**Arbeitsgemeinschaft
für angewandte
Humanpharmakologie e.V.**

Pitfalls in BA/BE

Attempts in beating Murphy's law: Learnings from failures in study design, bioanalytics and statistics

Helpful (?) quotations

If anything can go wrong, it will. — *Edward A. Murphy Jr.*

He who fails to plan is planning to fail. — *Winston Churchill*

You can't fix by analysis what you bungled by design. — *Richard J. Light, Judith D. Singer, John B. Willett*

100% of all disasters are failures of design, not analysis. — *Ronald G. Marks*

To propose that poor design can be corrected by subtle analysis techniques is contrary to good scientific thinking. — *Stuart J. Pocock*

To call the statistician after the experiment is done may be no more than asking him to perform a *postmortem* examination: He may be able to say what the experiment died of. — *Ronald A. Fisher*

If you think it's simple, then you have misunderstood the problem. — *Bjarne Stroustrup*

Whenever a theory appears to you as the only possible one, take this as a sign that you have neither understood the theory nor the problem which it was intended to solve. — *Karl R. Popper*

Study design

- In a crossover-study the washout between treatments has to be sufficiently long
  - Avoid pre-dose concentrations which are residuals of previous period(s)
  - In order to get an unbiased estimate of treatment differences the physiological state of subjects in higher period(s) has to be the same as in the (drug-naïve) first period
  - Cave
    - Never plan the washout (generally ≥5times the apparent half life) based on an average. Keep the distribution of half lives in mind. Some subjects might show a substantially longer half life – especially if the drug is subjected to polymorphism (poor and extensive metabolizers).
    - Think also about PD. If the drug is an auto-inducer or -inhibitor allow the body to return to its original state.

Case 1 | Study design

- Drug A: $t_{½}$ 60 – 100 h (literature)
  - BA study
    - 10 mg drug A hydrochloride p.o. *vs*. i.v.
    - 12 subjects
    - 2×2×2 crossover, washout 35 days
    - Sampling until 312 hours post dose
    - LC/MS-MS, LLOQ 1 ng/mL (drug A base / plasma)
    - Results considered important for designing other studies
      - $t_{½}$ 49.9 ± 13.0 h (harmonic mean ± jackknife standard deviation)
      - In none of the samples drawn at 312 h a concentration ≥LLOQ was measured
      - Extrapolated *AUC* 10.0% (median)
        3.8% – 13.9% (minimum – maximum)

Case 1 | Study design

- Drug A: $t_{½}$ 60 – 100 h (literature)
  - Comparative BA study aiming to demonstrate BE
    - 10 mg drug A hydrochloride
      (primary target T_2 *vs.* R, descriptive T_2 vs. T_1)
    - 36 subjects
    - 3×6×3 crossover (Williams' design), washout 14 days
      - Washout planned for a worst case $t_{½}$ of 66 h (covering >5 half lives)
    - Sampling until 216 hours post dose
      - No problems with extrapolated *AUC* expected (simulations)
    - GC/MS, LLOQ 0.117 ng/mL (drug A base / plasma)
  - Given that, what happened – and why?

Case 1 | Study design

- Pre-dose concentrations ≥LLOQ: n (% of subjects, geom. means)
  - Period 1: all <LLOQ
  - Period 2: 21 (58%, 0.226 ng/mL)
  - Period 3: 18 (50%, 0.222 ng/mL)

