

Pharmacokinetics

GENERAL

HOW TO CALCULATE THE PK PARAMETERS

PHARMACOKINETIC CALCULATIONS

PLOTS

EXAMPLE

REFERENCES

General

EquivTest/PK now has the added feature of simple-to-use, built-in pharmacokinetic functions. It provides a fast and easy means of calculating and plotting the basic and most commonly used pharmacokinetic parameters.

C_{max}, T_{max}, Lag Time, AUC(0-t), AUC(t-∞), AUC(0-∞), AUC(0-t)/AUC(0-∞), Mean Residence Time, AUMC(0-t), AUMC(0-∞), Half-Life, Elimination Rate Constant (λ).

All of the PK calculations in EquivTest/PK are based on noncompartmental methods of analysis for the estimation of pharmacokinetic parameters.

To access the pharmacokinetic functionality in EquivTest/PK, click on the **File** menu from the main EquivTest/PK screen and select **New**. From the list of File Types, select **EquivTest/PK – Datasheet**.

The Study Design setup screen appears.

Number of subjects : 1 Limit of Quantitation :

Number of groups : 1

Number of sampling points : 1 Advanced Settings

Group 1 :

Time	Value
T0	0.0

Ok Cancel

This screen allows you to set up the datasheet that is going to contain your concentration values and your subject, group and period identifiers.

Under **Number of Subjects**, either type in the total number of subjects in your study, or else use the up and down arrows to scroll to the desired number.

Under **Number of Groups**, either type in the total number of groups in your study, or else use the up and down arrows to scroll to the desired number of groups. For example, for a 2x2 crossover design you would enter 2 as the number of groups. Group names, or labels can be assigned to each of the groups, and when you enter the desired number of groups, the number of fields for entering the group names is updated accordingly. Numeric values or text values can be used as group names.

The **Number of sampling points** is the number of points at which a concentration value was measured. You can either type in the total number of sampling points in your study, or else you can use the up and down arrows to scroll to the desired number. When you have entered the number of sampling points, the table displaying the time values is updated accordingly. In most studies, concentration values are not measured at equal time intervals, so in this section the user specifies the time values at which measurements were taken. It is important to note that the unit of time used must be the same for all of the time values. You can enter integer values or decimal values here. Whatever number you enter as the time value will be prefixed by the letter T and will be used as a label for the column in the resulting datasheet.

The **Limit of Quantitation (LOQ)** can also be set in this screen. This value is stored as an attribute of the datasheet. Concentration values below the LOQ that occur *before* C_{max} (the maximum observed concentration for a particular subject) are set to zero in the calculations and concentration values that occur *after* C_{max} are set to the value of the LOQ.

If you click on the **Advanced Settings** button, you will see the following screen:

Advanced Settings

Select Spreadsheet type:

Multi Variables/Subject :

Multi Records/Subject :

Concentration value to be used in calculation of AUC:

Observed concentration value, C₀ :

Estimate C₀ :

Decimal Places:

Spreadsheet : 2

Results : 2

Missing values:

Linear interpolation :

No interpolation :

To be transferred for further analysis

All decimals :

Displayed decimals only :

Limit of Quantitation:

LOQ :

Units of measure:

Concentration units : milligram (mg)

Volume units : millilitre (mL)

Other : mg / mL

OK

Under **Select Spreadsheet type**, you can choose the format in which you want your data to appear. Data for these types of studies can be stored as “Multi Variables/Subject” – where concentration values for the different sampling points are stored in separate columns, so each row corresponds to one subject’s concentration/time profile.

Subject	Group	Period	T0	T1	T2	T3	T4
1	reference	1	0.00	0.00	0.43	1.23	1.80
2	reference	1	0.00	0.00	0.53	1.01	1.80
3	reference	2	0.00	0.00	0.60	1.41	2.31
4	reference	1	0.00	0.24	0.66	1.41	2.54
5	reference	2	0.00	0.00	0.00	0.45	0.81
6	reference	2	0.00	0.00	0.31	0.65	1.05
7	reference	1	0.00	0.00	0.75	1.16	1.70
8	reference	2	0.00	0.00	0.14	0.35	0.73
9	reference	1	0.00	0.00	0.00	0.25	1.04

Alternatively, the data can be stored as “Multi Records/Subject” – where the concentration values are stored in one column with another variable to identify the sampling point.

Subject	Group	Period	Conc	Time
1	reference	1	0.00	T0
1	reference	1	0.00	T1
1	reference	1	0.43	T2
1	reference	1	1.23	T3
1	reference	1	1.80	T4
1	reference	1	2.26	T5
1	reference	1	2.97	T6
1	reference	1	4.03	T7
1	reference	1	5.26	T8
1	reference	1	7.59	T9
1	reference	1	9.93	T12
1	reference	1	11.31	T14
1	reference	1	10.56	T16
1	reference	1	9.04	T18
1	reference	1	8.51	T20
1	reference	1	6.84	T22
1	reference	1	5.99	T24
1	reference	1	4.15	T28
1	reference	1	2.13	T36
1	reference	1	1.69	T40

For **Decimal Places**, the user can control the number of decimal places to be displayed in both the spreadsheet and the **Results** tab. The user also has control over the number of decimal places that will be transferred into the equivalence testing part of the program for further analysis. If you selected **All decimals**, then the entire number as stored in memory will be transferred, whereas if you select **Displayed decimals only**, then just the decimals that are displayed on screen will be transferred.

The **Units of Measure** section controls the units of measure that will be displayed in the concentration/time curves. EquivTest/PK offers the user a list of most of the commonly used units –

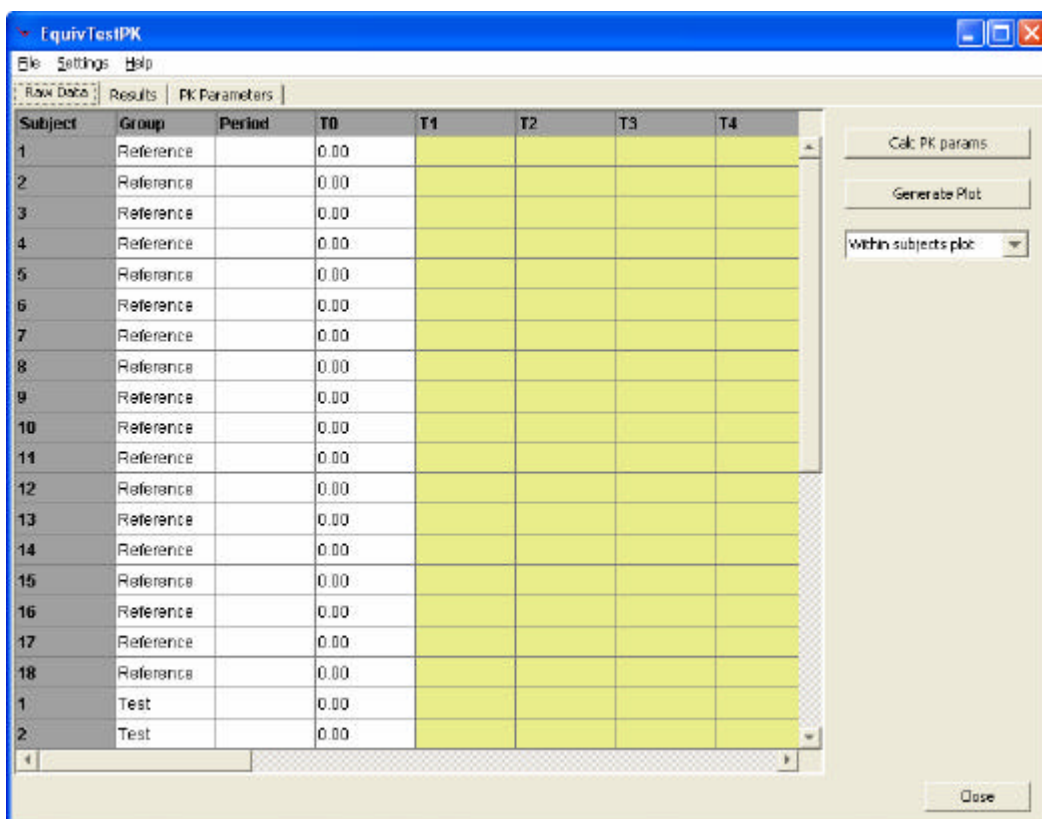
grams (g), milligrams (mg), micrograms (μg), nanograms (ng), picograms (pg), femtograms (fg), litre (L), decilitre (dL), millilitre (mL)

If you have some other unit that you want to specify you can simply enter it in the field labelled **Other** as free text. Whatever selection is made here will appear as the units on the Y-axis of the concentration/time curves.

