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

- **Advanced Topics**
  - Highly Variable Drugs (Add-On Designs, Reference-Scaled Average Bioequivalence, Replicate Designs)
  - Assessment of Metabolites
  - Chiral Drugs
  - Dose Proportionality

Bioavailability / Bioequivalence

- **Dose Proportionality**
  - EMEA
    - BE based on only one strength may be acceptable. However the choice of the strength used should be justified on analytical, pharmacokinetic and safety grounds. Furthermore all of the following conditions should be fulfilled:
      - the pharmaceutical products are manufactured by the same manufacturer and process;
      - the **drug input has been shown to be linear over the therapeutic dose range** (if this is not the case the strengths where the sensitivity is largest to identify differences in the two products should be used);
      - the qualitative composition of the different strengths is the same;

Bioavailability / Bioequivalence

- **Dose Proportionality**
  - EMEA
    - Conditions (cont.):
      - the ratio between amounts of active substance and exci-pients is the same, or, in the case of preparations containing a low concentration of the active substance (less than 5 %), the ratio between the amounts of excipients is similar;
      - the dissolution profile should be similar under identical condi-tions for the additional strengths and the strength of the batch used in the bioequivalence study.
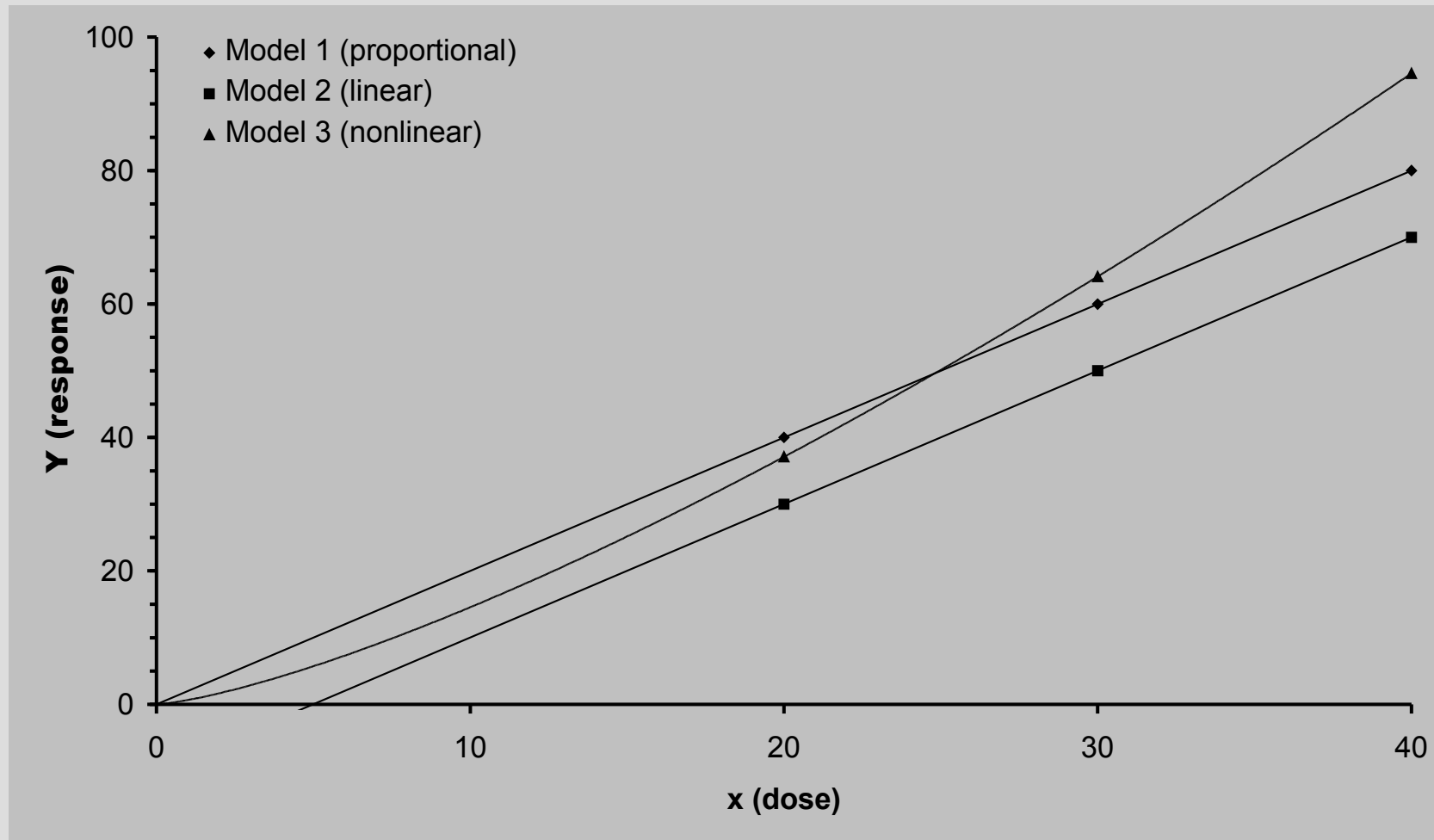
Bioavailability / Bioequivalence

