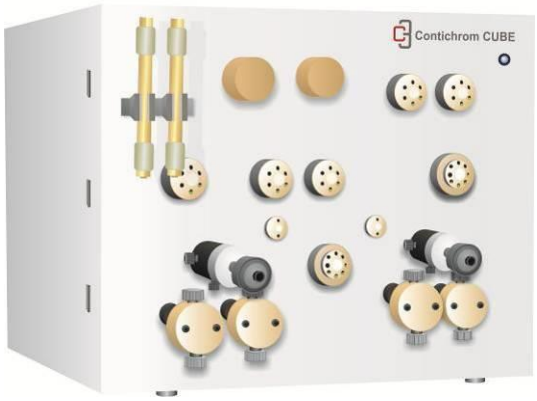
Contichrom® CUBE 30/100 (FPLC)
Contichrom® CUBE Combined 30/100 (FPLC)
Contichrom® HPLC 30/100

System Manual



Content

(to access a chapter click on the entry)

SAFETY SYMBOLS / SYMBOLES DE SÉCURITÉ

SAFETY INFORMATION

This manual contains WARNINGS and NOTICES concerning safety. These are defined below:

WARNING:

A WARNING is represented by the following symbol and warning information:

WARNING: Warning information

WARNING indicates a hazardous situation which, if not avoided, could result in harm to the system operator. It needs to be ensured that the hazardous situation is resolved before proceeding.

NOTICE:

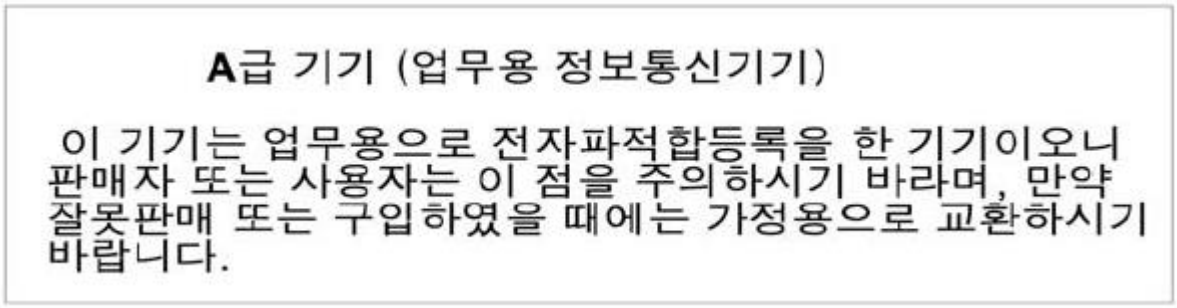
A NOTICE is represented by the following symbol and warning information:

NOTICE: Notice information

NOTICE indicates a situation which, if not avoided, could result in damage to the instrument. It needs to be ensured that the situation is resolved before proceeding.

REGULATORY INFORMATION

Class A: EMC Registration is done on this equipment for business use only (Class A). Product seller and user should notice that this equipment is not for household use.



INTRODUCTION TO THE CONTICHROM SYSTEM

INTRODUCTION

Contichrom is a modular laboratory scale chromatography platform designed for operation of single column and two column chromatography processes. The Contichrom platform is offered in different system configurations as shown in the table below. **This System Manual is not applicable to the Contichrom Discovery system.**

Contichrom Module Overview:

- CUBE: A module with 36 mL/min pumps, valves and detector system
- CUBE+: A module with 36 mL/min pumps, complementary with the CUBE module
- CUBE 100: A module with 100 mL/min pumps, valves and detector system
- CUBE+ 100: A module with 100 mL/min pumps, complementary with the CUBE 100 module
-

All modules are available with a pressure rating of 50 bar for Fast Protein Liquid Chromatography (FPLC) and with a pressure rating of 100 bar for High Performance Liquid Chromatography (HPLC).

The Contichrom system platform comprises the following components:

- Contichrom system according to the table below
- Laptop with the ChromIQ software installed
- Optional Fraction Collector
- Auxiliaries

Contichrom Systems Overview:

	Contichrom CUBE 30	Contichrom CUBE 100	Contichrom CUBE Combined 30	Contichrom CUBE Combined 100	Contichrom HPLC 30	Contichrom HPLC 100
CUBE module	CUBE	CUBE 100	CUBE	CUBE 100	CUBE	CUBE 100
CUBE+ module	n.a.	n.a.	CUBE+	CUBE+ 100	CUBE+	CUBE+ 100
Max. flow [mL/min]	36	100	36	100	36	100
Max. pressure [bar]	50	50	50	50	100	100
Tubing i.D. [mm], pressure side	0.75	1.00	0.75	1.00	0.75	1.00

The Contichrom systems are available with maximal flow rates of 36 mL/min and 100 mL/min. The Contichrom CUBE system is a stand-alone instrument, consisting of the Contichrom CUBE module and being capable of running multiple chromatographic processes. The CUBE+ upgrade module, in combination with the Contichrom CUBE module, forms the "Contichrom CUBE Combined" configuration that unlocks the full range of twin-column chromatography processes. "Contichrom HPLC" is a Contichrom CUBE Combined configuration with 100 bar pressure rating. The process capabilities for the individual configurations are shown in the table below:

Process Capabilities	Contichrom Systems					
	Contichrom CUBE 30	Contichrom CUBE 100	Contichrom CUBE Combined 30	Contichrom CUBE Combined 100	Contichrom HPLC 30	Contichrom HPLC 100
Batch (single column)	✓	✓	✓	✓	✓	✓
Integrated Batch (isocratic)	✓	✓	✓	✓	✓	✓
Integrated Batch (lin.gradient)			✓	✓	✓	✓
2C-PCC	✓	✓	✓	✓	✓	✓
MCSGP			✓	✓	✓	✓
N-Rich			✓	✓	✓	✓

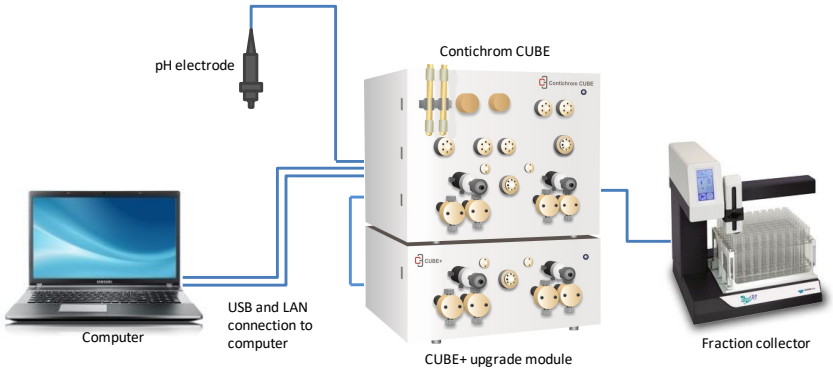
Columns with the same resin or different resins can be used.
Briefly, the different twin-column processes serve the following purposes:

- Integrated Batch chromatography combines two chromatography steps using different resins and automates the purification. The eluate from the first column is directly loaded on the e second column whereby inline dilution can be used.
- 2C-PCC (CaptureSMB) increases throughput and decreases resin consumption in affinity capture applications compared to single column batch capture chromatography.

- MCSGP is designed for difficult purifications where overlapping of impurities and the target molecule significantly lower the product pool purity. MCSGP produces target components with high yield and high purity at the same time.
- N-Rich isolates product-related impurities for characterization purposes. The process enriches the side-components while simultaneously depleting interfering main compounds, potentially saving dozens of HPLC runs.

CONTICHROM SYSTEM ILLUSTRATION

The following illustration is representative of Contichrom CUBE Combined 30/100 system and of Contichrom HPLC 30/100 system:



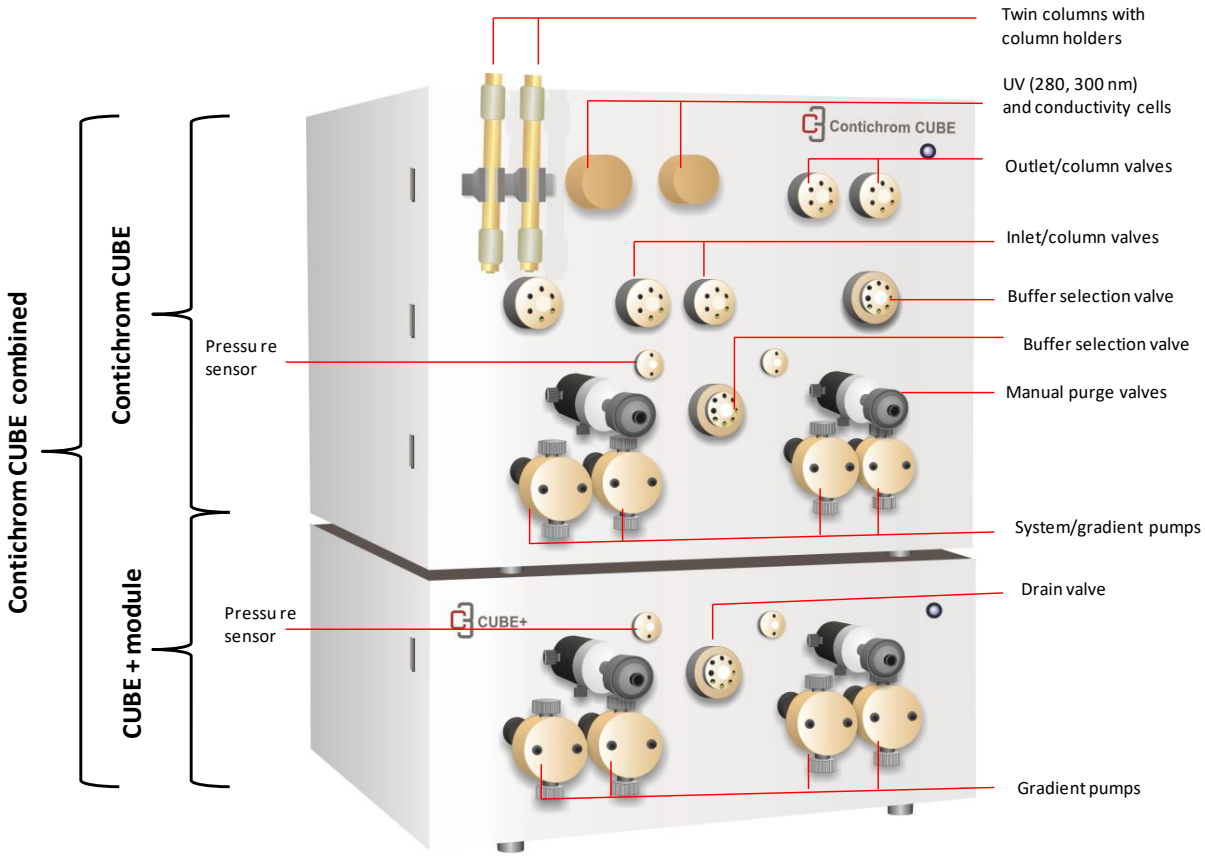
1. THE CONTICHROM SYSTEM

1.1. GENERAL INSTRUMENT OVERVIEW

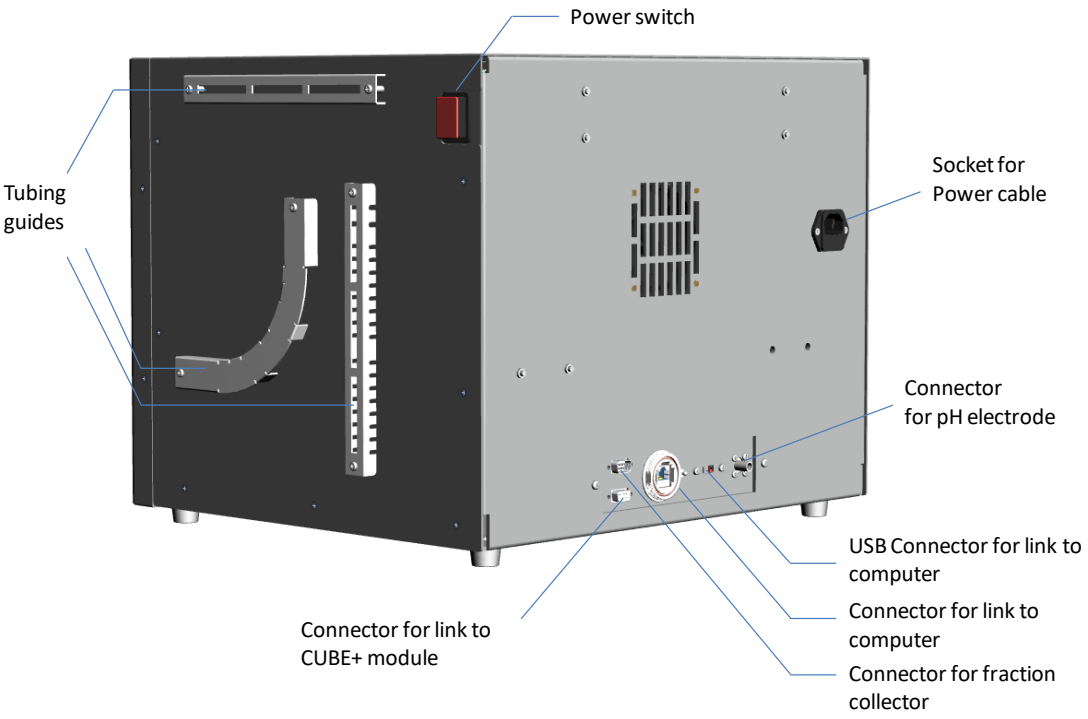
The Contichrom platform comprises the following main components:

- **Contichrom CUBE module (30/100)**, present in Contichrom CUBE 30/100 systems, Contichrom CUBE Combined 30/100 systems and Contichrom HPLC 30/100 systems
- **CUBE+ upgrade module (30/100)**, present in Contichrom CUBE Combined 30/100 systems and in the Contichrom HPLC 30/100 systems

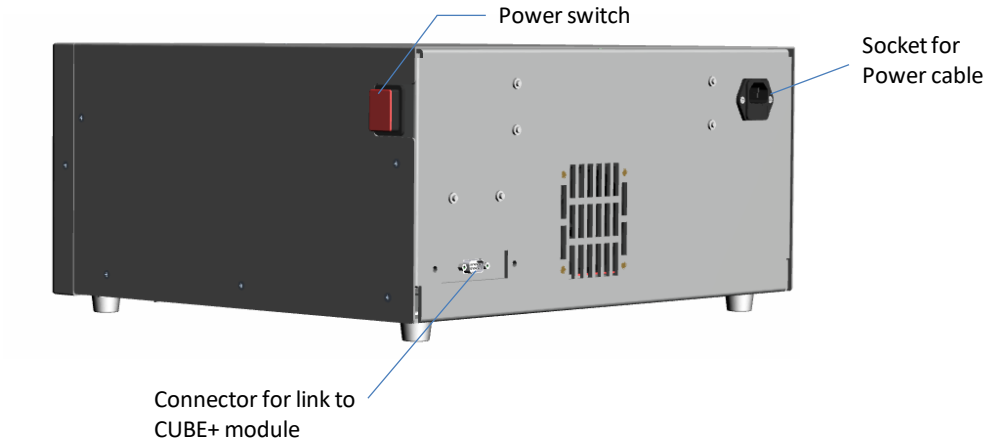
The CUBE+ upgrade module, in combination with the CUBE module, forms the "Contichrom CUBE Combined 30/100" systems or the Contichrom HPLC 30/100 systems, respectively.



Front view of the Contichrom CUBE Combined



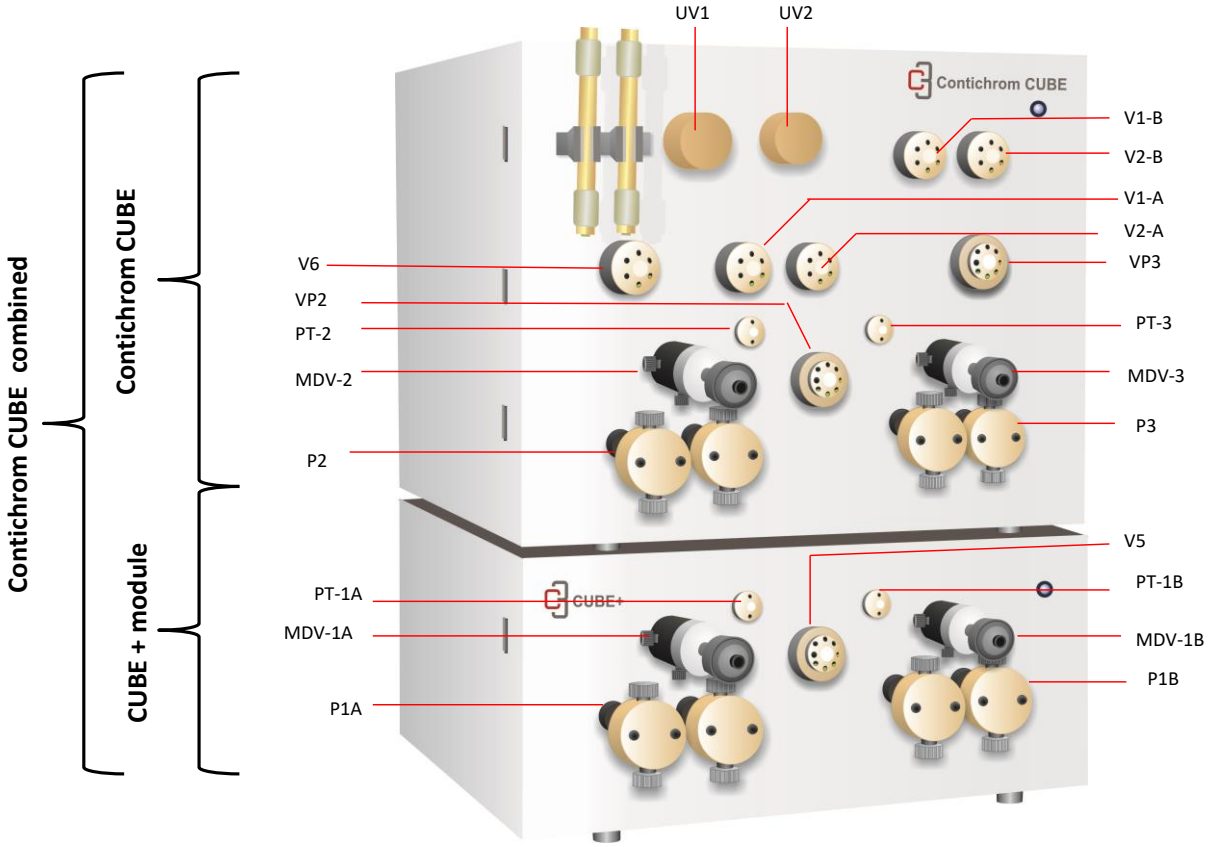
Side and back view of the CUBE module (top)



Side and back view of the CUBE+ module (bottom)

1.2. OVERVIEW OF WETTED SYSTEM COMPONENTS

The following illustration shows the system components that are in contact with liquid during operation of the the Contichrom system. The picture shows a “Contichrom CUBE Combined system” but is equally valid for the “Contichrom HPLC” systems.



	Description		Description
	CUBE + module	P3	Pump 3
P1A	Pump 1 A, channel A of gradient with P1B	MDV-3	Manual drain valve for P3
MDV-1A	Manual drain valve for P1A	PT-3	Pressure sensor for P3
PT-1A	Pressure sensor for P1A	VP3	Buffer selection valve for P3
P1B	Pump 1 B, channel B of gradient with P1A	V6	Automatic drain valve for P2 and P3
MDV-1B	Manual drain valve for P1B		
PT-1B	Pressure sensor for P1B	V1A	Inlet column valve for column position 1
V5	Automatic drain valve for P1A and P1B	V1B	Outlet column valve for column position 1
		V2A	Inlet column valve for column position 2
	CUBE module	V2B	Outlet column valve for column position 2
P2	Pump 2		
MDV-2	Manual drain valve for P2	UV1	Flow cell for fixed dual wavelength detector, conductivity, temperature
PT-2	Pressure sensor for P2	UV2	Flow cell for fixed dual wavelength detector, conductivity, temperature
VP2	Buffer selection valve for P2		

WARNING: Contichrom equipment must not be used with human contagious materials and/or radioactive materials.

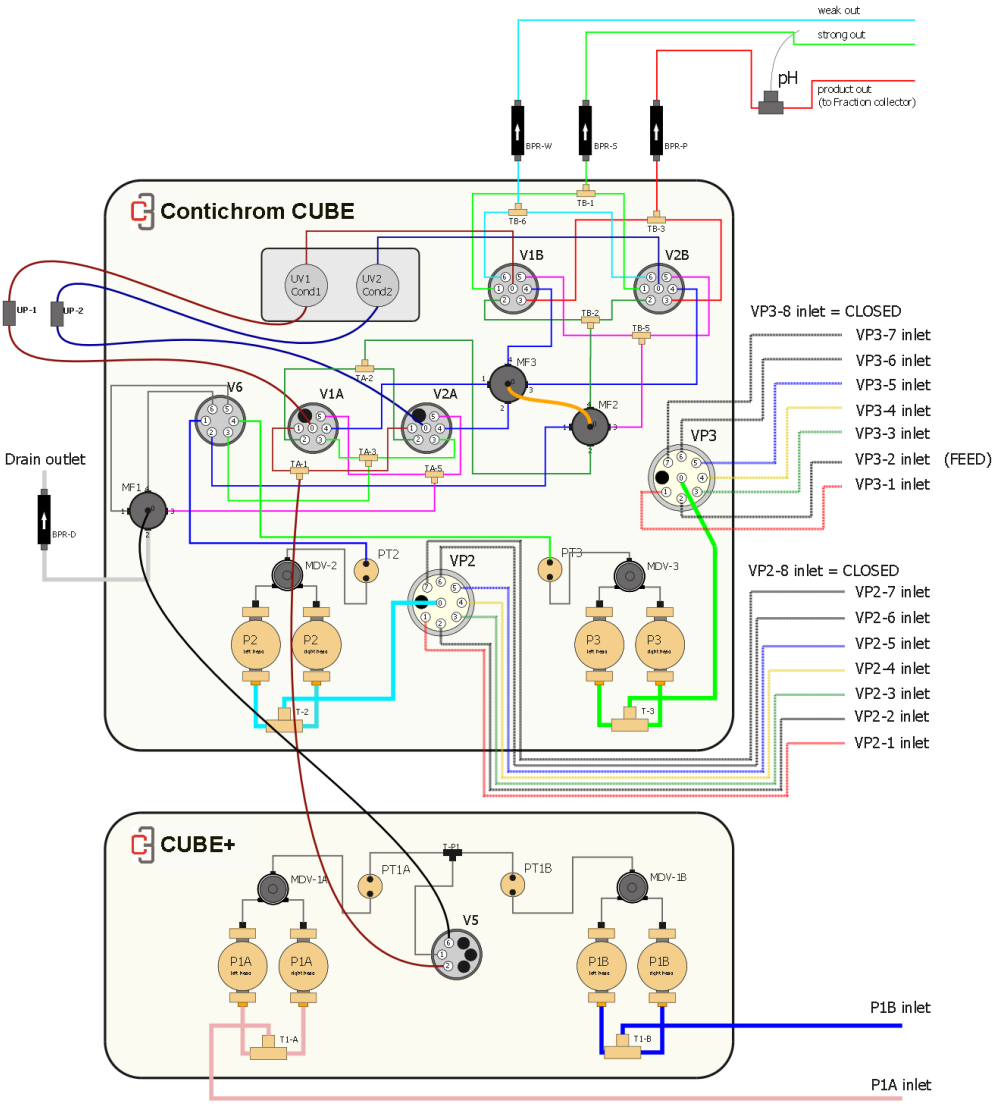
1.3. LIQUID FLUID PATH

1.3.1. OVERVIEW

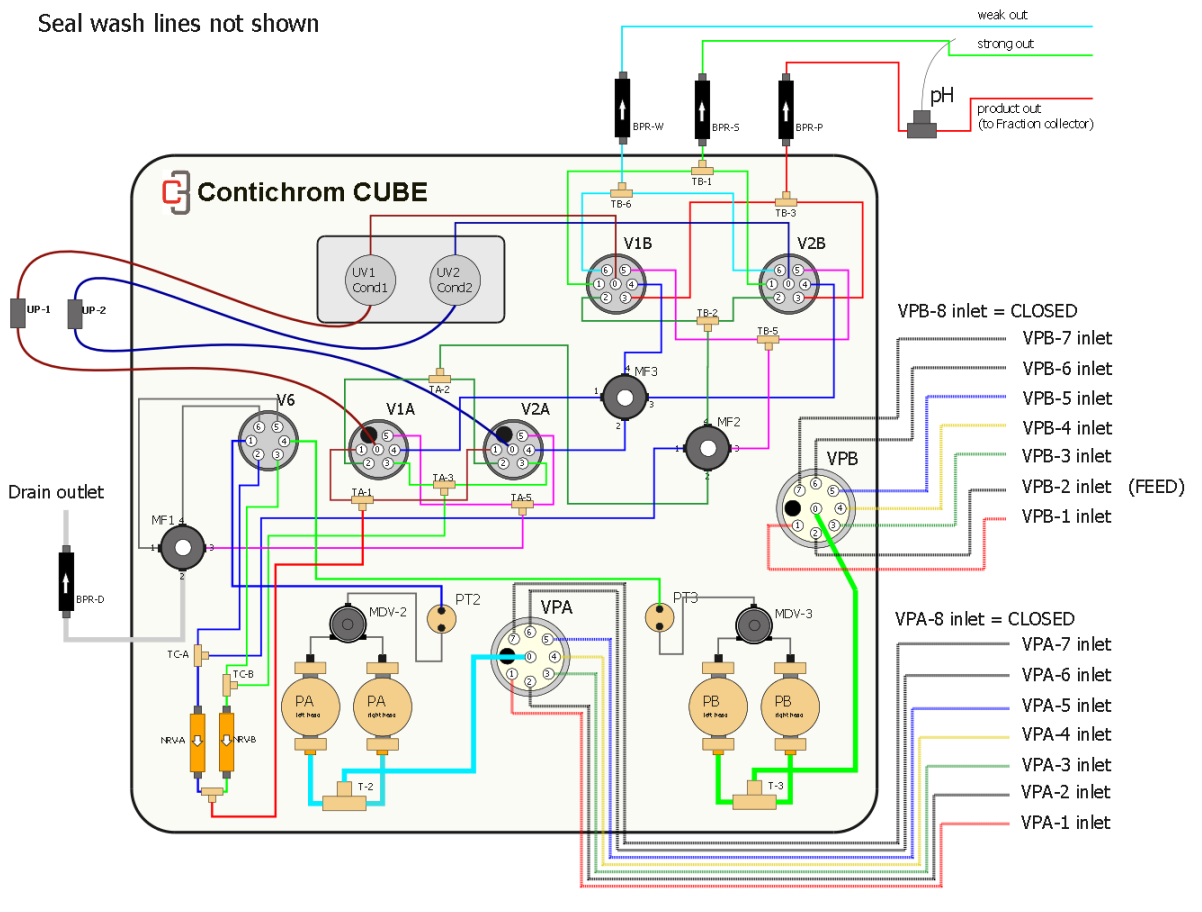
The illustrations below show the flow sheets of the Contichrom CUBE Combined system and of the CUBE system, respectively. The flow sheet of the Contichrom HPLC is identical to the flow sheet of the Contichrom CUBE Combined system.
In the CUBE module can utilize up to 16 different buffers (Pumps 2 and 3 each equipped with an 8-way buffer selection valve). In the CUBE+ module each pump is connected to one buffer, adding 2 more buffers for a total of 18 buffers that can be potentially used in a Contichrom CUBE Combined / Contichrom HPLC system.

