

Smartline

▶ Autosampler 3950 Manual

V1509A



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!

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Overview

Smartline Autosampler 3950 HPLC

The Smartline Autosampler 3950 has been developed to meet the demands of a modern analytical laboratory. It is robust and compact, easy to install and to operate, and designed for high throughput rates. For optimal results, sample cooling is possible. The autosampler is generally installed as a core element in a Smartline HPLC system.

Local area network and automatic configuration

- Remote control** Normally the autosampler is controlled by means of the chromatography software, via 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.

Laboratory use

- Biochemistry analyses
- Chiral analyses
- Food analyses
- Pharmaceutical analyses
- Environmental analyses

Where is it prohibited to use the device or system?



DANGER! Risk of explosion! Never use the device in potentially explosive atmospheres without appropriate protective equipment and approval by a notified body! Inform the technical support department of the manufacturer!

Safety

When the door of the autosampler is open, the syringe speed is reduced automatically. The auto-injection system can cause stab injuries when handled inappropriately!

Use of autosampler



▶ Always operate the autosampler with its front door closed!

DANGER! Risk of stab injuries! Never operate the autosampler when the front paneling is not in place!

Laboratory regulations

Adherence to laboratory regulations

- ▶ Observe national and international regulations pertaining to laboratory work!
 - Good Laboratory Practice (GLP) of the American Food & Drug Administration
 - For development of methods and validation of devices: Protocol for the Adoption of Analytical Methods in the Clinical Chemistry Laboratory, American Journal of Medical Technology, 44, 1, pages 30–37 (1978)
 - Accident prevention regulations published by the accident insurance companies for laboratory work

Solvents

Suitable solvents

Solvents suitable for use in HPLC:

- Acetone
- Acetonitrile
- Benzene
- Chloroform
- Acetic acid (10–50%), at 25 °C (77 °F)
- Ethyl acetate
- Ethanol
- Hexane/heptane
- Isopropanol
- Methanol
- Phosphoric acid
- Toluol
- Water

Note: Even small quantities of other substances, such as additives, modifiers, or salts can influence the durability of the materials. The list of selected solvents was compiled based on research in the pertinent literature and is only a recommendation by the manufacturer. In the event of doubt, contact the technical support of the manufacturer.

Solvent tray	To avoid damage from leaks, always place solvent bottles in a solvent tray on the device.
Toxicity	Organic solvents 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!
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!
Self-ignition point	Only use solvents that have a self-ignition point higher than 150 °C (302 °F) under normal ambient conditions!
Unsuitable solvents	The following solvents can attack the components of the pump and are therefore not suitable: <ul style="list-style-type: none">▪ Mineral and organic acids (except in buffer solutions)▪ Bases (except in buffer solutions)▪ Liquids containing particles
Suitable to only a limited extent	The following solvents are suitable to only a limited extent for use in the pump: <ul style="list-style-type: none">▪ Methylene chloride▪ Tetrahydrofuran (THF)▪ Dimethyl sulfoxide (DMSO)▪ Slightly volatile solvents▪ Fluorinated hydrocarbons
Leaks and clogged capillaries	► Regularly check for leaks and clogged capillaries – test back pressure without column!

Flushing solution

Note: Do not use a salt or buffer solution for flushing!

PEEK connections

- Disposable PEEK fittings
 - One-piece disposable polyetheretherketone fittings (PEEK) for easier installation of flexible, thin capillaries
 - Tightening torque of the PEEK screw:
Tightened by hand (approx. 0.5 Nm)

Protective measures

1. You are only permitted to perform the maintenance tasks described in this manual.
2. All other maintenance tasks are to be performed exclusively by the manufacturer or a company authorized by the manufacturer.

Without exception, the following applies to all maintenance tasks that can be performed by the user:

1. Switch off the device and pull the power plug!
2. Never open the device! High voltage poses a life-threatening risk!

Power supply and mains connection

The device is intended for use with AC power networks of 100–240 V. The supplied power cable is to be used to connect the device to the mains supply.

Note: To disconnect the device from the mains power, disconnect the power plug on the rear of the device.

Fuses

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

Target group

Operating the device or device system

The device can be operated by means of the chromatography software at the workstation, the touch screen or the membrane keyboard at the device.

To what should the user pay particular attention?

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

- | | |
|---|---|
| Avoiding additional dead volumes | <ol style="list-style-type: none">1. Once they have been used, never re-use capillaries in other areas of the HPLC or UHPLC system.2. 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. |
| Using special columns | ▶ Use special columns and follow the manufacturer's instructions on caring for the columns! |
| Checking for clogged capillaries | ▶ Regularly check for clogged capillaries – test back pressure without column! |

Using filtered solvents

1. Use ultra-pure, filtered solvents – *Gradient grade* – for the HPLC or UHPLC.
2. Filtration of substances to be analyzed.
3. Use of inline filters.

Devices are to be opened by the technical support department only

Note: Only allow the technical support department of the manufacturer or a company authorized by the manufacturer to open the devices for maintenance and repair work.



What expertise should users have to safely operate a HPLC device or device system?

- Completed degree as chemical laboratory technician or comparable vocational training
- Fundamental knowledge of liquid chromatography
- Participation in an installation of the system performed by the manufacturer or a company authorized by the manufacturer, or suitable training on the system and chromatography software
- Basic knowledge of Microsoft Windows®

Knowledge regarding substances that are suitable only to a limited extent for use in liquid chromatography

Symbols and labels

Explanations of symbols and labels on the autosampler

Symbol	Explanation
	CE (Conformité Européenne) mark for equipment that complies with the pertinent EU directives and comes with a declaration of conformity from the manufacturer.
	For your own safety, read the manual and always observe the warnings and safety information on the device and in the manual!

Installation

Packaging and transport

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

Checking for signs of damage during transport

Check the device for signs of damage that occurred during transport. If the shipment is incomplete or damaged, inform the manufacturing factory within three workdays. Also inform the freight carrier about transport damage.

Note: Without packaging, the autosampler weighs approx. 19 kg (21 kg with cooling option). Therefore, the autosampler should be transported and set up by two people.

Packaging material and shipping boxes

The device is held in place and protected by foam inserts at the top and bottom. Please keep the transport box and the foam inserts.

Removing the packaging material

► Remove the foam insert on the top of the device.

Remove device from packaging

► Grip the device at its sides near the front and lift it out of the packaging.

Scope of supply

- Autosampler with injection valve and 500 µl syringe

Accessories

- Device manual
- CD: *Smartline 3950 Autosampler Service Manager*
- PTFE tube, 300 cm
- Silicon drainage tube, 200 cm
- 2x sample plates for 48 vials
- 5x microtiter plate, 96 wells
- 2x 2.5 A fuses
- 1x flushing solution bottle
- Cables
 - Power supply cable
 - Network cable
 - I/O interface cable, 9-pin

Biocompatible version

- Inert sample needle with glass or titanium coating
- PEEK injection valve

Preparative version

- Syringe 2500 µl
- Sample loop 10 ml
- Preparative injection valve

Checking the scope of supply

1. Check whether the supplied devices and accessories are complete.
2. If anything is missing, consult with KNAUER Technical Support.

KNAUER Technical Support Hotline

European hotline

Languages: German and English
Available by telephone: 8 am to 5 pm (CET)
Phone: +49-(0)30-809727-0
Fax: +49-(0)30-8015010

E-mail: info@knauer.net

Space requirements

- Side clearance to other devices:
 - If there is a device on one side, min. clearance of 5 cm.
 - If there are devices on both sides, min. clearance of 10 cm.
- If cooling fan is present at rear of device, minimum clearance of 30 cm.

Installation site

Ambient conditions of the installation site

- Air humidity: below 90% (non-condensing)
- Temperature range: 4–40 °C; 39.2–104 °F
- Set up the autosampler so that it is protected against exposure to direct sunlight.

Features

Standard

- Can be flexibly equipped with microtiter plates or standard sample plates
- Maximum sample capacity 2x 384 or 96 standard autosampler vials
- Injection cycle <60 s, incl. cleaning
- Full loop or partial loop injection, as well as μl pick-up mode
- Sample priority function
- Quick-exchange injection valve
- High-resolution syringe controller

Optional

- Sample cooling

Biocompatible version

- Inert sample needle with glass or titanium coating
- PEEK injection valve

Preparative version

- Syringe 2500 μl
- Sample loop 10 ml
- Preparative injection valve

Startup

Note: Before starting up the autosampler for the first time, wait approximately one hour until the temperature of the device has adapted to the ambient temperature.

Front view of the device

The door of the autosampler can be pushed horizontally into the device. The front paneling can be completely removed. To replace the sample plate, remove the cooler cover.



DANGER! Risk of stab injuries! Never operate the autosampler while the door is open!

Opening the door ► Lift the door from the side and push it into the interior.

Legend

A LED status indicator

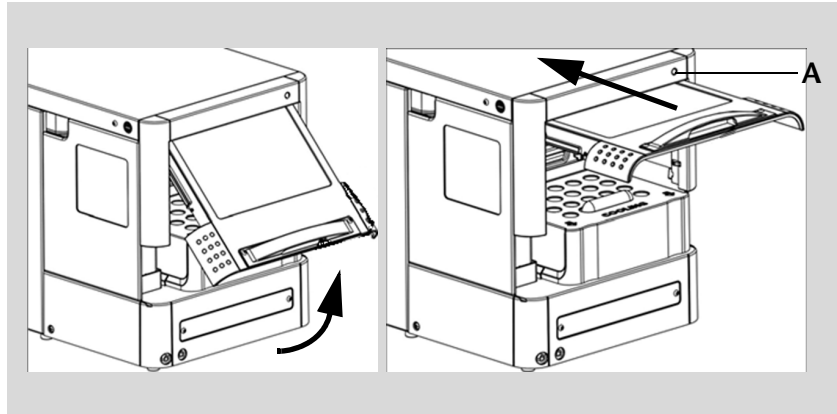


Fig. 1 Push the door into the interior

Removing the front panel ► Press both black buttons on either side (A) of the housing at the same time and remove the front paneling.

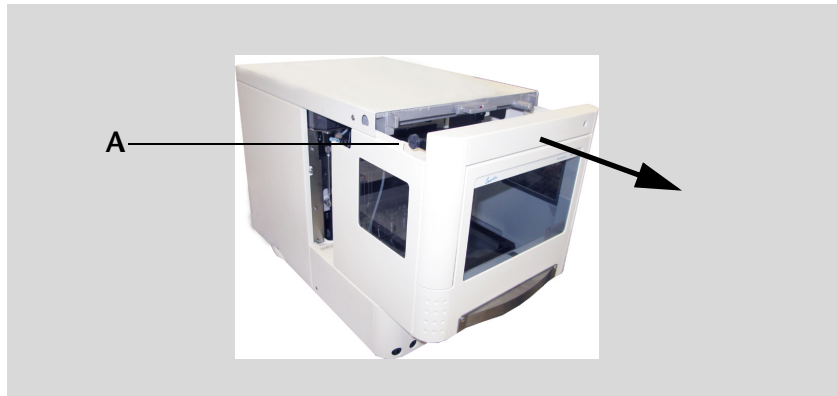


Fig. 2 Removing the front panel

Removing the cooler cover ► Pull out the cooler cover in the direction of the marked arrows.

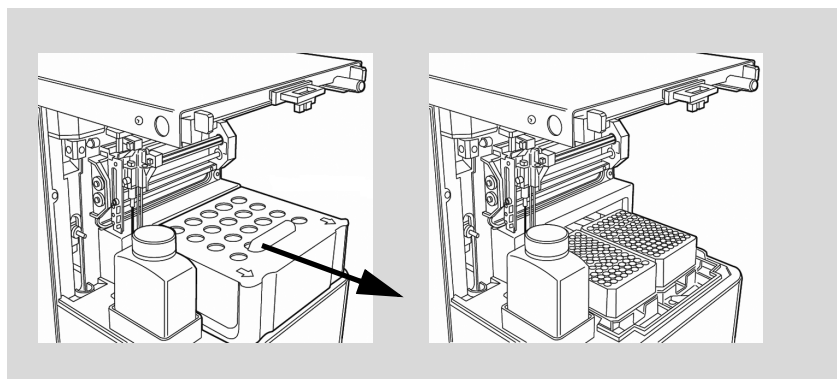


Fig. 3 Removing the cooler cover

Front view of device with sample compartment

Legend

- A Injection valve
- B Syringe
- C Needle guide
- D Collecting container
- E Flushing solution bottle
- F Cooler cover for sample plates
- G Tube connector for waste liquid
- H Tube connector for condensed water



Fig. 4 Front view of device with sample compartment

Rear view of the device

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

Legend

- A LAN connection
- B I/O connection (9-pin)
- C Warning A
- D Power switch
- E Warning B
- F CE mark
- G Fuse box
- H Mains connection
- I Cooling fan
- J Serial number and year of manufacture of device

