Using JMP for Statistical Analysis of Biological Assays and Test

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Analysis of Biological Assays

Introduction

- European Pharmacopoeia section 5.3 (EP5.3) provides detailed guidance for design, analysis, and validation of biological assays. It presents four approaches for working with dose-response curves:
  - Parallel Line Model
  - Slope-Ratio Model
  - Quantile Responses
  - Extended Sigmoid Dose Response Curves
- It is consistent with USP 1032-1034 and ICH Q2R, yet more thorough.

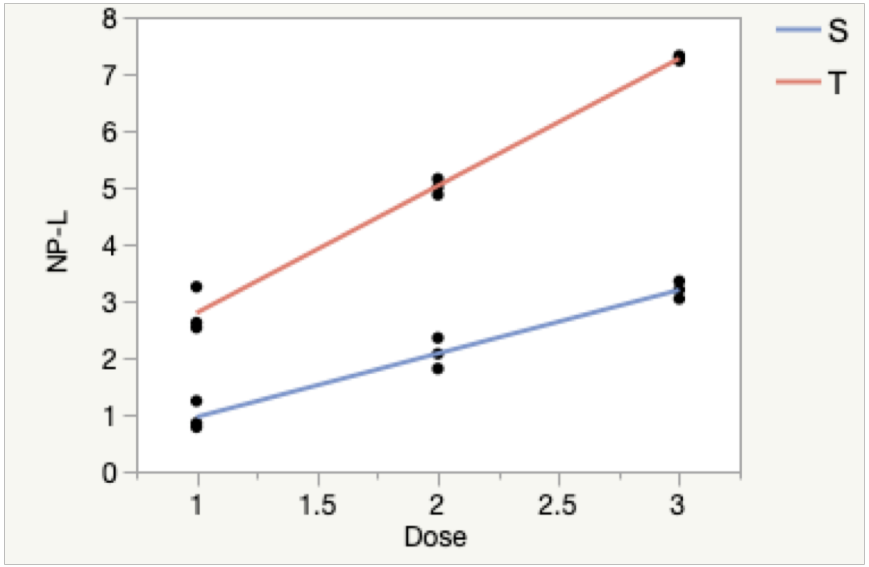
Analysis of Biological Assays

Introduction – Terminology

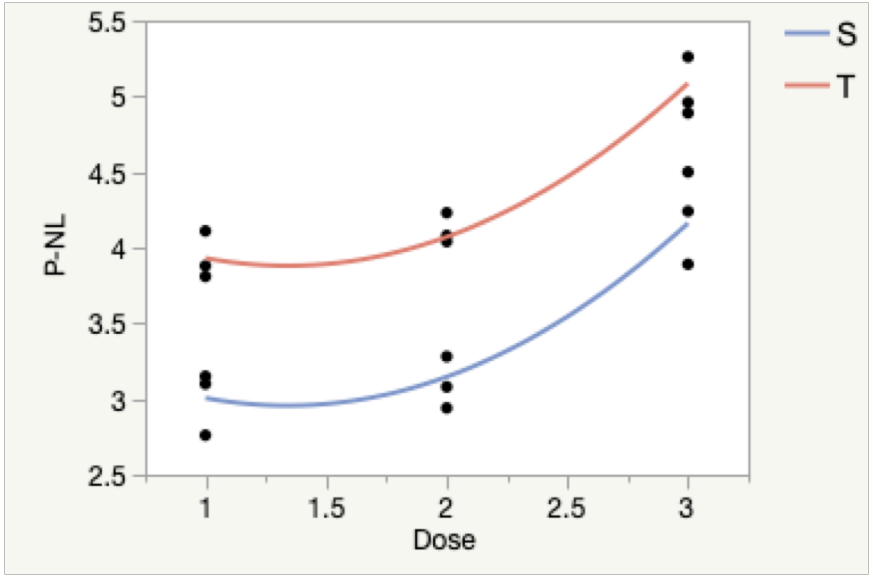
- Preparation – identifier of the formulation being tests, of which the standard is one.
- Treatments – unique combination of preparation and dose
- Regression – The change in response as a function of dose.
- Non-parallelism – comparison of the dose response curves across preparations. Using a parallel lines model, non-parallelism can be seen as the difference in slopes between preparations on the dose-response graph. Tested using the Preparation*Dose interaction.
- Non-linearity – differences in the shapes across preparations beyond that associated with linear slopes. Parallel preparations can be non-linear and vice versa.

