KNAUER

AZURA

Autosampler 3950
User Manual

V6818

HPLC

Table of Contents

Note For your own safety, **read** the manual and **always** observe the warnings and safety information on the device and in the manual!

Intended Use **7**
Device Overview 7
Features 8
Standard 8
Device Variants 8
Eluents 8
Unsuitable Eluents 8
Less Suitable Eluents 8
Suitable Eluents 9
Flushing Solution 9

Safety for Users **9**
Signal Words 10
Decontamination 10

Symbols and Signs **11**

Installation **11**
Scope of Delivery 11
Unpacking and Setup 11
Packaging and Transport 11
Contacting the Technical Support 11
Location Requirements 12
Space Requirements 12
Unpacking 12
System Adapter (A9863) 13
Startup 13
Opening the Door 14
Removing the Front Paneling and the Cooler Cover 14
Sample Compartment 15
Connecting the Device in a Local Area Network (LAN) to a Computer 15
Configuring the LAN Settings 16
Connecting the Cables 16
Configuring the Router 17
Integrating the LAN into a Company Network 17
Controlling Several Systems Separately in a LAN 17

Injection Principles **17**
ILD™ for analytical Autosamplers 17
PASA™ Loop Injection 18
Full Loop Filling 19
Partial Loop Filling 21
Microliter Pickup 24
Microliter Pickup with 84+3 Vial Plate 27

84+3 Vial Plate 28
Parameters for Microliter Pickup 29
Details on Programming 30

Air Needles 30
Standard Air Needle 30
Choosing the Correct Air Needle 31
Calculation Example for Air Needle 32
Handling the Sample Vials 32

Mixing and Diluting 33
Example: Add 33
Example: Mix 33
Sample Positions in Mixing Routine 34
Processing in Columns 34
Processing in Rows 34
Parameters for Mixing Method with 84+3 Vial Plate 34
Details on Programming the 84+3 Mixing Method 35

Capillary and Tubing Connections 36
Connecting the Injection Valve 36
Connecting the Syringe 37
Tube Guide for Flushing Solution 37
Connecting the Drainage Tubing 37
PEEK Connections 38

Connecting the Autosamplerwith other Devices 38
Controlling the Autosampler with Chromatography Software 38
Checking and Configuring the Parameters of the Autosampler 38
Configuration Window of ClarityChrom® 38
Autosampler Device Software 38
Flushing the System 39
System Flushing with Autosampler 3950 Service Manager 39
I/O Connection 39
Defining the TTL Inputs 40
Defining the Closed-Contact Output 40
Configuration of I/O Connection (9 pins) 40

Device Test 40
Test Intervals 40
Devices and Components for the Test 41
1. Reproducibility of Sample Volume 41
Standard Setting of Autosampler 41
Method Parameters of Pump 41
Method Parameters of Autosampler 41
Method Parameters of UV Detector 41
Configuring Repeat Runs of Autosampler 42
Starting Repeat Runs 42
Analyzing the Individual Chromatograms 42
Formula for Determining the Arithmetic Mean 42

2. Sample Carryover 42
Creating a Sequence with 6 Lines 42
Analyzing the Individual Chromatograms 42
Formula for Calculating Sample Carryover 43
3. Linearity 43
Analyzing the Individual Chromatograms 43
Formula for Determining the Correlation Coefficient 43
4. Mixture Test 43
Creating a Sequence with 2 Lines 44
Positioning Vials for Dilution 44
Analyzing the Individual Chromatograms 44
Archiving 44
Test Report 45
Operation Qualification Report 46

Maintenance and Care 46
Maintenance Contract 46
Which Type of Maintenance Tasks May Users Perform on the Device? 47
System Flushing 47
Taking out of Operation 47
Exchanging the Fuses 48
Exchanging the Injection Valve and Rotor Seal 48
Removing the Injection Valve and Rotor Seal 48
Installing the Injection Valve 49
Exchanging the Sample Loop 50
Exchanging the Sample Needle 50
Exchanging the Air Needle 51
Exchanging the Syringe 52
Exchanging the Syringe Plunger or Plunger Tip 52
Exchanging the Syringe Valve 53
Cleaning and Caring for the Device 53

Troubleshooting 54
Device Errors 54
Checking the Valve 54
LAN 54
Analytical Errors 55
System Messages in OpenLAB® 56

Technical Data 66
General Data 66
Analytical Versions 67
Preparative Versions 68

Repeat Orders 68

Legal Information **69**
Transport Damage 69
Warranty Conditions 70
Declaration of Conformity 70
Disposal 70

HPLC Glossary **71**

Index **72**

An alle, die es betrifft

Wenn Sie ein französischsprachiges Benutzerhandbuch zu diesem Produkt wünschen, senden Sie ihr Anliegen und die entsprechende Seriennummer per E-Mail oder Fax an KNAUER:

- support@knauer.net
- +49 30 8015010

Vielen Dank.

To whom it may concern

In case you prefer a French language user manual for this product, submit your request including the corresponding serial number via email or fax to KNAUER:

- support@knauer.net
- +49 30 8015010

Thank you.

A qui que ce soit

Si jamais vous préfériez un manuel en francais pour ce produit contacter KNAUER par email ou par fax avec le no. de série:

- support@knauer.net
- +49 30 8015010

Merci beaucoup.

Intended Use

Note: Only use the device for applications that fall within the range of the intended use. Otherwise, the protective and safety equipment of the device could fail.

Device Overview

Autosampler 3950

The Autosampler 3950 was developed for chromatographic analyses in the high pressure (HPLC) range. An injection valve guarantees precise injection volumes maximum 1000 or 700 bar for analytical autosamplers. There is an additional variant available offering temperature control. The device features fast sample injection, rapid flushing cycles and high sample throughput. The Autosampler is generally installed as a core element in an HPLC system.

Legend

① opening for capillary feed
② removable cover
③ inward sliding glass door
④ temperature control with sample cooler inside the Autosampler
⑤ tubing connectors

Fig. 1 front panel

The following components, connections and warnings can be found at the rear panel:

Legend

① LAN connector
② I/O connection (9-pin)
③ power switch
④ CE mark
⑤ fuse box
⑥ mains power connection
⑦ cooler fan
⑧ serial number and year of manufacture of device

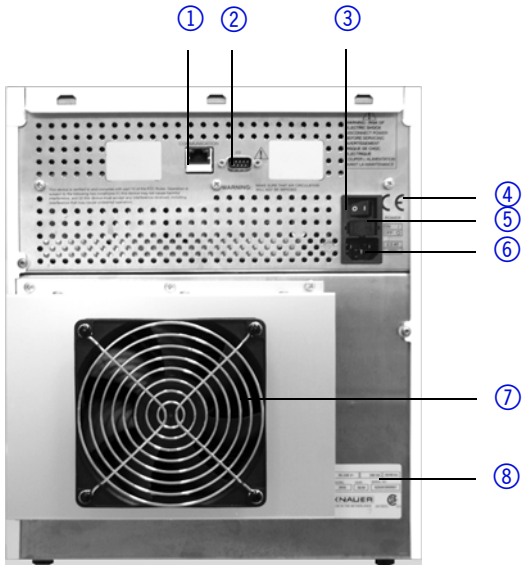


Fig. 2 rear panel

Operating ranges

The device can be used in the following areas:

- biochemical analysis
- chemical analysis
- food analysis
- pharmaceutical analysis
- environmental analysis

Features

To make your HPLC/UHPLC separations as efficient as possible, pay close attention to the following:

- Use ultra-pure, filtered solvents – Gradient grade – for the HPLC.
- Filtration of substances under analysis.
- Use of inline filters.

Standard

- parameters for analytical autosamplers:
  - injection valve with injection volume maximum of 1000 or 700 bar
  - Intermediate Loop Decompression technology (ILD™)[1]
- injection volume for HPLC between 0.1 µl and maximum 5000 µl (depending on model and equipment)
- temperature control 4-40 °C[2]
- flexibly equipped with microtiter plate or standard sample plate
- maximum sample capacity of 768 Wells or 108 standard Autosampler vials (depending on version)
- injection cycle < 60 s, 60 s incl. cleaning
- full loop or partial loop injection or microliter pick-up injection
- quick-exchange injection valve
- sample priority function
- high-resolution syringe controller

Device Variants

There are different variants of the device available

- analytical autosamplers 1000 bar A50070, A500701
- analytical autosamplers 700 bar A50080, A500811
- preparative autosamplers A50054-1, A50056-1
  - biocompatible versions A50052-1, A50053-1, A50055-1, A50055-2

Eluents

Even small quantities of other substances, such as additives, modifiers, or salts can influence the durability of the materials. If there is any doubt, contact the Technical Support of the manufacturer.

Note: The list of selected solvents was compiled based on research in the pertinent literature and is only a recommendation by KNAUER.

Unsuitable Eluents

- halogenated hydrocarbons, e.g. Freon®
- concentrated mineral and organic acids
- bases
- liquids containing particles

Less Suitable Eluents

- dimethyl sulfoxide (DMSO)
- fluorinated hydrocarbons
- slightly volatile eluents
- methylene chloride[3]
- tetrahydrofuran (THF)[3]

1. ILD™ is a Spark Holland trademark
2. Relevant to Autosampler 3950 with temperature control
3. not recommended in combination with PEEK small parts and PEEK capillaries

Suitable Eluents

- acetone
- acetonitrile[1]
- benzene
- chloroform
- acetic acid (10-50 %), at 25 °C (77 °C)
- ethyl acetate
- ethanol
- Hexane/heptane
- isopropanol
- methanol
- toluol
- water

Flushing Solution

Do not use a salt or buffer solution for flushing.

Safety for Users

Professional Group

The user manual addresses persons who are qualified as chemical laboratory technicians or have completed comparable vocational training.

The following knowledge is required:

- Fundamental knowledge of liquid chromatography
- Knowledge regarding substances that are suitable only to a limited extent for use in liquid chromatography
- Knowledge regarding the health risks of chemicals
- Participation during an installation of a device or a training by the company KNAUER or an authorized company.

If you do not belong to this or a comparable professional group, you may not perform the work described in this user manual under any circumstances. In this case, please contact your superior.

Safety Equipment

When working with the device, take measures according to lab regulations and wear protective clothing:

- Safety glasses with side protection
- Protective gloves
- Lab coat

What must be taken into account?

- All safety instructions in the user manual
- The environmental, installation, and connection specifications in the user manual
- National and international regulations pertaining to laboratory work
- Original spare parts, tools, and solvents made or recommended by KNAUER
- Good Laboratory Practice (GLP)
- Accident prevention regulations published by the accident insurance companies for laboratory work
- Filtration of substances under analysis
- Use of inline filters
- Once they have been used, never re-use capillaries in other areas of the HPLC system.
- Only use a given PEEK fitting for one specific port and never re-use it for other ports. Always install new PEEK fittings on each separate port.

1. not recommended in combination with PEEK components or PEEK capillaries

- Follow KNAUER or manufacturer's instructions on caring for the columns

More safety-relevant information is listed below:

- flammability: Organic solvents are highly flammable. Since capillaries can detach from their screw fittings and allow solvent to escape, it is prohibited to have any open flames near the analytical system.
- solvent tray: Risk of electrical shock or short circuit if liquids get into the device's interior. For this reason, place all bottles in a solvent tray.
- solvent lines: Install capillaries and tubing in such a way that liquids cannot get into the interior in case of a leak.
- leaks: Regularly check if any system components are leaking.
- power cable: Defective power cables are not to be used to connect the device and the power supply system.
- self-ignition point: Only use eluents that have a self-ignition point higher than 150 °C under normal ambient conditions.
- power strip: If several devices are connected to one power strip, always consider the maximum power consumption of each device.
- power supply: Only connect devices to voltage sources, whose voltage equals the device's voltage.
- toxicity: Organic eluents are toxic above a certain concentration. Ensure that work areas are always well-ventilated! Wear protective gloves and safety glasses when working on the device!

Where is use of the device prohibited?

Never use the system in potentially explosive atmospheres without appropriate protective equipment. For further information, contact the Technical Support of KNAUER.

Secure decommissioning

At any time, take the device completely out of operation by either switching off the power switch or by pulling the power plug.

Opening the Device

The device may be opened by the KNAUER Technical Support or any company authorized by KNAUER only.

Signal Words

Possible dangers related to the device are divided into personal and material damage in this user manual.

Lethal injuries will occur.

Serious or moderate injuries can occur.

Minor injuries can occur.

Device defects can occur.

Decontamination

Contamination of devices with toxic, infectious or radioactive substances poses a hazard for all persons during operation, repair, sale, and disposal of a device.

Life-threatening injuries

Health danger if getting in contact with toxic, infectious or radio-active substances.

➔ Before disposing of the device or sending it away for repair, you are required to decontaminate the device in a technically correct manner.

All contaminated devices must be properly decontaminated by a specialist company or the operating company before they can be recommissioned, repaired, sold, or disposed of. All materials or fluids used for decontamination must be collected separately and disposed of properly.

Decontaminatin Report

Devices without a completed Decontamination Report will not be repaired. If you would like to return a device to KNAUER, make sure to enclose a completed **Decontamination Report** with the device: http://www.knauer.net/en/downloads/service.html

Symbols and Signs

The following symbols and signs can be found on the device, in the chromatography software or in the user manual:

Warning Signs

Symbol	Meaning
	Electric shock hazard
	Electrostatic discharge hazard, damages to system, device, or components can occur.
CE Mark 	A device or system marked with CE fulfills the product specific requirements of European directives. This is confirmed in a Declaration of Conformity.
	Testing seals in Canada and the USA at nationally recognized testing centers (NRTL). The certified device or system has successfully passed the quality and security tests.

Installation

Scope of Delivery

Note: Only use original parts and accessories made by KNAUER or a company authorized by KNAUER.

- Installation Qualification
- accessories kit
- CD Autosampler
- user manual DE EN

Check

1. Check whether the device and accessories are complete.
2. If a part is missing, inform the technical support of KNAUER.

Unpacking and Setup

Packaging and Transport

At the factory, the device is carefully packed for safe transport.

Note: Check for damage caused during transportation. In case you notice any damage, contact the technical support and the forwarder company.

Contacting the Technical Support

You have various options to contact the Technical Support:

Phone +49 30 809727-111
Fax +49 30 8015010
Mail support@knauer.net

You can make your requests in English and German.

Location Requirements

Note: The autosampler is designed for indoor use only.

Requirements The location for the device must meet the following requirements:

- protect from heavy ventilation
- weight 19 kg (without temperature control)
- dimensions 300 × 377 × 510 mm (width × height × depth)
- line voltage 95–240 V AC
- air humidity 20–80 % relative humidity
- temperature 10–40 °C (50-104 °F)

Space Requirements

NOTICE

Device defect

The device overheats at exposure to sunlight and insufficient air circulation. Device failures are very likely.

➔ Set up the device in such a way that it is protected against exposure to direct sunlight.
➔ Keep at least 15 cm clear at the rear and 5–10 cm at each side for air circulation.

Clearance to other devices:

- at least 5 cm side clearance, if there is another device left or right
- at least 10 cm side clearance, if there are other devices left and right
- at least 30 cm for the cooler fan on the rear if present

Unpacking

Store all packing material. Retain included packing list carefully for repeat orders. The device is held in place and protected by foam inserts at the top and bottom.

Tools utility knife

⚠ WARNING

Back injuries

The device has a heavy weight. LIfting or carrying the device mean stress for the back.

➔ You should lift or carry the device with the help of a second person.
➔ Have a good posture to avoid putting unneccessary strain on your back.

Procedure

1. Setup the delivery in such a way that the label is in the correct position. Using the utility knife, cut the adhesive tape. Open the packaging.
2. Remove the foam insert. Take out the accessories kit and the manual.
3. Open the accessories kit and take out all accessories. Check the scope of delivery. In case any parts are missing, contact the technical support.
4. Clasp the device at its side panels and lift it out of the packaging.
5. Remove the foam inserts from the device.
6. Check for damage caused during transportation. In case you notice any damage, contact the technical support.
7. Set-up the device in its location.

8. Remove the adhesive tape from the door of the Autosampler that was used as transportation protection.

System Adapter (A9863)

The autosampler can be integrated into an HPCL system with the help of a system adapter. The system adapter is a metal sheet which can be mounted onto the top cover of the autosampler. Afterwards, additional AZURA devices can be set up on the autosampler.

Fig. 3 System adapter for autosampler

Prerequisite The lateral screws at top on the front side of the autosampler have been loosened and removed.

Tool Allen wrench, 2.5 mm

⚠ CAUTION

Device damage

Overloading the system adapter causes a breakdown, which can damage the autosampler.

➜ Pay attention to the maximum load capacity of 60 kg.

Process	Figure
1. Remove the screws from the autosampler left and right of the front ②. 2. Place the system adapter onto the autosampler. The bore holes point to the front of the autosampler ①. 3. Attach the system adapter by tightening the screws ②.	 Fig. 4 System adapter installed

Next steps Place the devices on the system adapter.

Startup

Note: Before initial startup, wait approximately one hour until the temperature of the device has adapted to the ambient temperature.

Auto-injection system The speed of the auto-injection system has been increased to fulfill the requirements of ultra-high performance liquid chromatography. However, be aware that the high speed of the auto-injection system can cause stab injuries when handled inappropriately. When the door of the autosampler is open, the syringe speed is reduced automatically.

Opening the Door

The door of the autosampler can be pushed horizontally into the sample compartment of the device.

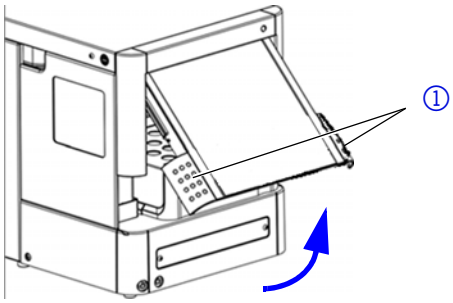
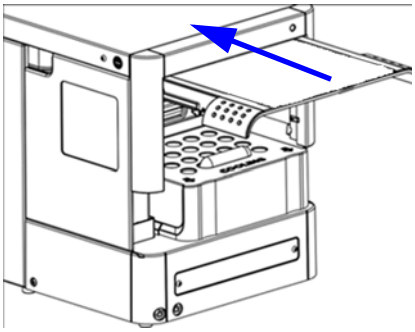
⚠ CAUTION

Stitching wounds

Behind the front panel is a chamber with a needle automatically transporting the sample during operation.

➜ Close the front panel during operation.

➜ Turn the device off before removing the front panel.

Process	Figure
1. Position your hands on both sides of the door ①. 2. LIft the door towards the front and upwards.	
3. Push the door into the sample compartment.	

Removing the Front Paneling and the Cooler Cover

The front paneling can be completely removed. In order to replace the vial plate, the cooler cover can be removed.

⚠ CAUTION

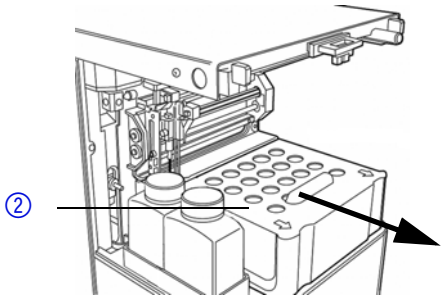
Stitching wounds

Behind the front panel is a chamber with a needle automatically transporting the sample during operation.

➜ Close the front panel during operation.

