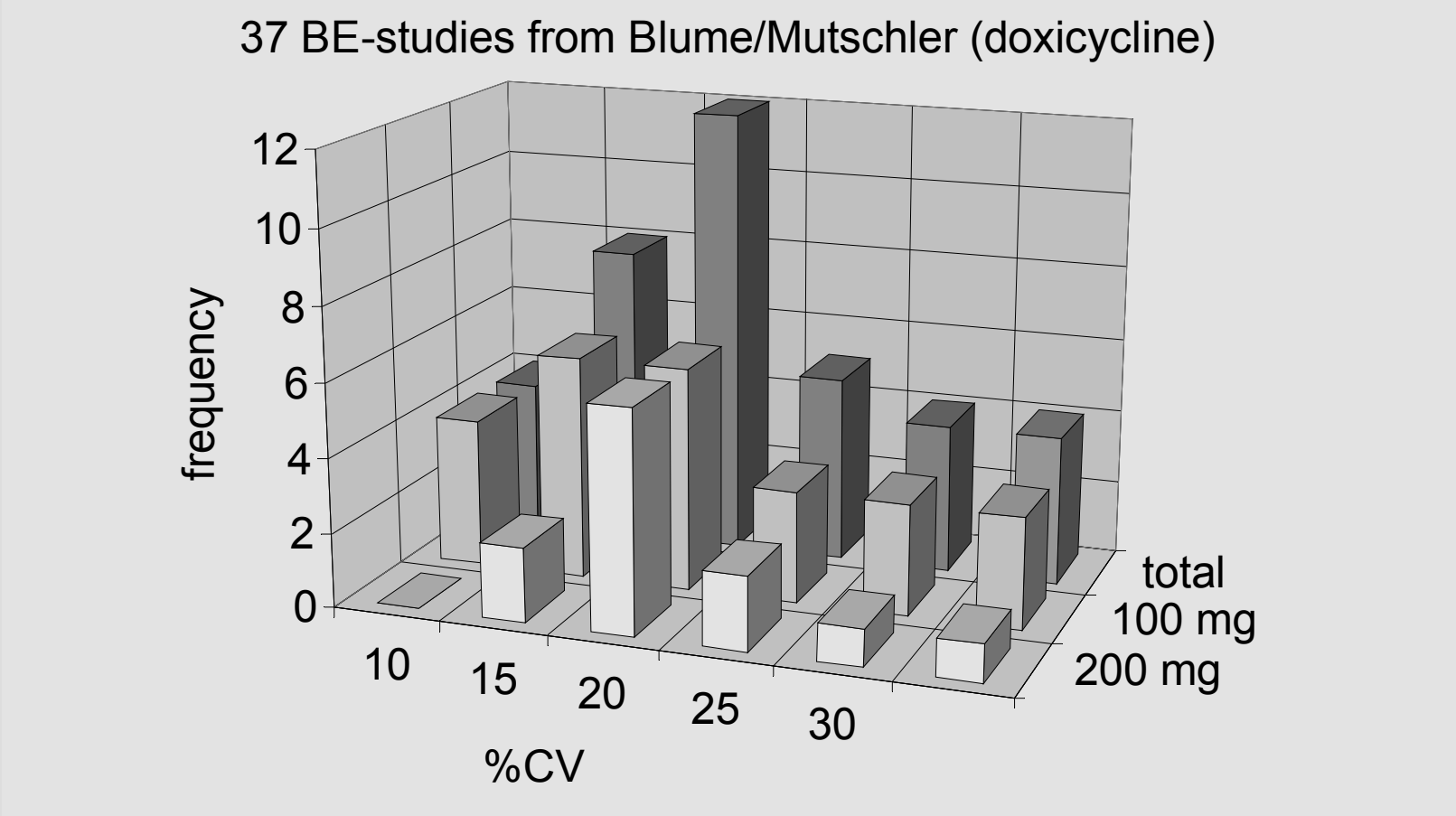
- **Sample Size Planning (Literature References)**
  - Literature References
    - Should be applied with caution (same dosage level and regimen, single dose / multiple dose, analytical method, log-transformed data, year of publication,…).
    - Preferable pooled data form a couple of studies.

