



Arbeitsgemeinschaft
für angewandte
Humanpharmakologie e.V.

How to measure what happens in pharmacokinetics

PK metrics of relevance!

Terminology



- Estimates obtained by a *PK model*:
PK parameters
 - Primary parameters
 V , CL , f , 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} , $t_{1/2}$, 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
($\lambda_z/t_{1/2}$, AUC_{0-t} , $AUC_{0-\infty}$, ...)

Noncompartmental Analysis



- 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 Analysis



- 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 Analysis



- 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

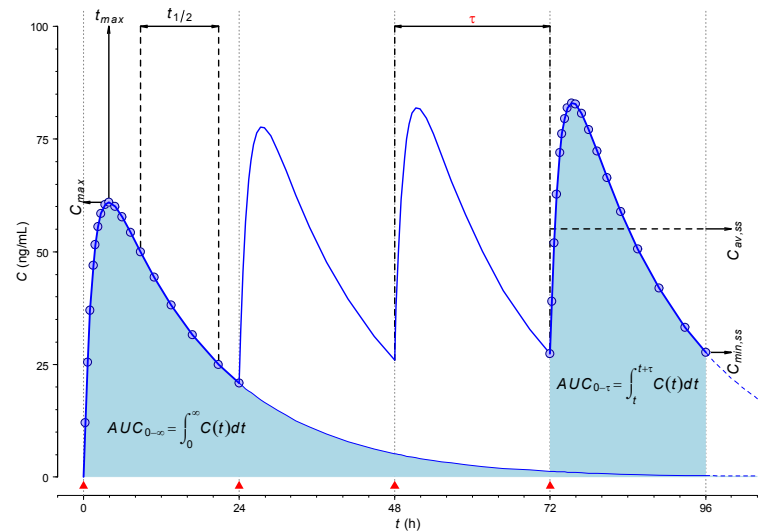
$$C(t) = \frac{f \cdot D}{V} \frac{k_a}{k_a - k_e} \left(e^{-k_e \cdot t} - e^{-k_a \cdot t} \right)$$

$$AUC_{0-\infty} = \int_0^{\infty} C(t) dt$$

$$= \frac{f \cdot D}{V} \frac{k_a}{k_a - k_e} \left(\frac{1}{k_e} - \frac{1}{k_a} \right)$$

$$= \frac{f \cdot D}{V \cdot k_e}$$

$$= \frac{f \cdot D}{CL}$$



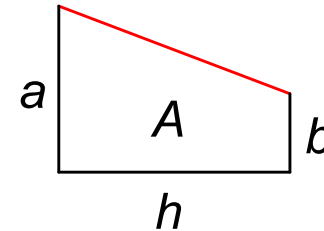
Superposition Principle of linear PK
 $AUC_{0-T} \approx AUC_{0-\infty}$