➜ Turn the device off before removing the front panel.

Process	Figure
1. Press the buttons on both sides ① simultaneously. 2. Remove the front paneling towards the front.	

Process	Figure
3. Remove the cooler cover ② towards the front.	

Sample Compartment

Legend

① syringe
② needle guide
③ flushing solution
④ tube connector for waste liquid
⑤ injection valve
⑥ collecting container
⑦ tube connector for condensed water

Fig. 5 sample compartment

Connecting the Device in a Local Area Network (LAN) to a Computer

The autosampler is operated exclusively using the chromatography software.

Remote control Normally, the autosampler is controlled by the chromatography software through a local network (LAN).

Automatic configuration The autosampler connected to the local area network (LAN) is automatically detected by the chromatography software.

Device status When used in a local area network (LAN), the system status of the autosampler can be checked using the chromatography software.

This section describes how to set up an HPLC system in a local area network (LAN) and how a network administrator can integrate this LAN into your company network. The description applies to the operating system Windows® and all conventional routers.

Note: To set up a LAN, we recommend to use a router. That means the following steps are required:

Process

1. On the computer, go to the control panel and check the LAN properties.
2. Hook up the router to the devices and the computer.

3. On the computer, configure the router to set up the network.
4. Install the chromatography software from the data storage device.
5. Switch on the device and run the chromatography software.

Configuring the LAN Settings

The LAN uses only one server (which is normally the router) from that the devices automatically receive their IP address.

Prerequisite

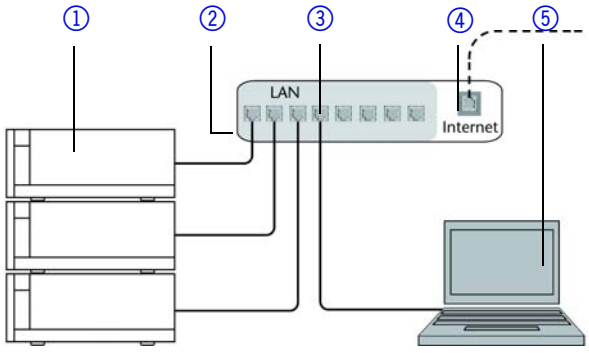
- In Windows®, power saving, hibernation, standby, and screen saver must be deactived.
- In case you use an USB-to-COM box, the option "Allow the computer to turn off ths device to save power" in the devicemanager must be deactivated for all USB hosts.
- Only for Windows 7: For the network adapter, the option "Allow the computer to turn off this device to save power" in the Device Manager must be deactivated.

Process

1. In Windows 7 choose *Start ⇒ Control Panel ⇒ Network and Sharing Center*.
2. Double-click on *LAN Connection*.
3. Click on the button *Properties*.
4. Select *Internet Protocol version 4 (TCP/IPv4)*.
5. Click on the button *Properties*.
6. Check the settings in the tab *General*. The correct settings for the DHCP client are:
   a) *Obtain IP address automatically*
   b) *Obtain DNS server address automatically*
7. Click on the button *OK*.

Connecting the Cables

A router ③ has several LAN ports ② and one WAN port ④ that can be used to integrate the LAN into a wide area network (WAN), e.g. a company network or the Internet. In contrast, the LAN ports serve to set up a network from devices ① and a computer ⑤. To avoid interference, we recommend operating the HPLC system separately from the company network.



You will find patch cables for each device and the router in the accessories kit. To connect the router to a WAN, an additional patch cable is required, which is not supplied within the scope of delivery.

Prerequisite

- The computer has been switched off.
- There is a patch cable for each device and the computer.

Process

1. Use the patch cable to connect the router and the computer. Repeat this step to connect all devices.
2. Use the power supply to connect the router to the mains power system.

Configuring the Router

The router is preset at the factory. You will find a label at the bottom side of the router, on which IP address, user name, and password are printed. These information help to open the router configuration.

Process
1. To open the router configuration, start your Internet browser and enter the IP address (not for all routers).
2. Enter user name and password.
3. Configure the router as DHCP server.
4. In the router configuration, check the IP address range and make changes if necessary.

Result Once the router has assigned IP addresses to all devices, the chromatography software can be used to remotely control the system.

Integrating the LAN into a Company Network

A network administrator can integrate the LAN into your company network. In this case you use the WAN port of the router.

Prerequisite There is a patch cable for the connection.

Process
1. Check that the IP address range of the router and of the company network do not overlap.
2. In case of an overlap, change the IP address range of the router.
3. Use the patch cable to connect the router WAN port to the company network.
4. Restart all devices, including the computer.

Controlling Several Systems Separately in a LAN

Devices connected to a LAN communicate through ports, which are part of the IP address. If more than one HPLC system is connected to the same LAN and you plan on controlling them separately, you can use different ports to avoid interference. Therefore, the port number for each device must be changed and this same number must be entered into the device configuration of the chromatography software. We recommend to use the same port number for all devices in the same system.

Note: The port is set to 10001 at the factory. You must use the same numbers in the device configuration of the chromatography software as in the device, otherwise the connection fails.

Process
1. Find out port number and change it on the device.
2. Enter the port number in the chromatography software.

Result The connection is established.

Injection Principles

The autosampler can be operated according to the following principles:

ILD™ for analytical Autosamplers

For injections in the high pressure range up to 1000 or 700 bar, the autosampler has an ILD™ valve (Intermediate Loop Decompression by Spark Holland). This valve consists of a rotor-stator combination and a central port for pressure release. For applications in the high pressure range, pressure is released from the sample loop to avoid diluting the sample by eluent. Extremely fast switching valves reduce pressure surges further on. The results are more exact analyses and long-lasting columns.

Legend
① sample loop
② syringe
③ sample vial
④ column
⑤ pump

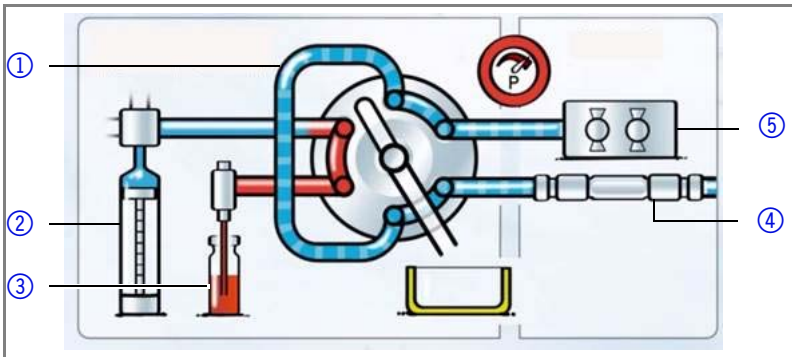


Fig. 6 ILD™ injection principle

PASA™ Loop Injection

Loop injection with pressure assistance (Pressure Assisted Sample Aspiration PASA™) features the following:

- Samples do not have to be degassed.
- No air bubbles in sample loop.
- No clogging or contamination of sample needle.
- precise control of syringe movement

Legend
① buffer tube
② syringe
③ sample needle
④ capillary to pump
⑤ capillary to tube
⑥ sample loop
⑦ connector for compressed air
⑧ air needle

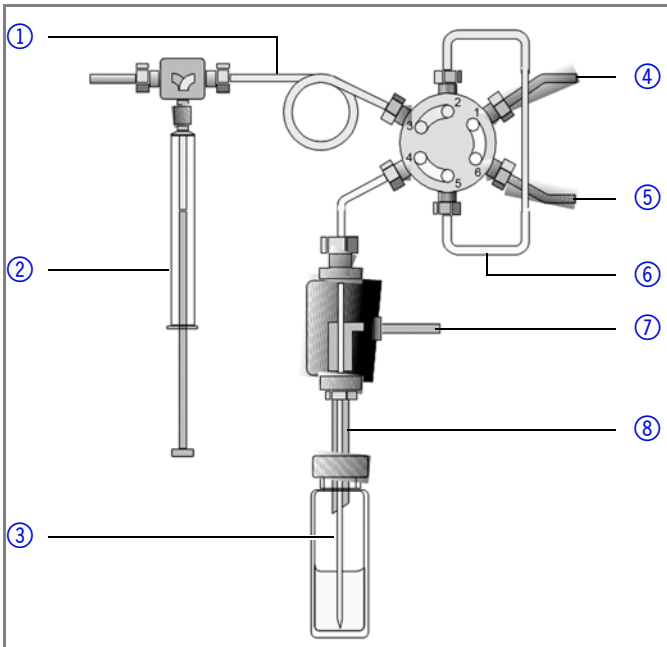


Fig. 7 PASA™ loop injection

Three different injection methods are available:

- full loop filling
- partial loop filling
- microliter pickup
- microliter pickup 84+3

Full loop filling

In full loop filling mode, the sample loop is completely filled with the sample. The maximum reproducibility but not the maximum precision is achieved because the size of the sample loop may have a deviation of ±10 %. The maximum injection volume equals the loop volume. The sample loop is filled with a multiple of the loop volume:

- 3 x loop volume for loops up to 100 µl
- 2 x loop volume for loops from 100 µl to 500 µl
- 1.5 x loop volume for loops of more than 500 µl

The sample loss per injection is the sum of the overfilling of the sample injection times x and the flush volume set for the needle used.

Partial loop filling

In partial loop filling mode, the sample loop is filled with both sample and mobile solvent. This ensures the highest precision of the sample volume with minimal loss of sample. The maximum injection volume equals 50 % of the loop volume. The sample loss per injection equals the adjusted flush volume plus three times the sample volume for the needle used.

Microliter pickup

In microliter pick-up mode, the sample loop is filled with a very small amount of sample and transport liquid (mobile phase). This ensures very high precision with no loss of sample. The maximum sample volume is (loop volume – 3 x needle volume)/2.

Microliter pickup 84+3

If the 84+3 sample plate is selected for the microliter pickup, the sample is transported by a separate transport liquid instead of the flushing fluid. The consumption of transport liquid depends on the needle volume and corresponds to the 2.5 needle volume for the segments before and after the sample.

The autosampler uses a system of two telescopic needles, one that pierces through the cap of the sample vial – the air needle – and one that extracts the sample – the sample needle.

Using a syringe, the sample is aspirated through the two needles out of the sample vial while under pressure and into the sample loop. To prevent the syringe from becoming contaminated, a buffer tube is situated between the syringe and the valve. Using washing solution, sample residue is removed from the sample needle and buffer tube.

Full Loop Filling

The sample loop is completely filled with sample. This kind of injection leads to outstanding reproducibility.

Reducing consumption

You can use an air segment of 5 µl to reduce the flush volume. The air segment precedes the flush segment and is not injected.

The flush volume must be at 30 µl in case of a standard needle and an injection with air segment; in case of an injection withouth air segment, the flush volume has to be 35 µl. You may need to insert a higher flush volume for extremely viscous samples to reduce the syringe speed and improve the performance.

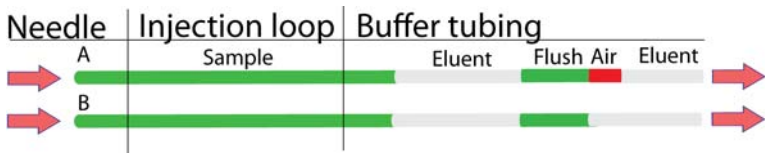
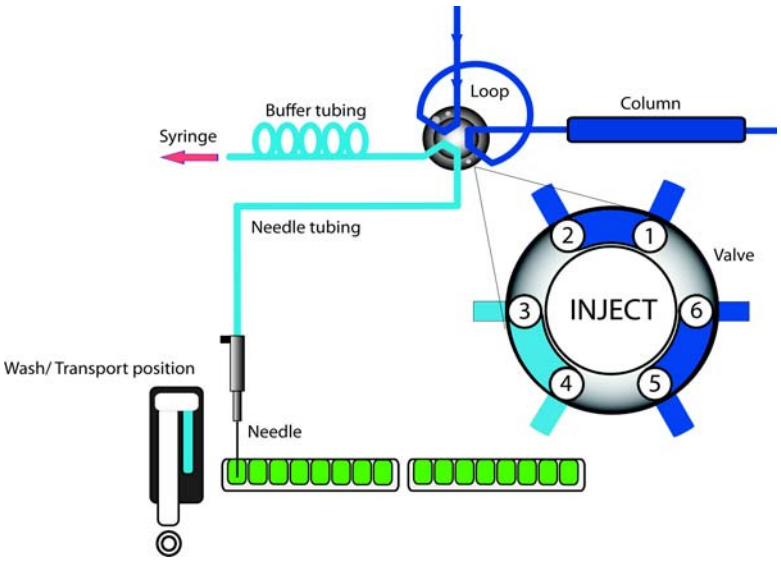
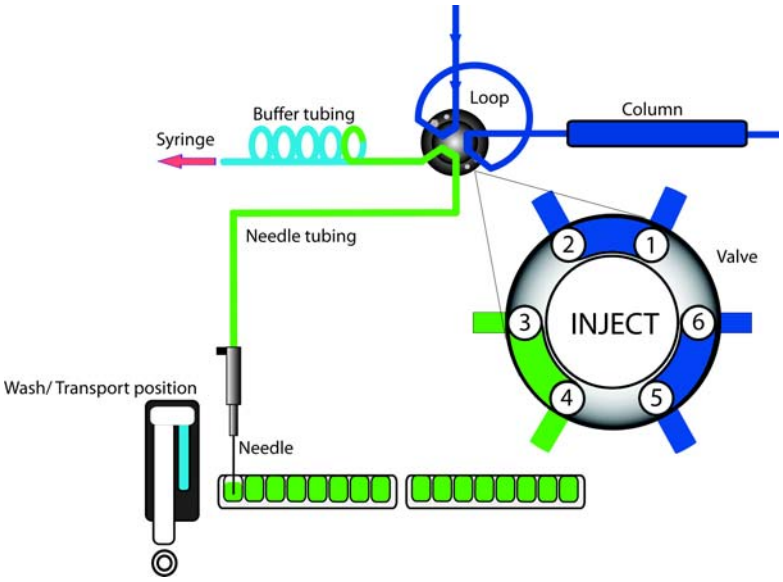
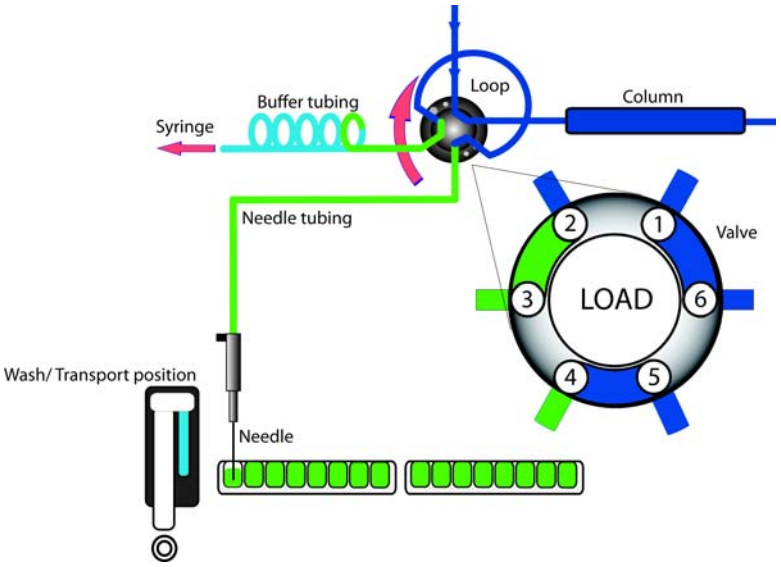
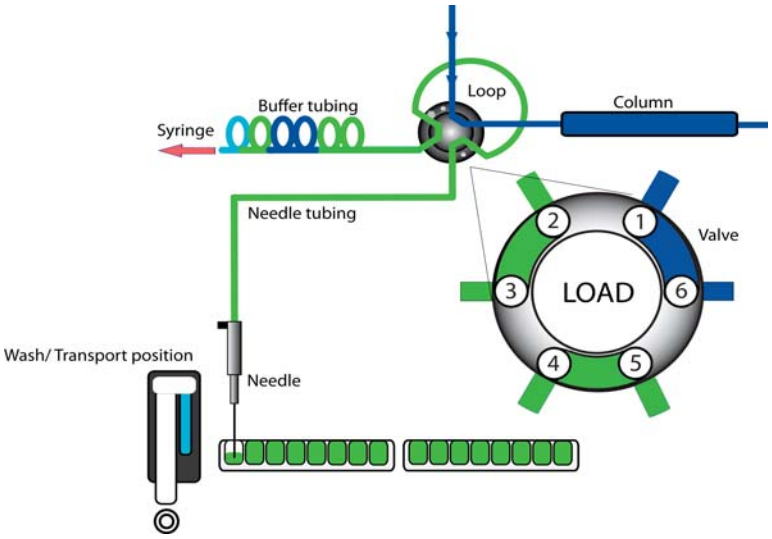
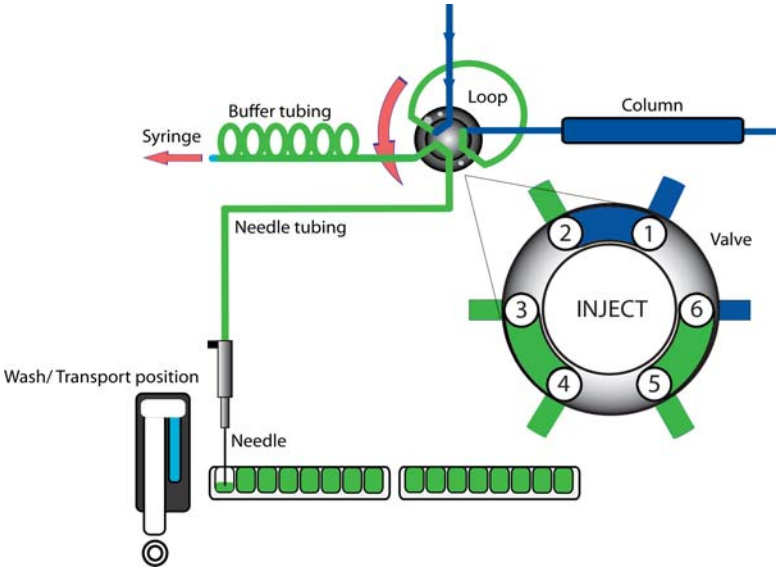


Fig. 8 air segment

Note: A flushing process takes places after every injection.

Explanations	Functional schematic
1. Initial conditions: The injection valve in INJECT position. Sample needle and air needle are inserted into the vial. The air needle creates pressure, which prevents air and steam bubbles from developing.	 Fig. 9 full loop filling initial conditions
2. The syringe aspirates the flush volume from the sample vial into the sample line and removes any washing solution.	 Fig. 10 full loop filling The needle and sample lines are flushed
3. The valve switches to LOAD position in order to transport the sample material to the inlet of the sample loop.	 Fig. 11 full loop filling: valve switches to *LOAD* position

Explanations	Functional schematic
4. The sample loop is filled by transporting a certain amount of the loop volume (depending on the volume of the loop) through the loop. 3 x loop volume for loops up to 100 µl	 Fig. 12 full loop filling: The sample loop is completely filled.
5. The valve switches to the INJECT position. From that moment, the sample loop is part of the HPLC flow of the mobile phase. The sample is transported to the column. The analysis starts.	 Fig. 13 full loop filling: injection valve switches to *INJECT* position

Next Steps Flush the needle after every injection.