Blume, H. and E. Mutschler (eds., in German only);
Bioäquivalenz. Qualitätsbewertung wirkstoffgleicher Fertigarzneimittel.
Anleitung – Methoden – Materialien.
GOVI, Frankfurt/Eschborn, loose-leaf-collection (1989-1996)

Steinijans, V.W. *et al.*;
Reference tables for the intrasubject coefficient of variation in bioequivalence studies.
Int. J. Clin. Pharm. Therap. 33(8), 427-430 (1995)

Bioavailability / Bioequivalence

- **Sample Size Planning (Literature References)**

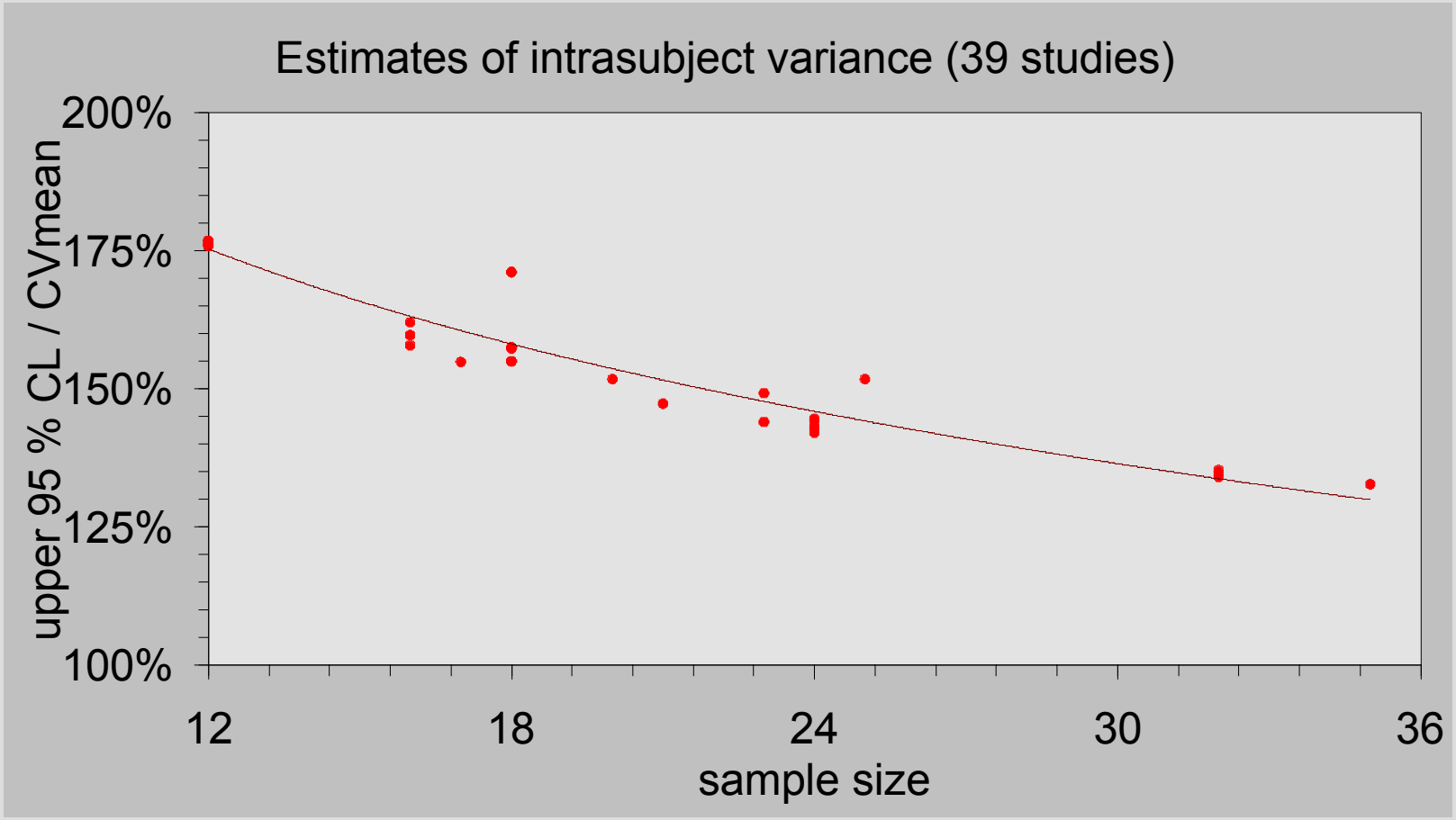


Bioavailability / Bioequivalence

- **Sample Size Planning (Pilot Studies)**
  - Pilot Studies
    - Only reasonably large study (*e.g.*, n=16) helpful for the selection of the ‘best’ of several similar formulations.
    - Estimates of PK parameters from small studies have large confidence intervals.
    - Estimate of the intrasubject variance even more uncertain.

