General Hurdles and Pitfalls in BE Studies

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Timing and project management

What to do if you have more studies to perform.

- Suggestions
  - Start with the most difficult one (*i.e.*, the one which most likely fails) *first*!
    - Variability in fed state commonly higher than in fasting state.
    - Due to potential different food effects of T and R the *GMR* may be worse.
      - Hence, fed study → fasting study.
  - MR: If the GL allows waiving the MD-study, perform the SD-study and assess the *additional* PK metrics (*e.g.*, early and terminal *pAUC*s) for BE.
    - If you fail these PK metrics (but still pass C_{max}, AUC_{0-t}, $AUC_{0-\infty}$) perform the MD-study.
      - If you have performed the SD- and MD-study and pass required PK metrics in both, the failing *pAUC*s in the SD-study are 'overruled'.
      - Since the purpose of *pAUC*s was only to justify waiving the MD-study (which *was* later performed) there is no reason for an assessor not accepting the application.

Timing and project management

What to do if you have more studies to perform.

- Suggestions
  - Variability in steady state is generally lower than after a single dose.
    - Estimate the *CV* from the SD-study.
    - Perform the MD-study in a Two-Stage-Design where the size of the first stage is ~75% of a fixed sample design.
      - » Reasonably high chance to pass already in the first stage (due to lower *CV*).
      - » If the CV is higher (unlikely!) you still get a second chance.
  - If ever possible try to perform studies in the same CRO.
    - If there are problems with the clincial capacity (→ different CROs), employ still the *same* bioanalytical CRO.
      - » If you face capacity problems in bioanalytics (→ different CROs) make sure (!) that the *same* validated method is used.
      - » If ever possible,
        (a) assure that the same type of instruments are used and
        (b) run a cross-validation between sites.

Timing and project management

Large studies – lacking capacity of the clinical site.

- Suggestions
  - Find a larger CRO – even if more expensive!
  - If you have to split the estimated sample size into groups:
    - Dose subjects within a limited time frame, *e.g.*, the groups only days apart (sometimes called the 'staggered approach').
      Group I : period 1, Mo – We → washout → period 2, Mo – We
      Group II: period 1, Th – Sa → washout → period 2, Th – Sa
    - Do *not* split groups into equal sizes.
      Perform at least one in the maximum capacity of the clinical site.
    - Some jurisdictions (Russian MoH and Saudi FDA always, FDA regularly, EMA sometimes) require a statistical test for the 'group-by-treatment interaction'.
      - » If this test is significant at the 0.1 level, one is *not* allowed to pool the data and is only free to demonstrate BE in the *largest* group.

Timing and project management

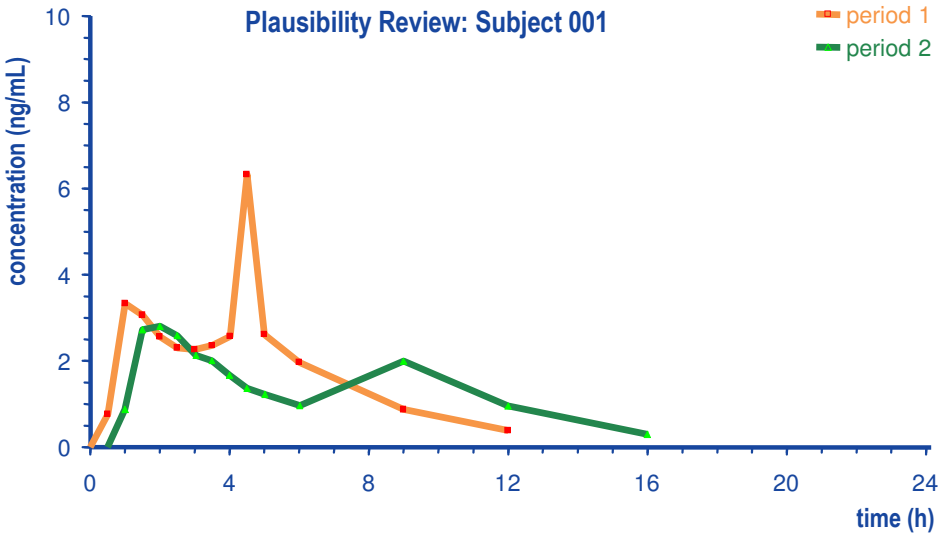
Large studies – lacking capacity of the clinical site.

