Pharmechanics

We help you analyze your pharmacology data

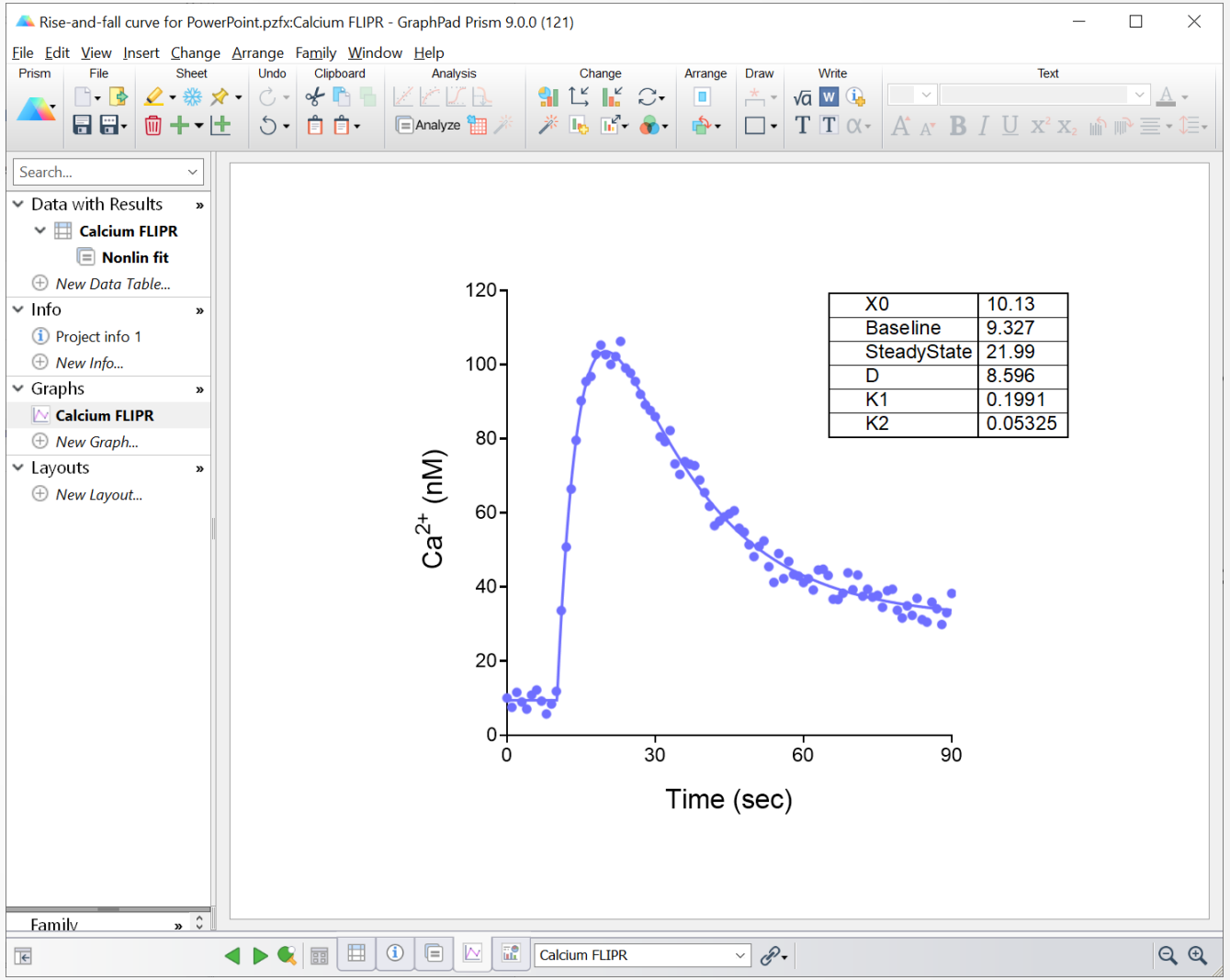
New time course equations for GraphPad Prism

Sam Hoare, PhD
sam.hoare@pharmechanics.com

October 30 2020

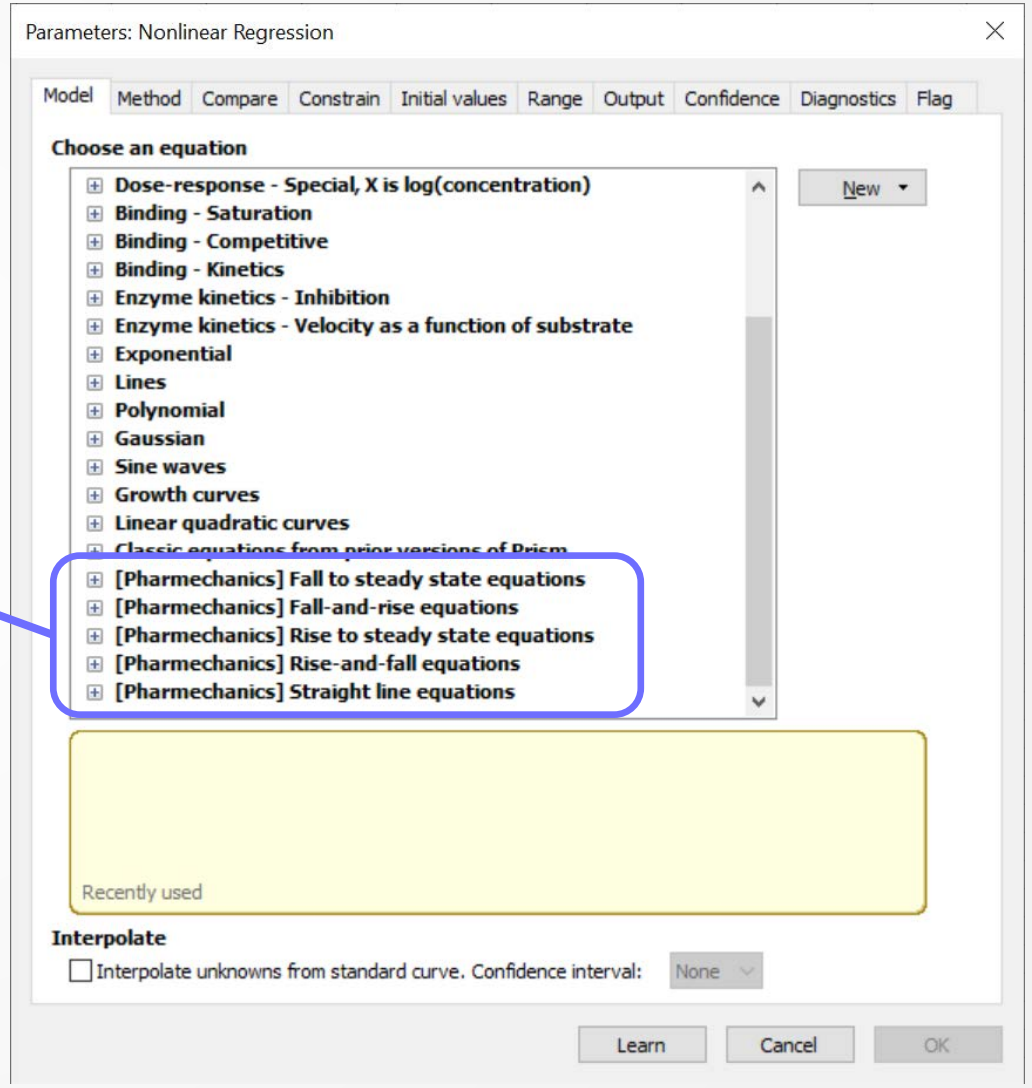
Outline

- Time course data are often described by unfamiliar equations such as the rise-and-fall equation.
- These equations have been loaded into GraphPad Prism in custom templates designed by Pharmechanics.
- We are grateful to Harvey Motulsky at GraphPad Software for guidance.

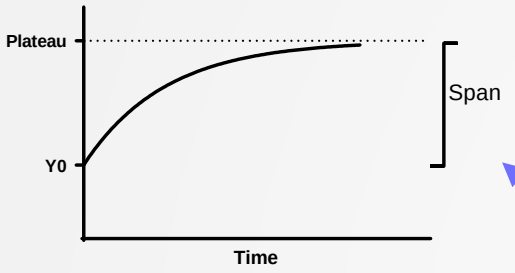


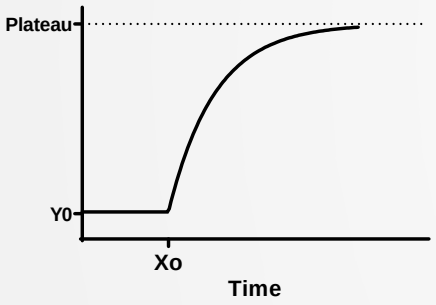
Overview

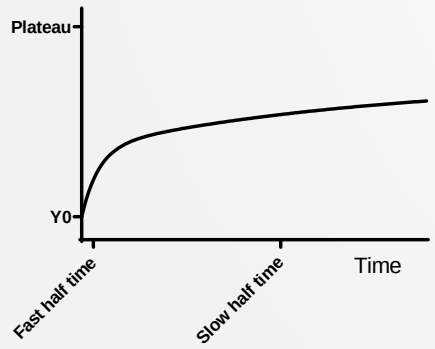
- Download the relevant Prism template from here.
- The equations can be loaded in batch or individually into your equation library as described here and on Slide 16.
- Questions? Email sam.hoare@pharmechanics.com
- The equations are supported by Pharmechanics, not by GraphPad.

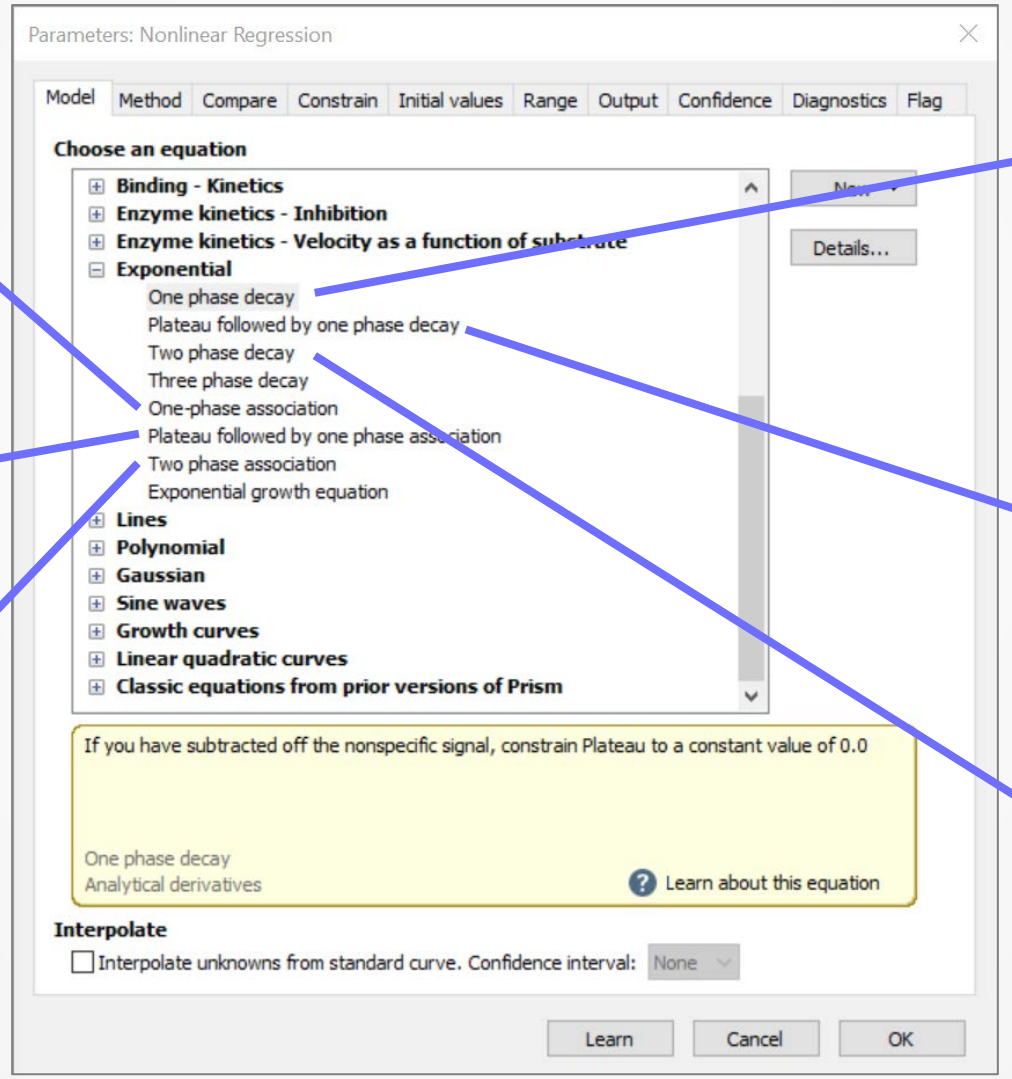


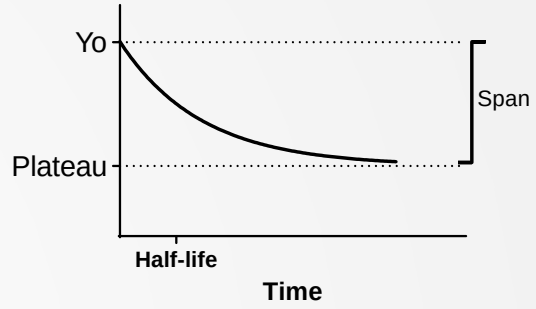
Familiar time course equations built into Prism

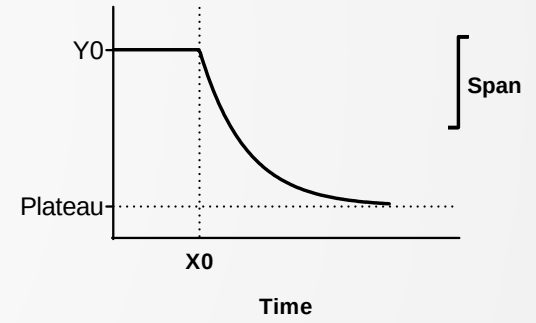


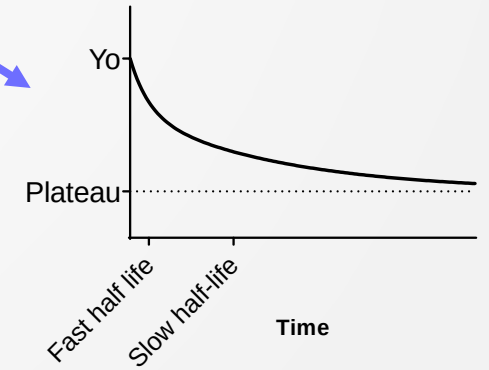






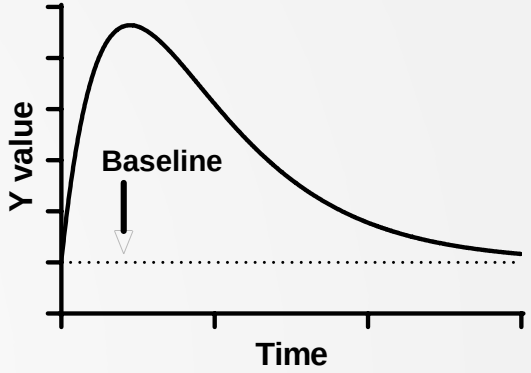


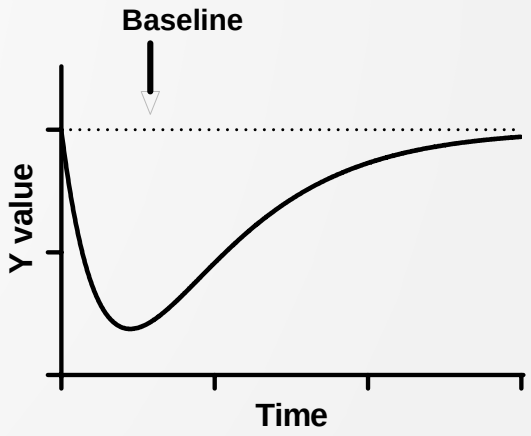




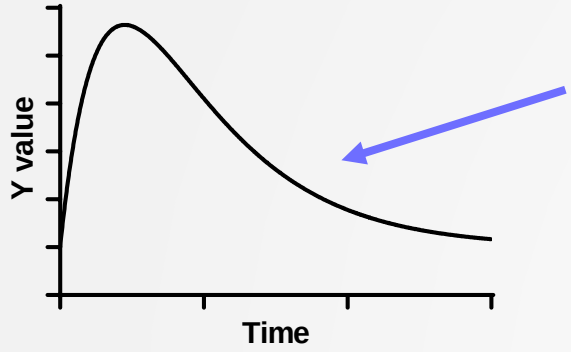
New time course equations from Pharmechanics

- New equations for rise-and-fall curves have been introduced by Pharmechanics.
- Upward and downward versions available.
- In addition, the familiar time course equations (straight line, association & dissociation curves) have been rewritten to be in the same format as these new equations.





Five files of equations

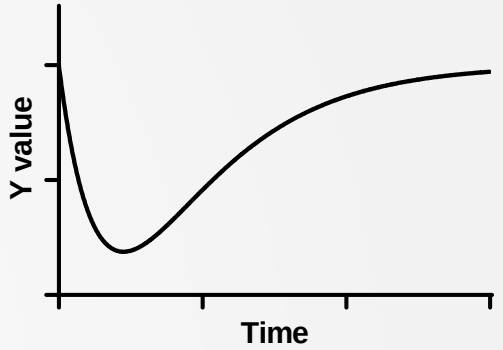


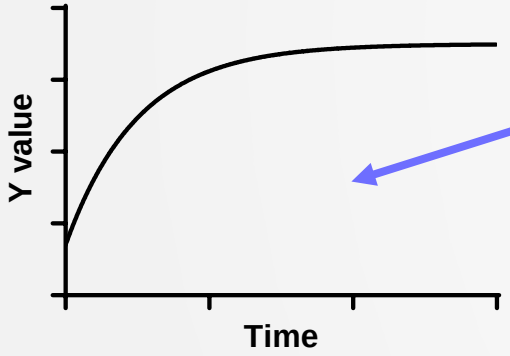
1. Rise-and-fall equations

Y increases over time, peaks, then falls back down.

2. Fall-and-rise equations

Y decreases over time, troughs, then rises back up.



