



Agilent 1260 Infinity Analytical- and Preparative-scale Fraction Collectors

User Manual



Agilent Technologies

Notices

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Manual Part Number

G1364-90014

Edition

03/2014

Printed in Germany

Agilent Technologies
Hewlett-Packard-Strasse 8
76337 Waldbronn

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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 contains technical reference information about the Agilent 1260 Infinity Fraction Collectors analytical and preparative scale. The manual describes the following:

1 Installing the Fraction Collector

This chapter provides information about the installation of the Agilent 1260 Infinity Fraction Collectors G1364B and C.

2 Configuration and Operation of the Fraction Collector

This chapter describes the configuration and operation of the fraction collector including guidelines to optimize the system and to avoid problems.

3 Troubleshooting and Test Functions

This chapter describes the modules built-in troubleshooting and test functions.

4 Repairing the Fraction Collector

This chapter contains instructions on simple repair and maintenance procedures.

5 Parts and Materials

This chapter contains lists for identification of common repair and maintenance parts.

6 Specifications

This chapter contains performance specifications of the fraction collectors.

A Safety Information

This appendix provides a safety summary.

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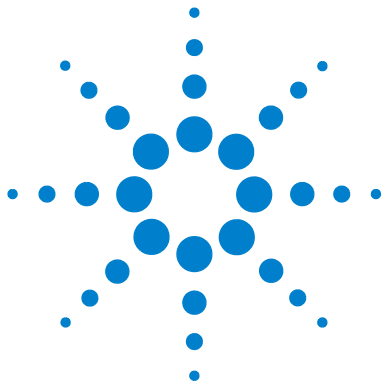
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Site Requirements

A suitable site environment is important to ensure optimum performance of the Fraction Collector.

Power Consideration

The Fraction Collector power supply has wide-ranging capability (see [Table 1](#) on page 8). Consequently there is no voltage selector in the rear of the Fraction Collector. There are also no externally accessible fuses, as automatic electronic fuses are integrated in the power supply.

The thermostatted fraction collector comprises two modules, the fraction collector (G1364B or G1364C) and the thermostat (G1330B). Both modules have a separate power supply and a power plug for the line connections. The two modules are connected by a control cable and both are turned on by the Fraction Collector module. The thermostat power supply has two externally accessible fuses.

WARNING

To disconnect the Fraction Collector from line power, unplug the power cord. The power supply still uses some power, even if the power switch on the front panel is turned off.

WARNING

To disconnect the thermostatted Fraction Collector from line power, unplug the power cord from the Fraction Collector and the thermostat. The power supplies still use some power, even if the power switch on the front panel is turned off. Please make sure that it is always possible to access the power plug.

WARNING

Shock hazard or damage of your instrumentation can result if the devices are connected to a line voltage higher than specified.

Power Cords

Your Fraction Collector is delivered with a power cord which matches the wall socket of your particular country or region. The plug on the power cord which connects to the rear of the instrument is identical for all types of power cord.

WARNING

Never operate your instrumentation from a power outlet that has no ground connection. Never use a power cord other than the power cord designed for your region.

WARNING

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

Bench Space

The Fraction Collector dimensions and weight (see [Table 1](#) on page 8) allow the instrument to be placed on almost any laboratory bench. The instrument requires an additional 2.5 cm (1.0 inch) of space on either side, and approximately 8 cm (3.1 inches) at the rear for the circulation of air, and room for electrical connections. Ensure the Fraction Collector is installed in a horizontal position.

The thermostatted Fraction Collector dimensions and weight (see [Table 2](#) on page 9) allow the instrument to be placed on almost any laboratory bench. The instrument requires an additional 25 cm (10 inches) of space on either side for the circulation of air, and approximately 8 cm (3.1 inches) at the rear for electrical connections. Ensure the Fraction Collector is installed in a horizontal position.

If a complete Agilent 1260 Infinity LC System is to be installed on the bench, make sure that the bench is designed to carry the weight of all the modules. For a complete system including the thermostatted Fraction Collector it is recommended to position the modules in two stacks, see “[Recommended Stack Configuration - Fraction Collector in an Analytical System \(Front View\)](#)” on page 14. Make sure that in this configuration there is 25 cm (10 inches) space on either side of the thermostatted Fraction Collector for the circulation of air.

Environment

Your Fraction Collector will work within specifications at ambient temperatures and relative humidity as described in [Table 1](#) and [Table 2](#) on page 9.

CAUTION

Do not store, ship or use your Fraction Collector under conditions where temperature fluctuations may cause condensation within the Fraction Collector. Condensation will damage the system electronics. If your Fraction Collector was shipped in cold weather, leave it in its box, and allow it to warm up slowly to room temperature to avoid condensation.

Table 1 Physical Specifications - Fraction Collectors (G1364B, G1364C)

Type	Specification	Comments
Weight	13.5 kg (29.8 lbs)	
Dimensions (height × width × depth)	200 × 345 × 440 mm (8 × 13.5 × 17 inches)	
Line voltage	100 – 240 VAC, ±10 %	Wide-ranging capability
Line frequency	50 or 60 Hz, ±5 %	
Power consumption (apparent power)	200 VA	Maximum
Power consumption (active power)	180 W	Maximum
Ambient operating temperature	4 – 45 °C (41 – 113 °F)	
Ambient non-operating temperature	-40 – 70 °C (-4 – 158 °F)	
Humidity	< 95 %, at 25 – 40 °C (77 – 104 °F)	Non-condensing
Operating Altitude	Up to 2000 m (6500 ft)	
Non-operating altitude	Up to 4600 m (14950 ft)	For storing the Fraction Collector
Safety standards: IEC, CSA, UL	Installation Category II, Pollution Degree 2. For indoor use only.	

Table 2 Physical Specifications - Thermostat (G1330B)

Type	Specification	Comments
Weight	18.5 kg (40.7 lbs)	
Dimensions (height × width × depth)	140 × 345 × 435 mm (5.5 × 13.5 × 17 inches)	
Line voltage	100 – 120 or 220 – 240 VAC, ± 10 %	Wide-ranging capability
Line frequency	50 or 60 Hz, ± 5 %	
Power consumption (active power)	210 W	Maximum
Power consumption (apparent power)	260 VA	Maximum
Ambient operating temperature	4 – 40 °C (41 – 131 °F)	
Ambient non-operating temperature	-40 – 70 °C (-4 – 158 °F)	
Humidity	< 95 %, at 25 – 40 °C (77 – 104 °F)	Non-condensing
Operating Altitude	Up to 2000 m (6500 ft)	
Non-operating altitude	Up to 4600 m (14950 ft)	For storing the Fraction Collector
Safety standards: IEC, CSA, UL, EN	Installation Category II, Pollution Degree 2. For indoor use only.	

Unpacking the Fraction Collector

CAUTION

If you need to ship the Fraction Collector at a later date, always use the shipping protection foam parts (see “[Transporting the Fraction Collector](#)” on page 37).

Damaged Packaging

Upon receipt of your Fraction Collector, inspect the shipping containers for any signs of damage. If the containers or cushioning material are damaged, keep them until the contents have been checked for completeness and the Fraction Collector has been mechanically and electrically checked. If the shipping container or cushioning material is damaged, notify the carrier and keep the shipping material for the carrier’s inspection.

CAUTION

If there are signs of damage to the Fraction Collector, please do not attempt to install the Fraction Collector.

Delivery Checklist

Ensure all parts and materials have been delivered with the Fraction Collector. 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.

Three models of Agilent 1260 Infinity fraction collectors are available:

- *G1364B* Fraction Collector, *preparative-scale*, designed for flow rates up to 100 ml / min. and for the use with vials, deep well plates and test tubes up to 100 mm height.
- *G1364C* Fraction Collector, *analytical-scale*, designed for flow rates below 10 ml / min. (can be modified for higher flow rates) and for the use with vials, test tubes of up to 75 mm height, well-plates and a 40-funnel tray connecting to external locations of any size

- *G1364C Thermostatted Fraction Collector, analytical scale*, can be created by additionally ordering and installing a G1330B Fraction Collector Thermostat.

The terms (thermostatted) *analytical scale* Fraction Collector and the *preparative scale* Fraction Collector refer to these Agilent 1260 Infinity modules in this manual. Unless otherwise stated all information in this section is valid for all models.

Table 3 Delivery Checklist for the G1364B (preparative-scale) and G1364C (analytical-scale) Fraction Collector

Description	Quantity
Fraction collector module with inlet / waste tubing assembly	1
Power cord, local (Matching the sockets in your country or region)	1
Accessory kit (see below)	1
Delay Calibrant G1946-85020	1

Table 4 Delivery Checklist for the G1330B Thermostat Module (optional)

Description	Quantity
Thermostat Module	1
Power cord, local (Matching the sockets in your country or region)	1
Accessory kit thermostat module (see below)	1

Accessory Kits

Each shipment contents an Accessory kit with the necessary tools to install the system and to have an operating system.

1 Installing the Fraction Collector

Unpacking the Fraction Collector

Table 5 Fraction Collector Accessory Kit Contents G1364-68725

Description	Quantity	Part Number
Wrench, open end, 4mm	1	8710-1534
Wrench, open end, 1/4-5/16 inch	1	8710-0510
Hex key 2.0 mm	1	8710-2476
Finger tight fittings, 1/16" f-120*	3	0100-1516
Waste tubing (1.2 m) [†]	1	5062-2463
Y-Type Fitting for waste tubing [‡]	1	5065-9971
CAN cable, 1 m	1	5181-1519
Air channel adapter	1	G1329-43200
Sticking clamp for corrugated waste tubing (large)**	3	5065-9976
Sticking clamp for waste tubing (small) ^{††}	3	5065-9976
Tray for 15 x 6 ml vials	1	G1313-44513
Tray for 40 x 2 ml vials	1	G1313-44512

* Reorder gives pack of 2

† Reorder gives 5 m

‡ Reorder gives pack of 10

** Reorder gives 10 clamps for corrugated waste tubing (large))

†† Reorder gives 10 clamps for waste tubing (small)

- The Accessory kit (G1330-68705) shown in [Table 6](#) is shipped with the (G1330B) thermostat module if the thermostat module was ordered.

Table 6 (Optional) Thermostat Module Accessory Kit Contents G1330-68705

Description	Quantity	Part Number
Waste tubing (1.2 m) [*]	1	5062-2463
Cable thermostat module to Fraction Collector	1	G1330-81600

* Reorder gives 5 m

Optimizing the Stack Configuration

If your Fraction Collector is part of a system, you can ensure optimum performance and minimum delay volume by installing the following configuration.

- [Figure 1](#) and [Figure 2](#) on page 15 show the configuration recommended for the Fraction Collector in an analytical scale system.
- [Figure 3](#) on page 16 and [Figure 4](#) on page 17 show the configuration recommended for the Fraction Collector with a preparative scale system.

NOTE

Agilent 1260 Infinity LC Purification Systems can comprise many different LC modules. This section describes some common stack configurations and general considerations. On more complex systems, e.g. with two or more fraction collectors or with mass selective detector, other stack configurations might show advantages.

1 Installing the Fraction Collector

Optimizing the Stack Configuration

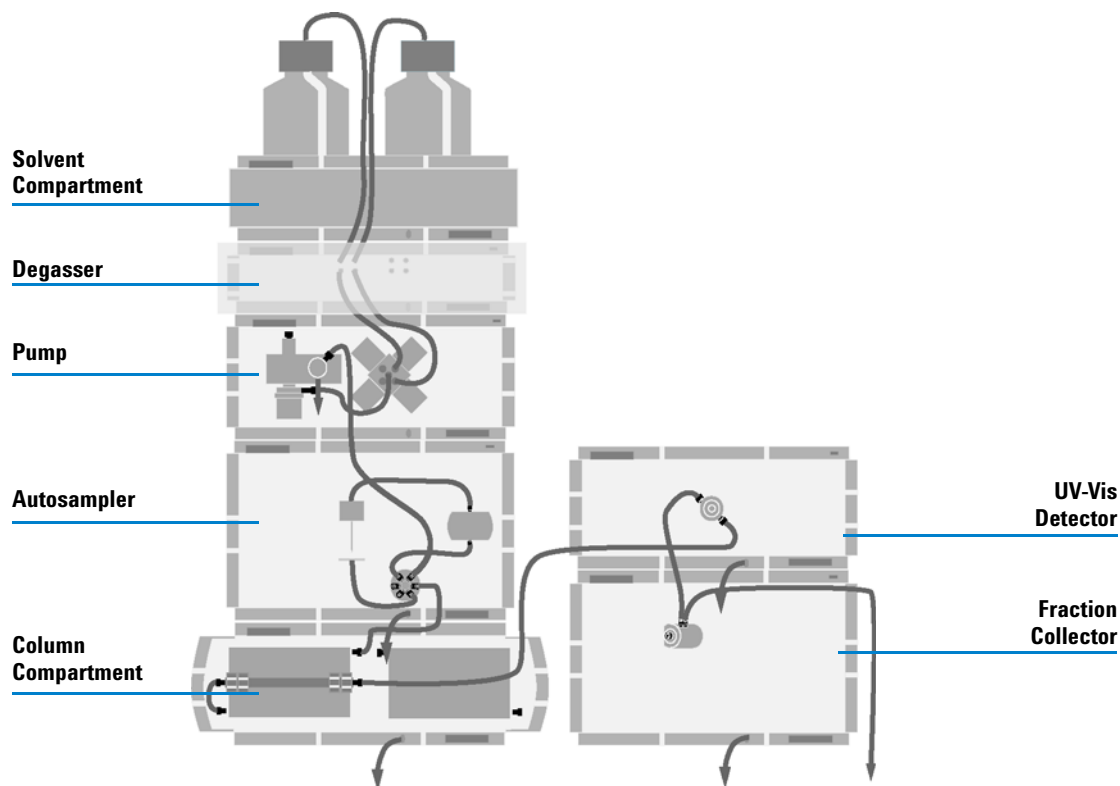


Figure 1 Recommended Stack Configuration - Fraction Collector in an Analytical System (Front View)

NOTE

For information about connecting an Agilent LC-MSD, a Non-1260 Infinity or Non-UV-Vis detector to the system, please refer to the "Agilent 1260 Infinity Purification Solution-System User Guide", PN M8368-90300. For configuration of an MSD with "Active Splitter Configuration", p/n G1968-90002, refer to the instructions delivered with the kit.

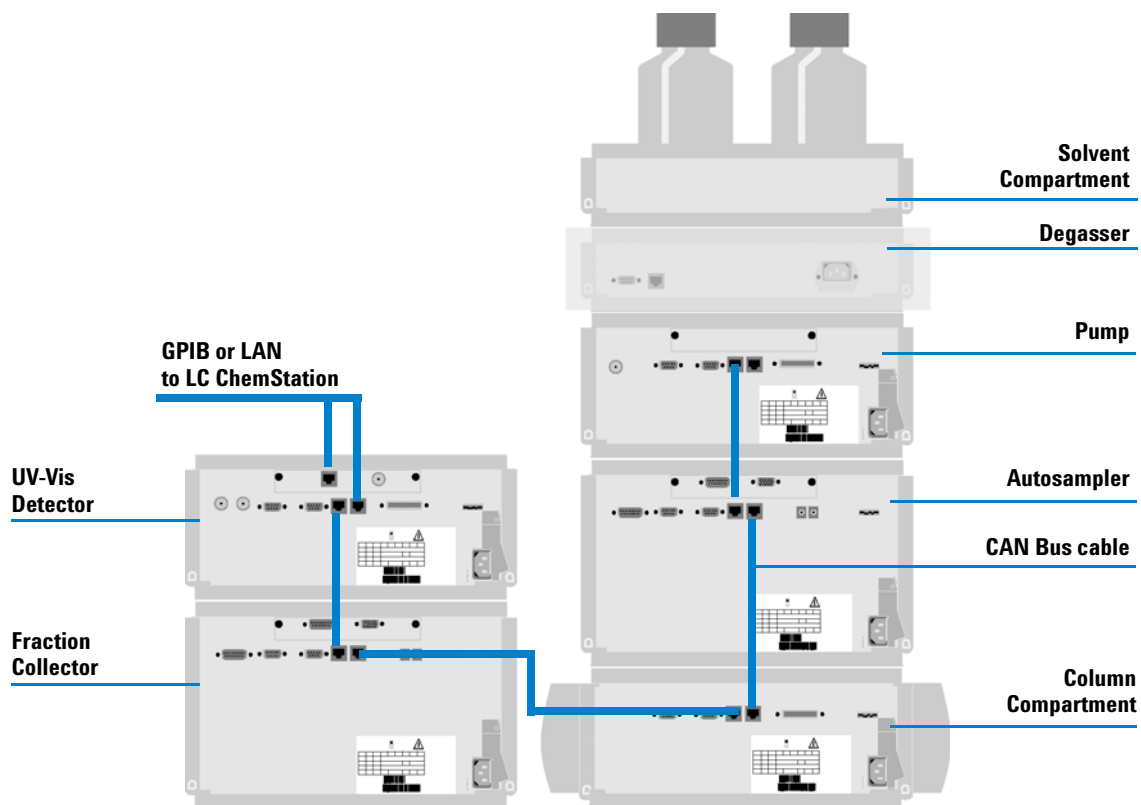


Figure 2 Recommended Stack Configuration - Fraction Collector in an Analytical System (Rear View)

NOTE

If a G1330B ALS thermostat is part of the system it must always be installed underneath the thermostatted injector or Fraction Collector (see “Installing a Thermostatted Fraction Collector” on page 22).

NOTE

The fraction collector should never be positioned on top of a module that generates heat. This could lead to an unwanted evaporation of fractions in the fraction collector (e.g. Agilent 1260 Infinity Thermostatted Column Compartment G1316A or Agilent 1260 Infinity Diode Array Detectors).

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Optimizing the Stack Configuration

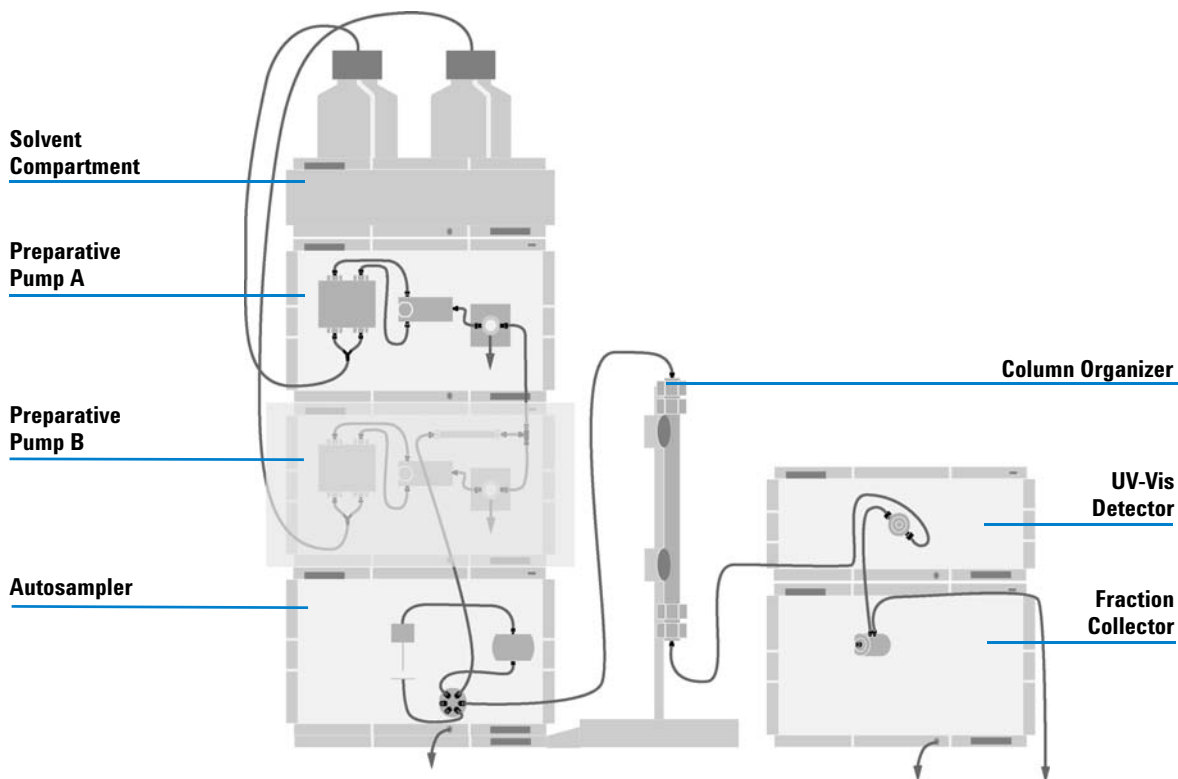


Figure 3 Recommended Stack Configuration - Fraction Collector in a Preparative System (Front View)

NOTE

For information about connecting an Agilent LC-MSD, a Non-1260 Infinity or Non-UV-Vis detector to the system, please refer to the "Agilent 1260 Infinity Purification Solution-System User Guide", PN M8368-90300. For configuration of an MSD with "Active Splitter Configuration", p/n G1968-90002, refer to the instructions delivered with the kit.

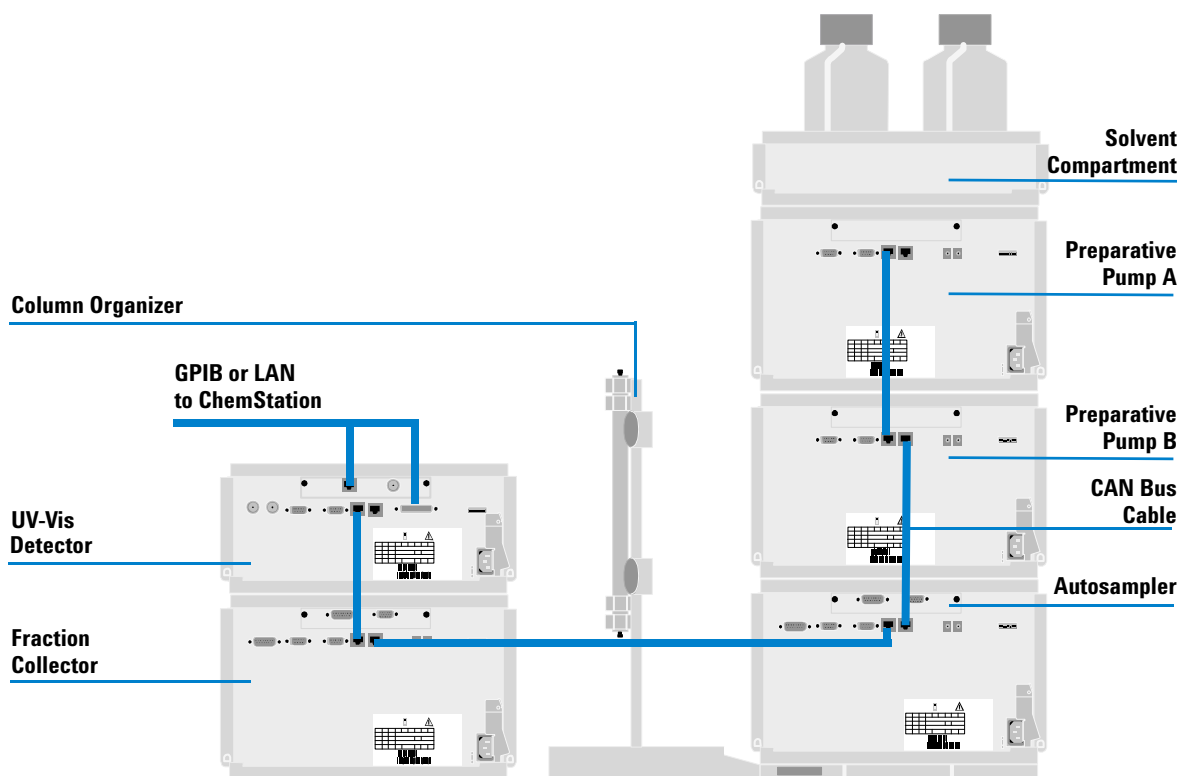


Figure 4 Recommended Stack Configuration - Fraction Collector in a Preparative System (Rear View)

NOTE

If a G1330B ALS thermostat is part of the system it must always be installed underneath the module, that is thermostatted injector or Fraction Collector (see [“Installing a Thermostatted Fraction Collector”](#) on page 22).

NOTE

The fraction collector should never be positioned on top of a module that generates heat, which can lead to an unwanted evaporation of fractions in the fraction collector (e.g. Agilent 1260 Infinity Thermostatted Column Compartment G1316A or Agilent 1260 Infinity Diode Array Detectors).

1 Installing the Fraction Collector

Installation Instructions for the Fraction Collector

Installation Instructions for the Fraction Collector

Preparation	Locate bench space Provide power connections Unpack the Fraction Collector
Parts required	Fraction Collector Power cord, Chemstation or Agilent Instant Pilot G4208A

WARNING

When opening capillary or tube fittings solvents may leak out. Please observe appropriate safety procedures (for example, goggles, safety gloves and protective clothing) as described in the material handling and safety data sheet supplied by the solvent vendor, especially when toxic or hazardous solvents are used.

WARNING

To avoid personal injury, keep fingers away from the needle area during Fraction Collector operation. Do not attempt to insert or remove a vial or a plate when the needle is positioned.

- 1 Install the LAN interface board in the Fraction Collector (if required).
- 2 Remove the adhesive tape which covers the side and front doors.
- 3 Open the front door and remove the left side door.
- 4 Remove the transport protection foam.
- 5 Install the corrugated waste tube in the plastic port at the front bottom center of the instrument. Slide the waste tubing coming from the internal tray (if present) through the plastic port and the corrugated waste tube (see [Figure 5](#) on page 19). Route the corrugated waste tubing into a waste container.

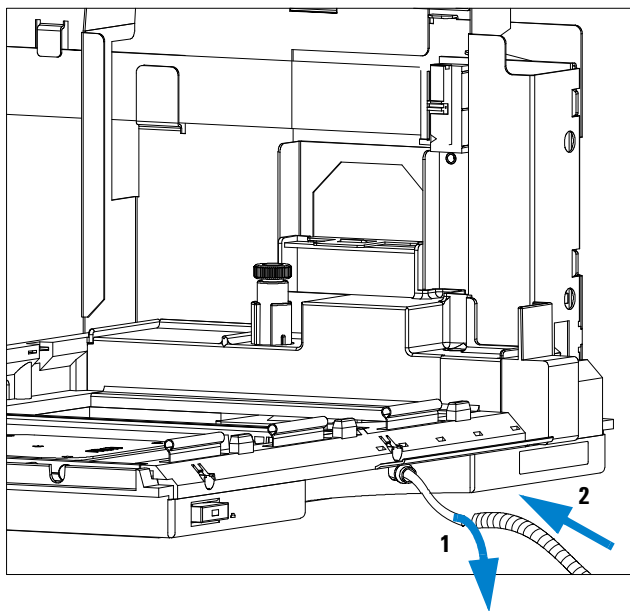


Figure 5 Installing the Corrugated Waste Tubing in the Plastic Port

- 6** Re-install the left side door (take care of the magnet at the back). Ensure the side door is correctly installed (its presence is sensed by a hall sensor, a missing side door will result in a NOT-READY state of the instrument).
- 7** Place the Fraction Collector in the stack or on the bench in all horizontal position.
- 8** Ensure the power switch at the front of the Fraction Collector is OFF.
- 9** Connect the power cable to the power connector at the rear of the Fraction Collector.
- 10** Connect the CAN cable to the other Agilent 1260 Infinity LC modules.
- 11** If an Agilent ChemStation is the controller, it must be connect to the LAN interface (should be installed to the detector).
- 12** Connect the APG remote cable (optional) for non Agilent 1260 Infinity instruments.
- 13** Install the tray that has been delivered for your fraction collector. The test tube trays can be adjusted in height, depending on the height of the used test tubes. To adjust the height of the trays, press against the snappers at

1 Installing the Fraction Collector

Installation Instructions for the Fraction Collector

the guides in the 4 corners of a tray and move the top plate of the tray up or down (see [Figure 6](#) on page 20).

NOTE

The preparative-scale fraction collector (G1364B) and the analytical-scale fraction collector (G1364C) are shipped with two trays (40x2 ml half tray and 15 x 6ml half tray).

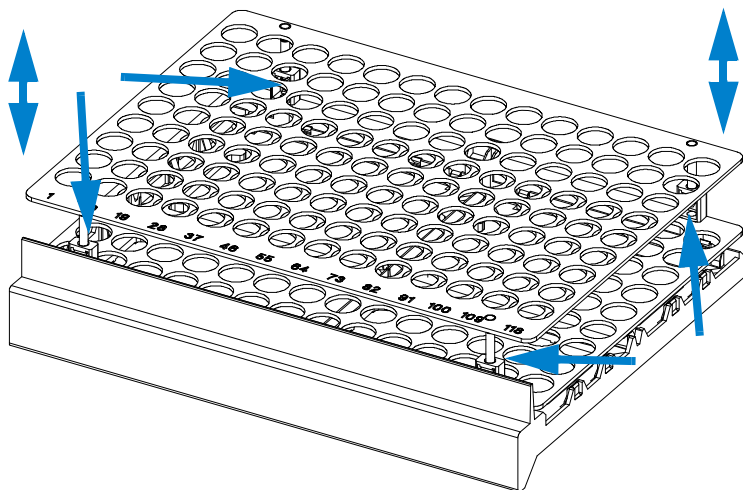


Figure 6 Adjusting the Height of the (Full) Test Tube Trays.

- 14** Turn ON power by pushing the button at the lower left hand side of the Fraction Collector.
- 15** The exhaust fan will turn ON and remove potential solvent vapor from the inside of the instrument. After 2 minutes close the front door. Then the Fraction Collector will start the hardware initialization process. At the end of this process the status LED should be green.

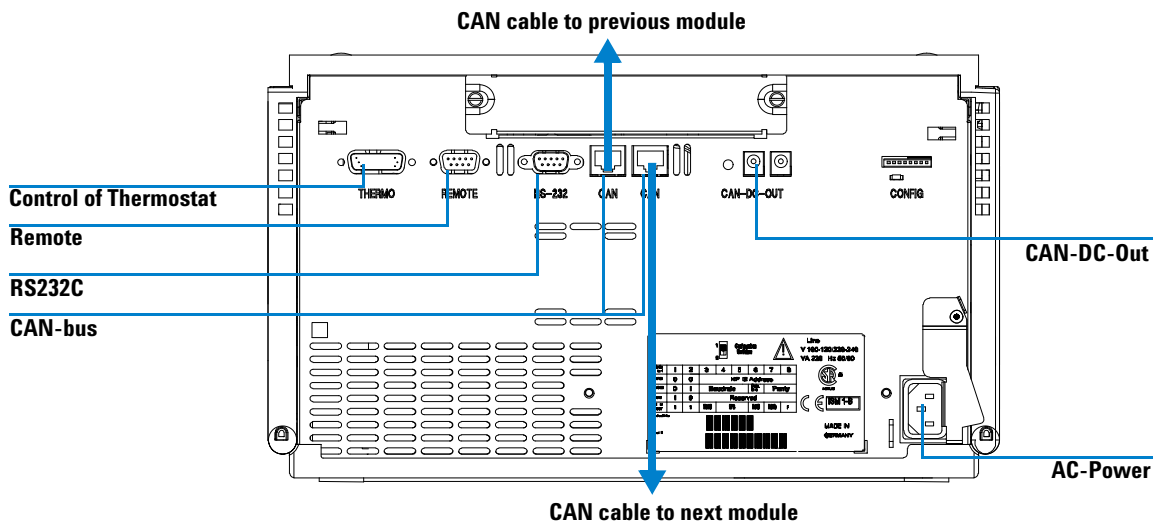


Figure 7 Cable Connections

NOTE

The Fraction Collector is turned ON when the line power switch is pressed and the green indicator lamp is illuminated. The detector is turned OFF when the line power switch is protruding and the green light is OFF.

WARNING

To disconnect the Fraction Collector from the line, unplug the power cord. The power supply still uses some power, even if the switch at the front panel is turned OFF.

Installing a Thermostatted Fraction Collector

Preparation	Locate bench space Provide power connections Unpack the Fraction Collector and the thermostat
Parts required	Fraction Collector and thermostat Power cords, ChemStation or Agilent Instant Pilot G4208A

- 1 Place the thermostat on the bench.

CAUTION

The fraction collector thermostat requires 25 cm (10 inch) space on each for sufficient air circulation.

- 2 Remove the front cover and transport protection foam.
- 3 Route the condensation drain tube to a waste container.

CAUTION

Make sure the condensation drain tube runs down into a waste container without any (upwards) bends or curves. Free and unrestricted flow of the condensation into a waste container must be guaranteed. Make sure that the condensation drain tube is always above the liquid level in the container. If the tube is located in liquid the condensed water cannot flow out of the tube and the outlet is blocked. Any further condensation will then remain in the instrument. This may damage the instruments electronics.

- 4 Install the LAN interface board in the Fraction Collector (if required).
- 5 Remove the adhesive tape which covers the side and front doors.
- 6 Open the front door and remove the left side door.
- 7 Install the corrugated waste tube in the plastic port at the front bottom center of the Fraction Collector and route down into a waste container.

- 8 Slide the waste tubing coming from the internal tray (if present) through the plastic port and the corrugated waste tube (see [Figure 5](#) on page 19). Route the corrugated waste tubing into a waste container.
- 9 Re-install the left side door (take care of the magnet at the back). Ensure the side door is correctly installed (its presence is sensed by a hall sensor, a missing side door will result in a NOT-READY state of the instrument).
- 10 Place the Fraction Collector on top of the thermostat. Make sure that the Fraction Collector is correctly engaged in the thermostat locks.