Under **Value of Ck to be used in calculation of AUC**, the user has the choice of either selecting the observed concentration value or the estimated concentration value. This setting relates to the concentration value that is used in the calculation of the AUC. See the “Calculation of Pharmacokinetic Parameters” section later in this chapter for further details.

Under **Missing Values**, the user can select **Linear Interpolation** or **No interpolation**. If you select Linear Interpolation, then the system will linearly interpolate between any missing concentration values when calculating the AUC. If you select No Interpolation then any missing values will be excluded from the calculation of the AUC.

When you are satisfied with the values you have entered for the number of subjects, groups etc., and you click **OK**, a spreadsheet corresponding in size to the dimensions that you specified will appear.



By default, all concentration values for time zero are set to zero and the remaining concentrations are blank, but the user has the choice of either performing the data entry directly into EquivTest/PK, or copying and pasting the concentration values from EXCEL or any other spreadsheet. Note that blank concentration values are highlighted in the datasheet – this makes it easier to spot missing values that occur in the data. The values for Period have been left blank, so the user will need to fill these in. In the screen above, the data appear in the format of “Multi Variables/Subject”, but this can be changed to “Multi Records/Subject” at any stage during the analysis by making the appropriate change in the **Advanced Settings** dialogue.

By default, the dataset is sorted by Group, and then by Subject. You can, however, change the sort order of the data at any stage by right-clicking on the column header of the variable on which you want to sort, and selecting either “Sort A scending” or “Sort Descending”.

Once the data has been entered (or copied and pasted from an external source), you can immediately look at some basic summary results for the data by clicking on the **Results** tab.

EquivTest 02.00

File Settings

Raw Data Results PK Parameters

reference

Subject	T0	T1	T2	T3	T4	T5	T6	T7	T8	T9	T12	T14	T16	T18
1	< 0.06	< 0.06	0.43	1.23	1.80	2.26	2.97	4.03	5.26	7.59	9.93	11.31	10.56	9.04
2	< 0.06	< 0.06	0.53	1.01	1.80	2.69	3.16	4.68	6.73	9.93	9.88	8.24	6.83	5.81
3	< 0.06	< 0.06	0.60	1.41	2.31	4.45	7.49	10.10	10.89	12.95	14.36	14.36	12.15	11.63
4	< 0.06	0.24	0.66	1.41	2.54	4.99	8.74	8.95	13.14	12.88	12.90	10.29	9.28	7.61
5	< 0.06	< 0.06	< 0.06	0.45	0.81	1.24	1.53	2.00	2.61	8.38	13.96	11.80	10.77	8.31
6	< 0.06	< 0.06	0.31	0.65	1.05	1.79	3.03	5.60	7.69	9.66	9.63	8.34	7.23	5.85
7	< 0.06	< 0.06	0.75	1.16	1.70	2.36	3.24	4.11	4.49	6.57	8.07	13.54	11.95	9.14
8	< 0.06	< 0.06	0.14	0.35	0.73	1.16	1.99	2.60	3.34	4.45	4.18	3.90	3.43	2.90
9	< 0.06	< 0.06	< 0.06	0.25	1.04	1.48	1.84	2.30	2.79	4.65	11.15	7.15	6.51	6.01
10	< 0.06	< 0.06	0.63	1.36	2.14	3.19	4.65	6.76	10.21	12.58	10.48	9.16	6.85	5.54
11	< 0.06	< 0.06	0.71	1.17	1.74	2.90	5.01	6.89	8.39	8.25	8.53	7.50	6.44	5.30
12	< 0.06	< 0.06	0.83	1.59	3.23	5.63	8.31	10.76	11.74	12.98	13.29	13.09	11.53	10.15
13	< 0.06	0.18	0.75	1.40	1.81	2.93	4.46	5.83	6.96	9.70	10.18	9.74	9.41	7.93
14	< 0.06	< 0.06	0.38	1.33	1.93	2.54	3.98	5.76	6.96	10.66	11.60	11.59	9.88	8.55
15	< 0.06	< 0.06	< 0.06	< 0.06	0.19	0.48	2.70	6.90	11.24	13.58	12.44	10.81	10.75	9.09
16	< 0.06	0.31	0.81	1.55	2.36	3.46	4.45	6.06	6.93	9.91	11.14	11.13	8.84	7.34
17	< 0.06	< 0.06	0.54	1.19	2.51	4.14	5.76	7.39	9.88	14.41	13.05	12.56	10.41	8.69
18	< 0.06	< 0.06	0.56	1.41	2.01	2.99	3.98	5.13	6.06	6.84	7.09	7.34	6.83	5.70
N		3	15	17	18	18	18	18	18	18	18	18	18	18
Geom. mean		0.24	0.53	0.99	1.52	2.44	3.83	5.34	6.80	9.27	10.28	9.70	8.50	7.15
Exp(mean +/- SD,		0.18	0.33	0.57	0.79	1.34	2.34	3.32	4.18	6.53	7.65	7.09	6.19	5.18
Ln-data)		0.31	0.85	1.74	2.94	4.46	6.29	8.59	11.07	13.16	13.83	13.27	11.69	9.88
N	18	18	18	18	18	18	18	18	18	18	18	18	18	18
Median	< 0.06	< 0.06	0.32	0.80	1.59	2.34	3.24	4.53	6.50	8.62	10.82	8.16	6.68	5.78
Min.	< 0.06	< 0.06	< 0.06	< 0.06	0.19	0.48	1.53	2.00	2.61	4.45	4.18	3.90	3.43	2.90
Max.	< 0.06	0.31	0.83	1.59	3.23	5.63	8.74	10.76	13.14	14.41	14.36	14.36	12.15	11.63

This screen shows a table of all the raw data values along with some summary statistics. Note that any values below the limit of quantitation will be displayed as "<X" where X is the value of the LOQ that was set.

For each sampling point within each group, the summary statistics that are given are:

N	The number of concentration values that are above the LOQ
Geom. Mean	The geometric mean of the N concentration values. The arithmetic mean of a set of numbers x_1, x_2, \dots, x_n is just $\sum_{i=1}^n x_i$. And the Geometric mean is $\exp[\frac{1}{n} \sum_{i=1}^n \ln x_i]$
Exp(mean +/- SD, Ln-data)	This is an interval of +/- one standard deviation of the geometric mean. The mean and standard deviation of the log transformed values to create the range (Mean-1SD, Mean+1SD) and then this range is transformed back to the original scale.
N	This is the total number of concentration values at each sampling point (including those that are <LOQ).
Median	The median of all the concentration values at each sampling point.
Min.	The minimum of all the concentration values at each sampling point.
Max.	The maximum of all the concentration values at each sampling point.

