

**Sepiatec SFC-250** Operation Manual



#### Imprint

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# 1 About this document

This operation manual is applicable for all variants of the instrument. Read this operation manual before operating the instrument and follow the instructions to ensure safe and trouble-free operation.

Keep this operation manual for later use and pass it on to any subsequent user or owner.

BÜCHI Labortechnik AG accepts no liability for damage, faults and malfunctions resulting from not following this operation manual.

If you have any questions after reading this operation manual:

► Contact BÜCHI Labortechnik AG Customer Service.

https://www.buchi.com/contact

### **1.1** Mark-ups and symbols



#### NOTE

This symbol draws attention to useful and important information.

- ☑ This character draws attention to a requirement that must be met before the instructions below are carried out.
- ▶ This character indicates an instruction that must be carried out by the user.

 $\Rightarrow$  This character indicates the result of a correctly carried out instruction.

Explanation	
Software Windows are marked-up like this.	
Tabs are marked-up like this.	
Dialogs are marked-up like this.	
Buttons are marked-up like this.	
Field names are marked-up like this.	
Menus or menu items are marked-up like this.	
Status is marked-up like this.	
Signals are marked-up like this.	

#### 1.2 Trademarks

Product names and registered or unregistered trademarks that are used in this document are used only for identification and remain the property of the owner in each case.

#### 1.3 Connected instruments

In addition to this operation manual, follow the instructions and specifications in the documentation for the connected instruments.

# 2 Safety

#### 2.1 Proper use

The instrument is designed for super fluid chromatography.

The instrument can be used in laboratories for the following tasks:

- Purification
- Separation of one or more compounds from a mixture

# 2.2 Use other than that intended

Use of any kind other than that described in Chapter 2.1 "Proper use", page 7 and any application that does not comply with the technical specifications (See Technical data) constitutes use other than intended. In particular, the following applications are not permissible:

- Use of the instrument in areas which require explosion-safe instruments.
- Use of the instrument with solvents containing peroxides.

# 2.3 Staff qualification

Unqualified persons are unable to identify risks and are therefore exposed to greater dangers.

The instrument may only be operated by suitably qualified laboratory staff. These operating instructions are aimed at the following target groups:

#### Users

Users are persons that meet the following criteria:

- They have been instructed in the use of the instrument.
- They are familiar with the contents of these operating instructions and the applicable safety regulations and apply them.
- They are able on the basis of their training or professional experience to assess the risks associated with the use of the instrument.

#### Operator

The operator (generally the laboratory manager) is responsible for the following aspects:

- The instrument must be correctly installed, commissioned, operated and serviced.
- Only suitably qualified staff may be assigned the task of performing the operations described in these operating instructions.
- The staff must comply with the local applicable requirements and regulations for safe and hazard-conscious working practices.
- Safety-related incidents that occur while using the instrument should be reported to the manufacturer (quality@buchi.com).

#### **BUCHI service technicians**

Service technicians authorized by BUCHI have attended special training courses and are authorized by BÜCHI Labortechnik AG to carry out special servicing and repair measures.

## 2.4 Personal protective equipment

Depending on the application, hazards due to heat and/or corrosive chemicals may arise.

- Always wear appropriate personal protective equipment such as safety goggles, protective clothing and gloves.
- Make sure that the personal protective equipment meets the requirements of the safety data sheets for all chemicals used.

# 2.5 Warning notices in this document

Warning notices warn you of dangers that can occur when handling the instrument. There are four danger levels, each identifiable by the signal word used.

Signal word	Meaning
DANGER	Indicates a danger with a high level of risk which could result in death or serious injury if not prevented.
WARNING	Indicates a danger with a medium level of risk which could result in death or serious injury if not prevented.
CAUTION	Indicates a danger with a low level of risk which could result in mi- nor or medium-severity injury if not prevented.
NOTICE	Indicates a danger that could result in damage to property.

# 2.6 Warning symbols

The following warning symbols are displayed in this operation manual or on the instrument.

Symbol	Meaning
	General warning
<u>x:</u>	Instrument damage
	Dangerous electrical voltage
	Hot surface

# 2.7 Residual risks

The instrument has been developed and manufactured using the latest technological advances. Nevertheless, risks to persons, property or the environment can arise if the instrument is used incorrectly.

Appropriate warnings in this manual serve to alert the user to these residual dangers.

#### 2.7.1 Faults during operation

If an instrument is damaged, sharp edges, glass splinters, moving parts or exposed electrical wires can cause injuries.

- ► Regularly check instruments for visible damage.
- ► If faults occur, switch off the instrument immediately, unplug the power cord and inform the operator.
- ▶ Do not continue to use instruments that are damaged.

#### 2.7.2 Hot surfaces

The column oven area and the heating elements of the device can become hot. If touched they can cause skin burns.

▶ Do not touch hot surfaces or else wear suitable protective gloves.

#### 2.7.3 Dangerous vapors

The use of the instrument can produce dangerous vapors that are capable of causing life-threatening toxic effects.

- ▶ Do not inhale any vapors produced during processing.
- Ensure that vapors are removed by a suitable fume hood.
- Only use the instrument in well ventilated areas.
- ▶ If vapors escape from connections, check the ferrules an fittings concerned and replace them if necessary.
- ▶ Do not process any unknown fluids.
- Observe the safety data sheets for all substances used.

#### 2.7.4 Dangerous particles

The use of the instrument can produce dangerous particles that can cause life-threatening toxic effects.

- ▶ Do not inhale any particles produced during processing.
- Ensure that particles are removed by a suitable fume hood.
- Only use the instrument in well ventilated areas.
- If particles escape from connections, check the ferrules an fittings concerned and replace them if necessary.
- Do not process any unknown fluids or solvent mixtures.
- Observe the safety data sheets for all substances used.

#### 2.7.5 Glass breakage

Broken glass can cause severe cuts.

Damaged glass components may implode if subjected to high pressure. Minor damage to the ground joints impairs the sealing effect and may therefore diminish performance.

- ▶ Handle the flask and other glass components carefully and do not drop them.
- Always visually inspect glass components for damage every time they are to be used.
- ▶ Do not continue to use glass components that are damaged.
- ► Always wear protective gloves when disposing of broken glass.

#### 2.7.6 Malfunction of a connected instrument (option)

A malfunction on a connected instrument can cause poisoning or death.

Make sure that the connected instrument is prepared and maintained according to the user documentation.

#### 2.7.7 Malware infection due to connections with other devices or network

Connections with other devices or a network can cause a malware infection to the instrument.

Install antivirus software and firewall on the instrument before connecting it to other devices or network.

#### 2.8 Modifications

Unauthorized modifications can affect safety and lead to accidents.

- ▶ Use only genuine BUCHI accessories, spare parts and consumables.
- ► Carry out technical changes only with prior written approval from BUCHI.
- Only allow changes to be made by BUCHI service technicians.

BUCHI accepts no liability for damage, faults and malfunctions resulting from unauthorized modifications.



# Warning signs

# 

#### **Risk of electric shock**

Risk of death by electrocution

- ► Never remove the housing.
- Never operate the instrument with removed housing or without the included pumps.
- Never use any electric connections or cables that were not supplied with the instrument.



# 

#### **Risk of cold burns**

Pressurized gases can generate low temperatures on depressurization.

 Always wear appropriate personal protective equipment such as safety goggles, protective clothing and gloves.



# ▲ DANGER

#### Hot surface

The column oven can reach temperatures of up to 70°C. The heating can heat up for some time even after switching off power, due to internally stored energy.

- Open door carefully so that the hot air can vent.
- Only change columns after complete cool down.
- Always wear appropriate personal protective equipment such as safety goggles, protective clothing and gloves.
- Make sure the ventilation slits and the oven heater are not covered.



# 

#### Pressurized gas

The operating instrument is pressurized. Venting of pressurized gases and solvents is possible.  $CO_2$  is suffocating in high concentrations.

