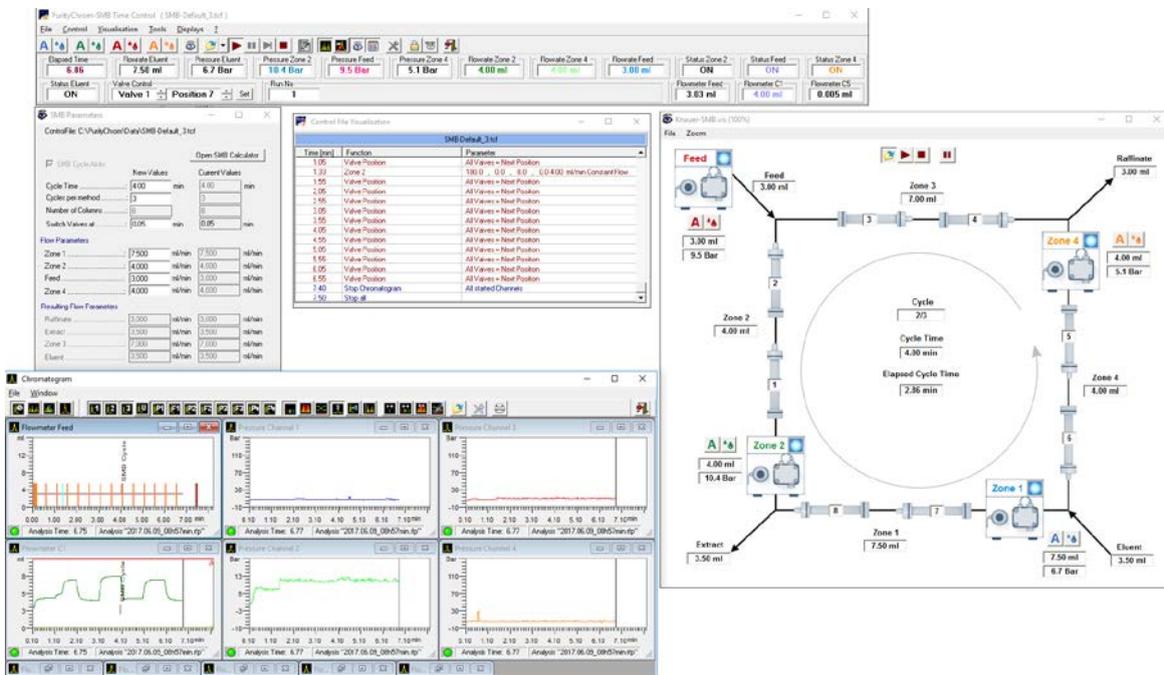


PurityChrom® MCC

Version 5.9.20

Software Instructions



SOFTWARE



Note: For your own safety, read the instructions and observe the warnings and safety information on devices and in the device instructions. Keep the instructions for future reference.

Technical Support:

Phone: +49 30 809727-111 (9-17h, Central European Time)

Fax: +49 30 8015010

E-Mail: support@knauer.net

Languages: German, English

Publisher:

KNAUER Wissenschaftliche Geräte GmbH

Hegauer Weg 38

D-14163 Berlin

Phone: +49 30 809727-0

Fax: +49 30 8015010

Internet: www.knauer.net

E-Mail: info@knauer.net

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For latest version of the instructions, check our website:

<https://www.knauer.net/en/Support/User-manuals>

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Introduction

PurityChrom® MCC (PurityChrom) version 5.9.20 is a chromatography data and control software especially designed for the area of continuous purification. Its superior flexibility and modular construction mean that the program can be adapted to suit to our standard AZURA® SMB configurations as well as to nearly any customized system. PurityChrom has the added advantages of a clearly structured interface and a user-friendly method construction. All the advantages of networks can be exploited without restrictions. National and international regulations, such as 21 CFR Part 11, are fully supported.

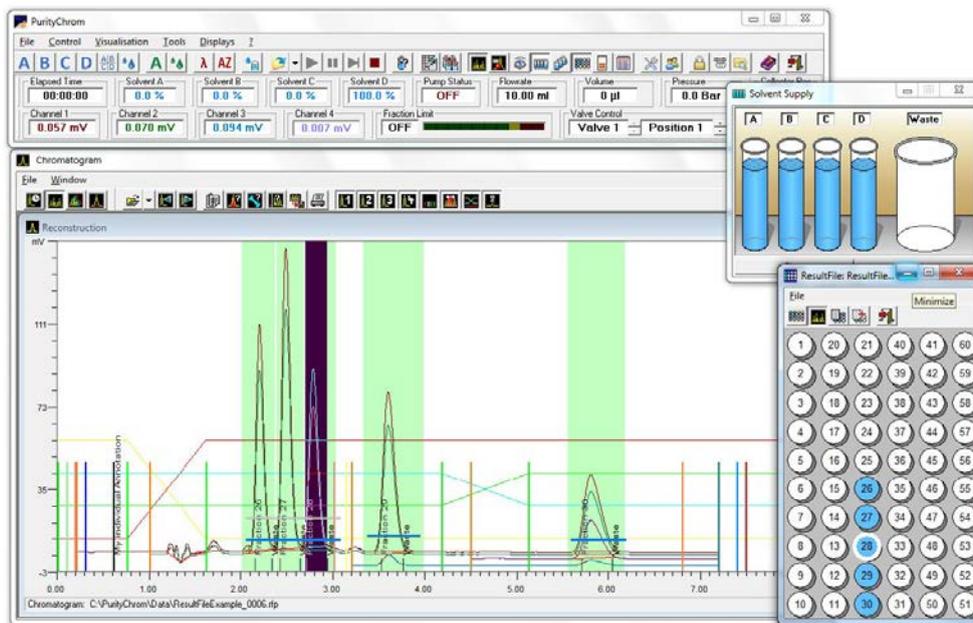


Fig. 1 PurityChrom

The program controls the SMB system via a parameter wizard or via the free programmable time table.

The entire run is displayed in a visualisation diagram of the system which can be individually formatted, and in a time control file which indicates the stage in progress at all times.

The display settings and function buttons in the main window can be personalized by the user.

The screen keys allow manual control of the pumps, the detector and the valves.

The system visualization and the graphic time control editor provide graphical representation and easy handling of the complex flow processes. Furthermore, each device which is displayed in the fluidic scheme can be manually controlled giving the opportunity to optimize and change and adapt your conditions spontaneously during the run.

Additionally, there is also a possibility to pause the separation during the run due to the hold function providing you with complete control over your chromatography process.

The results data from a run includes all the chromatograms recorded, with integration results, the time control tables and a protocol of the entire run with all changes. Thus, PurityChrom allows the user to re-establish all the relevant information needed to reproduce the run from the results data alone.

To verify supported devices, please go to the KNAUER webpage.

Main window



Fig. 2 Main window

General

The main window is made up of a toolbar with function buttons, plus a display bar in which the current system data is displayed. The arrangement of the function buttons and displays can be personalized by the user. The size and position of the window can also be changed and will be saved when the program is closed.



Fig. 3 Customize toolbar

To make changes to the toolbar, double-click on an empty space on the toolbar. The *CUSTOMIZE TOOLBAR* will open, where you can add and remove buttons. To hide a button, deselect the checkbox under it. The settings will be saved when you exit the program.

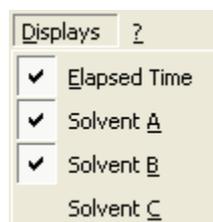


Fig. 4 Displays

To add or remove displays, select the relevant display from the *DISPLAYS* menu. The selected displays will be ticked in the menu. To change the display colour, right-click on a display and select the menu option *EDIT DISPLAY COLOUR*. The colour dialogue that appears will allow you to choose a new colour.

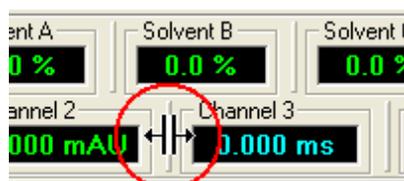


Fig. 5 Move displays

To move displays, click on the vertical line in front of the display and drag it to its new position. If you drag a display down and out of the window, a new display bar will appear. The arrangement of the toolbar and display bar is saved when you exit the program and will reappear this way the next time you open it.

Function buttons

Button	Name	Function
	100% Solvent A Eluent Pump	Starts with the currently set flow rate.
	Flow rate	Opens a window to show the flow rate. A change to the flow rate does not start the pumps, but it will take immediate effect if the pumps are running.
	100% Solvent A Zone 2 Pump	Starts with the currently set flow rate.
	Flow rate	Opens a window to show the flow rate. A change to the flow rate does not start the pumps, but it will take immediate effect if the pumps are running.
	100% Solvent A Feed Pump	Starts with the currently set flow rate.
	Flow rate	Opens a window to show the flow rate. A change to the flow rate does not start the pumps, but it will take immediate effect if the pumps are running.
	100% Solvent A Zone 4 Pump	Starts with the currently set flow rate.
	Flow rate	Opens a window to show the flow rate. A change to the flow rate does not start the pumps, but it will take immediate effect if the pumps are running.
	Wavelength 1	Opens a window where you can enter the wavelength your controlled UV detector. Using a DAD you cannot choose the wavelength here, but in the Time Control File (see page 41) or chromatogram view (Analysis Window).
	Wavelength 2	Opens a window where you can enter the second wavelength of a two-channel serial controlled detector.
	Autozero	Will return a serial controlled detector to zero.
	Set Reference Spectrum	Sets the current DAD spectrum as reference spectrum.
	Limiter Value	Opens a window where you can enter the maximum value for the fraction limiter.
	Load Time Control File or Autosampler Control File	Opens a data file selection window where you can load a time control file or autosampler control file. In the file type box of this window, you can select either *.tcf (Time Control File) or *.acf (Autosampler Control File). If the option Start Pumps after loading Time Control File is selected in the time control file, the pumps will begin once loading is complete, with the solvent composition and flow rate programmed for time point 0.00 minutes.
	Start Time Control File or Autosampler Control File	Starts the time control file or autosampler control file that has been loaded. If the function Hold Time Control File or Autosampler Control File was executed first, the run will resume.

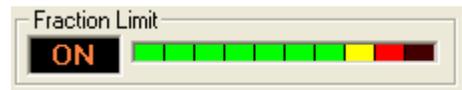
	Hold Time Control File or Autosampler Control File	Pauses the time flow of a time control file or autosampler table. If the option Stop Pumps at Time Control Hold is selected in the time control file, the pumps will have a flow rate of 0 ml. While the Hold function is activated, the flow rate of the pumps can be adjusted manually using the Flow rate function. Start Time Control File or Autosampler Control File resumes the run at the time point where it was paused.
	Jump to next Time Control Line	Jumps forward in time to the next line programmed in the time control table. The first time you use this function, you must confirm it in a dialogue box, but it is possible here to select the option Don't ask again, to avoid seeing this box in future.
	Stop all	Stops the pumps and the time control file from running. It is not possible to resume the run at the point where it was stopped.
	Manual Sample ID	Enables you to enter a sample identifier when starting a time control file. This option is not possible when running an autosampler control file, because the sample identifiers are derived from the accompanying sample identifier file.
	Create / Edit Time Control File	Opens the time control file editor to create or edit a time control file.
	Create / Edit Autosampler Control File	Opens the autosampler control file editor to create or edit an autosampler control file.
	Chromatogram	Opens the chromatogram window to show the analysis, compare, reconstruction and reintegration windows.
	Peak Detection	Opens the window with the automatic peak sampling displays.
	System Visualisation	Opens the system visualisation window.
	Solvent Supply	Opens the window with the solvent supply display. *
	Waste Management	Opens the window with the waste management display. *
	Fraction Rack	Opens the window with the fraction rack visualisation.
	Event Box Control	Opens the window with the visualisation and manual control of the event box.
	Control Visualisation	Opens the window with the visualisations of the time control file and autosampler control file in table form.
	Setup	Opens the setup window, in which communication settings, device parameters and device descriptions etc. can be set.
	User Administration	Opens the user administration window.

* not supported in PurityChrom MCC

	Keylock	Makes it possible for you to lock the program. After activation, a Keylock deactivation window appears on the task bar, which will unlock the program when you enter the user password. Please login with the current user login.
	Serial Communication	Opens a terminal window showing the serial communication, in which you can make entries in linked interfaces.
	Open current working folder	Opens an Windows Explorer Window showing the content of the current working folder.
	Online Help	Opens the program's online help. For further help please use the instructions.
	Exit	Ends the program.
	SMB Parameter	Open the SMB Parameter Window.

Displays

Display	Function
	<i>ELAPSED TIME</i> shows the time in hours, minutes, and seconds that has elapsed in a time control file. If the time control file is on <i>HOLD</i> , then the display will flash between the elapsed time and <i>HOLD</i> . During the injection phase, the display flashes between the elapsed time and <i>INJECTION</i> . Following the time control file command <i>WAIT FOR INPUT</i> , the display will flash between the elapsed time and <i>WAITING</i> .
	<i>SOLVENT A</i> shows the current percentage of eluent A being pumped.
	<i>SOLVENT B</i> shows the current percentage of eluent B being pumped.
	<i>SOLVENT C</i> shows the current percentage of eluent C being pumped.
	<i>SOLVENT D</i> shows the current percentage of eluent D being pumped.
	<i>PUMP STATUS</i> shows the current pump status. ON = pump is running, OFF = pump has been stopped, OVER = pump has stopped because the pressure was too high, UNDER = pump has stopped because the pressure was too low.
	<i>FLOW RATE</i> shows the pump's current flow rate.

	<p><i>VOLUME</i> indicates the volume pumped through since pumping began. When the pump is stopped, the volume display returns to 0. Depending on the volume pumped, the display unit changes automatically from μl through ml to l.</p>
	<p><i>PRESSURE</i> indicated the current pump pressure. You can change the display value in Setup, under <i>PRESETS</i>, using an offset and a factor.</p>
	<p><i>COLLECTOR POS</i> shows the current fraction collector position.</p>
	<p><i>FRACTION LIMIT</i> indicates the fraction limiter status. OFF = limiter is inactive, ON = Limiter is active. The LED band display indicates the volume pumped of the current fraction in steps of 10%. When the last red LED has lit up (100%), the limiter triggers a step in the fraction collector.</p>
	<p><i>VALVE CONTROL</i> shows the current position of the selected valve and is also a way of switching the valve manually. To do this, select the desired valve and the required position, and use the <i>SET</i> button to change the position.</p>
	<p><i>VIAL NO</i> indicates the current vial number of the current autosampler injection.</p>
	<p><i>RUN NO.</i> shows the number of the current run in association with the function <i>RESTART</i>.</p>
	<p><i>CHANNEL 1 - CHANNEL ...</i> show the values of the device connected to each analogue entry. In the Analysis window, under <i>CHANNEL SETUP</i> you can change the displays for each device using an offset, a factor and a dimension.</p>

Program settings (setup)

The function key or the menu option *SETUP*  in the main window opens the program settings window. In the *FILE* menu you have the possibility to save your configuration. The window contains 8 tabs, whose content is described below.

Communication

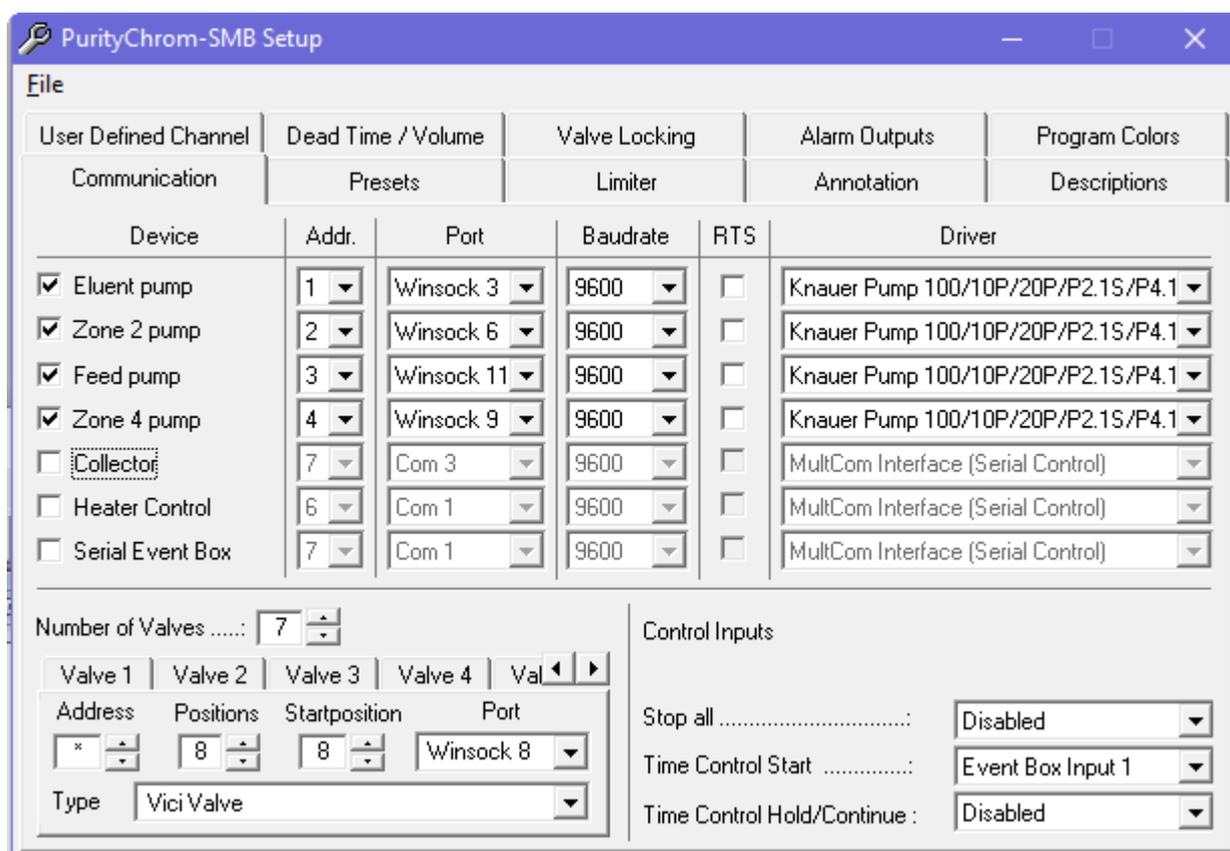


Fig. 1 PurityChrom setup, communication

The Communication setup tab is used to configure your system's serial and LAN control interfaces and valves.

ADDR.: In *ADDRESS* you can set the serial or LAN address which is set for the MultCom interfaces with the help of the round encoding switch (see illustration).

PORT: In *PORT* you can choose the serial port or *WINSOCK* port to which each interface is connected.

DRIVER: In *DRIVER* you can choose your driver for your connected interface. You are not allowed to choose your UV-Detector here, as it is automatically configured during installation of PurityChrom. You can configure your system's valves at the bottom of the page.

NUMBER OF VALVES allows you to enter the number of valves. Each valve appears on its own tab, where you can select the serial or LAN address, the number of positions and the port for this valve. Using a fraction collector, you have to configure the valve of the collector separately (Addr.: 1, Pos.: 2, Port: Driver 5, Type: Standard Valve). Using the P6.1L (high pressure gradient version) you have to configure the solvent selection valve separately by choosing two standard valves (Addr.: 1 and 2, Pos.: 2, Port: Driver 1).

The first valve can switch between A1 (position 1) and A2 (position 2) and the second between B1 (position 1) and B2 (position 2). In the tab *DESCRIPTION* from the setup (see chapter Description page 17) you can name these valves and positions in for example solvent selection A and B with the positions A1, A2, B1 and B2 for more clarity.

The standard configuration for the SMB with 8 port multi-position valves are shown in table 1.

Valve number	Adress	Position	Startposition	Port	Type
1	*	8	1	Winsock 2	Vici Valve
2	*	8	2	Winsock 7	Vici Valve
3	*	8	3	Winsock 1	Vici Valve
4	*	8	5	Winsock 4	Vici Valve
5	*	8	6	Winsock 10	Vici Valve
6	*	8	7	Winsock 5	Vici Valve
7	*	8	8	Winsock 8	Vici Valve

Table 1 Standard configuration for a SMB with 8 columns (values may differ, depends on SMB configuration).

In *CONTROL INPUTS* you can define event box inputs or game port inputs that stop a run (*STOP ALL*), start a run (*TIME CONTROL START*) or temporarily pause and then resume a run (*TIME CONTROL HOLD/CONTINUE*). Using a trigger cable, you have to choose the Event Box Input 1.

Besides if you want to start your pump dependent on the trigger, the PurityChrom.ini-file [Pumps → IOcontrol=1] (see appendix) has to be modified.

Presets

User Defined Channel	Dead Time / Volume	Valve Locking	Alarm Outputs	Program Colors
Communication	Presets	Limiter	Annotation	Descriptions
<div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <p>Eluent Zone 2 Feed Zone 4</p> <p>Default Flowrate : <input type="text" value="0"/> ml/min</p> <p>Max. Flowrate: <input type="text" value="50"/> ml/min</p> <p>Min. Pressure: <input type="text" value="0"/> bar</p> <p>Max. Pressure: <input type="text" value="150"/> bar</p> <p>Serial Cycle: <input type="text" value="1"/> ms</p> <p>Kompr. Korrektur <input type="text" value="1"/></p> <p>Kompr. Korr. B <input type="text" value="1"/></p> <p><input type="checkbox"/> Gradient Valves</p> <p><input type="checkbox"/> Different Pumps</p> </div> <div style="width: 35%;"> <p>Pressure Display</p> <p>Eluent</p> <p>Offset ...: <input type="text" value="0.00"/> Bar</p> <p>Factor ...: <input type="text" value="1.00"/></p> <p>Zone 2</p> <p>Offset ...: <input type="text" value="0.00"/> Bar</p> <p>Factor ...: <input type="text" value="1.00"/></p> <p>Feed</p> <p>Offset ...: <input type="text" value="0.00"/> Bar</p> <p>Factor ...: <input type="text" value="1.00"/></p> <p>Zone 4</p> <p>Offset ...: <input type="text" value="0.00"/> Bar</p> <p>Factor ...: <input type="text" value="1.00"/></p> </div> <div style="width: 30%;"> <p>Collector</p> <p>Startposition: <input type="text" value="1"/></p> <p>Endposition: <input type="text" value="36"/></p> <p>Temperatures</p> <p>Temperature 1: <input type="text" value="20"/> °C</p> <p>Temperature 2: <input type="text" value="20"/> °C</p> <p>UV Detector</p> <p>Wavelength 1.....: <input type="text" value="260"/> nm</p> <p>Wavelength 2.....: <input type="text" value="280"/> nm</p> <p>Wavelength 3.....: <input type="text" value="295"/> nm</p> <p>Wavelength 4.....: <input type="text" value="490"/> nm</p> </div> </div>				

Fig. 2 PurityChrom setup, presets

You can set standard and maximum values for the devices being controlled in the presets setup. You can enter the following values in the four pump tabs:

- *DEFAULT FLOW RATE*: the standard flow rate transmitted to the pumps at program start.
- *MAX. FLOW RATE*: maximum flowrate of the pump
- *MAX. PRESSURE*: maximum pressure, the point at which the pumps will stop.
- *MINIMALDRUCK OR MIN. PRESSURE*: minimum pressure, the point at which the pumps will stop.
- *SERIAL CYCLE*: time interval at which the status of serial controlled pumps is requested. This setting is given by the driver of KNAUER pumps.
- *KOMPR. KORREKTUR*: (compression factor) for used buffers. Use 0,46 for water and 1,10 for ethanol

Different pumps can be activated with voltage controlled pumps with different maximum flow rates. In the tabs which appear underneath, the controlling voltage and the maximum flow rate for each pump can be set separately.

COLLECTOR START POSITION indicates the starting position of the fraction collector when the program starts (for home position 0 has to be chosen).

TEMPERATURES indicates two system temperatures that are set when the program starts (e.g. column oven and sample heater, this feature is not supported by the SMB column oven!).

UV-DETECTOR WAVELENGTH 1 & 2 show the wavelengths of the UV detector when the program starts.

You can use *PRESSURE DISPLAY* to change the program's pressure display to match that of the pumps with the help of an offset and a factor.

If the setup is closed, this presets will be sent to the devices. For example, the pumps will start with the set *DEFAULT FLOWRATE*. Therefore, we recommend to set the *DEFAULT FLOWRATE* to 0 ml/min.

Fraction limiter

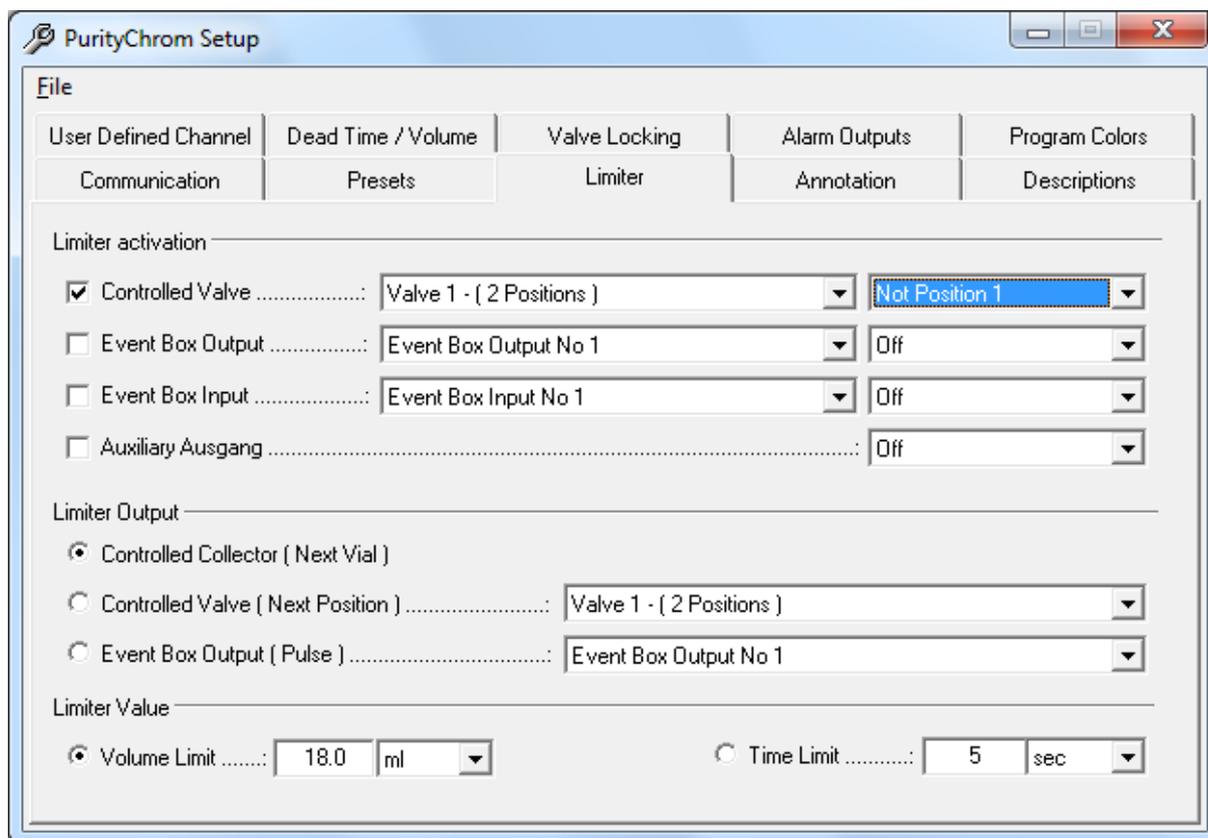


Fig. 3 PurityChrom setup, limiter

The fraction limiter is a feature which calculates the volume of your fraction vial dependent on the flow rate. In the limiter setup the fraction limiter can be configured. In *LIMITER ACTIVATION* you indicate when the fraction limiter should become active. As a rule, this is the point of switching between waste and the fraction. Using a fraction collector, you have to choose the valve built in the collector which is responsible for switching between waste and fraction.