The Contichrom systems are provided with a fully assembled fluid path. By default, the tubing on the low pressure side (before the pumps) consists of tubing with 1/8 inch outer diameter and 1.55 mm inner diameter (CUBE / CUBE Combined 30) and 2.0 mm inner diameter (CUBE / CUBE Combined 100), respectively.
The tubing on the high pressure side consists of capillaries with 1/16 inch outer diameter and 0.75 mm inner diameter (CUBE / CUBE Combined 30) and 1.0 mm inner diameter (CUBE / CUBE Combined 100), respectively.
The drain tubing and the pump seal wash tubing is of 8 inch outer diameter and 1.55 mm inner diameter (CUBE / CUBE Combined 30) and 2.0 mm inner diameter (CUBE / CUBE Combined 100), respectively.

Flow Sheet of the Contichrom CUBE Combined 30/100 systems (pump seal wash lines not shown). The flow sheet for the Contichrom HPLC 30/100 systems are identical:



Flow Sheet of the Contichrom CUBE 30/100 systems (pump seal wash lines not shown):

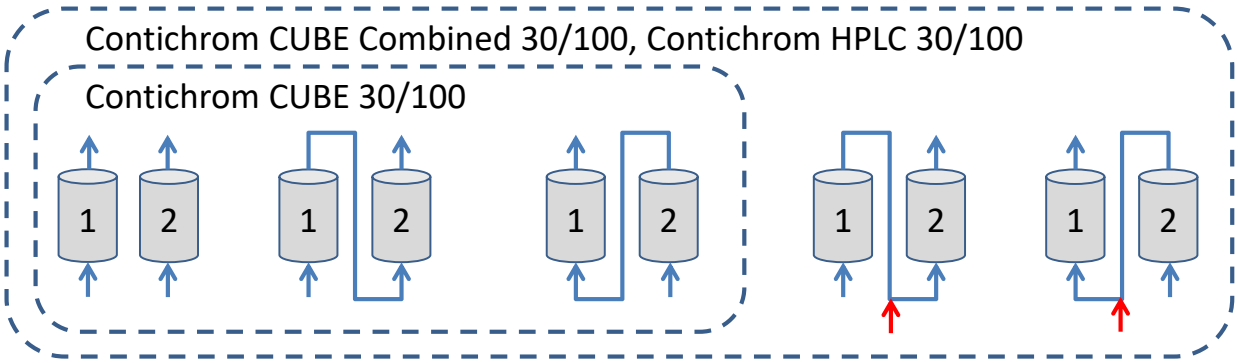


WARNING: Be aware that protruding capillaries and tubes may trap parts of the body or clothing causing abrasion and/or injury.

1.3.2. TWIN COLUMN FLUID PATHS

The Contichrom systems have been designed to support both single column batch chromatography and chromatography processes with two columns.
The fluid path allows running the columns in single column mode or in interconnected mode. When run in interconnected mode either column can be selected to be the most upstream one. Detectors are located at the outlet of each column.
In combination with the Contichrom CUBE 30/100 module, the CUBE+ modules add additional gradient capabilities and inline dilution capabilities. A linear gradient can run on one of the columns and the eluate can be diluted inline before being loaded onto a second column.

The following schematic illustrates the fluid paths for Contichrom CUBE 30/100 and Contichrom CUBE Combined 30/100 systems// Contichrom HPLC 30/100 systems used in the different single and multicolumn processes.



1.3.3. ADDING AND REMOVING TUBES

The Contichrom systems are either delivered with a pre-mounted set of tubing or the tubing is installed by service personnel.

NOTICE: The user is strongly advised not to modify the flow sheet of the Contichrom system on the high pressure side, apart from the modification that is necessary to connect the upper and the lower system modules. The ChromIQ operating software is based on the predefined flow sheet. Modifying the flow sheet may lead to dead-ending of pumps when using the process wizards, which may lead to column damage. It is recommended to contact ChromaCon service personnel before doing major changes of the flow path.

Tubing with 1/8 inch outer diameter and the colored buffer selection valve fittings may be added to increase the number of selectable buffers on the low pressure side at the buffer selection valves. See chapter “buffer selection valves”.

1.3.4. CONNECTING CUBE AND CUBE+ MODULES

Contichrom CUBE Combined 30/100 systems and Contichrom HPLC 30/100 systems comprise of an upper module (CUBE module) and a lower module (CUBE+ module). For mounting the tubing that connects the two modules please refer to the instructions “Contichrom installation guide” and “CUBE/CUBE+ connection guide” provided in separate documents.

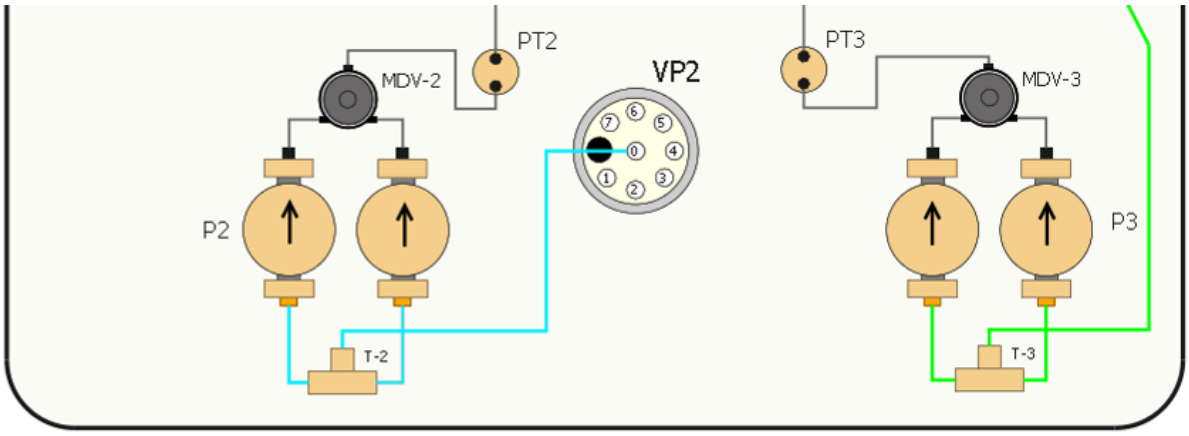
1.4. INSTRUMENT COMPONENTS

1.4.1. PUMPS

The Contichrom CUBE 30/100 modules are provided with two high precision double-head pumps (P2 and P3), each equipped with a buffer selection valve on the suction side (VP2, VP3). In the Contichrom CUBE 30/100 system, P2 and P3 are referred to as PA and PB, respectively, by the ChromIQ operating software. The pumps can operate independently in isocratic mode or may be joined to run a gradient. By default, P3 (PB) is used as the feed pump.

The Contichrom CUBE Combined 30/100 systems and the Contichrom HPLC 30/100 systems feature two additional pumps P1A, P1B that are joined on the high pressure side to provide additional linear gradient capabilities.

The flow from each pump passes through a manual purge valve that is suited for manual filling and purging of the pumps. Afterwards the fluids pass through the pressure sensors. The setup of pump, manual purge valves and pressure sensors is shown in the illustration below.



The pumps include a self-flush feature of the pump heads. The self-flushing provides continuous washing of the piston surface without the inconvenience of a manual flush or gravity feed arrangement. The self-flushing pump head uses a self-flush seal and secondary set of check valves to create a continuous and positive flow in the area behind the high-pressure pump seal.

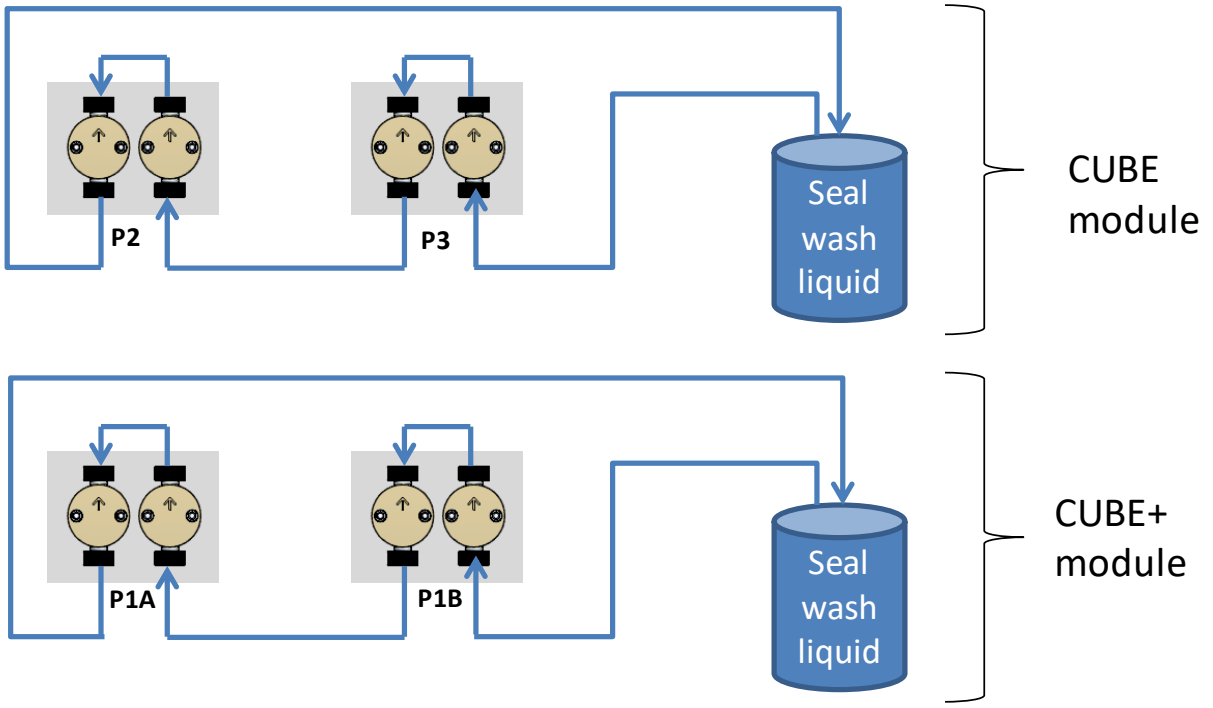
It is recommended that the self flush feature be used to improve seal life in a number of applications. In particular, (as stated above) if pumping buffers, acids/bases or any inorganic solution near saturation, the pump should utilize the self flush feature. With every piston stroke, an extremely thin film of solution is pulled back past the seal. If this zone is dry (without use of self flush) then crystals will form with continuous operation, which will ultimately damage the seal.

20% IPA/water mix or 30% Ethanol/water mix are good choices for the flush solution.

A schematic of the fluid connections of the seal wash of Contichrom CUBE Combined 30/100 systems are provided further below. The fluid connections of the Contichrom HPLC 30/100 systems are the same.

NOTICE: It is strongly recommended to ensure that the self-flushing feature is working properly. The flushing solution washes away any buffer salts that have precipitated onto the

piston. If not removed, these precipitates can abrade the high-pressure seal and cause premature seal failure, leakage, and can possibly damage the pump.



1.4.2. BACK PRESSURE REGULATORS

Back pressure regulators (40 psi, 2.75 bar) are mounted at all system outlets. The back pressure regulators suppress bubble formation in the system, thus increasing the accuracy. Moreover back pressure regulators prevent buffers leakage from the system due to hydrostatic pressure. The back pressure regulators are mounted at the following positions (also refer to flow sheet in chapter 1.3):

1. Drain outlet (at drain manifold on the drain line)
2. On the line originating from the joined outlets of V1B-6 and V2B-6 (also referred to as “W-outlet” or “strip outlet)
3. On the line originating from the joined outlets of V1B-3 and V2B-3 (also referred to as “P-outlet” or “product outlet”) after the pH electrode.
4. On the line originating from the joined outlets of V1B-1 and V2B-1 (also referred to as “S-outlet” or “strip outlet)

NOTICE: Make sure that the pH flow cell is connected such that outlet of the flow cell is open to the atmosphere without a back pressure regulator. The back pressure regulator must be located on the inlet side of the pH flow cell since the pH electrode is susceptible against high pressures

NOTICE: It is not recommended to use columns with a pre column pressure limit of 72 psi (5 bar) pressures when the back pressure regulators are mounted. For these columns, the backpressure regulators 2.-4. (see above) must be removed. The backpressure regulator on the drain outlet 1. (see above) must never be removed!

One pressure sensor is mounted at each pump after the manual drain valve. The pressure sensors record the pre-column pressure.

1.4.3. VALVES

Drain valves

The modules of the Contichrom system are equipped with the following automatic drain valves:

- V6 for CUBE 30/100 module
- V6 for the CUBE 30/100 module of the Contichrom CUBE Combined 30/100 systems and of the Contichrom HPLC 30/100 systems, respectively and
- V5 for the CUBE + 30/100 modules of the Contichrom CUBE Combined 30/100 systems and Contichrom HPLC 30/100 systems.
  These valves are used for rapidly filling the buffer lines and the pumps with new buffers at high flow rates. V6 is attributed to pumps P2 and P3 (PA and PB in CUBE 30/100 module) while V5 is attributed to P1A/B of the CUBE+ 30/100 module. The streams from P1A and P1B are united before entering V5.

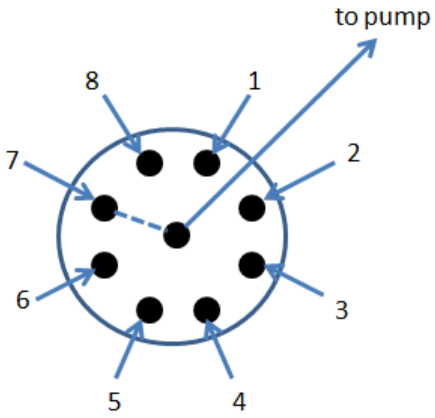
The drain valves have two positions:

1. Position 1, “to system”: the drain valves connect the pumps with the column valves

2. Position 2. “drain”: the drain valves connect the pumps with the drain outlet

Buffer selection valves

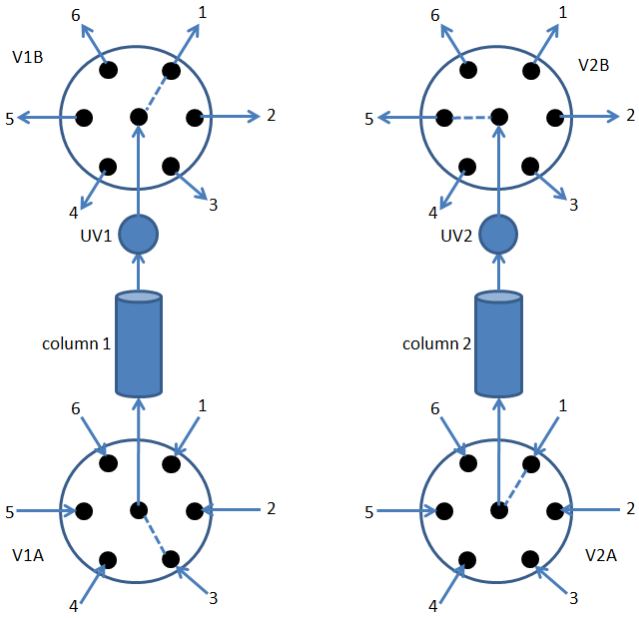
The pumps P2 and P3 of the Contichrom CUBE Combined 30/100 system and Contichrom HPLC 30/100 system (pumps PA and PB of the Contichrom CUBE 30/100 system) are equipped with automatic buffer selection valves on the suction side. Each buffer selection valve can select among 8 different buffer/feed solutions (see illustration below). By default, the feed stock solution containing the product to be purified is connected to VP3, inlet 2. The center position of the buffer selection valves leads to the pump. Buffer positions that are not in use should be closed using plugs. Tubing with 1/8 inch outer diameter and the colored buffer selection valve fittings may be added to increase the number of selectable buffers on the low pressure side at the buffer selection valves.



Column valves

A Contichrom system includes four column valves, one inlet column valve and one outlet column valve per column, see illustration below. The column valves are multi-position valves, connected as follows.

1. the center positions of the column inlet valves are connected to the column inlets
2. the center positions of the column outlet valves are connected to the outlets of the respective detector flow cells (denoted by “UV1” and “UV2” in the illustration below.



V1A is the inlet column valve of column 1, V1B is the outlet column valve of column 1.

V2A is the inlet column valve of column 2, V2B is the outlet column valve of column2.

The remaining valve inlets and outlets are connected as follows:

Example Inlet/outlet map for Contichrom CUBE Combined 30/100 and Contichrom HPLC 30/100

Position	V1A	V1B	V2A	V2B
1	From P1	Strip outlet	From P1	Strip outlet
2	Internal recycling from column 2, inline dilution by P2	Internal recycling to column 2	Internal recycling from column 1, inline dilution by P2	Internal recycling to column 1
3	From P3	Product outlet	From P3	Product outlet
4	Internal recycling from column 2, inline dilution by P2	Internal recycling to column 2	Internal recycling from column 1, inline dilution by P2	Internal recycling to column 1
5	From P2	CIP outlet	From P2	CIP outlet
6	Inlet plugged/ store position	Waste outlet	Inlet plugged/ store position	Waste outlet

1.4.4. DETECTORS

The Contichrom system contains a detector system measuring two fixed wavelengths at the outlet of each column. In its standard layout, the wavelengths are 280 nm and 300 nm. Moreover, the detector system measures the conductivity at the outlet of each column and the fluid temperature at the outlet of column 1. In flow cell 1 (left-hand side when facing CUBE) UV, conductivity and temperature are measured, in flow cell 2 (right-hand side when facing CUBE) UV and conductivity are measured. A separate flow cell is provided for the pH electrode. Refer to chapter 2.13 for mounting the pH electrode.

The detector system is pre-calibrated. However it is recommended to calibrate the pH before the first run.

1.4.5. FRACTION COLLECTOR

The Fraction collectors R1 and R2 are optional accessories for the Contichrom system (see picture below). The Fraction collectors can carry different rack types including 50 mL centrifuge tube, 15 mL centrifuge tubes, HPLC vials and 96-well plates. The R2 fraction collector can carry twice the number of racks and therefore has twice the capacity of the R1 fraction collector. The rack type is set in the ChromIQ operating software. Fractionation can be initiated based on time, volume, column volume and can be triggered through a number of different signals such as UV and conductivity.

By default, the fraction collector is connected to the product outlet. When installing the fraction collector make sure that the secondary drain of the fraction collector routes liquid from the drip tray of the fraction collector to a safe collection point.

Additional information on the fraction collector is provided in the R1/R2 fraction collector manual.

Information on the programming of the fraction collector is provided in the ChromIQ software manual.

R1 fraction collector

R2 fraction collector

WARNING: The drain outlet and the drip tray fluid outlet of the fraction collector must be routed to a waste container in order to avoid spillage. A hazardous situation may arise when liquids that are flammable or conductive are overflowing and get in contact with sources of ignition or electricity. Make sure that the secondary drain of the fraction collector routes liquid from the drip tray of the fraction collector to a safe collection point.

2. PREPARING THE CONTICHROM INSTRUMENT

2.1. STARTING THE SYSTEM

The Contichrom CUBE 30/100 system is switched on by plugging the power cable in and pressing the power switch. Contichrom CUBE Combined 30/100 systems and Contichrom HPLC 30/100 systems are switched on by plugging the power cables of the CUBE and the CUBE+ modules and by pressing their power switches.
The fraction collector is switched on by plugging in the power cable and by pressing the power symbol on its touchpad.

Once the power switches are on, acoustic signals will confirm that internal communications of the instrument have been launched.

WARNING: Only trained operators should set-up and operate the Contichrom system to avoid human injury.

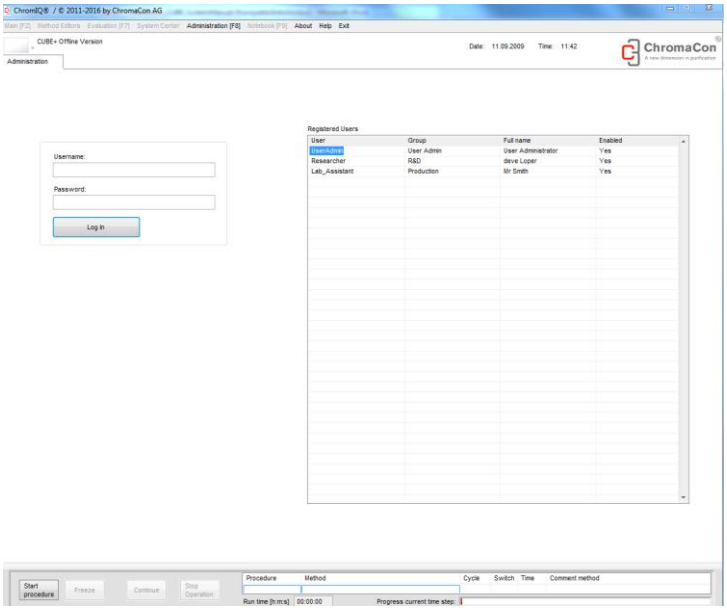
2.2. LAUNCHING CHROMIQ AND LOGGING IN

Contichrom instruments are operated using the ChromIQ operating software. The software is started using a desktop icon or the start menu of the control computer. After logging onto the control computer that is connected to the instrument, the ChromIQ software can be started by clicking on the ChromIQ icon on the desktop or selecting the software icon from the Windows start menu.

ChromIQ icon

As the software is being loaded, a window will appear indicating the software version and the software establishes the connection to the instrument and checks the availability of all hardware components.

When the software has finished loading, a login page appears where you can enter your user name and password at the prompt.

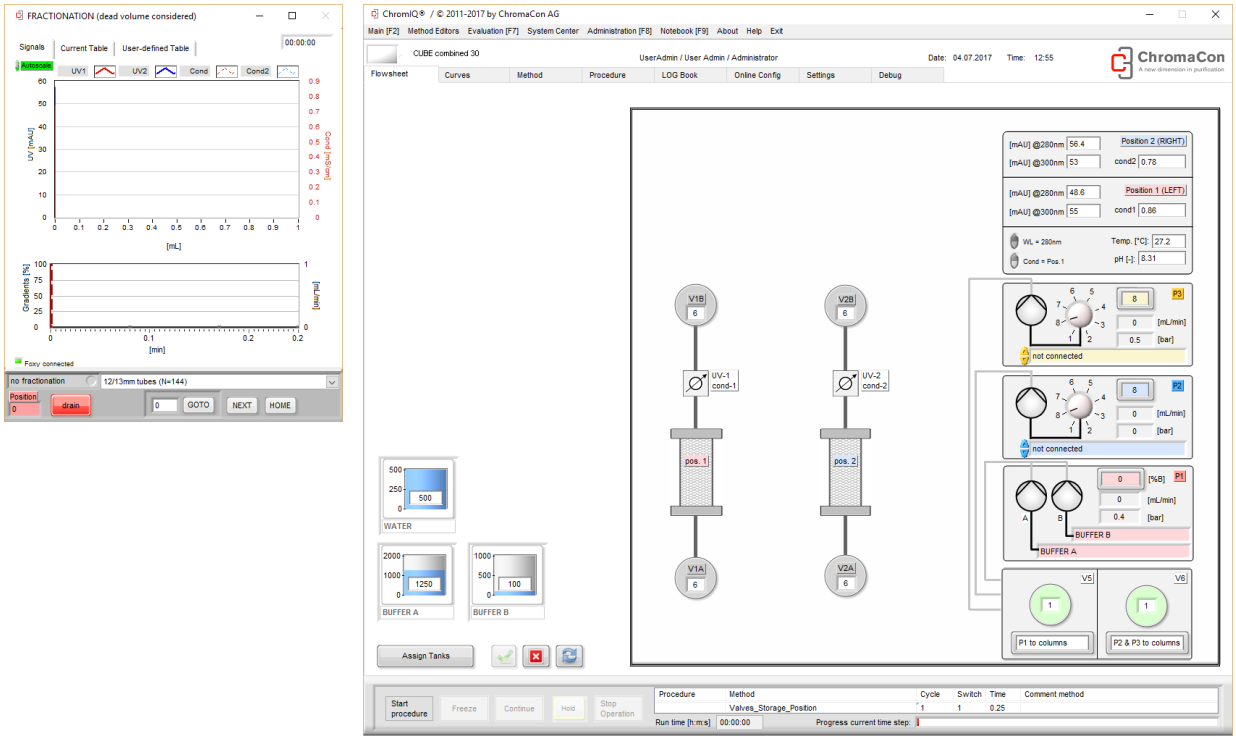


Screen shot of the Log on (Administration) tab.

The available user names are displayed at the right hand side of the login page. The ChromIQ software has three pre-installed user accounts:

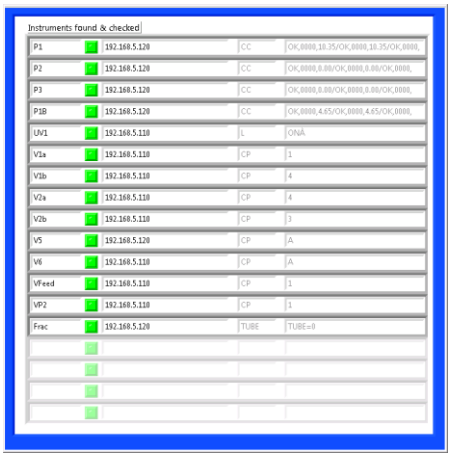
- UserAdmin (a regular administrator, password: U)
- Researcher (a R&D user, password: R)
- Lab_Assistant (a restricted production user, password: L)

Entering user name and password and confirming the entry will open the main window shown in the figure below. In addition, a fraction collector window will be shown in case a fraction collector is connected to the Contichrom system.



Screen shot of the main window (right) and the fractionation window (left)

After logging in, depending on computer and connection speed a communication check summary may be displayed for a few seconds. If you do not see the communication check summary, it means that the communication test was successful or that the software is run in demo mode.



Screen shot of the communications test window

2.1. BUFFER/SOLVENT PREPARATION

Prepare all buffers or solvents that are required to carry out the desired runs. Place the buffer containers/bottles in the buffer tray on top of the instrument. Insert and fix the desired inlet tubing using bottle caps with through-holes or parafilm such that the tubing does not slides out. Make sure that the inlet tubing reaches the bottom of the vessel.

NOTICE: Filter and degass all buffers/solvents used in the Contichrom system. Solids must not be present in the buffer bottles

WARNING: For handling chemicals laboratory goggles and gloves shall be worn at all times and a protective shield should be placed in front of the Contichrom system.

WARNING: Do not fill buffers overhead due to risk of spillage

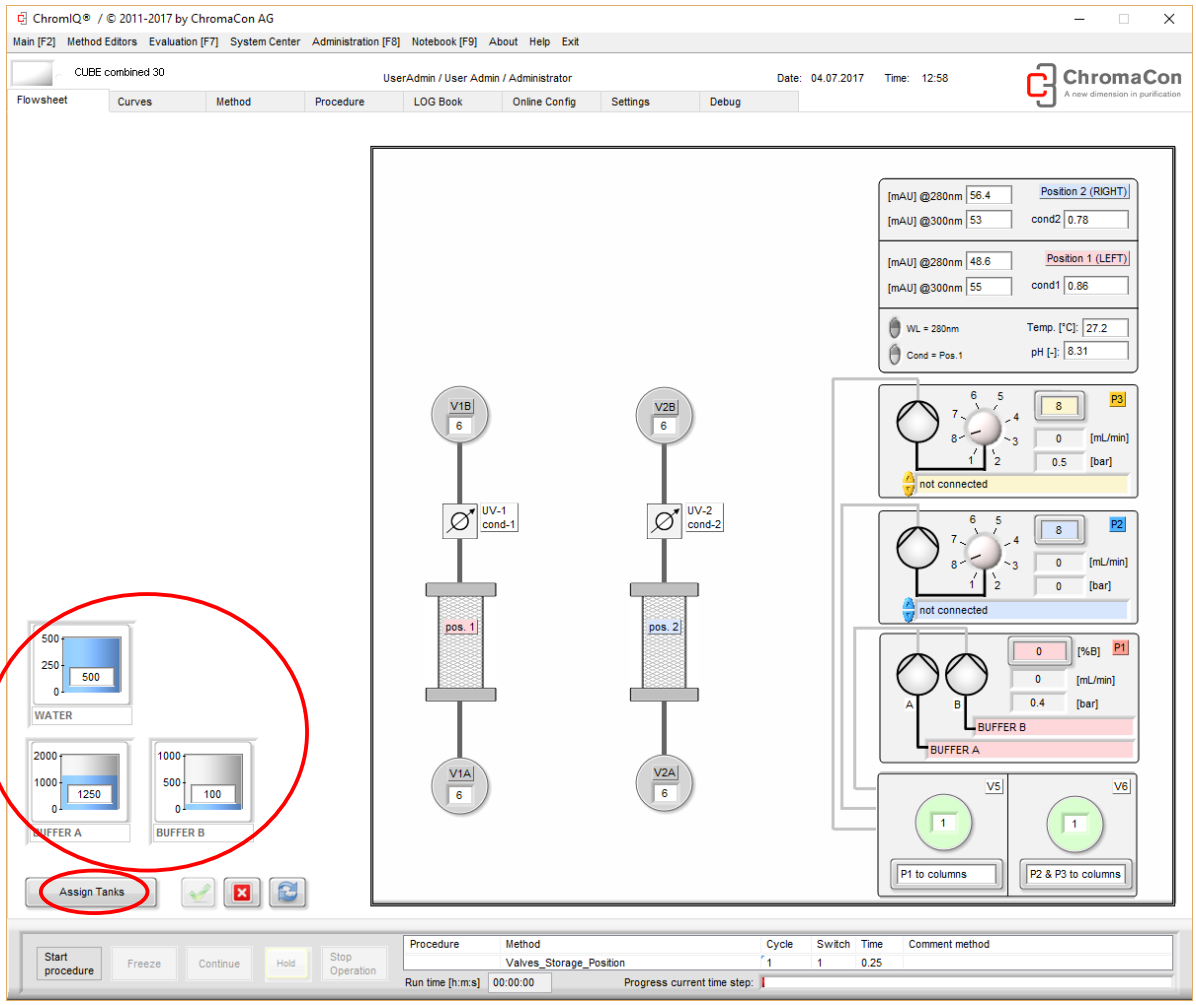
WARNING: Use a suitable, approved and safe aid to step-on when placing buffer containers into the buffer tray on the Contichrom system.

2.2. BUFFER TANK ASSIGNMENT AND TANK LEVELS

The following descriptions refer to both buffers and solvents which are referred to commonly as “buffers” in the following. The ChromIQ software includes a buffer management system that calculates and updates current buffer levels based on the pump flow rates. The buffer levels should be entered correctly and checked before starting a procedure. The inlets of the suction side tubing should be fully submerged in the buffer bottles and reach the bottom of the respective bottles.

NOTICE: It is strongly recommended to refill and enter buffer levels diligently and to ensure that the inlet tubing reaches the bottom of the vessel. This prevents the pumps from running dry and becoming damaged.

In order to set the buffer levels in the ChromIQ software navigate to the “Flow sheet” tab (see figure below) where the virtual tanks are displayed. The filling level is shown graphically as well as in numbers (unit mL).

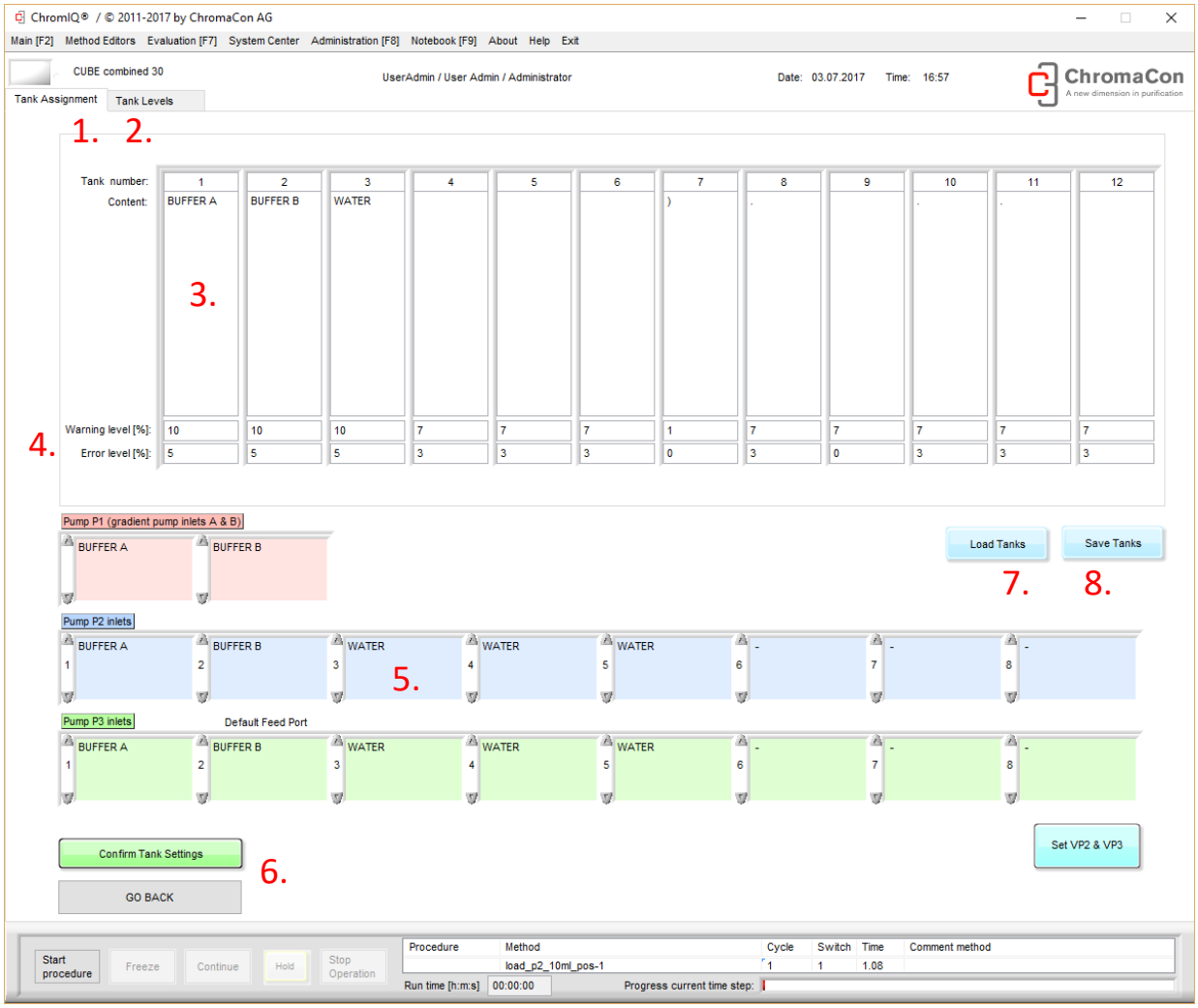


Screen shot of the Flow sheet tab. Here four tanks are assigned to the pumps. The maximum levels of all tanks are displayed as well as the actual levels.

The virtual tanks should be updated whenever the real tanks are refilled in order to display the correct filling levels and to trigger warning messages when appropriate.

Each tank can be assigned to one or more pumps and inlets. Tanks are only displayed if they are connected to a pump inlet.

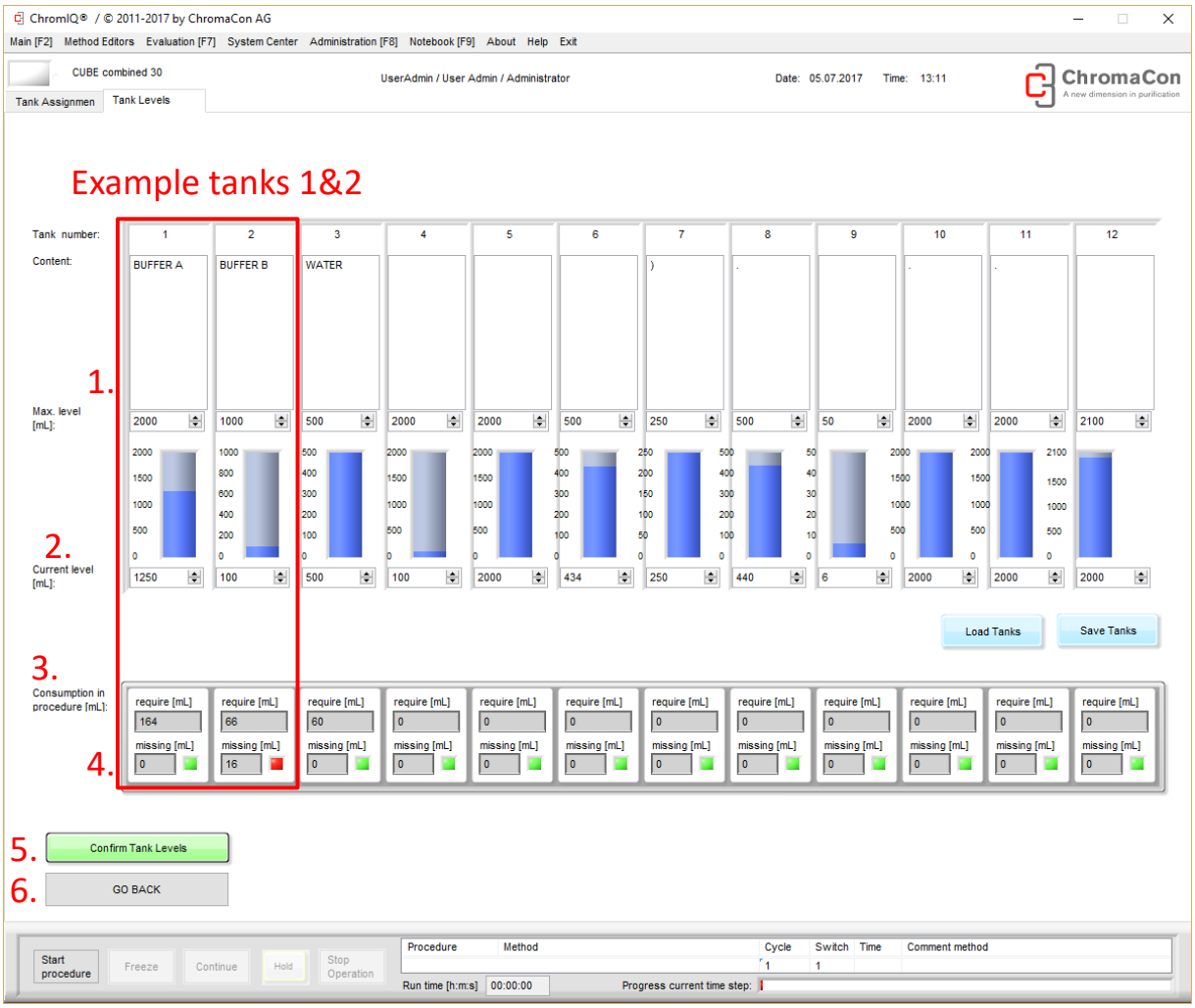
By pressing the button ”Assign Tanks” in the lower left of the flow sheet tab (see Figure below), a window with two tabs opens. The tabs are “Tank Assignment” (1.) and “Tank Levels” (2.) (see Figure below).



Screenshot of the tank assignment tab. The content of every single tank, an error level and a warning level can be set and the tanks can be assigned to pump inlets.

The tank assignment tab allows assigning the tanks to pump inlets and to name the tanks. A tank can be named by entering the name into the “content” field (3.). It is necessary to press “Confirm tank settings” (6.), to display the new name in the tank assignment table. Tanks are assigned to pump inlets by selecting the buffers for the individual pumps by means of the arrows in the buffer assignment field (5.). After tank assignment to the pump inlets the “Confirm tank settings” button needs to be pressed in order to activate the new data entries. Warning and error levels can be set in this tab as well (4.). They are given in % of the tank volume (defined in the “Tank Levels” tab). If a tank level drops below the warning level, a warning is displayed. If the tank level further drops below the error level, an error message is displayed and the system enters the “freeze” mode, i.e. the pumps are stopped to prevent them from running dry. Tank names can be changed in the “content” field. In the example shown above this example only the buffer 1, 2 and 6 are connected to the pumps. Accordingly these tanks are the only ones that are displayed in the flow sheet tab. A set of tank settings can be saved (8.) and loaded (7.) at a later stage.

Once the general parameters of the tanks have been set in the **Tank Assignment** tab the buffer levels can be set in the **Tank Level** tab. This tab is shown in the figure below.



"Tank Level" tab showing the (1.) the maximum level, (2.) actual level, (3.) buffer requirement for the current procedure and (4.) any 'missing' volumes. The "save" tank settings button (5.) and the "back to flowsheet" (6.) button are indicated.

Each column of the uppermost table in the **Tank Levels** screen shows the buffers that were set up earlier in the **Tank Assignment** screen. The maximum level (1. in figure above) corresponds to the original volume of buffer prepared, and the current level (2.) corresponds to the current volume of buffer currently in each tank. If a value is entered for the "current level" which is larger than the maximum level, the "current level" will be automatically reduced to the maximum level.

The lower table displays the total buffer requirement for all the methods in the current procedure (3.). If there is a lack of a particular buffer, the missing volume will be indicated in the lower box ("missing [mL]") next to a red warning square (4.), see "example buffer B" in the figure above. The buffer volume "missing" includes also takes the buffer added as safety margin into account (error level). In the example above buffer tank 2 "buffer B" contains 100 mL of buffer B. The buffer requirement is 66 mL and the software indicates that 16 mL are missing because the error level of the system was set to 5% which means that a safety volume of 50 mL is remaining in the tank (66 mL – 50 mL = 16 mL).

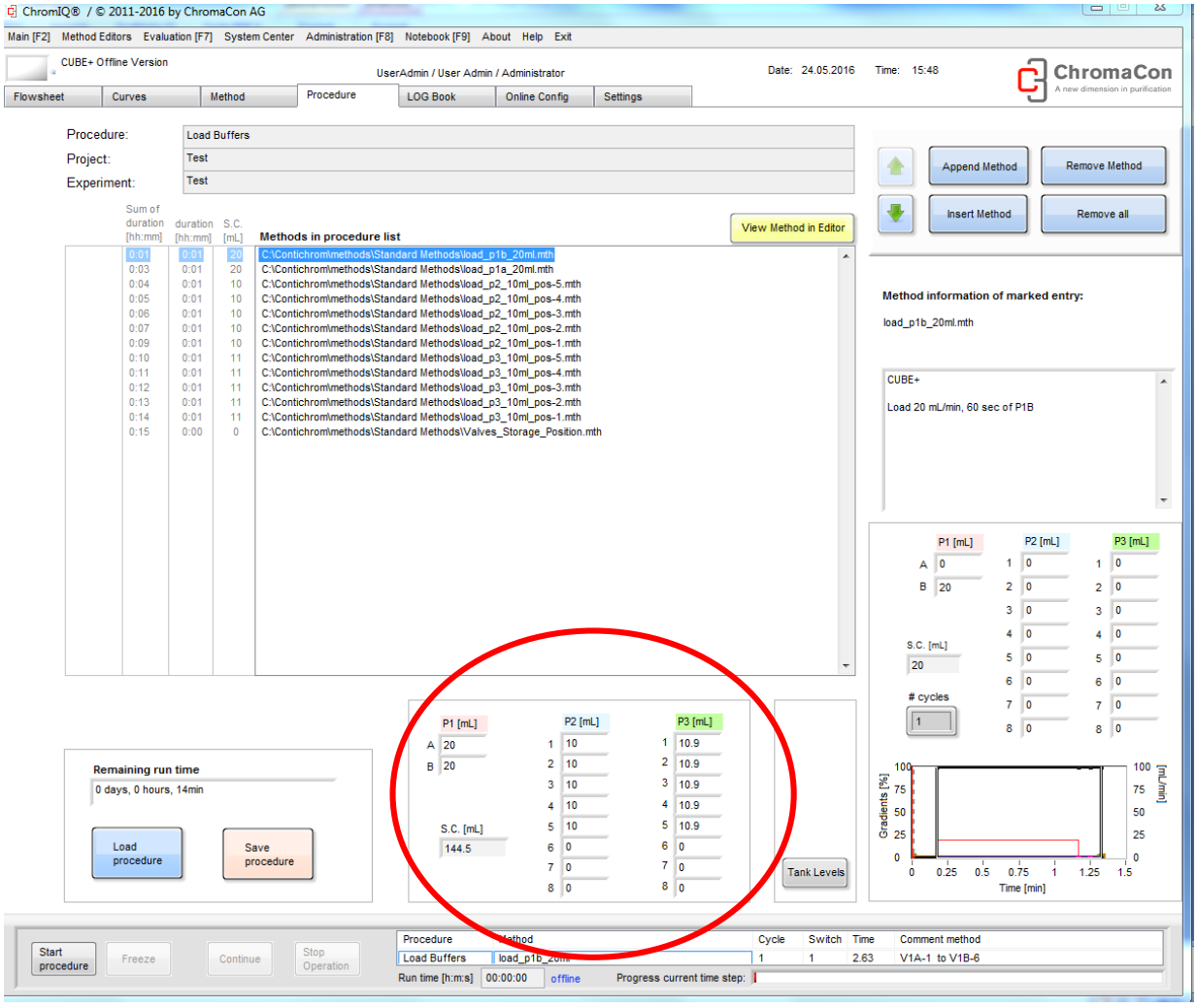
If there is sufficient buffer available, the missing amount is zero and a green square is displayed.

This information enables the user to estimate the buffer volumes required for the experiment. It is recommended to prepare sufficient buffers before starting a procedure and to fill up the buffer levels physically and in the ChromIQ software.

NOTICE: It is strongly recommended to refill and enter buffer levels diligently and to ensure that the inlet tubing reaches the bottom of the vessel. This prevents the pumps from running dry and becoming damaged.

2.3. PREPARING THE FLUID OUTLETS

All outlet / waste capillaries must be guided to collection vessels. The collection vessels must be large enough to hold the volumes of liquids accumulating during the procedure. The liquid volumes consumed in the procedure by pump channel can be viewed in the “procedure tab”, see red oval in screenshot below:



Screenshot of the procedure tab. The content of every single tank, an error level and a warning level can be set and the tanks can be assigned to pump inlets.

The fraction collection vessels should be large enough to accommodate the selected fraction volumes.

The fraction collector drain tube must be connected to a waste line/waste vessel capable of collecting all volumes entering the fraction collector during a run.

All waste containers must be placed such that the maximum filling level is at least 10” (25 cm) below the bottom of the Contichrom instrument.

WARNING: Choosing fraction collection or outlet vessels that are too small to accommodate the fluid volumes produced during the procedure results in overflow of the collection / outlet vessels. A hazardous situation may arise when liquids that are flammable or conductive are overflowing and get in contact with sources of ignition or electricity. Make sure that the secondary drain of the fraction collector routes liquid from the drip tray of the fraction collector to a safe collection point.

The inlet tubings for self-flushing of the pumps must be submerged in containers with flush solution. It is recommended to guide the outlet tubing of the self-flushing installation to the same container in order to prevent the self-flushing installation from running dry.