Partial Loop Filling

This kind of injection results in maximum conciseness of sample volume.

Reducing consumption An air segment of 5 µl preceding the flushing volume, which is not injected, can reduce the amount of sample dilution caused by the dispersion during aspiration.

- Choose the following flush volumes for a standard needle:
  - At least 30 µl for injections with air segment
  - 35 µl for injections without air segment
  - Increase the flush volume and reduce the syringe speed to achieve better results for highly viscous samples.

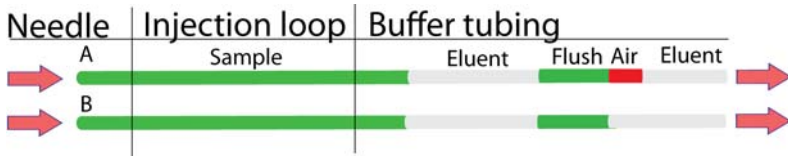


Fig. 14 air segment

Sample volume

In the Autosampler 3950, the syringe moves the sample into the sample loop. In partial loop filling mode, the sample volume is maximum 50 % of the loop volume.

Legend

① flushing solution
② tube connector for waste
③ syringe
④ tube
⑤ valve
⑥ sample loop
⑦ column
⑧ needle
⑨ microtiter plate with samples
⑩ Capillary

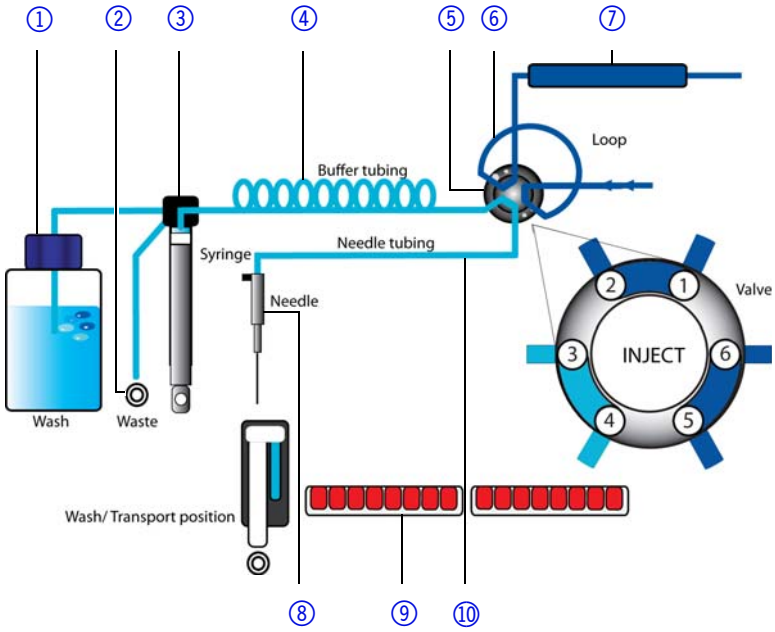


Fig. 15 scheme of initial position for partial loop filling

Note: Partial loop fill is processed automatically.

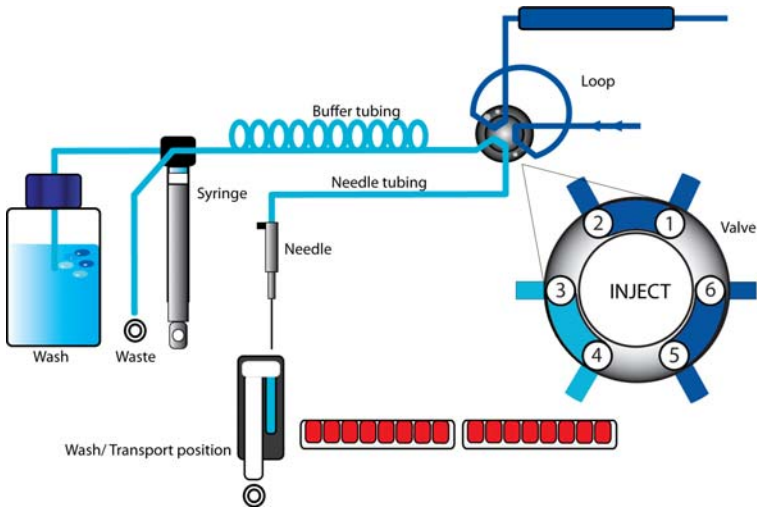
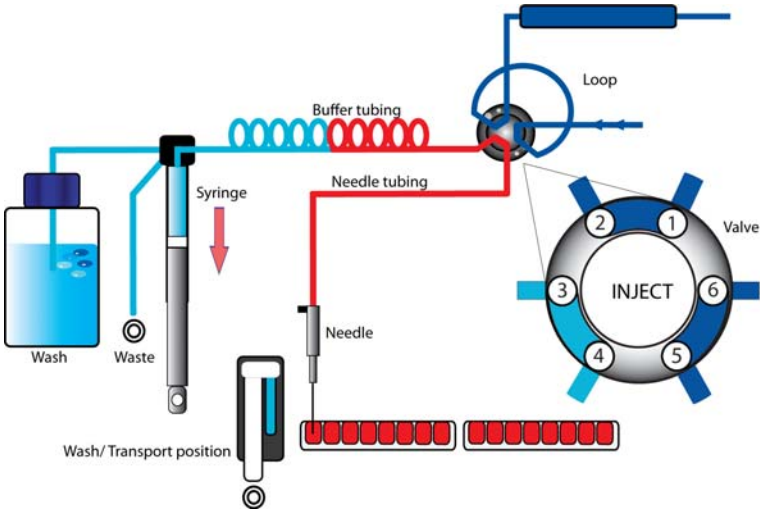
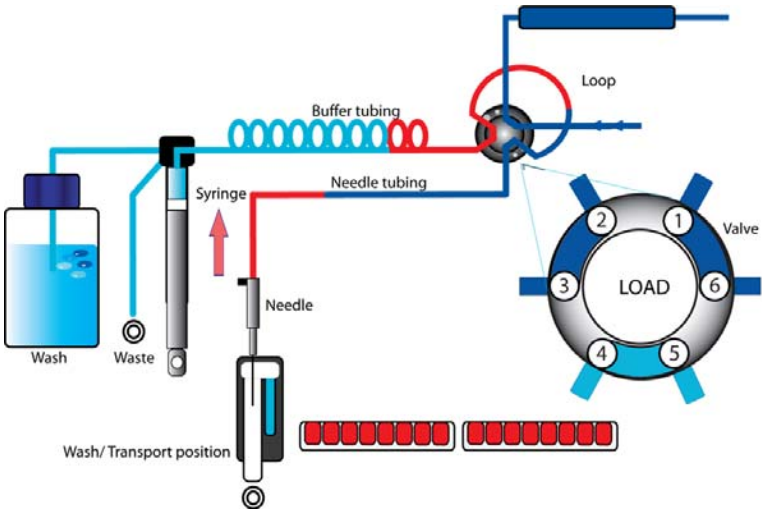
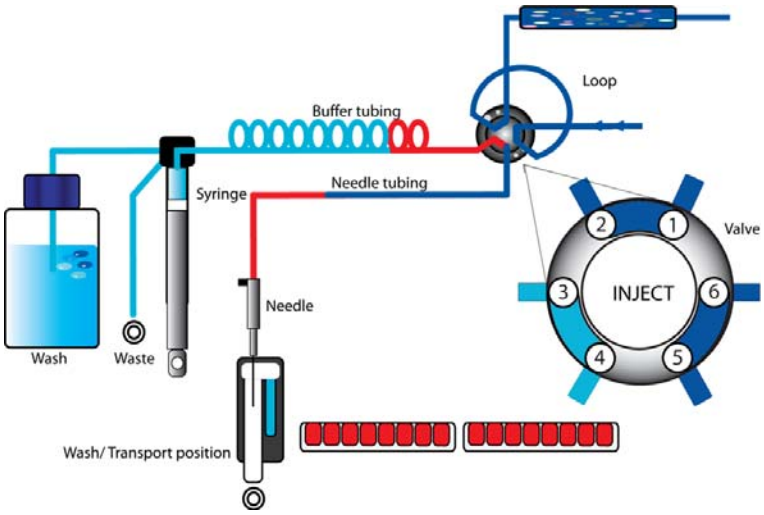
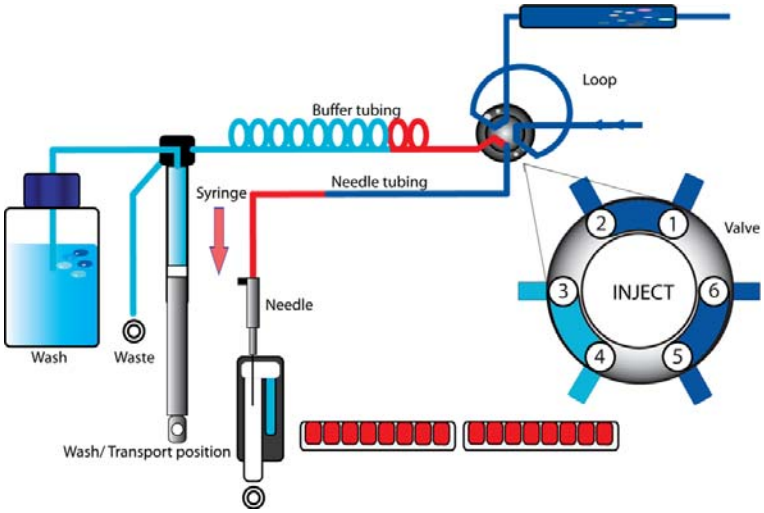
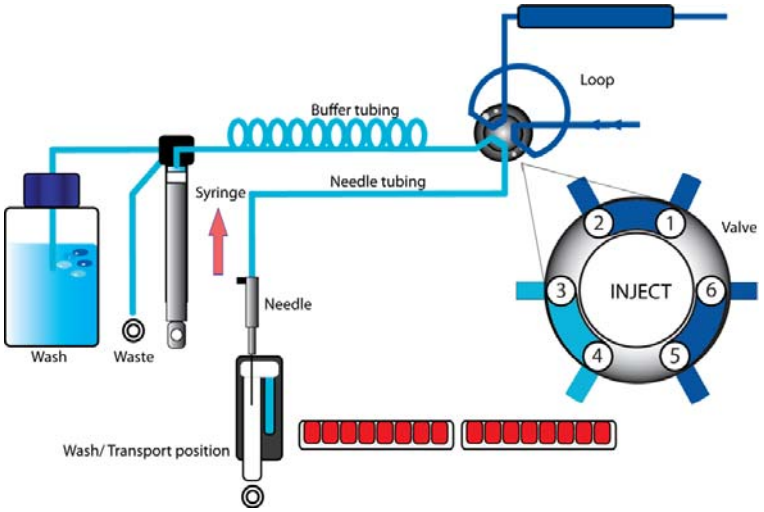
Process	Figure
1. The valve switches to the *INJECT* position. The tube and the capillary are filled with flushing solution.	

Fig. 16 partial loop filling valve position "Inject"

Process	Figure
2. The needle moves into the sample and the syringe aspirates the sample. The tube is partially and the capillary fully filled with sample.	 Fig. 17 partial loop filling aspirating the sample
3. The valve switches into the *LOAD* position and the syringe aspirates the sample into the sample loop. The tube is full of flushing solution and sample, the capillary of sample and eluent.	 Fig. 18 partial loop filling valve position "Load"
4. The valve switches to the *INJECT* position and the pump transports the sample and the eluent to the column.	 Fig. 19 partial loop filling injecting into the column

Process	Figure
5. The syringe aspirates the flushing solution from the bottle.	 Fig. 20 partial loop filling aspirating flushing solution
6. The syringe injects the flushing solution into tube and capillary. Both sample and eluent get flushed.	 Fig. 21 partial loop filling flushing sample and eluent

Microliter Pickup

For this kind of injection, the sample is delivered into the sample loop by flushing fluid. The process results in maximum conciseness of sample volume without sample loss.

Reducing consumption

If an air segment has been programmed, it will be inserted in front of the eluent area and in front of each sample.

The following conditions apply:

- The air segment is injected into the system in front of the sample segment.
- In this mode, the needle does not create pressure because the sample volume could be distorted due to the air expansion during the movement from sample vial to wash position.

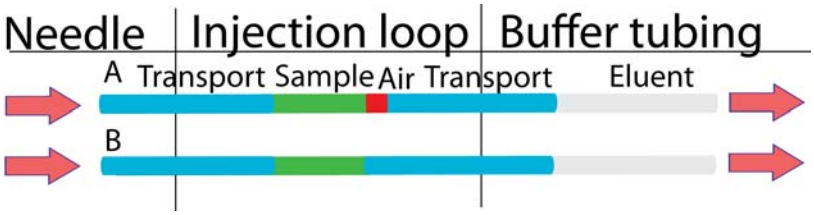
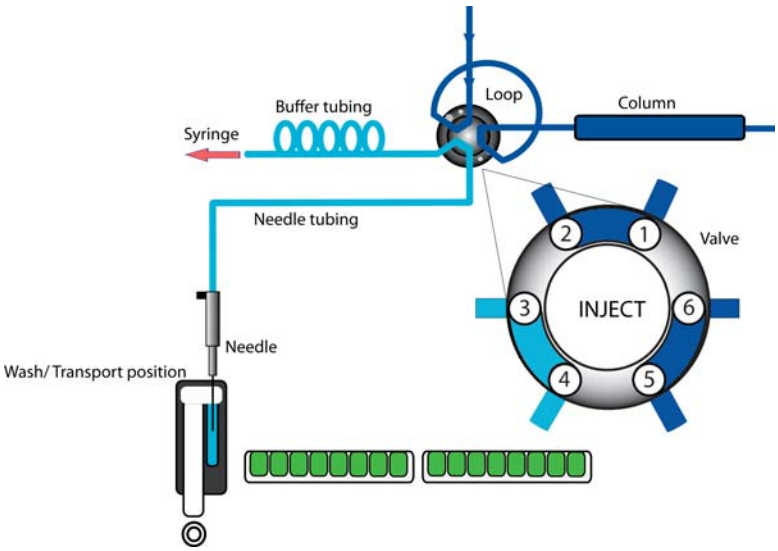
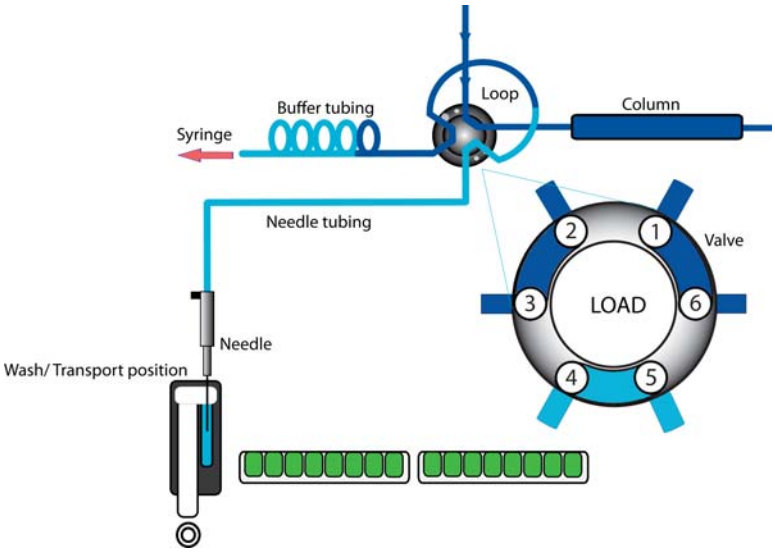
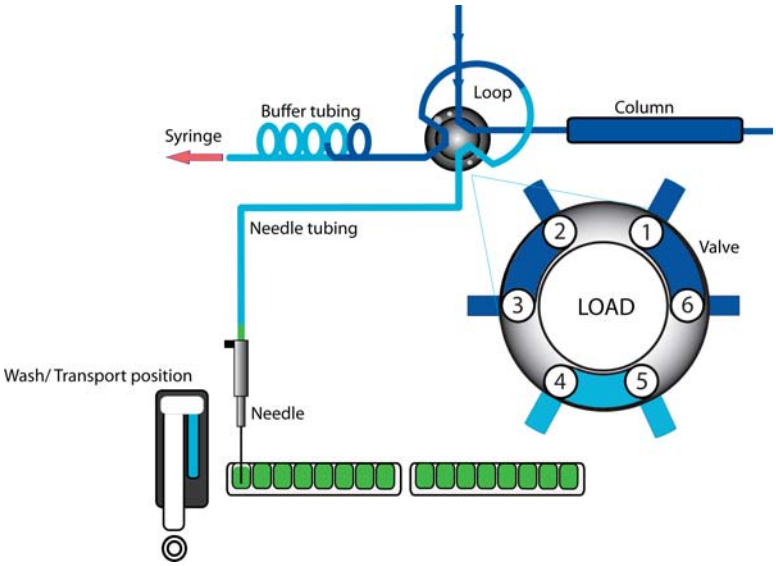
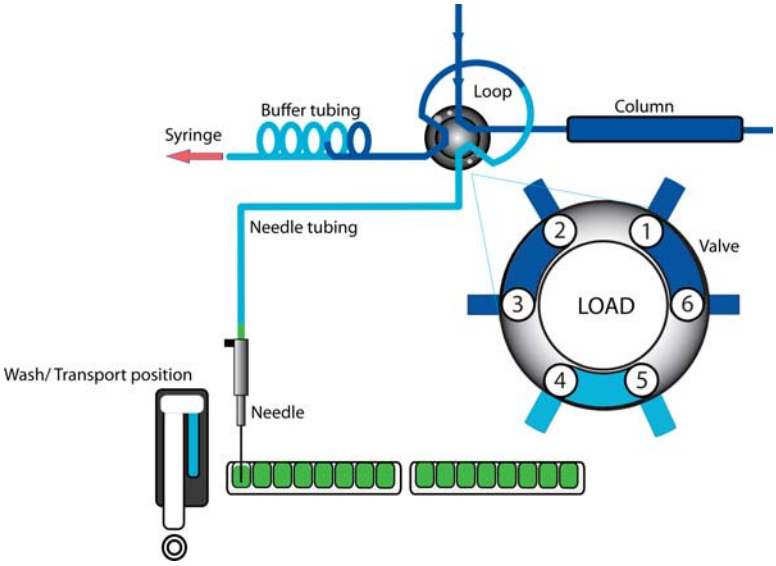
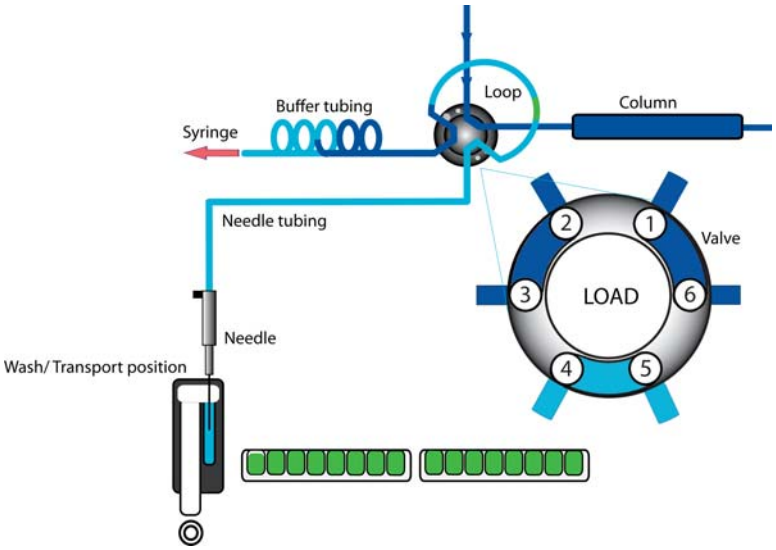
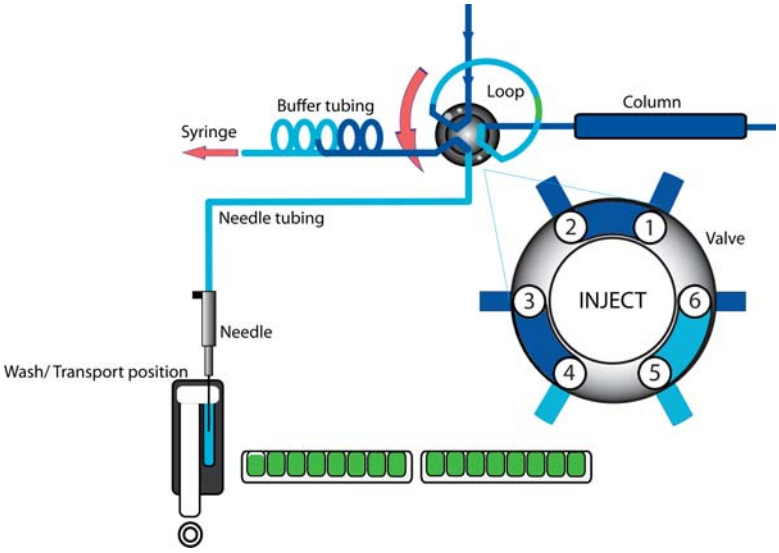


Fig. 22 Microliter pickup with air segment (A), without air segment (B)

Note: Make sure that washing liquid and eluent are compatible.