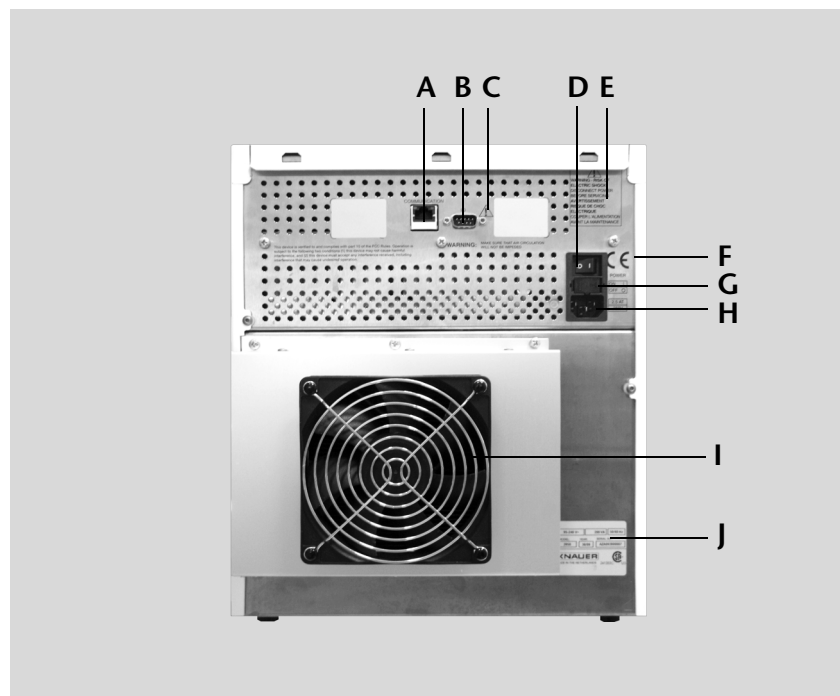


Fig. 5 Rear view of the autosampler

PASA™ loop injection principle

The autosampler uses loop injection with pressure assistance (*Pressure Assisted Sample Aspiration* PASA™) as a selectable option. PASA™ injection has the following features:

- 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

- A Buffer tube
- B Capillary to pump
- C Sample loop
- D Capillary to column
- E Syringe
- F Connector for compressed air
- G Air needle
- H Sample needle
- I Sample vial

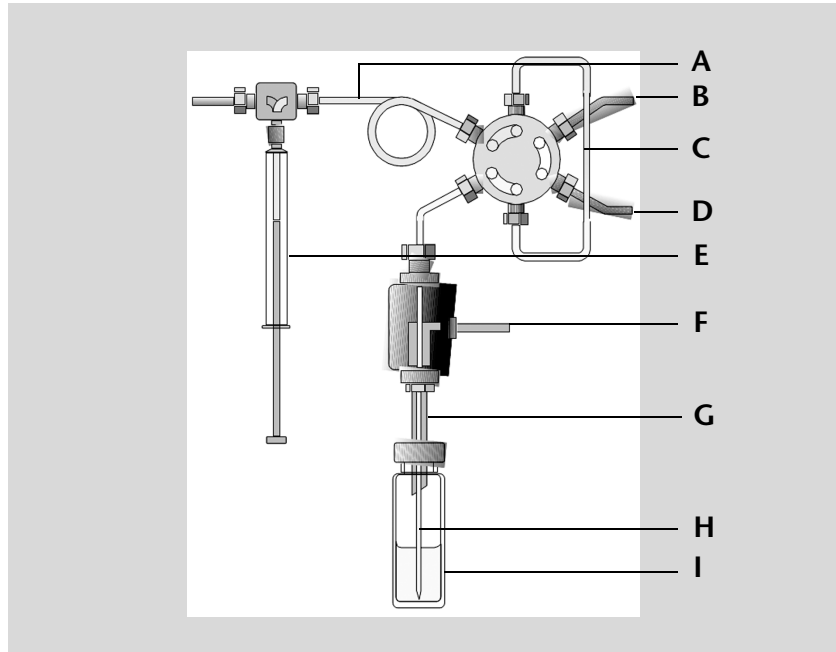


Fig. 6 PASA™ loop injection principle

Injection modes

Four different injection modes are available:

- Full loop injection
- Partial loop injection
- Microliter pick-up injection
- Microliter pick-up 84+3 injection

Full loop injection

In full loop injection 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 $\pm 10\%$. 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 x-time overfilling of the sample injection and the specific flush volume of the employed needle.

Partial loop injection In partial loop injection mode, the sample loop is only partially filled with the sample. 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 specific flush volume of the employed needle.

Microliter pick-up injection In microliter pick-up injection mode, the sample loop is filled with a very small amount of sample that is transported by washing solution. This ensures the highest precision of the sample volume without loss of sample. The maximum sample volume is 50% of the loop volume, minus 1.5-times the needle volume.

Microliter pick-up injection 84 + 3 If the 84 + 3 tray is selected for the microliter pick-up injection, the sample is transported by a separate transport liquid instead of the washing solution. The consumption of transport liquid depends on the needle volume and corresponds to 2.5-times the needle volume for the segments before and after the sample.

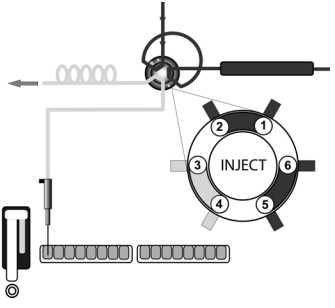
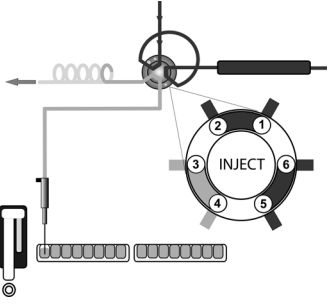
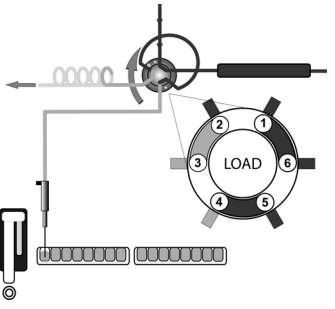
The autosampler uses a system of two telescopic needles, one that pierces through the cap of the sample vial – the air needle, – and one that extracts the sample – the sample needle.

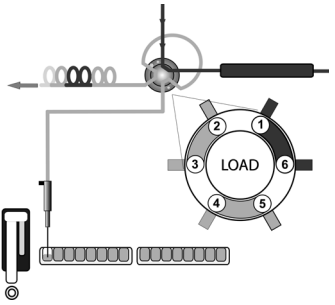
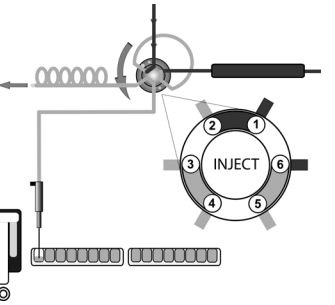
Using a syringe, the sample is aspirated through the two needles out of the sample vial while under pressure and into the sample loop. To prevent the syringe from becoming contaminated, a buffer tube is situated between the syringe and the valve. Using washing solution, sample residue is removed from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

Full loop injections

The sample loop is completely filled with sample. This type of injection results in extremely good reproducibility.

Full loop injection

Functional schematic	Explanation
 <p>Fig. 7 Full loop: Initial situation</p>	<p>1. The initial situation: the injection valve is in INJECT position. The sample needle with air needle has entered the well or vial. Headspace pressure, applied through the air needle, ensures that no air or vapor bubbles are formed during sample aspiration.</p>
 <p>Fig. 8 Full loop: The needle and sample lines are flushed</p>	<p>2. The syringe dispenser aspirates the flush volume from the sample well/vial to fill the sample line with sample and remove wash solvent.</p>
 <p>Fig. 9 Full loop: Injection valve switches to LOAD position</p>	<p>3. The injection valve switches to LOAD position, placing a distinct sample plug at the inlet of the sample loop.</p>

Functional schematic	Explanation
 <p data-bbox="582 571 1013 638">Fig. 10 Full loop: Sample loop is filled completely</p>	<p data-bbox="1050 257 1390 526">4. The sample loop is quantitatively filled by transporting a number of times the loop volume through the loop, depending on the volume of the loop.</p> <p data-bbox="1082 533 1358 600">3 x loop volume for loop 100 μL</p> <p data-bbox="1082 607 1374 674">2 x loop volume for loops 100 μL - 500 μL</p> <p data-bbox="1082 680 1369 748">1.5 x loop volume for loop 500 μL</p>
 <p data-bbox="582 1075 1013 1142">Fig. 11 Full loop: Injection valve switches to INJECT position</p>	<p data-bbox="1050 761 1390 1064">5. The injection valve switches to the INJECT position. The sample loop is now part of the HPLC mobile phase flow path: sample is transported to the column. The analysis starts.</p> <p data-bbox="1050 1075 1390 1176">Note: A wash routine is performed after each injection.</p>

Air segment with Full loop injections

An air segment of 5 μL can be used to reduce the amount of flush volume. This air segment is at the front of the flush volume and will not be injected.

With a standard needle, the flush volumes must be a minimum of 30 μL for injections with air segment, and 35 μL for injections without air segment. If samples are highly viscous it may be necessary to program larger flush volumes and reduce the syringe speed for better performance.

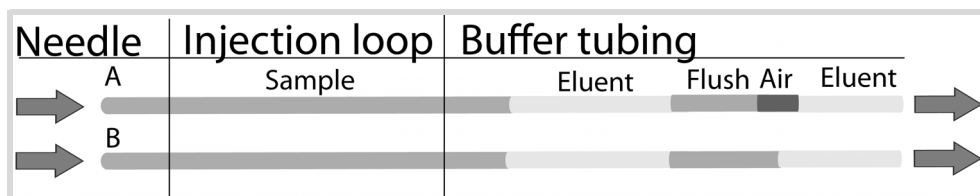
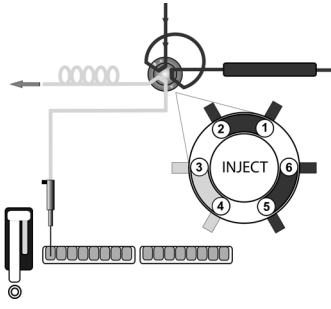
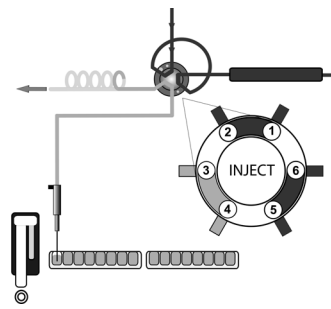
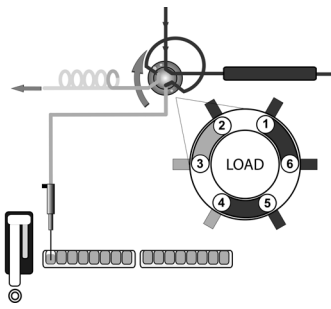


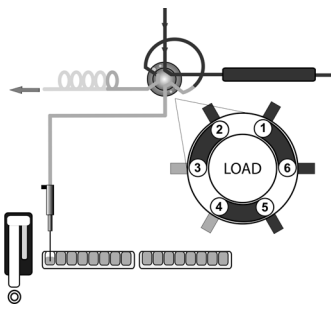
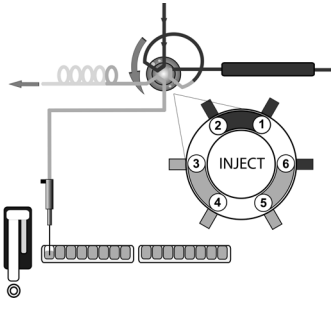
Fig. 12 Full loop injection with air segment (A), without air segment (B)

Partial loopfill injections

This type of injection results in maximum accuracy. The switching sequence for a partial loopfill injection is:

Patial loop injection

Functional schematic	Explanation
 <p data-bbox="582 795 938 869">Fig. 13 Partial loop: Initial situation</p>	<p data-bbox="1045 465 1385 913">1. The initial situation: the injection valve is in the INJECT position. The sample needle with air needle has entered the vial/well. Headspace pressure, applied through the outer air needle, ensures that no air or vapor bubbles are formed during sample aspiration.</p>
 <p data-bbox="582 1265 1008 1339">Fig. 14 Partial loop: The sample line is filled with sample</p>	<p data-bbox="1045 931 1374 1171">2. The syringe dispenser aspirates the flush volume from the sample vial/well to fill the sample line with sample and remove wash solvent.</p>
 <p data-bbox="582 1691 991 1794">Fig. 15 Partial loop: Injection valve switches to LOAD position</p>	<p data-bbox="1045 1361 1334 1570">3. The injection valve switches to LOAD, placing a distinct sample plug at the inlet of the sample loop.</p>

Functional schematic	Explanation
 <p data-bbox="584 591 963 663">Fig. 16 Partial loop: Sample loop is filled partially</p>	<p data-bbox="1050 253 1385 394">4. The programmed injection volume is now aspirated into the sample loop.</p>
 <p data-bbox="584 1016 1007 1120">Fig. 17 Partial loop: Injection valve switches to <i>INJECT</i> position</p>	<p data-bbox="1050 685 1374 987">5. The injection valve switches to <i>INJECT</i>. The sample loop is now part of the HPLC mobile phase flow path: sample is transported to the column. The analysis starts.</p>

If an injection from the same vial and no wash routine is programmed, the next injection sequence will start with a flush of 50% of the programmed flush volume. Otherwise, it will start with a flush of the programmed flush volume. If the withdrawal of sample for the next injection exceeds the total volume of the sample buffer tubing, the buffer tubing is rinsed before the next injection. The next injection will start with the programmed flush.

