AGAH

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

How to measure what happens in pharmacokinetics

PK metrics of relevance!

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Terminology

- Estimates obtained by a *PK model*:
  PK parameters
  - Primary parameters
    f, V, CL, micro rate constants (k_a, k_e, k_{12}, k_{21}, …),
    macro constants (A, B, C; α, β, γ, …), etc.
  - Secondary parameters derived from primary ones & the model
    C_{max}/t_{max}, $t_{½}$, AUC_{0-t}, $AUC_{0-\infty}$, …
- Results obtained by *Noncompartmental Analysis*:
  PK metrics
  - Either directly measured (C_{max}/t_{max}) or
  - calculated by – rather simple – numerical methods
    ($\lambda_z/t_{½}$, AUC_{0-t}, $AUC_{0-\infty}$, …)

Noncompartmental Analyis

- NCA aka SHAM (Shape, Height, Area, Moments)
  - PK metrics (plasma)
    - Single dose
      - Extent of Absorption (EEA, …), Total Exposure (USA): *AUC* (Area Under the Curve)
        - » In most jurisdictions the PK metric for BE is AUC_{0-t}, where t is the last time point with a quantifiable concentration
        - » EEA: For IR products with a long half life AUC_{0-72h} is sufficient
        - » USA and EEA (CR products only): additionally $AUC_{0-\infty}$
      - Rate of Absorption (EEA, …), Peak Exposure (USA): C_{max}
      - t_{max} (Russia, Eurasian Economic Area, …)
      - Rarely relevant
        - » $t_{75\%}$, POT-25 (Plateau time or peak occupancy time; time span where $C(t) \geq 75\%\ C_{max}$: Russia for modified release products)
        - » *MRT* (Mean of Residence Times)
        - » Therapeutic Occupancy Time (time span where $C(t) \geq$ some given limit, *e.g.*, the MIC)

Noncompartmental Analyis

- Multiple dose
  - Extent of Absorption (EU, …), Total Exposure (USA):
    $AUC_{0-\tau}$ (AUC covering the dosing interval τ)
    If chronopharmacological variation and more than o.a.d. regimen:
    $AUC_{ss,24h}$
    No extrapolation of *AUC* in any case
  - Rate of Absorption (EU, …), Peak Exposure (USA):
    $C_{ss,max}$
  - Minimum concentration
    $C_{ss,min}$ (C_{trough}: located anywhere within τ; originators)
    $C_{ss,min}$ (C_{τ}: concentration at the end of the dosing interval; generics)
  - *PTF* (Peak-to-Trough Fluctuation)
    $(C_{ss,max} - C_{ss,min}) / C_{ss,av}$, where $C_{ss,av} = AUC_{0-\tau} / \tau$
  - Mentioned in some GLs but practically obsolete due to its extreme variability
    Swing = $(C_{ss,max} - C_{ss,min}) / C_{ss,min}$

Noncompartmental Analyis

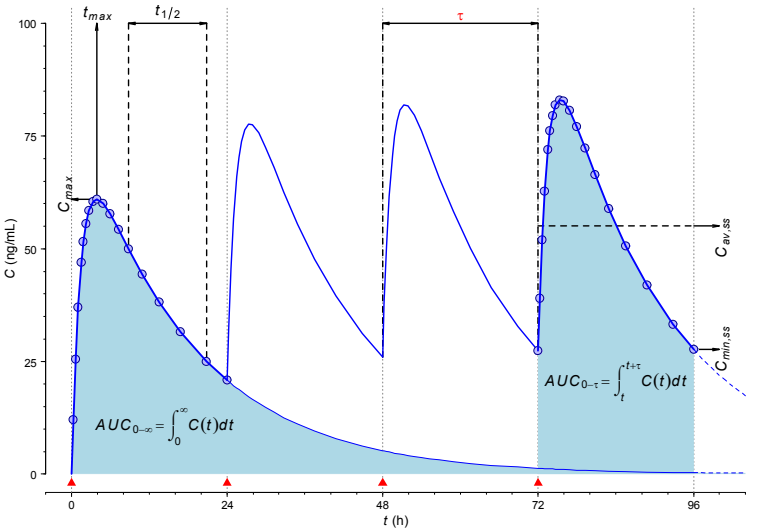
- PK metrics obtained by NCA depend much more on the sampling schedule than PK parameters
  - Examples
    - It is unlikely that one is able to 'catch' the true C_{max}/t_{max} in every subject. Hence, frequent sampling around t_{max} mandatory.
    - To obtain a reliable estimate of the apparent elimination λ_z, *at least* three samples required.
- According to all guidelines in BA/BE *only* NCA is acceptable
  - Rationale
    - PK models require exhaustive validation and documentation. The *same data set* does not necessarily give the *same results* with *different software*.
    - NCA is independent from software. Paper, pencil, brain…

PK model | *AUC*

- *AUC* is the integral of the concentration-time curve
  - One compartment, extravascular dose, no lag-time

$$C(t) = \frac{f \cdot D}{V} \frac{k_a}{k_a - k_e} \left(e^{-k_e \cdot t} - e^{-k_a \cdot t} \right)$$

$$AUC_{0-\infty} = \int_0^{\infty} C(t)\, dt = \frac{f \cdot D}{CL}$$



Superposition Principle of linear PK

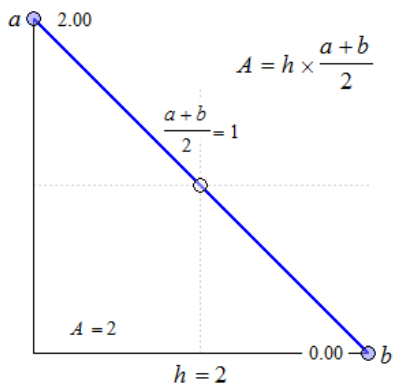
$AUC_{0-\tau} \approx AUC_{0-\infty}$

NCA | *AUC*

- In NCA numeric approximation of the integral is required
  - Linear trapezoidal method
  - Linear-up / logarithmic-down trapezoidal method
  - Of academic interest
    - Cubic splines
    - Lagrange polynomials
    - Simpson's rule