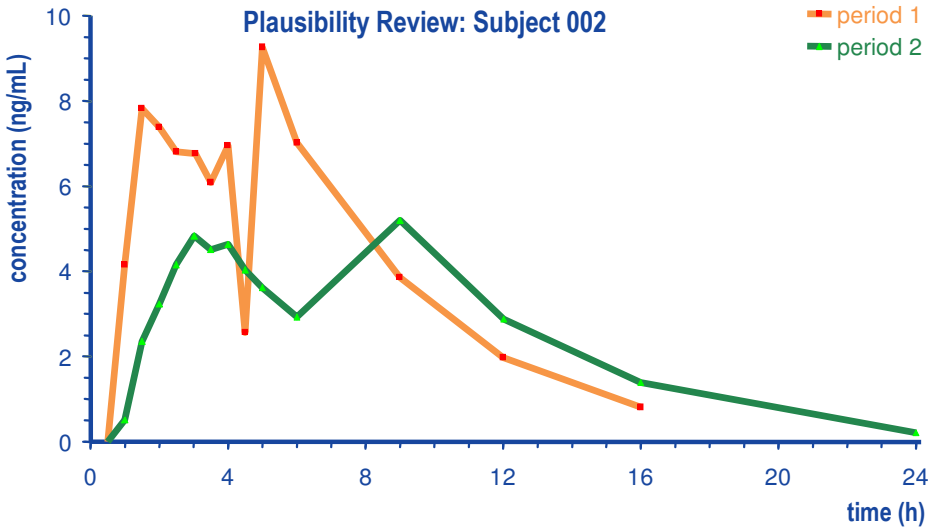
- Example
  - *CV* of *AUC* 30% (no scaling allowed), *GMR* 0.90, target power 90%, 2×2×4 (reference-scaling of C_{max} intended). Estimated sample size 54.
  - Maximum capacity 24 beds.
    - Option 1: Equal group sizes (3 × 18).
    - Option 2a: Two groups with the maximum size (24), the remaining one 6.
    - Option 2b: One group 24, the remaing ones as balanced as possible (16 | 14).
  - Let us assume that there are no drop-outs and pooling is not allowed (significant group-by-treatment interaction). Expected power:
    - Option 1: 51% in each of the groups.
    - Option 2a: 62% in the two largest groups (n = 24 each).
    - Option 2b: 62% in the largest group.
  - Which one would you prefer – and *why*?

Pitfalls: Case Study 1

Sample mix-up.

- Very large CRO (study performed in 2008). Common drug, biphasic MR formulations, pilot study (suboptimal sampling between 6 – 14 h).

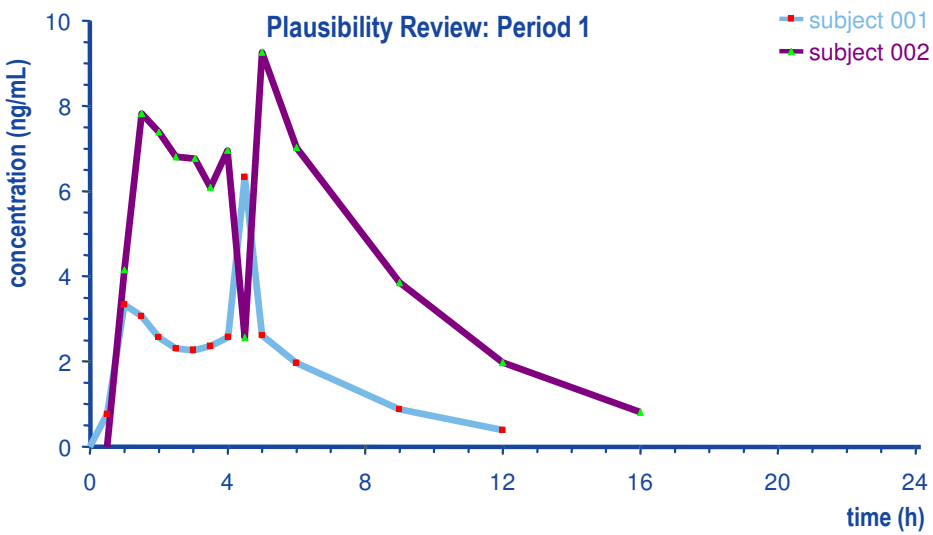




Pitfalls: Case Study 1

Sample mix-up.

- Barcode-system out of order in the first period of the study.
- No bail-out procedure (*e.g.*, four eyes principle).
- Suspected sample mix-up at 4.5 h.
- Concentrations confirmed.
- No deviation documented in clinical phase.
- Drug has very low intra-subject *CV* (*AUC* ≤10%, C_{max} 10–15%) and high inter-subject *CV* (>50%) due to polymorphism.
  Pivotal studies are generally performed in only 14 subjects.
- A single mixed-up sample close to t_{max} could ruin an entire study.