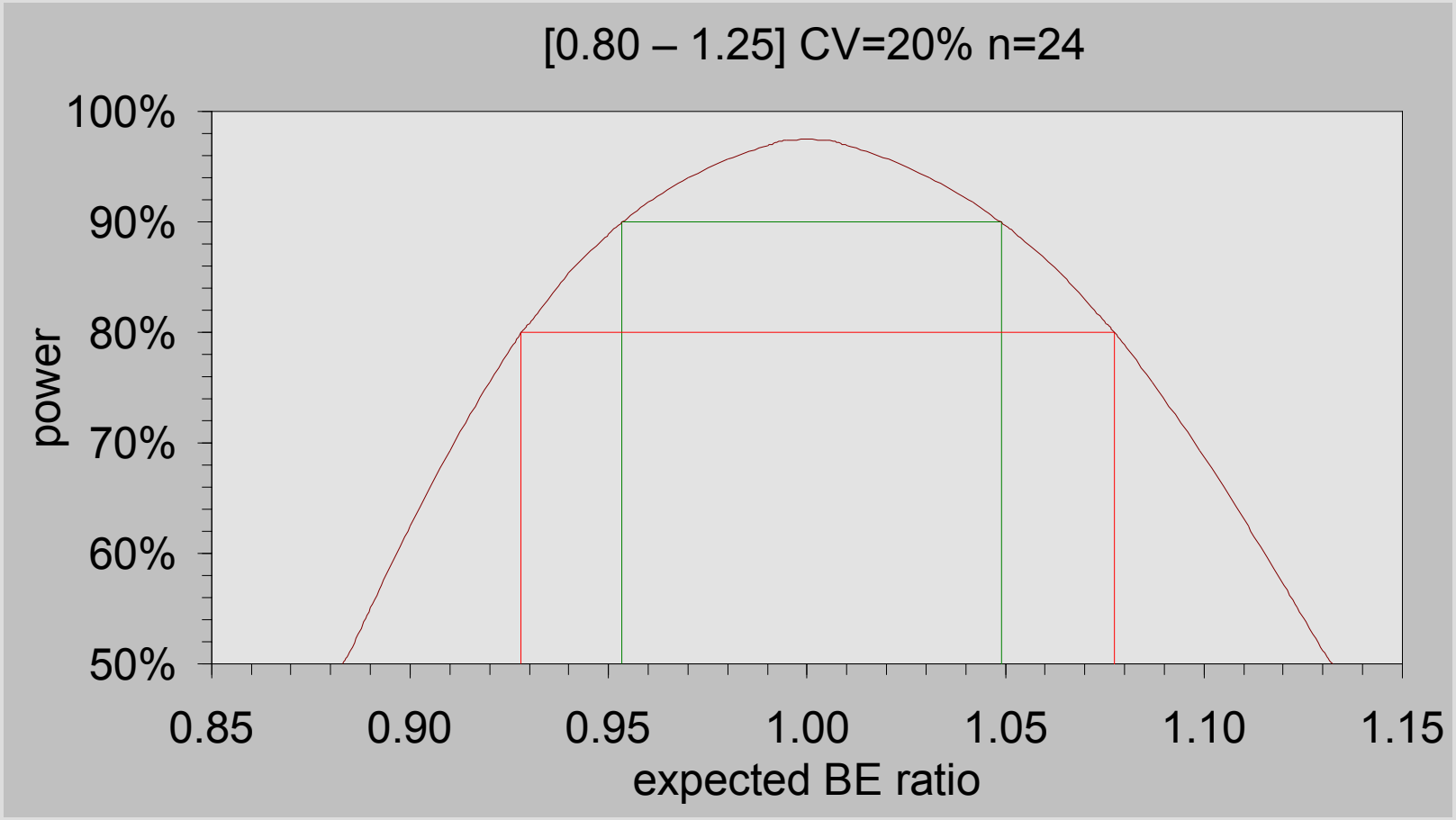
Bioavailability / Bioequivalence

- **Sample Size Planning (Pilot Studies)**



Bioavailability / Bioequivalence

- **Sample Size Planning (Pilot Studies)**



Bioavailability / Bioequivalence

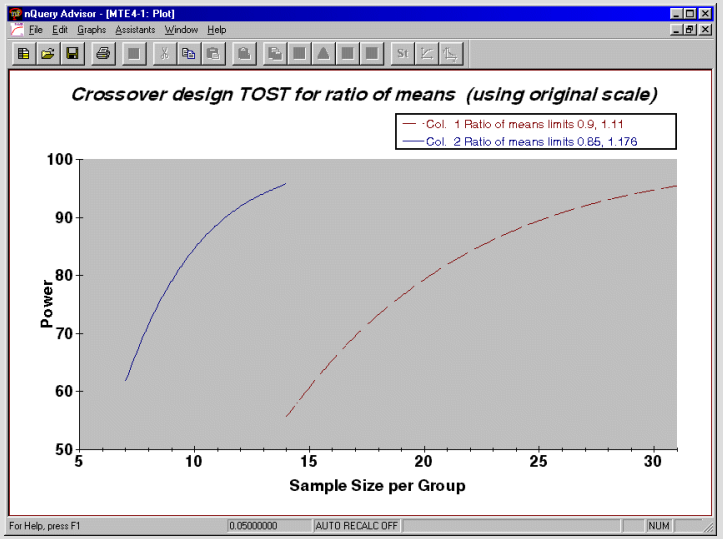
- **Sample Size Planning (Literature References, Pilot Studies)**
  - Estimation of Sample Size
    - Tables
      - Diletti, E., Hauschke, D. and V.W. Steinjans;
        Sample size determination for bioequivalence assessment by means of confidence intervals.
        Int. J. Clin. Pharm. Ther. Toxicol. 29(1), 1-8 (1991)
      - Diletti, E., Hauschke, D. and V.W. Steinjans;
        Sample size determination: Extended tables for the multiplicative model and bioequivalence ranges of 0.9 to 1.11 and 0.7 to 1.43.
        Int. J. Clin. Pharm. Ther. Toxicol. 30/Suppl.1, S59-62 (1992)

Bioavailability / Bioequivalence

- **Sample Size Planning (Literature References, Pilot Studies)**
  - Estimation of Sample Size
    - Approximations (may be implemented in a Spreadsheet)
      - Hauschke, D. *et al.*;
        Sample Size Determination for Bioequivalence Assessment Using a Multiplicative Model.
        J. Pharmacokin. Biopharm. 20(5), 557-561 (1992)
      - Chow, S.-C. and H. Wang;
        On Sample Size Calculation in Bioequivalence Trials.
        J. Pharmacokin. Pharmacodyn. 28(2), 155-169 (2001)
        Errata: J. Pharmacokin. Pharmacodyn.29(2), 101-102 (2002)

Bioavailability / Bioequivalence

- **Sample Size Planning (Literature References, Pilot Studies)**
  - Estimation of Sample Size
    - Programs
      - nQuery Advisor
        v6.01, Statistical Solutions (2005)
      - PASS
        v2005, NCSS (2005)
      - STATISTICA Power Analysis
        v7, StatSoft (2005)
      - StudySize
        v1.09, CreoStat (2004)
      - Formulas may be programmed in any language/package which supports the noncentral *t*-distribution (SAS, S+, R,…)



