Chapter 16

Non-compartmental Analysis

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Abstract

When analyzing pharmacokinetic data, one generally employs either model fitting using nonlinear regression analysis or non-compartmental analysis techniques (NCA). The method one actually employs depends on what is required from the analysis. If the primary requirement is to determine the degree of exposure following administration of a drug (such as AUC), and perhaps the drug’s associated pharmacokinetic parameters, such as clearance, elimination half-life, \( T_{\max} \), \( C_{\max} \), etc., then NCA is generally the preferred methodology to use in that it requires fewer assumptions than model-based approaches. In this chapter we cover NCA methodologies, which utilize application of the trapezoidal rule for measurements of the area under the plasma concentration–time curve. This method, which generally applies to first-order (linear) models (although it is often used to assess if a drug’s pharmacokinetics are nonlinear when several dose levels are administered), has few underlying assumptions and can readily be automated.

In addition, because sparse data sampling methods are often utilized in toxicokinetic (TK) studies, NCA methodology appropriate for sparse data is also discussed.

Key words: Non-compartmental, NCA, AUC, Toxicokinetic, TK, \( \lambda_z \)

1. Non-compartmental Analysis

1.1. Non-compartmental Versus Regression Analysis

Most current approaches to characterize a drug’s kinetics involve non-compartmental analysis, denoted NCA, and nonlinear regression analysis (1). The advantages of the regression analysis approach are the disadvantages of the non-compartmental approach and vice versa. NCA does not require the assumption of a specific compartmental model for either drug or metabolite. The method used involves application of the trapezoidal rule for measurements of the area under a plasma concentration–time curve. This method, which applies to first-order (linear) models, is rather assumption free and can readily be automated. Figure 1 gives a schematic picture of the NCA and nonlinear regression approaches. As can be seen, NCA deals with sums of areas whereas regression...
modeling uses a function with regression parameters. Both methods are applied for the characterization of the kinetics of a compound.

The time course of drug concentration in plasma can usually be regarded as a statistical distribution curve. The area under a plot of the plasma concentration versus time curve is referred to as the area under the zero moment curve $AUC$, and the area under the product of the concentration and time versus time curve is then called the area under the first moment curve $AUMC$. Only the areas of the zero and first moments are generally used in pharmacokinetic analysis, because the higher moments are prone to an unacceptable level of computational error.

This section focuses on NCA with regard to computational methods, strategies for estimation of $\lambda_z$, pertinent pharmacokinetic estimates, issues related to steady state, and how to tackle situations where $t_{1/2}$ is much less than input time.

1.2. Computational Methods: Linear Trapezoidal Rule

The areas can either be calculated by means of the linear trapezoidal rule or by the log-linear trapezoidal rule. The total area is then measured by summing the incremental area of each trapezoid (Fig. 2).

The magnitude of the error associated with the estimated area depends on the width of the trapezoid and the curvature of the true profile. This is due to the fact that the linear trapezoidal rule overestimates the area during the descending phase assuming elimination is first-order, and underestimates the area during the ascending part of the curve (Fig. 3). This over/underestimation error will be more pronounced if the sampling interval $\Delta t$ is large in relation to the half-life.

Using the linear trapezoidal method for calculation of the area under the zero moment curve $AUC$ from 0 to time $t_n$, we have

$$ Cl_{0} = \frac{Dose_{po}}{\sum_{i=0}^{n} A_i} $$

$$ AUMC = \sum_{i=0}^{n} \int_{0}^{t_i} C(t) \, dt $$

Fig. 1. Comparison of NCA (left) and nonlinear regression modeling (right). $K_a, K, V$ in the right-hand panel indicate the model parameters to be estimated by regressing the model to data.
where \( D_t = t_{i+1} - t_i \) and \( t_{\text{last}} \) denotes the time of the last measurable concentration. Unless one has sampled long enough in time so that concentrations are negligible, the AUC as defined above will underestimate the true AUC. Therefore it may be necessary to extrapolate the curve out to \( t \) equal to infinity (\( \infty \)). The extrapolated area under the zero moment curve from the last sampling time to infinity \( AUC_{t_{\text{last}}\infty} \) is calculated as
AUC_{t_{\text{last}}} = \int_{t_{\text{last}}}^{\infty} C_{\text{last}} \cdot e^{-\lambda_z (t-t_{\text{last}})} \, dt = C_{\text{last}} \left[ \frac{e^{-\lambda_z (t-t_{\text{last}})}}{-\lambda_z} \right]_{t_{\text{last}}}^{\infty} = C_{\text{last}} \left[ 0 - \frac{1}{-\lambda_z} \right] = \frac{C_{\text{last}}}{\lambda_z}, \quad (2)

where $C_{\text{last}}$ and $\lambda_z$ are the last measurable nonzero plasma concentration and the terminal slope on a loge scale, respectively. One may also use the predicted concentration at $t_{\text{last}}$ if the observed concentration deviates from the terminal regression line (Fig. 4).