Process	Figure
1. Initial conditions: The injection valve in INJECT position. The sample needle is in position wash/transport. The transport container is filled with washing liquid. 2. The sample line is filled with washing fluid until the fluid reaches the sample loop inlet. The valve remains in the INJECT position during filling/transportation.	 Fig. 23 microliter pickup initial conditions
3. The injection valve switches to LOAD. A transport segment of washing fluid is aspirated into the sample loop.	 Fig. 24 microliter pickup: sample line is filled with eluent
4. The needle moves from the transport position to the sample vial.	 Fig. 25 microliter pickup: sample is aspirated

Process	Figure
5. The sample is aspirated from the sample vial according to the amount of programmed injection volume.	 Fig. 26 microliter pickup: The injection volume is aspirated.
6. The needle moves back into transport position. A second washing liquid sample is aspirated. The sample is transported through the sample loop.	 Fig. 27 microliter pickup: sample is transported through the sample loop
7. The injection valve switches to INJECT. From that moment, the sample loop is part of the flow of the mobile phase. The sample is transported to the column. The analysis starts.	 Fig. 28 microliter pickup: The sample is transported to the column.

Microliter Pickup with 84+3 Vial Plate

If you choose to operate the microliter pickup with the 84+3 vial plate, three 10 ml vials are used automatically for transportation. In this case, the needle position wash/transport is only used for washing the needle.

Reducing consumption

If an air segment has been programmed, it is inserted in front of the transportation liquid segment and in front of each sample.

Details on this injection mode:

- The air segment is injected into the system in front of the sample segment.
- In this mode, the needle does not create pressure because the sample volume could be distorted due to the air expansion during the movement from sample vial to wash position.

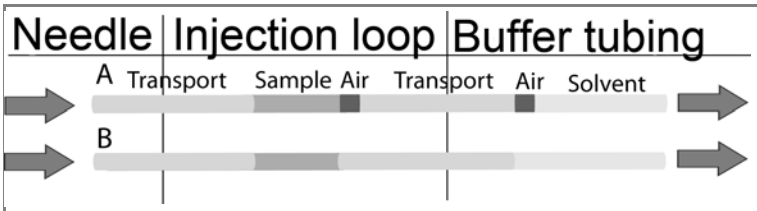
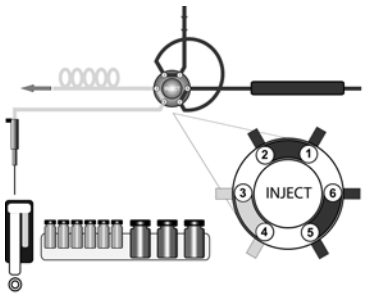
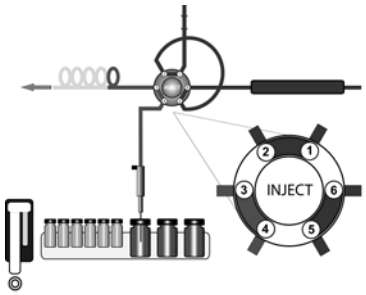
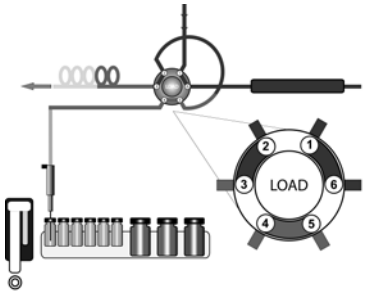
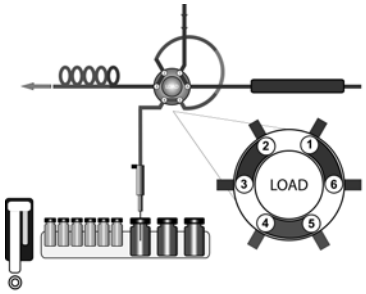
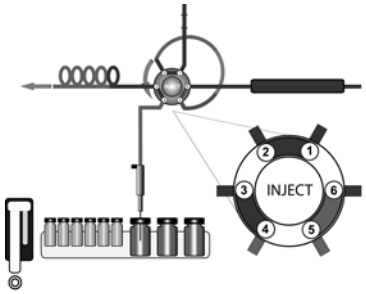


Fig. 29 microliter pickup 84+3 with air segment (A), without air segment (B)

Process	Figure
1. The sample needle begins with the wash/ transport position. The valve starts with the INJECT position.	 Fig. 30 microliter pickup 84+3: initial conditions
2. The first injection starts in the transport position with the syringe aspirating transport liquid from the vial to fill the sample line with transport liquid and to remove the flushing solution.	 Fig. 31 microliter pickup 84+3: sample line is filled with flushing solution.
3. The injection valve switches to LOAD. The programmed injection volume is aspirated out of the sample vial.	 Fig. 32 microliter pickup 84+3: Injection valve switches to *LOAD* position

Process	Figure
4. The needle moves back into the transport position. A second segment of transport liquid is aspirated. The sample is transported through the sample loop.	 Fig. 33 microliter pickup 84+3: The sample is transported through the sample loop.
5. The valve switches to INJECT. From that moment, the sample loop is part of the flow of the mobile phase. The sample is transported to the column. The analysis starts.	 Fig. 34 microliter pickup 84+3: The injection valve switches to position *INJECT*.

84+3 Vial Plate

The 84+3 vial plate is to be ordered separately. Position the plate on top of the plate holders inside the autosampler.

Note: Position 87 of the vial plate must always be located at the rear right corner of the sample compartment.

The vial plate offers space for maximum 84 vials with 1.5 ml and 3 vials with 10 ml. It was designed with the caps of all vials being level, independently from their unique heights, which means that one needle can be used for all vial sizes.

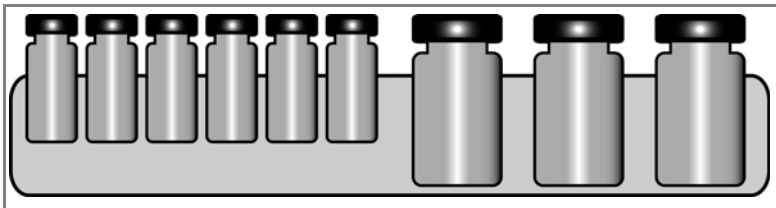


Fig. 35 Height of 84+3 vial plate

The length of the sample needle must be programmed at 1.5 ml. Relating to the liquid level in 10 ml vials, it is possible to program the needle at two different lengths.

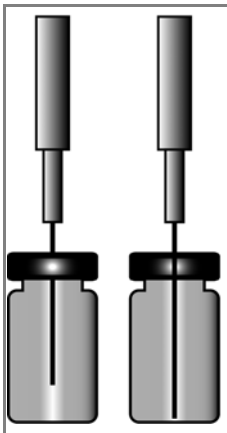


Fig. 36 Needle length and liquid level

Follow the numeric order if you are to program a sample sequence for a vial plate.

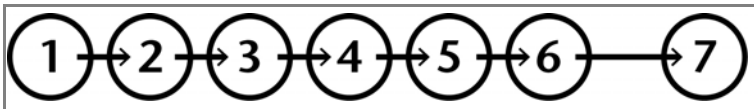


Fig. 37 Sequence

In case you are creating a sequence chart with only one sample per row (1 sample in each run), programming the sequence is free to you.

Parameters for Microliter Pickup

Before using the 84+3 vial plate, it is necessary to change the software settings.

Note: If you choose to operate the microliter pickup with the 84+3 vial plate, three 10 ml vials are used automatically for transportation. In this case, the needle position wash/transport is only used for washing the needle.

The vial positions for the 84+3 vial plate are as follows:

Position of the first sample:	Vial positions 1-84
Position of the final sample:	Vial positions 1-84
First destination position:	Vial positions 1-84
Transport position:	Vial positions 85-87

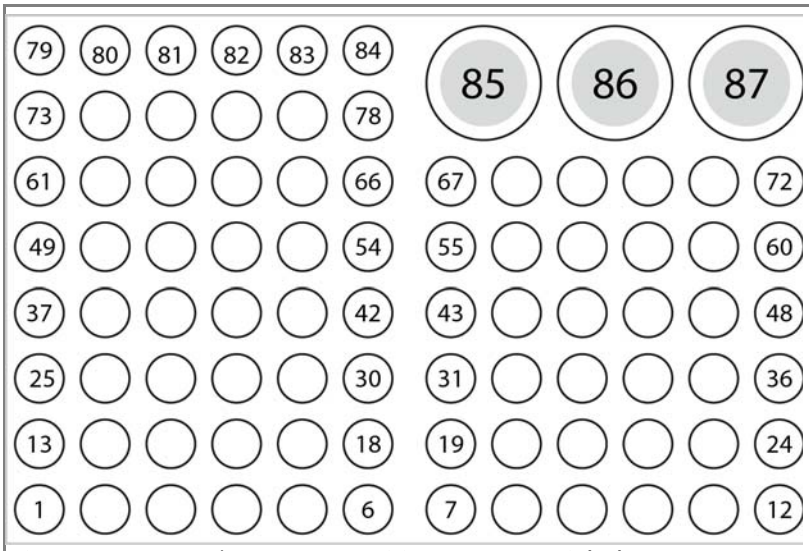


Fig. 38 Reagent/Transport position on 84+3 vial plate

You need to define the first and the final sample to enter a sample range. The transport positions are always positions 85, 86, 87.

Details on Programming

- The position of the transport vial can be programmed. Some possible positions are 85, 86, and 87. Position 85 is the standard position for transport vials. The system calculates the necessary transport volume. Make sure before system start that the vial is filled with at least 8000 µl of liquid.
- The liquid levels of the transportation liquids are not updated at system start. To avoid contamination of the air needle, the needle stops inside the transport vial in the up most position.
- If conducting a sample sequence or one sample per row, the autosampler takes the residual volume of the transport liquid into account. If the volume falls below 4000 µl, the needle moves deeper into the transport vial. If the amount of transport liquid reaches 0 µl, the autosampler sends out error message 369 (not enough transport liquid available).
- The liquid levels of the transportation liquid will reset after reprogramming the mode.
- The needle does not move automatically to the next transport vial. If transport liquid is to be taken from another position, you have to change the program settings.

Air Needles

Six different lengths of air needles from 50 to 80 mm are available for the autosampler. The needle holder allows you to further adjust the needle height by 6 mm.

Standard Air Needle

The standard air needle is 62 mm long and can be used for a wide range of deep and shallow vial plates.

When 10 ml sample vials are used, the needle deeply penetrates the sample vial. If this is not filled to more than 60%, the needle can be used in the typical manner. The same applies to deep microtiter plates.

For non-standard settings, use the corresponding needle types.

Legend
① 10 ml sample vials
② 2 ml sample vials

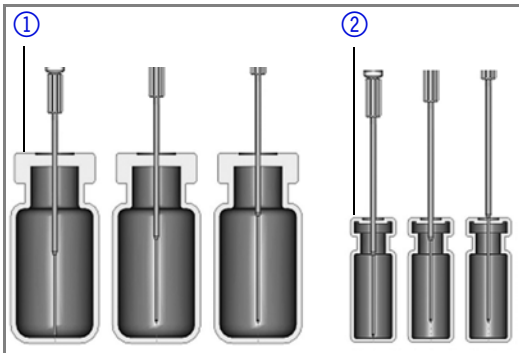


Fig. 39 Standard air needle with 10 and 2 ml sample vial

Note: The PASA™ loop injection principle is not suitable for shallow microtiter plates. The function of the air needle is only ensured when it pierces the closure to a sufficient degree.

Legend

① deep microtiter plate with closure

② shallow microtiter plate

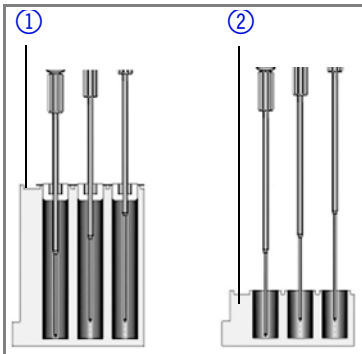


Fig. 40 standard air needle with microtiter plates

Choosing the Correct Air Needle

To choose the correct air needle, take the following dimensions into consideration:

Legend

H_t = height of vial plate

D_w = hole depth

C_d = thickness of closure

N_h = set needle height

A_c = distance from air needle tip to closure (min. 2 mm)

? = excess length

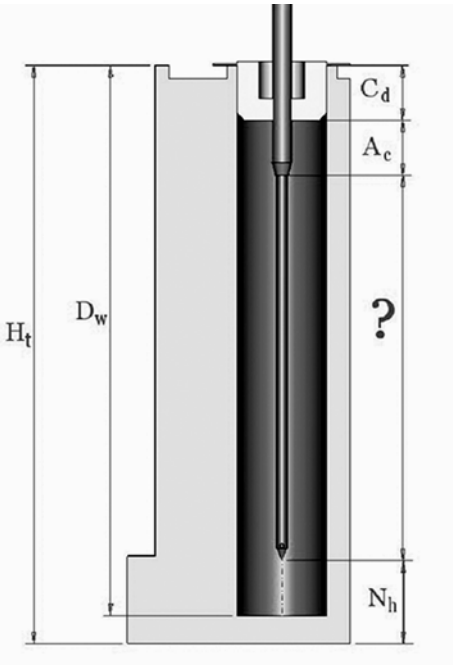


Fig. 41 Calculating the correct air needle

Condition: **H_t - D_w** = 2 to 6 mm

Excess length of the sample needle:
$H_t - C_d - N_h - A_c = ?$

Choose the correct needle type on the basis of the excess length.

Air needle type	Protrusion length
50 mm, yellow	34-40 mm
56 mm, red	28-34 mm
62 mm, natural (standard needle)	22-28 mm
68 mm, blue	16-22 mm
74 mm, green	10-16 mm
80 mm, black	4-10 mm

Legend

① 10 ml sample vial, 50 mm air needle

② 2 ml sample vial, 62 mm air needle

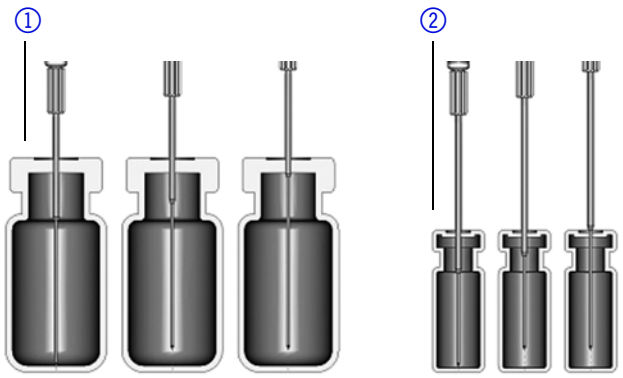


Fig. 42 Selected air needles with sample vials

Legend

① deep microtiter plate with closure, 56 mm air needle

② shallow microtiter plate, 80 mm air needle

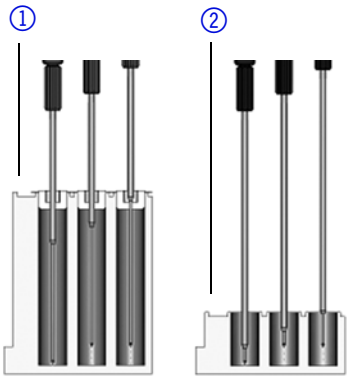


Fig. 43 selected air needles with microtiter plates

Calculation Example for Air Needle

Starting point:

- autosampler with standard setting for needle height.
- deep microtiter plate with closure

Dimensions:

$\mathbf{H_t}$ = 41.4 mm

$\mathbf{D_w}$ = 37.8 mm

$\mathbf{C_d}$ = 3.8 mm

$\mathbf{N_h}$ = 6.0 mm (standard)

$\mathbf{A_c}$ = 2.0 mm (minimum)

Condition:

$\mathbf{H_t}$ - $\mathbf{D_w}$ = between 2 and 6 mm

$\mathbf{H_t}$ - $\mathbf{D_w}$ = 41.4 mm - 37.8 mm = 3.6 mm. Condition has been met.

H_t - C_d - N_h - A_c = excess length

41.4 mm - 3.8 mm - 6.0 mm - 2.0 mm = 29.6 mm

Air needle type	Protrusion length
56 mm, red	28-34 mm

An air needle length of 56 mm is required.

Handling the Sample Vials

When handling the sample vials, observe the following:

- Fill the sample vials using a pipette to allow air to escape.

- To prevent the sample from contaminating the air needle, do not fill the sample vials to the very top.
- Do not use sample vials that are unclosed.
- Only use air-tight closure seals to prevent air bubbles from forming and volatile components from evaporating.
- Do not use sample vials with hard closures that the sample needle cannot pierce.

Mixing and Diluting

A mix method can be programmed for the autosampler to mix or dilute the sample fluid.

- Configure the mixing routine and syringe speeds using the chromatography software.
- A maximum of 15 steps can be programmed for a mix method.

Three types of actions are possible:

1. Add
2. Mix
3. Wait

Add When adding, the defined volume is aspirated from either the sample vial, the vial with *Reagent A* or *Reagent B* or flushing fluid and then dispensed into the destination vial.

Note: To prevent carryover, the autosampler removes 125% of the given volume from the corresponding sample vial and uses the additional 25% to flush the tube and needle.

Mix When mixing, the contents of a specific sample vial is mixed by aspirating and dispensing the defined volume n times. If a destination vial has not been defined, mixing is performed in the current sample vial.

Note: When entering the *Sample Vials*, the *Destination Vial* is used automatically.

Wait With the Wait command, the system waits until the programmed delay time has elapsed before executing the next line of the program.

Example: Add

The command *ADD 100 µl from Reagent A to Destination* involves the following steps:

1. An air segment of 5 µl is aspirated to separate the flushing solution in the buffer tube from *Reagent A*.
2. 50 µl of *Reagent A* are aspirated to flush the tube and needle.
3. Syringe is emptied into the waste container through the drainage tube.
4. 100 µl of *Reagent A* are aspirated and then dispensed into the destination vial.
5. Tube and needle are flushed with flushing solution.

Example: Mix

If this is preceded by an *ADD ... to Destination* command, mixing is performed in the destination vial. If this is preceded by an *ADD to Sample* command, mixing is performed in the sample vial.

The *MIX 3 times with 100 µl* command triggers the following steps:

1. An air segment of 50 µl is aspirated to separate the flushing solution in the buffer tube from the sample solution to be mixed.
2. Syringe is emptied into the waste container through the drainage tube.
3. 100 µl solution are aspirated and dispensed into the same sample vial.
4. Step 3 is repeated twice.

5. Tube and needle are flushed with flushing solution.

Sample Positions in Mixing Routine

When configuring a mix method, the positions of the sample vials depend on whether the vial plates are to be processed in rows or columns.

Processing in Columns

When column processing is used, the following positions are possible for the *Sample, Destination, Reagent A* and *Reagent B*:

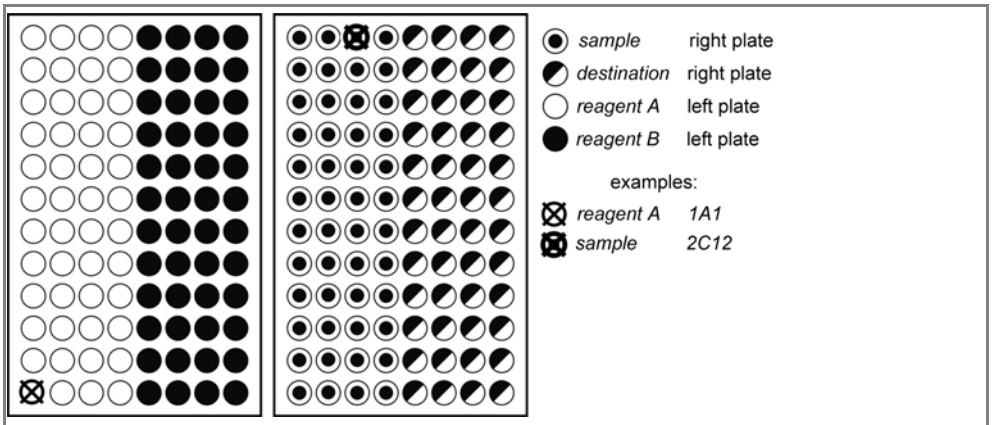


Fig. 44 Sample positions in columns

Processing in Rows

When row processing is used, the following positions are possible for the *Sample, Destination, Reagent A* and *Reagent B*:

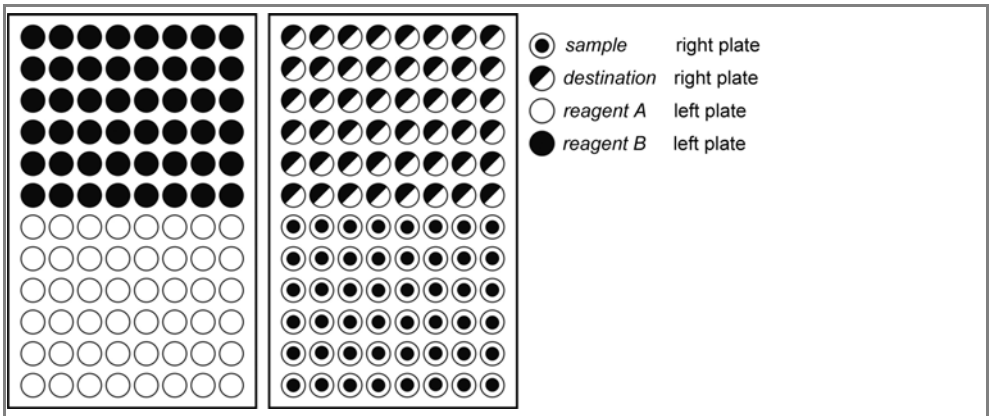


Fig. 45 Sample positions in rows

Parameters for Mixing Method with 84+3 Vial Plate

Before using the 84+3 vial plate, it is necessary to change the software settings.

The vial positions for the 84+3 vial plate are as follows:

Position of the first sample:	Vial positions 1-84
Position of the final sample:	Vial positions 1-84
First destination position:	Vial positions 1-84
Reagent position:	Vial positions 85-87

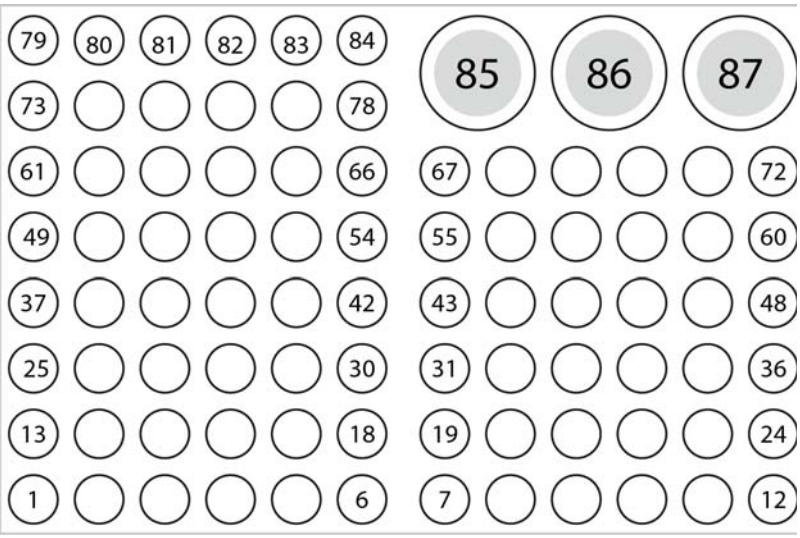


Fig. 46 Reagent/Transport position on 84+3 vial plate

You need to define the first and the final sample to enter a sample range. The same range can be used for the destination vials. The reagent positions are always positions 85, 86, 87.

Details on Programming the 84+3 Mixing Method

- The position of the reagent vial can be programmed. Some possible positions are 85, 86, and 87. The standard transport positions are 86 for Reagent A and 87 for Reagent B. The system calculates the necessary reagent volume. Make sure before system start that the vials are filled with at least 8000 µl of liquid.
- The liquid levels of the reagent liquids are not updated at system start. To avoid contamination of the air needle, the needle stops inside the reagent vial in the up most position.
- If conducting a sample sequence or one sample per row, the autosampler takes the residual volume of the reagent liquid into account. If the volume falls below 4000 µl, the needle moves deeper into the reagent vial. If the amount of reagent liquid reaches 0 µl, the autosampler sends out error message 370 (not enough reagent liquid available).
- The liquid levels of the reagent liquid will reset after reprogramming the mode.
- Reagent will only be taken from preprogrammed positions. The needle does not move automatically to the next reagent vial. You have to change the settings when reagent is to be taken from one of the other vials.

Capillary and Tubing Connections

Note: Flush the system before connecting the column.

The connection scheme inside the autosampler displays the correct connections:

Legend

① waste tubing
② flushing fluid container
③ buffer tube
④ sample loop
⑤ column
⑥ pump
⑦ Injection needle
⑧ Position: Injection
⑨ Position: Load

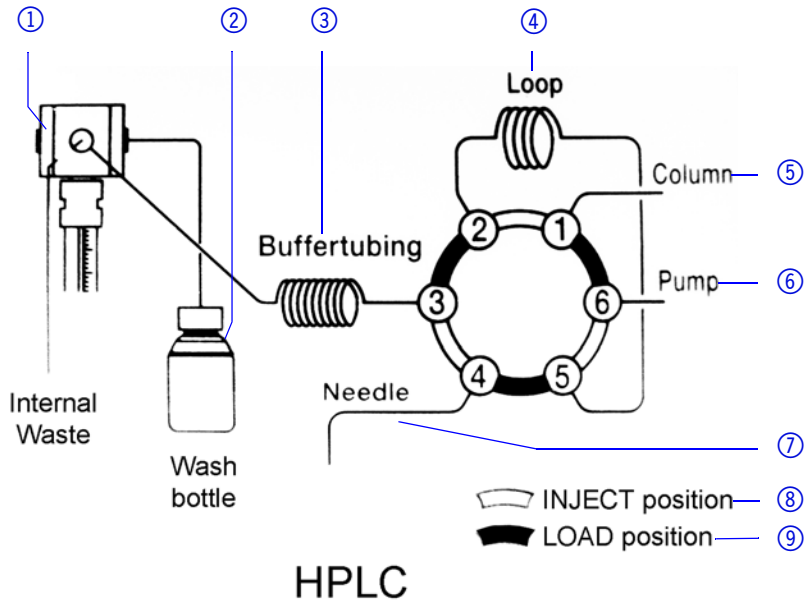


Fig. 47 connection scheme for capillaries and tubing

Connecting the Injection Valve

Legend

① connection tube buffer to syringe
② sample loop
③ stainless steel capillary to column
④ stainless steel capillary to pump
⑤ connection plastic capillary to injection needle
⑥ cleanout ILD™ valve (analytical autosamplers)

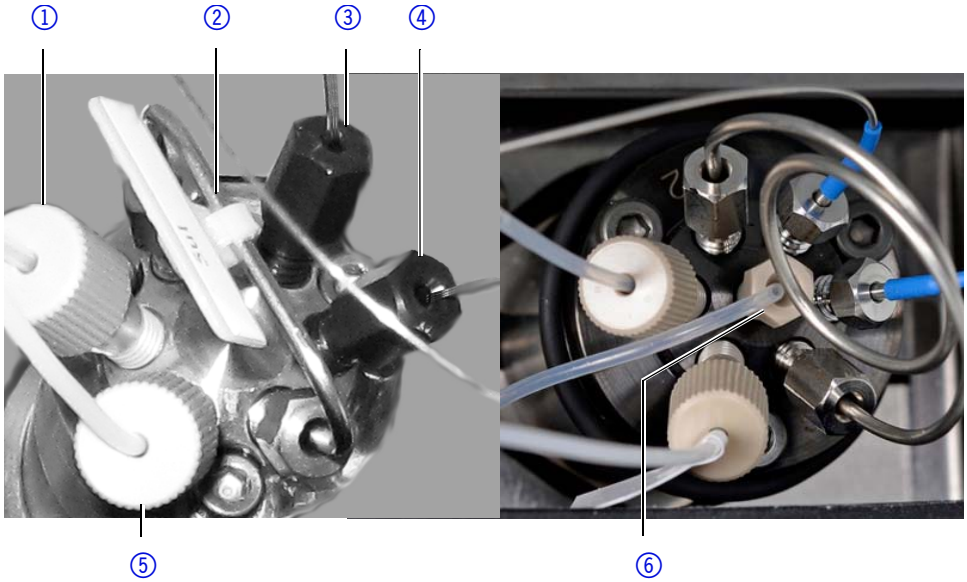


Fig. 48 left preparative injection valve, right analytical injection valve with central port

Connecting the Syringe

Legend

① connection transport liquid (not with all models available)

② connection buffer tube

③ connection flushing solution

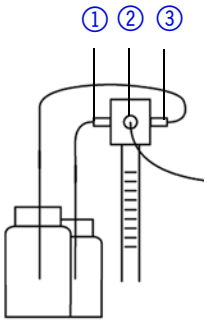


Fig. 49 Syringe connections

Tube Guide for Flushing Solution

Note: Use the tube guide in the collecting container for the flushing solution in order to not hinder horizontal movement of the needle.

Legend

① tube Guide for Flushing Solution

② collecting container with hole for tube guide (flushing fluid)

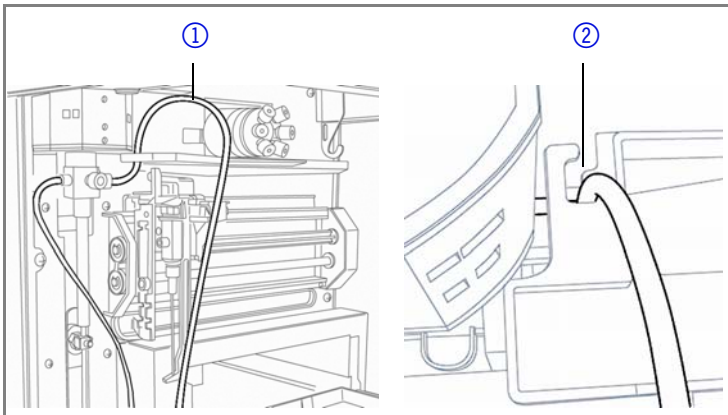


Fig. 50 Tube guide for flushing solution tube

Connecting the Drainage Tubing

The waste drainage removes all flushing fluids and non-injected sample solutions.

- Connect the condensed water and drainage tubing to the front panel of the device.
- Connect the adapter and insert the end of the tube into a container on the floor.

Note: Make sure that the drainage tubing is not pinched so that the liquid can drain.

Legend

① Tube for waste liquid

② Tube for condensed water

③ Adapter for drainage tube

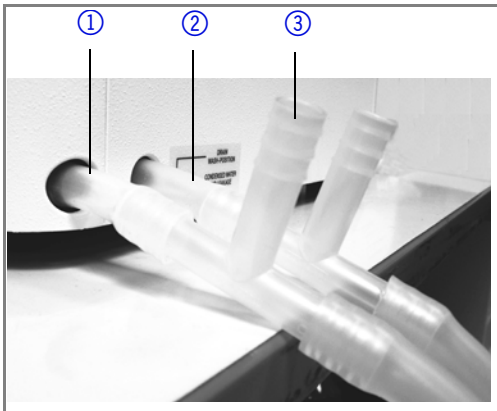


Fig. 51 Connecting the drainage tubing

PEEK Connections

Disposable PEEK fittings

- One-piece disposable polyetheretherketone fittings (PEEK) for easier installation of flexible, thin capillaries (outer diameter 0.5 mm)
- Tightening torque of the PEEK screw:Tightened by hand (about 0.5 Nm)

Connecting the Autosampler with other Devices

Controlling the Autosampler with Chromatography Software

You control the autosampler directly with software, e. g. OpenLAB® and ClarityChrom® by KNAUER.

Using the LAN connection at the rear panel of the device, connect the autosampler directly to the network.

Checking and Configuring the Parameters of the Autosampler

You can configure the autosampler parameters using the chromatography software, e.g. ClarityChrom®:

1. Select the autosampler in LAN.
2. Set the syringe volume to either 250 µl (default) or 500 µl.
3. Enter the serial number of the autosampler.
4. Choose the cooling option when your autosampler offers temperature control.

Configuration Window of ClarityChrom®

Legend

① serial number
② device detection in local network
③ manual search for device in network
④ volume of sample loop
⑤ volume of syringe

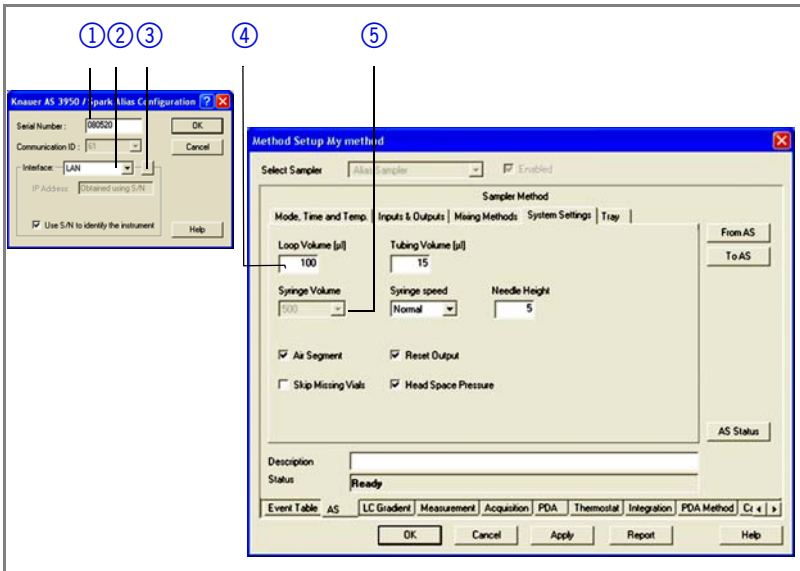


Fig. 52 Autosampler configuration with ClarityChrom®

Autosampler Device Software

A software CD with *Autosampler 3950 Service Manager* is included with the autosampler. Using this software, you can check and control the device. These settings can also be made with the chromatography software, so that it is not absolutely necessary to install the *Service Manager*.

Flushing the System

The system should be flushed before the column is connected. The flushing of the system can be controlled using the chromatography software or *Autosampler 3950 Service Manager*.

Note: KNAUER recommends using a mixture of water and isopropanol (80 %/20 %) or the mobile phase as the flushing solution.

The following steps are explained for when the *Service Manager* is used.

System Flushing with *Autosampler 3950 Service Manager*

1. Install *Autosampler 3950 Service Manager*.
2. Fill the flushing solution into a solvent bottle and degas it using helium or an ultrasonic bath.
3. Slide the tube for the washing liquid into the solvent bottle.
4. Select the *Alias* Þ ⇒*Direct Control* menu.
5. In the *Syringe* field, click *End*. One syringe volume is aspirated into the syringe through the flushing solution tube.
6. In the *Syringe* field, click *Home*. The syringe content is emptied into the drainage tube.
7. Repeat step 5 and 6 until the syringe and the flushing solution tube have been filled completely.
8. In the *Initial wash* field, click *Start*. All tubes that are connected to the syringe are flushed.
9. In the *Initial wash* field, click *Stop*.
10. Click *Close* to exit the *Direct Control* window.

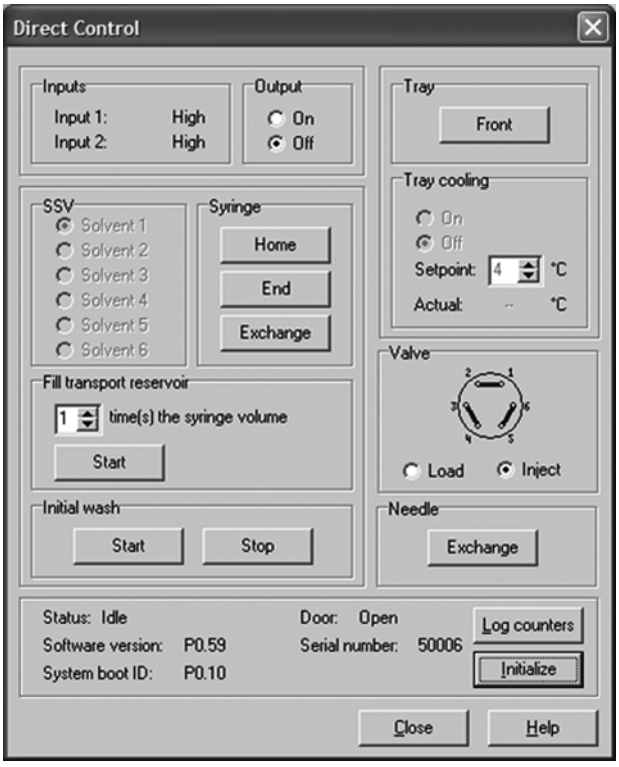


Fig. 53 System flushing with the *Service Manager* software

I/O Connection

By default, the autosampler has an I/O connection that supports TTL inputs (low-active) and a closed-contact output. Devices without LAN connection that require a trigger signal for the injection can be connected by means of the I/O connection.