- *SERIAL CONTROLLED VALVES* should be activated if you are using a serial or LAN controlled valve for this. Using a multi-position valve for fractionation the first position corresponds to the position *WASTE*. The following position must be the position *FRACTION*.
- *EVENT BOX OUTPUT* should be activated if you are using an event box output for this. The following output state must be the state *FRACTION*.
- *EVENT BOX INPUT* should be activated if you are recording the fraction position with an event box input. The following input state must be the one for the state *FRACTION*.
- *AUXILIARY OUTPUT* should be activated if you are using the auxiliary output of the pump interface for this. The following output state must be the one for the state *FRACTION*.

Enter in *LIMITER OUTPUT* what the limiter should do when the fraction limit is reached.

The device you are fractionating with should be activated here.

- *SERIAL CONTROLLED COLLECTOR* should be activated if you are using a serial or LAN controlled fraction collector. When the fraction limit is reached, a *STEP* is carried out.

- *SERIAL CONTROLLED VALVE* should be activated if you are using a serial or LAN controlled motor switching valve. When the fraction limit is reached, it switches to the next position.
- *EVENT BOX OUTPUT* should be activated if you are using a fraction collector controlled by the event box. When the fraction limit is reached, there is a pulse at the indicated output.

Enter the volume or time limit for the fractions in *LIMITER VALUE*. After reaching this volume or time the limiter will do what you set in *LIMITER OUTPUT*.

Annotation

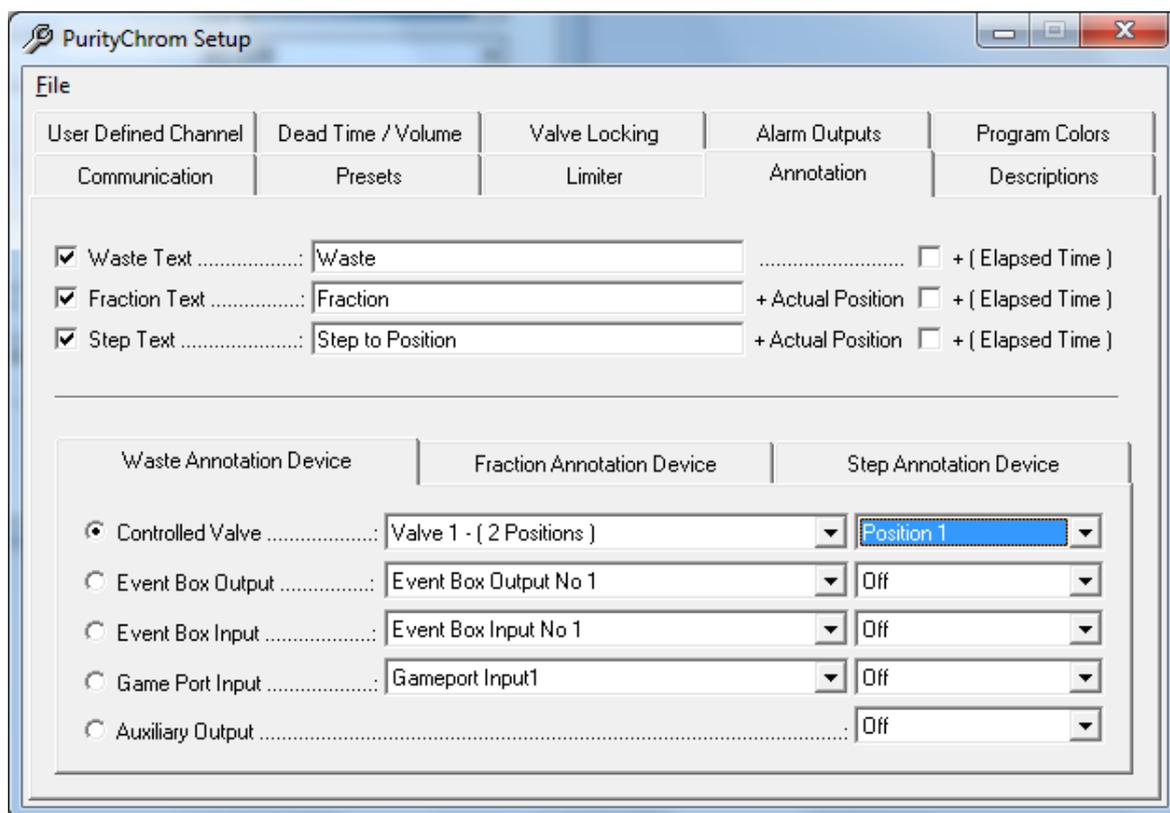


Fig. 4 PurityChrom setup, annotation

The automatic annotations in the chromatograms are configured in the annotation setup.

WASTE TEXT allows you to activate or deactivate an annotation when switching to waste. You are free to choose the text of the waste annotation. The option *+(ELAPSED TIME)* adds the elapsed time to the text. *FRACTION TEXT* allows you to activate or deactivate an annotation when switching to the *FRACTION* position. You are also free to choose the text of the fraction annotation.

The fraction number is automatically appended to the text. If you select the *+(ELAPSED TIME)* option, the elapsed time is also added.

STEP TEXT allows you to activate or deactivate an annotation when changing a position. You are also free to choose the text of the step annotation. The fraction number is automatically appended to the text. If you select the *+(ELAPSED TIME)* option, the elapsed time is also added.

In *WASTE ANNOTATION DEVICE* you can enter the device with the position or state that corresponds to the *WASTE* position. (Using a fraction collector you have to enter the valve

of the collector with the position that corresponds to the *WASTE* fraction.)

In *FRACTION ANNOTATION DEVICE* you can enter the device with its position or state that corresponds to the *FRACTION* position. (Using a fraction collector you have to enter the valve of the collector with the position that corresponds to the *FRACTION* fraction.)

In *STEP ANNOTATION DEVICE* you can enter the device with which you are fractionating or controlling the fractionating.

Descriptions

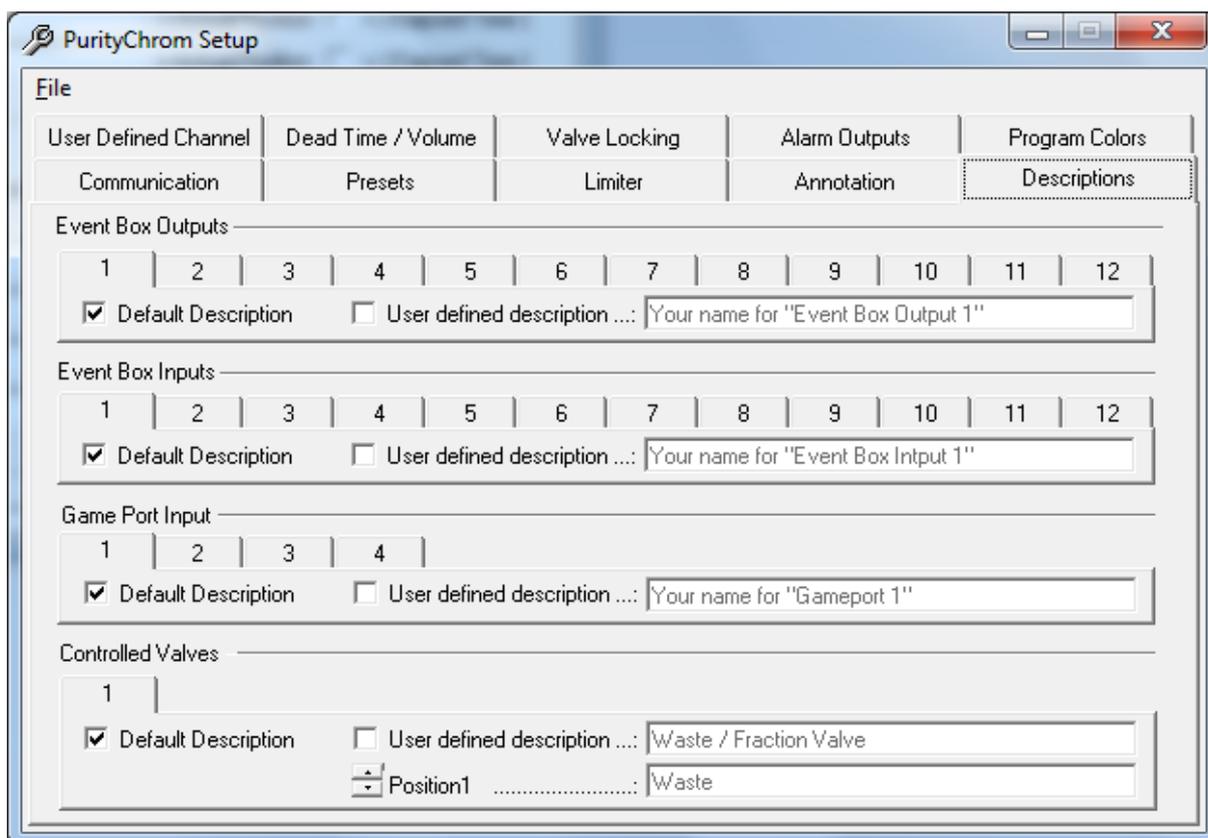


Fig. 5 PurityChrom setup, description

You can give your own names to the event box inputs and outputs, valves and the auxiliary output of the pump interface in the descriptions setup. This makes it easier to program time control files. It also makes the visualisations clearer, since the inputs, outputs and valves are can be listed by function.

The *DEFAULT DESCRIPTION* option gives these inputs, outputs and valves their standard description (*EVENT BOX OUTPUT ...*, *EVENT BOX INPUT ...*, *VALVE NO ...* and *AUXILIARY OUTPUT*).

The *USER DEFINED DESCRIPTION* gives you the possibility of entering your own descriptions in the adjacent text boxes.

User defined channel

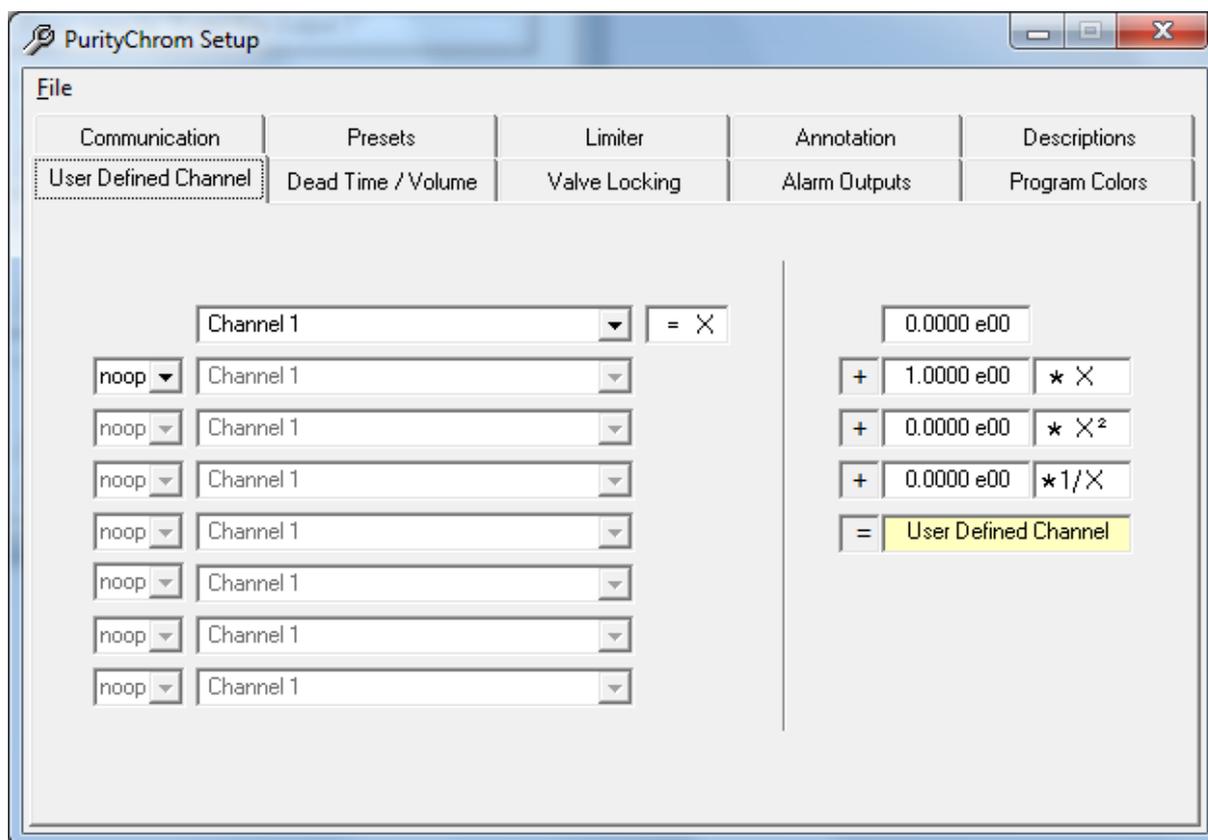


Fig. 6 PurityChrom setup, user defined channel

You can set up a user-defined data channel in the user defined channel setup. This data channel is used in the same way as one of your analogue channels, and carries the description *U*.

On the left hand side, you can activate one or more of your analogue signals as a data source. You have a choice of possible operations between these channels:

NOOP(No operation)

- + (Addition of the following channel)
- (Subtraction of the following channel)
- * (Multiplication by the following channel)
- / (Division by the following channel)

The result of these operations is on the right side available as value *X* to calculate the *USER DEFINED CHANNEL*. You can enter an offset in the first box. In the second box you can enter a factor to *X*. In the third box you can enter a factor to the square of *X* and in the fourth box, you can enter a factor to the reciprocal of *X*. The addition of the four lines gives the value of your *USER DEFINED CHANNEL*. One Example for the use of this feature is the calculation of the pressure on your column with two pressure monitors before and after your column. The User Defined Channel will be the subtraction of the pressure after your column und the pressure in front of the column.

Dead time / volume

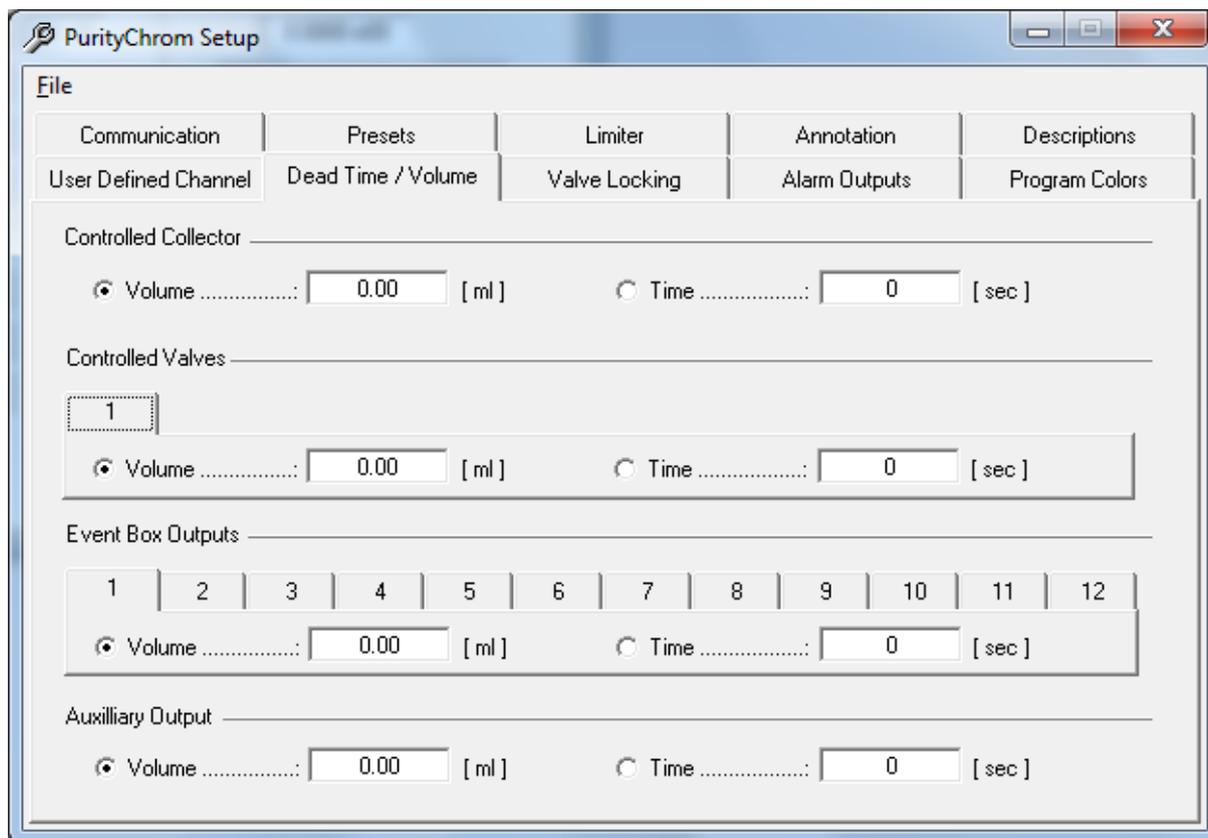


Fig. 7 PurityChrom setup, dead time / volume

A considerable dead volume can occur in the tubing and valves between the detector cell and the waste/fraction switching valve, which results in a time delay when fractionating, especially at low flow rates. In the Dead Time / Dead Volume setup, you can define the dead volume or dead time of your system. Using a fraction collector, you have to set the dead volume of your system in the section *SERIAL CONTROLLED COLLECTOR* and *SERIAL CONTROLLED VALVES*.

When you enter a dead volume, the program calculates the necessary dead time for each device according to the current flow rate. Functions triggered by threshold values, automatic peak detection as well as fractionation are also carried out with this delay. Therefore keep in mind that the run time have to be long enough to collect all the desired fractions.

When you enter a dead time, this is carried out on a 1:1 basis. Annotations are recorded in the chromatogram without the time delay

Alarm outputs

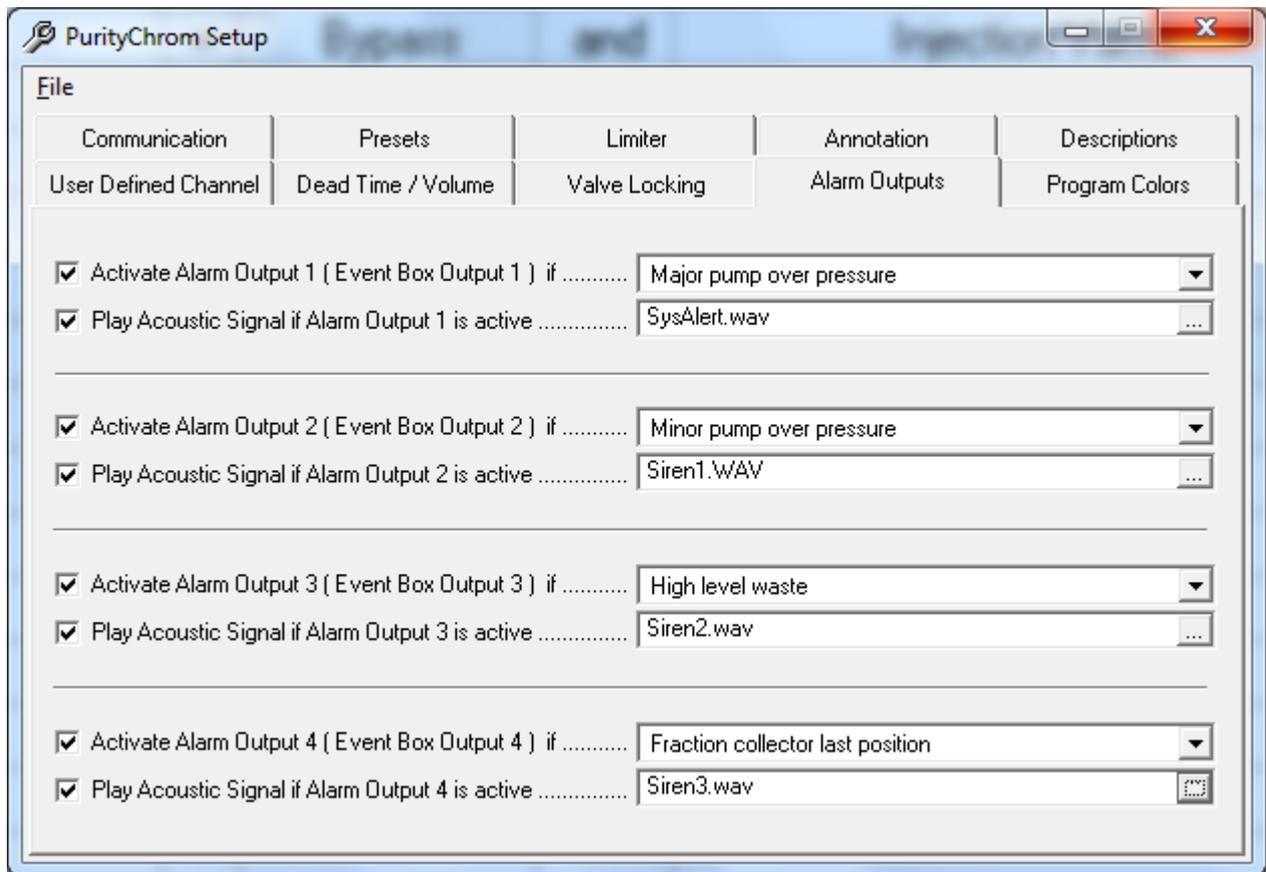


Fig. 9 PurityChrom setup, alarm outputs

Up to 4 alarm outputs can be activated in the alarm outputs set-up. The alarm outputs 1 to 4 are set using event box outputs.

Using the corresponding dropdown-lists you can configure the respective situations leading to an alarm by choosing from several predefined situations.

The option *PLAY ACOUSTIC SIGNAL IF ALARM OUTPUT N IS ACTIVE* activates an acoustic signal, which will be played while the alarm output is active. The signal file can be selected in the adjacent field.

Program colors

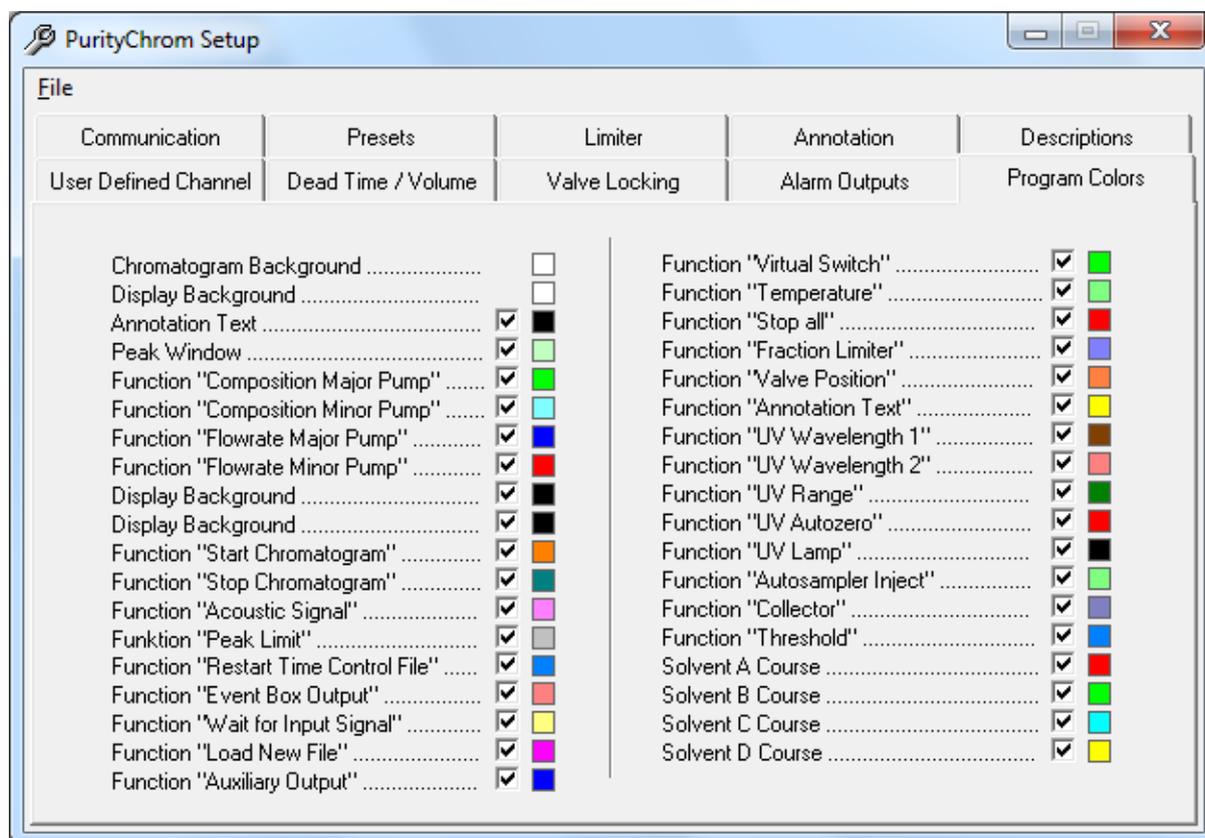


Fig. 10 PurityChrom setup, colors

The Program Colors set-up allows the configuration of colors for chromatogram background, annotation texts, peak windows and the graphic time table functions.

For each graphic element or function an adjacent checkbox determines whether the element or function will be displayed. Clicking on the colored boxes opens a color dialog window in which the corresponding display colors can be selected.

User administration

General

The user administration  allows you to set up administrators and users who can be allocated user authorities.

Working without user administration

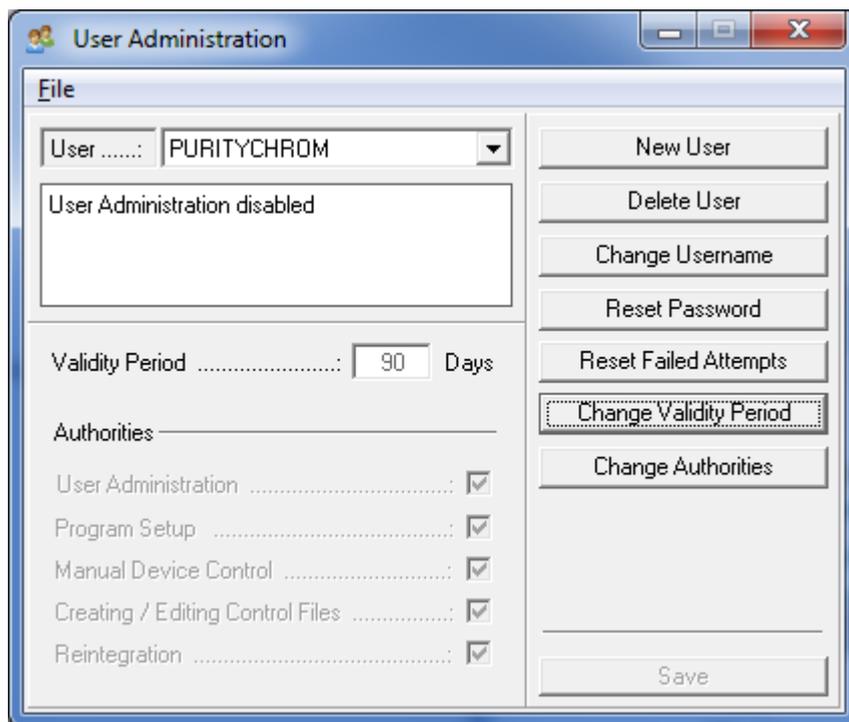


Fig. 11 User administration, disabled

At initial installation the user administration is deactivated. This setting can be re-established any time. To work without user administration name the administrator "PURITYCHROM" and allocate the password "KNAUER". This combination of name and password deactivates the user administration.