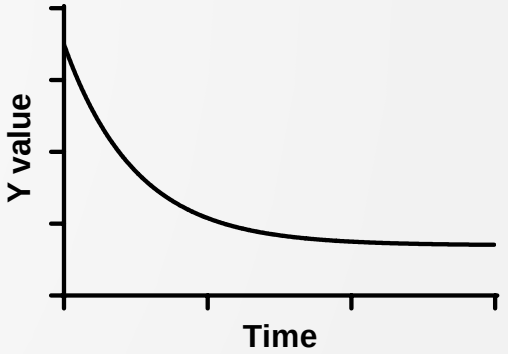


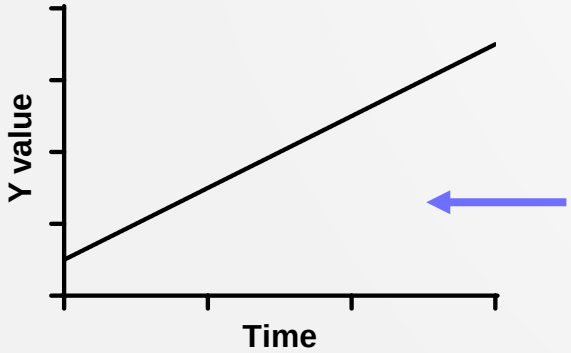
3. Rise to steady state equations

Y rises to a steady state plateau. Reformat of “One phase association” equation in Prism.

4. Fall to steady state equations

Y falls to a steady state plateau. Reformat of “One phase decay” equation in Prism.





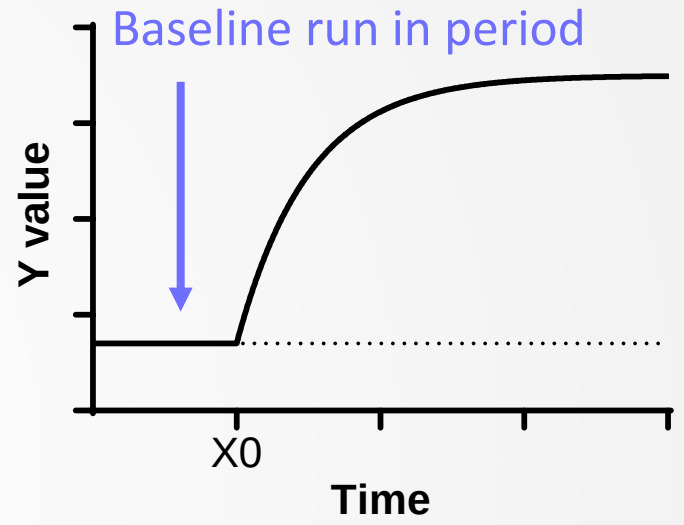
5. Straight line equations

Y rises or falls linearly over time. Reformat of “Straight line” equation in Prism.

Baseline considerations

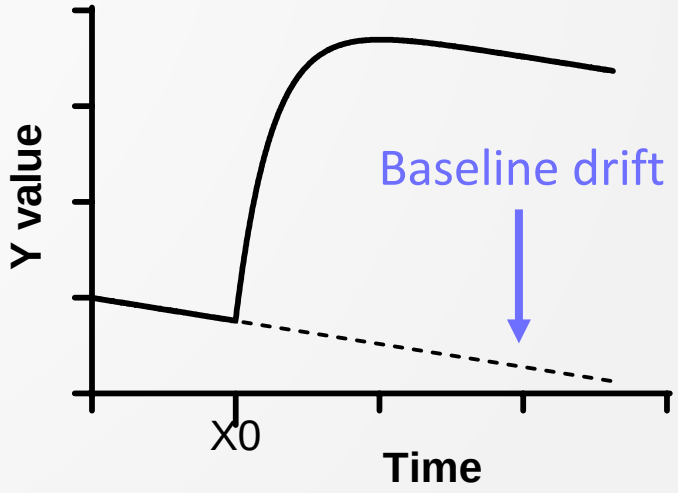
In some experiments, baseline signaling is recorded before compound is added.

Equations are available that incorporate this baseline run in period.



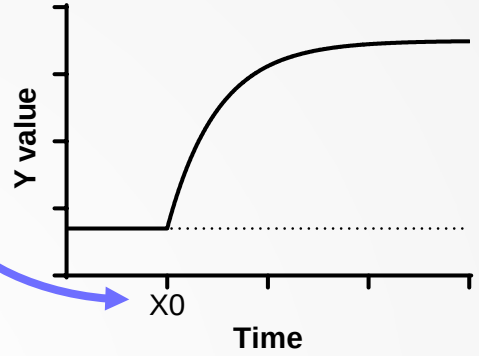
Occasionally, the baseline drifts over time.

Equations are available that incorporate this baseline drift

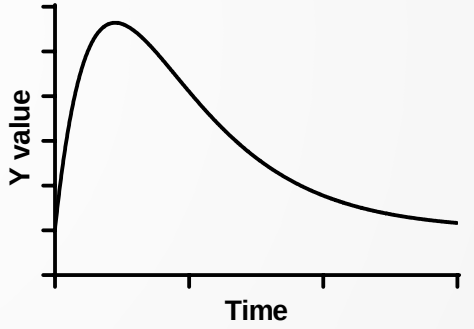


Analysis tips

When there is a baseline run in period, the initial value of the parameter X0 often needs to be entered manually for the fit to work. See here.



For the bell or U-shaped curves, K1 is the faster of the two rates and K2 is the slower. Usually, but not always, K1 is the rate of the first phase and K2 the rate of the second.



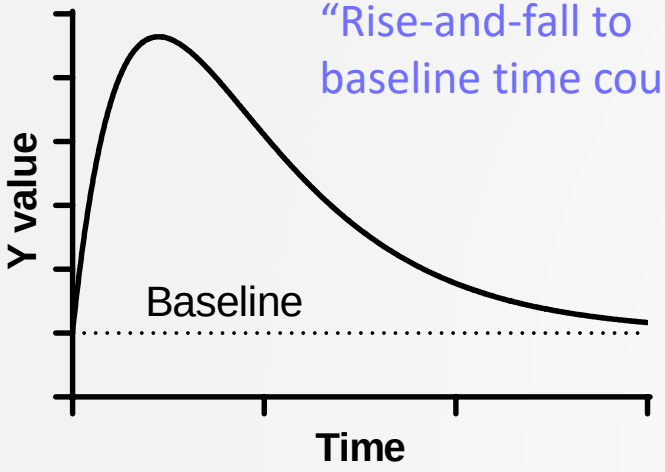
The equations are written out in the “Time course equation list” document here and can be viewed from the “Details” tab in the “Nonlinear regression” dialogue in Prism.

Time course equation list

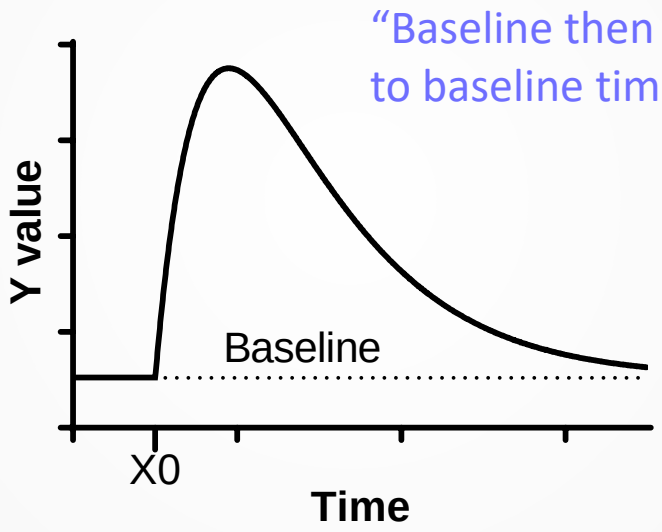
Rise-and-fall equations

In “Rise-and-fall equations” file

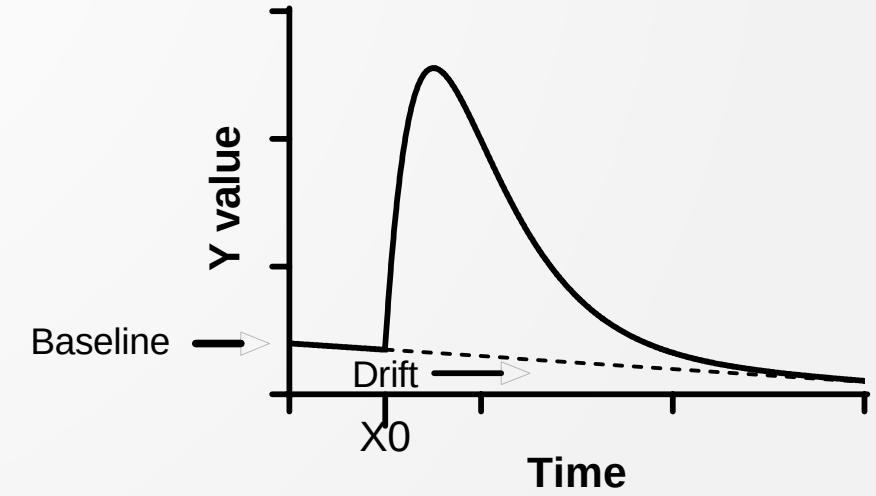
“Rise-and-fall to baseline time course”



“Baseline then rise-and-fall to baseline time course”



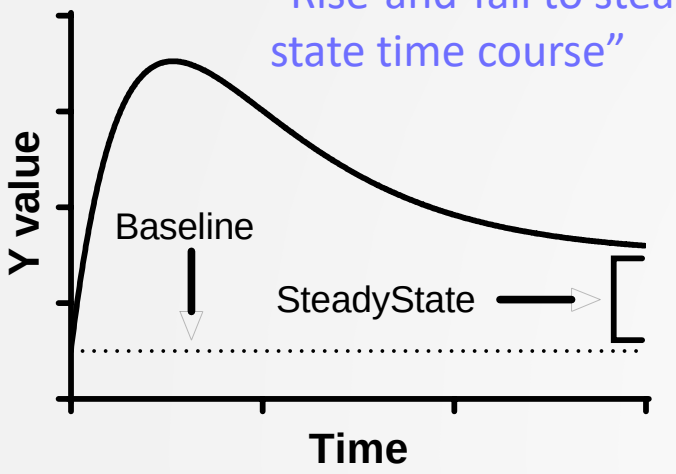
“Baseline then rise-and-fall to baseline with drift”



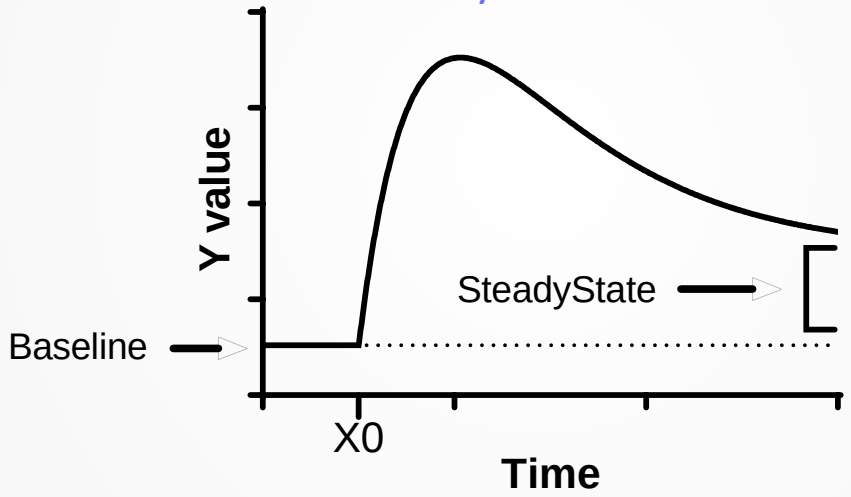
Rise-and-fall equations

In “Rise-and-fall equations” file

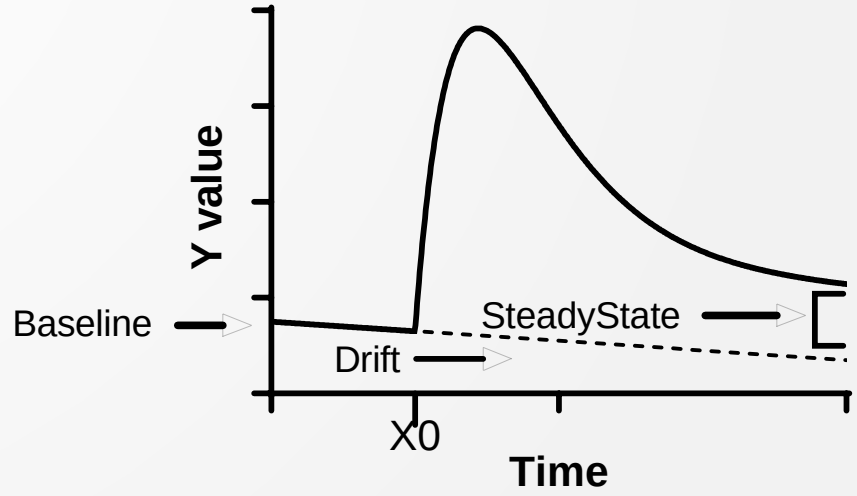
“Rise-and-fall to steady state time course”



“Baseline then rise-and-fall to steady state time course”



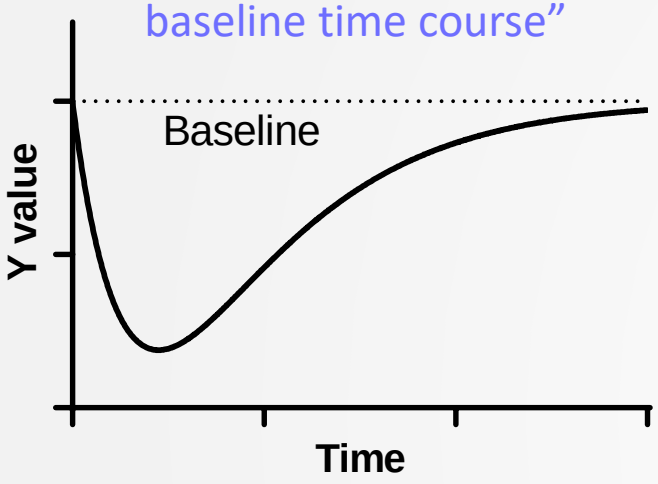
“Baseline then rise-and-fall to steady state with drift”



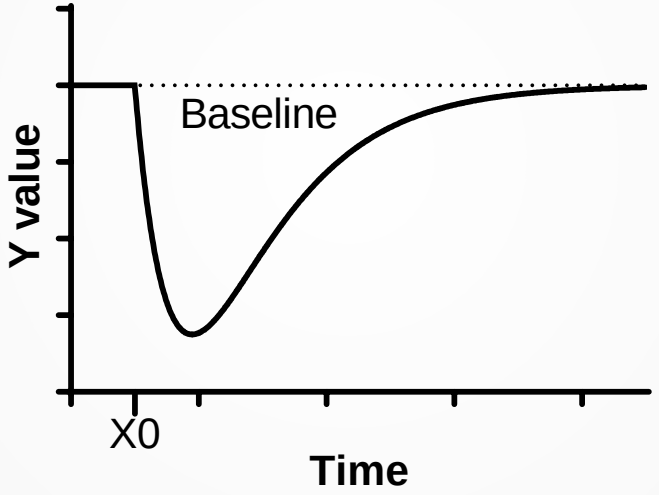
Fall-and-rise equations

In “Fall-and-rise equations” file

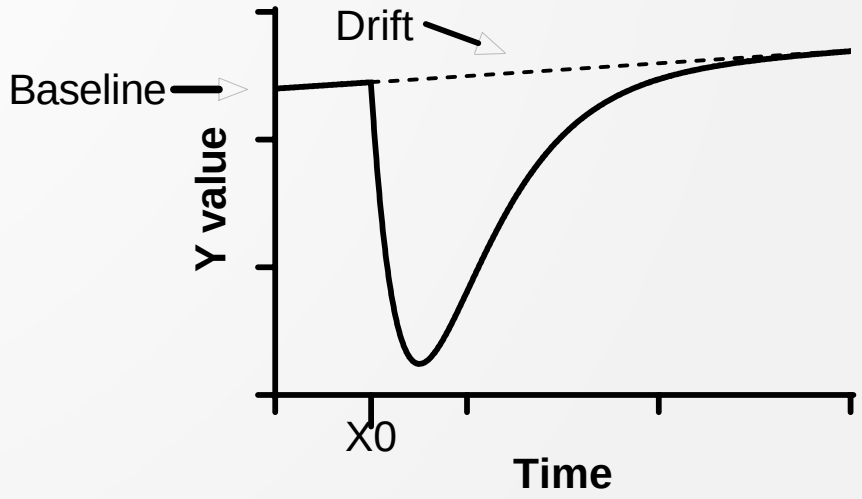
“Fall-and-rise to baseline time course”



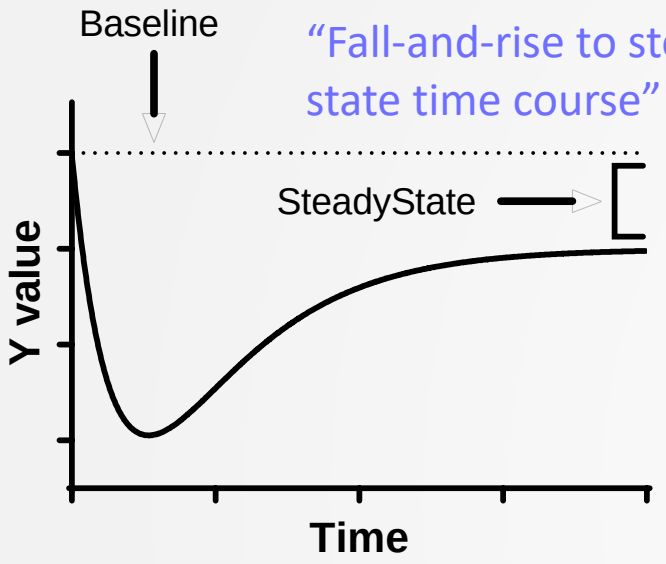
“Baseline then fall-and-rise to baseline time course”



“Baseline then fall-and-rise to baseline with drift”

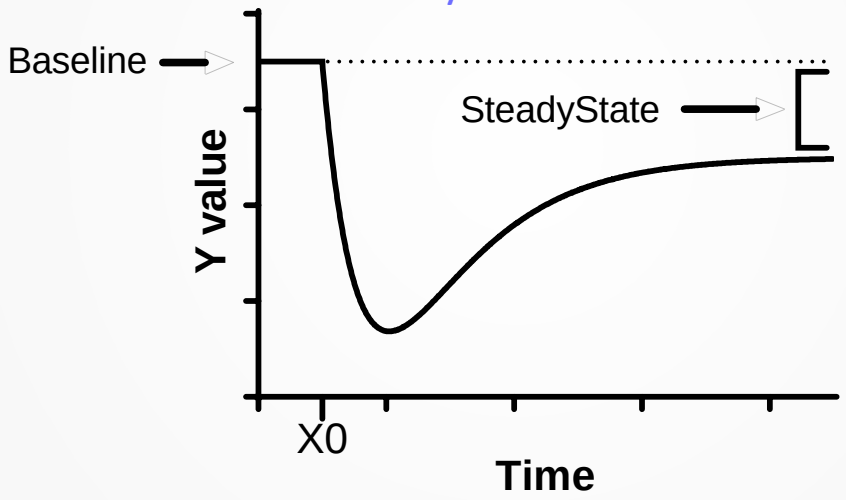


Fall-and-rise equations

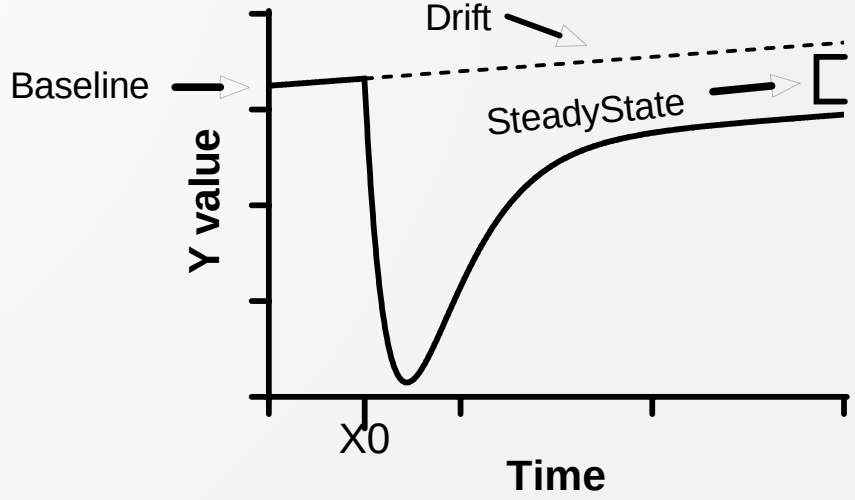


"Fall-and-rise to steady state time course"