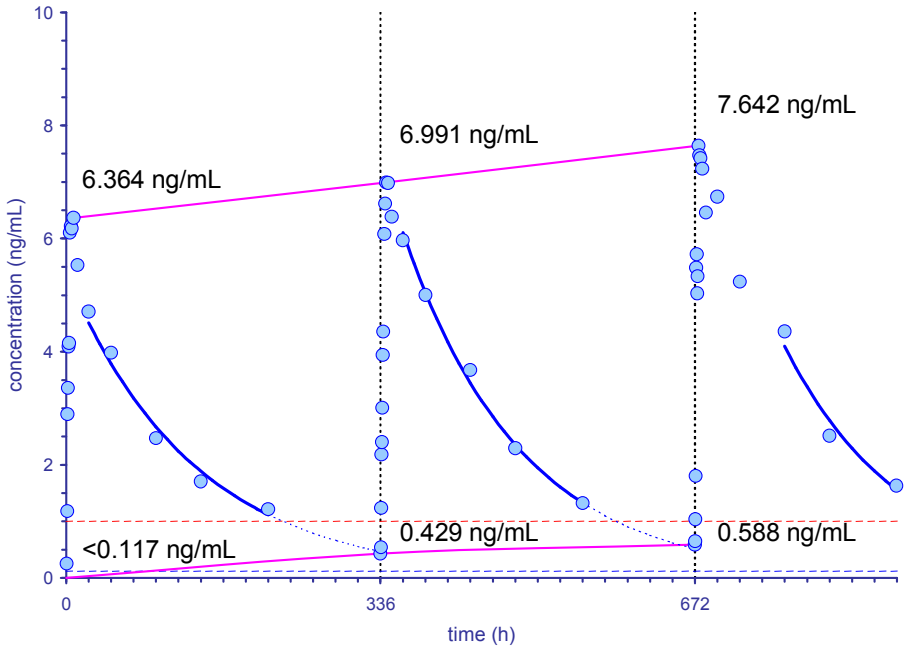
  } carry-over
- Half lives (harmonic means)
  - Period 1: 51.68 h
  - Period 2: 54.20 h
  - Period 3: 63.03 h

  } increasing with time

– Issues
- Improving the bioanalytical method (~9times lower LLOQ) was not a good idea
  - If we would have used the old method we would have seen not a single (!) pre-dose concentration >LLOQ
- The shorter washout (35 days → 14) was not a good idea as well
  - Only if the estimation of λ_z is performed *blinded for* treatment different half lives in the periods (due to accumulation) become evident – even with the less sensitive method

Case 1 | Study design

- Most statisticians unblind studies *before* performing NCA, which will cover potential problems
  - Half lives (harmonic means)
    - » T_1: 54.51 h
    - » T_2: 55.99 h
    - » R: 56.73 h
- Worst case Subject 23



