

PLA 1.2

Analysis of Parallel-Line Assays

- User Manual -

Version dated 08/04/2000

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2 How to read this manual?

For a general introduction to the program, read

- chapter 3 – “Introduction” and
- chapter 6 – “Terms and Concepts”.

If you also need some introduction to the practical work with PLA, refer to

- chapter 7 – “My first Parallel-Line Assay”.

If you want to install and configure PLA, read

- chapter 4 – “System Requirements and Installation” and
- chapter 5 – “PLA Administration”.

If you are working with PLA for the first time, read

- chapter 6 – “Terms and Concepts” and
- chapter 7 – “My first Parallel-Line Assay”.

For more in-depth information read

- chapter 8 – “Reference”.

If you want to work under GLP/GMP conditions, read

- chapter 9 – “GMP/GLP Settings in PLA”.

If you are interested in the mathematical background of PLA, read

- chapter 10 – “The Statistics Core of PLA”.

3 Introduction

3.1 Parallel-Line Assays

Parallel-line assays are used in biological and pharmaceutical research. A parallel-line assay determines the effectiveness of a prepared sample in comparison to a standard. The standard sample has a defined effectiveness of 1.0. The result of such an analysis is the so-called *relative potency* of the prepared sample. The *relative potency* is a dimensionless factor, so it can be given in percent. For example, a *relative potency* of 2.0 correlates to an effectiveness that is twice as big as the standard.

The calculation of the relative potency is based on measurable properties (observables) that correlate to the logarithm of the concentration. If the observable depends linearly on this function, a relative concentration can be calculated by a parallel-line assay.

An example is shown in Figure 3-1.

The relative potency can be calculated as the distance of the regression lines at a fixed value of the observable. We can use the following two equations for the calculation of the potency.

Both lines are supposed to be parallel; their slope m is equal. Thus, for every point y on the regression line

$$y = mx_p + b_p = mx_s + b_s$$

(P: prepared sample, S: standard sample)

where x is the logarithm of the concentration.

The relative potency (RP) is then given as

$$\log_2(RP) = \Delta x = x_s - x_p = \frac{b_p - b_s}{m}$$

In our example the intersection with the y-axis b of the preparation is 5.5 and for the standard 6.0. The common slope is 2.0. By solving the above equation, we obtain a relative potency of 0.84 for the preparation.

In biological and pharmaceutical research and quality control the analysis is often not as trivial as in the given example. Many possible sources of error require an analysis of the measurements that is statistically valid. Legislative bodies and regulatory authorities often require a proof of validity. The most important questions arising from this requirement are

- proof for the linearity of the measurements,
- proof for the parallelity of the measurements and
- the confidence interval of the relative potency.

These questions must be answered by the analysis.

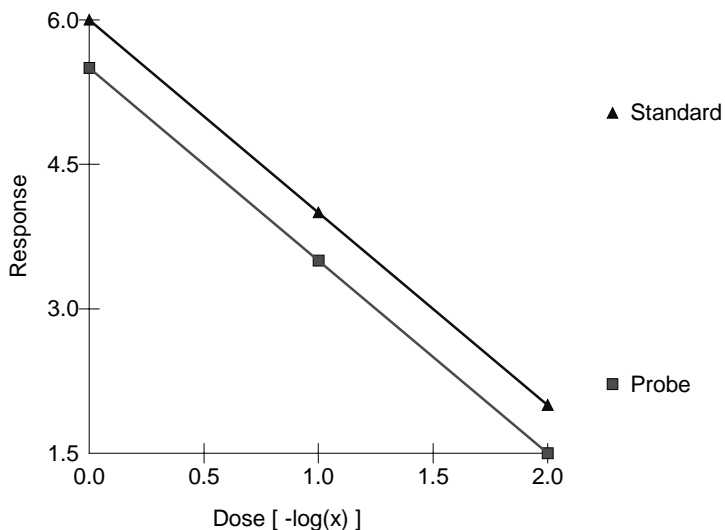


Figure 3-1 Schematic Analysis of a Parallel-Line Assay

The European Pharmacopoeia describes in its appendices the necessary preconditions for a valid analysis of the relative potency using parallel-line assays. These requirements cover the construction of the experimental design as well as the mathematical analysis.

The implemented mathematical methods in PLA 1.2 perform an analysis of parallel-line assays according to the European Pharmacopoeia.

The program can work with either the measurements of dilution series or with freely defined doses. It can be used for arbitrary measurement methods, e.g. ELISA measurements or flow cytometry.

Furthermore, PLA contains many additional functions for the efficient administration of measurements and the development of statistically valid parallel-line assays. Typically, measurements of biological systems show saturation effects at very high and very low concentrations. These regions cannot be analyzed with the parallel-line method. PLA offers methods for the automatic detection of linear regions to make analysis of measurement curves more efficient in product development.

3.2 PLA 1.2

PLA is the first commercially available program solution for the analysis of parallel-line assays. The actual version is the third, significantly improved release of the program.

Until the release of PLA biological assays had to be analyzed either with the help of extensive statistical program packages or with software that had been developed for the special system in question. The statistical programs had the disadvantage that they were not optimized for the analysis of biostatistical assays. They had to be adapted to the task with great effort. Normally, the results were not flexible enough and too slow to be used in day-to-day routine of development and quality assurance departments.

Furthermore, the need for modern program systems with user-friendly interfaces arose from the laboratory staff. Another aspect that was not taken care of by statistical programs was the electronic archiving of measurements. Especially in research departments the access to older measurements and analyses is essential for the development of new ideas or standard operating procedures. On the one hand, PLA is flexible enough to allow the user to vary the analysis side conditions quickly for development of new analysis procedures. On the other hand, PLA offers all possibilities for the work under GLP/GMP conditions in quality assurance. Older systems normally did not offer this flexibility.

The following list gives you an overview on the features of PLA:

- **Statistical analysis according to the European Pharmacopoeia.**
- A user-friendly and modern graphical user interface. Anyone who has ever worked with Windows applications will find his way around PLA quickly.
- Database approach for all data. You can access your assay data fast and efficiently.
- Efficient organization of data. You can structure your assays in projects and access them with the Navigator that allows you to find your data easily.

- Multi-document interface. You can work simultaneously on different measurements.
- Extended analysis functionality. The program finds linear and parallel regions in assays automatically. You can fine-tune this feature with many available program options.
- Management of additional assay information. You can manage all your measurement information with PLA.
- RTF reports are generated (including graphics). You can print out your reports on any printer that is supported by Microsoft Windows. You can load the reports in any standard text processors that support the RTF format (e. g. Microsoft Word). Report templates can be easily adapted for special requirements.
- Check function. You can immediately see a graphical representation of your data on screen with a preliminary analysis.
- Speed: The statistics core of PLA is highly optimized. Depending on the number of measurements, the analysis normally takes only a few seconds to complete. For example, the analysis of the Linder standard example (preparation and standard with three measurements each taken three times, overall 18 measurement values) takes less than one second on a standard PC (Pentium 133 MHz, 32 MB RAM, Windows NT 4.0).

We do our best to improve our software. If you have any suggestions for new features or improving existing features, please let us know. You can reach us under the following address:

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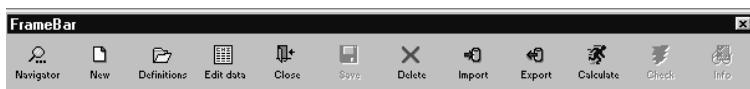
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3.3 What is new in this Version?

In comparison to previous versions PLA 1.2 offers a multitude of new important and productivity-increasing features. The following list gives you but a quick overview:

1. The graphical user interface has been redesigned. A task bar has been added to allow you even quicker access to the functions that are most important.



2. The function for the automatic detection of linear regions has been extended considerably. You can now limit the region in which the program searches for linearity and parallelity and the number of points in the linear region. Furthermore, you can enforce the inclusion of the 50% response value in the linear range.
3. The calculation of the 50% response value as well as the calculation of the 50% concentration of the standard (EC_{50}) are new features. In addition the new outputs contain mean values, standard deviations and coefficients of variation of the measured values.
4. In PLA 1.2 controls are now available as part of an assay. You can enter positive and negative controls as well as blanks.
5. The input of individual dilution steps (*direct dose input*) has been improved. You can now enter dilution steps already at project or assay level. These values will be passed to the samples.
6. Probably the most fundamental improvements are the import and export feature. This new feature allows you to transfer your data automatically into PLA.

The import interface is designed to easily migrate any data format. You can purchase individual import and export converters separately. If you do not find your system in the list of available converters, we can easily create a new import converter for any measurement instrument. The only requirement for the development of a new converter is

that you can supply us with the data format. Do not hesitate to contact the Stegmann Systemberatung.

7. GLP/GMP support. PLA now contains vastly improved functions for the work according to GLP/GMP regulations. PLA users are assigned access rights, as they are demanded by GLP/GMP.

4 System Requirements and Installation

4.1 System Requirements

PLA needs some RAM for the database management as well as for the generation of graphics and reports. Besides, the statistics core of PLA needs a certain amount of core memory for the efficient evaluation of the assays. This leads to the following minimum requirements for a PC running PLA:

- Intel 486 processor with 66 MHz.
- 32 MB of RAM.
- 30 MB free hard disk space.
- Screen resolution of 800 x 600 pixels.
- Microsoft Windows 95™, Windows 98™, Windows NT 4.0™ (SP4) or higher.

The application performance and speed of analysis is greatly enhanced with the following configuration:

- Intel Pentium ® processor with 133 MHz.
- 64 MB RAM.
- Ca. 30 MB of free hard disk space.
- Microsoft Windows NT 4.0 Workstation ™ (SP4).

Technical Note:

Additionally, the program installs the following necessary components:

- runtime module for Powersoft Powerbuilder 6.5 applications,
- runtime module for Heiler Graphics Server 5.0 OCX control,
- Sybase SQL Anywhere 5.5 desktop server.

The modules are protected products by their respective manufacturers, Powersoft, Sybase, Heiler Software and Microsoft. These components are only sold and may only be used in conjunction with PLA 1.2.

4.2 Installation

PLA comes on a CD-ROM or, upon request, on diskettes. The setup program on the CD or the first diskette installs PLA on your system. If you do not give any other drive letter PLA is installed on drive C: of your hard disk.

Note:

Please note that PLA must be configured after it has been installed. Refer to chapter 4.3 “First Time Configuration of PLA”.

4.2.1 Preparing the Installation

The installation consists of three steps:

1. Installation of the software validation module (SVM) if you have received one with your software.
2. Installation of the software.
3. First time configuration of PLA.

Note:

*If you are using **Windows NT 4.0** or **Windows 2000**, the installation have to be done by a system administrator.*

*A prerequisite for the installation of PLA 1.2 under **Windows 95** is the system component **DCOM 95**. This component is already installed if you are using the **Microsoft Internet Explorer 4** or higher. Otherwise you have to install this component, which is available on our CD-ROM in the subdirectory **DCOM95**.*

PLA is a so-called node-locked software. The program can only be installed on a single workstation. You can either get a SVM with the software, which is connected to the parallel port of your computer, or receive a license number, which is unique for the PC on which you have installed the software. If you decided to install a SVM, the installation of the module is described in chapter 4.2.2. Otherwise, continue with chapter 4.2.3.

4.2.2 Step 1 – Installation of the SVM

The SVM is a small device that has to be attached to the parallel port of your computer. If you did not receive a SVM with the software, simply skip this chapter.

Please attach the SVM to the parallel port of your PC. If you use a printer on your system, please remove the plug of the printer cable first and mount the SVM on the printer port with the side that is marked with PARALLEL. Attach the printer cable to the other side of the SVM that is marked with SERIAL. The SVM will not interfere with the printer and you can use the device normally.



4.2.3 Step 2 – Start the Installation Program

4.2.3.1 *Installation from Diskette*

On the first diskette you will find the program `setup.exe`. This program will install PLA on your PC.

Insert the diskette with the label

PLA 1.2 – Disk 1

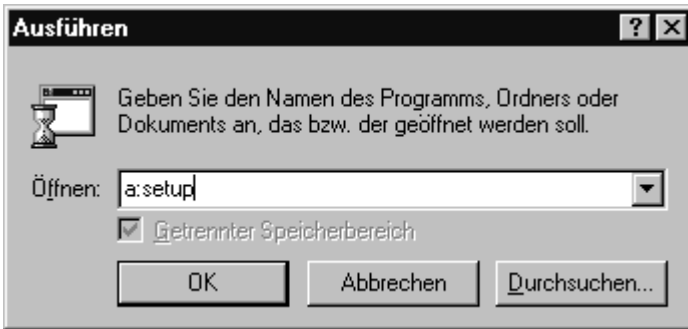
into drive a.

From the Windows **Start** menu choose the **Run** function.



In the appearing dialog, enter

A:SETUP



4.2.3.2 *Installation from CD-ROM*

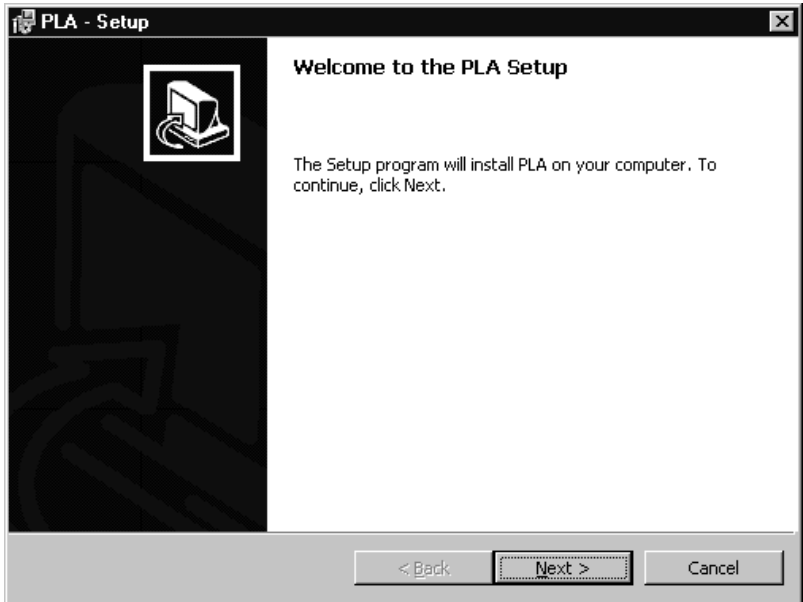
Insert the CD-ROM into your CD drive. The setup program should start automatically. If the program does not start you can call the **Run** function from the Windows **Start** menu and type

D:SETUP

where D: is the drive letter of your CD drive.

4.2.4 Step 3 – Welcome to the Setup Program

After the setup program has been started, you should see the following welcome screen:



You will always find the following option to navigate through the setup wizard on the following screens:

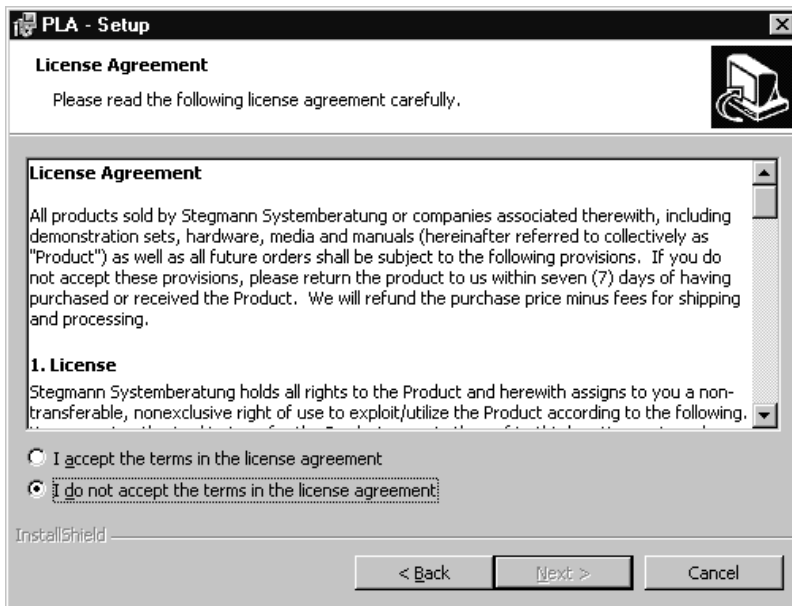
Next	Next step
Cancel	Abort the setup program
Back	Go to the previous step

The welcome screen introduces you shortly to the setup program. Press the **Next** button.

4.2.5 Step 4 – License Agreement

Please read the License Agreement carefully!

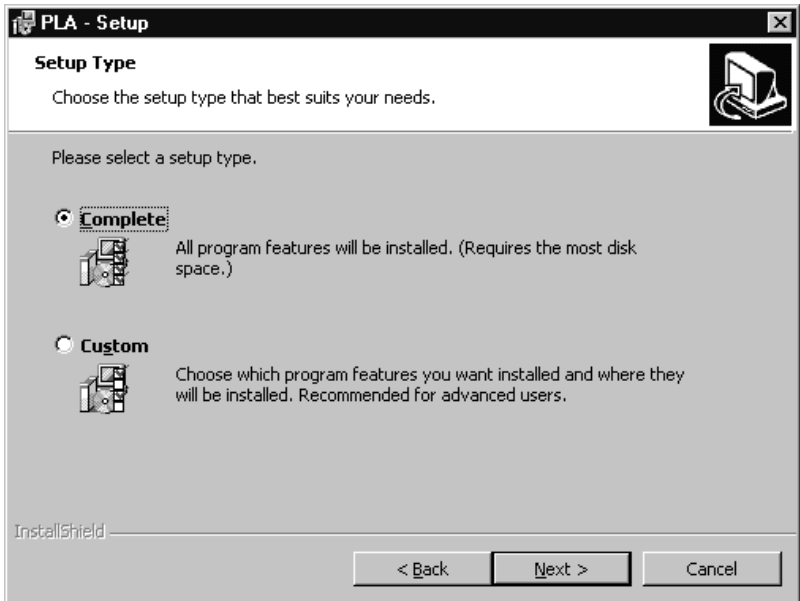
You have to agree to the licensing contract in order to install PLA. The setup program shows you the license agreement on the screen. You can use the scroll bar to move up and down the text. If you agree to the licensing conditions, select the **I accept the terms in the license agreement** button and go to the next screen. Otherwise, the setup program will terminate.



4.2.6 Step 5 – Choosing the Setup Type

On the following screen, you will be asked for the type of installation. If you choose the **Complete** option, all PLA program files are being installed in the default destination directory, which is

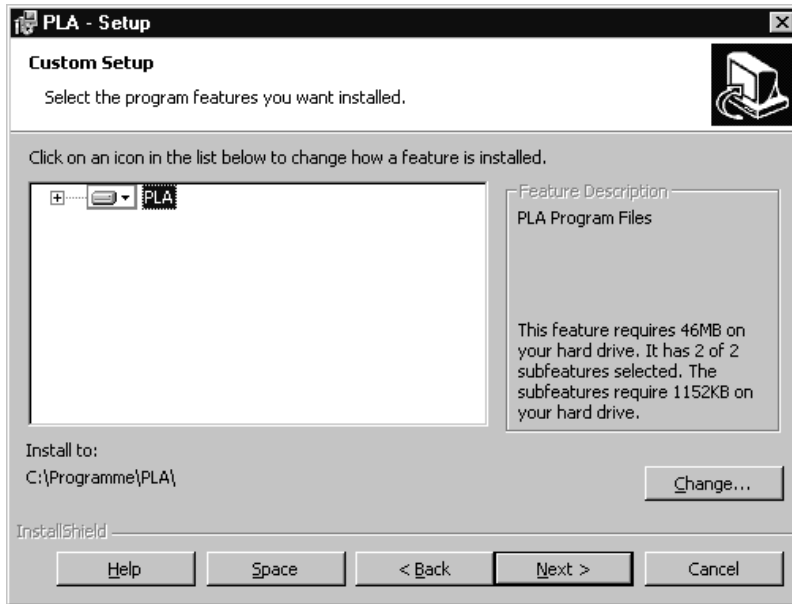
C:\Program Files\PLA\



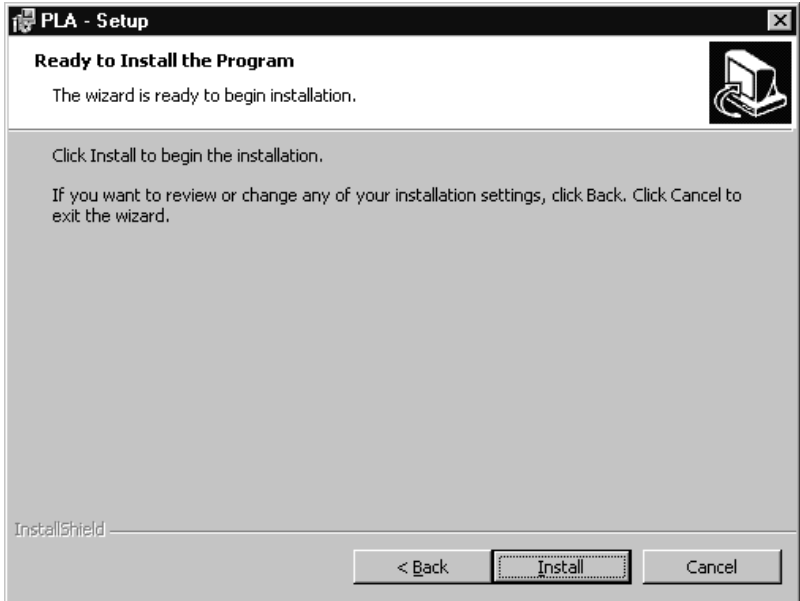
If you want to change the destination folder, choose the **Custom** option and press the **Next** button. You will see the following screen on which you can change the destination folder

System Requirements and Installation

Therefore you have to press the **Change** button and select a different destination directory.



When you press the **Next** button, the setup wizard has the complete information for starting the setup process. You will see the following screen:



If you press the **Install** button the setup program will start to copy the files to the destination folder. If you install the program from diskettes, you will be asked to change the installation medium for several times. The program will tell you when the installation is finished. You will see the following screen:



PLA is ready for use now. Read the following chapter to learn about the necessary steps to configure PLA.

4.3 First Time Configuration of PLA

After the successful installation of PLA, you have to configure the program before you will be able to work with it. These configuration steps must be completed when you start PLA for the first time. You have to perform the following steps for the successful configuration of PLA:

- Start PLA for the first time.

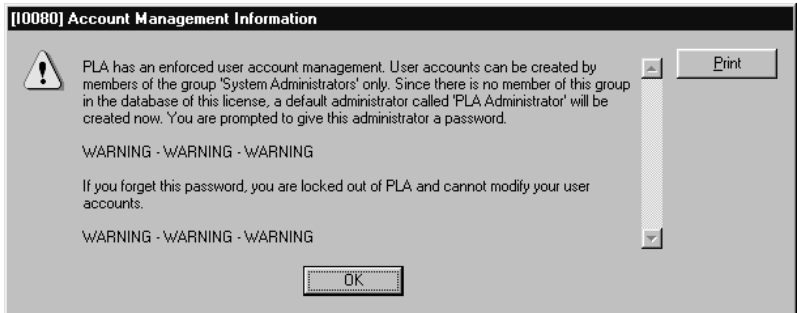
The next step is only necessary if you did not receive a SV module with PLA.

- Node lock PLA with the PLA Registration wizard.

4.3.1 Starting PLA for the first Time

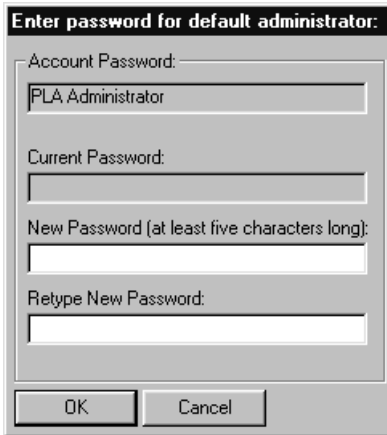
You can now start PLA for the first time. Choose PLA from the **Start|Programs** menu. The program might ask you for the conversion of existing data at start-up. This conversion is not destructive and can be readily done.

After the successful conversion, PLA will display a message for your information concerning the improved user management of this version. Only PLA administrators can now establish new user accounts.¹



At the time of the first start up a user account *PLA Administrator* is automatically created. You will be asked to enter and confirm a new password for this account. The password is case-sensitive.

¹ In previous versions any user was able to create new accounts.



The image shows a Windows-style dialog box titled "Enter password for default administrator:". It contains four text input fields: "Account Password:" (pre-filled with "PLA, Administrator"), "Current Password:", "New Password (at least five characters long):", and "Retype New Password:". At the bottom are "OK" and "Cancel" buttons.

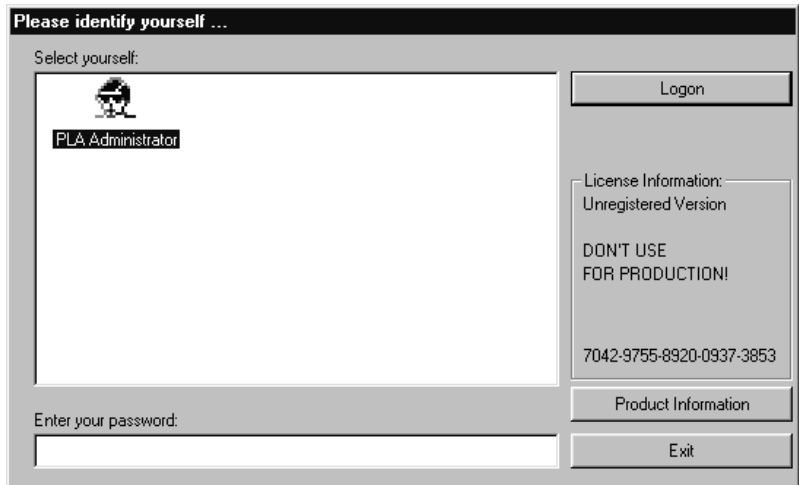
ATTENTION: Remember your password well! You will not be able to create new accounts or to manage existing accounts without the PLA Administrator password.

After you have created the account, you can log on to PLA.

4.3.2 Administrator Login

You have to identify yourself to the program in order to gain access to PLA. Choose one of the symbols on the left side - *PLA Administrator* for the first login – and enter the password in the lower input field. Please remember the password is case-sensitive.

Press ENTER or the **Logon** button to log in.



If your password is correct, you will gain access to the program.

4.3.3 The PLA Registration Wizard

If you have received a SV module with the software, you can skip this section.

As an alternative to installing a SVM, PLA supports the usage of a license number logic. This licensing technique is performed by the PLA Registration Wizard.

After a successful login to PLA, the PLA Registration Wizard will start up. You can choose either **OK** or **Cancel** to proceed. If you press the **Cancel** button, the program it will terminate immediately. If you press the **OK** button, you will get a 14-day trial access to the program. Within this period you must register PLA.



If you decide to use PLA the program will display the following screen until you register your program. Press the **OK** button.



The shown number (red background) is your registration code (red code). Please copy this number to the supplied registration form and send it to the Stegmann Systemberatung. We will send you an unlock code, which you enter in the registration wizard to lift the time limitation.


Note:

The registration code is unique for the computer. It will not work on any other machine! Before you send us the red code, please be sure you have installed PLA on the computer you want to use for production with PLA.

System Requirements and Installation

After you have received the unlock code (green code), press the **Next** button in the registration wizard. Enter the unlock code in the field below the registration code (green background). If the code is accepted, the PLA registration wizard will not show up again.

PLA Registration Wizard



PLA Registration

version 1.2
©1999 Stegmann Systemberatung

Thank you for trying out our product! Please follow these instructions for extending this trial.

- 1) Communicate the value in the red box to us.
- 2) Enter our response in the green box.
- 3) Press the 'OK' button.

If you have any questions please do not hesitate to contact us.

Registration Number: 1051-1164-9920-3268-0296

Unlock Code:

< Back Next >

OK Cancel

5 PLA Administration

This chapter describes the administrative functions of PLA. You will learn how to use the account management and where the program preferences are modified.

5.1 Account Management

One of the most important functions in PLA 1.2 is the completely revised account management. You have to identify yourself to the program with a user name and a password to access PLA 1.2 and the data stored in the program. The user management serves two purposes:

- Different access privileges depending on the status of the user must be assigned in order to establish GLP/GMP environments. The user management is a prerequisite for the operation of the program under GLP/GMP conditions.
- All changes of the data should be logged. PLA uses the account management to save the user name with the changes in the data. With this security feature it not only possible to determine when the data had been changed but also who has done the changes. However, the login mechanism does not prevent the manipulation of data (intended or unintended) but you are able to recognize modifications more easily.

Note: The identification mechanism itself does not guarantee the security of your data. You should use the security mechanisms of the operating system (e. g. Windows NT) to protect the access to the workstation with your data. If you fear that your data might be manipulated, you should end PLA and lock the workstation every time you leave it unattended.

The following sections will show you the usage of the PLA account management. Only PLA administrators have access to the user management.

5.1.1 Creating a New Account

The user management of PLA tries to depict the organization of an analytical laboratory. As you can see from Figure 5-1 PLA distinguishes between four groups of users:

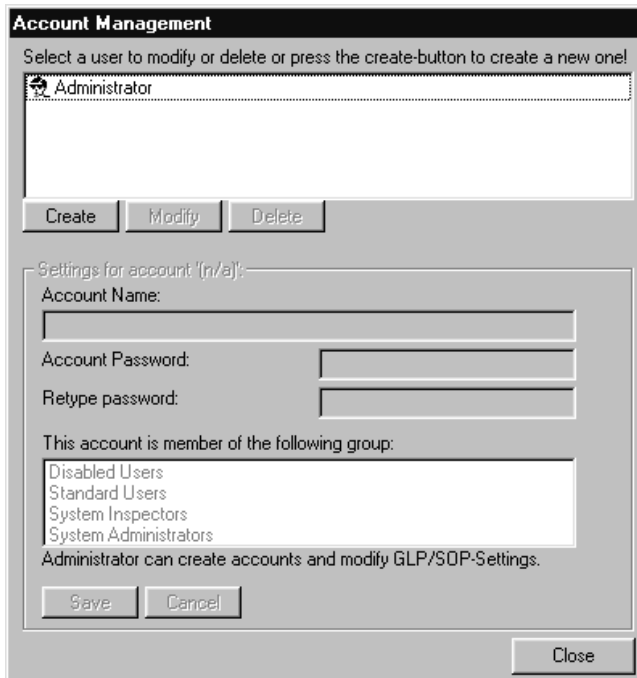
1. Standard users.
2. System administrators.
3. System inspectors.
4. Deactivated users.

The position of the PLA administrator is normally taken by the head of the laboratory or department. The PLA administrator has the highest position in the user hierarchy. Additionally to all the rights of a standard user an administrator has the possibility to manage accounts. This does include the creation of new account, changing passwords and deactivating users. Furthermore, the PLA administrator can use all the functions for the GLP/GMP management. With these functions he can define standard operating procedures for the standard user. For a description of the GMP/GLP settings refer to chapter 1.

The laboratory staff members that perform and analyze the biological tests are the standard users of PLA. Standard users can use all the functions of PLA except for the account management and the GMP/GLP settings. Administrators can limit the access to some functions, however. In this way, administrators can enforce standard operating procedures on the standard users.

The PLA inspector is the lowest level of the PLA user hierarchy. Inspectors can examine the settings and data within PLA but they are not allowed to change them.

To create new user accounts, choose **Preferences|Account Management** from the menu.



