Agilent 1260 Infinity Purification Solution

System User Guide

Agilent Technologies

Notices

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CAUTION

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

WARNING

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

In This Book

This user guide provides information on how to setup and run the Agilent 1260 Infinity Purification Solution.

1 Product Description

This chapter describes the supported configurations, main features and specifications of the Agilent 1260 Infinity Purification Solution. It also provides generic information on leak and waste handling.

2 Operating Instructions

This chapter provides information on how to use and operate the Agilent 1260 Infinity Purification Solution.

3 Required Practice

This chapter provides practical hints for optimizing the purification, avoiding common problems and optimizing the workflow.

4 Troubleshooting

Overview about troubleshooting.

5 Maintenance, Repair and Parts

For detailed information on maintenance, repair and parts, see the individual module manuals.

6 Appendix

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

Contents

1
Product Description

This chapter describes the supported configurations, main features and specifications of the Agilent 1260 Infinity Purification Solution. It also provides generic information on leak and waste handling.

The Purification Workflow

Purification is a core task for all industries or institutes that are interested in synthesizing or analyzing of pure compounds.

In these branches, HPLC (high-pressure liquid chromatography) is a key technology:

- Analytical HPLC is a standard tool to separate and identify compounds.
- Preparative HPLC is the only method that allows an automatic and easy-to-use purification of compounds.

The purification workflow with HPLC consists of several steps. In the whole process, analytical scouting, target confirmation, and method transfer from analytical to preparative scale HPLC are critical issues, regarding time and costs.

Thus, the combination of analytical and preparative HPLC has become a successful tool for many purification tasks.

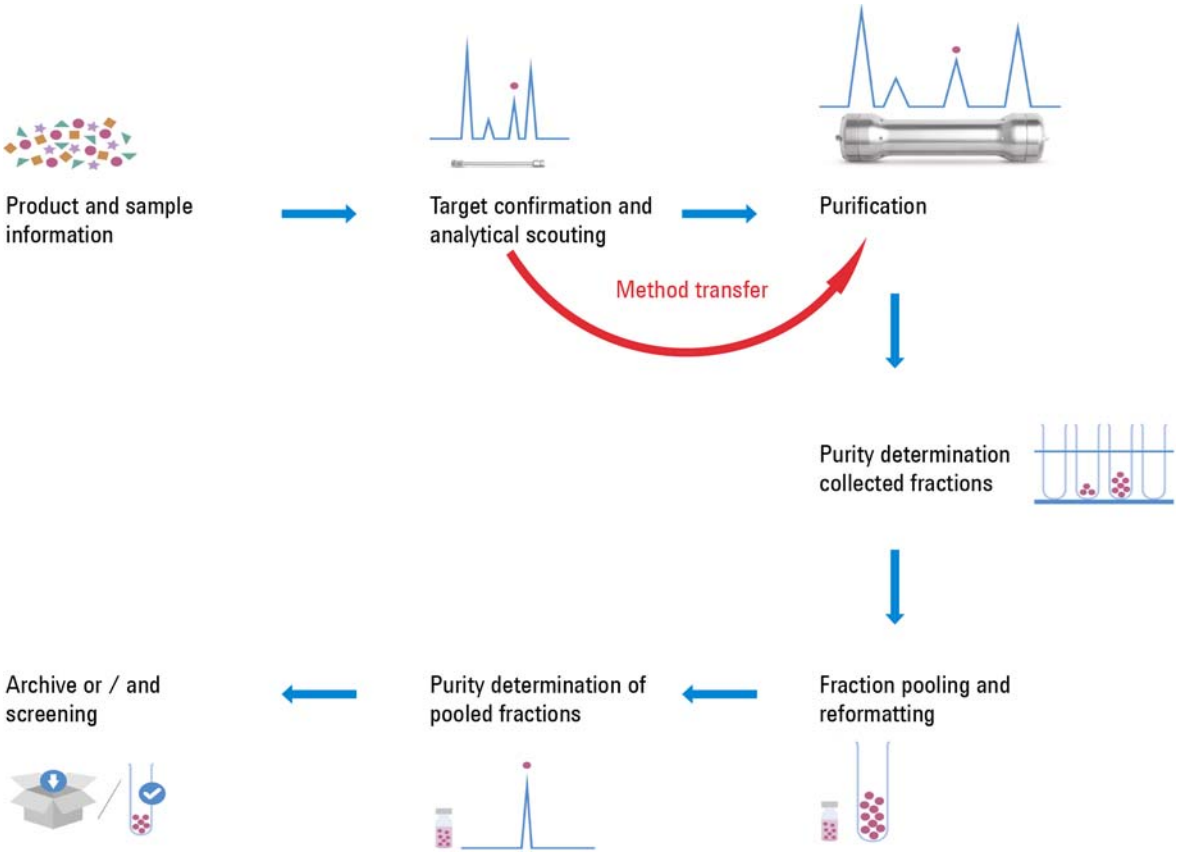

Figure 1 Overview on a typical purification workflow and the importance of method transfer from analytical to preparative scale HPLC