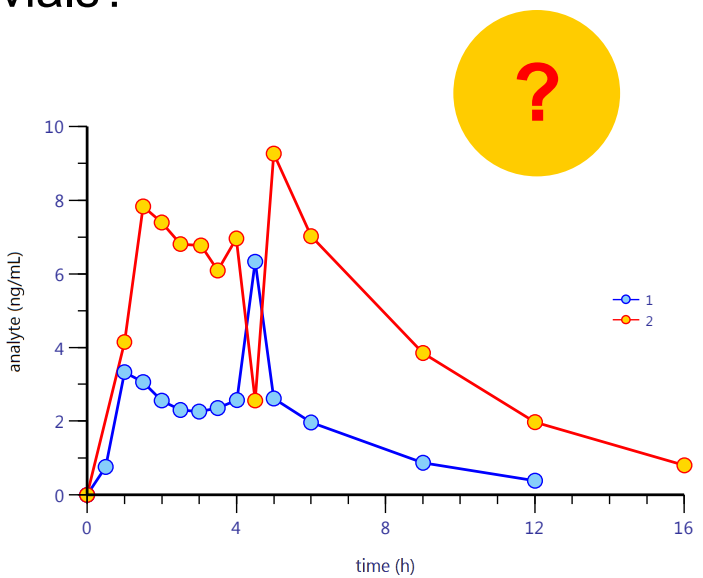
NCA

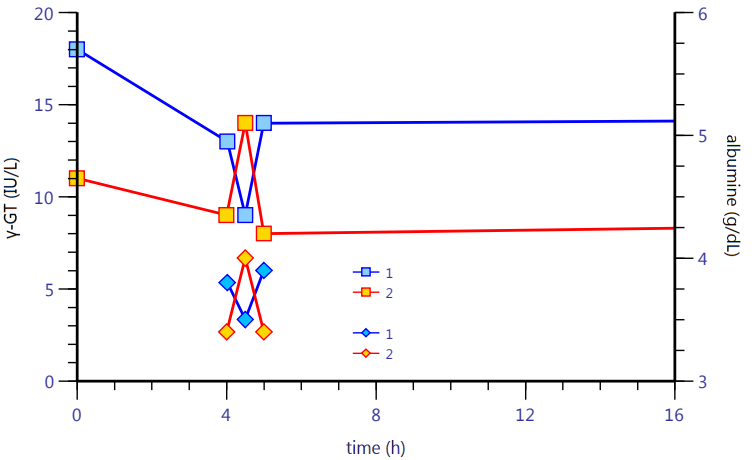
- Requirements for BA/BE studies
  - Bioanalytical method developed and validated *to serve the study's purpose*
    - Calibration range
      - LLOQ ≤5% C_{max} in any of the subjects
      - ULOQ ideally ≥C_{max} in any of the subjects
    - (In)accuracy and (im)precision
      - 15% throughout the range (20% for ligand-binding assays)
      - 20% at LLOQ (30% for ligand-binding assays)
  - Sampling long enough to obtain reliable estimates of
    - λ_z : at least three samples in the log/linear part
    - AUC_{0-t} : covering ≥80% of $AUC_{0-\infty}$ in ≥80% of observations
    - Note that both are *not required* if target metric is AUC_{0-72h} (IR single dose) or $AUC_{0-\tau}$ (steady state)

Case 2 | Sample handling

- Clinical phase
  - Biphasic modified release product of drug B
  - Mix-up in the transfer from sample vials after centrifu-gation to (plasma) sample vials?

Measurable values in clin. chemistry (limited, since anticoagulant citrate)

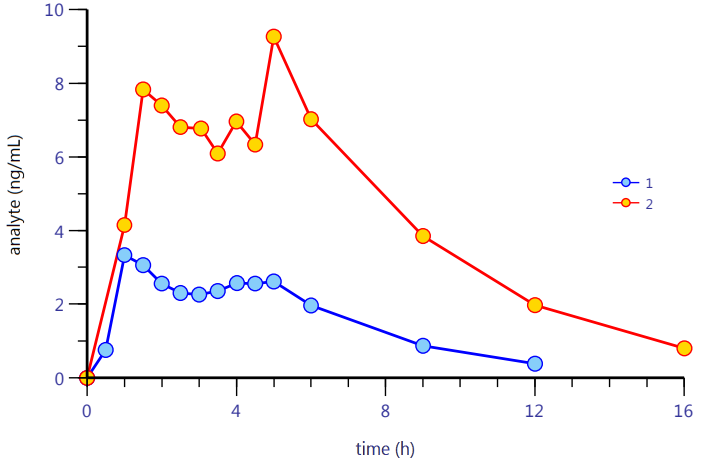


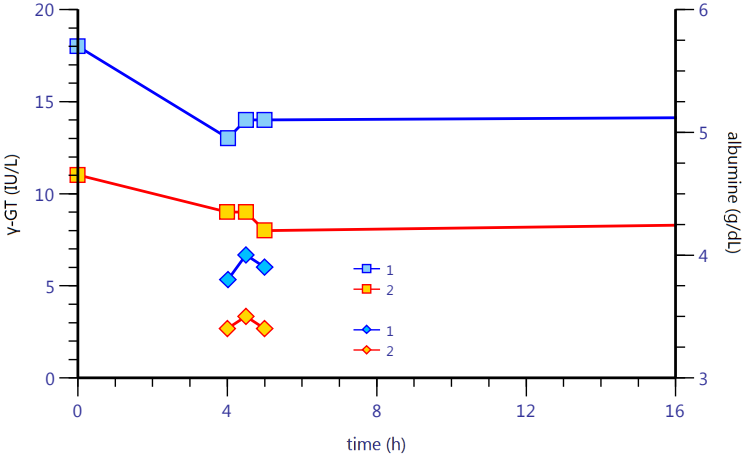


Case 2 | Sample handling

- Clinical phase
  - Biphasic modified release product of drug B
  - Exploratory: values swapped (both analyte and clin.chemistry)
  - Samples of subjects 1 & 2 both taken in the first period