Analysis of Biological Assays

Introduction – Terminology



Non-parallel/Linear



Parallel/Non-linear

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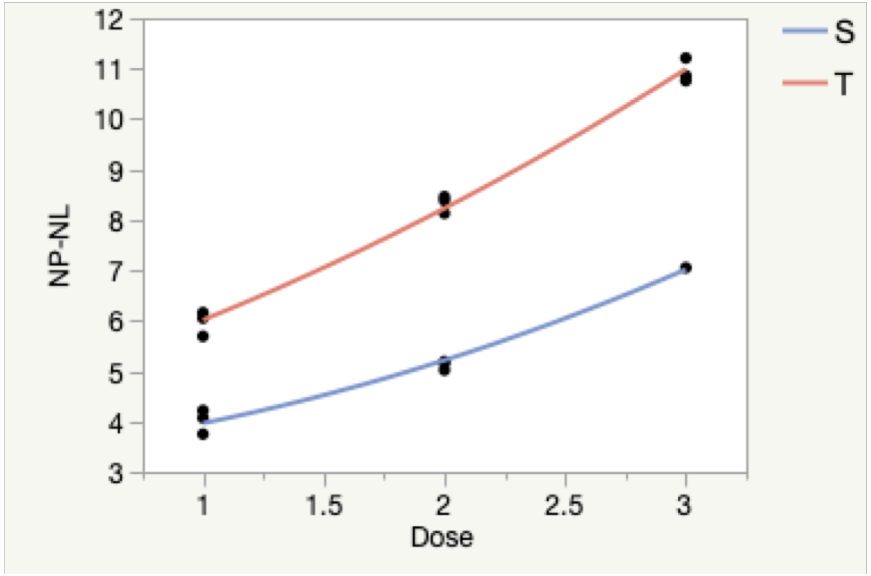
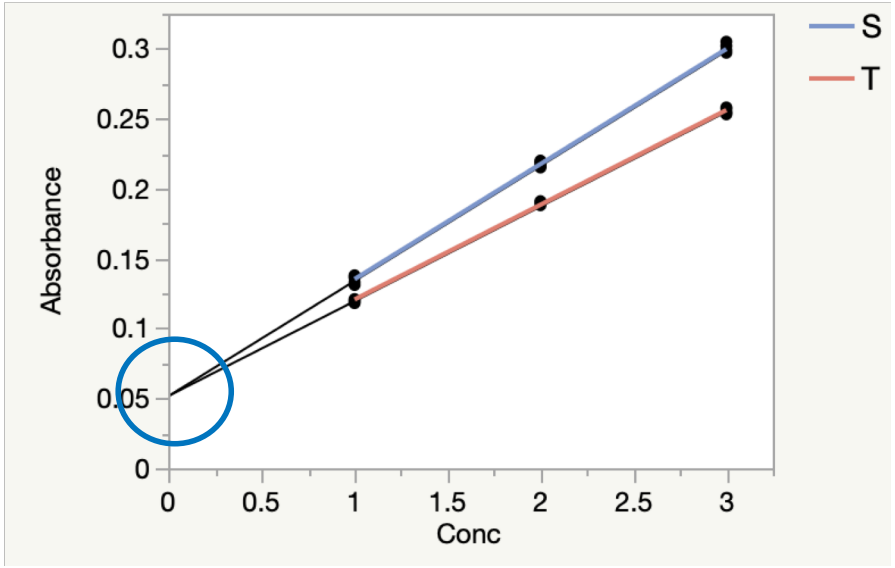
Introduction – Terminology



Non-parallel/Non-linear



Intersection

Analysis of Biological Assays

Introduction – Test of Validity/Relative Potency

- Tests of Validity
  - Regression is significant (Parallel Line only)
  - Non-linearity is not significant.
  - Non-parallelism is not significant (except Slope Ratio).
- Relative Potency
  - Parallel Line: (Preparation[Test] – Preparation[Standard])/Common Slope
  - Slope Ratio: Slope[Preparation]/Slope[Standard]
  - Quantile Responses: (Preparation[Test] – Preparation[Standard])/Common Slope
  - Sigmoidal Curves: curve dependent. For the 4 parameter logistic it is the difference between the test and standard inflection parameter estimates.

Analysis of Biological Assays

Introduction – Relative Potency

- There are multiple ways to calculate confidence intervals for relative potency. The script demonstrated below is based on Fieller's Theorem, a common general approach discussed in EP5.3 (and USP 1032-34).
  - An adjustment is made to relative potency relative to the correlation of the parameters used in the numerator and denominator. If they are uncorrelated, there is no adjustment.
- The application of Fieller's theorem to relative potency is a bit complicated but can be hand calculated using JMP output. The method for doing this is described in the Additional Material section.

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Parallel Line Model

- Assumes a linear relationship between dose and response over the measurement range. Transformation of the dose and/or response may be needed prior to analysis. Analyzed using standard regression methods.
  - Log transformation often used for dose
- Steps
  - Fit model shown on next page. Non-linearity can only be included if there are more than two levels of dose.
    - You can use Estimates > Custom Test to combine the non-linearity components (shown below).
    - Design components appear after the non-linearity terms.
  - If Dose (Regression) is non-significant stop. If non-parallelism or non-linearity are significant, you will need to find the preparations causing the significance and remove them before calculating relative potency.