Introduction to the Agilent 1260 Infinity Purification Solution

The Agilent 1260 Infinity Purification Solution is extremely versatile and very flexible. It enables method transfer and automated target identification from UHPLC-MS to preparative LC/MS using one simple yet powerful software package.

To fulfill the demands of modern laboratories, Agilent Automated Purification Software provides two modes of user access:

- *Expert* mode

  In this mode, the software supports the user to setting up the analytical and preparative system parameters, preparing the necessary Agilent OpenLAB CDS ChemStation Edition methods, and creating the master tasks for all system combinations.

- *Easy Prep* mode

  The Easy Prep mode supports walk-up chemists to view the analytical or preparative systems parameters. Users running the software in this mode cannot edit any of the purification parameters.

The solution consists of the following components:

- Purification software
- Preparative-scale LC-Instrument
- Analytical scouting mode of the preparative LC instrument (optional)
- Mass spectrometer (optional)
- Additional detectors (optional)
- Valve kits
- Capillary kits

Agilent Technologies offer help in the following areas, to ensure optimal performance of your system:

- Configuration
- Installation, familiarization, preventive maintenance
- Application specific consulting, training and support

For details, please contact your Agilent sales representative.

Features

Features of the Agilent 1260 Infinity Purification Solution:

- Designed for preparative flow rates up to 100 mL/min at 400 bar.
- Analytical scouting supported in gradient mode.
- Method transfer and automated target identification from UHPLC-MS to preparative LC/MS using one simple yet powerful software package.
- Automatic delay calibration ensures maximum recovery and purity of the compound.
- Flexible configurations for purification of milligrams to grams of pure product.
- Rugged scale up from analytical to preparative scale.

Automated Purification Software Overview

The Agilent Automated Purification Software supports the purification workflow from analytical scouting run, through the automated scale-up and formation of a focused gradient, to the scheduling of a corresponding preparative run. The software allows the analytical scouting run to be carried out on a separate instrument from the preparative instrument, or on a combined instrument comprising both analytical and preparative hardware.

The Agilent Automated Purification Software is an add-on to the Agilent OpenLAB CDS ChemStation Edition (revision C.01.07 SR1). When the Automated Purification Software is installed and licensed, Purification menus are added to the ChemStation Method and Run Control and Data Analysis views.

The basis of the Automated Purification Software is the Purification Task. This defines the complete purification experiment, and consists of specifications of the analytical system, preparative system, the scale-up parameters, and the analytical and preparative run parameters.

The analytical system parameters are a combination of instrument configuration and method parameters that characterize the analytical system that is used for the analytical scouting run. The parameters are required for the calculation of the scale-up to the preparative run. Several sets of such parameters are maintained, for example, related to different instruments or different operating conditions. The analytical system parameters can be stored and recalled for reuse.

The important characteristics and operation parameters of the instrument that is used for the preparative runs are stored as a preparative system, and can be used for various purification tasks.

The scale-up process is centered on the generation of a focused gradient, which defines a gradient around the elution point of the target compound. The focused gradient is designed to optimize the separation of the target compound from its neighbors. The Automated Purification Software calculates the focused gradient by considering the differences in column geometry, void volume and flow between the analytical instrument and the preparative instrument.

The generation of the focused gradient is carried out automatically, but the parameters can be fine-tuned by a user with the requisite permissions, who has full access to all parameters and results. However, once the purification task has been optimized, it can be run by an operator with limited permissions in a simplified user interface that allows minimal interaction.

NOTE Modifying the automatically generated focused gradient must be done by knowledgeable users only.