Suspected mix-up confirmed by clin. chemistry values





Case 2 | Sample handling

- Clinical phase
  - Barcode system failed in the first period
  - No bail-out procedure (*e.g.*, four-eye principle)
  - Sponsor monitored plasma separation only up to two hours (when the barcode stystem was still working)
  - Blinded review of data for irregular profiles?
    - EMA
      - According the Bioanalytical Method Validation Guidelines (EMA, ICH) measured results are ‘carved from stone’
        - » Exclusion of data only possible if documented error
        - » Not even repeated analysis acceptable
    - FDA
      - Was acceptable until 2022
        - » Exclusion after repeated analysis possible if defined by SOP
      - Currently like EMA and ICH

Case 3 | Sample handling

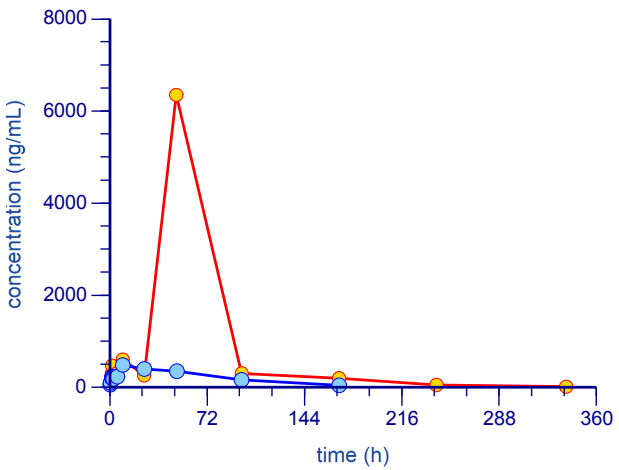
- Clinical phase
  - Liposome encapsulated drug C for infusion
  - Analytes
    - encapsulated drug C
    - unencapsulated drug C (*i.e.*, released from the liposomes)
    - total drug C (encapsulated + unencapsulated)
    - Metabolite (formed from unencapsulated drug C only)
  - Drug may be released from liposomes by
    - Shear forces (infusion pump, infusion needle with narrow diameter)
    - High temperature and long interval until centrifugation
    - High *g* force in centrifugation
    - Only the latter two can be avoided by proper stabilization
      - blood samples on ice
      - addition of DMSO

Case 3 | Sample handling

- Clinical phase
  - Multi-site study in cancer patients
  - Clinical staff educated about critical sample handling, but
    - unfamililar procedure esp. in small clinical sites
    - necessity of following SOPs and documentation of deviations in conformity with GCP not well understood

Case 3 | Sample handling

- Clinical phase
  - Surprises in bioanalytics
  - Extremely high concentrations of unencapsulated drug C observed in about 2% of samples
    - All values confirmed in repeated analyses (note: against guidelines)