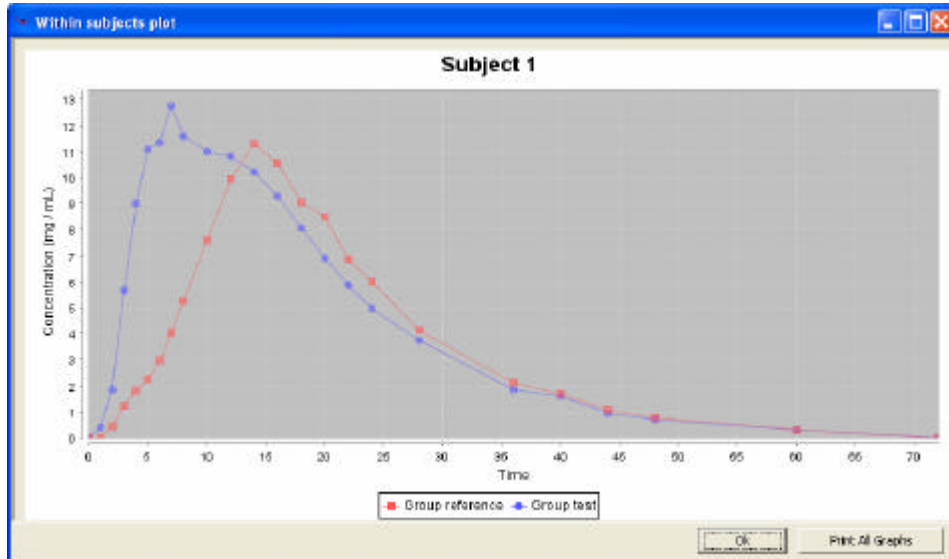
The data in this screen is in HTML format and so can be easily copied and pasted into a variety of word-processing and spreadsheet applications without losing the formatting on the screen. You can simply use your mouse to select sections to copy or alternatively hold down the CTRL key and press A to select all the text.

Back on the **Raw Data** tab, the user has a selection of summary plots available.

If you click on the drop down list of plots, you can select from the following:

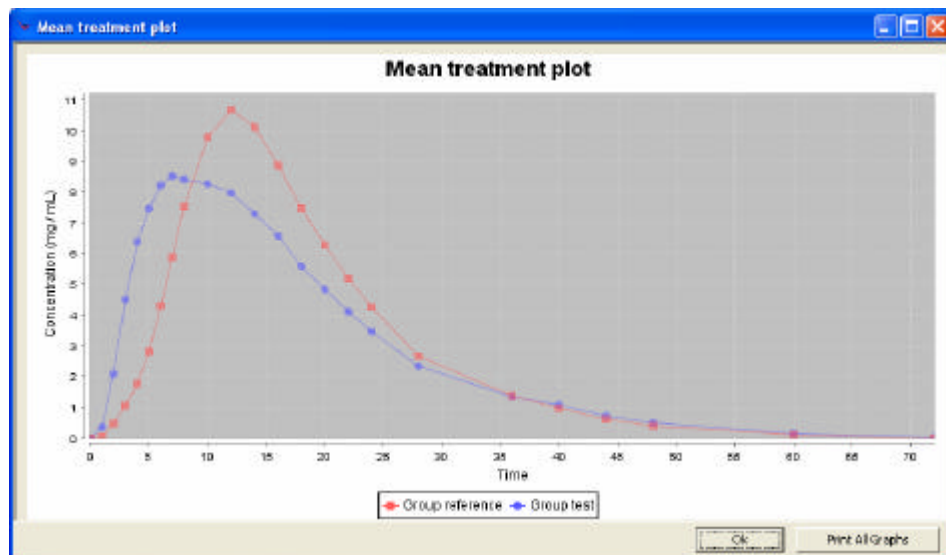
- **Within subjects plot**
- **Mean treatment plot**
- **Geometric means plot**
- **All subjects per treatment**
- **All subjects per period**

The **Within subjects plot** produces a plot that shows the concentration/time curve for each treatment group for a particular subject on one graph. You can create “Within subjects plots” for one subject, a subset of subjects, or for all of the subjects together. Simply select the subjects that you want plots for from the datasheet and click on the **Generate Plot** button.

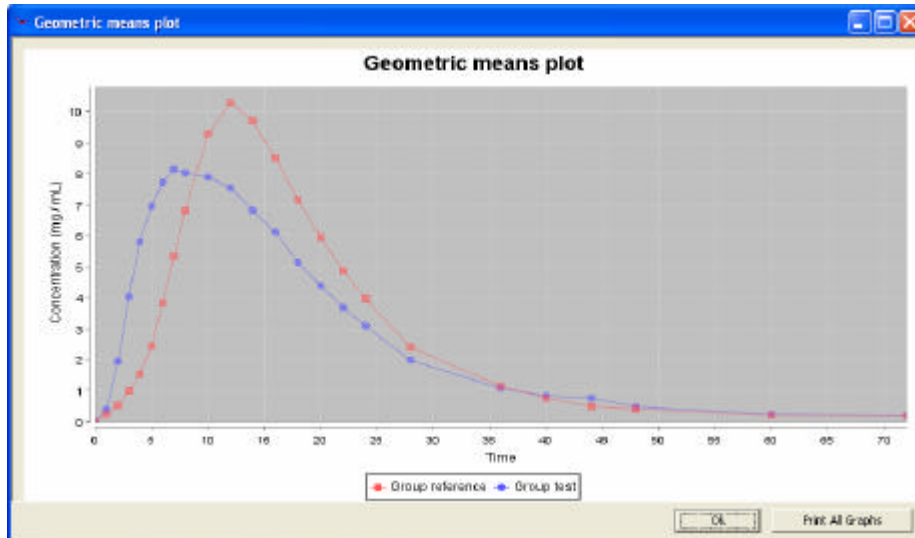


For each of the other 4 plot types, you just make your selection from the drop down list and click on the **Generate Plot** button.

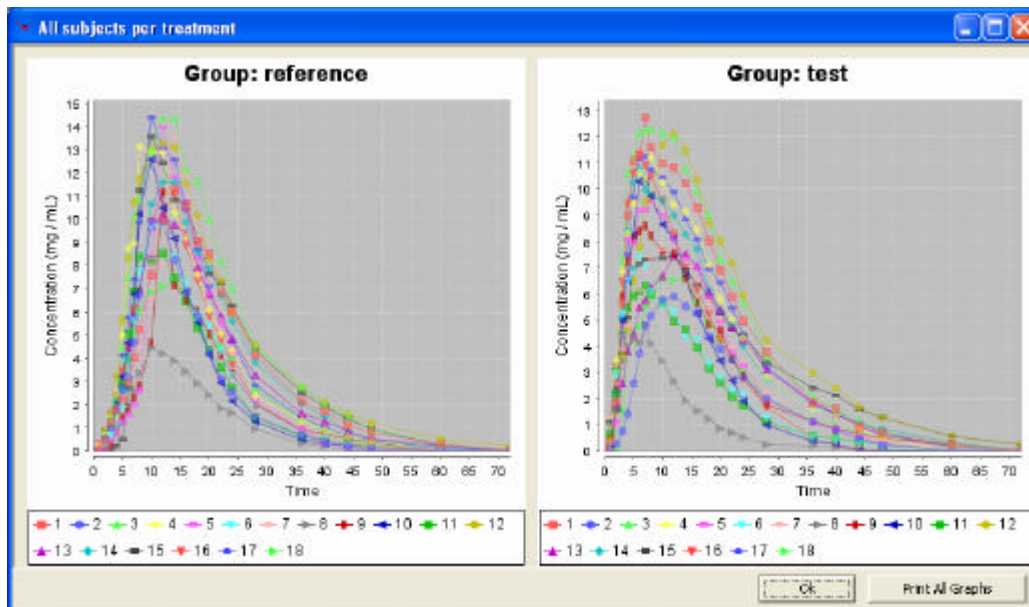
The **Mean treatment plot** shows the mean concentration value at each sampling point for each treatment.