- **Dose Proportionality**
  - Design of Study
    - ◆ 6-sequence 3-period Williams' design (3 dosage strengths)
    - ◆ Since the standard deviation of Y (AUC, C_{max}) increases

      with the dose, the primary assumption of dose proportiona-

      lity is that the standard deviation of Y is proportional to x

      (dose); that is Var(Y) = $x^2\sigma^2$ (σ^2 = total variance).
      - ➜ Model 1: E(Y|x) = $b \cdot$ x 'Dose Proportionality
      - ➜ Model 2: E(Y|x) = $a + b \cdot$ x, where $a{\neq}0$ 'Dose Linearity'
      - ➜ Model 3: E(Y|x) = $a \cdot x^b$, where $a{>}0$ and $b{\neq}0$ 'nonlinear'

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- ## Dose Proportionality

Bioavailability / Bioequivalence

- **Dose Proportionality**
  - Evaluation
    - Model 1:
      - PK responses (Ys) are normalized to the dose of the reference dose.
      - if 90 % CI of Ys are included in the Acceptance Range (*e.g.*, 0.80 – 1.25), Model 1 (Dose Proportionality) is proven, and the procedure stops,
      - if CIs are not included, Models 2 and 3 subsequently will be evaluated,
      - if bioequivalence to a reference has to be demonstrated, and Model is not proven, subsequent BE studies must be performed at each dose level!

Bioavailability / Bioequivalence

- **Dose Proportionality**
  - Evaluation
    - Model 2:
      - indicates that the relation between response and the dose follows a straight line with nonzero intercept (a).
      - Weighted linear regression with weights equal to x^{-1} with the original (untransformed) data (x,Y).
        - H_0: dose response curve goes through the origin
        - H_a: nonzero intercept
      - Evaluation by examining the 95 % confidence interval for the intercept a (*i.e.,* the null hypothesis will be rejected if zero is not included).
        - if the null hypothesis will be rejected, Dose Linearity is proven and Model 3 will additionally be evaluated.
        - if the null hypothesis will not be rejected, Model 1 may still hold, but the study was underpowered.

Bioavailability / Bioequivalence

- **Dose Proportionality**
  - Evaluation
    - ◆ Model 3:
      - ➔ indicates that the relation between response and the dose follows the form of a power curve with the exponent b.
      - ➔ Weighted nonlinear regression with weights equal to x^{-1} with the original (untransformed) data (x,Y). Alternatively the model may be linearized: $\log(\mathbb{E}(Y|x)) = \log(a) + b \cdot \log(x)$.
        - H_0: dose response curve follows a power curve
        - H_a: nonzero exponent
      - ➔ Evaluation by examining the 95 % confidence interval for the exponent b (*i.e.*, the null hypothesis will be rejected if zero is not included).
        - if the null hypothesis will be rejected, nonlinearity of PK in the dose range proven.