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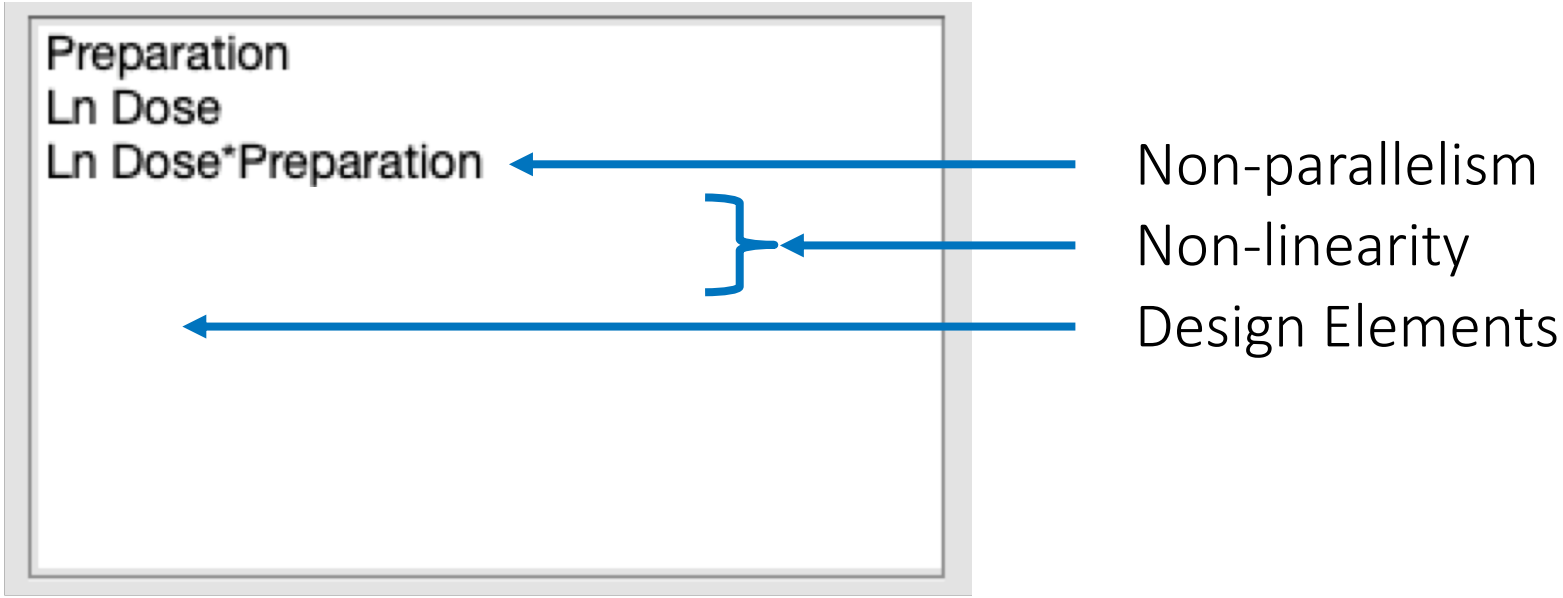
Parallel Line Model – Basic Setup



1. Cross dose with itself one fewer times than there are dose levels
2. Cross the results of 1. with Preparation

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Parallel Line Model – Combining Non-linearity Terms

Parameter						
Intercept	0	0	0	0	0	0
Preparation[A]	0	0	0	0	0	0
Preparation[B]	0	0	0	0	0	0
Dose	0	0	0	0	0	0
Preparation[A]*(Dose-2.5)	0	0	0	0	0	0
Preparation[B]*(Dose-2.5)	0	0	0	0	0	0
(Dose-2.5)*(Dose-2.5)	1	0	0	0	0	0
(Dose-2.5)*(Dose-2.5)*(Dose-2.5)	0	1	0	0	0	0
(Dose-2.5)*(Dose-2.5)*Preparation[A]	0	0	-1	0	0	0
(Dose-2.5)*(Dose-2.5)*Preparation[B]	0	0	0	-1	0	0
(Dose-2.5)*(Dose-2.5)*(Dose-2.5)*Preparation[A]	0	0	0	0	-1	0
(Dose-2.5)*(Dose-2.5)*(Dose-2.5)*Preparation[B]	0	0	0	0	0	-1
=	0	0	0	0	0	0

1. Start with #preparations*(# dose levels – 2) columns.
2. Leave these 0
3. (Dose with itself) 1s on the diagonal, 0 everywhere else
4. (Dose*Preparation terms) -1s on the diagonal, 0 everywhere else

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Parallel Line Model – Relative Potency

Expanded Estimates

Nominal factors expanded to all levels

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-0.525846	0.024496	-21.47	<.0001*
Preparation[S]	0.458218	0.018091	25.33	<.0001*
Preparation[T]	-0.246016	0.018091	-13.60	<.0001*
Preparation[U]	-0.054402	0.018091	-3.01	0.0040*
Preparation[V]	-0.1578	0.018091	-8.72	<.0001*
Log[Dose]	0.9084792	0.010655	85.26	<.0001*

- (Standard – Preparation)/Common Slope
- Example – preparation T
  - (0.458218 – (– 0.246016))/0.9084792 = 0.7752

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Parallel Line Model – Examples

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Parallel Line Model – Notes

- You may need to turn on Estimates > Sequential Tests to match the Type I sums of squares in EP5.3.
- Analysis for Treatments and plots are included with the JMP tables. While they are displayed in EP5.3, neither are mentioned in the Tests of Validity section (Section 3.2.4).
- The Parallel Line Model has drawback beyond the reliance on hand calculations (details at the end of the slide deck). Alternatively:
  - Set up the model as above except make any experimental components random.
  - Use JMP's Type III SS to test regression and non-linearity terms.
  - If non-parallelism and non-linearity are non-significant, remove them from the model before calculating relative potency.