AUC | linear trapezoidal method

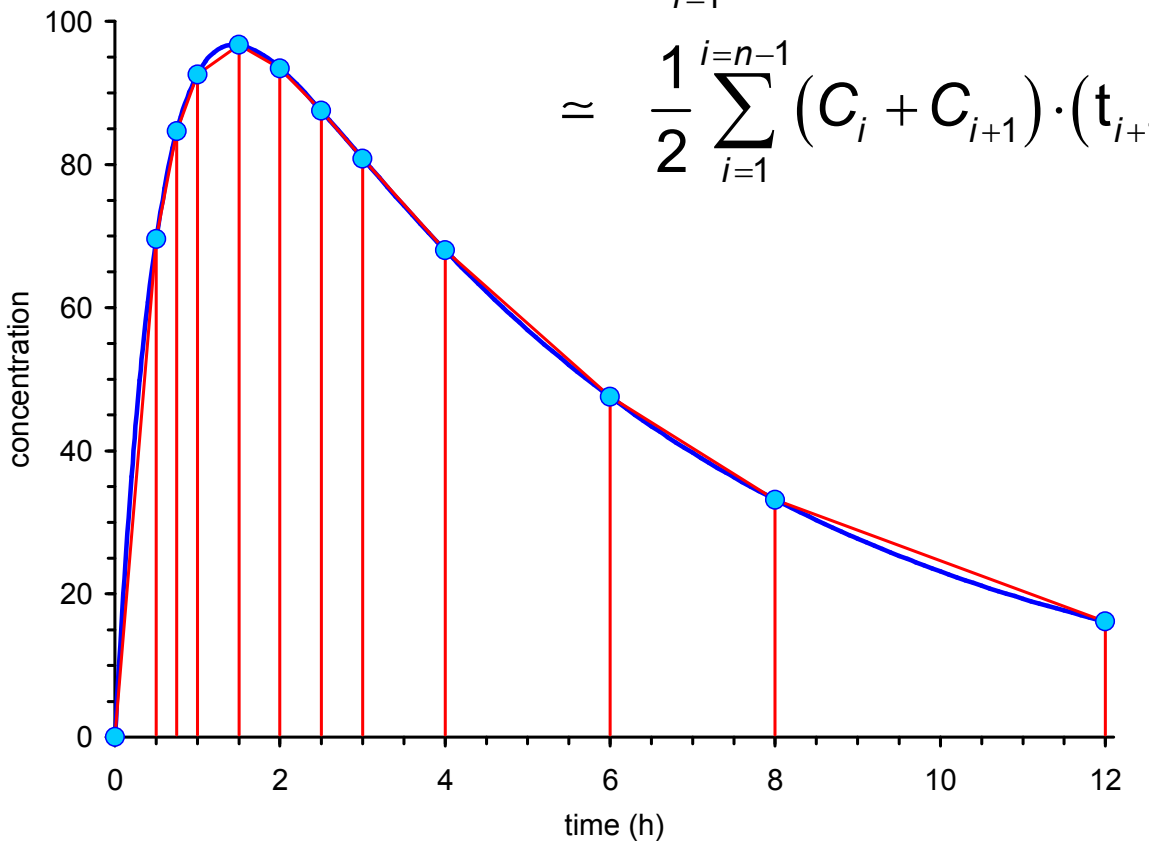
- Linear interpolation between data points
- Sections are represented by trapezoids
- Sides *a*, *b* are two neighbouring concentrations
- *h* is the time interval
- Area of one trapezoid $A = \frac{a+b}{2}h$