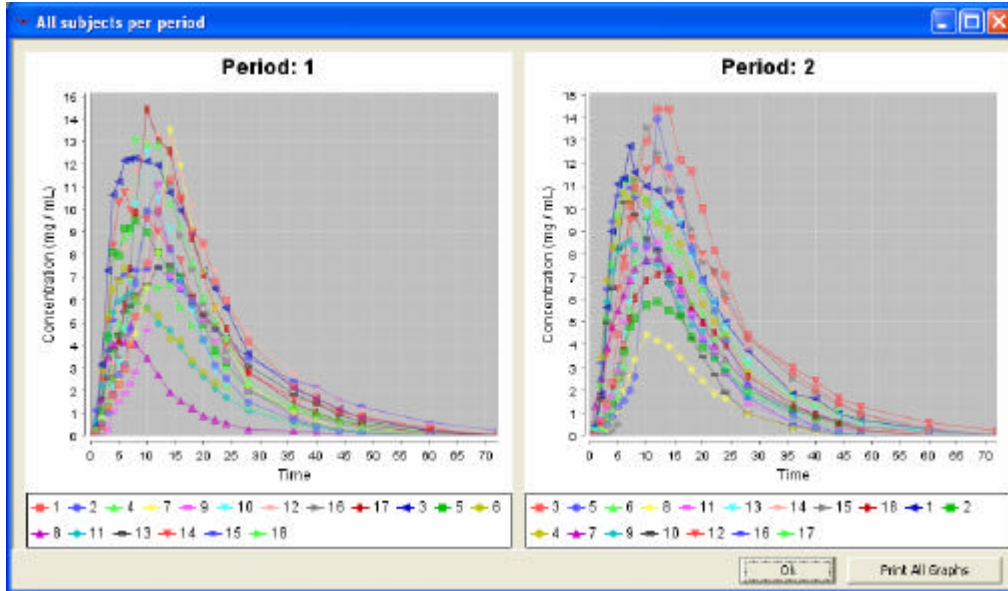
The **Geometric means treatment plot** shows the geometric mean of the concentration values at each sampling point for each treatment.



The **All subjects per treatment plot** shows a separate plot for each treatment group. The plot includes the concentration/time curves for every subject. Note you can use the right click menu on these plots to toggle on and off the plot legend.



The **All subjects per period plot** shows a separate plot for each period. The plot includes the concentration/time curve for every subject. Note you can use the right click menu on these plots to toggle on and off the plot legend.



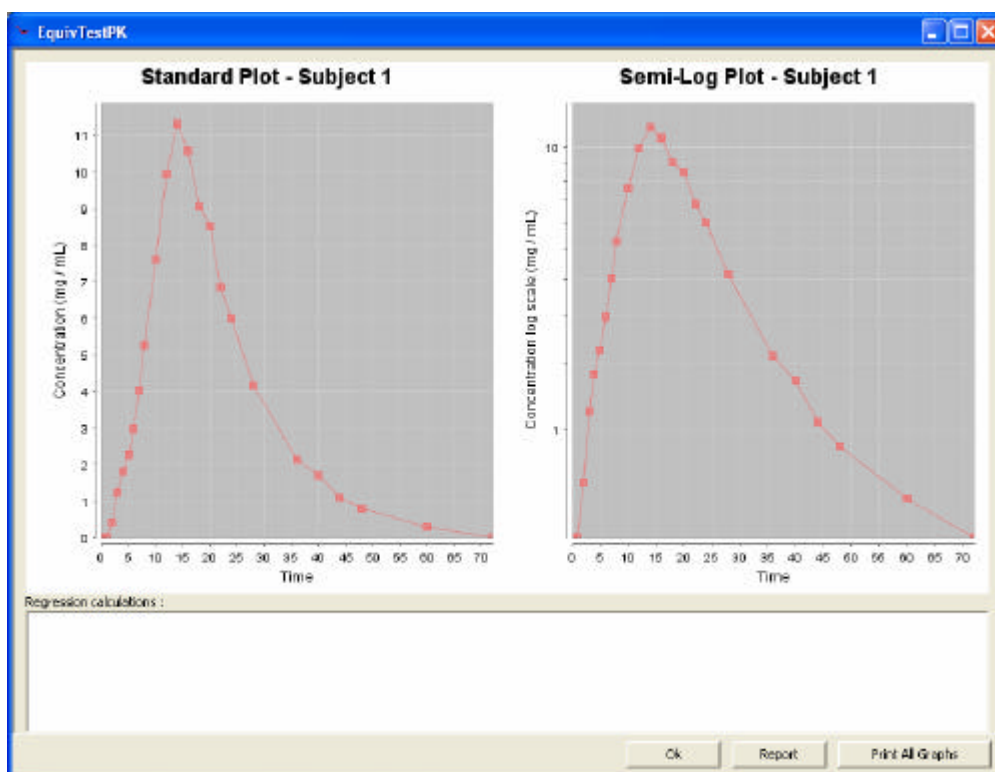
For further details on various plot options, see the **Plots** section later in this chapter.

How to calculate the PK Parameters

In order to calculate the PK parameters in EquivTest/PK, the user must first look at each subject's concentration/time curve and select the point from which the terminal elimination rate will be calculated. It is possible to view these profiles and perform the PK calculations on a subject by subject basis, or alternatively you can view the profiles for all of the subjects at the same time and do all of the PK calculations together.

To perform the PK calculations for one subject only, click anywhere in the row for the particular subject of interest and then click on the **Calc PK Params** button.

The concentration/time curve for that subject will appear.



This is made up of a Standard plot and a Semi-Log plot. The X-axis of both plots corresponds to the sampling points that were specified in the Design Setup screen. The Y-axis of the Standard plot corresponds to the concentration values for that subject and the Y-axis of the Semi-Log plot is just these same concentration values plotted on a logscale axis. The units of the concentration values are included on the Y-axis label, and these units can be set in the **Advanced Settings** screen.

{For further details on how to edit and save plots see the **Plot** section later in this chapter. }

You use these plots in the calculation of the AUC. In a single-dose study, the AUC (0- ∞) is usually calculated in two steps; the linear trapezoidal rule up to the last sampling point above the limit of quantitation, plus extrapolation to infinity. Linear regression of the natural logs of the observed concentration values during the terminal monoexponential phase versus time is used to estimate the terminal rate constant, λ . Let (t_k, C_k) denote the last sampling point above the LOQ, and let $\hat{\lambda}_k$ denote the estimate of the terminal rate constant and \hat{C}_k the estimate of the concentration at time t_k . By default, it is

this estimated concentration value \hat{C}_k rather than the observed value C_k that is used in the calculation of AUC(0- t_k) by linear trapezoidal formula up to (t_k, \hat{C}_k). This is to avoid a discontinuity between the measured concentration/time curve until t_k and the fitted monoexponential phase from t_k onwards. However, if you would rather use the observed concentration value C_k instead of the estimate \hat{C}_k , you can change this setting in the **Advanced Settings** screen.