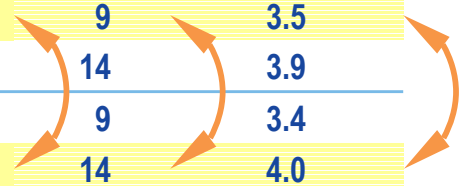


Pitfalls: Case Study 1

Sample mix-up.

- We tried to confirm the mix-up by comparing lab-values of the suspect samples (and each of the two neighbouring ones in each profile).
  - Anticoagulant was citrate for GC/MS.
  - With this anticoagulant the analyzer was validated only for γ-GT and albumine.

subject	time (h)	analyte (ng/mL)	γ-GT (U/L)	albumine (g/dL)
001	4.0	2.572	13	3.8
001	4.5	6.330	9	3.5
001	5.0	2.615	14	3.9
002	4.0	6.956	9	3.4
002	4.5	2.561	14	4.0
002	5.0	9.262	8	3.4



- γ-GT and albumine showed a similar pattern like the analyte.
  - Mean values of γ-GT in the pre- and post-study lab exams were 14 U/L (# 001) and 9 U/L (# 002). Means of albumine were 3.9 g/dL (# 001) and 3.4 g/dL (# 002).
  - Luckily subjects differed in their values. The pilot study was only supportive…

Pitfalls: Case Study 1

Sample mix-up.

- Before the current EMA GLs a blinded plausibility review was acceptable (and still is in many regulations like the FDA).
- According to the current EMA GLs re-analyzing of samples is not permitted.
  - Gerald Beuerle of TEVA/ratiopharm (joint EGA/EMA workshop, London 2010) presented an example were due to a single mix-up a study would *pass*.
    - The study would *fail* to show BE if the results were exchanged.
    - The study would *fail* to show BE if the two subjects were excluded.
    - Panelists of the EMA's PKWP confirmed that either procedure is not acceptable and the values have to be used *as the are* (*i.e.*, the study would *pass*).
  - Helmut Schütz: *'The EMA is a Serious Risk to Public Health!'*
- At the 2nd International Conference of the Global Bioequivalence Harmonization Initiative (Rockville, 15 – 16 September 2016) Session IV was devoted to the issue (*Exclusion of PK Data in the Assessment of IR and MR Products*).

Pitfalls: Case Study 1

Sample mix-up.

- Lessons learned:
  - The most critical phase is the transfer from centrifuged blood sample tubes to the vials containing the sample matrix used in bioanalytics.
  - When we installed a barcode-system in 1991, the rate of sample mix-ups dropped from 0.2% to zero.
  - A bail-out procedure must be in place (four eyes principle), an SOP at hand and followed by the personel!
    - I once audited a CRO where the SOP mandated that the centifuged samples and vials are scanned one after the other – immediately after the transfer.
      - The technician took four Eppendorf vials (centrifuged blood samples) in his left hand and scanned them.
      - Then he scanned four empty sample vials.
      - Next he pipetted the four samples one after the other.
      - *'Why are you do this in such a way?' –*
        'It saves time, and four vials fit nicely in my hand.'