- 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



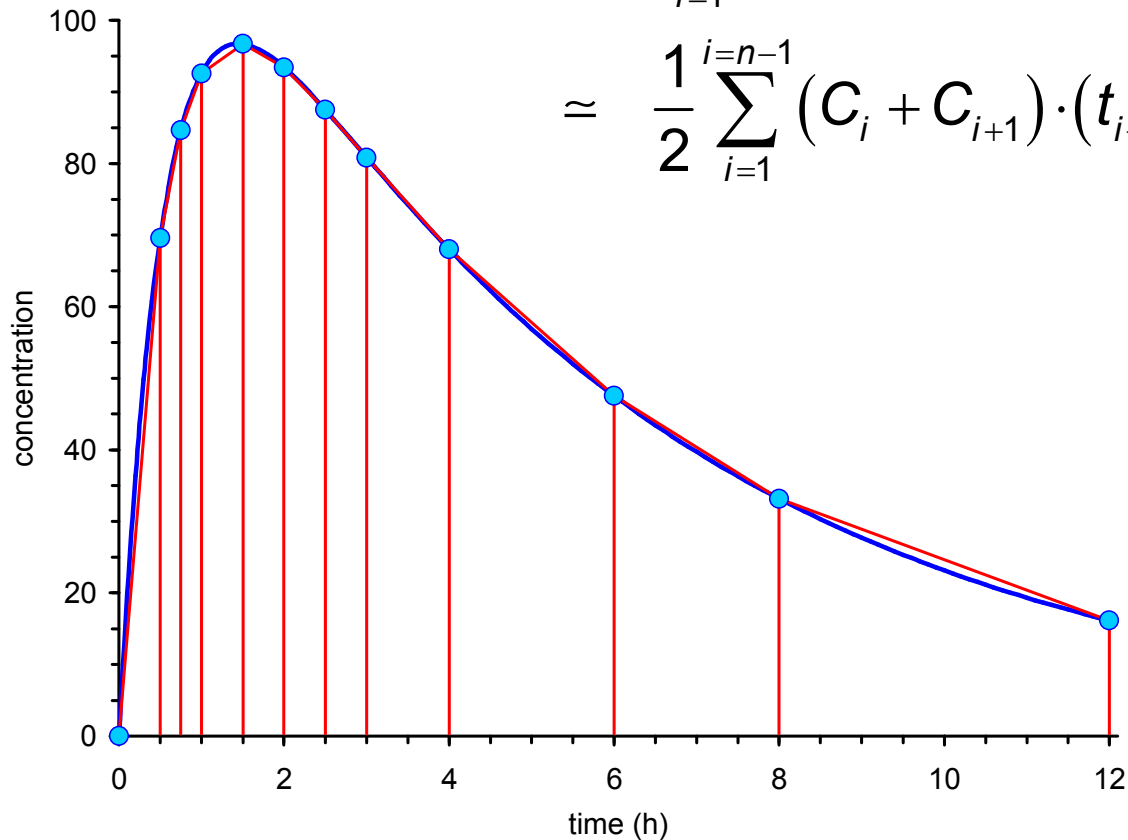
- Linear interpolation between data points
- Sections are represented by trapezoids
- Sides a , b are two neighbouring concentrations
- h is the time interval