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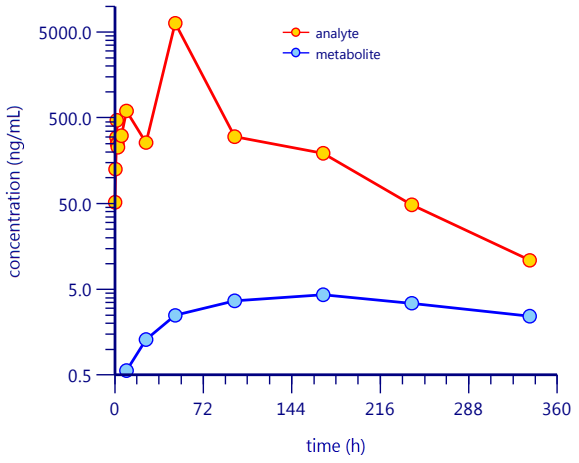
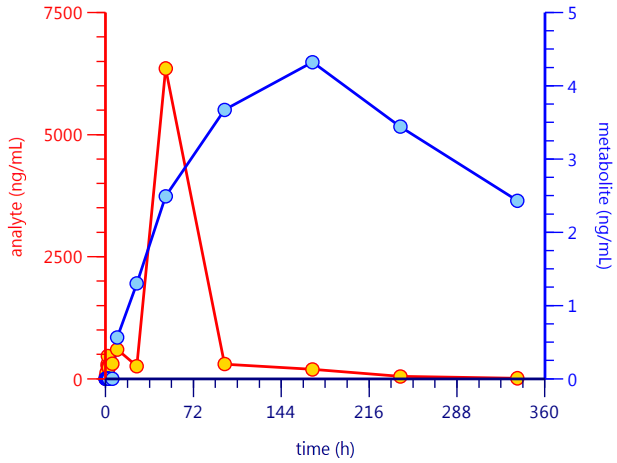
Case 3 | Sample handling

- Clinical phase
  - Extremely high concentrations of unencapsulated drug C observed in about 2% of samples
    - However, ‘normal’ concentrations of the metabolite
      - Since the metabolite can only be formed from the unencapsulated drug C, the analyte’s high concentrations were considered an artifact
      - No documentation about improper sample handling (temperature, time, stabilization)