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

Bioavailability / Bioequivalence

- **Steps in bioanalytical Validation (Validation Plan, Pre-Study Validation, In-Study Validation)**
  - Essential Documents

The European Agency for the Evaluation of Medicinal Products, Human Medicines Evaluation Unit;
CPMP/ICH/381/95: ICH Q2A Note for Guidance on Validation of Analytical Methods: Definitions and Terminology. Step 5 (November 1994)

The European Agency for the Evaluation of Medicinal Products, Human Medicines Evaluation Unit;
CPMP/ICH/281/95: ICH Q2B Note for Guidance on Validation of Analytical Methods: Methodology. Step 4 (November 1996)

Food and Drug Administration: Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM);
Guidance for Industry. Bioanalytical Method Validation. (May 2001)

Bioavailability / Bioequivalence

- **Steps in bioanalytical Validation (Validation Plan, Pre-Study Validation, In-Study Validation)**
  - Useful Documents

Cartwright, A.C. *et al.*;
International harmonization and consensus DIA meeting on bioavailability and bioequivalence testing requirements and standards.
Drug Information Journal 25, 471 (1991)

Shah, V.P. *et al.*;
Analytical methods validation: Bioavailability, bioequivalence and pharmacokinetic studies.
Int. J. Pharm. 82, 1-7 (1992)

Shah, V.P., *et al.*;
Bioanalytical Method Validation – A Revisit with a Decade of Progress.
Pharm. Res. 17, 1551-1557 (2000)

Bioavailability / Bioequivalence

- **Steps in bioanalytical Validation (Validation Plan, Pre-Study Validation, In-Study Validation)**
  - Useful Documents

Organisation for Economic Co-operation and Development, Environment Directorate / Chemicals Group and Management Committee;
OECD Principles on Good Laboratory Practice (as revised in 1997)
Document ENV/MC/CHEM(98)17
In: OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, Paris, 21-Jan-1998