The screenshot shows a window titled "Account Management". At the top, it says "Select a user to modify or delete or press the create-button to create a new one!". Below this is a list box containing "Administrator". Under the list box are three buttons: "Create", "Modify", and "Delete". Below these buttons is a section titled "Settings for account '[n/a]:'". This section contains four input fields: "Account Name:", "Account Password:", "Retype password:", and "This account is member of the following group:". The "This account is member of the following group:" field is a list box containing "Disabled Users", "Standard Users", "System Inspectors", and "System Administrators". Below the list box is a text label: "Administrator can create accounts and modify GLP/SOP-Settings.". At the bottom of the settings section are "Save" and "Cancel" buttons. At the bottom right of the window is a "Close" button.

Figure 5-1 User management of PLA

Existing users are displayed in the list in the upper part of the dialog. For now, only the user *Administrator* exists. Press the **Create** button to create a new user account. This will activate the fields in the center of the dialog.

Enter the user name in the input field *Account Name*. The password for the new account must be given in the input field *Account Password*. Retype the password in the appropriate entry field to ensure you did not make any typing mistakes. The password must have at least five characters. Tell the new user the initial password for his account. After the first time the new user has logged in he can, and ought to, change his password. Please refer to chapter 5.2 to learn how to change your password.

Note: Please keep in mind that the password is case-sensitive. The password “Test” is different from “test”. Changing your keyboard settings will change your keyboard layout. National characters might become unavailable and the keys for other characters might change (e. g. qwertz and qwerty keyboard layout).



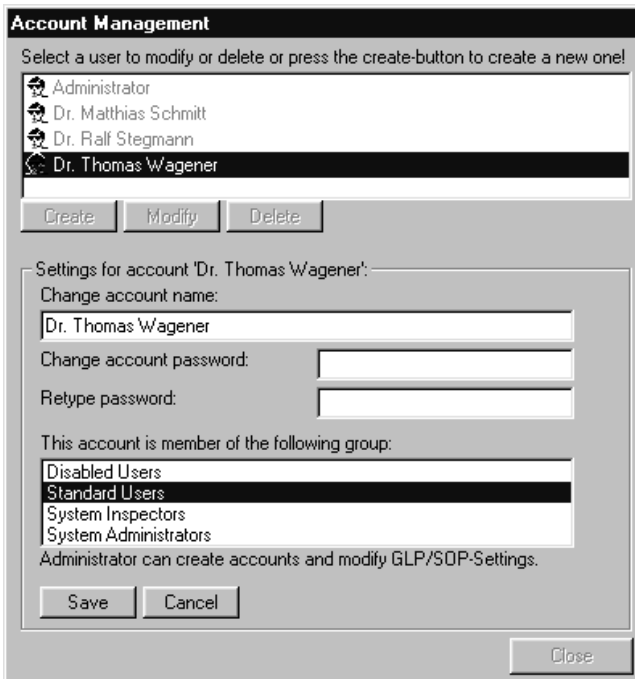
The image shows a Windows-style dialog box titled "Account Management". At the top, it says "Select a user to modify or delete or press the create-button to create a new one!". Below this is a list box containing a single entry: "Administrator" with a small user icon to its left. Underneath the list box are three buttons: "Create", "Modify", and "Delete". Below these buttons is a section titled "Please enter the setting of the new account:". It contains three input fields: "Enter account name:" with the text "Dr. Matthias Schmitt", "Enter account password:" with masked text "xxxx", and "Retype password:" also with masked text "xxxx". Below the password fields is a section titled "This account is member of the following group:" followed by a list box containing four entries: "Disabled Users", "Standard Users" (which is highlighted), "System Inspectors", and "System Administrators". Below the list box is the text "Administrator can create accounts and modify GLP/SOP-Settings.". At the bottom of the dialog are two buttons: "Create" and "Cancel". A "Close" button is located at the bottom right corner of the dialog box.

Select the user group of the new account from the pick list in the lower part of the dialog. Normally you will choose *Standard User*, *System Administrator* or *System Inspector*. Press the **Create** button to generate the new account. Press **Cancel** to abort the account generation.

The next time the login screen of PLA is displayed, the new user will be available.

5.1.2 Changing an Existing User Account

If you want to change an existing user account, you can do this with the account management dialog. Pick the user name from the list in the upper part of the dialog and press the **Modify** button.



The 'Account Management' dialog box has a title bar with the text 'Account Management'. Below the title bar is a subtitle: 'Select a user to modify or delete or press the create-button to create a new one!'. There is a list box containing four entries: 'Administrator', 'Dr. Matthias Schmitt', 'Dr. Ralf Stegmann', and 'Dr. Thomas Wagener'. The entry 'Dr. Thomas Wagener' is selected and highlighted. Below the list box are three buttons: 'Create', 'Modify', and 'Delete'. Below these buttons is a section titled 'Settings for account 'Dr. Thomas Wagener':'. This section contains three input fields: 'Change account name:' with the text 'Dr. Thomas Wagener', 'Change account password:', and 'Retype password:'. Below these fields is a section titled 'This account is member of the following group:' with a list box containing four entries: 'Disabled Users', 'Standard Users', 'System Inspectors', and 'System Administrators'. The entry 'Standard Users' is selected and highlighted. Below the list box is the text 'Administrator can create accounts and modify GLP/SOP-Settings.'. At the bottom of the dialog box are three buttons: 'Save', 'Cancel', and 'Close'.

You can now change the name, the password and the user group of this account. You will probably use this function most often because a user has forgotten his password. Press the **Save** button to activate the changes. To dismiss the changes press the **Cancel** button.

5.1.3 Deleting an Account

To delete a user account from the account management you have to login to PLA as an administrator. Perform the following three steps to delete the account:

1. Start the account management.
2. Select the user that you want to delete.
3. Press the **Delete** button.



To delete the user account finally press **Yes** in the pop-up dialog otherwise press **No**. In both cases, you will get back to the account management dialog.

5.2 Changing Your Password

PLA offers two options to change a password. The first option is available to administrators only and can be used if a user has forgotten his password (see 5.1.2).

The second possibility is available for any user. Select **Preferences|Change your Password...** from the start the following dialog:



The dialog box is titled "Change your password:". It contains four text input fields. The first field is labeled "Account Password:" and contains the text "Dr. Matthias Schmitt". The second field is labeled "Current Password:" and contains "xxxxxx". The third field is labeled "New Password (at least five characters long):" and contains "xxxxxx". The fourth field is labeled "Retype New Password:" and contains "xxxxxx". At the bottom of the dialog are two buttons: "OK" and "Cancel".

Enter your old password in the field *Current Password*.

Enter the new password in the input field *New Password* and re-type your new password in the field below.

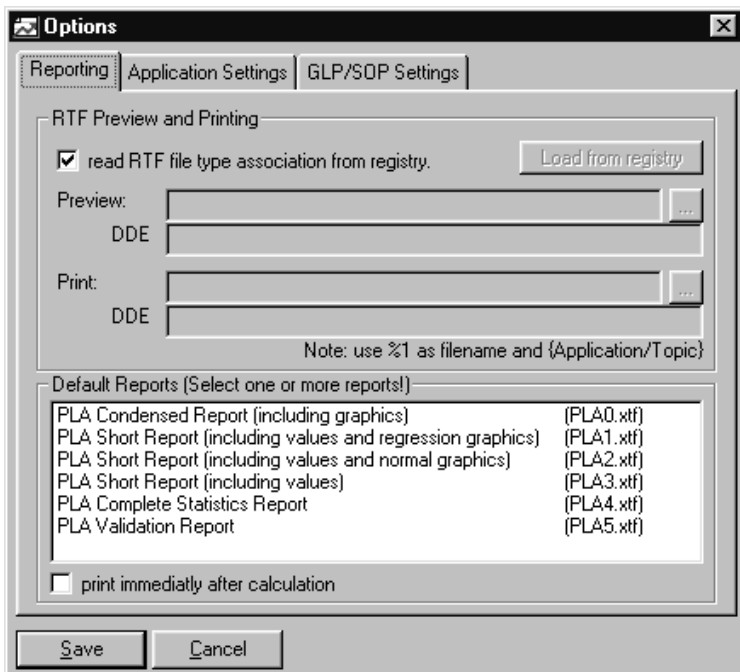
Press **OK** to change your password or **Cancel** to dismiss the changes.

5.3 Program Options Settings

The program options contain several settings that are important for the correct output of the analysis reports. For this some application paths must be set. You have to login as an administrator in order to be able to access these functions.

5.3.1 Report Settings

Select **Preferences|Options** from the menu. The following dialog will appear:



On the first tab of the options dialog, you have access to several options that are important for viewing and printing reports.

PLA creates reports as RTF files (rich text format) that can be viewed with various text processor applications. You have to define with which program you want to view and print your report files. The default setting is to *read RTF file association from registry*. PLA will find the program that is linked to RTF files. You do not need to change any of these settings.

If you deactivate the check box, you can enter DDE commands¹ for the preview and printing of RTF files. Only experienced users should manipulate these settings. If you press the **Load from Registry** button, the actual values from the registry are loaded into the input fields.

If you want to use the program WordView for the preview of your reports for example, you must give the exact path to the program. Alternatively you can press the button right of the input field to open up a standard file dialog. You must give the parameter “%1” to hand over the name of the RTF file to the program.

RTF Preview and Printing

☐ read RTF file type association from registry. Load from registry

Preview: ...

DDE

Print: ...

DDE

Note: use %1 as filename and {Application/Topic}

¹ The DDE commands must be given in the syntax {Application/Topic}[Command 1][Command 2]..., e.g. {Win-Word/System}[FileOpen \"%1\"]

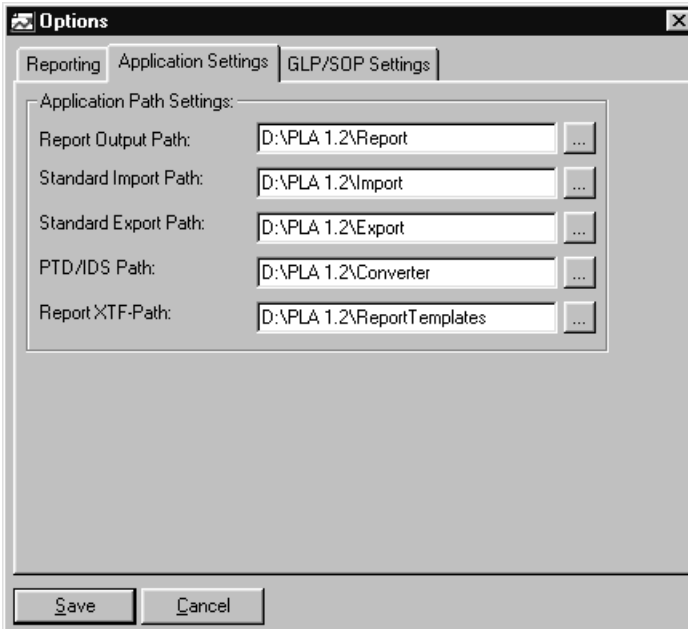
You can change the following settings in the lower part of the dialog:

1. **Default reports:** You can select one or more reports from the list. These reports will already be activated when you start a calculation. Together with the option **Print immediately after calculation** you can minimize the effort for an analysis. To choose more than one report keep the CTRL key pressed while selecting the report types from the list. (**Note:** The list of available reports may differ between installations.)
2. **Print immediately after Calculation:** This will activate the setting **Print immediately** in the calculation dialog as a default.

Press the **Save** button to confirm the new settings. Press the **Cancel** button otherwise.

5.3.2 Setting the Application Paths

You can change the PLA application paths on the next page of the options dialog. If you press the ellipsis buttons right of the input fields, you can open a standard file dialog to select the directories.



You can change the following paths:

1. **Report Output Path:** This is the default path to which your analysis reports will be saved. The default setting is the *Report* subdirectory of your PLA program directory.
2. **Standard Import Path:** The default path from which you want to import data files. The default setting is the *Import* subdirectory of your PLA installation.
3. **Standard Export Path:** The default path to which you want to write your exported data files. The default setting is the *Export* subdirectory of your PLA program directory.

4. **PTD/IDS Path:** The path where your input converters and your import definition schemes reside. The default setting is the *Converter* path of your PLA installation. (See chapter 8.12.2 for further details.)
5. **Report XTF Path:** The path where the program looks for the report template files. The default setting is the *Report Templates* subdirectory of your PLA program directory.

Confirm the changes by pressing the **Save** button. Otherwise, press the **Cancel** button.

The last page of the options dialog contains the GLP/GMP settings of PLA. These are not directly relevant for the installation of PLA. The whole chapter 9 is dedicated to the GLP/GMP settings. Please refer to this chapter for further information.

6 Terms and Concepts

This chapter will introduce you to the terms and concepts of PLA. You will learn about the structure and properties of the PLA objects. The goal of this chapter is to make working with PLA easier more efficient for you.

6.1 Objects

PLA knows five different kinds of objects. These objects carry out certain functions within the program. The five objects are the project, the assay, the standard, the preparation and the control.

Assay Is equal to the term of an assay in biostatistical literature. The assay consists of the measurements of a standard and at least one preparation. The measurements of the standard and the corresponding preparations and controls are analyzed collectively. A parallel-line assay calculates the relative potency of a preparation in comparison to a standard sample. Per definition, the relative potency of a standard is always 1.0 (100%).

Standard Refers to the measurements and properties of a standard graph of an assay. The standard consists of at least two point of measurement collected at least twice.

Preparation Is the term for the measurements and properties of the preparations of an assay. The preparation consists of at least two points of measurement from repeated readings.

The task of the program is to determine the relative potency of a preparation referring to the corresponding standard of the assay.

Control Is the term for the measurements and properties of an assay control. There are three different kinds of controls: positive controls, negative controls and blanks. In contrast to the preparation, the properties of all controls are managed in one object.

Controls are optional for the calculation of relative potencies. They serve as a check for the examined test system. The concept of controls is fundamentally different from that of standards or preparations. (Controls

are only measured as repetitions and not as dilution series.)

Project

The project is an object for the management of a group of assays. The organization of assays in projects allows you to keep track of a multitude of assays.

Examples:

Project „storing experiments“ contains all assays that were calculated during a storing experiment.

Project „measurements April, 1st“ contains all assays taken on April 1st.

PLA encourages you to manage all assays in projects. This allows you to organize your work better if you keep the hierarchy of the objects in mind. The object hierarchy is described in the following paragraph.

6.2 Object Hierarchy

Projects, assays and standards/preparations/controls are organized in a hierarchical way. The topmost level is the project level. You can see a part of the PLA navigator in Figure 6-1. You see several entries.



Figure 6-1: Navigator - Projects

Open the project ,Beispiel Nr. 2‘ by clicking on the ,+‘-symbol left of the project name. In this way you get access to assays, the second level objects. In Figure 6-2, you can see that project ,Beispiel Nr. 2‘ contains three assays, which are called ‘Assay 1’, ‘Assay 2’, and ,Assay 3‘.

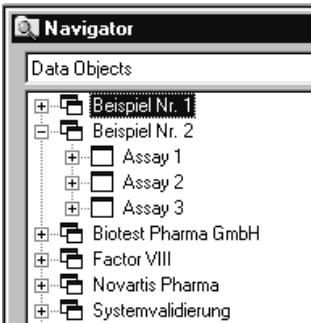


Figure 6-2: Navigator - Assays

Each of the assays contains another level of objects the standards, the preparations and the controls. Those objects are on the third and lowest

level of the object hierarchy. You can access the object from the assay level in the same way as you can access the assays from the project level. In Figure 6-3 you can see that the assay 2 contains one standard, one preparation and one control.

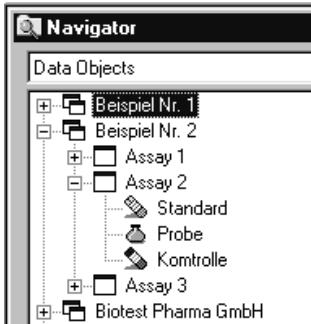
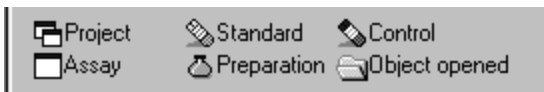


Figure 6-3: Navigator - standards/preparations/controls

All kinds of objects have a different icon as a distinctive feature.



The standard is depicted by an orange test tube, the control by a blue-green test tube and the preparation has a green reaction vessel icon. You can choose the name of all five types of objects freely. In our example, the objects are called preparation, standard and control only for reasons of better understandability.

Why using the object hierarchy?

Just to point out only two aspects:

1. After some time, you will probably have done a multitude of analyses with PLA. Of course can delete these measurements but especially in development departments the access to old data is often essential. With the help of the hierarchical organization in PLA you can structure your work. The project object should be regarded as a file for the assays.

2. You can save all your data about your measurements and further information already on the project level. If you create a new assay within the project afterwards all data is automatically added to your assay. You do not have to enter all common information on the project again. **Be careful:** If you change your project data in a project where you have already created assays the changes are **not** automatically copied to the existing assays. The project data is only copied on the creation of an assay.

6.3 Inheritance

A central element in the usage of PLA is the concept of inheritance. To illustrate this concept one might use the analogy to genetic inheritance:

„An ancestor passes its genetic information on to its descendants.“

Within PLA inheritance works in the following way:

The first generation (the topmost hierarchy level) is always a project. The second generation (second level) is always an assay. The third level is a standard, a preparation or a control. If you create an assay, it will ‘inherit’ the information of the project it belongs to. Analogously all the standards, preparations and controls will inherit the information of the parent assay on creation.

In this way you need to enter the common information for the assays or the standard/preparations/controls only once on the **next higher** level of the object hierarchy. If you later create a new object, all the information from the next higher level, in this case the assay level, will be copied to the new object automatically.

It is important to understand that the analogy to genetic inheritance is valid **only on the creation of new objects**. If you modify a higher-level object later, the changes will **not** occur in the existing descendants. Only newly created objects inherit the modified data.

However, you can influence the inheritance of a new object by giving the name of the object from which the new object will get its data explicitly. You can also use the *apply* function to transfer data, e.g. changed analysis settings, from a higher-level object to an existing object.

6.4 Object Properties

All objects in PLA (project, assay, standard and preparations) contain the same possibilities for the definition of properties. The program dialog for the input of the settings is the same for all object types. Controls are the only exception because they are described by a reduced set of information.

On the following pages, we will introduce you to all the settings. They are divided into seven categories that reflect the structure of the program.

Settings that are marked with a star (*) are optional. They are not necessary for a successful calculation.

6.4.1 General Settings

Setting	Description
<i>Code</i>	Abbreviation of the object's the full name. This name is mainly used for the screen display, e. g. in the navigator.
<i>Title</i>	Full name of the object. This name is used for the printout of the calculation results.
<i>Measured Substance</i>	Name of the examined substance.
<i>Batch identification of the Substance</i>	Batch identification number of the examined substance.
<i>Status finished</i>	If this field is marked, the object will be considered closed. The object is no longer displayed in the navigator but will not be deleted from the database.
<i>Date of measurement</i>	The creation date of the object. You cannot edit this field.

Setting	Description
<i>Switch Object Type</i>	This option is only active for standards and preparations. A preparation object will be switched to a standard and vice versa.
<i>History</i>	This field contains information about the creation, the last modification and the ancestors of the object.

6.4.2 Reagents Settings

The next index of the object definition gives you the opportunity to enter a list of the reagents in the sample. You can document the way the preparation was processed by filling out these fields. These settings are optional. They are not necessary for the analysis.

Property	Description
<i>Reagents*</i>	Name of the reagent.
<i>Batch*</i>	Batch id of the reagent.
<i>Concentration*</i>	You can only enter the concentration of the reagent as a numerical value. Thus, you should enter the dimension of the concentration in the name field.

6.4.3 Definition settings

Property	Description
<i>Concentration of the undiluted standard*</i>	The original concentration of the standard. (For the analysis this value is taken from the object definitions of the standard.)
<i>in units*</i>	Units (dimension) of the concentration.
<i>Predilution factor of the standard</i>	(For the analysis this value is taken from the object definitions of the standard.)
<i>Predilution factor of the Preparation</i>	(For the analysis this value is taken from the object definitions of the preparation.)
<i>Number of dilution steps in a data series</i>	Number of measurements in a dilution series, e. g. points on a titration curve.
<i>Number of data series</i>	Number of data sets taken, e. g. two titration curves.
<i>Additional predilution factor for the first step</i>	You can enter a predilution factor e. g. from the introduction of a prediluted medium to the object here. Values from 1:1 to 1:10 are possible.
<i>Dilution scale</i>	It is possible to switch between 1:2-dilution series and direct dose input here. The input of the dose can already be given at project level.

6.4.4 Analysis Settings

Property	Description
<i>Analysis according to Europ. Pharmacopoeia 97</i>	All analysis settings will be set according to the values given by the European Pharmacopoeia 1997.
<i>Dixon test for data outliers. (Up to 25 series.)</i>	Activation of the Dixon test for statistical outliers. This test is limited to a range from 3 to 25 data sets.
<i>Regression model</i>	Switch between a double logarithmic and a logarithmic analysis.
<i>Reverse response-effect relation</i>	By the activation of this function, the correct relative potency of a reverse response-effect calculation will be calculated. This setting is used for e.g. inhibition assays
<i>Dixon-test contamination (alpha)</i>	α -value of the Dixon test. Refer to chapter 10.1.2 "Dixon Test" for further details.
<i>Significance of the slope (%)</i>	Statistical confidence of the F-test of the significance of the slope.
<i>Significance of deviations from linearity (%)</i>	Statistical confidence of the F-test of the significance of deviation of the measurement points from linearity in the linear range.
<i>Significance of deviations from parallelity (%)</i>	Statistical confidence of the limits for the F-test of the deviation from parallelity in the linear range of the standard and the preparation. This value will always be taken from the object of which the relative potency is to be calculated.

Property	Description
<i>Fiducial limit of the potency estimation (%)</i>	Confidence interval for the relative potency of the preparation. This value will always be taken from the object of which the relative potency is to be calculated.
<i>Rejection criteria</i>	By default, the preparation will be rejected when the criteria for the slope, linearity or parallelity are not satisfied. You can define here which criteria are significant.
<i>EC50 Calculation Method</i>	Select the method for the calculation of the effective concentration of the 50% response. There are three options available: <ol style="list-style-type: none">1. No calculation of the EC50.2. Calculation of the EC50 by linear regression of the standard.3. Calculation of the EC50 by linear regression of the assay.
<i>Calculate EC50 by using</i>	Selection from which object(s) the EC50 will be calculated. You can choose from: <i>this object, standard, control</i> and <i>all objects</i> .

6.4.5 Range Selection Settings

You can choose the method for the selection of the linear and parallel range on this tab of the object definition dialog. The range can be allocated either manually or automatically. Both methods are mutually exclusive. By selecting one method, the input fields for the other method are grayed out.

Property	Description
<i>Linear Range</i>	<p>Selection of the linear range. There are four methods available for the selection of the linear and parallel range:</p> <ol style="list-style-type: none"> 1. <i>Fixed Range.</i> 2. <i>Automatic Detection (individual range).</i> 3. <i>Automatic Detection (common range for standard/individual for preparations).</i> 4. <i>Automatic Detection (common range for standard/identical for preparations).</i> <p>Refer to chapter 10.2 “Automatic Detection” for further details.</p>
<i>Fixed Range</i>	<p>Manual input of the linear and parallel range. (Inactive if automatic detection is activated.)</p>
<i>Allocation strategy</i>	<p>Evaluation criterion for the automatic selection of the linear and parallel range. The following choices are available:</p> <ol style="list-style-type: none"> 1. <i>Best Range.</i> 2. <i>Maximum Range.</i> 3. <i>Exact Range.</i> <p>(Inactive if manual detection is activated.)</p>
<i>Minimal # of steps - Exact # of steps</i>	<p>Depending on the selected allocation strategy either the minimum or the exact number of points is given.</p> <p>(Inactive if manual detection is activated.)</p>

Property	Description
<i>Allocation Region</i>	<p>You can restrict the interval in which the automatic detection tries to allocate the linear and parallel range. You must select <i>Maximum Allocation Range</i> in the <i>Allocation Strategy</i> field for this feature.</p> <p>(Inactive if manual detection is activated.)</p>
<i>Maximum Allocation Range</i>	<p>If you activate this option, the whole concentration range will be used for the automatic detection.</p> <p>(Inactive if manual detection is activated.)</p>
<i>Inclusion of 50% response</i>	<p>With this feature you can select if the 50% response of an object is to be taken into account for the determination of the linear and parallel range. The following choices are possible:</p> <ol style="list-style-type: none">1. <i>Ignore</i>2. <i>Inclusion if possible</i>3. <i>Inclusion is mandatory</i> <p>(Inactive if manual detection is activated.)</p>
<i>Calculate the 50% response by using</i>	<p>Choose the object of which the 50% response for the selection of the linear and parallel range is used. You can choose from: <i>this object, standard, control</i> and <i>all objects</i>.</p> <p>(Inactive if manual detection is activated.)</p>

6.4.6 Annotations

Property	Description
<i>Annotations*</i>	<p>Annotations and further information on the object. (This information is not passed on by inheritance.)</p>

6.4.7 GLP/GMP Settings

On this page of the object definitions you can select the settings for the protection of the object. These settings are used for the work under GLP/GMP conditions where well-defined operation procedures have to be followed.

A more detailed description of the GLP/GMP functions is given in chapter 9.

Property	Description
<i>Object uses the following scheme*</i>	Give the scheme for the object definitions here.
<i>Enable GLP/SOP Protection (Mandatory)</i>	If you activate this button, you can lock the selected protection levels for the user. Only PLA administrators can activate or deactivate this setting.
<i>Enable GLP/SOP Protection (User Level)</i>	This button activates the protection levels for the user. In contrast to the above option, PLA administrator as well as normal users can manipulate this function.
<i>Substance information protected</i>	By activating this option, you can protect the access to the substance information and prevent changes by the user.
<i>Reagent information protected</i>	Activate this option to protect the access to the reagent information and prevent changes by the user.
<i>Object definitions protected</i>	By activating this option, you can protect the access to the general object settings and prevent changes by the user.

Property	Description
<i>Analysis settings protected</i>	Activation of this option protects the access to the analysis settings and prevents changes by the user.
<i>Range selection settings protected</i>	By activating this option, you can protect the access to the linear selection settings and prevent changes by the user.
<i>Direct dose values protected</i>	By activating this option you can protect the access to the given dose values and prevent changes by the user.
<i>Data values not editable</i>	By activating this option, you can protect the access to the measurement points and prevent changes by the user.

6.4.8 Dose and Measurement Input

For the input of the dose values and measurements, the same input mask is used in PLA. While the dose values can be set on a higher object level the input of the measurement values is only possible on the lowest level of the object hierarchy (standards/preparations/controls). The following properties are available for each measurement:

Property	Description
<i>Concentration</i>	Concentration of the respective dilution step. If you have preselected 1:2 dilution series, the value will be given automatically and cannot be changed by the user.
<i>Series No</i>	Input of the measurement reading.
<i>Status indicator</i>	Manual exclusion of a reading from the analysis (technical outlier).

7 My first Parallel-Line Assay

This chapter will show you how to run a full parallel-line analysis of an assay. You should be able to do all the steps by yourself with your PLA installation. After reading this chapter, you should be able to run parallel-line assays with PLA 1.2.

The chapter does not introduce you to all functions of PLA, however. It just shows you the steps that are normally necessary to run the analysis. For a description of all functions, please refer to chapter 8.

7.1 Initial Situation

You have done a measurement with five preparations and one standard sample. The measured concentration range extends from 1:1 to 1:64. All dilution steps have been taken twice. The measurements are in Table 7-1.

No.	Dilution	Series 1	Series 2	Series 1	Series 2
		Standard		Preparation 1	
1	1:1	704	719	701	704
2	1:2	641	645	637	632
3	1:4	575	578	566	565
4	1:8	512	514	502	505
5	1:16	450	450	439	438
6	1:32	394	384	383	380
7	1:64	338	339	334	333
		Preparation 2		Preparation 3	
1	1:1	652	674	675	677
2	1:2	582	585	603	602
3	1:4	512	514	530	529
4	1:8	447	454	470	474
5	1:16	394	396	412	412
6	1:32	336	333	350	354
7	1:64	294	294	307	310
		Preparation 4		Preparation 5	
1	1:1	710	707	768	771
2	1:2	668	659	732	739
3	1:4	649	645	693	696
4	1:8	593	587	628	647
5	1:16	522	521	569	572
6	1:32	463	459	502	517
7	1:64	417	416	456	466

Table 7-1: Measurements of the example

Your task is to determine the relative potency of the preparation samples in comparison to the standard. The preparations cannot be analyzed over the full range of dilution steps because of saturation effects. You do not know which interval can be analyzed.

7.2 Creating a Project

First you should create a new project. This is not really necessary for only one assay but it will help you if decide to measure further assay later. These assays can then be added to the project.

Do you remember chapter 6.2 “Object Hierarchy” and 6.3 “Inheritance”? There you will find all essential information about the creation of objects and how they relate to each other.

Inheritance has the advantage that all settings of the higher order objects are passed on to a lower level object. You can immediately take advantage of this feature in our example.

Take a moment to think about which objects you will need!

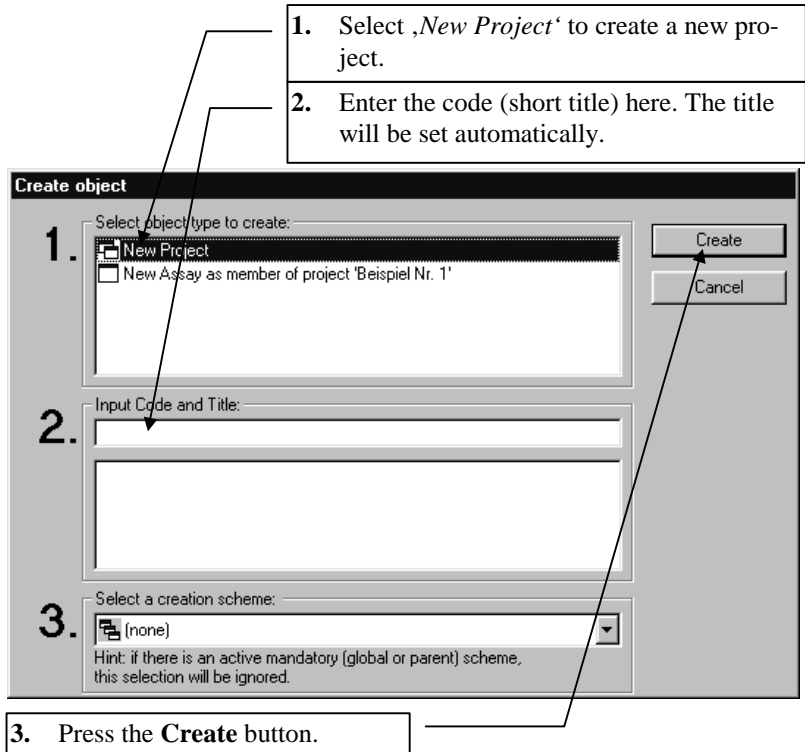
First, all assays that you create will be regarded as part of a project. Therefore, you will need a project. Second, all samples are part of the same assay. You will need one assay for the preparations and the standard.

Additionally you have six series of readings: one series for the standard and one for each of the five measurements. You will need one object for the standard sample and one for each preparation. The structure of the project should now look like this:

```
Project
  Assay
    Standard
    Preparation 1
    Preparation 2
    Preparation 3
    Preparation 4
    Preparation 5
```

Do you still remember how inheritance works? When you enter all settings that all subordinate objects have in common at the higher-level object, all settings will be copied to any newly created lower level objects.