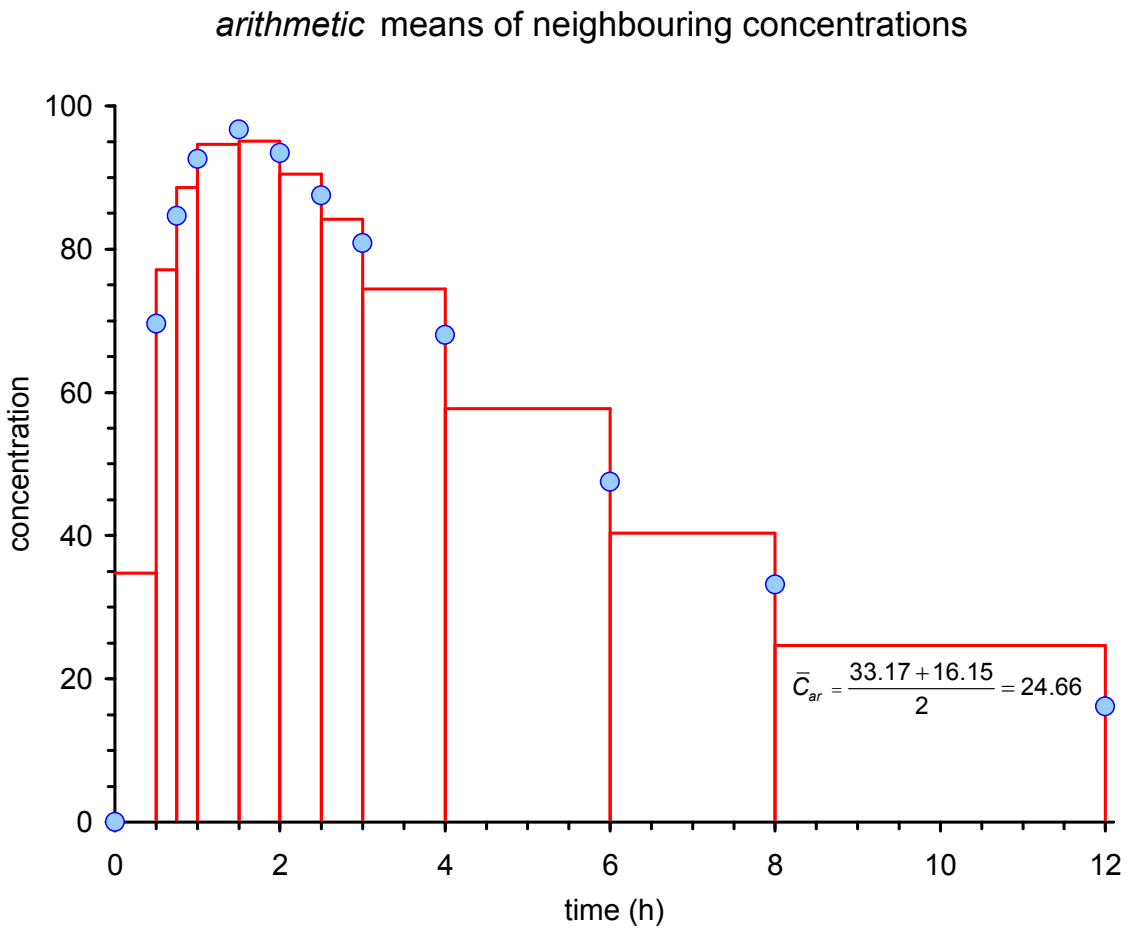
AUC | linear trapezoidal method

- Total Area $AUC_{0-t_n} \simeq \sum_{i=1}^{i=n-1} \frac{C_i + C_{i+1}}{2}\left(t_{i+1} - t_i\right)$

$$\simeq \frac{1}{2}\sum_{i=1}^{i=n-1}\left(C_i + C_{i+1}\right)\cdot\left(t_{i+1} - t_i\right)$$



AUC | linear trapezoidal method



AUC | linear trapezoidal method

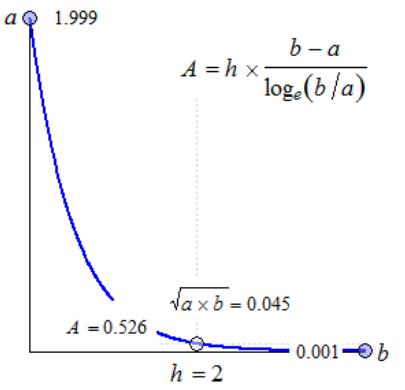
- Positive bias
  - Overestimates *AUC* in both the absorption and distribution / elimination phases
- Originated in the dark ages when profiles were plotted on paper, cut out, weighed on an analytical scale, and compared to the paper-weight of known area (*e.g.*, A4 of 80 g/m^2: 4.9896 g / 623.7 cm^2)
- Should have been thrown into the scientific trash can with the invention of pocket calculators decades ago
- In general elimination follows an exponential decrease

$$C(t) = \frac{f \cdot D}{V} \frac{k_a}{k_a - k_e} \left(e^{-k_e \cdot t} - e^{-k_a \cdot t} \right)$$