- Area of one trapezoid $A = \frac{a+b}{2} h$



AUC | linear trapezoidal method



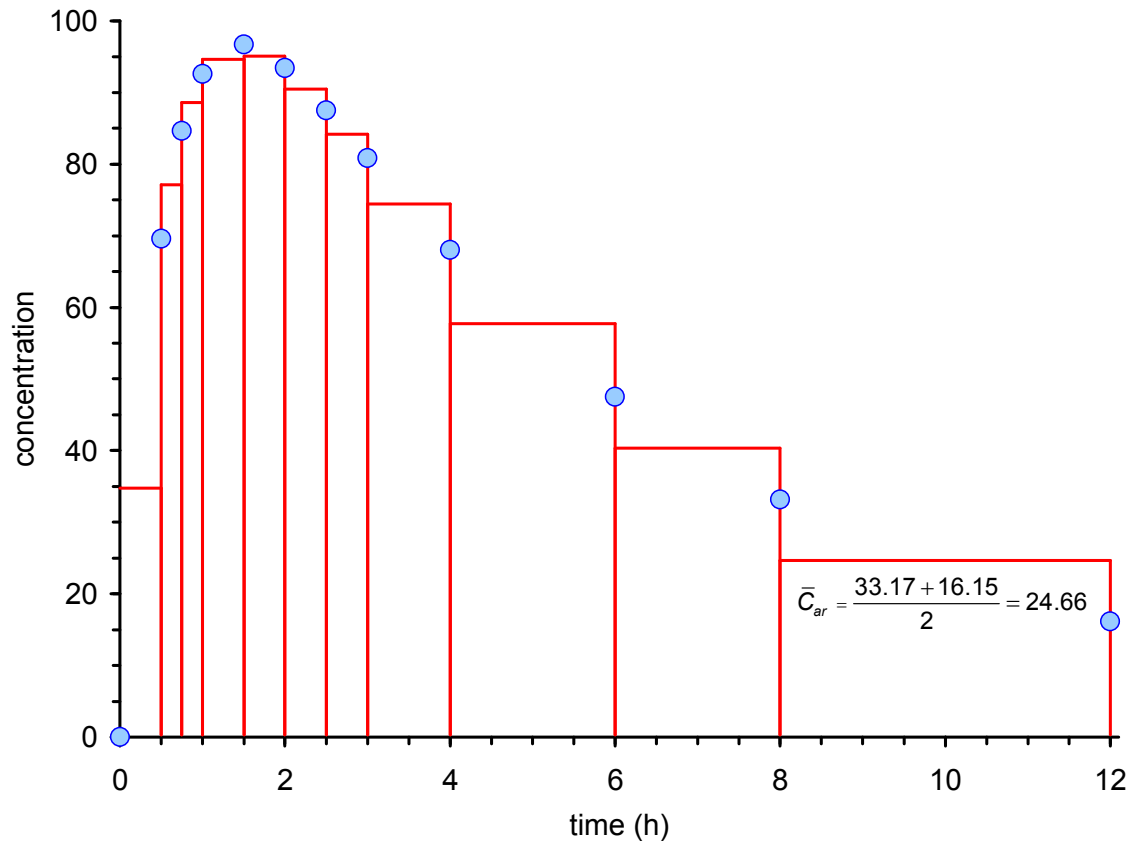
- Total Area $AUC_{0-t_n} \approx \sum_{i=1}^{i=n-1} \frac{C_i + C_{i+1}}{2} (t_{i+1} - t_i)$
 $\approx \frac{1}{2} \sum_{i=1}^{i=n-1} (C_i + C_{i+1}) \cdot (t_{i+1} - t_i)$