The TTL inputs allow you to control the autosampler using other devices and are defined using the instrument methods of the chromatography software.

Note: Only connect the autosampler to devices that fulfill the required safety standards!

Defining the TTL Inputs

- *Next Injection Input*: The injection sequence is started. After ending the injection sequence, the autosampler waits for the next start signal.
- *Freeze Input*: The analysis time is paused. The autosampler runs the configured program up to the filling of the sample loop. The injection is not performed until the input is deactivated.
- *Stop Input*: Immediately halts the analysis.

Defining the Closed-Contact Output

- *Inject Marker*: The closed-contact output is activated when the injection valve switches from *LOAD* to *INJECT*.
- *Alarm*: The closed-contact output is activated when there is an autosampler fault.
- Auxiliary: -

Configuration of I/O Connection (9 pins)

Explanation	Cable color
1. Output, start injection	Red in three-conductor cable
2. Output, start injection	Black in three-conductor cable
3. Input 1, programmable input for stopping injections (low-active)	Red in four-conductor cable
4. Input 2, programmable input for stopping injections (low-active)	Black in four-conductor cable
5. Not occupied.	-
6. Output	Brown in three-conductor cable.
7. Not occupied.	-
8. Ground, for inputs 1 and 2	Orange in four-conductor cable
9. Ground, for inputs 1 and 2	Brown in four-conductor cable

Device Test

The reproducibility of the sample volume is a critical factor for maintaining high-quality analysis results.

- Test the autosampler features with the chromatography software on a regular basis.
- If the device test determines that the autosampler does not fulfill the requirements, mark the device as defective and do not continue using it.
- Do not re-use the autosampler until it has been repaired and/or serviced.

Test Intervals

Run the device test at the following time intervals:

- Average use of 1 to 5 days/week: Device test every 6 months

- Average use of more than 5 days/week or 24 hours/day: Device test every 3 months
- Operation with buffer solutions or other salt solutions: Device test every 3 months

Devices and Components for the Test

- Autosampler 3950, standard version with 10 µl sample loop and 250 µl syringe
- pump, 1 ml/min flow rate
- UV detector (with a data rate of 50 Hz if possible, otherwise 10 Hz, flow cell: 10 mm path length)
- Chromatography software
- Eluent: 90 % water, 10 % methanol (HPLC quality)
- Test solution (sample):
  - *a:* 50 ppm uracil dissolved in water (HPLC quality)
  - *b:* 250 ppm uracil dissolved in water (HPLC quality)
- Flushing solution:
  - 80 % water, 20 % isopropanol (HPLC quality)
  - Alternative: 80 % water, 20 % methanol (HPLC quality)
- Restriction capillary:
  - Inner diameter 0.25 mm
  - Length 200 cm

Note: Degas the eluent to prevent malfunctions caused by the presence of air bubbles.

1. Reproducibility of Sample Volume

The variation coefficient must not exceed 0.5 %.

- Configure the pump, UV detector and autosampler using the chromatography software
- Inject 10 µl of test solution *a* (50 ppm uracil, dissolved in water)

Standard Setting of Autosampler

- Loop volume: 10 µl
- Tubing volume: 15 µl
- Syringe volume: 250 µl

Method Parameters of Pump

- Flow: 1 ml/min
- Time: 1 min

Method Parameters of Autosampler

- Injection method: Partial loopfill
- Syringe speed: normal
- Flush volume: 30 µl, (40 µl for microtiter plates)
- Needle wash: active 2 times
- Air segment: yes
- Headspace pressure: yes
- Injections/vial: 9 (7 for microtiter plates)
- Inj. volume: 2 µl
- Vial position: 1A1. Start the single run with

Method Parameters of UV Detector

- Wavelength: 254 nm
- Sampling rate: 50 Hz if possible, else 10 Hz

- Time: 0.5 min

Configuring Repeat Runs of Autosampler

- Injections/vial: 9x (7x for microtiter plates)
- Inj. volume: 1 µl

Starting Repeat Runs

1. Put a vial with at least 500 µl test solution on position *1A1* of the sample plate.
2. Start the repeat runs.

Analyzing the Individual Chromatograms

1. Calculate the average of the measuring values of the *Peak* areas.
2. Calculate the variation coefficient VK_1 .
3. Enter the results into the *Test Report* form.

Formula for Determining the Arithmetic Mean

$$\overline{Peakareas} = \frac{\sum_{i=1}^{n} Peakareas_i}{n}$$

- Formula for determining the standard deviation (i = 1-9):

$$\sigma_{n-1} = \sqrt{\frac{\sum (Peakarea_i - \overline{Peakareas})^2}{n-1}}$$

- Formula for determining the variation coefficient:

$$VK_1 [\%] = \frac{\sigma_{n-1}}{peakarea} \times 100$$

2. Sample Carryover

The percentage of sample carryover must not exceed 0.3 %.

- Alternately inject 10 µl of test solution *b* (250 ppm uracil, dissolved in water) followed by eluent.
- Position of sample vial: *1A1*
- Position of eluent: *1A2*
- Injection volume: 1 µl

Creating a Sequence with 6 Lines

- Test solution: Position *1A1*
- Eluent: Position *1A2*
- Injection volume: 1 µl
- Repeats: 1

Note: For microtiter plates, select 6 consecutive positions that are alternately to be filled with test solution and eluent.

Analyzing the Individual Chromatograms

1. Calculate the average of the measuring values of the *Peak* areas.

2. Put the average of the eluent injection in relation to the average of the test solution injection.
3. Enter the results into the *Test Report* form.

Formula for Calculating Sample Carryover

$$PV\,[\%] = \frac{\sum_i \frac{Peakarea_{i\,Fließmittel}}{3}}{\sum_i \frac{Peakarea_{i\,Testlösung}}{3}} \times 100$$

3. Linearity

To determine the linearity, the correlation coefficient of the regression lines is determined from the measured values for the *peak* areas and injection volume.

The correlation coefficient must not exceed 0.998 %.

- Inject 10, 20, 30, 40 and 50 µl of test solution *b* (250 ppm uracil, dissolved in water) respectively.
- Position of sample vial: *1A1*
- Injection volume: 10 µl, 20 µl, 30 µl, 40 µl, 50 µl
- Repetition: 3

Note: Fill consecutive positions on the microtiter plates with test solution.

Analyzing the Individual Chromatograms

1. Calculate the correlation coefficient *r* of the regression lines from the measured values for the *Peak* areas and the injection volume.
2. Enter the results into the *Test Report* form.

Formula for Determining the Correlation Coefficient

$$r = \frac{\sum x_i y_i - n\bar{x}\bar{y}}{\sqrt{\left(\sum x_i^2 - n\bar{x}^2\right)\left(\sum y_i^2 - n\bar{y}^2\right)}}$$

y_i = *Y* value of measured value *i* (injection volume)

x_i = *X* value of measured value *i* (peak area)

$\bar{y}$ y = arithmetic mean of *Y* across all *n* measured values

$\bar{x}$ x = arithmetic mean of *X* across all *n* measured values

n = number of measured value pairs

4. Mixture Test

To create a mixing method, follow the instructions in the manual of the chromatography software.

- The test solution variation coefficient *VK2* must not exceed 0.5 %.
- The dilution variation coefficient *VK2* must not exceed ≤ 0.5 %.
- The dilution factor *F10* has to be within the range of $9.85 < x < 10.25$.
- Test solution: 10 µl (50 ppm uracil, dissolved in water)
- For injecting the dilution, go to *Mix methods* and create a mixing method in which 40 µl of the test solution is mixed with 360 µl eluent.
  - Dilution: 10 µl, 5 ppm uracil, dissolved in deionized water
- Inject 10 µl test solution and und 10 µl dilution three times each.

Creating a Sequence with 2 Lines

- Injection volume: 1 µl
- Repeats: 3

Positioning Vials for Dilution

- To position the vials on the sample plates, select the *Columns* option in the chromatography software.
- Put a vial with test solution (*Sample*) at position *2A1* of the vial plate.
- Put an empty vial (*Destination*) at position *2A5* of the vial plate.
- Put a vial with eluent (*Reagent A*) at position *1A1* of the vial plate.

Note: Observe the plate assignments for the vials (*Sample*, *Reagent A, Reagent B*, *Destination*).

Analyzing the Individual Chromatograms

1. Calculate the mean of the measuring values of the *Peak* areas for the test solution and the dilution.
2. Calculate the variation coefficient VK_2 .
3. Calculate the variation coefficient VK_3 .
4. Calculate the dilution factor *F10* from the ratio of the mean of the *Peak* areas of the test solution and the dilution.
5. Enter the results into the Test Report form.

Archiving

- Enter all test results into the *Test Report* form.
- Enter the serial number, date of the test, date of the next test and name of the tester.
- File the *Test Report* form in the device logbook.

Test Report

Module	Autosampler	
Autosampler 3950	Standard ☐	Sample cooler ☐
serial number		

No.	Test	Setting	Specification	Result
1	Reproducibility	▪ Inject 10 µl test solution nine times. ▪ Microtiter plate: Inject 10 µl test solution seven times.	$VK_1 \leq 0.5$ %	
2	Carryover	Alternately inject 10 µl test solution and 10 µl eluent three times.	$PV \leq 0{,}3$ %	
3	linearity	Inject 10, 20, 30, 40 and 50 µl test solution three times each.	$r \geq 0{,}998$	
4	Mixture test	Inject 10 µl test solution and 10 µl of the dilution created by the autosampler, three times each.	$VK_2 \leq 0.5$ % $VK_3 \leq 0.5$ % F10: 9,85 < x < 10,25	

Date:

Date of the next device test:

Tester:

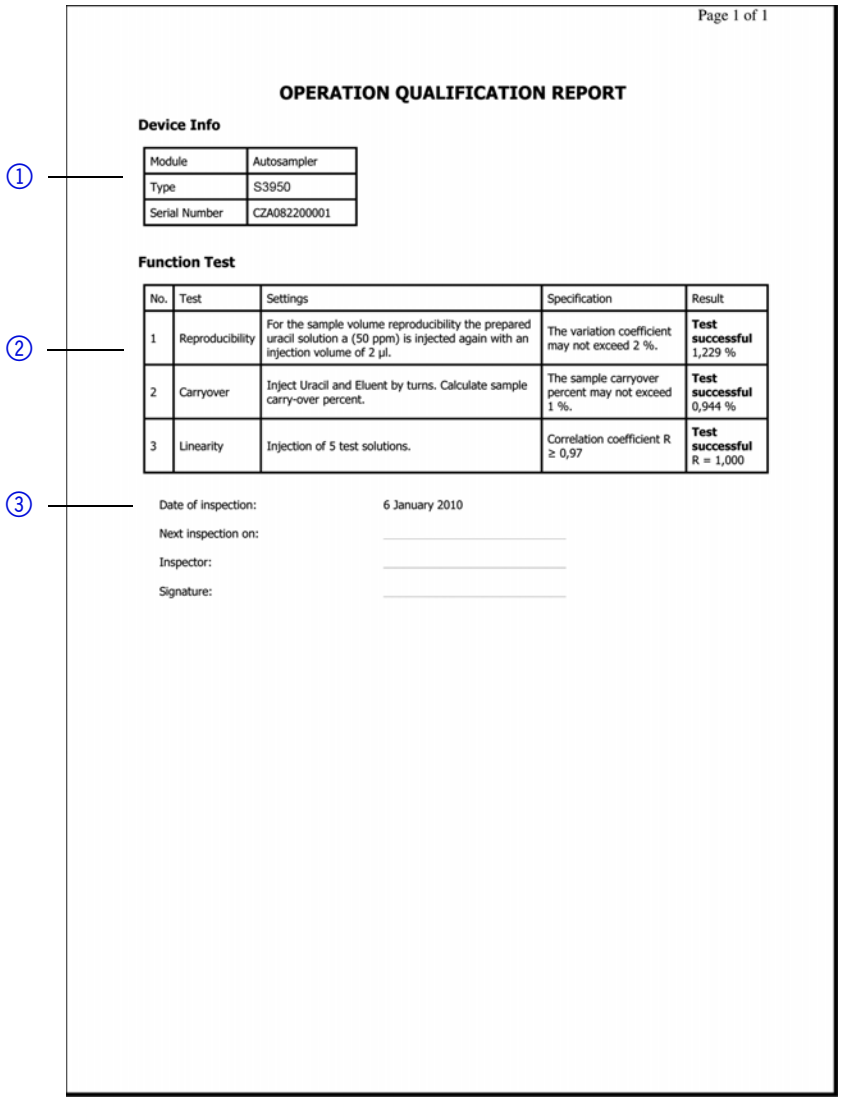
Operation Qualification Report

Legend

① Detailed information of the autosampler

② Detailed function test

③ Date and fields for signature and other information



Page 1 of 1

OPERATION QUALIFICATION REPORT

Device Info

Module	Autosampler
Type	S3950
Serial Number	CZA082200001

Function Test

No.	Test	Settings	Specification	Result
1	Reproducibility	For the sample volume reproducibility the prepared uracil solution a (50 ppm) is injected again with an injection volume of 2 µl.	The variation coefficient may not exceed 2 %.	**Test successful** 1,229 %
2	Carryover	Inject Uracil and Eluent by turns. Calculate sample carry-over percent.	The sample carryover percent may not exceed 1 %.	**Test successful** 0,944 %
3	Linearity	Injection of 5 test solutions.	Correlation coefficient R ≥ 0,97	**Test successful** R = 1,000

Date of inspection: 6 January 2010

Next inspection on:

Inspector:

Signature:

Fig. 54 Operation Qualification Report

Maintenance and Care

You have various options to contact the Technical Support:

Phone +49 30 809727-111

Fax +49 30 8015010

Mail support@knauer.net

You can make your requests in English and German.

Maintenance Contract

The following maintenance work on the device may only be performed by KNAUER or a company authorized by KNAUER and is covered by a separate maintenance contract:

- Opening the Module
- Removing the hood or the side panels.

⚠ DANGER

Electric shock
Danger of electric shock from voltage-carrying parts inside the device. The housing serves as a protective cover against voltages inside the device.

Which Type of Maintenance Tasks May Users Perform on the Device?

Users may perform the following maintenance tasks themselves:

- Regularly check for clogged capillaries – test back pressure without column.
- Exchanging the fuses
- Exchanging the air and sample needle
- Exchanging the injection valve
- Exchanging the sample loop
- Exchanging the rotor seals
- Exchanging the capillary and tubing

NOTICE

Electronic defect
Performing maintenance tasks on a switched on device can cause damage to the device.

➔ Switch off the device
➔ Pull the power plug.

Note: If leaks occur on the capillary screw fittings after maintenance and proper assembly, do not tighten them further, but instead replace them with new connection capillaries.

System Flushing

Procedure

1. Connect the autosampler to the power supply.
2. Establish a connection to the PC.
3. Start *Autosampler 3950 Service Manager*.
4. Select the *Alias⇒Direct Control* menu.
5. Click *Initialize* to check whether the valve is correctly positioned at the Inject position.
6. In the *Initial Wash* field, click *Start* to flush the system.
7. In the *Initial Wash* field, click *Stop* to stop flushing the system.

Taking out of Operation

The device is designed for the usage of different solvents. Solvent residue can damage the device or irritate the skin. That is why we recommend to flush components of the flow path in the autosampler before maintenance.

Process
1. Switch off the autosampler. 2. Pull the plug from the socket. 3. Pull the plug from the device.

Next Steps

Conduct any allowed maintenance tasks. For storage, select a location according to the requirements, which are listed in the according chapter of this user manual.

Exchanging the Fuses

Note: If the fuses blow repeatedly, consult with KNAUER Technical Support for replacements and help in identifying the cause.

Process
1. Switch off the autosampler and remove the power plug to completely disconnect the device from the power supply. 2. Remove the fuses from the fuse box at the rear of the device. 3. Insert new fuses (2 x 2.5 A). 4. Plug in the power plug.

Exchanging the Injection Valve and Rotor Seal

- Regularly clean the rotor seal of the injection valve.
- Regularly replace the rotor seal (approx. every three years).

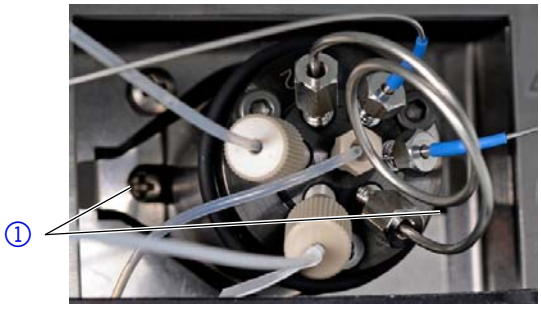
Removing the Injection Valve and Rotor Seal

Prerequisites

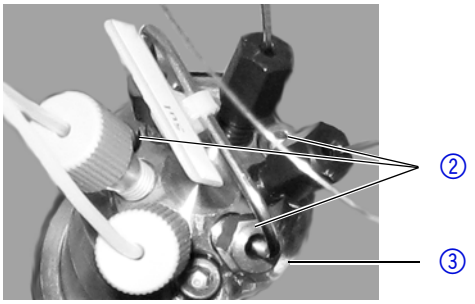
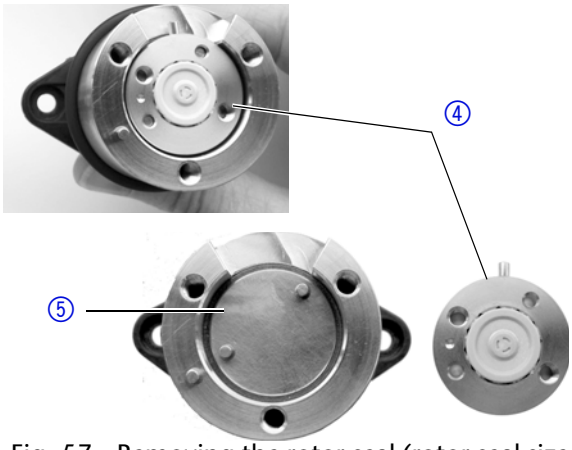
- Remove the front panel of the autosampler.
- Remove the capillary connections, except the sample loop, from the valve.
- During removal, consecutively loosen all screws by half a turn respectively, until they can be removed.

Note: Do not remove the screw in the cover plate hole diagonal to the valve.

Procedure

Process	Figure
1. With a screwdriver, remove screw ① on both sides of the analytical/preparative injection valvehousing. 2. Unscrewing the injection valve.	 Fig. 55 unscrewing the injection valve (figure shows analytical valve)

Procedure

Process	Figure
3. With a hexagon wrench key, remove the screws ② from the valve stator block 2. 4. Carefully remove the stator ③ .	 Fig. 56 unscrewing the stator (figure shows preparative valve)
5. Remove the rotor seal ④ from the rotor ⑤ . 6. Clean or exchange the rotor seal.	 Fig. 57 Removing the rotor seal (rotor seal size or type vary depending on autosampler equipment)

Installing the Injection Valve

- During installation, hold the injection valve in such a way that the bore hole for connecting the capillary to the pump (port 6) is facing upward.
- Alternately tighten all screws by half a turn, until all screws have been fully tightened.