Bioavailability / Bioequivalence

- **Dose Proportionality**
  - Evaluation
    - Model 3:
      - The departure from dose linearity can be evaluated by the confidence interval (L,U) for b according to the following decision criteria:
      - Weighted nonlinear regression with weights equal to x^{-1} with the original (untransformed) data (x,Y). Alternatively the model may be linearized: $\log(\mathbb{E}(Y|x)) = \log(a) + b \cdot \log(x)$.
        - H_0: dose response curve follows a power curve
        - H_a: nonzero exponent
      - Evaluation by examining the 95 % confidence interval for the exponent b (*i.e.*, the null hypothesis will be rejected if zero is not included).
        - if the null hypothesis will be rejected, nonlinearity of PK in the dose range proven.

Bioavailability / Bioequivalence

- **Dose Proportionality**
  - Evaluation
    - Model 3:
      - The departure from dose linearity can be evaluated by the 95 %confidence interval (L,U) for b according to the following decision criteria:

0.75 < L < 1 < 1.25	no departure from dose linearity (*i.e.*, Model 2 holds)
1 < L < U < 1.25 *or* 0.75 < L < U < 1	slight departure from dose linearity, but no practical significance from dose linearity
L > 1.25 *or* U < 0.75	reject hypothesis of dose linearity (*i.e.*, Model 3 holds)

Bioavailability / Bioequivalence

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Bioavailability / Bioequivalence

- **Avoiding Pitfalls**

  - Matrix Effects in LC/MS

  - Missing Plausibility Review of Data

  - Exclusion of Outliers / Re-testing of Subjects

  - Dealing with Deficiency Letters

  - Repetition of Studies

Bioavailability / Bioequivalence

- **Matrix Effects in LC/MS**
  - Despite its popularity in modern bioanalytics LC/MS needs some special attention
    - Although a method may fully pass its validation with spiked samples, application of the method on 'real world' clinical samples sometimes fails:
      - Co-eluting substances may compete for ionization with the analyte.
      - Such an influence on the ionization efficacy, which – mostly – supresses the signal in the ion source (but rarely also may enhance the signal) is called a 'Matrix Effect'.

Bioavailability / Bioequivalence

- **Matrix Effects in LC/MS**
  - Remedies
    - Matrix effects must be thoroughly assessed
      - As a general rule – and just opposite to assertions of instrument manufacturers (just protein precipitation, and injection…) – sample clean-up for LC/MS must be more stringent than for other methods!
      - Application of an stable isotope labelled internal standard (^{2}H, ^{13}C, ^{18}O) may by helpful.
      - The shorter the chromatographic run time, the higher the posibilities of suffering matrix effects.
      - The use of tandem MS does not assure the absence of these effects because they take place in the ion source during the ion evaporation step and not in the analyzer.