The $\lambda_z$ parameter is graphically obtained from the terminal slope of the semilogarithmic concentration–time curve as shown in Fig. 4, with a minimum of 3–4 observations being required for accurate estimation. The Y axis ln(C) denotes the natural logarithm (loge) of the plasma concentration $C$.

The linear trapezoidal method for calculation of the area under the first moment curve $AUMC$ from 0 to time $t_{\text{last}}$ is obtained from

$$AUMC_{0-t_{\text{last}}} = \sum_{i=1}^{n} \frac{t_i \cdot C_i + t_{i+1} \cdot C_{i+1}}{2} \cdot \Delta t. \quad (3)$$

Remembering that $\int x \cdot e^{-a \cdot x} \, dx = -\frac{xe^{-a \cdot x}}{a} - \frac{e^{-a \cdot x}}{a^2}$, the corresponding area under the first moment curve from time $t_{\text{last}}$ to infinity $AUMC_{t_{\text{last}}-\infty}$ is computed as

$$AUMC_{t_{\text{last}}-\infty} = \int_{t_{\text{last}}}^{\infty} t \cdot C \, dt = \int_{t_{\text{last}}}^{\infty} t \cdot C_{\text{last}} e^{-\lambda_z (t-t_{\text{last}})} \, dt$$

$$= C_{\text{last}} \cdot t_{\text{last}} \cdot \left[ \frac{e^{-\lambda_z (t-t_{\text{last}})}}{-\lambda_z} \right]_{t_{\text{last}}}^{\infty} + \left[ \frac{e^{-\lambda_z (t-t_{\text{last}})}}{-\lambda_z^2} \right]_{t_{\text{last}}}^{\infty}$$

$$= \frac{C_{\text{last}} \cdot t_{\text{last}}}{\lambda_z} + \frac{C_{\text{last}}}{\lambda_z^2} \cdot \lambda_z^2 \quad (4)$$
An alternative procedure that has been proposed is the log-linear trapezoidal rule. The underlying assumption is that the plasma concentrations decline mono-exponentially between two measured concentrations. However, this method applies only for descending data and fails when \( C_i = 0 \) or \( C_{i+1} = C_i \). In these instances one would revert to the linear trapezoidal rule. The principal difference between the linear and the log-linear trapezoidal method is demonstrated in Fig. 5.

Remember that when the concentrations decline exponentially

\[
C_{i+1} = C_i \cdot e^{-K(t_{i+1} - t_i)} = C_i \cdot e^{-K\Delta t},
\]

where \( t_{i+1} - t_i \) is the time step \( \Delta t \) between two observations and \( K \) is the elimination rate constant for a one-compartment system. Otherwise, \( \lambda_z \) should be used as the slope. The above expression when rearranged gives the elimination rate constant \( K \):

\[
K = \frac{\ln(C_i/C_{i+1})}{\Delta t}.
\]

The \( AUC \) within the time interval \( \Delta t \) is the difference between the concentrations divided by the slope \( K \):

\[
AUC_{i+1} = \frac{C_i - C_{i+1}}{K} = \frac{C_i - C_{i+1}}{\ln(C_i/C_{i+1})} \cdot \Delta t.
\]

Using the log-linear trapezoidal method from time zero to \( t_n \)

\[
AUC_{0}^{t_n} = \sum_{i=1}^{n} \frac{C_i - C_{i+1}}{\ln(C_i/C_{i+1})} \cdot \Delta t,
\]

while the corresponding equation for \( AUMC \) from time zero to \( t_n \) with this method yields

![Fig. 5. The principal difference between the linear (left) and the log-linear (right) trapezoidal methods. The shaded region represents the over-predicted area with the linear trapezoidal rule. Note that the log-linear approximation is only true if the decay is truly mono-exponential between \( t_i \) and \( t_{i+1} \).](image-url)
The extrapolated area under the zero moment curve from the last sampling time to infinity $AUC_{t_{\text{last}}-\infty}$ is calculated as

$$AUMC_{0\to\infty}^{t_{\text{last}}} = \sum_{i=1}^{n} t_i \cdot C_i - t_{i+1} \cdot C_{i+1} \cdot \Delta t - \frac{C_{i+1} - C_i}{\ln(C_i/C_{i+1})} \cdot \Delta t^2.$$  