Air segment with Partial loopfill injections

An air segment can be used to reduce the amount of flush volume. The air segment is at the front of the flush volume and will not be injected.

With a standard needle, the flush volumes must be a minimum of 30 μL for injections with air segment and 35 μL for injections without air segment. If the samples are highly viscous, it may be necessary to program larger flush volumes and reduce the syringe speed for better performance.

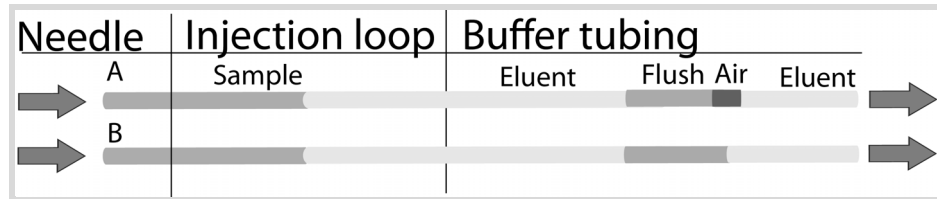


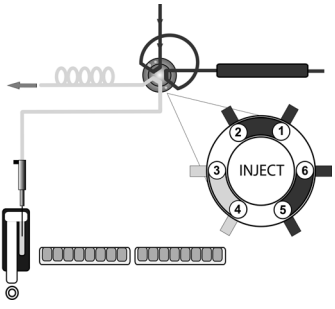
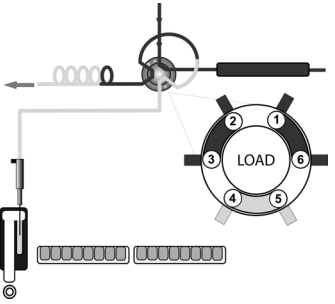
Fig. 18 Partial loop injection with air segment (A), without air segment (B)

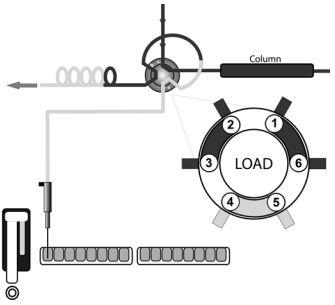
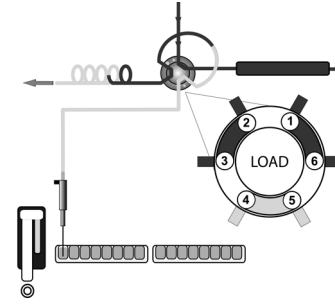
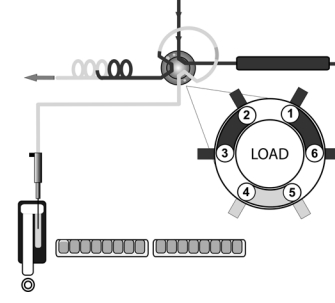
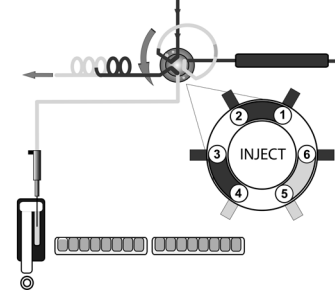
Microliter pick-up injection

In this injection mode sample is transported into the sample loop by washing liquid. This type of injection results in maximum accuracy without sample loss. If you select the 84+3 tray with μL pickup, the three 10 mL vials are automatically used for transport. The Needle Wash/Transport position in that case will only be used for the needle wash. That means that a different routine will be followed with first transport. Refer to the section on 84+3 tray (see " μL Pickup method parameters for the 84+3 tray" on page 31) for more information.

The switching sequence for μL pickup injections is:

Microliter pick-up injection

Functional schematic	Explanation
 <p data-bbox="582 1075 933 1176">Fig. 19 Microliter pick-up: Washing liquid is aspirated</p>	<ol style="list-style-type: none"> <li data-bbox="1045 757 1388 1030">1. In the initial situation, the injection valve is in INJECT position. The sample needle has entered the transport position. The transport container is filled with wash solvent. <li data-bbox="1045 1041 1388 1377">2. Washing liquid is aspirated into needle and buffer tubing to place a distinct sample plug at the inlet of the sample loop. Please note that the washing liquid needs to be compatible with eluent.
 <p data-bbox="582 1724 997 1825">Fig. 20 Microliter pick-up: Injection valve switches to LOAD position</p>	<ol style="list-style-type: none"> <li data-bbox="1045 1411 1388 1612">3. The injection valve switches to LOAD position. A transport plug of washing solution is aspirated into the sample loop.

Functional schematic	Explanation
 <p data-bbox="582 571 1013 672">Fig. 21 Microliter pick-up: The needle moves to sample vial/well</p>	<p data-bbox="1045 257 1380 392">4. The needle moves from the transport position to the sample vial/well.</p>
 <p data-bbox="582 1012 1013 1113">Fig. 22 Microliter pick-up: Injection volume of sample is aspirated</p>	<p data-bbox="1045 698 1380 833">5. The programmed injection volume is aspirated from the sample vial/well.</p>
 <p data-bbox="582 1453 1013 1554">Fig. 23 Microliter pick-up: Sample is transported into sample loop</p>	<p data-bbox="1045 1140 1380 1408">6. The sample needle moves back to the washing position. A second transport plug of washing solution is aspirated. The sample is transported into the sample loop.</p>
 <p data-bbox="582 1895 1013 1995">Fig. 24 Microliter pick-up: Sample is transported to column</p>	<p data-bbox="1045 1581 1380 1872">7. The injection valve switches to INJECT. The sample loop is now part of the HPLC mobile phase flow path: sample is transported to the column. The analysis timer starts.</p>

Air segment with μL Pickup injections

If an air segment has been programmed, it appears at the front of the first plug of transport liquid and at the front of every sample plug.

In this injection mode:

- The air segment at the front of the sample plug is injected into the HPLC system
- No headspace pressure can be applied on vials/wells in this mode to avoid sample errors due to air expansion during exchange from the sample vial/well to the transport position.

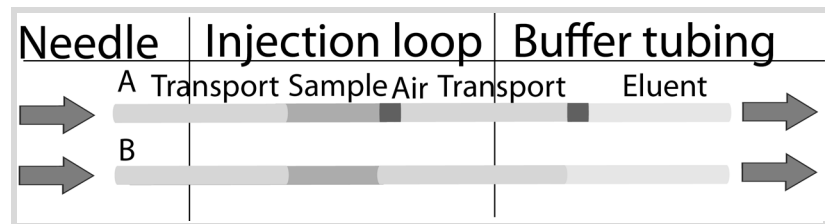


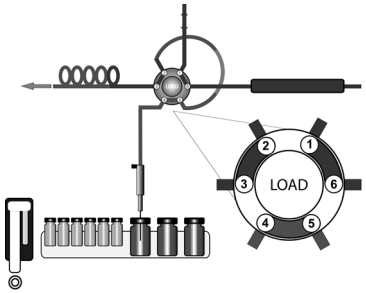
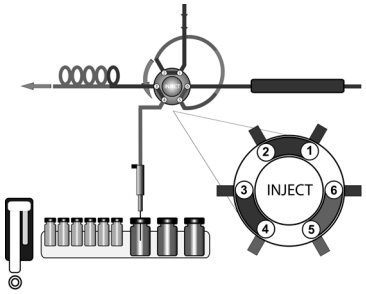
Fig. 25 Microliter pick-up injection with air segment (A), without air segment (B)

µL Pickup injections with 84+3

If you select the 84+3 tray with µL pickup, the three 10 mL vials are automatically used for transport. The Needle Wash position in that case will only be used for the needle wash. This results in the following injection routine:

Microliter pick-up injection with 84+3

Functional schematic	Explanation
<p>Fig. 26 Microliter pick-up 84+3: Initial situation</p>	<p>1. At the start of the routine, the needle is still at the Needle Wash position. The valve is in INJECT position.</p>
<p>Fig. 27 Microliter pick-up 84+3: Sample line is filled with transport liquid</p>	<p>2. The syringe dispenser aspirates a transport plug from the transport vial position to fill the sample line with transport liquid and remove wash solvents.</p>
<p>Fig. 28 Microliter pick-up 84+3: The sample is aspirated</p>	<p>3. The injection valve switches to LOAD position. The programmed injection volume is aspirated from the sample vial/well.</p>

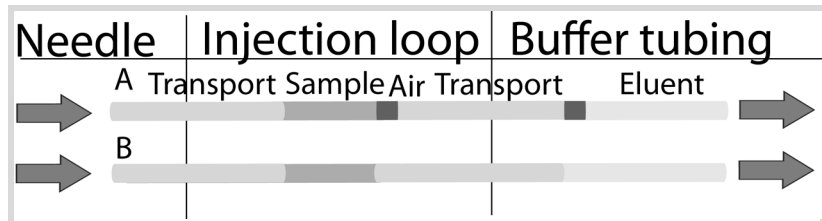
Functional schematic	Explanation
 <p data-bbox="582 571 997 660">Fig. 29 Microliter pick-up 84+3: Sample is transported into sample loop</p>	<p data-bbox="1045 257 1380 504">4. The sample needle moves back to the transport vial position. A second transport plug is aspirated. The sample is transported into the sample loop.</p>
 <p data-bbox="582 1003 997 1093">Fig. 30 Microliter pick-up 84+3: Injection valve switches to INJECT position.</p>	<p data-bbox="1045 689 1380 958">5. The valve switches to INJECT. The sample loop is now part of the HPLC mobile phase flow path: sample is transported to the column. The analysis timer starts.</p>

Air segment with μL Pickup injections 84+3

If an air segment has been programmed, it appears at the front of the first plug of transport liquid and at the front of every sample plug.

In this injection mode:

- The air segment at the front of the sample plug is injected into the HPLC system
- No headspace pressure can be applied on vials in this mode to avoid sample errors due to air expansion during exchange from the sample vial to the transport position.



84+3 tray

A 84+3 tray is available for the Alias autosampler. The tray is placed over both tray positions in the autosampler.

Note: Always place the tray with position 87 in the farmost right corner in the sampling compartment.

The tray may contain a maximum of 84 vials of 1.5 mL + 3 vials of 10 mL. The tray has been designed in such a manner that the caps of the 1.5 mL and of the 10 mL vials are at the same height to make sure that the same injection needle can be used for both vial types:

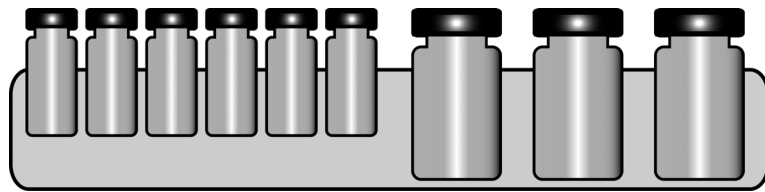


Fig. 31 Height 84+3 tray

That means that the sample needle height can only be programmed for the 1.5 mL vials. For the 10 mL vials two fixed needle height levels are available, depending on the transport liquid levels.

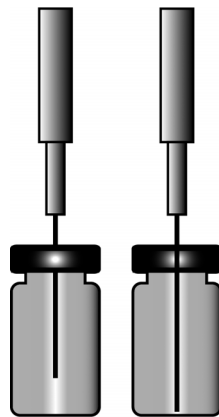


Fig. 32 Needle height and fluid levels

Please note that the processing order for this tray is fixed in numerical order if you program a series of samples:

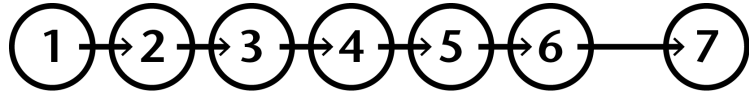


Fig. 33 Processing order

However, if you create sequence tables using one-line-one sample (1 sample in each run), the sample processing order can of course be programmed randomly.

µL Pickup method parameters for the 84+3 tray

To indicate that you wish to use the 84+3 tray, change the tray settings in the control software.

Note: If you select the 84+3 tray with µL pickup, the three 10 mL vials are automatically used for transport. The Needle Wash position in that case will only be used for the needle wash.

Sample positions are defined as follows for the 84+3 tray:

First sample position to be processed:	Vial positions 1 - 84
Last sample position to be processed:	Vial positions 1 - 84
First destination position to be processed:	Vial positions 1 - 84
Transport positions:	Vial positions 85 - 87

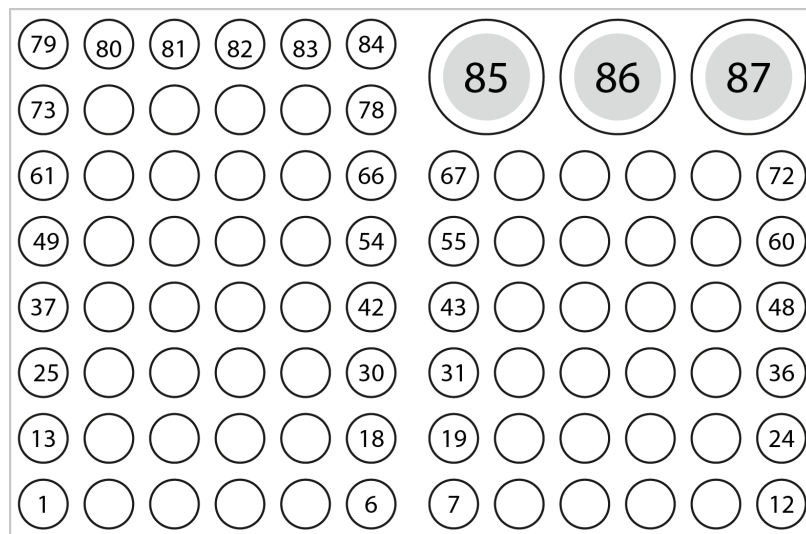


Fig. 34 Transport/Reagent positions on 84+3 tray

The range of samples is indicated by the parameters First Sample to Last Sample. Transport positions are fixed (vials 85, 86, 87).