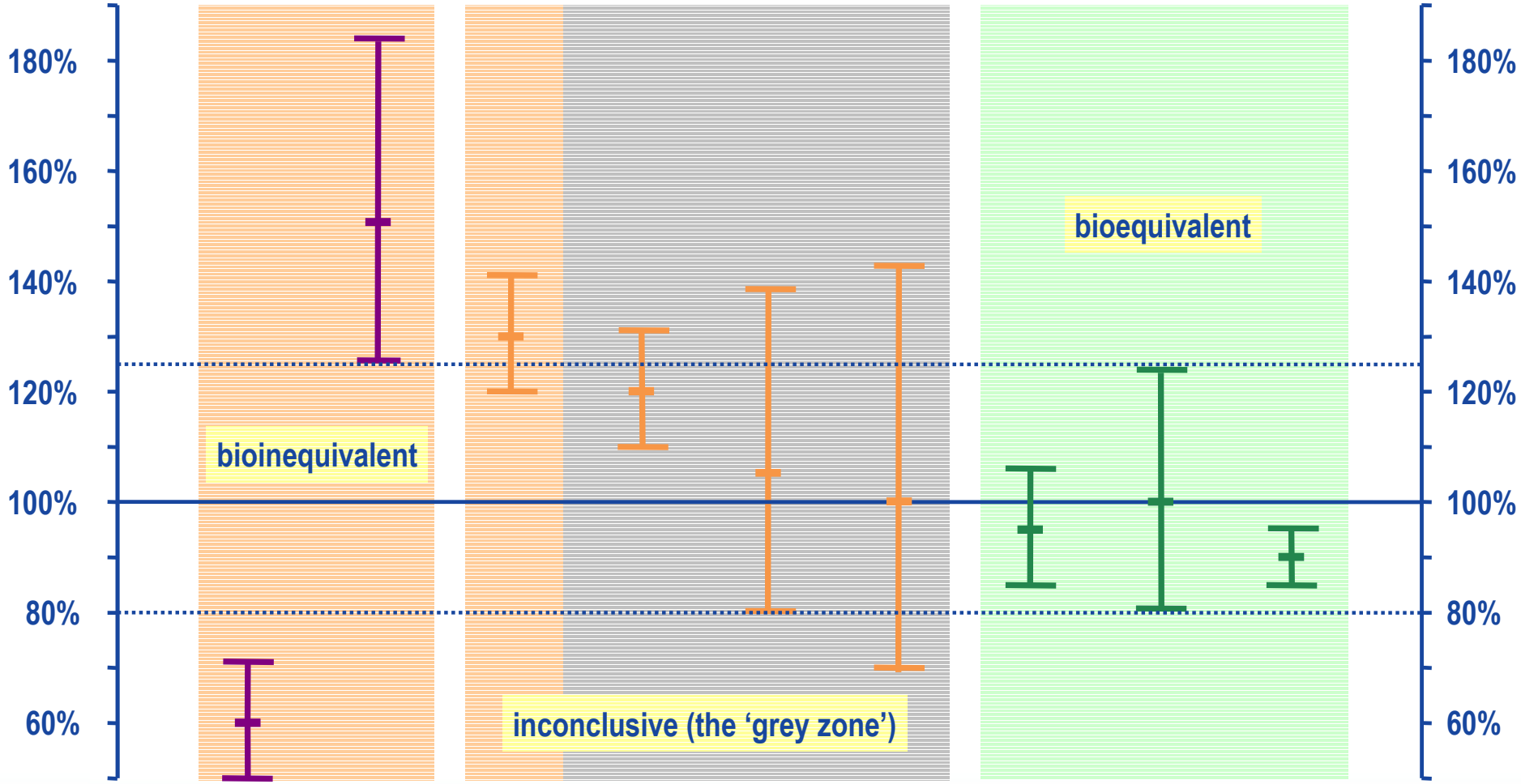
‘Lack’ of statistical power

Sometimes a properly planned study fails by pure chance.

- Power is *fixed* by design (in the sample size estimation)!
- It is unavoidable, that the producer’s risk (probability of Type I Error, where $\beta = 1 - \text{power}$) hits in actual studies.
  - If studies are planned with 80% power,
    one out of five studies will fail –
    even if products *are* bioequivalent.
  - *Post hoc* (aka *a posteriori*) power is a flawed statistical concept.
  - Reporting *post hoc* power is a bad habit and should be abandoned.
    - Either a study has demonstrated bioequivalence or not.
      - » As ‘high’ power does not further *support* the claim of already demonstrated BE,
      - » ‘low’ power does not *invalidate* the conclusion of BE!
- The only *realistic* remedy for a failed study is to repeat it in a larger sample size – *if the PE is promising*.

‘Lack’ of statistical power?

Some studies: Point estimates and their 90% CIs.



Are Add-on studies acceptable?

Add-on Designs

- In an Add-on Design (AOD) an initial group of subjects is treated and – if the result is inclusive (*i.e.*, although the point estimate is within the BE-limits, the CI is not) –
  - an additional group of subjects can be recruited and
  - the assessment of bioequivalence repeated in the pooled dataset.
- General conditions:
  - The intention to perform an AOD has to be stated in the protocol.
  - The same batches of products and the same clinical and bioanalytical methods have to be employed in both groups.
  - Additional requirements were stated in some jurisdictions.
- Somewhat popular in the 1990s and reflected in regulatory documents (HC 1992, NZ 1997) – and later abandoned. Currently still in Argentina (2006), Korea (2008), Japan (2012), Mexico (2013).

Are Add-on studies acceptable?

Add-on Designs

- Statistically questionable
  - Repeated testing without adjusting the level of the tests will inflate the Type I Error (patient's risk).
  - If *k* repeated test are performed at α 0.05, the TIE will approach $1 - (1 - \alpha)^k$ or 9.75% for two tests.
  - In naïve pooling of data, both the variance will be underestimated and the nominal level of the test will be exceeded.
  - Inflation of the TIE demonstrated in simulations (Potvin *et al.* 2008, Wonnemann *et al.* 2015, Schütz 2015).
- Preserving the consumer risk
  - Bonferroni correction (for two tests α 0.025 or a 95% CI) keeps the TIE at ≤4.94%.
    - Sample size penality compared to a fixed-sample design (20–30% more subjects).
    - n_2 should be $\geq n_1$ (Birkett and Day 1994).