Supported Configurations and Components

The Agilent 1260 Infinity Purification Solution supports the following configurations:

- “Preparative-Scale LC with UV-Detection” on page 21
- “Preparative-Scale LC with UV- and MS-Detection” on page 18
- “Combined Analytical-/Preparative-Scale LC with UV- and MS-Detection” on page 15

Combined Analytical-/Preparative-Scale LC with UV- and MS-Detection

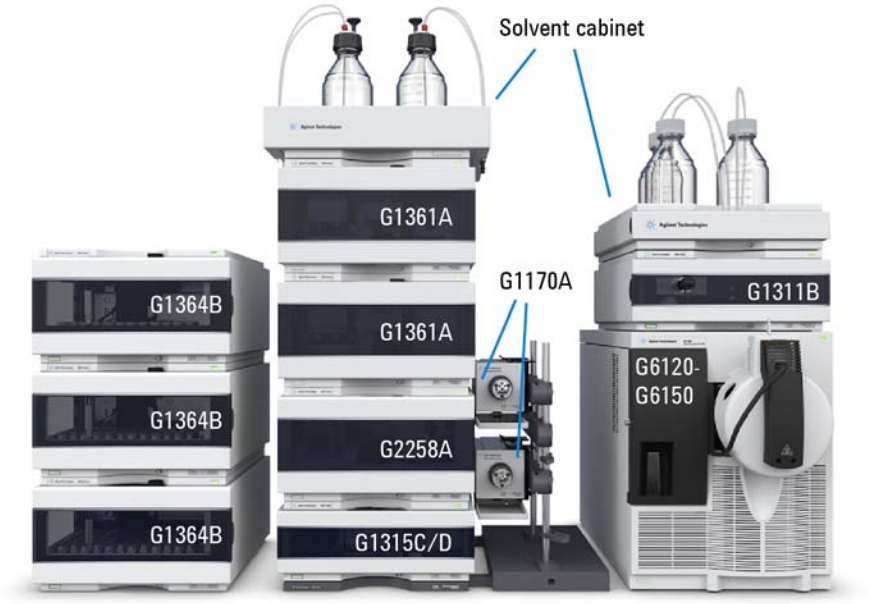

Figure 2 Stack configuration for Agilent 1260 Infinity Purification Solution, combined system with UV- and MS-Detection

The combined analytical-scale and preparative-scale LC instrument with UV- and MS-detection consists of the following modules:

- One preparative and one analytical column
- Two G1361A Preparative Pumps and G1391 Accessory kit as gradient system
- One G2258A Dual-loop Autosampler for sample injection
- One G1315C/D Diode Array Detector or G1365C/D Multiple Wavelength Detector for UV-Detection

- G1364B Fraction Collector (FC) for sample collection, in the following configurations:
  - One FC, or
  - Up to three FCs as cluster with G1170A Valve Drive/G4731A Valve Kit (option shown in Figure 2 on page 15)
- G1170-68705 Accessory Kit to configure the G1170A Valve Drive for plumbing the combined system (not shown)
- One G1311B Quaternary Pump as make-up pump and as analytical pump
- One G6120B - G6150B Mass Detector for Mass-Detection
- One G1968F Mass Based Purification Kit for the 6100 (not shown)
- One G1390B Universal Interface Box for electrical connections (not shown, part of G1968F)
- One 5067-6176 Capillary Kit for Mass-based Systems