Programming details for the 84 + 3 injection mode

- The transport vial position can be programmed. Valid positions are 85, 86 and 87. Position 85 is the default transport vial position. The system calculates the transport volume used. Make sure the vial contains at least 8000 µL whenever starting the system.
- Whenever a run is started, the transport fluid levels are not reset. In the transport vial, the needle stops at a high level in the vial, to prevent the air needle from being polluted.
- When a series of samples or one-line-one-sample is processed, the autosampler keeps track of the remaining transport volume during the run and moves deeper in the transport vial when transport volume level is lower than 4000 µL. When the amount of transport liquid reaches 0 µL, the autosampler reports error 369 (not enough transport liquid).
- The transport volume levels are reset by reprogramming the mode.
- The needle will not automatically move to a different transport vial. You will have to program a different transport position if you want to draw transport liquid from a different transport vial position for further runs.

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 broad range of deep and shallow sample plates.

When 10 ml sample vials are used, the needle penetrates deeply into 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 using deep microtiter plates.

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

Legend

- A 10 ml sample vial
- B 1.5 ml sample vial

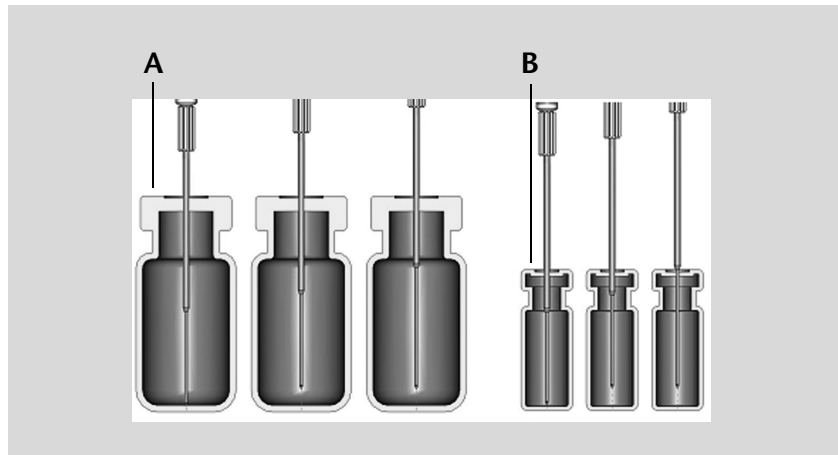


Fig. 35 Standard air needle with 10 and 1.5 ml sample vial

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 through the closure.

Legend

- A Deep Greiner microtiter plate with closure
- B Shallow Greiner microtiter plate

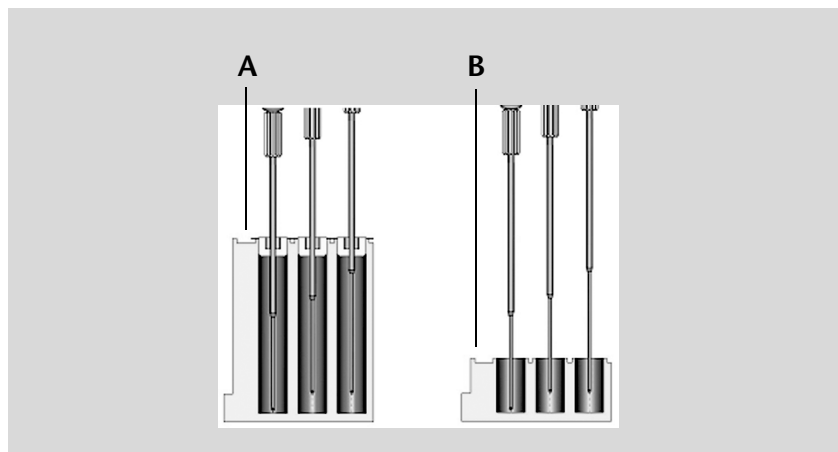


Fig. 36 Standard air needle with Greiner microtiter plates

Choosing the correct air needle

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

H_t = height of sample plate

D_w = hole depth

C_d = thickness of closure

N_h = set needle height

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

? = excess length

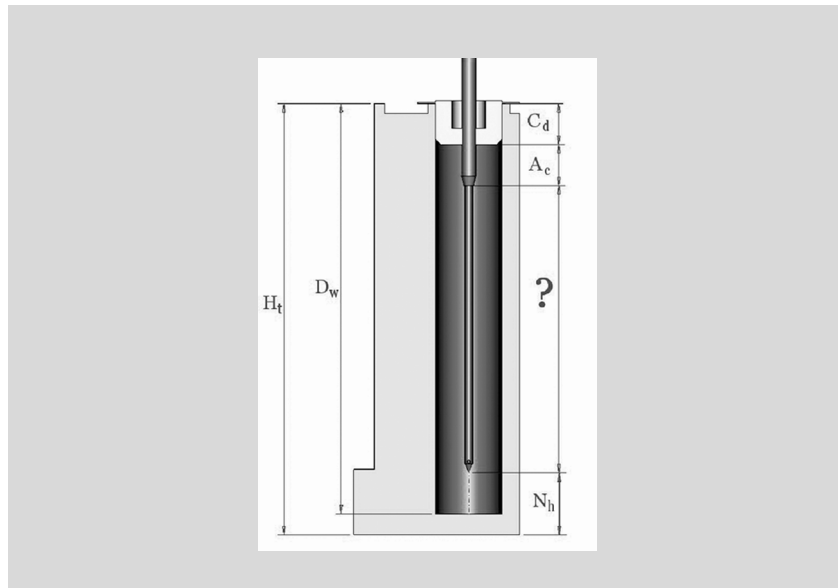


Fig. 37 Calculating the correct air needle

Condition:

$$H_t - D_w = 2 \text{ to } 6 \text{ mm.}$$

Protrusion length of the sample needle:

$$H_t - C_d - N_h - A_c = ?$$

- Choose the correct needle type on the basis of the protrusion length:

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

Legend

- A** 10 ml sample vials,
50 mm air needle
- B** 1.5 ml sample vials,
62 mm air needle

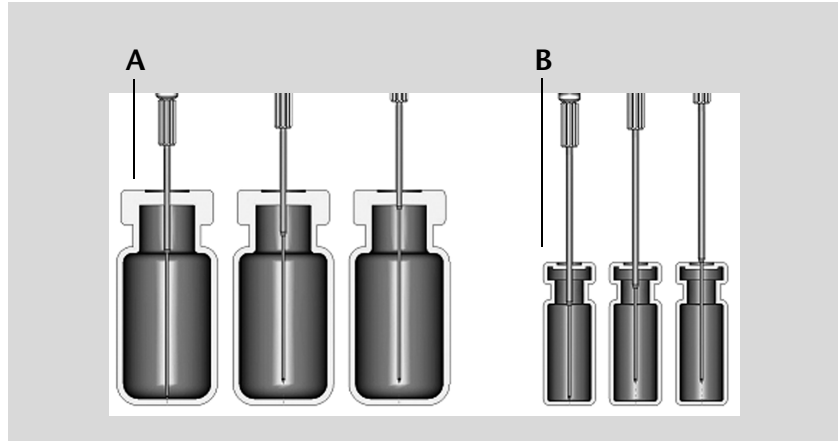


Fig. 38 Selected air needles with sample vials

Legend

- A** Deep Greiner microtiter plate with closure,
56 mm air needle
- B** Shallow Greiner microtiter plate,
80 mm air needle

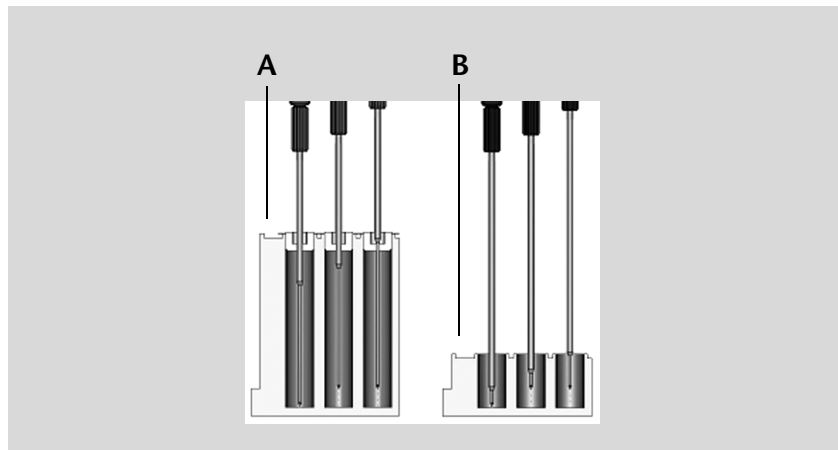


Fig. 39 Selected air needles with Greiner microtiter plates

Calculation example for air needle

Starting point:

- Autosampler with standard setting for needle height.
- Depth of Greiner microtiter plate with closure.

Dimensions:

$$H_t = 41.4 \text{ mm}$$

$$D_w = 37.8 \text{ mm}$$

$$C_d = 3.8 \text{ mm}$$

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

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

Condition:

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

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

$$H_t - C_d - N_h - A_c = \text{Protrusion length of the sample needle}$$

$$41.4 \text{ mm} - 3.8 \text{ mm} - 6.0 \text{ mm} - 2.0 \text{ mm} = 29.6 \text{ mm}$$

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 injection needle cannot pierce.

Mixing and diluting

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

- Configure the mix method 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 With the Mix command, the contents of a specific sample vial are 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.

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 *ADD 200 µl from Reagent A to Destination* command triggers 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. 200 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 250 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. 250 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 mix method

When configuring a mix method, the positions of the sample vials depend on whether the sample 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*:

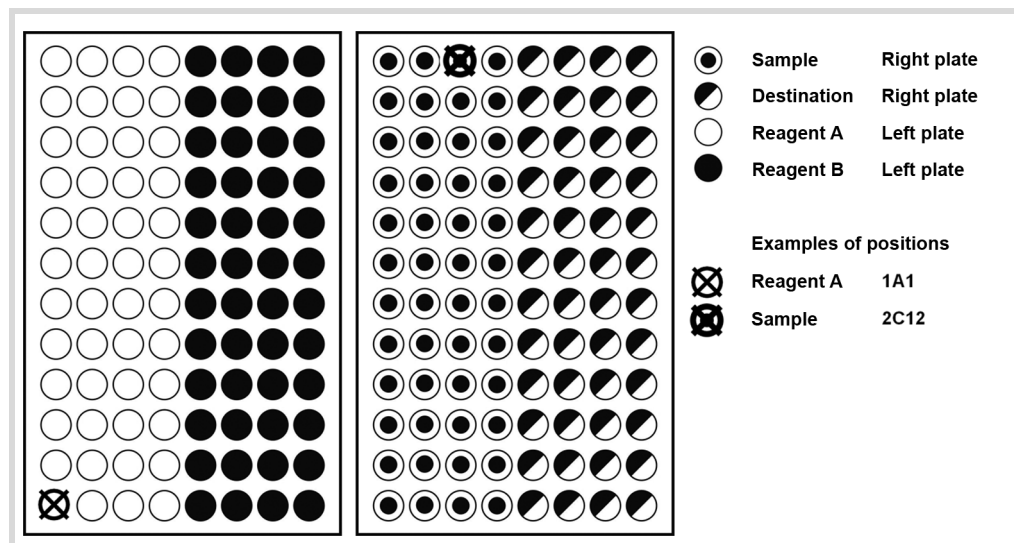


Fig. 40 Sample positions in columns

Processing in rows

When row processing is used, the following positions are possible for the *sample*, *destination*, *reagent A* and *reagent B*:

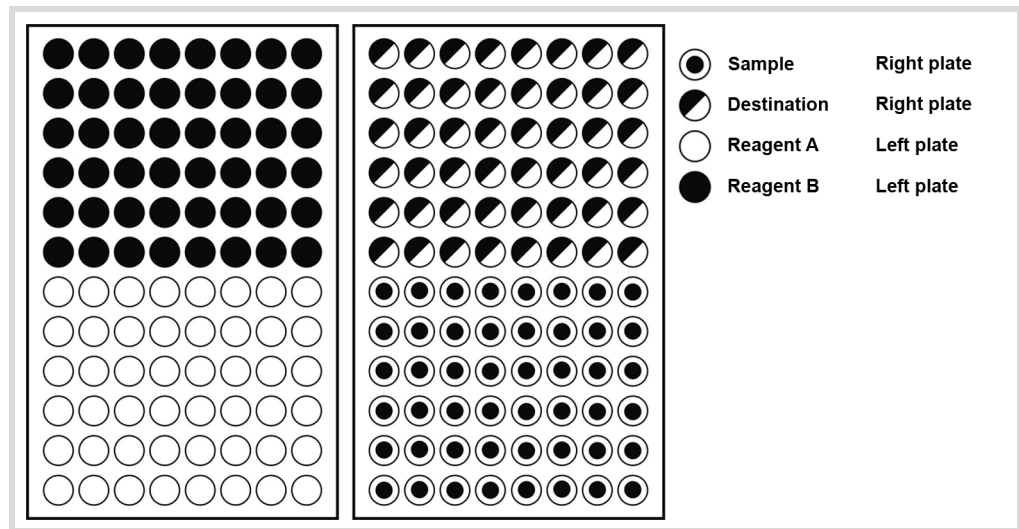


Fig. 41 Sample positions in rows

Mix method parameters for the 84+3 tray

To indicate that you wish to use the 84+3 tray, change the tray settings in the control software.