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Slope-Ratio Model

- Similar model set-up to the Parallel Line Model with some exceptions:
  - The Regression term includes both Dose and Dose*Preparation (non-parallelism). It is not part of the tests of validity (Section 3.3.4).
  - An Intersection term tests that all preparations are not statistically different from zero where they cross the y-axis. It is associated with the term for preparation.

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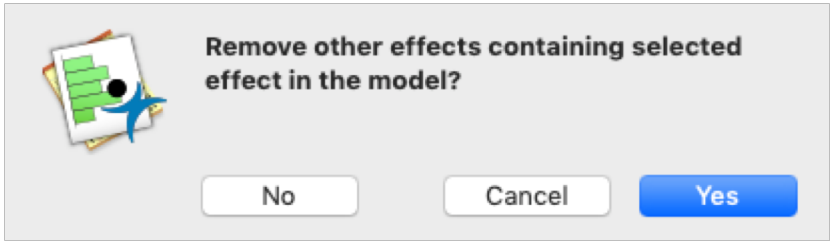
Slope-Ratio Model

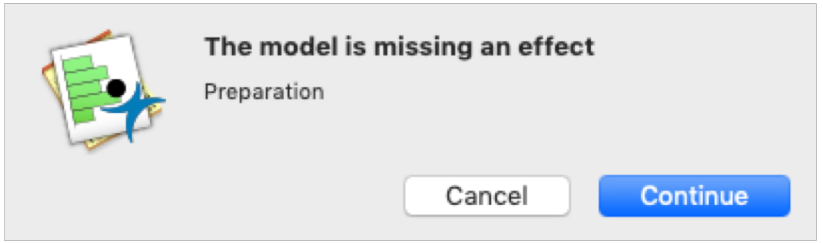
- Steps
  1. In the Fit Model dialog, build the model as you would for a Parallel Line Model.
  2. Under the hotspot button to the left of Model Specifications turn off Center Polynomials. Run the dialog and combine the non-linearity as above.
  3. Assuming non-significant non-linearity, rerun with the non-linearity terms removed. The preparation term is the test for Intersection.
     - The F and p values will be slightly different from the EP5.3 approach because the non-linearity terms are added to mean square error (denominator of the F statistic). If non-linearity is non-significant this difference should be small.
  4. If preparation (intersection) is not significant, rerun the model with the dose and dose*preparation terms only. This will give the regression term.

Analysis of Biological Assays

Slope-Ratio Model – Notes

- When fitting and building the model for the Regression term you may get warnings similar to those shown below. Answer the left query “No” and the right “Continue”. The Regression values correspond to the Model line in the Analysis of Variance outline box.





- Relative potency is calculated using these estimates:

$$\frac{dose + dose * preparation[Test]}{dose + dose * preparation[Standard]}$$

Analysis of Biological Assays

Slope-Ratio Model – Examples

Analysis of Biological Assays

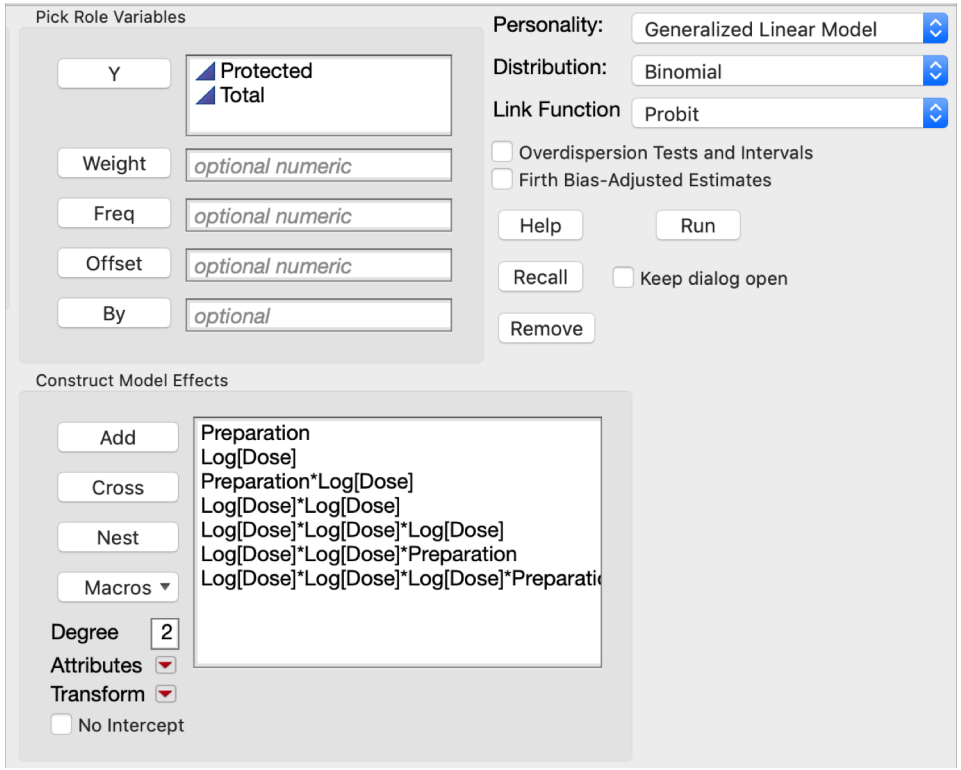
Slope-Ratio Model

- Advantages
  - Offers a viable approach when the slopes are different between standard and test preparation.
- Drawbacks
  - Assumes equally spaced concentrations
  - Requires the same number of aliquots for every preparation
  - Require balanced design

Analysis of Biological Assays

Quantile Response

- The probit and logit Quantile Response models are set up in a similar fashion to the Parallel Line model above. It uses the Generalized Linear Model personality under Fit Model. On the right is an example using the probit distribution. The logit distribution can be selected under Link Function. The data in the example is summarized and requires 2 Y variables.



Analysis of Biological Assays

Quantile Responses (Probit) – Notes

- You need at least three doses to use this approach.
- For unsummarized data, use a single nominal Y with 1 indicating positive results and 0 negative results.
- If needed, transform the dose using to natural logarithm scale.
- As data departs further from the typical s-shaped curve, convergence becomes more difficult. Adjusting starting values or increasing number of iterations may help.
- While the tests for non-linearity and non-parallelism will be consistent to that in EP5.3, the chi-square and p-values will not match exactly. See the appendix and included script for recreation of exact values using the algorithm given in EP5.3.

Analysis of Biological Assays

Quantile Response – Examples

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Quantile Responses – Notes

- To reproduce results from EP5.3, fit a model using the Nonlinear platform (detail below in Additional Material). Use the quantile response add-in with the full model.
  - For Example 5.3.3 use the attached script to summarize the data and set up the Nonlinear platform.

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Sigmoidal Dose-Response Curves

- This approach can be implemented with either the Nonlinear or Fit Curve platform. Fit Curve is the Fit Nonlinear platform with a number of commonly fitted curves.
- To set up Fit Curve use the response as Y, the dose as X, and the preparation as Group variables. In the resulting report window, select Sigmoid Curves>Logistic Curves>Fit Logistic 4P under the hotspot.
  - Under the hotspot to the left of Logistic 4P outline box, Test Parallelism tests for non-parallelism.
- Nonlinear requires a parametric formula. The example in the saved script forces all the parameters to be the same except the inflection point.
- The relative potency is Exp(Inflection[Standard] – Inflection[Test]).

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Sigmoidal Dose-Response Curves– Examples

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Sigmoidal Dose-Response Curves – Notes

- You need at least three doses for this approach.

Thanks!