After you have started PLA and logged on the navigator appears. Create a new object by selecting **New** or **Create Object**. You should see the following dialog on the screen now:

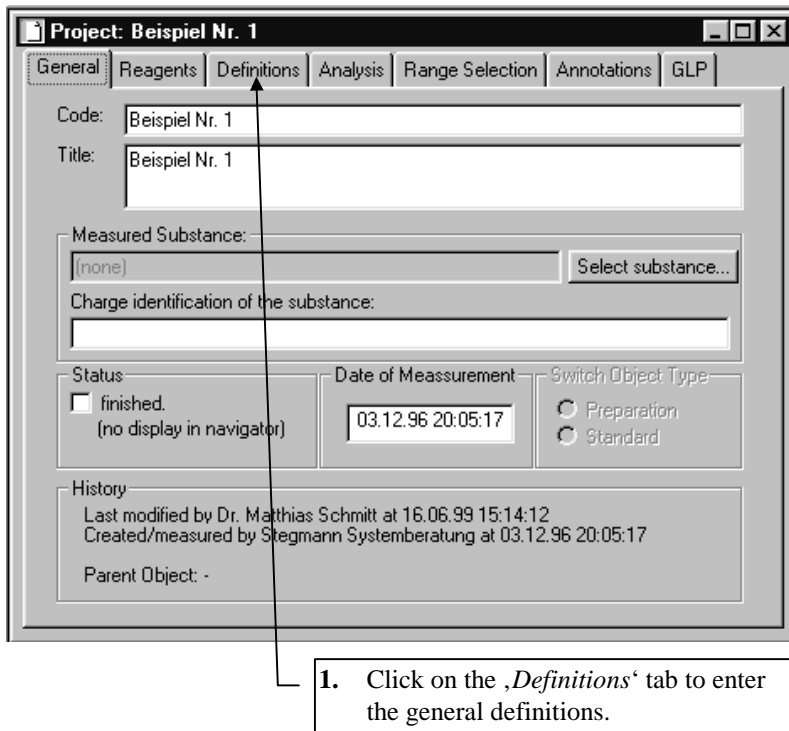


After you have performed all three actions, a new project will be created. You should see the following confirmation dialog on the screen.



Additionally you will get further information from which object the definitions have been copied (parent object or scheme).

After you have pressed the **OK** button, the object dialog appears. You should see the page with the general settings now. Most of the settings in this tab have no influence on the calculation. In the following steps we will limit ourselves to the settings that are important for the analysis. These are the definition of the dilution steps, the data series and the analysis settings.



When you click on the index tab 'Definitions', the appearance of the dialog changes. You can change the general settings of the project on the page you can see on the screen.

Keep in mind that the settings on the project level are not used immediately for the calculations. Instead, they serve as a template for the object you create in the next steps.

1. Set the number of dilution steps to 7.

Project: Beispiel Nr. 1

General Reagents Definitions **Analysis** Range Selection Annotations GLP

Concentrations and Predilutions

Concentration of the undiluted standard%: 1,0000

%in units:

Predilution factor of the standard: 1 : 1,0000

Predilution factor of the preparation: 1 : 1,0000

Dilution series

Number of dilution steps in a data series: 7

Number of data series: 2

Additional predilution factor for the first step: 1:1

Dilution scale: '1 in 2' series

2. Set the number of data series to 2.

3. Go to the analysis settings by clicking on the 'Analysis' tab.

All measurements in this example have the following settings in common: the number of dilution steps, seven in our case, and all data series have been taken twice. When you enter the dilution values the numbers will be copied to the assays, standards and preparations. Furthermore, the predilution factors in the preparations and standards are the same. In this case, the respective input fields can be left unchanged. (Note: If all the predilution factors of the standards and preparations are the same, the correct predilution factor will have no influence on the result of the calculation.)

Click on the ‘*Analysis Settings*’ page now. Before you fill out this dialog, consider which settings for the statistic parameters of the analysis you will need.

For the evaluation of the tests for the slope, linearity and parallelity always choose to work with a confidence level of 98.0%. Set the confidence interval for the relative potency to 95.0%. If one of the tests is not passed, the relative potency should not be calculated and the assay should be rejected. To achieve this your analysis settings should look like the following figure:

1. In the following 3 fields set the value to 98.0% and check the *rejection criterion* box.

2. Set the *fiducial limit* to 95.0%.

Project: Beispiel Nr. 1

General | Reagents | Definitions | Analysis | Range Selection | Annotations | GLP

General

☐ Analysis according to Europ. Pharmacopoeia §7

☐ Perform Dixon test for data outliers. (Up to 25 series.)

Regression Model:

lin/log ☐ Reverse Response-Effect Correlation.

Probabilities:

Dixon test - contamination (alpha): 0,2

Significance of the slope (%): 98,0 ☒ rejection criterion

Significance of deviations from linearity (%): 98,0 ☒ rejection criterion

Significance of deviations from parallelity* (%): 98,0 ☒ rejection criterion

Fiducial limit of potency estimation* (%): 95,0

Values marked * are used only by objects whose potency will be calculated.

EC50 Calculation

Method: Do not calculate

Calculate 50% Response by: This object.

3. Click on the ‘*Range Selection*’ tab to go to the range selection dialog.

Leave all other settings on the default values. On the tab '*Range Selection*' you can decide which method is used for the selection of the linear and parallel interval. For our example choose the following settings:

The linear and parallel range of our preparations and standards are to be determined automatically in a way that

1. the linear range for each preparation can be found in different intervals.
2. the linear range for the standard must always be in the same interval. (Each preparation is compared with the standard individually. With this restriction, you prevent that different linear regions are chosen for the standard in each comparison.)
3. in each preparation there must be at least three dilution steps in the linear range.
4. the range selected should be as large as possible, meaning as many measurements as possible should be within the region.

To achieve this you have to change the settings in the range selection dialog to the following:

1. Select *'Automatic Detection (common range for standard; individual range for preparations)'* from the drop-down box here. This will activate the automatic detection of the linear range.
2. Choose *'Maximum Range'* from the drop-down box to trigger an analysis that will try to find the linear range that will contain the maximum number of points.

Project: Beispiel Nr. 1

General Reagents Definitions Analysis Range Selection Annotations GLP

Linear Range

Automatic Detection (common range for standard/individual for preparations)

Manual Selection Options:

Fixed Range: 1 - 2

Automatic Selection Options (used by automatic detection methods)

Allocation Strategy: Maximum Range

Allocation region: 1 - 7

Minimal # of points: 3

☒ maximal allocation range.

Inclusion of 50% response: Calculate the 50% response by using the

ignore. this object.

3. Choose the value 3, to find a range with minimal 3 dilution steps.

After you have entered the range selection settings, the definition of the project is finished. Close the window and save the settings. The newly created project is shown in the navigator. You can now continue with the creation of the assays.

7.3 Creating an Assay

Now you can create the second level of the object definition: the assay. Assays structure your project. Later you will start calculations from this level. The procedure to create an assay is easy. Select the project you have just created and click **New**.

1. Select *'New Assay as member of project'*. The code of the project is shown in quotes.

2. Enter a code (short title) for the assay.

Create object

1. Select object type to create:

☒ New Project
☐ **New Assay as member of project 'Beispiel Nr. 1'**
2. Input Code and Title:
3. Select a creation scheme:

☒ (none)

Hint: if there is an active mandatory (global or parent) scheme, this selection will be ignored.

Create

Cancel

3. Press **Create** to create the new assay!

After you clicked *Create*, the object dialog for the assay appears on the screen. In this example, no changes are necessary for this object. Look at the properties of the assay. You will see that the project settings have been copied to the assay.

7.4 Creating the Preparations and the Standard

The next thing you will have to do is to create the preparations and the standard. Start with the standard. If you have not closed the creation dialog for the assay yet, please do so. In analogy to the creation of the assay select the newly created assay in the object navigator window and click **New** or **Create Object** from the menu.

Now you have the choice to create either a preparation or a standard. Create the standard first. Do always check that you have selected the correct type of object. For each of the five preparations select '*New Preparation as member of assay...*' If you want to create a standard, choose *New Standard as member of assay...!*

After you have executed the **Create** command for the standard the object dialog appears.

As you have seen in the previous chapter, all settings you have entered at project level have copied to the assay object. These settings are now copied to the standard. Take a look around the settings!

To enter the data for the standard you have to start the data editor by choosing **Edit Data** from the menu. The object dialog will now close and you should see the following screen:

The screenshot shows a software window titled "Standard: Standard". It contains a table with three columns: "Concentration", "Series N° 1", and "Series N° 2". The "Concentration" column lists seven ratios: 1:1, 1:2, 1:4, 1:8, 1:16, 1:32, and 1:64. The "Series N° 1" and "Series N° 2" columns each contain a text input field with the value ",00000" and a small checkbox to its right. All checkboxes are currently checked. Below the table is a horizontal scrollbar. At the bottom of the window, there is a "Toggle status indicator" checkbox which is checked, followed by a text label: "<-status indicator. Un-check the indicator at the right side of the value for exclusion."

Concentration	Series N° 1	Series N° 2
1:1	,00000 <input checked="" type="checkbox"/>	,00000 <input checked="" type="checkbox"/>
1:2	,00000 <input checked="" type="checkbox"/>	,00000 <input checked="" type="checkbox"/>
1:4	,00000 <input checked="" type="checkbox"/>	,00000 <input checked="" type="checkbox"/>
1:8	,00000 <input checked="" type="checkbox"/>	,00000 <input checked="" type="checkbox"/>
1:16	,00000 <input checked="" type="checkbox"/>	,00000 <input checked="" type="checkbox"/>
1:32	,00000 <input checked="" type="checkbox"/>	,00000 <input checked="" type="checkbox"/>
1:64	,00000 <input checked="" type="checkbox"/>	,00000 <input checked="" type="checkbox"/>

Toggle status indicator ☒ <-status indicator. Un-check the indicator at the right side of the value for exclusion.

This is the edit mask for the measurement values. Now enter your readings. The check box on the right side of the measured values is activated automatically as soon as a value is entered into the edit field. The check box is called the status indicator. It shows you if the respective value is used for the analysis. For further information on the status indicator, please refer to chapter 8. You can go to the next field by pressing the tab key.

After you have entered the data for the standard, your screen should look like this:

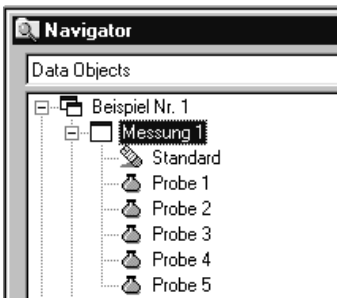
Standard: Standard

Concentration	Series N° 1		Series N° 2	
1:1	704,00000	<input checked="" type="checkbox"/>	719,00000	<input checked="" type="checkbox"/>
1:2	641,00000	<input checked="" type="checkbox"/>	645,00000	<input checked="" type="checkbox"/>
1:4	575,00000	<input checked="" type="checkbox"/>	578,00000	<input checked="" type="checkbox"/>
1:8	512,00000	<input checked="" type="checkbox"/>	514,00000	<input checked="" type="checkbox"/>
1:16	450,00000	<input checked="" type="checkbox"/>	450,00000	<input checked="" type="checkbox"/>
1:32	394,00000	<input checked="" type="checkbox"/>	384,00000	<input checked="" type="checkbox"/>
1:64	338,00000	<input checked="" type="checkbox"/>	339,00000	<input checked="" type="checkbox"/>

Toggle status indicator ☒ <-status indicator. Un-check the indicator at the right side of the value for exclusion.

Close the data editor and answer the question if the values should be saved with **Yes**. This will lead you back to the navigator.

Create the five preparation objects in the same manner. Keep in mind that you have to select *preparations* in the create dialog of the object. Enter the measurement values for all preparations. When you have finished the creation of all preparation samples your navigator window should look like this:



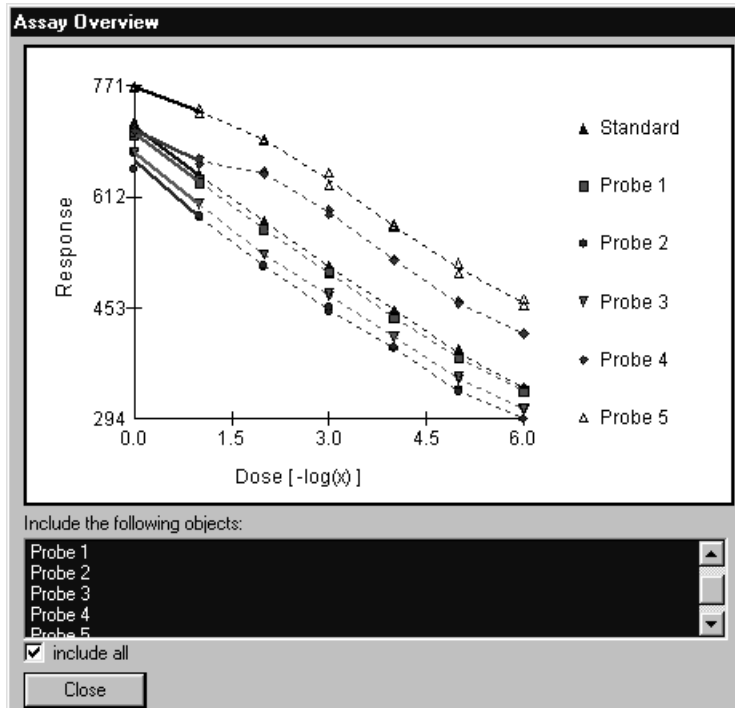
The identifiers you see in the navigator correspond to the names that you gave to the objects. Since you have finished the data input successfully, you can now analyze the assay.

7.5 Analysis

In this chapter you will do your first PLA analysis. The analysis settings are identical in all objects that you have created. You can start your analysis immediately.

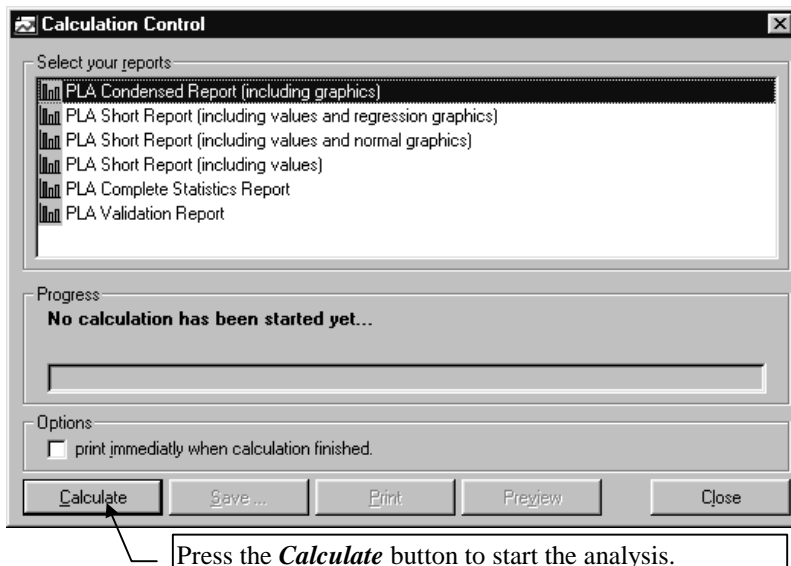
Before you start the analysis, you should check if your data is correct.

Select the assay you have created in the navigator. To get a plot of your data use the **Info** command from the menu. When you select this function, you should see the measurement values of the preparation and the standard on a graph. For our example, you should see the following graph on your screen:

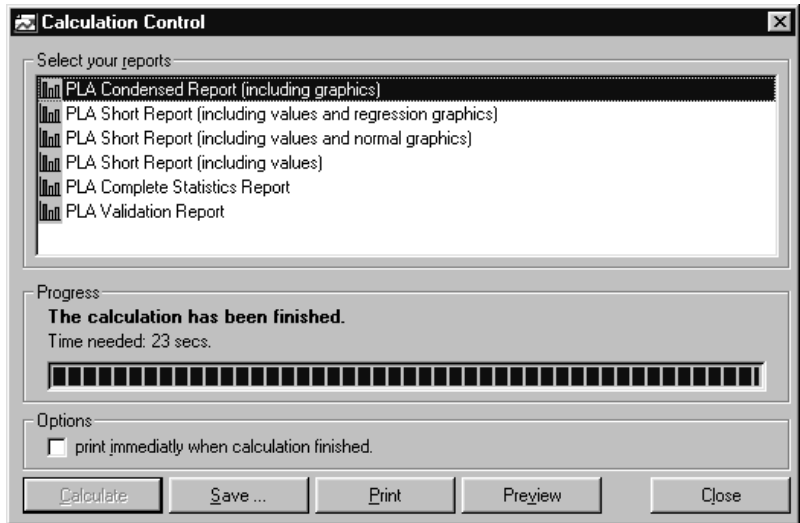


The purpose of this function is to give you a quick overview over the quality of your data. For the moment the visual control is sufficient. Please close the window.

Back in the navigator window, you select the function **Calculate** from the menu to start the analysis. You should see the following dialog on the screen now:



The calculation might take a moment. PLA now performs the automatic detection of the linear interval and analyses the assay afterwards. When the calculation is finished the print, save and preview buttons will be activated. Select one or more reports from the upper box in the dialog and press the preview button to view the report.



If you choose the '*PLA Condensed Report*', you should see the following results:

My first Parallel-Line Assay

Page 1 of 1
Beispiel Nr. 1

09.09.99 09:30:50

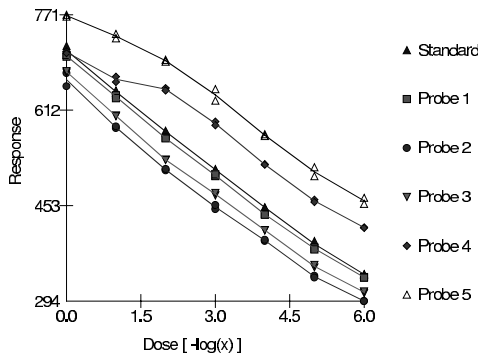
Project: **Beispiel Nr. 1**

Current user: Dr. Matthias Schmitt

.....
Date/Signature

Assay	Standard	Sample	Test of Linearity	Test of Slope	Test of Parallelity	Relative Potency
-------	----------	--------	-------------------	---------------	---------------------	------------------

Messung 1



Standard	PASSED	PASSED		
Probe 1	PASSED	PASSED	PASSED	0,892
Probe 2	PASSED	PASSED	PASSED	0,504
Probe 3	PASSED	PASSED	PASSED	0,633
Probe 4	PASSED	PASSED	PASSED	2,174
Probe 5	PASSED	PASSED	PASSED	3,664

PLA 1.2 has successfully analyzed the assay and has found linear and parallel intervals.

Note: PLA offers a plethora of functions and settings that modify the analysis of parallel line assays. You can change these settings to a very large extend. However, many of the settings address explicitly the use of PLA in research and product development. In this field, many of the parameters must be variable to develop standard procedures for the analysis of measurements using parallel line assays. Not every parallel line assay that is accepted by PLA is automatically valid. The changed settings have to be evaluated and approved as a part of the development process. Only those analyses that are performed in the framework of tested parameters are valid in a statistical sense.

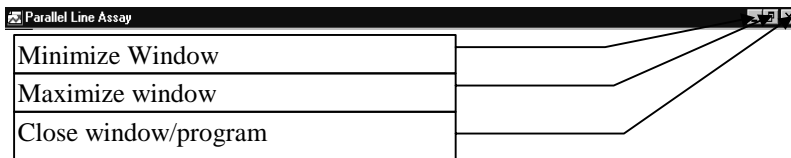
8 Reference

8.1 General Information

PLA 1.2 uses the common Windows user interface. If you have already used common Windows applications, you will be able to find your way around PLA quickly. All PLA functionalities can be accessed from the menu. Alternatively you can use the task bar for the most common tasks.

In this chapter, you will find a description of every element of PLA. The only exception is the user management that has already been introduced in chapter 4 “*System Requirements and Installation*”. Generally, you can operate PLA either with the mouse or with the keyboard. Use the tab key to move within the dialogs.

Nearly every window offers the following options:



If you have changed anything within the window, you will be asked if the changes should be saved when you close the window.

8.2 The Menu

You can access all functions of PLA from the menu bar. Many of these functions can also be accessed by short cut key combinations or via the task bar icons. Yet, some of the functions are only accessible from the menu. All functions available from the menu are listed in the following sections. A more detailed description is given in the following sections.

8.2.1 File Menu

Selection	Short Cut	Action
Navigator	F2	Open a navigator window.
Create Object	CTRL+N	Open a create dialog.
Open Object Definitions	CTRL+O	Open the object definitions dialog of the selected object.
Edit Object Data	CTRL+D	Open the data editor.
Open Substance Database		Open the substance database.
Open Reagent Database		Open the reagent database.
Close Window	CTRL+F4	Close the active window.
Save Object	CTRL+S	Save the settings for the active object without closing the dialog.
Delete Object Tree		Delete the selected object including all lower level objects.

Selection	Short Cut	Action
Apply...	CTRL+A	Open the apply function.
Import		Start the import module.
Export		Start the export module.
Logout		Log out from PLA.
Exit	ALT+F4	Terminate the program.

8.2.2 Action Menu

Selection	Short Cut	Action
Calculate & Report	CTRL+C	Open the calculate dialog.
Check	CTRL+K	Quick analysis.
Display Graphical Info	CTRL+I	Open a graphical representation of the assay for visual control.

8.2.3 Preferences Menu

Selection	Short Cut	Action
Customize Toolbars...		Menu for the customization of PLA.
Options		Open a dialog for application paths and the GLP/GMP settings of PLA.
Account Management		Open the account management dialog.
Change your Password		Open a dialog for changing the password.

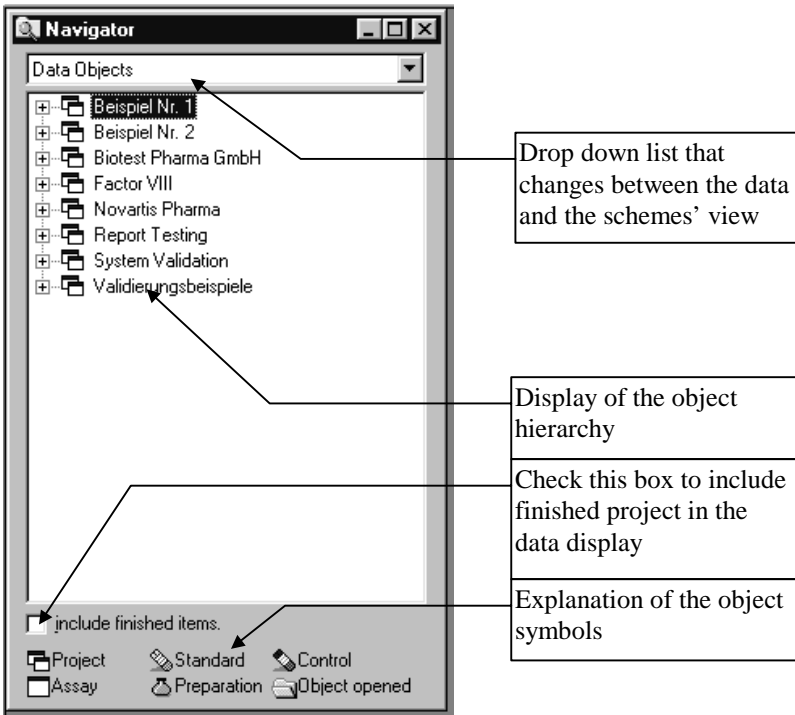
Selection	Short Cut	Action
Check Data- base Consis- tency		Perform a check of the database consistency.

8.2.4 Help Menu

Selection	Short Cut	Action
About		Open the about dialog.

8.3 The Navigator

The central element in PLA is the navigator. This window controls the access to all objects. It includes a hierarchical list of the different object types. To learn more about the hierarchical organization of PLA refer to chapter 6.2.



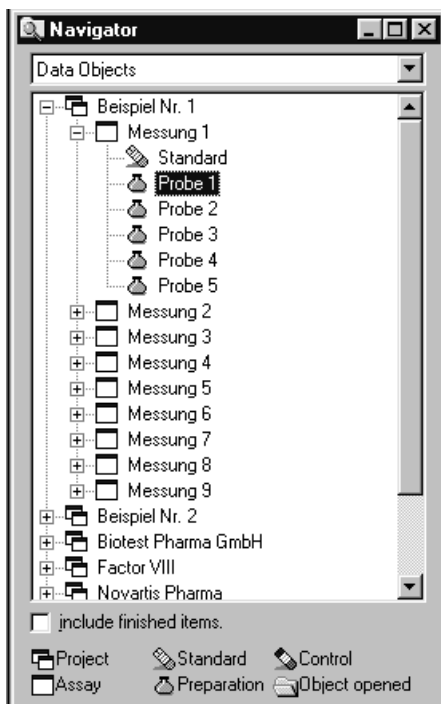
The list displays all objects within the database. The first level contains the project. Click on the + symbol on the left side of the project to access the assay level below the projects. You can access the samples and controls from the assay level in the same way.

With the help of the drop down box in the upper part of the navigator, you can switch between the view of the data objects and a view of the defined schemes. Schemes are templates for the generation of new data objects.

Furthermore, they are important for working under GMP/GLP conditions. Please refer to chapter 9.1 for a more detailed description of schemes.

8.3.1 Opening Objects

To open the object definition dialog select the object in the navigator by either clicking on it or highlighting it with the cursor keys.



You can open the object in four different ways:

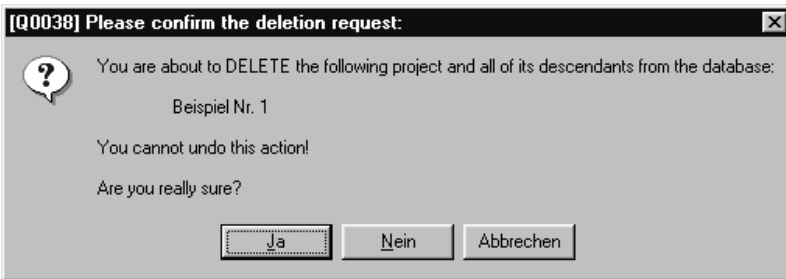
1. Choose **File|Open|Object Definitions** from the menu.
2. Select **Definitions** from the task bar.
3. Press CTRL+O.
4. Double click on the object.

8.3.2 Deleting Objects

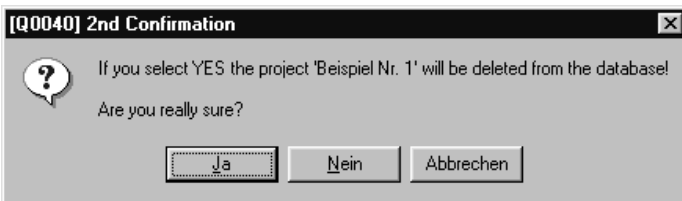
Click once on the object you want to delete.

ATTENTION: All lower level objects will be deleted as well. The deletion cannot be undone!

Once the object is selected, either press the DEL key or choose **File|Delete Object Tree** from the menu. You will be asked to affirm the deletion.



Press the **Yes** button to execute the deletion. A second dialog will appear to ask you again. If you press the **Yes** button again, the object and all subordinate objects will be deleted.

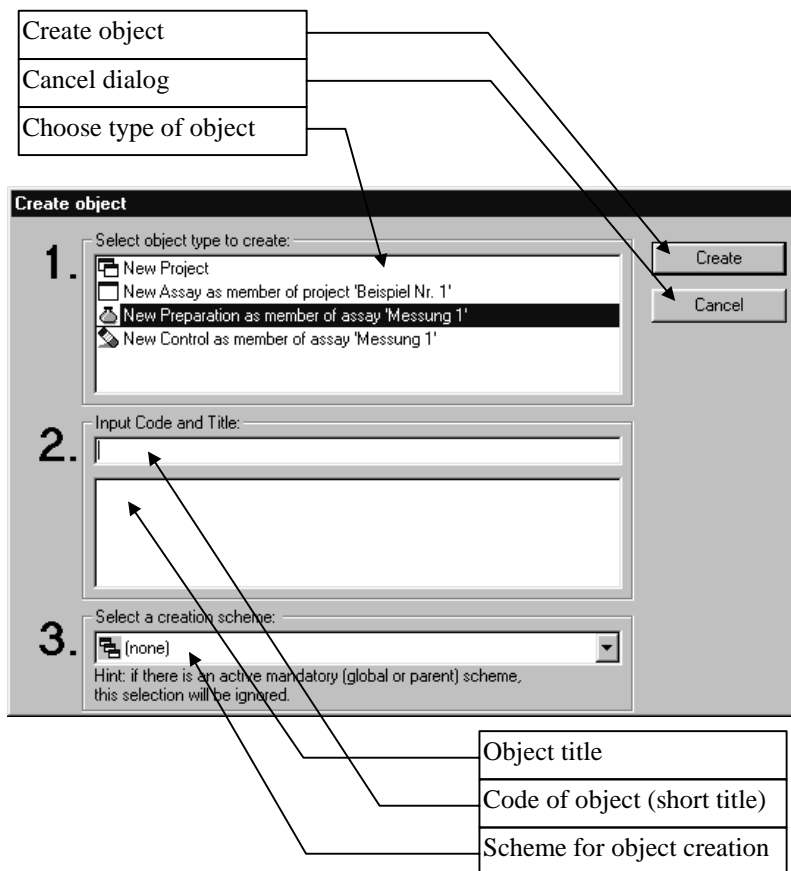


8.3.3 Showing Finished Items

If the checkbox *include finished items* is deactivated the navigator will only show active objects.

8.4 Creating Objects

To create a new object select the parent object from the navigator list first. E. g. if you want to create another sample in the assay *Messung 1* in the project *Beispiel 1*, select the assay and choose **New** from the task bar. Alternatively, you can select **Create Object...** from the menu or press CTRL+N. The following dialog will appear on the screen.



You can enter the essential properties of the new object in this dialog.

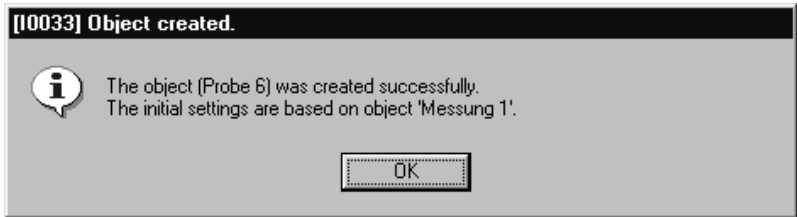
First, you can select the object type you want to create. Depending on the parent of the object some options might not be available. If you have not chosen any object from the navigator hierarchy, only projects can be generated. If you have chosen a project as parent, only assays can be created. If the selected parent is an assay, new preparations, standards and control can be generated.

New Object	Mandatory Parent Selection
Project	Any
Assay	Project
Preparation/Standard/Control	Assay

Second, you can enter a short title (*code*) for your new object. This name will be copied to the full name (*title*) but you can change this name afterwards. The input for the code and the title of the new object is mandatory.

Third, you can give the name of a template that is used for the creation of the object (*creation scheme*). Defining a scheme is a comfortable and efficient way to define default settings for the properties of you newly generated object. If you do not select a scheme, the settings of the parent will be copied to the new object. For the case that you have already created schemes, you can pick one of them from the drop down box. Please refer to chapter 9.1 if you want to know more about schemes.

When you are finished with your input, press the **Create** button to generate the object. If the operation is successful, you will see the following affirmative dialog.



Press the **OK** button to open the object setting dialog.

If you press the **Cancel** button instead of the **Create** button the dialog will be closed immediately without generating a new object.