Sample positions are defined as follows for the 84+3 tray:

First sample position to be processed:	Vial positions 1 - 84
Last sample position to be processed:	Vial positions 1 - 84
First destination position to be processed:	Vial positions 1 - 84
Reagent positions:	Vial positions 85 - 87

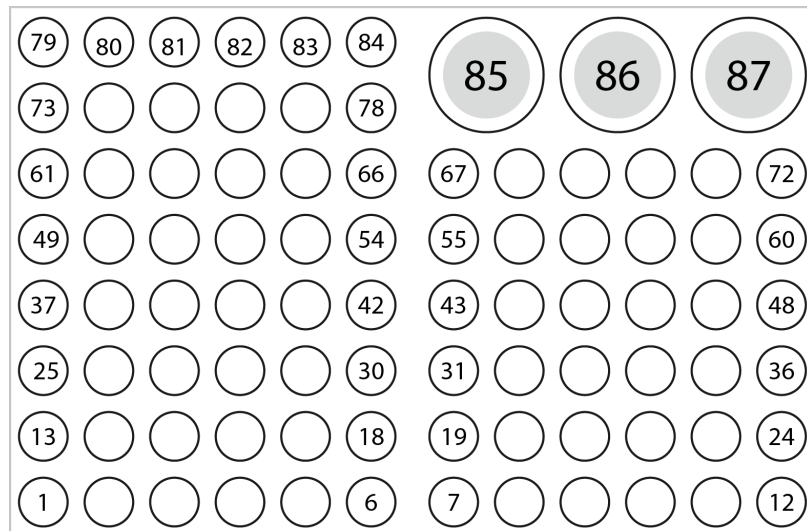


Fig. 42 Transport/Reagent positions on 84+3 tray

The range of samples is indicated by the parameters "First Sample to Last Sample". The same range can be used for "Destination Vials". Reagent positions are fixed (vials 85, 86, 87).

Programming details for the 84 + 3 mix method

- The transport vial position can be programmed. Valid positions are 85, 86 and 87. Position 85 is the default transport vial position. The system calculates the transport volume used. Make sure the vial contains at least 8000 μL whenever starting the system.
- Whenever a run is started, the reagent fluid levels are not reset. In the reagent vial, the needle stops at a high level in the vial, to prevent the air needle from being polluted.
- When a series of samples or one-line-one-sample is processed, the autosampler keeps track of the remaining reagent volume during the run and moves deeper in the

reagent vial when reagent volume level is lower than 4000 μL . When the amount of reagent liquid reached 0 μL , the autosampler reports error 370 (not enough reagent).

- The reagent volume levels are reset by reprogramming the mode.
- Reagent is used from the programmed reagent position only. The needle will not automatically move to a different reagent vial. If you want to use reagent from one of the other two vials, you will have to program that setting.

Capillary and tube connections

Note: Flush the system before connecting the column.

The schematic diagram inside the autosampler shows the correct connections.

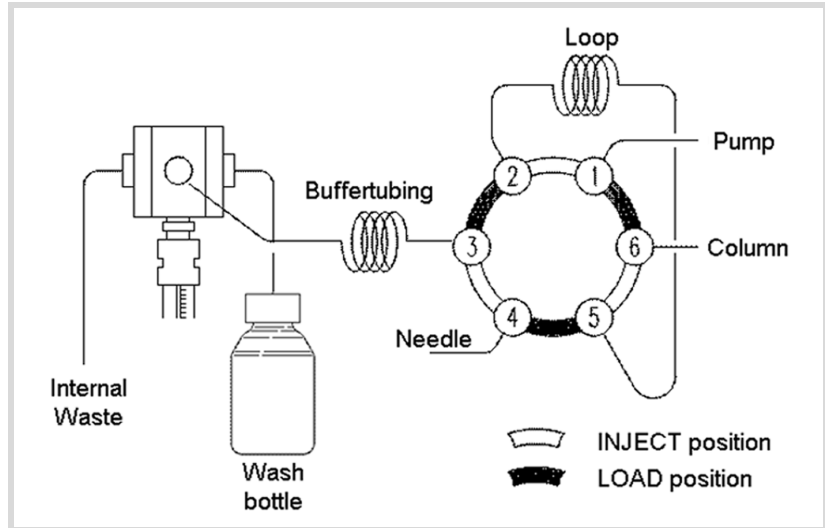


Fig. 43 Connection schematic for capillaries and tubing

Connections on the injection valve

Explanation	Component
A Stainless steel capillary to pump	
B Sample loop	
C Plastic capillary to syringe	
D Plastic capillary to injection needle	
E Sample loop	
F Stainless steel capillary to column	

Fig. 44 Connections on the injection valve

Syringe connections

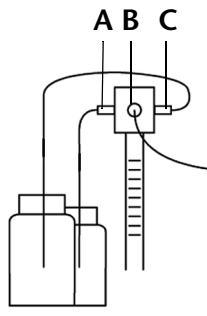
Explanation	Component
A For waste tube B For buffer tube C For washing solution tube	

Fig. 45 Syringe connections

Tube guide for flushing solution

Note: Use the tube guide in the collecting container for the flushing solution to prevent the needle unit from moving horizontally.

Legend

- A** Tube guide for flushing solution
- B** Hole in collecting container (top view)

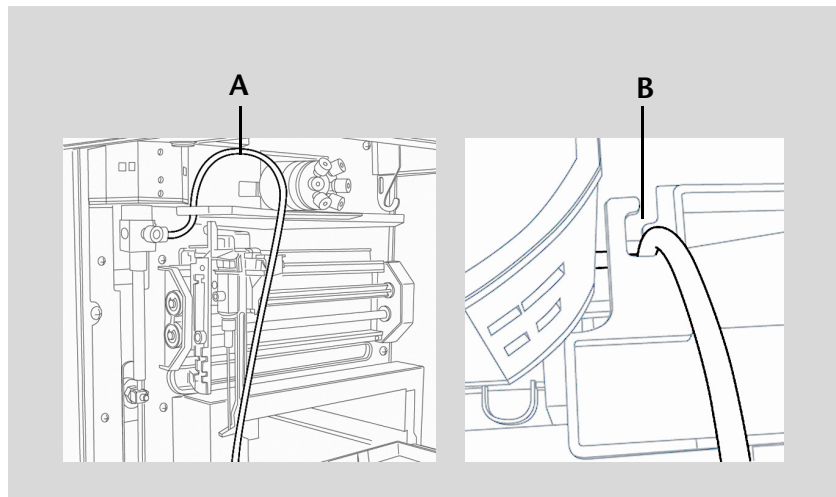


Fig. 46 Tube guide for flushing solution tube

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 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 flow away.

Legend

- A** Tube for waste liquid
- B** Tube for condensed water
- C** Adapter for drainage tube

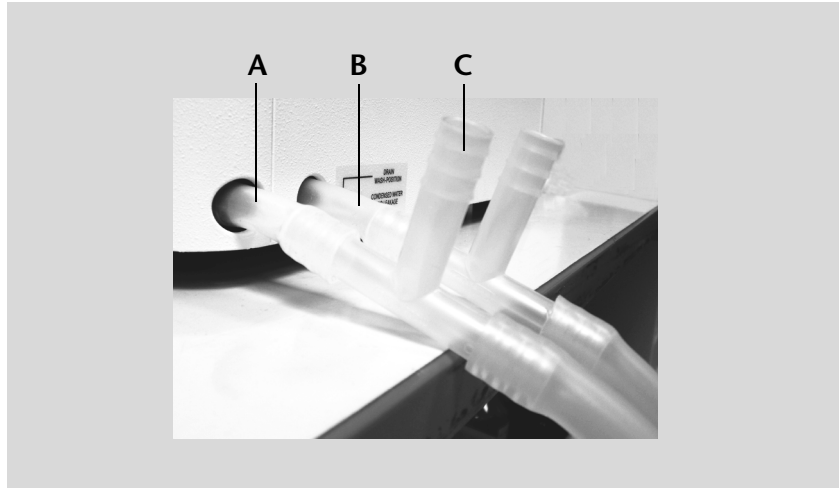


Fig. 47 Connecting the drainage tubing

Connecting the autosampler to other devices

Controlling the autosampler with chromatography software

The autosampler is controlled directly using chromatography software, e.g. ChromGate® and ClarityChrom® from KNAUER.

- Using the LAN connection at the rear of the device, connect the autosampler 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 autosampler in LAN.
2. Set the syringe volume to either 250 ml (default) or 500 ml.
3. Enter the serial number of the autosampler.
4. Select the cooling option if the autosampler is equipped with sample tray cooling.

Configuration window of ClarityChrom®

Legend

- A Serial number
- B Device detection in local network
- C Manual search for device in network
- D Volume of sample loop
- E Volume of syringe

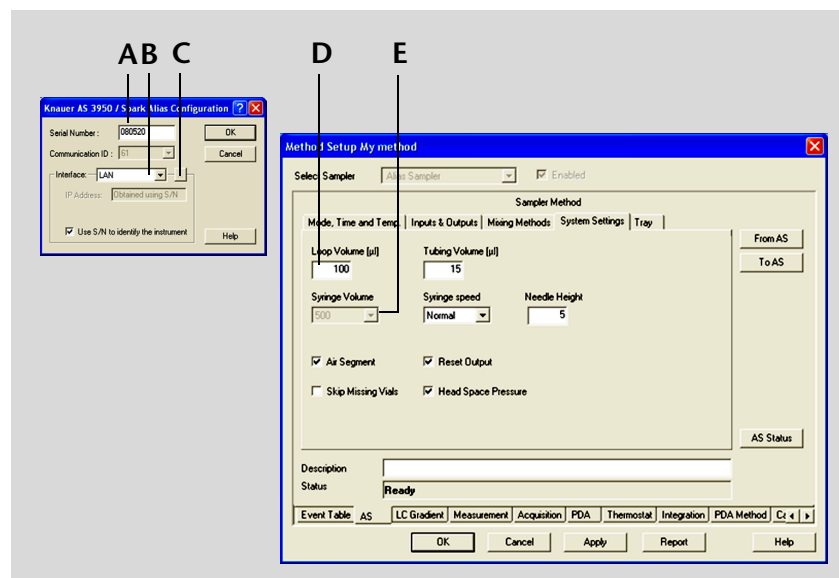


Fig. 48 Autosampler configuration with ClarityChrom®

Autosampler device software

A software CD with *Smartline 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*.

System flushing

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

1. Install *Smartline Autosampler 3950 Service Manager*.
2. Fill the flushing solution into a solvent bottle and degas it using helium or an ultrasonic bath.
3. Insert the flushing solution tube into the solvent bottle.
4. Select the *Alias* ⇒ *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.

Practical tip! Perform additional flushing to remove all of the air from the syringe.

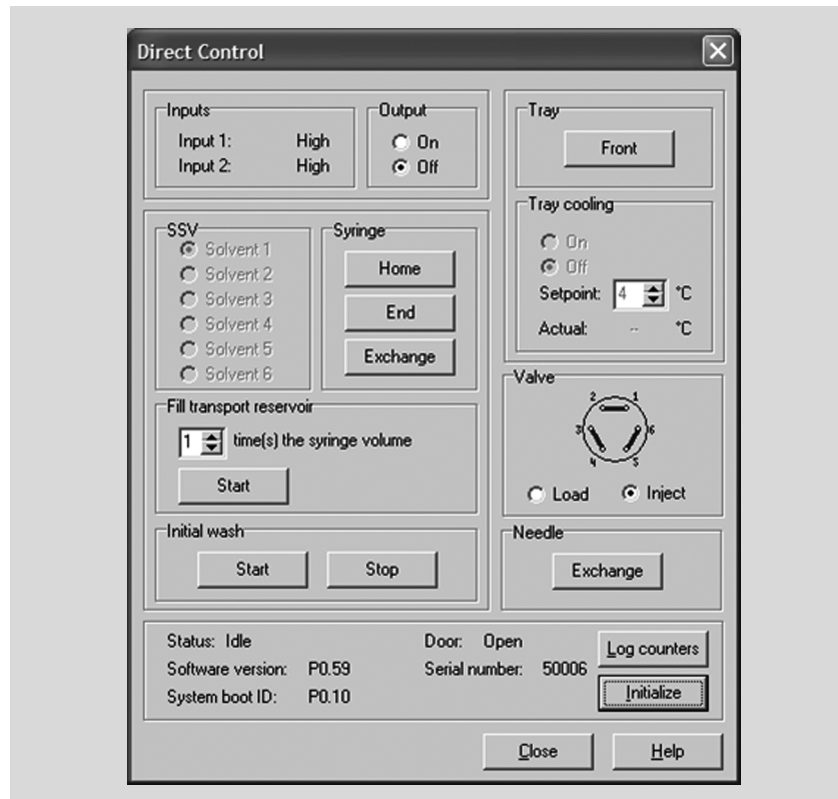


Fig. 49 System flushing with the Service Manager software

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-pin)

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 test

- Smartline Autosampler 3950, standard version with 100 µl sample loop and 500 µl syringe
- HPLC pump, 1 ml/min flow rate
- UV detector (with a data rate of 50 Hz if possible, else 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 HPLC 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: 100 μl
- Tubing volume: 15 μl
- Syringe volume: 500 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, otherwise 10 Hz
- Time: 0.5 min

Configuring repeat runs of autosampler

- Injections/vial: 9x (7x for microtiter plates)
- Inj. volume: 10 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 average

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

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

Creating a sequence with 6 lines

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

- Test solution: Position 1A1
- Eluent: Position 1A2
- Injection volume: 10 μ l
- Repeats: 1

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

$$PV [\%] = \frac{\sum_i \frac{Peakarea_i \text{ Solvent}}{3}}{\sum_i \frac{Peakarea_i \text{ Resolution test mixture}}{3}} \times 100$$

3. Linearity

To determine the linearity, the correlation coefficient of the regression lines is determined from the measured values for the *Peak* areas and the 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

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

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

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

\bar{y} = Arithmetic average of Y across all n measured values

\bar{x} = Arithmetic average of X across all n measured values

n = Number of measuring value pairs

4. Mixture test

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

- The variation coefficient VK_2 must not exceed 0.5%.
- The variation coefficient VK_3 for dilution must not exceed $\leq 0.5\%$.
- 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: 10 μl
- Repeats: 3

Positioning vials for dilution

Note: Observe the plate assignments for the vials (*sample, reagent A, reagent B, destination*).

- 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 sample plate.

- Put an empty vial (*destination*) at position 2A5 of the sample plate.
- Put a vial with eluent (*reagent A*) at position 1A1 of the sample plate.

Analyzing the individual chromatograms

1. Calculate the average 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 averages 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			
Smartline 3950	Standard <input type="checkbox"/>	Sample cooling <input type="checkbox"/>	Biocompatible version <input type="checkbox"/>	Preparative version <input type="checkbox"/>
Serial number				

No.	Test	Setting	Specification	Result
1	Reproducibility	<ul style="list-style-type: none"> ▪ 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:

Signature:

Operation Qualification (OQ) with ChromGate®

- Extensive functionality test** Extensive test of autosampler functionality using ChromGate® chromatography software.
- Start OQ**
1. Select *Control* ⇒ *Qualification procedures*. In the window that opens, select a performance qualification under *Available Procedures*.
 2. In the *Available procedures* window, choose an OQ test for the autosampler and choose *Begin New Qualification Session*.
 3. Select [Start Session]. The Introduction window opens for the function test.
 4. Select [Continue] to display the required materials in the chromatography software.
 5. Select [Continue] to display the settings for the OQ test in the chromatography software.
- Choose automatic test**
6. Select Perform all tests automatically.
 7. Select Active channel of the detector.
 8. Select Pump and click [Continue] to apply the settings.
- Start OQ**
9. Click [Continue] to perform the function test. The chromatography software sets the flow rate to 1 ml/min and the wavelength of the detector to 274 nm. You can print out the test results.
- Printing out the results of the function test**
10. Select [Print report] to print the function test of the autosampler.
- Quit OQ**
11. Select [Exit] to end the function test of the device.

Operation Qualification Report

Legend

- A** Detailed information on autosampler
- B** Detailed function test
- C** 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 μ l.	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 $R \geq 0,97$	Test successful $R = 1,000$

Date of inspection: 6 January 2010

Next inspection on: _____

Inspector: _____

Signature: _____

Fig. 50 Operation Qualification Report

Maintenance and care

KNAUER Technical Support

Contact data for KNAUER Technical Support

If you have any technical questions regarding KNAUER hardware or software, please use one of the contact options below:

KNAUER Technical Support Hotline

European hotline

Languages: German and English

Available by telephone: 8 am to 5 pm (CET)

Phone: +49-(0)30-809727-0

Fax: +49-(0)30-8015010

E-mail: info@knauer.net

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 device or removing housing parts

What maintenance tasks can users perform on the device?

Users may perform the following maintenance tasks themselves:

- Exchanging the injection valve
- Exchanging the rotor seals
- Exchanging the syringe on the autosampler
- Exchanging the sample needle
- Exchanging the air needle



DANGER! When covers are removed, potentially dangerous live components are exposed!
Switch off the device prior to maintenance tasks and pull the power plug to completely isolate it from the supply voltage!
The only time when the Smartline Autosampler 3950 need not be disconnected from the power supply is if it is being checked exclusively by means of the control software at the PC!

Leaks in the capillary screw fittings

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

Exchanging the fuses

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 (2x 2.5 A).
4. Plug in the power plug.

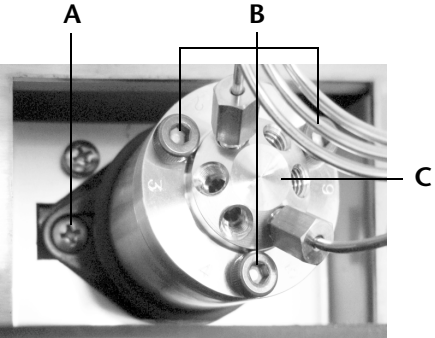
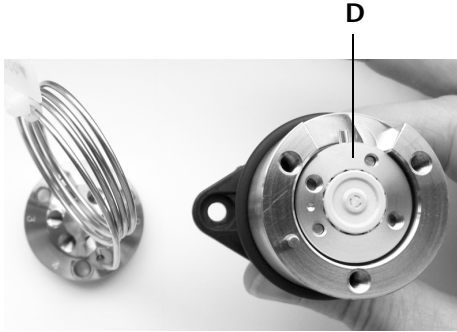
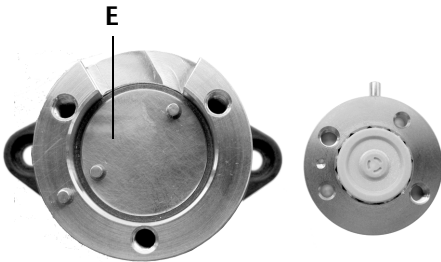
Exchanging the injection valve and rotor seal

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

Removing the injection valve and rotor seal

Removing the injection valve and rotor seal

Steps	Figure
<ol style="list-style-type: none">1. With a screwdriver, remove the screw (A) on both sides of the injection valve support.2. Remove the injection valve.3. With an Allen wrench, remove the screws (B) from the stator (C).4. Carefully remove the stator.5. Remove the rotor seal (D) from the rotor (E).6. Clean or exchange the rotor seal.	 <p data-bbox="916 757 1326 819">Fig. 51 Removing the injection valve</p>  <p data-bbox="916 1189 1337 1223">Fig. 52 Removing the rotor seal</p>  <p data-bbox="916 1536 1366 1570">Fig. 53 Valve stator and rotor seal</p>

Installing the rotor seal and injection valve

- During installation, hold the injection valve in such a way that the bore hole for connecting the steel capillary to the pump (port 1) is facing upward.
- Alternately tighten all screws by half a turn, until all screws have been fully tightened.

Legend

A Labeling, port 1

B Connection from capillary to pump

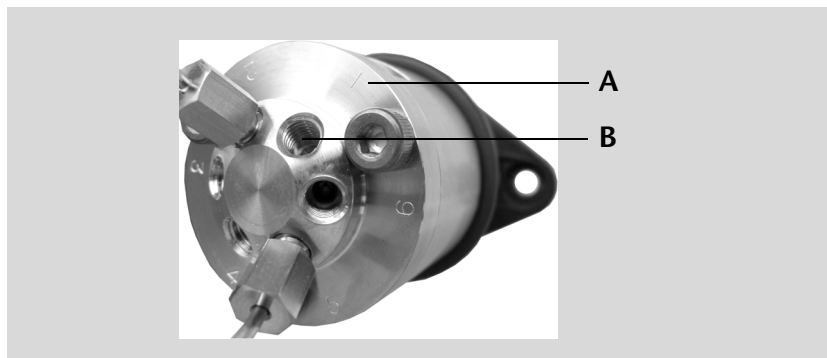


Fig. 54 Installing the injection valve

- Procedure**
1. Insert the rotor seal.
 2. Place the valve stator block on the valve rotor and use an Allen wrench 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.

System flushing

- Procedure**
1. Connect the autosampler to the power supply.
 2. Establish a connection to the PC.
 3. Start the *Smartline 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.

Exchanging the sample loop

By standard, the autosampler is equipped with a 100 µl 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.

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.
- Only tighten the screw fitting until it is finger-tight, to prevent the plastic capillary from becoming blocked.

Legend

- A Screw fitting
- B Plastic capillary
- C Union nut
- D Sample needle

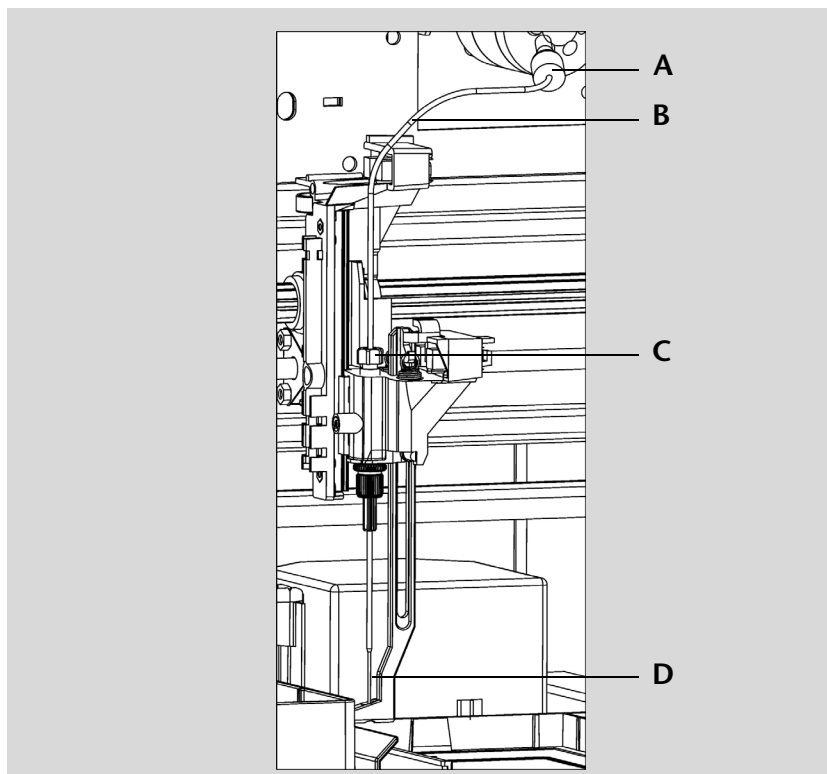


Fig. 55 Exchanging the sample needle

- Procedure**
1. Start the *Smartline Autosampler 3950 Service Manager*.
 2. Select the Alias \Rightarrow 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

- A Union nut
- B Retaining nut
- C Height adjustment screw
- D Air needle
- E Sample needle

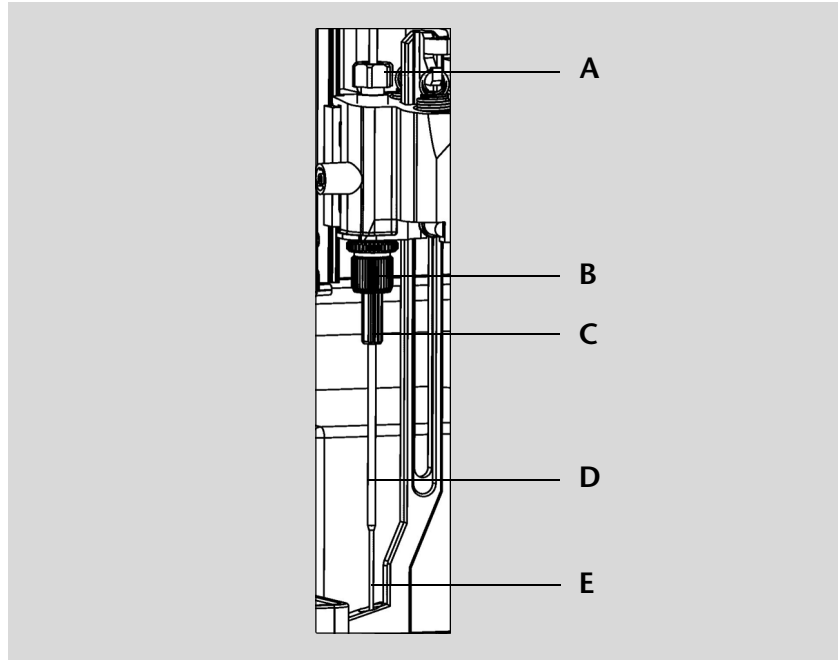


Fig. 56 Exchanging the air needle

- Procedure**
1. Start the *Smartline 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 500 µl syringe.

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

Legend

- A Syringe valve
- B Syringe
- C Syringe drive
- D Syringe plunger

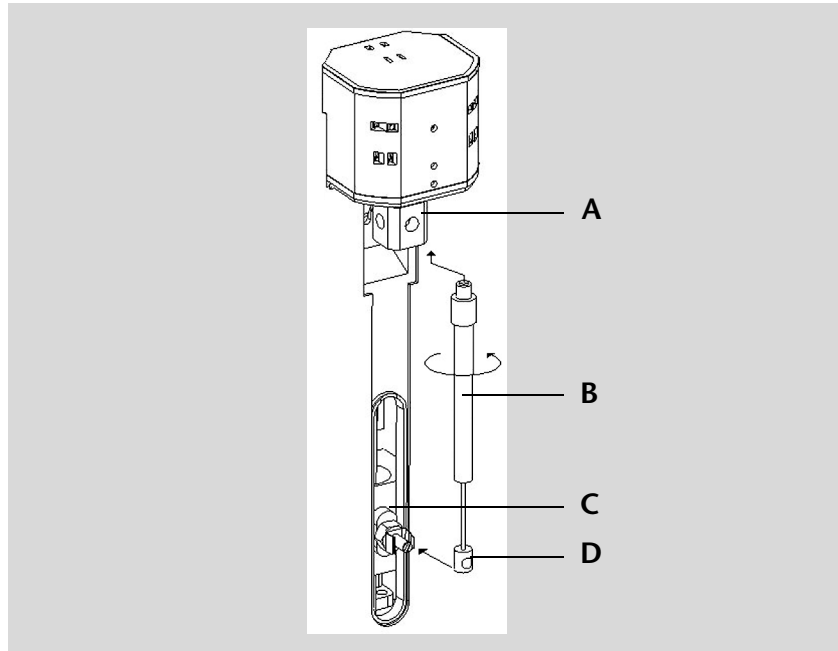


Fig. 57 Exchanging the syringe

- Procedure**
1. Start the *Smartline 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

- Procedure**
1. Start the *Smartline 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 Allen screws are only accessible if the valve is in this position.

Legend

- A** Upper Allen screw
- B** Lower Allen screw
- C** Connector for flushing solution tube (hidden)
- D** Connector for buffer tube
- E** Connector for syringe
- F** Unused connection

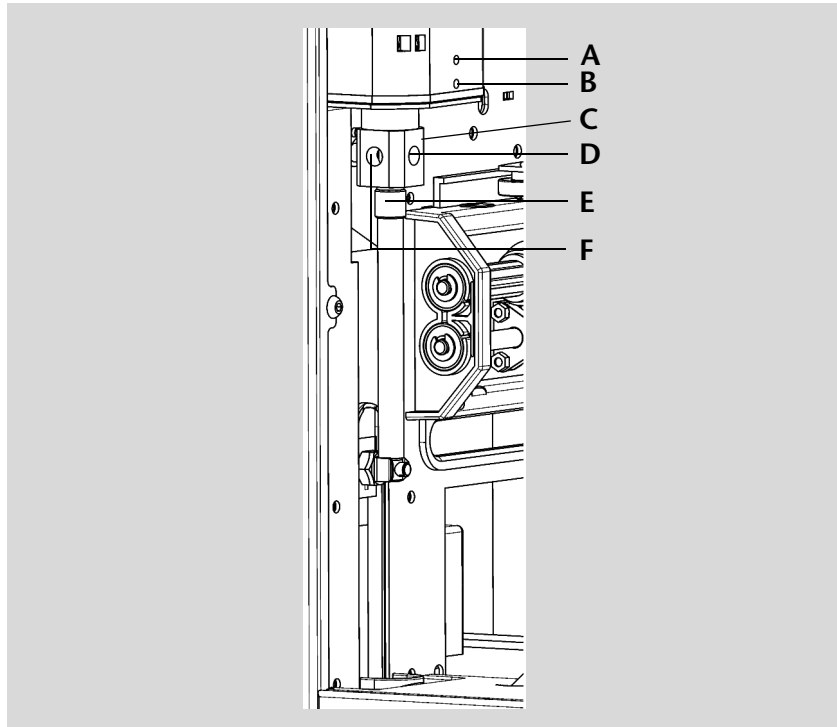


Fig. 58 Exchanging the syringe valve

- Procedure**
1. Start *Smartline 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 with two turns the lower Allen screw.
 5. Loosen with two turns the upper Allen screw.
 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 Allen screws.

Cleaning and caring for the device



DANGER! Risk of electrical shock or short circuit if cleaning solution enters the device's interior! Only lightly moisten the cleaning cloth!

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

- Use a soft cloth to clean the collecting container and to remove any spilled sample solution from the sample plate
- To remove deposits, clean the drainage tube regularly using solvent

Environmental protection

Disposal

Drop the devices off at the local municipal waste facilities or send the devices back to the manufacturer where it will be disposed of properly.

Decontamination

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



DANGER! Danger caused by toxic, infectious, or radio-active substances! A contaminated device must never be submitted for repairs, sold, or disposed of!

Contract a specialist company to decontaminate the device or perform the decontamination yourself if you have the required expertise!

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.

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.

Software fault

Software errors can occur due to faulty communication between the devices or incorrect installation of the software.

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

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 Smartline Autosampler 3950.
Leaking syringe	<ul style="list-style-type: none"> ▪ 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 tip.
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 <i>hardware</i>	<ul style="list-style-type: none"> ▪ Check <i>hardware</i>: <ul style="list-style-type: none"> ▪ Flush needle (inside and outside) or install a different needle type (steel, PEEK or with glass coating). ▪ Injection 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	<ol style="list-style-type: none"> 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 injection valve	<ol style="list-style-type: none"> 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. C Connect the HPLC pump to the injection valve. D Close the connection to the column at the injection valve. E Start the pump at a low flow rate. F Check the connections to the syringe and to the needle at the injection valve to ensure that they are tight. G If liquid escapes there, check the rotor seal. H If no liquid escapes there, check the HPLC with a manual valve.

System messages from ChromGate®

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

System message	Explanation
<i>Autosampler is in run mode.</i>	<ul style="list-style-type: none"> ▪ Quit the controller software and restart. ▪ Switch the devices off and on again.
<i>Autosampler is not responding. Please check communication settings and ensure the device is online.</i>	<ul style="list-style-type: none"> ▪ Switch the devices off and on again. ▪ Check the network settings. If the system message appears again, notify KNAUER Technical Support.
<i>Cannot run autosampler.</i>	<ul style="list-style-type: none"> ▪ Switch the devices off and on again. ▪ Check the network settings. If the system message appears again, notify KNAUER Technical Support.
<i>Cannot set destination vial to %d.</i>	Check parameters in controller software and correct entry.
<i>Cannot set first transport vial to %d.</i>	Check parameters in controller software and correct entry.
<i>Cannot set last transport vial to %d.</i>	Check parameters in controller software and correct entry.
<i>Cannot stop autosampler.</i>	Check the network settings. If the system message appears again, notify KNAUER Technical Support.
<i>Communication port for autosampler was not initialized. Please check the configuration settings.</i>	Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>Configuration settings do not match with the device. Run cannot start.</i>	Check configuration and settings.
<i>Destination position not reached.</i>	Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.

System message	Explanation
<i>Deviation of more than +/-2 mm towards home.</i>	<ul style="list-style-type: none"> ▪ Look for visible obstructions in area of sample plate. ▪ Check the belt tension of the tray.
<i>Dispenser error.</i>	Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>EEPROM error in adjustments.</i>	Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>EEPROM error in log counter.</i>	Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>EEPROM error in settings.</i>	Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>EEPROM write error.</i>	Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>Electronics error.</i>	Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>Error 369</i>	Not enough transport liquid. Fill up transport liquid.
<i>Error 370</i>	Not enough reagent. Fill up reagent.
<i>Error by setting Mix&Dilute vials.</i>	Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>Error occurred during initialization, the autosampler cannot start.</i>	Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>Error resetting output.</i>	Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.

System message	Explanation
<i>Error running user defines program.</i>	Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>Error setting injection mode.</i>	Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>Error setting injection mode.</i>	Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>Error setting needle height.</i>	Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>Error setting syringe speed.</i>	Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>Error setting the analysis time.</i>	Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>Error setting the auxiliaries.</i>	Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>Error setting the flush time.</i>	Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>Error setting the flush volume.</i>	Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>Error setting the injection volume.</i>	Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>Error setting the loop volume.</i>	Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.

System message	Explanation
<i>Error setting the prep. mode.</i>	Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>Error setting the syringe volume.</i>	Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>Error setting the tray configuration.</i>	Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>Error setting the tray temperature.</i>	Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>Error setting the vial number.</i>	Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>Error setting timed events.</i>	Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>Error setting tubing volume.</i>	Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>Error setting wash volume.</i>	Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>Flush volume error.</i>	Check parameters in controller software and correct entry.
<i>Home sensor activated when not expected.</i>	<ul style="list-style-type: none"> ▪ Check parameters in controller software and correct entry. ▪ Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.

System message	Explanation
<i>Home sensor not de-activated.</i>	<ul style="list-style-type: none"> ▪ Check whether there are visible obstructions impairing the sample plate tray. ▪ Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>Home sensor not reached.</i>	<ul style="list-style-type: none"> ▪ Check whether there are visible obstructions impairing the sample plate tray. ▪ Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>Horizontal: Home sensor activated when not expected.</i>	<ul style="list-style-type: none"> ▪ Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>Horizontal: Home sensor not de-activated.</i>	<ul style="list-style-type: none"> ▪ Check whether there are visible obstructions impairing the needle unit. ▪ Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>Horizontal: Home sensor not reached.</i>	<ul style="list-style-type: none"> ▪ Check whether there are visible obstructions impairing the needle unit. ▪ Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>Horizontal: Needle position is unknown.</i>	Initialize the needle unit using the controller software.
<i>Illegal sensor readout.</i>	Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>Incorrect first destination vial.</i>	Check parameters in controller software and correct entry.

System message	Explanation
<i>Injection needle unit error.</i>	<ul style="list-style-type: none"> ▪ Check whether there are visible obstructions impairing the needle unit. ▪ Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>Injection valve or ISS unit error.</i>	Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>Injection volume %.2f is invalid. For specified injection method, volume should be within the range %.2f µl-%.2f µl, with %.2f. µl increments.</i>	Check parameters in controller software and correct entry.
<i>Injection volume error.</i>	Check parameters in controller software and correct entry.
<i>Invalid %s vial position %02d. The vial position must be between 01 and %02d.\n.</i>	Check parameters in controller software and correct entry.
<i>Invalid combination of the trays. The combination of different trays for the Mix&Dilute mode is not allowed.</i>	<ul style="list-style-type: none"> ▪ Insert the correct sample plate. ▪ Check parameters in controller software and correct entry.
<i>Invalid combination of the trays. The combination of plates 384 low and 96 high is not allowed.</i>	<ul style="list-style-type: none"> ▪ Insert the correct sample plate. ▪ Check parameters in controller software and correct entry.
<i>Invalid configuration. ISS option not installed on autosampler. Please switch off this option in configuration dialog.</i>	Check parameters in controller software and correct entry.
<i>Invalid configuration. SSV option not installed on autosampler. Please switch off this option in configuration dialog.</i>	Check parameters in controller software and correct entry.

System message	Explanation
<i>Invalid flush volume %2f µl. The flush volume should be between 0 and %2f µl.</i>	Check parameters in controller software and correct entry.
<i>Invalid flush volume %d µl. The flush volume should be between 0 and %d µl.</i>	Check parameters in controller software and correct entry.
<i>Invalid input. Only values with increments of %.2f allowed.</i>	Check parameters in controller software and correct entry.
<i>Invalid instrument is detected.</i>	Check parameters in controller software and correct entry.
<i>Invalid integer number.</i>	Check parameters in controller software and correct entry.
<i>Invalid loop volume %2f µl. The loop volume should be between 0 and %2f µl.</i>	Check parameters in controller software and correct entry.
<i>Invalid loop volume %d µl. The loop volume should be between 0 and %d µl.</i>	Check parameters in controller software and correct entry.
<i>Invalid mix program: No Destination vial is specified in configuration dialog.</i>	Check parameters in controller software and correct entry.
<i>Invalid mix program: No reagent A vial is specified in configuration dialog.</i>	Check parameters in controller software and correct entry.
<i>Invalid mix program: No reagent B vial is specified in configuration dialog.</i>	Check parameters in controller software and correct entry.
<i>Invalid mix times. The time should be between 1 and 9.</i>	Check parameters in controller software and correct entry.
<i>Invalid needle height %d mm. The needle height should be between %d and %d mm.</i>	Check parameters in controller software and correct entry.
<i>Invalid time-based method. Several AUX events have the same time.</i>	Check parameters in controller software and correct entry.
<i>Invalid time-based method. Several SSV events have the same time.</i>	Check parameters in controller software and correct entry.
<i>Invalid tray configuration: two or more vial positions are the same.</i>	Check parameters in controller software and correct entry.

System message	Explanation
<i>Invalid tray temperature %d °C. The temperature should be between 4 and 22 °C.</i>	Check parameters in controller software and correct entry.
<i>Invalid tubing volume %2f µl. The tubing volume should be between %2f and %2f µl.</i>	Check parameters in controller software and correct entry.
<i>Invalid tubing volume %d µl. The tubing volume should be between 0 and %d µl.</i>	Check parameters in controller software and correct entry.
<i>Invalid volume %d µl. The volume should be between the 0 and the syringe volume (%d µl).</i>	Check parameters in controller software and correct entry.
<i>Invalid wait time. The time should be between 0 and 9 h 50 min 59 sec. Invalid wash volume %d µl. The wash volume should be between %d and %d µl</i>	Check parameters in controller software and correct entry.
<i>ISS valve error.</i>	Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>ISS-A option not installed on autosampler. Please switch off ISS-A option in configuration dialog.</i>	Check controller software configuration and correct entry.
<i>ISS-B option not installed on autosampler. Please switch off ISS-B option in configuration dialog.</i>	Check controller software configuration and correct entry.
<i>Missing destination vial.</i>	<ul style="list-style-type: none"> ▪ Check position of sample vial. ▪ Check parameters in controller software and correct entry.
<i>Missing reagent vial.</i>	<ul style="list-style-type: none"> ▪ Check position of sample vial. ▪ Check parameters in controller software and correct entry.

System message	Explanation
<i>Missing transport vial.</i>	<ul style="list-style-type: none"> ▪ Check position of sample vial. ▪ Check parameters in controller software and correct entry.
<i>Missing vial.</i>	<ul style="list-style-type: none"> ▪ Check position of needle unit. ▪ Switch the devices off and on again.
<i>Missing wash vial error.</i>	<ul style="list-style-type: none"> ▪ Check position of needle unit. ▪ Switch the devices off and on again.
<i>Needle movement error.</i>	<ul style="list-style-type: none"> ▪ Check position of needle unit. ▪ Switch the devices off and on again.
<i>No destination vial is specified in the configuration.</i>	Check parameters in controller software and correct entry.
<i>No reagent A vial is specified in the configuration.</i>	Check parameters in controller software and correct entry.
<i>No reagent B vial is specified in the configuration.</i>	Check parameters in controller software and correct entry.
<i>No transport vials are defined in the tray configuration. It is not possible to use the μl pick-up injection mode.</i>	Check parameters in controller software and correct entry.
<i>No user defined or mix program is running.</i>	Check parameters in controller software and correct entry.
<i>Not enough reagent liquid.</i>	Check volume of liquid and change as required.
<i>Not enough transport liquid available due to missing transport vials.</i>	Check volume of liquid and change as required.
<i>Oven option not installed on autosampler. Please switch off oven option in configuration dialog.</i>	Check controller software configuration and correct entry.
<i>Please specify inject marker or AUX event to be able to trigger the run.</i>	Check parameters in controller software and correct entry.

System message	Explanation
<i>Selecting transport position failed.</i>	Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>Serial number is not valid. Please check the configuration.</i>	Check parameters in controller software and correct entry.
<i>Setting mix program error.</i>	Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>Setting service mode failed.</i>	Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>Syringe dispenser unit error.</i>	Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>Syringe home sensor not de-activated.</i>	<ul style="list-style-type: none"> ▪ Needle flushing with controller software. ▪ Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>Syringe home sensor not reached.</i>	<ul style="list-style-type: none"> ▪ Needle flushing with controller software. ▪ Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>Syringe position is unknown.</i>	Initialize the syringe unit using the controller software.
<i>Syringe rotation error.</i>	<ul style="list-style-type: none"> ▪ Needle flushing with controller software. ▪ Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>Syringe valve did not find destination position.</i>	<ul style="list-style-type: none"> ▪ Needle flushing with controller software. ▪ Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.

System message	Explanation
<i>Temperature above 48 °C at cooling ON.</i>	<ul style="list-style-type: none"> ▪ Switch off the cooling and check whether ambient temperature sensor is properly functioning. ▪ If the system message appears again, notify KNAUER Technical Support.
<i>The 10 ml syringe cannot be used for standard injections.</i>	Exchange the syringe.
<i>The autosampler has detected another tray than that which is currently configured. Please select the correct tray in configuration dialog.</i>	Check controller software configuration and correct entry.
<i>The autosampler is not ready. Please try later.</i>	Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>The injection volume of %2f µl is invalid. For the specified injection method, volume should equal %2f µl.</i>	Check parameters in controller software and correct entry.
<i>The sample needle is not in the home position while the tray is rotating.</i>	Check parameters in controller software and correct entry.
<i>Trace from tray cooling cannot be acquired. Tray cooling is off.</i>	Check parameters in controller software and correct entry.
<i>Tray advance is not available at this time.</i>	Check parameters in controller software and correct entry.
<i>Tray error.</i>	Check parameters in controller software and correct entry.
<i>Valve error.</i>	Check parameters in controller software and correct entry.
<i>Vertical: Home sensor not de-activated.</i>	<ul style="list-style-type: none"> ▪ Check whether there are visible obstructions impairing the needle unit. ▪ Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.

System message	Explanation
<i>Vertical: Home sensor not reached.</i>	<ul style="list-style-type: none"> ▪ Check whether there are visible obstructions impairing the needle unit. ▪ Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>Vertical: Needle position is unknown.</i>	Initialize the instrument in the controller software.
<i>Vertical: stripper did not detect plate (or wash/waste). Missing vial.</i>	<ul style="list-style-type: none"> ▪ Check sample vial and plate. ▪ Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>Vertical: stripper stuck.</i>	Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>Vertical: The sample needle arm is at an invalid position.</i>	Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support.
<i>Vial number error.</i>	Check parameters in controller software and correct entry.
<i>Wear-out limit reached.</i>	Switch the devices off and on again. If the system message appears again, notify KNAUER Technical Support. The injection valve must be replaced.
<i>Wrong loop volume. The largest loop volume for standard injections is 1000 µl.</i>	Check parameters in controller software and correct entry.
<i>Wrong tubing volume. The largest tubing volume for standard injections is 200 µl.</i>	Check parameters in controller software and correct entry.

Technical data

Ambient conditions

Temperature range	10–40 °C; 39–104 °F
Air humidity	20–80% air humidity (non-condensing)

Smartline Autosampler 3950



Standard version

Sample capacity	Microtiter plates for max. 768 samples or sample plates for max. 96 vials
Injection volume	1–5000 µl
Standard sample loop	100 µl
Syringe	500 µl
Injection time	<60 s, incl. cleaning
Injection modes	PASA™ loop injection principle: <ul style="list-style-type: none"> ▪ Full loop injection ▪ Partial loop injection ▪ <i>Microliter pickup</i> injection
Reproducibility	RSD (Relative Standard Deviation): <ul style="list-style-type: none"> ▪ full loop injection: <0.3% ▪ partial loop injection at an injection volume >10 µl: <0.5% ▪ microliter pickup injection at an injection volume >10 µl: <1.0%
Carryover	<0.05% with needle cleaning
Sample cooling	4–22 °C
Weight	<ul style="list-style-type: none"> ▪ 19 kg ▪ With cooling: 21 kg
Dimensions (length x width x height)	<ul style="list-style-type: none"> ▪ 510 mm x 300 mm x 360 mm ▪ With cooling: 575 mm x 300 mm x 360 mm
Supply voltage range	95–240 V
Supply frequency	50–60 Hz

Preparative version

Sample capacity	2x sample plate with 12 vials respectively (10 ml)
Vial height	32–47 mm
Injection volume	1–10000 µl
Standard sample loop	10 ml
Syringe	2500 µl
Injection time	<60 s, incl. cleaning
Injection modes	PASA™ loop injection principle: Partial loop injection
Reproducibility	RSD: partial loop injection at an injection volume >10 µl: <1%
Carryover	<0.1% with needle cleaning
Weight	19 kg
Dimensions (length x width x height)	510 mm x 300 mm x 360 mm
Supply voltage range	100–240 V
Supply frequency	50–60 Hz

Delivery program

Devices and accessories

Name	Order number
Smartline Autosampler 3950, standard version, injection valve, accessories	A5005-1
Smartline Autosampler 3950, standard version incl. sample cooling, accessories	A50051-1
Smartline Autosampler 3950, biocompatible version, accessories	A50052-1
Smartline Autosampler 3950, biocompatible version incl. sample cooling, accessories	A50053-1
Smartline Autosampler 3950, preparative version, incl. vial plates for 12 x 10 vials, accessories	A50054-1
Manual	V1509A

Spare parts

Name	Order number
Autosampler accessory kit: <ul style="list-style-type: none"> ▪ Sample vial with closure and septa (1.5 ml, 1000 pcs.) ▪ Opening and closing pliers 	A0664
2x sample plate for 48 vials, 1.5 ml	A50050
Vial plate for 12 vials, 10 ml (preparative version)	M2072
Microtiter plate 96 wells, U sanitized, 0.35 ml	A1823
Microtiter plate 96 wells, U sanitized, 1.2 ml	A1823V1
PTFE tube, 3.2 mm AD 1.5 mm ID, 300 cm	A0732
Silicon tube 8.0 mm ID, 200 cm	M20702
Flushing solution bottle	M2054

Name	Order number
Syringe 500 µl	M2070
Syringe 2500 µl	M20701
Sample needle kit for SPARK valve 1/16"	A64700
Sample needle	A0646
Sample needle incl. tube and connector, biocompatible version	A15086
Air needle, white, 62 mm	A50058
Set of air needles	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
2x 2.5 A fuse	M2040
Network cable	A5255
RS-232 cable	A0895
Manual	V1509A

Accessories 84+3

Name	Order number
Vial tray for 84x1.5ml and 3x10ml vials	A500501
125 Vials 10 ml, 500 crimp caps and 500 septa, ø 22 mm	A1662

Legal information

Warranty conditions

The factory warranty for the device is valid for 12 months after the date of dispatch. All warranty claims shall expire in the event that any unauthorized changes are made to the device.

During the warranty period, any components with material or design-related defects will be replaced or repaired by KNAUER free of charge.

This warranty excludes the following:

1. Accidental or willful damage
2. Damage or errors caused by third parties that are not contractually related to KNAUER at the time the damage occurs
3. Wear parts, fuses, glass parts, columns, light sources, cuvettes and other optical components
4. Damage caused by negligence or improper operation of the devices and damage caused by clogged capillaries
5. Packaging and transport damage

In the event of device malfunctions, contact:

Manufacturer Wissenschaftliche Gerätebau
Dr. Ing. Herbert KNAUER GmbH
Hegauer Weg 38
14163 Berlin, Germany
Phone: +49-(0)30-809727-0
Fax: +49-(0)30-8015010
E-Mail: info@knauer.net
Internet: www.knauer.net

The packaging of our devices provides the best possible protection against transport damage. However, immediately inspect each delivery for signs of transport damage. If the shipment is incomplete or damaged, inform the manufacturing factory within three workdays. Also inform the freight carrier about transport damage.

Declaration of conformity

**Manufacturer name
and address**

Wissenschaftliche Gerätebau
Dr. Ing. Herbert KNAUER GmbH
Hegauer Weg 38
14163 Berlin, Germany

**Smartline
Autosampler 3950**

Order number A5005-1, A50051-1, A50052-1, A50053-1,
A50054-1

complies with the following requirements and product specifications:

- DIN EN 60799 (June 1999) Electrical accessories – Cord sets and interconnection cord sets
- DIN EN 61010-1 (August 2002) Safety requirements for electrical equipment for measurement, control and laboratory use
 - Low voltage directive (2006/95/EC)
- DIN EN 61000-3-2 (March 2010) Electromagnetic compatibility (EMC) Part 3-2
EN 61000-3-2:2006 + A1:2009 + A2:2009
 - EMC standards (2004/108/EC)
- DIN EN 61326-1 (October 2006) Electrical equipment for measurement, control and laboratory use – EMC requirements
 - DIN EN 61326-1 Corrigendum 2 (April 2011)
- Directives for an environmentally sound use of electrical and electronic equipment
 - RoHS directive 2002/95/EC (February 2003) on the restriction of the use of certain hazardous substances in electrical and electronic equipment
 - WEEE directive 2002/96/EC (February 2003) on waste electrical and electronic equipment

Date Berlin, 2012-01-04



Dr. Alexander Bünz (Managing Director)

The mark of conformity has been applied to the rear panel of the device.



Abbreviations and terminology

Here you can find information on the abbreviations and terminology used in this device manual for the Smartline Autosampler 3950.

Terminology	Explanations
GLP	Good Laboratory Practice – quality assurance for laboratories.
HPG	High Pressure Gradient (HPG). Operating mode of an HPLC or UHPLC system. The solvent is mixed on the high-pressure side of the pump.
HPLC	High Pressure Liquid Chromatography (HPLC).
IP address	Unique address of transmitter or receiver in local network or Internet (Internet protocol).
Solvent	Mobile phase (eluent) or carrier for liquid chromatography.
LPG	Low-pressure gradient, LPG. Operating mode of an HPLC or UHPLC system. The solvent is mixed on the low-pressure side of the pump.
Remote	The chromatography software controls the autosampler.
UHPLC	Ultra High-Pressure Liquid Chromatography (UHPLC). Ultra high-pressure liquid chromatography.

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