AUC | linear trapezoidal method



arithmetic means of neighbouring concentrations



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/m²: 4.9896 g / 623.7 cm²)
- Should have been thrown into the scientific waste-can with the invention of pocket calculators decades ago
- In general elimination follows an exponential decrease

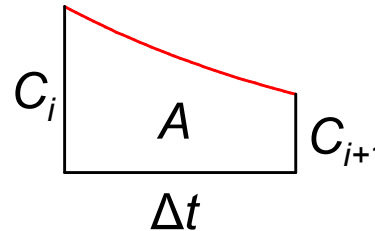
$$C(t) = \frac{f \cdot D}{V} \frac{k_a}{k_a - k_e} \left(e^{-k_e \cdot t} - e^{-k_a \cdot t} \right)$$

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

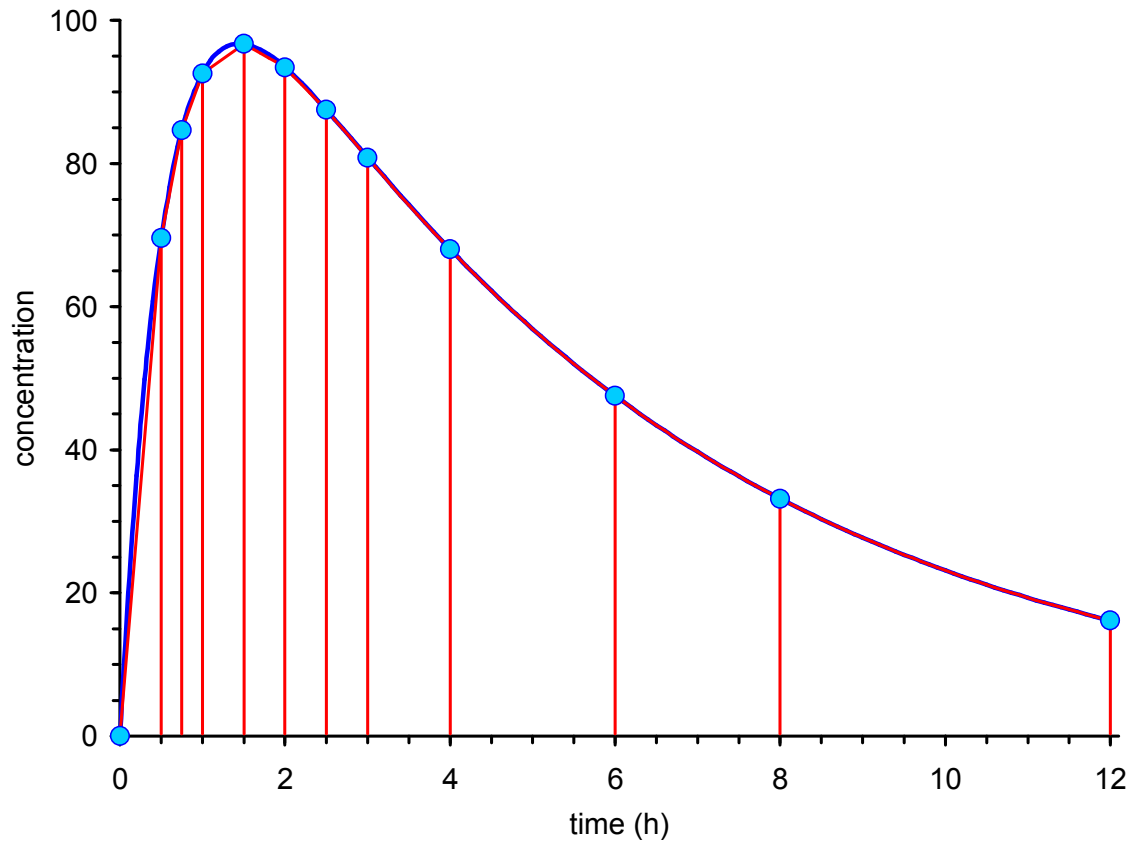


- Much better alternative:
Linear-up / logarithmic-down trapezoidal method
- Sections with *increasing or equal* concentrations ($C_{i+1} \geq C_i$) calculated by the linear trapezoidal method
- Sections with *decreasing* concentrations ($C_{i+1} < C_i$) calculated by the logarithmic-linear trapezoidal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

$$AUC_{t_i-t_{i+1}} \approx \frac{C_{i+1} - C_i}{\ln \frac{C_{i+1}}{C_i}} (t_{i+1} - t_i)$$



