Agilent 1290 Infinity Autosampler

User Manual

Agilent Technologies

Notices

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CAUTION

A **CAUTION** notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in damage to the product or loss of important data. Do not proceed beyond a **CAUTION** notice until the indicated conditions are fully understood and met.

WARNING

A WARNING notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a WARNING notice until the indicated conditions are fully understood and met.

In This Guide

This manual covers the Agilent 1290 Infinity Autosampler (G4226A)

1 Introduction

This chapter gives an introduction to the autosampler.

2 Site Requirements and Specifications

This chapter provides information on environmental requirements, physical and performance specifications.

3 Installing the Autosampler

This chapter provides information on unpacking, checking on completeness, stack considerations and installation of the autosampler.

4 Using the Module

This chapter provides information on how to set up the autosampler for an analysis and explains the basic settings.

5 Optimizing Performance

This chapter gives hints on how to optimize the performance or use additional devices.

6 Troubleshooting and Diagnostics

This chapter gives an overview about the troubleshooting and diagnostic features and the different user interfaces.

7 Error Information

This chapter describes the meaning of error messages, and provides information on probable causes and suggested actions how to recover from error conditions.

8 Test Functions

This chapter describes the tests for the module.

9 Maintenance

This chapter describes the maintenance of the Autosampler

10 Parts for Maintenance

This chapter provides information on parts material required for the module.

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This chapter provides information on cables used with the 1290 series of HPLC modules.

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Contents

1 Introduction

This chapter gives an introduction to the autosampler.

Features

The 1290 Infinity Autosampler features an increased pressure range enabling the use of today's column technology (sub-two-micron narrow bore columns) with the Agilent 1290 Infinity LC System. Increased robustness is achieved by optimized new parts, high speed with lowest carry-over by flow through design, increased sample injection speed for high sample throughput, increased productivity by using overlapped injection mode and flexible and convenient sample handling with different types of sample containers, such as vials and well plates. Using 384-well plates allows you to process up to 768 samples unattended.

For specifications, see "Performance Specifications" on page 28.

NOTE This 1290 Infinity Autosampler has been introduced together with the Agilent 1290 Infinity Liquid Chromatograph.

Overview of the Module

The Autosampler transport mechanism uses an X-Z-theta robot to optimize the positioning of the sampling arm on the well plate. Once the sampling arm is positioned over the programmed sample position, the programmed sample volume is drawn by the metering device into the sampling needle. The sampling arm then moves to the injection position where the sample is flushed onto the column.

The autosampler employ a vial/plate pusher mechanism to hold down the vial or the plate while the needle is drawn back from the sample vessel (a must in the case a septum is used). This vial/plate pusher employs a sensor to detect the presence of a plate and to ensure accurate movement regardless of plate used. All axes of the transport mechanism (x-,z-,theta-robot) are driven by stepper-motors. Optical encoders ensure the correct operation of the movement.

The standard metering device provides injection volumes from 0.1–20 µl. A 0.1-40 µl injection volume metering device is installed in the G4226A, with a 20 µl, low restriction loop capillary restricting the injection volume. The entire flowpath including the metering device is always flushed by the mobile phase after injection for minimum internal carry-over.

An additional needle flush station with a peristaltic pump is installed to wash the outside of the needle. This reduces the already low carry-over for very sensitive analysis.

The bottle containing the mobile phase for the wash procedure will be located in the solvent bottle cabinet. Produced waste during this operation is channeled safely away through a waste drain.

The six-port (only 5 ports are used) injection valve unit is driven by a high-speed hybrid stepper motor. During the sampling sequence, the valve unit bypasses the autosampler, and connects flow from the pump to the column directly. During injection and analysis, the valve unit directs the flow through the autosampler which ensures that all of the sample is injected onto the column, and that the metering unit and needle are always free from sample residue before the next sampling sequence begins.

Control of the vial/plate temperature in the thermostatted autosampler is achieved using an additional Agilent 1200 Series module; the Agilent 1200 Series thermostat for ALS/FC/Spotter. The thermostat contains Peltier-controlled heat-exchangers. A fan draws air from the area above the sample vial tray of the autosampler. It is then blown through the fins of the cooling/heating module. There it is cooled or heated according the temperature setting. The thermostatted air enters the autosampler through a recess underneath the special designed sample tray. The air is then distributed evenly through the sample tray ensuring effective temperature control, regardless of how many vials are in the tray. In cooling mode condensation is generated on the cooled side of the Peltier elements. This condensed water is safely guided into a waste bottle for condensed water.

Autosampler Principle

The movements of the autosampler components during the sampling sequence are monitored continuously by the autosampler processor. The processor defines specific time windows and mechanical ranges for each movement. If a specific step of the sampling sequence is not completed successfully, an error message is generated. Solvent is bypassed from the autosampler by the injection valve during the sampling sequence. The needle moves to the desired sample position and is lowered into the sample liquid in the sample to allow the metering device to draw up the desired volume by moving its plunger back a certain distance. The needle is then raised again and moved onto the seat to close the sample loop. Sample is applied to the column when the injection valve returns to the mainpass position at the end of the sampling sequence.

The standard sampling sequence occurs in the following order:

1. The injection valve switches to the bypass position.
2. The plunger of the metering device moves to the initialization position.
3. The needle lock moves up.
4. The needle moves to the desired sample vial (or well plate) position.
5. The needle lowers into the sample vial (or well plate).
6. The metering device draws the preset sample volume.
7. The needle lifts out of the sample vial (or well plate).
8. The needle is then moved onto the seat to close the sample loop.
9. The needle lock moves down.
10. The injection cycle is completed when the injection valve switches to the mainpass position.

If needle wash is required it will be done between step 7 and 8.

Injection Sequence

Before the start of the injection sequence, and during an analysis, the injection valve is in the mainpass position. In this position, the mobile phase flows through the autosampler metering device, sample loop, and needle, ensuring all parts in contact with sample are flushed during the run, thus minimizing carry-over.

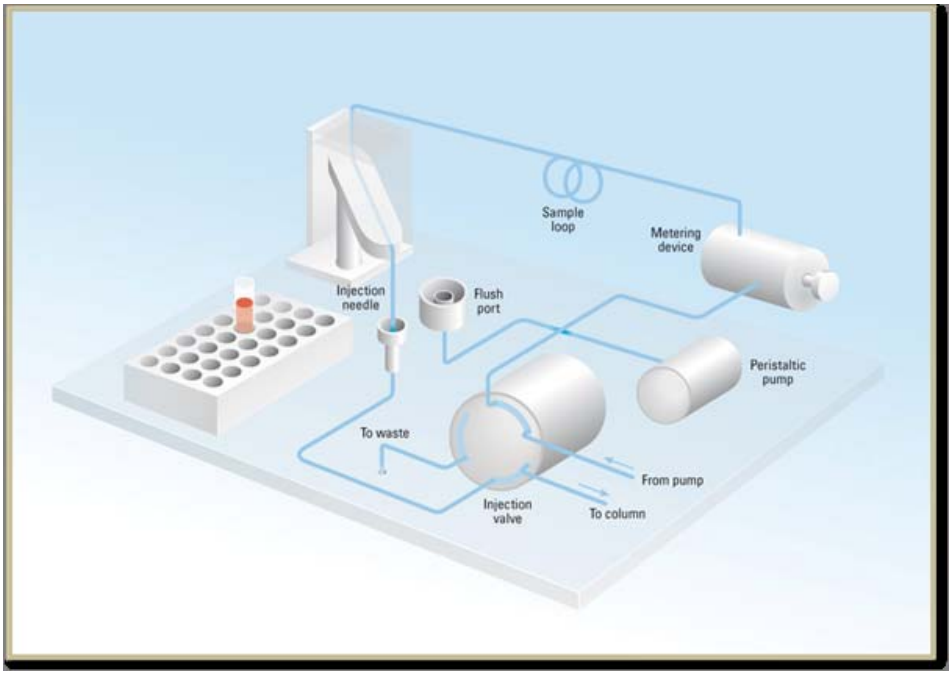


Figure 1 Mainpass Position

When the sample sequence begins, the valve unit switches to the bypass position. Solvent from the pump enters the valve unit at port 1, and flows directly to the column through port 6.

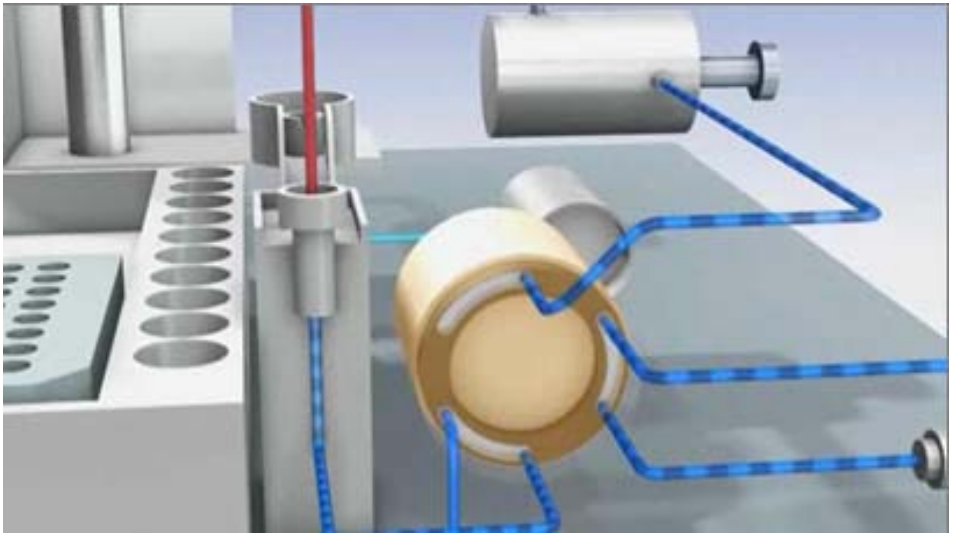


Figure 2 Bypass Position

The standard injection starts with *draw sample from vial*. In order to do this the needle moves to the desired sample position and is lowered into the sample liquid in the sample to allow the metering device to draw up the desired volume by moving its plunger back a certain distance. The needle is then raised again and moved onto the seat to close the sample loop. In case of an injector program several steps are interspersed at this point.

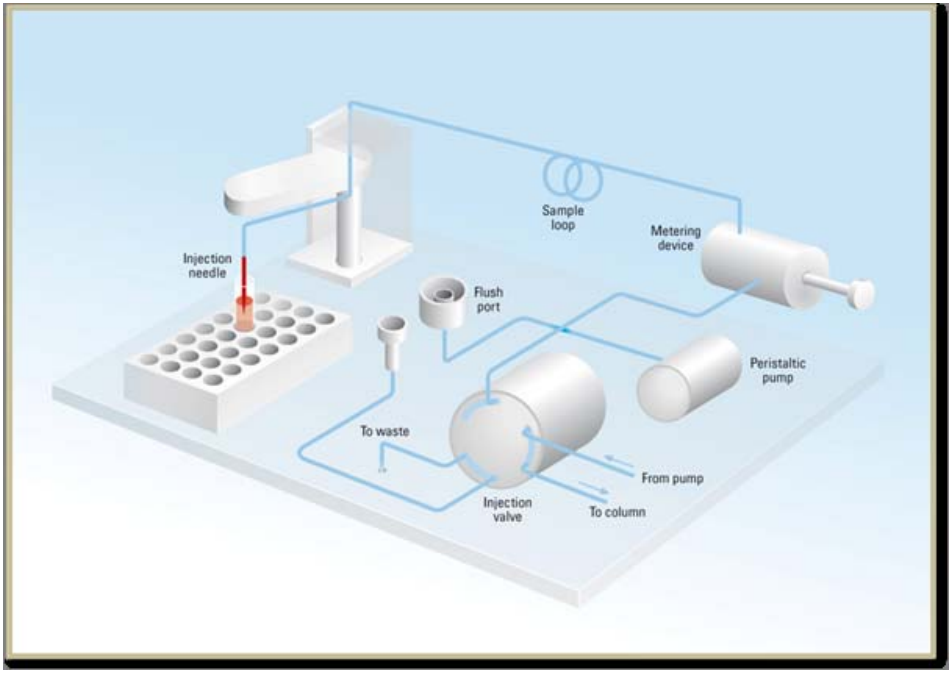


Figure 3 Drawing the Sample

Flush the Needle

Before injection and to reduce the carry-over for very sensitive analysis, the outside of the needle can be washed in a flush port located behind the injector port on the sampling unit. As soon as the needle is on the flush port a peristaltic pump delivers some solvent during a defined time to clean the outside of the needle. At the end of this process the needle returns to the injection port.

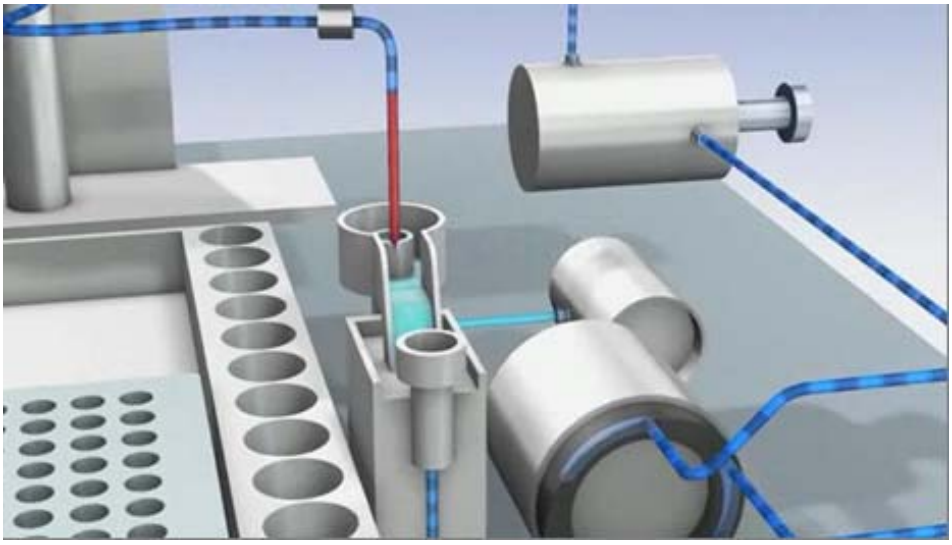


Figure 4 Flush the needle

Inject-and-Run The final step is the inject-and-run step. The six-port valve is switched to the main-pass position, and directs the flow back through the sample loop, which now contains a certain amount of sample. The solvent flow transports the sample onto the column, and separation begins. This is the beginning of a *run* within an analysis. In this stage, all major performance-influencing hardware is flushed internally by the solvent flow. For standard applications no additional flushing procedure is required.

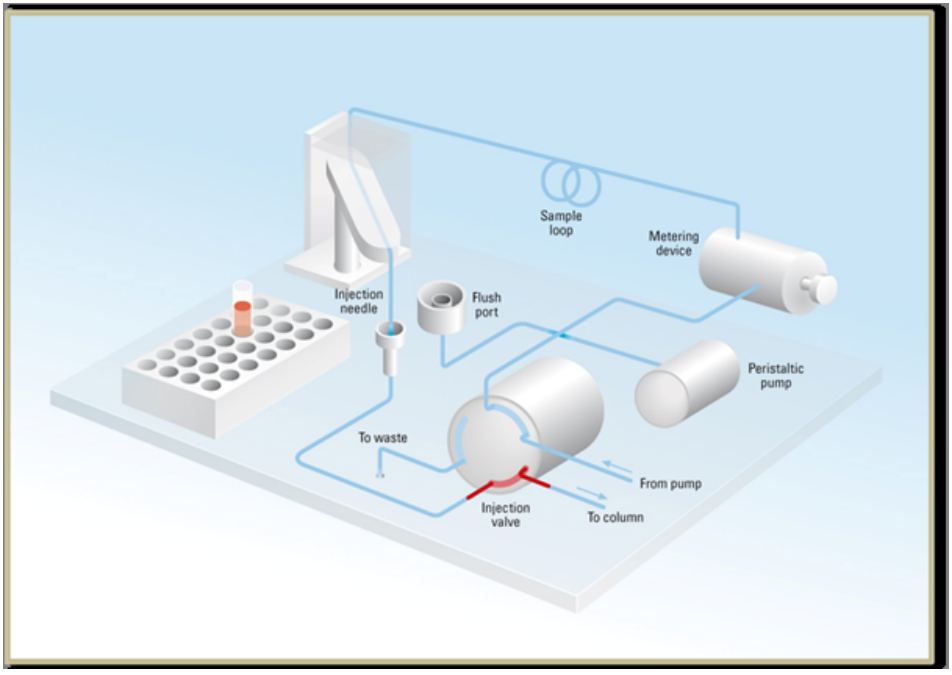


Figure 5 Inject and Run

System Overview

Leak and Waste Handling

The 1200 Infinity Series has been designed for safe leak and waste handling. It is important that all security concepts are understood and instructions are carefully followed.

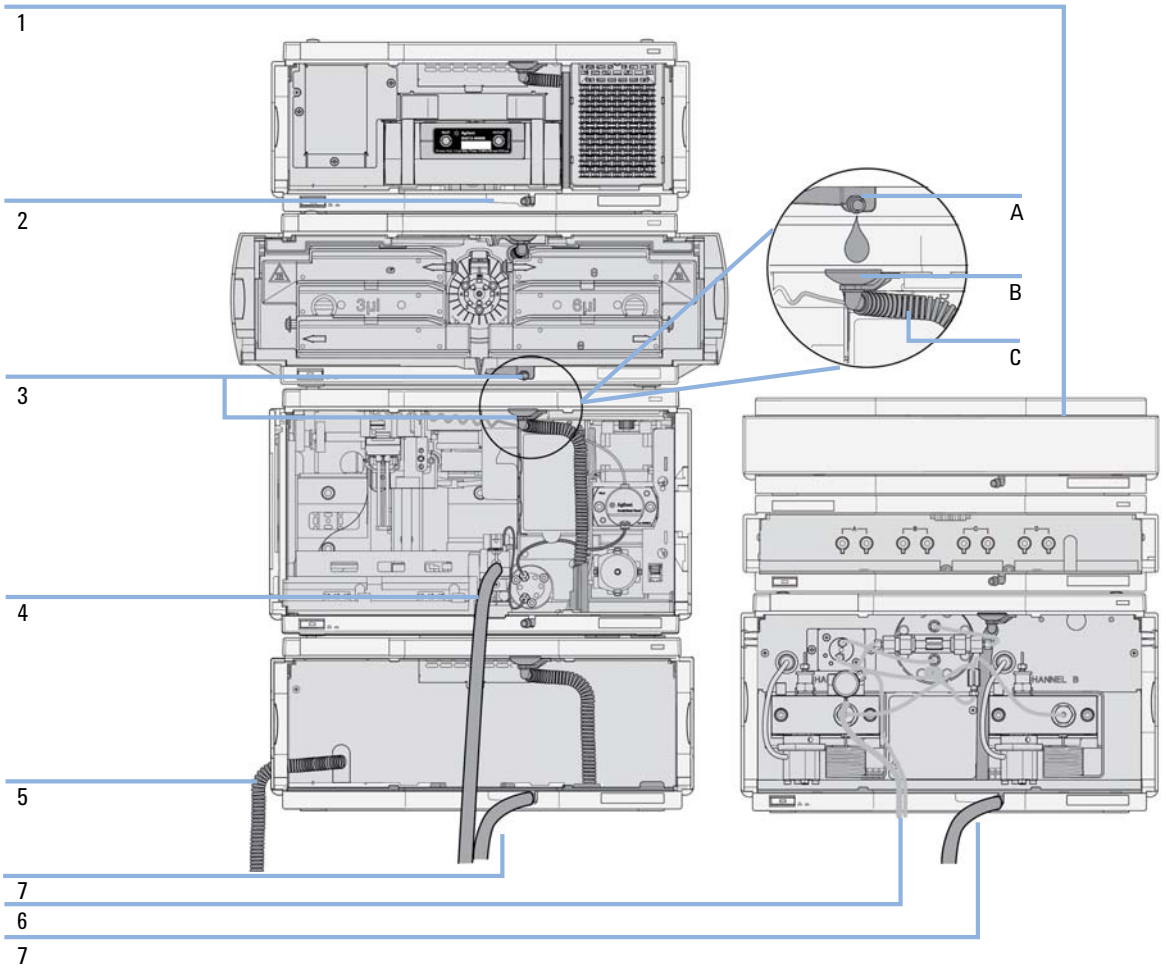


Figure 6 Leak and waste handling concept (overview - typical stack configuration as an example)

The solvent cabinet (1) is designed to store a maximum volume of 6 L solvent. The maximum volume for an individual bottle stored in the solvent cabinet should not exceed 2.5 L. For details, see the usage guideline for the Agilent 1200 Infinity Series Solvent Cabinets (a printed copy of the guideline has been shipped with the solvent cabinet, electronic copies are available on the Internet).

The leak pan (2) (individually designed in each module) guides solvents to the front of the module. The concept covers also leakages on internal parts (e.g. the detector's flow cell). The leak sensor in the leak pan stops the running system as soon as the leak detection level is reached.

The leak pan's outlet port (3, A) guides excessive overfill from one module to the next, as the solvent flows into the next module's leak funnel (3, B) and the connected corrugated waste tube (3, C). The corrugated waste tube guides the solvent to the next lower positioned module's leak tray and sensor.

The waste tube of the sampler's needle wash port (4) guides solvents to waste.

The condense drain outlet of the autosampler cooler (5) guides condensate to waste.

The waste tube of the purge valve (6) guides solvents to waste.

The waste tube connected to the leak pan outlet on each of the bottom instruments (7) guides the solvent to a suitable waste container.

2
Site Requirements and Specifications

This chapter provides information on environmental requirements, physical and performance specifications.

Site Requirements

A suitable environment is important to ensure optimal performance of the module.

Power Considerations

The module power supply has wide ranging capability. It accepts any line voltage in the range described in Table 1 on page 27. Consequently there is no voltage selector in the rear of the module. There are also no externally accessible fuses, because automatic electronic fuses are implemented in the power supply.

WARNING

Hazard of electrical shock or damage of your instrumentation

can result, if the devices are connected to a line voltage higher than specified.

➔ Connect your instrument to the specified line voltage only.

WARNING

The module is partially energized when switched off, as long as the power cord is plugged in.

Repair work at the module can lead to personal injuries, e.g. electrical shock, when the cover is opened and the module is connected to power.

➔ Always unplug the power cable before opening the cover.

➔ Do not connect the power cable to the instrument while the covers are removed.

CAUTION

Inaccessible power plug.

In case of emergency it must be possible to disconnect the instrument from the power line at any time.

➔ Make sure the power connector of the instrument can be easily reached and unplugged.

➔ Provide sufficient space behind the power socket of the instrument to unplug the cable.

Power Cords

Different power cords are offered as options with the module. The female end of all power cords is identical. It plugs into the power-input socket at the rear. The male end of each power cord is different and designed to match the wall socket of a particular country or region.

WARNING

Absence of ground connection or use of unspecified power cord

The absence of ground connection or the use of unspecified power cord can lead to electric shock or short circuit.

➔ Never operate your instrumentation from a power outlet that has no ground connection.

➔ Never use a power cord other than the Agilent Technologies power cord designed for your region.

WARNING

Use of unsupplied cables

Using cables not supplied by Agilent Technologies can lead to damage of the electronic components or personal injury.

➔ Never use cables other than the ones supplied by Agilent Technologies to ensure proper functionality and compliance with safety or EMC regulations.

WARNING

Unintended use of supplied power cords

Using power cords for unintended purposes can lead to personal injury or damage of electronic equipment.

➔ Never use the power cords that Agilent Technologies supplies with this instrument for any other equipment.

Bench Space

The module dimensions and weight (see Table 1 on page 27) allow you to place the module on almost any desk or laboratory bench. It needs an additional 2.5 cm (1.0 inches) of space on either side and approximately 8 cm (3.1 inches) in the rear for air circulation and electric connections.

If the bench shall carry a complete HPLC system, make sure that the bench is designed to bear the weight of all modules.

The module should be operated in a horizontal position.

Condensation

CAUTION

Condensation within the module

Condensation can damage the system electronics.

➔ Do not store, ship or use your module under conditions where temperature fluctuations could cause condensation within the module.

➔ If your module was shipped in cold weather, leave it in its box and allow it to warm slowly to room temperature to avoid condensation.

Physical Specifications

Table 1 Physical Specifications

Type	Specification	Comments
Weight	15.5 kg (34.2 lbs)	
Dimensions (height × width × depth)	200 x 345 x 440 mm (8 x 13.5 x 17 inches)	
Line voltage	100 – 240 V~, ± 10 %	Wide-ranging capability
Line frequency	50 or 60 Hz, ± 5 %	
Power consumption	200 VA / 200 W / 683 BTU	Maximum
Ambient operating temperature	4–55 °C (39–131 °F)	
Ambient non-operating temperature	-40 – 70 °C (-40 – 158 °F)	
Humidity	< 95 % r.h. at 40 °C (104 °F)	Non-condensing
Operating altitude	Up to 2000 m (6562 ft)	
Non-operating altitude	Up to 4600 m (15092 ft)	For storing the module
Safety standards: IEC, CSA, UL	Installation category II, Pollution degree 2	For indoor use only.

Performance Specifications

Table 2 Performance specifications G4226A

Type	Specification	Comment
Injection range	0.1 – 20 µL in 0.1 µL increments 0.1 – 40 µL in 0.1 µL increments if 40 µL loop is installed 0.1 – 120 µL in 0.1 µL increments with 1290 Infinity large volume injection kit (hardware modification required) pressure range up to 1200 bar 0.1 – 100 µL in 0.1 µL increments with 100 µL upgrade kit (G4214A) (hardware modification required) up to 600 bar	
Precision	Typically <0.25 % RSD from 5 – 20 µL, Typically <0.5 % RSD from 2 – 5 µL volume, Typically <0.7 % RSD from 1 – 2 µL volume.	Measured with injections of benzylalcohol.
Pressure range	Up to 1200 bar Up to 600 bar	with 1290 Infinity large volume injection kit installed with 100 µL upgrade kit (G4214A) installed
Sample viscosity range	0.2 – 5 cp	
Sample capacity	Capacity 2 x well plates (MTP) + 10 x 2 mL vials, 108 x 2 mL vials in 2 x 54 vial plate plus 10 additional 2 mL vials, 30 x 6 mL vials in 2 x 15 vial plate, 100 Micro vial tray, plus 10 additional 2 mL vials, 54 Eppendorf tubes (0.5/1.5/2 mL) in 2 x 27 Eppendorf tube plate.	Also compatible with the Agilent 1200 Series sample capacity extension for further expansion of the sample capacity.

Table 2 Performance specifications G4226A

Type	Specification	Comment
Injection cycle time	Typically <21 s using following standard conditions: Default draw speed: 100 µL/min Default eject speed: 100 µL/min Injection volume: 5 µL	

Carry Over	Typically <0.004 %	Using the following conditions:
		• Column: Agilent ZORBAX SB-C18, 2.1 x 50 mm 1.8 µm (827700-902)
		• Mobile Phase:
		  ◦ A: 0.1 % TFA in water
		  ◦ B: 0.1 % TFA in Acetonitrile
		• Isocratic : % B=35 %
		• Flow rate: 0.5 mL/min
		• Temperature: 25 °C
		• Wavelength: 257 nm
		• Sample: 1200 ng/µL Chlorhexidine for UV, 240 ng/µL Chlorhexidine for MS (dissolved with mobile phase A), 1 µL injected and measured both on Agilent 6410 QQQ and G4212A DAD
		• Wash solution: H_2O with 0.1 % TFA (5 s)

Control and data evaluation	Agilent ChemStation for LC EZChrom Elite Mass hunter Lab Advisor	B.04.02 or above 3.3.3 or above B.02.01 sp1 or above B.01.03 or above
Local Control	Agilent Instant Pilot (G4208A)	B.02.08 or above
Communications	Controller-area network (CAN), RS-232C, APG Remote: ready, start, stop and shut-down signals, optional four external contact closures and BCD vial number output.	

Table 2 Performance specifications G4226A

Type	Specification	Comment
Safety and maintenance	Extensive diagnostics can be done with the help of the Control Module and Agilent LabAdvisor Diagnostic Software, error detection and display (through Instant Pilot and Diagnostic Software), leak detection, safe leak handling, leak output signal for shutdown of pumping system. Low voltages in major maintenance areas.	
GLP features	Early maintenance feedback (EMF) for continuous tracking of instrument usage with user-settable limits and feedback messages. Electronic records of maintenance and errors.	
Housing	All materials recyclable.	
Metering device	Metering pump in high pressure flow path	

3
Installing the Autosampler

This chapter provides information on unpacking, checking on completeness, stack considerations and installation of the autosampler.

Unpacking the Autosampler

Damaged Packaging

If the delivery packaging shows signs of external damage, please call your Agilent Technologies sales and service office immediately. Inform your service representative that the instrument may have been damaged during shipment.

CAUTION

"Defective on arrival" problems

If there are signs of damage, please do not attempt to install the module. Inspection by Agilent is required to evaluate if the instrument is in good condition or damaged.

➔ Notify your Agilent sales and service office about the damage.

➔ An Agilent service representative will inspect the instrument at your site and initiate appropriate actions.

Delivery Checklist

Ensure all parts and materials have been delivered with the autosampler. For this compare the shipment content with the checklist included in each instrument box. Please report missing or damaged parts to your local Agilent Technologies sales and service office.

Table 3 Agilent 1290 Infinity Autosampler

Description	Quantity
Autosampler	1
Power Cable	1
User Manual on Documentation CD (part of the shipment - not module specific)	1 per order
Accessory Kit	1

Autosampler Accessory Kit Contents

Item	p/n	Description
1	G4226-68705	Accessory kit
2	5067-4659	SS Capillary 340x0.12 ps-ns
3	5042-1386	96 well plate 0.5 ml, PP (pack of 10)
4	5063-6527	Tubing assembly, i.d. 6 mm, o.d. 9 mm, 1.2 m (to waste)
5	5181-1516	CAN cable, Agilent module to module, 0.5 m
6	8710-0510	Wrench open 1/4 — 5/16 inch

Optimizing the Stack Configuration

If your module is part of a complete Agilent 1290 Infinity Liquid Chromatograph, you can ensure optimum performance by installing the following configurations. These configurations optimize the system flow path, ensuring minimum delay volume.

For other possible configurations, please refer to the Agilent 1290 Infinity System Manual.

One Stack Configuration

Ensure optimum performance by installing the modules of the Agilent 1290 Infinity Binary LC System in the following configuration (See Figure 7 on page 35 and Figure 8 on page 36). This configuration optimizes the flow path for minimum delay volume and minimizes the bench space required.

The Agilent 1290 Infinity Binary Pump should always be installed at the bottom of the stack.

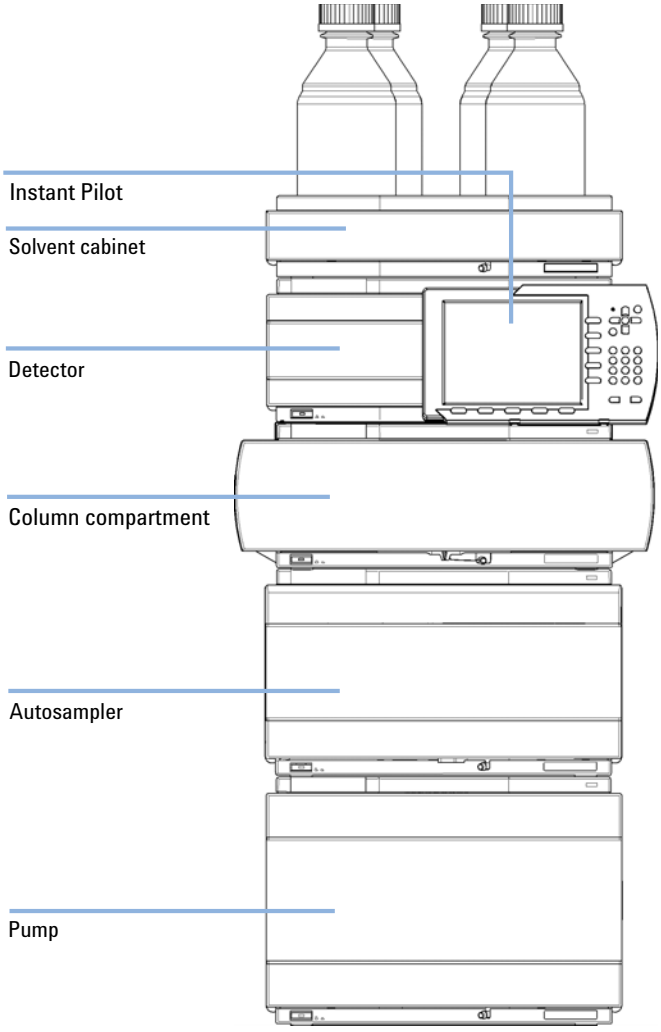


Figure 7 Recommended stack configuration for 1290 Infinity (front view)

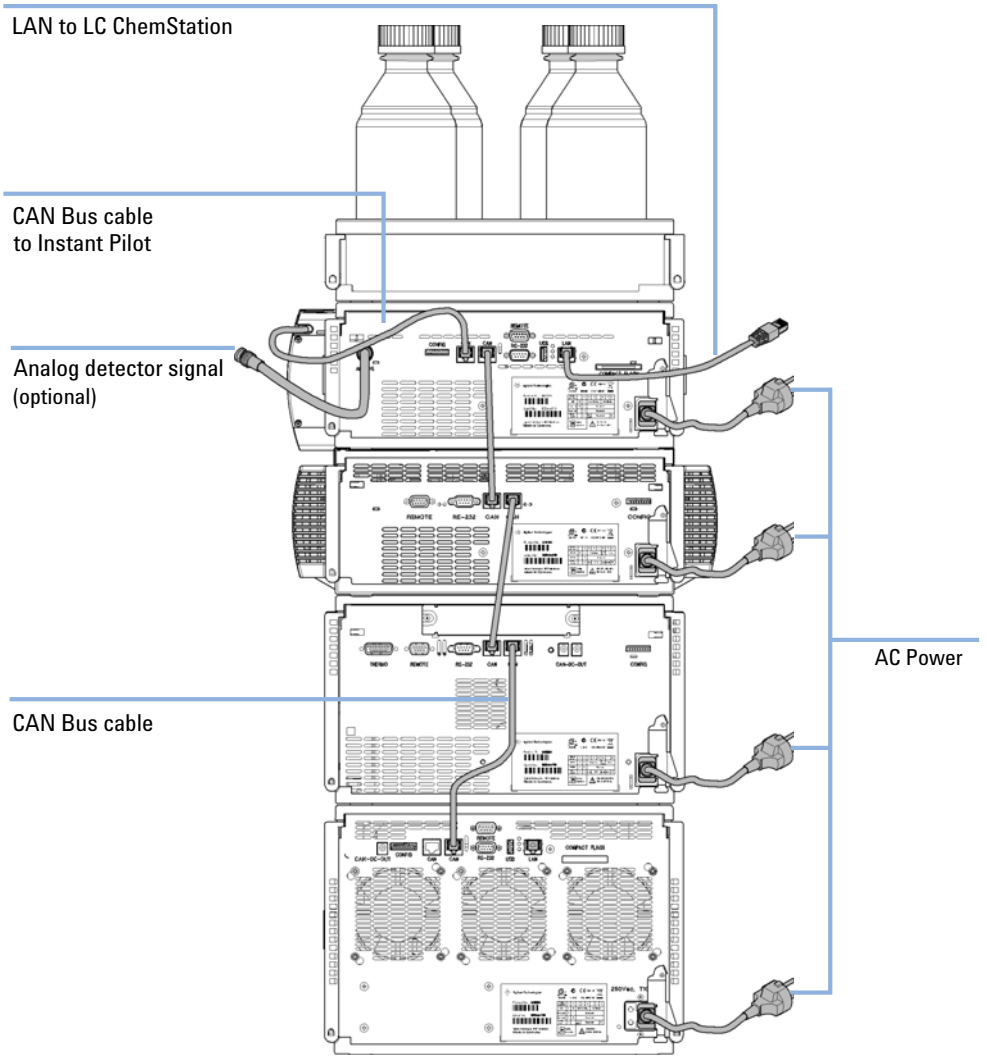


Figure 8 Recommended stack configuration 1290 Infinity (rear view)

Two Stack Configuration

In case the autosampler thermostat is added to the system, a two-stack configuration is recommended, which places both heavy modules (1290 Infinity pump and thermostat) at the bottom of each stack and avoids high stacks. Some users prefer the lower height of this arrangement even without the autosampler thermostat. A slightly longer capillary is required between the pump and autosampler. (See Figure 9 on page 37 and Figure 10 on page 38).