8.5 The Object Dialog

Every object in PLA except controls has a full set of properties. These have already been introduced in chapter 6.4. You can edit the settings in the object dialog. The dialog contains seven different tabs structuring the information on the object.

If you change any of these settings, the program will ask you to save the changes upon closing the dialog.

In the following sections, every object setting is described in detail. To bring a particular dialog page to the front simply click on the respective tab with the mouse.

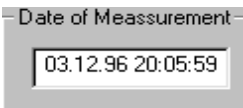
8.5.1 General Settings

The first dialog page contains the general settings for the object. You can change the object's *code* (short title) and *title* here. Furthermore, objects can be declared *finished*. Checking this box has the effect that the object and all its descendants will not be displayed in the navigator. To display finished objects in the navigator you have to check the box *include finished items* there.

The *date of measurement* will always be set to the creation date of the object in PLA but you can change the date on this dialog page. The format of the time stamp input is

dd.mm.yy hh.mm.ss

with dd for day, mm for the month and yy for the year in the date input and with hh for the hour, mm for the minute and ss for the second in the time input. E. g.



will set the time stamp to December 3rd, five minutes past 8 a.m..

Preparations and standards (on the lowest object hierarchy) can be converted into each other. By changing the setting in the *switch object type* group box, you can change a standard into a preparation and vice versa. In this way, it is possible to compare different standards against each other.

Code of the object (short title)
Title (full name of the object)
Measured substance name
Select substance from data base
Batch number from which the substance was taken

Assay: Europ. Pharmacop. 1993

General | Reagents | Definitions | Analysis | Range Selection | Annotations | GLP

Code: Europ. Pharmacop. 1993

Title: Europ. Pharmacopeia 1993, VIII. 13, Beispiel 3.2.8.1

Measured Substance: [none] Select substance...

Batch identification of the substance:

Status: ☐ finished. (no display in navigator)

Date of Measurement: 16.06.99 15:14:55

Switch Object Type: ☒ Preparation ☐ Standard

History:

Last modified by Administrator at 02.08.99 09:21:37
Created/measured by Dr. Matthias Schmitt at 16.06.99 15:14:55

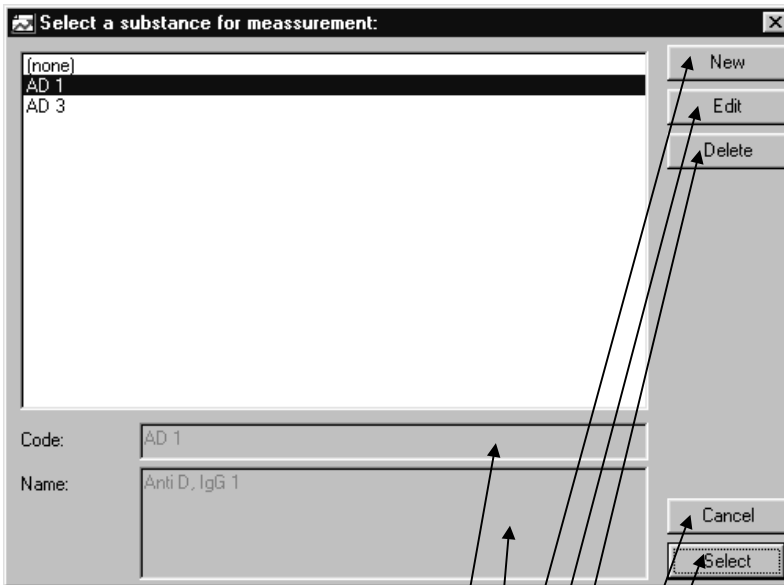
Parent Object: Validierungsbeispiele

Object type (change to convert)
Time stamp of measurement
Declare object finished
Object history (last modifier/generator)

8.5.1.1 The Substance Database

Substances often have long and complicated names. PLA offers a substance database to facilitate the substance input for you. In this database, the program keeps a list of all substances that have been measured already. You only need to enter a substance name only once. After that, you can always pick the substance from database list.

Pressing the **Select Substance** button will open the substance dialog. You can now either pick a substance name from the list or add a new substance to the database. You cannot enter the substance name directly.



Input the substance code (short name)
Input field for the substance title
Add a substance to the database
Edit a substance
Delete a substance from the database
Cancel the dialog
Apply changes and close dialog

In analogy to preparations and standards, PLA uses a substance code and a substance name. For the analysis you can use the code to corresponding to the labels, e. g. identification numbers, you normally use in your laboratory while using the correct name for the printed output.

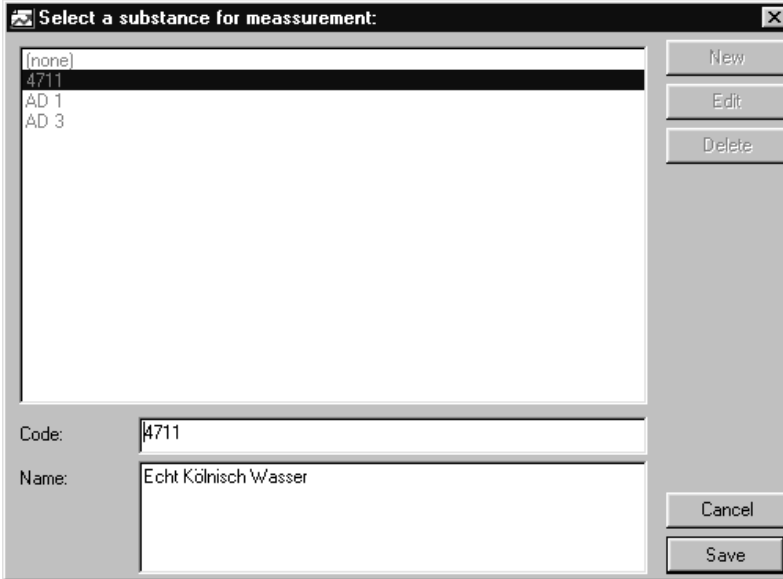
Choose a code from the list and press the **Select** button to pick a substance from the list. You will also get back to the object settings.

Click on the **New** button to add a new substance to the database. Enter the substance code and name in the appropriate input fields. Press the **Save** button to apply the changes to the database. The substance is now displayed in the pick list.

If you do not supply a code and a name of the substance, a warning message will appear.



Choose the code from the list and press the **Edit** button to change a substance name.



The dialog box titled "Select a substance for measurement:" contains a list box with the following items: [none], 4711, AD 1, and AD 3. The item "4711" is selected. To the right of the list box are three buttons: "New", "Edit", and "Delete". Below the list box are two input fields: "Code:" with the value "4711" and "Name:" with the value "Echt Kölnisch Wasser". At the bottom right are two buttons: "Cancel" and "Save".

You can change the input fields *code* and *name* now. To apply the changes to the database press the **Save** button.

To delete an entry from the list select the substance code with the mouse and press the **Delete** button. If the substance is used in any object, you will see the following error message and the procedure will terminate without any changes to the database.

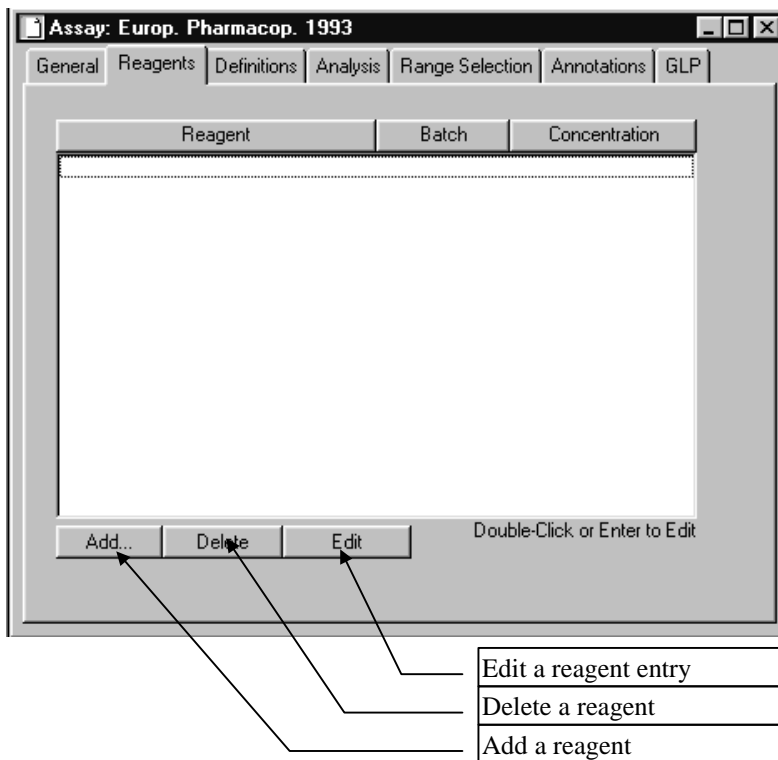


The error dialog box has a title bar that reads "[E0192] Request Denied!". It features a circular icon with a cross (X) on the left. To the right of the icon is the text: "Sorry the entry is in use. You cannot delete entries while they are in use by other objects." At the bottom center is an "OK" button.

Generally, you can only delete substances that are not in use by any object. However, this only applies to objects that have already been saved.

8.5.2 Reagents

The second page of the object dialog serves only informative purposes. You can specify the reagents that you have used for the preparation of the samples, standards and controls on this page. The information on the reagents you can specify is the name, the batch number and the concentration. Again, you can give a *code* and a *name* for the reagent. You can add as many reagents as you like.



You cannot edit the list directly. Instead, you have to use the three buttons under the list field. Press the **Add...** button to add a reagent to the list. Press the **Edit** button to edit an entry. Both functions will open the reagent database. With the **Delete** button, you can delete entries from the list.

The image shows a software dialog box titled "Reagent selection and definition". It contains the following elements:

- Select a reagent:** A section with an entry field for a code and a list of reagents. The list currently shows "Anti-IgG 1" and "Ethanol".
- Batch:** An entry field for the batch number.
- Concentration:** An entry field showing the value ".00000".
- Buttons:** Four buttons at the bottom: "Ok", "Cancel", "Delete", and "Edit description".

Annotations with arrows point to the following components:

- Entry field for code
- Pick list for reagents
- Batch number
- Concentration
- Edit reagent entry
- Delete reagent
- Cancel dialog
- Apply changes and close dialog

You can add, delete and edit reagents in this dialog,. In principle, the name, batch number and concentration characterize every reagent. The concentration input accepts only numerical values. The dimension of the concentration should be given with the description of the reagent.

Description for the selected reagent:

Please enter a code:

Please enter a description:


Note: the description is displayed in reports instead of the short one shown in the user interface of the program.

After you have supplied all the necessary information, the reagent definition is saved in the reagent database. The reagent will be available the next time the database is opened.

If you want to edit an existing reagent, select the entry from the list and press the **Edit Description** button. The reagent description dialog appears and you can change the code as well as the name of the reagent.

To delete a reagent from the list select the reagent code and press the **Delete** button. The following dialog appears and you will be asked to confirm the deletion.

[Q0170] Please Confirm Deletion

 You have selected to delete Reagent (Anti-IgG 1) from the database. (The deletion will be rejected if the reagent is used by any object). Are you sure?

If no other object uses the reagent, it will be deleted from the list. Otherwise, the deletion process will be terminated.

8.5.3 Definition Settings

The third page of the object definitions contains the fundamental settings for the data. The dilution steps and the number of readings must be given here.

Concentration standard	
Concentration units	
Predilution standard	
Predilution preparation	
Number of measurements	
Number of data series	
Predilution factor	
Dose input	

Assay: Europ. Pharmacop. 1993

General | Reagents | **Definitions** | Analysis | Range Selection | Annotations | GLP

Concentrations and Predilutions

Concentration of the undiluted standard*: 1,0000

*in units:

Predilution factor of the standard: 1 : 1,0000

Predilution factor of the preparation: 1 : 1,0000

Dilution series

Number of dilution steps in a data series: 3

Number of data series: 10

Additional predilution factor for the first step: 1:1

Dilution scale: 1 in 2' series

The first three input fields are related to the settings for the standard. The input of the *concentration of the undiluted standard* is optional. The dimension of the standard concentration should be given in the *units* field. The input of the *predilution of the standard* is mandatory. The predilution is given as a ratio 1:X. If your predilution is 1:100, enter 100 in this field.

The predilution of the preparation is given in the same way. The input for this field is compulsory, too.

In the following input fields the *number of dilution steps* and the *number of data series* must be given. E.g. if your measurement has four dilution steps and has been repeated three times, enter 4 as the number of dilution steps and 3 as the number of data series.

In addition to the given predilution, you can enter an *additional predilution factor for the first step* because many measurement procedures contain an extra dilution step by bringing the preparation into a medium for the measurement. You can only enter integer ratios from 1:1 to 1:10 here.

The last entry concern with the input of the dose values. The default setting is '*1 in 2*' series. If you want to enter other dose values, you have to select *direct dose input*. With this setting you can enter the dose values directly in the data editor.

ATTENTION: If you change the dilution scale from direct dose input to '*1 in 2*' series you loose the already entered dose values.

8.5.4 Analysis properties

The settings on the fourth page of the object dialogs refer exclusively to the analysis definitions. These settings are divided into three sections:

Dixon p-value

Slope hypothesis

Linearity hypothesis

Parallelity hypothesis

Confidence interval

EP 1997 accordance

Dixon test activation

Regression model

Inverse response-effect relation

Assay: Europ. Pharmacop. 1993

General | Reagents | Definitions | Analysis | Range Selection | Annotations | GLP

General

☒ Analysis according to Europ. Pharmacopoeia 9?

☒ Perform Dixon test for data outliers (Up to 25 series.)

Regression Model:
lin/log

☐ Reverse Response-Effect Correlation.

Probabilities:

Dixon test - contamination (alpha): 0,02

Significance of the slope (%): 98,0

rejection criterion

Significance of deviations from linearity (%): 98,0

rejection criterion

Significance of deviations from parallelity* (%): 98,0

rejection criterion

Fiducial limit of potency estimation* (%): 95,0

Values marked * are used only by objects whose potency will be calculated.

EC50 Calculation

Method: Do not calculate

Calculate 50% Response by: This object.

Slope is rejection criterion

Linearity is rejection criterion

Parallelity is rejection criterion

Object for the EC₅₀ response

Method for the calculation of the EC₅₀ response

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The general section allows you to set all the analysis properties according to the European Pharmacopoeia 1997. If you check this option, the whole dialog page will be deactivated. The option facilitates the control of objects. The second option in the general analysis box is the activation of the Dixon test, which performs a check for statistical outliers. Please refer to chapter 10.1.2 for further information on the Dixon test.

PLA can do the analysis of parallel-line assays with two different *regression models*. You can choose between a simple logarithmic (*lin/log*) and a double logarithmic analysis (*log/log*). The simple logarithmic option, in which only the dose inputs are treated logarithmically, is the default. In the double logarithmic version the dose inputs as well as the responses are transformed. The logarithm is always calculated to a base of 2. **Note:** Never use different regression models within the same assay.

Another feature of the general analysis settings is the ability of PLA to calculate *reverse response-effect correlation*. Check this feature if an inverse dose-response relation exists in your assay. This option is necessary for the measurement of e.g. inhibition assays where the increase of the dose leads to a decrease of the reading. The relative potency of these assays would be the inverse of the potency for 'normal' assays. PLA will give the right value for this kind of assays if you check this option.

The second section of this dialog page relates to the statistical analysis of the assays. You can set the necessary statistical parameters and the rejection criteria for the assay here.

In the input field *Dixon-test contamination (alpha)* the confidence level of the Dixon test can be given. You can enter values from 0.005 to 0.3 here. The input has no effect, if you have deactivated the function *Perform Dixon test for data outliers*.

The following three fields, *significance of the slope*, *significance of deviations from linearity* and *significance of deviations from parallelity*, refer to give the probabilities for the F-value for achieving the respective hypothesis for the slope, linearity and parallelity. You can enter values from 90% to 99.99% here.

If you want to reject the calculation of assays that do not fulfill the hypothesis, mark the rejection criterion check box. In the case the option is activated, the calculation of the assay will immediately stop after failing the test. If you checked the field *Analysis according to Europ. Pharmacopoeia 1997*, all three hypotheses would be rejection criteria.

The value for the *Fiducial limit of potency estimation* determines the confidence interval for the calculation of the relative potency according to Fieller's Theorem. You choose from 90%, 95%, 99% and 99.9% here.

The last section on this page covers the calculation of the EC₅₀. The EC₅₀ is the effective concentration of a test substance at 50% response. The calculation of this value is done with the equation for a regression line ($y = mx + b$). Two variables must be known for the calculation of the EC₅₀: the slope and the 50% response. You can choose one of the following options:

Selection	Effect
Do not calculate	The EC ₅₀ will not be calculated.
Linear regression of the standard	The EC ₅₀ is calculated from the linear regression of the standard.
Common regression of the assay	The EC ₅₀ value will be calculated from the linear regression of the whole assay. For the calculation, the common slope of the standard and the preparations will be used. This slope is also used for the calculation of the relative potency.

In the input field calculate *50% response b*, you can specify from which object the 50% response will be calculated. Essentially, the 50% response is the difference between the maximum and the minimum reading divided by 2. The following objects can be chosen:

Selection	Effect
This object	The 50% response will be calculated from the respective object.
Standard	Calculates the 50% response of the standard.
Control	Calculates the 50% response from a positive and negative control.
All objects	Calculates the 50% response from all objects.

8.5.5 Linear Selection

The fifth page of the object definition dialog contains the settings for the selection of the linear and parallel range of the measurements.

End of linear range
Linear range start
Selection method

Assay: Europ. Pharmacop. 1993

General | Reagents | Definitions | Analysis | Range Selection | Annotations | GLP

Linear Range

Automatic Detection (individual range)

Manual Selection Options:

Fixed Range: 1 3

Automatic Selection Options (used by automatic detection methods)

Allocation Strategy: Best Range

Allocation region: 1 3 maximal allocation range.

Minimal # of points: 3

Inclusion of 50% response: Calculate the 50% response by using the ignore. this object.

First point of allocation range
Use maximum number of points for allocation range
Last point of allocation range

Calculate 50% response of the following object
Minimum number of points
Allocation strategy
Include 50% response

On the top of the page you can pick the selection method you would like to use from the list. You can choose from four different methods: three automatic approaches and one manual scheme. By selecting an automatic mechanism, all input fields for the manual method will be grayed out and vice versa.

Selection	Effect
Fixed range	Manual method. You must give a linear interval from which the relative potency will be calculated.
Automatic detection (individual range)	Automatic method. The linear range of preparations and standards will be determined individually for every combination.
Automatic detection (common range for standard/ individual for preparations)	Automatic method. The linear range of the standard will be determined once, while the linear interval of the preparations will be calculated individually.
Automatic detection (common range for standard/ identical for preparations)	Automatic method. The linear range of the standard will be determined once. The same interval is used for the preparations.

Please consult chapter 10.2 for further information on the selection methods.

If you choose the manual selection method, you gain access to the manual selection options where you can set the linear range.

Reference

If you choose the automatic method instead, you can modify the automatic selection options. You can set several criteria for finding a linear interval here.

First, you should select an allocation strategy from one of the available options.

Selection	Effect
Best Range	Does a best fit of the linear and parallel range where all hypotheses are fulfilled.
Maximum Range	Looks for the largest number of points for which the hypotheses are fulfilled.
Exact Range	Finds the best linear interval with exactly the number of points given.

By giving the *minimum number of points*, you can specify the minimum interval of the linear range. **Note:** The bigger the given value the faster is the automatic method. For normal analyses, you should leave the value on the default of three. If you have selected the exact range allocation strategy, this input defines the exact number of point for the linear range.

With the settings for the *allocation region* you can limit the search for a linear range to a particular interval. Set the allocation region by deactivating the field maximum allocation region and giving the interval manually.

Another option for automatic methods is the possibility to include the 50% response in the linear region. You can again choose from three options.

Selection	Effect
ignore	The 50% response is ignored for the selection of the linear range.

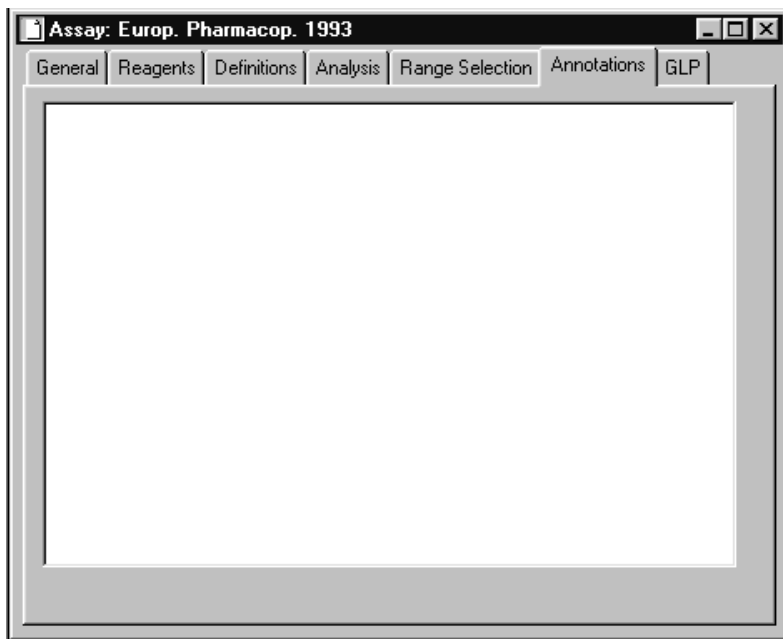
Selection	Effect
include if possible	The program tries to include the 50% response if possible. However, if the range containing the 50% response is not valid, the 50% is ignored.
inclusion is mandatory	The 50% response must be included in the linear range even if no valid linear range is found and the assay is rejected.

The same four options as for the calculation of the EC50 are available for the calculation of the 50% response, namely *this object*, *standard*, *control* and *all objects*.

Selection	Effect
This object	The 50% response will be calculated from the respective object.
Standard	Calculate the 50% response of the standard.
Control	Calculate the 50% response from a positive and negative control.
All objects	Calculate the 50% response from all objects.

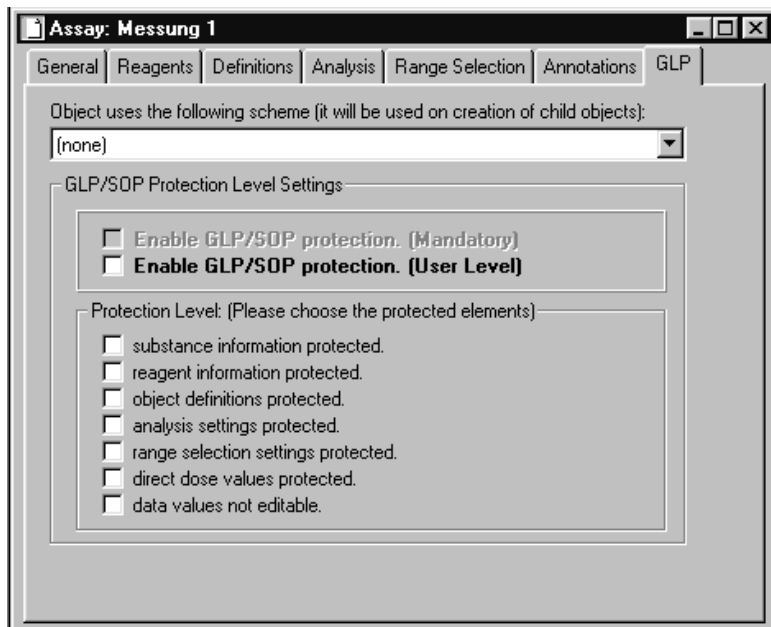
8.5.6 Annotations

The sixth page of the object settings allows you to add text to the comment or evaluate the object.



8.5.7 GLP/GMP Settings

The last page of the object settings allows you to define the protection level of the respective object. Since the GLP/GMP settings are described later, please refer to chapter 9.2 for further information.



8.6 The Data Editor

The PLA data editor is used for the data input. You can enter measurements as well as dose values here if you have selected direct dose input. While the input of the measurements is only possible at the lowest level of the object hierarchy, you can enter dose values on all hierarchy levels. If you have selected a 1:2 dilution series (*1 in 2 series*) as the dilution scale the dose values will already be filled in when you open the data editor.

Concentration	Series N° 1	Series N° 2
1:1	667,00000 ✓	672,00000 ✓
1:2	623,00000 ✓	617,00000 ✓
1:4	572,00000 ✓	562,00000 ✓
1:8	513,00000 ✓	506,00000 ✓
1:16	454,00000 ✓	447,00000 ✓
1:32	390,00000 ✓	383,00000 ✓
1:64	339,00000 ✓	345,00000 ✓

Legend:

- Dose
- # of series
- Data input field
- Status of the measurement

Toggle status indicator ☒ -status indicator. Un-check the indicator at the right side of the value for exclusion.

Graphical explanation
Change the status of the focussed measurement

You can open the data input window by pressing CTRL+D, by selection **Edit Data** from the PLA task bar or by choosing **File|Open|Edit Object Data** from the menu. The dimension of the input for the *number of dilution steps* and the *number of data series* is given in the object dialog.

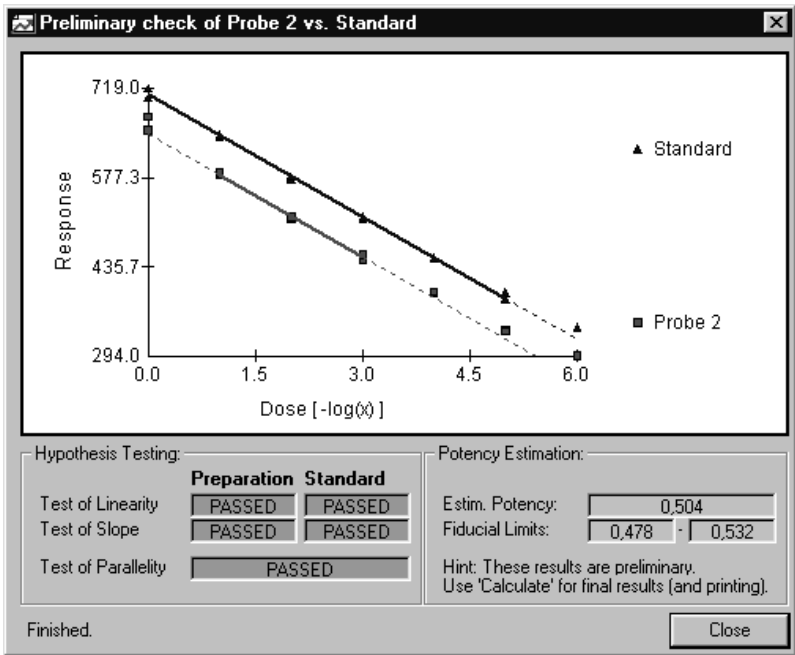
The data is entered in the input field of the respective dilution step. When you leave the input field, the check box for the measurement status will be activated. By checking or unchecking this option you control if the step is used for the analysis. E. g. if you discovered a technical outlier in your measurement data you can exclude this value from the analysis by unchecking the box right of the input field. You can change the status of the box by pressing the **Toggle status indicator** button.

Hint: You can use the TAB and RETURN key to move between the input fields.

Close the input dialog or return to the object dialog to save your data.

8.7 The Check Function

You can start the check function from the PLA task bar or from the menu. It can only be applied to standards and preparations. The purpose of the function is to give you a quick overview on a single object. The check function performs a test of the respective object against itself.



When you call the check function the calculation starts immediately. After it is finished, you should see the above overview screen of the results. The results shown are always with regard to the chosen confidence interval for the relative potency, the slope, linearity and parallelity hypothesis. The graph of the mean values is shown as a solid line.

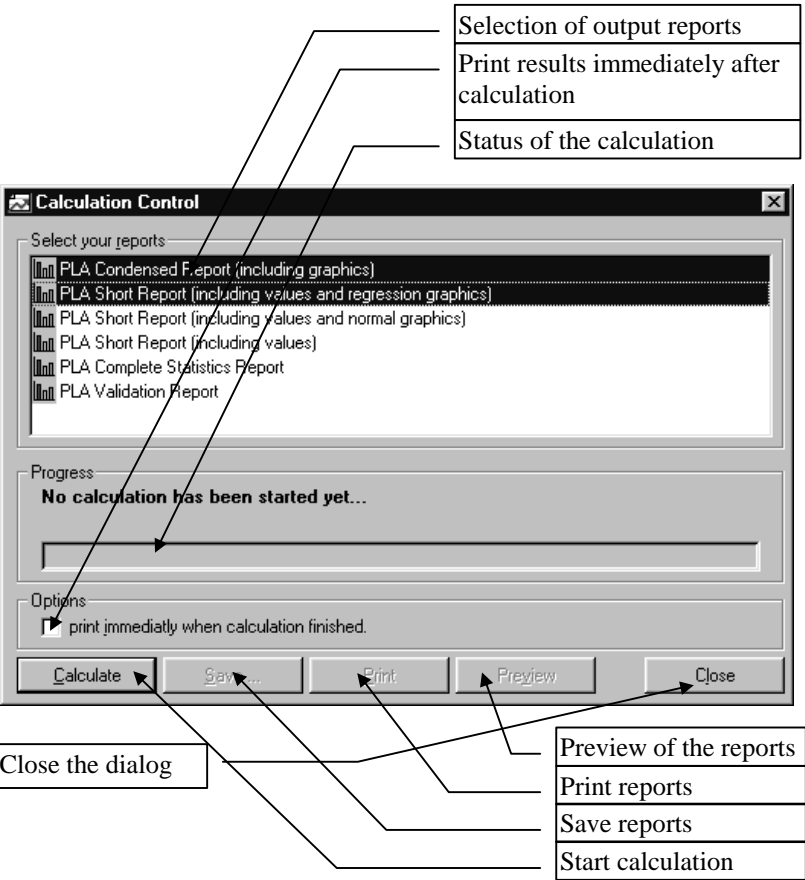
Press the **Close** button to return to the main window.

Note: If you have selected an automatic method for the detection of the linear range the results can be different from those of the calculate function! The check function always tests one preparation against one standard

while the calculate function might test several preparations against a standard. For the latter situation, you might obtain a different linear range that is optimized with regard to all preparations. You should only use the check function to test if your input data is reasonable. You should always use the calculate function for the complete analysis.

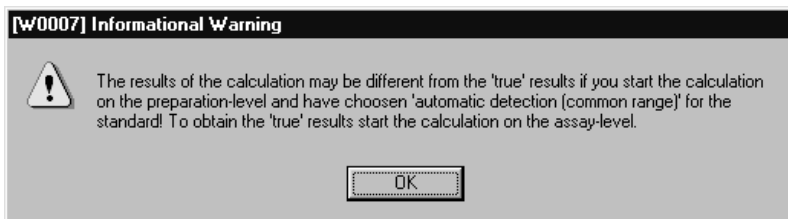
8.8 The Calculate Function

The calculate dialog's purpose is the control of the analysis. Select an assay from the navigator window and choose **Calculate** from the task bar. The following dialog will appear on the screen.



If you have selected an automatic method for the detection of linear ranges, it is significant from which object you start the calculation. In the case you have chosen the setting *Automatic detection. Common range for*

the standard and you start the calculation from a preparation object in an assay that contains several preparations, the linear range might differ from the one that the program would have found, if you had started the calculation from the assay level. Nevertheless, the results are nevertheless correct and valid. They may not be the best possible, however. In the above case, PLA will show the following warning.