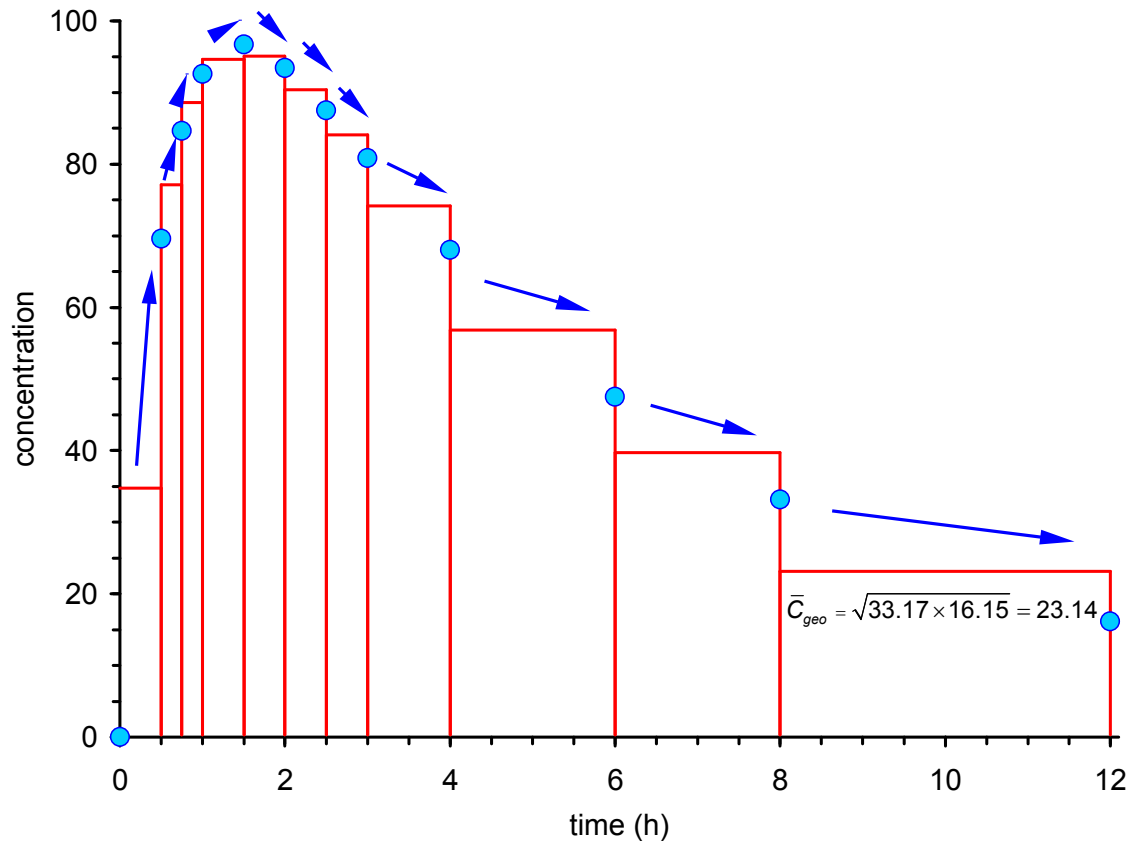
AUC | lin-up / log-down trap. method



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



arithmetic / geometric means of neighbouring concentrations



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



- 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 standard PK software for decades
- Only exception where the method performs worse than the linear trapezoidal
 - Drugs following Michaelis-Menten PK (e.g., alcohol)