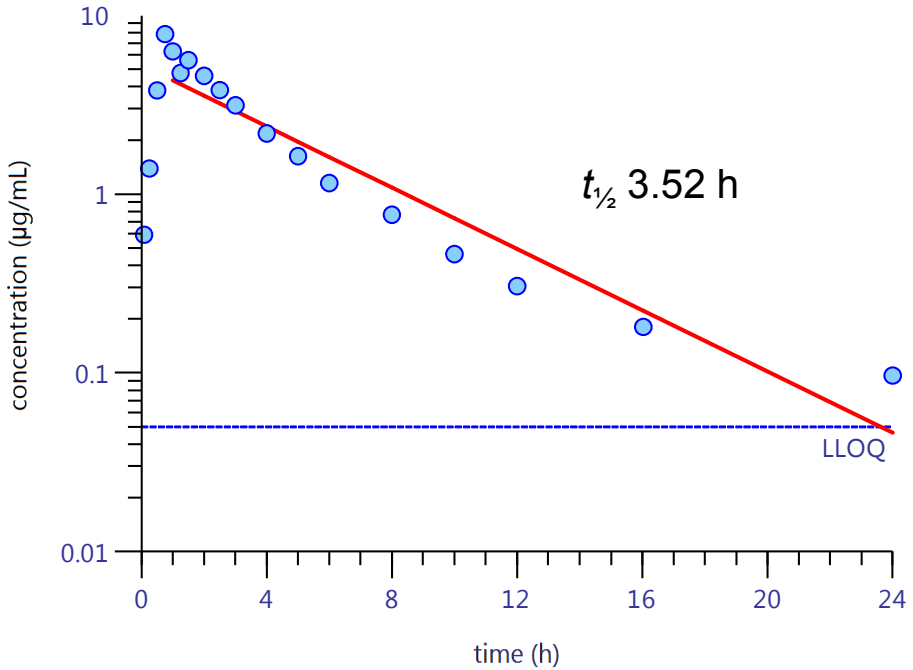


Case 4 | NCA

- Drug D: $t_{½}$ 2 – 3 h (literature)
  - BE study (500 mg D component of a three drug FDC)
    - liquid formulations, T *vs*. R
    - 27 subjects
    - TRR | RTR | RRT replicate design, washout seven days
    - Sampling until 24 hours post dose
    - LC/MS-MS, LLOQ 50 ng/mL
  - Drug D passed ABE with ease
    - $t_{½}$ 3.92 ± 0.88 h (T), 4.98 ± 1.24 h (R)
    - Extrapolated *AUC* (median, minimum – maximum)
      T: 1.76 (0.87% – 3.61%), R: 2.42% (1.14% – 6.19%)
  - Sponsor developed a 4 drug FDC
    - Data of the BE study should be used in a PopPK model to optimize the sampling schedule for a new study

Case 4 | NCA

- Drug D: $t_{½}$ 2 – 3 h (literature)
  - No individual λ_z or $t_{½}$ (as well as the time range used in estimation) given in the report, only $AUC_{0–t}$ and $AUC_{0–\infty}$
  - Reproduced the CRO's results by trial and error. Example:



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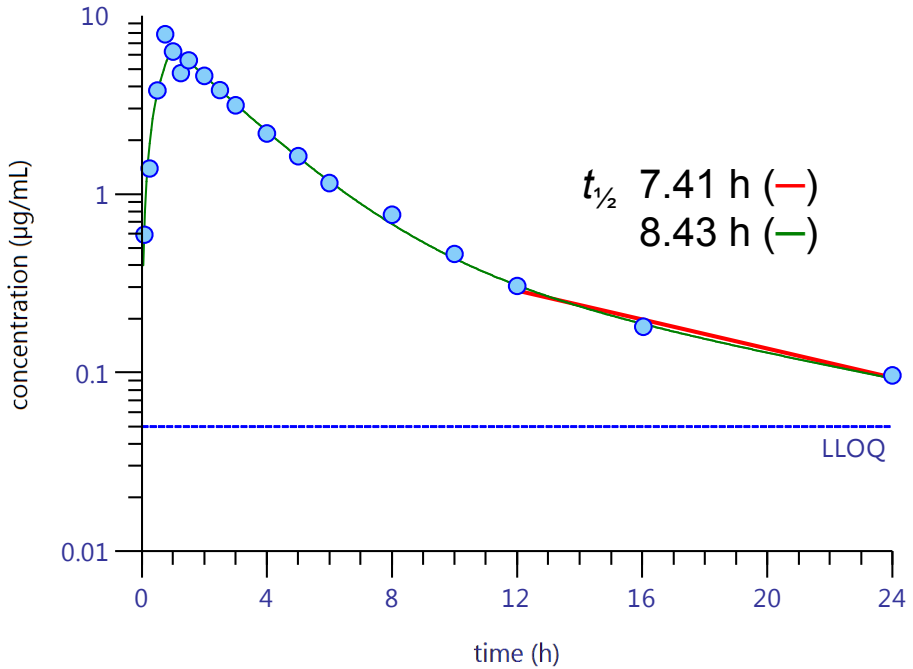
Case 4 | NCA

- Drug D: $t_{½}$ 2 – 3 h (literature)
  - Obviously the time range for the estimation of λ_z was wrong
    - Two-compartment model!
  - What I obtained in NCA (—) and by the PK model (—)