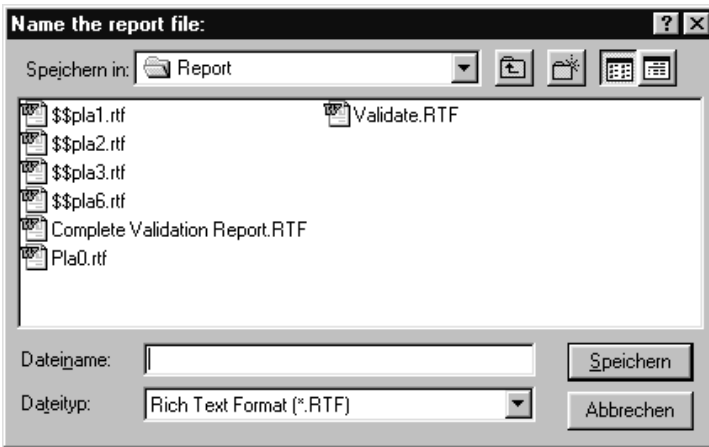
The following table correlates the type of calculation performed to the object from which the calculation is started.

Start calculation from	Calculation of
Project	all assays in the project
Assay	the whole assay
Standard	the whole assay
Preparation	the selected preparation in comparison to the standard

Calculate is a two-step function. First the calculation is performed. The time that is required depends on the selected method, the performance of your computer and the complexity of your assay. Before the calculation has finished the **Save**, **Print** and **Preview** buttons are deactivated. When the calculation is finished, these functions will become available while the **Calculate** button will be grayed out.

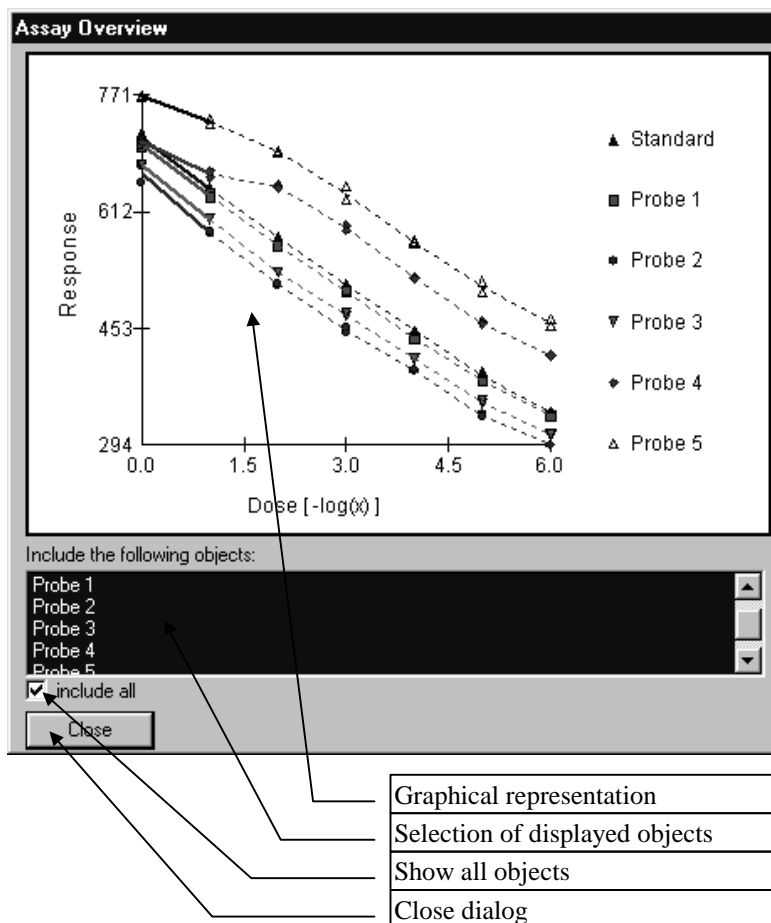
If you have activated the **print immediately when calculation finished** function, your report will be printed automatically after the completion of the analysis. Otherwise, you can check the report on-screen by pressing the **Preview** button and send the output to your standard printer with the **Print** button afterwards.

Furthermore, you can archive your reports on hard disk by pressing the **Save...** button and use the typical file dialog shown to save your results to a rich text format (RTF) file.



8.9 The Info Function

This dialog shows you a graphical representation of your assay. You can use the info function to gain an overview on the measurements and control them visually.



From the pick list *Include the following objects*, you can select one or more objects to be displayed. If you want to select more than one object,

keep the CTRL-key pressed during the selection. If you would like to see all objects that are displayed, activate the *include all* field.

Hint for the graphical representation: Linear regions of the mean value's graph are displayed as solid lines. Non-linear regions are shown as dashed lines.

8.10 The Apply Function

Eventually, you will be in a situation where you want to copy information from higher level to lower level objects. For this purpose, PLA offers you the apply function. You can call this function from the menu by choosing **File|Apply...** or by pressing CTRL+A.

You should be very careful with the apply function because the settings will be copied without any check of the information's validity. E. g. if you copied the assay definitions, the apply function would copy, among other things, the number of dilution steps and the number of data series. A very common way of using this function is the actualization of changed analysis and linear selection method settings.

The apply function needs two inputs:

1. Which information is to be copied?
2. Where should the information be copied?

Enter the selection of the information, which is to be copied in the input field *Apply what?* You can select six different groups of information that can be copied:

- Substance information
(refer to chapter 8.5.1.1 "*The Substance Database*")
- Reagent information
(refer to chapter 8.5.2 "*Reagents*")
- Definitions information
(refer to chapter 8.5.3 "*Definition Settings*").
Please be very careful with this option!
- Analysis information
(refer to chapter 8.5.4 "*Analysis properties*")
- Linear selection information
(refer to chapter 8.5.5 "*Linear Selection*").
Most common application of this function.
- GMP/GLP definitions
(refer to chapter 1 "to chapter 1")

- *GMP/GLP Settings in PLA")*

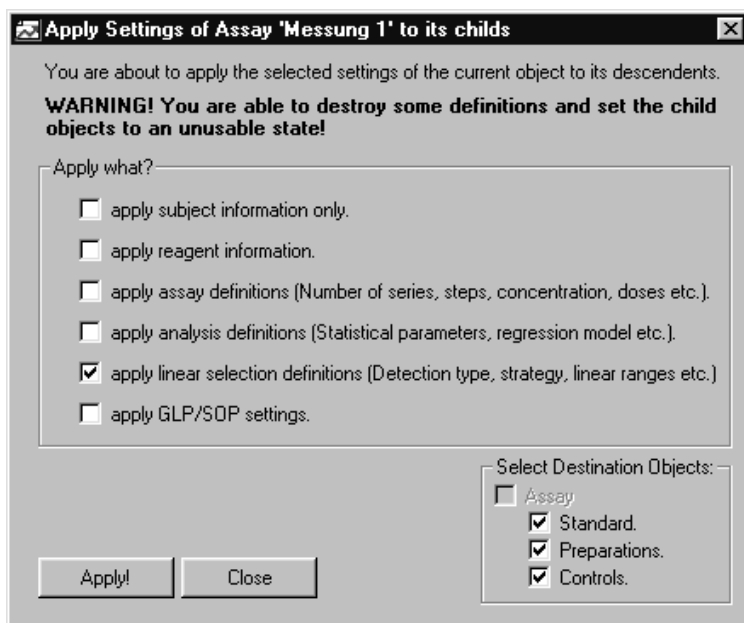
The screenshot shows a dialog box titled "Apply Settings of Project 'Beispiel Nr. 1' to its childs". The dialog contains a warning message, a list of settings to apply, and a section for selecting destination objects. Callout boxes provide detailed descriptions for various elements:

- Copy the substance information**: Points to the "apply subject information only." checkbox.
- Copy the reagent information**: Points to the "apply reagent information." checkbox.
- Copy the assay definitions**: Points to the "apply assay definitions (Number of series, steps, concentration, doses etc.)." checkbox.
- Copy the analysis definitions**: Points to the "apply analysis definitions (Statistical parameters, regression model etc.)." checkbox.
- Copy the linear selection definitions**: Points to the "apply linear selection definitions (Detection type, strategy, linear ranges etc.)." checkbox.
- Copy the GLP/GMP definitions**: Points to the "apply GLP/SOP settings." checkbox.
- Copy to assays**: Points to the "Assay" checkbox in the "Select Destination Objects:" section.
- Copy to standards**: Points to the "Standard." checkbox.
- Copy to preparations**: Points to the "Preparations." checkbox.
- Copy to controls**: Points to the "Controls." checkbox.
- Close dialog**: Points to the "Close" button.
- Apply settings**: Points to the "Apply" button.

After you have chosen what information is to be copied, you have to define the destination of the copy command. Enter your destination in the input field *Select Destination Objects*. In principle, the Apply function does only copy the information to lower level objects.

If you called the apply function from the project level the default setting is to copy the information only to assay level and not beyond. Since PLA uses only information from the lowest object level, this option prevents the assay from being damaged. If you want to pass down the information through the hierarchy tree, activate the Standard, Preparation or Controls check box.

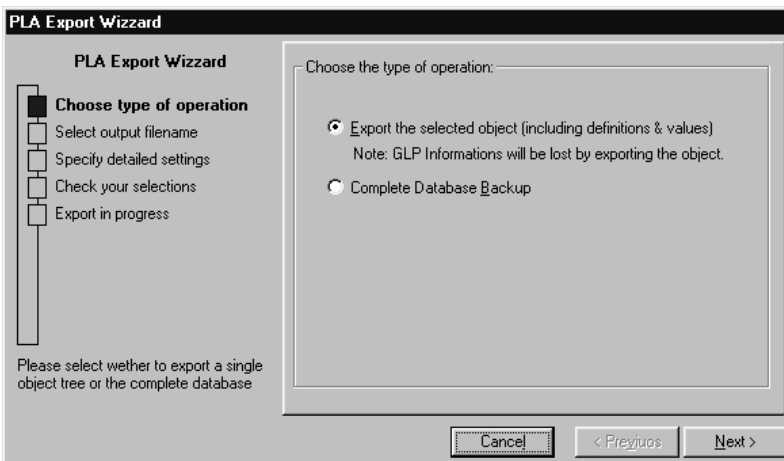
For the case you invoked the function on the assay level, you can steer the copying process by selecting the target objects in the box *Select Destination Objects*.



As a default, all three object types are selected. To start the copying process presses the **Apply** button. To cancel the process presses the **Close** button.

8.11 Data Export

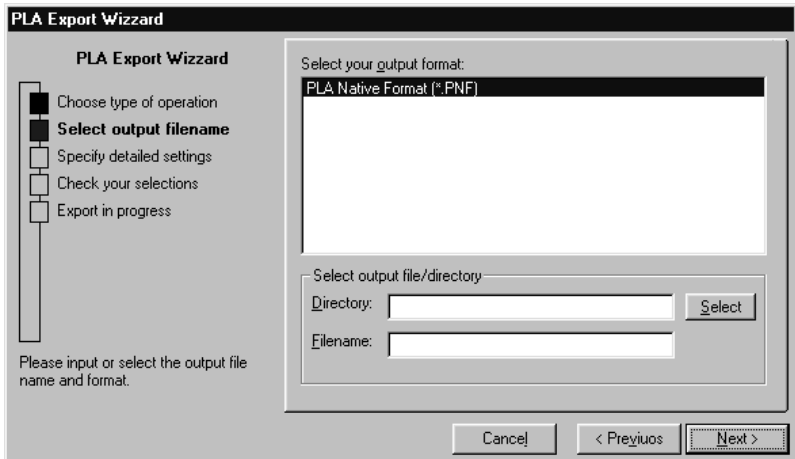
In this version, PLA offers you two new, very helpful functions: the import and export of data. With these functions you can easily exchange data between PLA and other applications. The export function is useful for backup purposes because it allows you to export the complete PLA database as well as single objects. Select **Export** from the task bar to activate the export wizard.



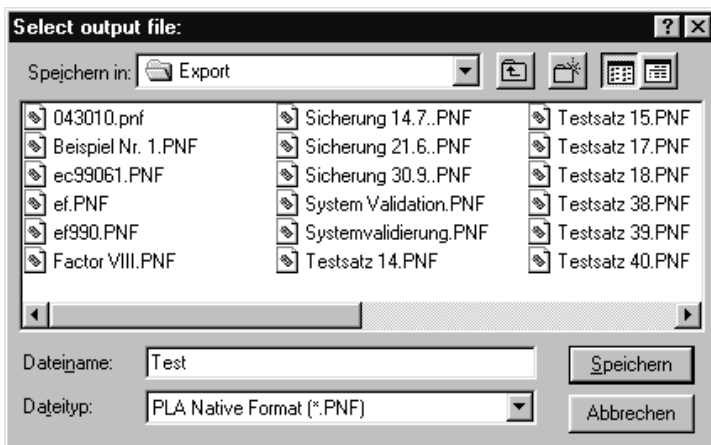
The PLA export wizard will guide you through the data export step-by-step. The blue square on the left always shows you the actual step of the process.

The first thing that you have to decide is if you want to export the whole database (*Complete Database Backup*) or a single object from the database (*Export the Selected Object*). To export a single object you have to select that object in the navigator before you call the export function.

After you have decided which type of export you want to do, press the **Next** button to get to the next page of the wizard. In the second step, you select the output format, the file name and the directory to which the data will be saved.



Select the output format from the list. Normally, the only option available is the PNF format. Press **Select** to choose file name and directory.



In the file dialog, switch to the directory in which you want to save the data. Enter a file name in the input field and press the **Save** button to take over the changes to the export wizard. Back in the export wizard, press the

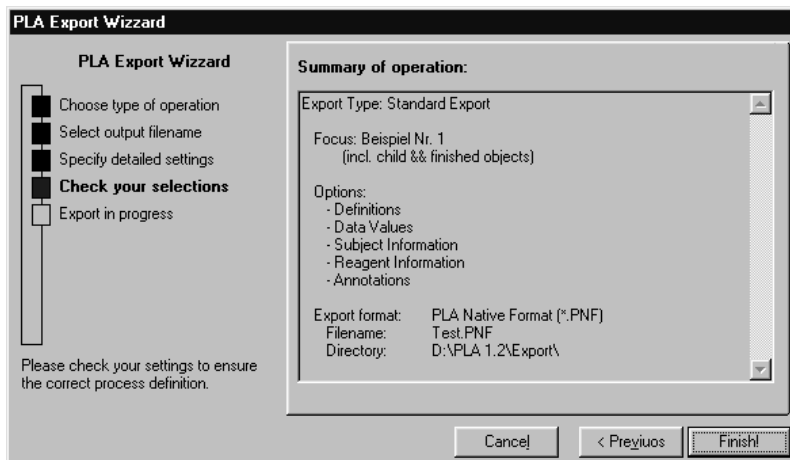
Next button to get to step three of the export process (*Specify detailed settings*).

The screenshot shows the 'PLA Export Wizard' dialog box. On the left, a vertical pane lists five steps: 'Choose type of operation', 'Select output filename', 'Specify detailed settings' (which is highlighted with a black square), 'Check your selections', and 'Export in progress'. Below this list, a text box says 'Please choose the elements that will be exported.' The main area of the dialog is titled 'Specify detailed settings' and contains two sections. The first section, 'Object(s) to export', has a dropdown menu showing 'Beispiel Nr. 1' and two checked checkboxes: 'include child objects' and 'include finished objects'. The second section, 'Output options', has five checkboxes: 'include data values' (checked), 'include assay definition data' (checked), 'include subject information' (checked), 'include reagent information' (checked), 'include annotations' (checked), and 'include administrative information' (unchecked). At the bottom of the dialog are three buttons: 'Cancel', '< Previous', and 'Next >'. The 'Next >' button is highlighted with a dotted border.

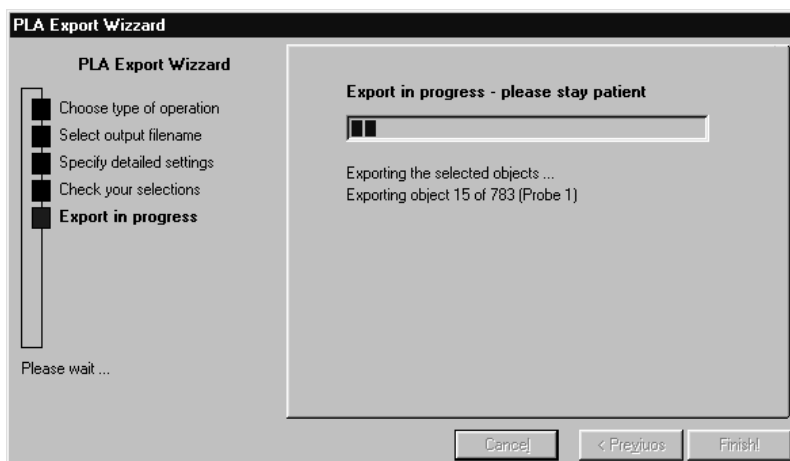
In the upper part of the dialog, you can again choose the object that is to be exported. On this page, you have two choices. Either you keep the object that you have marked in the navigator or you can choose *all objects*, which essentially does the same as a complete database backup but does not export any user information. In the check boxes below the drop-down field you can choose if you want to export the lower hierarchy levels as well and if you want to include finished objects.

On the lower part of the page, you can define which data of the object shall be exported. The meaning of the check boxes is the same as with those of the apply function. E. g. if you only want to export the measurements of the object, uncheck all other fields.

Again, press the **Next** button to get to the next page of the export wizard. There you will see a summary of all the settings you have selected so you can check them before you export your data.



If all the settings are correct, start the data export by pressing the **Finish** button.



The blue progress bar shows you how far the export process has succeeded. After the export has been successfully finished, press the **Close** button to leave the export wizard.

8.12 Data Import

The import interface is a new function in PLA 1.2 with which you can migrate external data from various sources into the program.

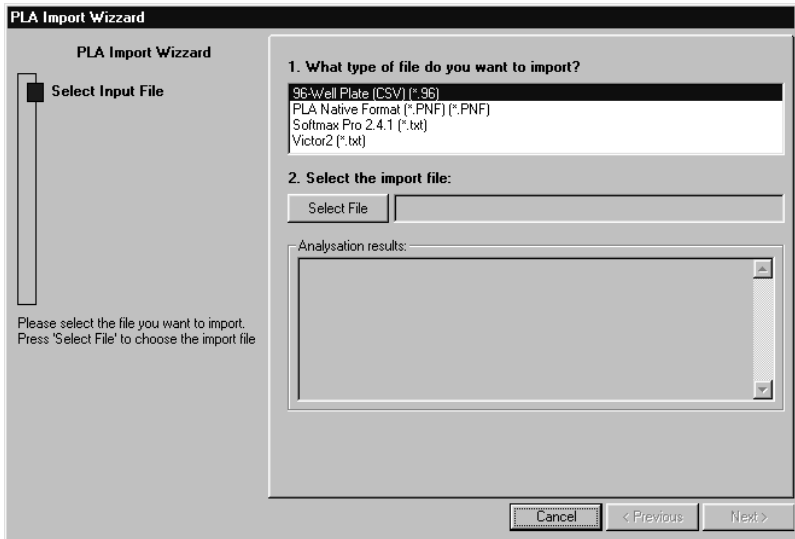
On the one hand, PLA can import PNF files that have been exported from PLA. Users can exchange data from different PLA installations in this way.

On the other hand, PLA can read virtually data from any measurement system that is able to write files. You will need import modules, which you can purchase from the Stegmann Systemberatung, for the import of third-party data formats. The standard installation contains an import module for 96 well plates in CSV (comma separated values) format.

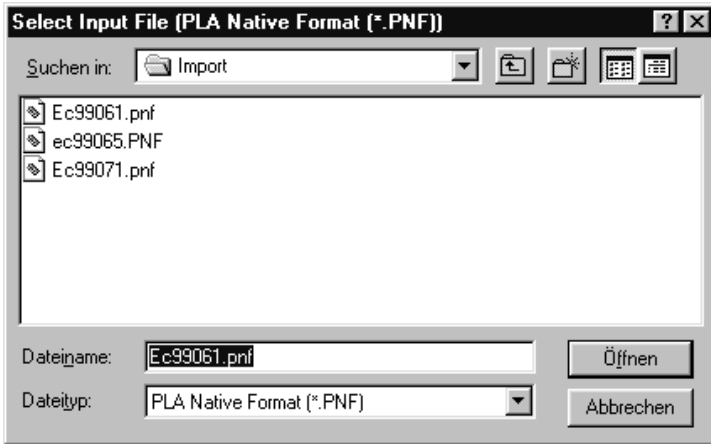
Depending on the type of the object, import as PNF file or by an external import module, that is imported into PLA the steps in the PLA Import Wizard are different. We will introduce you to the procedures in the next chapters.

8.12.1 Importing PNF Files

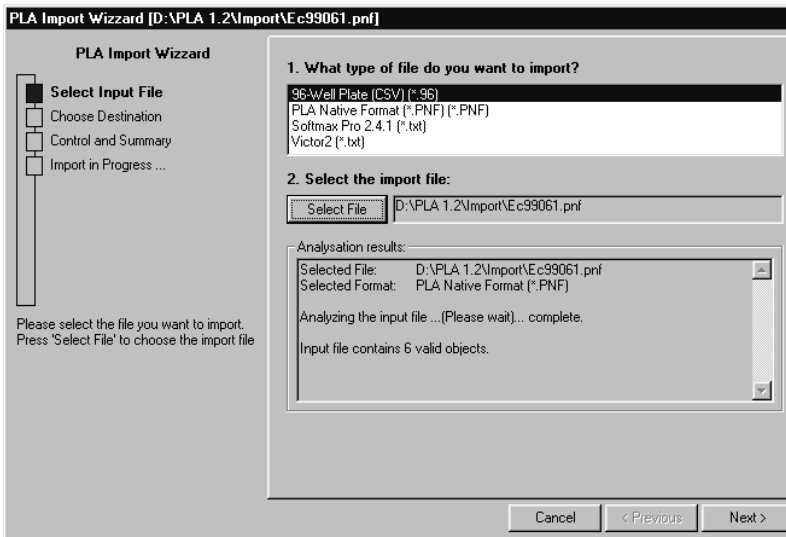
In this section the import of an existing PNF file is described. First select *PLA Native Format* in the upper part of the first page of the dialog.



Second, choose the directory and the name of the file you want to import into PLA. By pressing the **Select File** button a standard file dialog is opened where you can choose the file to import.

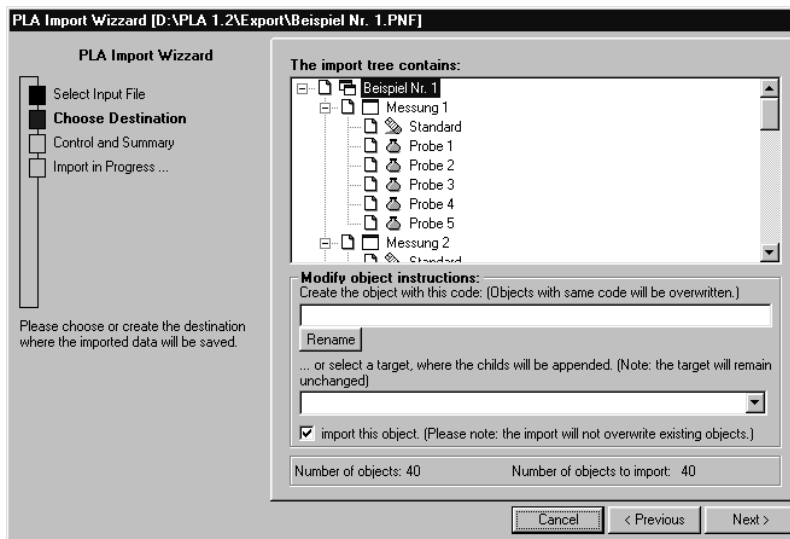


Select the input file that contains the data you would like to import into PLA and press the **Open** button to get back to the PLA Import wizard. The program will check the file structure and data in the file. A summary of the analysis results will be displayed in the import wizard.



Press the **Next** button to change to the next dialog page of the import wizard. Choose the location to which the object is imported on this page. Of course the location is dependent on the type of object you want to import.

If you are trying to import a project, the program checks if a project of the same name already exists in the PLA navigator. If this is the case, all subordinate objects (assays, preparations, standards and controls) are inspected in turn. If all subordinate objects are identical, PLA terminates the import process with an error message. Otherwise, you will get to the next page of the import wizard.



In the upper part of the window, you will see the structure of the import object displayed. You should be familiar with this representation from the navigator window. Besides, the usual icons for the object types (assays, standards, preparations and controls) several other symbols might be shown. These symbols have the following meaning:

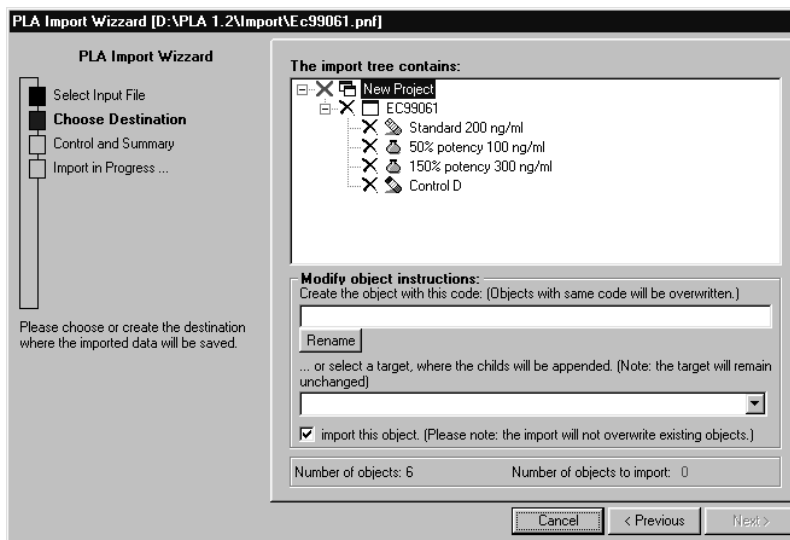
Symbol	Meaning
Cross	The object already exists in the database. You will have to rename the object if you want to continue the import process.
Bend arrow	The object already exists but you can import the lower level objects. This symbol is used for assays and projects only.

Symbol	Meaning
Rectangle	The object does not exist in the database. It can be imported without limitations.

If all objects are marked with a rectangle, you can continue the import process without limitations. You can change the name of the project if you want to. Select the project you want to rename and enter a new name in the field *Create the object with this code*. If you confirm the change by pressing the **Rename** button, the name of the project will be changed in the object tree.

Furthermore, you can exclude objects from the import by selecting the object and unchecking the box *Import this object*. If you exclude an assay or project from the import all the lower level objects will also be excluded.

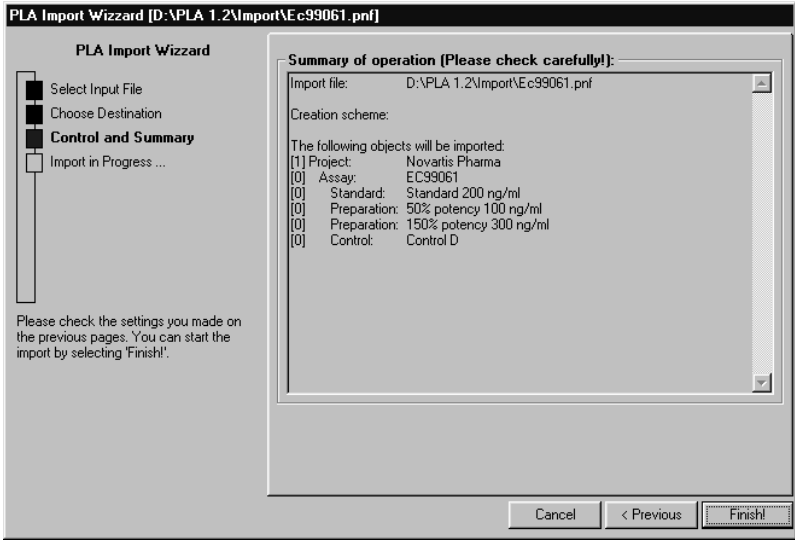
If your object, is an assay you will first see the following dialog.



The object is displayed in the object tree but it is marked with a red cross. Before you can import the assay, you must select the project to which the assay will belong. You have two options to continue the import process: either you create a new object or you append the assay to an existing project.

Select a project from the drop-down list *Select a target where the childs will be appended* to join the assay to an existing project. All projects in the database are displayed in this list. The program will check if the same assay already exists in the project. If this is not the case, you can continue with the import process.

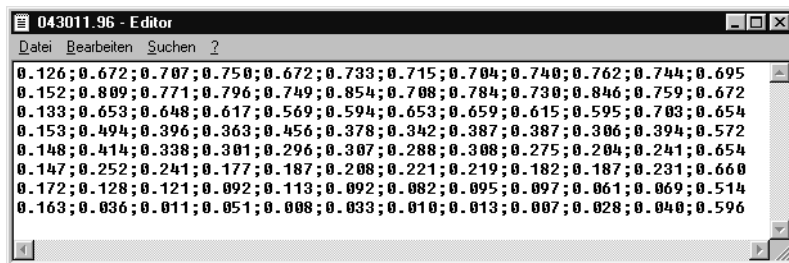
When you have finished the entries on this page, press the **Next** button to see a summary of the settings you have chosen. If you want to change settings, use the **Previous** button to get back to the import wizard.



If all the settings are correct, press the **Finish** button to finally impot the data into the PLA database.

8.12.2 Importing External Data

The import module of PLA enables you to migrate your measurements with other systems to the PLA database. Because the data format is different for every system, you must install an import module for your format. In the standard installation, PLA comes with an import module for measurements of 96-well plates in CSV (comma separated values) format. The following example shows you a file with the correct data format.



The measurements are arranged in a 8x12 matrix. The values are separated by semicolons. The example shows the measurements of a micro titer plate. The samples are arranged according to the following scheme:

NC	S1	P1	S1	P1	S1	P1	S1	P1	S1	P1	PC
NC	S2	P2	S2	P2	S2	P2	S2	P2	S2	P2	PC
NC	S3	P3	S3	P3	S3	P3	S3	P3	S3	P3	PC
NC	S4	P4	S4	P4	S4	P4	S4	P4	S4	P4	PC
NC	S5	P5	S5	P5	S5	P5	S5	P5	S5	P5	PC
NC	S6	P6	S6	P6	S6	P6	S6	P6	S6	P6	PC
NC	S7	P7	S7	P7	S7	P7	S7	P7	S7	P7	PC
NC	S8	P8	S8	P8	S8	P8	S8	P8	S8	P8	PC

Where

NC negative control

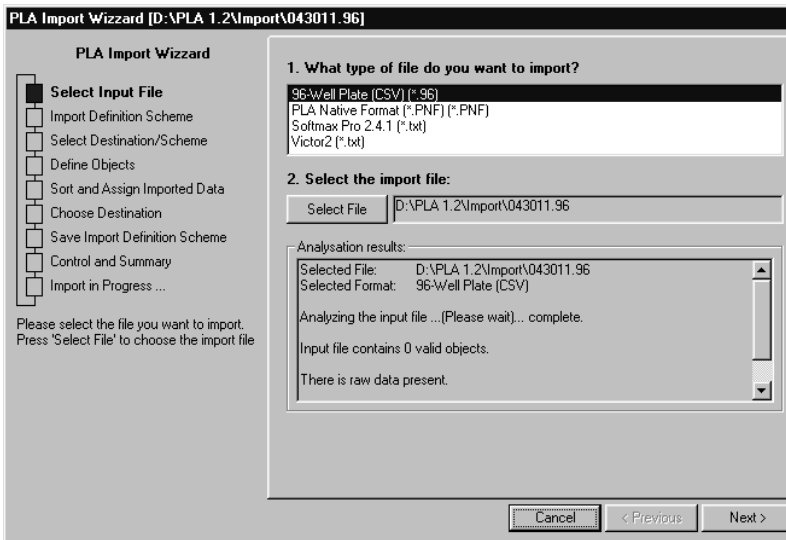
PC positive control

S1-S8 standard dilution step 1 – dilution step 8

P1-P8 preparation dilution step 1 – dilution step 8

A preparation and a standard with eight dilution steps were taken five times. Additionally, a negative and a positive control were taken with each dilution step. With the shown example, we will now demonstrate how the import wizard works with an external import module.