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Additional Material

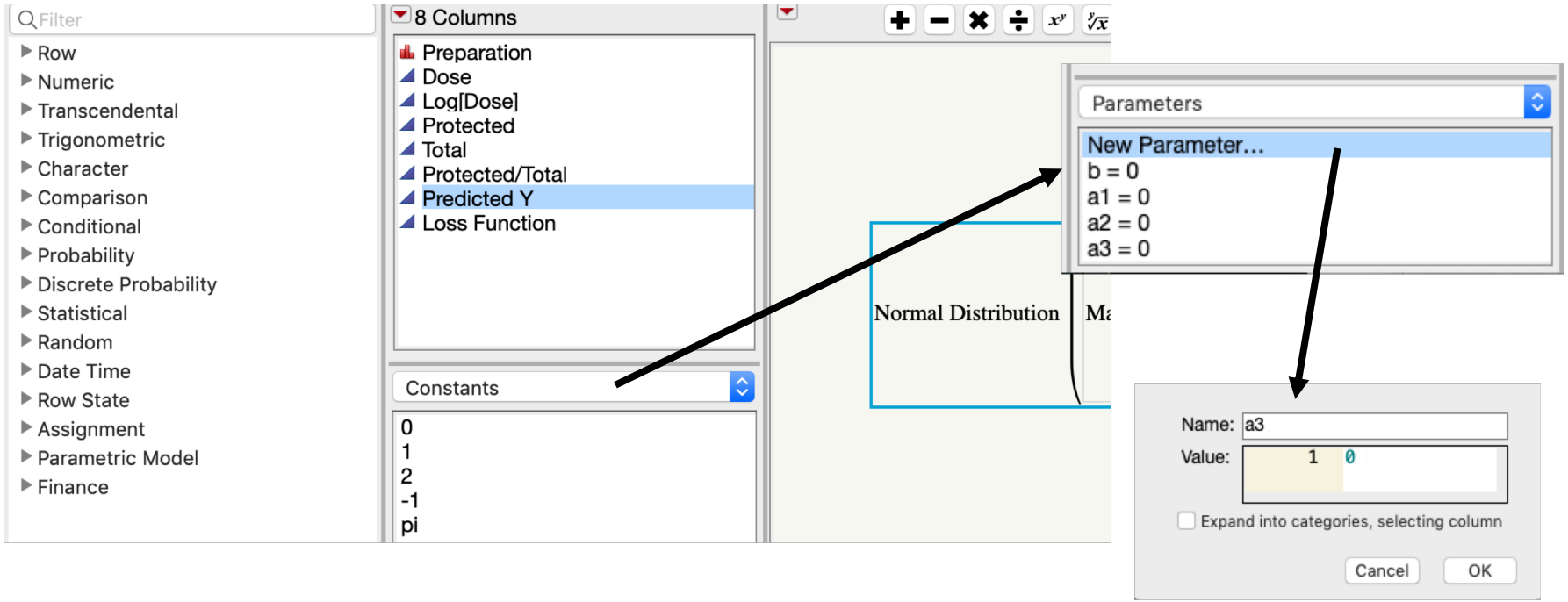
Analysis of Biological Assays

Building Parametric Formulas

- A parametric formula is different from a regular formula in that the parameter values (which appear in bold) start with initial values but can be changed based on results from Nonlinear.
- By default, the lower center area of Formula Editor window is set to Constants. To create parameters, set this to Parameters, click New Parameters …, then give the parameter a name and starting value. You can edit or delete parameters by right clicking them.
- Drag the parameters into the formula where you want them. The parametric formula can contain other formula elements, such as columns, operators, and functions.

Analysis of Biological Assays

Building Parametric Formulas



Analysis of Biological Assays

Parallel Line Model – Notes

- Drawbacks with the Parallel Line Model
  - Assumes balanced design. Missing values replaced using design specific approximations.
  - Details are only provided for four designs (completely randomized, randomized complete block, crossover, and Latin square). Not guaranteed to be extendible to other designs.
  - Statistical tests use Type I SS. Values may be affected by the order in which the sources of variation are fitted.

Analysis of Biological Assays

Parallel Line Model – Notes

- Should the model be refit after removing non-linearity and non-parallelism terms before calculating relative potency (RP)?
  - RP is a function of the ratio of the Preparation estimate to the Regression estimate. The estimates are correlated with the non-linearity and non-parallelism estimates.
  - The Preparation and Regression estimates are different depending whether non-linearity and non-parallelism are removed or retained.
  - RP is also a function of the MSE, which is also affected by removing or retaining non-linearity and non-parallelism.
  - EP5.3 includes non-linearity and non-parallelism for estimating Preparation and Regression, but excludes them for MSE.

Analysis of Biological Assays

Parallel Line Model – Notes

- When additional design components are used should the be fixed or random? EP5.3 treats them as fixed, but they may be more naturally considered random since they are sampled from a larger population.
- If none of the non-linearity components are significant, it is extremely likely, though not guaranteed, the combined test will not be significant. However, there may be one or more significant components, but the overall test may be non-significant.
- A general linear models approach can be used to estimate RP (EP5.3 Section 7.1). This is what is used in the JSL script. If non-linearity and non-parallelism are not statistically different from zero, it may make sense to remove them before calculating RP.

Analysis of Biological Assays

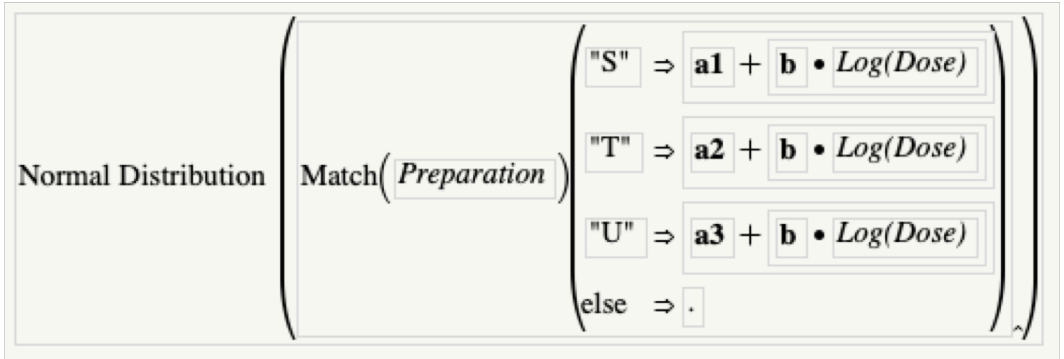
Parallel Line Model – Crossover Designs

- More difficult to set up and analyze because there are multiple treatments and multiple time periods. You need to account for who is given which treatment in which period and if there are carryover effects.
- The example in EP 5.3 is very basic, two treatments and two periods. It is assumed there is no carryover effect.
- In addition to the extra design components you need a random effect uniquely identifying the subject and the order in which the treatments were applied.
  - Day, Preparation*Day, Log(Level)*Day, and Preparation*Log(Level)*Day are the design components.
  - Subject[Sequence] identifies who got what treatment in what period.
- See the end of my Discovery 2012 talk for another analysis example.

Analysis of Biological Assays

Quantile Responses (Probit)

- The set-up for Probit analysis of quantile responses using the Nonlinear platform requires a parametric formula for the predicted response and a loss function formula.
  - An example of the predicted response formula is shown below. You need one Match value for each preparation. The slope parameter **b** is shared by all preparations, but the intercept parameters (**a**'s) are different.
  - With only two preparations, an If() function can be used instead of Match().



Analysis of Biological Assays

Quantile Responses (Probit)

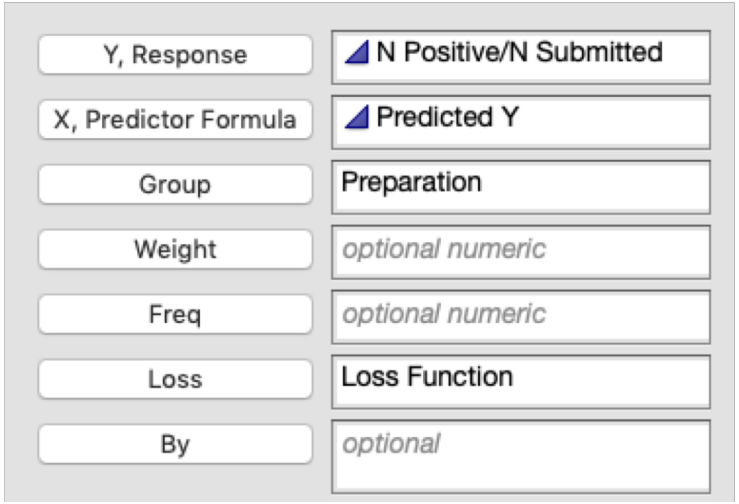
- The loss function will be similar to that shown at the bottom
  - *Predicted Y* is the parametric formula for predicted response
  - *N Positive* is the number of positive outcomes
  - *N Submitted* is the number units submitted to treatment.

$$-\left(N\,Positive \bullet \mathrm{Log}\left(Y\,Predicted \right) + \left(N\,Submitted - N\,Positive \right) \bullet \mathrm{Log}\left(1 - Y\,Predicted \right) \right)$$

Analysis of Biological Assays

Quantile Responses (Probit)

- The Nonlinear dialog will look similar to that below.
  - For **Y, Response** use the ratio of the number positive to number submitted per preparation (likely a formula).
  - The parametric formula is used for **X, Predictor Formula**
  - The preparation ID column is used for the **Group** variable
  - The loss function formula is used for **Loss**.



Analysis of Biological Assays

Quantile Responses (Probit) – Notes

- If needed, summarize the data so each row corresponds to a unique treatment/dose combination. Include the number of units submitted and the positive responses for each combination.
- If needed, transform the dose using the natural logarithm (the Log() function in JMP).
- Validity tests, Calculations for RP
- Starting parameter values, convergence, iteration options.

Analysis of Biological Assays

Confidence Intervals for Relative Potency

$$\frac{R - \frac{gv_{12}}{v_{22}} \pm \frac{t_{\alpha,df}}{b}\sqrt{(1-g)v_{11} + R^2 v_{22} - 2Rv_{12} + \frac{gv_{12}^2}{v_{22}}}}{1-g}$$

$$g = \frac{t_{\alpha,df}^2 s^2 v_{22}}{b^2}$$

The formulas for R, v_{11}, v_{12}, v_{22}, and b depend on the models. Parallel Line and Quantile Response will differ from Slope Ratio. The Sigmoidal Dose-Response model uses a different approach to estimating confidence intervals.

Analysis of Biological Assays

Confidence Intervals for Relative Potency – Parallel Line Model

$$R = \frac{a_S - a_T}{b^2}$$

$$g = \frac{t^2_{\alpha,df} MS_e v_{22}}{b^2}$$

$$v_{11} = \frac{SE^2_{Std} + SE^2_{Test} - 2r_{st} SE^2_{Std} SE^2_{Test}}{MS_e}$$

$$v_{12} = \frac{r_{ds} SE^2_{Dose} SE^2_{Std} - r_{dt} SE^2_{Dose} SE^2_{Test}}{MS_e}$$

$$v_{22} = \frac{SE^2_{Dose}}{MS_e}$$

a_S – Estimate for standard

a_T – Estimate for test preparation

b – Common slope estimate

$t_{\alpha,df}$ – Student's t $1 - \alpha/2$ quantile, error degrees of freedom df 2-sided (1- α)% CI

