

Agilent 1200 Series Analytical and Preparative Scale Fraction Collectors



# **User Manual**

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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 1200 Series 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 1200 Series fraction collectors AS and PS.

#### 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 Control Module Screens of the Fraction Collector**

The reference to the screens of the Agilent 1200 Series control module is provided in the chapter.

#### 4 Troubleshooting and Test Functions

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

#### 5 Repairing the Fraction Collector

This chapter contains instructions on simple repair and maintenance procedures.

#### 6 Parts and Materials

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

#### 7 Specifications

This chapter contains performance specifications of the fraction collectors.

#### A Safety Information

This appendix provides a safety summary.

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Agilent 1200 Series Fraction Collectors User Manual

# **Installing the Fraction Collector**

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1 Installing the Fraction Collector Site Requirements

### **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 14). 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 (G1330A). 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 14) 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 15) 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 1200 Series 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 21. 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. **Site Requirements** 

### Environment

Your fraction collector will work within specifications at ambient temperatures and relative humidity as described in Table 1 and Table 2 on page 15.

### 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.

Туре	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 – 55 °C (41 – 131 °F)	
Ambient non-operating temperature	-40 - 70 °C (-4 - 158 °F)	
Humidity	< 95 %, at 25 $-$ 40 $^{\circ}$ C (77 $-$ 104 $^{\circ}$ 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.	

Туре	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, $\pm$ 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 $^{\circ}$ C (77 $-$ 104 $^{\circ}$ 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.	

### Table 2 Physical Specifications - Thermostat (G1330B)

1 Installing the Fraction Collector Unpacking the Fraction Collector

### **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 43).

### **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 1200 Series fraction collector 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 1200 Series 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.

**Unpacking the Fraction Collector** 

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 <sup>*</sup>	3	0100-1516
Waste tubing (1.2 m) <sup>†</sup>	1	5062-2463
Y-Type Fitting for waste tubing <sup>‡</sup>	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) <sup>††</sup>	3	5065-9976
ESD wrist strap	1	9300-1408
Tray for 15 x 6 ml vials	1	G1313-44513
Tray for 40 x 2 ml vials	1	G1313-44512

#### Table 5 Fraction Collector Accessory Kit Contents G1364-68725

\* Reorder gives pack of 2

- † Reorder gives 5 m
- ‡ Reorder gives pack of 10
- \*\* Reorder gives 10 clamps for corrugated waste tubing (large))
- tt 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) <sup>*</sup>	1	5062-2463
Cable thermostat module to fraction collector	1	G1330-81600

#### Installing the Fraction Collector 1 Unpacking the Fraction Collector

\* Reorder gives 5 m

1 Installing the Fraction Collector Optimizing the Stack Configuration

### **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 22 show the configuration recommended for the fraction collector in an analytical scale system.
- Figure 3 on page 23 and Figure 4 on page 24 show the configuration recommended for the fraction collector with a preparative scale system.

### NOTE

Agilent 1200 Series 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.

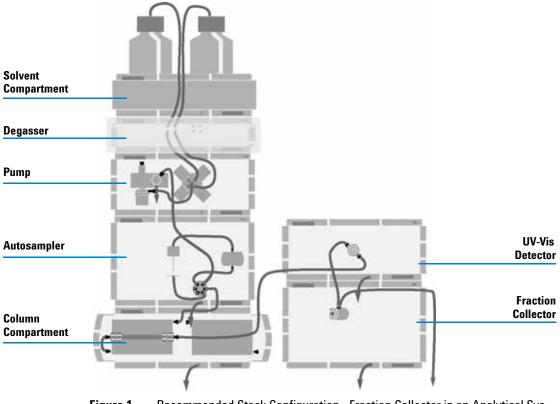


Figure 1 Recommended Stack Configuration - Fraction Collector in an Analytical System (Front View)

### NOTE

For information about connecting an Agilent LC-MSD, a Non-1200 or Non-UV-Vis detector to the system, please refer to the "User's Guide for Purification / High Throughput System", PN G2262-90010 or to the "Installation Note for the Mass Based Fraction Collection Kit".

**Optimizing the Stack Configuration** 

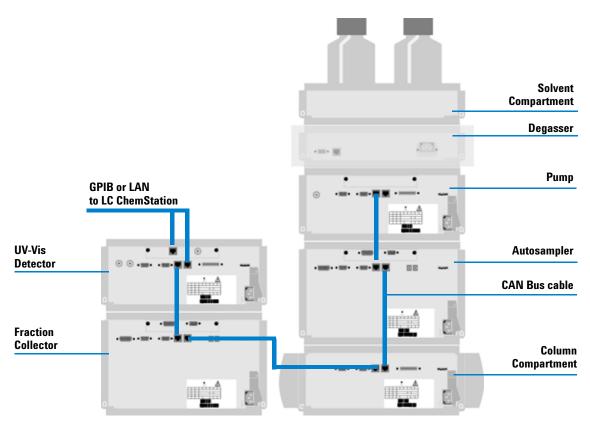
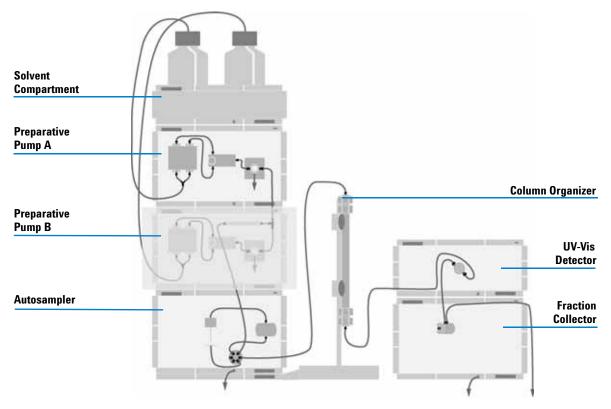


Figure 2 Recommended Stack Configuration - Fraction Collector in an Analytical System (Rear View)

**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 29).

### 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 1200 Series thermostatted column compartment G1316A/B or Agilent 1200 Series diode-array detectors G1315B/C.

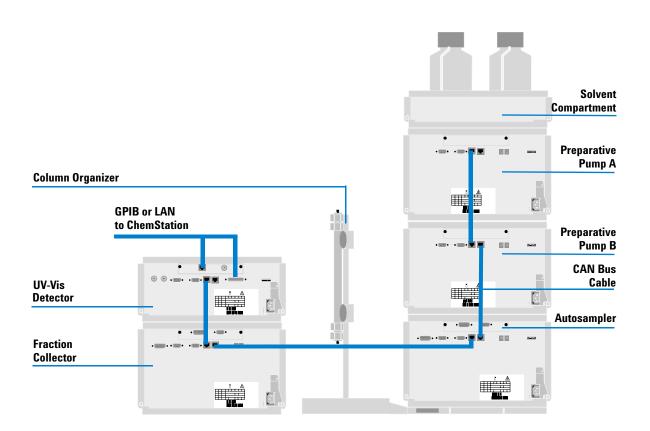


**Figure 3** Recommended Stack Configuration - Fraction Collector in a Preparative System (Front View)

### NOTE

For information about connecting an Agilent LC-MSD, a Non-1200 or Non-UV-Vis detector to the system, please refer to the "User's Guide for Purification / High Throughput System", PN G2262-90010 or to the "Installation Note for the Mass Based Fraction Collection Kit".

**Optimizing the Stack Configuration** 



**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 29).

#### 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 1200 Series thermostatted column compartment G1316A/B or Agilent 1200 Series diode-array detectors G1315B/C.

Preparation	Locate bench space Provide power connections Unpack the fraction collector
Parts required	Fraction Collector Power cord, Chemstation and/or Control Module G1323B

### 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 26). Route the corrugated waste tubing into a waste container.

**Installing the Fraction Collector** 

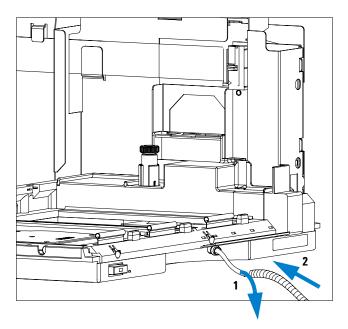


Figure 5 Installing the Corrugated Waste Tubing in the Plastic Port

- **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 1200 Series 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 1200 Series 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

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 27).

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).

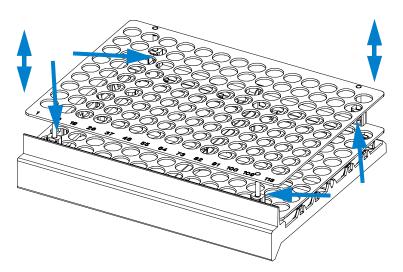


Figure 6 Adjusting the Height of the (Full) Test Tube Trays.

- **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.

NOTE

**Installing the Fraction Collector** 

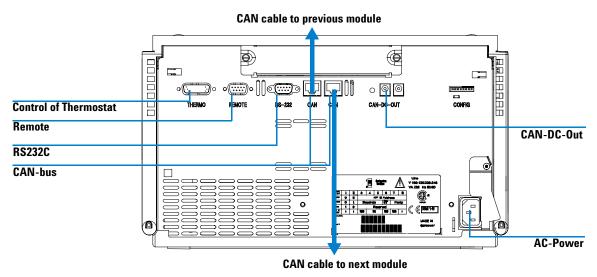


Figure 7 Cable Connections

**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 and/or Control Module G1323B

- **1** Place the thermostat on the bench.
- **2** Remove the front cover and route the condensation drain tube to a waste container.

### WARNING

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.

### CAUTION

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

- **3** Install the LAN interface board in the fraction collector (if required).
- **4** Remove the adhesive tape which covers the side and front doors.
- **5** Open the front door and remove the left side door.
- **6** Remove the transport protection foam.
- 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. Slide the waste tubing coming from the internal tray (if present) through the

Installing a Thermostatted Fraction Collector

plastic port and the corrugated waste tube (see Figure 5 on page 26). Route the corrugated waste tubing into a waste container.

- 8 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).
- **9** Place the fraction collector on top of the thermostat. Make sure that the fraction collector is correctly engaged in the thermostat locks.
- **10** 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.

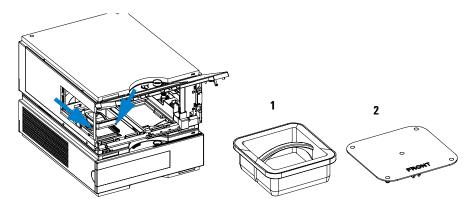


Figure 8 Installation of Thermostat and Fraction Collector

**11** 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.

**Installing a Thermostatted Fraction Collector** 

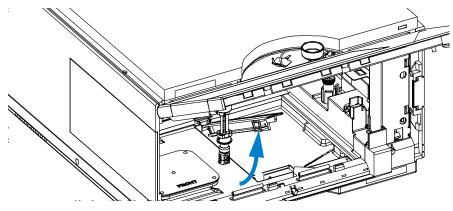


Figure 9 Removing the Tray Compartment Divider

**12** 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

**Installing a Thermostatted Fraction Collector** 

guides in the 4 corners of a tray and move the top plate of the tray up or down (see Figure 6 on page 27).

- **13** Ensure the power switch on the front of the fraction collector is 0FF and the power cables are disconnected.
- **14** Connect the cable between the fraction collector and the thermostat, see Figure 10 on page 33.

# **WARNING** 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.

- **15** Connect the power cables to the power connectors.
- 16 Connect the CAN cable to other Agilent 1200 Series 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 1200 Series 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 0FF when the line power switch is protruding and the green light is 0FF.

### 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.

#### 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.

Installing a Thermostatted Fraction Collector

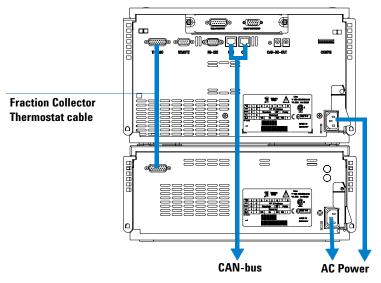


Figure 10 Connections at the Rear of the Thermostatted Fraction Collector

**Flow Connections to the Fraction Collector** 

# **Flow Connections to the Fraction Collector**

	Preparation Parts required	Fraction Collector is installed in the LC system Parts from the accessory kit, see "Fraction Collector Accessory Kit Contents G1364-68725" on page 18	
WARNING	capillary or tube fittings, solvents may leak out. Please observe ety procedures (for example, goggles, safety gloves and protective scribed in the material handling and safety data sheet supplied by the 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	inlet / waste tu or fraction loss	ow the described installation procedures to maximize the lifetime of bing assembly the valve to needle tubing and to avoid potential spills es. Regularly inspect the tubings and exchange them if they are worn ible signs of damage.	
WARNING	This instrument higher than 200	s should only be used with solvents that have an ignition temperature ${}^{\mathrm{o}}\mathrm{C}!$	

**Flow Connections to the Fraction Collector** 

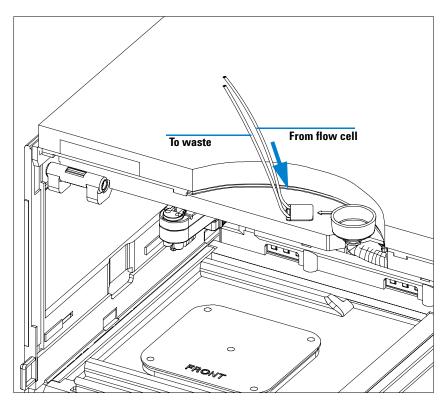


Figure 11 Flow Connections to and from the Fraction Collector.

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.

Flow Connections to the Fraction Collector

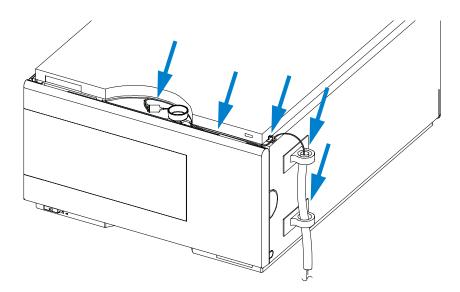


Figure 12 Fixing and Routing the Waste Tubings

# **Fraction Collector Trays**

Installing	g the Fraction Collector Trays
1	<b>1</b> Press the front door latch-holding button located at the front of the right-side cover.
2	<b>2</b> Lift the front door.
:	<b>3</b> Adjust the top plate of the test tube tray for the correct tube height if required.
1	<b>4</b> Load the fraction collector tray with fraction collector well-plates, test tubes or vials as required.
ļ	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.
(	<b>6</b> Press the front of the fraction collector tray down to secure the tray in the fraction collector.
7	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

	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.

1 Installing the Fraction Collector Configure Well-plate Types

# **Configure Well-plate Types**

If the plate you are using is not found on the "List of Recommended Plates and Closing Mats" on page 168 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 Control Module.