WARNING

To disconnect the Fraction Collector from the line, unplug the power cord. The power supply still uses some power, even if the power switch at the front panel is turned OFF.

- 11 Remove the plastic cover from the tray base, place the air channel adapter (1) into the Fraction Collector tray base. Make sure the adapter is fully pressed down. This assures that the cold airstream from the thermostat is correctly guided to the tray area of the Fraction Collector. Place the plug channel (2) on top of the air channel adapter. Both devices must be installed correctly, to assure proper operation of the instrument.

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Installing a Thermostatted Fraction Collector

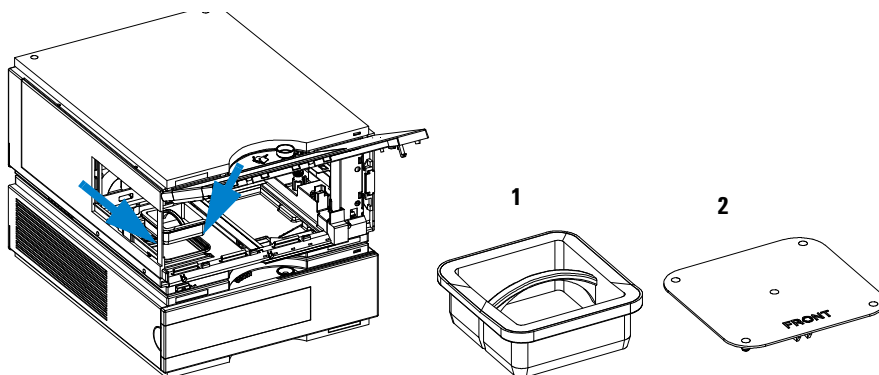


Figure 8 Installation of Thermostat and Fraction Collector

12 The *analytical scale* Fraction Collector is delivered with a pre-installed tray compartment divider. This divider should only be used with the thermostatted Fraction Collector, if a (half or std.) tray is installed into the left and center positions of the instrument. If any tray is installed to the right side of the instrument, remove the tray compartment divider. The tray compartment divider optimizes the cooling performance of the instrument, if only the left and center position of the Fraction Collector are in use.

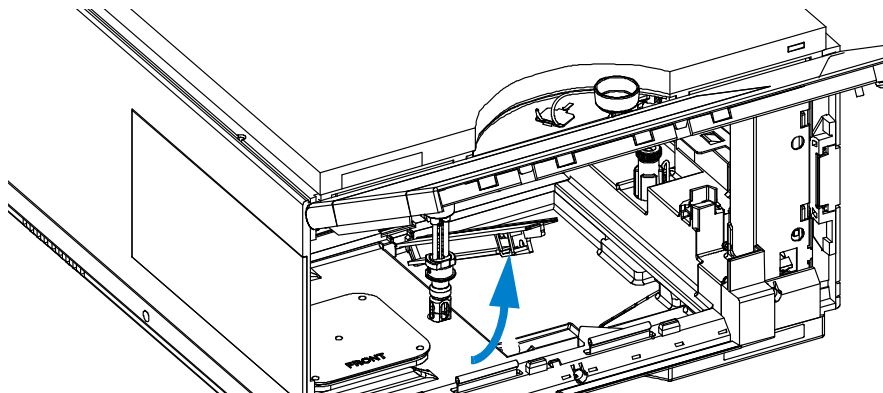


Figure 9 Removing the Tray Compartment Divider

WARNING

To avoid personal injury, keep fingers away from the needle area during Fraction Collector operation. Do not attempt to insert or remove a vial or a plate when the needle is positioned.

13 Install the tray you have ordered for your fraction collector. The test tube trays can be adjusted in height, depending on the height of the used test tubes. To adjust the height of the trays, press against the snappers at the guides in the 4 corners of a tray and move the top plate of the tray up or down (see [Figure 6](#) on page 20).

14 Ensure the power switch on the front of the Fraction Collector is OFF and the power cables are disconnected. Connect the cable between the Fraction Collector and the thermostat

CAUTION

Do not disconnect or reconnect the Fraction Collector to thermostat cable when the power cords are connected to either of the two modules. This will damage the electronics of the modules.

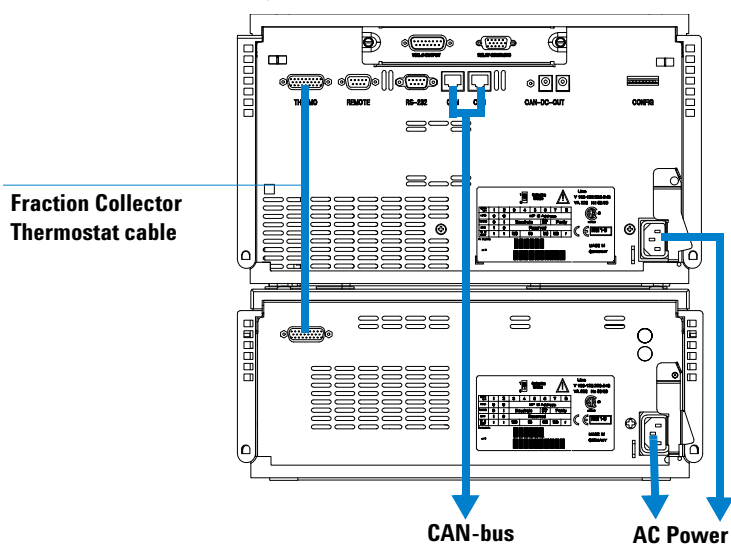


Figure 10 Connections at the Rear of the Thermostatted Fraction Collector

1 Installing the Fraction Collector

Installing a Thermostatted Fraction Collector

- 15** Connect the power cables to the power connectors.
- 16** Connect the CAN cable to other Agilent 1260 Infinity LC modules.
- 17** If an Agilent ChemStation is the controller, it must be connect to the LAN interface (should be installed to the detector).
- 18** Connect the APG remote cable (optional) for non Agilent 1260 Infinity instruments.
- 19** Turn ON power by pushing the button at the lower left hand side of the Fraction Collector.
- 20** The exhaust fan will turn ON and remove potential solvent vapor from the inside of the instrument. After 2 minutes close the front door. Then the Fraction Collector will start the hardware initialization process. At the end of this process the status LED should be green.
- 21** The Fraction Collector is turned ON when the line power switch is pressed and the green indicator lamp is illuminated. The detector is turned OFF when the line power switch is protruding and the green light is OFF.

Flow Connections to the Fraction Collector

Preparation	Fraction Collector is installed in the LC system
Parts required	Parts from the accessory kit, see " Fraction Collector Accessory Kit Contents G1364-68725 " on page 12

WARNING

When opening capillary or tube fittings, solvents may leak out. Please observe appropriate safety procedures (for example, goggles, safety gloves and protective clothing) as described in the material handling and safety data sheet supplied by the solvent vendor, especially when toxic or hazardous solvents are used.

WARNING

Regularly inspect the inlet / waste tubing assembly and the valve to needle tubing and exchange them if they are worn out or show visible signs of damage.

WARNING

Thoroughly follow the described installation procedures to maximize the lifetime of inlet / waste tubing assembly the valve to needle tubing and to avoid potential spills or fraction losses. Regularly inspect the tubings and exchange them if they are worn out or show visible signs of damage.

WARNING

This instrument should only be used with solvents that have an ignition temperature higher than 200°C!

1 Installing the Fraction Collector

Flow Connections to the Fraction Collector

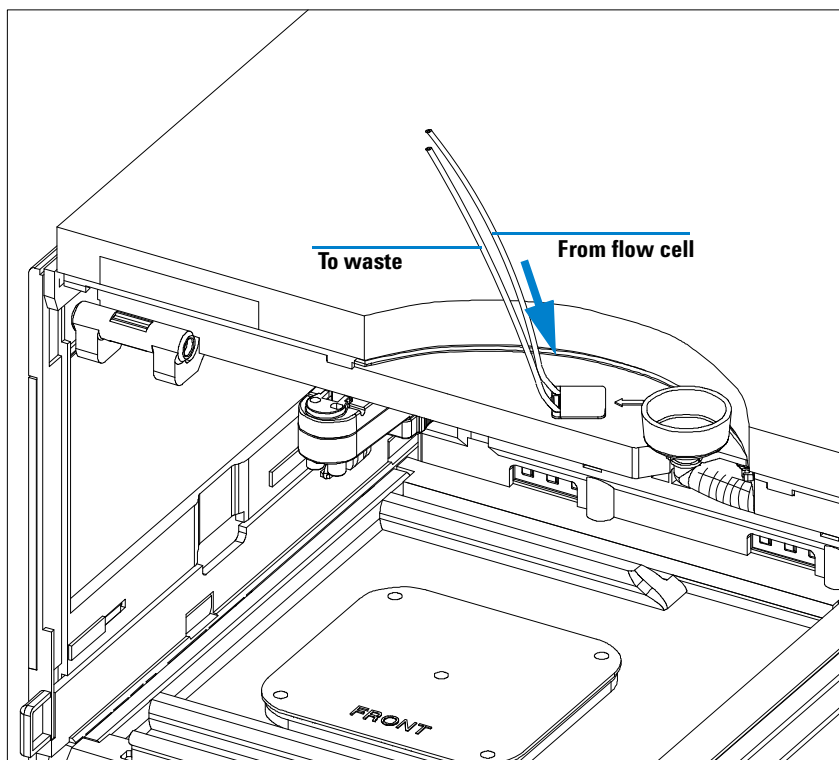


Figure 11 Flow Connections to and from the Fraction Collector.

Route the waste outlet around the instrument as shown below and into a corrugated waste tubing. Fix the waste outlet tubing and the corrugated waste tubing to the instrument with the sticking holders (part of the G1364-68725 accessory kit) as shown below. Route the corrugated waste tubing into a waste container.

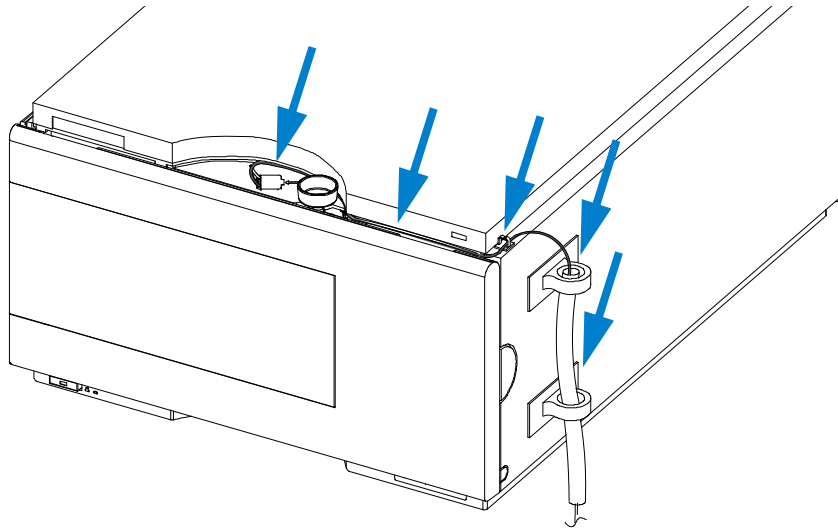


Figure 12 Fixing and Routing the Waste Tubings

Fraction Collector Trays

Installing the Fraction Collector Trays

- 1 Press the front door latch-holding button located at the front of the right-side cover.
- 2 Lift the front door.
- 3 Adjust the top plate of the test tube tray for the correct tube height if required.
- 4 Load the Fraction Collector tray with Fraction Collector well-plates, test tubes or vials as required.
- 5 Slide the Fraction Collector tray into the Fraction Collector so that the rear of the tray is seated firmly against the rear of the tray area.

NOTE

Installed trays are automatically detected and identified.

- 6 Press the front of the Fraction Collector tray down to secure the tray in the Fraction Collector.
- 7 Close the front door.

NOTE

If the tray pops out of position the air channel adapter is not correctly inserted.

NOTE

Before starting a run, the instrument has to be correctly configured in the user interface.

Numbering of Vial, Test Tube and Well-plate Positions

With the 4 plates full tray

Plate in the left front position: P1

Plate in the left back position: P2

Plate in the right front position: P3

Plate in the right back position: P4

Vessel: A1; A2;... B1; B2;

With the 2 plates / 10 x 2ml vials or 10 funnels std. trays

Plate in the front position: P1

Plate in the back position: P2

Vessel: A1; A2;... B1; B2;...

Vials / funnels: 1 - 10

With the 100 vials std. tray

Vial: 1 - 100

With the half-trays

Left-hand 40-position tray: 1 - 40

Center 40-position tray: 101-140

Right-hand 40-position tray: 201 - 240

or

Left-hand 15-position tray: 1 - 15

Center 15-position tray: 101-115

Right-hand 15-position tray: 201 - 215

1 Installing the Fraction Collector

Fraction Collector Trays

With the 40, 60, 125 or 215 position test tube full trays

Numbering starts in front left corner in columns to the back and then to the right.

WARNING

If you are using flammable solvents, remove the plates from the Fraction Collector after turning it OFF. You avoid the risk of building explosive gas mixtures in the instrument.

WARNING

If you are using flammable solvents, cover the plates. You avoid the risk of building explosive gas mixtures in the instrument.

WARNING

Closing mats with adhesive can give some contamination in the system. The adhesive is soluble in most of the solvents used in HPLC.

WARNING

In general do not use closing mats with adhesive. The Fraction Collector has no prepunch needle, therefore the adhesive will clog the needle after several injections.

Configure Well-plate Types

If the plate you are using is not found on the “[List of Recommended Plates and Closing Mats](#)” on page 162 you may configure a custom plate. Measure the exact dimensions of the plate as marked below and enter the values in the plate configuration table of the ChemStation or the Agilent Instant Pilot G4208A.

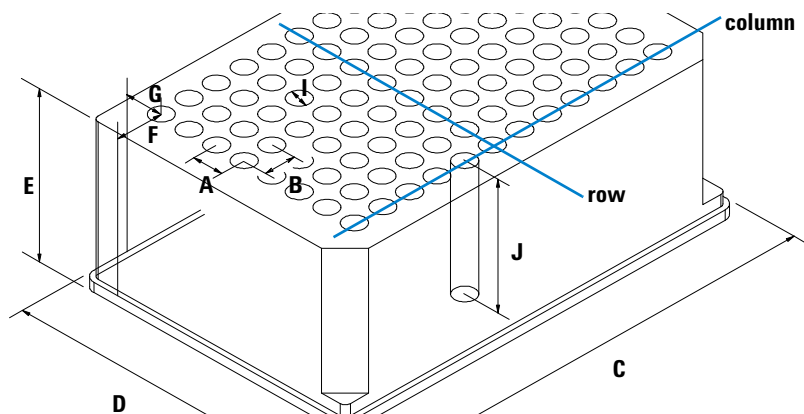


Figure 13 Well-plate Dimensions (straight)

1 Installing the Fraction Collector
Configure Well-plate Types

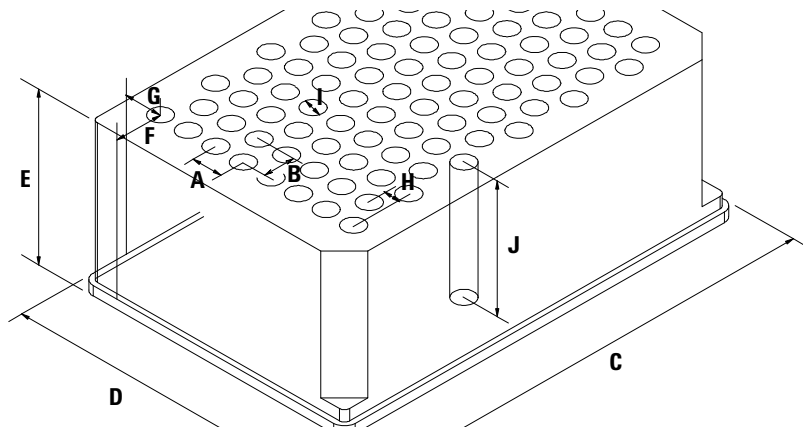


Figure 14 Well-plate Dimensions (staggered)

Table 7 Configuring Well-plate Types

Location	Description	Definition	Limits
	Rows	Number of rows on the plate	up to 16
	Columns	Number of columns on the plate	up to 24
	Volume	Volume (in μl) of a sample vessel	
A	Row distance	Distance (in mm) between the center of two rows	
B	Column distance	Distance (in mm) between the center of two columns	
C	Plate length	X size (in mm) at the bottom of the plate	127.75+/- 0.25 mm (SBS Standard)
D	Plate width	Y size (in mm) at the bottom of the plate	85.50+/-0.25 mm (SBS Standard)
E	Plate height	Size (in mm) from the bottom to the top of the plate. If you use well plates with inlets, caps and septa, you have to specify the distance from the bottom to the top of the caps.	up to 47 mm
F	Column offset	Distance (in mm) from the left edge (bottom) to the center of the first hole (A1)	
G	Row offset	Distance (in mm) from the back edge (bottom) to the center of the first hole (A1)	
H	Column shift	Offset (in mm) to Y when the rows are not straight but staggered	
J	Well depth	Distance (in mm) from the top of the plate to the bottom of the well. If you use well plates with inlets, caps and septa, you have to specify the distance from the top of the septa to the bottom of the inlets.	up to 45 mm

1 Installing the Fraction Collector

Configure Well-plate Types

Table 7 Configuring Well-plate Types (continued)

Location	Description	Definition	Limits
	Well X size	Size of the well in x direction (Plate length) If you use well plates with inlets, caps and septa, you have to specify the x size of the septa.	min. 3.7 mm min. 3.0 mm with position accuracy alignment (micro scale)
	Well Y size	Size of the well in y direction (Plate width). If you use well plates with inlets, caps and septa, you have to specify the y size of the septa.	down to 3.7 mm min. 3.0 mm with position accuracy alignment (micro scale)
	Bottom size	For round wells, the relative of the top and bottom of the well	1.0: cylindrical well 0.0: conical well
	Square	Click in the field to specify whether the well is rectangular or round	Yes: rectangular No: round /oval
	Is well plate	Click in this field to specify if this is a well plate or not. Relevant for continuous flow operation.	Yes: well plate or MALDI Target No: Vial Tray or Eppendorf tray

NOTE

The distances need to be measured with high precision. It is recommended to use a caliper. If possible contact the vendor of non-predefined well plates to obtain these dimensions.

Transporting the Fraction Collector

When moving the Fraction Collector inside the laboratory, no special precautions are needed. However, if the Fraction Collector needs to be shipped to another location via carrier, ensure:

- ✓ The transport assembly is in the park position. Use the ChemStation or the Agilent Instant Pilot G4208A for this command.

To move the arm to the park position:

- 1 Switch to the **Diagnosis** view of the ChemStation and select **Fraction Collector > Maintenance Positions.** from the **Maintenance** menu
 - 2 In the upcoming dialog box click **Park Arm.**
- ✓ The vial tray and the sample transport mechanism is secured with the transport protection foam.

1 Installing the Fraction Collector
Transporting the Fraction Collector



2 Configuration and Operation of the Fraction Collector

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Configuration of the Fraction Collector

Configuration of the Fraction Collector in the ChemStation

NOTE

The section “*Configuration of the Fraction Collector in the ChemStation*” is only valid for ChemStation Rev. B.xx.xx.

If you are using a newer revision of ChemStation (Rev. C.xx.xx), or EZChrom, or any 3rd party software to control the Fraction Collector, you need the Agilent Lab Advisor software to run the Delay Time and Delay Volume Calibration procedure. Refer to “[Delay Time and Volume Calibration of Fraction Collector for UV \(Lab Advisor\) and MSD Signals](#)” on page 70.

CAUTION

Before using the preparative-scale fraction collector G1364B the delay calibration adapter G1364-87301 has to be removed from the needle carrier assembly.

In order to setup or change the configuration parameters of your fraction collector select **More Fraction Collector > Configuration** from the Instrument menu or right-click on the fraction collector icon in the graphical user interface. In the resulting **Fraction Collector Configuration** dialog box (Figure 15 on page 41) the configuration of the **Trays**, the **Fraction Delay Volumes**, the **Collection Order**, the **Needle Movement** and the **Well-Plates** can be specified.

Trays

In the online mode of the Agilent ChemStation the type of the installed tray is recognized automatically. In the off-line mode an appropriate tray type can be chosen from a dropdown list. For a selection of supported trays see “[Supported Trays for Fraction Collectors](#)” on page 156.

Tube volume [ml] Volume of the test tube should be specified 10% less than the maximum fill volume to avoid overflow.

Tube height [mm] The exact test tube height has to be specified. For preparative scale fraction collector the minimum height is 48 mm and the maximum height is 100 mm. For the analytical scale fraction collector with 50 mm needle (standard) the maximum height is 48 mm. By changing to

the short 20 mm needle the maximum tube height can be increased to 75 mm. For the part numbers of the different needle assemblies see “Needle Assemblies” on page 166.

NOTE

In the online ChemStation the installed tray is recognized and the default settings for tube volume and tube height are loaded and displayed.

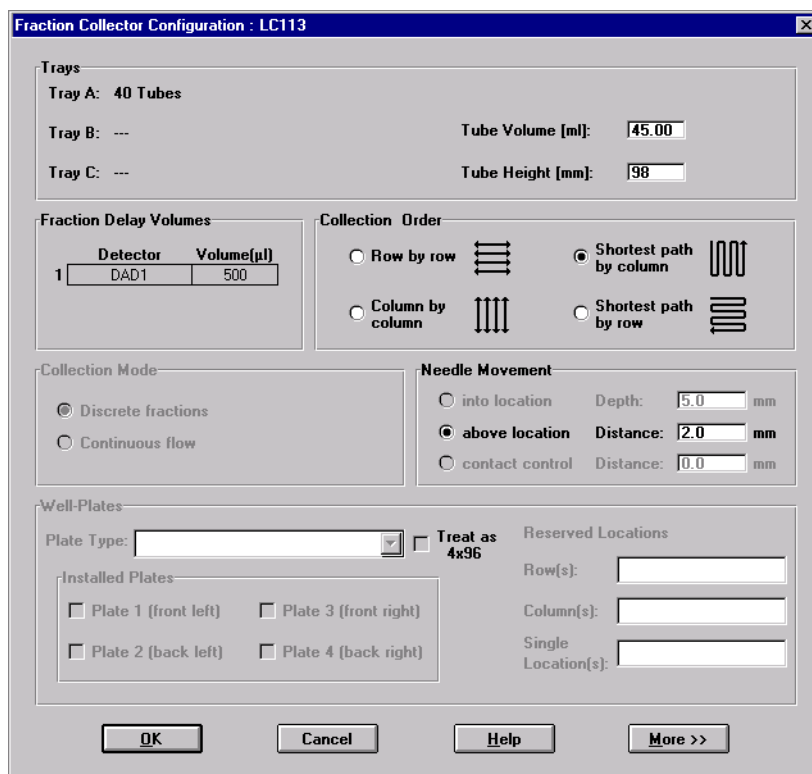


Figure 15 Fraction Collector Configuration dialog

Use for Sample Recovery This checkbox only appears, if multiple fraction collectors are configured (see Figure 41 on page 109). Then the last fraction collector can be selected for sample recovery. If four fraction collectors are configured, the fourth one will automatically used for sample recovery. To change the order of your fraction collectors select

2 Configuration and Operation of the Fraction Collector

Configuration of the Fraction Collector

Configure 1260 Access from the **Instrument** menu. In the **Configuration** dialog box use the **Up** or **Down** button to change the configuration order and restart your ChemStation.

Fraction Delay Volumes

The Fraction Delay Volume specifies the volume between the detector cell and fraction collector diverter valve. This volume has to be determined during the delay calibration. See “[Delay Volumes and Delay Calibration](#)” on page 46 for a detailed description.

Collection Order

The Collection Order describes the way of capillary movement during fraction collection. Four different settings are possible:

- row-by-row
- column-by-column
- shortest way by row
- shortest way by column

Shortest way by row/column is recommend, if **Continuous flow** is selected as Collection mode.

Collection Mode

The Collection Mode defines the triggering of the diverter valve. For **Discrete fractions** the diverter valve switches to waste while the needle moves to the next test tube, vial or well. For **Continuous flow** the diverter valve is not switching during needle movements, except for long movements in the row-by-row or column-by-column mode. In addition the needle cannot move **Into location** during continuous flow operation. **Continuous flow** is only available for well plates.

Needle Movement

Into location In the Into Location mode the needle tip moves into the well to the specified **Depth** (in mm). This option is only available for the analytical scale fraction collector in the **Discrete fractions** mode.

Above location In the Above Location mode the needle tip stays at the specified **Distance** (in mm) above the well during fraction collection.

Contact Control In this mode the needle tip moves down to the well bottom until it reaches the specified **Distance** (in mm) between needle tip and the vial/well bottom. This ensures that the forming droplet is in contact to the vial/well bottom. During the continuing filling process the needle tip moves upwards while staying in contact with the surface of the collected liquid. This option is recommended for low flow rates and small fraction volumes to avoid air bubbles and accomplish an accurate fraction volume. It is only available for the analytical scale fraction collector.

Well-Plates

In the **Well-Plates** section the type of well plates used in a well plate tray can be configured. The well plate type can be chosen from the Plate Type dropdown list. More detailed information about all pre-configured well plates can be found in the Instrument menu. Only one type of well plate can be used on the well plate tray.

The checkbox **Treat as 4 x 96** is available only for 384 well plates and allows to split the 384 well plate virtually into 4 separate 96-well plates. This only changes the collection order. The numbering of the wells remains as indicated on the plate. The four virtual 96 well plates start at locations A1, A13, I1 and I13, respectively.

The filling order of each quarter is as specified in the Collection Order section. When the 384 well plate is split into four equal quarters the order of the four plates is the same as displayed in the Installed Plates section.

In the **Reserved Locations**, you have the possibility to specify locations that will not be used for Fraction Collection (see [Table 8](#)).

Table 8 Syntax for the definition of Reserved Locations

Location	Syntax	Description
Rows	A	Row A can't be used
	A,B	Rows A and B can't be used
	A-D	A, B, C and D can't be used
	A-D,F	Rows A, B, C, D and F can't be used
Columns	1	Column 1 can't be used
	1,2	Columns 1, and 2 can't be used

2 Configuration and Operation of the Fraction Collector

Configuration of the Fraction Collector

Table 8 Syntax for the definition of Reserved Locations (continued)

Location	Syntax	Description
	1-4	Columns 1, 2, 3 and 4 can't be used
	1-4,12	Columns 1, 2, 3, 4 and 12 can't be used
Single locations	G12,H12	Locations G12 and H12 can't be used

Configuring Multiple Fraction Collectors

To increase the capacity of the systems up to three fraction collectors can be configured by using the Agilent 1260 Infinity 12-position 13-port valve G1160A. In addition a fourth fraction collector can be configured for recovery collection.

- The **Configuration** has to be edited for all fraction collectors. The last fraction collector in the configuration can be selected for sample recovery. To configure the recovery fraction collector please read [“Sample Recovery”](#) on page 109.
- With multiple fraction collectors a fraction start location has to be specified to define the starting position. For further details see [“Assignment of Start Location for Fraction Collection”](#) on page 101. Never used the valve settings to define a start location on systems with multiple fraction collectors.
- We recommend to use inlet tubing of the same length for all fraction collectors. Otherwise a **Delay Calibration** has to be performed for each of those fraction collectors. For the recovery fraction collector the delay volume parameter will be ignored.

NOTE

The **Delay Calibration** can only be performed for fraction collector 1 of your configured system.

The calculated delay volume is used for all fraction collectors; therefore, the volume (tubing) between the selection valve and each installed fraction collector must be identical.

Otherwise fraction collector 2 and fraction collector 3 have to be temporarily configured as fraction collector 1 during the delay calibration.

- The fraction collector inlet tubings have to be connected to port 1-3 of the selection valve in the same order as configured in the ChemStation. The recovery fraction collector is always connected to the waste tubing of the main fraction collectors. The waste tubing from multiple fraction collectors and the inlet tubing to the recovery collector have to be connected through a T-Piece.
- Fraction Collector 4 will always be used as a recovery fraction collector.

Delay Volumes and Delay Calibration

Delay Times and Volumes

Figure 16 shows a schematic drawing of the flow path between the detector and the fraction collector with the two delay volumes V_{D1} and V_{D2} . For peak-based fraction collection the system delay times t_{D1} and t_{D2} can be calculated by dividing the delay volumes by the flow rate \dot{v} .

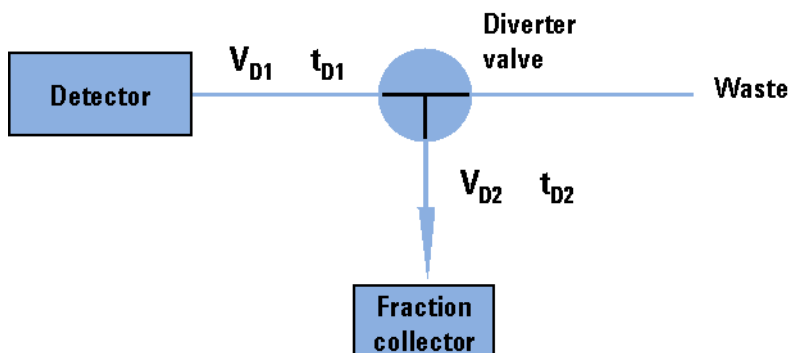


Figure 16 Delay Volumes and delay times

The delay volume V_{D2} is a system parameter, it depends on the installed fraction collector tubing and the fraction collector needle. It is approximately 23 μl for the fraction collector AS and approximately 120 μl for the fraction collector PS. Delay volume V_{D1} , which is specified in the **Fraction Collector Configuration** window, is determined using the **Delay Volume Calibration** feature of the ChemStation software.

When a peak is detected during a purification run (Figure 17) the diverter valve is triggered using the following delay time calculations:

$$\text{Start of fraction collection: } t = t_0 + t_{D1}$$

$$\text{End of fraction collection: } t = t_E + t_{D1} + t_{D2}$$

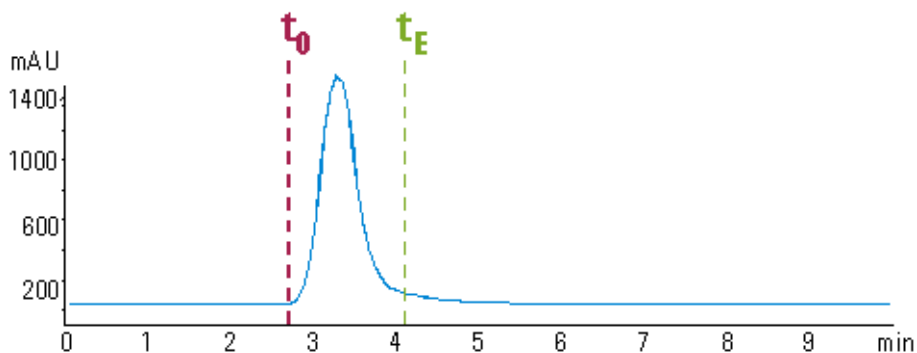


Figure 17 Chromatogram with peak start t_0 and peak end t_E

Detector Signal Delay

Every Agilent 1260 Infinity detector that is used for triggering fractions has an internal signal delay caused by filtering the raw data. The signal delay depends on the *Peakwidth* setting of the detector and is accounted for when the diverter valve is triggered. [Table 9](#) and [Table 11](#) list the internal signal delay times for different **Peakwidth** settings.

Table 9 Signal delay times for the Agilent 1260 Infinity DAD/MWD (G1315D/G1365D)

Peakwidth (min)	Response time (sec)	Signal delay (sec)
<0.01	0.1	0.05
>0.01	0.2	0.15
>0.03	0.5	0.5
>0.05	1.0	1.25
>0.10	2.0	2.75
>0.20	4.0	5.9
>0.40	8.0	11.9
>0.85	16.0	23.9

2 Configuration and Operation of the Fraction Collector

Delay Volumes and Delay Calibration

Table 10 Signal Delay Times for the Agilent 1260 Infinity DAD/MWD (1315C/G1365C)

Peakwidth (min)	Response time (sec)	Signal delay (sec)
<0.0025	0.02	0.0375
>0.0025	0.05	0.0625
>0.005	0.1	0.144
>0.01	0.2	0.294
>0.03	0.5	0.619
>0.05	1.0	1.27
>0.1	2.0	2.57
>0.2	4.0	5.17
>0.4	8.0	10.4
>0.85	16.0	20.8

Table 11 Signal Delay Times for the Agilent 1260 Infinity VWD G1314B

Peakwidth (min)	Response time (sec)	Signal delay (sec)
<0.005	<0.12	0.07
>0.005	0.12	0.14
>0.01	0.25	0.29
>0.025	0.5	0.58
0.05	1	1.3
0.1	2	2.8
0.2	4	6.0
0.4	8	12.3

Table 12 Signal Delay Times for the Agilent 1260 Infinity VWD G1314C

Peakwidth (min)	Response time (sec)	Signal delay (sec)
<0.00125	<0.02	0.0182
>0.00125	0.02	0.0364
>0.0025	0.05	0.0728
>0.005	0.12	0.146
>0.01	0.25	0.328
>0.025	0.5	0.710
>0.05	1	1.49
>0.1	2	3.08
>0.2	4	6.26
>0.4	8	12.6

Table 13 Signal Delay Times for the Agilent 1260 Infinity FLD G1321A

Peakwidth (min)	Response time (sec)	Signal delay (sec)
<0.005	<0.12	0.018
>0.005	0.12	0.073
>0.01	0.25	0.18
>0.03	0.5	0.44
>0.05	1	0.96
>0.1	2	2.0
>0.2	4	4.2
>0.4	8	8.6

2 Configuration and Operation of the Fraction Collector

Delay Volumes and Delay Calibration

CAUTION

If the internal signal delay is longer than the delay time t_{D1} some of the peak will be lost. The maximum allowed signal delay time can be calculated using the following equation:

$$\text{Signal delay time}_{(max)} = \frac{V_{D1}}{\dot{v}} \quad \dot{v} = \text{Flow rate}$$

After calculating the maximum signal delay time a **Peakwidth** setting can be selected that gives a signal delay time, which is shorter than the calculated maximum signal delay time. This **Peakwidth** setting should then be used for the LC purification run.

NOTE

We recommend to set the **Peakwidth** always to > 0.01 for the DAD and MWD or to > 0.005 for the VWD.

If the **Peakwidth** setting cannot be reduced and the signal delay time is longer than t_{D1} it is also possible to increase V_{D1} by adding additional tubing. However this higher delay volume will increase the peak dispersion between detector and fraction collector.

The stop-time of the run in the ChemStation must be set to at least:

Total duration of time table (time of last entry *OFF*) + fraction collector delay time $(V_{D1}/\dot{v}) + 0.1min$ for time-based fraction collection.

End of last peak (t_E) + fraction collector delay time $(V_{D1}/\dot{v}) + 0.1min$ for peak-based fraction collection

Performing a Delay Calibration with an UV Detector

- 1 Place a vial containing the Delay Sensor Calibrant (G1946-85020) in position 1 of the Autosampler.
- 2 Remove the installed column and connect the capillaries with a zero-dead-volume connector.
- 3 Install the 40 x 2ml vial tray (analytical scale) or the 15 x 6ml vial tray (preparative scale) in the fraction collector.
- 4 Connect a bottle of water to Channel A.
- 5 Switch to Diagnosis View (if necessary).
- 6 Open the AFC Delay Volume Calibration status window (see [Figure 18](#)) from the Fraction Collector sub-menu of the Maintenance menu:

Maintenance > Fraction Collector > Delay Volume Calibration

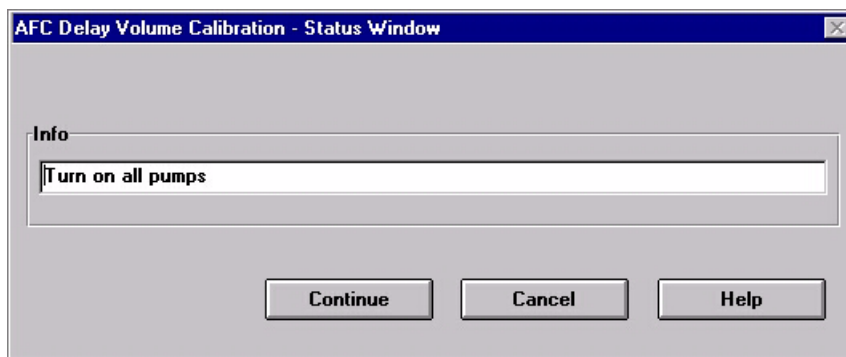


Figure 18 Delay Volume Calibration status window

- 7 The Info field gives the instruction to turn ON the pumps (see [Figure 18](#)). Click **Continue**.

2 Configuration and Operation of the Fraction Collector

Delay Volumes and Delay Calibration

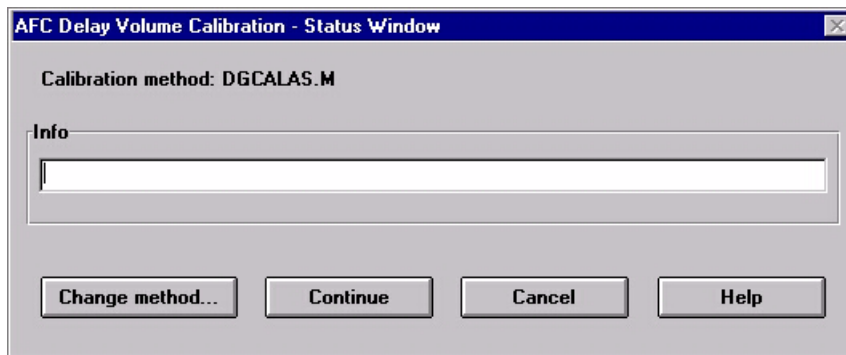


Figure 19 AFC Delay Volume Calibration Window

When you have turned ON the pumps, click **Continue** again to start the delay volume calibration.

The method to be used for the delay volume calibration is displayed (DGCALAS.M is the default calibration method for the analytical scale fraction collector and DGCALPS.M is the default method for the preparative fraction collector); if you want to change to a different method, click the Change Method button and select a new method. See also “[Making Your Own Calibration Method](#)” on page 60

The Info field shows the current status of the delay volume calibration, and gives instructions and warnings.

- 8 Install the delay calibration adapter G1364-87301 by attaching it to the needle carrier assembly (G1364B preparative-scale only).

The selected method is loaded, and the delay volume calibration sample is injected.

- 9 After the delay calibration has been finished, you are prompted to remove the delay calibration adapter (G1364B preparative-scale only).
- 10 When the calibration run has finished, click **OK**. The Calibration Results panel (see [Figure 20](#)) is displayed.

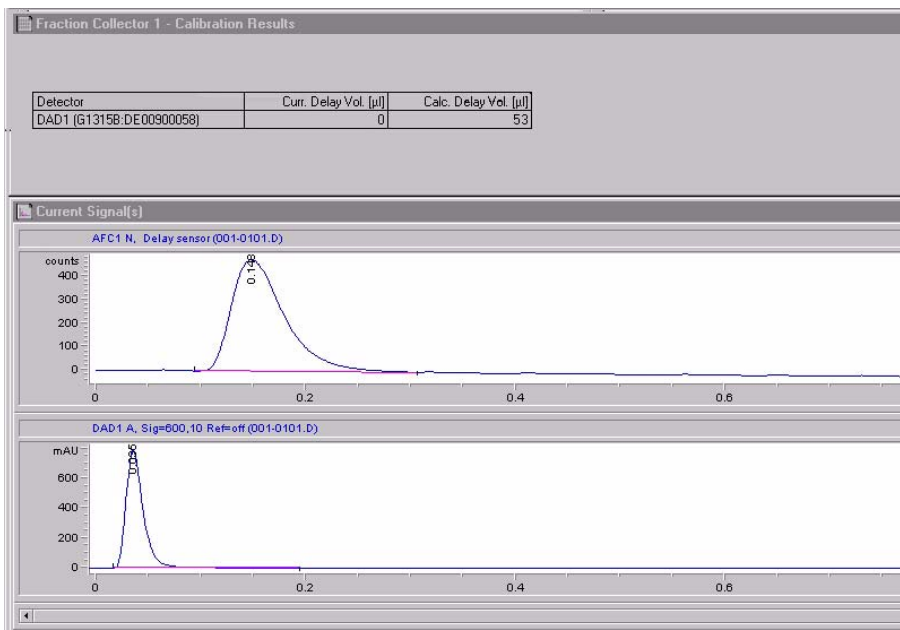


Figure 20 Delay Calibration Results Panel

The Calibration Results panel contains a results table and the acquired chromatograms from the detector(s) and the fraction collector's flow delay sensor. The results table contains

- the name and serial number of the detector,
- the current delay volume (stored in the fraction collector configuration),
- the calculated delay volume from the calibration.

To print the Calibration Results panel click **Print**.

- 11** If you want to store the calculated delay volume in the fraction collector configuration, click **Calibrate**. A confirmation dialog box is displayed when the delay volume has been stored; to close the confirmation click **OK**.

2 Configuration and Operation of the Fraction Collector

Delay Volumes and Delay Calibration

If you do not want to store the calculated delay volume in the fraction collector configuration, click the Close button. A confirmation dialog box is displayed; click **OK** to close, the **Confirmation** or **Cancel** to return to the Calibration Results panel.

NOTE

The **Delay Calibration** can only be performed for fraction collector 1 of your configured system.

The calculated delay volume is used for all fraction collectors; therefore, the volume (tubing) between the selection valve and each installed fraction collector must be identical.

Otherwise fraction collector 2 and fraction collector 3 have to be temporarily configured as fraction collector 1 during the delay calibration.

CAUTION

Before using the preparative-scale fraction collector G1364B the delay calibration adapter G1364-87301 has to be removed from the needle carrier assembly.

Performing a Delay Calibration with an MSD

- 1 Place a vial with the Delay Sensor Calibrant (G1946-85020) in position 1 of the autosampler.
- 2 Remove the installed column and connect the capillaries with a zero-dead-volume connector or a mixer (Part No. 79835-87330).
- 3 Install the 40 x 2ml vial tray (analytical scale) or the 15 x 6ml vial tray (preparative scale) in the fraction collector.
- 4 Connect a bottle of water to channel A of the main pump.
- 5 Connect a bottle of water with 0.1% acetic acid or ammonium formate to the make-up pump.
- 6 Load the method DGCALAS.M (analytical scale) or DGCALPS.M (preparative scale) and adjust the flow in the main pump to the flow you will be using for the analysis.

CAUTION

Unlike for UV detectors, the delay calibration for the MSD needs to be performed whenever the flow rate is changed.

- 7 Set the flow of the make-up pump to the flow rate you will be using for your analysis.
- 8 Set the active splitter to the split ratio as used during operation.
- 9 Save the method to a new name.
- 10 Go to Diagnostics menu; select Delay volume Calibration from the Fraction Collector Sub-menu within the Maintenance menu.
- 11 Press **Change method...** to select the method you created. The new method name appears on the info line.
- 12 Start the active splitter.
- 13 Press **Continue** to execute the delay volume calibration

2 Configuration and Operation of the Fraction Collector

Delay Volumes and Delay Calibration

14 Install the delay calibration adapter G1364-87301 by attaching it to the needle carrier assembly (G1364B preparative-scale only).

The selected method is loaded and the delay calibration sample is injected.

15 After the delay calibration has been finished, you are prompted to remove the delay calibration adapter (G1364B preparative-scale only).

16 When the calibration run is finished, press **OK**.

17 Examine the value for the MSD Calculated Delay time.

The delay time should be at least 5 seconds but may, in fact, be negative (see [Figure 21](#)).

Detector	Curr. Delay Vol. [µl]	Calc. Delay Vol. [µl]
DAD1 (G1315A:US64400562)	883	349.14

Detector	Curr. Delay Time [sec]	Calc. Delay Time [sec]
MSD	?	-0.294

Figure 21 Delay Volume Calibration results

18 If the measured delay time is greater than 5 seconds, you can skip to [step 23](#), otherwise, continue with [step 19](#).

CAUTION

If the measured delay time is significantly greater than 5 seconds, you should continue with the calibration at [step 16](#) to avoid losing fractions at the end of the run.

- 19** Determine how much additional time is needed to create a 5-second delay. For example, if the time displayed is -1 sec, a six-second delay is needed.
- 20** Using the main pump flow rate, determine the desired additional delay volume. For example, at 3 ml/min and a six-second delay, an additional $3.0 \times 6/60$ or 0.3 ml is required.
- 21** Cut off a sufficient piece of the supplied ETFE tubing (G1968-60500) to provide the needed volume. The tubing's volume is 8.1 µl/cm. In this case, 37 cm would be required.
- 22** Add the tubing between the splitter and the fraction collector or, if multiple fraction collectors are installed, between the splitter and the G1160A valve.
- 23** Perform the calibration again. Verify the time is sufficient (>5 seconds, see [Figure 22](#)).

2 Configuration and Operation of the Fraction Collector

Delay Volumes and Delay Calibration

Detector	Curr. Delay Vol. [µl]	Calc. Delay Vol. [µl]
DAD1 (G1315A:US64400562)	883	937.14

Detector	Curr. Delay Time [sec]	Calc. Delay Time [sec]
MSD	?	11.991

Figure 22 Delay time greater than 5 seconds

24 Press **Calibrate** if you want to store the new calculated delay volume for the UV detector in the Fraction Collector Configuration (see also “[Configuration of the Fraction Collector in the ChemStation](#)” on page 40).

25 Press **Print** to get a hard copy of the report.

26 Stop the splitter.

27 Return to the **Method and Run Control View** and load the method you will be using to run your samples. Select Fraction Collection from the **More MSD...** sub-menu under the **Instrument** menu

Press **Parameters** and enter the final calculated delay time that appears in the report in the Collector Delay field, first converting it to minutes. In this case you would enter $11.991/60 = 0.20$ minutes (see [Figure 23](#)).

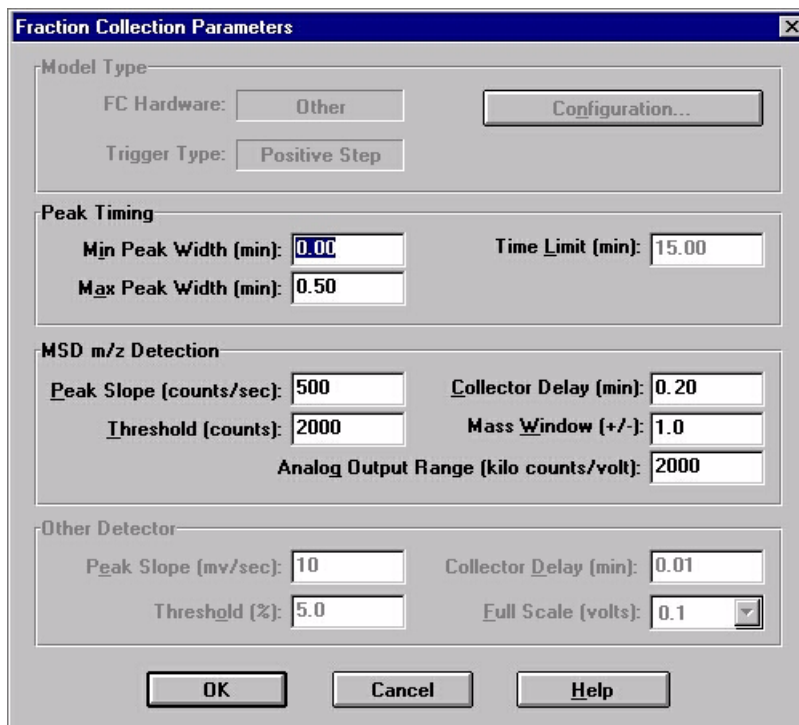


Figure 23 Fraction Collection Parameters dialog box with delay volume entry

28 Save the method.

NOTE

If you anticipate running samples at a variety of flow rates, it is best to calibrate at the highest likely flow rate first and install the appropriate length tubing. This way, there will be sufficient delay for the lower flow rates and new lengths will not need to be cut.

CAUTION

Before using the preparative-scale fraction collector G1364B the delay calibration adapter G1364-87301 has to be removed from the needle carrier assembly.

Making Your Own Calibration Method

You make your own calibration method by editing one of the default calibration methods:

- 1** Load the default calibration method: DGCALAS.M in the case of an analytical system and DGCALPS.M in the case of a preparative system.
- 2** Change the method parameters (flow, runtime, solvent composition, injection volume or detector parameters) to suit your adapted calibration procedure.
- 3** Save the method with a new name in the method folder for your instrument.
- 4** Follow the appropriate procedure as described in the previous sections for running the method.

Delay Volumes and Delay Calibration for Fraction Collection (Lab Advisor)

NOTE

This section describes the standard Delay Volume Calibration procedure with Agilent Lab Advisor software, Rev. B.02.04 or higher.

If you are running your *preparative-scale* fraction collector as a component of the Agilent 1260 Infinity Purification Solution with Automated Purification Software, follow the specific flavor of this procedure as described in the Purification Solution's documentation.

The delay calibration procedure determines the delay time between the detector and the fraction collector in the system. The delay time is used to compensate the time a compound needs to travel between the point of detection in the detector and the point of collection in the fraction collector.

This delay is a system parameter which depends on the installed fraction collector tubing (t_{D1}) and the fraction collector needle (t_{D2}). The delay volume corresponding of the delay t_{D2} is automatically taken in consideration on the different types of fraction collectors:

- Analytical scale fraction collector: approximately 23 μL
- Preparative scale fraction collector: approximately 120 μL

With the Agilent 1260 Infinity Fraction Collector, the delay calibration procedure is performed using the flow delay sensor (FDS), a very simple detector built into the fraction collector. Together with the signal from the detector, the signal from the FDS facilitates determination of the delay time between detector and fraction collector as shown in [Figure 24](#) on page 62.

2 Configuration and Operation of the Fraction Collector

Delay Volumes and Delay Calibration for Fraction Collection (Lab Advisor)

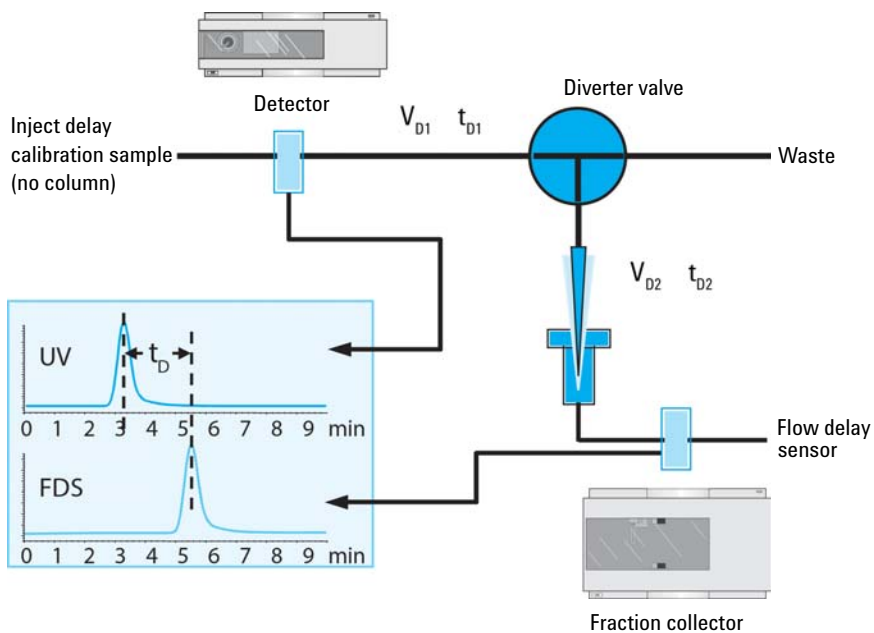


Figure 24 Scheme of the delay volume between UV detector and fraction collection

Delay Volume with a Detector After the Splitter

If the detector is placed *after* a flow splitter, the resulting volume does not represent a real tubing volume between the UV detector and fraction collector, but a virtual delay volume. Two cases can be distinguished:

- If an Agilent Splitter Kit is used, the resulting delay volume measured during the delay calibration is compatible with other flow rates. The delay volume will be converted to a correct delay time between detector and fraction collector.

Examples with the Agilent Splitter Kit #5023-2258:

- Method 1:
Prep flow path: Capillary volume of 2.69 mL with a flow rate of 50 mL/min. The time a compound needs to reach the fraction collector is 3 seconds
Split flow path: Capillary volume of 7.5 μ L with a flow rate of 0.5 mL/min. The time a compound needs to reach the fraction collector is 1 second.
The delay time is 2 seconds, corresponding to a delay volume of 1.67 mL.
- Method 2:
Prep flow path: Capillary volume of 2.69 mL with a flow rate of 25 mL/min. The time a compound needs to reach the fraction collector is 6 seconds.
Split flow path: Capillary volume of 7.5 μ L with a flow rate of 0.25 mL/min. The time a compound needs to reach the fraction collector is 2 seconds.
The delay time is 4 seconds, corresponding to a delay volume of 1.67 mL.
For the Agilent Splitter kits, the delay volume measured for one flow rate is compatible with any other preparative flow rate.

2 Configuration and Operation of the Fraction Collector

Delay Volumes and Delay Calibration for Fraction Collection (Lab Advisor)

- In the particular case of an active splitter or a passive splitter combined with a make-up pump, the delay calibration must be performed using the preparative and make-up flows, which will be used for the preparative method. If one of the flows is changed (or if a HPLC capillary after the UV detector is changed), then the resulting delays cannot be simply recalculated to a new condition. The delay calibration needs to be measured for the new combination of flow rates.

Examples with the Active Splitter G1968D:

- Method 1:

Prep flow path: Capillary volume of 2.69 mL with a flow rate of 50 mL/min. The time a compound needs to reach the fraction collector is 3 seconds.

Split flow path: Capillary volume of 7.5 μ L with a flow rate of 1 mL/min. The time a compound needs to reach the fraction collector is 0.5 seconds.

The delay time is 2.5 seconds, corresponding to a delay volume of 2.08 mL.

- Method 2:

Prep flow path: Capillary volume of 2.69 mL with a flow rate of 25 mL/min. The time a compound needs to reach the fraction collector is 6 seconds.

Split flow path: Capillary volume of 7.5 μ L with a flow rate of 1 mL/min. The time a compound needs to reach the fraction collector is 0.5 seconds.

The delay time is 7 seconds, corresponding to a delay volume of 2.94 mL.

With a make-up flow splitter, the delay volume measured in Lab Advisor cannot simply be used for any other flow rate.

NOTE

There are combinations of main and make-up flows that cannot be used. If in the same example as above, the main flow used is 60 mL/min (5 s splitter to FC delay) and the make-up flow is 0.5 mL/min (6 s splitter to detector delay), the resulting delay time and volume become negative.

Delay Volume with an Additional Detector

Despite the Mass Spectrometers detectors, for additional detectors, the delay calibration procedure is similar. To each detector, corresponds one delay volume. The delay calibration procedure can be performed from any detector connected to a Universal Interface Box (UIB). The signal collected by the UIB will be then used for the delay calibration.

Peak Triggering in Consideration of the Delay Volume

After correct completion of the delay volume calibration, when a peak is detected during a purification run (see [Figure 25](#) on page 65) the diverter valve is triggered using the following delay time calculations:

Start of fraction collection: $t = t_0 + t_{D1}$

End of fraction collection: $t = t_E + t_{D1} + t_{D2}$

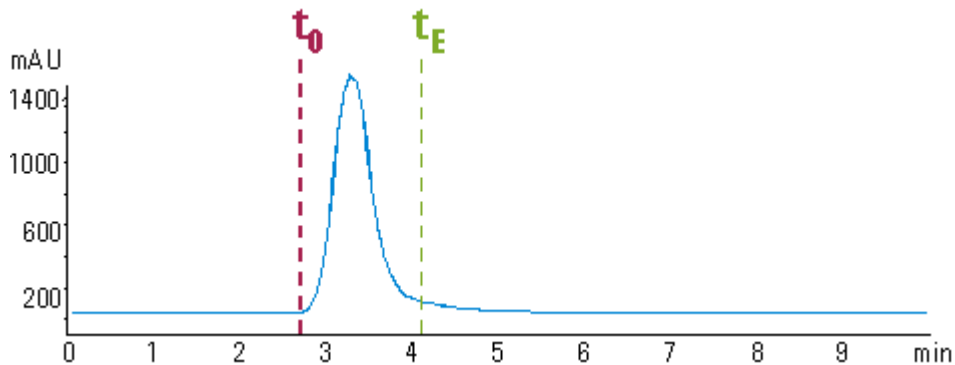


Figure 25 Chromatogram from a UV-detector with peak starting at t_0 and ending at t_E

t_{D1} is the delay time corresponding to the delay volume measured during the delay calibration.

Detector Signal Delay

Every Agilent 1260 Infinity detector that is used for triggering fractions has an internal signal delay caused by filtering the raw data. The signal delay depends on the **Peakwidth** setting of the detector and is accounted for when the diverter valve is triggered. [Table 14](#) on page 66 to [Table 18](#) on page 68 list the internal signal delay times for different **Peakwidth** settings.

Table 14 Signal Delay Times for the Agilent 1260 Infinity DAD/MWD (G1315D; G4212B/G1365D)

Peakwidth (min)	Response time (sec)	Signal delay (sec)
<0.01	0.1	0.05
>0.01	0.2	0.15
>0.03	0.5	0.5
>0.05	1.0	1.25
>0.10	2.0	2.75
>0.20	4.0	5.9
>0.40	8.0	11.9
>0.85	16.0	23.9

Table 15 Signal Delay Times for the Agilent 1260 Infinity DAD/MWD SL (G1315C/G1365C)

Peakwidth (min)	Response time (sec)	Signal delay (sec)
<0.0025	0.02	0.0375
>0.0025	0.05	0.0625
>0.005	0.1	0.144
>0.01	0.2	0.294
>0.03	0.5	0.619
>0.05	1.0	1.27
>0.1	2.0	2.57
>0.2	4.0	5.17
>0.4	8.0	10.4
>0.85	16.0	20.8

Table 16 Signal Delay Times for the Agilent 1260 Infinity VWD G1314B

Peakwidth (min)	Response time (sec)	Signal delay (sec)
<0.005	<0.12	0.07
>0.005	0.12	0.14
>0.01	0.25	0.29
>0.025	0.5	0.58
0.05	1	1.3
0.1	2	2.8
0.2	4	6.0
0.4	8	12.3

2 Configuration and Operation of the Fraction Collector

Delay Volumes and Delay Calibration for Fraction Collection (Lab Advisor)

Table 17 Signal Delay Times for the Agilent 1260 Infinity VWD SL G1314C

Peakwidth (min)	Response time (sec)	Signal delay (sec)
<0.00125	<0.02	0.0182
>0.00125	0.02	0.0364
>0.0025	0.05	0.0728
>0.005	0.12	0.146
>0.01	0.25	0.328
>0.025	0.5	0.710
>0.05	1	1.49
>0.1	2	3.08
>0.2	4	6.26
>0.4	8	12.6

Table 18 Signal Delay Times for the Agilent 1260 Infinity FLD G1321A

Peakwidth (min)	Response time (sec)	Signal delay (sec)
<0.005	<0.12	0.018
>0.005	0.12	0.073
>0.01	0.25	0.18
>0.03	0.5	0.44
>0.05	1	0.96
>0.1	2	2.0
>0.2	4	4.2
>0.4	8	8.6

CAUTION

If the internal signal delay is longer than the delay time t_{D1} some of the peak will be lost. The maximum allowed signal delay time can be calculated using the following equation:

$$\text{Signal delay time}_{(max)} = \frac{V_{D1}}{\dot{v}} \quad \dot{v} = \text{Flow rate}$$

After calculating the maximum signal delay time a **Peakwidth** setting can be selected that gives a signal delay time, which is shorter than the calculated maximum signal delay time. This **Peakwidth** setting should then be used for the LC purification run.

NOTE

We recommend to set the **Peakwidth** always to > 0.01 for the DAD and MWD or to > 0.005 for the VWD.

If the **Peakwidth** setting cannot be reduced and the signal delay time is longer than t_{D1} it is also possible to increase V_{D1} by adding additional tubing. However this higher delay volume will increase the peak dispersion between detector and fraction collector.

Delay Time and Volume Calibration of Fraction Collector for UV (Lab Advisor) and MSD Signals

Performing a Delay Calibration with an UV Detector

The delay time calibration determines a delay of a signal between a fraction collector (FC) and a detector. In the UV to FC delay volume calibration the measured delay time (t_{delay}) is recalculated to a delay volume (V_{delay}) using a preparative flow (F): $V_{\text{delay}} = t_{\text{delay}} * F$.

In case the detector is placed after a flow splitter with a make-up pump, the delay volume is measured each time the combination of preparative and make-up flows or the make-up flow needs to be re-adjusted, see [Table 19](#) on page 70.

The delay volume between the UV detector and the FC is determined using Agilent Lab Advisor software. A delay calibration mixture (p/n 5190-6887) is injected as a marker.

For the preparative Fraction collector, the delay calibration is performed using a column, and for the analytical fraction collector, a zero dead volume union is used.

Table 19 Recommendations for fraction collector delay calibration

Scale	Column	Flow rate	Injection volume
Analytical	Use the zero dead volume union instead of a column	1 – 5 mL/min	25 µL
Preparative	9.4 mm id	6 mL/min	25 µL
	21.2 mm id	25 mL/min	50 µL
	30 mm id	45 mL/min	75 µL

Delay Time and Volume Calibration of Fraction Collector for UV (Lab Advisor) and MSD Signals

Parts required	Description
G1946-85020	Delay Sensor Calibrant Calibration time solution For delay calibration with analytical scale fraction collector
0100-0900	1/16 in union, zero dead volume, stainless steel For delay calibration with analytical scale fraction collector
5190-6887	Agilent Standard #2 (Delay time calibration solution for 1260 LC-MS) For delay calibration with preparative scale fraction collector
5190-5108	Regenerated cellulose filters, 15 mm diameter, 0.2 µm pore size For delay calibration with preparative scale fraction collector

Preparations

Solvents:

- Solvent A: water with 0.1 % formic acid.
- Solvent B: acetonitrile with 0.1 % formic acid.
- In the case the UV detector is after splitter and a make-up pump is used:

Recommended make-up solvent: 80 % acetonitrile in water with 0.1 % formic acid .

Delay volume UV/FC calibration procedure:

- 1 Install a column (for UV/FC calibration at preparative scale).
OR
Install a zero dead volume union (for UV/FC calibration at analytical scale)
- 2 Start OpenLAB CDS ChemStation Edition and prepare your instrument:
 - a Load a preparative method containing the system parameters desired and considered for the delay calibration. If a make-up pump is used, ensure the correct flow set.
 - b If using a column (for UV/FC calibration at analytical scale) purge the column with 70 % of solvent B using the recommended flow rate for 2 min.

2 Configuration and Operation of the Fraction Collector

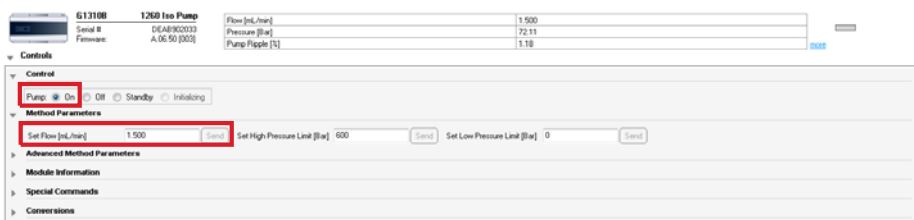
Delay Time and Volume Calibration of Fraction Collector for UV (Lab Advisor) and MSD Signals

- c Switch the preparative or analytical pumps off after column equilibration (in order to save solvent)
- 3 Exit OpenLAB CDS ChemStation Edition
- 4 If using MSD, remove *remote start cable* from LC stack
- 5 Make sure that both standard outlet and Delay Sensor waste tubing of FC go to a waste container
- 6 Place at least 500 μL of the corresponding delay calibration solution to 2 mL vial and place it in the sample tray – use single numbered vial positions on the tray (1, 2, ...)

NOTE

Filter Agilent Standard #2 (5190-6887) sample before use with Regenerated cellulose filters, 15 mm diameter, 0.2 μm pore size (5190-5108).

- 7 Start the Agilent Lab Advisor software.
- 8 If a make-up pump is used, the flow can be checked on the **Instrument Control** and under **Controls** the make-up pump. The pump must be in operation (if not, click **On**)



- 9 Go to **Service & Diagnostics**, select the target fraction collector (FC) and start **Delay Volume Calibration**.

NOTE

If more than one FC is used and if in the last working instrument configuration were fraction collectors clustered, perform the calibration only with the first clustered FC (the order of FCs in the FC cluster can be found in the instrument configuration in OpenLab Control Panel software). The clustered FC has one extra section **Clustering** in the **Delay Volume Calibration** window (see [Figure 26 on page 73](#)). If FCs are clustered but not the first one FC is selected for the calibration (incorrect one), then the calibration window is the same as for FCs without clustering.

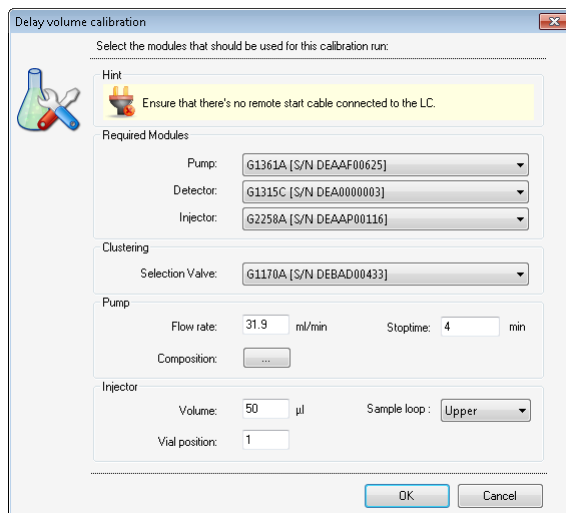


Figure 26 Delay volume calibration of fraction collector in Lab Advisor: Clustered fraction collector with extra section Clustering.

2 Configuration and Operation of the Fraction Collector

Delay Time and Volume Calibration of Fraction Collector for UV (Lab Advisor) and MSD Signals

Delay volume calibration

Select the modules that should be used for this calibration run:

Hint
Ensure that there's no remote start cable connected to the LC.

Required Modules

Pump: G1361A [S/N DEAAF00625]

Detector: G1315C [S/N DEA0000003]

Injector: G2258A [S/N DEAAP00116]

Pump

Flow rate: 31.9 ml/min Stoptime: 4 min

Composition: ...

Injector

Volume: 50 µl Sample loop: Upper

Vial position: 1

OK Cancel

Figure 27 Delay volume calibration of fraction collector in Lab Advisor: Window of non-clustered fraction collector.