Brazilian Sanitary Surveillance Agency (ANVISA);
Manual for Good Bioavailability and Bioequivalence Studies.
Volume 1, Module 2: Analytical Step.
https://www.anvisa.gov.br/eng/bio/manual/volume1.zip
Brasília (2000)

Bioavailability / Bioequivalence

- **Steps in bioanalytical Validation: Validation Plan**
  - Method used for quantitative measurement of analytes in a given biological matrix must be reliable and reproducible for the intended use.
    - Accurracy
    - Precision
    - Selectivity
    - Sensitivity
    - Reproducibility
    - Stability

Bioavailability / Bioequivalence

- **Steps in bioanalytical Validation: Validation Plan**
  - Full Validation
    - Developing and implementing a new bioanalytical method, or adding metabolites to an existing assay.
      - **Selectivity**
        Lack of interferences above the Lower Limit of Quantitation (LLOQ) in ≥6 different sources of matrix.
      - **Accuracy**
        Replicate (≥5) analysis of known concentrations measured at ≥3 levels (low, intermediate, high).
        Mean values within ±15 % of expected (except at LLOQ, where ±20 % is acceptable).

Bioavailability / Bioequivalence

- **Steps in bioanalytical Validation: Validation Plan**
  - Full Validation
    - Developing and implementing a new bioanalytical method, or adding metabolites to an existing assay.
      - **Precision**
        Replicate (≥5) analysis of known concentrations measured at ≥3 levels (low, intermediate, high).
        Coefficient of Variation (CV) ≤15 % at each concentration (except at LLOQ, where ≤20 % is acceptable).
        - **intra-batch**
          within analytical run.
        - **inter-batch**
          between analytical runs (aka repeatability).

Bioavailability / Bioequivalence

- **Steps in bioanalytical Validation: Validation Plan**
  - Full Validation
    - Developing and implementing a new bioanalytical method, or adding metabolites to an existing assay.
      - **Recovery**
        The detector response obtained from an amount of the analyte added to and extracted from the biological matrix, compared to the detector response obtained for the true concentration of the pure authentic standard. Recovery of the analyte need not be 100 %, but the extent of recovery of an analyte and of the internal standard should be consistent, precise, and reproducible..
        Measured at low/intermediate/high level.

Bioavailability / Bioequivalence

- **Steps in bioanalytical Validation: Validation Plan**
  - Full Validation
    - Developing and implementing a new bioanalytical method, or adding metabolites to an existing assay.
      - **Calibration/Standard Curve**
        Same matrix as the samples in the intended study spiked with known concentrations. Number of standards: function of the anticipated range of analytical values, nature of the analyte/response relationship. Concentrations of standards chosen on basis of the concentration range expected.
        - Blank sample (matrix sample processed without internal standard),
        - Zero sample (matrix sample processed with internal standard),
        - 6 – 8 non-zero samples covering the expected range, including LLOQ.

Bioavailability / Bioequivalence

- **Steps in bioanalytical Validation: Validation Plan**
  - Full Validation
    - Developing and implementing a new bioanalytical method, or adding metabolites to an existing assay.
      - → **Calibration/Standard Curve**
        - Simplest model that adequately describes the concentration-response relationship should be used.
        - Selection of weighting and use of a complex regression equation should be justified.
        - Response at LLOQ ≥5 times response of blank.
        - Response at LLOQ: precision ≤20 %, accuracy ±20 % from nominal concentration.
        - Response at other levels: accuracy ±15 % from nominal concentration.

Bioavailability / Bioequivalence

- **Steps in bioanalytical Validation: Validation Plan**
  - Full Validation
    - Developing and implementing a new bioanalytical method, or adding metabolites to an existing assay.
      - → **Calibration/Standard Curve**
        - At least four out of six non-zero standards should meet the above criteria, including the LLOQ and the calibration standard at the highest concentration.
        - Excluding the standards should not change the model used.

Bioavailability / Bioequivalence

- **Steps in bioanalytical Validation: Validation Plan**
  - Full Validation
    - Developing and implementing a new bioanalytical method, or adding metabolites to an existing assay.
      - **Stability**
        Stability of the analytes during sample collection and handling.
        - **Three freeze-thaw cycles**
          ≥3 aliquots at low and high levels stored for 24 hours and thawed at room temperature. When completely thawed, refrozen for 12 – 24 hours. This cycle two more times repeated, then analyzed after the third cycle. If instable: samples should be frozen at -70 °C during another FT-cylce.

Bioavailability / Bioequivalence

- **Steps in bioanalytical Validation: Validation Plan**
  - Full Validation
    - Developing and implementing a new bioanalytical method, or adding metabolites to an existing assay.
      - **Stability**
        Stability of the analytes during sample collection and handling.
        - **Short-Term Storage** (bench top, room temperature):
          Three aliquots of each of the low and high concentrations should be thawed at room temperature and kept at this temperature from 4 – 24 hours (based on the expected duration that samples will be maintained at room temperature in the intended study) and analyzed.

Bioavailability / Bioequivalence

- **Steps in bioanalytical Validation: Validation Plan**
  - Full Validation
    - Developing and implementing a new bioanalytical method, or adding metabolites to an existing assay.
      - **Stability**
        Stability of the analytes during sample collection and handling.
        - **Long-Term Storage** (frozen at the intended storage temperature) should exceed the time between the date of first sample collection and the date of last sample analysis. Determined by storing ≥3 aliquots of low/high levels under the same conditions as the study samples. Volume should be sufficient for analysis on 3 separate occasions. Concentrations of all the stability samples should be compared to the mean of back-calculated values for the standards at the appropriate concentrations from the first day of long-term stability testing.

Bioavailability / Bioequivalence

- **Steps in bioanalytical Validation: Validation Plan**
  - Full Validation
    - Developing and implementing a new bioanalytical method, or adding metabolites to an existing assay.
      - **Stability**
        Stability of the analytes during sample collection and handling.
        - **Stock Solution Stability** of drug and the internal standard should be evaluated at room temperature for ≥6 hours. If the stock solutions are refrigerated or frozen for the relevant period, the stability should be documented. After completion of the desired storage time, the stability should be tested by comparing the instrument response with that of freshly prepared solutions.

Bioavailability / Bioequivalence

- **Steps in bioanalytical Validation: Validation Plan**
  - Full Validation
    - Developing and implementing a new bioanalytical method, or adding metabolites to an existing assay.
      - **Stability**
        Stability of the analytes during sample collection and handling.
        - **Post-Preparative Stability**
          Stability of processed samples, including the resident time in the autosampler, should be determined. The stability of the drug and the internal standard should be assessed over the anticipated run time for the batch size in validation samples by determining concentrations on the basis of original calibration standards.

Bioavailability / Bioequivalence

- **Steps in bioanalytical Validation: Validation Plan**
  - Partial Validation
    - Method transfers between laboratories (or analysts).
    - Change in analytical methodology (*e.g.*, change in detection systems).
    - Change in anticoagulant in harvesting biological fluid.
    - Change in matrix within species (*e.g.*, human plasma to human urine).
    - Change in sample processing procedures.
    - Change in species within matrix (*e.g.*, rat plasma to mouse plasma).