The import wizard starts with the same dialog as for the import of a PNF file. You first have to select which data format you want to import.

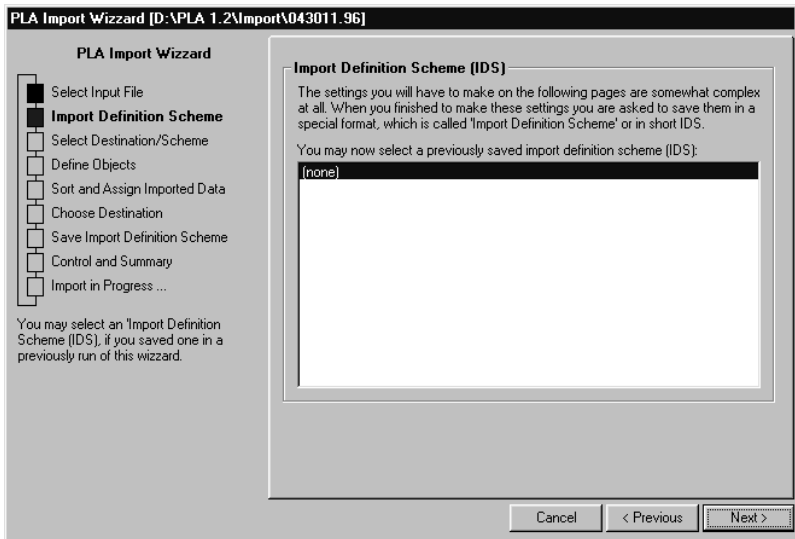


Select the data type *96-Well Plate* and choose the file and the directory by pressing the **Select File** button. Please note that the files must have the extension *.96* for PLA to recognize the data format.

In the field *Analysis Results* a summary of the selected object is shown. Most important, the program will report if the data format was recognized successfully. Click the **Next** button to proceed to the following dialog page.

Here you can choose a so-called import definition scheme. This scheme is a template in which all the information on the arrangement of the data can be saved. If you import a file for the first time this field will be empty.

Import definition schemes will be generated at the end of the import procedure after all other settings of the import have been selected.



Because no import definition schemes exist you can directly go on to the next dialog page. Here, you can give the information into which PLA object the data is imported. The object will be imported to a new project by default. You can change this setting by giving an existing project in the input field *Destination Project*.

The second input field allows you to attach object definitions to the imported data from an object definition scheme. This feature is important if you want to add GLP/GMP information to the project. (For further information on the GLP/GMP settings refer to chapter 9)

PLA Import Wizzard [D:\PLA 1.2\Import\043011.96]

PLA Import Wizzard

- Select Input File
- Import Definition Scheme
- Select Destination/Scheme**
- Define Objects
- Sort and Assign Imported Data
- Choose Destination
- Save Import Definition Scheme
- Control and Summary
- Import in Progress ...

Objects in PLA can be created by using schemes. You use these schemes in every creation process. Please select the one you want to use here. Please also select the destination project of your imported data.

Select the Destination Project:

Destination Project:
[New Project]

Default Scheme for Creation of Objects:

[none]

Cancel < Previous Next >

Switch to the next dialog page on which you have to supply general information on the number and type of the objects that you want to import.

PLA Import Wizzard [D:\PLA 1.2\Import\043011.96]

PLA Import Wizzard

- Select Input File
- Import Definition Scheme
- Select Destination/Scheme
- Define Objects**
- Sort and Assign Imported Data
- Choose Destination
- Save Import Definition Scheme
- Control and Summary
- Import in Progress ...

Please define the type, count, and general settings (number of steps and series) of the objects you want to create

Raw data objects - general settings

Number of objects: 0 ☐ number of steps identical for all objects
☐ number of series identical for all objects

Apply general settings

List of raw data objects:

ID	Code	# steps	# series
----	------	---------	----------

Selected object:

Type of object: # steps: # series:
Standard 0 0

Apply

Hint: You have to create the objects that will contain the raw data!

Cancel < Previous Next >

Let us discuss this dialog page with the data from our example. In the first section of the page you have to give the number of object that are contained in the import data. In our example we have three objects: a prepara-

tion, a standard and a control. Because all controls (positive and negative controls) are treated by PLA as one object each assay can only have one control. Enter 3 in the input field *Number of Objects*. With the check boxes left of the spin control you can define that the number of dilution steps and the number of repetitions are the same for all objects. This true for the preparation and the standard but not for the controls¹ in our example. Nevertheless, let us check the two boxes. Two activate the settings press the button **Apply General Settings**.

PLA Import Wizard [D:\PLA 1.2\Import\043011.96]

PLA Import Wizard

- ☒ Select Input File
- ☒ Import Definition Scheme
- ☒ Select Destination/Scheme
- ☒ **Define Objects**
- ☐ Sort and Assign Imported Data
- ☐ Choose Destination
- ☐ Save Import Definition Scheme
- ☐ Control and Summary
- ☐ Import in Progress ...

Please define the type, count, and general settings (number of steps and series) of the objects you want to create

Raw data objects - general settings

Number of objects: 3 ☒ number of steps identical for all objects
☒ number of series identical for all objects

Apply general settings

List of raw data objects:

ID	Code	# steps	# series
A	Standard A	2	1
B	Preparation B	2	1
C	Preparation C	2	1

Selected object:

Type of object: # steps: # series:

As you can see, all three objects are created automatically (standard A, preparation B and C). The number of steps and series is identical for all objects. Since the numbers are not correct, you have to edit the entries. You can edit the settings of the related object in the lower fields of the dialog page. Change name as you like, e. g. Standard, and change the number of steps and the number of series according to our example (eight steps, five series).

¹ Controls are special compared to preparations and standards because three objects (positive, negative control and blank) are combined within one object. The number of repetitions is thus given as the number of series. The number of steps is always three. The first step is always a positive control, the second a negative control and the last step is a blank.

Selected object:
Standard

Type of object: # steps: # series:
Standard 8 5

Apply

Press the **Apply** button to confirm your changes. The changed settings will be transferred to the upper list. When you edit the preparation B the number of steps and the number of series are already set to the correct values.

Finally, edit the settings for the last object, preparation C. The last object is not a sample like the standard and the preparation but a control. Change the object type accordingly in the *Type of Object* input field.

Selected object:
Control

Type of object: # steps: # series:
Control 8 8

Apply

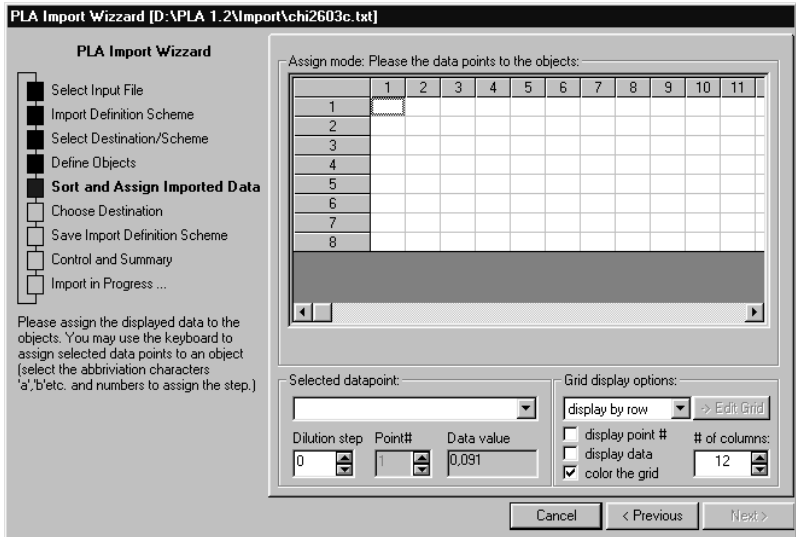
When you choose a control object the field for the number of steps will be grayed out. You can only give the number of series, which is the number of repetitions for controls. Enter eight into the input field.¹ Before you confirm the changes you must uncheck the two boxes in the first section of the dialog page because otherwise the number of series will be set to eight for all objects. Press the **Apply** button to confirm the changes. You should see the following list of objects now:

ID	Code	# steps	# series
A	Standard	8	5
B	Probe	8	5
C	Control	8	8

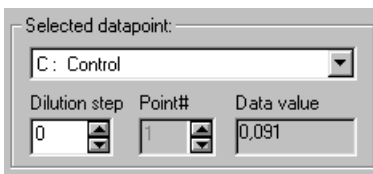
You can proceed to the next dialog page of the import wizard now. On this page, the data values of the import file, which correspond to the pods on the micro titer plate, are assigned to the data objects. The upper part of the dialog page is arranged like a 96-well plate to give you a better over-

¹ If the number of repetitions is not the same for all types of controls you must give the maximum number of repetitions.

view on the data. With the help of the matrix you can assign every pod of the plate to the corresponding dilution step. You can work with the matrix, as you would use a Microsoft Excel data sheet. To select a complete row or column, click on the corresponding number.



If you click on an arbitrary field on the matrix, you will see its properties displayed in the lower sections of the dialog page. The left dialog field is actually relevant for the designation of the data objects. Additionally, you can see the information on the measurement of the corresponding pod.



Mark column 1 and select *Control* in the input field **Selected Datapoint** to assign the measurements. Alternatively, you can press the key C because this letter was given to the control object in the previous dialog. You can assign the columns 2, 4, 6, 8 and 10 to the standard (key A) and the columns 3, 5, 7, 9 and 11 to the preparation (key B) in the same manner. The columns will be displayed in different colors according to their assigned object.

After these steps your matrix should now look like this:

PLA Import Wizzard [D:\PLA 1.2\Import\chihi2603c.txt]

PLA Import Wizzard

- Select Input File
- Import Definition Scheme
- Select Destination/Scheme
- Define Objects
- Sort and Assign Imported Data**
- Choose Destination
- Save Import Definition Scheme
- Control and Summary
- Import in Progress ...

Please assign the displayed data to the objects. You may use the keyboard to assign selected data points to an object (select the abbreviation characters 'a', 'b' etc. and numbers to assign the step.)

Assign mode: Please the data points to the objects:

	1	2	3	4	5	6	7	8	9	10	11	12
1	C	A	B	A	B	A	B	A	B	A	B	C
2	C	A	B	A	B	A	B	A	B	A	B	C
3	C	A	B	A	B	A	B	A	B	A	B	C
4	C	A	B	A	B	A	B	A	B	A	B	C
5	C	A	B	A	B	A	B	A	B	A	B	C
6	C	A	B	A	B	A	B	A	B	A	B	C
7	C	A	B	A	B	A	B	A	B	A	B	C
8	C	A	B	A	B	A	B	A	B	A	B	C

At least one point was assigned to a step or object only (and not to both)

Selected datapoint: C: Control

Grid display options:

display by row ☐ display point # ☐ display data ☒ color the grid

Dilution step: 0 Point#: 1 Data value: 0,091

of columns: 12

Cancel < Previous Next >

The data input is not finished yet as you can see from the red error message that is displayed and the grey **Next** button. Since the measurements are defined by their object type and the dilution step you have to assign a dilution step to each data point in the second step of the input. Mark all the fields that belong to the same dilution step, e. g. the first row in our example. Either press the key 1 or enter 1 in the *Dilution Step* input field to assign the dilution step with the highest concentration to the data points. Assign the dilution steps 2 to 8 in the same way.

Your data matrix should look like this afterwards:

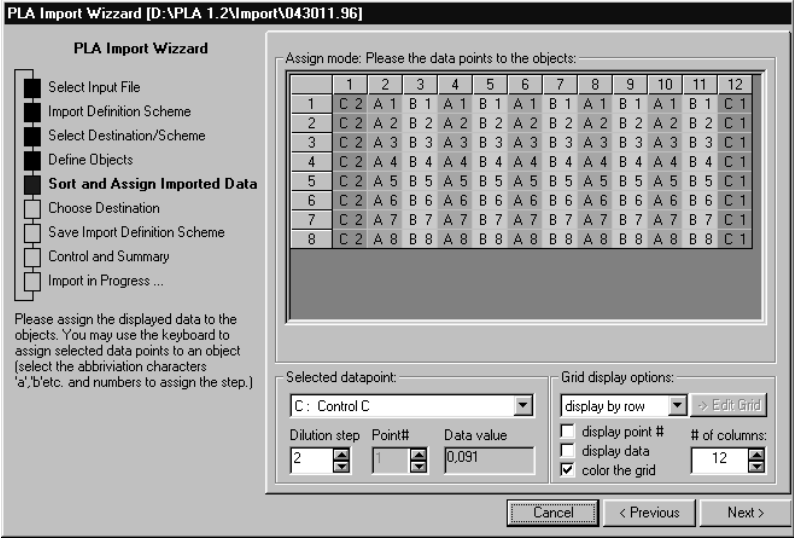
Assign mode: Please the data points to the objects:

	1	2	3	4	5	6	7	8	9	10	11	12
1	C	A 1	B 1	A 1	B 1	A 1	B 1	A 1	B 1	A 1	B 1	C
2	C	A 2	B 2	A 2	B 2	A 2	B 2	A 2	B 2	A 2	B 2	C
3	C	A 3	B 3	A 3	B 3	A 3	B 3	A 3	B 3	A 3	B 3	C
4	C	A 4	B 4	A 4	B 4	A 4	B 4	A 4	B 4	A 4	B 4	C
5	C	A 5	B 5	A 5	B 5	A 5	B 5	A 5	B 5	A 5	B 5	C
6	C	A 6	B 6	A 6	B 6	A 6	B 6	A 6	B 6	A 6	B 6	C
7	C	A 7	B 7	A 7	B 7	A 7	B 7	A 7	B 7	A 7	B 7	C
8	C	A 8	B 8	A 8	B 8	A 8	B 8	A 8	B 8	A 8	B 8	C

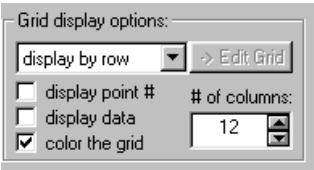
At least one point was assigned to a step or object only (and not to both)

As a last step, you have to assign the controls in the first and last column. The logic of control objects is somewhat different from standards and preparations.

Column 1 contains a negative control, column 12 a positive control. According to the PLA logic, positive controls are assigned to dilution step one, negative controls are dilution step 2. Blanks would have been assigned to dilution step 3 if they had been taken in the assay. In our example, you have mark column 1 and assign the value 2 for the dilution step. Process column 12 in the same way but assign the column to dilution step 1. The data assignment is now finished and you can go to the next page. (The **Next** button will be available now)



The dialog box *Grid Display Option* has been explained yet. Let us leave the example for a moment to discuss the available options. With the options in this dialog field, you change the display of the data matrix in the upper part of the dialog page. The best way to learn the effect of these settings is to play with them and observe the changes in the data display.

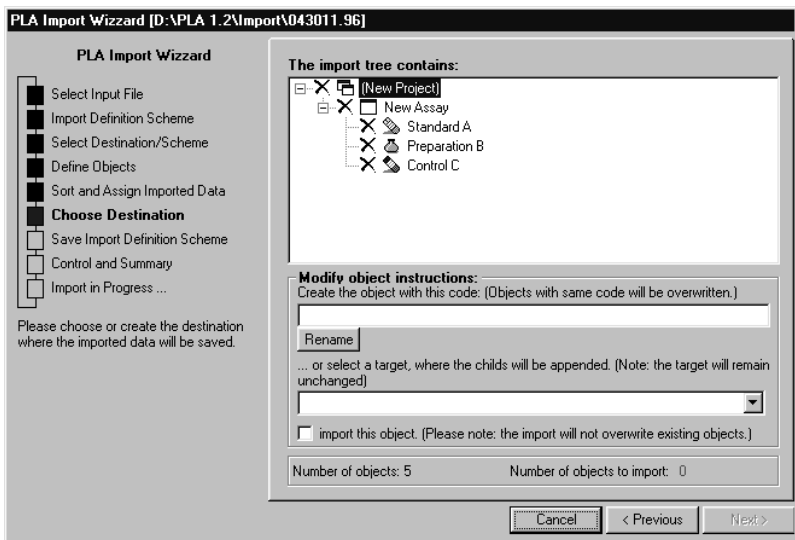


The following table gives you a short description of the available options

Function	Effect
display by ...	Select one of the three possible data display arrangements.
display point #	Show the counter of every data field.
display data	Show the measurement of every data field.

Function	Effect
color the grid	The coloring of the table can be switched on or off with this option.
#of columns	Limit the number of columns that are displayed to the given value.

Probably, you will rarely use these functions. You will possibly recognize the next dialog page from the previous chapter. You have to give the destination and the name of the object that will be imported.

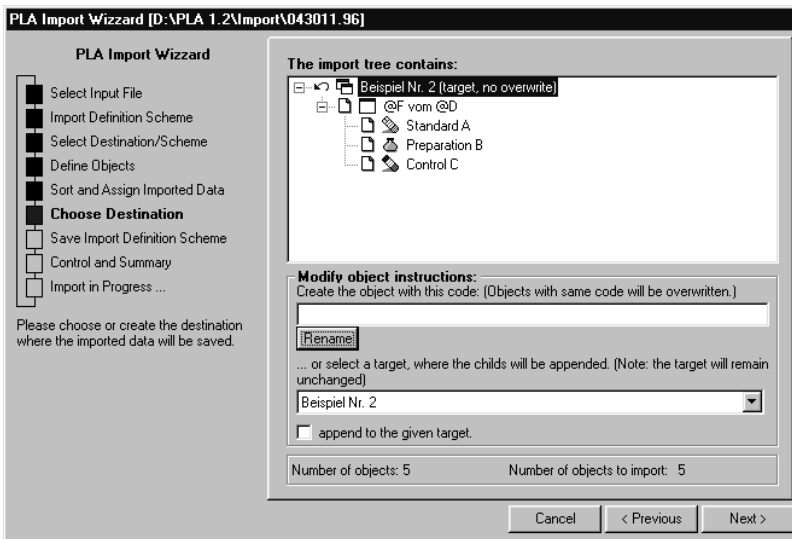


Because we have selected *New Project* in step 3 of the import process, the first thing we must do is to create a new project or to assign an existing project to the assay. (See section 8.12.1 for details)

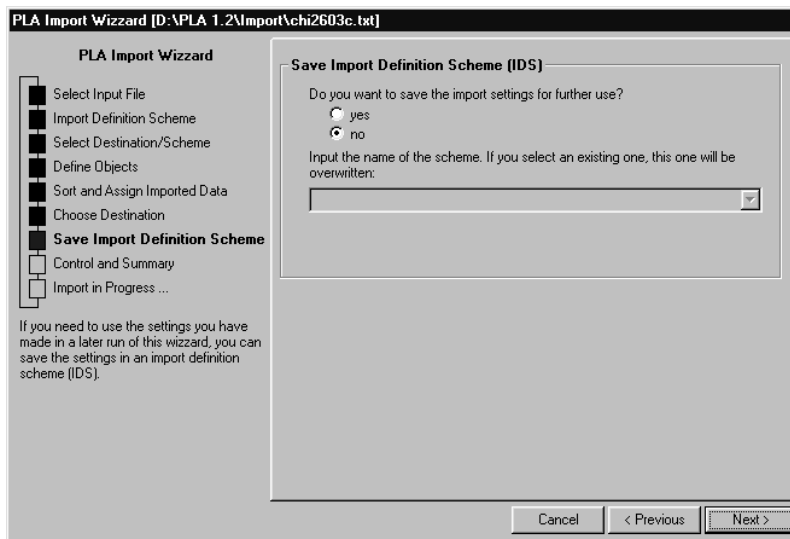
For the automated import of assays, macros are available. Instead of a fixed name, you can give a macro value as assay name. The following macros are available.

Macro	Return Value
@U	Will give the user name.
@D	Will give the actual date.
@T	Will give the actual time.
@F	Will give the name and directory of the imported file.

You can combine macros. In this manner, you easily generate unique assay names. E. g. if you enter an assay name “@F from @D”, the full file name with a date extension will be the assigned name of the assay.



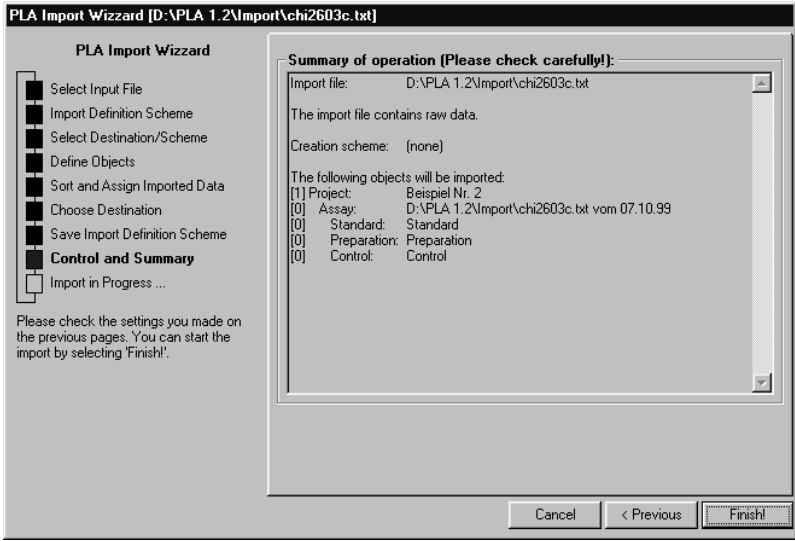
Macros only make sense if you want to use import definition schemes. These templates are used for the automated import of external data. Change to the next page where you can save your settings in an import definition scheme.



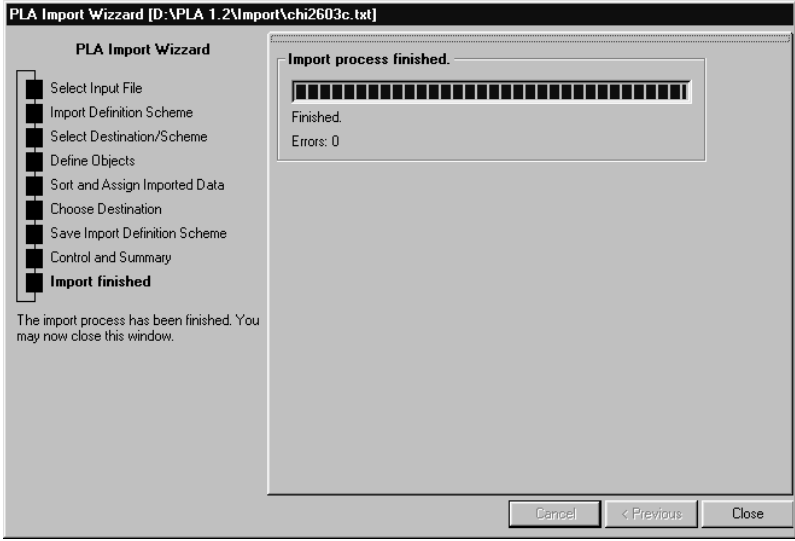
The purpose of import definition schemes is to facilitate the import process. The settings you have to manipulate during the import process are quite complex. In most laboratories the assays are measured in a standard arrangement, e. g. of the 96-well plates. However, the import settings are normally the same for all assays. With the import definition schemes you can save all your import settings so you can use them for the import. In this way, import definition schemes will save you a lot of time because you load all your settings from a file. Furthermore, this also will avoid errors because you only have to check you import settings once.

Choose **Yes** on the dialog page and enter a name for you scheme in the input field below. The next time you call the import function the scheme will be available.

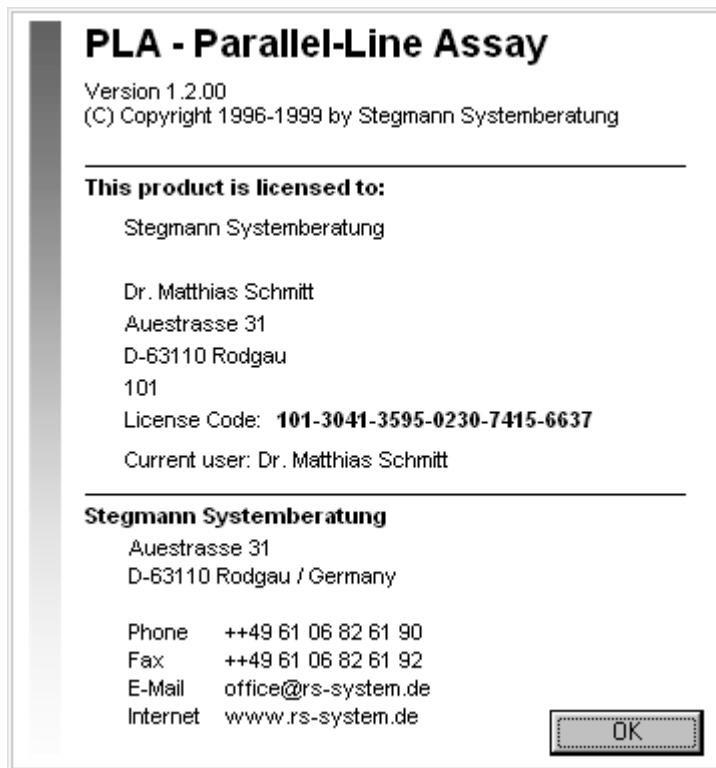
On the next dialog page, you will see a summary of your input data. Check if your settings are correct. If you find an error on this page, you can always go back using the **Previous** button and do changes on the according dialog page.



If you are content with your settings start the import procedure by pressing the **Finish** button. When the import process has finished successfully press the **Close** button to leave the import wizard.



8.13 About



9 GMP/GLP Settings in PLA

During the development of this version of PLA, special emphasis was laid on the implementation of GMP/GLP compliant standards, as regulatory authorities demand them. For this reason, an account management has been closely revised. All PLA users now belong to a user group (c f. chapter 5.1 “Account Management”). In the following chapter we will introduce you to additional functionalities of PLA that will allow you to work under GLP/GMP conditions.

9.1 Schemes

Another tool for the implementation of GMP/GLP requirements is the usage of schemes. Originally, schemes in PLA were developed to facilitate working procedures that are repeated regularly. A new aspect within the GMP/GLP framework is their suitability for the definition of standard operating procedures.

Schemes are used as templates for the creation of new objects (projects, assays, standards, preparations and controls). In comparison to data objects, templates are defined by a set of object settings but they do not contain any measurement data. When you start the 'create object' dialog, you have the opportunity to select an object definition scheme (see topic 3 in Figure 9-1). By the use of a scheme you bypass the normal way of inheritance. The created object is not based on the definitions of the parent object. It is based on the settings of the selected scheme.

Create object

1. Select object type to create:

- ☒ New Project
- ☐ New Assay as member of project 'Test'

2. Input Code and Title:

3. Select a creation scheme:

(none)

Hint: if there is an active mandatory (global or parent) scheme, this selection will be ignored.

Create Cancel

Figure 9-1 The create object dialog

For the usage of PLA, you should always define a scheme for every assay. In this scheme all information on the number of dilution steps, the number of data series, the statistical parameters as well as the analysis method is saved.

Create a new scheme by clicking on the drop down box in the upper part of the navigator. Select the schemes' view from the list (see Figure 9-2).

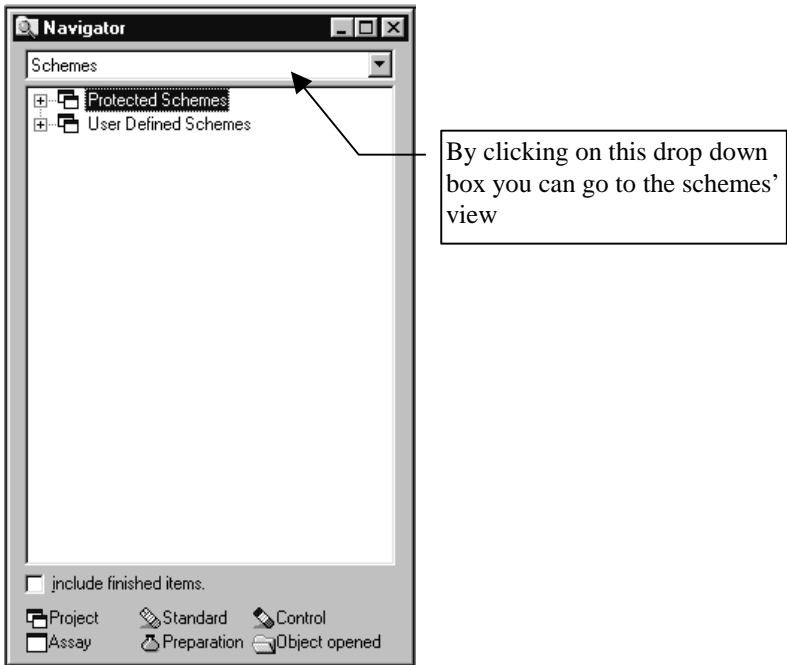


Figure 9-2 Schemes' view in PLA

On this page, you see two objects:

1. Protected schemes can only be created and modified by administrators. The schemes are used to enforce GMP/GLP requirements.
2. User defined schemes can be created and modified by every user. These schemes can be used as templates for the creation of new data objects.

Administrators can create protected as well as user-defined schemes while users can only create the latter.

Creating a scheme works exactly like generating a data object via the create object dialog. Enter all the settings as you would do for a data object.

Compared to former versions of PLA an important novelty is that the object hierarchy has been transferred to the usage of schemes. You can now define schemes for standards, preparations and controls that belong to an assay scheme. This new feature seemed to be reasonable because the settings for standard, preparation and control within an assay do not necessarily have to be identical.

When you create a new data object based on an assay scheme, PLA checks if the scheme contains subordinate schemes. In this case the definitions of lower level objects will be taken into account. Otherwise, all definitions will be taken from the assay scheme.

9.1.1 An Example on Using Schemes

Let us elucidate the usage of schemes with the example given in chapter 8.12.2. The data is from a standard 96 well plate containing a standard, a preparation and a control (with a positive and negative control). The preparation and standard are identical regarding their object definition. Both consist of eight dilution steps taken five times. The control differs in this regard because positive and negative controls were taken eight times.

Because the number of repetitions of the standard/preparation and the control are different is reasonable to define two schemes. For this proceed in the following manner:

1. Create a scheme that contains the definitions for the standard and the preparation.
2. Assign a specific control scheme as a sub-object to the created scheme that accounts for the special settings of the controls (different number of data series).

To create the higher level object select **Create Object**.

Create object

1. Select object type to create:

☐ New Protected Scheme

2. Input Code and Title:

3. Select a creation scheme:

(none)

Hint: if there is an active mandatory (global or parent) scheme, this selection will be ignored.

Create Cancel

From topic 1 of the dialog, you see that we will create a protected scheme. You must be logged in as an administrator user to be able to perform this task. If you belong to the standard user group, you can only create user-

defined schemes. However, user defined schemes are not compliant with GLP/GMP regulations.