How to activate the user administration

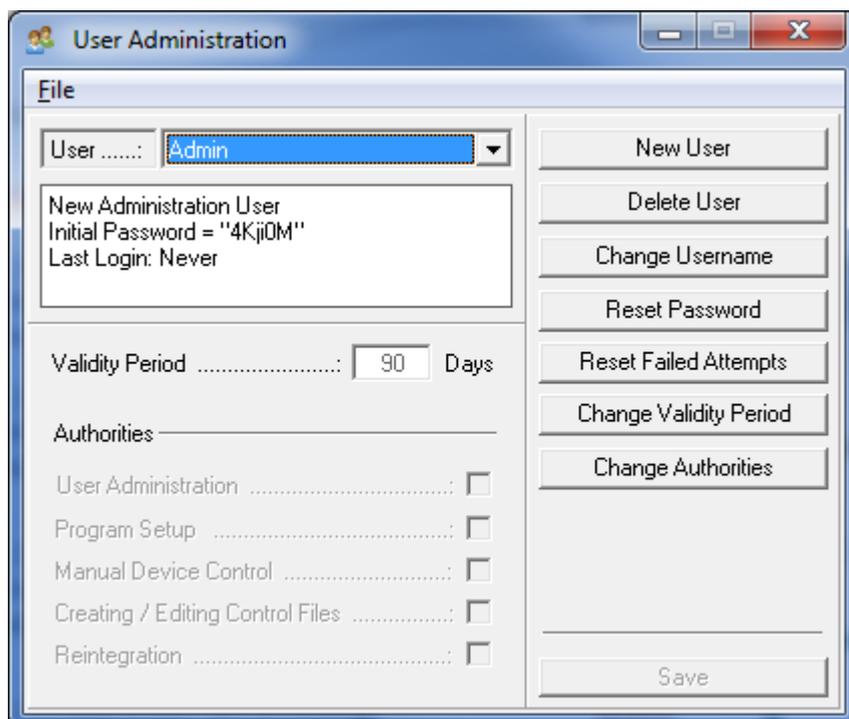


Fig. 12 User administration, adding an administrator

The user administration will be activated when the password or the user name of the administrator is changed. To change the name of the administrator, press the button *CHANGE USERNAME*. When entering a new name, you need to respect following restrictions:

1. Case sensitivity
2. No blanks
3. At least 3 characters

To confirm the input of the new user name, press the *SAVE* button. In the status window you will get an *INITIAL PASSWORD* which you need to use at your first login.

Adding a user

To create a new user profile, click on the button *NEW USER*. Into the new user interface, you can enter the user name, the validity period of the password and assign user authorities. To confirm the input, press the *SAVE* button.

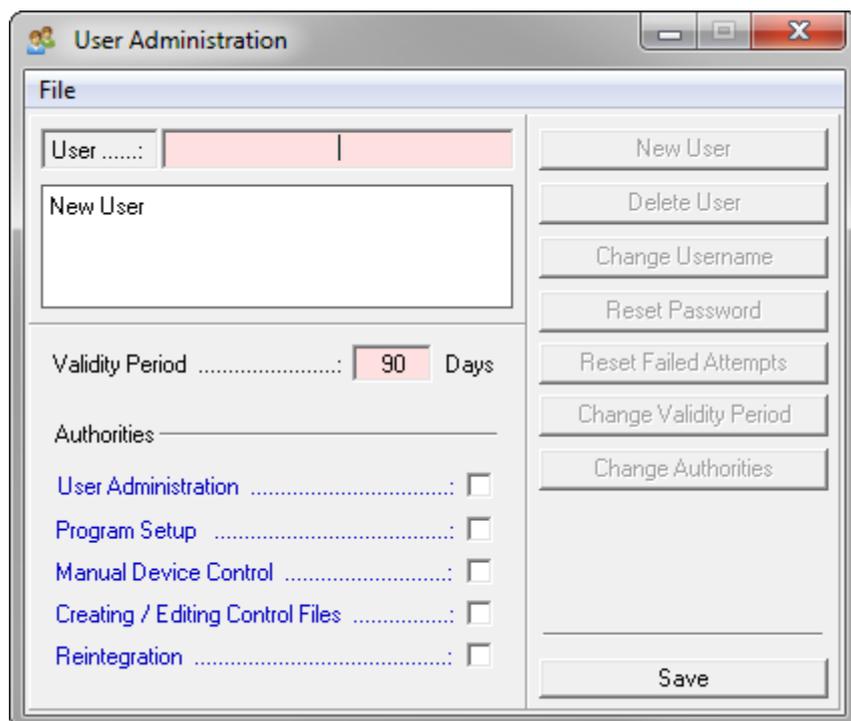


Fig. 13 User administration, adding a user

- *USER NAME* Need to have at least 3 characters, no blanks and attend to case sensitivity.
- *VALIDITY PERIOD* Defines the duration when the password is valid. After the entered period of time the user needs to enter a new password. The validity period can be between 7 and 180 days long.
- *USER ADMINISTRATION* Allows access to the user administration

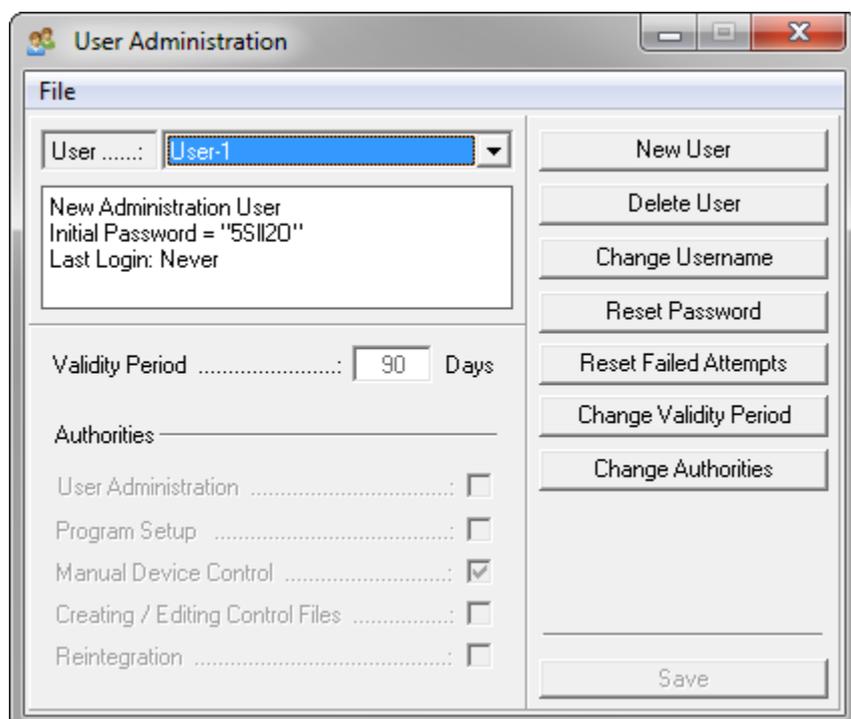


Fig. 14 User administration, adding a user

- *PROGRAM SETUP* Allows access to the program setup

- *MANUAL DEVICE CONTROL* Allows manual operation of the system using the manual control functions in the main window or the visualisation
- *CREATING / EDITING CONTROL FILES* Allows the creating and editing of time control and autosampler control files
- *REINTEGRATION* Allows the belated integration of the result files

By pressing the button of the combination-field behind the user name you can switch between the different user interfaces. The initial password for the first login you will find in the status window of the user interface of the new registered operator.

It also shows the administrator some additional information (last login, expiry date of the password).

Resetting a password

The administrator can reset a forgotten or unsafe password for the affected user. When pressing the "Reset Password" button a new initial password will be generated. The operator needs to use this password for the next login.

Resetting failed attempts

After three failed attempts to login the account of the operator will be locked. The administrator has the possibility to reset these attempts to give the operator another try to login.

The administrator will get the information, if a user is locked due to failed attempts, in the status window of the user interface.

User login



Fig. 15 User login

When the user administration is activated the operator will get a request window for the login when starting PurityChrom.

The operator needs to identify by entering user name and password.

At the first login after activation or resetting of the password the operator need to use the assigned initial password which was generated when creating the user profile or resetting the password.

After the successful input of the initial password the operator is requested to define and enter a new password.

For safety reasons the operator needs to confirm the password with a second entry.

At the end of the validity period of the password the operator is also asked to enter a new password.

The previously used password will not be accepted.

Locked account



Fig. 16 User login, new password

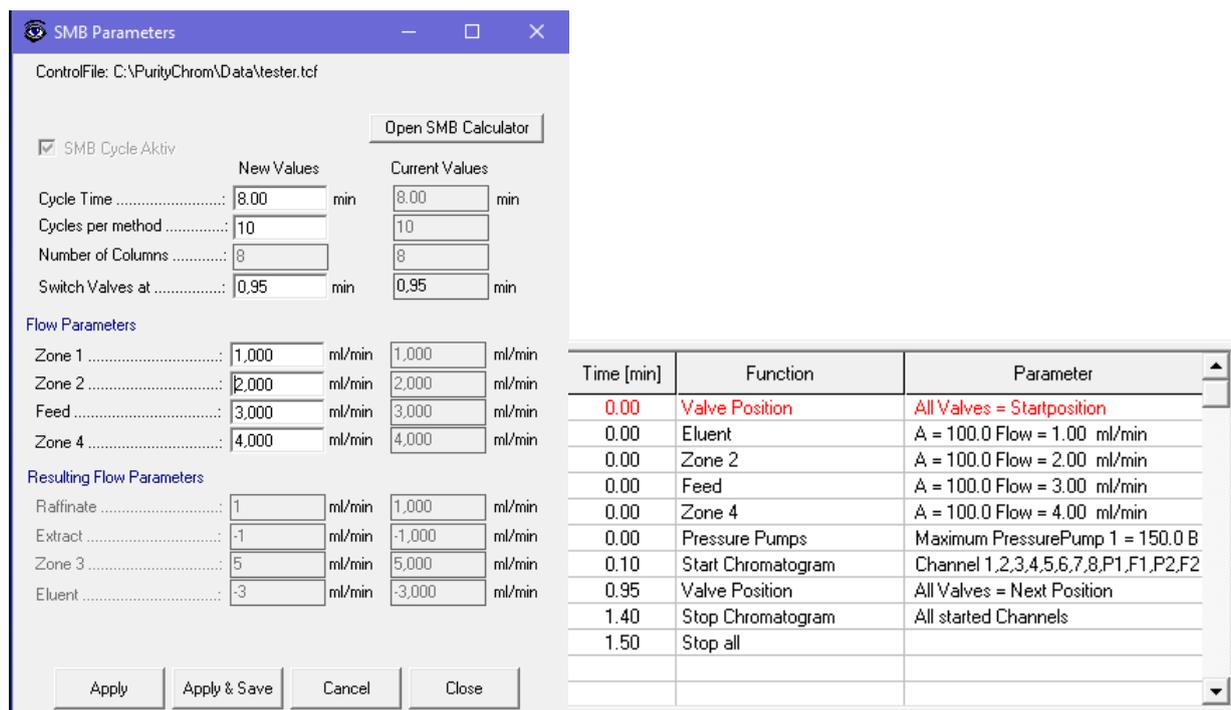
After three attempts with a wrong password the account of the user will be locked. The administrator can reset the attempts or reset the password to generate a new initial password.

Creating time control files

SMB Parameter Wizard

PurityChrom MCC has included a unique SMB Parameter Wizard that allows the easy setup of a SMB process.

To start the wizard, the  button in the main toolbar has to be clicked. If no "TimeControlFile" is loaded, you are asked to create a default SMB TimeControlFile. If a file with this name ("SMB-Default.tcf") already exists, this file can be overwritten or the file can be saved with a new name.



Time [min]	Function	Parameter
0.00	Valve Position	All Valves = Startposition
0.00	Eluent	A = 100.0 Flow = 1.00 ml/min
0.00	Zone 2	A = 100.0 Flow = 2.00 ml/min
0.00	Feed	A = 100.0 Flow = 3.00 ml/min
0.00	Zone 4	A = 100.0 Flow = 4.00 ml/min
0.00	Pressure Pumps	Maximum PressurePump 1 = 150.0 B
0.10	Start Chromatogram	Channel 1,2,3,4,5,6,7,8,P1,F1,P2,F2
0.95	Valve Position	All Valves = Next Position
1.40	Stop Chromatogram	All started Channels
1.50	Stop all	

The SMB Parameter Wizard creates a new method file with following entries:

Function	Parameter	Description
valve position	Valve Position: All Valves = Startposition	Set all Valves to the position defined in the preset window (page 7)
Pumps (Eluent, Zone2, Feed, Zone4)	A = 100.0 Flow = X ml/min	Set the flow rate. The composition is always A = 100.
pressure pumps	Maximum Pressure Pump X	Set the Maximum pressure to the value entered in the preset options (page 10)
start chromatogram	Channel 1,2,3...	Initializes the data acquisition for all channels available
Valve Position	All valves = Next Position	Switches all Valves to the next position. Switching time is defined by the Parameter Wizard.
stop chromatogram	All Started Channels	Stops the data acquisition
stop all		Stop every device.

The "Cycle Time" and the "Cycles per method" defines the length of the SMB separation. For example: 10 cycles of 8 minutes will result in 8x8x10 valve switches and a run of 80 minutes length (plus the time to the "Stop all" value). For every cycle, the valves will be switched eight times (in an eight-column setup).



Time [min]	Function	Parameter
0.00	Valve Position	All Valves = Startposition
0.00	Eluent	100.0 , 0.0 , 0.0 , 0.0 1.00 ml/min Constant Flow
0.00	Zone 2	100.0 , 0.0 , 0.0 , 0.0 2.00 ml/min Constant Flow
0.00	Feed	100.0 , 0.0 , 0.0 , 0.0 3.00 ml/min Constant Flow
0.00	Zone 4	100.0 , 0.0 , 0.0 , 0.0 4.00 ml/min Constant Flow
0.00	Pressure Pumps	Maximum Pressure Pump 1 = 150.0 Bar
0.10	Start Chromatogram	Channel 1,2,3,4,5,6,7,8,P1,F1,P2,F2,P3,F3,P4,F4 (500 ms)
0.95	Valve Position	All Valves = Next Position
1.40	Stop Chromatogram	All started Channels
1.50	Stop all	

General

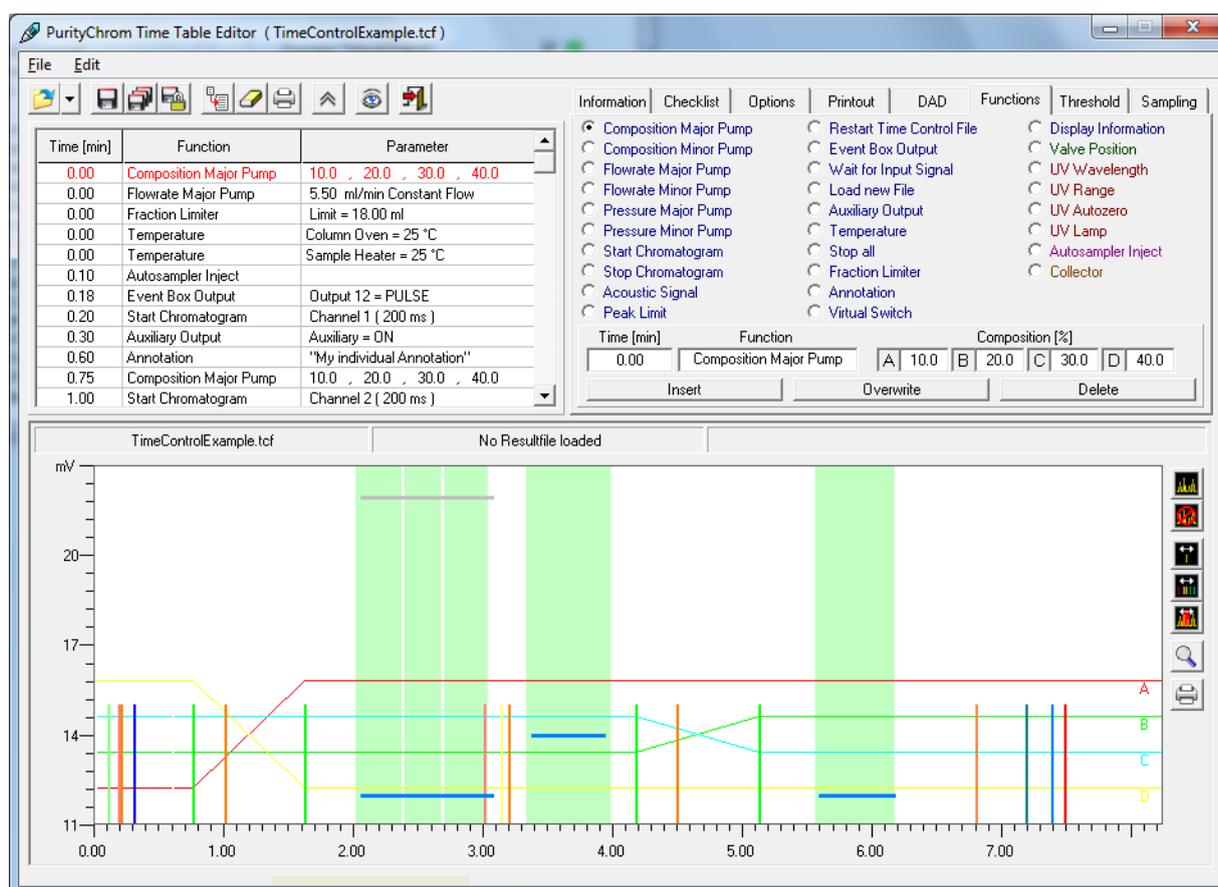


Fig. 17 Time table editor

You can open the time control file editor with the function button  or the menu option *CREATE / EDIT TIME CONTROL FILE* in the main window. The editor shows the time control file in both table and graphic form. The graphic display also allows you to load a chromatogram as a background image, with the help of which the functions of the time control file can be adjusted and checked along the time and intensity axes. The graphic presentation of the functions, particularly the display of the gradient, ensures increased

clarity when programming and thus helps you to avoid making mistakes.

By creating time control files based on time, keep in mind that seconds are indicated as hundredth of minutes.

The functions of the time table need not be programmed in chronological order. They are sorted automatically in the right order on inserting into the time table.

Programming for volume and column volume (cv) is not supported for continuous process and the PurityChrom MCC version.

Time [min]	Function	Parameter
0.30	Auxiliary Output	Auxiliary = ON
0.60	Annotation	"My individual Annotation"
0.75	Composition Major Pump	10.0 , 20.0 , 30.0 , 40.0
1.00	Start Chromatogram	Channel 2 (200 ms)
1.62	Composition Major Pump	40.0 , 20.0 , 30.0 , 10.0
2.06	Threshold	1.00 min / 14.00 mV
2.06	Peak Limit	Cut / Insert Time
3.01	Event Box Output	Your name for "Event Box Output 1"
3.14	Wait for Input Signal	Your name for "Event Box Input 1"
3.20	Start Chromatogram	Channel 3 (200 ms)
3.38	Threshold	0.58 min / 14.00 mV
4.18	Composition Major Pump	40.0 , 20.0 , 30.0 , 10.0

1.00	Start Chromatogram
1.62	
2.06	
2.06	Peak Limit

Fig. 18 Time table

If you want to move more than one function of the time table to another time, click with the right mouse key into the first line of the functions to be moved. On clicking into *CUT/INSERT TIME* an entry box appears, where a positive or a negative time can be entered. A positive time moves all following functions to a later time, a negative time moves all functions to an earlier time.

There are certain things you should keep in mind when creating or editing a time control file.

It is a basic principle that every time control file must begin at a time point of 0.00 minutes with an eluent composition and a flow rate, so that the initial conditions of the pumps are set.

All the functions programmed for time point 0.00 minutes are carried out when the time control file is loaded. For this reason, it is sensible use this point in the program to set all the controlled valves to a basic position and to set the event box exits used, so that the system is not left in an undefined state because of previous use or manual operation.

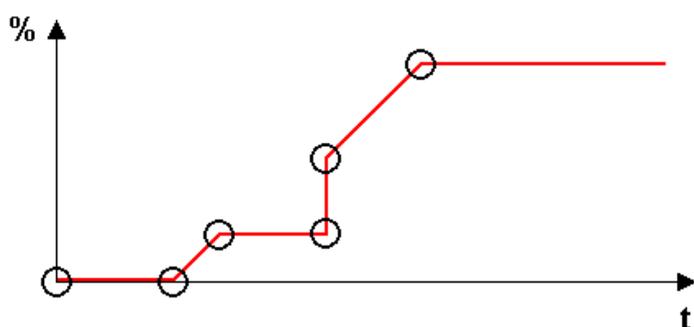


Fig. 19 Programming a gradient

If you want to program a gradient, you should take care that you include all the angles of this gradient as eluent compositions in the time control file. A linear gradient will be calculated between two different eluent compositions at two different times. If you want to program a stepped gradient using our P2.1L, add start and end points with a time difference of at least 0.06 minutes. The last eluent composition programmed will run isocratically until the time control file is ended (by the program or manually).

Creating a time control file

On the right hand side of the window is the toolbar for the time control file editor. The functions are listed here in the order which corresponds to the display:

-  *OPEN TIME CONTROL FILE* loads an available time control file into the editor. The types of file you can select to load are: *TIME CONTROL FILE* (*.tcf), *Result File* (*.rfp) or *PURITYCHROM FILE* (*.lgr). The *Result File* option allows you to load a time control file from results data. *PURITYCHROM FILE* allows you to import time control files created with PurityChrom
-  *SAVE TIME CONTROL FILE* saves the time control file under its existing name.
-  *SAVE TIME CONTROL FILE AS ...* saves the time control file under a new name.
-  *SAVE TIME CONTROL FILE AS VALIDATED* saves the time control file as a validated file. This file cannot be modified any more.
-  *IMPORT TIME CONTROL PART* is used to import an element of a time control file. The content of the selected tab can be imported from an existing time control file.
-  *CLEAR ALL* deletes the content of a time control file. In order to prevent the file from being deleted by mistake, this function requires a further confirmation from you before being carried out.
-  *PRINT TIME CONTROL FILE* opens a printer dialogue to print out the time control file.
-  *HIDE GRAFIC / SHOW GRAFIC* hides or shows the graphic time table editor. The window of the time table editor will be reduced or enlarged.
-  *SIMULATION MODE* activates a simulation mode for the loaded time table in the time table editor. By clicking a line in the editor the correspondent situation is displayed in the system visualisation.

5.60	Threshold	0.60 min / 12.00 mV
▶ 6.81	Valve Position	Valve 1 = Position 1 aste ◀
7.20	Stop Chromatogram	All started Channels

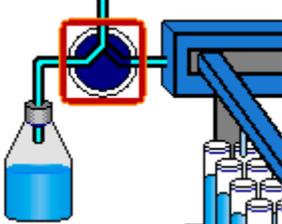


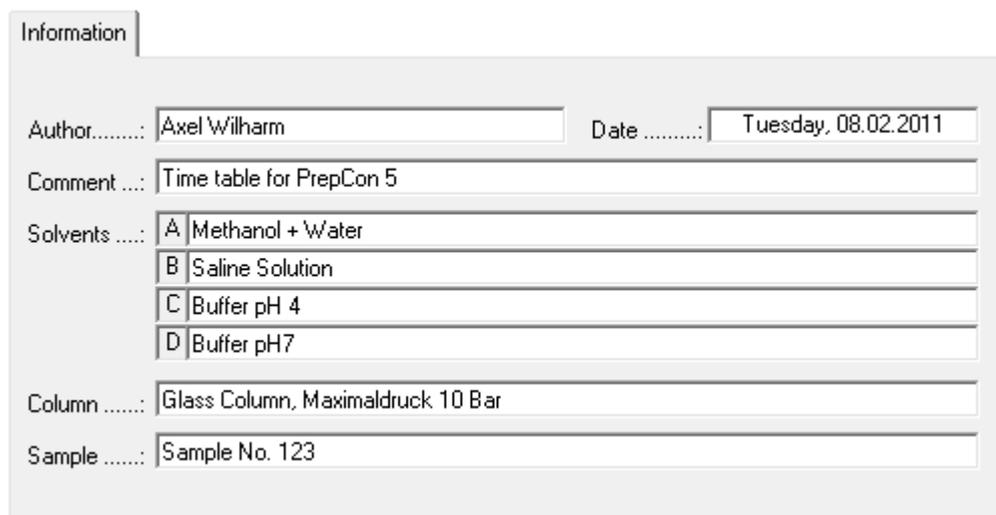
Fig. 20 Functions of time tables

The selected line is marked in the table with two arrows. With the arrow keys on the keyboard the next or the previous line can be selected. Using this simulation, basic functions of time tables can be tested. In the system visualisation the appropriate graphic object is marked by a red blinking frame. In addition value labels are showing the parameters of the selected line (solvent compositions, flow rates, UV wavelengths, etc.)

-  *EXIT* closes the time control file editor.

Storing of general information

In the *INFORMATION* tab, you can enter information about the author of the file, the eluents used, the column use, a sample identifier and a general comment. The date is automatically generated when you save the file.



Information

Author.....: Axel Wilharm Date: Tuesday, 08.02.2011

Comment ...: Time table for PrepCon 5

Solvents: A | Methanol + Water
B | Saline Solution
C | Buffer pH 4
D | Buffer pH7

Column: Glass Column, Maximaldruck 10 Bar

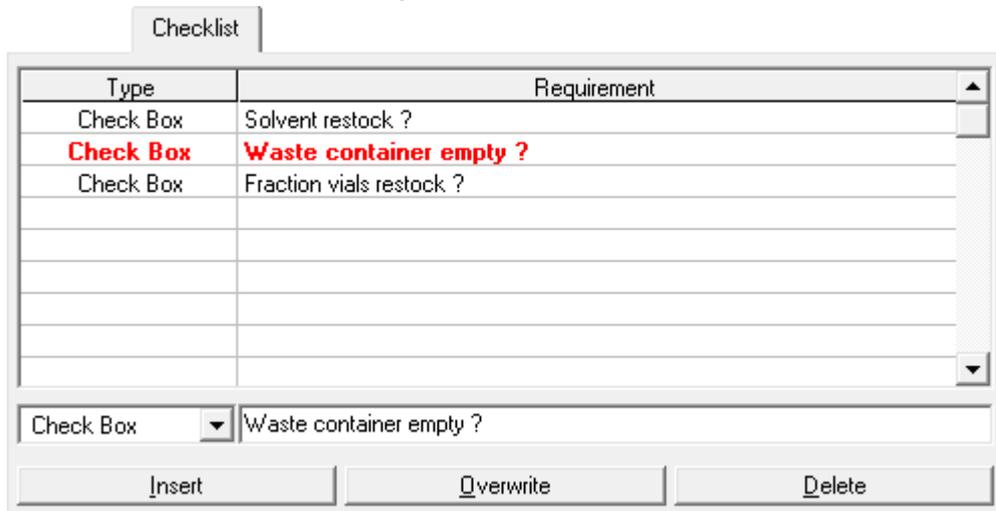
Sample: Sample No. 123

Fig. 21 General information

The sample identifier is not included when using an autosampler or when the *MANUAL SAMPLE ID* option is activated. The information entered is saved in the results file generated and can be reopened later.

Creation of a checklist

The *CHECKLIST* tab allows you to create an instructionlist or a checklist. The editor bar is at the bottom of the tab, where you can choose between *CHECKBOX* or *USER INPUT*:



Type	Requirement
Check Box	Solvent restock ?
Check Box	Waste container empty ?
Check Box	Fraction vials restock ?

Check Box Waste container empty ?

Insert Overwrite Delete

Fig. 22 Creation of a checklist

CHECKBOX is an instruction or a control statement. Enter the text of the statement in the input field on the right. This item has to be ticked off by the user before he can start this time control file.

USER INPUT is an input prompt. Enter the text of the prompt in the input field on the right. The user has to enter the demand before he can start this time control file.

The *INSERT* button adds the content of the current editor bar to the checklist. The *OVERWRITE* button overwrites the content of the line you have selected with the contents of the editor bar. The *DELETE* button deletes the line you have selected. To change the order of the lines, left-click on a line and while keeping it pressed move it to the desired place.

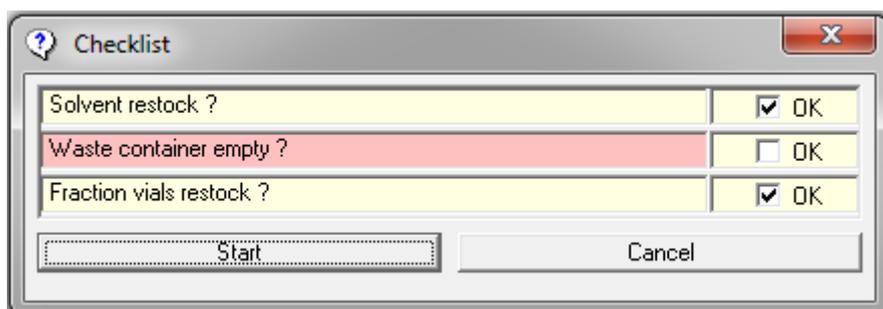


Fig. 23 Creation of a checklist

If the checklist of a time control file contains rows, the checklist window will open before starting this file. The user has to ticked off all checkboxes and enter all demands before the run begins. A forgotten line will be highlight red and prevents the start. The checklist will be logged in the result file.

Optional settings

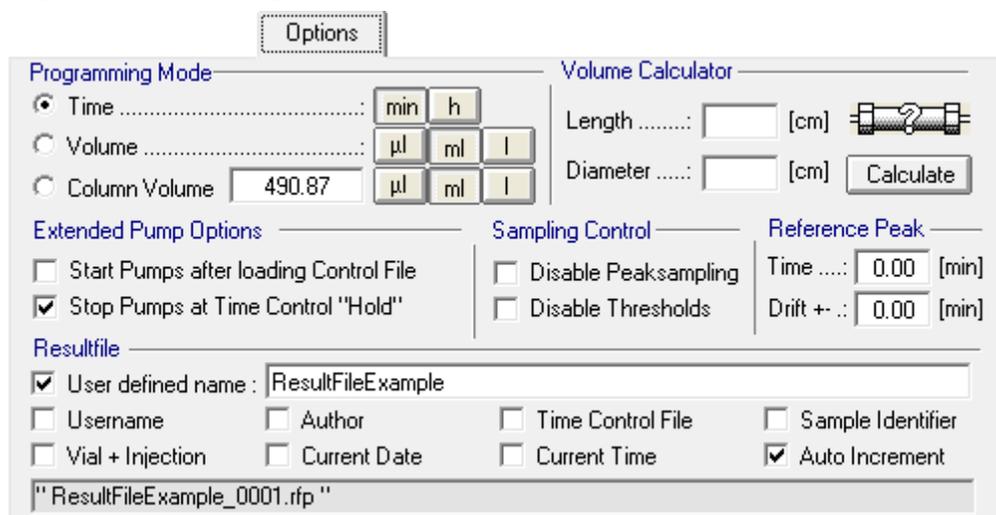


Fig. 24 Optional settings

The *OPTIONS* tab allows you to set the dimensions of the x-axis in *PROGRAMMING MODE*. You have a choice between units of time, volume or column volume **Note: The volume and column volume mode is not supported by the PurityChrom MCC.** You can also select the size of the units beside each programming mode. To help you determine the column volume when programming in units of column volume, you can use the mini-calculator on the right. To do this, enter the length and internal diameter of the column and click on the *CALCULATE* button. The column volume will then appear in the *COLUMN VOLUME* box.

In *EXTENDED PUMP OPTIONS*, you can use the *START PUMPS AFTER LOADING TIME CONTROL FILE* option to determine whether the pumps should start after loading the time control file, with the parameters programmed for time point 0.00 minutes. The *STOP PUMPS AT TIME CONTROL HOLD* lets you determine whether the pumps should stop when the *HOLD TIME CONTROL FILE* occurs at a flow rate of 0 ml.

SAMPLING CONTROL allows you to deactivate the peak sampling programmed by selecting the *DISABLE PEAK SAMPLING* option. *DISABLE THRESHOLDS* deactivates the thresholds programmed for this time control file. These options allow you to deactivate the collective functions without needing to delete the threshold values or peak samples from a valuable program you may need later.

For the function *REFERENCE PEAK* you will need a reference chromatogram with a reference peak that elutes at a specific time point. This time you can set here.

If you program a threshold for this peak you can choose the function reference peak. If the peak elutes in your next run after a different time period all the function you programmed after the threshold will be postponed due to your set reference time. You can also program a drift. This means all the functions will be postponed due to your reference time plus the drift time. *RESULT FILE* allows you to enter the names of the result files to be generated by this time control file. You can set the file names using the following 9 name segments:

1. *USER-DEFINED NAME* adds a user-defined text to the file name. It should be short enough to fit into the text box.
2. *USERNAME* adds the name of the current user to the file name.
3. *AUTHOR* adds the name of the author of the time control file to the file name.
4. *TIME CONTROL FILE* adds the name of the time control file to the file name.
5. *SAMPLE IDENTIFIER* adds the sample identifier to the file name.
6. *VIAL + INJECTION* adds the number of the current sample vial and the current injection to the file name.
7. *ACTUAL DATE* adds the current date to the file name.
8. *ACTUAL TIME* adds the current time to the file name.
9. *AUTO INCREMENT* Adds a four-digit consecutive number to the end of the file name, in order to stop result files with the same name from being overwritten. If you do not want to lose your last file, you should always select this name segment.

Printout options

The *PRINTOUT* tab allows you to setup the content and design of your report file for automatic printing.

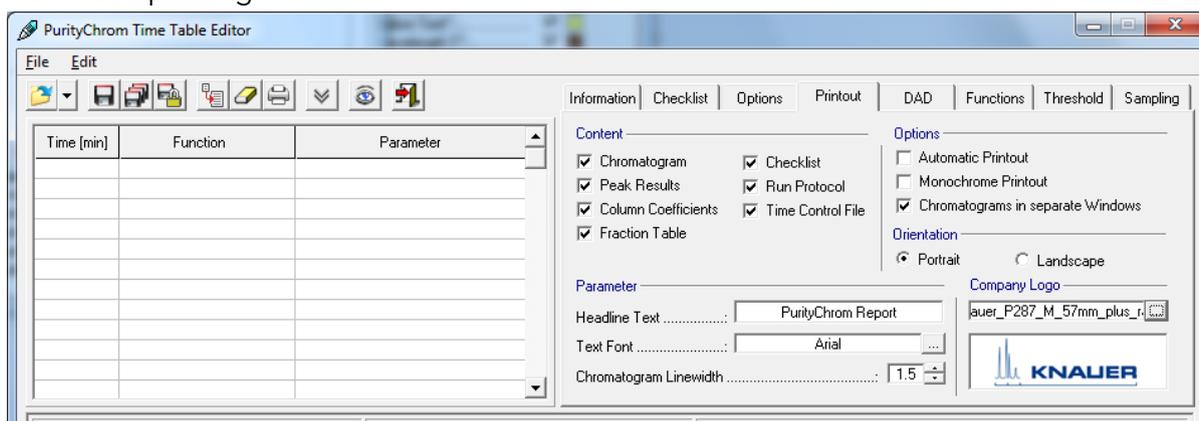


Fig. 25 Print options

In the section *CONTENT* you can define which data you want to include in your printout. Besides the chromatogram you can choose different data, tables and protocols.

In the section *OPTIONS* you can checkmark, if the printout should be executed automatically after each run. It is also possible to choose the option to have each data channel in a separate window and the option for a monochrome printout.

The layout can be varied between portrait and landscape in the section *ORIENTATION*.

In the section *PARAMETER* and *COMPANY LOGO* you can adapt your individual design with an individual headline, text font, line width of the data traces and a company logo.

The size of the logo should be 154 to 42 pixels and the type Bitmap.

Settings for diode array detectors

The parameters of the diode array detector are defined in the *DAD* tab. To use a DAD detector, you need to scale up the full license with the DAD license. Select the source of the data channel under *DATA SELECTION*. You can choose either the A/D converter board by *A/D CONVERTER* for the conductivity, pH or analog signals or the data of the DAD by *DAD*. Choose the wavelength range of the DAD channels in the adjacent entry boxes. For using DAD, you cannot choose the wavelength by using the function button "Wavelength" from the main window. Channels are chosen in this window or in the chromatogram view (see Data Channel Setting, page 66). The selection of data channels is automatically adopted when loading the time table. Choose the slicewidth (data rate) and bandwidth, otherwise the preset values from PurityChrom.ini file will be taken. A maximum data rate of 10 Hz (100 ms) is supported.

Fig. 26 Settings for DAD detector

Enter the slice width of the DAD channels in ms in the *SLICEWIDTH* entry box.

The *BANDWIDTH* parameter is used for averaging the measured values in order to reduce signal noise. For a bandwidth of e.g. 5 nm the signal of each wavelength is averaged over a range of ± 2 nm. No average value is calculated when a bandwidth of 1 nm is entered.

By choosing the *SAVE 2D-DATA* option the spectra data set of the DAD is saved in the ChromStar DAD data format.

Time control file commands

You will find a list of all the programmable functions of the time control file in the *FUNCTIONS* tab. The editor bar is at the bottom of the tab, where all the functions are to be entered with their time and parameters. The parameter boxes in the editor bar change depending on the function selected. The *INSERT* button adds the content of the current editor bar to the time control file. The order in which you make your entries is irrelevant, since all the lines of program are ordered chronologically. The *OVERWRITE* button overwrites the content of the line you have selected with the contents of the editor bar. The *DELETE* button deletes the line you have selected.

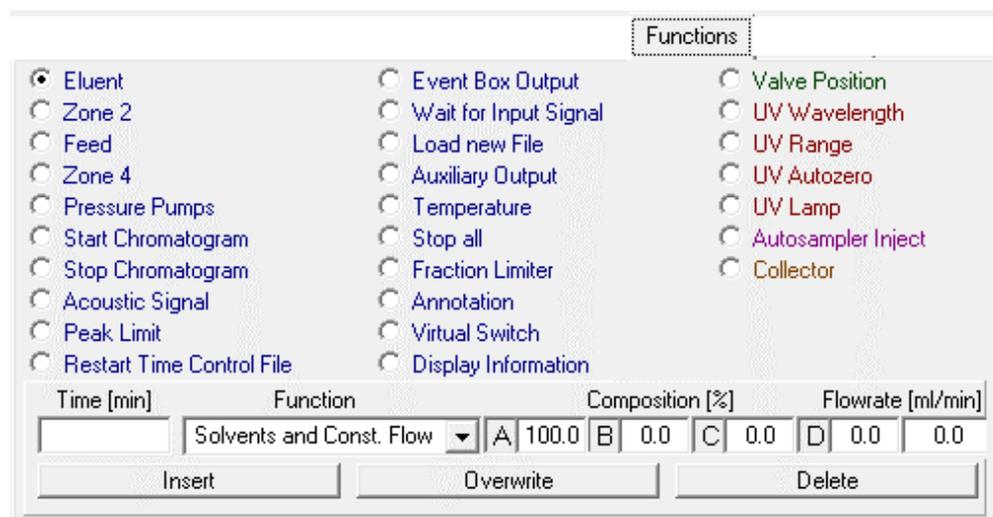


Fig. 27 Time control file functions

Pumps (Eluent/Zone 2/ Feed/Zone 4) determines the eluent composition and the flowrate for the pumps in the system. Entries are made in percent, and while you are making the entries, the entry in the following box always makes up the difference to 100%. For isocratic pumps A has to have a value of 100%.

PRESSURE determines the behavior of the pump at the inscribed values. The set value will be sent to the pump and will be remembered. By opening and saving the setup or restarting the software the set maximum and minimum pressure in the tab *PRESETS* from the setup will be sent to the pump.

- Minimum Pressure: Pausing of the method and stopping of the pump when the pressure was constantly below the appointed value for 20 seconds.
- Maximum Pressure: Pausing of the method and stopping of the pump when the pressure when the appointed value is exceeded.
- Pressure Limit (not supported by KNAUER pumps): automatic flow rate control starts working when the appointed value is achieved.

START CHROMATOGRAM starts plotting the chromatogram for the channels selected, with the data point density entered under *SLICE WIDTH* (max. 60 ms). Channel 1 will record the UV signal, channel 2 the conductivity signal, channel 3 the pH signal and channel 4 the temperature. More UV-channels will occupy the first channels and the conductivity signal, the pH signal and the temperature will follow in this order. The basic license includes three data channels plus flow rate and pressure recording. The full license supports eight data channels plus flow rate and pressure recording. When using the detector 50 D with four wavelengths the full license is needed.

STOP CHROMATOGRAM stops plotting the chromatogram for the analogue channels selected or, if *ALL ACTIVE* is selected, stops plotting for all the analogue channels started.

ACOUSTIC SIGNAL initiates a sound reproduction of the wave file selected with an interval of 4 seconds. The test button  allows you to hear the file. The sound will end when the time control file is stopped.

PEAK LIMIT is used to monitor peak heights and peak times. It works in conjunction with the *RESTART* command and takes effect when the time control file is restarted during cyclic effluents. The end time in minutes (*END*) and the limit value in mV (*VALUE*) must be entered as parameters. A peak limit only allows the time control file to restart when one or more peaks exceeds the limit value and then falls back below this value during the time duration of the time control file. If one or more peak limits fail, the *RESTART* command will be ignored and you will receive an error message on screen.

RESTART TIME CONTROL FILE is used to restart the time control file. You can select the parameter *Loop* for an endless loop, or *Repeats* in order to enter a certain number of repeats. Once all the repeats have been carried out, this command will be ignored. Therefore you have to program this function at the end of your time table and before the function *STOP ALL*. Before you restart your time control file use the function *STOP CHROMATOGRAM* to restart your data recording.

EVENT BOX OUTPUT is used to switch one of the 12 event box outputs. The required event box output and the state to be switched to (*Off*, *On*, *Pulse*) must be selected as parameters. *PULSE* means that the event has to be switched on and off again.

WAIT FOR INPUT interrupts the continuation of the time control file through time (*HOLD FUNCTION*) until the selected event box input or the game port has the state programmed (*OFF*, *ON* or *PULSE*). *Off*: The time table will continue if the trigger stays off. *On*: The time table will continue if the trigger switches to the state on and produces a short-circuit. *Pulse*: The time table will continue if the trigger switches to the state on and off again. This function can be used when injecting by means of a manual injection valve, for example. You cannot use this function at the time/volume/column volume 0.00. If you want to use the trigger to start a time table you have to enable the respective event box input or game port input in the input field *TIME CONTROL START* (see chapter Communication page 13). If you use a trigger cable, you have to choose the Event Box Input 1. Besides if you want to start your pump dependent on the trigger, the PurityChrom.ini-file [Pumps → IOcontrol=1] (see appendix) has to be modified.

LOAD NEW FILE is used to load and start the time control file selected. This allows you to link several time control files together.

AUXILIARY OUTPUT is used to switch the pump interface's auxiliary output. This function allows you to control a relay contact. You can select *ON* or *OFF* as parameters.

TEMPERATURE is used to program up to two system temperatures. The parameters are the temperatures in °C.

STOP ALL is used to end a run and stop the pumps.

FRACTION LIMITER is used to program fraction limits. You can enter a maximum volume in ml or a maximum time in seconds. The value programmed will not change the limiter setup, but will be adopted as a current value.

ANNOTATION is used to add manual text annotations to the chromatograms.

VIRTUAL SWITCH is used to program up to 10 virtual switches. This virtual switch is only a variable that can be set to 0 or 1. Example: You can link this switch with a button in your visualisation. In your time control file you can program to wait for the virtual switch set to 1 using the function *WAIT FOR INPUT*. Then your time control file will be hold until you switch the virtual switch to 1 by pressing the button in your visualisation. These virtual switches can have the state *ON* or *OFF*.

DISPLAY INFORMATION is used to display user defined information which are shown in the Information label of the system visualisation.

VALVE POSITION is used to switch valves. Select the desired valve and position as parameters.

UV-WAVELENGTH 1 is used to program the first wavelength of a UV detector. Enter the desired wavelength in nm as the parameter.

UV-WAVELENGTH 2 is used to program the second wavelength of a UV detector. Enter the desired wavelength in nm as the parameter.

UV-RANGE is used to program the range value of a UV detector.

UV-AUTOZERO makes the UV detector automatically return to zero.

UV-LAMP is used to switch on or off the lamp from the UV-detector. (Only when supported)

AUTOSAMPLER INJECT is used to carry out an injection from an autosampler. You need the full license to use an autosampler. This function can only be used in conjunction with an autosampler control file. The course of the time control file is paused during the injection phase and the *ELAPSED TIME* display in the main window shows the time passed and Injection alternately.

COLLECTOR is used to program a fraction collector position. The parameters can be a return to the home position (*HOME*), a move to a further position (*STEP*) or a position number (*POSITION*).

Using the P6.1L (high pressure gradient version)

Using the P 6.1L (high pressure gradient version) you have to configure the solvent selection valve separately by choosing two standard valves (see chapter Presets page 13). The first valve can switch between A1 (position 1) and A2 (position 2) and the second between B1 (position 1) and B2 (position 2). Use the starting point of your time table control file to set these valves to the position that corresponds to your wanted solvent. During a run it is not recommended to switch these valves.

Fractionating with a fraction collector

First you have to configure the valve of your fraction collector in the PurityChrom setup (see chapter Fraction Limiter page 15), the annotations (see chapter Annotation page 16), the dead volume or time (see chapter Dead Time/Dead Volume page 19) and your rack that you want to use (see chapter Fraction Rack Setup page 72). At the respective time point at which you want to start fractionating you have to choose the function collector and tell the collector arm the position at which it should start fractionating. If you choose *STEP*, it will switch to the next position in the rack. The actual position of the fraction collector arm will be remembered until the program is switched off and on again. By programming the function *COLLECTOR STEP* at the start of the time table control file, the arm will step to the next position when the time control file is loaded and again when it is started. To avoid that you can program that function at for example 0.01. Next you have to switch the valve of the fraction collector from waste to fraction and set a volume or time limit, but only if it is different from the set limit in the setup (see chapter Fraction Limiter page 15).

Fractionating with a multi-position valve

First you have to configure the valve in the PurityChrom setup (see chapter Fraction Limiter page 15), the annotations (see chapter Annotation page 16), the dead volume or time (see chapter Dead Time/Dead Volume page 19). At the respective time point at which you want to start fractionating you have to switch multi-position valve from waste to fraction and set a volume or time limit, but only if it is different from the set limit in the setup (see chapter Fraction Limiter page 15). You can choose distinct position, or you choose the command *NEXT STEP* to go automatically to the next position. The positions of your collected fractions will not be remembered by the software after changing to waste position.

Programming thresholds

THRESHOLD: The *THRESHOLD* tab allows you to program thresholds, meaning that a series of device control functions can be executed if a value exceeds or drops below the threshold. The threshold values allow individual fractionating or system monitoring possibilities, thanks to their selectable data source, programmable time frames for activities and programmable threshold levels. Up to 50 threshold values can be active at any one time.

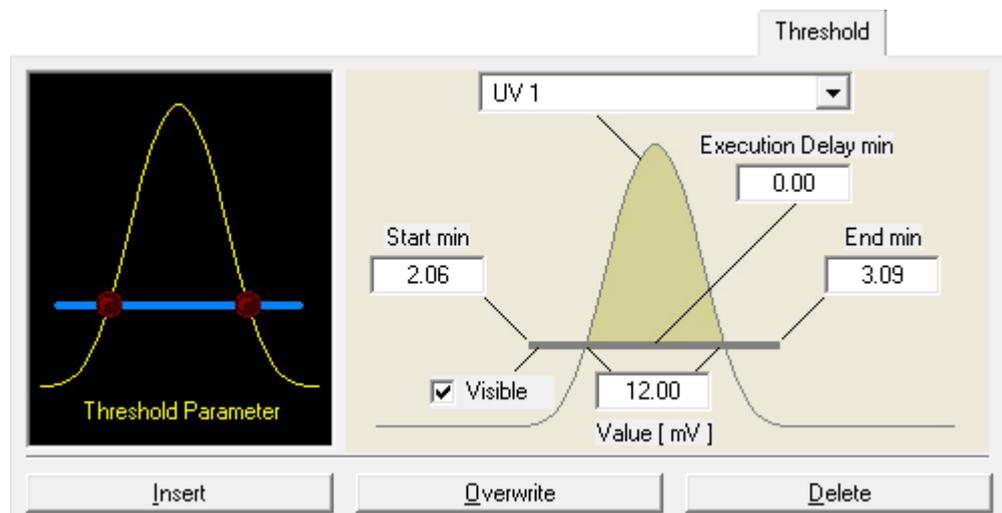


Fig. 28 Programming thresholds

The graph on the left of the tab is used to select the threshold parameters. Click on the graph next to the LEDs and the boxes for the general threshold parameters will appear. You can select the data source of a threshold value in the upper box. You can choose between all the analogue channels, the system pressure, the event box inputs and the game port inputs.

START TIME and **END TIME:** The boxes *START TIME* and *END TIME* set the time frame for the activity of the threshold

VALUE: The *VALUE* box is for the threshold value. When using event box or game port inputs, this parameter is not available, since the data source only has two possible states (Low = Threshold Under Event, High = Threshold Over Event).

EXECUTION DELAY: The *EXECUTION DELAY* parameter delays the execution of all programmed functions in this Threshold.

AREA DELAY: The *AREA DELAY* parameter delays the *OVER EVENT FUNCTIONS* in dependency on the peak area. The input value is equivalent to the product of the peak area over the threshold and the time.

VISIBLE: By choosing the *VISIBLE* option the graphic line of this Threshold will be displayed in the analysis windows. Otherwise it will be invisible. Click on the left-hand LED in the graph and the function table of the *OVER EVENT FUNCTIONS* will appear, in other words the functions that are carried out if the threshold value is exceeded.

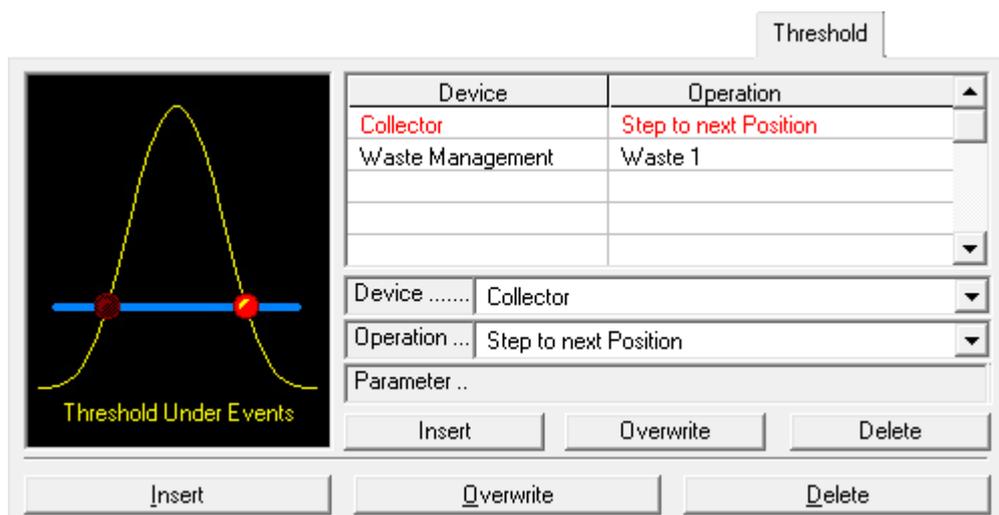


Fig. 29 Programming thresholds

The selection boxes *DEVICE* and *OPERATION* allow you to set the desired function, and the *INSERT* button adds these functions to the time control file.

You can use the *OVERWRITE* button to overwrite a selected line, and *DELETE* to delete it. If you click on the right-hand LED, the function table of the *UNDER EVENT FUNCTIONS* appears, i.e. the functions that are carried out when the value is less than the threshold. Functions can be set in exactly the same way here as for *OVER EVENTS*.

Programming peak sampling

The *SAMPLING* tab allows you to program peak sampling. Peak sampling is a way of carrying out a series of device control functions on the basis of automatically recognised peak situations. Peak sampling takes place in peak windows, the start and end times of which can be set by the user. You can select the data source, sensitivity of peak recognition and the functions to be carried out separately for each peak window. This allows you to react to single peaks on an individual basis. When fractionating in combination with threshold values, the thresholds and peak sampling always combine to form one fractionation event. This means that as long as the threshold is still exceeded, or the peak sampling has not yet recognised the end of a peak, fractionating continues.

The graph of peak situations is used to select the peak sampling parameters and peak situations. Click on the graph next to the LEDs and the boxes where you can enter the peak sampling parameters will appear. You can choose the peak window you require from the graph.

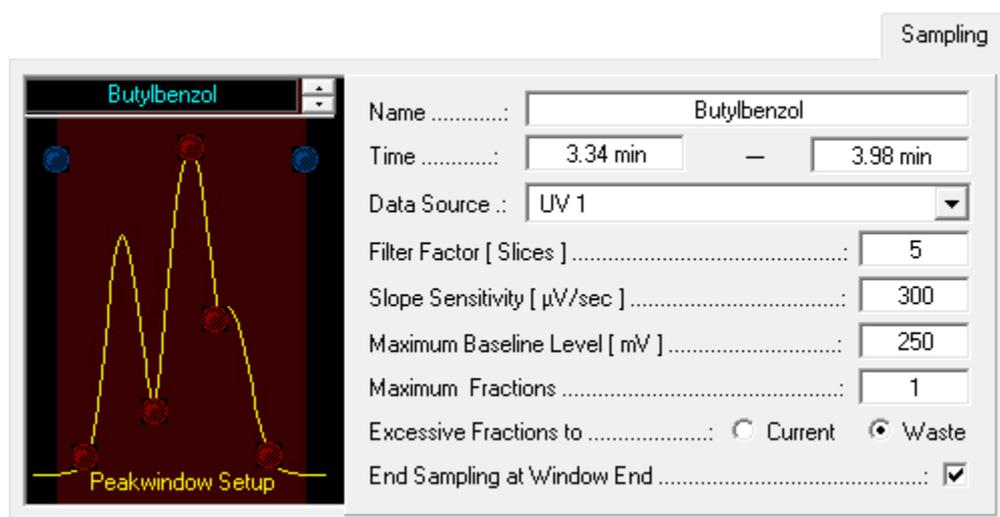


Fig. 30 Programming peak sampling

NAME: The name of the peak window can be entered in the *NAME* box.

TIME: The *TIME* boxes are for the time point where the window starts and ends.

DATA SOURCE: In *DATA SOURCE* you can select the data sources for these time windows.

FILTER FACTOR: The *FILTER FACTOR* parameter indicates the number of data points used for the first derivation when calculating the gradient.

SLOPE SENSITIVITY: The *SLOPE SENSITIVITY* indicates the size of the gradient above which the start of a peak is recognised.

MAXIMUM BASELINE LEVEL is used as a threshold value for peak sampling. No peak end is recognised above the value entered.

MAXIMUM FRACTIONS: The *MAXIMUM FRACTIONS* parameter is only relevant to the *DEFINED BY PEAK WINDOW* setting, which is a possible position setting for motorised switching valves. It indicates the maximum number of fractions that can arise in this peak window.

EXCESSIVE FRACTIONS: The *EXCESSIVE FRACTIONS TO* option determines where the fractions that exceed the maximum number of fractions should be pumped. *CURRENT* pumps them in the current position, and *WASTE* pumps them into the waste.

END SAMPLING AT WINDOW END: The *END SAMPLING AT WINDOW END* option indicates whether fractionating should continue after the end of the time period of a peak window, or whether the system should switch to waste.

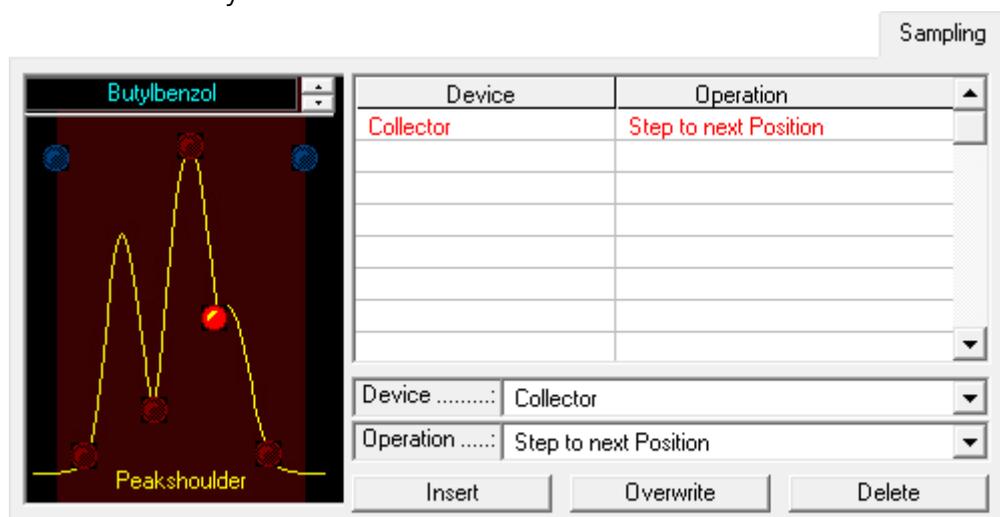


Fig. 31 Programming peak sampling

To program peak sampling functions, click on the LED at the desired location. A function table will then appear on the right-hand side, which you can fill in with functions. The selection boxes *DEVICE* and *OPERATION* allow you to select the required function and add it to the time control file with the *Insert* button. The *OVERWRITE* button allows you to overwrite a selected line, and the *DELETE* button deletes it.

The possible peak sampling situations are:

- *PEAK WINDOW START* (Blue LED on the left) is the time when a peak window begins.
- *PEAK WINDOW END* (Blue LED on the right) is the time when a peak window ends.
- *PEAK START* is the start of a peak as recognised by the peak sampling.
- *PEAK VALLEY* is a dip in a peak recognised by the peak sampling.
- *PEAK SHOULDER* is the shoulder of a peak recognised by the peak sampling.
- *PEAK END* is the end of a peak as recognised by the peak sampling. The following rules apply to the sensitivity of peak sampling:
 - The smaller the data point density (slice width), the faster and more sensitive the peak sampling. (However, bear in mind that very small slice widths lead to large results files)
 - The smaller the *FILTER FACTOR*, the faster and more sensitive the peak sampling. (Bear in mind that the factor range is from 5 to 15, and the value entered must be an odd number)
 - The smaller the *SLOPE SENSITIVITY* value, the more sensitive the peak sampling
- A peak start will be recognized if the measured value exceeds the set value for the *SLOPE SENSITIVITY*. The recognition of the peak start is independent from the *MAXIMUM BASELINE LEVEL*.
- A peak end will only be recognized if the measured value will fall below the set value for the *SLOPE SENSITIVITY* and the *MAXIMUM BASELINE LEVEL*.

Graphic time control file editor

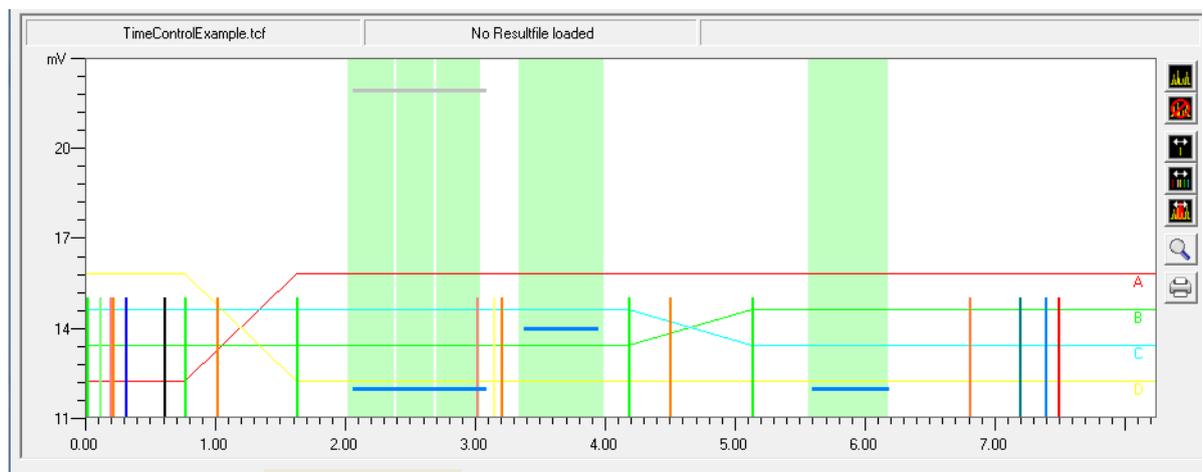


Fig. 32 The graphic time control file editor

The graphic time control file editor is used to create a graphic presentation of a time control file and allow visual editing. All the functions of the time control file are shown as coloured lines which can be moved horizontally (movement in time) and vertically (changing the threshold value or peak limit) with the mouse. A highlighted function in the table representation of the time control file will flash on the graph. Clicking on a function on the graph will likewise highlight the corresponding line of the table. Peak windows are also represented graphically and can be created and edited with the mouse.

A programmed gradient development is also represented by coloured lines, whereby 0% is the y-axis minimum and 100% is the y-axis maximum.

You can load a chromatogram as a background image, which you can then use to adjust the functions of your time control file. The display colours of all the functions can be selected in the graphic time control file editor setup, and any functions you do not want to be shown can be deactivated.



Fig. 33 The graphic time control file editor, status bar

The status bar in the graphic time control file editor shows the name of the time control file loaded, the name of the chromatogram loaded and an information box indicating the following:

The time control file function over which your mouse cursor is positioned, or which you are moving by means of the *SINGLE FUNCTION MOVING* function.

The time by which you are shifting the functions when using the *MULTI FUNCTION MOVING* function.

The name and times of the peak window over which your mouse cursor is positioned when using the *CREATE / EDIT PEAK WINDOW* function.

The graphic time editor tool bar is on the right-hand side of the window. Its functions are listed here in the same order they appear on the screen:



LOAD CHROMATOGRAM opens a chromatogram being used as a background image.



CLEAR CHROMATOGRAM removes the loaded chromatogram from the graph.



SINGLE FUNCTION MOVING allows single functions to be moved.



MULTI FUNCTION MOVING allows multiple functions to be used. To do this, begin by drawing a time window with the left mouse button held down. All the functions within this window can then be moved with the mouse at the same time. Holding down the right mouse button will adopt the time shift.



CREATE / EDIT PEAK WINDOW allows peak windows to be created and edited. To create a peak window, simply hold down the left mouse button and draw a new window on screen. When you release the mouse button, you will be asked for the name of the new window. You can edit an existing peak window by moving the mouse cursor over the window. The cursor will change, depending on its position in the window, from an arrow pointing left (on the left-hand side of the window) to an arrow pointing right (on the right-hand side of the window) or an arrow pointing both ways (in the centre of the window). By holding down the left mouse button, you can now extend or move the window to the left and right. If you right-click over a peak window, menu options to rename or delete the window will appear.



ZOOM GRAPHIC is used to enlarge part of a graph. To do this, drag out a window with the left mouse button held down to include the area you wish to enlarge. In order to return to the original graph size, right-click with the mouse.

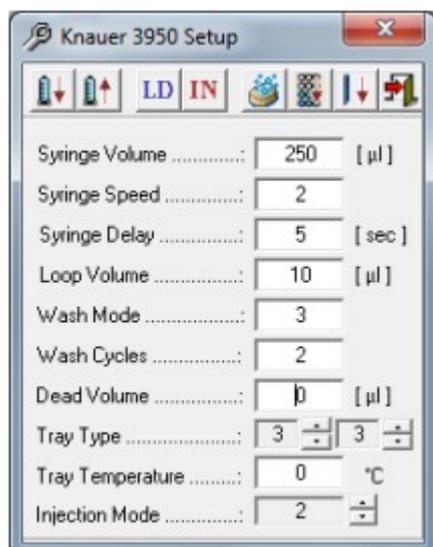


PRINT GRAPHIC prints the graph shown.

Creating autosampler control files

General

In the menu option *FILE* in the main window of PurityChrom® MCC you will find the Autosampler Setup:



Syringe Volume	The volume of the autosampler syringe.
Syringe Speed	1: slow, 2: middle, 3: fast
Syringe Delay	Leave the syringe delay at 5 sec.
Loop Volume	The volume of your sample loop.
Wash Mode	0: washing cycle is turned off 2: between vials 3: between injection
Wash Cycles	How many wash cycles (1 - 3 times).
Dead Volume	Not important for KNAUER 3950.
Tray Type	0: Wellplate 96 low 1: Wellplate 96 high, 2: Wellplate 384 low 3: 48 vials 4: 12 vials
Tray Temperature	0°C: off Sample tray cooling for KNAUER 3950 with cooling option: 4 - 40 °C
Injection Mode	0: None 1: Partial Loop Fill 2: Flushed Loop 3: Microliter Pickup

Partial Loop Fill: maximum injection volume is 50 % of the sample loop volume
Microliter Pickup: minimum sample loop volume is 50 µl
maximum injection volume is:
(sample loop volume - 3 x tubing volume)/2

The function key and the menu option *CREATE / EDIT AUTOSAMPLER CONTROL FILE*  in the main window opens the autosampler control file editor. The full version of PurityChrom is needed to use an autosampler.

An autosampler control file works on the principle of a series of time control files, each of which is allocated an automatic sample injection. The command for the sample injection is programmed into the corresponding time control file with the command *INJECT*.

During injection, the time progression is paused (*TIME CONTROL HOLD*). The parameters for the injections are programmed into the autosampler control file. These parameters include the vial numbers, the number of injections per vial, the injection volume and the column oven temperature. However certain values for the time control file can also be pre-set. To do this, the parameter *AS* must be entered in the time control file. Then the parameter *AS* will act as a variable and obtain its values from the current line of the autosampler control file.

Possible commands include:

- *SOLVENT*(eluent composition for the pump)
- *FLOWRATE*(flow rate for the pumps)
- *THRESHOLD*(level of a programmed threshold value)
- *WAVELENGTH*(UV detector wavelength)
- *LIMITER VALUE*(maximum size of the fractions obtained)

The running time programmed into the autosampler control table determines the time after which the current or a new time control file is started for the next injection. In other words, the time progression of an autosampler control file determines which time control file is started and how long it runs for. Restarting a time control file will only occur for as long as further injections take place. After the last injection, the corresponding time control file will therefore continue running until the system stops. This function can be useful if you program a *STOP ALL* in the time control file after the programmed running time of the autosampler control file. For as long as there are further injections waiting, this command will not be carried out, because the time control file will always restart first. It is only after the last injection that the time control file will reach this line of the program, and the system will stop automatically.

Autosampler injections can be programmed either singly or as a sequence. The sample identifiers of the individual vials can be entered in a sample table. For each injection, the identifier corresponding to the sample number will be read from this table.

Creating an autosampler control file

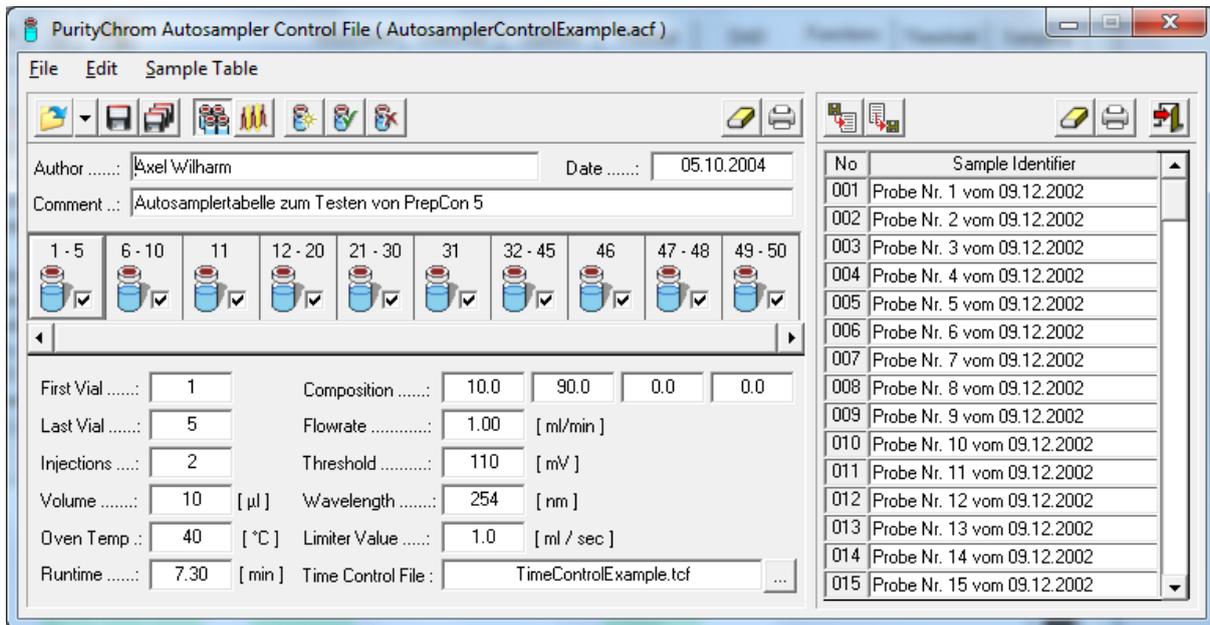


Fig. 34 Autosampler control file

The editor toolbar is in the upper part of the window. The functions are listed in the order they appear on screen:

-  *OPEN AUTOSAMPLER CONTROL FILE* loads an existing autosampler control file into the editor. The possible file types to be loaded are *AUTOSAMPLER CONTROL FILE*(* .acf) or *RESULT FILE*(* .rpf). *RESULT FILE* allows you to load an autosampler control file from a result file.
-  *SAVE AUTOSAMPLER CONTROL FILE* saves the autosampler control file under its existing name.
-  *SAVE AUTOSAMPLER CONTROL FILE AS ...* saves the autosampler control file under a new name.
-  *INJECTION PARAMETER* opens the panel with the input fields of the injection Parameter.
-  *STACKED INJECTION PARAMETER* opens the panel with the input fields of the stacked injection Parameter.
-  *STACKED INJECTION ENABLE /DISABLE* activates or deactivates the stacked injection-function of the autosampler control file.
-  *INSERT AUTOSAMPLER SEQUENCE* adds the autosampler sequence in the editor box to the autosampler control file.
-  *OVERWRITE AUTOSAMPLER SEQUENCE* overwrites the highlighted autosampler sequence with the one in the editor box.
-  *DELETE AUTOSAMPLER SEQUENCE* deletes the highlighted autosampler sequence.
-  *CLEAR ALL* deletes the contents of the autosampler control file. In order to prevent accidental deletion, this function is only carried out after you have confirmed it.
-  *PRINT AUTOSAMPLER CONTROL FILE* opens a printer dialogue box to print out the autosampler control file.
-  *EXIT* closes the editor.

You can enter your name and a comment on the autosampler control file in the Author and *COMMENT*. The date is generated automatically when you save the file.

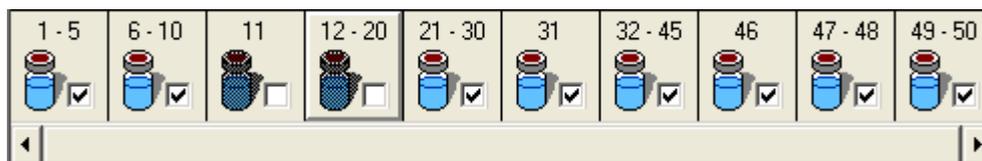


Fig. 35 Autosampler control file, sequence bar

The sequence bar shows all the autosampler sequences. The highlighted line is shown as raised. If there are more than 10 sequences, you can scroll along the sequence bar using the scroll bar underneath. Each sequence is shown with the vial numbers programmed. You can tick the sequence icons to activate or deactivate them for processing. When you resume an interrupted run, you can use this function to deactivate the sequences that have already been processed.

Beneath the sequence bar is the editor box with entry boxes for a sample sequence. The parameters to be entered are the following:

FIRST VIAL is the number of the first vial in a sample sequence.

LAST VIAL is the number of the last vial in a sample sequence. If you are only injecting from one vial, enter the same number in *FIRST VIAL* and *LAST VIAL*.

INJECTIONS indicates the number of injections per vial.

VOLUME indicates the injection volume in μl .

OVEN TEMP indicates the temperature of the column oven.

RUNTIME indicates the running time after which the current or a new time control file will begin with the next injection. The running time should always be shorter than the function *STOP ALL* in the corresponding time control file that still has to be carried out. For multiple injections use the function *STOP CHROMATOGRAM* to restart your data recording. You need to program a minimum time difference of 0,2 min between the function *STOP CHROMATOGRAM* in your time control and the *RUNTIME* of your autosampler. Furthermore a minimum time gap of 0,2 min has to be programmed between the *RUNTIME* of your autosampler and the function *STOP ALL* in your time control file.

SOLVENT indicates an eluent composition entered in the solvent lines of a time control file with the eluent composition *AS* as a parameter.

FLOW RATE indicates a flow rate entered in the flow rate lines of the time control file with the flow rate parameter *AS*.

THRESHOLD indicates a threshold level entered in the threshold lines of the time control file with the threshold value *AS* as a parameter.

WAVELENGTH indicates a wavelength entered in the wavelength lines of the time control file with the wavelength parameter *AS*.

LIMITER VALUE indicates a maximum volume entered in the limiter lines of the time control file with the maximum volume parameter *AS*.

TIME CONTROL FILE indicates the time control file which is started for every injection in this sequence.

Stacked injection

Stacked injection is not supported with KNAUER 3950.

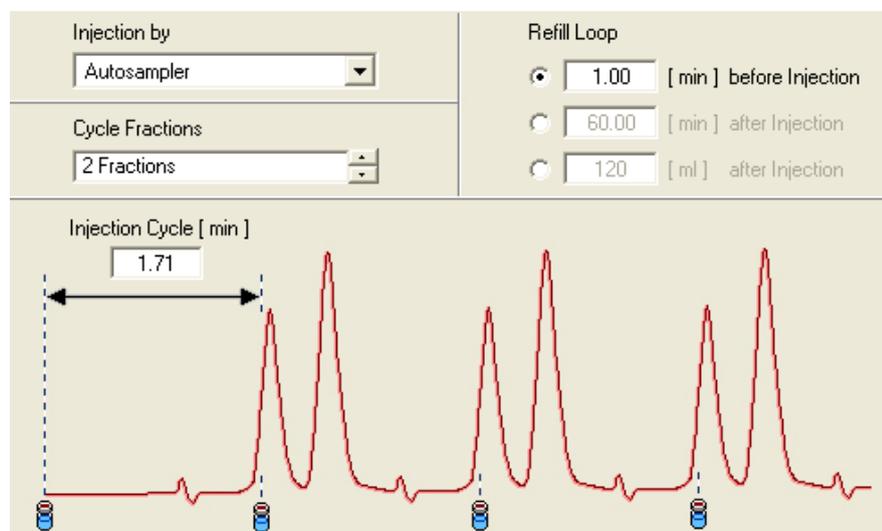


Fig. 36 Stacked injection

The function *STACKED INJECTION* allows for multiple nested injections of identical probes in order to save solvents and time. During the elution time of one sample the following sample is already charged onto the column. If the function *STACKED INJECTION* is activated, all entries of the sample table will be executed within one run. In the select list under the item *INJECTION BY...* one can choose whether samples are automatically injected by an autosampler or by a valve. In case of injection by valve, the main pump aspirates sample and switches the injection valve into position 2 until the required amount of sample is pumped into the system.

The Parameter *FRACTIONS PER CYCLE* defines the number of fractions collected within one cycle. After this number is reached the cycle is repeated and the following fraction is again collected into the start position.

In case of injections using an autosampler the parameter *SAMPLE LOOP FILL* defines at what time the sample loop is refilled. Possible options are *TIME BEFORE INJECTION*, *TIME AFTER INJECTION* and *VOLUME AFTER INJECTION*.

The time table for a run using stacked injections corresponds to that of a single injection but will be automatically extended for each additional injection (see example tables below). The parameter *INJECTION CYCLE* defines the time between two consecutive injections. The injection times are indicated by sample vials in the chromatogram as can be seen in the upper figure.

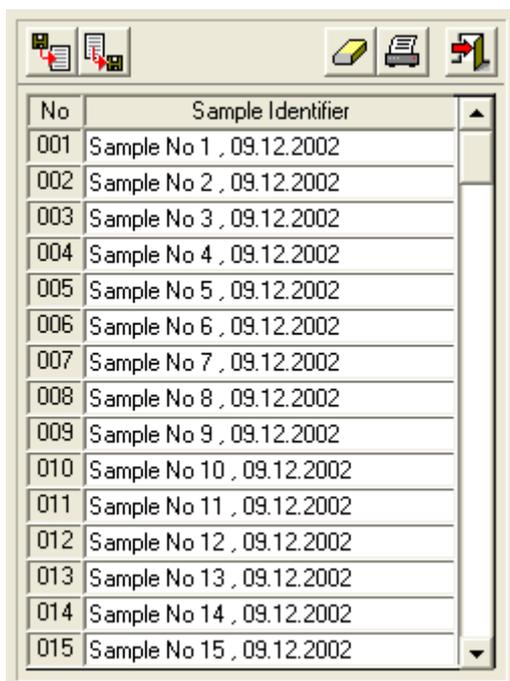
Zeit [Min]	Funktion	Parameter
0.00	Composition Major Pump	100.0 , 0.0 , 0.0 , 0.0
0.00	Flowrate Major Pump	100.00 ml/min
0.15	Autosampler Inject	
0.20	Start Chromatogram	Channel 1 (200 ms)
1.00	Threshold	4.90 min / 25.00 mV
6.00	Stop Chromatogram	All started Channels
6.10	Stop all	

Fig. 37 Initial time table

Zeit [Min]	Funktion	Parameter
0.00	Composition Major Pump	100.0 , 0.0 , 0.0 , 0.0
0.00	Flowrate Major Pump	100.00 ml/min
0.15	Autosampler Inject	
0.20	Start Chromatogram	Channel 1 (200 ms)
1.00	Threshold	20.29 min / 25.00 mV
1.86	Autosampler Inject	
3.57	Autosampler Inject	
5.28	Autosampler Inject	
6.99	Autosampler Inject	
8.70	Autosampler Inject	
10.41	Autosampler Inject	
12.12	Autosampler Inject	
13.83	Autosampler Inject	
15.54	Autosampler Inject	
21.39	Stop Chromatogram	All started Channels
21.49	Stop all	

Fig. 38 Extended time table after additional Injections

Creating a sample identifier table



No	Sample Identifier
001	Sample No 1 , 09.12.2002
002	Sample No 2 , 09.12.2002
003	Sample No 3 , 09.12.2002
004	Sample No 4 , 09.12.2002
005	Sample No 5 , 09.12.2002
006	Sample No 6 , 09.12.2002
007	Sample No 7 , 09.12.2002
008	Sample No 8 , 09.12.2002
009	Sample No 9 , 09.12.2002
010	Sample No 10 , 09.12.2002
011	Sample No 11 , 09.12.2002
012	Sample No 12 , 09.12.2002
013	Sample No 13 , 09.12.2002
014	Sample No 14 , 09.12.2002
015	Sample No 15 , 09.12.2002

Fig. 39 Sample identifier table

The sample identifier table is located on the right hand side of the editor. The sample numbers are displayed in the first column of the table, and the sample identifiers in the second column. The sample identifiers can be written directly into the table.

Before each injection, the identifier corresponding to the sample number is read from this table and used as the *SAMPLE IDENTIFIER*. The number of rows in the table can be set in the file *PurityChrom.ini* with the command *[MAXIMUMSAMPLES]*. 100 sample identifiers are possible as standard.

Above the table is the sample identifier table toolbar. Its buttons have the following functions:



IMPORT SAMPLE TABLE imports a sample identifier table from a text file.



EXPORT SAMPLE TABLE exports the sample identifier table as a text file.



CLEAR SAMPLE TABLE deletes the content of the sample identifier table. In order to avoid accidental deletion, this function will only be carried out after confirmation from the user.



PRINT SAMPLE TABLE opens a printer dialogue box to print out the sample identifier table.

Control file visualisation

AutosamplerControlExample-en.acf				
Vial	Inj	Volume	Time	Sample
1	1	10	7.30	Sample No. 1 from 09.12.2002
1	2	10	7.30	Sample No. 1 from 09.12.2002
2	1	10	7.30	Sample No. 2 from 09.12.2002
2	2	10	7.30	Sample No. 2 from 09.12.2002
3	1	10	7.30	Sample No. 3 from 09.12.2002
3	2	10	7.30	Sample No. 3 from 09.12.2002
4	1	10	7.30	Sample No. 4 from 09.12.2002
4	2	10	7.30	Sample No. 4 from 09.12.2002
5	1	10	7.30	Sample No. 5 from 09.12.2002
5	2	10	7.30	Sample No. 5 from 09.12.2002
6	1	20	7.30	Sample No. 6 from 09.12.2002
7	1	20	7.30	Sample No. 7 from 09.12.2002

TimeControlExample.tcf		
Time [min]	Function	Parameter
0.00	Composition Major Pump	10.0 , 20.0 , 30.0 , 40.0
0.00	Flowrate Major Pump	5.50 ml/min Constant Flow
0.00	Fraction Limiter	Limit = 18.00 ml
0.00	Temperature	Column Oven = 25 °C
0.00	Temperature	Sample Heater = 25 °C
0.10	Autosampler Inject	Vial 1 , Injection 1
0.18	Event Box Output	Output 12 = PULSE
0.20	Start Chromatogram	Channel 1 (200 ms)
0.30	Auxiliary Output	Auxiliary = ON
0.60	Annotation	"My individual Annotation"
0.75	Composition Major Pump	10.0 , 20.0 , 30.0 , 40.0
1.00	Start Chromatogram	Channel 2 (200 ms)

Fig. 40 control file visualisation

The function key and the menu option *CONTROL FILE VISUALISATION*  in the main window open the window where you can visualise the time and autosampler control files. When you are running a time control file, it will be displayed in the form of a table. All the lines that have already been worked through will be displayed in red, and those still to come will be in blue. The blue line above the table shows the name of the time control file loaded. When you are running an autosampler control file, another table shows all the injections in the sequences programmed. Here also, the injections that have already been made are in red and those still to come are in blue. The columns of the table show you the number of each vial, the injection number, the sample volume injected, the running time of the time control file and the sample identifier. The yellow field above this table shows the name of the autosampler control file. The tables automatically scroll down in such a way that the current line is always visible.

Data recording (Analysis)

General

The function key and the menu option *CHROMATOGRAM*  in the main window opens the chromatogram window. The first area is data recording (Analysis).

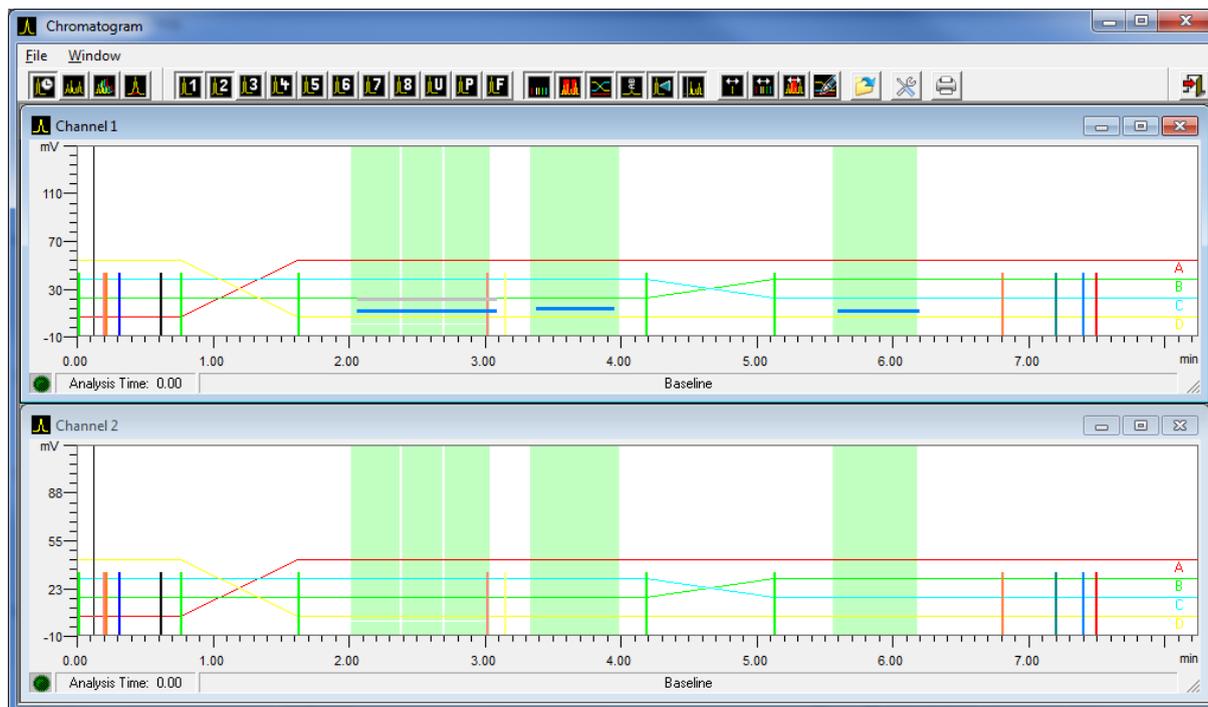


Fig. 41 Data recording, chromatogram

The data-recording window is used to display and record analogue signals. The requirement for this is an analogue/digital converter card in your PC. Up to 8 analogue signals can be recorded at once. The automatic peak sampling can only work with these analogue signals.

Data recording is started in the time control file with the command *START CHROMATOGRAM* and stopped with *STOP CHROMATOGRAM*. Chromatograms can be started simultaneously or with a delay.

While a time control file is running, the graphic display of the time control file, the current chromatogram trace, the programmed peak windows, the gradient and the current chromatogram labels are displayed in the data recording windows. Just like in the graphic time control file editor, you have the possibility to edit time control file functions and peak windows in the data recording windows. These changes take effect on the current run and can also be saved in the time control file loaded. This means you have the possibility of intervening in a process *ONLINE*. The buttons 100% Solvent A-D and flowrate in the main window can be enabled during a time control run by changing the PurityChrom.ini-file [Pumps → Parameter Change=1] (see appendix). This enables you to change the flowrate and gradient composition during the run. All changes except for changed fraction collector or fraction valve positions which were performed during a run are recorded and can be viewed in *VIEW PROTOCOL* by opening the chromatogram in the reconstruction window (see chapter Function Buttons in the Reconstruction Window page 56).

Function buttons



CHANNEL... is used to open or close a data channel window. Only the buttons for the channels that can be selected are shown. The *U* button belongs to a user-defined channel (see chapter User Defined Channel page 18). Data channels which are programmed into a time control file are automatically opened upon loading. If no data recording is active, the data track selected runs in base line mode, i.e. from the left to the right edge in about 8 minutes as an endless loop. Data recording is started with the time control file command *START CHROMATOGRAM* and stopped with the command *STOP CHROMATOGRAM*.



PRESSURE CHANNEL shows the pressure data of the pump. This channel can be used like the data channels described above.



FLOW RATE CHANNEL shows the actual value of the flow rate performed by the major pump system. It can be used like the data channels described above. It is especially useful when using the option *PRESSURE LIMIT* in a time control file programming. Then it will show when and in which intensity the automatic flow rate control was active.



SPECTRUM CHANNEL is used to show or hide the spectrum window of the DAD.



SHOW FUNCTIONS is used to show or hide the time control file functions. When the functions are hidden, the contents of the window are scaled to fit the area of the data recording.



SHOW PEAK WINDOWS is used to show or hide the peak windows.



SHOW GRADIENT is used to show or hide the programmed



gradients. *SHOW ANNOTATIONS* is used to show or hide the current annotations.



SHOW LAST CHROMATOGRAM automatically loads the last chromatogram as a background image in the data channel window.



SHOW ELAPSED TIME LINE is used to show or hide the vertical line indicating the elapsed time.



SINGLE FUNCTION MOVING enables you to move single functions (see chapter The Graphic Time Control File Editor page 42). Changes take immediate effect in the current run, and after deselecting these functions you will be asked if they should be saved in the time control file as well.



MULTI FUNCTION MOVING enables you to move multiple functions (see chapter The Graphic Time Control File Editor page 42). Changes take immediate effect in the current run, and after deselecting these functions you will be asked if they should be saved in the time control file as well.



CREATE / EDIT PEAK WINDOW allows you to create and edit peak windows (see chapter The Graphic Time Control File Editor page 42). Changes take immediate effect in the current run, and after deselecting these functions you will be asked if they should be saved in the time control file as well.



EDIT GRADIENT opens a window where you can edit the running Gradient. Changes take immediate effect in the current run, and after closing the window you will be asked if they should be saved in the time control file as well.

-  *LOAD LAST CHROMATOGRAM* is used to load the last chromatogram manually, as a background image for the data channel window.
-  *CHANNEL SETUP* opens the window with the settings for the individual data channels. The different parameters will be described in chapter Data Channel Settings page 53.
-  *AUTOMATIC PRINT* activates or inactivates the automatic printing of the report after the recent run.

Status bar

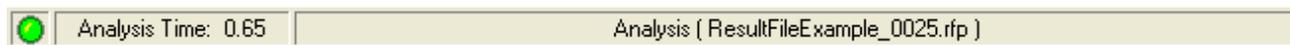


Fig. 42 Data recording, status bar

The data recording status is displayed with a graphic in the left-hand box of the status bar. The meaning of the symbols is as follows:

-  *BASELINE* (Baseline is displayed)
-  *READY* (data channel is in use, but has not yet started)
-  *CHROMATOGRAM RUNNING* (data recording is active)
-  *CHROMATOGRAM FINISHED* (data recording has finished)

The central box shows the chromatogram running time, and the right-hand box shows the current status, with the name of the results file.

Data channel settings

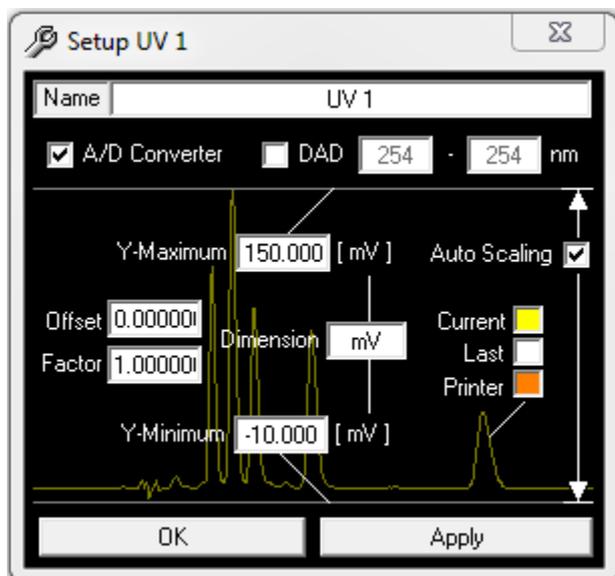


Fig. 43 Fig. 44 Data channel settings

The window always shows the data of the selected channel, i.e. the analysis window in focus. *NAME*: You can change the name of the channel in the *NAME* window.

A/D CONVERTER: You can use the options *A/D CONVERTER* or *DAD* to determine the data source of the channel. Select *A/D CONVERTER* for the data of the A/D conversion cards and *DAD* for the data of the DAD. You can set the DAD wavelength range in the adjacent text boxes.

OFFSET: The *OFFSET* parameter is used to compensate for a detector offset. The value entered will be added to the analogue signal, and can be positive or negative.

FACTOR: The *FACTOR* parameter is used to correct the size of the analogue signal indicated. A factor of 1 shows the analogue signal at its original size.

Y-MAXIMUM and *Y-MINIMUM*: The *Y-MAXIMUM* and *Y-MINIMUM* values set the scale of the y-axis.

AUTO SCALING: The *AUTO SCALING* option automatically corrects the scaling when these values exceed or drop below the corresponding maximum or minimum signal.

DIMENSION: You can set the measurement unit of each channel in the *DIMENSION* box.

CURRENT, *LAST* or *PRINTER*: Clicking on the *CURRENT*, *LAST* or *PRINTER* box opens a colour selection dialogue, which you can use to set the colour of each chromatogram trace.

CURRENT is the colour of the current chromatogram and *LAST* is the colour of the last chromatogram, which is displayed if you use the *SHOW LAST CHROMATOGRAM* function and *PRINTER* is the colour of the printed chromatogram.

The *APPLY* button applies the settings, and the *OK* button closes the window at the same time.

Choose the type of the displayed spectrum in the setup window for the DAD spectrum under *SPECTRUM TYPE*.

DARK CURRENT is the remaining current without exposing the diode array.

LAMP SPECTRUM: The *LAMP SPECTRUM* is the measured spectrum of the diode array minus the dark current.

REFERENCE SPECTRUM: The *REFERENCE SPECTRUM* is a saved lamp spectrum. A *REFERENCE SPECTRUM*

can be set manually by the *SET REFERENCE SPECTRUM* function key. Otherwise it is set automatically when PurityChrom is started.

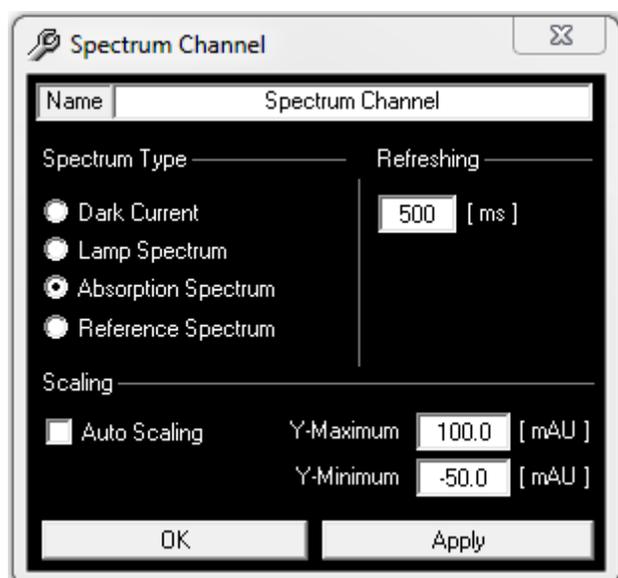


Fig. 45 Data channels settings, Spectrum Channel

ABSORPTION SPECTRUM: The *ABSORPTION SPECTRUM* of the DAD is the logarithm of the reference spectrum minus the logarithm of the actually measured lamp spectrum.

REFRESHING: The *REFRESHING* parameter defines after which time the spectrum display is actualized.

Y-MAXIMUM and *Y-MINIMUM*: The *Y-MAXIMUM* and *Y-MINIMUM* values define the scaling of the y-axis.

AUTO SCALING: The *AUTO SCALING* option automatically fits the scaling according to the signal maximum and minimum.

Displaying chromatograms (Reconstruction)

General

The function key and the menu option *CHROMATOGRAM*  in the main window open the chromatogram window. The second area displays the chromatograms (reconstruction).

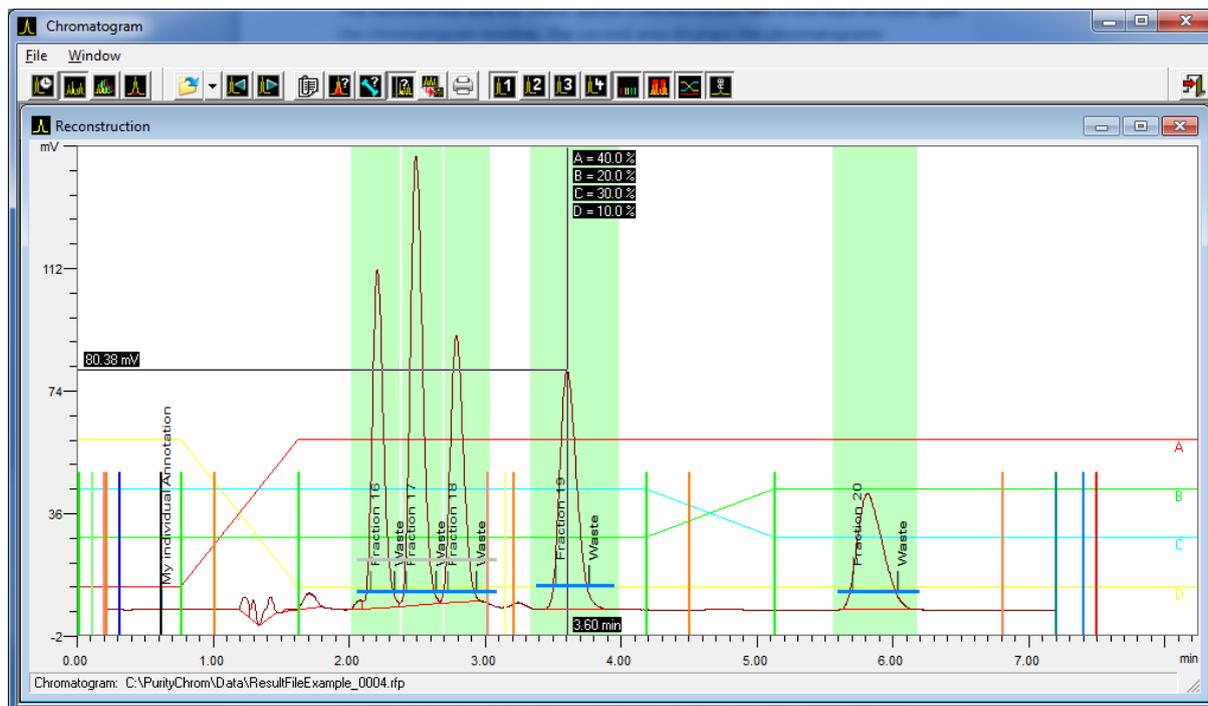


Fig. 46 Displaying chromatograms, general

You can load and display result files in the reconstruction window. Chromatograms, time control file functions, peak windows, gradients, chromatogram labels and the protocol of the run can all be displayed. The chromatograms can be displayed singly or, in the case of multiple channel data recording, superimposed. To enlarge the display of certain areas, you can drag out a zoom window by holding down the left mouse button. When you release the mouse button, the display will automatically zoom in on this area. You can repeat this process up to the maximum zoom level. To return to the original size, click on the chart with the right mouse button.

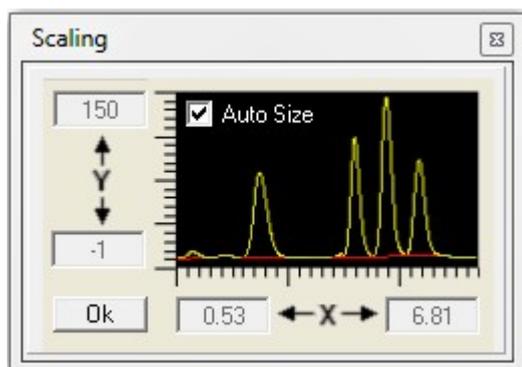


Fig. 47 Displaying chromatograms, scaling

Double-clicking on the chromatogram scaling opens a window, in which you can fix the scaling by values. After this, the scaling will be displayed highlight. The option *AUTO-SIZE* automatically fits the chromatogram in the reconstruction window.

The status bar at the bottom of the window shows the path and name of the result file loaded. If you move the mouse cursor over a time control file function, the time and parameter values of this function will be displayed in the status bar.

Function buttons



OPEN RESULT FILE opens a results file (*.rfp). Temporary results files (*(H).tmp) can also be opened. The temporary results files are created at the end of a method run and usually are deleted after they have been summarized to the results file. On an unexpected end of a run such as power failure or system crash the temporary files are still existing. On opening a temporary results file a normal results file is automatically created. The maximum loss of data will be 100 data points. This corresponds to a loss of 50 seconds of chromatographic time when using a slice width of 500 ms.



PREVIOUS RESULT FILE loads the result file that comes before the loaded file in the order of their names.



NEXT RESULT FILE loads the result file that comes after the loaded file in the order of their names.



VIEW PROTOCOL opens a window that displays the run protocol ordered by time. All changes which were performed during the run are listed here.



VIEW PEAK RESULTS opens the window with the display of the integration results in table form.



VIEW COLUMN COEFFICIENTS opens the window with the display of the column coefficients in table form. To calculate the capacity enter the column dead Time in the field *COLUMN DEAD TIME*. Instead of the asymmetry the tailing factor can be calculated as well. Therefore the PurityChrom.ini-file [IntegrationPresets → TailingFactor=1] (see appendix) has to be modified.



VIEW PEAK VALUE is used to register the peak values. After selecting this function, cross hairs will appear on the graph, which you can move along the chromatogram trace. The time and intensity will be displayed on the cross hair lines. In a multiple channel result file, only one chromatogram can be selected to register peak values.

At any time in the chromatogram the appropriate spectrum can be shown when the chromatogram is recorded by a DAD detector and a DAD result file (2D data) exists. Click with the right mouse key in the chromatogram and choose *SHOW SPECTRUM*. A new window appears showing the spectrum belonging to the chosen time. This operation can be repeated by clicking again with the right mouse key or by moving the mouse with pressed right key.



EXPORT CHROMATOGRAM is used to export one or more chromatograms. The export formats can be selected in the file selection window as file types. Possible export formats are *COMMA SEPARATED VALUE FILE* for export to Excel, *AIA/ANDI FILE* for export in Andi file format or *SLICEFILE* for export to ChromStar.



PRINT RESULT FILE is used to print the result file loaded. After selecting this function, a window opens in which you activate or deactivate elements of the results data to appear on the printout (see chapter Printout Options page 33).

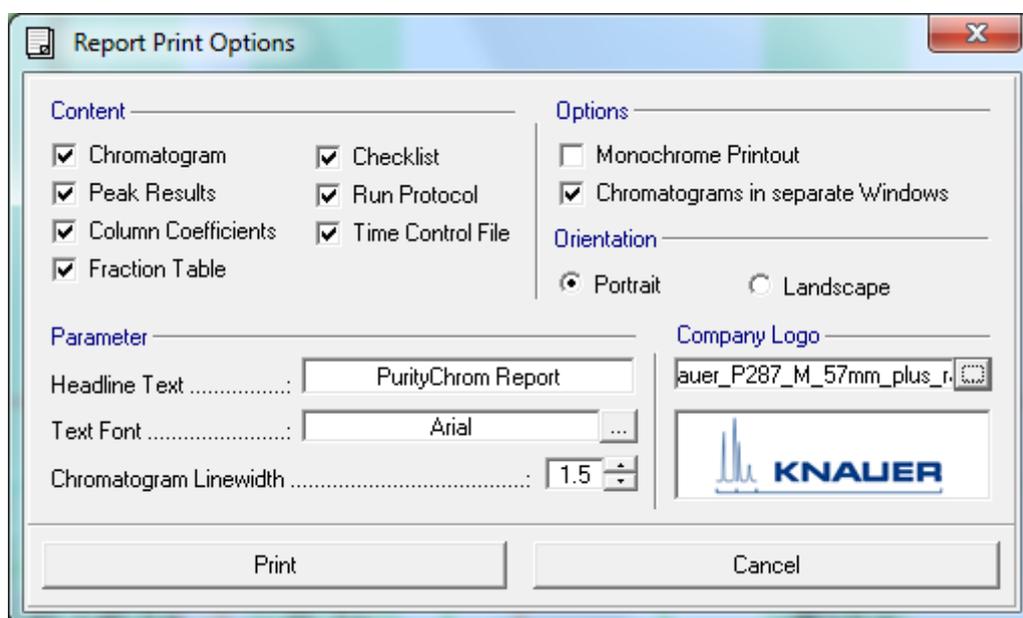


Fig. 48 Displaying chromatograms, Report Print Options



SHOW CHANNEL ... shows or hides each chromatogram. Hidden chromatograms are not displayed on screen, and they do not appear on the printout either.



SHOW FUNCTIONS shows or hides the time control file functions. When the functions are hidden, the window contents are scaled to fit the area of the data recording.



SHOW PEAK WINDOWS shows or hides the peak windows.



SHOW GRADIENT shows or hides the gradient.



SHOW ANNOTATIONS shows or hides the annotations.

Comparing chromatograms (Compare)

General

The chromatogram window is opened in the main window via function key or menu

procedure *CHROMATOGRAM* . The third function key opens the compare chromatograms window (*COMPARE*).

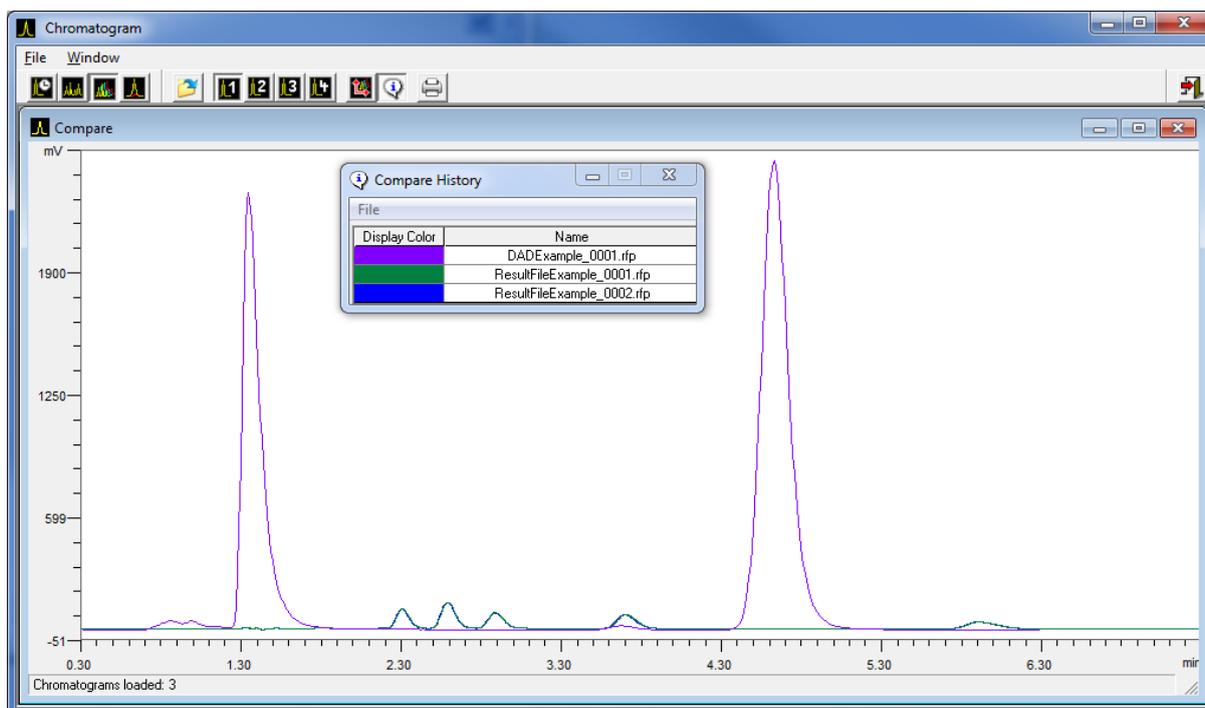


Fig. 49 Comparing chromatograms, general

Several result files (.rfp) can be loaded. Only the chromatogram is displayed.

A zoom window can be opened by pressing the left mouse button and dragging the mouse. On unpressing the mouse button, the zoomed window is shown. This procedure can be repeated until a maximum of magnification is reached. By clicking the right mouse button, the original size is restored.

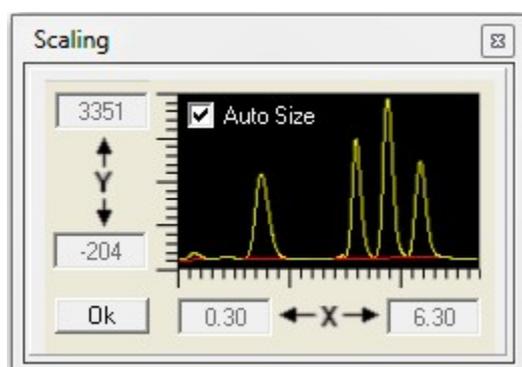


Fig. 50 Comparing chromatograms, scaling

Double-clicking on the chromatogram scaling opens a window, in which you can fix the scaling by values. After this, the scaling will be displayed highlight. The option *AUTO-SIZE* automatically fits the chromatogram in the reconstruction window.

The status bar in the bottom shows the number of loaded result files.

Function keys



OPEN RESULTFILES opens one or more result files. Multiple selection of several result files can either be done by using the *STRG*-key or the *SHIF*-key and the result files simultaneously.



CHANNEL ... chooses the channel the chromatograms of which are to be shown in the Compare window.



CHROMATOGRAM OFFSET is used to offset the loaded Chromatograms. By using the cursor keys the chromatograms will be moved on x axis and y axis. The status bar shows the offset in pixel.

Display Color	Name
Yellow	ResultFileExample_0001.rfp
Green	ResultFileExample_0002.rfp
Cyan	ResultFileExample_0003.rfp
Red	ResultFileExample_0004.rfp
Blue	ResultFileExample_0005.rfp
Magenta	ResultFileExample_0006.rfp
Orange	ResultFileExample_0007.rfp
Light Green	ResultFileExample_0008.rfp
White	ResultFileExample_0009.rfp
Yellow	ResultFileExample_0010.rfp

Fig. 51 Comparing chromatograms, Compare History



COMPARE HISTORY opens a window which shows the list of the result files and the corresponding colors. The column *DISPLAY COLOR* shows the color and the column *NAME* shows the name of the result file.

Using the menu procedure *FILE* and *DISPLAY COLOR* the colors in the display can be chosen. By using *PRINTER COLOR* the colors in the print-out can be chosen. A double click in one of the colors opens a dialog box in which the color can be changed.



PRINT RESULTFILES prints out the graphic representation of the loaded result files and the color references.

Reintegrating chromatograms

General

The function key and the menu option Chromatogram in the main window open the chromatogram window. The fourth area displays the reintegration of chromatograms. You can reintegrate the chromatograms from result files in the integration window.

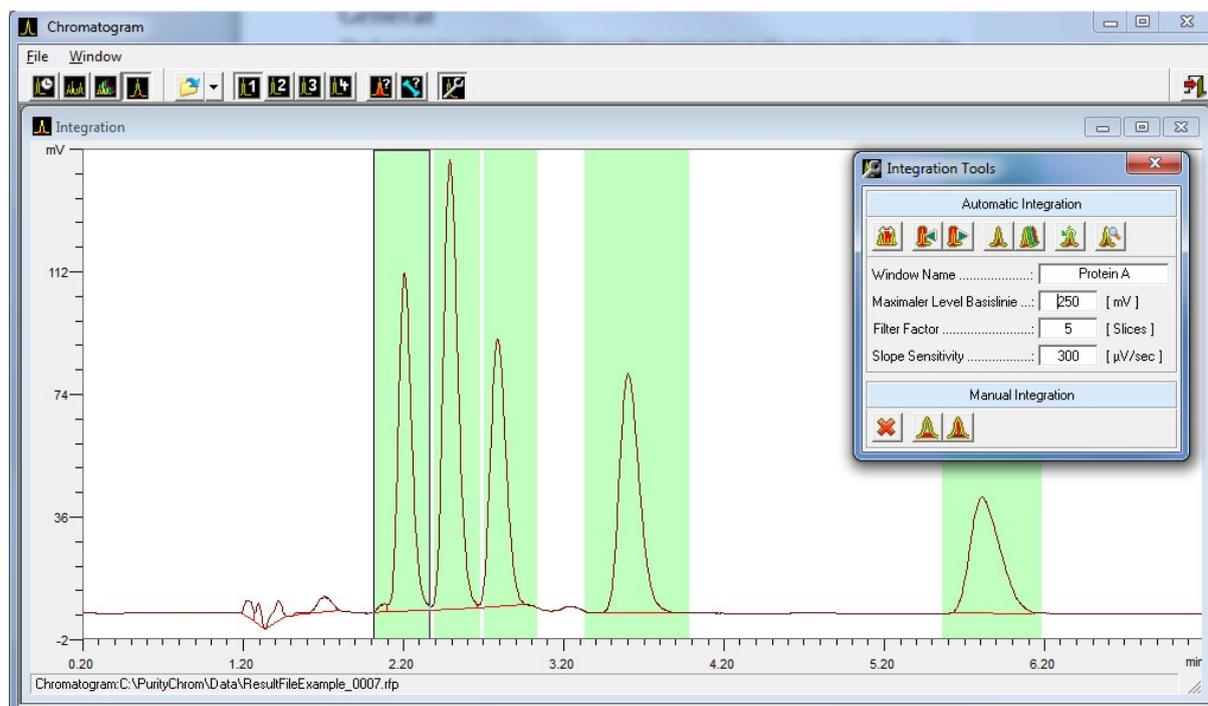


Fig. 52 Reintegrating chromatograms

The chromatograms can be reintegrated singly, or collectively in the case of multiple channel data recording. Once reintegration has been carried out, it is saved automatically. The original integration remains in the result file, so that you always have the possibility of reverting to the original.

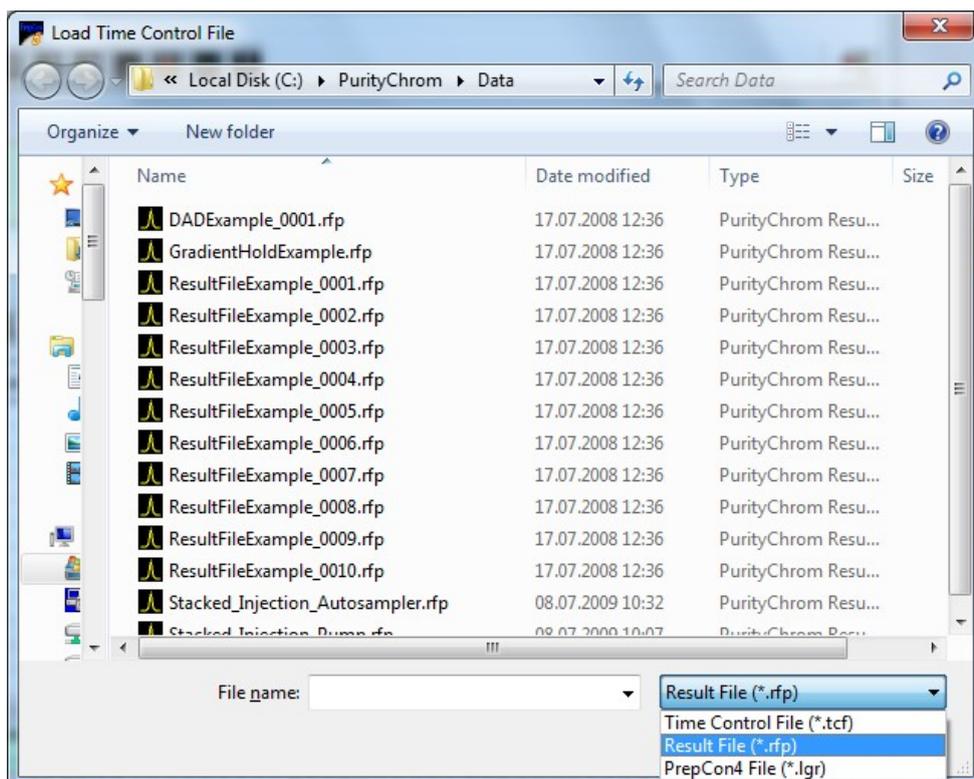


Fig. 53 Loading reintegration file

Reintegration allows you to optimise the integration of your chromatograms later. If you load a reintegration results file (.rpf file, see figure) into the time control file editor (instead of a time control file), the program will recognise the reintegration and ask you if you want to load the reintegration parameters. This time control file will be identical to the run completed, but will contain the integration parameters of the reintegration. You can then save this time control file under a new name, or overwrite the old file.

To enlarge the display of certain areas, you can drag out a zoom window by holding down the left mouse button. When you release the mouse button, the display will automatically zoom in on this area. You can repeat this process up to the maximum zoom level. To return to the original size, click on the chart with the right mouse button.

The status bar at the bottom of the window shows the path and name of the result file loaded.

Function buttons



OPEN RESULT FILE opens a result file.



CHANNEL ... selects the chromatogram shown in the reintegration window.



VIEW PEAK RESULTS opens the window with display of integration results in table form.



INTEGRATION TOOLS opens the window with the function buttons and parameter boxes for reintegration.

The Integration Tools window contains the tools needed for reintegration. The integration parameters are saved with the peak windows. Carrying out a reintegration always concerns the part of the chromatogram within the peak window selected. Outside of the peak windows the default parameters are applied as defined in *PurityChrom.ini* (see chapter The Entries in the File PurityChrom page 75). The buttons in the tools window have the following functions:

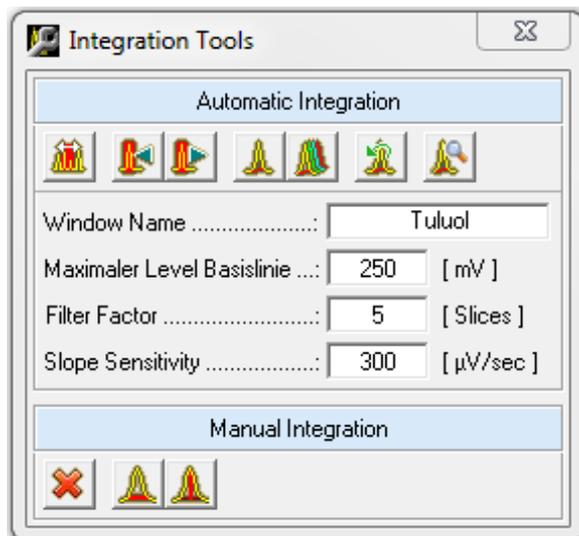


Fig. 54 Integration Tools

-  Create / *EDIT PEAK WINDOW* enables you to create and edit peak windows (see chapter The Graphic Time Control File Editor page 42).
 -   *PREVIOUS PEAKWINDOW* and *NEXT PEAKWINDOW* are used to select the peak window. The peak window selected is framed in the graph in the reintegration window. The name of the window is displayed in the box below the toolbar.
 -  *INTEGRATE SELECTED CHANNEL* reintegrates the selected chromatogram.
 -  *INTEGRATE ALL CHANNELS* reintegrates all the chromatograms of the result file loaded.
 -  *ORIGINAL INTEGRATION* reverts to the original integration.
- The reintegration parameters have the following meanings

MAXIMUM BASELINE LEVEL functions as a threshold for peak sampling. No more peak ends are recognised above the value entered in mV.

FILTER FACTOR: The parameter *FILTER FACTOR* shows the number of slices used to form the first derivative of the gradient calculation.

SLOPE SENSITIVITY: The parameter *SLOPE SENSITIVITY* indicates the size of the gradient where a peak beginning is recognised in $\mu\text{V}/\text{sec}$.

Changing this parameters will affect the integration of your chosen peaks.

-  *PEAKSAMPLING PREVIEW* displays the result of the online peak sampling. The peaks that will be recognised by the online peak sampling correspond to the filled areas under the chromatogram. Different peak situations are indicated by light vertical lines. When moving the mouse over the recognised peak situations the area between two successive peak situations is highlighted. The corresponding start and end time as well as the respective situation names are displayed in the statusbar.

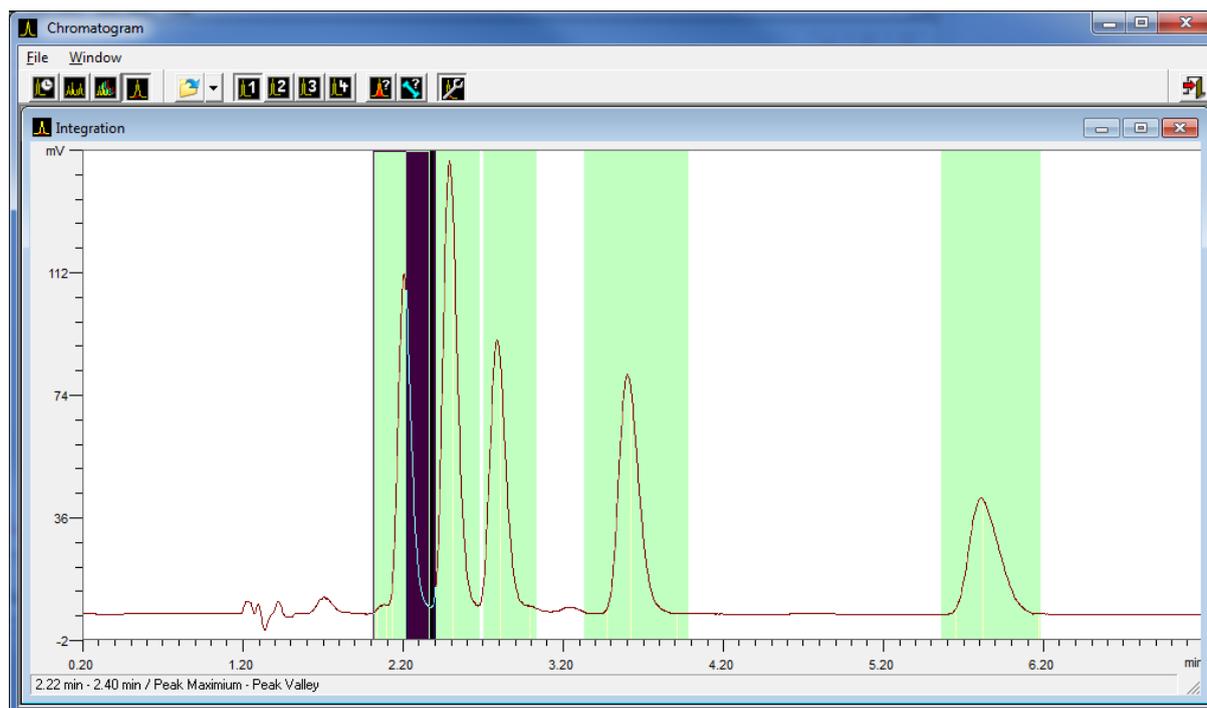


Fig. 55 Reintegrating chromatograms, highlighted peak area



CLEAR INTEGRATION deletes all integration results of the displayed channel.



SET MANUAL BASELINE allows to insert a baseline manually. Starting point and end point can be fixed by left mouse button. Placed points can still be shifted by left mouse button. After unpressing the key the baseline will be used automatically. For each situation you have to select the button *SET MANUAL BASELINE* again.



SET MANUAL DROP allows to insert a drop line manually. The time of the drop can be fixed by left mouse button. The placed drop line can still be shifted by left mouse button. After unpressing the key the drop line will be used automatically. For each situation you have to select the button *SET MANUAL DROP* again.

System visualization

General

The function key or the menu option *SYSTEM VISUALISATION*  in the main window opens the system visualisation window.

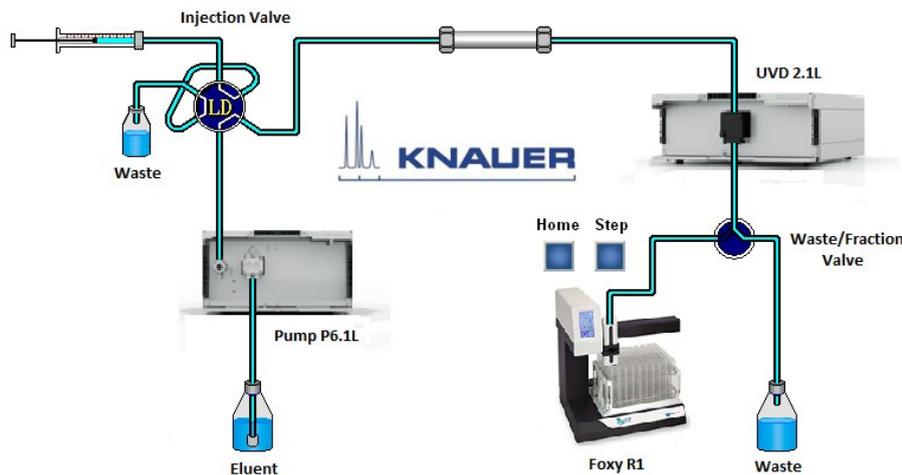


Fig. 56 Visualisation

The system visualisation is used to display the flow process graphically, as well as to operate manually all the devices that can be controlled by means of the visualisation. What is more, the display boxes can show data relating to the current pressure, eluent composition, flow rate, detector signals, autosampler vial numbers, fraction collector position and temperature.

A library of graphic objects and the use of background images in Windows Bitmap Format (*.bmp) allow the user to create an individually configured system visualisation.

Each of the devices that can be visualised displays its current position or state at all times.

The visualisation window can be scaled freely using the menu option *Zoom* or by dragging the borders of the window.

System visualisation functions

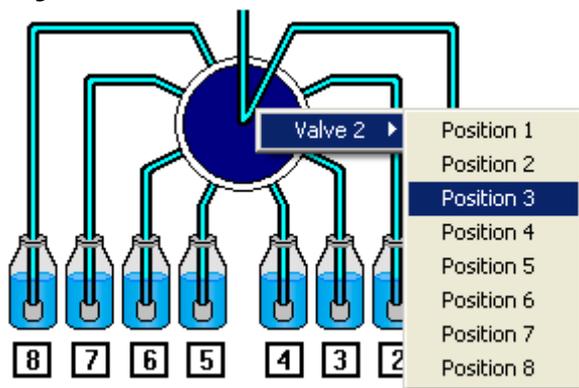


Fig. 57 Visualisation, direct control menu

All the devices that can be visualised can be controlled within the visualisation. To see a menu, right-click the mouse on the device required. The first menu option in the popup menu that appears is the device name, followed by the positions or states to which the device in question can be switched. By left-clicking on the menu options, the device is switched to the corresponding position or state. The *BUTTON* object can be used like a function button by left-clicking with the mouse. Right-clicking on a box (value label) shows the value label's data source.

Creating a system visualisation

The menu option *SETUP* in the visualisation window opens the system visualisation setup window.

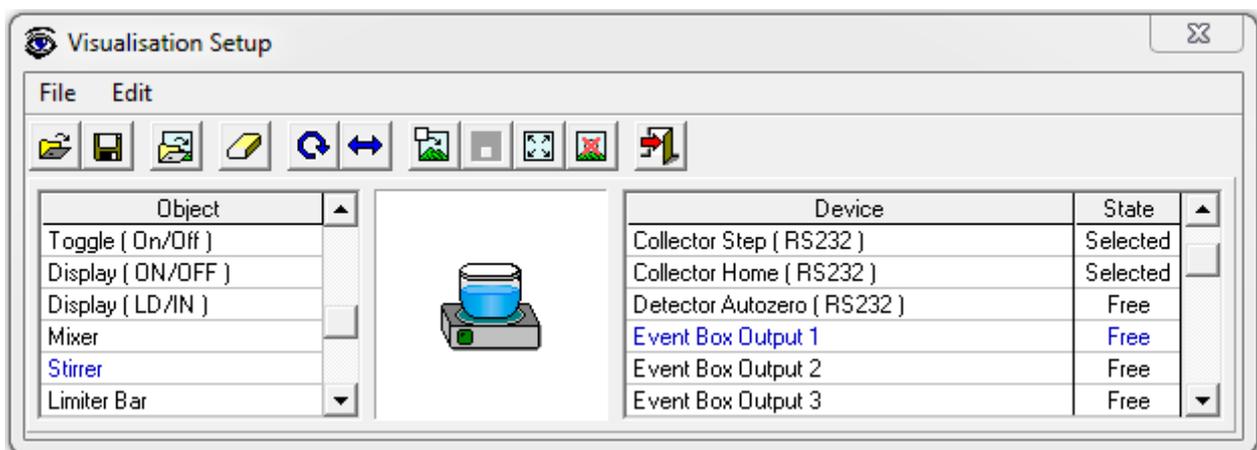


Fig. 58 Visualisation Setup

The function buttons in the toolbar have the following functions:

-  *LOAD VISUALISATION* opens a previously created visualisation. All the visualisations are to be found in the *VISUALISATION FILES* folder. This folder can be found in the program directory in the subfolder *VISUALISATION*.
-  *SAVE VISUALISATION* saves a newly created visualisation.
-  *LOAD BACKGROUND PICTURE* opens a background image in Windows Bitmap Format. All background images are stored in the *VISUALISATION BACKGROUNDS* folder. This folder can be found in the program directory in the subfolder *VISUALISATION*.
-  *CLEAR VISUALISATION* deletes the loaded visualisation and all its objects. To avoid accidental deletion, this function is only carried out after confirmation from the user.
-  *ROTATE OBJECT 90°* rotates the selected object 90° clockwise.
-  *FLIP OBJECT* flips the selected object horizontally.
-  *INSERT OBJECT* adds the selected object to the chosen arrangement in the visualisation window. All objects are inserted in their basic position (e.g. valves in position 1).



PLACE OBJECT places the inserted object at the chosen position in the visualisation.



MOVE OBJECT moves an already placed object.



DELETE OBJECT deletes an already placed object.



EXIT SETUP closes the visualisation setup window.

To create a new visualisation, first draw a background image that corresponds to the devices and tubing in your system. You can use the windows program *PAINT* for this. You will find a number of models for the single elements in the system in the *BITMAP LIBRARY* folder. You must then save the background image in the *VISUALISATION BACKGROUNDS* folder, and load it as a background image using the *LOAD BACKGROUND PICTURE* function.

Now you have to position the appropriate graphic objects for each of the devices to be visualised. The left-hand table in the setup window contains all the graphic objects and the right-hand table contains all the devices in your system that can be visualised. If you choose a value label as an object, all the values that can be visualised will be the only thing shown in the device table.

The graphic objects are displayed in the preview box in the centre of the setup window. Check here that the object is the same way up as the corresponding element in your background image.

Use the *ROTATE OBJECT 90°* and *FLIP OBJECT* functions to adjust the alignment. Once you have chosen an appropriate device for your system in the right-hand table, you can add the object to the visualisation window with the *INSERT OBJECT* function. It will appear in the top right corner, and can be put in the right place with the mouse or the cursor keys. When you have done this, use the *PLACE OBJECT* function to add it to the visualisation.

When all the objects have been added, you must save the visualisation using the *SAVE VISUALISATION* function. All the visualisations must be saved in the *VISUALISATION FILES* folder.

Displaying the solvent supply

General

The function button or the menu option *SOLVENT SUPPLY*  in the main window opens the solvent supply display window.

The solvent supply display is used to control the supply of solvents. To prevent a column from running dry, you have the possibility of setting two volume limits in percent. When the volume drops below the first limit, an alarm will sound, and when it drops below the second limit, the pumps will stop.

The values displayed are based on the calculated used volume of solvent at the current flow rate and time passed. This means that there is no provision for pump tolerance in this calculation. For this reason, the volume limits for the warning and turning off the pumps should allow a certain leeway. Regular control of the supply containers also helps to prevent a column from running dry.

Solvent visualisation

The supply of solvents A to D and the level of the waste container are shown as a graphic on the left-hand side of the window. They are intended to allow the user to make instant checks.

On the right-hand side, the solvent names are displayed, with the current values as volumes and percentages next to them.

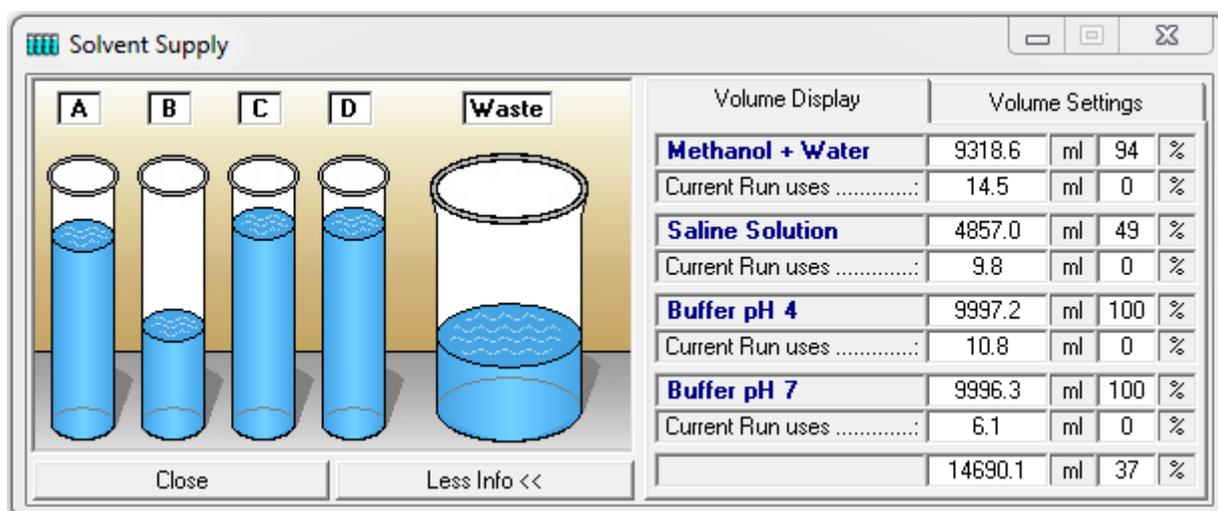


Fig. 59 Solvent supply

The values next to *CURRENT RUN USES* show the solvent consumption of the time control file loaded as a volume and percentage. This display is intended as a check of whether the solvent supplies are sufficient for the current run.

A solvent that has dropped below the minimum percent level set, or a waste volume that has exceeded the maximum percent level, will be marked with a red exclamation mark. You can open the right-hand side of the window with the *MORE INFO* button. You can then use the *LESS INFO* button to close it again. The right-hand side of the window displays the solvent names and the numerical solvent quantities. It also shows the solvent consumption for the current run. The values are shown as absolute volumes and as percentages of the total volume.

Solvent supply display settings

Volume Settings		
Name	Total [ml]	Current [ml]
Methanol + Water	10000.0	9318.6
Saline Solution	10000.0	4857.0
Buffer pH 4	10000.0	9997.2
Buffer pH 7	10000.0	9996.3
Waste	40000.0	14690.1
<input checked="" type="checkbox"/> Acoustic Warning at		10 %
<input checked="" type="checkbox"/> Hold with Pump stop		5 %
<input type="button" value="Save"/> <input type="button" value="Refill all"/> <input type="button" value="[ml]"/>		

Fig. 60 Solvent supply display settings

In the *VOLUME SETTINGS* part, you can enter your own names for the solvents A to D under *NAME*. Enter the total volume of the individual solvent containers in the boxes under *TOTAL* and enter the current solvent volume in the containers under *CURRENT*. The pumps should be stopped when you enter the current solvent volume, since the values in the boxes are constantly updated when the pumps are running, meaning that your entries will be overwritten. Use the *[U]* to switch the unit of volume between [ml] and [l]. The *REFILL ALL* button returns all the current solvent volumes to the total volume of the corresponding containers and the waste container to zero. The *SAVE* button applies and saves the settings. The option *ACOUSTIC WARNING AT* sets a percentage threshold at which the alarm will sound.

You can define the sound in the PurityChrom.ini-file [SolventSupply] (see appendix). The second threshold with the option *STOP ALL* or *HOLD WITH PUMP STOP* is used to stop the pumps, in order to prevent the column from running dry. *STOP ALL* stops the time control file and the pumps, and *HOLD WITH PUMP STOP* pauses the running of the time control file and sets the pumps to a flow rate of 0 ml. When the solvent containers have been refilled, you can resume the time control file using *CONTINUE*. If you don't want to use the Solvent Supply function you will have to deselect the option *ACOUSTIC WARNING AT* and *STOP ALL* or *HOLD WITH PUMP STOP*.

Additionally, you have the possibility to use a real level indicator with analog outputs which can be defined in the PurityChrom.ini-file [SolventSupply] (see appendix).

Waste management

General

The function key and menu option *WASTE MANAGEMENT*  in the main window opens the window where you can visualise the waste management display.

In the PurityChrom.ini-file [WasteManagement] (see appendix) you can define the valve which should function as the waste management valve. The number of the possible containers complies with the number of valve positions. Additionally you have the possibility to bypass the waste management and collect your fractions in the normal waste in defined situations by configuring the bypass valve in the PurityChrom.ini-file [WasteManagement] (see appendix). This valve should have two positions. The first one can switch to the normal waste and the second one can switch to the valve used for waste management.

Display

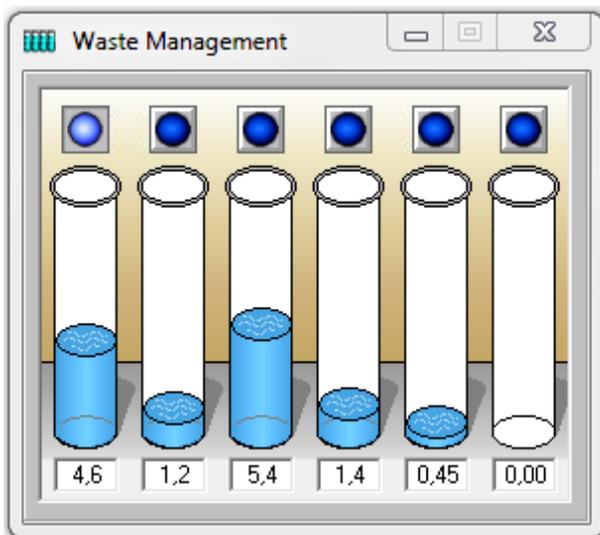


Fig. 61 Waste management

The window will show the number of containers in order of the configured valve positions. The LED above the containers shows if the position on the valve is chosen. At the same time, it is also a button for setting the valve position. Below the containers a value display will show the calculated volume inside the container. With a right-button click you can *EMPTY* the container

How to use the waste management

A typical usage of the waste management is the automatic sample. In such an application you can collect the non-valuable fractions of each injection separately in case a sample did not bind to the column or the elution went wrong. In a cyclic time control file programming, the position will switch to the next position after each run or when the set volume for the container is reached. A new run will only start when the container of the designated position is marked as *EMPTY*. Otherwise the method will switch to *HOLD*. In that case you will get an error message on the screen.

The rack display of the fraction collector (fraction rack)

General

The function key or the menu option *FRACTION RACK*  in the main window opens the window with the rack display of the fraction collector.

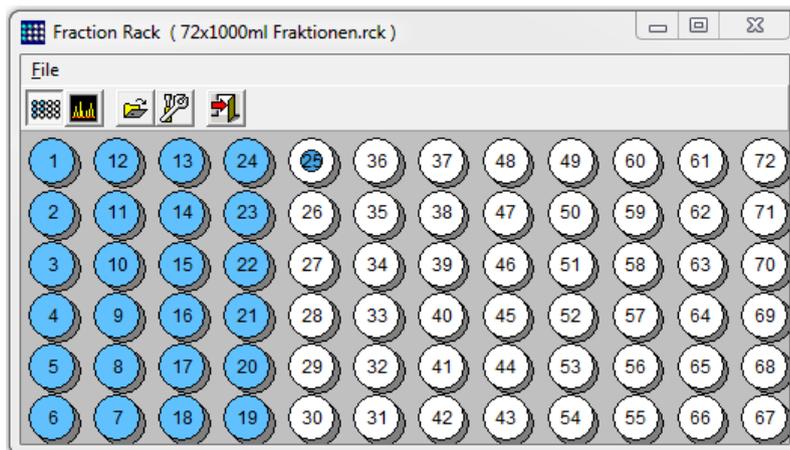


Fig. 62 Fraction rack, current view

The fraction rack can be displayed in two ways:

- The current view shows the fraction collector rack with all the positions. The filled fractions are displayed in blue and the empty ones in white. The current position is indicated by the blue dot.
- The reconstructed view shows the fractions produced by the result file loaded into the reconstruction window.