MS_e – Mean square error

SE_{Std} – Standard error for a_S

SE_{Test} – Standard error for a_T

SE_{Dose} – Standard error for b

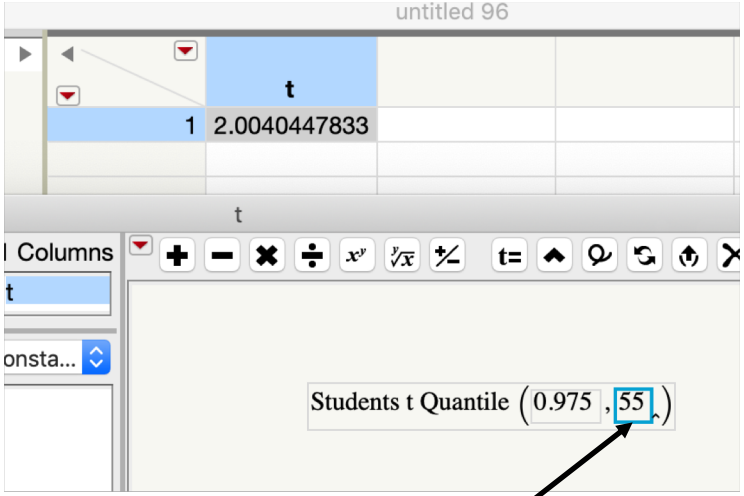
r_{st} – Correlation between a_S & a_T

r_{ds} – Correlation between b& a_S

r_{dt} – Correlation between b& a_T

Analysis of Biological Assays

Confidence Intervals for Relative Potency – Parallel Line Model



a_S, SE_{std}

Estimates and standard errors for tests directly below standard

Expanded Estimates

Nominal factors expanded to all levels

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-0.525846	0.024496	-21.47	<.0001*
Preparation[S]	0.458218	0.018091	25.33	<.0001*
Preparation[T]	-0.246016	0.018091	-13.60	<.0001*
Preparation[U]	-0.054402	0.018091	-3.01	0.0040*
Preparation[V]	-0.1578	0.018091	-8.72	<.0001*
Log[Dose]	0.9084792	0.010655	85.26	<.0001*

MS_e

Analysis of Variance

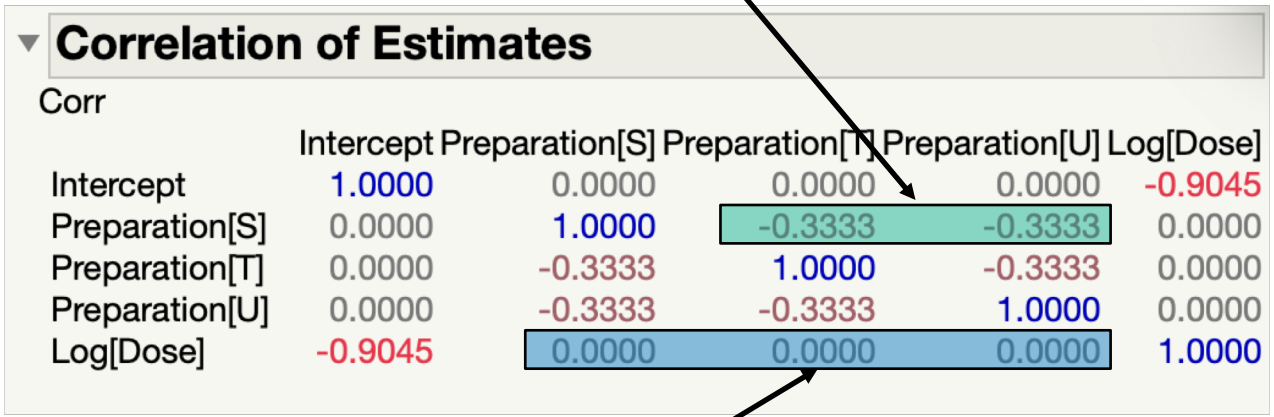
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	4	52.059348	13.0148	1988.239
Error	55	0.360025	0.0065	Prob > F
C. Total	59	52.419373		<.0001*

b, SE_{Dose}

Analysis of Biological Assays

Confidence Intervals for Relative Potency – Parallel Line Model

Correlations between standard and test preparations



Correlation of Estimates

Corr

	Intercept	Preparation[S]	Preparation[T]	Preparation[U]	Log[Dose]
Intercept	1.0000	0.0000	0.0000	0.0000	-0.9045
Preparation[S]	0.0000	1.0000	-0.3333	-0.3333	0.0000
Preparation[T]	0.0000	-0.3333	1.0000	-0.3333	0.0000
Preparation[U]	0.0000	-0.3333	-0.3333	1.0000	0.0000
Log[Dose]	-0.9045	0.0000	0.0000	0.0000	1.0000

Correlations between dose and preparations