- ▶ Install gas detectors at site.
- Create sufficient ventilation.



# 

Risk due to falling objects or material

Personal injury or property damage

▶ Never place solvent bottles or other items on the top of the instrument.



#### NOTE

The pumps switch off automatically when reaching 400 bar.

The detector flow cell is stable up to a pressure of 300 bar. To prevent damage to the flow cell, the back pressure regulator is programmed to switch off at 300 bar.



#### NOTE

The system my be pressurized after switching off.

Keep in mind, the escape of pressurized gases and solvents are possible.

# 3 Product description

# **3.1** Description of function

The instrument is for preparative separations on small columns by means of supercritical fluid chromatography (SFC).

- Supercritical fluid chromatography has the ability to separate gram size samples.
- The integrated gas-liquid-separators (GLS) allow fast and efficient removal of CO<sub>2</sub> from separated components.

The instrument allows:

- A mix of supercritical CO<sub>2</sub> and one solvent.
  With the optional solvent selection valve, the solvent can be chosen from four different solvents.
- Injection of liquid or solid sample
- Separation on a column
- Detection of the components by using a UV, ELSD or MS detector.
- Collecting the desired fractions



- 1 Pumps
- 3 Detector, flow cell
- 5 Post-heater
- 7 Fractions (8 pcs.)
- 9 Pre-cooling
- 11 Modifier pump
- 13 Modifier stream injection
- 15 BPR pressure value 80-250 bar
- 17 Low pressure
- 19 Depressurized fraction collection

- 2 Column oven
- 4 Back pressure regulator
- 6 Gas-liquid-separator
- 8 Liquid CO<sub>2</sub> 60 75 bar
- 10 CO<sub>2</sub> pump
- 12 Sample
- 14 Supercritical CO<sub>2</sub> 80 250 bar
- 16 Add-on pump
- 18 CO<sub>2</sub> exhaust

The mobile phase in supercritical fluid chromatography consists of a supercritical fluid and a liquid solvent. This fluid is a condensed gas above the critical temperature and pressure. Its properties are those of a gas and liquid. Carbon dioxide  $(CO_2)$  is mostly used.

The  $CO_2$  is transported by the pump in a cooled state. The fluid gets heated above the supercritical temperature in the column oven and mixed with the modifier. The back pressure regulator maintains an elevated pressure to keep the mobile phase above the critical point of  $CO_2$ . The sample is then transported over the separation column by the supercritical fluid. A modifier can be added to improve the elution of organic substances.

After detection, the  $CO_2$  is separated from the eluted substances. The Detection and Fraction valve position decides in which GLS the stream is going. The GLS separates most of the  $CO_2$  and removes it from the system by an exhaust pipe and fed into the ventilation system.

The collected fractions contain the dissolved substances in the modifier and a small amount of residual  $CO_2$  and optional in the organic from the add-on pump. The fractions are available in highly concentrated form for further analysis.

# 3.2 Configuration

#### 3.2.1 Front view



- 1 Touchscreen
- 3 Fraction bottles
- 5 Detector flap
- 7 Column oven
- 9 Add-on pump (optional)

- 2 Gas-liquid-separator
- 4 CO<sub>2</sub> and modifier pump
- 6 Fraction collector valve
- 8 Back pressure regulator cover

# â 1 2) 3 2 (4)2 8 5 6 $\overline{(7)}$ 7)

#### Column oven

- 1 Flow cell with optic fibre cable
- 3 Injection valve
- 5 Heating module
- 7 Check valves

- 2 Leak sensor
- 4 Column selector valve
- 6 Filter
- 8 Column holder



# 3.2.2 Electric and electronic connections

- 1 Fuses and FI-circuit breaker
- 3 USB connection
- 5 LAN external connection
- 7 Emergency switch connection
- 2 Main switch
- 4 HDMI connection
- 6 LAN internal connection



#### 3.2.3 CO<sub>2</sub>- and coolant connections

- 1 Connection for cooling water supply 2 Outlet CO<sub>2</sub> safety relief valve
- 3 Connection for CO<sub>2</sub> supply

#### 3.2.4 Exhaust connections

There are three exhaust connections on this instrument:

- On the left side is the exhaust of the fractionation compartment.
- On the right side is the outlet of the CO<sub>2</sub> safety exhaust valve.
- On the rear side is the GLS CO<sub>2</sub> exhaust.

#### 3.3 Scope of delivery

NOTE



The scope of delivery depends on the configuration of the purchase order.

Accessories are delivered as per the purchase order, order confirmation, and delivery note.

#### 3.4 Technical data

#### 3.4.1 Sepiatec SFC-250

	720 mm × 600 mm × 880 mm
Weight	130 kg
Power consumption	Max. 1,300 W
Connection voltage	Europe: 230 ± 10% VAC
	Asia: 230 ± 10% VAC
	USA: 120 ± 10% VAC
Frequency	50 / 60 Hz
Fuse	15 A
Over voltage category	II
IP Code	IP 20
Pollution degree	2
Minimum clearance on all sides	200 mm
Controller	15.6" touchscreen, 16:9 format
System	Integrated Windows 10
Control software	Prep SFC control software
Operating temperature range	15 – 25 °C
Operating pressure	Max. 300 bar
Injection valve	Electrically operated 6-port / 2-ways valve
Column selection valve	Electrically operated 11-port / 10-ways valves
Back pressure regulator valve	Electromagnetic check valve
Fractionation valve	Electrically operated 11-port / 10-ways valve
Tubing	Stainless steel capillary tubes OD: 1/16 <sup>~′</sup> ID: 1.0 mm or 0.5 mm or 0.25 mm
Pump head temperature control	Chiller
Method development	On analytical columns (ID: 4 – 4.6 mm)
Column dimension	ID: 4 – 16 mm
Column length	Max. 250 mm
Column oven temperature	Ambient to 70 °C

Standard column capacity	2
Max column capacity	8 (optional)
Fraction collection number	1 - 8 pcs.
Volumes	unlimited
Pressure-less collection	Standard
Syringe size	1.0 ml
	Other volumes on request
Loop	0.5 ml
Stack injection	Standard
Certificate	CSA / CE

#### 3.4.2 Ambient conditions

For indoor use only.

Max. altitude above sea level	2,000 m
Ambient and storage temperature	15 – 25 °C
Maximum relative humidity	45 – 75% not condensing

#### 3.4.3 Solvents

CO <sub>2</sub>	Liquid
Organic modifier solvents	4 pcs.
	Only with the optional solvent selector
	valve.

#### 3.4.4 Detectors

UV wavelengths range	190 – 500 nm
UV wavelengths selectable	1
DAD	Standard
ELSD	Optional
MS	Optional

#### 3.4.5 Pumps

•	
CO <sub>2</sub> Pump	150 ml/min
Flow rate	
CO <sub>2</sub> Pump	Max. 400 bar
Pressure	
Modifier pump	2 pcs.
Modifier pump	30 ml/min
Flow rate	
Modifier pump	Max. 400 bar
Pressure	
Add-on pump	Standard

Flow rate accuracy	±2% at 2 – 50% of the flow range with methanol (water 80/20 v/v)
Precision	0.25% from 1 ml/min to 20 ml/min at 20 °C

# 4 Transport and storage

## 4.1 Transport

# NOTICE

#### Risk of breakage due to incorrect transportation

- ▶ Make sure that the instrument is fully dismantled.
- Pack all instrument components properly to prevent breakage. Use the original packaging whenever possible.
- ► Avoid sharp movements during transit.
- ► After transporting, check the instrument and all glass components for damage.
- ▶ Damage that has occurred in transit should be reported to the carrier.
- ► Keep packaging for future transportation.

#### 4.2 Storage

- Make sure that the ambient conditions are complied with (see Chapter 3.4 "Technical data", page 18).
- ▶ Wherever possible, store the device in its original packaging.
- After storage, check the device, all glass components, seals and tubing for damage and replace if necessary.