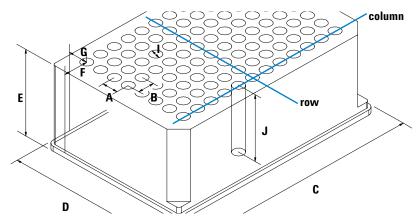


Figure 13 Well-plate Dimensions (straight)

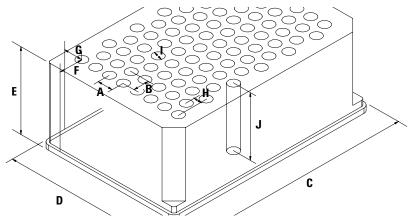


Figure 14 Well-plate Dimensions (staggered)

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	
В	Column distance	Distance (in mm) between the center of two columns	
С	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)	
Н	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

### Table 7 Configuring Well-plate Types

#### **1** Installing the Fraction Collector

**Configure Well-plate Types** 

ocation	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
	ls 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

#### Table 7 Configuring Well-plate Types (continued)

## 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 Control Module for this command.

To move the arm to the park position:

- 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** 



Agilent 1200 Series Fraction Collectors User Manual

# 2 Configuration and Operation of the Fraction Collector

Configuration of the Fraction Collector 46 Delay volumes and delay calibration 52 Setting up a Fraction Collector Method 67 Starting your run with fraction collection 74 Viewing your Results 78 Special Applications 80 Limitations and how to avoid problems 85 Check-out Procedures 86 Application Notes 91



# **Configuration of the Fraction Collector**

# **Configuration of the fraction collector in the ChemStation**

### 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 47) 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 162.

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

**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 in creased to 75 mm. For the part numbers of the different needle assemblies see "Needle Assemblies" on page 173.

#### NOTE

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

**Configuration of the Fraction Collector** 

Tray A: 40 Tubes	
Tray B:	Tube Volume (ml): 45.00
Tray C:	Tube Height (mm): 98
raction Delay Volumes	Collection Order
Detector Volume(µI)	C Row by row
1 0AD1 300	C Column by IIII O Shortest path
Collection Mode	Needle Movement
Discrete fractions	C into location Depth: 5.0 mm
C Continuous flow	above location Distance: 2.0 mm     O contact control Distance: 0.0 mm
≪ell-Plates	
Plate Type:	Treat as Reserved Locations
Installed Plates	Row(s):
Plate 1 (front left) Plate 1	ate 3 (front right) Column(s):
Plate 2 (back left)	ate 4 (back right) Single Location(s):

Figure 15 Fraction Collector Configuration dialog

**Use for Sample Recovery** This checkbox only appears, if multiple fraction collectors are configured (see Figure 34 on page 81). 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 **Configure 1200 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.

#### 2 Configuration and Operation of the Fraction Collector Configuration of the Fraction Collector

**Configuration of the Fraction Collector** 

#### **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 52 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 is 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 recommend 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).

Syntax	Description
А	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
olumns 1	Column 1 can't be used
1,2	Columns 1, and2 can't be used
1-4	Columns 1, 2, 3 and 4 can't be used
	A A,B A-D A-D,F 1 1,2

 Table 8
 Syntax for the definition of Reserved Locations

**Configuration of the Fraction Collector** 

Location	cation Syntax Description	
	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

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

# **Configuring multiple fraction collectors**

To increase the capacity of the systems up to three fraction collectors can be configured by using the Agilent 1200 Series 12-position13-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 81.
- 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 74. 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  $\nu$ .

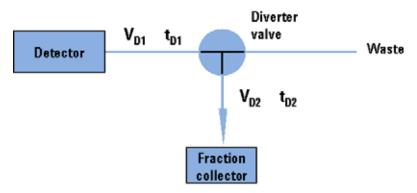


Figure 16 Delay Volumes and delay times

The delay volume  $V_{\rm D2}$  is a system parameter, it depends on the installed fraction collector tubing and the fraction collector needle. It is approximately 23  $\mu l$  for the fraction collector AS and approximately 120  $\mu l$  for the fraction collector PS. Delay volume  $V_{\rm D1},$  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:

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

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

Delay volumes and delay calibration

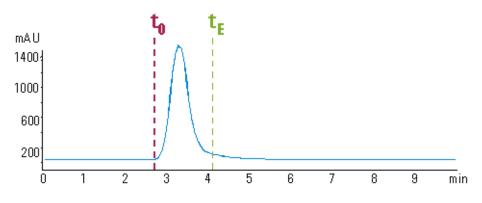


Figure 17 Chromatogram with peak start t0 and peak end tE

# **Detector signal delay**

Every Agilent 1200 Series 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.

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 9
 Signal delay times for the Agilent 1200 Series DAD/MWD (G1315B/G1365B)

Delay volumes and delay calibration

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 10
 Signal delay times for the Agilent 1200 Series DAD/MVD SL (1315C/G1365C)

 Table 11
 Signal delay times for the Agilent 1200 Series 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

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 12
 Signal delay times for the Agilent 1200 Series VWD SL G1314C

 Table 13
 Signal delay times for the Agilent 1200 Series 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

**Delay volumes and delay calibration** 

### CAUTION

If the internal signal delay is longer than the delay time t<sub>D1</sub> some of the peak will be lost. The maximum allowed signal delay time can be calculated using the following equation:

Signal delay time<sub>(max)</sub> =  $\frac{V_{D1}}{\dot{v}}$   $\dot{v}$  = 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 > 0.005for the VWD.

If the **Peakwidth** setting cannot be reduced and the signal delay time is longer than t<sub>D1</sub> it is also possible to increase V<sub>D1</sub> 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  $(V_{D1}/v) + 0.1$  min for time-based fraction collection.

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

# Performing a delay calibration with an UV detector

- **1** Place a vial containing the Delay Sensor Calibrant (Part No. 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

AFC Delay Volume Calibra	ation - Status Window		×
Info			
Turn on all pumps			
	Continue	Cancel	Help

Figure 18 Delay Volume Calibration status window

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

**Delay volumes and delay calibration** 

AFC Delay Volume Calibration - Status Window	
Calibration method: DGCALAS.M	
Info	
I.	
Change method Continue	Cancel Help

Figure 19 AFC Delay Volume Calibration Window

When you have turned ON the pumps, click  $\ensuremath{\textit{Continue}}$  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 66

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.

Delay volumes and delay calibration

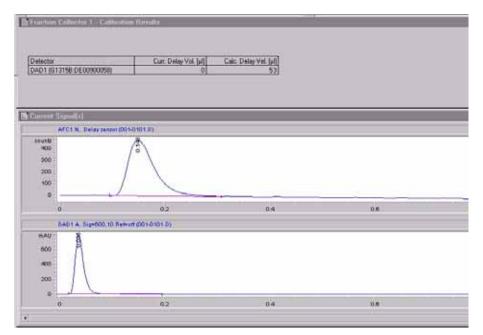


Figure 20 Delay Calibration Results Panel

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

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.

**Delay volumes and delay calibration** 

# **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 (Part No. 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
- **14** Install the Delay Calibration Adapter G1364-87301 by attaching it to the needle carrier assembly (G1364B preparative scale only).

Delay volumes and delay calibration

The selected method is loaded and the Delay Calibration Sample 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.

**Delay volumes and delay calibration** 

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

Print	Close	Calibrate
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	2	-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 x 6/60 or 0.3 ml is required.
- **21** Cut off a sufficient piece of the supplied ETFE tubing (Part No. G1968-60500) to provide the needed volume. The tubing's volume is 8.1  $\mu$ /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).

**Delay volumes and delay calibration** 

Print	Close	Calibrate	
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	2	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 46).
- **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).

Delay volumes and delay calibration

action Collection Parameters		
Model Type FC Hardware: Trigger Type: Pos	Other sitive Step	Co <u>n</u> figuration
Peak Timing		· · · · · · · · · · · · · · · · · · · ·
M <u>i</u> n Peak Width (min): M <u>a</u> x Peak Width (min):		Time <u>L</u> imit (min): 15.00
MSD m/z Detection		·
Peak Slope (counts/sec):	500	<u>Collector Delay (min):</u> 0.20
Threshold (counts):	2000	Mass <u>W</u> indow (+/-): 1.0
A	nalog Outpu	t Range (kilo counts/volt): 2000
Other Detector		
P <u>e</u> ak Slope (mv/sec):	10	Collector <u>D</u> elay (min): 0.01
Thresh <u>o</u> ld (%):	5.0	Eull Scale (volts): 0.1
Γοκ		ncel <u>H</u> elp

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.

**Delay volumes and 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.

# **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.

# **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 24. In the **Setup Fraction Collector** dialog box general method settings are specified.

raction	Trigger	Mode						
0.01	0	Use Tineta	de .	Peak-based		Max. Pea	k Duration 0.5 min	
eak De	tectors							
De	tector	Working Ho	de U	p Slope [Unit/s]	Down Slope [Unit/s]	Threshold [Unit]	Upper Threshold [Unit	Unit
1 0	DAD1	Threshold/Slo	pe	5.00	10.00	10.000	3000.000	mAU
	(i) at le	elected peak aast one selec		ak detector				Intert
	(i) at le	ast one selec	ed pea	ak detector	stion [min] # of Fract	tions Timesfices		Insert Append
	(i at le	ast one selec	ed pea	ak detector	ation [min] # of Fract	ions Timeslices		Linsert Append Cut Copy
met abl	(i at le	ast one selec	ed pea	sk detector Max. Peak Dure	tion (min) # of Fract			Insert Append Cut
met abl	(Fatle	Trigger	ed pea	ik detector Max. Peak Dura Rinse F	Fraction Collection Ne	edie	[min] Auxiliary Max. fill volume per	Insert Append Cut Copy Paste
met abl	(Fatle	as Pump	ed pea	ik detector Max. Peak Dura Rinse F		edie	[min] Auxiliary Max. fill volume per as configured 🚍	Insert Append Cut Copy Paste
met abl	(i at le Time ine:	as Pump	led pea	A detector Max. Peak Dura (Rinse F	Fraction Collection Ne	eda	[min] Auxiliary Max. fill volume per as configured	Insert Append Cut Copy Paste Iocation nl e signal
inetabl ine <u>S</u> topti	(i at le Time ine:	as Pump	nd pea	A detector Max. Peak Dura (Rinse F	fraction Collection Ne	eda	[min] Auxiliary Max. fill volume per as configured 🚍	Insert Append Cut Copy Paste Iocation m e signal or signal

Figure 24 Set up Fraction Collector dialog box

#### 2 Configuration and Operation of the Fraction Collector Setting up a Fraction Collector Method

# **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 1200 Series 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 25. The 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.

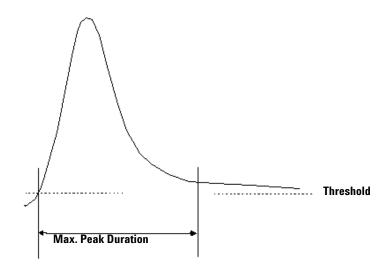


Figure 25 Maximum Fraction Duration

#### **Peak Detectors**

In the **Peak Detectors** section a list of all Peak Detectors that are connected to the system is displayed. Agilent 1200 Series 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 72.

**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  $\ensuremath{\mathsf{Setup}}\xspace$  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.

#### **Rinse Fraction Collection Needle (Analytical Scale only)**

If Discrete Fractions is selected as Collection Mode (see also"Collection Mode" on page 48), 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**.

Setting up a Fraction Collector Method

# **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 26) in the Peak Detectors section.

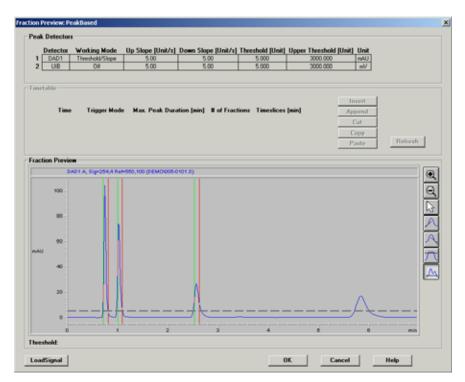


Figure 26 Fraction Preview dialog box

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 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 1200 Series 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 27 will be displayed. Click on **Yes** to reset all fill levels from the previous run.





## CAUTION

The window (Figure 27) 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 overfill 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 28) dialog box in the **RunControl** menu, in the **Sequence Parameters** (Figure 29) dialog box or in the **Sequence Table** in the **Sequence** menu. All fraction start locations are entered in the following formats.

#### 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.

sle Info: Instrum	ent 1		
perator Name:	Bio Chemist		
lata File	·		
Prefix/Counter	@ Manual	Filename DEFAULT.D	
Subdirectory: P	ROTEOME	_	
Path: C:\HPCHE	M\1\DATA\		
ample Paramete	rs		
		Logation: Vier a Salank run if no entry)	
	Ere	action Start Location: 1-P1-A-01	
Sample <u>N</u> ame:	Sample_v.	Plate ID:	
Sample <u>A</u> mount:	0	Multiplier: 1	
STD Amount	0	Dilution: 1	

Figure 28 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.

#### 2 Configuration and Operation of the Fraction Collector

Starting your run with fraction collection

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.

Data File	< Bur Carbo Dender
Pata / Profix/Counter	E Une le Sequenze
Prete: Counter.	Ou is how route minorettek P injurit unyway
Subdirectory: PROTEOME Path: C1/HPCHEM(11/DATA)	# Dav't spert
Part of methods to run According to Runtime Checklist	Shuldown © Post-Sequence Cred / Macro
Files Sequence Table intermeter	macro "SHUTDOWN MAC".go 💌
WailTime min (nher loading a new method)	nRdy Timeout. imin min
Fraction Information Eraction Start Locatio	
equence Comment	

Figure 29 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.
- 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 click on the **Change** button in your **Online Plot** window. Then check **Show fraction collection ticks** in the **Edit Signal Plot** window (Figure 30).