AUC_{0-t} | Problem 1

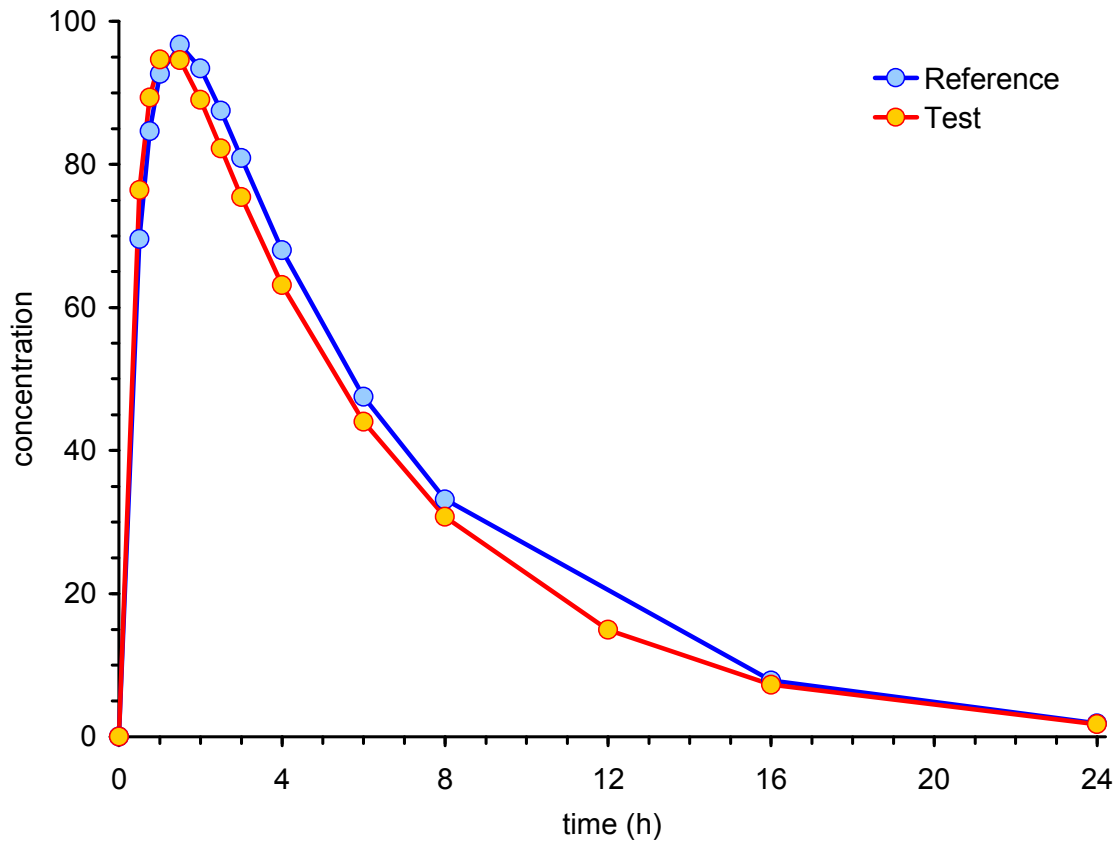


- Recap: In most jurisdictions the PK metric for BE is AUC_{0-t} , where t is the last time point with a quantifiable concentration
- Ideally we are able to calculate AUC_{0-t}
 - for all treatments
 - in all subjects
- What if
 - a sample was missing (e.g., vial broken in centrifugation)?
- Example
 - 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



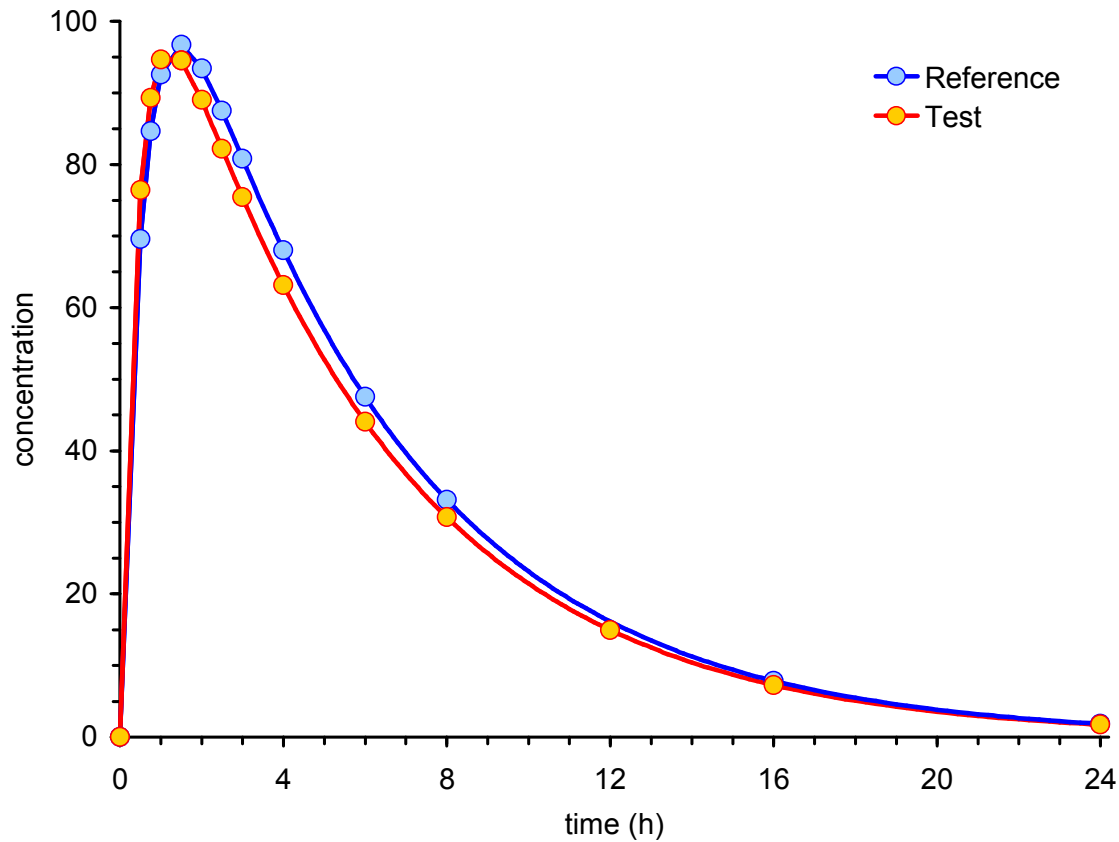
AUC_{∞} (R) 725, AUC_{∞} (T) 671, T/R 92.5%, bias -2.60%