#### 4.3 Lifting the instrument



# 

#### Danger due to incorrect transportation

The possible consequences are crushing injuries, cuts and breakages.

- ▶ The instrument should be transported by two persons at the same time.
- ▶ Lift the instrument at the points indicated.
- Lift the instrument this requires two persons each lifting at one of the points indicated on the bottom of the instrument.

# 5 Installation

# 5.1 Installation site



#### NOTE

Please never unpack the shipment unless expressly agreed.



# NOTE

Make sure that the power supply can be disconnected at any time in an emergency.

A BUCHI service engineer or an authorized representative will unpack and check the consignment carefully to ensure that all modules and accessories are in proper condition. This has to be done prior to installation.

The installation site must meet the following requirements:

- Firm, level surface.
- Take into account the maximum product dimensions and weight. See Chapter 3.4 "Technical data", page 18
- Clearance on each side of the instrument must be at least 200 mm.
- Do not expose the instrument to any external thermal loads, such as direct solar radiation.
- Do not expose the instrument to increased electromagnetic emissions. Electromagnetic fields in the frequency range between 200 to 300 MHz can cause the instrument to operate incorrectly.
- Make sure that the installation site meets the requirements of the safety data sheets for all solvents and samples used.
- It is recommended to install the instrument in a fume hood that remove solvent vapours and gases directly from the proximity (minimum air exchange 200 m<sup>3</sup>/ hour).
- The instrument is designed for use a laboratory bench. The instrument can be connected to a venting system by the connection nozzle on the left side.
- The Exhaust line inner diameter for the CO<sub>2</sub> gas waste must be at least 10 mm.
- The instrument must be at least 200 mm away from adjacent walls or other instruments. The distance at the reverse side should be at least 100 mm. This ensures adequate ventilation and unrestricted access to the main switch and fuses.
- Please make an external chiller available to keep the CO<sub>2</sub> pump head at a constant temperature of about 10 °C independent of the ambient temperature. Required cooling capacity (Ethanol) 0.26 kW, connection: G1/4 inch or G1/8 inch.
- The power line must be free from electromagnetic interference (EMI) and grounded. The device should not be connected to a power supply system that is vulnerable to sudden changes in power demand. If significant fluctuations in voltage occur, a constant voltage transformer may be required.
- Liquid CO<sub>2</sub> supply, pressure 60 75 bar.
- 1/8 inch connector (Swagelok)
- A CO<sub>2</sub> alarm should be set up at the site to warn of high levels of CO<sub>2</sub> in the air.
- The associated emergency switch, which should be installed within the user's accessibility and outside the fume hood. It is used to switch off the pumps, heating elements and CO<sub>2</sub> supply in the event of danger.
- For the operation of a mass spectrometer nitrogen supply must be available at the workplace (laboratory supply, nitrogen generator or cylinder).



#### Before installation

#### NOTICE

#### Instrument damage due to switching it on too early.

Switching on the instrument too early after transportation can cause damage.

• Climatize the instrument after transportation.

**Establishing electrical connections** 

#### 5.3

#### NOTICE

#### Risk of instrument damage because of not suitable power supply cables.

Not suitable power supply cables can cause bad performance or an instrument damage

► Use only BUCHI power supply cables.

#### Precondition:

- $\blacksquare$  The electrical installation is equipped with a proper grounding system.
- ☑ The electrical installation is equipped with suitable fuses and electrical safety features.
- ☑ The installation site is as specified in the technical date. See Chapter 3.4 "Technical data", page 18.
- ► Connect the mains plug to an own mains outlet socket.

# 6 Software

#### 6.1 Introduction

The instrument is controlled by the Prep SFC control software. The software is operated by the integrated touchscreen. All the information needed for a correct separation run (separation parameters, system settings, fractionation conditions) is selected by means of this software.

All parameters are entered by touching the relevant display fields. This opens a dialog box to enter and edit the data. To confirm the data, tap the [OK] button. The dialog box closed automatically and the data is visible in the display field. All display fields are shown in this operation manual. Depending on the configuration the display fields are shown in grey. This grey display fields are inactive or not included.

#### 6.1.1 Starting the software

Precondition:

 $\boxdot$  Instrument is switched on.

- Tap the [Prep SFC] symbol on the touchscreen to start the software.
- ⇒ Start window opens.



6.00 6.00							MANUAL IN	ECTION		Pressure (law)	MANUAL
2 2 2 2										*****	PARAMETER
1 = † 4.4 UV Abs	orbance [AU]	RESSURE	ů.		1	RATURE		1 LANP ON		Time(min)	COLLECTION
POSITIC Delay:	ON FRACTS	ON VALVE			WA	ISTE		FELL SYRINGE	CO2/few Notifier Plow Pressee Pang	nijnin nijnin bar	SYSTEM
1	2	3	4	5	6	7		PUECT	Pressare Modifier Dump Address Flows Pressare Address Pump	ber nijmin ber	SISILI
				VE	RIPY	AUT	IDZERO		Pressare (69) Wavelength UV VV Risorbance	ber All	
					ат	-	RVICE	START PUMP	Islandian Volume Leak Value CO2 Islat Pressure	0,0	RUN METHO

► Tap the [VERIFY] button.

⇒ The system checks if all the functional elements are responding properly.

#### 6.1.2 Entering values

#### Enter numbers

- ► Tap on an entry field.
- ⇒ The display shows a dialog box with a numeric input box.

7		,	
4	5	6	BACK
1	2	3	
			1

#### ▶ Enter the value.

- ► Tap the [OK] button to confirm.
- $\Rightarrow$  The value is saved.
- $\Rightarrow$  The dialog box closes.
- ► Tap the [CANCEL] button to leave the dialog box
  - without changing the values.
- $\Rightarrow$  The dialog box closes.

#### **Enter names**

- ► Tap on an entry field.
- ⇒ The display shows a dialog with an alphanumeric input box.
- ► Enter the value.
- ► Tap the [OK] button to confirm.
- $\Rightarrow$  The value is saved.
- $\Rightarrow$  The dialog box closes.

#### 6.1.3 Exiting the software

Precondition:

 $\boxdot$  Run is finished.

- $\square$  Pumps are switched off.
- Tap the [EXIT] button to exit the software.



 $\Rightarrow$  Prep SFC control software closes.

#### 6.2 Layout

	MANUAL INJE	CTION.		Pressure (low)	2
439. 439.				· · · · · · · · · · · · · · · · · · ·	PI 3 ER
† 4.9 uii UV Absorbance (Aii)	i ú	,	.0 →	Time (min)	cc 4 ON
BACK PRESSURE	TEMPERATURE	LAMP ON	CO2 VALV	E	
POSITION FRACTION VALVE	WASTE	FBLL SYRINGE	CO2 Plaw Rodfer Plaw Pressere Parts	nijnin nijnin ber	5
1 2 3 4	5 6 7 8	INJECT	Pressure Hoddler Pump Add on How Pressure Add on Pump	bar mijinim bar	5
(10)	AUTOZERO	START PUMP	Pressure BPR Wavelength UV UV Absorbance Injection Volume	ber All All All	RU 6 00
	8 9			0,0 ber	
No. Name		Description			

1 *Chromatogram* display To display the measured values.

No.	Name	Description
2	MANUAL tab	To start and stop a run manually. See MAN- UAL tab.
3	PARAMETER tab	To set, load and edit parameter. See PARAM- ETER tab.
4	COLLECTION tab	To configurate fractionation conditions, num- ber of peaks to be collected and the fraction- ation method. See COLLECTION tab.
5	SYSTEM tab	To configurate system settings. The system parameters are set in this window. They do not depend on the method which has been loaded and can be adjusted individually for each run. See SYSTEM tab.
6	RUN METHOD tab	To start a loaded method and view online. This window is used to start the current run, which can be monitored online. See RUN METHOD tab.
7	[VERIFY] button	To check if all the functional elements are re- sponding properly.
8	[EXIT] button	To exit the control software.
9	[SERVICE] button	To open the service settings of the system.
10	[Windows] button	To exit the control software. Opens the win- dows home screen.