10 In the Delay Volume Calibration window set following:

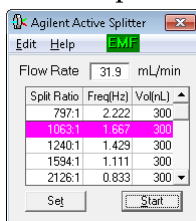
- **Required Modules:**
 - **Pump:** Choose the preparative pump, or the analytical pump used.
 - **Detector:** UV detector for calibration.
 - **Injector:** Autosampler for calibration.
- **Clustering** (appears only for the first FC of the FC cluster):
 - **Selection Valve:** Select the valve that was clustered in the FC cluster (the serial number is labelled on the valve).
- **Pump:**
 - **Flow rate:** Set target flow of the main pump.
 - **Stoptime:** 4 min.
 - **Composition** (for G1361A pumps): Click on “...” button and set composition to 70 % of solvent B.
- **Injector:**
 - **Volume:** See [Table 19](#) on page 70.
 - **Sample Loop** (for G2258A dual-loop autosampler): Upper loop.
 - **Vial Position:** Enter position corresponding to the vial with the Agilent Standard #2 (5190-6887).

- If an Agilent *Active Splitter G1968D* is used:
 - If using Active Splitter Software (OpenLAB CDS Chemstation Edition, installation disk 6):
 - Set the Agilent Active Splitter to **Remote** (button on the front side of the splitter).

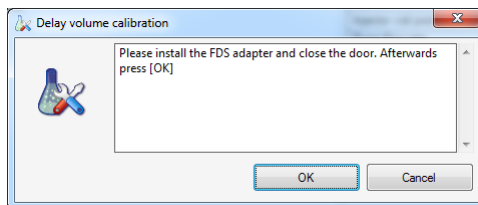
Start (and configure) Active Splitter Software.

In software enter the target main pump flow and set a split ratio (typically around 1:1000).

Start the splitter.



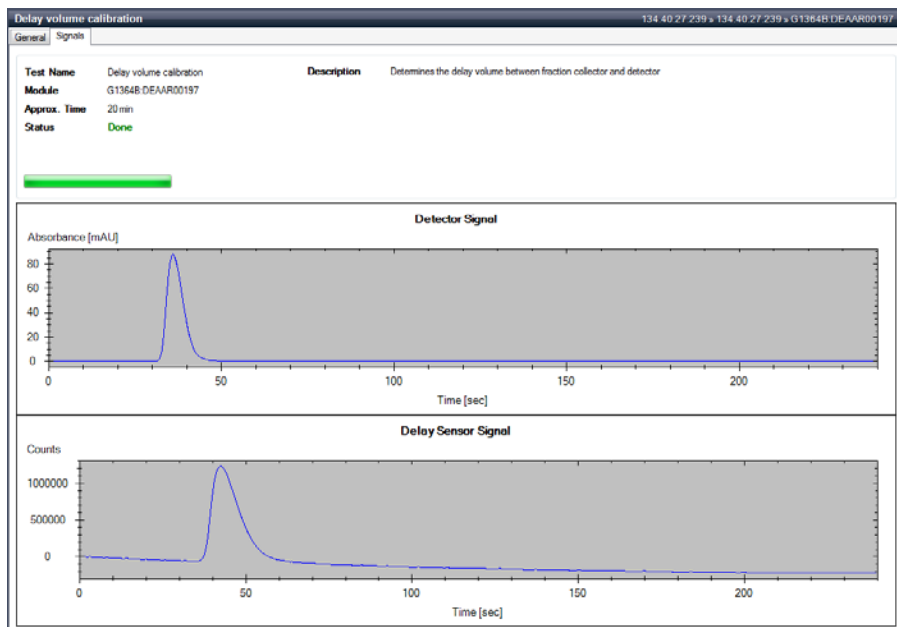
- Otherwise perform manual setup (see *MRA Operating Manual*, which you can find e.g. on the *Agilent Purification & Preparative LC User Documentation DVD*):
 - Set Agilent Active Splitter to **Local**.
 - Use Split Factor table on page 5 and Calculating split ratio equation on page 6 of the *MRA Operating Manual* to set the target split ratio (typically 1:1000).
 - Start the splitter (to start or stop the splitter press simultaneously both **SET** and **↑** buttons).
 - Click **OK** button to start calibration.
- 11** During initialization, which can take several minutes, the FC arm moves forward and the procedure asks to install the FDS adapter and close the FC door.



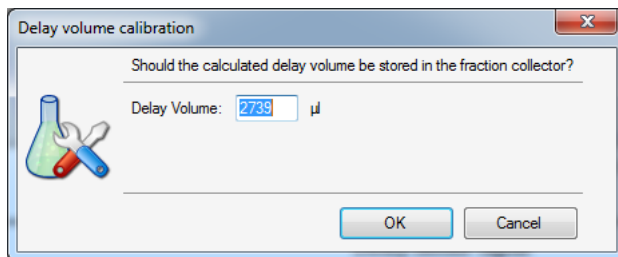
2 Configuration and Operation of the Fraction Collector

Delay Time and Volume Calibration of Fraction Collector for UV (Lab Advisor) and MSD Signals

- 12 After the sample is injected it is possible to click on **Signals** tab to see UV detector and delay sensor signals.



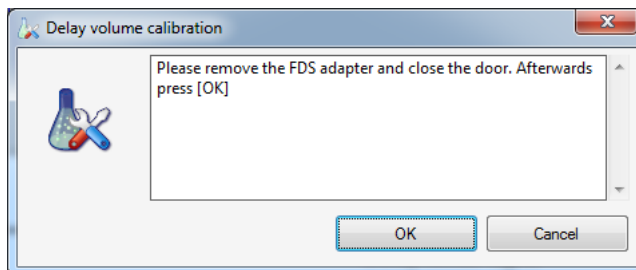
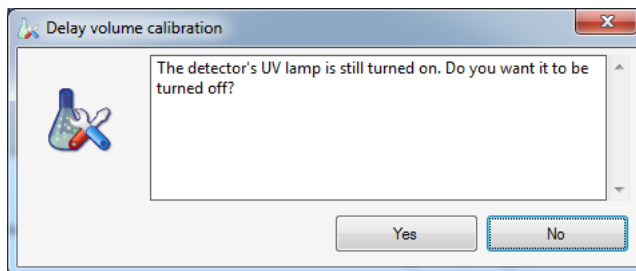
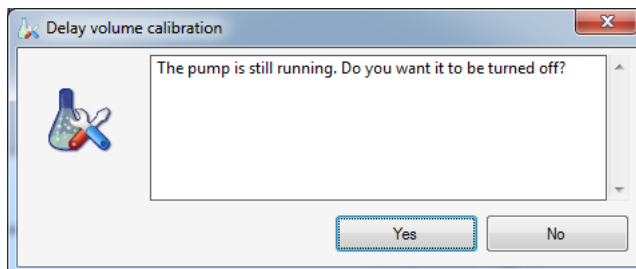
- 13 The results are displayed after completion.



NOTE

Keep a record of this delay volume, for further use in Automated Purification Software for example.

- 14 After the end of calibration, the procedure offers to stop main flow (recommended), switch off UV lamp and remove the FDS adapter.



- 15 Connect back the remote start cable to LC stack (if used).
- 16 Stop the splitter, go to **Instrument Control**, and stop the make-up pump.
- 17 The delay volume must be positive otherwise the calibration is invalid. In such a case see [“Insufficient UV to FC delay volume”](#) on page 91 or [“Insufficient MSD to FC delay time”](#) on page 91.
- 18 The delay volume is written to the FC firmware and the value is coupled with the used UV detector and will be used for fraction collection. To check or change the value, go to **Service & Diagnostics > select FC > Delay Volume Tool**.
- 19 If applicable, set the same delay volumes for other fraction collectors by **Service & Diagnostics > select the other FCs > Delay Volume Tool**. In case of

2 Configuration and Operation of the Fraction Collector

Delay Time and Volume Calibration of Fraction Collector for UV (Lab Advisor) and MSD Signals

different lengths of capillaries between the FC and UV detector execute Delay Volume Calibration for each FC in order to calibrate them individually but if FC clustering is used, remove it first from the instrument configuration.

Performing a Delay Calibration with an Additional Detector

For theory of delay calibration, see [“Performing a Delay Calibration with an UV Detector”](#) on page 51.

Parts required

	Description
5190-6886	Agilent Standard #1 (Spec out solution for 1260 LC-MS)
5190-6887	Agilent Standard #2 (Delay time calibration solution for 1260 LC-MS)
5190-5084	PTFE syringe filter, 0.2 µL, 15 mm

Performing a Delay Calibration with an Additional Detector

- 1 Connect the additional detector (ELSD, RID, FLD, ...) to an Universal Interface Box (UIB) (G1390B)
- 2 Install a preparative column (recommended SB-C18 21.2 x 50 mm, 5 µm or 21.2 x 150 mm, 5 µm)
- 3 Start OpenLab CDS ChemStation and prepare instrument:
 - Load a preparative method containing the system parameters desired and considered for the delay calibration. If a make-up pump is used, ensure the correct flow set.
 - Purge th column with 70 % of solvent B using 30 mL/min for 2 min.

- Switch the preparative pumps off after column equilibration (in order to save solvent).

4 Exit OpenLab CDS ChemStation

5 If using MSD, remove *remote start cable* from LC stack

6 Make sure that both standard outlet and Fraction Collector’s flow delay sensor waste tubing of FC go to a waste container

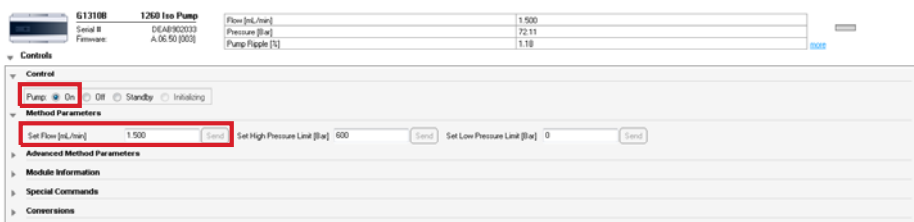
7 Place at least 500 µL of Agilent Standard #2 (5190-6887) to a 2 mL vial and place it in the sample tray - use single numbered vial positions on the tray (1, 2, ...)

NOTE

Filter Agilent Standard #2 (5190-6887) sample before use with PTFE syringe filter, 0.2 µL, 15 mm (5190-5084).

8 Start Lab Advisor.

9 If a make-up pump is used, the flow can be checked on the Instrument Control and under Controls the make-up pump. The pump must be in operation (if not, click On)



10 Go to **Service & Diagnostics**, select the target fraction collector (FC) and start **Delay Volume Calibration**.

NOTE

If more than one FC is used and if in the last working instrument configuration were fraction collectors clustered, perform the calibration only with the first clustered FC (the order of FCs in the FC cluster can be found in the instrument configuration in OpenLab Control Panel software). The clustered FC has one extra section **Clustering** in the **Delay Volume Calibration** window (see [Figure 28 on page 80](#)). If FCs are clustered but not the first one FC is selected for the calibration (incorrect one), then the calibration window is the same as for FCs without clustering.

2 Configuration and Operation of the Fraction Collector

Delay Time and Volume Calibration of Fraction Collector for UV (Lab Advisor) and MSD Signals

Delay volume calibration

Select the modules that should be used for this calibration run:

Hint
Ensure that there's no remote start cable connected to the LC.

Required Modules

Pump: G1361A [S/N DEAAF00625]
Detector: G1315C [S/N DEA0000003]
Injector: G2258A [S/N DEAAP00116]

Clustering

Selection Valve: G1170A [S/N DEBAD00433]

Pump

Flow rate: 31.9 ml/min Stoptime: 4 min
Composition: ...

Injector

Volume: 50 µl Sample loop: Upper
Vial position: 1

OK Cancel

Figure 28 Delay volume calibration of fraction collector in Lab Advisor: Clustered fraction collector with extra section **Clustering**.

Delay volume calibration

Select the modules that should be used for this calibration run:

Hint
Ensure that there's no remote start cable connected to the LC.

Required Modules

Pump: G1361A [S/N DEAAF00625]
Detector: G1315C [S/N DEA0000003]
Injector: G2258A [S/N DEAAP00116]

Pump

Flow rate: 31.9 ml/min Stoptime: 4 min
Composition: ...

Injector

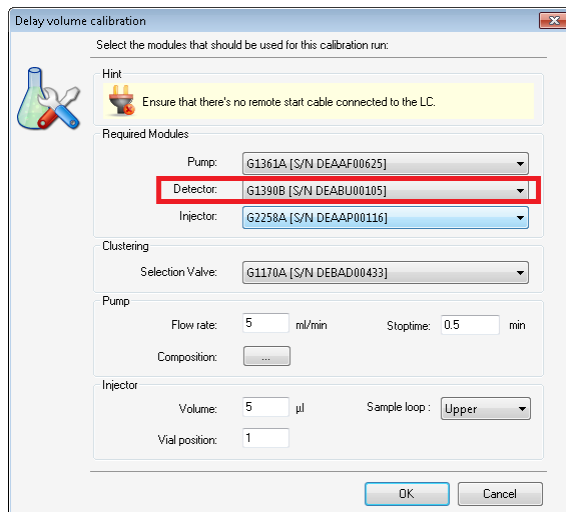
Volume: 50 µl Sample loop: Upper
Vial position: 1

OK Cancel

Figure 29 Delay volume calibration of fraction collector in Lab Advisor: Window of non-clustered fraction collector.

11 In the **Delay Volume Calibration** window set the following:

- **Required Modules:**
 - **Pump:** Preparative pump that corresponds to solvent A.
 - **Detector:** G1390B

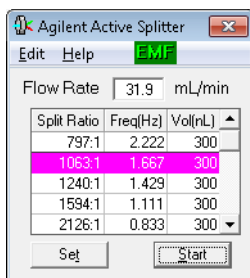


- **Injector:** Autosampler for calibration.
- **Clustering** (appears only for the first FC of the FC cluster):
 - **Selection Valve:** Select the valve that was clustered in the FC cluster.
- **Pump:**
 - **Flow rate:** Set target flow of the main pump.
 - **Stoptime:** 4 min.
 - **Composition:** Click on “...” button and set composition to 70 % of solvent B.
- **Injector:**
 - **Volume:** 50 µL
 - **Sample Loop:** Upper loop.
 - **Vial Position:** Enter position corresponding to the vial with the Prep LC Standards #2.

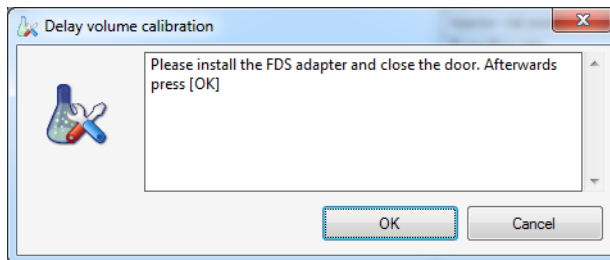
2 Configuration and Operation of the Fraction Collector

Delay Time and Volume Calibration of Fraction Collector for UV (Lab Advisor) and MSD Signals

- If an *Agilent Active Splitter G1968D* is used:
 - If using Active Splitter Software (OpenLAB CDS Chemstation installation disk 6):
 - Set the Agilent Active Splitter to **Remote** (button on the front side of the splitter).
Start (and configure) Active Splitter Software.
In software enter the target main pump flow and set a split ratio (typically around 1:1000).
Start the splitter.

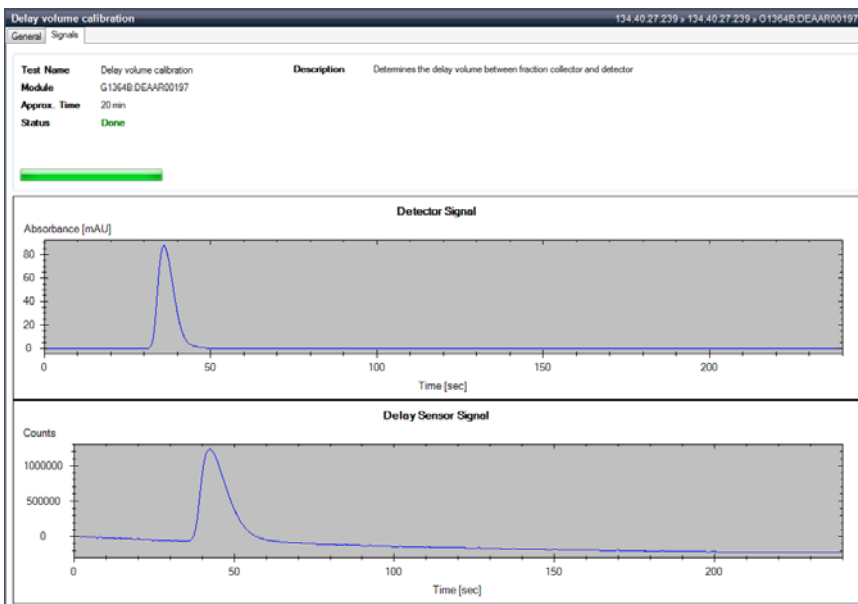


- Otherwise perform manual setup (see *MRA Operating Manual* on the *Agilent Purification & Preparative LC User Documentation DVD*):
 - Set Agilent Active Splitter to **Local**.
 - Use Split Factor table on page 5 and Calculating split ratio equation on page 6 of the *MRA Operating Manual* to set the target split ratio (typically 1:1000).
 - Start the splitter (to start or stop the splitter press simultaneously both **SET** and **↑** buttons).
 - Click **OK** button to start calibration.
- 12** During initialization, which can take several minutes, the FC arm moves forward and the procedure asks to install the FDS adapter and close the FC door.

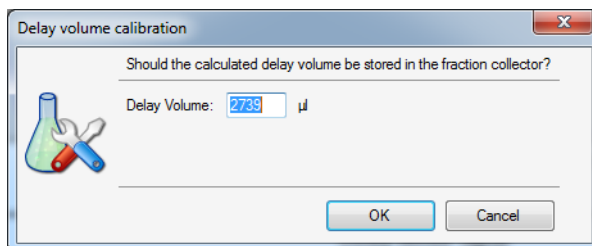


Delay Time and Volume Calibration of Fraction Collector for UV (Lab Advisor) and MSD Signals

13 After the sample is injected it is possible to click on Signals tab to see UV detector and delay sensor signals.



14 The results are displayed after completion.



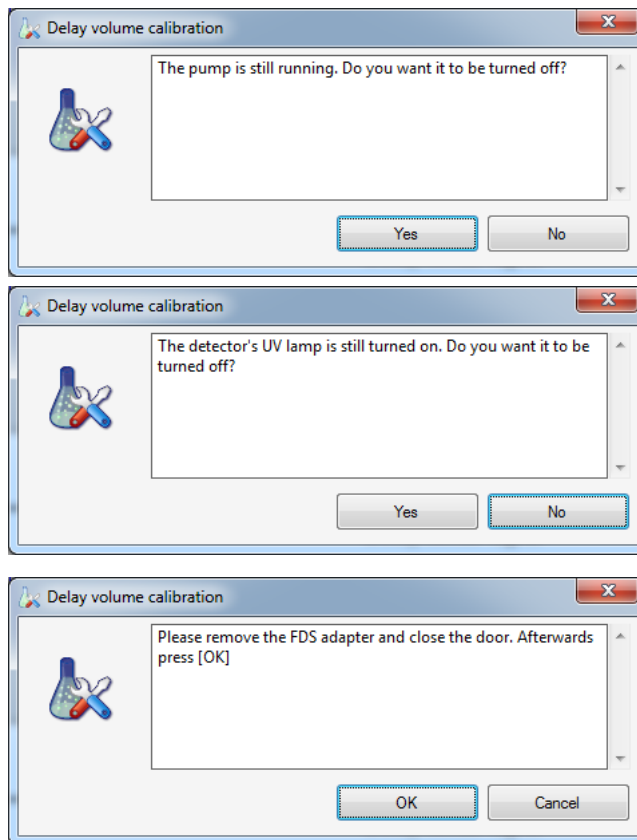
NOTE

Make a note on this delay volume, for further use in Automated Purification Software for example.

2 Configuration and Operation of the Fraction Collector

Delay Time and Volume Calibration of Fraction Collector for UV (Lab Advisor) and MSD Signals

- 15** After the end of calibration, the procedure offers to stop main flow (recommended), switch off UV lamp and remove the adapter.



- 16** Connect back the remote start cable to LC stack (if used).
- 17** Stop the splitter, go to **Instrument Control**, and stop the make-up pump.
- 18** The delay volume must be positive otherwise the calibration is invalid. In such a case see “[Insufficient UV to FC delay volume](#)” on page 91 or “[Insufficient MSD to FC delay time](#)” on page 91.

- 19** The delay volume is written to the FC firmware and the value is coupled with the used UV detector and will be used for fraction collection. To check or change the value, go to **Service & Diagnostics > select FC > Delay Volume Tool**.
- 20** If applicable, set the same delay volumes for other fraction collectors by **Service & Diagnostics > select the other FCs > Delay Volume Tool**. In case of different lengths of capillaries between the FC and UV detector execute Delay Volume Calibration for each FC in order to calibrate them individually but if FC clustering is used, remove it first from the instrument configuration.

Performing a Delay Calibration with an MSD

The procedure calibrates the delay time between the UV detector and MSD using a standard run in Agilent OpenLab CDS ChemStation software. As explained above (see [“Performing a Delay Calibration with an UV Detector”](#) on page 70). When a flow splitter is used, the calibration can be used only for runs with identical tubing and settings of preparative and make-up pump flow.

Table 20 Recommendations for fraction collector delay calibration

Scale	Column	Flow rate	Injection volume
Analytical	Use the zero dead volume union instead of a column	1 – 5 mL/min	25 µL
Preparative	9.4 mm id	6 mL/min	25 µL
	21.2 mm id	25 mL/min	50 µL
	30 mm id	45 mL/min	75 µL

2 Configuration and Operation of the Fraction Collector

Delay Time and Volume Calibration of Fraction Collector for UV (Lab Advisor) and MSD Signals

Parts required	p/n	Description
	G1946-85020	Delay Sensor Calibrant Calibration time solution For delay calibration with analytical scale fraction collector
	0100-0900	1/16 in union, zero dead volume, stainless steel For delay calibration with analytical scale fraction collector
	5190-6887	Agilent Standard #2 (Delay time calibration solution for 1260 LC-MS) For delay calibration with preparative scale fraction collector
	5190-5108	Regenerated cellulose filters, 15 mm diameter, 0.2 µm pore size For delay calibration with preparative scale fraction collector

Preparations

- Solvent A: water with 0.1 % formic acid.
- Solvent B: acetonitrile with 0.1 % formic acid.
- Make-up solvent: 80 % acetonitrile in water with 0.1 % formic acid.
- Needle purge and wash solution for the dual loop autosampler (degassed in ultrasonic bath): 80 % acetonitrile or another suitable solution (methanol should not be used with dual-loop autosampler due to peristaltic pump tubing – check the dual-loop autosampler manual)

Performing a Delay Calibration with an MSD

- 1 Switch off all pumps
- 2 Fill at least 700 µL of the sample to a vial and place it to the injector

NOTE

Filter Agilent Standard #2 (5190-6887) sample before use with PTFE syringe filter, 0.2 µm, 15 mm (5190-5084).

- 3 Place a tray into the fraction collector with a valid collection position (for example a tube tray or a plate tray with a plate and set the plate up in the fraction collector's **Assign WellPlates...** window – accessible via right-click on the fraction collector instrument icon).
- 4 Set-up the method:

- Use a Default Purification Method.
- Save the Method as **UV_MSD_Calibration**.
- Disable fraction collector triggering:
 - In the Method parameters of the fraction collector, select **Off** on the **Fraction Trigger Mode**.
- Disable the Mass fraction collector triggering:
 - In the Fraction Collector settings of the MSD, select **None** on **FC Mode**.
- Set **Stop Time** to **No Limit** in all modules (infinite run time).
- For the analytical or preparative pumps:
 - **Set Solvents: 70 % B**.
 - Clear Time Table.
- Injection volume, see [Table 20](#) on page 85.
- If using dual loop autosampler:
 - Set **Injection Loop** to **Upper**.
 - Set **Partial loop filling**.
- UV detector:
 - Set UV detector to 600 nm wavelength with 4 nm bandwidth and no reference.

NOTE

Alternative wavelength setting is 280 nm with 4 nm bandwidth and no reference

- Mark **Vis lamp** in Lamps on required for acquisition if available.
 - Set the active splitter to **Local** if available (in order to execute external contacts timetable in the UIB method).
 - Save the Method changes.
- 5** If using the dual loop autosampler:
- Make sure that the Upper loop is in the main flow path (right-click on the autosampler diagram and check if a valve command refers to **Switch Valve to Lower Loop**. If not select **Switch Valve to Upper Loop** command).

2 Configuration and Operation of the Fraction Collector

Delay Time and Volume Calibration of Fraction Collector for UV (Lab Advisor) and MSD Signals

- Wash needle (right-click on **autosampler diagram** > **Wash Needle** > **Flush Port**, 15 s).
 - Purge needle (right-click on **autosampler diagram** > **Start Purging...** 3x).
- 6** If using a column (for preparative fraction collector), purge the column with 70 % of solvent B using the recommended flow rate for 2 min.
- 7** Adjust the preparative and make-up using the same combination of flow rates as during the delay calibration in Lab Advisor.
- 8** Switch on all modules.
- 9** Monitor 600 nm UV signal and positive scan MSD signal in **Online Plot**.
- 10** Go to Sample Info window:
- Set **Vial/Location** of the sample vial.
 - Enter a run name.
 - **Run Method**.
- 11** Stop the run after the delay marker peak is eluted.
- 12** Clean the dual-loop autosampler if used:
- Purge needle (right-click on **autosampler diagram** > **Start purging...** 3x).
 - Wash needle (right-click on **autosampler diagram** > **Wash Needle** > **Flush Port**, 15 s).

13 Evaluate data (in mL and min units):

- Load MSD positive scan data and read time at the peak apex (signal maximum), t_{MSD} .
- Load UV 600 nm data and read time at the apex of the peak, t_{UV} .
- UV / MSD delay time: subtract MSD and UV time, $t_{UV/MSD} = t_{MSD} - t_{UV}$

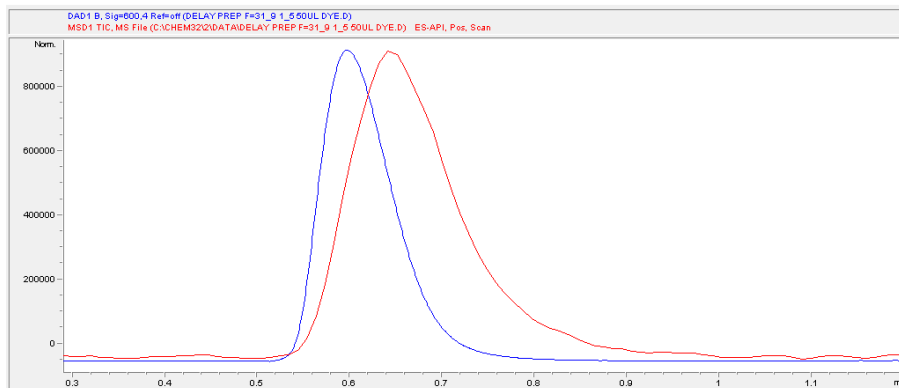


Figure 30 UV / MSD delay time calibration as a time offset between MSD (positive scan, red curve) and UV detector (600 nm, blue curve) at the apex of peaks.

14 Check value of MSD to FC delay time ($t_{MSD/FC}$):

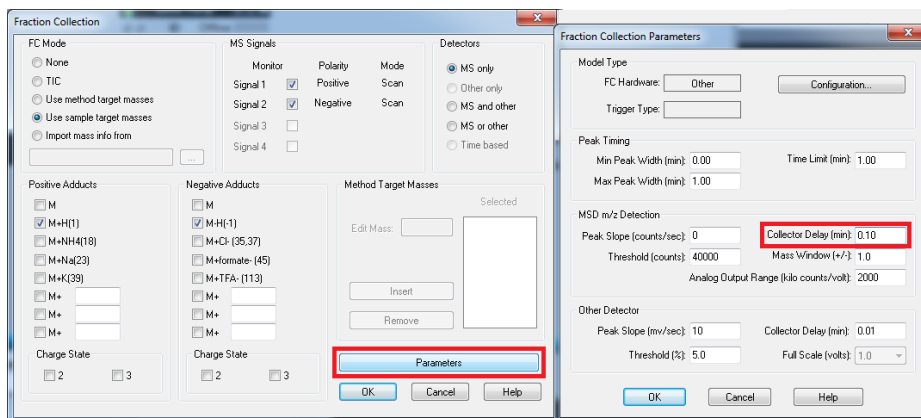
- Recalculate UV to FC delay volume from the Lab Advisor calibration to the delay time using applied flow F: $t_{UV/FC} = V_{UV/FC}/F$
- Calculate **MSD to FC delay time** as a difference between UV to FC and UV to MSD delays: $t_{MSD/FC} = t_{UV/FC} - t_{UV/MSD}$
- Calculate **MSD cycle time** from measured UV/MSD data: Load MSD positive scan data again, select Integration mode (first icon below the list of files) and use Analysis Time and Datapoints in the File Information table below the graph to calculate the cycle time:
- Cycle Time (sec/cycle) = (Analysis Time in min) * 60 / Datapoints
- Calculate a ratio of the MSD/FC delay time and cycle time:
- Ratio = $t_{MSD/FC} * 60 / \text{cycle time}$.

2 Configuration and Operation of the Fraction Collector

Delay Time and Volume Calibration of Fraction Collector for UV (Lab Advisor) and MSD Signals

- The ratio of MSD/FC delay time and the cycle time must be at least three but recommended is four ($t_{\text{MSD/FC}} > 3x$ or better $4x$ cycle time). If not, then the MSD-based collection may not be performed correctly (insufficient MSD datapoints for a correct FC triggering decision) - see “Insufficient UV to FC delay volume” on page 91 or “Insufficient MSD to FC delay time” on page 91.

If the $t_{\text{MSD/FC}}$ is sufficient, the value need to be reported under the MS Fraction collection settings. Once in the MS **Fraction Collection**, click on **Parameters** and change if required the **Collector Delay (min)** to the value calculated during the delay time calibration.



Tips and Tricks (Lab Advisor)

Insufficient UV to FC delay volume

There are several reasons why the UV to FC delay volume may be too low:

- The volume of delay tubing in front of FC is too low.
- The make-up flow is too low for the applied preparative flow. There are limitations for combinations of preparative and make-up flows, see [“Performing a Delay Calibration with an UV Detector”](#) on page 51.
- The volume of tubing between the splitter and detector(s) is too high.

There are several ways how to increase the UV to FC delay volume but each of them has a certain disadvantage so consider the most suitable option for the given situation:

- Increase the volume of delay tubing in front of FC (increases peak volume in FC).
- Decrease the preparative flow (influences chromatography).
- Increase the make-up flow (increases speed of analytes when passing through detectors that results in narrower and smaller peaks so consider sufficiency of the signal response and the detector data rate).
- Decrease the volume of tubing between the splitter and detector(s).

After one or more solutions are applied, repeat the FC delay calibration from the beginning.

Insufficient MSD to FC delay time

Since the MSD / FC delay time is the difference: $UV/FC - UV/MSD$ (see [“Evaluate data \(in mL and min units\):”](#) on page 89), the both sources influence the MSD / FC value. Reasons for insufficient MSD / FC delay time may be following:

- The volume of delay tubing in front of FC is too low.
- MSD cycle time is too high.

2 Configuration and Operation of the Fraction Collector

Tips and Tricks (Lab Advisor)

- The make-up flow is too low for the applied preparative flow. There are limitations for combinations of preparative and make-up flows - see “Performing a Delay Calibration with an UV Detector” on page 70.
- The volume of tubing between the splitter and detector(s) is too high.

The following suggestions are options how to increase the MSD / FC delay time but each of them has a certain disadvantage so consider the most suitable way for the given situation:

- Optimize the MSD cycle time in the MSD method:
 - Use only one polarity if suitable (requires detailed knowledge of collected compounds).
 - Optimize peakwidth parameter – decrease it until MSD signal noise is still acceptable.
 - Decrease MW range or increase MW step size if suitable - it will not typically directly decrease the cycle time but it allows further decreasing of the peakwidth parameter (short MW range hides potential contaminants).
 - Performing MSD maintenance can also help in optimization of the noise level.
- Increase the volume of delay tubing in front of FC (increases peak volume in FC).
- Decrease the preparative flow (influences chromatography).
- Increase the make-up flow (increases speed of analytes when passing through detectors that results in narrower and smaller peaks so consider sufficiency of the signal response and the detector data rate – especially the MSD data rate is limiting).
- Decrease the volume of tubing between the splitter and detector(s).

NOTE

Repeat calibrations:

- If only the MSD cycle time was changed, repeat only the Delay time UV / MSD calibration.
- In other cases repeat both UV / FC and UV / MSD calibrations.

Setting up a Fraction Collector Method

In order to setup or change the method parameters of your fraction collector select **Setup Fraction Collector** from the **Instrument** menu or right-click on the fraction collector icon in the graphical user interface. This will open the **Setup Fraction Collector** dialog box as displayed in [Figure 31](#). In the **Setup Fraction Collector** dialog box general method settings are specified.

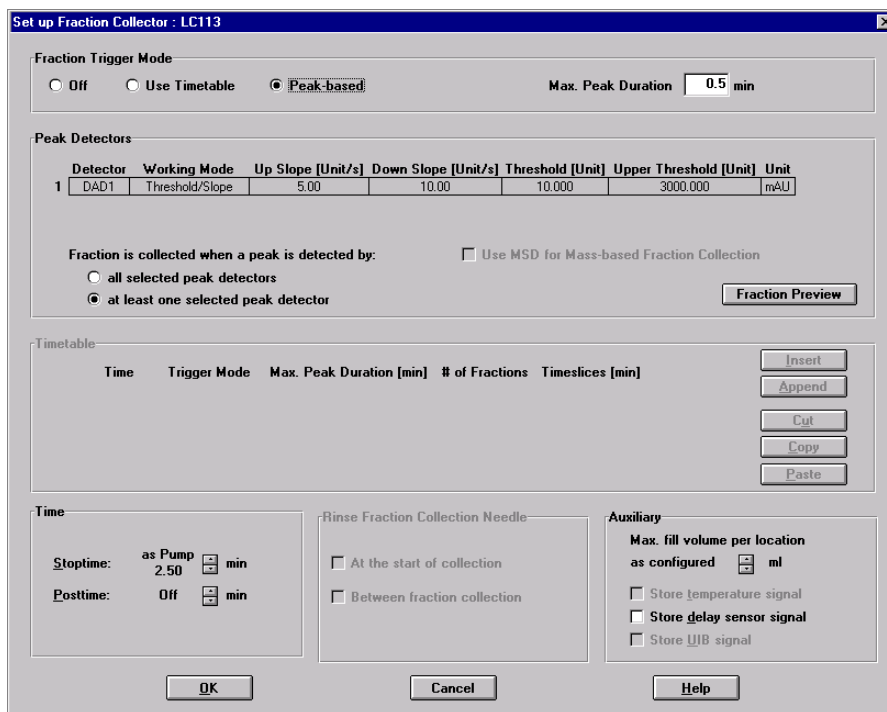


Figure 31 Set up Fraction Collector dialog box

Fraction Trigger Mode

Use Timetable Enables the **Timetable**

Peak-based If Peak-based is selected, the collection of a fraction is triggered by the signal of the detector, e.g. the Agilent 1260 Infinity Diode Array Detector or variable wavelength detector. The detailed trigger conditions are specified in the **Peak Detectors** table. In the peak-based trigger mode all entries in the Timetable are ignored.

Max. Peak Duration Defines a maximum collection time in case that the signal does not reach the condition to cut the fraction as exhibited in [Figure 32](#). This could be caused by tailing peaks or if the baseline is drifting during gradient runs. The default value is set to 0.5 minutes. If broad peaks are expected, this value should be increased without exceeding the run time.

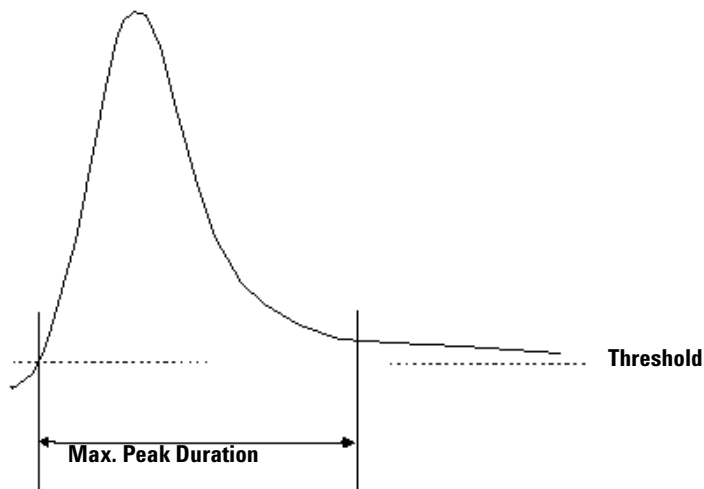


Figure 32 Maximum Fraction Duration

Peak Detectors

In the **Peak Detectors** section a list of all Peak Detectors that are connected to the system is displayed. Agilent 1260 Infinity Diode Array Detectors, multi-wavelength detectors, variable wavelength detectors and fluorescence detectors are recognized automatically. Other Detectors, e.g. Agilent 6000 Series mass-selective detectors or HP1050 Detectors, are connected through the Universal Interface Box (UIB).

The Peak detector table contains seven columns:

Working Mode For each peak detector **Threshold only**, **Threshold/Slope** or **Slope only** are possible.

In the **Threshold only** mode the settings for Up Slope, Down Slope and Upper Threshold in the subsequent columns are ignored. Fraction collection is triggered whenever the detector signal exceeds the specified threshold value. When the signal drops below the threshold value fraction collection is stopped.

In the **Slope only** mode fraction collection is triggered on the slope of the detector signal. Adequate values for Up Slope and Down Slope can be specified in the corresponding fields.

In the **Threshold/Slope** mode fraction collection is triggered on the corresponding values for threshold and slope. The fraction collection is started if the detector signal exceeds both the threshold and the Up Slope value. The fraction collection is stopped if the detector signal drops either below the threshold or the Down Slope value.

To specify the trigger values **Up Slope**, **Down Slope**, **Threshold** and **Upper Threshold** we recommend to use the **Fraction Preview** tool as described in “[Fraction Preview](#)” on page 99.

Upper Threshold At high absorbance values the light intensity on the detector is extremely low and consequently detector noise will be superimposed on the detector signal. In this case the detector noise might trigger fraction collection. To avoid false fraction collection triggering, we recommend setting an **Upper Threshold** well below the limit where this false triggering effect might occur. As soon as the detector signal exceeds the **Upper Threshold**, settings for Up Slope or Down Slope will be ignored until the signal drops again below the Upper Threshold.

When using more than one peak detector fraction collection can be triggered either when **all selected peak detectors** detect a peak or when **at least one selected peak detector** detects a peak basing on the settings in the Peak Detectors table above.

If an MSD is used for mass-based fraction collection, **Use MSD for mass-based Fraction Collection** must be checked.

Timetable

The **Timetable** can be used to program changes in the Fraction Trigger Mode during the analysis by entering a **Time** and specifying the trigger settings.

Trigger Mode Off, Peak Based and Time Based can be selected. If the Off is selected, no fractions are collected. The last entry in the timetable has to be the command Off.

Whenever the **Peak Based** mode is specified fractions will be collected based on the peak detection parameters given in the **Peak Detector** table. Additionally a **Maximum Peak Duration** in minutes has to be specified. This parameter is mandatory if you use Peak Controlled fraction collection, but is disabled for Time Based fraction collection.

When the **Time Based** mode is chosen two different options are available:

- The **# of Fractions** can be edited to collect a fixed number of equal fractions in a give time interval. This time interval is defined by the time value in the current and following timetable line.
- **Timeslices [min]** can be edited to collect fractions with a defined collection time. With this option the collection time of the last fraction can be shorter. This depends on the overall runtime.

For editing the Timetable the functions **Insert**, **Append**, **Cut**, **Copy** and **Paste** are offered.

To access the additional sections in the **Setup Fraction Collector** dialog box click **More**.

Time

In the time section of the dialog box the **Stoptime** and the **Posttime** for the fraction collector can be specified. By default the Stoptime is set to as pump and the posttime is switched OFF.

2 Configuration and Operation of the Fraction Collector

Setting up a Fraction Collector Method

Rinse Fraction Collection Needle (Analytical Scale only)

If Discrete Fractions is selected as Collection Mode (see also “Collection Mode” on page 42), you can setup a needle rinse step before the fraction collection and/or between fractions. Then the needle will move to the funnel on the internal tray and the diverter valve will switch to flush the needle in order to avoid carry over from the previous fraction. The instrument will determine, if it is possible to rinse the needle before the next fraction is expected.

If you have recovery positions in your fraction collector or if you are using one fraction collector for sample recovery in a multiple fraction collector configuration, the function **rinse between fractions** is ignored.

Auxiliary

In the Auxiliary section the **Maximum fill volume** per location can be specified. If **as configured** is selected, the pre-configured volume (see **Instrument > Pre-configured Wellplate Types**) is used. This ensures that the location (well, vial or tube) cannot be overfilled during fraction collection. This volume can be further reduced by defining a customized volume.

Additional check boxes in this section provide the opportunity to **Store** the **temperature signal** and the **UIB signal**.

Fraction Preview

To determine the appropriate fraction collection parameters the Agilent ChemStation provides a valuable tool that becomes accessible by pushing the button labelled Fraction Preview Tool (Figure 33) in the Peak Detectors section.

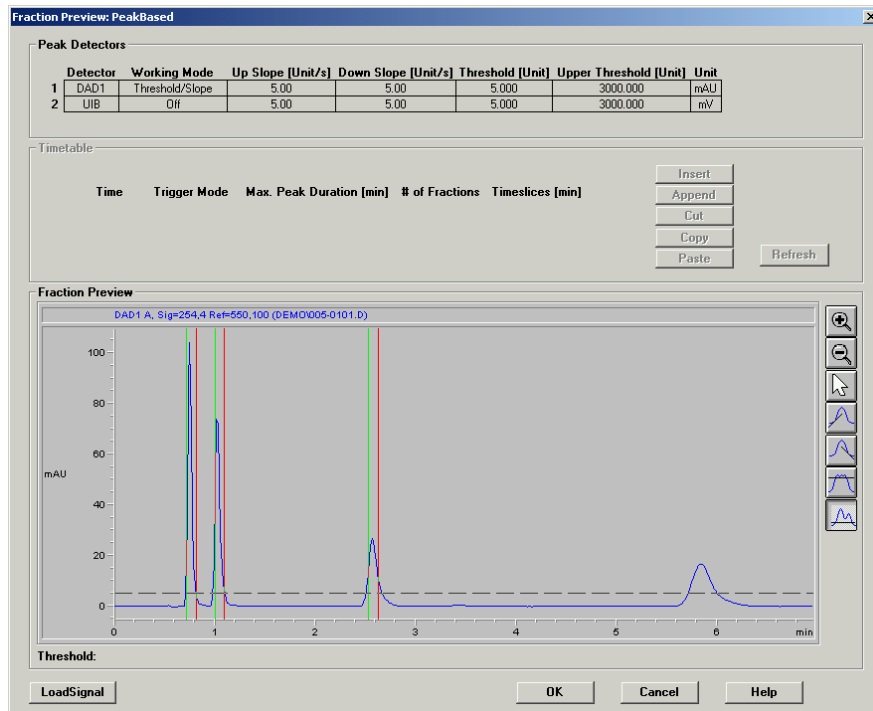


Figure 33 Fraction Preview dialog box

The Fraction Preview screen allows to test the fraction collection parameters against an example chromatogram. It can also be used to optimize the fraction collection parameters interactively. With the help of this tool values for up and down slope as well as for upper and lower threshold can easily be graphically specified. To load a chromatogram e.g. a pilot run click **Load Signal**. Parameters can now be changed either manually in the Detector Table and Timetable or graphically in the Fraction Preview screen. By pushing the desired buttons on the right hand

2 Configuration and Operation of the Fraction Collector

Setting up a Fraction Collector Method

side of **Fraction Preview** screen the chromatogram can be zoomed, the values for up and down slope can be specified and the upper and lower threshold level can be set-up. The graphically specified values are automatically transferred to the Peak Detector Table.

Starting Your Run with Fraction Collection

Resetting the Current Fill Levels

To protect the collected fractions against contaminations and to avoid overfilling of single positions the Agilent 1260 Infinity Fraction Collector stores current fill levels. It will block used positions, if no fraction start location has been specified.

If a new tray of the same type is inserted in the fraction collector, all fill levels can be reset (not just the well plate, vial or test tube). After re-installation of the tray a pop-up window as displayed in [Figure 34](#) will be displayed. Click on **Yes** to reset all fill levels from the previous run.



Figure 34 Reset fill volumes

CAUTION

The window ([Figure 34](#)) to reset current fill levels is not displayed, if the well plate, the vial or the test tube is removed without removing the complete tray. Consequently contaminated fractions, unwanted overflow events or split fractions might be observed during the following LC run.

Assignment of Start Location for Fraction Collection

The start location for fraction collection can either be assigned in the **Sample Info** ([Figure 35](#)) dialog box in the **RunControl** menu, in the **Sequence Parameters** ([Figure 36](#)) dialog box or in the **Sequence Table** in the **Sequence** menu. All fraction start locations are entered in the following formats.

2 Configuration and Operation of the Fraction Collector

Starting Your Run with Fraction Collection

For vials and test tubes:

fraction collector # - vessel number, e.g. **1-Vial 1**

For well plates:

fraction collector # - plate number - row - column, e.g. **1-P1-A-01**

with

fraction collector # = 1,2; plate number = P1-P4; row is A to H and column = 1-12 for 96-well plates.

NOTE

Note the orientation of the well plate. The starting position A1 is always in the back left corner of the plate.

Sample Info: Instrument 1

Operator Name: Bio Chemist

Data File

Prefix/Counter Manual

Filename: DEFAULT.D

Subdirectory: PROTEOME

Path: C:\HPCHEM\1\DATA\

Sample Parameters

Location: Vial 1 (blank run if no entry)

Fraction Start Location: 1-P1-A-01

Sample Name: Sample_1 Plate ID:

Sample Amount: 0 Multiplier: 1

ISTD Amount: 0 Dilution: 1

Figure 35 Sample Info dialog box

All settings made in the Sequence Table overrule the settings made in the **Sequence Parameters** screen. In addition to the exact position for fraction collection start in the **Sequence Table** it is also possible to specify Next Plate and Next Location. In the former case fraction collection is started at the next free plate and in the latter case fraction collection is started at the next free location.

If a fraction start location is specified, the collector will start at this location, even if this vessel has been used during a previous run.

If no fraction start location has been specified, the fraction collection starts at the first empty vessel or location.

CAUTION

With multiple fraction collectors a fraction start location must be specified, e.g. 1-P1-A-01 or 2-Vial 10. Otherwise the fraction collection of the next run will start at the fraction collector, where the last run stopped. Never used the valve settings to define a start location.

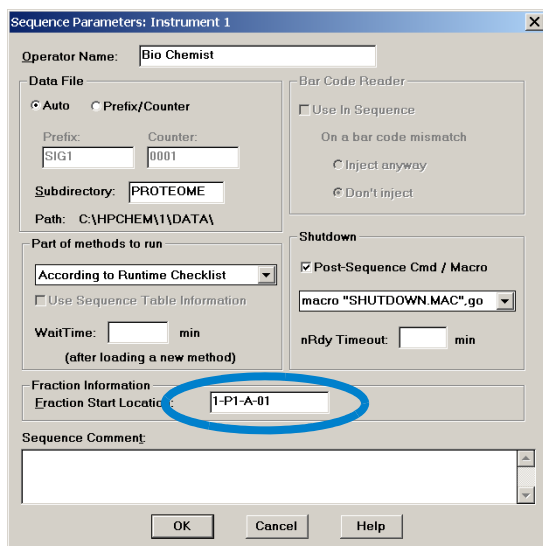


Figure 36 Start Location in Sequence Parameters dialog box

To start a single run

- click on the Start button in the graphical user interface of the ChemStation.
- select **Run Method** from the **RunControl** menu.
- press **F5**

To start the sequence

- click on the Start button in the graphical user interface of the ChemStation.

2 Configuration and Operation of the Fraction Collector

Starting Your Run with Fraction Collection

- select **Run Sequence** from the **RunControl** menu.
- press **F6**

Online Tick Marks

To display tick marks for fraction start and stop events in you Online Plot window, click on the **Change** button in your **Online Plot** window. Then check **Show fraction collection ticks** in the **Edit Signal Plot** window (Figure 37).

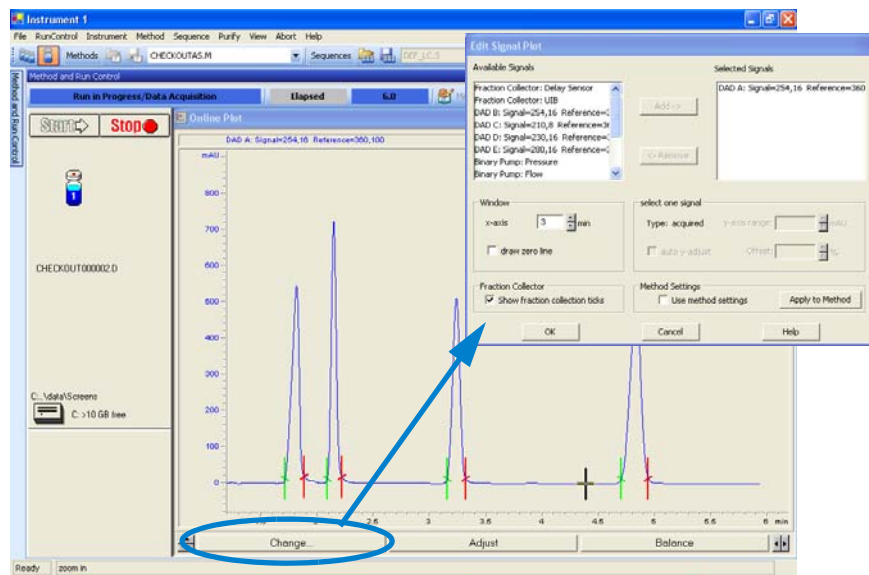


Figure 37 Online Tick Marks

Viewing Your Results

Data Analysis

In order to display the tick marks for the collected fractions on the screen, click **Signal options** from the **Graphics** menu. Then choose **Separated** in the **Layout dropdown list**.

To review your chromatograms, file information and a fraction list, select the **Data Analysis** view and press **Fraction Task** as displayed in [Figure 38](#).

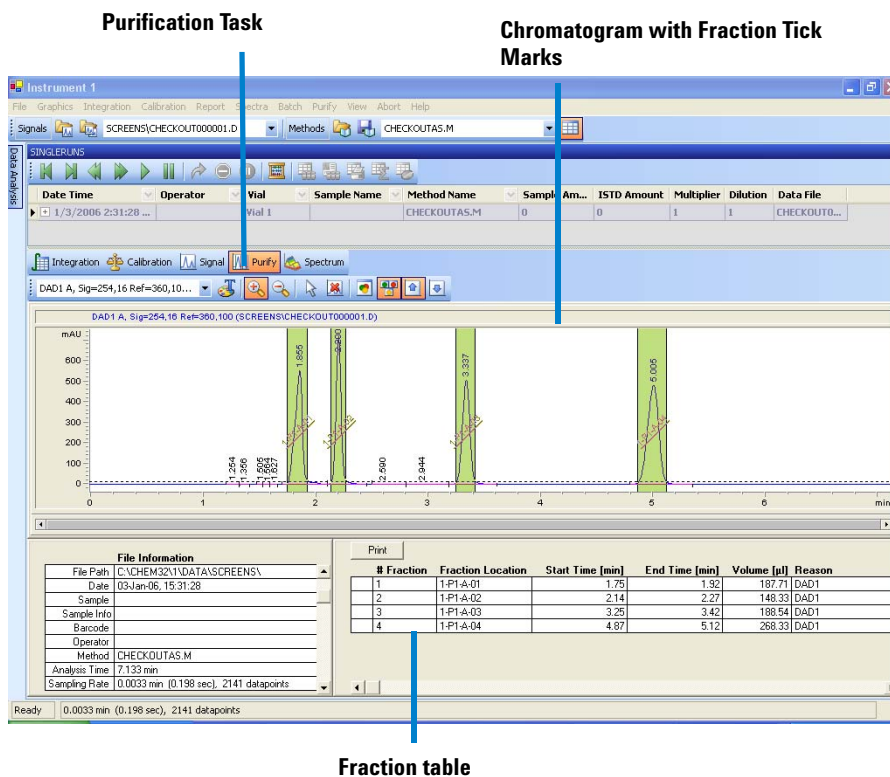


Figure 38 Fraction Task button

In order to display the tick marks for the collected fractions on the screen, click **Signal options** from the **Graphics** menu. Then choose **Separated** in the Layout dropdown list.

Report

In order to create reports with a fraction table and tick marks the Specify Report box the item Add Fraction Table and Tick Marks has to be checked.

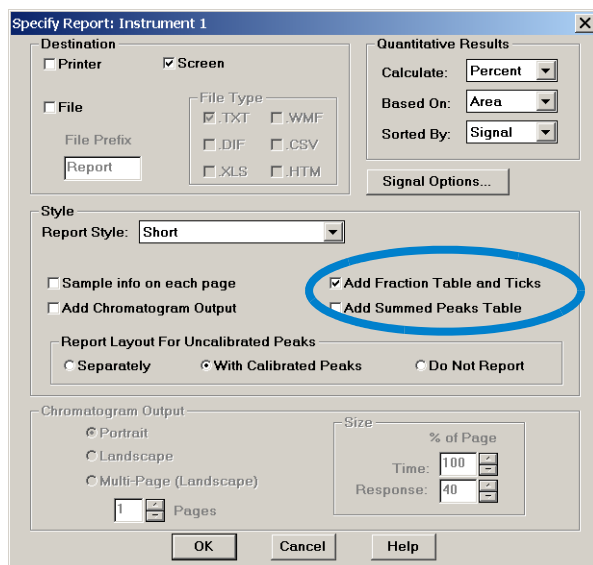


Figure 39 Fraction collection report setup

Special Applications

Pooling

We define Pooling as collecting fractions from multiple injections into the same fraction locations. In the Agilent Chemstation, there are two ways to initiate pooling:

- If you want to collect multiple injections from the one sample vial or sample well into the same fraction location, specify multiple injections in the Sequence Table.
- In case you want to collect multiple injections from the several sample vials or sample wells into the same fraction location, specify the same **Fract. Start** for multiple samples in the **Sequence Table**. An example is displayed in [Figure 40](#) on page 108. In this sequence the Sample 1 will be injected from Plate 1 Position A1 of the Agilent 1260 Infinity High Performance Autosampler and fraction collection will start at Plate 1 Position A1 of the fraction collector. For the next Sample 2, which is injected from a different location in the high performance autosampler, the fraction collection will start again at Plate 1 Position A1 of the fraction collector.

Line	Location	Sample Name	Dilution	Datafile	Inj Volume	Frac. Start
1	P1-A-01	Sample 1				1-P1-A-01
2	P1-A-02	Sample 2				1-P1-A-01
3	P1-A-03	Sample 3				1-P1-A-01
4						

Figure 40 Pooling Sequence

CAUTION

If you specify multiple injections for pooling, the overflow protection is not activated. It is the user's responsibility to make sure that all fraction collection locations are large enough to completely collect all pooled fractions. If a fraction collection location is overflowed, an error message occurs and the pump is shut OFF.

Sample Recovery

The Agilent 1260 Infinity Fraction Collectors offer different possibilities for sample recovery:

- The preferred recovery strategy is to install multiple fraction collectors in your LC systems and use the last of those fraction collectors for recovery. This recovery fraction collector can be selected in the Configuration dialog box as displayed in [Figure 41](#). The fourth fraction collector in your systems will always be used for sample recovery. Also read “[Configuring Multiple Fraction Collectors](#)” on page 44.

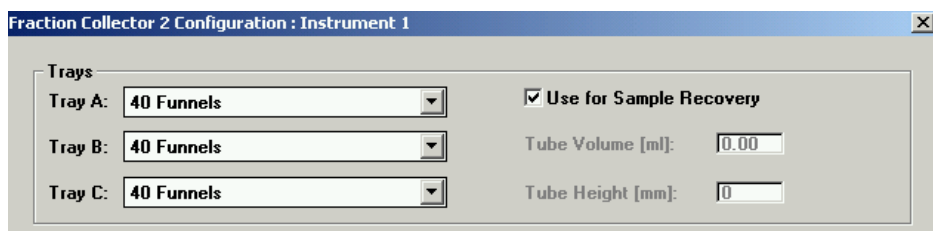


Figure 41 Sample Recovery Fraction Collector

- For the following tray configurations fixed recovery location will be assigned from the ChemStation. In order to disable the recovery the tray configuration has to be changed.
 - 1 Standard tray for two well plates + 10 2ml x 2 ml vials (G2258-60011) and half tray for 40 x 2 ml vials (G1313-44512).**
In this configuration the forty 2 ml vials on the half will automatically be used for recovery.
 - 2 Standard tray for two well plates + 10 x 2ml vials (G2258-60011) and half tray for 15x 6 ml vials (G1313-44513).**
In this configuration the fifteen 6 ml vials on the half will automatically be used for recovery.
 - 3 Standard tray for two well plates + 10 x 2ml vials (G2258-60011) and half tray with 40 funnels (G1364-84532).**
In this configuration the funnels on the half will automatically be used for recovery.
- The **Standard tray for two well plates + 10 funnels (G1364-84522)** allows two modes of operation.

2 Configuration and Operation of the Fraction Collector Special Applications

- 1 If well-plates are configured in the fraction collector configuration the (checkbox **Plate 1** or **Plate 2** are selected), the 10 funnel locations will automatically be used for recovery.
- 2 If the checkbox **Plate 1** and **Plate 2** are not selected, the 10 funnel position can be used for regular fraction collection.

CAUTION

With each start of a new sequence the recovery will start at the same positions. In order to avoid contamination the vessels that contain the recovery fractions have to be exchanged.

CAUTION

The number of recovery locations automatically defines the maximum number of injections. When using the standard tray for two well plates and 10 funnels, only ten injections per sequence are executed.

Semi-preparative Operation

The analytical-scale fraction collector G1364C can be modified for semi-preparative operation. This allows to use of external collection vessels through funnels at high flow rates above 10 ml/min. The maximum flow rate depends on the viscosity of the solvent.

In order to modify the analytical fraction collector the following parts have to be installed:

- 20 mm needle assembly (G1364-87202)
- preparative tubing kit (G1364-68711)
- internal tray preparative scale (G1364-63123)

The procedures to exchange these parts are described in [“Replacing the Inlet/Waste Tubings”](#) on page 135, [“Replacing the Valve to Needle Tubing”](#) on page 139, [“Exchanging the Analytical Needle Assembly”](#) on page 145, and [“Exchanging the Internal Tray”](#) on page 150.

In addition the wellplate adapter has to be removed for operation with the shorter needle.

In the ChemStation Software the new configuration has to be specified in order to consider the new volumes for the delay calibration. In the **Configuration** dialog box of the fraction collector select **More**. Then change the **Inner Capillary Diameter** to 0.8 mm as well as the **Needle Type** to semi-prep needle as displayed in [Figure 42](#) on page 111. For further information on delay volumes and delay volume calibration see “[Delay Volumes and Delay Calibration](#)” on page 46.

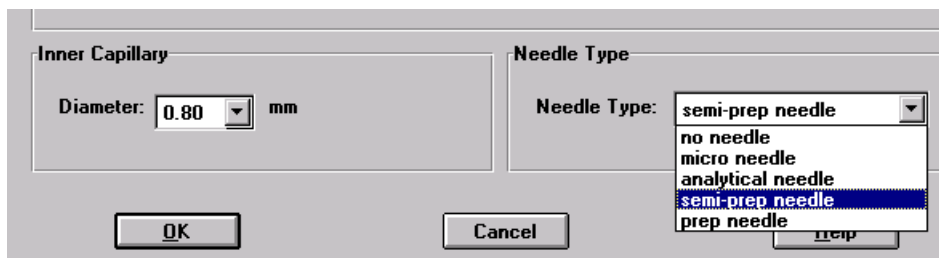


Figure 42 Fraction collector configuration parameters for semi-prep operation

Using High Test Tubes in the Analytical Scale Fraction Collector

In the standard configuration the analytical-scale fraction collector G1364C the maximum test tube height is limited to 48 mm. This limitation can be extended to 75 mm if

- the standard 50 mm needle assembly (G1367-87200) is replaced by the 20 mm needle assembly (G1364-87202)
- the wellplate adapter is removed.

Optimizing Fraction Collection

- Time-based fraction collection**
 - *Time slices* must have a length of at least 0.05 min.
 - Set # of *Fractions* such that length of resulting fractions is at least 0.05 min.
- Peak-based fraction collection**
 - Set threshold and slope values such that length of fractions is at least 0.05 min.
 - Unresolved peaks can be separated using appropriate threshold and slope values. If two unresolved peaks are to be collected as one fraction, collect based on threshold only.
 - If the baseline of the chromatogram is below or above 0 mAU, this offset is not accounted for when triggering peaks using a threshold value. The threshold value is always added to 0 mAU.

Limitations and How to Avoid Problems

- Rinse Fraction Collection Needle** If *Rinse Fraction Collection Needle* is set to *Between fraction collection*, at least 0.3 min are required to perform this task. When doing time-based fraction collection rinsing the needle is only possible between two time table entries, which must have a gap of at least 0.3 min. For peak-based fraction collection a time gap of also at least 0.3 min is required. If a new peak is detected during the rinse process, it is aborted and the needle moves back to the next free fraction position. Depending on flow rate and delay volume V_{D1} the beginning of this peak may be lost.
- If you have recovery positions in your fraction collector or if you are using one fraction collector for sample recovery in a multiple fraction collector configuration, the rinse function **between fraction collection** is ignored.
- Needle Movement** The option **into location** under **Needle Movement** in the fraction collector configuration must only be used for capped 2 or 5 ml vials or well-plates. Using other or open vials with this command can lead to a **Movement failed** error.
- Replacing fraction containers** When replacing filled tubes, vials or well-plates from the fraction collector make sure to remove and re-insert the complete tray. Otherwise the fraction collector will not recognize that the fraction containers were emptied.
- Pooling**
- When pooling fractions, overflow protection no longer exists. It is the user's responsibility to make sure that all fraction collection locations are large enough to completely collect all pooled fractions. If a fraction collection location is overfilled, an error message occurs and the pump is shut OFF.

Check-out Procedures

The purpose of the check-out injection is the verification of the correct installation and configuration of the Agilent 1260 Infinity Purification System. In addition the checkout procedure can be used for troubleshooting, if the Agilent 1260 Infinity Fraction Collector doesn't operate as expected.

The checkout procedure depends on the flow range (analytical scale AS or preparative scale PS) and on the peak trigger (UV-based or mass-based).

In the following sections the check-out procedures for the UV based trigger are described. For the mass-based system refer to the Agilent 1260 Infinity Purification System User Manual G2262-90011.

Check-out Procedure for UV-triggered Fraction Collection AS

Prerequisites

- ✓ The Agilent 1260 Infinity LC System has been flushed thoroughly with the liquid phase as described in [Table 21](#) and the lamp(s) of the UV detector have been switched on for at least 15 minutes to provide a stable baseline.
- ✓ The Agilent 1260 Infinity Fraction Collector has been installed and configured. The delay volume has been calibrated as described in [“Performing a Delay Calibration with an UV Detector”](#) on page 51.
- ✓ The 40-vial tray (G1313-44512) with at least four 2ml vials is installed.
- ✓ All wells are empty and the fill levels, which are stored on the module, have been reset. To verify this remove and insert the 40-vial tray. Then click on **Yes** in displayed dialog box.

Table 21 Method Parameters for UV-triggered Fraction Collection Check-out AS

Agilent 1260 Infinity modules	<ul style="list-style-type: none"> • standard or micro degasser • isocratic, quaternary or binary pump • autosampler/ well plate sampler • column compartment • diode array detector, multi-wavelength detector or variable wavelength detector with analytical flow cell • fraction collector analytical scale
Column	ZORBAX Eclipse XDB-C18, 5 µm, 150 x 4.6mm (993967-906) or equivalent
Sample	Check-out sample (01080-68704)
Flow	0.8 ml/min
Stoptime	6 minutes
Solvent A	10% (HPLC grade water)
Solvent B	90% (HPLC grade acetonitrile)
Wavelength VWD	254 nm
Wavelength DAD/MWD	Signal A: 254/16nm, Reference: 360/80nm
Peakwidth (Responsetime)	> 0.1min (2s)
Injection volume	1 µl
Column temperature	40.0 °C
Fraction trigger mode	Peak-based
Max. peak duration	0.5 min
DAD/MWD working mode	Threshold/slope
Upslope	5 mAU/s
Downslope	10 mAU/s
Threshold/Upper threshold	10 mAU/ 3000 mAU

2 Configuration and Operation of the Fraction Collector Check-out Procedures

Expected Result

Run a single injection under the conditions as described in Table 21. You should observe a chromatogram with four peaks as displayed in Figure 43 on page 116. Four fractions should have been collected in the fraction collector vials.

NOTE

The retention times of the peaks could be different depending on the system configuration (e.g. flow cell type, capillary volumes, type and age of column).

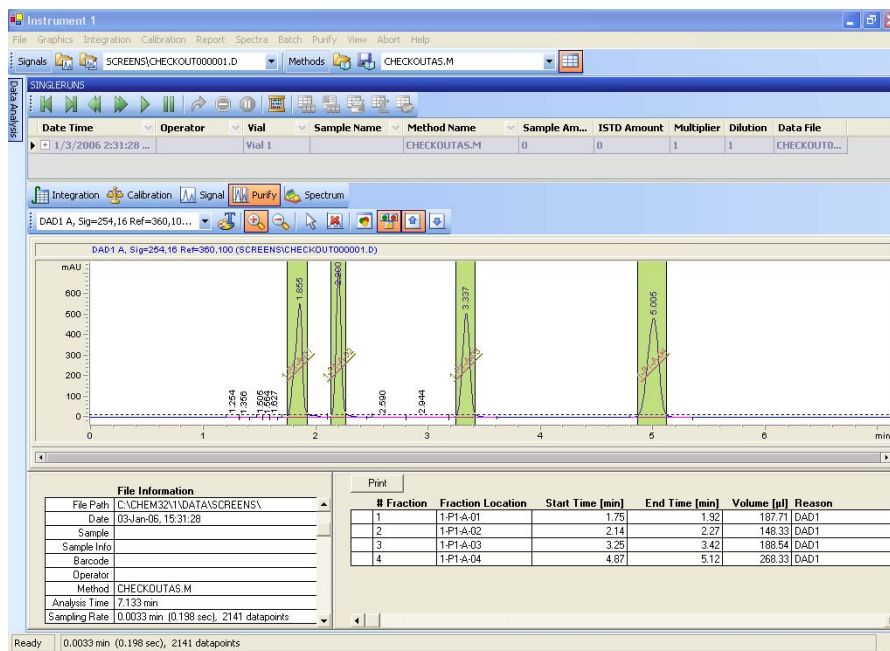


Figure 43 Chromatogram of the check-out injection with UV-based trigger

Check-out Procedure for UV-triggered Fraction Collection PS

Prerequisites

- ✓ The Agilent 1260 Infinity LC System has been flushed thoroughly with the liquid phase as described in [Table 21](#) and the lamp(s) of the UV detector have been switched on for at least 15 minutes to provide a stable baseline.
- ✓ The Agilent 1260 Infinity Fraction Collector has been installed and configured. The delay volume has been calibrated as described in [“Performing a Delay Calibration with an UV Detector”](#) on page 51.
- ✓ The 15-vial tray (G1313-44513) with at least four 6ml vials is installed.
- ✓ All wells are empty and the fill levels, which are stored on the module, have been reset. To verify this remove and insert the 15-vial tray. Then click on **Yes** in displayed dialog box.

Table 22 Method Parameters for UV-triggered Fraction Collection Check-out PS

Agilent 1260 Infinity modules	<ul style="list-style-type: none"> • preparative pump plus gradient extension (optional) • preparative autosampler/ dual-loop autosampler • diode array detector or multi-wavelength detector with preparative flow cell* • fraction collector preparative scale
Column	ZORBAX SB-C18, 50 mm x 9.46mm (846975-202) or equivalent
Sample	Check-out sample (01080-68704)
Flow	10 ml/min
Stoptime	4 minutes
Solvent A	35% (HPLC grade water)
Solvent B	65% (HPLC grade acetonitrile)
Wavelength DAD/MWD	Signal A: 254/16nm, Reference: 360/80nm
Peakwidth (Responsetime)	> 0.1 min (2s)
Injection volume	50 µl
Column temperature	ambient
Fraction trigger mode	Peak-based

2 Configuration and Operation of the Fraction Collector

Check-out Procedures

Table 22 Method Parameters for UV-triggered Fraction Collection Check-out PS

Max. peak duration	0.5 min
DAD/MWD working mode	Threshold/slope
Upslope	5 mAU/s
Downslope	10 mAU/s
Threshold/Upper threshold	10 mAU/ 3000 mAU

* The checkout method is tailored for the 3mm stainless steel flow cell G1315-60016. For other preparative flow cells some parameters (e.g. flow rate, injection volume, threshold/slope), should be adjusted.

Expected Result

Run a single injection under the conditions as described in [Table 22](#). You should observe a chromatogram with four peaks as displayed in [Figure 43](#) on page 116. Four fractions should have been collected in the fraction collector vials.

NOTE

The retention times of the peaks could be different depending on the system configuration (e.g. flow cell type, capillary volumes, type and age of column).

Application Notes

More information about the Agilent 1260 Infinity Fraction Collectors and Purification Systems are available from a series of application notes. Printed versions can be ordered from Agilent or pdf-files can be downloaded from the Agilent Website

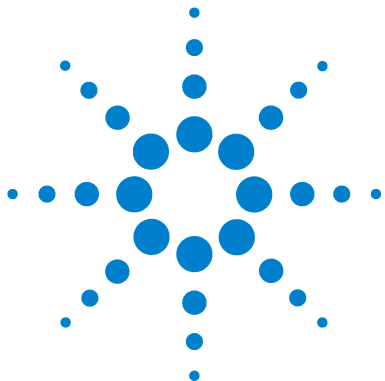
<http://www.chem.agilent.com>

Table 23 Selected Agilent Technologies Application Notes

Title	Publication Number
An optimized system for analytical and preparative work	5988-9649EN
Recovery collection with the Agilent LC purification system	5988-9650EN
Solutions for preparative HPLC-Application Compendium	5988-9646EN
Innovative fraction collection with the Agilent LC purification platform	5988-9250EN
Automated fraction re-analysis - does it really make sense?	5988-8653EN
Injection of high-concentration samples with the Agilent LC purification system	5988-8654EN
Sophisticated peak-based fraction collection - working with up and down slope	5988-7895EN
Strategies for purification of compounds from non-baseline separated peaks	5988-7460EN
Method scale-up from analytical to preparative scale with the Agilent LC purification system PS	5988-6979EN
Peak-based fraction collection with the Agilent LC purification system AS - Influence of delay volume on recovery	5988-5747EN

2 Configuration and Operation of the Fraction Collector

Application Notes



3 Troubleshooting and Test Functions

Status Indicators	123
Maintenance Functions	125
Maintenance Functions	125
Transport Alignment	127
Step Commands	129



Status Indicators

The Fraction Collector is provided with two status indicators which indicate the operational state (prerun, not ready, run, and error states) of the instrument. The status indicators provide a quick visual check of the operation of the Fraction Collector (see [“Status Indicators”](#) on page 123).

Maintenance Functions

The maintenance functions position the transport unit and needle carrier assembly on certain positions for maintenance, homing, parking or delay calibration (see [“Maintenance Functions”](#) on page 125).

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.

Status Indicators

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

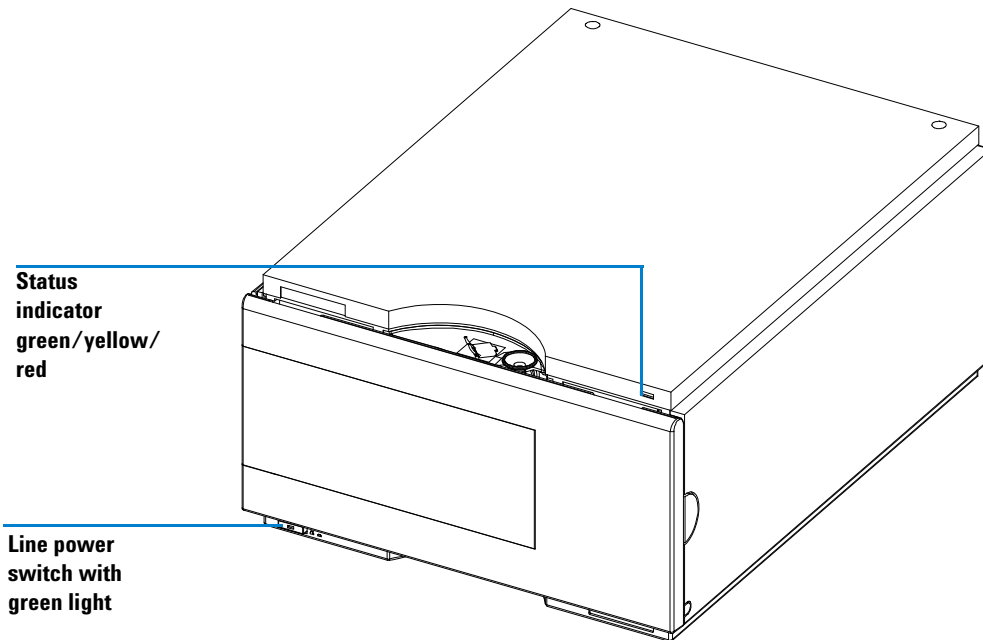


Figure 44 Location of Status Indicators

Power Supply Indicator

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

Instrument Status Indicator

The instrument status indicator indicates one of four possible instrument conditions:

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

Maintenance Functions

Some maintenance procedures require the needle arm, 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 ChemStation the fraction collector maintenance positions can be selected from the **Maintenance menu** in the **Diagnosis** view (see [Figure 45](#)). In the Agilent Instant Pilot G4208A the functions can be selected in the **Test** screens of the fraction collector.



Figure 45 Fraction Collector Maintenance Positions dialog box

Change Parts.

If you click **Start** the transport unit will move upwards, the needle carrier assembly will move to the front center and then turn OFF the theta motor to allow free rotation of the theta arm. This position enables easy access to the transport unit to change:

3 Troubleshooting and Test Functions

Maintenance Functions

- The needle assembly
- The needle carrier assembly
- The valve to needle tubing.
- The diverter valve.

After the maintenance or repair task has been finished, click **End** to move the transport assembly to the **Home** position

Home Position

This maintenance function moves the arm up and to the right rear for better access and exchange of the trays.

Park Arm

This maintenance position moves the arm to the park position at the upper rear left side of the tray for transporting or shipping the Fraction Collector.

Transport Alignment

The transport alignment is required to compensate for larger deviations in positioning the needle carrier assembly.

When is a Transport Alignment Necessary?

The transport alignment is required after disassembling the module or when you exchange:

- the transport unit,
- the needle/capillary carrier assembly,
- the MTP main board.

The transport alignment is a function in Lab Advisor B.02.04 or higher.

This function is also available in the **Diagnose** screen of the ChemStation (Rev. B.xx.xx) and with the Agilent Instant Pilot G4208A.

CAUTION

The transport alignment requires the 4-wellplate tray (G1364-84501) to be installed, but wellplates MUST NOT be installed.

If the transport alignment is started with wellplates on the tray, the alignment procedure aborts WITHOUT error message.

How to perform a Transport Alignment?

Steps	Comments
1 If the transport unit has been exchanged or if it is very badly misaligned, set the 8-bit configuration switch to the Forced Cold Start Configuration.	For details see: "Forced Cold Start" in the Fraction Collectors Service Manual.
2 Install the 4-wellplate tray (G1364-84501)	IMPORTANT: Remove all wellplates!
3 Ensure that the wellplate adapter is correctly assembled	On a G1364B preparative-scale fraction collector install the delay calibration adapter G1364-87301

3 Troubleshooting and Test Functions

Transport Alignment

How to perform a Transport Alignment?

Steps	Comments
4 Select the Maintenance menu in the Diagnosis view of Agilent ChemStation (Rev. B.xx.xx).	
5 Choose Fraction Collector > Transport Alignment... to start the automated procedure.	The Transport Alignment procedure takes approximately 10-15 minutes
6 Set the 8-bit configuration switch to the default setting.	See "8-bit configuration switch" in the Fraction Collectors Service Manual.

Step Commands

Some movements of the fraction collection sequence can be done under **Manual control**. This is useful during troubleshooting where close observation of each of the fraction collection step is required to confirm a specific failure mode or verify successful completion of a repair.

Each step command actually consists of a series of individual commands which move the Fraction Collector components to predefined positions enabling the specific step to be done.

In the ChemStation the step commands can be selected from the “**Test Selection Box**” (see [Figure 46](#)) in the **Diagnosis** display. In the Agilent Instant Pilot G4208A the step commands can be accessed from the pull-down menu in the Fraction Collector “**Test**”.

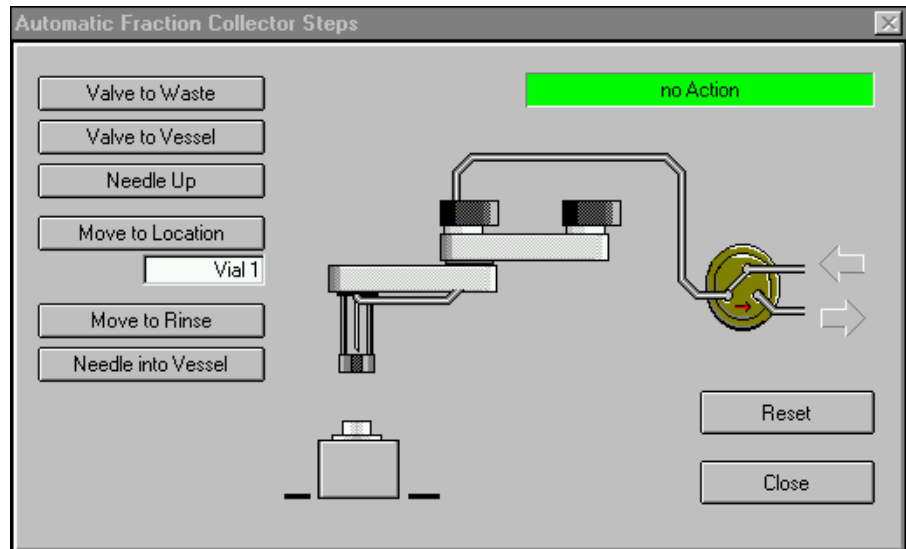


Figure 46 Fraction Collector Step Commands

3 Troubleshooting and Test Functions

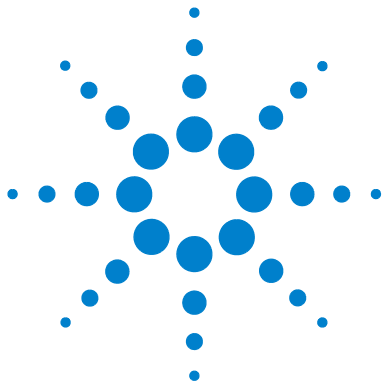
Step Commands

Table 24 Step Commands

Step	Action	Comments
Needle Up	Lifts the needle arm to the upper position.	Command also switches the diverter valve to waste if it is not already in that position.
Needle into vessel	Lowers the needle into the specified vessel.	only analytical-scale G1364C
Needle to rinse / flush port	Moves the needle to the rinse / flush port.	only analytical scale G1364C
Switch valve to needle	Switches the diverter valve to the needle.	
Switch valve to waste	Switches the diverter valve from needle to waste.	

Troubleshooting

If the Fraction Collector is unable to perform a specific step due to a hardware failure, an error message is generated. You can use the step commands to perform a fraction collection sequence, and observe how the Fraction Collector responds to each command.



4 Repairing the Fraction Collector

Cleaning the Fraction Collector [132](#)

Overview of Main Repair Procedures [133](#)

Simple Repairs [134](#)



Introduction into Repairing the Fraction Collector

Simple Repairs

The Fraction Collector is designed for easy repair. The most frequent repairs such as changing a needle assembly or tubings can be done from the front of the instrument with the instrument in place in the system stack. These repairs are described in “Simple Repairs” on page 134.

WARNING

When opening capillary or tube fittings solvents may leak out. Please observe appropriate safety procedures (for example, goggles, safety gloves and protective clothing) as described in the material handling and safety data sheet supplied by the solvent vendor, especially when toxic or hazardous solvents are used.

WARNING

To avoid personal injury, keep fingers away from the needle area during Fraction Collector operation. Do not bend the safety flap away from its position, or attempt to insert or remove a vial from the gripper when the gripper is positioned below the needle.

WARNING

Regularly inspect the inlet / waste tubing assembly, and the valve to needle tubing and exchange them if they are worn out or show visible signs of damage.

Cleaning the Fraction Collector

The Fraction Collector covers should be kept clean. Cleaning should be done with a soft cloth slightly dampened with water or a solution of water and a mild detergent. Do not use an excessively damp cloth from which liquid could drip into the Fraction Collector.

WARNING

Do not let liquid drip into the Fraction Collector. It could cause a shock hazard or damage to the Fraction Collector.

Overview of Main Repair Procedures

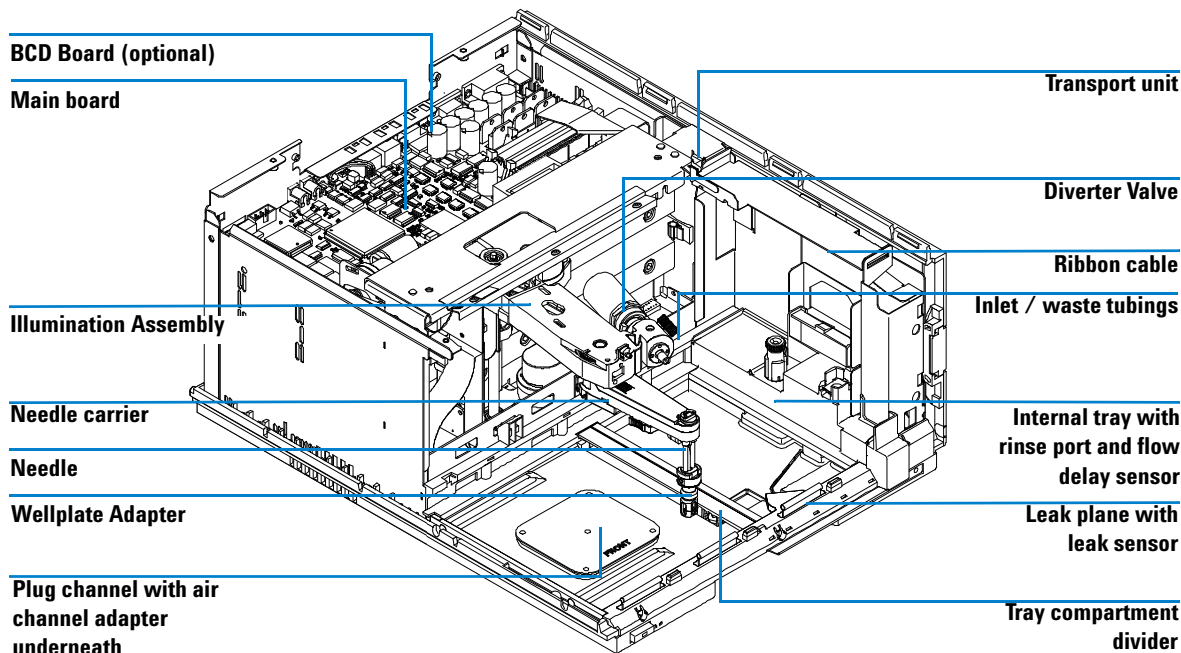


Figure 47 Main Assemblies (Example displays the analytical-scale module G1364C. The preparative-scale fraction collector G1364B contains a different needle)

Simple Repairs

The procedures described in this section can be done with the Fraction Collector in place in the stack. These procedures can be done on a more frequent basis.

Table 25 Simple Repair Procedures

Procedure	Typical Frequency	Notes
Replacing the Inlet / waste tubings	When worn out, when showing visual signs of damage, typically once per year.	See “Replacing the Inlet/Waste Tubings” on page 135
Replacing the valve to needle tubing	When worn out, when showing visual signs of damage, typically once per year	See “Replacing the Valve to Needle Tubing” on page 139
Exchanging the preparative needle assembly	When needle shows indication of damage or blockage	See “Exchanging the Preparative Needle Assembly” on page 143
Exchanging the analytical needle assembly	When needle shows indication of damage or blockage Or when using the short needle assembly for operation with high test tubes (>45mm)	See “Exchanging the Analytical Needle Assembly” on page 145
Exchanging the diverter valve	When defective (internal / external leak, valve not switching any more)	See “Exchanging the Diverter Valve” on page 147
Exchanging the internal tray	When flow delay sensor defective	See “Exchanging the Internal Tray” on page 150
Repairing or exchanging a funnel of the internal tray or funnel tray	When defective (leaky, blocked or contaminated)	See “Repairing or Exchanging a Funnel of the Internal Tray” on page 152

Replacing the Inlet/Waste Tubings

Frequency	When contaminated, worn out or visibly damaged Typically once every year
Tools required	None
Parts Required	Inlet / waste tubing assembly in tubing kit preparative scale 0.8 mm ID , PN G1364-68711, analytical scale 0.25 mm ID , PN G1364-68712 or analytical-scale 0.15 mm ID , G1364-68723

WARNING

To avoid personal injury, keep fingers away from the needle area during Fraction Collector operation.

WARNING

Follow the described installation procedure exactly to maximize the lifetime of the inlet / waste tubings and to avoid potential spills or fraction losses. Regularly inspect the tubings and exchange them if they are worn out or show visible signs of damage.

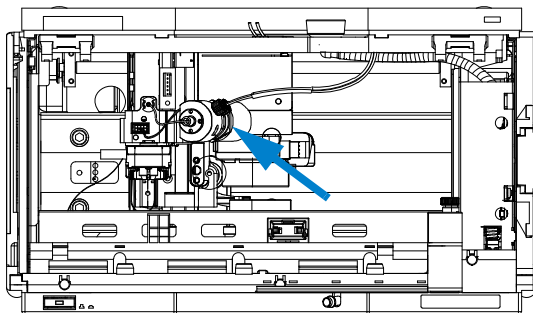
4 Repairing the Fraction Collector

Simple Repairs

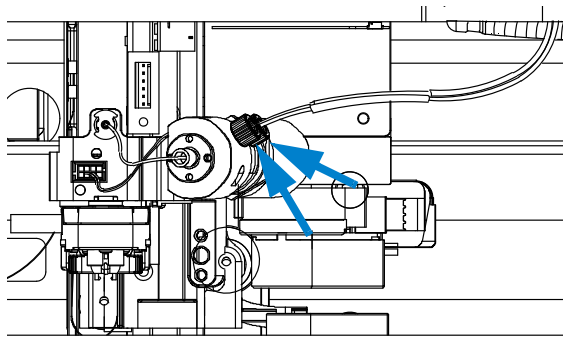
Before beginning this procedure:

- Position the transport unit of the Fraction Collector in the “**Home Position**” (see “**Maintenance Functions**” on page 125).
- Remove all installed trays from the tray base.
- Position the transport unit of the Fraction Collector in the “**Change Parts Position**” (see “**Maintenance Functions**” on page 125).
- Turn OFF the instrument.
- Remove the rear end of the Fraction Collector’s waste tubing from the waste container, unscrew the front end of the Fraction Collector’s inlet tubing from the flow cell of the detector.

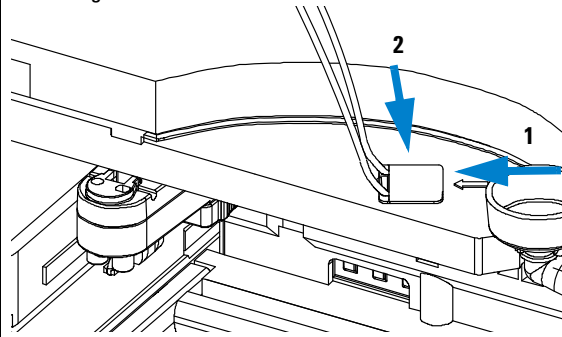
- 1** Locate the diverter valve with the finger-tight fittings of the inlet / waste tubing assembly (the figure shows the open Fraction Collector seen from the front).



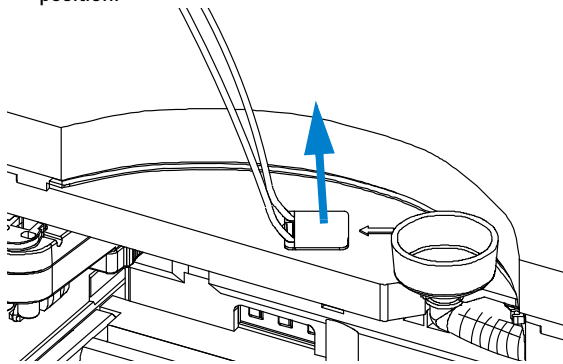
- 2** Unscrew the 2 finger-tight fittings of the inlet / waste tubing assembly at the diverter valve.



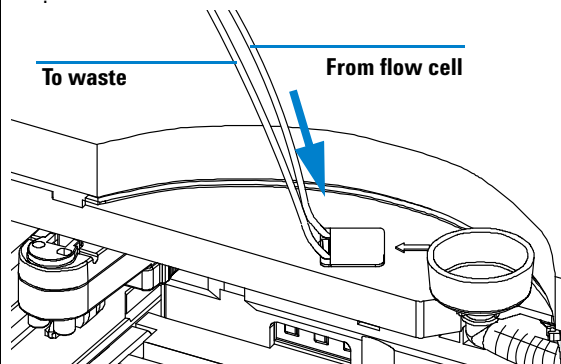
- 3** Unplug the inlet / waste tubing assembly from the bolt carrier (located on the top center of the front cover) by moving the snapper as indicated by the arrows. Slide the tubings out to the bottom.



4 Install the new inlet / waste tubing assembly into the bolt carrier as shown below. Slide in the long ends of the tubings from bottom to top and let the snapper click into position.

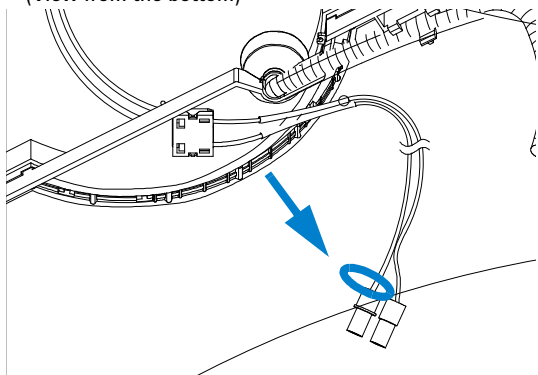


5 The rear tubing (with the label with the arrow) must be connected to the flow cell of the detector. The front tubing must be inserted into the waste container

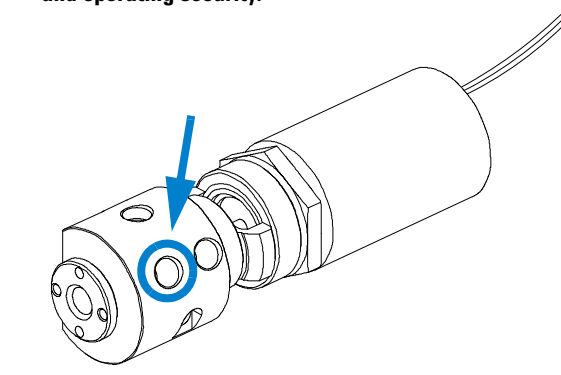


6 Connect the finger-tight fittings of the inlet / waste tubing assembly to the ports of the diverter valve.

IMPORTANT: The tubings must not be bent up- or downwards. The cables must not be twisted.
(View from the bottom)



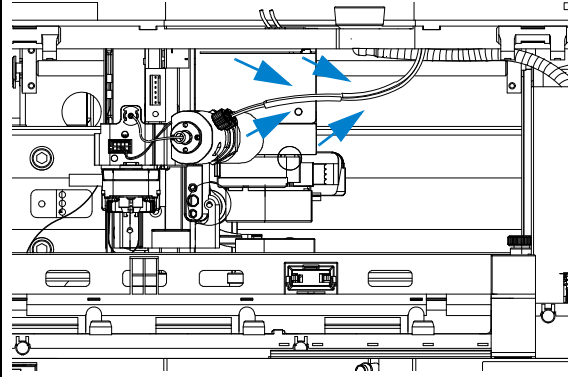
7 A color coded ring on one of the tubings and the valve body indicates, which cable belongs to which port.
IMPORTANT: It is absolutely vital to connect these tubings as described, in order to maximize their lifetime and operating security.



4 Repairing the Fraction Collector

Simple Repairs

8 IMPORTANT: The cables must run into the ports of the diverter valve in lines parallel to the horizon.



On completion of this procedure:

- Re-install the tray(s) in the tray base.
- Start the instrument.
- Close the front cover.

Replacing the Valve to Needle Tubing

Frequency	When contaminated, worn out or visibly damaged Typically once every year
Tools required	Wrench, open end, 4mm, PN 8710-1534 (supplied in accessory kit) Wrench, open end, 1/4 – 5/16 inch, PN 8710-0510 (supplied in accessory kit)
Parts Required	Valve to needle tubing assembly included in tubing kit preparative scale 0.8 mm ID , PN G1364-68711, analytical scale 0.25 mm ID , PN G1364-68712 or analytical scale 0.15 mm ID , G1364-68723

WARNING

To avoid personal injury, keep fingers away from the needle area during Fraction Collector operation.

WARNING

Follow the described installation procedure exactly to maximize the lifetime of the valve to needle tubing and to avoid potential spills or fraction losses. Regularly inspect the tubings and exchange them if they are worn out or show visible signs of damage.

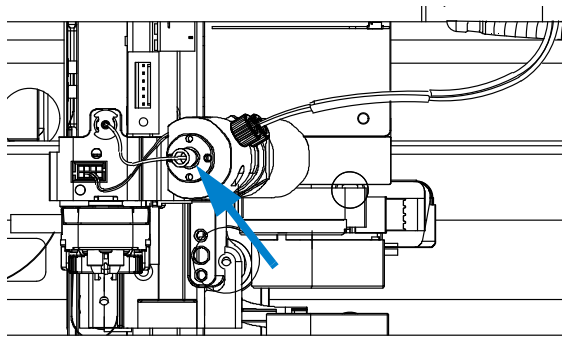
4 Repairing the Fraction Collector

Simple Repairs

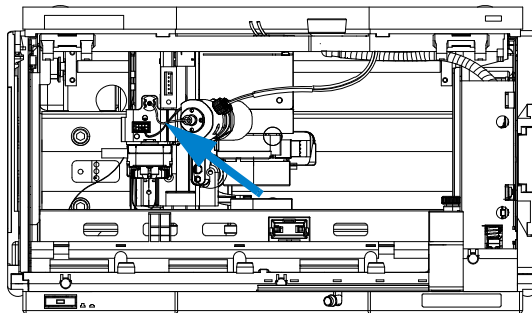
Before beginning this procedure:

- Position the transport unit of the Fraction Collector in the “**Home Position**” (see “**Maintenance Functions**” on page 125).
- Remove all installed trays from the tray base.
- Position the transport unit of the Fraction Collector in the “**Change Parts Position**” (see “**Maintenance Functions**” on page 125) and turn off the instrument.
- It might be more convenient to remove the needle from its carrier before unscrewing the needle tubing.

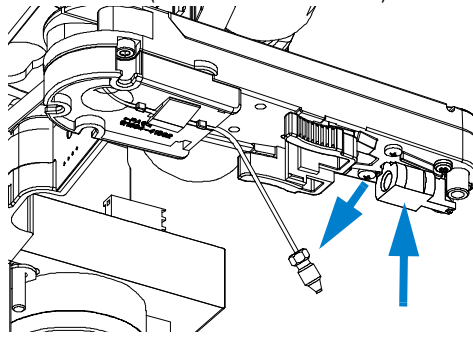
2 Unscrew the finger-tight fitting of the valve to needle tubing assembly at the diverter valve.



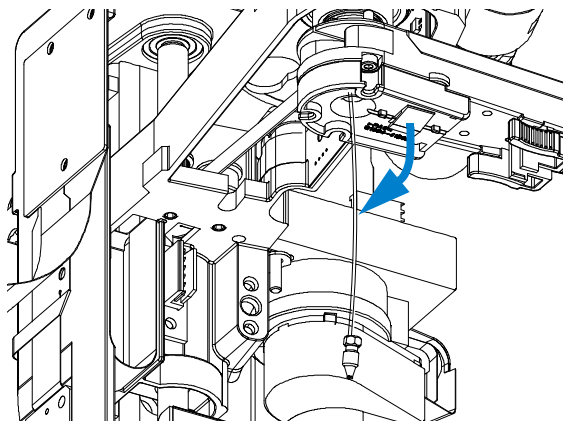
1 Locate the diverter valve with the finger-tight fittings of the valve to needle tubing assembly (the figure shows the open Fraction Collector seen from the front).



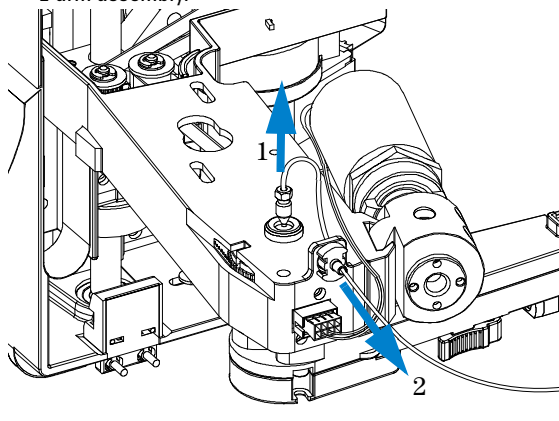
3 Using the 4 mm wrench and the 5/16" wrench for counter-holding unscrew the valve to needle tubing from the needle. (Viewed from the bottom)



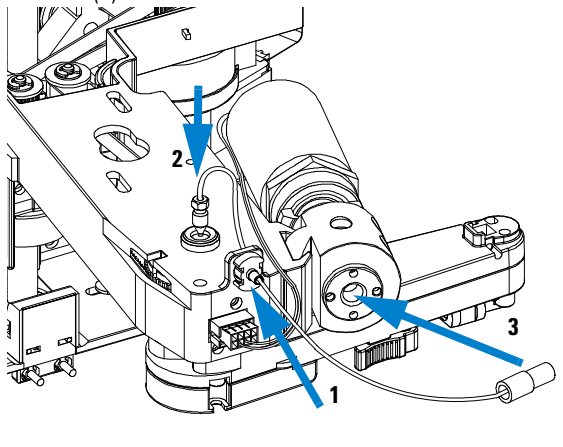
4 Un-clip the tubing from the bottom of the needle carrier assembly.



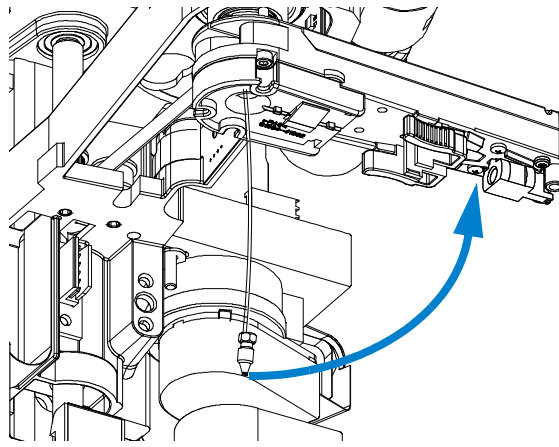
5 Slide the tubing through the hole in the needle carrier assembly (from bottom to top) and out of the holder in the z-arm assembly.



6 Install the new valve to needle tubing assembly by clipping it in to the holder in the z-arm assembly (1), **Important!!!** and slide it through the hole in the z-arm (2) and out on the bottom of the needle carrier assembly (top to bottom). Screw the finger-tight fitting into the port of the diverter valve (3).



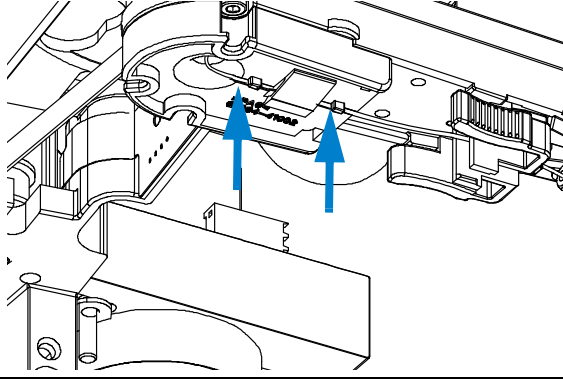
7 Using the 4 mm wrench and the 5/16" wrench for counter-holding connect the valve to needle tubing to the needle. (Viewed from the bottom)



4 Repairing the Fraction Collector

Simple Repairs

8 IMPORTANT: After fixing the screw clip the tubing into the guide on the bottom of the needle carrier assembly. It is absolutely vital that the tubing is installed as described, to maximize the lifetime of the tubing.



On completion of this procedure:

- Re-install the needle to the needle carrier assembly, if you previously removed it. Make sure to slide the needle all the way to the front of the needle carrier assembly (clicks into position).
- Re-install the tray(s) in the tray base.
- Start the instrument.
- Close the front cover.

Exchanging the Preparative Needle Assembly

Frequency	When the needle is leaky or visibly damaged When the needle is blocked or contaminated
Tools required	Wrench, open end, 4mm, PN 8710-1534 (supplied in accessory kit) Wrench, open end, 1/4 – 5/16 inch, PN 8710-0510 (supplied in accessory kit)
Parts required	Preparative needle assembly, G1364-87201

WARNING

To avoid personal injury, keep fingers away from the needle area during Fraction Collector operation.

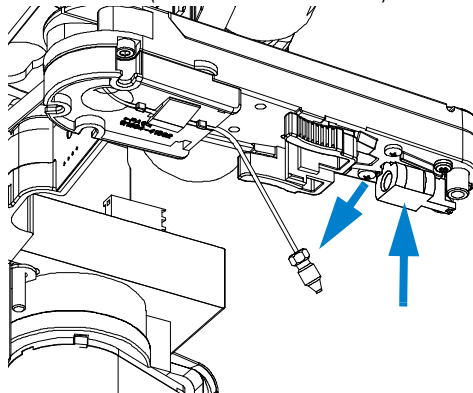
CAUTION

Regularly inspect the inlet / waste tubing assembly and the valve to needle tubing and exchange them if they are worn out or show visible signs of damage.

Before beginning this procedure:

- Position the transport unit of the Fraction Collector in the “Home Position” (see “[Maintenance Functions](#)” on page 125).
- Remove all installed trays from the tray base.
- Position the transport unit of the Fraction Collector in the “Change Parts Position” (see “[Maintenance Functions](#)” on page 125).
- Turn OFF the instrument.
- It might be more convenient to remove the needle from its carrier before unscrewing the needle tubing.

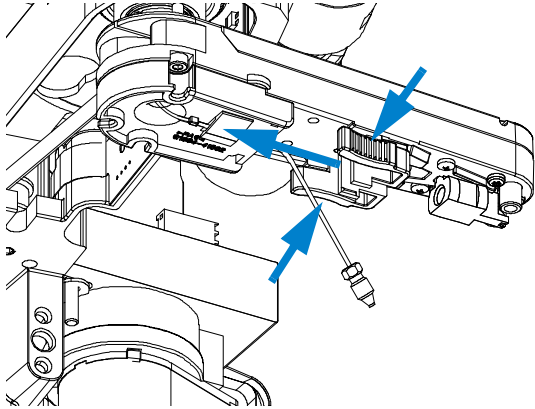
1 Using the 4 mm wrench and the 5/16” wrench for counter-holding unscrew the valve to needle tubing from the needle. (Viewed from the bottom)



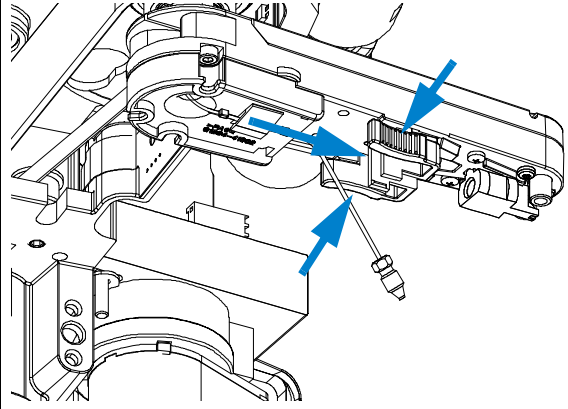
4 Repairing the Fraction Collector

Simple Repairs

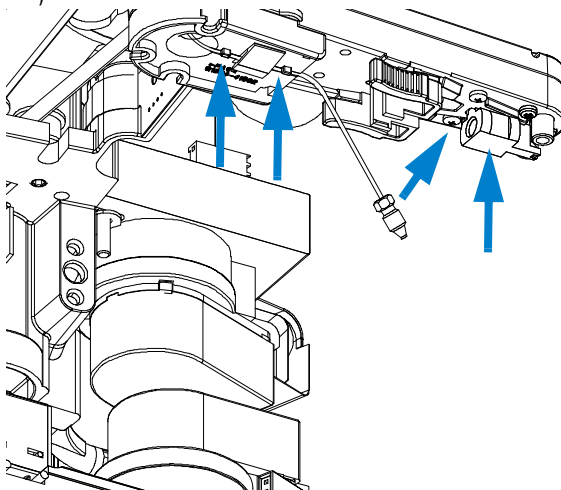
2 Holding the needle assembly between your thumb and forefinger, slide out the assembly towards the rear of the needle carrier assembly.



3 Insert the new needle assembly into the holder of the needle carrier assembly. Make sure to push it all the way to the front.



4 Using the 4 mm wrench and the 5/16" wrench for counter-holding connect the valve to needle tubing to the needle. (Viewed from the bottom)



On completion of this procedure:

- Re-install the needle to the needle carrier assembly, if you previously removed it. Make sure to slide the needle all the way to the front of the needle carrier assembly (clicks into position).
- **IMPORTANT:** After fixing the needle in the needle carrier clip the tubing into the guide on the bottom of the needle carrier assembly in case it slipped out of this guide (see 2 left arrows on the figure to the left).
It is absolutely vital that the tubing is installed as described, to maximize the lifetime of the tubing.
- Re-install the tray(s) in the tray base.
- Start the instrument.
- Close the front cover.

Exchanging the Analytical Needle Assembly

Frequency	When the needle is visibly damaged When the needle is blocked or contaminated
Tools required	Wrench, open end, 4mm, PN 8710-1534 (supplied in accessory kit) Wrench, open end, 1/4 – 5/16 inch, PN 8710-0510 (supplied in accessory kit)
Parts required	Analytical needle assembly, G1367-87200

WARNING

To avoid personal injury, keep fingers away from the needle area during Fraction Collector operation.

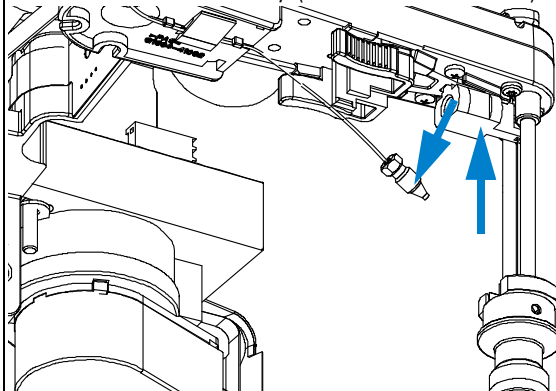
CAUTION

Regularly inspect the inlet / waste tubing assembly and the valve to needle tubing and exchange them if they are worn out or show visible signs of damage.

Before beginning this procedure:

- Position the transport unit of the Fraction Collector in the “Home Position” (see “[Maintenance Functions](#)” on page 125).
- Remove all installed trays from the tray base.
- Position the transport unit of the Fraction Collector in the “**Change Parts Position**” (see “[Maintenance Functions](#)” on page 125).
- Turn OFF the instrument.
- It might be more convenient to remove the needle from its carrier before unscrewing the needle tubing.

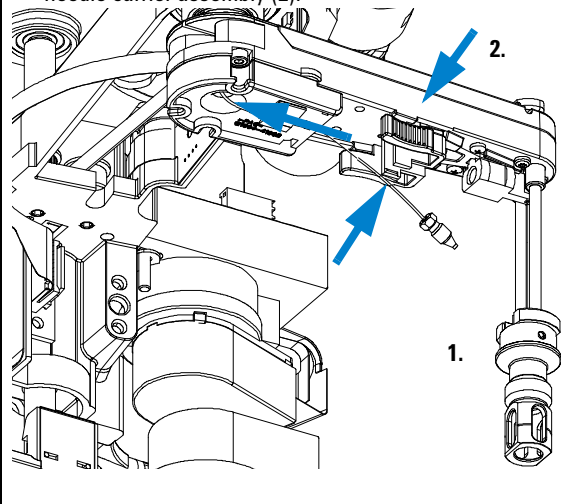
1 Use the 4 mm wrench and the 5/16” wrench for counter-holding to unscrew the valve-to-needle tubing from the needle assembly. (Viewed from the bottom)



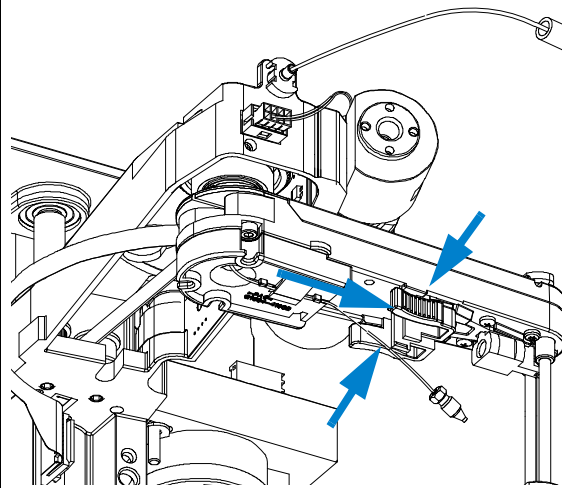
4 Repairing the Fraction Collector

Simple Repairs

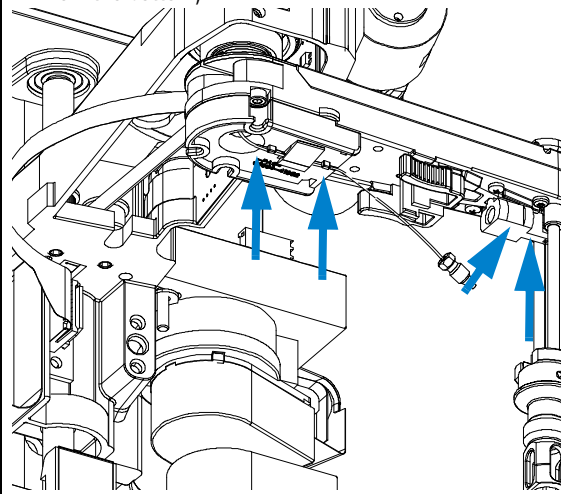
2 Remove the wellplate adapter by turning it clockwise (1). Hold the needle assembly between your thumb and forefinger, slide out the assembly towards the rear of the needle carrier assembly (2).



3 Insert the new needle assembly into the holder of the needle carrier assembly. Make sure to push it all the way to the front. Reassemble the wellplate adapter.



4 Use the 4 mm wrench and the 5/16" wrench for counter-holding to connect the valve to needle tubing to the needle. Reassembly the wellplate adapter. (Viewed from the bottom)



On completion of this procedure:

- **IMPORTANT:** After fixing the needle in the needle carrier clip the tubing into the guide on the bottom of the needle carrier assembly in case it slipped out of this guide (see 2 left arrows on the figure to the left). It is absolutely vital that the tubing is installed as described, to maximize the lifetime of the tubing.
- Re-install the tray(s) in the tray base.
- Start the instrument.
- Close the front cover.

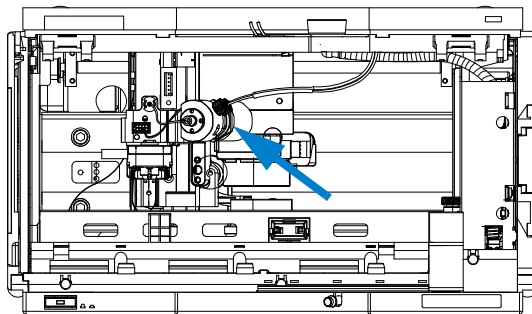
Exchanging the Diverter Valve

Frequency	When leaky or defective
Tools required	Hex key 2.0 mm, PN 8710-2438 (supplied in accessory kit)
Parts required	Diverter Valve, PN G1364-61901

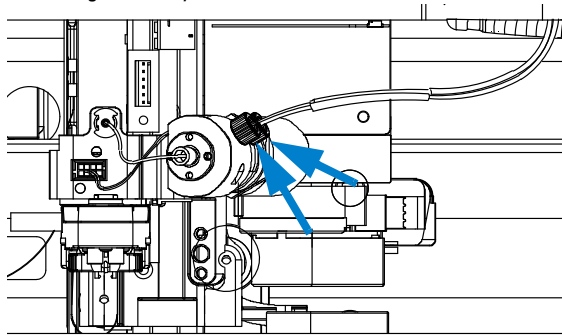
Before beginning this procedure:

- Position the transport unit of the Fraction Collector in the **“Home Position”** (see **“Maintenance Functions”** on page 125).
- Remove all installed trays from the tray base.
- Position the transport unit of the Fraction Collector in the **“Change Parts Position”** (see **“Maintenance Functions”** on page 125).
- Turn **OFF** the instrument.

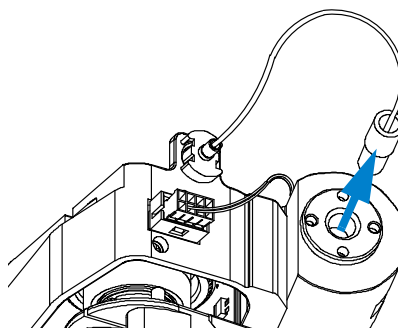
- 1** Locate the diverter valve with the finger tight fittings of the inlet / waste tubing assembly (the figure shows the open Fraction Collector seen from the front).



- 2** Unscrew the 2 finger tight fittings of the inlet / waste tubing assembly at the diverter valve.



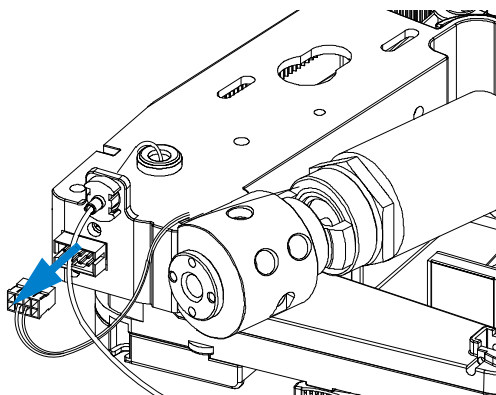
- 3** Unscrew the finger tight fitting of the valve to needle tubing assembly at the diverter valve.



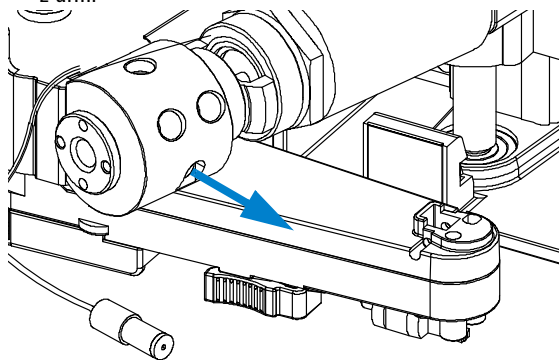
4 Repairing the Fraction Collector

Simple Repairs

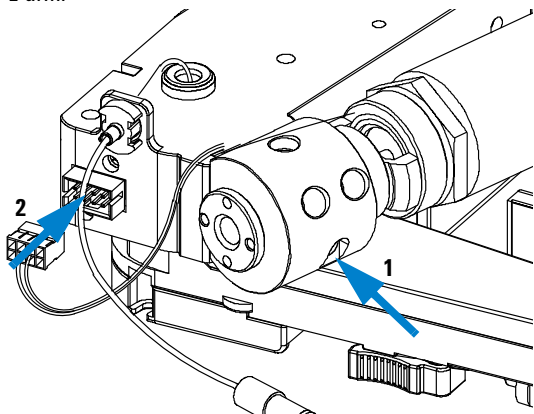
4 Disconnect the power cable of the diverter valve from the connector on the front of the z-arm



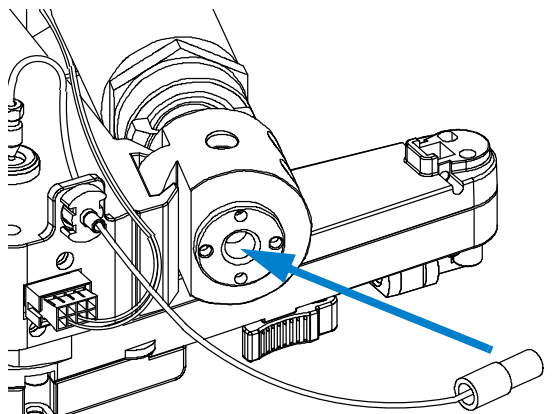
5 Using the 2 mm hex key loosen the holding screw of the diverter valve and remove the diverter valve from the z-arm.



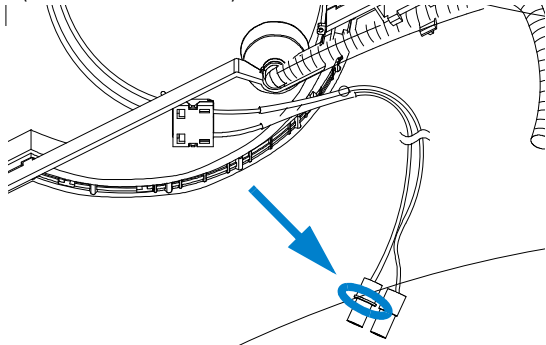
6 Install the new diverter valve to the z-arm with its holding screw. Plug in the new diverter valve power cable to the z-arm.



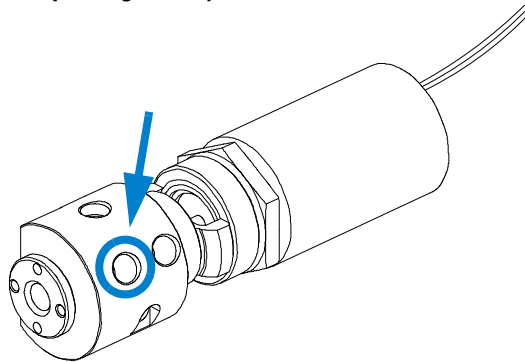
7 Install the valve to needle tubing at the new diverter valve.



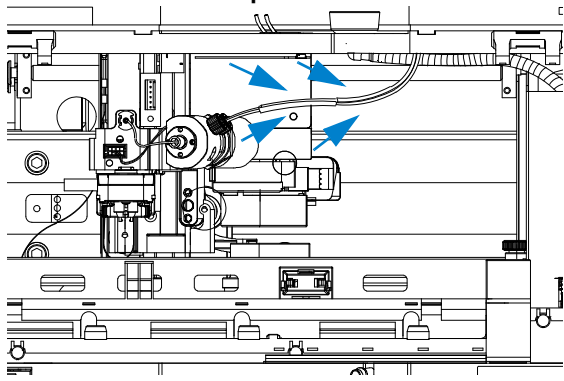
8 Connect the finger tight fittings of the inlet / waste tubing assembly to the ports of the diverter valve.
IMPORTANT: The tubings must not be bent up- or downwards. The cables must not be twisted.
(View from the bottom)



9 A color coded ring on one of the tubings and the valve body indicates, which cable belongs to which port.
IMPORTANT: It is absolutely vital to connect these tubings as described, in order to maximize their lifetime and operating security.



10 IMPORTANT: The cables must run into the ports of the diverter valve in lines parallel to the horizon.



On completion of this procedure:

- Re-install the tray(s) in the tray base.
- Start the instrument.
- Close the front cover.

4 Repairing the Fraction Collector

Simple Repairs

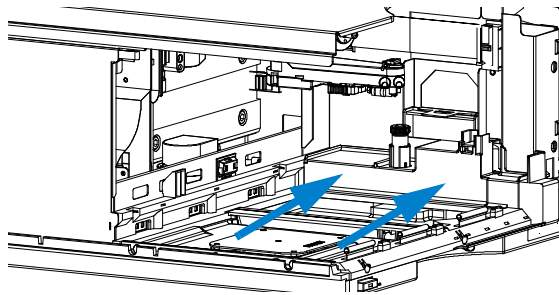
Exchanging the Internal Tray

Frequency	When defective
Tools required	None
Parts required	Internal tray analytical scale , (G1364-63124) Internal tray preparative scale ,(G1364-63123)

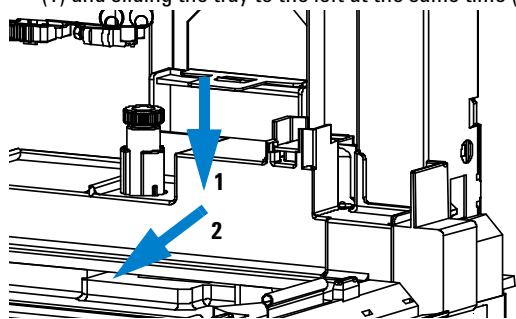
Before beginning this procedure:

- Position the transport unit of the Fraction Collector in the “**Home Position**” (see “[Maintenance Functions](#)” on page 125).
- Remove all installed trays from the tray base.
- Turn OFF the instrument.

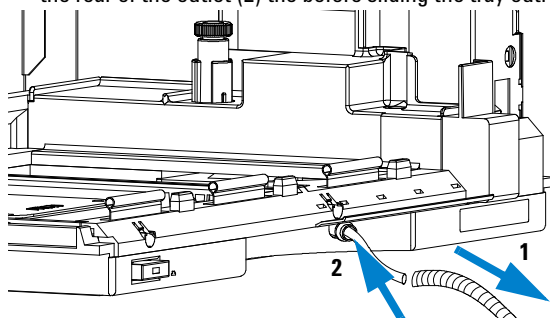
- 1 Locate the internal tray assembly with the rinse funnel and flow delay sensor in the bottom of the right front corner of the instrument.



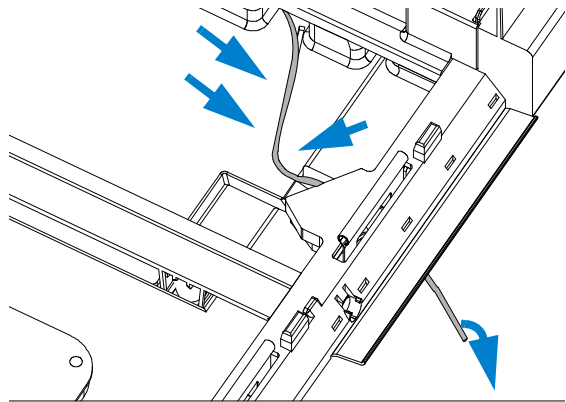
2 Remove the internal tray by pushing down the plastic holder that holds it in position underneath the metal latch (1) and sliding the tray to the left at the same time (2).



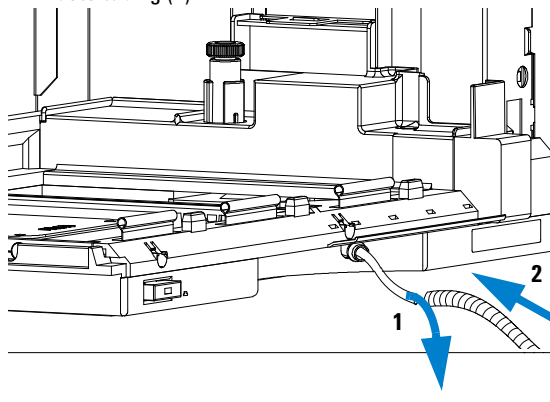
3 Remove the corrugated waste tubing from the front of the instrument (1) and slide the internal tray's waste tubing to the rear of the outlet (2) before sliding the tray out.



4 Install the new tray by sliding it into position underneath the metal latch that holds it. The waste tubing from the internal tray should be guided as shown.



5 Make sure that the waste tubing is slid all the way through the outlet (1). Its end should be over the edge and below the level of the laboratory desk that the system stands on to avoid any back flow of solvent. Re-install the corrugated waste tubing (2).



On completion of this procedure:

- Re-install the tray(s) in the tray base.
- Start the instrument.
- Close the front cover.

4 Repairing the Fraction Collector

Simple Repairs

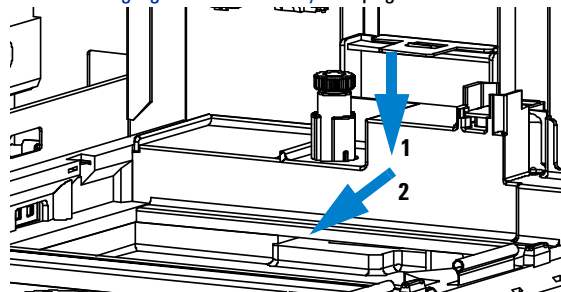
Repairing or Exchanging a Funnel of the Internal Tray

Frequency	When leaky or contaminated
Tools required	None
Parts required	Funnel assembly, waste tubing assembly, seals (for part numbers see “Internal Tray Assembly” on page 169)

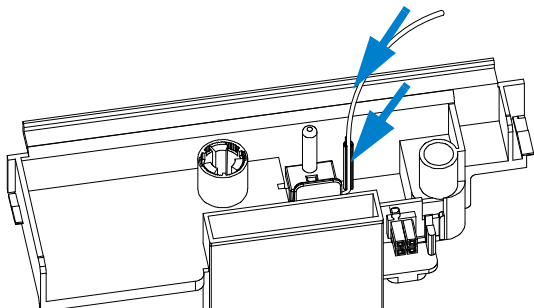
Before beginning this procedure:

- Position the transport unit of the Fraction Collector in the “Home Position” (see “Maintenance Functions” on page 125) and remove all installed trays from the tray base.
- Turn OFF the instrument.
- The procedure is almost identical for both, the preparative and the analytical scale internal tray, but it requires different parts.

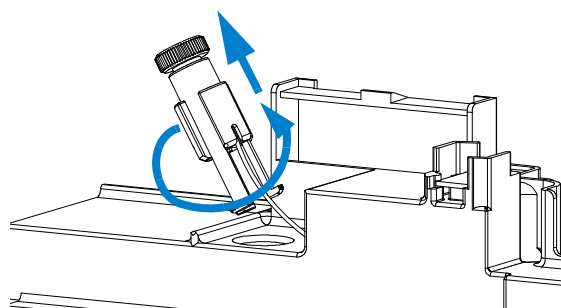
1 Remove the internal tray from the instrument, see “Exchanging the Internal Tray” on page 150.



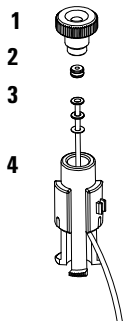
2 Turn the internal tray upside down and remove the funnel’s waste tubing through the flow delay sensor.



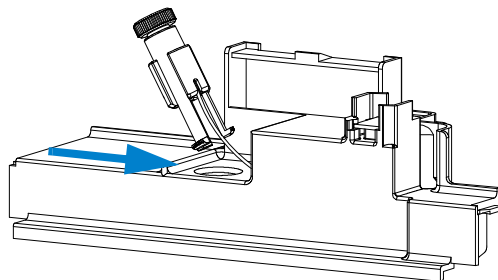
3 Remove the funnel assembly from the internal tray by screwing it counter clockwise and lifting it out.



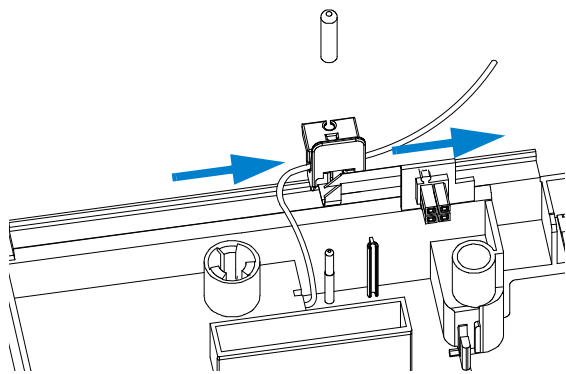
4 Exploded view for the parts of the internal tray. Once the funnel has been removed from the internal tray, it can be disassembled and defective parts can be replaced (funnel screw (1), seals (2), tubing (3) or funnel holder (4)).



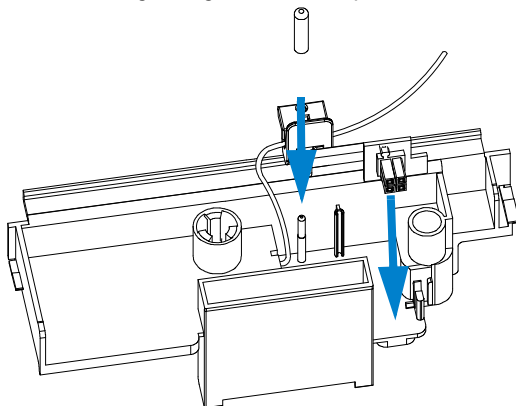
5 Slide the waste tubing of the newly assembled funnel assembly through the slit beside the funnel in the internal tray assembly



6 Turn the internal tray upside down. Slide the funnel's waste tubing through the flow delay sensor.



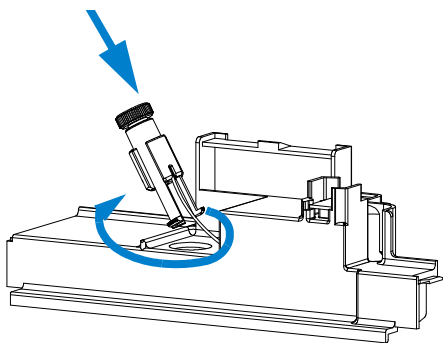
7 Turn the internal tray upside down. Slide the funnel's waste tubing through the flow delay sensor.



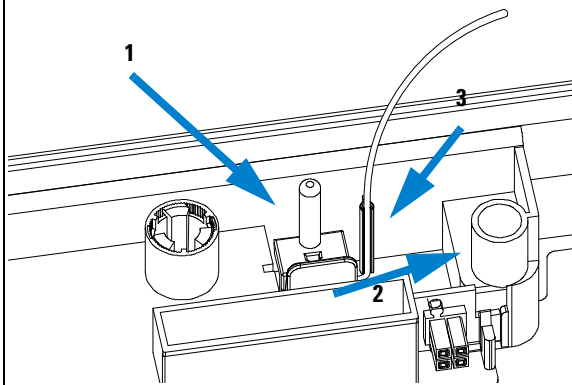
4 Repairing the Fraction Collector

Simple Repairs

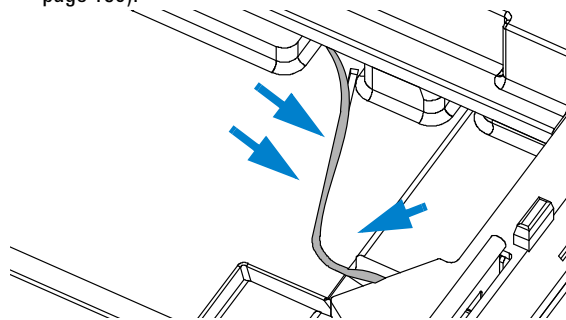
8 Insert the funnel into the tray and screw it tight (clockwise)



9 Re-install the plug that holds the flow delay sensor (1), pull the waste tubing tight through the flow delay sensor (2) and mount the waste tubing (3).

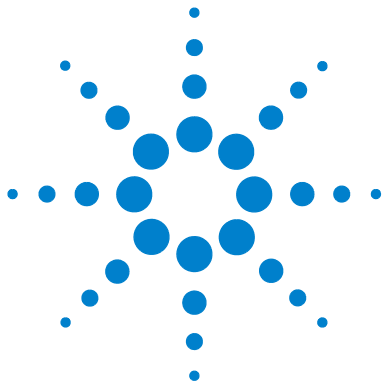


10 Re-install the internal tray and make sure to route the waste tubing out through the outlet of the instrument, correctly (see “Exchanging the Internal Tray” on page 150).



On completion of this procedure:

- Re-install the tray(s) in the tray base.
- Start the instrument.
- Close the front cover.