Bioavailability / Bioequivalence

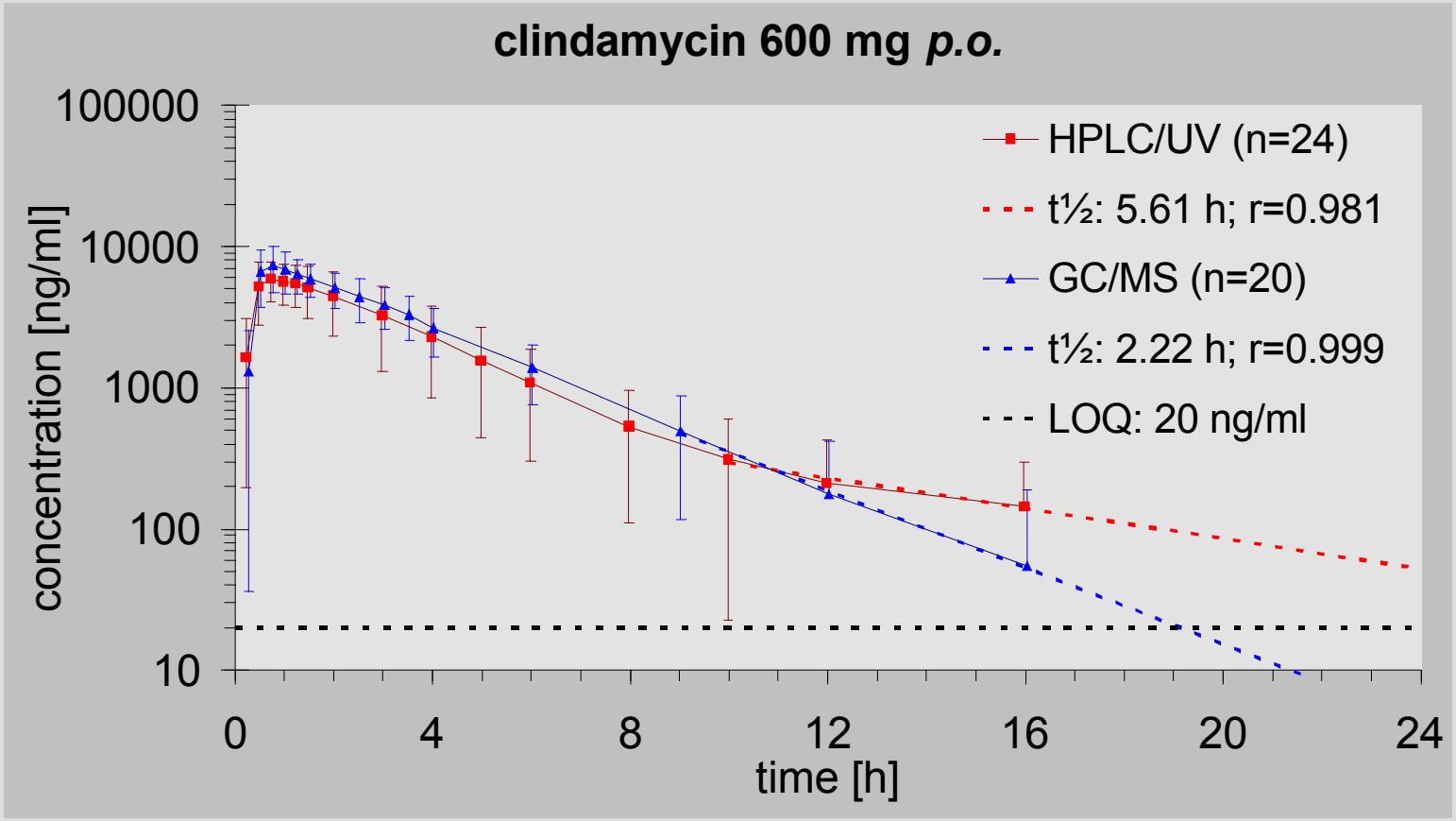
- **Steps in bioanalytical Validation: Validation Plan**
  - Partial Validation
    - Change in relevant concentration range.
    - Changes in instruments and/or software platforms.
    - Limited sample volume (*e.g.*, pediatric study).
    - Rare matrices.
    - Selectivity demonstration of an analyte in the presence of concomitant medications and/or specific metabolites.

Bioavailability / Bioequivalence

- **Steps in bioanalytical Validation: Validation Plan**
  - Cross-Validation
    - Comparison of validation parameters when two or more bioanalytical methods are used to generate data within the same study or across different studies. An example of cross-validation would be a situation where an original validated bioanalytical method serves as the *reference* and the revised bioanalytical method is the *comparator*.
    - Cross-validation should also be considered when data generated using different analytical techniques (*e.g.*, LC-MS/MS *vs.* ELISA) in different studies are included in a regulatory submission.