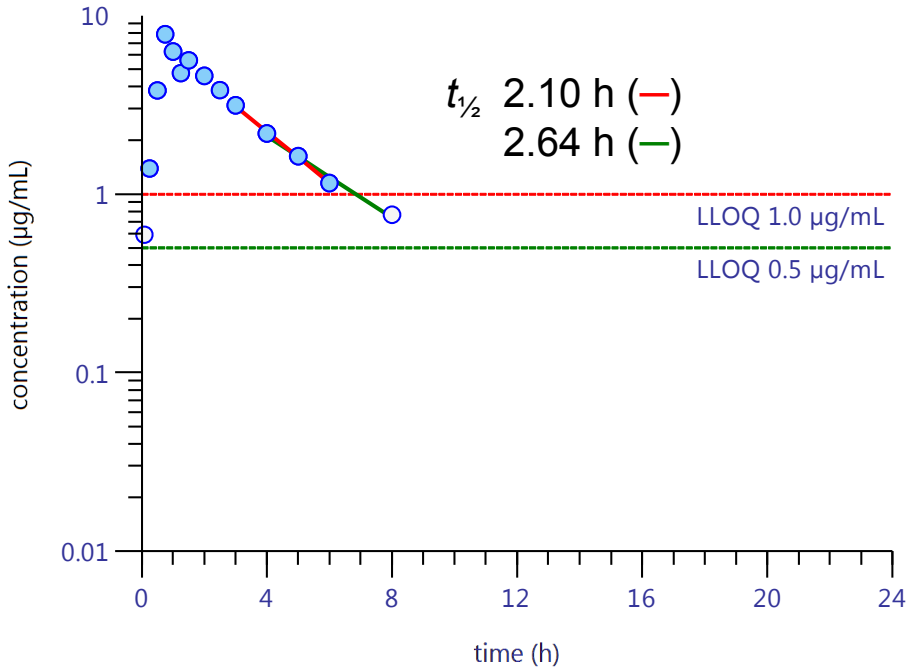


Case 4 | NCA

- Drug D: $t_{½}$ 2 – 3 h (literature)
  - Why? No problems with correct estimation of λ_z
    - $t_{½}$ 4.63 ± 1.07 h (T), 5.59 ± 1.19 h (R)
    - Extrapolated *AUC* (median, minimum – maximum)
      T: 2.08% (1.06% – 4.32%), R: 2.84% (1.47% – 6.19%)
  - Possible explanations
    - 'Push-the-button-pharmacokineticist' at work
      - Relied on an automatic algorithm?
      - No visual inspection of fits?
    - Anticipatory obedience ('vorauseilender Gehorsam')?
      - The bioanalytical method was at least 10times more sensitive than ones used in the past (drug D approved in 1955)
      - Maybe the CRO wanted to avoid a single sentence in the discussion section explaining why a second phase is apparent – explaining a half life longer than the one known from literature

Case 4 | NCA

- Drug D: $t_{½}$ 2 – 3 h (literature)
  - Estimation of λ_z by bioanalytical methods with a LLOQ of 1.0 or 0.5 µg/mL explains short half lives given in ‘old’ literature



Case 4 | NCA

- Drug D: $t_{½}$ 2 – 3 h (literature)
  - Lessons learned
    - Insist that the (draft!) PK report allows independent assessment
    - Mandatory[1,2]
      - All raw data
      - λ_z and/or $t_{½}$ as well as time ranges used in estimation
      - All derived PK metrics
    - Desirable
      - Data in a machine-readable format (CSV, SAS transport, CDISC)
  - Unacceptable
    - A 500+ page PDF generated by SAS
    - As above but a scanned print

1 Schulz H-U, Steinijans, VW. *Striving for standards in bioequivalence assessment: a review*. Int J Clin Pharm Ther Toxicol. 1991;29(8):293–8. PMID 1743802.

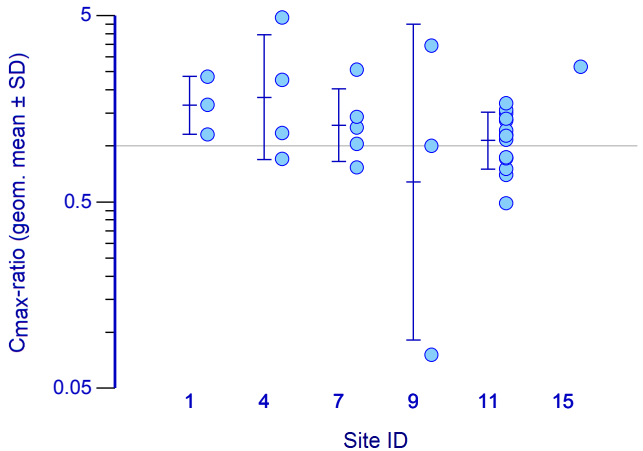
2 Sauter R, Steinijans VW, Diletti E, Böhm E, Schulz H-U. *Presentation of results from bio-equivalence studies*. Int J Clin Pharm Ther Toxicol. 1992;30(Suppl.1):S7–30. PMID 1601535.