NOTICE: All waste containers must be placed such that the maximum filling level is at least 10” (25 cm) below the bottom of the CUBE / CUBE Combined instrument.

2.4. FILLING THE PUMP SEAL WASH

In order to fill the pump seal wash prepare:

- For CUBE 30/100 system: one bottle (recommended 500mL) with seal wash solution
- For the CUBE Combined 30/100 and Contichrom HPLC 30/100 system: two bottles (recommended 500mL) with seal wash solution.

Use 20% IPA/water mix or 30% Ethanol/water mix as seal wash flush solution.

WARNING: Do not use flammable solutions for the pump seal wash.

The pump seal wash is self-priming, however, when the seal wash lines are filled with air, e.g when installing the system for the first time, or after relocation, the lines need to be manually primed.

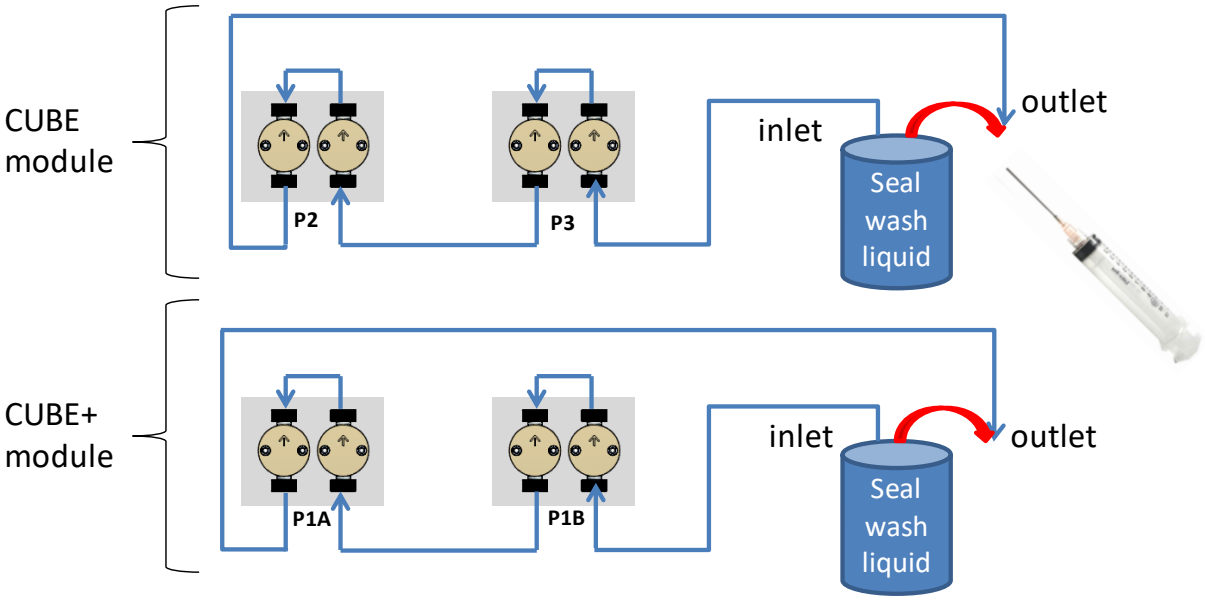
In order to manually fill the pump seal wash, proceed as follows:

1. Place the inlet tubing of the pump seal wash in the bottles and leave the outlet of the pump seal wash outside. Make sure that the inlets are fully submerged in the washing solution. (see schematic below).
2. Place a syringe with blunt needle in one of the outlets and draw liquid out the air and liquid into the line.

3. CUBE Combined 30/100 and Contichrom HPLC 30/100 system: Repeat the above procedure for the second pump seal wash outlet.

4. Place the pump seal wash outlets back in the respective bottles.

NOTICE: It is strongly recommended to ensure that the self-flushing feature is working properly. The flushing solution washes away any buffer salts that have precipitated onto the piston. If not removed, these precipitates can abrade the high-pressure seal and cause premature seal failure, leakage, and can possibly damage the pump.

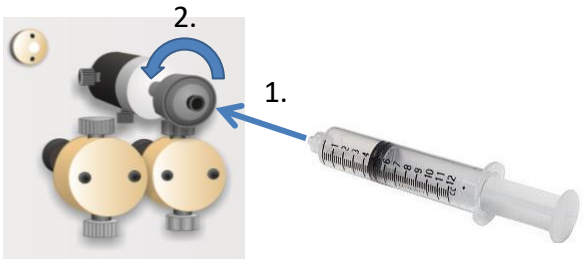


Schematic of the pump seal wash fluid path of a CUBE Combined 30/100 and Contichrom HPLC 30/100

2.5. FILLING THE PUMPS

When the Contichrom system is used for the first time, or when significant portions of the inlet tubing are filled with air, the pumps need to be filled with buffers or feed solution manually.

For manual filling, connect a syringe of at least 10 mL volume to the priming valve (1.). Open the prime/purge valve 1 to 2 turns (counter-clockwise). Prime the pump by pulling liquid and any air bubbles through the system and into the syringe (a minimum of 10 mL). Close the prime/purge valve and remove the syringe.

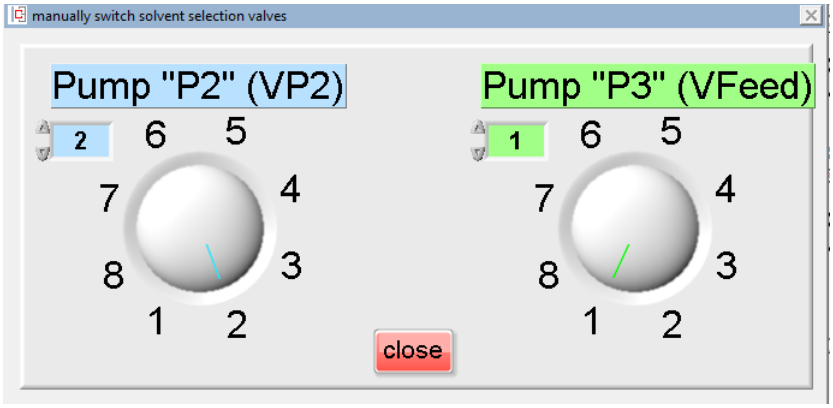


Pumps P1A and P1B of the bottom module of the CUBE Combined 30/100 and Contichrom HPLC 30/100 system, respectively, have only one suction side fluid inlet each that is to be primed as described above.

Pumps P2 and P3 (corresponding to pumps PA and PB of the CUBE 30/100 system) can have multiple buffers connected to them on the suction side through the buffer selection valves. In order to prime each of the inlets,

use the manual valve control (see picture below for CUBE Combined 30/100 and Contichrom HPLC 30/100 system, respectively) to switch through the buffer selection valve positions that are in use and repeat the above described procedure for every inlet.

Make sure that the last buffer to be primed is a buffer compatible with the initial steps of the following chromatography runs (typically equilibration buffer).



NOTICE: When priming the pumps, make sure that no liquids that are incompatible and/or lead to precipitation are mixed or are primed consecutively. Precipitates can lead to pump damage and tubing / capillary blocking.

2.6. STANDARD METHODS FOR PRIMING PUMPS

The ChromIQ software includes a number of standard methods for loading of buffers and priming of the pumps. The methods are located in the folder **Standard_Methods**. (The methods in the folder **System_Qualification** are for system qualification and for maintenance).

NOTICE: The standard software methods for priming the pumps are only functional if the pumps and the tubes on the suction side are filled with liquid. Air must be removed by manual purging before filling the pumps using the software methods.

METHOD "LOAD_BUFFERS_5-POSITIONS.MTH"

Contichrom CUBE Combined 30/100, Contichrom HPLC 30/100: This method primes the inlets on the low pressure side of the pumps P1, P2 (inlets 1-5) and P3 (inlets 1-5). The method consists of seven time steps. The flow rate is 30 mL/min for the pumps P2 and P3 in all time steps and 10 mL/min for the gradient pump P1. P1A and P1B are loaded with 6.6 mL each while each of the inlets 1 to 5 of P2 and P3 are filled with 10 mL of new buffer. At the end of the method the pump heads (except P1B) are primed with the buffer connected to inlet A (P1A) or inlet 1 (P2, P3), respectively. The last step sets all pumps are set to a flow rate of 0 mL/min.

Contichrom CUBE 30/100: This method primes the inlets on the low pressure side of the pumps P1A, P1B (inlets 1-5). The method consists of seven time steps. The flow rate is 30 mL/min in all time steps. Each of the inlets 1 to

5 of P1A and P1B are filled with 10 mL of new buffer. At the end of the method the pump heads are primed with the buffer connected to inlet 1 respectively. The last step sets all pumps are set to a flow rate of 0 mL/min.

The total duration of the method is approximately 2 min.

METHOD "LOAD_BUFFERS_7-POSITIONS.MTH"

Contichrom CUBE Combined 30/100, Contichrom HPLC 30/100: This method primes the inlets on the low pressure side of the pumps P1, P2 (inlets 1-7) and P3 (inlets 1-7). The method consists of seven time steps. The flow rate is 30 mL/min for the pumps P2 and P3 in all time steps and 10 mL/min for the gradient pump P1. P1A and P1B are loaded with 6.6 mL each while each of the inlets 1 to 7 of P2 and P3 are filled with 10 mL of new buffer. At the end of the method the pump heads (except P1B) are primed with the buffer connected to inlet A (P1A) or inlet 1 (P2, P3), respectively. The last step sets all pumps are set to a flow rate of 0 mL/min.

Contichrom CUBE 30/100: This method primes the inlets on the low pressure side of the pumps P1A, P1B (inlets 1-7). The method consists of seven time steps. The flow rate is 30 mL/min in all time steps. Each of the inlets 1 to 7 of P1A and P1B are filled with 10 mL of new buffer. At the end of the method the pump heads are primed with the buffer connected to inlet 1 respectively. The last step sets all pumps are set to a flow rate of 0 mL/min.

The total duration of the method is approximately 3 min.

METHODS TO FILL ONE INLET OF A PUMP ONLY

The following methods fill the specified inlets of the pumps and the pumps themselves. Note that a standard procedure "Load_buffers.prc" is available that includes all the methods listed below (see chapter "standard procedures"), **except for the ones related to inlets 6 and 7**.

The total duration of each method is approximately 1.5 min.

Methods for flushing single inlets of pumps

Contichrom CUBE Combined 30/100 Contichrom HPLC 30/100			Contichrom CUBE 30/100		
method name	pump	inlet	method name	pump	inlet
Load_P1_A_20mL.mth	1	A			
Load_P1_B_20mL.mth	1	B			
Load_P2_10mL-pos1.mth	2	1	Load_P1A_10mL-pos1.mth	1A	1
Load_P2_10mL-pos2.mth	2	2	Load_P1A _10mL-pos2.mth	1A	2
Load_P2_10mL-pos3.mth	2	3	Load_P1A _10mL-pos3.mth	1A	3
Load_P2_10mL-pos4.mth	2	4	Load_P1A _10mL-pos4.mth	1A	4
Load_P2_10mL-pos5.mth	2	5	Load_P1A _10mL-pos5.mth	1A	5
Load_P2_10mL-pos6.mth	2	6	Load_P1A _10mL-pos6.mth	1A	6
Load_P2_10mL-pos7.mth	2	7	Load_P1A _10mL-pos7.mth	1A	7
Load_P3_10mL-pos1.mth	3	1	Load_P1B_10mL-pos1.mth	1B	1
Load_P3_10mL-pos2.mth	3	2	Load_P1B _10mL-pos2.mth	1B	2
Load_P3_10mL-pos3.mth	3	3	Load_P1B _10mL-pos3.mth	1B	3
Load_P3_10mL-pos4.mth	3	4	Load_P1B _10mL-pos4.mth	1B	4
Load_P3_10mL-pos5.mth	3	5	Load_P1B _10mL-pos5.mth	1B	5
Load_P3_10mL-pos6.mth	3	6	Load_P1B _10mL-pos6.mth	1B	6
Load_P3_10mL-pos7.mth	3	7	Load_P1B _10mL-pos7.mth	1B	7

2.7. METHOD FOR CAPILLARY FILLING AND SYSTEM STORAGE

METHOD FILL_CAPILLARIES_WITH_BUFFER_A.MTH

NOTICE: Before running this procedure, please remove the columns!

This method fills all capillaries of the system on the high pressure side (behind the pumps) with the buffer connected to inlet A (P1A/P1B) and inlet 1 (P2 and P3). The method is used to clean the instrument and to prime the system with equilibration buffer before starting a new series of experiments.

The total duration of the method is approximately 15 min.

METHOD VALVES_STORAGE_POSITION.MTH

This method sets the valves to positions that are suited for long time storage. The positions are:

- V1A: 6
- V1B: 6
- V2A: 6
- V2B: 6
- V5: 1
- V6: 1

The drain valves are switched for connecting the pumps to the system (not in “drain” position). The valves before the column positions are set to a closed position so the pumps cannot empty themselves through the static pressure in this position. All pump flow rates are zero in this method.

The total duration of the method is approximately 15 sec.

2.8. METHODS FOR UV-SENSORS

METHOD UV AUTOZERO.MTH

This method performs an auto zero on both UV detectors.

The total duration of the method is approximately 3 sec.

METHOD BACKFLUSH_DETECTORS.MTH

NOTICE: Before running this procedure, please remove the columns!

This method back-flushes the UV detector using the buffer on pump P2 (pump P1A in Contichrom CUBE 30/100), inlet 1. It is used to remove persisting air bubbles from the detector.

Each flow cell is flushed for 30 sec at 10 mL/min top-down and subsequently for 30 sec at 10 mL/min bottom-up.

The total duration of the method is approximately 5 min.

2.9. OTHER METHODS

METHOD PAUSE.MTH

This method sets the valves to storage position and pauses the system, i.e. the run time is halted and the pump flow rate set to zero. The run can be continued by pressing the “continue” button or aborted by pressing “stop the run”.

FRAC_HOME.MTH

This method sends the fractionators back to its home position.

The total duration of the method is approximately 3 sec.

2.10. STANDARD PROCEDURES

The standard procedures are intended for frequently recurring operations such as priming with new buffers, cleaning and storage.

LOAD_BUFFERS.PRC

The procedure for loading buffers/solvents “load_buffers.prc” in the folder “standard procedures” includes all methods listed in the sub-chapter “METHODS TO FILL ONE CHANNEL OF A PUMP ONLY” and is concluded by the method “Valves_storage_position.mth” except for the methods related to inlets 6 and 7. It is used for priming the system with new buffers. The columns may remain mounted during this procedure. To prime also the lines of inlet 6 and 7, the corresponding methods have to be amendend manually to the procedure. Use a binding buffer or solvent as last method in the procedure to ensure that the pump heads are filled with a buffer/solvent, favoring adsorption.

CLEANING.PRC

NOTICE: Before running this procedure, please remove the columns!

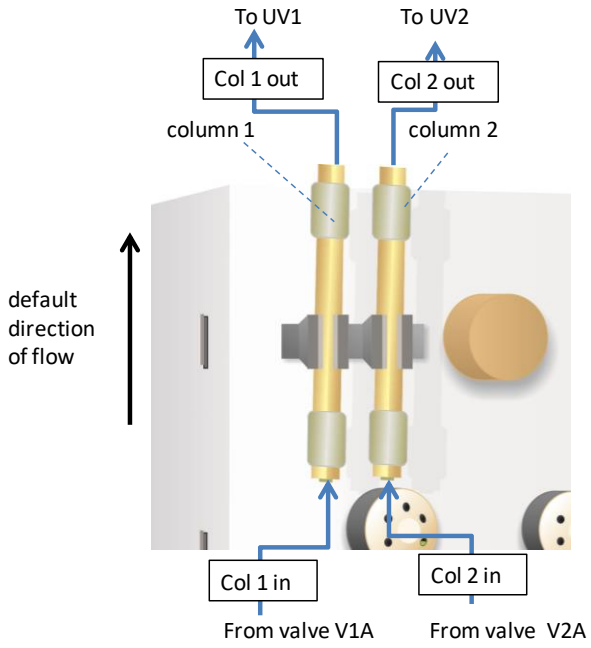
The procedure for loading buffers “cleaning.prc” in the folder “cleaning” includes 2x the method “load_buffers_5-position.mth”, followed by “fill_all_capillaries_with_buffer_A.mth” and “Valves_storage_position.mth”, It Is generally used to prime, clean and / or store the system. The columns have to be removed for this procedure.

2.11. CREATING METHODS AND PERFORMING RUNS

The ChromIQ software uses wizards to facilitate method generation. Details on the method programming are provided in the ChromIQ software manual

2.12. PREPARING AND MOUNTING THE COLUMNS

Columns of an outer diameter of equal or less than 10 mm (0.4”) can be mounted using the column clamps provided with the Contichrom system. The column clamps can be clicked into the rail located on the upper left side of the system (looking from the front, see below).



Columns of outer diameter of > 10 mm have to be mounted using a separate stand and clamps (see below).

Union pieces (see picture below) should be used as placeholders when no columns are used and when system storage methods are carried out.

NOTICE: When the columns are not in use, union pieces must be placed at their positions instead in order to prevent the capillaries from drying out, which could leave solids behind.

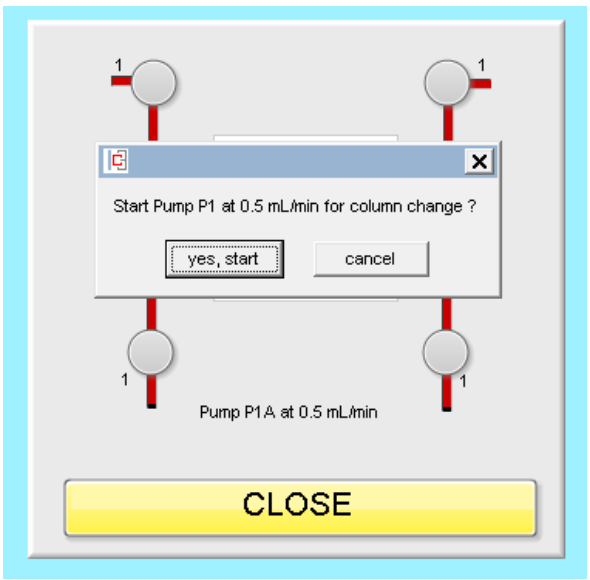
Generally the direction of flow in the Contichrom system and in the columns is bottom-up. For the twin-column chromatography processes 2C-PCC (CaptureSMB), MCSGP, and N-Rich, two columns, packed with the same resin must be used. The columns should be packed under the same conditions and to similar bed heights. Generally a difference of less than 10% in bed height is acceptable, a bed height difference of less than 5% is preferred.

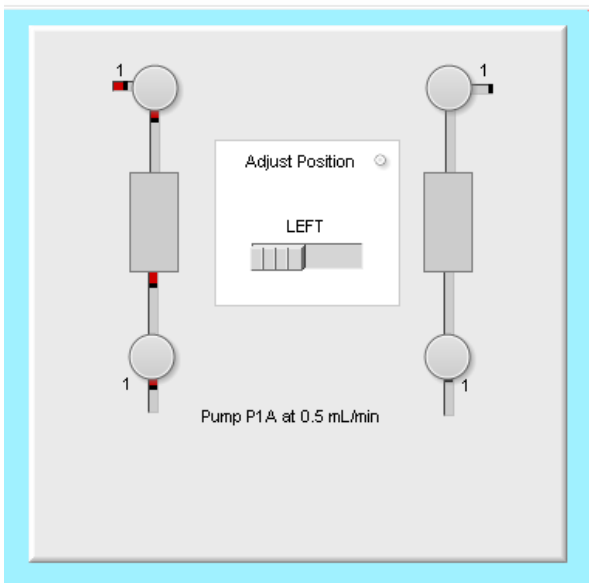
By definition, in the ChromIQ operating software, column 1 is connected to the left detector flow cell (when facing the Contichrom system front) and column 2 is connected to the right detector flow cell (when facing the Contichrom system front).

For mounting column 1, remove the placeholder (union piece) for column 1. Connect the capillary originating in the center position of V1A to the inlet of column 1. Connect the capillary originating in the bottom inlet of UV1 to the column outlet.

Likewise, for mounting column 2, remove the placeholder (union piece) for column 2. Connect the capillary originating in the center position of V2A to the inlet of column 2. Connect the capillary originating in the bottom inlet of UV2 to the column outlet.

For mounting the columns under flow, click on the columns in the flow sheet tab of the ChromIQ software. A window will occur allowing starting a flow of 0.5 mL/min and, once started, selection of left or right column position (see illustration below).





2.13. MOUNTING THE PH FLOW CELL AND ELECTRODE

The pH flow cell holder can be clicked into the rail located on the right side of the Contichrom system (looking from the front) or be placed into a tray (2017 version onwards), with the flow cell inlet facing the back of the Contichrom system. The pH electrode is mounted on the flow cell by means of a screw ring provided with the electrode. The pH electrode should point upwards.

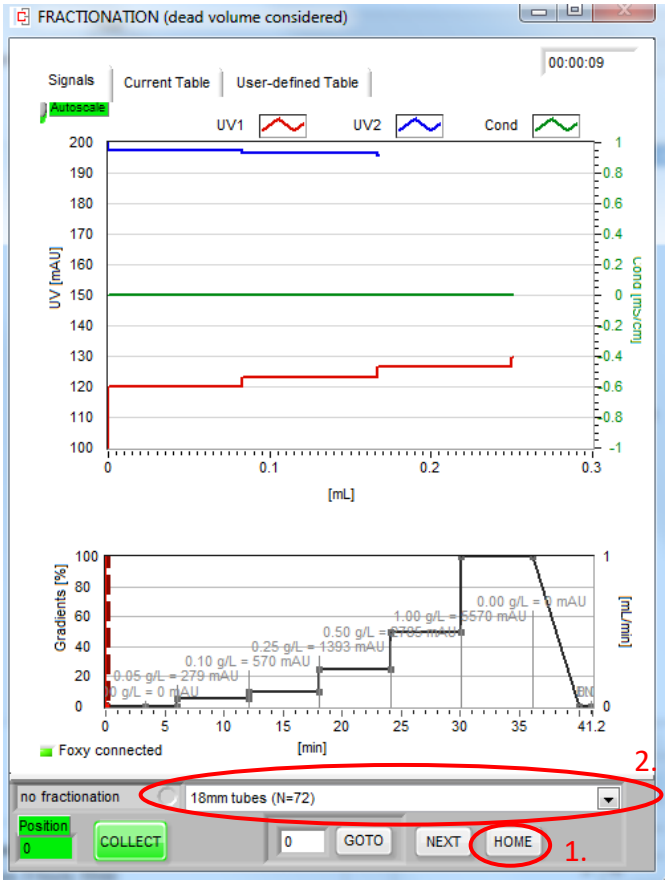
The pH of the waste (flow-through) outlet, the product outlet, or the strip outlet may be monitored by connecting the respective outlet capillaries to the pH flow cell inlet. The pH flow cell outlet must then be connected to an appropriate collection vessel using 1/16” capillaries (green).

NOTICE: It is strongly recommended to install the pH electrode/flow cell at a liquid outlet. The pH electrode should not be placed in between column valves due to the maximum operating pressure of 5 bar (70 psi) of the pH electrode

2.14. PREPARING THE FRACTION COLLECTOR

In the fraction collector window of the ChromIQ user interface, send the fractionator to its “home” position (see picture below, (1.)), so that the fractionator head doesn’t interfere with the preparation of the fraction collector.

Select the rack type from the pull down menu in the fraction collector window (see picture below, (2.)). The selection is saved automatically for future runs until another rack type is selected.



Insert a rack on the fraction collector capable of carrying the collection vessels required for the desired fractionation. Populate the rack with sufficient tubes.

The fraction collector drain tube must be connected to a waste line/waste vessel capable of collecting all volumes entering the fraction collector during a run.

For 15 mL centrifuge tubes choose the setting “18 mm tubes (N=72)”, for 50 mL centrifuge tubes select “25/28 mm vials (N=36)”.

WARNING: Choosing fraction collection or outlet vessels that are too small to accommodate the fluid volumes produced during the procedure results in overflow of the collection / outlet vessels. A hazardous situation may arise when liquids that are flammable or conductive are overflowing and get in contact with sources of ignition or electricity.

WARNING: The drain outlet and the drip tray fluid outlet of the fraction collector must be routed to a waste container in order to avoid spillage. A hazardous situation may arise when liquids that are flammable or conductive are overflowing and get in contact with sources of ignition or electricity.

2.15. OPERATION IN COLD CABINET OR COLD ROOM

NOTICE: When keeping the Contichrom system in a cold room or cold cabinet, leave it switched on permanently to avoid condensation.

NOTICE: The Contichrom system must not be operated in a cold cabinet when the cold cabinet cooling is not operational, due to the risk of overheating

NOTICE: When operating the Contichrom system in a cold room or cold cabinet, use a cold-room compatible computer or keep the computer outside the cold room / cold cabinet

3. OPERATING THE CONTICHROM SYSTEM

Chromatographic runs to be carried out on the Contichrom system are edited and saved as “methods”. A method is a sequence of commands for the individual hardware components.

A list of methods to be carried out sequentially is called a “procedure”. A comprises at least one method.

3.1. RECOMMENDED COLUMN SIZES

The following table provides a guideline to the recommended column dimensions and shows the typical loads for the individual processes. Below the specified minimum column inner diameters it is possible that under certain operating conditions the pumps are operating below their accuracy limit of 0.1 mL/min. With the listed maximum diameter the process can be run at a linear flow rate of 300 cm/h. The calculations for the feed requirement are based on a run duration of 5 cycles for 2C-PCC (CaptureSMB), N-Rich and MCSGP. Using 10 cm bed height is

recommended for scalability reasons. A 10 cm bed may be assembled by connecting 2x 5 cm columns in series with a low dead volume connection.

Recommended column sizes for Contichrom CUBE 30, Contichrom CUBE Combined 30 and Contichrom HPLC 30

Process	inner diameter [cm]		Bed height [cm]	Column vol. [mL]		typical load [g/L]	typical amount per run [mg]	
	min.	max.		min.	max.	per cycle	min.	max.
Batch	0.5	2.7	10	2.0	11.2	20.0	200	1100
2C-PCC (CaptureSMB)*	0.8	2.7	10	5.0	28.8	40.0	1000	6000
N-Rich	0.8	2.7	10	5.0	28.8	10.0	250	1400
MCSGP	0.8	2.7	10	5.0	28.8	10.0	250	1400
* monoclonal antibody captured by Protein A affinity chromatography								

Recommended column sizes for Contichrom CUBE 100, Contichrom CUBE Combined 100 and Contichrom HPLC 100.

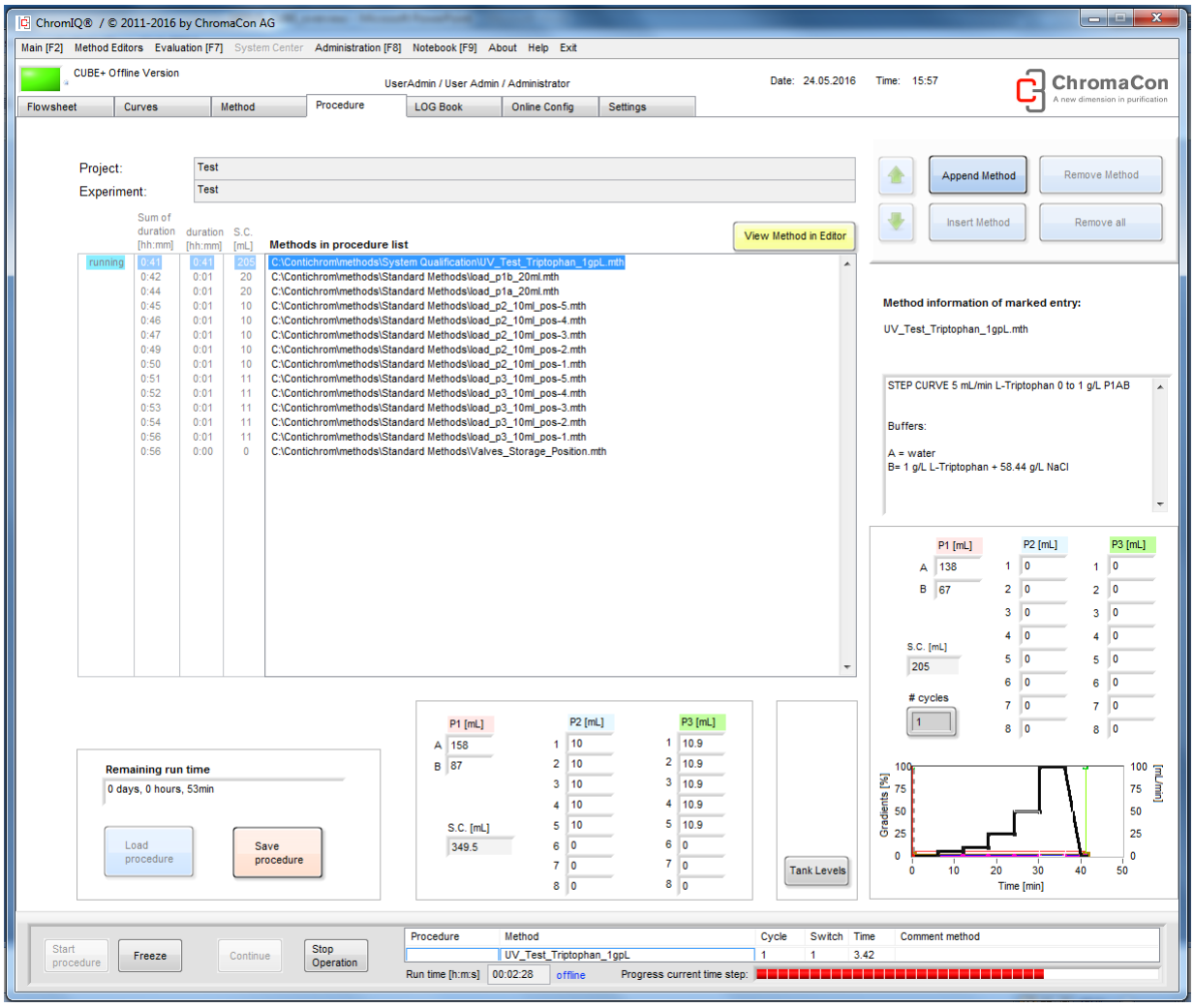
Process	inner diameter [cm]		Bed height [cm]	Column vol. [mL]		typical load [g/L]	typical amount per run [mg]	
	min.	max.		min.	max.	per cycle	min.	max.
Batch	0.5	5.0	10	2.0	38.6	20.0	200	4000
2C-PCC (CaptureSMB)*	0.8	5.0	10	5.0	98.7	40.0	1000	20000
N-Rich	0.8	5.0	10	5.0	98.7	10.0	250	5000
MCSGP	0.8	5.0	10	5.0	98.7	10.0	250	5000
* monoclonal antibody captured by Protein A affinity chromatography								

3.1. PREPARING A PROCEDURE TO BE RUN

In order to run a previously programmed method, navigate to the “Procedure” tab. Click on the button “Insert method” to load a method into the procedure. Additional methods can be added by clicking the “append method” button. The methods will be carried out sequentially starting with the first method. To remove a method from the list, select the method and click on “Remove Method”. In order to remove all methods, click on “Remove all”

In order to load a previously saved procedure, click on the “load procedure” button.

For a detailed description of the procedure tab, refer to the ChromIQ software manual.



Screenshot of the "Procedure" tab.

3.2. PROCEDURE FOR LOADING BUFFERS

A standard procedure for loading buffers "load_buffers.prc" is available and can be loaded using the "load procedure" button. This procedure includes methods for flushing pumps P1A and P1B with 20 mL buffers each and methods for flushing pumps 2 and 3, inlets 1-5 each. This procedure can be used for automated loading of new buffers, provided that no air is in the lines.

3.3. STARTING A RUN

In order to run a procedure, press the "start procedure" button located in the lower left corner of the ChromIQ user interface. It is recommended to include the method "Fill_all_capillaries_with_bufferA.mth". This method uses the inlets 1.

After having clicked the "start procedure" button a dialog appears, prompting for "project name" and "experiment name". In the ChromIQ measurement results folder structure, entering new names will generate a new folder with the project name and a new subfolder with the experiment name. Alternatively, existing project and experiment names can be used.

WARNING: The Contichrom system is generally operating under pressure. Wear safety glasses when operating it.

3.4. MANAGING A RUN

The **control bar** at the bottom of the ChromIQ user interface (see image below) allows execution of direct commands related to the procedure by four buttons:

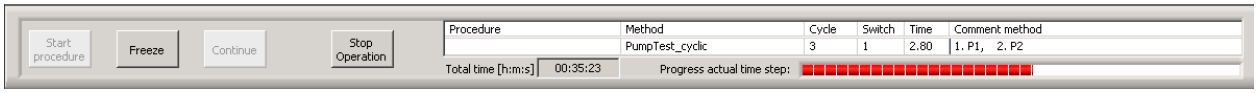
- Start procedure
- Freeze a procedure
- Continue a procedure
- Stop operation

Upon “Start procedure” a file browser opens (see above chapter “starting a run”). If a procedure is already running, the “Start procedure” button is disabled.

A running procedure can be paused using the “Freeze” command. Upon “Freeze” a prompt will occur asking for confirming the freeze mode. When “Freeze” is executed, all pumps immediately decrease their flow rates to zero. Valve positions and other settings remain unchanged. The run time of the method does not change. The time-point of the “Freeze” is logged and automatically a marker is generated and shown in the online signal tab.

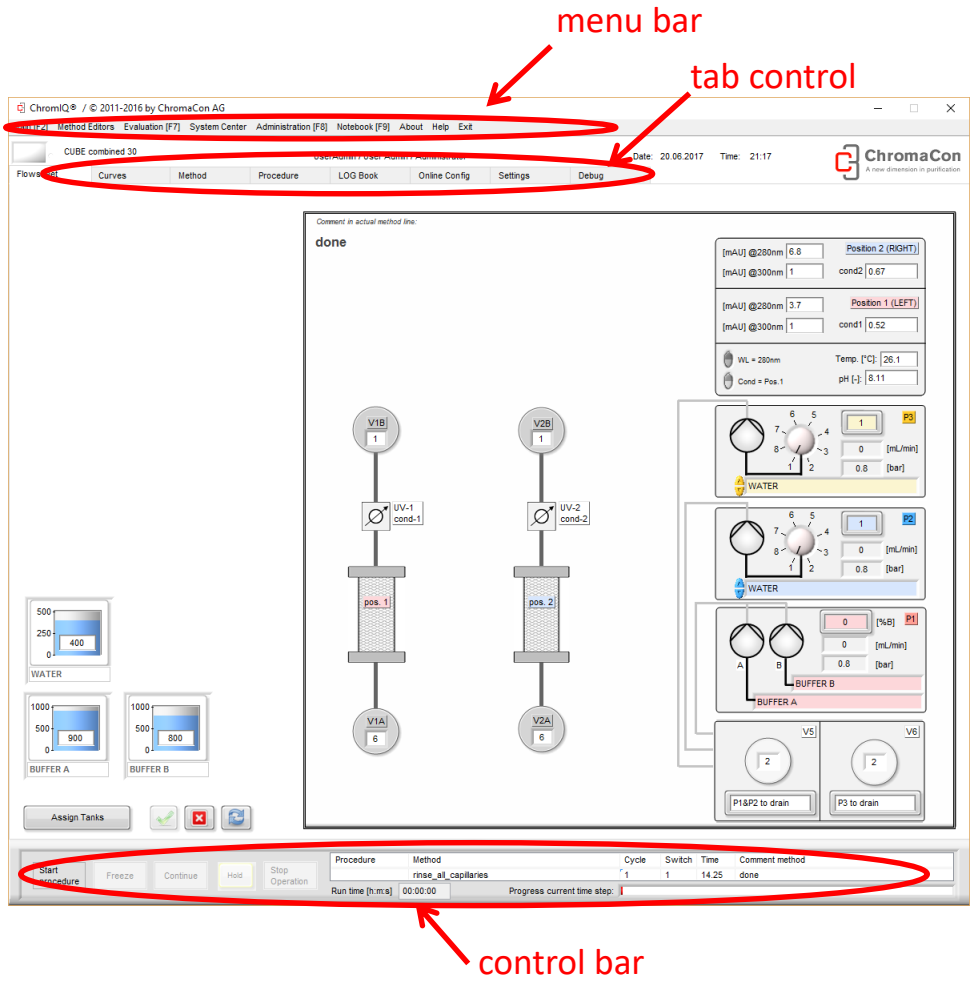
Upon “Continue” the procedure is continued after a “Freeze” command. When “Freeze” is not active, the “Continue” button is disabled.

Upon “Stop” a prompt will occur asking for confirming the stopping of the procedure. When “Stop” is executed, the pumps immediately decrease to zero flow. The procedure cannot be continued and the “Continue” and “Freeze” buttons become disabled.



Screenshot of the ChromIQ control bar. Since a procedure is already running “start procedure” and “continue” are disabled.

An example for the full ChromIQ user interface including menu bar, tab register and control bar is shown below:



Screenshot of the ChromIQ user interface with menu bar (top), tab register (top) and control bar (bottom), (Contichrom CUBE Combined 30/100 and Contichrom HPLC 30/100).

REFILLING BUFFERS DURING A RUN

Buffers may be refilled anytime during a run. However it must be ensured that the inlet tubing does not take in air during the refill operation. After having refilled the buffers, it must be checked that the inlet tubing reaches the bottom of the respective liquid vessel. The buffer levels must be updated in the tank management system (See chapter buffer tank assignment and tank levels")

NOTICE: It is strongly recommended to refill and enter buffer levels diligently and to ensure that the inlet tubing reaches the bottom of the vessel. This prevents the pumps from running dry and potentially becoming damaged.

During a run, the warnings "low buffer" or "buffers empty" may be displayed.

While the "low buffer" warning is displayed, the pumps continue to run.

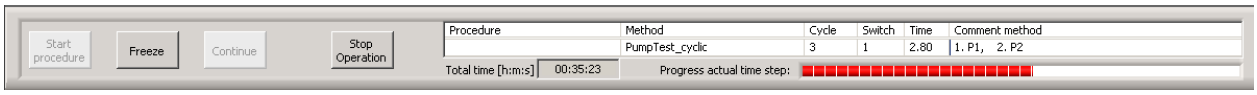
While the "tank empty" warning is displayed, the system is in freeze mode.

3.5. MONITORING A RUN

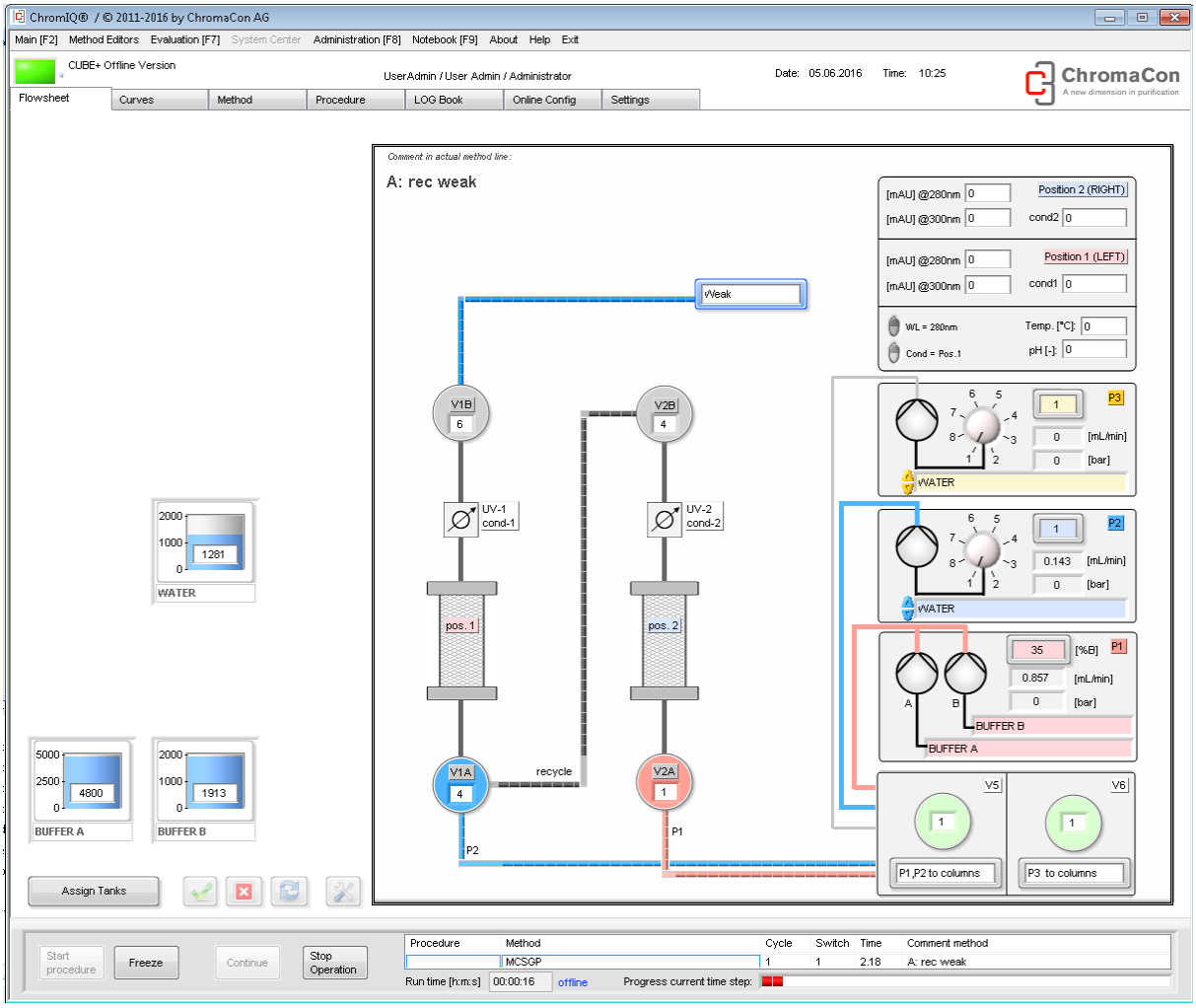
In case a procedure is running the control bar also shows:

- Procedure name
- Method name of method that is currently
- The cycle and switch number
- The progress of the current switch (= progress of current run if a single column batch method is carried out)
- The comment entered in the method for the respective time step

An example control bar from the ChromIQ software is shown below.

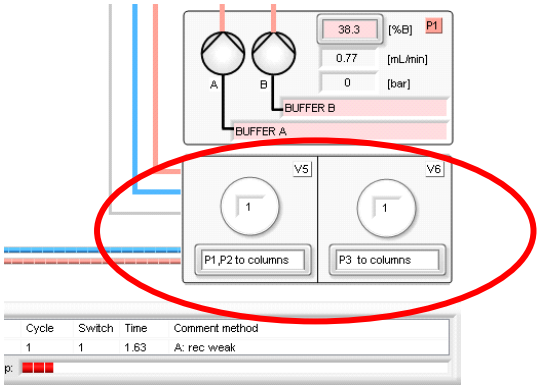


The flow sheet tab of the ChromIQ operating software shows the currently active flow paths of the Contichrom instrument and the currently set values of the valves and pumps, as well as the currently measured values of UV, conductivity, temperature, pH and pressure. In the example shown below there are physical connections between Pump P2 and valve V1A (through drain valve V5), between Pump P1 and valve V2A (through drain valve V5). There is also a connection between V2B and V1A and from V1B to the waste outlet (“weak”)

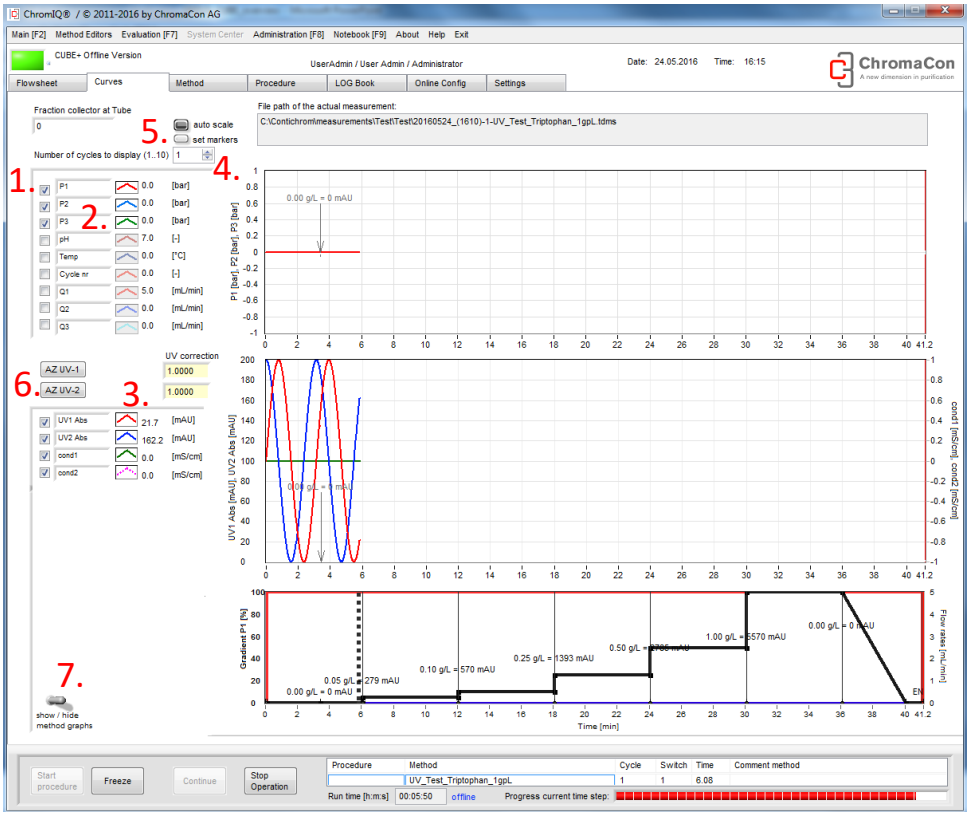


Screenshot of the Flowsheet tab. (Contichrom CUBE Combined 30/100 and Contichrom HPLC 30/100)

Flow paths in the Contichrom system become active / inactive through switching of the multiposition valves V1A, V1B, V2A and V2B. Moreover the drain multiposition valve V5 can direct the flow of the pumps P1A and P1B into the system (position 1), or to the drain (position 2), (Contichrom CUBE Combined 30/100 and Contichrom HPLC 30/100 only). The drain multiposition valve V6 can direct the flow of the pumps P2 and P3 (PA and PB for Contichrom CUBE 30/100) into the system (position 1), or to the drain (position 2), see screenshot below.



The “Curves”-tab of the ChromIQ user interface shows the online signals and the traces of the currently running method. The file path of the current measurement is displayed on the top of the curves as well as in the “control bar”.



Screenshot of the Curves tab

It is possible to choose the curves to be displayed in the upper and lower graph in the legend on the left by activating the check boxes (see screenshot above): (1.) and choosing the signal channel (2.). Typically, the top graph is used to show pump and pressure related information.

By pressing the right mouse button on the legend (3.), details can be adjusted for every single graph. These details are saved in the user setting of the software, so that the software keeps a set of user-defined line colors. The lower graph can display two signals on the left axis and two signals on the right axis.

When a cyclic process such as MCSGP is in operation, the number of cycles to be displayed in superposition, counting back from the last cycle, can be adjusted by setting the number of cycles in the upper left corner of the curves tab (4.). Up to 10 cycles can be superimposed during an experiment.

Markers are shown automatically in the curve charts. They can be deselected/displayed at (5.). A manual autoscale of both UV can be set directly from the curves tab (6.).

Zooming of the curves is possible by changing the values on the X and Y- axes as well as by simply left mouse button clicking on the graphs and moving the mouse while the button remains pressed. Unzooming can be performed by pressing the autoscale button (5.) or by left clicking on the graph.

It is possible to display a graph that shows all flow rates and gradient compositions of the currently running method as function of time below the two data-curves tab (7.). This graph contains markers defined in the method and a continuously updated cursor, which indicates the current time position of the run.

3.6. PROGRAMMING METHODS

The ChromIQ operating software offers a number of wizards for simple method programming of batch and twin-column processes. Also advanced method programming using the method expert mode editor is available. Refer to the ChromIQ Software Manual for detailed information on method programming.

WARNING: Do not exceed the maximum pressure of the columns

NOTICE: Columns and detectors may be damaged due to a pressure spike if a method is run that includes a step where a pump runs against a closed valve with high flow rate. Use the wizards for method programming to prevent these situations from happening.

3.7. EVALUATING A RUN

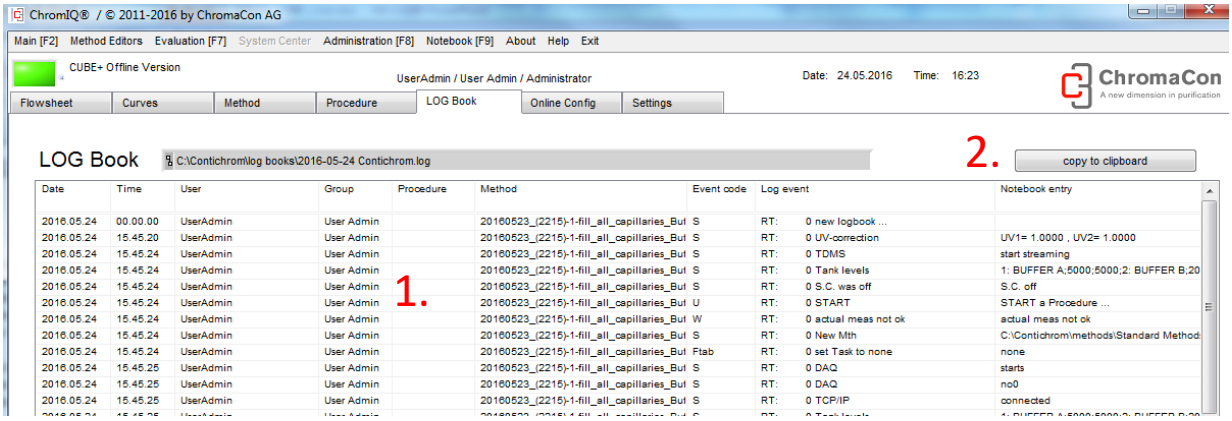
Previous, completed runs can be evaluated using the evaluation center of ChromIQ. The evaluation center is a sub-program of ChromIQ that is described in the evaluation center manual. The evaluation center is called from from the menu bar.