{For further details on the calculation of the AUC see the **Calculation of Pharmacokinetic Parameters** section later in this chapter.}

You use this concentration/time curve to determine the time interval to be used in the calculation of the terminal monoexponential phase. You can click on any point on the concentration/time curve, and this point is used as the starting point of the terminal monoexponential phase. The endpoint is always going to be the last observed concentration value >0. When you make your selection, a list of results appears in the area just below the plots. These results allow you to evaluate your chosen starting point and compare it with other selections. When you click on another point on the plot, the results are updated appropriately. The results listed here are:

Regression calculations :				
$\ln(C_i) = 3.84 + (-0.08) Ti$	Subject:	1	Group:	reference
Selected Time: 36.0	Sum χ^2:	10736.0	Pearsons R	-1.00
AUC - T 223.00	AUC - ∞:	226.41	$\frac{AUC - T}{AUC - \infty}$:	0.98
R-Squared 1.00				

- The regression equation resulting from a linear regression of the natural log of the concentration values versus time.
- The selected time value.
- The AUC up to the last observed sampling point above the limit of quantitation.
- The R-Squared value of the linear regression.
- The subject number.
- The sum of squares from the regression.
- The AUC to infinity.
- The Group that the specific subject belongs to.
- Pearson's correlation coefficient.
- The ratio of AUC(0- t_k)/ AUC (0- ∞). This should exceed 0.80 for every subject (i.e. the fraction of the curve that is extrapolated should not exceed 0.2 or 20% of the total AUC).

Once you are happy with the time point that you selected here, you can click on the **Report** button to send these results to a Rich Text File (RTF), or you can **Print** the results directly from within EquivTest/PK, or you can click on the **OK** button. When you click **OK**, you are returned to the datasheet and if you click on the **PK Parameters** tab you will notice that the various PK parameters have been filled to the datasheet for the particular subject that was selected.

You can also select a subset of subjects and perform the PK calculations for those specific cases together. You can highlight consecutive rows by just clicking and dragging your mouse, or to highlight non-consecutive rows, hold down the CTRL key on your keyboard and click on the rows you wish to select.

Alternatively, you can select all subjects by clicking on the Subject column heading, or by just clicking and dragging the mouse to highlight all rows. If you select all subjects and click on the **Calc PK Params** button, the concentration/time curves for each subject will appear. You can then go through them one-by-one specifying the point for calculating the slope of the terminal monoexponential phase.

Calculation of the Pharmacokinetic Parameters

For a specific subject, let C_0, C_1, \dots, C_k be their plasma or blood concentrations obtained at time $0, t_1, \dots, t_k$, respectively. Where C_k is the last observed concentration above the Limit of Quantitation (LOQ) and t_k is the corresponding time point.

C_{max} – is the maximum observed concentration from time 0 to the last observed concentration for each subject.

T_{max} – is the time point at which the maximum concentration occurs.

Lag Time - is the time to the first positive concentration value.

The Area under the Curve (AUC) is the area under the concentration/time curve calculated using the linear trapezoidal rule. In a single-dose study, the $AUC(0-\infty)$ is usually calculated in two steps – the linear trapezoidal rule up to the last sampling point above the limit of quantitation, plus extrapolation to infinity.

The AUC from 0 to t_k , denoted by $AUC(0-t_k)$, is obtained by

$$AUC(0-t_k) = \sum_{i=1}^k \left(\frac{C_{i-1} + C_i}{2} \right) (t_i - t_{i-1})$$

{Note that the last C value to be used here could either be C_k (the observed concentration value) or \hat{C}_k (the concentration estimated at t_k). By default, the estimate \hat{C}_k is used, but this can be changed in the **Advanced Settings** dialogue box. }

The AUC *should* be calculated from zero to infinity, and not just to the time of the last blood sample. The AUC from zero to infinity is denoted by $AUC(0-\infty)$, and can be estimated as follows

$$AUC(0-\infty) = AUC(0-t_k) + \hat{C}_k / \hat{I}_k$$

where \hat{I}_k is the estimated elimination rate constant.

For the estimation of the elimination rate \hat{I}_k , the user must specify the point from which the slope is to be calculated – this can be different for each subject. The slope is based on a linear regression of the natural log of the observed concentrations, where the resultant regression line would be

$$\ln(C_i) = \ln(a) + I_k(t_i)$$

AUC(0- t_k)/AUC(0- ∞) is used as an indicator of the fraction of the concentration/time curve that is extrapolated. The ratio of these two values should be greater than 0.80 for every subject. In other words, the fraction extrapolated should not exceed 20% of the total AUC.

Area Under the Moment Curve (AUMC) calculates the area under the moment curve, i.e. under the curve for (concentration*time) versus time using the linear trapezoidal rule.

$$\text{AUMC}(0-t_k) = \sum_{i=1}^k \left(\frac{C_{i-1} * t_{i-1} + C_i * t_i}{2} \right) (t_i - t_{i-1})$$

{Again, note that the last C value to be used here could either be C_k (the observed concentration value) or \hat{C}_k (the concentration estimated at t_k). By default, the estimate \hat{C}_k is used, but this can be changed in the **Advanced Settings** dialogue box.}

$$\text{AUMC}(0-\infty) = \text{AUMC}(0-t_k) + (\hat{C}_k * t_k / \hat{I}_k) + (\hat{C}_k / \hat{I}_k^2)$$

$$\text{Mean Residence Time (MRT)} = \text{AUMC}(0-\infty) / \text{AUC}(0-\infty)$$

Half Life– during the elimination phase, the elimination half-life ($t_{1/2}$) is often studied. The plasma elimination half life is the time taken for the plasma concentration to fall by one half.

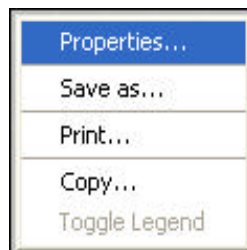
$$\text{Half Life, } t_{1/2} = \frac{\ln(2)}{I_k}$$

Plots

EquivTest/PK has 6 different plot types;

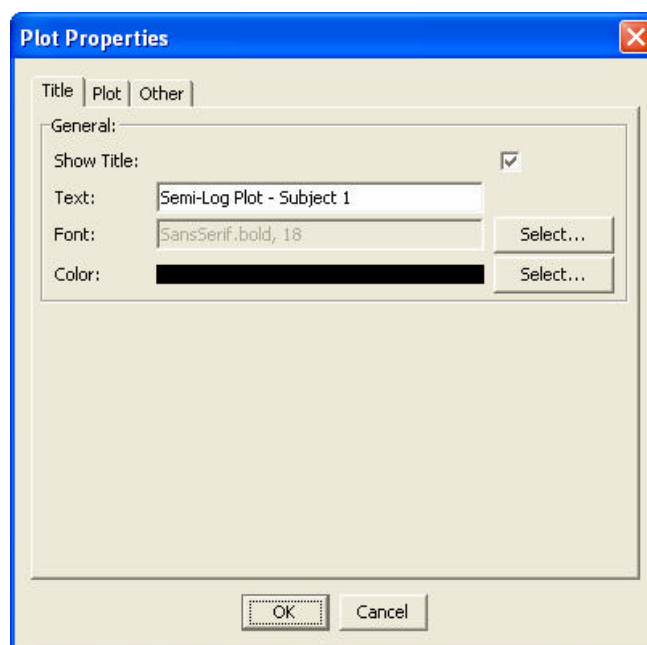
- The individual concentration/time curves that are used in calculating the AUC.
- Within subjects plot.
- Mean treatment plot.
- Geometric means plot.
- All subjects per treatment plot.
- All subjects per period plot.

The options for printing, editing etc. are the same for all of the plot types. If you right click anywhere on any of the plots you will see the following menu:



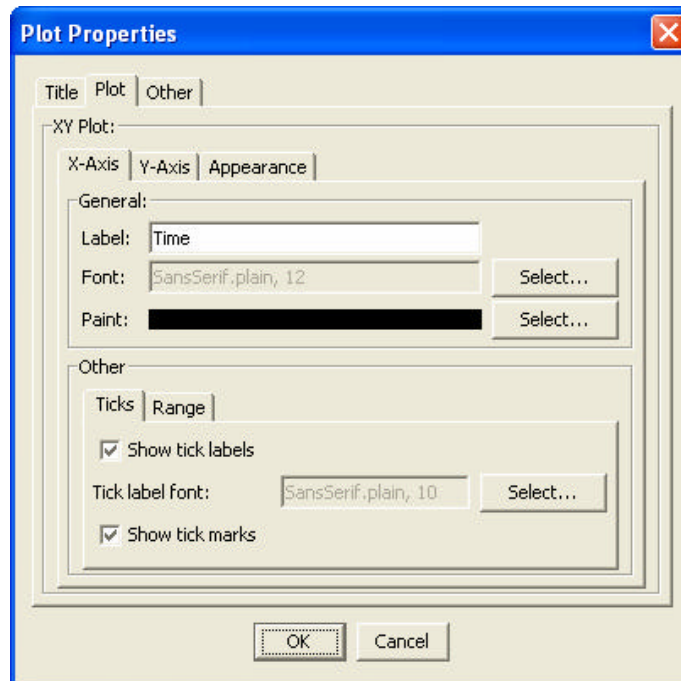
Plot Properties

If you select **Properties** from the right-click menu you will see the following dialogue box.



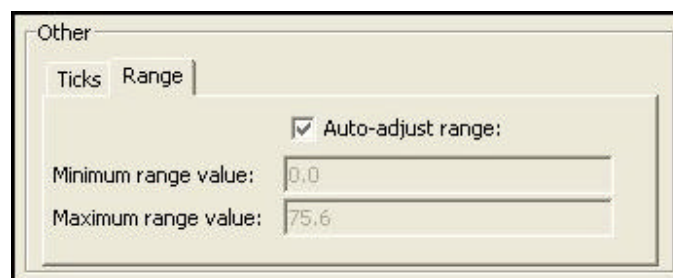
You can use this dialogue box to edit aspects of the plot, including axis titles, symbols, background colours etc.

On the **Title** tab, you can change the title of your plot and you can also specify the font and colour. You can also switch off the plot title completely by clicking in the tick box opposite “Show Title”.



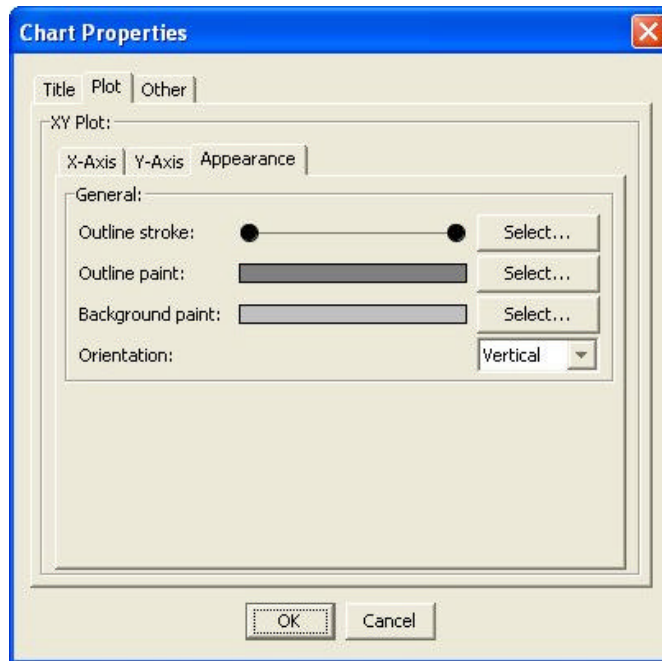
On the **Plot** tab, you can change the labels of the X and Y axes and also change the font and colour that is to be used. You can choose whether or not you want to have tick labels and tick marks displayed on the plots.

If you click on the **Range** tab in the lower part of the dialogue box, you will see the following screen:

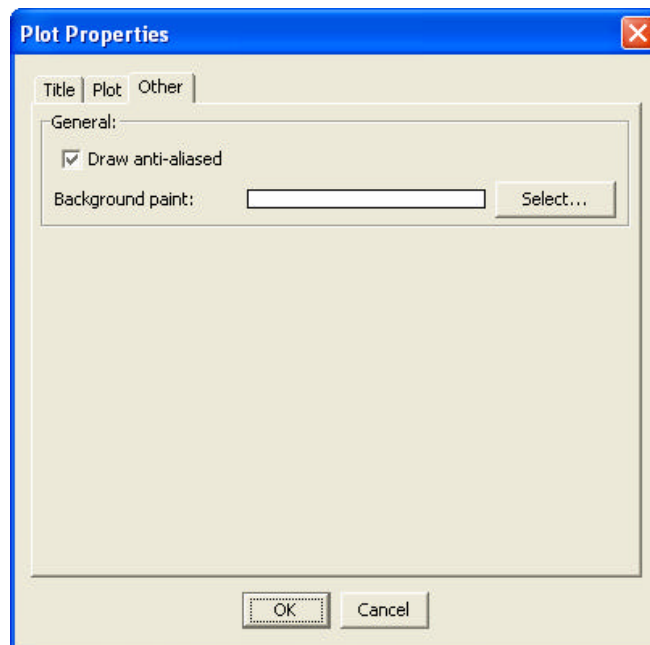


By default, EquivTest/PK automatically sets the axes ranges based on the actual data, but you can switch off the **Auto-adjust** option and specify your own minimum and maximum values if you wish.

On the **Appearance** tab, you can change the width of the line, the outline paint colour, the background paint colour, and you can change the orientation of the plot from vertical to horizontal.



By default, the plot is Anti-aliased. This means that the edges of the lines, dots, etc. have been smoothed. You can turn this option off by unchecking the tick box labelled "Draw anti-aliased" that appears on the **Other** tab. You can also change the background colour of the plots on this tab.

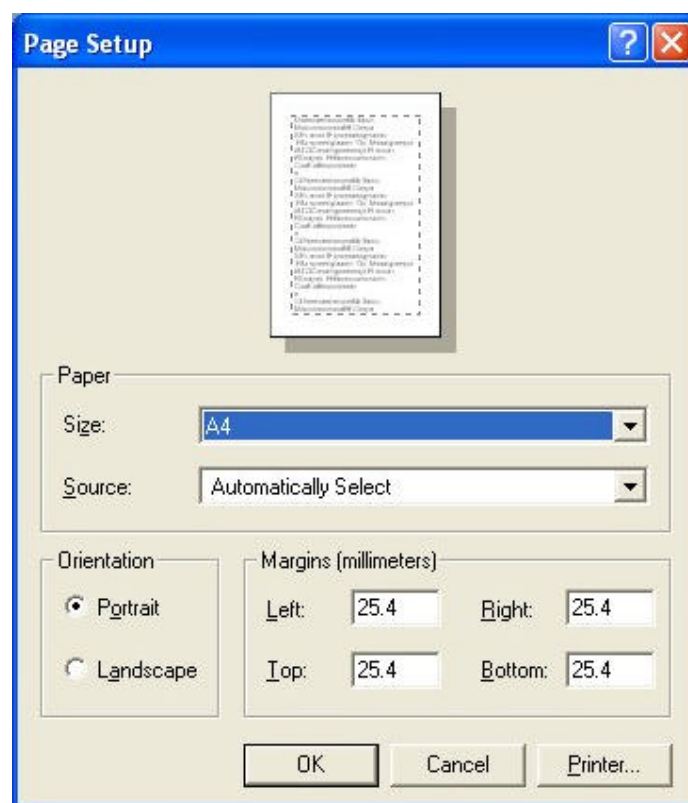


Saving Plots

If you right click on a plot you can select **Save as ...** and save the plot as a PNG (Portable Network Graphics) Image File. These are compressed bitmap files and are readable by a wide variety of applications, including Microsoft Word.

Printing Plots

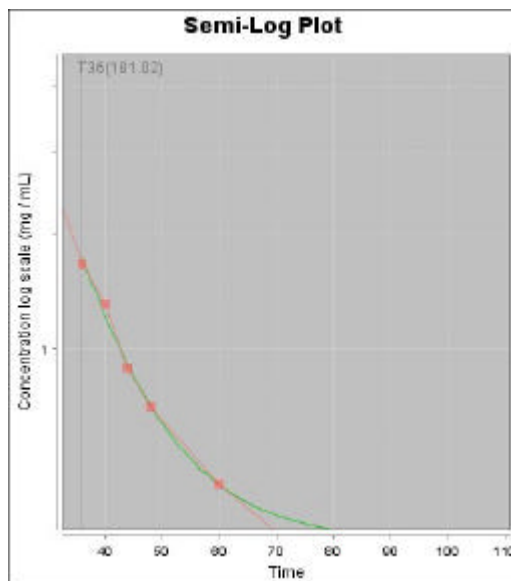
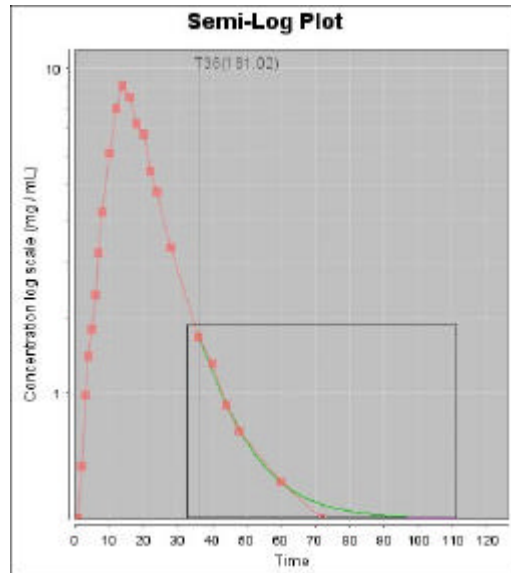
To print a plot directly from within EquivTest/PK you can right click on the specific plot and select **Print**.



The print options allow you to select the paper size, the orientation, and the margins for printing.

Zooming

You can zoom in on a specific area on a plot by simply highlighting the particular area with your mouse. To highlight an area, click and hold down the left mouse button and drag to create the selection. Note that to make the selection you must drag downwards and to the right.



To zoom back out to the original view, just click anywhere on the graph and drag the mouse up and to the left.

Example

A sample dataset has been provided with the program. The file is called EXAMPLE.EQA and is located in the SAMPLES folder. This file contains blood concentration values measured at 24 sampling points, for 18 subjects in a 2x2 crossover study. There are two treatment groups – Reference and Test, and two periods – 1 and 2.

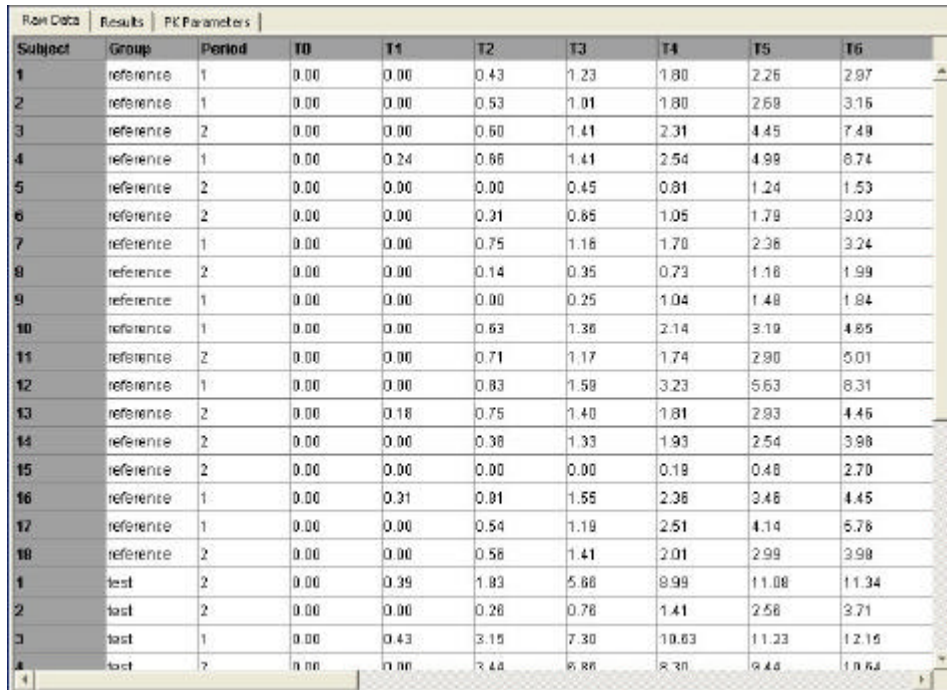
When you launch EquivTest/PK, click on the **File** menu and select **Open**, and from the list of File Types, select Equiv Test PK – Datasheet (*.eqa)

Browse to the SAMPLES subdirectory of the folder where you installed EquivTest/PK. Note that if you selected the default installation location, this would be

C:\Program Files\Statistical Solutions Ltd\EquivTestPK\Samples\

And select the file **Example.eqa**.

When the file opens, you should see the following spreadsheet:



Subject	Group	Period	T0	T1	T2	T3	T4	T5	T6
1	reference	1	0.00	0.00	0.43	1.23	1.80	2.26	2.97
2	reference	1	0.00	0.00	0.53	1.01	1.80	2.68	3.16
3	reference	2	0.00	0.00	0.60	1.41	2.31	4.45	7.49
4	reference	1	0.00	0.24	0.66	1.41	2.54	4.99	6.74
5	reference	2	0.00	0.00	0.00	0.45	0.81	1.24	1.53
6	reference	2	0.00	0.00	0.31	0.85	1.05	1.79	3.03
7	reference	1	0.00	0.00	0.75	1.16	1.70	2.36	3.24
8	reference	2	0.00	0.00	0.14	0.35	0.73	1.18	1.99
9	reference	1	0.00	0.00	0.00	0.25	1.04	1.48	1.84
10	reference	1	0.00	0.00	0.63	1.36	2.14	3.19	4.85
11	reference	2	0.00	0.00	0.71	1.17	1.74	2.90	5.01
12	reference	1	0.00	0.00	0.83	1.59	3.23	5.63	8.31
13	reference	2	0.00	0.18	0.75	1.40	1.81	2.93	4.46
14	reference	2	0.00	0.00	0.38	1.33	1.93	2.54	3.98
15	reference	2	0.00	0.00	0.00	0.00	0.19	0.48	2.70
16	reference	1	0.00	0.31	0.81	1.55	2.36	3.46	4.45
17	reference	1	0.00	0.00	0.54	1.19	2.51	4.14	5.78
18	reference	2	0.00	0.00	0.58	1.41	2.01	2.99	3.98
1	test	2	0.00	0.39	1.83	5.68	8.99	11.08	11.34
2	test	2	0.00	0.00	0.26	0.76	1.41	2.58	3.71
3	test	1	0.00	0.43	3.15	7.30	10.63	11.23	12.15
4	test	2	0.00	0.00	2.44	6.88	8.30	9.44	10.64