5 Parts and Materials

Supported Trays for Fraction Collectors	156
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List of Recommended Vials and Caps	159
List of Recommended Plates and Closing Mats	162
Transport Unit Assembly (Preparative Scale)	164
Transport Unit Assembly (Analytical Scale)	165
Needle Assemblies	166
Diverter-Valve Assembly	167
Tubing Kits	168
Internal Tray Assembly	169
Fraction Collector Accessory Kit	170



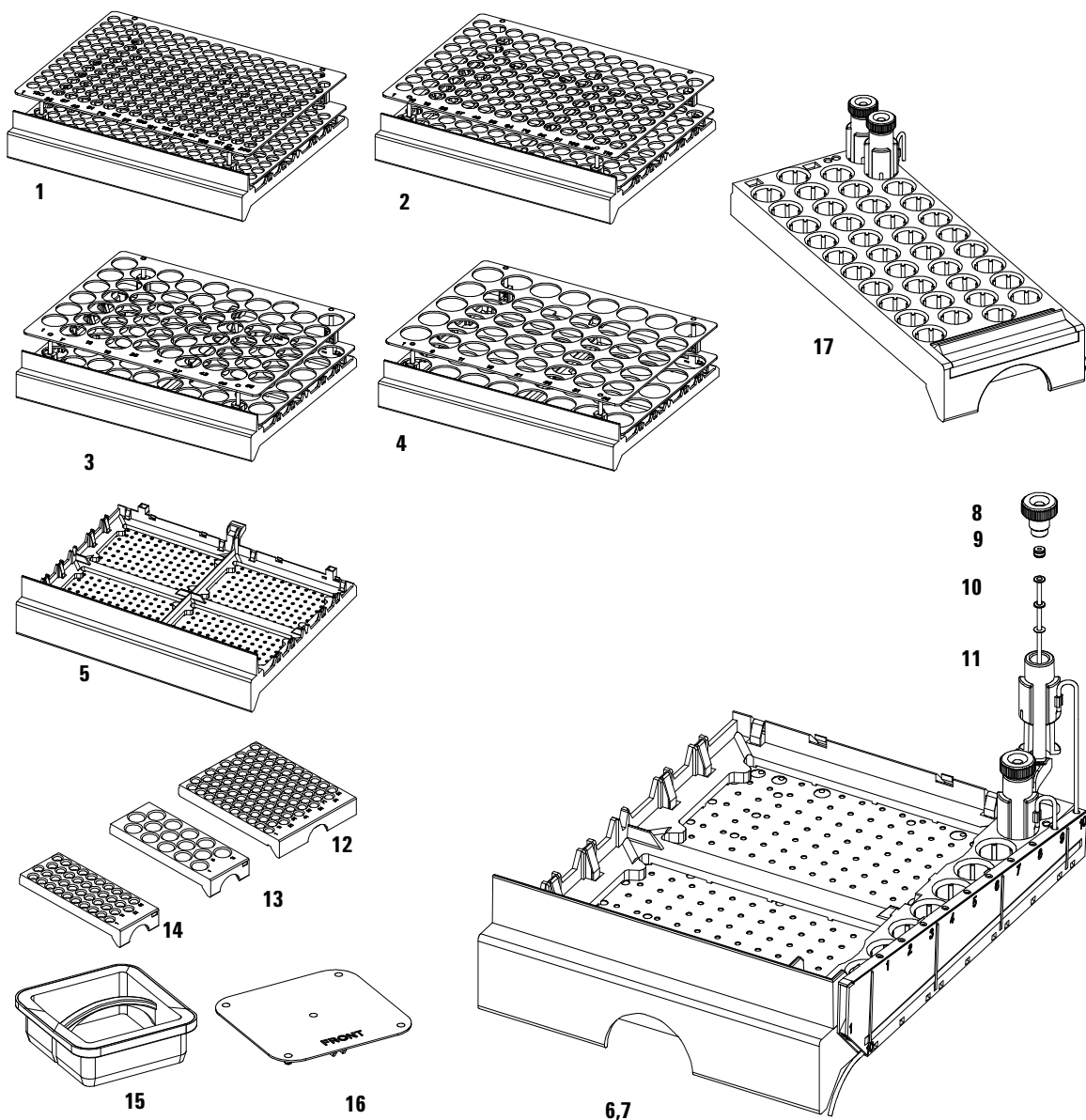
Supported Trays for Fraction Collectors

Table 26 Trays for the Fraction Collector

Item	Description	Part Number
1	Full tray for 215 test tubes, 12x100 mm	G1364-84516
2	Full tray for 126 test tubes, 16x100 mm	G1364-84525
3	Full tray for 60 round bottom tubes, 25x100 mm	G1364-84524
4	Full tray for 40 round bottom tubes, 30x100 mm	G1364-84523
5	Full tray for 4 well plates	G1364-84521
6	Std. tray for 2 well plates + 10 2ml vials	G2258-60011
7	Std. tray for 2 well plates + 10 collecting funnels	G1364-84522
8	Funnel for item 7	5022-2200
9	Funnel seal kit for item 7 (pack. of 10)	G1364-68730
10	Tubing kit 10T for item 7 (pack of 10)	G1364-86707
11	Funnel coupler for item 7	G1364-43201
12	Std. tray for 100 x 2 ml vials Std. tray for 100 x 2 ml vials, thermostatable	G1313-44510 G1329-60011
13	Half tray for 15 x 6 ml vials	G1313-44513
14	Half tray for 40 x 2 ml vials	G1313-44512
15	Adapter air channel (installed underneath plug channel, if the fraction collector is used with the thermostat)	G1329-43200
16	Plug channel	G1364-47200
17	Half Tray for 40 Funnels	G1364-84532

NOTE

Only one type of well-plates can be used at a time in one tray.



The items in this figure are displayed in different scaling factors!

Figure 48 Trays

List of Recommended Test Tubes

Table 27 Round Bottom Test Tubes

outer diameter	height	Volume	recommended tray type	Part Number
30 mm	100 mm	45 ml	G1364-84523 (40 tubes)	5042-6459 (100/pk)
30 mm	48 mm	20 ml;	G1364-84523 (40 tubes)	5042-6458 (100/pk)
25 mm	100 mm	35 ml	G1364-84524 (60 tubes)	5042-6470 (100/pk)
16 mm	100 mm	19 ml	G1364-84525 (126 tubes)	5022-6532 (250/pk)
16 mm	48 mm	9 ml	G1364-84525 (126 tubes)	5022-6533 (100/pk)
12 mm	100 mm	11 ml	G1364-84516 (215 tubes)	5022-6431 (250/pk)
12 mm	48 mm	5 ml	G1364-84516 (215 tubes)	5022-6435 (100/pk)

List of Recommended Vials and Caps

Table 28 Crimp Top Vials (Caps for Use with the Analytical Scale Fraction Collector, only!)

Description	Volume (ml)	100/Pack	1000/Pack	100/Pack (silanized)
Clear glass	2	5181-3375	5183-4491	
Clear glass, write-on spot	2	5182-0543	5183-4492	5183-4494
Amber glass, write-on spot	2	5182-3376	5183-4493	5183-4495

Table 29 SnapTop Vials (Caps for Use with the Analytical Scale Fraction Collector, only!)

Description	Volume (ml)	100/Pack	1000/Pack	100/Pack (silanized)
Clear glass	2	5182-0544	5183-4504	5183-4507
Clear glass, write-on spot	2	5182-0546	5183-4505	5183-4508
Amber glass, write-on spot	2	5182-0545	5183-4506	5183-4509

Table 30 Screw Top Vials (Caps for Use with the Analytical Scale Fraction Collector, only!)

Description	Volume (ml)	100/Pack	1000/Pack	100/Pack (silanized)
Clear glass	2	5182-0714	5183-2067	5183-2070

5 Parts and Materials

List of Recommended Vials and Caps

Table 30 Screw Top Vials (Caps for Use with the Analytical Scale Fraction Collector, only!)
(continued)

Description	Volume (ml)	100/Pack	1000/Pack	100/Pack (silanized)
Clear glass, write-on spot	2	5182-0715	5183-2068	5183-2071
Amber glass, write-on spot	2	5182-0716	5183-2069	5183-2072

Table 31 Crimp Caps (Caps for Use with the Analytical Scale Fraction Collector, only!)

Description	Septa	100/Pack
Silver aluminum	Clear PTFE/red rubber	5181-1210
Silver aluminum	Clear PTFE/red rubber	5183-4498 (1000/Pack)
Blue aluminum	Clear PTFE/red rubber	5181-1215
Green aluminum	Clear PTFE/red rubber	5181-1216
Red aluminum	Clear PTFE/red rubber	5181-1217

Table 32 Snap Caps (Caps for Use with the Analytical Scale Fraction Collector, only!)

Description	Septa	100/Pack
Clear polypropylene	Clear PTFE/red rubber	5182-0550
Blue polypropylene	Clear PTFE/red rubber	5182-3458
Green polypropylene	Clear PTFE/red rubber	5182-3457
Red polypropylene	Clear PTFE/red rubber	5182-3459

Table 33 Screw Caps (Caps for Use with the Analytical Scale Fraction Collector, only!)

Description	Septa	100/Pack
Blue polypropylene	Clear PTFE/red rubber	5182-0717
Green polypropylene	Clear PTFE/red rubber	5182-0718
Red polypropylene	Clear PTFE/red rubber	5182-0719
Blue polypropylene	Clear PTFE/silicone	5182-0720
Green polypropylene	Clear PTFE/silicone	5182-0721
Red polypropylene	Clear PTFE/silicone	5182-0722

List of Recommended Plates and Closing Mats

Table 34 Recommended Plates and Closing Mats (Std. Well Plates and Closing Mats for Use with the Analytical Scale Fraction Collector, only!)

Description	Volume (ml)	Package	Part Number
96 polypropylene well-plate*	0.5	10	5042-1386
96 polypropylene well-plate*	0.5	120	5042-1385
96 polypropylene deep well plate	1.0	50	5042-6454
96 polypropylene deep well-plate with glass inserts, caps and septa pre assembled*	0.35	1	5065-4402
glass inserts for 5065-4402*	0.35	1000	5188-5321
384 polypropylene well-plate*	0.1	30	5042-1388
96 polypropylene conical-well plate*	0.18	25	5042-8502
54 x 2ml vial plate*	1.5	6	G2255-68700
15 x 6ml vial plate	4.5	1	5022-6539
Plate for 27 self lock Eppendorf tubes*	0.5, 1.5, 2	1	5022-6538
Silicon Closing mats for 96 well-plate*		50	5042-1389

* For use with the **analytical scale** Fraction Collector, only!

NOTE

Only one type of well-plates can be used at a time in one tray.

WARNING

If you are using flammable solvents, remove the plates from the Fraction Collector after turning it OFF. You avoid the risk of building explosive gas mixtures in the instrument.

WARNING

If you are using flammable solvents, cover the plates. This action avoids the risk of building explosive gas mixtures in the instrument.

WARNING

Closing mats with adhesive can give some contamination in the system. The adhesive is soluble in most of the solvents used in HPLC.

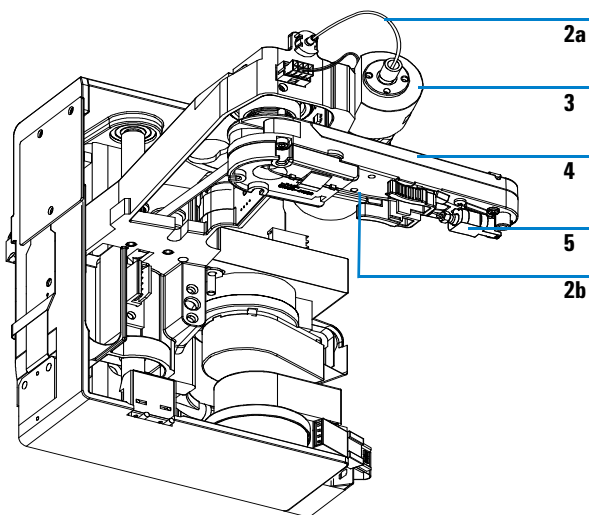
WARNING

In general do not use closing mats with adhesive. The Fraction Collector has no prepunch needle, therefore the adhesive will clog the needle after several injections.

Transport Unit Assembly (Preparative Scale)

Table 35 Transport Unit Assembly (Preparative Scale)

Item	Description	Part Number
1	Transport Unit Assembly (Preparative Scale), includes items 2 and 4	G1364-60018
2a, b	Tubing - diverter valve to needle, 0.8 mm ID	G1364-86711
3	Diverter valve assembly	G1364-61901
4	Needle carrier assembly preparative scale	G1364-60011
5	Needle assembly preparative scale	G1364-87201
6	Delay Calibration Adapter (not shown)	G1364-87301

**Figure 49** Transport Unit Assembly (Preparative Scale), partly displayed

Transport Unit Assembly (Analytical Scale)

Table 36 Transport Unit Assembly (Analytical Scale)

Item	Description	Part Number
1	Transport unit assembly (analytical scale), includes items 2 and 4	G1364-60019
2a, b	Tubing - diverter valve to needle, 0.25 mm ID (order full kit)	G1364-68712
3	Diverter valve assembly	G1364-61901
4	Needle carrier assembly analytical scale	G1364-60022
5	Needle assembly analytical scale (50mm needle) Needle assembly semi-preparative scale (20mm needle)	G1367-87200 G1364-87202
6	Wellplate adapter	G1364-60021

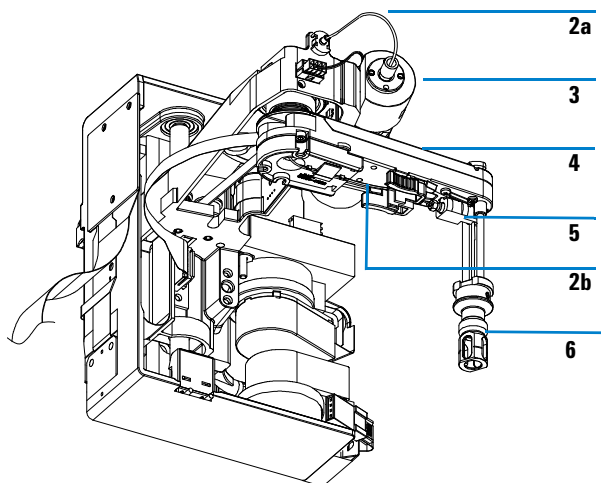


Figure 50 Transport Unit Assembly (Analytical Scale), partly displayed

Needle Assemblies

Table 37 Needle Assemblies

Item	Description	Part Number
1	Needle assembly preparative scale	G1364-87201
2	Needle assembly semi preparative scale (20 mm needle) For use in the analytical scale fraction collector at flow rates > 10 ml/min. or with high test tubes (48 mm - 75mm).	G1364-87202
3	Needle assembly analytical scale (50 mm needle)	G1367-87200
	Delay Calibration Adapter (not shown)	G1364-87301

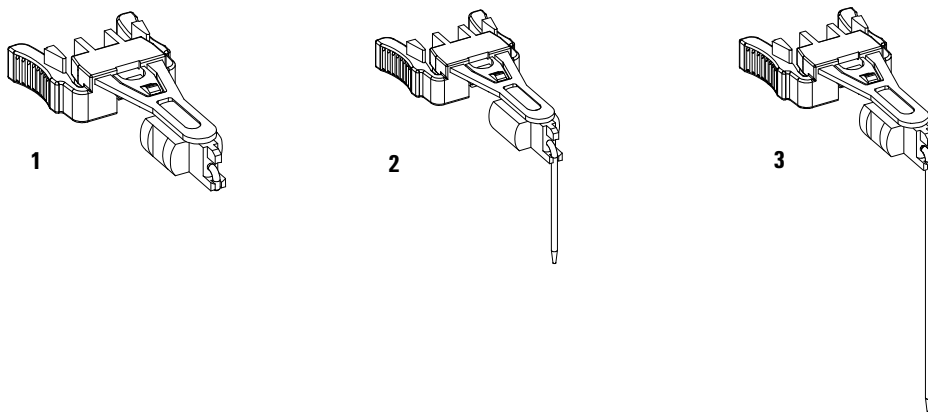


Figure 51 Needle Assemblies

Diverter-Valve Assembly

Table 38 Diverter-Valve Assembly (preparative and analytical scale)

Item	Description	Part Number
1	Diverter-valve assembly	G1364-61901
2	PIN screw	0515-1211

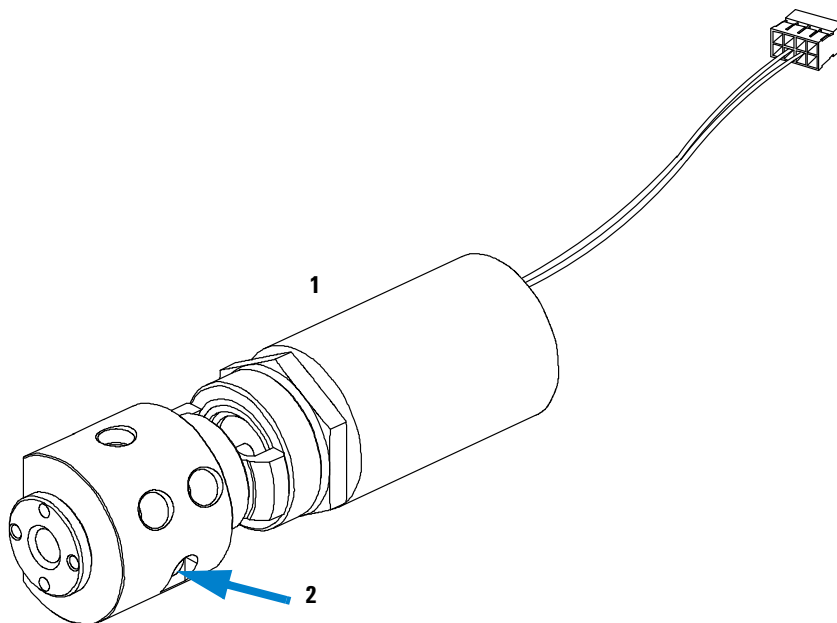


Figure 52 Diverter-Valve Assembly

Tubing Kits

Table 39 Tubing Kits

Item	Description	Part Number
1	Tubing kit preparative scale 0.8 mm ID (consists of items 2, and 3)	G1364-68711
2	Inlet/waste tubing assembly preparative scale 0.8 mm ID	Order Item 1
3	Diverter valve to needle tubing preparative scale 0.8 mm ID	Order Item 1
4	Tubing kit analytical scale 0.25 mm ID (consists of items 5, and 6)	G1364-68712
5	Inlet/waste tubing assembly analytical scale 0.25 mm ID	Order Item 4
6	Injection valve to needle tubing analytical scale 0.25 mm ID	Order Item 4
7	Low dispersion tubing kit analytical scale 0.15 mm ID for flow rates < 1 ml/min	G1364-68723
8	Inlet/waste tubing assembly analytical scale 0.15 mm ID	Order Item 7
9	injection valve to needle tubing analytical scale 0.15 mm ID	Order Item 7
10	Finger tight fitting (pack of 2)	0100-1516

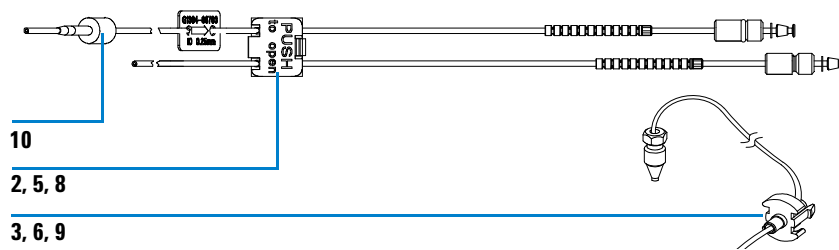


Figure 53 Tubing Kits

Internal Tray Assembly

Table 40 Internal Tray Assembly (Analytical and Preparative Scale)

Item	Description	Part Number
1	Internal tray assembly analytical scale , includes items 2 – 7 Internal tray assembly preparative scale , includes items 2 – 7	G1364-63124 G1364-63123
2	Screw-seat-adapter	5022-2200
3	Seal funnel (reorder No., pack. of 10)	G1364-68730
4a	Waste Tubing Kit 0.5T (analytical scale)	G1364-86708
4b	Waste Tubing Kit 0.8T (preparative scale)	G1364-86719
5	Funnel coupler	G1364-43201
6	Tray internal	Order Item 1
7	Flow delay sensor (not shown)	Order Item 1

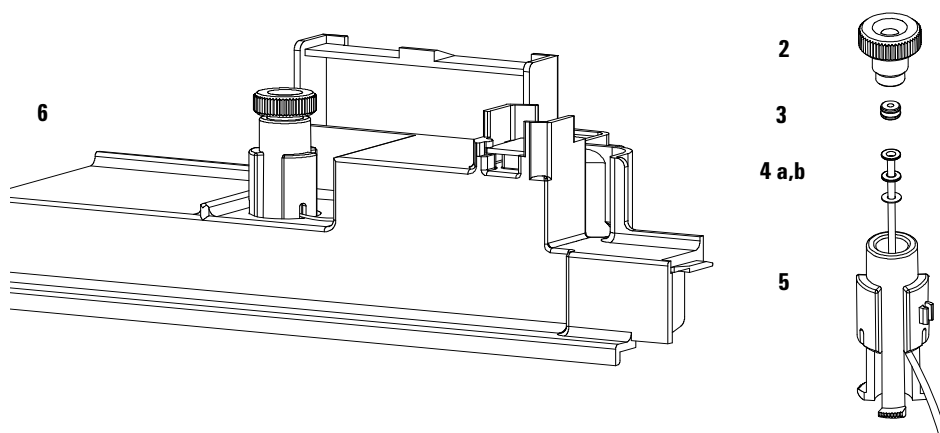


Figure 54 Internal Tray Assembly (Analytical Scale)

Fraction Collector Accessory Kit

Table 41 Fraction Collector Accessory Kit Contents G1364-68725

Description	Quantity	Part Number
Wrench, open end, 4mm	1	8710-1534
Wrench, open end, 1/4-5/16 inch	1	8710-0510
Hex key 2.0 mm	1	8710-2476
Finger tight fittings, 1/16" f-120*	3	0100-1516
Waste tubing (1.2 m) [†]	1	5062-2463
Y-Type Fitting for waste tubing [‡]	1	5065-9971
CAN cable, 1 m	1	5181-1519
Air channel adapter	1	G1329-43200
Sticking clamp for corrugated waste tubing (large)**	3	5065-9976
Sticking clamp for waste tubing (small) ^{††}	3	5065-9976
Tray for 15 x 6 ml vials	1	G1313-44513
Tray for 40 x 2 ml vials	1	G1313-44512

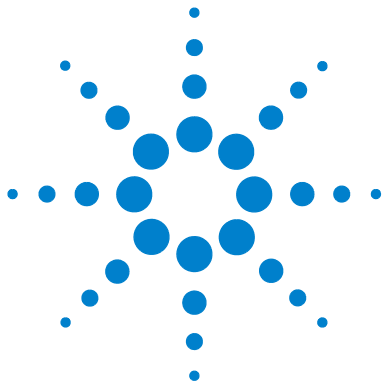
* Reorder gives pack of 2

† Reorder gives 5 m

‡ Reorder gives pack of 10

** Reorder gives 10 clamps for corrugated waste tubing (large))

†† Reorder gives 10 clamps for waste tubing (small)



6 Specifications

Performance Specifications for the Fraction Collectors [172](#)



Performance Specifications for the Fraction Collectors

Table 42 performance Specifications Agilent 1260 Infinity Preparative-scale Fraction Collector (G1364B)

Type	Specification
Trigger modes	Time slices, Peak (threshold, up- / downslope), Timetable (combination of time intervals and peak) and Manual trigger (supported only with Agilent Instant Pilot G4208A) Agilent 1260 Infinity DAD/MWD detectors (G1315C,D and G4212B/G1365C,D), the Agilent 1260 Infinity Fluorescence Detector (G1321A) and the Agilent G1946C/D, G1956A/B LC-MSD are fully supported other detectors can be used but are not supported for fraction collection.
Operating modes	Discrete fractions: default mode for all vessels. The flow is diverted to waste, while moving from one vessel position to the next vessel position Continuous flow: optional, available only when using well plates. It is possible to move from one well plate position to the next one without diverting the flow into the well plate to waste
Fraction capacities and trays	4 x well-plates full tray (MTP)* (for use with deep well plates, only) 2 x well-plates std. tray (MTP) (for use with deep well plates, only) + 10 x 2 ml vials* (+ 1 half tray) 100 x 2 ml in std. tray (+ 1 half tray)* 3 x 40 x 2 ml in half tray* 3 x 15 x 6 ml in half tray* Full tray with 40 test tubes (30 mm OD, max. height 100 mm, ~45 ml / tube) Full tray with 60 test tubes (25 mm OD, max. height 100 mm, ~25 ml / tube) Full tray with 126 test tubes (16 mm OD, max. height 100 mm, ~12 ml / tube) Full tray with 215 test tubes (12 mm OD, max. height 100 mm, ~7 ml / tube) Installed trays are automatically detected and identified. For the with uncapped vials, tests tubes and well plates, only!
Test tube / plate sizes	Minimum 48 mm to 100 mm maximum
Maximum tube volume	ca. 45 ml

Table 42 performance Specifications Agilent 1260 Infinity Preparative-scale Fraction Collector (G1364B) (continued)

Type	Specification
Maximum flow rate	100 ml / min (depending on viscosity and generated back pressure, max. 6 bar at the diverter valve)
Delay volumes [μl]	Fraction collector inlet to diverter valve: ~500 (typical, depends on length of the tubing) Diverter valve: ~15 Diverter valve to needle: ~110 Needle: ~5
Delay calibration sensor	Single wavelength absorbance detector working at 654 nm, consisting of a LED and a photo diode
Diverter valve	3/2 Diverter valve with low internal volume (15 μl), switching time < 100 ms, maximum operating pressure 6 bar
cooling	Optional (with additional G1330B), performance depending on ambient conditions and the volume of collected fractions
maximum capacity	3 fraction collectors in parallel plus one recovery fraction collector connected via 12-Position, 13-Port Selector valve (PN G1160A)
GLP features	Early maintenance feedback (EMF), electronic records of maintenance and errors
Interfaces	Controller-area network (CAN), optional; LAN or external contacts interface RS232C, APG-remote (for remote start / stop signals to / from other modules) Interface to G1330B Thermostat CAN-DC-out for operation of Agilent approved external devices like valves
Safety features	Leak detection and safe leak handling, error detection and display, exhaust fan for fume extraction of hazardous vapors

* Vials can be used as recommended by Agilent Technologies (see “[List of Recommended Vials and Caps](#)” on page 159 and “[List of Recommended Plates and Closing Mats](#)” on page 162) **but must be uncapped**. Only the 96 deep well-plates can be used (**without closing mats**, see “[List of Recommended Plates and Closing Mats](#)” on page 162)

NOTE

Only one type of well-plates can be used at a time in one tray.

Table 43 Performance Specifications Agilent 1260 Infinity Analytical-scale Fraction Collector (G1364C)

Type	Specification
Trigger modes	Time slices, Peak (threshold, up- / downslope), Timetable (combination of time intervals and peak) and Manual trigger (supported only with Agilent Instant Pilot G4208A) Agilent 1260 Infinity DAD/MWD detectors (G1315C,D; G4212B/ G1365 C,D), the Agilent 1260 Infinity Fluorescence Detector (G1321A) and the Agilent G1946C/D, G1956A/B LC-MSD are fully supported other detectors can be used but are not supported for fraction collection.
Operating modes	Discrete fractions: default mode for all vessels. The flow is diverted to waste, while moving from one vessel position to the next vessel position Continuous flow: optional, available only when using the deep well plates. It is possible to move from one well plate position to the next one without diverting the flow into the well plate to waste Needle into location: Needle pushes into the vessel as deep as specified, for the use with capped vials and test tubes and well plates with closing mats Droplet setup mode: The tip of the fraction collector needle will initially move down to the bottom of the well. Then it will slowly move upwards while the fraction is collected. The droplet setup mode enables the fraction collector to collect small fractions without bubbles.

Table 43 Performance Specifications Agilent 1260 Infinity Analytical-scale Fraction Collector (G1364C) (continued)

Type	Specification
Fraction vessel capacities and trays	<ul style="list-style-type: none"> • 4 x well-plates full tray (MTP)[*] • 2 x well-plates std. tray + 10 funnels with external containers[*] (+ 1 half tray) • 2 x well-plates std. tray (MTP) + 10 x 2 ml vials[*] (+ 1 half tray) • 100 x 2 ml in std. tray (+ 1 half tray)[*] • 3 x 40 x 2 ml in half tray[*] • 3 x 40 funnels in half tray[*] • 3 x 15 x 6 ml in half tray[*] • Full tray with 40 test tubes (30 mm OD, max. height 48 mm, ~20 ml vol.) • Full tray with 60 test tubes (25 mm OD, max. height 48 mm) • Full tray with 126 test tubes (16 mm OD, max. height 48 mm) • Full tray with 215 test tubes (12 mm OD, max. height 48 mm) <p>Installed trays are automatically detected and identified. Installed plates and vials can be detected when operating in the needle into location mode</p> <p>[*] max. height can be extended by using the short needle assembly G1364-87202</p>
Maximum tube / plate height	48 mm with long needle assembly G1367-87200 75 mm with short needle assembly G1364-87202
Maximum tube volume	ca. 20 ml with 48 mm test tubes, ca. 30 ml with 75 mm test tubes or unlimited, if funnels are used with external containers.
Maximum flow rate	10 ml / min (depending on viscosity and generated back pressure, max. 6 bar at the diverter valve). The analytical scale fraction collector can be modified for flow rates > 10 ml/min.
Delay volumes [μl]	Fraction collector inlet to diverter valve: ~50 (typical, depends on the length of the tubing) Diverter valve: ~15 Diverter valve to needle: ~10 Needle: ~4
Delay calibration sensor	Single wavelength absorbance detector working at 654 nm, consisting of a LED and a photo diode
Diverter valve	3/2 Diverter valve with low internal volume (15 μl), switching time < 100 ms, maximum operating pressure 6 bar

6 Specifications

Performance Specifications for the Fraction Collectors

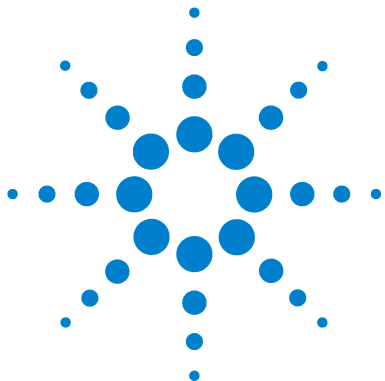
Table 43 Performance Specifications Agilent 1260 Infinity Analytical-scale Fraction Collector (G1364C) (continued)

Type	Specification
Cooling	Optional (with additional G1330B), performance depending on ambient conditions and the volume of collected fractions
Maximum capacity	3 fraction collectors in parallel plus one recovery fraction collector connected via 12-Position, 13-Port Selector valve (PN G1160A)
GLP features	Early maintenance feedback (EMF), electronic records of maintenance and errors
Interfaces	<ul style="list-style-type: none">- Controller-area network (CAN).- optional; LAN or external contacts interface- RS232C,- APG-remote (for remote start / stop signals to / from other modules)- Interface to G1330B Thermostat- CAN-DC-out for operation of Agilent approved external devices like valves
Safety features	Leak detection and safe leak handling, error detection and display, exhaust fan for fume extraction of hazardous vapors

* Vials and well-plates and capped vials and well plates with closing mats can be used as recommended by Agilent Technologies (see “[List of Recommended Vials and Caps](#)” on page 159 and “[List of Recommended Plates and Closing Mats](#)” on page 162)

NOTE

Only one type of well-plates can be used at a time in one tray.



A **Safety Information**

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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.

General

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

WARNING

If you are using flammable solvents, remove the well-plates from the tray when you turn off the sampler. You avoid the risk of building explosive gas mixtures in the tray compartment.

WARNING

If you are using flammable solvents, cover the well-plates with closing mats to avoid the risk of building explosive gas mixtures.

WARNING

After a leak in the sampler, make sure the leak plane is cleaned and dry.

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 fuseholders must be avoided.

WARNING

Any adjustment, maintenance, and repair of the opened instrument under voltage is forbidden.

WARNING

Disconnect the instrument from the line and unplug the power cord before maintenance.

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.

Safety Symbols

Table 44 shows safety symbols used on the instrument and in the manuals.

Table 44 Safety Symbols

Symbol	Description
	The apparatus is marked with this symbol when the user should refer to the instruction manual in order to prevent risk of harm to the operator and to protect the apparatus against damage.
	Indicates dangerous voltages.
	Indicates a protected conductor terminal.
	Eye damage may result from directly viewing the light produced by the Xenon flash lamp used in this product. Always turn the xenon flash lamp off before removing it.

WARNING

A warning alerts you to situations that could cause physical injury or damage to the equipment. 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 a possible loss of data. Do not proceed beyond a caution until you have fully understood and met the indicated conditions.

Lithium Batteries Information

WARNING

Danger of explosion if battery is incorrectly replaced. Replace only with the same or equivalent type recommended by the equipment manufacturer. 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. Discharged Lithium batteries shall be disposed off locally according to national waste disposal regulations for batteries.

WARNING

Lithiumbatteri - Eksplosionsfare ved fejlagtig handling. Udskiftning må kun ske med batteri af samme fabrikat og type. Lever det brugte batteri tilbage til leverandoren.

WARNING

Lithiumbatteri - Eksplosionsfare. Ved udskiftning benyttes kun batteri som anbefalt af apparatfabrikanten. Brukt batteri returneres apparatleverandoren.

NOTE

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



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

Solvent Information

Observe the following recommendations on the use of solvents.

WARNING

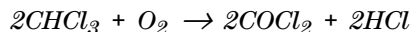
This instrument should only be used with solvents that have an ignition temperature higher than 200°C!

Solvents

Brown glass ware can avoid growth of algae.

Always filter solvents, small particles can permanently block the capillaries. 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 nitric acid, sulfuric acid 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:



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.

Agilent Technologies on Internet

For the latest information on products and services visit our worldwide web site on the Internet at:

<http://www.agilent.com>

Select **“Products”** - **“Chemical Analysis”**

It will provide also the latest firmware of the Agilent 1260 Infinity modules for download.

A Safety Information
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In This Book

This manual contains technical reference information about the Agilent 1260 Infinity Fraction Collectors. The manual describes the following:

- configuration and operation of the fraction collector,
- troubleshooting and test functions,
- simple repairs
- parts and materials,
- specifications,
- safety information.

© Agilent Technologies 2010, 2013-2014
Printed in Germany
03/2014



G1364-90014