In "Fall-and-rise equations" file



"Baseline then fall-and-rise to steady state time course"

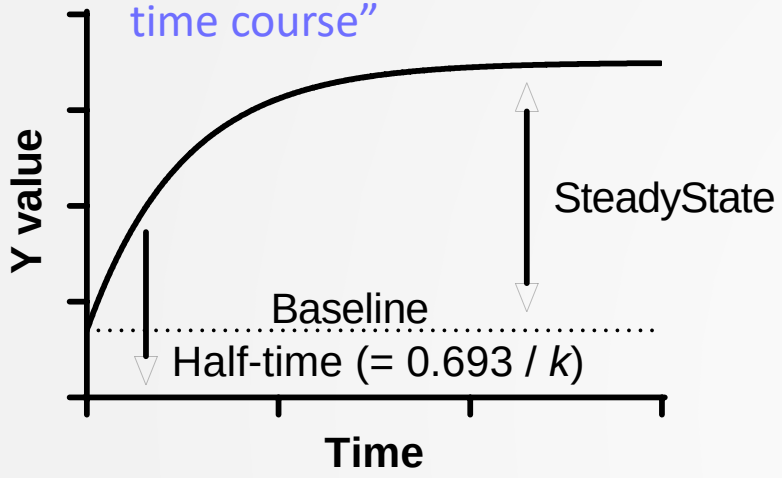


"Baseline then fall-and-rise to steady state with drift"

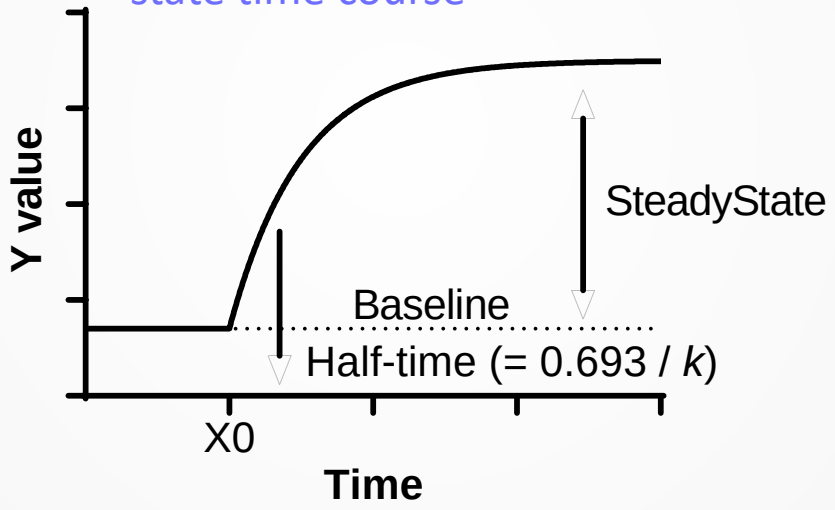
Rise to steady state equations

In “Rise to steady state equations” file

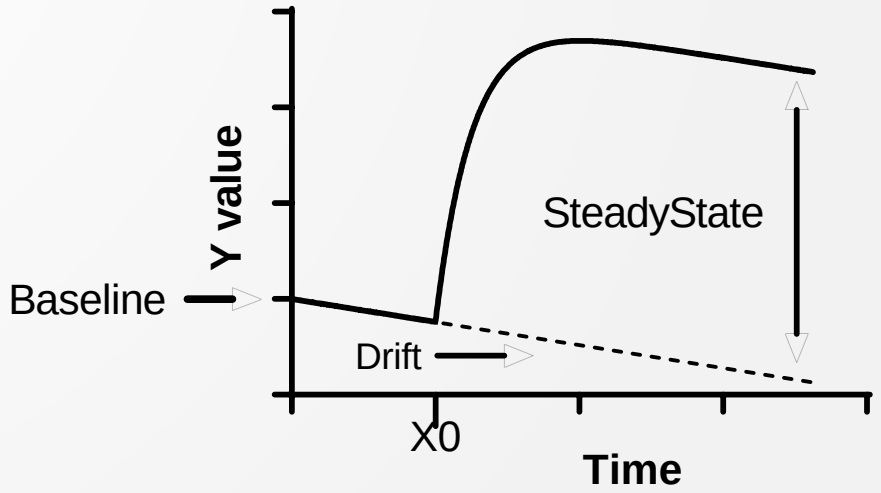
“Rise to steady state time course”



“Baseline then rise to steady state time course”



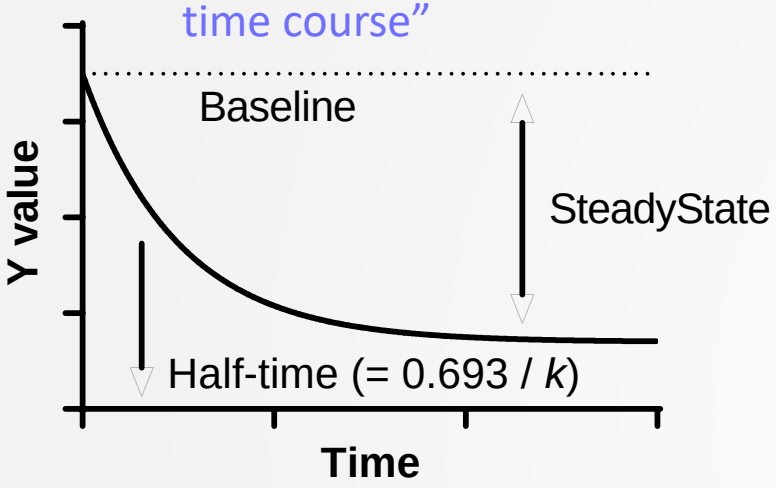
“Baseline then rise to steady state with drift”



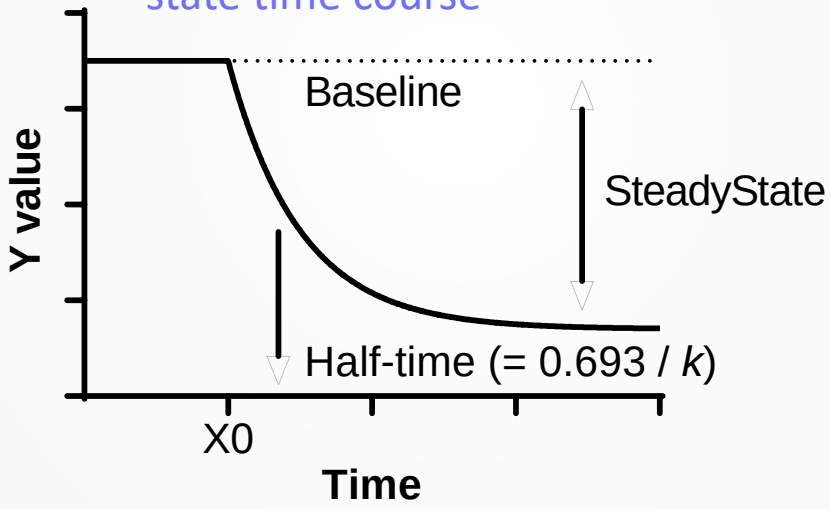
Fall to steady state equations

In “Fall to steady state equations” file

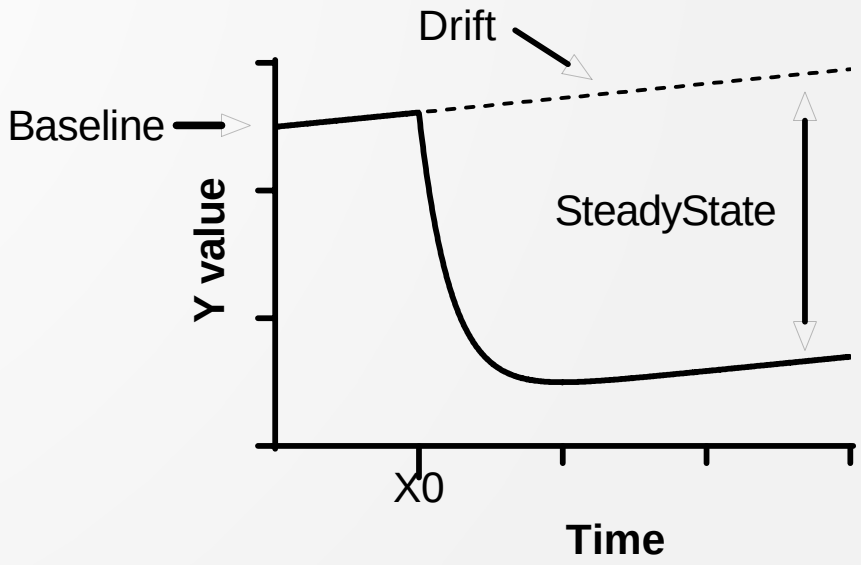
“Fall to steady state time course”



“Baseline then fall to steady state time course”



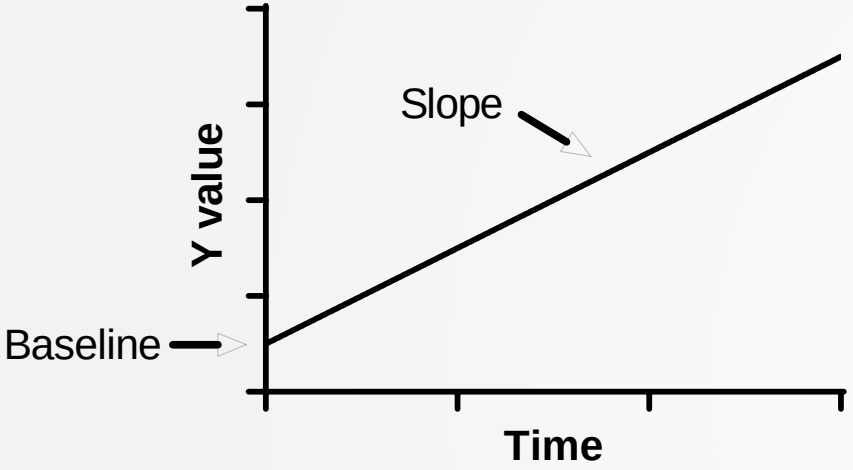
“Baseline then fall to steady state with drift”



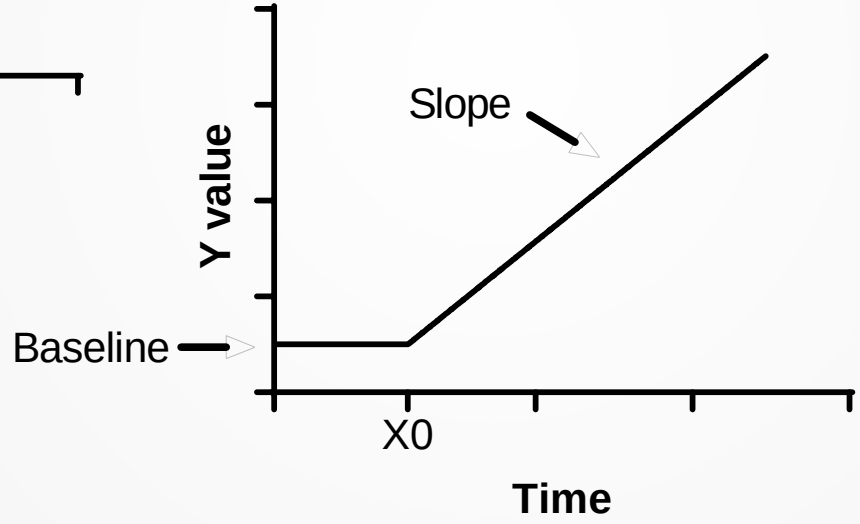
Straight line equations

In “Straight line equations” file

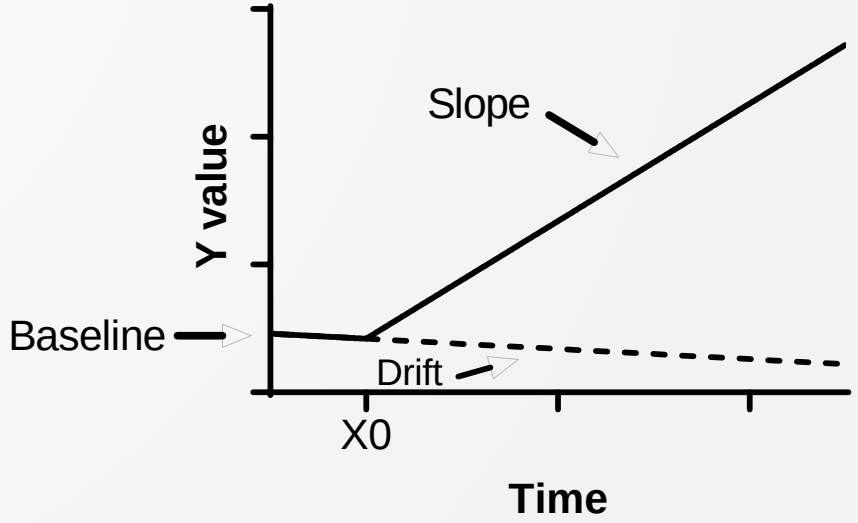
“Straight line time course”



“Baseline then straight line time course”



“Baseline then straight line with drift”

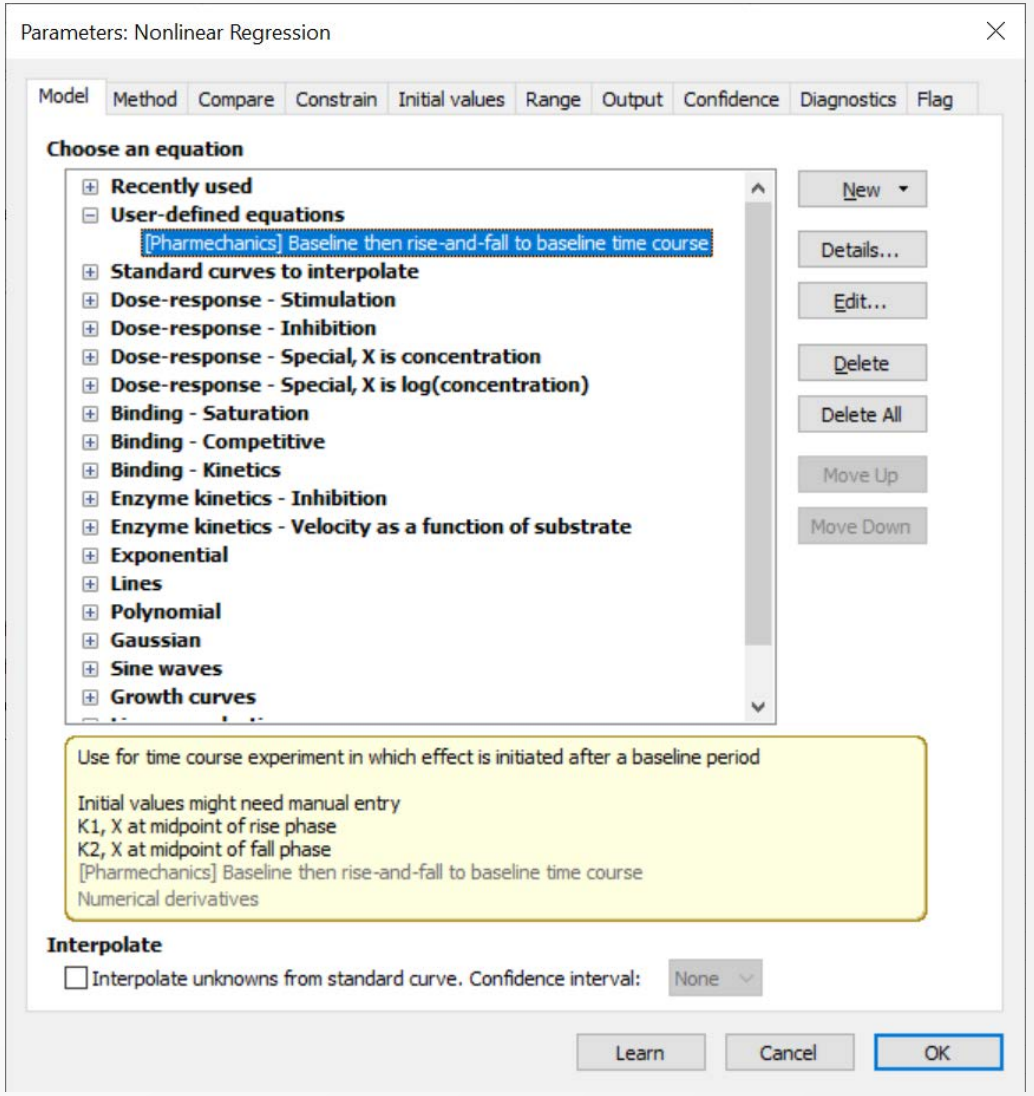


Loading equations into Prism from a file

GraphPad Prism contains an equation editor for the input of user-defined equations.

There are sharing methods that simplify the loading of equations written by other users.