Bioavailability / Bioequivalence

- **Matrix Effects in LC/MS**
  - Remedies
    - Matrix effects must be thoroughly assessed
      - Column switching may be helpful, because a lower quantity of the plasma or urine endogeneous products are entering into the ionization source.
      - The fact that you use QC samples does not assure that your results are correct because the matrix of the unknown samples will never be the same than yours.
      - The combination of two preparation techniques (*e.g.*, protein precipitation + on line SPE, off line + on line SPE) is also recommended.
      - If you want to have a robust and reliable method you need
        - a clean extract,
        - a suitable chromatographic separation, and
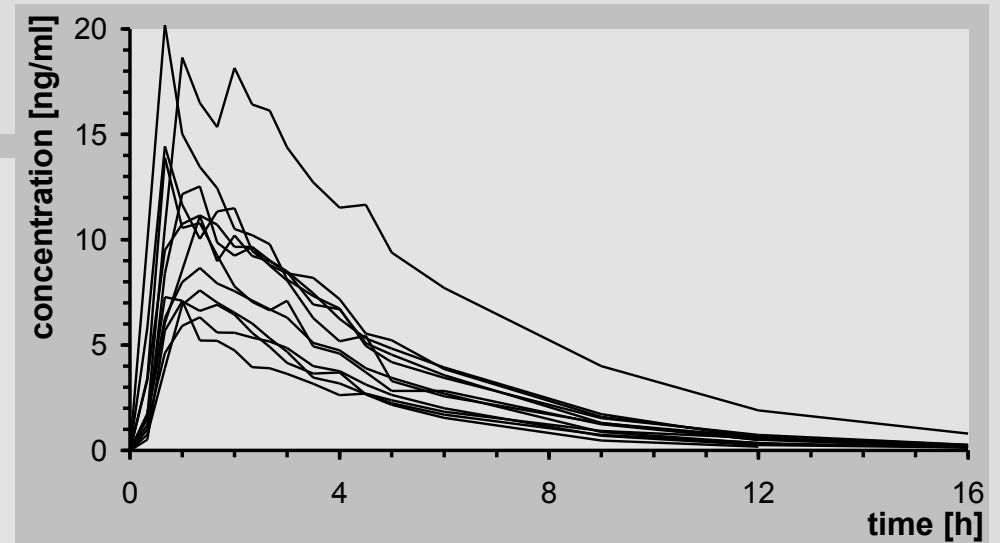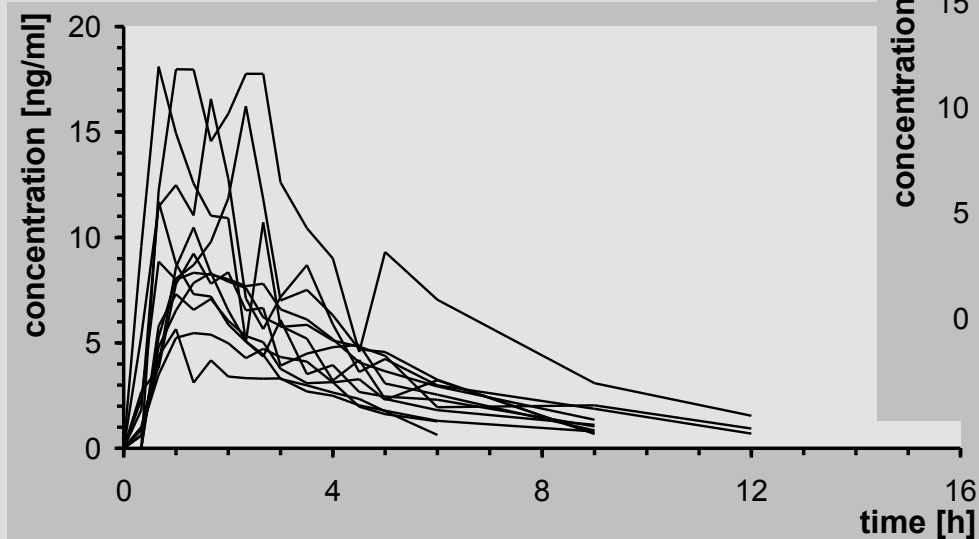   - a good internal standard (stable labelled if possible).

Bioavailability / Bioequivalence

- **Matrix Effects in LC/MS**
  - Methylphenidate
    - LC-MS/MS (LLOQ 220 pg/ml), GC/MS (LLOQ 143 ng/ml)
    'true LLOQ' in LC-MS/MS for some subjects >1.5 ng/ml!

Bioavailability / Bioequivalence

- **Avoiding Pitfalls**
  - Matrix Effects in LC/MS
  - Missing Plausibility Review of Data
  - Exclusion of Outliers / Re-testing of Subjects
  - Dealing with Deficiency Letters
  - Repetition of Studies

Bioavailability / Bioequivalence

- **Missing Plausibility Review of Data**
  - A Plausibility Review may prevent you from using 'false' data which may invalidate your entire study.
    - Suggested by Shah *et al.*
      - Suspected 'pharmacokinetic outliers' should be re-analyzed.