Legend

① connector port 1
② connection from capillary to pump

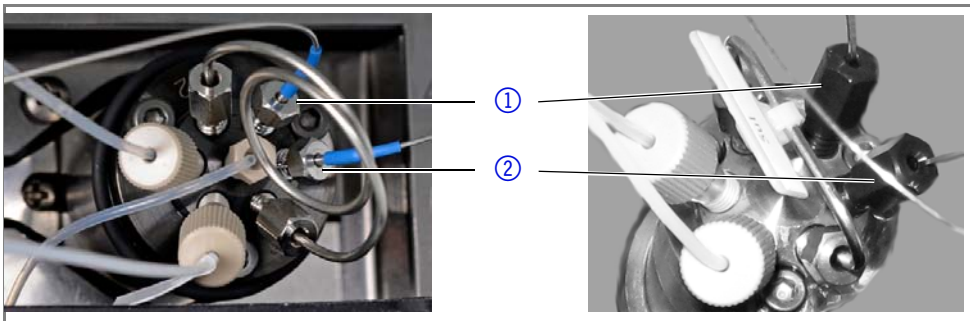


Fig. 58 Installing the valve

Process
1. Insert the rotor seal. 2. Place the stator onto the rotor and use a hexagon wrench key to tighten the screws. 3. Insert the injection valve and use a screwdriver to tighten the screw on both sides of the valve housing. 4. Connect the capillary again.

Exchanging the Sample Loop

By standard, the analytical autosampler is equipped with a 10 µl sample loop. The preparative autosampler is equipped with a 10 ml sample loop.

- When assembling a sample loop with a different injection volume, make sure to use the correct combination of syringe and capillaries and configure the controller software appropriately.
- Always connect the sample loop to ports 2 and 5 of the injection valve.
- Calculate the maximum injection volume according to the following formula:
  - full loop filling:
    Maximum injection volume = 3 times sample volume for loops with 100 µl, 2 times loop volume for loops with 100-500 µl, 1.5 times loop volume for loops above 500 µl
  - partial loop filling
    Maximum injection volume = 50 % loop volume
  - microliter pickup:
    Maximum injection volume = (loop volume – 3 x needle volume)/2

Exchanging the Sample Needle

- When using sample plates with 12 or 48 sample vials, make sure that the needle height setting is >2 mm to prevent the needle from contacting the bottom of the sample vial.
- Tighten the screw fitting until it is finger-tight to prevent the plastic capillary from becoming blocked.

Legend

① screw fitting
② plastic capillary
③ nut
④ sample needle

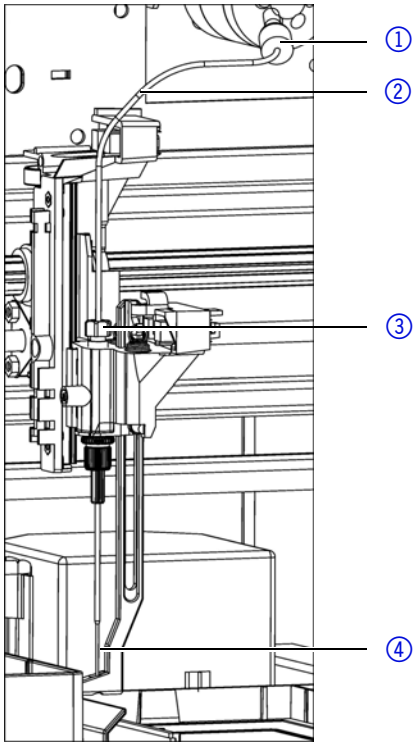


Fig. 59 Exchanging the sample needle

Procedure

1. Start *Autosampler 3950 Service Manager*.
2. Select the *Alias⇒Direct Control* menu.
3. In the *Needle* field, click *Exchange*. The needle moves to the replacement position.
4. Loosen the union nut ③ .
5. Loosen the screw fitting ① of the plastic capillary ② on the injection valve.
6. Remove the sample needle ④ with the plastic capillary.

7. Install a new sample needle unit. Make sure that the air seal fully surrounds the sample needle.
8. Fasten the sample needle with the union nut.
9. Fasten the plastic capillary using the screw fitting on the injection valve.
10. In the *Direct Control* window, click *Initialize*. The needle moves to the initial position.
11. In the *Initial Wash* field, click *Start* to flush the system.
12. In the *Initial Wash* field, click *Stop* to stop flushing the system.
13. Click *Close* to exit the *Direct Control* window.
14. Select the *Alias⇒Adjustments* menu.
15. On the *Needle-Tray* tab, update the settings for the sample plates.

Exchanging the Air Needle

- When exchanging the air needle, make sure that the thread of the new height adjustment screw is flush with the lower edge of the retaining nut.
- Make sure that the sealing ring is located in the retaining nut.

Legend

① nut
② retaining nut
③ height adjustment screw
④ air needle
⑤ sample needle

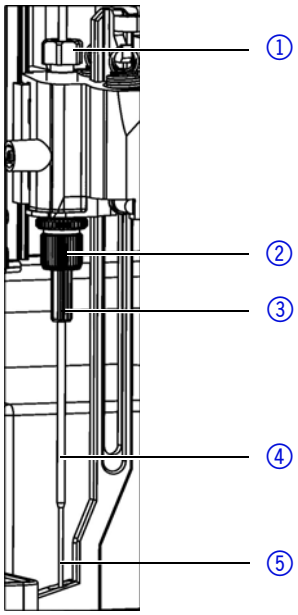


Fig. 60 Exhanging the air needle

Procedure

1. Start *Autosampler 3950 Service Manager*.
2. Select the *Alias⇒Direct Control* menu.
3. In the *Needle* field, click *Exchange*. The needle moves to the replacement position.
4. Loosen the union nut ① .
5. Loosen the screw fitting of the plastic capillary on the injection valve.
6. Remove the sample needle ⑤ with the plastic capillary.
7. Loosen the retaining nut ② and pull it downwards together with the air needle ④ .
8. Unscrew the retaining nut from the height adjustment screw ③ .
9. Screw a new air needle with a new height adjustment screw into the retaining nut.
10. Screw in the retaining nut.
11. Insert the sample needle and fasten with the union nut.
12. Fasten the plastic capillary using the screw fitting on the injection valve.
13. In the *Direct Control* window, click *Initialize*. The needle moves to the initial position.
14. In the *Initial Wash* field, click *Start* to flush the system.
15. In the *Initial Wash* field, click *Stop* to stop flushing the system.

16. Click *Close* to exit the *Direct Control* window.
17. Select the *Alias⇒Adjustments* menu.
18. On the *Needle-Tray* tab, update the settings for the sample plates.

Exchanging the Syringe

By standard, the autosampler is equipped with a 250 µl syringe.

Use isopropanol as flushing solution to remove air bubbles from the new syringe.

Legend

① Syringe valve
② syringe
③ Syringe drive
④ Syringe plunger

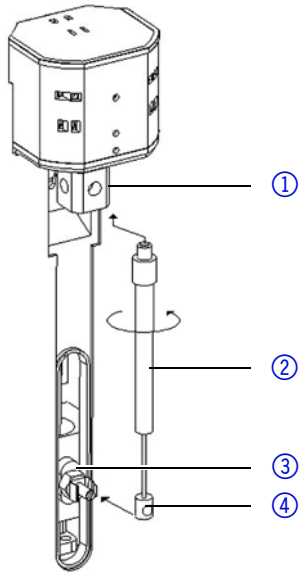


Fig. 61 Exchanging the syringe

Procedure

1. Start *Autosampler 3950 Service Manager*.
2. Select the *Alias⇒Direct Control* menu.
3. In the *Syringe* field, click *Exchange*. The syringe plunger is lowered.
4. Unscrew the syringe ② by rotating it counterclockwise; leave the adapter in the syringe valve ① .
5. Remove the syringe plunger ④ from the syringe drive ③ .
6. Fill new syringe with flushing solution.
7. Insert the syringe plunger into the syringe drive.
8. Tighten the syringe in the syringe valve by rotating it clockwise.
9. In the *Syringe* field, click *Home*. The syringe content is emptied into the drainage tube.
10. If there is still air in the syringe, click *End* in the *Syringe* field. One syringe volume is aspirated into the syringe through the flushing solution tube.
11. In the *Syringe* field, click *Home*. The syringe content is emptied into the drainage tube.
12. Slightly tap the body of the syringe if it still contains air. If necessary, repeat step 10 and 11.
13. In the *Initial Wash* field, click *Start* to flush the system.
14. In the *Initial Wash* field, click *Stop* to stop flushing the system.
15. Click *Close* to exit the *Direct Control* window.

Exchanging the Syringe Plunger or Plunger Tip

1. Start *Autosampler 3950 Service Manager*.
2. Select the *Alias⇒Direct Control* menu.
3. In the *Syringe* field, click *Exchange*. The syringe plunger is lowered.
4. Remove the syringe (see above).
5. Pull the syringe plunger out of the glass cylinder of the syringe.

6. Use a pair of tweezers to remove the plunger tip.
7. Wet the new plunger tip with isopropanol.
8. Mount the new plunger tip onto the syringe plunger.
9. Push the syringe plunger into the glass cylinder of the syringe.
10. Install the syringe (see above.)
11. In the *Syringe* field, click *Home*. The syringe content is emptied into the drainage tube.

Exchanging the Syringe Valve

The syringe valve has four connections, one of them remains unused.

- Hand-tighten all fittings on the connections to the syringe valve.
- To exchange the valve, set it to the Waste position, because the hexagon socket screws are only accessible if the valve is in this position.

Legend

① upper hexagon socket screw
② lower hexagon socket screw
③ connection flushing solution tube (hidden)
④ connection buffer tube
⑤ connection syringe
⑥ unused connection

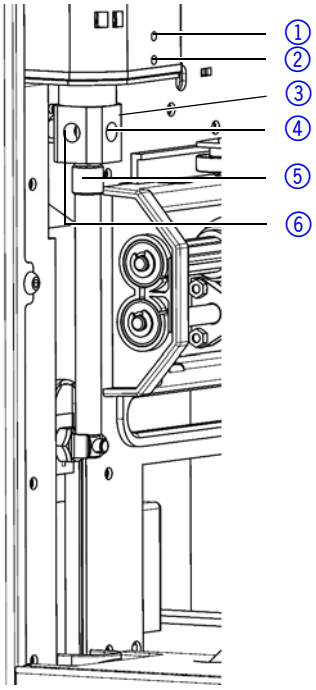


Fig. 62 Exchanging the syringe valve

Procedure

1. Start *Autosampler 3950 Service Manager*.
2. Select the *Alias⇒Direct Control* menu.
3. In the *Syringe* field, click *Exchange*. The syringe plunger is lowered.
4. Loosen the lower hexagon socket screw by 2 turns.
5. Loosen the upper hexagon socket screw by 2 turns.
6. Pull out the upper part of the syringe.
7. Remove the syringe.
8. Exchange the syringe valve.
9. Insert a new syringe.
10. Tighten the hexagon socket screws.

Cleaning and Caring for the Device

NOTICE

Device defect

Intruding liquids can cause damage to the device.

➔ Place solvent bottles next to the device or in a solvent tray.
➔ Moisten the cleaning cloth only slightly.

All smooth surfaces of the device can be cleaned with a mild, commercially available cleaning solution, or with isopropanol.

- Clean collecting container and vial plates with a soft cloth.
- To remove deposits, flush the drainage tube regularly using solvent.

Troubleshooting

Device Errors

One possible cause of device errors is a malfunctioning valve.

Checking the Valve

Remove the valve and check all parts for wear and contamination. After the problem has been eliminated and the valve reinstalled, perform the following steps:

Procedure

1. Select the *Alias⇒Direct Control* menu.
2. In the *Direct Control* window, click *Initialize*. The needle moves to the initial position.
3. In the *Initial Wash* field, click *Start* to flush the system.
4. In the *Initial Wash* field, click *Stop* to stop flushing the system.
5. Click *Close* to exit the *Direct Control* window.

LAN

Software faults can occur due to flawed communications between the devices or incorrect installation of the software.

Procedure

1. Check the cable connections.
2. Start *Autosampler 3950 Service Manager*.
3. Select the *Alias⇒Direct Control* menu.
4. In the *Direct Control* window, click *Initialize*.

Go through the following steps, in case no connection between the computer and the devices can be established. Check after each step if the problem is solved. If the problem cannot be located, call the Technical Support.

1. Check the status of the LAN connection in the Windows task bar:
   -  Connected
   -  Connection not established

   If no connection was established, test the following:
   - Is the router switched on?
   - Is the patch cable connected correctly to the router and the computer?
2. Check the router settings:
   - Is the router set to DCHP server?
   - Is the IP address range sufficient for all the connected devices?
3. Check all connections:
   - Are the patch cable connected to the LAN ports and not the WAN port?
   - Are all cable connections between devices and router correct?
   - Are the cables plugged in tightly?
4. If the router is integrated into a company network, pull out the patch cable from the WAN port.
   - Can the devices communicate with the computer, even though the router is disconnected from the company network?
5. Turn off all devices, router, and computer. Firstly, turn on the router and secondly turn on the devices and the computer.
   - Has this been successful?
6. Replace the patch cable to the device with that no connection could be established.

  - Has this been successful?
7. Make sure that the IP port of the device matches the port in the chromatography software.

Analytical Errors

Possible causes:

- Wear due to errors in the injection and method settings.
- Unsuitable combination of sample loop, buffer tube and syringe.
- External effects such as temperature, and light-sensitive samples being exposed to light.

Solutions:

- Check whether the application has run previously without errors and that no changes have been made to the analytical system.
- Determine whether the fault is caused by the autosampler or other devices in the system.

If the required degree of reproducibility is not achieved, check the following possible sources of error and instigate steps to eliminate them:

Cause of fault	Elimination
Air in liquid path	Initialize the Autosampler 3950.
Leaking syringe	▪ If the syringe is leaking at the top, check whether it has been installed correctly. ▪ If the syringe is leaking at the bottom, exchange the syringe plunger.
Leaking syringe valve	Check valve and exchange if required.
Rotor seal worn	Exchange the rotor seal and check the stator block of the valve.
Dead volume in capillary connections	Install new fittings onto capillary connections.

If an empty sample run returns an excessively large peak, check the following possible causes of error and instigate steps to eliminate them:

Cause of fault	Elimination
Solubility problems	Either modify sample or accept carryover.
Interaction between the empty sample and the *hardware*	▪ Check *hardware*: - Flush needle (inside and outside) or install a different needle type (steel, PEEK or with glass coating). - Valve: Exchange rotor seal (other material). - Capillaries and tubing: Use other connections between the autosampler and the columns (steel, PEEK) or other flushing solutions.
Empty sample contaminated	Use new empty sample.
Cause unknown.	Attempt to solve problem by using different solvents and eluents.

If no injection is performed:

Cause of fault	Elimination
Liquid path blocked	1. Disconnect the plastic capillary of the needle from the injection valve. 2. Start system flushing. 3. If solvent escapes at the injection valve connection to the needle, check the needle. 4. If no solvent escapes at the injection valve connection to the needle, disconnect the buffer tube from the injection valve. 5. Start system flushing. 6. If solvent flows out at the open end of the buffer tube, check the rotor seal. 7. If no solvent flows out of the open end of the buffer tube, disconnect the buffer tube from the syringe valve. 8. Start system flushing. 9. If solvent flows out of syringe valve, check the buffer tube. 10. If no solvent flows out of the syringe valve, check whether the connections of the liquid path have been tightened too much.
Leaking valve	1. Unscrew the plastic capillary leading to the needle from the injection valve. 2. Disconnect the plastic capillary leading to the syringe from the injection valve. 3. Connect the pump to the injection valve. 4. Close the connection to the column at the injection valve. 5. Start the pump at a low flow rate. 6. Check the connections to the syringe and to the needle at the injection valve to ensure that they are tight. 7. If liquid escapes there, check the rotor seal. 8. If no liquid escapes there, check the system with a manual valve.

System Messages in OpenLAB®

The various system messages of the OpenLAB® chromatography software from KNAUER are explained below. The system messages are sorted alphabetically.

System message	Explanation
Autosampler is in run mode.	▪ Quit the control software and restart. ▪ Switch the device off and on.

System message	Explanation
Autosampler is not responding. Please check communication settings and ensure the device is online.	Switch the device off and on. Check the network settings. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Cannot run autosampler.	Switch the device off and on. Check the network settings. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Cannot set destination vial to %d.	Check parameters in control software and correct entry.
Cannot set first transport vial to %d.	Check parameters in control software and correct entry.
Cannot set last transport vial to %d.	Check parameters in control software and correct entry.
Cannot stop autosampler.	Check the network settings. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Communication port for autosampler was not initialized. Please check the configuration settings.	Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Configuration settings do not match with the device. Run cannot start.	Check configuration and settings.
Destination position not reached.	Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Deviation of more than +/- 2 mm towards home.	▪ Look for visible obstructions in area of vial plate. ▪ Check the belt tension of the vial plate.
Dispenser error.	Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Electronics error.	Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
EEPROM error in adjustments.	Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
EEPROM error in log counter.	Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.

System message	Explanation
EEPROM error in settings.	Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
EEPROM write error.	Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Error 369	Not enough transport liquid in store. Refill transport liquid.
Error 370	Not enough reagent in store. Refill reagent.
Error by setting Mix&Dilute vials.	Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Error occurred during initialization, the Autosampler AS-1 cannot start.	Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Error resetting output.	Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Error running user defines program.	Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Error setting injection mode.	Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Error setting needle height.	Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Error setting injection mode.	Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Error setting syringe speed.	Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Error setting the analysis time.	Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Error setting the auxiliaries.	Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Error setting the flush time.	Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Error setting the flush volume.	Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.

System message	Explanation
Error setting the injection volume.	Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Error setting the loop volume.	Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Error setting the prep. mode.	Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Error setting the syringe volume.	Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Error setting timed events.	Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Error setting the tray configuration.	Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Error setting the tray temperature.	Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Error setting the vial number.	Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Error setting tubing volume.	Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Error setting wash volume.	Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Flush volume error.	Check parameters in control software and correct entry.
Home sensor activated when not expected.	▪ Check parameters in control software and correct entry. ▪ Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Home sensor not de-activated.	▪ Check whether there are visible obstructions impairing the vial plate. ▪ Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Home sensor not reached.	▪ Check whether there are visible obstructions impairing the vial plate. ▪ Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.

System message	Explanation
Horizontal: home sensor activated when not expected.	▪ Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Horizontal: home sensor not de-activated.	▪ Check whether there are visible obstructions impairing the needle unit. ▪ Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Horizontal: home sensor not reached.	▪ Check whether there are visible obstructions impairing the needle unit. ▪ Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Horizontal: needle position is unknown.	Initialize the needle unit using the control software.
Illegal sensor readout.	Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Incorrect first destination vial.	Check parameters in control software and correct entry.
Injection needle unit error.	▪ Check whether there are visible obstructions impairing the needle unit. ▪ Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Injection valve or ISS unit error.	Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Injection volume %.2f is invalid. For specified injection method, volume should be within the range %.2f µl-%.2f µl, with %2f. µl increments.	Check parameters in control software and correct entry.
Injection volume error.	Check parameters in control software and correct entry.
Invalid %s vial position %02d. The vial position must be between 01 and %02d.\n.	Check parameters in control software and correct entry.
Invalid combination of the trays. The combination of different trays for the Mix&Dilute mode is not allowed.	▪ Insert the correct vial plate. ▪ Check parameters in control software and correct entry.