# 6.3 MANUAL tab

15	MANUAL INJ	ECTION		2		1
68 68						PARAMETER
v Alexandres (AU)	1	(8)	*	+ Time	a a a a a a a a a a a a a a a a a a a	COLLECTION
SETION FRACTION VALVE	WASTE	12	CO2 How Modifier Flow Pressure Pump	0.0 0.0 132	m(min m(min far	SYSTEM
1 2 3 4	<b>5 1 6 1 7 1</b>	6	Pressure Hodfler Pump Add-on Flow Pressure Add-on Pump	- 112	tar nijnin tar	Sister
	vestry 4	51 (5)	Pressure BPR. Wavelength UV UV Absorbance Enjection Volume	132 254 0.000	ber An AU	RUN METHO
	ват (3)		Leak Value CO2 Intel Pressure	32.0	la la	

No.	Name	Description
1	MANUAL tab	To start and stop a run manually.
2	[FILL SYRINGE] button	To rinse the tubing to the syringe.

No.	Name	Description
3	[SERVICE] button	To open the service settings of the system. Parameters, sensor signals and valve posi- tions of the recycling module are displayed.
4	[AUTO ZERO] button	To set the detector signal to zero.
5	[START PUMP] button	To start the pump.
	[STOP PUMP] button	To stop the pump.
6	[INJECTION] button	To start the injection after starting the pump.
	[END INJECTION] button	To end injection.
7	CO <sub>2</sub> VALVE (grey)	Valve is closed
	CO <sub>2</sub> VALVE (green)	Valve is open
	CO <sub>2</sub> VALVE (red)	The $CO_2$ inlet pressure is too high, the system cannot be started.
	CO <sub>2</sub> VALVE (yellow/orange blink)	The CO <sub>2</sub> inlet pressure is too low. The pumps can be manual started for a flush. Starting an automated run is not possible
8	[LAMP ON] button	See RUN METHOD tab.
9	[TEMPERATURE] button	_
10	[BACK PRESSURE] button	

#### 6.3.1 Rinsing the tubing and syringe

#### Navigation path:

- → MANUAL
- ► Tap the [FILL SYRINGE] button to rinse the tubing to the syringe.
- ⇒ The display shows a dialog box with a numeric input box.
- ► Enter the value.
- ► Tap the *[OK]* button to confirm.
- $\Rightarrow$  The value is saved.
- $\Rightarrow$  The dialog box closes.



#### 6.3.2 Starting the pump



#### NOTE

Before or during an isocratic method run in after starting the manual mode. By tapping the *[STOP PUMP]* button a new window opens and allows you to change the parameters or to stop the pump.

→ MANUAL	
► Tap the [START PUMP] button.	
⇒ A new window opens.	
·	Do you next to state the Turne or change the Twometern 7
	Canol Deep Families Step
► Tap the [CHANGE PARAMETERS] button.	Corgo human for the hory
A new window opens to change the flow and	Res (related)      100.0        Roder (n)      1.0
the modifier percentage.	. The second sec
► Enter the value.	
► Tap the [OK] button to confirm.	CANCEL
$\Rightarrow$ The value is saved.	
⇒ The dialog box closes.	
► Tap the [INJECTION] button.	
► Enter the value.	
► Tap the [OK] button to confirm.	Enter the volume mi
$\Rightarrow$ The value is saved.	Nec. 1 Nec. 5
$\Rightarrow$ The dialog box closes.	7      8      9        4      5      4
	CANCEL

► Tap the [STOP PUMP] to stop the pump.

# NOTICE! An automatic run can be started, while the pump is still running but the injection has to be finished.

The chromatogram can then be called up in the *COLLECTION* menu. The fractionation parameters can be adjusted for automated further separations.

#### 6.3.3 Setting detector signal to zero

#### Navigation path:

→ Λ	IANUAL	$\rightarrow$	[SERVICE]
-----	--------	---------------	-----------

► Tap the [AUTO ZERO] button.

 $\Rightarrow$  The detector signal is set to zero.

#### 6.3.4 Changing CO<sub>2</sub> recycling module

#### Navigation path:

→ MANUAL → [SERVICE]



#### NOTE

Activated buttons have a green color.

- ► Tap the *[SERVICE]* button.
- A new window opens. Parameters, sensor signals and valve positions of the recycling module are displayed.
- ► View parameter table.
- ▶ Tap the button to activate or deactivate.



- ► Tap the [BACK TO MAIN VIEW] button to close the service view window.
- $\Rightarrow$  The window closes automatically.

#### 6.4 PARAMETER tab

The parameter menu has two isocratic modes depending on the configuration:

- UV detector
- DAD detector (optional)

50 ml 5% A		METH	OD NAME			LOAD S	AVE SAVE AS	MANUAL
Isocratic	Grad	ient	Wavelength (	nm				
Equilibration 2	) min	1,0	254 190	210		Column	-	PM 1 ER
Run Time	) min	10,0				Chiral NR Bu	SiO2 250x25mm 12u	
now (4	ml/min	150	ELSD Galei (10			not available	not available	COLLECTION
Modifier 5	) *	5	Injection (1	1 "	0,1	not available	not available	
6	)		No. of Inject	2	3	not available	not available	SYSTEM
Use Add-On 7	)	Off	Modifier (1	3)		not available	FlowControl	
Use GLS tan	)	Off	A: Methanol IPA	c		Setup XIC		RUN METHOD

No.	Name	Description
1	PARAMETER tab	To set, load and edit parameter.
2	Equilibration	Column equilibration time in minutes.
3	Run Time	Run time for the separation in minutes.

No.	Name	Description
4	Flow	Flow rate in ml/min.
5	Modifier	Modifier concentration in percentage.
6	Add-On Flow	Flow rate in ml/min of the Add-On pump
7	Use Add-On	Determines if the third pump is used as Add- On pump (On) or works together with the modifier pump (Off).
8	Use GLS tandem mode	Combines two GLS for higher flow rates
9	Wavelength	Wavelength at which detection takes place in nm.
10	ELSD Gain	Number of ELSD gain.
11	Injection	Injection volume in ml.
12	No. of Injections	Number of injections to be carried out one af- ter the other.
13	Modifier	Selection of pump inlet at the modifier pump.

#### 6.4.1 Setting new methods

#### Navigation path:

- → PARAMETER
- ▶ Tap the [METHOD NAME] button.
- ➡ The display shows a dialog with an alphanumeric input box.
- ► Enter the value.
- ► Tap the [OK] button to confirm.
- $\Rightarrow$  The value is saved.
- $\Rightarrow$  The dialog box closes.
- ► Tap the COLUMN [...] button to enter a column designation.
- A new window opens to choose a column from a list or add a new one.
- ► Tap the appropriate [A], [B], [C], [D] button to select the solvent connection.
- ► Tap the MODIFIER [...] button to enter solvent designation.
- A new window opens to choose a solvent from a list or add a new one.
- Set the parameters on the left part of the window.

#### Optional detector

► Tap the WAVELENGTH [...] button to set the number of wavelengths.

#### 6.4.2 Setting gradient mode

#### Navigation path:

- → PARAMETER
- ► Tap the [GRADIENT] button.
- A new window opens displaying the gradient table.
- ► Tap into the gradient table.
- A new window opens to edit the gradient table.
- Tap on the field on the control panel with a blue or white background to enter time, modifier and flow values.



- Enter the value.
- ► Tap the [OK] button to confirm.
- $\Rightarrow$  The value is saved.
- $\Rightarrow$  The dialog box closes.

#### NOTICE! The necessary volume of $CO_2$ is calcu-

#### lated automatically.

- ► Tap the [ADD] button to insert a row at the bottom of the table.
- Select a row and tap the [DELETE] button to remove a row.

NOTICE! The course of the gradient is shown in the upper left part of the gradient window.