(9)

The extrapolated area under the zero moment curve from the last sampling time to infinity $AUC_{t_{\text{last}}-\infty}$ is calculated as

$$AUC_{t_{\text{last}}-\infty}^{\infty} = \frac{C_{\text{last}}}{\lambda_z},$$  

(10)

where $C_{\text{last}}$ and $\lambda_z$ are as defined earlier. The corresponding area under the first moment curve from time zero to infinity $AUMC_{t_{\text{last}}-\infty}$ is

$$AUMC_{t_{\text{last}}-\infty}^{\infty} = \frac{C_{\text{last}} \cdot t_{\text{last}}}{\lambda_z} + \frac{C_{\text{last}}}{\lambda_z^2}.$$  

(11)

As previously pointed out, the linear trapezoidal method gives approximate estimates of $AUC$ during both the ascending and descending parts of the concentration–time curve, although the bias is usually negligible for the upswing. The log-linear trapezoidal method may also give somewhat biased results, though to a lesser extent. Some people argue that the log-linear trapezoidal method may therefore be preferable for drugs with long half-lives relative to the sampling interval. From a practical point of view this still needs to be proven. However, our own experience is that the difference between the two methods is negligible as long as a reasonable sampling design has been used. We generally use a mixture of the two methods, which means that the linear trapezoidal method is applied for increasing and equal concentrations, e.g., at the peak or a plateau, and the log-linear trapezoidal method for decreasing concentrations. This is demonstrated in Fig. 6.

Note that NCA is often used in crossover studies comparing two formulations and 12–36 subjects. Thus, since the error associated

Fig. 6. NCA using a combination of the linear and log-linear trapezoidal methods. The linear method is used for consecutively increasing or consecutively equal concentrations. The log-linear method is used for decreasing concentrations.
with an individual patient’s $AUC$ is generally small, the (average) error associated with the average $AUC$ for a formulation will generally be negligible, regardless of the method used. The choice of method is thus up to the discretion of the modeler, as long as one can explain why a particular method provides a more accurate estimate of $AUC$.

The linear trapezoidal method will work excellently in situations of zero-order kinetics since plasma concentrations decline linearly with time. Hence, even large sampling intervals will be acceptable. The log-linear trapezoidal rule may in some instances be more optimal within the first-order concentration range. The linear method will then overpredict the areas particularly when half-life is short relative to the sampling interval.

Direct integration of the function for the drug’s kinetics in plasma is discussed under the introductory section on mono- and multi-exponential models and will therefore not be further elaborated here.

1.4. Strategies for Estimation of $\lambda_z$

When estimating $\lambda_z$, we recommend that data from each individual are first plotted in a semilog diagram. Ideally, to obtain a reliable estimate of the terminal slope, 3–4 half-lives would need to have elapsed. However, sometimes this is not possible. A minimum requirement is then to have 3–4 observations for the terminal slope (Fig. 7). By means of log-linear regression of those observations, the estimate of $\lambda_z$ is obtained. This is then used for calculation of the extrapolated area as shown below:

$$AUC_{\text{last (observed)}}^{\infty} = \frac{C_{\text{last}}}{\lambda_z} \quad (12)$$

or

$$AUC_{\text{last (predicted)}}^{\infty} = \frac{\hat{C}_{\text{last}}}{\lambda_z}. \quad (13)$$

In Fig. 8 the last observed concentration $C_{\text{obs}}$ deviates somewhat from the regression line. The extrapolated area, if based on

![Fig. 7. The ideal situation (left) for estimation of the terminal slope $\lambda_z$. Another and perhaps more commonly encountered situation (right) is where one only has an indication of an additional slope.](image-url)
C_{obs, would} be disproportionately large as compared to the area
based on the predicted concentration.

The total area is obtained by summing the individual areas
obtained by means of the trapezoidal rule to the last time (t_{last}),
and adding the extrapolated area according to

\[ AUC_{total} = AUC_0^\infty = AUC_0^{inf} + AUC_{t_{last}}^\infty. \]  

(14)

The fraction of AUC_{extr} to AUC_0^\infty is calculated as

\[ \% \text{ extrapolated area} = \frac{AUC_{t_{last}}^\infty}{AUC_0^\infty} \times 100. \]  

(15)

The extrapolated area should ideally be as small as possible in
comparison to the total area. We believe that AUC_{t_{last-\infty}} should not
exceed 20–25% of AUC_{total}, unless it is only used as a preliminary
estimate for further study refinement.

