Prism 8 curve fitting to rise-and-fall to baseline exponential equation with baseline drift



"Fit height" enables page flipping in PDF viewer

Here we are going to fit biosensor time course data to the rise-and-fall to baseline equation.

In this example there is baseline drift, i.e. the baseline falls slightly over time. This is handled using a modified equation.

The curve-fitting program is Prism 8, from GraphPad Software, Inc. <u>https://www.graphpad.com/scientific-software/prism/</u>

The example will use is Ca2+ mobilization via the AT1 receptor, by five ligands – AngII and four synthetic ligands. A maximally-stimulating concentration was used (32 μ M).



- First we need to load the equation.
- The rise-and-fall equation with drift is not a built-in equation in Prism.
- Instead it is loaded as a User-defined equation.
- This can be done easily from a template.
- This is shown starting on page 21.
- The equation is called: "[Pharmechanics] Baseline then rise-and-fall to baseline with drift"

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| | A Prism 8 curve fitting to rise-and-fall to | o bas | seline equati | on with drif | t.pzfx:Ca2 | 2+, AT1 r | eceptor - (| GraphPa | d Prism 8. | 3 — | | \times |
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| | Ca2+, AT1 receptor | V 4 | < l | | | | | | | | | > |

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Here is the graph of the data.

Notes

- Lead-in phase before addition of ligand, where we are measuring the baseline signal.
- 2. Response at the end is slightly lower than response at the beginning. This indicates the baseline has drifted downwards.
- Data are expressed as △F / F. This is the fluorescence intensity after ligand addition divided by the average baseline fluorescence intensity before addition, calculated using a spreadsheet.
- 4. Error bars are SEM.







This brings up the "Parameters: Nonlinear Regression" dialogue.

Now we select the equation.

Click "User-defined equations" checkbox.

Note this will only appear if user-defined equations have been loaded. See <u>page 21</u>.

| odel | Method | Compare | Constrain | Initial values | Range | Output | Confidence | Diagnostics | Flag |
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Select ""[Pharmechanics] Baseline then rise-and-fall to baseline with drift"

Note this will only appear if the equation has been loaded. See <u>page 21</u>.

Click on the "Initial values" tab

| odel | Method | Compare | Constrain | Initial values | Range | Output | Confidence | Diagnostics | Flag |
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Now we are going to change a setting to make the analysis run efficiently.

Prism uses a rigorous method to compute the error associated with the fitted parameter values. For complicated equations this can greatly increase the fitting time.

The rigorous method can be turned off to make the analysis run faster.

Click on the "Confidence" tab

| lodel | Method | Compare | Constrain | Initial values | Range | Output | Confidence | Diagnostics | Fla |
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| | | К1 | | | 2 | [| 0,1478743068 | 39 [| |
| | | К2 | | | 2 | 1 | 0.0044362292 | 205 | |
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"Asymmetrical" is the rigorous error calculation method

To turn it off, click the "Symmetrical" radio button

| Confide | nce interval | s (CI) of param | neters | | | | |
|--------------------------|-----------------|----------------------|--------------------|------------|-------------|-----------------|------------|
| Calc | culate CI of pa | arameters | | | | | |
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| Out | put Format: | Range ("1.23 b | o 4.56") | ~ | | | |
| O | Asymmetrical | (profile-likelihood) | CT | | | | |
| F | Recommended | because they ar | e more accurat | e. Can be | e slow. | | |
| ſ | | ven when the fit i | is ambiguous ar | nd the CI | s would be | difficult to in | terpret. |
| - 0 | Symmetrical (a | symptotic) appro | ximate CI | | | | |
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| E | Show SE of | parameters | | | | | |
| Confide | nce or predi | ction bands | | | | | |
| Plot | confidence/p | rediction bands | | | | | |
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| | Confidence ba | nds | | | | | |
| (| Confidence ba | nds show you the | e likely location | of the TR | UE curve. | | |
| OF | Prediction ban | ds | | | | | |
| F | Prediction ban | ds show you the l | likely location ol | f addition | ial data po | ints. | |
| Ambigu | ous fits and | unstable para | meters | | | | |
| Ider | ntify "ambiguo | ous" fits. Matches | Prism 8.1 and | earlier. | | | |
| | ntify "unstable | e"parameters. A | new (8.2) feat | ure from | Prism Labs | i. | |
| OIde | | | | | | | |