- ► Tap the [OK] button to confirm and return to parameter menu.
- ► Tap the [CANCEL] button to discard the changes and return to parameter menu.
- $\Rightarrow$  The programmed gradient is displayed.

NOTICE! In the isocratic mode and gradient mode, the values for equilibration time, wavelength and injection volume and modifier inlet are determined in this window.



- ► Tap the [SAVE AS] button to save the parameter in a different name.
- ⇒ The display shows a dialog box with an alphanumeric input box.
- ► Tap the [SAVE] button to set the parameter in the methods.

NOTICE! The number of injections to be carried out in sequence is not saved in the method and must be specified again whenever reloading the method or starting the system.

#### 6.4.3 Loading saved methods

#### Navigation path:

- → PARAMETER
- ▶ Tap the [Load] button to open saved methods.
- $\Rightarrow$  A list of all methods appears.
- ► Select the needed method.
- ► Tap the [Load] button to open the selected method.

#### 6.4.4 Editing saved methods

A method that has been saved and opened can be processed as described under Chapter 6.4.1 "Setting new methods", page 31. This method must be saved again before starting it.

# 6.5 COLLECTION tab

(#1) 10		SAMPLE					2)	MANUAL
136 136 136						s	3)	PARAMETER
t and a state of the state of t		ů.	1	19	→ Time [mi	n]		CO ON
Stack Injectio	· (4)	P(7) V(8)	1 2	3 4	5	6 7	8	SYSTEM
Scort Peak Control (14)	off 0	\$9 \$10						RUN METHOD

No.	Name	Description
1	COLLECTION tab	To configurate fractionation conditions, num- ber of peaks to be collected and the fraction- ation method.
2	[Load Run]button	To open completed runs.
3	[Save Report]button	Allows the operator to save a report in PDF format.
4	[TIME] button	Chooses the time based fractionation.
5	[PEAK] button	Chooses the peak based fractionation.
6	<i>[T/P]</i> button	Switches on the combined fractionation. In this mode, the peaks are searched inside the programmed intervals.
7	Peak No.	Number of peaks to be collected.
8	Vial	Fractionation bottle in which the fraction is collected.
9	Start min	Start of fractionation.
	Start AU	This value is the threshold for the start of col- lection.
10	Stop min	End of fractionation.
	Stop AU	End of peak detection.
11	Stack injection	To start and stop stack injection.
12	Stack time	The time between one injection and the next.
13	Start peak detect time	To starts the detection mode and control the peaks.
14	Peak control	To set parameters for multiple injections.

No.	Name	Description		
	Trace	Optional detectors		
		Determines which UV wavelength is used for		
		the peak detection.		
		This value is used to determine which chro- matogram line is shown in the overview chart on the RUN menu.		
	V	Intermediate part between the peaks, the Valley.		

# i

#### NOTE

Up to eight fractions in total can be collected. All parts of a run that are not to be collected will be captured in the waste container.

#### Peak control window



No.	Name	Description
1	Use Peak Control	Switch peak control on and off.
2	Controlled Peak	Number of the controlled peak.

No.	Name	Description
3	Automatic Shift ON	The collection window is automatically cor- rected.
	Automatic Shift OFF	The shift of the peak is just observed to be smaller than max. shift.
4	Max. Shift	The maximum shift difference is in seconds. The maximum allowed shift from the reten- tion time observed during the first injection.
5	Min. Height	The minimum height is in percentage. Com- pare the minimum height the controlled peak with the fist injection peak. If the peak is smaller, the run is automatically stopped. The minimum height is in percentage. If the peak is smaller, the run is automatically stopped.


### **Injection Diagram**

No.	Name	Description
1	First injection	Chromatogram of the first injection.
2	Multiple injections	Chromatogram of multiple injections.
3	Stack injections	Chromatogram of stack injection.
4	Stack time	The time between one injection and the next.
5	Start second injection	Time when the second injection takes place.

### 6.5.1 Setting peak control

When using a fractionation mode with peak detection the programmed number of peaks are compared with the actual detected number of peaks at the end of each injection. The run stops automatically if the peak detection is not successful.

The number of peaks to be collected is determined by touching the appropriate peak number. The operator can specify the fractionation start and stop time and the sample bottle in which the fractions are collected for the number selected. The start time of the following peak must be higher than the stop time of the preceding peak. The stop time of the final fraction must be lower than the total separation run time. Tap the *[Peak No.]* button if no peaks are to be collected. This deactivates the input fields.

The peak control feature allows the system to recognize automatically when a peak is outside the collection area and either stop the run or correct the collection window accordingly.

If a mode with peak detection is used, the number of detected peaks has to be the same as the number of programmed peaks, otherwise the run will stop. This test is performed even if the Peak Control is switched off.

#### Navigation path:

- → COLLECTION → [Peak control]
- ► Tap the [OFF] button to deactivate peak control.

► Tap the [ON] button to activate peak control.

- A new window will open to set the control conditions.
- ► Set the control conditions.

Use Peak Control		On
Controlled Peak		0
Automatic Shift		or
Max, Shift	985	600
Mini, Height		0

CANCEL OK

► Tap the [OK] button to confirm.

### 6.5.2 Setting stack injection

The stack injection in isocratic mode allows time saving fractionation of large samples. The sample portions are injected after the elution of a peak.

#### Navigation path:

→ COLLECTION

► Tap the [Stack injection] button to choose the stack injection option.

Stack Injection	On
Stack time [min]	1.0
	Stack Injection

### 6.6 SYSTEM tab

	Back Pressa 2 bar	150	122	BPR	BPR is active	MANUAL
	Thermostat 3 %	6	1	CONTROL	RELEASE	MANUAL
	Frahester 4 *C	40	39			
	Column Core 5 +C	40	40	CO2 VALVE	Closed	PARAMETER
	Footbaaler 6 +c	40	40	OPEN	CLOSE	
	Fraction Mod 7 *C	35	34			COLLECTION
			$\square$			
	Springer Scale 9 ent	25.0				
	Loop Size 10 =1	5.0				
	un and (11)			PURG	E PUMP	RUN METHOD
No.	Name			Description		
1	SYSTEM tab			To configurate	e system setti	ngs. The system
				parameters ar	e set in this w	vindow. They do
				not depend or	n the method	which has been
					n be adjusted	individually for
				each run.		
2	Back Pressure				back pressure	in the system in
2	Back Pressure Thermostat			Indicates the l		
				Indicates the l bar.	in the heat ex	changer in °C.

No.	Name	Description
5	Column Compartment	Temperature in the column oven in °C.
6	Postheater	Temperature at the gas-liquid-separators in °C.
7	Fraction Module	Temperature at the gas-liquid-separators in °C.
8	ELSD Temperature	Temperature for the ELSD in °C.
9	Syringe Size	Syringe volume in ml or $\mu$ l .
10	Loop Size	Injection loop volume in ml.
11	Use ELSD	Switches the ELSD on or off.

#### 6.6.1 Controlling the back pressure

The button controlling the back pressure regulator is in the top right-hand corner. The white field shows the status of the back pressure regulator.

Navigation path:				
→ SYSTEM				
Precondition:				
<ul> <li>Instrument is running. Displayed BPR status:</li> <li>BPR is active.</li> </ul>	BPR	BPR is active		
► Tap the [ <i>RELEASE</i> ] button.				
⇒ The valve opens and release the pressure in the system. BPR status changes to BPR is open.	CONTROL	RELEASE		
Precondition:				
☑ Displayed BPR status: BPR is open.	BPR	BPR is open		
► Tap the [CONTROL] button.				
$\Rightarrow$ The valve close and activates the back	CONTROL	RELEASE		

#### 6.6.2 Controlling the CO<sub>2</sub> valve

The button controlling the CO<sub>2</sub> valve is located in the middle. The white field shows the status of the CO<sub>2</sub> valve.