Are Add-on studies acceptable?

Add-on Designs

- Only if unavoidable!
  - If you apply in Argentina, Korea, Japan, or Mexico – aim for a scientific advice suggesting a Two-Stage Design (Session 4, part I) instead.
  - If you do not succeed:
    - Employ Bonferroni's adjustment (95% confidence interval).
    - Adjust the sample size accordingly.

Failing a fed or fasting part of the study

MR products (EMA 2014) and some product-specific guidance by the FDA

- Fasting and fed in the *same* study in the EMA's approaches 1 and 2.
- Fasting and fed in *separate* studies (fasting, fed) in the EMA's approach 3 and recommended by the FDA.
- Suggestions
  - Educated guess whether the study failed *only* by lacking power (too small sample size) or a 'bad' point estimate (slides 11–12).
    - If the PE is promising, repeat the study in a larger sample size.
      - » If fasting/fed was nested in a design (EMA #1 and #2) it will be difficult. If you repeat the entire study due to pure chance the respective other comparision may fail this time due to pure chance.
      - » For EMA #3 and the FDA repeat the respective study.

Failing a fed or fasting part of the study

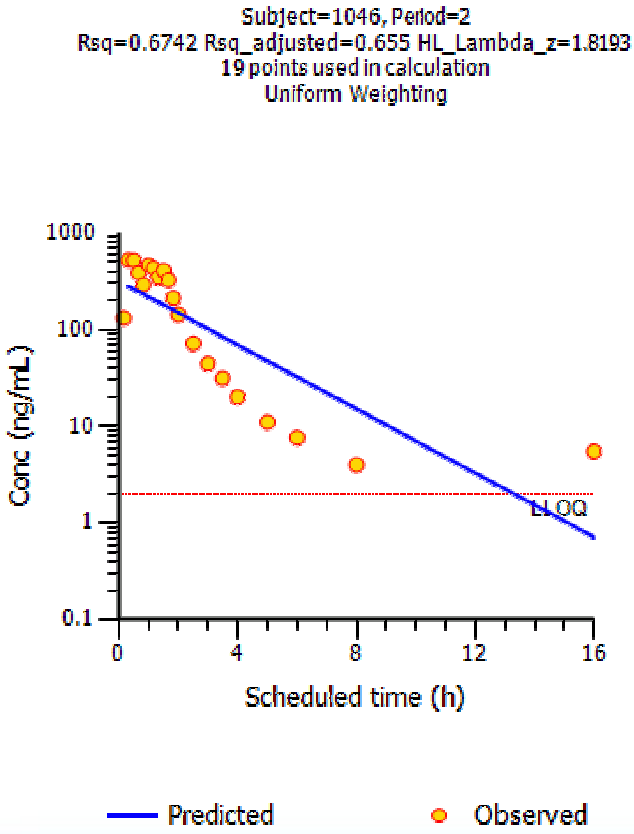
MR products (EMA 2014) and some product-specific guidance by the FDA

- **Suggestions**
  - If products are inequivalent (CI completely outside the BE-limits) or if the PE is not promising (*e.g.*, close to or even outside the BE-limits) modify the formulation.
    - » If you did not do that before, consult with an expert in IVIVC and explore new dissolution methods (maybe biorelevant).
    - » Development of candidate formulations with different release charcteristics.
    - » Pilot *in vivo* studies and development of a discriminatory dissolution method which allows selection of a test formulation which matches the reference *in vitro*.
    - » Repeat the entire pivotal BE-program.

Pitfalls: Case Study 2

NCA (estimating λ_z).

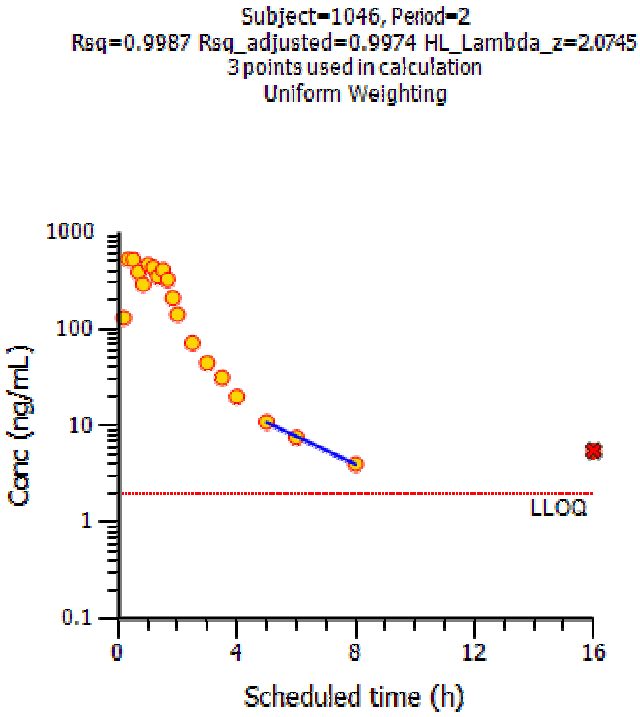
- Large CRO (study performed in 2013). 4-period full replicate; the double peak is specific for the formulation.
  - In four cases the last concentration was *increasing*. The CRO followed EMA's GLs and did not re-analyze samples (PK reason alone not sufficient). Obviously the CRO tried to 'save' the profiles by including more data points…
  - To the right the most extreme case.
    - Two samples (at 10 & 12 h) were BLQ.
    - 5.47 ng/mL (~2.7× LLOQ) at 16 h.
    - The first time point for the estimation of λ_z was t_{max}.



Pitfalls: Case Study 2

NCA (estimating λ_z).

- What I would do (if an SOP allows that). Two options:
  - Exclude the doubtful value from the estimation of λ_z. Justifications:
    - The estimated half-life of 2.07 h is consistent with the ones of the same subject in the other periods (2.12, 2.00, 2.16 h).
    - Two values before the doubtful value were BLQ – which agrees with the predicted λ_z.
  - Drop the profile from the *AUC* comparison, but keep C_{max} (higher variability anyway and reference-scaling intended in the protocol).



Pitfalls: Case Study 2

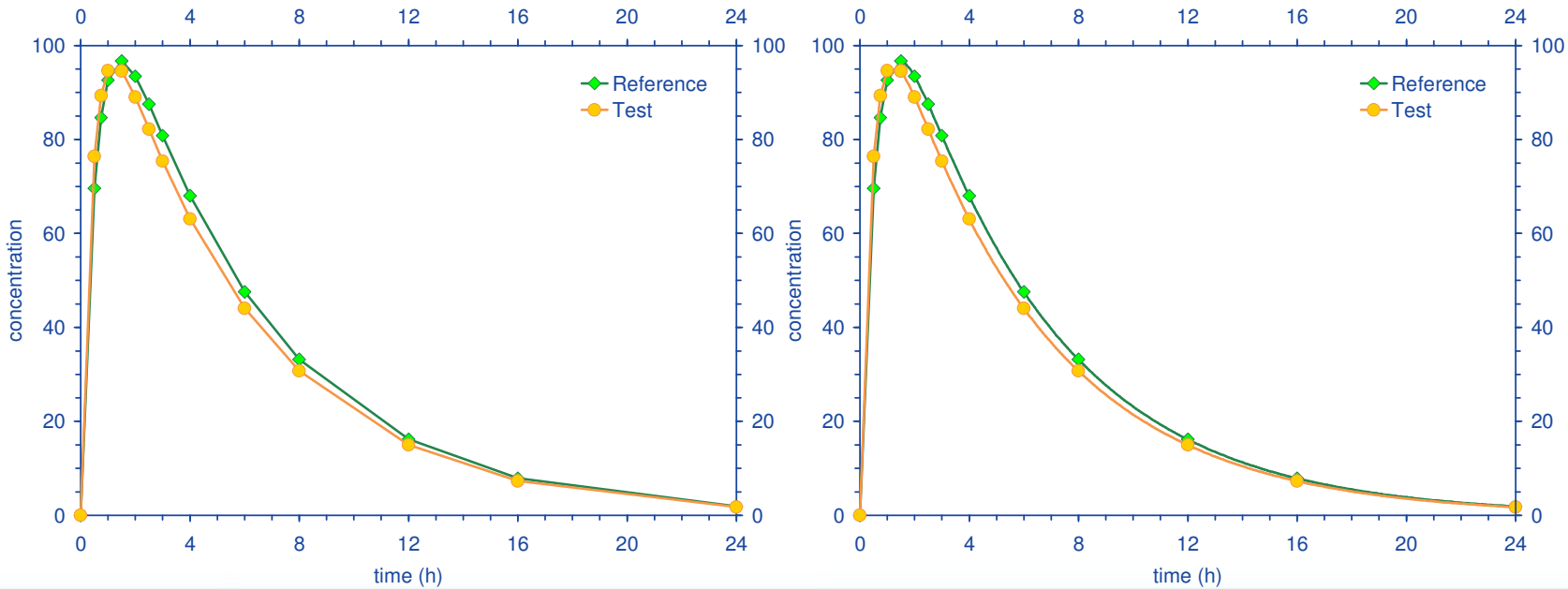
NCA (estimating λ_z).