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 \times Parameters: Nonlinear Regression Model Method Compare Constrain Initial values Range Output Confidence Diagnostics Flag Confidence intervals (CI) of parameters Calculate CI of parameters Confidence level: 95% Output Format: Range ("1.23 to 4.56") V Asymmetrical (profile-likelihood) CI Recommended because they are more accurate. Can be slow. Compute even when the fit is ambiguous and the CIs would be difficult to interpret. Symmetrical (asymptotic) approximate CI Less accurate so not recommended. Matches Prism 1-6 and most programs. Faster to calculate. Show SE of parameters Click "OK" to run the analysis Confidence or prediction bands Plot confidence/prediction bands Confidence level: 95% Confidence bands Confidence bands show you the likely location of the TRUE curve. Prediction bands Prediction bands show you the likely location of additional data points. Ambiguous fits and unstable parameters Identify "ambiguous" fits. Matches Prism 8.1 and earlier. O Identify "unstable" parameters. A new (8.2) feature from Prism Labs. O Neither. Just show the best-fit values even when the fit is problematic. Make these choices the default for future fits. Cancel OK Learn



Reviewing the results

Click "Nonlin fit" to bring up the results table



A Prism 8 curve fitting to rise-and-fall to baseline equation with drift.pzfx:Nonlin fit of Ca2+, AT1 receptor - GraphPad Prism 8.3.0 (538)

<u>File Edit View Insert Change Arrange Family Window Help</u>

| Prism File Sheet | Undo | Clipboard | Analysis | Interpret | Change | Draw Write | • | Text | | Export Print Send LA | Help | | | |
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| Info | » | 4 Base | eline | | | | | 0.994 | 0.996 | 0.995 | 1.00 | 0.997 | | |
| Project info 1 | | - Bust | cime | | | | | -0.000724 | -0.000379 | -0.000569 | -0.000339 | -0.000463 | | |
| (1) Project info 1 | | . 1 | | | . 1 | | | 1.88 | 2.19 | 0.625 | 0.373 | 0.176 | | |
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| | | | | | C C · · | | | 0.0475 | 0.0492 | 0.0589 | 0.0638 | 0.0548 | | |
| 🕀 New Info | de | termi | ine the go | odne | ess of fit | | | 0.449 | 0.457 | 1.09 | 1.38 | 2.57 | | |
| Graphs | | | 0 | | |) | | 14.6 | 14.1 | 11.8 | 10.9 | 12.6 | | |
| Ca2+, AT1 receptor | | 11 Std. Er | rror | | | | | | | | | | | |
| A New Graph | | 12 X0 | | | | | | 0.0201 | 0.00774 | 0.0281 | 0.0287 | 0.0447 | | |
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| | | 10 KI | | | | | | 0.0094 | 0.0325 | 0.0207 | 0.0149 | 0.00001 | | |
| | | 19 95% C | (asymptotic) | | | | | 0.000432 | 0.000200 | 0.000703 | 0.000024 | 0.000030 | | - |
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| | | 21 Drift | | | | | | -0.000791 to -0.000658 | -0.000426 to -0.000332 | -0.000623 to -0.000516 | -0.000377 to -0.000300 | -0.000498 to -0.000428 | | † |
| | | 22 C | | | | | | 1.72 to 2.04 | 2.10 to 2.28 | 0.590 to 0.661 | 0.355 to 0.392 | 0.168 to 0.183 | | 1 |
| | | 23 K1 | | | | | | 1.41 to 1.68 | 1.45 to 1.58 | 0.598 to 0.679 | 0.474 to 0.532 | 0.254 to 0.285 | | |
| | | 24 K2 | | | | | | 0.0466 to 0.0484 | 0.0487 to 0.0498 | 0.0575 to 0.0603 | 0.0621 to 0.0654 | 0.0531 to 0.0566 | | |
| | | 25 Half- | -time K1 | | | | | 0.413 to 0.493 | 0.438 to 0.477 | 1.02 to 1.16 | 1.30 to 1.46 | 2.43 to 2.73 | | 1 |
| | | 26 Half- | -time K2 | | | | | 14.3 to 14.9 | 13.9 to 14.2 | 11.5 to 12.0 | 10.6 to 11.2 | 12.3 to 13.1 | | - |
| | | 27 Goodn | ness of Fit | | | | | 4000 | 4000 | 4000 | 4000 | 4000 | | - |
| | | 28 Degi | rees of Freedom | | | | | 1620 | 1620 | 1620 | 1620 | 1620 | | - |
| | | 29 R Sq 20 Sum | and Squares | | | | | 2.25 | 1.60 | 2.00 | 0.971 | 0.971 | | - |
| | | 30 Sum | i oi oquales | | | | | 0.0448 | 0.0315 | 0.0359 | 0.0262 | 0.903 | | |
| | | 32 Constr | raints | | | | | 0.0110 | 0.0010 | 0.0000 | 0.0202 | 0.0200 | | |
| | | 33 C | | | | | | C > 0 | C > 0 | C > 0 | C > 0 | C > 0 | | |
| | | 34 K2 | | | | | | K2 > 0 | K2 > 0 | K2 > 0 | K2 > 0 | K2 > 0 | | |
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| 😑 Nonlin fit | ~ | < | | | | | | | | | | | > | |
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A Prism 8 curve fitting to rise-and-fall to baseline equation with drift.pzfx:Nonlin fit of Ca2+, AT1 receptor - GraphPad Prism 8.3.0 (538)

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| | | | analyze | #.# • 1.25 | | $A A' \mathbf{D} I \cup X' X$ | 2 ₩1'₩1* = * ↓=* | | | | | |
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| Data with Results | » | **** | | Nonlin fit | | A | В | С | D | E | F | ^ |
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| | | 1 [Pharm | t values | en rise-and-fail to baseline time cours | se with drift | | | | | | | |
| Thew Data Table | | 2 Dest-1 | t values | | | 15.6 | 15.5 | 16.6 | 17.2 | 17 1 | | |
| r Info | >> | 4 Base | eline | | | 0.994 | 0.996 | 0.995 | 1.00 | 0.997 | | |
| Project info 1 | | 5 Drift | | | | -0.000724 | -0.000379 | -0.000569 | -0.000339 | -0.000463 | | |
| Project info 1 | | 6 C | | | | 1.88 | 2.19 | 0.625 | 0.373 | 0.176 | | |
| | | 7 K1 | | | | 1.54 | 1.52 | 0.638 | 0.503 | 0.269 | | |
| | | | | | | 0.0475 | 0.0492 | 0.0589 | 0.0638 | 0.0548 | | |
| (±) New Info | Data | ana fi | | the equation. | | 0.449 | 0.457 | 1.09 | 1.38 | 2.57 | | |
| Graphs | Data | aren | it well by | the equation: | | 14.6 | 14.1 | 11.8 | 10.9 | 12.6 | / | |
| 🗠 Ca2+, AT1 receptor | | П | anuarad | \mathbf{b} | | 0.0004 | 0.00774 | 0.0004 | 0.0007 | 0.0117 | | |
| New Graph | | K | squared | > 0.9 | | 0.0201 | 0.00774 | 0.0281 | 0.0287 | 0.0447 | | |
| | | | | | | 2.400.005 | 2.400.005 | 0.00200 | 1.060.005 | 1.00174 | | |
| Layouts | >> | 14 Dim | | | | 0.0813 | 0.0449 | 0.0170 | 0.00032 | 0.00386 | | |
| 🕀 New Layout | | 16 K1 | | | | 0.0694 | 0.0325 | 0.0207 | 0.00332 | 0.00300 | | |
| | | 17 K2 | | \mathbf{i} | | 0.000452 | 0.000280 | 0.000703 | 0.000824 | 0.000890 | | |
| | | 18 95% C | I (asymptotic) | | | | 0.000200 | | | | | |
| | | 19 X0 | | | | 15.6 to 15.6 | 15.5 to 15.5 | 16.6 to 16.7 | 17.1 to 17.2 | 17.1 to 17.2 | | |
| | | 20 Base | eline | | | 0.988 to 1.00 | 0.991 to 1.00 | 0.990 to 1.00 | 0.996 to 1.00 | 0.994 to 1.00 | | |
| | | 21 Drift | | | | -0.000791 to -0.000658 | -0.000426 to -0.000332 | -0.000623 to -0.000516 | -0.000377 to -0.000300 | -0.000498 to -0.000428 | | |
| | | 22 C | | | | 1.72 to 2.04 | 2.10 to 2.28 | 0.590 to 0.661 | 0.355 to 0.392 | 0.168 to 0.183 | | |
| | | 23 K1 | | | | 1.41 to 1.68 | 1.45 to 1.58 | 0.598 to 0.679 | 0.474 to 0.532 | 0.254 to 0.285 | | |
| | | 24 K2 | | | | 0.0466 to 0.0484 | 0.0487 to 0.0498 | 0.0575 to 0.0603 | 0.0621 to 0.0654 | 0.0531 to 0.0566 | | |
| | | 25 Half- | time K1 | | \mathbf{i} | 0.413 to 0.493 | 0.438 to 0.477 | 1.02 to 1.16 | 1.30 to 1.46 | 2.43 to 2.73 | | |
| | | 26 Half- | time K2 | | | 14.3 to 14.9 | 13.9 to 14.2 | 11.5 to 12.0 | 10.6 to 11.2 | 12.3 to 13.1 | | |
| | | 27 Goodn | less of Fit | | | 4620 | 1600 | 4600 | 4600 | 4600 | | |
| | | 28 Deg | rees of Freedom | | — | 0.077 | 0.001 | 0.072 | 0.071 | 0.071 | | |
| | | 29 K Su 30 Sum | n of Squares | | | 3.25 | 1.60 | 2.09 | 1 11 | 0.971 | | |
| | | 31 SV X | ror oquares | | | 0.0448 | 0.0315 | 0.0359 | 0.0262 | 0.0236 | | |
| | | 32 Constr | raints | | | | 0.0010 | 0.0000 | 0.0202 | 0.0200 | | |
| | | 33 C | | | | C > 0 | C > 0 | C > 0 | C > 0 | C > 0 | | |
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| (c | <► | | 1 🗉 🗠 📰 | Nonlin fit of Ca2+, AT1 receptc ~ | | sults - | | | | Q – | Ð | |

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Calculating the initial rate and k_{τ}

A Prism 8 curve fitting to rise-and-fall to baseline equation with drift.pzfx:Nonlin fit of Ca2+, AT1 receptor - GraphPad Prism 8.3.0 (538)

| Eile Edit Prism | The initial rate and k_{τ} are easy to determine. | ge Draw Write √a w ↓ 1 123 - T T α A | $\overrightarrow{\mathbf{B} \ I \ \underline{U} \ \mathbf{X}^2 \ \mathbf{X}_2}$ | ► ▲ • | Print Send LA | Help Prism8 | | |
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| ✓ Data wit | Deculte v Madia 6 | 5 | Angl | TRV055 | C TRV045 | D TRV026 | E SI | F |
| ✓ III C III C III C | The initial rate is equal to the | eline time course with drift | | | | | | |
| ⊻ Info | value of the parameter C | | 15.6 | 15.5 | 16.6 | 17.2 | 17.1 | |
| | | | 0.994 | 0.996 | 0.995 | 1.00 | 0.997 | |
| Projec | t Info i j 5 Drift | | -0.000724 | -0.000379 | -0.000569 | -0.000339 | -0.000463 | - |
| 1 Projec | | • | 1.66 | 1.52 | 0.625 | 0.503 | 0.176 | J |
| (1) Proje | Since we are using a maximally- | | 0.0475 | 0.0492 | 0.0589 | 0.0638 | 0.0548 | |
| 🕀 New | | | 0.449 | 0.457 | 1.09 | 1.38 | 2.57 | |
| 🗸 Graphs | stimulating concentration of | | 14.6 | 14.1 | 11.8 | 10.9 | 12.6 | |
| 🗠 Ca2+ | licensed by is served to the initial | | 0.0004 | 0.00774 | 0.0004 | 0.0007 | 0.0447 | |
| ① New | ligand, κ_{τ} is equal to the initial | | 0.0201 | 0.00774 | 0.0281 | 0.0287 | 0.0447 | |
| | rate and as is sound to C | | 3 40e-005 | 2 40e-005 | 2 71e-005 | 1.96e-005 | 1.77e-005 | |
| | rate, and so is equal to C. | | 0.0813 | 0.0449 | 0.0179 | 0.00932 | 0.00386 | |
| (†) New | | | 0.0694 | 0.0325 | 0.0207 | 0.0149 | 0.00801 | |
| | | | 0.000452 | 0.000280 | 0.000703 | 0.000824 | 0.000890 | |
| | | | | | | | | |
| | Angli = 1.88 NFU.sec * | | 15.6 to 15.6 | 15.5 to 15.5 | 16.6 to 16.7 | 17.1 to 17.2 | 17.1 to 17.2 | |
| | | | 0.988 to 1.00 | 0.991 to 1.00 | 0.990 to 1.00 | 0.996 to 1.00 | 0.994 to 1.00 | |
| | $10055 = 2.19 \text{ NFU.sec}^{-1}$ | | 1 72 to 2 04 | -0.00042010-0.000332 | 0.590 to 0.661 | -0.00037710-0.000300 | -0.000498 to -0.000428 | |
| | | | 1.41 to 1.68 | 1.45 to 1.58 | 0.598 to 0.679 | 0.474 to 0.532 | 0.254 to 0.285 | |
| | 1KVU45 = 0.63 NFU.sec * | | 0.0466 to 0.0484 | 0.0487 to 0.0498 | 0.0575 to 0.0603 | 0.0621 to 0.0654 | 0.0531 to 0.0566 | |
| | | | 0.413 to 0.493 | 0.438 to 0.477 | 1.02 to 1.16 | 1.30 to 1.46 | 2.43 to 2.73 | |
| | 1 RVU26 = 0.37 NFU.sec \pm | | 14.3 to 14.9 | 13.9 to 14.2 | 11.5 to 12.0 | 10.6 to 11.2 | 12.3 to 13.1 | |
| | | | 1620 | 1600 | 1600 | 1600 | 1620 | |
| | SII = 0.18 NFU.sec ⁺ | | 0.977 | 0.991 | 0.972 | 0.971 | 0.971 | |
| | | | 3.25 | 1.60 | 2.09 | 1.11 | 0.905 | |
| | | | 0.0448 | 0.0315 | 0.0359 | 0.0262 | 0.0236 | |
| | | | | | | | | |
| | | | C > 0 | C > 0 | C > 0 | C > 0 | C > 0 | |
| | NFU, normalized fluorescence units |) | K2 > 0 | K2 > 0 | K2 > 0 | K2 > 0 | K2 > 0 | |
| | onlin fit | | | | | | + | ~ |

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Loading equations into Prism from a file

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

There is a sharing method that simplifies the loading of equations written by other users.

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

This process is described in this presentation.

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| Us the | e for time ere is base | course expe line drift. | eriment in wi | hich effect is ini | tiated aff | ter a base | line period , a | and where | |
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We are going to load the equation from the Results sheet.

The equation we need is called "Baseline then rise-and-fall to baseline with drift"

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| 🖃 Nonlin fit 🔅 | 36 Number of points | |
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| | [Pharmechanics] Baseline then rise-and-fall to baseline time course with drift | (| 0.05000 to 0.05000 | |
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🔺 Rise-and-fall time course equations for AGM.pzf:Nonlin fit of Baseline then rise-and-fall to baseline with drift - GraphPad Prism 8.3.0 (538)

Click "Details" for guidance on how to use the equation.

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| | Dose-response - Stimulation <u>Edit</u> | | 15.00 |
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| 👻 拱 Baseline then rise-a | Dose-response - Special, X is concentration Delete | | -0.001500 |
| 🗐 Nonlin fit | Dose-response - Special, X is log(concentration) | | 2.000 |
| ✓ □ P seune then rise-; | Binding - Saturation Delete All | | 1.500 |
| 🕞 Manlin fit | Binding - Competitive | | 0.05000 |
| | Binding - Kinetics Move Up | | 0.4621 |
| 👻 🖽 Baseline then rise | Enzyme kinetics - Inhibition | | 13.86 |
| 📃 Nonlin fit | Enzyme kinetics - Velocity as a function of substrate Move Down | | 6.0000.016 |
| 🕀 New Data Table | Exponential | | 1 170e-016 |
| Info | ± Lines | | 1.165e-018 |
| | | | 2.496e-015 |
| 1 Project info 1 | | | 1.954e-015 |
| 🕀 New Info | Sine waves | | 1.493e-017 |
| 🗸 Graphs | Growth curves | | |
| 应 Curve: Nonlin fit of Ris | | | 15.00 to 15.00 |
| Curve: Nonlin fit of Ris | Use for time course experiment in which effect is initiated after a baseline period , and where there is baseline drift | | 1.000 to 1.000 |
| Curve: Nonlin fit of Do | | | 2 000 to 2 000 |
| | Initial values might need to be entered manually: | | 1.500 to 1.500 |
| 🗠 Curve: Nonlin fit of Ba | K1, 1/t-half rise phase | | 0.05000 to 0.05000 |
| 应 Curve: Nonlin fit of I | [Pharmechanics] Baseline then rise-and-fall to baseline time course with drift | | 0.4621 to 0.4621 |
| 🕀 New Graph | Numerical derivatives | | 13.86 to 13.86 |
| Lavouts | Interpolate | | |
| A New Levent | ☐ Interpolate unknowns from standard curve. Confidence interval: None ∨ | | 536 |
| Thew Layout | | | 1.000 |
| | | _ | 4.090e-028 |
| | Learn Cancel OK | | 8.7358-016 |
| | 1.33 | | C > 0 |
| , | 34 K2 | | K2 > 0 |
| <u> </u> | 35 | | |
| 🖃 Nonlin fit | 36 Number of points | | |



本 Rise-and-fall time course equations for AGM.pzf:Nonlin fit of Baseline then rise-and-fall to baseline with drift - GraphPad Prism 8.3.0 (538) X <u>File Edit View Insert Change Arrange Family Window Help</u> Text Prism Shee Х Parameters: Nonlinear Regression 🗟 🗃 - 🗙 🕂 Model Method Compare Constrain Initial values Range Output Confidence Diagnostics Flag Search... Click OK then close file. Now Choose an equation Recently used Data with Results New • the equation is available every User-defined equations ✓ ☐ Rise-and-fall to bas [Pharmechanics] Baseline then rise-and-fall to baseline time course wit Details... Nonlin fit rift time you open Prism, in the Standard curves to interpolate ✓ ☐ Rise-and-fall to ste Dose-response - Stimulation Edit ... 15.00 "User-defined equations" list. Nonlin fit Dose-response - Inhibition 1.000 Dose-response - Special, X is concentration ✓ III Baseline then rise-a Delete -0.001500 Dose-response - Special, X is log(concentration) 2.000 🔲 Nonlin fit Binding - Saturation Delete All 1.500 ✓ ☐ Baseline then rise-a Binding - Competitive 0.05000 🔲 Nonlin fit Binding - Kinetics 0.4621 Move Up 13.86 Baseline then rise Enzyme kinetics - Inhibition Enzyme kinetics - Velocity as a function of substrate Move Down Nonlin fit 6.099e-016 Exponential 1.170e-016 ± Lines ✓ Info 1.165e-018 Polynomial 2.496e-015 Project info 1 Gaussian 1.954e-015 ine waves New Info... 1.493e-017 curves Graphs 15.00 to 15.00 Curve: Nonlin fit of Rise ment in which effect is initiated after a baseline period , and where Use for time coun 1.000 to 1.000 Curve: Nonlin fit of Ris there is baseline drift. -0.001500 to -0.001500 2.000 to 2.000 Curve: Nonlin fit of Bas Initial values might need to be ena pually: 1.500 to 1.500 Curve: Nonlin fit of Bas K1, 1/t-half rise phase 0.05000 to 0.05000 time course with drift [Pharmechanics] Baseline then rise-and-fall to Curve: Nonlin fit of B 0.4621 to 0.4621 Numerical derivatives 13.86 to 13.86 🕀 New Graph... Interpolate Layouts 536 Interpolate unknowns from standard curve. Confidence interval: ① New Layout... 1.000 4.090e-028 8.735e-016 Cancel OK Learn C > 0 55 K2 34 K2 > 0 < > 35 🔲 Nonlin fit 36 Number of points < > Σ < (\mathbf{i}) Q€ € Nonlin fit of Baseline then rise- 🖂 *∂* ▼ Table of results