#### Navigation path:

→ SYSTEM		
Precondition:		
<ul> <li>✓ Displayed CO₂ VALVE status: Closed.</li> <li>▶ Tap the [OPEN] button.</li> </ul>	CO2 VALVE	Closed
⇒ The valve opens. CO <sub>2</sub> VALVE status changes to Open.	OPEN	CLOSE

Precondition:

 $\square$  Displayed CO<sub>2</sub> VALVE status: Open.

- ► Tap the *[CLOSE]* button.
- $\Rightarrow$  The valve closes. The CO<sub>2</sub> VALVE status changes to Closed.



### 6.6.3 Controlling the purge pump

#### Navigation path:

$\rightarrow$ SYSTEM $\rightarrow$ [Purge Pump]	
---	--

#### Precondition:

- $\square$  Instrument is running.
- $\square$  Displayed CO<sub>2</sub> VALVE status: Closed.
- ► Tap the [Purge Modifier Pump] or [Purge Addon Pump] button.
- Non France

   Maine Name

   M
- $\Rightarrow$  A new window opens to set modifier pump.
- ► Tap the [Open] button to release the purge valve from the pumps.

NOTICE! The Purge Add-on Pump has just one inlet. The modifier inlet can not be selected. NOTICE! If the pressure at the pump is higher than the maximum pressure for the purge pump, it will not start to purge.

- ► Select the modifier inlet and the flow rate.
- ► Tap the [Pump Start] button to start the process.
- ► Tap the [*Pump Stop*] button to close the purge valve at the pump and exit purge.

### 6.7 RUN METHOD tab



No.	Name	Description
1	Chromatogram	The current chromatogram is shown in the upper section of the window.
2	Title bar	The method name and sample name are dis- played in the title bar. The injection number can be seen in the title bar.
3	Process overview graph	In the process overview graph in the bottom left-hand corner, chromatograms are dis- played overlaid in case of multiple injections. In case of stack injection, the collected frac- tions are displayed overlaid.
4	Parameter status	The status of individual system parameters and method parameters during the run is shown in the table in the bottom right-hand area.
5	[ <i>PEAK CONTROL</i> ] button (grey)	Peak control is off.
	<i>[PEAK CONTROL]</i> button (green)	Peak control is on. The position of the peaks has not changed compared to the first injection.
	[PEAK CONTROL] button (or- ange)	Peak control is on. The peak being monitored has moved and the collection window was adjusted automatically.
	<i>[PEAK CONTROL]</i> button (red)	Peak control is on. The peak being monitored is outside the collection range and the run was stopped.

No.	Name	Description
6	[LAMP OFF] button (grey)	Lamp is off.
	<i>[LAMP HEATING]</i> button (yellow)	Lamp is heating up.
	[LAMP ON] button (green)	Lamp is on.
7	<i>[TEMPERATURE]</i> button (red)	The temperatures in all monitored parts (col- umn department, preheating and fractiona- tion module) have not been reached. The run cannot be started.
	<i>[TEMPERATURE]</i> button (yel- low)	The column department and the preheating have reached the set temperature. The run can be started.
	<i>[TEMPERATURE]</i> button (green)	The fractionation module as well as the col- umn department and the preheating have reached their temperature intervals. Injec- tions can be done.
8	[BACK PRESSURE] button (orange)	The back pressure regulator valve is active. The pumps can be started. A run can also been started, but the equilibration time only starts counting after the back pressure has been reached.
	[BACK PRESSURE] button (green)	The back pressure set in the system has been reached. The run can be started.
	<i>[BACK PRESSURE]</i> button (yellow)	The back pressure set in the system has not been reached within 2 minutes after the start, or the pressure has been 10 bars lower than the set pressure for more than 30 sec during the run, or the pressure has dropped for more than 50 bar during the run. The pumps are switched off as there may be a leak.
9	[CHANGE] button	The button allows changing parameters dur- ing the run.

### 6.7.1 Before starting a run

#### Navigation path:

- → RUN METHOD
- ► Tap the *[Start]* button.
- ➡ The display shows a dialog with an alphanumeric input box.
- ▶ Enter sample name.

- ► Tap the [OK] button to confirm.
- $\Rightarrow$  The value is saved.
- $\Rightarrow$  The dialog box closes.
- Start a run. See Chapter 7.5 "Starting a run", page 53.

### 6.8 Changing during a run

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The minimum number of injections is the current injection or 1, if the equilibration is still running.

The number of injections can be changed for all injection types.

### NOTE

NOTE

With multiple injection, the injection volume and the run time for isocratic runs can be adjusted.

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### NOTE

A change of the injection volume is effective on the next not yet started aspiration of sample volume. If the start of the injection process is later than it should be for a new increased volume, the next injection will wait the necessary amount of time. This will not change the run time.



### NOTE

The fractionation parameters can be adjusted starting with the second fractionation.

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1	

#### NOTE

When thresholds are changed the effect is simulated on the already recorded and shown in the graph to show the effect. The increasing tail slope (arrows to the right) will move the line to the left and vice versa.



#### NOTE

If collection intervals are changed the originally used values are shown in a less vibrant colour, the now used values will be shown in the known color.

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### NOTE

If runs are loaded on the collection menu, the original values for the collection are shown, if only one injection is shown, the values used in this injection are shown.

### 6.8.1 Change No. of Injections

#### Navigation path:

 $\rightarrow$  RUN METHOD  $\rightarrow$  [Change]

NOTICE! This window close automatically after 10 seconds if no selection is made.

- ► Tap the [No. of Injections] button to change numbers of injection.
- $\Rightarrow$  A new window opens.

No. of Irgentions	3	
legetion est	1.0	
Run Time min	6.0	
Colector Parameters		

► Tap a [negative number] button to set a minimum number.



- ► Tap the [CANCEL] button to close the window without any changes.
- ► Tap the [OK] button to update the version of the solvent amount.
- $\Rightarrow$  The window closes automatically.

#### 6.8.2 Change injection and run time parameter

#### Navigation path:

 $\rightarrow$  RUN METHOD  $\rightarrow$  [Change]

NOTICE! This window close automatically after 10 seconds if no selection is made.

- ► Tap the [Injection] or [Run Time] button to change parameters.
- ⇒ The display shows a dialog with a numeric input box.

Olanga parameters	during the run		
No. of Ispectors		3	
Injection	**	10	
Run Time	min	- 60	
Collection Param			

- ► Enter the value.
- ► Tap the [OK] button to confirm.
- $\Rightarrow$  The value is saved.
- $\Rightarrow$  The dialog box closes.

Min: G	8		Mac 200		
2		,	MCK	Overfil Loop	
	,		BACK		
		,			

NOTICE! The used maximum volume is not only determined by the injection loop or the size of the syringe but also by the time between injections.

- ► Tap the [CANCEL] button to close the window without any changes.
- ► Tap the [OK] button to update the version of the solvent amount.
- $\Rightarrow$  The window closes automatically.

#### 6.8.3 Change fraction parameter

#### Navigation path:

 $\rightarrow$  RUN METHOD  $\rightarrow$  [Change]

#### NOTICE! This window close automatically after 10 seconds if no selection is made.

- ▶ Tap the [Collection Parameters] button.
- $\Rightarrow$  A new window opens.

Ownge parameters during the run	
No. of Dejections	3
lipition ml	1,0
Run Time min	6,0
Colection Parameters	

► Tap the [Show Last] button.

Allows to show and toggle between the last, the last two or the last three injections (if these are already recorded).



NOTICE! The method for the fractionation (time and/or peak based) as well as the Start peak detect time can not be changed after the run is started. These buttons are just for information.

- ▶ Tap one of the value button for Start or Stop to change the fraction parameter.
- $\Rightarrow$  The value will be shown in blue.



- ► Tap the [>] button to change the value + 0.01.
- ► Tap the [<] button to change the value 0.01.
- ▶ Tap the [>>] button to change the value + 0.05.
- ▶ Tap the [<<] button to change the value 0.05.
- ▶ Tap the [OK] button to make the changes effective.