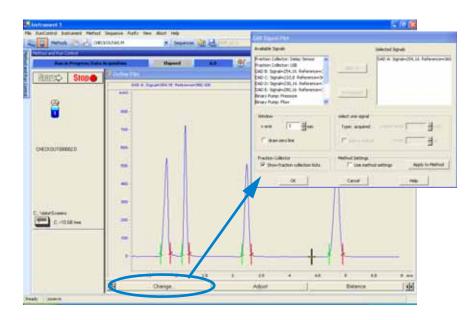


Figure 30 Online Tick Marks

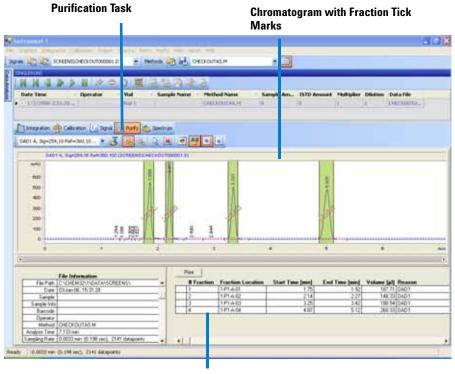
2 Configuration and Operation of the Fraction Collector Viewing your Results

# **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 31.



Fraction table

Figure 31 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.

lestination —		Quantitative Results
Printer	I Screen	
r miller	- Gereen	Calculate: Percent -
	-File Type	Based On: Area 💌
File	F.TXT F.WMF	Based On: Area
File Prefix		Sorted By: Signal 💌
	E.DIF E.CSV	
Report	T XLS T.HTM	Signal Options
tyle		college of second
keport Style: S		
		d Fraction Table and Ticks
Add Chromato	gram Output	d Fraction Table and Ticks d Summed Peaks Table
Add Chromato	gram Output Add	
Add Chromato Report Layout C Separately	gram Output	d Summed Peaks Table
	gram Output For Uncalibrated Peaks © With Calibrated Peaks	C Do Not Report
Add Chromato Report Layout C Separately hromatogram Or Portrait	gram Output Add For Uscalibrated Peaks © With Calibrated Peaks stput	C Do Not Report
Add Chromato Report Layout C Separately bromatogram Or Portrait C Landsca	gram Output Add For Uncalibrated Peaks & With Calibrated Peaks stput Size	C Do Not Report
Add Chromato Report Layout C Separately bromatogram Or Portrait C Landsca	gram Output Add For Uscalibrated Peaks © With Calibrated Peaks stput Sin pe or (Lendecape)	C Do Not Report

Figure 32 Fraction collection report setup

2 Configuration and Operation of the Fraction Collector Special Applications

# **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 33 on page 80. In this sequence the Sample 1 will be injected from Plate 1 Position A1 of the Agilent 1200 Series 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						



## CAUTION

If you specify multiple injections for pooling, the overfill 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 overfilled, an error message occurs and the pump is shut OFF.

## **Sample Recovery**

The Agilent 1200 Series fraction collectors offers 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 34. The fourth fraction collector in your systems will always be used for sample recovery. Also read "Configuring multiple fraction collectors" on page 50.

Fraction Colle	ctor 2 Configuration : Instrument 1		×
Trays Tray A:	40 Funnels	▼ Use for Sample Recovery	
Tray B:	40 Funnels	Tube Volume [ml]: 0.00	
Tray C:	40 Funnels	Tube Height [mm]: 0	

Figure 34 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 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

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 (G1364-63113)

The procedures to exchange these parts are described in "Replacing the Inlet/Waste Tubings" on page 141, "Replacing the Valve to Needle Tubing" on page 145, "Exchanging the Analytical Needle Assembly" on page 150, and "Exchanging the Internal Tray" on page 156.

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 35 on page 83. For further information on delay volumes and delay volume calibration see "Delay volumes and delay calibration" on page 52.

Inner Capillary	Needle Type	
Diameter: 0.80 💌 mm	Needle Type:	semi-prep needle 💌 no needle micro needle
<u><u> </u></u>	Cancel	analytical needle semi-prep needle prep needle <u>neep</u>

Figure 35 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.

2 Configuration and Operation of the Fraction Collector Optimizing fraction collection

# **Optimizing fraction collection**

Time-based fraction collection	<ul> <li><i>Time slices</i> must have a length of at least 0.05 min.</li> <li>Set # of <i>Fractions</i> such that length of resulting fractions is at least 0.05 min.</li> </ul>
Peak-based fraction collection	<ul> <li>Set threshold and slope values such that length of fractions is at least 0.05 min.</li> <li>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.</li> </ul>
	• 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 <i>Rinse Fraction Collection Needle</i> is set to <i>Between fraction collection</i> , 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 $V_{\rm D1}$ 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 <b>between fraction collection</b> is ignored.
Needle Movement	The option <b>into location</b> under <b>Needle Movement</b> 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 <b>Movement failed</b> 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, overfill 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.

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

# **Check-out Procedures**

The purpose of the check-out injection is the verification of the correct installation and configuration of the Agilent 1200 Series purification system. In addition the checkout procedure can used for troubleshooting, if the Agilent 1200 Series 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 1200 Series Purification system manual G2262-90010.

## Check-out procedure for UV-triggered fraction collection AS

#### Prerequisites

- ✓ The Agilent 1200 Series LC system has been flushed thoroughly with the liquid phase as described in Table 14 and the lamp(s) of the UV detector have been switched on for at least 15 minutes to provide a stable baseline.
- ✓ The Agilent 1200 Series 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 57.
- ✓ 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.

Agilent 1200 Series modules	<ul> <li>standard or micro degasser</li> <li>isocratic, quaternary or binary pump</li> <li>autosampler/ well plate sampler</li> <li>column compartment</li> <li>diode-array detector, multi-wavelength detector or variable wavelength detector with analytical flow cell</li> <li>fraction collector analytical scale</li> </ul>
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 μΙ
Column temperature	40.0 <sup>0</sup> 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

 Table 14
 Method parameters for UV-triggered fraction collection check-out AS

# 2 Configuration and Operation of the Fraction Collector

**Check-out Procedures** 

#### **Expected result**

Run a single injection under the conditions as described in Table 14. You should observe a chromatogram with four peaks as displayed in Figure 36 on page 88. 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).

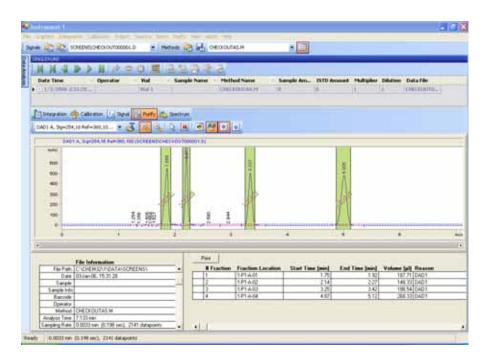


Figure 36 Chromatogram of the check-out injection with UV-based trigger

# **Check-out procedure for UV-triggered fraction collection PS**

#### Prerequisites

- ✓ The Agilent 1200 Series LC system has been flushed thoroughly with the liquid phase as described in Table 14 and the lamp(s) of the UV detector have been switched on for at least 15 minutes to provide a stable baseline.
- ✓ The Agilent 1200 Series 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 57.
- ✓ 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.

Agilent 1200 Series modules	<ul> <li>preparative pump plus gradient extension (optional)</li> <li>preparative autosampler/ dual-loop autosampler</li> <li>diode-array detector or multi-wavelength detector with preparative flow cell<sup>*</sup></li> <li>fraction collector preparative scale</li> </ul>
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)	> 0.1min (2s)
Injection volume	50 µl
Column temperature	ambient
Fraction trigger mode	Peak-based

 Table 15
 Method parameters for UV-triggered fraction collection check-out PS

#### 2 Configuration and Operation of the Fraction Collector

**Check-out Procedures** 

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

 Table 15
 Method parameters for UV-triggered fraction collection check-out PS

\* 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 15. You should observe a chromatogram with four peaks as displayed in Figure 36 on page 88. 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**

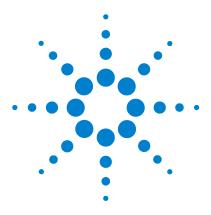
More information about the Agilent 1200 Series fraction collectors and Purification Systems are available from a series of application notes. Printed versions can be ordered from Agilent or pdf-files can be downloaded from the Agilent Website

http://www.chem.agilent.com

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

#### Table 16 Selected Agilent Technologies Application Notes

#### 2 **Configuration and Operation of the Fraction Collector Application Notes**



Agilent 1200 Series Fraction Collectors User Manual

# 3 Control Module Screens of the Fraction Collector

Major keys on the Agilent 1200 Series control module 94 Screens available from the Analysis view 95 Screens available from the System view 109

This chapter is intended to introduce an operator to the functionality available for operation of the Agilent 1200 Series fraction collector with the Agilent 1200 Series control module.

Please use the manual of Control module for further detailed reference.



# Major keys on the Agilent 1200 Series control module

ESC	SC Return to previous screen, abort any change of parameters and toggle betwee the last two top layer views					
m	Open context sensitive menus					
i	Information/Help					
Enter	Store changed parameters or execute a choice					
Done	(If available) Activate settings of current screen					
On/Off	Switch on individual Instrument(s) or complete System					
Start	Start a location range or sequence					
Plot	View online signals					
Views Change between analysis - (samples)- status - system views						

## NOTE

The screens shown on the next pages are based on: Control Module (G1323B) firmware revision B.03.0x and higher LC Module firmware revision A.05.06 and higher

## NOTE

In case the control module's display seems to be frozen (hang-up due to a communication problem on the CAN bus), unplug the control module from the HPLC module and reconnect.

# Screens available from the Analysis view

**Analysis view** This is the wake-up screen, if the Agilent 1200 Series fraction collector or thermostatted fraction collector is the only configured Agilent 1200 Series LC module.

Analysis	Line 🛛 Loc	o. <u>O</u> lnj.	# 🛛 Time	0.00 Idle	e Ready
Mon 10:55	31 - 1	# 1)	<b>★ACTUAL</b>	-*\$) 🕲 10	0.00 Start
	4	Manual Trigge	). F		10
					On/Off
		P1A:01			
Settings	Timetable	Metho	od 👔 Se	quence	Views
Analysis	Line 🛛 Loc	o. Olinj.	# 0 Time	0.00 Idle	Ready
Mon 10:55 [	31 - 1	# 1)	À *ACTUAL	-*\$ 🚯 10	0.00 Start
		Manual Trigge	эг		1 0
	Ter		-		On/Off
		▶ 17.5 °i P1 A:01	0		<u>    </u> Plot
Settings	Timetable	Metho	od 🚺 Se	quence	Views

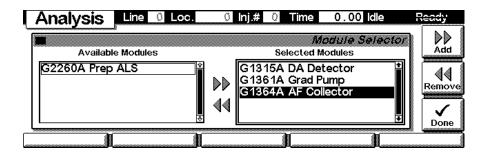
**On/Off** (**F7**) allows you to turn on the Thermostat Module of the thermostatted versions of the fraction collector.

The **m**-key allows access to the context sensitive menus. **Date&Time** allows you to change time settings. **Print Screen** gives access to the print screen. **About** tells you the current firmware revision and the serial# of your control module. **Setup view** leads you to analysis view configuration for additional Agilent 1200 Series LC modules. **Restart** re-boots the control module. If multiple instruments are configured in the Analysis screen the context menu contents will vary, depending on the cursor position, when the m-key is pressed (depending on highlighted module).

Screens available from the Analysis view

Analysis	Line 🛛 Loc.	0 Inj.# 0	Time 0	.00 Idle	Ready
Mon 10:56 🖪	1 - 1 ;	₩ 1 📾 + Δ Analvsis	≏TUAL * \$	③ 10.0	00 🛋
		1Date&Time			
	(	2Print Scree 3 About	n		On/Off
		4 Setup View	<i>י</i>		
	F	5 Restart			
Settings	Timetable	Method	Sequer		Views

In the Setup view, modules can be added or removed to the analysis view. Select a module, you want to move, with the help of the **selection**-keys. Use function keys **F7/F8** (**Remove/Add**) to move the highlighted module. Changes must be activated with **Done** (**F6**).



Here, e.g. the binary pump, the injector and the detector parameters are shown on the display as well. The number of parameters for each module is reduced as additional modules are added to the display. A maximum of 4 modules can be shown simultaneously. If more modules are connected to the system, you have to choose 4 of them in **Setup view**.

Screens available from the Analysis view

Analysis	Line 🕴 Loc.	0 Inj.# 0 Time	0.00 Idle	Ready
Mon 10:57	1] - 1#	1) 📸 * ACTUAI	.** 🕲 10.0	0
	× 000 0			Start
Flow [11/4]	μl <u>900.0</u> μl	<u>Α</u> λ Β₩ Samp 254 4	Manual Trigge	
0.000		Ref 360 100		
① 1 bar	0.0µl	A ~6.735	P1A:01	<u>     _1</u>    Plot
Settings	Timetable		equence	Views

# **Settings** With the **F1**-key (**Settings**) you open a pull-down menu where you can select the fraction collector module.

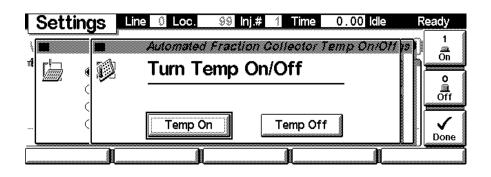
Analysis	Line 🛛 Loc.	0 Inj.# 0 Time	0.00 ldle	Ready
Mon 10:59	1- 1#	1 🗟 * ACTUA	L*\$ 🚯 10.0	
8		X Ready	)	Start
1HPLC System	<b>γ 🖉 900.0</b> μ	I <mark>A</mark> 入 В₩	Manual Trigge	म 10
2 Grad Prep Pump		Samp 254 4	-	On/Off
<u> ≗</u> Prep ALS		Ref 360 100		
<u>4</u> DA Detector				
5 AF Collector	0.0µl	13.022℃	P1A:01	Plot
Settings	Timetable	Method S	equence	Views

Within **Settings** you can change the fraction collector parameters like the type of fraction collection (**Off, Timetable, or Peak Controlled**). There are different sets of parameters available through the **F1-5** navigation-keys for setting path operation of the fraction collector. **F7** (**Default**) resets the fraction collector to default values. Changes must be acknowledged with **F6** (**Done**).

S	ettings	Line 🛛 Loc.	0 Inj.# 0	Time 0.	00 Idle	Ready
^		Auto	ma <b>led</b> Fracti	on Collect	or Setting	<b>.</b> 1 2
Ħ.		⊖ Off				On/Off
n n	raction	○ Timetable				Default
	Mode	Peak Controlled	Max. Peak D	uration 0.	50 min	Done
1	Nore	Timetable	Detectors	Thermos	tat 👔 🛛 F	Runtimes

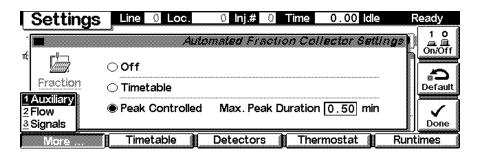
Settings screen with thermostat connected. **On/Off (F8)** allows you to switch the thermostat on or off.

Screens available from the Analysis view



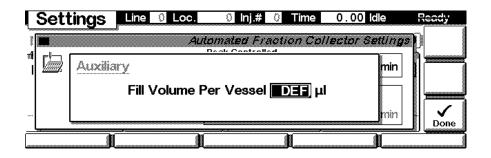
Use the **m**-key for the context sensitive menu. The **Status** command pulls up a module specific screen displaying details of configured trays. **Reset** will initialize the fraction collector.

Settings More... The F1-key (More) opens a pull-down menu.

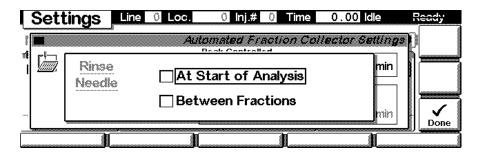


By selecting **More...Auxiliary** on the pull-down menu you can enter the fraction collector **Fill Volume Per Vessel** for the currently installed trays and vessels. Leaving this value at the **"DEF"** value will completely fill the vessels according to their specified size. Changes must be acknowledged with **F6** (**Done**).