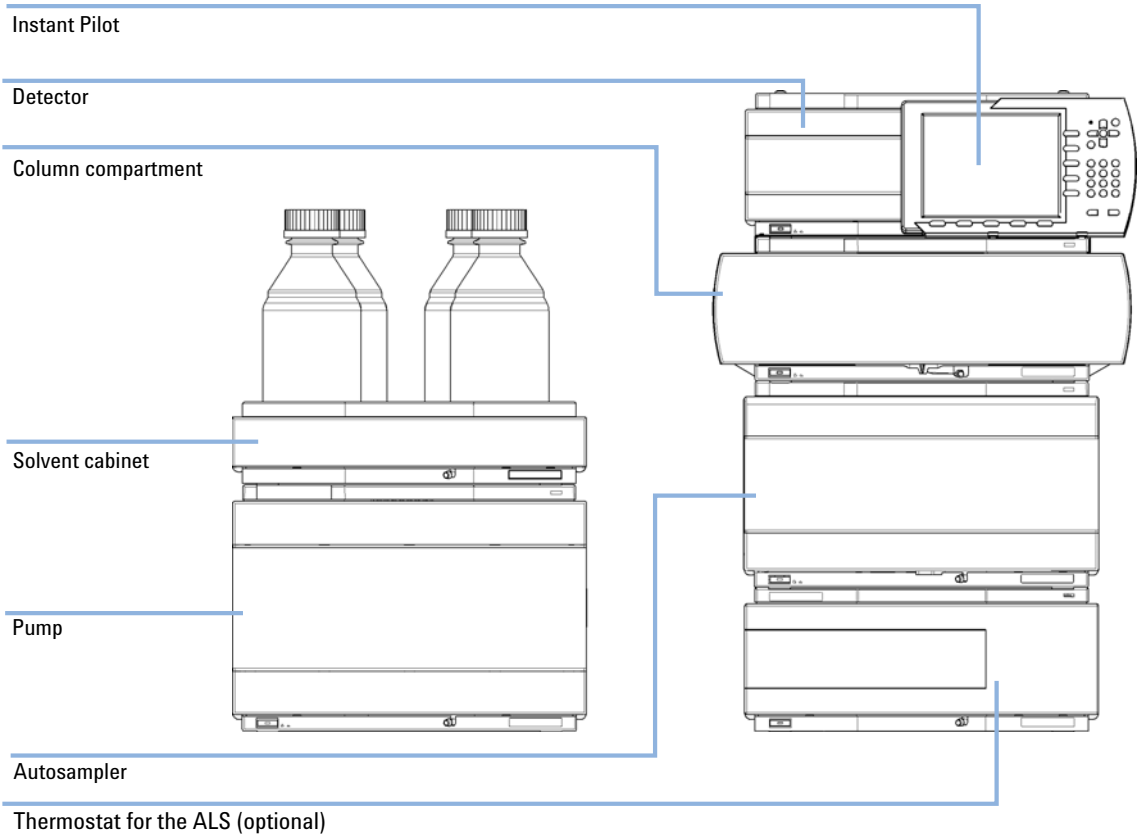


Figure 9 Recommended two stack configuration for 1290 Infinity (front view)

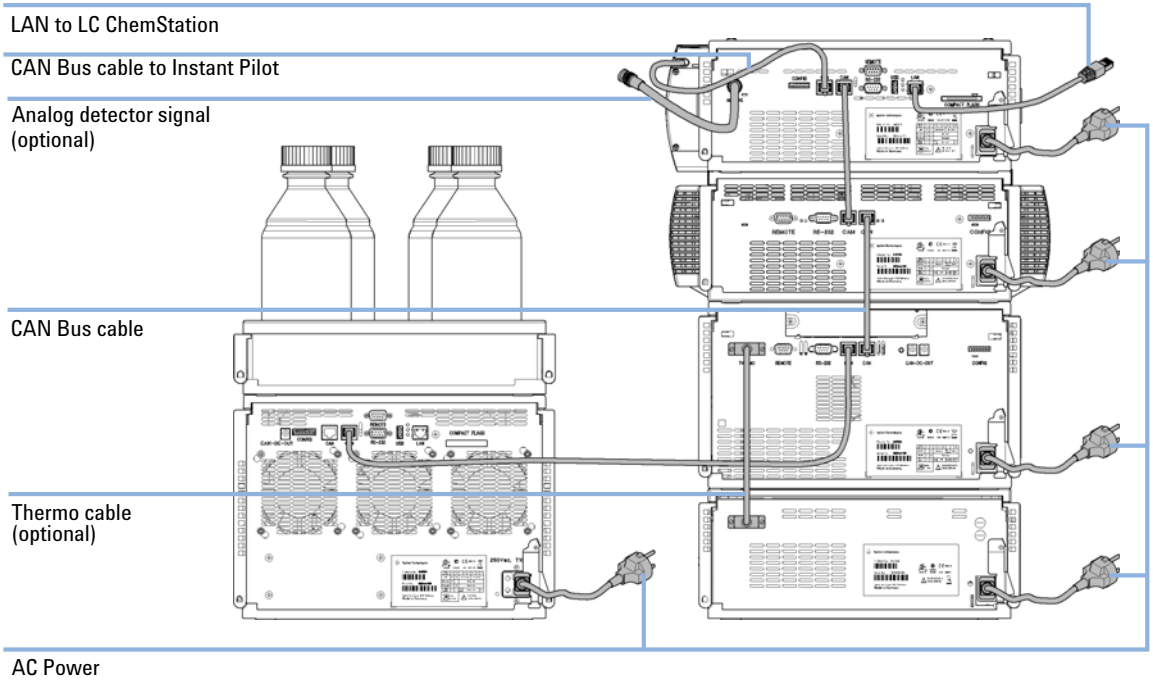


Figure 10 Recommended two stack configuration for 1290 Infinity (rear view)

Installation Information on Leak and Waste Handling

The Agilent 1200 Infinity Series has been designed for safe leak and waste handling. It is important that all security concepts are understood and instructions are carefully followed.

WARNING

Toxic, flammable and hazardous solvents, samples and reagents

The handling of solvents, samples and reagents can hold health and safety risks.

- ➔ When working with these substances observe appropriate safety procedures (for example by wearing goggles, safety gloves and protective clothing) as described in the material handling and safety data sheet supplied by the vendor, and follow good laboratory practice.
- ➔ The volume of substances should be reduced to the minimum required for the analysis.
- ➔ Never exceed the maximal permissible volume of solvents (6 L) in the solvent cabinet.
- ➔ Do not use bottles that exceed the maximum permissible volume as specified in the usage guideline for the Agilent 1200 Infinity Series Solvent Cabinets.
- ➔ Arrange the bottles as specified in the usage guideline for the solvent cabinet.
- ➔ A printed copy of the guideline has been shipped with the solvent cabinet, electronic copies are available on the Internet.

NOTE

Recommendations for Solvent Cabinet

For details, see the usage guideline for the Agilent 1200 Infinity Series Solvent Cabinets.

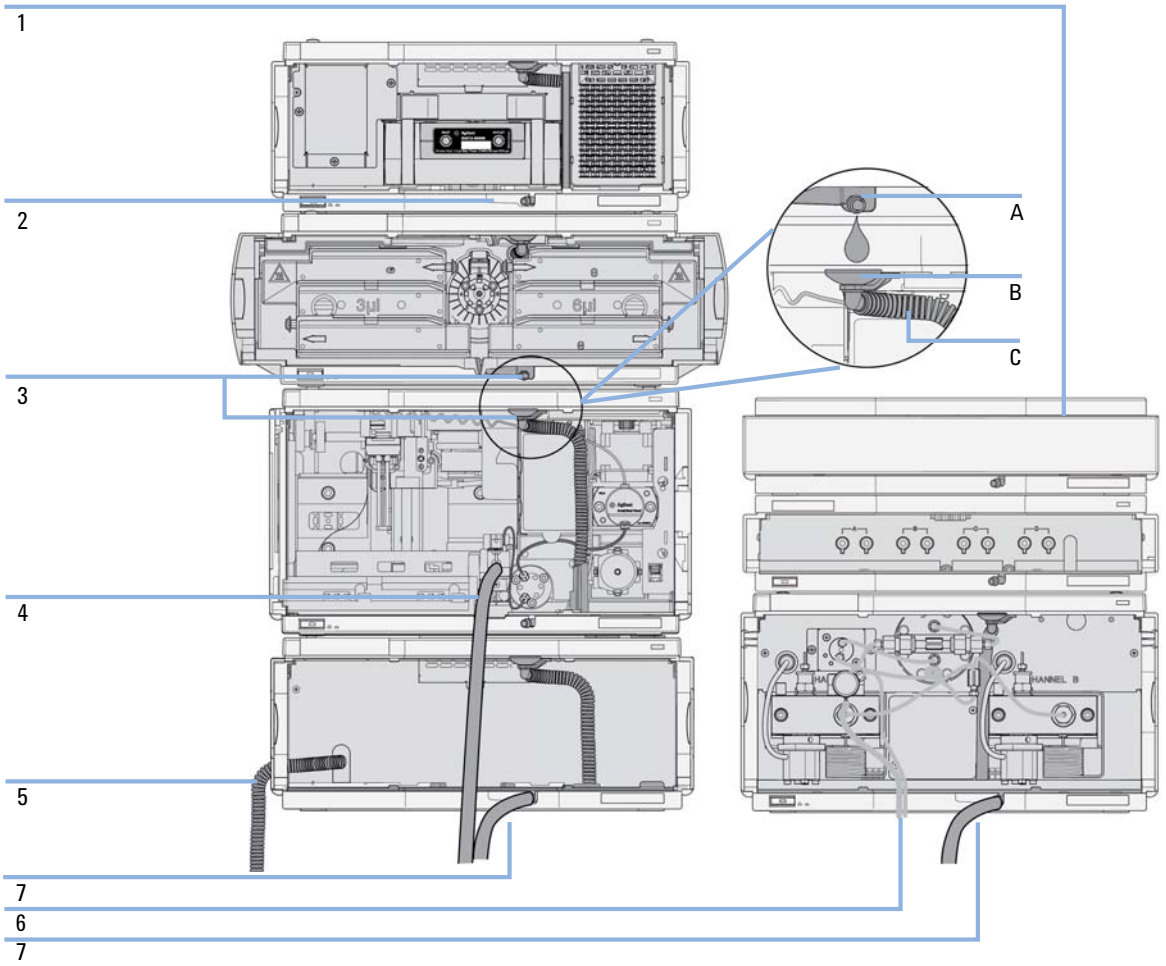


Figure 11 Leak and waste handling (overview - typical stack configuration as an example)

1	Solvent cabinet
2	Leak pan
3	Leak pan's outlet port (A), leak funnel (B) and corrugated waste tube (C)
4	Waste tube of the sampler's needle wash
5	Condense drain outlet of the autosampler cooler
6	Waste tube of the purge valve
7	Waste tube

1 Stack the modules according to the adequate stack configuration.

The leak pan outlet of the upper module must be vertically positioned above the leak tray of the lower module, see Figure 11 on page 40.

2 Connect data and power cables to the modules, see section *Installing the Module* below.

3 Connect capillaries and tubes to the modules, see section *Flow Connections to the module* below or the relevant system manual.

WARNING

Toxic, flammable and hazardous solvents, samples and reagents

➔ Keep solvent path free from blockages.

➔ Keep the flow path closed (in case the pump in the system is equipped with a passive inlet valve, solvent may leak out due to hydrostatic pressure, even if your instrument is off).

➔ Avoid loops.

➔ Tubes must not sag.

➔ Do not bend tubes.

➔ Do not immerse tube end in waste liquid.

➔ Do not intubate tubes in other tubes.

➔ For correct tubing follow instructions on label attached to the module.

Figure 12 Warning label (illustration for correct waste tubing)

Installing the Autosampler

Parts required	#	Description
	1	Autosampler Power cord
	1	Other cables see below and section "Cable Overview" on page 174.
	1	ChemStation and/or Instant Pilot G4208A with the appropriate revisions, see "Performance Specifications" on page 28.

Preparations

- Locate bench space
- Provide power connections.

Unpack the module.

CAUTION

"Defective on arrival" problems

If there are signs of damage, please do not attempt to install the module. Inspection by Agilent is required to evaluate if the instrument is in good condition or damaged.

➔ Notify your Agilent sales and service office about the damage.

➔ An Agilent service representative will inspect the instrument at your site and initiate appropriate actions.

1 Place the Autosampler in the stack, see "Optimizing the Stack Configuration" on page 34.

2 Ensure the power switch on the front of the module is OFF (switch stands out).

3 Connect the power cable to the power connector at the rear of the module.

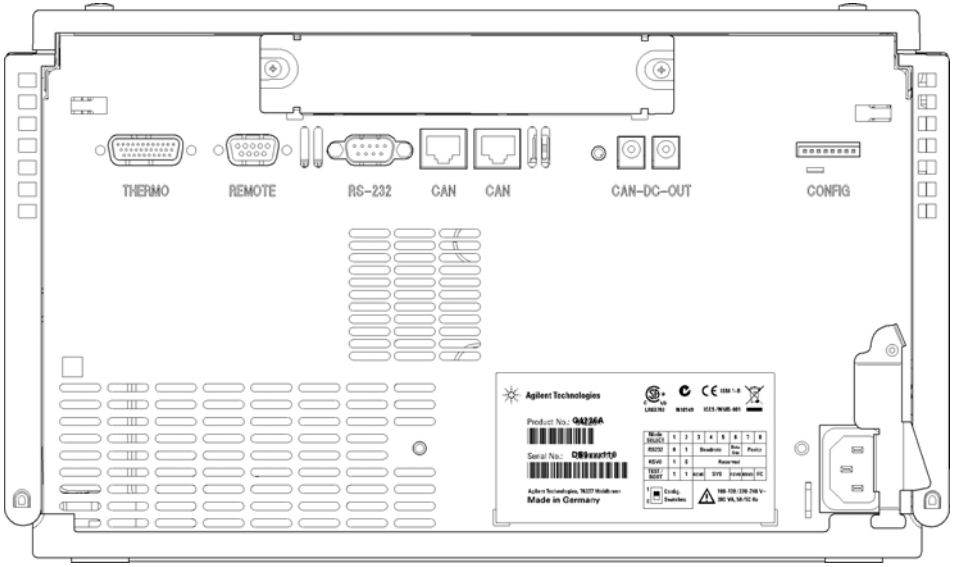


Figure 13 Rearview of Autosampler

4 Connect the CAN cable to other Agilent 1290 modules.

5 Connect the APG remote cable (optional) for non-Agilent instruments.

6 Turn on the power by pushing the button at the lower left hand side of the module.

The power button stays pressed in and the status LED should be green.

NOTE When the line power button stands out and the green light is off, the module is turned off.

NOTE The module was shipped with default configuration settings. For changing these settings, refer to section *Setting the 8-bit configuration switch*.

Flow Connections to the Autosampler

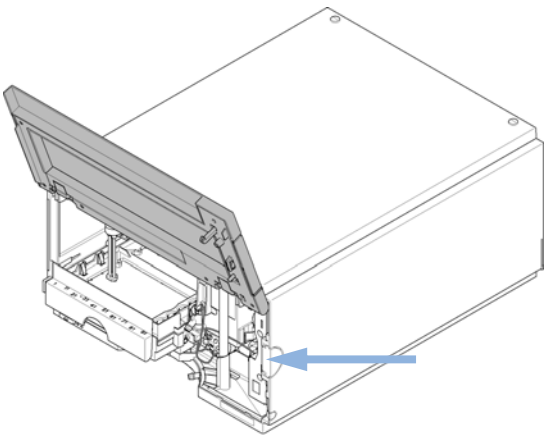
Parts required	#	Description
	1	System, Capillaries and tubing from Accessory Kit.
	1	ChemStation and/or Instant Pilot G4208A with the appropriate revisions, see "Performance Specifications" on page 28.

Preparations

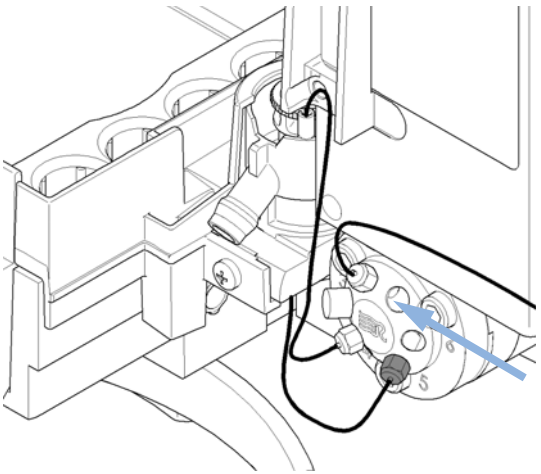
- Autosampler is installed in system.

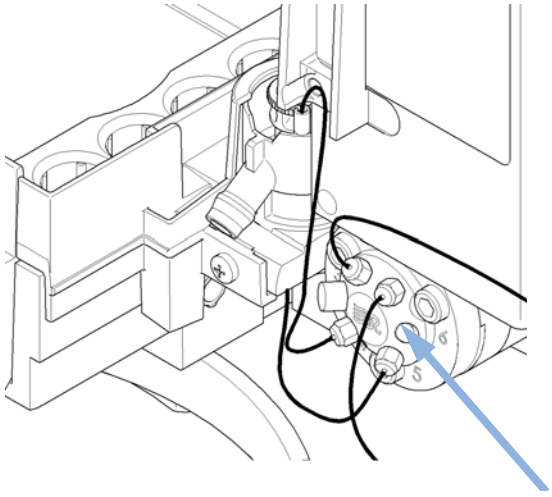
NOTE This procedure shows the Autosampler outside of a system. In an Agilent 1290 Infinity LC System, the Autosampler is located between a G4220A Binary pump (below) and the G1316C TCC-SL+ (above), see "Optimizing the Stack Configuration" on page 34.

1 Open the front cover by pressing the button on the right side of the module.



2 Install the capillary from the pump outlet into the port 1 of the injection valve.



3 Install the capillary from the port 6 of the injection valve to the TCC. 	

NOTE The Autosampler can only be operated with the front and side covers closed.

4
Using the Module

This chapter provides information on how to set up the autosampler for an analysis and explains the basic settings.

Leak and Waste Handling

WARNING

Toxic, flammable and hazardous solvents, samples and reagents

The handling of solvents, samples and reagents can hold health and safety risks.

- When working with these substances observe appropriate safety procedures (for example by wearing goggles, safety gloves and protective clothing) as described in the material handling and safety data sheet supplied by the vendor, and follow good laboratory practice.
- The volume of substances should be reduced to the minimum required for the analysis.
- Do not operate the instrument in an explosive atmosphere.
- Never exceed the maximal permissible volume of solvents (6 L) in the solvent cabinet.
- Do not use bottles that exceed the maximum permissible volume as specified in the usage guideline for the Agilent 1200 Infinity Series Solvent Cabinets.
- Arrange the bottles as specified in the usage guideline for the solvent cabinet.
- A printed copy of the guideline has been shipped with the solvent cabinet, electronic copies are available on the Internet.
- Ground the waste container.
- The residual free volume in the appropriate waste container must be large enough to collect the waste liquid.
- Check the filling level of the waste container regularly.
- To achieve maximal safety, check the correct installation regularly.
- Do not use solvents with an auto-ignition temperature below 200 °C (392 °F).

NOTE

Recommendations for Solvent Cabinet

For details, see the usage guideline for the Agilent 1200 Infinity Series Solvent Cabinets.

For details on correct installation, see section *Installation Information on Leak and Waste Handling* in the service manual.

Preparing the Autosampler

For best performance of the autosampler

- When using the Autosampler in a system with a vacuum degassing unit, shortly degas your samples before using them in the autosampler.
- Filter samples before use in 1290 system. Use the high pressure filter kit (High pressure filter kit (5067-4638)) for inline filtering.
- When using buffer solutions, flush the system with water before switching it off.
- Check the autosampler plungers for scratches, grooves and dents when changing the piston seal. Damaged plungers cause micro leaks and will decrease the lifetime of the seal.
- Solvent Information - Observe recommendations on the use of solvents.
  - Always filter solvents through 0.4 µm filters. Small particles can permanently block the capillaries and valves. Avoid the use of the following steel-corrosive solvents:
    - Solutions of alkali halides and their respective acids (for example, lithium iodide, potassium chloride, and so on).
    - High concentrations of inorganic acids like sulfuric and nitric acid, especially at higher temperatures (replace, if your chromatography method allows, by phosphoric acid or phosphate buffer which are less corrosive to stainless steel).
    - Halogenated solvents or mixtures which form radicals and/or acids, for example:

      $2CHCl_3 + O_2 \rightarrow 2COCl_2 + 2HCl$

      This reaction, in which stainless steel probably acts as a catalyst, occurs quickly with dried chloroform if the drying process removed the stabilizing alcohol.
    - Chromatographic grade ethers, which can contain peroxides (for example, THF, dioxane, di-isopropylether). Such ethers should be filtered through dry aluminium oxide which adsorbs the peroxides.
    - Solvents containing strong complexing agents (e.g. EDTA).
    - Mixtures of carbon tetrachloride with 2-propanol or THF dissolve stainless steel.

- Priming and Purging the System - When the solvents have been exchanged or the system has been turned off for a certain time (for example, overnight) oxygen will re-diffuse into the solvent channel. Therefore priming and purging of the system is required before starting an application.

Table 4 Choice of Priming Solvents for Different Purposes

Activity	Solvent	Comments
After an installation	Isopropanol	Best solvent to flush air out of the system
When switching between reverse phase and normal phase (both times)	Isopropanol	Best solvent to flush air out of the system
After an installation	Ethanol or methanol	Alternative to isopropanol (second choice) if no isopropanol is available
To clean the system when using buffers	Bidistilled water	Best solvent to re-dissolve buffer crystals
After a solvent change	Bidistilled water	Best solvent to re-dissolve buffer crystals

Setting up the Autosampler with Agilent ChemStation

The setup of the Autosampler is shown with the Agilent ChemStation B.04.02. Depending on the controller (e.g. Agilent Instant Pilot, EZChrom Elite) the screens look different. For the Instant Pilot refer to "Main Screens of the Autosampler with Agilent Instant Pilot (G4208A)" on page 61.

NOTE This section describes the autosampler settings only. For information on the Agilent ChemStation or other 1290 Infinity modules refer to the corresponding documentation or the 1290 Infinity System Manual.

After successful load of the ChemStation, you should see the module as an active item in the graphical user interface (GUI).

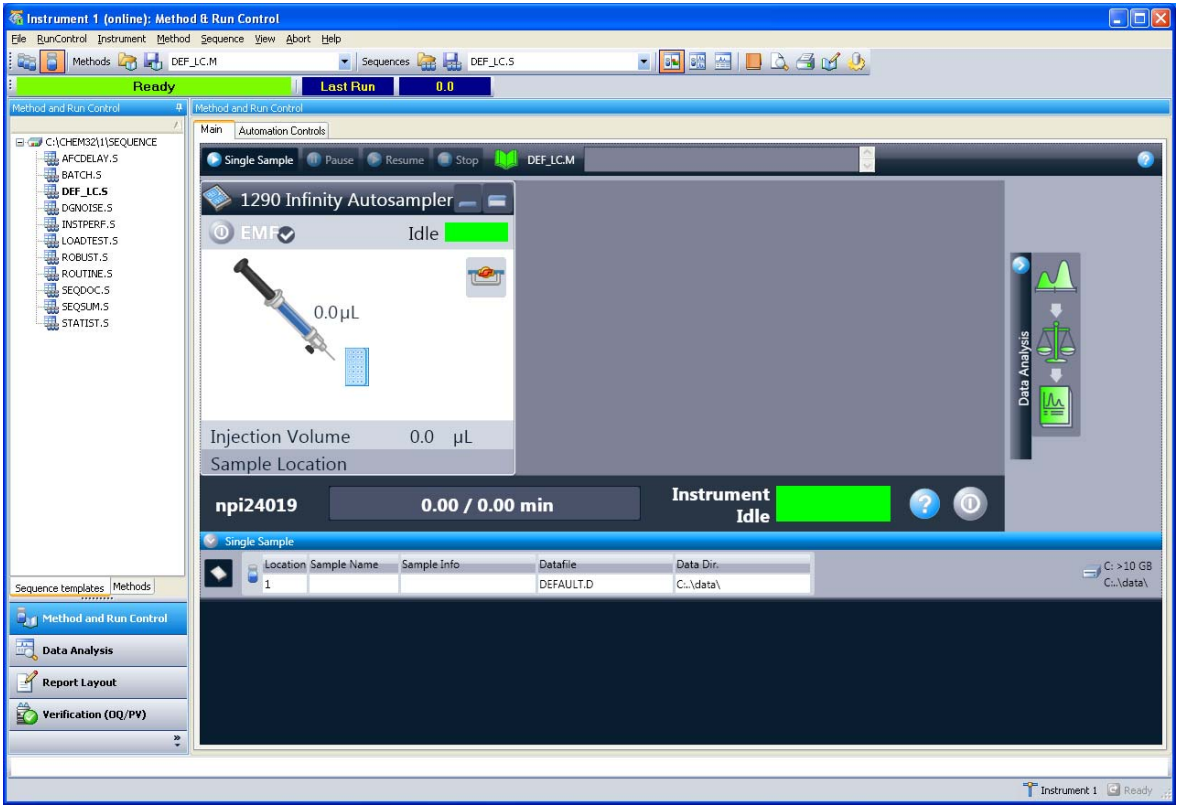
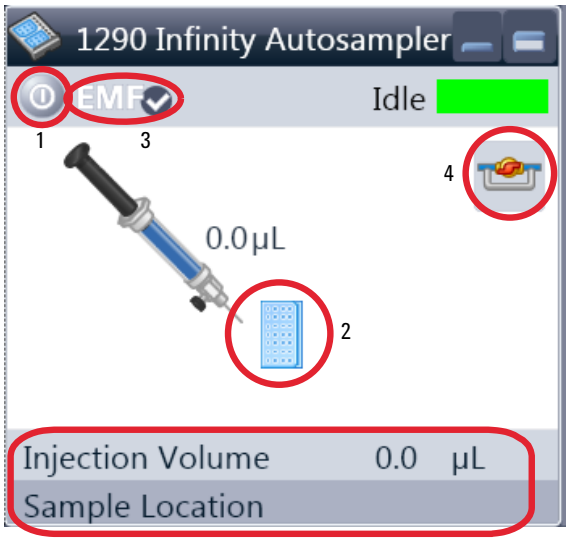


Figure 14 ChemStation Method & Run Control

The Autosampler User Interface



Within the Autosampler user interface, there are active areas. If you move the mouse cursor across the icons (tray, EMF button), the cursor will change and you may click on the icon to

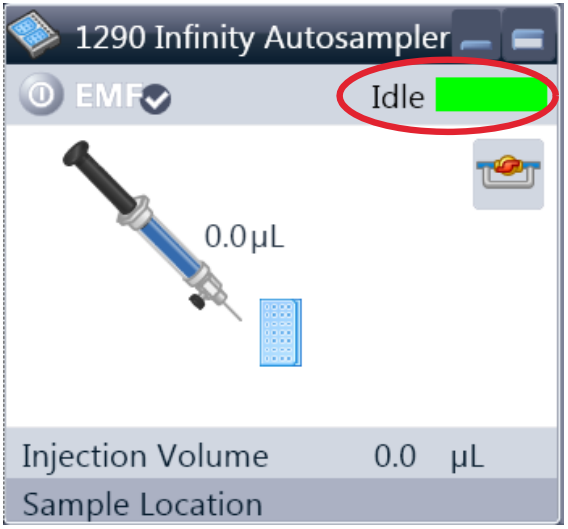
- Turn on/off the autosampler (1)
- Configure the sample tray (2)
- Get the status of the EMF (Early Maintenance Feature) (3)
- Switch injection valve to Mainpass / Bypass (4)

Instrument actuals Information

- Injection volume
- Sample location

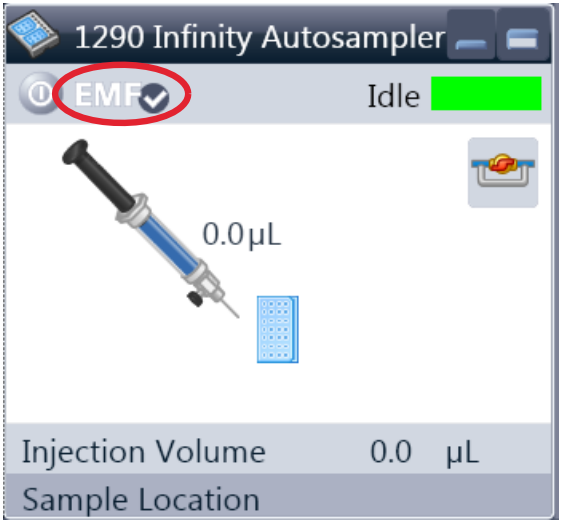
A right-click into the **Active Area** will open a menu to

- Show the **Control** User Interface (special module settings)
- Show the **Method** User interface (same as via menu Instrument – Setup G4226A)
- **Set Error Method**
- **Identify Device**
- **Home Arm**
- **Reset Sampler**
- **Wash Needle**
- **Needle Up**
- Valve Mainpass / Bypass (same as click on the valve icon)
- **Switch on Tray Illumination**
- **Edit Well Plate Types**
- Wellplate Configuration (same as click on the Tray icon)



Module Status shows Run / Ready / Error state and "Not Ready text" or "Error text".

- Error (Red)
- Not ready (yellow)
- Ready (green)
- Pre run, Post run (purple)
- Run (blue)
- Idle (green)
- Offline (dark gray)
- Standby (light gray)

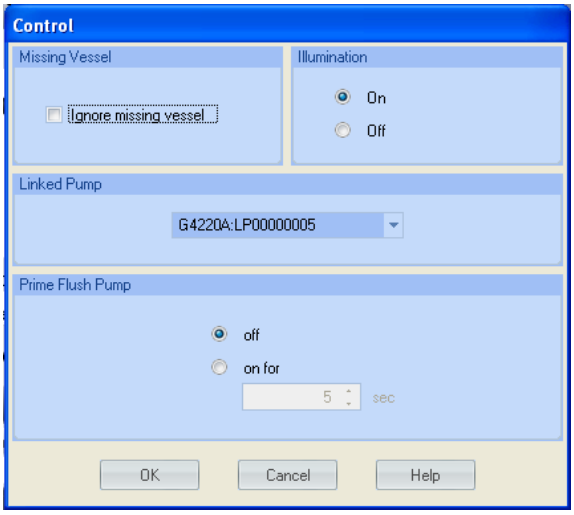


EMF Status shows Run / Ready / Error state and "Not Ready text" or "Error text".