Under topic 2, you can enter a name for the scheme that you want to create. Press the **Create** button to open the object dialog where you can enter the desired definitions. Change to the definitions tab. Enter the number of data series (5) and dilution steps (8). These are all the settings we will need for our example.

Create the scheme for the controls now. Select the scheme you have just created in the navigator and choose **New** from the task bar.

Create object

1. Select object type to create:

- ☒ New Protected Scheme
- ☐ Special Standard Scheme as Part of Protected Scheme
- ☐ Special Preparation Scheme as Part of Protected Scheme
- ☐ Special Control Scheme as Part of Protected Scheme

2. Input Code and Title:

Kontrolle

Kontrolle

3. Select a creation scheme:

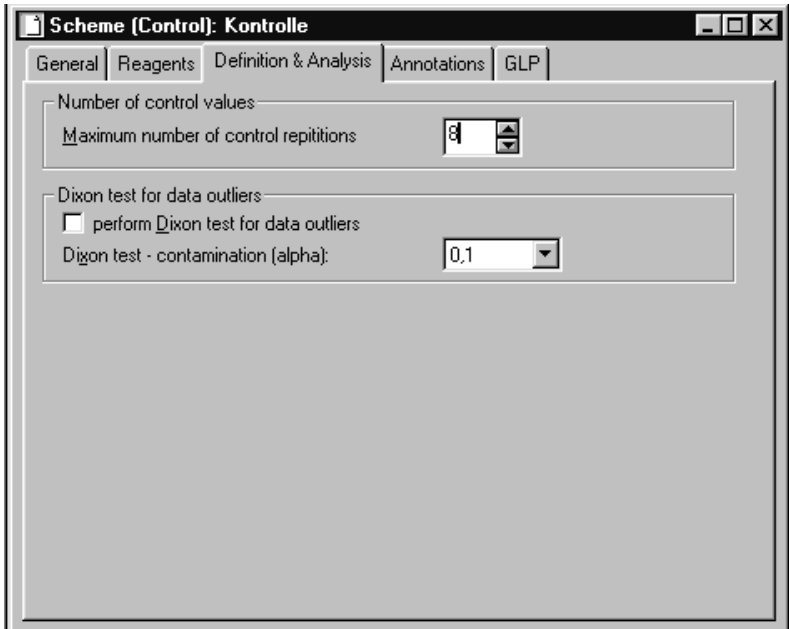
(none)

Hint: if there is an active mandatory (global or parent) scheme, this selection will be ignored.

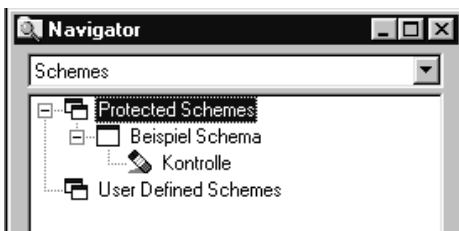
Create

Cancel

Select „*Special Control Scheme as Part of Protected Scheme*“ under topic 1 because we want to create a scheme especially for controls. After you have entered a name, press the **Create** button to open the object dialog.



On the tab Definition & Analysis enter the number of repetition in the appropriate input field. This is all you need for the control scheme. You should see the following hierarchy in the navigator window:



If you create a new object using your scheme, the default settings will be taken from your scheme settings. However, if you create a new control object the settings will be taken from the subordinate control scheme. In this way, you do not have to create a scheme for every object type but only for those that differ from the default settings.

9.2 GLP/GMP Settings of the Object Definition

The access protection is attached directly to the objects. Change to the last tab of the object definitions (see Figure 9-3). It does not matter if you define a data object or a scheme because both input masks are the same.

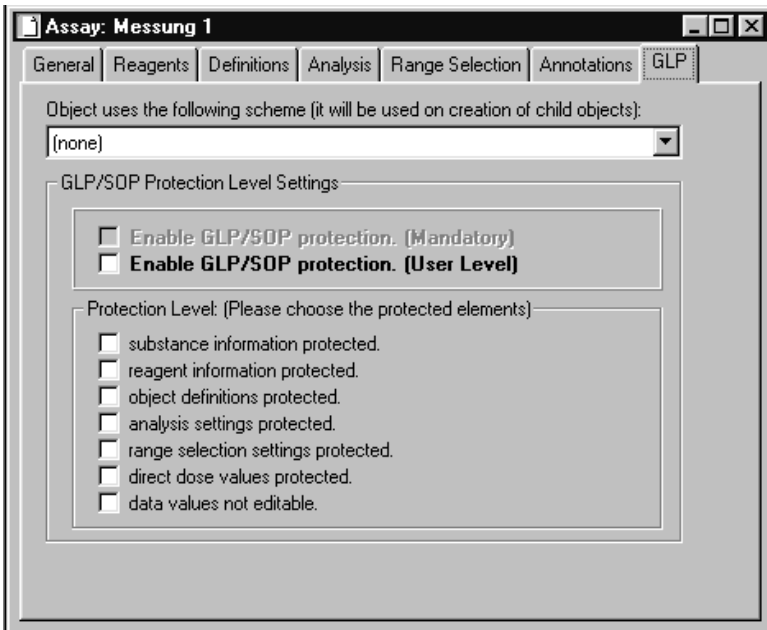
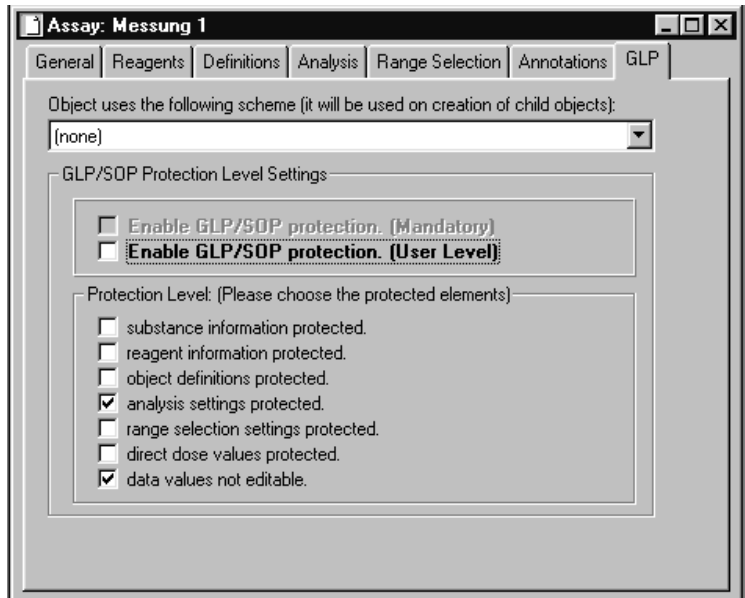


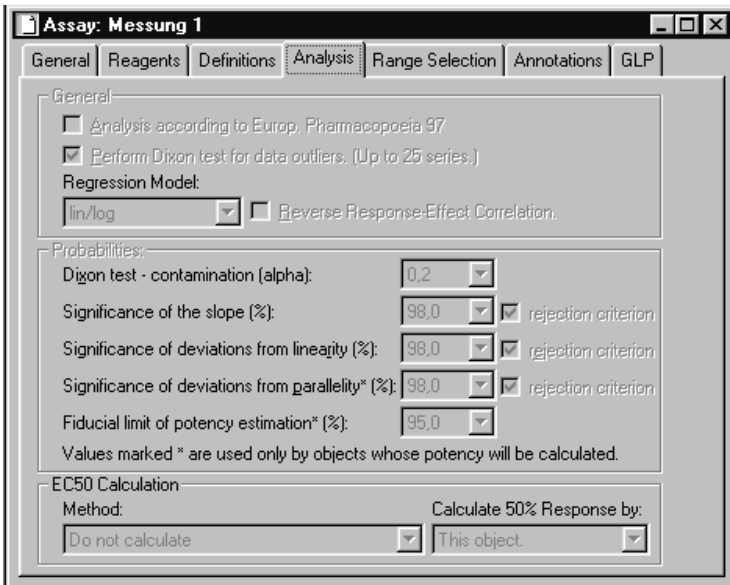
Figure 9-3 GLP settings within the object definitions

The protection of an object is a two-step process.

1. First, you have to select the protection level you want to apply to the project. For example, if you would like to prevent access to the analysis settings you should chose the respective field. If you want to protect the access to the measurements, you should check the '*data values not editable*' field. **Note that the protection of the object has not been activated yet.**



2. To activate the protection level you have to press the 'enable GLP/SOP protection' button first. If you select one of the protected pages you will see that you cannot change the settings on that page.



The kind of GMP/GLP protection you can activate is dependent on whether you are logged on as user or administrator. As a standard user you can only activate the object access protection on a user level. Any other user can lift this protection.

If you activate the access protection on the administrator level (*mandatory*) standard users will not be able to change any of the GLP settings. Thus, for the realization of GLP/GMP requirements especially the administrator protection is important.

9.3 Global GLP/GMP-Options

In the previous sections you have learned how to protect objects or how to create new objects with the help of schemes. But how can you lead your coworkers to work with PLA according to standard operation procedures (SOP)?

In this regard, PLA offers several possibilities that you can achieve with the help of the GMP/GLP settings. Select **Preferences|Options** from the menu. From the dialog, choose the ‘*GLP/SOP settings*’ tab.

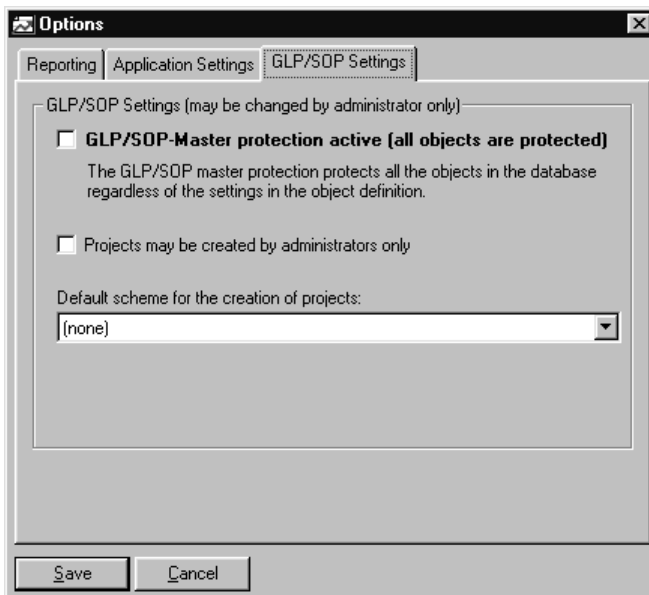


Figure 9-4 GMP settings in the options dialog. These options are only accessible for PLA administrators.

The global GMP/GLP settings are shown in Figure 9-4. Access to these options is limited to users with administrator privileges. You can set the following options:

1. *GLP/SOP-Master protection active.* By activating this function the field 'Enable GLP/SOP protection (mandatory)' on the GLP tab will be activated in all PLA objects within the PLA database. All fields checked under the protection level topic will be locked. If there are no fields marked the protection will have no further effect. The standard users cannot unlock these settings.
2. *Projects may be created by administrators only.* This option forbids the standard users to create new projects. The administrator has the opportunity to enforce GMP/GLP settings in every project that cannot be changed by the user. Every assay in the project will automatically inherit the protected information.
3. *Default scheme for the creation of projects.* With the help of this option you can choose the basis on which scheme the user can create new projects and assays. With the GLP settings of the selected scheme, the protected object properties are already defined. **Note:** The protection of the object created with this scheme is not activated automatically. Either you will have to initiate the protection manually or you must set the option 'GLP/SOP master protection active' to automatically activate the protection.

As you can see, administrators have several options at hand to selectively limit the access privileges of the standard users.

With the flexible interpretation of the GMP/GLP restrictions PLA is suitable for the use in product development on the one hand as well as for the GMP/GLP conform use in quality assurance on the other.

10 The Statistics Core of PLA

The topic of this chapter is the statistical methods for the calculations of PLA are. In the first part, we will give you an overview on the execution of the calculations. The operating procedures are oriented towards those

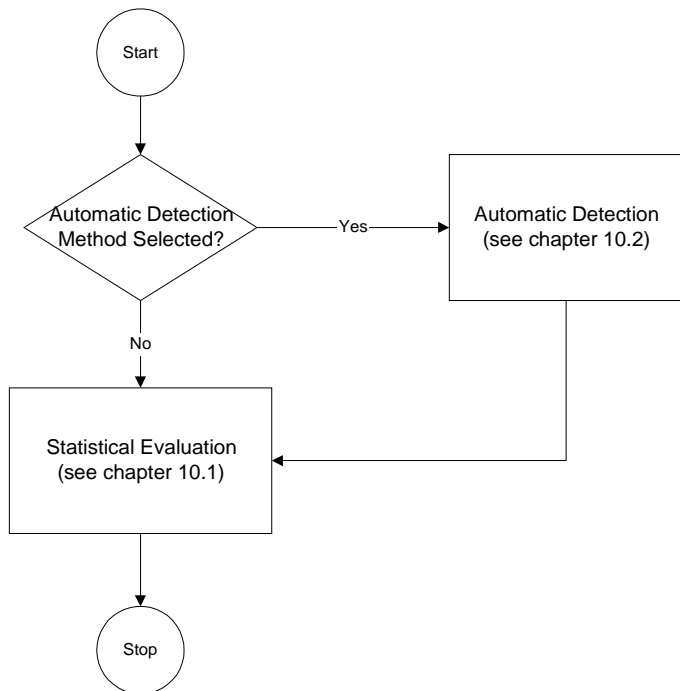


Abb. 10-1 Flow diagram

proposed by the European Pharmacopoeia 1997.

Please note that some of the requirements of the European Pharmacopoeia refer to the design of the experiment. If you are in doubt of the correctness of your experimental setup, please check those issues separately. If you meet the requirements, e. g. the normal distribution of the measurements, you will get a correct and valid analysis of your assay provided that you validation parameters are correct.

Section 10.1 describes the analysis of a parallel-line assay according to the European Pharmacopoeia with fixed values for the linear and parallel regions of your measured values. If you have chosen an automatic detection method the program will first do a selection of the measured values as described in section 10.2. As a result, all preparations and standards will be assigned a linear and parallel region, which will be analyzed statistically according to the following section. The methods for the automatic detection partially utilize the functionality depicted in section 10.1 but the technical implementation is completely separated from the statistical calculation. Therefore, the analysis is independent from detection method and in full accordance with the European Pharmacopoeia.

10.1 Statistical Calculations

In figures Figure 10-1 and Figure 10-2 the flow chart of a statistical analysis in PLA is shown. To some extent the analysis settings have effect on the course of the calculation. The analysis corresponds to the EP procedure although the sequence of steps is slightly altered for technical rea-

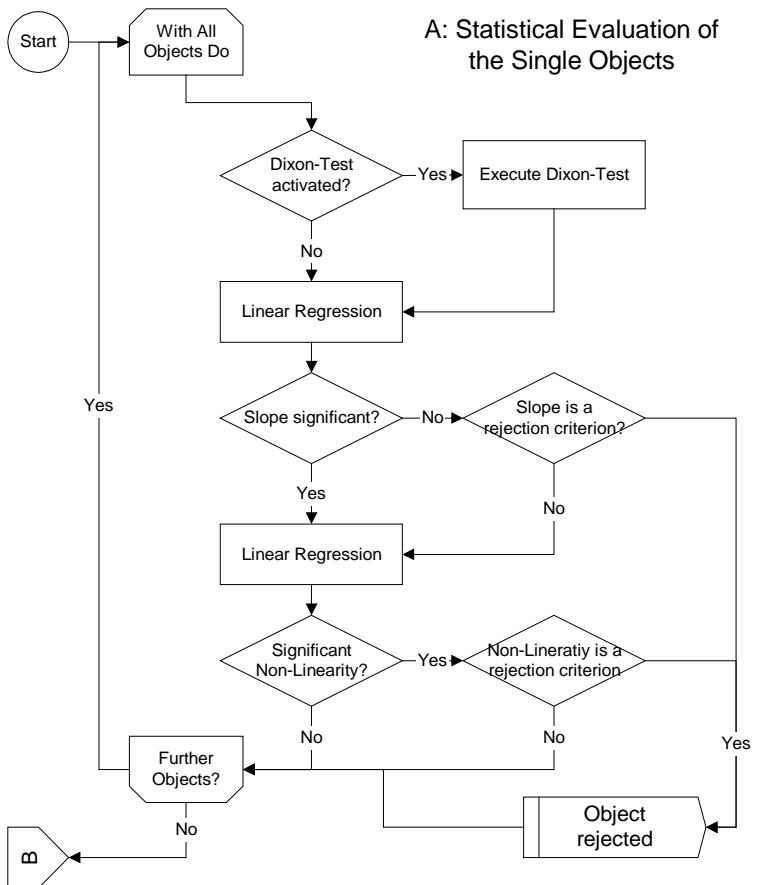


Figure 10-1 Statistical Analysis (Part A)

sons. First, PLA inspects all objects that are necessary for the calculation. For every object a Dixon test is performed, if this option has been activated. Subsequently all measurement values within the linear region are tested for significant slope and non-significant deviation from linearity. If one criterion is not fulfilled, the object and the assay as a whole is rejected.

Depending on the analysis settings, PLA may nevertheless proceed with the calculation (see *rejection criterion* in the analysis settings). However, the assay is **not valid** in a statistical sense because one of the starting hypotheses is not fulfilled.

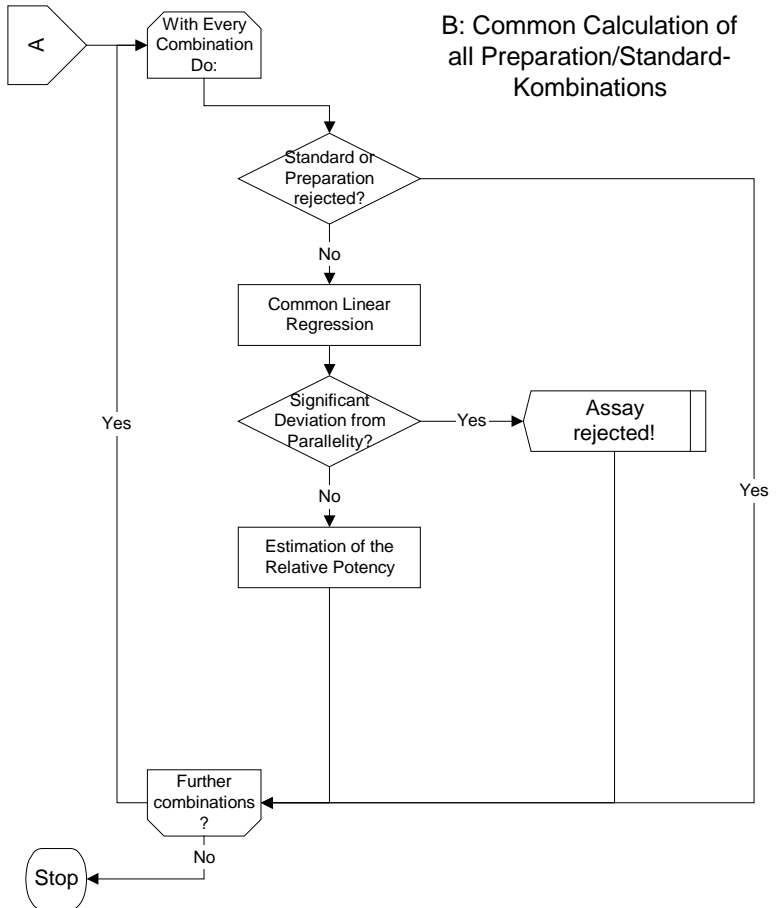


Figure 10-2 Statistical Analysis (Part B)

10.1.1 Transformation of the Measurement Values

PLA supports two different kinds of regression schemes for the analysis of parallel-line assays. The default method is based on a simple logarithmic transformation of the values. The logarithm of the dose values is calculated while the response values are not transformed. As an alternative scheme, PLA can use a double logarithmic method by calculation the dose as well as the response on a logarithmic scale. PLA works corresponding

to the EP and common biostatistical literature with the logarithm to the base 2.

10.1.2 Dixon Test

With samples that have been measured more than twice, a so-called Dixon test¹ can be performed to rule out statistical errors. The test is applied to every value. In PLA the two-sided version of the test is implemented. PLA supports contamination levels (α) of 0.3, 0.2, 0.1, 0.05, 0.02, 0.01 and 0.005. These levels can be set for each object separately in the analysis definitions. The smaller you choose the estimated contamination level, the stricter every value is examined. PLA can test up to 25 repetitions of measurement with the Dixon test.

To perform the Dixon test the readings are sorted by value. In a second step the value with the largest deviation from the average is determined (either the largest or the smallest value). Referring to this extreme value the r-quotient is calculated and then compared to a critical r-value from the literature. If the r-quotient is greater than the critical r-value, the measurement value will be marked as a statistical outlier. These values will not be considered in further calculations. This procedure is repeated until there are only two values left or the extreme value is accepted.

Depending on whether the largest or the smallest value is chosen the r-quotient is calculated as follows:

¹ W.J. Dixon, Processing Data For Outliers, *Biometrics*, 1953, 9, 74-89

Number of measurements N	Extreme is the largest value	Extreme is the smallest value
3-7	$r = \frac{x_N - x_{N-1}}{x_N - x_1}$	$r = \frac{x_2 - x_1}{x_N - x_1}$
8-10	$r = \frac{x_N - x_{N-1}}{x_N - x_2}$	$r = \frac{x_2 - x_1}{x_{N-1} - x_1}$
11-13	$r = \frac{x_N - x_{N-2}}{x_N - x_2}$	$r = \frac{x_3 - x_1}{x_{N-1} - x_1}$
14-25	$r = \frac{x_N - x_{N-2}}{x_N - x_3}$	$r = \frac{x_3 - x_1}{x_{N-2} - x_1}$

$\{x\}$ are the measurements sorted by value. Thus, x_N is the largest and x_1 is the smallest value.

10.1.3 Linear Regression

In order to be able to perform a valid calculation of the relative potency several criteria have to be fulfilled. These criteria are determined by a variance analysis with each object (standards and preparations) and by a variance analysis with each preparation and the standard together.

The three criteria that must be fulfilled are

- The regression line of each object must have a significant slope.
- The deviation from linearity must not be significant in any object.
- The deviation from parallelity of a preparation in comparison to the standard must not be significant.

The checks for the above criteria are done with the help of several F-tests. PLA supports statistical reliabilities $(1-\alpha)$ of 90%, 95%, 98%, 99%, 99.5%, 99.8%, 99.9%, 99.95%, 99.98% and 99.99% in every F-test. These values correspond to a significance level of 0.1, 0.05, 0.02, 0.01,

0.005, 0.002, 0.001, 0.0005, 0.0002 and 0.0001. The critical F-values (quantiles) are calculated within PLA.

10.1.3.1 Significance of the Slope

PLA does a variance analysis (ANOVA) to prove the significance of the slope. The parameters of a linear regression over

$$y = a + bx$$

are determined. y is the (transformed) measured value, x the logarithmic concentration, a the intersection with the abscissa and b the slope of the regression line. PLA always uses the logarithm to the base 2 in logarithmic calculations.

A F-test is performed in which the F-value of the model ($y = a + bx$) must be greater than the critical F-value. If the F-value is greater, the linear regression has a significant slope and the validation criterion is fulfilled.

10.1.3.2 Significant Deviation from Linearity

PLA performs a variance analysis (ANOVA) for each object that contains more than two dilution steps to determine if the measurement values deviate significantly from linearity. The model for the analysis is

$$y = a + bx + cx^2$$

y corresponds to the (transformed) values, x is the logarithmic concentration, a , b and c are the regression coefficients. The deviation from linearity is checked by comparing the F-value of the quadratic regression coefficient to the critical F-value. If the calculated F-value is less than the critical F-value, one can assume that the measurements do have a significant deviation from linearity and that the validation criterion is fulfilled.

10.1.3.3 Significant Deviation from Parallelity

The proof for the parallelity is based on two variance analyses over the measurement of all objects. The first analysis uses the model

$$y = a_n + bx_n$$

(n = 1 for the standard, n = 2 for the preparation). With this model, the slope of both regression lines is calculated.

The second analysis is done with the model

$$y = a_n + b_n x_n$$

With this variance analysis, both regression lines are estimated together. The deviation from parallelity is determined by the F-value of the difference between the square sums of both models. If the F-value is less than the critical F-value, both regions are assumed to be parallel.

10.1.4 Calculation of the Relative Potency according to Fieller

The calculation of the relative potency and of the confidence intervals is done according to Fieller's theorem. The theorem exceeds the quality that is demanded by the European Pharmacopoeia. The calculation can be performed with a level of significance of 90.0%, 95.0%, 99.0% and 99.9%.

The logarithm of the relative potency M is given by the following equation:

$$M = \frac{Y_P - Y_S}{b}$$

Where Y_P and Y_S are the intersections with the y-axis and b is the common slope of both samples. According to Fieller,¹ one obtains the following confidence interval with a defined error probability:

$$M_{u/l} = \bar{x}_S - \bar{x}_P + \frac{t(f, p)s}{b} \pm \frac{t(f, p)s}{b} \sqrt{(1-g)\left(\frac{1}{n_S} + \frac{1}{n_P}\right) + \frac{(M - \bar{x}_S + \bar{x}_P)^2}{\sum S_{xx}}}$$

$$g = \frac{t_{(f, p)}^2 s^2}{b^2 \sum S_{xx}}$$

¹ according to D. J. Finney, Statistical Method in Biological Assay, London, 1978

Where:

x_s	averaged \log_2 dose of the standard
x_p	averaged \log_2 dose of the preparation
s^2	mean square of errors (MSE)
S_{xx}	sum of square of errors
$t(f,p)$	value of the student t-distribution for f degrees of freedom and a confidence interval of p
n_s	number of measurements of the standard
n_p	number of measurements of the preparation

The value of the student t-distribution is calculated within PLA.

10.2 Automatic Detection

The crucial novelty in PLA is the possibility to automatically detect the linear and parallel regions of assays. This function enables you to find the valid intervals for the parallel-line analysis quickly and efficiently. Assays of biological systems often show saturation effects in the region of high and low concentration. These regions are unsuitable for a parallel-line assay. The automatic detection method has been developed to facilitate the analysis of such measurements. The purpose of this chapter is to help you to utilize this method efficiently.

Overall, there are four methods for the determinations of the linear and parallel region: one manual (*fixed range* and *graphical selection*) and three automatic approaches. These methods can be combined arbitrarily. You can for example detect the linear region of the standard with a manual method and the linear region of the preparations with an automatic detection scheme to optimize with respect to the manually selected range.

There are two groups of automatic methods for each the standard and the preparations. Take into account that the analysis with PLA always takes place at the assay level. The program always focuses on the standard and all preparations at the same time. This leads to the problem that the automatic optimization of the linear range of the standard can be done either with respect to all preparations or for each probe individually.

It possible to select if the linear range of the standard should be optimized for all preparations together (*Automatic detection. Common range for standards*) or for each preparation individually (*Automatic detection. Individual range*).

For the preparations, you can choose likewise, if the linear range should be optimized freely or if the same dilution steps as for the linear range of the standard should be used. The following matrix will give you an overview on the results of combination of the different choices.

	Standard fixed	Standard common	Standard individual
Preparation fixed	No optimization.	The same region of the standard is used for all preparations.	The linear region of the standard is optimized for each preparation separately.
Preparation identical	The linear region of the preparation will be set to the same interval as the standard.	The linear region of the standard is optimized with respect to all preparations. The region of the preparations is set to the same interval.	For each standard/preparation combination the best common range is determined.
Preparation individual	The preparation is optimized individually within the linear range of the standard.	The preparations are optimized with respect to the common linear range of the standard.	Preparations and standards are optimized freely without any restrictions.

Overall, you have nine possibilities to optimize the linear range(s) with side conditions of varying strength.

Once you know the valid region of the standard, you should define the fixed range in the definitions of the standard. Depending on whether you want to allow a free choice of the linear region for the preparations you should set the allocation strategy to *Automatic detection. Individual range* or to *Automatic detection. Identical range* if you want to keep the linear range of the preparation within the bounds of the standard.

Besides, you have the possibility to influence the results of the analysis by changing the evaluation criteria. The allocation strategy allows you to

choose between the linear range with the best agreement (*Best Range*), the range with the maximum number of points (*Maximum Range*) and a range with a given number of points. Additionally you can enter a lower limit for the number of points in the linear region (*Minimum # of points*). You can narrow the interval in which PLA tries to detect the linear region by giving an allocation region. Furthermore, you can include the 50% response within the linear region. You can either force the program to do so (*inclusion is mandatory*) or propose the program to include the value (*include if possible*). With the latter option, the program will include the 50% response only if the assay is still valid.

11 Appendices

11.1 Check A - Linder

The textbook on statistical methods in science by A. Linder¹ contains a complete analysis of a parallel-line assay. The test data set has been recalculated with PLA to check the program results.

In Table 11-1 the results of Linder and PLA are both shown. The values of PLA have been rounded for this comparison.

You see that for preparation 2 the F-value of the slope and the upper limit of the confidence interval of the relative potency differ slightly. Both differences are due to rounding errors in the Linder calculation. While Linder rounds all the sums of squares and the mean sums of squares to integer numbers PLA uses double precision numbers (8 byte double precision number format compliant to IEEE). Linder himself mentions the possibility of deviations from computer results because of rounding errors.

The table shows that PLA reproduces the literature values.

Annotations on the table:

Linder's sample 2 is used as the standard in PLA; Linder's sample 1 is used as the preparation. SOS is the sum of squares, MS is the mean square. For each sample the SOS and MS for the applied model are shown. SOS error and MS error are the respective residual errors. SOS quadratic and MS quadratic are the quadratic regression coefficients. SOS model and MS model are the square sums of the regression. SOS difference and MS difference are the square sum of the model differences in the parallelity analysis.

Table 11-1 Comparison between the Linder and PLA results

¹ A. Linder, Statistische Methoden, Basel und Stuttgart, 1964, 4. Auflage, S. 162-169

Range	Value	Linder	PLA
Standard (Sample 2)			
Linearity	SOS quadratic	35.363	35.363
	MS quadratic	35.363	35.363
	SOS error	113.848	113.848
	MS error	9.487	9.487
	F-value	3,73	3,73
Slope	SOS model	475.240	475.240
	MS model	475.240	475.240
	SOS error	149.211	149.211
	MS error	11.478	11.478
	F-value	41,40	41,41
Preparation (Sample 1)			
Linearity	SOS quadratic	832	832
	MS quadratic	832	832
	SOS error	116.620	116.620
	MS error	9.718	9.718
	F-value	0,09	0,09
Slope	SOS model	670.810	670.810
	MS model	670.810	670.810
	SOS error	117.453	117.453
	MS error	9.035	9.035
	F-value	74,25	74,25
Parallelity of Sample 1 and Sample 2			
	SOS difference	8.405	8.405
	MS difference	8.405	8.405
	SOS error	266.664	266.664
	MS error	10.256	10.256
	F-value	0,82	0,82
Relative Potency of Sample 1 in Comparison to Sample 2			
	RP	2,04	2,04
	RP upper limit	1,63	1,63
	RP lower limit	2,56	2,57

11.1.1 PLA Output (PLA Short Report)

The following output is generated as a so called PLA Short Report. It contains the shown values.