Screens available from the Analysis view

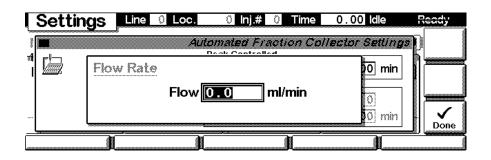


By selecting **More...Rinse Needle** on the pull-down menu you can specify when the needle is rinsed. Settings can be **Off** (both tick marks not selected), **At Start of Analysis or Between Fractions**. Rinsing the needle between fractions is only available, for peak-based fraction collection with the analytical scale fraction collector G1364C. Changes must be acknowledged with **F6** (**Done**).

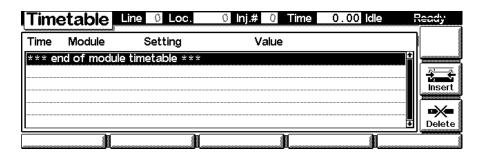


By selecting **More...Flow** on the pull-down menu you can specify the flow rate of the connected pump, in case the pump is a non-1200 Series pump or if the pump is not connected to the fraction collector via CAN.

Screens available from the Analysis view



# Settings -With the F2-key (Timetable) in the Settings screen you can list the timetableTimetablefor the fraction collector. Press F7-key (Insert) to edit the selected entry orF6-key (Delete) to remove the selected/highlighted entry.



Edit each entry line according to your needs, by entering a time for a Setting and by selecting a Setting with the help of the pull-down menu. Use **F7** (**Enter**) to insert the current settings. Changes must be acknowledged with **F6** (**Done**).

	Timetable	Line 🚺 Loc.	99 Inj.# 1 Time	0.00 Idle	F	Ready
Ē		<i>k</i>	7;	metable Inser	ลา	
lī	Time [min]	Module	Setting			
ľ	0.00	AF Collector	C Trigger Mod	e 🗄		
ŀ		Mode Off	t O	.00		Enter
ľ	- কি	fields change v	vith setting selected	-D	÷	Done
	Ĭ		Ĭ			_

Use the  $\mathbf{m}$ -key for the context sensitive menu. It gives you additional tools for the timetable.

In the timetable several trigger modes can be selected. Peak-based trigger can be specified for defined time frames during the run. For **time-based** trigger either the **duration of a fraction** or the **number of fractions per time interval** can be chosen.

Time	Module	Setting	Value	
0.00	AF Collector	Trigger Mode	1.00 min each fra	ction 🏻 🏳
10.00	) AF Collector	Trigger Mode	Off	
*** er	id of module ti	metable ***		

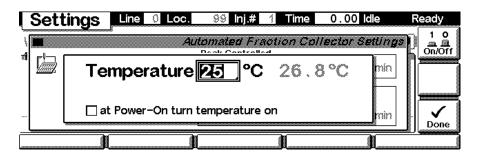
Time	etable	Line 🛛 🛛	Loc.	0 inj.#	0 Ti	ne (	).00 Idle	F	Ready *
Time	Module	Set	ting	v	alue				
1.00	AF Collec	ctor Trig	ger Mode	5	fractic	ns per	run	ŧ	
10.00	) AF Collec	ctor Trig	ger Mode	0	ff				<u></u>
*** er	d of modu	ule timeta	ble ***						Insert
									⋼⋈⋹
								ŧ	Delete
	1		1				1		

Screens available from the Analysis view

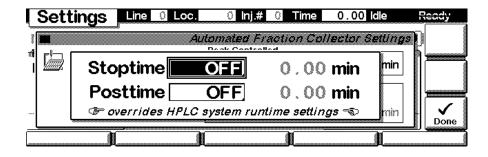
Settings - With the F3-key (Detectors) in the Settings screen you can configure and
 Detectors Enable (F8) or Disable (F7) any detector that is part of a system for peak detection. Changes must be acknowledged with F6 (Done).

Settings	s Line O	Loc.	0 Inj.#	i 🛛 Time	0.00 dle	Ready		
	Detector	Use	Upslope	Downslope	Threshold			
	DA Detector	$\checkmark$	OFF	OFF	5.000			
Peak Detectors						Disable		
Use MSD for Mass-based Fraction Collection								
Fraction collected, v				tect a peak ector detects	a peak	Done		

Settings -With the F4-key (Thermostat) in the Settings screen you can set the fractionThermostatcollector thermostat temperature. Changes must be acknowledged with F6<br/>(Done).



Settings -With the F5-key (Runtimes) in the Settings screen you can change the stopRun timestime and the post-run time for the selected module individually. Changes must<br/>be acknowledged with F6 (Done).



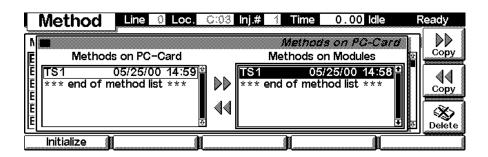
Method screens Use Esc to return to the Analysis screen. Use the F3-key (Method) to view the parameters in a method. Use the F8-key (Save As) to save the method in the module(s). The PC-Card-key is only active when a PCMCIA card is inserted in the control module. Module (F1) and Method (F2) give access to methods stored on the Agilent 1200Series LC module or the PC-Card. Timetable (F3) gives access to the timetable of a method (if specified).

## NOTE

The PCMCIA card must have been inserted into the control module prior to the last start-up in order to be recognized.

Metho	Line 🛛 Loc.	99 Inj.# 1 Time	0.00 Idle	Ready
Module	Setting	<b>Value</b>	à	
Grad Pump	Stoptime	OFF		Save As
Grad Pump	Posttime	OFF		
Grad Pump	Flow	0.000 ml/min		∎ <b></b>
Grad Pump	Lower Limits	0 bar		
Grad Pump	Upper Limit	400 bar		
Grad Pump	%B	0.0 %		■ Print
Module	PC-Card	Timetable		

After pressing **F1** or **F2** use the **Right/Left** selection keys to switch between PC-Card and Instrument window.Use the **Up/Down** selection keys to select the method. Use **F7/F8** (**Copy**) to copy a selected method from one window to the other one. Use **F6** (**Delete**) to delete a selected method.



Press the **m**-key to open the context sensitive menu for printing the method directory.

Method	Line	Loc.	C:03 Inj.#	1 Time	0.00 Idle	Ready
Name TS1	Date 05/25/00 method list	14:5°	User Comme Methoa 1Print Direc	ent	on Module ⊕	S Load Delete

sequence In the sequence view (F4 from the Analysis Screen) you can specify the sequence's End Actions (F1), load sequences from PC-Card (F2) (if present) or proceed to a method table (F3). F6 (Delete) and F7 (Insert) allow you to edit lines in the sequence table.

Se	quen	ce Lin	e 🛛 L	oc.	99 Inj.#	1 Time	0.00 <b>id</b> le	Ready
Line	From	То	#	lnj.Vol	Method	Wait	Calibration	
***	end of	sequence	***					
								Insert
<u> </u>		- 410 - 124	0.0					
End	l Actior		C-Card		iviethod	U	U	LOGDOOK

K	Sequence 🛄	ne 🛛 Loc. 🛛 🖇	9 Inj.# 1 Time	0.00 ldle	Ready
٦	•			End Action	a) (
Ē	On end of		Method 🛛 🖈 A	CTUAL * 🏻	
	■ Sequence	🖲 Turn	off Nothing		
	Timeout 💽	min O Turn	off complete l	HPLC system	
	1	l	l		

Parameters that can be entered into a line in the sequence table.

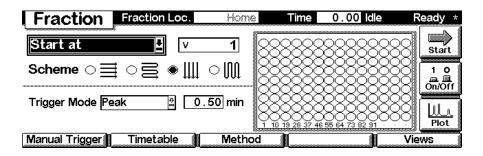
Sequence	Line 🛛 🛛 L	.oc. 99	nj.# 1 Time	0.00 Idle	Ready
			Sequence	e Insert Line 1	<u>ה</u>
Vial 🚺	]to 🗌	1 #lnj. 🗌	1 Vol	DEF 🕄 µl	
Method 🛛 A	CTUAL *	∗ ⊉ Wait	after loading	0.00 min	Enter
j 🖉 use 'i	Salibration	r' to apply d	alibration set	ings 🐨	Done
Calibration			Ĩ	1	

Screens available from the Analysis view

#### **Fractions** From the Analysis View press the **F5**-key (Views) and select **Fractions**.

Analysis 🛄	ne 🛛 Loc.	99 Inj.# 1	Time 0.00 ld	le Ready
Wed 09:10 🖪 9	9 - 100 #	1) 🗟 * AC	TUAL*:	1.00
Flow 0.000	🗟 Inj Vol [	0.0µl	<u>Α</u> λ	1 Analysis
0.000   5	<u></u>		Sample 25	2 Samples
%B0.0	×в)		Reference 36	3 Fractions
0 bar	<b>8</b> 99;	0.0µl	🔒 -69.496	5 System lot
Settings T	ïmetable	Method	Sequence	Views

This is an example if an Agilent 1200 Series fraction collector is configured stand-alone. Here you can define a **Start** position for a fraction, select a collection **Scheme**, choose a **Trigger Mode** etc.



status From the Analysis View press the F5-key (Views) and select Status.

Analysis Line	0 Loc.	99 Inj.# 1	Time 0.00 k	dle Ready
Wed 09:10 🗄 99	) - 100 #	1) 📸 * AC	TUAL * 🕄 🚯	1.00
Flow 0.000 ml 🛣	Inj Vol	0.0µl	Α λ	
		<u> </u>	Sample 25	
%B_0.0	J		Reference 30	3 Fractions
① 0 bar	<b>8</b> 99;	0.0µ1	1 -69.49	5 <b>System</b> ot
Settings 📔 Tin	netable	Method	Sequence	Views

This is an example if an Agilent 1200 fraction collector is configured stand-alone.

St	atus	Line	0 Loc.	0 Inj.#	0 Time	0.00 Idle	Ready
	Locat	ion	<b>P1</b>	A:1			Start
	Trigge	er			Vol.	μ	
	Installed <sup>-</sup>	Trays ′	126 Tubes				Plot
			Elapse	ed 0.00 mi	n		

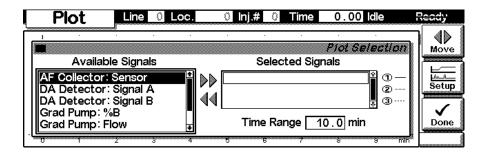
**Signal plot** Press **F6**-key (**Plot**) to enter the plot screen (available also from all other screens). Here you can observe the on-line signal(s). If more than one signal is configured, use the 1-2-3 alphanumeric keys to switch between the signals.

	Plot	Lir	ne Ö	Loc.	Û	nj.# 🛛 🕄	Time	0.00	ldle	Ready
1	•									
8-					÷			-	÷	Cursor
61										
2	:	:	:		1	:	:	:	:	
0		÷			÷			÷		Rescale
-2-										
-6-			÷		÷		:	:	÷	
-8-	:		:					:		Select
-10 1-	·····	2	3	4	5	6	····· <del>/</del> ·····	8		min

Press the **m**-key to open the context sensitive menu.

	Plot	Lir	ne 🛛 L	oc.	0 In	j.# 🛛	Time	0.0	ldle	Ready
1	:		:	:		:	:	:	:	– lut
81 61		-	-	:	:	:		:	÷	Cursor
4	· · · · · · · · · · · · · · · · · · ·		••••	· · · · · · · · ·	<i>P1</i> ,		••••	••••	• • • • • • • • •	····   I 1
ō-	:	-	:	:	<u>1</u> Clear 2 Print			÷	:	Rescale
-2-					≟ 3 Maxi					
-6-	÷	-	-					÷	÷	Select
-10		· ·	····;····	····;····	·····	····	·····	····;····	·····	nin

Press **F6**-key (**Select**). Here you can add additional on-line signals (maximum are 3). Additional signals could be also pressure or temperature signals from other modules. Use the **Right/Left** selection keys to switch between 'Available Signals' and 'Selected Signals'. Use the **F8**-key (**Move**) to enter available signals into the box for selected signals or vice versa. Use the **F7**-key (**Setup**) to enter the display range for the highlighted/selected signal. Use **F6** (**Done**) to activate changes and to return to the Plot screen.



# Screens available from the System view

**System view** Use the **Esc**-key until you receive **Views** on the **F5**-key. Choose **System** from the pull-down menu. This screen shows the last activities in the system.

Analysis	Line	Loc.	99 <b>Inj.#</b> 1	Time	0.00	ldle	Ready
Wed 09:10	<b>99</b> -	100 #	1) 🗟 * A(	TUA	L * \$ 🕲	1.1	00 start
X				) 🖾 🚃			
Flow 0.000	nl/ nin 8A 100	lnj Vol [	0.0µl	<u>A</u>		λ <u>1</u> Ar	alysis
0.000				S	ample 🗌		mples off
%B_0.0	(%B) 0			Refe	rence 🗌		actions
🗘 0 b	ar ]	3 99;	0.0µI	]	-69.4	.9€ <u>5</u> Sy	stem lot
Settings	Timeta	able 👔	Method	Se Se	equence		Views
System	Pump [		Lamp 🗌	Time	0.00	ldle	Ready
Joystein					0.00		intectury
Wed 09:25							EMF Start
Module	Message			ld	Date	Time	
Prep Pump 2	Gradient	prep pum	p partner	STR	TE 07/11	09:19:2	
Prep Pump 1			p solvent A	STR	TE 07/11	09:10:2	24 On/Off
AF Collector		changed		STR	TE 07/11	08:51:2	
Prep Pump 2	Purge va					09:16:5	
Prep Pump 1	Purge va	lve off		STR	TE 07/11	09:07:5	53 FIOT

Tests

Records

Views

System - ControlUse the F1-key (Control) to select the fraction collector. Here you receive<br/>information about the not-Ready conditions, if needed. Press F2 to Home the<br/>transport unit (e.g. for replacing trays), press F3 to Park the carrier for<br/>transportation (also requires a protective foam and cardboard holder to avoid<br/>damage to the transport unit during transportation). F7 (Reset) does a<br/>re-initialization of the fraction collector. Use the F8-key (On/Off) to turn on<br/>the thermostat (if present). Changes must be acknowledged with F6 (Done).

Configure

Control

Screens available from the System view

System	Pump 🗌	Lamp 🗖	Time	0.0	0 Idle	Ready
Mon 11:04	LAMP OFF,LAMP VIS	S OFF				EXF Start
1 HPLC System	Message		ld	Date	Time	
2 Prep Pump 1 🖡	Tray changed: 126 T	Tubes	INFO	07/09		
🗄 Prep Pump 2 📔	Initialization done		=	E 07/09		:49 On/Off
4Prep ALS	Setpoint changed			E 07/09		
5 DA Detector	Side door closed				10:54	
6 AF Collector	Front door closed		5111	07/09	10:54	:06 🕁 🔜 Plot
			20			6 df
Control	Configure	Tests	R	ecords		Views
Control Control	Configure	Tests	Time		0 Idle	Ready
	Pump 🗆		Time	0.0		Ready
Control	Pump	Lamp 🗖	Time	0.0		Ready
	Pump	Lamp 🗖	Time	0.0		Ready
	Pump	Lamp 🗖	Time	0.0		Ready []
	Pump	Lamp 🗖	Time	0.0		Ready
	Pump	Lamp 🗖	Time	0.0		Ready []
	Pump	Lamp 🗖	Time	0.0		Ready []

Manual Trigger
 In this screen you can view the online signal of any connected detector. Press
 F6 (Select) to choose, which detector signal will be displayed for identifying
 the starting point and the end point of a fraction. The Manual Trigger Mode
 allows to manually start a fraction collection by pressing 1-Trigger (F8).
 Pressing 0-Trigger, again, will then stop the collection of a fraction. The
 fraction collector will wait for the delay-time, specified for the chosen
 detector, before it starts or stops the collection of a fraction.

Tri	gger	Pu	mp 🗖		Lan	np 🗌	Time	0.0	0 Idle	Ready
8	: : : :			N	lo Delay	-		-		1 Trigger
4 · · · · 2 · 0 ·	· · · · · · · · · · · · · · · · · · ·	· · · · · · · · · · · · · · · · · · ·			· · · · · · · · · · · · · · · · · · ·					
-2 - -4 - · · · -6 - -8 -		· · · · · · · · · · · · · · · · · · ·						<u>1 No I</u> 2 DA	Delay Detecto	
-10	·····	······ 2	·····	4		6	·····	8	9	min

System -Use the Esc-key to receive Views on the F5-key in the Analysis Screen. ChooseConfigureSystem from the pull-down menu. Use the F2-key (Configure) to select the<br/>fraction collector. Here you define further special set points for the fraction<br/>collector operation. Changes must be acknowledged with F6 (Done).

System	Pump 🗌	Lamp 🗖	Time 0.0	0 Idle	Ready
Mon 11:06	LAMP OFF,LAM	VIP VIS OFF			EMF Start
Module	1HPLC System		ld Date	Time	
AF Collector	2 Prep Pump 1		INF0 07/09	0 10:55:0	
	3Prep Pump 2	he	5TRIE 07/09		
AF Collector	4 Prep ALS	)	STRIE 07/09	) 10:54:(	D6   🕂
AF Collector	5 DA Detector	d	5TRTE 07/09	) 10:54:0	06    <u>[Jll.r.</u> ]
AF Collector	6 AF Collector	ed	5TRTE 07/09	) 10:54:0	06 <sub>⊕</sub> Plot
Control	Configure	Tests	Records	1	Views

,	Co	nfig	Pump 🗖	Lamp 🗌	Time	0.00 ldla	e Ready
"   		Options	Automat	ed Fraction Co	illector	Configur	
	Inter	faces	Trays	Fraction	Ther	mostat 👔	Needle Move

Use the **F1**-key (**Interfaces**) to access the interface settings (if required). Changes must be acknowledged with **F6** (**Done**).

Config	Pump 🗌	Lamp 🗌	Time	0.00 Idle	Ready
	Automat	ed Fraction Co	liector C	ontiguratio	<b>n</b> ]
Options					] [   ]
<u>2 Serial</u> ∂ MIO					
4BCD Output					Done
Interfaces	Trays	Fraction	JI	Ne	edle Move 🥤

Screens available from the System view

**Configure - trays** Use the **F2**-key (**Trays**) to view and configure the (automatically) detected sample trays. Different views will come up depending on the installed trays. Enter the **Vessel Volume** and the **Vessel Height** for the vessels used with your installed tray(s). Changes must be acknowledged with **F6** (**Done**).

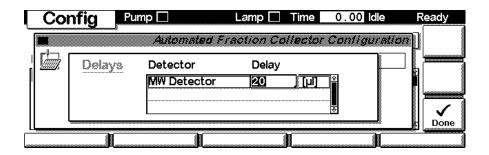
Config Pump Lamp	Time 0.00 Idle	Ready
Plate Formats     Active Plates       *384Agilent*     P4: *96Agilent*		
*384Corning* *384DeepCorning* *384DeepGreiner* *384DeepGreiner* \$		
*384Greiner* *96Agilent*		Done
Definition 👔 Delete Def. 👔 Rotate Plat	te	
Config Pump Lamp	Time 0.00 Idle	Ready
Config Pump Lamp Installed Tray	Time 0.00 Idle	Ready
	□ Time 0.00 Idle	Ready
Installed Tray		Ready
Installed Tray 126 Tubes		Ready Done

Select/highlight a tray configuration with the help of the selection keys. **F7/F8** (**Add/Remove**) allows you to move configurations from available Plate Formats to the Active Plates and vice versa. Press **F2** (**Delete Def.**) to delete a user defined definition. Press **F3** (**Rotate Plate**) to rotate the selected plate. Use **F1** (**Definition**) to edit the user defined definition of a well-plate. Changes must be acknowledged with **F6** (**Done**). Configure -<br/>fractionF3 (Fraction) in the main Configuration screen allows you to access the Delay<br/>volume definition screen, to specify Filling Order and Reserved or Recovery<br/>Locations.

Co	nfig	Tem	р 🗹 🛛 Т	ime	0.00 Idle	Ready *
	-	* Automat	ed Fraction Colli	ector	Configurati	on]]
	Options	Thermostat,Pre			Collector	
			2 Filling Order 3 Reserved Location	000		
			4 Recovery Locati			
			5 Collection Mode			
			6 Flow Path			Done
 Inter	faces	Trays	Fraction	Ther	mostat	

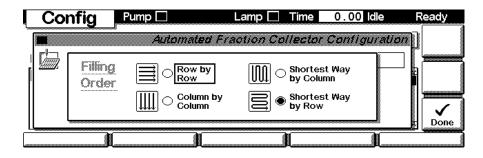
Configure -<br/>fraction - delaysDelays in the Fraction Configuration screen allows you to configure the delay<br/>volume between each connected detector and the fraction collector. Edit (F8)<br/>allows to enter new values for the selected detector. Changes must be<br/>acknowledged with F6 (Done).

	Co	nfig	Pump 🗖	Lamp 🗖	Time	0.00 Idle	Ready
L				led Fraction Co	ollector C	ontiguratio	
 [		Dela	ys Detector	Delay		_	
ľ			MW Deteo	ctor O	FF (μl) <sup>1</sup>		
Ľ		Pump	1	) I			Done



Screens available from the System view

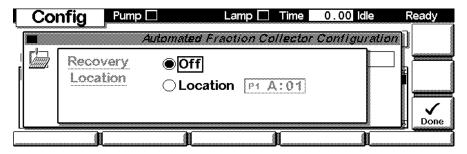
Configure -<br/>fraction -Filling Order in the Fraction Configuration screen allows you to configure the<br/>filling order of the trays in the fraction collector. Edit (F8) allows to enter new<br/>values for the selected detector. Changes must be acknowledged with F6<br/>(Done)



Configure -<br/>reservedReserved Locations allows you to specify reserved locations by Rows,<br/>Columns or Single Locations, that are not used (filled) during the collection.<br/>Changes must be acknowledged with Done (F6).

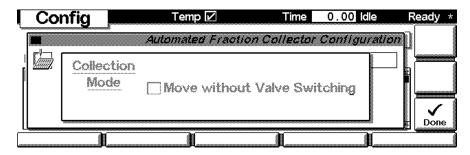
	Col	nfig	Pump		Lamp 🗌	Time	0.00 Idle	Ready
		Rese Frac Local	rved tion tions	Automated	(s)	0//8010/		
Ц			(	;>	<->	1	<:>	

**Configure -** The **Recovery Location** allows you to specify a location for the collection of any mobile phase or sample that are not collected with the fractions during the study. Changes must be acknowledged with **Done (F6)**.



#### - Configure Collection Mode

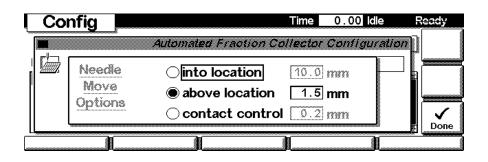
In the **Collection Mode** View it can be specified if the valve switches to waste when the needle moves to the next vessel or if the needle **Moves without Valve Switching**.



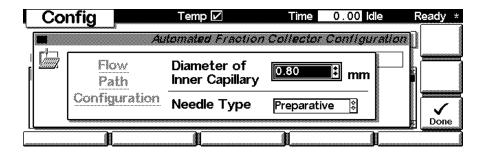
# Configure -<br/>NeedleThe Needle Movement allows you to specify the position of the needle tip<br/>during fraction collection. With into location the needle tip moves to the<br/>specified depth in the well. With above location the needle tip stays at the<br/>specified distance above the well.

In the **contact control** mode the needle tip moves to the specified distance above the bottom of the well. When the fraction is collected the needle will move upwards while staying in contact with the liquid surface of the collected fraction. This movement types enables the Agilent 1200 Series fraction collector to collect low volumes without carry-over or air bubbles at the bottom of the well. Changes must be acknowledged with **Done (F6)**.

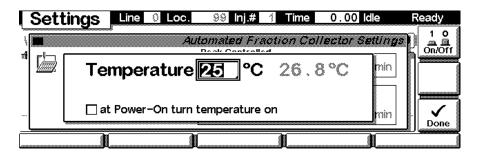
Screens available from the System view



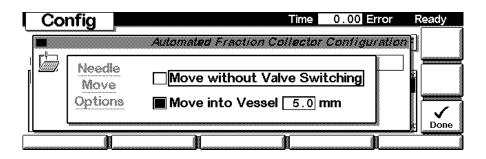
Configure -In the Flow Path configuration the diameter fraction collector capillary andFlow Paththe Needle Type can be defined.



Configure -F4 (Therm) in the main Configuration screen allows you to configure the<br/>temperature and turn on conditions of the thermostatted fraction collector.<br/>Changes must be acknowledged with F6 (Done).



Configure -Press F5 from the Configure Screen and choose how the fraction collector's<br/>needle carrier arm moves from one location to the next one. This can either be<br/>with diverting the flow to waste (with valve switching) while moving (default)<br/>or without diverting the flow (without valve switching, optional only when<br/>neighbored well plate positions are used). With the analytical scale fraction<br/>collector it is also possible to insert the needle into a capped vial or through a<br/>closing mat of a well plate with a specified depth (Move into vessel).



**System - tests** Press **F3** from the **System** View and choose the fraction collector to access the fraction collector **Tests** screen.

System	Pump 🗌	Lamp 🗌	Time (	0.00 <b>idi</b> e	Ready
Wed 09:20					ESSF
Module	Message	1HPLC System	ld Da	te Time	Start
Prep Pump 2	Gradient prep p	2 Prep Pump 1	SIRIE 07	711 09:19	:20
Prep Pump 1	Gradient prep p	<u>3</u> Prep Pump 2	STRIE 07	711 09:10	
AF Collector	Statechange (0		STRIE 07	711 0 <b>8</b> :51	:20
Prep Pump 2	Purge valve off	5 MW Detector		711 09:16	
Prep Pump 1	Purge valve off	<u>6</u> AF Collector	STRIE 07	711 09:07	:53 😛 🛛 Plot
Control	Configure	Tests	Reco	rds	Views

The Tests screen gives you access to multiple tests and features such as the automatic transport unit **Alignment** (F1) tool, maintenance procedures to **Change** (F2) parts on the fraction collector or additional test **Signals** (F3) to monitor various parameters during the fraction collector operation. **Calibration** (F4) gives you access to the delay calibration procedure. **Parts Info** (F5) gives you information about the type and revision of the installed transport unit assembly. The pull-down menu in the center of the screen gives you access to various step commands.

Screens available from the System view

Test	S 📕	-	Temp [		Time	0.00 <b>i</b> dle	Ready
			A	nomated	Fraction	Collector T	ests
	Functi	on 4 Sv	vitch	valve to	needle	-	
							Reset
Align Trans	sp.	Change		Signals	) Cal	bration	Part Info

Use the **F1**-key (**Align Trans**) to perform an automatic alignment of the fraction collector transport unit.

# WARNINGOnly the empty 4-plate tray base (G1364-84501) should be installed, when<br/>performing the alignment. The well plates must be removed from the fraction<br/>collector tray base before performing the transport unit alignment. If the well plates<br/>are installed during the alignment procedure, the transport unit might be damaged.

The alignment process may take several minutes. Changes must be acknowledged with **F6** (**Done**).

EMF (Early<br/>Maintenance<br/>Feedback)Use the F1-key (EMF) to set EMF limits. Choose Setup limits to select the<br/>number of injection valve cycles or number of needle movements into the<br/>needle seat at which you want to receive a warning. Changes must be<br/>acknowledged with F6 (Done).

Screens available from the System view

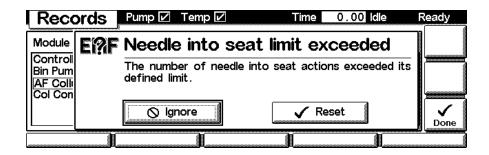
Records	Pump 🗌	Lam	o 🔲 Time	e 0.00 d	lle F	Ready
Module	Product#	Serial#	Version	On-Time	EMF	-☆- Identifv
Controller	G1323B	DE92900000	T.04.12	0d 00:20ł	1 <sup>2</sup>	
Prep Pump 1	G1361A	PR03500059	T.04.35	0d 00:21h	ר 🔳	
Prep Pump 2	G1361A	PR03500060	T.04.35	0d 00:21h	ר 🔳	
Autosampler	G1313A	DE53600103	T.04.30	0d 00:21h	ר 🔳	
1 Setup Limits	G1365A*	DE00000001	T.04.35	0d 00:21h	ר 🔳	3
2 Show Events	G1364A	DE00000000	T.00.43	0d 01:20h	J Š	Print
ENF	System Lo	g 📔 Error Lo	og 📔 I	Maint Log 🥼	FW U	pdate
Records		Temp 🗖	Time	9 0.00 ld	le F	Ready
	A	nomated Frac	tion Colli	ector EMF L	imits	
🛛 📇 G1364A	Automated	Fraction Collec	tor Seria	al# PR0000	0065 🛓	

		G1364A		<i>utomated F</i> I Fraction Co			o <i>r EMF Limi</i> PR00000065	
	<u> </u>	eedle int	o Seat		0	Limit	10000	 Reset
Ē		Ì		1		1	1	

If a set limit has been exceeded, a message box will pop up. If you press **Reset**, the limits will be removed. **Ignore** will continue to keep the EMF flag set.

### NOTE

An exceeded limit will not stop a sequence or run (information only, to plan maintenance activities).

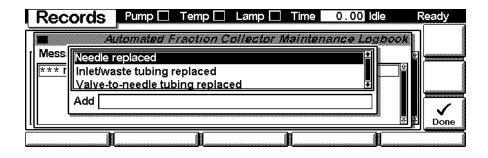


Screens available from the System view

System /Use the F2-key (System Log) or F3-key (Error Log) to look for errors. ForError Logtroubleshooting reasons these Logbooks can be printed (F6) or saved to a file<br/>on the PCMCIA card (pressing the m-key).

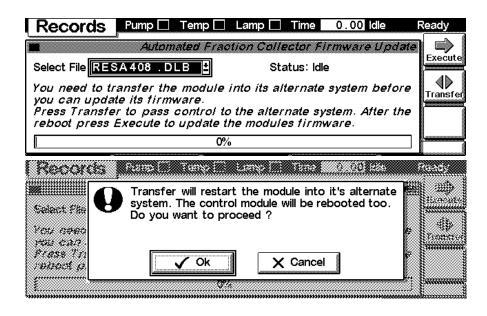
Records	Pump 🗖	Temp 🗌	Lamp 🗌	Time	0.00 Idle	Ready
	Autom	ated Fract	ion Colle	••••••	stem Logboc	<b>X</b>
Message				Date	Time	
Setpoint chang	ged				/01 08:30:25	
Side door close					/01 08:30:25 /01 08:30:25	
Setpoint change					/01 08:30:25 /01 08:30:25	
Setpoint change					/01 08:30:25	
				1		
Records	Pump 🗌	Temp 🗌	Lamp 🗌	Time	0.00 idle	Ready
r Message	AUNON	imea riac	uon com	eotor 3) Date	<i>vstem Logbo</i> Time	OK
	<u> </u>					
Setpoint char		<u>_</u>	ogbook	7/11	1/01 08:30:20 1/01 08:30:20	
Front door clo		2Des		07/1	1/01 08:30:20	5 <b>• • • • • • • • • • • • • • • • • • •</b>
Setpoint char		2000	cenu		1/01 08:30:20	
Setpoint char	nged			07/11	1/01 08:30:25	5 Print
	I			J		
Records	Pump 🗖	Temp 🗌	Lamp 🗌	Time	0.00 idle	Ready
	Auto	omated Fra	action Co	liector	Error Logbo	OR
[ Message				Date	Time	
*** no entrie	S ***					
						J I I I I I I I I I I I I I I I I I I I
		41		30		
	4					

Maintenance LogUse the F4-key (Maintenance Log) to look for maintenance activities<br/>performed on the instrument. A list of possible events are listed in the scroll<br/>screen (Needle replaced, Inlet/waste tubing replaced, Valve-to-needle<br/>tubing replaced, Funnel seals replaced, Rinse port seal replaced). Select the<br/>activity you have performed and press Done to create an entry in the logbook.<br/>Editing the add line with the help of the alphanumeric keys allows you to enter<br/>your own maintenance function. For troubleshooting reasons this Logbook can<br/>be printed or saved to a file on the PCMCIA card (pressing the m-key).<br/>Changes must be acknowledged with Done (F6).



Screens available from the System view

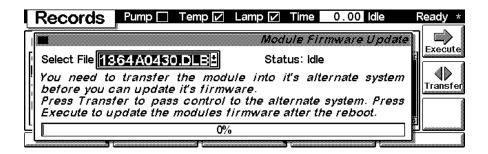
**Firmware Update** Use the **Esc**-key to receive **Views** on the **F5**-key. Choose **System** from the pull-down menu. Use the **F3**-key (**Records**) to select the fraction collector. Use the **F5**-key (**FW Update**) to enter the Update section. If you want to update the resident firmware (together with specific main firmware revisions), select the a file from the PCMCIA card (RESnnnn.DLB) and press execute. If you want to update the main firmware, press **F7**-key (**Transfer**) to turn the module into the resident mode (LED on module should blink yellow).



Use the **Esc**-key to receive **Views** on the **F5**-key. Choose **System** from the pull-down menu. Use the **F3**-key (**Records**) to select the fraction collector. Use the **F5**-key (**FW Update**) to enter the Update section. Select the a file from the PCMCIA card (1364nnn.DLB for the fraction collector) and press execute. When the update has finished the update, press **F8**-key (**Transfer**) to return the module into the normal mode (LED on module should stay yellow).

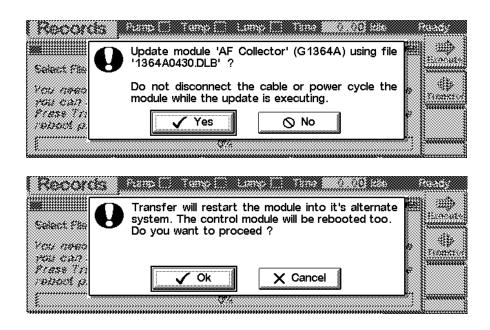
Record	S		Ti	me 0.00 I	dle	Ready
Module	Product#	Serial#	Version	On-Time	EMF	☆- Identifv
Controller	G1323B*	DE01704451	B.03.01	0d 00:00h	1	
Generic	G1364A -R	* DE01700107	A.04.30	0d 00:00h		
						Print
EMF	System	Log Error	Log	Maint Log		Jodate

Use the **F5**-key (**FW Update**) to enter the Update section. Select the a file from the PCMCIA card (1364nnnn.DLB for the fraction collector) and press execute. When the update has finished, press **F7**-key (**Transfer**) to return the module into the normal mode (LED on module should stay yellow).



If you have not saved your methods, please do it before continuing. Otherwise they will be overwritten during the update process.

Screens available from the System view



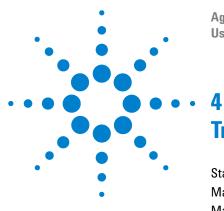
Changing the<br/>serial numberIn case the serial number of the module has to be added, use the m-key to open<br/>the menu Enter Serial#. The serial number becomes active after restart of the<br/>module.

Records Pump Temp Lamp Time 0.00 Idle	Ready
Automated Fraction Collector Firmware Update	
Select File 1364A0430.DLB	Execute
You need to transfer the mc Update rnate system before you can update its firmwark <b>1Enter Serial#</b> Press Transfer to pass control to the anernate system. After the	Transfer
Press Transfer to pass cont <del>ror to the altern</del> ate system. After the reboot press Execute to update the modules firmware.	
0%	

Type in the module's Serial# as indicated on the front or rear label of the module using the alphanumeric keys. Changes must be acknowledged with  ${\bf F6}$  (Done).

Reco	Ords Pump Temp Lamp Time 0.00 Idle	Ready
	Automated Fraction Collector Firmware Upo	iate
Select F		
You n	Product# G1364A	ore
you c Press	Serial# DE01700107	the
reboc	restart the module to activate changes 🖘	
	0%	

Screens available from the System view



Agilent 1200 Series Fraction Collectors User Manual

# **Troubleshooting and Test Functions**

Status Indicators 129 Maintenance Functions 131 Maintenance Functions 131 Transport Unit Self Alignment 133 Step Commands 135



# **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 129).

### **Error Messages**

In the event of an electronic, mechanical or hydraulic failure, the instrument generates an error message in the user interface. For a detailed description of the failure, a list of probable causes of the problem, and a list of suggested actions refer to the *Agilent 1200 Series Fraction Collectors Service Manual G1364-90110*.

# **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 131).

## **Transport Unit Self Alignment**

The transport unit alignment with the transport unit and the well-plate tray is required to compensate for larger deviations in positioning the needle carrier assembly.

The transport unit self alignment is required after disassembling the system or when you exchange the transport unit, the needle carrier assembly or the MTP main board. This function is in the **Diagnose** screen of the ChemStation or the Control Module.

#### WARNING

The sample transport self alignment requires one of the 4-well-plate trays (Part Number: G1364-84501), but well plates MUST NOT be installed!

# **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.

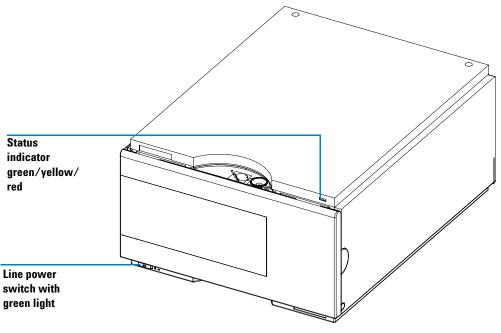


Figure 37 Location of Status Indicators

# **Power Supply Indicator**

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

# **Instrument Status Indicator**

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

- When the status indicator is OFF (and power switch light 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 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 38). In the Control Module the functions can be selected in the **Test** screens of the fraction collector.

Fraction collector Maintenance Positions	×
Change Parts	1
Ch-4	
Start	
End	
Move Arm Home Park Arm for Transport	]
Home Park Arm	
No tray Close	]

Figure 38 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:

#### 4 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  ${\sf End}$  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 Unit Self Alignment**

The transport unit alignment with the transport unit and the well-plate tray is required to compensate for larger deviations in positioning the needle carrier assembly.

The transport unit self alignment is required after disassembling the system or when you exchange the transport unit, the needle carrier assembly or the MTP main board.

This function is in the **Diagnose** screen of the ChemStation or the Control Module.

# WARNING The sample transport self alignment requires one of the 4-well-plate trays (Part Number: G1364-84501), but well plates MUST NOT be installed.

If the Transport Unit Self Alignment is started with well plates on the tray, the alignment procedure is aborted WITHOUT error message.

#### When is a Transport Unit Self Alignment Necessary?

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

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

#### How to perform a Transport Unit Self Alignment?

Steps	Comments	
I 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-well-plate tray (G1364-84501)	IMPORTANT: Remove all plates!	

#### How to perform a Transport Unit Self Alignment?

St	eps	Comments	
3	Ensure that the wellplate adapter is correctly assembled	On a G1364B preparative scale fraction collector install the delay calibration adapter G1364-87301	
4	Select the <b>Maintenance</b> menu in the <b>Diagnosis</b> view of the Agilent ChemStation.		
5	In the menu choose <b>Fraction Collector &gt;</b> <b>Transport Alignment</b> 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.	

## NOTE

If the Transport Unit Self Alignment is started with well plates on the tray, the alignment procedure is aborted without any error message.

# **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 39) in the **Diagnosis** display. In the Control Module the step commands can be accessed from the pull-down menu in the fraction collector **"Test**".

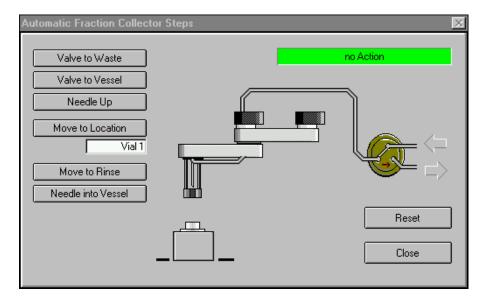


Figure 39 Fraction Collector Step Commands

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.	

 Table 17
 Step Commands

# 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.



Agilent 1200 Series Fraction Collectors User Manual

# **5 Repairing the Fraction Collector**

Cleaning the Fraction Collector 138 Overview of Main Repair Procedures 139 Simple Repairs 140



# **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 140.

#### 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.

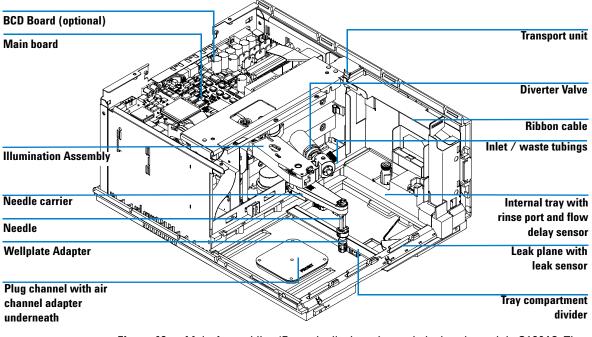
#### WARNING

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

# Repairing the Fraction Collector 5

**Overview of Main Repair Procedures** 

# **Overview of Main Repair Procedures**



**Figure 40** 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 18
 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 141
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 145
Exchanging the <b>preparative</b> needle assembly	When needle shows indication of damage or blockage	See "Exchanging the Preparative Needle Assembly" on page 148
Exchanging the <b>analytical</b> 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 150
Exchanging the diverter valve	When defective (internal / external leak, valve not switching any more)	See "Exchanging the Diverter Valve" on page 153
Exchanging the internal tray	When flow delay sensor defective	See "Exchanging the Internal Tray" on page 156
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 158

# **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 <b>preparative scale 0.8 mm ID</b> , PN G1364-68711, <b>analytical scale 0.25 mm ID</b> , PN G1364-68712 or <b>analytical scale</b> <b>0.15 mm ID</b> , G1364-68723

# WARNING

To avoid personal injury, keep fingers away from the needle area during fraction collector operation.

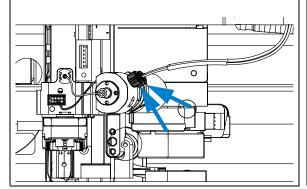
## WARNING

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.

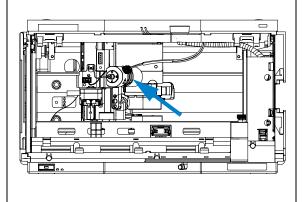
#### Before beginning this procedure:

- Position the transport unit of the fraction collector in the "Home Position" (see "Maintenance Functions" on page 131).
- 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 131).
- 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.

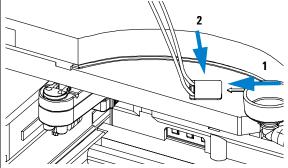
**2** Unscrew the 2 finger-tight fittings of the inlet / waste tubing assembly at the diverter valve.



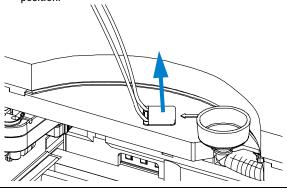
1 Locate the diverter valve with the finger-tight fittings of the inlet / waste tubing assembly (the figure shows the open fraction collector seen from the front).



**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.

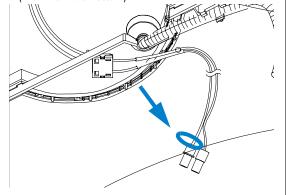


**4** Install the new inlet / waste tubing assembly into the bolt carrier as shown below. Slide in the long ends of the tubings from bottom to top and let the snapper click into position.

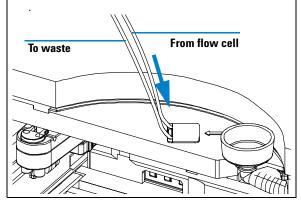


6 Connect the finger-tight fittings of the inlet / waste tubing assembly to the ports of the diverter valve.
IMPORTANT: The tubings must not be bent up- or downwards. The cables must not be twisted.

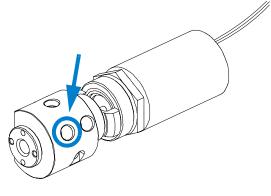
(View from the bottom)

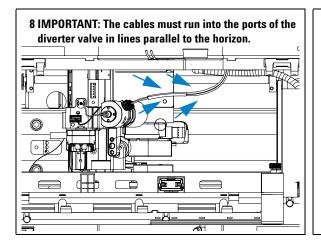


5 The rear tubing (with the label with the arrow) must be connected to the flow cell of the detector. The front tubing must be inserted into the waste container



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.





#### 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 <b>preparative scale 0.8</b> 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.

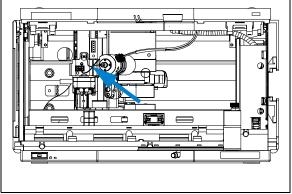
### WARNING

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.

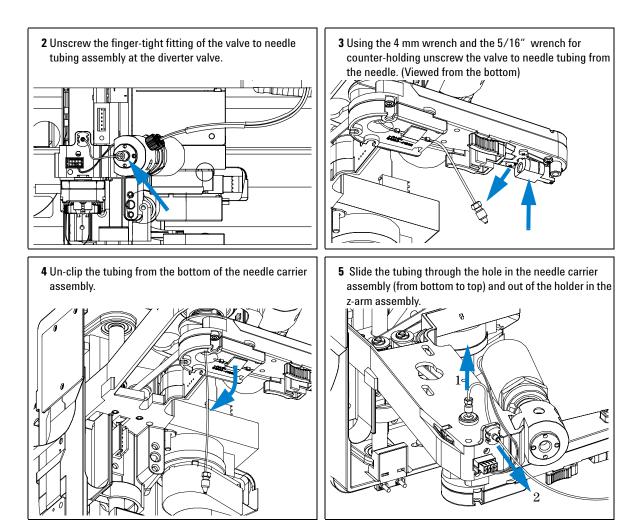
#### Before beginning this procedure:

- Position the transport unit of the fraction collector in the "**Home Position**" (see "Maintenance Functions" on page 131).
- 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 131) and turn off the instrument.
- It might be more convenient to remove the needle from its carrier before unscrewing the needle tubing.

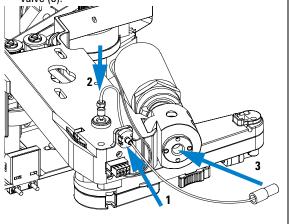
**1** Locate the diverter valve with the finger-tight fittings of the valve to needle tubing assembly (the figure shows the open fraction collector seen from the front).



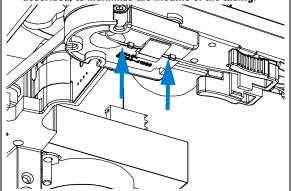
**Simple Repairs** 



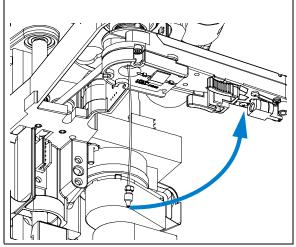
6 Install the new valve to needle tubing assembly by clipping it in to the holder in the z-arm assembly (1), Important!!!) and slide it through the hole in the z-arm (2) and out on the bottom of the needle carrier assembly (top to bottom). Screw the finger-tight fitting into the port of the diverter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



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.



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)



#### 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.

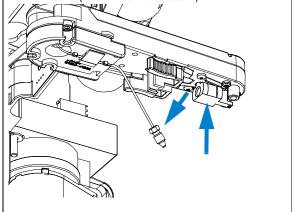
### CAUTION

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.

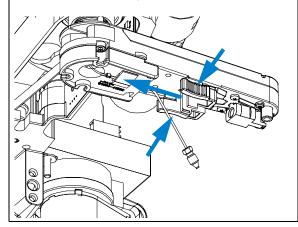
#### Before beginning this procedure:

- Position the transport unit of the fraction collector in the "Home Position" (see "Maintenance Functions" on page 131).
- 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 131).
- Turn OFF the instrument.
- It might be more convenient to remove the needle from its carrier before unscrewing the needle tubing.

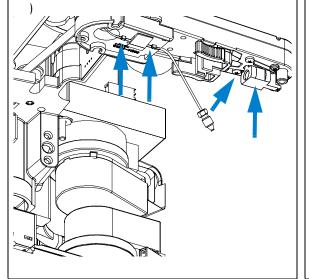
1 Using the 4 mm wrench and the 5/16" wrench for counter-holding unscrew the valve to needle tubing from the needle. (Viewed from the bottom)



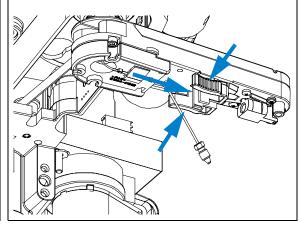
2 Holding the needle assembly between your thumb and forefinger, slide out the assembly towards the rear of the needle carrier assembly.



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



**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.



#### 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

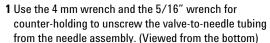
To avoid personal injury, keep fingers away from the needle area during fraction collector operation.

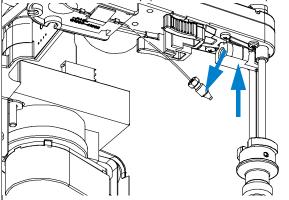
### CAUTION

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.

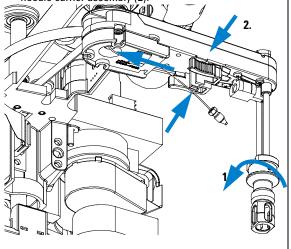
#### Before beginning this procedure:

- Position the transport unit of the fraction collector in the "Home Position" (see "Maintenance Functions" on page 131).
- 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 131).
- Turn OFF the instrument.
- It might be more convenient to remove the needle from its carrier before unscrewing the needle tubing.

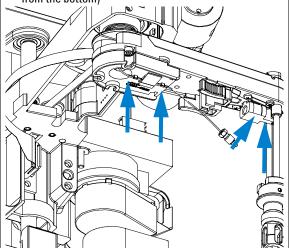




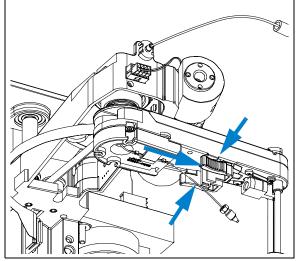
2 Remove the wellplate adapter by turning it clockwise (1). Hold the needle assembly between your thumb and forefinger, slide out the assembly towards the rear of the needle carrier assembly (2).



**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)



**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.



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.

5 Repairing the Fraction Collector

Simple Repairs

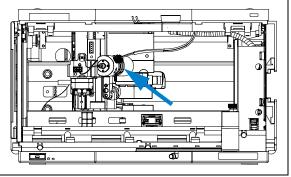
### **Exchanging the Diverter Valve**

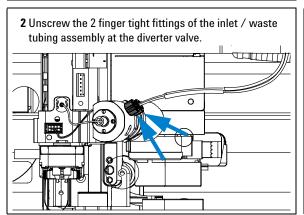
Frequency	When leaky or defective
Tools required	Hex key 2.0 mm, PN 8710-2438 (supplied in accessory kit)
Parts required	Diverter Valve, PN G1364-61901

#### Before beginning this procedure:

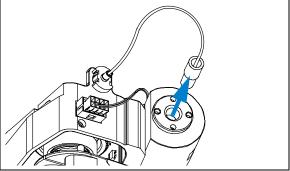
- Position the transport unit of the fraction collector in the "**Home Position**" (see "Maintenance Functions" on page 131).
- Remove all installed trays from the tray base.
- Position the transport unit of the fraction collector in the "Change Parts Position" (see "Maintenance Functions" on page 131).
- 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).

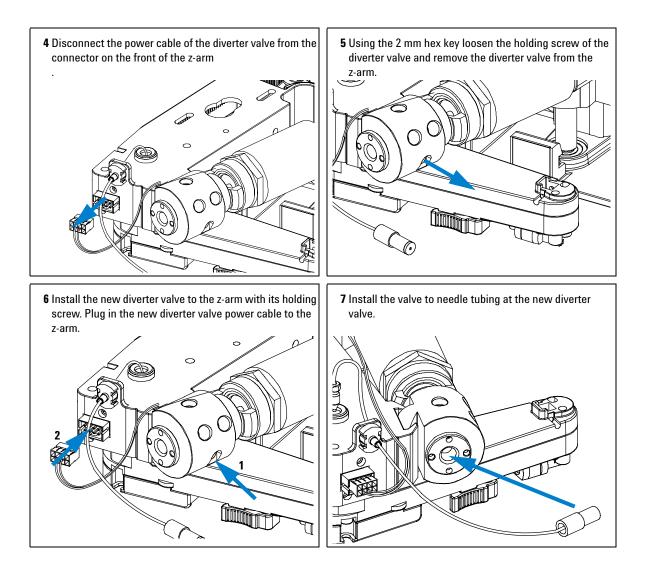


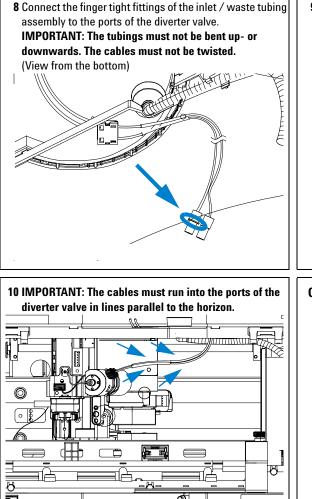


**3** Unscrew the finger tight fitting of the valve to needle tubing assembly at the diverter valve.

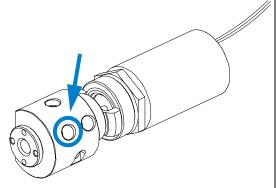


**Simple Repairs** 





 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.



#### On completion of this procedure:

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

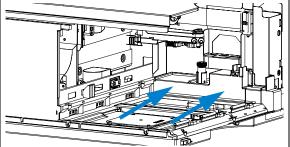
### **Exchanging the Internal Tray**

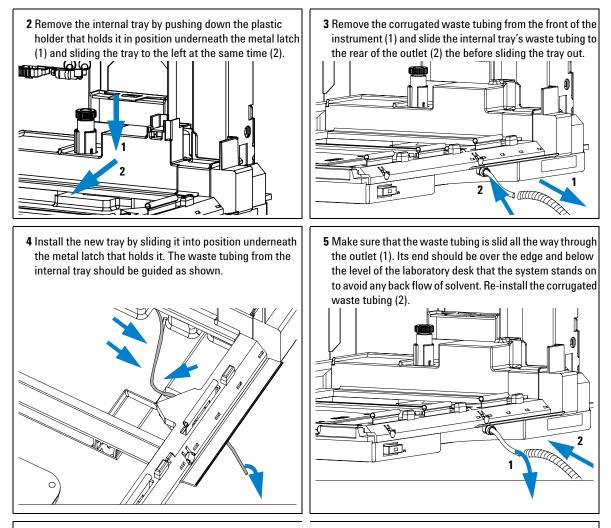
Frequency	When defective
Tools required	None
Parts required	Internal tray <b>analytical scale</b> , (G1364-63113) Internal tray <b>preparative scale</b> ,(G1364-63114)

#### Before beginning this procedure:

- Position the transport unit of the fraction collector in the "Home Position" (see "Maintenance Functions" on page 131).
- 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.





#### On completion of this procedure:

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

### 5 Repairing the Fraction Collector

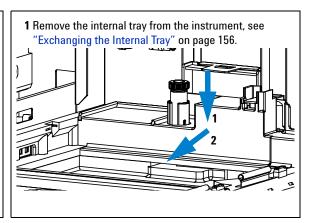
**Simple Repairs** 

### **Repairing or Exchanging a Funnel of the Internal Tray**

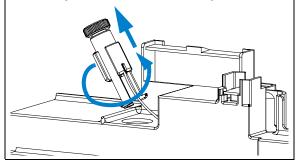
Frequency	When leaky or contaminated
Tools required	None
Parts required	Funnel assembly, waste tubing assembly, seals (for part numbers see "Internal Tray Assembly" on page 176)

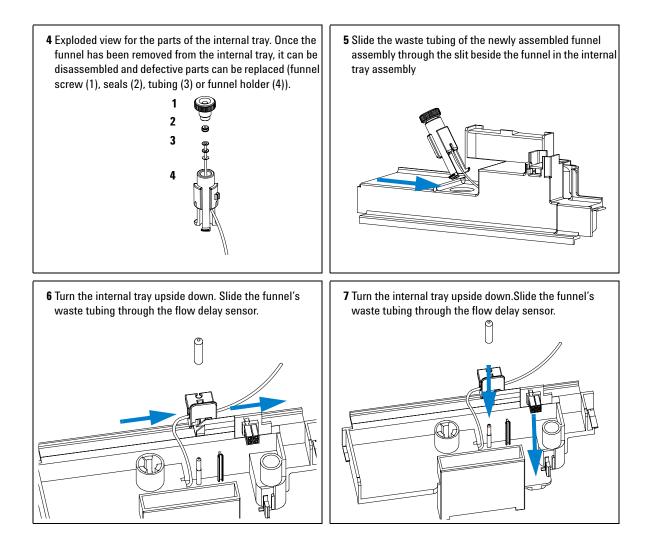
#### Before beginning this procedure:

- Position the transport unit of the fraction collector in the "**Home Position**" (see "Maintenance Functions" on page 131) 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.
- 2 Turn the internal tray upside down and remove the funnel's waste tubing through the flow delay sensor.

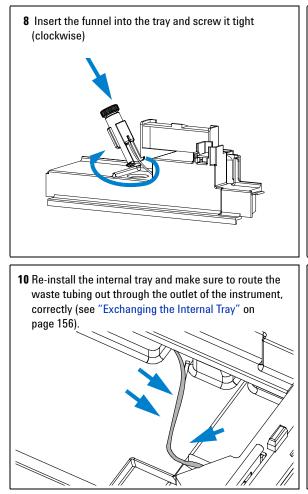


**3** Remove the funnel assembly from the internal tray by screwing it counter clockwise and lifting it out.

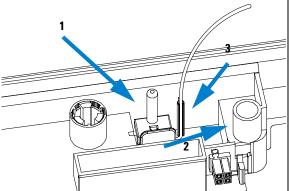




**Simple Repairs** 

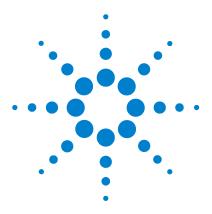


**9** Re-install the plug that holds the flow delay sensor (1), pull the waste tubing tight through the flow delay sensor (2) and mount the waste tubing (3).



### On completion of this procedure:

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



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# **Parts and Materials**

6

Supported Trays for Fraction Collectors 162 List of Recommended Test Tubes 164 List of Recommended Vials and Caps 165 List of Recommended Plates and Closing Mats 168 Transport Unit Assembly (Preparative Scale) 170 Transport Unit Assembly (Analytical Scale) 171 Needle Assemblies 173 Diverter-Valve Assembly 174 Tubing Kits 175 Internal Tray Assembly 176 Fraction Collector Accessory Kit 177



6

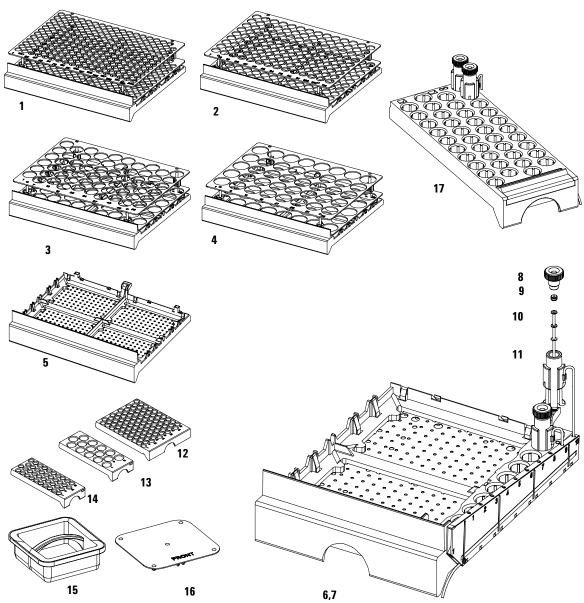
# **Supported Trays for Fraction Collectors**

ltem	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, thermostattable	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

### Table 19 Trays for the Fraction Collector

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 41 Trays

### 6 Parts and Materials

List of Recommended Test Tubes

# **List of Recommended Test Tubes**

outer diameter	height	Volume	recommended tray type	Part Number
30 mm	100 mm	45 ml	G1364-84503 (40 tubes)	5042-6459 (100/pk)
30 mm	48 mm	20 ml;	G1364-84503 (40 tubes)	5042-6458 (100/pk)
25 mm	100 mm	35 ml	G1364-84504 (60 tubes)	5042-6470 (100/pk)
16 mm	100 mm	19 ml	G1364-84505 (126 tubes)	5022-6532 (250/pk)
16 mm	48 mm	9 ml	G1364-84505 (126 tubes)	5022-6533 (100/pk)
12 mm	100 mm	11 ml	G1364-84506 (215 tubes)	5022-6431 (250/pk)
12 mm	48 mm	5 ml	G1364-84506 (215 tubes)	5022-6435 (100/pk)

Table 20Round Bottom Test Tubes

### **List of Recommended Vials and Caps**

Description	Volume (ml)	l00/Pack	l000/Pack	l00/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 21
 Crimp Top Vials (Caps for Use with the Analytical Scale Fraction Collector, only!)

 Table 22
 SnapTop Vials (Caps for Use with the Analytical Scale Fraction Collector, only!)

Description	Volume (ml)	l00/Pack	l000/Pack	l00/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 23
 Screw Top Vials (Caps for Use with the Analytical Scale Fraction Collector, only!)

Description	Volume (ml)	l00/Pack	l000/Pack	l00/Pack (silanized)
Clear glass	2	5182-0714	5183-2067	5183-2070

#### **6** Parts and Materials

List of Recommended Vials and Caps

Description	Volume (ml)	IOO/Pack	l000/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 23
 Screw Top Vials (Caps for Use with the Analytical Scale Fraction Collector, only!) (continued)

 Table 24
 Crimp Caps (Caps for Use with the Analytical Scale Fraction Collector, only!)

Clear PTFE/red rubber	5181-1210
Clear PTFE/red rubber	5183-4498 (1000/Pack)
Clear PTFE/red rubber	5181-1215
Clear PTFE/red rubber	5181-1216
Clear PTFE/red rubber	5181-1217
	Clear PTFE/red rubber Clear PTFE/red rubber Clear PTFE/red rubber

 Table 25
 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

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

 Table 26
 Screw Caps (Caps for Use with the Analytical Scale Fraction Collector, only!)

**List of Recommended Plates and Closing Mats** 

### **List of Recommended Plates and Closing Mats**

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 pate	1.0	50	5042-6454
96 polypropylene deep well-plate with glass inserts, caps and septa pre assembled <sup>*</sup>	0.35	1	5065-4402
glass inserts for 5065-4402 <sup>*</sup>	0.35	1000	5188-5321
384 polypropylene well-plate <sup>*</sup>	0.1	30	5042-1388
96 polypropylene conical-well plate <sup>*</sup>	0.18	25	5042-8502
54 x 2ml vial plate <sup>*</sup>	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 <sup>*</sup>		50	5042-1389

 
 Table 27
 Recommended Plates and Closing Mats (Std. Well Plates and Closing Mats for Use with the Analytical Scale Fraction Collector, only!)

\* 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)

# Transport Unit Assembly (Preparative Scale)

ltem	Description	Part Number
1	Transport Unit Assembly ( <b>Preparative Scale)</b> , 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

### Table 28 Transport Unit Assembly (Preparative Scale)

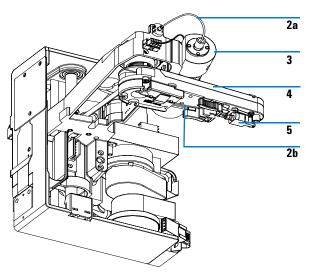


Figure 42 Transport Unit Assembly (Preparative Scale), partly displayed

# Transport Unit Assembly (Analytical Scale)

ltem	Description	Part Number
1	Transport unit assembly <b>(analytical scale)</b> , 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 <b>analytical scale (50mm needle)</b> Needle assembly <b>semi-preparative scale (20mm needle)</b>	G1367-87200 G1364-87202
6	Wellplate adapter	G1364-60021

### Table 29 Transport Unit Assembly (Analytical Scale)

### 6 Parts and Materials

Transport Unit Assembly (Analytical Scale)

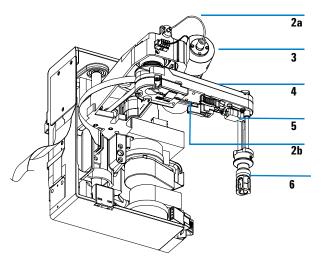


Figure 43 Transport Unit Assembly (Analytical Scale), partly displayed

## **Needle Assemblies**

ltem	Description	Part Number
1	Needle assembly preparative scale	G1364-87201
2	Needle assembly <b>semi preparative scale (20 mm needle)</b> 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

Table 30Needle Assemblies

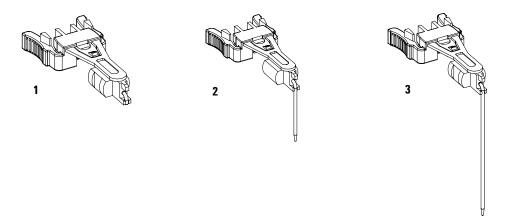


Figure 44 Needle Assemblies

# **Diverter-Valve Assembly**

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

**Table 31** Diverter-Valve Assembly (preparative and analytical scale)

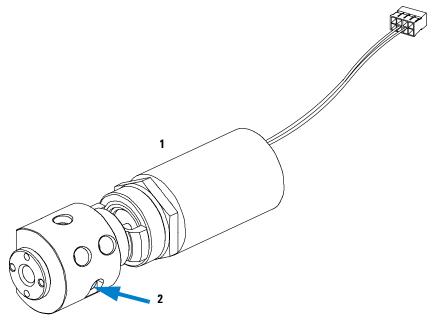
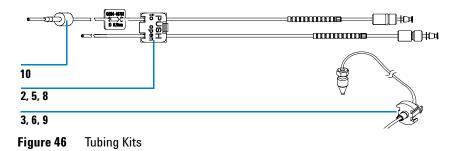


Figure 45 Diverter-Valve Assembly

# **Tubing Kits**

ltem	Description	Part Number
1	Tubing kit <b>preparative scale 0.8 mm ID</b> (consists of items 2,and 3)	G1364-68711
2	Inlet/waste tubing assembly <b>preparative scale 0.8 mm ID</b>	Order Item 1
3	Diverter valve to needle tubing preparative scale 0.8 mm ID	Order Item 1
4	Tubing kit <b>analytical scale 0.25 mm ID</b> (consists of items 5,and 6)	G1364-68712
5	Inlet/waste tubing assembly <b>analytical scale 0.25 mm ID</b>	Order Item 4
6	Injection valve to needle tubing analytical scale 0.25 mm ID	Order Item 4
7	Low dispersion tubing kit <b>analytical scale 0.15 mm ID</b> 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

### Table 32 Tubing Kits



# **Internal Tray Assembly**

ltem	Description	Part Number
1	Internal tray assembly <b>analytical scale</b> , includes items 2 – 7 Internal tray assembly <b>preparative scale</b> , includes items 2 – 7	G1364-63114 G1364-63113
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

**Table 33** Internal Tray Assembly (Analytical and Preparative Scale)

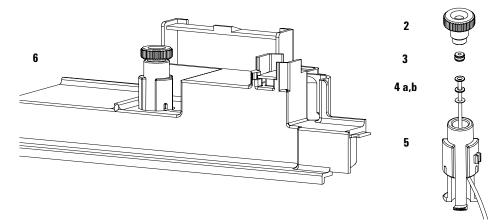


Figure 47 Internal Tray Assembly (Analytical Scale)

### **Fraction Collector Accessory Kit**

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 <sup>*</sup>	3	0100-1516
Waste tubing (1.2 m) <sup>†</sup>	1	5062-2463
Y-Type Fitting for waste tubing <sup>‡</sup>	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) $^{\dagger\dagger}$	3	5065-9976
ESD wrist strap	1	9300-1408
Tray for 15 x 6 ml vials	1	G1313-44513
Tray for 40 x 2 ml vials	1	G1313-44512

### Table 34 Fraction Collector Accessory Kit Contents G1364-68725

\* Reorder gives pack of 2

† Reorder gives 5 m

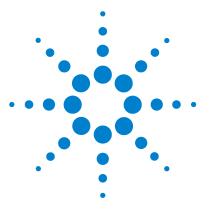
‡ Reorder gives pack of 10

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

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

### 6 Parts and Materials

**Fraction Collector Accessory Kit** 



Agilent 1200 Series Fraction Collectors User Manual

# 7 Specifications

Performance Specifications for the Fraction Collectors 180



# **Performance Specifications for the Fraction Collectors**

Туре	Specification
trigger modes	Time slices, Peak (threshold, up- / downslope), Timetable (combination of time intervals and peak) and Manual trigger (supported only with G1323B Control Module) Agilent 1200 Series DAD/MWD detectors (G1315A/B/C, G1365 A/B/C), the Agilent 1200 Series fluorescence detector 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) <sup>*</sup> (for use with deep well plates, only) 2 × well-plates std. tray (MTP) (for use with deep well plates, only) + 10 × 2 ml vials <sup>*</sup> (+ 1 half tray) 100 x 2 ml in std. tray (+ 1 half tray) <sup>*</sup> 3 x 40 x 2 ml in half tray <sup>*</sup> 3 x 15 x 6 ml in half tray <sup>*</sup> 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 35 performance Specifications Agilent 1200 Series PREPARATIVE SCALE Fraction Collector (G1364B)

7

**Performance Specifications for the Fraction Collectors** 

Туре	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 o 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 $\mu$ l), 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 G1330A 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

 
 Table 35
 performance Specifications Agilent 1200 Series PREPARATIVE SCALE Fraction Collector (G1364B) (continued)

\* Vials can be used as recommended by Agilent Technologies (see "List of Recommended Vials and Caps" on page 165 and "List of Recommended Plates and Closing Mats" on page 168) 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 168)

### 7 Specifications

NOTE

**Performance Specifications for the Fraction Collectors** 

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

**Performance Specifications for the Fraction Collectors** 

Туре	Specification
trigger modes	Time slices, Peak (threshold, up- / downslope), Timetable (combination of time intervals and peak) and Manual trigger (supported only with G1323B Control Module) Agilent 1200 Series DAD/MWD detectors (G1315A/B/C, G1365 A/B/C), the Agilent 1200 Series fluorescence detector 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 36 Performance Specifications Agilent 1200 Series ANALYTICAL SCALE Fraction Collector (G1364C)

#### 7 Specifications

**Performance Specifications for the Fraction Collectors** 

Туре	Specification
fraction vessel capacities and trays	<ul> <li>4 x well-plates full tray (MTP)<sup>*</sup></li> <li>2 x well-plates std. tray + 10 funnels with external containers<sup>*</sup> (+ 1 half tray)</li> <li>2 × well-plates std. tray (MTP) + 10 × 2 ml vials<sup>*</sup> (+ 1 half tray)</li> <li>100 x 2 ml in std. tray (+ 1 half tray)<sup>*</sup></li> <li>3 x 40 x 2 ml in half tray<sup>*</sup></li> <li>3 x 40 funnels in half tray<sup>*</sup></li> <li>3 x 40 funnels in half tray<sup>*</sup></li> <li>Full tray with 40 test tubes (30 mm OD, max. height 48 mm, ~20 m vol.)</li> <li>Full tray with 60 test tubes (16 mm OD, max. height 48 mm)</li> <li>Full tray with 126 test tubes (12 mm OD, max. height 48 mm)</li> <li>Full tray are automatically detected and identified. Installed plates and vials can be detected when operating in the needle into location mode</li> <li>* max. height can be extended by using the short needle assembly G1364-87202</li> </ul>
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

#### Table 36 Performance Specifications Agilent 1200 Series ANALYTICAL SCALE Fraction Collector (G1364C) (continued)

**Performance Specifications for the Fraction Collectors** 

Туре	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> <li>Controller-area network (CAN).</li> <li>optional; LAN or external contacts interface</li> <li>RS232C,</li> <li>APG-remote (for remote start / stop signals to / from other modules)</li> <li>Interface to G1330A Thermostat</li> <li>CAN-DC-out for operation of Agilent approved external devices like valves</li> </ul>
safety features	Leak detection and safe leak handling, error detection and display, exhaust fan for fume extraction of hazardous vapors

Table 36	Performance Specifications Agilent 1200 Series ANALYTICAL SCALE Fraction
	Collector (G1364C) (continued)

 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 165 and "List of Recommended Plates and Closing Mats" on page 168)

# NOTE

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

### 7 Specifications

**Performance Specifications for the Fraction Collectors** 



Agilent 1200 Series Fraction Collectors User Manual

# Safety Information

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A Safety Information Safety Information

# **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 37 shows safety symbols used on the instrument and in the manuals.

Symbol	Description
$\triangle$	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.
4	Indicates dangerous voltages.
	Indicates a protected conductor terminal.
Þ	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 fejlagtic handtering. Udskiftning ma 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 av apparatfabrikanten. Brukt batteri returneres appararleverandoren.

#### NOTE

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





# **Radio Interference**

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

## **Test and Measurement**

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

# **Sound Emission**

# **Manufacturer's Declaration**

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

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

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

# **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:

 $2CHCl_3 + O_2 - 2COCl_2 + 2HCl$ 

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 1200 Series modules for download.

#### A Safety Information

**Agilent Technologies on Internet** 

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# In This Book

This manual contains technical reference information about the Agilent 1200 Series 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.

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