AUC_{0-t} | Solution



AUC^∞ (R) 694, AUC^∞ (T) 658, T/R 94.9%, bias -0.15%



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



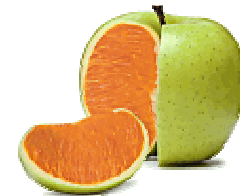
- In BE we administer the same molar doses and assume constant inter-occasion clearances. Hence,

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

with $D_T = D_R$ and $CL_T = CL_R$ 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

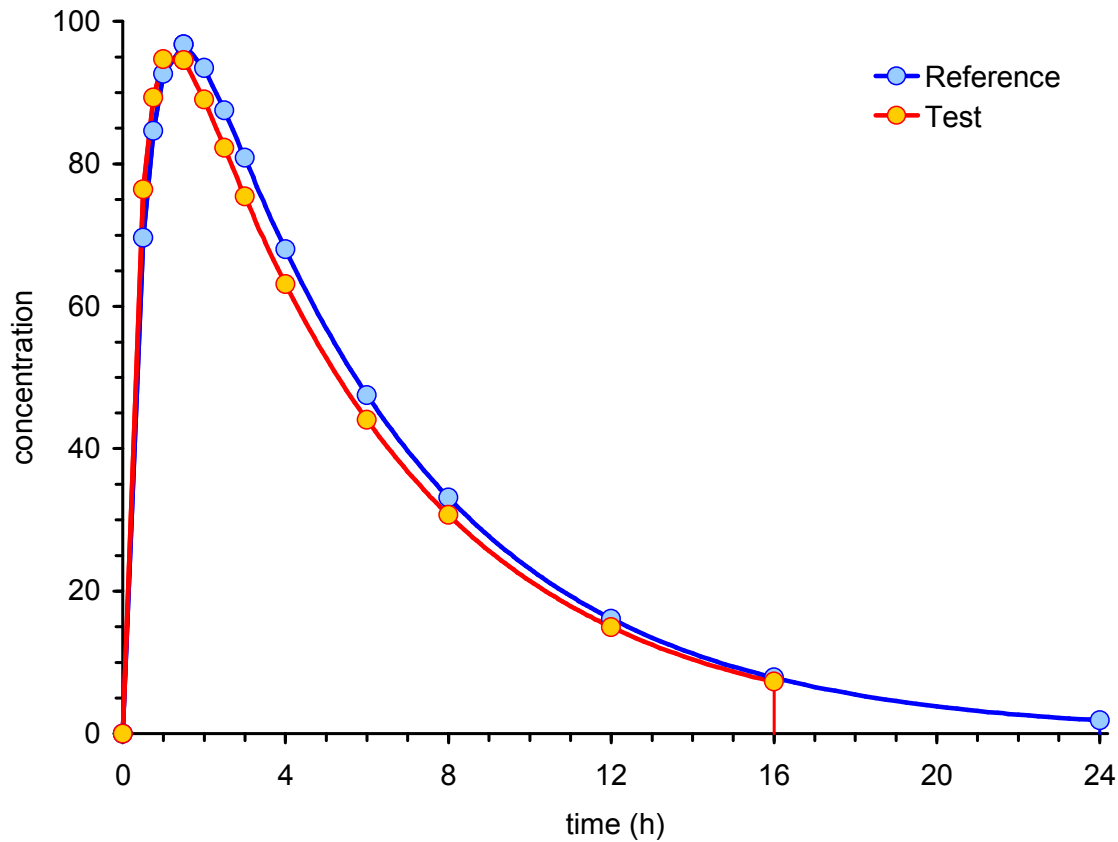


- Only if the true relative BA-ratio is *exactly* 1, the chance to observe concentrations at $t < \text{LLOQ}$ is similar for all treatments and the estimate will be unbiased
- If the true BA-ratio is $\neq 1$, the estimate will be biased away from one (the difference between treatments will be exaggerated)
 - 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