- Lessons learned:
  - Never solely rely on automatic methods (maximum R^2_{adj}) implemented in software.
    - Visual inspection of the fit (and correction if necessary) recommended (Hauscke *et al.* 2007, Scheerans *et al.* 2008).
    - For IR products absorption is essentially complete after two times t_{max}. Hence, $\geq 2 \times t_{max}$ is good starting point to get an unbiased estimate of λ_z (not substantially contaminated by absorption).
    - In WinNonlin 5.3 (Pharsight) and Kinetica 5.0 (Thermo Scientific) t_{max} can be included by the automatic method. Update the software (Phoenix/WinNonlin ≥6.0) or rule it out in an SOP.
    - Have an SOP in place which allows
      - visual inspection of fits / correction (mandatory),
      - exclusion of a subject from the AUC comparison if no reliable fit can be established (good) or
      - exclusion of data points (much better).

Pitfalls: Case Study 3

NCA (trapezoidal methods).

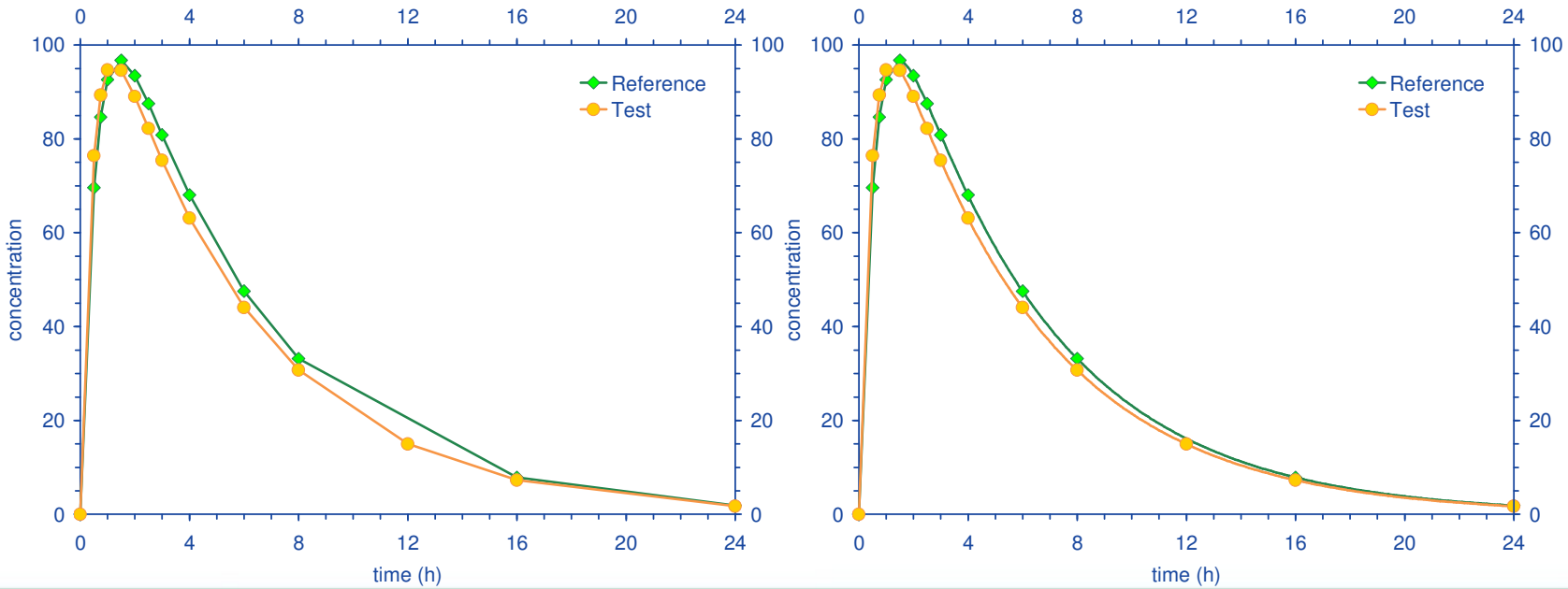
- If all samples are available, there is practically no difference between algorithms.
  - Simulated data. AUC_{∞} 697.8 (Reference), 662.9 (Test), true *GMR* 95.00%.
    - Linear trapezoidal: 707.6 (R), 670.9 (T); *GMR* 94.85% (bias –0.20%).
    - Lin-up / log-down trapezoidal: 693.7 (R), 658.0 (T); *GMR* 94.89% (bias –0.16%).