- Offline (gray)
- Ok. No Maintenance required (green)
- EMF warning. Maintenance might be required (yellow)
- EMF warning. Maintenance required (red)

Control Settings

These settings are available via right click on the Active Area of the ALS GUI.



Missing Vessel: The handling of missing vessels can be configured.
Illumination: Can be turned on / off
Linked Pump: To configure which pump delivers flow to the Autosampler.
Prime Flush Pump: Priming the Needle wash flush pump.

Method Parameter Settings

These settings are available via **Menu > Instrument > Setup Agilent 1290 Infinity Autosampler** or via right click on the Active area.

NOTE The signal window in the lower part is not shown when opening the parameter settings via right mouse on the Autosampler user interface.

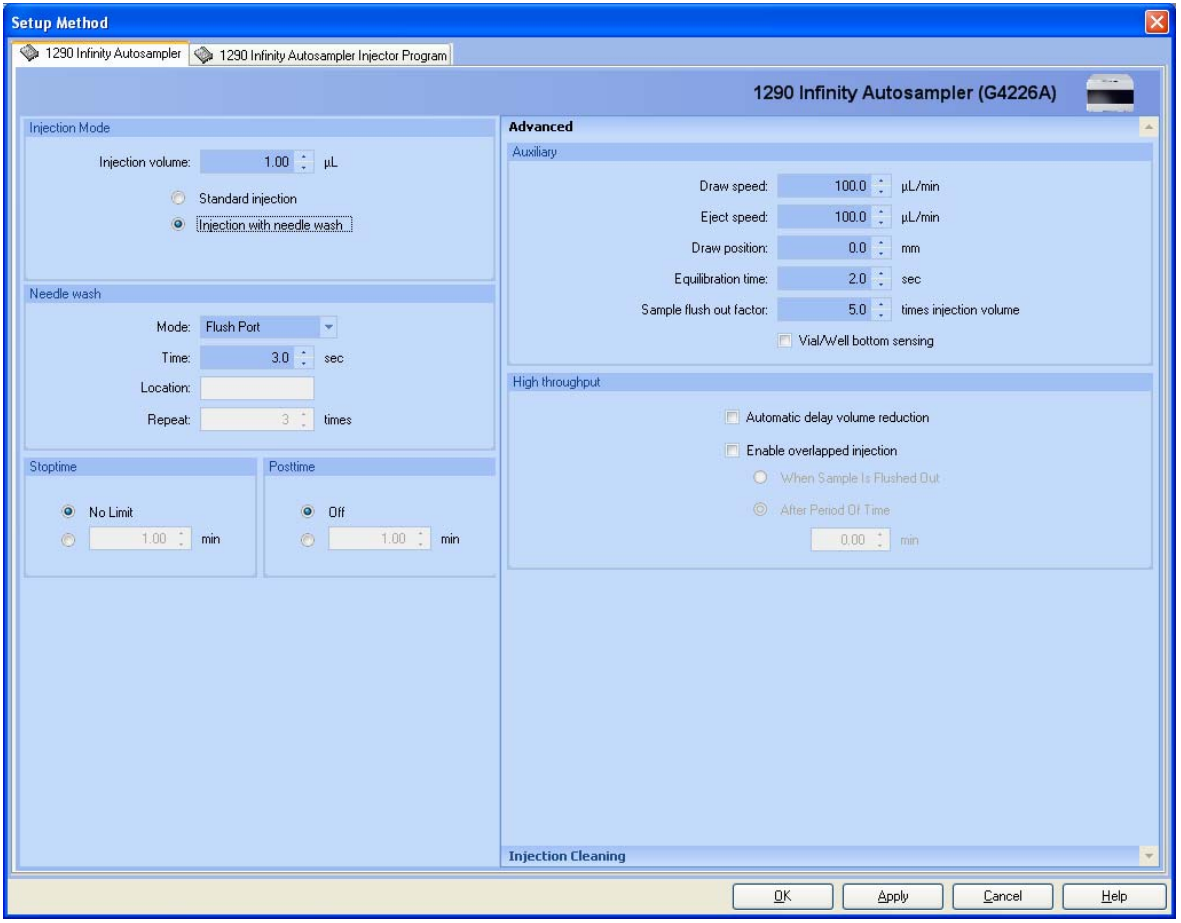
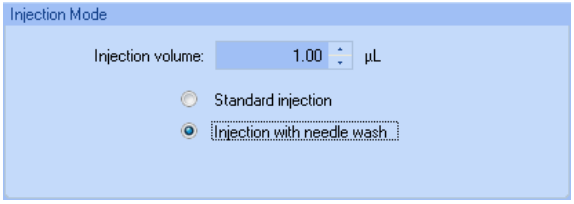


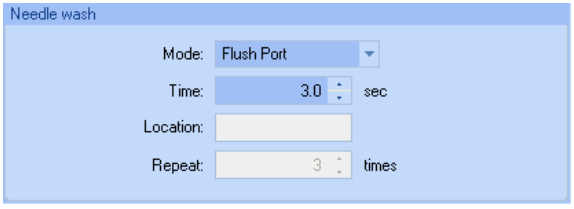
Figure 15 Method Parameter Settings

Injection Mode



The settable **Injection volume** range is from 0.1 – 20.0 µL. Select to use **Standard injection** or **Injection with Needle wash**.

Needle wash

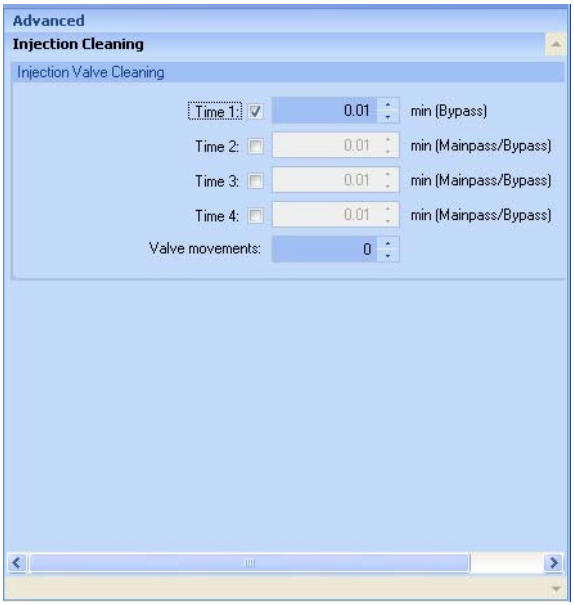


It is possible to select between using the built in flush port of the Autosampler or using a non-capped vial. Using **Needle wash** is required to obtain minimum carry-over.

Stop Time

An autosampler **Stop Time** can be set.

Injection Cleaning

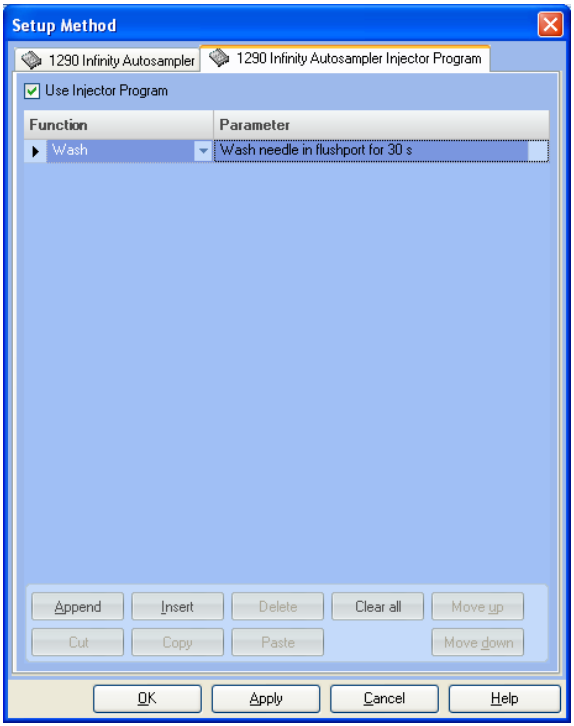


The **Injection Valve Cleaning** section allows you to specify the valve switching times at the end of overlap or sample flush.

Times 1 ... 4 are the times when the valve switches to bypass (for time 1) or to mainpass and bypass (for times 2, 3 and 4).The times must be specified in ascending order. You can also switch the times to off. Between the first and second, and second and third valve switches, a rinse is executed using the rinse volumes specified in the Injector Cleaning section.

Valve movements specifies the number of times that the valve switches from mainpass to bypass at times 2, 3 and 4 in the field.The maximum value is 2; default is 1.

Injection Program



The pretreatment/injector program comprises a series of numbered lines, each specifying an operation that the autosampler carries out sequentially. When you activate a pretreatment/injector program, it replaces the standard injection cycle.

Select **Append** to add the contents of the edit line to the end of the table.

Select **Insert** to insert the contents of the edit line above the currently-selected line.

Select **Delete** to delete the currently selected line.

Select **Clear All** to clear all pretreatment/injector program functions from the table.

Select **Move up** to move the currently selected line one position up in the order of execution.

Select **Move down** to move the currently selected line one position down in the order of execution.

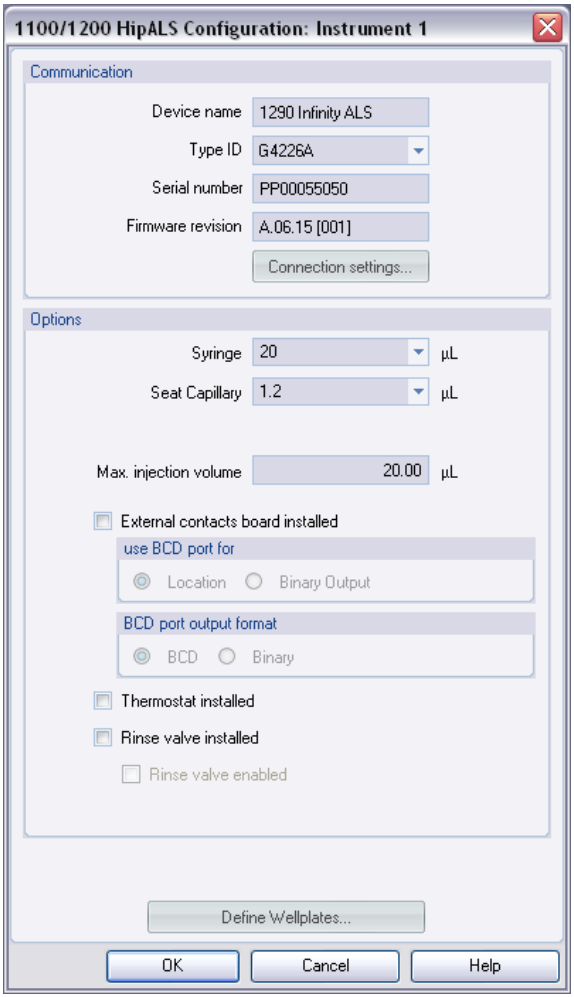
Select **Cut** to delete the currently-selected line and place it on the clipboard.

Select **Copy** to copy the currently selected line to the clipboard.

Select **Paste** to paste the line on the clipboard at the current position.

Module Configuration

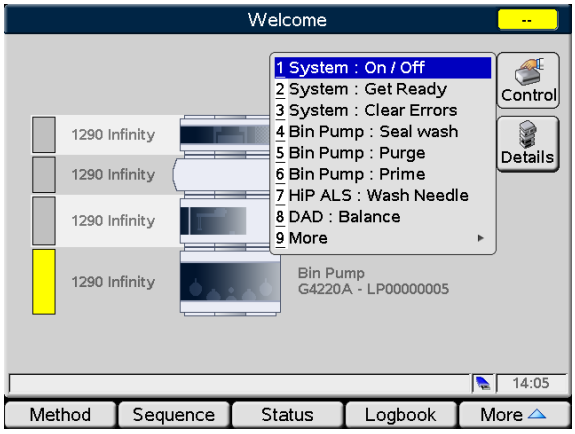
These settings are available via menu **Instrument > More Agilent 4220A > Autosampler Configuration**.



Device name: based on the module.
Type ID: based on the module (product number). Some modules may allow changing the type based on hardware/firmware. This results in a change of features and functions.
Serial number: based on the module.
Firmware revision: based on the module.
Options: lists installed options.

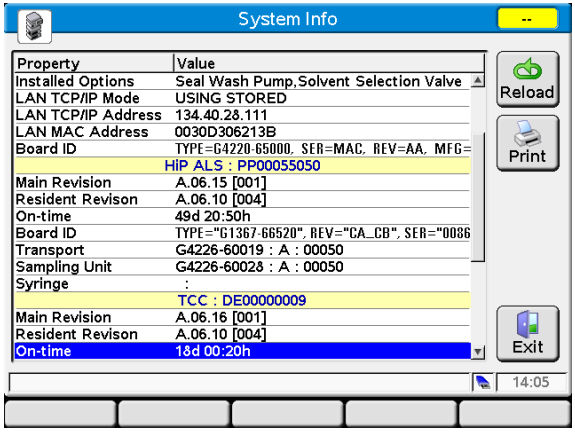
Main Screens of the Autosampler with Agilent Instant Pilot (G4208A)

Below the main screens for the use of the autosampler are shown.



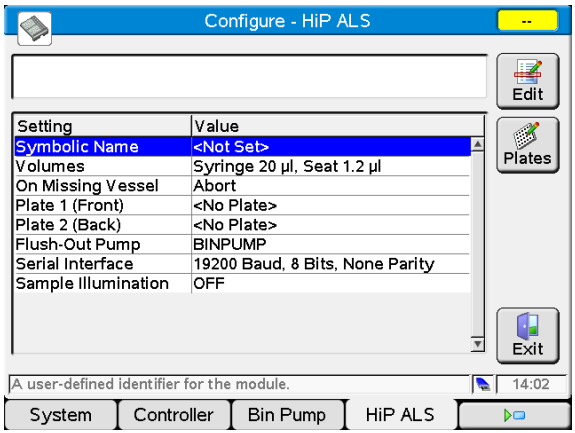
The **Control** screen allows

- System: On/Off
- System: Get Ready
- System: Clear Errors
- HIP ALS: Wash needle



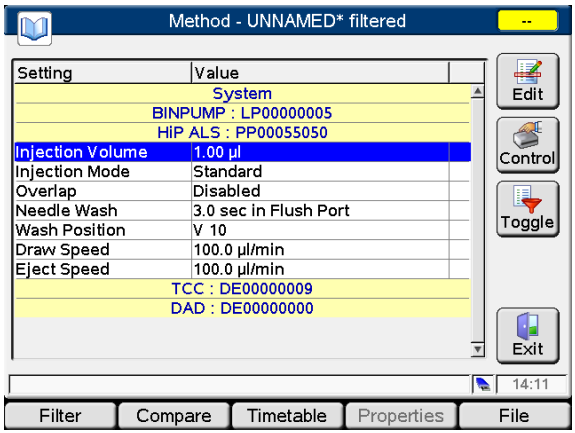
The **System Info** screen lists details of the autosampler

- Firmware revision
- On-time
- Main Board information
- Transport assembly information
- Sampling unit information
- Syringe information

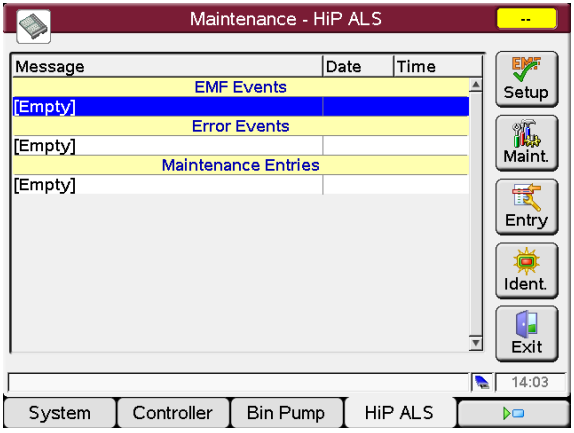


The **Configure** screen allows to configure

- Symbolic Name of module
- Volumes
- On Missing Vessel behaviour
- Plate configuration
- Flush-Out Pump
- Serial Interface configuration
- Sample Illumination



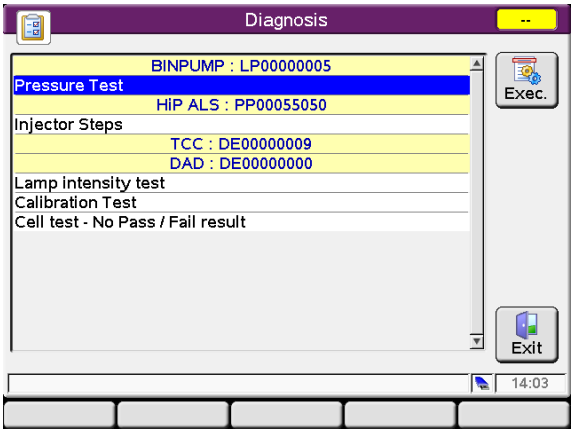
The **Method** screen lists all method parameters of the autosampler. These can be edited.



The **Maintenance** screen allows

- EMF setup
- logging of maintenance activities
- module identification (blinking LED)

Firmware updates can be done via the System Maintenance screen.



The **Diagnosis** screen provides access to module specific tests.

- Injector steps

Solvent Information

Observe the following recommendations on the use of solvents.

- Follow recommendations for avoiding the growth of algae, see pump manuals.
- Small particles can permanently block capillaries and valves. Therefore, always filter solvents through 0.4 µm filters.
- Avoid or minimize the use of solvents that may corrode parts in the flow path. Consider specifications for the pH range given for different materials like flow cells, valve materials etc. and recommendations in subsequent sections.

Material Information

Materials in the flow path are carefully selected based on Agilent's experiences in developing highest quality instruments for HPLC analysis over several decades. These materials exhibit excellent robustness under typical HPLC conditions. For any special conditions, please consult the material information section or contact Agilent.

Disclaimer

Subsequent data were collected from external resources and are meant as a reference. Agilent cannot guarantee the correctness and completeness of such information. Data is based on compatibility libraries, which are not specific for estimating the long-term life time under specific but highly variable conditions of UHPLC systems, solvents, solvent mixtures and samples. Information can also not be generalized due to catalytic effects of impurities like metal ions, complexing agents, oxygen etc. Apart from pure chemical corrosion, other effects like electro corrosion, electrostatic charging (especially for non-conductive organic solvents), swelling of polymer parts etc. need to be considered. Most data available refers to room temperature (typically 20 – 25 °C, 68 – 77 °F). If corrosion is possible, it usually accelerates at higher temperatures. If in doubt, please consult technical literature on chemical compatibility of materials.

PEEK

PEEK (Polyether-Ether Ketones) combines excellent properties regarding biocompatibility, chemical resistance, mechanical and thermal stability. PEEK is therefore the material of choice for UHPLC and biochemical instrumentation.

It is stable in a pH range between 1 – 12, and inert to many common solvents.

There is still a number of known incompatibilities with chemicals such as chloroform, methylene chloride, THF, DMSO, strong acids (nitric acid > 10 %, sulphuric acid > 10 %, sulfonic acids, trichloroacetic acid), halogenes or aequous halogene solutions, phenol and derivatives (cresols, salicylic acid etc.).

Polyimide

Agilent uses semi-crystalline polyimide for rotor seals in valves and needle seats in autosamplers. One supplier of polyimide is DuPont, which brands polyimide as Vespel, which is also used by Agilent.

Polyimide is stable in a pH range between 1 and 10 and in most organic solvents. It is incompatible with concentrated mineral acids (e.g. sulphuric acid), glacial acetic acid, DMSO and THF. It is also degraded by nucleophilic substances like ammonia (e.g. ammonium salts in basic conditions) or acetates.

Polyethylene (PE)

Agilent uses UHMW (ultra-high molecular weight)-PE/PTFE blends for yellow piston and wash seals, which are used in 1290 Infinity pumps and for normal phase applications in 1260 Infinity pumps.

Polyethylene has a good stability for most common inorganic solvents including acids and bases in a pH range of 1 to 12.5. It is compatible to many organic solvents used in chromatographic systems like methanol, acetonitrile and isopropanol. It has limited stability with aliphatic, aromatic and halogenated hydrocarbons, THF, phenol and derivatives, concentrated acids and bases. For normal phase applications, the maximum pressure should be limited to 200 bar.

Tantalum (Ta)

Tantalum is inert to most common HPLC solvents and almost all acids except fluoric acid and acids with free sulfur trioxide. It can be corroded by strong bases (e.g. hydroxide solutions > 10 %, diethylamine). It is not recommended for the use with fluoric acid and fluorides.

Stainless Steel (ST)

Stainless steel is inert against many common solvents. It is stable in the presence of acids and bases in a pH range of 1 to 12.5. It can be corroded by acids below pH 2.3. It can also corrode in following solvents:

- Solutions of alkali halides, their respective acids (for example, lithium iodide, potassium chloride, and so on) and aqueous solutions of halogens.
- High concentrations of inorganic acids like nitric acid, sulfuric acid and organic solvents especially at higher temperatures (replace, if your chromatography method allows, by phosphoric acid or phosphate buffer which are less corrosive against stainless steel).
- Halogenated solvents or mixtures which form radicals and/or acids, for example:

  $2\ CHCl_3 + O_2 \rightarrow 2\ COCl_2 + 2\ HCl$

  This reaction, in which stainless steel probably acts as a catalyst, occurs quickly with dried chloroform if the drying process removes the stabilizing alcohol.
- Chromatographic grade ethers, which can contain peroxides (for example, THF, dioxane, di-isopropylether). Such ethers should be filtered through dry aluminium oxide which adsorbs the peroxides.
- Solutions of organic acids (acetic acid, formic acid, and so on) in organic solvents. For example, a 1 % solution of acetic acid in methanol will attack steel.
- Solutions containing strong complexing agents (for example, EDTA, ethylene diamine tetra-acetic acid).
- Mixtures of carbon tetrachloride with 2-propanol or THF.

Diamond-Like Carbon (DLC)

Diamond-Like Carbon is inert to almost all common acids, bases and solvents. There are no documented incompatibilities for HPLC applications.

Fused silica and Quartz (SiO_2)

Fused silica is used in 1290 Infinity Flow Cells and capillaries. Quartz is used for classical flow cell windows. It is inert against all common solvents and acids except hydrofluoric acid and acidic solvents containing fluorides. It is corroded by strong bases and should not be used above pH 12 at room temperature. The corrosion of flow cell windows can negatively affect measurement results. For a pH greater than 12, the use of flow cells with sapphire windows is recommended.

Gold

Gold is inert to all common HPLC solvents, acids and bases within the specified pH range. It can be corroded by complexing cyanides and concentrated acids like aqua regia.

Zirconium Oxide (ZrO_2)

Zirconium Oxide is inert to almost all common acids, bases and solvents. There are no documented incompatibilities for HPLC applications.

Platinum/Iridium

Platinum/Iridium is inert to almost all common acids, bases and solvents. There are no documented incompatibilities for HPLC applications.

Fluorinated polymers (PTFE, PFA, FEP, FFKM)

Fluorinated polymers like PTFE (polytetrafluorethylene), PFA (perfluoroalkoxy) and FEP (fluorinated ethylene propylene) are inert to almost all common acids, bases, and solvents. FFKM is perfluorinated rubber, which is also resistant to most chemicals. As an elastomer, it may swell in some organic solvents like halogenated hydrocarbons.

TFE/PDD copolymer tubings, which are used in all Agilent degassers except G1322A, are not compatible with fluorinated solvents like Freon, Fluorinert, or Vertrel. They have limited life time in the presence of

Hexafluoroisopropanol (HFIP). To ensure the longest possible life with HFIP, it is best to dedicate a particular chamber to this solvent, not to switch solvents, and not to let dry out the chamber. For optimizing the life of the pressure sensor, do not leave HFIP in the chamber when the unit is off.

Sapphire, Ruby and Al_2O_3-based ceramics

Sapphire, ruby and ceramics based on aluminum oxide Al_2O_3 are inert to almost all common acids, bases and solvents. There are no documented incompatibilities for HPLC applications.

5 Optimizing Performance

This chapter gives hints on how to optimize the performance or use additional devices.

Delay Volume and Extra-Column Volume

The *delay volume* is defined as the system volume between the point of mixing in the pump and the top of the column.

The *extra-column volume* is defined as the volume between the injection point and the detection point, excluding the volume in the column.

Delay Volume

In gradient separations, this volume causes a delay between the mixture changing in the pump and that change reaching the column. The delay depends on the flow rate and the delay volume of the system. In effect, this means that in every HPLC system there is an additional isocratic segment in the gradient profile at the start of every run. Usually the gradient profile is reported in terms of the mixture settings at the pump and the delay volume is not quoted even though this will have an effect on the chromatography. This effect becomes more significant at low flow rates and small column volumes and can have a large impact on the transferability of gradient methods. It is important, therefore, for fast gradient separations to have small delay volumes, especially with narrow bore columns (e.g., 2.1 mm i.d.) as often used with mass spectrometric detection.

The delay volume in a system includes the volume in the pump from the point of mixing, connections between pump and autosampler, volume of the flow path through the autosampler and connections between autosampler and column.

How to Configure the Optimum Delay Volume

For very fast gradients over 0.5 min, which can only be achieved in the Agilent 1290 Infinity Binary LC System, the delay volume of the system can be easily reduced without changing the physical configuration of the system. This change is achieved by changing the behavior of the autosampler.

The 80 µl delay volume of the Agilent 1290 Infinity Autosampler is due to the flow path from the injection valve through the metering device, needle, needle seat and connecting capillaries back to the injection valve (see Figure 16 on page 72). To make an injection the valve switches from mainpass to bypass so that the metering device can draw the sample into the needle capillary. The injection is made when the valve switches back to mainpass and the sample is flushed onto the column. The valve remains in this position during analysis so that the autosampler is continually flushed and hence the gradient has to flow through this delay volume to reach the column. This can be eliminated by switching the injection valve from mainpass to bypass after the injection has been made and the injected sample has been flushed onto the column. In practice this can be done a few seconds after injection and is activated by selecting the "Automatic Delay Volume Reduction" (ADVR) function in the autosampler setup menu. The Flush-out Factor (typically 5 times injection volume) ensures that enough time is allowed to flush the sample out of the injector before switching to bypass. This effectively reduces the system delay volume from 125 µl to 50 µl.

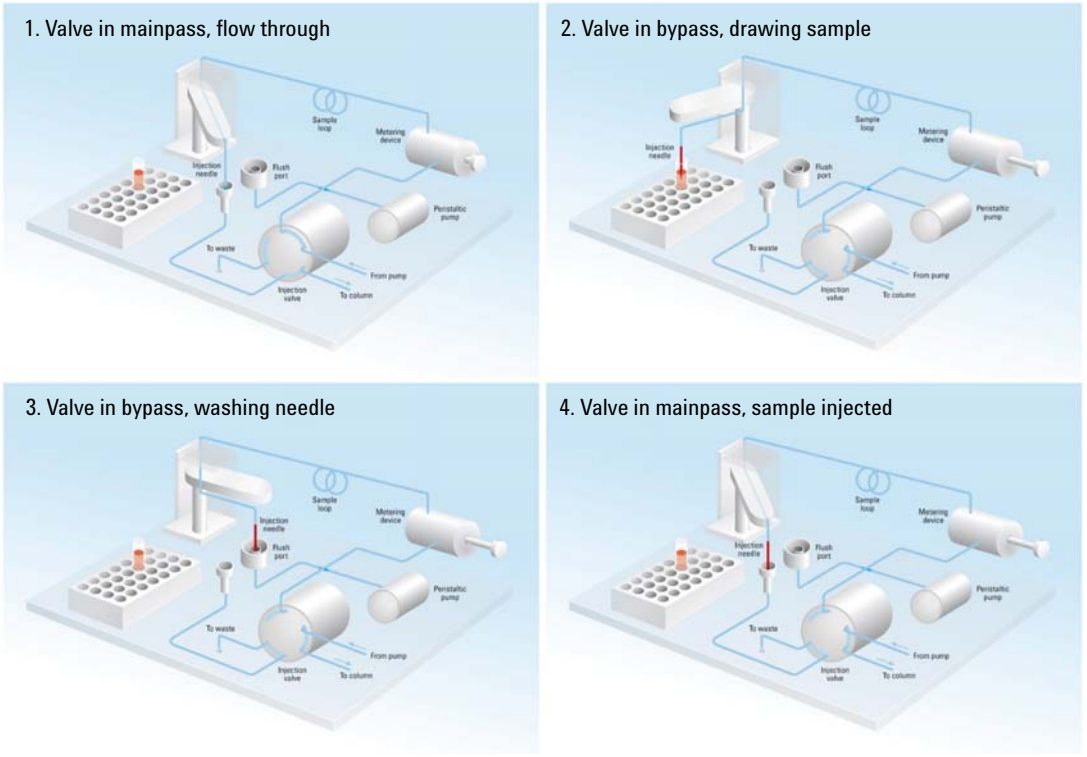


Figure 16 Schematic of injection steps in 1290 Infinity Autosampler

When using ADVR it should be noted that the gradient has already started at the pump at the instant of injection. The question should be asked whether the gradient has already reached the autosampler, in which case a small step in the gradient will result. This happens when the delay volume is less than the flush-out volume and is not necessarily a problem but may be a factor to be considered in a method transfer. With a flush-out factor of 5 and an injection volume of 10 µl, the autosampler will allow 50 µl to pass through before switching to bypass which, with a delay volume of 50 µl, means the gradient just reached the injection valve. Smaller injection volumes will have no effect but for larger injection volumes this will introduce a small step in the gradient. The flow rate in use will also have an impact on the decision to use ADVR or not. At 0.2 ml/min the delay time saved is 21 seconds while at 1.0 ml/min it is 4 seconds.

The ADVR function is unlikely to be suitable for applications involving compounds which are known to cause carry-over problems.

How to Achieve Higher Injection Volumes

The standard configuration of the Agilent 1290 Infinity Autosampler includes a variable volume sample loop for up to 20 µl injections. The metering device can inject a maximum volume of 40 µl and the sample loop cartridge can be exchanged to allow this. The system delay volume due to the autosampler will increase accordingly.

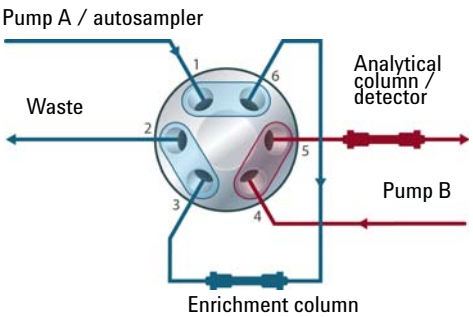
To extend the injection range once again you can either use a 1290 Infinity large volume kit (G4266-68714) which increases the range of the injection volume up to 100 µL or 120 µL depending which loop size is installed or install the 100 µL Upgrade Kit (G4214A) which is reducing the pressure limit to 600 bar.

Whenever a method is scaled down from a larger column to a smaller column it is important that the method translation makes an allowance for reducing the injection volume in proportion to the volume of the column to maintain the performance of the method. This is to keep the volume of the injection at the same percentage volume with respect to the column. This is particular important if the injection solvent is stronger (more eluotropic) than the starting mobile phase and any increase will affect the separation particularly for early running peaks (low retention factor). In some cases it is the cause of peak distortion and the general rule is to keep the injection solvent the same or weaker than the starting gradient composition. This has a bearing on whether, or by how much, the injection volume can be increased and the user should check for signs of increased dispersion (wider or more skewed peaks and reduced peak resolution) in trying to increase the injection size. If an injection is made in a weak solvent then the volume can probably be increased further because the effect will be to concentrate the analyte on the head of the column at the start of the gradient. Conversely if the injection is in a stronger solvent than the starting mobile phase then increased injection volume will spread the band of analyte down the column ahead of the gradient resulting in peak dispersion and loss of resolution.

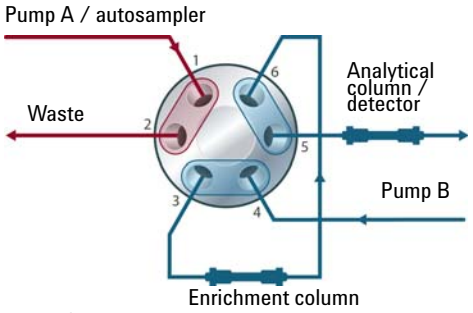
Perhaps the main consideration in determining injection volume is the diameter of the column as this will have a big impact on peak dispersion. Peak heights can be higher on a narrow column than with a larger injection on a wider column because there is less peak dispersion. With 2.1 mm i.d. columns typical injection volumes might range up to 5 to10 µl but it is very dependent on the chemistry of the analyte and mobile phase as discussed above. In a gradient separation injection volumes of about 5 % of the column volume might be achieved whilst maintaining good resolution and peak dispersion.

One way to achieve larger injections is to use a trapping column selected by a switching valve to capture and concentrate the injection before switching it, i.e. injecting it, onto an analytical column, see "Sample Enrichment" on page 74. The valve can be conveniently located in the thermostatted column compartment or in the flexible cube.

Sample Enrichment



Position 1



Position 2

How to Achieve High Throughput

The injection can be optimized for speed remembering that drawing the sample too fast can reduce the reproducibility. Marginal gains are to be made here as the sample volumes used tend towards the smaller end of the range in any case. A significant portion of the injection time is the time taken with the needle movements to and from the vial and into the flush port. These manipulations can be performed while the previous separation is running. This is known as "overlapped injection" and it can be easily turned on from the autosampler setup screen in the control software. The autosampler can be told to switch the flow through the autosampler to bypass after the injection has been made and then after, for example, 3 minutes into a 4 minutes run to start the process of aspirating the next sample and preparing for injection. This can typically save 0.5 to 1 minute per injection.

How to Achieve Higher Resolution

Increased resolution in a separation will improve the qualitative and quantitative data analysis, allow more peaks to be separated or offer further scope for speeding up the separation. This section explains how resolution can be increased by examining the following points:

- Optimize selectivity
- Smaller particle-size packing
- Longer Columns
- Shallower gradients, faster flow

Resolution between two peaks is described by the resolution equation:

$$Rs = \frac{1}{4}\sqrt{N}\,\frac{(\alpha - 1)}{\alpha}\,\frac{(k_2 + 1)}{k_2}$$

where

- R_S=resolution,
- N=plate count (measure of column efficiency),
- α=selectivity (between two peaks),
- k_2=retention factor of second peak (formerly called capacity factor).

The term that has the most significant effect on resolution is the selectivity, α, and practically varying this term involves changing the type of stationary phase (C18, C8, phenyl, nitrile etc.), the mobile phase and temperature to maximize the selectivity differences between the solutes to be separated. This is a substantial piece of work which is best done with an automated method development system which allows a wide range of conditions on different columns and mobile phases to be assessed in an ordered scouting protocol. This section considers how to get higher resolution with any chosen stationary and mobile phases. If an automated method development system was used in the decision on phases it is likely that short columns were used for fast analysis in each step of the scouting.

The resolution equation shows that the next most significant term is the plate count or efficiency, N, and this can be optimized in a number of ways. N is inversely proportional to the particle size and directly proportional to the length of a column and so smaller particle size and a longer column will give a higher plate number. The pressure rises with the inverse square of the particle size and proportionally with the length of the column. This is the reason that the 1290 Infinity LC system was designed to go to 1200 bar so that it can run sub-two-micron particles and column length can be increased to 100 mm or 150 mm. There are even examples of 100 mm and 150 mm columns linked to give 250 mm length. Resolution increases with the square root of N so doubling the length of the column will increase resolution by a factor of 1.4. What is achievable depends on the viscosity of the mobile phase as this relates directly to the pressure. Methanol mixtures will generate more back pressure than acetonitrile mixtures. Acetonitrile is often preferred because peak shapes are better and narrower in addition to the lower viscosity but methanol generally yields better selectivity (certainly for small molecules less than about 500 Da). The viscosity can be reduced by increasing the temperature but it should be remembered that this can change the selectivity of the separation. Experiment will show if this leads to increase or decrease in selectivity. As flow and pressure are increased it should be remembered that frictional heating inside the column will increase and that can lead to slightly increased dispersion and possibly a small selectivity change both of which could be seen as a reduction in resolution. The latter case might be offset by reducing the temperature of the thermostat by a few degrees and again experiment will reveal the answer.

The van Deemter curve shows that the optimum flow rate through an STM column is higher than for larger particles and is fairly flat as the flow rate increases. Typical, close to optimum, flow rates for STM columns are: 2 ml/min for 4.6 mm i.d.; and 0.4 ml/min for 2.1 mm i.d. columns.

In isocratic separations, increasing the retention factor, k, results in better resolution because the solute is retained longer. In gradient separations the retention is described by k^* in the following equation:

$$k^* = \frac{t_G}{\Delta\%B} \cdot \frac{F}{V_m} \cdot \frac{100}{S}$$

where:

- k^* = mean k value,
- t_G = time length of gradient (or segment of gradient) (min),
- F = flow (ml/min),
- V_m = column delay volume,
- Δ%B = change in fraction of solvent B during the gradient,
- S = constant (ca. 4-5 for small molecules).

This shows that k and hence resolution can be increased by having a shallower gradient (2 to 5 %/min change is a guideline), higher flow rate and a smaller volume column. This equation also shows how to speed up an existing gradient – if the flow is doubled but the gradient time is halved, k^* remains constant and the separation looks the same but happens in half the time. Recently published research has shown how a shorter STM column (at temperatures above 40 °C) can generate higher peak capacity than a longer STM column by virtue of running it faster. (Refer to *Petersson et al., J.Sep.Sci, 31, 2346-2357, 2008, Maximizing peak capacity and separation speed in liquid chromatography*).

How to Achieve Higher Sensitivity

The sensitivity of a separation method is linked to the choice of stationary and mobile phases as good separation with narrow peaks and a stable baseline with minimal noise are desirable. The choice of instrument configuration will have an effect and a major impact is the setup of the detector. This section considers how sensitivity is affected by:

- Pump mixer volume
- Narrower columns
- Detector flow cell
- Detector parameters

In addition, the discussion on detector parameters also mentions the related topics of selectivity and linearity.

Columns

Sensitivity is specified as a signal-to-noise ratio (S/N) and hence the need to maximize peak height and minimize baseline noise. Any reduction in peak dispersion will help to maintain peak height and so extra-column volume should be minimized by use of short, narrow internal diameter, connection capillaries and correctly installed fittings. Using smaller inner diameter columns should result in higher peak height and is therefore ideal for applications with limited sample amounts. If the same sample amount can be injected on a smaller i.d. column, then the dilution due to column diameter will be less and the sensitivity will increase. For example, decreasing the column i.d. from 4.6 mm to 2.1 mm results in a theoretical gain in peak height of 4.7 times due to the decreased dilution in the column. For a mass spectrometer detector, the lower flow rates of narrow columns can result in higher ionization efficiencies and therefore higher sensitivity.

How to Achieve Higher Sensitivity for Detector

The detector has a number of parameters that are used to optimize performance. The following sections describe how the detector parameters affect performance characteristics:

- Flow cell affects sensitivity,
- Wavelength and bandwidth affect sensitivity, selectivity and linearity,
- Slit Width affects sensitivity, spectral resolution and linearity,
- Peak Width affects sensitivity and resolution.

Flow Cell

The Max-Light cartridge flow cell has a standard 10 mm path length and is optimized for minimal volume and dispersion (σ volume 1.0 µL). It has high light transmission minimizing noise to reduce noise due to the optofluidic waveguide. It is suitable for use with a wide range of analytical columns from short narrow-bore columns to long standard diameter (4.6 mm) columns. Generally the peak dispersion volume (calculated from peak width x flow rate) should be greater than about 2 µL for this cell (for example 0.02 min x 200 µL/min = 4 µL).

The Max-Light high sensitivity cell has a path length of 60 mm and this will give between three and five times increase in signal-to-noise values depending on the application conditions. The dispersion volume is fractionally increased compared to the standard cell.

Wavelength and Bandwidth

The detector measures absorbance simultaneously at wavelengths from 190 nm to 640 nm using diode-array detection. A UV-lamp provides good sensitivity over the whole wavelength range. The diode-array detector (DAD) can simultaneously compute and send to the data system up to eight chromatographic signals and the full-range spectra at every time point.

A UV chromatogram or signal is a plot of absorbance data versus time and is defined by its wavelength and bandwidth.

- The wavelength indicates the center of the detection band.
- The bandwidth defines the wavelength range over which the absorbance values are averaged to give the result at each time point.

For example, a signal at wavelength 250 nm with a bandwidth of 16 nm will be an average of the absorbance data from 242 nm to 258 nm. Additionally, a reference wavelength and reference bandwidth can be defined for each signal. The average absorbance from the reference bandwidth centered on the reference wavelength will be subtracted from its equivalent value at the signal wavelength to produce the output chromatogram.

The signal wavelength and bandwidth can be chosen so that they are optimized for:

- Broad band universal detection
- Narrow band selective detection
- Sensitivity for a specific analyte.

Broad band or universal detection works by having a wide bandwidth to detect any species with absorbance in that range. For example, to detect all absorbing molecules between 200 nm and 300 nm set a signal at 250 nm with a bandwidth of 100 nm. The disadvantage is that sensitivity will not be optimal for any one of those molecules. Narrow band or selective detection is used most often. The UV spectrum for a particular molecule is examined and an appropriate absorbance maximum is selected. If possible, the range where solvents absorb strongly should be avoided (below 220 nm for methanol, below 210 nm for acetonitrile). For example, in Figure 17 on page 83, anisic acid has a suitable absorbance maximum at 252 nm. A narrow bandwidth of 4 nm to 12 nm generally gives good sensitivity and is specific for absorbance in a narrow range.

The narrow band can be optimized for sensitivity for a specific molecule. As the bandwidth is increased the signal is reduced but so is the noise and there will be an optimum for best S/N. As an approximate guide, this optimum is often close to the natural bandwidth at half-height of the absorption band in the UV spectrum. In the anisic acid example this is 30 nm.

The analytical wavelength is usually set at a wavelength maximum to increase sensitivity to that molecule. The detector is linear up to 2 AU and beyond for many applications. This offers a wide linear range for concentration. For high concentration analysis the concentration linear range can be extended by setting the wavelength to one with a lower absorbance such as a wavelength minimum or by taking a wider bandwidth which usually includes lower absorbance values. The use of wavelength maxima and minima for quantitation dates back to

conventional UV detectors which because of mechanical tolerances in moving gratings needed to avoid steeply sloping parts of the spectrum. Diode-array based detectors do not have this limitation but for reasons of convention maxima and minima are chosen in preference to other parts of the spectrum.

The reference bandwidth is normally set on a region of the UV spectrum in which the analyte has no absorbance. This is shown in the spectrum for anisic acid in Figure 17 on page 83. This spectrum is typical of many small molecules containing a UV chromophore. For best results the reference has been set so that it is a wide band as close to the signal wavelength as possible but on a zero absorbance region. Reference bandwidths of 60 nm to 100 nm are commonly used. The default reference is 360 nm with a bandwidth of 100 nm. A wide bandwidth is used because this reduces the noise in the reference signal (from statistical theory, the error, i.e. noise in this case, is reduced by the square root of the number of determinations). It is important that the reference bandwidth does not extend to a part of the spectrum that has some absorbance as this would then reduce the resulting signal and sensitivity would be reduced. The use of a reference wavelength can help to reduce drift or wander in the chromatogram caused by refractive index changes due to room temperature fluctuation or gradient operation. The effect of a reference signal can be easily tested by setting two otherwise identical signals, one with and one without a reference signal. If there is no part of the spectrum with zero absorbance then it will be better to have the reference signal turned off.

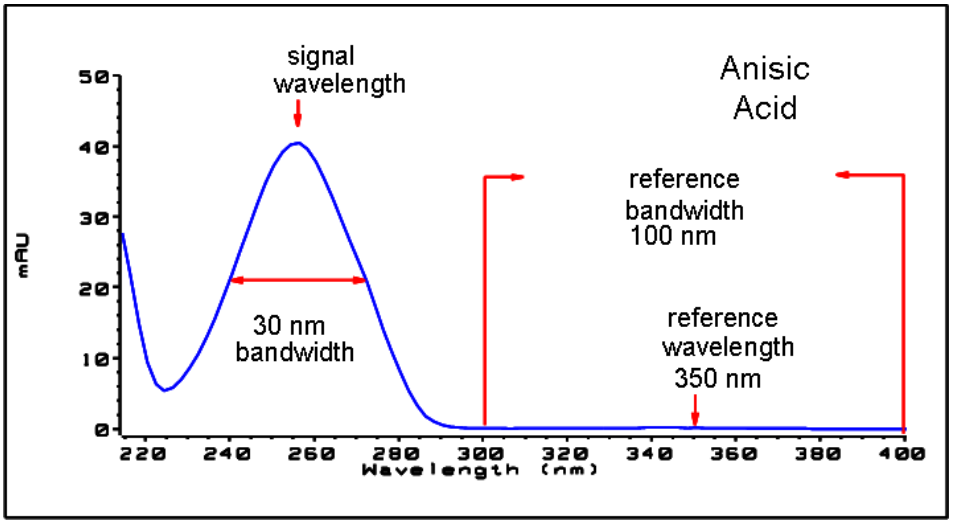


Figure 17 Spectrum of Anisic Acid

Slit Width (G4212A only)

Light transmission into the spectrograph and the optical bandwidth are controlled by the variable aperture entrance slit. The default setting for the slit width is 4 nm which is appropriate for most applications as it gives good all-round performance. The performance characteristics affected are sensitivity, spectral resolution and linearity. Considering a particular wavelength entering the spectrograph, its light will effectively fall onto a small band of diodes, the width of which is proportional to the width of the entrance slit. The description of the slit as 4 nm describes this behavior – the light falls on the number of diodes that detect a bandwidth of 4 nm. It follows that the minimum optical resolution will be 4 nm and therefore the diode-array (or digital) bandwidth should be set to 4 nm or greater. For optimum sensitivity the 8 nm setting will allow most light in and will minimize noise but spectral resolution is at its lowest. This is not usually a problem with UV spectra as their natural bandwidths are usually greater than 25 nm without any fine structure. The optical bandwidth at 8 nm reduces the linearity range compared to 4 nm slit so it is important that a validated method always uses the slit width that was used for validation. For optimum spectral resolution the 1nm setting is best. This will enable fine structure such as in the benzene spectrum to be resolved (see Figure 18 on page 84). Very few compounds display such fine detail in solution spectra. The light level will be lower so the signal will have more noise - the noise level depends on the wavelength and mobile phase solvents used.

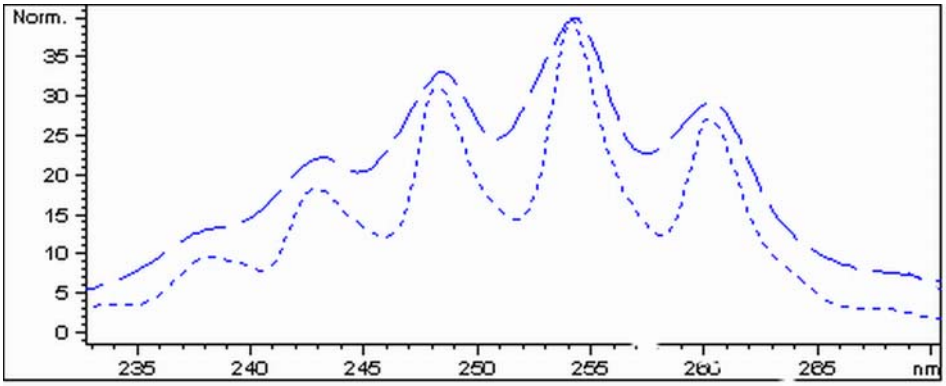


Figure 18 Benzene at 1 and 4 nm Slit Width (Principle)

The injection volume and the sample dissolution solvent are important in controlling dispersion. Care must be taken that the compounds are focused at the top of the column, to avoid peak dispersion due to the injection, which would cause a reduced peak height. To achieve this, the sample should be dissolved in a solvent composition of lower elution strength than the mobile phase. It may be possible to increase the injection volume to have a greater concentration of analyte on the column and hence increased peak height.

Peak Width, Response Time and Data Collection Rate

The peak width setting, response time and data rate in the detector are all linked. The available settings are shown in Table 5 on page 86. It is important to set this correctly for optimum sensitivity and to preserve the resolution achieved in the separation.

The detector internally acquires data points faster than is needed for a chromatogram and processes them to produce the signal seen by the data system. Part of the processing reduces the data to an appropriate data rate which allows the chromatographic peaks to be accurately drawn. As with most analytical determinations groups of readings are effectively averaged to reduce error in the result. The detector bunches raw data points and produces the output signal data at the required data collection rate by an electronic filtering process. If the resulting data rate is too slow (over filtering) the peak heights will be reduced and the resolution between them reduced; too fast and the data is noisier than it need be to accurately profile narrow peaks.

The *peak width* setting in the detector allows the user to correctly set these parameters without needing any knowledge other than sight of the chromatogram integration results to see how wide the peaks are. The peak width setting should be set for the narrowest peak width observed in the chromatogram. If it is set too wide it will make the peaks appear lower in height and wider (and potentially less resolved) and if it is set too narrow it will increase the baseline noise unnecessarily. Essentially the software uses this value to set the *data collection rate* such that it collects enough data points over the narrowest peaks and it is aiming for 15 to 25 points across a peak. The 1290 Infinity DAD can collect at a maximum 160 Hz if required which would allow enough data points to be collected over a peak that is only 0.1 s wide. The *response time* setting is another way of indicating how this filtering is set. It is measured in seconds and is about one-third of the peak width value (which is measured in minutes). It effectively shows how quickly the plotted signal responds to a step change in the input signal.

NOTE

The full spectra is not available under all conditions.

Based on the data points, the scan data rate is reduced, see Table 5 on page 86.

Table 5 Peak Width — Response Time — Data Rate

Peak width at half height [min][1]	Response [s]	Signal data rate [Hz]	Scan data rate [HZ] ≤126 pts/scan	Scan data rate [HZ] ≤251 pts/scan	Scan data rate [HZ] ≤501 pts/scan	Scan data rate [HZ] >501 pts/scan
< 0.0016	0.016	160[2]	160[2]	80	40	20
> 0.0016	0.03	160[2]	160[2]	80	40	20
> 0.003	0.062	80	80	80	80	40
> 0.006	0.12	40	40	40	40	40
> 0.012	0.25	20	20	20	20	20
> 0.025	0.5	10	10	10	10	10
> 0.05	1.0	5	5	5	5	5
> 0.10	2.0	2.5	2.5	2.5	2.5	2.5
> 0.20	4.0	1.25	1.25	1.25	1.25	1.25
> 0.40	8.0	0.625	0.62	0.625	0.625	0.625
> 0.85	16.0	0.3125	0.31	0.3125	0.3125	0.3125

1 Values in the User Interface may be rounded.

2 G4212A only

How to Achieve Lowest Carry Over

Carryover is measured when residual peaks from a previous active-containing injection appear in a subsequent blank solvent injection. There will be carry over between active injections which may lead to erroneous results. The level of carryover is reported as the area of the peak in the blank solution expressed as a percentage of the area in the previous active injection. The Agilent 1290 Infinity autosampler is optimized for lowest carryover by careful design of the flow path and use of materials in which sample adsorption is minimized. A carryover figure of 0.002 % should be achievable even when a triple quadrupole mass spectrometer is the detector. Operating settings of the autosampler allow the user to set appropriate parameters to minimize carryover in any application involving compounds liable to stick in the system.

The following functions of the autosampler can be used to minimize carryover:

- Internal needle wash
- External needle wash
- Needle seat backflush
- Injection valve cleaning

The flow path, including the inside of the needle, is continuously flushed in normal operation, providing good elimination of carryover for most situations. Automated delay volume reduction (ADVR) will reduce the delay volume but will also reduce the flushing of the autosampler and should not be used with analytes where carryover might be a problem.

The outside of the needle can be washed using a wash vial in a specific location or the needle can be washed using the flush port. If a wash vial in a tray location specified by the user is chosen then this vial should have no septum and should contain a solvent suitable for washing the sample from the needle. The septum is not used to avoid wiping contamination off the needle on the downstream only to re-apply it on the upstroke. The needle can be dipped into the vial multiple times. This will be effective in removing a small degree of carryover but for more effective washing of the outside of the needle use the flushport.

The flush port is located above and behind the needle seat and a peristaltic pump delivers the wash solvent. It has a volume of 0.68 ml and the peristaltic pump delivers 6 ml/min, which means the flush port volume is completely refilled with fresh solvent in 7 s. If the flush port is selected, the user can set how long the outside of the needle is to be washed with fresh solvent. This may be as low as two or three seconds in routine situations where carryover is less of a problem and 10 to 20 s for more complete washing. It is recommended that washing the outside of the needle in the flush port should be standard procedure to avoid contaminating the needle seat. If the needle seat becomes contaminated it will have to be back-flushed, by manually changing the flow connections, to clean it. This is one of the tasks that can be automated using the Flexible Cube module.

The flush port and its solvent delivery pump and tubing should be regularly flushed to ensure the lowest carryover. For example, before using the system each day, prime the flush pump for three minutes with appropriate solvent.

When other measures have failed to eliminate carryover it might be that analyte is sticking inside the injector valve. The injector valve can be set to make additional switching movements to clean out the flow path in the valve if problems occur here with carryover. If the problem compounds need a high percentage of organic phase for elution, it is recommended to switch the injection valve at the high percentage of organic phase after the last peak has eluted. It is also recommended to switch the injection valve again after the initial conditions for the mobile phase have stabilized. This ensures that the bypass groove in the rotor seal of the valve contains the gradient start conditions, which is especially important for flow rates below 0.5 ml/min.

For samples where the outside of the needle cannot be cleaned sufficiently with water or alcohol from the flush pump use wash vials with an appropriate solvent. With an injector program several wash vials can be used for cleaning.

6
Troubleshooting and Diagnostics

This chapter gives an overview about the troubleshooting and diagnostic features and the different user interfaces.

Overview of the Module's Indicators and Test Functions

Status Indicators

The module is provided with two status indicators which indicate the operational state of the module. The status indicators provide a quick visual check of the operation of the module.

Error Messages

In the event of an electronic, mechanical or hydraulic failure, the module generates an error message in the user interface. For each message, a short description of the failure, a list of probable causes of the problem, and a list of suggested actions to fix the problem are provided (see chapter Error Information).

Test Functions

A series of test functions are available for troubleshooting and operational verification after exchanging internal components (see Tests and Calibrations).

Status Indicators

Two status indicators are located on the front of the module. The lower left indicates the power supply status, the upper right indicates the module status.

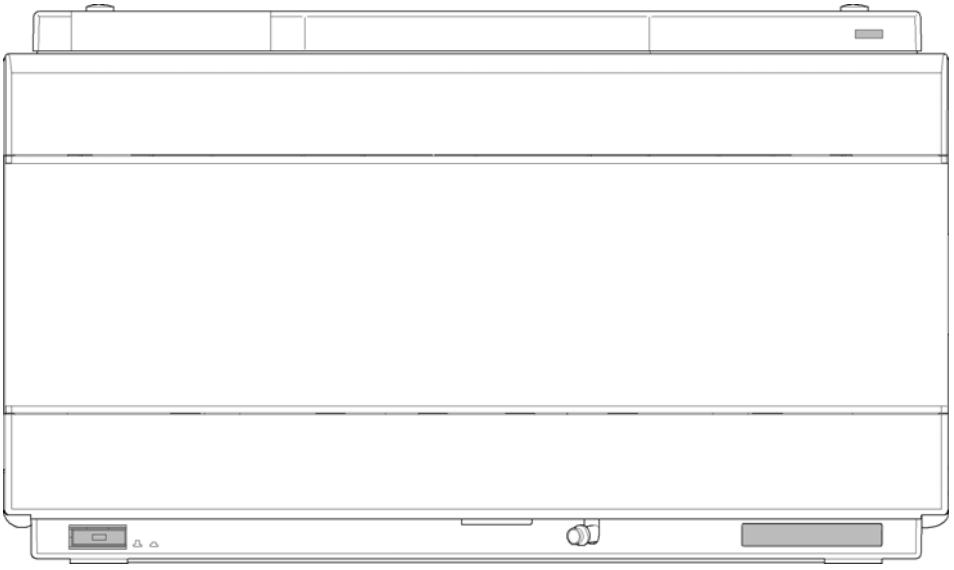


Figure 19 Location of Status Indicators

Power Supply Indicator

The power supply indicator is integrated into the main power switch. When the indicator is illuminated (*green*) the power is *ON*.

Module Status Indicator

The module status indicator indicates one of six possible module conditions:

- When the status indicator is *OFF* (and power switch light is on), the module is in a *prerun* condition, and is ready to begin an analysis.
- A *green* status indicator, indicates the module is performing an analysis (*run* mode).
- A *yellow* indicator indicates a *not-ready* condition. The module is in a not-ready state when it is waiting for a specific condition to be reached or completed (for example, immediately after changing a set point), or while a self-test procedure is running.
- An *error* condition is indicated when the status indicator is *red*. An error condition indicates the module has detected an internal problem which affects correct operation of the module. Usually, an error condition requires attention (e.g. leak, defective internal components). An error condition always interrupts the analysis.

  If the error occurs during analysis, it is propagated within the LC system, i.e. a red LED may indicate a problem of a different module. Use the status display of your user interface for finding the root cause/module of the error.
- A *blinking* indicator indicates that the module is in resident mode (e.g. during update of main firmware).
- A *fast blinking* indicator indicates that the module is in a low-level error mode. In such a case try to re-boot the module or try a cold-start (see "Special Settings" on page 204. Then try a firmware update (see "Replacing the Module Firmware" on page 158). If this does not help, a main board replacement is required.

User Interfaces

- Depending on the user interface, the available tests and the screens/reports may vary.
- Preferred tool should be Agilent Lab Advisor Software, see "Agilent Lab Advisor Software" on page 94.
- The Agilent ChemStation B.04.02 and above do not include any maintenance/test functions.
- Screenshots used within these procedures are based on the Agilent Lab Advisor Software.

Agilent Lab Advisor Software

The Agilent Lab Advisor Software is a standalone product that can be used with or without chromatographic data system. Agilent Lab Advisor helps to manage the lab for high-quality chromatographic results by providing a detailed system overview of all connected analytical instruments with instrument status, Early Maintenance Feedback counters (EMF), instrument configuration information, and diagnostic tests. By the push of a button, a detailed diagnostic report can be generated. Upon request, the user can send this report to Agilent for a significantly improved troubleshooting and repair process.

The Agilent Lab Advisor software is available in two versions:

- Lab Advisor Basic
- Lab Advisor Advanced

Lab Advisor Basic is included with every Agilent 1200 Infinity Series and Infinity II Series pump.

The Lab Advisor Advanced features can be unlocked by purchasing a license key, and include real-time monitoring of instrument actuals, all various instrument signals, and state machines. In addition, all diagnostic test results, calibration results, and acquired signal data can be uploaded to a shared network folder. The Review Client included in Lab Advisor Advanced allows to load and examine the uploaded data no matter on which instrument it was generated. This makes Data Sharing an ideal tool for internal support groups and users who want to track the instrument history of their analytical systems.

The optional Agilent Maintenance Wizard Add-on provides an easy-to-use, step-by-step multimedia guide for performing preventive maintenance on Agilent 1200 Infinity and Infinity II Series instruments.

The tests and diagnostic features that are provided by the Agilent Lab Advisor software may differ from the descriptions in this manual. For details, refer to the Agilent Lab Advisor software help files.

7
Error Information

This chapter describes the meaning of error messages, and provides information on probable causes and suggested actions how to recover from error conditions.

What are Error Messages

Error messages are displayed in the user interface when an electronic, mechanical, or hydraulic (flow path) failure occurs which requires attention before the analysis can be continued (for example, repair, or exchange of consumables is necessary). In the event of such a failure, the red status indicator at the front of the module is switched on, and an entry is written into the module logbook.

If an error occurs outside a method run, other modules will not be informed about this error. If it occurs within a method run, all connected modules will get a notification, all LEDs get red and the run will be stopped. Depending on the module type, this stop is implemented differently. For example, for a pump the flow will be stopped for safety reasons. For a detector, the lamp will stay on in order to avoid equilibration time. Depending on the error type, the next run can only be started, if the error has been resolved, for example liquid from a leak has been dried. Errors for presumably single time events can be recovered by switching on the system in the user interface.

Special handling is done in case of a leak. As a leak is a potential safety issue and may have occurred at a different module from where it has been observed, a leak always causes a shutdown of all modules, even outside a method run.

In all cases, error propagation is done via the CAN bus or via an APG remote cable (see documentation for the APG interface).

General Error Messages

General error messages are generic to all Agilent series HPLC modules and may show up on other modules as well.

Timeout

Error ID: 0062

The timeout threshold was exceeded.

Probable cause	Suggested actions
1 The analysis was completed successfully, and the timeout function switched off the module as requested.	Check the logbook for the occurrence and source of a not-ready condition. Restart the analysis where required.
2 A not-ready condition was present during a sequence or multiple-injection run for a period longer than the timeout threshold.	Check the logbook for the occurrence and source of a not-ready condition. Restart the analysis where required.

Shutdown

Error ID: 0063

An external instrument has generated a shutdown signal on the remote line.

The module continually monitors the remote input connectors for status signals. A LOW signal input on pin 4 of the remote connector generates the error message.

Probable cause	Suggested actions
1 Leak detected in another module with a CAN connection to the system.	Fix the leak in the external instrument before restarting the module.
2 Leak detected in an external instrument with a remote connection to the system.	Fix the leak in the external instrument before restarting the module.
3 Shut-down in an external instrument with a remote connection to the system.	Check external instruments for a shut-down condition.

Remote Timeout

Error ID: 0070

A not-ready condition is still present on the remote input. When an analysis is started, the system expects all not-ready conditions (for example, a not-ready condition during detector balance) to switch to run conditions within one minute of starting the analysis. If a not-ready condition is still present on the remote line after one minute the error message is generated.

Probable cause	Suggested actions
1 Not-ready condition in one of the instruments connected to the remote line.	Ensure the instrument showing the not-ready condition is installed correctly, and is set up correctly for analysis.
2 Defective remote cable.	Exchange the remote cable.
3 Defective components in the instrument showing the not-ready condition.	Check the instrument for defects (refer to the instrument's documentation).

Lost CAN Partner

Error ID: 0071

During an analysis, the internal synchronization or communication between one or more of the modules in the system has failed.

The system processors continually monitor the system configuration. If one or more of the modules is no longer recognized as being connected to the system, the error message is generated.

Probable cause	Suggested actions
1 CAN cable disconnected.	• Ensure all the CAN cables are connected correctly. • Ensure all CAN cables are installed correctly.
2 Defective CAN cable.	Exchange the CAN cable.
3 Defective main board in another module.	Switch off the system. Restart the system, and determine which module or modules are not recognized by the system.

Leak Sensor Short

Error ID: 0082

The leak sensor in the module has failed (short circuit).

The current through the leak sensor is dependent on temperature. A leak is detected when solvent cools the leak sensor, causing the leak sensor current to change within defined limits. If the current increases above the upper limit, the error message is generated.

Probable cause	Suggested actions
1 Defective leak sensor.	Please contact your Agilent service representative.
2 Leak sensor incorrectly routed, being pinched by a metal component.	Please contact your Agilent service representative.

Leak Sensor Open

Error ID: 0083

The leak sensor in the module has failed (open circuit).

The current through the leak sensor is dependent on temperature. A leak is detected when solvent cools the leak sensor, causing the leak-sensor current to change within defined limits. If the current falls outside the lower limit, the error message is generated.

Probable cause	Suggested actions
1 Leak sensor not connected to the main board.	Please contact your Agilent service representative.
2 Defective leak sensor.	Please contact your Agilent service representative.
3 Leak sensor incorrectly routed, being pinched by a metal component.	Please contact your Agilent service representative.

Compensation Sensor Open

Error ID: 0081

The ambient-compensation sensor (NTC) on the main board in the module has failed (open circuit).

The resistance across the temperature compensation sensor (NTC) on the main board is dependent on ambient temperature. The change in resistance is used by the leak circuit to compensate for ambient temperature changes. If the resistance across the sensor increases above the upper limit, the error message is generated.

Probable cause	Suggested actions
1 Defective main board.	Please contact your Agilent service representative.

Compensation Sensor Short

Error ID: 0080

The ambient-compensation sensor (NTC) on the main board in the module has failed (open circuit).

The resistance across the temperature compensation sensor (NTC) on the main board is dependent on ambient temperature. The change in resistance is used by the leak circuit to compensate for ambient temperature changes. If the resistance across the sensor falls below the lower limit, the error message is generated.

Probable cause	Suggested actions
1 Defective main board.	Please contact your Agilent service representative.

Fan Failed

Error ID: 0068

The cooling fan in the module has failed.

The hall sensor on the fan shaft is used by the main board to monitor the fan speed. If the fan speed falls below a certain limit for a certain length of time, the error message is generated.

Depending on the module, assemblies (e.g. the lamp in the detector) are turned off to assure that the module does not overheat inside.

Probable cause	Suggested actions
1 Fan cable disconnected.	Please contact your Agilent service representative.
2 Defective fan.	Please contact your Agilent service representative.
3 Defective main board.	Please contact your Agilent service representative.

Leak

Error ID: 0064

A leak was detected in the module.

The signals from the two temperature sensors (leak sensor and board-mounted temperature-compensation sensor) are used by the leak algorithm to determine whether a leak is present. When a leak occurs, the leak sensor is cooled by the solvent. This changes the resistance of the leak sensor which is sensed by the leak-sensor circuit on the main board.

Probable cause	Suggested actions
1 Loose fittings.	Ensure all fittings are tight.
2 Broken capillary.	Exchange defective capillaries.

Module Error Messages

These errors are autosampler specific.

Exhaust Fan Failed

Error ID: 4456, 4457

The exhaust fan in the module has failed.

The hall sensor on the fan shaft is used by the main board to monitor the fan speed. If the fan speed falls below a certain value the error message is generated and the module shuts down.

Probable cause	Suggested actions
1 Fan cable disconnected.	Please contact your Agilent service representative.
2 Defective fan.	Please contact your Agilent service representative.
3 Defective main board.	Please contact your Agilent service representative.

Front Door Error

Error ID: 4350, 4352, 4458

The front door and/or the SLS board are damaged.

Probable cause	Suggested actions
1 The sensor on the SLS board is defective.	Please contact your Agilent service representative.
2 The door is bent or the magnet is misplaced/broken.	Please contact your Agilent service representative.

Side Door Error

Error ID: 4355, 4459

The side door and/or the main board are damaged.

Probable cause	Suggested actions
1 The door is bent or the magnet is misplaced/broken.	Please contact your Agilent service representative.
2 The sensor on the main board is defective.	Please contact your Agilent service representative.

Arm Movement Failed or Arm Movement Timeout

Error ID: 4002

The transport assembly was unable to complete a movement in one of the axes.

The processor defines a certain time window for the successful completion of a movement in any particular axis. The movement and position of the transport assembly is monitored by the encoders on the stepper motors. If the processor does not receive the correct position information from the encoders within the time window, the error message is generated.

Axes identification:

- Arm Movement 0 Failed: X-axis.
- Arm Movement 1 Failed: Z-axis.
- Arm Movement 2 Failed: Theta (needle carrier rotation).

Probable cause	Suggested actions
1 Mechanical obstruction.	Ensure unobstructed movement of the transport assembly.
2 High friction in the transport assembly.	Please contact your Agilent service representative.
3 Defective motor assembly.	Please contact your Agilent service representative.
4 Defective sample transport assembly flex board.	Please contact your Agilent service representative.
5 Defective main board.	Please contact your Agilent service representative.

Valve to Bypass Failed

Error ID: 4014, 4701

The injection valve failed to switch to the bypass position.

The switching of the injection valve is monitored by two microswitches on the valve assembly. The switches detect the successful completion of the valve movement. If the valve fails to reach the bypass position, or if the microswitch does not close, the error message is generated.

Probable cause	Suggested actions
1 Valve in an intermediate position between the bypass and mainpass positions.	Turn the Autosampler main power OFF and ON.
2 Defective injection valve.	Please contact your Agilent service representative.
3 Defective main board.	Please contact your Agilent service representative.

Valve to Mainpass Failed

Error ID: 4015

The injection valve failed to switch to the mainpass position.

The switching of the injection valve is monitored by two microswitches on the valve assembly. The switches detect the successful completion of the valve movement. If the valve fails to reach the mainpass position, or if the microswitch does not close, the error message is generated.

Probable cause	Suggested actions
1 Valve in an intermediate position between the bypass and mainpass positions.	Turn the Autosampler main power OFF and ON.
2 Defective injection valve.	Please contact your Agilent service representative.
3 Defective main board.	Please contact your Agilent service representative.

Needle Lock Failed

Error ID: 4702, 4703

The lock assembly on the sampling unit failed to move successfully.

The upper and lower positions of the needle lock are monitored by position sensors on the sampling unit flex board. The sensors detect the successful completion of the needle lock movement. If the needle lock fails to reach the end point, or if the sensors fail to recognize the needle lock movement, the error message is generated.

Probable cause	Suggested actions
1 Defective or dirty position sensor.	Clean the position sensor.
2 Sticking spindle assembly.	Please contact your Agilent service representative.
3 Defective needle drive motor	Please contact your Agilent service representative.
4 Defective main board.	Please contact your Agilent service representative.

Needle to Needle Seat Position

Error ID: 4510, 4511, 4714

The needle failed to reach the end position in the needle seat.

The position of the needle is monitored by a position encoder on the needle carrier. If the needle fails to reach the end point, or if the encoder fails to recognize the needle carrier movement, the error message is generated.

Probable cause	Suggested actions
1 Bad sample transport/sampling unit alignment	Do an auto-alignment
2 Bent needle.	Check and exchange the needle assembly if necessary.
3 Missing needle.	Exchange the needle carrier assembly.
4 Blocked seat.	Clean or change the needle seat assembly if necessary.
5 Defective position sensor in the needle carrier assembly.	Please contact your Agilent service representative.
6 Defective main board.	Please contact your Agilent service representative.

Needle Carrier Failed

The needle carrier on the Sample Transport Assembly failed to move correctly.

Probable cause	Suggested actions
1 Defective Z-motor.	Please contact your Agilent service representative.
2 Vial pusher blocked.	Please contact your Agilent service representative.
3 Bad needle carrier positioning in X or Theta.	Please contact your Agilent service representative.
4 Defective vial pusher sensor.	Please contact your Agilent service representative.
5 Defective main board.	Please contact your Agilent service representative.

Missing Vial or Missing Wash Vial

Error ID: 4019, 4034, 4035, 4541, 4542, 4706, 4707

No vial was found in the position defined in the method or sequence.

When the needle carrier moves to a vial and the needle goes into the vial, the position of the needle is monitored by an encoder behind the vial pusher. If no vial is present, the encoder detects an error and the message “missing vial” is generated.

Probable cause	Suggested actions
1 No vial in the position defined in the method or sequence.	Install the sample vial in the correct position, or edit the method or sequence accordingly.
2 Defective needle carrier assembly.	Please contact your Agilent service representative.
3 Defective transport assembly flex board.	Please contact your Agilent service representative.
4 Defective main board.	Please contact your Agilent service representative.

Initialization Failed

Error ID: 4020

The autosampler failed to complete initialization correctly.

The autosampler initialization procedure moves the needle arm and transport assembly to their home positions in a predefined routine. During initialization, the processor monitors the position sensors and motor encoders to check for correct movement. If one or more of the movements is not successful, or is not detected, the error message is generated.

Probable cause	Suggested actions
1 Side door not installed correctly.	• Check if the side door is installed correctly. • Check if the magnet is in place in the side door.
2 Sample transport/sampling unit not aligned correctly.	Do an auto-alignment
3 Mechanical obstruction.	Ensure unobstructed movement of the transport assembly.
4 Defective sampling unit flex board.	Please contact your Agilent service representative.
5 Defective transport assembly flex board.	Please contact your Agilent service representative.
6 Defective sampling unit motor.	Please contact your Agilent service representative.
7 Defective main board.	Please contact your Agilent service representative.

Metering Home Failed

Error ID: 4054, 4704

The metering piston has failed to move back to the home position.

The home position sensor on the sampling unit flex board monitors the home position of the piston. If the piston fails to move to the home position, or if the sensor fails to recognize the piston position, the error message is generated.

Probable cause	Suggested actions
1 Defective sensor or main board.	Please contact your Agilent service representative.
2 Broken plunger.	Exchange the metering plunger and seal.
3 Defective metering-drive motor.	Please contact your Agilent service representative.
4 Defective main board.	Please contact your Agilent service representative.

Motor Temperature

Error ID: 4027, 4040, 4261, 4451

One of the motors of the transport assembly has drawn excessive current, causing the motor to become too hot. The processor has switched off the motor to prevent damage to the motor.

Motor identification:

- Motor 0 temperature: X-axis motor.
- Motor 1 temperature: Z-axis motor.
- Motor 2 temperature: Theta motor.

The processor monitors the current drawn by each motor and the time the motor is drawing current. The current drawn by the motors is dependent on the load on each motor (friction, mass of components etc.). If the current drawn is too high, or the time the motor draws current is too long, the error message is generated.

Probable cause	Suggested actions
1 Mechanical obstruction.	Ensure unobstructed movement of the transport assembly.
2 High friction in the transport assembly.	Please contact your Agilent service representative.
3 Motor belt tension too high.	Switch off the module at the power switch. Wait at least 10 minutes before switching on again.
4 Defective motor.	Please contact your Agilent service representative.
5 Defective transport assembly flex board.	Please contact your Agilent service representative.

Invalid Vial Position

Error ID: 4042

The vial position defined in the method or sequence does not exist.

The reflection sensors on the transport assembly flex board are used to automatically check which sample trays are installed (coding on tray). If the vial position does not exist in the current sample tray configuration, the error message is generated.

Probable cause	Suggested actions
1 Incorrect tray installed.	Install the correct trays, or edit the method or sequence accordingly.
2 Incorrect tray definition.	Install the correct trays, or edit the method or sequence accordingly.
3 Incorrect vial positions defined in the method or sequence.	Install the correct trays, or edit the method or sequence accordingly.
4 Tray recognition defective (dirty sample tray or defective transport assembly flex board).	• Ensure the coding surfaces of the sample tray are clean (located at the rear of the sample tray). • Please contact your Agilent service representative.

Peristaltic Pump Error

Error ID: 4514

The peristaltic pump motor in the autosampler has failed.

The current on the motor is used by the MTP board to monitor the speed of the peristaltic pump motor. If the current falls below a certain value, the error message is generated.

Probable cause	Suggested actions
1 Defective motor.	Please contact your Agilent service representative.
2 Defective SUD board.	Please contact your Agilent service representative.
3 Defective main board.	Please contact your Agilent service representative.

Vessel or Wash Vessel Error

Error ID: 4540, 4544, 4545, 4705, 4712

The needle does not reach the target position in the vial or in the vessel of the well plate.

The sensor behind the vial pusher in the needle carrier assembly detects the successful completion of the needle movement to the vessel. If the needle fails to reach the end point, the sensor fails to recognize the needle movement and the error message is generated.

Probable cause	Suggested actions
1 Bad vessel definition in the plate configuration.	Check the vessel definition in the plate configuration.
2 Closing mat to rigid/thick.	Check that the closing mat is not too thick.
3 Bad X or Theta positioning.	Please contact your Agilent service representative.
4 Defective encoder on the needle carrier assembly.	Please contact your Agilent service representative.

Vessel Stuck to Needle

Error ID: 4453

The vessel sticks to the needle when the needle moves up.

Probable cause	Suggested actions
1 Closing mat to rigid/thick.	Check that the closing mat is not too thick.
2 Bad X or Theta positioning and the needle sticks into the wall between two holes.	Please contact your Agilent service representative.
3 Defective encoder on the needle carrier assembly.	Please contact your Agilent service representative.

Rear Blind Seat Missing

Error ID: 4724

Rear blind seat is missing although claimed to exist by main board information – occurs during initialization or if the blind seat location has to be used.

Probable cause	Suggested actions
1 Blind seat is missing.	Install blind seat.

8
Test Functions

This chapter describes the tests for the module.

Introduction

All tests are described based on the Agilent Lab Advisor Software B.01.03 or above. Other user interfaces may not provide any test or just a few.

Table 6 Interfaces and available test functions

Interface	Comment	Available Function
Agilent Instrument Utilities	Maintenance tests available	• System Pressure test • Sample transport Self Alignment
Agilent Lab Advisor	All tests are available	• System Pressure test • Sampler leak test • Sample transport Self Alignment
Agilent ChemStation	No tests available Adding of pressure to chromatographic signals possible	• Pressure • Pressure ripple • Temperature mainboard
Agilent Instant Pilot	Some tests are available	• System Pressure test • Sample transport Self Alignment

For details on the use of the interface refer to the interface documentation.

System Pressure Test

The test determines the leak rate of the system between pump outlet valves and a blank nut. The blank nut can be positioned at different locations in the system before the flow cell, to determine and verify the leak rate of individual modules and components. The test allows for setting the pressure at which the test is performed. The leak rate of high pressure parts are not always a linear function and therefore it is recommended to perform the test at a pressure that correspond to the normal operating pressure of the system.

When In case of a suspected leak. To verify successful execution of maintenance tasks.

Parts required

#	p/n	Description
1	01080-83202	Blank nut

Preparations Solvents must be present in both channels.

1 Run the **System pressure test** with the Agilent Lab Advisor (for further information see Online-Help of user interface).

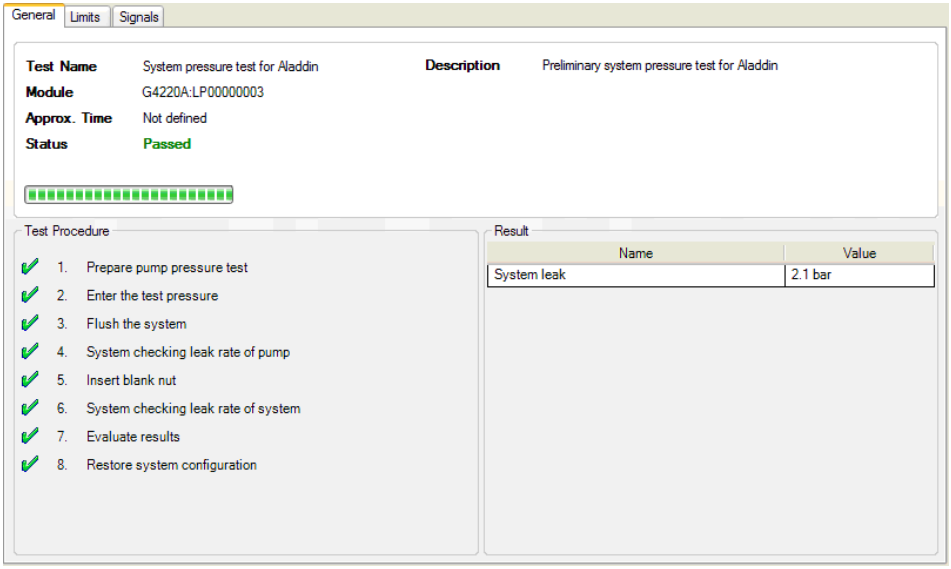


Figure 20 System Pressure Test – Result

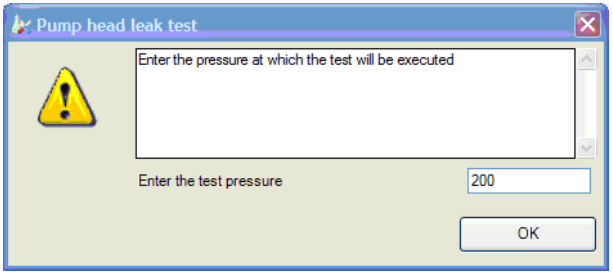


Figure 21 System Pressure Test – Dynamic pressure input

System Pressure Test Evaluation

`System Pressure Test Failed`

	Probable cause	Suggested actions
1	Pump leakages	Perform the Pump Head Leak test.
2	Loose or leaky fittings	Tighten the fittings or replace capillaries.
3	Autosampler leakages	Perform the Autosampler Leak test.
4	Thermostatted Column Compartment valve leakages	Replace the TCC valve rotor seal.

NOTE

- Notice the difference between *error* in the test and a *failed* result! An *error* is caused by an abnormal termination during the operation of the test, whereas a *failed* result indicates that the test results were not within the specified limits.
- Often it is only a damaged blank nut (poorly shaped from over tightening) that causes the test to fail. Before investigating any other possible sources of failure make sure that the blank nut you are using is in a good condition and properly tightened.

Sampler Leak Test

The test determines the specific leak rates of rotor seal, metering device, needle/seat and system by performing a series of pressure tests. This is done with the injection valve in different positions and by using a blocked needle seat positioned at the rear of the module to block certain parts of the flow path. The test allows for setting the pressure at which the test is performed. The leak rate of high pressure parts are not always a linear function and therefore it is recommended to perform the test at a pressure that correspond to the normal operating pressure of the system.

When By suspected Autosampler performance problems.

Preparations Solvents must be present in both channels.

1 Run the **Autosampler Leak Test** with the Agilent Lab Advisor (for further information see Online-Help of user interface).

Figure 22 Sampler Leak Test – Results

Sampler Leak Test Evaluation

`Sampler Leak Test Failed`

	Probable cause	Suggested actions
1	Leaky metering device seal	Change metering device seal.
2	Damaged needle and/or needle seat	Change needle and needle seat.
3	Damaged rotor seal in the injection valve	Change Rotor seal.
4	Leaky fittings	Tighten fittings or replace capillaries.

NOTE Notice the difference between *error* in the test and a *failed* result! An *error* is caused by an abnormal termination during the operation of the test, whereas a *failed* result indicates that the test result were not within the specified limits.

Sample Transport Self Alignment

The sample transport self alignment uses predefined positions on the well plate tray to calibrate the positioning of the needle. The sample transport self alignment is required to compensate for larger deviations in positioning the needle carrier. The sample transport self alignment is required after disassembling the system or when you exchange the sample transport, the sampling unit, the tray or the MTP main board. This function is in the calibration screen of the Lab Advisor.

When After disassembling the module or by larger deviations in the positioning of the needle.

Preparations Well plate tray needs to be installed and empty.

1 Run the **Transport Alignment** with the Agilent Lab Advisor (for further information see Online-Help of user interface).

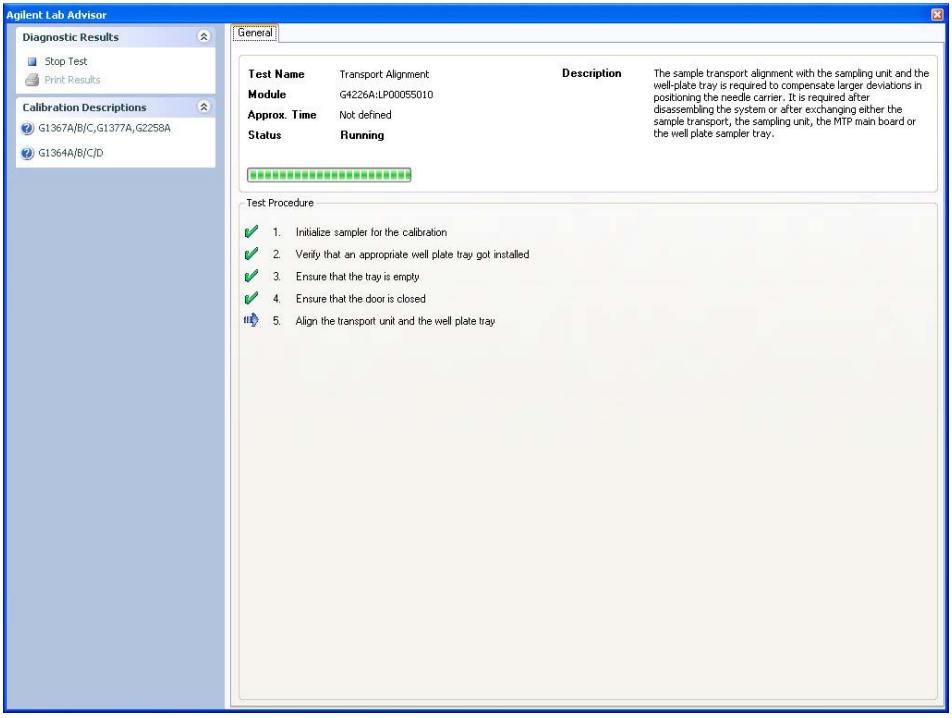


Figure 23 Sample Transport Self Alignment– Running

Maintenance Positions

Some maintenance procedures require the needle arm, metering device, and needle carrier to be moved to specific positions to enable easy access to components. The maintenance functions move these assemblies into the appropriate maintenance position. In the Agilent Lab Advisor Software the maintenance positions can be selected from the **Tools** icon.

When When performing Maintenance on the module.

1 Run the **Maintenance Positions** with the Agilent Lab Advisor (for further information see Online-Help of user interface).

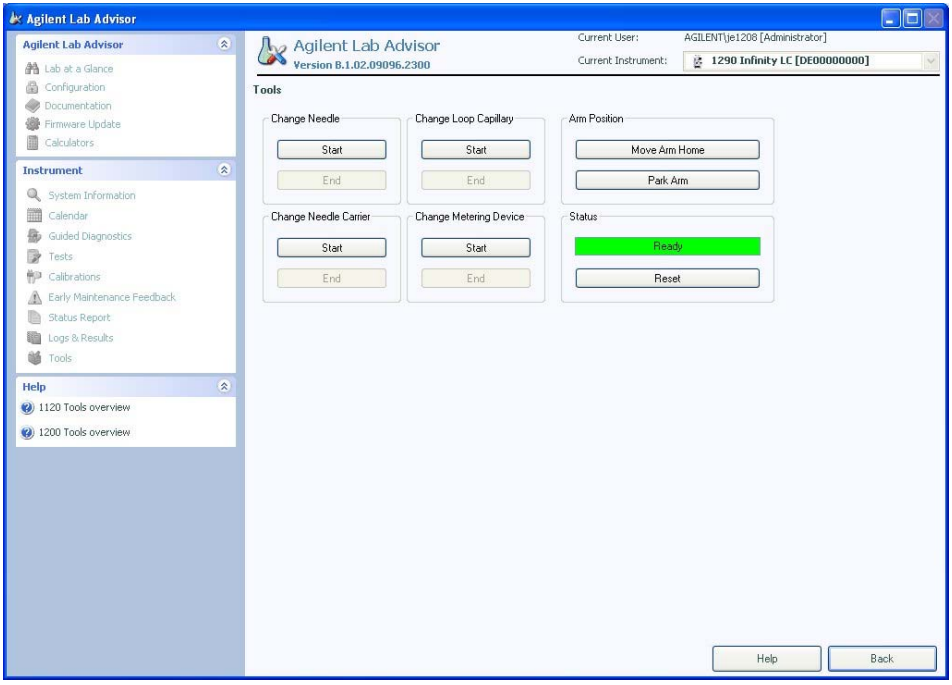


Figure 24 Maintenance Positions– Running

Change Needle

The position is positioning the needle carrier so that there is easy access for changing needle or needle seat. The position is to the far left, and the current to the motors are off, so that the arm can be turned while servicing the module.

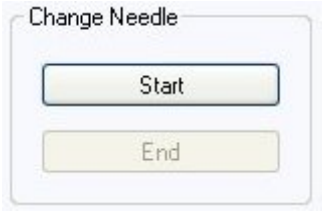


Figure 25 Maintenance Positions– Change Needle

Change Loop Capillary

The **Change Loop Capillary** command positions the arm in the middle of the tray at half height to enable easy exchange of the loop cartridge.

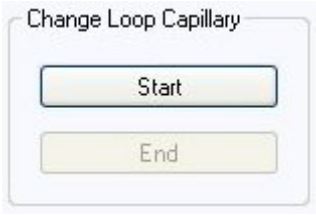


Figure 26 Maintenance Positions– Change Loop Capillary

Arm Position

The home position of the autosampler ensures a better access to the tray area and for exchanging trays. When transporting the module it is highly recommended to use the **Park Arm** command, in order to place the Arm in a position for safe transport.

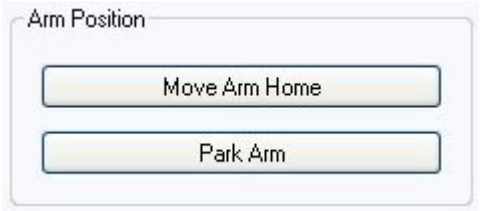


Figure 27 Maintenance Positions– Arm Position

Change Needle Carrier

The **Change Needle Carrier** function moves the needle to the front of the autosampler, enabling easy access to the needle carrier mechanism.

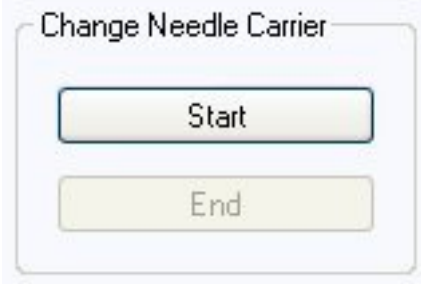


Figure 28 Maintenance Positions - Needle Carrier

- **Start** moves the needle to the front of the sample-tray area.
- **End** resets the autosampler after the needle carrier has been changed.

Change Metering Device

When removing the metering device is necessary (by exchanging the metering seal for instance), the metering drive needs to be moved to a position at the far back, in order to prevent seal and/or piston damage.

Figure 29 Maintenance Positions– Change Metering device

Injector Steps

Each movement of the sampling sequence can be done under manual control. This is useful during troubleshooting, where close observation of each of the sampling steps is required to confirm a specific failure mode or verify successful completion of a repair. Each injector step command actually consists of a series of individual commands that move the autosampler components to predefined positions, enabling the specific step to be done.

When When troubleshooting the module.

1 Run the **Injector steps** with the Agilent Lab Advisor (for further information see Online-Help of user interface).

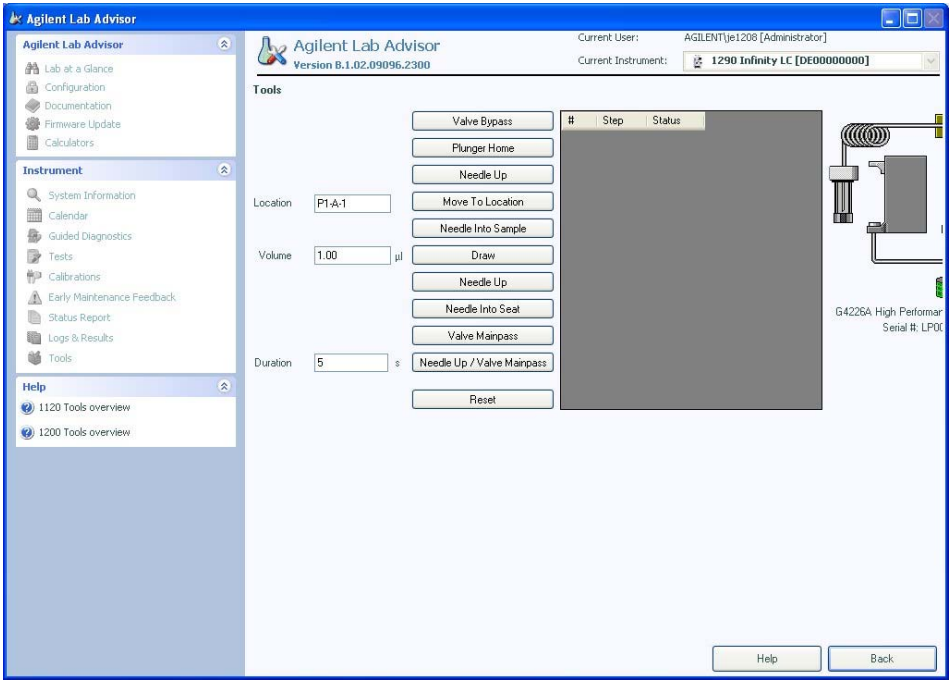


Figure 30 Injector Steps– Running

Step Commands

Table 7 Step Commands

Step	Action	Comments
Valve Bypass	Switches injection valve to the bypass position.	
Plunger Home	Moves the plunger to the home position.	
Needle Up	Lifts the needle arm to the upper position.	Command also switches the valve to bypass if it is not already in that position.
Move to Location	Move the needle arm to the vial location on the plate.	
Needle into Sample	Lowers the needle into the vial.	
Draw	Metering device draws the defined injection volume.	Command lifts the needle, and lowers the needle into the sample. Command can be done more than once, maximum draw volume of 20 µL (for 40 µL and 120 µL hardware changes are required see multi-draw) cannot be exeeded. Use **Plunger Home** to reset the metering device.
Needle Up	Lifts the needle out of the vial.	
Needle into Seat	Lowers the needle arm into the seat.	
Valve Mainpass	Switches the injection valve to the mainpass position.	
Needle Up/Mainpass	Moves needle arm to waste position and switches the injection valve to the mainpass position.	

9 Maintenance

This chapter describes the maintenance of the Autosampler

Introduction to Maintenance

Figure 31 on page 134 shows the main user accessible assemblies of the autosampler. These parts can be accessed from the front (simple repairs) and don't require to remove the autosampler from the system stack.

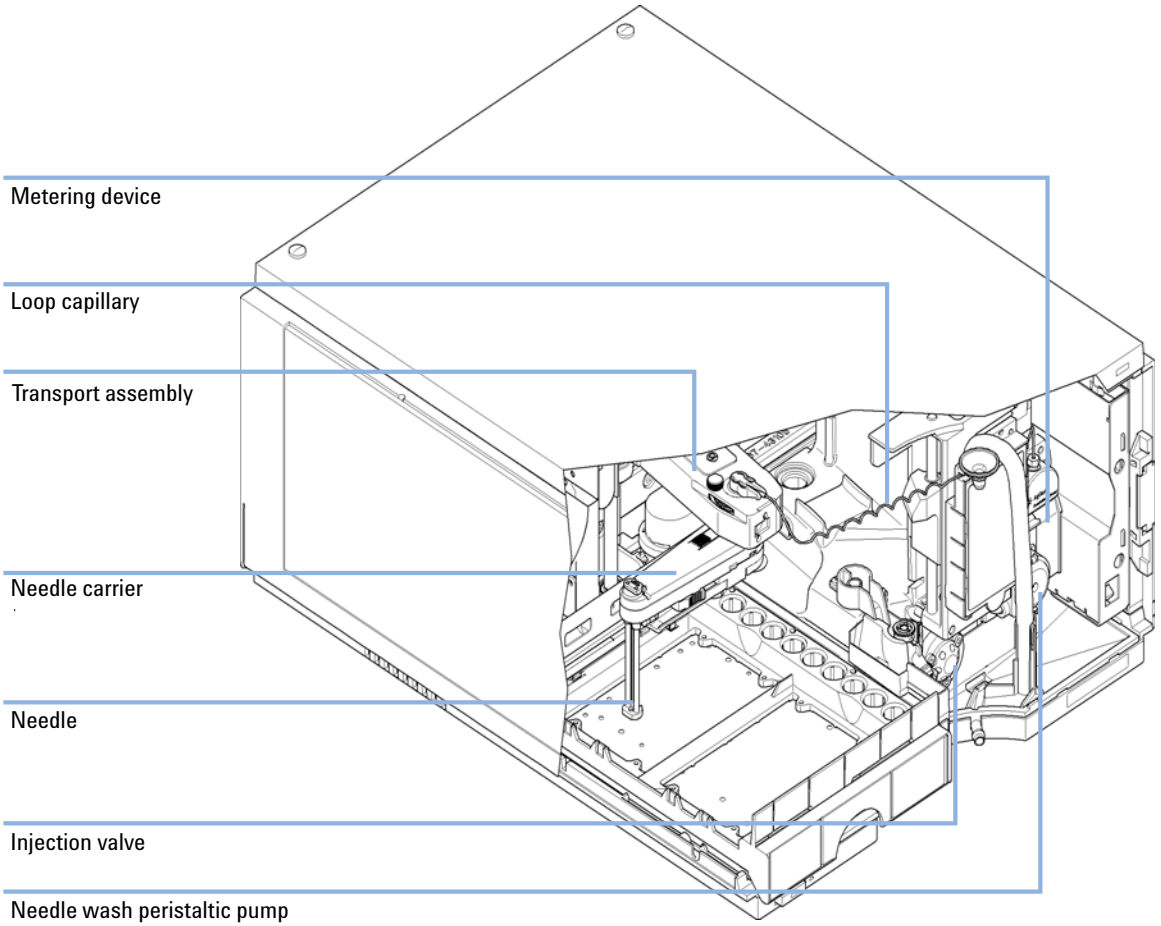


Figure 31 Main user accessible assemblies

Warnings and Cautions

WARNING

Toxic, flammable and hazardous solvents, samples and reagents

The handling of solvents, samples and reagents can hold health and safety risks.

- ➔ When working with these substances observe appropriate safety procedures (for example by wearing goggles, safety gloves and protective clothing) as described in the material handling and safety data sheet supplied by the vendor, and follow good laboratory practice.
- ➔ The volume of substances should be reduced to the minimum required for the analysis.
- ➔ Do not operate the instrument in an explosive atmosphere.

WARNING

Electrical shock

Repair work at the module can lead to personal injuries, e.g. shock hazard, when the cover is opened.

- ➔ Do not remove the cover of the module.
- ➔ Only certified persons are authorized to carry out repairs inside the module.

WARNING

Personal injury or damage to the product

Agilent is not responsible for any damages caused, in whole or in part, by improper use of the products, unauthorized alterations, adjustments or modifications to the products, failure to comply with procedures in Agilent product user guides, or use of the products in violation of applicable laws, rules or regulations.

- ➔ Use your Agilent products only in the manner described in the Agilent product user guides.

CAUTION

Safety standards for external equipment

- ➔ If you connect external equipment to the instrument, make sure that you only use accessory units tested and approved according to the safety standards appropriate for the type of external equipment.

Overview of Maintenance

The following pages describe maintenance (simple repairs) of the autosampler that can be carried out without opening the main cover.

Table 8 Overview of Maintenance

Procedure	Typical Frequency	Notes
Change needle/needle seat	60.000 needle into seat	
Change metering seal	30.000 injections	
Peristaltic pump cartridge	3000 hours on-time	
Change rotor seal	30.000 injections	

Cleaning the module

To keep the module case clean, use a soft cloth slightly dampened with water, or a solution of water and mild detergent.

WARNING **Liquid dripping into the electronic compartment of your module can cause shock hazard and damage the module**

➔ Do not use an excessively damp cloth during cleaning.

➔ Drain all solvent lines before opening any connections in the flow path.

Removing the needle assembly

When

When the limit in the needle into seat counter in the EMF is exceeded or when needle shows indications of damage, blockage or leaks.

Tools required

p/n	Description
8710-0510	Wrench open 1/4 — 5/16 inch

Parts required

p/n	Description
G4226-87201	Needle assembly

Preparations

In order to avoid leaks, close the shutoff valves in the pump or remove tubings from solvent bottles.

WARNING

Risk of injury by uncovered needle

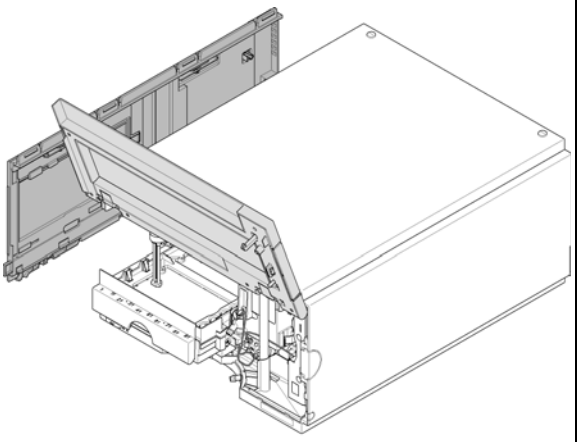
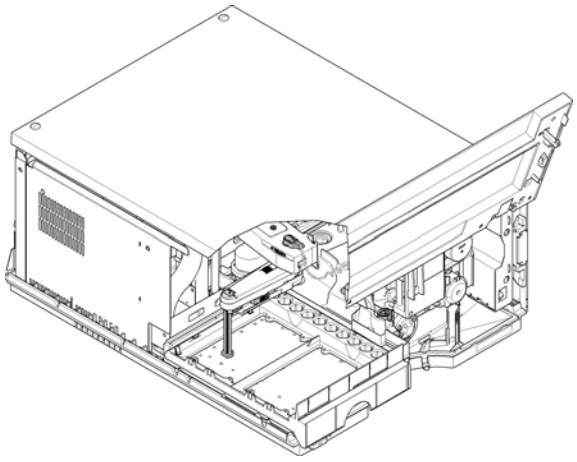
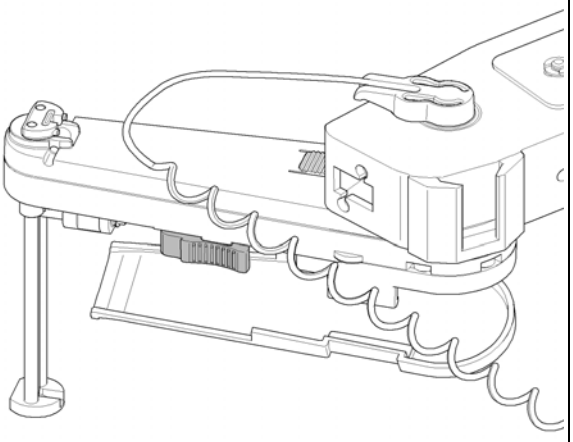
An uncovered needle is a risk of harm to the operator.

➔ Be careful working at the needle carrier assembly.

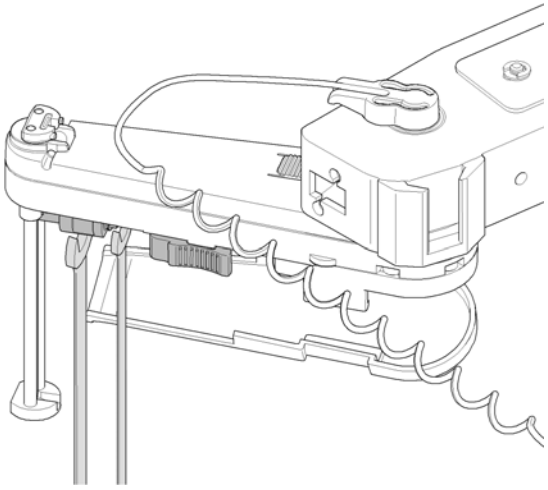
➔ Use the silicon safety tube supplied with every new needle.

NOTE

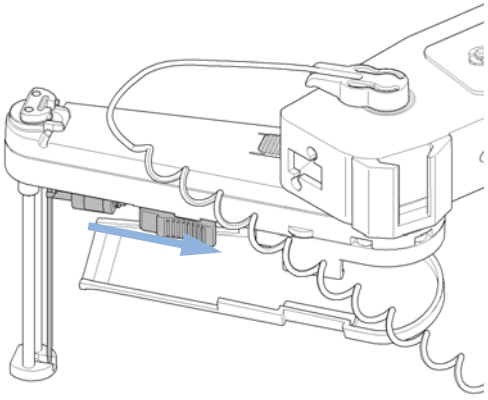
It is recommended to always exchange the needle assembly and the needle seat at the same time to prevent premature leakage.

1 In the user interface start the maintenance mode and select **Change needle/seat** function. In the Agilent Lab Advisor software the **Change needle/seat** function can be found in the **Tools** section.	**2** Open the front door and remove the side door. 
3 Turn the Needle carrier 90 ° clockwise. 	**4** Flip the Leak guide open. 

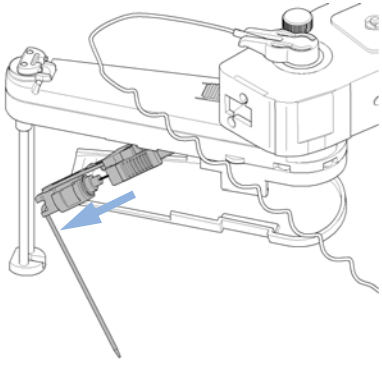
5 Attach a 5/16 inch wrench to hold the position at the needle assembly. Use a 1/4 inch wrench to loosen the fitting of the loop capillary.



6 Pinch the holder clamp, pull back and remove the loop capillary from the needle assembly.



7 Remove the needle assembly.



Installing the needle assembly

When	When the limit in the needle into seat counter in the EMF is exceeded or when needle shows indications of damage, blockage or leaks.	
Tools required	**p/n**	**Description**
	8710-0510	Wrench open 1/4 — 5/16 inch
Parts required	**p/n**	**Description**
	G4226-87201	Needle assembly
Preparations	In order to avoid leaks, close the shutoff valves in the pump or remove tubings from solvent bottles.	

WARNING

Risk of injury by uncovered needle

An uncovered needle is a risk of harm to the operator.

➔ Be careful working at the needle carrier assembly.

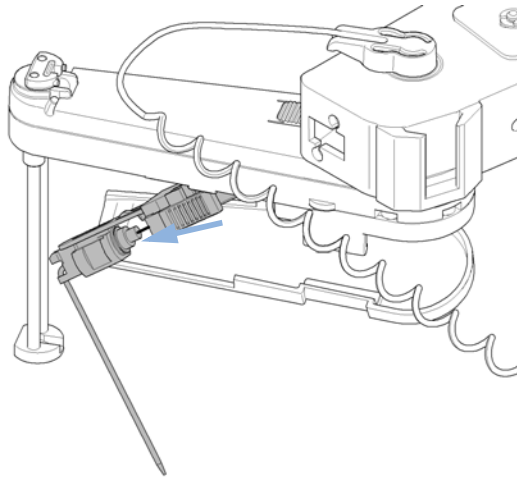
➔ Use the silicon safety tube supplied with every new needle.

NOTE

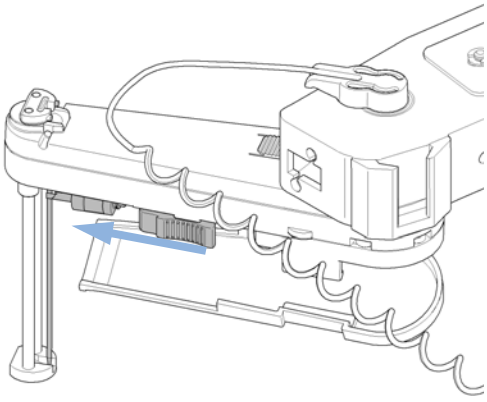
It is recommended to always exchange the needle assembly and the needle seat at the same time to prevent premature leakage.

1 Push the silicon safety tube delivered with every needle, over the needle.

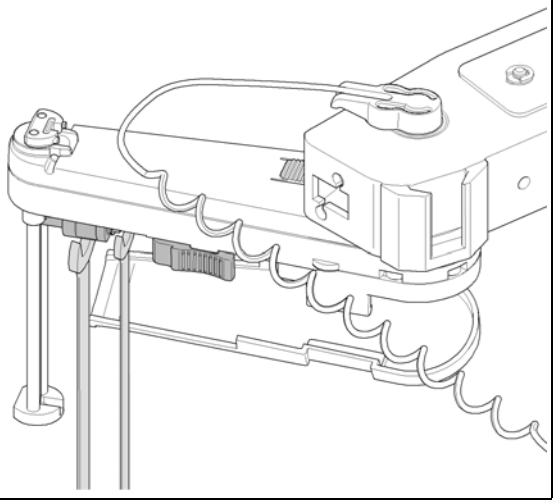
2 Insert the loop capillary into the needle assembly and tighten the fitting hand tight.



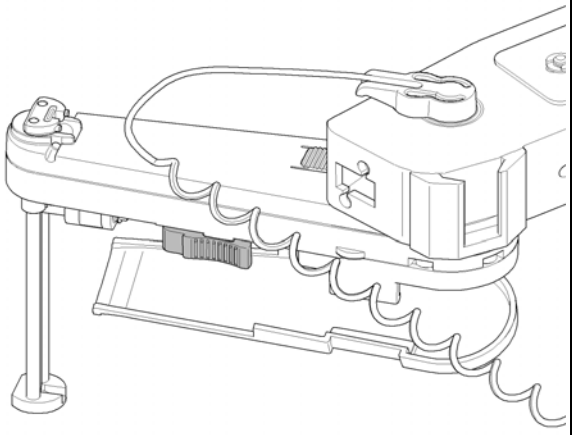
3 Pinch the holder clamp and reinsert the needle assembly into the needle carrier.



4 Attach a 5/16 inch wrench to hold the position at the needle assembly. Use a 1/4 inch wrench to tighten the fitting of the loop capillary.



5 Close the leak guide



Next Steps:

6 Check the alignment of the needle in the needle pusher of the needle carrier by viewing from several directions to see that it is aligned in the center of the needle pusher.

NOTE

The needle must be centered in the needle pusher as all alignment by the Autosampler is calculated from the needle pusher position.

7 Remove the silicon safety tube from the needle.

8 In the user interface exit the **Change needle/seat** function and exit the maintenance mode. In the Lab Advisor software the **Change needle/seat** function can be found in the **Tools** section.

9 Re-install the side door and close the front door.

Exchanging the Needle Seat

When	When seat is visibly damaged, blocked or leaks.		
Tools required	**p/n**	**Description**	
	8710-0510	Wrench open 1/4 — 5/16 inch	
		Flat head screwdriver	
Parts required	**#**	**p/n**	**Description**
	1	G4226-87012	Needle seat
Preparations	In order to avoid leaks, close the shutoff valves in the pump or remove tubings from solvent bottles.		

WARNING

Risk of injury by uncovered needle

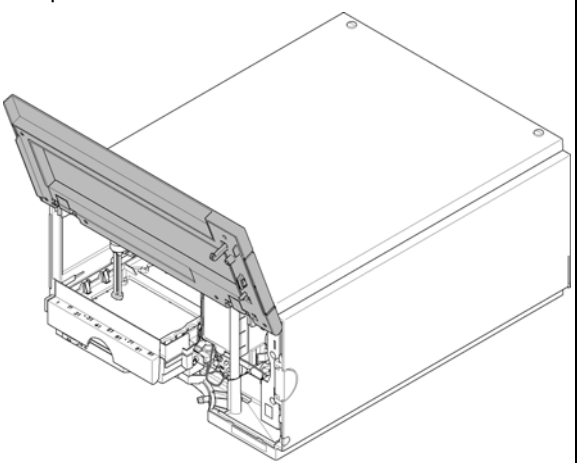
An uncovered needle is a risk of harm to the operator.

➔ Be careful working at the needle carrier assembly.

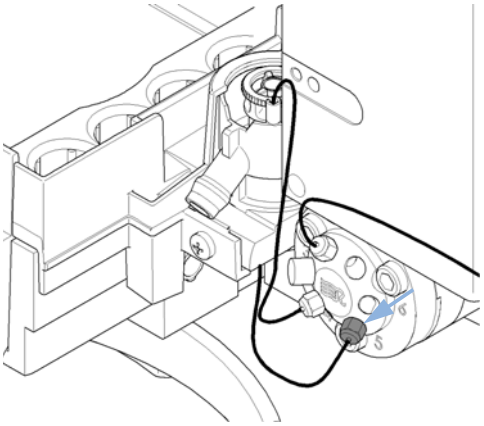
➔ Use the silicon safety tube supplied with every new needle.

1 In the user interface start the maintenance mode and select **Change needle/seat** function. In the Agilent Lab Advisor software the **Change needle/seat** function can be found in the **Tools** section.

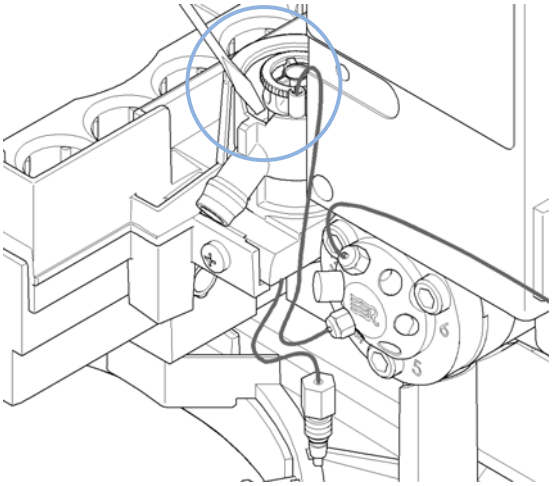
2 Open the front door.



3 Disconnect the seat capillary from the Injection valve.



4 With a flat head screw driver carefully lift out the needle seat from the holder.



Next Steps:

5 Insert the new needle seat. Press it firmly in position.

6 In the user interface exit the **Change needle/seat** function and exit the maintenance mode. In the Lab Advisor software the **Change needle/seat** function can be found in the **Tools** section.

Replacing the Rotor seal

When When poor injection volume reproducibility or when injection valve is leaking.

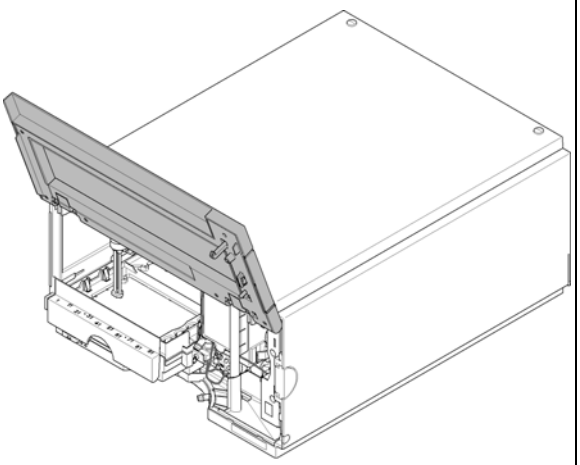
Tools required

p/n	Description
8710-0510	Wrench open 1/4 — 5/16 inch
8710-2394	Hex key 9/64 inch 15 cm long T-handle

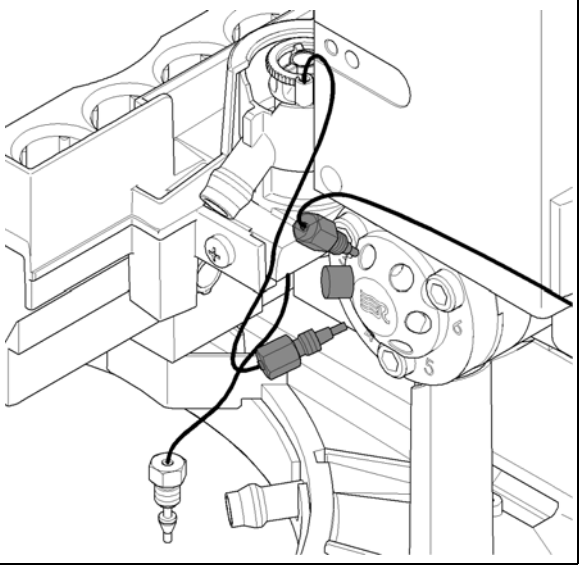
Parts required

#	p/n	Description
1	5068-0007	Injection valve rotor seal

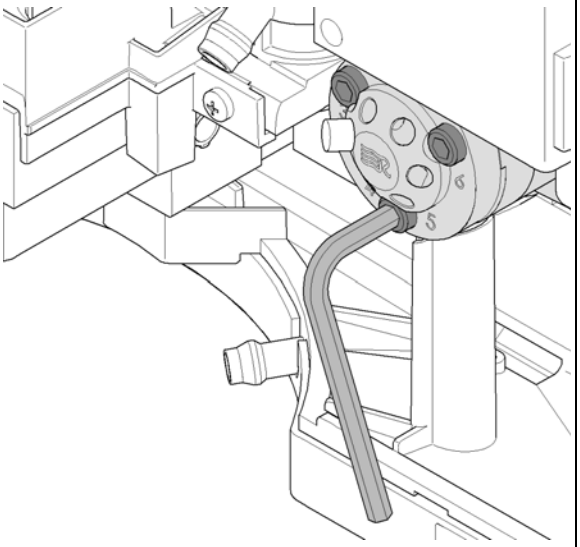
1 Open the front door.



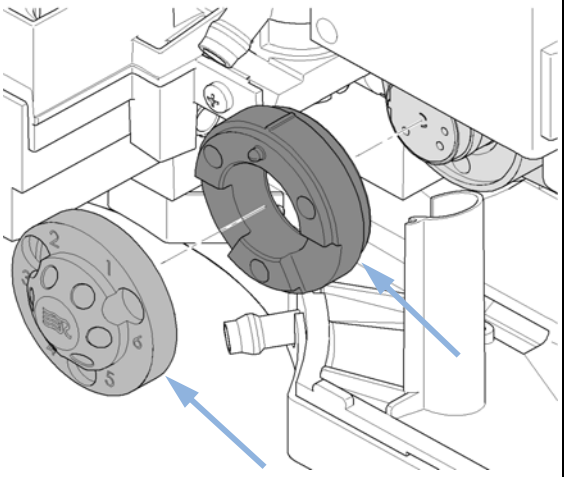
2 Remove all capillaries from the Injection valve with a 1/4 inch wrench.



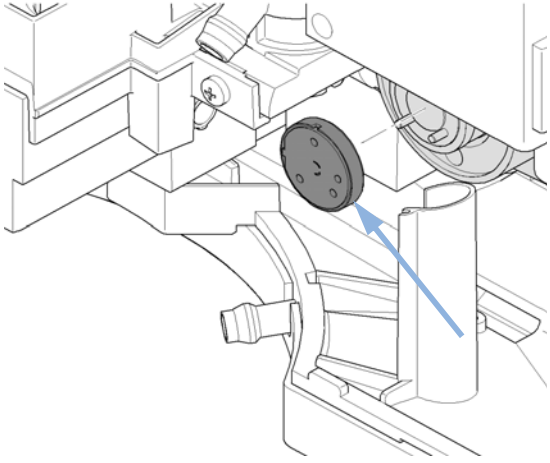
3 Unscrew and remove the three stator screws from the stator head with a 9/64 inch hex key.



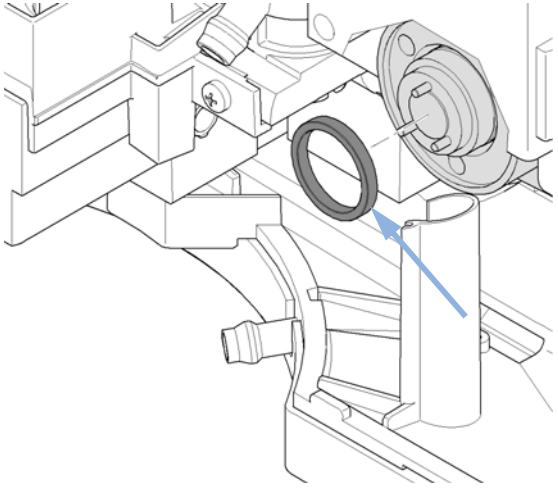
4 Remove the stator head and the stator ring.



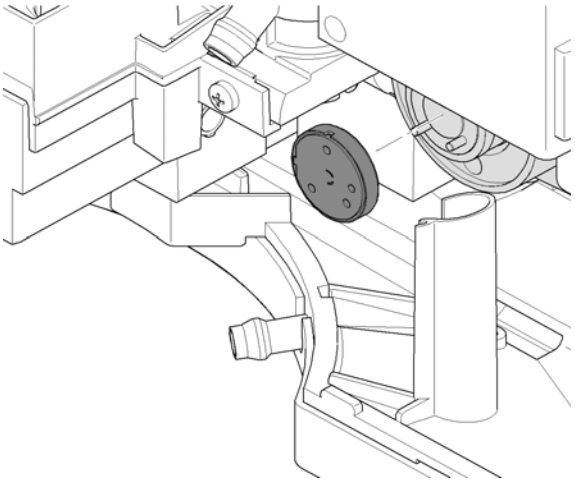
5 Remove the rotor seal.



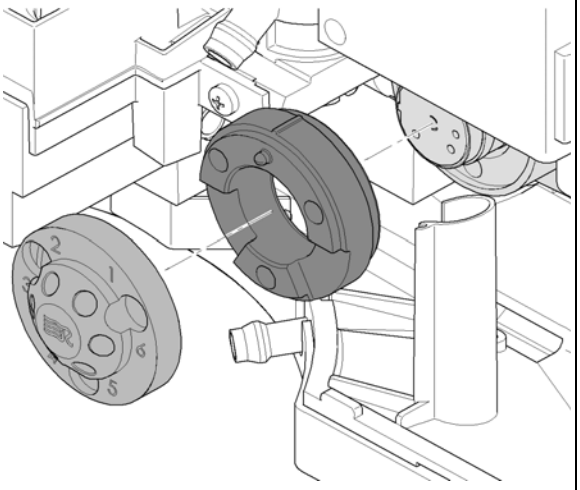
6 Remove the Isolation seal.



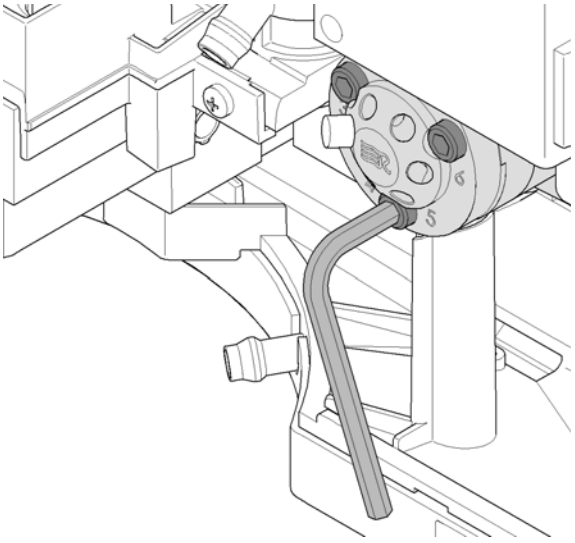
7 Install new rotor seal and isolation seal.



8 Reinstall the stator ring and stator head. The pins on stator ring and stator head must engage in the corresponding holes.



9 Insert and tighten the stator screws alternating with the 9/64 inch hex key, until the stator head is secure.



Next Steps:

10 Reconnect all capillaries to the injection valve ports with a 1/4 inch wrench. The positions of the individual fittings can be seen on the sticker on the sampling unit.

11 Close the front door.

Removing the Metering Seal

When When poor injection volume reproducibility or when metering device / analytical head is leaking.

Tools required

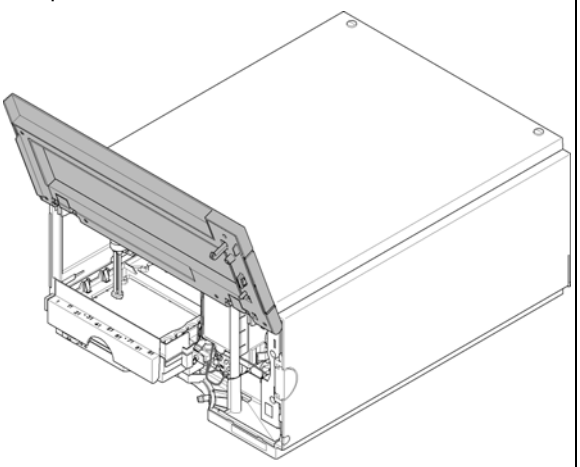
p/n	Description
8710-0510	Wrench open 1/4 — 5/16 inch
8710-2392	4 mm Hex key
G4226-43800	Seal insert tool

Parts required

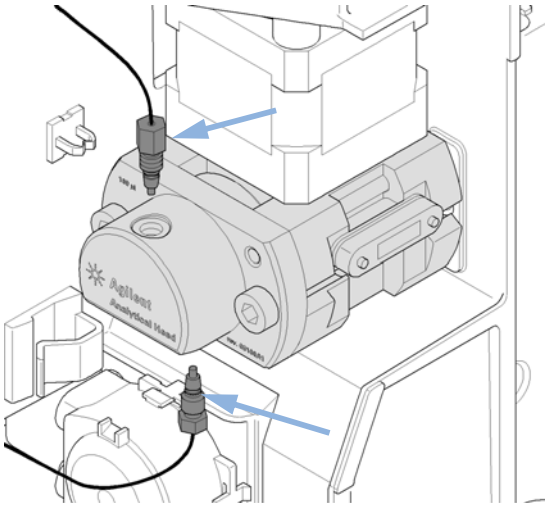
#	p/n	Description
1	0905-1717	Metering seal

1 In the LabAdvisor User Interface start **Service & Diagnostic** and select **Maintenance Positions** function. In this section the **Change Metering Device** function can be found.

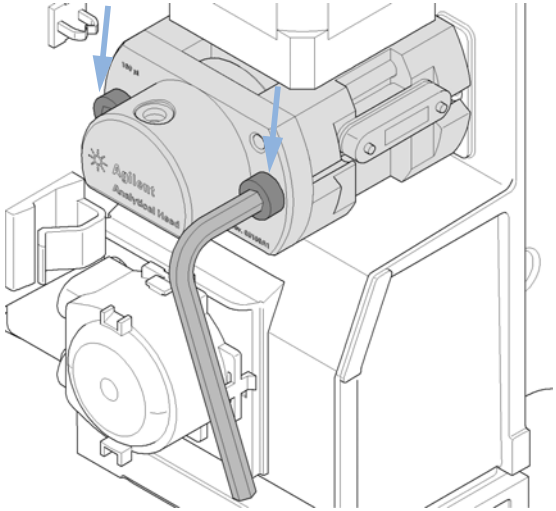
2 Open the front door.



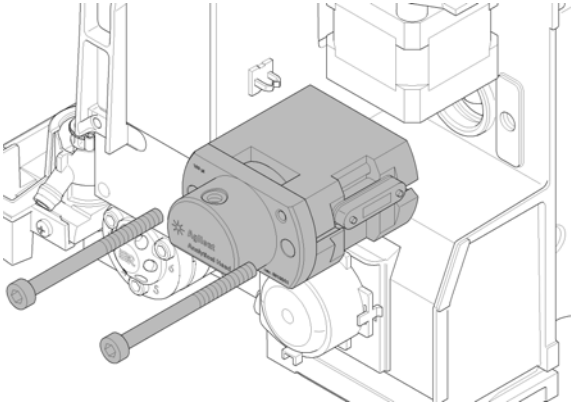
3 Remove the two attached capillaries with a ¼ inch wrench.



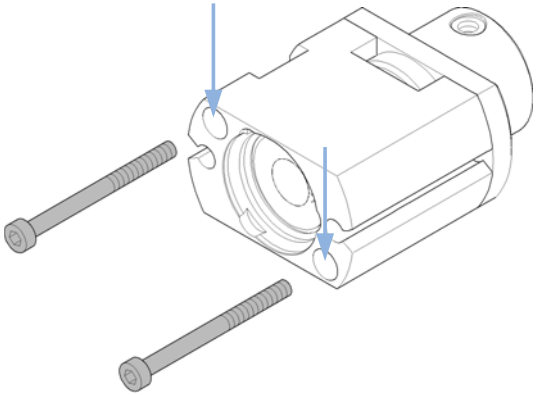
4 Unscrew alternately the two fixing screws with a 4 mm hex key.



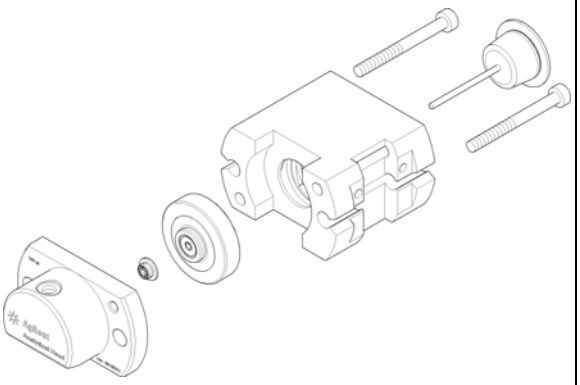
5 Pull the metering device / analytical head away from the sampling unit.



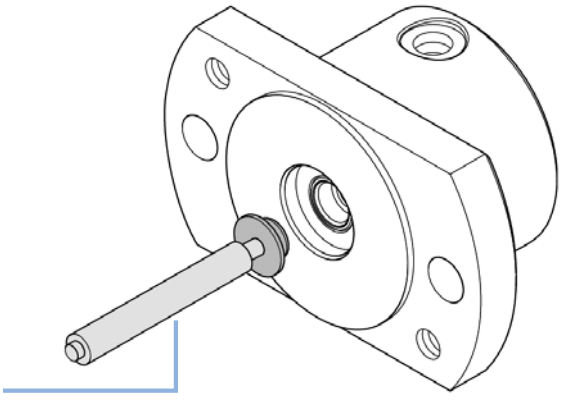
6 Remove the two fixing screws at the base of the metering device / analytical head.



7 Remove the head body.



8 Carefully remove the metering seal using the steel side of the insert tool. Clean the chamber and ensure all particulate matter is removed.



Installing the Metering Seal

When After removing the metering seal.

Tools required

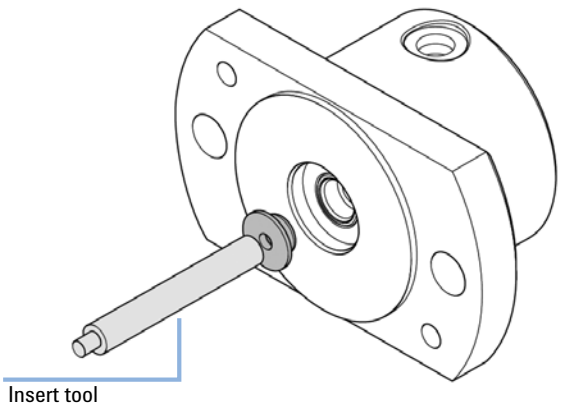
p/n	Description
8710-0510	Wrench open 1/4 — 5/16 inch
8710-2392	4 mm Hex key
G4226-43800	Seal insert tool

Parts required

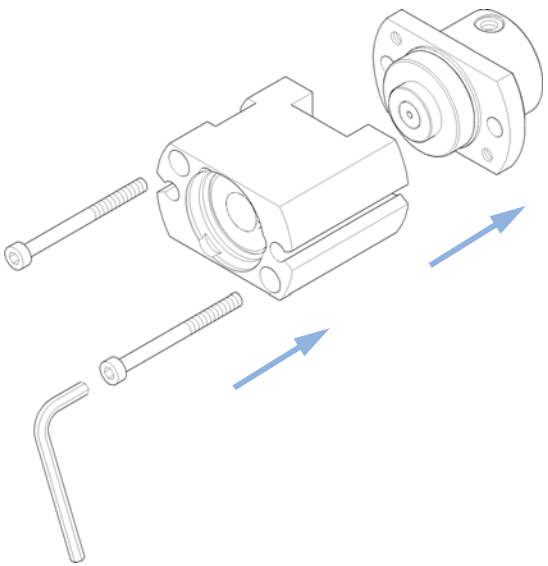
#	p/n	Description
1	0905-1717	Metering seal

Preparations Removing the metering seal, see "Removing the Metering Seal" on page 149.

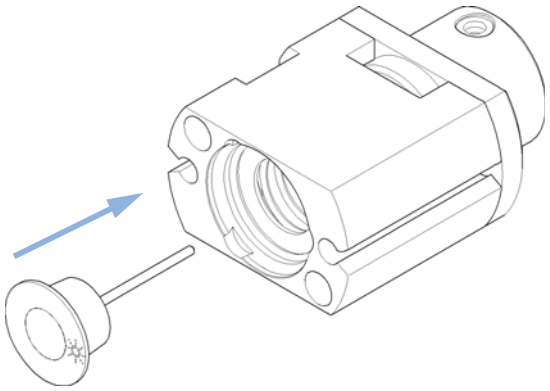
1 Install the new metering seal using the plastic side of the insert tool. Press it firmly into position. Avoid any offset angle as it might deform the seal.



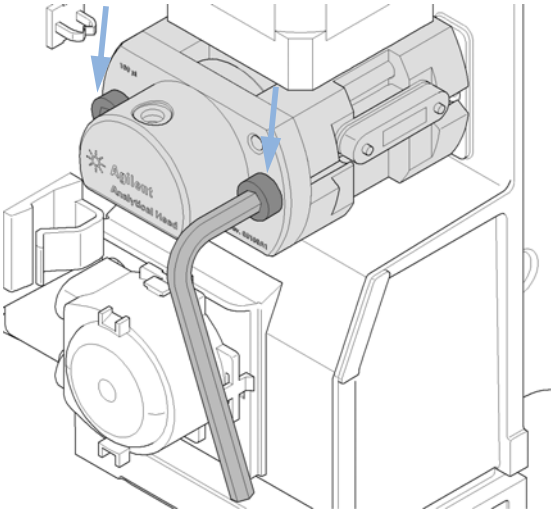
2 Reassemble the metering device / analytical head. Make sure to tighten screws firmly and have the tag on the right side looking from the front.



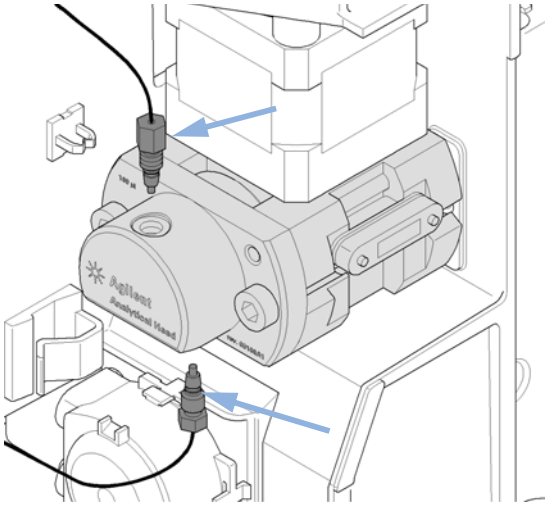
3 Press the piston into the seal.



4 Reinstall the metering device / analytical head to the sampling unit by tightening the two fixing screws alternately with a 4 mm hex key.



5 Connect the two capillaries to the metering device using a 1/4 inch wrench.



Next Steps:

6 Close the front door.

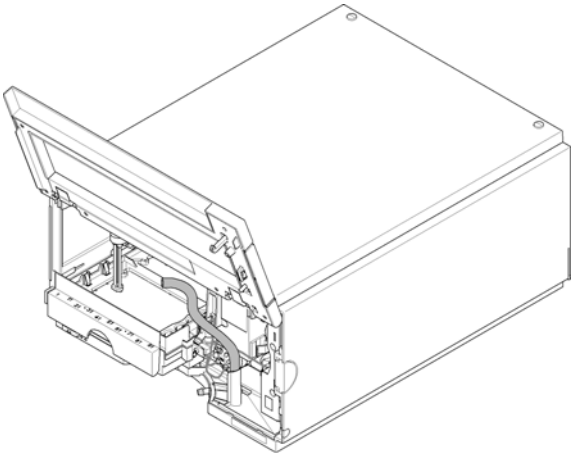
7 In the user interface exit the **Change Metering device** function and exit the maintenance mode. In the Lab Advisor software the **Change Metering device** function can be found in the **Tools** section.

Replacing Peristaltic Pump Cartridge

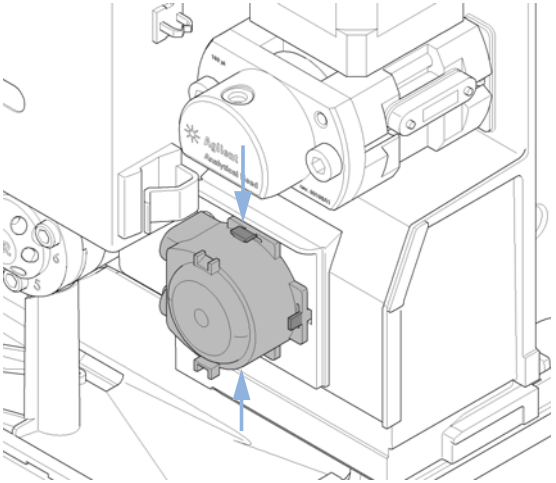
When	Tubing blocked or broken		
Parts required	**#**	**p/n**	**Description**
	1	5065-4445	Peristaltic pump with Pharmed tubing

NOTE The peristaltic pump cartridge is a replaceable unit. The tubing inside the pump is not replaceable.

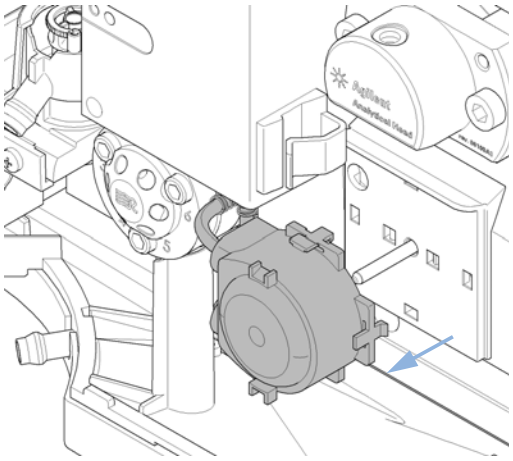
1 Remove the corrugated leak tubing.



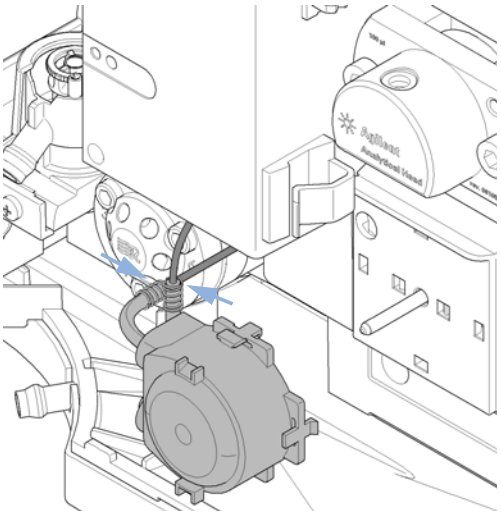
2 Press the two clips on the front of the peristaltic pump cartridge.



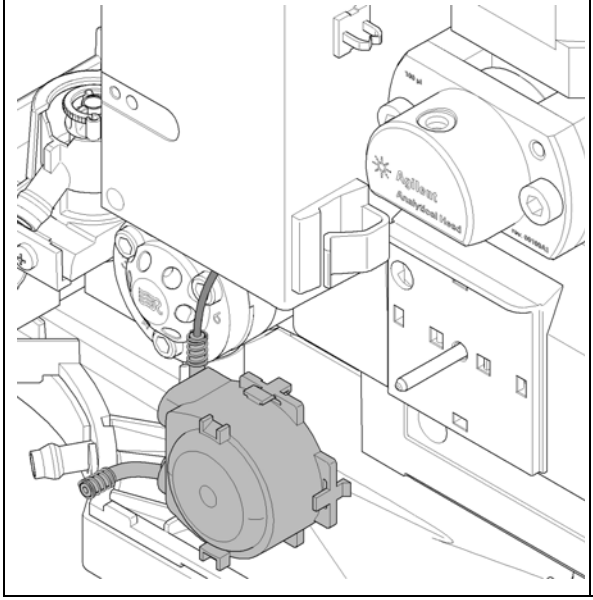
3 Pull the cartridge forward off the motor shaft.



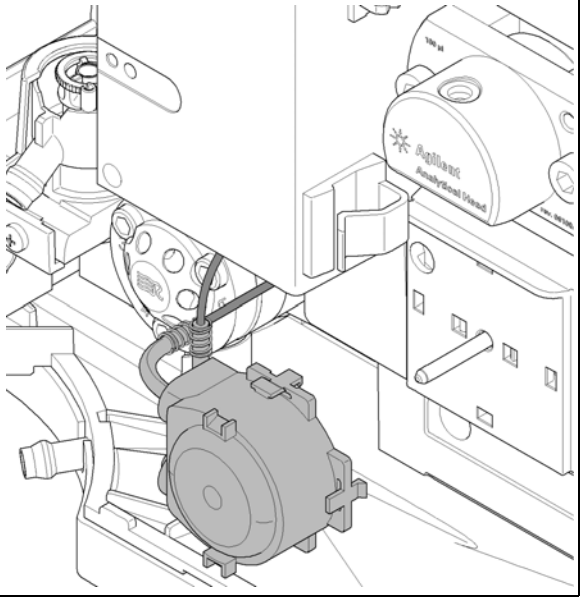
4 Disconnect the tubing leading to the wash port and the tubing coming from the solvent bottle.



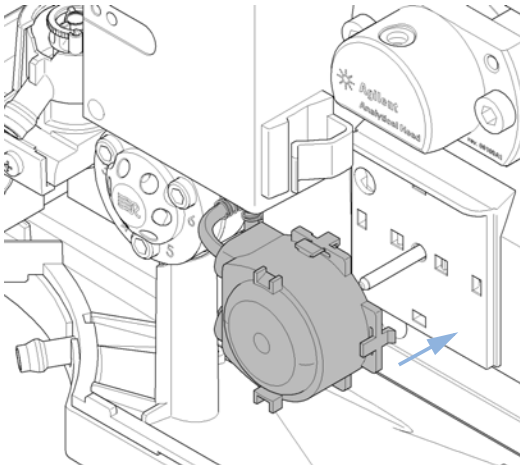
5 Connect the wash port tubing to the upper tubing of the new cartridge (use sand paper to get a good grip on the tubing).



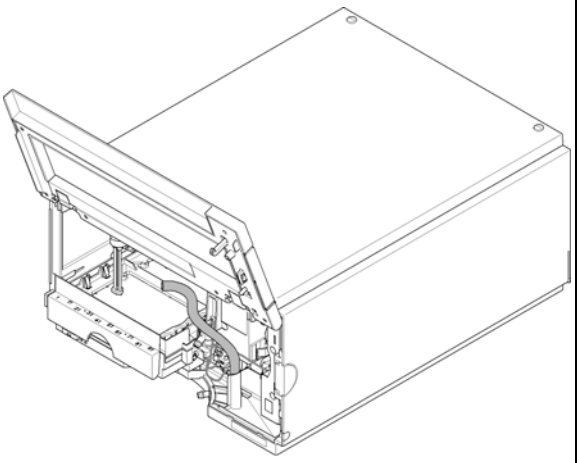
6 Connect the tubing coming from the solvent bottle to the lower tubing of the new cartridge.



7 Push the cartridge onto the motor shaft until the clips click into place.



8 Reinstall the corrugated leak tubing.



Installing the Interface Board

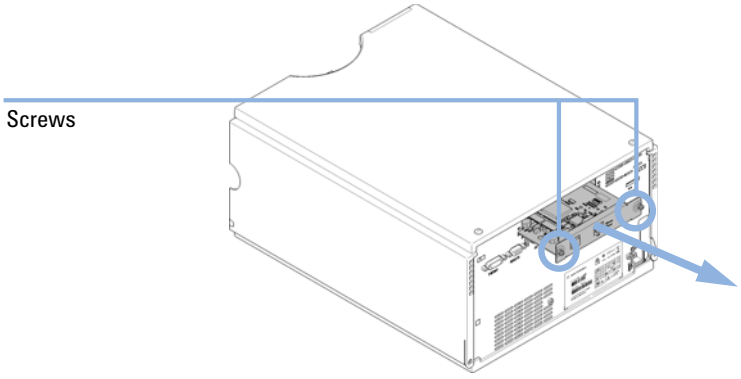
When	At installation or when defective.
Tools required	**Description** Flat head screwdriver
Parts required	**# Description** 1 Interface board

CAUTION Electronic boards are sensitive to electrostatic discharge (ESD) and should be handled with care so as not to damage them. Touching electronic boards and components can cause electrostatic discharge.

ESD can damage electronic boards and components.

➔ Be sure to hold the board by the edges and do not touch the electrical components. Always use an ESD protection (for example, an ESD wrist strap) when handling electronic boards and components.

1 Switch OFF the autosampler at the main power switch.
2 Disconnect cables from the interface board connectors.
3 Loosen the screws. Slide out the interface board from the autosampler.
4 Install the interface board. Secure the screws.
5 Reconnect the cables to the board connectors.



Replacing the Module Firmware

When

The installation of newer firmware might be necessary
- if a newer version solves problems of older versions or
- to keep all systems on the same (validated) revision.

The installation of older firmware might be necessary
- to keep all systems on the same (validated) revision or
- if a new module with newer firmware is added to a system or
- if third party control software requires a special version.

Tools required

	Description
	LAN/RS-232 Firmware Update Tool
OR	Agilent Lab Advisor software
OR	Instant Pilot G4208A (only if supported by module)

Parts required

#	Description
1	Firmware, tools and documentation from Agilent web site

Preparations

Read update documentation provided with the Firmware Update Tool.

To upgrade/downgrade the module's firmware carry out the following steps:

1 Download the required module firmware, the latest LAN/RS-232 FW Update Tool and the documentation from the Agilent web.
   - http://www.chem.agilent.com/_layouts/agilent/downloadFirmware.aspx?whid=69761

2 For loading the firmware into the module follow the instructions in the documentation.

Module Specific Information

There is no specific information for this module.

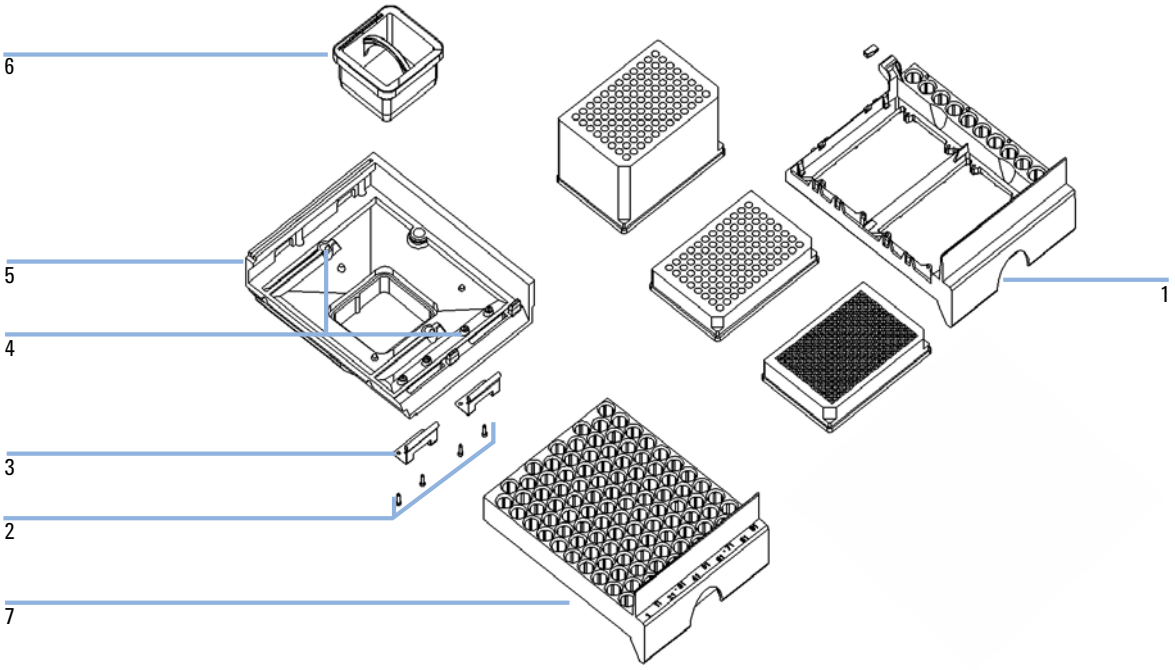
10
Parts for Maintenance

This chapter provides information on parts material required for the module.

Overview of Maintenance Parts

p/n	Description
0905-1717	Metering seal
5068-0007	Injection valve rotor seal
G4226-87201	Needle assembly
G4226-87012	Needle seat
G4226-60310	Loop cartridge 20 µL
G4226-60013	40 µL analytical head
5067-4703	40 µL Flex loop kit

Vial Trays



Item	p/n	Description
1	G2258-60011	Tray for 2 plates + 10 x 2 mL vials
2	0515-0866	Screws for springs
3	G1313-09101	Spring
4	0570-1574	Spring stud
5	G4226-60000	Tray Support
6	G1329-43200	Adapter air channel
	G1367-47200	Plug channel
7	G4226-60021	Tray for 100 micro vials

Recommended Plates and Closing Mats

Table 9 Recommended plates and closing mat

Description (Part Number)	Rows	Columns	Plate height	Volume (µL)	Package
384Agilent (5042-1388)	16	24	14.4	80	30
384Corning (No Agilent PN)	16	24	14.4	80	
384Nunc (No Agilent PN)	16	24	14.4	80	
96 well plate 0.5 ml, PP (pack of 10) (5042-1386) 96 well plate 0.5 ml, PP (pack of 120) (5042-1385)	8	12	14.3	500	10 120
96Agilent conical (5042-8502)	8	12	17.3	150	25
96CappedAgilent (5065-4402)	8	12	47.1	300	1
96Corning (No Agilent PN)	8	12	14.3	300	
96CorningV (No Agilent PN)	8	12	14.3	300	
96DeepAgilent31mm (5042-6454)	8	12	31.5	1000	50
96DeepNunc31mm (No Agilent PN)	8	12	31,5	1000	
96DeepRitter41mm (No Agilent PN)	8	12	41.2	800	
96Greiner (No Agilent PN)	8	12	14.3	300	
96GreinerV (No Agilent PN)	8	12	14.3	250	
96Nunc (No Agilent PN)	8	12	14.3	400	
Closing mat for all 96 Agilent plates (5042-1389)	8	12			50

NOTE Using vessels higher than 41 mm, will result in needle not being able to reach bottom of vessel.

Recommended Vial Plates

p/n	Description
G2255-68700	Vial plate for 54 x 2 mL vials (6/pk)
5022-6539	Vial plate for 15 x 6 mL vials (1/pk)
5022-6538	Vial plate for 27 Eppendorf tubes (1/pk)

Accessory Kit

p/n	Description
G4226-68705	Accessory kit
5181-1519	CAN cable, Agilent module to module, 1 m
5182-0716	Screw Cap Vial, 2 mL, amber glass, write-on spot, 100/pk
5182-0717	Blue screw caps 100/pk
8710-0510 (2x)	Wrench open 1/4 — 5/16 inch
8710-2391	Rheotool socket wrench ¼ inch
8710-2392	Hex key 4 mm15 cm long T-handle
8710-2394	Hex key 9/64 inch 15 cm long T-handle
8710-2411	Hex key 3 mm12 cm long
5065-9978	Tubing, 1 mm i.d., 3 mm o.d., silicone, 5 m
5067-4659	SS Capillary 340x0.12 ps-ns
G1329-43200	Adapter air channel
n/a (2x)	Tubing clips
G4226-43800	Seal insert tool

Analytical Head Assembly

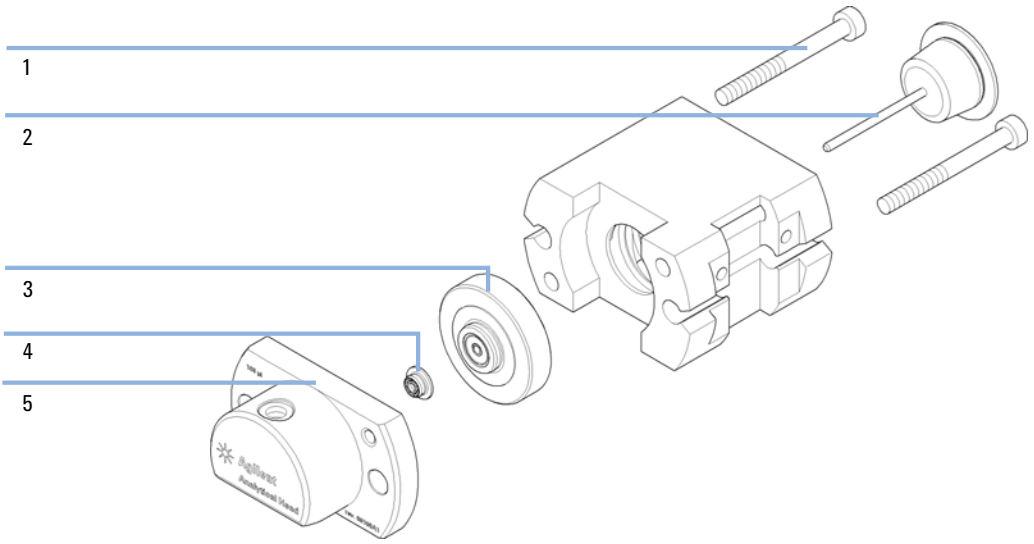
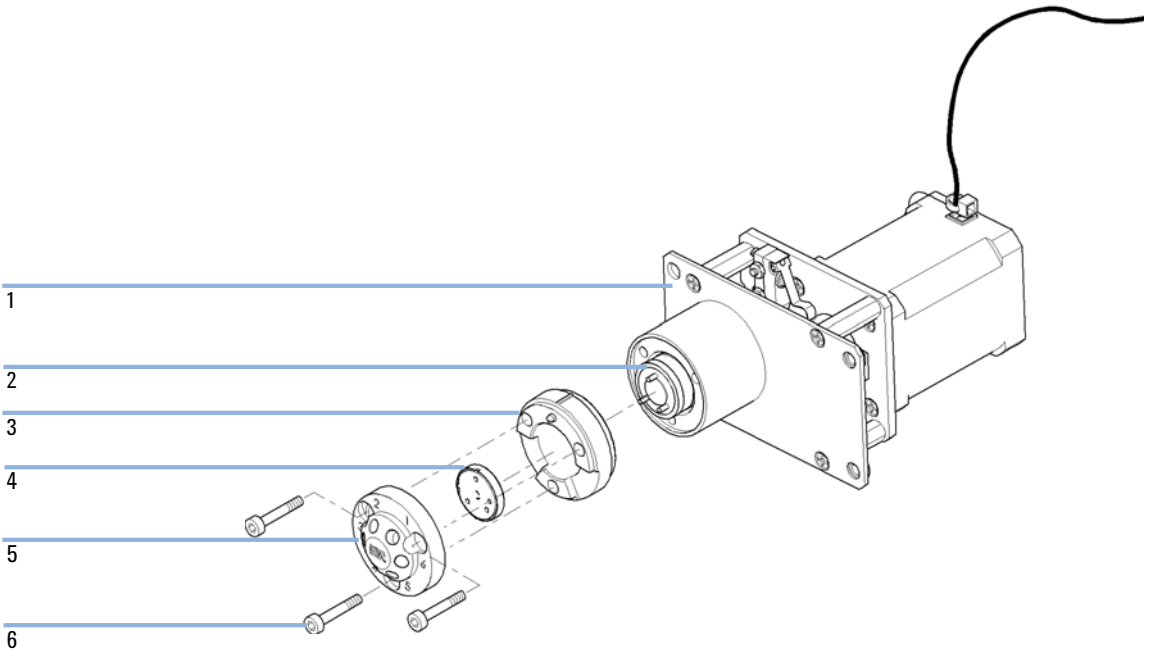


Figure 32 Analytical Head Assembly

Item	p/n	Description
	G4226-60013	40 µL analytical head
1	0515-0850	Screws
2	5064-8293	Micro Plunger assembly
3	G1377-60012	Micro seal support
4	0905-1717	Metering seal
5	G4226-27701	Head body
6	G4226-60301	Metering capillary SST Cap. 0.17 mm i.d. 160 mm pre-swaged (not shown)

Injection Valve Assembly



Item	p/n	Description
1	5067-4114	Injection valve actuator
2	1535-4045	Isolation seal
3	5068-0118	Stator ring
4	5068-0007	Injection valve rotor seal
5	5068-0006	Stator head
6	5068-0018	Stator screws

Cover Parts

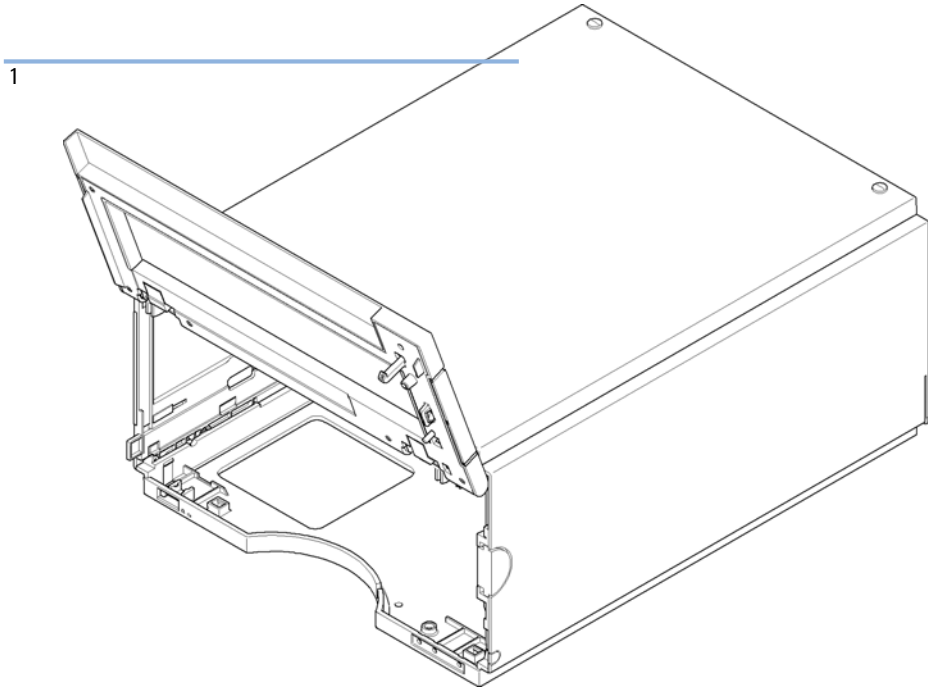


Figure 33 Cover parts

Item	p/n	Description
1	5067-4662	Cabinet kit (base, sides and top)
	5042-9964	Name plate for Agilent 1290 series
	G4226-67001	Door repair kit, includes the front door

Leak System Parts

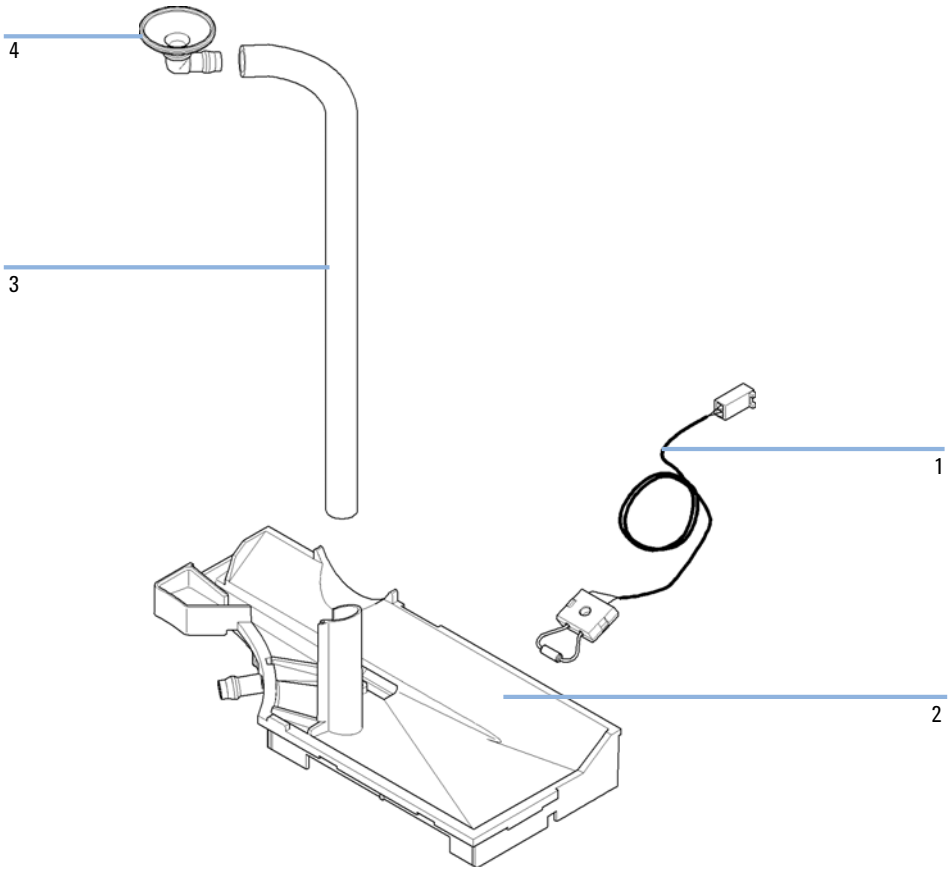


Figure 34 Leak system parts

Item	p/n	Description
1	5061-3356	Leak sensor
2	G4226-44511	Leak plane
3	0890-1711	Leak tubing 185 mm
4	5041-8388	Leak funnel

Upgrade Kits

p/n	Description
5067-4703	40 µL Flex loop kit
G4214A	100 µL Injection Kit, includes 100 µL Flex Loop Kit (5067-4710) and analytical head (G1367-60003), only for 600 bar

Large Volume Injection Kit (multi-draw)

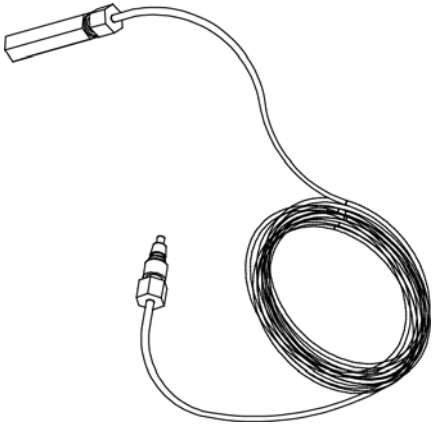


Figure 35 Extension Seat Capillary, 80 µL

Item	p/n	Description
1	G4216-68711	Large Volume Injection Kit (multi-draw) contains the following 2 items:
2	G4216-90000	1290 Infinity 1200 bar Multi-draw Tech Note ENG
3	G4226-87303	Extension Seat Capillary, 80 µL, 0.5 mm ID (0.9 mm OD)

11
Identifying Cables

This chapter provides information on cables used with the 1290 series of HPLC modules.

Cable Overview

NOTE Never use cables other than the ones supplied by Agilent Technologies to ensure proper functionality and compliance with safety or EMC regulations.

Analog cables

p/n	Description
35900-60750	Agilent module to 3394/6 integrators
35900-60750	Agilent 35900A A/D converter
01046-60105	Analog cable (BNC to general purpose, spade lugs)

Remote cables

p/n	Description
03394-60600	Agilent module to 3396A Series I integrators
	3396 Series II / 3395A integrator, see details in section "Remote Cables" on page 178
03396-61010	Agilent module to 3396 Series III / 3395B integrators
5061-3378	Remote Cable
01046-60201	Agilent module to general purpose

BCD cables

p/n	Description
03396-60560	Agilent module to 3396 integrators
G1351-81600	Agilent module to general purpose

CAN cables

p/n	Description
5181-1516	CAN cable, Agilent module to module, 0.5 m
5181-1519	CAN cable, Agilent module to module, 1 m

LAN cables

p/n	Description
5023-0203	Cross-over network cable, shielded, 3 m (for point to point connection)
5023-0202	Twisted pair network cable, shielded, 7 m (for point to point connection)

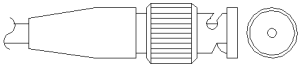
External Contact Cable

p/n	Description
G1103-61611	General Purpose Cable

RS-232 cables

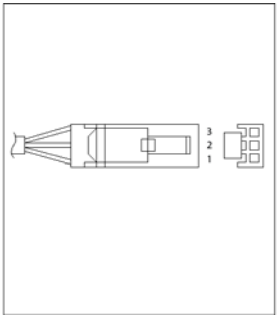
p/n	Description
G1530-60600	RS-232 cable, 2 m
RS232-61601	RS-232 cable, 2.5 m Instrument to PC, 9-to-9 pin (female). This cable has special pin-out, and is not compatible with connecting printers and plotters. It's also called "Null Modem Cable" with full handshaking where the wiring is made between pins 1-1, 2-3, 3-2, 4-6, 5-5, 6-4, 7-8, 8-7, 9-9.
5181-1561	RS-232 cable, 8 m

Analog Cables

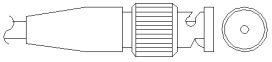


One end of these cables provides a BNC connector to be connected to Agilent modules. The other end depends on the instrument to which connection is being made.

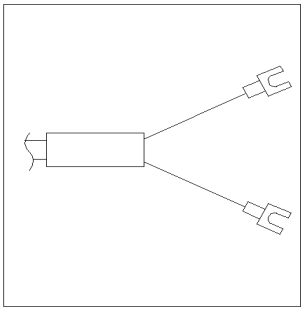
Agilent Module to 3394/6 Integrators

p/n 35900-60750	Pin 3394/6	Pin Agilent module	Signal Name
	1		Not connected
	2	Shield	Analog -
	3	Center	Analog +

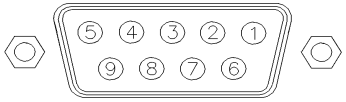
Agilent Module to BNC Connector

p/n 8120-1840	Pin BNC	Pin Agilent module	Signal Name
	Shield	Shield	Analog -
	Center	Center	Analog +

Agilent Module to General Purpose

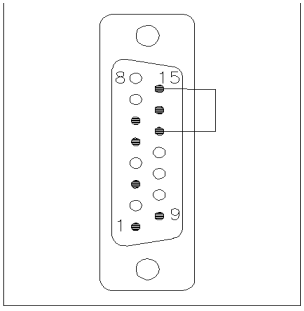
p/n 01046-60105	Pin	Pin Agilent module	Signal Name
	1		Not connected
	2	Black	Analog -
	3	Red	Analog +

Remote Cables



One end of these cables provides a Agilent Technologies APG (Analytical Products Group) remote connector to be connected to Agilent modules. The other end depends on the instrument to be connected to.

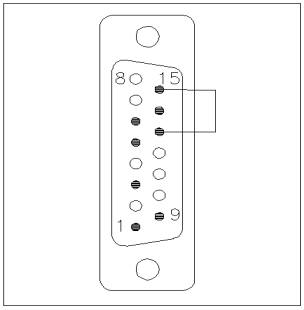
Agilent Module to 3396A Integrators

p/n 03394-60600	Pin 3396A	Pin Agilent module	Signal Name	Active (TTL)
	9	1 - White	Digital ground	
	NC	2 - Brown	Prepare run	Low
	3	3 - Gray	Start	Low
	NC	4 - Blue	Shut down	Low
	NC	5 - Pink	Not connected	
	NC	6 - Yellow	Power on	High
	5,14	7 - Red	Ready	High
	1	8 - Green	Stop	Low
	NC	9 - Black	Start request	Low
	13, 15		Not connected	

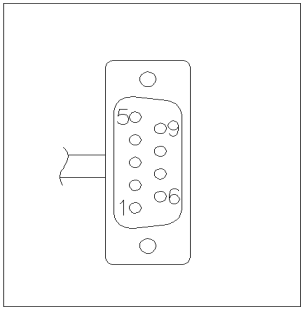
Agilent Module to 3396 Series II / 3395A Integrators

Use the cable Agilent module to 3396A Series I integrators (03394-60600) and cut pin #5 on the integrator side. Otherwise the integrator prints START; not ready.

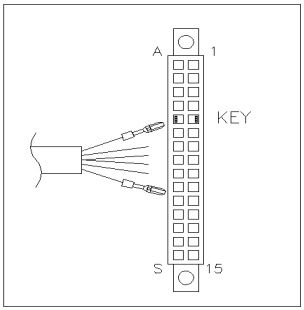
Agilent Module to 3396 Series III / 3395B Integrators

p/n 03396-61010	Pin 33XX	Pin Agilent module	Signal Name	Active (TTL)
	9	1 - White	Digital ground	
	NC	2 - Brown	Prepare run	Low
	3	3 - Gray	Start	Low
	NC	4 - Blue	Shut down	Low
	NC	5 - Pink	Not connected	
	NC	6 - Yellow	Power on	High
	14	7 - Red	Ready	High
	4	8 - Green	Stop	Low
	NC	9 - Black	Start request	Low
	13, 15		Not connected	

Agilent Module to Agilent 35900 A/D Converters

p/n 5061-3378	Pin 35900 A/D	Pin Agilent module	Signal Name	Active (TTL)
	1 - White	1 - White	Digital ground	
	2 - Brown	2 - Brown	Prepare run	Low
	3 - Gray	3 - Gray	Start	Low
	4 - Blue	4 - Blue	Shut down	Low
	5 - Pink	5 - Pink	Not connected	
	6 - Yellow	6 - Yellow	Power on	High
	7 - Red	7 - Red	Ready	High
	8 - Green	8 - Green	Stop	Low
	9 - Black	9 - Black	Start request	Low

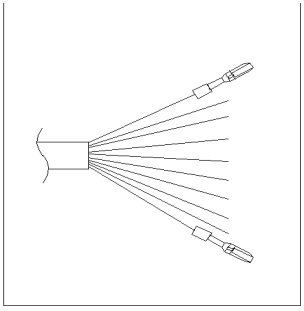
Agilent Module to General Purpose

p/n 01046-60201	Wire Color	Pin Agilent module	Signal Name	Active (TTL)
	White	1	Digital ground	
	Brown	2	Prepare run	Low
	Gray	3	Start	Low
	Blue	4	Shut down	Low
	Pink	5	Not connected	
	Yellow	6	Power on	High
	Red	7	Ready	High
	Green	8	Stop	Low
	Black	9	Start request	Low

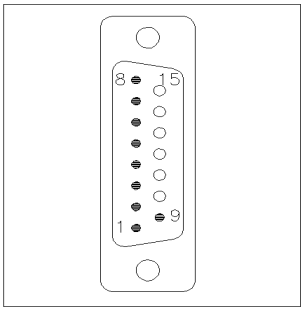
BCD Cables

One end of these cables provides a 15-pin BCD connector to be connected to the Agilent modules. The other end depends on the instrument to be connected to

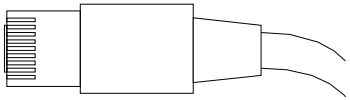
Agilent Module to General Purpose

p/n G1351-81600	Wire Color	Pin Agilent module	Signal Name	BCD Digit
	Green	1	BCD 5	20
	Violet	2	BCD 7	80
	Blue	3	BCD 6	40
	Yellow	4	BCD 4	10
	Black	5	BCD 0	1
	Orange	6	BCD 3	8
	Red	7	BCD 2	4
	Brown	8	BCD 1	2
	Gray	9	Digital ground	Gray
	Gray/pink	10	BCD 11	800
	Red/blue	11	BCD 10	400
	White/green	12	BCD 9	200
	Brown/green	13	BCD 8	100
	not connected	14		
	not connected	15	+ 5 V	Low

Agilent Module to 3396 Integrators

p/n 03396-60560	Pin 3396	Pin Agilent module	Signal Name	BCD Digit
	1	1	BCD 5	20
	2	2	BCD 7	80
	3	3	BCD 6	40
	4	4	BCD 4	10
	5	5	BCD0	1
	6	6	BCD 3	8
	7	7	BCD 2	4
	8	8	BCD 1	2
	9	9	Digital ground	
	NC	15	+ 5 V	Low

CAN/LAN Cables



Both ends of this cable provide a modular plug to be connected to Agilent modules CAN or LAN connectors.

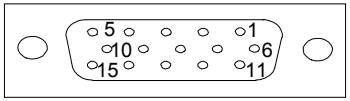
CAN Cables

p/n	Description
5181-1516	CAN cable, Agilent module to module, 0.5 m
5181-1519	CAN cable, Agilent module to module, 1 m

LAN Cables

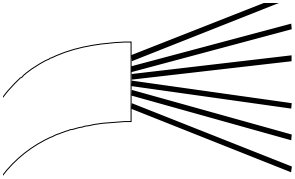
p/n	Description
5023-0203	Cross-over network cable, shielded, 3 m (for point to point connection)
5023-0202	Twisted pair network cable, shielded, 7 m (for point to point connection)

External Contact Cable



One end of this cable provides a 15-pin plug to be connected to Agilent modules interface board. The other end is for general purpose.

Agilent Module Interface Board to general purposes

p/n G1103-61611	Color	Pin Agilent module	Signal Name
	White	1	EXT 1
	Brown	2	EXT 1
	Green	3	EXT 2
	Yellow	4	EXT 2
	Grey	5	EXT 3
	Pink	6	EXT 3
	Blue	7	EXT 4
	Red	8	EXT 4
	Black	9	Not connected
	Violet	10	Not connected
	Grey/pink	11	Not connected
	Red/blue	12	Not connected
	White/green	13	Not connected
	Brown/green	14	Not connected
	White/yellow	15	Not connected

Agilent Module to PC

p/n	Description
G1530-60600	RS-232 cable, 2 m
RS232-61601	RS-232 cable, 2.5 m Instrument to PC, 9-to-9 pin (female). This cable has special pin-out, and is not compatible with connecting printers and plotters. It's also called "Null Modem Cable" with full handshaking where the wiring is made between pins 1-1, 2-3, 3-2, 4-6, 5-5, 6-4, 7-8, 8-7, 9-9.
5181-1561	RS-232 cable, 8 m

Agilent 1200 Module to Printer

p/n	Description
5181-1529	Cable Printer Serial & Parallel, is a SUB-D 9 pin female vs. Centronics connector on the other end (NOT FOR FW UPDATE). For use with G1323 Control Module.

12
Hardware Information

This chapter describes the autosampler in more detail on hardware and electronics.

Firmware Description

The firmware of the instrument consists of two independent sections:

- a non-instrument specific section, called *resident system*
- an instrument specific section, called *main system*

Resident System

This resident section of the firmware is identical for all Agilent 1100/1200/1220/1260/1290 series modules. Its properties are:

- the complete communication capabilities (CAN, LAN and RS-232C)
- memory management
- ability to update the firmware of the 'main system'

Main System

Its properties are:

- the complete communication capabilities (CAN, LAN and RS-232C)
- memory management
- ability to update the firmware of the 'resident system'

In addition the main system comprises the instrument functions that are divided into common functions like

- run synchronization through APG remote,
- error handling,
- diagnostic functions,
- or module specific functions like
  - internal events such as lamp control, filter movements,
  - raw data collection and conversion to absorbance.

Firmware Updates

Firmware updates can be done using the following tools (latest version should be used):

- Agilent Lab Advisor software with files on the hard disk (*)
- Firmware Update Tool with local files on the hard disk (*)
- Instant Pilot (G4208A) with files on a USB Flash Disk

(*) Required tools, firmware and documentation are available from the Agilent web:
http://www.chem.agilent.com/_layouts/agilent/downloadFirmware.aspx?whid=69761

The file naming conventions are:

PPPP_RVVV_XXX.dlb, where

PPPP is the product number, for example, 1315B for the G1315B DAD,

R the firmware revision, for example, A for G1315B or B for the G1315C DAD,

VVV is the revision number, for example 650 is revision 6.50,

XXX is the build number of the firmware.

For instructions on firmware updates refer to section *Replacing Firmware* in chapter *"Maintenance"* or use the documentation provided with the *Firmware Update Tools*.

NOTE

Update of main system can be done in the resident system only. Update of the resident system can be done in the main system only.

Main and resident firmware must be from the same set.

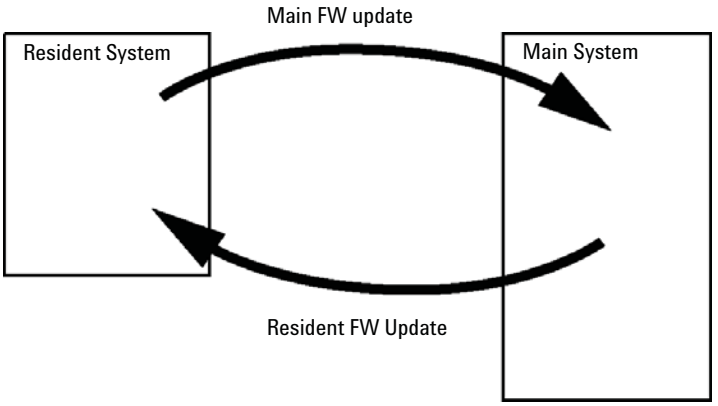


Figure 36 Firmware Update Mechanism

NOTE

Some modules are limited in downgrading due to their main board version or their initial firmware revision. For example, a G1315C DAD SL cannot be downgraded below firmware revision B.01.02 or to a A.xx.xx.

Some modules can be re-branded (e.g. G1314C to G1314B) to allow operation in specific control software environments. In this case the feature set of the target type are use and the feature set of the original are lost. After re-branding (e.g. from G1314B to G1314C), the original feature set is available again.

All these specific informations are described in the documentation provided with the firmware update tools.

The firmware update tools, firmware and documentation are available from the Agilent web.

- http://www.chem.agilent.com/_layouts/agilent/downloadFirmware.aspx?whid=69761

Boot-up and Initialization Process

CAUTION

Obstruction of transport unit

Any obstruction of the transport unit during the initialization process will result in a wrong transmission ratio and thus wrong needle positions.

➔ Make sure no vials or other material gets into the X-slide.

1 Firmware Boot Process.
   a Start Boot Loader.
   b Boot main firmware.

      OR

      Boot resident firmware (if set in VRAM, by DIP switch or if no/wrong main FW is found).
2 Initialize Transport Unit.
   a Switch injection valve to bypass position.
   b Find initial positions for X,Z and theta motors.
   c Check belt tension of theta motor.
   d Determine transmission ratio for X and theta axes.
      - Turn needle carrier fully counter-clockwise (= theta min).
      - Move X-slide into left end-stop (= X min).
      - Move X-slide into right end-stop (= X max).
      - Rotate needle carrier fully clockwise (= theta max, happens at the same time as step iii.).
3 Read RFID tag of Sampling Unit.
4 Read RFID tag of sample tray (if tray is different from last time).
5 Move needle into needle seat to determine the seat depth.
6 Move needle into seat (use depth value from step 5).
7 Lower the needle lock.
8 Switch the injection valve to mainpass.

Electrical Connections

- The CAN bus is a serial bus with high speed data transfer. The two connectors for the CAN bus are used for internal module data transfer and synchronization.
- One analog output provides signals for integrators or data handling systems.
- The REMOTE connector may be used in combination with other analytical instruments from Agilent Technologies if you want to use features such as start, stop, common shut down, prepare, and so on.
- With the appropriate software, the RS-232C connector may be used to control the module from a computer through a RS-232C connection. This connector is activated and can be configured with the configuration switch.
- The power input socket accepts a line voltage of 100 – 240 VAC ± 10 % with a line frequency of 50 or 60 Hz. Maximum power consumption varies by module. There is no voltage selector on your module because the power supply has wide-ranging capability. There are no externally accessible fuses, because automatic electronic fuses are implemented in the power supply.

NOTE Never use cables other than the ones supplied by Agilent Technologies to ensure proper functionality and compliance with safety or EMC regulations.

Rear view of the module

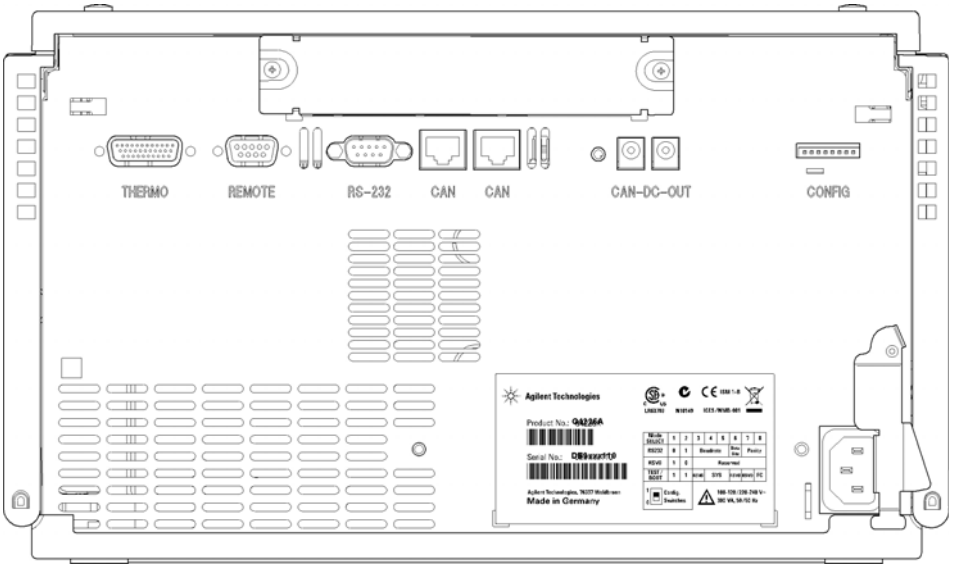


Figure 37 Rear view of the module

Interfaces

The Agilent 1200 Infinity Series modules provide the following interfaces:

Table 10 Agilent 1200 Infinity Series Interfaces

Module	CAN	LAN/BCD (optional)	LAN (on-board)	RS-232	Analog	APG Remote	Special
Pumps							
G1310B Iso Pump G1311B Quat Pump G1311C Quat Pump VL G1312B Bin Pump K1312B Bin Pump Clinical Ed. G1312C Bin Pump VL 1376A Cap Pump G2226A Nano Pump G5611A Bio-inert Quat Pump	2	Yes	No	Yes	1	Yes	
G4220A/B Bin Pump G4204A Quat Pump	2	No	Yes	Yes	No	Yes	CAN-DC- OUT for CAN slaves
G1361A Prep Pump	2	Yes	No	Yes	No	Yes	CAN-DC- OUT for CAN slaves
Samplers							
G1329B ALS G2260A Prep ALS	2	Yes	No	Yes	No	Yes	THERMOSTAT for G1330B/K1330B
G1364B FC-PS G1364C FC-AS G1364D FC-µS G1367E HiP ALS K1367E HiP ALS Clinical Ed. G1377A HiP micro ALS G2258A DL ALS G5664A Bio-inert FC-AS G5667A Bio-inert Autosampler	2	Yes	No	Yes	No	Yes	THERMOSTAT for G1330B/K1330B CAN-DC- OUT for CAN slaves
G4226A ALS	2	Yes	No	Yes	No	Yes	

Table 10 Agilent 1200 Infinity Series Interfaces

Module	CAN	LAN/BCD (optional)	LAN (on-board)	RS-232	Analog	APG Remote	Special
Detectors							
G1314B VWD VL G1314C VWD VL+	2	Yes	No	Yes	1	Yes	
G1314E/F VWD K1314F Clinical Ed.	2	No	Yes	Yes	1	Yes	
G4212A/B DAD K4212B DAD Clinical Ed.	2	No	Yes	Yes	1	Yes	
G1315C DAD VL+ G1365C MWD G1315D DAD VL G1365D MWD VL	2	No	Yes	Yes	2	Yes	
G1321B FLD K1321B FLD Clinical Ed. G1321C FLD	2	Yes	No	Yes	2	Yes	
G1362A RID	2	Yes	No	Yes	1	Yes	
G4280A ELSD	No	No	No	Yes	Yes	Yes	EXT Contact AUTOZERO
Others							
G1170A Valve Drive	2	No	No	No	No	No	1
G1316A/C TCC K1316C TCC Clinical Ed.	2	No	No	Yes	No	Yes	
G1322A DEG K1322A DEG Clinical Ed.	No	No	No	No	No	Yes	AUX
G1379B DEG	No	No	No	Yes	No	Yes	
G4225A DEG K4225A DEG Clinical Ed.	No	No	No	Yes	No	Yes	

Table 10 Agilent 1200 Infinity Series Interfaces

Module	CAN	LAN/BCD (optional)	LAN (on-board)	RS-232	Analog	APG Remote	Special
G4227A Flex Cube	2	No	No	No	No	No	CAN-DC- OUT for CAN slaves [1]
G4240A CHIP CUBE	2	Yes	No	Yes	No	Yes	CAN-DC- OUT for CAN slaves THERMOSTAT for G1330A/B (NOT USED), K1330B

[1] Requires a HOST module with on-board LAN (e.g. G4212A or G4220A with minimum firmware B.06.40 or C.06.40) or with additional G1369C LAN Card

NOTE The detector (DAD/MWD/FLD/VWD/RID) is the preferred access point for control via LAN. The inter-module communication is done via CAN.

- CAN connectors as interface to other modules
- LAN connector as interface to the control software
- RS-232C as interface to a computer
- REMOTE connector as interface to other Agilent products
- Analog output connector(s) for signal output

Overview Interfaces

CAN

The CAN is inter-module communication interface. It is a 2-wire serial bus system supporting high speed data communication and real-time requirement.

LAN

The modules have either an interface slot for an LAN card (e.g. Agilent G1369B/C LAN Interface) or they have an on-board LAN interface (e.g. detectors G1315C/D DAD and G1365C/D MWD). This interface allows the control of the module/system via a PC with the appropriate control software. Some modules have neither on-board LAN nor an interface slot for a LAN card (e.g. G1170A Valve Drive or G4227A Flex Cube). These are hosted modules and require a Host module with firmware B.06.40 or later or with additional G1369C LAN Card.

NOTE If an Agilent detector (DAD/MWD/FLD/VWD/RID) is in the system, the LAN should be connected to the DAD/MWD/FLD/VWD/RID (due to higher data load). If no Agilent detector is part of the system, the LAN interface should be installed in the pump or autosampler.

RS-232C (Serial)

The RS-232C connector is used to control the module from a computer through RS-232C connection, using the appropriate software. This connector can be configured with the configuration switch module at the rear of the module. Refer to *Communication Settings for RS-232C*.

NOTE There is no configuration possible on main boards with on-board LAN. These are pre-configured for

- 19200 baud,
- 8 data bit with no parity and
- one start bit and one stop bit are always used (not selectable).

The RS-232C is designed as DCE (data communication equipment) with a 9-pin male SUB-D type connector. The pins are defined as:

Table 11 RS-232C Connection Table

Pin	Direction	Function
1	In	DCD
2	In	RxD
3	Out	TxD
4	Out	DTR
5		Ground
6	In	DSR
7	Out	RTS
8	In	CTS
9	In	RI

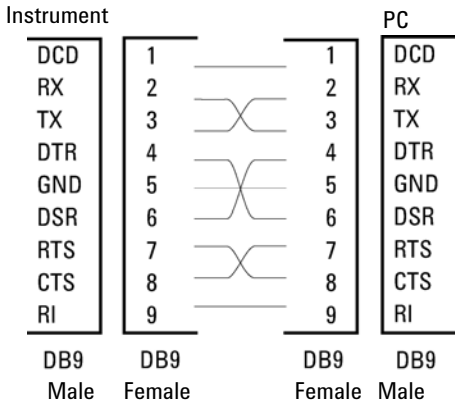


Figure 38 RS-232 Cable

Analog Signal Output

The analog signal output can be distributed to a recording device. For details refer to the description of the module's main board.

APG Remote

The APG Remote connector may be used in combination with other analytical instruments from Agilent Technologies if you want to use features as common shut down, prepare, and so on.

Remote control allows easy connection between single instruments or systems to ensure coordinated analysis with simple coupling requirements.

The subminiature D connector is used. The module provides one remote connector which is inputs/outputs (wired- or technique).

To provide maximum safety within a distributed analysis system, one line is dedicated to **SHUT DOWN** the system's critical parts in case any module detects a serious problem. To detect whether all participating modules are switched on or properly powered, one line is defined to summarize the **POWER ON** state of all connected modules. Control of analysis is maintained by signal readiness **READY** for next analysis, followed by **START** of run and optional **STOP** of run triggered on the respective lines. In addition **PREPARE** and **START REQUEST** may be issued. The signal levels are defined as:

- standard TTL levels (0 V is logic true, + 5.0 V is false),
- fan-out is 10,
- input load is 2.2 kOhm against + 5.0 V, and
- output are open collector type, inputs/outputs (wired- or technique).

NOTE All common TTL circuits operate with a 5 V power supply. A TTL signal is defined as "low" or L when between 0 V and 0.8 V and "high" or H when between 2.0 V and 5.0 V (with respect to the ground terminal).

Table 12 Remote Signal Distribution

Pin	Signal	Description
1	DGND	Digital ground
2	PREPARE	(L) Request to prepare for analysis (for example, calibration, detector lamp on). Receiver is any module performing pre-analysis activities.
3	START	(L) Request to start run / timetable. Receiver is any module performing run-time controlled activities.
4	SHUT DOWN	(L) System has serious problem (for example, leak: stops pump). Receiver is any module capable to reduce safety risk.
5		Not used
6	POWER ON	(H) All modules connected to system are switched on. Receiver is any module relying on operation of others.
7	READY	(H) System is ready for next analysis. Receiver is any sequence controller.
8	STOP	(L) Request to reach system ready state as soon as possible (for example, stop run, abort or finish and stop injection). Receiver is any module performing run-time controlled activities.
9	START REQUEST	(L) Request to start injection cycle (for example, by start key on any module). Receiver is the autosampler.

Special Interfaces

There is no special interface for this module.

Setting the 8-bit Configuration Switch (without On-board) LAN

The 8-bit configuration switch is located at the rear of the module.

This module does not have its own on-board LAN interface. It can be controlled through the LAN interface of another module, and a CAN connection to that module.

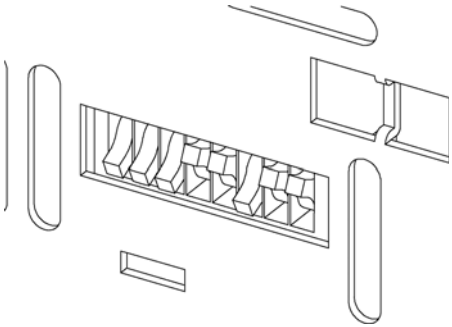


Figure 39 Configuration switch (settings depend on configured mode)

All modules without on-board LAN:

- default should be ALL DIPS DOWN (= best settings)
  - Bootp mode for LAN and
  - 19200 baud, 8 data bit / 1 stop bit with no parity for RS-232
- DIP 1 DOWN and DIP 2 UP allows special RS-232 settings
- for boot/test modes DIPS 1+2 must be UP plus required mode

NOTE For normal operation use the default (best) settings.

Switch settings provide configuration parameters for serial communication protocol and instrument specific initialization procedures.

NOTE With the introduction of the Agilent 1260 Infinity, all GPIB interfaces have been removed. The preferred communication is LAN.

NOTE The following tables represent the configuration switch settings for the modules without on-board LAN only.

Table 13 8-bit Configuration Switch (without on-board LAN)

Mode Select	1	2	3	4	5	6	7	8
RS-232C	0	1	Baudrate			Data Bits	Parity	
Reserved	1	0	Reserved					
TEST/BOOT	1	1	RSVD	SYS		RSVD	RSVD	FC

NOTE The LAN settings are done on the LAN Interface Card G1369B/C. Refer to the documentation provided with the card.

Communication Settings for RS-232C

The communication protocol used in the column compartment supports only hardware handshake (CTS/RTR).

Switches 1 in down and 2 in up position define that the RS-232C parameters will be changed. Once the change has been completed, the column instrument must be powered up again in order to store the values in the non-volatile memory.

Table 14 Communication Settings for RS-232C Communication (without on-board LAN)

Mode Select	1	2	3	4	5	6	7	8
RS-232C	0	1	Baudrate			Data Bits	Parity	

Use the following tables for selecting the setting which you want to use for RS-232C communication. The number 0 means that the switch is down and 1 means that the switch is up.

Table 15 Baudrate Settings (without on-board LAN)

Switches			Baud Rate	Switches			Baud Rate
3	**4**	**5**		**3**	**4**	**5**	
0	0	0	9600	1	0	0	9600
0	0	1	1200	1	0	1	14400
0	1	0	2400	1	1	0	19200
0	1	1	4800	1	1	1	38400

Table 16 Data Bit Settings (without on-board LAN)

Switch 6	Data Word Size
0	7 Bit Communication
1	8 Bit Communication

Table 17 Parity Settings (without on-board LAN)

Switches		Parity
7	8	
0	0	No Parity
0	1	Odd Parity
1	1	Even Parity

One start bit and one stop bit are always used (not selectable).

Per default, the module will turn into 19200 baud, 8 data bit with no parity.

Special Settings

The special settings are required for specific actions (normally in a service case).

Boot-Resident

Firmware update procedures may require this mode in case of firmware loading errors (main firmware part).

If you use the following switch settings and power the instrument up again, the instrument firmware stays in the resident mode. It is not operable as a module. It only uses basic functions of the operating system for example, for communication. In this mode the main firmware can be loaded (using update utilities).

Table 18 Boot Resident Settings (without on-board LAN)

Mode Select	SW1	SW2	SW3	SW4	SW5	SW6	SW7	SW8
TEST/BOOT	1	1	0	0	1	0	0	0

Forced Cold Start

A forced cold start can be used to bring the module into a defined mode with default parameter settings.

CAUTION

Loss of data

Forced cold start erases all methods and data stored in the non-volatile memory. Exceptions are calibration settings, diagnosis and repair log books which will not be erased.

➔ Save your methods and data before executing a forced cold start.

If you use the following switch settings and power the instrument up again, a forced cold start has been completed.

Table 19 Forced Cold Start Settings (without on-board LAN)

Mode Select	SW1	SW2	SW3	SW4	SW5	SW6	SW7	SW8
TEST/BOOT	1	1	0	0	0	0	0	1

Early Maintenance Feedback

Maintenance requires the exchange of components which are subject to wear or stress. Ideally, the frequency at which components are exchanged should be based on the intensity of usage of the module and the analytical conditions, and not on a predefined time interval. The early maintenance feedback (**EMF**) feature monitors the usage of specific components in the instrument, and provides feedback when the user-selectable limits have been exceeded. The visual feedback in the user interface provides an indication that maintenance procedures should be scheduled.

EMF Counters

EMF counters increment with use and can be assigned a maximum limit which provides visual feedback in the user interface when the limit is exceeded. Some counters can be reset to zero after the required maintenance procedure.

Using the EMF Counters

The user-settable **EMF** limits for the **EMF Counters** enable the early maintenance feedback to be adapted to specific user requirements. The useful maintenance cycle is dependent on the requirements for use. Therefore, the definition of the maximum limits need to be determined based on the specific operating conditions of the instrument.

Setting the EMF Limits

The setting of the **EMF** limits must be optimized over one or two maintenance cycles. Initially the default **EMF** limits should be set. When instrument performance indicates maintenance is necessary, take note of the values displayed by the **EMF counters**. Enter these values (or values slightly less than the displayed values) as **EMF** limits, and then reset the **EMF counters** to zero. The next time the **EMF counters** exceed the new **EMF** limits, the **EMF** flag will be displayed, providing a reminder that maintenance needs to be scheduled.

Instrument Layout

The industrial design of the module incorporates several innovative features. It uses Agilent's E-PAC concept for the packaging of electronics and mechanical assemblies. This concept is based upon the use of expanded polypropylene (EPP) layers of foam plastic spacers in which the mechanical and electronic boards components of the module are placed. This pack is then housed in a metal inner cabinet which is enclosed by a plastic external cabinet. The advantages of this packaging technology are:

- virtual elimination of fixing screws, bolts or ties, reducing the number of components and increasing the speed of assembly/disassembly,
- the plastic layers have air channels molded into them so that cooling air can be guided exactly to the required locations,
- the plastic layers help cushion the electronic and mechanical parts from physical shock, and
- the metal inner cabinet shields the internal electronics from electromagnetic interference and also helps to reduce or eliminate radio frequency emissions from the instrument itself.

13
LAN Configuration

This chapter provides information on connecting the autosampler to the Agilent ChemStation PC.

Setting up the module in a LAN environment

It is not recommended to connect an Agilent 1290 Infinity system via the G4226A Autosampler. The G4212A Diode Array Detector is producing the most data in the stack, followed by the G4220A Binary pump, and it is therefore highly recommended to use either of these modules for the LAN connection.

Connecting the module via LAN

If the module is being operated as a standalone module or if a connection via LAN is required regardless of above mentioned recommendation, a G1369B/C LAN card has to be used. For installation and configuration, see the G1369B/C documentation.

14
Appendix

This chapter provides addition information on safety, legal and web.

General Safety Information

General Safety Information

The following general safety precautions must be observed during all phases of operation, service, and repair of this instrument. Failure to comply with these precautions or with specific warnings elsewhere in this manual violates safety standards of design, manufacture, and intended use of the instrument. Agilent Technologies assumes no liability for the customer's failure to comply with these requirements.

WARNING

Ensure the proper usage of the equipment.

The protection provided by the equipment may be impaired.

➔ The operator of this instrument is advised to use the equipment in a manner as specified in this manual.

Safety Standards

This is a Safety Class I instrument (provided with terminal for protective earthing) and has been manufactured and tested according to international safety standards.

Operation

Before applying power, comply with the installation section. Additionally the following must be observed.

Do not remove instrument covers when operating. Before the instrument is switched on, all protective earth terminals, extension cords, auto-transformers, and devices connected to it must be connected to a protective earth via a ground socket. Any interruption of the protective earth grounding will cause a potential shock hazard that could result in serious personal injury. Whenever it is likely that the protection has been impaired, the instrument must be made inoperative and be secured against any intended operation.

Make sure that only fuses with the required rated current and of the specified type (normal blow, time delay, and so on) are used for replacement. The use of repaired fuses and the short-circuiting of fuse holders must be avoided.

Some adjustments described in the manual, are made with power supplied to the instrument, and protective covers removed. Energy available at many points may, if contacted, result in personal injury.

Any adjustment, maintenance, and repair of the opened instrument under voltage should be avoided whenever possible. When inevitable, this has to be carried out by a skilled person who is aware of the hazard involved. Do not attempt internal service or adjustment unless another person, capable of rendering first aid and resuscitation, is present. Do not replace components with power cable connected.

Do not operate the instrument in the presence of flammable gases or fumes. Operation of any electrical instrument in such an environment constitutes a definite safety hazard.

Do not install substitute parts or make any unauthorized modification to the instrument.

Capacitors inside the instrument may still be charged, even though the instrument has been disconnected from its source of supply. Dangerous voltages, capable of causing serious personal injury, are present in this instrument. Use extreme caution when handling, testing and adjusting.

When working with solvents, observe appropriate safety procedures (for example, goggles, safety gloves and protective clothing) as described in the material handling and safety data sheet by the solvent vendor, especially when toxic or hazardous solvents are used.

Safety Symbols

Table 20 Safety Symbols

Symbol	Description
	The apparatus is marked with this symbol when the user should refer to the instruction manual in order to protect risk of harm to the operator and to protect the apparatus against damage.
	Indicates dangerous voltages.
	Indicates a protected ground terminal.
	Indicates eye damage may result from directly viewing the light produced by the deuterium lamp used in this product.
	The apparatus is marked with this symbol when hot surfaces are available and the user should not touch it when heated up.

WARNING

A WARNING

alerts you to situations that could cause physical injury or death.

➔ Do not proceed beyond a warning until you have fully understood and met the indicated conditions.

CAUTION

A CAUTION

alerts you to situations that could cause loss of data, or damage of equipment.

➔ Do not proceed beyond a caution until you have fully understood and met the indicated conditions.

Lithium Batteries Information

WARNING **Lithium batteries may not be disposed-off into the domestic waste. Transportation of discharged Lithium batteries through carriers regulated by IATA/ICAO, ADR, RID, IMDG is not allowed.**

Danger of explosion if battery is incorrectly replaced.

➔ Discharged Lithium batteries shall be disposed off locally according to national waste disposal regulations for batteries.

➔ Replace only with the same or equivalent type recommended by the equipment manufacturer.

WARNING **Lithiumbatteri - Eksplosionsfare ved fejlagtig håndtering.**

Udskiftning må kun ske med batteri af samme fabrikat og type.

➔ Lever det brugte batteri tilbage til leverandøren.

WARNING **Lithiumbatteri - Eksplosionsfare.**

Ved udskiftning benyttes kun batteri som anbefalt av apparatfabrikanten.

➔ Brukt batteri returneres appararleverandoren.

NOTE Bij dit apparaat zijn batterijen geleverd. Wanneer deze leeg zijn, moet u ze niet weggooien maar inleveren als KCA.

The Waste Electrical and Electronic Equipment (WEEE) Directive (2002/96/EC)

Abstract

The Waste Electrical and Electronic Equipment (WEEE) Directive (2002/96/EC), adopted by EU Commission on 13 February 2003, is introducing producer responsibility on all Electric and Electronic appliances from 13 August 2005.

NOTE

This product complies with the WEEE Directive (2002/96/EC) marking requirements. The affixed label indicates that you must not discard this electrical/electronic product in domestic household waste.

Product Category: With reference to the equipment types in the WEEE Directive Annex I, this product is classed as a “Monitoring and Control instrumentation” product.

Do not dispose off in domestic household waste

To return unwanted products, contact your local Agilent office, or see www.agilent.com for more information.

Radio Interference

Never use cables other than the ones supplied by Agilent Technologies to ensure proper functionality and compliance with safety or EMC regulations.

Test and Measurement

If test and measurement equipment is operated with equipment unscreened cables and/or used for measurements on open set-ups, the user has to assure that under operating conditions the radio interference limits are still met within the premises.

Sound Emission

Manufacturer's Declaration

This statement is provided to comply with the requirements of the German Sound Emission Directive of 18 January 1991.

This product has a sound pressure emission (at the operator position) < 70 dB.

- Sound Pressure Lp < 70 dB (A)
- At Operator Position
- Normal Operation
- According to ISO 7779:1988/EN 27779/1991 (Type Test)

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http://www.agilent.com

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In This Book

This manual contains technical reference information about the Agilent 1290 Infinity autosampler G4226A.

- introduction and specifications,
- installation,
- using and optimizing,
- troubleshooting and diagnose,
- maintenance,
- parts identification,
- hardware information,
- safety and related information.

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