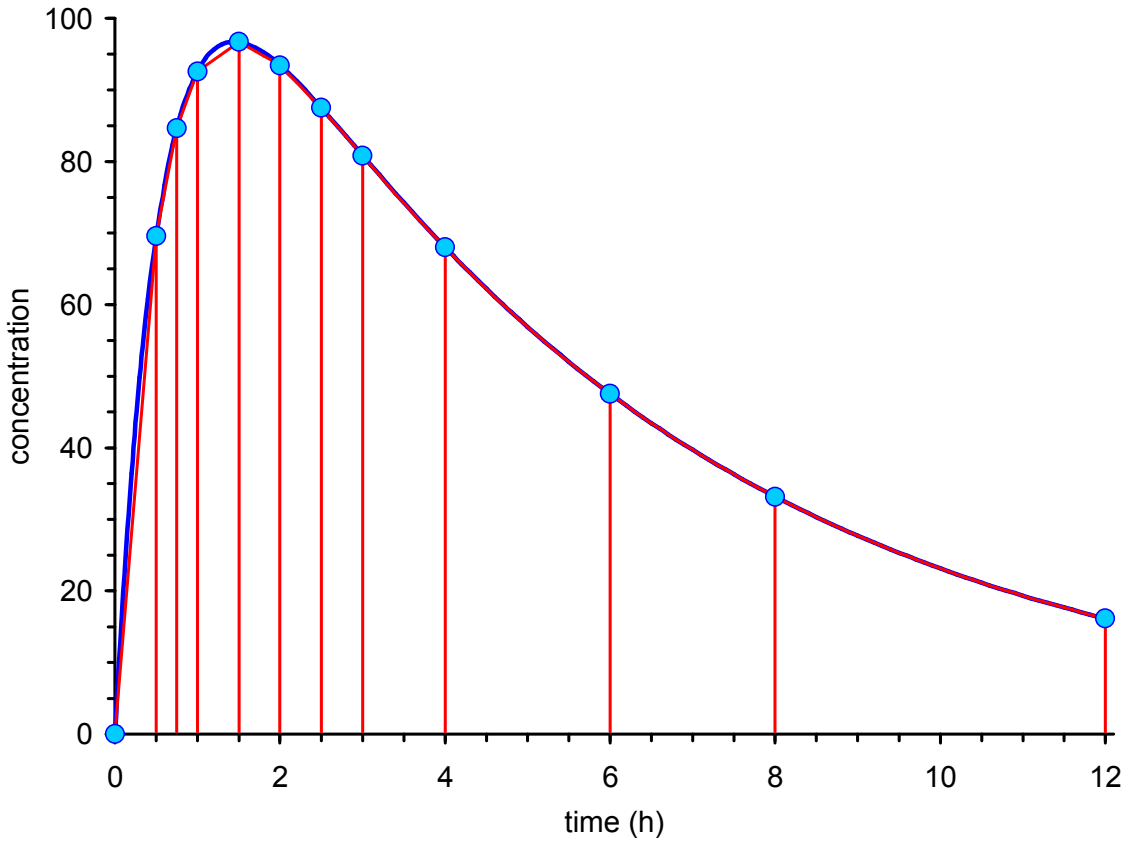
AUC | lin-up / log-down trap. method

- Much better alternative:
  Linear-up / logarithmic-down trapezoidal method
- Sections with *increasing or equal* concentrations
  ($C_{i+1} \geq C_i$) calculated by the linear trapezoidal method
- Sections with *decreasing* concentrations
  ($C_{i+1} < C_i$) calculated by the logarithmic-linear trapezoidal method, *i.e.*,

$$AUC_{t_i - t_{i+1}} \simeq \frac{C_{i+1} - C_i}{\log_e \frac{C_{i+1}}{C_i}} \left(t_{i+1} - t_i \right)$$