Pitfalls: Case Study 3

NCA (trapezoidal methods).

- If a sample is missing (*e.g.*, vial broken in centrifugation), the chosen algorithm matters. 12 h sample (R) removed.
  - Simulated data. AUC_{∞} 697.8 (Reference), 662.9 (Test), true *GMR* 95.00%.
    - Linear trapezoidal: 725.1 (R), 670.9 (T); *GMR* 92.53% (bias –2.60%).
    - Lin-up / log-down trapezoidal: 693.7 (R), 658.0 (T); *GMR* 94.89% (bias –0.15%).



Pitfalls: Case Study 3

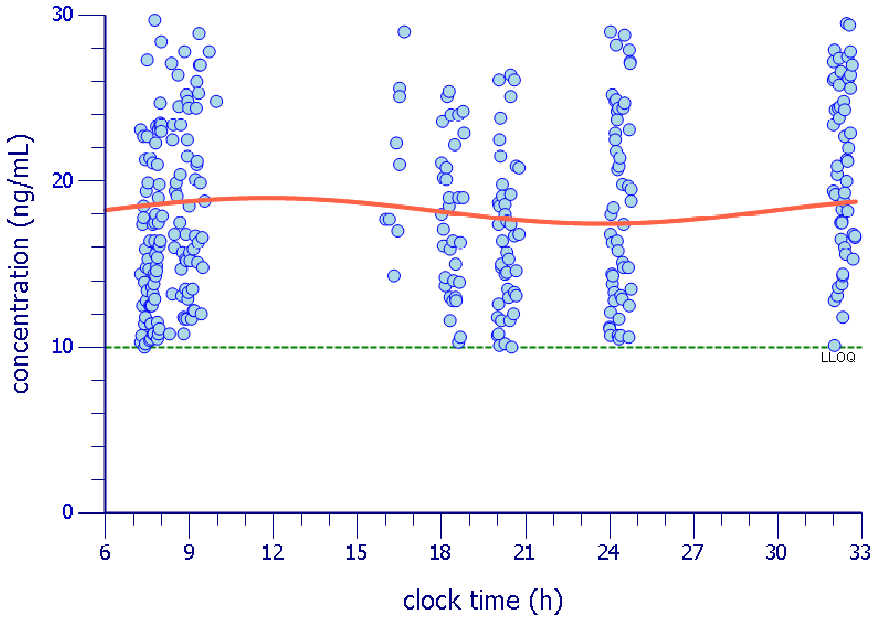
NCA (trapezoidal methods).

- Lessons learned:
  - Trapezoidal methods
    - The linear trapezoidal method goes back to the times were we drew profiles on millimeter paper, clipped them, and weighed them on an analytical balance.
    - I never saw anybody using a curve template in order to approximate an exponential decrease. Connecting data points by straight lines was state-of-the-art.
    - With a few exceptions (ethanol, Michaelis-Menten PK) we know [*sic*] that concentrations decrease exponentially.
      Therefore, the most suitable NCA-method for calculating the AUC is the lin-up / log-down trapezoidal method.
  - Missing samples are not uncommon.
    - Only with the lin-up / log-down trapezoidal method we get unbiased estimates of the AUC.
    - The linear trapezoidal method should be abandoned.

Pitfalls: Case Study 4

The 'perfect' bioanalytical method.

- Endogenous drug (basal levels BQL to 30 ng/mL; circadian rhythm), average C_{max} 5,400 µg/mL (MR), 26,200 µg/mL (IR), half life 45 min, sampling for 24 hours, method validated for 10 ng/mL to 50 µg/mL.
  - In the estimation of λ_z I had to exclude *all* time points >12 hours since concentrations were consistently *increasing*.
  - Although the protocol and my SOP allowed that, it *looks* fishy.
  - I developed a full-blown PopPK model to explain the diurnal variations in basal levels.
  - Justification accepted by the agency.



Pitfalls: Case Study 4

The 'perfect' bioanalytical method.

- Lessons learned:
  - Well-intentioned is often the opposite of well done.
  - The bioanalytical method should be validated *for the intended use* (Session 9).
    - It does not make sense that the LLOQ of the method was 0.19% and 0.04% of C_{max} (after MR and IR, respectively).
  - In later studies
    - the LLOQ was set to 50 ng/mL (*i.e.*, five times higher),
    - sampling performed only up to 12 hours;
    - no more problems with basal levels (below the LLOQ) and increasing concentrations, and
    - the extrapolated fraction of the *AUC* was still below 1%.

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Thank You!

Open Questions?

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