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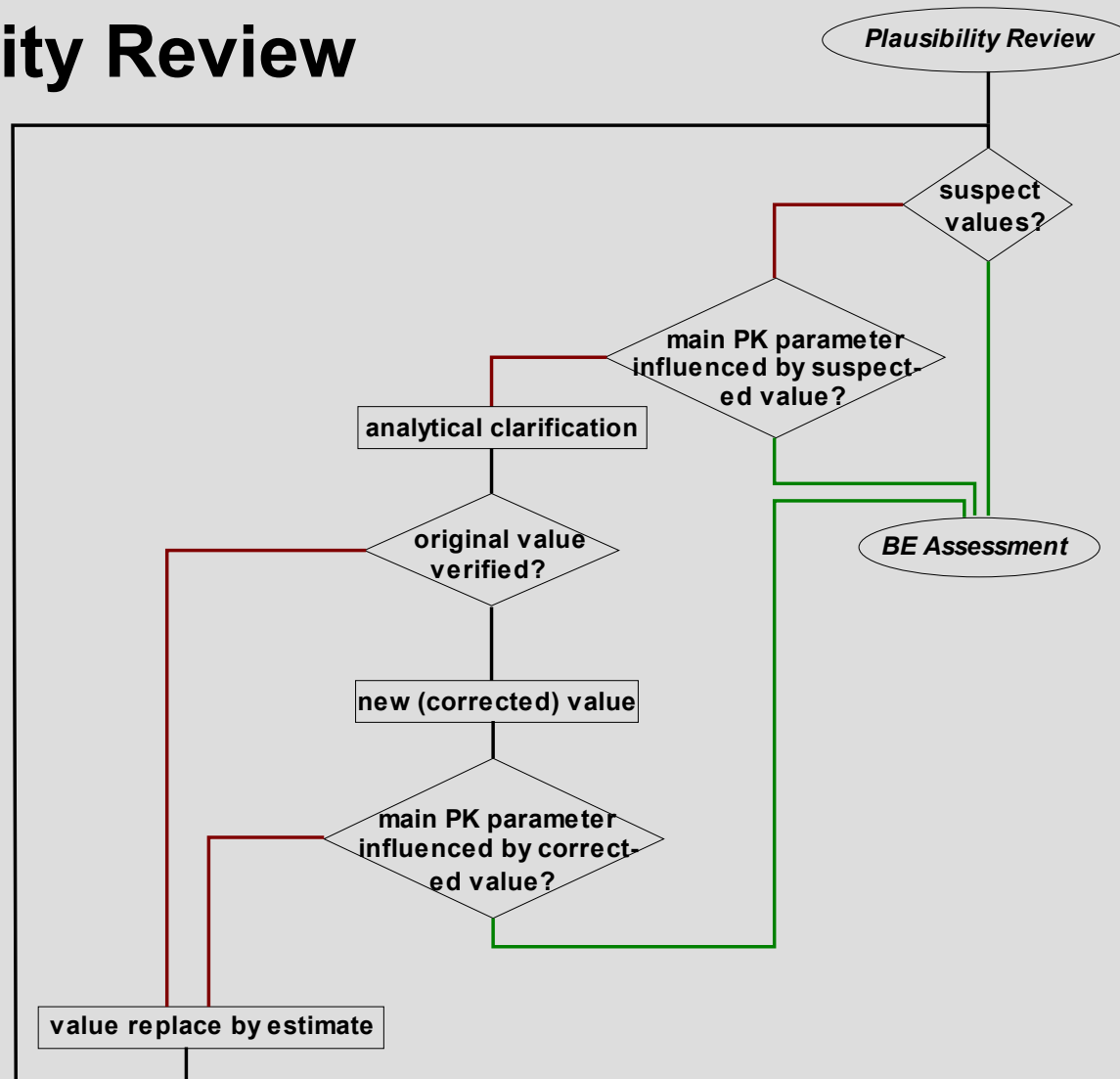   Bioanalytical Method Validation – A Revisit with a Decade of Progress.
   Pharm. Res. 17, 1551-1557 (2000)

Bioavailability / Bioequivalence

- **Missing Plausibility Review of Data**
  - Plausibility Review
    - If values would be analytically justified by repeated analysis, outliers may be substituted by estimates, if
      - ➔ pharmacokinetic characteristics would be directly and pronouncedly influenced, and/or.
      - ➔ their calculation would be impossible, *e.g.*,
        - 'sawtooth'-profiles in the range of tmax,
        - rising concentrations in the elimination phase leading to AUC=∞).
      - ➔ Since such a decision is not based on statistical methods but scientific knowledge and experience, great care should be taken rejecting and / or substituting questionable values. Any rejection is only allowed if the randomisation seal has not been broken. Any rejection / substitution has to be fully documented and justified in the biostatistical report.

Bioavailability / Bioequivalence

- **Plausibility Review**

Bioavailability / Bioequivalence

- ## Plausibility Review