#### 6.9 CO<sub>2</sub> recycling module

Navigation path:  $\rightarrow$  [SERVICE] → MANUAL 5)<sup>₅ off</sup> (15) Switch Ter (1)EME(2) BACK (3)VIEW 6) Piston Flu (16) •• 7 Detector (17)PARAMETER (18) Lamp On (8) Close 4 Waste Bottle Se Lamp Off (9)Naste Bottle Se COLLECTION Flush Fra Syringe Ex (11)Inject Va Inject Valv (12) (13)<sup>nject</sup> RUN METHOD Version: 3.1.0.8 Expansi 

No.	Name	Description
1	MANUAL tab	To start and stop a run manually.
2	[EMERGENCY STOP] button	To perform the EMERGENCY STOP.
3	[BACK TO MAIN VIEW] but- ton	To go back to the MANUAL tab view.



No.	Name	Description
4	Parameter table	To see several parameters about the recycling module.
5	[Switch Temperature OFF] button	To switch off all temperature controllers.
6	[Piston Flush 10 sec] button	To flush the piston for 10s.
7	[Detector Calibration] button	To manually start the calibration of the UV detector.
8	[Lamp ON] button	To switch on the lamp.
9	[Lamp OFF] button	To switch off the lamp.
10	[Syringe Exchange] button	To move the syringe pump into a position that allows the exchange of the glass syringe.
11	[Inject Valve to Inject] but- ton	To switch the injection valve into the manual position.
12	[Inject Valve to Load] button	To switch the injection valve into the load po- sition.
13	[Expansion Valve to Inject] button	To switch the expansion valve into the injec- tion mode.
14	[Expansion Valve to Expand] button	To switch the expansion valve into the expan- sion mode.
15	[Stop Flush Fraction immedi- ately] button	This button cancels the fractionation cycles of all 10 GLS channels.
16	<i>[Manual Flush Fractions]</i> button	This button starts the fractionation cycle of a GLS channel that can be selected in a new window.
17	[Flush condensate] button	This button simulates a liquid detection in the first cylinder, so that any residue condensate is flushed out.
18	[Close all Fraction Valves] button	This button closes all fractional valves for a service on the recycling module.
19	Software Version	Displays the current software version.

#### **Parameter table**

Fraction Ir 1 essure	46.9	bar	
Recycling 2 Pressure	72.3	bar	
Recycling 3 Pressure	47.1	bar	
Waste Bott 4 isor	Off		
Waste Bott 5 Isor Count	0		
Flush Frad 6	Off		
Fraction O 7 Ime	0.0	sec	

No.	Name	Description
1	Fraction Inlet Pressure	The pressure at the input of gas-liquid separa- tors
2	Recycling Inlet Pressure	The pressure from the $CO_2$ supply that goes into the recycling module
3	<b>Recycling Outlet Pressure</b>	The pressure of the recycled $CO_2$
4	Waste Bottle Sensor	The sensor is set to detect liquid [ON] or not [OFF]
5	Waste Bottle Sensor Count	This value indicates how often the sensor has detected liquid. This value is an indication how stable the recycling and the gas-liquid separators are working. A low value means stable conditions. A high value means a lot of liquid is entrained. This value is set to zero at every start in the Run Method window.
6	Flush Fraction	This value indicates whether the fraction valve out is closed OFF or open ON.
7	Fraction Open Time	This value indicates that fractionation is in progress and how long the fraction valve in is still open.

### **Functional description**

If the Recycling Inlet Pressure and Recycling Outlet Pressure are approximately equal, the  $CO_2$  pump is supplied with recycled  $CO_2$ .

In the first cylinder of the recycling module dissolved modifier can condense. The liquid collects at the bottom of the cylinder, where a valve-sensor-valve combination is installed. When the sensor detects liquid, the upper valve is closed and the lower valve is opened to drain the condensate.

Due to the recycling module, the gas-liquid separators (GLS) are also under pressure. The fractionated liquid is fed to the recycling module via pressure-stable flexible lines. A valve-storage tube-valve combination is available for each of the ten GLS. When a GLS is switched active for fractionation in the software, the upper valve (fraction valve in) opens and the storage tube fills with the fractionated liquid. The software uses the flow rate of the modifier pump to calculate how long the valve can remain open and when it is full. It will close and open the lower valve (fraction valve out) to transfer the fractionated liquid to the collection container.

## 7 Operation

### 7.1 System start - up

- Switch on the external thermostat. The recommended temperature is 5 °C.
- ▶ Switch on the chiller.
- ▶ Push the *ON/OFF-switch*.
- ➡ This powers up the remaining hardware.
- Switch on the system with the main switch.
- ⇒ This also starts the computer and the touchscreen.
- ▶ Release the emergency switch button.
- Press the green button next to the emergency switch.
- ► Tap the [Prep SFC] symbol on the touchscreen to start the software.
- ▷ Prep SFC control software window opens.



6.00 6.00							MANUAL INJ	IBCTION .				MANUAL
												PARAMETER
† 44	orbance (AU)							,		- Tene (nin)	10 H	COLLECTION
	BACKP	RESSURE		TEMPERATURE			TEMPERATURE LAMP ON		CO2 VALVE			
POSTING	ION FRACTION VIEWE					CO2 Play		(min )				
		AL DELLE		WASTE				FBLL SYRINGE	Notifer Flow		Code	
Delay: -						Presson Partici		ber	SYSTEM			
									Pressare Hoddler Pump		ber	
1	2	3	4	5	6	7		INDECT	Add on Flow		Ceals	
									Pressare Add on Pump		ber	
				-					Pressare (898)		bir	
				VE	RIFY	AUT	DZERO		Wavelength UV		Alb	
								START PUMP	W Reating		AU .	RUN METHO
				-		1000		STRATTONE	Standism Volume		m	
				e	TD	50	NOCE		Lesk Table	0,0		
									CO2 bird Presserv		bar .	

Tap the [Verify] button to ensure that all modules are communicating correctly.

### 7.2 Positioning of the sample



### NOTE

The capillary tubes should descend in a straight line and without tension.

- ▶ Place the sample bottle into the sample holder.
- ► Feed the capillary tubes through the fittings of the holder until it reach the base of the sample bottle.



### 7.3 Positioning the fractionation bottles



### NOTE

The number of the GLS corresponds to the outlet number of the fractionation valve.

- Place the fraction bottles in the positions specified in the collection menu.
- Attach the appropriate capillary tubes of the gas-liquid-separators to the fraction bottle.
- Make sure the waste bottle is in the central position (position 9).



### 7.4 Method selection

#### Navigation path:

- → PARAMETER
- ► Tap the [Load] button to select the method you intend to use.
- Specify the number of injections which have to take place in sequence.
- Check if the correct column is in place at the selected column position.
- ▶ Tap the *COLLECTION* menu button.

- Define the number of fractions to be collected and the fractionation time-scale.
- Check the system settings in the system window.

See Software, for more detailed information about setting up methods, adjusting fractionation conditions and specifying system settings.

### 7.5 Starting a run



NOTE

Depending on the selected parameters, an equilibration time of several minutes is required to adjust to the set temperature profile. The shortest recommended time period is 5 minutes. If a stable temperature profile has not yet been established during this time, the equilibration time is automatically extended until either a stable temperature profile has been established or a maximum duration of 15 minutes has been reached. If a stable temperature profile has not been established after 15 minutes, the run is automatically stopped.

### Navigation path:

→ PARAMETER

Precondition:

- $\square$  Make sure there is enough  $CO_2$  and solvents to complete the separation run of the sample. The eluent consumption is highly dependent on the used method. To prevent damage to the pumps, it is necessary to avoid running them dry.
- ☑ Make sure the volume of the fraction bottles and the waste container are large enough for the whole separation to avoid overfilling.
- ☑ Make sure the collection bottle is empty and the collection bottle cap is tightly screwed.
- $\ensuremath{\boxdot}$  Make sure instrument is ready for a run.
- $\ensuremath{\boxdot}$  Make sure the correct method and fraction conditions are selected.
- ► Select the correct column, gradient and detection wavelength.
- ► Tap the [Pump Start] button.
- $\Rightarrow$  The pumps are starting with the starting conditions of the selected gradient.
- ► Tap the [Injection] button.
- $\Rightarrow$  The injection can be started when the set temperature conditions are reached.