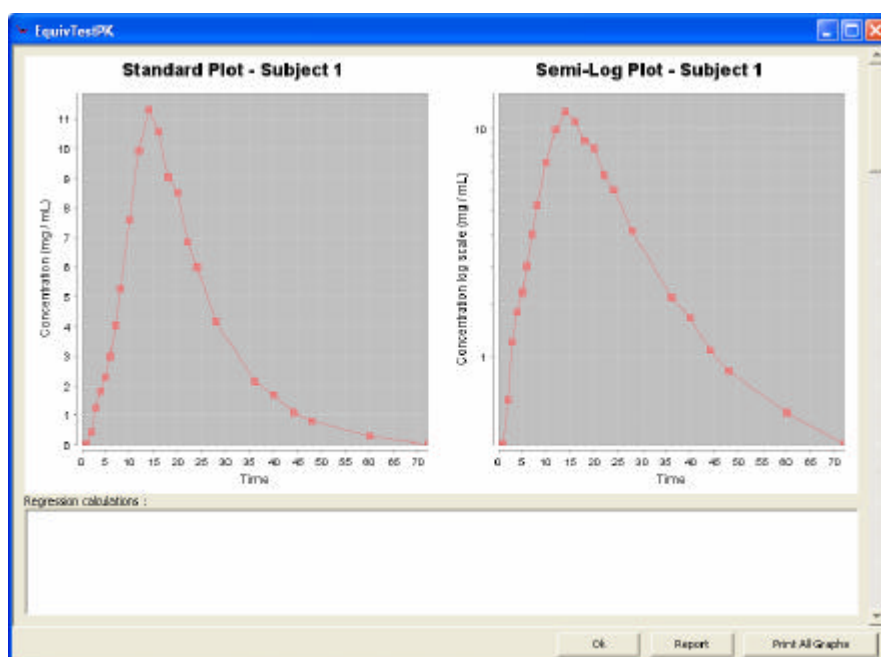
This dataset is stored in the format of multiple variables per subject, i.e. the concentration values for every subject are stored in separate columns. EquivTest/PK also handles data in the format of multiple records per subject, i.e. concentration values for every subject are stored in one column with a separate variable to identify the sampling point. You can change between these two formats at any stage by clicking on the **Settings** menu and selecting **Advanced Settings**, and from the **Select Spreadsheet type** section, select either “**Multi Variables/Subject**” or “**Multi Records/Subject**”.

You can immediately look at some summary statistics for this data by clicking on the Results tab. This will give the Minimum, Maximum, Mean, Median etc., concentration value at each of the sampling points for each of the treatment groups.

Raw Data	RESUM	PK Parameters												
16	< 0.06	0.31	0.81	1.55	2.36	3.46	4.45	6.06	6.93	9.91	11.14	11.13	8.84	7.34
17	< 0.06	< 0.06	0.54	1.19	2.51	4.14	5.76	7.39	9.88	14.41	13.05	12.56	10.41	8.69
18	< 0.06	< 0.06	0.56	1.41	2.01	2.99	3.98	5.13	6.06	6.84	7.09	7.34	6.83	5.70
N	3	15	17	18	18	18	18	18	18	18	18	18	18	18
Geom. mean	0.24	0.53	0.89	1.52	2.44	3.83	5.34	6.80	9.27	10.28	9.70	8.50	7.15	
Exp.(mean +/- SD,	0.18	0.33	0.57	0.79	1.34	2.34	3.32	4.18	6.53	7.65	7.09	6.19	5.18	
Ln-data)	0.31	0.85	1.74	2.94	4.46	6.29	8.59	11.07	13.16	13.83	13.27	11.69	9.88	
N	18	18	18	18	18	18	18	18	18	18	18	18	18	18
Median	< 0.06	< 0.06	0.32	0.80	1.50	2.34	3.24	4.53	6.50	8.62	10.92	8.16	6.68	5.78
Min.	< 0.06	< 0.06	< 0.06	< 0.06	0.19	0.48	1.53	2.00	2.61	4.45	4.18	3.90	3.43	2.90
Max	< 0.06	0.31	0.83	1.59	3.23	5.63	8.74	10.76	13.14	14.41	14.36	14.36	12.15	11.63
test														
Subject	T0	T1	T2	T3	T4	T5	T6	T7	T8	T9	T12	T14	T16	T18
1	< 0.06	0.30	1.83	5.66	8.00	11.08	11.34	13.75	13.58	10.00	10.83	10.70	8.78	8.04

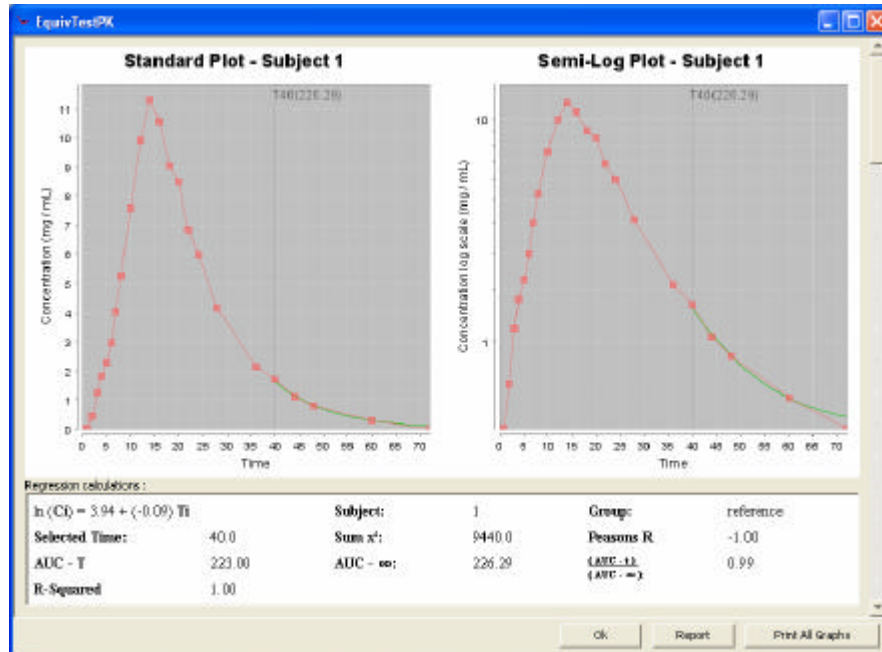
To obtain the actual pharmacokinetic results, you must first look at the concentration/time curves for each subject and select the point from which you want to calculate the terminal elimination rate. To view all the concentration/time curves, click on the Subject column heading to select all of the rows in the datasheet, and then click on the **Calc PK Params** button.

This opens a new screen which contains two plots for each treatment for every subject. The plot on the left hand side is a standard plot of the observed concentration values versus time, and the plot on the right hand side is a semi-log plot (where the concentration values have been plotted on a logscale axis).



You can click on any of the points on the curve to set it as the point from which the terminal elimination rate will be calculated. Once you have made your selection here, EquivTest/PK will fill out the area directly below the plots with the resulting AUC values, and with the details of the regression line that was used to determine the terminal elimination rate, λ . You can change your selection here by simply clicking on

another point on the curve and the results section will be updated appropriately so the effect of including or excluding extra data points can be evaluated immediately.



Scroll through the entire list of concentration/time curves for each subject and select a point on each of the plots for calculation of the elimination rate.

When you have completed this for each subject, you can click on the **Report** button to send all plots and results to an RTF file (Rich Text File) for printing or saving. Alternatively you can print all the plots and results directly from within EquivTest/PK.

When you are satisfied with your selections, click the **OK** button to view the actual PK results. This will bring you back to the datasheet view and if you click on the **PK Parameters** tab you will see that all the various PK parameters have been calculated and filled into a separate spreadsheet.

If you click on the **FINISH** button at the bottom of the PK Parameters tab, the program will convert the PK parameters datasheet into an EquivTest datasheet (*.etd) and you can proceed to perform equivalence testing in the main EquivTest program – see the EquivTest User Reference manual for further details on the equivalence testing options available.

References

Sauter, R., Steinijs, V.W., Diletti, E., Böhm, A., and Schulz, H.-U. 1992 Presentation of results from bioequivalence studies. *International Journal of Clinical Pharmacology, Therapy and Toxicology*, Vol. 30, Suppl. No. 1, ppS7-30.

Chow, S-C., Liu, J-P. (1992): *Design and Analysis of Bioavailability and Bioequivalence Studies*. Published by Marcel Dekker Inc., New York.