Results of Assay: **Linder**
Project: **Validierungsbeispiele**

Current user: Dr. Matthias Schmitt

.....
Date/Signature

1 Data Input: (Data index: x = technical outlier, o = outlier found by Dixon test)

1.1 Standard: Standard

Step	X	log ₂ (X)	Series 1	Series 2	Series 3	Series 4	Series 5
1	1,0000	0,0000	487	525	585	600	715
2	0,5000	-1,0000	150	350	275	122	410
3	0,2500	-2,0000	164	255	64	95	154

Step	X	log ₂ (X)	Mean	S.D.	C.V. [%]
1	1,0000	0,0000	582,400	87,028	14,9
2	0,5000	-1,0000	261,400	124,458	47,6
3	0,2500	-2,0000	146,400	73,473	50,2

50% Response: **364,400**

1.2 Sample: Probe 1

Step	X	log ₂ (X)	Series 1	Series 2	Series 3	Series 4	Series 5
1	0,5000	-1,0000	710	516	620	510	650
2	0,2500	-2,0000	560	268	372	247	185
3	0,1250	-3,0000	83	84	90	84	75

Step	X	log ₂ (X)	Mean	S.D.	C.V. [%]
1	0,5000	-1,0000	601,200	86,817	14,4
2	0,2500	-2,0000	326,400	146,933	45,0
3	0,1250	-3,0000	83,200	5,357	6,4

50% Response: **342,200**

2 Definitions:

	Standard	Probe 1
Perform Dixon Test	YES	YES
Regression Model	lin/log	lin/log
Reverse Response Effect Correl.	NO	NO
Selected Method	Fixed range	Fixed range
Allocation Strategy	-	-
Minimal No. of Points	-	-
Maximal Allocation Range	-	-
Allocation region	Step 1 to Step 3	Step 1 to Step 3
Inclusion of 50% response	-	-
50% Response of	-	-

3 Results:

	Standard	Probe 1
Selected linear Range	Step 1 to Step 3	Step 1 to Step 3
Test of Linearity	PASSED	PASSED
F _{quadratic}	3,7274	0,0856
F _{critical}	4,7472	4,7472
P	95,0 %	95,0 %
r ²	0,8177	0,8521
Test of Slope	PASSED	PASSED
F _{model}	41,4053	74,2470
F _{critical}	4,6672	4,6672
P	95,0 %	95,0 %
r ²	0,7611	0,8510
Test of Parallelism	PASSED	
F _{diff of models}	0,819	
F _{critical}	4,225	
P	95,00 %	
r ²	0,9439	
Probe 1 vs. Standard		
Relative Potency	2,040	
95 % fiducial Limits	1,635 – 2,567	
Relative fiducial limits	80,1 % – 125,8 % (45,6 %)	

4 Calculation of EC50:

	Standard	Probe 1
Method of EC50 calculation	n/c	n/c
50% Response of	-	-
EC50 concentration	-	-

Appendices

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Project: Validierungsbeispiele

08.10.99 10:06:47
Assay: Linder

Additional Annotations:

11.1.2 PLA Output (PLA Complete Statistics Report)

The PLA Complete Statistics Report of PLA contains all the variance analyses' data. It is used for the comparison with the Linder results.

Results of Assay: **Linder**
Project: **Validierungsbeispiele**

Current user: Dr. Matthias Schmitt

.....
DateSignature

1 Complete List of the Object Definitions:

	Standard	Sample
General settings:		
Code:	Standard	Probe 1
Title:	Standard	Probe 1
Measured substance:		
Batch identification:		
Object status declared closed:	NO	NO
Object was created by:	Dr. Matthias Schmitt	Dr. Matthias Schmitt
Creation timestamp:	16.06.99 15:14:50	16.06.99 15:14:52
Last modification by:		
Modification timestamp:	06.10.99 12:53:13	06.10.99 12:53:13
Definition settings:		
Concentration of undiluted standard:	1	-
Concentration units:		-
Predilution of standard/sample:	1:1	1:1
Number of dilution steps:	3	3
Number of data series:	5	5
Additional predilution factor:	1:1	1:2
Dilution scale:	1:2	1:2
Analysis settings:		
Analysis according to EP:	NO	NO
Dixon test enabled:	YES	YES
Regression model:	lin/log	lin/log
Reverse response effect correlation:	NO	NO
Dixon contamination:	0,1	0,1
F-Test of the slope:	95	95
F-Test of linearity:	95	95
F-Test of parallelity:	95	95
Fiducial limit percents:	95	95
Slope as rejection criterion:	NO	NO
Linearity as rejection criterion:	NO	NO
Parallelity as rejection criterion:	NO	NO
EC50 calculation:	NO	NO
by using:	-	-
Range Selection Settings		
Linear detection type:	Fixed range (1)	Fixed range (1)
Allocation Strategy:	-	-
Minimal/Exact # of Points:	-	-
Maximal Allocation Range:	-	-
Allocation Range:	Step 1 to Step 3	Step 1 to Step 3
Inclusion of 50% Response:	-	-
50% Response of:	-	-

2 Data Input: (Data index: x = technical outlier, o = outlier found by Dixon test)

2.1 Standard: Standard

Step	X	log ₂ (X)	Series 1	Series 2	Series 3	Series 4	Series 5
1	1,0000	0,0000	487	525	585	600	715
2	0,5000	-1,0000	150	350	275	122	410
3	0,2500	-2,0000	164	255	64	95	154

Step	X	log ₂ (X)	Mean	S.D.	C.V. [%]
1	1,0000	0,0000	582,400	87,028	14,9
2	0,5000	-1,0000	261,400	124,458	47,6
3	0,2500	-2,0000	146,400	73,473	50,2

50% Response: 364,400

2.2 Sample: Probe 1

Step	X	log ₂ (X)	Series 1	Series 2	Series 3	Series 4	Series 5
1	0,5000	-1,0000	710	516	620	510	650
2	0,2500	-2,0000	560	268	372	247	185
3	0,1250	-3,0000	83	84	90	84	75

Step	X	log ₂ (X)	Mean	S.D.	C.V. [%]
1	0,5000	-1,0000	601,200	86,817	14,4
2	0,2500	-2,0000	326,400	146,933	45,0
3	0,1250	-3,0000	83,200	5,357	6,4

50% Response: 342,200

3 Range Selection

	Standard	Sample
Linear detection type:	Fixed range (1)	Fixed range (1)
Selected Linear Range:	Step 1 to Step 3	Step 1 to Step 3

4 Test of Linearity

Regression equation: $y = a + b \cdot \lg(x) + c \cdot \lg(x)^2$

4.1 Standard: Standard

Total number of observations: 15

Source	d.f.	Sum of Squares	Mean Square	F-Value
Model	2	510,603,3333	255,301,6667	26,9098
Error	12	113,847,6000	9,487,3000	
Total	14	624,450,9333		
Linearity	1	475,240,0000	475,240,0000	50,0922
Quadratic	1	35,363,3333	35,363,3333	3,7274

	Param. Estimates	Error	Quality of regression	
Intercept a	582,4000	43,5598	r^2	0,817684
Slope b	424,0000	111,0562	r^2_{adj}	0,787298
Quadratic c	103,0000	53,3497	Root MSE	97,4028

Test passed if $F_{\text{quadratic}} < F_{\text{crit}} (P=95\%) = 4,7472$

Test PASSED!

4.2 Sample: Probe 1

Total number of observations: 15

Source	d.f.	Sum of Squares	Mean Square	F-Value
Model	2	671,642,1333	335,821,0667	34,5552
Error	12	116,620,8000	9,718,4000	
Total	14	788,262,9333		
Linearity	1	670,810,0000	670,810,0000	69,0247
Quadratic	1	832,1333	832,1333	0,0856

	Param. Estimates	Error	Quality of regression	
Intercept a	907,6000	192,1716	r^2	0,852053
Slope b	322,2000	218,2204	r^2_{adj}	0,827396
Quadratic c	15,8000	53,9956	Root MSE	98,5819

Test passed if $F_{\text{quadratic}} < F_{\text{crit}} (P=95\%) = 4,7472$

Test PASSED!

5 Test of Slope

Regression equation: $y = a + b \cdot \lg(x)$

5.1 Standard: Standard

Total number of observations: 15

Source	d.f.	Sum of Squares	Mean Square	F-Value
Model	1	475,240,0000	475,240,0000	41,4053
Error	13	149,210,9333	11,477,7641	
Total	14	624,450,9333		

	Param. Estimates	Error	Quality of regression	
Intercept a	548,0667	43,7374	r^2	0,761053
Slope b	218,0000	33,8788	r^2_{adj}	0,742672

Test passed if $F_{\text{model}} > F_{\text{crit}} (P=95\%) = 4,6672$

Test PASSED!

5.2 Sample: Probe 1

Total number of observations: 15

Source	d.f.	Sum of Squares	Mean Square	F-Value
Model	1	670,810,0000	670,810,0000	74,2470
Error	13	117,452,9333	9,034,8410	
Total	14	788,262,9333		

	Param. Estimates	Error	Quality of regression	
Intercept a	854,9333	64,9327	r^2	0,850998
Slope b	259,0000	30,0580	r^2_{adj}	0,839536

Test passed if $F_{\text{model}} > F_{\text{crit}} (P=95\%) = 4,6672$

Test PASSED!

6 Test of Parallelity

6.1 Estimation of Common Slope

Total number of observations: 30

Source	d.f.	Sum of Squares	Mean Square	F-Value
Model	3	4,474,666,1333	1,491,555,3778	
Error	27	275,068,8667	10,187,7358	
Total	30	4,749,735,0000		

	Param. Estimates	Error	Quality of regression	
Intercept a1	813,9333	52,1223	r ²	0,942088
Intercept a2	568,5667	34,4756	Root MSE	100,9343
Slope b	238,5000	22,5696		

6.2 Estimation of error due to non-parallelity

Total number of observations: 30

Source	d.f.	Sum of Squares	Mean Square	F-Value
Model	3	4,474,666,1333	1,491,555,3778	
Difference of Models	1	8,405,0000	8,405,0000	0,8195
Model (Total)	4	4,483,071,1333	1,120,767,7833	
Error	26	266,663,8667	10,256,3026	
Total	30	4,749,735,0000		

	Param. Estimates	Error	Quality of regression	
Intercept a1	854,9333	69,1829	r ²	0,943857
Intercept a2	548,0667	41,3447	Root MSE	101,2734
Slope b1	259,0000	32,0255	g	0,0079
Slope b2	218,0000	32,0255		

Test passed if F_{crit, of models} < F_{crit} (P=95%) = 4,2252

Test PASSED!

7 Estimation of the Relative Potency

Relative potency of sample **Probe 1** in comparison to standard **Standard**

Probe 1 vs. Standard	
Estimated relative potency:	2,04
95% Fiducial Limits:	1,64 - 2,57
Relative fiducial limits:	80,1 % - 125,8 % (45,6%)

8 Calculation of EC50:

	Standard	Probe 1
Method of EC50 calculation	n/c	n/c
50% Response of	-	-
EC50 concentration	-	-

9 Annotations:

Appendices

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Project: Validierungsbeispiele

08.10.99 10:07:28
Assay: Linder

Additional Annotations:

11.2 Check B - European Pharmacopoeia

For the second check of the PLA results the examples of the European Pharmacopoeia 1997 section 5.3 'Statistical Analysis', example 3.2.8.1 are recalculated with PLA. Because the EP does not include dilution series but only measurements 1:1 (*1.0 unit per 100g of body mass*) and 1:4 (*0.25 units per 100g of body mass*) a test for the standard S and the preparation U will be defined with three dilution steps. All values for the dilution step 2, the missing 1:2 step, will be excluded from the analysis, however. A test for linearity is not possible in this case because the test does only contain two concentration values.

In the EP the slope and parallelity are calculated with a slightly different scheme of the variance analysis. For that reason a direct comparison of the numbers is not possible for all values.

Annotations on the table:

SOS is the sum of squares; MS is the mean sum of squares. The SOS and MS for the respective models are shown. SOS error and MS error are the residual errors. SOS difference and MS difference are the sums of squares of the differences between the models in the parallelity analysis.

Range	Value	EP 1997	PLA
Parallelity of Preparation U and Standard S			
SOS difference		34,2	34,2
MS difference		34,2	34,2
SOS error		26587,3	26587,3
MS error		738,5	738,5
F-value		0,05	0,05
Relative Potency of Preparation U in Comparison Standard S			
RP		1,11	1,11
RP upper limit		0,82	0,83
RP lower limit		1,51	1,50

11.2.1 PLA Output (PLA Short Report)

The following output is generated as a so called PLA Short Report. It contains the shown values.

Results of Assay: **Europ. Pharmacop. 1993**
Project: **Validierungsbeispiele**

Current user: Dr. Matthias Schmitt

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Date/Signature

1 Data Input: (Data index: x = technical outlier, o = outlier found by Dixon test)

1.1 Standard: Standard S

Step	X	log ₂ (X)	Series 1	Series 2	Series 3	Series 4	Series 5
1	1,0000	0,0000	289	221	267	236	250
2	0,2500	-2,0000	300	310	330	290	364

Step	X	log ₂ (X)	Series 6	Series 7	Series 8	Series 9	Series 10
1	1,0000	0,0000	231	229	269	233	259
2	0,2500	-2,0000	328	390	360	342	306

Step	X	log ₂ (X)	Mean	S.D.	C.V. [%]
1	1,0000	0,0000	248,400	21,996	8,9
2	0,2500	-2,0000	332,000	32,042	9,7

50% Response: 290,200

1.2 Sample: Preparation U

Step	X	log ₂ (X)	Series 1	Series 2	Series 3	Series 4	Series 5
1	1,0000	0,0000	290	210	280	261	241
2	0,2500	-2,0000	310	290	360	341	321

Step	X	log ₂ (X)	Series 6	Series 7	Series 8	Series 9	Series 10
1	1,0000	0,0000	290	223	254	216	235
2	0,2500	-2,0000	370	303	334	295	315

Step	X	log ₂ (X)	Mean	S.D.	C.V. [%]
1	1,0000	0,0000	244,000	26,808	11,0
2	0,2500	-2,0000	323,900	26,926	8,3

50% Response: 263,950

2 Definitions:

	Standard S	Preparation U
Perform Dixon Test	YES	YES
Regression Model	lin/log	lin/log
Reverse Response Effect Correl.	NO	NO
Selected Method	Fixed range	Fixed range
Allocation Strategy	-	-
Minimal No. of Points	-	-
Maximal Allocation Range	-	-
Allocation region	Step 1 to Step 2	Step 1 to Step 2
Inclusion of 50% response	-	-
50% Response of	-	-

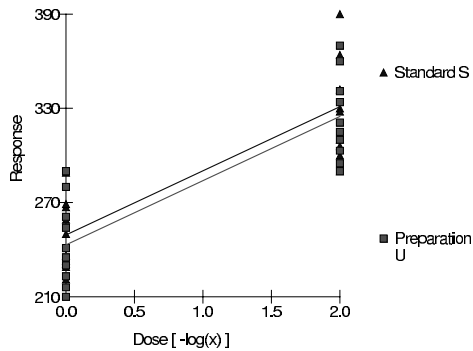
3 Results:

	Standard S	Preparation U
Selected linear Range	Step 1 to Step 2	Step 1 to Step 2
Test of Linearity	Linearity is not calculated!	Linearity is not calculated!
F _{quadratic}	-	-
F _{critical}	-	-
P	-	-
r ²	-	-
Test of Slope	PASSED	PASSED
F _{model}	46,2695	44,2211
F _{critical}	6,5146	6,5146
P	98,0 %	98,0 %
r ²	0,7199	0,7107
Test of Parallelism	PASSED	
F _{diff of models}	0,046	
F _{critical}	5,927	
P	98,00 %	
r ²	0,9922	
Preparation U vs. Standard S		
Relative Potency	1,112	
95 % fiducial Limits	0,830 – 1,497	
Relative fiducial limits	74,6 % – 134,7 % (80,0 %)	

4 Calculation of EC50:

	Standard S	Preparation U
Method of EC50 calculation	n/c	n/c
50% Response of	-	-
EC50 concentration	-	-

5 Figure:



6 Annotations:

Results of Assay: **Europ. Pharmacop. 1993**
Project: **Validierungsbeispiele**

Current user: Dr. Matthias Schmitt

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Date/Signature

1 Data Input: (Data index: x = technical outlier, o = outlier found by Dixon test)

1.1 Standard: Standard S

Step	X	log ₂ (X)	Series 1	Series 2	Series 3	Series 4	Series 5
1	1,0000	0,0000	289	221	267	236	250
2	0,2500	-2,0000	300	310	330	290	364

Step	X	log ₂ (X)	Series 6	Series 7	Series 8	Series 9	Series 10
1	1,0000	0,0000	231	229	269	233	259
2	0,2500	-2,0000	328	390	360	342	306

Step	X	log ₂ (X)	Mean	S.D.	C.V. [%]
1	1,0000	0,0000	248,400	21,996	8,9
2	0,2500	-2,0000	332,000	32,042	9,7

50% Response: **290,200**

1.2 Sample: Preparation Z

Step	X	log ₂ (X)	Series 1	Series 2	Series 3	Series 4	Series 5
1	1,0000	0,0000	236	213	283	269	251
2	0,2500	-2,0000	250	268	273	240	307

Step	X	log ₂ (X)	Series 6	Series 7	Series 8	Series 9	Series 10
1	1,0000	0,0000	294	223	250	216	265
2	0,2500	-2,0000	270	317	312	320	265

Step	X	log ₂ (X)	Mean	S.D.	C.V. [%]
1	1,0000	0,0000	250,000	28,012	11,2
2	0,2500	-2,0000	282,200	29,234	10,4

50% Response: **266,100**

2 Definitions:

	Standard S	Preparation Z
Perform Dixon Test	YES	YES
Regression Model	lin/log	lin/log
Reverse Response Effect Correl.	NO	NO
Selected Method	Fixed range	Fixed range
Allocation Strategy	-	-
Minimal No. of Points	-	-
Maximal Allocation Range	-	-
Allocation region	Step 1 to Step 2	Step 1 to Step 2
Inclusion of 50% response	-	-
50% Response of	-	-

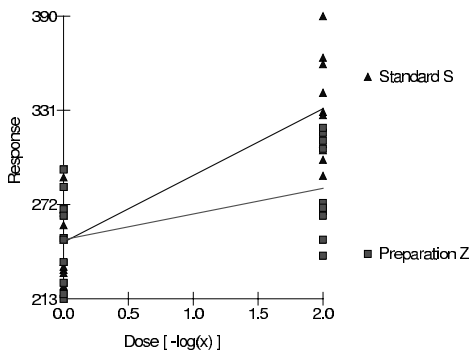
3 Results:

	Standard S	Preparation Z
Selected linear Range	Step 1 to Step 2	Step 1 to Step 2
Test of Linearity	Linearity is not calculated!	Linearity is not calculated!
F _{quadratic}	-	-
F _{critical}	-	-
P	-	-
r ²	-	-
Test of Slope	PASSED	FAILED
F _{model}	46,2695	6,3249
F _{critical}	6,5146	6,5146
P	98,0 %	98,0 %
r ²	0,7199	0,2600
Test of Parallelism	Parallelity is not calculated!	
F _{diff of models}	-	-
F _{critical}	-	-
P	-	-
r ²	-	-
Preparation Z vs. Standard S		
Relative Potency	Assay Rejected!!	
95 % Fiducial Limits	Relative Potency is not calculated	
Relative fiducial limits		

4 Calculation of EC50:

	Standard S	Preparation Z
Method of EC50 calculation	n/c	n/c
50% Response of	-	-
EC50 concentration	-	-

5 Figure:



6 Annotations:

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Project: Validierungsbeispiele

08.10.99 10:07:56
Assay: Europ. Pharmacop. 1993

Additional Annotations:

11.2.2 PLA Output (PLA Complete Statistics Report)

The PLA Complete Statistics Report of PLA contains all the variance analyses' data. It is used for the comparison with the EP results.

Results of Assay: **Europ. Pharmacop. 1993**
Project: **Validierungsbeispiele**

Current user: Dr. Matthias Schmitt

.....
Date/Signature

1 Complete List of the Object Definitions:

	Standard	Sample
General settings:		
Code:	Standard S	Preparation U
Title:	Standard S	Preparation U
Measured substance:		
Batch identification:		
Object status declared closed:	NO	NO
Object was created by:	Dr. Matthias Schmitt	Dr. Matthias Schmitt
Creation timestamp:	16.06.99 15:14:55	16.06.99 15:14:57
Last modification by:		
Modification timestamp:	06.10.99 12:53:12	06.10.99 12:53:12
Definition settings:		
Concentration of undiluted standard:	1	-
Concentration units:		-
Predilution of standard/sample:	1:1	1:1
Number of dilution steps:	2	2
Number of data series:	10	10
Additional predilution factor:	1:1	1:1
Dilution scale:	direct input	direct input
Analysis settings:		
Analysis according to EP:	YES	YES
Dixon test enabled:	YES	YES
Regression model:	lin/log	lin/log
Reverse response effect correlation:	NO	NO
Dixon contamination:	0,02	0,02
F-Test of the slope:	98	98
F-Test of linearity:	98	98
F-Test of parallelity:	98	98
Fiducial limit percents:	95	95
Slope as rejection criterion:	YES	YES
Linearity as rejection criterion:	YES	YES
Parallelity as rejection criterion:	YES	YES
EC50 calculation:	NO	NO
by using:	-	-
Range Selection Settings		
Linear detection type:	Fixed range (1)	Fixed range (1)
Allocation Strategy:	-	-
Minimal/Exact # of Points:	-	-
Maximal Allocation Range:	-	-
Allocation Range:	Step 1 to Step 2	Step 1 to Step 2
Inclusion of 50% Response:	-	-
50% Response of:	-	-

2 Data Input: (Data index: x = technical outlier, o = outlier found by Dixon test)

2.1 Standard: Standard S

Step	X	log ₂ (X)	Series 1	Series 2	Series 3	Series 4	Series 5
1	1,0000	0,0000	289	221	267	236	250
2	0,2500	-2,0000	300	310	330	290	364

Step	X	log ₂ (X)	Series 6	Series 7	Series 8	Series 9	Series 10
1	1,0000	0,0000	231	229	269	233	259
2	0,2500	-2,0000	328	390	360	342	306

Step	X	log ₂ (X)	Mean	S.D.	C.V. [%]
1	1,0000	0,0000	248,400	21,996	8,9
2	0,2500	-2,0000	332,000	32,042	9,7

50% Response: 290,200

2.2 Sample: Preparation U

Step	X	log ₂ (X)	Series 1	Series 2	Series 3	Series 4	Series 5
1	1,0000	0,0000	230	210	280	261	241
2	0,2500	-2,0000	310	290	360	341	321

Step	X	log ₂ (X)	Series 6	Series 7	Series 8	Series 9	Series 10
1	1,0000	0,0000	290	223	254	216	235
2	0,2500	-2,0000	370	303	334	295	315

Step	X	log ₂ (X)	Mean	S.D.	C.V. [%]
1	1,0000	0,0000	244,000	26,808	11,0
2	0,2500	-2,0000	323,900	26,926	8,3

50% Response: 283,950

3 Range Selection

	Standard	Sample
Linear detection type:	Fixed range (1)	Fixed range (1)
Selected Linear Range:	Step 1 to Step 2	Step 1 to Step 2

4 Test of Linearity

Regression equation: $y = a + b \cdot \lg(x) + c \cdot \lg(x)^2$

4.1 Standard: Standard S

LINEARITY IS NOT CALCULATED!!

4.2 Sample: Preparation U

LINEARITY IS NOT CALCULATED!!

5 Test of Slope

Regression equation: $y = a + b \cdot \lg(x)$

5.1 Standard: Standard S

Total number of observations: 20

Source	d.f.	Sum of Squares	Mean Square	F-Value
Model	1	34,944,8000	34,944,8000	46,2695
Error	18	13,594,4000	755,2444	
Total	19	48,539,2000		

	Param. Estimates	Error	Quality of regression	
Intercept a	248,4000	8,6905	r^2	0,719929
Slope b	-41,8000	6,1451	r^2_{adj}	0,704370

Test passed if $F_{model} > F_{crit}$ ($P=98\%$) = 6,5146

Test PASSED!

5.2 Sample: Preparation U

Total number of observations: 20

Source	d.f.	Sum of Squares	Mean Square	F-Value
Model	1	31,920,0500	31,920,0500	44,2211
Error	18	12,992,9000	721,8278	
Total	19	44,912,9500		

	Param. Estimates	Error	Quality of regression	
Intercept a	244,0000	8,4960	r^2	0,710709
Slope b	-39,9500	6,0076	r^2_{adj}	0,694638

Test passed if $F_{model} > F_{crit}$ ($P=98\%$) = 6,5146

Test PASSED!

6 Test of Parallelity

6.1 Estimation of Common Slope

Total number of observations: 40

Source	d.f.	Sum of Squares	Mean Square	F-Value
Model	3	3,363,703,4750	1,121,234,4917	
Error	37	26,621,5250	719,5007	
Total	40	3,390,325,0000		

	Param. Estimates	Error	Quality of regression	
Intercept a1	243,0750	7,3459	r^2	0,992148
Intercept a2	249,3250	7,3459	Root MSE	26,8235
Slope b	-40,8750	4,2412		

6.2 Estimation of error due to non-parallelity

Total number of observations: 40

Source	d.f.	Sum of Squares	Mean Square	F-Value
Model	3	3,363,703,4750	1,121,234,4917	
Difference of Models	1	34,2250	34,2250	0,0463
Model (Total)	4	3,363,737,7000	840,934,4250	
Error	36	26,587,3000	738,5361	
Total	40	3,390,325,0000		

	Param. Estimates	Error	Quality of regression	
Intercept a1	244,0000	8,5938	r^2	0,992158
Intercept a2	248,4000	8,5938	Root MSE	27,1760
Slope b1	-39,9500	6,0767	g	0,0221
Slope b2	-41,8000	6,0767		

Test passed if $F_{\text{diff. of models}} < F_{\text{crit}}$ ($P=98\%$) = 5,9268

Test PASSED!

7 Estimation of the Relative Potency

Relative potency of sample **Preparation U** in comparison to standard **Standard S**

Preparation U vs. Standard S	
Estimated relative potency:	1,11
95% Fiducial Limits:	0,83 - 1,50
Relative fiducial limits:	74,6 % - 134,7 % (60,0%)

8 Calculation of EC50:

	Standard S	Preparation U
Method of EC50 calculation	n/c	n/c
50% Response of	-	-
EC50 concentration	-	-

9 Annotations:

Results of Assay: **Europ. Pharmacop. 1993**
Project: **Validierungsbeispiele**

Current user: Dr. Matthias Schmitt

.....
Date/Signature

1 Complete List of the Object Definitions:

	Standard	Sample
General settings:		
Code:	Standard S	Preparation Z
Title:	Standard S	Preparation Z
Measured substance:		
Batch identification:		
Object status declared closed:	NO	NO
Object was created by:	Dr. Matthias Schmitt	Dr. Matthias Schmitt
Creation timestamp:	16.06.99 15:14:55	16.06.99 15:14:58
Last modification by:		
Modification timestamp:	06.10.99 12:53:12	06.10.99 12:53:12
Definition settings:		
Concentration of undiluted standard:	1	-
Concentration units:		-
Predilution of standard/sample:	1:1	1:1
Number of dilution steps:	2	2
Number of data series:	10	10
Additional predilution factor:	1:1	1:1
Dilution scale:	direct input	direct input
Analysis settings:		
Analysis according to EP:	YES	YES
Dixon test enabled:	YES	YES
Regression model:	lin/log	lin/log
Reverse response effect correlation:	NO	NO
Dixon contamination:	0,02	0,02
F-Test of the slope:	98	98
F-Test of linearity:	98	98
F-Test of parallelity:	98	98
Fiducial limit percents:	95	95
Slope as rejection criterion:	YES	YES
Linearity as rejection criterion:	YES	YES
Parallelity as rejection criterion:	YES	YES
EC50 calculation:	NO	NO
by using:	-	-
Range Selection Settings		
Linear detection type:	Fixed range (1)	Fixed range (1)
Allocation Strategy:	-	-
Minimal/Exact # of Points:	-	-
Maximal Allocation Range:	-	-
Allocation Range:	Step 1 to Step 2	Step 1 to Step 2
Inclusion of 50% Response:	-	-
50% Response of:	-	-

2 Data Input: (Data index: x = technical outlier, o = outlier found by Dixon test)

2.1 Standard: Standard S

Step	X	log ₂ (X)	Series 1	Series 2	Series 3	Series 4	Series 5
1	1,0000	0,0000	289	221	267	236	250
2	0,2500	-2,0000	300	310	330	290	364

Step	X	log ₂ (X)	Series 6	Series 7	Series 8	Series 9	Series 10
1	1,0000	0,0000	231	229	269	233	259
2	0,2500	-2,0000	328	390	360	342	306

Step	X	log ₂ (X)	Mean	S.D.	C.V. [%]
1	1,0000	0,0000	248,400	21,996	8,9
2	0,2500	-2,0000	332,000	32,042	9,7

50% Response: 290,200

2.2 Sample: Preparation Z

Step	X	log ₂ (X)	Series 1	Series 2	Series 3	Series 4	Series 5
1	1,0000	0,0000	236	213	283	269	251
2	0,2500	-2,0000	250	268	273	240	307

Step	X	log ₂ (X)	Series 6	Series 7	Series 8	Series 9	Series 10
1	1,0000	0,0000	294	223	250	216	265
2	0,2500	-2,0000	270	317	312	320	265

Step	X	log ₂ (X)	Mean	S.D.	C.V. [%]
1	1,0000	0,0000	250,000	28,012	11,2
2	0,2500	-2,0000	282,200	29,234	10,4

50% Response: 266,100

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3 Range Selection

	Standard	Sample
Linear detection type:	Fixed range (1)	Fixed range (1)
Selected Linear Range:	Step 1 to Step 2	Step 1 to Step 2

4 Test of Linearity

Regression equation: $y = a + b \cdot \lg(x) + c \cdot \lg(x)^2$

4.1 Standard: Standard S

LINEARITY IS NOT CALCULATED!!

4.2 Sample: Preparation Z

LINEARITY IS NOT CALCULATED!!

5 Test of Slope

Regression equation: $y = a + b \cdot \lg(x)$

5.1 Standard: Standard S

Total number of observations: 20

Source	d.f.	Sum of Squares	Mean Square	F-Value
Model	1	34,944,8000	34,944,8000	46,2695
Error	18	13,594,4000	755,2444	
Total	19	48,539,2000		

	Param. Estimates	Error	Quality of regression	
Intercept a	248,4000	8,6905	r^2	0,719929
Slope b	-41,8000	6,1451	r^2_{adj}	0,704370

Test passed if $F_{model} > F_{crit}$ ($P=98\%$) = 6,5146

Test PASSED!

5.2 Sample: Preparation Z

Total number of observations: 20

Source	d.f.	Sum of Squares	Mean Square	F-Value
Model	1	5,184,2000	5,184,2000	6,3249
Error	18	14,753,6000	819,6444	
Total	19	19,937,8000		

	Param. Estimates	Error	Quality of regression	
Intercept a	250,0000	9,0534	r^2	0,260019
Slope b	-16,1000	6,4017	r^2_{adj}	0,218909

Test passed if $F_{model} > F_{crit}$ ($P=98\%$) = 6,5146

Test FAILED!

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6 Test of Parallelity

PARALLELITY IS NOT CALCULATED!!

7 Estimation of the Relative Potency

Relative potency of sample Preparation Z in comparison to standard Standard S

Preparation Z vs. Standard S	
Estimated relative potency: 95% Fiducial Limits: Relative fiducial limits:	ASSAY REJECTED RELATIVE POTENCY IS NOT CALCULATED!!

8 Calculation of EC50:

	Standard S	Preparation Z
Method of EC50 calculation	n/c	n/c
50% Response of	-	-
EC50 concentration	-	-

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Additional Annotations:

