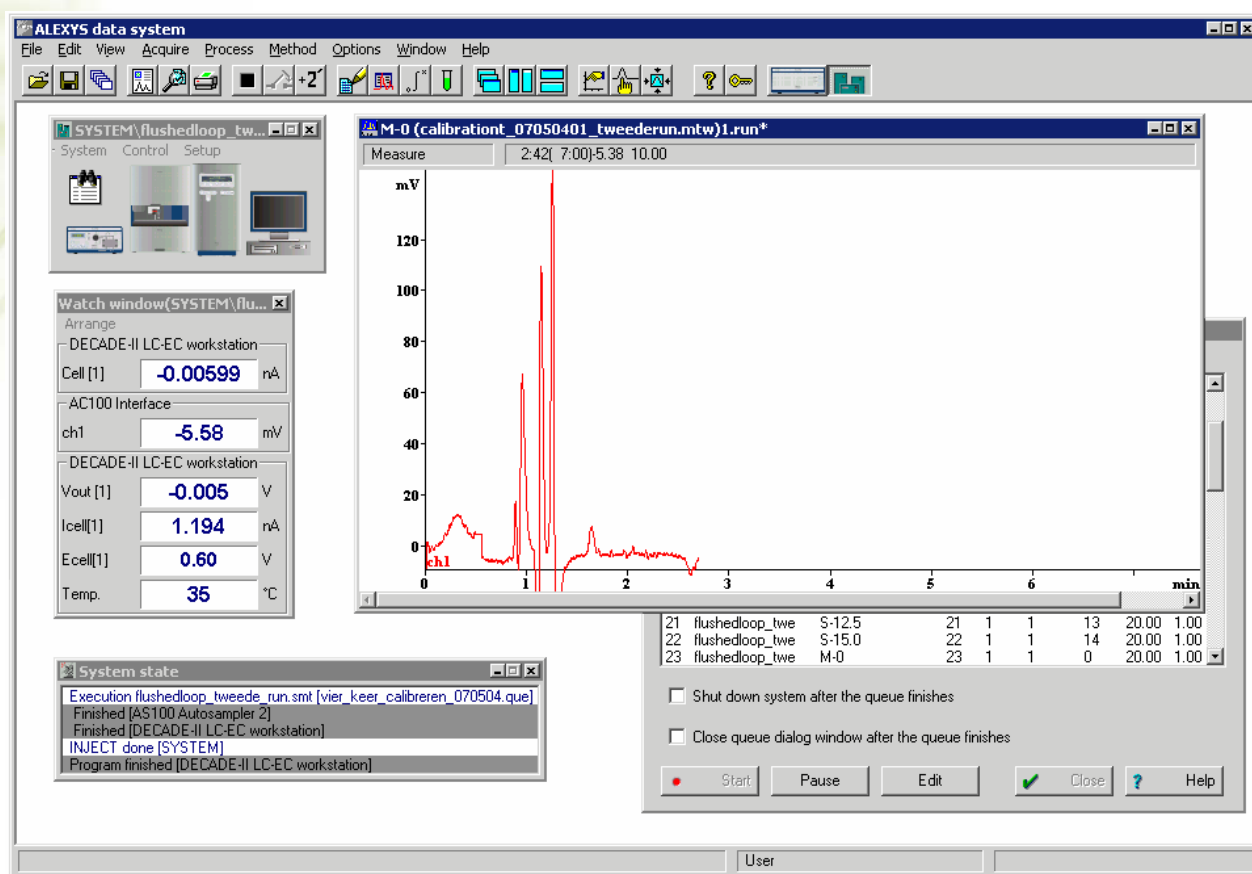# ALEXYS data system

user manual

Symbols

The following pictogram is used in the DECADE II manual:

 Caution / warning

Please read sections with a caution/warning sign carefully, they contain important information about the use of the software and its functionality.

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Table of contents

The purpose of this manual

This manual is intended to help learning and properly using the ALEXYS data system. It includes *Installation*, *Tutorial*, and *Procedures Reference* chapters. You will find a detailed description of the software capabilities here.

A short description of operations and dialogue boxes is available in the context-sensitive *Help* directory. If you are interested in the meaning of a parameter or menu item, just press **[F1]** key if the dialogue box is opened and you will find a detailed description on the subject. You can also open the **Help** menu and select the *Index* option or just press **[Shift-F1]**. The *Help Index* will pop-up then.

Please contact us in case of any difficulty you cannot overcome yourself. Write us about any problems you met in studying and using the software. It is important for us to reveal difficulties and wishes of our users to solve them in the updated versions of the software.

System Overview

C H A P T E R   1

Introduction

ALEXYS data system

The ALEXYS data system is the control centre for the ALEXYS 100 and ALEXYS 110 LC-EC system and can also be used to control the ALEXYS jr. The ALEXYS data system supplies a simple and intuitive user interface with full data processing functionality.
Specific features are:

- Two independent time bases, with up to 4 analogue channels
- Up to 16 devices for data acquisition and instrument control
- Additional device channels for temperature, pressure, cell current, % solvent A/B etc.
- Drivers for all ALEXYS components
- Scanning Voltammetry support
- 21CFR part 11 support
- Sequence programming ('sample queues')
- Batch processing
- Easy report generator

The software has been designed with Good Laboratory Practice (GLP) and Good Automated Laboratory Practice (GALP) standards in mind. The software has 21CFR Part11 support options available.
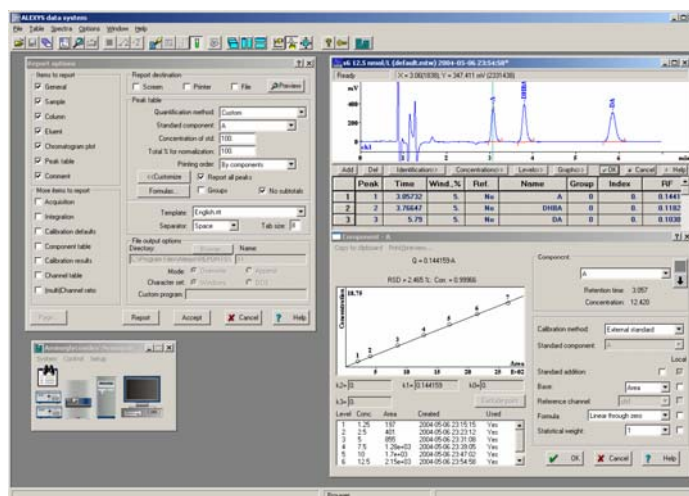
Fig. 1. Top: ALEXYS 100 LC-EC System. Lower left-side: ALEXYS 110 system. Lower right-side": ALEXYS jr.

Note that besides the ALEXYS 100, 110 and jr device drivers (DECADE II, AS 100, AS 110, AC 100, 24-bit AD converter LC 100 device driver) also a large number of third-party device drivers for auto samples, optical detectors and pumps are available in the ALEXYS data system software.

The third-party device drivers are provided "as is" and are not tested by Antec Leyden. These drivers could contain bugs and proper & reliable operation of these drivers in the ALEXYS data system cannot be guaranteed.

C H A P T E R   2

Hardware overview

For proper operation all instruments must be either connected directly to the computer via available COM or USB ports or through the AC 100 acquisition controller (ALEXYS 100 LC-EC system only).

AC 100 acquisition controller

Up to two independent LC systems can be served. The AC 100 contains 4 analogue inputs (24-bits ADC) for data acquisition. Up to 16 devices can be connected through RS232 for digital data acquisition and instrument control. In addition, 2 inputs and 14 outputs are provided to deal with external events.

The ADC channels are shared: any ADC channel can be used by either controlled system, i.e. systems with 1, 2, 3, or 4 analogue channels can be defined. The AC 100 is connected with the computer via a single RS232 port.
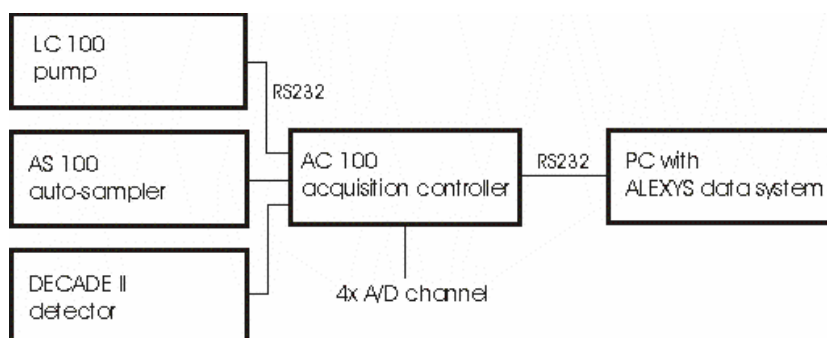
Scheme 1. ALEXYS 100 LC-EC system with AC 100 controller.

IMPORTANT: ALEXYS components must be connected to the first 3 ports of the AC 100 in D – A – P order: detector, autosampler, pump to be compatible with pre-configured systems on the CD-ROM supplied.

The ALEXYS data system is compatible with Windows 98, Windows Me, Windows NT, Windows 2000, Windows XP and Vista. The 21CFR Part 11 support options are only compatible with *Windows 2000, Windows* XP (Professional Edition) and Vista and requires the NTFS format.

USB connection kit

By means of one USB connection kit (p/n 190.0200) and a free USB port on the PC a complete ALEXYS 110/jr system can be served. The USB hub contains 4 ports to which up to 4 USB-to-Serial connectors (p/n 250.0123) can be connected. In case additional ADC channels are required an optional 24-bit A/D converter can be connected.
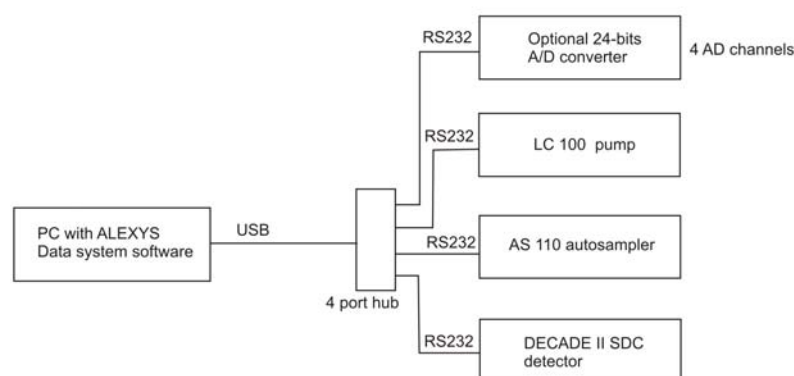
Scheme 2. ALEXYS 110 LC-EC system with USB connection kit.

Analogue-to-Digital Conversion (ADC)

An *Analogue-to-Digital Converter* (ADC) is used to convert analogue signal of e.g. a detector into a digital format to enable computer processing. The *dynamic range* of an ADC is the ratio of the maximum input signal that can be measured and the minimum signal change that can be detected and is usually expressed in orders of 2 ('bits'). Thus, a dynamic range of 20 bits equals to $2^{20} = 1,048,576$, while 24 bits equals to $2^{24} = 16,777,216$. On a full scale of +/- 1 Volt this means that steps as small as 2/16.7 M = 0.12 µV can be measured.

The *sampling rate* must ensure 20 up to 30 measurements on half-width of the narrowest peak in the chromatogram. A typical sampling rate is 5 - 10 Hz for capillary gas chromatography and 1 - 2 Hz for liquid chromatography. In some cases a higher rate is necessary.

Many detectors are equipped with a digital data output. To enable digital data collection, the detector must have a serial port and a detector-specific device driver must be available in the data system. The ALEXYS 100 data system contains all necessary ALEXYS 100, 110 and jr drivers.

C H A P T E R 3

Systems

The ALEXYS data system uses the S**ystems** and **Methods** concept for instrument control, data acquisition and processing. Systems define the hardware configuration and settings. Methods comprise the information required for data processing (analysis description, calibration, quantisation, report parameters, etc.). In this chapter systems are dealt with. If all ALEXYS hardware is connected to the AC 100 in a pre-defined way, a system is installed by loading the system file from the CD-ROM. The same holds for an ALEXYS 110 system. Only in this case the AC 100 is not present and the ALEXYS hardware is directly connected to PC COM ports in a pre-defined way.

Equipment control

Usually a driver belongs to a physical device (such as pump, detector, AD converter, etc.), controls it and interfaces it with the main program. Some drivers are computer created *virtual devices,* such as *Recorder*, *Watch window*, *Timer*.

A driver has a default parameter set to be sent to a real device when its job starts. The driver can give information (data channels) or resources (COM ports, control lines) to other drivers, and can be in turn controlled (started, stopped, filled with parameters, etc.) by other drivers.

Drivers can be organised in a group, called *System*, being a driver set describing a hardware configuration and synchronised hardware control actions. There is a special wizard that helps to create a *System*. A typical *System* consists of the following drivers: *Watch window*, *Recorder*, *Solvent delivery unit*(s) for one or more pumps, and *detector*. If a *System* is opened, a special *System window* appears.

Example of ALEXYS 100 system file.

Example of ALEXYS 110 system file.

A device driver can be opened by double-clicking the left mouse button or via a context menu that pops up by clicking the right mouse button. A typical driver includes dialogue pages for manual control. It also includes an initial parameter set, i.e. parameters loaded to the device at the *System* initialisation and time programming.
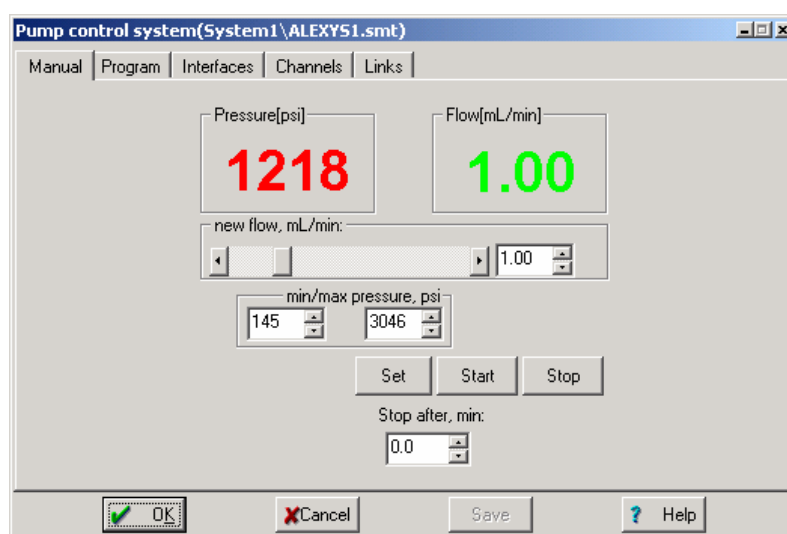
«Channels» and «Links» pages are only accessible at the *Administrator* level. The «Channels» page contains information on all values that are measured and reported by the driver. The «Links» page reports all resources used by the driver.
The devices in a *System* can be divided into two groups: those with the '*Start with determination'* event (immediately after the System starts) and those starting with the 'S*tart with inject'* event.
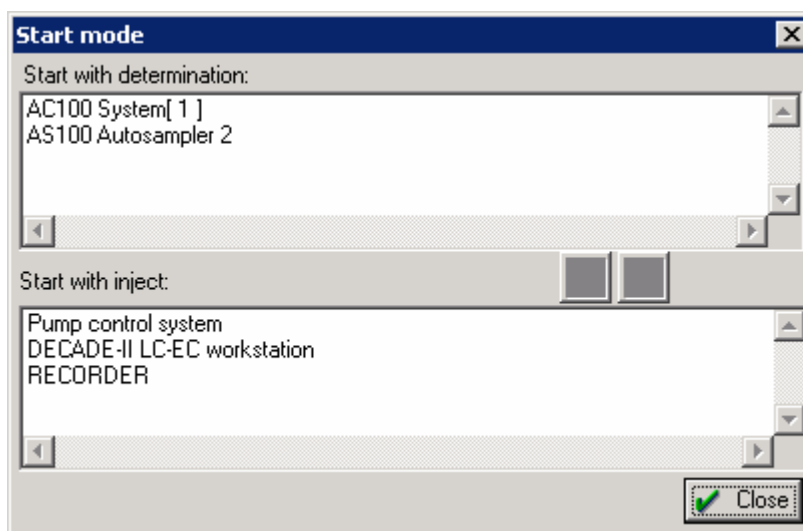
The '*Start with inject'* event is usually evoked by the ADC device or auto sampler. Thus, the AC 100 interface for instance is able to generate an *inject* event if either the «*System 1*» or «*System 2*» button on the front panel is pressed or by an external Start/Stop event. Devices like the AS 100, the DECADE II detector can also be used to generate the *inject* event.

If the *inject* event is activated, it is transferred to all other devices in the *System*, both physical and virtual. If the *chromatogram* was started, a repeated *inject* event signal stops data acquisition.

Some drivers are installed *extra-system* (i.e. can be *shared* between several *Systems* or other drivers). Normally these are AD converters and interfaces providing data channels and/or COM ports to other devices. The AC 100 interface is a shared device.

System window functions

A *'System'* appears on the desktop as a *System window*. A system only controls instruments if *'connected to workspace'*. If '***Control/Connect to workspace'*** operation is performed, the System checks all connections and resources and is able to control equipment on-line. If a System is loaded from disk, ALEXYS data system tries to connect it automatically.

If everything is <OK> (meaning that all necessary COM ports, data chan-
nels and hardware were found), the System is ready (Messages in **yellow**
background appear in the **«***System state***»** window for all drivers that are
being checked. When checking is completed, a message with a grey
background like "*On-line AC 100 interface*" is shown). Otherwise such
messages as '*No response'* or '*Detection of hardware failed'* are shown
with a **red** background. All successive operations are plotted in grey and
are removed from the "System state" window. Some important messages
such as *Waiting to inject* are given on a **green** background.
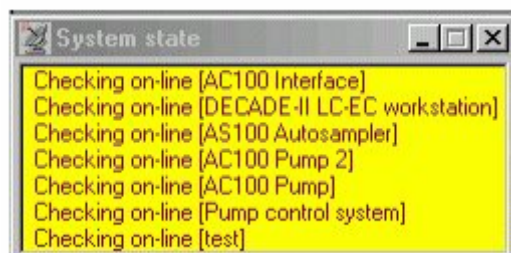
If a System is connected to workspace, a special icon is put in the
icon bar of the main window. This icon allows to close the System and to
re-open it without parameter loss. Several Systems can be opened at the
same time.
It is impossible to connect two identical Systems or Systems to workplace
that use at least one common device or port: all connected Systems (and
devices) must use different resources!

System window menu

System submenu

Save: saves *System* configuration (and settings of all its drivers) in «***.SMT**» file.

Change: loads a new *System* to the current *System window*.

Open other: opens an additional *System window* (operates similar to **File / Open / System** function in the main menu). If the new System uses the same resources or equipment as the existing one, it is left unlinked.

Sample Queue: opens an existing *Sample queue* or creates a new one (if a new filename is entered).

Close: Closes the current System. Both the *System window* is closed and the corresponding *System* icon is removed from the icon bar. If the System has been modified, software prompts for saving.

Control submenu

Start determination: starts a new run, opens (a) *chromatogram window*(s), and plots a baseline while waiting for '*inject event*'.
If the «*Verify sample*» item is checked in the current submenu, the «*Edit sample description*» dialogue is opened. If the sample description is accepted, the system changes its status to «*Waiting for inject*».
If the «*Watch window*» item is checked in the **Setup** submenu, *watch window* is opened.
All drivers that were set as «Start with determination» in the **Setup**/*Start mode* box, start their time programs. All other drivers are set to their initial parameters.

Stop determination: finishes the current chromatogram and aborts all running device programs.

Stop data acquisition: finishes the current chromatogram (stops data acquisition) only. All running device programs are continued.

Start-up hardware: starts all devices within the system without starting any device programs, starts pumps, switches on lamps, etc.

Shutdown hardware: stops all device programs, stops pumps, switches off lamps, etc.

✓ *Auto restart*: if set, this switch restarts the system immediately after the current run is over. This option performs an automatic «Start determination» procedure.

✓ *Verify sample*: if set, this switch evokes an «*Edit sample description*» dialogue box to enter a sample description manually before the run.

✓ *Connect to workspace*: Only a connected system can control equipment so a system must be connected before it can start a run. The connection procedure implies that the system verifies all its connections and resources, and if found *<OK>* (all necessary COM ports and data channels were found) it switches to the «Connected» state. If the *System* is connected to workspace, its icon is placed to the *icon bar*.

✓ *Disconnect from workspace*: breaks all connections and frees resources for use by other systems. Disconnected (off-line) system allows modifying start-up values and programs of devices, but it does not allow controlling any devices directly.

Setup submenu

✓ *Drag icons*: a switch that allowing to change position and size of bitmaps inside the *System window* as well as the *System window* size itself. So, the System can be organised similarly as on the bench. Use the left mouse button to perform these operations.

Zoom in / Zoom out: these items allow to change size of the entire *System window*. Each operation can be performed several times.

✓ *Watch window*: this switch shows, whether *Watch window* should be opened every time the *System* starts. If this switch is off, *Watch window* can be opened later manually, by double-clicking *Watch* bitmap.
Start mode: open the two-pane window where all devices of the system are classified into two groups: those having *time programs* start *with Inject*, and those, which start time programs *with determination*. Any selected item can be moved between groups, using the drag-and-drop procedure or by clicking arrow buttons.

New devices: a wizard, that allows adding new devices to a System.

Install new device: adds an additional new driver to your System. Choose this item to start «New device» wizard.

Link to existing device: to select a shared device and to add it to a System. Shared devices are available for all Systems opened on the desktop. As a rule, these devices are ADC channels and COM ports.

Parameters: a submenu, that allows printing initial settings of all devices and corresponding time programs (in parts or all together).

Print: prints a report on actual system settings. The WordPad or NotePad text editor is opened for this purpose.

Start-up hardware: shows initial hardware parameters (i.e. parameters that are set when «Start determination» command is executed).

Start with determination: a list of drivers whose programs are started *with determination*.

Start with inject: a list of drivers whose programs are started *with inject event*.

Links: this window lists all resources that are currently used by the *System*. Double click on the desired item opens a corresponding device driver.

C H A P T E R   4

Methods and chromatograms

The ALEXYS data system uses the **systems** and **methods** concept for instrument control, data acquisition and processing. Systems define the hardware configuration and settings. Methods comprise the information required for data processing (analysis description, calibration, quantisation, report parameters, etc.). In this chapter methods are dealt with.

Chromatograms, Methods and Systems

A system stores information on the hardware configuration, including the parameters used for hardware control and data acquisition. It also determines the relation between device drivers and stores equipment settings and control programs. *Systems* are stored as ***.SMT** files. As a rule, each *.SMT file corresponds to a separate chromatographic system or its configuration needed for a series of analyses. *Systems* do not contain information on the data processing algorithms but include a reference to the *Method* file that will be used for data processing.

It is allowed to change the Method attached to the current System. This does not change the System hardware settings. Each *System* can include more than one *Recorder* driver, i.e. can start more than one chromatogram from different detectors[1]. Each chromatogram in this case is started in its own window and is processed by its own *Method*. Each opened *System* is present on the desktop as a separate *System window* that can be used to edit hardware configuration and parameters, and/or to control chromatographic processing.

The analysis Method comprises all information required for data processing (analysis description, calibration, quantification and report parameters, etc.). *Methods* are stored as ***.MTW** files. If a *Method* is loaded, a *chromatogram window* is opened on the desktop. Initially the Method only includes data processing information. *Method* in the current ALEXYS data system version (version 1.73 and up) also includes a complete local copy of the System used for its development. These data are needed for identification and calibration purposes and are valid if equipment configuration and equipment control parameters are fixed. This Method's local *System* can be opened and restarted.

---

1 It is possible to start two chromatograms from the single data source (for example, they can have different scales)

All data and relevant information for each chromatographic run are stored in the corresponding Chromatogram file. A *Chromatogram* includes both the *Method* and the *System* used for data acquisition, hardware control and data processing. The Filename of the chromatogram is created automatically on the basis of date and time of the start of the run and has a ***.CHW** extension. Management of data files is provided through the **File/Open/Chromatogram** menu. You can choose the desired chromatogram from the list by its description and open it for inspection and reprocessing. This can be done while chromatograms are running. *Systems* and *Methods* used for data acquisition, chromatogram processing, and equipment control are attached to raw data, and can differ from that saved in the corresponding ***.MTW** and ***.SMT** files. By loading a chromatogram from disk you will get the same *Method* and the same *System* that was used last time to measure and process it. This feature is in full compliance with the *GLP* requirements, providing capability to reinvestigate data after analysis and to repeat the analysis if necessary.

Systems, Methods or chromatograms can be used to start new analyses. A separate System (*.SMT) file is created for each hardware configuration. For example, it is possible to exclude instruments that are not used in the current series of analyses, and to save the resulting configuration under a new name.
Within a single hardware configuration it is possible to have several Methods. Each Method is dedicated to a specific analytical task, and it contains all necessary hardware and data processing parameters. Methods are used to restart a series of similar analyses, with the same settings and calibration.

Method structure

A *Method* is divided into several sections. Each section corresponds to one item of the **Method** menu.

- Passport *(Operator's notebook).* It includes *header*, *sample description*, *user's name*, *column, eluent description*, etc.; note that some *Passport* parameters are used for quantification or calibration procedures.

- Method setup. Allows to select a *noise filtering method*, to set *post-run operations*, to select common equations, etc.

- Channels setup. This section contains description of all data channels and calculated channels. They can be edited here.

- Integration. This section describes the algorithm for peak detection; *integration events* are also set here.

- Calibration. In this section *Component table*, *Concentrations table* and *Calibration plot* for each component can be created and edited. Parameters for component identification and quantification are included as well.

- Report options. Parameters to customise the report appearance are listed here.

- System. This section enables tuning the *System* used in the current chromatogram. Note that a copy of the *System* is stored in the *Chromatogram* and in the *Method*.
  A copy of the *System* that is stored in the chromatogram (*Method*), is local, i.e. it only holds for this chromatogram . This local System can be used to restart the chromatogram (or *Method*). In this case the new chromatogram will use exactly the same *System* and *Method* parameters as the parent chromatogram.

ALEXYS data system desktop and main menu

Once you have started the ALEXYS data system and are "Logged-in", the
Desktop will appear on the screen.
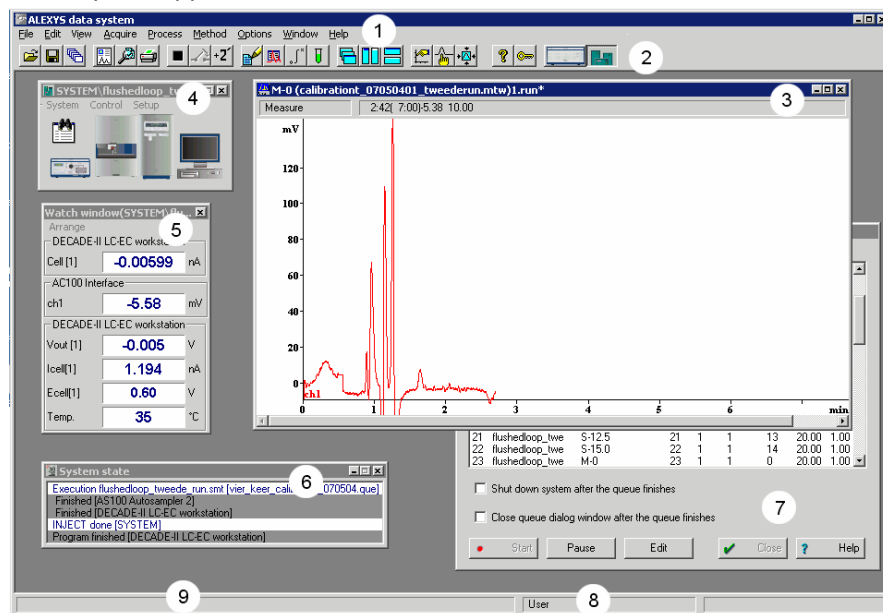
*Fig. 2. Main window with menu (1), button bar (2), chromatogram window
(3), system window (4), watch window (5), system state window (6), queue
window (7), current user (8) and comments (9).*

The Desktop has several fields. The upper line is the standard Windows
header. The second line contains the Main Menu. All control functions are
accessible via this menu. A detailed description of the *Main menu* is given
in the context-sensitive *Help* (press F1 to start Help).
The most common operations are grouped on the icon bar. Click on the
appropriate icon to activate the desired function or operation. There are
bitmaps of active *Systems* and *shared interfaces* on the right side of the
Icon bar.
The bottom line of the desktop includes two fields. The *Help line* field con-
tains a short description of the selected menu item, system's explanation
or advice for the user. The right field shows the current user's name.

All other space of the desktop is the working area and can contain one or more Chromatogram Windows. Their number is restricted by available memory and normally cannot exceed 100[2].

Each chromatogram settles down in its window. One of them is *active* (current). All manipulations are only possible with a chromatogram in the active window (several chromatograms can also be processed simultaneously by using *Batches*). To make a window active, place the cursor inside the desired window and click the left mouse button. It is also possible to switch between *chromatogram windows* by pressing the keyboard combination [Ctrl] + [F6].

System window, Watch window, and System state window can also be opened in the desktop working area. The *System window* shows the current hardware configuration and is used to control it. The *Watch window* displays current values of device parameters and detector signals. The user can define a set of such parameters. The *System state window* contains a list of active System drivers. Operations that failed are marked red; correct operations are marked yellow. If the System is ready for injection, a green colour appears.

 There are three standard ways of windows layout on the screen: cascade, vertical mosaic and horizontal mosaic. Each one can be selected through appropriate icons or commands of the **Window** menu. These operations arrange all opened *chromatogram windows*, *System windows*, and *Watch windows*.

---

2 Windows uses disk space to increase amount of available memory (it is called "virtual" memory). The maximum number of simultaneously opened chromatogram files depends on default Windows settings.

Cascade

(**Cascade**) - cascades all opened windows except for *System state window*. It is a default setting. The last active window also stays the last in the cascade. Opened *System windows* are cascaded also.

Horizontal mosaic

(**Tile horizontal**) - arranges all opened *chromatogram windows* as a horizontal mosaic. The last active chromatogram will be the lowest. If more than 3 chromatogram windows are opened, a combination of horizontal and vertical mosaic is used. The procedure does not influence the position of the System window.

Vertical mosaic

(**Tile vertical**)- arranges all opened chromatogram windows as a vertical mosaic. It does not interfere with the position of the System window.

A horizontal or vertical mosaic is especially convenient to visually match similar chromatograms. If using the combo box **View** / *Appearance* / *Set All* it allows a common scale, colour or other appearance attribute setting for all chromatograms simultaneously. The *System state window* can be minimized in this case.

The chromatogram window
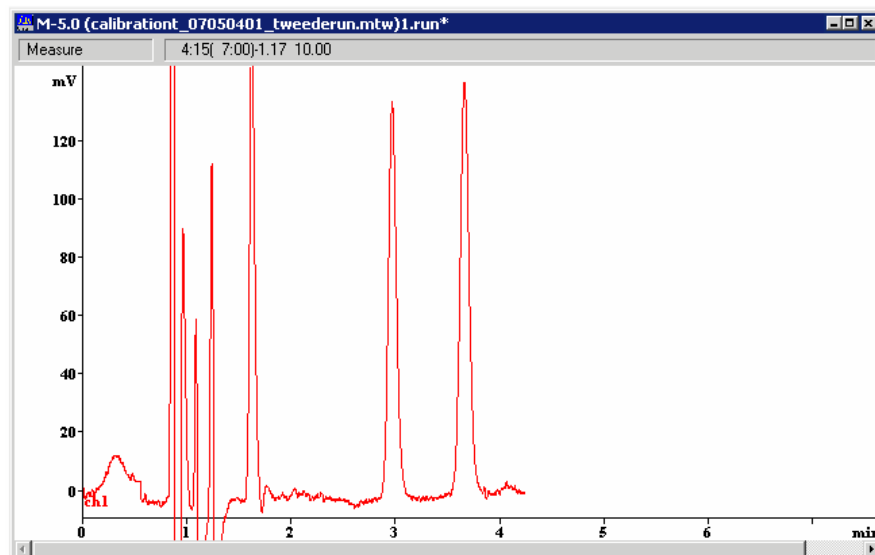
Each *Chromatogram window* contains one chromatogram (it can be either currently running or read from disk). All data processing and manipulation functions are only available for the chromatogram in the current window. Each *Chromatogram window* has a Header, Status and Channels bar and a Chromatogram area.

The Header bar comprises:

1) The System menu button opens the standard Windows system menu allowing to *move, size, minimize* and *close* the window.

2) The name of the chromatogram name is composed of passport header, working processing methods and name of the chromatogram file.

(*Close, Minimize* and *Maximize* buttons) are standard Window buttons.

The Status bar consists of a *Status* field and an *Information* field. The Status field displays the status of the chromatogram and some error conditions.

The Information field contains the following information:

1) when data acquisition is active, the detector output on the *current channel*, run time, time left to the chromatogram end, and preset data acquisition time are shown.

2) If the Peak editor mode is active, coordinates of the cursor are shown. The position on the X axis is given in selected retention units and number of measurements. The position on the Y axis is given for the current channel.

The scroll bar appears if only a part of the chromatogram is displayed in the window. Use arrows or mouse to scroll through the chromatogram.

To switch between chromatogram windows, click the desired one by mouse or enter the **Window** menu and select the desired one. Press [Ctrl-F6] to list windows.

The current *Chromatogram window* can be moved or resized. To change size of the window, use mouse and drag the corner or side of the window in any direction. To move a window to the different position drag its title bar to another position.

It is possible to *minimise* the current chromatogram window by clicking the ▬ button in the upper right corner. A running chromatogram can also be minimised. The minimised chromatogram windows are located at the bottom of the desktop area as icons.

Each icon contains a short header of the chromatogram. To see the complete header its necessary to place mouse cursor on it.

You can *restore* or *maximise* any one by clicking buttons 🔲 or 🔲 with mouse or by double-clicking the header of the minimized window.

Dialogue boxes

Dialogue boxes are used throughout the ALEXYS data system to provide access to user-selectable parameters. Some dialogue boxes are composed of several *dialogue pages*. Each page has a bookmark with its name. Click on the desired bookmark to choose the corresponding dialogue page.

Basic elements of the dialogue box are *text fields*, *numerical fields, lists*, *check boxes*, *switches*, and *command buttons*.

Text fields accept input of arbitrary text and are descriptive. Numerical fields accept input of numbers only. To accept the entered value just pass to the following field. It is possible to move to the field of interest by pressing the [Tab] key, or by mouse clicking on the desired field.

Lists have the button ▼ on the right and allow choosing a value among acceptable ones. Click on the button ▼ and select a value from the list.

(☑,☐) Check boxes accept only two values: on / off. The check boxes are marked by a grey or white square ▢. Each check box is set irrespective of

a state of other check boxes. Click on a box to invert its value. If the check box is set, a tick ✔ appears inside.

(⊙, ○) Switches allow selecting only one of the given variants. If a switch is set it is marked by a dot inside.

Each dialogue box contains command buttons located in the lower or right part of the dialogue window. At pressing such button the appropriate operation will be executed. Some buttons can execute other dialogue boxes.

The following *command buttons* are frequently used:

✔ OK	It accepts all changes and exits the dialogue box. The [Enter] key usually does the same as *<OK>* button.
✖ Cancel	Closes the dialogue box and discards all changes. [*Esc*] key or ⊠ button at the upper right corner of the dialogue box does the same.
Apply	Accepts all changes without exiting the dialogue box. This button can be replaced by the *Save as* button, in case of **Options/*Global preferences*/☑ *Save object to file* flag is set
? Help	Invokes a context-sensitive help (the same as [*F1*] key pressing).
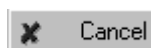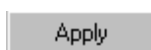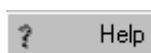
Method setup

General | Measure | Filters | Processing | Export | Math

Title: Standaard 2.5 nM   Duration : 7 ⬍ min

METHOD: D:\temp\040001JdJ070504\METHOD\calibrationt_07050401_tweederun

DATA: D:\temp\040001JdJ070504\DATA\

Date/time: 07/05/2004 19:04:13   Last update:

Calibration level: 0

User: User

Detector: Antec Decade - II

1

Run: 243

Batch: 0/1

✔ OK   ✖ Annuleren   Toepassen   ?   Help
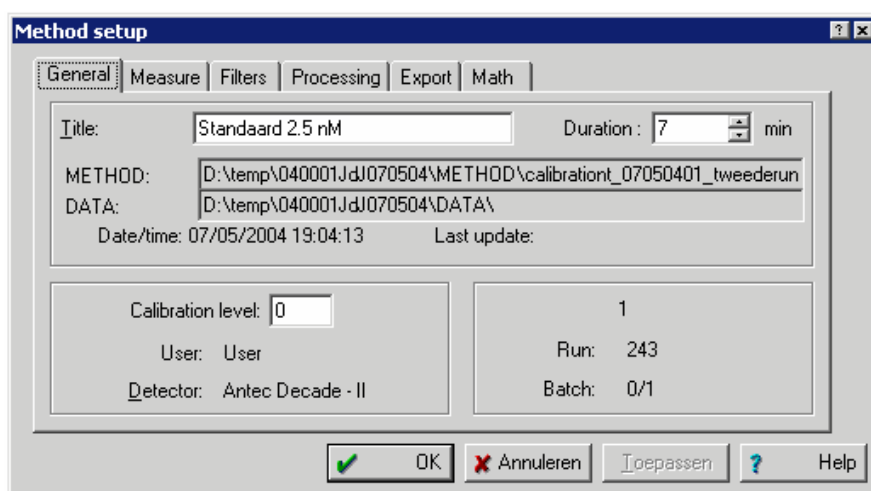
Data processing

Data processing features include common procedures like *Noise filtering*, *Peak detection*, *Peak identification and Quantification*, *Reporting* and special procedures, being described later. Most of the data processing options are performed automatically when a chromatogram finishes. In addition, some data processing procedures can be performed at any moment on the user request. Acquired data are stored in the computer's memory as raw data points. *Noise filtering* procedures may be performed to improve the signal-to-noise ratio. There are several powerful filtering algorithms in the ALEXYS data system*: Spikes filter, Median filter* and *Gaussian filter*.

Peak detector uses the value of the first derivative to detect peaks. It can be tuned both by default *integration parameters* and by *integration events.* If the results of peak detection are not satisfactory to tune integration tuning is not wished, the *Peak editor* can be used. This feature allows easy manual peak handling.

Peak identification is executed in the *Component table* that includes component names, retention times, calibration coefficients, retention indexes, etc. A *Component table* is created on the basis of a *Calibration* procedure. Both *Single-* and *Multi-level* calibrations are possible in using *Absolute*, *Relative* or *Tabulated response factors*. The *Component table* is part of the *Method*, and it is stored in a chromatogram file.

The Report function allows extensive customisation. The report can be directed to a file (and further executed by any word processor) or exported to popular spreadsheet or database programs. Peak grouping is also possible for advanced reporting. The *Preview* function is available for convenience. Some advanced procedures such as processing of multi-channel chromatograms, *batch reprocessing*, etc. can be executed as well.

Installation and configuration

C H A P T E R   5

Installation and Configuration

Hardware and System Requirements

Computer Requirements

Minimum specifications for basic configuration
- 800 MHz Pentium computer with 256 Mb memory
- Windows 98, Windows NT, Windows 2000, Windows XP or Vista operating system. The 21CFR part 11 support version only operates under Windows 2000 or Windows XP Professional Edition using an NTFS disk partition
- 15 Mb free space on hard disk (is needed for installation only)
- 100 Mb free space on hard disk drive for data storage
- Monitor resolution 1024x768 with 16-bit colour depth
- One free RS232 port to connect the AC 100 interface or 3 - 4 free RS232 ports to connect all equipment to the PC directly*
- One free USB port to connect a USB license dongle

* The serial ports can either be true COM ports (9-pins sub-D) or virtual COM ports created by Serial-to-COM converters which can be purchased from Antec Leyden (p/n 250.0123). In that case free available USB ports are required.

> **Only use Serial-to-COM converters purchased from Antec Leyden to avoid incompatibility problems. Although the use of Serial-to-COM connectors is tested thoroughly, we cannot guarantee that it works properly will all PC configurations & Operating Systems. In that case install true COM ports by means of serial PCI cards.**

Shipment review

The ALEXYS data system set includes following parts:

- Installation CD with ALEXYS data system and drivers
- USB license dongle
- User manual

It is not possible to use the software without the provided USB license dongle. Always install the dongle drivers first prior to installation of the ALEXYS data system. The drivers can be found on the installation CD. Plug the key into a free USB slot.

Software upgrade to version 1.2xxx

Users which work with older versions of ALEXYS (1.1xxx) and want to upgrade to ALEXYS version 1.2xxx need to get a new activation key for their dongle. Without activation key it is not possible to work with ALEXYS 1.2xxx. This activation key is free and can be requested on the Antec web site on the following location:

http://www.antecleyden.com/cs/activate.shtml

Please follow the procedure below to obtain your free key:

Step 1 - read out the dongle serial number
Install the new software on another PC. Open your new ALEXYS 1.2 software, goto menu Help/About and read the license dongle serial number. The serial number is 1 character followed by 10 digits. This 10 digit serial number can not be read using the old ALEXYS software (the old ALEXYS shows another 5 digit number). Fill in the request form on the above-mentioned web location (user support account is required, please register for free if you do not have an account yet). The activation key will be returned to you by e-mail within a few working days.
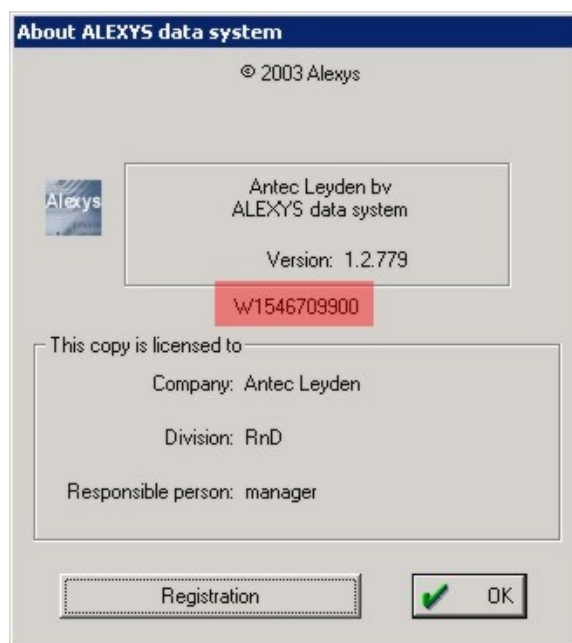
Step 2 - activate dongle with activation key
You will receive an activation key by email. Click 'Registration' button and
import the license key to activate your dongle.
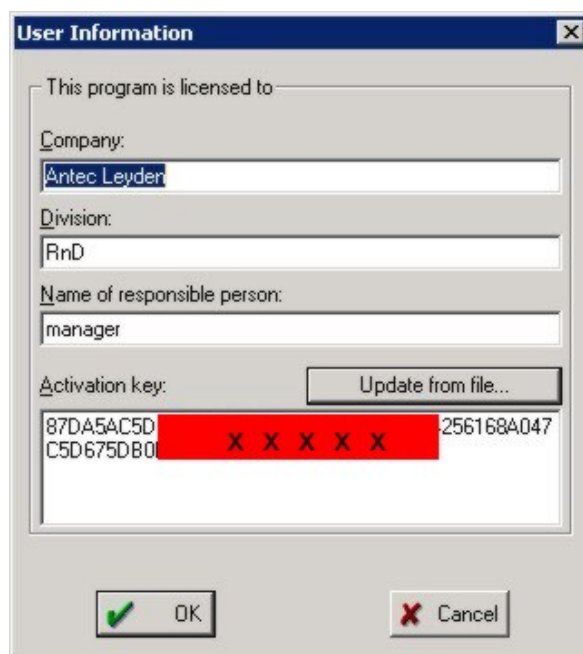
The activation key will be stored in your dongle. Once activated, this don-
gle can be used on any PC with ALEXYS version 1.2xxx.

Hardware Installation

Installing ALEXYS system with the AC 100 controller

The hardware must be installed as described in the respective user manuals. Installation of the AC 100 acquisition controller is described in the AC 100 user manual. Important for successful installation of pre-defined system drivers is the DAP order of connecting devices.

Fig. 3. Rear panel connectors AC 100. D - A - P refers to detector, auto sampler and pump. 'PC' is connection with computer; 'mains' is connected to OR 100.

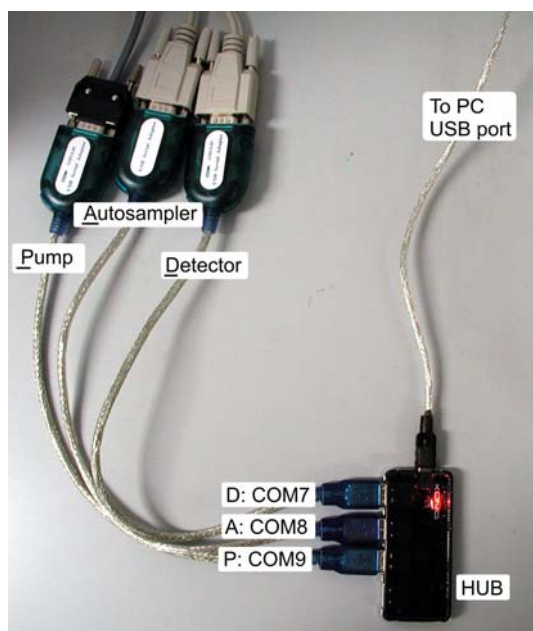Use Antec-supplied RS232 cables to connect different devices:

Device	RS232 cable (p/n)	AC 100 port
PC	183.0502	PC
DECADE II	250.0122	Dev 1/5
AS 100 autosampler	181.0596	Dev 2/6
LC 100 pump	182.0544	Dev 3/7

Installing ALEXYS system with direct PC connection

The hardware must be installed as described in the corresponding user manuals of the ALEXYS system components. Installation of an ALEXYS system directly to (virtual) PC com ports is described in the USB connection kit manual (p/n 190.7004). Important for successful installation of pre-defined system drivers is the DAP order of connecting devices.

Check for D – A – P (Detector – Autosampler – Pump) order of connecting devices to the USB hub.

Use only Antec-supplied RS232 cables to connect different devices:

Device	RS232 cable (p/n)	PC port
DECADE II SDC	250.0122	COM7
AS 110 autosampler	191.0528	COM8
LC 100 pump	182.0544	COM9

In case of an ALEXYS 110 system with two pumps, connect the second pump to COM10 (D-A-P-P configuration).

Software Installation

The ALEXYS data system runs under Microsoft Windows 98, *Windows NT*, *Windows* 2000, Windows XP or Vista. Note that the NTFS file system is mandatory in case the ALEXYS data system is installed with the 21CFR part 11 support option. The license is protected by a USB dongle.

The installation procedure must be started with loading the dongle drivers. Do not plug-in the dongle before installation of its drivers.

Installation of USB dongle:
1. Run the program HDD32.exe
2. Reboot the computer
3. After reboot plug-in the USB dongle to complete the installation.

To install the ALEXYS data system on your computer's hard disk:
- Before installation, make sure that the Windows operating system is properly installed including all relevant Service Packs. Installation must be done using an account with administrator rights.
- Run **Setup.exe** from the installation CD-ROM and follow instructions during the installation. If necessary, check ☑ *21CFR Part 11 support* flag to install this option. Note that working under *21CFR Part 11* includes a number of restrictions. For further information about 21CFR part 11 see chapter 6.


If not required, it is recommended NOT to install the 21CFR Part 11 option as it comes with a number of restrictions.

If the installation procedure is completed you have access to the ALEXYS data system directory with several sub-directories containing all necessary system files. The new program folder "Antec Leyden Software/ALEXYS data system" with ALEXYS data system icon will appear in the *Start* menu / *Programs* folder.

Check www.antecleyden.com (download & support section) for updates of software & drivers.

Software Qualification

To ensure that the software is correctly installed and the end-user(s) is/are adequately instructed there is a Installation Qualification procedure available at Antec Leyden:
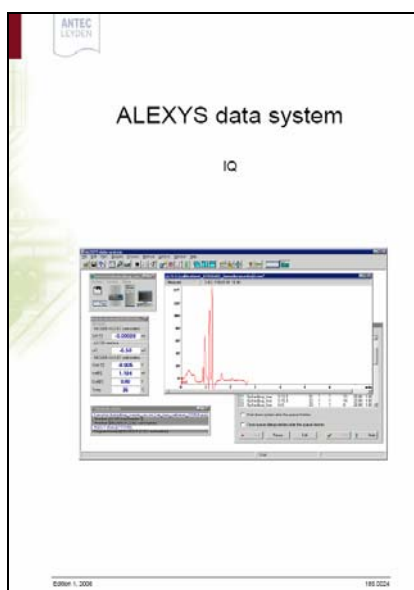
p/n 185.0022   ALEXYS data system IQ

Furthermore there is also Operational Qualification procedure available at Antec Leyden. This procedure checks the proper operation (correct integration) of the ALEXYS data system software.

p/n 185.0022   ALEXYS data system OQ

The objective of this Operational Qualification of the HPLC software is to ensure correct integration of measured chromatography data. The test is based on an EU reference chromatogram (TestA), which was issued for the integrator test for project EU-2026 (HPLC reference column). Integrator test according Quality Manual Part II SOP, EU SM&T CT95 No. 2026. The test does not check any hardware.

For more information about Qualification procedures please visit our web site: www.antecleyden.com

IQ software utility

Upon installation the ALEXYS data system automatically installs a software Installation Qualification utility to verify that all software components are correctly installed. A report is generated and can be printed out (see screen dump below).
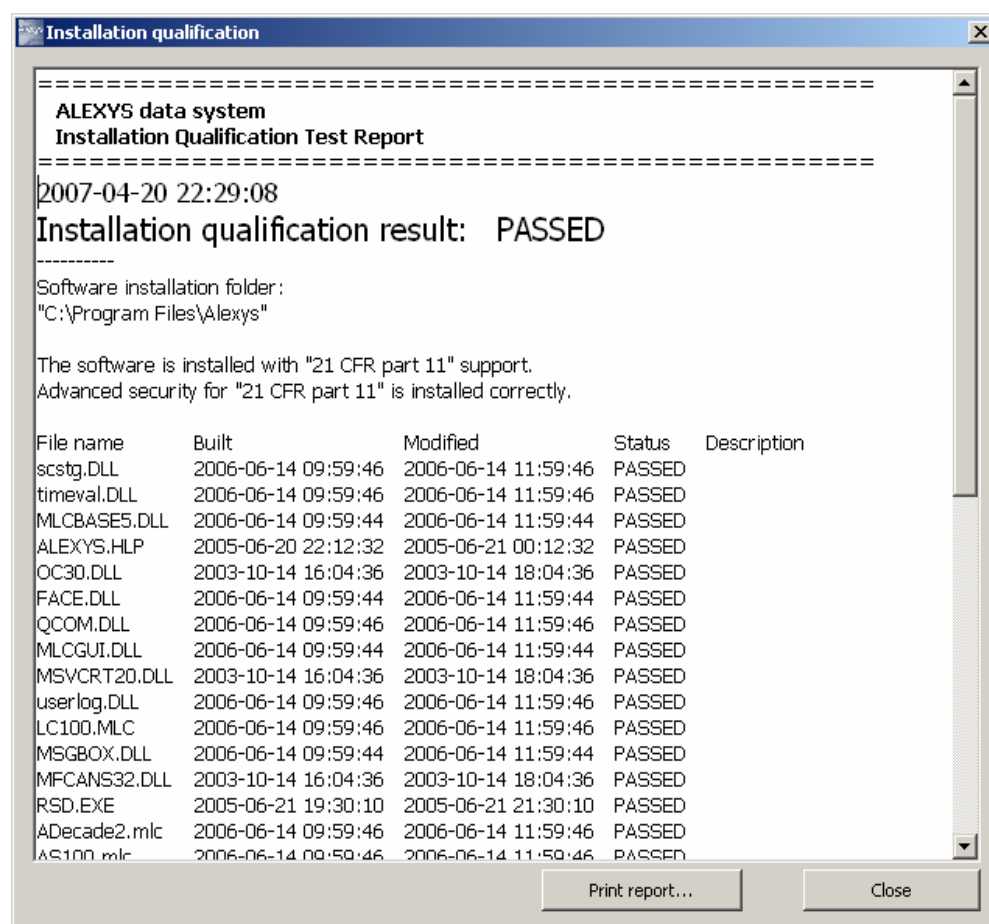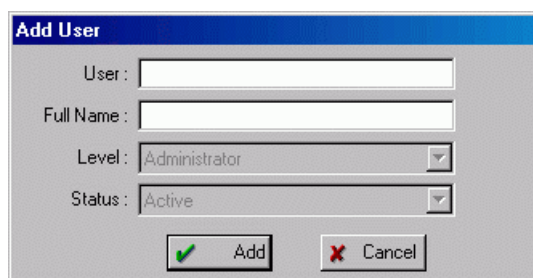

The IQ utility checks if the ALEXYS system files are correctly installed and than only appropriate and supported device drivers are used by the software. The IQ utility can be started at any point in time by the end-user to check the integrity of the installation.

System Start-up and Configuration

Prior to use of the ALEXYS data system power up all the hardware of the ALEXYS 100 or 110 LC-EC System. The LC 100 pump must be set to remote: stop the pump using the "Start/Stop" button and select "Remote". All other devices go into remote automatically.

Starting the ALEXYS data system

- Activate the <*Start*> menu button, find and click the ALEXYS data system icon in the Antec Leyden Software / ALEXYS data system folder.

- The following "*Add user*" window will appear[3]. Enter short and full name of the user and click <Add> to add a new operator to the *list of users*:
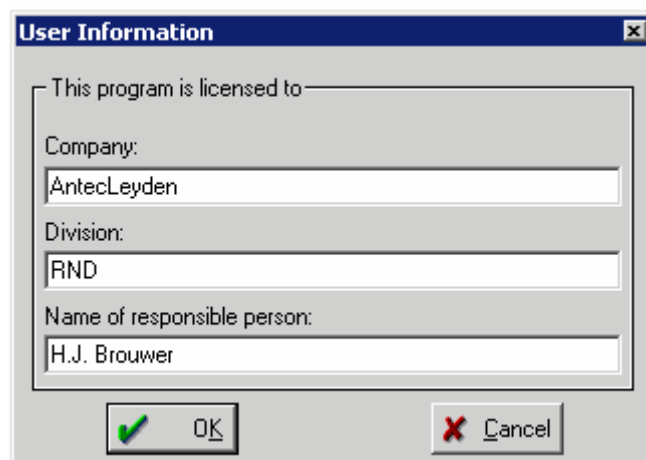

Note that the first user always gets an *Administrator Access level*.
The short name will be asked for each time at login. The full name is the official name of the user. It will be used to sign reports, chromatograms, methods, etc.

Enter the password and confirm it once more. The length of the password must be at least 6 characters for safety reasons, but can be altered later. Note that the password is case sensitive.

Important Note: remember the password and short user name otherwise it will be impossible to login next time. If this information is lost by any reason, de-install/re-install the program. All data are preserved, but the list of users is erased.

---

3 "*Add user*" window will be opened when the program is started for the first time only while *user list* is empty.

Fill-in the *User Information Card*:

The '*User Information'* card is shown each time at program start, until the "*Company*" field is filled in. This window can be invoked and edited at any time by using [Ctrl]+[F9] hotkeys.
Accept this User information or by-pass it to see an empty *desktop* .

When the ALEXYS data system is started again, the "*Log in*" dialog box[4] will be opened. Enter '*short user name'* and corresponding '*password'* to enter the program.
Note: if an incorrect password/user combination has been entered three times, access for this user will be *blocked* (i.e. become temporary un-available). Another user with an administrator access level can reactivate such a user again.

It is advised to create at least two administrator accounts. In case one administrator account is deactivated the other is able to enter the system and reactivate the account again.

---

4 To skip the "Log-in" dialogue box, it is possible to start the *ALEXYS data system* with **/password=...** key. Click the right mouse button on the *ALEXYS data system Control Centre* icon, choose *Properties* menu item and add the mentioned above key string with your password to the program name.
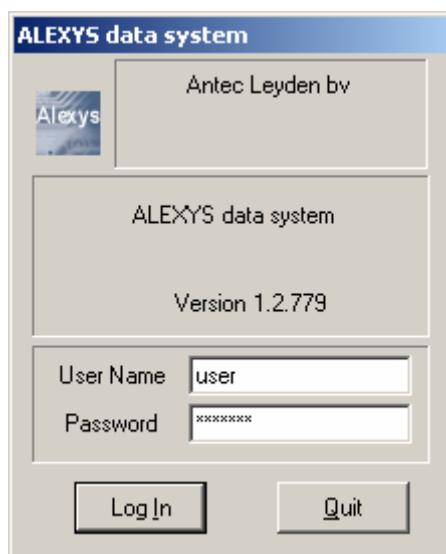
The "*Log in*" window also appears if there has not been any user activity for a defined period of time. The default Log out time is set to 30 min and can be changed by the user. It can be changed via the Options/Lock System menu item.

Security system

If the ALEXYS data system is used in a multi-user environment, it is possible to configure the system such that only designated users have access. This can be established in the '*Security system'*.

The *'Security system'* defines the access of an authorized user consistent with his skill and responsibility, and protects the system from unauthorized intervention. The users are displayed in the '*List of users'*. Each user has a unique '*Password'* and '*Access level'*.
The '*Password'* is used to identify the user and define his access level.
The *'User name'* is attached to the chromatogram and can be printed in the report.
The access level restricts the parameter set that the user can deal with. Three access levels are available:

- *'Novice'* - only the most common parameters can be modified. This level is recommended for routine users.

- *'Master'* - most parameters are available for modification with exception of hardware configuration parameters. This level is recommended for method development.

- *'Administrator'* - all system parameters, including hardware configuration, are available for editing. The privilege of the Administrator is:
  a) to edit the '*List of users'*;
  b) to change the hardware configuration.

Set '*Password'* and '*User name'* if you wish the system to stamp your name automatically in the passport and in reports.

List of users

The '*List of users*' and corresponding access levels are available through the **Options** / **Security...** item of the *Main menu*. In this case a password protected "Log in" window appears prior to access to *Security options*. Note that only a user with *Administrator* access can enter here. Also note that several users may have administrator access.
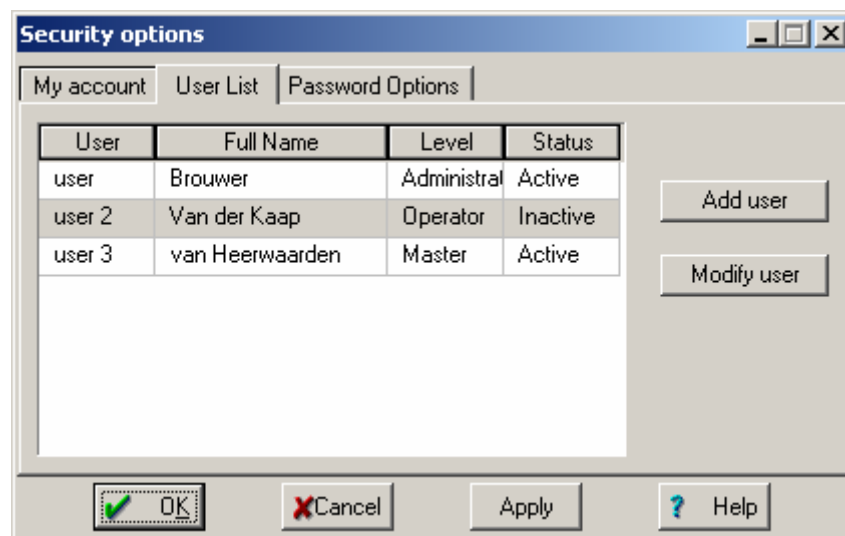
This dialogue box allows adding a new user to the list or to modify name and password of any existing user.

If you have forgotten an Administrator's password the ALEXYS data system must be de- and reinstalled. Note, that all data and methods will be preserved. However, the list of users will be erased.

To add a new user to the list, click <**Add user**> and fill in his nickname, full name and access level and the new user will appear in the list. If wished, a password or access level can be modified by pressing <**Password**> or <**Modify user**>.

Note: If a new user is entered, his password is the users' short name. If then the «Log-in» dialog box is invoked, the short name must be entered as a parole. The software will then request to re-enter a password. Please note that it's the password must be longer than 6 characters.
The Access level, is linked with a Status:

'Active': Name and password of the current user can be used to login to the system.
'Deactivated': Name and password of the current user cannot be used for system log-in. A warning message is given if these data are entered.
'Removed': The *S*ystem "is unaware of" name and password, but they can be used again for new accounts. The old record is not removed from the list, but cannot be re-activated.

When the list of users is completed, click <**OK**> button. The program will request a password each time the system starts and will link the user name with the password entered. This user name stamps methods, chromatograms and reports created during the current working session.

Password options
The *'Password option'* page allows tuning of some additional parameters of the security system.

Minimum password length
This is the minimum length of the password. The default value is 6 characters with a range of 2 - 10 characters.

Maximum number of login attempts
if this number is exceeded, the current user account is *deactivated*. It is only possible to enter system under another existing name. If Administrators are deactivated as well, the only way to enter the program is to reinstall the ALEXYS data system. In this case all data (chromatograms, methods, program settings, etc.) are preserved, but the list of users is erased. Default value: 6.

Password validity
The default validity of a password is 365 days. After this period the software requests another password.

Automatic logout After
The period (default 30 min) that the system is 'not used' (via keyboard or mouse) leads to an automatic log-out. The user name and keyword are required to re-enter. Default value: 30 min.

Lock system

The data system can be locked by clicking the 🔑 icon or through the **Lock system** item in the **Options** menu. Your *Password* must be entered to "log-in" again. Note that only access to the data system is controlled here. Access to the computer, operation system, and other applications is still possible. This option can be used to change the current user as well. The *Lock system* function is activated automatically if there has not been any user activity for a pre-defined time. This parameter is called *'Automatic logout time'* and can be tuned in the *'Password options'* window.

Configuring your system

For proper operation the ALEXYS data system requires details about the devices to be controlled and their *Interface* with the computer and software. These details are present in manufacturer defined subroutines called *drivers,* which are stored in special driver files. These files are named after the controlled device and have ***.DEV** as file extension. By default, driver files are stored in the ALEXYS data system*/Devices* directory. The data system uses a special wizard to install new drivers.
To operate properly, drivers should be organized in a group, called *System*.

A '*System'* is a set of drivers and operational ties between them. Some drivers can be shared by more than one system, i.e. so-called shared drivers. Normally such drivers provide ADC channels and COM ports to other devices (the *AC 100* supports both). Shared drivers can be used by several systems or drivers simultaneously.
Each *System* includes a section responsible for chromatogram *processing* and system configuration. This part is called '*Method'* and is saved as a separate file with ***.MTW** extension. It is possible to change a *Method* that is attached to a *System*. Each chromatogram file includes complete information on Method settings used for data processing and on the System configuration. So, any previously obtained chromatogram can be opened and reprocessed using these conditions. These Method and System settings are stored within the chromatogram file and can be different from the global ones that are stored in the corresponding *.MTW and *.SMT files.

A typical *System* consists of a *Watch window*, *Recorder*, *Pump, Autosampler* and *detector*.
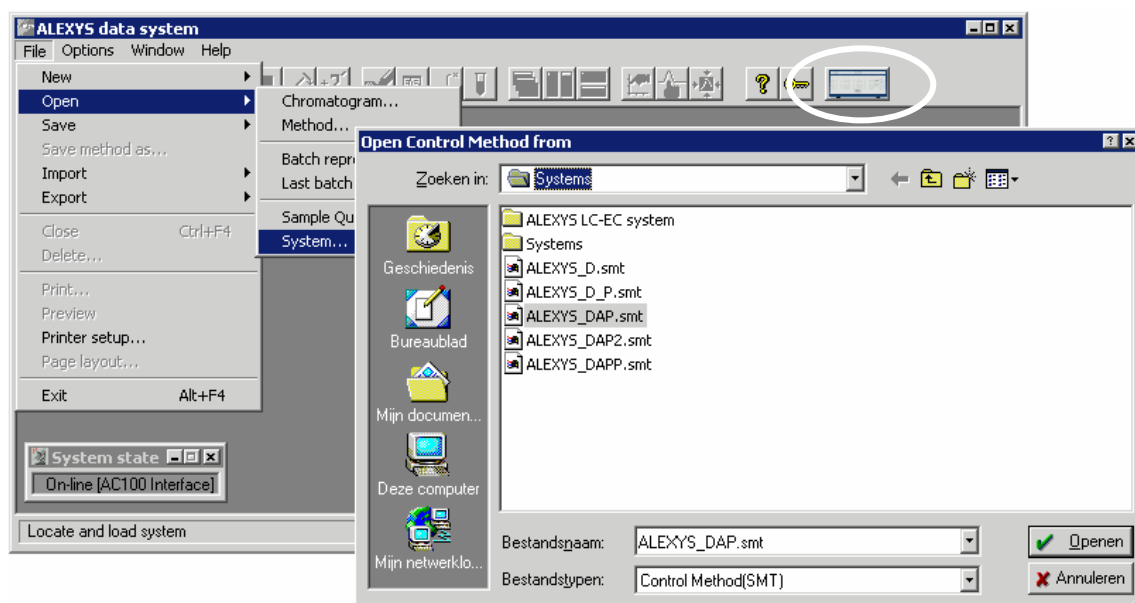
Quick system installation

One of the attractive features of the ALEXYS data system is exchangeability of system configurations. If hardware is connected in a pre-defined way (see p 30) installation of software drivers is as simple as opening a system file and connecting it to workplace.
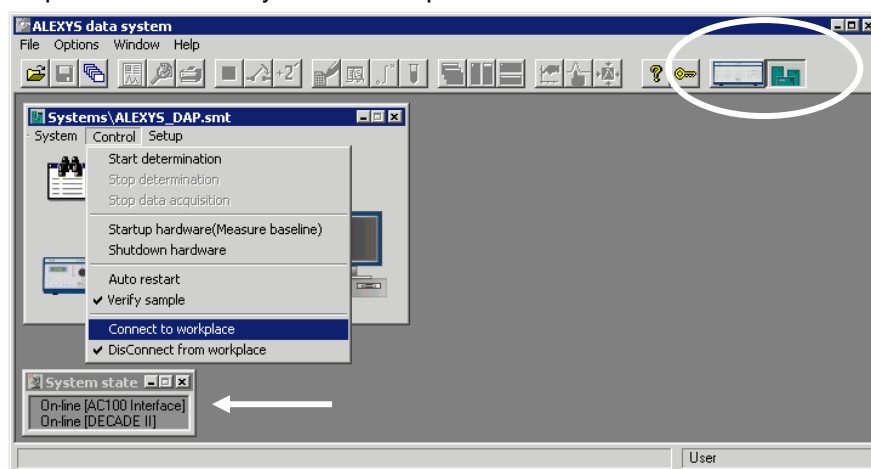
Step 1: Load the AC 100 device driver:
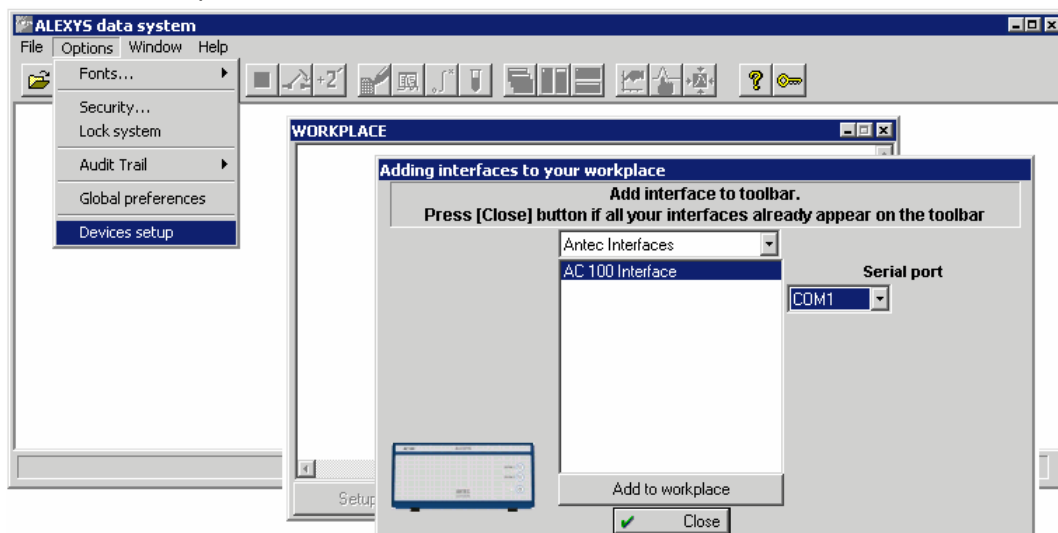
Ensure that the AC 100 driver icon is in the top right corner.
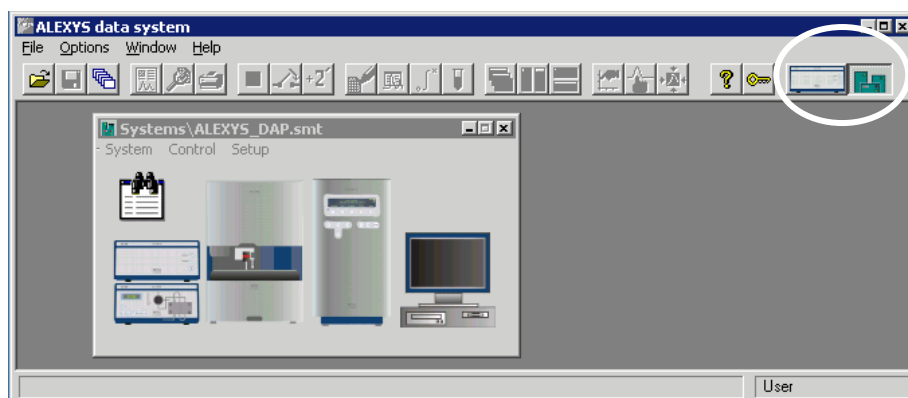
Step 2: In File/Open/System select one of the pre-defined ALEXYS systems:

Step 3: Connect the system to workplace.

Note the appearance of the System icon and check whether the 'on-line' messages to be expected are in the *'System state'* window.

The installation is completed now. Note that the buttons for opening the AC 100 and System window are added to the task bar (top right corner). If multiple (unwanted) icons are shown see 'Removing a system' on page 51.

Different pre-defined systems are present in the Systems folder. DAP stands for <u>d</u>etector, <u>a</u>utosampler, and <u>p</u>ump. If two independent pumps are used, select the DAPP system, for binary gradients select DAP2. External devices must be connected in DAP order otherwise the pre-defined drivers will not work.

Note that for the ALEXYS 110 system no AC 100 is required. After loading the relevant system file and "connecting to workplace" the system is online.

### *Creating a new system with a New System Wizard*

There is a special Wizard to create a new *System*. As an example the creation of a ALEXYS 100 system is explained here.

The ALEXYS 100 LC system includes:

- AC 100 acquisition controller
- LC 100 pump;
- AS 100 auto-sampler
- DECADE II EC workstation
- Watch Window and Recorder drivers

All devices are addressed by means of the RS232 ports of the AC 100.

- Click **File** / *New* / *System*. The 'New system wizard' window will appear. Enter the name of the new system folder ("ALEXYS 100 LC-EC system" in the current example) and click **<Next>**. All systems that use the same equipment configuration «*ALEXYS 100 LC-EC system*», but differ in processing methods, can be stored here.
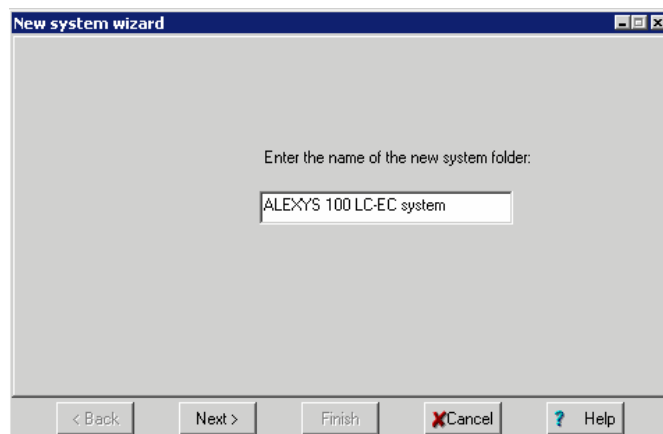
A new *System window* appears on the screen. It contains just two drivers by default:

The *Watch Window driver* is a special window that shows current state and parameters of all devices in the system. By default basic parameters of all drivers in the *System* are shown. It is possible to define the list of parameters: open *Watch* window and click the right mouse button somewhere inside to call the appropriate dialogue box.

The *Recorder driver* contains data acquisition channels and the data processing method. Chromatogram processing is performed via the *Method* attached to the *System*. Any data channel available in the current

System can be used by *Recorder* (detector signal(s), pressure, flow profile, etc). Note that detector data channel(s) must be selected first.

Watch Window and *Recorder* make up a simple *System* that does not perform equipment control (manual settings on the device panels are used), but allows data acquisition and processing.

The '*Next'* window enables installation of *shared devices* (this operation is called «*add a shared device to workplace»*). To add a device to the workplace, select it and click <***Add to workplace***>. All shared devices are installed only once and are available for any *System* afterwards. If a shared device is installed (connected to workplace), its bitmap appears in the *icon bar*. The following shared devices are available now: Timer, AC 100 acquisition controller.

- The timer device allows activating system actions such as *start-up*, *run*, *shutdown*, *open*, *close*, according to a user-defined event schedule. The timer is a shared device; it can control several *Systems* at the same time. It is possible to install several shared timers (for example, it is convenient to have one timer for each active *System*). Besides, a *local* timer can exist inside each *System*.
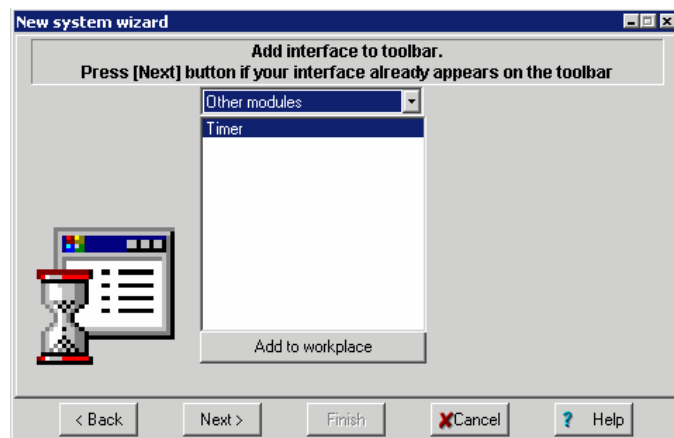
- Select the «*Antec Interfaces*» item and select «*AC 100 interface*». Choose the computers' serial port (COM1 in this example) to which the AC 100 is connected to. Click <***Add to workspace***>.
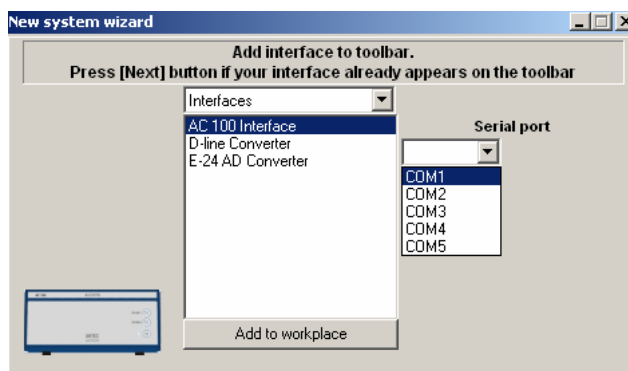
The *AC 100 interface bitmap* will be created in the *icon bar* of the main data system window and 16 additional COM ports will be available via the "*Serial port*" list. These additional ports are designated as:

AC 100 port[COM? / X(Y)]

Where:

COM? The computers' serial port, which is connected to the *AC 100* acquisition controller.

X System number (*AC 100* can provide two independent systems: «System 1» or «System 2»)

Y COM port number. Each *System* is able to control 8 devices through four RS232 sockets. Each socket can be used to control two devices when using the special RS232 splitter cable. So, if a splitter cable is plugged into the first socket of the *System 1* area, AC 100 port[COM1 / 1(1)] and AC 100 port[COM2 / 1(5)] are available. The second plug gives AC 100 port[COM1 / 1(2)] and AC 100 port[COM1 / 1(6)], respectively and so on.

- Click **<Next>** to continue. The third wizard window configures devices that will be included in the *System*. All devices are grouped according to their function: detectors, solvent delivery systems, auto-samplers, etc.

- In the «*Antec detectors*» group select DECADE-II driver, choose a COM port where the DECADE-II device is to be connected to. It is the AC 100 port [COM1/1(1)] in this example and click the <Add to System> button.
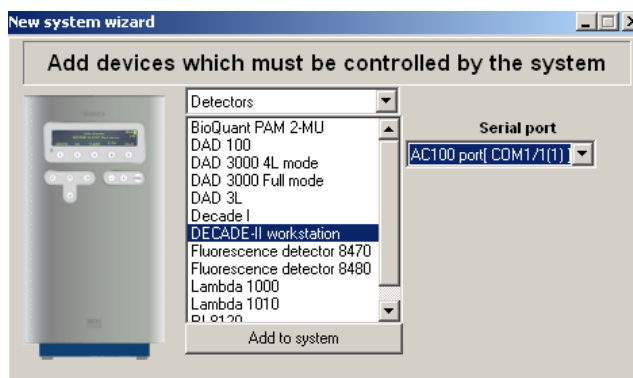
- The DECADE II icon will be added to the System.

- Select *Antec autosamplers /AS 100 Autosampler* device and set the *Serial port* to be connected «AC 100 port [COM1/1(2)]» here. Click <*Add to system*> button. The AS 100 icon will appear in the system window. These devices must be physically connected to the corresponding ports of the AC 100 acquisition controller.
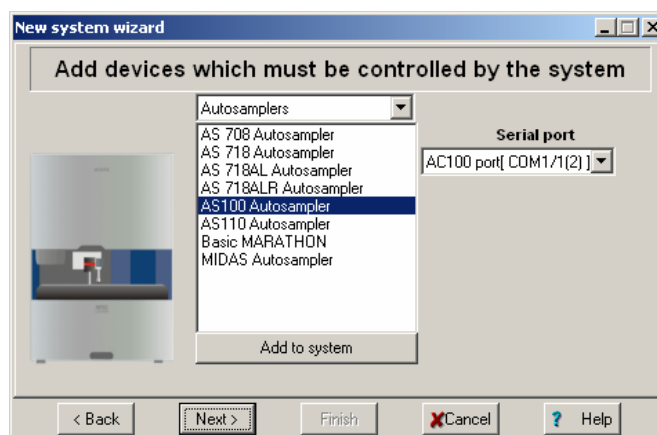
- Select *Antec Solvent delivery systems / LC 100 with one pumps* driver and set correct s*erial port* to «AC 100 port [COM1/1(3)]». Click <*Add to system*> button.
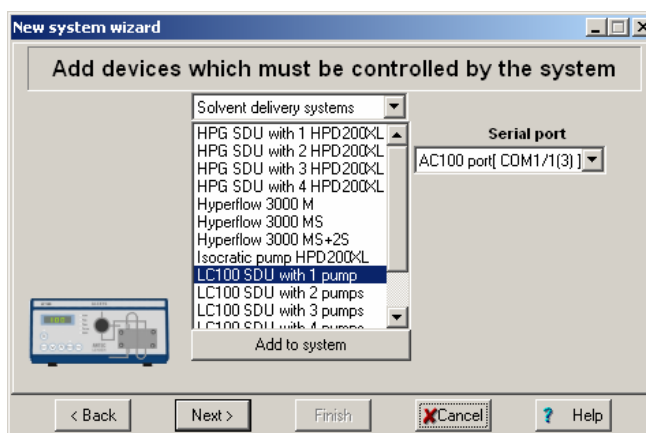
- Select **Other modules / Timer** and click <**Add to system**>.
- Click **Next>** button to continue. The final page of the wizard allows the configuration of the *Recorder*.
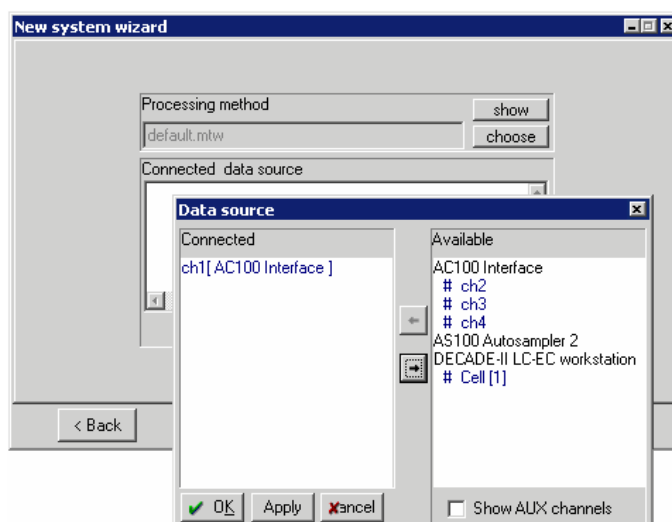
- This window enables selection of the data processing Method to be used by the *System*. It is possible to select the desired *Method* from the list (click <**Choose**> button in the "*Processing method*" area)[5]. The «Default.mtw» method is proposed as the default one.

- Set the data source (analogue input number, or channel number of the AC 100 interface to be used for data acquisition). To configure the

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5 Any appropriate method can be used as a template: open it by the <**Show**> button, edit it and save it under a new name.

data source, click the <***Choose***> button "*Connected data source*"
area. Shared devices are listed first (they are highlighted in dark-blue).
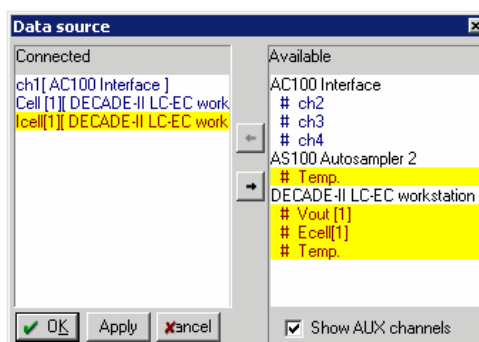
- Any channel can be connected or disconnected by clicking the arrow
  buttons [←] and [→] in the middle of the "Data source" window. In
  case of the DECADE I select the *Cell1[DECADE II]* data source. Sev-
  eral channels can be connected to allow *multi-channel chroma-
  tograms*. Click <***OK***> to accept data source settings.

- If other data sources are needed like column pressure, gradient or flow
  profile, column temperature, check the ☑ *Show AUX channels* box
  and select the desired channels. Auxiliary channels are highlighted on
  a yellow background.

- Click <***Finish***> to complete the procedure. Enter a name for the new
  System, Configuration 01 and click <***OK***>.

The following "*Test \ Configuration 01*" system must be created as a result
and the corresponding System bitmap will appear in the *icon bar* of the
ALEXYS data system main window[6]. The connected System is not re-
moved from desktop if its System window is closed using the ⊠ button, it
is hidden only. Click the bitmap in the *Icons bar* to open the *System win-
dow*.

---

6 The software automatically tries to connect each newly created *System* or loaded from disk
to workplace. This operation is only possible if all necessary resources and equipment are
available (are not used by another System). Otherwise the System is left unconnected. Each
connected System is presented by the *System icon* in the *Icons bar* on the Desktop.

If another *System* (i.e. changed equipment configuration) must be used, it can be created as described previously. Another *System bitmap* will be added to the icon bar in this case.

If the data system is shut down, the system asks if interface changes must be saved or not. If so, save the *System* to maintain the configuration for future sessions. If the system was modified after the last saving, an asterisk (*****) appears at the end of the header bar indicating that the new modification is not saved yet.

Using shared devices within a System

All spare resources of shared devices (such as COM ports, ADC channels, timers, etc.) can be used by any *System*. If a shared device is used by a system, it is convenient to add its bitmap to the *System window*. This local device copies control functions that are used by this System only. In some cases additional functions can be used. For example, a local duplicate of the AC 100 interface gives the opportunity to define and control *external programmed events*.

To add a local AC 100 duplicate to the *System*, the following operations are necessary:

- Open the desired *System*.
- Choose the **Setup** / *New devices* / **Link to existing device** option in the *System window*.
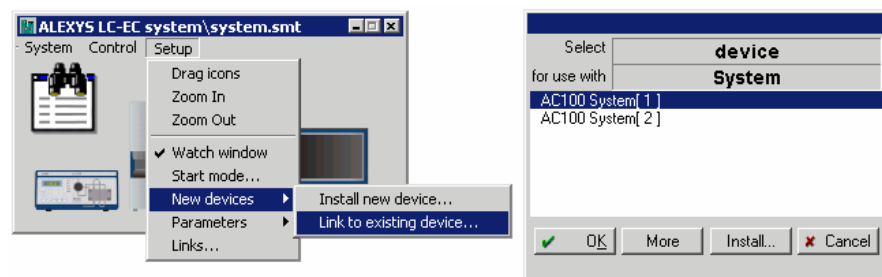
- Select "*AC 100 System(1)*" in the list and click **<OK>**. The *AC 100* bit-
map will be added to the *System*.

In order to remove a device from the *System*, click the right mouse button
on the bitmap and execute the ***Unlink*** function of the context menu.

In order to move a device icon within the current System window, set
SYSTEM MENU**/Setup/✓ Drag icons** tick. In this case it is also possible
to change the dimensions of the System window.

### Removing a system

Removing a System from the data system completely is done by 'discon-
necting from workplace' followed by 'deleting device'.
Step 1: in System window select 'Control/Disconnect from workplace' .
Step 2: in Main window select 'Options/Devices setup'
Step 3: select top level device and click 'Delete device'
Note that the AC 100 button will also disappear from the button bar.
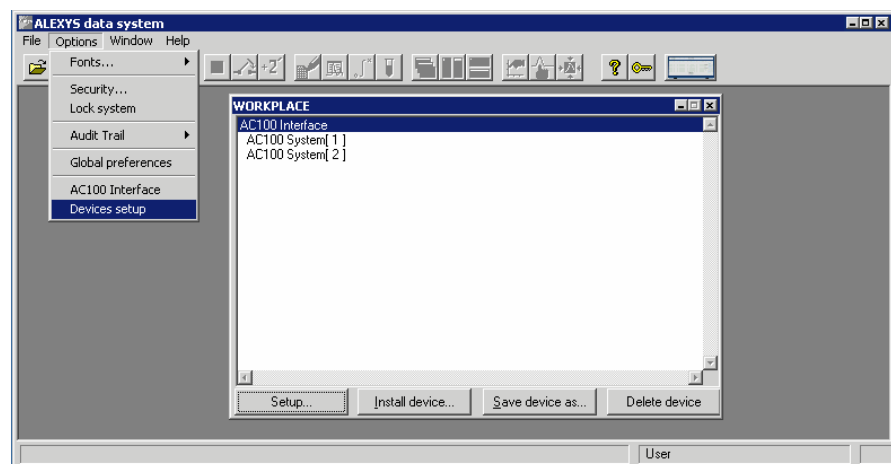
Device drivers

A typical driver consists of page(s) with *initial parameter settings* (includes manual control of the device as a rule) and *time programmed parameters*. *Initial parameters* are uploaded to the device at the moment of system initialization, and *time programmed parameters* start either immediately or after an *Inject* event.

Double click on the bitmap of the desired device. A *Device driver window* opens[7]. The *Device driver window* contains several dialogue pages, their number and appearance depending on the type of device. As a rule, the «Manual» page contains parameters that can be changed immediately and at any time. Most of these parameters are *initial settings* of the device (i.e. settings that are sent to the device at system initialisation). If the device supports time programming for some parameter, the «Program» page is added to the *Device driver window*. If *Administrator access level* is set, additional pages are available: «Interfaces», «Channels»[8], and «Links»[9]. These pages are used to configure a *System*. ALEXYS device drivers are described in detail in the *Appendix D*. However, some important drivers and questions will be discussed here.

Watch window
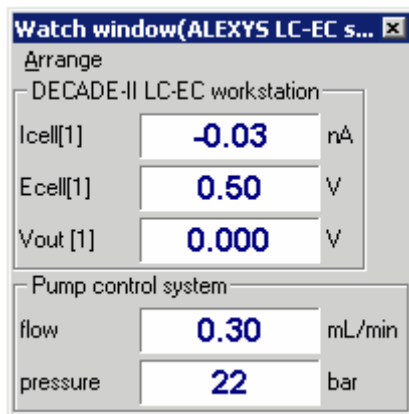
The Watch Window provides a special window that displays current state and parameters of devices. By default all drivers of the *System* are shown here. The *Watch window* is opened by clicking the *Watch icon*

() in the System window. To show the *Watch Window* automatically at every *System* start, check the **Setup/Watch** window menu item (the flag is set by default).

It is possible to tune the structure of the *Watch Window* manually. Device parameters can be individually selected and altered (change of the precision of displayed values). The configuration utility can be opened by the

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7 It is also possible to click the right mouse button on the device bitmap and to select the «*Open*» menu item.

8 This page enables parameter editing of data channels provided by the current device. This option is available for an Administrator only. Sometimes it is useful to change «Precision» (number of decimal digits for the value in the Watch window) or «Units» parameter of the channel.

9 The «*Links*» page shows all resources, interfaces or other drivers, used by the current device. Double click the desired interface in the list to change its settings.

right mouse button clicking inside the window frame and choosing the
«Manual arrange» item. Normally there is no need to configure the *Watch
window*. The «Auto-arrange» option of the same menu sets the most im-
portant parameters. For details see also: *Appendix D.*

Recorder

The *Recorder driver (* *)* is used to organize data flows from various
data sources and their subsequent processing. It enables to select data
sources and to define a data processing *Method* that is attached to the
System. The driver has not any *initial* or *time program* parameters. Click
the right mouse button on the *Recorder bitmap* and choose the **Setup**
menu item (this option is available if the *System* has been already *con-
nected to workplace*, but has not been started yet).
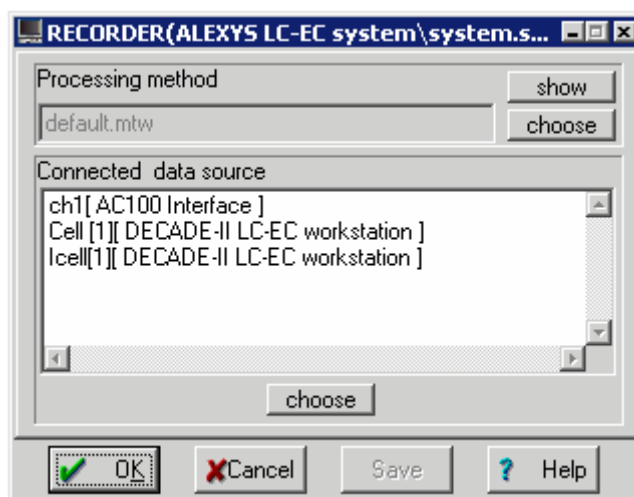
The upper box of the window contains the name of the data processing
Method attached to the System; the lower box lists data channels to be
used for acquisition and they are shown on the chromatogram plot.
The *Recorder* is normally started *with the inject event*. Prior to injection the
recorder shows a baseline plot just after the start of a determination. After
the injection event all preliminary baseline data are discarded, the window
background colour is changed (Blue colour to white by default) and the
chromatogram plot is started.

- To change a *Method* that is used to process chromatographic data and
  is attached to the *System*, click <***Choose***> button and select a desired
  file from the list.

- To change the data source, click <***Choose***> in the bottom part of the window.
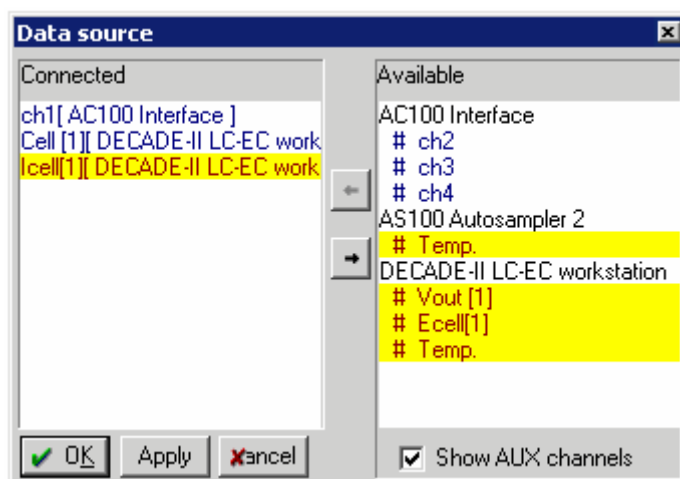
It is assumed that the data source has been selected correctly. If not, transfer the correct data channel into the "Connected" area and move the ones, which are not needed to the "Available" list. Use the drag-and-drop technique or the arrow buttons. All available channels are divided into two groups: *Shared* and *Local (Auxiliary).*

Shared channels belong to devices that can be used by several *Systems.* However, if the channel is "busy" by some *System*, another one cannot use it at the same time. As a rule, AD channels are declared shared de-vices. Channels of shared devices are highlighted on a green background.

Local channels are only available within the current *System.* As a rule, local channels are used to control device parameters like pressure, flow, solvent composition, etc. Each device has its own set of local channels. Channels of local devices are highlighted on a yellow background. Check the ☑ *Show AUX channels* box to display local channels.

Start mode

All system devices are divided into two groups: those, which programs start immediately at *System* start (when «Start determination» function is activated), and those, which start with the *Inject event*. Groups are visible by clicking the **Setup** */ Start mode* item of the *System window* menu. Any selected device can be moved between groups. By default all drivers are placed in the «Start with inject» group. Double-click a device to edit its parameters.
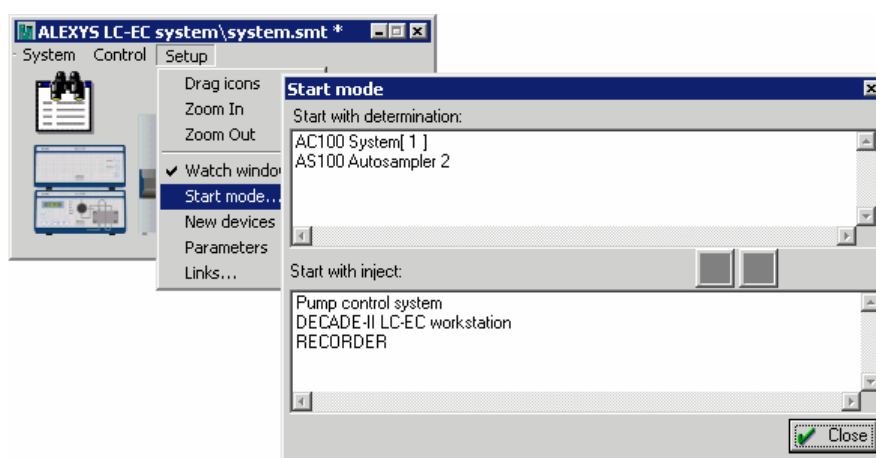
The *Inject event* defines the starting moment of time programs of different devices. It is usually generated by an AD device such as the AC 100. The AC 100 has special IO's (External start) for each channel socket that can detect the moment of injection by contact closure. Some devices that are not data sources (e.g. autosamplers, but the DECADE as well) can also generate this signal, which is immediately transmitted to all other system devices. Also the AC 100 is able to generate an *inject event*, which is separate for each of the two systems.

The *Initial parameters* (i.e. those set in the «Manual» page of the driver) are sent to the device immediately after *System* initialisation, while time programs can be activated either at the system initialisation or with the *inject event* (according to the settings of the "Start mode" window). The "Start with inject" parameter is usually selected for Recorder and all devices with defined *Time programs*.

Checking System's Start-up parameters

It is possible to check the System's start-up configuration.

- Click **Setup /** *Parameters / Start-up hardware* to check initial parameters:
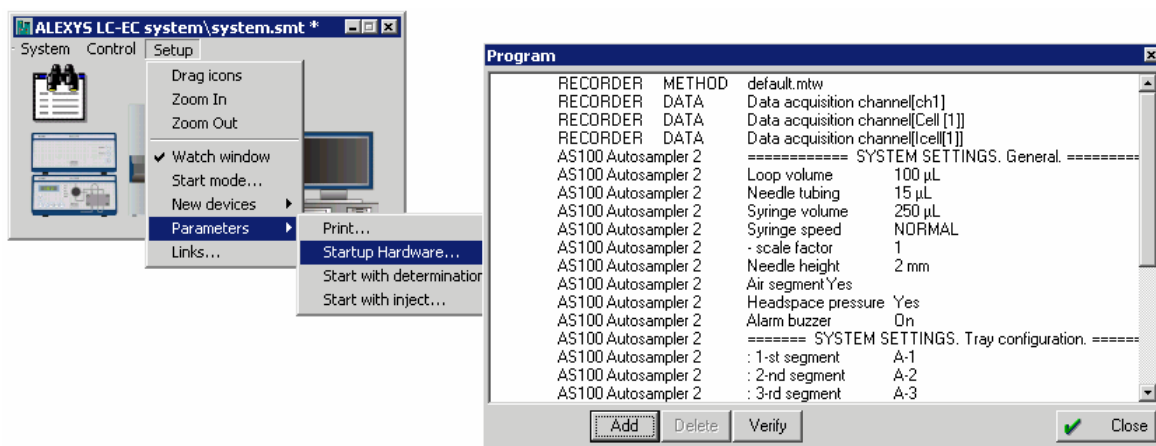
Saving a System

To save a system Click the **System / Save as** menu item in the *System window*, confirm the existing name or enter a new name for the modified *System* and press <**OK**>.
If the *System* is saved under the same name, just select the **System / Save** menu item in the *System window*. The current system file is rewritten without prompt.

- Click the right mouse button on the autosampler icon in the *System window* and choose the **Unlink** option in the context-sensitive menu.

- Click the **System / Save as** menu item, and save the "simplified" System without the AS 100 under **Configuration 02.smt** filename.

Verification of connections and settings

- Switch on all devices being part of the System. Check that the LED "External control" is on (LC 100).

- Open the desired *System* by clicking the System's bitmap on the icon bar. Be sure that the System is connected correctly to workplace (a corresponding bitmap in the Icons bar must exist or the **«System»/ Control / ✓ Connect to workplace** flag must be set).

- Check the **«***System state***»** window. If all devices are switched on and connected correctly, all messages in the window should become grey. Failed operations are highlighted in red, and currently not tested operations are on a yellow background.

- Open the "Watch Window" driver by double-clicking the bitmap .
If all connections and settings are correct, current parameters of devices (they are showed on their LED displays on front panels) will correspond to values in the *Watch window*:
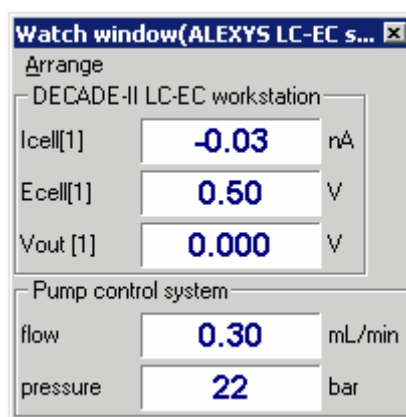
Additional diagnostic or error messages can also be displayed in the *System state* window. Additional information of how to configure your system is given in the *Appendix D*.

C H A P T E R   5

21 CFR part 11 support

Introduction

21 CFR (Code of Federal Regulations) part 11 is an European standards adopted in 1997 by FDA (Food and Drug Administration of the Department of Health and Human Services). It provides basic demands for data safety and declares principals of electronic signatures and electronic records, records as equivalent to paper records and handwritten signatures executed on paper. Although use of electronic records is voluntary, it has become common practice. The final 21 CFR part 11 rule is available via Internet: http://www.fda.gov.

21 CFR part 11 regulations is an attempt to create "a practical and workable approach for criteria to ensure that records can be stored in electronic form and are reliable, trustworthy, secure, accurate, confidential, and authentic".

In ALEXYS the 21 CFR part 11 support can be enabled during the installation procedure. It is also possible to install the ALEXYS for the current user only, i.e. other users of the Windows operational system will not have access to this software Installation of the ALEXYS with 21 CFR part 11 is described in the chapter "Installation and configuration".
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> ⚠️ **21 CFR part 11 features are available if NT-core system (Windows NT, Windows 2000 Windows XP or Vista) is installed and NTFS file system is used on the partition where ALEXYS software installed and data are stored.**

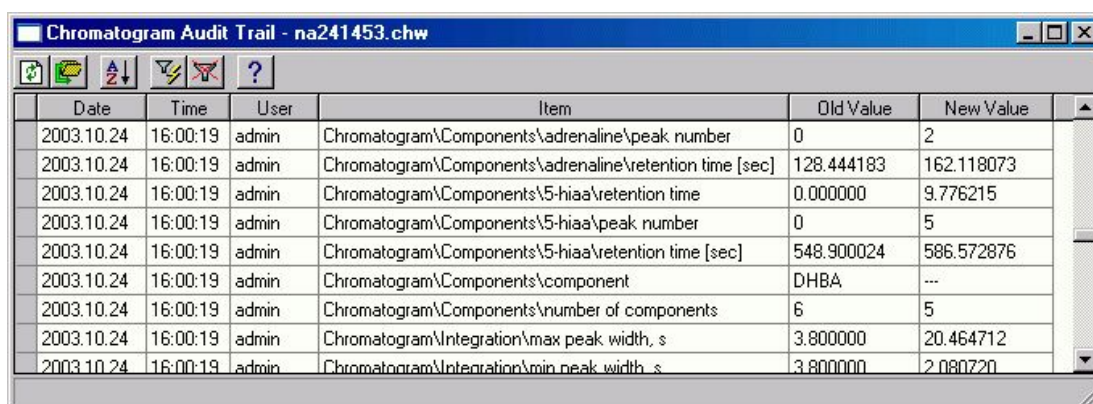The following features of the 21 CFR part 11 are supported by ALEXYS:

- All folders with chromatograms become locked for Windows file operations. Hence, chromatogram files can be handled only within ALEXYS software.
- All changes made in chromatograms and methods, are reflected in Chromatogram trail and History trail. These records can not be changed by the user.

- Each chromatogram run gets a serial number that can not be corrected later.
- Each chromatogram file can be signed by the user. Each user gets a unique electronic signature that can be used to detect data authenticity and integrity. Moreover, several levels of electronic signatures are supported that can be used by responsible persons for authorship, approval, responsibility and other purposes.
- On the other hand side, it is possible to load a chromatogram file and repeat exactly the same analysis because all information, including hardware control and data processing parameters, is stored in a single file. This feature fits GLP demands.
- In combination with Adobe Acrobat software (full version) or PDF Factory Pro software it is possible to print PDF documents and in such a way create electronically sealed reports. These PDF report files preserve the contents and formatting of the ALEXYS data system reports and can be protected against any modification by applying the appropriate encryption and password protection.

Achieving and maintaining compliance to this rule necessitates Standard Operating Procedures (SOPs) that support and complement the functionality of electronic systems, this means that no product alone can ensure compliance. However, products with integrated functions supporting 21 CFR Part 11 can make the task of achieving and maintaining full compliance with the rule significantly easier. This document describes which 21 CFR part 11 options are available in the ALEXYS software.

Chromatogram audit trail

The "Chromatogram audit trail" window traces all user activity concerned with method and chromatogram processing. It is opened via ALEXYS /Options/Audit trail/Chromatogram menu item. Chromatogram Audit Trail is stored in the chromatogram file. While working, a virtual copy of the trail is created on disk for each opened chromatogram, and new records are added there. The modified trail is added to the definite chromatogram file when saving is performed. If chromatogram is saved under a new name, the old trail is discarded, and comment from the current user is required.

The "Chromatogram audit trail" window includes *table of events* and *pictographic menu*.

All records are added automatically and are stamped with *date*, *time*, and name of the *current user*, and cannot be cleared or edited. Besides user activity, each record includes changes in parameter values (*old values* and *new values* are listed).

In addition to automatic log, a free-text user comments can be entered. ☑ *Require comment on changes* flag should be set in the **Options/Global preferences** window to allow this option. User comments are entered in the "comments on changes" window that appears before the chromatogram saving. The chromatogram saving is not performed until user comments has been entered.

Pictographic menu is used to control the following functions:
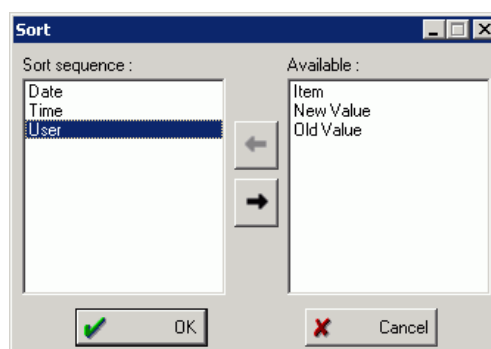
 Refresh information of the window. By default the information is refreshed at the window opening.

 Save log to disk in text format. Name of the file is selected by the user.

 Sort the log table according to rules defined in the "Sort" window:

Sorting is performed by column in increasing order. It is possible to select several columns to sort. In this case sort operations are applied consecutively, as listed in the "Sort sequence" area. Any keys from "Available" area can be selected. Use arrow buttons ⇦ and ⇨ to move sorting keys between lists.

![filter icon]	Use the current cell as a filter to sort records. Records with the same cells are found and listed while others are discarded. All filters set in the "*SORT*" window are ignored.
![reset filter icon]	Reset the filter. Entire table is shown. All filters set in the "Sort" window are restored.
![help icon]	Call context-sensitive help topic.

History Audit Trail

"History audit trail" window automatically traces all common user activity such as opening of a window, pushing a button, parole changing, etc.. It is opened via ALEXYS /Options/Audit trails/History menu item. Unlike Chromatogram Trail, it does not connected to the definite chromatogram, and traces all actions of all users. History Audit Trail is stored in a special catalog Flog which files are unavailable for editing.

> **Note: History Audit Trail is never deleted in the current ALEXYS version. Its size grows rather slowly, and modern hard disk capacity allows ignoring this fact.**

In addition to automatic log, a free-text user comments can be entered similar to *Chromatogram Audit Trail*. ☑ *Require comment on changes* flag should be set in the **Options/Global preferences** window to allow this option. User comments are entered in the "comments on changes" window that appears before the chromatogram saving. The chromatogram saving is not performed until user comments has been entered. A *pictographic menu* acts similar as in the "chromatogram audit trail" window.

Electronic signature

Electronic signature is generated automatically on the basis of the chromatogram data using cryptographic methods and rules so that the identity of the signer and the integrity of the data can be verified. Electronic signature is a part of the *21 CFR Part 11* regulations.

Electronic signature itself is a long string that is generated automatically by the software and added to or inserted in the chromatogram file.It performs the following functions:

- Ensure an authenticity of the chromatogram data. Nobody can change a signed chromatogram and to preserve validity of the sign.
- Check identity of the sign to be sure that it was designed and processed by the responsible person.

⚠ **Once a chromatogram is signed it cannot be altered (all parameters and descriptions can be only watched, and no processing can be performed). In order to alter the signed chromatogram, its signature has to be revoked first.**

"*Electronic signature*" window is used to sign (or to alter the existing signature) of the current chromatogram. "*Electronic signature"* window is invoked by *ALEXYS / **Process / Electronic signature*** menu item. Once a chromatogram is signed it cannot be altered. To alter the signed chromatogram its signature has to be revoked.

In order to sign the current chromatogram, user has to enter *Short user name*, *password* and to select a *signature meaning*:
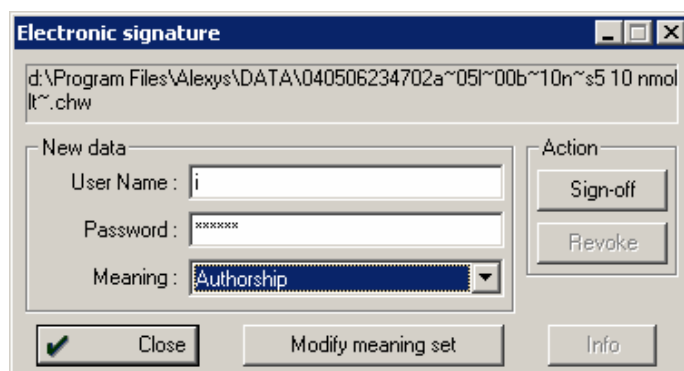
When *<Sign-off>* button is clicked, *User name* and *password* are verified, and if they correspond each other, the current chromatogram becomes signed. In addition to user name and meaning, each signature bears *date and time* when it was executed. When the chromatogram is signed, *<Info>* button becomes available.

Available operations with electronic signatures depends on user's *access level*. The following rules are valid:
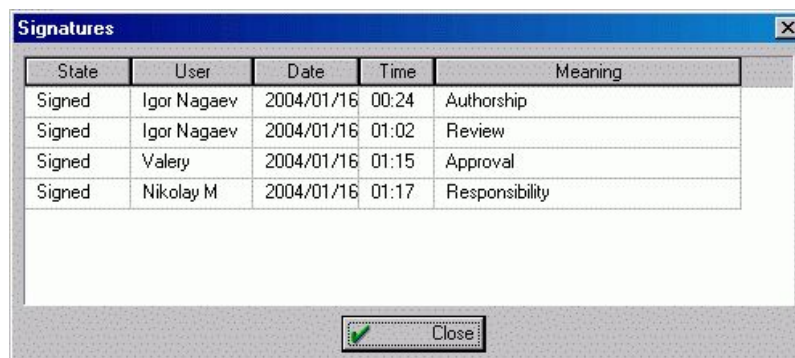
Administrator	Is able to revoke signatures applied by Master or Administrator. For signing and revoking Administrator can specify a value from both "*Common*" and "*Administrator*" meaning set.
Master	Is able to revoke a signature applied by Master only. For signing and revoking Master can specify a meaning only from "*Common*" meaning set.
Operator	Is not allowed to sign or revoke chromatograms.

Signature meaning is a description of the function that the signature carries out. The current ALEXYS version does not offer any restrictions (each user can select any signature meaning), however such responsibility policy is always defined in any company or lab.

It is possible to add several signatures for each chromatogram. For example, a hierarchy of three responsible persons exists in some company:

Person	Available signature meanings
A	authorship, review, rejection
V	review, approval, rejection
N	rejection, responsibility

Of course, all three persons have a unique *user name* and *password* in the *list of users*. The following order of signatures can appear:

State	User	Date	Time	Meaning
Signed	Igor Nagaev	2004/01/16	00:24	Authorship
Signed	Igor Nagaev	2004/01/16	01:02	Review
Signed	Valery	2004/01/16	01:15	Approval
Signed	Nikolay M	2004/01/16	01:17	Responsibility

There is no a priority of signature meanings within ALEXYS software. So it is impossible to revoke a definite signature from the list. Any responsible user can revoke *all existing signatures*. To revoke a list of signatures, the user has to enter his name and password, and click ***<Revoke>*** button In addition, user-defined signatures can be added to the list or existing meaning can be edited or deleted. Click ***<Modify meaning set>*** button to edit *signature meaning set*:
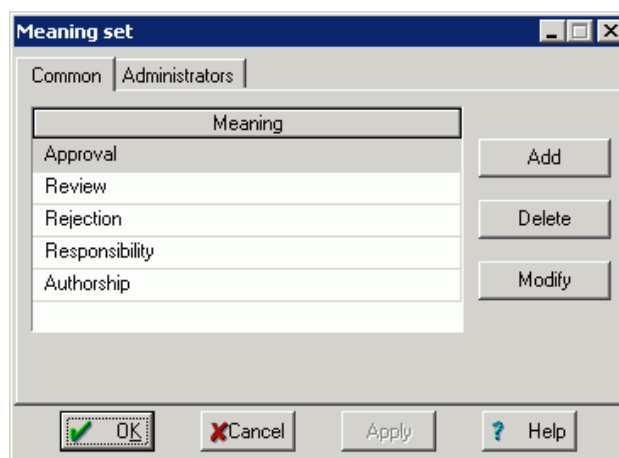
It is possible to add a new meaning, delete or modify an exisiting one. There are two sets of signature meaning: *Common* and *Administrators*.

⚠ | **Meaning sets can be edited by Administrators only**

Common meaning set is available for *Masters* and *Administrators* and contains by default a predefined list of signature meanings recommended in 21 CFR part11 regulations: {approval, review, rejection, responsibility, and authorship}. *Administrators meaning set* is available for *Administrators* only and is empty by default.

⚠ | **For proper operations with signatures *Certificates* are required. *ALEXYS* software automatically generates a default certificate for each user. However, true certificates issued by *Certificate Authorities* can be used as described in the next chapter.**

Certificates

Basic definitions

Certificates and *Public Keys* are regulated by *21 CFR part 11* requirements
Certificate Services is one foundation for the *Public Key Infrastructure (PKI)* that provides the means for safeguarding and authenticating information. The relationship between a certificate holder, the certificate holder's identity, and the certificate holder's public key is a critical portion of PKI.

This infrastructure is made up of the following parts:

- The Public/Private Key Pair
- The Certificate Request
- The Certification Authority
- The Certificate
- Your Public Key Used for Signature Verification

The Public/Private Key Pair

PKI requires the use of *public/private key pairs.* The mathematics of public/private key pairs is beyond the scope of this documentation, but it is important to note the functional relationship between a public and a private key. PKI *cryptographic algorithms* use the public key of the receiver of an encrypted message to encrypt data, and the related private key and only the related private key to decrypt the encrypted message.

Similarly, a *digital signature* of the content, described in greater detail below, is created with the signer's private key. The corresponding public key, which is available to everyone, is used to verify this signature. The secrecy of the private key must be maintained because the framework falls apart after the private key is compromised.

Given enough time and resources, a *public/private key pair* can be compromised, that is, the private key can be discovered. The longer the key, the more difficult it is to use brute force to discover the private key. In practice, sufficiently strong keys can be used to make it unfeasible to determine the private key in a timely manner, making the Public Key Infrastructure a viable security mechanism.

The *private key* can be stored, in protected format, on a disk, in which case it can only be used with that specific computer unless it is physically moved to another computer. An alternative is to have a key on a smart card that can be used on a different computer provided it has a smart card reader and supporting software.

The *public key*, but not the private key, of the subject of a digital certificate is included as part of the certificate request. (Hence, a public/private key pair must exist before making the certificate request.) That public key becomes part of the issued certificate.

The Certificate Request

Before a certificate is issued, a *certificate request* must be generated. This request applies to one entity, for example, an end-user, a computer, or an application. For discussion, assume that the entity is you. Details of your identity are included in the certificate request. After the request is generated, it is submitted to a *Certification Authority* (CA). The CA then uses your identity information to determine whether the request meets the CA's criteria for issuing a certificate. If the CA approves the request, it issues a certificate to you, as the entity named in the request.

Certification Authority

Before issuing your certificate, the CA verifies your identity. When the certificate is issued, your identity is bound to the certificate, which contains your public key. Your certificate also contains the CA's *digital signature* (which can be verified by anyone who receives your certificate).

Because your certificate contains the identity of the issuing CA, an interested party that trusts this CA can extend that trust to your certificate. The issuance of a certificate does not establish trust, but transfers trust. If the certificate consumer does not trust the issuing CA, it will not (or at least should not) trust your certificate.
A chain of signed certificates allows trust to be transferred to other CAs as well. This allows parties who use different CAs to still be able to trust certificates (provided there is a common CA in the chain, that is, a CA that is trusted by both parties).

The Certificate path

In addition to your public key and the identity of the issuing CA, the issued certificate contains information about the purposes of your key and certificate. Furthermore, it includes the path to the CA's list of revoked certifi-

cates, and it specifies the certificate validity period (beginning and ending dates).

Assuming the certificate consumer trusts the issuing CA for your certificate, the certificate consumer must determine if the certificate is still valid by comparing the certificate's beginning and ending dates with the current time and by checking that your certificate in not on the CA's list of revoked certificates.

Your Public Key Used for Signature Verification

A *digital signature* is used as confirmation that a message has not been altered and as confirmation of the message sender's identity. This digital signature is dependent on your private key and the message contents. Using the message as input and your private key, cryptographic algorithms create the digital signature. The contents of the message are not changed by the signing process. A recipient can use your public key (after checking your certificate's validity, issuing CA, and revocation status) to determine whether the signature corresponds to the message contents and to determine whether the message was sent by you.

If a third party intercepts the intended message, alters it (even slightly), and forwards it and the original signature to the recipient, the recipient, upon examination of the message and signature, will be able to determine that the message is suspect. Similarly, if a third party creates a message and sends it with a bogus digital signature under the guise that it originated from you, the recipient will be able to use your public key to determine that the message and signature do not correspond to each other.

Non-repudiation is also supported by digital signatures. If the sender of a signed message denies sending the message, the recipient can use the signature to refute that claim. Note that the bulk of the activities listed here are handled by the software, not directly by the user.

Certificates in ALEXYS

ALEXYS software uses Certificates to create and validate electronic signatures. *ALEXYS* software supports *X.509 certificates. X.509* is an internationally recognized standard for certificates that defines their required parts. X.509 digital certificates include not only a user's name and public key, but also other information about the user.

These certificates are more than stepping stones in a digital hierarchy of trust. They enable the CA to give a certificate's receiver a means of trusting not only the public key of the certificate's subject, but also that other information about the certificate's subject. That other information can include, among other things, an e-mail address, an authorization to sign documents of a given value, or the authorization to become a CA and sign other certificates.

In order to use personal certificate issued and signed by Certification Authority it is necessary to import it from disk file.

The following certificate services are supported by *ALEXYS* software:

- Software automatically generates "Self Signed" certificates with public/private key pair for each new user. If organization requires official Certificates issued by Certification Authorities, user is able to import them from disk file.
- Software supports export and import of certificates. Private and public keys as well as whole certificates are transferred through *Personal Information Exchange -PKCS #12 (.PFX)* files. To maintain security, only user himself is able to export and import his certificate.*Personal Information Exchange - PKCS #12* means the Personal Information Exchange Syntax Standard, developed and maintained by RSA Data Security, Inc. This syntax standard specifies a portable format for storing or transporting certificate's private keys, certificates themselves, and miscellaneous secrets.
- Certificates and private keys are stored in the secured place and could not be accessed by usual means.
- Private keys are encrypted. That ensures that only user himself could use his private key.
- When user makes signing operation, the software copies public part of the user's certificate and stores it with the digital signature.

Software enables to view certificate corresponding to each signature.

ALEXYS software uses Certificate Store services offered by MS Windows to store and handle Certificates. On this reason local Windows language is used for some messages and dialog boxes.

Operations with certificates

ALEXYS software allows the following operations with Certificates:

- View
- Export
- Import

View certificate

This option shows the certificate assigned to the current user or to the selected signature meaning. Subject and issuer names, certificate expiration date and other information can be viewed here.

Certificates are available for viewing for Masters and Administrators only

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It can be a so called "self-signed" certificate generated automatically by *ALEXYS* software for each user or "genuine" certificate issued and signed by *Certification Authority*.

There are two places to view certificates:

- In *"My account"* window that is opened with **Options** */ Security* menu item. Certificate import and export operations are also available here. Certificate of the current user only is available here.
- In *"Signatures"* window that is opened with **Process/Electronic signature/Security/*<Info>*** option. Certificate for each signature meaning is available here

The "*View certificate*" window is divided into three pages:

General	General information on the current certificate such as trust status, Certification authority, period of validity, etc
Details	Provides more detailed information including version, serial number, public key, subject, etc.
Certification path	Hierarchy of the current certificate up to root certificate

Export certificate

Certificate export wizard is available from *"Security options" / "My account"* page.
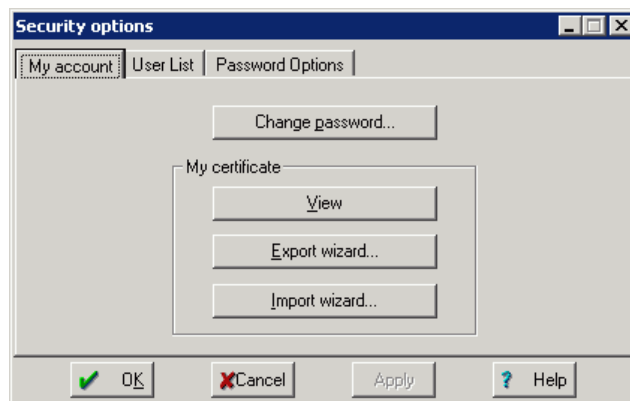
- Select "*Options/Security"* menu item to open the "*Security options"* window, and click **<Export wizard>** button on *"My Account"* page. This option executes *Certificate Export Wizard* allowing exporting the current certificate from a *Certificate Store* to disk file. *PKCS#12 specification* (Personal Information Exchange) is used for import-export of certificates. Certificate files have **.PFX** extension.
- Follow *Certificate Export Wizard steps* to export securely your current certificate and optionally your private key. Exported certificate could be imported by you to another PC with different installation of ALEXYS. In such case you are able to create identical electronic signatures on different ALEXYS installations.

Certificate Export Wizard consists of the following steps:

- *Welcome message*. Read the information and click *<Next>* button to continue.
- If needed, select "⊙ *Yes, export the private key*" option. In this case the private key will be exported as well. As it is password protected, password will be asked on the next page. Click *<Next>* button to continue.
- Type and confirm the password. No limitations for password length or its coincidence with operator's password, etc., exist here. Click *<Next>* button to continue.
- Enter filename to export certificate to. It is possible to use *<Browse>* button to select filename and catalog for storage. Click *<Next>* to continue and then *<Finish>* to complete operation.

Import certificate

Certificate import wizard is available from "*Security options*" / "*My account*" page and allows to export the current certificate from the Certificate Store to disk file. Certificate files have .PFX extension.

> **Warning: import procedure will destroy your current certificate! Use export procedure to preserve the current certificate on disk.**

Select **Options/*Security*** menu item to open the *"Security options"* window, and click *<Import wizard>* button on *"My Account"* page.

Certificate Import Wizard consists of the following steps:

- *Welcome message*. Read the information and click *<Next>* button to continue.
- Enter filename to export from. It is possible to use *<Browse>* button to select the desired filename and catalog. Click *<Next>* to continue.
- If certificate file contains the private key, type the password used for export of the certificate file. Click *<Next>* to continue and then *<Finish>* to complete operation.

Secure PDF reports in ALEXYS

For submission of electronic records the Portable Document Format (PDF) is recommended. To create PDF files from the electronic records of the ALEXYS data system a PDF printer is required. Good PDF printers can be for instance purchased from Adobe (Adobe Acrobat) or FinePrint (pdfFactory Pro).

> **A PDF printer is not part of the ALEXYS data system package and has to be purchased by the end-user separately.**

Below the procedure is shown how to generate a secure PDF report in Fine Print pdfFactory Pro as an example:

- Configure Adobe Acrobat, Fine Print pdfFactory Pro (or other suitable printer purchased) as standard printer of Windows.
- Open the Report options window with Process/Make report…, if you would like to create a report of a existing chromatogram, or Method/Report options…, to set the report options of a method.


- Check Printer as the Report destination in the Report options window.

- Click on <Preview> to display a preview of the report.
- Click on <Accept> to accept the setting and close the window without printing.
- Click on <Report> to print the PDF file of the report.
- Fine Print pdfFactory Pro shows a preview of the PDF file.
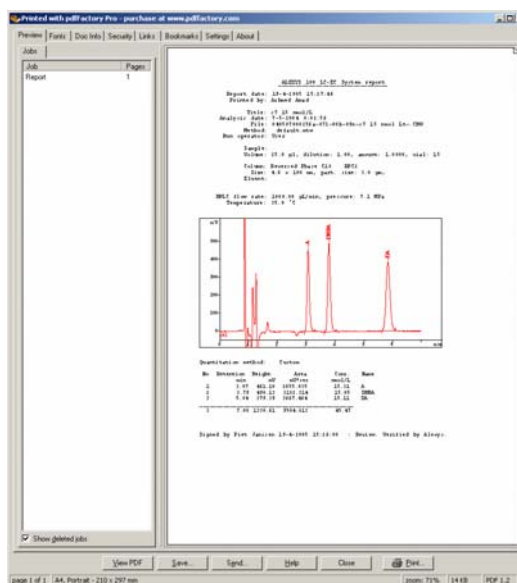
- Click the tab Doc Info and fill the required fields.
- Click the tab Security and check the options shown to create a secure encrypted PDF document that cannot be altered.
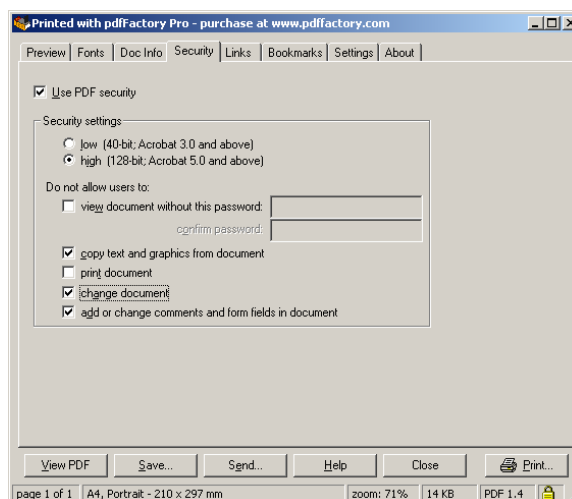
- Click <Save> and enter directory and file name for the PDF report.

Tutorial

Introduction

This tutorial is a step-by-step instruction of how to use the ALEXYS data system. The most essential operations and parameters will be discussed here: how to create a simple *Method* and to start a *Chromatogram*, how to optimise *Integration,* create a *Calibration* table, generate a *Report*, run a *Queue*, etc. The tutorial is designed to be followed in the order presented here.

For details, please, refer to the next session, *Procedures reference*. Processing of multi-channel chromatograms is described in a separate chapter.

Additional information is available through a context-sensitive *Help* file.

Press F1 key or click the ⚿ icon to get help on the current subject. Press [*Shift-F1*] key combination to call up the *Help Index*.

The *Tutorial* assumes that you have properly installed the data system and defined the system configuration as described previously. If you have not configured your system yet, please follow the instructions in the *System Start-up and Configuration* section.

T U T O R I A L   1

System start-up

In this section all necessary preparations are listed to start up the ALEXYS LC-EC 100 system. The steps taken are: loading System and Method, setting device parameters, and configuring the 'Start mode'.

1. Start up the data system.

2. If not already loaded automatically, start with loading a System using File/Open/System. The system contains and controls all relevant *hardware* settings.

3. Select 'Control/Connect to workplace' in the system window. If correctly configured the main window should show:
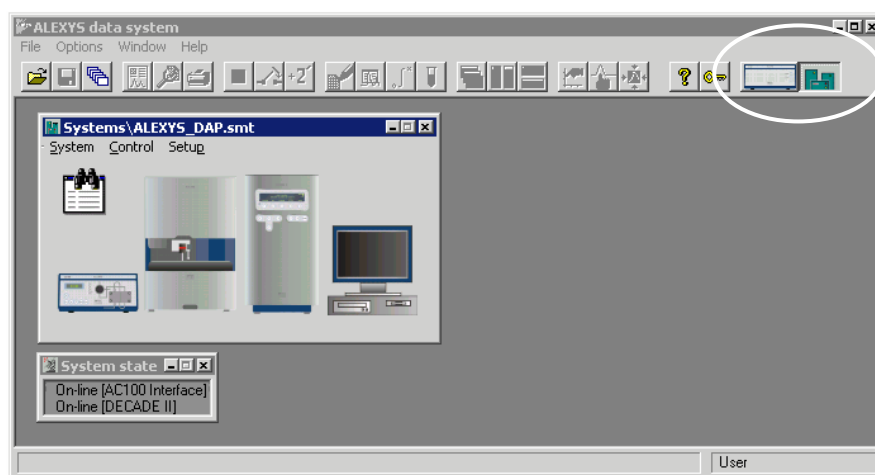

Note both icons of System and AC 100 (top right corner of window). If multiple (unwanted) icons are shown, remove them. 'Removing a system' is described on page 51.

4. Open the default method by double clicking on icon in the system window. An empty chromatogram opens. Note that the main window title bar shows a number of additional menus: Edit, View, Acquire, Process, Method.
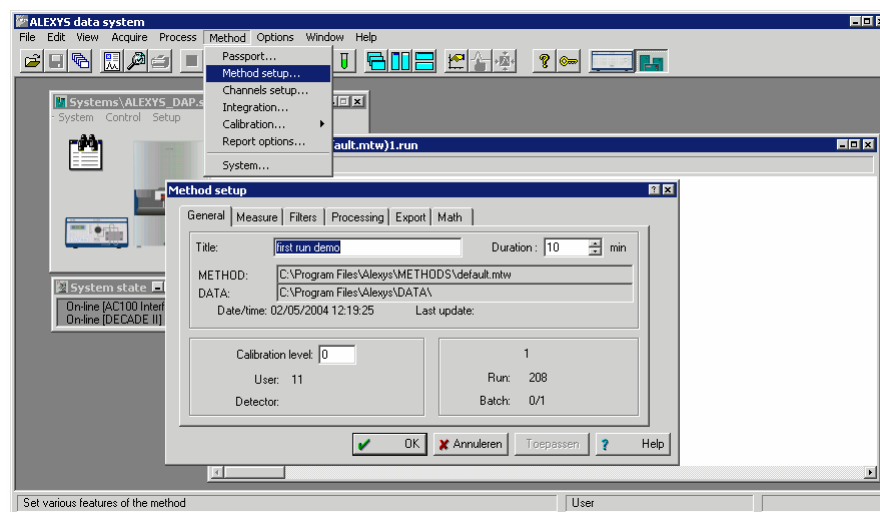
5. Select 'Method/Method set-up' and set the following parameters:
    - General: set title and run duration (10 min)
    - Measure: set frequency divisor of AC 100. For example: to 5 = 2 Hz on ADC channels, which is sufficient for most applications.
    - Filters: all values to zero, deselect spike filter.
    - Processing: set data path (for example: C:\Program Files\ALEXYS\Data\)

6. Select 'Method/Passport'. The method and passport menus contain much information to be printed in Reports. It is therefore recommended to fill in all appropriate fields.
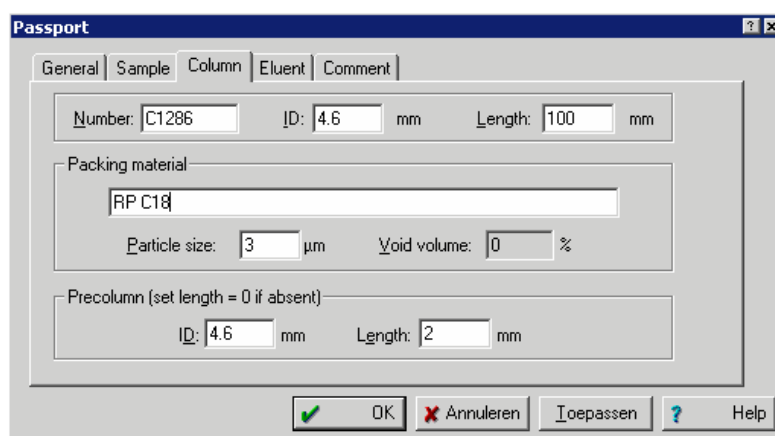
7. Set the AS 100 device parameters by double clicking on the device pictogram. If the AS 100 autosampler is used read the AS 100 manual for details on program injections and washing steps. In this example a 25 µL injection and a washing step is programmed.
8. Set 'System settings' for the standard configuration:

9. Set the AS 100 'Methods programming' menu:

10. Injection settings:

11. Wash settings:

12. Template settings:
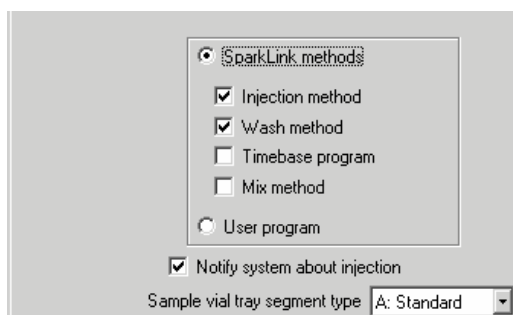
13. 'The template' tells the system which steps to perform upon injection. Leave all other AS 100 menu settings to default.

14. Open LC 100 device menu by double clicking on the corresponding icon. Set the flow rate, by clicking the 'Set' button. Note that in the main menu 'Options/Global preferences' the units for flow and pressure can be changed.
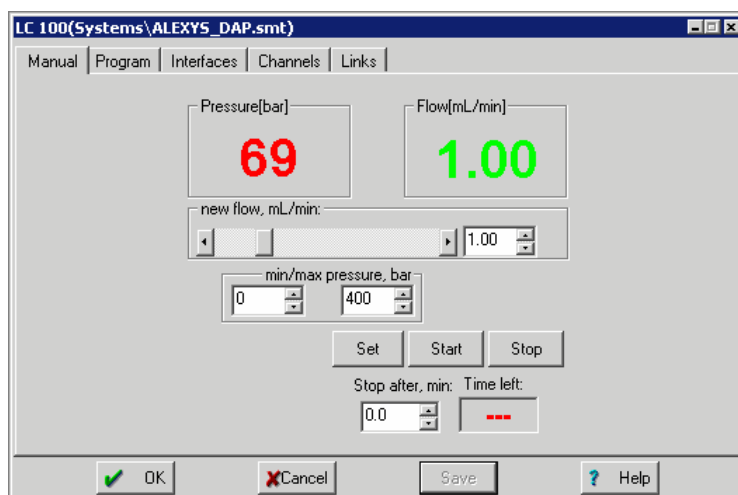
15. Open the DECADE II menu by double clicking on the icon. Set relevant parameters and confirm by clicking 'Set' button. See DECADE II manual for details on settings.
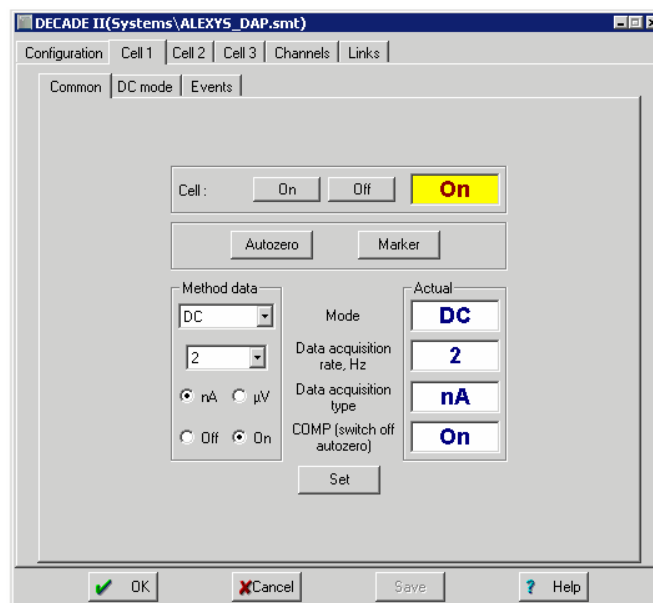
In our example: Mode DC, Data rate 2 Hz, Range 50 nA, filter 0.1 Hz, Ecell 0.60 V (vs. ISAAC REF)

16. Open RECORDER menu by a right mouse click on the [icon] icon and select 'Setup'.
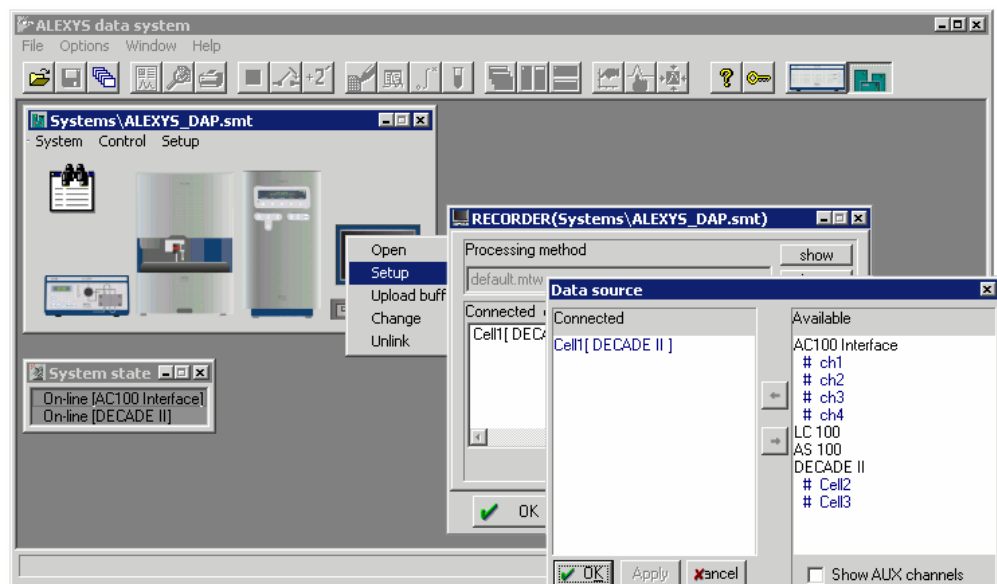
The upper box of the RECORDER Setup window contains the name of the data processing *Method* attached to the System. The lower box lists data channels that will be used for acquisition and are shown on the chromatogram plot. Clicking 'Choose' opens the data source window, and available channels can be selected. 'Show AUX channels' enables selection of system parameters such as cell current, temperature and pump pressure.

17. Open Watch Window menu by double clicking on its icon: . Click on 'Manual' and select the channels for display. Check 'AUX' to see all auxiliary channels.
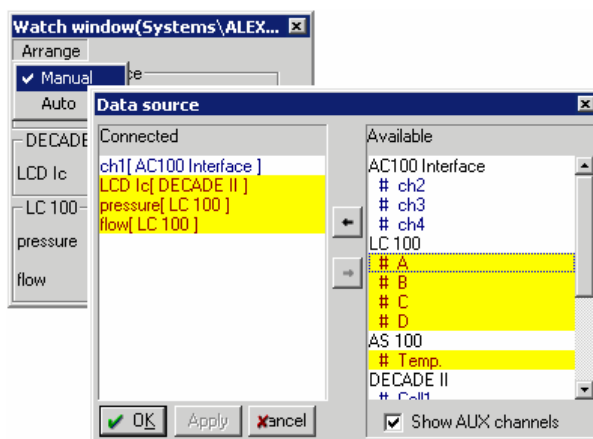
18. In the Setup/Start mode set the AS 100 to the upper window, and all other devices to the bottom window. This ensures that when starting a determination, only the AS 100 will start. When the valve switches from Load to Inject the Recorder will start acquisition. Timed devices for any other programmed event in the bottom window will start.
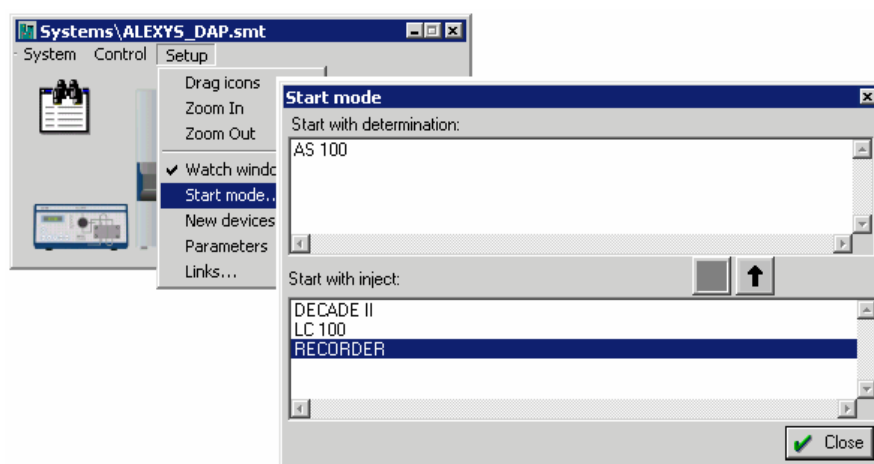
The system is now configured and ready to start with injections. Before running samples select Control/Startup Hardware to monitor the baseline.
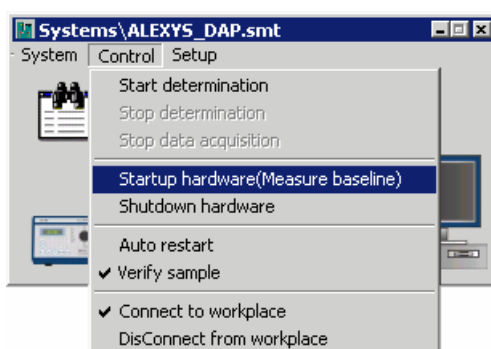
T U T O R I A L   2

Running samples

In this section performing single injections with the ALEXYS LC-EC 100 system are described. In the previous tutorial section all necessary preparations are made.

Starting a single run

1. Place sample vial in position 1 of the AS 100.
2. Select 'Control/Start determination' in the System window. Enter relevant parameters in Edit sample description. Note that 'Volume' and 'Dilution' are used for calculations in data analysis.

Title:	first run demo		Calibration level:	0
Info 1:	Catecholamine mix of standards			
Info 2:				
Volume:	25 µL	Dilution: 1	Vial number:	1
	Amount: 1		Internal standard amount:	1

Date/time when sample was collected (if different from injection time):
0 / 0 / 0   0 : 0 : 0

⚠ **Make sure to enter the correct <u>vial number</u> and <u>volume</u>. Values must correspond with the AS 100 settings otherwise unexpected results may be found.**

3. To edit the *analysis duration* click the 📝 icon or the **Method / Passport** menu item. If the chromatogram is running, its duration can be increased or decreased arbitrarily. Use <**+2'**> button in Icons bar for "hot" prolongation of the running chromatogram for 2 min. A running chromatogram can be finished by the user at any time.

   Press **<OK>** in the «*Edit sample description*» window to accept all settings and to start data acquisition.

   If "Start with inject" mode is set for the *Recorder* in the **Control / Start mode** window, the system waits for the *inject event* signal (the *Status line*

contains a word "*Measure (baseline)*") and a pre-analysis plot is running in the window allowing to control the baseline stability before injection. All devices with the "*Start with determination*" status are activated at system start and their *Time programs* are started. On the contrary, devices with the "*Start with inject*" status, will start at the *inject event signal.*

If the chromatogram has started successfully, the colour of the window background will change and you will see the chromatogram started. By default a white colour is used for running baseline and also white for running a chromatogram.
While the chromatogram is running it is possible to read old data files from disk and to reprocess them. Another chromatogram can be started as well if a different ADC channel is used for acquisition and another chromatographic system is used for the analysis.

Finishing a chromatogram

The software automatically finishes data acquisition when the preset runtime is elapsed. *Integration* and *Post-run processing* are performed according to the *processing Method* attached to the *System*. Note that *time programs* of System devices continue[10, 11]. This is normal.
To interrupt both the chromatogram and running time programs, choose the **Control** / *Stop determination* menu item in the *System window*. By ticking this function programs of devices are finished, the software automatically performs *Integration*, and *Post-run processing* as usual.
Upon data acquisition completion the ALEXYS data system automatically executes a number of operations, the so called *Post-run processing*. Some operations, such as *noise estimation*, *peak searching*, *identification*, and *quantification*, are always executed.
Other operations, such as *data smoothing*, *chromatogram saving*, *report output* etc. are executed optionally, at the user's choice (**Method** / *Method setup* / *Processing*).
When *data acquisition* and *Post-run processing* are completed, save the chromatogram by choosing the **File / Save** / *Chromatogram* menu item or just click 🖫 (*Save*) icon.

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10 For example, it is possible to regenerate the column before the next run. When time program is completed, device settings that were used last (just before time programs stop), are active.
11 Some devices have additional opportunities: for example, pump flow can be stopped if ☑ *Stop pumps after run* flag is set in the **Pump control system /** «Program» dialogue page. If this flag is reset, flow composition and flow rate that existed at the end of the run are preserved.

The name of a chromatogram is composed automatically on the basis of date and start of analysis time.
The chromatogram file will be stored in the directory set in the method.

(choose **Method / *Method setup* / *Processing*** dialogue sheet or click 🖼 (*Method setup*) icon to change a working folder).

When chromatogram has been saved, the software prompts for Method file modification[12].

Printing a report with a chromatogram is very simple; see page 125: 'Report generation'.

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12 Check the «*If Method changed/Ask to save*» switch in the **Options/<**Global preferences> dialogue box to perform this option.

T U T O R I A L   3

Scaling and channels

Zooming in and out

The ALEXYS data system provides many capabilities for chromatogram scaling. Graphs can be controlled by keyboard and mouse. Both provide access to practically the same operations. Scaling functions work on running chromatograms as well.

Using a mouse
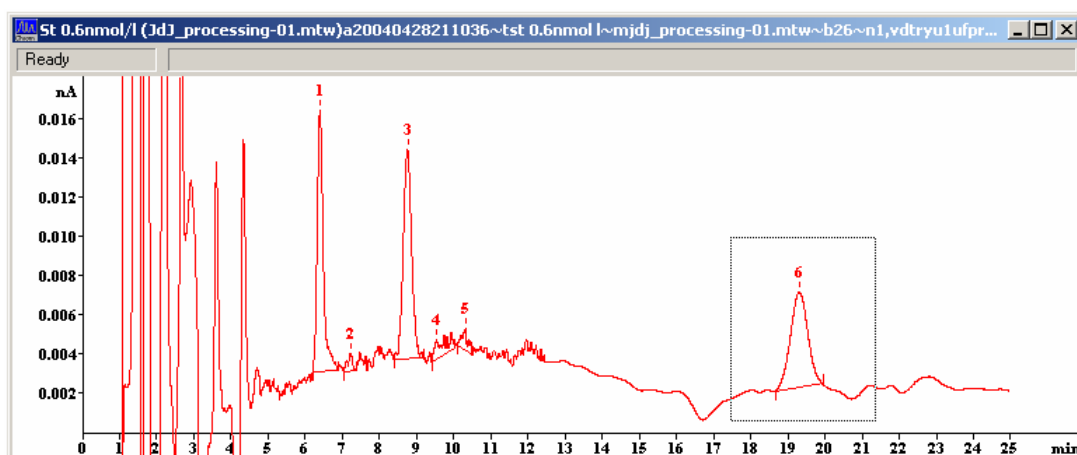
A part of the chromatogram can be zoomed in by clicking left mouse button at the upper left corner of the area of interest and dragging the cursor to the lower right corner of that area. You can move along the chromatogram by dragging the pointer on the horizontal scroll bar (that is in the bottom of the window).

Click the ⊡ (*View all)* icon in the *Icons bar* to restore a complete chromatogram in the current window. The same result can be achieved by:

- double clicking the left mouse button in the working area,
- Selecting the **View / *View all*** menu item,
- Pressing the [**Alt**]+[**V**] keyboard combination.

Using the keyboard

You can also change the horizontal and vertical scale and execute other operations in the current window. Depending whether the cursor is active

or not (the cursor is activated by [**Alt**]+[**C**] keyboard combination or by

mouse clicking on 🖑 (*Peak editor*) button), the same keys cause different actions.

The combinations printed in **bold** in the table below, work in both modes.

Basic keyboard combinations for image management:

Combination	Result
Cursor is inactive (Peak Editor not active)	
[↑]	increase sensitivity on the Y axis
[↓]	reduce sensitivity on the Y axis
[→]	expand a chromatogram on the X axis
[←]	shrink a chromatogram on the X axis
[Ctrl]+[Home]	auto scale procedure on the X axis (shows all on X)
[Ctrl]+[End]	auto scale procedure on the Y axis (shows all on Y)
[Shift]+[↑]	increase the distance between channels of the chromatogram
[Shift]+[↓]	reduce the distance between channels of a chromatogram
[PageUp]	shift a chromatogram plot 10% of the screen upwards
[PageDown]	shift a chromatogram 10% of the screen downwards
[Z] or [0]	set zero on the last point of a chromatogram
Only a part of the chromatogram is on the screen	
[→]	expands a chromatogram on the X axis
[←]	shrinks a chromatogram on the X axis
[Ctrl]+[→]	moves one window to the right (without change of scale on X and Y axes)
[Ctrl]+[←]	moves one window to the left (without change of scale on X and Y axes)
[Home]	shows beginning of the chromatogram (without change of scale on X and Y)
[End]	shows end of the chromatogram (without change of scale on X and Y)
[Z] or [0]	sets zero at the lowest point of the visible part of the chromatogram
Cursor is active (Peak editor mode)	
[Z]	sets a zero at cursor position
[→]	moves cursor to the right
[Shift]+[→]	moves cursor from left to right fast
[←]	moves cursor to the left
[Shift]+[←]	moves cursor from right to left fast

[Home]	moves cursor to the beginning of the window
[End]	moves the cursor to the end of the window
[Shift]+ [Home]	sets the end of a window at cursor position
[Shift]+ [End]	sets the beginning of a window at cursor position

Zero level

For screen display one of the data points is accepted as zero point. This point is located at approximately 10% of the window height. The position of the lowest channel on the chromatogram plot is set, and positions of all other channels are related to this. The distance between channels is changed by the **[Shift]+[↑]** and **[Shift]+[↓]** hotkeys.

A new baseline zero point is set by pressing the **[Z]** or **[0]** key. If a cursor is active, the zero level will be set at cursor position, otherwise at the lowest point of the chromatogram shown in the window. When a chromatogram is running, the data system sets *Zero level* at the last data point. If a chromatogram is loaded from disk, its previous *Appearance* settings are used.

When the "*View all*" operation is executed, the lowest point in the chromatogram is accepted as a zero level, horizontal and vertical scales are automatically chosen such, that the entire chromatogram fits the window. This operation can be executed in several ways:

- by choosing the **View** / *View all* menu item

- by clicking the (*View all*) icon

- by pressing the [**Alt**]+[**V**] key combination

- by double clicking the left mouse button inside the chromatogram area.

Autoscale option

The running chromatogram can be shown on the screen in the *Autoscale* mode. The Autoscale mode is selected by checking the ✓ *Recorder Autoscale* option in the **View** menu**.**

While data acquisition is in progress and *Autoscale* option is checked (default mode), every time the last point acquired is outside the window, *Autoscale is activated*. If the last point of a chromatogram is outside the window limits

- to the right, the window is shifted a half-screen right

- downwards, *Autozero* is performed

- upwards, the sensitivity on a vertical axis decreases 50%.

Thus, in the *Autoscale* mode the last acquired point of the running chromatogram is always within the window limits. If this option is switched off, any part of the running chromatogram can be scaled arbitrarily.

To increase the sensitivity during data acquisition, press **[Z]** or **[0]** key first for setting a zero on the last acquired point of a chromatogram, and then several times press the **[↑]** key. If necessary, decrease the inter-channel distance by the **[Shift]+[↓]** hotkeys.

Selection of channels

A chromatogram can be run on several channels. These may be data channels of several detectors or channels of a dual-channel detector like the DECADE-II DCC. Also, several user-defined *calculated channels* can be added (at least one calculated channel is created by default). It may be inconvenient to watch all channels in one window. A Channels bar exists in the upper part of each chromatogram window is available allowing to control the number of channels displayed and to select desired channels. All calculated channels become available when the chromatogram is finished while primary data channels are available when the chromatogram is running.

- In order to show the desired channel press the corresponding button, to hide it, depress . All: show all available data channels (as on the plot below)
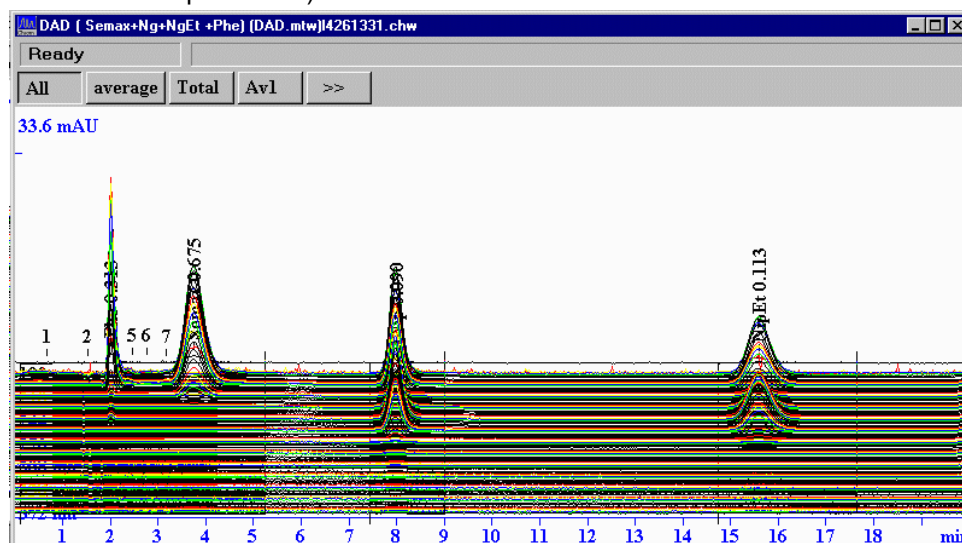
- Average: default channel of "average" type that is calculated on the basis of all available data channels.
- Total, Av1: calculated channels; names and types are user defined.
- >> (<<): this button is used to show (>>) or hide (<<)   available data channels.
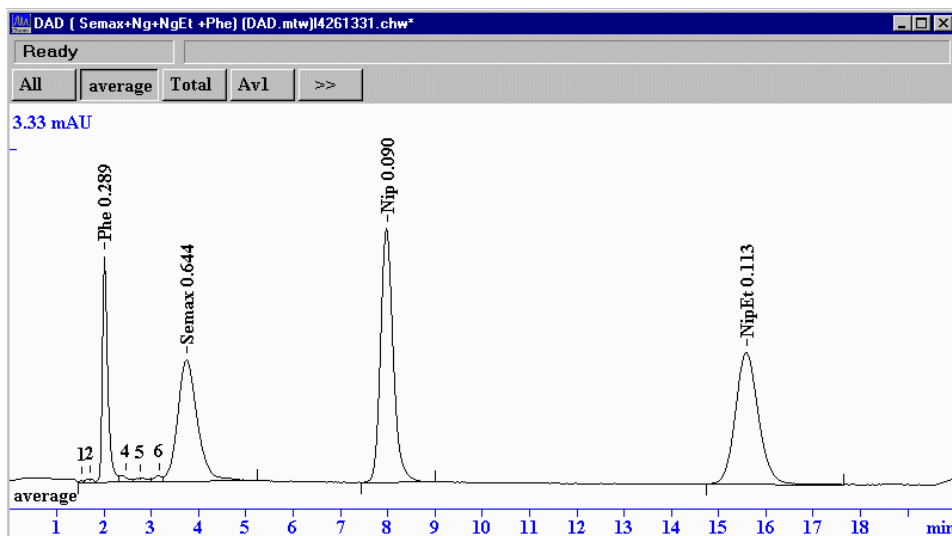
To select an arbitrary set of channels:

- depress <All> button (in order to hide all channels)
- click >> button (to show available data channels)
- push buttons of desired channels (use ◀ or ▶ buttons to move along the row)
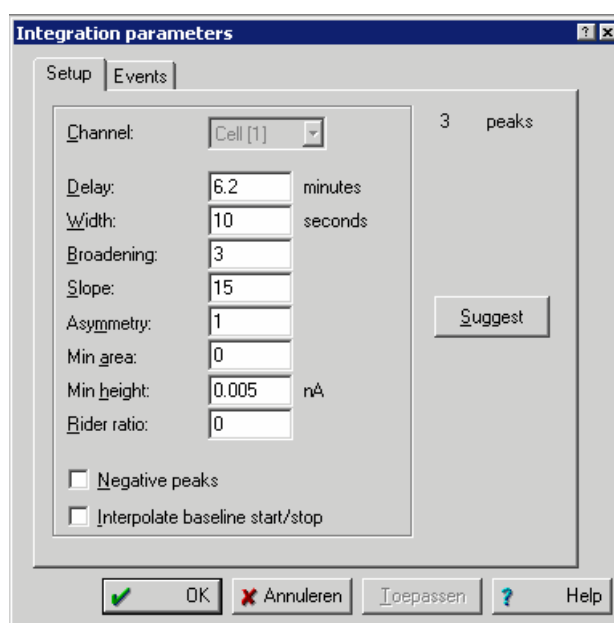
T U T O R I A L   4

Integration, peak editor and acquisition rate

When the chromatogram is finished, inspect whether peaks and baseline meet the requirements.

- If there are several channels, it is possible to select the one that is the most suited for integration of all desired peaks[13].
- Set the correct value of *Width* (expected peak width in the beginning of the chromatogram, in seconds) and *Broadening* (how many times peaks at the end of the chromatogram are broader than in the beginning). Press <*Apply*> button to see results without closing the dialogue box. A good idea is to try the <*Suggest*> - <*Apply*> combination 2 or 3 times to define the optimal parameters.
- Vary the *Slope* parameter improvement is wished. Reasonable values of *Slope* are between 0.5 to 5. A smaller slope value results in more peaks, a higher value less peaks. The default is 3. Press <*Apply*> to evaluate the results.

---

13 In the case of a multi-channel chromatogram it is possible to select one channel for integration. However, it is often difficult or impossible to find a common channel for all peaks. The solution is using a *calculated channel* that is defined as the sum of responses or the sum of signal-to-noise ratios.

An «*average*» channel can be calculated as the sum of responses for all channels. It is recommended to use it as a reference channel for integration for multi-channel chromatograms.

- Set the *Min. Height*[14] parameter to exclude small, unwanted peaks. Set a higher value than the peak height you want to exclude. Click <***Apply>*** to check.

- There are more capabilities to tune integration: you can use the ***Peak editor*** (manual editing) and ***Integration events***. *Integration events* are used if a series of similar chromatograms with the same properties is expected. The *Peak editor* is used to correct integration results for a single chromatogram.

- Save optimised integration parameters for future analyses as a method (**File** / ***Save / Method)***;

- Save the modified chromatogram (**File** / ***Save / Chromatogram*** menu option or click on an icon 🖫).

Peak editor

Along with the automatic *Peak detector*, the ALEXYS data system includes a *Peak editor* allowing to manually edit peaks like include or remove a peak, to change the start, end or top of a peak.
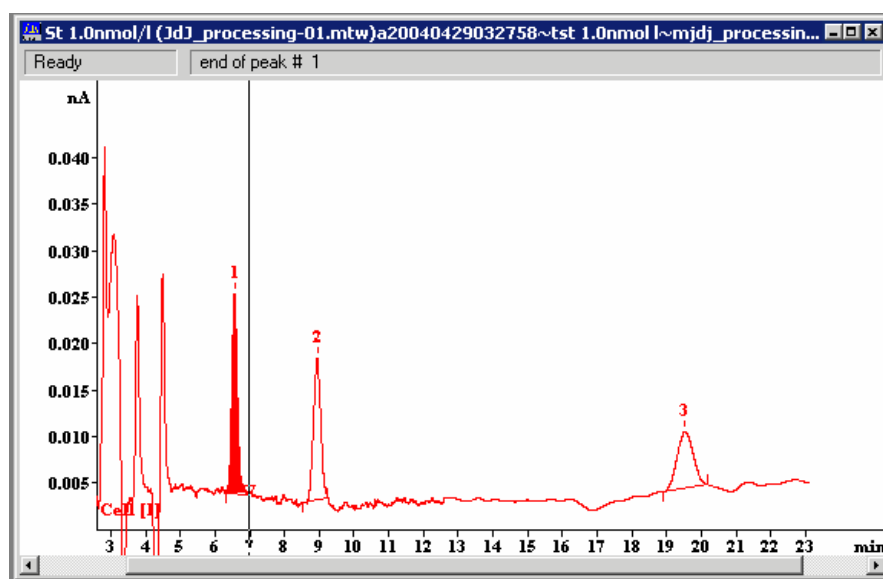
- Hide all channels depressing <All> button in the channels bar and then select the desired channel. As a rule, the "*reference for integration*" channel or the"*average*" channel is chosen.

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14 *Min. Area* parameter can be used for this purpose as well. However, it is more difficult to estimate the correct value of this parameter.

- Activate the *Peak editor* by clicking the icon or keyboard combination [Alt]+[C]. The main icon bar will be substituted by the Peak editor icon bar, and the cursor will appear on the screen. The *Peak* item is added to the *Main menu*.
  It is possible to move the cursor with arrow keys (shift/arrow for coarse movements), but it is more convenient to drag it with the **right button of the mouse pressed**.
- Each peak on the chromatogram has several *Cardinal* (*or Special*) *points*: *beginning*, *top*, and *end*. Neighbouring peaks can possess a *valley* point as well.

Let's assume, that the peak in the chromatogram already exists and it is required to move its beginning, top and end or valley point.

- Place the cursor inside the peak to be edited. Depending on what special point should be moved, click one of the icons:

- - to select beginning of the peak,

- - to select top of the peak

- - to select end of the peak

- - to select a valley between two peaks

The peak (or two adjacent peaks), to which this point belongs, will be selected by a black colour, and the type of the selected point will be printed on the *information line*.

Click icon to reset selection. Place cursor on the position, to where the selected *special point* should be transferred.

Click on the (*Move selected point*) icon or press a key [**-**].

- To destroy the selected peak, click on the icon (*Delete peak*) or press the [**Del**] key.

- To create a new peak with the top at cursor position, click (*Insert peak*) icon or press the [**Ins**] key. Note, that even a new peak can be evoked on a position where no peak is present.

- Click the icon to exit *Peak editor*.

A confirmation to accept the changes is required. Click the <**Yes>** button to accept all changes, <**No>** to exit and ignore changes, and <**Cancel>** to stay in the *Peak editor*.

Basic functions of the Peak editor:

Mouse	Keyboard	Peak editor function
	[←] or [→]	moves cursor to the left or to the right one step
	[Shift]+[←] [Shift]+[→]	moves cursor to the left or to the right 5 steps
	[Ctrl]+[Enter]	selects the *Cardinal point*, closest to the cursor position
	[←] +[Ctrl] +[Enter]	selects a point belonging to the left peak only (for *merged* peaks);
	[→] +[Ctrl] +[Enter]	selects a point belonging to the right peak only (for *merged* peaks);
		selects *Start* peak point closest to the cursor location
		selects *Top* peak point closest to the cursor location
		selects *End* peak point closest to the cursor location
		selects *Valley* peak point closest to the cursor position
		unselects a previously selected *Cardinal point*
	[-]	moves a selected point to the cursor position
	[*]	fuses the beginning of the previous and the end of the next peak at the cursor position
	[+]	merges two neighbouring peaks into a single peak
	[/]	splits peak into two peaks at the cursor position
	[Ins]	creates a new peak with its top at the cursor position
	[Del]	deletes the selected peak
		deletes all peaks left of the cursor position
		deletes all peaks right of the cursor position

Note that in some cases using the keyboard is more convenient than mouse operations.

Acquisition rate

The ADC of the AC 100 provides data sampling frequencies of 10 Hz or higher. For the majority of chromatographic applications such high frequencies are superfluous. Selecting the frequency divisor can set acquisition rates lower than 10 Hz. This value can be calculated or entered in 'Method/Method setup/Measure'. The calculation assumes that the narrowest peak contains at least 30 data points; a corresponding value is suggested.

- Select the **Process** menu / *More...* / *Compress* option. If the compression factor offered by the software exceeds 1, you may accept it or adjust it manually to an acceptable value.
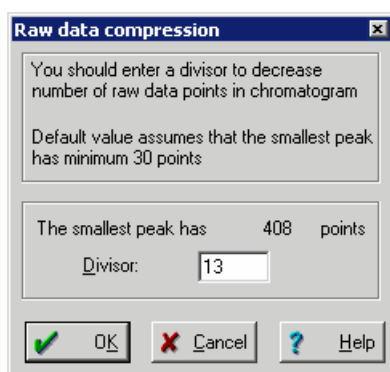
- Save the method (**File** / *Save* / *Method* menu item) and the chroma-

    togram (**File** / *Save* / *Chromatogram* menu item or 🖫 icon).
All future chromatograms obtained by the modified *Method* will use this corrected *Frequency divisor* value. This setting does not affect the hardware sampling frequency, but it changes the sampling frequency of the saved chromatogram. In 'Method/Method setup/Measure' the new sampling rate can be found.

For standard liquid chromatography we advise to use a sampling rate of 1 or 2 Hz.

Restart a local Method

Each chromatogram (***.chw**) or Method (***.mtw**) file contains a local copy of the System configuration with its corresponding control parameters (control method) and data processing method.

So it is possible to load old *chromatogram* or *Method* from disk and to *restart* it (i.e. to repeat the analysis with the same conditions as before). It is also possible to change settings in the loaded *chromatogram* or *Method* before restarting it. In order to avoid confusion, such a System that was embedded in the chromatogram or *.mtw file, gets the name of the parent chromatogram file.

This approach can be recommended in specific cases only, for example if an analysis is to be repeated only once. Otherwise, it is more convenient to create a separate *System* and *Method* for the analysis.

Each chromatogram or Method stores a *hardware configuration* with a complete set of *device parameters*, as well as a *data processing method* (and data itself for the chromatogram).

- Open the *chromatogram* (**File/*Open/Chromatogram***) or *Method* (**File/*Open/Method***) that is restarted.

- Choose the **Method */ System** menu item. The *System window* will open that has the name of the parent chromatogram in the header.

- Connect the *System* to workspace by choosing the **Control/*Connect to workspace*** menu item.
  Note that all other systems that use the same hardware must be *closed* or *disconnected*.
  Moreover, one must be sure that all devices in the old system are connected to COM ports properly.

- If necessary, modify the working parameters of the *devices.*

- If necessary, modify the *processing Method* (in the current chromatogram window).

- If the analysis will be repeated more than once, save the System (*.smt) and the Method (*.mtw) as a new file.

- Start the chromatogram. Before start, the software opens a new chromatogram window and invokes the «Start run» dialogue box.

TUTORIAL 5

Calibration

Introduction

The *Calibration* procedure provides the system with all necessary data for peak *Identification* and *Quantification*. *Calibration* has two basic functions:

1. It defines *retention times* (volumes, indexes) of analysed components. This information is required for the subsequent identification of components in mixtures of unknown composition (i.e. for *qualitative analysis* of the sample).

2. It defines *calibration coefficients* that link the detector response (peak height or area) and the concentration for each component to be analysed. This information is used to calculate the concentration of identified components (i.e. for *quantitative analysis* of the sample).

By obtaining calibration chromatograms for mixtures with known qualitative and quantitative composition both objectives can be achieved simultaneously. All calibration data and results of calibration are stored in the *Method* (and in each chromatogram as well).

All steps for successful calibration will be described shortly, and each step will be explained in detail.

Calibration procedure: short description of steps

In this procedure we assume that a number of samples with unknown concentrations is analysed. A few standards are used for calibration. The System is initialised and optimised for analysis. The chromatography and detection are reproducible. The Autosampler is filled with standards and samples. The following steps must be taken for successful calibration:

Step 1: calibration chromatogram

First we must know where the relevant peaks are in the chromatogram. This information is obtained from a calibration chromatogram. It can be a newly acquired chromatogram, or you can open a previous run. Important is that **all relevant peaks** are present in this calibration chromatogram. The Integration parameters are tuned such that all peaks are automatically found. Be careful with minimum area/peak height that small peaks are not overlooked.

Step 2: component table

On basis of the calibration chromatogram a Component table is created that contains the information on analysed components: name, expected retention time, retention index, and response factor for each component as well as some other information, necessary for peak identification and quantification.

> **Don't forget:**
> **- always save your method!**
> **- make sure the correct method is connected to your System**

Step 3: concentrations table

The concentrations table of the calibration chromatogram contains the concentrations of all standards to be used for calibration. The table is a template made before calibration and will be filled with actual data later during calibration. It is important to realise that **each chromatogram** obtained with this method contains the concentrations table. So if changes need to be made afterwards, these become only effective after **all chromatograms** are reprocessed in a batch.
Note that 'levels' must be considered as a counter for calibration points. If a given standard concentration must be analysed twice just add a new

level (increment) with the same concentration. Because concentrations of standards are calculated from volume, dilution and amount, these data fields must be entered correctly.

> ⚠ **It is very important to save the method. A method contains all calibration information for new chromatograms to be acquired.**

Step 4: running calibrations

Running calibrations can be done:
- one by one followed by manual integration and calibration
- one by one followed by automatic integration: automatic level filling
- in a sample queue with autosampler

Step 5: viewing calibration graphs

After running all standards it is possible to view your Calibration curve (graph) for each component and to edit it (erase some points, change Calibration formula, etc.). This is an important step to have reliable calibration results.

Step 6: analysis of unknown sample

Running samples is done in the same way as standards; the only difference is that samples have **calibration level 0 (zero)**.

Step 7: reporting results

Each chromatogram can be printed including (calculated) raw concentration, calibration data and many more integration and analysis details. Multiple chromatograms can be reported **batch wise in a peak table** including average and standard deviation. This is described in the next chapter.

Programming calibration

All calibration operations are available from the **Method** / *Calibration* submenu, that includes the following items. Each item activates an appropriate dialogue box or procedure.

Components	creates or edits the *Component table*, includes component names, data on their retention times and *calibration results*[15]
Identification	selects an identification scheme
Concentrations	creates or edits the *Concentrations table*, contains calibration data for all components and for all calibration points
Graphs	performs operations on calibration curves of separate components (to view and to edit)
Update	updates the retention time, indexes or calibration coefficients for all components of *Component table*, all in accordance with the current chromatogram
Load from method	reads the calibration results from the current method to the current chromatogram
Save to method	Saves the calibration results from the current chromatogram to the current method
Import calibration	reads the *Component table* and calibration parameters from a file
Export calibration	Saves the *Component table* and calibration parameters to a file

The complete calibration procedure is not needed for some *quantification methods*: for example, calibration of retention times is performed only in case of the *Response normalization* method.

In case of multi-channel chromatograms additional options appear. It is possible to select any data channel or any pre-defined calculated channel as a default for quantification. The default channel is used for quantification of all components except for that declared as *local* (or special). Any component can be declared as a *local*, and the channel for its quantification can be selected individually.

Step 1: calibration chromatogram

The first obtained *calibration chromatogram*[16] is used to create the *Components table* and to fill the first *calibration level* in the *Concentrations table*.

- Prepare your first calibration sample. Inject it and start the first calibration chromatogram.

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15 Calibration results - a set of *calibration coefficients* for all components analysed. Calibration coefficients are used to calculate concentrations of components starting from peak heights or areas.
16 The calibration chromatogram is that to be used for calibration procedure.

- Fill in the «Start run» dialogue box as described previously. Pay attention to correct filling of "*Duration*", "*Volume*", and "*Dilution*" fields that are used for further calculations. "*Vial number*" and "*Volume*" parameters are used to control the autosampler.
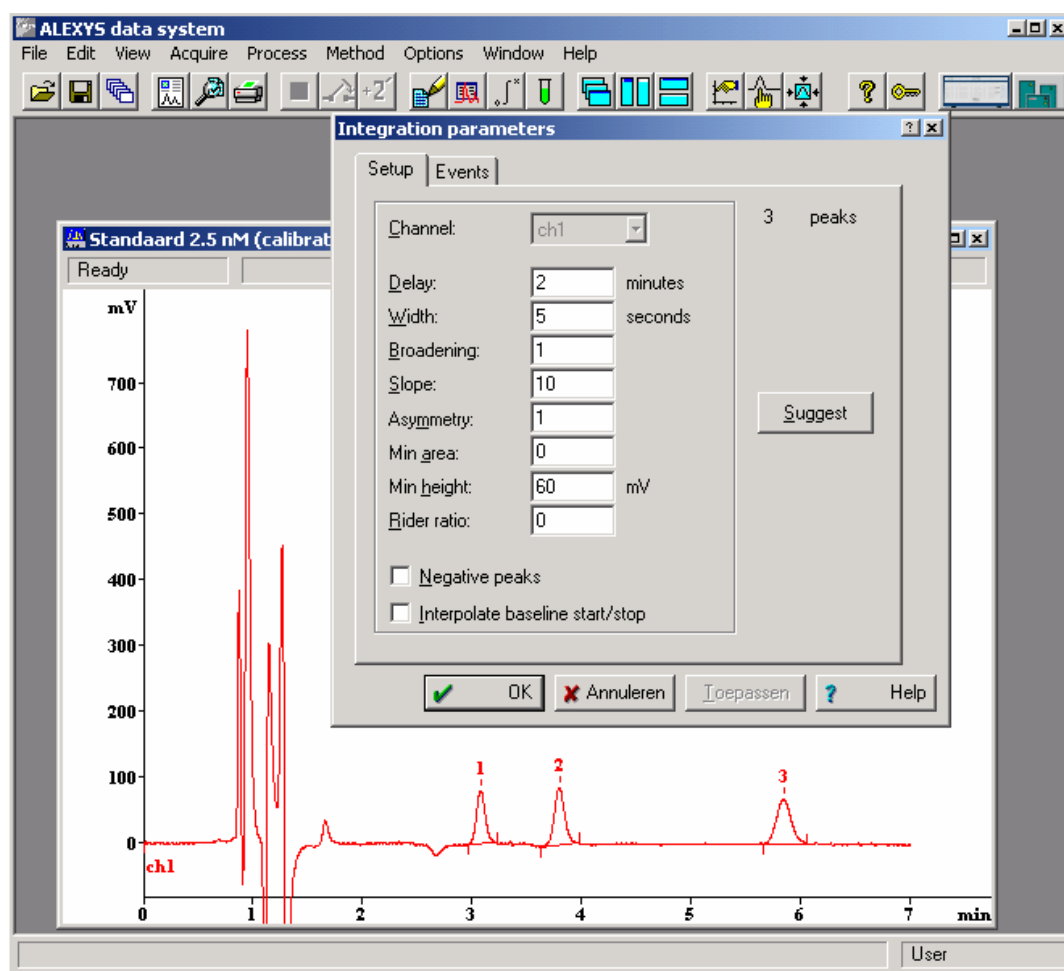
- When the chromatogram is completed, check if the integration results are fine. Make corrections if needed.
- Save the chromatogram on disk (**File** / *Save / Chromatogram* menu

  item or click ![save icon] icon).
- If the *processing Method* has been modified, the software prompts to save changes. It is possible to modify the current *Method*, to save it as a new file[17], and not to save any changes at all.

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17 Even if a new ***.mtw** file is created, the old Method remains attached to the current System. If necessary, change the attached Method as described in the *Installation and configuration* section,.

Step 2: component table

The *Component table* stores the information on analysed components. It contains *name*, *expected retention time*, *retention index* and *response factor*[18] for each component as well as some other information, necessary for peak identification and quantification.

The *Component table* is created on the basis of a *Calibration chromatogram*, which is a chromatogram of the mixture of all components of interest, with known concentrations as a rule[19].
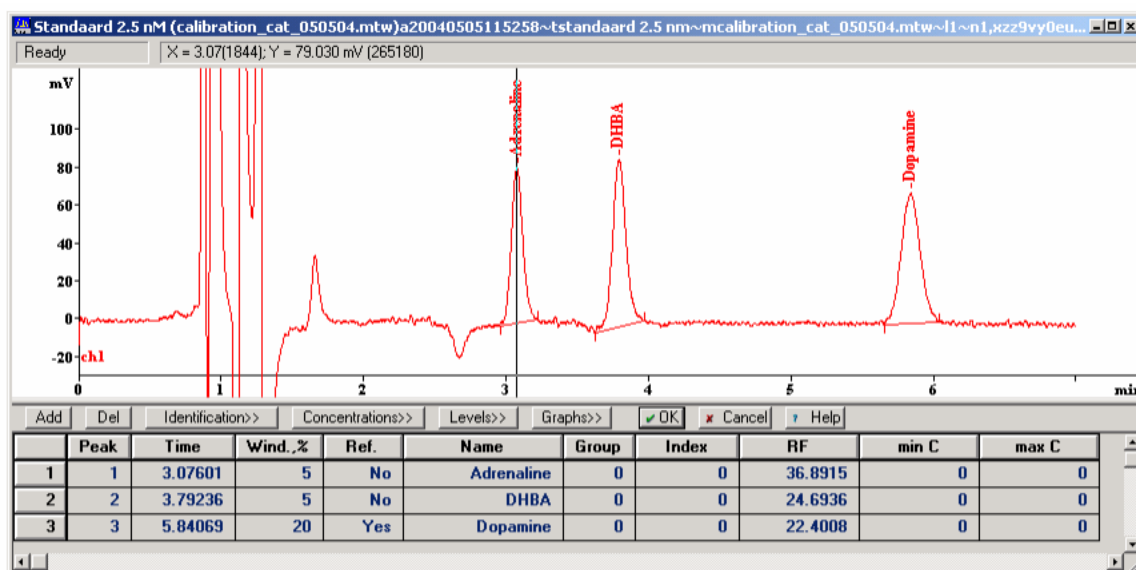
- Open the previously obtained calibration chromatogram.
- Execute the **Method** / *Calibration...* / *Components* menu item or click
  icon. An empty *Component table* will appear in the lower half of the window.

If an old *Component table* already exists and you would like to erase it as a whole, perform the *Clear all table* procedure from the **Table** menu.
- Click the <*Add*> button in the line above the *Component table* (or choose **Table menu** / *Add component* item). A new row will appear in the table.

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18 All calibration coefficients are stored here, but the first one (i.e. the response factor) is visible.
19 The component table can be created based on qualitative composition of the calibration mixture.

- Enter the peak number you wish to name, into the "*Peak*" column. Press the right arrow. The cursor will jump to the chosen peak apex and its retention time will appear in the "*Time*" column.
- Enter the identification *Window* (typically 2 -10% depending on precision of your chromatographic system, 5% is the default). The *Identification window* is the range where the current component is expected, expressed as a percent of its retention time.
- Enter the component's *Name*.

Nameless components are erased from the *Component table* when editing is completed!
Use the mouse to zoom in on any desired part of the chromatogram. Double click the left mouse button to view the complete chromatogram. Click inside the *Components' table* to resume editing.

- Press the <***Add***> button and fill in a new line for every component you want to add. You can delete the current line (the line where the cursor is in) from the *Component table* by pressing the <***Del***> button. For components with a known *retention index* fill in the "*Index*" column.
- Select one or more peaks that stand separately or are in all subsequent chromatograms the largest within their identification windows. Declare them as *Reference*[20] by clicking the <***Yes***> trigger in *Ref.* cell of the *Component table*. Set the *Identification window* parameter for these components twice as high as others.

If you wish to report all unrecognised peaks just create a so-called *Universal component* with zero retention time (the component *Unknown* in the first line of the *Component table* on the figure below). Its name and calibration coefficient will be applied for all unidentified peaks.

- Accept all changes and close the *Component table* by pressing the <***OK***> button. It is possible to reject all changes by clicking the <***Cancel***> button.
- Save the method (**File** / *Save / Method* menu item) and the chromatogram (**File** / *Save* / *Chromatogram* or 💾 icon).

Now your *Method* is ready to execute identification of components and their quantification in the simplest way available (i.e. by the *Response normalization* method).

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20 The reference peak algorithm enables to correct a possible drift in retention.
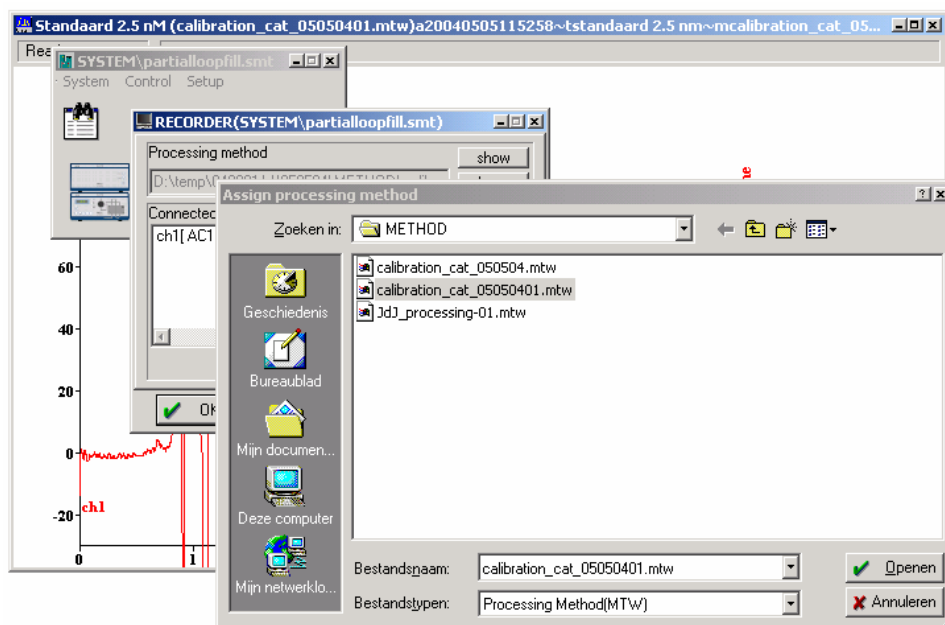
It is important that the new Method is also the Method that is attached to the System. This is done by clicking on the recorder icon and the 'choose' button to assign the Method to the System.

Multi channel chromatogram: select reference channel

Before the calibration procedure is started, the default reference channel used for quantification of all components, except for local (special), must be selected. Any data channel or calculated channel can be selected. However, for reliable calibration results it is recommended to use either a data channel or a calculated channel of the «average» type. If there is one data channel only, it automatically becomes the reference one.

Open the Components table (**Method** / *Calibration...* / *Components* menu item or click the [icon] icon). Click <Graphs> button.

Note that the calibration data have not been acquired completely and data points 2 – 5 are not used (plotted with x = 0).

- Set the following parameters (which are global, i.e. valid for all components):
  Calibration method: "*External standard*" (the «external standard» calibration method will be used).
  Base: "*Area*" (the peak area will be used to calculate the detector response)
  Reference: name of the channel to be used for quantification.
  The «Average» channel can be used if it is difficult to select a suitable channel for quantification of all peaks. This does not hold for a single-channel chromatogram.
- If some global parameter has been changed, the user will be prompted <Yes> to accept changes

Select local reference channel

If a component must be calculated using its individual channel, select the desired component desired. List components by arrow buttons or select from the list, then check the box for "Reference" line and select the desired channel.

- Click *<OK>* to accept the settings and close the «*COMPONENT GRAPH*» window. No additional prompt is needed.
- Click *<OK>* to accept settings and close the «*COMPONENTS TABLE*» window. Don't perform this operation if a next step is to be taken.

Step 3: concentrations table

Now we are going to create a template from the *Concentrations table.* The *Concentrations table* contains the concentrations of all components in all samples to be used for calibration. Each calibration chromatogram corresponds to a separate column of the Concentrations table, the so called *Calibration level* or *calibration point*.

It is recommended to create the *Concentrations table* template directly after the first calibration run, i.e. when *Component table* has been created. The software takes the component names from the *Component table*. Peak areas (or heights) and actual adjusted volumes will be added later, i.e. during the calibration procedure.

The *Concentrations table* is always created on the basis of the existing *Component table*!

- Open the *Concentrations table* (choose the **Method** / ***Calibrate*** / ***Concentrations*** item or click the <***Concentrations***> button in the *Component table* window). A new *Concentration table* contains two columns: *Name* (name of the components, taken from *Component table*) and *This run* (concentrations of recognized components in the current chromatogram, calculated from their peak areas and calibration coefficients[21]). Both columns are unavailable for editing here.

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21 Response factors (RF's) for all un-calibrated components are considered as 1 by default.
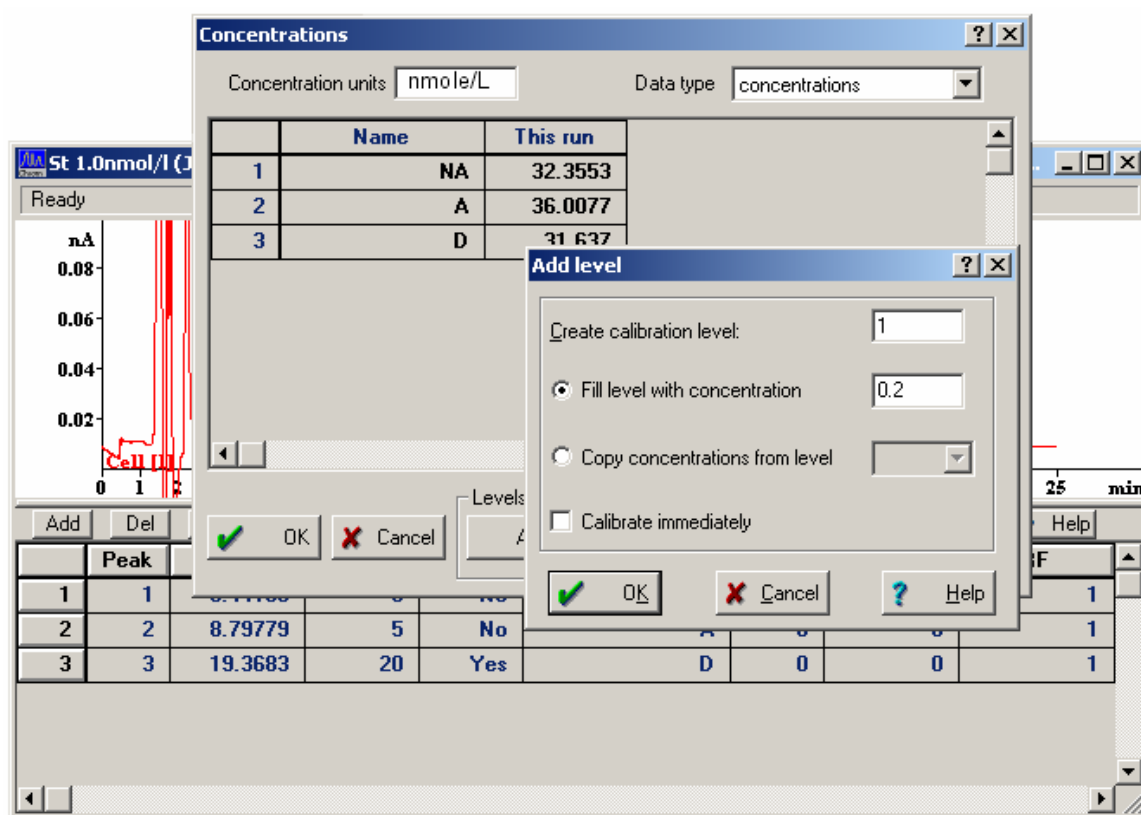
- Press the <***Add***> button (or the keyboard [**Alt**]+[**A**] combination).
- Enter a number for the *Calibration level* to create ("1" in this case) and press the <***OK***> button or the [**Enter**] key. A new column *Level # 1* will be created.
- Fill in the column with concentrations for every component in the first calibration mixture.
- Fill in the level for every calibration mixture you are going to use. If you are planning 3 calibration runs for the same mixture, create three levels with the same concentrations of components. In order to obtain a more precise and visual curve, you can inject different volumes of the same calibration solution or use dilutions of the same sample.
- Enter the *Concentration units*[22] you are going to use.
- Click the <***Calibrate***> button to couple peak areas and heights from the current calibration chromatogram to the first calibration level[23].

---

22 These *Concentration units* are used for reference only.

23 This operation can fill any desired calibration level number. The only requirement is that the of component concentrations in the sample correspond to that in the *Concentrations table*

- Accept changes and close the *Concentrations table* by pressing the <*OK*> button or [**Enter**] key (or click <*Cancel*> to loose them).
- Save the method (**File** / *Save* / *Method as*) and the chromatogram (**File** / *Save* / *Chromatogram* menu item or ![save icon] icon).

Other operations on *calibration levels* can also be performed here. You can *delete* the current level (i.e. the column where cursor is placed) by pressing <*Delete*> or [Alt]+[D] keyboard combination.

Step 4: running calibrations

A calibration procedure consists of the following steps:
- obtaining the calibration chromatogram;
- filling[24] in the appropriate calibration level in the *Concentrations table* template (that must be created in advance as described previously).
As a rule, the first calibration chromatogram is used both for the *Component table* creation and filling the first *Calibration level*.

- Obtain the next calibration chromatogram or load it from disk.
- Check that peak integration results and identification are satisfactory; make corrections if necessary.

Hint: choose the **View** / *Appearance* /*Peak label* / *Component name* option for visual control of identification results. The peak names will be plotted for all identified peaks.

- Select the **Process** menu / *Calibrate* item.
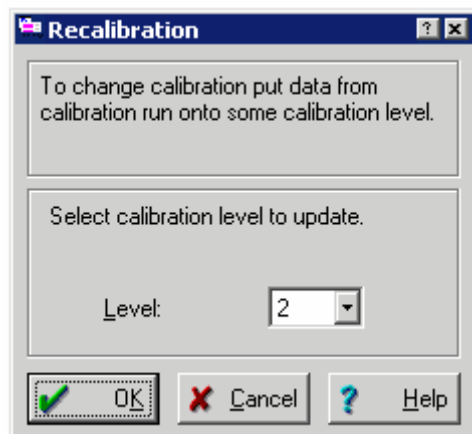
- Click the ![dropdown] button in the "*Level:*" input field and fill in the level number. Press <*OK*>. The chosen level in the *Concentrations table* will be

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24 To fill in the calibration level means to put data from the current calibration chromatogram to the appropriate column in the *Concentrations table*

coupled to the peak areas and heights; the calibration coefficients will be calculated for all identified components.

An arbitrary sampling order of calibration mixtures is acceptable. Nevertheless, to avoid mixing-up it is recommended to analyse calibration samples in the order listed in the *Concentrations table*,. In any case, the calibration sample and calibration level number (i.e. concentration of components) must correspond.

- Run all other calibration chromatograms and fill in the corresponding *Calibration levels* as described.

Automatic level filling

If the integration results of calibration chromatograms are fine and do not need further correction, the calibration levels can be filled in automatically. This must be done after finishing the chromatogram.

Note that *Automatic level filling* still is a way of manual calibration. To perform this operation, start a new calibration analysis and specify a number of calibration levels in the «Start run» window:

When the chromatogram is finished, the specified level will be automatically filled with calibration data taken from the current chromatogram.

Two conditions are needed to perform successful automatic level filling:

- integration parameters must be tuned to avoid additional manual correction;

- The *Concentrations table* template must exist before the start of analyses.

Note that if the autosampler is used, complete automation via *Queue* is available (see next).

Automated calibration using the sample queue

Working with an autosampler and a sample queue is the most convenient way of running calibrations. This procedure is described in the last chapter (page 129). Briefly, it allows assigning levels to standards and autosampler vial numbers in a convenient queue editor.

	System	Ident	Vial	Volume	Dilution	Amount	Internal Standard Amount	Level	Injections	Done	
29	*flushedloop_tweede_run.smt*	*M-12.5*	29	20.0	1.0	1.0	100.0	0	1	1	
30	*flushedloop_tweede_run.smt*	*M-15.0*	30	20.0	1.0	1.0	100.0	0	1	1	
31	*flushedloop_derde_run.smt*	*S-1.25*	1	20.0	1.0	1.0	100.0	15	1	0	
32	*flushedloop_derde_run.smt*	*S-2.5*	2	20.0	1.0	1.0	100.0	16	1	0	
33	*flushedloop_derde_run.smt*	*S-5.0*	3	20.0	1.0	1.0	100.0	17	1	0	
34	*flushedloop_derde_run.smt*	*S-7.5*	4	20.0	1.0	1.0	100.0	18	1	0	
35	*flushedloop_derde_run.smt*	*S-10.0*	5	20.0	1.0	1.0	100.0	19	1	0	

Step 5: viewing calibration graphs

The aim of the calibration procedure is to construct a *Calibration curve*. After the method has been calibrated, it is possible to view the *Calibration curve (graph)* for each component and to edit it (erase some points, change *Calibration formula*, etc.).
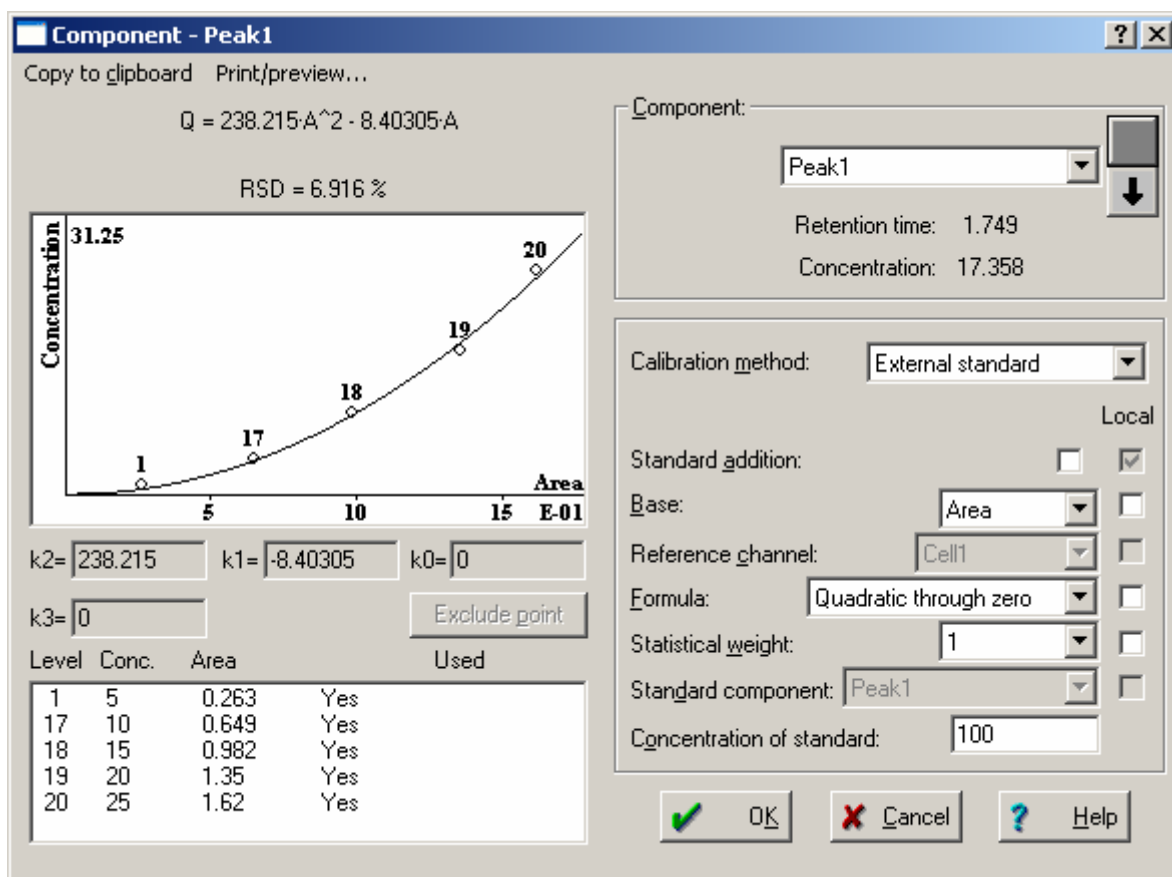
- Open a *Calibration graph* window by the **Method** / *Calibration...* / *Graphs* menu option. If the *Component table* is opened, click `Graphs>>` button.
- Only the graph of the current component is shown. The name of the current component is printed as window header. List the components by [↑] and [↓] buttons or choose any one from the list by clicking [▼] in the «*Component*» field.
- Set the *External standard* calibration method.

If some point of *Calibration curve* for the current component drops out, it can be excluded. To exclude a point from the list, select it by the mouse clicking and execute <*Exclude point*> operation. The **Yes / No** trigger in the "Used" column switches to the opposite. Repeated operation includes the point. The Calibration curve is recalculated automatically when if this point is excluded (included). Watch for «*RSD*» (Relative Squared Deflection) and «Corr.» (Correlation Factor) values over the graph during this procedure. This operation only affects the current component, not the *Calibration level* as a whole!

- Click the <*OK*> button to confirm editing.
- Now the calibration process is completed.
- Save the method (**File** / *Save / Method)*.

Tabulated calibration method

Sometimes relative response coefficients of the detector are known (they can be taken from reference literature, calculated by any means or obtained experimentally). In such cases only the calibration of the component retention is carried out (i.e. the mixture with the known qualitative and unknown quantitative composition can be used) and the simplified *tabulated* way of calibration is applied.

Relative calibration coefficients are known to be much more stable than absolute ones. So, in most cases it is sufficient to update an absolute calibration for the standard only in order to refresh the entire calibration for all other components. However, additional systematic errors may occur. Therefore, it is recommended to update relative calibration coefficients experimentally on a regular basis, especially in case of recurring service or repair of detectors.

The ALEXYS data system allows using a tabulated way of calibration to calculate absolute concentration of components. It is then necessary to declare one of the components as a standard and to obtain its calibration dependence by the external method, as has been described above. In this case calibration graphs for other components can be nonlinear (but similar to a curve of the standard component!). If absolute calibration of the standard is unknown, linear calibration graphs only can be used for all components.

«Normalized concentrations» quantification method

The *Normalized concentrations* quantification method calculates concentrations of components using their predefined relative calibration coefficients. All concentrations are normalized to the user-defined normalization coefficient (100% is the default value).

A *quantification method* can be chosen in the "*Report options*" dialogue box.

- Choose the **Method** / *Calibration …* / *Graphs* item (or click the ⬇
  icon to invoke *Components' table* and press the `Graphs>>` button).
- Set the *Tabulated* calibration method; select a component to be used as *calibration standard*; click <*OK*> to close the dialogue box and to accept editing.
- Obtain a *calibration chromatogram*.

- Create and fill in the *Component table*.
- Enter *relative response factors*[25] for all components of interest into the "RF" (*Response factor*) column. Click the <***OK***> button to accept changes and close the *Components' table*.
- Open the "*Report options*" dialogue box (**Method** / ***Report options*** menu item or ⊞ icon) and set the *Normalized concentrations* quantification method. Accept editing by clicking <***OK***>.
- Save the method (**File** / ***Save / Method***) and the chromatogram (**File** / ***Save*** / ***Chromatogram*** menu item or the 🖫 icon).

«Absolute concentrations» quantification method

The *Tabulated calibration method* can be used to calculate *absolute concentrations* of components as well. In this case an absolute calibration curve for the component, used as a calibration standard, is required.

- Open a chromatogram of the calibration mixture, create the *Component table,* and enter *relative calibration coefficients* of components, as described above.
  The response factor of the standard component must be set to 1.

- Select the **Method** / ***Calibration …*** / ***Graphs*** item (or click the 🔻 icon to open the *Components' table,* press the Graphs>> button) and set a *Tabulated* calibration method.
- Create a *Concentrations table* for the standard component as described in "*Step 14...*" paragraph. Use as many calibration levels as needed. Note: Only the concentrations table for the standard component must be filled in. Concentrations for all other components must be zero.
- Carry out a calibration procedure for the standard component[26] as described in the "*Step 14...*" paragraph.
- View and edit the resulting calibration graph for the standard as described in the "*Step 15...*" paragraph. Note, that the relative response factor of the standard component (always 1) was replaced by its absolute value, while response factors of other components stayed relative.
- Save the method on disk as LEARNTAB.MTW (**File / Save / Method** menu item).

Now it should be possible to use any quantification method offered by the ALEXYS software. An absolute calibration dependence for any component is obtained by multiplying the calibration dependence of the standard

---

25 *Relative response factors* can be obtained experimentally or taken from reference literature. The response factor of the standard component is taken as 1.
26 Calibration samples containing a standard component only are usually used.

by relative response factor of the given component. In other words, the calibration curves of all components are similar to that of the standard.

- Don't forget to change the *processing Method*, attached to the current System, to the modified one. This operation is essential because our *Method* was saved under a new name while *Method* attached to the *System* was left unchanged.

How to obtain relative response factors

It is possible to determine relative response factors for the *Tabulated* quantification method experimentally. It is then necessary to have a calibration mixture, containing all components of interest, with known concentrations.
The following sequence is recommended.
- Create an absolute calibration dependence for all components of interest, including the calibration standard, using appropriate calibration mixtures (as it is described in "*Stage 9...*" - "*Stage 13...*" paragraphs).
- Open the *Component table* (**Method** / **Calibration...** / **Components** or click icon ⬇).
- Open the "*Graphics*" dialogue box (click on the **Graph>>** button inside the *Components' table*).
   - set the *Tabulated* calibration method. Agree with the inquiry to change the global parameter hereinafter;
   - select the standard component for calibration;
   - Click <*OK*> to return to the *Component table* again and enter a value of 1 to the "RF" cell for the standard component.
- Open the "Graphics" dialogue again and set the *Internal standard* calibration method[27]. Relative response factors will be calculated for all components (RF for standard = 1).
   Click <*OK*> or press [*Enter*] to see the results.
- Open the "*Graphics*" dialogue again and set the *Tabulated* calibration method. The absolute RF value for the standard will be set while relative RF values will be preserved for all other components.
- Click <*OK*> or press [*Enter*] to accept results and to close the *Components' table*.
   (All changes can be discarded here by clicking <*Cancel*>).
- Save the calibration results in the LEARNTAB.MTW method (**File** / ***Save / Method)***.

---

27 The *RF* of the standard component in this case will be absolute, whereas the RF of other components will be relative (i.e. calculated against a standard component). If the "*Internal standard*" calibration method will be left, all RF values in the *Components' table* will be relative.

The data system is able to calculate both relative and absolute concentrations using the *Tabulated* calibration method. The *Relative response factors* of components are quite stable and need no frequent updates. It is sufficient to perform an absolute calibration (to update absolute response factor) periodically for a standard component only.

Step 6: analysis of unknown samples

Now you are ready to analyse "unknown" samples and perform their identification and quantification based on previously created *System* and *processing methods.*

- Click the *System bitmap* in the *icon bar* of the *main window* to open . the *System window.*
- Click **Control / *Start determination*** in the *System window*.
- Fill in the analysis description fields in the "Start run" dialogue box. Check sample *Volume*, sample *Dilution,* and analysis *Duration*. Click <***OK***> button[28].
  Set «*Calibration level*» parameter (must be **0** for analytical runs).
- Inject the sample, and immediately press *Start* button on the AC 100 front panel.
- When the preset analysis time is over, the software finishes the chromatogram and *post-run operations* are automatically performed. You can interrupt data acquisition at any moment by **Control / *Stop data acquisition*** menu option.
  Activate **Control / *Stop determination*** to cancel both data acquisition and device time programs.
- Inspect results of integration and, if necessary, tune them.
- Save the chromatogram (**File / Save / Chromatogram** or 🖫 icon).
- Save the current processing method (**File / Save / Method**), and save the current control method («System» / System / Save), if corrections were made.

Step 7: reporting results

For step 7 see next chapter.

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28 If *Recorder* device is situated in the "Start with determination" area of the **Setup / *Start mode*** window, data acquisition will start immediately. If *Recorder* device is situated in the "Start with inject" area, the software will wait for the *inject event* (i.e. external start knob pressing, injector contacts closure, auto-sampler start relay, etc.).
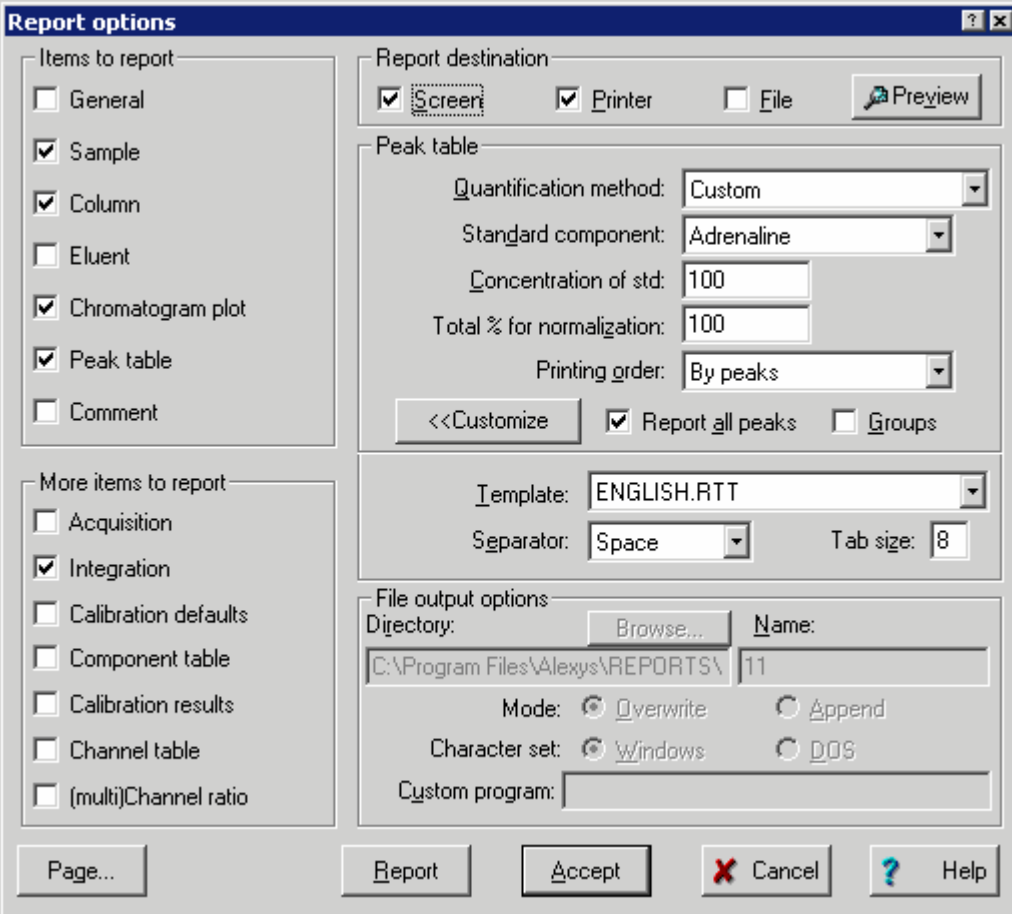
> **For more information about calibration and the mathematical background information, see the ALEXYS data system help file in the software itself.**

T U T O R I A L   6

Report generating

- Load the desired chromatogram from disk or obtain it as described above.
- Open the "Report options" dialogue box (**Method /** *Report options*, **Process /** *Make report* menu item, or ⌗ icon).

- Tick all items in the left part of the screen you wish to include in the report. A standard report usually includes items from «*Items to report*» area, while «*More items to report*» area can be used for an extended report.

- Select the desired *Quantification method* that determines the structure of the *Peak table* ("*Absolute concentration*" in our example).
- Click <***Preview***> in the *Report destination* area and check the results.
- If the results are not satisfactory, correct where required and repeat evaluation.
- Choose desired devices for report output (for example, screen and printer). Click <***Page...***> and set page setup options such as margins and plot size.
- Press <***Report***> to start output.
- Save the chromatogram (**File / Save / Chromatogram** menu item or 🖫 icon)
  Save the *Method* to make selected report options permanent (**File / Save / Method**).

The report can be directed to the ***Screen***, ***Printer***, and ***File*** simultaneously, in any combination.

Quantification methods

Any standard *quantification method* of the data system can be used if the external standard calibration is executed. The current chromatogram can be processed by several methods. Moreover, several quantification methods can be combined in a single report by using the *Custom* method. The *Quantification method* is chosen in the "Report options" dialogue box. However, additional parameters may be required in this case.
The following quantification methods need additional parameters:

Normalized concentration method.
- Choose "*Normalized concentration*" quantification method.
- Set a desired "*Total % for normalization*" parameter to the desired value (100% is default).
- Generate a report as described above.

Internal standard method
- Choose the "*Internal standard*" quantification method.
- Select the component to be the *internal standard*.
- Enter the concentration of the internal standard in the sample.
- Generate a report as described above.

Custom report

The ALEXYS data system provides a set of default report templates that correspond to common quantification methods: *Response normalization*, *Normalized concentrations*, *Absolute concentrations*, *Relative concentrations*, *Index* and *Column test.*

In addition, it is possible to create your own, completely customised report format and use it in your *Method.*

- Choose the «*Custom*» quantification method in the "Report options" dialogue box.
- Click **<<*Customize>>*:**

- Choose desired columns to be included in the *peak table.*
- If «*rel. concentration*» or «*rel. concentration%*» items are selected, the following parameters must also be defined:
  - Standard component;
  - Concentration of std.
- Click <*Preview*> button and check the results.

- If the results are fine, select the ☑ *Printer* checkbox, click <***Page...***> and adapt chromatogram plot size and print margins if required.
- Click <***Report***>.
- Save the modified *Method* and *Chromatogram* as usual.

T U T O R I A L   7

Creating a Queue

You have now learned how to create a *System* and a data processing *Method*, make a calibration and run a single chromatogram. To automate data acquisition and processing, you can run or reprocess a sequence of chromatograms.

A *Sequence of chromatograms* can be used to automate:

- The data acquisition: a *Sample queue.*

- Reprocessing of previously obtained chromatograms: a *Batch.*

1. A *Sample queue* is in fact a list of *Systems* to be run in a sequence plus some processing instructions that run automatically. A *Sample queue* can be used for automation of data acquisition and calibration and is especially useful if an autosampler is part of the system. A *Sample queue* is stored on disk as a ***.que** file and can be re-executed repeatedly.

2. A *Batch* is composed of several chromatograms that have been al-ready obtained and saved on disk. This approach is used to reprocess a number of chromatograms similarly. A *Batch* is stored on disk as a ***.bar** file. It can be re-processed repeatedly but cannot be used for data acquisition like a *Sample queue*!

Creating a *Sample Queue*

The following example of the *Sample queue* shows how to prepare and run a sequence of chromatograms to automate the calibration procedure and analysis of "unknown" samples.

The "Test / Learn system" *System*, that has been prepared in previous steps, will be used.

- Open the *System window* (click *System bitmap* in the *icon bar* of the *main window*).

- Click **System / *Sample queue...*** in the *System window*.

- Enter the name of the *Sample queue* (QUEUE EXAMPLE) and click ***Open***.

- A new *Sample queue* will be created with the "QUEUE EXAMPLE" name in the "DAD"[29] directory, and the *Sample queue control window* will be opened.

---

29 Systems are stored in the default catalogue "/ALEXYS data system/Systems/". When the "New system" wizard creates a new System, a new subdirectory is created. Each subdirectory
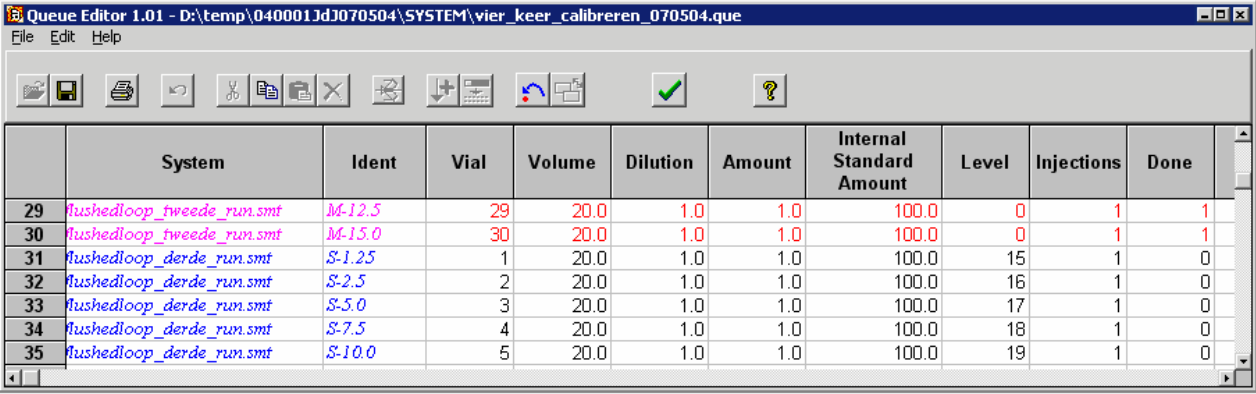
Long file names can be used for *Queue* files according to *Windows 98*, Windows Me, *Windows NT, Windows 2000 or Windows XP* rules.

```
D:\temp\040001JdJ070504\SYSTEM\vier_keer_calibreren_070504.que
File   Control
 14  flushedloop_eer    M-12.5      14   1   1      0    20.00  1.00
 15  flushedloop_eer    M-15.0      15   1   1      0    20.00  1.00
 16  flushedloop_twe    S-1.25      16   1   1      8    20.00  1.00
 17  flushedloop_twe    S-2.5       17   1   1      9    20.00  1.00
 18  flushedloop_twe    S-5.0       18   1   1     10    20.00  1.00
 19  flushedloop_twe    S-7.5       19   1   1     11    20.00  1.00
 20  flushedloop_twe    S-10.0      20   1   1     12    20.00  1.00
 21  flushedloop_twe    S-12.5      21   1   1     13    20.00  1.00
 22  flushedloop_twe    S-15.0      22   1   1     14    20.00  1.00
 23  flushedloop_twe    M-0         23   1   1      0    20.00  1.00
 24  flushedloop_twe    M-1.25      24   1   1      0    20.00  1.00
 25  flushedloop_twe    M-2.5       25   1   1      0    20.00  1.00
 26  flushedloop_twe    M-5.0       26   1   1      0    20.00  1.00
 27  flushedloop_twe    M-7.5       27   1   1      0    20.00  1.00
 28  flushedloop_twe    M-10.0      28   1   1      0    20.00  1.00
 29  flushedloop_twe    M-12.5      29   1   1      0    20.00  1.00
 30  flushedloop_twe    M-15.0      30   1   1      0    20.00  1.00

 □  Shut down system after the queue finishes

 □  Close queue dialog window after the queue finishes

  ●    Start    |   Pause   |    Edit    |   ✔   Close  |  ?    Help
```

- Click <***Edit***> to enter the *Sample queue* editing screen.
- The *Queue editor*[30] is activated and the dialogue box is displayed. Note that it looks like a spreadsheet and some editing functions are similar to Microsoft "Excel".

---

corresponds to a different equipment configuration, and in turn stores *System* files of the same equipment structure, but including various data processing *Methods*.
30 The queue editor in the current ALEXYS data system version is designed as a separate application QUEBAR.EXE. The same executable is used to edit either *.que or *.bar files.

	System	Ident	Vial	Volume	Dilution	Amount	Internal Standard Amount	Level	Injections	Done
29	flushedloop_tweede_run.smt	M-12.5	29	20.0	1.0	1.0	100.0	0	1	1
30	flushedloop_tweede_run.smt	M-15.0	30	20.0	1.0	1.0	100.0	0	1	1
31	flushedloop_derde_run.smt	S-1.25	1	20.0	1.0	1.0	100.0	15	1	0
32	flushedloop_derde_run.smt	S-2.5	2	20.0	1.0	1.0	100.0	16	1	0
33	flushedloop_derde_run.smt	S-5.0	3	20.0	1.0	1.0	100.0	17	1	0
34	flushedloop_derde_run.smt	S-7.5	4	20.0	1.0	1.0	100.0	18	1	0
35	flushedloop_derde_run.smt	S-10.0	5	20.0	1.0	1.0	100.0	19	1	0

- Fill in the first row of the *Sample queue table* (edit "System", "Title", "Volume", "Dilution", and "Level" columns; accept default settings for other columns). Press *F2* key or double click the left mouse button to edit the existing value; otherwise enter it as new.

    System[31]: the *System* to be used for equipment control, data acquisition and processing (Gradient x2/Learn.smt).

    The *System* can be changed in the current row: click ⊞ button, and select the desired *System* from the list.

    Title : the title of the chromatogram. Will be put into the appropriate *Passport* field of the future chromatogram and will be used as its window title. It is recommended to use a short but unique title to distinguish each chromatogram in the series and it will facilitate subsequent chromatogram handling and reprocessing.

    Volume: volume of the sample to be injected. Will be put into appropriate *Passport* field and used for auto sampler control and calculations.

    Dilution[32]: dilution of the sample prior to analysis. Note that the same standard solution is used for all calibration runs, but different dilutions are used .

    Level: number of the *Calibration level*. Enables to put data to the *Concentrations tab*le and to calculate Calibration coefficients automatically. If the sample is a calibration standard, enter level number; otherwise enter "0".

- *Done*: this parameter indicates if the chromatogram has been executed or not. If the sample is to be run more than once[33], "Done"

---

31 Different Systems can be included into a single queue.

32 Dilution is a convenient mean to prepare several calibration samples starting from a single calibration mixture. A more precise calibration with time saving is achieved in this case.
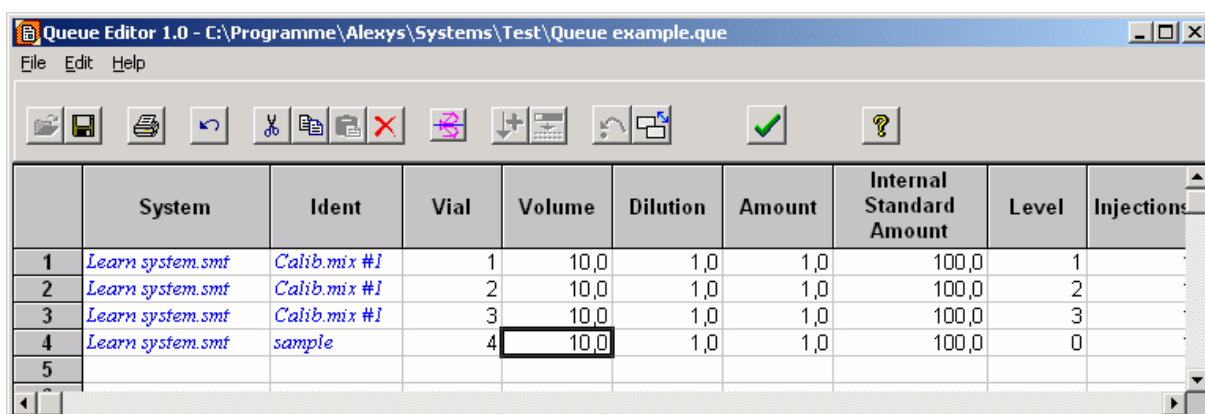
33 "Injection" parameter is greater than "1" for multiple injections. This item is useful for repeated analytical runs (with "Level"=0).

shows the number of the last completed run of the sample.
"0": sample has not run yet or is running now.
"1" or greater: sample run is finished.

- Select **Edit** / *Duplicate row(s)* in the *Queue editor* or click [icon] (Duplicate rows) button. A new row appears in the *Sample Queue table* that is a copy of the current one.

- Create and edit as many rows as you intend to include in the *Sample Queue* (each row corresponds to one sample). Note that several injections of each sample can be done.
- Save the *Sample queue* and exit the *Queue Editor* without execution (QUEUE EDITOR: **File** / *Save & exit* menu item or [icon] icon)[34].
- <*Cancel*> allows to exit the *Queue Editor* and to discard all changes.
- Check the «☑ *Shut down system after the queue finishes*» box. In this case all equipment will be shut down when the queue is completed (for example, pumps are stopped, the flow cell of the detector is switched of, column oven is off, etc.). You can use this option to leave the system working overnight.

The above *Sample Queue* example contains three calibration runs and one analytical sample run. All runs will be processed automatically, provided that the appropriate *Component table* and *Concentrations table* with three *Calibration levels* have been created in the LEARN SYSTEM.SMT *System*.

---

34 It is possible also to start the *Sample queue* without leaving the *Queue Editor*.

> ⚠️ **It is important to pay highest attention to the *Method* development and tuning in order to avoid any problems with integration and identification of peaks.**

The ALEXYS data system uses the ".QUE" extension for *Sample queue* files.

Running a Queue

Now you are ready to run the *Sample Queue*. Prepare three standard solutions for the *Calibration* procedure and one "unknown" sample.
Using the *Sample Queue*, it is critical to inject standards and samples in the exact order as they are specified in the *Sample Queue table*. Sample *Volume* and *Dilution* values must be in accordance as well.

To start batch acquisition:
- Choose **File** / *Open* / *Sample queue* in main menu.
  or open the *System window* and select **System / *Sample queue***.
- Choose the desired name of the *Sample Queue* file ("QUEUE EXAMPLE.QUE") and click <*Open*> to activate the *Sample queue control window*.
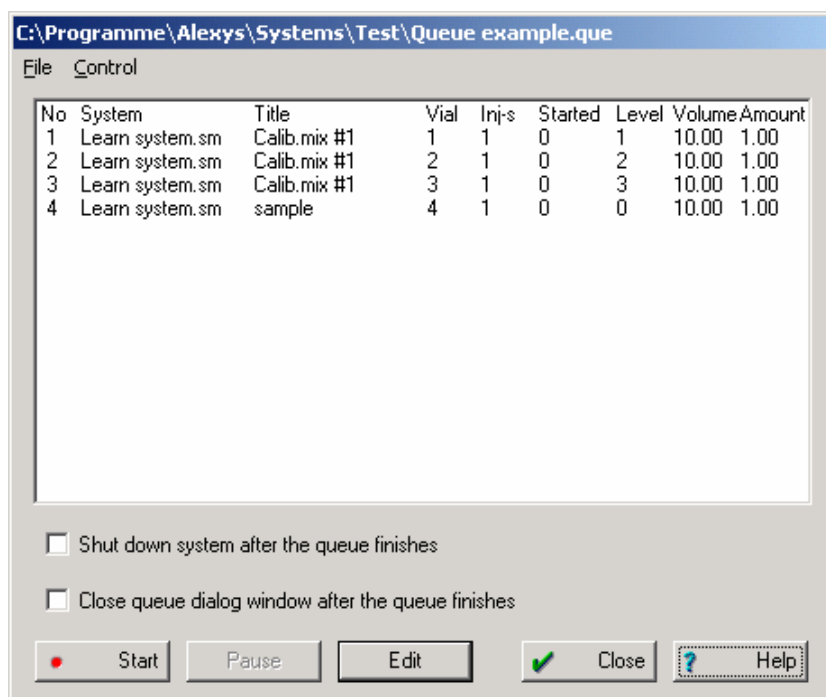
- Click <***Start***> to begin execution. The line of the *Sample Queue table* that corresponds to the running chromatogram will be selected.
  If necessary, open the *Queue editor* by clicking <***Edit***> button, make corrections to the *Queue table*, save the Sample queue, and exit the editor, as described earlier.
- The *chromatogram window* will be created and the pre-run baseline plot will be displayed. Inject the first sample and press button to generate the *inject event*[35]. The corresponding parameter "Started" in the *Sample Queue table* will be increased by 1.
- When a preset run time is elapsed, the chromatogram will be finished, processed and saved on disk. If driver time programs were run, they are continued according with the System settings.
  When all *device time programs* are completed, a new *chromatogram window* will be created and the data system will be ready for the next analysis («*Waiting for inject*» message of green background will be displayed in the *System state* window).
- Inject other samples as described (if the AS 100 autosampler is used, no user intervention is needed).

While the *Sample Queue* is running it is possible to load and process other chromatograms.
Each completed chromatogram in the *Sample Queue* will be automatically saved whether the ☑ *Save chromatogram after run* item in the **Method setup** / <Processing> dialogue sheet is set or not. If the chromatogram is interrupted by the user, a prompt for chromatogram saving will be generated.

Basic operations to control *Sample Queue*:

|　　●　　Start　| *(Control* / *Start)*

starts execution of the *Sample Queue* from the first chromatogram with the "Started"=0. It also resumes the paused *Sample Queue* from the next chromatogram.

| Pause | *(Control* / *Pause)*

makes a pause the running *Sample Queue* in order to prepare all necessary operations on the chromatogram such as integration, identification control, to look at results or to change some *Method* parameters. The current chromatogram will be finished in the normal way, in accordance with the preset time, but the start of the next one will be delayed.

---

35 If the autosampler operates independently (is not controlled by the **ALEXYS data system**), the chromatogram *duration* must not exceed duration of the autosampler cycle. In this case the special 'Start' contacts of the *AC 100* interface can be used to synchronize devices.

(**Control** / *Reset*)

finishes the current chromatogram, executes post-run operations and saves them on disk.

(**Control** / *Cancel last run*)

Cancels the last run and clears the "Done" flag for this row. If the queue is restarted, this row is executed again.

T U T O R I A L   8

Batch processing

How to create a Batch

Sometimes it is necessary to reprocess a set of similar chromatograms. This approach is used, for instance, to update calibration, to change integration results, a quantification method, report options, and appearance for several chromatograms simultaneously.

Creating a *Batch* for data reprocessing

A example is given here of reprocessing three calibrations and one sample obtained in the previous section.

- Choose **File** / *Open* / *Chromatogram* or click 📂 to invoke "*Chromatogram open*" window.
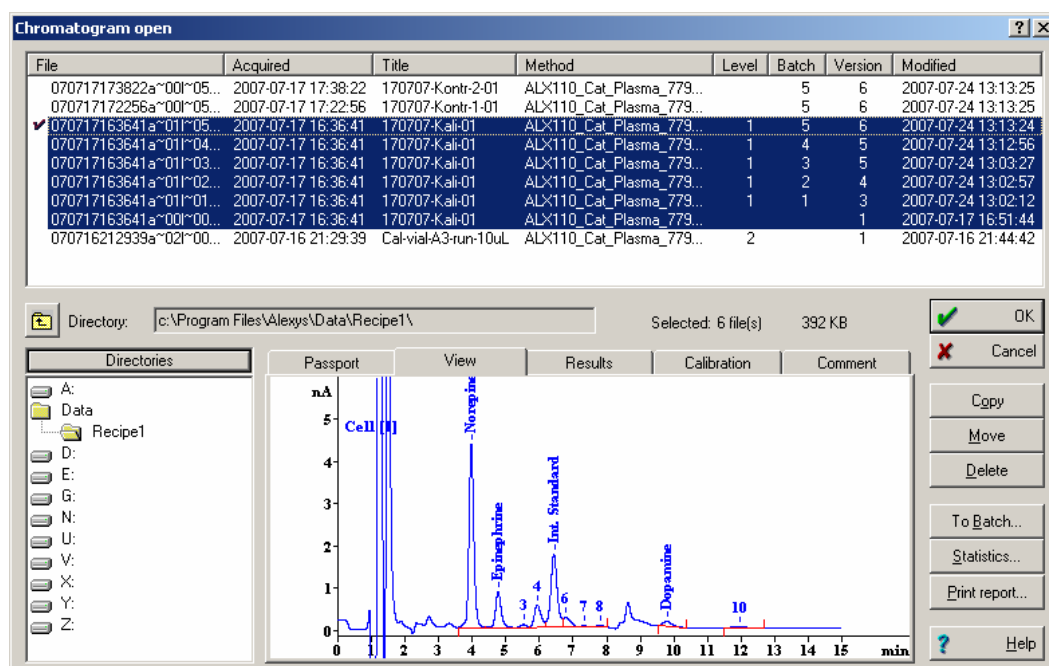
- Select chromatograms that must be included in the *Batch*. Press and hold the [*Shift*] key and use the left mouse button or arrow keys to select consecutive chromatograms. Alternatively, press and hold the [*Ctrl*] key and use the left mouse button to select several chromatogram files in arbitrary order.

> **Note: The same *Batch* can contain chromatograms that use different *Methods* for data processing. However, In this case the same Method will reprocess all chromatograms in the batch!**

The *Processing Method* is taken from one of the chromatograms in the current batch.

- Click <***To Batch***>.
- Enter the name of the *Batch* (BATCH EXAMPLE) and press <**OK>**. The "BATCH EXAMPLE.BAR" batch file will be saved on disk and the *Reprocess window* will appear. Long file names can be used for batches!
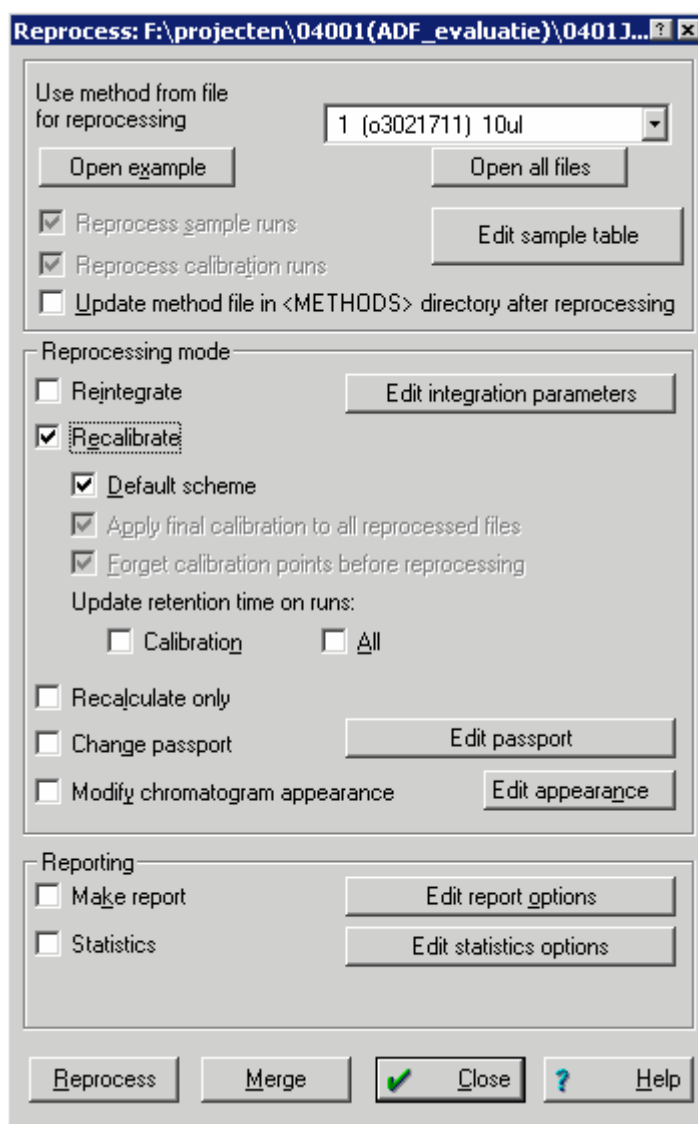
Reprocessing a Batch

Batch reprocessing can be used for:
- reintegration;
- making reports;
- recalibration;
- modifying appearance of chromatograms;
- modifying passport fields.

These operations can be executed either separately or all together. In the first two cases the software will allow modification of the appropriate dialogue box just before reprocessing. In all other cases, except for calibration, it is possible to open appropriate dialogue boxes manually for correction of processing parameters.

- Choose **File** / ***Open / Batch reprocessing*** and open the BATCH EXAMPLE.BAR file created in the last step.
  It is also possible to open the *last batch* (i.e. batch that was edited last)

  by choosing **File** / ***Open*** / ***Last batch*** or clicking icon.

- In the "*Use method from file for reprocessing*" field it is necessary to
  choose a chromatogram, which *Method* will be used for recalculation
  of all other chromatograms in the *Batch*. It is possible to open it click-
  ing the <***Open example***> button. However, to edit the Method, it is
  necessary to close the «Reprocess» window. When editing is com-

  pleted, open the batch again (use the [icon] icon in the *main window*).
  If the "*Update method file in <METHODS> directory after reprocessing*"
  box is checked, all changes in the *Passport*, *Integration parameters*, *Re-
  port options*, *Appearance properties*, and *Calibration* will be saved in the
  Method after reprocessing is finished. Besides, parameters such as cali-

bration level, sample volume, dilution, and auto-sampler vial number will be taken from the *Sample table* (click <*Edit sample table*> to invoke).

Editing the Batch processing table

The *Batch processing table* is edited by the special application - *Queue editor*[36]. The structure of the *Batch processing table* is similar to the *Sample Queue table*. The difference is that the "File Name" column is added, the "Done" column is removed, and other control editor functions are used. When editing is completed, the *Batch reprocessing table* must be saved and the *Queue editor* program must be closed (use the ✓ *Save and Exit* button).

Fields like "File Name" and "Method" are read-only fields and cannot be edited by the user, while all other columns contain data that will replace the corresponding values in the chromatograms during the reprocessing procedure.

The most important columns are:

Title: User-defined identifier for a chromatogram. Will replace the appropriate field of the Passport.

Volume: Injected volume of sample, in micro litres. Will replace the appropriate field of the Passport.
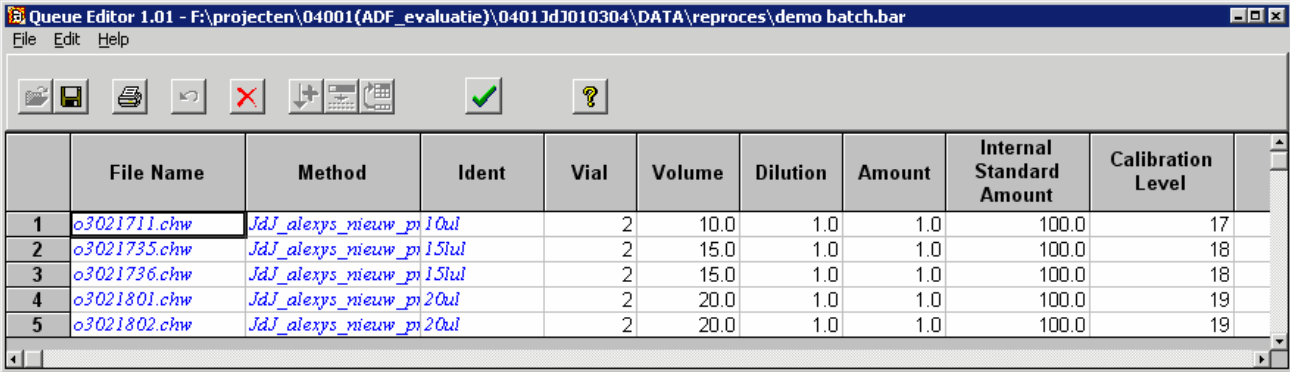
Dilution: Sample dilution prior to injection. Will replace the appropriate field of the Passport.

Calibration level: Calibration level for a given sample. Level "0" holds for a normal analysis, levels "1" and greater hold for calibrations. Correct filling in of this column enables data input to the *Concentration table* and to calculate *calibration coefficients* automatically.

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36 The *Queue Editor* is executed independently from the parent *ALEXYS data system* application. It is possible to switch between the *Queue Editor* and main program by a standard [Alt] + [Tab] combination. Note that the second copy of the *Queue Editor* can be active if a *Sample Queue* is running.

Reintegration

This option enables to reintegrate chromatograms according to *Integration parameters* settings.
As to *sample chromatograms* concentrations of components are updated, and as to *calibration chromatograms* recalibration is performed as well.
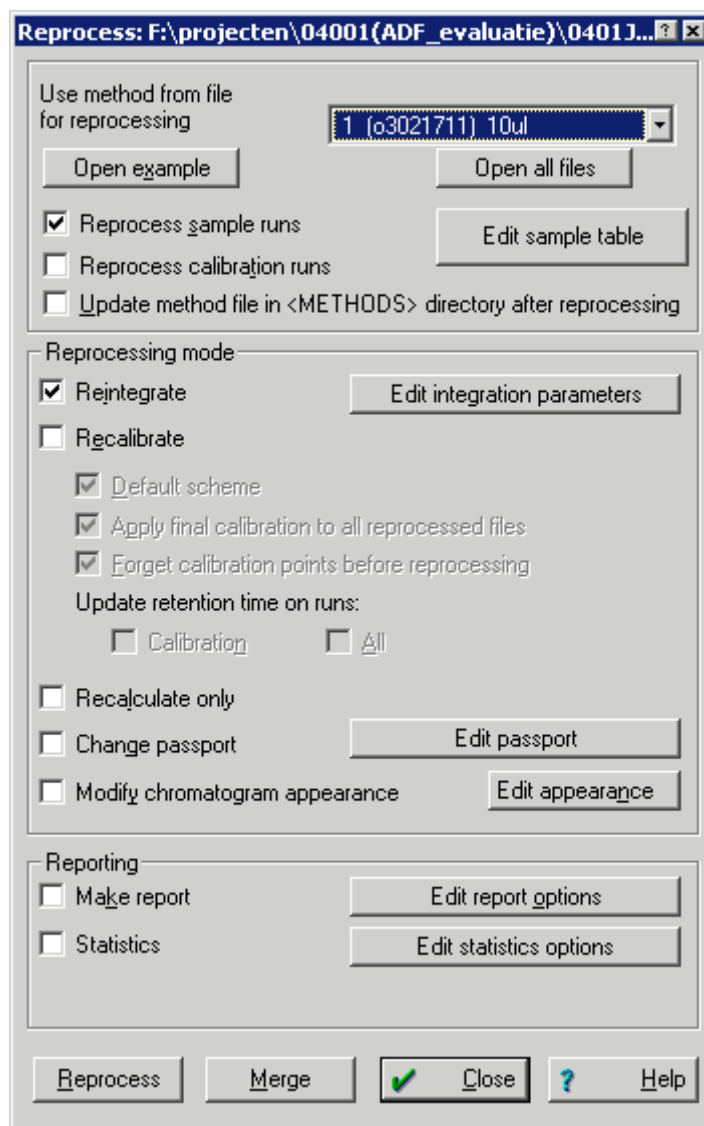
- To reintegrate sample chromatograms only, set checkboxes as in the figure above.

- Click <***Reprocess***>. The "Integration parameters" dialogue box will appear[37].
  Edit it if necessary, and click <***OK***> to start.

Recalibration

The Recalibration procedure allows updating levels of the *Concentrations table*, *calibration coefficients*, and, using the updated calibration, to recalculate concentration of components for sample runs. In some cases the recalibration procedure may require a slight modification of the method.

Modifying a method

- Load the chromatogram (**File** / ***Open / Chromatogram***) that will be used as the prototype method. It is possible also to click <***Open example***>[38].
- Make the required corrections as follows:
  - ♦ Update estimated retention times (if they are far from the observed ones) using the last calibration chromatogram; change the *Identification parameters* and make corrections, if necessary.
  - ♦ Edit the *Concentrations table* (it should contain the *correct* values of concentrations);
  - ♦ Change the *Calibration method*, if necessary.
    It is possible to change the reference channel, to select local components and tune their parameters
  - ♦ Edit *Integration parameters* and *Report options* (these sections of the method will be offered automatically before reprocessing).
- Save the chromatogram.
- To perform recalibration and to recalculate sample runs on the basis of the obtained calibration, set checkboxes in the "Reprocess" dialogue box as shown below and click <***Reprocess***>.

---

37 Integration parameters are taken from the example chromatogram selected ("Use method from file for reprocessing")

38 It is necessary to close the <Reprocess> window in order to edit the opened chromatogram.

☑ *Update method file in <METHODS> directory after reprocessing* saves the final calibration to the method that was used in the example chromatogram.

Default scheme of recalibration implies that two flags are set:

☑ *Forget calibration before reprocessing*

erases existing calibration points and constructs a new calibration.

☑ *Apply final calibration to all runs of the queue*

clears so called the "historical" calibration and applies the updated calibration to all sample and calibration runs.

Reporting

This option prints report for each chromatogram in the *Batch*, in accordance with *Report options* setting in the example run.

- In order to make reports for sample runs only, set checkboxes as shown below.

- Click <***Reprocess***>.
- The "Report options"[39] dialogue box will be opened. Edit if necessary.
- Click <***Report***> or <***Accept***> to continue.

39 Report options parameters are taken from the example chromatogram selected.

Statistics report

A statistics report is generated as follows:
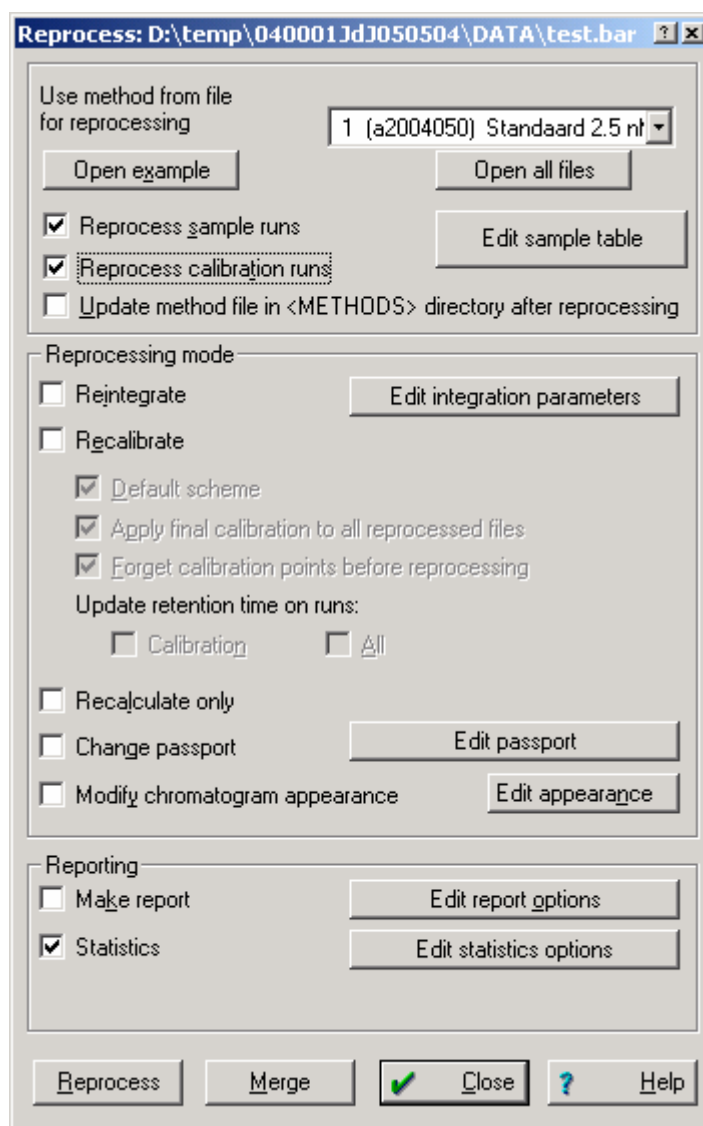
Click Edit statistics options to set the report fields. A 'statistics options' window opens.
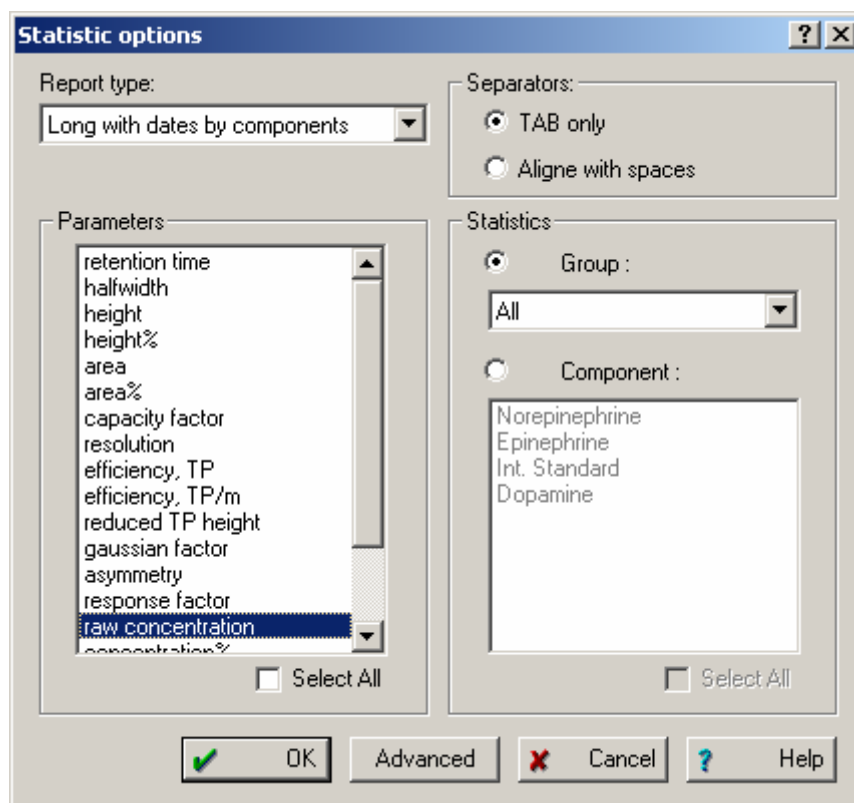
In the statistics window select the components and the report properties. Commonly used properties are retention times, height, area and raw concentration. Click Advanced to set the destination of the ASCII report file.
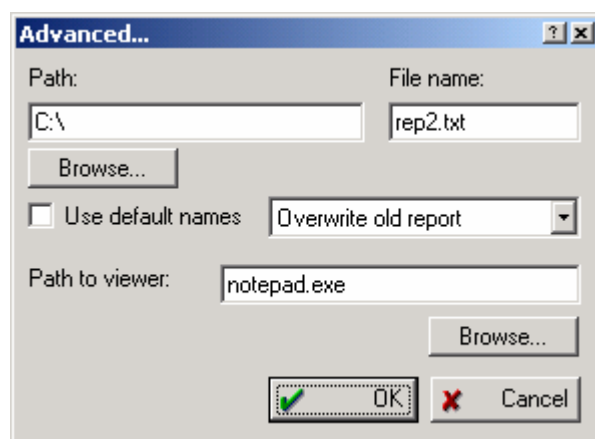
The output will show the concentrations in the selected chromatograms, complete with average value and standard deviation.

```
5-5-2004  14:46
Conc.,
Group: ALL
Components: Dopamine.

                                    Dopamine
1        Standaard 2.5 nM    05/05/2004 12:45:21        2.5
2         Standaard 5 nM     05/05/2004 12:53:19      4.9347
3         Standaard 10 nM    05/05/2004 13:01:16       10.01
4        Sample1,  7.5 nM    05/05/2004 13:09:13      7.6693

                                  Max:               10.01
                                  Min:                 2.5
                                 Mean:              6.2785
                               StdDev:               3.263
                                 %RSD:                  52
```

To have all statistics results grouped per parameter choose "Long with dates per parameter" in the statistics option window.

It is also possible to view the statistics results directly in Excel after batch reprocessing. This can be achieved by setting "path to viewer" to excel.
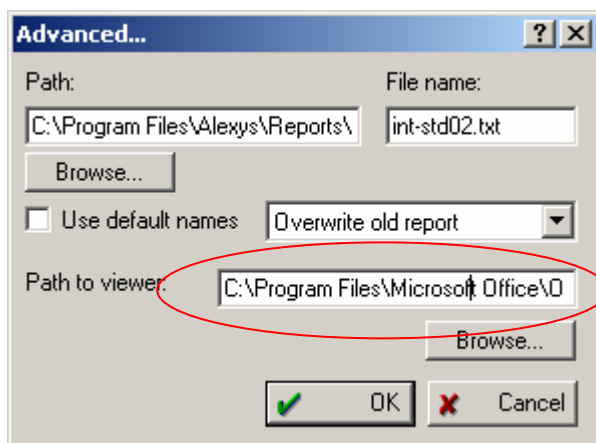
T U T O R I A L   9

Data export

It may be necessary to transfer data into another program for further processing. The ALEXYS data system enables to transfer data to both text editors and spreadsheets.
There are two ways: to use Clipboard and to use files.

Data export via Clipboard

Windows includes a clipboard function to transfer pictures and text between applications. The ALEXYS data system supports this option.

- To copy the current chromatogram plot to the Clipboard[40], just click **Edit** / *Copy to Clipboard*, switch to the desired application and paste the picture. In spite of the chromatogram window colours the white background will be used , while channel colours are preserved.
- To copy a report to the Clipboard, choose **Process** / *Make report*, set the ☑ *Screen* report destination option and click <*Report*>. The *Report* window will open. Select any part of the text (press left mouse button and drag it to the desired position). Click **Edit** /*Copy to Clipboard* (or click right mouse button and choose *Copy* item). Switch to the desired application and paste the text.
- To copy a calibration graph to the Clipboard, open the **Method** / *Calibration… / Graphs* dialogue box. Choose the desired component and click *Copy to Clipboard* in the window.

Data export via files

You are able to save a chromatogram picture and report, in order to use it for other applications.

- Choose the **Process** menu / *Make report* option, set the ☑ *File* report destination option.
- Enter the file output options

Name: File name. The report will be saved as you specify, the chromatogram plot - as a *.WMF (Windows metafile format) file.
Mode: Overwrite or *Append* existing report file.
Character set: The Windows (ANSI) or DOS (ASCII) character set is chosen for report.
<Browse>: Click it to change the report directory

---

40 In addition, it is possible to use the Windows™ command [**Print Screen**] and [**Alt**]+[**Print Screen**] to put the screen copy to the Clipboard.
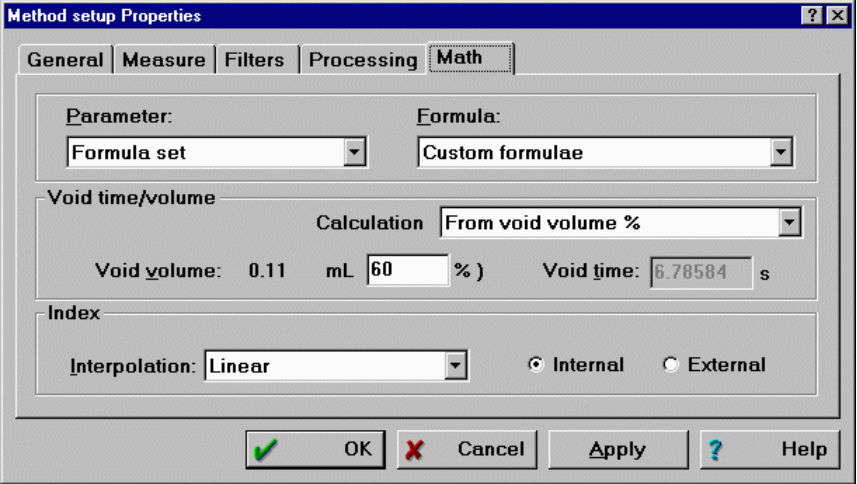
Custom program: Name of the program to be executed after the report output. The name of the report file can be transferred as a parameter '@'. If the software detects the symbol (preceded by *space*) in the command line, it will replace it with a full path and name of the report file. The report template "EXPORT.RTT" is designed specifically to facilitate data export to other applications.

Additional information on commands, parameters and terms is also available in the context-sensitive *Help*.

R E F E R E N C E

Mathematical formulas

"*MATH*" page (choose **Method** / *Method setup* menu item or click ![icon] icon to call) allows to select common formulas used to calculate column efficiency, dead time (volume), retention indexes, peak asymmetry, and resolution. A complete formula set that corresponds to *US Pharmacopoeia* or *European Pharmacopoeia* can also be chosen.

"Parameter"	this field enables to choose formula type to be changed
"Formula"	this field contains a list of available formulas to calculate the chosen *Parameter*
Formula set	chooses a predefined formula set according to *US* or *European Pharmacopoeia*. *Custom* formula set can also be chosen. In this case each formula can be set independently.

Theoretical plates	a method to calculate column's performance (effectivity).
$2 \cdot \pi \cdot (T \cdot H / A)^2$	**Eff = $2 \cdot \pi \cdot (H \cdot t_r / A)^2$**. This formula can be used for poorly resolved peaks
$5.54 \cdot (T/W)^2$	**Eff = $5.54 \cdot (t_r / W_{1/2})^2$**. The equation is used in European Pharmacopoeia.
$16 \cdot (T/Wb)^2$	**Eff = $16 \cdot (t_r / W_b)^2$**. The equation is used in US Pharmacopoeia.

Where t_r - retention time of the peak, H - height, A - area, $W_{1/2}$ - width at the half-height, W_b - width at the baseline

Resolution — a method to calculate column's resolution for two neighboring peaks.

_(T2-T1) / (W2+W1) |60.7%_ — $R_s = (t_2 - t_1) / (W_2^{0.607} + W_1^{0.607})$. This formula can be used for poorly resolved peaks

_1.177 * (T2-T1) / (W2+W1) |50%_ — $R_s = 1.177 \cdot (t_2 - t_1) / (W_2^{0.5} + W_1^{0.5})$. The equation is used in _European Pharmacopoeia_.

_2 * (T2-T1) / (Wb2+Wb1)_ — $R_s = 2 \cdot (t_2 - t_1) / (W_2^b + W_1^b)$. The equation is used in _US Pharmacopoeia_

where t_2 and t_1 - retention time of two neighboring peaks, W_i^b, $W_i^{0.5}$, and $W_i^{0.607}$ - their width at the baseline, half-height, and 0.607 of the height, respectively. **Rs** -resolution for the peaks.

Asymmetry — a method to calculate peak's asymmetry.

Width before / Width after|10% — $A_s = W_2^{0.1} / W_1^{0.1}$. The equation is used in _European Pharmacopoeia_.

Width before+after/2* Width after|5% — $A_s = (W_2^{0.05} + W_1^{0.05}) / (2 \cdot W_1^{0.05})$. The equation is used in _US Pharmacopoeia_

where $W_2^{0.1}$ and $W_2^{0.05}$ - back semi-width of the peak, $W_1^{0.1}$ and $W_1^{0.05}$ - front semi-width of the peak, measured at 10% or 5% of the height, respectively. See also: _Asymmetry_.

Total channel — a way of the total channel calculation (setting is available for _multi-channel chromatograms_ only).

None — total channel is not calculated.

Sum of s/n — total channel is defined as a sum of signal-to-noise ratios for all channels. It can be effectively used for integration of multi-channel chromatograms.

Sum of responses — total channel is computed as a sum of detector responses for all channels. It can be used for integration and quantification procedures. It is not so good for integration as _sum of s/n_, but can be used for quantification.

Void time/volume:

Calculation method   a method used for _void (dead) time_ calculation

None	void time is not calculated, it is entered manually and is considered as a constant.
1st component	the first component of the current chromatogram is taken to define *void time*. If the first component is not found, its *estimated retention time* is taken from the *Components table*.
1st peak	the first integrated peak is used to mark a *void time*.
From void volume %	dead volume is calculated as a percent of volume of the empty column. Dead time equals to dead volume divided to an eluate flow rate.
Void volume	this value is calculated from "*Void time*" or "*Void volume %*" value.
Void time	this value can be entered manually if "*None*" void time calculation method is set or calculated according to the chosen *Void time calculation method*.

Index:

Interpolation	using linear or logarithmic interpolation scale for *indices*.
⊙ *Internal*	index scale is constructed using the current chromatogram.
⊙ *External*	index scale is constructed on the basis of another, calibration chromatogram.

Calibration

External standard (Absolute) calibration

External standard calibration is a key way of calibration. Basically this procedure calculates dependence of injected component's quantity versus area (or height) of the corresponding peak. Usually this dependence is showed as a calibration plot with injected quantity along Y axis and peak area along X axis. *Injected quantity* is calculated as product of component's concentration (**C**) to reduced injected sample volume (**V'**):

$$Q_i = C_i \cdot V' = C_i \cdot V \cdot A / D$$

where **V** is a sample volume, **D** - sample dilution, and **A** - amount of the sample.

Calibration dependence $W_i(R)$ for each component is calculated

$$W_i(R) = RMS(Q_{ij}, R_{ij}) = RMS((C_{ij} \cdot V'_j), R_{ij}),$$

where **RMS** stands for *root-mean-squared* procedure that computes coefficients of calibration dependence, V'_j - reduced volume of injected sample in the j-th calibration run, R_{ij} - detector response for i-th component in the j-th calibration run.

When quantification procedure is performed, a concentration of i-th component is determined as a ratio of computed quantity (determined by means of calibration curve starting from the corresponding peak area or height) to a corrected volume of the sample injected:

$$C_i = W_i(R_i) / V'$$

Absolute calibration can be easily converted to the *Internal standard* calibration. Any component can be selected as a standard if its concentration in the calibration mixture is exactly known.

Tabulated relative response factors calibration

This simplified way of *External standard* calibration can be used in the case of reliable and reproducible results provided by the detector. It is assumed, that detector relative responses of different components (relative to the chosen standard component) are constant and can be tabulated. Relative response factor of the standard component is taken to be 1.

Calibration of the *Standard component* only is performed by *External standard* (*Absolute) calibration* method:

$$\mathbf{W_s(R) = RMS((C_{sj} \cdot V'_j), R_{sj}).}$$

Other components are not calibrated, and their calibration curves assumed to be similar to the calibration curve of the *standard component*:

$$\mathbf{W_i(R_i) = W_s(R_i) \cdot K_{1i},}$$

where K_{1i} - relative response factor for i-th component in the *Components table*.

Relative response factors may be obtained experimentally by *Internal standard calibration* assuming K_1 coefficient of standard equal to 1, taken from a reference literature, or calculated from theoretical models.

Internal standard method

The main reason of error in the *Quantification* procedure is run-to-run variation of component quantity that reaches a detector. This happens because injected volume differs from injection to injection, even in the case of a very good auto-sampler or syringe. Another reason is substance losses during the sample preparation or separation process itself. *Internal standard* technique is used to increase the accuracy of calculations with the help of *a priory* information, namely that concentration and calibration of some component, called the *Internal Standard*, is known exactly and in advance. This additional information appears to be very important, as it allows to use a calculation procedure that decreases expected errors of determined concentrations.

This *a priory* information can be used both on the *Quantification* and *Calibration* steps. *Quantification* accepts new information in *Relative concentration* formulas, and *Calibration* can increase its prediction power by implementing the *Internal standard* method.

Internal standard (Relative) calibration
The calibration curve is created similar to the *External standard* procedure, but amount of the substance is corrected according to the declared concentration of the *standard component*:

$$W_i(R) = RMS(Q'_{ij}, R_{ij}),$$

where $\quad Q'_i = C_i \cdot W_S(R_S) / C_S$

Calibration of the standard component can be carried out using the same calibration mixtures, as for other components:

- Just receive calibration dependencies for all components by the *External standard* method.
- Then select a standard components
- Set the *Internal standard* calibration method. The software will make necessary calculations. Thus all points on a calibration graph of the standard component visually fall on the selected type of a calibration curve, and absolute concentration of all components can be correctly calculated.

The *ALEXYS* software uses an *Internal standard* method that is more universal and common [2] than a "classical" one [3]. The difference is in that

instead of C/C_s versus R/R_s calibration graphs we use absolute - type curves. Net result is that user can apply our *Internal standard* method in the case of nonlinear calibration dependencies without compromise. In the case of linear calibration dependencies our scheme is indistinguishable from a "classical" one and both methods provide the same results.
One idea is essential for understanding of our version of *Internal standard* calibration. That is:

CALIBRATION DEPENDENCE OF THE STANDARD COMPONENT ITSELF CANNOT BE CALCULATED BY RELATIVE CALIBRATION METHOD.
THIS DEPENDENCE HAS TO BE KNOWN IN ADVANCE OR OBTAINED BY THE ABSOLUTE CALIBRATION PROCEDURE!

Relative(Internal standard) calibration **essentially improves reproducibility of the analysis and the accuracy of relative response factors calculation.**
However, an errors of absolute concentrations of components are determined by an errors of calibration dependence of a standard component, obtained by an *External standard* calibration method.

Quantification by the Internal standard method

Calculations are performed with assumption that concentration of the *Internal standard* in the sample solution C_s is precisely known. Another value known from calibration curve of the standard component is the amount of substance that has reached a detector $Q_s = W_s(R_s)$. From these two values effective volume of the injected sample can be calculated:

$$V'_e = W_S(R_S)/C_S,$$

It accounts for all injection volume errors, sample loss during preparation etc. This effective volume is different for every calibration sample and it is used to correct calibration curves of another components:

$$Q'_{ij} = C_{ij} \cdot V' = C_{ij} \cdot W_s(R_{sj}) / C_{sj}$$

Calibration curve for target component is created in the same way as in the case of *External standard* calibration (*Absolute calibration*) method, but with corrected quantity values:

$$W_i(R) = RMS(Q'_{ij}, R_{ij}),$$

If calibration graph of the standard component is not known, it is assumed to be linear, to go through coordinate origin and to have a response factor equal to one (**RF$_s$ = 1**). This makes system to behave analogously to the "classical" *Internal standard* calibration method. If the dependency is linear, this approach provides correct *Relative concentration*, *Relative concentration percent* and *Normalized concentration* values. **Absolute concentration is not available in this case**.

The best way of accurate work in the case of nonlinear calibration dependencies is to calibrate standard component in advance (or even with the same set of calibration points as for other components) by *Absolute calibration* method and apply *Relative calibration* method after that. In this case system outputs correct absolute concentrations for all components.

Report columns

Different quantification methods include various sets of report columns into the *Peaks table*. Lack of initial data may result in that *ALEXYS* software will not be able to calculate some values. In this case default value or zero will be placed into the column.

Number   Peak number, in accordance with enumeration on the chromatogram plot. Total equals total number of peaks.

Retention time   Peak retention time or volume, depending on the retention units. Total value stands for the chromatogram duration.

Half width   Half-width (width of the peak at its half height). Total is weighted average of half-widths of all listed peaks.

Height   Peak height. Total value in the column equals sum of peak heights.

Height %   Height, normalized to NORM value.

$$\mathbf{H_i\% = NORM \cdot H_i / \Sigma(H_i).}$$

Area   Peak area. Total value equals sum of areas for all peaks.

Area %   Peak area, normalized to NORM value. Total equals NORM.

$$\mathbf{A_i\% = NORM \cdot A_i / \Sigma(A_i).}$$

Capacity factor   Capacity factor is widely used in LC relative retention of the component. Requires known "dead time" ($\mathbf{t_0}$) for calculation.
$k' = (t-t_0) / t_0$
Total for this column equals capacity factor for the last peak of the chromatogram.

Resolution

*Resolution* (**R**) for two neighboring peaks can be calculated by three ways:

$R = (T_{i+1} - T_i) / (W_i^{0.607} + W_{i+1}^{0.607})$. A popular equation to calculate resolution.

$R = 1.177 \cdot (T_{i+1} - T_i) / (W_i^{0.5} + W_{i+1}^{0.5})$. This equation is used in *European Pharmacopoeia*.

$R = 2 \cdot (T_{i+1} - T_i) / (W_i^{b} + W_{i+1}^{b})$. This equation is used in *US Pharmacopoeia*.

where T - retention time of a peak,
$W^{0.5}$, $W^{0.607}$, and W^{b} are peak widths on the half-height, on the 0.607 of its height, or on the baseline,

(**i**) and (**i+1**) indexes refer to neighboring peaks. All equations give similar results. So, any one can be chosen if special standards are not required. Nevertheless, first formula is preferred in the case of poorly resolved tailing peaks. Equation type can be changed in the **Method** / *Method setup* / *Math* dialogue sheet.
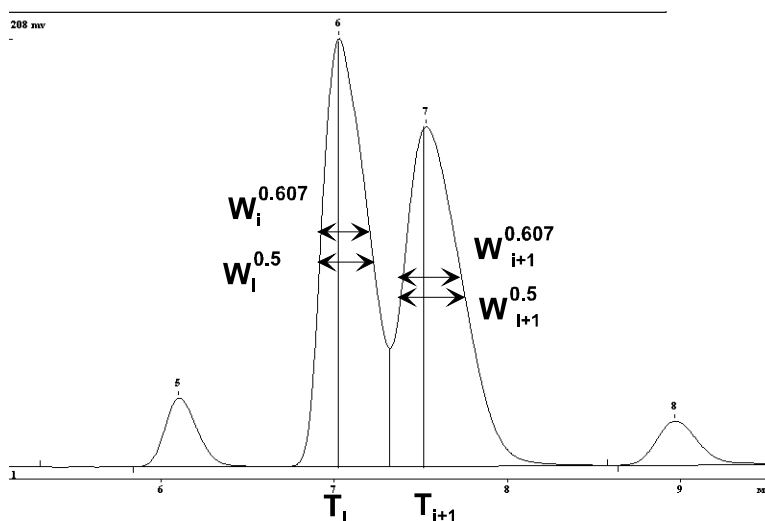
Efficiency, TP

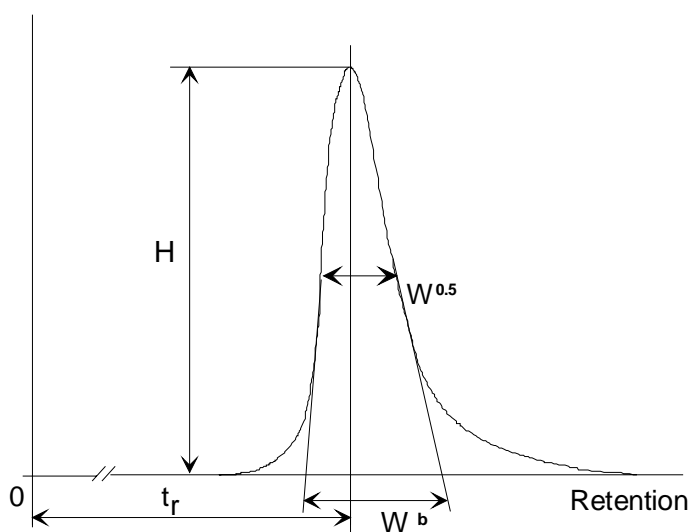Number of theoretical plates (N_i) per column (column's performance) for i-th peak can calculated as:

$N_i = 2 \cdot \pi \cdot (H_i \cdot T_i / A_i)^2$. This formula gives reasonable estimate of plate count for poorly resolved peaks

$N_i = 5.54 \cdot (T_i / W_i^{0.5})^2$. The equation is used in the *European Pharmacopoeia*.

$N_i = 16 \cdot (T_i / W_i^b)^2$. The equation is used in the *US Pharmacopoeia*.

Where T_i - retention time, H_i - height, A_i - area, $W_i^{0.5}$ - width at the half-height, W_i^b - width at the baseline for i-th peak (is determined by means of tangent lines at inflection points of the peak), and π is 3.1415926...

These formulas provide similar estimates for stand-alone peaks Nevertheless, the first one offers better estimates for fused or unresolved peaks, because half-width or width error for those peaks are much greater than height or area errors. Total for this column includes average value for the peaks listed.

Effectivity, TP/m   Number of theoretical plates per meter N_i' is calculated as

$$N_i' = N_i \cdot 1000 / L,$$

where L - length of the column in mm,
Ni - number of theoretical plates per column.
Total for this column includes average value for t
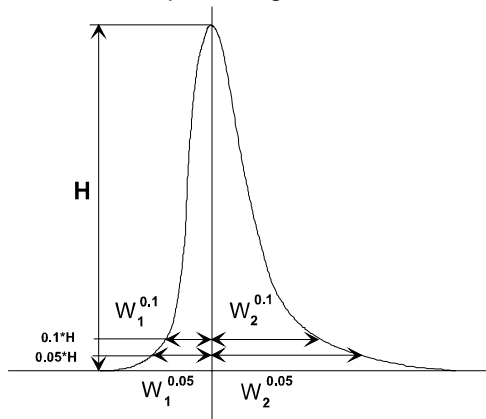he peaks listed.

Reduced TP height   Reduced height, equivalent to theoretical plate H_i is calculated by formula:

$$H_i = 1000 \cdot L / (N_i \cdot dp).$$

where L - length of the column in mm, dp - particle diameter in µm. Total for this column includes averaged H_i value for the peaks listed.

Peak asymmetry   Peak asymmetry (A_s) is calculated at 10% or 5% of the peak height.

$A_s = W_2^{0.1} / W_1^{0.1}$. This equation is used in *European Pharmacopoeia*
$A_s = (W_2^{0.05} + W_1^{0.05}) / (2 \cdot W_1^{0.05})$. This equation is used in *US Pharmacopoeia*

where $W_i^{0.1}$ and $W_i^{0.05}$ - half-widths measured on the 10% or 5% of the peak height. As a rule, A_s values are greater than 1 (i.e. peak tailing exists).

Response factor (RF) The most common for *ALEXYS* software cubic approximation of the calibration curve includes 4 coefficients (K_0, K_1, K_2, and K_3):

$$W_i(R_i) = K_3 \cdot R_i^3 + K_2 \cdot R_i^2 + K_1 \cdot R_i + K_0,$$

where $W_i(R_i)$ - calibration dependence for i-th component as a function of the its detector response R_i.

As calibration curves are linear in most cases, the only meaningful calibration coefficient for those cases is K_1 (*Response factor*, RF). Other coefficients can be printed via *Calibration results* section of the report or reviewed in the *Graphs* dialogue box.

Total column lists weighted average response factor for the group with weight equal to response (area or height). It can be used as a RF for the *universal component*.

Raw concentration *Raw concentration* column lists absolute concentrations of components.

$$C_i = W_i(R_i) / V',$$

where V' - corrected volume of the sample Total value for the column lists sum of concentrations in the column.

Concentration % Concentration, normalized for user-defined value

$$C_i\% = NORM \cdot W_i(R_i) / \Sigma(W_i(R_i)),$$

where NORM is normalization coefficient, entered by the user, default value equals 100.

Total value for the column equals NORM for the main *Peaks table*, and the sum of concentrations in the group for *Groups table*.

Relative concentration

This column lists concentration of the component relative to standard component (in most cases this column should be considered as an analog of "classical" Internal standard method concentration). It works in the case of any calibration method. Concentration of component is calculated by the formula:

$$C'_i = W_i(R_i) / V' = W_i(R_i) \cdot C_s / W_s(R_s),$$

where $V' = W_s(R_s)/C_s$ - effective volume of injected sample (for explanation see *Internal standard calibration* description).

Standard component concentration C_s is entered by the user. Total value for relative concentration column does not include concentration of the standard component.

Total value for "Relative concentration" column does not include the concentration of the *Standard component*.

Relative conc %

"*Relative concentration percent*" differs from "*Concentration percent*" by exclusion of Standard component(s) from summation, so that sum of all concentrations, excluding standard, equals to NORM. *Total* value for the column equals to NORM value for the main peaks table, and to the sum of concentrations in the case of group tables.

Retention index

Chromatogram has a primary retention scale, measured as time or volume of retention and a secondary scale, that emerges after peak identification. This scale is called *Index*. Typical case is Kovatch' or linear indices in gas chromatography. Total value for the column equals

$$\textbf{Total} = \Sigma(I_i \cdot C_i) / \Sigma C_i$$

Type

Types of components are designated by six letters divided in two parts looking like **BBH:SN?**.
First three letters code stands for peak separation code (unneeded codes are replaced by spaces):

B   baseline separation (BB - baseline to baseline),
D   dropline separation,
H   horse peak,
R   rider peak

For example:

BBH means baseline-to-baseline separated horse peak,
DBR - dropline-to-baseline separated rider peak

In the second part the following letter codes can be used:

R   *reference* component,
S   *standard* component
C   *calibration standard* in the case it is differ ent from the quantification standard
?   some points of corresponding peak are *out of ADC range*
!   component concentration is outside of lim its, set in the *components table*
A   *autoselected standard component* (in the case when standard have not been selected by the user)
N   component is outside of the calibrated region
p   component is *special*.

Group

Components can be combined into groups. All components that belong to one group will be re-ported in the same style as in the *Peaks table* and subtotals will be calculated. This group report will follow main *Peaks table*.

Spectral ratio

> This column lists spectral ratios for all reported components. The option is valid only for *multi-channel chromatograms*. Spectral ratios are calculated relatively to *Reference channel* that is set in the *Calibration defaults* dialogue box.

Name

> This column lists component's name.

File name

> This column includes filename of the chromatogram. It can be useful in the case of data export and following processing. So, each line in the baseline possess a reference for a file-origin. This is convenient in sorting and data base handling operations.

Title

> This column contains a title of the chromatogram. It facilitates reports export to spreadsheets.

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