Phoenix WinNonlin®
User’s Guide

Applies to:
Phoenix WinNonlin 8.3

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Phoenix WinNonlin

Phoenix WinNonlin is a non-compartmental analysis (NCA), pharmacokinetic/pharmacodynamic (PK/PD), and toxicokinetic (TK) modeling tool, with integrated tools for data processing, post-analysis processing including statistical tests and bioequivalence analysis, table creation, and graphics. It is highly suited for PK/PD modeling and noncompartmental analysis used to evaluate data from bioavailability and clinical pharmacology studies.

Phoenix WinNonlin includes a large library of pharmacokinetic, pharmacodynamic, noncompartmental, PK/PD linked, and indirect response models. It also supports creation of custom models to enable fitting and analysis of clinical data. It generates the graphs, tables, and output worksheets required for regulatory submission.

Operational objects that are part of WinNonlin include:

NCA
Bioequivalence
Linear Mixed Effects
Crossover
Convolution
Deconvolution
NonParametric Superposition
Semicompartmental Modeling
Modeling (Dissolution, Indirect Response, Linear, Michaelis-Menten, Pharmacodynamic, Pharmacokinetic, PK/PD Linked, User-defined ASCII)

References for the WinNonlin operational objects are provided in their descriptions. Additional references are provided below.

Pharmacokinetics References

Hooker, Staatz, and Karlsson (2007). Conditional Weighted Residuals (CWRES): A Model Diagnostic for the FOCE Method, *Pharmaceutical Research*, DOI:10.1007/s11095-007-9361-x.

Brown and Manno (1978). ESTRIP, A BASIC computer program for obtaining initial polyexponential parameter estimates. *J Pharm Sci* 67:1687–91.

Chan and Gilbaldi (1982). Estimation of statistical moments and steady-state volume of distribution for a drug given by intravenous infusion. *J Pharm Bioph* 10(5):551–8.

Cheng and Jusko (1991). Noncompartmental determination of mean residence time and steady-state volume of distribution during multiple dosing, *J Pharm Sci* 80:202.

Dayneka, Garg and Jusko (1993). Comparison of four basic models of indirect pharmacodynamic responses. *J Pharmacokin Biopharm* 21:457.

Endrenyi, ed. (1981). *Kinetic Data Analysis: Design and Analysis of Enzyme and Pharmacokinetic Experiments.* Plenum Press, New York.

Gabrielsson and Weiner (2016). *Pharmacokinetic and Pharmacodynamic Data Analysis: Concepts and Applications*, 5th ed. Apotekarsocieteten, Stockholm.

Gibaldi and Perrier (1982). *Pharmacokinetics*, 2nd ed. Marcel Dekker, New York.

Gouyette (1983). Pharmacokinetics: Statistical moment calculations. *Arzneim-Forsch/Drug Res* 33 (1):173–6.

Holford and Sheiner (1982). Kinetics of pharmacological response. *Pharmacol Ther* 16:143.

Jusko (1990). Corticosteroid pharmacodynamics: models for a broad array of receptor-mediated pharmacologic effects. *J Clin Pharmacol* 30:303.

Koup (1981). Direct linear plotting method for estimation of pharmacokinetic parameters. *J Pharm Sci* 70:1093–4.

Kowalski and Karim (1995). A semicompartmental modeling approach for pharmacodynamic data assessment. *J Pharmacokinet Biopharm* 23(3):307–22.

Metzler and Tong (1981). Computational problems of compartmental models with Michaelis-Menten-type elimination. *J Pharmaceutical Sciences* 70:733–7.

Nagashima, O'Reilly and Levy (1969). Kinetics of pharmacologic effects in man: The anticoagulant action of warfarin. *Clin Pharmacol Ther* 10:22.

Wagner (1975). *Fundamentals of Clinical Pharmacokinetics.* Drug Intelligence, Illinois.

Regression and modeling References

Akaike (1978). Posterior probabilities for choosing a regression model. *Annals of the Institute of Mathematical Statistics* 30:A9–14.

Allen and Cady (1982). *Analyzing Experimental Data By Regression*. Lifetime Learning Publications, Belmont, CA.

Bard (1974). *Nonlinear Parameter Estimation*. Academic Press, New York.

Bates and Watts (1988). *Nonlinear Regression Analyses and Its Applications.* John Wiley & Sons, New York.

Beck and Arnold (1977). *Parameter Estimation in Engineering and Science.* John Wiley & Sons, New York.

Belsley, Kuh and Welsch (1980). *Regression Diagnostics.* John Wiley & Sons, New York.

Corbeil and Searle (1976). Restricted maximum likelihood (REML) estimation of variance components in the mixed models, *Technometrics*, 18:31–8.

Cornell (1962). A method for fitting linear combinations of exponentials. *Biometrics* 18:104–13.

Davies and Whitting (1972). A modified form of Levenberg's correction. Chapter 12 in *Numerical Methods for Non-linear Optimization.* Academic Press, New York.

DeLean, Munson and Rodbard (1978). Simultaneous analysis of families of sigmoidal curves: Application to bioassay, radioligand assay and physiological dose-response curves. *Am J Physiol* 235(2):E97–E102.

Draper and Smith (1981). *Applied Regression Analysis*, 2nd ed. John Wiley & Sons, NY.

Fai and Cornelius (1996). Approximate f-tests of multiple degree of freedom hypotheses in generalized least squares analysis of unbalanced split-plot experiments. *J Stat Comp Sim* 554:363–78.

Fletcher (1980). Practical methods of optimization, Vol. 1: *Unconstrained Optimization.* John Wiley & Sons, New York.

Foss (1970). A method of exponential curve fitting by numerical integration. *Biometrics* 26:815–21.

Giesbrecht and Burns (1985). Two-stage analysis based on a mixed model: Large sample asymptotic theory and small-sample simulation results. *Biometrics* 41:477–86.

Gill, Murray and Wright (1981). *Practical Optimization.* Academic Press.

Gomeni and Gomeni (1979). AUTOMOD: A Polyalgorithm for an integrated analysis of linear pharmacokinetic models. *Comput Biol Med* 9:39–48.

Hartley (1961). The modified Gauss-Newton method for the fitting of nonlinear regression functions by least squares. *Technometrics* 3:269–80.

Jennrich and Moore (1975). Maximum likelihood estimation by means of nonlinear least squares. *Amer Stat Assoc Proceedings Statistical Computing Section* 57–65.

Kennedy and Gentle (1980). *Statistical Computing.* Marcel Dekker, New York.

Koch (1972). The use of nonparametric methods in the statistical analysis of the two-period change-over design. *Biometrics* 577–84.

Kowalski and Karim (1995). A semicompartmental modeling approach for pharmacodynamic data assessment, *J Pharmacokinet Biopharm* 23:307–22.

Leferink and Maes (1978). STRIPACT, An interactive curve fit programme for pharmacokinetic analyses. *Arzneim Forsch* 29:1894–8.

Longley (1967). *Journal of the American Statistical Association*, 69:819–41.

Nelder and Mead (1965). A simplex method for function minimization. *Computing Journal* 7:308–13.

Parsons (1968). Biological problems involving sums of exponential functions of time: A mathematical analysis that reduces experimental time. *Math Biosci* 2:123–8.

PCNonlin. Scientific Consulting Inc., North Carolina, USA.

Peck and Barrett (1979). Nonlinear least-squares regression programs for microcomputers. *J Pharmacokinet Biopharm* 7:537–41.

Ratkowsky (1983). *Nonlinear Regression Modeling.* Marcel Dekker, New York.

Satterthwaite (1946). An approximate distribution of estimates of variance components. *Biometrics Bulletin* 2:110–4.

Schwarz (1978). Estimating the dimension of a model. *Annals of Statistics* 6:461–4.

Sedman and Wagner (1976). CSTRIP, A FORTRAN IV Computer Program for Obtaining Polyexponential Parameter Estimates. *J Pharm Sci* 65:1001–10.

Shampine, Watts and Davenport (1976). Solving nonstiff ordinary differential equations - the state of the art. *SIAM Review* 18:376–411.

Sheiner, Stanski, Vozeh, Miller and Ham (1979). Simultaneous modeling of pharmacokinetics and pharmacodynamics: application to d-tubocurarine. *Clin Pharm Ther* 25:358–71.

Smith and Nichols (1983). Improved resolution in the analysis of the multicomponent exponential signals. *Nuclear Instrum Meth* 205:479–83.

Tong and Metzler (1980). Mathematical properties of compartment models with Michaelis-Menten-type elimination. *Mathematical Biosciences* 48:293–306.

Wald (1943). Tests of statistical hypotheses concerning several parameters when the number of observations is large. *Transaction of the American Mathematical Society* 54.

Bioequivalence

Defined as relative bioavailability, ***bioequivalence*** involves comparison between test and reference drug products, where the test and reference products can vary, depending upon the comparison to be performed. Although bioavailability and bioequivalence are closely related, bioequivalence comparisons rely on a criterion, a predetermined bioequivalence limit, and calculation of a interval for that criterion.

Use one of the following to add the object to a Workflow:

Right-click menu for a Workflow object: **New > Computation Tools > Bioequivalence**.
Main menu: **Insert > Computation Tools > Bioequivalence**.
Right-click menu for a worksheet: **Send To > Computation Tools > Bioequivalence**.

Note: To view the object in its own window, select it in the Object Browser and double-click it or press **ENTER**. All instructions for setting up and execution are the same whether the object is viewed in its own window or in Phoenix view.

This section contains information on the following topics:

Bioequivalence user interface
Results
Bioequivalence overview
Covariance structure types
Data limits and constraints
Average bioequivalence study designs
Population and individual bioequivalence
References
Bioequivalence model examples

Bioequivalence user interface

Main Mappings panel
Model tab
Fixed Effects tab
Variance Structure tab
Options tab
General Options tab

The Bioequivalence model object is based on a mixed effects model. For more see “General linear mixed effects model”.

Main Mappings panel

Use the Main Mappings panel to identify how input variables are used in a bioequivalence model. A separate analysis is performed for each profile, or unique level of soft key(s). Required input is highlighted orange in the interface.

None: Data types mapped to this context are not included in any analysis or output.

Sort: Categorical variable(s) identifying individual data profiles, such as subject ID and treatment in a crossover study. A separate analysis is done for each unique combination of sort variable values.

Subject: The subjects in a dataset.

Sequence: The order of drug administration.

Period: The washout period, or the time period between two treatments needed for drug elimination. Only applicable in a crossover study.

Formulation: The treatment and reference drug formulations used in a study.

Dependent: The dependent variables, such as drug concentration, that provides the values used to fit the model.

Classification: Classification variables or factors that are categorical independent variables, such as formulation, treatment, and gender.

Regressors: Regressor variables or covariates that are continuous independent variables, such as gender or body weight. The regressor variable can also be used to weight the dataset.

Input data considerations

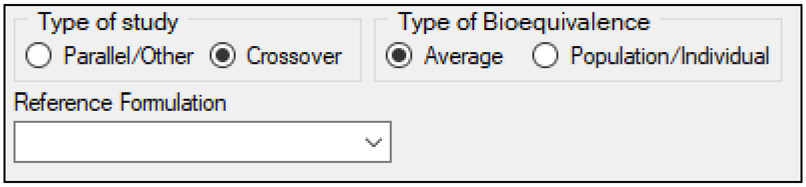
Missing data: For population and individual bioequivalence, the application assumes complete data for each subject. If a subject has a missing observation, that subject is not included in the analysis. If the data have many missing observations, consider imputing estimated values to produce complete records per subject. Phoenix does not impute missing values.

Variable name and data limits: See "Data limits and constraints".

Note: Be sure to finalize column names in your input data before sending the data to the Bioequivalence object. Changing names after the object is set up can cause the execution to fail.

Model tab

The Model tab allows users to specify the bioequivalence model.



Type of study: ○ Parallel/Other ● Crossover

Type of Bioequivalence: ● Average ○ Population/Individual

Reference Formulation

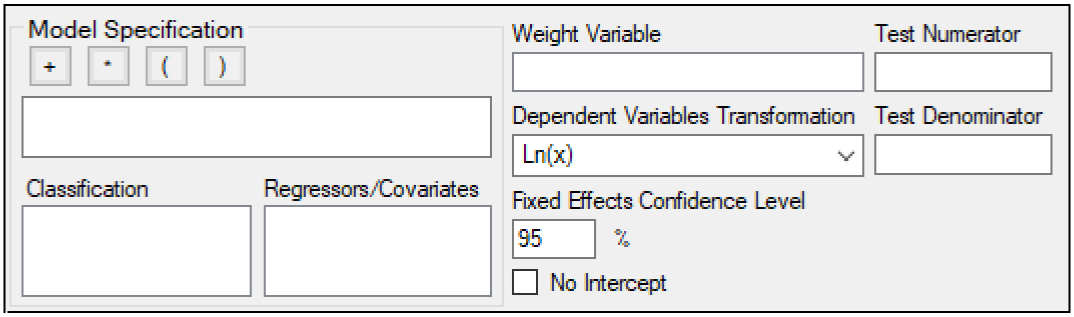
- Under **Type of study**, select the **Parallel/Other** or **Crossover** option buttons to select the type of study.
- Under **Type of Bioequivalence**, select the **Average** or **Population/Individual** option buttons to select the type of bioequivalence. Crossover studies are the only study type permitted when population or individual are the types of bioequivalence being determined.
- In the **Reference Formulation** menu, select the reference formulation or treatment. The menu is only available after a formulation variable is mapped.

The Bioequivalence object automatically sets up default fixed effects, random effects, and repeated models for average bioequivalence studies depending on the type of study design: replicated crossover, nonreplicated crossover, or parallel. For more details on the default settings, see, see "Average bioequivalence study designs".

For more information on population and individual bioequivalence, see "Population and individual bioequivalence".

Fixed Effects tab

The Fixed Effects tab allows users to specify settings for study variables used in an average bioequivalence model. Population and individual bioequivalence models do not use fixed effects, so most options in the Fixed Effects tab are unavailable for population or individual bioequivalence models.



Dependent Variables Transformation: Ln(x)

Fixed Effects Confidence Level: 95 %

☐ No Intercept

Average bioequivalence

For average bioequivalence models the **Model Specification** field automatically displays an appropriate fixed effects model for the study type. Edit the model as needed.

Phoenix automatically specifies average bioequivalence models based on the study type selected and the dataset used. These default models are based on US FDA Guidance for Industry (January 2001).

See the following for details on the models used in a particular study type:

Replicated crossover designs
Nonreplicated crossover designs
Parallel designs

Study variables in the **Classification** box and the **Regressors/Covariates** box can be dragged to the **Model Specification** field to create the model structure.

- Drag variables from the **Classification** and the **Regressors/Covariates** boxes to the **Model Specification** field and click the operator buttons to build the model or type the names and operators directly in the field.
  + addition,
  ***** multiplication,
  ( ) parentheses for indicating nested variables in the model

  Below are some guidelines for using parentheses:
  - Parentheses in the model specification represent nesting of model terms.
  - `Seq+Subject(Seq)+Period+Form` is a valid use of parentheses and indicates that Subject is nested within Seq.
  - `Drug+Disease+(Drug*Disease)` is not a valid use of parentheses in the model specification.
- Select a weight variable from the **Regressors/Covariates** box and drag it to the **Weight Variable** field.

  To remove the weight variable, drag the variable from the **Weight Variable** field back to the **Regressors/Covariates** box.

  The **Regressors/Covariates** box lists variables mapped to the Regressors context (in the Main Mappings panel). If a variable is used to weight the data then the variable is displayed in the **Regressors/Covariates** box. Below are some guidelines for using weight variables:

  - The weights for each record must be included in a separate column in the dataset.
  - Weight variables are used to compensate for observations having different variances.
  - When a weight variable is specified, each row of data is multiplied by the square root of the corresponding weight.
  - Weight variable values should be proportional to the reciprocals of the variances. Typically, the data are averages and weights are sample sizes associated with the averages.
  - The Weight variable cannot be a classification variable. It must be declared as a regressor/covariate before it can be used as a weight variable. It can also be used in the model.

- In the **Dependent Variables Transformation** menu, select one of the transformation options:

  None
  Ln(x): Linear transformation
  Log10(x): Logarithmic base 10 transformation
  Already Ln-transformed: Select if the dependent variable values are already transformed.
  Already Log10-transformed: Select if the dependent variable values are already transformed.

- In the **Fixed Effects Confidence Level** box, type the level for the fixed effects model. The default value is 95%.

- By default, the intercept term is included in the model (although it is not shown in the Model Specification field), check the **No Intercept** checkbox to remove the intercept term.

- Use the **Test Numerator** and **Test Denominator** fields to specify an additional test of hypothesis in the case of a model with only fixed effects.

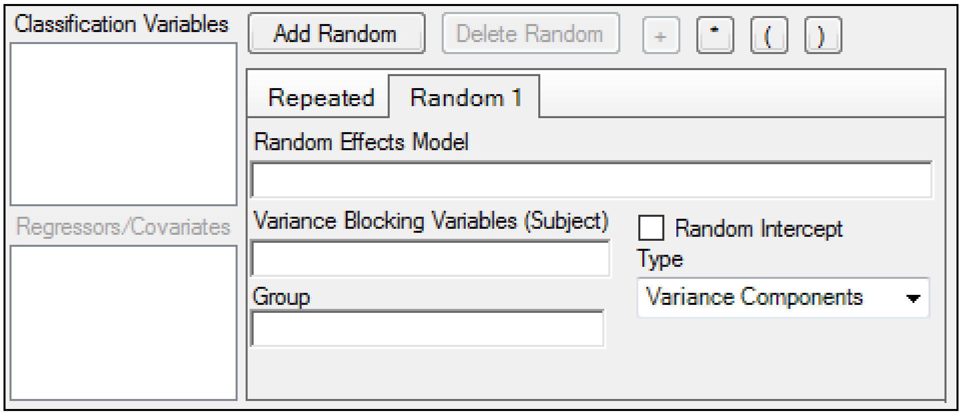
  For this case, the default error term (denominator) is the residual error, so an alternate test can be requested by entering the fixed effects model terms to use for the numerator and denominator of the F-test. The terms entered must be in the fixed effects model and the random/repeated models must be empty for the test to be performed. (See "Tests of hypotheses" for additional information.)

- In the **Dependent Variables Transformation** menu, select **Ln(x)** or **Already Ln-transformed**.

  Ln(x): Linear transformation
  Already Ln-transformed: Select if the dependent variable values are already transformed.

Variance Structure tab

The Variance Structure tab allows users to set random effects and repeated specification for the bioequivalence model. Users can also set traditional variance components and random coefficients. The Variance Structure tab is only available for average bioequivalence models.



☐ Random Intercept

Type: Variance Components

Users can specify none, one, or multiple random effects. The random effects specify Z and the corresponding elements of G=Var(γ). Users can specify only one repeated effect. The repeated effect specifies the R=Var(ε).

Phoenix automatically specifies random effects models and repeated specifications for average bioequivalence models. For more on the default models and specifications, see “Recommended models for average bioequivalence”.

The random effects model can be created using the classification variables.

- Use the pointer to drag the variables from the **Classification Variables** box to Random 1 tab.
- Users can also type variable names in the fields in the Random 1 tab.

The random effects model can be created using the regressor or covariate variables.

- Use the pointer to drag the variables from the **Regressors/Covariates** box to Random 1 tab.
- Users can also type variable names in the fields in the Random 1 tab.

Random 1 and Repeated tabs

The Random 1 tab is used to add random effects to the model. The random effects are built using the classification variables, the regressors/covariates variables, and the operator buttons.

The Repeated tab is used to specify the R matrix in the mixed model. If no repeated statement is specified, R is assumed to be equal to $\sigma^2 I$. The repeated effect must contain only classification variables.

The default repeated model depends on whether the crossover study is replicated or not.

Caution: The same variable cannot be used in both the fixed effects specification and the random effects specification unless it is used differently, such as part of a product. The same term (single variables, products, or nested variables) must not appear in both specifications.

- Drag variables from the boxes on the left to the fields in the tab and click the operator buttons to build the model or type the names and operators directly in the fields.
  + addition (not available in the Repeated tab or when specifying the variance blocking or group variables),
  ***** multiplication,
  ( ) parentheses for indicating nested variables in the model.
- The **Variance Blocking Variables (Subject)** field is optional and, if specified, must be a classification model term built from the items in the **Classification Variables** box. This field is used to identify the subjects in a dataset. Complete independence is assumed among subjects, so the subject variable produces a block diagonal structure with identical blocks.
- The **Group** field is also optional and, if specified, must be a classification model term built from items in the **Classification Variables** box. It defines an effect specifying heterogeneity in the covariance structure. All observations having the same level of the group effect have the same covariance parameters. Each new level of the group effect produces a new set of covariance parameters with the same structure as the original group.
- (Random 1 tab only) Check the **Random Intercept** checkbox to set the intercept to random.

  This setting is commonly used when a subject is specified in the **Variance Blocking Variables (Subject)** field. The default setting is no random intercept.
- If the model contains random effects, the covariance structure type must be specified from the **Type** menu.

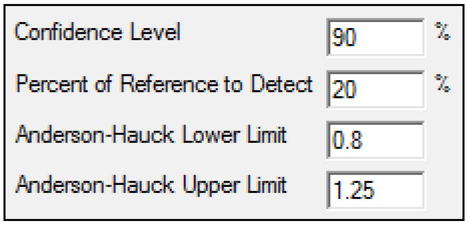
If **Banded Unstructured (b)**, **Banded No-Diagonal Factor Analytic (f)**, or **Banded Toeplitz (b)** is selected, type the number of bands in the **Number of bands(b)** field (default is 1).

The number of factors or bands corresponds to the dimension parameter. For some covariance structure types this is the number of bands and for others it is the number of factors. For explanations of covariance structure types, see "Covariance structure types".

- Click **Add Random** to add additional variance models.
- Click **Delete Random** to delete a variance model.

Options tab

Settings in the Options tab change depending on whether the model type is average or population/ individual.



Confidence Level 90 %
Percent of Reference to Detect 20 %
Anderson-Hauck Lower Limit 0.8
Anderson-Hauck Upper Limit 1.25

Average bioequivalence options

- In the **Confidence Level** field, type the level for the bioequivalence model (default is 90%).
- In the **Percent of Reference to Detect** field, type the percentage of reference treatment to detect (default is 20%).
- In the **Anderson-Hauck Lower Limit** field, type the lower limit for the Anderson-Hauck test (default is 0.8).
- In the **Anderson-Hauck Upper Limit** field, type the upper limit for the Anderson-Hauck test (default is 1.25 for log-transformed data and 1.2 for non-transformed data).

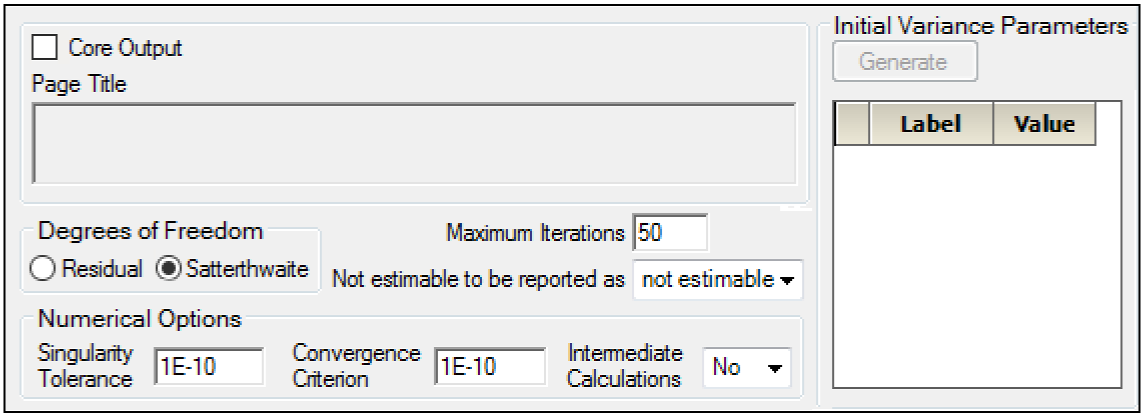
For more on the Anderson-Hauck test, see "Anderson-Hauck test".

Population/Individual bioequivalence options

- In the **Confidence Level** field, type the level for the bioequivalence model (default is 95%).
- In the **Percent of Reference to Detect** field, type the percentage of reference treatment to detect (default is 20%).
- In the **Total SD Standard** field in the **Population Limits** group, type the value of the total slope distance (default is 0.2).
- In the **Epsilon** field in the **Population Limits** group, type an epsilon value for the population limits (default is 0.02).
- In the **Within Subject SD Standard** field in the **Individual Limits** group, type the value of the within subject slope distance (default is 0.2).
- In the **Epsilon** field in the **Individual Limits** group, type an epsilon value for the individual limits (default is 0.05).

General Options tab

The General Options tab is used to set output and calculation options for a bioequivalence model. The options change depending on whether the model is average bioequivalence or population/individual bioequivalence.



□ Core Output

Page Title

Degrees of Freedom: ○ Residual ● Satterthwaite

Maximum Iterations: 50

Not estimable to be reported as: not estimable

Numerical Options

Singularity Tolerance: 1E-10

Convergence Criterion: 1E-10

Intermediate Calculations: No

Average bioequivalence options

- Check the **Core Output** checkbox to include the Core Output text file in the results.
- In the **Page Title** field, type a title for the Core Output text file.
- Choose the degrees of freedom calculation method:

  Residual: The same as the calculation method used in a purely fixed effects model.

  Satterthwaite: The default setting and computes the df base on X^2 approximation to distribution of variance.
- In the **Maximum Iterations** field, type the number of maximum iterations. This is the number of iterations in the Newton fitting algorithm. The default setting is 50.
- Use the **Not estimable to be reported as** menu to determine how output that is not estimable is represented.
  - not estimable
  - 0 (zero)
- In the **Singularity Tolerance** field, type the tolerance level. The columns in X and Z are eliminated if their norm is less than or equal to this number. (Default tolerance value is 1E–10.)
- In the **Convergence Criterion** field, type the criterion used to determine if the model has converged (default is 1E–10).
- In the **Intermediate Calculations** menu, select whether (**Yes**) or not (**No**) to include the design matrix, reduced data matrix, asymptotic covariance matrix of variance parameters, Hessian, and final variance matrix in the Core Output text file.
- In the **Initial Variance Parameters** group, click **Generate** to edit the initial variance parameters values.

Note: The **Generate** initial variance parameters option is available only if the model uses random effects.

- Select a cell in the Value column and type a value for the parameter (default is 1).
  If the values are not specified, then Phoenix uses the method of moments estimates.

To delete one or more of the parameters from the table:

- Highlight the row(s).
- Select **Edit >> Delete** from the menubar or press X in the main toolbar.
- Click the **Selected Row(s)** option button and click **OK**.

Population/Individual bioequivalence options

- Select the **Core Output** checkbox to include the Core Output text file in the results.
- In the **Page Title** field, type a title for the Core Output text file.

Results

The Bioequivalence object creates several output worksheets. Each type of bioequivalence model also creates a text file with model settings. If the Core Output checkbox is selected, then a Core Output text file that contains model output is added to the results.

Average bioequivalence output
Population/Individual bioequivalence output
Text output
Ratios test

Average bioequivalence output

- **Average Bioequivalence**: Output from the bioequivalence analysis. Columns include:

  Dependent: Input data column mapped to the Dependent context.
  Units: Units, if specified in the input dataset.
  FormVar: Input data column mapped to the Formulation context.
  FormRef: Reference formulation.
  RefLSM: Reference least squares mean.
  RefLSM_SE: Standard error computed for reference least squares mean.
  RefGeoLSM: Geometric reference least squares mean.
  Test: Test formulation.
  TestLSM: Test least squares mean.
  TestLSM_SE: Standard error computed for test least squares mean.
  TestGeoLSM: Geometric test least squares mean.
  Difference: Difference between test and reference LSM values.
  Diff_SE: Standard error of the difference in LSM values.
  Diff_DF: Degrees of freedom for the difference in LSM values.
  Ratio_%REF_: Percent ration of test LMS to reference LSM values.
  (See "Least squares means" for more details.)
  CI_xx_Lower(Upper): Confidence interval computations. (See "Classical intervals" for more details.)
  t1_TOST: Left-tail test result.
  t2_TOST: Right-tail test result.
  Prob_80(125)_00: Probability results for the two one-sided t-tests.
  MaxProb: Largest value of the probability results.
  TotalProb: Sum of the probability results.
  (See "Two one-sided t-tests" for more details.)
  Power_TOST: Power of the two one-sided t-tests. (See "Power of the two one-sided t-tests procedure" for more details.)
  AHpval: Anderson-Hauck test statistic. (See "Anderson-Hauck test" for more details.)
  Power_80_20: Power to detect a difference in least square means equal to 20% of the reference LSM. (See "Power for 80/20 Rule" for more details.)
  Prob_Eq_Var: Probability of equal variance. (See "Tests for equal variances" for more details.)

- **Ratios Test**: Ratios of reference to test mean computed. One worksheet for each test formulation.

- **User Settings**: User-specified settings

For convenience, the Linear Mixed Effects Modeling output is also included with Average Bioequivalence, but note that, since there is no specification of test or reference in Linear Mixed Effects Modeling, the LSM differences are computed alphabetically, which could correspond to reference minus test formulation rather than test minus reference.

- **Diagnostics**: Number of observations, number of observations used, residual sums of squares, residual degrees of freedom, residual variance, restricted log likelihood, AIC, SBC, and Hessian eigenvalues
- **Final Fixed Parameters**: Sort variables, dependent variable(s), units, effect level, parameter estimate, t-statistic, p-value, intervals, etc.
- **Final Variance Parameters**: Final estimates of the variance parameters
- **Initial Variance Parameters**: Parameter and estimates for variance parameters
- **Iteration History**: Iteration, Obj_function, and column for each variance parameter
- **Least Squares Means**: Sort variable(s), dependent variable(s), units, and the least squares means for the test formulation
- **LSM Differences**: Difference in least squares means
- **Parameter Key**: Variance parameter names assigned by Phoenix
- **Partial SS**: Only for 2x2 crossover designs using the default model: ANOVA sums of squares, degrees of freedom, mean squares, F-tests and p-values for partial test
- **Partial Tests**: Sort Variable(s), Dependent Variable(s), Units, Hypothesis, Hypothesis degrees of freedom, Denominator degrees of freedom, F statistic, and p-value
- **Residuals**: Sort Variable(s), Dependent Variable(s), Units, and information on residual effects vs. predicted and observed effects
- **Sequential SS**: Only for 2x2 crossover designs using the default model: ANOVA sums of squares, degrees of freedom, mean squares, F-tests and p-values for sequential test
- **Sequential Tests**: Sort Variable(s), Dependent Variable(s), Units, Hypothesis, Hypothesis degrees of freedom, Denominator degrees of freedom, F statistic, and p-value

Population/Individual bioequivalence output

The worksheets that are created depend on which model options are selected.

- **Population Individual**: Output from the bioequivalence analysis. Columns include:

  Dependent: Input data column mapped to the Dependent context.
  Units: Units, if specified in the input dataset.
  Statistic: Name of the statistic.
  Value: Computed value of the statistic.
  Upper_CI: Upper bound (an upper bound < 0 indicates bioequivalence).
  Conclusion: Bioequivalence conclusion.
- **Ratios Test**: Ratios of reference to test mean. One worksheet for each test formulation.
- **User Settings**: User-specified settings

The population/individual bioequivalence statistics include:

Difference (Delta): Difference in sample means between the test and reference formulations.

Ratio(%Ref): Ratio of the test to reference means expressed as a percent. This is compared with the percent of reference to detect that was specified by the user, to determine if bioequivalence has been shown. The ratio is expected to be 100% if both formulations are exactly equal. If the values of the two dependents are both over 100, it indicates that the test formulation resulted in higher average exposure than the reference. Values <100 indicate the reverse. For example, if the user specified a percent of reference to detect of 20%, and also specified a ln-transform, then Ratio(%Ref) needs to be in the interval (80%, 125%) to show bioequivalence for the ratio test.

See “Least squares means” for more details.

SigmaR: value that is compared with sigmaP, the Total SD Standard, to determine whether mixed scaling for population bioequivalence will use the constant-scaling values or the reference-scaling values.

SigmaWR: value that is compared with sigmaI, the Within Subject SD Standard, to determine whether mixed scaling for individual bioequivalence will use the constant-scaling values or the reference-scaling values.

See “Bioequivalence criterion” for more details.

Computed bioequivalence statistics include:

Ref_Pop_eta: Test statistic for population bioequivalence with reference scaling
Const_Pop_eta: Test statistic for population bioequivalence with constant scaling
Mixed_Pop_eta: Test statistic for population bioequivalence with mixed scaling
Ref_Indiv_eta: Test statistic for individual bioequivalence with reference scaling
Const_Indiv_eta: Test statistic for individual bioequivalence with constant scaling
Mixed_Indiv_eta: Test statistic for individual bioequivalence with mixed scaling

Because population/individual bioequivalence only allows crossover designs, the output also contains the ratios test, described under “Ratios test”.

Text output

The Bioequivalence object creates two types of text output: a Settings file that contains model settings, and an optional Core Output file. The Core Output text file is created if the **Core Output** checkbox is selected in the General Options tab, then the Core Output text file is created. The file contains a complete summary of the analysis, including all output as well as analysis settings and any errors that occur.

Ratios test

For any bioequivalence study done using a crossover design, the output also includes for each test formulation a table of differences and ratios of the original data computed individually for each subject.

Let *test* be the value of the dependent variable measured after administration of the test formulation for one subject. Let *ref* be the corresponding value for the reference formulation, for the case in which only one dependent variable measurement is made. If multiple test or reference values exist for the same subject, then let *test* be the mean of the values of the dependent variable measured after administration of the test formulation, and similarly for *ref*, except for the cases of ‘ln-transform’ and ‘log10-transform’ where the geometric mean should be used:

$$ln\_transform: test = \exp\left(\sum_i \frac{\ln(X_i)}{N}\right)$$

$$log10\_transform: test = 10^{N^{-1}\sum_i \log_{10} X_i}$$

where X_i are the measurements after administration of the test formulation, and similarly for *ref*. For each subject, for the cases of no transform, ln-transform, or log10-transform, the ratios table contains:

$Difference=test - ref$
$Ratio(\%Ref)=100*test/ref$

For data that was specified to be 'already ln-transformed,' these values are back-transformed to be in terms of the original data:

$Difference=exp(test) - exp(ref)$
$Ratio(\%Ref)=100*exp(test - ref)=100*exp(test)/exp(ref)$

Similarly, for data that was specified to be 'already log10-transformed':

$Difference=10^{\mathrm{test}} - 10^{\mathrm{ref}}$
$Ratio(\%Ref)=100*10^{\mathrm{test}} - ref=100*10^{\mathrm{test}}/10^{\mathrm{ref}}$

Note: For 'already transformed' input data, if the mean is used for *test* or *ref*, and the antilog of *test* or *ref* is taken above, then this is equal to the geometric mean.

Bioequivalence overview

Bioequivalence is said to exist when a test formulation has a bioavailability that is similar to that of the reference. There are three types of bioequivalence: Average, Individual, and Population. Average bioequivalence states that average bioavailability for test formulation is the same as for the reference formulation. Population bioequivalence is meant to assess equivalence in prescribability and takes into account both differences in mean bioavailability and in variability of bioavailability. Individual bioequivalence is meant to assess switchability of products, and is similar to population bioequivalence.

The US FDA Guidance for Industry (January 2001) recommends that a standard ***in vivo*** bioequivalence study design be based on the administration of either single or multiple doses of the test and reference products to healthy subjects on separate occasions, with random assignment to the two possible sequences of drug product administration. Further, the 2001 guidance recommends that statistical analysis for pharmacokinetic parameters, such as area under the curve (AUC) and peak concentration (Cmax) be based on a test procedure termed the two one-sided tests procedure to determine whether the average values for pharmacokinetic parameters were comparable for test and reference formulations. This approach is termed average bioequivalence and involves the calculation of a 90% confidence interval for the ratio of the averages of the test and reference products. To establish bioequivalence, the calculated interval should fall within a bioequivalence limit, usually 80–125% for the ratio of the product averages. In addition to specifying this general approach, the 2001 guidance also provides specific recommendations for (1) logarithmic transformations of pharmacokinetic data, (2) methods to evaluate sequence effects, and (3) methods to evaluate outlier data.

It is also recommended that average bioequivalence be supplemented by population and individual bioequivalence. Population and individual bioequivalence include comparisons of both the averages and variances of the study measure. Population bioequivalence assesses the total variability of the measure in the population. Individual bioequivalence assesses within-subject variability for the test and reference products as well as the subject-by-formulation interaction.

Bioequivalence studies

Bioequivalence between two formulations of a drug product, sometimes referred to as relative bioavailability, indicates that the two formulations are therapeutically equivalent and will provide the same therapeutic effect. The objectives of a bioequivalence study is to determine whether bioequivalence exists between a test formulation and reference formulation and to identify whether the two formulations are pharmaceutical alternatives that can be used interchangeably to achieve the same effect.

Bioequivalence studies are important because establishing bioequivalence with an already approved drug product is a cost-efficient way of obtaining drug approval. Bioequivalence studies are also useful for testing new formulations of a drug product, new routes of administration of a drug product, and for a drug product that has changed after approval.

There are three different types of bioequivalence: average bioequivalence, population bioequivalence, and individual bioequivalence. All types of bioequivalence comparisons are based on the calculation of a criterion (or point estimate), on the calculation of an interval for the criterion, and on a predetermined acceptability limit for the interval.

A procedure for establishing average bioequivalence involves administration of either single or multiple doses of the test and reference formulations to subjects, with random assignment to the possible sequences of drug administration. Then a statistical analysis of pharmacokinetic parameters such as AUC and Cmax is done on a log-transformed scale to determine the difference in the average values of these parameters between the test and reference data, and to determine the interval for the difference. To establish bioequivalence, the interval, usually calculated at the 90% level, should fall within a predetermined acceptability limit, usually within 20% of the reference average. An equivalent approach using two, one-sided ***t***-tests is also recommended. Both the interval approach and the two one-sided ***t***-tests are described further below.

The average bioequivalence should be supplemented by population and individual bioequivalences. These added approaches are needed because average bioequivalence uses only a comparison of averages, and not the variances, of the bioequivalence measures. In contrast, population and individual bioequivalence approaches include comparisons of both the averages and variances of the study measure. The population bioequivalence approach assesses the total variability of the study measure in the population. The individual bioequivalence approach assesses within-subject variability for the test and reference products as well as the subject-by-formulation interaction.

The concepts of population and individual bioequivalence are related to two types of drug interchangeability: prescribability and switchability. Drug prescribability refers to when a physician prescribes a drug product to a patient for the first time, and must choose from a number of bioequivalent drug products. Drug prescribability is usually assessed by population bioequivalence. Drug switchability refers to when a physician switches a patient from one drug product to a bioequivalent product, such as when switching from an innovator drug product to a generic substitute within the same patient. Drug switchability is usually assessed by individual bioequivalence.

Covariance structure types

The covariance types used in the Bioequivalence model object are the same as those used in the Linear Mixed Effects model object. For more on variance structures see "Variance structure" in the LinMix section. Covariance types must be specified if the model contains random effects.

The Variance Structure tab in the Bioequivalence object allows users to select the covariance type used in the model. The variances of the random-effects parameters become the covariance parameters for a bioequivalence model that contains both fixed effect and random effect parameters.

The choices for covariance structures are listed below. The list uses the following notation:

n: number of groups. n=1 if the group option is not used.
t: number of time points in the repeated context.
b: dimension parameter: for some variance structures, the number of bands; for others, the number of factors.
1(expression): 1 if the expression is true, 0 if the expression is false.

Variance Components have n parameters; i^{th}, j^{th} element:

$\sigma_k^2 1(i = j)$

i corresponds to k^{th} effect

Unstructured has n x t x (t + 1)/2 parameters; i^{th}, j^{th} element:

σ_{ij}

in output, $\sigma_{ij} = un(i,j)$

Banded Unstructured (b) has n x b/2(2t – b +1) parameters, if $b < t$; otherwise, same as Unstructured; i^{th}, j^{th} element:

$\sigma_{ij}(|i - j| < n)$

Compound Symmetry has n x 2 parameters; i^{th}, j^{th} element:

$\sigma^2{}_1 + \sigma^2 1(i = j)$

in output, $\sigma^2 = csDiag$, $\sigma^2{}_i = csBlock$

Heterogeneous Compound Symmetry has n x (t + 1) parameters; i^{th}, j^{th} element:

$\sigma_i\sigma_j[\rho 1(i \neq j) + 1(i = j)]$

in output, $\sigma_i = cshSD(i)$, $\rho = cshCorr$

Autoregressive (1) has n x 2 parameters; ith, jth element:

$$\sigma^2\rho^{|i-j|}$$

in output, σ^2 = *arVar,* ρ = *arCorr*

Heterogeneous Autoregressive (1) has n x (t + 1) parameters; ith, jth element:

$$\sigma_i\sigma_j\rho^{|i-j|}$$

in output, σ_i = *arhSD(i),* ρ = *arhCorr*

No-Diagonal Factor Analytic has n x $t(t + 1)/2$ parameters; ith, jth element:

$$\sum_{k=1}^{min(i,j,n)} \lambda_{ik}\lambda_{jk}$$

in output, λ_{ik} = *lambda(i,k)*

Banded No-Diagonal Factor Analytic (b) has n x $b/2(2t - b + 1)$ parameters if $b < t$; otherwise, same as No-Diagonal Factor Analytic; ith, jth element:

$$\sum_{k=1}^{min(i,j,b)} \lambda_{ik}\lambda_{jk}$$

in output, λ_{ik} = *lambda(i,k)*

Data limits and constraints

The limits and constraints discussed here are for Bioequivalence and Linear Mixed Effects modeling.

Cell data may not include question marks (?) or either single (') or double (“) quotation marks.

Variable names must begin with a letter. After the first character, valid characters include numbers and underscores (my_file_2). They cannot include spaces or the following operational symbols: +, –, *, /, =. They also cannot include parentheses (), question marks (?), semicolons (;), single or double quotes.

Titles (in Contrasts, Estimates, or ASCII) can have single or double quotation marks, but not both.

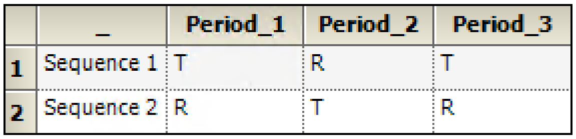
The following are maximum counts for variables.

model terms 30
factors in a model term 10
sort keys 16
dependent variables 128
covariate/regressor variables 255
variables in the dataset 256
levels per variable 1,000
random and repeated statements 10
contrast statements 100
estimate statements 100
combined length of all variance parameter names (total characters) 10,000

combined length of all level names (total characters) 10,000
combined length of input data line (total characters in line of data or column headers) 2,500

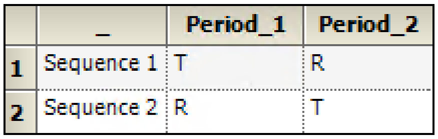
Average bioequivalence study designs

The most common designs for bioequivalence studies are replicated crossover, nonreplicated crossover, and parallel. In a parallel design, each subject receives only one formulation in randomized fashion, whereas in a crossover design each subject receives different formulations in different time periods. Crossover designs are further broken down into replicated and nonreplicated designs. In nonreplicated designs, subjects receive only one dose of the test formulation and only one dose of the reference formulation. Replicated designs involve multiple doses. A bioequivalence study should use a crossover design unless a parallel or other design can be demonstrated to be more appropriate for valid scientific reasons. Replicated crossover designs should be used for individual bioequivalence studies, and can be used for average or population bioequivalence analysis.



	_	Period_1	Period_2	Period_3
1	Sequence 1	T	R	T
2	Sequence 2	R	T	R

An example of a nonreplicated crossover design is the standard 2x2 crossover design described below.



	_	Period_1	Period_2
1	Sequence 1	T	R
2	Sequence 2	R	T

Information about the following topics is available:

Recommended models for average bioequivalence
Least squares means
Classical intervals
Two one-sided t-tests
Power of the two one-sided t-tests procedure
Anderson-Hauck test
Power for 80/20 Rule
Tests for equal variances

Recommended models for average bioequivalence

The default fixed effects, random effects, and repeated models for average bioequivalence studies depends on the type of study design: replicated crossover, nonreplicated crossover, or parallel.

Replicated crossover designs

Replicated data is defined as data for which, for each formulation, there exists at least one subject with more than one observation of that formulation. The default models depend on the type of analyses and the main mappings. For replicated crossover designs, the default model used in the Bioequivalence object is as follows:

Fixed effects model: **Sequence + Formulation + Period**

Random effects model: **Subject(Sequence)** and Type: **Variance Components**

Repeated specification: **Period**
Variance Blocking Variables: **Subject**
Group: **Treatment**
Type: **Variance Components**

Nonreplicated crossover designs

Nonreplicated data is defined as data for which there exists at least one formulation where every subject has only one observation of that formulation. The default models depend on the type of analyses, the main mappings, and a preference called **Default for 2x2 crossover set to all fixed effects** (set under **Edit > Preferences > LinMixBioequivalence**). For nonreplicated crossover designs, the default model is as follows.

Fixed effects model: **Sequence + Formulation + Period**

> Unless the bioequivalence preference **Default for 2x2 crossover set to all fixed effects** is turned on in the *Preferences* dialog (**Edit > Preferences > LinMixBioequivalence**), in which case the model is **Sequence + Subject(Sequence) + Formulation + Period**.

Random effects model: **Subject(Sequence)** and Type: **Variance Components**

> Unless the bioequivalence preference **Default for 2x2 crossover set to all fixed effects** is turned on, in which case the model is not specified (the field is empty).

Repeated model is not specified.

Since there is no repeated specification, the default error model $\varepsilon \sim N(0, \sigma^2 I)$ is used. This is equivalent to the classical analysis method, but using maximum likelihood instead of method of moments to estimate inter-subject variance. Using Subject as a random effect this way, the correct standard errors will be computed for sequence means and tests of sequence effects. Using a fixed effect model, one must construct pseudo-***F*** tests by hand to accomplish the same task.

Note: If Warning 11094 occurs, "Negative final variance component. Consider omitting this VC structure.", when Subject(Sequence) is used as a random effect, this most likely indicates that the within-subject variance (residual) is greater than the between-subject variance, and a more appropriate model would be to move Subject(Sequence) from the random effects to the fixed effects model, i.e., Sequence+Subject(Sequence)+Formulation + Period.

When this default model is used for a standard 2x2 crossover design, Phoenix creates two additional worksheets in the output called Sequential SS and Partial SS, which contain the degrees of freedom (DF), Sum of Squares (SS), Mean Squares (MS), F-statistic and p-value, for each of the model terms. These tables are also included in the text output. Note that the F-statistic and p-value for the Sequence term are using the correct error term since Subject(Sequence) is a random effect.

If the default model is used for 2x2 and the data is not transformed, the *intrasubject CV* parameter is added to the Final Variance Parameters worksheet:

$$intrasubject\ CV = \text{sqrt}(Var(Residual))/RefLSM$$

where *RefLSM* is the Least Squares Mean of the reference treatment.

If the default model is used for 2x2 and the data is either ln-transformed or log10-transformed, the *intra-subject CV* and *intrasubject CV* parameters are added to the Final Variance Parameters work-sheet:

For ln-transformed data:

$$intersubject\ CV = \text{sqrt}(\exp(Var(Sequence*Subject)) - 1)$$
$$intrasubject\ CV = \text{sqrt}(\exp(Residual) - 1)$$

For log10-transformed data:

$$intersubject\ CV = \text{sqrt}(10\text{^}(\ln(10)*Var(Sequence*Subject)) - 1)$$
$$intrasubject\ CV = \text{sqrt}(10\text{^}(\ln(10)*Var(Residual)) - 1)$$

Note that for this default model $Var(Sequence*Subject)$ is the intersubject (between subject) variance, and $Residual$ is the intrasubject (within subject) variance.

Parallel designs

For parallel designs, whether data is replicated or nonreplicated, the default model is as follows.

Fixed effects model: **Formulation**

There is no random model and no repeated specification, so the residual error term is included in the model.

Note: In each case, users can supplement or modify the model to suit the analysis needs of the dataset. For example, if a Subject effect is appropriate for the Parallel option, as in a paired design (each subject receives the same formulation initially and then the other formulation after a washout period), set the Fixed Effects model to Subject+Formulation.

Note: When mapping a new input dataset to a Bioequivalence object, if the mappings are identical for the new mapped dataset, then the model will remain the same. If there are any mapping changes other than mapping an additional dependent variable, then the model will be rebuilt to the default model since the existing model will not be valid anymore. This occurs for mapping changes either made by the user or made automatically due to different column names in the dataset matching the mapping context.

The user can reset to the default model at any time by making any mapping change, such as unmapping and remapping a column.

Least squares means

In determining the bioequivalence of a test formulation and a reference formulation, the first step is the computation of the least squares means (LSM) and standard errors of the test and reference formulations and the standard error of the difference of the test and reference least squares means. These quantities are computed by the same process that is used for the Linear Mixed Effects module. See “Least squares means” in the LinMix section.

To simplify the notation for this and the following sections, let:

$RefLSM$: reference least squares mean,
$TestLSM$: test least squares mean,
$fractionToDetect$: (user-specified percent of reference to detect)/100,
$DiffSE$: standard error of the difference in LSM,
$RatioSE$: standard error of the ratio of the least squares means,
df: degrees of freedom for the difference in LSM.

The geometric LSM are computed for transformed data. For ln-transform or data already ln-transformed,

$$RefGeoLSM = \exp(RefLSM)$$
$$TestGeoLSM = \exp(TestLSM)$$

For log10-transform or data already log10-transformed,

$$RefGeoLSM = 10^{RefLSM}$$
$$TestGeoLSM = 10^{TestLSM}$$

The difference is the test LSM minus the reference LSM,

$$Difference = TestLSM - RefLSM$$

The ratio calculation depends on data transformation. For non-transformed,

$$Ratio(\%Ref) = 100\left(\frac{TestLSM}{RefLSM}\right)$$

For ln-transform or data already ln-transformed, the ratio is obtained on arithmetic scale by exponentiating,

$$Ratio(\%Ref) = 100\exp(Difference)$$

Similarly for log10-transform or data already log10-transformed, the ratio is

$$Ratio(\%Ref) = 100 \text{ x } 10^{Difference}$$

Classical intervals

Output from the Bioequivalence module includes the classical intervals for confidence levels equal to 80, 90, 95, and for the confidence level that the user gave on the Options tab if that value is different than 80, 90, or 95. To compute the intervals, first the following values are computed using the students-***t*** distribution, where 2**alpha*=(100 – *Confidence Level*)/100, and *Confidence Level* is specified in the user inter-face.

$$\begin{aligned} Lower &= (t_{(1-\alpha,df)})DiffSE \\ Upper &= Difference + (t_{(1-\alpha,df)})DiffSE \end{aligned}$$

These values are included in the output for the no-transform case. These values are then transformed if necessary to be on the arithmetic scale, and translated to percentages. For ln-transform or data already ln-transformed,

$$\begin{aligned} CI\_Lower &= 100\exp(Lower) \\ CI\_Upper &= 100\exp(Upper) \end{aligned}$$

For log10-transform or data already log10-transformed,

$$\begin{aligned} CI\_Lower &= 100 \text{ x } 10^{Lower} \\ CI\_Upper &= 100 \text{ x } 10^{Upper} \end{aligned}$$

For no transform,

$$CI\_Lower = 100\left(1 + \frac{Lower}{RefLSM}\right)$$

$$= 100\left(1 + \left(\frac{TestLSM - RefLSM}{RefLSM}\right) - (t_{(1-\alpha,df)})\frac{DiffSE}{RefLSM}\right)$$

$$= 100\left(\frac{TestLSM}{RefLSM} - (t_{(1-\alpha,df)})RatioSE\right)$$

where the approximation *RatioSE=DiffSE/RefLSM* is used. Similarly,

$$CI\_Upper = 100\left(1 + \frac{Upper}{RefLSM}\right)$$

Concluding whether bioequivalence is shown depends on the user-specified values for the level and for the percent of reference to detect. These options are set on the Options tab in the Bioequivalence object. To conclude whether bioequivalence has been achieved, the *CI_Lower* and *CI_Upper* for the user-specified value of the level are compared to the following lower and upper bounds. Note that the upper bound for log10 or ln-transforms or for data already transformed is adjusted so that the bounds are symmetric on a logarithmic scale.

LowerBound
= 100 – (*percentReferenceToDetect*)

UpperBound (ln-transforms)
= 100 exp(–ln(1 – *fractionToDetect*))
= 100 (1/(1 – *fractionToDetect*))

UpperBound (log10-transforms)
= 100*10^(–log10(1 – *fractionToDetect*))
= 100 (1/(1 – *fractionToDetect*))

UpperBound (no transform)
= 100+(*percentReferenceToDetect*)

If the interval (*CI_Lower*, *CI_Upper*) is contained within *LowerBound* and *UpperBound*, average bioequivalence has been shown. If the interval (*CI_Lower*, *CI_Upper*) is completely outside the interval (*LowerBound*, *UpperBound*), average bioinequivalence has been shown. Otherwise, the module has failed to show bioequivalence or bioinequivalence.

Two one-sided t-tests

For ln-transform or data already ln-transformed, the first t-test is a left-tail test of the hypotheses:

H_0: *trueDifference* < ln(1 – *fractionToDetect*) (*bioinequivLeftTest*)
H_1: *trueDifference* ≥ ln(1 – *fractionToDetect*) (*bioequivLeftTest*)

The test statistic for performing this test is:

t_1=((*TestLSM* – *RefLSM*) – ln(1 – *fractionToDetect*))/*DiffSE*

The p-value is determined using the t-distribution for this t-value and the degrees of freedom. If the p-value is <0.05, then the user can reject H_0 at the 5% level, i.e. less than a 5% chance of rejecting H_0 when it was actually true.

For log10-transform or data already log10-transformed, the first test is done similarly using log10 instead of ln.

For data with no transformation, the first test is (where Ratio here refers to the true ratio of the Test mean to the Reference mean):

H_0: *Ratio* < 1 – *fractionToDetect*
H_1: *Ratio* ≥ 1 – *fractionToDetect*

The test statistic for performing this test is:

t_1=[(*TestLSM*/*RefLSM*) – (1 – *fractionToDetect*)]/*RatioSE* where the approximation $RatioSE=DiffSE/RefLSM$ is used.

The second t-test is a right-tail test that is a symmetric test to the first. However for $\log_{10}$ or ln-transforms, the test will be symmetric on a logarithmic scale. For example, if the percent of reference to detect is 20%, then the left-tail test is Pr(<80%), but for ln-transformed data, the right-tail test is Prob(>125%), since ln(0.8) = –ln(1.25).

For ln-transform or data already ln-transformed, the second test is a right-tail test of the hypotheses:

H_0: $trueDifference > -\ln(1 - fractionToDetect)$ $(bioinequivRightTest)$
H_1: $trueDifference \leq -\ln(1 - fractionToDetect)$ $(bioequivRightTest)$

The test statistic for performing this test is:

$$t_2=((TestLSM - RefLSM)+\ln(1 - fractionToDetect))/DiffSE$$

For log10-transform or data already log10-transformed, the second test is done similarly using $\log_{10}$ instead of ln.

For data with no transformation, the second test is:

H_0: $Ratio > 1+fractionToDetect$
H_1: $Ratio \leq 1+fractionToDetect$

The test statistic for performing this test is:

$$t_2=((TestLSM/RefLSM) - (1+fractionToDetect))/RatioSE$$

where the approximation $RatioSE=DiffSE/RefLSM$ is used.

The output for the two one-sided t-tests includes the t_1 and t_2 values described above, the p-value for the first test described above, the p-value for the second test above, the maximum of these p-values, and total of these p-values. The two one-sided t-tests procedure is operationally equivalent to the classical interval approach. That is, if the classical (1 – 2 x alpha) x100% confidence interval for difference or ratio is within LowerBound and UpperBound, then both the H_0s given above for the two tests are also rejected at the alpha level by the two one-sided t-tests.

Power of the two one-sided t-tests procedure

Power_TOST in the output is the power of the two one-sided t-tests procedure described in the previous section. Refer to the previous section for the definitions of t_1 and t_2 for the transformation that was used and recall from previous sections that df is the degrees of freedom for the difference of the test and reference least squares means. Define the critical t-value as:

$$t_{crit} = t_{(1-\alpha),df}$$

where 2α=(100 – Confidence Level/100) and Confidence Level is specified in the user interface.

In general:

$PowerOfTest=1 - (probTypeIIError) = probRejectingH_0$, when H_1 is true

For the two one-sided t-tests procedure, H_1 is bioequivalence (concluded when both of the one-sided tests pass) and H_0 is nonequivalence. The power of the two one-sided t-tests procedure is (Phillips, K. F. (1990), or Diletti, E., Hauschke, D., and Steinijans, V. W. (1991)):

$$Power = Prob\{t_1 \geq t_{crit} \text{ and } t_2 \leq -t_{crit} \mid bioequivalence\}$$

This power is closely approximated by the difference of values from a non-central t-distribution (Owen, D. B. (1965)):

$$Power\_TOST = p(-t_{crit}, df, t_2) - p(t_{crit}, df, t_1)$$

This is the formula used in the Bioequivalence module (power is set to zero if this results in a negative), and the non-central t should be accurate to 1e–8. If it fails to achieve this accuracy, the non-central t-distribution is approximated by a shifted central t-distribution (again, power is set to zero if this results in a negative):

$$Power\_TOST = p(-t_{crit}, -t_2, df) - p(t_{crit}, -t_1, df)$$

Power of the two one-sided t-tests procedure is more likely to be of use with simulated data, such as when planning for sample size, rather than for drawing conclusions for observed data.

Anderson-Hauck test

See Anderson and Hauck (1983), or page 99 of Chow and Liu (2000). Briefly, the Anderson-Hauck test is based on the hypotheses:

H_{01}: $\mu_T - \mu_R < \theta_L$ vs H_{A1}: $\mu_T - \mu_R > \theta_L$

H_{02}: $\mu_T - \mu_R < \theta_U$ vs H_{A2}: $\mu_T - \mu_R > \theta_U$

where θ_L and θ_U are the natural logarithm of the Anderson-Hauck limits entered in the bioequivalence options tab. Rejection of both null hypotheses implies bioequivalence. The Anderson-Hauck test statistic is t_{AH} given by:

$$t_{AH} = \frac{\bar{Y}_T - \bar{Y}_R - (\theta_L + \theta_U)/2}{DiffSE}$$

where $DiffSE$ is the standard error of the difference in means. Under the null hypothesis, this test statistic has a noncentral ***t***-distribution.

Power for 80/20 Rule

Power_80_20 in the output is the power to detect a difference in least square means equal to 20% of the reference least squares mean. Percent of Reference to Detect on the Options tab should be the default value of 20%, and the desired result is that Power_80_20 is greater than 0.8 or 80%. (See pg. 142–143 of Chow and Liu (2000).) In general:

$PowerOfTest$=1 – ($probTypeIIError$) = $probRejectingH_0$, when H_1 is true

Let μ_T and μ_R be the true (not observed) values of TestLSM and RefLSM. For this type of power calculation, for the no-transform case, the power is the probability of rejecting: $H_0(\mu_T = \mu_R)$ given H_1:

$|\mu_T - \mu_R| = fractionToDetect(\mu_R)$

For ln-transform, and data already ln-transformed, this changes to:

$|\mu_T = -\mu_R| = -\ln(1 - fractionToDetect)$

and similarly for log10-transform and data already log10-transformed.

For the default $fractionToDetect$=0.2, the default α=0.05 (2*α=(100 – $Confidence\_Level$) /100), with no transform on the data and $\mu_R > 0$:

$Power = Pr$

(rejecting H_0 at the alpha level given the true difference in means = 0.2 x μ_R)

$$= Pr\left|\frac{TestLSM - RefLSM}{DiffSE}\right| > t_{(\alpha,df)}$$

given $|\mu_T - \mu_R| = 0.2(\mu_R)$

Let:

$$t_1 = t_{(1-\alpha,df)} - 0.2\left(\frac{RefLSM}{DiffSE}\right)$$

$$t_2 = -t_{(1-\alpha,df)} - 0.2\left(\frac{RefLSM}{DiffSE}\right)$$

Then:

$Power \approx 1 - [Pr(T > t_1) - Pr(T > t_2)]$

where T has a central *t* distribution with df=Diff_DF. Note that the second probability may be negligible.

For ln-transform or data already ln-transformed, this changes to:

$$t_1 = t_{(1-\alpha,df)} - \left(\frac{-\ln(0.8)}{DiffSE}\right)$$

$$t_2 = -t_{(1-\alpha,df)} - \left(\frac{-\ln(0.8)}{DiffSE}\right)$$

Tests for equal variances

For the default parallel and 2x2 crossover models, some tests in Bioequivalence (e.g., the two one-sided t-tests) rely on the assumption that the observations for the group receiving the test formulation and the group receiving the reference formulation come from distributions that have equal variances, in order for the test statistics to follow a t-distribution. There are two tests in Bioequivalence that verify whether the assumption of equal variances is valid:

The ***Levene test*** is done for a parallel design that uses the default model (formulation variable with intercept included). See Snedecor and Cochran (1989) for more information.

The ***Pitman-Morgan*** test is done for a 2-period, 2-sequence, 2-formulation crossover design that uses either Variance Components or all fixed effects model. See Chow and Liu (2nd ed. 2000 or 3rd ed. 2009) for more information. In addition to the required column mappings, Sequence and Period must also be mapped columns.

For replicated crossover designs, the default model in Bioequivalence already adjusts for unequal variances by using Satterthwaite Degrees of Freedom and by grouping on the formulation in the repeated model, so a test for equality of variances is not done.

The results of the Levene test and Pitman-Morgan test are given in the Average Bioequivalence output worksheet and at the end of the Core Output. Both tests verify the null hypothesis that the true variances for the two formulations are equal by using the sample data to compute an F-distributed test statistic. A p-value of less than 0.05 indicates a rejection of the null hypotheses (and acceptance that the variances are unequal) at the 5% level of significance.

If unequal variances are indicated by the Levene or Pitman-Morgan tests, the model can be adjusted to account for unequal variances by using Satterthwaite Degrees of Freedom on the General Options tab and using a ‘repeated’ term that groups on the formulation variable as follows.

For a parallel design:

- Map the formulation variable as **Formulation**.
- Use the default **Fixed Effects** model (the formulation variable).
- Use the default **Degrees of Freedom** setting of **Satterthwaite**.
- Map the **Dependent** variable, and map the **Subject** and **Period** variables as **Classification** variables.

  If the data does not contain a Period column, a column that contains all ones can be added as the Period variable.
- Set up the Repeated sub-tab of the Variance Structure tab as:

  Repeated Specification: **Period**
  Variance Blocking Variables (Subject): **Subject**
  Group: **Formulation**
  Type: **Variance Components**

For a 2x2 crossover design:

- Map the **Sequence**, **Subject**, **Period**, **Formulation**, and **Dependent** variables accordingly.
- Use the default model (**Fixed Effects** is Sequence+Formulation+Period, **Random** is Subject(Sequence)).
- Use the default **Degrees of Freedom** setting of **Satterthwaite**.
- Set up the Repeated sub-tab of the Variance Structure tab as:

  Repeated Specification: **Period**
  Variance Blocking Variables (Subject): **Subject**
  Group: **Formulation**
  Type: **Variance Components**

Population and individual bioequivalence

Population and individual bioequivalence are used to assess switchability and prescribability. Because individual bioequivalence relies on estimates of within-subject, within-formulation variation, replicated crossover designs are required. This algorithm was developed by Francis Hsuan. Designs that can be appropriately analyzed include, but are not limited to the following list, which shows the Sequence (Period) Design.

2 (4) TRTR/RTRT
4 (4) TRTR/RTRT/TRRT/RTTR
4 (2) TT/RR/TR/RT
4 (3) TRT/RTR/TRR/RTT
2 (5) TRRTT/RTTRR
3 (3) TRR/RTR/RRT
2 (3) RTR/TRT
6 (3) TRR/RTT/TRT/RTR/TTR/RRT
2 (4) TRRR/RTTT
6 (4) TTRR/RRTT/TRRT/RTTR/TRRR/RTTT

The algorithm works for balanced and unbalanced data with an equal number of periods in each sequence and one measurement per subject in each period.

Bioequivalence criterion
Computational details

Bioequivalence criterion

Population bioequivalence (PBE) criteria are:

$$\frac{E(Y_{jT}-Y_{j'R})^2 - E(Y_{jR}-Y_{j'R})^2}{\frac{1}{2}E(Y_{jR}-Y_{j'R})^2} < \theta_P, \text{ if } \sigma_R > \sigma_P$$

$$\frac{E(Y_{jT}-Y_{j'R})^2 - E(Y_{jR}-Y_{j'R})^2}{\frac{1}{2}\sigma_P^2} < \theta_P, \text{ if } \sigma_R \le \sigma_P$$

where σ_P defaults to 0.2 and θ_P=(ln(1 – PercentReference)+ε_P) /σ_P^2. The default value for PercentReference is 0.20. In the Bioequivalence object, σ_P is called the Total SD standard. σ_R^2 is computed by the program and is the total variance of the reference formulation, i.e., the sum of within- and between-subject variance. The criteria take the linearized form:

$$\eta_{P1} = E(\mu_T-\mu_R)^2 + \sigma_T^2 - (1+\theta_P)\sigma_R^2 < 0, \text{ if } \sigma_R > \sigma_P$$
$$\eta_{P2} = E(\mu_T-\mu_R)^2 + \sigma_T^2 - \sigma_R^2 - \theta_P\sigma_P^2 < 0, \text{ if } \sigma_R \le \sigma_P$$

Individual bioequivalence (IBE) criteria are:

$$\frac{E(Y_{jT}-Y_{jR})^2-E(Y_{jR}-Y_{j'R})^2}{\frac{1}{2}E(Y_{jR}-Y_{j'R})^2}<\theta_I,\text{ if }\sigma_{WR}>\sigma_I$$

$$\frac{E(Y_{jT}-Y_{jR})^2-E(Y_{jR}-Y_{j'R})^2}{\frac{1}{2}\sigma_I^{\,2}}<\theta_I,\text{ if }\sigma_{WR}\le\sigma_I$$

where σ_I defaults to 0.2 and θ_I=(ln(1 – PercentReference)+ε_I) /σ_I^2. The default value for Percent Reference is 0.20, and the default value for ε_I is 0.05. In the Bioequivalence object, σ_I is called the within-subject SD standard. σ_{WR} is computed by Phoenix, and its square, σ_{WR}^2, is the within-subject variance of the reference formulation.

The IBE criteria take the linearized form:

$$\eta_{I1} = E(\mu_T-\mu_R)^2+\sigma_D^{\;*}+\sigma_{WT}^{\;2}-(1+\theta_I)\sigma_{WR}^{\;2}<0,\text{ if }\sigma_{WR}>\sigma_I$$

$$\text{and}$$

$$\eta_{I2} = E(\mu_T-\mu_R)^2+\sigma_D^{\;*}+\sigma_{WT}^{\;2}-\sigma_{WR}^{\;2}-\theta_I\sigma_I^{\;2}<0,\text{ if}\sigma_{WR}\le\sigma_I$$

$$\text{where }\sigma_D^{\;*}\text{ will be defined below.}$$

For reference scaling, use η_{P1} or η_{I1}. For constant scaling, use η_{P2} or η_{I2}. For mixed scaling, use one of the following.

Population

If σ_R > σ_{Pv}, use η_{P1}
If $\sigma_R \le \sigma_P$, use η_{P2}

Individual

If σ_{WR} > σ_I, use η_{I1}
If $\sigma_{WR} \le \sigma_I$, use η_{I2}

If the upper bound on the appropriate η is less than zero, then the product is bioequivalent in the chosen sense. The interval is set in the Bioequivalence object's Options tab. The method of calculating that upper bound follows.

Computational details

Let:

Y_{ijkt} be the response of subject j in sequence i at time t with treatment k.

$\underset{\sim}{Y}_{ij}'$ be a vector of responses for subject j in sequence i. The components of Y are arranged in the ascending order of time.

$\underset{\sim}{Z}_{iT}$ .be the design vector of treatment T in sequence i.

$\underset{\sim}{Z}_{iR}$ be a design vector for treatment R in sequence i.

$n_i > 1$ be the number of subjects in sequence i.

Assume the mean responses of $\underset{\sim}{Y}_{ij}$, $\underset{\sim}{\mu}_i$, follow a linear model:

$$\underset{\sim}{\mu}_i = \underset{\sim}{Z}_{iT}\mu_T + \underset{\sim}{Z}_{iR}\mu_R$$

where μ_T, μ_R are the population mean responses of Y with treatments T and R respectively, and X_i are design/model matrices.

Let:

$$p_{iT} := sum(\underset{\sim}{Z}_{iT})$$

be the number of occasions T is assigned to the subjects in sequence i.

$$p_{iR} := sum(\underset{\sim}{Z}_{iR})$$

be the number of occasions R is assigned to the subjects in sequence i.

Then:

$$\begin{aligned}
\underset{\sim}{u}_{iT} &:= p_{iT}^{-1}\underset{\sim}{Z}_{iT} \\
\underset{\sim}{u}_{iR} &:= p_{iR}^{-1}\underset{\sim}{Z}_{iR} \\
\underset{\sim}{u}_{iI} &:= (\underset{\sim}{u}_{iT} - \underset{\sim}{u}_{iR}) \\
\text{and } \underset{\sim}{d}_i &:= s_T^{-1}\underset{\sim}{u}_{iT} - s_R^{-1}\underset{\sim}{u}_{iR}
\end{aligned}$$

Assume that the covariances:

$$(\underset{\sim}{Y}_{ijkt}, \underset{\sim}{Y}_{ijk't'})$$

follow the model:

$$\begin{aligned}
\Sigma_i = {} & \sigma_{WT}^2 diag(\underset{\sim}{Z}_{iT}) + \sigma_{WR}^2 diag(\underset{\sim}{Z}_{iR}) + \sigma_{TT}\underset{\sim}{Z}_{iT}\underset{\sim}{Z}'_{iT} \\
& + \sigma_{RR}\underset{\sim}{Z}_{iR}\underset{\sim}{Z}'_{iR} + \sigma_{TR}\{\underset{\sim}{Z}_{iT}\underset{\sim}{Z}'_{iR} + \underset{\sim}{Z}_{iR}\underset{\sim}{Z}'_{iT}\}
\end{aligned}$$

where the parameters:

$$\underset{\sim}{\sigma} = \{\sigma_{WT}^2, \sigma_{WR}^2, \sigma_{TT}, \sigma_{RR}, \sigma_{TR}\}$$

are defined as follows:

$$\begin{aligned}
\sigma_T^2 &= \sigma_{TT} + \sigma_{WT}^2 \text{ is } varY_{ijTt} \\
\sigma_R^2 &= \sigma_{RR} + \sigma_{WR}^2 \text{ is } varY_{ijRt}
\end{aligned}$$

intra-subject correlation coefficients are:

$$\rho_{TT} = (\sigma_{TT}/\sigma_T^{\;2}) \text{ is } corr(Y_T, Y_T{}'), \; 1 > \rho_{TT} > 0$$
$$\rho_{RR} = (\sigma_{RR}/\sigma_R^{\;2}) \text{ is } corr(Y_R, Y_R{}'), \; 1 > \rho_{RR} > 0$$
$$\rho_{TR} = (\sigma_{TR}/(\sigma_T\sigma_R)) \text{ is } corr(Y_T, Y_R), \; 1 > \rho_{TR} > -1$$

intra-subject covariances are:

$$\sigma_{TT} \; = \; \rho_{TT}\sigma_T^{\;2}$$
$$\sigma_{RR} = \rho_{RR}\sigma_R^{\;2}$$
$$\sigma_{TR} = \rho_{TR}\sigma_T\sigma_R$$

intra-subject variances are:

$$\sigma_{WT}^{\;2} \; = \; \sigma_T^{\;2} - \sigma_{TT} \text{ is } var(Y_T - Y_T{}')/2$$
$$\sigma_{WR}^{\;2} = \sigma_R^{\;2} - \sigma_{RR} \text{ is } var(Y_R - Y_R{}')/2$$

For PBE and IBE investigations, it is useful to define additional parameters:

$$\sigma_D^{\;*} \; = \; \sigma_{TT} + \sigma_{RR} - 2\sigma_{TR}$$
$$\sigma_{iT}^{\;*2} = (\sigma_T^{\;2} - (1 - p_{iT}^{\;-1})\sigma_{WT}^{\;2})$$
$$\sigma_{iR}^{\;*2} = (\sigma_R^{\;2} - (1 - p_{iR}^{\;-1})\sigma_{WR}^{\;2})$$
$$\sigma_{iI}^{\;*2} = (\sigma_D^{\;*} + p_{iT}^{\;-1}\sigma_{WT}^{\;2} + p_{iR}^{\;-1}\sigma_{WR}^{\;2})$$

Except for σ_D^*, all the quantities above are non-negative when they exist. It satisfies the equation:

$$\mathrm{var}(Y_{ijT} - Y_{ijR}) \; = \; \sigma_D^{\;*} + \sigma_{WT}^{\;2} + \sigma_{WR}^{\;2}$$

In general, this σ_D^* may be negative. This method for PBE/IBE is based on the multivariate model. This method is applicable to a variety of higher-order two-treatment crossover designs including ***TR/RT/TT/RR*** (the Balaam Design), ***TRT/RTR***, or ***TxRR/xRTT/RTxx/TRxx/xTRR/RxTT*** (Table 5.7 of Jones and Kenward, page 205).

Given the ***i***th sequence, let:

$$\hat{\Delta} = \sum_i \underset{\sim}{d}'_i \bar{Y}_{i\bullet}$$

$$(S_{iT} = \underset{\sim}{u}'_{iT} V_i \underset{\sim}{u}_{iT}),\ (S_{iR} = \underset{\sim}{u}'_{iR} V_i \underset{\sim}{u}_{iR}),\ (S_{iI} = \underset{\sim}{u}'_{iI} V_i \underset{\sim}{u}_{iI})$$

$$S_{iWT} = (1 - p_{iT}^{-1})^{-1} (\mathrm{tr}\{\mathrm{diag}(\underset{\sim}{u}_{iT}) V_i\} - \underset{\sim}{u}'_{iT} V_i \underset{\sim}{u}_{iT})$$

$$S_{iWR} = (1 - p_{iR}^{-1})^{-1} (\mathrm{tr}\{\mathrm{diag}(\underset{\sim}{u}_{iR}) V_i\} - \underset{\sim}{u}'_{iR} V_i \underset{\sim}{u}_{iR})$$

where:

$\bar{Y}_{i\bullet}$ is the sample mean of the ith sequence.

V_i is the within-sequence sample covariance matrix.

It can be shown that:

$$\hat{\Delta} \sim N(\mu_T - \mu_R, \sum_i n_i^{-1} \underset{\sim}{d}'_i \sum_i \underset{\sim}{d}_i)$$

$$S_{iT} \sim \sigma_{iT}^{*2} (n_i - 1)^{-1} \chi^2_{n_i - 1}$$

$$S_{iR} \sim \sigma_{iR}^{*2} (n_i - 1)^{-1} \chi^2_{n_i - 1}$$

$$S_{iI} \sim \sigma_{iI}^{*2} (n_i - 1)^{-1} \chi^2_{n_i - 1}$$

$$S_{iWT} \sim \sigma_{WT}^{2} (p_{iT} - 1)^{-1} (n_i - 1)^{-1} \chi^2_{(p_{iT} - 1)(n_i - 1)}$$

$$S_{iWR} \sim \sigma_{WR}^{2} (p_{iR} - 1)^{-1} (n_i - 1)^{-1} \chi^2_{(p_{iT} - 1)(n_i - 1)}$$

Furthermore, it can be shown that $\{S_{iT}, S_{iR}\}$ (for PBE) are statistically independent from $\{\Delta\}$ and $\{S_{iWT}, S_{iWR}\}$, and that the four statistics Δ, S_{iI}, S_{iWT}, S_{iWR} (for IBE) are statistically independent.

Let α_i be sets of normalized weights, chosen to yield the method of moments estimates of the η. Then define the estimators of the components of the linearized criterion by:

$$E_0 = \Delta^2$$

$$E_{P1} = \sum_i \alpha_{iT} S_{iT}$$

$$E_{P2} = \left(1 - \sum_i \alpha_{iT} p_{iT}^{-1}\right) \sum_i \alpha_{iWT} S_{iWT}$$

$$E_{P3} = \sum_i \alpha_{iR} S_{iR}$$

$$E_{P4} = \left(1 - \sum_i \alpha_{iR} p_{iR}^{-1}\right) \sum_i \alpha_{iWR} S_{iWR}$$

$$E_{I1} = \sum_i \alpha_{iI} S_{iI}$$

$$E_{I2} = \left(1 - \sum_i \alpha_{iI} p_{iT}^{-1}\right) \sum_i \alpha_{iWT} S_{iWT}$$

$$E_{I3a} = \left(1 + \theta_I + \sum_i \alpha_{iI} p_{iR}^{-1}\right) \sum_i \alpha_{iWR} S_{iWR}$$

$$E_{I3b} = \left(1 + \sum_i \alpha_{iI} p_{iR}^{-1}\right) \sum_i \alpha_{iWR} S_{iWR}$$

Using the above notation, one may define unbiased moment estimators for the PBE criteria:

$$\hat{\eta}_{P1} = \hat{\Delta}^2 + E_{P1} + E_{P2} - (1 + \theta_P)E_{P3} - (1 + \theta_P)$$

$$\hat{\eta}_{P2} = \hat{\Delta}^2 + E_{P1} + E_{P2} - E_{P3} - E_{P4} - \theta_P \sigma_P^2$$

and for the IBE criteria:

$$\eta_{I1} = \hat{\Delta}^2 + E_{I1} + E_{I2} - E_{I3a}$$

$$\left(\eta_{I2} = \hat{\Delta}^2 + E_{I1} + E_{I2} - E_{I3b} - \theta_I \sigma_I^2\right)$$

Construct a 95% upper bound for η based on the ***TRTR/RTRT*** design using Howe's approximation I and a modification proposed by Hyslop, Hsuan and Holder (2000). This can be generalized to compute the following sets of n_q, H, and U statistics:

$$H_0 = \left(\left|\hat{\Delta}\right| + t_{.95,n_0}(\hat{\mathrm{var}}(\hat{\Delta}))^{1/2}\right)^2$$

$$H_q = \left(n_q E_q / \chi^2_{.05,n_q}\right) \text{ for } q = P1, P2, I1, I2$$

$$H_q = n_q E_q / \chi^2_{.95,n_q} \text{ for } q = P3, P4, I3a, I3b$$

$$U_q = (H_q - E_q)^2 \text{ for all } q$$

where the degrees of freedom n_q are computed using Satterthwaite's approximation. Then, the 95% upper bound for each η is:

$$H_\eta = \hat{\eta} + \left(\sum U_q^*\right)^{1/2}$$

$$U_q^* = (1 + \theta_P)^2 U_q \text{ for } q = P3, P4$$

$$U_q^* = U_q \text{ for all other } q$$

If $H_q < 0$, that indicates bioequivalence; $H_q > 0$ fails to show bioequivalence.

References

Anderson and Hauck (1983). A new procedure for testing equivalence in comparative bioavailability and other clinical trials. *Commun Stat Theory Methods*, 12:2663–92.

Chow and Liu (2nd ed. 2000 or 3rd ed. 2009). *Design and Analysis of Bioavailability and Bioequivalence Studies*, Marcel Dekker, Inc.

Hauschke, Steinijans, Diletti, Schall, Luus, Elze and Blume (1994). Presentation of the intrasubject coefficient of variation for sample size planning in bioequivalence studies. *Int J Clin Pharm Ther* 32(7): 376–378.

Hyslop, Hsuan and Holder (2000). A small sample on interval approach to assess individual bioequivalence. *Statist Medicine* 19:2885–97.

Schuirmann (1987). A comparison of the two one-sided tests procedure and the power approach for assessing the equivalence of average bioavailability. *J Pharmacokinet Biopharm* 15:657–680.

Snedecor and Cochran (1989). *Statistical Methods*, 8th edition, Iowa State Press.

US FDA Guidance for Industry (March 2003). *Bioavailability and Bioequivalence Studies for Orally Administered Drug Products—General Considerations.*

US FDA Guidance for Industry (January 2001). *Statistical Approaches to Establishing Bioequivalence.*

Phillips, K. F. (1990). Power of the Two One-Sided Tests Procedure in Bioequivalence. *Journal of Pharmacokinetics and Biopharmaceutics* 18: 137–144.

Diletti, E., Hauschke, D., and Steinijans, V. W. (1991). Sample size determination for bioequivalence assessment by means of intervals. *Int. J. of Clinical Pharmacology, Therapy and Toxicology* 29: 1–8.

Owen, D. B. (1965). A Special case of a bivariate noncentral t-distribution. *Biometrika* 52: 437–446.

Bioequivalence model examples

Knowledge of how to do basic tasks using the Phoenix interface, such as creating a project and importing data, is assumed.

Analyzing average bioequivalence of 2x2 crossover study example
Analyzing average bioequivalence of a replicated crossover design example
Evaluating individual and population bioequivalence example

Analyzing average bioequivalence of 2x2 crossover study example

The objective of this study is to compare a newly developed tablet formulation to the capsule formulation that was being used in Phase II studies. Both had a label claim of 25 mg per dosing unit.

A 2x2 crossover design was chosen for this study. Twenty subjects were randomly assigned to one of two sequence groups. Within each sequence group, each subject took both formulations, with a washout period between. Drug concentrations in plasma were measured, and the AUClast (area under a curve computed to the last observation) was calculated.

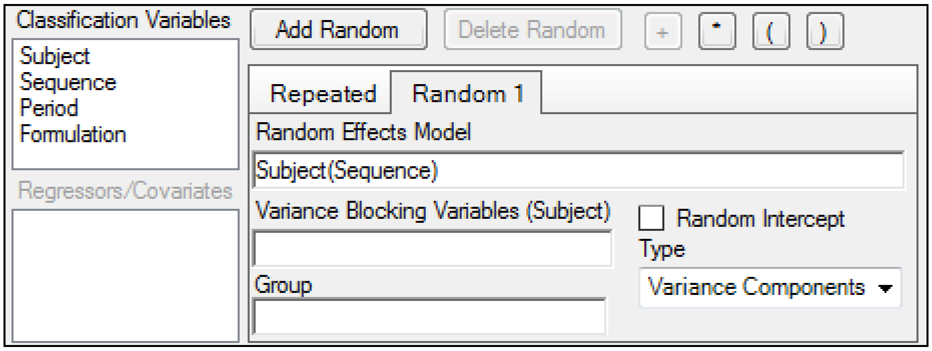
Data for this example are provided in …`\Examples\WinNonlin\Supporting files`. The dataset used is `Data 2x2.CSV`.

The completed project (`Bioequivalence_2x2.phxproj`) is available for reference in …`\Examples\WinNonlin`.

Set up the object

1. Create a new project named `Bioequivalence_2x2`.
2. Import the file …`\Examples\WinNonlin\Supporting files\Data 2x2.CSV`.
3. Right-click **Data 2x2** in the Data folder and then select **Send To > Computation Tools > Bioequivalence**.
4. In the Main Mappings panel:
   Leave **Sequence** mapped to the **Sequence** context.
   Leave **Subject** mapped to the **Subject** context.
   Leave **Period** mapped to the **Period** context.
   Leave **Formulation** mapped to the **Formulation** context.
   Map **AUClast** to the **Dependent** context.
5. In the Model tab below the Setup panel, make sure that:
   Crossover is selected as the **Type of study**,
   Average is selected as the **Type of Bioequivalence**, and
   Capsule is selected as the **Reference Formulation**.
6. Select the **Fixed Effects** tab, make sure that:
   `Sequence+Formulation+Period` appears in the **Model Specification** field.
   Ln(x) is selected in the **Dependent Variables Transformation** menu.
7. Select the **Variance Structure** tab.

   The random effects are already specified in the Variance Structure tab. If they are not, type `Subject(Sequence)` in the Random Effects Model field.



Classification Variables

Subject
Sequence
Period
Formulation

Random Effects Model

Subject(Sequence)

Variance Blocking Variables (Subject)

□ Random Intercept

Type

Variance Components

Execute and view the results

1. Click (**Execute** icon) to execute the object.

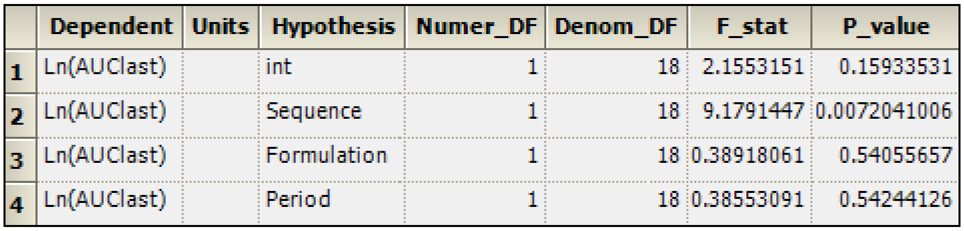
   The Average Bioequivalence worksheet indicates that the difference in ln(AUClast) between formulations is 0.046±0.073 (Difference±Diff_SE). The 90% confidence interval for the ratio is 92.216 (CI_90_Lower) to 118.780 (CI_90_Upper).

   Since the interval is completely contained between 80 and 125, one can conclude that the formulations are bioequivalent.

2. Select the **Partial Tests** worksheet and compare with the **Sequential Tests** worksheet.

   Because the data are balanced, the sequential and partial tests are identical. Note that, in the tests, Sequence is statistically significant, but no other factor is.

   Select any cell with a numerical value in the Bioequivalence worksheet output and look in the value display bar above to see the full precision of 15 decimal places.



	Dependent	Units	Hypothesis	Numer_DF	Denom_DF	F_stat	P_value
1	Ln(AUClast)		int	1	18	2.1553151	0.15933531
2	Ln(AUClast)		Sequence	1	18	9.1791447	0.0072041006
3	Ln(AUClast)		Formulation	1	18	0.38918061	0.54055657
4	Ln(AUClast)		Period	1	18	0.38553091	0.54244126

Figure 8-1. Sequential Tests worksheet for 2x2 crossover study

This concludes the Bioequivalence example of analyzing a 2x2 crossover study.

Analyzing average bioequivalence of a replicated crossover design example

The objective of this study is to compare a newly developed tablet formulation to a capsule formulation that was used in Phase II studies. Both formulations have the same label claim per dosing unit.

A RTRT/TRTR replicated crossover design was chosen for this study. Twenty subjects were randomly assigned to one of two sequence groups. Concentrations of the drug were measured in plasma, and the AUClast (area under the time-concentration curve, computed to the last observation) was calculated.

Note: The completed project (`Bioequivalence_replicated.phxproj`) is available for reference in …`\Examples\WinNonlin`.

Set up the object

1. Create a project called `Bioequivalence_replicated`.
2. Import the file …`\Examples\WinNonlin\Supporting files\Data 2x4.CSV`.

1. Right-click **Data 2x4** in the Data folder and select **Send To > Computation Tools > Bioequivalence**.
2. In the Mappings panel:
   Leave **Sequence** mapped to the **Sequence** context.
   Leave **Subject** mapped to the **Subject** context.
   Leave **Period** mapped to the **Period** context.
   Leave **Formulation** mapped to the **Formulation** context.
   Map **AUClast** to the **Dependent** context.
3. In the Model tab below the Setup panel, make sure that:
   Crossover is selected as the **Type of study**,
   Average is selected as the **Type of Bioequivalence**, and
   Capsule is selected as the **Reference Formulation**.
4. Select the **Fixed Effects** tab and make sure that:
   `Sequence+Formulation+Period` appears in the **Model Specification** field.
   Ln(x) is selected in the **Dependent Variables Transformation** menu.

Note: Phoenix has automatically selected a model specification and classification variables based on the model for replicated crossovers.

5. Select the **Variance Structure** tab.

   Notice that the default variance structure for a replicated crossover design is substantially different from and more complex than that for the 2x2 crossover design. As a result, the model fitting is more difficult as well.

   In the **Random 1** sub-tab, make sure that:
   `Formulation` appears in the Random Effects Model field.
   `Subject` appears in the Variance Blocking Variables field.
   Banded No-Diagonal Factor Analytic(f) is selected in the **Type** menu.
   2 is specified as the **Number of factors**.

   In the **Repeated** sub-tab, make sure that:
   `Period` appears in the Repeated Specification field.
   `Subject` appears in the Variance Blocking Variables field.
   `Formulation` appears in the Group field.
   Variance Components is selected in the **Type** menu.

Execute and view the results

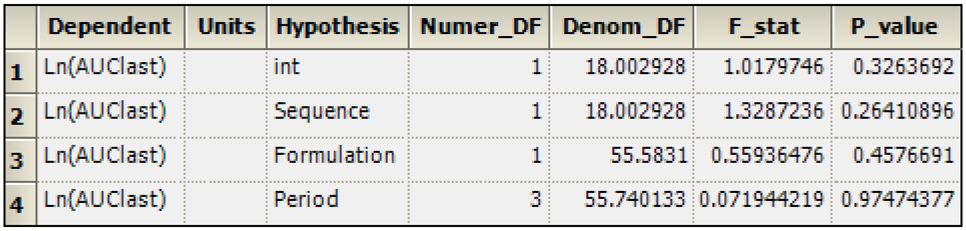
A user can expect that about 50% of datasets analyzed will produce a non-positive definite G matrix. This does not imply that the model-fitting is invalid, but only that a user must be careful not to over-interpret the variance estimates. The interval on the formulation difference will still have the expected statistical properties.

1. Execute the object.

The Average Bioequivalence worksheet indicates that the analysis just failed to show bioequivalence since the 90% confidence interval=91.612 (CI_90_Lower) and 125.772 (CI_90_Upper).

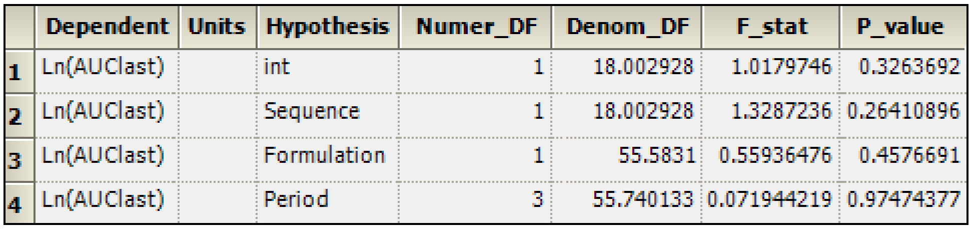
2. Select the **Partial Tests** worksheet and compare with the **Sequential Tests** worksheet.

   Because the data are balanced, the sequential and partial tests are identical.



	Dependent	Units	Hypothesis	Numer_DF	Denom_DF	F_stat	P_value
1	Ln(AUClast)		int	1	18.002928	1.0179746	0.3263692
2	Ln(AUClast)		Sequence	1	18.002928	1.3287236	0.26410896
3	Ln(AUClast)		Formulation	1	55.5831	0.55936476	0.4576691
4	Ln(AUClast)		Period	3	55.740133	0.071944219	0.97474377

Figure 8-2. Partial Tests worksheet for replicated crossover study



	Dependent	Units	Hypothesis	Numer_DF	Denom_DF	F_stat	P_value
1	Ln(AUClast)		int	1	18.002928	1.0179746	0.3263692
2	Ln(AUClast)		Sequence	1	18.002928	1.3287236	0.26410896
3	Ln(AUClast)		Formulation	1	55.5831	0.55936476	0.4576691
4	Ln(AUClast)		Period	3	55.740133	0.071944219	0.97474377

Figure 8-3. Sequential Tests worksheet for replicated crossover study

This concludes the Bioequivalence example of analyzing a replicated crossover study.

Evaluating individual and population bioequivalence example

Phoenix can handle a wide variety of model designs suitable for assessing individual and population bioequivalence, including:

TRTR/RTRT/TRRT/RTTR
TT/RR/TR/RT
TRT/RTR/TRR/RTT
TRRTT/RTTRR
TRR/RTR/RRT
RTR/TRT
TRR/RTT/TRT/RTR/TTR/RRT
TRRR/RTTT
TTRR/RRTT/TRRT/RTTR/TRRR/RTTT

where T=Test formulation and R=Reference formulation.

Note: Each sequence must contain the same number of periods. For each period, each subject must have one measurement.

A bioequivalence example, included as part of “Testing the Phoenix installation”, shows results for a RTR/TRT design. This example demonstrates an analysis of a TT/RR/TR/RT design.

Note: The completed project (`Bioequivalence_IndPop.phxproj`) is available for reference in …`\Examples\WinNonlin`.

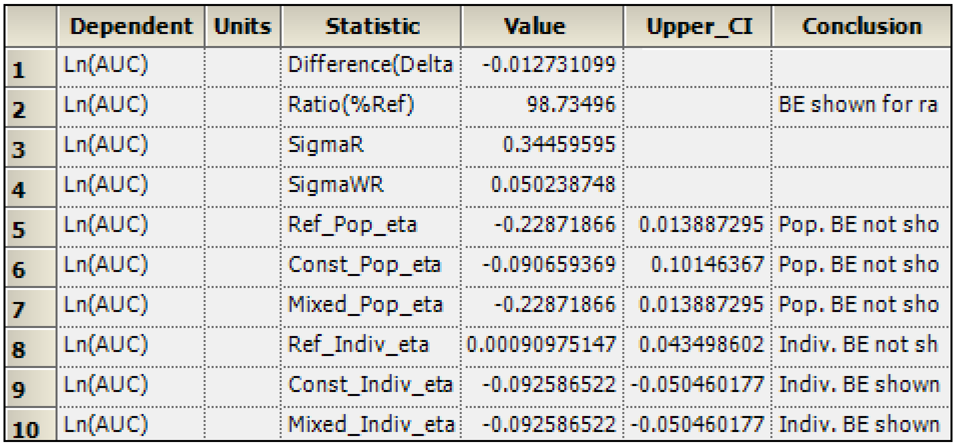
Set up the population/individual model

1. Create a project called `Bioequivalence_IndPop`.
2. Import the file …`\Examples\WinNonlin\Supporting files\TT RR RT TR.DAT`.

   Notice that the number of subjects is not the same in each sequence group. TT, RR, TR, and RT each have 4 subjects, whereas RT has 5.
3. Right-click **TT RR RT TR** in the Data folder and select **Send To > Computation Tools > Bioequivalence**.
4. In the Model tab below the Setup panel, select the **Population/Individual** option button in the **Type of Bioequivalence** area.
5. Map the data types to the following contexts:
   Leave **Sequence** mapped to the **Sequence** context.
   Leave **Subject** mapped to the **Subject** context.
   Leave **Period** mapped to the **Period** context.
   Leave **Formulation** mapped to the **Formulation** context.
   Map **AUC** to the **Dependent** context.
6. In the Model tab, make sure that:
   Crossover is selected in the **Type of study** area. Crossover studies are the only permitted type for Population/Individual bioequivalence analysis.
   Population/Individual is set as the **Type of Bioequivalence**.
   R is selected in the **Reference Value** menu.
7. Select the **Fixed Effects** tab and make sure that **Ln(x)** is set as the **Dependent Variables Transformation**. The values will be log-transformed before the analysis.
8. Select the **Options** tab and enter 95 as the **Confidence Level**.

Execute and view the Population/Individual model results

1. Execute the object.
2. Select the **Population Individual** worksheet in the Results list.



	Dependent	Units	Statistic	Value	Upper_CI	Conclusion
1	Ln(AUC)		Difference(Delta	-0.012731099		
2	Ln(AUC)		Ratio(%Ref)	98.73496		BE shown for ra
3	Ln(AUC)		SigmaR	0.34459595		
4	Ln(AUC)		SigmaWR	0.050238748		
5	Ln(AUC)		Ref_Pop_eta	-0.22871866	0.013887295	Pop. BE not sho
6	Ln(AUC)		Const_Pop_eta	-0.090659369	0.10146367	Pop. BE not sho
7	Ln(AUC)		Mixed_Pop_eta	-0.22871866	0.013887295	Pop. BE not sho
8	Ln(AUC)		Ref_Indiv_eta	0.00090975147	0.043498602	Indiv. BE not sh
9	Ln(AUC)		Const_Indiv_eta	-0.092586522	-0.050460177	Indiv. BE shown
10	Ln(AUC)		Mixed_Indiv_eta	-0.092586522	-0.050460177	Indiv. BE shown

Inspect the results for mixed scaling. For population bioequivalence, the upper limit is 0.014 > 0, and therefore population BE has not been shown. For individual bioequivalence, the upper limit is –0.05 < 0, and so individual BE has been shown.

Compare average bioequivalence

1. Right-click the **Bioequivalence** object in the Object Browser and select **Copy**
2. Right-click **Workflow** in the Object Browser and select **Paste**.
3. In the Model tab of the copied object, select **Average** as the **Type of Bioequivalence** and make sure that:
   Crossover is selected as the **Type of study**, and
   R is selected as the **Reference Formulation**.
4. Select the **Fixed Effects** tab and make sure that:
   `Sequence+Formulation+Period` appears in the **Model Specification** field.
   Ln(x) is selected in the **Dependent Variables Transformation** menu.
5. Select the **Variance Structure** tab.

   In the **Random 1** sub-tab, make sure that:
   `Formulation` appears in the Random Effects Model field.
   `Subject` appears in the Variance Blocking Variables field.
   Banded No-Diagonal Factor Analytic(f) is selected in the **Type** menu.
   2 is in the **Number of factors** field.

   Select the Variance Structure's **Repeated** tab and make sure that:
   `Period` appears in the **Repeated Specification** field.
   `Subject` appears in the **Variance Blocking Variables** field.
   `Formulation` appears in the **Group** field.

Execute and view the average bioequivalence results

1. Execute the object.

   Using the model for average bioequivalence on replicated crossover designs resulted in a 90% lower interval of 87.277% (CI_90_Lower) and a 99.715% upper interval (CI_90_Upper) for the ratio of average AUC. Therefore a user can also conclude average bioequivalence is achieved. This is not always the case. Data can pass individual BE and fail average BE, and data can also pass average BE and fail individual BE.

This concludes the Bioequivalence individual/population evaluation example.

Convolution

The Convolution object allows users to generate a predicted response to a known input (like a product dissolution profile) given a known response to an impulse input (e.g., IV bolus). The response to the impulse is called a unit impulse response (UIR).

The Convolution object requires two datasets: the input profile as either a rate or cumulative amount; and a UIR profile or function. Either profile may be entered as either a polyexponential function or an arbitrary set of points. A polyexponential function is defined by a set of up to nine pairs of coefficient (A) and exponent (alpha) values. An arbitrary profile will be broken down into either a series of step functions or linear splines. Typical usage is to specify an input as an arbitrary profile (e.g., cumulative drug dissolution) and the UIR as a polyexponential function.

Note: Only numeric values are valid for input data and any non-numeric values should be filtered out prior to execution. If non-numeric values are encountered, results will not be generated for that profile.

A separate convolution is computed for each profile, that is, for each unique combination of Sort variable values. The two datasets are joined on any sort variables that have identical names in both datasets. Phoenix uses the cross-product of any unmatched sort keys. If the input contains profiles for subjects “A” and “B”, and the UIR data contains parameters for subjects “A”, “B”, and “C”, then the output will contain profiles for “A”, “B”, “AC”, and “BC”.

Use one of the following to add the object to a Workflow:

Right-click menu for a Workflow object: **New > Computation Tools > Convolution**.
Main menu: **Insert > Computation Tools > Convolution**.
Right-click menu for a worksheet: **Send To > Computation Tools > Convolution**.

Note: To view the object in its own window, select it in the Object Browser and double-click it or press **ENTER**. All instructions for setting up and execution are the same whether the object is viewed in its own window or in Phoenix view.

The following topics are discussed:

Input Mappings panel
UIR panel
Options tab
Plots tab (See the “Plots tab” description in the NCA section.)
Results
Convolution methodology

Input Mappings panel

Use the Input Mappings panel to identify how input variables are used in the convolution process. A separate analysis is performed for each profile, or unique level of sort key(s). Context associations change depending on the function type selected in the Options tab. Required input is highlighted orange in the interface.

None: Data types mapped to this context are not included in any analysis or output.

Sort: Categorical variable(s) identifying individual data profiles, such as subject ID.

Time: Nominal or actual time collection points in a study.

Cumulative_Input: The integral of the drug input rate, or the amount of a drug delivered to a subject.

Input_Rate: Rate of drug input.

Carry Alongs: Variables that are not required for the current analysis, but are copied and included in the output dataset. Note that time-dependent data variables (those that change over the course of a profile) are not carried over to time-independent output (e.g., Final Parameters), only to time-dependent output (e.g., Summary).

Parameter: Data variable(s) to include in the output worksheets.

Value: A and Alpha parameter values.

Note: When dosing input involves very large numeric values, it is recommended that they be converted to a larger unit of measure (ng to mg, for example). Using a very large value can result in a small fraction Input result on deconvolution, and in turn can prevent convolution in the prediction stage from producing non-zero concentrations, which can lead to inaccurate Validation and Prediction results.

UIR panel

Use the UIR Mappings panel to identify how unit impulse response data are used in the Convolution object. The context associations for the UIR Mappings panel change depending on the UIR function option selected in the Options tab. Required input is highlighted orange in the interface.

None: Data types mapped to this context are not included in any analysis or output.

Sort: Categorical variable(s) identifying individual data profiles, such as subject ID. (If an external source is used for UIR data, only the UIR sorts that match the input source will be used.)

Carry Alongs: Variables that are not required for the current analysis, but are copied and included in the output dataset. Note that time-dependent data variables (those that change over the course of a profile) are not carried over to time-independent output (e.g., Final Parameters), only to time-dependent output (e.g., Summary).

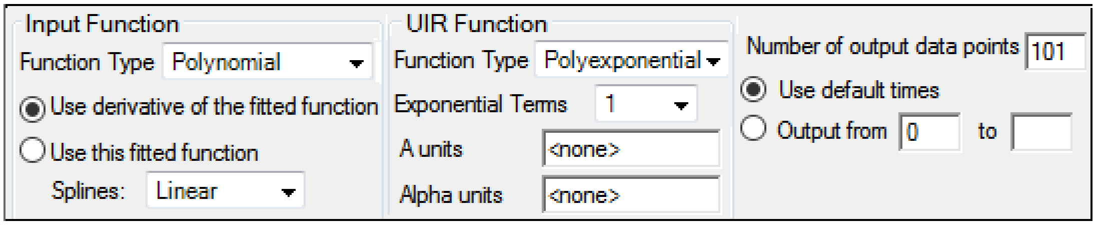
Parameter: Data variable(s) to include in the output worksheets.

Value: A and Alpha parameter values.

Note: When creating a copy of the Convolution object where an internal source is set for UIR and there is a sort variable, publish the internal UIR source first, then make a copy of the object. The object will then point to the external source for the UIR data. Otherwise, the UIR internal source will be reset in the copy of the object.

Options tab

Use the Options tab to select the appropriate function type and make settings for the Input function and Unit Impulse Response (UIR) function. To use an arbitrary profile select "polynomial" or select "polyexponential" to specify values for coefficients (A) and exponents (Alphas).



Input Function

Function Type: Polynomial

● Use derivative of the fitted function

○ Use this fitted function

Splines: Linear

UIR Function

Function Type: Polyexponential

Exponential Terms: 1

A units: <none>

Alpha units: <none>

Number of output data points: 101

● Use default times

○ Output from 0 to

The Options tab is used to define the functions *f(t)* and *g(t)* described in "Convolution methodology". *f(t)* and *g(t)* are interchangeable, so either could represent the input function or unit impulse response function.

Input Function area

In the **Function Type** menu, select whether to use a **Polynomial** or a **Polyexponential** function.

If **Polynomial** is selected, then the Convolution object finds the polynomial coefficients and users can specify the following options:

> Select the **Use derivative of the fitted function** option button to fit the data with the derivative of the piecewise polynomial. This method uses the first of two polynomials only. Selecting this option requires users to have cumulative input values in the Input dataset.
>
> Select the **Use this fitted function** option button to fit the data with the piecewise polynomial. Selecting this option requires users to have input rate values in the Input dataset.
>
> In the **Splines** menu, select the degree of polynomial splines, either **Linear** or **Constant**. Users are required to select linear splines for cumulative input data.

If **Polyexponential** is selected, users can select the following options:

> In the **Exponential Terms** menu, select the number of exponential terms to use (one to nine sets of coefficient (A) and exponent (Alpha) values per profile).
>
> In the **A units** field, type the units to use with the coefficient values.
>
> In the **Alpha units** field, type the units to use with the exponent values.

Note: If two polyexponentials are convolved, then none of the alpha values for the first polyexponential can be the same as any of the alpha values for the second polyexponential.

UIR Function area

In the **Function Type** menu, select whether to use a **Polynomial** or a **Polyexponential** function.

If **Polynomial** is selected, then the Convolution object finds the polynomial coefficients and users can specify the following options:

> The **Use this fitted function** option button is automatically selected for UIR polynomial functions.
>
> In the **Splines** menu, select the degree of polynomial splines, either **Linear** or **Constant**. Users are required to select linear splines for cumulative input data.

If **Polyexponential** is selected, then users can specify the following options:

> In the **Exponential Terms** menu, select the number of exponential terms to use (one to nine sets of coefficient (A) and exponent (Alpha) values per profile).
>
> In the **A units** field, type the units to use with the coefficient values.
>
> In the **Alpha units** field, type the units to use with the exponent values.

Note: If two polyexponentials are to be convolved, none of the alpha values for the first polyexponential can be the same as any of the alpha values for the second polyexponential.

Output and time options

- In the **Number of output data points** field, type the total number of output data points to use in convolution output. (If the Input and UIR functions are both **Polyexponentials**, then users cannot select to use the default times.)
- Select the **Use default times** option button Output will include time points from 0 (zero) to one of the following:
  - If the input and UIR functions are both **Polynomial**, then the last time value in one of the datasets plus the last time value in the other dataset.
  - If only one function is **Polynomial**, twice the last time value in the dataset.
- Select the **Output from** option button to spread the number of data points evenly across times between the first specified time point to the second specified time point.

  If the **Output from** option button is selected, type the first time point in the first field and the last time point in second field.

Results

The Convolution object generates worksheet, plot, and text output. The Convolution worksheet contains two columns: Time and the convolved data points for each level of the sort variables. The charts are a plot of the convolved data over time for each profile and a summary plot of all profiles. The text file contains user settings and input datasets.

Convolution units

Splines and polyexponential, with option “use this fitted function”

> If the Time and Input Rate columns have units, and there are units for A, then the convolved data have the following units. Note that the alpha units must equal 1/(Time units).
> *Time units*Input Rate units*A units*

Splines and polyexponential, with option “use derivative of this fitted function”

> If the Time and Cumulative Input columns have units, and there are units for A, then the convolved data have units:
> *Cumulative Input units*A units*

Splines and splines, with option “use this fitted function”

> The two Time columns must use the same units (if any). If all input columns have units, then the convolved data has units:
> *Time units*Input Rate units*Y units*

Splines and splines, with option “use derivative of this fitted function”

> The two Time columns must use the same units (if any). If all input columns have units, then the convolved data has units:
> *Cumulative Input units*Y units*

Polyexponential and polyexponential

> The alpha units must equal 1/(Time units). All alpha units must be the same; all A units must also be the same. If alpha and A units are provided, the convolved data has units:
> *Time units from alpha units*A units (1st poly.)*A units (2nd poly.)*

Convolution methodology

The Phoenix Convolution tool supports analytic evaluation of the convolution integral,

$$(f \cdot g)(t) = \int_0^t f(t-u)g(u)du$$

where $f(t)$ and $g(t)$ are constrained to be either polyexponentials or piecewise polynomials (splines), in any combination. Polyexponentials must take the form:

$$f(t) = \sum_{i=1}^{N} A_i \exp(-(\alpha_i \cdot t))$$

where N <= 9 and t >= 0. To specify a polyexponential, you must supply the polyexponential coefficients, i.e., the A and alpha.

Splines must be piecewise constant or linear splines, i.e., they must take the form:

$$f(t) = \sum_{i=1}^{N} b_{ij}(t-t_j)^{i-1}, t\varepsilon(t_j, t_j+1), j = 1, 2, \ldots, m$$

$$= 0, \text{otherwise}$$

where N is restricted to be 1 or 2.

For a spline function, you must supply a dataset to be fit with the splines. You can choose to convolve either this spline function, e.g., input rate data, or the derivative of this spline function, e.g., cumulative input data. For the first case, the Convolution tool will find the spline with the requested degree of polynomial splines, and will use these polynomial splines in the convolution. For the derivative of a spline, the Convolution tool will fit the data with the requested degree of polynomial splines, differentiate the splines to obtain polynomial splines of one degree lower, and use the derivatives in the convolution. For example, if cumulative input data is fit with linear splines, then the derivatives are piecewise constant, so the piecewise constant function is convolved with the user's other specified function.

Linear splines are the lines that connect the (x,y) points. For $t\ \varepsilon\ (t_{i,}\ t_{i+1})$,

$$y = y_i + m_i(t - t_i)$$

where m_i is the slope:

$$\frac{(y_{i+1} - y_i)}{(t_{i+1} - t_i)}$$

The derivative of a linear spline function is a piecewise constant function, with the constants being the slopes m_i. For input rate data, each piecewise constant spline will have the value that is the average of the y-values of the endpoints. This option is not available for cumulative input data since the derivative will be zero.

Crossover

Phoenix's Crossover object performs statistical analysis of data arising from subjects when variance homogeneity and normality may not necessarily apply. This tool performs nonparametric statistical tests on variables such as Tmax and provides two categories of results. First, it calculates intervals for each treatment median. It then calculates the median difference between treatments and the corresponding intervals. Finally, it estimates the relevance of direct, residual, and period effects.

Use one of the following to add the object to a Workflow:

Right-click menu for a Workflow object: **New > Computation Tools > Crossover**.
Main menu: **Insert > Computation Tools > Crossover**.
Right-click menu for a worksheet: **Send To > Computation Tools > Crossover**.

Note: To view the object in its own window, select it in the Object Browser and double-click it or press **ENTER**. All instructions for setting up and execution are the same whether the object is viewed in its own window or in Phoenix view.

Main Mappings panel
Options tab
Results
Crossover methodology
Data and assumptions
References
Crossover design example

For a brief description of datasets used with the Crossover object, see "Data and assumptions".

Main Mappings panel

Use the Main Mappings panel to identify how input variables are used in a Crossover object. The mapping options in the Main Mappings panel change depending on whether the dataset used with the object contains treatment data in a stacked or separate layout. Required input is highlighted orange in the interface.

None: Data types mapped to this context are not included in any analysis or output.
Sort: Categorical variable(s) identifying individual data profiles, such as subject ID and treatment in a crossover study. A separate analysis is performed for each unique combination of sort variable values.
Subject: The subjects in a dataset.
Sequence: The order of drug formulation administration.

Stacked Treatment Data

Response: The measured response to a drug formulation.
Treatment: The treatment drug formulations used in a study, presented in a stacked format.

Separated Treatment Data

Test Treatment: The treatment drug formulation being tested in a study.
Reference Treatment: The treatment drug formulation used as a reference in a study.

Options tab

- In the **Treatment Data Layout** menu, select the method used to store treatment data in a dataset.
  Stacked: The drug formulations are arranged in the same column in a dataset.

Separate: The drug formulations are separated into two columns in a dataset, Test and Reference.

- In the **Reference Treatment** menu, select the treatment to serve as the reference. Available for stacked data only.

Results

The Crossover object creates two worksheets and one text file in the Results tab. The worksheets are called Confidence Intervals and Effects. The text file is called Settings.

The Confidence Intervals worksheet lists the intervals at 80%, 90%, and 95% for the treatment medians in the crossover data and the treatment difference median.

The Effects worksheet lists the test statistic and p-value associated with each of four tests. It includes tests for an effect of sequence, treatment and period, as well as treatment and residual simultaneously.

The Settings text file lists the user-specified settings in the Crossover object.

Units associated with the Response variable in the input dataset units are carried through to the Crossover object output. The output worksheets display units in the median, lower, and upper value parameters.

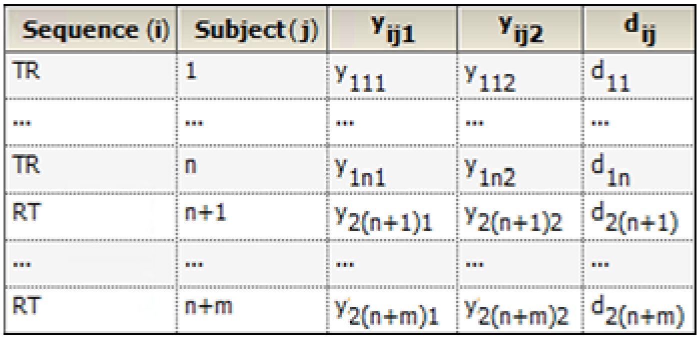
Crossover methodology

The two-period crossover design is common for clinical trials in which each subject serves as his or her own control. In many situations the assumptions of variance homogeneity and normality, relied upon in parametric analyses, may not be justified due to small sample size, among other reasons. The nonparametric methods proposed by Koch (1972) (The use of nonparametric methods in the statistical analysis of the two-period change-over design. *Biometrics* 577–83.) can be used to analyze such data.

Data and assumptions

Consider a trial testing the effects of two treatments, Test (T) and Reference (R). Suppose that n subjects are randomly assigned to the first sequence, TR, in which subjects are given treatment T first and then treatment R following an adequate washout period. Similarly, m subjects are assigned to the RT sequence. Y represents the outcome of the trial (for example Cmax or Tmax) and y_{ijk} is the value observed on sequence i $(i=1,2)$, subject j $(j = 1,2,\ldots,n, n+1,\ldots n+m)$ and period k $(k=1,2)$. Treatment is implied by sequence i and period k, for example i=1 and k=1 is treatment T; i=1 and k=2 is treatment R. The data are listed in columns 1 through 4 of the following table, and column 5 gives the within-subject difference in Y between the two periods, where:

$$d_{ij} = y_{ij1} - y_{ij2}$$



Sequence (i)	Subject(j)	y_{ij1}	y_{ij2}	d_{ij}
TR	1	y_{111}	y_{112}	d_{11}
...	...	...	...	...
TR	n	y_{1n1}	y_{1n2}	d_{1n}
RT	n+1	$y_{2(n+1)1}$	$y_{2(n+1)2}$	$d_{2(n+1)}$
...	...	...	...	...
RT	n+m	$y_{2(n+m)1}$	$y_{2(n+m)2}$	$d_{2(n+m)}$

Then the $n*m$ crossover differences $(d_{1i} - d_{2j})/2$ are computed, along with the median of these $n*m$ points. This median is the Hodges-Lehmann estimate of the difference between the median for T and the median for R.

Crossover Design supports two data layouts: stacked in same column or separate columns. For “stacked” data, all measurements appear in a single column, with one or more additional columns flagging which data belong to which treatment. The data for one treatment must be listed first, then all the data for the other. The alternative is to place data for each treatment in a separate column.

Descriptive analysis

The median response and its interval are calculated for ***Y*** of each treatment and the crossover difference between treatments, C. Let $X_{(1)}, X_{(2)},\ldots, X_{(N)}$ be the order statistic of samples $X_1,\ X_2,\ldots,\ X_N$. The median X_m is defined as:

$$X_m = \frac{\left[X\left(\frac{N}{2}\right) + X\left(\frac{N}{2}+1\right)\right]}{2}\text{, if } N \text{ is even}$$

$$= X\left[\frac{(N+1)}{2}\right]\text{, if } N \text{ is odd}$$

The 100% confidence interval (CI) of Xm is defined as follows.

- For N <= 20, the exact probability value is obtained from a Mann-Whitney U-distribution.
- For N > 20, normal approximation is used:

$$X_l = X_{(L)}\text{, where } L = \left(\text{int}\left(\frac{N}{2} - z_{\frac{1+p}{2}}\frac{\sqrt{N}}{2}\right) + 1\right)$$

$$X_u = X_{(U)}\text{, where } U = N - \text{int}\left(\frac{N}{2} - z_{\frac{1+p}{2}}\frac{\sqrt{N}}{2}\right)$$

where int(X) returns the largest integer less than or equal to X.

Hypothesis testing

Four hypotheses are of interest:

- no sequence effect (or drug residual effect);
- no treatment effect given no sequence effect;
- no period effect given no sequence effect; and
- no treatment and no sequence effect.

Hypothesis 1 above can be tested using the Wilcoxon statistic on the sum S. If $R(S_l)$ is the rank of S_{ij} in the whole sample, $l = 1,\ldots, n+m$. The test statistic is:

$$T = min\left(\sum_{l=1}^{n} R_l, \sum_{l=n+1}^{n+m} R_l\right)$$

The p-value is evaluated by using normal approximation (Conover, 1980):

$$[T - n(n+m+1)/2]/(\sqrt{nm(n+m+1)/12}) \sim N(0,1)$$

Similarly, hypothesis 2 can be tested using the Wilcoxon statistic on the difference D; hypothesis 3 can be tested using the Wilcoxon statistic on the crossover difference C. The statistics are in the form described above.

Hypothesis 4 can be tested using the bivariate Wilcoxon statistic on (Y_{ij1}, Y_{ij2}). For each period k, let R_{ijk} equal:

$$rank\{y_{ijk}:y_{11k},\ldots,y_{1nk},y_{2(n+1)k},\ldots,y_{2(n+m)k}\}$$

The average rank for each sequence is:

$$\bar{R}_{ik} = \sum_{j=1}^{n_i} R_{ijk}/n_i$$

where j=1,…, n_i, n_i=n for i=1, and n_i=m for i=2

Thus, the statistic to be used for testing hypothesis 4 is:

$$L = (n+m-1)[nU_1^T S^{-1} U_1 + mU_2^T S^{-1} U_2]$$

where U_i is a 2x1 vector:

$$[\bar{R}_{i1} - M, \bar{R}_{i2} - M]^T, M = (n+m+1)/2$$

S is the 2x2 covariance matrix:

$$S = \Sigma_i \Sigma_j \begin{bmatrix} (R_{ij1} - M)^2 & (R_{ij1} - M)(R_{ij2} - M) \\ (R_{ij1} - M)(R_{ij2} - M) & (R_{ij2} - M)^2 \end{bmatrix}$$

and $L \sim X^2$, so the p-value can be evaluated.

References

Conover (1980). *Practical Nonparametric Statistics* 2nd ed. John Wiley & Sons, New York.

Dixon and Massey (1969). *Introduction to Statistical Analysis*, 3rd ed. McGraw-Hill Book Company, New York.

Koch (1972). The use of non-parametric methods in the statistical analysis of the two-period change-over design. *Biometrics* 577–84.

Kutner (1974). Hypothesis testing in linear models (Eisenhart Model 1). *The American Statistician*, 28(3):98–100.

Searle (1971). *Linear Models*. John Wiley & Sons, New York.

Steel and Torrie (1980). *Principles and Procedures of Statistics; A Biometrical Approach*, 2nd ed. McGraw-Hill Book Company, New York.

Winer (1971). *Statistical Principles in Experimental Design*, 2nd ed. McGraw-Hill Book Company, New York, et al.

Crossover design example

Crossover design supports two data formats: data for both treatments stacked in one column, or each treatment placed in a separate column. An example of each follows.

Knowledge of how to do basic tasks using the Phoenix interface, such as creating a project and importing data, is assumed.

The completed project (`Crossover.phxproj`) is available for reference in …`\Examples\WinNonlin`.

Handle data stacked in one column

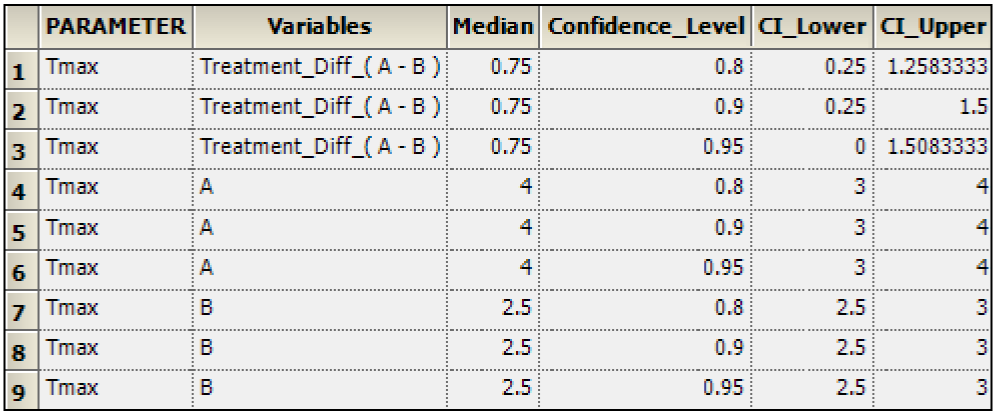
For this type of data, all the data for one treatment must be displayed in the first rows, followed by all the data for the other treatment.

1. Create a project called `Crossover`.
2. Import the file …`\Examples\WinNonlin\Supporting files\stacked.CSV`.
3. Right-click **stacked** in the Data folder and select **Send To > Computation Tools > Crossover**.
4. In the Main Mappings panel:
   Leave **TREATMENT** mapped to the **Treatment** context.
   Leave **SUBJECT** mapped to the **Subject** context.
   Map **PARAMETER** to the **Sort** context.
   Map **ESTIMATE** to the **Response** context.

Leave **PERIOD** mapped to **None**.
Leave **SEQUENCE** mapped to the **Sequence** context.

5. Click ▶ (**Execute** icon) to execute the object.

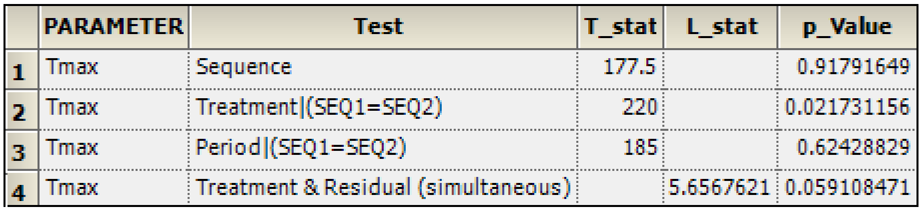
   The Crossover object computes intervals for treatment medians and median difference between treatments, the results of which are displayed in the Confidence Intervals worksheet.



	PARAMETER	Variables	Median	Confidence_Level	CI_Lower	CI_Upper
1	Tmax	Treatment_Diff_(A - B)	0.75	0.8	0.25	1.2583333
2	Tmax	Treatment_Diff_(A - B)	0.75	0.9	0.25	1.5
3	Tmax	Treatment_Diff_(A - B)	0.75	0.95	0	1.5083333
4	Tmax	A	4	0.8	3	4
5	Tmax	A	4	0.9	3	4
6	Tmax	A	4	0.95	3	4
7	Tmax	B	2.5	0.8	2.5	3
8	Tmax	B	2.5	0.9	2.5	3
9	Tmax	B	2.5	0.95	2.5	3

6. Select **Effects** in the Results list.

The Crossover object also estimates the relevance of direct, residual, and period effects as well as treatment and residual effects. These results are displayed in the Effects worksheet.



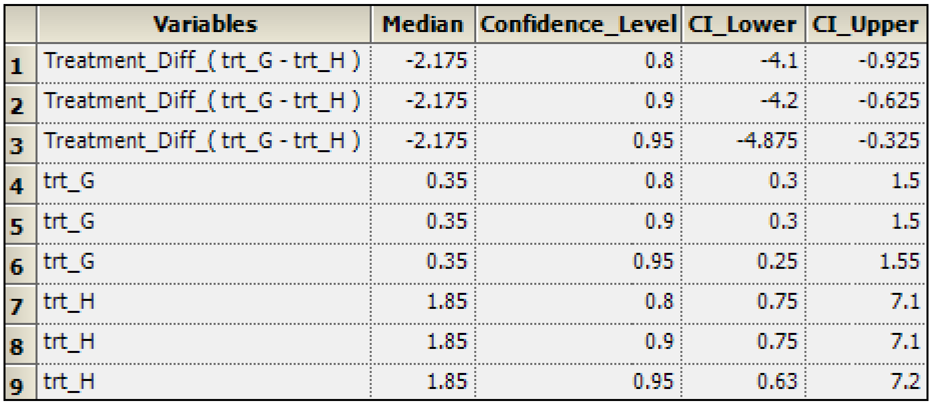
	PARAMETER	Test	T_stat	L_stat	p_Value
1	Tmax	Sequence	177.5		0.91791649
2	Tmax	Treatment|(SEQ1=SEQ2)	220		0.021731156
3	Tmax	Period|(SEQ1=SEQ2)	185		0.62428829
4	Tmax	Treatment & Residual (simultaneous)		5.6567621	0.059108471

Handle data in separate columns

1. Import the file ...`\Examples\WinNonlin\Supporting files\separate.CSV`.
2. Right-click **separate** in the Data folder and select **Send To > Computation Tools > Crossover**.

   The treatment data layout must be specified before the data can be mapped to the contexts for the Crossover 1 object.
3. In the Options tab below the Setup panel, select **Separate** in the **Treatment Data Layout** menu.
4. In the Main Mappings panel:
   Leave **Sequence** mapped to the **Sequence** context.
   Leave **Subject** mapped to the **Subject** context.
   Map **trt_G** to the **Test Treatment** context.
   Map **trt_H** to the **Reference Treatment** context.
5. Execute the object.

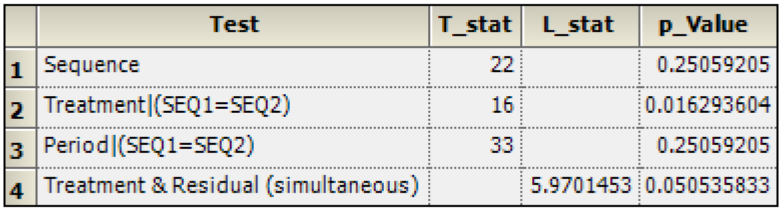
   The Confidence Intervals results worksheet contains treatment medians, median differences between treatments, and intervals for those estimates.



	Variables	Median	Confidence_Level	CI_Lower	CI_Upper
1	Treatment_Diff_(trt_G - trt_H)	-2.175	0.8	-4.1	-0.925
2	Treatment_Diff_(trt_G - trt_H)	-2.175	0.9	-4.2	-0.625
3	Treatment_Diff_(trt_G - trt_H)	-2.175	0.95	-4.875	-0.325
4	trt_G	0.35	0.8	0.3	1.5
5	trt_G	0.35	0.9	0.3	1.5
6	trt_G	0.35	0.95	0.25	1.55
7	trt_H	1.85	0.8	0.75	7.1
8	trt_H	1.85	0.9	0.75	7.1
9	trt_H	1.85	0.95	0.63	7.2

6. Select **Effects** in the Results list.

   The Effects worksheet provides statistics for direct, residual, and period effects, as well as the effect of treatment and residual simultaneously.



	Test	T_stat	L_stat	p_Value
1	Sequence	22		0.25059205
2	Treatment|(SEQ1=SEQ2)	16		0.016293604
3	Period|(SEQ1=SEQ2)	33		0.25059205
4	Treatment & Residual (simultaneous)		5.9701453	0.050535833

This concludes the Crossover example.

Deconvolution

Deconvolution is used to evaluate in vivo drug release and delivery when data from a known drug input are available. Phoenix's Deconvolution object can estimate the cumulative amount and fraction absorbed over time for individual subjects, using PK profile data and dosing data per profile.

Use one of the following to add the object to a Workflow:

Right-click menu for a Workflow object: **New > Computation Tools > Deconvolution**.
Main menu: **Insert > Computation Tools > Deconvolution**.
Right-click menu for a worksheet: **Send To > Computation Tools > Deconvolution**.

Note: To view the object in its own window, select it in the Object Browser and double-click it or press **ENTER**. All instructions for setting up and execution are the same whether the object is viewed in its own window or in Phoenix view.

User interface description
Results
Deconvolution methodology
Deconvolution example

User interface description

Main Mappings panel
Exp Terms panel
Dose panel
Observed Times panel
Options tab
Plots tab (See the "Plots tab" description in the NCA section.)

Main Mappings panel

Use the Main Mappings panel to identify how input variables are used in the Deconvolution object. Deconvolution requires a dataset containing time and concentration data, and sort variables to identify individual profiles. Required input is highlighted orange in the interface.

None: Data types mapped to this context are not included in any analysis or output.
Sort: Categorical variable(s) identifying individual data profiles, such as subject ID in a deconvolution analysis. A separate analysis is performed for each unique combination of sort variables.
Time: The relative or nominal dosing times used in a study.
Concentration: The measured amount of a drug in blood plasma.

Exp Terms panel

The Deconvolution object assumes a unit impulse response function (UIR) of the form:

$$c_\delta(t) = \sum_{j=1}^{N} A_j e^{-\alpha_j t}$$

where N is the number of exponential terms.

Use the Exponential Terms panel to enter values for the A and alpha parameters. For oral administration, the user should enter values such that:

$$\sum_{j=1}^{N} A_j = 0$$

Required input is highlighted orange in the interface.

None: Data types mapped to this context are not included in any analysis or output.
Sort: Categorical variable(s) identifying individual data profiles, such as subject ID.
Parameter: Data variable(s) to include in the output worksheets.
Value: A (coefficients) and Alpha (exponential) parameter values.

A and alpha parameters are listed sequentially based on the number of exponential terms selected. If only one exponential term is selected, each profile has an A1 and an Alpha1 parameter. If two exponential terms are selected, each profile has an A1 and an A2 parameter, and an Alpha1 and an Alpha2 parameter.

Rules for using an external exponential terms worksheet:

- The sort variables must match the sort variables used in the main input dataset.
- The worksheet's parameter column must match the number of exponential terms selected in the Exponential Terms menu. For example, if three exponential terms are selected, then each profile in the exponential terms worksheet must have three A parameters and three alpha parameters.
- The units used for the A and alpha parameters must match the units used for the concentration and dosing data. For example, If the concentration data has units ng/mL and the dosing data has units mg, then the A units must be ng/mL/mg, or the input data or dosing data should be converted (using Data Wizard Properties) to have consistent units. If the time units are hr, then the alpha units are 1/hr.
- If the UIR parameters are unknown, but a dataset is available that represents the impulse response, the parameters can be estimated by fitting the data in Phoenix with PK model 1, 8, or 18 (for N=1, 2, or 3, respectively). Since the UIR is the response to one dose unit, for model 1 (one-compartment), the inverse of the model parameter V is used for the UIR parameter, i.e., A1=1/V. For model 8 (two-compartment), the model parameters A and B should be divided by the stripping dose to obtain A1 and A2 for the UIR, and similarly for model 18 (three-compartment). However, if the dose units are different than the concentration mass units, the 'A' parameters must be adjusted so that the units are in concentration units divided by dose units. For the example in the prior bullet, if A=1/V from model 1 has units 1/mL, then 'A' must be converted to ng/mL/mg before it is used for the UIR in the Deconvolution object, A=10^6/V.

Dose panel

Supplying dosing information using the Dose panel is optional. Without it, the Deconvolution object runs correctly by assuming a dose at time zero time-concentration data with the fraction absorbed approaching a final value of one. The dose time is assumed to be zero for all profiles.

Note: The sort variables in the dosing data worksheet must match the sort variables used in the main input dataset.

Required input is highlighted orange in the interface.

None: Data types mapped to this context are not included in any analysis or output.
Sort: Categorical variable(s) identifying individual data profiles, such as subject ID.
Dose: The amount of drug administered.

Observed Times panel

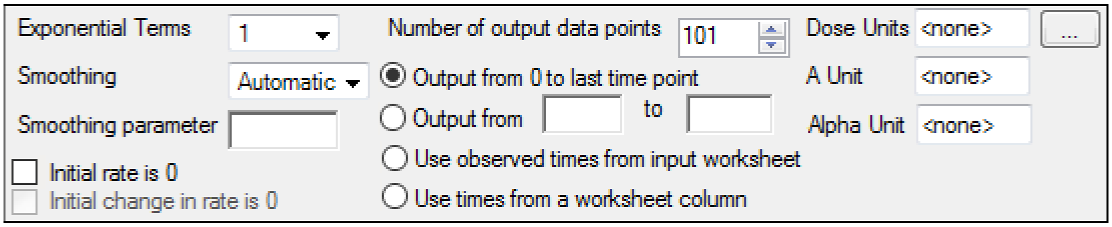
If the **Use times from a worksheet column** option button is selected in the Options tab, then the Observed Times panel is displayed in the Setup list. Required input is highlighted orange in the interface.

None: Data types mapped to this context are not included in any analysis or output.
Time: Time values.
Sort: Sort variables.

Note: The observed times worksheet cannot contain more than 1001 time points.

Options tab

The Options tab allows users to specify settings for exponential terms, parameters, and output time points.



Exponential Terms 1
Smoothing Automatic
Smoothing parameter
□ Initial rate is 0
□ Initial change in rate is 0
Number of output data points 101
● Output from 0 to last time point
○ Output from to
○ Use observed times from input worksheet
○ Use times from a worksheet column
Dose Units <none> ...
A Unit <none>
Alpha Unit <none>

- In the **Exponential Terms** menu, select the number of exponential terms in the unit impulse response to use per profile (up to nine exponential terms can be added).
- In the **Smoothing** menu, select the method Phoenix uses to determine the dispersion parameter.

  Automatic tells Phoenix to find the optimal value for the dispersion parameter delta.

  None disables smoothing that comes from the dispersion function. If selected, the input function is the piecewise linear precursor function.

  User allows users to type the value of the dispersion parameter delta as the smoothing parameter. The delta value must be greater than zero. Increasing the delta value increases the amount of smoothing.
- In the **Smoothing parameter** field, enter the value for the smoothing parameter.
  This option is only available if **User** is selected in the **Smoothing** menu.
- Select the **Initial rate is 0** checkbox to set the estimated input rate to zero at the initial time or lag time.
- Select the **Initial Change in Rate is 0** checkbox to set the derivative of the estimated input rate to zero at the initial time or lag time.
  This option is only available if the **Initial rate is 0** checkbox is selected and is always disabled when **Smoothing** is set to **None** (due to instabilities that occur in this case).
- In the **Dose Units** field, type the dosing units to use with the dataset.
  - Click the **Units Builder […]** button to use the *Units Builder* dialog to add dosing units.
  - See “Using the Units Builder” for more details on this tool.

- In the **Number of output data points** box, type or select the number of output data points to use per profile.
  The maximum number of time points allowed is 1001.
- Select the **Output from 0 to last time point** option button to have Phoenix generate output at even intervals from time zero to the final input time point for each profile.
- Select the **Output from _ to _** option button to have Phoenix generate output at even intervals between user-specified time points for each profile.
  - Type the start time point in the first field and type the end time point in the last field.
- Select the **Use observed times from input worksheet** option button to have Phoenix generate output at each time point in the input dataset for each profile.
- Select the **Use times from a worksheet column** option button to use a separate worksheet to provide ***in vivo*** time values.
  - Selecting this option creates an extra panel called **Observed Times** in the Setup tab list.
  - Users must map a worksheet containing time values used to generate output data points to the Observed Times panel.
  - The observed times worksheet cannot contain more than 1001 time points.

Results

The Deconvolution object generates worksheets, plots, and a text file.

Worksheet

Fitted Values: Predicted data for each profile.
Parameters: The smoothing parameter $delta$ and absorption lag time for each profile.
Values: Time, input rate, cumulative amount (Cumul_Amt, using the dose units) and fraction input (Cumul_Amt/test dose or, if no test doses are given, then fraction input approaches one) for each profile.

Plot

Cumulative Input: Cumulative drug input vs. time.
Fitted Curves: Observed time-concentration data vs. the predicted curve.
Input Rates: Rate of drug input vs. time.

Text File

Settings: Input settings for smoothing, number of output points and start and end times for output.

Users can double-click any plot in the Results tab to edit it. (See the menu options discussion in the Plots chapter of the Data Tools and Plots Guide for plot editing options.)

Deconvolution methodology

Deconvolution is used to evaluate in vivo drug release and delivery when data from a known drug input are available. Depending upon the type of reference input information available, the drug transport evaluated will be either a simple in vivo drug release such as gastro-intestinal release, or a composite form, typically consisting of an in vivo release followed by a drug delivery to the general systemic circulation.

One common deconvolution application is the evaluation of drug release and drug absorption from orally administered drug formulations. In this case, the bioavailability of the drug is evaluated if the reference formulation uses vascular drug input. Similarly, gastro-intestinal release is evaluated if the reference formulation is delivered orally. However, the Deconvolution object can also be used for other types of delivery, including transdermal or implant drug release and delivery.

Deconvolution provides automatic calculation of the drug input. It also allows the user to direct the analysis to investigate issues of special interest through different parameter settings and program input.

Linearity assumptions
Deconvolution through convolution methodology
References

Linearity assumptions

The methodology is based on linear system analysis with linearity defined in the general sense of the linear superposition principle. It is well recognized that classical linear compartmental kinetic models exhibit superposition linearity due to their origin in linear differential equations. However, all kinetic models defined in terms of linear combinations of linear mathematical operators, such as differentiation, integration, convolution, and deconvolution, constitute linear systems adhering to the superposition principle. The scope and generality of the linear system approach ranges far beyond linear compartmental models.

To fully appreciate the power and generality of the linear system approach, it is best to depart from the typical, physically structured modeling view of pharmacokinetics. To cut through all the complexity and objectively deal with the present problems, it is best to consider the kinetics of drug transport in a non-parametric manner and simply use stochastic transport principles in the analysis.

Convolution/deconvolution — stochastic background
The function representation

Convolution/deconvolution — stochastic background

The convolution/deconvolution principles applied to evaluate drug release and drug input (absorption) can be explained in terms of point A to point B stochastic transport principles. For example, consider the entry of a single drug molecule at point A at time zero. Let B be a sampling point (a given sampling space or volume) anywhere in the body (e.g. a blood sampling) that can be reached by the drug molecule entering at A. The possible presence of the molecule at B at time t is a random variable due to the stochastic transport principles involved. Imagine repeating this one molecule experiment an infinite number of times and observing the fraction of times that the molecule is at B at time t. That fraction represents the probability that a molecule is at point B given that it entered point A at time zero. Denote this probability by the function $g(t)$.

Next, consider a simultaneous entry of a very large number (N) of drug molecules at time zero, like in a bolus injection. Let it be assumed that there is no significant interaction between the drug molecules, such that the probability of a drug molecule's possible arrival at sampling site B is not significantly affected by any other drug molecule. Then the transport to sampling site B is both random and independent. In addition the probability of being at B at time t is the same and equals $g(t)$ for all of the molecules. It is this combination of independence and equal probability that leads to the superposition property that in its most simple form reveals itself in terms of dose-proportionality. For example, in the present case the expected number of drug molecules to be found at sampling site B at time t $(N_B(t))$ is equal to $Ng(t)$. It can be seen from this that the concentration of drug at the sampling site B at the arbitrary time t is proportional to the dose. N is proportional to the dose and $g(t)$ does not depend on the dose due to the independence in the transport.

Now further assume that the processes influencing transport from A to B are constant over time. The result is that the probability of being at point B at time t depends on the elapsed time since entry at A, but is otherwise independent of the entry time. Thus the probability of being at B for a molecule that enters A at time t_A is $g(t - t_A)$. This assumed property is called time-invariance. Consider again the simultaneous entry of N molecules, but now at time t_A instead of zero. Combining this property with those of independent and equal probabilities, i.e., superposition, results in an expected number of molecules at B at time t given by $N_B(t)=Ng(t - t_A)$.

Suppose that the actual time at which the molecule enters point A is unknown. It is instead a random quantity t_A distributed according to some probability density function $h(t)$, that is,

$$Pr(t_A \geq t) = \int_0^t h(u)du$$

The probability that such a molecule is at point B at time *t* is the average or expected value of $g(t - t_A)$ where the average is taken over the possible values of t_A according to:

$$\bar{g}(t) = \int_{-\infty}^{\infty} g(t - t_A)h(t_A)dt_A$$

Causality dictates that $g(t)$ must be zero for any negative times, that is, a molecule cannot get to B before it enters A. For typical applications drug input is restricted to non-negative times so that $h(t)$=0 for *t* < 0. As a result the quantity $g(t - t_A)h(t_A)$ is nonzero only over the range from zero to *t* and the equation becomes:

$$\bar{g}(t) = \int_0^t g(t - t_A)h(t_A)dt_A$$

Suppose again that N molecules enter at point A but this time they enter at random times distributed according to $h(t)$. This is equivalent to saying that the expected rate of entry at A is given by $N'_A(t)=Nh(t)$. As argued before, the expected number of molecules at B at time *t* is the product of N and the probability of being at B at time *t*, that is,

$$\begin{aligned} N_B(t) &= N\bar{g}(t) \\ &= N\int_0^t g(t - t_A)h(t_A)dt_A \\ &= \int_0^t g(t - t_A)N'_A(t_A)dt_A \end{aligned}$$

Converting from number of molecules to mass units:

$$M_B(t) = \int_0^t g(t - t_A)f(t_A)dt_A$$

where $M_B(t)$ is the mass of drug at point B at time t and $f(t)$ is the rate of entry in mass per unit time into point A at time t.

Let V_s denote the volume of the sampling space (point B). Then the concentration, $c(t)$, of drug at B is:

$$c(t) = \int_0^t f(t-u)c_\delta(u)du \equiv f(t)*c_\delta(t)$$

where "*" is used to denote the convolution operation and $c_\delta(t) = g(t)/V_s$

The above equation is the key convolution equation that forms the basis for the evaluation of the drug input rate, $f(t)$. The function $c_\delta(t)$ is denoted as the unit impulse response, also known as the characteristic response or the disposition function. The process of determining the input function $f(t)$ is called deconvolution because it is required to deconvolve the convolution integral in order to extract the input function that is embedded in the convolution integral.

The unit impulse response function (c_δ) provides the exact linkage between drug level response $c(t)$ and the input rate function $f(t)$. c_δ is simply equal to the probability that a molecule entering point A at t=0 is present in the sampling space at time t divided by the volume of that sample space.

The function representation

Phoenix models the input function as a piecewise linear "precursor" function $f_p(t)$ convolved with an exponential "dispersion" function $f_d(t)$. The former provides substantial flexibility whereas the latter provides smoothness. The piecewise linear component is parameterized in terms of a sum of hat-type wavelet basis functions, $h_j(t)$:

$$f_p(t) = \sum x_j h_j(t) \quad x_j \ge 0$$

$$h_j(t) = \frac{t-T_j}{T_{j+1}-T_j}, \quad T_j < t < T_{j+1}$$

$$h_j(t) = \frac{T_{j+2}-t}{T_{j+2}-T_{j+1}}, \quad T_{j+1} < t < T_{j+2}$$

$$h_j(t) = 0, \text{otherwise}$$

where x_j is the dose scaling factor within a particular observation interval and T_j are the wavelet support points. The hat-type wavelet representation enables discontinuous, finite duration drug releases to be considered together with other factors that result in discontinuous delivery, such as stomach emptying, absorption window, pH changes, etc. The dispersion function provides the smoothing of the input function that is expected from the stochastic transport principles governing the transport of the drug molecules from the site of release to subsequent entry to and mixing in the general systemic circulation.

The wavelet support points (T_j) are constrained to coincide with the observation times, with the exception of the very first support point that is used to define the lag-time, if any, for the input function. Furthermore, one support point is injected halfway between the lag-time and the first observation. This point is simply included to create enough capacity for drug input prior to the first sampling. Having just one support point prior to the first observation would limit this capacity. The extra support point is well suited to accommodate an initial "burst" release commonly encountered for many formulations.

The dispersion function $f_d(t)$ is defined as an exponential function:

$$f_d(t) = \frac{e^{-t/\delta}}{\delta}$$

where δ is denoted the dispersion or smoothing parameter. The dispersion function is normalized to have a total integral (t=0 to ∞) equal one, which explains the scaling with the δ parameter. The input function, $f(t)$, is the convolution of the precursor function and the dispersion function:

$$f(t) = f_p(t)*f_d(t) \equiv \int_0^t f_p(t-u)f_d(u)du$$

The general convolution form of the input function above is consistent with stochastic as well as deterministic transport principles. The drug level profile, $c(t)$, resulting from the above input function is, according to the linear disposition assumption, given by:

$$c(t) = f_p(t)*f_d(t)*c_\delta(t)$$

where "*" is used to denote the convolution operation.

Deconvolution through convolution methodology

Phoenix deconvolution uses the basic principle of deconvolution through convolution (DTC) to determine the input function. The DTC method is an iterative procedure consisting of three steps. First, the input function is adjusted by changing its parameter values. Second, the new input function is convolved with $c_\delta(t)$ to produce a calculated drug level response. Third, the agreement between the observed data and the calculated drug level data is quantitatively evaluated according to some objective function. The three steps are repeated until the objective function is optimized. DTC methods differ basically in the way the input function is specified and the way the objective function is defined. The objective function may be based solely on weighted or unweighted residual values, or observed–calculated drug levels. The purely residual-based DTC methods ignore any behavior of the calculated drug level response between the observations.

The more modern DTC methods, including Phoenix's approach, consider both the residuals and other properties such as smoothness of the total fitted curve in the definition of the objective function. The deconvolution method implemented in Phoenix is novel in the way the regularization (smoothing) is implemented. Regularization methods in some other deconvolution methods are done through a penalty function approach that involves a measurement of the smoothness of the predicted drug level curve, such as the integral of squared second derivative.

The Phoenix deconvolution method instead introduces the regularization directly into the input function through a convolution operation with the dispersion function, $f_d(t)$. In essence, a convolution operation acts like a "washout of details", that is a smoothing due to the mixing operation inherent in the convolution operation. Consider, for example, the convolution operation that leads to the drug level response $c(t)$. Due to the stochastic transport principles involved, the drug level at time t is made up of a mixture of drug molecules that started their journey to the sampling site at different times and took different lengths of time to arrive there. Thus, the drug level response at time t depends on a mixture of prior input. It is exactly this mixing in the convolution operation that provides the smoothing. The convolution operation acts essentially as a low pass filter with respect to the filtering of the input information. The finer details, or higher frequencies, are attenuated relative to the more slowly varying components, or low frequency components.

Thus, the convolution of the precursor function with the dispersion function results in an input function, $f(t)$, that is smoother than the precursor function. Phoenix allows the user to control the smoothing through the use of the smoothing parameter δ. Decreasing the value of the dispersion function parameter δ results in a decreasing degree of smoothing of the input function. Similarly, larger values of δ provide more smoothing. As δ approaches zero, the dispersion function becomes equal to the so-called Dirac delta "function", resulting in no change in the precursor function.

The smoothing of the input function, $f(t)$, provided by the dispersion function, $f_d(t)$, is carried forward to the drug level response in the subsequent convolution operation with the unit impulse response function (c_δ). Smoothing and fitting flexibility are inversely related. Too little smoothing (too small a δ value) will result in too much fitting flexibility that results in a "fitting to the error in the data." In the most extreme case, the result is an exact fitting to the data. Conversely, too much smoothing (too large a δ value) results in too little flexibility so that the calculated response curve becomes too "stiff" or "stretched out" to follow the underlying true drug level response.

Cross validation principles
User control over execution
The extent of drug input

Cross validation principles

Phoenix's deconvolution function determines the optimal smoothing without actually measuring the degree of smoothing. The degree of smoothing is not quantified but is controlled through the dispersion function to optimize the "consistency" between the data and the estimated drug level curve. Consistency is defined here according to the cross validation principles. Let r_j denote the differences between the predicted and observed concentration at the j-th observation time when that observation is excluded from the dataset. The optimal cross validation principle applied is defined as the condition that leads to the minimal predicted residual sum of squares ($PRESS$) value, where $PRESS$ is defined as:

$$\mathrm{PRESS} = \sum_{j=1}^{m} r_j^2$$

For a given value of the smoothing parameter δ, $PRESS$ is a quadratic function of the wavelet scaling parameters x. Thus, with the non-negativity constraint, the minimization of $PRESS$ for a given δ value is a quadratic programming problem. Let $PRESS$ (δ) denote such a solution. The optimal smoothing is then determined by finding the value of the smoothing parameter δ that minimizes $PRESS$ (δ). This is a one variable optimization problem with an embedded quadratic-programming problem.

User control over execution

Phoenix's deconvolution function permits the user to override the automatic smoothing by manual setting of the smoothing parameter. The user may also specify that no smoothing should be performed. In this case the input rate function consists of the precursor function alone.

Besides controlling the degree of smoothing, the user may also influence the initial behavior of the estimated input time course. In particular the user may choose to constrain the initial input rate to zero ($f(0)$=0) and/or constrain the initial change in the input rate to zero ($f'(0)$=0). By default Phoenix does not constrain either initial condition. Leaving $f(0)$ unconstrained permits better characterization of formulations with rapid initial "burst release," that is, extended release dosage forms with an immediate release shell. This is done by optionally introducing a bolus component or "integral boundary condition" for the precursor function so the input function becomes:

$$f(t) = f_p(t)*f_d(t) + x_d f_d(t)$$

where the $f_p(t)*f_d(t)$ is defined as before.

The difference here is the superposition of the extra term $x_d\, f_d(t)$ that represents a particularly smooth component of the input. The magnitude of this component is determined by the scaling parameter x_d, which is determined in the same way as the other wavelet scaling parameters previously described.

The estimation procedure is not constrained with respect to the relative magnitude of the two terms of the composite input function given above. Accordingly, the input can "collapse" to the "bolus component" $x_d\, f_d(t)$ and thus accommodate the simple first-order input commonly experienced when dealing with drug solutions or rapid release dosage forms. A drug suspension in which a significant fraction of the drug may exist in solution should be well described by the composite input function option given above. The same may be the case for dual release formulations designed to rapidly release a portion of the drug initially and then release the remaining drug in a prolonged fashion. The prolonged release component will probably be more erratic and would be described better by the more flexible wavelet-based component $f_p(t)*f_d(t)$ of the above dual input function.

Constraining the initial rate of change to zero ($f'(0)$=0) introduces an initial lag in the increase of the input rate that is more continuous in behavior than the usual abrupt lag time. This constraint is obtained by constraining the initial value of the precursor function to zero ($f_p(0)$=0). When such a constrained precursor is convolved with the dispersion function, the resulting input function has the desired constraint.

The extent of drug input

The extent of drug input in the Deconvolution object is presented in two ways. First, the amount of drug input is calculated as:

$$\text{Amount input} = \int_0^t f(t)\,dt$$

Second, the extent of drug input is given in terms of fraction input. If test doses are not supplied, the fraction is defined in a non-extrapolated way as the fraction of drug input at time t relative to the amount input at the last sample time, t_{end}:

$$\text{Fraction input} = \int_0^t f(t)\,dt \Big/ \int_0^{t_{\text{end}}} f(t)\,dt$$

The above fraction input will by definition have a value of one at the last observation time, t_{end}. This value should not be confused with the fraction input relative to either the dose or the total amount absorbed from a reference dosage form. If dosing data is entered by the user, the fraction input is relative to the dose amount (i.e., dose input on the dosing sheet).

References

Charter and Gull (1987). *J Pharmacokinet Biopharm* 15, 645–55.

Cutler (1978). Numerical deconvolution by least squares: use of prescribed input functions. *J Pharmacokinet Biopharm* 6(3):227–41.

Cutler (1978). Numerical deconvolution by least squares: use of polynomials to represent the input function. *J Pharmacokinet Biopharm* 6(3):243–63.

Daubechies (1988). Orthonormal bases of compactly supported wavelets. *Communications on Pure and Applied Mathematics* 41(XLI):909–96.

Gabrielsson and Weiner (2001). *Pharmacokinetic and Pharmacodynamic Data Analysis: Concepts and Applications*, 3rd ed. Swedish Pharmaceutical Press, Stockholm.

Gibaldi and Perrier (1975). *Pharmacokinetics*. Marcel Dekker, Inc, New York.

Gillespie (1997). Modeling Strategies for In Vivo-In Vitro Correlation. In Amidon GL, Robinson JR and Williams RL, eds., *Scientific Foundation for Regulating Drug Product Quality*. AAPS Press, Alexandria, VA.

Haskel and Hanson (1981). Math. *Programs* 21, 98–118.

Iman and Conover (1979). *Technometrics*, 21,499–509.

Loo and Riegelman (1968). New method for calculating the intrinsic absorption rate of drugs. *J Pharmaceut Sci* 57:918.

Madden, Godfrey, Chappell MJ, Hovroka R and Bates RA (1996). Comparison of six deconvolution techniques. *J Pharmacokinet Biopharm* 24:282.

Meyer (1997). IVIVC Examples. In Amidon GL, Robinson JR and Williams RL, eds., *Scientific Foundation for Regulating Drug Product Quality*. AAPS Press, Alexandria, VA.

Polli (1997). Analysis of In Vitro-In Vivo Data. In Amidon GL, Robinson JR and Williams RL, eds., *Scientific Foundation for Regulating Drug Product Quality*. AAPS Press, Alexandria, VA.

Treitel and Lines (1982). Linear inverse — theory and deconvolution. *Geophysics* 47(8):1153–9.

Verotta (1990). Comments on two recent deconvolution methods. *J Pharmacokinet Biopharm* 18(5):483–99.

Deconvolution example

Perhaps the most common application of deconvolution is in the evaluation of drug release and drug absorption from orally administered drug formulations. In this case, the bioavailability is evaluated if the reference input is a vascular drug input. Similarly, gastrointestinal release is evaluated if the reference is an oral solution (oral bolus input). Both are included here.

This example uses the dataset `M3tablet.dat`, which is located in the Phoenix examples directory. The analysis objectives are to estimate the following for a tablet formulation:

Knowledge of how to do basic tasks using the Phoenix interface, such as creating a project and importing data, is assumed.

- Absolute bioavailability and rate and cumulative extent of absorption over time (see “Evaluate absolute bioavailability”)
- ***In vivo*** dissolution and the rate and cumulative extent of release over time (see “Estimate dissolution”)

The completed project (`Deconvolution.phxproj`) is available for reference in …`\Examples\WinNonlin`.

Evaluate absolute bioavailability

To estimate the absolute bioavailability, the mean unit impulse response parameters A and alpha have already been estimated from concentration-time data following instantaneous input (IV bolus) for three subjects, using PK model 1. The data in `M3tablet.dat` includes those parameter estimates and plasma drug concentrations following oral administration of a tablet formulation. This example shows how to estimate the rate at which the drug reaches the systemic circulation, using deconvolution.

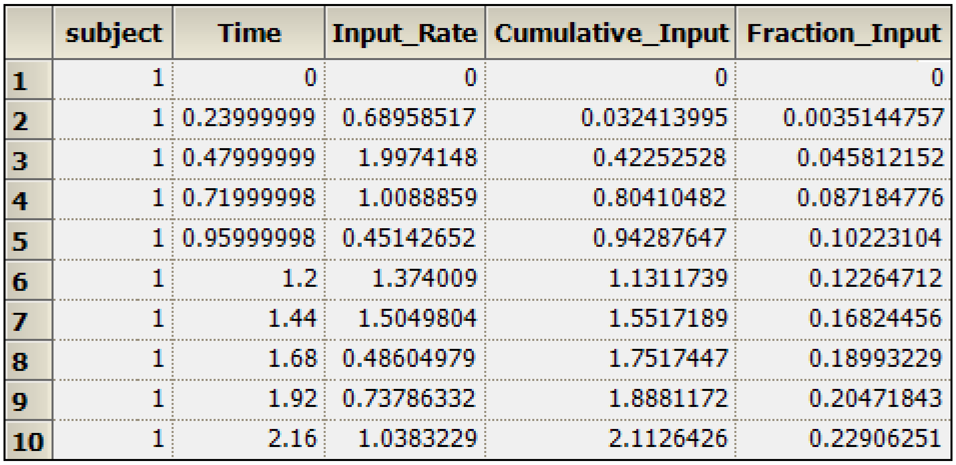
For this type of data, all the data for one treatment must be displayed in the first rows, followed by all the data for the other treatment.

1. Create a project called `Deconvolution`.
2. Import the file …`\Examples\WinNonlin\Supporting files\M3tablet.dat`.
3. Right-click **M3tablet** in the Data folder and select **Send To > Computation Tools > Deconvolution**.
4. In the Main Mappings panel:
   Map **subject** to the **Sort** context.
   Leave **time** mapped to the **Time** context.
   Map **conc** to the **Concentration** context.
   Leave all the other data types mapped to **None**.
5. Select **Exp Terms** in the Setup list.
6. Select the **Use Internal Worksheet** checkbox.
7. In the **Value** column type `100` for each row with A1 in the Parameter column.
8. In the **Value** column type `0.98` for each row with Alpha1 in the Parameter column.

   There are no dose amounts for this example. The calculated fractional input approaches a value of 1 rather than being adjusted for dose amount.

9. Click ▶ (**Execute** icon) to execute the object.

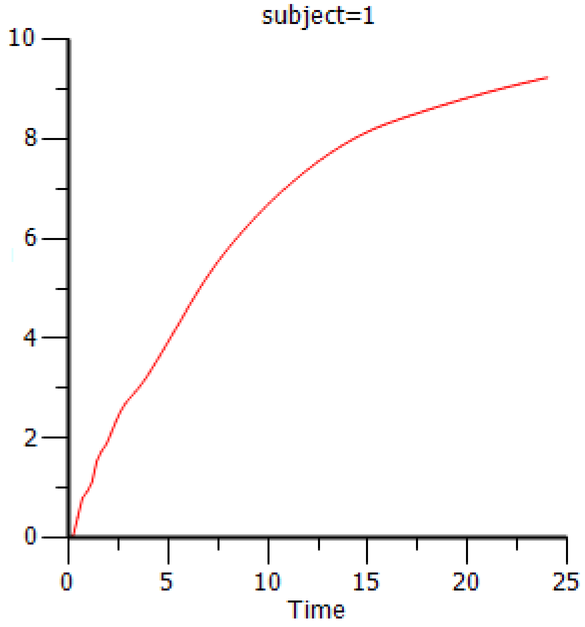
   Phoenix generates worksheets and plots for the output. Partial results for subject 1 are displayed below.



	subject	Time	Input_Rate	Cumulative_Input	Fraction_Input
1	1	0	0	0	0
2	1	0.23999999	0.68958517	0.032413995	0.0035144757
3	1	0.47999999	1.9974148	0.42252528	0.045812152
4	1	0.71999998	1.0088859	0.80410482	0.087184776
5	1	0.95999998	0.45142652	0.94287647	0.10223104
6	1	1.2	1.374009	1.1311739	0.12264712
7	1	1.44	1.5049804	1.5517189	0.16824456
8	1	1.68	0.48604979	1.7517447	0.18993229
9	1	1.92	0.73786332	1.8881172	0.20471843
10	1	2.16	1.0383229	2.1126426	0.22906251

Figure 11-1. Part of the Values worksheet

10. Select **Cumulative Input Plot** in the Results list.



Estimate dissolution

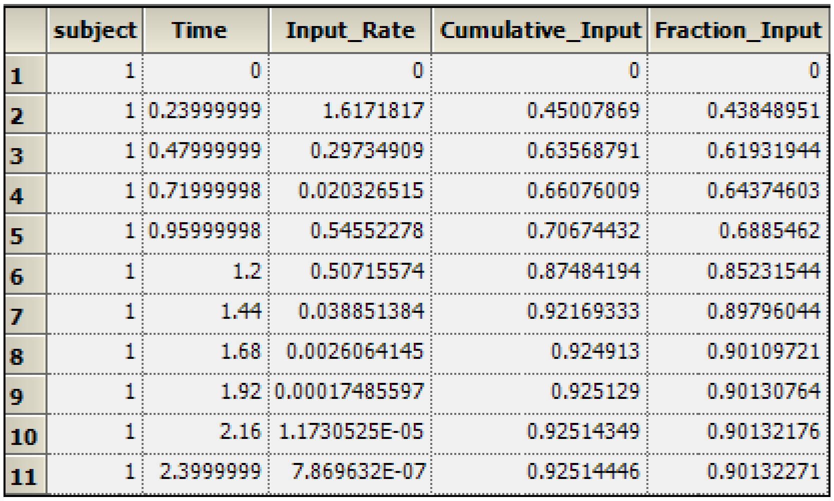
To estimate the ***in vivo*** dissolution from the tablet formulation, the mean unit impulse response parameters A and alpha have already been estimated from concentration-time data following instantaneous input into the gastrointestinal tract by administration of a solution, using PK model 3. The steps below show how to use deconvolution to estimate the rate at which the drug dissolves.

For the rest of this example an oral solution (oral bolus) is used to estimate the unit impulse response. In this case, the deconvolution result should be interpreted as an ***in vivo*** dissolution profile, not as an absorption profile. The oral impulse response function should have the property of the initial value being equal to zero, which implies that the sum of the 'A's must be zero. The alphas should all still be positive, but at least one A will be negative.

1. Right-click **M3tablet** in the Data folder and select **Send To > Computation Tools > Deconvolution**.
2. In the Main Mappings panel:
   Map **subject** to the **Sort** context.
   Leave **time** mapped to the **Time** context.
   Map **conc** to the **Concentration** context.
   Leave all the other data types mapped to **None**.
3. In the Options tab below the Setup panel, select **2** in the **Exponential Terms** menu.
4. Select **Exp Terms** in the Setup list.
5. Check the **Use Internal Worksheet** checkbox.
6. Fill in the **Value** column as follows:
   Type `-110` for each row with A1 in the Parameter column.
   Type `110` for each row with A2 in the Parameter column.
   Type `3.8` for each row with Alpha1 in the Parameter column.
   Type `0.10` for each row with Alpha2 in the Parameter column.

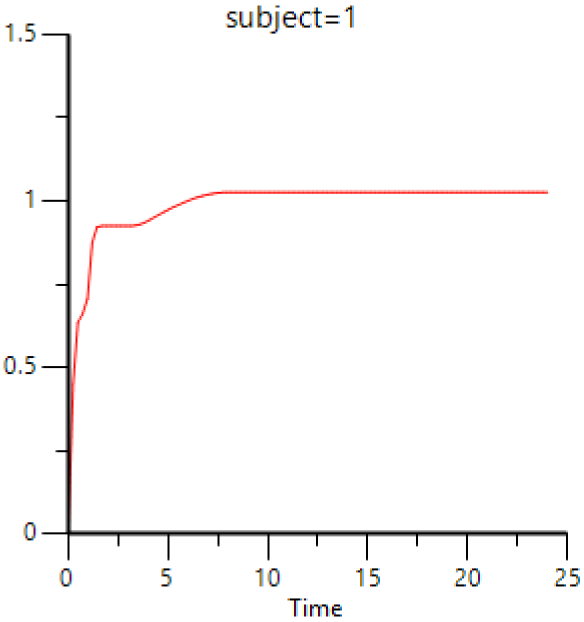
7. Execute the object.

   Results for subject 1 are displayed below:



	subject	Time	Input_Rate	Cumulative_Input	Fraction_Input
1	1	0	0	0	0
2	1	0.23999999	1.6171817	0.45007869	0.43848951
3	1	0.47999999	0.29734909	0.63568791	0.61931944
4	1	0.71999998	0.020326515	0.66076009	0.64374603
5	1	0.95999998	0.54552278	0.70674432	0.6885462
6	1	1.2	0.50715574	0.87484194	0.85231544
7	1	1.44	0.038851384	0.92169333	0.89796044
8	1	1.68	0.0026064145	0.924913	0.90109721
9	1	1.92	0.00017485597	0.925129	0.90130764
10	1	2.16	1.1730525E-05	0.92514349	0.90132176
11	1	2.3999999	7.869632E-07	0.92514446	0.90132271

8. Select **Cumulative Input Plot** under Plots in the Results list.



This concludes the Deconvolution example.

Linear Mixed Effects

The Linear Mixed Effects operational object (LinMix) is a statistical analysis system for analysis of variance for crossover and parallel studies, including unbalanced designs. It can analyze regression and covariance models, and can calculate both sequential and partial tests. LinMix is discussed in the following sections.

Use one of the following to add the object to a Workflow:

Right-click menu for a Workflow object: **New > Computation Tools > Linear Mixed Effects**.
Main menu: **Insert > Computation Tools > Linear Mixed Effects**.
Right-click menu for a worksheet: **Send To > Computation Tools > Linear Mixed Effects**.

Note: To view the object in its own window, select it in the Object Browser and double-click it or press **ENTER**. All instructions for setting up and execution are the same whether the object is viewed in its own window or in Phoenix view.

User interface description
Results
General linear mixed effects model
Linear mixed effects computations
Linear mixed effects model examples

User interface description

Main Mappings panel
Fixed Effects tab
Variance Structure tab
Random 1 and Repeated tabs
Contrasts tab
Contrast # 1 tab
Estimates tab
Estimate # 1 tab
Least Squares Means tab
General Options tab

Main Mappings panel

Use the Main Mappings panel to identify how input variables are used in a linear mixed effects model. A separate analysis is performed for each profile. Required input is highlighted orange in the interface.

None: Data types mapped to this context are not included in any analysis or output.

Sort: Categorical variable(s) identifying individual data profiles, such as subject ID and treatment. A separate analysis is done for each unique combination of sort variable values. If a sort variable has missing values, then the analysis is performed for the missing level and MISSING is printed as the sort variable value.

Classification: Classification variables or factors that are categorical independent variables, such as formulation, treatment, and gender.

Regressors: Regressor variables or covariates that are continuous independent variables, such as temperature or body weight. The regressor variable can also be used to weight the dataset.

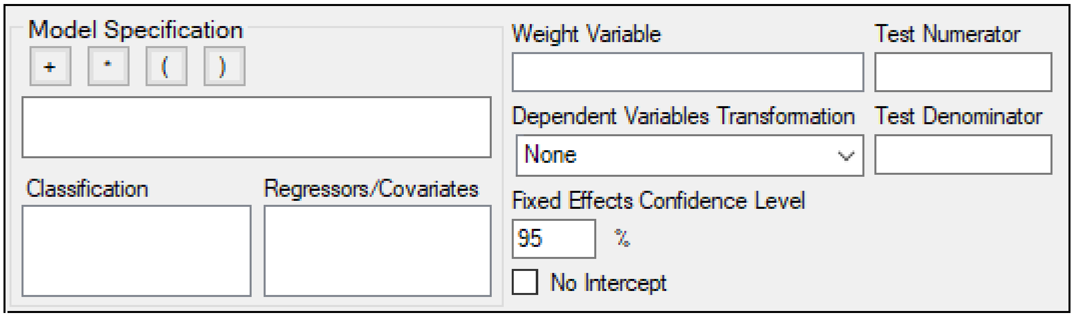
Dependent: The dependent variable, such as drug concentration, that provides the values used to fit the model.

For information on variable naming constraints and data limits, see "Data limits and constraints" in the Bioequivalence section.

Note: Be sure to finalize column names in your input data before sending the data to the Linear Mixed Effects object. Changing names after the object is set up can cause the execution to fail.

Fixed Effects tab

The Fixed Effects tab allows users to specify settings for study variables used in linear mixed effects model. The Model Specification field is used to categorize variables in a dataset for the linear mixed effects model. For more on fixed effects in the linear mixed effects model, see "Fixed effects".



- Drag variables from the **Classification** and the **Regressors/Covariates** boxes to the **Model Specification** field and click the operator buttons to build the model or type the names and operators directly in the field.
  + addition,
  ***** multiplication,
  ( ) parentheses for indicating nested variables in the model

  Below are some guidelines for using parentheses:
  - Parentheses in the model specification represent nesting of model terms.
  - `Seq+Subject(Seq)+Period+Treatment` is a valid use of parentheses and indicates that Subject is nested within Seq.
  - `Drug+Disease+(Drug*Disease)` is not a valid use of parentheses in the model specification.
- Select a weight variable from the **Regressors/Covariates** box and drag it to the **Weight Variable** field.

  To remove a weight variable, drag the variable from the **Weight Variable** field back to the **Regressors/Covariates** box.

  The **Regressors/Covariates** box lists variables that are mapped to the Regressors context (in the Main Mappings panel). If a variable is used to weight the data then the variable is displayed in the **Regressors/Covariates** box. Below are some guidelines for using weight variables:
  - The weights for each record must be included in a separate column in the dataset.
  - Weight variables are used to compensate for observations having different variances.
  - When a weight variable is specified, each row of data is multiplied by the square root of the corresponding weight.
  - Weight variable values should be proportional to the reciprocals of the variances. Typically, the data are averages and weights are sample sizes associated with the averages.

  - The Weight variable cannot be a classification variable. It must be declared as a regressor/covariate before it can be used as a weight variable. It can also be used in the model.

- In the **Dependent Variables Transformation** menu, select one of three options:

  None
  Ln(x): Linear transformation
  Log10(x): Logarithmic base 10 transformation

- In the **Fixed Effects Confidence Level** box, type the level for the fixed effects model. The default value is 95%.

- By default, the intercept term is included in the model (although it is not shown in the **Model Specification** field), check the **No Intercept** checkbox to remove the intercept term.

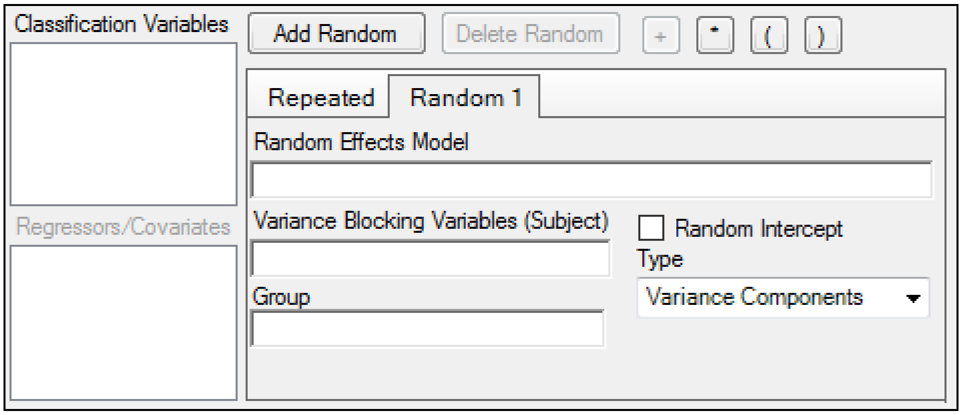
  Removing the intercept term changes the parameterization of the classification variables. See "Construction of the X matrix" for more information.

- Use the **Test Numerator** and **Test Denominator** fields to specify an additional test of hypothesis in the case of a model with only fixed effects.

  For this case, the default error term (denominator) is the residual error, so an alternate test can be requested by entering the fixed effects model terms to use for the numerator and denominator of the F-test. The terms entered must be in the fixed effects model and the random/repeated models must be empty for the test to be performed. (See "Tests of hypotheses" for additional information.)

Variance Structure tab

The Variance Structure tab allows users to set random effects and the repeated specification for the linear mixed effects model. Users can also set traditional variance components and random coefficients.



Users can specify none, one, or multiple random effects. The random effects specify Z and the corresponding elements of $G=Var(\gamma)$. Users can specify only one repeated effect. The repeated effect specifies the $R=Var(e)$.

For more on variance structures in the linear mixed effects model, see "Variance structure".

- Select a variable in the **Classification Variables** box and drag it to the Random 1 tab or type variable names in the fields.

- Select a variable in the **Regressors/Covariates** box and drag the variable to the Random 1 tab or type variable names in the fields.

Random 1 and Repeated tabs

The Random 1 tab is used to add random effects to the model. The random effects are built using the classification variables, the regressors/covariates variables, and the operator buttons.

The Repeated tab is used to specify the R matrix in the mixed model. The Repeated tab is also used to specify covariance structures for repeated measurements on subjects. If no repeated statement is specified, R is assumed to be equal to $\sigma^2 I$. The repeated effect must contain only classification variables.

Caution: The same variable cannot be used in both the fixed effects specification and the random effects specification unless it is used differently, such as part of a product. The same term (single variables, products, or nested variables) must not appear in both specifications.

- Drag variables from the boxes on the left to the fields in the tab and click the operator buttons to build the model or type the names and operators directly in the fields.
  + addition (not available in the Repeated tab or when specifying the variance blocking or group variables),
  ***** multiplication,
  ( ) parentheses for indicating nested variables in the model.

This **Variance Blocking Variables (Subject)** field is optional and, if specified, must be a classification model term built from items in the **Classification Variables** box. This field is used to identify the subjects in a dataset. Complete independence is assumed among subjects, so the subject variable produces a block diagonal structure with identical blocks.

This **Group** field is also optional and, if specified, must be a classification model term built from items in the **Classification Variables** box. It defines an effect specifying heterogeneity in the covariance structure. All observations having the same level of the group effect have the same covariance parameters. Each new level of the group effect produces a new set of covariance parameters with the same structure as the original group.

- (Random 1 tab only) Check the **Random Intercept** checkbox to include a random intercept.

  This setting is commonly used when a subject is specified in the **Variance Blocking Variables (Subject)** field. The default setting is no random intercept.
- If the model contains random effects, a covariance structure type must be selected from the **Type** menu.

  If **Banded Unstructured (b)**, **Banded No-Diagonal Factor Analytic (f)**, or **Banded Toeplitz (b**) is selected, type the number of bands in the **Number of bands(b)** field (default is 2).

  The number of factors or bands corresponds to the dimension parameter. For some covariance structure types this is the number of bands and for others it is the number of factors.

  For explanations of covariance structure types, see “Covariance structure types in the Linear Mixed Effects object”.
- Click **Add Random** to add additional variance models.
- Click **Delete Random** to delete a variance model.

Contrasts tab

The Contrasts tab provides a mechanism for creating custom hypothesis tests. For example, users can compare different treatments or treatment combinations to see if the mean values are the same.

Contrasts can only be computed using the fixed effect model terms set in the Model Specification field. For more on contrasts in the linear mixed effects model, see “Contrasts”.

The **Fixed Effects Model Terms** box lists all the fixed effect model terms specified in the Fixed Effects tab. Users can drag a term from the **Fixed Effect Model Terms** box to the **Effect** field to compute the contrasts for that term.

The conditions for using model terms as effect variables are:

- If all values of the dependent variable are missing or excluded for an effect variable level, then that level of the effect variable is not used in the Contrast vector.
- Effect variables can be either numeric or alphabetic, but they cannot be both. If a column contains both numeric and alphabetic data, the column cannot be used as an effect variable when building contrasts.
- Interactions can be used as contrasts only if the interaction is a model term.
- Nested terms can be used as contrasts only if the nested term is a model term.

Contrast # 1 tab

- Drag a model term from the **Fixed Effect Model Terms** box to the **Effect** field.

  To remove a model term, use the pointer to drag a model term from the **Effect** field back to the **Fixed Effects Model Terms** box.
- In the **Title** field, type a title for the contrast. The title is displayed in the Contrasts and Contrasts Coefficients worksheets.
- In the **Coefficients** column, type the coefficient value for each level of the model term in the contrast.

Caution: The coefficients for contrasts must sum to zero.

Add extra columns to enter multiple coefficients for each model term in the contrast.

- In the **Number of columns for contrast** box, select the number of coefficient columns to use with each contrast (default is 1).

  If multiple coefficient columns are selected, then Phoenix simultaneously tests the multiple linearly independent combinations of coefficients.
- To set the degrees of freedom, check the **User specified degrees of freedom** checkbox and type a value greater than one (1) in the field.

Caution: Only select the **User specified degrees of freedom** option if the Phoenix engine does not seem to use the appropriate choices for the degrees of freedom.

Note: The Univariate Confidence Intervals use an approximation for the t-value that is very accurate when the degrees of freedom value is at least five, but loses accuracy as the degrees of freedom value approaches one. The degrees of freedom are the number of observations minus the number of parameters being estimated (not counting parameters that are within the singularity tolerance, i.e., nearly completely correlated).

- Check the **Show coefficients of contrasts** checkbox to display the actual coefficients used in the Contrasts Coefficients results worksheet.

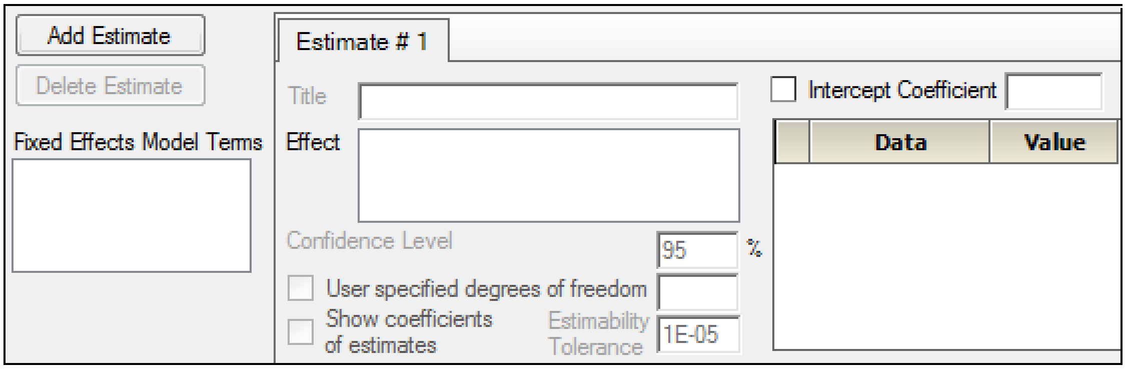
- In the **Estimability Tolerance** field, type a value for the zero vector.

  The estimability tolerance value indicates the closeness of the zero vector before the Phoenix engine is used to make it estimable. The default value is 1E–05. Users do not typically need to change this value.
- Click **Add Contrast** to add another contrast.

  Each contrast is tested independently of other contrasts. Users can enter up to 100 contrasts.
- Click **Delete Contrast** to delete a contrast.

Estimates tab

The Estimates tab provides a mechanism for creating custom hypothesis tests. Estimates can only be computed using the fixed effect model terms set in the Model Specification field. Since the Estimates tab produces estimates instead of contrasts, the coefficients do not have to sum to zero and more than one model term can be added to the Effect field. The marginal interval is generated for each estimate.



For more on estimates in the linear mixed effects model, see "Estimates".

The **Fixed Effects Model Terms** box lists all the fixed effect model terms specified in the Fixed Effects tab. Users can drag a term from the **Fixed Effect Model Terms** box to the **Effect** field to compute the estimates for that term. The conditions for using model terms as effect variables are:

- Interaction terms and nested terms can be used if they are used in the model.
- If the fixed effects model includes an intercept, which is the default setting, then the intercept can be used to produce an estimate.
- If the intercept term is used as an effect for an estimate it works like a regressor, which means only one coefficient value is used for the intercept.

Estimate # 1 tab

- Drag a model term from the **Fixed Effect Model Terms** box to the **Effect** field.
  - Multiple model terms can be dragged from the **Fixed Effect Model Terms** box to the **Effect** field.
  - To remove a model term, use the pointer to drag a model term from the **Effect** field back to the **Fixed Effects Model Terms** box.
- In the **Title** field, type a title for the estimate. The title is displayed in the Estimates and Estimates Coefficients worksheets.
- In the Coefficients column, type the coefficient value for each level of the model term in the estimate.

- Select the **Intercept Coefficient** checkbox to include the intercept in the estimate.
- In the **Intercept Coefficient** field, type the coefficient value for the intercept.
- In the **Confidence Level** field, type the level percentage. The default level is 95%. Users do not typically need to change this value.
- To set the degrees of freedom, check the **User specified degrees of freedom** checkbox and type a value greater than one (1) in the field.

Caution: Only select the **User specified degrees of freedom** option if the Phoenix engine does not seem to use the appropriate choices for the degrees of freedom.

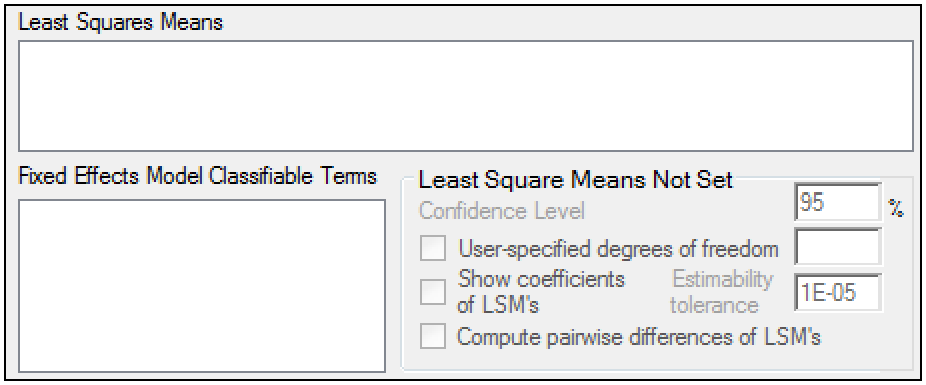
- Check the **Show coefficients of estimates** checkbox to display the actual coefficients used in the Estimates Coefficients results worksheet.
- In the **Estimability Tolerance** field, type a value for the zero vector.

  The estimability tolerance value indicates the closeness of the zero vector before the Phoenix engine is used to make it estimable. The default value is 1E–05. Users do not typically need to change this value.
- Click **Add Estimate** to add another contrast.

  Each estimate is tested independently of other estimates. Users can enter up to 100 estimates.
- Click **Delete Estimate** to delete a contrast.

Least Squares Means tab

Least squares means are generalized least-squares means of the fixed effects. They are estimates of what the mean values would have been had the data been balanced, which means these are the means predicted by the ANOVA model. If a dataset is balanced, the least squares means will be identical to the raw, or observed, means. Least Squares Means can be computed for any classification model term.



Least Squares Means

Fixed Effects Model Classifiable Terms

Least Square Means Not Set

Confidence Level 95 %

□ User-specified degrees of freedom

□ Show coefficients of LSM's Estimability tolerance 1E-05

□ Compute pairwise differences of LSM's

For more on least squares means in the linear mixed effects model, see “Least squares means”.

- Drag a model term from the **Fixed Effect Model Classifiable Terms** box to the **Least Squares Means** field.

  To remove a model term, drag a model term from the **Least Squares Means** field back to the **Fixed Effect Model Classifiable Terms** box.

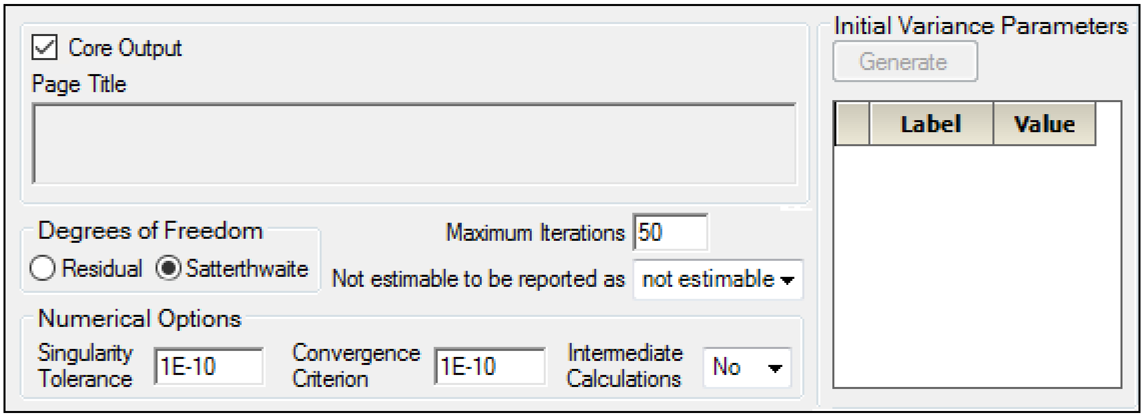
- In the **Confidence Level** field, type the level percentage. The default level is 95%. Users do not typically need to change this value.
- To set the degrees of freedom, check the **User specified degrees of freedom** checkbox and type a value greater than one (1) in the **User specified degrees of freedom** field.
- Check the **Show coefficients of LSMs** checkbox to display the actual coefficients used in the LSM Coefficients results worksheet.
- In the **Estimability Tolerance** field, type a value for the zero vector.

  The estimability tolerance value indicates the closeness of the zero vector before the Phoenix engine is used to make it estimable. The default value is 1E–05. Users do not typically need to change this value.
- Select the **Compute pairwise differences of LSMs** checkbox to display the differences of intervals and LSMs in the LSM Differences results worksheet.

  This function tests $\eta_1 = \eta_2$.

General Options tab

The General Options tab is used to set output and calculation options for a linear mixed effects model.



☑ Core Output

Page Title

Degrees of Freedom: ○ Residual ● Satterthwaite

Maximum Iterations: 50

Not estimable to be reported as: not estimable

Numerical Options

Singularity Tolerance: 1E-10

Convergence Criterion: 1E-10

Intermediate Calculations: No

Initial Variance Parameters

	Label	Value

- Check the **Core Output** checkbox to include the Core Output text file in the results.
- In the **Page Title** field, type a title for the Core Output text file.
- Choose the degrees of freedom calculation method:

  Residual: The same as the calculation method used in a purely fixed effects model
  Satterthwaite: The default setting and computes the df base on χ^2 approximation to distribution of variance.
- In the **Maximum Iterations** field, type the number of maximum iterations. This is the number of iterations in the Newton fitting algorithm. The default setting is 50.
- Use the **Not estimable to be reported as** menu to determine how output that is not estimable is represented.
  - not estimable
  - 0 (zero)
- In the **Singularity Tolerance** field, type the tolerance level. The columns in X and Z are eliminated if their norm is less than or equal to this number (default is1E–10).

- In the **Convergence Criterion** field, type the criterion used to determine if the model has converged (default is 1E–10).
- In the **Intermediate Calculations** menu, select whether or not to include the design matrix, reduced data matrix, asymptotic covariance matrix of variance parameters, Hessian, and final variance matrix in the Core Output text file.

Note: The **Generate** initial variance parameters option is available only if the model uses random effects.

- In the **Initial Variance Parameters** group, click **Generate** to edit the initial variance parameters values.
- Select a cell in the Value column and type a value for the parameter. The default value is 1. If the values are not specified, then Phoenix uses the method of moments estimates.

  To delete one or more of the parameters from the table:

  - Highlight the row(s).
  - Select **Edit >> Delete** from the menubar or click X in the main toolbar.
  - Click the **Selected Row(s)** option button and click **OK**.

Results

The Linear Mixed Effects object creates several output worksheets in addition to a text file that lists the model settings. The specific output created depends on the options set in the Linear Mixed Effects object.

Worksheet output
Text output
Output explanations

For more on how the Linear Mixed Effects object creates output, see "Linear mixed effects computations".

Worksheet output

The Output Data section in the Results tab contains linear mixed effects output in worksheet format. The worksheets that are created depends on which model options are selected. All possible worksheets are listed in the following table. For a comprehensive explanation of results worksheets, see "Output explanations".

Contrasts: Hypothesis, df, F-statistic, and p-value

Contrasts Coefficients: Shows the actual coefficients used in the contrasts

Diagnostics: Model-fitting information

Estimates: Estimate, standard error, t-statistic, pvalue, and interval

Estimates Coefficients: Shows the actual coefficients used in the estimates

Final Fixed Parameters: Estimates, standard error, t-statistic, p-value, and intervals

Final Variance Parameters: Estimates and units of the final variance parameters

Initial Variance Parameters: Initial values for variance parameters

Iteration History: Data on the variable estimation in each iteration

Least Squares Means: Least squares means, standard error, df, t-statistic, p-value, and interval

LSM Coefficients: Shows the actual coefficients used in the least squares means test

LSM Differences: Shows the differences of intervals and LSMs

Parameter Key: Information about the variance structure

Partial SS: Similar to Partial Tests but includes SS and MS

Partial Tests: Tests of fixed effects, but each term is tested last

Residuals: Dependent variable(s), units, observed and predicted values, standard error and residuals

Sequential SS: Similar to Sequential Tests but includes SS and MS

Sequential Tests: Tests of fixed effect for significant improvement in fit, in sequence. Depends upon the order that model terms are written.

User Settings: Information about model settings

Text output

The Linear Mixed Effects object creates two types of text output: a `Settings` file that contains model settings, and an optional `Core Output` file. The Core Output text file is created if the **Core**

Output checkbox is checked in the General Options tab. The file contains a complete summary of the analysis, including all output as well as analysis settings and any errors that occur.

Output explanations

The following sections provide more detail about the Linear Mixed Effects object's worksheet output, including how the results are derived.

Tests of hypotheses
Sequential Tests worksheet
Partial Tests worksheet
Sequential SS and Partial SS worksheets
ANOVA
Diagnostics worksheet
Residuals worksheet and predicted values
Final fixed parameters estimates
Coefficients worksheets
Iteration History worksheet
Parameter Key worksheet

Tests of hypotheses

The tests of hypotheses are the mixed model analog to the analysis of variance in a fixed model. The tests are performed by finding appropriate L matrices and testing the hypotheses H_0: Lb=0 for each model term.

If an additional test was requested for a fixed model, the test is performed by computing the ratio of the MS values of the specified fixed effects terms for numerator and denominator. LinMix does not check any assumptions about the user-specified test; the user should understand if the test is valid.

Sequential Tests worksheet

The Sequential Tests worksheet is created by testing each model term sequentially. The first model term is tested to determine whether or not it should enter the model. Then the second model term is tested to determine whether or not it should enter the model, given that the first term is in the model. Then the third model term is tested to determine whether or not it should enter the model, given that the first two terms are in the model. The model term tests continue until all model terms are exhausted.

The tests are computed using a QR factorization of the XY matrix. The QR factorization is segmented to match the number of columns that each model term contributes to the X matrix.

Partial Tests worksheet

The Partial Tests worksheet is created by testing each model term given every other model term. Unlike sequential tests, partial tests are invariant under the order in which model terms are listed in the Fixed Effects tab. Partial tests factor out of each model term the contribution attributable to the remaining model terms.

This is computed by modifying the basis created by the QR factorization to yield a basis that more closely resembles that found in balanced data.

Sequential SS and Partial SS worksheets

For models with only fixed effects, the Sequential Tests and Partial Tests are also presented in a form that includes SS (Sum of Squares) and MS (Mean Square).

ANOVA

For fixed effects models, certain properties can be stated for the two types of ANOVA. For the sequential ANOVA, the sums of squares are statistically independent. Also, the sum of the individual sums of squares is equal to the model sum of squares; which means the ANOVA represents a partitioning of the model sum of squares. However, some terms in ANOVA may be contaminated with undesired effects. The partial ANOVA is designed to eliminate the contamination problem, but the sums of squares are correlated and do not add up to the model sums of squares. The mixed effects tests have similar properties.

Diagnostics worksheet

Akaike Information Criterion (AIC)

The Linear Mixed Effects object uses the smaller-is-better form of Akaike's Information Criterion:

$$AIC = -2L_R + 2s$$

where:

L_R is the restricted log-likelihood function evaluated at the final fixed parameter estimates $\hat{\beta}$ and the final variance parameter estimates $\hat{\theta}$.

s is the rank of the fixed effects design matrix X plus the number of parameters in θ (i.e., s=rank(X)+dim(θ)).

Schwarz Bayesian Criterion (SBC)

The Linear Mixed Effects object uses the smaller-is-better form of Schwarz's Bayesian Criterion:

$$SBC = -2L_R + s\log(N - r)$$

where:

L_R is the restricted log-likelihood function evaluated at the final estimates $\hat{\beta}$ and $\hat{\theta}$.
N is the number of observations used.
r is the rank of the fixed effects design matrix X.
s is the rank of the fixed effects design matrix X plus the number of parameters in θ (i.e., s=rank(X)+dim(θ)).

Note: AIC and SBC are only meaningful during comparison of models. A smaller value is better, negative is better than positive, and a more negative value is even better.

Hessian eigenvalues

The eigenvalues are formed from the Hessian of the restricted log likelihood. There is one eigenvalue per variance parameter. Positive eigenvalues indicate that the final parameter estimates are found at a maximum.

Residuals worksheet and predicted values

In the Linear Mixed Effects object, predicted values for the dependent variables are computed at all of the input data points. To obtain predicted values for data points other than the observed values, the user should supply the desired points in the input dataset with missing dependent variables. Predicted values are estimated by using the rows of the fixed effects design matrix X as the L matrices for estimation, and therefore the predicted values for the input data are equal to:

$$\hat{y} = X\hat{\beta}$$

where $\hat{\beta}$ are the final fixed parameters estimates.

If a user selects a log-transform, then the predicted values are also transformed. The residuals are then the observed values minus the predicted values, or in the case of transforms, the residuals are the transformed values minus the predicted values. The variance of the prediction is as follows:

$$Var(\hat{y}) = X(X^T\hat{V}X)^{-1}X^T$$

The standard errors are the square root of the diagonal of the matrix above. T-type intervals for the predicted values are also computed.

The Lower_CI and Upper_CI are the bounds on the predicted response. The level is selected in the Fixed Effects tab of the Linear Mixed Effects object.

Final fixed parameters estimates

In the Linear Mixed Effects object, the final fixed effects parameter estimates are computed by solving:

$T_{00}\beta = y_0$

where:

T_{00} and y_0 are elements of the reduced data matrix as defined in the section on “Restricted maximum likelihood”.

$\hat{\beta}$ are the final fixed parameters estimates.

When there are multiple solutions, a convention is followed such that if any column of T_{00} is a linear combination of preceding columns, then the corresponding final fixed parameters estimate element is set to zero.

Coefficients worksheets

If Show Coefficients is selected on any tab in the Linear Mixed Effects object, then the coefficients of the parameters used to construct the linear combination is displayed in the output. If the linear combination is estimable, then the coefficients are the same as those entered. If the linear combination is not estimable, then the estimable replacement is displayed. This provides the opportunity to inspect what combination is actually used to see if it represents the intentions.

Iteration History worksheet

This worksheet lists the fitting algorithm results. If the initial estimates are the REML estimates, then zero iterations are displayed.

Parameter Key worksheet

If the model includes random or repeated effects, the Parameter column in the Parameter Key worksheet contains the variance parameter names assigned by the Linear Mixed Effects object. If the Group option is not used in the model or if there is only one random or repeated model, then indices are not used in the parameter names.

Otherwise, two indices are appended to the variance parameter names in order to clarify which random or repeated model, or group, the variance parameter addresses. The first index indicates the random model or repeated model to which the parameter applies. An index of 1 indicates the model on the Random 1 tab in the Variance Structure tab. The highest index indicates the repeated model if there was one. The second index is the group index indicating that the parameters within the same group index are for the same group level.

Source: Random if the parameter is from a random model. Repeated if the parameter is from a repeated specification. Assumed if the variance parameter is the residual.

Type: Variance type specified by the user, or Identity, if the residual variance.
Bands: Number of bands or factors if appropriate for the variance type.
Subject_Term: Subject term for this variance structure, if there was a subject.
Group_Term: Group term for this variance structure, if there was a group.
Group_Level: Level of the group that this parameter goes with, if there was a group.

General linear mixed effects model

Phoenix's Linear Mixed Effects object is based on the general linear mixed effects model, which can be represented in matrix notation as:

$y = X\beta + Z\gamma + \varepsilon$

where:

X is the matrix of known constants and the design matrix for β
β is the vector of unknown (fixed effect) parameters
γ is the random unobserved vector (the vector of random-effects parameters), normally distributed with mean zero and variance G
Z is the matrix of known constants, the design matrix for γ
ε is the random vector, normally distributed with mean zero and variance R

γ and ε are uncorrelated. In the Linear Mixed Effects object, the Fixed Effects tab is used to specify the model terms for constructing X; the Random tabs in the Variance Structure tab specifies the model terms for constructing Z and the structure of G; and the Repeated tab specifies the structure of R.

In the linear mixed effects model, if $R=\sigma^2 I$ and Z=0, then the LinMix model reduces to the ANOVA model. Using the assumptions for the linear mixed effects model, one can show that the variance of y is: $V = ZGZ^T + R$.

If Z does not equal zero, i.e., the model includes one or more random effects, the G matrix is determined by the variance structures specified for the random models. If the model includes a repeated specification, then the R matrix is determined by the variance structure specified for the repeated model. If the model doesn't include a repeated specification, then R is the residual variance, that is $R = \sigma^2 I$.

It is instructive to see how a specific example fits into this framework. The fixed effects model is shown in the "Linear mixed effects scenario" section. Listing all the parameters in a vector, one obtains: $\beta^T - [\mu,\pi_1,\pi_2,\delta_1,\delta_2,\tau_1,\tau_2]$

For each element of b, there is a corresponding column in the X matrix. In this mode, the X matrix is composed entirely of ones and zeros. The exact method of constructing it is discussed under "Construction of the X matrix", below.

For each subject, there is one $S_{k(j)}$. The collection can be directly entered into γ. The Z matrix will consist of ones and zeros. Element (i, j) will be one when observation i is from subject j, and zero otherwise. The variance of γ is of the form

$$G \equiv Var(\gamma) = Var\{S_{k(j)}\} I_{n(1)+n(2)}$$

where I_b represents a $b \times b$ identity matrix.

The parameter to be estimated for G is Var{$S_{k(j)}$}. The residual variance is:

$$R = Var\{\varepsilon_{ijkm}\} = \sigma^2 I_{2[n(1)+n(2)]}$$

The parameter to be estimated for R is σ^2.

A summary of the option tabs and the part of the model they generate follows.

Fixed Effects tab specifies the X matrix and generates β.

Variance Structure tab specifies the Z matrix
The Random sub-tab(s) generates G = var(γ).
The Repeated tab generates R=var(ε).

Additional information is available on the following topics regarding the linear mixed effects general model:

Construction of the X matrix

A term is a variable or a product of variables. A user specifies a model by giving a sum of terms. For example, if A and B are variables in the dataset: $A + B + AB$.

Linear Mixed Effects takes this sum of terms and constructs the X matrix. The way this is done depends upon the nature of the variables A and B.

By default, the first column of X contains all ones. The LinMix object references this column as the intercept. To exclude it, select the **No Intercept** checkbox in the Fixed Effects tab. The rules for translating terms to columns of the X matrix depend on the variable types, regressor/covariate or classification. This is demonstrated using two classification variables, Drug and Form, and two continuous variables, Age and Weight, the "Indicator variables and product of classification with continuous variables" table. A term containing one continuous variable produces a single column in the X matrix.

An integer value is associated with every level of a classification variable. The association between levels and codes is determined by the numerical order for converted numerical variables and by the character collating sequence for converted character variables. When a single classification variable is specified for a model, an indicator variable is placed in the X matrix for each level of the variable. The "Indicator variables and product of classification with continuous variables" table demonstrates this for the variable Drug.

The model specification allows products of variables. The product of two continuous variables is the ordinary product within rows. The product of a classification variable with a continuous variable is obtained by multiplying the continuous value by each column of the indicator variables in each row. For example, the "Indicator variables and product of classification with continuous variables" table shows the product of Drug and Age. The "Two classification plus indicator variables" table shows two classification and indicator variables. The "Product of two classification variables" table gives their product.

The product operations described in the preceding paragraph can be succinctly defined using the Kronecker product. Let A=(a_{ij}) be a $m{\times}n$ matrix and let B=(b_{ij}) be a $p{\times}q$ matrix. Then the Kronecker product $A \otimes B$=($a_{\text{ij}}B$) is an $mp \times nq$ matrix expressible as a partitioned matrix with $a_{\text{ij}}\,B$ as the (i, j)th partition, i=1,…, m and j=1,…,n.

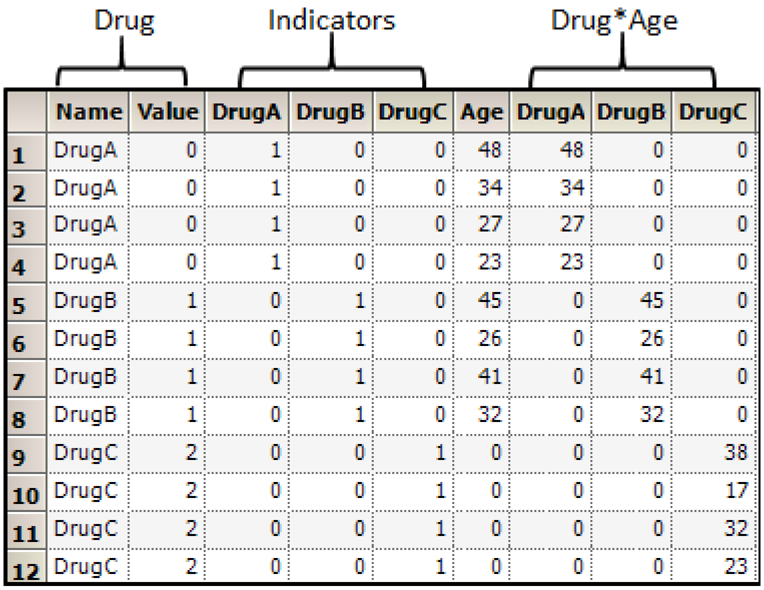
For example, consider the fifth row of the "Indicator variables and product of classification with continuous variables" table. The product of Drug and Age is:

$$\begin{bmatrix} 0 & 1 & 0 \end{bmatrix} \otimes \begin{bmatrix} 45 \end{bmatrix} = \begin{bmatrix} 0 & 45 & 0 \end{bmatrix}$$

Now consider the fifth row of the "Two classification plus indicator variables" table. The product of Drug and Form is:

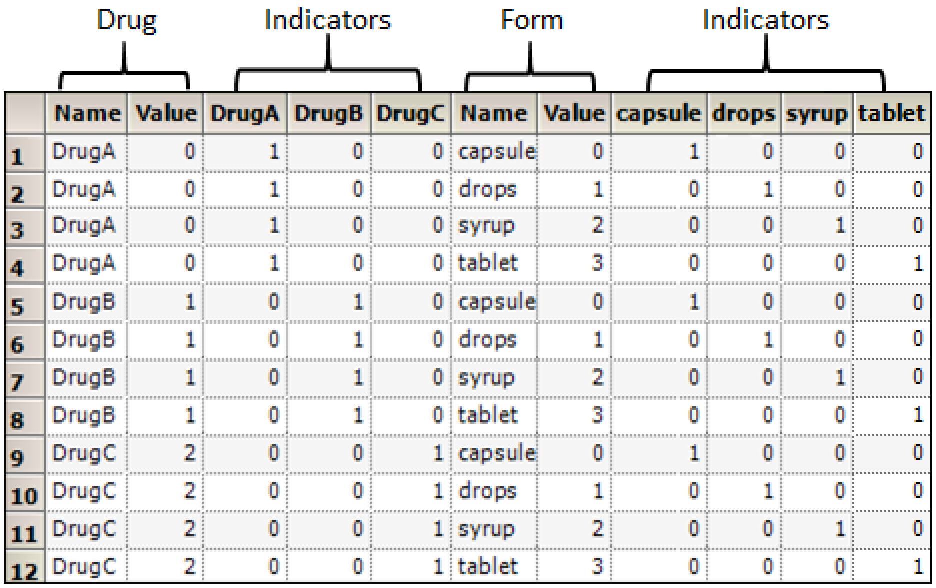
$$\begin{bmatrix} 0 & 1 & 0 \end{bmatrix} \otimes \begin{bmatrix} 1 & 0 & 0 & 0 \end{bmatrix} = \begin{bmatrix} 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{bmatrix}$$

The result is the fifth row of the “Product of two classification variables” table.


Drug
Indicators
Drug*Age

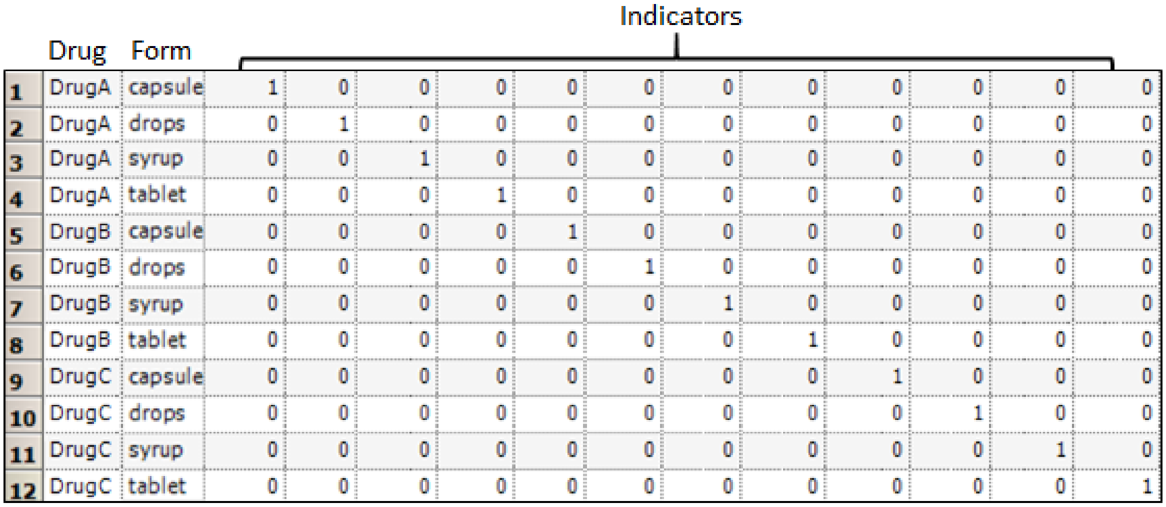
	Name	Value	DrugA	DrugB	DrugC	Age	DrugA	DrugB	DrugC
1	DrugA	0	1	0	0	48	48	0	0
2	DrugA	0	1	0	0	34	34	0	0
3	DrugA	0	1	0	0	27	27	0	0
4	DrugA	0	1	0	0	23	23	0	0
5	DrugB	1	0	1	0	45	0	45	0
6	DrugB	1	0	1	0	26	0	26	0
7	DrugB	1	0	1	0	41	0	41	0
8	DrugB	1	0	1	0	32	0	32	0
9	DrugC	2	0	0	1	0	0	0	38
10	DrugC	2	0	0	1	0	0	0	17
11	DrugC	2	0	0	1	0	0	0	32
12	DrugC	2	0	0	1	0	0	0	23

Figure 14-1. Indicator variables and product of classification with continuous variables


Drug
Indicators
Form
Indicators

	Name	Value	DrugA	DrugB	DrugC	Name	Value	capsule	drops	syrup	tablet
1	DrugA	0	1	0	0	capsule	0	1	0	0	0
2	DrugA	0	1	0	0	drops	1	0	1	0	0
3	DrugA	0	1	0	0	syrup	2	0	0	1	0
4	DrugA	0	1	0	0	tablet	3	0	0	0	1
5	DrugB	1	0	1	0	capsule	0	1	0	0	0
6	DrugB	1	0	1	0	drops	1	0	1	0	0
7	DrugB	1	0	1	0	syrup	2	0	0	1	0
8	DrugB	1	0	1	0	tablet	3	0	0	0	1
9	DrugC	2	0	0	1	capsule	0	1	0	0	0
10	DrugC	2	0	0	1	drops	1	0	1	0	0
11	DrugC	2	0	0	1	syrup	2	0	0	1	0
12	DrugC	2	0	0	1	tablet	3	0	0	0	1

Figure 14-2. Two classification plus indicator variables



	Drug	Form	Indicators											
1	DrugA	capsule	1	0	0	0	0	0	0	0	0	0	0	0
2	DrugA	drops	0	1	0	0	0	0	0	0	0	0	0	0
3	DrugA	syrup	0	0	1	0	0	0	0	0	0	0	0	0
4	DrugA	tablet	0	0	0	1	0	0	0	0	0	0	0	0
5	DrugB	capsule	0	0	0	0	1	0	0	0	0	0	0	0
6	DrugB	drops	0	0	0	0	0	1	0	0	0	0	0	0
7	DrugB	syrup	0	0	0	0	0	0	1	0	0	0	0	0
8	DrugB	tablet	0	0	0	0	0	0	0	1	0	0	0	0
9	DrugC	capsule	0	0	0	0	0	0	0	0	1	0	0	0
10	DrugC	drops	0	0	0	0	0	0	0	0	0	1	0	0
11	DrugC	syrup	0	0	0	0	0	0	0	0	0	0	1	0
12	DrugC	tablet	0	0	0	0	0	0	0	0	0	0	0	1

Figure 14-3. Product of two classification variables

Linear combinations of elements of beta

Most of the inference done by LinMix is done with respect to linear combinations of elements of β. Consider, as an example, the model where the expected value of the response of the j^{th} subject on the i^{th} treatment is:

$\mu + \tau_i$

where μ is a common parameter and $\mu + \tau_i$ is the expected value of the i^{th} treatment.

Suppose there are three treatments. The X matrix has a column of ones in the first position followed by three treatment indicators.

The β vector is: $\beta = [\beta_0\ \beta_1\ \beta_2\ \beta_3] = [\mu\ \tau_1\ \tau_2\ \tau_3]$

The first form is for the general representation of a model. The second form is specific to the model equation shown at the beginning of this section. The expression:

$$\sum_j l_j \beta_j$$

with the l_j constant, is called a linear combination of the elements of β.

The range of the subscript j starts with zero if the intercept is in the model and starts with one otherwise. Most common functions of the parameters, such as $\mu+\tau_1$, $\tau_1 - \tau_3$, and τ_2, can be generated by choosing appropriate values of l_j. Linear combinations of β are entered in LinMix through the Estimates and Contrasts tabs. A linear combination of β is said to be estimable if it can be expressed as a linear combination of expected responses. In the context of the model equation (shown at the beginning of this section), a linear combination of the elements of β is estimable if it can be generated by choosing c_1, c_2, and c_3.

$$c_1(\mu + \tau_1) + c_2(\mu + \tau_2) + c_3(\mu + \tau_3)$$

Note that c_1=1, c_2=0, and c_3=0 generates $\mu+\tau_1$, so $\mu+\tau_1$ is estimable. A rearrangement of the expression is:

$$(c_1 + c_2 + c_3)\mu + c_1\tau_1 + c_2\tau_2 + c_3\tau_3$$

This form of the expression makes some things more obvious. For example, l_j=c_j for j=1,2,3. Also, any linear combination involving only τs is estimable if, and only if, $l_1+l_2+l_3=0$. A linear combination of effects, where the sum of the coefficients is zero, is called a contrast. The linear combination $\tau_1 - \tau_3$ is a contrast of the τ's and thus is estimable. The linear combination τ_2 is not a contrast of the τ's and thus is not estimable.

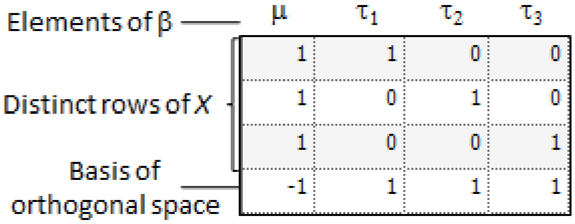
Determining estimability numerically

The X matrix for the model has three distinct rows, and these are shown in the table below. Also shown in the same table is a vector (call it z) that is a basis of the space orthogonal to the rows of X. Let l be a vector whose elements are the l_j of the linear combination expression. The linear combination in vector notation is $l^T b$ and is estimable if $l^T Z$=0. Allow for some rounding error in the computation of z. The linear combination is estimable if:

$$\frac{l^T Z}{\| l \|_\infty \sqrt{Z^T Z}} < tol$$

where $\| l \|_{\yen}$ is the largest absolute value of the vector, and ***tol*** is the Estimability Tolerance.

In general, the number of rows in the basis of the space orthogonal to the rows of X is equal the number of linear dependencies among the columns of X. If there is more than one z, then previous equation must be satisfied for each z. The following table shows the basis of the space orthogonal to the rows of X.


Elements of β
Distinct rows of X
Basis of orthogonal space

μ	τ1	τ2	τ3
1	1	0	0
1	0	1	0
1	0	0	1
-1	1	1	1

Substitution of estimable functions for non-estimable functions

It is not always easy for a user to determine if a linear combination is estimable or not. LinMix tries to be helpful, and if a user attempts to estimate a non-estimable function, LinMix will substitute a "nearby" function that is estimable. Notice is given in the text output when the substitution is made, and it is the user's responsibility to check the coefficients to see if they are reasonable. This section is to describe how the substitution is made.

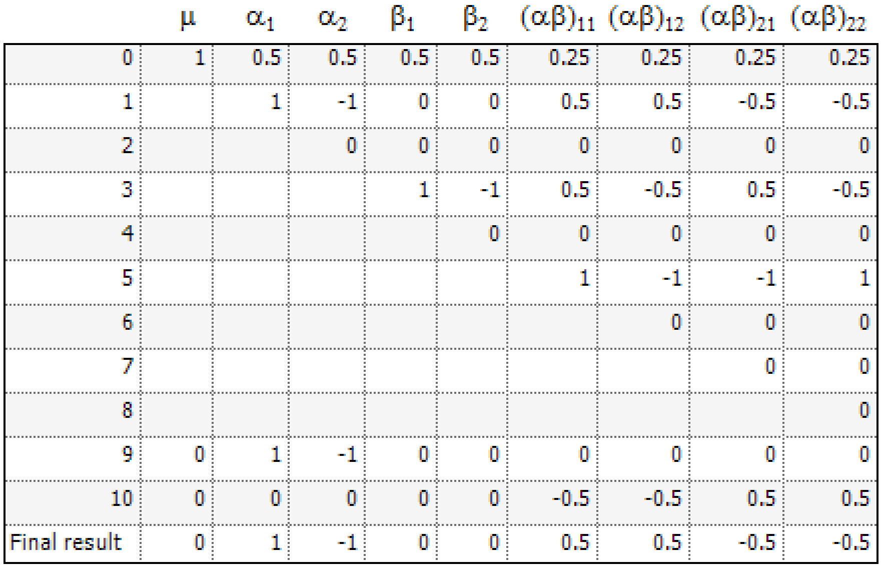
The most common attempt to estimate a non-estimable function of the parameters is probably trying to estimate a contrast of main effects in a model with interaction, so this will be used as an example. Consider the model:

$$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

where μ is the over-all mean; α_i is the effect of the i-th level of a factor A,i=1, 2; j is the effect of the j-th level of a factor B, j=1, 2; $(\alpha\beta)_{ij}$ is the effect of the interaction of the i-th level of A with the j-th level of B; and the ε_{ijk} are independently distributed $N(0, \sigma^2)$. The canonical form of the QR factorization of X is displayed in rows zero through eight of the table below. The coefficients to generate $\alpha_1 - \alpha_2$ are shown in row 9. The process is a sequence of regressions followed by residual computations. In the μ column, row 9 is already zero so no operation is done. Within the α columns, regress row 9 on row 3. (Row 4 is all zeros and is ignored.) The regression coefficient is one. Now calculate residuals: (row 9) – 1× (row 3). This operation goes across the entire matrix, i.e., not just the α columns. The result is shown in row 10, and the next operation applies to row 10. The numbers in the β columns of row 10 are zero, so skip to the $\alpha\beta$ columns. Within the $\alpha\beta$ columns, regress row 10 on row 5. (Rows 6 through 8 are all zeros and are ignored.). The regression coefficient is zero, so there is no need to compute residuals. At this point, row 10 is a deviation of row 9 from an estimable function. Subtract the deviation (row 10) from the original (row 9) to get the estimable function displayed in the last row. The result is the expected value of the difference between the marginal A means. This is likely what the user had in mind.

This section provides a general description of the process just demonstrated on a specific model and linear combination of elements of β. Partition the QR factorization of X vertically and horizontally corresponding to the terms in X. Put the coefficients of a linear combination in a work row. For each vertical partition, from left to right, do the following:

- Regress (i.e., do a least squares fit) the work row on the rows in the diagonal block of the QR factorization.
- Calculate the residuals from this fit across the entire matrix. Overwrite the work row with these residuals.
- Subtract the final values in the work row from the original coefficients to obtain coefficients of an estimable linear combination.



	μ	α_1	α_2	β_1	β_2	$(\alpha\beta)_{11}$	$(\alpha\beta)_{12}$	$(\alpha\beta)_{21}$	$(\alpha\beta)_{22}$
0	1	0.5	0.5	0.5	0.5	0.25	0.25	0.25	0.25
1		1	-1	0	0	0.5	0.5	-0.5	-0.5
2			0	0	0	0	0	0	0
3				1	-1	0.5	-0.5	0.5	-0.5
4					0	0	0	0	0
5						1	-1	-1	1
6							0	0	0
7								0	0
8									0
9	0	1	-1	0	0	0	0	0	0
10	0	0	0	0	0	-0.5	-0.5	0.5	0.5
Final result	0	1	-1	0	0	0.5	0.5	-0.5	-0.5

Figure 14-4. QR factorization of Z scaled to canonical form

Fixed effects

The specification of the fixed effects (as defined in “General linear mixed effects model” introduction) can contain both classification variables and continuous regressors. In addition, the model can include interaction and nested terms.

Output parameterization
Crossover example continued

Output parameterization

Suppose X is $n \times p$, where n is the number of observations and p is the number of columns. If X has rank p, denoted rank(X), then each parameter can be uniquely estimated. If rank(X) < p, then there are infinitely many solutions. To solve this issue, one must impose additional constraints on the estimator of β.

Suppose column j is in the span of columns 0, 1,..., $j - 1$. Then column j provides no additional information about the response y beyond that of the columns that come before. In this case, it seems sensible to not use the column in the model fitting. When this happens, its parameter estimate is set to zero.

As an example, consider a simple one-way ANOVA model, with three levels. The design matrix is:

$$\left[\begin{array}{cccc} X_0 & X_1 & X_2 & X_3 \\ 1 & 1 & 0 & 0 \\ 1 & 0 & 1 & 0 \\ 1 & 0 & 0 & 1 \\ 1 & 1 & 0 & 0 \\ 1 & 0 & 1 & 0 \\ 1 & 0 & 0 & 1 \end{array}\right]$$

X_0 is the intercept column. X_1, X_2, and X_3 correspond to the three levels of the treatment effect. X_0 and X_1 are clearly linearly independent, as is X_2 linearly independent of X_0 and X_1. But X_3 is not linearly independent of X_0, X_1, and X_2, since $X_3 = X_0 - X_1 - X_2$. Hence β_3 would be set to zero. The degrees of freedom for an effect is the number of columns remaining after this process. In this example, treatment effect has two degrees of freedom, the number typically assigned in ANOVA.

See “Residuals worksheet and predicted values” for computation details.

Singularity tolerance

If intercept is in the model, then center the data. Perform a Gram-Schmidt orthogonalization process. If the norm of the vector after GS divided by the norm of the original vector is less than δ, then the vector is called singular.

Transformation of the response

The Dependent Variables Transformation menu provides three options: No transformation, ln transformation ($\log_e$), or log transformation ($\log_{10}$). Transformations are applied to the response before model fitting. Note that all estimates using log will be the same estimates found using ln, only scaled by ln 10, since log(x)=ln(x)/ln(10).

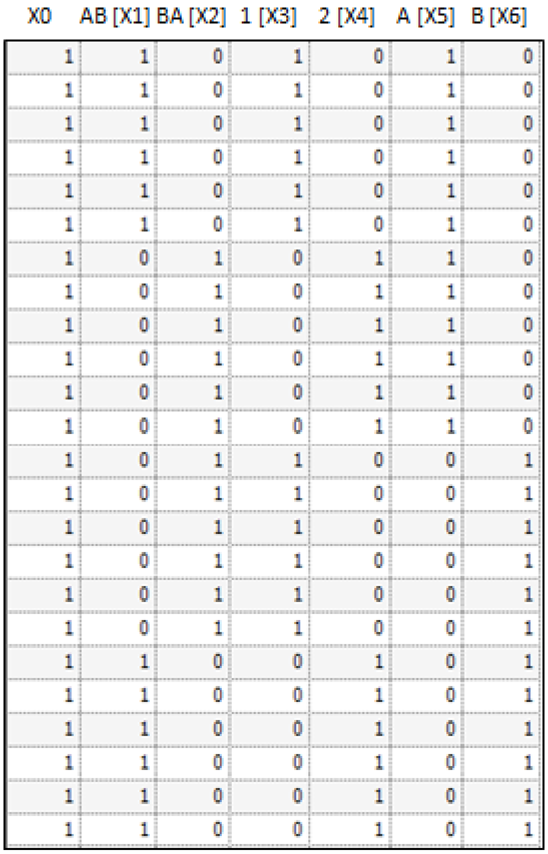
If the standard deviation is proportional to the mean, taking the logarithm often stabilizes the variance, resulting in better fit. This implicitly assumes that the error structure is multiplicative with lognormal distribution.

Crossover example continued

The fixed effects are given in the linear combination expression. Using that model, the design matrix X would be expanded into that presented in the following table. The vector of fixed effects corresponding to that design matrix would be: $\beta = (\beta_0,\beta_1,\beta_2,\beta_3,\beta_4,\beta_5,\beta_6)$.

Notice that $X_2=X_0 - X_1$. Hence set $\beta_2=0$. Similarly, $X_4=X_0 - X_3$ and $X_6=X_0 - X_5$, and hence set $\beta_4=0$ and $\beta_6=0$.

The table below shows the design matrix expanded for a the fixed effects model Sequence + Period +Treatment.



X0	AB [X1]	BA [X2]	1 [X3]	2 [X4]	A [X5]	B [X6]
1	1	0	1	0	1	0
1	1	0	1	0	1	0
1	1	0	1	0	1	0
1	1	0	1	0	1	0
1	1	0	1	0	1	0
1	1	0	1	0	1	0
1	0	1	0	1	1	0
1	0	1	0	1	1	0
1	0	1	0	1	1	0
1	0	1	0	1	1	0
1	0	1	0	1	1	0
1	0	1	0	1	1	0
1	0	1	1	0	0	1
1	0	1	1	0	0	1
1	0	1	1	0	0	1
1	0	1	1	0	0	1
1	0	1	1	0	0	1
1	0	1	1	0	0	1
1	1	0	0	1	0	1
1	1	0	0	1	0	1
1	1	0	0	1	0	1
1	1	0	0	1	0	1
1	1	0	0	1	0	1
1	1	0	0	1	0	1

Variance structure

The LinMix Variance Structure tab specifies the Z, G, and R matrices defined in "General linear mixed effects model" introduction. The user may have zero, one, or more random effects specified. Only one repeated effect may be specified. The Repeated effect specifies the R=Var(ε). The Random effects specify Z and the corresponding elements of G=Var(γ).

Repeated effect
Random effects
Multiple random effects vs. multiple effects on one random effect
Covariance structure types in the Linear Mixed Effects object

Repeated effect

The repeated effect is used to model a correlation structure on the residuals. Specifying the repeated effect is optional. If no repeated effect is specified, then $R=\sigma^2 I_N$ is used, where N denotes the number of observations, and I_N is the $N \times N$ identity matrix.

All variables used on this tab of the Linear Mixed Effects object must be classification variables.

To specify a particular repeated effect, one must have a classification model term that uniquely identifies each individual observation. Put the model term in the Repeated Specification field. Note that a model term can be a variable name, an interaction term (e.g., Time*Subject), or a nested term (e.g., Time(Subject)).

The variance blocking model term creates a block diagonal R matrix. Suppose the variance blocking model term has b levels, and further suppose that the data are sorted according to the variance blocking variable. Then R would have the form:

$$R = I_b \otimes \Sigma$$

where Σ is the variance structure specified.

The variance Σ is specified using the Type pull-down menu. Several variance structures are possible, including unstructured, autoregressive, heterogeneous compound symmetry, and no-diagonal factor analytic. See the Help file for the details of the variance structures. The autoregressive is a first-order autoregressive model.

To model heterogeneity of variances, use the group variable. Group will accept any model term. The effect is to create additional parameters of the same variance structure. If a group variable has levels g=1, 2,…, n_g, then the variance for observations within group g will be Σ_g.

An example will make this easier to understand. Suppose five subjects are randomly assigned to each of three treatment groups; call the treatment groups T_1, T_2, and T_3. Suppose further that each subject is measured in periods t=0, 1, 2, 3, and that serial correlations among the measurements are likely. Suppose further that this correlation is affected by the treatment itself, so the correlation structure will differ among T_1, T_2, and T_3. Without loss of generality, one may assume that the data are sorted by treatment group, then subject within treatment group.

First consider the effect of the group. It produces a variance structure that looks like:

$$R = \begin{bmatrix} R_1 & 0 & 0 \\ 0 & R_2 & 0 \\ 0 & 0 & R_3 \end{bmatrix}$$

where each element of R is a 15×15 block. Each R_g has the same form. Because the variance blocking variable is specified, the form of each R_g is:

$$I_5 \otimes \Sigma_g$$

I_5 is used because there are five subjects within each treatment group. Within each subject, the variance structured specified is:

$$\sigma_g^2\begin{bmatrix} 1 & \rho_g & \rho_g^2 & \rho_g^3 \\ \rho_g & 1 & \rho_g & \rho_g^2 \\ \rho_g^2 & \rho_g & 1 & \rho_g \\ \rho_g^3 & \rho_g^2 & \rho_g & 1 \end{bmatrix}$$

This structure is the autoregressive variance type. Other variance types are also possible. Often compound symmetry will effectively mimic autoregressive in cases where autoregressive models fail to converge.

The output will consist of six parameters: σ^2 and ρ for each of the three treatment groups.

Random effects

Unlike the (single) repeated effect, it is possible to specify up to 10 random effects. Additional random effects can be added by clicking **Add Random**. To delete a previously specified random effect, click **Delete Random**. It is not possible to delete all random effects; however, if no entries are made in the random effect, then it will be ignored.

Model terms entered into the Random Effects model produce columns in the Z matrix, constructed in the same way that columns are produced in the X matrix. It is possible to put more than one model term in the Random Effects Model, but each random page will correspond to a single variance type. The variance matrix appropriate for that type is sized according to the number of columns produced in that random effect.

The Random Intercept checkbox puts an intercept column (that is, all ones) into the Z matrix.

The Variance Blocking Variables has the effect of inducing independence among the levels of the Variance Blocking Variables. Mathematically, it expands the Z matrix by taking the Kronecker product of the Variance Blocking Variables with the columns produced by the intercept checkbox and the Random Effects Model terms.

The Group model term is used to model heterogeneity of the variances among the levels of the Group model term, similarly to Repeated Effects.

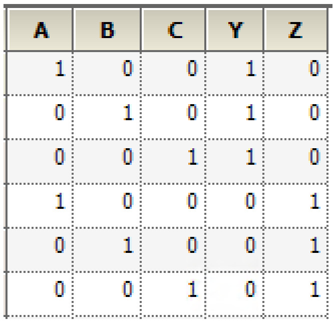
Multiple random effects vs. multiple effects on one random effect

Suppose one has two random effect variables, $R1$ and $R2$. Suppose further that $R1$ has three levels, A, B, and C, while $R2$ has two levels, Y and Z. The hypothetical data are shown as follows.

- R1 values: A, B, C, A, B, C
- R2 values: Y, Y, Y, Z, Z, Z

Specifying a random effect of $R1$+$R2$ will produce different results than specifying $R1$ on Random 1 and $R2$ on Random 2 pages. This example models the variance using the compound symmetry variance type. To illustrate this, build the resulting Z and G matrices.

For $R1+R2$ on Random 1 page, put the columns of each variable in the Z matrix to get the following.



A	B	C	Y	Z
1	0	0	1	0
0	1	0	1	0
0	0	1	1	0
1	0	0	0	1
0	1	0	0	1
0	0	1	0	1

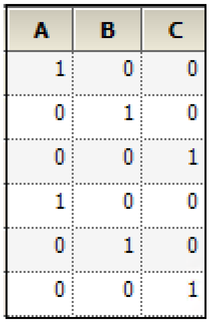
The random effects can be placed in a vector: $\gamma = (\gamma_1,\gamma_2,\gamma_3,\gamma_4,\gamma_5)$

$R1$ and $R2$ share the same variance matrix G where:

$$G = \begin{bmatrix} \sigma_1^2 & \sigma_2^2 & \sigma_3^2 & \sigma_4^2 & \sigma_5^2 \\ \sigma_2^2 & \sigma_1^2 & \sigma_2^2 & \sigma_3^2 & \sigma_4^2 \\ \sigma_3^2 & \sigma_2^2 & \sigma_1^2 & \sigma_2^2 & \sigma_3^2 \\ \sigma_4^2 & \sigma_3^2 & \sigma_2^2 & \sigma_1^2 & \sigma_2^2 \\ \sigma_5^2 & \sigma_4^2 & \sigma_3^2 & \sigma_2^2 & \sigma_1^2 \end{bmatrix}$$

Now, consider the case where $R1$ is placed on Random 1 tab, and $R2$ is placed on Random 2 tab. For $R1$, the Z matrix columns are:

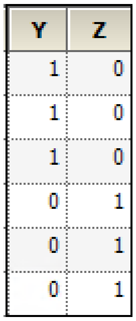
Variance Matrix



A	B	C
1	0	0
0	1	0
0	0	1
1	0	0
0	1	0
0	0	1

$$G_1 = \begin{bmatrix} \sigma_1^2 & \sigma_2^2 & \sigma_3^2 \\ \sigma_2^2 & \sigma_1^2 & \sigma_2^2 \\ \sigma_3^2 & \sigma_2^2 & \sigma_1^2 \end{bmatrix}$$

For $R2$, the columns in the Z matrix and the corresponding G is:



Y	Z
1	0
1	0
1	0
0	1
0	1
0	1

Variance Matrix

$$G_2 = \begin{bmatrix} \sigma_4^2 & \sigma_5^2 \\ \sigma_5^2 & \sigma_4^2 \end{bmatrix}$$

In this case:

$$G = \begin{bmatrix} G_1 & 0 \\ 0 & G_1 \end{bmatrix}$$

Covariance structure types in the Linear Mixed Effects object

The Type menu in the Variance Structure tab allows users to select a variety of covariance structures. The variances of the random-effects parameters become the covariance parameters for this model. Mixed linear models contain both fixed effects and random effects parameters. For more on variance structures see "Variance structure" for an explanation of variance in linear mixed effects models. See "Covariance structure types" in the Bioequivalence section for descriptions of covariance structures.

Contrasts

A contrast is a linear combination of the parameters associated with a term where the coefficients sum to zero. Contrasts facilitate the testing of linear combinations of parameters. For example, consider a completely randomized design with four treatment groups: Placebo, Low Dose, Medium Dose, and High Dose. The user may wish to test the hypothesis that the average of the dosed groups is the same as the average of the placebo group. One could write this hypothesis as:

$$H_0\colon \mu_{Placebo} - \frac{(\mu_{Low} + \mu_{Medium} + \mu_{High})}{3} = 0$$

or, equivalently:

$$\mu_{Placebo} - (\mu_{Low} + \mu_{Medium} + \mu_{High}) = 0$$

Both of these are contrasts since the sum of the coefficients is zero. Note that both contrasts make the same statement. This is true generally: contrasts are invariant under changes in scaling.

Contrasts are tested using the Wald (1943) test statistic:

$$(L\hat{\beta})^T \left(L(X^T \hat{V} X)^{-1} L^T \right)^{-1} (L\hat{\beta})$$

where:

- $\hat{\beta}$ is the estimator of β.
- $\hat{V}$ is the estimator of V.
- L must be a matrix such that each row is in the row space of X.

This requirement is enforced in the wizard. The Wald statistic is compared to an F distribution with rank(L) numerator degrees of freedom and denominator degrees of freedom estimated by the program or supplied by the user.

Joint versus single contrasts
Nonestimability of contrasts
Degrees of freedom
Other options

Joint versus single contrasts

Joint contrasts are constructed when the number of columns for contrast is changed from the default value of one to a number larger than one. Note that this number must be no more than one less than the number of distinct levels for the model term of interest. This tests both hypotheses jointly, i.e., in a single test with a predetermined level of the test.

A second approach would be to put the same term in the neighboring contrast, both of which have one column. This approach will produce two independent tests, each of which is at the specified level.

To see the difference, compare Placebo to Low Dose and Placebo to Medium Dose. Using the first approach, enter the coefficients as follows.

Title = Join tests
Effect = Treatment
Contrast = Treatment
#1 Placebo –1 –1
#2 Low Dose 1 0
#3 Medium Dose 0 1
#4 High Dose 0 0

This will produce one test, testing the following hypothesis.

$$H_0 : \begin{bmatrix} -1 & 1 & 0 & 0 \\ -1 & 0 & 1 & 0 \end{bmatrix} \beta = \begin{bmatrix} 0 \\ 0 \end{bmatrix}$$

Now compare this with putting the second contrast in its own contrast.

Contrast #1

Title Low vs. Placebo
Effect Treatment
Contrast Treatment
#1 Placebo –1
#2 Low Dose 1
#3 Medium Dose 0
#4 High Dose 0

Contrast #2

Title Medium vs. Placebo
Effect Treatment
Contrast Treatment
#1 Placebo –1

#2 Low Dose 0
#3 Medium Dose 1
#4 High Dose 0

This produces two tests:

$$H_{01}: \begin{bmatrix} -1 & 1 & 0 & 0 \end{bmatrix} \beta = \begin{bmatrix} 0 \end{bmatrix} \qquad H_{02}: \begin{bmatrix} -1 & 0 & 1 & 0 \end{bmatrix} \beta = \begin{bmatrix} 0 \end{bmatrix}$$

Note that this method inflates the overall Type I error rate to approximately 2α, whereas the joint test maintains the overall Type I error rate to α, at the possible expense of some power.

Nonestimability of contrasts

If a contrast is not estimable, then an estimable function will be substituted for the nonestimable function. See “Substitution of estimable functions for non-estimable functions” for the details.

Degrees of freedom

The **User specified degrees of freedom** checkbox available on the Contrasts, Estimates, and Least Squares Means tabs, allows the user to control the denominator degrees of freedom in the F approximation. The numerator degrees of freedom is always calculated as rank(L). If the checkbox is not selected, then the default denominator degrees of freedom will be used. The default calculation method of the degrees of freedom is controlled on the General Options tab and is initially set to **Satterthwaite**.

Note: For a purely fixed effects model, Satterthwaite and residual degrees of freedom yield the same number. For details of the Satterthwaite approximation, see “Satterthwaite approximation for degrees of freedom”.

To override the default degrees of freedom, select the checkbox and type an appropriate number in the field. The degrees of freedom must greater than zero.

Other options

To control the estimability tolerance, enter an appropriate tolerance in the Estimability Tolerance field. For more information see “Substitution of estimable functions for non-estimable functions”.

The Show Coefficients checkbox produces extra output, including the coefficients used to construct the tolerance. If the contrast entered is estimable, then it will repeat the contrasts entered. If the contrasts are not estimable, it will enter the estimable function used instead of the nonestimable function.

Estimates

The Estimates tab produces estimates output instead of contrasts. As such, the coefficients need not sum to zero. Additionally, multiple model terms may be included in a single estimate.

Unlike contrasts, estimates do not support joint estimates. The intervals are marginal. The level must be between zero and 100. Note that it is entered as a percent: Entering 0.95 will yield a very narrow interval with coverage just less than 1%.

All other options act similarly to the corresponding options in the Contrasts tab.

Least squares means

Sometimes a user wants to see the mean response for each level of a classification variable or for each combination of levels for two or more classification variables. If there are covariables with unequal means for the different levels, or if there are unbalanced data, the subsample means are not estimates that can be validly compared. This section describes least squares means, statistics that make proper adjustments for unequal covariable means and unbalanced data in the linear mixed effects model.

Consider a completely randomized design with a covariable. The model is:

$$y_{ij} = \alpha_i + x_{ij}\beta + \varepsilon_{ij}$$

where y_{ij} is the observed response on the j^{th} individual on the i^{th} treatment; α_i is the intercept for the i^{th} treatment; x_{ij} is the value of the covariable for the j^{th} individual in the i^{th} treatment; β is the slope with respect to x; and ε_{ij} is a random error with zero expected value. Suppose there are two treatments; the average of the x_{1j} is 5; and the average of the x_{2j} is 15. The respective expected values of the sample means are α_1+5β and α_2+15β. These are not comparable because of the different coefficients of β. Instead, one can estimate α_1+10β and α_2+10β where the overall mean of the covariable is used in each linear combination.

Now consider a 2 × 2 factorial design. The model is:

$$y_{ijk} = \mu + \alpha_i + \beta_j + \varepsilon_{ijk}$$

where y_{ijk} is the observed response on the k^{th} individual on the i^{th} level of factor A and the j^{th} level of factor B; μ is the over-all mean; α_i is the effect of the i^{th} level of factor A; β_j is the effect of the j^{th} level of factor B; and ε_{ijk} is a random error with zero expected value. Suppose there are six observations for the combinations where $i=j$ and four observations for the combinations where $i \neq j$. The respective expected values of the averages of all values on level 1 of A and the averages of all values on level 2 of A are μ + (0.6 β_1 + 0.4 β_2) + α_1 and μ + (0.4 β_1 + 0.6 β_2) + α_2. Thus, sample means cannot be used to compare levels of A because they contain different functions of β_1 and β_2. Instead, one compares the linear combinations:

$$\mu + \frac{(\beta_1 + \beta_2)}{2} + \alpha_1 \text{ and } \mu + \frac{(\beta_1 + \beta_2)}{2} + \alpha_2$$

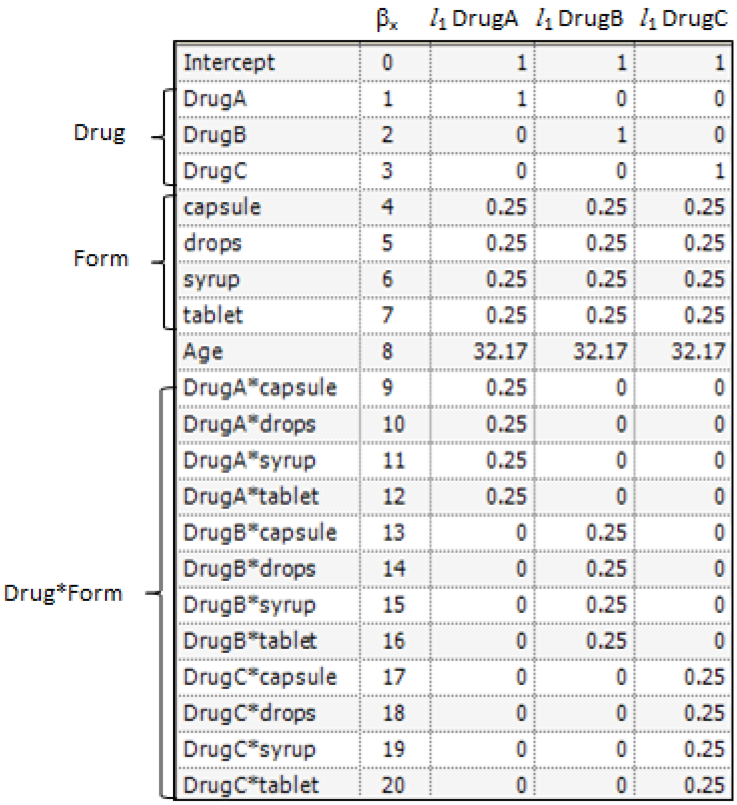
The preceding examples constructed linear combinations of parameters, in the presence of unbalanced data, that represent the expected values of sample means in balanced data. This is the idea behind least squares means. Least squares means are given in the context of a defining term, though the process can be repeated for different defining terms for the same model. The defining term must contain only classification variables and it must be one of the terms in the model. Treatment is the defining term in the first example, and factor A is the defining term in the second example. When the user requests least squares means, LinMix automatically generates the coefficients l_j of the linear combination expression and processes them almost as it would process the coefficients specified in an estimate statement. This chapter describes generation of linear combinations of elements of β that represent least squares means. A set of coefficients are created for each of all combinations of levels of the classification variables in the defining term. For all variables in the model, but not in the defining term, average values of the variables are the coefficients. The average value of a numeric variable (covariable) is the average for all cases used in the model fitting. For a classification variable with k levels, assume the average of each indicator variable is 1/k. The value 1/k would be the actual average if the data were balanced. The values of all variables in the model have now been defined. If some terms in the model are products, the products are formed using the same rules used for constructing rows of the X matrix as described in the "Fixed effects" section. It is possible that some least squares means are not estimable.

For example, suppose the fixed portion of the model is: Drug + Form + Age + Drug*Form

To get means for each level of Drug, the defining term is Drug. Since Drug has three levels, three sets of coefficients are created. Build the coefficients associated the first level of Drug, DrugA. The first coefficient is one for the implied intercept. The next three coefficients are 1, 0, and 0, the indicator variables associated with DrugA. Form is not in the defining term, so average values are used. The next four coefficients are all 0.25, the average of a four factor indicator variable with balanced data. The next coefficient is 32.17, the average of Age. The next twelve elements are:

$$\begin{aligned} &\begin{bmatrix}1 & 0 & 0\end{bmatrix} \otimes \begin{bmatrix}0.25 & 0.25 & 0.25 & 0.25\end{bmatrix} \\ &= \begin{bmatrix}0.25 & 0.25 & 0.25 & 0.25 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0\end{bmatrix} \end{aligned}$$

The final result is shown in the DrugA column in the following table. The results for DrugB and DrugC are also shown in the table. No new principles would be illustrated by finding the coefficients for the Form least squares means. The coefficients for the Drug*Form least squares means would be like representative rows of X except that Age would be replaced by the average of Age.



		β_x	l_1 DrugA	l_1 DrugB	l_1 DrugC
	Intercept	0	1	1	1
Drug	DrugA	1	1	0	0
	DrugB	2	0	1	0
	DrugC	3	0	0	1
Form	capsule	4	0.25	0.25	0.25
	drops	5	0.25	0.25	0.25
	syrup	6	0.25	0.25	0.25
	tablet	7	0.25	0.25	0.25
	Age	8	32.17	32.17	32.17
Drug*Form	DrugA*capsule	9	0.25	0	0
	DrugA*drops	10	0.25	0	0
	DrugA*syrup	11	0.25	0	0
	DrugA*tablet	12	0.25	0	0
	DrugB*capsule	13	0	0.25	0
	DrugB*drops	14	0	0.25	0
	DrugB*syrup	15	0	0.25	0
	DrugB*tablet	16	0	0.25	0
	DrugC*capsule	17	0	0	0.25
	DrugC*drops	18	0	0	0.25
	DrugC*syrup	19	0	0	0.25
	DrugC*tablet	20	0	0	0.25

Comparing the Linear Mixed Effects object to SAS PROC MIXED

The following outlines differences between LinMix and PROC MIXED in SAS.

Degrees of freedom

LinMix: Uses Satterthwaite approximation for all degrees of freedom, by default. User can also select Residual.

PROC MIXED: Chooses different defaults based on the model, random, and repeated statements. To match LinMix, set the SAS '`ddfm=satterth`' option.

Multiple factors on the same random command

LinMix: Effects associated with the matrix columns from a single random statement are assumed to be correlated per the variance specified. Regardless of variance structure, if random effects are correlated, put them on the same random tab; if independent, put them on separate tabs.

PROC MIXED: Similar to LinMix, except it does not follow this rule in the case of Variance Components, so LinMix and SAS will differ for models with multiple terms on a random statement using the VC structure.

REML

LinMix: Uses REML (Restricted Maximum Likelihood) estimation, as defined in Corbeil and Searle (1976), does maximum likelihood estimation on the residual vector.

PROC MIXED: Modifies the REML by 'profiling out' residual variance. To match LinMix, set the '`noprofile`' option on the PROC MIXED statement. In particular, LinMix `–log(likelihood)` may not equal twice SAS '`–2 Res Log Like`' if `noprofile` is not set.

Akaike and Schwarz's criteria

LinMix: Uses the smaller-is-better form of AIC and SBC in order to match Phoenix.

PROC MIXED: Uses the bigger-is-better form. In addition, AIC and SBC are functions of the likelihood, and the likelihood calculation method in LinMix also differs from SAS.

Saturated variance structure

LinMix: If the random statement saturates the variance, the residual is not estimable. Sets the residual variance to zero. A user-specified variance structure takes priority over that supplied by LinMix.

PROC MIXED: Sets the residual to some other number. To match LinMix, add the SAS residual to the other SAS variance parameter estimates.

Convergence criteria

LinMix: The convergence criterion is $g^T H^{-1} g$ < tolerance with H positive definite, where g is the gradient and H is the Hessian of the negative log-likelihood.

PROC MIXED: The default convergence criterion options are '`convh`' and '`relative`'. The LinMix criterion is equivalent to SAS with the '`convh`' and '`absolute`' options set.

Modified likelihood

LinMix: Tries to keep the final Hessian (and hence the variance matrix) positive definite. Determines when the variance is over-specified and sets some parameters to zero.

PROC MIXED: Allows the final Hessian to be nonpositive definite, and hence will produce different final parameter estimates. This often occurs when the variance is over-specified (i.e., too many parameters in the model).

Keeping VC positive

LinMix: For VC structure, LinMix keeps the final variance matrix positive definite, but will not force individual variances > 0.

PROC MIXED: If a negative variance component is estimated, 0 is reported in the output.

Partial tests of model effects

LinMix: The partial tests in LinMix are not equivalent to the Type III method in SAS though they coincide in most situations. See "Partial Tests worksheet".

PROC MIXED: The Type III results can change depending on how one specifies the model. For details, consult the SAS manual.

Not estimable parameters

LinMix: If a variable is not estimable, 'Not estimable' is reported in the output. LinMix provides the option to write out 0 instead.

PROC MIXED: SAS writes the value of 0.

ARH, CSH variance structures

LinMix: Uses the standard deviations for the parameters, and prints the standard deviations in the output.

PROC MIXED: Prints the variances in the output for final parameters, though the model is expressed in terms of standard deviations.

CS variance structure

LinMix: Does not estimate a Residual parameter, but includes it in the csDiag parameter.

PROC MIXED: Estimates Residual separately. $csDiag = Variance + Residual$ in SAS.

UN variance structure

LinMix: Does not estimate a Residual parameter, but includes it in the diagonal term of the UN matrix.