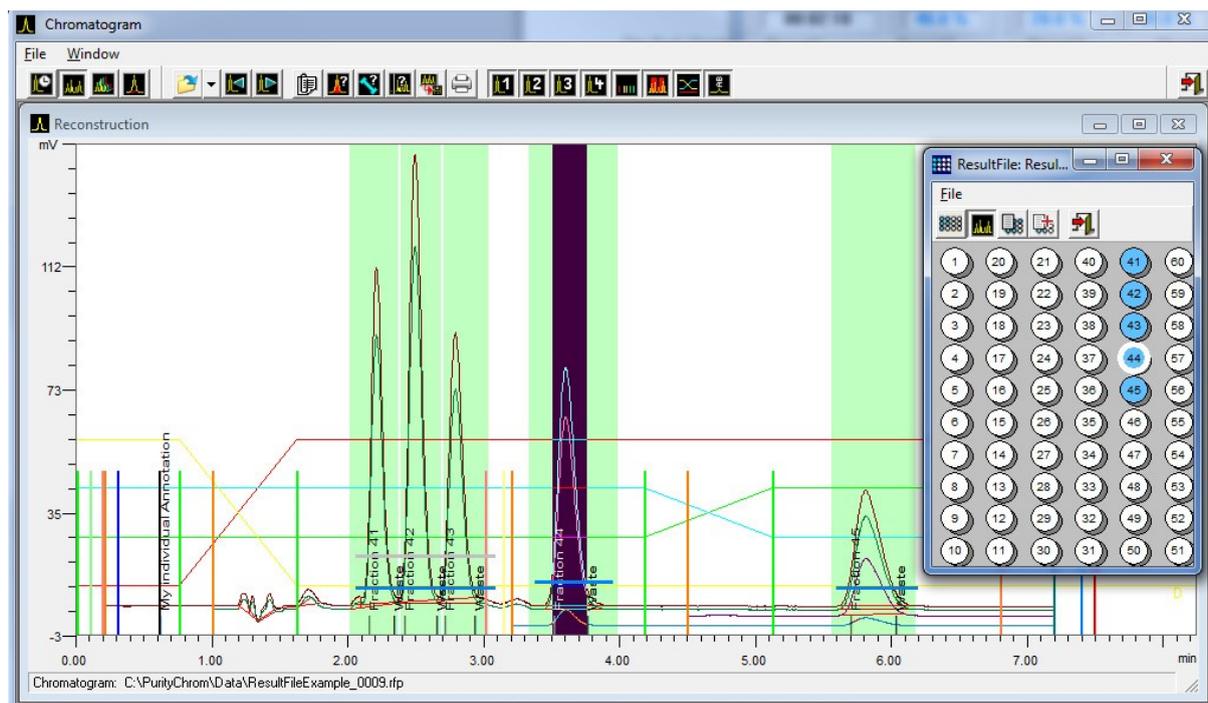


Fig. 63 Fraction rack, reconstructed view

If you left-click on one of the filled fractions, this part of the chromatogram will be displayed inverted. If you left-click on a peak, you will see the position of this fraction in the rack. This will give you a direct graphic relation between the chromatogram and the fraction produced in the fraction collector rack. If you move the mouse over one of the filled fractions, the fraction number and the volume fractionated will be displayed.

Fraction collector rack functions

-  *SHOW RACK* changes to the current view of the fraction collector rack.
-  *SHOW RECONSTRUCTION* changes to the reconstruction view of the fraction collector rack, displaying the result file loaded into the reconstruction window.
-  *LOAD RACK FILE* opens a rack file to display a fraction collector rack. You can also open a rack file out of a result file. The rack file is the same that was used in this run.
-  *RACK SETUP* opens the window to create a rack file to display a fraction collector rack.
-  *OPEN FRACTION TABLE* opens a window where a fraction table can be created.
-  *ADD FRACTIONS TO FRACTION TABLE* adds all fractions of the loaded results file into the fraction table.
-  *EXIT* closes the rack display of the fraction collector.



Fig. 64 Fraction rack, changing the fraction collector position

The rack display allows you to change the fraction collector positions directly. To do this, click with the right mouse button on a fraction and then select the menu option *STEP TO POSITION...* with the left mouse button.

Fraction table

No	Sample	Injection	Result File
1	Sample No. 1 from 09.12.2002	1	C:\PrepCon5\Data\2014.03.21_TimeControlExample-en_0001.rfp
2	Sample No. 1 from 09.12.2002	1	C:\PrepCon5\Data\2014.03.21_TimeControlExample-en_0001.rfp
3	Sample No. 1 from 09.12.2002	1	C:\PrepCon5\Data\2014.03.21_TimeControlExample-en_0001.rfp
4	Sample No. 1 from 09.12.2002	1	C:\PrepCon5\Data\2014.03.21_TimeControlExample-en_0001.rfp
5	Sample No. 1 from 09.12.2002	1	C:\PrepCon5\Data\2014.03.21_TimeControlExample-en_0001.rfp
6	Sample No. 1 from 09.12.2002	2	C:\PrepCon5\Data\2014.03.21_TimeControlExample-en_0002.rfp
7	Sample No. 1 from 09.12.2002	2	C:\PrepCon5\Data\2014.03.21_TimeControlExample-en_0002.rfp
8	Sample No. 1 from 09.12.2002	2	C:\PrepCon5\Data\2014.03.21_TimeControlExample-en_0002.rfp
9	Sample No. 1 from 09.12.2002	2	C:\PrepCon5\Data\2014.03.21_TimeControlExample-en_0002.rfp
10	Sample No. 1 from 09.12.2002	2	C:\PrepCon5\Data\2014.03.21_TimeControlExample-en_0002.rfp

Fig. 65 Fraction table

The fraction table contains a list of fractions, which the user has selected manually in the fractions rack window. Fractions can be inserted into the fraction table by clicking into a

fraction of the rack display with the right mouse key and choosing the menu point *ADD TO FRACTION TABLE*. All fractions of a fractionation run can be added into the fraction table by clicking the button *ADD FRACTIONS TO FRACTION TABLE* in the fractions rack window. The table contains the following columns: the fraction number, a sample description, the injection number, the name and the path of the corresponding result file.

The toolbar of the fraction table contains the following function buttons:



IMPORT FRACTION TABLE imports an already existing fraction table.



EXPORT FRACTION TABLE converts the fraction table into the CSV format (*Comma separated Value*).



PRINT FRACTION TABLE opens a dialog window for the print-out of the fraction table.



CLEAR FRACTION TABLE deletes the content of the fraction table.



EXIT closes the fraction table.

Fraction rack setup

The function button *RACK SETUP* opens the window to create or edit a rack file. Rack files contain information about the geometry of the rack and the relation between the number and the corresponding absolute co-ordinates of a fraction.

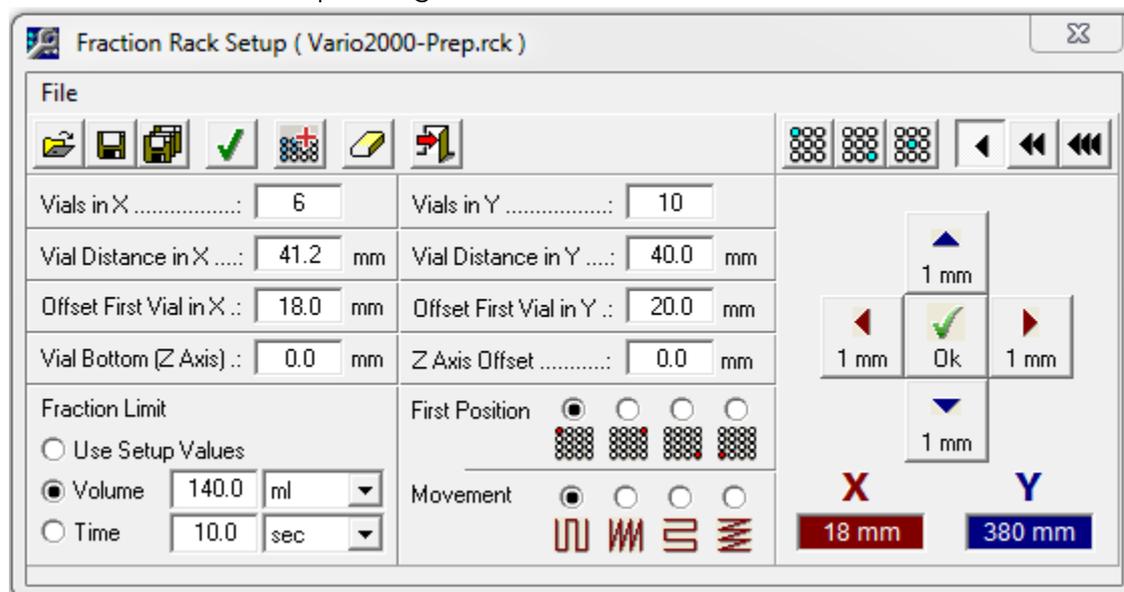


Fig. 66 Fraction rack setup

VIALS IN X and *VIALS IN Y* indicate the number of fractions in the x and y axes. You can enter the distances between the midpoints of the fraction vials in the x and y axes in the boxes *VIAL DISTANCE IN X* and *VIAL DISTANCE IN Y*. You can use *OFFSET FIRST VIAL IN X* and *OFFSET FIRST VIAL IN Y*

to define the x and y distance of the midpoint of the first fraction from the home position.

VIAL BOTTOM determines the depth of the fraction vials when a Z Axis is used and *Z AXIS OFFSET*

sets the offset of the Z Axis during a fraction step.

FIRST POSITION determines the angle for the first position *MOVEMENT* sets the procedure for fractionating.

You can add a maximum fraction volume or fraction time for the fraction limiter in

FRACTION LIMIT. The values from the limiter setup will be replaced by the rack file values when loading this rack file. If the option *USE SETUP VALUES* is selected, the values in the limiter setup will be retained.

The tool bar on the left side of the window contains the following function buttons:



LOAD RACK FILE to load a previously created rack file.



SAVE RACK FILE to save a new or changed rack file.



SAVE RACK FILE AS ... to save a rack file under a new name.



CREATE SYMMETRICAL RACK creates with the help of given data a symmetrical



Rack. *APPEND RACKFILE* appends an already created Rackfile to the current



loaded one. *CLEAR RACK FILE* to clear the content of the boxes.



EXIT to close the rack file setup window.

The teaching module of the setup is on the right hand side of the window. This module, allows the positions of the individual fractions to be determined and corrected step by step when using fraction collectors that are controlled by co-ordinates.

The tool bar in the teaching module contains the following function buttons:



TEACHING TOP LEFT POSITION selects the mode to move to the top left-hand fraction. The co-ordinates are automatically entered in the boxes *OFFSET FIRST VIAL IN X* and *OFFSET FIRST VIAL IN Y*.



TEACHING BOTTOM RIGHT POSITION selects the mode to move to the bottom right-hand fraction. The co-ordinates express the X and Y distances of the fractions one above the other. They are automatically entered in the boxes *VIAL DISTANCE IN X* and *VIAL DISTANCE IN Y*.



TEACHING SINGLE POSITION selects the mode for the corrector of the fraction that is indicated in the rack display as the current fraction.



TEACHWIDTH 1 MM sets the step distance when moving to a fraction at 1 mm.



TEACHWIDTH 10 MM sets the step distance when moving to a fraction at 10 mm.



TEACHWIDTH 50 MM sets the step distance when moving to a fraction at 50 mm.



A click on one of the *TEACHING BUTTONS* moves the arm of the fraction collector by the distance set in the corresponding direction.

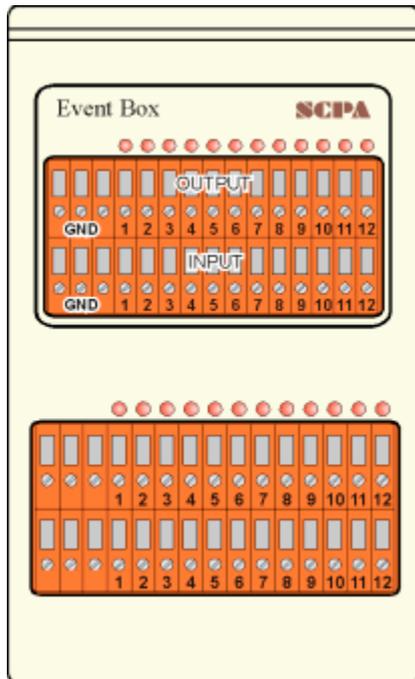


When it has reached the position required, the co-ordinates can be recorded by clicking on the *Set* button.



The current position of the fraction collector is shown in the X and Y co-ordinate displays.

Event box



The event box consists of 12 outputs, which are used to control system components, and 12 inputs used to capture statistical system signals. It is driven with the help of an interface card in the computer. The outputs are open collector outputs and switch the signals to ground (GND). The inputs are low active, i.e. they are considered active when they are connected to ground or when the input voltage is around 0 volts. The three connections on the left of the inputs and outputs are the mass connections. To switch devices such as magnetic valves, an external source of voltage is required. The maximum alternating voltage is 30 volts and the maximum current is 500 mA. Examples of applications that require an event box include: switching smaller magnetic valves, autozero on detectors, reporting back from an injection valve, controlling devices with TTL inputs etc.

Fig. 67 Event box

Manual control of the event box

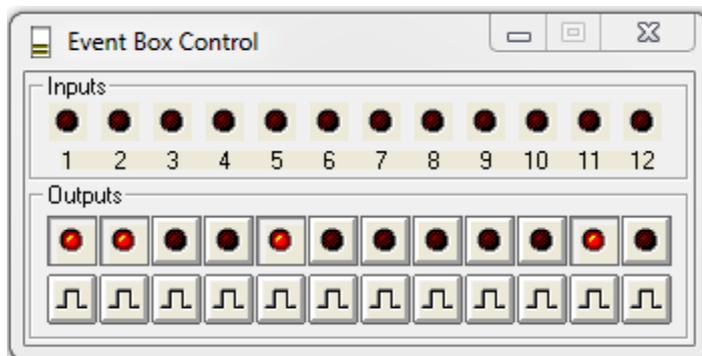


Fig. 68 Event box control

The function button or the menu option *EVENT BOX* in the main window opens the event box window. The upper row of LEDs represents the inputs. An active input is shown by a lit-up LED. The buttons in the lower part of the window are used to switch the outputs manually. The upper row of buttons switches the outputs on or off statically. The LEDs in the buttons show the status of each output. The lower row of buttons switches the outputs with a pulse lasting one second.

Appendix

Location of the connections on A/D conversion cards

Pin	1 Channel LP	2 Channel LP	2 Channel HP	4 Channel LP
1	- channel 1	- channel 1	- channel 1	- channel 1
2	+ channel 1	+ channel 1	+ channel 1	+ channel 1
3	n.c.	- channel 2	screening	- channel 2
4	n.c.	+ channel 2	- channel 2	+ channel 2
5	n.c.	n.c.	+ channel 2	- channel 3
6	n.c.	n.c.	screening	+ channel 3
7	n.c.	start channel 2	start channel 1	- channel 4
8	n.c.	GND	GND	+ channel 4
9	start channel 1	start channel 1	start channel 2	start channels 1-4
10	GND	GND	GND	GND

The entries in the file PurityChrom.ini

You can find the PurityChrom.ini file in C:\Windows

[MainWindow]

AlwaysOnTop=0	[0 or 1]	The main window is always in the foreground.
SolventConfirm=0	[0 or 1]	Starting the pump with the eluent buttons must always be confirmed by the user.
DataPathSelection=0	[0 or 1]	After loading a time control file the user can select a folder for the result files.
MultiLingual=1	[0 or 1]	The program is corresponding to the operating-system multilingual (German / English)
DateFormat=YYYY.MM.DD	[]	The programs date format
WavelengthRequest=0	[0 or 1]	Before starting a time control file, the user will be asked for a UV wavelegth.

[Boards]

Order=PCIFIRST [PCIFIRST or ISAFIRST]

Order of the analog channels if ISA and PCI cards are used combined.

[WinsockPorts]

Port1=	[IP,Port]	IP-Address und port number of the used Winsock-Ports. The port number will be appended with a comma after the IP-address.
Port2=		Winsock- Ports
Port3=		Winsock- Ports
Port4=		Winsock-Ports which are not used should not have parameters.
Port5=		Freestanding devices have the port number 10001.
Port6=		The left module in an assistant has the port number 10002, the middle module the port number 10003 and the right module the port number 10004.
Port7=		The middle module the port number 10003 and the right module the port number 10004.
Port8=		The Foxy R1 or R2 has the port number 23.

[AutomaticDataFiles]

CSVFile=0	[0 or 1]	A chromatogram file in *.csv format will be saved automatically at the end of a run.
FractionTable=0	[0 or 1]	A fraction table in ASCII format will be saved automatically at the end of a run.

[User Company]

Name=KNAUER	[Text]	User defined company name for all prints.
FontName=Arial	[Text]	Name of the font for the company name.
FontSize=18	[Value]	Font size for the company name.
FontBold=0	[0 or 1]	Bold font for the company name.
FontItalic=0	[0 or 1]	Italic font for the company name.
FontColor=128	[Value]	Font color for the company name.

[Pumps]

ConstantModeMajor=1	[0 or 1]	The pump system supports a constant pressure
ConstantModeMinor=0		mode, i.e. a flow rate control for working at a constant pressure.
OnlineMoving=1	[0 or 1]	The pump system supports the online gradient shift.
Autozero=0	[0 or 1]	The pump interface supports a potential free switch for a UV-autozero function.
MaxPressFactor=1	[0 or 1]	The maximum pressure value will be scaled appropriate to the pressure range.
PressureChannel=0	[0 or 1-8]	The pump pressure signal will be acquired by the specified A/D-conversion channel.
ParameterChange=0	[0 or 1]	The buttons 100% Solvent A-D and flowrate are enabled during a time control run.
FlowDimension=ml	[μ l , ml , l]	Dimension of the pump flowrate.
IOControl=0	[0 or 1]	Pump interface supports an emulation of an eventbox
SoftwareHPG=0	[1, 2, 3]	The high pressure gradient pump system is used isocratically or as binary or ternary gradient.
	[0]	An LPG is used.
Name1="Eluent"		To change the default pump names, change e.g. "Eluent" to "XXX" etc.
Name2="Zone 2"		
Name3="Feed"		
Name4 = "Zone 4"		

[Autosampler]

TempPrecision=3	[°C]	Precision of set temperature
MaximumSamples=100	[Value]	Maximum sample capacity of the autosampler
VolumeDimension=µl	[µl]	Dimension of the injection volume. Please don't change this entry.
AS3000=0	[0 or 1]	The autosampler is a Labomatic AS3000.
VarioPrep=0	[0 or 1]	The autosampler is a SCPA VarioPrep.
Alias=0	[0 or 1]	The autosampler is a Spark Alias.
Knauer3950=0	[0 or 1]	The autosampler is a KNAUER 3950.
Wash=0	[0 or 1]	1: washing cycle begins when starting the software 0: washing cycle after starting the software is turned off
TubingVolume=15	[µl]	Standard tubing volume (volume between needle and valve)
FlushVolume=30	[µl]	Standard flush volume (2x tubing volume)
[BronkFlow]		
Enabled=0	[0/1]	Disable or enable Bronkhorst flow meter
Port 2/3/4=	[Value]	Set serial port for connected flow meter
Channel=1	[1,2,3,4]	Set number of flow meter
Message=1	[0 or 1]	Disable or enable a message box on start up
Logfile=0	[0 or 1]	Disable or enable the logfile for all flow meters

[Collector]

Cetac=0	[0 or 1]	Activates the direct control of Cetac fraction collector
StepAtWaste=0	[0 or 1]	Automatic fraction collector step when switching to waste.
HomeOutput=0	[1 - 12]	Event box output for home fraction collector.
CollectorArray=0	[0 or 1]	Serial switching of several fraction collectors.
Collectors=0	[2 - ...]	Number of fraction collectors in the series.
CollectorValve=0	[1 - ...]	Number of the waste/fraction valve when several collectors are switched in series.
Autozero=0	[0 or 1]	The collector supports a potential free switch for a uv-autozero function.

[FractionCleaning]

Enabled=0	[0 or 1]	Automatic fraction cleaning enabled/disabled.
WashTime=2	[Value]	Wash time in the washing station.
XOffset=71	[Value]	X-offset to reach the fraction coordinate.
YOffset=-20	[Value]	Y-offset to reach the fraction coordinate.
ZOffset=0	[Value]	Z-offset to reach the fraction coordinate.

[Aliquotation]

Enabled=0	[0 or 1]	Automatic aliquotation enabled / disabled.
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[Detector]

AutozeroAnnotation=1	[0 or 1]	On each autozero an annotation will be written into the chromatogram.
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[KnauerUVD]

Enabled=1	[0/1]	UV is disabled/enabled
IP/IP2/IP3/IP4=	[IP]	IP-address for detector number 1, 2, 3 and 4
Port/Port2/Port3/Port4=	[Port]	Port number for detector number 1, 2, 3 or 4
NDetector=1	[1,2,3,4]	Number of used detectors
BaudRate=9600	[Value]	Baudrate
Wavelength1=254	[Value]	Preset wavelength
DataRate=2	[Value]	Data rate of the detector
Channels=1	[Value]	Number of channels
Integration=100	[Value]	Preset integration value
Type=UVD2.1S	[Type]	Your detector type (UVD2.1S or UVD 2.1L)

[KnauerRID21L]

Enabled=1	[0/1]	RID is disabled/enabled
NDetectors=1	[1/2/3/4]	Number of detectors
IP/IP2/IP3/IP4=	[IP]	IP-address for detector number 1, 2, 3 and 4
Port/Port2/Port3/Port4=	[Port]	Port number for detector number 1, 2, 3 or 4
DataRate=2	[Value]	Default data rate value
Channels=1	[Value]	Number of channels
Temperature=	[Value]	Temperature for detector number 1
Temperature 2/3/4=	[Value]	Temperature for detector number 2,3 and 4
TempZone=1	[Value]	
TimeConst=2000	[Value]	Time constant default value
SignalMode=0	[0/1]	Signal mode off/on
AnswerTimeOut=1	[Value]	Answer time out default value
ConnectionTimeOut=1	[Value]	Connection time out default value

[KnauerDAD61L]

Enabled=1	[0/1]	DAD is disabled/enabled
IP=	[IP]	IP address
Port =	[Port]	Port number
Integration=100	[Value]	Preset value for DAD integration time.

[KnauerIFU21]

Port=	[Port]	Portnumber
DataRate=2	[Value]	Datarate of the IFU
Channels=1	[Value]	Number of channels

[KnauerCM21S]

IP=	[IP]	IP-adress
Port=	[Port]	Portnumber
DataRate=2	[Value]	Datarate of CM2.1S
Channels=1	[Value]	Number of channels

[ThermoControl]

IP=	[IP]	IP-adress
Port=	[Port]	Portnumber

Channels=1	[Value]	Number of channels
[MMS]		
Port=COM2	[COM2 - ...]	8
(This entry is only necessary when the DAD is not connected to COM 1)		
[DAD]		
Enabled=0	[0 or 1]	Enables the DAD program functions.
Diodes=1024	[Value]	Number of diodes in the diode array.
ScanStart=200	[Value]	Spectrum start of ECOM-DAD.
ScanEnd=600	[Value]	Spectrum end of ECOM-DAD.
SliceWidth=100	[Value]	Preset value for DAD slicewidth.
IntegrationTime=100	[Value]	Preset value for DAD integration time.
IntegrationBandwidth=1	[Value]	Preset value for DAD integration bandwidth.
AutomaticReference=1	[0 or 1]	Automatic reference spectrum after starting a time control file.

[Smartline2600]

Integration=20 [Value] Integration time of Knauer Smartline DAD.

[Calibration]

p0=1.900000000E+002 [Value] Calibration factor 0 of the DAD.

p1=2.000000000E+000 [Value] Calibration factor 1 of the DAD.

p2=0.000000000E+000 [Value] Calibration factor 2 of the DAD.

p3=0.000000000E+000 [Value] Calibration factor 3 of the DAD.

p4=0.000000000E+000 [Value] Calibration factor 4 of the DAD.

[Eventbox]

Pulselength=0.5 [0.1 - ...] Pulse length of the event box outputs in seconds.

[Signal Inverting]

EventBox=0 [1 - 12] Listed EventBox or Gameport inputs will be inverted.

Gameport=0 [1 - 4]

[Temperature]

FunctionName1=... [Text] User defined name of the function „Temperature 1“.

FunctionName2=... [Text] User defined name of the function „Temperature 2“.

StirrerSpeed=150 [Value] Heater stirrer speed.

[MultiSystem]

Enabled=0 [0 or 1] To run the software several times on one computer. This is not supported with PurityChrom[®] Bio.

DataChannel1=1 [1 - 8] Number of the data channel that is allocated to the first system in a multiple system.

DataChannel2=2 [1 - 8] Number of the data channel that is allocated to the second system in a multiple system.

DataChannel3=3 [1 - 8] Number of the data channel that is allocated to the Third system in a multiple system.

DataChannel4=4 [1 - 8] Number of the data channel that is allocated to the fourth system in a multiple system.

[IntegrationPresets]

IntegrateInhibit=0	[0 or ...]	Data channels that are not to be automatically integrated, are to be entered separated by commas.
MaxBaselineLevel=250	[...]	Preset values for integration outside
FilterFactor=5	[5 - 15]	peak windows (explanation in Chapter Reintegrating Chromatograms page 60).
SlopeSensitivity=500	[...]	
AreaDelay=0	[0 or 1]	Enables an input field in the thresholdprogramming where a delay time for crossing execution can be entered.
TailingFactor=0	[0 or 1]	Instead of Assymetry the tailing factor will be calculated.

[SolventSupply]

AnalogA=0	[1 - 8]	Number of the channel that records the level signal of solvent A.
AnalogB=0	[1 - 8]	Number of the channel that records the level signal of solvent B.
AnalogC=0	[1 - 8]	Number of the channel that records the level signal of solvent C.
AnalogD=0	[1 - 8]	Number of the channel that records the level signal of solvent D.
AnalogWaste=0	[1 - 8]	Number of the channel that records the level signal of waste.
SoundfileWaste=*.wav	[*.wav]	Sound wave file that plays automatically when the waste level is upper maximum.
SoundfileSolvents=*.wav	[*.wav]	Sound wave file that plays automatically when a solvent level is under minimum.

[WasteManagement]

ValveNo=1	[1 - ...]	Number of the waste/fraction valve when using waste management.
BypassValve=0	[1 - ...]	Number of the bypass valve when using waste management.
Volume=10000	[...]	Volumes of waste containers when using waste management.
ErrorInput=0	[1 - ...]	Number of the EventBox-Input for a error signal.
Soundfile=Fehler.wav	[*.wav]	Sound wave file that plays automatically when an error occurs.

[LeakageInput]

LeakageInput=0	[1 - 12]	Event box input for leakage sensors.
LeakageCountdown=120	[0 - ...]	Countdown in seconds until the system is stopped following the detection of a leak.
Soundfile=Fehler.wav	[*.wav]	Sound wave file that plays automatically when a leak has been detected.
EluentValveA=2	[1 - ...]	Valve number for sealing off eluent Solvent A.
EluentValveB=3	[1 - ...]	Valve number for sealing off eluent Solvent B.
EluentValveC=4	[1 - ...]	Valve number for sealing off eluent Solvent C.
EluentValveD=5	[1 - ...]	Valve number for sealing off eluent Solvent D.

[LC-MS]

ReadyInput=0	[0 or 1]	Number of the event box input where the MS- signal of the searched mass is connected.
StartAnnotation=...	[Text]	Annotation text in the chromatogram when the MS-signal is switched on.
EndAnnotation=...	[Text]	Annotation text in the chromatogram when the MS-signal is switched off.
MS-Converter=0	[0 or 1]	A program to convert MassLynx traces will be started automatically at the end of a run.

[DemoMode]

Chromatogram=*.rfp	[*.rfp]	Result file for a simulated chromatogram signal in demo mode.
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[Visualisation]

ValvePartitioning=0	[1 - ...]	Number of the fraction valve that will be partitioned in horizontal segments. Double-clicking on a segment switches the valve to the matching position number instead of the next position.
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[TandemValve]

Index=0	[1 - ...]	Number of the tandem valve
SecondWinsockPort=1	[1 - ...]	Number of the Winsock-Port of the 2 nd valve
PositionRequest=0	[0 oder 1]	Before starting the time control file, the user will be asked for a valve position

PurityChrom file types

- *.tcf PurityChrom-Time Control File.
- *.acf PurityChrom-Autosampler Control File.
- *.rfp PurityChrom-Result File. Includes the result data of a run.
- *.dad PurityChrom-DAD-File. Includes the spectra of a DAD run.
- *.rck PurityChrom-Fraction Collector Rack File. Includes the geometric data of a fraction collector rack.
- *.vis PurityChrom-Visualisation File. Includes the data of a system visualisation.
- *.cfg PurityChrom-Configuration File. Includes the data of the program configuration.