Using Global Curve Fitting to Determine Dose Response Parallelism

Keywords: global curve fitting, dose response, parallelism, EC50, parameter sharing

Introduction

Dose response curves are parallel if they are only shifted right or left on the concentration (X) axis. So if you were to fit a 4 parameter logistic function to multiple dose response curves then, for curves which are parallel, only the EC50 parameters would be significantly different. If the data is normalized then the min, max and Hill slope parameters would not be significantly different.

A simple procedure to determine parallelism is to use global curve fitting to fit the dose response curve data two ways - 1) with no shared parameters and 2) with min, max and Hill slope shared for all data sets. Then use an F test to see if the two fits are significantly different. If there is no difference between the fits then only differences in the EC50 values caused the different data sets and the dose response curves would be considered to be parallel.

An Example

The data in the graph below has 3 replicates for each concentration. There are two data sets to be compared – a standard and a sample. A global curve fit of both data sets using a 4 parameter logistic function was performed with no shared parameters. The fit lines are shown in the left panel below.

To determine parallelism you perform a second global curve fit but, in this case, share the 3 parameters min, max and Hill slope. The results are shown in the right panel below.

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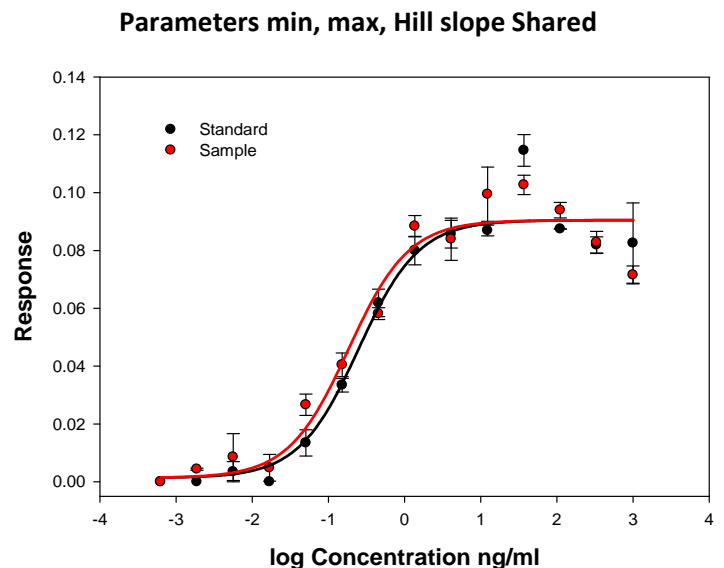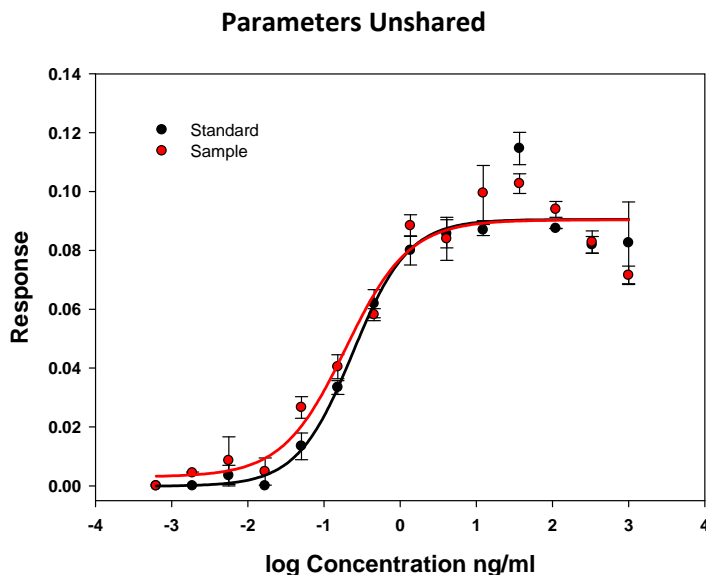
The two curves with shared parameters are clearly more uniformly shifted horizontally but are these fits significantly different from the previous? To determine this use the F test

$$F = \frac{\dfrac{SS_{shared} - SS_{unshared}}{DF_{shared} - DF_{unshared}}}{\dfrac{SS_{unshared}}{DF_{unshared}}}$$

where the residual degrees of freedom are the number of data points in the fit minus the number of fitted parameters (this definition applies for n data sets not just the two in this example). The residual degrees of freedom (Residual DF), required for the F test, are obtained from the ANOVA tables in the curve fit reports. They are 76 and 79 for the no-sharing and sharing cases, respectively.

Analysis of Variance - No Shared Parameters:

	DF	SS	MS
Regression	8	0.3630	0.0454
Residual	76	0.0090	0.0001
Total	84	0.3720	0.0044

Analysis of Variance - min, max, Hillslope Shared:

	DF	SS	MS
Regression	5	0.3629	0.0726
Residual	79	0.0091	0.0001
Total	84	0.3720	0.0044

The residual sum of squares for shared parameters shown in the tables above are larger than for unshared (0.0091 vs. 0.0090) – but only slightly.

A transform is used to compute the F and associated P value to determine if there is a significant difference. The curve fit option for placing the fit residuals in the worksheet was set and the resulting column numbers are specified in the transform.

```
'F test for Dose Response Parallelism
'   Enter the degrees of freedom and the residuals columns (one
'   for each data set) from the unshared and shared global fits.

'Input
DFnoshared = 76                          'unshared DOF
DFshared = 79                            'shared DOF
UnsharedResidualsColumns = {11,14}  'worksheet columns with unshared residuals
SharedResidualsColumns = {21,24}    'worksheet columns with shared residuals
co=30                                    'first output column

'Program
cw = 1000  'working column
cell(cw,1) = 0
cell(cw,2) = 0
for i = 1 to size(UnsharedResidualsColumns) do
    cell(cw,1) = cell(cw,1) + total(col(UnsharedResidualsColumns[i])^2)
    cell(cw,2) = cell(cw,2) + total(col(SharedResidualsColumns[i])^2)
end for
SSnoshared = cell(cw,1)
SSshared = cell(cw,2)
DFnum=DFshared-DFnoshared
DFdenom=DFnoshared
Fnumerator= (SSshared-SSnoshared)/(DFshared-DFnoshared)
Fdenominator=SSnoshared/DFnoshared
F=Fnumerator/Fdenominator
Pvalue=1-fdist(F,DFnum,DFdenom)
'Remove working area
cell(cw,1) = ""
cell(cw,2) = ""

'Output
col(co)={"F", "DFnum","DFdenom","P"}
col(co+1)={F, DFnum, DFdenom, Pvalue}
```
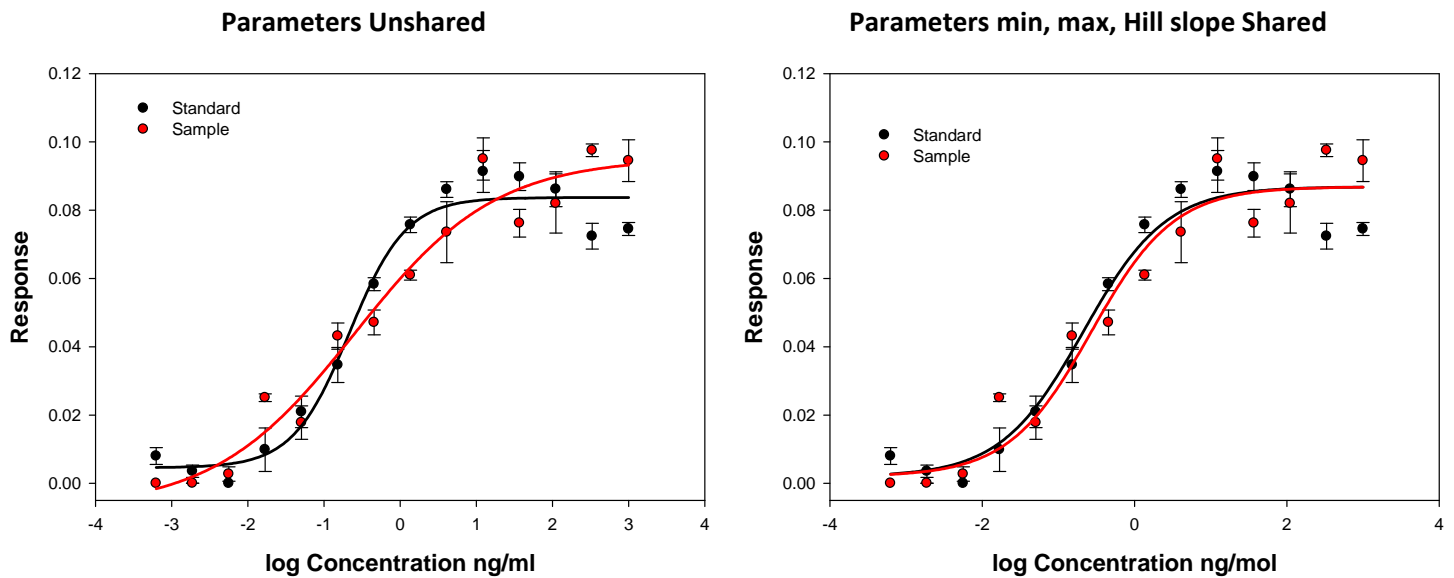
The results of running this transform were:

F	0.4075
DFnum	3
DFdenom	76
P	0.748

Since P > 0.05; the conclusion is that there is no reason to suspect that the data is not parallel.

A Second Example

A second data set pair shows a significant difference with P = 0.0039 indicating that these data sets are not parallel.

Parameters Unshared	Parameters min, max, Hill slope Shared

| F	4.8382
DFnum	3
DFdenom	76
P	0.0039

The Procedure

To repeat the second analysis shown above, do the following:

1. Download the notebook containing the data in the examples above, Dose Response Parallelism.jnb, and open the third worksheet "Demonstration". This contains the data for the second example.
2. Select the Global Fit Wizard under Nonlinear Regression.
3. Select the Ligand Binding Equation Category, and the equation "sigmoidal dose-response (variable slope)", then Click Next.
4. In the Shared Parameters panel do not select any parameters, Click Next.
5. In the Variables Panel, in "Data From" select the data format "X Many Y Replicates".
6. Select the following worksheet columns and click Next.
    a. column 1 for x (x: 1-log Conc.)
    b. column 2 for the first of the standard group y replicates (First y 1: 2-Standard 1)
    c. column 4 for the last of the standard group y replicates (Last y 1: 4-Standard 3)
    d. column 5 for the first of the sample group y replicates (First y 2: 5-Sample 1)
    e. column 7 for the last of the sample group y replicates (Last y 2: 7-Sample 3)
7. Scroll down the Fit Results panel to see that both data sets have been fit by the Global Fit Wizard (independently in this case since no parameters were shared), then Click Next.
8. Select the following options in the Numeric Output Options panel and then click Next.

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Results

☑ Parameters: First Empty
☑ Predicted: First Empty
☑ Residuals: First Empty

☑ Create Report
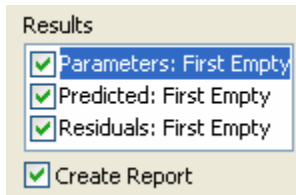
9. Select the "Create new graph" checkbox and click Finish.
10. Repeat steps 2 through 8 in exactly the same way except in the Shared Parameters Panel share the min, max and Hill slope parameters.

Shared Parameters

☑ min
☑ max
☐ logEC50
☑ Hillslope
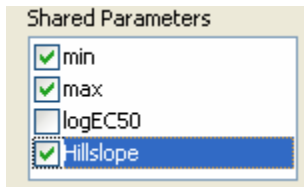
11. Select the Transform, User-Defined and open the transform that you downloaded from this site.
12. Enter values for the two degrees of freedom and the column numbers for the four Residuals columns in your worksheet and run the transform to produce the P value equal to 0.0039 shown above.