PROC MIXED: Estimates Residual separately. $UN\,(1{,}1) = UN(1{,}1) + Residual$ in SAS, etc. (If a weighting variable is used, the Residual is multiplied by 1/weight.)

Repeated without any time variable

LinMix: Requires an explicit repeated model. To run some SAS examples, a time variable needs to be added.

PROC MIXED: Does not require an explicit repeated model. It instead creates an implicit time field and assumes data are sorted appropriately.

Group as classification variable

LinMix: Requires variables used for the subject and group options to be declared as classification variables.

PROC MIXED: Allows regressors, but requires data to be sorted and considers each new regressor value to indicate a new subject or group. Classification variables are treated similarly.

Singularity tolerance

LinMix: Define d_{ii} as the i-th diagonal element of the QR factorization of X, and D_{ii} the sum of squares of the i-th column of the QR factorization. The i-th column of X is deemed linearly dependent on the proceeding columns if d_{ii}^2 < (singularity tolerance)×D_{ii}. If intercept is present, the first row of D_{ii} is omitted.

PROC MIXED: Define d_{ii} as the diagonal element of the $X^{\text{T}}X$ matrix during the sweeping process. If the absolute diagonal element $|d_{\text{ii}}|$ < (singularity tolerance)×D_{ii}, where D_{ii} is the original diagonal of $X^{\mathsf{T}}X$, then the parameter associated with the column is set to zero, and the parameter estimates are flagged as biased.

References

Allen and Cady (1982). *Analyzing Experimental Data By Regression*. Lifetime Learning Publications, Belmont, CA

Corbeil and Searle (1976). Restricted maximum likelihood (REML) estimation of variance components in the mixed models, *Technometrics*, 18:31–8

Fletcher (1980). Practical methods of optimization, Vol. 1: *Unconstrained Optimization.* John Wiley & Sons, New York

Giesbrecht and Burns (1985). Two-stage analysis based on a mixed model: Large sample asymptotic theory and small-sample simulation results. *Biometrics* 41:477–86

Gill, Murray and Wright (1981). *Practical Optimization*. Academic Press

Wald (1943). Tests of statistical hypotheses concerning several parameters when the number of observations is large. *Transaction of the American Mathematical Society* 54

Linear mixed effects computations

The following sections cover the methods the Linear Mixed Effects object uses to create model results.

Restricted maximum likelihood
Newton algorithm for maximization of restricted likelihood
Starting values
Convergence criterion
Satterthwaite approximation for degrees of freedom
Linear mixed effects scenario

Restricted maximum likelihood

For linear mixed effects models that contain random effects, the first step in model analysis is determining the maximum likelihood estimates of the variance parameters associated with the random effects. This is accomplished in LinMix using Restricted Maximum Likelihood (REML) estimation.

The linear mixed effects model described under "General linear mixed effects model" is:

$$\begin{aligned} y &= X\beta + Z\gamma + \varepsilon \\ V &= Variance(y) = ZGZ^T + R \end{aligned}$$

Let θ be a vector consisting of the variance parameters in G and R. The full maximum likelihood procedure (ML) would simultaneously estimate both the fixed effects parameters β and the variance parameters θ by maximizing the likelihood of the observations y with respect to these parameters. In contrast, restricted maximum likelihood estimation (REML) maximizes a likelihood that is only a function of the variance parameters θ and the observations y, and not a function of the fixed effects parameters. Hence for models that do not contain any fixed effects, REML would be the same as ML.

To obtain the restricted likelihood function in a form that is computationally efficient to solve, the data matrix $[X \mid Z \mid y]$ is reduced by a QR factorization to an upper triangular matrix. Let $[R_0 \mid R_1 \mid R_2]$ be an orthogonal matrix such that the multiplication with $[X \mid Z \mid y]$ results in an upper triangular matrix:

$$\begin{bmatrix} R_0^T \\ R_1^T \\ R_2^T \end{bmatrix} [X|Z|y] = \begin{bmatrix} T_{00} & T_{01} & y_0 \\ 0 & T_{11} & y_r \\ 0 & 0 & y_e \end{bmatrix}$$

REML estimation maximizes the likelihood of θ based on y_r and y_e of the reduced data matrix, and ignores y_0. Since y_e has a distribution that depends only on the residual variance, and since y_r and y_e are independent, the negative of the log of the restricted likelihood function is the sum of the separate negative log-likelihood functions:

$$\begin{aligned} \log(L(\theta;y_r,y_e)) &= \log(L(\theta;y_r)) + \log(L(\theta;y_e)) \\ &\quad - \frac{N - rank(X)}{2\log(2\pi)} \end{aligned}$$

where:

$\log(L(\theta; y_r))=-½ \log(|V_r|) - ½ y_r^T V_r^{-1} y_r$

$\log(L(\theta; y_e))=-n_e/2 \log(\theta_e) - ½ (y_e^T y_e/\theta_e)$

V_r is the Variance(y_r)

θ_e is the residual variance

n_e is the residual degrees of freedom

N is the number of observations used

For some balanced problems, y_e is treated as a multivariate residual: $[y_{e1} | y_{e2} | \ldots | y_{en}]$

in order to increase the speed of the program and then the log-likelihood can be computed by the equivalent form:

$$\log(L(\theta;y_e)) = \frac{n}{2}\log(|V^{-1}|) - \frac{1}{2}\sum_{i=1}^{n} y_{ei}^T V^{-1} y_{ei}$$

where V is the dispersion matrix for the multivariate normal distribution.

For more information on REML, see Corbeil and Searle (1976).

Newton algorithm for maximization of restricted likelihood

Newton's algorithm is an iterative algorithm used for finding the minimum of an objective function. The algorithm needs a starting point, and then at each iteration, it finds the next point using:

$$\theta_{i+1} = \theta_i - H^{-1}(\theta_i)g(\theta_i)$$

where H^{-1} is the inverse of the Hessian of the objective function, and g is the gradient of the objective function.

The algorithm stops when a convergence criterion is met, or when it is unable to continue.

In the Linear Mixed Effects object, a modification of Newton's algorithm is used to minimize the negative restricted log-likelihood function with respect to the variance parameters θ, hence yielding the variance parameters that maximize the restricted likelihood. Since a constant term does not affect the minimization, the objective function used in the algorithm is the negative of the restricted log-likelihood without the constant term.

$$\text{objective function} = -\log(L(\theta;y_r)) - \log(L(\theta;y_e))$$

Newton's algorithm is modified by adding a diagonal matrix to the Hessian when determining the direction vector for Newton's, so that the diagonal matrix plus the Hessian is positive definite, ensuring a direction of descent.

$$\theta_{i+1} = \theta_i - (H_i + D_i)^{-1} g_i$$

The appropriate D_i matrix is found by a modified Cholesky factorization as described in Gill, Murray and Wright (1981). Another modification to Newton's algorithm is that, once the direction vector, $d = -(H_i + D_i)^{-1} g_i$, has been determined, a line search is done as described in Fletcher (1980) to find an approximate minimum along the direction vector.

Note from the discussion on REML estimation that the restricted log-likelihood is a function of the variance matrix V_r and the residual variance, which are in turn functions of the parameters θ. The gradient and Hessian of the objective function with respect to θ are therefore functions of first and second derivatives of the variances with respect to θ. Since the variances can be determined through the types specified for G and R (e.g., Variance Components, Unstructured, etc.), the first and second derivatives of the variance can be evaluated in closed-form, and hence the gradient and Hessian are evaluated in closed-form in the optimization algorithm.

Starting values

Newton's algorithm requires initial values for the variance parameter θ. The user can supply these in the Linear Mixed Effects object's General Options tab, by clicking the **Generate** button under Initial Variance Parameters. More often, LinMix will determine initial values. For variance structures that are linear in θ (Variance Components, Unstructured, Compound Symmetry, and Toeplitz), the initial values are determined by the method of moments. If the variance structure is nonlinear, then LinMix will calculate a "sample" variance by solving for random effects and taking their sums of squares and cross-products. The sample variance will then be equated with the parametric variance, and the system of equations will be solved to obtain initial parameter estimates. If either method fails, LinMix will use values that should yield a reasonable variance matrix, e.g., 1.0 for variance components and standard deviations, 0.01 for correlations.

Sometimes the method of moments will yield estimates of θ that are REML estimates. In this case the program will not need to iterate.

Convergence criterion

The convergence criterion used in the Linear Mixed Effects object is that the algorithm has converged if: $g^T(\theta)H^{-1}(\theta)g(\theta) < \iota$, where ι is the convergence criterion specified on the General Options tab. The default is 1×10^{-10}.

The possible convergence outcomes from Newton's algorithm are:

- Newton's algorithm converged with a final Hessian that is positive definite, indicating successful convergence at a local, and hopefully global, minimum.
- Newton's algorithm converged with a modified Hessian, indicating that the model may be over-specified. The output is suspect. The algorithm converged, but not at a local minimum. It may be in a trough, at a saddle point, or on a flat surface.
- Initial variance matrix is not positive definite. This indicates an invalid starting point for the algorithm.
- Intermediate variance matrix is not positive definite. The algorithm cannot continue.
- Failed to converge in allocated number of iterations.

Satterthwaite approximation for degrees of freedom

The linear model used in linear mixed effects modeling is: $Y \sim N(Xb,V)$

$$Y \sim N(Xb,V)$$

where X is a matrix of fixed known constants, b is an unknown vector of constants, and V is the variance matrix dependent on other unknown parameters. Wald (1943) developed a method for testing hypotheses in a rather general class of models. To test the hypothesis H_0: $L\beta$=0, the Wald statistic is:

$$(L\hat{\beta})^T\left[L(X^T\hat{V}^{-1}X)^{-1}L^T\right]^{-1}(L\hat{\beta})$$

where:

- $\hat{\beta}$ is the estimator of β.
- $\hat{V}$ is the estimator of V.
- L must be a matrix such that each row is in the row space of X.

The Wald statistic is asymptotically distributed under the null hypothesis as a chi-squared random variable with q=rank(L) degrees of freedom. In common variance component models with balanced data, the Wald statistic has an exact F-distribution. Using the chi-squared approximation results in a test procedure that is too liberal. LinMix uses a method based on analysis techniques described by Allen and Cady (1982) for finding an F distribution to approximate the distribution of the Wald statistic.

Giesbrecht and Burns (1985) suggested a procedure to determine denominator degrees of freedom when there is one numerator degree of freedom. Their methodology applies to variance component models with unbalanced data. Allen derived an extension of their technique to the general mixed model.

A one degree of freedom (df) test is synonymous with L having one row in the previous equation. When L has a single row, the Wald statistic can be re-expressed as:

$$\frac{(L\hat{\beta})^2}{L(X^T\hat{V}^{-1}X)^{-1}L^T} = \frac{(L\hat{\beta})^2}{L(X^TV^{-1}X)^{-1}L^T} \div \frac{L(X^T\hat{V}^{-1}X)^{-1}L^T}{L(X^TV^{-1}X)^{-1}L}$$

Consider the right side of this equation. The distribution of the numerator is approximated by a chi-squared random variable with 1 df. The objective is to find ***n*** such that the denominator is approximately distributed as a chi-squared variable with ν df. If:

$$\frac{L(X^T\hat{V}^{-1}X)^{-1}L^T}{L(X^TV^{-1}X)^{-1}L} \sim \frac{\chi^2\nu}{\nu}$$

is true, then:

$$Var\left[\frac{L(X^T\hat{V}^{-1}X)^{-1}L^T}{L(X^TV^{-1}X)^{-1}L}\right] = \frac{2}{\nu}$$

The approach is to use this equation to solve for ν.

Find an orthogonal matrix R and a diagonal matrix Λ such that:

$$L(X^T \hat{V}^{-1} X)^{-1} L^T = R \Lambda R^T$$

The Wald statistic equation can be re-expressed as:

$$W = (R^T L \hat{\beta})^T \Lambda^{-1} (R^T L \hat{\beta}) = \sum_{u=1}^{q} \frac{(r_u L \hat{\beta})^2}{\lambda_u}$$

where r_u is the u-th column of R, and λ_u is the u-th diagonal of Λ.

Each term in the previous equation is similar in form to the Wald statistic equation for a single row. Assuming that the u-th term is distributed $F(1, \nu_u)$, the expected value of W is:

$$E(W) = \sum_{u-1}^{q} \frac{\nu_u}{\nu_u - 2}$$

Assuming W is distributed $F(q, \nu)$, its expected value is $q\nu/(\nu - 2)$. Equating expected values and solving for ν, one obtains:

$$\nu = \frac{2E\{W\}}{E\{W\} - q}$$

Linear mixed effects scenario

The following example is used as a framework for discussing the functions and computations of the Linear Mixed Effects object.

An investigator wants to estimate the difference between two treatments for blood pressure, labeled A and B. Because responses are fairly heterogeneous among people, the investigator decided that each study subject will be his own control, which should increase the power of the study. Therefore, each subject will be exposed to treatments A and B. Since the treatment order might affect the outcome, half the subjects will receive A first, and the other half will receive B first. For the analysis, note that this crossover study has two periods, 1 and 2, and two sequence groups AB and BA.

The model will be of the form:

$$y_{ijkm} = \mu + \pi_i + \delta_j + \tau_q + S_{k(j)} + \varepsilon_{ijkm}$$

where:

i is the period index (1 or 2)
j is the sequence index (AB or BA)
k is the subject within sequence group index ($1\ldots n_j$)
m is the observation within subject index (1, 2)
q is the treatment index (1, 2)
μ is the intercept

π_i is the effect due to period

δ_j is the effect due to sequence grouping

τ_q is the effect due to treatment, the purpose of the study

$S_{k(j)}$ is the effect due to subject

ε_{ijkm} is the random variation

Typically, ε_{ijkm} is assumed to be normally distributed with mean zero and variance $\sigma^2 > 0$. The purpose of the study is to estimate $\tau_1 - \tau_2$ in all people with similar inclusion criteria as the study subjects. Study subjects are assumed to be randomly selected from the population at large. Therefore, it is desirable to treat subject as a random effect. Hence, assume that subject effect is normally distributed with mean zero and variance $\mathrm{Var}(S_{k(j)}) \geq 0$.

This mixing of the regression model and the random effects is known as a mixed effect model. The mixed effect model allows one to model the mean of the response as well as its variance. Note that in the model equation above, the mean is:

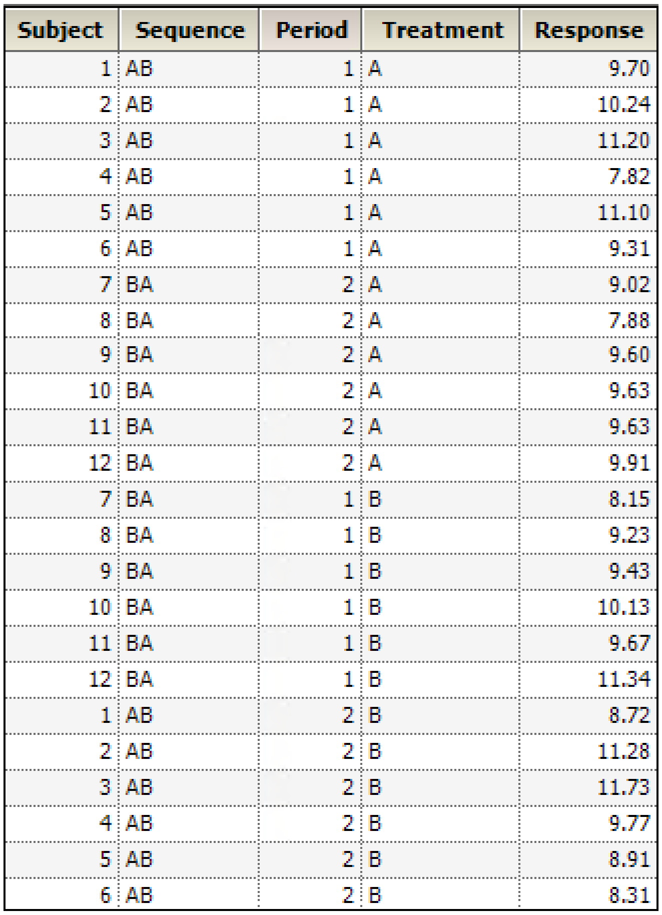
$$E\{y_{ijkm}\} = \mu + \pi_i + \delta_j + \tau_q$$

and the variance is given by:

$$Var\{y_{ijkm}\} = Var\{S_{k(j)}\} + \sigma^2$$

$E\{y_{ijkm}\}$ is known as the fixed effects of the model, while $S_{k(j)}+\varepsilon_{ijkm}$ constitutes the random effects, since these terms represent random variables whose variances are to be estimated. A model with only the residual variance is known as a fixed effect model. A model without the fixed effects is known as a random effect model. A model with both is a mixed effect model.

The data are shown in the table below. The Linear Mixed Effects object is used to analyze these data. The fixed and random effects of the model are entered on different tabs of the Diagram view. This is appropriate since the fixed effects model the mean of the response, while the random effects model the variance of the response.



Subject	Sequence	Period	Treatment	Response
1	AB	1	A	9.70
2	AB	1	A	10.24
3	AB	1	A	11.20
4	AB	1	A	7.82
5	AB	1	A	11.10
6	AB	1	A	9.31
7	BA	2	A	9.02
8	BA	2	A	7.88
9	BA	2	A	9.60
10	BA	2	A	9.63
11	BA	2	A	9.63
12	BA	2	A	9.91
7	BA	1	B	8.15
8	BA	1	B	9.23
9	BA	1	B	9.43
10	BA	1	B	10.13
11	BA	1	B	9.67
12	BA	1	B	11.34
1	AB	2	B	8.72
2	AB	2	B	11.28
3	AB	2	B	11.73
4	AB	2	B	9.77
5	AB	2	B	8.91
6	AB	2	B	8.31

The Linear Mixed Effects object can fit linear models to Gaussian data. The mean and variance structures can be simple or complex. Independent variables can be continuous or discrete, and no distributional assumption is made about them.

This model provides a context for the linear mixed effects model.

Linear mixed effects model examples

Knowledge of how to do basic tasks using the Phoenix interface, such as creating a project and importing data, is assumed.

Analyzing treatment effects
Analyzing variance structure effects

Analyzing treatment effects

This example uses the Linear Mixed Effects (LinMix) capability in Phoenix to test for differences among treatment groups in a parallel study. Twenty-eight subjects were randomly assigned to four treatment groups. One observation of drug effect was measured from each subject for a total of seven observations per treatment. If statistically significant differences are observed between treatments, then the estimates, with intervals, are desired.

The model for these data is as follows.

$$y_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

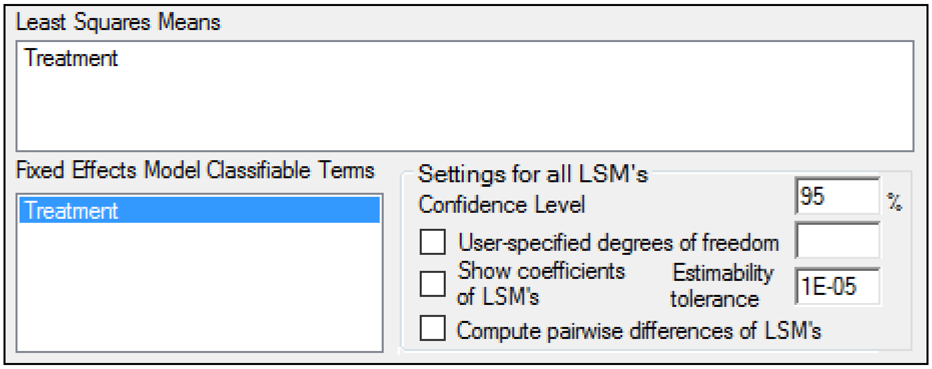
where:

i is the treatment index, 1, 2, 3, 4
j is the subject index within treatment, 1, 2, …, 7
y_{ij} is the observation value for treatment i, subject j
μ is the overall mean
τ_i is the effect of treatment i
ε_{ij} is the random error term for observation y_{ij}

Note: The completed project (`LinMix_TreatmentEffects.phxproj`) is available for reference in …`\Examples\WinNonlin`.

Set up the object

1. Create a new project with the name `LinMix_TreatmentEffects`.
2. Import the file …`\Examples\WinNonlin\Supporting files\OneWayData.CSV`.
3. Right-click **OneWayData** in the Data folder and select **Send To > Computation Tools > Linear Mixed Effects**.
4. In the Main Mappings panel:
   Map **Treatment** to the **Classification** context.
   Map **Response** to the **Dependent** context.
5. In the Fixed Effects tab below the Setup panel, drag **Treatment** from the Classification list up to the Model Specification field (or type `Treatment` in the Model Specification field).
6. **None** is selected by default in the **Dependent Variables Transformation** menu. Do not change this setting.
7. Select the **Least Squares Means** tab.
8. Drag **Treatment** from the Fixed Effects Model Classifiable Terms list to the Least Squares Means field.



Least Squares Means

Treatment

Fixed Effects Model Classifiable Terms

Treatment

Settings for all LSM's

Confidence Level 95 %

□ User-specified degrees of freedom

□ Show coefficients of LSM's

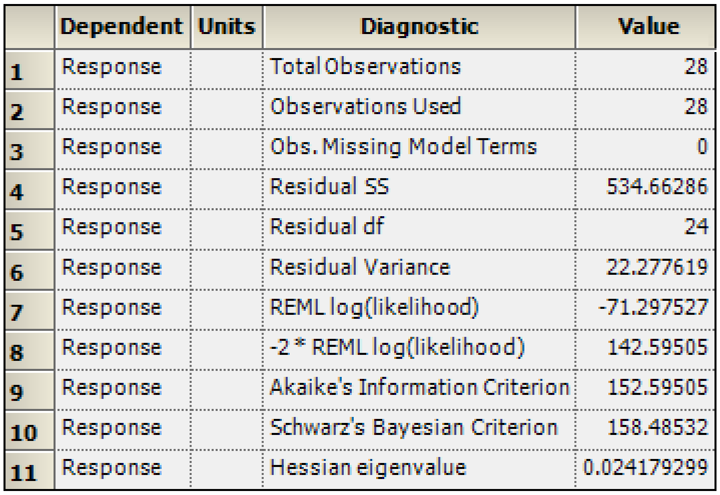
Estimability tolerance 1E-05

□ Compute pairwise differences of LSM's

Execute and evaluate the results

1. Click ▶ (**Execute** icon) to execute the object.

   The Diagnostics results worksheet is opened by default in the Results tab.



	Dependent	Units	Diagnostic	Value
1	Response		Total Observations	28
2	Response		Observations Used	28
3	Response		Obs. Missing Model Terms	0
4	Response		Residual SS	534.66286
5	Response		Residual df	24
6	Response		Residual Variance	22.277619
7	Response		REML log(likelihood)	-71.297527
8	Response		-2 * REML log(likelihood)	142.59505
9	Response		Akaike's Information Criterion	152.59505
10	Response		Schwarz's Bayesian Criterion	158.48532
11	Response		Hessian eigenvalue	0.024179299

2. Select **Final Fixed Parameters** in the Results list.

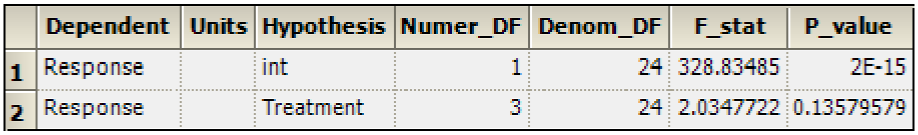
   This model is over parameterized. There are five parameters, μ, τ_1, τ_2, τ_3, τ_4, but there are only four means. The last parameter is removed from the model and is not estimated, resulting in the output of "Not estimable" for the Placebo group. When that happens, each of the other τ parameters represents the difference between the treatment mean and the last treatment mean. Note that subtracting the Least Squares Means for high dose group from the mean for placebo produces the same number as τ_1. The parameter μ is then the mean of the omitted treatment group, the placebo group in this case.

3. Select **Least Squares Means** in the Results list.

   On the Least Squares Means (LSM) tab, Estimate, for balanced data, is the average of the observations within each treatment group. Also listed are the standard error of each mean, p value for the hypothesis that the true mean equals zero, and interval.

4. Select **Partial Tests** in the Results list.

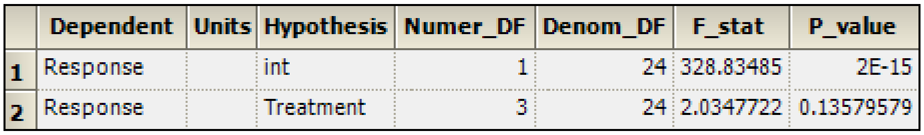
   In this case, the partial tests have the same value as the sequential test. This is always true for balanced datasets. For unbalanced data, these results can differ. Refer to "Least squares means" for more information on unbalanced data.



	Dependent	Units	Hypothesis	Numer_DF	Denom_DF	F_stat	P_value
1	Response		int	1	24	328.83485	2E-15
2	Response		Treatment	3	24	2.0347722	0.13579579

5. Select **Sequential Tests** in the Results list.

   The p-value is shown as 0.1358, indicating that differences among treatment groups were not statistically significant.



	Dependent	Units	Hypothesis	Numer_DF	Denom_DF	F_stat	P_value
1	Response		int	1	24	328.83485	2E-15
2	Response		Treatment	3	24	2.0347722	0.13579579

This concludes the LinMix treatment effects analysis example.

Analyzing variance structure effects

This analysis is concerned with the precision components of assay validation to estimate contributions due to assay variation, assay-to-assay variation, and analyst-to-analyst variation. A single QC sample was prepared containing, theoretically, 65 ng/mL of analyte. Five analysts were recruited for this study. Each analyst ran five aliquots of the sample on four assay runs. The data are available in the dataset `AssayVal1.CSV` in the Phoenix examples directory.

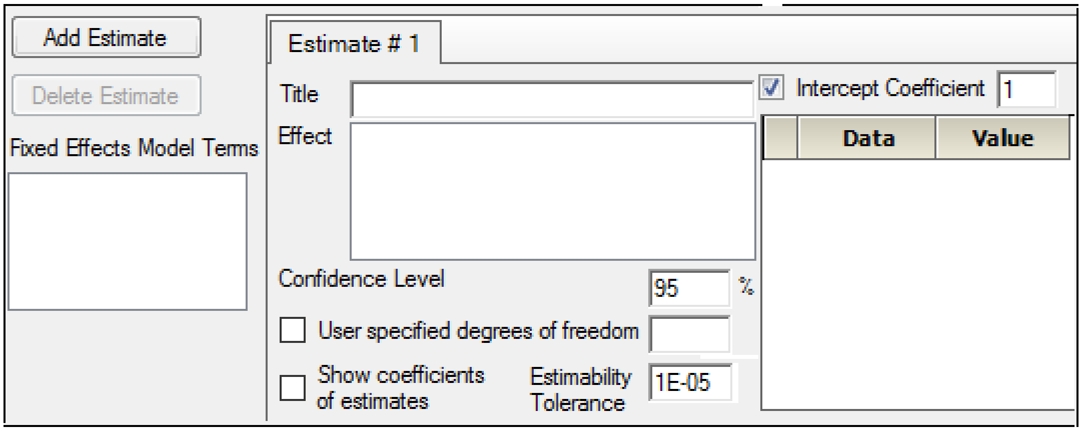
Note: The completed project (`LinMix_StructureEffects.phxproj`) is available for reference in …`\Examples\WinNonlin`.

Set up the object

1. Create a project called `LinMix_StructureEffects`.
2. Import the file …`\Examples\WinNonlin\Supporting files\AssayVal1.CSV`.

   Units must be added to the Determination column before the dataset can be used in a Linear Mixed Effects model.
3. In the Columns tab below the table, select the **Determination** column header in the Columns list.
4. Clear the **Unit** field, type `ng/mL`, and press the **Enter** key.
5. Right-click **Workflow** in the Object Browser and select **New > Computation Tools > Linear Mixed Effects**.
6. Drag the **AssayVal1** worksheet from the Data folder to the Main Mappings panel.
   Map **Analyst** to the **Classification** context.
   Map **Assay** to the **Classification** context.
   Map **Determination** to the **Dependent** context.
7. Select the **Variance Structure** tab below the Setup panel.
8. Drag **Analyst** from the Classification Variables list to the Random Effects Model field in the **Random 1** sub-tab (or type `Analyst` in the field).
9. Click **Add Random** to add another Random effect.
10. Drag **Assay** from the Classification Variables list to the Random Effects Model field in the **Random 2** sub-tab (or type `Assay` in the field).

11. Select the **Estimates** tab.
12. Check the **Intercept Coefficient** checkbox and type `1` in the **Intercept Coefficient** field.



☑ Intercept Coefficient 1

Confidence Level 95 %

☐ User specified degrees of freedom

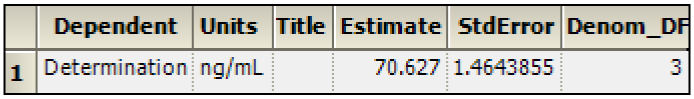
☐ Show coefficients of estimates

Estimability Tolerance 1E-05

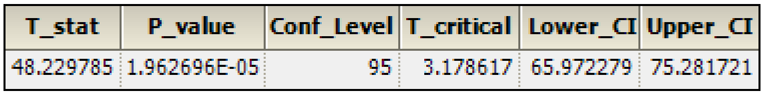
Execute and view the results of the first model

1. Execute the object.
2. Select **Estimates** in the Results list.

   Statistical accuracy values are located in the Estimates worksheet. The mean response is the intercept, which is estimated at 70.6 ng/mL with a 95% confidence interval. The lower interval is 65.97 and the upper interval is 75.28. Since the theoretical analyte concentration of 65 ng/mL is not within the interval, one can conclude that the bias is statistically significant. The method has a bias of approximately 5 ng/mL.

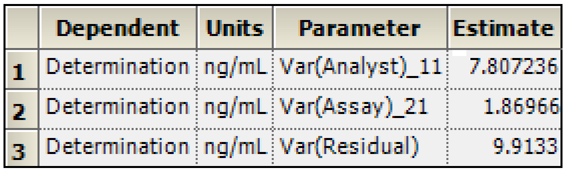


	Dependent	Units	Title	Estimate	StdError	Denom_DF
1	Determination	ng/mL		70.627	1.4643855	3



T_stat	P_value	Conf_Level	T_critical	Lower_CI	Upper_CI
48.229785	1.962696E-05	95	3.178617	65.972279	75.281721

3. Select the **Final Variance Parameters** worksheet to view precision estimates and variance components.



	Dependent	Units	Parameter	Estimate
1	Determination	ng/mL	Var(Analyst)_11	7.807236
2	Determination	ng/mL	Var(Assay)_21	1.86966
3	Determination	ng/mL	Var(Residual)	9.9133

Based on these results, most of the variation is coming from analyst-to-analyst variation and from within-assay variation. Assay-to-assay noise is quite small. The units on the variances are (ng/mL).

Apply model to a different dataset

Now fit the same model to the data in the dataset `AV3.CSV`.

1. Import the file …`\Examples\WinNonlin\Supporting files\AV3.CSV`.

   Units must be added to the Determination column before the dataset can be used in a Linear Mixed Effects model.

2. In the Columns tab, select the **Determination** column header in the Columns box.
3. Clear the **Unit** field, type `ng/mL`, and press the **Enter** key.
4. Right-click the **Linear Mixed Effects** object in the Object Browser and select **Copy**.
5. Right-click **Workflow** and select **Paste**.

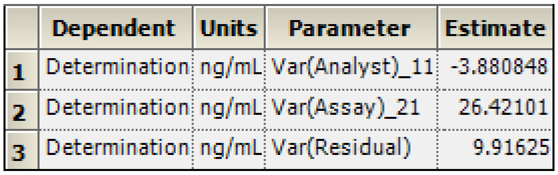
   A new Linear Mixed Effects object named Copy of Linear Mixed Effects is added to the Workflow. The LinMix object copy contains the same settings as the original object.

6. Map the **AV3** dataset to **Copy of Linear Mixed Effects**. Do not change the data mappings in the Main Mappings panel.

Execute and view the results from the second model

1. Execute the object.
2. Select **Final Variance Parameters** in the Results list.

   The Final Variance Parameters worksheet contains the following variance components:



	Dependent	Units	Parameter	Estimate
1	Determination	ng/mL	Var(Analyst)_11	-3.880848
2	Determination	ng/mL	Var(Assay)_21	26.42101
3	Determination	ng/mL	Var(Residual)	9.91625

   This table indicates that analyst-to-analyst variation is negative. Since variances cannot be negative, it is customary to replace the value with zero. A negative variance component indicates that the corresponding term should be removed from the model, which means that the contribution from that term is minimal compared to the contribution due to the other terms and it cannot be distinguished from the residual term.

   From the variance components it is clear that the largest contribution to noise in the method is from run-to-run variation. Within-run variation also contributes to the noise. There is very little variation among analysts, indicating that the method is robust.

   The Linear Mixed Effects object warns user about negative final variances.

3. In the Results tab, select the **Warnings and Errors** text file.

   The text file states: “Warning 11094: Negative final variance component. Consider omitting this VC structure.” Problems associated with a linear mixed effects model are written to this file during execution.

This concludes the LinMix structure effects analysis example.

NCA

Phoenix's noncompartmental analysis (NCA) engine computes derived measurements from raw data by using methods appropriate for either serially- or sparsely-sampled data. Phoenix provides the following NCA models for different types of input data:

200 Plasma/blood data type, extravascular dose type, requires constants for time of last dose and dose interval (Tau)[a]

201 Plasma/blood data type, IV Bolus dose type, requires constants for time of last dose and dose interval (Tau)[a]

202 Plasma/blood data type, constant infusion dose type, requires constants for length of infusion, time of last dose, dose interval (Tau)[a]

210 Urine data type, extravascular dose type, requires constants for time of last dose

211 Urine data type, IV Bolus dose type, requires constant for time of last dose

212 Urine data type, constant infusion dose type, requires constant for time of last dose

220 Drug effect data type, any dose type, requires constants for time, baseline effect, and threshold (optional)

[a] Tau is required for steady-state data only.

Models 200–202 can be used for either single-dose or steady-state data. For steady-state data, the computation assumes equal dosing intervals (Tau) for each profile, and that the data are from a “final” dose given at steady-state.

Models 210–212 (urine concentrations) assume single-dose data, and the final parameters do not depend on the dose type.

The plasma and urine models support rich datasets as well as sparsely-sampled studies such as toxicokinetic studies. The drug effect model is for analysis of slope, height, areas, and moments in richly-sampled time-effect data.

Use one of the following to add the object to a Workflow:

Right-click menu for a Workflow object: **New > NonCompartmental Analysis > NCA**.
Main menu: **Insert > NonCompartmental Analysis > NCA**.
Right-click menu for a worksheet: **Send To > NonCompartmental Analysis > NCA**.

Note: To view the object in its own window, select it in the Object Browser and double-click it or press **ENTER**. All instructions for setting up and execution are the same whether the object is viewed in its own window or in Phoenix view.

This section contains information on the following topics:

NCA user interface description
Results
NCA computation rules
NCA parameter formulas
NCA examples

NCA user interface description

Main Mappings panel
Dosing panel
Slopes Selector panel
Slopes panel

Partial Areas panel
Therapeutic Response panel
Units panel
Parameter Names panel
Options tab
User Defined Parameters tab
Rules tab
Plots tab

Main Mappings panel

Use the Main Mappings panel to identify how a dataset is used with an NCA object by mapping data types in a dataset to the appropriate contexts. Context associations change depending on the selected NCA model. Required input is highlighted orange in the interface.

None: Data types mapped to this context are not included in any analysis or output.
Sort: Categorical variable(s) identifying individual data profiles, such as subject ID in an NCA study. A separate analysis is done for each unique combination of sort variable values.
Carry: Data variable(s) to include in the output worksheets. Note that time-dependent data variables (those that change over the course of a profile) are not carried over to time-independent output (e.g., Final Parameters), only to time-dependent output (e.g., Summary).

Plasma study

Time: Nominal or actual time collection points in a plasma study.
Concentration: Drug concentration values in the blood of a plasma study.
(Plasma Models with Sparse Sampling also require a single Subject mapping.)

Urine study

Start Time: Starting times for individual collection intervals during a urine study.
End Time: Ending times for individual collection intervals during a urine study.
Concentration: Dependent variable, drug concentration in urine.
Volume: Volume of urine collected per time interval.

Drug effect study

X: Time values for the drug effect data.
Y: Drug effect or response values.

Dosing panel

The Dosing panel allows users to type or map dosing data for the different NCA models.

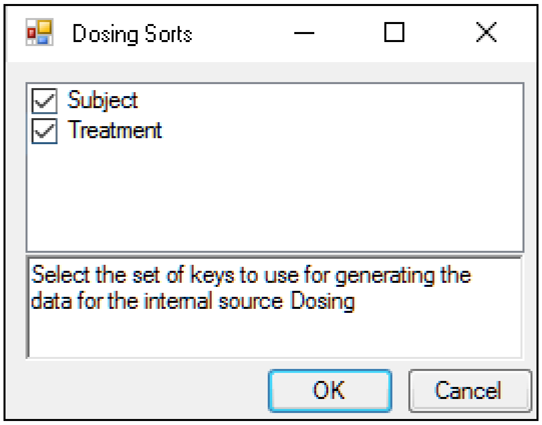
Models 200–202 (plasma) assume that data were taken after a single dose or after a final dose at steady state. For steady state Phoenix also assumes equal dosing intervals.

Models 210–212 (urine) assume single-dose urine data. If dose time is not entered, Phoenix uses a time of zero.

Model 220 (drug effect) uses the same time scale and units as the input dataset. Users enter the time of the most recent dose for each profile.

The Dosing panel columns change depending on the model type and dose type selected in the Options tab.

The first time a user selects the Dosing panel, if sort keys are defined in the Main Mappings panel, Phoenix displays the *Dosing sorts* dialog.



☑ Subject
☑ Treatment

Select the set of keys to use for generating the data for the internal source Dosing

The dialog has all of the currently specified sort variables selected by default. The selected sort variables will be used when creating the internal dosing worksheet. To select a subset of the sort variables, clear the checkbox beside the unwanted variable and click **OK**.

Context associations change depending on the selected NCA model. Required input is highlighted orange in the interface.

None: Data types mapped to this context are not included in any analysis or output.
Sort: Categorical variable(s) identifying individual data profiles. A separate computation is done for each unique combination of sort variable values.
Time: Time of dose administration.
Dose: Amount of drug per profile.
Tau: The (assumed equal) dosing interval for steady-state data. TAU must be a positive number for steady-state profiles and blank for non-steady-state profiles.
Infusion_Length: Total amount of time for an IV infusion.
Dose_Type: Dosing route, if not defined in the Options tab.

Note: The time units for the dosing data must be the same as the time units for the time/concentration data.

Slopes Selector panel

Phoenix attempts to estimate the rate constant, Lambda Z, associated with the terminal elimination phase for concentration data. If Lambda Z is estimable, parameters for concentration data will be extrapolated to infinity. For drug effect models, Phoenix estimates the two slopes at the beginning and end of the data. NCA does not extrapolate beyond the observed data for drug effect models.

The observed times for each profile are displayed in a graph on separate tabs in the Slopes Selector panel. Below are usage instructions. For descriptions of how the NCA object determines Lambda Z or slope estimation settings, see “Lambda Z or Slope Estimation settings”.

Note: Any changes to the settings available in the Slopes Selector panel also affect the Slopes panel and vice versa.

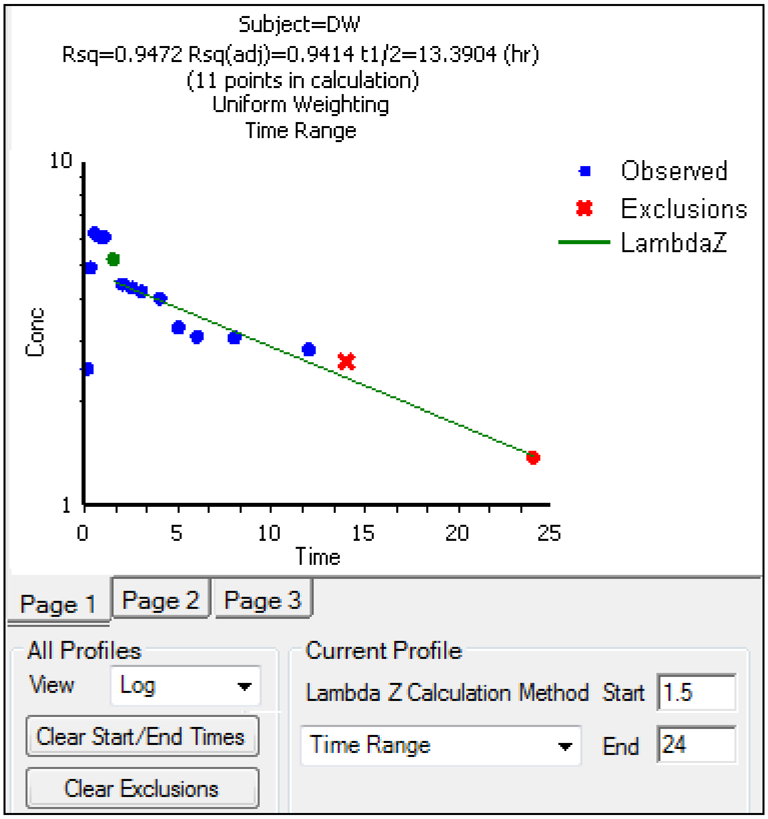
- Use the **View** menu to select a linear (**Lin**) or logarithmic (**Log**) axis scale.
- Use the **Lambda Z Calculation Method** menu to select a method.
  If **Best Fit** is selected, Phoenix calculates the points for Lambda Z estimation for each profile.
  If **Time Range** is selected, users must enter the start and end times for Lambda Z estimation.

- To turn off Lambda Z or slope estimation for all profiles, select the **Disable Curve Stripping** checkbox in the Options tab. For more information see step 4 in "Model settings".

Users can manually select start times, end times, and excluded time points by selecting them on the graph for each profile (this action automatically sets the **Lambda Z Calculation Method** to **Time Range**.

Click a data point on a graph to select the start time.
SHIFT+click a data point on a graph to select the end time.
CTRL+click a data point on a graph to exclude the time point.
Change the start time, end time, and exclusions by selecting new points on the graph using the same key combinations listed above.

When the start time, end time, and exclusions are manually selected, the graph title is updated to show the new R^2 calculation, the graph is updated to show the new slope, and the legend is updated to show the new slope and exclusions, as shown below.



Subject=DW
Rsq=0.9472 Rsq(adj)=0.9414 t1/2=13.3904 (hr)
(11 points in calculation)
Uniform Weighting
Time Range

All Profiles
View: Log

Current Profile
Lambda Z Calculation Method: Time Range
Start: 1.5
End: 24

Note: Excluded data points apply only to Lambda Z or slope calculations. The excluded data points are still included in the computation of AUCs, moments, etc.

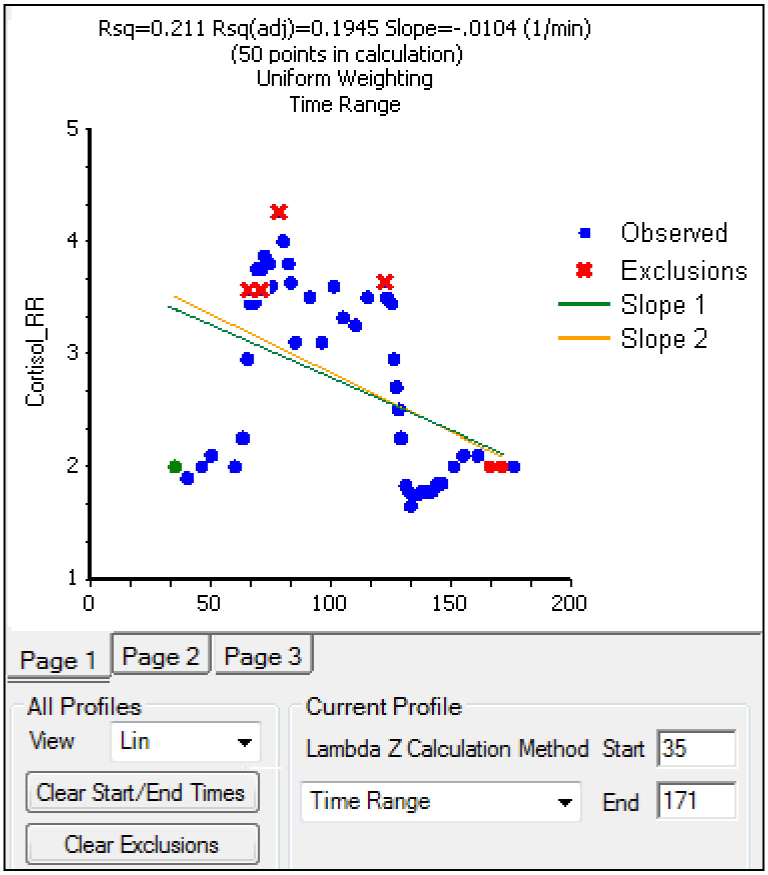
Note: Avoid having excluded or zero-valued points at the beginning or end of the Lambda Z range as this can result in inconsistent reporting of the Lambda Z range.For example, if values range from 0.8 to 2 and points before 1.4 are excluded, some of the output reports the range as (0.8, 2), whereas other output lists the range as (1.4, 2).

- Use the **Clear Start/End Times** button to have previously selected start time and end time points removed from the graphs in the Slopes Selector panel and from the worksheet in the Slopes panel.

- Use the **Clear Exclusions** button to have previously excluded time points included in the graphs in the Slopes Selector panel and in the worksheet in the Slopes panel.

The Drug Effect (220) model calculates two slopes per profile

- Under **Slope Calculation** in the Slopes Selector panel, select **Slope 1** or **Slope 2**.
- Select **Linear** or **Log** to set the slope calculation method for slope 1 or slope 2.
- Use the instructions listed above to select the start times, end times, and exclusions.



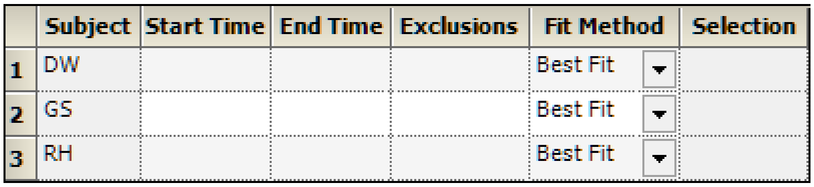
Rsq=0.211 Rsq(adj)=0.1945 Slope=-.0104 (1/min)
(50 points in calculation)
Uniform Weighting
Time Range

All Profiles
View: Lin

Current Profile
Lambda Z Calculation Method: Time Range
Start: 35
End: 171

Slopes panel

The start time, end time, and exclusions used in the calculation of Lambda Z for each profile are defined in the Slopes panel. Users can type the time points for each profile.



	Subject	Start Time	End Time	Exclusions	Fit Method	Selection
1	DW				Best Fit	
2	GS				Best Fit	
3	RH				Best Fit	

Below are usage instructions. For descriptions of how the NCA object determines Lambda Z or slope estimation settings, see “Lambda Z or Slope Estimation settings”.

Note: Any changes to the settings available in the Slopes panel also affect the Slopes Selector panel and vice versa.

- Type the start and end time values in the **Start Time** and **End Time** columns for each profile.
- Type excluded time points in the **Exclusions** column for each profile.
- Exclude multiple time points for a profile by typing the time points in the same cell and separating the time points with a semicolon.
- Use the **Fit Method** menu to specify the method.
  If **Best Fit** is selected, Phoenix calculates the points for Lambda Z estimation.
  If **Time Range** is selected, users must enter the start and end times for Lambda Z estimation.
- To turn off Lambda Z or slope estimation for all profiles, select the **Disable Curve Stripping** checkbox in the Options tab. For more information see step 4 in "Model settings".

The Slopes panel for the **Drug Effect (220)** model also includes options for the slope calculation method.

- In the **Lin/Log** column, select **Linear** or **Log** to set the slope calculation method.
- Use the same instructions listed above to select the start times, end times, and exclusions.

Partial Areas panel

The Partial Areas panel includes settings for the computation of partial areas under the curve. Partial area computations are optional. It is not necessary to enter or add any start times or end times in this panel. For descriptions of how the NCA object computes partial areas, see "Partial area calculation".

None: Data types mapped to this context are not included in any analysis or output.
Sort: Categorical variable(s) identifying individual data profiles, such as subject ID in an NCA study. A separate analysis is done for each unique combination of sort variable values.
Area #: Number to identify the defined partial area.
Label: Title to use as a label for the defined partial area.
Start Time: The time at which to begin the partial area calculation.
End Time: The time at which to end the partial area calculation.

Some additional notes on partial areas:

- Partial area computations are optional. To skip the computations leave the **Start Time** and **End Time** columns empty.
- Up to 127 partial areas can be computed per subject.
- The start times and end times can be after the last observed time if Lambda Z is estimable.

Therapeutic Response panel

The Therapeutic Response panel allows users to determine the time spent within a therapeutic range, and the AUC within the therapeutic range, by using the lower and upper therapeutic response values. For descriptions of how the NCA object handles therapeutic response data, see "Therapeutic response".

Setting the therapeutic response values is optional for plasma (200–202) and urine (210–212) models. Setting the baseline is recommended for drug effect models (220). The Min Response and Max Response columns show the minimum and maximum concentrations, rates, or responses contained in the datasets.

Caution: When entering or mapping the lower and upper therapeutic ranges of profiles in an NCA urine model, users must enter or map the rate of excretion.

None: Data types mapped to this context are not included in any analysis or output.

Sort: Categorical variable(s) identifying individual data profiles, such as subject ID and treatment in a crossover study. A separate analysis is performed for each unique combination of sort variable values.

Lower (Plasma and Urine study) / **Baseline** (Drug Effect study): The lower concentration, rate, or response for defining the lower boundary of the target range. This is the lower effect value for each profile. It is required when an external worksheet is mapped. If not specified for the drug effect model, a baseline of zero is used.

Upper (Plasma and Urine study) / **Threshold** (Drug Effect): The upper concentration, rate, or response for defining the upper boundary of the target range.

Threshold is the effect value used to calculate additional times and areas. See the “Drug effect data model 220” section.

Units panel

An NCA object’s display units can be changed to fit a user’s preferences. Each parameter used in a model and the parameter’s default units are listed in the Units panel. Required input is highlighted orange in the interface.

For **plasma models** (200–202), the time and concentration data must contain units before users can set preferred units.

For **plasma models** (200–202), the dosing unit must be set before users can set preferred units.

For **urine models** (210–212), the start time, end time, concentration, and volume data must contain units before users can set preferred units.

For the **drug effect model** (220), the time and effect data must contain units before users can set preferred units.

- **None**: Data types mapped to this context are not included in any analysis or output.
- **Name**: Model parameters associated with the units.
- **Default**: The model object’s default units.
- **Preferred**: The user’s preferred units for the parameter.

Note: if you see an “Insufficient units” message, check that units are defined for time and concentration in your input.

Parameter Names panel

Phoenix provides the option to specify the names for NCA model parameters.

None: (In mapped worksheet only) Rows mapped to this context are not included in any analysis or output.

Parameter Name: In the internal worksheet, Phoenix’s default parameter names are listed in this column. For a mapped worksheet, click in the cell to indicate that the name in that row is a parameter name.

Preferred (Name): In the internal worksheet, edit the name that will appear in the output. For a mapped worksheet, click in the cell to indicate that the name in that row is a preferred name.

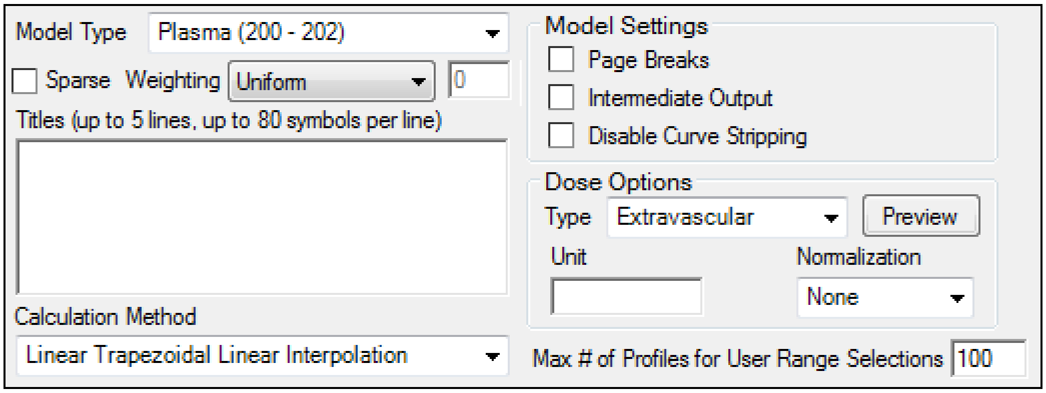
Include in Workbook: Indicate whether or not a parameter is included as final parameter in the workbook output.

Note: Parameter names cannot contain empty spaces. The case of each preferred parameter name is preserved.

For more on NCA output parameters see "NCA parameter formulas".

Options tab

The Options tab allows users to select the NCA model and set options for the selected model.



Model Type: Plasma (200 - 202)
□ Sparse
Weighting: Uniform
0
Titles (up to 5 lines, up to 80 symbols per line)
Calculation Method: Linear Trapezoidal Linear Interpolation
Model Settings
□ Page Breaks
□ Intermediate Output
□ Disable Curve Stripping
Dose Options
Type: Extravascular
Unit
Normalization: None
Max # of Profiles for User Range Selections: 100

- Use the **Model Type** menu to select the NCA model type (i.e., whether the input data are from plasma, urine, or drug effect measurements). Select **Plasma (200-202)**, **Urine (210-212)**, or **Drug Effect (220)**.
- Check the **Sparse** checkbox to use analysis methods for sparse datasets. See "Sparse sampling calculation" for more information on using sparse datasets with NCA models.
- Use the **Weighting** menu to select the regression that estimates Lambda Z or slopes. Select **User Defined**, **Uniform**, **1/Y**, or **1/(Y*Y)**.

Note: The relative proportions of the weights are important, not the weights themselves. See "Weighting" for more on weighting schemes.

Rules for using the **Weighting** menu:

- If **User Defined** is selected then users can enter their own Observed to Power N value. The value of N must be typed in the **Weighting** text field.
- **Sparse** data requires **Uniform** weighting.
- When a log-linear fit is done (**Uniform** weighting for Lambda Z), then the fit is implicitly using a weighting approximately equal to 1/Yhat^2.

Note: If **1/Y** and the **Linear Log Trapezoidal** calculation method are selected, a user might assume that the weighting scheme is 1/LogY, rather than 1/Y. This is not the case, however, since concentrations between zero and one would have negative weights and could not be included in the analysis.

- Use the **Titles** text box to type a title for the analysis. The title is displayed at the top of each page in the Core Output and can include up to 5 lines of text.
- Select a method for calculating the area under the curve from the **Calculation Method** menu.

The chosen method applies to all AUC, AUMC, and partial area computations. All methods reduce to the log trapezoidal rule, the linear trapezoidal rule, or both. The methods differ based on when the rules are applied. See “Partial area calculation” for descriptive equations of the calculation methods. Select from:

Linear Log Trapezoidal. This method uses the linear trapezoidal rule up to Cmax and then the log trapezoidal rule for the remainder of the curve, to compute AUCs. Points for partial areas are inserted using the logarithmic interpolation rule after Cmax, or after C0 for IV bolus, if C0 > Cmax. Otherwise, the linear trapezoidal rule is used. If Cmax is not unique, then the first maximum is used.

Linear Trapezoidal Linear Interpolation. This is the default method and recommended for **Drug Effect Data (220)**. It uses the linear trapezoidal rule, which is applied to each pair of consecutive points in the dataset that have non-missing values, and sums up these areas to compute AUCs. If a partial area is selected that has an endpoint that is not in the dataset, then the linear interpolation rule is used to insert a concentration value for that endpoint.

Linear Up Log Down. The linear trapezoidal rule is used to compute AUCs any time that the concentration data is increasing, the logarithmic trapezoidal rule is used any time that the concentration data is decreasing. Points for partial areas are inserted using the linear interpolation rule if the surrounding points show that concentration is increasing, and the logarithmic interpolation rule if the concentration is decreasing.

Linear Trapezoidal Linear/Log Interpolation. This method is the same as **Linear Trapezoidal Linear Interpolation** except when a partial area is selected that has an endpoint that is not in the dataset. In that case, the logarithmic interpolation rule is used to insert points after Cmax, or after C0 for IV bolus, if C0 > Cmax. Otherwise, the linear interpolation rule is used. If Cmax is not unique, then the first maximum is used.

Note: The **Linear Log Trapezoidal**, the **Linear Up Log Down**, and the **Linear Trapezoidal Linear/Log Interpolation** methods all apply the same exceptions in area calculation and interpolation. If a Y value (concentration, rate, or effect) is less than or equal to zero, Phoenix defaults to the linear trapezoidal or linear interpolation rule for that point. If adjacent Y values are equal to each other, Phoenix defaults to the linear trapezoidal or linear interpolation rule.

Model settings

- Check the **Page Breaks** checkbox to include page breaks in the ASCII text output.
- Check the **Intermediate Output** checkbox to add to the text Core Output the values of each iteration during estimation of Lambda Z and for each of the sub-areas in partial area computations.
- Check the **Disable Curve Stripping** checkbox to turn off Lambda Z or slope estimation for all profiles. When this option is selected Lambda Z or slopes are not estimated, parameters that are extrapolated to infinity are not calculated, and the Rules tab is disabled.
- Disable curve stripping for one or more individual profiles:
  - Select **Slopes** in the Setup tab.
  - Enter a start time that is greater than the last time point in a given profile and an end time greater than the start time.

Dose options

Note: Dose Options are not available for the **Drug Effect (220)** model.

- Use the **Type** menu to select the dosing route. Dose type selections determine two things: specific model type and columns available in the Dosing panel. Select **Extravascular**, **IV Bolus**, **IV Infusion**, or **Dosing Defined**.

- Set the dosing unit by clicking the **Units Builder - Dose […]** button or typing the dosing unit into the **Unit** text field. Only applicable when an internal worksheet is being used for the Dosing data.

  See "Using the Units Builder" for more details.
- Click **Preview** to see a preview of dose option selections in a separate window. Click **OK** to close the preview window.
- Use the **Normalization** menu to select the appropriate factor if the dose amount is normalized by subject body weight or body mass index. Select **None**, **kg**. **g**, **mg**, **m**2**, or **1.73 m**2**.

  If doses are in milligrams per kilogram of body weight, select **mg** as the dosing unit and **kg** as the dose normalization.

  The **Normalization** menu affects the output parameter units. For example, if dose volume is in liters, selecting **kg** as the dose normalization changes the units to L/kg.

  Dose normalization affects units for all volume and clearance parameters in NCA models, as well as AUC/D in NCA plasma models, and Percent_Recovered in NCA urine models.

Other options

- Enter the **Max # of Profiles for User Range Selections** in the field (default is 100).

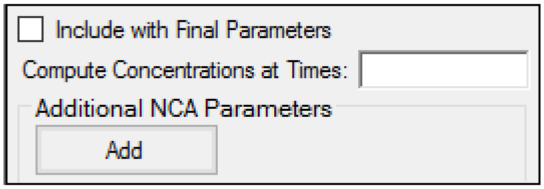
  If the number of profiles exceeds this value, then the user selection of slopes will be disabled and the engine will calculate the slopes using the Best Fit method. Selecting the Slopes Selector worksheet or Slopes worksheet will display a message notifying the user that the limit has been exceeded and the Best Fit method will be used for all profiles.

User Defined Parameters tab

The User Defined Parameters tab allows users to include concentrations/Y values at specified times, as well as additional NCA parameters.

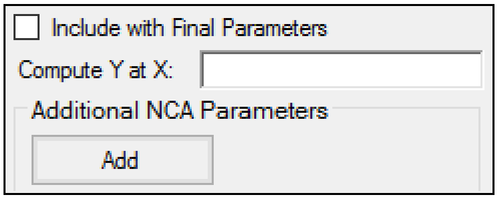
- Check the **Include with Final Parameters** box to append the user-defined parameters (both the computed concentrations/Ys and any additional user-defined parameters) to the Final Parameters worksheets, both the pivoted and non-pivoted versions.
- Enter time or X values at which to compute the concentration or Y value.

  For Plasma models, enter a value(s) in the field to have the NCA object compute the concentration (or the mean concentration, if the **Sparse Sampling** box in the Options tab is checked) at that time and include the result in the output.


☐ Include with Final Parameters
Compute Concentrations at Times:
Additional NCA Parameters
Add

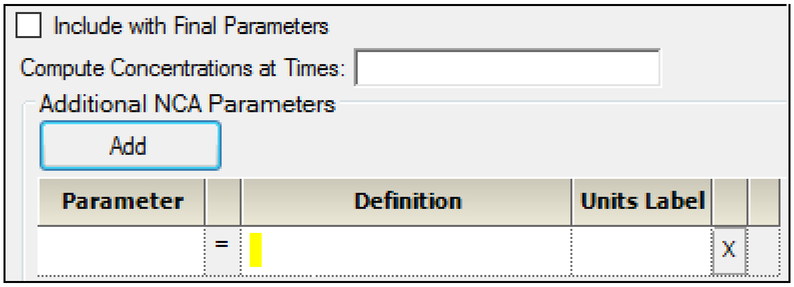
  For Drug Effect models, enter a value(s) in the field to have the NCA object compute the Y value at that X value and include the result in the output.

  Up to 30 values can be entered as a comma-separated list. Values can also be specified by using multiple '`seq`' statements with the format `seq(first_time,end_time,increment)`. For example, `seq(0,6,1),8,12,seq(18,36,6)`.



Note: If a concentration/drug effect value does not exist in the data set for the specified time/X value, it is calculated following the same rules as for computing Y-values for partial area endpoints, see “Partial area calculation”.

- Click **Add** to define other NCA parameters to include in the output.


□ Include with Final Parameters
Compute Concentrations at Times:
Additional NCA Parameters
Add
Parameter
Definition
Units Label
=
X

- Enter a name for the parameter in the Parameter column.

  The name is checked for validity as it is entered and a message is displayed in the last column of the table if it is not valid. Since the names become column headers in the output, they must also follow the rules for column headers: start with a letter or underscore; contain only letters, numbers, underscores, and ‘%’; and not contain spaces, periods, or symbols. The name cannot match any of the following:
  - default names for NCA final parameters
  - preferred names for NCA final parameters
  - previously defined additional NCA parameters
  - names that will be generated by the **Compute concentrations at times** option
  - the words “Dose” and “Sort”
  - names of columns defined as Carry variables
- Enter an equation that defines how the parameter is to be computed in the Definition column. The math function operators +, –, *, /, and ^ can be used in the Definition, as well as ln(x), log(x,base), log10(x), exp(x), and sqrt(x). The parameter can be a function of the following:
  - default parameter names (even if selected to be excluded from the results)
  - preferred names specified on the Parameter Names setup tab (even if selected to be excluded from the results)
  - partial areas
  - if an external dosing worksheet is used, the name of the column mapped to Dose and the name of the column mapped to Tau
  - if an internal dosing worksheet is used, the “Dose” and “Tau” columns
  - Carry variables (names of columns mapped as Carry)
  - previously defined user-defined parameter names (those that are above the current name in the user interface)

Note that:

- If more than one dose amount is specified for a profile, the last dose value will be used when computing the parameter.
- If a carryalong is a time-dependent variable (that is, if the carryalong takes on different values per profile), any user-defined parameters defined in terms of this carryalong will be undefined.
- If any of the values to be used when computing the user-defined parameter are missing (for example, an equation that uses Rsq when the Lambda Z fitting fails or was disabled), the value of the user-defined parameter will be missing (blank) in the results.
- Any names used are case-sensitive (they must be correct in terms of uppercase and lowercase).
- To assist with entering final parameter names in the equation, when the user starts to type a name, a dropdown list provides a list of final parameters that match. **Double-click** an entry from the list to auto-complete typing that name. The list contains only final parameters, even though other names, such as dosing and carryalong names, can be used. The list contains all possible final parameters in NCA, but some may not be applicable to the current selection of model and dosing options.

- In the Units Label column enter the unit name that is to appear when the parameter values are reported. No conversion of units is done for user-defined parameters.
- Click the red X in the row to remove that parameter.

Rules tab

The Rules tab contains optional settings that apply to the Lambda Z calculation in Plasma and Urine models. (The Rules tab does not apply to Slope1 and Slope2 in Drug Effect models.)

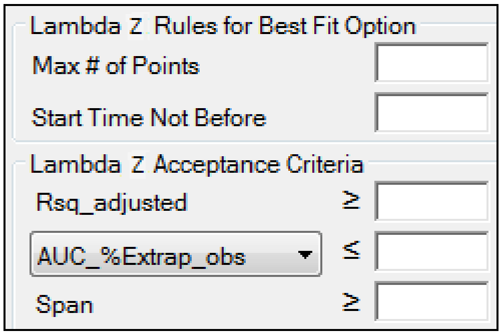


Figure 17-1. Rules tab for NCA object

Lambda Z Rules for Best Fit method: These optional rules are used only in the automatic selection of Lambda Z.

- In the **Max # of points** field, specify the maximum number of points that can be used in the best-fit method for Lambda Z. If a value is specified, the NCA engine will not consider time ranges that contain more than the specified number of points when finding the best Lambda Z. The entered value must be at least 3.
- In the **Start Time Not Before** field, specify the minimum start time that can be used in the best-fit method for Lambda Z. If a value is specified, the NCA engine will not consider time ranges that start before this minimum start time when finding the best Lambda Z.

Lambda Z Acceptance Criteria: These optional acceptance criteria apply to both the Best Fit method and the Time Range method. These rules are used to flag profiles (described further below) where the Lambda Z final parameter does not meet the specified acceptance criteria.

- Specify the minimum value of Rsq_adjusted that indicates an acceptable fit for Lambda Z in the **Rsq_adjusted** field. Value must be between 0 and 1.
- Use the pull-down menu to select whether to use **AUC_%Extrap_obs** or **AUC_%Extrap_pred** (or, for Urine models, **AURC_%Extrap_obs** or **AURC_%Extrap_pred**) when indicating the acceptable fit for Lambda Z. In the field, enter the maximum value to use. Value must be between 0 and 100.
- In the **Span** field, specify the minimum span or number of half-lives needed for the Lambda Z range to be acceptable. Values must be positive.

If a profile does not have an acceptable Lambda Z fit as specified by the acceptance criteria, an output flag value of ‘Not_Accepted’ is used to flag each of the criterion that is not met by that profile. In addition, a flag value of ‘Missing’ is used to flag all profiles where the parameter used for acceptance cannot be computed. Note that all computed results for flagged profiles are still included in the output, but the final parameters for these profiles will be marked by the flags. All profiles that have an acceptable Lambda Z fit (i.e., meet the acceptance criterion) will have the flag value of ‘Accepted’.

The flags appear in columns in the Final Parameters Pivoted output worksheet immediately after the column for the parameter used for the acceptance criterion. The flag column names are ‘Flag’ appended with the parameter name used for acceptance, e.g., Flag_Rsq_adjusted, Flag_Span. If the user wants to remove the profiles failing to meet the acceptance criteria from the output, the Final Parameters Pivoted worksheet can be processed by the Data Wizard to delete these profiles, by filtering on the flag values of ‘Not_Accepted’ and excluding these rows. Note that the Flag columns do not appear in the output when there are no flagged profiles, that is, when all profiles meet the acceptance criterion or when the acceptance criterion is not set.

In the Final Parameters output worksheet and in the Core Output text, where the final parameter output is stacked, the flag names and values appear below the acceptance parameter. If the acceptance parameter is selected to not be included in the worksheet output in the “Parameters Names” setup, the corresponding flag will also not be included in the worksheet.

See “Data checking and pre-treatment” for a list of cases that also produce flagged output.

Plots tab

The Plots tab allows users to select individual plots to include in the output.

- Use the checkboxes to toggle the creation of graphs.
- Click **Reset Existing Plots** to clear all existing plot output.

  Each plot in the Results tab is a single plot object. Every time a model is executed, each object remains the same, but the values used to create the plot are updated. This way, any styles that are applied to the plots are retained no matter how many times the model is executed. Clicking **Reset Existing Plots** removes the plot objects from the Results tab, which clears any custom changes made to the plot display.
- Use the **Enable All** and **Disable All** buttons to check or clear all checkboxes for all plots in the list.

Results

After an NCA object is executed, the output is displayed on the Results tab in Phoenix.

- Worksheet output: worksheets listing input data, output parameters, as well as execution summary.
- Plot output: plots of observed and predicted data.
- Core Output: text version of all model settings and output, including any errors that occurred during modeling.
- The Settings text file lists the user-specified settings in the NCA object.

Worksheet output

Worksheet output contains summary tables of the modeling data and a summary of the information in the Core Output. The worksheets present the output in a form that can be used for reporting and further analyses. The results worksheets are listed on the Results tab underneath Output Data.

Note: The worksheets produced depend on the analysis type and model settings.

Dosing Used: The dosing regimen specified in the Dosing panel.

Exclusions: Any excluded data points specified in the Slopes panel.

Final Parameters: Estimates of the final parameters for each level of the sort variable, including times and areas above (“TimeHigh”), in (“TimeBetween”) and below (“TimeLow”) the therapeutic response (AUCHigh, AUCLow, etc.). Parameter names that include “Inf” are extrapolated to infinity using estimated Lambda Z.

Final Parameters Pivoted: The same as Final Parameters, but with one parameter per column, in order to conveniently perform further analysis on individual parameters.

Partial Areas: Lists start and end times used to define the partial areas under the curve.

Plot Titles: The title of each graph in the output.

Slopes Settings: The settings for the user specified for the Terminal elimination phase.
It includes the start and end time for each defined time range, excluded points, fit method used and whether the time range was set by the System or the User.

Summary Table: The sort variables, X variable, points included in the regression for Lambda Z (noted with *), Y variable, predicted Y for the regression, residual for the regression, area under the curve (AUC), area under the moment curve AUMC and the weight used for the regression.

Therapeutic Response: Lists the lower and upper boundaries used to define the therapeutic response windows.

User Defined Computed Y: List of user-entered time or X values and the concentration or Y values. The Type column indicates if the value was Observed or Computed by interpolation.

User Defined Parameters: List of any parameters defined by the user in the User Defined Parameters tab.

User Defined Parameters Pivoted: The same as User Defined Parameters, but with one parameter per column, in order to conveniently perform further analysis on individual parameters.

Plot output

Executing an NCA object generates an ‘Observed Y and Predicted Y vs X’ plot for each subject. Plot output is listed underneath Plots in the Results tab.

Note: Hovering over a regression line in the NCA plot result (Observed Y and Predicted Y vs X plot) displays [time, conc] value pairs. However only coordinates of the predicted value at the nearest observed time points are displayed. In other words, hovering over the regression line does not give continuous predicted coordinates.

NCA computation rules

Computational rules for the NCA engine are discussed in sections:

Time deviations in steady-state data
Data checking and pre-treatment

NCA computations are covered under the headings:

Lambda Z or Slope Estimation settings
Therapeutic response
Partial area calculation
Sparse sampling calculation
Drug effect calculation
Weighting

Time deviations in steady-state data

When using steady-state data, Phoenix computes AUC_TAU from dose time to dose time+tau, based on the tau value set in the Dosing panel. However, in most studies, there are sampling time deviations. For example, if dose time=0 and tau=24, the last sample might be at 23.975 or 24.083 hours. In this instance, the program will estimate the AUC_TAU based on the estimated concentration at 24 hours, and not the concentration at the actual observation time. For steady state data, Cmax, Tmax, Cmin and Tmin are found using observations taken at or after the dose time, but no later than dose time+tau.

Data checking and pre-treatment

Prior to calculation of pharmacokinetic parameters, Phoenix institutes a number of data-checking and pre-treatment procedures as follows.

- **Sorting**: Prior to analysis, data within each profile is sorted in ascending time order. (A profile is identified by a unique combination of sort variable values.)
- **Inserting initial time points**: If a PK profile does not contain an observation at dose time, Phoenix inserts a value using the following rules. (Note that, if there is no dosing time specified, the dosing time is assumed to be zero.)

  Extravascular and **Infusion** data: For single dose data, a concentration of zero is used; for steady-state, the minimum observed during the dose interval (from dose time to dose time +tau) is used.

  IV Bolus data: Phoenix performs a log-linear regression of first two data points to back-extrapolate C0. If the regression yields a slope >= 0, or at least one of the first two y-values is zero, or if one or both of the points is viewed as an outlier and excluded from the Lambda Z regression, then the first observed y-value is used as an estimate for C0. If a weighting option is selected, it is used in the regression.

  Urine data: A rate value of zero is used at dose time.

  Drug Effect data: An effect value equal to the user-supplied baseline is inserted at dose time (assumed to be zero if none is supplied).

  The inserted point at dose time is needed to compute the initial trapezoid from dose time to the first observation for the AUC final parameters and to compute partial areas or therapeutic response parameters that depend on times from the dose time to the first observation. The inserted point is never used in Lambda Z computations.

Only one observation per profile at each time point is permitted. If multiple observations at the same time point are detected, the analysis is halted with an error message. No output is generated.

- **Data exclusions**: NCA automatically excludes unusable data points meeting the following criteria:

  Missing values: For plasma models, if either the time or concentration value is missing or non-numeric, then the associated record is excluded from the analysis. For urine models, records with missing or non-numeric values for any of the following are excluded: collection interval start or stop time, drug concentration, or collection volume. Also for urine models, if the collection volume is zero, the record is excluded.

  Data points preceding the dose time: If an observation time is earlier than the dosing time, then that observation is excluded from the analysis. For urine models, the lower time point is checked.

  Data points for urine models: In addition to the above rules, if the lower time is greater than or equal to the upper time, or if the concentration or volume is negative, the data must be corrected before NCA can run.

- **Flagging data deficiencies in the Final Parameters output**: After exclusion of unusable data points as described in the prior section, profiles that result in no non-missing observations, and profiles that result in only one non-missing observation where a valid point at dose time cannot be inserted, have a flag called “Flag_N_Samples” in the Final Parameters output. More specifically, these cases are:
  - Profile with all missing observations (N_Samples=0) for any NCA Model Type. No Final Parameters can be reported.
  - Urine model profile with all urine volumes equal to zero. This is equivalent to no measurements being taken (N_Samples=0). No Final Parameters can be reported.
  - Profile with all missing observations, except one non-missing observation at dose time, for Plasma or Drug Effect models (N=1 and a point cannot be inserted at dose time because the one observation is already at dose time). All Final Parameters that can be defined, e.g., Cmax, Tmax, Cmin, Tmin, are reported. (This case does not apply to Urine models — the midpoint of an acceptable observation would be after dose time.)
  - Bolus-dosing plasma profile with all missing observations, except one non-missing observation after dose time (N=1 and a point cannot be inserted at dose time because NCA does not back-extrapolate C0 when there is only one non-missing observation). All Final Parameters that can be defined, e.g., Cmax, Tmax, Cmin, Tmin, are reported.

  Each of these cases will have a ‘Flag_N_Samples” value in the Final Parameters output that is ‘Insufficient’. This flag allows these profiles to be filtered out of the output worksheets if desired, using the Data Wizard.

Note: A non-bolus profile with all missing observations, except one non-missing positive observation after dose time is not a data deficiency, because a valid point is inserted at dose time – (dose time, 0), or (dose time, Cmin) for steady state – which provides a second data point.

Lambda Z or Slope Estimation settings

This section pertains to NCA and IVIVC objects.

Phoenix will attempt to estimate the rate constant, Lambda Z, associated with the terminal elimination phase for concentration data. If Lambda Z is estimable, parameters for concentration data will be extrapolated to infinity. For NCA drug effect models, Phoenix estimates the two slopes at the beginning and end of the data. NCA does not extrapolate beyond the observed data for drug effect models.

Lambda Z or slope range selection

Phoenix will automatically determine the data points to include in Lambda Z or slope calculations as follows. (The exception to this is if the time range to use in the calculation of Lambda Z or slopes is not specified in an NCA object and curve stripping is not disabled, as described under “Options tab”.)

For concentration data: To estimate the best fit for Lambda Z, Phoenix repeats regressions of the natural logarithm of the concentration values using the last three points with non-zero concentrations, then the last four points, last five points, etc. Points with a concentration value of zero are not included since the logarithm cannot be taken. Points prior to Cmax, points prior to the end of infusion, and the point at Cmax for non-bolus models, are not used in the Best Fit method (they can only be used if the user specifically requests a time range that includes them). For each regression, an adjusted R^2 is computed:

$$Adjusted\ R^2 = 1 - \frac{(1 - R^2) \times (n - 1)}{(n - 2)}$$

where n is the number of data points in the regression and R^2 is the square of the correlation coefficient.

Lambda Z is estimated using the regression with the largest adjusted R^2 and:
– If the adjusted R^2 does not improve, but is within 0.0001 of the largest adjusted R^2 value, the regression with the larger number of points is used.
– Lambda Z must be calculated from at least three data points.
– The estimated slope must be negative, so that its negative Lambda Z is positive.

For sparse sampling data: For sparse data, the mean concentration at each time (plasma or serum data) or mean rate for each interval (urine data) is used when estimating Lambda Z. Otherwise, the method is the same as for concentration data.

For drug effect data: Phoenix will compute the best-fitting slopes at the beginning of the data and at the end of the data using the same rules that are used for Lambda Z (best adjusted R-square with at least three points), with the exception that linear or log regression can be used according to the user's choice and the estimated slope can be positive or negative. If the user specifies the range only for Slope1, then in addition to computing Slope1, the best-fitting slope for Slope 2 will be computed at the end of the data. If the user specifies the range only for Slope2, then the best-fitting slope for Slope 1 will be computed at the beginning of the data.

The data points included in each slope are indicated on the Summary table of the output workbook and text for model 220. Data points for Slope1 are marked with “1” in workbook output and footnoted using “#” in text output; data points for Slope2 are labeled “2” and footnoted using an asterisk, “*”.

Calculation of Lambda Z

Once the time points being used in the regression have been determined either from the Best Fit method or from the user’s specified time range, Phoenix can estimate Lambda Z by performing a regression of the natural logarithm of the concentration values in this range of sampling times. The estimate slope must be negative, Lambda Z is defined as the negative of the estimated slope.

Note: Using this methodology, Phoenix will almost always compute an estimate for Lambda Z. It is the user’s responsibility to evaluate the appropriateness of the estimated value.

Calculation of slopes for effect data

Phoenix estimates slopes by performing a regression of the response values or their natural logarithm depending on the user's selection. The actual slopes are reported; they are not negated as for Lambda Z.

Limitations of Lambda Z and slope estimation

It is not possible for Phoenix to estimate Lambda Z or slope in the following cases.

- There are only two non-missing observations and the user requested automatic range selection (Best Fit).
- The user-specified range contains fewer than two non-missing observations.
- Automatic range selection is chosen, but there are fewer than three positive concentration values after the Cmax of the profile for non-bolus models and fewer than two for bolus models or the slope is not negative.
- For infusion data, automatic range selection is chosen, and there are fewer than three points at or after the infusion stop time.
- The time difference between the first and last data points in the estimation range used is approximately < 1e –10.

In these instances, the curve fit for that subject will be omitted. The parameters that do not depend on Lambda Z (i.e., Cmax, Tmax, AUClast, etc.) will still be reported, but the software will issue a warning in the text output, indicating that Lambda Z was not estimable.

Therapeutic response

For non-compartmental analysis of concentration data, Phoenix computes additional parameters for different time range computations, as listed below. The parameters included depend on whether upper, lower, or both limits are supplied. See "Therapeutic response windows" for additional computation details.

Time range from dose time to last observation
Additional parameters:

TimeLow: Total time below lower limit. Included when lower or both limits are specified.
TimeBetween: Total time between lower/upper limits. Included when both limits are specified.
TimeHigh: Total time above upper limit. Included when lower or both limits are specified.
AUCLow: AUC that falls below lower limit: Included when lower or both limits are specified.
AUCBetween: AUC that falls between lower/upper limits. Included when both limits are specified.
AUCHigh: AUC that falls above upper limit. Included when lower or both limits are specified.

Time range from dose time to infinity using Lambda Z (if Lambda Z exists)
Additional parameters:

TimeInfBetween: Total time (extrapolated to infinity) between lower/upper limits. Included when both limits are specified.
TimeInfHigh: Total time (extrapolated to infinity) above upper limit. Included when lower or both limits are specified.
AUCInfLow: AUC (extrapolated to infinity) that falls below lower limit: Included when lower or both limits are specified.
AUCInfBetween: AUC (extrapolated to infinity) that falls between lower/upper limits. Included when both limits are specified.
AUCInfHigh: AUC (extrapolated to infinity) that falls above upper limit. Included when lower or both limits are specified.

Therapeutic response windows

For therapeutic windows, one or two boundaries for concentration values can be given, and the program computes the time spent in each window determined by the boundaries and computes the area under the curve contained in each window. To compute these values, for each pair of consecutive data points, including the inserted point at dosing time if there is one, it is determined if a boundary crossing occurred in that interval. Call the pair of time values from the dataset (t_i, t_{i+1}) and called the boundaries y_{lower} and y_{upper}.

If no boundary crossing occurred, the difference t_{i+1}, t_i is added to the appropriate parameter $TimeLow$, $TimeBetween$, or $TimeHigh$, depending on which window the concentration values are in. The AUC for (t_i, t_{i+1}) is computed following the user's specified AUC calculation method as described in the section on the "Options tab". Call this $AUC*$. The parts of this $AUC*$ are added to the appropriate parameters $AUCLow$, $AUCBetween$, or $AUCHigh$. For example, if the concentration values for this interval occur above the upper boundary, the rectangle that is under the lower boundary is added to $AUCLow$, the rectangle that is between the two boundaries is added to $AUCBetween$ and the piece that is left is added to $AUCHigh$. This is equivalent to these formulae:

$$AUCLow = AUCLow + y_{lower}(t_{i+1} - t_i)$$

$$AUCBetween = AUCBetween + (y_{upper} - y_{lower})(t_{i+1} - t_i)$$

$$AUCHigh = AUCHigh + AUC* - y_{upper}(t_{i+1} - t_i)$$

If there was one boundary crossing, an interpolation is done to get the time value where the crossing occurred; call this $t*$. The interpolation is done by either the linear rule or the log rule following the user's AUC calculation method as described in the section on the "Options tab", i.e., the same rule is used as when inserting a point for partial areas. To interpolate to get time $t*$ in the interval (t_i, t_{i+1}) at which the concentration value is y_w:

Linear interpolation rule for time:

$$t* = t_i + \left[\frac{y_w - y_i}{y_{i+1} - y_i}\right](t_{i+1} - t_i)$$

Log interpolation rule for time:

$$t* = t_i + \left[\frac{\ln(y_w) - \ln(y_i)}{\ln(y_{i+1}) - \ln(y_i)}\right](t_{i+1} - t_i)$$

The AUC for (t_i, $t*$) is computed following the user's specified AUC calculation method. The difference $t* - t_i$ is added to the appropriate time parameter and the parts of the AUC are added to the appropriate AUC parameters. Then the process is repeated for the interval ($t*$, t_{i+1}).

If there were two boundary crossings, an interpolation is done to get $t*$ as above for the first boundary crossing, and the time and parts of AUC are added in for the interval (t_i, $t*$) as above. Then another interpolation is done from t_i to t_{i+1} to get the time of the second crossing $t**$ between ($t*$, t_{i+1}), and the time and parts of AUC are added in for the interval ($t*$, $t**$). Then the times and parts of AUC are added in for the interval ($t**$, t_{i+1}).

The extrapolated times and areas after the last data point are now considered for the therapeutic window parameters that are extrapolated to infinity. For any of the AUC calculation methods, the AUC rule after tlast is the log rule, unless an endpoint for the interval is negative or zero in which case the linear rule must be used. The extrapolation rule for finding the time $t*$ at which the extrapolated curve would cross the boundary concentration y_w is:

$$t* = \left(\frac{1}{Lambda\_z}\right)(Lambda\_z\_intercept - \ln(y_w))$$

If the last concentration value in the dataset is zero, the zero value was used in the above computation for the last interval, but now ylast is replaced with the following if Lambda Z is estimable:

$$y_{last} = \exp(Lambda\_z\_intercept - Lambda\_z(t_{last}))$$

If ylast is in the lower window: $InfBetween$ and $InfHigh$ values are the same as $Between$ and $High$ values. If Lambda Z is not estimable, $AUCInfLow$ is missing. Otherwise:

$$AUCInfLow = AUCLow + (y_{last} / Lambda\_z)$$

If ylast is in the middle window for two boundaries: $InfHigh$ values are the same as $High$ values. If Lambda Z is not estimable, $InfLow$ and $InfBetween$ parameters are missing. ***Otherwise:*** Use the extrapolation rule to get $t*$ at the lower boundary concentration. Add time from $tlast$ to $t*$ into $TimeInfBetween$. Compute $AUC*$ from $tlast$ to $t*$, add the rectangle that is under the lower boundary into $AUCInfLow$, and add the piece that is left into $AUCInfBetween$. Add the extrapolated area in the lower window into $AUCInfLow$. This is equivalent to:

$$t* = (1/Lambda\_z)(Lambda\_z\_intercept - \ln(y_{lower}))$$

$$TimeInfBetween = TimeBetween + (t* - t_{last})$$

$$AUCInfBetween = AUCBetween + AUC* - y_{lower}(t* - t_{last})$$

$$AUCInfLow = AUCLow + y_{lower}(t* - t_{last}) + y_{lower} / Lambda\_z$$

If ylast is in the top window when only one boundary is given, the above procedure is followed and the $InfBetween$ parameters become the $InfHigh$ parameters.

If there are two boundaries and y_{last} is in the top window: If Lambda Z is not estimable, all extrapolated parameters are missing. Otherwise, use the extrapolation rule to get $t*$ at the upper boundary concentration value. Add time from tlast to $t*$ into $TimeInfHigh$. Compute $AUC*$ for tlast to $t*$, add the rectangle that is under the lower boundary into $AUCInfLow$, add the rectangle that is in the middle window into $AUCInfBetween$, and add the piece that is left into $AUCInfHigh$. Use the extrapolation rule to get $t**$ at the lower boundary concentration value. Add time from $t*$ to $t**$ into $TimeInfBetween$. Compute $AUC**$ from $t*$ to $t**$, add the rectangle that is under the lower boundary into $AUCInfLow$, and add the piece that is left into $AUCInfBetween$. Add the extrapolated area in the lower window into $AUCInfLow$. This is equivalent to:

$$t* = (1/Lambda\_z)(Lambda\_z\_intercept - \ln(y_{upper}))$$

$$t** = (1/Lambda\_z)(Lambda\_z\_intercept - \ln(y_{lower}))$$

$$TimeInfHigh = TimeHigh + (t* - t_{last})$$

$$TimeInfBetween = TimeBetween + (t** - t*)$$

$$AUCInfHigh = AUCHigh + AUC^* - y_{upper}(t^* - t_{last})$$

$$\begin{aligned} AUCInfBetween &= AUCBetween \\ &+ AUC^* + (y_{upper} - y_{lower})(t^* - t_{last}) \\ &+ AUC^{**} - y_{lower}(t^{**} - t^*) \end{aligned}$$

$$\begin{aligned} AUCInfLow &= AUCLow + y_{lower}(t^* - t_{last}) \\ &+ y_{lower}(t^{**} - t^*) + y_{lower} / Lambda\_z \end{aligned}$$

Partial area calculation

Linear trapezoidal rule

$$AUC\Big|_{t_1}^{t_2} = \delta t \times \frac{C_1 + C_2}{2}$$

$$AUMC\Big|_{t_1}^{t_2} = \delta t \times \frac{t_1 \times C_1 + t_2 \times C_2}{2}$$

Logarithmic trapezoidal rule

$$AUC\Big|_{t_1}^{t_2} = \delta t \times \frac{C_2 - C_1}{\ln\left(\frac{C_2}{C_1}\right)}$$

$$AUMC\Big|_{t_1}^{t_2} = \delta t \times \frac{t_2 \times C_2 - t_1 \times C_1}{\ln\left(\frac{C_2}{C_1}\right)} - \delta t^2 \times \frac{C_2 - C_1}{\ln\left(\frac{C_2}{C_1}\right)^2}$$

where δt is $(t_2 - t_1)$.

Note: If the logarithmic trapezoidal rule fails in an interval because $C_1 <= 0$, $C_2 <= 0$, or $C_1 = C_2$, then the linear trapezoidal rule will apply for that interval.

Linear interpolation rule (to find C^* at time t^* for $t_1 < t^* < t_2$)

$$C^* = C_1 + \left|\frac{t^* - t_1}{t_2 - t_1}\right|(C_2 - C_1)$$

Logarithmic interpolation rule

$$C^* = \exp\left(\ln(C_1) + \left|\frac{t^* - t_1}{t_2 - t_1}\right| \times (\ln(C_2) - \ln(C_1))\right)$$

Note: If the logarithmic interpolation rule fails in an interval because C_1 <= 0 or C_2 <= 0, then the linear interpolation rule will apply for that interval.

Extrapolation (to find C^* after the last numeric observation)

Additional rules for plasma and urine models

The following additional rules apply for plasma and urine models (not model 220 for Drug Effect):

- If a start or end time falls before the first observation and after the dose time, the corresponding Y value is interpolated between the first data point and $C0$. $C0$=0 except in IV bolus models (model 201), where $C0$ is the dosing time intercept estimated by Phoenix, and except for models 200 and 202 at steady state, where $C0$ is the minimum value between dose time and tau.
- If a start or end time falls within the range of the data but does not coincide with an observed data point, then a linear or logarithmic interpolation is done to estimate the corresponding Y, according to the AUC Calculation method selected in the NCA Options. (See "Options tab".) Note that logarithmic interpolation is overridden by linear interpolation in the case of a non-positive endpoint.
- If a start or end time occurs after the last numeric observation (i.e., not "missing" or "BQL") and Lambda Z is estimable, Lambda Z is used to estimate the corresponding Y:

  $$\begin{aligned} Y &= \exp(Lambda\_z\_intercept - Lambda\_z(t)) \\ &= [\exp(Lambda\_z\_intercept - Lambda\_z(t_{last}))] \\ &\quad [\exp(-Lambda\_z(t - t_{last}))] \\ &= (\text{predictedConcAtTlast})\exp(-Lambda\_z(t - t_{last})) \end{aligned}$$

  The values $Lambda\_z\_intercept$ and $Lambda\_z$ are those values found during the regression for Lambda Z. Note that a last observation of zero will be used for linear interpolation, i.e., this rule does not apply prior to a last observation of zero.
- If a start or end time falls after the last numeric observation and Lambda Z is not estimable, the partial area will not be calculated.
- If both the start and end time for a partial area fall at or after the last positive observation, then the log trapezoidal rule will be used. However, if any intervals used in computing the partial area have non-positive endpoints or equal endpoints (for example, there is an observation of zero that is used in computing the partial area), then the linear trapezoidal rule will override the log trapezoidal rule.
- If the start time for a partial area is before the last numeric observation and the end time is after the last numeric observation, then the log trapezoidal rule will be used for the area from the last observation time to the end time of the partial area. However, if the last observation is non-positive or is equal to the extrapolated value for the end time of the partial area, then the linear trapezoidal rule will override the log trapezoidal rule.

The end time for the partial area must be greater than the start time. Both the start and end time for the partial area must be at or after the dosing time.

Sparse sampling calculation

The NCA object provides special methods to analyze concentration data with few observations per subject. The NCA object treats this sparse data as a special case of plasma or urine concentration data. It first calculates the mean concentration curve of the data, by taking the mean concentration value for each unique time value for plasma data, or the mean rate value for each unique midpoint for urine data. For this reason, it is recommended to use nominal time, rather than actual time, for these analyses. The standard error of the data is also calculated for each unique time or midpoint value.

Using the mean concentration curve, the NCA object calculates all of the usual plasma or urine final parameters listed under "NCA parameter formulas". In addition, it uses the subject information to calculate standard errors that will account for any correlations in the data resulting from repeated sampling of individual animals.

The NCA sparse methodology calculates PK parameters based on the mean profile for all the subjects in the dataset. For batch designs, where multiple time points are measured for each subject, this methodology only generates unbiased estimates if equal sample sizes per time point are present. If this is not the case, then bias in the parameter estimates is introduced.

Note: In order to create unbiased estimates, the sparse sampling routines used in the NCA object require that the dataset does not contain missing data.

For plasma data (models 200–202), the NCA object calculates the standard error for the mean concentration curve's maximum value (Cmax), and for the area under the mean concentration curve from dose time through the final observed time (AUCall). Standard error of the mean Cmax will be calculated as the sample standard deviation of the y-values at time Tmax divided by the square root of the number of observations at Tmax, or equivalently, the sample standard error of the y-values at Tmax. Standard error of the mean AUC will be calculated as described in Nedelman and Jia (1998), using a modification in Holder (2001), and will account for any correlations in the data resulting from repeated sampling of individual animals. Specifically:

$$\mathrm{SE}(A\hat{U}C) = \sqrt{\mathrm{Var}(A\hat{U}C)}$$

Since AUC is calculated by the linear trapezoidal rule as a linear combination of the mean concentration values,

$$A\hat{U}C = \sum_{i=0}^{m} w_i \bar{C}_i$$

where:

$$\bar{C}_i = \text{the sample mean at time i}$$

$$w_i = \begin{cases} (t_1 - t_0)/2, & i = 0 \\ (t_{i+1} - t_{i-1})/2, & i = 1, \ldots, m-1 \\ (t_m - t_{m-1})/2, & i = m \end{cases}$$

m=last observation time for AUCall, or time of last measurable (positive) mean concentration for AUClast it follows that:

$$Var(A\hat{U}C) = \sum_{i=0}^{m} \frac{w_i^2 s_i^2}{r_i} + 2\sum_{i<j} \frac{w_i w_j r_{ij} s_{ij}}{r_i r_j}$$

where:

r_{ij} = number of animals sampled at both times i and j

r_i = number of animals sampled at time i

s_i^2 = sample variance of concentrations at time i

s_{ij} = sample covariance between concentrations c_{ik} and c_{jk} for all animals k that are sampled at both times i and j

The above equations can be computed from basic statistics, and appear as equation (7.vii) in Nedelman and Jia (1998). When computing the sample covariances in the above, NCA uses the unbiased sample covariance estimator, which can be found as equation (A3) in Holder (2001):

$$s_{ij} = \sum_{k=1}^{r_{ij}} \frac{(C_{ik} - \overline{C}_i)(C_{jk} - \overline{C}_j)}{(r_{ij} - 1) + \left(1 - \frac{r_{ij}}{r_i}\right)\left(1 - \frac{r_{ij}}{r_j}\right)}$$

For urine models (models 210–212), the standard errors are computed for Max_Rate, the maximum observed excretion rate, and for AURC_all, the area under the mean rate curve through the final observed rate.

For cases where a non-zero value $C0$ must be inserted at dose time t_0 to obtain better AUC estimates (see “Data checking and pre-treatment”), the AUC estimate contains an additional constant term: $C^* = C0(t_1 - t_0)/2$. In other words, w_0 is multiplied by $C0$, instead of being multiplied by zero as occurs when the point (0,0) is inserted. An added constant in AÛC will not change $Var(A\hat{U}C)$, so *SE_AUClast* and *SE_AUCall* also will not change. Note that the inserted C0 is treated as a constant even when it must be estimated from other points in the dataset, so that the variances and covariances of those other data points are not duplicated in the $Var(A\hat{U}C)$ computation.

For the case in which r_{ij}=1, and r_i=1 or r_j=1, then s_{ij} is set to 0 (zero).

The AUCs must be calculated using one of the linear trapezoidal rules. Select a rule using the instructions listed under “Options tab”.

Drug effect calculation

NCA for drug effect data requires the following input data variables:

Dependent variable, i.e., response or effect values (continuous scale)

Independent variable, generally the time of each observation. For non-time-based data, such as concentration-effect data, take care to interpret output parameters such as “Tmin” (independent variable value corresponding to minimum dependent variable value) accordingly.

It also requires the following constants:

Dose time (relative to observation times), entered in the Dosing worksheet

Baseline response value, entered in the Therapeutic Response tab of the Model Properties or pulled from a Dosing worksheet as described below

Threshold response value (optional), entered in the Therapeutic Response tab of the NCA Diagram

If the user does not enter a baseline response value, the baseline is assumed to be zero, with the following exception. When working from a Certara Integral study that includes a Dosing worksheet, if no baseline is provided by the user, Phoenix will use the response value at dose time as baseline, and if the dataset does not include a response at dose time, the user will be required to supply the baseline value.

If there is no response value at dose time, or if dose time is not given, see "Data checking and pretreatment" for the insertion of the point (dosetime, baseline).

Instead of Lambda Z calculations, as computed in NCA PK models, the NCA PD model can calculate the slope of the time-effect curve for specific data ranges in each profile. Unlike Lambda Z, these slopes are reported as their actual value rather than their negative. See "Lambda Z or Slope Estimation settings" for more information.

Like other NCA models, the PD model can compute partial areas under the curve, but only within the range of the data (no extrapolation is done). If a start or end time does not coincide with an observed data point, then interpolation is done to estimate the corresponding Y, following the equations and rules described under "Partial area calculation".

For PD data, the default and recommended method for AUC calculation is the Linear Trapezoidal with Linear Interpolation (set as described under "Options tab".) Use caution with log trapezoidal since areas are calculated both under the curve and above the curve. If the log trapezoidal rule is appropriate for the area under a curve, then it would underestimate the area over the curve. For this reason, Phoenix uses the linear trapezoidal rule for area where the curve is below baseline when computing AUC_Below_B, and similarly for threshold and AUC_Below_T.

Weighting

Phoenix provides flexibility in performing weighted nonlinear regression and using weighted regression. Weights are assigned using menu options or by adding weighting values to a dataset. Each operational object in Phoenix that uses weighted values has instructions for using weighting.

There are three ways to assign weights, other than uniform weighting, through the Phoenix user interface:

Weighted least squares: weight by a power of the observed value of Y.
Iterative reweighting: weight by a power of the predicted value of Y.
Reading weights from the dataset: include weight as a column in the dataset.

Weighted least squares

When using weighted least squares, Phoenix weights each observation by the value of the dependent variable raised to the power of n. That is, WEIGHT=Y^n. For example, selecting this option and setting n= –0.5 instructs Phoenix to weight the data by the square root of the reciprocal of observed Y:

$$1/\sqrt{Y}$$

If n has a negative value, and one or more of the Y values are less than or equal to zero, then the corresponding weights are set to zero.

The application scales the weights such that the sum of the weights for each function equals the number of observations with non-zero weights. See "Scaling of weights".

Iterative reweighting

Iterative Reweighting redefines the weights for each observation to be F^n, where F is the predicted response. For example, selecting this option and setting n= –0.5 instructs Phoenix to weight the data by the square root of reciprocal of the predicted value of Y, i.e.,

$$1/(\sqrt{\hat{Y}})$$

As with Weighted least squares, if N is negative, and one or more of the predicted Y values are less than or equal to zero, then the corresponding weights are set to zero.

Iterative reweighting differs from weighted least squares in that for weighted least squares the weights are fixed. For iteratively re-weighted least squares the parameters change at each iteration, and therefore the predicted values and the weights change at each iteration.

For certain types of models, iterative reweighting can be used to obtain maximum likelihood estimates. For more information see the article by Jennrich and Moore (1975). Maximum likelihood estimation by means of nonlinear least squares. *Amer Stat Assoc Proceedings Statistical Computing Section* 57–65.

Reading weights from the dataset

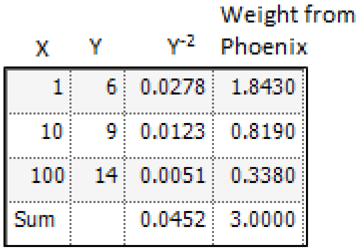
It is also possible to have a variable in the dataset that has as its values the weights to use. The weights should be the reciprocal of the variance of the observations. As with Weighted Least Squares, the application scales the weights such that the sum of the weights for each function equals the number of observations with non-zero weights.

Scaling of weights

When weights are read from a dataset or when weighted least squares is used, the weights for the individual data values are scaled so that the sum of the weights for each function is equal to the number of data values with non-zero weights.

The scaling of the weights has no effect on the model fitting as the weights of the observations are proportionally the same. However, scaling of the weights provides increased numerical stability.

Consider the following example:



X	Y	Y^{-2}	Weight from Phoenix
1	6	0.0278	1.8430
10	9	0.0123	0.8190
100	14	0.0051	0.3380
Sum		0.0452	3.0000

Suppose weighted least squares with the power –2 was specified, which is the same as weighting by 1/Y*Y. The corresponding values are as shown in the third column. Each value of Y^{-2} is divided by the sum of the values in the third column, and then multiplied by the number of observations, so that:

(0.0277778/.0452254)*3=1.8426238, or 1.843 after rounding.

Note that 0.0278/0.0051 is the same proportion as 1.843/0.338.

NCA parameter formulas

Plasma or serum data
Urine data
Sparse sampling (pre-clinical) data
Drug effect data model 220
User defined parameters
References

See also "NCA" and, for additional reading, see "References".

Plasma or serum data

Data structure: NCA for blood concentration data requires the following input data:

- Time of each sample
- Plasma or serum concentrations

Output: Models 200–202 estimate the parameters in the following lists.

Plasma parameters that do not require Lambda Z estimation

Plasma parameters that are estimated when Lambda Z is estimated

Plasma parameters that are estimated when at steady-state

Plasma parameters that do not require Lambda Z estimation

Dosing time: Available as 'Time' in the Dosing Used results. Time of last administered dose. It is assumed to be zero unless otherwise specified. This parameter is used mainly with steady-state data, where time may be coded as the time elapsed since the first dose, or the elapsed time since the time of the first dose.

N_Samples: This parameter reports the number of non-missing observations used in the analysis of the profile (time is at or after dosing time, the observation is numeric, and the volume is positive for urine models). It does not count points inserted by engine, e.g., inserted at dosing time.

Dose: Amount of last administered dose. This is assumed to be zero if not specified.

No_points_Lambda_z: Number of points used in computing Lambda Z. If Lambda Z is not estimable, zero.

Tlag: Time of observation prior to the first observation with a measurable (non-zero) concentration. For plasma models, Tlag is only computed when the dosing type is extravascular.

Tmax: Time of maximum observed concentration. For non-steady-state data, the entire curve is considered. If the maximum observed concentration is not unique, then the first maximum is used.

Cmax: Maximum observed concentration, occurring at time Tmax, as defined above.

Cmax_D: = $Cmax/Dose$

C0: Initial concentration. Given only for IV Bolus dosing. It is equal to the first observed concentration value if that value occurs at the dose time. Otherwise, it is estimated by back-extrapolating (see AUC_%Back_Ext below).

Tlast: Time of last measurable (positive) observed concentration.

Clast: Observed concentration corresponding to Tlast.

AUClast: Area under the curve from the time of dosing to the time of the last measurable (positive) concentration (Tlast).

AUClast_D: = $AUClast/Dose$

AUCall: Area under the curve from the time of dosing to the time of the last observation. If the last concentration is positive, AUClast=AUCall. Otherwise, AUCall will not be equal to AUClast, as it includes the additional area from the last measurable (positive) concentration down to zero or negative observations.

AUMClast: Area under the moment curve from the time of dosing to the last measurable (positive) concentration.

MRTlast: Mean residence time from the time of dosing to the time of the last measurable concentration.

For non-infusion models: = $AUMClast/AUClast$
For infusion models: = $(AUMClast/AUClast) - (Tinf/2)$
where $Tinf$ is the length of infusion.

Plasma parameters that are estimated when Lambda Z is estimated

The following list includes several parameters that are extrapolated to infinity. These parameters are calculated two ways: based on the last observed concentration (indicated by "_obs" appended to the parameter name), or based on the last predicted concentration (indicated by "_pred" appended to the parameter name), where the predicted value is based on the linear regression performed to estimate Lambda Z.

Rsq: Goodness of fit statistic for the terminal elimination phase.

Rsq_adjusted: Goodness of fit statistic for the terminal elimination phase, adjusted for the number of points used in the estimation of Lambda Z.

Corr_XY: Correlation between time (X) and log concentration (Y) for the points used in the estimation of Lambda Z.

Lambda_z: First-order rate constant associated with the terminal (log-linear) portion of the curve. Estimated by linear regression of time vs. log concentration.

Lambda_z_intercept: Intercept on log scale estimated via linear regression of time vs. log concentration.

Lambda_z_lower: Lower limit on time for values to be included in the calculation of Lambda Z.

Lambda_z_upper: Upper limit on time for values to be included in the calculation of Lambda Z.

HL_Lambda_z: Terminal half-life: = $\ln(2)/\lambda_z$

Span: = $(Lambda\_z\_upper - Lambda\_z\_lower)/HL\_Lambda\_z$

Clast_pred: Predicted concentration at $Tlast$:

$$= exp(Lambda\_z\_intercept - Lambda\_z*Tlast)$$

AUCINF(_obs, _pred): AUC from time of dosing extrapolated to infinity, based on the last observed concentration (_obs) or last predicted concentration (_pred).

$$= AUClast + (Clast/Lambda\_z)$$

AUCINF_D(_obs, _pred): = $AUCINF/Dose$

AUC_%Extrap(_obs, _pred): Percentage of AUCINF(_obs, _pred) due to extrapolation from Tlast to infinity:

$$= 100[(AUCINF - AUClast)/AUCINF]$$

AUC_%Back_Ext(_obs, _pred): Computed for IV Bolus models. Percentage of AUCINF that was due to back extrapolation to estimate C0 when the first measured concentration is not at dosing time.

Vz(_obs, _pred), **Vz_F(_obs, _pred)**[a]: Volume of distribution based on the terminal phase.

For non-steady-state data: = $Dose/[Lambda\_z(AUCINF)]$

Cl(_obs, _pred), **Cl_F(_obs, _pred)**[a]: Total body clearance for extravascular administration.

= $Dose/AUCINF$

AUMCINF(_obs, _pred): Area under the first moment curve (AUMC) extrapolated to infinity, based on the last observed concentration (obs) or the last predicted concentration (pred).

$$= AUMClast + \frac{tlast \cdot Clast}{Lambda\_z} + \frac{Clast}{Lambda\_z^2}$$

AUMC_%Extrap(_obs, _pred): Percent of AUMCINF(_obs, _pred) that is extrapolated.

= $100[(AUMCINF - AUMClast)/AUMCINF]$

MRTINF(_obs, _pred): Mean residence time (MRT) extrapolated to infinity. For non-steady-state data:

For non-infusion models: = $AUMCINF/AUCINF$
For infusion models: = $(AUMCINF/AUCINF) - (Tinf/2)$
where $Tinf$ is the length of infusion.
(Note that, for extravascular dosing (oral model 200), MRTINF includes Mean Input Time as well as time in systemic circulation.)

Vss(_obs, _pred): For non-steady-state data: An estimate of the volume of distribution at steady-state based on the last observed (obs) or last predicted (pred) concentration.

= $(MRTINF)(CL)$

Computed for IV Bolus and Infusion dosing only. Not computed for extravascular dosing (oral model 200), as MRTINF for oral models includes Mean Input Time as well as time in systemic circulation and therefore is not appropriate to use in calculating Vss.

[a]For extravascular models (model 200), the fraction of dose absorbed cannot be estimated; therefore Volume and Clearance for these models are actually Volume/F or Clearance/F where F is the fraction of dose absorbed.

Plasma parameters that are estimated when at steady-state

Tau: Available in the Dosing Used results worksheet for steady-state data. The (assumed equal) dosing interval for steady-state data.

Tmax: Time of maximum observed concentration. For steady-state data, based on observations collected during the dosing interval, that is, at or after the dosing time, but no later than the dosing time plus Tau, where Tau is the dosing interval. If the maximum observed concentration is not unique, then the first maximum is used.

Cmax: Maximum observed concentration, occurring at time Tmax, as defined above.

Tmin: Time of minimum observed concentration. For steady-state data, based on observations collected during the dosing interval (i.e., after the dosing time, but no later than dosing time plus Tau, where Tau is the dosing interval). If the minimum observed concentration is not unique, then the first minimum is used.

Cmin: Minimum observed concentration occurring at time Tmin as defined above.

> ***Note:*** Regulatory agencies differ on the definition of Cmin: some agencies define Cmin the same as Ctau is defined below. Both Cmin and Ctau are included in the output so that users can use the correct parameter for their situation.

Ctau: Concentration at dosing time plus Tau. Observed concentration if the value exists in the input data; otherwise, the predicted concentration value. Predicted concentrations are calculated following the same rules as for computing inserting missing endpoints needed for partial areas, see "Partial area calculation".

Cavg: Average concentration, computed = AUC_TAU/Tau

Swing: = $(Cmax - Cmin)/Cmin$

Swing_Tau: = $(Cmax - Ctau)/Ctau$

Fluctuation%: = $100[(Cmax - Cmin)/Cavg]$ where $Cmin$ and $Cmax$ were obtained between dosing time and dosing time plus Tau.

Fluctuation%_Tau: = $100[(Cmax - Ctau)/Cavg]$

CLss, **CLss_F**^{a}: An estimate of the total body clearance, computed for IV Bolus and Infusion dosing only.

$$= \frac{Dose}{AUC|_0^\tau}$$

MRTINF(_obs, _pred): Mean residence time (MRT) extrapolated to infinity based on AUCINF(_obs, _pred).

For non-infusion: $$\frac{AUMC|_0^\tau + \tau(AUCINF - AUC|_0^\tau)}{AUC|_0^\tau}$$

For infusion: $$\frac{AUMC|_0^\tau + \tau(AUCINF - AUC|_0^\tau)}{AUC|_0^\tau} - \frac{TI}{2}$$

where TI represents infusion duration. (Note that, for oral model 200, MRTINF includes Mean Input Time as well as time in systemic circulation.)

Vz, Vz_F^{a}: = $$\frac{Dose}{\lambda_z \cdot AUC|_0^\tau}$$

Vss(_obs, _pred): An estimate of the volume of distribution at steady-state based on the last observed (obs) or last predicted (pred) concentration. Computed for IV Bolus and infusion dosing only.

= $MRTINF(CLss)$

Not computed for extravascular dosing (oral model 200), as MRTINF for oral models includes Mean Input Time as well as time in systemic circulation and therefore is not appropriate to use in calculating **Vss**.

Accumulation Index: = $\dfrac{1}{[1 - e^{-Lambda\_z\tau}]}$

AUC_TAU: The partial area from dosing time to dosing time plus Tau. See "Partial area calculation" for information on how it is computed.

AUC_TAU_D: = $AUC\_TAU/Dose$

AUC_TAU_%Extrap: Percentage of AUC_TAU that is due to extrapolation from Tlast to dosing time plus Tau.

$$= 100 \cdot \frac{AUC\_TAU - AUClast}{AUC\_TAU}, \text{ if } Dosing\_Time + \tau > Tlast$$

$$= 0, \text{ if } Dosing\_Time + \tau \le Tlast$$

AUMC_TAU: Area under the first moment curve from dosing time to dosing time plus Tau. See "Partial area calculation" for information on how it is computed.

AUClower_upper: (Optional) User-requested area(s) under the curve from time "lower" to "upper".

[a] For extravascular models (model 200), the fraction of dose absorbed cannot be estimated; therefore Volume and Clearance for these models are actually Volume/F or Clearance/F where F is the fraction of dose absorbed.

Urine data

Data structure: NCA for urine data requires the following input data:

Starting and ending time of each urine collection interval
Urine concentrations
Urine volumes

From this data, models 210–212 compute the following for the analysis:

Midpoint of each collection interval=(Starting time+Ending time)/2
Excretion rate for each interval (amount eliminated per unit of time)=(Concentration*Volume)/(Ending time-Starting time)

Output: Models 210–212 estimate the following parameters.

The worksheet will include the Sort(s), Carry(ies), parameter names, units, and computed values. A User Defined Parameters Pivoted worksheet will include the pivoted form of the User Defined Parameters worksheet.

Urine parameters that do not depend on Lambda Z

Dosing time: Available as 'Time' in the Dosing Used results. Time of last administered dose. It is assumed to be zero unless otherwise specified. This parameter is used mainly with steady-state data, where time may be coded as the time elapsed since the first dose, or the elapsed time since the time of the first dose.

Dose: Amount of last administered dose. This is assumed to be zero if not specified.

N_Samples: Number of non-missing observations in the analysis. Does not include missing or non-numeric observations, observations before dosing time, or urine observations where volume is zero. Does not include the point at dosing time if it was not observed but was inserted by the engine.

No_points_lambda_z: Number of points used in the computation of Lambda Z. If Lambda Z cannot be estimated, this is set to zero.

Tlag: Midpoint of the collection interval prior to the first collection interval with measurable (non-zero) rate. Computed for all urine models.

Tmax_Rate: Midpoint of the collection interval associated with the maximum observed excretion rate. If the maximum observed excretion rate is not unique, then the first maximum is used.

Max_Rate: Maximum observed excretion rate, at time Tmax_Rate as defined above.

Mid_Pt_last: Midpoint of collection interval associated with last measurable (positive) observed excretion rate.

Rate_last: Last observed measurable (positive) rate at time Mid_Pt_last.

AURC_last: Area under the urinary excretion rate curve from time of dosing to Mid_Pt_last.

AURC_last_D: = $AURC\_last/Dose$

Vol_UR: Sum of Urine Volumes (urine)

Amount_Recovered: Cumulative amount eliminated.

$$= \sum Concentration\_Volume$$

Percent_Recovered: = $100(Amount\_Recovered/Dose)$

AURC_all: Area under the urinary excretion rate curve from the time of dosing to the midpoint of the interval with the last rate. If the last rate is positive, AURC_last=AURC_all.

Urine parameters that are estimated when Lambda Z is estimated

The following list includes some parameters that are extrapolated to infinity. These parameters are calculated two ways: based on the last observed excretion rate: Rate_last (indicated by “_obs” appended to the parameter name), or based on the last predicted excretion rate: Rate_last_pred (indicated by “_pred” appended to the parameter name), where the predicted value is based on the linear regression performed to estimate Lambda Z.

Rsq: Goodness of fit statistic for the terminal elimination phase.

Rsq_adjusted: Goodness of fit statistic for the terminal elimination phase, adjusted for the number of points used in the estimation of Lambda Z.

Corr_XY: Correlation between midpoints and log excretion rates for the points used in the estimation of Lambda Z.

Lambda_z: First-order rate constant associated with the terminal (log-linear) portion of the curve. This is estimated via linear regression of midpoints vs. log excretion rates.

Lambda_z_intercept: Intercept on log scale estimated via linear regression of midpoints vs. log excretion rates.

Lambda_z_lower: Lower limit on midpoint for values to be included in Lambda Z estimation.

Lambda_z_upper: Upper limit on midpoint for values to be included in Lambda Z estimation.

HL_Lambda_z: Terminal half-life=ln(2)/$Lambda\ Z$

Span: = $(Lambda\_z\_upper - Lambda\_z\_lower)/HL\_Lambda\_z$

Rate_last_pred: Predicted rate at Mid_Pt_last.

AURC_INF(_obs, _pred): Area under the urinary excretion rate curve extrapolated to infinity, based on the last observed excretion rate (_obs) or the last predicted rate (_pred), i.e., the excretion rate at the final midpoint estimated using the linear regression for Lambda Z. Note that AURC_INF is theoretically equal to Amount_Recovered, but will differ due to experimental error.

AURC_%Extrap(_obs, _pred): Percent of AURC_INF(_obs, _pred) that is extrapolated.

Sparse sampling (pre-clinical) data

When an NCA model is loaded with the Sparse Sampling option (see "Options tab"), the data are treated as a special case of plasma or urine concentration data. The NCA engine computes the mean concentration or rate at each unique time value or interval. Using the mean concentration curve across subjects, it estimates the same parameters normally calculated for plasma or urine data, plus those listed below. See "Sparse sampling calculation" for additional details of NCA computations with sparse data.

Note: The names of some of the output worksheets change when the data is sparse: Final Parameters becomes Mean Curve Final Parameters, and Summary Table becomes Mean Curve Summary Table.

Plasma or serum concentration parameters

Sparse sampling methods for plasma data (models 200–202) compute the following parameters in addition to those listed in "Plasma or serum data".

SE_Cmax: Standard error of data at Tmax (time of maximum mean concentration).

SE_AUClast: It is the standard error of the area under the mean concentration curve from dose time to Tlast, where Tlast is the time of last measurable (positive) mean concentration.

SE_AUCall: Standard error of the area under the mean concentration curve from dose time to the final observation time.

Note: SE_AUClast and SE_AUCall provide a measurement of the uncertainty for AUClast and AUCall, respectively, and are usually the same. Differences between these parameter values will only be observed if some of the measurements were flagged as BQL (below the quantitation limit).

Note: With Sparse Sampling, since SE for AUC computations depend on AUC being a linear combination of the mean concentrations, SE_AUClast and SE_AUCall are not included in the output when the output when log trapezoidal rules are specified as the method for computing AUC.

Urine excretion rate parameters

Sparse sampling methods for urine data (models 210–212) compute the following parameters in addition to those listed under "Urine data".

SE_Max_Rate: Standard error of the data at the time of maximum mean rate.

SE_AURC_last: Standard error of the area under the mean urinary excretion rate curve from dose time through the last interval that has a measurable (positive) mean rate.

SE_AURC_all: Standard error of the area under the mean urinary excretion rate curve from dose time through the final interval.

Individuals by time

This sheet includes the individual subject data, along with N (number of non-missing observations), the mean and standard error for each unique time value (plasma data) or unique midpoint value (urine data).

References

Holder (2001). Comments on Nedelman and Jia's extension of Satterthwaite's approximation applied to pharmacokinetics. *J Biopharm Stat* 11(1-2):75–9.

Nedelman, Gibiansky and Lau (1995). Applying Bailer's method for AUC intervals to sparse sampling. *Pharm Res* 12:124–8.

Nedelman and Jia (1998). An extension of Satterthwaite's approximation applied to pharmacokinetics. *J Biopharm Stat* 8(2):317–28.

Yeh (1990). Estimation and Significant Tests of Area Under the Curve Derived from Incomplete Blood Sampling. *ASA Proceedings of the Biopharmaceutical Section* 74–81.

Drug effect data model 220

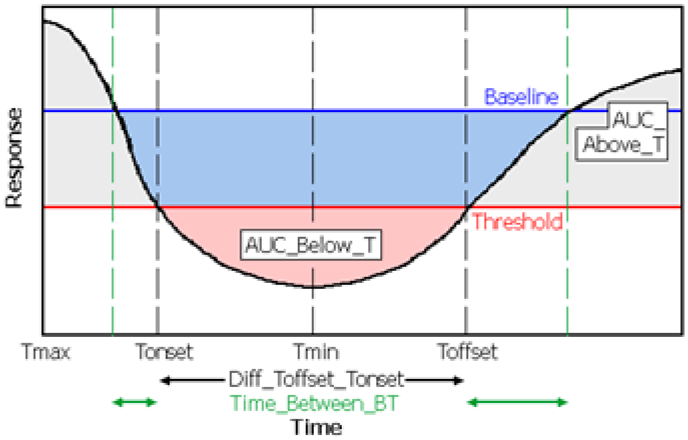


Figure 20-1. Illustration of a time-effect curve with AUCs highlighted

Output parameter names use the following conventions:

B is the baseline effect value (discussed above).
T is the user-supplied threshold effect value.
“Above” means towards increasing Y values, even for inhibitory effects.

Estimated parameters for model 220

N_Samples: This parameter reports the number of non-missing observations used in the analysis of the profile (time is at or after dosing time, the observation is numeric, and the volume is positive for urine models). It does not count points inserted by engine, e.g., inserted at dosing time.

Slope1 (or **2**): Slope of the first (or second) segment of the curve. See “Lambda Z or Slope Estimation settings”.

Rsq_Slope1 (or **2**): Goodness of fit statistic for slope 1 or 2.

Rsq_adj_Slope1 (or **2**): Goodness of fit statistic for slope 1 or 2, adjusted for the number of points used in the estimation.

Corr_XY_Slope1 (or **2**): Correlation between time (X) and effect (or log effect, for log regression) (Y) for the points used in the slope estimation.

No_points_Slope1 (or **2**): The number of data points included in calculation of slope 1 or 2.

Slope1_lower or **Slope2_lower**: Lower limit on Time for values to be included in the slope calculation.

Slope1_upper or **Slope2_upper**: Upper limit on Time for values to be included in the slope calculation.

Tmin: Time of minimum observed response value (Rmin).

Rmin: Minimum observed response value.

Tmax: Time of maximum observed response value (Rmax).

Rmax: Maximum observed response value.

Baseline: Baseline response (Y) value supplied by the user, (assumed to be zero if none is supplied) or, for Certara Integral studies with no user-supplied baseline value, effect value at dose time.

AUC_Above_B: Area under the response curve that is above the baseline (dark gray areas in the above diagram).

AUC_Below_B: Area that is below the baseline and above the response curve (combined blue and pink areas in the above diagram).

AUC_Net_B: = $AUC\_Above\_B - AUC\_Below\_B$. This is likely to be a negative value for inhibitory effects.

Time_Above_B: Total time that Response is greater than or equal to Baseline.

Time_Below_B: Total time that Response is less than Baseline.

Time_%Below_B: = 100*$Time\_Below\_B/(Tfinal - Tdose)$, where $Tfinal$ is the final observation time and $Tdose$ is dosing time.

When a threshold value is provided, model 220 also computes the following.

Threshold: Threshold value used.

AUC_Above_T: Area under the response curve that is above the threshold value (combined light and dark gray areas in the above diagram).

AUC_Below_T: Area that is below the threshold and above the response curve (pink area in the above diagram).

AUC_Net_T: = $AUC\_Above\_T - AUC\_Below\_T$

Time_Above_T: Total time that Response >= Threshold.

Time_Below_T: Total time that Response < Threshold.

Time_%Below_T: = 100*$Time\_Below\_T/(Tlast - Tdose)$
where Tlast is the final observation time and Tdose is dosing time.

Tonset: Time that the response first crosses the threshold coming from the direction of the baseline value, as shown in the above diagram. $Tonset$=$Tdose$ if the first response value is across the threshold, relative to baseline. The time will be interpolated using the calculation method selected in the model options. (See "Options tab".)[a]

Toffset: Time greater than Tonset at which the curve first crosses back to the baseline side of threshold, as shown in the diagram above.[a]

Diff_Toffset_Tonset: = $Toffset - Tonset$

Time_Between_BT: Total time spent between baseline and threshold (sum of length of green arrows in diagram).

AUClower_upper: (Optional) user-requested area(s) under the curve from time “lower” to time “upper”.

[a]Use caution in interpreting $Tonset$ and $Toffset$ for noisy data if Baseline and Threshold are close together.

User defined parameters

Any user defined parameters will be computed and reported in the User Defined Parameters and User Defined Parameters Pivoted worksheets. The worksheets include entries for Sort, Carry, parameter name, unit, and estimated value. A User Defined Parameters Pivoted worksheet will include the pivoted form of the User Defined Parameters worksheet.

C<time value>: For Plasma models with user-defined times only, one column per requested time value. (For plasma models, a computed concentration at time zero will be reported as the parameter C0_0, since C0 is already used for the initial concentration measurement.)

Y<x-value>: For Drug Effect models with user-defined x-values only, one column per requested x-value.

<user-defined name>: Parameter defined using the User Defined Parameters tab.

References

Holder (2001). “Comments on Nedelman and Jia's extension of Satterthwaite's approximation applied to pharmacokinetics.” *J Biopharm Stat* 11(1–2):75–9.

Nedelman and Jia (1998). “An extension of Satterthwaite's approximation applied to pharmacokinetics.” *J Biopharm Stat* 8(2):317–28.

NCA examples

This section presents several examples of the NCA operational object usage within Phoenix. Knowledge of how to do basic tasks using the Phoenix interface, such as creating a project and importing data, is assumed.

The examples include:

Analysis of three profiles using NCA
Exclusion and partial area NCA example
Sparse sampling NCA example
Urine study NCA example
Drug effect NCA example
Multiple profile analysis using NCA

Analysis of three profiles using NCA

Suppose a researcher has obtained time and concentration data following oral administration of a test compound to three subjects, and wants to perform noncompartmental analysis and summarize the results.

The completed project (`NCA.phxproj`) is available for reference in …`\Examples\WinNonlin`.

Set up the project and data for the three profiles

1. Create a new project named NCA.
2. Import the file …`\Examples\WinNonlin\Supporting files\Bguide1 single dose and steady state.dat`.

Note: Units must be added to the time and concentration columns before the dataset can be used in a noncompartmental analysis.

3. With **Bguide1 single dose and steady state** selected in the Data folder, go to the Columns tab (lower part of the Phoenix window) and select the **Time** column header in the Columns box.
4. Clear the **Unit** field and type `hr`.
5. Select the **Conc** column header in the Columns box.
6. Clear the **Unit** field and type `ng/mL`.
   Or
   Click the **Units Builder** button and use the *Units Builder* dialog tools

Note: Units added to ASCII datasets can be preserved if the datasets are saved in `.dat` or `.csv` file format. Phoenix adds the units to a row below the column headers. To import a `.dat` or `.csv` file with units, select the **Has units row** option in the *File Import Wizard* dialog.

Set up the NCA object for analysis of the three profiles

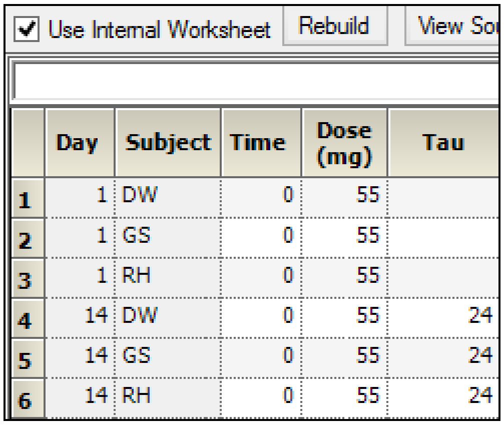
Noncompartmental analysis for extravascular dosing is available as Model 200 in the Phoenix model library. Phoenix displays the model type (Plasma, Urine, or Drug Effect) in the Options tab of an NCA object.

Note: The exact model used is determined by the dose type. Extravascular Input uses Model 200, IV-Bolus Input uses Model 201, and Constant Infusion uses Model 202.

1. Select **Workflow** in the Object Browser and then select **Insert > NonCompartmental Analysis > NCA**.
2. Drag the **Bguide1 single dose and steady state** worksheet from the Data folder to the NCA object’s Main Mappings panel.
3. Map the data types as follows:
   Day to the **Sort** context (make sure to map **Day** first)
   Subject to the **Sort** context
   Time mapped to the **Time** context
   Conc to the **Concentration** context

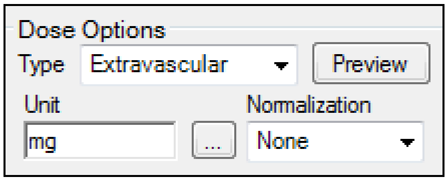
Prepare the dosing information from the profiles

1. Select **Dosing** in the NCA object's Setup list.
2. In the Dosing panel, select the **Use Internal Worksheet** checkbox.
3. Click **OK** in the *Select sorts* dialog to accept the default sort variables.
4. For each row:
   In the Time column, enter `0`.
   In the Dose column, enter 55.
   In the Tau column, enter 24 for the three Day 14 rows (rows 4, 5, and 6).



	Day	Subject	Time	Dose (mg)	Tau
1	1	DW	0	55	
2	1	GS	0	55	
3	1	RH	0	55	
4	14	DW	0	55	24
5	14	GS	0	55	24
6	14	RH	0	55	24

5. In the Dose Options area of the Options tab, type `mg` in the **Unit** field and press the **Enter** key. The units are immediately added to the column header.
6. **Extravascular** is selected by default in the **Type** menu. Do not change this setting.



Dose Options
Type: Extravascular
Unit: mg
Normalization: None

Set up the terminal elimination phase for the analysis

Phoenix attempts to estimate the rate constant Lambda Z, associated with the terminal elimination phase. Although Phoenix is capable of selecting the times to be used in the estimation of Lambda Z, this example provides Phoenix with the time range.

Specify the times to be included.

1. Select **Slopes** in the Setup list.
2. For each row:
   In the Start Time column, type 8.
   In the End Time column, type 24.
   Do not type any values into the **Exclusions** column.
3. Select **Slopes Selector** in the Setup list.

   The **Time Range** is selected in the **Lambda Z Calculation Method** menu. The **Start** and **End** times have been specified for each subject. A line is displayed on each graph that shows the Lambda Z time range.

   In this example, no points are excluded from the specified Lambda Z time range. The example "Exclusion and partial area NCA example" demonstrates Lambda Z exclusions.

Specify therapeutic response options

The next step is to define a target concentration range to enable calculation of the time and area located above, below, and within that range.

Note: See "Exclusion and partial area NCA example" for an NCA example that includes computation of partial areas under the curve.

1. Select **Therapeutic Response** in the Setup list.
2. Select the **Use Internal Worksheet** checkbox.
3. Click **OK** in the sorts dialog to accept the default sort variables.
4. For each row:
   Type 2 in the **Lower** column.
   Type 4 in the **Upper** column.

Set preferred units for the analysis

The next step in setting options is to specify preferred output units. The independent variable, dependent variable, and dosing regimen must have units before preferred output units can be set.

1. Select **Units** in the Setup list.

   The Units worksheet lists both the Default units and the Preferred units for each parameter. The new preferred volume unit needs to be set to L (liter).
2. Select the cell in the **Preferred** column for Volume (Vz, Vz/F, Vss).
3. Type L and press **ENTER**.

Specify NCA model options for the analysis

Four methods are available for computing the area under the curve. The default method is the linear trapezoidal rule with linear interpolation. This example uses the **Linear Log Trapezoidal** method: linear trapezoidal rule up to Tmax, and log trapezoidal rule for the remainder of the curve.

Use the Options tab to specify settings for the NCA model options. The Options tab is located underneath the Setup tab.

1. Select **Linear Log Trapezoidal** in the **Calculation Method** menu.
2. In the **Titles** field type `Example of Noncompartmental Analysis`.

Set an acceptance criteria rule

Acceptance criteria for Lambda Z, which are applicable to both the **Best Fit** method and the **Time Range** method, can be specified on the Rules tab. These rules are used to flag profiles where the Lambda_z final parameter does not meet the specified acceptance criteria.

1. Select the **Rules** tab.
2. In the **Rsq_adjusted** field, type `0.97` to flag any profile with an Rsq_adjusted value greater than or equal to this value.

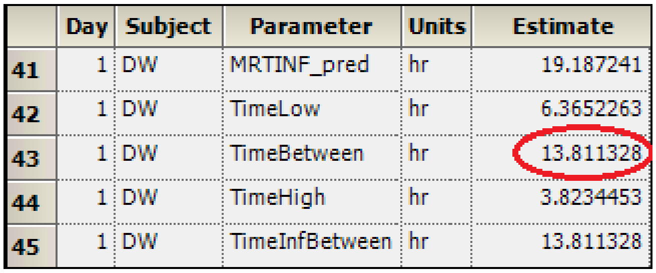
Profiles that break the rule are flagged in the output and can be quickly filtered out of the results. The process will be illustrated later in this example.

Execute and view the results of the analysis

At this point, all of the necessary commands have been specified.

1. Click ▶ (**Execute** icon) to execute the object.
2. In the Results tab, select **Final Parameters** in the list.

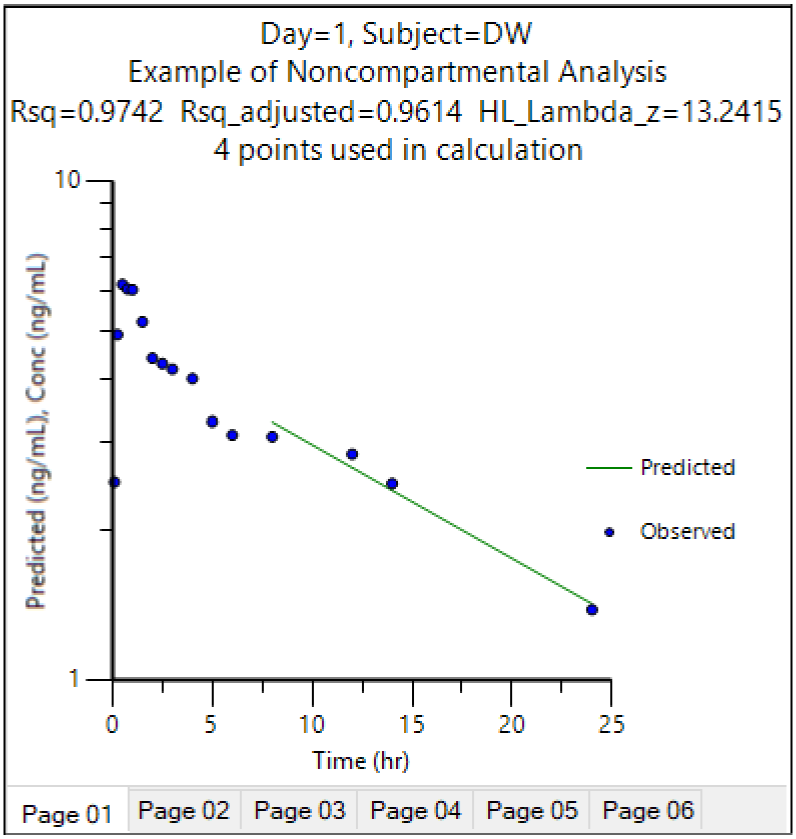
   Subject DW's concentrations were within the theoretical therapeutic range for just over 13.8 hours, as reflected in the parameter TimeBetween.



	Day	Subject	Parameter	Units	Estimate
41	1	DW	MRTINF_pred	hr	19.187241
42	1	DW	TimeLow	hr	6.3652263
43	1	DW	TimeBetween	hr	13.811328
44	1	DW	TimeHigh	hr	3.8234453
45	1	DW	TimeInfBetween	hr	13.811328

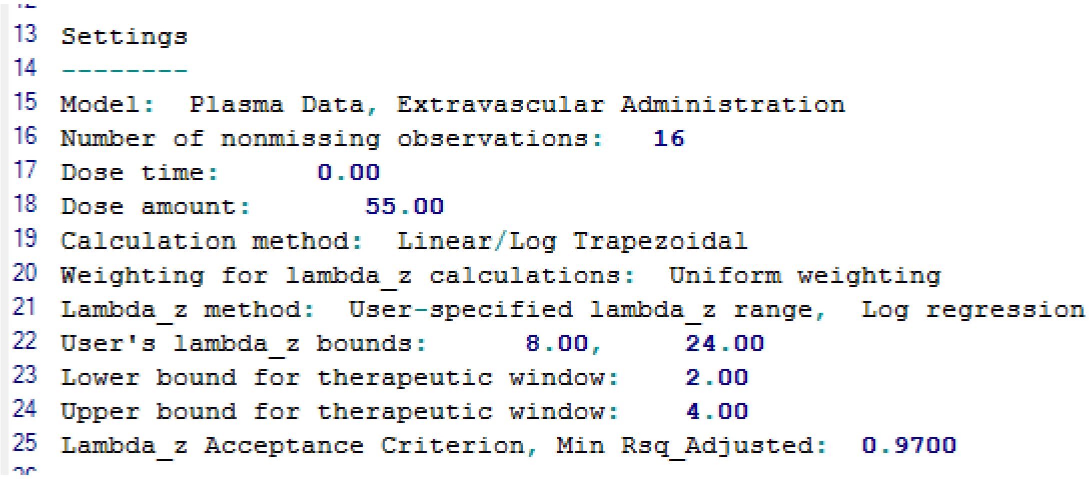
3. In the Results tab, select **Observed Y and Predicted Y vs X** in the list.

   The NCA object's plot output includes Observed Y and Predicted Y vs X graphs for each subject (switch between the plots using the tabs below the graph).



4. In the Results tab, select **Core output** in the list.

   The NCA object's Core Output text file contains user settings, a brief summary table, and final parameters output for each subject.

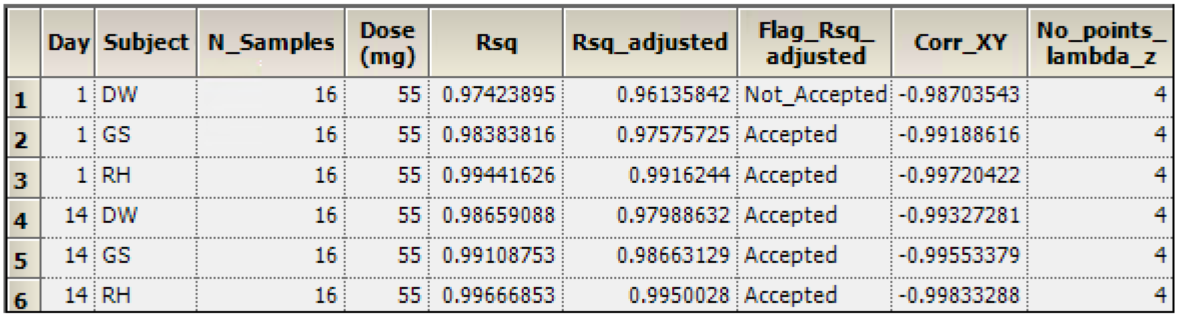


```
Settings
--------
Model:  Plasma Data, Extravascular Administration
Number of nonmissing observations:   16
Dose time:       0.00
Dose amount:       55.00
Calculation method:  Linear/Log Trapezoidal
Weighting for lambda_z calculations:  Uniform weighting
Lambda_z method:  User-specified lambda_z range,  Log regression
User's lambda_z bounds:      8.00,     24.00
Lower bound for therapeutic window:    2.00
Upper bound for therapeutic window:    4.00
Lambda_z Acceptance Criterion, Min Rsq_Adjusted:  0.9700
```

Figure 21-1. Settings portion of the Core Output

Filter out flagged profiles

1. In the Results tab, select **Final Parameters Pivoted** in the list.



	Day	Subject	N_Samples	Dose (mg)	Rsq	Rsq_adjusted	Flag_Rsq_ adjusted	Corr_XY	No_points_ lambda_z
1	1	DW	16	55	0.97423895	0.96135842	Not_Accepted	-0.98703543	4
2	1	GS	16	55	0.98383816	0.97575725	Accepted	-0.99188616	4
3	1	RH	16	55	0.99441626	0.9916244	Accepted	-0.99720422	4
4	14	DW	16	55	0.98659088	0.97988632	Accepted	-0.99327281	4
5	14	GS	16	55	0.99108753	0.98663129	Accepted	-0.99553379	4
6	14	RH	16	55	0.99666853	0.9950028	Accepted	-0.99833288	4

Figure 21-2. Part of the Final Parameters Pivoted worksheet

For this example, a rule was set for the value of Rsq_adjusted (see “Set an acceptance criteria rule”). The output indicates that the profile for DW broke this rule with a value of ‘Accepted’ in the Flag_Rsq_adjusted column.

To remove any profiles failing to meet acceptance criteria from the output, the Final Parameters Pivoted worksheet can be processed by the Data Wizard to delete these profiles. The worksheet is filtered on the flag values of 'Not Accepted' and identified rows are then excluded.

Note: The `NCA.phxproj` file does not include processing by the Data Wizard, but the information is included here as a reference.
a) Insert a Data Wizard object.
b) In the Options tab, set Action to **Filter** and click **Add**.
c) Click the **Select Source** icon in the Mappings panel and select the NCA Final Parameter Pivoted worksheet.
d) In the Options tab, click the **Add** button to the right of the **Specify Filter** field.
e) In the *Filter Specification* dialog, define the filter to **Exclude** rows that have a value of **Not Accepted** in the **Flag_Rsq_adjusted** column (or other “Flag_” column on which you want to filter profiles).
Once the filter is defined, execute the Data Wizard object.

Summarize the analysis output with statistics

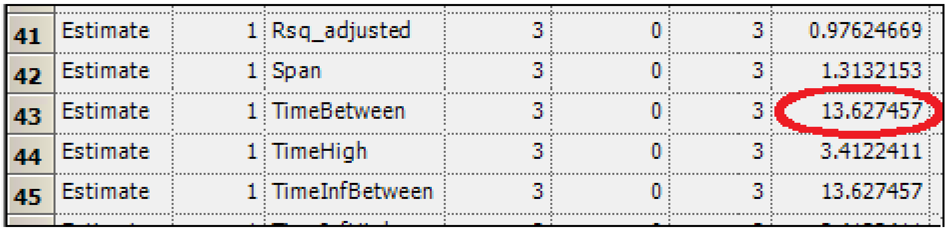
At this point, it is convenient to summarize the results of the noncompartmental analysis using a Descriptive Statistics object. This example summarizes parameter estimates across subjects.

1. Select **Workflow** in the Object Browser and then select **Insert > Computation Tools > Descriptive Statistics**.
2. In the Descriptive Statistic’s Main Mappings panel click the **Select Source** icon.
3. Under the **NCA** node, select the **Final Parameters** worksheet and click **OK**.
4. In the Main Mappings panel, map the data types to the following contexts:
   Map **Day** to the **Sort** context (make sure to map **Day** first).
   Leave **Subject** mapped to **None**.
   Map **Parameter** to the **Sort** context.
   Leave **Units** mapped to **None**.
   Map **Estimate** to the **Summary** context.

Note: Mapping Day and Parameter to Sort computes statistics on the parameter estimates for each day and mapping Estimate to Summary computes one statistic per parameter per day.

5. In the Options tab, check the **Confidence Intervals** and **Number of SD Statistics** checkboxes, but do not change the default values for these two items.
6. Execute the object.

The three subjects spent an average of 13.6 hours within the therapeutic concentration range on Day 1, as shown by the parameter TimeBetween.

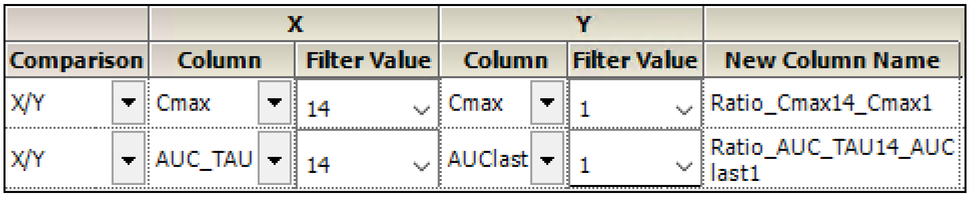


41	Estimate	1	Rsq_adjusted	3	0	3	0.97624669
42	Estimate	1	Span	3	0	3	1.3132153
43	Estimate	1	TimeBetween	3	0	3	13.627457
44	Estimate	1	TimeHigh	3	0	3	3.4122411
45	Estimate	1	TimeInfBetween	3	0	3	13.627457

Use ratios to compare data

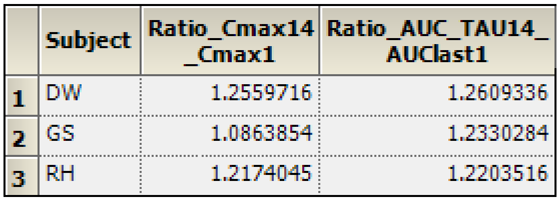
The Ratios and Differences object can be used to quickly setup ratios and/or differences between parameter values in order to compare data. A description of the object can be found in "Ratios and Differences".

1. In the Object Browser, click the NCA object.
2. In the Results list, right-click **Final Parameters Pivoted** and select **Send To > Computation Tools > Ratios and Differences**.
3. In the Main Mappings panel of the Ratios and Differences object, map the data types to the following contexts:
   Map **Day** to the **Filter** context.
   Map **Subject** to the **Sort** context.
   Map the rest of the data types to the **Carry** context by first mapping **N_Samples** to **Carry** and then drag the corner of the selected cell all the way to the bottom of the grid. It may take a few seconds.
4. In the Options tab, set up two ratios as shown in the following image. Use the **Add** button to add the second row of options to the table.



	X		Y		
Comparison	Column	Filter Value	Column	Filter Value	New Column Name
X/Y	Cmax	14	Cmax	1	Ratio_Cmax14_Cmax1
X/Y	AUC_TAU	14	AUClast	1	Ratio_AUC_TAU14_AUClast1

5. Execute the object.
6. In the Results tab, select **Ratios Differences** and compare the ratios for each subject.



	Subject	Ratio_Cmax14_Cmax1	Ratio_AUC_TAU14_AUClast1
1	DW	1.2559716	1.2609336
2	GS	1.0863854	1.2330284
3	RH	1.2174045	1.2203516

This concludes the multiple profile example.

Exclusion and partial area NCA example

This example demonstrates the exclusion of points in the terminal elimination phase and computation of partial area under the curve in the Phoenix NCA object. The time-concentration data is for a single subject and the data is provided in `NCA2.csv`, which is located in the Phoenix examples directory.

Noncompartmental analysis for extravascular dosing is available as model 200 in Phoenix's noncompartmental analysis object. Phoenix always displays the model type in the NCA object's Options tab.

Note: The exact model used is determined by the dose type. Extravascular Input uses Model 200, IV-Bolus Input uses Model 201, and Constant Infusion uses Model 202.

The completed project (`NCA_PartialAreas.phxproj`) is available for reference in …`\Examples\WinNonlin`.

Set up the NCA object

1. Create a project called `NCA_PartialAreas`.
2. Import the file …`\Examples\WinNonlin\Supporting files\NCA2.csv`.
   In the *File Import Wizard* dialog, make select the **Has units row** option.
   Click **Finish**.
3. Select **Workflow** in the Object Browser and then select **Insert > NonCompartmental Analysis > NCA**.
4. Drag the **NCA2** worksheet from the Data folder to the NCA object's Main Mappings panel to map it as the input source.
   Leave **Time** mapped to the **Time** context.
   Map **Conc** to the **Concentration** context.

Prepare the dosing information

In this example, one dose of 70 mg was administered at time zero.

1. Select **Dosing** in the Setup list.
2. In the Dosing panel, check the **Use Internal Worksheet** checkbox.
3. In the first cell in the **Time** column type `0`.
4. In the first cell in the **Dose** column type `70`.
5. Do not enter any values in the **Tau** column.
6. **Extravascular** is selected by default in the **Type** menu in the Options tab. Do not change this setting.
7. In the **Unit** field type `mg` and press the **Enter** key.

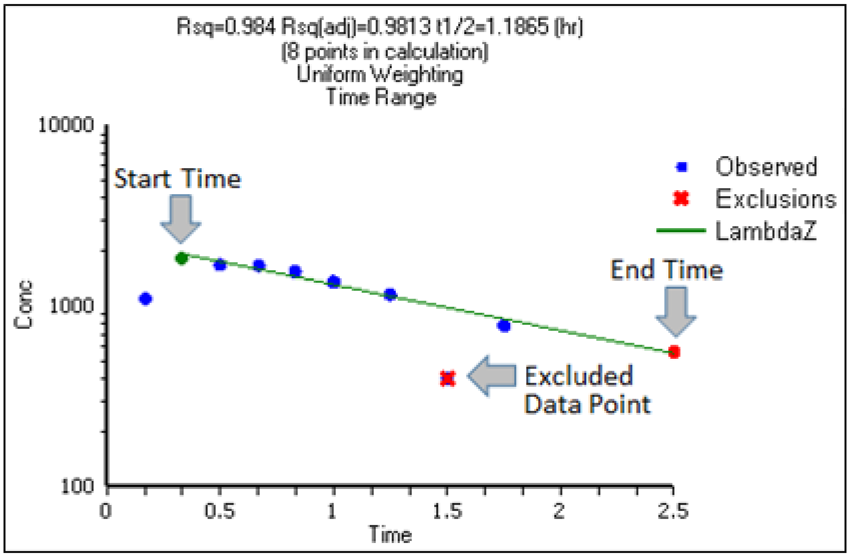
Set up the terminal elimination phase

Phoenix attempts to estimate the rate constant Lambda Z associated with the terminal elimination phase. Although Phoenix is capable of selecting the times to be used in the estimation of Lambda Z, this example provides Phoenix with the time range.

1. Select **Slopes** in the Setup list.
2. In the Slopes panel, type `0.33` in the **Start Time** column.

3. Type `2.5` in the **End Time** column.
4. Exclude the data point at 1.5 by typing `1.5` in the **Exclusions** column and press the **Enter** key.
5. Select **Slopes Selector** in the Setup list.

The Start and End times and the Exclusion are marked on the graph display.



Specify the AUCs to calculate

Partial areas under the curve are computed for zero to 3.0 hours and 1.25 to 2.5 hours.

1. Select **Partial Areas** in the Setup list.
2. Select the **Use Internal Worksheet** checkbox.
3. In the Options tab, change the **Max # of Partial Areas** pull-down menu to **2**.
4. In the first row, type `0` in the **Start Time** column and type 3 in the **End Time** column.
5. In the second row, type `1.25` in the **Start Time** column, type `2.5` in the **End Time** column and press the **Enter** key.

Specify the NCA model options

This example includes titles in the graph output and uses the Linear Log Trapezoidal method to calculate areas under the curve.

Use the Options tab to specify settings for the NCA model options.

1. The default setting for **Model Type** is **Plasma (200-202)**. Do not change this setting.

Note: The exact model type (200, 201, or 202) is determined by the dose type.

2. Select **Linear Log Trapezoidal** in the **Calculation Method** menu.
3. In the **Titles** field type `A Second NCA Example`.

Request a concentration at a specified time

Set up a request to calculate the concentration at time 0.25 and include the information in the results.

1. Select the User Defined Parameters tab.
2. Check the **Include with Final Parameters** check box.
3. In the **Compute Concentrations at Times** field, enter `0.25`.

Define a new parameter

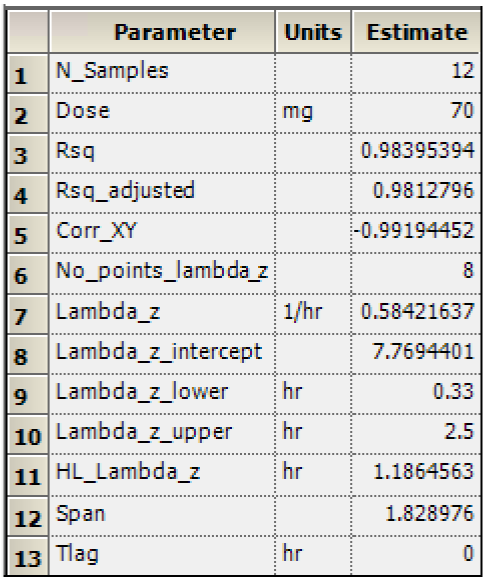
Add a new dose-normalized parameter.

1. In the User Defined Parameters tab, click the **Add** button.
2. Enter `AUCall_D` in the **Parameter** column.
3. Enter `AUCall/Dose` in the **Definition** column.
4. Enter `hr*ng/mL/mg` in the **Units Label** column.

Execute and view the NCA results

All necessary settings are complete.

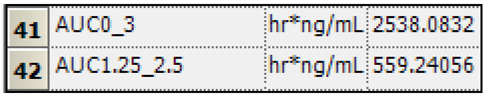
1. Click ▶ (**Execute** icon) to execute the object.
2. In the Results tab, click **Final Parameters**.



	Parameter	Units	Estimate
1	N_Samples		12
2	Dose	mg	70
3	Rsq		0.98395394
4	Rsq_adjusted		0.9812796
5	Corr_XY		-0.99194452
6	No_points_lambda_z		8
7	Lambda_z	1/hr	0.58421637
8	Lambda_z_intercept		7.7694401
9	Lambda_z_lower	hr	0.33
10	Lambda_z_upper	hr	2.5
11	HL_Lambda_z	hr	1.1864563
12	Span		1.828976
13	Tlag	hr	0

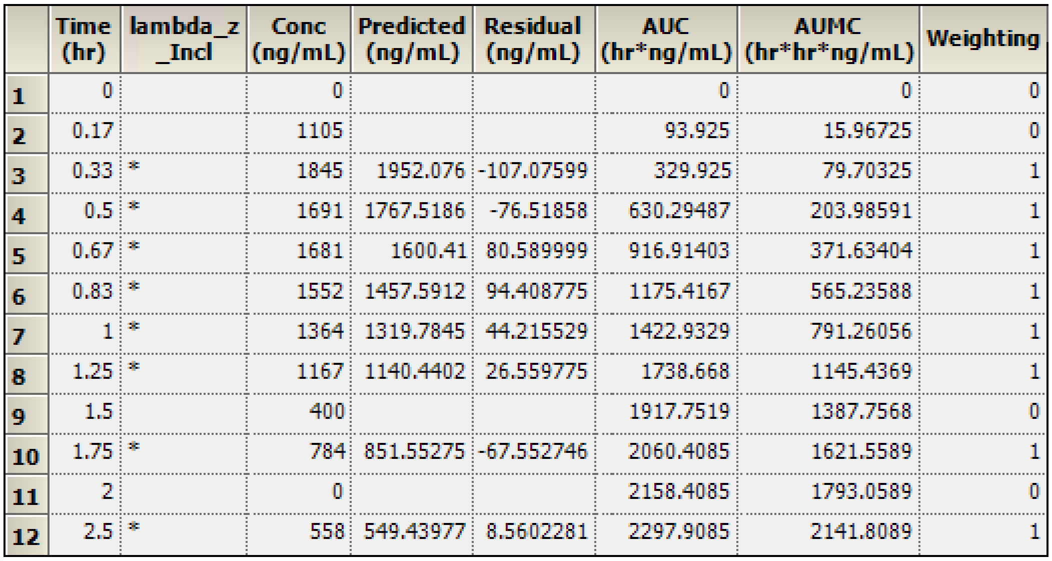
Figure 22-1. Part of the Final Parameters worksheet

Note the partial area estimates near the bottom of the Final Parameters.



41	AUC0_3	hr*ng/mL	2538.0832
42	AUC1.25_2.5	hr*ng/mL	559.24056

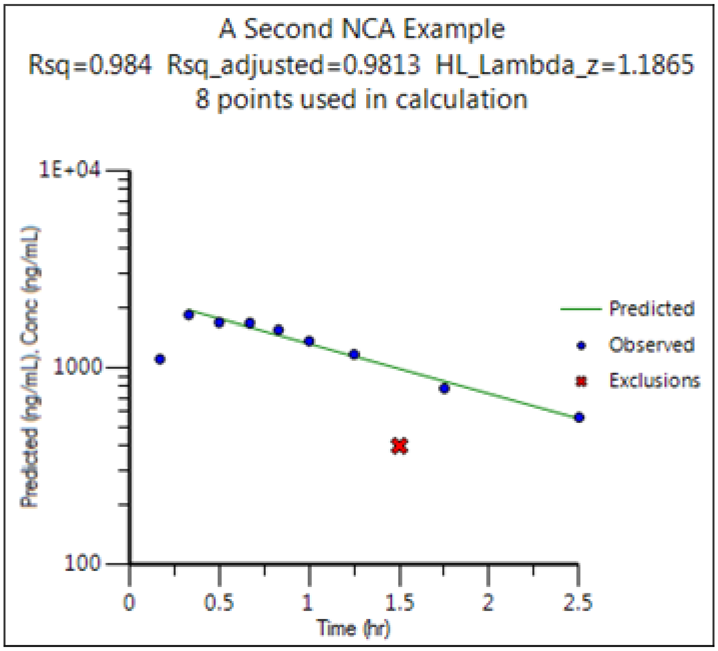
3. Click **Summary Table** in the Results list.



	Time (hr)	lambda_z _Incl	Conc (ng/mL)	Predicted (ng/mL)	Residual (ng/mL)	AUC (hr*ng/mL)	AUMC (hr*hr*ng/mL)	Weighting
1	0		0			0	0	0
2	0.17		1105			93.925	15.96725	0
3	0.33	*	1845	1952.076	-107.07599	329.925	79.70325	1
4	0.5	*	1691	1767.5186	-76.51858	630.29487	203.98591	1
5	0.67	*	1681	1600.41	80.589999	916.91403	371.63404	1
6	0.83	*	1552	1457.5912	94.408775	1175.4167	565.23588	1
7	1	*	1364	1319.7845	44.215529	1422.9329	791.26056	1
8	1.25	*	1167	1140.4402	26.559775	1738.668	1145.4369	1
9	1.5		400			1917.7519	1387.7568	0
10	1.75	*	784	851.55275	-67.552746	2060.4085	1621.5589	1
11	2		0			2158.4085	1793.0589	0
12	2.5	*	558	549.43977	8.5602281	2297.9085	2141.8089	1

Note that values used in the calculation of Lambda Z are marked with an asterisk in the column Lambda_z_Incl. The data point corresponding to time 1.5, which was excluded manually, is not marked with an asterisk. The observation at time 2.0, with a value of zero, was automatically excluded.

4. Click **Observed Y and Predicted Y vs X**.



The excluded data point is marked on the Observed Y and Predicted Y vs X plot output of observed and predicted data.

This concludes the partial areas example.

Sparse sampling NCA example

This example demonstrates how to model with sparse sampling. The time-concentration data is provided in `SparseSamplingChaioYeh.xls`. The dosing and therapeutic response data are stored in `SparseSamplingChaioYeh_sources.xls`. These files are located in the Phoenix examples directory.

The completed project (`NCA_SparseSampling.phxproj`) is available for reference in …`\Examples\WinNonlin`.

Set up the NCA object

1. Create a project called `NCA_SparseSampling`.
2. Import the two files …`\Examples\WinNonlin\Supporting files\SparseSamplingChaioYeh.xls` and `SparseSamplingChaioYeh_sources.xls`.
   In the *File Import Wizard* dialog, select the **Has units row** option for Sheet 1 and Dosing worksheets.
3. Select **Workflow** in the Object Browser and then select **Insert > NonCompartmental Analysis > NCA**.
4. Rename the NCA object just added as `SparseSamplingChaioYeh`.
5. Drag the **SparseSamplingChaioYeh** worksheet from the Data folder to the SparseSamplingChaioYeh object's Main Mappings panel. Map the context as follows:
   Subject set to **None** context
   Time to **Time** context
   Conc to **Concentration** context

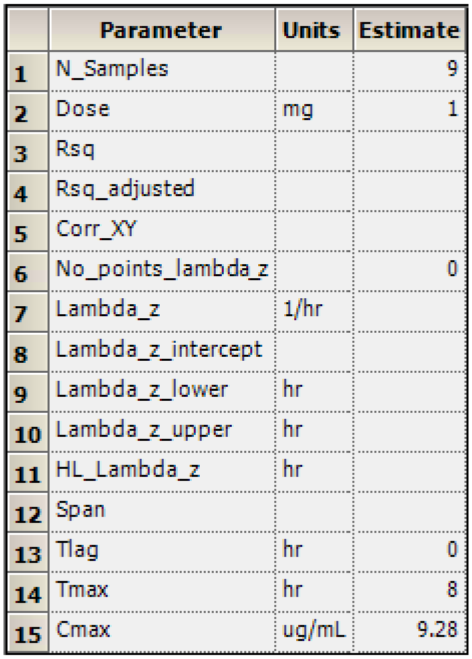
Set up for sparse sampling

1. Select **Dosing** in the Setup list.
2. Expand the **SparseSamplingChaioYeh_sources** item in the Object Browser Data folder and drag the **Dosing** item to the Dosing panel.
   Type is already mapped to the **None** context.
   Dose is already mapped to the **Dose** context.
   Time is already mapped to the **Time** context.
3. Select **Therapeutic Response** in the Setup list.
4. In the expanded **SparseSamplingChaioYeh_sources** item in the Object Browser Data folder, drag the **TherapeuticResponse** item to the Therapeutic Response panel.
   Lower is already mapped to the **Lower** context.
5. In the Options tab, check the **Sparse** check box.

Execute and view the NCA results

All necessary settings are complete.

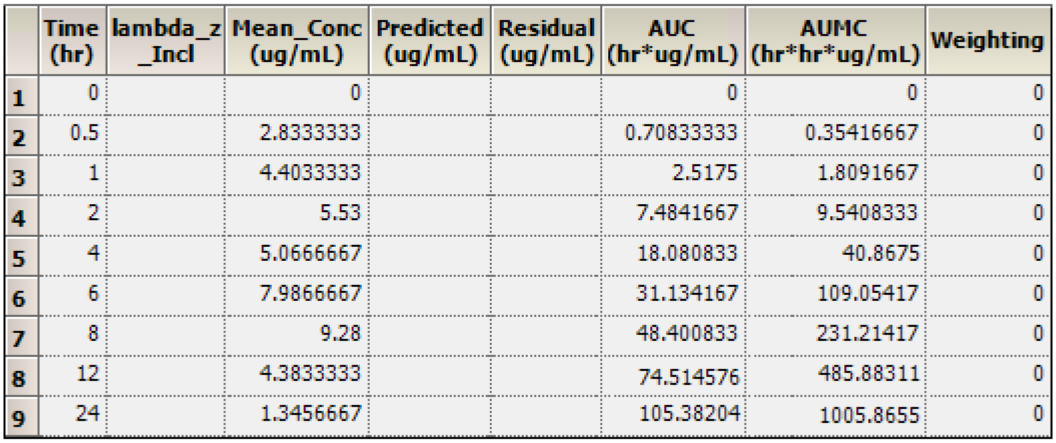
1. Click ▶ (**Execute** icon) to execute the object.
2. In the Results tab, click **Final Parameters**.



	Parameter	Units	Estimate
1	N_Samples		9
2	Dose	mg	1
3	Rsq		
4	Rsq_adjusted		
5	Corr_XY		
6	No_points_lambda_z		0
7	Lambda_z	1/hr	
8	Lambda_z_intercept		
9	Lambda_z_lower	hr	
10	Lambda_z_upper	hr	
11	HL_Lambda_z	hr	
12	Span		
13	Tlag	hr	0
14	Tmax	hr	8
15	Cmax	ug/mL	9.28

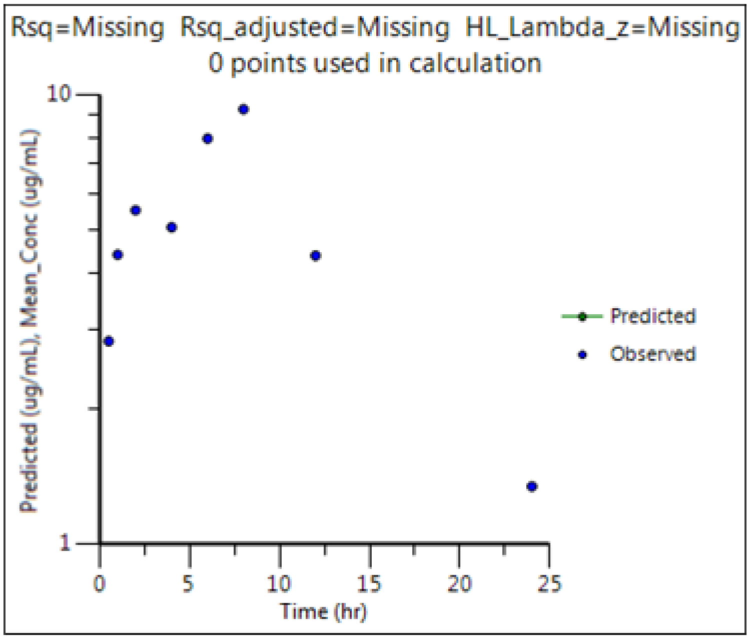
Figure 23-1. Part of the Final Parameters worksheet

3. Click **Summary Table**.



	Time (hr)	lambda_z _Incl	Mean_Conc (ug/mL)	Predicted (ug/mL)	Residual (ug/mL)	AUC (hr*ug/mL)	AUMC (hr*hr*ug/mL)	Weighting
1	0		0			0	0	0
2	0.5		2.8333333			0.70833333	0.35416667	0
3	1		4.4033333			2.5175	1.8091667	0
4	2		5.53			7.4841667	9.5408333	0
5	4		5.0666667			18.080833	40.8675	0
6	6		7.9866667			31.134167	109.05417	0
7	8		9.28			48.400833	231.21417	0
8	12		4.3833333			74.514576	485.88311	0
9	24		1.3456667			105.38204	1005.8655	0

4. Click **Observed Y and Predicted Y vs X**.



This concludes the sparse sampling example.

Urine study NCA example

This example demonstrates how to model a urine study. The time range, concentration, and data is provided in `urine.xls`. The dosing data are stored in `urine_sources.xls`. These files are located in the Phoenix examples directory.

The completed project (`NCA_UrineStudy.phxproj`) is available for reference in …`\Examples\WinNonlin`.

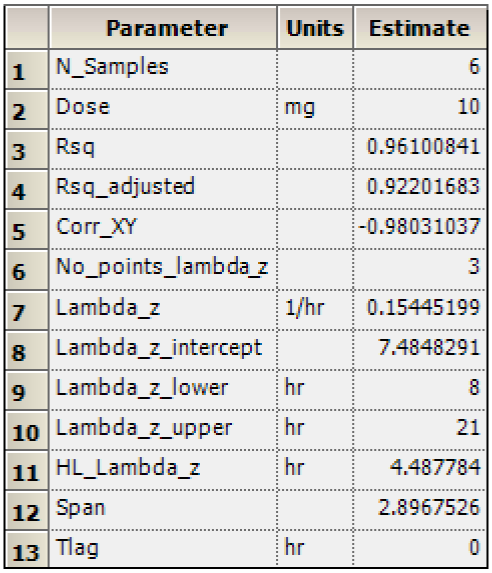
Set up the NCA object

1. Create a project called `NCA_UrineStudy`.
2. Import the two files …`\Examples\WinNonlin\Supporting files\urine.xls` and `urine_sources.xls`.
   In the *File Import Wizard* dialog, select the **Has units row** option for both worksheets.
3. Select **Workflow** in the Object Browser and then select **Insert > NonCompartmental Analysis > NCA**.
4. Rename the NCA object just added as `urine`.
5. In the Options tab, set the Model Type to **Urine (210-212)**.
6. Drag the **urine** worksheet from the Data folder to the urine object's Main Mappings panel to map it as the input source.
7. In the Main Mappings panel, map the data types to the following contexts:
   Map **Lower** to the **Start Time** context.
   Map **Upper** to the **End Time** context.
   Leave **Concentration** mapped to the **Concentration** context.
   Leave **Volume** mapped to the **Volume** context.
8. Select **Dosing** in the Setup list.
9. Drag the **urine_sources** item from the Data folder to the Dosing panel.
   Type is already mapped to the **None** context.
   Dose is already mapped to the **Dose** context.
   Time is already mapped to the **Time** context.
10. Select **Therapeutic Response** in the Setup list.
11. In the Therapeutic Response panel, check the **Use Internal Worksheet** checkbox.
12. In the first cell of the **Lower** column, enter 4.

Execute and view the NCA results

All necessary settings are complete.

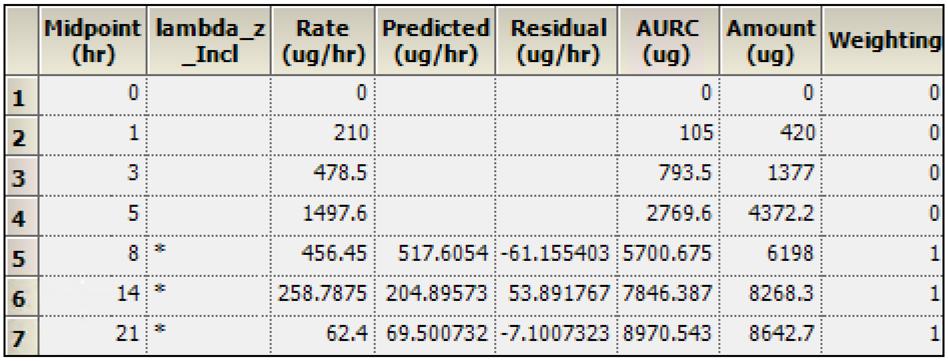
1. Click ▶ (**Execute** icon) to execute the object.
2. In the Results tab, click **Final Parameters**.



	Parameter	Units	Estimate
1	N_Samples		6
2	Dose	mg	10
3	Rsq		0.96100841
4	Rsq_adjusted		0.92201683
5	Corr_XY		-0.98031037
6	No_points_lambda_z		3
7	Lambda_z	1/hr	0.15445199
8	Lambda_z_intercept		7.4848291
9	Lambda_z_lower	hr	8
10	Lambda_z_upper	hr	21
11	HL_Lambda_z	hr	4.487784
12	Span		2.8967526
13	Tlag	hr	0

Figure 24-1. Part of the Final Parameters worksheet

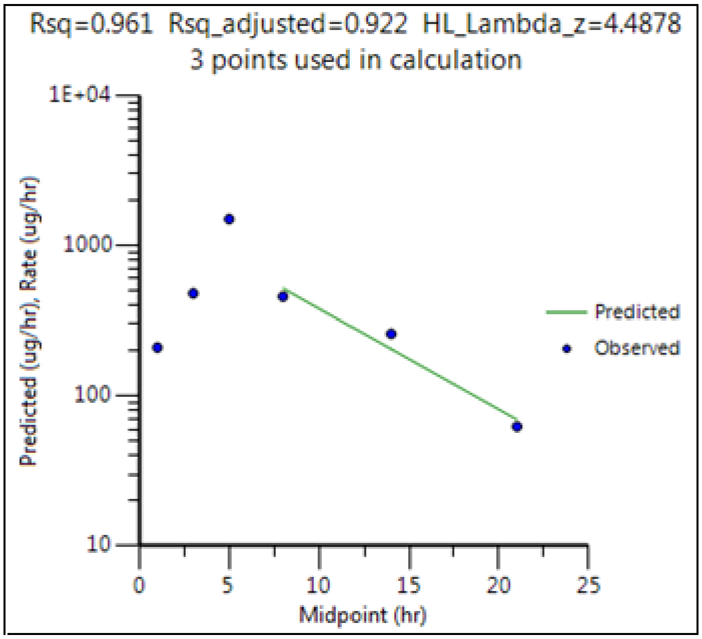
3. Click **Summary Table**.



	Midpoint (hr)	lambda_z _Incl	Rate (ug/hr)	Predicted (ug/hr)	Residual (ug/hr)	AURC (ug)	Amount (ug)	Weighting
1	0		0			0	0	0
2	1		210			105	420	0
3	3		478.5			793.5	1377	0
4	5		1497.6			2769.6	4372.2	0
5	8	*	456.45	517.6054	-61.155403	5700.675	6198	1
6	14	*	258.7875	204.89573	53.891767	7846.387	8268.3	1
7	21	*	62.4	69.500732	-7.1007323	8970.543	8642.7	1

Values used in the calculation of Lambda Z are marked with an asterisk in the Lambda_z_Incl column.

4. Click **Observed Y and Predicted Y vs X**.



This concludes the urine study example.

Drug effect NCA example

This example demonstrates how to model a urine study. The time range, concentration, and data are provided in `nca_pd.xls`. The dosing data are stored in `nca_pd_sources.xls`. These files are located in the Phoenix examples directory.

The completed project (`NCA_DrugEffect.phxproj`) is available for reference in …`\Examples\WinNonlin`.

Set up the NCA object

1. Create a project called `NCA_DrugEffect`.
2. Import the two files …`\Examples\WinNonlin\Supporting files\nca_pd.xls` and `nca_pd_sources.xls`.
   In the *File Import Wizard* dialog, select the **Has units row** option for Sheet 1.
3. Select **Workflow** in the Object Browser and then select **Insert > NonCompartmental Analysis > NCA**.
4. Rename the NCA object just added as `nca_pd`.
5. In the Options tab, set the Model Type to **Drug Effect (220)**.
6. Drag the **nca_pd** worksheet from the Data folder to the nca_pd object's Main Mappings panel to map it as the input source.
   Map **Time** to the **X** context.
   Map **Cortisol_RR** to the **Y** context.
7. Select **Dosing** in the Setup list.
8. Expand the **nca_pd_sources** item in the Object Browser Data folder and drag the **Dosing** item to the Dosing panel.
   Type is already mapped to the **None** context.
   Time is already mapped to the **Time** context.
9. Select **Partial Areas** in the Setup list.
10. In the expanded **nca_pd_sources** item in the Object Browser Data folder, drag the **PartialAreas** item to the Partial Areas panel.
    Map **Curve** to the **Area #** context.
    Map **Lower** to the **Start Time** context.
    Map **Upper** to the **End Time** context.
11. Select **Parameter Names** in the Setup list.
12. In the expanded **nca_pd_sources** item in the Object Browser Data folder, drag the **Names** item to the Parameter Names panel.
    Map **Name** to the **Parameter Name** context.
    Leave **Preferred** mapped to the **Preferred** context.
    Map **Include** to the **Include in Workbook** context.
13. Select **Slopes** in the Setup list and enter the following information:
    For the first row:
    `60` for **Start Time**
    `67` for **End Time**
    Time Range for **Fit Method**
    Linear for **Lin/Log**

    For the second row:

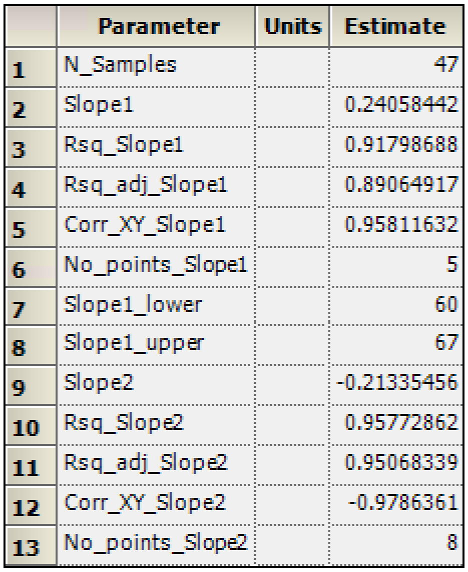
125 for **Start Time**
133 for **End Time**
Time Range for **Fit Method**
Linear for **Lin/Log**

14. Select **Therapeutic Response** in the Setup list, check the **Use Internal Worksheet** box and enter the following information:
    2.5 for **Baseline**
    5 for **Threshold**

Execute and view the NCA results

All necessary settings are complete.

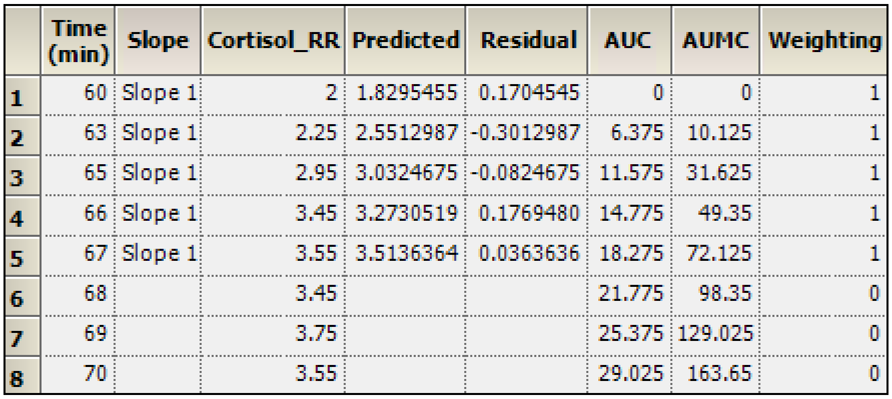
15. Click ▶ (**Execute** icon) to execute the object.
16. In the Results tab, click **Final Parameters**.



	Parameter	Units	Estimate
1	N_Samples		47
2	Slope1		0.24058442
3	Rsq_Slope1		0.91798688
4	Rsq_adj_Slope1		0.89064917
5	Corr_XY_Slope1		0.95811632
6	No_points_Slope1		5
7	Slope1_lower		60
8	Slope1_upper		67
9	Slope2		-0.21335456
10	Rsq_Slope2		0.95772862
11	Rsq_adj_Slope2		0.95068339
12	Corr_XY_Slope2		-0.9786361
13	No_points_Slope2		8

Figure 25-1. Part of the Final Parameters worksheet

17. Click **Summary Table**.



	Time (min)	Slope	Cortisol_RR	Predicted	Residual	AUC	AUMC	Weighting
1	60	Slope 1	2	1.8295455	0.1704545	0	0	1
2	63	Slope 1	2.25	2.5512987	-0.3012987	6.375	10.125	1
3	65	Slope 1	2.95	3.0324675	-0.0824675	11.575	31.625	1
4	66	Slope 1	3.45	3.2730519	0.1769480	14.775	49.35	1
5	67	Slope 1	3.55	3.5136364	0.0363636	18.275	72.125	1
6	68		3.45			21.775	98.35	0
7	69		3.75			25.375	129.025	0
8	70		3.55			29.025	163.65	0

This concludes the drug effect example.

Multiple profile analysis using NCA

Data for noncompartmental analyses can include one or more ***sort variables***. Sort variables have discrete values that identify time-concentration profiles to be analyzed individually. Input datasets should be stacked (long and skinny) rather than unstacked (short and wide).

Stacking simply means moving information stored in column headings into the rows. For example, matrix data such as plasma or urine can be placed in one row, and all associated data are arranged in rows beside the matrix data. This means that all measurements appear in a single column, with one or more additional columns flagging which data belong to which matrix. The data for one matrix must be listed first, then all the data for the other matrix.

For noncompartmental analysis data, this means that time (the independent variable) and concentration (the dependent variable) data for all individuals should occupy only one column each, with one or more additional columns (sort variables) used to identify individual profiles.

This example demonstrates the general steps to summarize a dataset using noncompartmental analysis. The dataset contains time-concentration profiles from a two period crossover study with six subjects.

The study data for this example are contained in `Profiles.CSV`, which is located in the Phoenix examples directory. This crossover study includes two sort variables: Subject (subject identifiers) and Form (formulation). There are six subjects, each of whom was tested with two formulations, for a total of twelve profiles.The completed project (`Multiple_Profiles.phxproj`) is available for reference in ...`\Examples\WinNonlin`.

Set up the project

1. Create a new project called `Multiple Profiles`.
2. Import the file ...`\Examples\WinNonlin\Supporting files\Profiles.CSV`.
   In the *File Import Wizard* dialog, select the **Has units row** option.

Review profile plots

Before analyzing the data, examine a plot of each profile to confirm the model and scan for outlying data points.

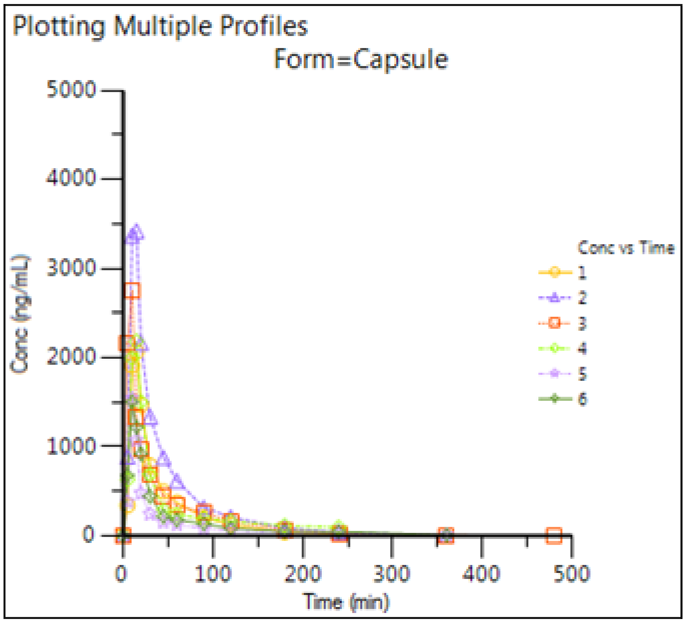
1. Right-click **Profiles** in the Data folder and then select **Send To > Plotting > XY Plot**.
2. In the XY Data Mappings panel:
   Map **Subject** to the **Group** context.
   Map **Form** to the **Lattice Conditions Page (Sort)** context.
   Map **Time** to the **X** context.
   Map **Conc** to the **Y** context.

Note: The plot display options are located in the XY Plot's Options tab. Expand items in the Options menu tree by clicking the (+) signs.

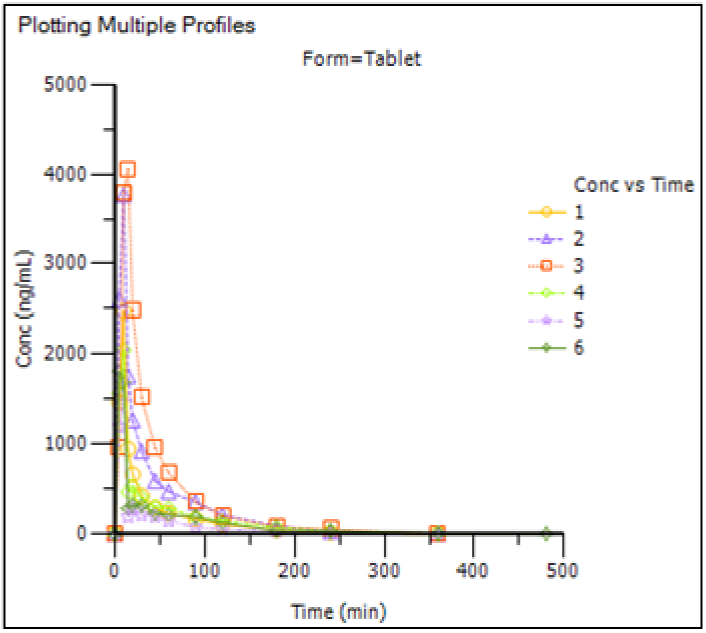
3. In the Options tab, with **Plot** selected in the menu tree, click the **Title** tab.
4. In the Title field type `Plotting Multiple Profiles`.
5. Click ▶ (**Execute** icon) to execute the object.

   There are two formulations in the input dataset. Since Form (formulation) was mapped to the Page

(Sort) Lattice Condition context, two plots are generated, each representing a formulation, and each on a separate tab. The plot for the first formulation (Capsule) is displayed automatically.



6. Click the Page 02 tab to view the plot for the second formulation (Tablet).



Set up the NCA object for the multiple profile analysis

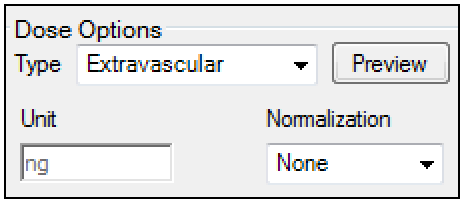
The noncompartmental analysis (NCA) plasma model 200 (extravascular dosing) is suitable for this data. All subjects had a dose of 100 ng at time zero for each formulation. All profiles use uniform weighting, and allow Phoenix to select the terminal elimination phase. The linear trapezoidal method with linear interpolation is used to compute the areas under the curve.

1. Right-click **Profiles** in the Data folder and select **Send To > NonCompartmental Analysis > NCA**.

2. In the Main Mappings panel:
   Map **Subject** to the **Sort** context.
   Map **Form** to the **Sort** context.
   Leave **Time** mapped to the **Time** context.
   Map **Conc** to the **Concentration** context.

Prepare the dosing information from the multiple profiles

1. Select **Dosing** in the Setup list.
2. Check the **Use Internal Worksheet** checkbox.
   Click **OK** in the *Dosing sorts* dialog to accept the default sort variables.
   For each cell in the Time column, enter `0`.
   For each cell in the Dose column, enter `100`.
   Do not enter any value in the Tau column.
3. In the Dose Options area of the Options tab, type `ng` in the **Unit** field and press the **Enter** key.
4. **Extravascular** is selected by default in the **Type** menu. Do not change this setting.



Type: Extravascular
Unit: ng
Normalization: None

Add a partial area calculation

1. Select **Partial Areas** in the Setup list.
2. Check the **Use Internal Worksheet** checkbox.
   Click **OK** in the *Partial Areas sorts* dialog to accept the default sort variables.
   For each cell in the Start Time column, enter `0`.
   For each cell in the End Time column, enter `120`.

Specify the NCA model options for the multiple profile analysis

1. In the Options tab, the default setting for **Model Type** is **Plasma (200-202)**. Do not change this setting.

Note: The exact plasma model type (200, 201, or 202) is determined by the dose type.

2. The default setting for **Calculation Method** is **Linear Trapezoidal Linear Interpolation**. Do not change this setting.
3. In the **Titles** field type `Processing Multiple Profiles with Model 200`.

Set up a user-defined parameter

1. Go to the **User Defined Parameters** tab.
2. Check the **Include with Final Parameters** checkbox.
3. Enter 75 in the field to compute the concentration at 75 minutes.

Execute and view the results of the multiple profile analysis

At this point, all of the necessary mappings and options have been specified.

1. Execute the object.

   In each Results worksheet, the sort variables Subject and Form are included as columns in the data grid, and the output is presented for each level of the sort variables. See the NCA "Results" for descriptions of the output.
2. Select the **Observed Y and Predicted Y vs X** plot in the Results tab and double-click it. The plot is opened in its own window.
3. In the Options menu tree below the plot, select **Lattice** under **Plot**.
4. Clear the **Bind Lattice to Data** checkbox.
5. Click the up and down arrows in the **Lattice Rows** and **Lattice Columns** boxes to change the number of rows/columns, respectively, that form the lattice.
6. Close the Observed Y and Predicted Y vs X window.

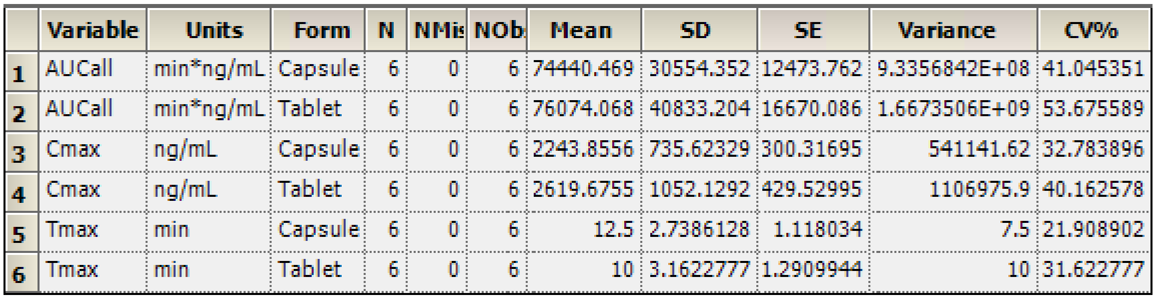
Phoenix can display a maximum of 15 latticed rows and 15 latticed columns, but no more than 200 charts per page. The number of plots that can be displayed per page depends on the monitor size and resolution. If too many plots are placed on one page the axes labels, legends, and other plot information can be difficult to read. Additional information on lattices can be found in "More on latticed plots in Phoenix".

Summarize the multiple profile analysis output with statistics

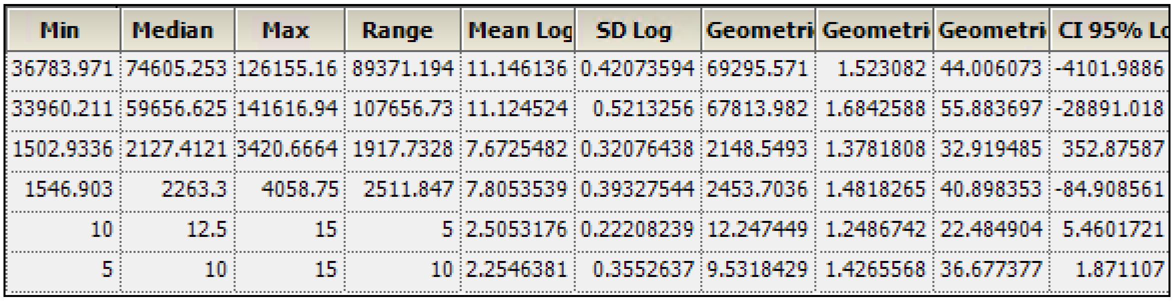
Phoenix's Descriptive Statistics object is used to summarize several of the output parameters in the Final Parameters Pivoted worksheet. The Descriptive Statistics object generates separate statistics for each formulation.

1. Right-click **Workflow** in the Object Browser and select **New > Computation Tools > Descriptive Statistics**.
2. In the Descriptive Statistics Main Mappings panel click 📂 (**Select Source** icon).
3. In the dialog, under the NCA node, select the **Final Parameters Pivoted** worksheet and click **OK**.
4. Map:
   Form to the **Sort** context.
   Tmax, **Cmax**, and **AUCall** to the **Summary** context.
   Leave all other data types mapped to **None**.
5. In the Options tab, check the **Confidence Intervals** and **Number of SD Statistics** checkboxes, but do not change the default values for these two items.
6. Execute the object.

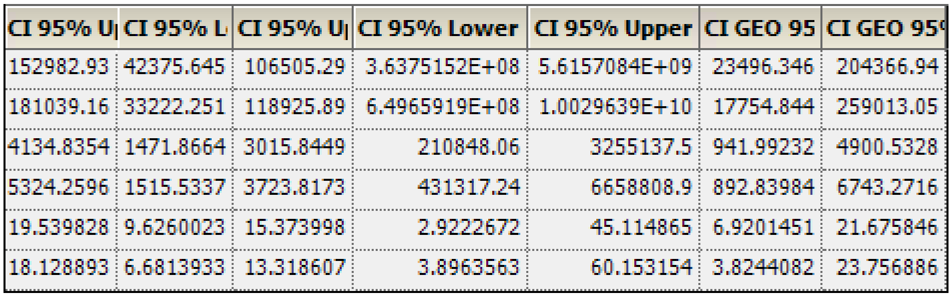
This example summarizes $AUCall$, the area under the curve through the last measured value, $Cmax$, the maximal concentration of drug in the blood, and $Tmax$, the time at maximal concentration.



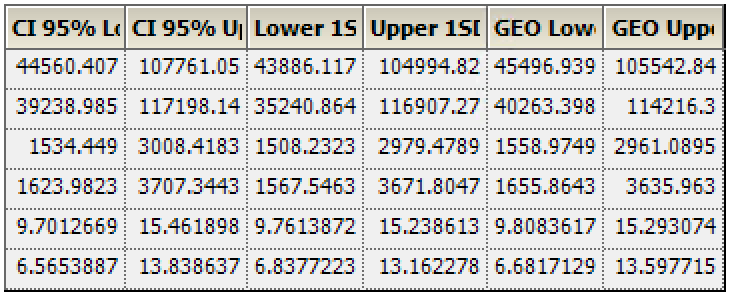
	Variable	Units	Form	N	NMis	NOb	Mean	SD	SE	Variance	CV%
1	AUCall	min*ng/mL	Capsule	6	0	6	74440.469	30554.352	12473.762	9.3356842E+08	41.045351
2	AUCall	min*ng/mL	Tablet	6	0	6	76074.068	40833.204	16670.086	1.6673506E+09	53.675589
3	Cmax	ng/mL	Capsule	6	0	6	2243.8556	735.62329	300.31695	541141.62	32.783896
4	Cmax	ng/mL	Tablet	6	0	6	2619.6755	1052.1292	429.52995	1106975.9	40.162578
5	Tmax	min	Capsule	6	0	6	12.5	2.7386128	1.118034	7.5	21.908902
6	Tmax	min	Tablet	6	0	6	10	3.1622777	1.2909944	10	31.622777



Min	Median	Max	Range	Mean Log	SD Log	Geometri	Geometri	Geometri	CI 95% Lo
36783.971	74605.253	126155.16	89371.194	11.146136	0.42073594	69295.571	1.523082	44.006073	-4101.9886
33960.211	59656.625	141616.94	107656.73	11.124524	0.5213256	67813.982	1.6842588	55.883697	-28891.018
1502.9336	2127.4121	3420.6664	1917.7328	7.6725482	0.32076438	2148.5493	1.3781808	32.919485	352.87587
1546.903	2263.3	4058.75	2511.847	7.8053539	0.39327544	2453.7036	1.4818265	40.898353	-84.908561
10	12.5	15	5	2.5053176	0.22208239	12.247449	1.2486742	22.484904	5.4601721
5	10	15	10	2.2546381	0.3552637	9.5318429	1.4265568	36.677377	1.871107



CI 95% U	CI 95% L	CI 95% U	CI 95% Lower	CI 95% Upper	CI GEO 95	CI GEO 95
152982.93	42375.645	106505.29	3.6375152E+08	5.6157084E+09	23496.346	204366.94
181039.16	33222.251	118925.89	6.4965919E+08	1.0029639E+10	17754.844	259013.05
4134.8354	1471.8664	3015.8449	210848.06	3255137.5	941.99232	4900.5328
5324.2596	1515.5337	3723.8173	431317.24	6658808.9	892.83984	6743.2716
19.539828	9.6260023	15.373998	2.9222672	45.114865	6.9201451	21.675846
18.128893	6.6813933	13.318607	3.8963563	60.153154	3.8244082	23.756886



CI 95% Lo	CI 95% U	Lower 1S	Upper 1SI	GEO Low	GEO Upp
44560.407	107761.05	43886.117	104994.82	45496.939	105542.84
39238.985	117198.14	35240.864	116907.27	40263.398	114216.3
1534.449	3008.4183	1508.2323	2979.4789	1558.9749	2961.0895
1623.9823	3707.3443	1567.5463	3671.8047	1655.8643	3635.963
9.7012669	15.461898	9.7613872	15.238613	9.8083617	15.293074
6.5653887	13.838637	6.8377223	13.162278	6.6817129	13.597715

Create a Cmax plot with error bars

This section will illustrate how to use the means and SDs of data, computed by the Descriptive Statistics object, to create an overlaid plot with error bars. The Descriptive Statistics results obtained in the previous section will be filtered so that the output for only one variable (Cmax) remains. The data will then be used to create the error bars for a plot of Cmax values.

First filter the Descriptive Statistics results:

1. Right-click **Workflow** in the Object Browser and select **New > Data Management > Data Wizard**.
2. In the Options tab, select **Filter** from the Action menu and click **Add** right below the menu.
3. Click the **Select Source** icon in the Mappings panel.
4. In the *Select Source* dialog, under the **Descriptive Statistics** node, select **Statistics**, and click **OK**.

5. In the Options tab, click the **Add** button to the right of the **Specify Filter** field.
6. In the *Filter Specification* dialog, select **Include** as the Action.
7. Type `Cmax` in the **Select Column or Enter Value** field.
8. Make sure the **Apply to entire row** box is checked.
9. Click **OK**.
10. Execute the object.
    The Result worksheet now only contains the rows of Cmax data.

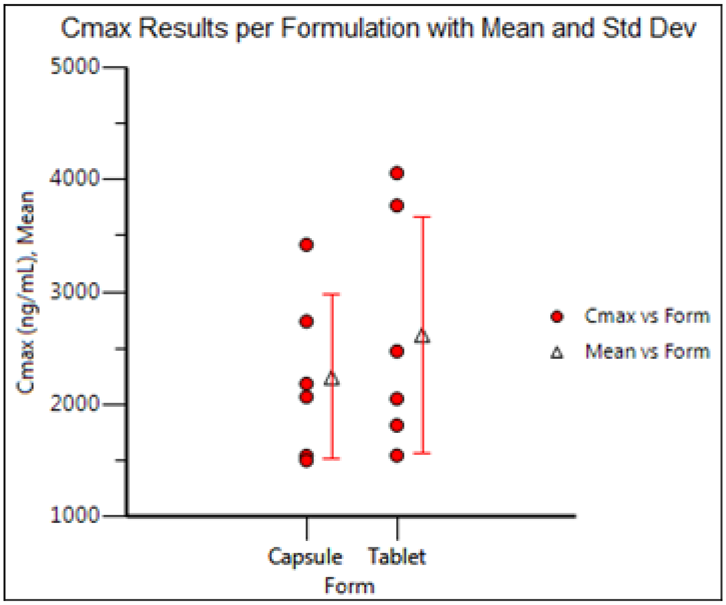
Now set up the X-Categorical XY Plot object:

1. Right-click **Workflow** in the Object Browser and select **New > Plotting > X-Categorical XY Plot**.
2. Click the **Select Source** icon in the Mappings panel and select the **NCA Final Parameters Pivoted** worksheet and click **OK**.
3. Map:
   Form to the **X** context.
   Cmax to the **Y** context.
   Leave all other data types mapped to **None**.
4. In the Options tab, select **Plot** in the menu tree and then the **Graphs** sub-tab.
5. Click the **Add** button.
6. Select the **CategoricalX 1 Data** item in the Setup tab, click the **Select Source** icon.
7. In the *Select Source* dialog, under the **Data Wizard** node, select **Result**, and click **OK**.
8. Map:
   Form to the **X** context.
   Mean to the **Y** context.
   SD to both the **Error Bars Lower** and **Upper** contexts.

Adjust the appearance of the plot:

1. In the Options tab, with **Plot** selected in the menu tree, go to the **Title** sub-tab.
2. Enter `Cmax Results per Formulation with Mean and Std Dev` in the field and click the ☰ icon to center the plot title.
3. In the menu tree, select **Cmax vs Form** under the Graphs node.
4. Change the Marker Color to **Red** and set the Marker Size to **7**.
5. Select **Mean vs Form** under the Graphs node.
6. In the **Content** sub-tab, check the **Offset** checkbox and enter `14` for the number of pixels.
7. Select the **Appearance** sub-tab and set the Marker Shape to **Triangle**.
8. In the menu tree, expand the **Mean vs Form** node and select **Error Bars**.
9. Click the **Appearance** sub-tab and set the Color to **Red** and the Cap Width to **9**.
10. Execute the object.

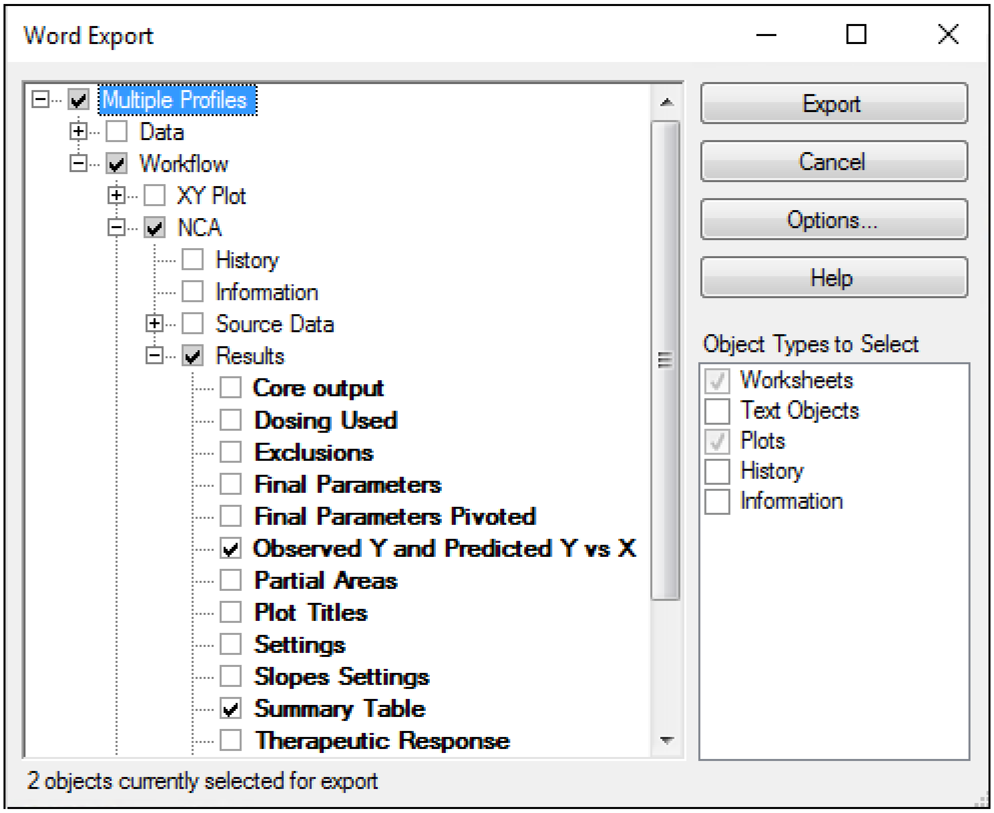
The output is an overlay of two graphs (Cmax vs Form and Mean vs Form) that includes error bars.



Export results to Microsoft Word

The results of any operational object can be exported to a Microsoft Word document. This example shows how to format plot output and export it to Microsoft Word. By default, plots are exported at a resolution of 1024 by 768 pixels.

1. Select **File > Word Export**.
2. In the *Word Export* dialog, click the (+) signs beside **Workflow > NCA > Results** to expand the menu tree.
3. Select the **Observed Y and Predicted Y vs X** checkbox.
4. Select the **Summary Table** checkbox.



- ☑ Multiple Profiles
  - □ Data
  - ☑ Workflow
    - □ XY Plot
    - ☑ NCA
      - □ History
      - □ Information
      - □ Source Data
      - ☑ Results
        - □ Core output
        - □ Dosing Used
        - □ Exclusions
        - □ Final Parameters
        - □ Final Parameters Pivoted
        - ☑ Observed Y and Predicted Y vs X
        - □ Partial Areas
        - □ Plot Titles
        - □ Settings
        - □ Slopes Settings
        - ☑ Summary Table
        - □ Therapeutic Response

Object Types to Select

- ☑ Worksheets
- □ Text Objects
- ☑ Plots
- □ History
- □ Information

2 objects currently selected for export

5. Click **Options**.
6. Select the **Landscape** option button in the Document tab.
7. Make sure the **Add source line to objects** checkbox is cleared and click **Finished**.
8. Click **Export**.
   Phoenix creates a new Microsoft Word document and exports the selected objects into the document.
9. In the *Export Complete* dialog, click **OK**.
10. Save the Word file and exit Microsoft Word.
11. Close the project by right-clicking the project in the Object Browser and selecting **Close Project**.

NonParametric Superposition

In pharmacokinetics it is often desirable to predict the drug concentration in blood or plasma after multiple doses, based on concentration data from a single dose. This can be done by fitting the data to a compartmental model with some assumptions about the absorption rate of the drug. An alternative method is based on the principle of superposition, which does not assume any pharmacokinetic (PK) model.

Phoenix's nonparametric superposition object is used to predict drug concentrations after multiple dosing at steady state, and is based on noncompartmental results describing single dose data. The predictions are based upon an accumulation ratio computed from the terminal slope (Lambda Z). The feature allows predictions from simple (the same dose given in a constant interval) or complicated dosing schedules. The results can be used to help design experiments or to predict outcomes of clinical trials when used in conjunction with the semicompartmental modeling function.

Use one of the following to add the object to a Workflow:

Right-click menu for a Workflow object:
New > NonCompartmental Analysis > Nonparametric Superposition
Main menu:
Insert > NonCompartmental Analysis > Nonparametric Superposition
Right-click menu for a worksheet:
Send To > NonCompartmental Analysis > Nonparametric Superposition

Note: To view the object in its own window, select it in the Object Browser and double-click it or press **ENTER**. All instructions for setting up and execution are the same whether the object is viewed in its own window or in Phoenix view.

User interface description
Results
NonParametric Superposition methodology
NonParametric Superposition example

User interface description

Main Mappings panel
Administered Dose panel
Terminal Phase panel
Dosing panel
Options tab
Plots tab (See the "Plots tab" description in the NCA section.)

Main Mappings panel

Use the Main Mappings panel to identify how input variables are used in the NonParametric object. NonParametric superposition requires a dataset containing time and concentration data, and sort variables to identify individual profiles. Required input is highlighted orange in the interface.

None: Data types mapped to this context are not included in any analysis or output.
Sort: Categorical variable(s) identifying individual data profiles, such as subject ID in a nonparametric analysis. A separate analysis is performed for each unique combination of sort variables.
Time: The relative or nominal sampling times used in a study.
Concentration: The measured amount of a drug in blood plasma.

Administered Dose panel

Using the Administered Dose panel is optional. When this panel is not used, it is assumed that the administered dose, or the dose associated with the data, is the same as the Loading Dose specified in the Regular Dosing tab, or the same as the dose at time zero specified in the Variable Dosing tab. Required input is highlighted orange in the interface.

Note: The sort variables in the dosing data worksheet must match the sort variables used in the main input dataset.

None: Data types mapped to this context are not included in any analysis or output.
Sort: Categorical variable(s) identifying individual data profiles, such as subject ID in a nonparametric analysis. A separate analysis is performed for each unique combination of sort variables.
Administered Dose: The amount of drug given.

Terminal Phase panel

A dataset containing terminal phase information is options. If the dataset is available, use the Terminal Phase panel for mapping the start and end times for the terminal elimination phase for each profile. Use an internal worksheet to set the range (NPS does not use an extern worksheet mapping for Lambda Z setup.)

Note: The sort variables in the dosing data worksheet must match the sort variables used in the main input dataset.

None: Data types mapped to this context are not included in any analysis or output.
Sort: Categorical variable(s) identifying individual data profiles, such as subject ID in a nonparametric analysis. A separate analysis is performed for each unique combination of sort variables.
Start and **End**: Start and end times for the terminal elimination phase. These contexts are generally not required. However, if the user maps an external worksheet to Terminal Phase, then the Start and End columns are required.

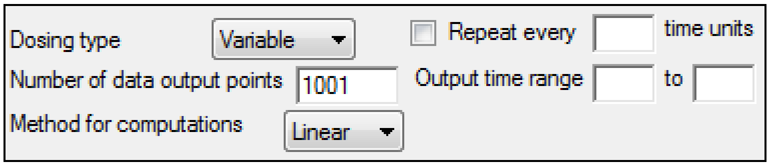
Dosing panel

The Dosing panel is only available if Variable is selected in the Dosing type menu. If the main input dataset used with the nonparametric superposition object contains variable times between doses, then use the Dosing panel to enter the separate time and dose values. Required input is highlighted orange in the interface.

None: Data types mapped to this context are not included in any analysis or output.
Time: Time that the drug is administered.
Dose: The amount of drug administered.

Options tab

The Options tab allows users to select the dosing type, specify options related to regular and variable dosing, and enter dosing values for regular dosing.



Dosing type: Variable
□ Repeat every ____ time units
Number of data output points: 1001
Output time range ____ to ____
Method for computations: Linear

- In the **Dosing Type** menu, select the dosing interval type.

  Regular: For dosing at a regular time interval.
  Variable: For dosing at variable time intervals.

  If variable dosing is selected then the Dosing panel is displayed in the Setup tab list. The Dosing panel is used to enter separate doses and times.

  Selecting different dosing type changes the options in the Options tab. For regular dosing type options see "Regular dosing options". For variable dosing type options see "Variable dosing options".

- In the **Number of data output points** field, type the number of data output points.
  The default number of data points for regular dosing is 101. The default number of data points for variable dosing is 1001.

- In the **Method for computations** menu, select the method used for interpolation and extrapolation of untransformed data.

  Linear: Uses only Linear interpolation. Use when data after Tmax is not necessarily exponentially declining.
  Linear/Log: For each dosing interval, uses Linear interpolation through the Tmax of that interval, and Log interpolation after Tmax.

Regular dosing options

- In the **Loading Dose** field, type the initial dose used to calculate the AUC (area under the curve).
- In the **Maintenance dose** field, type the maintenance dose used to calculate the AUC.
- In the **Tau** field, type the dosing interval value.

Note: The value entered in the Tau field must match the time values used in the dataset. For example, if the time in the dataset is measured in hours and a dose is given once every day, type 24 in the Tau field.

- Select the **Display at steady state** option button to have Phoenix generate the Predictions vs Time plot at steady state.
- Select the **Display Nth dose** option button to have Phoenix generate the Predictions vs Time at a particular dose.
- In the **Display Nth dose** field, type the dose used to generate the Predictions vs Time plot.

Variable dosing options

- Select the **Repeat every N time units** checkbox to specify a repeating dosing regimen.
- In the **Repeat every N time units** field, type the repeat time for one dosing cycle.

Note: The time units entered in the **Repeat every N time units** field must match the time units used in the Dosing panel. For example, if the dosing cycle repeats every 24 hours, type 24 in the **Repeat every N time units** field.

- In the **Output time range** fields, type the start and end times used to create the predicted output data.

Results

NonParametric superposition generates worksheets containing predicted concentrations and Lambda Z values, as well as plots of predicted concentration over time for each sort level, and a summary plot.

- The Concentrations worksheet contains times and predicted concentrations for each level of the sort variables. If a regular dosing schedule is selected, this output represents times and concentrations between two doses at steady state. If a variable dosing schedule is selected, the output includes the times and concentrations within the supplied output range.
- The Lambda Z worksheet contains the Lambda Z value and the half-life for each sort key.

Note: NPS and NCA Best-Fit Lambda Z calculations can generate slightly different Lambda Z values (at the sixth significant digit) when the input data contains eight significant digits or more.

NPS stops if the last three points fail to compute Lambda Z (such as the last three points going uphill). If this situation occurs, you can execute NCA on the data using the default settings, and then map the NCA Slopes result to the Terminal Phase setup in the NPS object. The NCA execution will check further back in the dataset to see if a larger group of points ending with the last point will yield a valid Lambda Z.

NonParametric Superposition methodology

NonParametric superposition assumes that each dose of a drug acts independently of every other dose; that the rate and extent of absorption and average systemic clearance are the same for each dosing interval; and that linear pharmacokinetics apply, so that a change in dose during the multiple dosing regimen can be accommodated.

In order to predict the drug concentration resulting from multiple doses, one must have a complete characterization of the concentration-time profile after a single dose. That is, it is necessary to know $C(t_i)$ at sufficient time points t_i, (i=1,2,…,n), to characterize the drug absorption and elimination process. Two assumptions about the data are required: independence of each dose effect, and linearity of the underlying pharmacokinetics. The former assumes that the effect of each dose can be separated from the effects of other doses. The latter, linear pharmacokinetics, assumes that changes in drug concentration will vary linearly with dose amount.

The required input data are the time, dosing, and drug concentration. The drug concentration at any particular time during multiple dosing is then predicted by simply adding the concentration values as shown in the next section ("Computation method").

Note: User-defined terminal phases apply to all sort keys. In addition, dosing schedules and doses are the same for all sort keys.

Computation method

Given the concentration data points $C(t_i)$ at times t_i, (i=1,2,…,n), after a single dose D, one may obtain the concentration $C(t)$ at any time t through interpolation if $t_1 < t < t_n$, or extrapolation if $t > t_n$. The extrapolation assumes a log-linear elimination process; that is, the terminal phase in the plot of log($C(t)$) versus t is approximately a straight line. If the absolute value of that line's slope is λ_Z and the intercept is ln(β), then $C(t)$ = βexp(–$\lambda_Z t$) for ($t > t_n$).

The slope λ_Z and the intercept are estimated by least squares from the terminal phase; the time range included in the terminal phase may be specified by the user or, if not specified, estimates from the best linear fit (based on adjusted R^2 as in the Best Fit method in Noncompartmental Analysis) will be used. The half life is: $\ln(2)/\lambda_Z$

Suppose there are m additional doses D_j, $j = 1,\ldots,m$, and each dose is administered after τ_j time units from the first dose. The concentration due to dose D_j will be:

$$C_j(t) = \left(\frac{D_j}{D}\right)(C(t-\tau_j))$$

where t is time since the first dose and $C(t - \tau_j) = 0$ for $t \le \tau_j$.

The total predicted concentration at time t will be:

$$Conc(t) = \sum_j C_{j(t)}, \quad j = 1,2,\ldots,m$$

If the same dose is given at constant dosing intervals τ, and τ is sufficiently large that drug concentrations reflect the post-absorptive and post-distributive phase of the concentration-time profile, then steady state can be reached after sufficient time intervals. Let the concentration of the first dose be $C_1(t)$ for $0 < t < \tau$, so τ is greater than t_n. Then the concentration at time t after n^{th} dose (i.e., t is relative to dose time) will be:

$$\begin{aligned} C_n(t) &= C_1(t) + \beta\exp[-\lambda(t+\tau)] + \beta\exp[-\lambda(t+2\tau)] \\ &\quad + \ldots + \beta\exp[-\lambda(t+(n-1)\tau)] \\ &= C_1(t) + \beta\exp[-\lambda(t+\tau)]\frac{1-\exp[-\lambda(n-1)\tau]}{1-\exp(-\lambda\tau)} \end{aligned}$$

As $n \to \infty$, the steady state (ss) is reached:

$$C_{SS}(t) = C_1(t) + \beta\exp\frac{-\lambda(t+\tau)}{1-\exp((-\lambda)\tau)}$$

To display the concentration curve at steady state, Phoenix assumes steady state is at ten times the half life.

For interpolation, Phoenix offers two methods: linear interpolation and log-interpolation. Linear interpolation is appropriate for log-transformed concentration data and is calculated by:

$$C(t) = C(t_{i-1}) + [C(t_1) - C(t_{i-1})]\frac{t - t_{i-1}}{t_i - t_{i-1}},$$

$$t_{i-1} < t < t_i$$

Log-interpolation is appropriate for original concentration data, and is evaluated by:

$$C(t) = \exp\left\{ \log(C(t_{i-1})) + [\log(C(t_1)) - \log(C(t_{i-1}))]\frac{t - t_{i-1}}{t_i - t_{i-1}} \right\},\ t_{i-1} < t < t_i$$

For additional information see Appendix E of Gibaldi and Perrier (1982). *Pharmacokinetics*, 2nd ed. Marcel Dekker, New York.

NonParametric Superposition example

This example uses the output from the semicompartmental modeling example, detailed under "Semi-compartmental model example". The study data are from an early Phase I PK/PD trial. Quick input is sought for the design of a seven day multiple dose study. However, the profiles are irregular, and it is not easy to apply a compartmental modeling approach to the data.

This section uses the NonParametric Superposition object to predict plasma concentrations and effect-site concentrations at steady-state based on single-dose data. This feature allows for predictions on data that are otherwise difficult to model.

Knowledge of how to do basic tasks using the Phoenix interface, such as creating a project and importing data, is assumed.

The completed project (`NPS.phxproj`) is available for reference in …`\Examples\WinNonlin`.

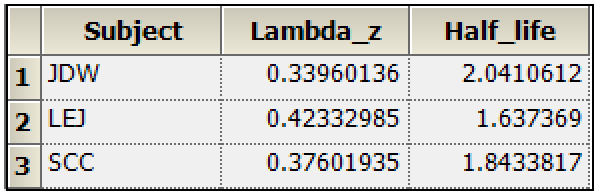
Set up an estimation of steady-state plasma concentrations

1. Create a project called NPS.
2. Import the files …`\Examples\WinNonlin\Supporting files\SCM_Results.xls`.
   In the *File Import Wizard* dialog, select the **Has units row** option.
1. Right-click **SCM_Results** in the Data folder and select **Send To > NonCompartmental Analysis > NonParametric Superposition**.
2. In the Main Mappings panel:
   Map **Subject** to the **Sort** context.
   Leave **Time** mapped to the **Time** context.
   Map **Conc** to the **Concentration** context.
   Leave **Ce** mapped to **None**.
   Leave **Effect** mapped to **None**.
3. In the Options tab below the Setup panel, type `50` in the **Loading Dose** field.
4. In the **Maintenance Dose** field type `50`.
5. In the **Tau** (dosing interval) field type `4`.

Execute and view the plasma estimation results

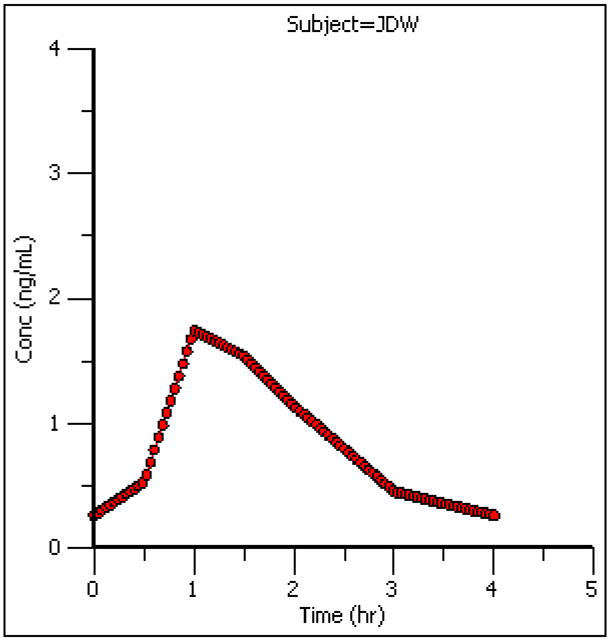
1. Click ▶ (**Execute** icon) to execute the object.

The Concentration worksheet provide predicted steady-state plasma concentrations. The Lambda z worksheet lists the Lambda Z and half-life estimates.



	Subject	Lambda_z	Half_life
1	JDW	0.33960136	2.0410612
2	LEJ	0.42332985	1.637369
3	SCC	0.37601935	1.8433817

The plot output shows predicted steady state concentrations over time for each subject. The first subject's plot is shown below.



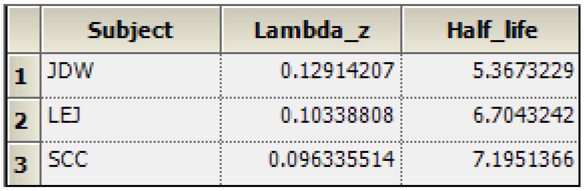
Set up an estimation of steady-state effect-site concentrations

1. Select the NonParametric object's Setup panel.
2. In the Main Mappings panel, re-map the data types to the following contexts:
   Leave **Subject** mapped to the **Sort** context.
   Leave **Time** mapped to the **Time** context.
   Map **Conc** to **None**.
   Map **Ce** to the **Concentration** context.
   Leave **Effect** mapped to **None**.
3. Select **Terminal Phase** from the Setup list.
4. Check the **Use Internal Worksheet** checkbox.
5. For each row:
   In the **Start** column, type 4.
   In the **End** column, type 8.

Execute and view the effect-site concentration estimation results

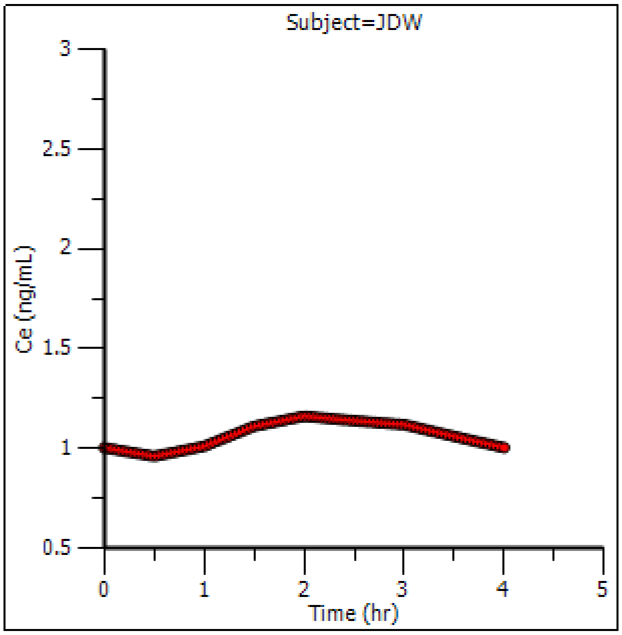
1. Execute the object.

The new NonParametric worksheet results provide predicted effect site concentrations at steady-state and Lambda Z and half-life estimates.



	Subject	Lambda_z	Half_life
1	JDW	0.12914207	5.3673229
2	LEJ	0.10338808	6.7043242
3	SCC	0.096335514	7.1951366

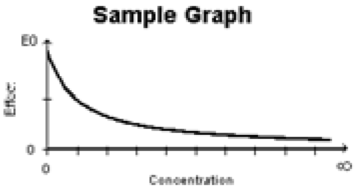
The plot output shows predicted effect site concentrations at steady-state over time for each subject. The first subject's graph is shown below.



Compute the steady-state effect

Now it is possible to compute the steady-state effect from the predicted steady-state concentrations at the effect site.

In the Semicompartmental example, the PD model 103 was used (its sample graph is shown below). The effect formula for model 103 is E=E0*(1 – (C/(C+IC50))). This formula will be adjusted for each subject in the study.



1. In the Results tab, right-click the **Concentrations** (effect site concentrations) worksheet and select **Copy to Data Folder**.

   The worksheet is added to the project's Data folder and renamed "Concentrations from NonParametric".
2. In the Object Browser, select **Concentrations from NonParametric** in the Data folder.
3. In the Columns tab below the table, click **Add** under the Columns list.

4. In the *New Column Properties* dialog, the **Numeric** option button is selected by default. Do not change this setting.
5. In the **Column Name** field type `Effect` and click **OK**.

   The Effect column is added in the Columns list and in the table in the Grid tab.
6. Use the **Down Arrow** button beside the Columns list to move the Effect column header to the bottom of the Columns list.
7. In the Object Browser, right-click **Concentrations from NonParametric** and select **Edit in Excel**.

   Phoenix displays a message warning users that changes made in Excel are not recorded in Phoenix.
8. Click **OK**.

   The worksheet is opened in Excel. If you see a pop-up dialog stating that the format and extension of the file do not match, click **Yes** to continue as the file is safe to open.

   In Excel, enter the PD model 103 effect formula in the Effect column for each subject at time zero. Use the E0 and IC50 values from the PD Model object's Final Parameters worksheet.
9. Select the cell in the **Effect** column at time zero for the first subject, JDW.
10. Type the effect formula shown below in the Effect column cell at time 0 (zero) for subject JDW, which is row 3.
    `= 102.93*(1-(C3/(C3+0.09)))` ***(for subject JDW)***
11. Repeat for the second and third subjects, LEJ (row 104) and SCC (row 205).
    `= 100.17*(1-(C104/(C104+0.09)))` ***(for subject LEJ)***
    `= 100.45*(1-(C205/(C205+0.08)))` ***(for subject SCC)***
12. After the Effect value formula is set up at time zero for each subject copy the formula to the other time points for each subject.

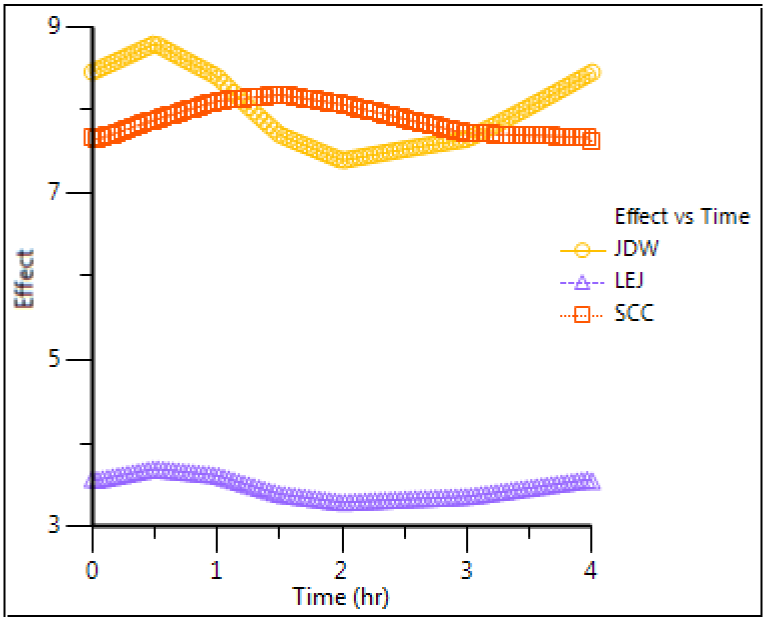
    Because of the way Phoenix handles its interactions with Excel, users cannot use the **Save As** option in Excel to save the worksheet with a different name or to a different location. The **Save** option must be used.
13. Select **File > Save**.
14. Close Excel. Be sure to save the worksheet before closing Excel, or all changes are lost.
    An *Apply Changes* message dialog is displayed.
15. Click **Yes** to apply the changes. An entry is written in the worksheet History tab noting that it was edited in Excel.
16. When asked whether to save formulas, select **No** so that the worksheet is editable in Phoenix.

    The Concentrations from NonParametric worksheet now has Effect values derived from the equations used in the Excel edit and can still be used with operational objects.

Plot time vs effect

Once the steady-state effects and concentrations are generated it is possible to use the modified concentrations from NonParametric worksheet to plot time vs. effect for each subject by mapping the worksheet to an XY Plot object.

1. Right-click **Concentrations from NonParametric** in the Data folder and select **Send To > Plotting > XY Plot**.
2. In the XY Data Mappings panel:
   Map **Subject** to the **Group** context.
   Map **Time** to the **X** context.
   Leave **Ce** mapped to **None**.
   Map **Effect** to **Y** context.
3. Execute the object.



This concludes the Nonparametric superposition example.

Semicompartmental Modeling

Semicompartmental modeling was proposed by Kowalski and Karim (1995) for modeling the temporal aspects of the pharmacokinetic-pharmacodynamic relationship of drugs. Their model was based on the effect-site link model of Sheiner, Stanski, Vozeh, Miller and Ham (1979) to estimate effect-site concentration C_e, but uses a piecewise linear model for plasma concentration C_p rather than specifying a PK model for C_p. The potential advantage of this approach is reducing the effect of model misspecification for C_p when the underlying PK model is unknown.

Use one of the following to add the object to a Workflow:

Right-click menu for a Workflow object:
New > NonCompartmental Analysis > Semicompartmental Modeling.
Main menu:
Insert > NonCompartmental Analysis > Semicompartmental Modeling.
Right-click menu for a worksheet:
Send To > NonCompartmental Analysis > Semicompartmental Modeling.

Note: To view the object in its own window, select it in the Object Browser and double-click it or press **ENTER**. All instructions for setting up and execution are the same whether the object is viewed in its own window or in Phoenix view.

User interface description
Results
Semicompartmental calculations
Semicompartmental model example

User interface description

Main Mappings panel
Options tab
Plots tab (See the "Plots tab" description in the NCA section.)

Main Mappings panel

Use the Main Mappings panel to identify how input variables are used in the Semicompartmental object. Semicompartmental modeling requires a dataset containing time and concentration data, and sort variables to identify individual profiles. Required input is highlighted orange in the interface.

None: Data types mapped to this context are not included in any analysis or output.

Sort: Categorical variable(s) identifying individual data profiles, such as subject ID in a semicompartmental analysis. A separate analysis is performed for each unique combination of sort variables.

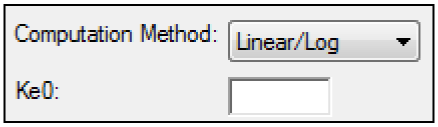
Time: The relative or nominal dosing times used in a study.

Concentration: The measured amount of a drug in blood plasma.

Effect: The measured effect data.

Options tab

The Options tab allow users to select the computation method and the Ke0 value.



Computation Method: Linear/Log

Ke0:

- In the **Computation Method** menu, select the method Phoenix uses to perform the semicompartmental analysis.

  Linear uses a linear piecewise PK model.
  Log uses a log-linear piecewise PK model.
  Linear/Log start with linear to Tmax and then log-linear after Tmax; this is also the default option.

- In the **Ke0** field, type the value for the equilibrium rate constant.

Results

The Semicompartmental model object generates four charts, one worksheet, and one text file.

Up to four plots are created for each profile. Each profile's plot is displayed on its own page in the Results tab. Click the page tabs at the bottom of each plot panel to view the plots for individual profiles. If drug effect data is included in the dataset, *Effect vs Ce* and *Effect vs Cp* plots are also created.

Ce vs Time: Estimated effect site concentration vs. time.

Cp vs Time: Plasma concentration vs. time.

Effect vs Ce: Drug effect vs. the estimated effect site concentration.

Effect vs Cp: Drug effect vs. plasma concentration.

A text file called **Settings** is also created. It lists the user-specified settings in the Semicompartmental object

The Semicompartmental object creates a worksheet called **Results**. This worksheet contains: sort variables (if any are used), time points used in the study, drug concentration levels in blood plasma, Ce (estimated effect site concentration), effect data (if included). Any units associated with the time and concentration columns in the input data are carried through to the semicompartmental modeling output.

Semicompartmental calculations

Phoenix's semicompartmental modeling estimates effect-site concentrations for given times and plasma concentrations and an appropriate value of K_{e0}. This function should be used when a counterclockwise hysteresis is observed in the graph of effect versus plasma concentrations. The hysteresis loop collapses with the graph of effect versus effect-site concentrations.

A scientist developing a PK/PD link model based upon simple IV bolus data can use this function to compute effect site concentrations from observed plasma concentrations following more complicated administration regimen without first modeling the data, i.e., semicompartmental modeling determines if the hysteresis can be collapsed using an effect compartment, without requiring a full compartmental model. The results can then be compared to the original datasets to determine if the model suitably describes pharmacodynamic action after the more complicated regimen.

Data and assumptions

Drug concentration in plasma C_p for each subject is measured at multiple time points after the drug is administrated. To minimize the bias in estimating C_e, the time points need to be adequately sampled such that accurate estimation of the AUC by noncompartmental methods can be obtained. Effect-site link model is used and the value of the equilibration rate constant k_{e0} that accounts for the lag between the C_p and the C_e curves is required. The default option is for a piecewise Linear/Log model to be assumed for C_p.

Computation method

In the effect-site link model proposed by Sheiner, Stanski, Vozeh, Miller and Ham (1979), a hypothetical effect compartment was proposed to model the time lag between the PK and PD responses. The effect site concentration C_e is related to C_p by first-order disposition kinetics and can be obtained by solving the differential equation:

$$\frac{dC_e}{dt} = k_{e0}(C_p - C_e)$$

where k_{e0} is a known constant. In order to solve this equation, one needs to know C_p as a function of time, which is usually given by compartmental PK models.

Here, use a piecewise linear model for C_p:

$$C_p(t) = C_{p_{j-1}} + \lambda_j(t - t_{j-1}) \qquad (t_{j-1} < t < t_j)$$

where:

$$\lambda_j = (C_{p_j} - C_{p_{j-1}})/(t_j - t_{j-1})$$

and:

$$C_{p_{j-1}} = C_p(t_{j-1})$$

Using the above two equations can lead to a recursive equation for C_e:

$$C_{e_j} = C_{e_{j-1}} e^{-k_{e0}(t_j - t_{j-1})} + \left(C_{P_{j-1}} - \frac{\lambda_j}{k_{e0}}\right)[1 - e^{-k_{e0}(t_j - t_{j-1})}]$$

$$+ \lambda_j(t_j - t_{j-1})$$

The initial condition is $C_p(0)=C_e(0) = 0$.

One can also assume a log-linear piecewise PK model for C_p:

$$C_p(t) = C_{p_{j-1}} \exp[-\lambda_j(t - t_{j-1})] \qquad (t_{j-1} < t < t_j)$$

where:

$$\lambda_j = \frac{(\ln C_{p_{j-1}} - \ln C_{p_j})}{(t_j - t_{j-1})}$$

and:

$$C_{e_j} = C_{e_{j-1}} e^{-k_{e0}(t_j - t_{j-1})} + C_{P_{j-1}} \frac{k_{e0}}{k_{e0} - \lambda_j} [e^{-\lambda_j (t_j - t_{j-1})} - e^{-k_{e0}(t_j - t_{j-1})}]$$

λ_1 and C_{e_1} are estimated by a linear regression, assuming $C_e(0) = C_p(0) = 0$.

Phoenix provides three methods. The 'linear' method uses linear piecewise PK model; the 'log' method uses log-linear piecewise PK model; the default 'log/linear' method uses linear to Tmax and then log-linear after Tmax. The Effect field is not used for the calculation of C_e but when it is provided, the E vs C_p and E vs C_e plots will be plotted.

References

Kowalski ad Karim (1995). A semicompartmental modeling approach for pharmacodynamic data assessment, *J Pharmacokinet Biopharm* 23:307–22.

Sheiner, Stanski, Vozeh, Miller and Ham (1979). Simultaneous modeling of pharmacokinetics and pharmacodynamics: application to d-tubocurarine. *Clin Pharm Ther* 25:358–71.

Semicompartmental model example

This example uses the dataset in the file PK.CSV, which is located in the Phoenix examples directory. The data are from an early Phase I PK/PD trial. Quick input is sought for the design of a seven day multiple dose study. However, the profiles are irregular, and it is not easy to apply a compartmental modeling approach to the data. A semicompartmental approach will be attempted here. This example then continues with a NonParametric Superposition prediction of plasma concentrations and effect-site concentrations at steady-state, using the Semicompartmental results (see "NonParametric Superposition example".

Knowledge of how to do basic tasks using the Phoenix interface, such as creating a project and importing data, is assumed.

The completed project (`SCM.phxproj`) is available for reference in …`\Examples\WinNonlin`.

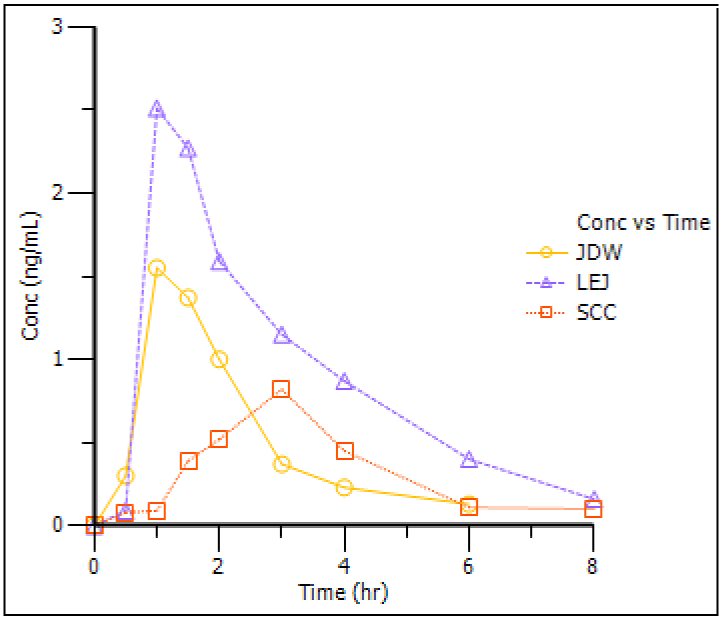
Explore the input data

1. Create a new project called SCM.
2. Import the file …`\Examples\WinNonlin\Supporting files\PK.CSV`.
   In the *File Import Wizard* dialog, select the **Has units row** option.

   The braces in the Effect column header indicate that the units are nonstandard and will be carried throughout the analysis but they cannot be used in unit conversions.

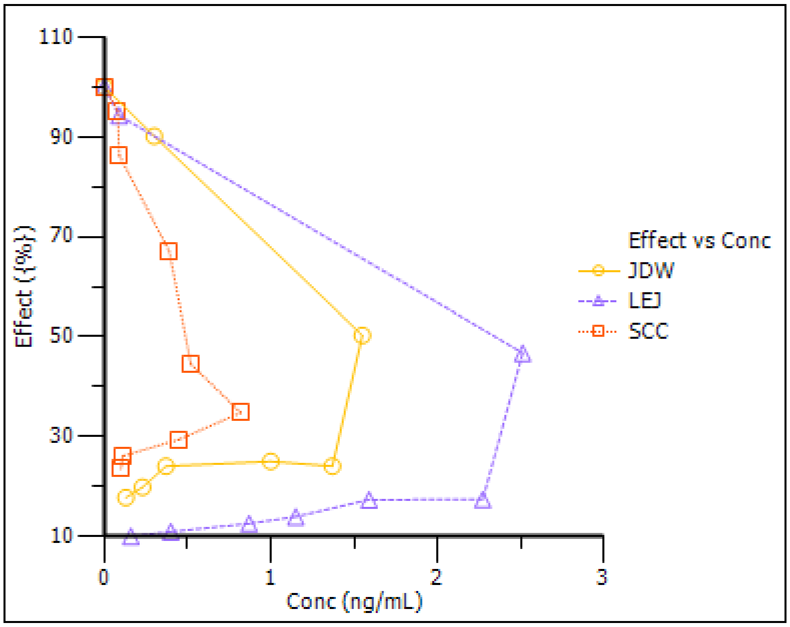
3. Right-click **PK** in the Data folder and select **Send To > Plotting > XY Plot**.
4. In the XY Data Mappings panel:
   Map **Subject** to the **Group** context.
   Map **Time** to the **X** context.
   Map **Conc** to the **Y** context.
   Leave **Effect** mapped to **None**.
5. Click ▶ (**Execute** icon) to execute the object.

   The plot indicates that compartmental modeling might be problematic. The data are highly variable.



6. Return to the Setup panel and re-map the input data:
   Leave **Subject** mapped to the **Group** context.
   Map **Time** to **None**.
   Map **Conc** to the **X** context.
   Map **Effect** to the **Y** context.
7. In the Options tab below the Setup panel, select **Graphs > Effect vs Conc** in the menu tree.
8. Clear the **Sort X Values** checkbox.

   Clearing the **Sort X Values** checkbox tells Phoenix to not sort the dataset by ascending concentration values before creating the XY plot.
9. Execute the object.



Notice the hysteresis in the plot. Semicompartmental modeling supports calculation of effect-site concentrations based on Ke0. In this example, pre-clinical studies indicated that the Ke0 is between 0.2 and 0.3 per hour in rats and dogs.

Set up the Semicompartmental object

This example estimates effect-site concentrations using semicompartmental modeling:

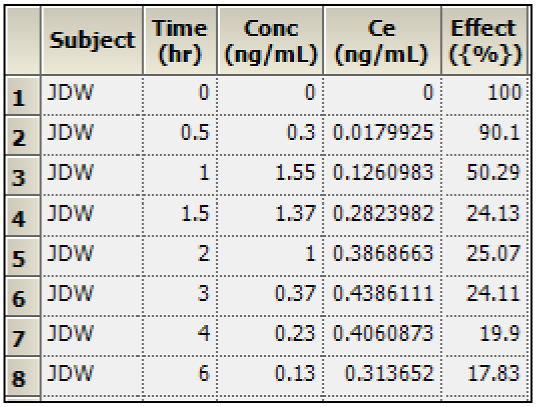
1. Right-click **PK** in the Data folder and select **Send to > NonCompartmental Analysis > Semi-compartmental Modeling**.
2. In the Main Mappings panel:
   Map **Subject** to the **Sort** context.
   Leave **Time** mapped to the **Time** context.
   Map **Conc** to the **Concentration** context.
   Leave **Effect** mapped to the **Effect** context.
3. In the Options tab below the Setup panel, type `0.25` in the **Ke0** field.

Execute and view the Semicompartmental results for the PK data

1. Execute the object.

The Semicompartmental Model provides both workbook and graph output.

The Results worksheet shows the calculated concentration of the drug in the effect compartment, Ce, at each Time in the input dataset, along with the input Conc and Effect data, for each subject.



	Subject	Time (hr)	Conc (ng/mL)	Ce (ng/mL)	Effect ({%})
1	JDW	0	0	0	100
2	JDW	0.5	0.3	0.0179925	90.1
3	JDW	1	1.55	0.1260983	50.29
4	JDW	1.5	1.37	0.2823982	24.13
5	JDW	2	1	0.3868663	25.07
6	JDW	3	0.37	0.4386111	24.11
7	JDW	4	0.23	0.4060873	19.9
8	JDW	6	0.13	0.313652	17.83

Figure 28-1. Part of the Semicompartmental Results worksheet

The Semicompartmental object generated four plots for each subject.

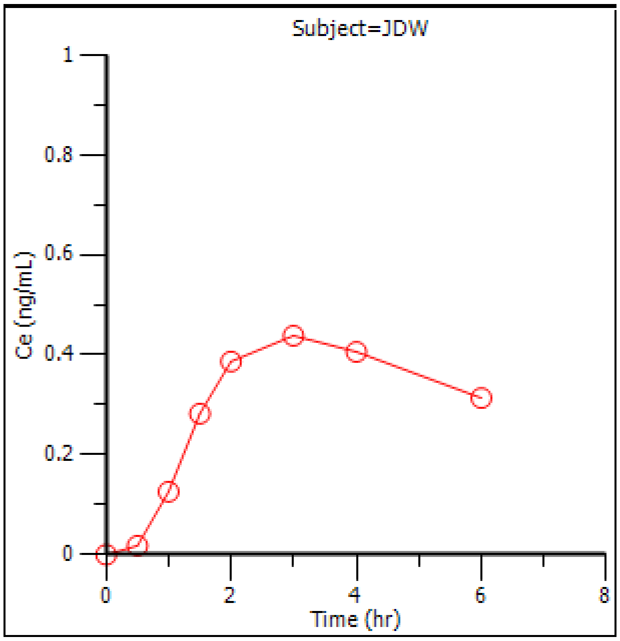


Figure 28-2. Effect-compartment concentration (Ce) over time (Ce vs Time)

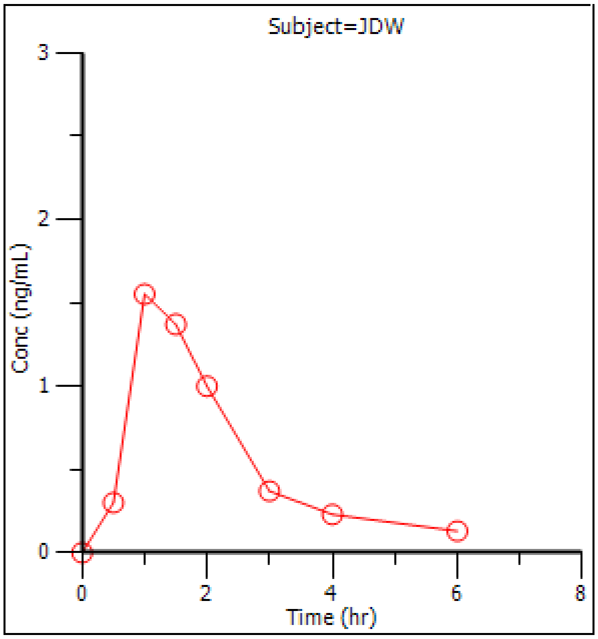


Figure 28-3. Concentration over time (Cp vs Time)

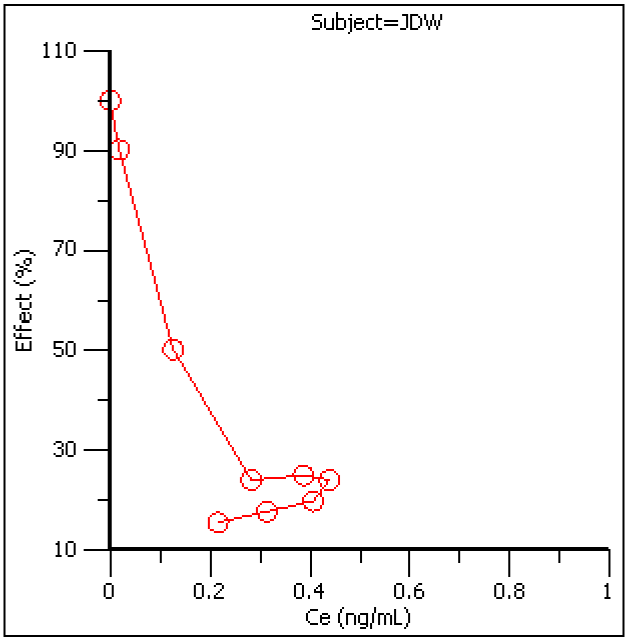


Figure 28-4. Effect as a function of Ce (Effect vs Ce)

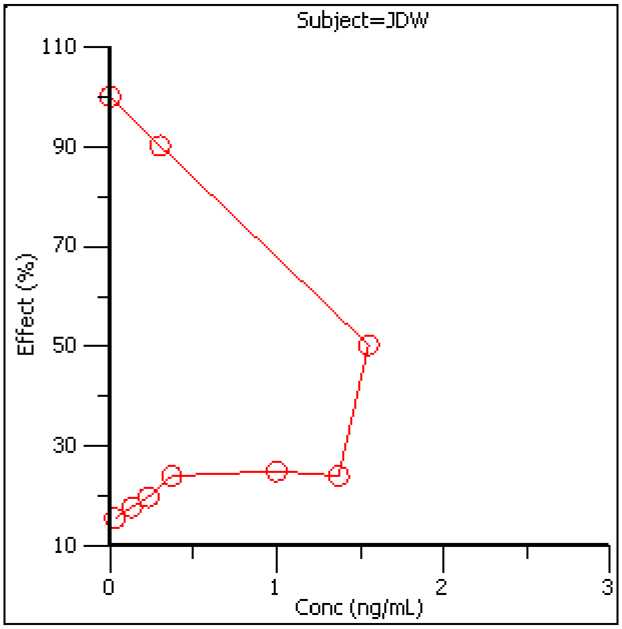


Figure 28-5. Effect over concentration (Effect vs Cp)

Based on the plots, PD model 103, an Inhibitory Effect E0 model, is appropriate to use to model the concentration in the effect compartment (Ce) versus effect relationship.

Model the pharmacodynamics

1. Right-click **Workflow** in the Object Browser and select **New > Modeling > Least Squares Regression Models > PD Model**.
2. Use the **Select Source** icon to map the Semicompartmental Results worksheet as the input source for the PD Model object.
3. In the Mappings panel:
   Map **Subject** to the **Sort** context.
   Leave **Time** mapped to **None**.
   Leave **Conc** mapped **None**.
   Map **Ce** to the **X** variable context.
   Map **Effect** to the **Y** variable context.
4. In the Model Selection tab below the Setup panel, check the model Number **103** checkbox.

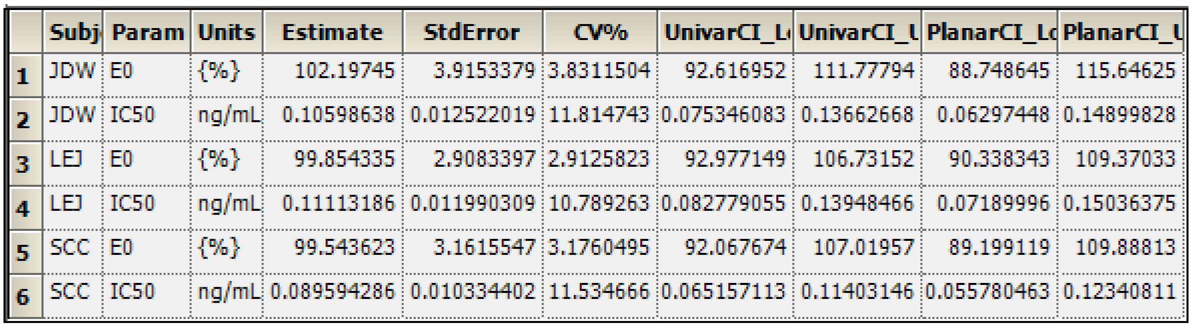
   The default model parameter options are used. Phoenix generates initial parameter values and parameter bounds. To view the parameter option settings, select the Parameter Options tab.

Execute and view the PD Model results for the Semicompartmental output

1. Execute the object.

   Phoenix analyzes each subject separately and includes all time points per subject.

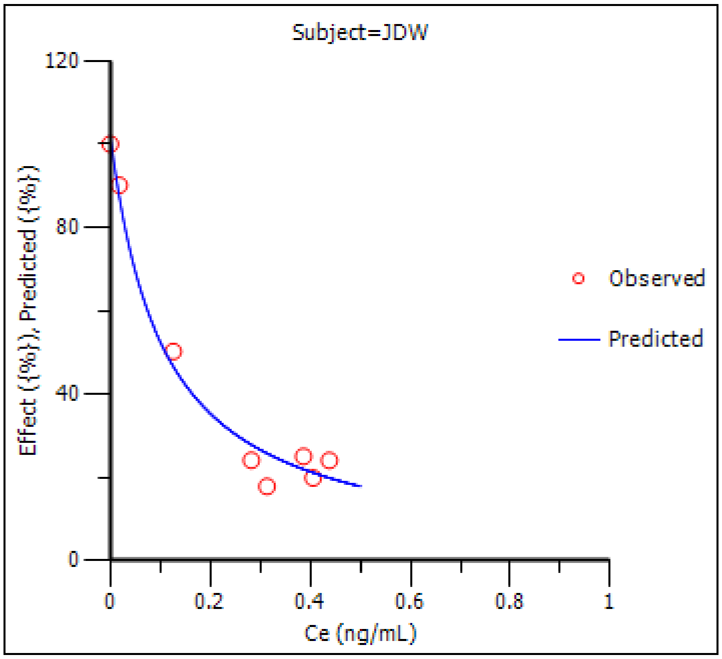
   The Final Parameters output provides estimates for E0 and IC50 for each subject. These are used later to predict steady-state effect values.



	Subj	Param	Units	Estimate	StdError	CV%	UnivarCI_L	UnivarCI_U	PlanarCI_L	PlanarCI_U
1	JDW	E0	{%}	102.19745	3.9153379	3.8311504	92.616952	111.77794	88.748645	115.64625
2	JDW	IC50	ng/mL	0.10598638	0.012522019	11.814743	0.075346083	0.13662668	0.06297448	0.14899828
3	LEJ	E0	{%}	99.854335	2.9083397	2.9125823	92.977149	106.73152	90.338343	109.37033
4	LEJ	IC50	ng/mL	0.11113186	0.011990309	10.789263	0.082779055	0.13948466	0.07189996	0.15036375
5	SCC	E0	{%}	99.543623	3.1615547	3.1760495	92.067674	107.01957	89.199119	109.88813
6	SCC	IC50	ng/mL	0.089594286	0.010334402	11.534666	0.065157113	0.11403146	0.055780463	0.12340811

2. Click **Observed Y and Predicted Y vs X** under Plots.

   The Observed Y and Predicted Y vs X plot illustrates the fit of PD model 103 to the effect data when Ce from Semicompartmental modeling is used as the measure of exposure. The Observed Y and Predicted Y vs X plot for the first subject is shown below.



   The other plots address model fit.

The NonParametric superposition example uses the output from this example. You may wish to save this project or leave the project open if you are continuing with the NonParametric superposition example.

Modeling

Least-Squares Regression Models interface
Least-Squares Regression Models Results
Nonlinear Regression Overview
Least-Squares Regression Model calculations

Least-Squares Regression models include the following. Many of the models can be run using the NLME engine (even if you do not have an NLME license). This is done by setting up a Maximum Likelihood Models object for individual modeling and using the **Set WNL Model** button to select the model. See "PK model options" in the Phoenix NLME documentation for more information. Refer to "An example of individual modeling with Maximum Likelihood Model object" for an illustration of how a dataset can be fitted to a two-compartment model with first-order absorption in the pharmacokinetic model library using either the Least-Squares Regression PK Model or a Maximum Likelihood Model object GUI.

Dissolution Models

Choose from Hill, Weibull, Double Weibull, or Makoid-Banakar dissolution models.

Indirect Pharmacodynamic Response Models

Four basic models have been developed for characterizing indirect pharmacodynamic responses after drug administration. These models are based on the effects (inhibition or stimulation) that drugs have on the factors controlling either the input or the dissipation of drug response. See "Indirect Response models" for more details.

Linear Models

Phoenix includes a selection of models that are linear in the parameters. See "Linear models" for more details on available models. Refer to "Linear Mixed Effects" for more sophisticated linear models.

Michaelis-Menten Models

Phoenix's Michaelis-Menten models are one-compartment models with intravenous or 1st order absorption, and can be used with or without a lag time to the start of absorption. For more on Phoenix's Michaelis-Menten models, see "Michaelis-Menten models". Information on required constants is available in "Dosing constants for the Michaelis-Menten model".

Pharmacodynamic Models

Phoenix includes a library of eight pharmacodynamic (PD) models. The PD models include simple and sigmoidal Emax models, and inhibitory effect models. For more on Phoenix's PD models, see "Pharmacodynamic models".

Pharmacokinetic Models

Phoenix includes a library of nineteen pharmacokinetic (PK) models. The PK models are one to three compartment models with intravenous or first-order absorption, and can be used with or without a lag time to the start of absorption. For more on Phoenix's PK models, see "Pharmacokinetic models". See also the "PK model examples".

PK/PD Linked Models

When pharmacological effects are seen immediately and are directly related to the drug concentration, a pharmacodynamic model is applied to characterize the relationship between drug concentrations and effect. When the pharmacologic response takes time to develop and the observed response is not directly related to plasma concentrations of the drug a ***linked model*** is usually applied to relate the pharmacokinetics of the drug to its pharmacodynamics.

The PK/PD linked models can use any combination of Phoenix's Pharmacokinetic models and Pharmacodynamic models. The PK model is used to predict concentrations, and these concentrations are then used as input to the PD model. This means that the PK data are not modeled, so the linked PK/PD models treat the pharmacokinetic parameters as fixed, and generate concentrations at the effect site to be used by the PD model. Model parameter information is required for the PK model in order to simulate the concentration data. Refer to "PD output parameters in a PK/PD model" for parameter details.

User-Defined ASCII Models

Phoenix does not support the creation of ASCII models. ASCII models have been deprecated in favor of the Phoenix Modeling Language (PML). However, users can still import and run legacy WinNonlin ASCII models. For more on PML, see "Phoenix Modeling Language". Refer to "ASCII Model dosing constants" for details on required constants.

Note: There can be a loss of accuracy in Least-Squares Regression Modeling univariate confidence intervals for small sample sizes (NDF < 5). The Univariate CIs in use an approximation for the t-value which is very accurate when the degrees of freedom is at least five, but loses accuracy as the degrees of freedom approaches one. The degrees of freedom are the number of observations minus the number of parameters being estimated (not counting parameters that are within the singularity tolerance, i.e., nearly completely correlated).

Note: In extremely rare instances, the nonlinear modeling core computational engine may get into an infinite loop during the minimization process. This infinite looping will cause Phoenix to "hang" and the application must be shutdown using the Task Manager. The process `wnlpk32.exe` may also need to be shutdown. The problem typically occurs when the parameter space in which the program is working within is very flat. To work around the problem, it is first suggested that the user change the minimization algorithm found on the Engine Settings tab to **Nelder-Mead** and retry the problem. If this fails to correct the problem, varying the initial estimates and/or using bounds on the parameters may allow processing to complete as expected.

Least-Squares Regression Models interface

Use one of the following to add the object to a Workflow:

Right-click menu for a Workflow object: **New > Least Squares Regression Models >** [object name].
Main menu: **Insert > Least Squares Regression Models >** [object name].
Right-click menu for a worksheet: **Send To > Least Squares Regression Models >** [object name].

Note: To view the object in its own window, select it in the Object Browser and double-click it or press **ENTER**. All instructions for setting up and execution are the same whether the object is viewed in its own window or in Phoenix view.

Main Mappings panel
Dosing panel
Initial Estimates panel
PK Parameters panel
Units panel
Stripping Dose panel

Constants panel
Format panel
Linked Model tab
Weighting/Dosing Options tab
Parameter Options tab
Engine Settings tab
Plots tab (See the "Plots tab" description in the NCA section.)

Main Mappings panel

Use the Main Mappings panel to identify how input variables are used in a model. A separate analysis is performed for each profile. Required input is highlighted orange in the interface.

None: Data types mapped to this context are not included in any analysis or output.

Sort: Categorical variable(s) identifying individual data profiles, such as subject ID and treatment in a crossover study. A separate analysis is done for each unique combination of sort variable values.

X: For Dissolution, Indirect Response, Linear, PD, and PK/PD Linked Models. The independent variable in a dataset.

Y: For Dissolution, Indirect Response, Linear, PD, and PK/PD Linked Models. The dependent variable a dataset. Not needed if the **Simulation** checkbox is selected.

Time: For Michaelis-Menten, PK, and ASCII Models. Nominal or actual time collection points in a study.

Concentration: For Michaelis-Menten, PK Models, and ASCII Models. Drug concentration values in the blood. Not needed if the Simulation checkbox is selected.

Function: For ASCII Models. Model function.

Carry: Data variable(s) to include in the output worksheets. Note that time-dependent data variables (those that change over the course of a profile) are not carried over to time-independent output (e.g., Final Parameters), only to time-dependent output (e.g., Summary).

Weight: For Indirect Response, PK, PD, and PK/PD Linked Models. Used if weighting data is contained in the dataset. This column is only available if **User Defined** and **Source** are selected in the Weighting/Dosing Options tab.

Note: When using a PK operational object, external worksheets for stripping dose, units and initial estimates can be accessed in different ways. The differences will occur if there is more than 1 row of information on these external worksheets that correspond to one or more individual profiles of data of the Main input worksheet. In such cases, the stripping dose for PK models will be determined as the first value found on that external worksheet whereas the units and initial parameters will be based on the last row found on those external worksheets (for any given profile). To avoid any confusion stemming from these differences, it is suggested that external worksheets maintain a one-to-one row-based correspondence to the Main input profiles whenever possible.

Dosing panel

Available for Indirect Response, PK, and PK/PD Linked Models only, the Dosing panel allows users to type or map dosing data for the different models. The Dosing panel mapping columns change depending on the PK model type selected in the Model Selection tab. Required input is highlighted orange in the interface.

If multiple sort variables have been mapped in the Main Mappings panel, the *Select sorts* dialog is displayed so that the user can select the sort variables to include in the internal worksheet. Required input is highlighted orange in the interface.

None: Data types mapped to this context are not included in any analysis or output.

Sort: Sort variables.

Time: The time of dose administration.

Dose: The amount of drug administered. Dosing units are used.

End Time: The end time of the infusion.

Infusion Length: The total amount of time for an IV infusion. Only used in conjunction with a bolus dose.

Bolus: Amount of the bolus dose.

Amount Infused: Total amount of drug infused. Dosing units are used.

Note: If the dosing data is entered using the internal dosing worksheet, and different profiles require different numbers of doses, then leave the **Time**, **End Time**, **Dose**, **Infusion Length**, **Bolus**, or **Amount Infused** cells blank for profiles that require less than the highest number of doses.

When using an internal worksheet, click the **Rebuild** button to reset the worksheet to its default state and delete all entered values.

Initial Estimates panel

The Initial Estimates panel allows users to type or map initial values and lower and upper boundaries for different indirect response parameters.

Note: Multiple-dose datasets ***require*** users to provide initial parameter values. For more on setting initial parameter estimates, see the "Parameter Estimates and Boundaries Rules" section.

If initial estimates and parameter boundaries for ASCII models are not set in the code, then they ***must*** be set in the Initial Estimates panel.

In datasets containing multiple sort variables, the initial estimates must be provided for each level of the sort variables unless the **Propagate Final Estimates** checkbox is selected. Checking this box applies the same types of parameter calculations and boundaries to all sort levels. Required input is highlighted orange in the interface.

If multiple sort variables have been mapped in the Main Mappings panel, the *Select sorts* dialog is displayed so that the user can select the sort variables to include in the internal worksheet.

None: Data types mapped to this context are not included in any analysis or output.

Sort: Sort variables.

Parameter: Model parameters such as v (volume) or km (Michaelis constant).

Initial: Initial parameter estimate values.

Fixed or Estimated: For Dissolution models. Whether the initial parameter is fixed or estimated.

Lower: Lower parameter boundary.

Upper: Upper parameter boundary.

The lower and upper values limit the range of values the parameters can take on during model fitting. This can be very valuable if the parameter values become unrealistic or the model will not converge. Although bounds are not always required, it is recommended that Lower and Upper bounds be used routinely. If they are used, every parameter must be given lower and upper bounds.

The Phoenix default bounds are zero for one bound and ten times the initial estimate for the other bound. For models with parameters that may be either positive or negative, user-defined bounds are preferred.

Note: For the **WinNonlin Generated Initial Parameter Values** option with Dissolution models, to avoid getting pop-up warnings that “WinNonlin will determine initial estimate” when using the Initial Estimates internal worksheet setup, delete the initial values and change the menu option from **Estimated** to **Fixed** before entering the initial estimates. Once the dropdown is changed from **Fixed** to **Estimated**, the initial value entered cannot be deleted and the warning pop-up will be displayed. Note that this situation does not affect the estimation process, as the entered initial value will not be used and WinNonlin will estimate the initial value as requested.

When using an internal worksheet, click the **Rebuild** button to reset the worksheet to its default state and delete all entered values.

PK Parameters panel

This panel is available for Indirect Response and PK/PD Linked models only.

Note: Users are ***required*** to enter initial PK parameter values in the PK Parameters panel in order for the model to run.

To display the units in the PK Parameters panel, units must be included in the time, concentration, and dose input data and the concentration units entered in the **PK Units** text field in the Model Selection tab.

If multiple sort variables have been mapped in the Main Mappings panel, the *Select sorts* dialog is displayed so that the user can select the sort variables to include in the internal worksheet. Required input is highlighted orange in the interface.

None: Data types mapped to this context are not included in any analysis or output.
Sort: Sort variables.
Parameter: Name of the parameter.
Value: Value of the parameter.

When using an internal worksheet, click the **Rebuild** button to reset the worksheet to its default state and delete all entered values.

Units panel

For all Least-Squares Regression Models except Michaelis-Menten, an object’s display units can be changed to fit a user’s preferences. Required input is highlighted orange in the interface.

Depending on the type of model, there are some prerequisites for setting preferred units:

- For Indirect Response and PK Models, the **Time**, **Concentration**, and **Dose** data must all contain units before users can set preferred units.

- For PD and PK/PD Linked Models, the data mapped to the **X** and **Y** contexts must all contain units before users can set preferred units.
- For ASCII Models, units must be set in the ASCII model code.

Each parameter used in a model and the parameter's default units are listed in the Units panel.

None: Data types mapped to this context are not included in any analysis or output.
Name: Model parameters associated with the units.
Default: The model object's default units.
Preferred: The user's preferred units for the parameter.

When using an internal worksheet, click the **Rebuild** button to reset the worksheet to its default state and delete all entered values.

Note: if you see an "Insufficient units" message in the table, check that units are defined for time and concentration in your input.

Stripping Dose panel

The Stripping Dose panel is available when one of the macro constant PK models that use a stripping dose is specified in the Model Selection tab (i.e., models 8, 13, 14, 17, or 18). This panel is used to enter the stripping dose amount, which is the dose associated with initial parameter values for macro constant models.

If a user selects a macro constant PK model and provides user-specified initial estimates, then the user must specify the associated dose. If a user chooses to have Phoenix generate initial parameter values, then the stripping dose is identical to the administered dose.

None: Data types mapped to this context are not included in any analysis or output.
Sort: Sort variables.
Dose: The stripping dose.

If multiple sort values have been mapped in the Main Mapping panel, the *Select sorts* dialog is displayed so that the user can select the sort variables to include in the internal worksheet.

Constants panel

The Constants panel is available only for Michaelis-Menten and ASCII Models and allows users to type or map dosing data for the model. Specific dosing information is determined using dosing constants. For more on how constants relate to dosing in Michaelis-Menten models, see "Dosing constants for the Michaelis-Menten model". For more on how constants relate to dosing in ASCII models, see "ASCII Model dosing constants".

When using an external worksheet for Constants, the Number of Constants will be determined from the number of rows per profile after mapping the following:

None: Data types mapped to this context are not included in any analysis or output.
Sort: Sort variables.
Order: The number of dosing constants used.
Value: The value for each dosing constant. For example, the value for CON[0] is 1 if the model is single-dose.

For an internal worksheet, set the **Number of Constants** on the Options panel, or specify `NCON` in the model text, to expand the internal worksheet for entering the Constants.

Format panel

The Format panel is only available for ASCII Models and is used to map ASCII code to an ASCII model. Users can view and edit ASCII model code in this panel.

- Check the **Use internal Text Object** checkbox to edit the ASCII code.

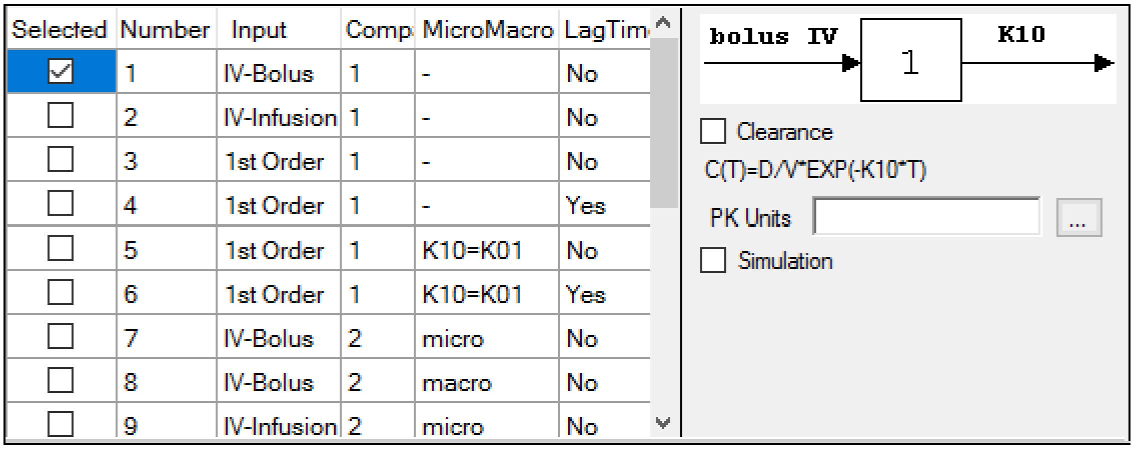
  Phoenix displays a message box asking users if they want to copy the ASCII code to an internal source:
  - Click **Yes** to have the ASCII code copied to the internal text editor.
  - Click **No** to remove the ASCII code and start with a blank internal text editor.

If ASCII code was previously mapped to the User ASCII Model panel then that code is displayed in the internal text editor, instead of a blank panel.

More information on the text editor is available on the Syncfusion website.

Model Selection tab

The Model Selection tab for most of the Least-Squares Regression Models allows users to select a model and whether or not the model uses simulated data/clearance parameter. (For the User ASCII Model, the Model Selection tab contains weighting options as described in the “Weighting/Dosing Options tab” description.)



Selected	Number	Input	Comp	MicroMacro	LagTim
☑	1	IV-Bolus	1	-	No
□	2	IV-Infusion	1	-	No
□	3	1st Order	1	-	No
□	4	1st Order	1	-	Yes
□	5	1st Order	1	K10=K01	No
□	6	1st Order	1	K10=K01	Yes
□	7	IV-Bolus	2	micro	No
□	8	IV-Bolus	2	macro	No
□	9	IV-Infusion	2	micro	No

□ Clearance

C(T)=D/V*EXP(-K10*T)

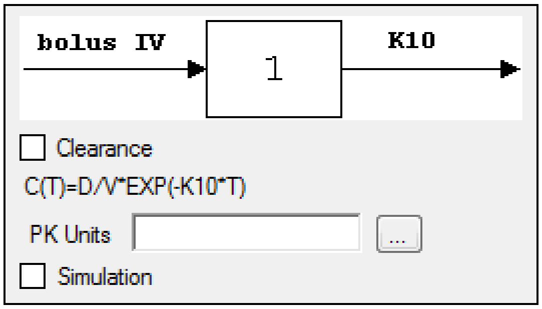
PK Units

□ Simulation

- Check the checkbox beside the model number to choose it. For Dissolution models, click an option button to select a model.

  Refer to any of the following sections for model details:
  Linear models
  Michaelis-Menten models
  Pharmacodynamic models
  Pharmacokinetic models

  Selecting a model displays a diagram beside the model selection menu that describes the model’s functions. The model’s equation is listed beneath the diagram.



□ Clearance

C(T)=D/V*EXP(-K10*T)

PK Units [ ] ...

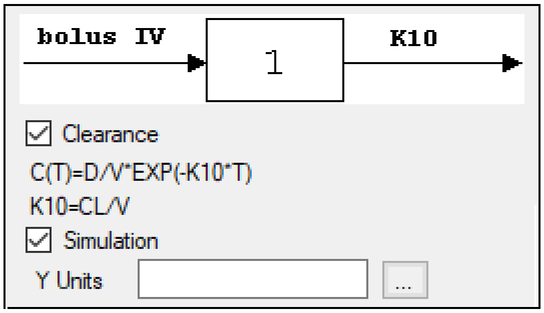
□ Simulation

- Set the options for the selected model.
  - For Indirect Response, PK, and PK/PD Linked models, select the **Clearance** checkbox to add a clearance parameter to the model. (The clearance parameter option is not available for PK models 8, 10, 13, 14, 17, 18, or 19.)
  - For Michaelis-Menten Models, use the **Number of Constants** box to type or select the number of dosing constants used per profile. (For more on how the number of constants corresponds to the number, amount, and time of doses, see "Dosing constants for the Michaelis-Menten model".)
  - For PK/PD Linked Models, enter concentration units in the **PK Units** text field or click the **Units Builder […]** button to use the *Units Builder* dialog.

Note: For PK/PD Linked Models, to view all PK parameter units in the PK Parameters panel, users must supply concentration units in the **PK Units** text field in the Model Selection tab and dose units in the Weighting/Dosing Options tab.

- Check the **Simulation** checkbox to simulate concentration data. Enter units for the simulated data in the **Y Units** text field or click the **Units Builder […]** button to use the *Units Builder* dialog.

  If the **Simulation** checkbox is selected then no concentration variable is required to run the model.



☑ Clearance

C(T)=D/V*EXP(-K10*T)

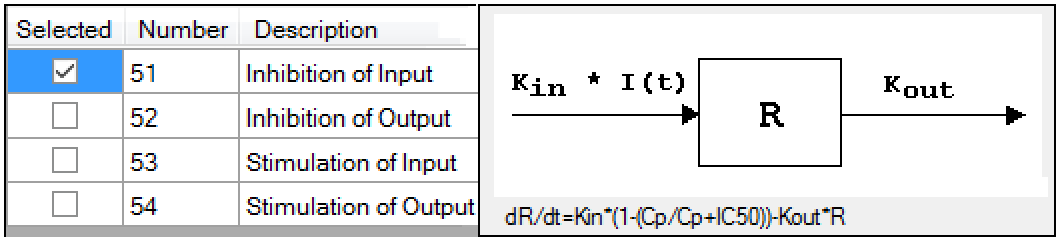
K10=CL/V

☑ Simulation

Y Units [ ] ...

Linked Model tab

The Linked Model tab allows users to select the model to link with model specified in the Model Selection tab. It is available only for the Indirect Response and PK/PD Linked Models.



Selected	Number	Description
☑	51	Inhibition of Input
□	52	Inhibition of Output
□	53	Stimulation of Input
□	54	Stimulation of Output

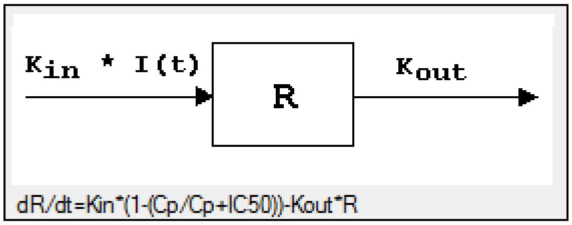
dR/dt=Kin*(1-(Cp/Cp+IC50))-Kout*R

- Check the checkbox beside the model number to choose it.

For more on these models, see “Indirect Response models”. For PK/PD Linked Models, see “Pharmacodynamic models”.

For a list of PD output parameters used in linked PK/PD models, see “PD output parameters in a PK/PD model”.

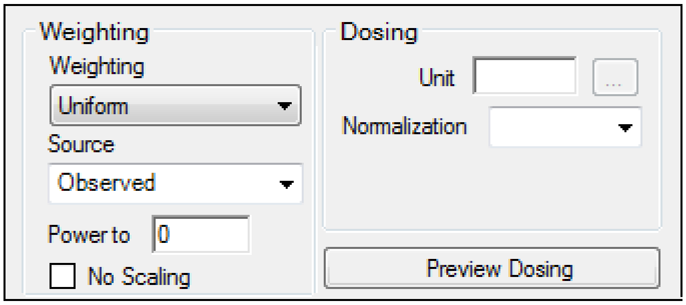
Selecting a model displays a diagram beside the model selection menu that describes the model’s functions. The model’s equation is listed beneath the diagram.



dR/dt=Kin*(1-(Cp/Cp+IC50))-Kout*R

Weighting/Dosing Options tab

The Weighting Options tab (in Dissolution, Linear, M-M, and PD Model objects) and the Weighting/Dosing Options tab (in Indirect Response, PK, and PK/PD Linked Model objects) allows users to select a weighting scheme and specify and preview dosing options.



Weighting: Uniform

Source: Observed

Power to: 0

□ No Scaling

Unit:

Normalization:

Weighting options

- Use the **Weighting** menu to select one of six weighting schemes:

  User Defined: Weights are read from a column in the dataset.
  Uniform: Users can enter custom observed to or predicted to the power of N values. If **s**elected, then users must select **Observed** or **Predicted** in the **Source** menu and type the power value in the **Power to** text field.
  1/Y: Weight the data by 1/observed Y.
  1/Yhat: Weight the data by 1/predicted Y (iterative reweighting).
  1/(Y*Y): Weight the data by 1/observed Y^2.
  1/(Yhat*Yhat): Weight the data by 1/predicted Y^2 (iterative reweighting).

- Use the **Source** menu to select one of three weighting sources:

  Source: Selecting this option sets the weighting to **User Defined** and adds a Weight column to the Main Mappings panel. If selected, users must map the weighting column in the dataset to the **Weight** context in the Main Mappings panel.

  Observed: Select to use weighted least squares. This is the default selection for **1/Y** and **1/(Y*Y)**. The default power for **1/Y** is –1 and for **1/(Y*Y)** it is –2.

  Predicted: Select to use iterative reweighting. This is the default selection for **1/Yhat** and **1/(Yhat*Yhat)**. The default power for **1/Yhat** is –1 and for **1/(Yhat*Yhat)** it is –2.

- Type a power value in the **Power to** text field. (This option is disabled if **Source** is set to **Source**.)

  Entering `-1` automatically sets the weighting to **1/Y** (if **Observed** is the source) or **1/Yhat** (if **Predicted** is the source).

  Entering `-2` automatically sets the weighting to **1/(Y*Y)** (if **Observed** is the source) or **1/(Yhat*Yhat)** (if **Predicted** is the source).

- Check the **No Scaling** checkbox to not scale the weighting for observed weighting values.

  When weights are contained in a dataset or **Observed** is selected as the source, the weights for individual data values are scaled so that the sum of weights for each function is equal to the number of data values. Weights must not be 0 (zero) in order to scale.

  Scaling has no effect on the model fitting, because the weights of the observations are proportionally the same. However, scaling weights provides increased numerical stability.

Dosing options

Available for Indirect Response, PK, and PK/PD Linked models.

- Enter the dosing unit in the **Unit** text field or click the **[…]** button to use the *Units Builder* dialog to set the dosing unit.

Note: For Indirect Response Models, to view all PK parameter units in the PK Parameters panel, users must supply units for the time and concentration data in the input and specifying dose units in the Weighting/Dosing Options tab.

For PK/PD Linked Models, to view all PK parameter units in the PK Parameters panel, users must supply concentration units in the **PK Units** text field in the Model Selection tab and dose units in the Weighting/Dosing Options tab.

- Use the **Normalization** menu to select the appropriate factor, if the dose amount is normalized by subject body weight or body mass index. Options include: **None**, **kg**, **g**, **mg**, **m**2**, **1.73 m**2**.

  Dose normalization usage:

  - If doses are in milligrams per kilogram of body weight, select **mg** as the dosing unit and **kg** as the dose normalization.
  - The **Normalization** menu affects the output parameter units. For example, if dose volume is in liters, selecting **kg** as the dose normalization changes the units to `L/kg`.
  - Dose normalization affects units for all volume and clearance parameters in PK models.

- Type the number of doses per profile into the **# Doses** text field. (Only enabled if an internal worksheet is used for Dosing.)

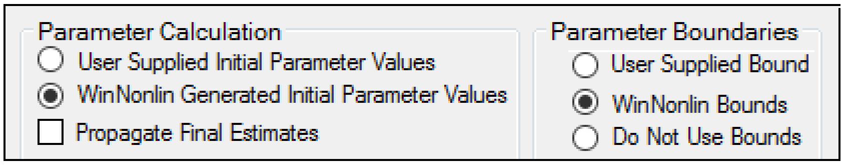
If different profiles require different numbers of doses, enter the highest number of doses.

- Click **Preview Dosing** to view a preview of dose option selections.

  If an external dosing data worksheet is used to provide dosing data, then the preview window will show the number of doses and dosing time points per profile. Click **OK** to close the preview window.

Parameter Options tab

All iterative estimation procedures require initial estimates of the parameter values. Phoenix computes initial estimates via curve stripping for single-dose models. For all other situations, including multiple-dose models, users must provide initial estimates or boundaries to be used by Phoenix in creating initial estimates. Parameter boundaries provide a basis for grid searching initial parameter estimates, and also limit the estimates during modeling. This is useful if the values become unrealistic or the model does not converge. For more on setting initial parameter estimates, refer to “Parameter Estimates and Boundaries Rules”.



Parameter Calculation

○ User Supplied Initial Parameter Values

● WinNonlin Generated Initial Parameter Values

□ Propagate Final Estimates

Parameter Boundaries

○ User Supplied Bound

● WinNonlin Bounds

○ Do Not Use Bounds

Set the parameter calculation method:

Note: The default minimization method, **Gauss-Newton (Hartley)** (located in the Engine Settings tab), and the Parameter Boundaries option **Do Not Use Bounds** are recommended for all Linear models.

- Select the **User Supplied Initial Parameter Values** option button to enter initial parameter estimates in the Initial Estimates panel.
  Or
  Select the **WinNonlin Generated Initial Parameter Values** option button to have Phoenix determine the initial parameter values.
  - If the **User Supplied Bounds** option button is selected, Phoenix uses curve stripping to provide initial estimates. If curve stripping fails, then Phoenix uses the grid search method.
  - If the **WinNonlin Bounds** option button is selected, Phoenix uses curve stripping to provide initial estimates, and then applies boundaries to the model parameters for model fitting. If curve stripping fails, the model fails because Phoenix cannot use grid search for initial estimates without user-supplied boundaries.
- Check the **Propagate Final Estimates** checkbox to propagate initial parameter estimates across all sort levels.
  This option is available when more than one sort variable is used in the main dataset.

  If this option is selected, then initial estimates and boundaries are entered or mapped only for the first sort level. The final parameter estimates from the first sort level provide the initial estimates for each consecutive sort level.

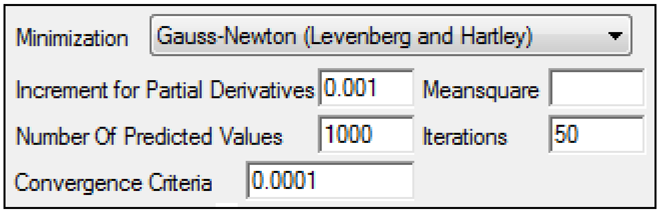
Set the boundary calculation method:

Parameter boundaries provide a basis for grid searching initial parameter estimates, and also limit the estimates during modeling. This is useful if the values become unrealistic or the model does not converge. For more on using parameter boundaries, refer to "Parameter Estimates and Boundaries Rules".

- Select the **User Supplied Initial Bounds** option to enter parameter boundaries in the Initial Estimates panel.
  Or select the **WinNonlin Bounds** option to have Phoenix determine the parameter boundaries.
  Or select the **Do No Use Bounds** option to not use parameter boundaries.

Engine Settings tab

The Engine Settings tab provides control over the model fitting algorithm and related settings.



Minimization: Gauss-Newton (Levenberg and Hartley)

Increment for Partial Derivatives: 0.001

Meansquare:

Number Of Predicted Values: 1000

Iterations: 50

Convergence Criteria: 0.0001

- Use the **Minimization** menu to select the method to use with the Indirect Response model.

  Method 1: The **Nelder-Mead** algorithm does not require the estimated variance-covariance matrix as part of its algorithm, so it often performs very well on ill-conditioned datasets. ***Ill-conditioned*** indicates that, for the given dataset and model, there is not enough information contained in the data to precisely estimate all of the parameters in the model or that the sum of squares surface is highly curved. Although the procedure works well, it can be slow, particularly when used to fit a system of differential equations.

  Method 2: **Gauss-Newton (Levenberg and Hartley)** is the default algorithm and performs well on a wide class of problems, including ill-conditioned datasets. Although the Gauss-Newton method does require the estimated variance-covariance matrix of the parameters, the Levenberg modification suppresses the magnitude of the change in parameter values from iteration to iteration to a reasonable amount. This enables the method to perform well on ill-conditioned datasets.

  Method 3: **Gauss-Newton (Hartley)** is another Gauss-Newton method. It is not as robust as Methods 1 and 2 but is extremely fast. Method 3 is recommended when speed is a consideration or when maximum likelihood estimation or linear regression analysis is to be performed. It is not recommended for fitting complex models.

Note: The use of bounds is recommended with Methods 2 and 3. For linear regressions, use Method 3 without bounds.

- In the **Increment for Partial Derivatives** text field, type the incremental value that the parameter value is to be multiplied by.

  Nonlinear algorithms require derivatives of the models with respect to the parameters. The program estimates these derivatives using a difference equation. For example (Δ = the increment with which the parameter value is multiplied)

$$\frac{\partial F}{\partial P(1)} = \frac{F(P(1)+\Delta)-F(P(1))}{\Delta}$$

- In the **Number of Predicted Values** text field, type the number of predicted values used to determine the number of points in the predicted data.

Use this option to create a dataset that will plot a smooth curve. When fitting or simulating multiple dose data, the predicted data plots may be much improved by increasing the number of predicted values. The minimum allowable value is 10; the maximum is 20,000.

If the number of derivatives is greater than zero (i.e., differential equations are used), this command is ignored and the number of predicted points is equal to the number of points in the dataset.

For compiled models without differential equation, the default value is 1000. The default for user models is the number of data points in the original dataset. This value may be increased only if the model has no more than one independent variable.

Note: To better reflect peaks (for IV dosing) and troughs (for extravascular, IV infusion and IV bolus dosing), the predicted data for the built-in PK models includes dosing times, in addition to the concentrations generated. For all three types, concentrations are generated at dosing times; in addition, for infusion models, data are generated for the end of infusion.

- In the **Convergence Criteria** text field, type the criterion value used to determine convergence.

  The default is 0.0001. Convergence is achieved when the relative change in the residual sum of squares is less than the convergence criterion.
- Leave the **Meansquare** text field blank, unless the mean square needs to be a fixed value in order to compute the variance in a model.

  The variance is normally a function of the weighted residual SS/df, or the Mean Square. For certain types of problems, when computing the variance, the mean square needs to be set to a fixed value.
- Leave the **Iterations** text field set to its default value, unless the purpose of the model is to evaluate it, and not fit it.

  The default iteration values are:
  500 for Nelder-Mead minimization. Each iteration is a reflection of the simplex.
  50 for Gauss-Newton (Levenberg and Hartley) or Gauss-Newton (Hartley) minimizations.
- Type a value of `0` (zero) in the **Iterations** text field if the purpose of the model is evaluation.

  A value of `0` requires users to supply their own initial parameter values and all output will use the initial estimates as the final parameters.

Least-Squares Regression Models Results

Note: This section is meant to provide guidance and references to aid in the interpretation of modeling output, and is not a replacement for a PK or statistics textbook.

After a Classic model is run, the output is displayed on the Results tab in Phoenix. The output is discussed in the following sections.

Core Output: text version of all model settings and output, including any errors that occurred during modeling. See “Core Output File” for a full description.

Settings: test version of all user-defined settings.

Worksheet output: worksheets listing input data, modeling iterations and output parameters, as well as several measures of fit.

Plot output: plots of observed and predicted data, residuals, and other quantities, depending on the model run.

Worksheet output

Worksheet output contains summary tables of the modeling data and a summary of the information in the Core Output. The worksheets generated depend on the analysis type and model settings. They present the output in a form that can be used for reporting and further analyses and are listed on the Results tab underneath Output Data.

Condition Numbers: Rank and condition number of the matrix of partial derivatives for each iteration.
The matrix is of full rank, since Rank is equal to the number of parameters. If the Rank were less than three, that would indicate that there was not enough information in the data to estimate all three parameters. The condition value is the square root of the ratio of the largest to the smallest eigenvalue and values should be less than 10^n where n is the number of parameters.

Correlation Matrix: A correlation matrix for the parameters, for each sort level. If any values get close to 1 or –1, there may be too many parameters in the model and a simpler model may work better.

Diagnostics: Diagnostics for each function in the model and for the total:

CSS: corrected sum of squared observations
WCSS: weighted corrected sum of squared observations
SSR: sum of squared residuals
WSSR: weighted sum of squared residuals
S: estimate of residual standard deviation
DF: degrees of freedom
CORR_(OBS,PRED): correlation between observed Y and predicted Y
WT_CORR_(OBS,PRED): weighted correlation
AIC: Akaike Information Criterion goodness of fit measurement
SBC: Schwarz Bayesian Criterion goodness of fit measurement[a]

Differential Equations: The value of the partial derivatives for each parameter at each time point for each value of the sort variables.

Dosing Used: The dosing regimen specified for the modeling.

Eigenvalues: Eigenvalues for each level of the sort variables. (An eigenvalue of matrix A is a number λ, such that $Ax=\lambda x$ for some vector x, where x is the eigenvector. Eigenvalues and their associated eigenvectors can be thought of as building blocks for matrices.)

Final Parameters and **Final Parameters Pivoted**: Parameter names, units, estimates, standard error of the estimates, CV% (values < 20% are generally considered to be very good), univariate intervals, and planar intervals for each level of the sort variables.

Fitted Values: (Dissolution models) Predicted data for each profile.

Initial Estimates: Parameter names, initial values, and lower and upper bounds for each level of the sort variables.

Minimization Process: Iteration number, weighted sum of squares, and value for each parameter, for each level of the sort variables. This worksheet shows how parameter values converged as the iterations were performed. If the number of iterations is approaching the specified limit, there may be some problems with the model.

Parameters: (Dissolution models) The smoothing parameter *delta* and absorption lag time for each profile.

Partial Derivatives and **Stacked Partial Derivatives**: Values of the differential equations at each time in the dataset.

Predicted Data: Time and predicted Y for multiple time points, for each sort level.

Secondary Parameters and **Secondary Parameters Pivoted**: Available for Michaelis-Menten, PK, PD, PK/PD Linked and ASCII models. Secondary parameter name, units, estimate, standard error of the estimate, and CV% for each sort level.

Summary Table[b]: The sort variables, X, Y, transformed X, transformed Y, predicted Y, residual, weight, standard error of predicted Y, standardized residuals, for each sort level. For link models, also includes C_P and C_e. For indirect response models, also includes C_P.

Values: (Dissolution models) Time, input rate, cumulative amount (Cumul_Amt, using the dose units) and fraction input (Cumul_Amt/test dose or, if no test doses are given, then fraction input approaches one) for each profile.

Variance Covariance Matrix: A variance-covariance matrix for the parameters, for each sort level.

User Settings: Model number, minimization method, convergence criterion, maximum number of iterations allowed, and the weighting scheme.

[a] AIC and SBC are only meaningful during comparison of models. A smaller value is better, negative is better than positive, and a more negative value is even better. AIC is computed as: AIC=N log ($WRSS$)+2P, where N is the number of observations with positive weight, log is the natural logarithm, $WRSS$ is the weighted residual sum of squares, P is the number of parameters.

[b] If there are no statements to transform the data, then X and Y will equal X(obs) and Y(obs).

Plot output

Analysis produces up to eight graphs that are divided by each level of the sort variable. Plot output is listed underneath Plots in the Results tab.

Cumulative Rates: (Dissolution models) Cumulative drug input vs. time.

Fitted Curves: (Dissolution models) Observed time-concentration data vs. the predicted curve.

Input Rates: (Dissolution models) Rate of drug input vs. time.

Observed Y and Predicted Y vs X: plots the predicted curves as a function of X, with the Observed Y overlaid on the plot. Used for assessing the model fit.

Partial Derivatives Plot: plots the partial derivative of the model with respect to each parameter as a function of the x variable. If f(x; a, b, c) is the model as a function of x, based on parameters a, b, and c, then $df(x; a, b, c)/da$, $df(x; a, b, c)/db$, $df(x; a, b, c)/dc$ are plotted versus x. Each derivative is evaluated at the final parameter estimates. Data taken at larger partial derivatives are more influential than those taken at smaller partial derivatives for the parameter of interest.

Predicted Y vs Observed Y: plots weighted Y against observed Y. Scatter should lie close to the 45 degree line.

Residual Y vs Predicted Y: used to assess whether error distribution is appropriately modeled throughout the range of the data.

Residual Y vs X: used to assess whether error distribution is appropriately modeled across the range of the X variable.

- **Weighted Predicted Y vs Observed Y**: plots weighted predicted Y against observed Y. Scatter should lie close to the 45 degree line; only produced if a non-Uniform weighting scheme is used.
- **Weighted Residual Y vs Observed Y**: used to assess whether error distribution is appropriately modeled across the range of the observed Y variable; only produced if a non-Uniform weighting scheme is used.
- **Weighted Residual Y vs Predicted Y**: used to assess whether error distribution is appropriately modeled across the range of the predicted variable; only produced if a non-Uniform weighting scheme is used.
- **Weighted Residual Y vs Weighted Predicted Y**: used to assess whether error distribution is appropriately modeled throughout the range of the data; only produced if a non-Uniform weighting scheme is used.
- **Weighted Residual Y vs X**: used to assess whether error distribution is appropriately modeled across the range of the X variable; only produced if a non-Uniform weighting scheme is used.

Users can double-click any plot in the Results tab to edit it. (See the "Plot Options tab" description for editing options.)

Nonlinear Regression Overview

Modeling and nonlinear regression
Model fitting algorithms and features of Phoenix WinNonlin

Modeling and nonlinear regression

The value of mathematical models is well recognized in all of the sciences — physical, biological, behavioral, and others. Models are used in the quantitative analysis of all types of observations or data, and with the power and availability of computers, mathematical models provide convenient and powerful ways of looking at data. Models can be used to help interpret data, to test hypotheses, and to predict future results. The main concern here is with “fitting” models to data — that is, finding a mathematical equation and a set of parameter values such that values predicted by the model are in some sense “close” to the observed values.

Most scientists have been exposed to linear models, models in which the dependent variable can be expressed as the sum of products of the independent variables and parameters. The simplest example is the equation of a line (simple linear regression):

$$Y = A + BX + \varepsilon$$

where Y is the dependent variable, X is the independent variable, A is the intercept and B is the slope. Two other common examples are polynomials such as:

$$Y = A_1 + A_2X + A_3X^2 + A_4X^3 + \varepsilon$$

and multiple linear regression.

$$Y = A_1 + A_2X_1 + A_3X_2 + A_4X_3 + \varepsilon$$

These examples are all “linear models” since the parameters appear only as coefficients of the independent variables.

In nonlinear models, at least one of the parameters appears as other than a coefficient. A simple example is the decay curve:

$$Y = Y_0 \exp(-BX)$$

This model can be linearized by taking the logarithm of both sides, but as written it is nonlinear.

Another example is the Michaelis-Menten equation:

$$V = \frac{V_{max}C}{K_m + C}$$

This example can also be linearized by writing it in terms of the inverses of V and C, but better estimates are obtained if the nonlinear form is used to model the observations (Endrenyi, ed. (1981), pages 304–305).

There are many models which cannot be made linear by transformation. One such model is the sum of two or more exponentials, such as:

$$Y = A_1 \exp(-B_1X) + A_2 \exp(-B_2X)$$

All of the models on this page are called nonlinear models, or nonlinear regression models. Two good references for the topic of general nonlinear modeling are Draper and Smith (1981) and Beck and Arnold (1977). The books by Bard (1974), Ratkowsky (1983) and Bates and Watts (1988) give a more detailed discussion of the theory of nonlinear regression modeling. Modeling in the context of kinetic analysis and pharmacokinetics is discussed in Endrenyi, ed. (1981) and Gabrielsson and Weiner (2016).

All pharmacokinetic models derive from a set of basic differential equations. When the basic differential equations can be integrated to algebraic equations, it is most efficient to fit the data to the integrated equations. But it is also possible to fit data to the differential equations by numerical integration. Phoenix WinNonlin can be used to fit models defined in terms of algebraic equations and models defined in terms of differential equations as well as a combination of the two types of equations.

Given a model of the data, and a set of data, how is the model "fit" to the data? Some criterion of "best fit" is needed, and many have been suggested, but only two are much used. These are maximum likelihood and least squares. With certain assumptions about the error structure of the data (no random effects), the two are equivalent. A discussion of using nonlinear least squares to obtain maximum likelihood estimates can be found in Jennrich and Moore (1975). In least squares fitting, the "best" estimates are those which minimize the sum of the squared deviations between the observed values and the values predicted by the model.

The sum of squared deviations can be written in terms of the observations and the model. In the case of linear models, it is easy to compute the least squares estimates. One equates to zero the partial derivatives of the sum of squares with respect to the parameters. This gives a set of linear equations with the estimates as unknowns; well-known techniques can be used to solve these linear equations for the parameter estimates.

This method will not work for nonlinear models, because the system of equations which results by setting the partial derivatives to zero is a system of nonlinear equations for which there are no general methods for solving. Consequently, any method for computing the least squares estimates in a nonlinear model must be an iterative procedure. That is, initial estimates of the parameters are made and then, in some way, these initial estimates are modified to give better estimates, i.e., estimates which result in a smaller sum of squared deviations. The iteration continues until hopefully the minimum (or least) sum of squares is reached.

In the case of models with random effects, such as population PK/PD models, the more general method of maximum likelihood (ML) must be employed. In ML, the likelihood of the data is maximized with respect to the model parameters. The LinMix operational object fits models of this variety.

Since it is not possible to know exactly what the minimum is, some stopping rule must be given at which point it is assumed that the method has converged to the minimum sum of squares. A more complete discussion of the theory underlying nonlinear regression can be found in the books cited previously.

Model fitting algorithms and features of Phoenix WinNonlin

Phoenix WinNonlin estimates the parameters in a nonlinear model, using the information contained in a set of observations. Phoenix WinNonlin can also be used to simulate a model. That is, given a set of parameter values and a set of values of the independent variables in the model, Phoenix WinNonlin will compute values of the dependent variable and also estimate the variance-inflation factors for the estimated parameters.

A computer program such as Phoenix WinNonlin is only an aid or tool for the modeling process. As such it can only take the data and model supplied by the researcher and find a "best fit" of the model to the data. The program cannot determine the correctness of the model nor the value of any decisions or interpretations based on the model. The program does, however, provide some information about the “goodness of fit” of the model to the data and about how well the parameters are estimated.

It is assumed that the user of Phoenix WinNonlin has some knowledge of nonlinear regression such as contained in the references mentioned earlier. Phoenix WinNonlin provides the “least squares” estimates of the model parameters as discussed above. The program offers three algorithms for minimizing the sum of squared residuals: the simplex algorithm of Nelder and Mead (1965), the Gauss-Newton algorithm with the modification proposed by Hartley (1961), and a Levenberg-type modification of the Gauss-Newton algorithm (Davies and Whitting (1972)).

The simplex algorithm is a very powerful minimization routine; its usefulness has formerly been limited by the extensive amount of computation it requires. The power and speed of current computers make it a more attractive choice, especially for those problems where some of the parameters may not be well-defined by the data and the sum of squares surface is complicated in the region of the minimum. The simplex method does not require the solution of a set of equations in its search and does not use any knowledge of the curvature of the sum of squares surface. When the Nelder-Mead algorithm converges, Phoenix WinNonlin restarts the algorithm using the current estimates of the parameters as a “new” set of initial estimates and ting the step sizes to their original values. The parameter estimates which are obtained after the algorithm converges a second time are treated as the “final” estimates. This modification helps the algorithm locate the global minimum (as opposed to a local minimum) of the residual sum of squares for certain difficult estimation problems.

The Gauss-Newton algorithm uses a linear approximation to the model. As such it must solve a set of linear equations at each iteration. Much of the difficulty with this algorithm arises from singular or near-singular equations at some points in the parameter space.Phoenix WinNonlin avoids this difficulty by using singular value decomposition rather than matrix inversion to solve the system of linear equations. (See Kennedy and Gentle (1980).) One result of using this method is that the iterations do not get “hung up” at a singular point in the parameter space, but rather move on to points where the problem may be better defined. To speed convergence, Phoenix WinNonlin uses the modification to the Gauss-Newton method proposed by Hartley (1961) and others; with the additional requirement that at every iteration the sum of squares must decrease.

Many nonlinear estimation programs have found Hartley’s modification to be very useful. Beck and Arnold (1977) compare various least squares algorithms and conclude that the Box-Kanemasu method (almost identical to Hartley’s) is the best under many circumstances.

As indicated, the singular value decomposition algorithm will always find a solution to the system of linear equations. However, if the data contain very little information about one or more of the parameters, the adjusted parameter vector may be so far from the least squares solution that the linearization of the model is no longer valid. Then the minimization algorithm may fail due to any number of numerical problems. One way to avoid this is by using a ‘trust region’ solution; that is, a solution to the system of linear equations is not accepted unless it is sufficiently close to the parameter values at the current iteration. The Levenberg and Marquardt algorithms are examples of ‘trust region’ methods.

Note: The Gauss-Newton method with the Levenberg modification is the default estimation method used by Phoenix WinNonlin.

Trust region methods tend to be very robust against ill-conditioned data sets. There are two reasons, however, why one may not want to use them. (1) They require more computation, and thus are not efficient with data sets and models that readily permit precise estimation of the parameters. (2) More importantly, the trust region methods obtain parameter estimates that are often meaningless because of their large variances. Although Phoenix WinNonlin gives indications of this, it is possible for users to ignore this information and use the estimates as though they were really valid. For more information on trust region methods, see Gill, Murray and Wright (1981) or Davies and Whitting (1972).

In Phoenix WinNonlin, the partial derivatives required by the Gauss-Newton algorithm are approximated by difference equations. There is little, if any, evidence that any nonlinear estimation problem is better or more easily solved by the use of the exact partial derivatives.

To fit those models that are defined by systems of differential equations, the RKF45 numerical integration algorithm is used (Shampine, Watts and Davenport (1976)). This algorithm is a 5th order Runge-Kutta method with variable step sizes. It is often desirable to set limits on the admissible parameter space; this results in "constrained optimization." For example, the model may contain a parameter which must be non-negative, but the data set may contain so much error that the actual least squares estimate is negative. In such a case, it may be preferable to give up some of the properties of the unconstrained estimation in order to obtain parameter estimates that are physically realistic. At other times, setting reasonable limits on the parameter may prevent the algorithm from wandering off and getting lost. For this reason, it is recommend that users always set limits on the parameters (the means of doing this is discussed in "Parameter Estimates and Boundaries Rules" and "Modeling"). In Phoenix WinNonlin, two different methods are used for bounding the parameter space. When the simplex method is used, points outside the bounds are assigned a very large value for the residual sum of squares. This sends the algorithm back into the admissible parameter space.

With the Gauss-Newton algorithms, two successive transformations are used to affect the bounding of the parameter space. The first transformation is from the bounded space as defined by the input limits to the unit hypercube. The second transformation uses the inverse of the normal probability function to go to an infinite parameter space. This method of bounding the parameter space has worked extremely well with a large variety of models and data sets.

Bounding the parameter space in this way is, in effect, a transformation of the parameter space. It is well known that a reparameterization of the parameters will often make a problem more tractable. (See Draper and Smith (1981) or Ratkowsky (1983) for discussions of reparameterization.) When encountering a difficult estimation problem, the user may want to try different bounds to see if the estimation is improved. It must be pointed out that it may be possible to make the problem worse with this kind of transformation of the parameter space. However, experience suggests that this will rarely occur. Reparameterization to make the models more linear also may help.

Because of the flexibility and generality of Phoenix WinNonlin, and the complexity of nonlinear estimation, a great many options and specifications may be supplied to the program. In an attempt to make Phoenix WinNonlin easy to use, many of the most commonly used options have been internally specified as defaults.

Phoenix WinNonlin is capable of estimating the parameters in a very large class of nonlinear models. Fitting pharmacokinetic and pharmacodynamic models is a special case of nonlinear estimation, but it is important enough that Phoenix WinNonlin is supplied with a library of the most commonly used pharmacokinetic and pharmacodynamic models. To speed execution time, the main Phoenix WinNonlin library has been built-in, or compiled. However, ASCII library files corresponding to the same models as the built-in models, plus an additional utility library of models, are also provided.

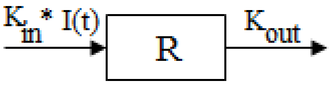
Least-Squares Regression Model calculations

Indirect Response models
Linear models
Michaelis-Menten models
Pharmacodynamic models
Pharmacokinetic models
PD output parameters in a PK/PD model
ASCII Model dosing constants
Parameter Estimates and Boundaries Rules
References

Indirect Response models

When the pharmacologic response takes time to develop and the observed response is not directly related to plasma concentrations of the drug a link model can be applied to relate the pharmacokinetics of the drug to its pharmacodynamics. Phoenix contains a group of indirect pharmacodynamic response (IPR) models proposed by Jusko (1990) and Dayneka, Garg, and Jusko (1993). The indirect response models differ from other models in Phoenix in that they use a PK model to predict concentrations, and then use these concentrations as input to the indirect PD response model.

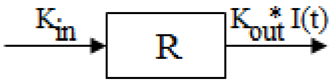
Model 51: Inhibition of input.



$$\frac{dR}{dt} = k_{in}\left(1 - \frac{C_p}{C_p + IC_{50}}\right) - k_{out}R$$

Estimated parameters: Kin Kout IC_{50}

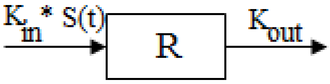
Model 52: Inhibition of output.



$$\frac{dR}{dt} = k_{in} - k_{out}\left(1 - \frac{C_p}{C_p + IC_{50}}\right)R$$

Estimated parameters: Kin Kout IC_{50}

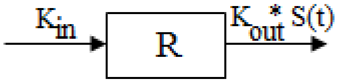
Model 53: Stimulation of input.



$$\frac{dR}{dt} = k_{in}\left(1 + \frac{Emax \cdot C_p}{C_p + EC_{50}}\right) - k_{out}R$$

Estimated parameters: Kin Kout EC_{50} Emax

Model 54: Stimulation of output.



$$\frac{dR}{dt} = k_{in} - k_{out}\left(1 + \frac{Emax \cdot C_p}{C_p + EC_{50}}\right)R$$

Estimated parameters: Kin Kout EC_{50} Emax

Model notation

R: Measured response to a drug.
k_{in}: The zero-order constant for the production of response.
k_{out}: The first-order rate constant for loss of response.
Cp: Plasma concentration of a drug.
Ce: Drug concentration at the effect site.
IC_{50}: Drug concentration required to produce 50% of the maximal inhibition.
Emax: Maximum drug effect.
EC_{50}: Concentration in plasma that achieves 50% of predicted maximum effect in an Emax model.

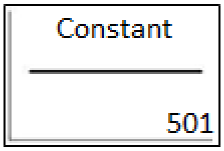
Linear models

Phoenix contains four Linear models used to perform linear regression. In the following table the y-unit is the preferred unit of the y (concentration) variable and the t-unit is the preferred unit of the t (time) variable. See “Units panel” to set preferred units for the output parameters.

The linear models do not require dosing information.

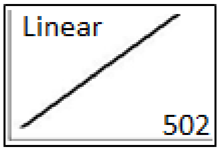
Note: The default minimization method, **Gauss-Newton (Hartley)** (located in the Engine Settings tab), and the Parameter Boundaries option **Do Not Use Bounds** are recommended for all linear models.

Linear models include:



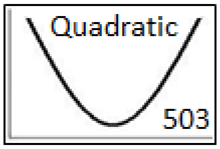
$y(t)=CONSTANT$

Estimated Parameters: $CONSTANT$ (y-unit)



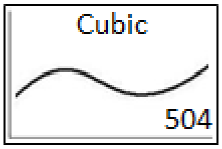
$y(t)=INT+SLOPE*t$

Estimated Parameters: INT (y-unit), $SLOPE$ (y-unit/t-unit)



$y(t)=A0+A1*t+A2*t^2$

Estimated Parameters: $A0$ (y-unit), $A1$ (y-unit/t-unit), $A2$ (y-unit/t-unit^2)



$y(t)=A0+A1*t+A2*t^2+A3*t^3$

Estimated Parameters: $A0$ (y-unit), $A1$ (y-unit/t-unit), $A2$ (y-unit/t-unit^2), $A3$ (y-unit/t-unit^3)

Michaelis-Menten models

The Michaelis-Menten models describe the relationship between the rate of substrate conversion by an enzyme to the concentration of the substrate. These kinetics are valid only when the concentration of substrate is higher than the concentration of enzyme, and in the case of steady-state, where the concentration of the complex enzyme-substrate is constant.

The Phoenix library contains four Michaelis-Menten models that use constants to supply the dosing information. The required number of constants are listed below under each model number. For an explanation of dosing constants see "Dosing constants for the Michaelis-Menten model".

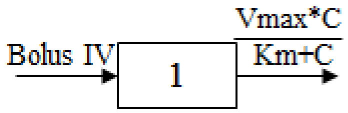
Phoenix assumes that the time of the first dose is zero. For these models, times in the dataset must correspond to the dosing times, even if these times contain no observations. In this case, include a column for Weight in the dataset, and weight the observations as zero.

These models parameterize $Vmax$ in terms of concentration per unit of time.

For a discussion of the difficulties in fitting the Michaelis-Menten models see Tong and Metzler (1980) and Metzler and Tong (1981).

Model 301

One-compartment with bolus input and Michaelis-Menten output.



$C(t)$ is the solution to the differential equation:

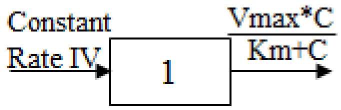
$$\frac{dC}{dt} = -\frac{Vmax(C)}{Km + C}$$

with initial condition $C(0)=D/V$.

Required constants: Number of doses (N), Dose amount for dose N, Time of dose for dose N
Estimated parameters: V is the volume, Vmax is the max elimination rate, Km is the Michaelis constant
Secondary parameters: AUC=(D/V)(D/V/2+Km)/ Vmax

Model 302

One-compartment with constant IV input and Michaelis-Menten output.



$C(t)$ is the solution to the differential equations:

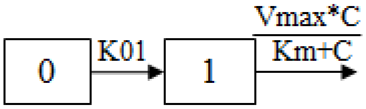
$$\frac{dC}{dt} = \frac{D}{tinf(V)} - \frac{Vmax(C)}{Km + C} \quad \text{for } t \le Tinf$$
$$\frac{dC}{dt} = -\frac{Vmax(C)}{Km + C} \quad \text{for } t > Tinf$$

with initial condition $C(0)=0$.

Required constants: Number of doses (N), Dose amount for dose N, Start time of dose for dose N, End time for dose N
Estimated parameters: V is the volume, Vmax is the max elimination rate, Km is the Michaelis constant
Secondary parameters: None

Model 303

One-compartment with first-order input, Michaelis-Menten output and no lag time.



$C(t)$ is the solution to the differential equation:

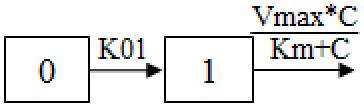
$$\frac{dC}{dt} = K01\frac{D}{V}\exp(-K01(t)) - \frac{Vmax(C)}{Km+C}$$

with initial condition $C(0)=0$.

Required constants: Number of doses (N), Dose amount for dose N, Time of dose for dose N
Estimated parameters: V_F, K01 is the absorption rate, Vmax is the max elimination rate, Km is the Michaelis constant
Secondary parameters: K01 half-life

Model 304

One-compartment with first-order input, Michaelis-Menten output and lag time.



$C(t)$ is the solution to the differential equation:

$$\frac{dC}{dt} = K01\frac{D}{V}\exp(-K01(t)) - \frac{Vmax(C)}{Km+C} \quad \text{for } t \geq Tlag$$
$$\frac{dC}{dt} = -\frac{Vmax(C)}{Km+C} \quad \text{for } t < tlag$$

with initial condition $C(0)=0$.

Required constants: Number of doses (N), Dose amount for dose N, Time of dose for dose N
Estimated parameters: V_F, K01 is the absorption rate, Vmax is the max elimination rate, Km is the Michaelis constant, Tlag is the lag time
Secondary parameters: K01 half-life

Dosing constants for the Michaelis-Menten model

The number of constants in the model corresponds to the dosing route for the model.

Bolus and first-order input models require at least three dosing constants per profile:

CON[0]: Number of doses (N)
CON[1]: Dose amount for dose N
CON[2]: Time of dose for dose N
CON[3]: Dose amount for dose N+1 (if multiple doses are used)
CON[4]: Time of dose for dose N+1 (if multiple doses are used)

Constant IV infusion models require at least four dosing constants per profile:

CON[0]: Number of doses (N)
CON[1]: Dose amount for dose N
CON[2]: Start time for dose N
CON[3]: End time for dose N
CON[4]: Dose amount for dose N+1 (if multiple doses are used)
CON[5]: Start time for dose N+1 (if multiple doses are used)
CON[6]: End time for dose N+1 (if multiple doses are used)

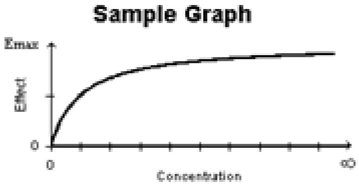
Pharmacodynamic models

When pharmacological effects are seen immediately and are directly related to the drug concentration, a pharmacodynamic model is applied to characterize the relationship between drug concentrations and effect. Phoenix includes the following PD models:

Notation definitions

Emax: Maximum drug effect.
Imax: Maximum drug inhibition.
E_0: Baseline effect (effect at C=0).
EC_{50}: Concentration in plasma that achieves 50% of predicted maximum effect in an Emax model.
IC_{50}: Drug concentration required to produce 50% of the maximal inhibition.
Rmax: Effect at infinity (includes maximum drug effect/inhibition and baseline).
F_Emax: Fractional change in effect from baseline E_0
Gamma: Shape parameter

Model 101: Simple Emax model.

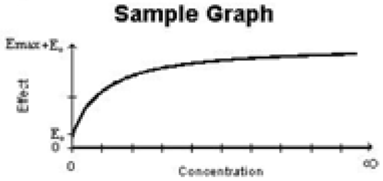


$$E = \frac{Emax(C)}{C + EC_{50}}$$

Estimated parameters: Emax, EC_{50}

Effect = 0 at C=0 and Emax at C=infinity

Model 102: Simple Emax model with a baseline effect parameter.



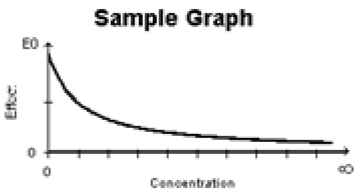
$$E = E_0 + \frac{Emax(C)}{C + EC_{50}}$$

Estimated parameters: Emax, EC_{50}, E_0

Secondary parameters: Rmax=Emax+E_0, F_Emax=Emax/E_0

Effect = E0 at C=0 and Emax + E0 at C=infinity

Model 103: Inhibitory effect model.

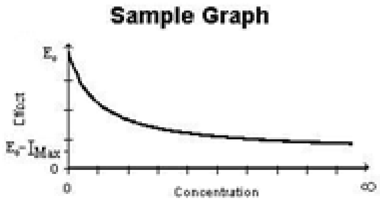


$$E = E_0\left(1 - \frac{C}{C + IC_{50}}\right)$$

Estimated parameters: E_0, IC_{50}

Effect = 0 at C=0 and Emax at C=infinity

Model 104: Inhibitory effect model with a baseline effect parameter.



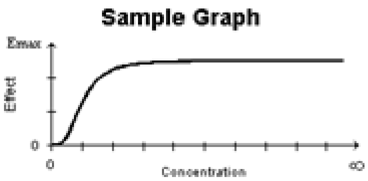
$$E = E_0 - \frac{Imax(C)}{C + IC_{50}}$$

Estimated parameters: Imax, $IC_{50,}$ E_0

Secondary parameters: Rmax=E_0 – Imax, F_Imax=Imax/E_0

Effect = E0 at C=0 and E0 – Imax at C=infinity

Model 105: Sigmoid Emax model.

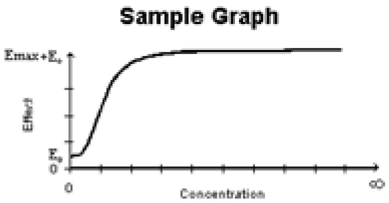


$$E = \frac{Emax(C^{\gamma})}{C^{\gamma} + EC^{\gamma}{}_{50}}$$

Estimated parameters: Emax, EC_{50}, Gamma

Effect = 0 at C=0 and Emax at C=infinity

Model 106: Sigmoid Emax model with a baseline effect parameter.



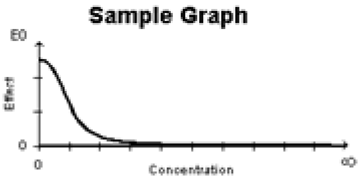
$$E = E_0 + \frac{Emax(C^{\gamma})}{C^{\gamma} + EC^{\gamma}{}_{50}}$$

Estimated parameters: Emax, EC_{50}, E_0, Gamma

Secondary parameters: Rmax=Emax+E_0, F_Emax=Emax/E_0

Effect = E0 at C=0 and Emax + E0 at C=infinity

Model 107: Sigmoid inhibitory effect model.

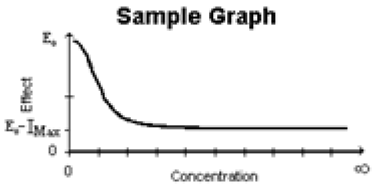


$$E = E_0\left(1 - \frac{C^{\gamma}}{C^{\gamma} + IC^{\gamma}{}_{50}}\right)$$

Estimated parameters: E_0, IC_{50}, Gamma

Effect = E0 at C=0 and 0 at C=infinity

Model 108: Sigmoid inhibitory effect model with a baseline effect parameter.



$$E = E_0 - \frac{Imax(C^{\gamma})}{C^{\gamma} + IC^{\gamma}{}_{50}}$$

Estimated parameters: Imax, IC_{50}, E_0, Gamma

Secondary parameters: Rmax=E_0 – Imax, F_Imax=Imax/E_0

Effect = E0 at C=0 and E0 – Imax at C=infinity

Pharmacokinetic models

Phoenix includes nineteen pharmacokinetic (PK) models. Each is available in the PK Model object.

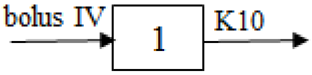
Model 1: IV-bolus input, 1-compartment, no lag, 1st order elimination
Model 2: IV-infusion input, 1-compartment, no lag, 1st order elimination
Model 3 1st order input, 1-compartment, no lag, 1st order elimination
Model 4 1st order input, 1-compartment, yes lag, 1st order elimination
Model 5 1st order input, 1-compartment, K10=K01 parameterization, no lag, 1st order elimination
Model 6 1st order input, 1-compartment, K10=K01 parameterization, yes lag, 1st order elimination
Model 7 IV-bolus input, 2-compartment, micro parameterization, no lag, 1st order elimination
Model 8 IV-bolus input, 2-compartment, macro parameterization, no lag, 1st order elimination
Model 9 IV-infusion input, 2-compartment, micro parameterization, no lag, 1st order elimination
Model 10 IV-infusion input, 2-compartment, macro parameterization, no lag, 1st order elimination
Model 11 1st order input, 2-compartment, micro parameterization, no lag, 1st order elimination
Model 12 1st order input, 2-compartment, micro parameterization, yes lag, 1st order elimination
Model 13 1st order input, 2-compartment, macro parameterization, no lag, 1st order elimination
Model 14 1st order input, 2-compartment, macro parameterization, yes lag, 1st order elimination
Model 15 IV-bolus + IV-infusion input, 1-compartment, micro parameterization, no lag, 1st order elimination
Model 16 IV-bolus + IV-infusion input, 2-compartment, micro parameterization, no lag, 1st order elimination
Model 17 IV-bolus + IV-infusion input, 2-compartment, macro parameterization, no lag, 1st order elimination
Model 18 IV-bolus input, 3-compartment, macro parameterization, no lag, 1st order elimination
Model 19 IV-infusion input, 3-compartment, macro parameterization, no lag, 1st order elimination

Note: All models except models 15–17 accept multiple dose data.

The models shown in this section give equations for compartment 1 (central).

Model 1

One-compartment with IV-bolus input and first-order output.



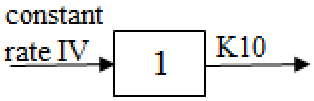
$$C(t) = \frac{D}{V}$$

$$C(t) = \frac{D}{V}\exp(-K10(t))$$

Required constants: N doses, dose N, time of dose N (Repeat for each dose)
Estimated parameters: V (volume), K10 (elimination rate)
Secondary parameters: AUC=D/V/K10, K10 half-life, Cmax=D/V, AUMC, MRT, Vss
Clearance estimated: V, CL
Clearance secondary: AUC, K10 half-life, Cmax, AUMC, MRT, VSS

Model 2

One-compartment with constant IV input, first-order absorption.



$$C(t) = \frac{D}{(Tinf)VK10}[\exp(-K10t^*) - \exp(-K10t)]$$

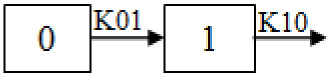
where:

$Tinf$ = infusion length
$t^* = t - Tinf$ for $t > Tinf$
$t^* = 0$ for $t \leq Tinf$

Required constants: N doses, dose N, start time N, end time N (Repeat for each dose)
Estimated parameters: V (volume), K10 (elimination rate)
Secondary parameters: AUC=D/V/K10, K10 half-life, Cmax=C(Tinf), CL, AUMC, MRT, Vss
Clearance estimated: V, CL
Clearance secondary: AUC, K10 half-life, Cmax, K10, AUMC, MRT, VSS

Model 3

One-compartment with first-order input and output, no lag time.



$$C(t) = \frac{DK01}{V(K01 - K10)}[\exp(-K10t) - \exp(-K01t)]$$

Required constants: N doses, dose N, time of dose N (Repeat for each dose)
Estimated parameters: V_F, K01 (absorption rate), K10 (elimination rate)
Secondary parameters: AUC=D/V/K10, K01 half-life, K10 half-life, CL_F, Tmax=time of Cmax = [ln(K01/K10)] / [(K01 – K10)]
Clearance estimated: V_F, K01, CL_F
Clearance secondary: AUC, K01 half-life, K10 half-life, K10, Tmax, Cmax

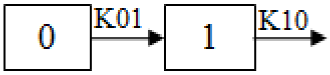
Model 4

One-compartment with first-order input and output with lag time.

Identical to Model 3, with an additional estimated parameter: $Tlag$=lag time. In the Model 3 equation, substitute $(t - Tlag)$ for t.

Model 5

One-compartment, equal first-order input and output, no lag time.



$$C(t) = \frac{D}{V}K(t)\exp[-Kt]$$

Required constants: N doses, dose N, time of dose N (Repeat for each dose)
Estimated parameters: V_F, K (absorption and elimination rate)
Secondary parameters: AUC=D/V/K, K half-life, CL_F, Tmax=time of Cmax=1/K
Clearance estimated: V_F, CL_F
Clearance secondary: AUC, K half-life, K, Tmax, Cmax

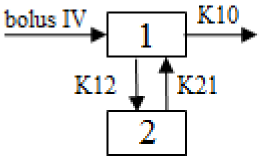
Model 6

One-compartment, equal first-order input and output with lag time.

Identical to Model 5, with an additional estimated parameter: $Tlag$=lag time. In the Model 5 equation, substitute $(t - Tlag)$ for t.

Model 7

Two-compartment with bolus input and first-order output; micro-constants as primary parameters.



$$C(t) = A\exp[-\alpha t] + B\exp[-\beta t]$$

where:

$$A = \frac{D}{V}\left(\frac{\alpha - K21}{\alpha - \beta}\right)$$

$$B = -\frac{D}{V}\left(\frac{\beta - K21}{\alpha - \beta}\right)$$

$-\alpha$ and $-\beta$ ($\alpha > \beta$) are the roots of the quadratic equation:
$r^2 + (K12 + K21 + K10)r + K21(K10) = 0$

Required constants: N doses, dose N, time of dose N, (Repeat for each dose)
Estimated parameters: V1 is Volume1, K10 (elimination rate), K12 (transfer rate, 1 to 2), K21 (transfer rate, 2 to 1)
Secondary parameters: AUC=A/Alpha+B/Beta, K01 half-life, Alpha, Beta, Alpha half-life, Beta half-life, A, B, Cmax=D/V, CL, AUMC, MRT, Vss, V2, CLD2
Clearance estimated: V1, CL, V2, CLD2

Clearance secondary: AUC, K10 half-life, Alpha, Beta, Alpha half-life, Beta half-life, A, B, Cmax, K10, AUMC, MRT, Vss, K12, K21

Model 8

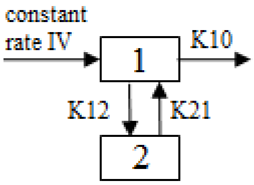
Two-compartment with bolus input and first-order output; macro-constants as primary parameters.

As Model 7 with macroconstants as the primary (estimated) parameters. Clearance parameters are not available for Model 8.

Required constants: Stripping dose, N doses, dose N, time of dose N (Repeat for each dose)
Estimated parameters: A, B, Alpha, Beta
Secondary parameters: AUC=A/Alpha+B/Beta, K10 half-life, Alpha half-life, Beta half-life, K10, K12, K21, Cmax, V1, CL, AUMC, MRT, Vss, V2, CLD2

Model 9

Two-compartment with constant IV input and first-order output; micro-constants as primary parameters.



$$C(t) = \frac{A}{Tinf(\alpha)}[\exp(-\alpha t^*) - \exp(-\alpha t)] + \frac{B}{Tinf(\beta)}[\exp(-\beta t^*) - \exp(-\beta t)]$$

where:

$Tinf$ = infusion length
$t^* = t - Tinf$ for $t > Tinf$
$t^* = 0$ for $t \le Tinf$

$$A = \frac{D}{V1}\left(\frac{\alpha - K21}{\alpha - \beta}\right)$$

$$B = -\frac{D}{V1}\left(\frac{\beta - K21}{\alpha - \beta}\right)$$

$-\alpha$ and $-\beta$ ($\alpha > \beta$) are the roots of the quadratic equation:
$r^2 + (K12 + K21 + K10)r + K21(K10) = 0$

Required constants: N doses, dose N, start time N, end time N,
Estimated parameters: V1 is Volume1, K10 (elimination rate), K12 (transfer rate, 1 to 2), K21 (transfer rate, 2 to 1) (Repeat for each dose)
Secondary parameters: AUC=D/V/K10, K10 half-life, Alpha, Beta, Alpha half-life, Beta half-life, A, B, Cmax=C(T_{inf}), AUMC, MRT, Vss, V2, CLD2
Clearance estimated: V1, CL, V2, CLD2,

Clearance secondary: AUC, K10 half-life, Alpha, Beta, Alpha half-life, Beta half-life, A, B, Cmax, AUMC, MRT, Vss, K12, K21

Model 10

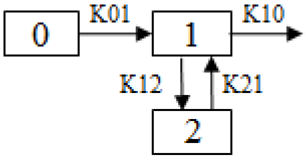
Two-compartment with constant IV input and first-order output; macro-constants as primary parameters.

As Model 9 with macroconstants as the primary (estimated) parameters. Clearance parameters are not available in Model 10.

Required constants: N doses, dose N, start time N, end time N (Repeat for each dose)
Estimated parameters: V1, K21, Alpha, Beta
Secondary parameters: K10, K12, K10 half-life, AUC, Alpha half-life, Beta half-life, A, B, Cmax, CL, AUMC, MRT, Vss, V2, CLD2

Model 11

Two-compartment with first-order input, first-order output, no lag time and micro-constants as primary parameters.



$$C(t) = A\exp[-\alpha t] + B\exp[-\beta t] + C\exp[-K01t]$$

where:

$$A = \frac{D}{V}\frac{K01(K21-\alpha)}{(\alpha-\beta)(\alpha-K01)}$$

$$B = -\frac{D}{V}\left(\frac{K01(K21-\beta)}{(\alpha-\beta)(\beta-K01)}\right)$$

$$C = \frac{D}{V}\left(\frac{K01(K21-K01)}{(\beta-K01)(\alpha-K01)}\right)$$

$-\alpha$ and $-\beta$ ($\alpha > \beta$) are the roots of the quadratic equation:
$r^2 + (K12 + K21 + K10)r + K21(K10) = 0$

Required constants: N doses, dose N, time of dose N (Repeat for each dose)
Estimated parameters: V1_F, K01 (absorption rate), K10 (elimination rate), K12 (transfer rate, 1 to 2). K21 (transfer rate, 2 to 1)
Secondary parameters: AUC=D/V/K10, K01 half-life, K10 half-life, Alpha, Beta, Alpha half-life, Beta half-life, A, B, CL_F, V2_F, CLD2_F, Tmax[a], Cmax[a]
Clearance estimated: V1_F, K01, CL_F, V2_F, CLD2_F
Clearance secondary: AUC, K01 half-life, K10 half-life, Alpha, Beta, Alpha half-life, Beta half-life, A, B, K10, K12, K21, Tmax, Cmax

[a] Estimated for the compiled model only

Model 12

Two-compartment with first-order input, first-order output, lag time and micro-constants as primary parameters.

As Model 11, with an additional estimated parameter: $Tlag$=lag time. In the Model 11 equation, substitute $(t - Tlag)$ for t.

Model 13

Two-compartment with first-order input, first-order output, no lag time and macro-constants as primary parameters.

As Model 11, with macroconstants as the primary (estimated) parameters. Clearance parameters are not available for Model 13.

Required constants: Stripping dose, N doses, dose N, time of dose N (Repeat for each dose)
Estimated parameters: A, B, K01 (absorption rate), Alpha, Beta, Note: C=–(A+B)
Secondary parameters: K10, K12, K21, AUC=D/V/K10, K01 half-life, K10 half-life, Alpha half-life, Beta half-life, V1_F, CL_F, V2_F, CLD2_F, Tmax[a], Cmax[a]

[a]Estimated for the compiled model only

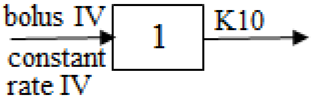
Model 14

Two-compartment with first-order input, first-order output, lag time and macro-constants as primary parameters.

As Model 13, with an additional estimated parameter: $Tlag$=lag time. In the Model 3 equation, substitute $(t - Tlag)$ for t.

Model 15

One-compartment with simultaneous bolus IV and constant IV infusion.



$$C_B(t) = \left(\frac{D_b}{V}\right)\exp[-K10t]$$

$$C_{IV}(t) = \left(\frac{D_{IV}}{Tinf}\right)\frac{1}{V(K10)}[\exp(-K10t^*) - \exp(-K10t)]$$

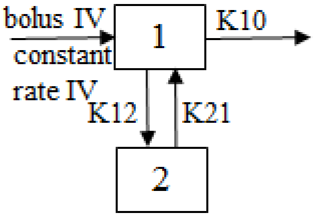
$$C(t) = C_B(t) + C_{IV}(t)$$

where:

$Tinf$ = infusion length
$t^* = t - Tinf$ for $t > Tinf$
$t^* = 0$ for $t \leq Tinf$

Model 16

Two-compartment with simultaneous bolus IV and constant infusion input; micro constants as primary parameters.



$$C_B(t) = A_1 \exp(-\alpha t) + B_1 \exp(-\beta t)$$

where:

$$A = \frac{D_B}{V}\left(\frac{\alpha - K21}{\alpha - \beta}\right)$$

$$B = -\frac{D_B}{V}\left(\frac{\beta - K21}{\alpha - \beta}\right)$$

and

$$C_{IV}(t) = A_2[\exp(-\alpha t) - \exp(-\alpha t^*)] + B_2[\exp(-\beta t) - \exp(-\beta t^*)]$$

where:

$$A = \frac{D_{IV}}{Tinf(V)}\left(\frac{K21 - \alpha}{(\alpha - \beta)\alpha}\right)$$

$$B = -\frac{D_{IV}}{Tinf(V)}\left(\frac{K21 - \beta}{(\alpha - \beta)\beta}\right)$$

$Tinf$ = infusion length
$t^* = t - Tinf$ for $t > Tinf$
$t^* = 0$ for $t \le Tinf$

$-\alpha$ and $-\beta$ ($\alpha > \beta$) are the roots of the quadratic equation:
$r^2 + (K12 + K21 + K10)r + K21(K10) = 0$

$$C(t) = C_B(t) + C_{IV}(t)$$

Required constants: Bolus dose, IV dose, length of infusion (= Tinf)
Estimated parameters: V1, K10, K12, K21
Secondary parameters: K10 half-life, Alpha, Beta, Alpha half-life, Beta half-life, A, B, CL, V2, CLD2
Clearance estimated: V1, CL, V2, CLD2
Clearance secondary: K10 half-life, Alpha, Beta, Alpha half-life, Beta half-life, A, B, K10, K12, K21

Model 17

Two-compartment with simultaneous bolus IV and constant infusion input and macro constants as primary parameters.

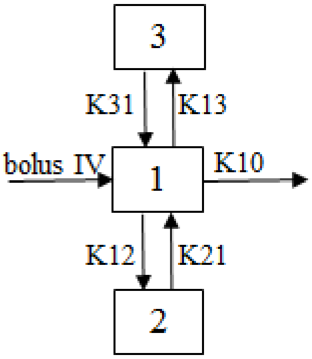
As Model 10 with macroconstants as the primary (estimated) parameters.

Required constants: Stripping dose, bolus dose, IV dose, length of infusion (= Tinf)
Estimated parameters: A, B, Alpha, Beta
Secondary parameters: K10, K12, K21, K10 half-life, Alpha half-life, Beta half-life, V1, CL, V2, CLD2

Clearance parameters are not available in Model 17.

Model 18

Three-compartment with bolus input, first-order output; macro constants as primary parameters.



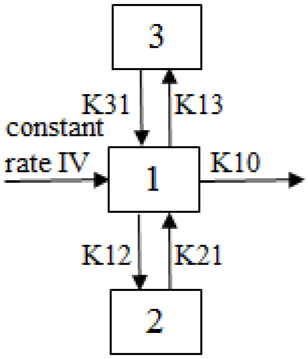
$$C(t) = A\exp(-\alpha t) + B\exp(-\beta t) + C\exp(-\Gamma t)$$

Required constants: Stripping dose, N doses, dose N, time of dose N (Repeat for each dose)
Estimated parameters: A, B, C, Alpha, Beta, Gamma
Secondary parameters: Cmax, V1, K21, K31, K10, K12, K13, K10 half-life, Alpha half-life, Beta half-life, Gamma half-life, AUC, CL, AUMC, MRT, Vss, V2, CLD2, V3, CLD3

Clearance parameters are not available in Model 18.

Model 19

Three compartment model with constant IV infusion; macro constants as primary parameters.



$$\begin{aligned} C(t) = {} & A_1[\exp(-\alpha t^*) - \exp(-\alpha t)] \\ & + B_1[\exp(-\beta t^*) - \exp(-\beta t)] \\ & + C_1[\exp(-\gamma t^*) - \exp(-\gamma t)] \end{aligned}$$

where:

$$A_1 = \frac{D}{Tinf}\left(\frac{(K21 - \alpha)(K31 - \alpha)}{V(\alpha)(\gamma - \alpha)(\beta - \alpha)}\right)$$

$$B_1 = \frac{D}{Tinf}\left(\frac{(K21 - \beta)(K31 - \beta)}{V(\beta)(\gamma - \beta)(\alpha - \beta)}\right)$$

$$C_1 = \frac{D}{Tinf}\left(\frac{(K21 - \gamma)(K31 - \gamma)}{V(\gamma)(\alpha - \gamma)(\beta - \gamma)}\right)$$

$Tinf$ = infusion length
$t^* = t - Tinf$ for $t > Tinf$
$t^* = 0$ for $t \leq Tinf$

Required constants: N doses, dose N, start time and end time of dose N (Repeat for each dose)
Estimated parameters: V1, K21, K31, Alpha, Beta, Gamma
Secondary parameters: Cmax, K10, K12, K13, A, B, C, K10 half-life, Alpha half-life, Beta half-life, Gamma half-life, AUC, CL, AUMC, MRT, Vss, V2, CLD2, V3, CLD3

A, B, and C are the zero time intercepts following an IV injection.

Clearance parameters are not available in Model 19.

PD output parameters in a PK/PD model

The output parameters for PD models in a linked PK/PD model differ from the parameters for PD models that are not linked. The following list shows the PD model parameters used in linked PK/PD models.

101 Simple Emax model

Estimated parameters: Emax, EC_{50}, Ke0

102 Simple Emax model with a baseline effect

Estimated parameters: Emax, $EC_{50,}$ E0, Ke0
Secondary parameters: Rmax, F_Emax

103 Inhibitory effect E0 model

Estimated parameters: E0, IC_{50}, Ke0

104 Inhibitory effect Imax model with a baseline effect

Estimated parameters: Imax, IC_{50}, E0, Ke0
Secondary parameters: Rmax, F_Emax

105 Sigmoid Emax model

Estimated parameters: Emax, EC_{50}, Gamma, Ke0

106 Sigmoid Emax model with a baseline effect

Estimated parameters: Emax, EC_{e50}, E0, Gamma, Ke0
Secondary parameters: Rmax, F_Emax

107 Inhibitory effect sigmoid E0 model

Estimated parameters: E0, IC_{50}, Gamma, Ke0

108 Inhibitory effect sigmoid Imax model with a baseline effect

Estimated parameters: Imax, IC_{50}, E0, Gamma, Ke0
Secondary parameters: Rmax, F_Emax

Below are definitions for the estimated and secondary parameters.

Emax: Maximum drug effect.

Imax: Maximum drug inhibition.

EC_{50}: Concentration in plasma that achieves 50% of predicted maximum effect in an Emax model.

ECe$_{50}$: For effect compartment models, the concentration at the effect site that achieves 50% of predicted maximum effect in an Emax model.

IC_{50}: Drug concentration required to produce 50% of the maximal inhibition.

E_0: Baseline effect (effect at 0). In models 103 and 107, E0 could also be named Imax, the maximum drug inhibition.

Gamma: Shape parameter.

Ke0: Exit rate constant from effect compartment.

Rmax: Observed or predicted maximum response.

F_Emax: Fractional change in response from baseline E_0.

ASCII Model dosing constants

The number of constants in the model corresponds to the dosing route for the model.

Bolus and first-order input models require at least three dosing constants per profile.

CON[0]: Number of doses (N)
CON[1]: Dose amount for dose N
CON[2]: Time of dose for dose N
CON[3]: Dose amount for dose N+1 (if multiple doses are used)
CON[4]: Time of dose for dose N+1 (if multiple doses are used)

Constant IV infusion models require at least four dosing constants per profile.

CON[0]: Number of doses (N)
CON[1]: Dose amount for dose N
CON[2]: Start time for dose N
CON[3]: End time for dose N
CON[4]: Dose amount for dose N+1 (if multiple doses are used)
CON[5]: Start time for dose N+1 (if multiple doses are used)
CON[6]: End time for dose N+1 (if multiple doses are used)

Parameter Estimates and Boundaries Rules

Rules for using parameter estimates and boundaries in Indirect Response, Linear, Michaelis-Menten, PD, PK, PK/PD, and ASCII models include the following:

- Using boundaries, either user- or WinNonlin-generated, is recommended.
- Phoenix uses curve stripping only for single-dose data. If multiple dose data is fit to a PK model and Phoenix generates initial parameter estimates, a grid search is performed to obtain initial estimates. In this case, boundaries are required.
- For linear regressions, it is recommended that users keep the **Do Not Use Bounds** option checked in the Parameter Options tab and select minimization method 3 (**Gauss-Newton (Hartley)**) in the Engine Settings tab.
- The grid used in grid searching uses three values for each parameter. If four parameters are estimated, the grid contains 3^4=81 possible points. The point on the grid associated with the smallest sum of squares is used as an initial estimate for the estimation algorithm. This option is included for convenience, but it might greatly slow the parameter estimation process if the model is defined in terms of differential equations.
- If the data are fitted to a micro-constant model, Phoenix performs curve stripping for the corresponding macro-constant model then uses the macro-constants to compute the micro-constants. It is recommended that users specify the **Lower** and **Upper** boundaries, even when Phoenix computes the initial estimates.
- If the **Gauss-Newton** methods (minimization methods 2 and 3) are used to estimate the parameters, then the boundaries are determined by applying a normit transform to the parameter space.
- If the **Nelder-Mead** method (minimization method 1) is selected, the residual sum of squares is set to a large number if any of the parameter estimates go outside the specified constraints at any iteration, which forces the parameters back within the specified bounds.
- Unlike the linearization methods, the use of bounds does not affect numerical stability when using the Nelder-Mead method. Using bounds with the Nelder-Mead method will keep the parameters within the bounds. The use of bounds, either user-supplied or Phoenix-supplied, is always recommended with the Gauss-Newton methods.

References

Beck and Arnold (1977). *Parameter Estimation in Engineering and Science.* John Wiley & Sons, New York.

Bard (1974). *Nonlinear Parameter Estimation*. Academic Press, New York.

Bates and Watts (1988). *Nonlinear Regression Analyses and Its Applications.* John Wiley & Sons, New York.

Davies and Whitting (1972). A modified form of Levenberg's correction. Chapter 12 in *Numerical Methods for Non-linear Optimization.* Academic Press, New York.

Dayneka, Garg and Jusko (1993). Comparison of four basic models of indirect pharmacodynamic responses. *J Pharmacokinet Biopharm* 21:457.

Draper and Smith (1981). *Applied Regression Analysis*, 2nd ed. John Wiley & Sons, NY.

Endrenyi, ed. (1981). *Kinetic Data Analysis: Design and Analysis of Enzyme and Pharmacokinetic Experiments.* Plenum Press, New York.

Gabrielsson and Weiner (2016). *Pharmacokinetic and Pharmacodynamic Data Analysis: Concepts and Applications*, 5th ed. Apotekarsocieteten, Stockholm.

Gill, Murray and Wright (1981). *Practical Optimization.* Academic Press.

Hartley (1961). The modified Gauss-Newton method for the fitting of nonlinear regression functions by least squares. *Technometrics* 3:269–80.

Jennrich and Moore (1975). Maximum likelihood estimation by means of nonlinear least squares. *Amer Stat Assoc Proceedings Statistical Computing Section* 57–65.

Jusko (1990). Corticosteroid pharmacodynamics: models for a broad array of receptor-mediated pharmacologic effects. *J Clin Pharmacol* 30:303.

Kennedy and Gentle (1980). *Statistical Computing.* Marcel Dekker, New York.

Metzler and Tong (1981). Computational problems of compartmental models with Michaelis-Menten-type elimination. *J Pharmaceutical Sciences* 70:733–7.

Nelder and Mead (1965). A simplex method for function minimization. *Computing Journal* 7:308–13.

Ratkowsky (1983). *Nonlinear Regression Modeling.* Marcel Dekker, New York.

Shampine, Watts and Davenport (1976). Solving nonstiff ordinary differential equations - the state of the art. *SIAM Review* 18:376–411.

Tong and Metzler (1980). Mathematical properties of compartment models with Michaelis-Menten-type elimination. *Mathematical Biosciences* 48:293–306.

PK model examples

Knowledge of how to do basic tasks using the Phoenix interface, such as creating a project and importing data, is assumed.

Fit a PK model to data example
Simulation and study design of PK models example
More nonlinear model examples

Fit a PK model to data example

This example is about creating and saving PK models in Phoenix and supposes that a researcher has obtained concentration data from one subject after oral administration of a compound, and now wishes to fit a pharmacokinetic (PK) model to the data.

The completed project (`PK_Model.phxproj`) is available for reference in …`\Examples\WinNonlin`.

Explore the PK input data

1. Create a new project called `PK Model`.
2. Import the file …`\Examples\WinNonlin\Supporting files\study1.CSV`.
   In the *File Import Wizard* dialog, select the **Has units row** option and click **Finish**.
3. Right-click **study1** in the Data folder and select **Send To > Plotting > XY Plot**.
4. In the XY Data Mappings panel:
   Leave **Subject** mapped to the **None** context.
   Map **Time** to the **X** context.
   Map **Conc** to the **Y** context.
5. Click ▶ (**Execute** icon) to execute the object.
6. In the Options tab below the plot, select **Axes > Y** from the menu tree.
7. Select the **Logarithmic** option button in the Scale area. Leave the logarithmic base set to **10**.
   The XY Plot is automatically updated to reflect the scale change.

Set up the object

The plots suggests that the system might be adequately modeled by a one-compartment, 1st order absorption model. This model is available as Model 3 in the pharmacokinetic models included in Phoenix. Set up a PK Model object and a Maximum Likelihood Model object, for comparison.

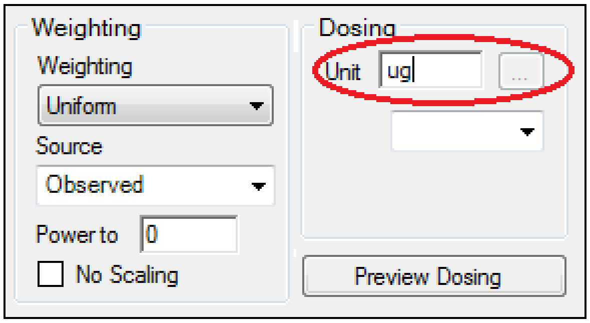
PK Model object

1. Right-click **study1** in the Data folder and select **Send To > Modeling > Least Squares Regression Models > PK Model**.
2. In the Main Mappings panel:
   Map **Subject** to the **Sort** context.
   Leave **Time** mapped to the **Time** context.
   Map **Conc** to the **Concentration** context.
3. In the Model Selection tab below the Setup panel, check the **Number 3** model checkbox.

Entering the units for dosing data makes it possible to view and adjust units for the model parameters.

In this example, a single dose of 2 micrograms was administered at time zero.

1. Select the **Dosing** panel in the Setup tab.
2. Check the **Use Internal Worksheet** checkbox.
3. Click **OK** in the *Select sorts* dialog to accept the default sort variable.
4. In the cell under **Time** type `0`.
5. In the cell under **Dose** type `2`.
6. In the Weighting/Dosing Options tab below the Setup panel, type `ug` in the **Unit** field.



Weighting

Weighting: Uniform

Source: Observed

Power to: 0

□ No Scaling

Dosing

Unit: ug

All model estimation procedures benefit from initial estimates of the parameters. While Phoenix can compute initial parameter estimates using curve stripping, this example will provide user values for the initial parameter estimates.

1. Select the **Parameter Options** tab below the Setup panel.
2. Select the **User Supplied Initial Parameter Values** option.
   The **WinNonlin Bounds** option is selected by default as the Parameter Boundaries. Do not change this setting.
3. Select **Initial Estimates** in the Setup panel list.
4. Check the **Use Internal Worksheet** checkbox.
5. Click **OK** to accept the default sort variable in the *Select sorts* dialog.
6. Enter the following information in the table:
   For row 1 (V_F), enter `0.25` in the Initial column.
   For row 2 (K01), enter `1.81` in the Initial column.
   For row 3 (K10), enter `0.23` in the Initial column.

Maximum Likelihood Models object

1. Right-click the **study1** worksheet in the Data folder and select **Send To > Modeling > Maximum Likelihood Models**.
2. In the Structure tab, uncheck the **Population** box.
3. Click the **Set WNL Model** button.
   The contents of the Structure tab changes. The first of the two untitled menus allows users to select a PK model, and the second allows users to select a PD model.
4. In the first untitled menu, select **3 (1cp extravascular)**.
5. Click **Apply** to set the WinNonlin model.
6. Use the option buttons in the Main Mappings panel to map the data types to the following contexts:
   Subject to the **Sort** context.
   Time to the **Time** context.
   Conc to the **CObs** context.

The WARNINGS tab at the bottom highlights potential issues. If there are no issues, the tab will be labeled “no warnings”. Click the **WARNINGS** tab and note the message that **Aa** values are missing. This will be taken care of in the next few steps.

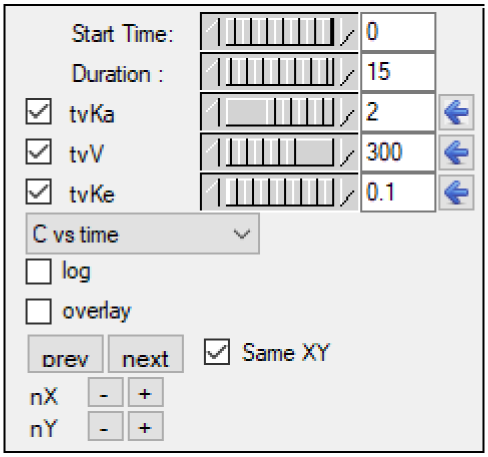
In this example, a single dose of 2 micrograms was administered at time zero. However, since the concentration units were ng/mL, the dose should be entered as 2000ng so the units are equivalent.

1. Select the **Dosing** panel in the Setup tab.
2. Check the **Use Internal Worksheet** checkbox.
3. In the cell under **Aa** type `2000`.
4. In the cell under **Time** type `0`.
5. Click **View Source** above the worksheet.
6. In the **Columns** tab below the table, select **Aa** from the Columns list and enter `ng` in the **Unit** field.
7. Click X in the upper right corner to close the source window (the units are added to the column header).

Notice that the warning about Aa values is gone and the tab label now says “no warnings.”

The Maximum Likelihood Models engine will not generate its own initial estimates, like the Least-Squares Regression Model engine can, so it is important to consider reasonable starting values.

1. Select the **Initial Estimates** tab in the lower panel.
2. Extend the **Duration** to `15`, since the last observed timepoint is 14 hours.
3. Enter values `2`, `300`, and `0.1` for **tvKa**, **tvV**, and **tvKe**, respectively.



Start Time: 0

Duration : 15

☑ tvKa 2

☑ tvV 300

☑ tvKe 0.1

C vs time

□ log

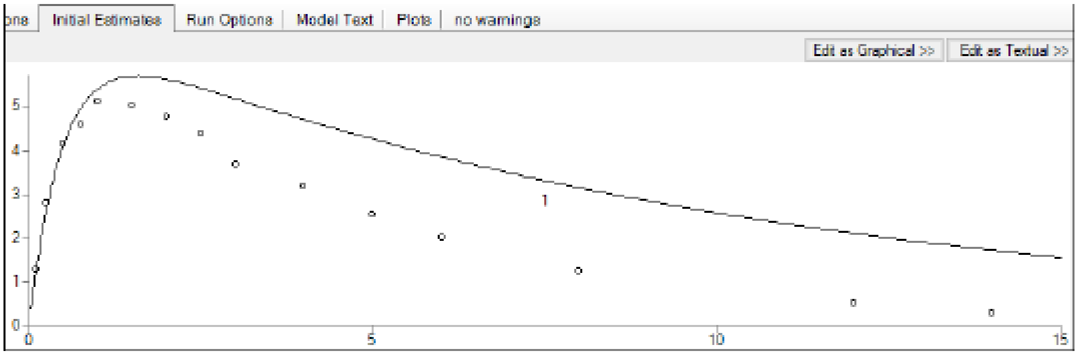
□ overlay

☑ Same XY

Note: Make sure the **tvKe** box is checked after entering the value. Entering a leading “0” for a value causes the corresponding checkbox to automatically be cleared.

The y axis can also be set to log scale and, if there is more than one profile, the curves can be overlaid by checking the **log** and **overlay** boxes, respectively.

Note how the predicted curve roughly follows the observed data now.



4. Click the blue arrows to submit these values to the main model engine (if the arrow is blue, then the value will not be used).

Execute and view the results

At this point, all of the necessary commands and options have been specified.

1. Execute each object in the workflow.

The Results tab contains three types of model output:

Worksheets (descriptions of the worksheets are located in the "Worksheet output" section)
Plots
Text output

The six plots generated by the PK Model object are shown below. The NLME object generates more plots, however the ones corresponding to the WinNonlin output are listed in parentheses.

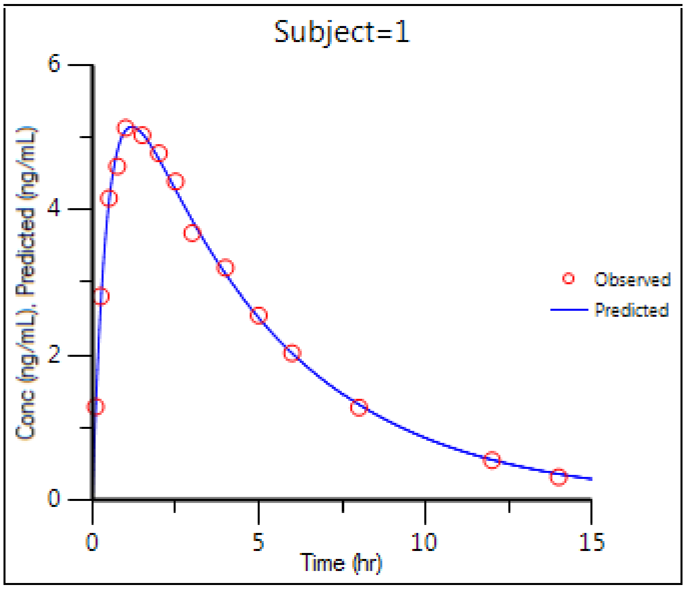


Figure 39-1. Observed Y and Predicted Y vs X (Ind DV, IPRED vs TAD)

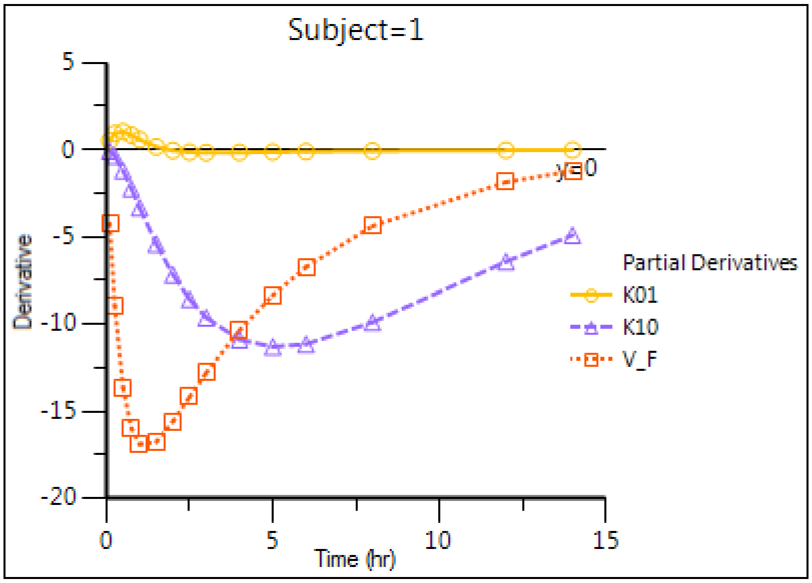


Figure 39-2. Partial Derivatives plot (Ind Partial Derivatives)

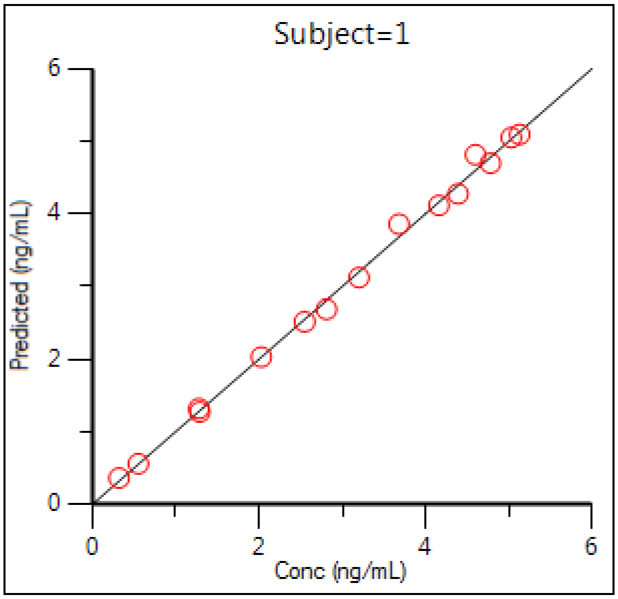


Figure 39-3. Predicted Y vs Observed Y (Ind DV vs IPRED, axes swapped)

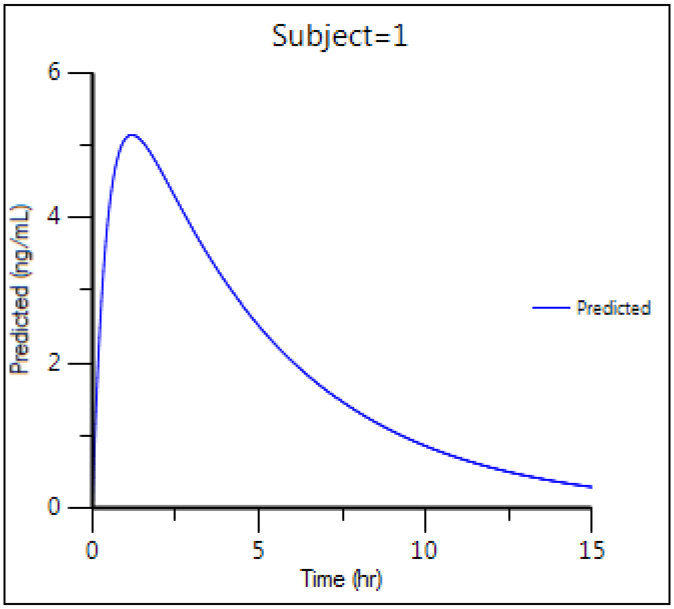


Figure 39-4. Predicted Y vs X

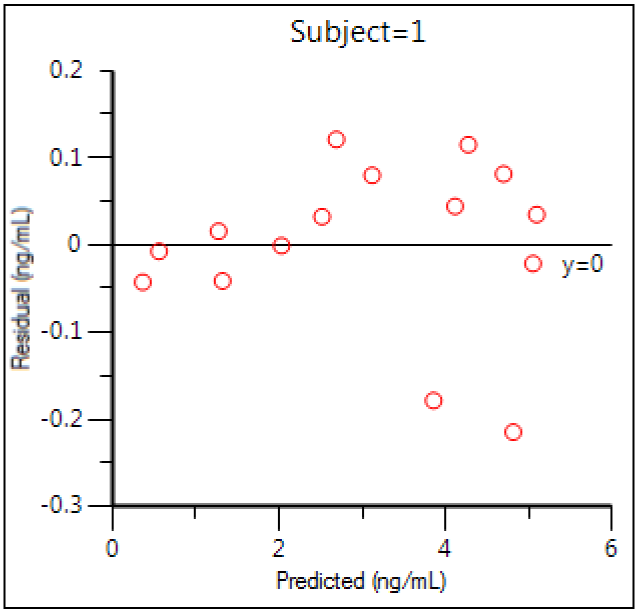


Figure 39-5. Residual Y vs Predicted Y (Ind IWRES vs IPRED)

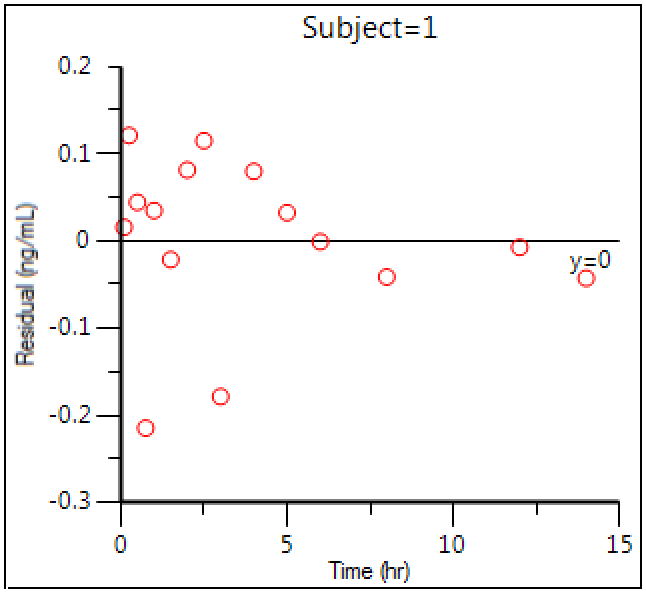
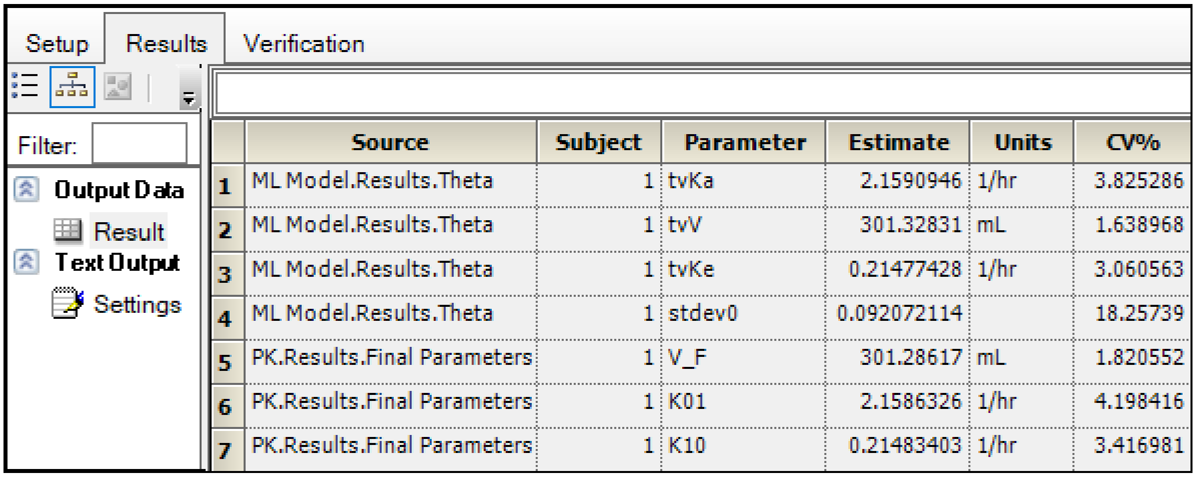


Figure 39-6. Residual Y vs X (Ind IWRES vs TAD)

The tables generated by the ML Model object summarize useful information.

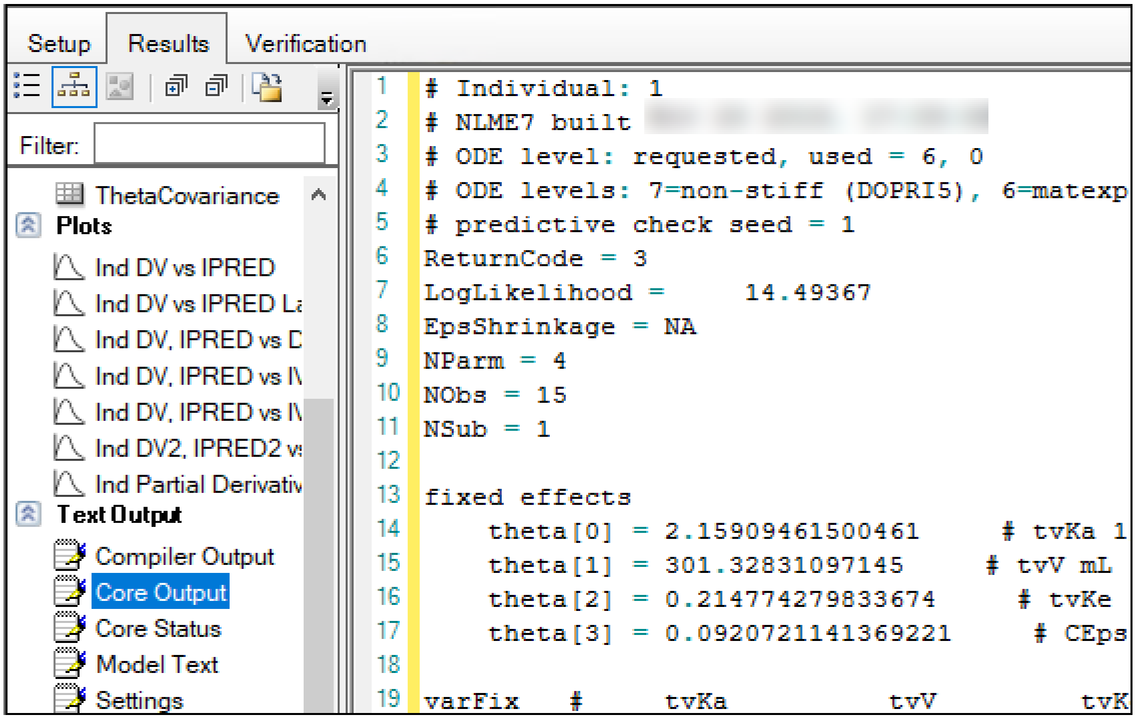
Overall: Contains -2LL (the objective function) and other goodness of fit information
Theta: Final parameter estimates
Residuals: Analogous to Summary Table of Least-Squares Regression and NCA models

To compare the two models, create a worksheet by appending the Final Parameters worksheet from the PK model to the Theta worksheet from the ML Model object. (See "Append Worksheets" for specifics on how to append worksheets.)



	Source	Subject	Parameter	Estimate	Units	CV%
1	ML Model.Results.Theta	1	tvKa	2.1590946	1/hr	3.825286
2	ML Model.Results.Theta	1	tvV	301.32831	mL	1.638968
3	ML Model.Results.Theta	1	tvKe	0.21477428	1/hr	3.060563
4	ML Model.Results.Theta	1	stdev0	0.092072114		18.25739
5	PK.Results.Final Parameters	1	V_F	301.28617	mL	1.820552
6	PK.Results.Final Parameters	1	K01	2.1586326	1/hr	4.198416
7	PK.Results.Final Parameters	1	K10	0.21483403	1/hr	3.416981

The Core Output text file contains all model settings and output in plain text format. Below is part of the Core Output file from the ML Model object run.



```
# Individual: 1
# NLME7 built [redacted]
# ODE level: requested, used = 6, 0
# ODE levels: 7=non-stiff (DOPRI5), 6=matexp
# predictive check seed = 1
ReturnCode = 3
LogLikelihood =      14.49367
EpsShrinkage = NA
NParm = 4
NObs = 15
NSub = 1

fixed effects
    theta[0] = 2.15909461500461     # tvKa 1
    theta[1] = 301.32831097145     # tvV mL
    theta[2] = 0.214774279833674     # tvKe
    theta[3] = 0.0920721141369221     # CEps

varFix   #     tvKa         tvV         tvK
```

Save the project and the results

Projects and their results can be saved as a project file or loaded into Integral.

To save the project as a file, select **File > Save Project**.

For details on adding the project to Certara Integral, see "Adding a project to Integral".

Simulation and study design of PK models example

Considerable research has been done in the area of optimal designs for linear models. Most methods involve computation of the variance covariance matrix. The "optimal" design is usually one in which replicate samples are taken at a limited number of combinations of experimental conditions. Unfortunately, these methods are of little or no value when designing experiments involving nonlinear models for a number of reasons, including:

- It can be difficult or, in the case of a pharmacokinetic study, impossible to obtain replicate observations.
- The primary interest often is not in the model parameters but in some functions of the model parameters such as AUC, $t_{1/2}$, etc.

When Phoenix performs a simulation, the output includes information on precisely how parameters in the model can be estimated for specified values of the independent variables, such as time.

Assume that a study is being planned and that the data produced by this study should be consistent with Phoenix PK model 3. Assume also that the parameter values should be approximately: V_F=10, K01=3, K10=0.05 and one of the following study designs, or sampling times, will be used:

0, 1.5, 3, 6, 9, 12, 15, 18, and 24 hours
or
0, 0.5, 1, 2, 4, 8, 12, 24, and 36 hours.

Simulation can be used to determine which set of sampling times would produce the more precise estimates of the model parameters. This example will use Phoenix to simulate the model with each set of sampling times, and compare the variance inflation factors for the two simulations.

The completed project (`Study_Design.phxproj`) is available for reference in …`\Examples\WinNonlin`.

Create the input dataset

Follow the following steps to create the dataset. Alternatively, the data can be imported from …`\Examples\WinNonlin\Supporting files\Example Data.csv`.

1. Create a new project with the name `Study Design`.
2. Right-click the Data folder in the Object Browser and select **New > Worksheet**.
3. Name the new worksheet `Example Data`.
4. In the Columns tab, add a column identifying the group number by clicking **Add** underneath the Columns list.
5. In the *New Column Properties* dialog, type `Group` in the Column Name field. Leave the data type set to **Numeric**, and click **OK**.
6. In the first cell under **Group**, type `1` and press **ENTER**. Continue to enter `1` for rows 2–9.
7. In rows 10–18 type `2` in the **Group** column.
8. Add a column of time data by clicking **Add** underneath the Columns list.
9. In the Column Name field, type `Times`. Leave the data type set to **Numeric** and click **OK**.
10. Type the values 0, 1.5, 3, 6, 9, 12, 15, 18, and 24 in the Times column for rows 1–9 and values 0, 0.5, 1, 2, 4, 8, 12, 24, and 36 for rows 10–18.

Set up the object

This model is available as Model 3 in the pharmacokinetic models included in Phoenix.

1. Right-click **Example Data** in the Data folder and select **Send To > Modeling > Least Squares Regression Models > PK Model**.
2. In the Main Mappings panel:
Map **Group** to the **Sort** context.
Map **Times** to the **Time** context.
3. In the Model Selection tab below the Setup panel, specify the PK model that Phoenix will use in the analysis by selecting the **Number 3** model checkbox.
4. Select the **Simulation** checkbox on the right side of the Model Selection tab (notice that the Concentration mapping is changed from required (orange) to option (gray)).
5. In the **Y Units** field, type `ng/mL`.
6. Enter the dosing data by selecting the PK Model's **Dosing** panel in the Setup tab.
7. Check the **Use Internal Worksheet** checkbox.
8. Click **OK** in the *Select sorts* dialog to accept the default sort variable.
9. In the **Time** column type `0` for both groups.
10. In the **Dose** column type `100` for both groups.

Note: The number of rows in the Group column corresponds to the number of doses received. For example, if group 1 had 10 doses, there would be 10 rows of dosing information for group 1. In Phoenix this grouping of data is referred to as stacking data.

11. In the Weighting/Dosing Options tab below the Setup panel, type `mg` in the **Unit** field.
12. Select the **Parameter Options** tab.

    Parameter values must be specified for simulations. The **User Supplied Initial Parameter Values** option is selected and cannot be changed. The **Do Not Use Bounds** option is selected by default and cannot be changed.

    Selecting the **Simulation** checkbox makes the parameter calculation and boundary selection options unavailable. If the **Simulation** checkbox is selected, then users must supply initial parameter values, and parameter boundaries are not used.
13. Select **Initial Estimates** in the Setup list.
14. Check the **Use Internal Worksheet** checkbox.
15. In the *Select sorts* dialog, click **OK** to accept the default sort variable.
16. Enter the following initial values for each group: V_F=`10`, K01=3, K10=`0.05`.

Execute and view the results

All the settings are complete and the model can be executed.

1. Execute the object.

The variance inflation factors (VIF) for each dosing scheme (groups 1 and 2) are located in the Final Parameters worksheet and are summarized (with values rounded) below.

V_F
 Estimate = 10
 Group 1 VIF = 0.779
 Group 2 VIF = 0.657

K01
 Estimate = 3
 Group 1 VIF = 68.48
 Group 2 VIF = 1.176

K10
 Estimate = 0.05
 Group 1 VIF = 0
 Group 2 VIF = 0

In practice, it is useful to vary the values of V_F, K01, and K10 and repeat the simulations to determine if the first set of sampling times consistently yields less precise estimates than the second set.

Design the sampling plan

Note that, for the parameters V_F and K10, the estimated variances would be approximately 15% lower using the second set of times, while the difference is much more dramatic for the parameter K01. These sets of variance inflation factors indicate that the second set of sampling times would provide tighter estimates of the model parameters.

The partial derivatives plots for this model explain this result. The locations at which the partial derivative plots reach a maximum or a minimum indicate times the model is most sensitive to changes in the model parameters, so one approach to designing experiments is to sample where the model is most sensitive to changes in the model parameters.

1. Click **Partial Derivatives Plot** in the Results tab.

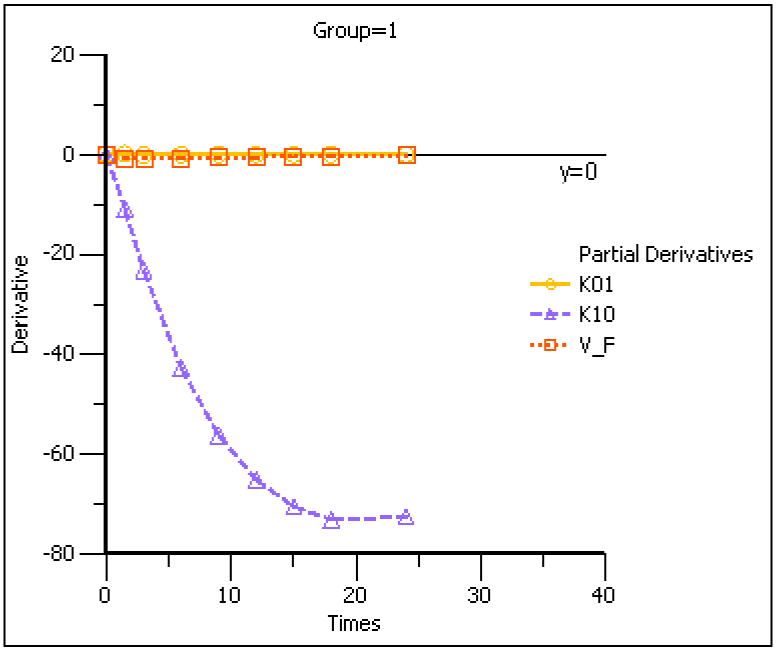
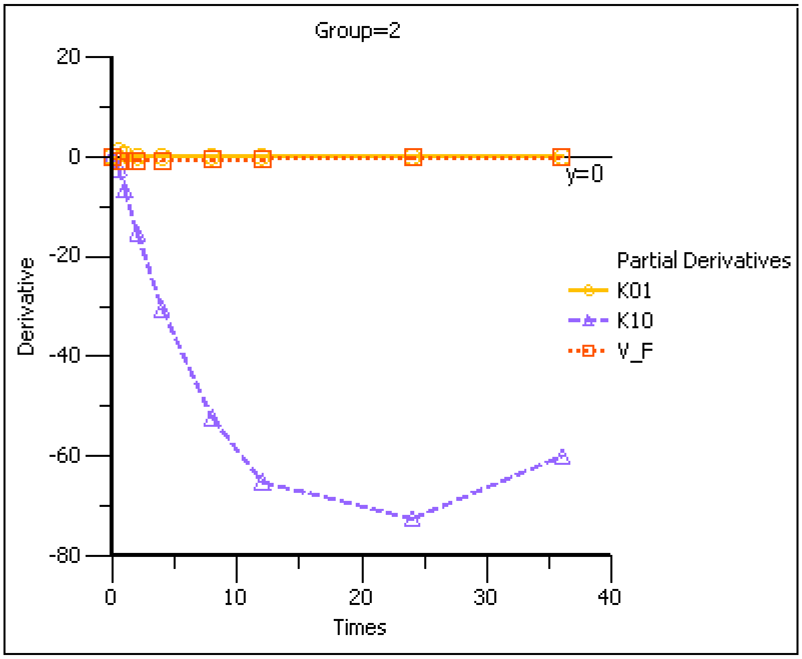


Figure 39-7. Partial Derivatives plot Group 1

2. Select the **Page 02** tab below the plot.



Note that in the first plot of the partial derivatives the model is most sensitive to changes in K10 at about 20 hours. Both sampling schemes included times near 20 hours, so therefore the two sets of sampling times were nearly equivalent in the precision with which K10 would be estimated.

For both V_F and K01 the model is most sensitive to changes very early, at about 0.35 hours for K01 and about 1.4 hours for V_F. The first set of sampling times does not include any post-zero points until hour 3, long past these areas of sensitivity. Even the second set of times could be improved if samples could be taken earlier than 0.5 hours.

This same technique could be used for other models in Phoenix or for user-defined models.

3. Close this project by right-clicking the project and selecting **Close Project**.

More nonlinear model examples

Knowledge of how to do basic tasks using the Phoenix interface, such as creating a project and importing data, is assumed.

The examples project `Model_Examples.phxproj` located in …`\Examples\WinNonlin` involve Least-Squares Regression modeling objects. Each model object within this project contains the appropriate default mappings and settings needed to run the model.

To run these example objects:

1. Load the project …`\Examples\WinNonlin\Model_Examples.phxproj` into Phoenix.

   Each example model has the following items associated with it:
   - A dataset in workbook form.
   - Many have datasets in workbook form for dosing.
   - A PK, PKPD, Indirect Response, or User ASCII model object.
2. Explore each model object and its mappings and settings.
3. Execute each model.

Explanations for each model object in the example is given below.

Pharmacokinetic model (Exp1)

In the Exp1 example, a dataset was fit to PK model 13 in the pharmacokinetic model library. Four constants are required for model 13: the stripping dose associated with the parameter estimates, the number of doses, the dose, and the time of dosing.

This example uses weighted least squares (1/observed Y). Phoenix determines initial estimates via curve stripping and then generates bounds for the parameters.

Pharmacokinetic model with multiple doses (Exp2)

In the Exp2 example, a dataset obtained following multiple dosing is fit to model 13 in the pharmacokinetic model library, which is a two-compartment open model. This model has five parameters: A, B, K01, Alpha, and Beta and uses the user-supplied initial values `20`, `5`, `3`, `2`, and `0.05`. Phoenix generates bounds for the parameters.

Probit analysis: maximum likelihood estimation of potency (Exp3)

The Exp3 example demonstrates how to use the NORMIT and WTNORM functions to perform a probit regression (parallel line bioassay or quantal bioassay) analysis. Note that a probit is a normit plus five. There are several interesting features used in this example:

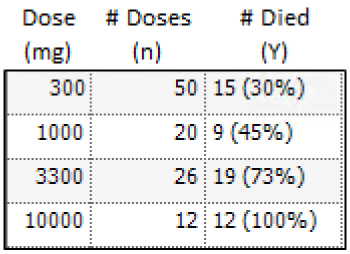
- The transform capability was used to create the response variable.
- The logarithm of the relative potency is estimated as a secondary parameter.

- Maximum likelihood estimates were obtained by iteratively reweighting and turning off the halvings and convergence criteria. Therefore, instead of iterating until the residual sum of squares is minimized, the program adjusts the parameters until the partial derivatives with respect to the parameters in the model are zero. This will normally occur after a few iterations.
- Since there is no σ^2 in a problem such as this, variances for the maximum likelihood estimates are obtained by setting S^2=1 (MEANSQUARE=1).
- The following modeling options are used:
  - Method 3 is selected, which is recommended for Maximum Likelihood estimation (MLE), and iterative reweighting problems.
  - Convergence Criterion is set to 0. This turns off convergence checks for MLE.
  - Iterations are set to 10. Estimates should converge after a few iterations.
  - Meansquare is set to 1. Sigma squared is 1 for MLE.

For further reading regarding use of nonlinear least squares to obtain maximum likelihood estimates, refer to Jennrich and Moore (1975). Maximum likelihood estimation by means of nonlinear least squares. *Amer Stat Assoc Proceedings Statistical Computing Section* 57–65.

Logit regression (bioassay) (Exp4)

The following data were obtained in a toxicological experiment:



Dose (mg)	# Doses (n)	# Died (Y)
300	50	15 (30%)
1000	20	9 (45%)
3300	26	19 (73%)
10000	12	12 (100%)

In the Exp4 example, assume that the distribution of Y is binomial with:

mean = np
variance = npq, and q=1 – p

where:

$$p = \frac{\exp[\alpha + \beta X]}{1 + \exp[\alpha + \beta X]}$$

and X = $\log_e$ $dose$

Maximum likelihood estimates of α and β for this model are obtained via iteratively reweighted least squares. This is done by fitting the mean function (np) to the Y data with weight $(npq)^{-1}$.

The modeling commands needed to fit this model to the data are included in an ASCII model file. Note that the $\log_e$ LD50 and $\log_e$ LD001 are also estimated as secondary parameters.

This model is really a linear logit model in that:

$$\log\frac{p}{1-p} = \alpha + \beta X$$

Note: For this type of problem, the final value of the residual sum of squares is the Chi-square statistic for testing heterogeneity of the model. If this example is run a user obtains X^2 (heterogeneity)=2.02957, with 4 – 2=2 degrees of freedom (number of data points minus the number of parameters that were estimated).

For a more in-depth discussion of the use of nonlinear least squares for maximum likelihood estimation, see Jennrich and Moore (1975). Maximum likelihood estimation by means of nonlinear least squares. *Amer Stat Assoc Proceedings Statistical Computing Section* 57–65.

In the Engine Settings tab, the Convergence Criteria is set to zero to turn off the halving and convergence checks. Meansquare is set to 1 (the residual mean square is redefined to be 1.00) in order to estimate the standard errors of a, b, and the secondary parameters.

Survival analysis (Exp5)

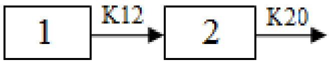
Exp5 is another maximum likelihood example and is very similar to Model Exp4. It is included to show that models arising in a variety of disciplines, such as case survival or reliability analysis, can be fit by nonlinear least squares.

The dosing constant is defined in this model as the denominator for the proportions, that is, N.

In the Engine Settings tab the Convergence Criteria is set to zero to turn off the halving and convergence checks. Meansquare is set to 1 (redefines the residual mean square to be 1.00) in order to estimate the standard errors of the primary and secondary parameters.

Two differential equations with data for both compartments (Exp6)

Exp6 involves the following model:



where K12 and K20 are first-order rate constants. This model may be described by the following system of two differential equations.

$$\frac{dZ_1}{dt} = -K12 \times Z_1 \text{ compartment 1}$$

$$\frac{dZ_2}{dt} = K12 \times Z_1 - K20 \times Z_2 \text{ compartment 2}$$

with initial conditions Z_1=D, and Z_2=0.

In addition to obtaining estimates of K12 and K20, it is also desirable to estimate D and the half-lives of K12 and K20.

A sample solution for this example is given here. Note that, for this example, the model is defined as an ASCII file. Data corresponding to both compartments are available.

Column C in the dataset for this example contains a function variable, which defines the separate functions.

Two differential equations with data on one-compartment (Ex7)

The model for the Exp7 example is identical to that for Exp6. However, in this example, it is assumed that data are available only for compartment two.

Multiple linear regression (Exp8)

Linear regression models are a subset of nonlinear regression models; consequently, linear models can also be fit using Phoenix. To illustrate this, a sample dataset (taken from *Analyzing Experimental Data By Regression* by Allen and Cady (1982), Lifetime Learning Publications, Belmont, CA) was analyzed in Exp8. Note that linear models can always be written as:

$$Y = B_0 + \sum_{i=1}^{n} X_i B_i$$

This example is also interesting in that the model was initially defined in such a way to permit several different models to be fit to the data.

In the ASCII model panel, note that the number of parameters to be estimated is defined in CONS in order to make the model specification as general as possible. Note also the use of a DO loop in the model text.

Note: When using Phoenix to fit a linear regression model:
- use arbitrary initial values
- make sure the **Do Not Use Bounds** option is checked
- select the Gauss-Newton minimization method with the Levenberg and Hartley modification

The dosing constant for this example is the number of terms to be fit in the regression.

Cumulative areas under the curve (Exp9)

The Exp9 example uses the TRANSFORM block of commands to output cumulative area under the curve values calculated by trapezoidal rule. It computes cumulative urine excretion then fits it to a one-compartment model. The use of the LAG function is demonstrated.

Mitscherlich nonlinear model (Exp10)

In the Exp10 example, a dataset is fit to the Mitscherlich model. The data were taken from Allen and Cady (1982), *Analyzing Experimental Data By Regression*. Lifetime Learning Publications, Belmont, CA. Fitting data to this model involves the estimation of three parameters; b_1, b_2, and α.

$$y = b_1 - b_2 \times \exp(-\alpha \times x)$$

Four parameter logistic model (Exp11)

The Exp11 example illustrates how to fit a dataset to a general four parameter logistic function. The function is often used to fit radioimmunoassay data. The function, when graphed, depicts a sigmoidal (S-shaped) curve. The four parameters represent the lower and upper asymptotes, the ED50, and a measure of the steepness of the slope. For further details see DeLean, Munson and Rodbard (1978). Simultaneous analysis of families of sigmoidal curves: Application to bioassay, radioligand assay and physiological dose-response curves. *Am J Physiol* 235(2):E97–E102. The model, with parameters a, b, c, and d is as follows:

$$y = \frac{(a-d)}{1+(x/c)^b} + d$$

Linear regression (Exp12)

The Exp12 example is based on work published by Draper and Smith (1981). *Applied Regression Analysis*, 2nd ed. John Wiley & Sons, NY.

Note: When doing linear regression in Phoenix, enter arbitrary initial estimates and make sure the **Do Not Use Bounds** option is checked. Use the Gauss-Newton minimization method with the Levenberg and Hartley modification

Indirect response model (IR)

The IR example is PD8 from the textbook: Gabrielsson and Weiner (2016). *Pharmacokinetic and Pharmacodynamic Data Analysis: Concepts and Applications*, 5th ed. Apotekarsocieteten, Stockholm. It uses an indirect response model, linking Phoenix pharmacokinetic model 11 to indirect response model 54. The PK data were fit in a separate run and are linked to the Indirect Response model. This can be done via the PKVAL command when using an ASCII model or via the PK parameters panel. Model 11 is a two-compartment micro constant model with extravascular input.

Ke0 link model (PKPD)

The PKPD example is PD10 from the textbook: Gabrielsson and Weiner (2016). *Pharmacokinetic and Pharmacodynamic Data Analysis: Concepts and Applications*, 5th ed. Apotekarsocieteten, Stockholm. It uses an effect compartment PK/PD link model. The drug was administered intravenously, and a one-compartment model is assumed (PK Model 1). The PD data is fit to a simple Emax model (PD Model 101). The pharmacokinetic data were fit in a separate run and are linked to the pharmacodynamic model.

Pharmacokinetic/pharmacodynamic link model (Exp15)

Rather than fitting the PK data to a PK model, an effect compartment is fitted and Ke0 estimated in the Exp15 example using the observed Cp data. Therefore it is a type of nonparametric model. The collapsed Ce values are then used to model the PD data. The example also illustrates how to mix differential equations and integrated functions. This approach was proposed by Dr. Wayne Colburn.

Core Output File

The Core Output is an ASCII text file that contains a complete summary of the model commands, options, parameters, and values for a model, as well as any errors that occurred during modeling. This file is generated as output for Indirect Response, Linear, Michaelis-Menten, PD, PK, PKPD, and ASCII Model objects.

Sections of the Core Output file include:

List of input commands
Minimization process
Final parameters
Variance-covariance matrix, correlation matrix, and eigenvalues
Residuals
Secondary parameters

List of input commands

The first section lists the input commands used to run the model. See "Commands, Arrays, and Functions" for an alphabetic listing of commands.

Minimization process

This section varies depending on the selected minimization method. If a Gauss-Newton method is used (methods 2 or 3), this page shows the parameter values and the computed weighted sum of squared residuals at each iteration. In addition, the following two values are listed for each iteration:

RANK: The rank of the matrix of partial derivatives of the model parameters. If the matrix is of full rank, then the rank equals the number of parameters. If the rank is less than the number of parameters, then the problem is ill-conditioned. That means there is not enough information contained in the data to precisely estimate all of the parameters in the model.

CONDITION NO.: Condition number of the matrix of partial derivatives. The condition number is the square root of the ratio of the largest to the smallest eigenvalue of the matrix of partial derivatives. If the condition number gets to be very large, for example greater than 10E+06, then the estimation problem is very ill-conditioned. If minimization methods 2 or 3 are used, then using lower and upper parameter boundaries can help reduce the condition number.

If the Nelder-Mead method is used, this section only shows the parameter values and the weighted sum of squares for the initial point, final point (the best or smallest sum of squares), and the next-to-best point.

Final parameters

This section lists the parameter estimates, the asymptotic estimated standard error of each estimate, and two intervals based on this estimated standard error. The intervals labeled UNIVAR_CI_LOW and UNIVAR_CI_UPP are the parameter estimates plus and minus the product of the estimated standard error and the appropriate value of the t-statistic. Univariate intervals should be applied individually to the relevant single parameters, and have the interpretation that there is a 95% probability that the interval contains the true value of the individual parameter.

The intervals labeled PLANAR_CI_LOW and PLANAR_CI_UPP are obtained from the tangent planes to the joint 95% ellipsoid of all the parameter estimates. The intervals are defined by the parameter estimates plus and minus the product of the standard error and the appropriate value of an F-statistic. The PLANAR intervals in general are larger than the corresponding UNIVARIATE intervals and jointly define a region in the shape of a rectangular box that contains the joint ellipsoid. This PLANAR region has the interpretation there is at least a 95% probability that this box contains the true parameter vector formed from the individual parameter values.

For an introductory discussion of the issues involved in UNIVARIATE and PLANAR intervals see page 95 (Draper and Smith (1981)). Details of the appropriate F-distribution statistic used in the PLANAR ellipsoidal and box region computations can be found in most advanced statistical texts.

The estimated standard errors and intervals are only approximate, because they are based on a linearization of a nonlinear model. The closer the model is to being linear, the better the approximation. See Chapter 10 of Draper and Smith (1981) for a complete discussion of the true intervals for parameter estimates in nonlinear models.

The estimated standard errors are valuable for indicating how much information about the parameters is contained in the data. Many times a model provides a good fit to the data in the sense that all of the deviations between observed and predicted values are small, but one or more parameter estimates have standard errors that are large relative to the estimate.

Variance-covariance matrix, correlation matrix, and eigenvalues

The next three sections of the Core Output show the correlation matrix of the estimates and the eigenvalues of the linearized form of the model. High correlations among one or more pairs of the estimates indicate that the UNIVAR limits are underestimates of the uncertainty in the parameter estimates and, although the data may be well fit by the model, the estimates may not be very reliable. Also, datasets that result in highly correlated estimates are often difficult to fit, in the sense that the Gauss-Newton algorithm will have trouble finding the minimum residual sum of squares.

The eigenvalues are another indication of how well the data define the parameters. If the parameter estimates are completely uncorrelated, then the eigenvalues are equal. A large ratio between the largest and smallest eigenvalue may indicate that there are too many parameters in the model. However, it is usually not possible to remove one parameter from a nonlinear model. For discussion of the use of eigenvalues in modeling see (Belsley, Kuh and Welsch (1980)).

Residuals

This section of the output lists the observed data, calculated predicted values of the model function, residuals, weights, the standard deviations (S) of the calculated function values and standardized residuals. Runs of positive or negative deviations indicate non-random deviations from the model and are indicators of an incorrect model and/or choice of weights.

Also in this section are the sum of squared residuals, the sum of weighted squared residuals, an estimate of the error standard deviation, and the correlation between observed and calculated function values. In nonlinear models the correlation is not a particularly good measure of fit. In most problems it is greater than 0.9, and anything less than 0.8 probably indicates serious problems with the data and/or model. Two statistical criterion for model selection and comparison, the AIC (Akaike (1978)) and SBC (Schwarz (1978)), are also listed in this section.

If the data is fit to a model for extravascular or constant infusion input, area under the curve (AUC) is listed at the end of this section of the Core Output. The AUC is computed by the linear trapezoidal rule. If the first time value is not zero, then WinNonlin generates a data point with time and concentration values of zero, to compute AUC from zero to the last time. Note that this AUC in the Core Output differs from the AUC in the Secondary Parameters results worksheet. The secondary parameter AUC is calculated using the model parameters.

Note: AUC values are always associated with the first dose and might not be meaningful if the data were obtained after multiple dosing or IV dosing, due to time zero extrapolation problems.

Secondary parameters

Any secondary parameters and their estimated asymptotic standard errors are listed in the last section. The secondary parameters are functions of the primary parameters listed in the Core Output. In the case of multiple-dose data, secondary parameters that depend on dose use the first dose in their computation. The standard errors of the secondary parameters are obtained by computing the linear term of a Taylor series expansion of the secondary parameters. Secondary parameters are the third level of approximation and their accuracy should be regarded with caution.

References

Akaike (1978). Posterior probabilities for choosing a regression model. *Annals of the Institute of Mathematical Statistics* 30:A9 –14.

Belsley, Kuh and Welsch (1980). *Regression Diagnostics.* John Wiley & Sons, New York.

Draper and Smith (1981). *Applied Regression Analysis*, 2nd ed. John Wiley & Sons, NY

Schwarz (1978).Estimating the dimension of a model. *Annals of Statistics* 6:461–4.

Commands, Arrays, and Functions

BASELINE command: Sets the (optional) baseline value for use analyzing effect data with NCA model 220.

BEGIN command: Indicates that all commands have been specified and processing should start.

BTIME command: Selects points within the Lambda Z time range to be excluded from computations of Lambda Z. In the following example, Lambda Z is to be computed using times in the range of 12 to 24, excluding the concentrations at 16 and 20 hours. These "excluded" points will, however, still be used in the computation of the pharmacokinetic parameters.

```
BTIME 12, 24, 2, 16, 20
```

If there are no times to be excluded, set `numexcl` to zero or leave it blank.
Do not mark a point for exclusion by setting the case weight to zero as that will also exclude this data point from the calculation of the PK parameters.

CARRYALONG command: Specifies variables from the input data grid to be copied into each of the output worksheets. These variables are not required for the analysis.

```
CARRY 3
CARR 1
CARRYALONG 2
```

See also the NCARRY command.

How Carry Along Data is Migrated to Output Multigrids

The data for carry along columns is moved to all output worksheets from PK (PK/PD) or NCA analyses, with the exception of the last worksheet, named Settings.

For all output worksheets other than the Summary Table, the carry along data will appear in the right-most column(s) of the worksheet. The first cell of data for any given profile within a carry along column will be copied over the entire profile range. There is one exception: generation of the "Summary Table" worksheets. The carry along columns will be the first columns, preceded only by any sort key columns.

Also, as there are almost the same number of observations in the raw dataset as in the Summary Table, all the cells within a profile of a carry along column are copied over to the Summary Table—not just the first value. There is one special case here as well: an NCA without a first data point of t=0, c=0. When such a data point does not exist on the raw dataset, depending on which NCA model is selected, the core program may generate a pseudo-point of t=0, c=0 to support all necessary calculations. In this event, the first cell within a carry along column is copied 'up' to the core-generated data value of 0,0.

COMMANDS (model block): The `COMMANDS` block allows Phoenix commands to be permanently inserted into a model. Following the `COMMANDS` statement, any Phoenix command such as `NPARAMETERS`, `NCONSTANTS`, `PNAME`, etc., may be given. To end the `COMMAND` section use the `END` statement.

```
COMMANDS
NCON 2
NPARM 4
PNAME 'A', 'B', 'Alpha', 'Beta'
END
```

CON (array): Contains the values of the constants, such as dosing information, used to define the model. The index N indicates the Nth constant.

```
CON(3)
```

The values specified by the CONSTANT command, described below, correspond to CON(1), CON(2), etc. respectively.

CONSTANTS command: Specifies the values of the constants (such as dosing information) required by the model. The arguments C1, C2, …, CN are the values.

```
CONSTANTS 20, 2.06, -1
CONS are 5, 2
```

The NCONSTANTS command must appear before CONSTANTS.

CONVERGENCE command: Specifies the convergence criterion, C. 0 < C < 0.1.
The default value is 10^{-4}.

```
CONVERGENCE=1.e-6
CONV .001
```

The test for convergence is:

$$\left|\frac{SSnew - SSold}{SSold}\right| < C$$

where SS is the residual sum of squares. When CONVERGENCE=0, convergence checks and halvings are turned off (useful for maximum likelihood estimates).

DATA command: Begins data input and optionally specifies an external data file. If arguments are omitted, the data are expected to follow the DATA statement. If the arguments are used, path is the full path of the dataset, up to 64 characters.

```
DATA
DATA 'C:\MYLIB\MYFILE.DAT'
```

DATE command: Places the current date in the upper right corner of each page in Core Output.

```
DATE
```

DHEADERS command: Statement indicating that data included within the model file contain variable names as the first row.

```
DHEADERS
```

If a DNAMES command is also included, it will override the names in the data.

DIFFERENTIAL model block: Statements in the DIFFERENTIAL-END block define the system of differential equations to fit. The values of the equations are assigned to the DZ array.

```
DIFFERENTIAL
DZ(1)=-K1+KE)*Z(1)+K2*Z(2)
DZ(2)=K1*Z(1)-K2*Z(2)
END
```

NDERIVATIVES must be used to set the number of differential equations to fit. NFUNCTIONS specifies the number of differential equations and/or other functions with associated data.

DINCREMENT command: Specifies the increment to use in the estimation of the partial derivatives. The argument D is the increment. 0 < D < 0.1. The default value is 0.0001

```
DINCREMENT is 0.001
DINC 0.005
```

DNAMES command: Assigns names to the columns on the dataset. The arguments 'name1', 'name2',…,'nameN' are comma- or space-separated variable names. Each may use up to eight letters, numbers or underscores, and must begin with a letter.

```
DNAMES 'Subject', 'Time', 'Conc'
```

The names appear in the output and may also be used in the modeling code. If both DNAMES and DHEADERS are included, DNAMES takes precedence.

DTA array: Returns the value of the Nth column for the current observation.

```
T=DTA(1)
W=DTA(3)
```

DTIME command: Specifies the time of the last administered dose. This value will appear on the Final Parameters table as Dosing_time. Computation of the final parameters will be adjusted for this dosing time.

```
DTIME 48
```

DZ array: Contains the current values of the differential equations. The index N specifies the equation, in the order listed in the DIFFERENTIAL block.

```
DZ(3)=-P(3)*Z(3)
```

The maximum value of N is specified by the NDERIVATIVES command.

F variable: Returns the value of the function for the current observation.

```
F=A*exp(-B*X)
```

FINISH command: Included for compatibility with PCNonlin Version 4. FINISH should immediately follow the BEGIN command.

```
BEGIN
FINISH
```

FNUMBER command: Indicates the column in the dataset that identifies data for the FUNCTION block when NFUNCTIONS > 1. The function variable values must correspond to the FUNCTION numbers, i.e., 1 for FUNC 1, 2 for FUNC 2, etc.

```
FNUMBER is 3
FNUM 3
```

FNUMBER should be specified prior to DATA.

FUNCTION model block: The FUNCTION-END block contains statements to define the function to be fit to the data. The argument N indicates the function number.

```
FUNC 1
F=A*EXP(B)+C*EXP(D)
END
FUNC 1
F=A*EXP(B)+C*EXP(D)
END
FUNC 2
F=Z(1)
END
```

A function block must be used for every dataset to be fit. NFUNCTIONS specifies the number of function blocks. The variable F must be assigned the function value. WT can be assigned a value to use as the weight for the current observation.

INITIAL command: Specifies initial values for the estimated parameters. The arguments I1, I2, I3, …, IN are the initial values, separated by commas or spaces.

```
INITIAL=0.01, 5.e2, -2.0
INIT are 0.02, 10, 1.3e-5
INIT 0.1 .05 100
```

INITIAL must be preceded by NPARAMETERS.

ITERATIONS command: Specifies the maximum number of iterations to be executed, N.

```
ITERATIONS are 30
ITER 20
```

If ITERATIONS=0, the usual output is produced using the initial estimates as the final values of the parameters.

LOWER command: Specifies lower bounds for the estimated parameters. The arguments L1, L2, L3, …, LN are the lower bounds, separated by commas or spaces.

```
LOWER 0 0 10
```

If LOWER is used, a lower bound must be provided for each parameter, and UPPER must also be used. LOWER must be preceded by NPARAMETERS.

MEANSQUARE command: Allows a specified value other than the residual mean square to be used in the estimates of the variances and standard deviations. The argument M replaces the residual sum of squares in the estimates of variances and standard deviations. If given, MEANSQUARE must be greater than zero.

```
MEANSQUARE=100
MEAN=1.0
```

METHOD command: In modeling, the METHOD command specifies the type of fitting algorithm Phoenix is to use. The argument N takes the following values:

1 Nelder-Mead Simplex
2 Levenberg and Hartley modification of Gauss-Newton (default)
3 Hartley modification of Gauss-Newton

In noncompartmental analysis, METHOD specifies the method to compute area under the curve. The argument N takes on the following values:

1 Lin/Log-Linear trapezoidal rule up to Tmax, and log trapezoidal rule after
2 Linear trapezoidal rule (Linear interpolation) (default)
3 Linear up/Log down rule: Uses the linear trapezoidal rule any time the concentration data is increasing and the logarithmic trapezoidal rule any time that the concentration data is decreasing.
4 Linear Trapezoidal (Linear/log Interpolation)

```
METHOD is 2
METH 3
```

MODEL command: In a command file, MODEL specifies the compiled or source model to use. If MODEL N is specified, model N is used from the built-in library. If MODEL N, PATH is used, model N from the source file path is used.

```
MODEL 5
MODEL 7, 'PK'
MODEL 3 'D:\MY.LIB'
```

MODEL LINK command: MODEL with the option LINK specifies that compiled models are to be used to perform PK/PD or Indirect Response modeling. M1 is the PK model number, and M2 is the model number of the PD model or Indirect Response model. When this option is used a PKVAL command must also be used.

```
MODEL LINK 4 101
PKVAL 10 1.5 1.0 1.0
MODE LINK 1, 53
PKVA 10, 2.5
```

See also PKVALUE.

NCARRY command: Specifies the number of carry-over variables specified. The argument N is the number of carry-over variables.

```
NCAR are 3
NCAR 1
```

NCARRY must be specified before CARRYALONG.

NCATRANS command: When using NCA, NCATRANS specifies that the Final Parameters output will present each parameter value in a separate column, rather than row.

```
NCATRANS
NCAT
```

NCONSTANTS command: The NCONSTANTS command specifies the number of constants, N, required by the model. If used, NCONSTANTS must precede CONSTANTS.

```
NCONSTANTS are 3
NCON 1
```

NDERIVATIVES command: Indicates the number, N, of differential equations in the system being fit.

```
NDERIVATIVES are 2
NDER 5
```

NFUNCTIONS command: Indicates the number, N, of functions to be fit (default=1).
There must be data available for each function. If used, NFUNCTIONS must precede DATA.

```
NFUNCTIONS are 2
NFUN 3
```

The functions can contain the same parameters so that different datasets can contribute to the parameter estimates. When fitting multiple functions one must delineate which observations belong to which functions. To do this, include a function variable in the dataset or use NOBSERVATIONS. See FNUMBER or NOBSERVATIONS.

NOBOUNDS command: Tells Phoenix not to compute bounds during parameter estimation.

```
NOBOUNDS
NOBO
```

Unless a NOBOUNDS command is supplied Phoenix will either use bounds that the user has supplied, or compute bounds.

NOBSERVATIONS command: Provides backward compatibility with PCNonlin command files. When more than one function is fit simultaneously, NOBS can indicate which observations apply to each function. The arguments N_1, N_2,…, N_F are the number of observations to use with each FUNCTION block. The data must appear in the order used: FUNC 1 data first, then FUNC 2, etc. Note that this statement requires counting the number of observations for each function, and updating NOBS if the number of observations changes.
NOBS must precede DATA.

```
NOBS 10, 15, 12
```

The recommended approach is to include a function variable whose values indicate the FUNCTION block to which the data belong. (See FNUMBER.)

NOPAGE command: Tells Phoenix to omit page breaks in the Core Output file.

```
NOPAGE
NOPAGE BREAKS ON OUTPUT
```

NOPLOTS command: Specifies that no plots are to be produced.

```
NOPLOTS
```

This command turns off both the high resolution plots and Core Output.

NOSCALE command: Turns off the automatic scaling of weights when WEIGHT is used. By default, the weights are scaled such that the sum of the weights equals the number of observations with non-zero weights.

```
NOSCALE
```

This command will have no effect unless WEIGHT is used.

NOSTRIP command: Turns off curve stripping for all profiles.

```
NOSTRIP
```

NPAUC command: Specifies the number of partial area under the curve computations requested.

```
NPAUC 3
```

See PAUC.

NPARAMETERS command: Sets the number of parameters, N, to be estimated. NPARAMETERS must precede INITIAL, LOWER or UPPER. (LOWER and UPPER are optional.)

```
NPARAMETERS 5
NPAR 3
```

See PNAMES.

NPOINTS command: Determines the number of points in the Predicted Data file. Use this option to create a dataset to plot a smooth curve. 10 <= N <= 20,000.

```
NPOINTS 200
```

For compiled models without differential equations the default value is 1000. If NDERIVATES> 0 (i.e., differential equations are used) this command is ignored and the number of points is equal to the number in the dataset. The default for user models is the number of data points. This value may be increased only if the model has only one independent variable.

NSECONDARY command: Specifies the number, N, of secondary parameters to be estimated.

```
NSECONDARY 2
NSEC=3
```

See SNAMES.

NVARIABLES command: Specifies the number of columns in the data grid. N is the number of columns (default=2). If used, NVARIABLES must precede DATA.

```
NVARIABLES 5
NVAR 3
```

P array: Contains the values of the estimated parameters for the current iteration. The index N specifies the Nth estimated parameter.

```
P(1)
T=P(3)-A
```

The values of the INITIAL command correspond to P(1), P(2), etc., respectively. NPARAMETERS specifies the value of N. PNAMES can be used to assign aliases for P(1), P(2), etc.

PAUC command: Lower, upper values for the partial AUC computations. If a specified time interval does not match times on the dataset, then Phoenix will generate concentrations corresponding to those times.

```
PAUC 0, 5, 17, 24
```

See NPAUC.

PKVALUE command: Used to assign parameter values to the PK model when using MODEL LINK.

```
MODEL LINK 4 101
PKVAL 10 1.5 1.0 1.0
MODE LINK 1, 53
PKVA 10, 2.5
```

PNAMES command: Specifies names associated with the estimated parameters. The arguments 'name1', 'name2', …,'nameN' are names of the parameters. Each may have up to eight letters, numbers, or underscores, and must begin with a letter. These names appear in the output and may be used in the modeling code.

```
PNAMES are 'ALPHA', 'BETA', 'GAMMA'
PNAM 'K01', 'K10', 'VOLU
```

The number of names specified must equal that for NPARAMETERS.

PUNITS command: Specifies the units associated with the estimated parameters. The arguments 'unit1', 'unit2', …,'unitN' are units and may be up to eight letters, numbers, operators or underscores in length. These units are used in the output.

```
PUNITS are 'hr', 'ng/mL', 'L/hr'
PUNI 'hr'
```

The number of units specified must equal that for NPARAMETERS.

REMARK command: Allows comments to be placed in a command file.

```
REMARK Y transformed to log(y)
REMA GAUSS-NEWTON
```

If a command takes no arguments or a fixed number of arguments, comments may be placed on the same line as a command after any required arguments.

REWEIGHT command: In the PK modeling module, REWEIGHT specifies that iteratively reweighted least squares estimates of the parameters are to be computed. The weights have the form WT=FW where W is the argument of REWEIGHT and F is the current value of the estimated functions. If W < 0 and F < 0 then the weight is set to zero.

```
REWEIGHT 2
REWE -.5
```

REWEIGHT is useful for computing maximum likelihood estimates of the estimated parameters. The following statement in a model is equivalent to a REWEIGHT command: WT=exp(W log(F)), or WT=F**W. This command is not used with noncompartmental analysis.

S array: Contains the estimated secondary parameters. The index N specifies the Nth secondary parameter.

```
S(1)=-P(1)/P(2)
```

The range of N is set by NSECONDARY. Aliases may be assigned for S(1), S(2), etc. using SNAMES. The secondary parameters are defined in the SECONDARY section of the model.

SECONDARY model block: The secondary parameters are defined in the SECONDARY-END block. Assignments are made to the array S.

```
SECONDARY
S(1)=0.693/K10
END
```

Or, if the command SNAMES 'K10_HL' and a PNAMES command with the alias K10 have been included:

```
SECONDARY
K10_HL=0.693/K10
END
```

NSECONDARY must be used to set the number of secondary parameters.

SIMULATE command: Causes the model to be estimated using only the time values in the dataset and the initial values. It must precede the DATA statement, if one is included.

```
SIMULATE
```

SIMULATE may be placed in any set of Phoenix commands. When it is encountered, no data fitting is performed, only the estimated parameters and predicted values from the initial estimates are computed. All other output such as plots and secondary parameters are also produced.

SIZE command: N is the model size. Model size sets default memory requirements and may range from 1 to 6. The default value is 2.

```
SIZE 3
```

Applicable only when using ASCII models.

SNAMES command: Specifies the names associated with the secondary parameters. The arguments 'name1', 'name2', …, 'nameN' are names of the secondary parameters, aliases for S(1), S(2), etc. Each may be up to eight letters, numbers or underscores in length, and must begin with a letter. They appear in the output and may also be used in the programming statements.

```
SNAMES are 'AUC', 'Cmax', 'Tmax'
SNAM 'K10_HL' 'K01_HL'
```

SORT command: Causes Phoenix to segment a large data file into smaller subsets. Each subset will be run through the modeling separately. Arguments s1 and the options s2, s3, etc. are the column numbers of the sort keys. The 1 that follows the command SORT has no function. It is included to provide compatibility with PCNonlin version 4.

```
SORT 1, 1
SORT 1 3 2
SORT 1, 1, 2
```

SORT must be on one line, i.e., it can not use the line continuation symbol '&.’

SPARSE command: Causes Phoenix to use sparse data methods for noncompartmental analysis. The default is to not use sparse methods. See “Sparse sampling calculation”.

STARTING model block: Statements in the `STARTING-END` block specify the starting values of the differential equations. Values are placed in the Z array. Parameters can be used as starting values.

```
START
Z(1)=CON(1)
Z(2)=0
END
```

STEADY STATE command: Informs Phoenix that data were obtained at steady state, so that different PK parameters are computed. # is Tau, the (assumed equal) dosing interval.

```
STEADY STATE 12
STEA 12
```

SUNITS command: Specifies the units associated with the secondary parameters. The arguments 'unit1', 'unit2', …,'unitN' are units and may be up to eight letters, numbers, operators or underscores in length. These units are used in the output.

```
SUNITS are 'ml', 'mL/hr'
SUNI '1/hr'
```

The number of units specified must equal `NSECONDARY`.

TEMPORARY model block: Statements placed on the `TEMPORARY-END` block define global temporary variables that can be used in the `MODEL` statements.

```
TEMP
T=X
DOSE1=CON(1)
TI=CON(2)
K21=(A*BETA+B*ALPHA)/(A+B)
K10=ALPHA*BETA/K21
K12=ALPHA+BETA-K21-K10
END
```

THRESHOLD command: Sets the (optional) threshold value for use analyzing effect data with NCA model 220. See “Drug effect data model 220” for details.

```
THRE 0
```

TIME command: Places the current time in the upper right hand corner of each output page.

```
TIME
```

TITLE command: Indicates that the following line contains a title to be printed on each page of the output. Up to five titles are allowed per run. To specify any title other than the first, a value N must be included with `TITLE`. 1 <= N <= 5.

```
TITLE
Experiment EXP-4115
```

TRANSFORM model block: Statements placed in the `TRANSFORM-END` block are executed for each observation before the estimation begins.

```
TRANSFORM
Y=exp(Y)
END
```

UPPER command: Sets upper bounds for the estimated parameters. The arguments U_1, U_2, U_3, …, U_N are the bounds, separated by commas or spaces.

```
UPPER 1, 1, 200
```

If UPPER is used, an upper bound must be provided for each parameter, and LOWER must also be specified. UPPER must be preceded by NPARAMETERS.

URINE command: Used to specify variables when doing noncompartmental analysis for urine data (Models 210–212). The user must supply: the beginning time of the urine collection interval, the ending times of the urine collection interval, the volume of urine collected, and the concentration in the urine. The four keywords LOWER UPPER VOLUME, and CONCENTRATION identify the required data columns; C1–C4 give their column numbers.

```
URINE LOWER=C1 UPPER=C2 VOLUME=C3 CONC=C4
URINE CONC C1 VOLU C2 LOWE C3 UPPE C4
```

The keywords may appear in any order, and only the first four letters of each keyword is required. Variables must be specified for all four keywords.

WEIGHT command: Activates weighted least squares computations. The argument W sets the weight value: YW. If W is not set, a column in the dataset provides weights—by default, the third column. Another column may be set using WTNUMBER.

WEIGHT	Uses the values in Column 3 as the weights
WEIGHT -.5	Uses as the weight 1/sqrt(Y)
WEIGHT WTNUM 7	Uses the values in Column 7 as the weights

The weights are scaled so that their sum = the number of observations with non-zero weights. See also WTNUMBER, WT, REWEIGHT and NOSCALE.

WT variable: In PK modeling, WT contains the weight for the current observation.

```
WT=1/(F*F)
```

If the variable WT is included in a model, then any weighting indicated via the *Variables and Weighting* dialog will be ignored.

WTNUMBER command: Specifies which column of the dataset contains weights. Not used for NCA.

```
WTNUMBER 4
WTNU is 7
```

If WEIGHT is used and WTNUMBER is not, the default column for weights is 3.

X variable: Contains the current value of the independent variable.

```
F=exp(-X)
```

By default, when used in a command file, the values of X are taken from the first column of the dataset unless the XNUMBER command has been used. If the variable is raised to a negative power, enclose the power in parentheses or write the expression as a fraction. For example, X**–2 must be written as X**(–2) or 1/X**2.

XNUMBER command: Sets the column number, N, of the independent variable. If differential equations are being fit, XNUMBER indicates the column number that the differential equations are integrated with respect to. The default value is 1.

```
XNUMBER is 3
XNUM 1
```

XNUMBER should precede DATA.

Y command: Current value of the dependent variable.

```
Y=log(Y)
```

By default, Y is assumed to be in the 2nd column unless YNUMBER is used.

YNUMBER command: The YNUMBER command specifies the column number, N, of the dependent variable. When running Version 4 command files, the default value is 2.

```
YNUMBER is 4
YNUM 3
```

The YNUMBER command should precede the DATA command.

Z array: Contains the current values of the solutions of the differential equations. The index N specifies the Nth differential equation.

```
B=A*Z(2)
```

The maximum value of N is set by NDERIVATIVES.

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