Statistics

- Requirements for BA/BE studies
  - Design should allow accurate (unbiased) assessment of the treatment effect
  - EMA (2010), ICH M13A (2024)
    - The study should be designed in such a way that the formulation effect can be distinguished from other effects.
    - The precise model to be used for the analysis should be pre-specified in the protocol. The statistical analysis should take into account sources of variation that can be reasonably assumed to have an effect on the response variable.

Case 5 | Statistics

- Continuing Case 3
  - Extreme C_{max}-ratios of unencapsulated drug C observed only in small clinical sites



  - Pooling data of sites *only* if
    - similar variances
    - no treatment-by-site interaction

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Case 5 | Statistics

- Planned evaluation of unencapsulated drug C
  - Statistical model *did not* take the multi-site nature of the study into account (*i.e.*, data of all sites were naïvely pooled);
    - Failed: PE 121.01% (90% CI: 96.13 – 152.33%), CV_w 55.6%
- Sensitivity analysis
  - Statistical model suggested by the FDA including the site-by-treatment interaction
    - Significant (p 0.00063)
    - Hence, pooling of sites is *not* justified
  - Therefore, analysis of largest site #11 only
    - Passed: PE 103.80% (90% CI: 89.87% – 119.90%), CV_w 21.4%

Statistics

- Multi-Group Studies
  - ICH M13A (2024)
    BE should be determined based on the overall treatment effect in the whole study population. The statistical model should take into account the multi-group nature of the BE study, *e.g.*, by using a model including terms for group, sequence, sequence × group, subject within sequence × group, period within group and formulation. The group × treatment interaction term should not be included in the model. However, applicants should evaluate potential for heterogeneity of treatment effect across groups and discuss its potential impact on the study data, *e.g.*, by investigation of group × treatment interaction in a supportive analysis and calculation of descriptive statistics by group.

Statistics

- Multi-Group Studies
  - The test of a Group × Treatment interaction has low power (performed *between* subjects)
  - The test will 'detect' a G×T interaction in α% of studies though there is none (false positive rate)
  - Considered a statistical artifact in meta-analyses of well-controlled studies[1,2]
  - Conclusions[1] questioned by authors of the FDA[3] and refuted[4]

1 Schütz H *et al*. *Group-by-Treatment Interaction Effects in Comparative Bioavailability Studies.* AAPS J. 2024; 26(3): 50. doi:10.1208/s12248-024-00921-x.

2 Schütz H. Group 'Effect'. *To Pool or Not to Pool?* July 27, 2025. https://bebac.at/articles/Group-Effect.phtml

3 Sun W *et al*. *Letter to the Editor on "Group-by-Treat-ment Interaction Effects in Comparative Bioavailability Studies"*. AAPS J. 2024; 26(5): 101. doi:10.1208/s12248-024-00972-0.

4 Schütz H *et al. Rejoinder to the 'Letter to the Editor' on "Group-by-Treatment Interaction Effects in Comparative Bio-availability Studies".* AAPS J. 2025; 27(1): 14. doi:10.1208/s12248-024-01008-3.

Case 6 | Statistics

- Study in two groups (19 and 18 subjects)
  - Analysis by the group-model according to the guideline
    - *AUC*: 116.57% (90% CI: 110.83 – 122.62%)
    - Supportive analysis
      - G×T not significant (p 0.8785 > 0.05)
      - What is meant by »calculation of descriptive statistics by group«?