System message	Explanation
Invalid combination of the trays. The combination of plates 384 low and 96 high is not allowed.	▪ Insert the correct vial plate. ▪ Check parameters in control software and correct entry.
Invalid configuration. ISS option not installed on autosampler. Please switch off this option in configuration dialog.	Check parameters in control software and correct entry.
Invalid configuration. SSV option not installed on autosampler. Please switch off this option in configuration dialog.	Check parameters in control software and correct entry.
Invalid flush volume %d µl. The flush volume should be between 0 and %d µl.	Check parameters in control software and correct entry.
Invalid flush volume %2f µl. The flush volume should be between 0 and %2f µl.	Check parameters in control software and correct entry.
Invalid input. Only values with increments of %.2f allowed.	Check parameters in control software and correct entry.
Invalid integer number.	Check parameters in control software and correct entry.
Invalid instrument is detected.	Check parameters in control software and correct entry.
Invalid loop volume %d µl. The loop volume should be between 0 and %d µl.	Check parameters in control software and correct entry.
Invalid loop volume %2f µl. The loop volume should be between 0 and %2f µl.	Check parameters in control software and correct entry.
Invalid mix program: no Destination vial is specified in the configuration dialog.	Check parameters in control software and correct entry.
Invalid mix program: no Reagent A vial is specified in the configuration dialog.	Check parameters in control software and correct entry.
Invalid mix program: no Reagent B vial is specified in the configuration dialog.	Check parameters in control software and correct entry.
Invalid mix times. The time should be between 1 and 9.	Check parameters in control software and correct entry.

System message	Explanation
Invalid needle height %d mm. The needle height should be between %d and %d mm.	Check parameters in control software and correct entry.
Invalid time-based method. Several AUX events have the same time.	Check parameters in control software and correct entry.
Invalid time-based method. Several SSV events have the same time.	Check parameters in control software and correct entry.
Invalid tray temperature %d ˚C. The temperature should be between 4 and 22 ˚C.	Check parameters in control software and correct entry.
Invalid tray configuration: two or more vial positions are the same.	Check parameters in control software and correct entry.
Invalid tubing volume %d µl. The tubing volume should be between 0 and %d µl.	Check parameters in control software and correct entry.
Invalid tubing volume %2f µl. The tubing volume should be between %2f and %2f µl.	Check parameters in control software and correct entry.
Invalid wait time. The time should be between 0 and 9 h 50 min 59 sec.Invalis wash volume %d µl. The wash volume should be between %d and %d µl	Check parameters in control software and correct entry.
Invalid volume %d µl. The volume should be between the 0 and the syringe volume (%d µl).	Check parameters in control software and correct entry.
ISS valve error.	Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Missing destination vial.	▪ Check position of sample vial. ▪ Check parameters in control software and correct entry.
Missing reagent vial.	▪ Check position of sample vial. ▪ Check parameters in control software and correct entry.
Missing transport vial.	▪ Check position of sample vial. ▪ Check parameters in control software and correct entry.

System message	Explanation
Needle movement error.	▪ Check position of needle unit. ▪ Switch the device off and on.
Missing vial.	▪ Check position of needle unit. ▪ Switch the device off and on.
Missing wash vial error.	▪ Check position of needle unit. ▪ Switch the device off and on.
No destination vial is specified in the configuration.	Check parameters in control software and correct entry.
No reagent A vial is specified in the configuration.	Check parameters in control software and correct entry.
No reagent B vial is specified in the configuration.	Check parameters in control software and correct entry.
No transport vials are defined in the tray configuration. It is not possible to use the µl pick-up injection mode.	Check parameters in control software and correct entry.
No user defined or mix program is running.	Check parameters in control software and correct entry.
Not enough reagent liquid.	Check volume of liquid and change as required.
Not enough transport liquid available due to missing transport vials.	Check volume of liquid and change as required.
Please specify inject marker or AUX event to be able to trigger the run.	Check parameters in control software and correct entry.
Selecting transport position failed.	Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Serial number is not valid. Please check the configuration.	Check parameters in control software and correct entry.
Setting mix program error.	Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Setting service mode failed.	Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Syringe dispenser unit error.	Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.

System message	Explanation
Syringe home sensor not de-activated.	▪ Needle flushing with control software. ▪ Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Syringe home sensor not reached.	▪ Needle flushing with control software. ▪ Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Syringe position is unknown.	Initialize the syringe unit using the control software.
Syringe rotation error.	▪ Needle flushing with control software. ▪ Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Syringe valve did not find destination position.	▪ Needle flushing with control software. ▪ Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Temperature above 48 °C at cooling ON.	▪ Switch off the cooling and check whether ambient temperature sensor is properly functioning. ▪ Inform the Technical Support of the manufacturer in case the system message repeats itself.
The 10 ml syringe cannot be used for standard injections.	Exchange the syringe.
The autosampler has detected another tray than that which is currently configured. Please select the correct tray in configuration dialog.	Check control software configuration and correct entry.
ISS-A option not installed on autosampler. Please switch off ISS-A option in configuration dialog.	Check control software configuration and correct entry.
ISS-B option not installed on autosampler. Please switch off ISS-B option in configuration dialog.	Check control software configuration and correct entry.
Oven option not installed on autosampler. Please switch off oven option in configuration dialog.	Check control software configuration and correct entry.
The autosampler is not ready. Please try later.	Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.

System message	Explanation
The injection volume of %2f µl is invalid. For the specified injection method, volume should equal %2f µl.	Check parameters in control software and correct entry.
The sample needle is not in the home position while the tray is rotating.	Check parameters in control software and correct entry.
Trace from tray cooling cannot be acquired. Tray cooling is off.	Check parameters in control software and correct entry.
Tray advance is not available at this time.	Check parameters in control software and correct entry.
Tray error.	Check parameters in control software and correct entry.
Valve error.	Check parameters in control software and correct entry.
Vertical: home sensor not de-activated.	▪ Check whether there are visible obstructions impairing the needle unit. ▪ Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Vertical: home sensor not reached.	▪ Check whether there are visible obstructions impairing the needle unit. ▪ Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Vertical: needle position is unknown.	Initialize the instrument in the control software.
Vertical: stripper did not detect plate (or wash/ waste). Missing vial.	▪ Check sample vial and plate. ▪ Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Vertical: stripper stuck.	Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Vertical: The sample needle arm is at an invalid position.	Switch the device off and on. Inform the Technical Support of the manufacturer in case the system message repeats itself.
Vial number error.	Check parameters in control software and correct entry.
Wear-out limit reached.	Switch the device off and on. If the system message appears again, notify KNAUER Technical Support. The valve must be replaced.

System message	Explanation
Wrong loop volume. The largest loop volume for standard injections is 1000 µl.	Check parameters in control software and correct entry.
Wrong tubing volume. The largest tubing volume for standard injections is 200 µl.	Check parameters in control software and correct entry.

Technical Data

General Data

Sample injection

max. plate/vial height	47 mm (incl. septa or capmat)
injection volume range	see “Analytical Versions” on page 67 see “Preparative Versions” on page 68
sample loop	see “Analytical Versions” on page 67 see “Preparative Versions” on page 68
dispenser syringe	see “Analytical Versions” on page 67 see “Preparative Versions” on page 68
headspace pressure	built-in compressor, only for sample vials with septum
switching time inj. valve	< 100 ms
piercing needle precision	± 0.6 mm
sample tray cooling	with cooling function 4-40 °C
vial detection	missing vial/well plate detection by sensor
Needle flushing	programmable: wash between injections and wash between vials
wetted materials	Tefzel®, Vespel®, glass, Teflon® (PTFE) standard: stainless steel, PEEK bio: PEEK
injection modes	see “Analytical Versions” on page 67 see “Preparative Versions” on page 68
injection precision	RSD (Relative Standard Deviation): ▪ full loop filling < 0.3 % ▪ partial loop filling at injection volumes > 10 µl: < 0.5 % ▪ microliter pickup at injection volumes > 10 µl: < 1.0 %
injection volumes	see “Analytical Versions” on page 67 see “Preparative Versions” on page 68
sample carryover	see “Analytical Versions” on page 67 see “Preparative Versions” on page 68
injections per vial	max. 9 injections

	injection cycle time	see “Analytical Versions” on page 67 see “Preparative Versions” on page 68
	analysis time	max. 9 h, 59 min, 59 s
Communication	inputs	2 programmable TTL inputs (next injection, freeze, stop)
	outputs	1 programmable relay output (inject marker, auxiliary, alarm)
	control	Ethernet (LAN)
Ambient conditions	temperature range	10–40 ˚C; 50–104 ˚F
	air humidity	20–80 % relative humidity
General	power supply	95–240 V AC
	dimensions	see “Analytical Versions” on page 67 see “Preparative Versions” on page 68
	weight	see “Analytical Versions” on page 67 see “Preparative Versions” on page 68
	installation category	II
	pollution degree	2

Analytical Versions

sample capacity	max. 768 samples (microtiter plates) or 96 standard autosampler vials
injection volume range	0.1-5000 µl programmable
sample loop	10 µl
dispenser syringe	250 µl
injection modes	full lloop filling, partial loop filling and microliter pickup, PASA™ (pressure-assisted sample aspiration)
injection volumes	▪ full loop filling: max. 5000 µl ▪ partial loop filling: 2500 µl (50 % of loop volume) ▪ microliter pickup: max. 2455 µl (50 % loop volume - 1.5× needle volume) ▪ 0.1 µl increment for all injection modes
sample carryover	< 0.05 % with needle cleaning
injection cycle time	min. 7 s from the same vial, 14 s from different vials; <60 s for =100 µl sample injection in all injection modes, incl. 300 µl needle wash
dimensions	377x300x510 mm (width × height × depth)
weight	19 kg

Preparative Versions

sample capacity	24 vials, 10 ml each (LSV)
injection volume range	1-5000 µl programmable
sample loop	100 µl/10 ml
dispenser syringe	500 µl/2500 µl
injection modes	partial loop filling
injection volumes	1–5000 µl, 1 µl steps
sample carryover	< 0.1 % with needle cleaning RSD ≤ 1.0 % for partial loop filling, injection volumes > 10 µl up to 50 % of installed sample loop
injection cycle time	min. 7 s from the same vial, 14 s from different vials; <60 s for =100 µl sample injection in all injection modes, incl. 300 µl needle wash
dimensions	377×300×575 mm (width × height × depth)
weight	21 kg

Repeat Orders

Up-to-date information on spare parts and accessories can be found online: www.knauer.net. This list for repeat orders is valid for the time the document has been published. Deviations afterwards are possible.

Note: For repeat orders of spare parts use the enclosed packing list. Contact the Technical Support in case there are any questions on spare parts or accessories.

	Name	Order number
Device	Autosampler 3950, standard, 1000 bar incl. accessories	A50070
	Autosampler 3950, cool/heat, 1000 bar incl. accessories	A500701
	Autosampler 3950, standard, 700 bar incl. accessories	A50080
	Autosampler 3950, cool/heat, 700 bar incl. accessories	A500801
	Autosampler 3950, bio version	A50052-1
	Autosampler 3950, biocompatible and cool version	A50053-1
	Autosampler 3950, preparative version	A50054-1
	Autosampler 3950, biocompatible preparative version	A50055-1
	Autosampler 3950, biocompatible preparative cool version	A50055-2
	Autosampler 3950, preparative version, cool	A50056-1
Vial plate	vial plates for 1.5 ml vials	A50050
	vial plate for 84x1.5ml and 3x10ml vials	A500501

	Name	Order number
	prep. vial adapter plate for 12×10 ml	A500502
	96 well plate, U sanitized, 0.35 ml	A1823
	96 well plate, U sanitized, 1.2 ml	A1823V1
Tubing	PTFE tubing, 3.2 mm (1/8") OD, 1.5 mm ID, 300 cm length	A0732
	silicone tubing for waste line 8.0 mm ID, 2 m	A0991-69
Syringe	syringe 500 µl	M2070
	syringe 2500 µl	M20701
Sample needle	sample needle for autosampler	A0646
	sample needle kit for SPARK valve 1/16"	A64700
	bio inert silicone sample needle including tubing, nut and ferrule	A15086
Air needle	air needle, natural, 62 mm	A50058
	set with air needles (includes one piece of M20401, M20402, M20403, M20404, M20405)	A50059
	air needle, yellow, 50 mm	M20401
	air needle, red, 56 mm	M20402
	air needle, blue, 68 mm	M20403
	air needle, green, 74 mm	M20404
	air needle, black, 80 mm	M20405
Accessories	2 x 2.5 A fuse	M2067
	network cable	A5255
	RS-232 cable	A0895
	wash bottle 250 ml rectangular	M2054
	accessory kit with vials caps, septum, and pliers for opening and closing	A0664
	125 Vials 10 ml, 500 crimp caps and 500 septa, ø 22 mm	A1662
	system adapter	A9863

Legal Information

Transport Damage

The packaging of our devices provides the best possible protection against transport damage. Check the devices for signs of transport damage. In case you notice damages, contact the Technical Support and the forwarder company within three workdays.

Warranty Conditions

The factory warranty for the device is stipulated by contract. During the warranty period, any components with material or design-related defects will be replaced or repaired by the manufacturer free of charge. Please connect to our website for further information on terms and conditions.

All warranty claims shall expire in the event that any unauthorized changes are made to the device. This warranty also excludes the following:

- accidental or willful damage
- damage or errors caused by third parties that are not contractually related to the manufacturer at the time the damage occurs
- wear parts, fuses, glass parts, columns, light sources, cuvettes and other optical components
- damage caused by negligence or improper operation of the device and damage caused by clogged capillary
- packaging and transport damage

In the event of device malfunctions, directly contact the manufacturer.

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Declaration of Conformity

The Declaration of Conformity is part of the delivery and accompanies the product as a separate document.

Disposal

Hand in old devices or disassembled old components at a certified waste facility, where they will be disposed of properly.

AVV Marking in Germany

According to the German "Abfallverzeichnisverordnung" (AVV) (January, 2001), old devices manufactured by KNAUER are marked as waste electrical and electronic equipment: 160214.

WEEE Registration

KNAUER as a company is registered by the WEEE number DE 34642789 in the German "Elektroaltgeräteregister" (EAR). The number belongs to category 8 and 9, which, among others, comprise laboratory equipment.

All distributors and importers are responsible for the disposal of old devices, as defined by the WEEE directive. End-users can send their old devices manufactured by KNAUER back to the distributor, the importer, or the company free of charge, but would be charged for the disposal.

Solvents and Other Operating Materials

All solvents and other operating materials must be collected separately and disposed of properly.

All wetted components of a device, e. g. flow cells of detectors or pump heads and pressure sensors for pumps, have to be flushed first with isopropanol and then with water before being maintained, disassembled or disposed.

HPLC Glossary

Here you find definitions for abbreviations and technical terms, which are used in liquid chromatography.

Term	Definition
analytical	analysis and determination in terms of volume for HPLC samples (see: preparative).
chromatogram	Record of a detector signal, depending on output volume of mobile phase and time
detector	device measuring the composition or the quantity of a substance.
eluent	mobile phase transporting substances to be separated or isolated through the column
GLP	Good Laboratory Practice, quality assurance system for laboratories
HPLC	High-Pressure Liquid Chromatography (HPLC)
capillary	thin metal or PEEK pipe that connects components and devices within the chromatography system
solvent	mobile solvent transporting substances to be separated or isolated through the column
peak	deflection of an analyte by the detector in a differential chromatogram
sample	Mixture of different components, which are to be separated using chromatography. They are transported by the mobile phase and dissolved from the column.
sample loop	A loop, which is separated from the system by the valve, that contains sample first. After switching the valve, the eluent flows through the loop and is flushed to the column.
pump	device that pumps the mobile phase at a controlled volume flow into the chromatography system
column	Pipe with final closures, which allow the mobile phase to pass. The pipe contains the packing materials.
valve	mechanism to insert the sample into the eluent flow

Index

A

accessories **11**
additives **8**
air needle **19, 30**
 calculation example **32**
 choose correctly **31**
 standard **30**
 type **32**
analytical **7, 8, 17, 36, 50, 55**
auto-injection system **13**
automatic configuration **15**
AVV marking **70**

B

biocompatability
 features PEEK **38**
Biokompatibilität **8**

C

capillary **71**
care **46, 53**
chromatogram **71**
cleaning **53**
computer connection **15**
contamination **10, 11**
cooler fan **7, 12**
cover
 figure **7**

D

decontamination **10**
device overview **7**
device test **40**
 archiving **44**
 linearity **43**
 mixture test **43**
 reproducibility **41**
 sample carryover **42**
 test interval **40**
disposal **10, 11**
door
 figure **7**
 open **14**
drainage tube **52, 54**
drainage tubing
 connect **37**

E

eluents **8**

F

features **8**
flushing solution **41**
front paneling
 remove **14**
full loop filling **19**

G

GLP **71**

H

HPLC **71**

I

I/O connection **39**
ILD™ **17**
initial startup **13**
injection principles **17**
 ILD™ **17**
 PASA™ **18**
injection valve **7, 20–21, 25, 26–28**
 analytical **36**
 closed-contact output **40**
 connect **36**
 exchange **48**
 figure **15, 36**
 injection pressure **8**
 leak **56**
 preparative **36**
 switching **66**
installation **11**

L

LAN
 port **17**
 problems **54**
 router **17**
 settings **16**
 setup **16**
leak **10**
legal information **69**
location **12**

M

maintenance **46**
 air needle **51**
 contract **46**
 fuses **48**
 syringe **52, 53**
 valve and rotor seal **48**
markings **9**
microliter pickup **19, 24**
mixing and diluting **33**
 mix **33**
 sample positions **34**

mixing and thinning
 Add **33**
modifiers **8**

N

needle flushing **21**

O

operating ranges **7**

P

partial loop filling **21**
PASA™ **18**
peak **71**
port (LAN) **17**
power cable **10**
power strip **10**
power supply **10**
preparative **8, 36, 50, 68**
professional group **9**

R

repair **10, 11**
repeat orders **68**
router (LAN) **17**

S

safety **9**
safety equipment **9**
salts **8**
sample **71**
sample loop **17–19, 20–23, 24–25, 71**
 analytical **67**
 exchange **50**
 loop volume **18**
 preparative **68**
sample needle **19**
 exchange **50**
Sample vial **32**
setup **11**
software **38**
 ClarityChrom® **38**
 errors **54**
 Service Manager **38, 39**
solvent **71**
 flammability **10**
 flushing solution **9**
 line **10**
 self-ignition point **10**
 tray **10**
space requirement **12**
spare parts **11**
storage **48**
support **11, 46**
symbols **9**
syringe
 connect **37**
system flushing **39**

T

technical data **66**
temperature control **7, 8**
 cooler **14, 38, 45, 66**
transport
 damage **11**
 packaging **11**
 protection **13**
transport damage **69**
troubleshooting **54**
 analytical errors **55**
 OpenLAB® system messages **56**
TTL input **40**
Tube **39**
tubing
 connection **7, 36**
 flushing solution **53**
 repeat orders **69**

U

unpacking **11, 12**

W

warning signs **11**
warranty **70**

Translation of the original edition of this manual, version 3.2
Last update: 2016-08-17
Printed in Germany on environmentally friendly paper from sustainable forests.

www.knauer.net/downloads

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