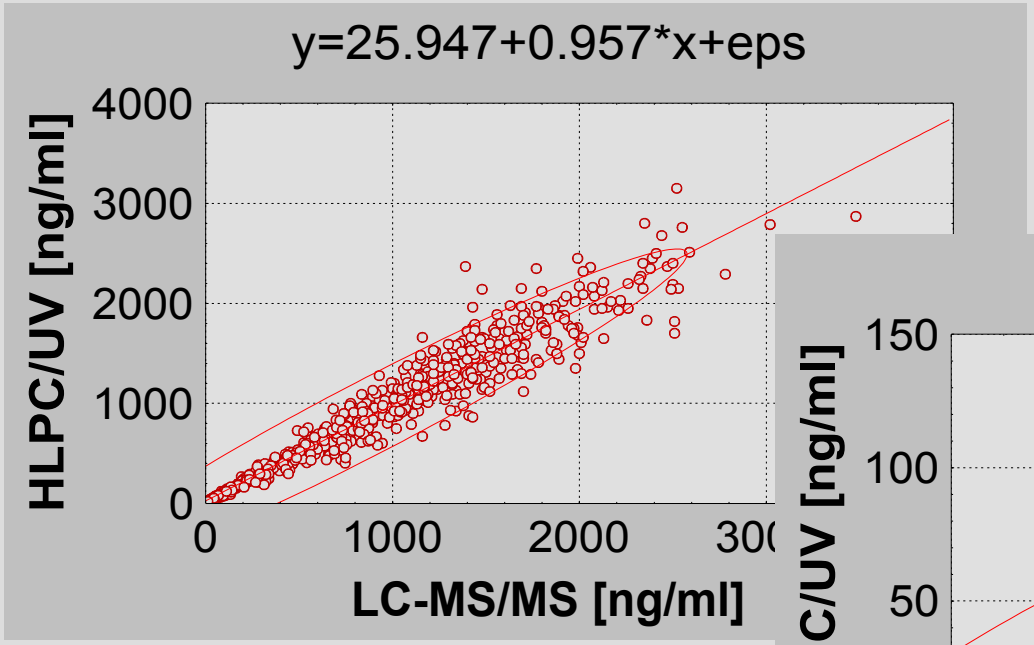
Bioavailability / Bioequivalence

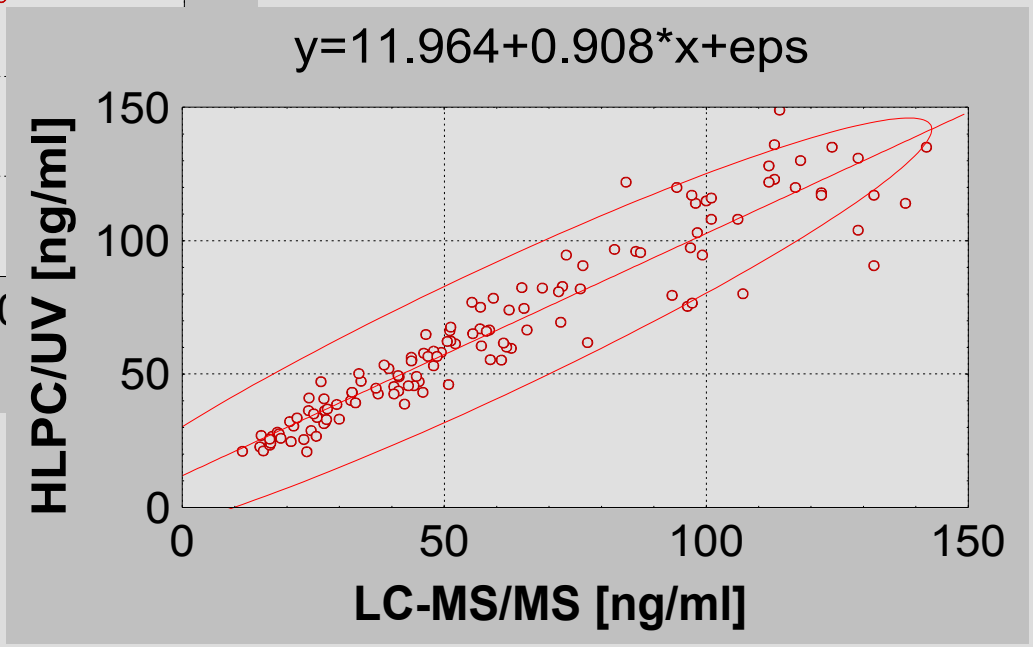
- **Steps in bioanalytical Validation: Validation Plan**
  - Cross-Validation, Example: Clindamycin



Bioavailability / Bioequivalence

- **Steps in bioanalytical Validation: Validation Plan**
  - Cross-Validation, Example: Clindamycin





Bioavailability / Bioequivalence

- **Steps in bioanalytical Validation: Validation Plan**
  - Written Document describing which steps will be performed in the Validation.
    - Purpose of Validation (*e.g.*, ‘Validation of bioanalytical method X for the determination of Y in human plasma’).
    - Reference to already established method (SOP).

Bioavailability / Bioequivalence

- **Steps in bioanalytical Validation: Pre-Study Validation**
  - Performance of Validation according to the Validation Plan.
    - Results must comply with limits set in the Validation Plan.
  - Report of Results
    - ‘Method Validation Report’.
    - Will be referred in the Analytical Protocol of BA/BE-studies.

Bioavailability / Bioequivalence

- **Steps in bioanalytical Validation: In-Study Validation**
  - Study Samples should be analyzed according to the Analytical Protocol.
    - Quality Control Samples (QCs) should be analyzed together with Calibrators and study samples.
      - **Low / intermediate / high concentration levels**
        At least Duplicates at each level.
        Low: within 3times the LLOQ.
        Intermediate: near the center of the calibration range.
        High: near the upper boundary.

Bioavailability / Bioequivalence

- **Steps in bioanalytical Validation: In-Study Validation**
  - Study Samples should be analyzed according to the Analytical Protocol.
    - **Acceptance Criteria for an analytical run**
      - **QCs**
        85 % – 115 % accuracy for single determinations of QCs; not more than two different of six per run should be out of range.
      - **Standard Curve**
        85 % – 115 % accuracy for 75 % of standard points, except for LLOQ (80 % – 120 %). Values outside can be discarded, provided they do not change the established model.