AUCt (R) 683, AUCt (T) 618, T/R 90.4%, bias -4.87%



- 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

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

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

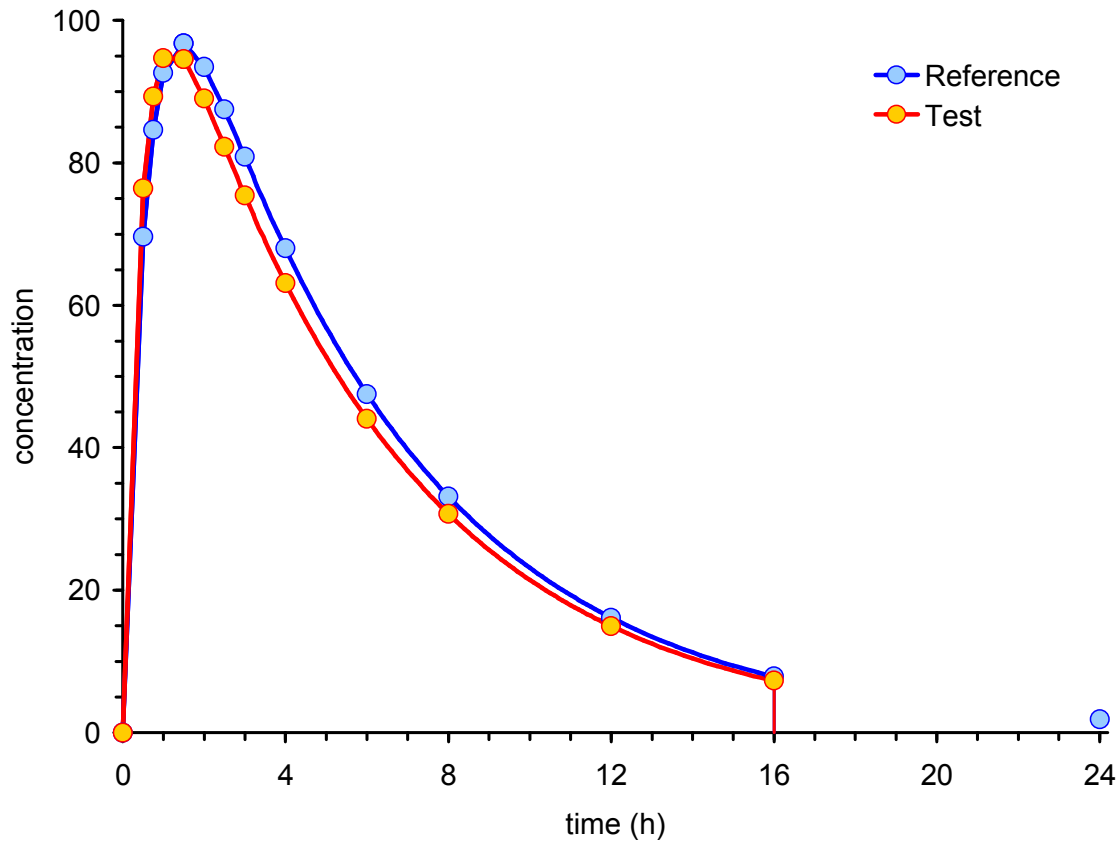
$$\frac{f_T}{f_R} = \frac{AUC_{0-16,T}}{AUC_{0-16,R}}$$

* 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](https://doi.org/10.1002/jcph.663). [Open access](#).

AUC_{0-t} | Solution



AUCt.comm (R) 650, AUCt.comm (T) 618, T/R 95.0%, bias 0.00%



- 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 $\leq 20\%$ of $AUC_{0-\infty}$
 - However, regulations \neq 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 already 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](https://doi.org/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 $AUCs$

AUC_{0-t} | Solution



- For wary ones
 - Exclude the subject from the comparison of $AUCs$ 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 affected
- 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 $AUCs$

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 courageous 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

- 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