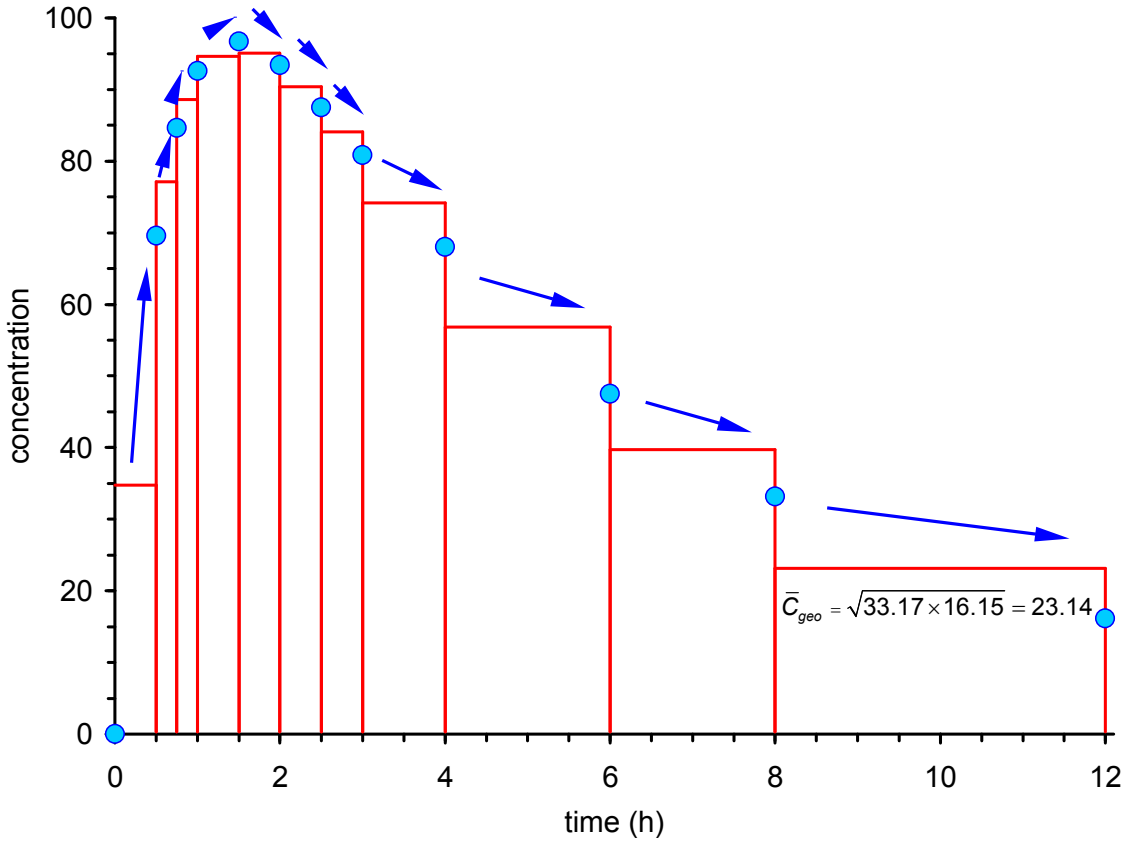


AUC | lin-up / log-down trap. method



AUC | lin-up / log-down trap. method

arithmetic / *geometric* means of neighbouring concentrations



AUC | lin-up / log-down trap. method

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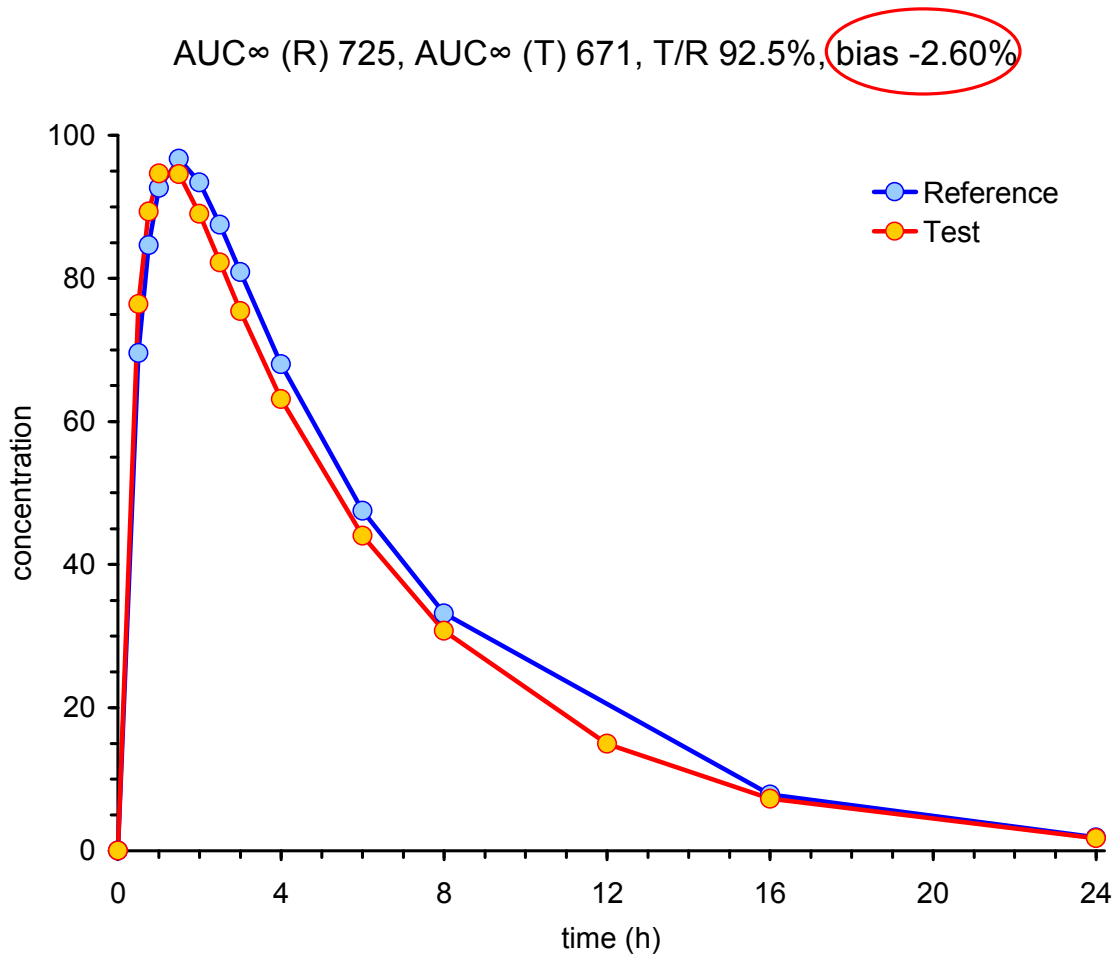
- Avoids positive bias in distribution / elimination phases
- Suitable for both i.v. and e.v. administrations
- Suitable for multiphasic profiles
  - Secondary peaks due to enterohepatic recycling
  - Pulsatile release products
  - If *AUC* of more than one profile has to be calculated (*e.g.*, two doses with τ 12 h and $AUC_{0–24h}$ is required due to circadian variation in PK)
- Implemented in PK software since 1993 (!)
- Only exception where the method performs worse than the linear trapezoidal
  - Drugs following Michaelis-Menten PK (*e.g.*, alcohol)

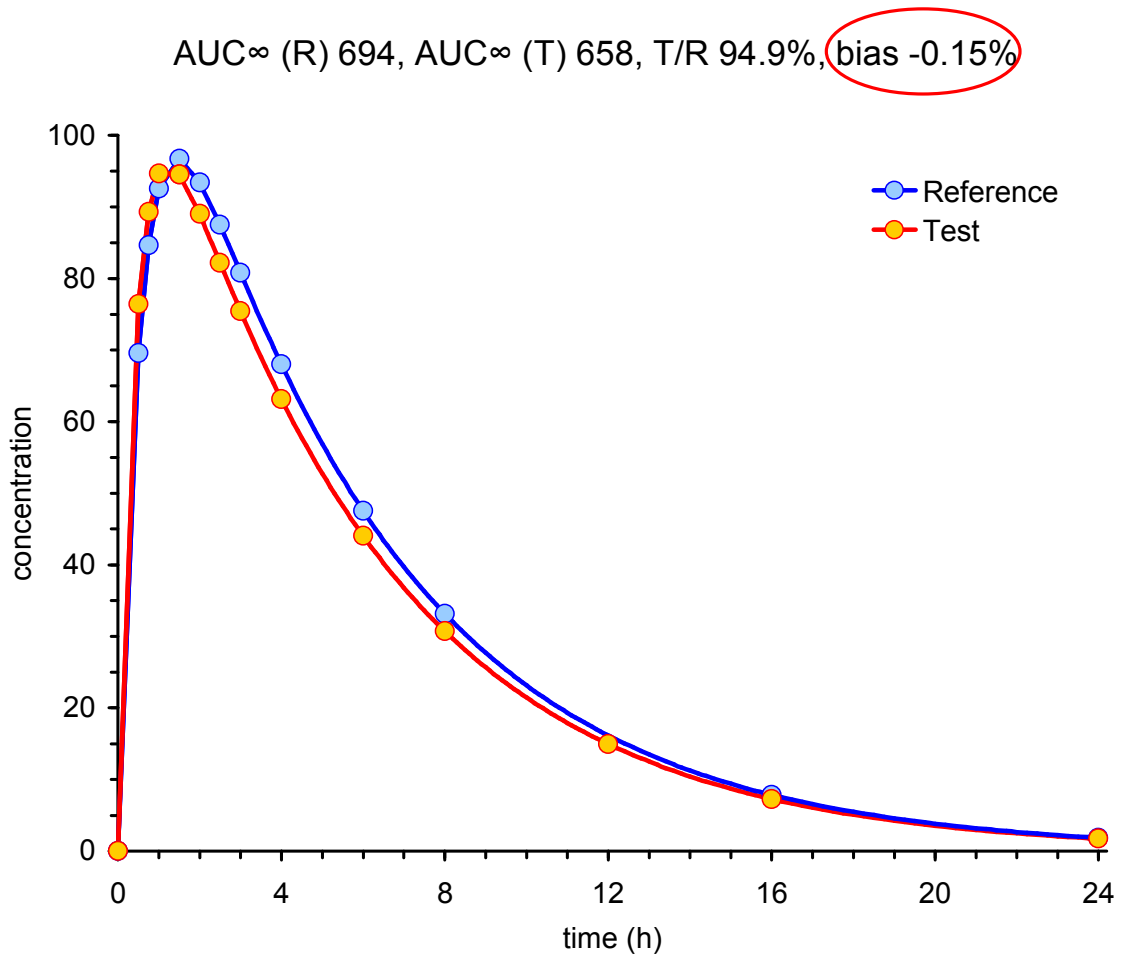
AUC_{0-t} | Problem 1

- Recap: In most jurisdictions the PK metric for BE is AUC_{0-t}, where t is the last time point with a quantifiable concentration
- Ideally we are able to calculate AUC_{0-t}
  - for all treatments
  - in all subjects
- What if
  - a sample was missing (*e.g.*, vial broken in centrifugation)?
- Example
  - True T/R-ratio 95%, 12 h sample (R) missing
  - Comparison of linear and lin-up / log-down trapezoidal methods

AUC_{0-t} | Problem 1



AUC_{0-t} | Solution



AUC_{0-t} | Problem 2

- What if
  - The bioanalytical method was sensitive enough to measure *all* concentrations but a sample at the last time point (t) was missing (*e.g.*, vial broken in centrifugation)?
  - The bioanalytical method was sensitive enough to measure *most* low concentrations but there were a few values at *t* below the LLOQ (lower limit of quantification)?

AUC_{0-t} | Problem 2

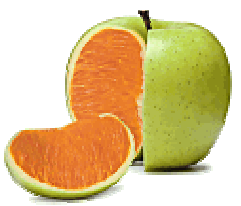
- In BE we administer the same molar doses and assume constant inter-occasion clearances. Hence,

$$AUC_{0-t,T} = \frac{f_T \cdot D_T}{CL_T} \text{ and } AUC_{0-t,R} = \frac{f_R \cdot D_R}{CL_R}$$

$$\text{with } D_T = D_R \text{ and } CL_T = CL_R \text{ we get } \frac{f_T}{f_R} = \frac{AUC_{0-t,T}}{AUC_{0-t,R}}$$

  - Example: t for one product is 24 h but due to missingness for the other one occasionally 16 h. If we follow guidelines blindly, the estimate will be biased because