1.5. Pertinent Pharmacokinetic Estimates

Moment analysis has been widely used in recent years as a non-
compartmental approach to the estimation of clearance Cl, mean
residence time MRT, steady-state volume of distribution V_{ss}, and
volume of distribution during the terminal phase V_z (also called V_d\beta
for a bi-exponential system). A general treatment for the aforementioned parameters has been presented, which includes the possibility
of input/exit from any compartment in a mammillary model
(2, 3). This approach also defines exit site-dependent and exit
site-independent parameters. We will, however, assume in the
following examples that input/output occurs to the central com-
partment. Assuming a simple case with a one-compartment bolus
system, the shape of the concentration–time and t-concentration–
time profiles will take the form depicted in Fig. 9.

The extrapolated area from the last sample at t_{last} to infinity is in
this case small. However, the corresponding area under the first
moment curve has an altogether different shape. Clearly, the extrapolated area from last sampling point to infinity will generally contribute to a much larger extent under the first moment curve as compared to the area under the zero moment curve.

Pharmacokinetics has moved almost completely from parameterizing elimination in terms of rate constants, with the more physiologically relevant use of clearance now being widely recognized. To put even more focus on clearance, Holford suggested that \( AUC \) no longer be used as a pharmacokinetic parameter. Clearance \( Cl \) or clearance over bioavailability \( Cl/F \) also denoted that \( Cl_0 \) is easily computed from \( AUC \) and dose, and \( Cl \) and \( CL/F \) can immediately be interpreted in a physiological context. On the other hand, \( AUC \) can be viewed as a parameter that confounds clearance and dose, and that has no intrinsic merit. While we agree with those ideas, \( AUC \) is still useful as a measure of exposure in toxicological studies and when dose is unknown.

Clearance is calculated from the dose and the area under the zero moment curve:

\[
Cl = \frac{D_{iv}}{AUC_0^\infty}.
\]  

Oral clearance \( Cl_0 \) or \( Cl/F \) is calculated from the oral dose and the area under the zero moment curve:

\[
Cl_0 = \frac{Cl}{F} = \frac{D_{po}}{AUC_0^\infty}.
\]  

Using the areas obtained from systemic, e.g., intravenous and extravascular, e.g., oral, dosing, the bioavailability \( F \) is calculated, after dose-normalization, according to

\[
F = \frac{AUC_{ev}}{AUC_{iv}} \cdot \frac{D_{iv}}{D_{ev}},
\]
where $AUC_{ev}$ and $AUC_{iv}$ denote area under the extravascular and intravenous concentration–time profiles, respectively. $D_{ev}$ and $D_{iv}$ are the respective extravascular and intravenous doses.

If the drug is given at a constant rate over a period of $T_{inf}$, then one also needs to adjust $MRT$ for the infusion time by means of subtracting $T_{inf}/2$ (infusion time/2) as follows:

$$MRT = \frac{AUMC_{0}^{\infty}}{AUC_{0}^{\infty}} - \frac{T_{inf}}{2}.$$  \hspace{1cm} (19)

$T_{inf}/2$ originates from the average time a molecule stays in the infusion set (e.g., syringe, catheter, line). Half of the dose is infused when the piston has traveled half of the intended distance. $T_{inf}/2$ is the mean input time, $MIT$. Similarly for first-order input,

$$MRT = \frac{AUMC_{0}^{\infty}}{AUC_{0}^{\infty}} - \frac{1}{K_a}.$$  \hspace{1cm} (20)

Remember that $K_a$ is the apparent first-order absorption rate constant derived from plasma data. This parameter may also contain processes parallel to the true absorption step of drug in the gastrointestinal tract, e.g., chemical degradation ($k_d$). Consequently, the mean absorption time, $MAT$, is the sum of several processes including absorption and chemical degradation:

$$MAT = \frac{1}{K_a(\text{apparent})} = \frac{1}{K_a(\text{true}) + k_d}.$$  \hspace{1cm} (21)

The $MRT$ of the central compartment $MRT(1)$ is the sum of the inverse of the initial $\alpha$ and terminal $\beta$ slopes corrected for the inverse of the sum of the exit rate constants from the peripheral compartment:

$$MRT_{iv}(1) = \frac{1}{\alpha} + \frac{1}{\beta} - \frac{1}{E_2}.$$  \hspace{1cm} (22)

Assuming that there is only one exit rate constant from the peripheral compartment, which then is $k_{21}$, the $MRT_{iv}$ is

$$MRT_{iv} = \frac{1}{\alpha} + \frac{1}{\beta} - \frac{1}{k_{21}}.$$  \hspace{1cm} (23)