### 7.6 Finishing a run



#### NOTE

After a run is finished, the pumps stop automatically and the  $CO_2$  stop valve is closed automatically. The temperature controllers and the back pressure regulator will remain active.

- ► Tap the *[STOP]* button.
- $\Rightarrow$  A new window opens.

want to stop the Run ?				
	Cancel	Freah Departmen	Inmediately	

- ► Tap the *[Cancel]* button.
- ⇒ The *RUN METHOD* menu opens but the
  - run continues.
- ▶ Tap the [Finish Injection] button.
- All injections that are already on the column (stack injection) will be finished. Then the system stops. The caption of the automatic start button changes to stopped.
- ► Tap the [Immediately] button.
- $\Rightarrow$  The run stops immediately.

### 7.7 Shutting down the system

#### Navigation path:

- → MANUAL
- Tap the [EXIT] button to close the Prep SFC control software.
- ▶ Shut down the windows computer.
- Switch off the systems with the main switch to shut down all the modules.

### 7.8 Using the emergency switch

- Press the *emergency switch* button to turn off the pumps, the detector and all temperaturecontrolled modules.
- $\Rightarrow$  This closes the CO<sub>2</sub> stop valve that no more CO<sub>2</sub> can emerge.
- ⇒ The windows computer is still running. No data gets lost by the emergency switch off.

### 8 Cleaning and servicing



### NOTE

- ▶ Carry out only the service and cleaning operations described in this section.
- Do not carry out any servicing and cleaning operations that involve opening the housing.
- Use only genuine BUCHI spare parts in order to ensure correct operation and preserve the warranty.
- Carry out the service and cleaning operations described in this section to extend the lifetime of the instrument.

### 8.1 Pumps

For the pump maintenance please refer to the manufacturer documentation.

### 8.2 Servicing the injection valves

The Rheodyne injection valves can be opened when they are blocked. If this happens, it is recommended that the seals and stator be cleaned in an ultrasound bath using methanol. Seals should be replaced when they are worn. The parts required are contained in the Rhebuild kit.

### 8.3 Cleaning the UV detector flow cell

Precondition:

- ☑ The detector baseline starts to get noisy. Possible sample residues are in the flow cell.
- Remove the columns.
- ▶ Replace the columns by a stainless steel capillary tube.
- ▶ Rinse the stainless steel capillary tube with CO<sub>2</sub> modifier mixture.

For further instructions please consult the manufacturer's manual for the Knauer Detector UVD 2.1S.

### 8.4 Back pressure regulator valve

Please contact the BUCHI Customer Service if any problems occur with the back pressure regulator valve.

### 8.5 Fractionation valve

#### 8.5.1 Cleaning the valve and replacing the rotor

The valve can be cleaned by rinsing through all the channels with a suitable solvent. If this does not resolve the fault, the valve can be dismantled so that the parts can be cleaned more thoroughly and checked for damage.

The valves have polished, sealed surfaces which must be protected during dismantling and cleaning. Work in a clean environment and place the parts on a soft cloth or clean paper.



Motor		2

3 Rotor

Body
 Cap/Stator

### 8.5.2 Dismantling

1

- Use the 3/32" hex key provided to remove the five hex socket screws that connect the stator to the valve body.
- Place the stator on its outer side to prevent damage to its sealed surface.
- ► Carefully pull the rotor away from the motor.
- Check the rotor surface for scratches. Replace the rotor if there is any damage.
- Check the surface of the stator.
- Replace it if there are any scratches between the connections. Contact BUCHI Customer Service to have the part examined to ascertain whether it can be reconditioned.
- Use suitable solvents to clean all parts and take care to avoid scratching the surfaces. The rotor does not need to be dried.

### 8.5.3 Assembling

- Put the rotor back on the motor. Make sure the rotor surface with the notch indicating the flow is facing outwards.
- ▶ Put the stator back on the valve.

- Put the five hex socket screws back on but only tighten them slightly.
- Tighten the screws diagonally one by one until all the screws are tight. Take care not to overtighten the screws as they only hold the structure together and do not affect seal strength.
- ▶ Test the valve in the system under pressure.
- Please contact BUCHI Customer Service if problems persist.

### 8.6 Gas-liquid-separators (GLS)

### NOTE

To avoid contamination in the collected fractions, it is recommended to flush the gas-liquid separators with a  $CO_2$  modifier mixture after each run.

- Purged each GLS for 30 seconds with a total flow and the modifier content.
- $\, \Rightarrow \,$  If this is not sufficient clean them manually.
- Remove the GLS cups and clean them manually with an ultrasound bath using a suitable solvent.
- ► Loosen the green union nut.
- ▶ Remove the flask cap.



- Loosen the brown PEEK capillary tube fittings on the GLS.
- Remove the brown PEEK capillary tube fittings on the GLS.



Carefully pull the blue insulation vertically downwards from the GLS.



- Use the GLS key provided to open the GLS by turning it to the left.
- ► Hold the GLS with one hand and take it out.



## 9 Help with faults

### 9.1 Troubleshooting

Possible cause	Action		
Mains plug is not connected	Connect the mains plug		
	<ul> <li>Switch on fuse or FI-circuit breaker</li> </ul>		
Computer or touchscreen defective	<ul> <li>Contact BUCHI Customer Service</li> </ul>		
Mains plug is not connected	Connect the mains plug		
Fuse or FI-circuit breaker are tripped	<ul> <li>Switch on fuse or FI-circuit breaker</li> </ul>		
Computer or touchscreen defective	<ul> <li>Contact BUCHI Customer Service</li> </ul>		
Pump defective	Contact BUCHI Customer		
Blockages in the capillary system or separation column	Service		
Valve malfunction			
Pump suction not operating	<ul> <li>Secure fittings to suction capillary tube, open purge valve and pump at high flow rate</li> </ul>		
Leaks in the system	Secure fittings		
High-pressure pump seals are worn	<ul> <li>Replace high-pressure pump seals</li> </ul>		
Check valves are dirty or worn out	<ul> <li>Clean or replace check valves</li> <li>See pump manual.</li> </ul>		
Defective heating elements	Contact BUCHI Customer		
Defective temperature con-	Service		
	Mains plug is not connectedFuse or FI-circuit breaker are trippedComputer or touchscreen defectiveMains plug is not connectedFuse or FI-circuit breaker are trippedComputer or touchscreen defectivePump defectiveBlockages in the capillary system or separation columnValve malfunctionPump suction not operatingLeaks in the systemHigh-pressure pump seals are wornCheck valves are dirty or worn outDefective heating elements		

### 10 Taking out of service and disposal

### 10.1 Taking out of service

- ▶ Remove all solvents and coolants.
- ▶ Switch off the instrument and disconnect it from the mains power supply.
- Clean the instrument.
- ▶ Remove all tubing and communication cables from the device.

### 10.2 Disposal

The operator is responsible for proper disposal of the instrument.

- ► When disposing the equipment observe the local regulations and statutory requirements regarding waste disposal.
- ▶ When disposing, observe the disposal regulations of the materials used. Materials used see Chapter 3.4 "Technical data", page 18.

### **10.3** Returning the instrument

Before returning the instrument, contact the BÜCHI Labortechnik AG Service Department. https://www.buchi.com/contact

## 11 Appendix

### **11.1** Spare parts and accessories

Use only genuine BUCHI consumables and spare parts in order to ensure correct, safe and reliable operation of the system.



### NOTE

Any modifications of spare parts or assemblies are only allowed with the prior written permission of BUCHI.

We are represented by more than 100 distribution partners worldwide. Find your local representative at:

www.buchi.com

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