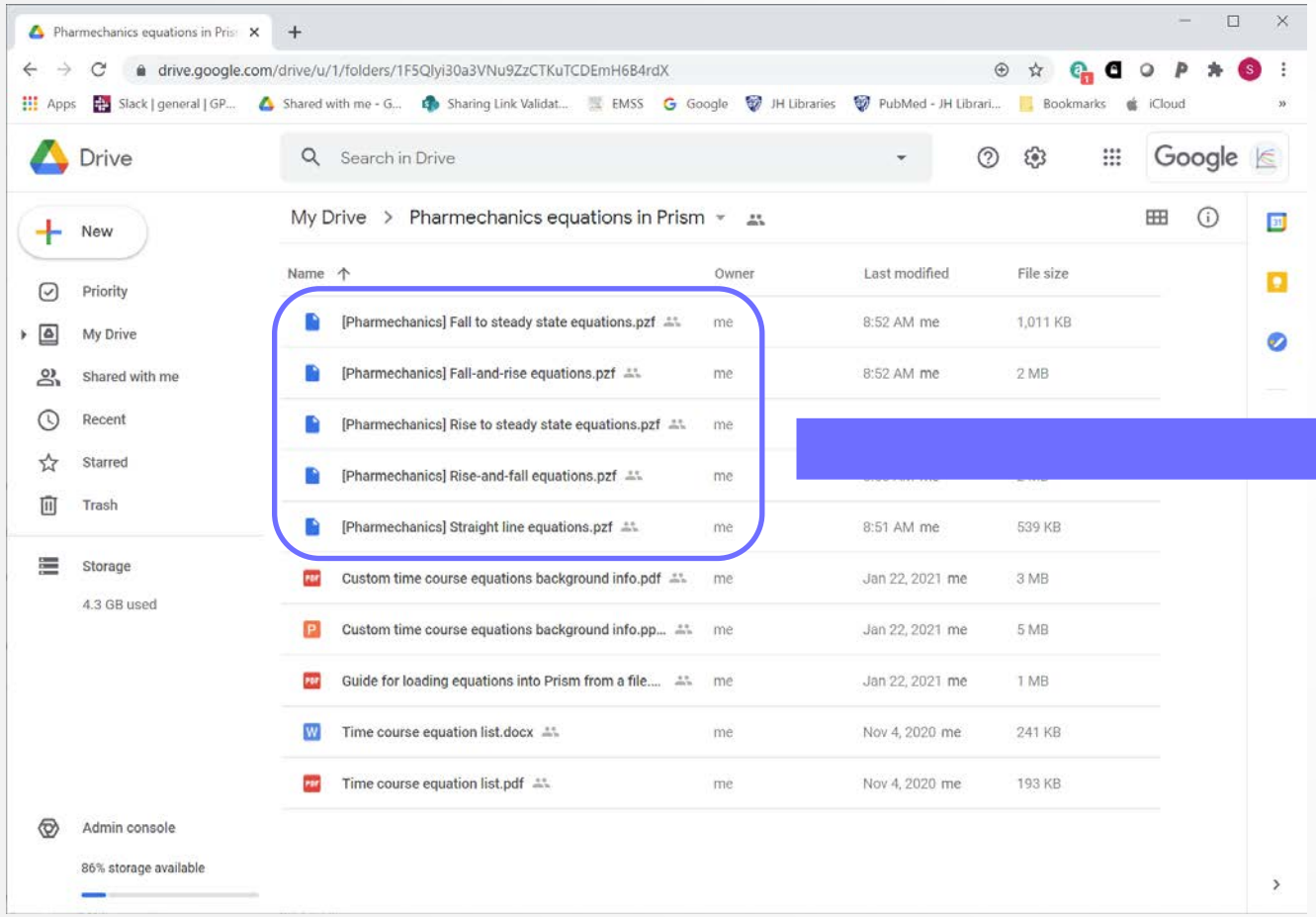
This avoids the need to write in the equation and all the fit settings.

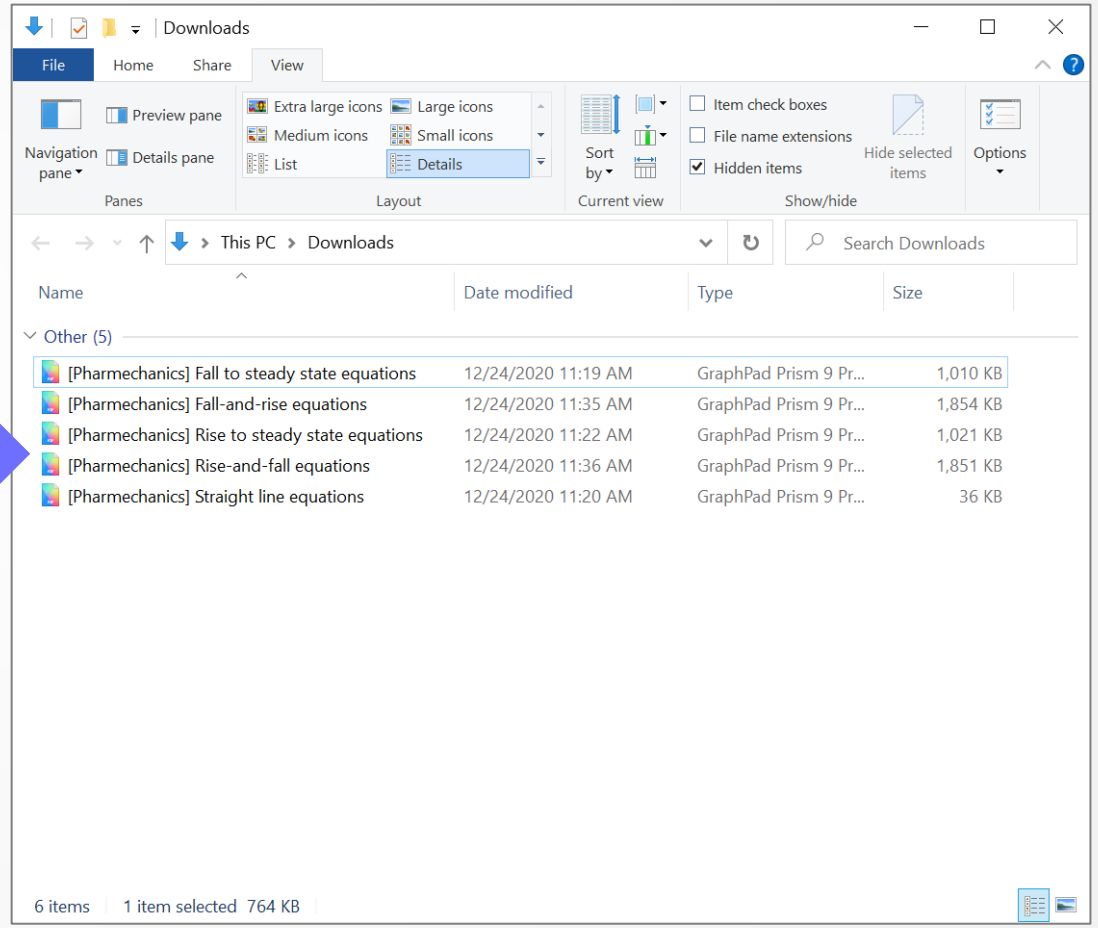


First, download the files containing the equations to your computer. Here we are going to do all of the collections but you can select only the collection you need.

The files are located on an open-access Google Drive at:

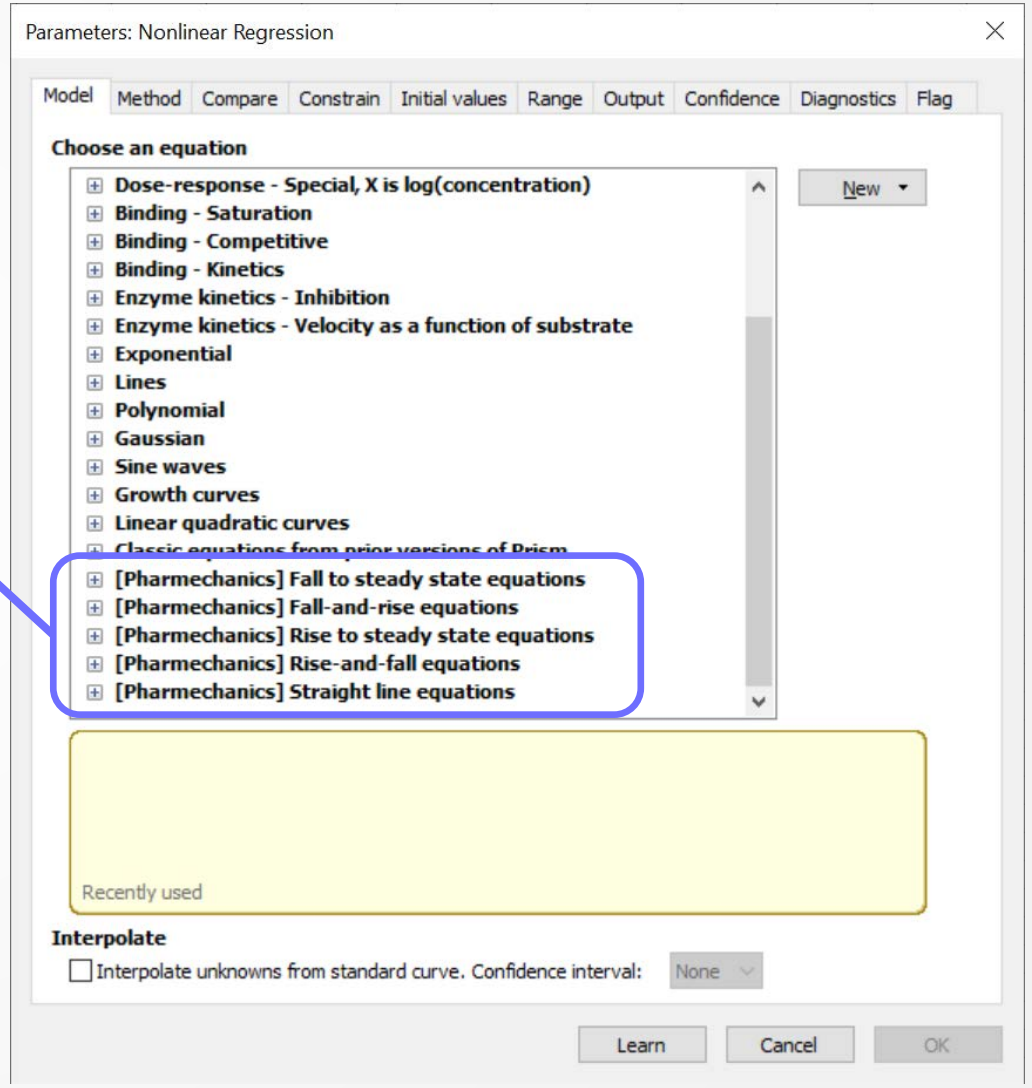
https://drive.google.com/drive/folders/1F5Qlyi30a3VNu9ZzCTKuTCDEmH6B4rdX?usp=sharing





Loading equations in batch

This will load a collection of equations into subfolders in the analysis dialogue.



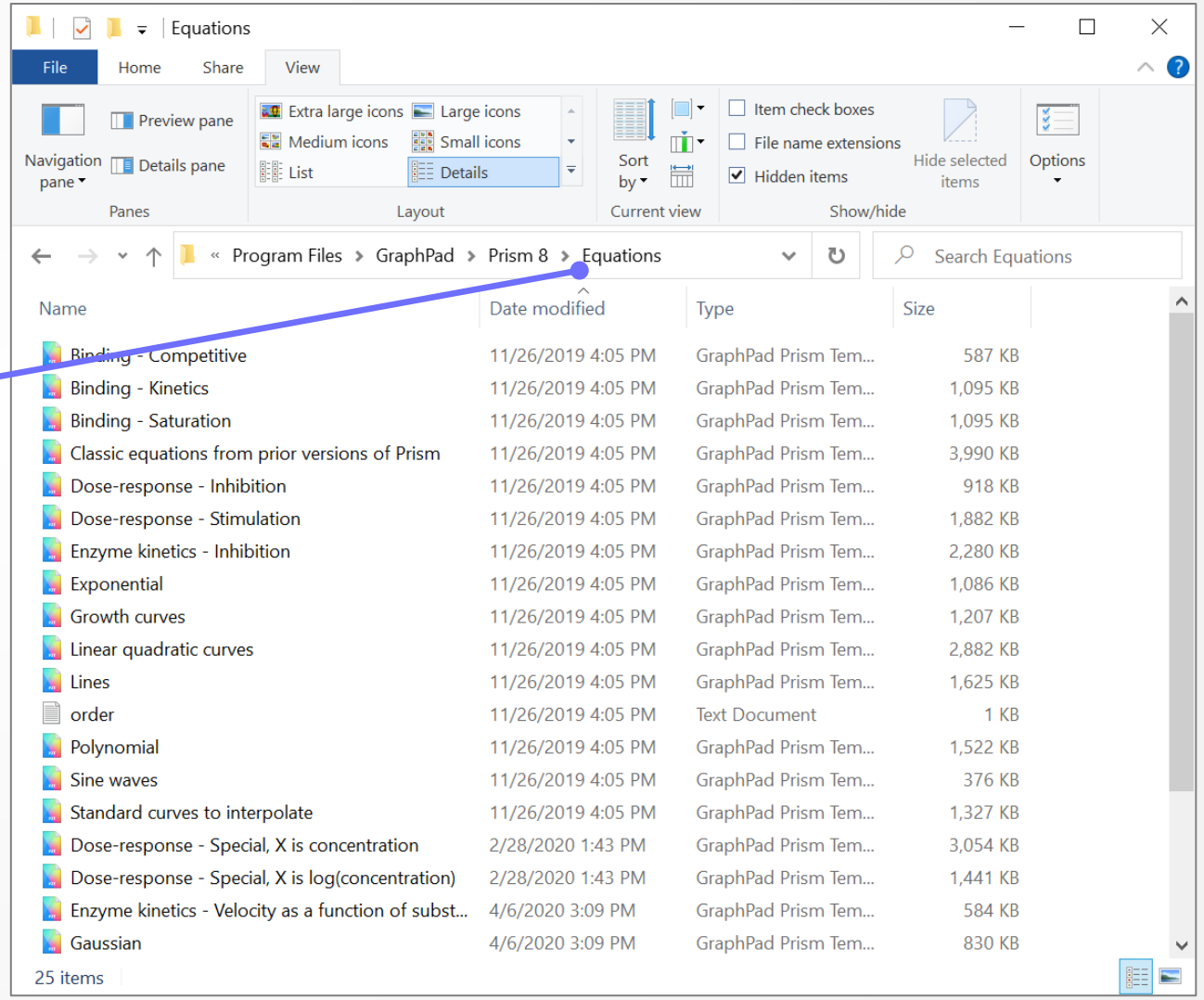
Loading equations in batch

First, if Prism is open, close it.

Navigate to the Prism program files location on your computer.

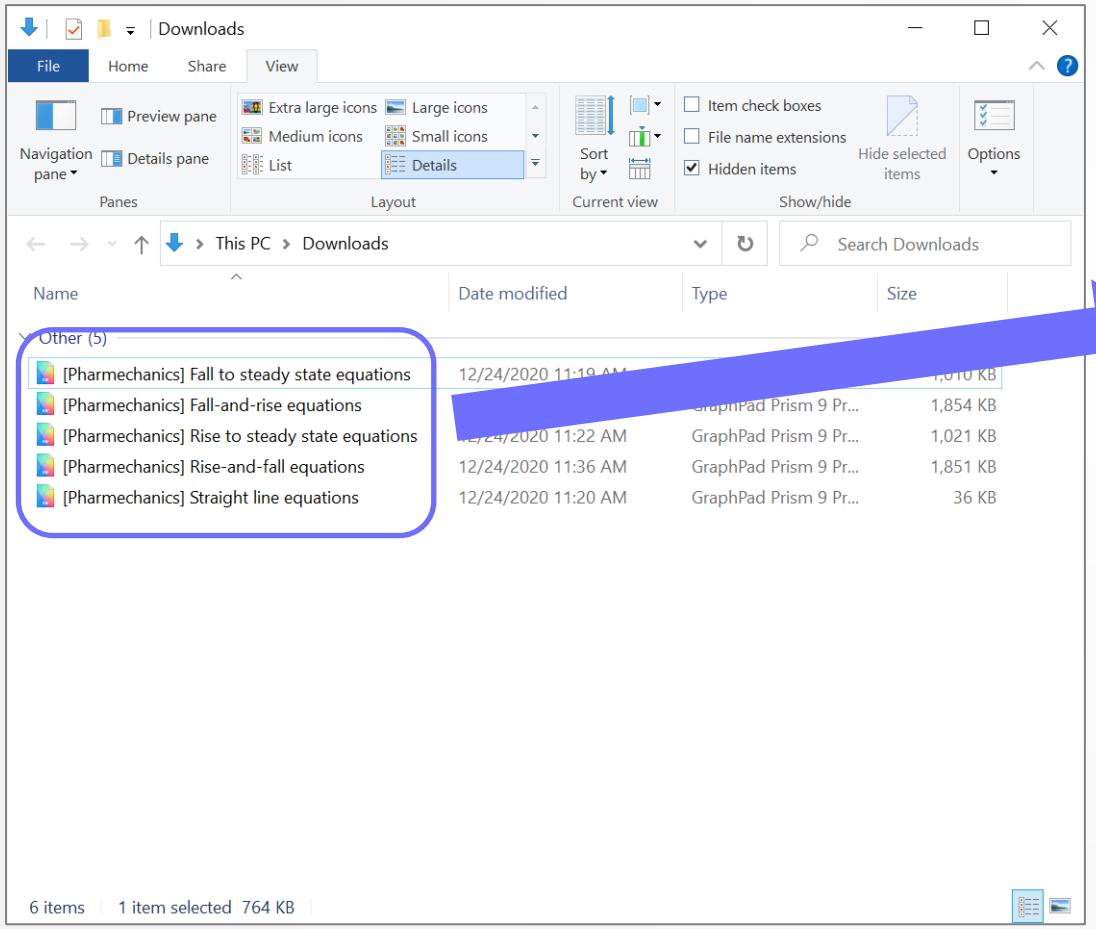
Open the “Equations” folder.

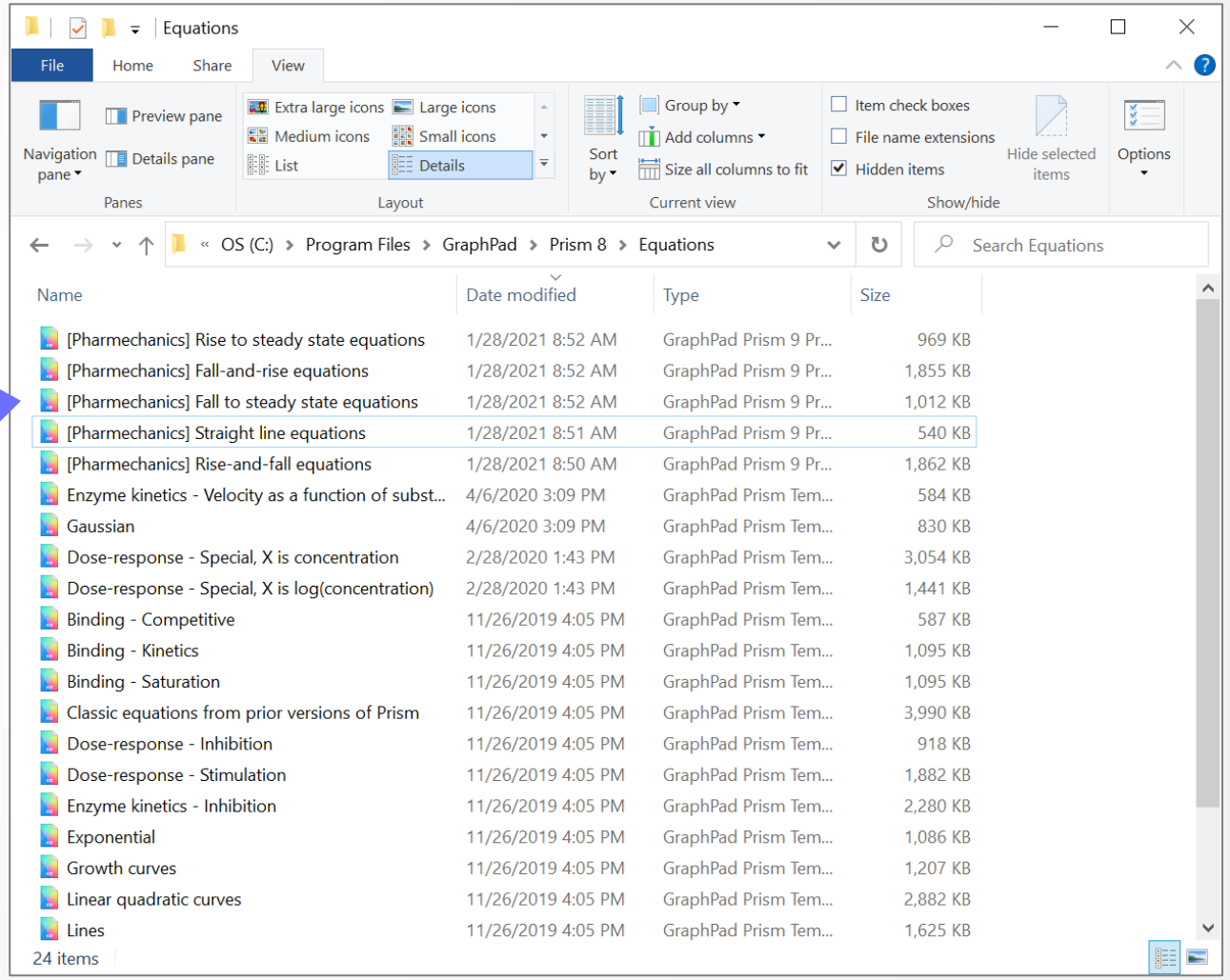
Be very careful not to delete any of the files here – this is the equation library Prism uses.



Loading equations in batch

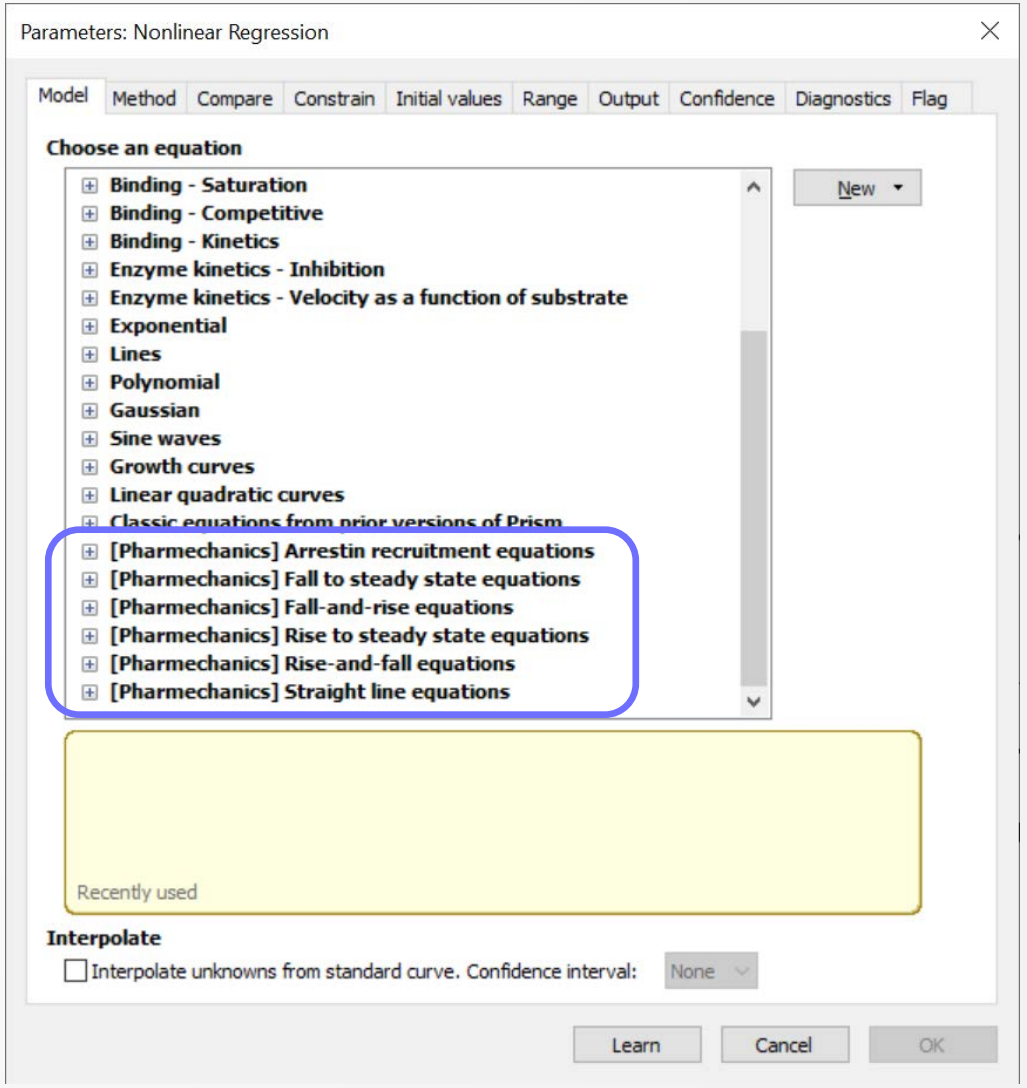
Now copy the equations files into the “Equations” folder.





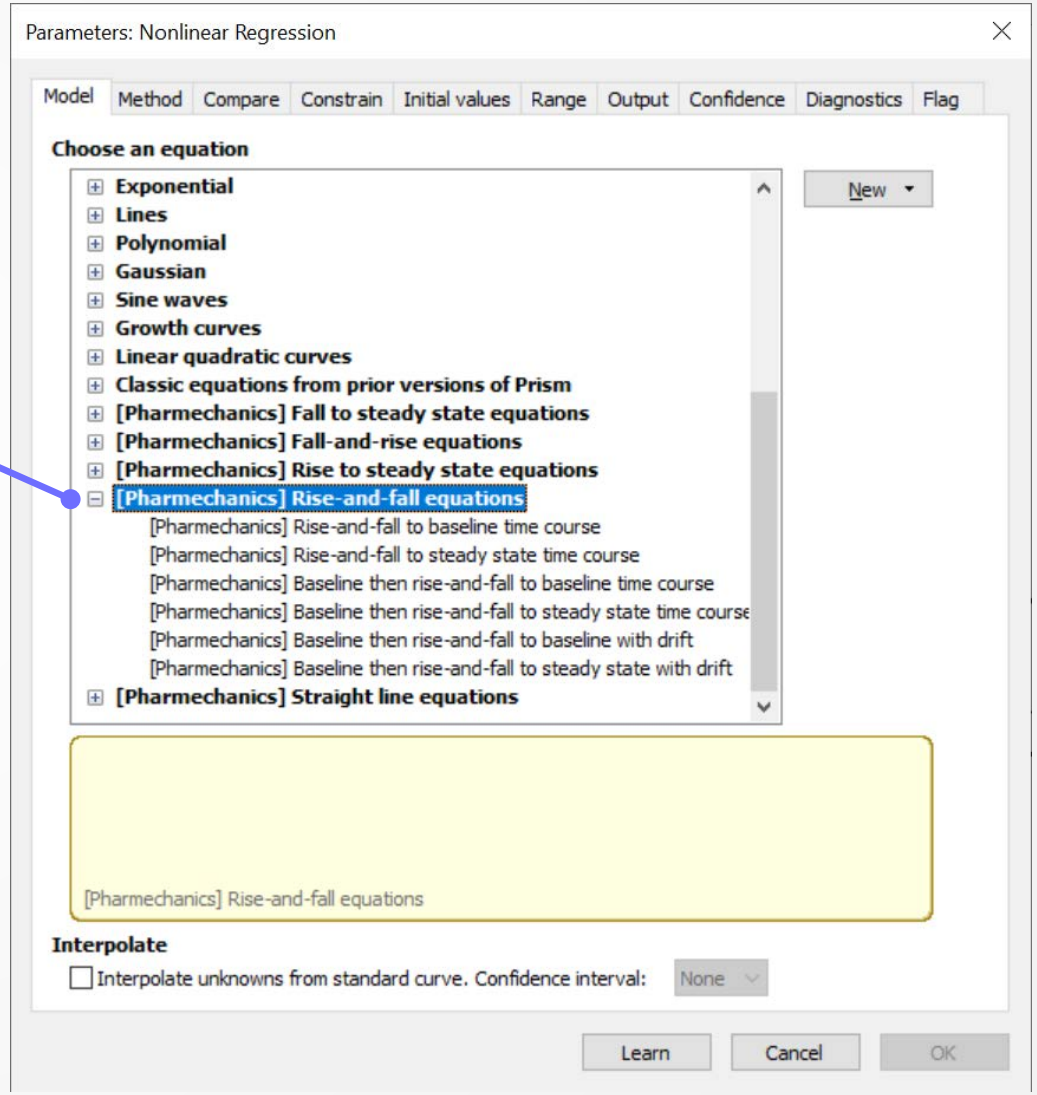
Loading equations in batch

Open Prism, navigate to the “Nonlinear Regression” dialogue and in the “Model” tab the equation folders will appear at the bottom of the list.



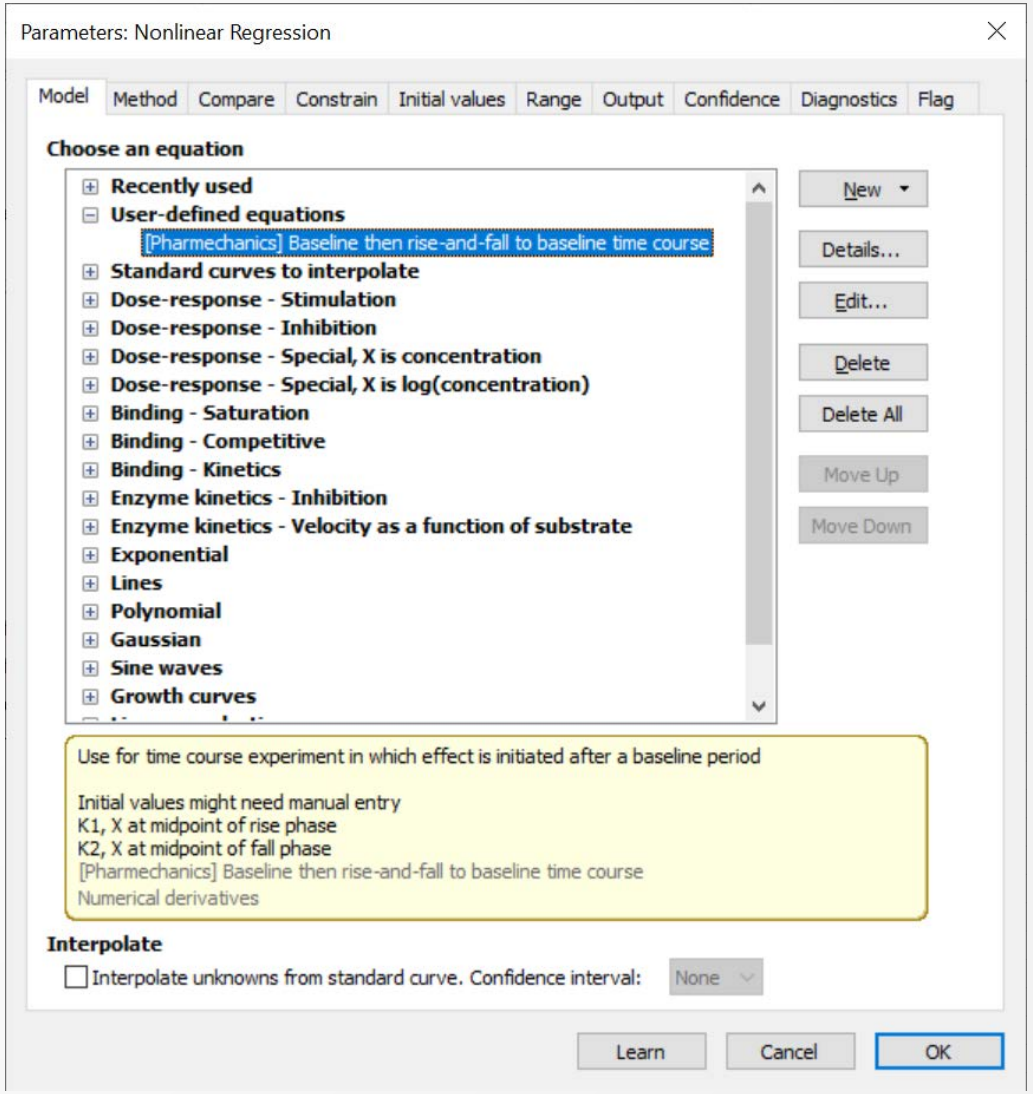
Loading equations in batch

Click the check box to see the equations in the folder.

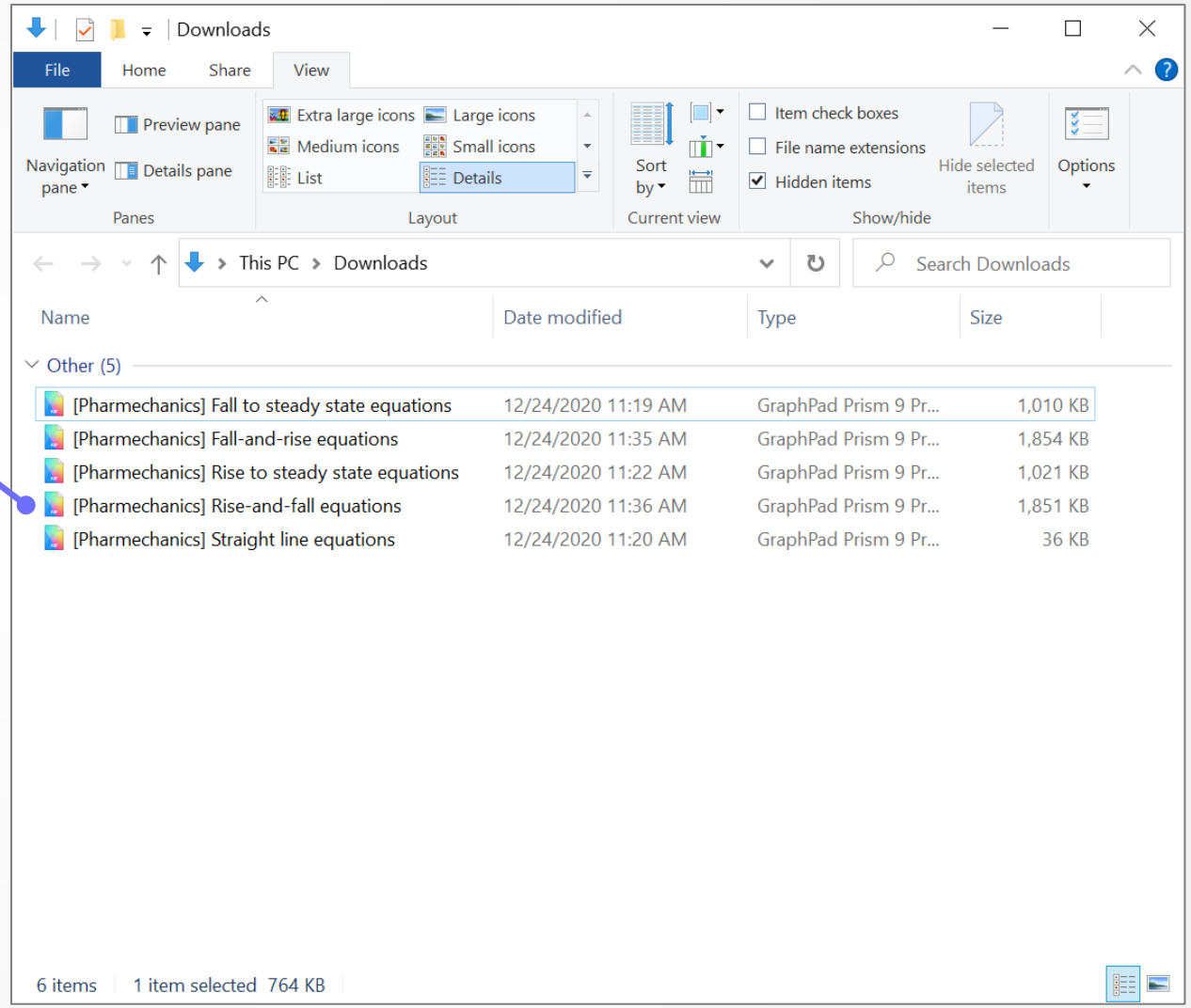


Loading equations individually

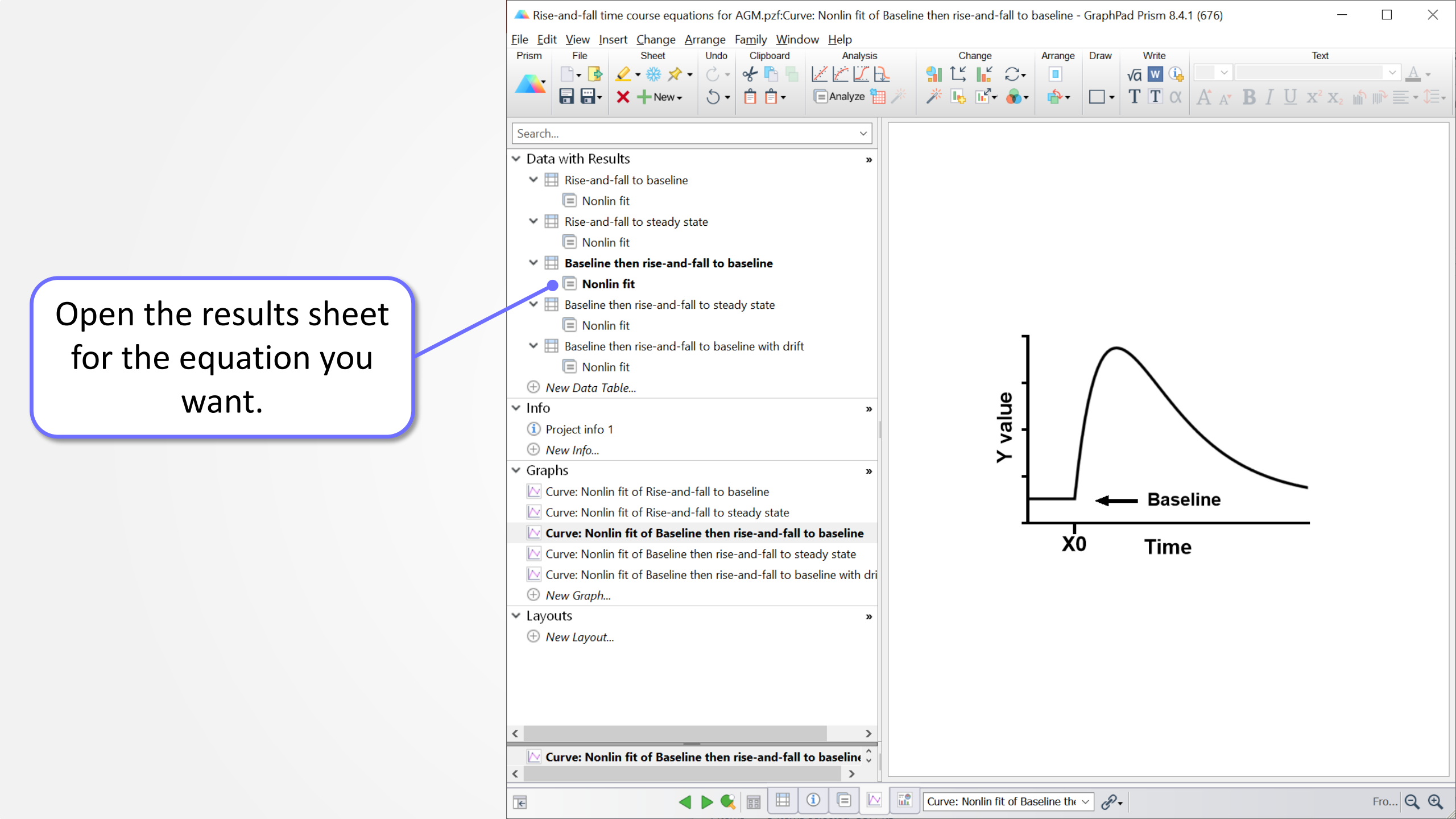
An equation can be loaded individually from the Analysis results page on any file.

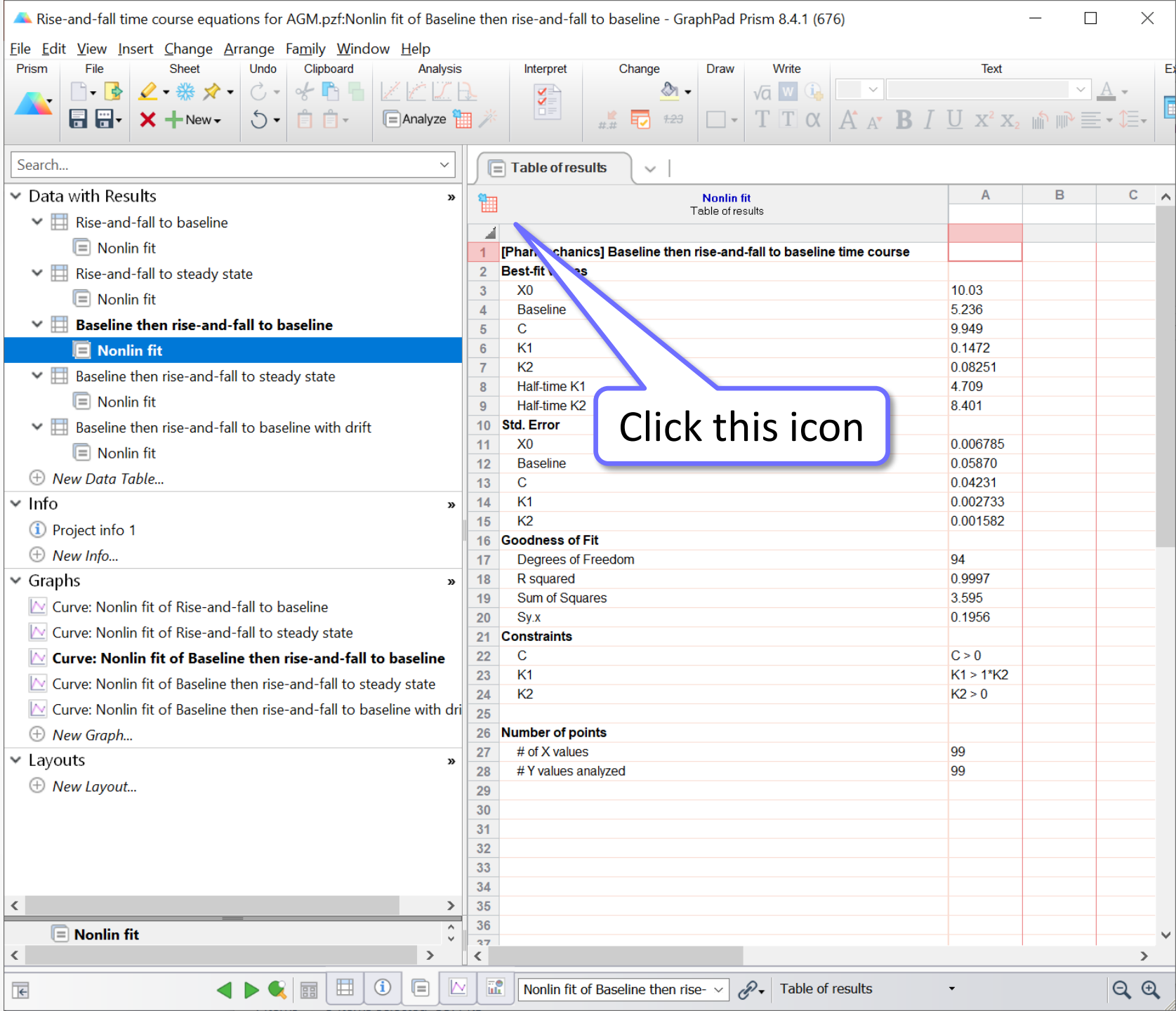


Open the file containing the equation you want.

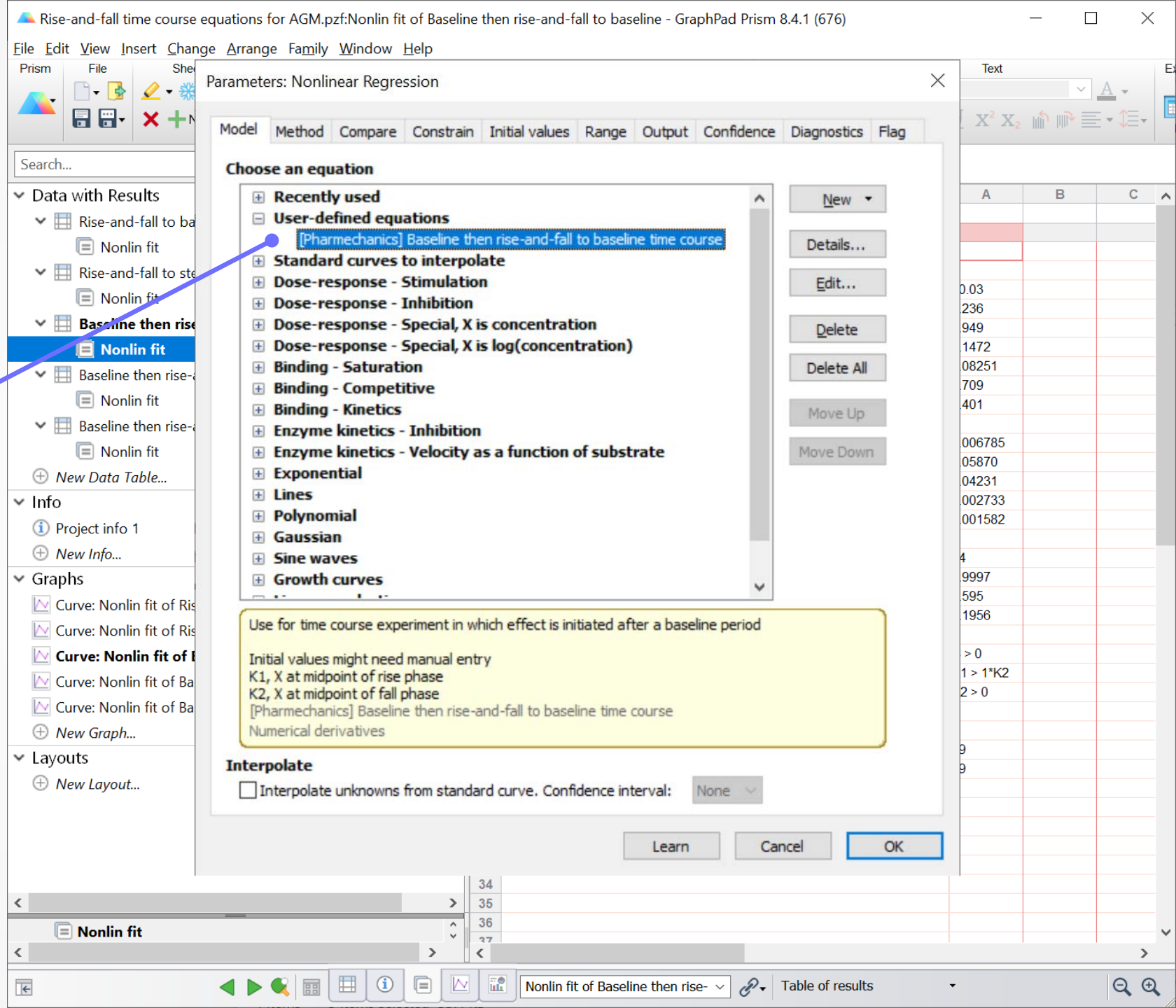


Open the results sheet for the equation you want.

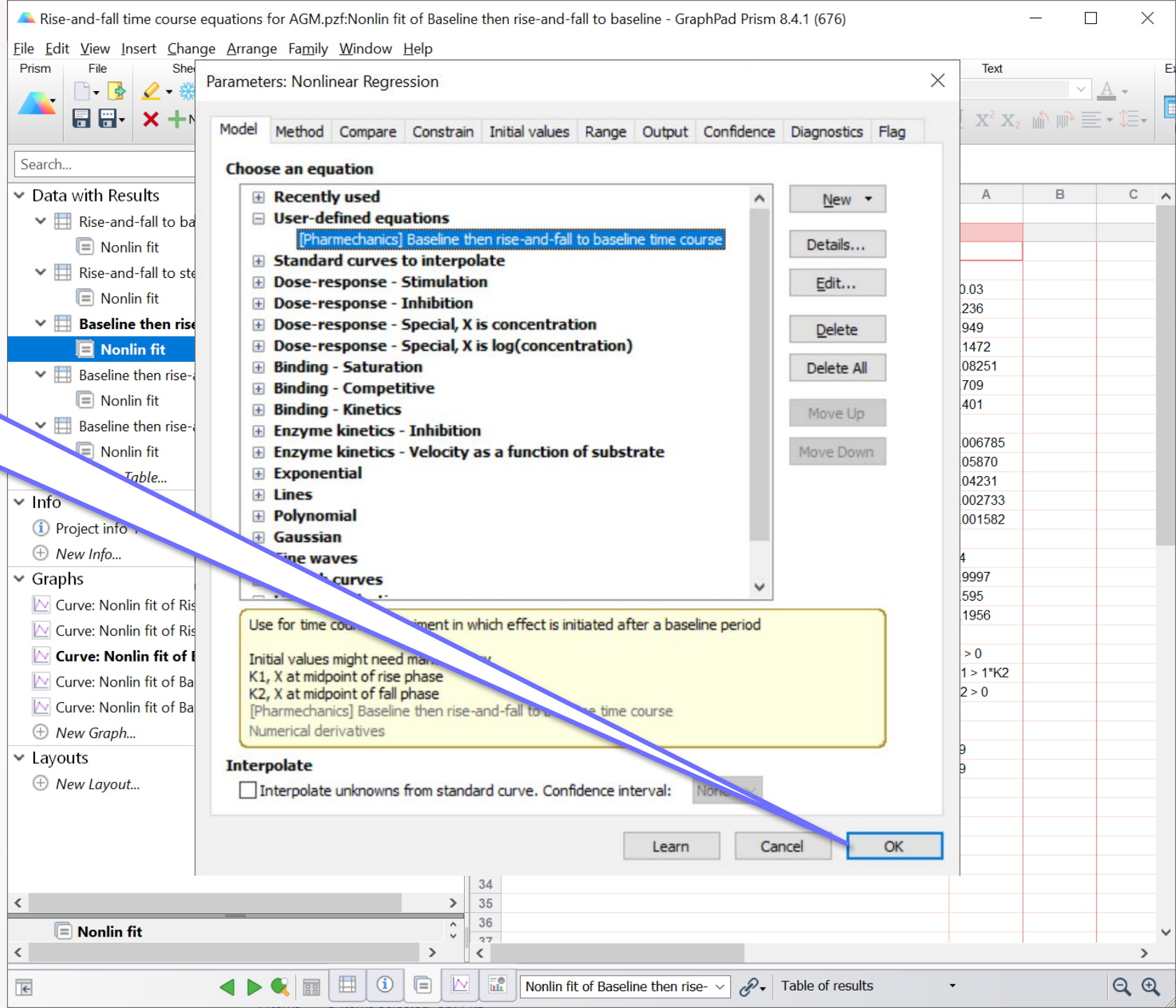



Rise-and-fall time course equations for AGM.pzf:Nonlin fit of Baseline then rise-and-fall to baseline - GraphPad Prism 8.4.1 (676)
File Edit View Insert Change Arrange Family Window Help
Prism File Sheet Undo Clipboard Analysis Interpret Change Draw Write Text
New
Analyze
Search...
Data with Results
Rise-and-fall to baseline
Nonlin fit
Rise-and-fall to steady state
Nonlin fit
Baseline then rise-and-fall to baseline
Nonlin fit
Baseline then rise-and-fall to steady state
Nonlin fit
Baseline then rise-and-fall to baseline with drift
Nonlin fit
New Data Table...
Info
Project info 1
New Info...
Graphs
Curve: Nonlin fit of Rise-and-fall to baseline
Curve: Nonlin fit of Rise-and-fall to steady state
Curve: Nonlin fit of Baseline then rise-and-fall to baseline
Curve: Nonlin fit of Baseline then rise-and-fall to steady state
Curve: Nonlin fit of Baseline then rise-and-fall to baseline with dri
New Graph...
Layouts
New Layout...
Nonlin fit
Table of results
Nonlin fit
Table of results
A B C
[Pharmacokinetics] Baseline then rise-and-fall to baseline time course
Best-fit values
X0 10.03
Baseline 5.236
C 9.949
K1 0.1472
K2 0.08251
Half-time K1 4.709
Half-time K2 8.401
Std. Error
X0 0.006785
Baseline 0.05870
C 0.04231
K1 0.002733
K2 0.001582
Goodness of Fit
Degrees of Freedom 94
R squared 0.9997
Sum of Squares 3.595
Sy.x 0.1956
Constraints
C C > 0
K1 K1 > 1*K2
K2 K2 > 0
Number of points
# of X values 99
Y values analyzed 99
Click this icon
Nonlin fit of Baseline then rise-
Table of results

This process loads the equation into the “User-defined equations” list. It only needs to be done once. After that, the new equation will be available every time you open Prism.



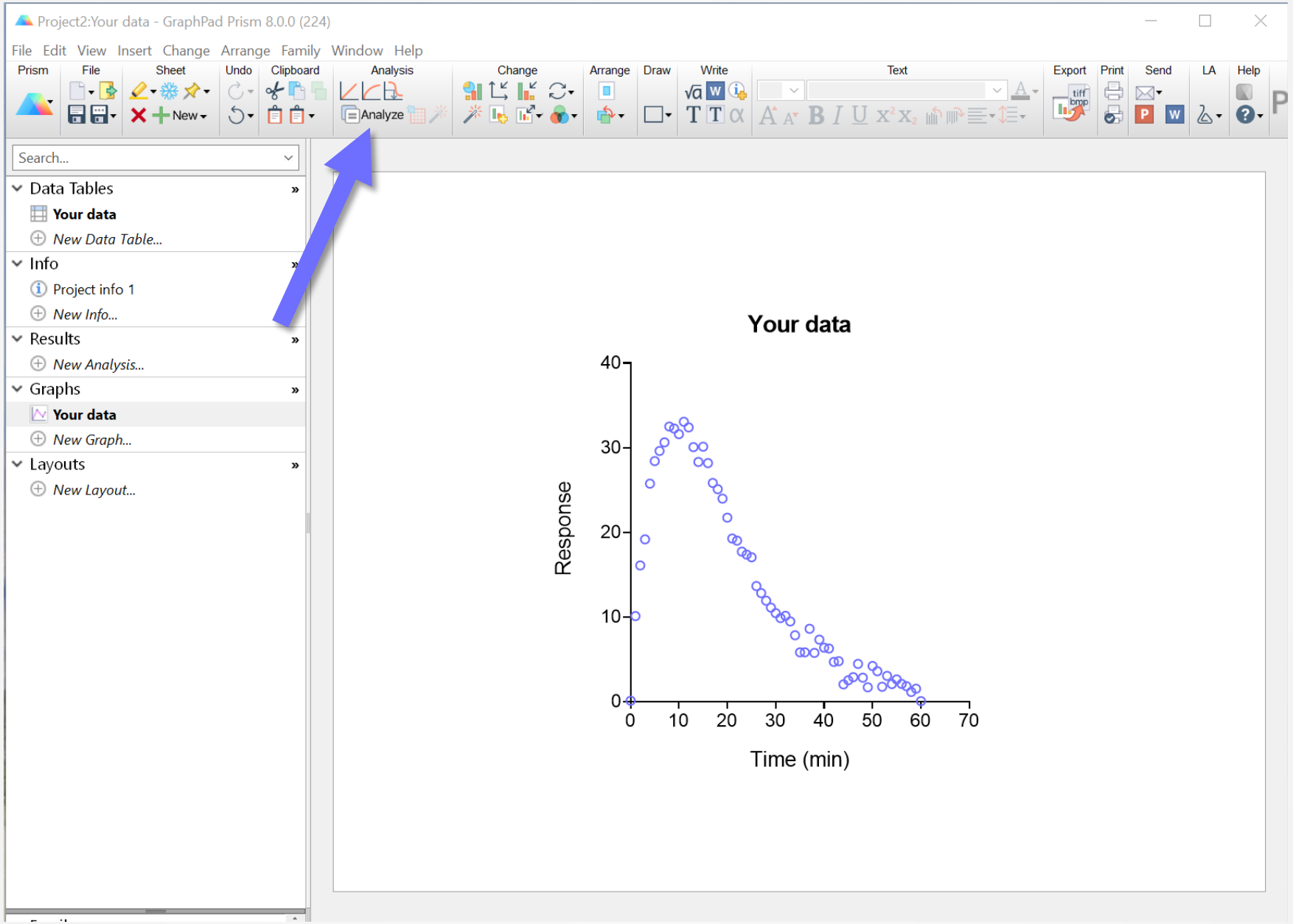
Click OK then close file. Now the equation is available every time you open Prism, in the “User-defined equations” list.

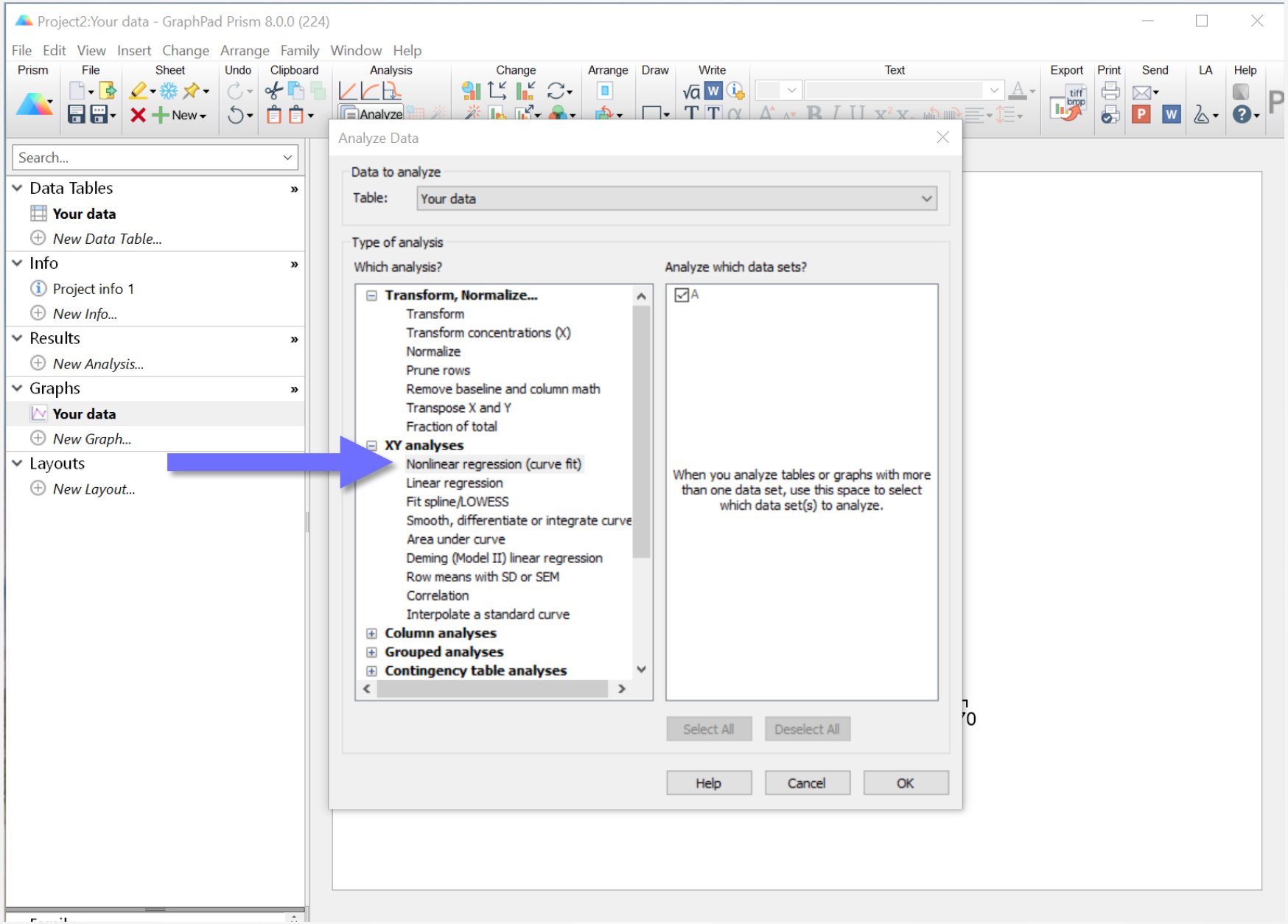


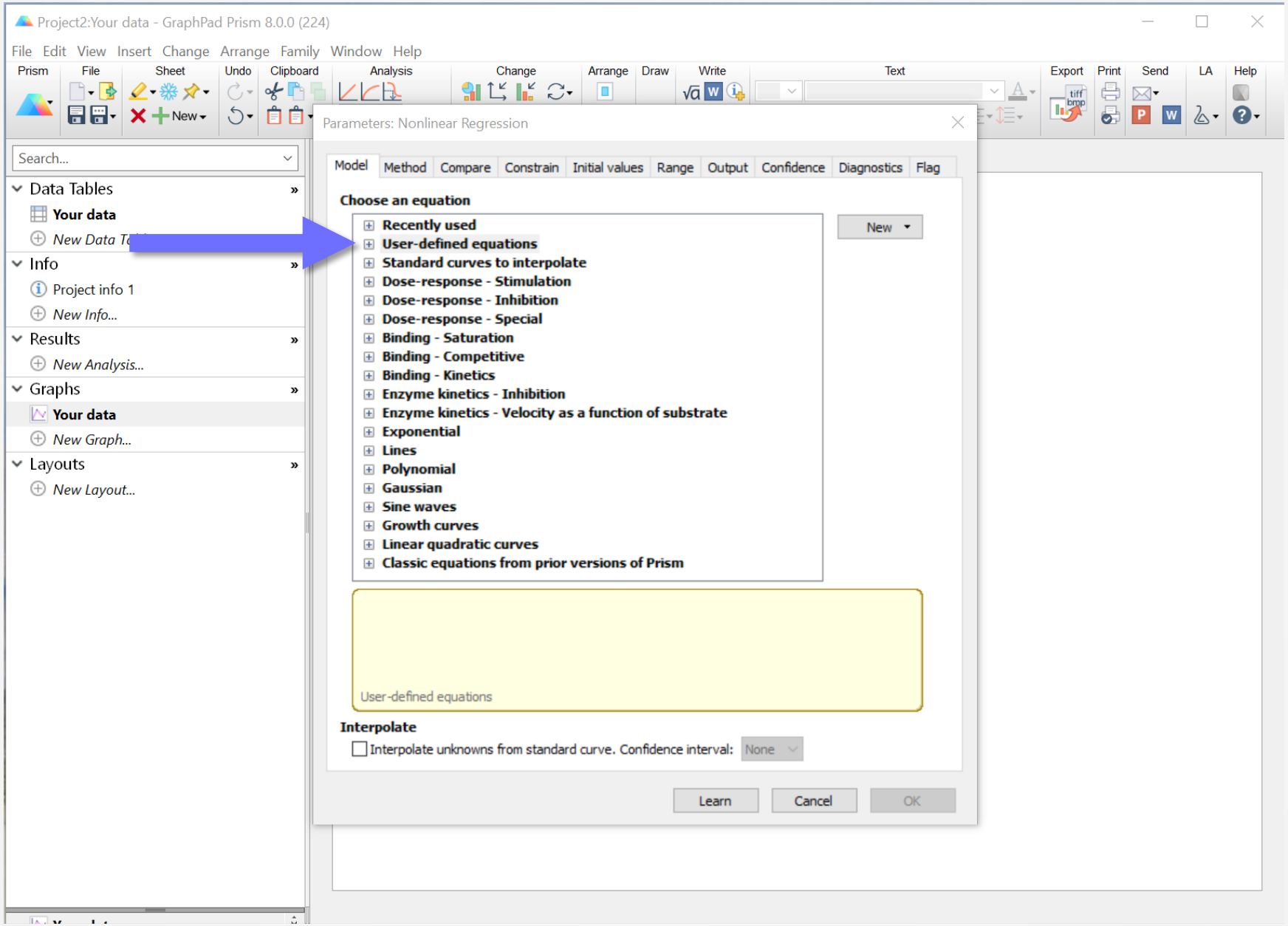
Using the new equations

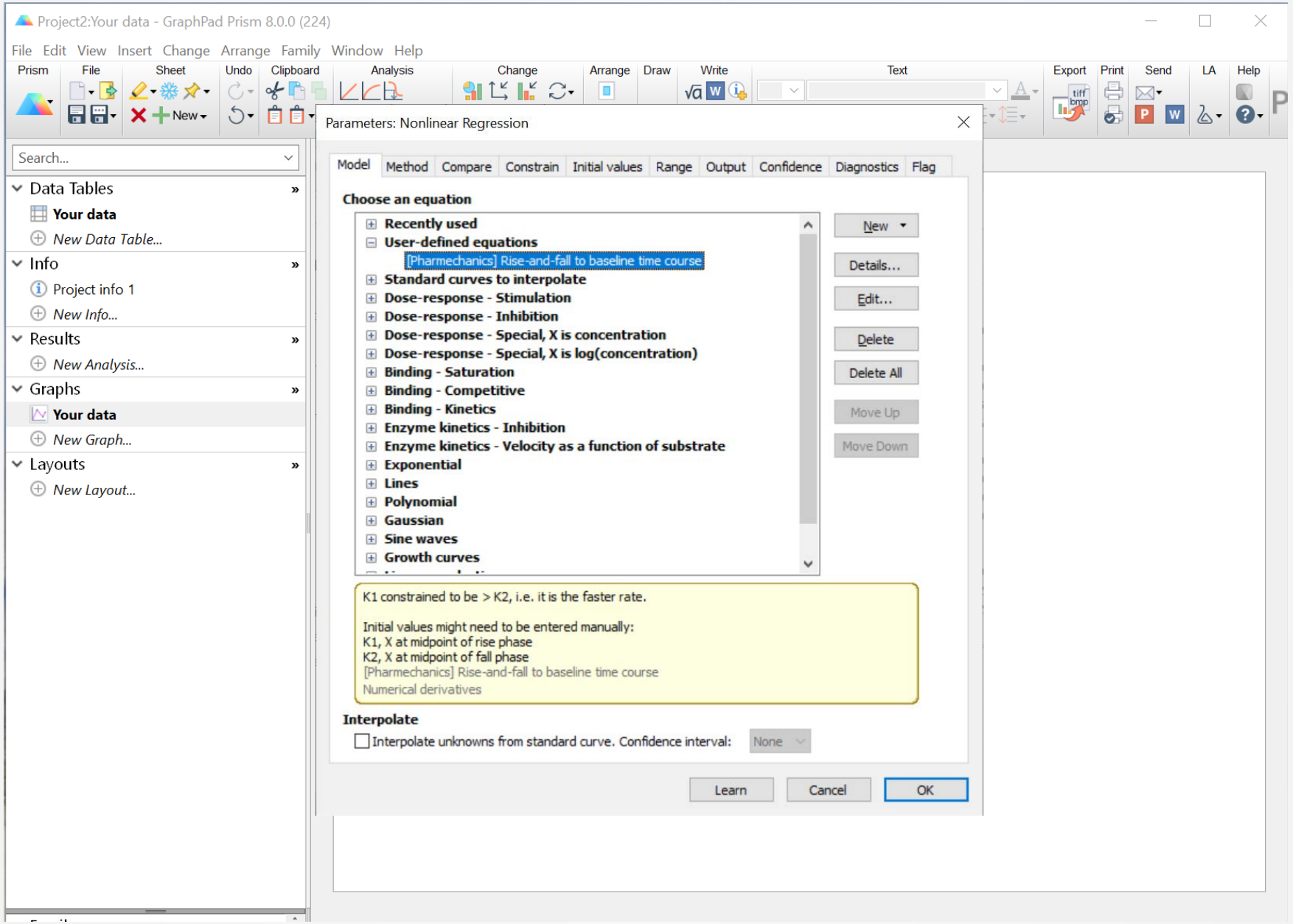
- **The new equations are used in the same way as the “Exponential” equations built into Prism.**
- **See following slides for screen clippings**

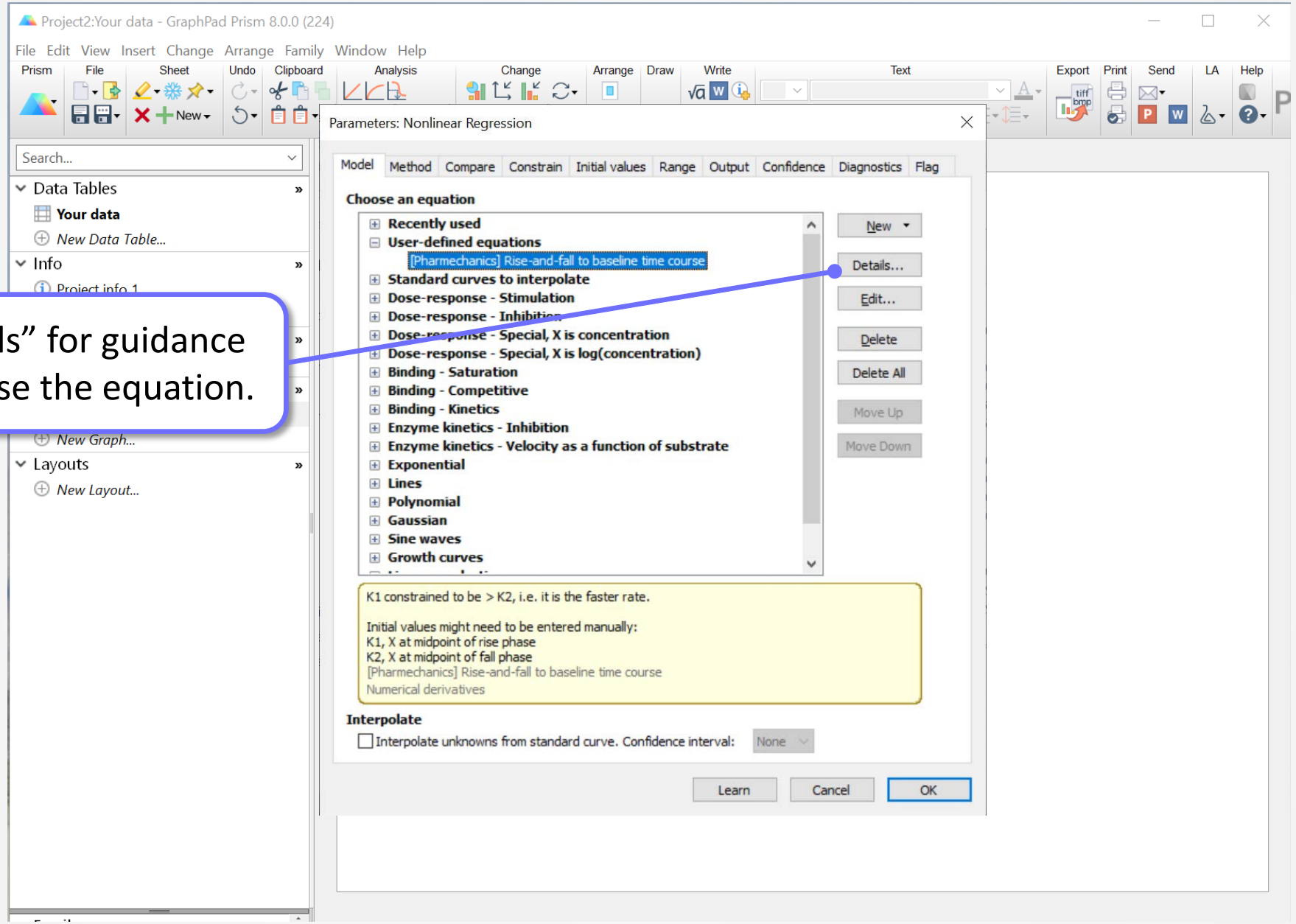
Open your file



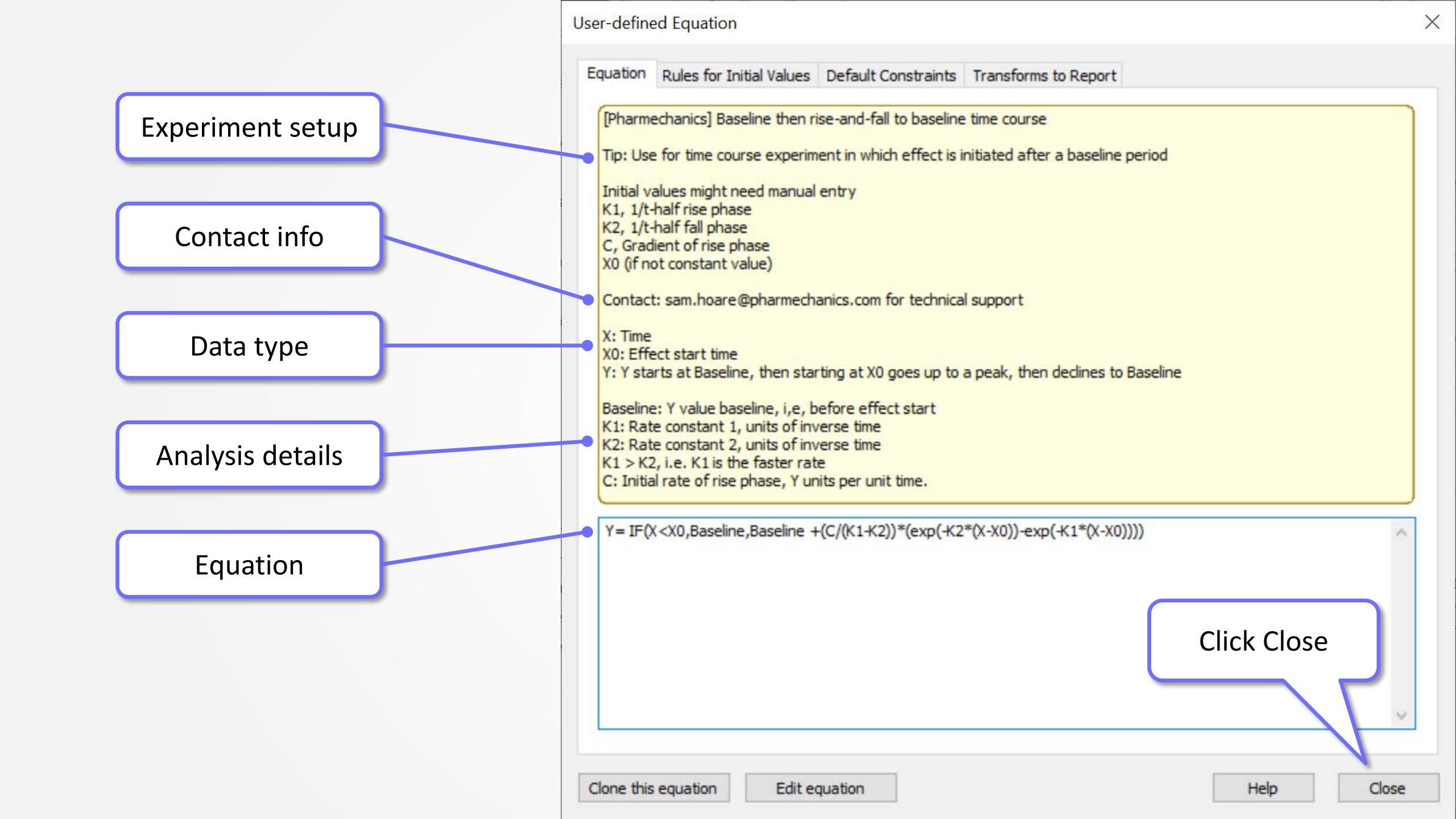

Project2:Your data - GraphPad Prism 8.0.0 (224)
File Edit View Insert Change Arrange Family Window Help
Prism
File
Sheet
Undo
Clipboard
Analysis
Change
Arrange
Draw
Write
Text
Export
Print
Send
LA
Help
New
Analyze
Search...
Data Tables
Your data
New Data Table...
Info
Project info 1
New Info...
Results
New Analysis...
Graphs
Your data
New Graph...
Layouts
New Layout...
Analyze Data
Data to analyze
Table:
Your data
Type of analysis
Which analysis?
Analyze which data sets?
Transform, Normalize...
Transform
Transform concentrations (X)
Normalize
Prune rows
Remove baseline and column math
Transpose X and Y
Fraction of total
XY analyses
Nonlinear regression (curve fit)
Linear regression
Fit spline/LOWESS
Smooth, differentiate or integrate curve
Area under curve
Deming (Model II) linear regression
Row means with SD or SEM
Correlation
Interpolate a standard curve
Column analyses
Grouped analyses
Contingency table analyses
A
When you analyze tables or graphs with more than one data set, use this space to select which data set(s) to analyze.
Select All
Deselect All
Help
Cancel
OK

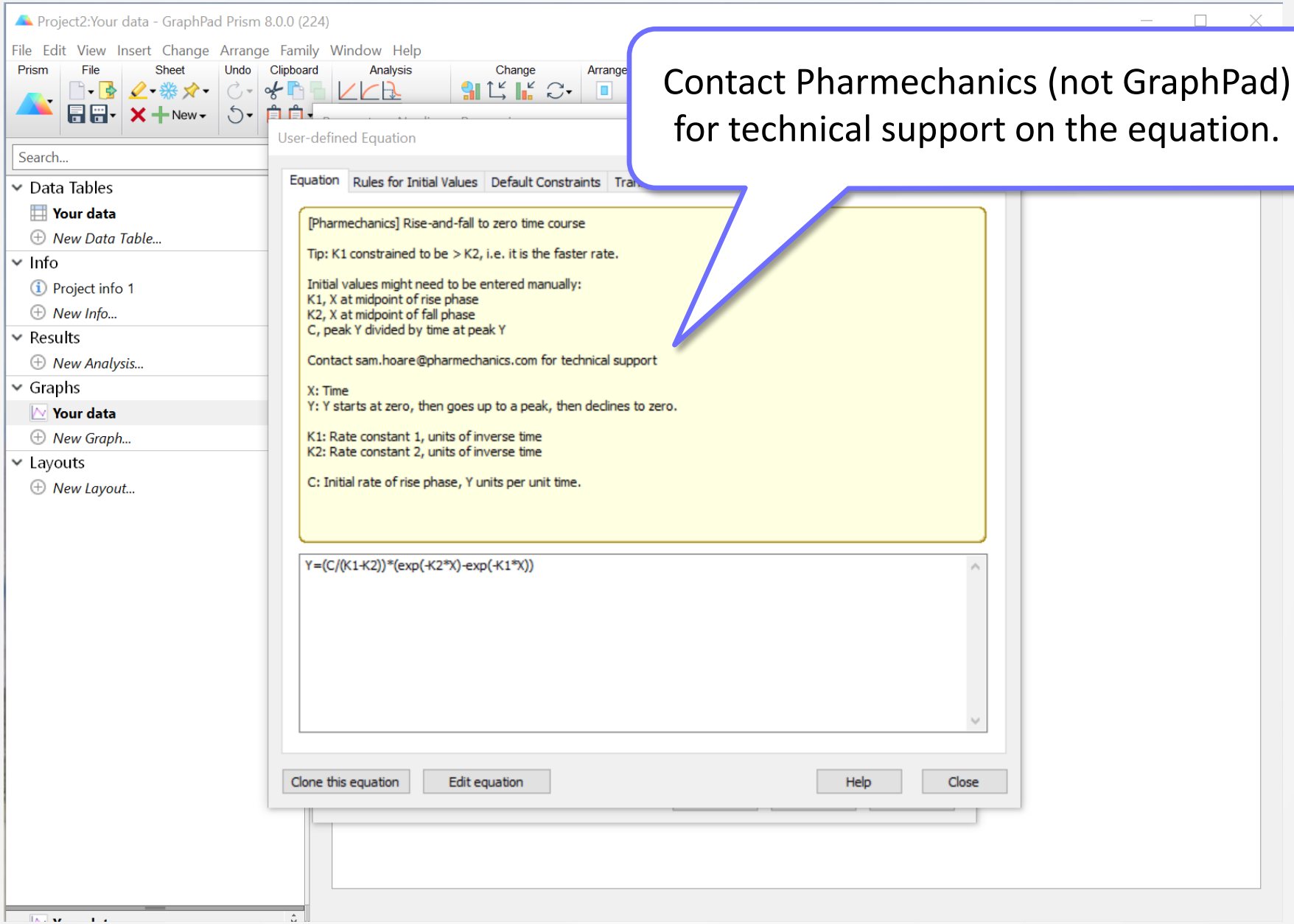

Project2:Your data - GraphPad Prism 8.0.0 (224)
File Edit View Insert Change Arrange Family Window Help
Prism File Sheet Undo Clipboard Analysis Change Arrange Draw Write Text Export Print Send LA Help
New
Search...
Data Tables
Your data
New Data Table...
Info
Project info 1
New Info...
Results
New Analysis...
Graphs
Your data
New Graph...
Layouts
New Layout...
Parameters: Nonlinear Regression
Model Method Compare Constrain Initial values Range Output Confidence Diagnostics Flag
Choose an equation
Recently used
User-defined equations
Standard curves to interpolate
Dose-response - Stimulation
Dose-response - Inhibition
Dose-response - Special
Binding - Saturation
Binding - Competitive
Binding - Kinetics
Enzyme kinetics - Inhibition
Enzyme kinetics - Velocity as a function of substrate
Exponential
Lines
Polynomial
Gaussian
Sine waves
Growth curves
Linear quadratic curves
Classic equations from prior versions of Prism
New
User-defined equations
Interpolate
Interpolate unknowns from standard curve. Confidence interval:
None
Learn
Cancel
OK

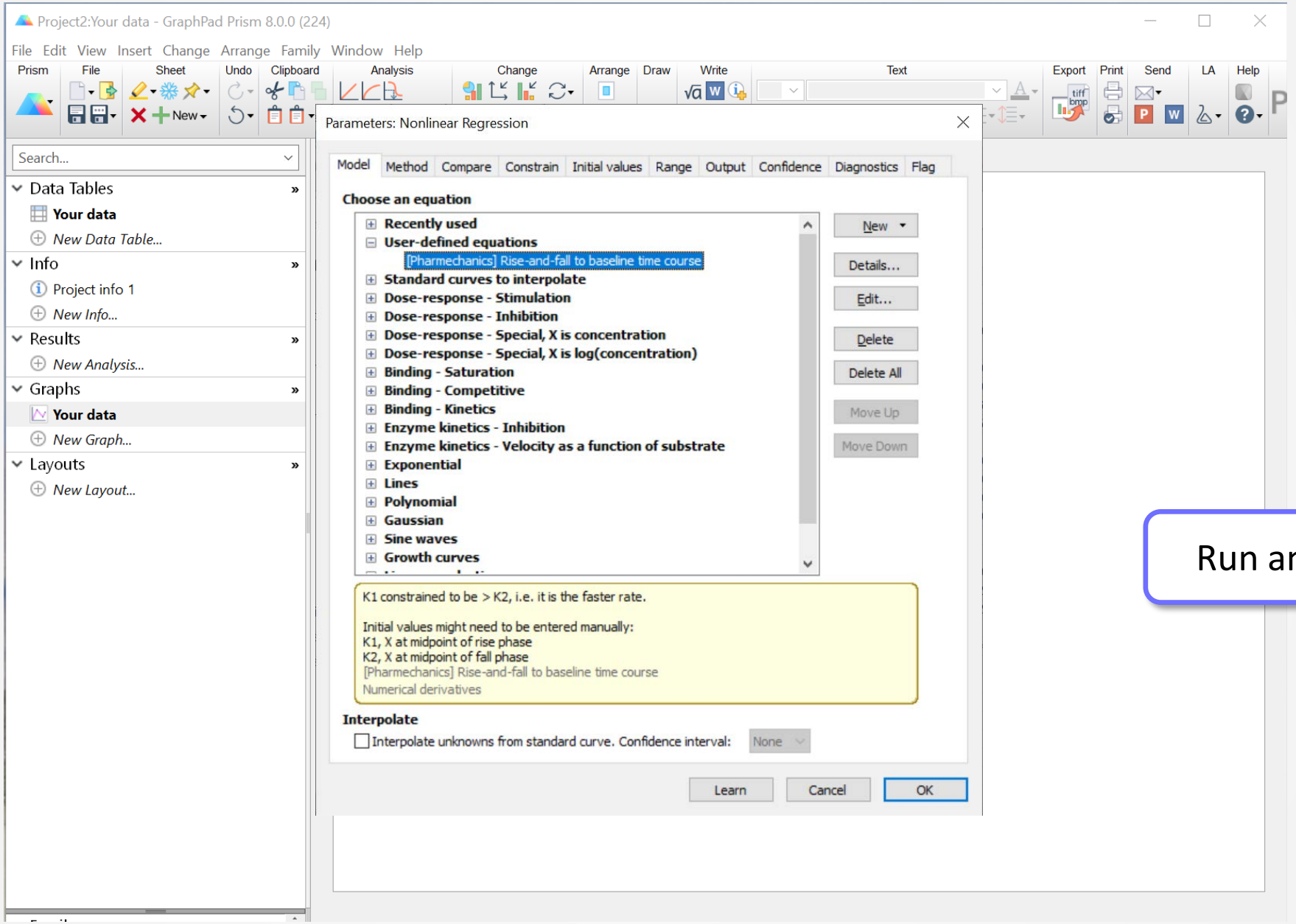

Project2:Your data - GraphPad Prism 8.0.0 (224)
File Edit View Insert Change Arrange Family Window Help
Prism File Sheet Undo Clipboard Analysis Change Arrange Draw Write Text Export Print Send LA Help
New
Search...
Data Tables
Your data
New Data Table...
Info
Project info 1
New Info...
Results
New Analysis...
Graphs
Your data
New Graph...
Layouts
New Layout...
Parameters: Nonlinear Regression
Model Method Compare Constrain Initial values Range Output Confidence Diagnostics Flag
Choose an equation
Recently used
User-defined equations
[Pharmechanics] Rise-and-fall to baseline time course
Standard curves to interpolate
Dose-response - Stimulation
Dose-response - Inhibition
Dose-response - Special, X is concentration
Dose-response - Special, X is log(concentration)
Binding - Saturation
Binding - Competitive
Binding - Kinetics
Enzyme kinetics - Inhibition
Enzyme kinetics - Velocity as a function of substrate
Exponential
Lines
Polynomial
Gaussian
Sine waves
Growth curves
New
Details...
Edit...
Delete
Delete All
Move Up
Move Down
K1 constrained to be > K2, i.e. it is the faster rate.
Initial values might need to be entered manually:
K1, X at midpoint of rise phase
K2, X at midpoint of fall phase
[Pharmechanics] Rise-and-fall to baseline time course
Numerical derivatives
Interpolate
Interpolate unknowns from standard curve. Confidence interval:
None
Learn
Cancel
OK

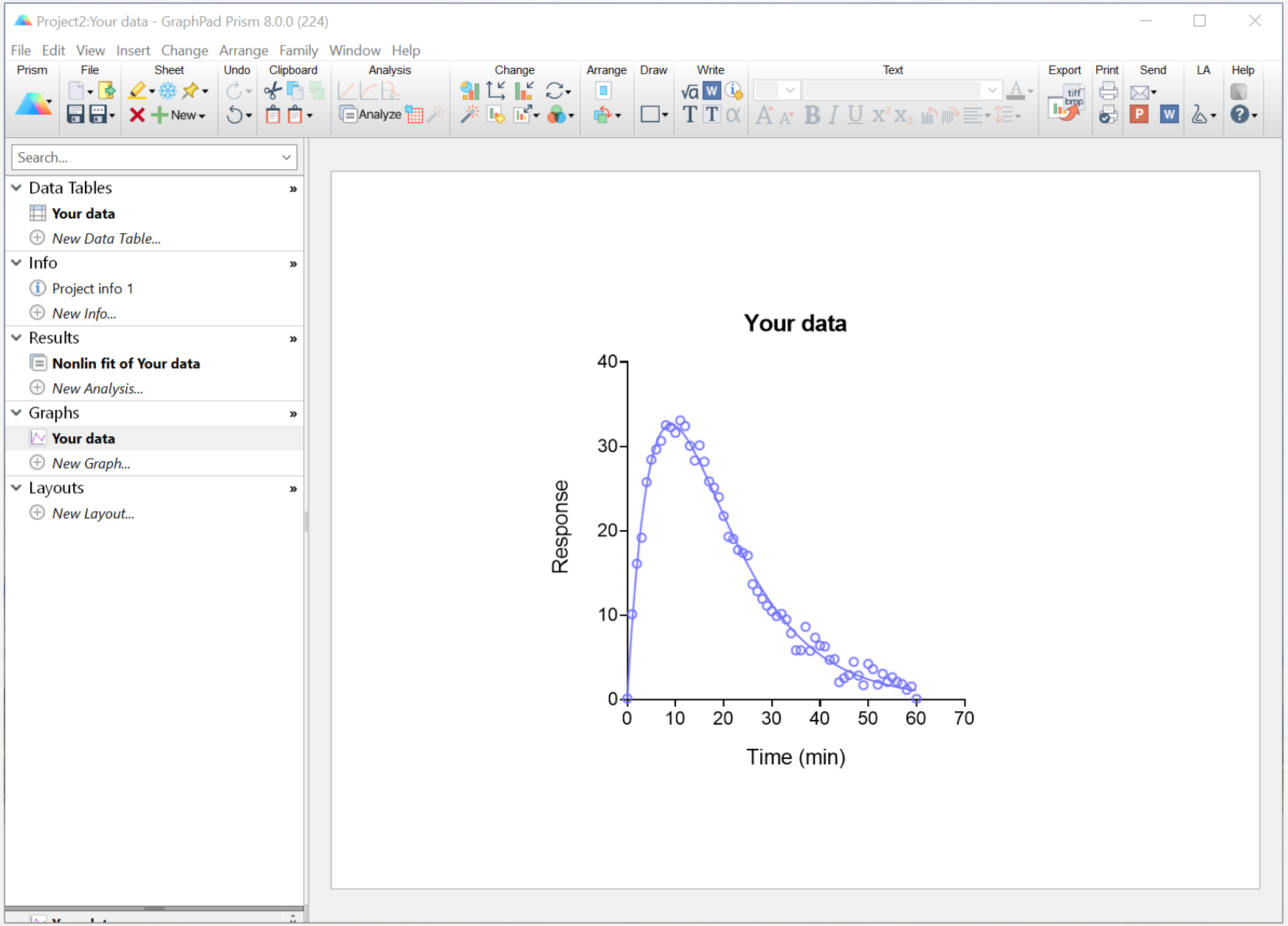


Click “Details” for guidance on how to use the equation.


Experiment setup
Contact info
Data type
Analysis details
Equation
User-defined Equation
Equation
Rules for Initial Values
Default Constraints
Transforms to Report
[Pharmechanics] Baseline then rise-and-fall to baseline time course
Tip: Use for time course experiment in which effect is initiated after a baseline period
Initial values might need manual entry
K1, 1/t-half rise phase
K2, 1/t-half fall phase
C, Gradient of rise phase
X0 (if not constant value)
Contact: sam.hoare@pharmechanics.com for technical support
X: Time
X0: Effect start time
Y: Y starts at Baseline, then starting at X0 goes up to a peak, then declines to Baseline
Baseline: Y value baseline, i,e, before effect start
K1: Rate constant 1, units of inverse time
K2: Rate constant 2, units of inverse time
K1 > K2, i.e. K1 is the faster rate
C: Initial rate of rise phase, Y units per unit time.
Y= IF(X<X0,Baseline,Baseline +(C/(K1-K2))*(exp(-K2*(X-X0))-exp(-K1*(X-X0))))
Click Close
Clone this equation
Edit equation
Help
Close


Project2:Your data - GraphPad Prism 8.0.0 (224)
File Edit View Insert Change Arrange Family Window Help
Prism File Sheet Undo Clipboard Analysis Change Arrange
New
Search...
Data Tables
Your data
New Data Table...
Info
Project info 1
New Info...
Results
New Analysis...
Graphs
Your data
New Graph...
Layouts
New Layout...
User-defined Equation
Equation
Rules for Initial Values
Default Constraints
[Pharmechanics] Rise-and-fall to zero time course
Tip: K1 constrained to be > K2, i.e. it is the faster rate.
Initial values might need to be entered manually:
K1, X at midpoint of rise phase
K2, X at midpoint of fall phase
C, peak Y divided by time at peak Y
Contact sam.hoare@pharmechanics.com for technical support
X: Time
Y: Y starts at zero, then goes up to a peak, then declines to zero.
K1: Rate constant 1, units of inverse time
K2: Rate constant 2, units of inverse time
C: Initial rate of rise phase, Y units per unit time.
Y=(C/(K1-K2))*(exp(-K2*X)-exp(-K1*X))
Clone this equation
Edit equation
Help
Close
Contact Pharmechanics (not GraphPad) for technical support on the equation.


Project2:Your data - GraphPad Prism 8.0.0 (224)
File Edit View Insert Change Arrange Family Window Help
Parameters: Nonlinear Regression
Model Method Compare Constrain Initial values Range Output Confidence Diagnostics Flag
Choose an equation
Recently used
User-defined equations
[Pharmechanics] Rise-and-fall to baseline time course
Standard curves to interpolate
Dose-response - Stimulation
Dose-response - Inhibition
Dose-response - Special, X is concentration
Dose-response - Special, X is log(concentration)
Binding - Saturation
Binding - Competitive
Binding - Kinetics
Enzyme kinetics - Inhibition
Enzyme kinetics - Velocity as a function of substrate
Exponential
Lines
Polynomial
Gaussian
Sine waves
Growth curves
New
Details...
Edit...
Delete
Delete All
Move Up
Move Down
K1 constrained to be > K2, i.e. it is the faster rate.
Initial values might need to be entered manually:
K1, X at midpoint of rise phase
K2, X at midpoint of fall phase
[Pharmechanics] Rise-and-fall to baseline time course
Numerical derivatives
Interpolate
Interpolate unknowns from standard curve. Confidence interval:
None
Learn
Cancel
OK
Data Tables
Your data
New Data Table...
Info
Project info 1
New Info...
Results
New Analysis...
Graphs
Your data
New Graph...
Layouts
New Layout...
Run analysis


Project2:Your data - GraphPad Prism 8.0.0 (224)
File Edit View Insert Change Arrange Family Window Help
Prism File Sheet Undo Clipboard Analysis Change Arrange Draw Write Text Export Print Send LA Help
New
Analyze
Search...
Data Tables
Your data
New Data Table...
Info
Project info 1
New Info...
Results
Nonlin fit of Your data
New Analysis...
Graphs
Your data
New Graph...
Layouts
New Layout...
Your data
Response
Time (min)
0
10
20
30
40
50
60
70