The observed $MRT$ after extravascular dosing becomes

$$\frac{AUMC_{0}^{\infty}_{\text{measured}}}{AUC_{0}^{\infty}_{\text{measured}}} = MRT + MIT,$$  \hspace{1cm} (24)

which is the sum of the true $MRT$ and $MIT$. $MIT$ can also be obtained from the input function according to Eq. 25 below:

$$MIT = \frac{\int_{0}^{\infty} \text{input function} \cdot t \, dt}{\int_{0}^{\infty} \text{input function} \, dt} = \frac{\int_{0}^{\infty} \text{input function} \cdot t \, dt}{\int_{0}^{\infty} \text{input function} \, dt} \cdot F \cdot \text{Dose},$$  \hspace{1cm} (25)
provided the input function is known,
\[ A_{\text{gut}} = F \cdot D_{po} \cdot e^{-K_{s,t}} , \]
and \( MIT \) can be derived:
\begin{align*}
MIT &= \int_{0}^{\infty} F \cdot D_{po} \cdot e^{-K_{s,t}} \cdot t \, dt \\
   &= \frac{\int_{0}^{\infty} F \cdot D_{po} \cdot e^{-K_{s,t}} \cdot t \, dt}{\int_{0}^{\infty} F \cdot D_{po} \cdot e^{-K_{s,t}} \, dt} \\
   &= \frac{F \cdot D_{po} / K_{a}^{2}}{F \cdot D_{po} / K_{a}} = \frac{1}{K_{a}}. 
\end{align*}

The volume of distribution at steady-state \( V_{ss} \) is computed as
\[ V_{ss} = MRT \cdot Cl = \frac{AUMC_{0}^{\infty}}{AUC_{0}^{\infty}} \cdot \frac{D_{iw}}{AUC_{0}^{\infty}} = \frac{D_{iw} \cdot AUMC_{0}^{\infty}}{[AUC_{0}^{\infty}]^{2}}. \]

The volume of distribution during the terminal phase \( V_{z} \) is computed as
\[ V_{z} = \frac{Cl}{\lambda_{z}} = \frac{D_{iw} \cdot 1}{AUC_{0}^{\infty}}. \]

The corresponding volume for a bi-exponential function is computed as
\[ V_{d\beta} = \frac{Cl}{\beta} = \frac{D_{iw} \cdot 1}{AUC_{0}^{\infty}}. \]

The terminal half-life \( t_{1/2z} \) is readily estimated from the slope \( \lambda_{z} \) as
\[ t_{1/2z} = \frac{\ln(2)}{\lambda_{z}}. \]

The half-life of the initial \( \alpha \)-phase is
\[ t_{1/2\alpha} = \frac{\ln(2)}{\alpha}. \]

The half-life of the terminal \( \beta \)-phase of a bi-exponential function is
\[ t_{1/2\beta} = \frac{\ln(2)}{\beta}. \]

Note that the \( t_{1/2z} \) parameter is referred to as \( t_{1/2\beta} \) in a bi-exponential function and simply \( t_{1/2} \) in a mono-exponential system.

1.6. NCA Approaches for Sparse Data

In some instances it may not be possible to obtain sufficient samples from each subject so as to completely characterize the plasma concentration–time curve. This may be due to the need to sacrifice the animal to obtain the samples, general concerns over blood loss (such as in human neonates or small rodents), or cost concerns. In these situations it is necessary to pool the data from multiple
subjects to characterize the full time–plasma concentration curve. Generally these approaches are recommended only when the data are being collected from populations that do not exhibit extensive subject-to-subject variation, such as in highly inbred strains of animals.

One such approach is an extension of the NCA analysis for rich data described previously, and enables one to derive an estimated standard error (se) for AUC for sparse data (4–6). This procedure is implemented in Phoenix® WinNonlin®.

Another approach has also been proposed that involves non-linear mixed effects modeling (also denoted as population modeling). In this instance a structural pk model is specified and fit to the data. This approach has the advantage of incorporating covariates (e.g., age, gender, body weight, etc.). That is, the ability (e.g.) to model changes in clearance as a function of age or body weight. It also has a limitation of possibly not being able to adequately identify the underlying structural model unless the sparse data can be pooled with rich data from some other cohort (7).

1.7. Suggested Reading
For further reading on basic pharmacokinetic principles, we refer the reader to Benet (8), Benet and Galeazzi (9), Gibaldi and Perrier (10), Nakashima and Benet (2, 3), Jusko (11), and Rowland and Tozer (12). Houston (13) and Pang (14) provide excellent texts on metabolite kinetics.

Benet and Galeazzi (9), Watari and Benet (15), and Nakashima and Benet (3) have elaborated on the theory of NCA, while Gillespie (16) discussed the pros and cons of NCA versus compartmental models.

References
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