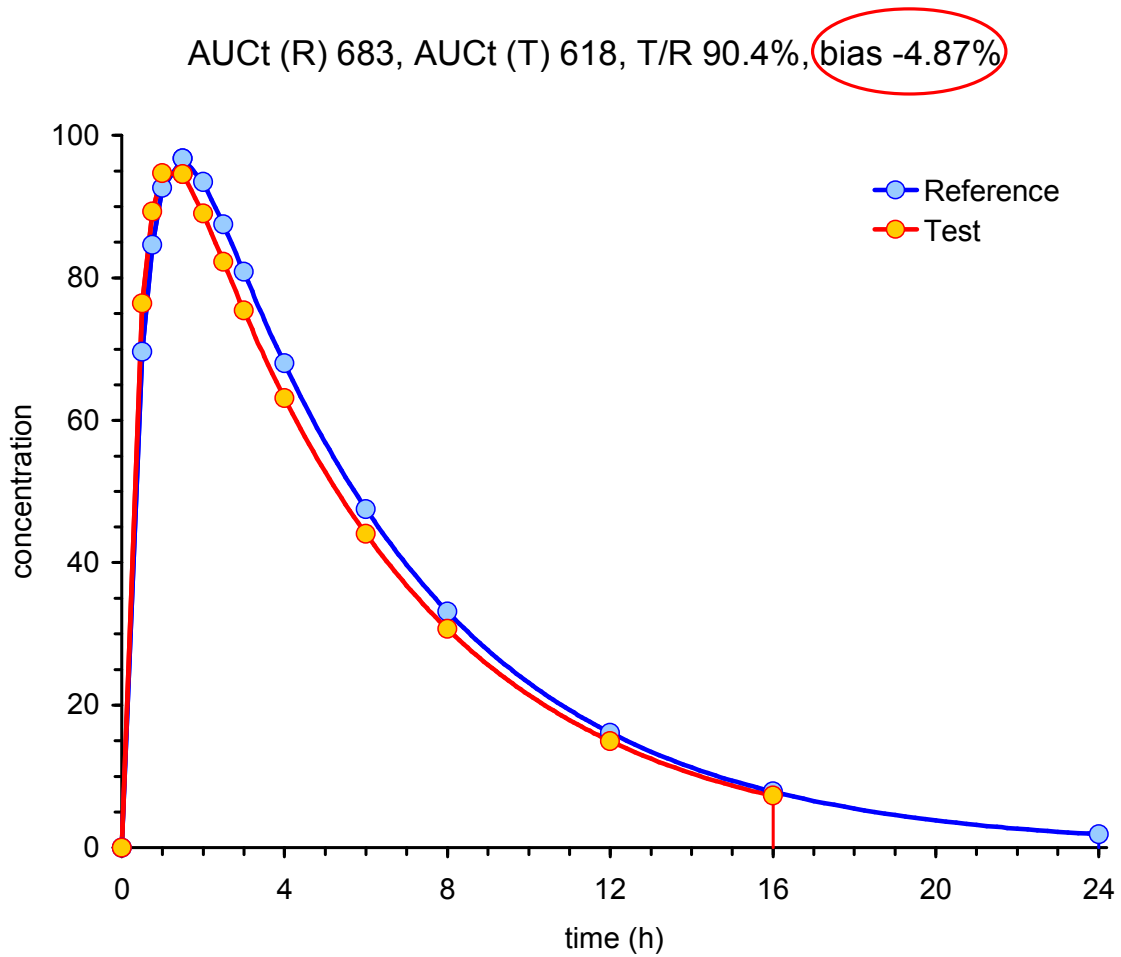
$$\frac{f_T}{f_R} \neq \frac{AUC_{0-16,T}}{AUC_{0-24,R}}$$



AUC_{0-t} | Problem 2

- Only if the true relative BA-ratio is *exactly* 1, the chance to observe concentrations at t <LLOQ is similar for all treatments and the estimate will be unbiased
- If the true BA-ratio is ≠ 1, the estimate will be biased away from one (the difference between treatments will be exaggerated)
  - Regulators don't care because the patient's risk is not affected and the chance to demonstrate BE *decreases*
  - Applicants should care since the producer's risk of failure *increases*

AUC_{0-t} | Problem 2



AUC_{0-t} | Solutions

- Impute missings or BQLs by their estimates
  - Requires reliable estimate of λ_z
  - Implemented only in the current release of Phoenix/WinNonlin
  - In other software or 'by hand' according to

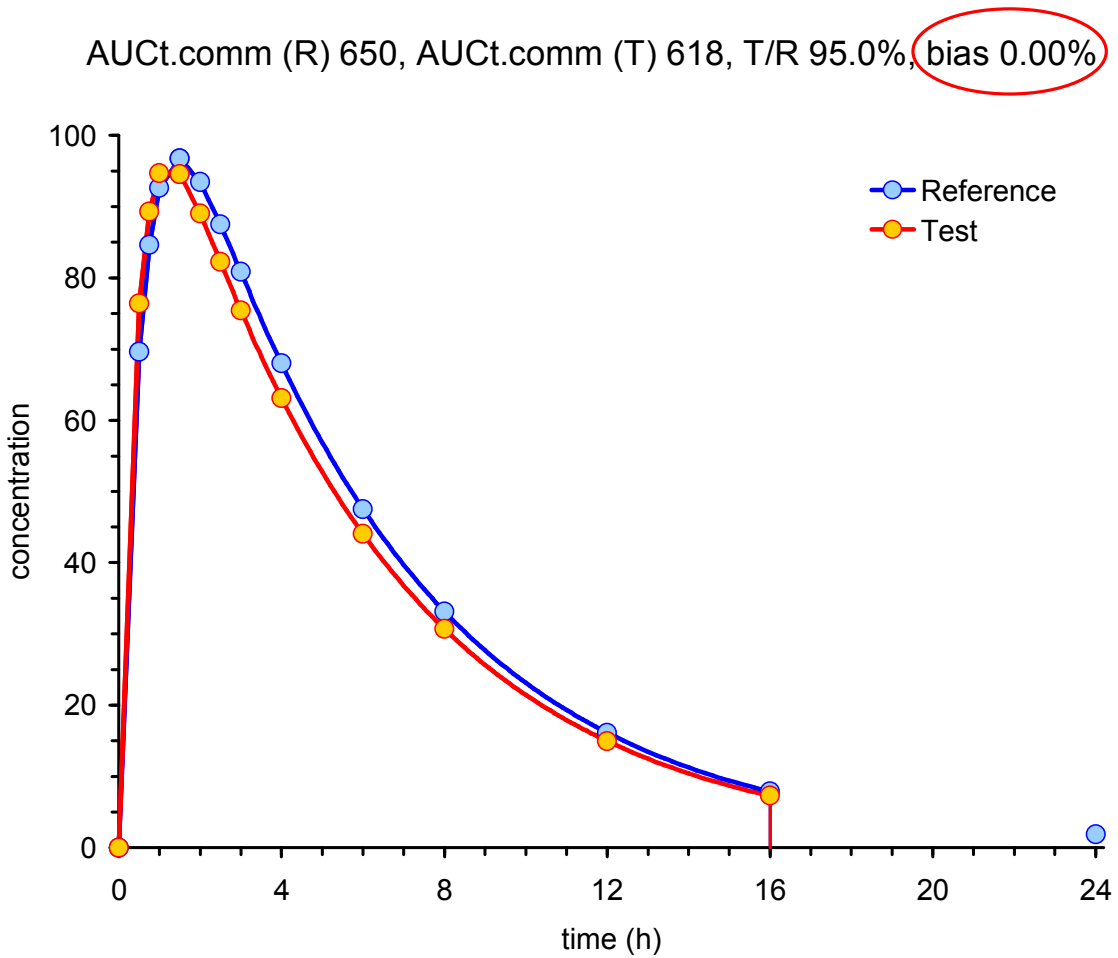
$$C_t = e^{\ln\left(\hat{C}_0\right)-\hat{\lambda}_z \cdot t}$$