3.8. LOG BOOK

The LOG book tab (see Figure below) shows the log of system information for the current day generated automatically by ChromIQ and the user entries made using the notebook function (1.). The entries are sorted by date, time, user name, user group, procedure, method, event code, log event and notebook entry.

A new Log Book file is created each day that the system is in use and the complete daily log is saved to the folder C:\Contichrom\log books.

Log Book files can be reviewed in the Evaluation Center and exported (2.). More information is provided in the software manual



Screenshot of the top part of the "LOG book" tab

4. CLEANING THE SYSTEM

4.1. SIMPLE CLEANING

Simple cleaning of the system is recommended when the system is left un-operated for a period of 1-3 days (e.g. weekend). The simple cleaning flushes all buffers and feed solutions out of the system using de-ionized water.

It is recommended to carry out system cleaning in the following steps:

1. Store columns
2. Remove columns, insert union fittings instead
3. Insert all inlet tubing into a bottle filled with at least 1L of de-ionized water
4. Load the procedure "cleaning.prc". This procedure will prime all inlet tubing and fill all capillaries with de-ionized water. It assumes that on pump 2 and pump 3 the inlets 1-5 are in use. When using a larger number of inlets, the cleaning procedures must be adjusted by methods that load buffer through the respective inlet of the respective pump. For example "load_p2_10ml_pos6" must be inserted in case inlet 6 of pump 2 is in use. Likewise, if fewer inlets are in use, the respective methods must be removed after having loaded the procedure.
5. Run the procedure "cleaning.prc".

4.2. THOROUGH CLEANING

Thorough cleaning of the system is recommended in regular intervals in order to ensure proper operation and high product quality. Thorough cleaning will also clean the fraction collector, and all flow cells that are connected to the system (UV, conductivity, pH). Extraordinary cleanings should be performed at product/project changeover, before storing the system for a longer period (weeks) or before relocating the system.

WARNING: The cleaning procedure uses 1M NaOH and 20% Acetic Acid. Wear protective gear and use a protection shield

WARNING: Chemicals and substances present in the system due to incomplete or missing instrument cleaning may harm the receiving operators and lead to instrument damage due to deposit formation.

System cleaning must be carried out according to the following steps:

1. Store the columns
2. Remove columns, insert union fittings instead
3. Insert all inlet tubing into a bottle filled with at least 2L of de-ionized water
4. Insert all outlet tubing into a waste bottle of at least 1L volume
5. Load the procedure “cleaning.prc” in the folder “cleaning”. This procedure includes 2x the method “load_buffers_5-position.mth”, followed by “fill_all_capillaries_with_buffer_A.mth” and “Valves_storage_position.mth”, The columns have to be removed for this procedure. It will purge all inlet tubing and fill all capillaries with de-ionized water. In this procedure on pump 2 and pump 3 the inlets 1-5 are in use. When using a larger number of inlets, the cleaning procedures must be adjusted by methods that load buffer through the respective inlet of the respective pump. For example “load_p2_10ml_pos6” must be inserted in case inlet 6 of pump 2 is in use. Likewise, if fewer inlets are in use, the respective methods must be removed after having loaded the procedure
6. Run the procedure “cleaning.prc”
7. Insert all inlet tubing into a bottle filled with at least 1L of 1M NaOH
8. Run the procedure “cleaning.prc”. This procedure purges all inlet tubing and fills all capillaries with 1M NaOH. Adjust the procedure for the number of inlets if necessary.
9. Leave the system in 1M NaOH for 15-60min. The NaOH will hydrolyze any protein in the system and sanitize the system
10. Insert all inlet tubing into a bottle filled with at least 2L of de-ionized water
11. Run the procedure “cleaning.prc”. This procedure purges all inlet tubing and fills all capillaries with de-ionized water, removing the NaOH. Adjust the procedure for the number of inlets if necessary
12. Insert all inlet tubing into a bottle filled with at least 1L of 20% Acetic Acid
13. Run the procedure “cleaning.prc”. This procedure purges all inlet tubing and fills all capillaries with 20% Acetic Acid. Adjust the procedure for the number of inlets if necessary
14. Leave the system in 20% Acetic Acid for 15-30min
15. Insert all inlet tubing into a bottle filled with at least 2L of de-ionized water

16. Run the procedure “cleaning.prc”. This procedure purges all inlet tubing and fills all capillaries with de-ionized water, removing the Acetic Acid. Adjust the procedure for the number of inlets if necessary
17. Insert all inlet tubing into a bottle filled with at least 1L of 20% Ethanol
18. Run the procedure “cleaning.prc”. This procedure purges all inlet tubing and fills all capillaries with 20% Ethanol. Adjust the procedure for the number of inlets if necessary
19. Leave the system in 20% Ethanol for 15-30min. After this step the system may be stored. For preparation of a new run, refer to steps 20. and 21.
20. Insert all inlet tubing into a bottle filled with at least 2L of de-ionized water
21. Run the procedure “cleaning.prc”. This procedure purges all inlet tubing and fills all capillaries with de-ionized water. Adjust the procedure for the number of inlets if necessary

For storing the system after cleaning, refer to the following chapter “storing the system”.

5. STORING THE SYSTEM

Storing the Contichrom system prevents microbial and/or algal growth in the system when the system is not operated for a longer time.

The recommended system storage solution is 20 vol% Ethanol in de-ionized water.

NOTICE: Run a cleaning procedure before running the storage procedure. High salt buffers may form precipitates when getting in contact with the storage solution and must be removed by a cleaning procedure.

After having completed the system cleaning run the storage procedure as follows:

1. Insert all inlet tubing into a bottle filled with at least 1L of storage solution
2. Run the procedure “cleaning.prc” for the CUBE Combined system. This procedure purges all inlet tubing and fills all capillaries with the storage solution. It assumes that on pump 2 and pump 3 the inlets 1-5 are in use. When using a larger number of inlets, the cleaning procedures must be adjusted by methods that load buffer through the respective inlet of the respective pump. For example “load_p2_10ml_pos6” must be inserted in case inlet 6 of pump 2 is in use. Likewise, if fewer inlets are in use, the respective methods must be removed after having loaded the procedure

After the system storage procedure has been completed, the system may be shut down.

In order to shut down the system, switch off the CUBE module using the power switch on the side of the instrument. Also turn off the CUBE+ module using the power switch on the side if present.

NOTICE: When keeping the Contichrom system in a cold room or cold cabinet, leave it switched on permanently to avoid condensation.

5.1. STORING THE PH ELECTRODE

If the pH electrode is not used for a week or longer, storage of the pH electrode is recommended.

To store the pH electrode proceed as follows:

1. Prepare about 20 mL of a pH electrode storage solution (50:50 mixture of 4 M potassium chloride and pH 4 standard buffer)
2. Fill the storage solution into a 50 mL centrifuge tube and place the tube in a stand. Alternatively use a tube with standing ring bottom.
3. Replace the pH electrode by a dummy electrode and place the pH electrode in the centrifuge tube such that the tip is submerged in the storage solution. Avoid contact of the tip with the inner walls of the tube. Fix the electrode in place by wrapping parafilm around it.
4. If a dummy electrode is not available, the flow cell has to be removed. Do this, by untightening the connectors at the pH flow cell and placing a union fitting at the position of the flow cell instead. (See below)

5. Then place the pH electrode in the centrifuge tube such that the tip is submerged in the storage solution. Avoid contact of the tip with the inner walls of the tube. Fix the electrode in place by wrapping parafilm around it.

NOTICE: Avoid contact of the pH-electrode tip and solid surfaces since this may damage the pH electrode.

6. MAINTENANCE

WARNING: To avoid electric shock, do not open the Contichrom module metal enclosures. Maintenance on the interior of the Contichrom modules may only be performed by authorized service personnel

WARNING: Before performing maintenance on parts of the Contichrom system on the outside of the sheet metal case, turn off the Contichrom modules and unplug the power cables

6.1. RECOMMENDED MAINTENANCE INTERVALS

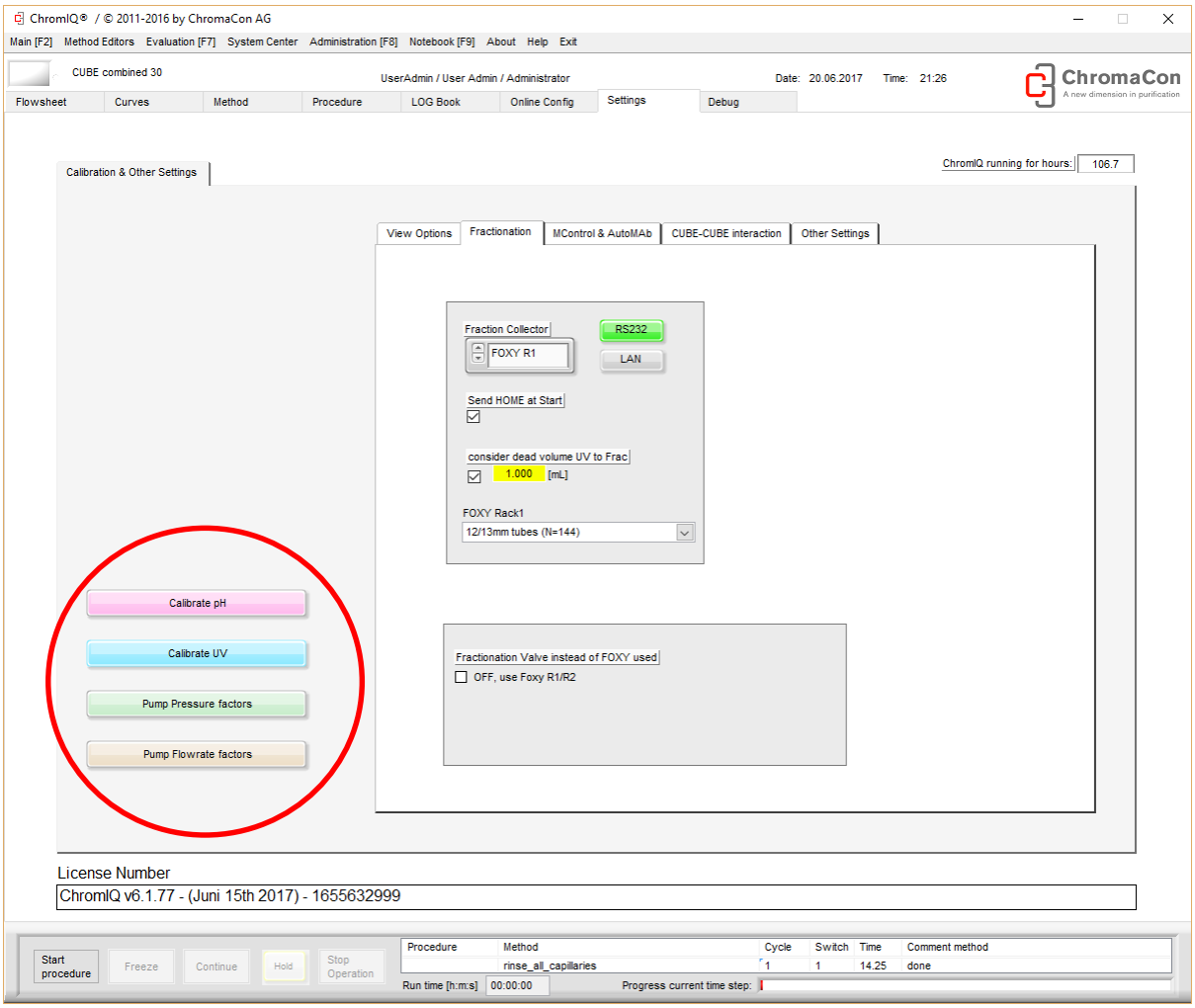
The following maintenance actions and intervals are recommended:

Interval	Action	Reference
Weekly	Exchange pump seal wash solution	Chapter 2.4
Weekly	Calibrate pH sensor	Chapter 6.2
Semi-Annually	Perform through system cleaning	Chapter 4.2.
Semi-Annually	Check for ChromIQ operating software update	Contact Service Support
Annually	Replace pump seals and pistons	Chapter 6.3
Annually	Replace valve rotor and stator	Chapter 6.4
When required	Exchange Capillaries	Chapter 6.5

6.2. CALIBRATING THE PH SENSOR

The pH sensor calibration is started by accessing the “settings” tab and then the “calibration” tab. If the “calibration” tab is not visible, check the box saying “Calibration tab hidden” to display the calibration tab (see screen shot below).

Make sure to have three calibration solutions ready (typical calibration solutions are pH 4.0, pH 7.0 and pH 10.0).



Screenshot of the Settings tab, the calibration tab is highlighted

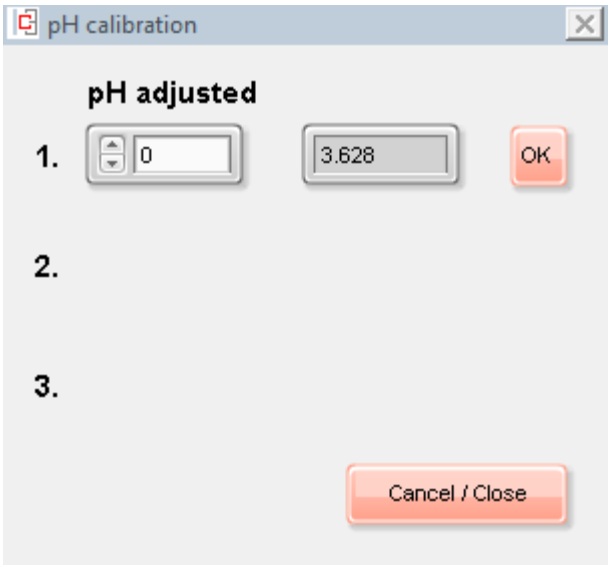
After entering the calibration tab, clicking the “Calibrate pH” button and confirming that a pH calibration is desired, an interface for pH calibration is displayed (see image below). Make sure that the pH electrode is connected to the Contichrom system and rinsed with purified water. Then:

1. Place the pH electrode in the first calibration solution. Enter the pH value of one of the calibration solutions in the “pH adjusted” field next to “1.” and wait for the value in the grey field next to it to stabilize. Then click on the “OK” button and rinse the pH electrode with purified water.

2. Repeat step 1. for the second calibration solution

3. Repeat step 1. for the third calibration solution

Afterwards confirm and save the calibration. Rinse the pH electrode and mount it according to chapter 2.13.



6.3. PUMP MAINTENANCE

PUMP SEAL WASH SOLUTION REPLACEMENT

It is recommended to replace the pump seals wash solution every week. When exchanging the seal wash solution make sure that no large amounts of air enter the seal wash inlet lines. Individual bubbles are usually not problematic.
When the inlet line has run partially dry during exchange of the seal wash liquid, manual filling of the lines is need. (Refer to chapter “Filling the Pump Seal Wash”)

Use 20% IPA/water mix or 30% Ethanol/water mix as seal wash solution.

PUMP HEAD MAINTENANCE

WARNING: When working with aggressive or toxic solvents, residual amounts of these chemicals could be present in the system. Wear protective gear.

Removing the Pump Head Assembly

The self flushing pump head assembly is shown in the figure below.

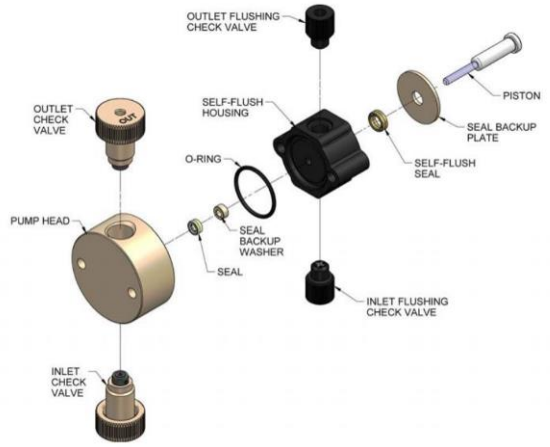


Figure: Self flushing pump head assembly

To remove the pump head:
1. Turn OFF the power of the Contichrom system modules (one module for Contichrom CUBE 30/100, two modules for Contichrom CUBE Combined 30/100 and Contichrom HPLC 30/100).

2. Unplug the power cords of the CUBE/CUBE+ modules.

3. Remove the inlet lines from the buffer reservoirs.

4. Remove the inlet line from the inlet check valve.

5. Remove the outlet line from the outlet check valve.

6. Remove the inlet and outlet self-flush lines.

7. Carefully remove the two Allen nuts at the front of the pump head with a 3/16“ allen wrench.

CAUTION: Be careful not to break the piston when removing the pump head. Twisting the pump head can cause the piston to break.

---

8. Carefully separate the pump head from the pump.

a. Move the pump head straight out from the pump and remove it from the piston. **Be careful not to break or damage the piston.**

b. Remove the seal and seal backup washer from the piston if they did not stay in the pump head.

c. Remove the O-ring.

9. Carefully separate the self-flush housing from the pump. Move the flush housing straight out from the pump and remove it from the piston. Also remove the self-flush seal or guide bushing

CLEANING THE PUMP HEAD ASSEMBLY

Note: If the piston seal or self-flush seal are going to be removed, it is recommended to have a new set on hand to install after cleaning. It is not recommended to reinstall the used piston seal or self-flush seal since they are likely to be scratched and damaged during removal and would not provide a reliable seal if reused. If the seal is removed, use only the flanged end of the plastic seal removal tool supplied with the seal replacement kit. Avoid scratching the sealing surface in the pump head.

Inspect the piston seal cavity in the pump head. Remove any foreign material using a cotton swab or equivalent, and avoid scratching the sealing surfaces. Be sure no fibers from the cleaning swab remain in the components. The pump head, check valves, and flushing housing may be further cleaned using a laboratory grade detergent solution in an ultrasonic bath for at least 30 minutes, followed by rinsing for at least 10 minutes in distilled water. Be sure that all particles loosened by the above procedures have been removed from the components before reassembly.

REPLACING THE PUMP HEAD

1. Carefully align the flush housing and gently slide it into place on the pump. Make sure that the Inlet self-flush check valve is on the bottom and the Outlet self-flush check valve is on the top. If misalignment with the piston occurs, gently realign the piston holder.

2. Install the O-ring in its grove.

3. Line up the pump head and carefully slide it into place. Be sure that the Inlet valve is on the bottom and the Outlet valve is on the top. Do not force the pump head into place.

4. Finger tighten the allen nuts into place. To tighten firmly, alternately turn nuts 1/4 turn with a suitable tool (alternating side-to-side) while gently rotating the pump head to center it.

5. Torque the Allen nuts to 30 in-lbs using a suitable torque wrench and 3/16 allen wrench adaptor.

6. Reattach the inlet and outlet lines. Reattach the self-flush lines. Change the flushing solution.

PISTON SEALS

Lower than normal pressure, pressure variations, and leaks in the pumping system can all indicate possible problems with the piston seal. Depending on the fluid or mobile phase used, piston seal replacement is often necessary after 1000 hours of running time.

Removing the Seals

1. Remove the pump head and self-flush assemblies as described above.

2. Remove the backup washer if it is present in the pump head.

3. Insert the flanged end of the seal insertion/removal tool into the seal cavity on the pump head. Tilt it slightly so that flange is under the seal and pull out the seal.

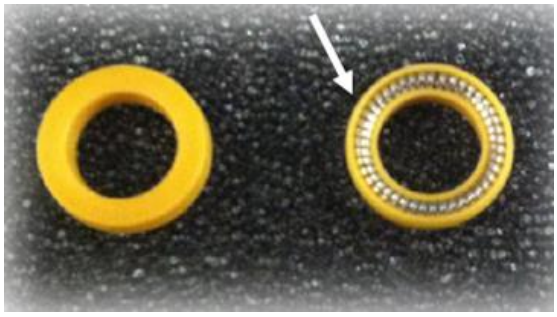
CAUTION: Using any other "tool" will scratch the finish of the sealing surface and create a leak.

4. Repeat the procedure for the low-pressure seal in the flush housing.

5. Inspect, and if necessary, clean the pump head as described above.

REPLACING THE SEALS

1. Place a **high pressure replacement seal** (see Figure below) on the rod-shaped end of the seal insertion/removal tool so that the energizer is visible when the seal is fully seated on the tool. Insert the seal into the pump head. Be careful to line up the seal with the cavity while inserting. Then, withdraw the tool, leaving the seal in the pump head. When looking into the pump head cavity, only the polymer side of the seal should be visible.



Example of polymer side vs. energizer side of seal. Note stainless steel energizer shown. Seal could have fluoropolymer o-ring energizer instead (black o-ring).

2. Place a **self-flush replacement seal** on the seal insertion/removal tool so that the energizer in the seal is visible when the seal is on the tool. As in the previous step, insert the tool and seal into the seal cavity on the flushing housing, taking care to line up the seal with the cavity, and then withdraw the tool. When the seal is fully inserted, only the polymer side of the seal will be visible in the seal cavity.

3. Place the seal back-up washer over the high-pressure seal in the pump head.

4. Replace the self-flush and pump head assemblies.

5. Condition the new seals as described below.

Conditioning New Seals

New seals should be conditioned prior to use. Conditioning is the process of running the seals wet under controlled conditions to allow surfaces to seat and to prepare the seal for operation.
It is recommended to remove the columns from the system and to install restrictor coil, a backpressure regulator (see below) or a suitable column that has a certain backpressure in place of one of the columns.

The other column should be replaced by a union fitting (see below)

Then use the System Center (see chapter on System Center to select a flow path) that uses the pump with the new seals and the position with the pressure increaser.
For instance, use “P2 left” if the pressure increaser is mounted on the left position and for running in the seals of pump 2. For Pump P1 there are two channels A, B. Make sure to run in the seals belonging to the channel on which maintenance has been performed.

NOTICE: Use only organic solvents to condition new seals. Buffer solutions and salt solutions should never be used to condition new seals. Recommended solvents are HPLC-grade methanol and isopropanol, and water mixtures of either.

NOTICE: When running in the seals of Pump P1 using the System Center, make sure to run in the correct channel of the pump by selecting the gradient concentration (100% A or 100%B). .

Suggested Conditioning Parameters:

Using a restrictor coil, a backpressure regulator or a suitable column, run the pump with a 50:50 solution of isopropanol and water for 30 minutes at the back pressure and flow rate listed under PHASE 1 below, depending on the pump head type. Make sure that the pump outlet where the backpressure increasers are mounted, are disconnected from the rest of the system. Make sure that the flow is guided to a vessel after having passed through the backpressure regulator.
Then, run the pump for 15 minutes at a back pressure and flow rate listed under PHASE 2 below, depending on the pump head type.

Contichrom CUBE 30, Contichrom CUBE Combined 30, Contichrom HPLC 30:

PHASE 1 **Pump Type**	**Pressure**	**Flow Rate**	**Duration**
36 mL/min pump	1,000 psi	<3 mL/min	30 min

PHASE 2 **Pump Type**	**Pressure**	**Flow Rate**	**Duration**
36 mL/min pump	1,500 psi	<4 mL/min	15 min

Contichrom CUBE 100, CUBE Combined 100, Contichrom HPLC 100

PHASE 1 **Pump Type**	**Pressure**	**Flow Rate**	**Duration**
100 mL/min pump	500 psi	10 mL/min	15 min

PISTONS

Cleaning the Piston

1. After the pump head and self-flush housing are removed, gently remove the backup seal plate from the pump housing, using either a small screwdriver or toothpick in the slot on top of the pump housing.

2. Grasp the metal base of the piston assembly to avoid exerting any side load on the sapphire rod, and remove the piston from the slot in the carrier by sliding it up.

3. Use the scouring pad included in the seal replacement kit to clean the piston. Gently squeeze the piston within a folded section of the pad and rub the pad along the length of the piston. Rotate the pad frequently to assure

the entire surface is scrubbed. Do not exert pressure perpendicular to the length of the piston, as this may cause the piston to break. After scouring, use a lint-free cloth, dampened with alcohol, to wipe the piston clean.

4. To reinstall the piston, grasp the metal base of the piston assembly and insert it into the slot in the piston carrier until it bottoms in the slot.

Replacing the Piston

Remove the pump head and self-flush assemblies.
1. Grasp the metal base of the piston assembly to avoid exerting any side load on the sapphire rod, and remove the piston from the slot in the carrier by sliding it up.

2. Grasp the metal base of the replacement piston assembly, and insert it into the slot in the piston carrier until it bottoms in the slot.

3. Replace the pump head as described below.

CHECK VALVE CLEANING AND REPLACEMENT

Many check valve problems are the result of small particles interfering with the operation of the check valve. As a result, simply cleaning the pump head with the appropriate laboratory apparatus may resolve any issues.

Check Valve Cleaning

1. To clean pump check valves, remove the pump head and immerse the entire head into a laboratory ultrasonic cleaner.

2. Sonicate for about 30 minutes using a standard cleaning solution. Rinse the pump head thoroughly with distilled water.

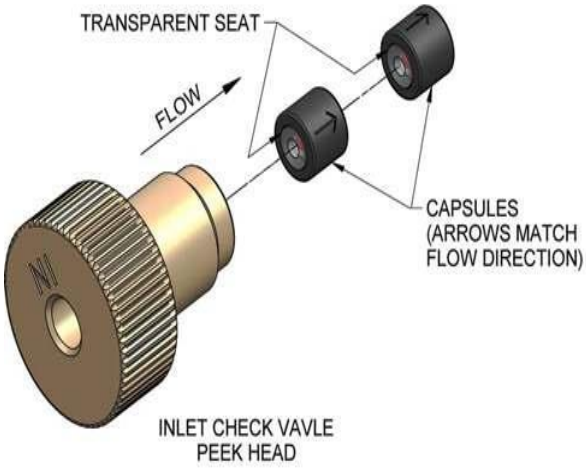
3. Replace the pump head assembly.

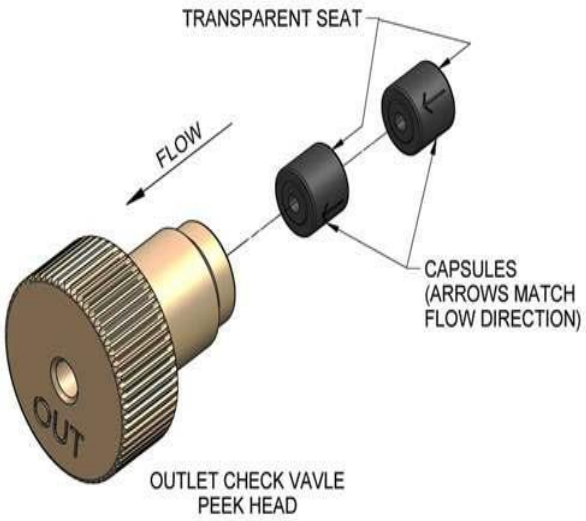
4. Run the pump at 3 mL/min // 5 mL/min for a 36 mL/min // 100 mL/min pump head) with distilled water for 15 minutes. Always direct the output directly to a waste beaker during cleaning (do not recycle).

If this procedure does not return the pump to proper performance, the check valves should be replaced. An example of new check valves from their package can be seen in the Figure below.

Check Valve Replacement

1. The check valves can be replaced without removing the pump head of self-flush assembly, and do not require any tools. When installing new check valves, notice the outlet has a transparent washer, and the Inlet has a cross ball retainer. Also, the words INLET and OUTLET should be visible on the top of the self-flush check valves. (see figure below):





NOTICE: Be careful not to break the piston when removing the pump head. Twisting the pump head can cause the piston to break.

NOTICE: Make sure check valve is kept in the above position to avoid losing parts

SEAL WASH CHECK VALVE REPLACEMENT

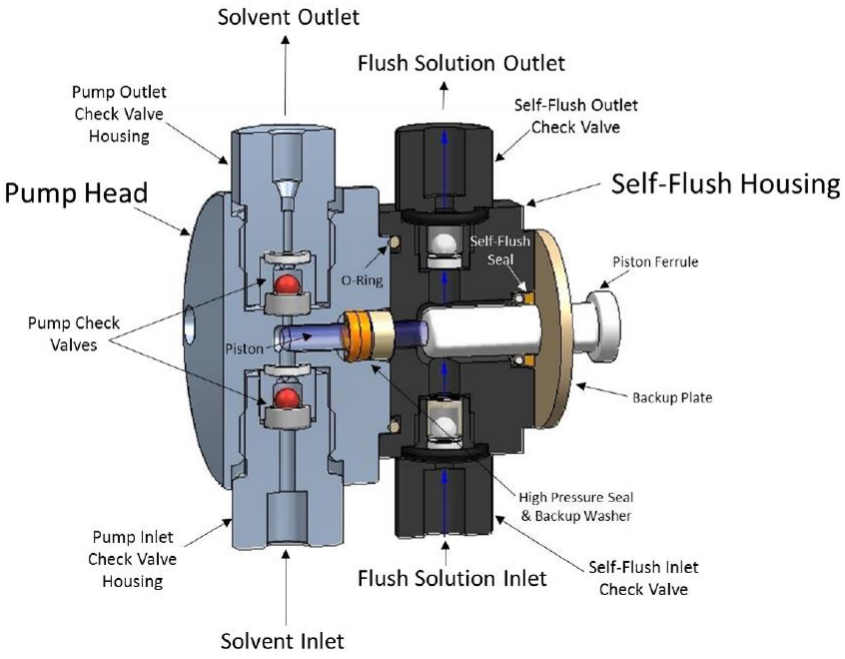
Self-flush check valves can be replaced without removing the pump head of self-flush assembly, and do not require any tools. When installing new check valves, notice the outlet has a transparent washer, and the Inlet has a cross ball retainer. Also, the words INLET and OUTLET should be visible on the top of the self-flush check valves.

Note: The Sapphire Seat is an opaque white ring. The red ruby ball can be seen through the ring. Flow is always away from the sapphire seat, as shown by the directional arrows etched on the capsules.

Note: The **INLET** check valve has a **LARGER** opening (1/4”-28, flat bottom seat) for the 1/8” inlet tubing;
The **OUTLET** check valve has a **SMALLER** opening (#10-32, cone seat) for the 1/16” outlet tubing.

NOTICE: It is strongly recommended to ensure that the self-flushing feature is working. The flushing solution washes away any buffer salts that have precipitated onto the piston. If not removed, these precipitates can abrade the high-pressure seal and cause premature seal failure, leakage, and can possibly damage the pump.

Refer to the figure below for detailed drawing of a self-flushing pump head.



Long Term Pressure Calibration Accuracy
This note applies if the pump is equipped with an electronic pressure transducer. The transducer has been zeroed and calibrated at the factory. Over the life of the pump, some drift may occur.
If pressure calibration and/or drift are a concern, contact the service.

6.4. VALVE MAINTENANCE

The only possible maintenance operations are the exchange of the rotor and the exchange of the stator.

NOTICE: When exchanging the stator the rotor should be exchanged as well.

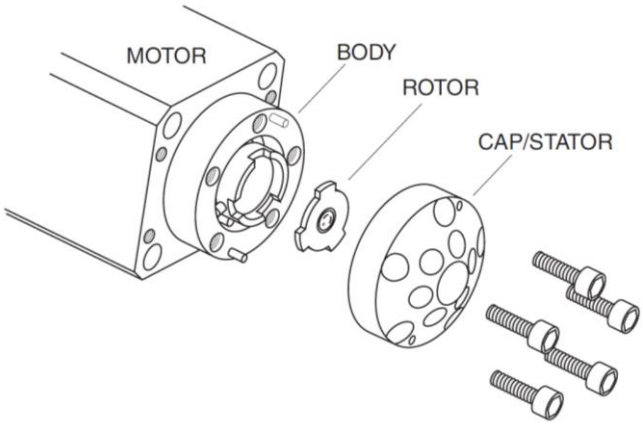
CLEANING AND ROTOR REPLACEMENT

The multi-position valves used in the Contichrom platform have polished sealing surfaces which must be protected during any disassembly or cleaning procedure. Work in a clean environment and always set parts on a soft tissue or clean paper. Cleaning a valve can often be accomplished by flushing all the lines with appropriate solvents.
Do not disassemble the valve unless system malfunction is definitely isolated to the valve.

Disassembly (Refer to **Figure 7**)

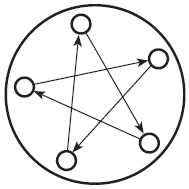
1. Use a 3/32" hex driver to remove the 5-40 socket head screws that secure the stator to the valve body.
2. To insure that the sealing surface of the stator is not damaged, rest it on its outer face. Or, if the tubing is still connected, leave it suspended by the tubing.
3. With your fingers or a small tool, gently pry the rotor away from the driver.
4. Examine the rotor sealing surface for scratches. If you see any, the rotor should be replaced.
5. Examine the stator sealing surfaces. If scratches are visible between the ports, that part should be replaced or resurfaced. Call support for help in determining if resurfacing is feasible.
6. Clean all the parts thoroughly with an appropriate solvent, taking care that no surfaces get scratched. (A common problem with HPLC is the formation of buffer crystals, which are usually water-soluble.)

It is not necessary to dry the rotor.



Reassembly

1. Replace the rotor in the driver, making sure that the rotor sealing surface with its engraved flow passages is facing out. The tabs on the rotor have an asymmetrical pattern to prevent assembly with improper orientation.
2. Replace the stator. Insert the five socket head screws and tighten them gently. As they begin to get snug, tighten them gradually in the order indicated in the figure below until all are snug. Do not overtighten them – the screws simply hold the assembly together and do not affect the sealing force, which is automatically set as the screws pull the stator against the rotor.
3. Test the valve by pressurizing the system. If it doesn't hold pressure, contact technical service for repair.

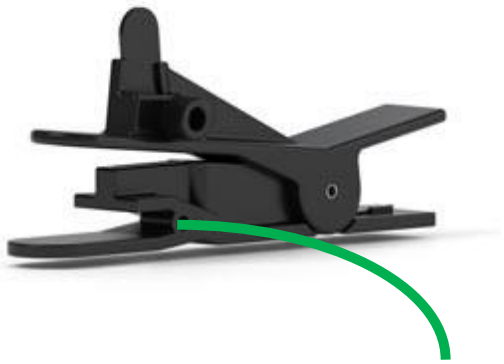


Tightening pattern of the valve stator screws

6.5. REPLACING TUBING CONNECTIONS

Occasionally, tubing of the Contichrom system may become clogged. Possible causes for clogging are: Leaching of resin particles into the system, product precipitation, use of incompatible solvents leading to precipitation.

Use a suitable tube cutter to cut new tubing to the required length (see image below). The tube cutter allows a straight cut, which is essential for a good tube connection.

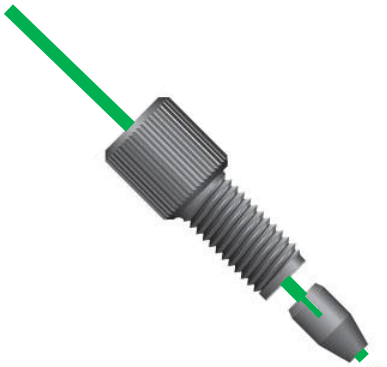


WARNING: The tube cutter has a sharp blade that can lead to injury

WARNING: When exchanging tubes make sure that no hazardous solutions are present in the system. When exchanging a tube on the low pressure side, make sure that its inlet is not immersed in liquid to avoid siphoning the liquid and spillage/

For replacement of tubing on the high pressure side (1/16” outer diameter) of the system use the appropriate connection:

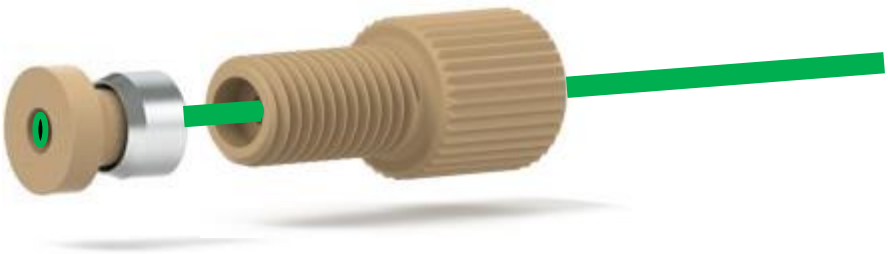
1. Tight seal short connection



In order to mount the tube, first slide the fitting and then the nut over the tubing as indicated above.

Ensure that the tube is extending slightly beyond the nut when inserting the tube into the hole for mounting.

2. Flat bottom connection



In order to mount the tube, first slide the fitting and then the metal ring over the tubing as indicated above. Take special care about the orientation of the metal ring. Then slide the ferrule over the tube. Make sure that the end of the tube and the flat bottom of the ferrule are aligned.
Then insert the tube into the target hole and tighten the fitting. Note that the ferrule and the ring cannot be removed from the fitting after tightening.

For replacement of tubing on the low pressure side (1/8” outer diameter) of the system the following connections are available:

1. Flat bottom connection (Pump inlets)

In order to mount the tubing, first slide the fitting and then the yellow nut over the tubing as indicated above. Take special care about the orientation of the nut. Make sure that the end of the tube and the flat bottom of the nut are aligned.

2. Flat bottom connection (buffer selection valves)

The mounting of the tubing for the buffer selection valves is in analogy to the mounting of the tubing for the pump inlets (see above). Note that the fittings are colored and the nuts are transparent for the buffer inlet valves.

6.6. DETECTOR MAINTENANCE

The detector system is designed for long-term stability. Contact Service in the event that the detector is not performing as desired.

6.7. CHROMIQ SOFTWARE INSTALLATION

The Contichrom system is delivered together with the following components related to the operating software:

- A laptop that has the ChromIQ operating software pre-installed.
- An installation CD that can be used to install ChromIQ on a different computer.

The ChromIQ operating software is only capable of controlling the Contichrom system if the computer is connected to the Contichrom system using both a network cable and a USB cable. This computer is called the control computer. When installing the ChromIQ software on a different computer and connecting the computer by USB cable and network cable to the control computer, this computer becomes the control computer.

From the computer delivered with the Contichrom system, after logon, the ChromIQ software can be started by clicking on the ChromIQ icon or launching the software from the Windows start menu.

ChromIQ icon

For installation of the ChromIQ software on a new computer, refer to the **ChromIQ software manual**.

If ChromIQ is installed on a computer without a USB connection to the Contichrom system, ChromIQ will automatically run in demo mode without establishing a link to the Contichrom system. The Contichrom system cannot be controlled without the USB connection, however, method and procedure generation is possible and the evaluation software module can be used.

6.8. CHROMIQ SOFTWARE UPDATE

Check for ChromIQ operating software updates every six months with the Service support. You will be contacted by Service in case critical updates are required.

7. TROUBLESHOOTING

7.1. GENERAL TROUBLESHOOTING PROCEDURE

In case of problems a number of general items should be checked before entering detailed troubleshooting:

Tubing and columns

- Is there a leakage of the system?
- Are all inlet tubes connected to the correct buffer and submerged, reaching out to the bottle bottom?
- Is air visible in any of the inlet tubing? (See section on purging the pumps)
- Are the columns mounted on the correct positions?
- Have all columns been cleaned and equilibrated before starting the separation?
- Have the samples been conditioned to binding conditions?
- Have buffers and feed been filtered prior to use?
- Are the correct buffers used for the chromatographic separation and are the buffers assigned correctly to the pumps in the ChromIQ tank management system?

Monitor

- Are the desired signals selected to be shown in the “curves” tab?

Purification checks

- Have all columns been cleaned and prepared according to the column recommendations?
- Have the samples been adjusted to binding buffer conditions?
- Have the samples been clarified by centrifugation and/or filtration prior to sample loading?
- Are the correct buffers used for the chosen columns and proteins?

WARNING: When working with aggressive or toxic solvents, residual amounts of these chemicals could be present in the system. Wear protective gear d troubleshooting.

WARNING: Equipment must be cleaned after stoppage to avoid formation of deposits which might lead to built-up of pressure during subsequent operation and potential detachment of capillaries and potential spraying of chemicals/buffer.

7.2. DETAILED TROUBLESHOOTING PROCEDURE

Communication

Problem	Possible cause and Solution
A system component is not found	• The firewall settings of the computer interfere with the connection between the ChromIQ operating software and the Contichrom hardware ➔ Adjust firewall settings / turn off firewall ➔ Check if the system components are found by starting the system center. The system

	center performs an instrument check and displays a message in case of missing components. • The network settings of the computer are incorrect ➔ Refer to ChromIQ software manual for correct settings • The instrument component or instrument connection is temporarily interrupted ➔ Close the ChromIQ software. Turn the Contichrom system off and on again. Then restart the ChromIQ software. • The instrument component or instrument connection is physically broken/interrupted ➔ Contact service
All instrument components are not found	• The Contichrom system or one of its modules (top/bottom) is turned off → turn on Contichrom system, restart ChromIQ • The network cable connection between computer and Contichrom system is interrupted. → plug in network cable / replace network cable. • The USB cable connection between computer and CUBE is interrupted. → plug in USB cable / replace USB cable

Tank Levels too low

Problem	Possible cause and Solution
Tank Levels too low / System has entered Freeze mode	• When the calculated tank levels drop below the error level, an error message is displayed and the system enters the “freeze” mode, i.e. the pumps are stopped to prevent them from running dry. ➔ Refill the buffers, ensuring that the inlet tubes remain fully submerged and reach out to the bottom of the buffer vessel. Then update the buffer levels in the tank management tabs according to the refilled buffer volume (See chapter 2.2 (buffer tank assignment and tank levels). Click on the “continue” button to resume the run. ➔ If the buffer lines contain air, manually purge the lines before resuming the run. If the system has not been in freeze mode for too long (typically in the range of minutes up to a few hours) cyclic runs typically may be resumed without having the freeze mode affect the performance of the run for more than the current cycle.

UV-Monitor

Problem	Possible cause and Solution
The monitor UV1 is not found by the system	• See communication troubleshooting
Signal intensity of UV1 or UV2 is lower than expected	• The UV1 or UV2 cell is dirty. ➔ Perform system cleaning (see section on system cleaning)
The baseline of the UV signal is elevated	• The UV was not autozeroed correctly . ➔ Equilibrate the system with binding buffer and perform an autozero
The UV signals do not return to baseline after cleaning or re-equilibration	• The pump used to drive down the UV signal (e.g. by elution, cleaning, re-equilibration) was not working at the correct flow rate ➔ see pump troubleshooting • The UV was not autozeroed correctly . ➔ Equilibrate the system with binding buffer and perform an autozero
Ghost peaks	• Air is present in the UV flow cell ➔ Check if air-bubbles are present in buffers. If this is the case, degass the buffers and manually purge the inlet tubes with the degassed buffers. ➔ Replace the columns by union fittings. Use the system center or a programmed method to flush through the detector with equilibration buffer at 30 mL/min to remove the air • The UV1 or UV2 cell is dirty or the flow path is dirty ➔ Perform system cleaning (see section on system cleaning) • The buffers are dirty ➔ Check if buffers are dirty and replace them if necessary, then flush the affected lines.
Noisy signal or baseline drift	• Air is present in the UV flow cell ➔ Check if air-bubbles are present in buffers. If this is the case, degass the buffers and manually purge the inlet tubes with the degassed buffers. ➔ Replace the columns by union fittings. Use the system center or a programmed method to flush through the detector with equilibration buffer at 30 mL/min and to remove the air • The pump used to produce the flow is not working properly ➔ see pump troubleshooting • The UV1 or UV2 cell is dirty or the flow path is dirty

	➔ Perform system cleaning (see section on system cleaning) • The buffers are dirty ➔ Check if buffers are dirty and replace them if necessary, then flush the affected lines.
Waves in UV signal	• The pump used to produce the flow is not working properly ➔ see pump troubleshooting
The UV signals are cut-off at the top (curves tab, observed during a run)	• A too sensitive wavelength was shown to be displayed ➔ Select the second wavelength to be displayed • The detector calibration does not cover the required range ➔ Contact service about possibility to extent calibration for detector
A UV signal is not displayed although the detector signal has been selected to be shown and the detector has been found by the system.	• UV lamp broken ➔ Contact service

Conductivity Monitor

Problem	Possible cause and Solution
The conductivity signal is unstable	• Air is present in the flow cell ➔ Check if air-bubbles are present in buffers. If this is the case, degass the buffers and manually purge the inlet tubes with the degassed buffers. ➔ Replace the columns by union fittings. Use the system center or a programmed method to flush through the detector with equilibration buffer at 30 mL/min to remove the air • The pump used to produce the flow is not working properly ➔ see pump troubleshooting • The flow cell is dirty or the flow path is dirty ➔ Perform system cleaning (see section on system cleaning) • The buffers are dirty ➔ Check if buffers are dirty and replace them if necessary, then flush the affected lines.
Conductivity signal appears to drift or conductivity signal appears to increase or decrease from cycle to cycle in cyclic processes.	• The flow cell is dirty. ➔ Perform thorough system cleaning (see chapter on system cleaning) • The temperature of the environment is not constant ➔ provide constant temperature environment • Calibration is required ➔ Check calibration with a standard solution of known conductivity

	➔ Perform conductivity calibration if necessary
Conductivity shows a step increase although a step gradient or a linear gradient has been programmed	• The pump channel of the gradient pump containing the low conductivity buffer didn't work properly due to air in the pump or a non-functional check valve ➔ See section on pump troubleshooting.
Conductivity stays low although a linear gradient has been programmed	• The pump channels of the gradient pump containing the high conductivity buffer didn't work properly due to air in the pump or a non-functional check valve ➔ See section on pump troubleshooting.
The conductivity signal does not return to baseline after cleaning or re-equilibration	• The re-equilibration time is too short / the re-equilibration flow rate is too low. ➔ Increase the re-equilibration time/ re-equilibration flow rate • The pump used to drive down the conductivity signal (e.g. by elution, cleaning, re-equilibration) was not working at the correct flow rate ➔ see pump troubleshooting

pH Monitor

Problem	Possible cause and Solution
The pH signal is noisy	• Air is present in the flow cell ➔ Check if air-bubbles are present in buffers. If this is the case, degass the buffers and manually purge the inlet tubes with the degassed buffers. ➔ Replace the columns by union fittings. Use the system center or a programmed method to flush through the flow cell with equilibration buffer at 30 mL/min to remove the air • The pump used to produce the flow is not working properly ➔ see pump troubleshooting • The flow cell is dirty or the flow path is dirty ➔ Perform system cleaning (see section on system cleaning) • The buffers are dirty ➔ Check if buffers are dirty and replace them if necessary, then flush the affected lines.
The pH signal does not return to baseline after cleaning or re-equilibration	• The re-equilibration time is too short / the re-equilibration flow rate is too low. ➔ Increase the re-equilibration time/ re-equilibration flow rate for the next run. • The pump used for pH re-equilibration (e.g. by elution, cleaning, re-equilibration) was not working at the correct flow rate ➔ see pump troubleshooting • The buffer used for pH re-equilibration is too week ➔ Use stronger buffer of higher molarity
The pH is not correct	• Calibration is required ➔ Check calibration with a standard solution of known pH ➔ Perform conductivity calibration if necessary • pH electrode broken / expired ➔ Replace pH electrode

Pressure sensors

Problem	Possible cause and Solution
Overpressure (system in freeze mode)	• Column fouling, characterized usually by pressure values that increases gradually over time ➔ remove the columns and put union pieces at the column positions as placeholders and click on the “continue” button in order to resume the run. If the backpressure warning does not occur again, the overpressure cause was most likely a blocked column. • Blocking of a tube or a valve, characterized usually by a spontaneous steep increase in backpressure ➔ locate the blockage (valve, tube) by viewing the flow sheet (see flow sheet tab in ChromIQ software) and identifying the active flow path. By successively loosening tubing connections in the active fluid path, starting closest to the pumps and continuing the run after loosening the connection, the blocked tube or valve can be identified. Do not use this method if potentially harmful fluids are in the active fluid path (e.g, caustic soda). If the blockage was traced back to a valve, refer to the section on valve maintenance. Make sure to re-tighten all connections firmly after testing. ➔ An alternative method to identify potentially blocked connections is to run the system qualification test. The system qualification test systematically tests each tubing connection. The test documentation provides acceptance criteria for the backpressure of each connection. Prior to running the system qualification test, the columns must be removed. • Blocking of a backpressure regulator (BPR) ➔ To locate the blockage remove the BPRs one by one and see if the problem persists when continuing the run. Replace the blocked BPR if necessary.
Pressure signals drop to zero from a higher level temporarily or permanently in spite of a constant set flow.	• Air in the pump ➔ Prime the pump (see section on manual purging of the pumps) • Inlet check valves are blocked ➔ Based on the pressure signal, identify the affected pump. Clean or replace inlet check valves of that pump (see section on pump maintenance)

Long-term pressure drift of one or more sensors	• The pressure sensor(s) is/are decalibrated ➔ Contact service

NOTICE: When loosening capillaries, liquid may leak out of the system at the location of the loose capillary. Ensure that any leaking liquid is taken up, e.g. by paper wipes.

WARNING: Do not loosen connections of the active fluid path if harmful fluids are in the active fluid path (e.g, caustic soda).