- Compare *AUC*s in each subject where *both* treatments showed concentrations ≥LLOQ*
  - Example: $t_{last,T}$ = 16 h, $t_{last,R}$ = 24 h, t_{last} *(Common)* = 16 h

$$\frac{f_T}{f_R} = \frac{AUC_{0-16,T}}{AUC_{0-16,R}}$$

* Fisher D, Kramer W, Burmeister Getz E. *Evaluation of a Scenario in Which Estimates of Bioequivalence Are Biased and a Proposed Solution: t_{last} (Common).* J Clin Pharm. 2016;56(7):794–800. doi:10.1002/jcph.663. Open access.

AUC_{0-t} | Solution



$AUC_{0–t}$ | Problem 3

- What if
  - a substantial number of samples in the late part of a profile is missing?
  - Such a case might happen if a subject drops out from a study
  - $AUC_{0–t(common)}$ will not necessarily help because according to most GLs a ‘reliable estimate’ of the extent of absorption is given if $AUC_{t–\infty}$ is ≤20% of $AUC_{0–\infty}$
  - However, regulations ≠ science
    - For IR products ($k_a \gg k_e$) already at $2\times t_{max}$ absorption is practically complete (93.75%); at $4\times t_{max}$ 99.61% are absorbed*
    - In the late part of the profile distribution / elimination prevails – which is drug-specific and not relevant for detecting differences between treatments

      * Scheerans C, Derendorf H, Kloft C. *Proposal for a Standardised Identification of the Mono-Exponential Terminal Phase for Orally Administered Drugs.* Biopharm Drug Dispos. 2008;29(3):145–57. doi:10.1002/bdd.596.

$AUC_{0–t}$ | Solution

- EMA BE-GL Section 4.1.8 (2010)
  - Subjects should not be excluded from the statistical analysis if $AUC_{(0–t)}$ covers less than 80% of $AUC_{(0–\infty)}$, but if the percentage is less than 80% in more than 20% of the observations then the validity of the study may need to be discussed.
- For optimistic ones
  - Cross fingers and prepare for the discussion
- For very brave ones
  - Give a justification in the protocol that absorption is already complete even at very early time points
  - Use $AUC_{0–t(common)}$
- For brave ones
  - As above but state in the protocol a limit for the earliest acceptable truncation time; if earlier, exclude the subject from the comparison of *AUC*s

AUC_{0-t} | Solution

- For wary ones
  - Exclude the subject from the comparison of *AUC*s but – if C_{max} is well defined (*e.g.*, a couple of decreasing concentrations after t_{max}) – keep the subject in the comparison of C_{max}
  - Rationale
    - In general the variability of C_{max} is substantially higher than the one of *AUC* and therefore, likely the study was powered for C_{max}
    - Although power to show BE will slightly decrease for *AUC*, the overall power of the study will not be compromised
- Prolonged (aka sustained) release formulations
  - By their biopharmaceutical design (flip-flop PK: $k_a \leq k_e$) the *late part* of the profile represents *absorption*
  - Exclude the subject from the comparison of *AUC*s

C_{max} | Problem & Solutions

- What if
  - samples in the area of t_{max} are missing?
- Exclude the subject from the comparison of C_{max}
  - Power depends on the *CV* (coefficient of variation), the *GMR* (geometric mean ratio), and *n* (sample size) where the rank order of their influence on power is $GMR \gg CV > n$
  - Power will be compromised but to a much lesser degree than many people expect
- For curageous ones
  - Keep the observed C_{max} (which potentially is lower than the true one)
  - Impute the highest concentration observed in any of the other subjects (irrespective of the treatment) and perform a sensitivity analysis

NCA | λ_z

- Recap: To obtain a reliable estimate of the apparent elimination λ_z, *at least* three samples required.
  - The automatic algorithm based on maximizing R^2_{adj} is known to be 'greedy' (*i.e.*, reaches for too early time points) and
    - has difficulties with 'flat' profiles (*e.g.*, ill-defined C_{max} of CR products) and
    - regularly fails completely for multiphasic release products
  - Alternative: TTT method (Scheerans *et al*. 2008)
    - Implemented in the open source package bear for R.
    - Two-step procedure in Phoenix/WinNonlin
      - Estimate t_{max} in one run of the NCA module
      - Set $2\times t_{max}$ as the start time in a second run
  - Visual inspection of fits by a pharmacokineticist (with optional correction) is mandatory in all methods