Fraction collector

Problem	Possible cause and Solution
Fraction collector not found	• Fraction collector powered off or not connected ➔ Check if the power cable is plugged in and the communication cable between fraction collector and Contichrom system is firmly connected. The communication cable must be connected to upper one of the two serial ports on the backside of the Contichrom system. • Wrong Baud rate setting of fraction collector ➔ The IP address of the Fraction Collector must be set to 9600. The setting must be done on the fraction collector itself. Use the touchpad on the fraction collector to navigate to its network settings and to set the baud rate. Refer to the fraction collector manual for more details the IP address.
No fractions have been collected despite a fractionation being part of the method and the fractionator having advanced one or more positions	• Wrong rack type selected (a spill should be visible in the drip tray of the fraction collector, as the fractionator head outlet missed some tubes) ➔ set the correct rack type at the bottom of the fraction collector window (window is opening by default at ChromIQ startup). Changes become effective for the next sample being collected. • Fraction collector powered off or not connected ➔ Check the power and network connection of the fraction collector

	• A pump was not working (e.g. due to air in the pump, effects should also be visible in the pressure/conductivity signals) ➔ Check pump function using the system center. If necessary, manually purge the pump to remove air (see chapter on filling the pumps).
Fractions do not have correct volumes / volumes differ among the fractions	• Fraction collector outlet not centered on the fractions, tubes were partially missed ➔ Use the wing screw on the front of the fractionator head to fine-adjust the position of the fractionator head. • A pump was not working (e.g. due to air in the pump, effects should also be visible in the pressure/conductivity signals) ➔ Check pump function using the system center. If necessary, manually purge the pump (see chapter on filling the pumps).
Tubes are overflowing	• Wrong rack type selected ➔ Enter correct rack type in fractionation window
Fractionator does not move forward	• Fractionator ignores “NEXT” command ➔ Turn fractionator on / off again

7.3. DISPOSAL OF THE SYSTEM

WARNING: Equipment must be cleaned before dismantling and disabling/scrapping to avoid accidental contact with chemicals.

7.4. SYSTEM QUALIFICATION TESTS

For priming the system and to test functionality of the individual components, refer to the **Contichrom system qualification** document.

8. APPENDIX

APPENDIX A - TECHNICAL SPECIFICATIONS

Technical specifications: Contichrom CUBE 30 system / Contichrom CUBE 100 system for FPLC

The Contichrom CUBE 30 system / Contichrom CUBE 100 system comprises the Contichrom CUBE module / Contichrom CUBE 100 module, respectively.

Technical specifications: Contichrom CUBE module / Contichrom CUBE 100 module for FPLC

Main system components	• 2x dual-head pump • 2x UV sensors • 2x conductivity sensor • 1x temperature sensor • 1x pH sensor • 4x multiposition valve (actuated) • 1x drain valve (actuated) • 2x buffer selection valve (actuated) • 2x manual drain valve • 2x pressure transducer
Communication and Control	• 1x laptop computer (Windows, 64 bit, resolution 1920 x 1080 or higher) • 1x ChromIQ® operating software
Maximum system pressure	• 50 bar / 725 psi
System Dimensions	• 450 mm wide, 456 mm deep, 370 mm high, weight 30 kg (67 lbs)
Power specifications	• Supply voltage 100-240 VAC • Max consumption: 90 W • Fuses: 2x T2AL
Pump description	• dual-head pump design for low pulsation • biocompatible • sapphire pistons • PEEK pump heads • active piston seal wash • flow rate 0.1-36 mL/min / 0.1-100 mL/min (CUBE 30, CUBE 100) • accuracy better than 2% across flow range • binary high pressure gradient mixing capability by combining both pumps
UV detector description	• spectrophotometer for routine HPLC and other flow-through detection tasks • Two fixed wavelengths, 280 nm and 300 nm recorded simultaneously
Multiport valve description	• electrical valve drive • Materials: PPS, PAEK, PEEK, PTFE seals
conductivity / temperature sensor description:	• conductivity 0-150 mS/cm • temperature sensor • Sensors are incorporated in UV flow cell
pH sensor description	• external pH probe with flow cell • pH measurements 1-14 • pressure rating 7 bars / 100 psi
Materials	• all biocompatible • high pressure side tubing: PEEK, 0.75 / 1.0 mm i.D.(CUBE 30 /CUBE 100) • low pressure side tubing: PTFE, 1/8”, 1.6 / 2.0 mm i.D.(CUBE 30 /CUBE 100) • Fittings: PEEK
Fraction Collector (optional)	• Fraction collector Foxy R1 with integrated diverter valve • Fraction capacity: All standard formats including 96-well plates, 15 mL and 50 mL tubes
Operation	• Cold room (4°C) compatible • 16 buffer inlets (8 per buffer selection valve)
Operating software description	• ChromIQ® operating software (in English)

Technical specifications: Contichrom CUBE Combined 30 / Contichrom CUBE Combined 100 system for FPLC

The Contichrom CUBE Combined 30 system / Contichrom CUBE Combined 100 system comprises the Contichrom CUBE module / Contichrom CUBE 100 module (see specifications above) and a CUBE+ module / CUBE+ 100 module, respectively.

Technical specifications: CUBE+ module / CUBE+ 100 module

Modularity	• The CUBE+ upgrade module is not stand-alone and requires a Contichrom CUBE module to be operational
Main module components	• 2x dual-head pump • 1x drain valve (actuated) • 2x manual drain valve • 2x pressure transducer
Maximum system pressure	• 50 bar / 725 psi
System Dimensions	• 450 mm wide, 509 mm deep, 214 mm high, weight 17 kg (38 lbs)
Voltage	• Supply voltage 100-240 VAC • Max consumption: 80 W • Fuses: 2x T2AL
Pump description	• dual-head pump design for low pulsation • PEEK pump heads • biocompatible • sapphire pistons • active piston seal wash • flow rate 0.1-36 mL/min / 0.1-100 mL/min (CUBE+ 30, CUBE+ 100) • accuracy better than 2% across flow range • binary high pressure gradient pump
Multiport valve description	• electrical valve drive • Materials: PAEK, PEEK, PTFE seals
Materials	• all biocompatible • high pressure side tubing: PEEK, 0.75 / 1.0 mm i.D.(CUBE+ 30 /CUBE+ 100) • low pressure side tubing PTFE,1/8",1.6 / 2.0 mm i.D.(CUBE+ 30 /CUBE+ 100) • Fittings: PEEK
Operation	• Cold room (4°C) compatible • 2 buffer inlets (1 per pump)

Technical specifications: Contichrom HPLC 30 / Contichrom HPLC 100

The Contichrom CUBE HPLC 30 system / Contichrom CUBE HPLC 100 system comprises the Contichrom CUBE module / Contichrom CUBE 100 module (see specifications above) and a CUBE+ module / CUBE+ 100 module, respectively.

Technical specifications: Contichrom CUBE module / Contichrom CUBE 100 module for HPLC

Main system components	• 2x dual-head pump • 2x UV sensors • 2x conductivity sensor • 1x temperature sensor • 1x pH sensor • 4x multiposition valve (actuated) • 1x drain valve (actuated) • 2x buffer selection valve (actuated) • 2x manual drain valve • 2x pressure transducer
Communication and Control	• 1x laptop computer (Windows, 64 bit, resolution 1920 x 1080 or higher) • 1x ChromIQ® operating software
Maximum system pressure	• 100 bar / 1450 psi
System Dimensions	• 450 mm wide, 456 mm deep, 370 mm high, weight 30 kg (67 lbs)
Power specifications	• Supply voltage 100-240 VAC • Max consumption: 90 W • Fuses: 2x T2AL
Pump description	• dual-head pump design for low pulsation • biocompatible • sapphire pistons • PEEK pump heads • active piston seal wash • flow rate 0.1-36 mL/min / 0.1-100 mL/min (CUBE 30, CUBE 100) • accuracy better than 2% across flow range • binary high pressure gradient mixing capability by combining both pumps
UV detector description	• spectrophotometer for routine HPLC and other flow-through detection tasks • Two fixed wavelengths, 280 nm and 300 nm recorded simultaneously
Multiport valve description	• electrical valve drive • Materials: PPS, PAEK, PEEK, PTFE seals
conductivity / temperature sensor description:	• conductivity 0-150 mS/cm • temperature sensor • Sensors are incorporated in UV flow cell
pH sensor description	• external pH probe with flow cell • pH measurements 1-14 • pressure rating 7 bars / 100 psi
Materials	• all biocompatible • high pressure side tubing: PEEK, 0.75 / 1.0 mm i.D.(CUBE 30 /CUBE 100) • low pressure side tubing: PTFE, 1/8”, 1.6 / 2.0 mm i.D.(CUBE 30 /CUBE 100) • Fittings: PEEK
Fraction Collector (optional)	• Fraction collector Foxy R1 with integrated diverter valve • Fraction capacity: All standard formats including 96-well plates, 15 mL and 50 mL tubes
Operation	• Cold room (4°C) compatible • 16 buffer inlets (8 per buffer selection valve)
Operating software description	• ChromIQ® operating software (in English)

Technical specifications: CUBE+ module / CUBE+ 100 module for HPLC

Modularity	• The CUBE+ upgrade module is not stand-alone and requires a Contichrom CUBE module to be operational
Main module components	• 2x dual-head pump • 1x drain valve (actuated) • 2x manual drain valve • 2x pressure transducer
Maximum system pressure	• 100 bar / 1450 psi
System Dimensions	• 450 mm wide, 509 mm deep, 214 mm high, weight 17 kg (38 lbs)
Voltage	• Supply voltage 100-240 VAC • Max consumption: 80 W • Fuses: 2x T2AL
Pump description	• dual-head pump design for low pulsation • PEEK pump heads • biocompatible • sapphire pistons • active piston seal wash • flow rate 0.1-36 mL/min / 0.1-100 mL/min (CUBE+ 30, CUBE+ 100) • accuracy better than 2% across flow range • binary high pressure gradient pump
Multiport valve description	• electrical valve drive • Materials: PAEK, PEEK, PTFE seals
Materials	• all biocompatible • high pressure side tubing: PEEK, 0.75 / 1.0 mm i.D.(CUBE+ 30 /CUBE+ 100) • low pressure side tubing PTFE,1/8",1.6 / 2.0 mm i.D.(CUBE+ 30 /CUBE+ 100) • Fittings: PEEK
Operation	• Cold room (4°C) compatible • 2 buffer inlets (1 per pump)

APPENDIX B - LIST OF WETTED PARTS

Category	Detailed Description	material
Pumps (Dual piston 36 mL/min)	Pump heads	PEEK
	Pump pistons	sapphire
	Check valve	PTFE
	Check valve balls	synthetic ruby
	Seals	UHMW-PE
Prime/purge valve	Manual valve, 3 way	PEEK
Pressure sensor	Titanium in PEEK tee	titanium / PEEK
Detector system	UV and conductivity flow cell	quartz, sapphire, platinum, titanium
Valves	Buffer selection valvesy 1/8" (low pressure side), Material Stator // Rotor	PPS // PEEK/PTFE
	Column valves 1/16" (high pressure), Material Stator // Rotor	PAEK, carbon-fortified // PEEK/PTFE
	Automatic drain valves 1/16" (high pressure), Material Stator // Rotor	PAEK, carbon-fortified // PEEK/PTFE
Fittings	Ferrules	PEEK
tubing	1/8" low pressure side tubing , i.d. 1.55 mm	PTFE
capillaries	1/16" high pressure side capillaries	PEEK
pH electrode	pH Electrode	Glass
	pH flow cell	Titanium and Perfluoroelastomer (FFKM)
fraction collector (optional)	fraction collector diverter valve	PEEK and Perfluoroelastomer (FFKM)
	ferrules	ETFE
	valve tubing	PTFE
	drain tubing	Vinyl

APPENDIX C – CHEMICAL RESISTANCE GUIDE

Introduction

This section specifies the chemical resistance of the Contichrom systems to some of the most commonly used chemicals in liquid chromatography. The following information on chemical resistance does not take into account combinations of chemicals. It is valid for 20-25° C.

Biocompatibility

The Contichrom systems are designed for maximum biocompatibility, exploiting a highly inert fluid path. The entire fluid path is free of stainless steel to minimize the contribution of potentially deactivating metal ions such as iron, nickel and chromium. The fluid path is primarily constructed of polyetheretherketone (PEEK), highly resistant fluoropolymers and other highly resistant plastics, other non-metallic materials and titanium which also considered being biocompatible.

Cleaning chemicals

Thorough cleaning of the system works well with 1M NaOH, 20% acetic acid and ethanol, following the steps outlined in Section 4.

Some materials used in the Contichrom system are extremely sensitive to acids (including some Lewis acids) and acid halides. System cleaning with any amount of hydrochloric acid should be avoided.

Organic Solvents

Reverse phase chromatography of proteins works well with 100% acetonitrile and additives trifluoroacetic acid (TFA) up to 0.2% or formic acid up to 5%. The following chemicals may be used at the indicated concentrations for up to 2 hours contact time at room temperature:

- Ethanol CAS no 75-08-1 (100%)
- Isopropanol CAS no 67-63-0 (100%)
- Methanol CAS no 67-56-1 (100%)
- Acetonitrile CAS No 109-99-9 (100%)

A user can be exposed to large volumes of chemical substances over a long time period. The flow rate for the Contichrom systems can be chosen to assume maximal 100 mL/min. This may result in a calculated 6L solvent waste/hour. Therefore, it will be important to

- dispose organic solvent waste generated in conjunction with HPLC operation to prevent accumulation and unwanted human health effects,
- operate the Contichrom equipment in a laboratory environment with air ventilation to avoid accumulation of organic solvent vapors/mists,
- have Material Safety Data Sheets (MSDS) available which provide the user with information of characteristics of substances, human and environmental risks and preventive measures. MSDS are available from the chemical distributor or databases in the public domain.

Organic solvents can penetrate weaknesses in PEEK tubing walls more easily than water based buffers. Special care should therefore be taken with prolonged use of organic solvents close to pressure limits.

The seals included with the Contichrom systems are machined from a formulated Ultra-High-Molecular-Weight Polyethylene (UHMW-PE) and are widely used for many HPLC applications. It is recommended to replace the UHMW-PE seals with the highly resistant graphite-filled Polytetrafluorethylen (PTFE), when the system is mostly in contact with organic solvents or high concentration of organic acids, such as acetic acid and formic acid for a longer period of time. Contichrom systems with "organic seals" can will be delivered based on customer request.

Chemicals to be specifically avoided

NOTICE: Some of these materials used in the Contichrom system are extremely sensitive to acids (including some Lewis acids) and acid halides. Avoid using solvents that contain any amount of hydrochloric acid.

Some solvents to specifically avoid are: Aqua Regia, Hydrochloric Acid, Bromine Hydrofluoric Acid, Chlorine Anhydrous Hydrofluorsilicic Acid, Copper Chloride, Hydrogen Peroxide, Ferric Chloride, Iodine Ferrous Chloride, Mercuric Chloride, Freon 12 (wet), Guanidine Hydrobromic Acid 7. In addition, some users of HPLC systems have observed that chloroform and carbon tetrachloride slowly decompose to liberate hydrochloric acid, which, as noted above, shall be avoided.
It is also recommended to avoid ammonium hydroxide. Although ammonium hydroxide will not harm the pump itself, it is likely to damage the stator and rotor in the valves.
The use of 100% ethyl acetate, 100% hexane and 100% tetrahydrofuran is contra-indicated, since the pH detector is sensitive to these solvents.

Strong organic solvents like ethyl acetate, 100% acetone, or chlorinated organic solvents should be avoided. These might cause swelling of plastic material and reduce the pressure tolerance of PEEK Tubing. For this reason, flash chromatography and straight (normal) phase chromatography is generally not recommended on the system.
Aqueous and nonaqueous solutions containing free hydrofluoric acid (HF) cannot be allowed to make contact with the flow cell, even intermittently. HF is a weak acid and will form in any acidic solution which contains

fluoride anions (like NaF + HCl or even TBAF + Acetic Acid). Fluorinating agents like DAST (dimethylaminosulfur trifluoride), SF4 solutions, and the like, are all incompatible with the flow cell.
Immediate severe and irreparable damage to the flow cell will result upon exposure to any of these chemicals.

Concentrated (>10% vol) hydrogen peroxide, especially in the presence of strongly chelating compounds, such as EDTA or glycolic acid, slowly dissolves titanium and must not be used with the system.

Strong hot aqueous alkaline solutions (like 40% NaOH at 50 degree C) will cause corrosion of quartz and titanium surfaces and thus are not recommended for use with the system.

Fuming nitric acid may react with titanium and must not be used with the system.

APPENDIX D - ROOM REQUIREMENT FOR OPERATION AND STORAGE

The system must only be operated in the following range:

- +4°C to +35°C, +39°F to +95°F
- Relative humidity 20-95%, non-condensing

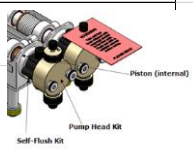
The system should not be exposed to direct sunlight and operated in an atmosphere with minimized dust.
The Contichrom system has the following electrical requirements:

- Supply voltage: 100-240 VAC, frequency 50-60 Hz
- 1 Power outlet for Contichrom CUBE module / Contichrom CUBE 100 module
- 1 Power outlet for CUBE+/ CUBE+ 100 module
- 1 Power outlet for laptop computer
- 1 Power outlet for optional fraction collector

Maximum Power consumption:

- Contichrom CUBE module: 90 W
- Contichrom CUBE 100 module: 90 W
- CUBE+ module: 80 W
- CUBE+ 100 module: 80 W

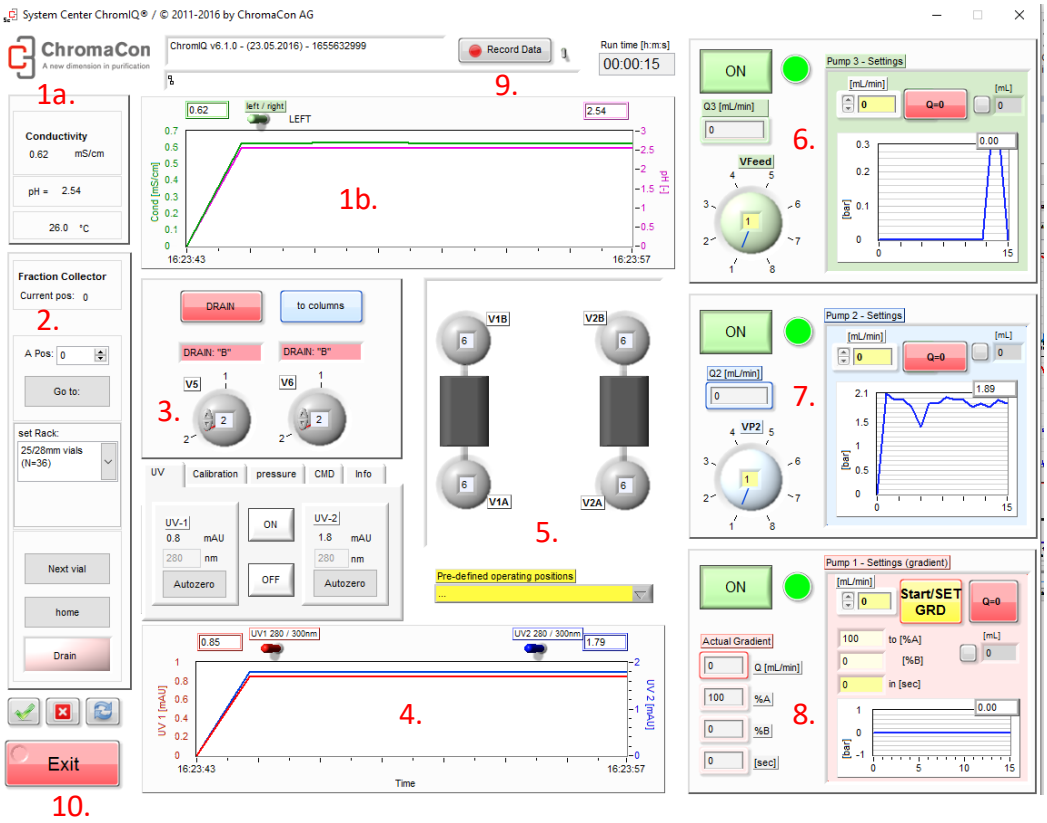
APPENDIX E - LIST OF SPARE PARTS

Spare Parts List for Contichrom CUBE/CUBE+		
Part Number	**Description**	**Picture**
880836CC	Complete Start Up / Tubing Kit for CUBE	
880837CC	Complete Start Up / Tubing Kit for CUBE+	
880838CC	PEEK Tubing, 1/16" OD x .030" ID, Green, 100 ft	
880839CC	FEP Tubing, 1/8" OD x 1/16" ID, Clear, 100 ft	
881053CC	Fittings Tightening Tool kit	
881042CC	Biocompatible Pressure Regulator (40 psi)	
890904CC	Anti Slip Mat	
890908CC	Bracket to mount column holders	
890909CC	brackets to connect CUBE and CUBE+	
890905CC	PH Electrode Bracket	
880203CC	Seal Kit (1 req'd per pump head)	
880253CC	Seals, 10 Pack	
880354CC	Pump Piston (1 req'd per pump head)	
880402CC	PEEK Check Valve Kit (1 req'd per pump head)	
880452CC	PEEK Check Valve Capsules, 10 Pack	
890370	Pump Head Kit (1 req'd per pump head)	
890371	Pump Self-Flush Kit (1 req'd per pump head)	

Spare Parts List for Contichrom CUBE/CUBE+		
Part Number	**Description**	
890601CC	Prime/Purge Valve	
880652	Prime/Purge Valve Rebuild Kit	
881032	Valve Rotor for 6-port valve	
881033	Valve Stator for 6-port valve	
881034	Valve Stator for 7-port valve	
881035	Valve Rotor for 7-port valve	
881036	Valve Stator for 9-port valve	
881037	Valve Rotor for 9-port valve	
881043CC	Buffer Tray	
880807CC	Fuse (2 req'd per CUBE or CUBE+)	
881049CC	CUBE / CUBE+ connection cable	
881050CC	Foxy R1 Fraction Collector Communication Cable	
881051CC	PH Detector	
881052CC	USB 2.0 Cable	

APPENDIX F - MANUAL CONTROL USING SYSTEM CENTER

The system center is suited to control individual hardware components (pumps, valves, detectors) separately. The "**System Center**" can be accessed only when no procedure is running. In order to start the "System Center" press the menu entry "System Center" in the main application. Upon startup of the System Center the software checks for the presence of all hardware components and will issue a warning if one or more hardware components are not found.



Screenshot of the System Center

In the System Center the following displays, graphs, buttons and settings are available (see Figure):

- The conductivity and pH values are displayed as current values (1a.) and as graph over time (1b.)
- The fraction collector position is shown; the fraction collector arm can be ordered to move to a certain position (button "Go to:" and menu "Pos"); the fractionator rack type can be defined (menu "set Rack"); the fractionator can be ordered to advance by one position (button "next vial"), to move to the home position (button "home"), to collect/drain (button "Drain" or "Collect") (2.)
- The drain valves can be order to both drain ("DRAIN" button) or to both direct the flow to the columns (button "to columns"). Using the arrows in the valve symbols, the valves can also be switched individually to drain (position 2) or to direct the flow to the columns (position 1) (3.)
- The UV lamps can be switched on or off (standby); wavelengths and autozeros can be set individually; and the UV signals over time are shown in a graph (4.)
- Pre-defined valve/column positions can be selected by clicking into the yellow field ("…"). The corresponding flow sheet is shown in the valve/column schematic above the yellow selection field (5.)
- Pump 3 can be turned on/off, a green button next to the "ON" button lights up, when the pump is on; the current flow rate is shown in the field below the "ON" button. The flow rate can be set (yellow field, [mL/min]). The red button "Q=0" immediately sets the flow rate to zero. The small grey button next to the "Q=0" button starts/resets a counter [mL], show on the right side of the grey button. The buffer

selection valve can be controlled by entering the desired position into the yellow field in the center. The pressure recorded at the pump is shown in the graph next to the buffer selection valve symbol (6.)

- Pump 2 (7.) can be controlled in analogy to pump 3 (6.)
- Pump 1 is a binary gradient pump. Pressure graph, flow rate settings, volume counter and “Q=0” functions are identical to the ones of pumps 2 and 3. In order to set a concentration gradient it is recommended to first set the gradient flow rate by entering the desired flow rate in the yellow field [mL/min] and pressing the “set grd” button (with duration of 0 seconds, yellow field above the pressure graph). Once the flow rate is active (check “actual gradient” display below the “ON” button). A concentration gradient can be run by typing a %B number in the “[% B]” field, entering a gradient duration in seconds underneath and clicking on the “SET GRD” button. The progress of the gradient is displayed in the “actual gradient” display below the “ON” button. Step gradients can be run by entering gradient durations of 0 seconds. When clicking the “SET GRD” button starting from a different flow rate level, a flow rate gradient is run. (8.)
- The data generated using the System Center may be recorded by clicking the “Record Data” button (9.). The data file name and path is displayed below the “Record Data” button.
- The System Center is closed using the “Close” button (10.)