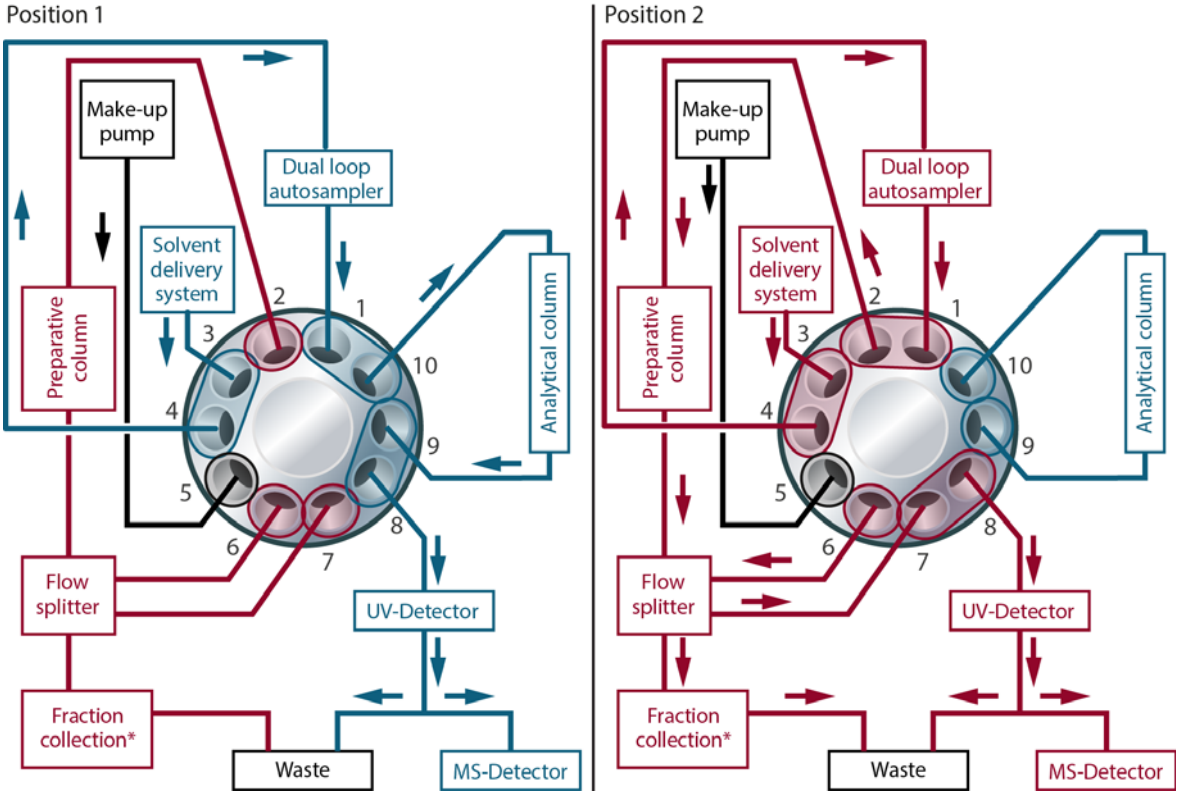

Figure 3 Schematic overview for a UV-MS detection based combined analytical to preparative scale system

* Up to three fraction collectors (FC) as cluster with one G1170A Valve drive/G4731A Valve kit and one G1170A Valve drive/G4730A Valve kit

Preparative-Scale LC with UV- and MS-Detection

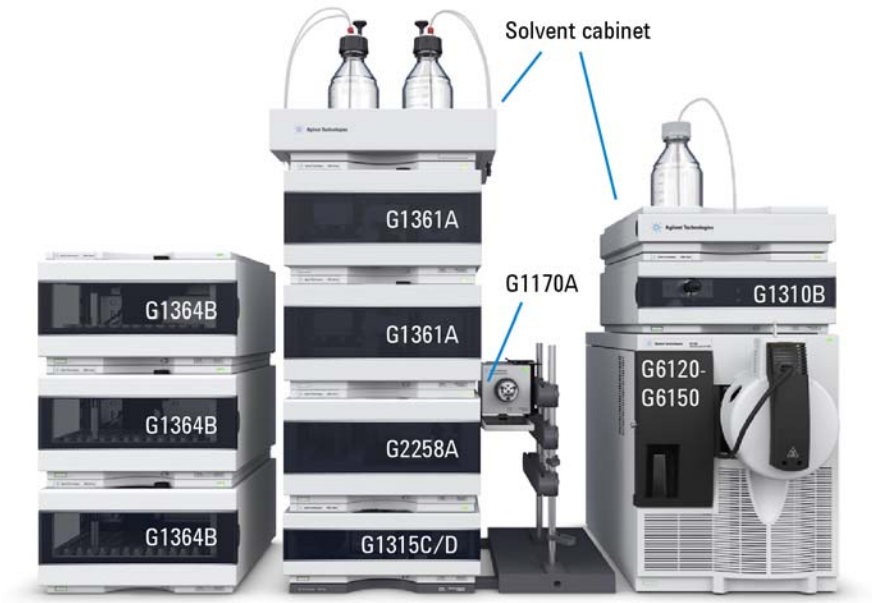

Figure 4 Stack configuration for Agilent 1260 Infinity Purification Solution with UV and MS detection

The preparative-scale LC instrument with UV- and MS-detection consists of the following modules:

- Two G1361A Preparative Pumps and G1391 Accessory kit as gradient system
- One G2258A Dual-loop Autosampler for sample injection
- One G1315C/D Diode Array Detector or G1365C/D Multiple Wavelength Detector for UV-Detection

- G1364B Fraction Collector (FC) for sample collection, in the following configurations:
  - One FC, or
  - Up to three FCs as cluster with G1170A Valve Drive/G4731A Valve Kit (option shown in Figure 4 on page 18)
- G1170-68705 Accessory Kit to configure the G1170A Valve Drive for plumbing the combined system (not shown)
- One G1310B Isocratic Pump as make-up pump
- One G6120B - G6150B Mass Detector for Mass-Detection
- One G1968F Mass Based Purification Kit for the 6100 (not shown)
- One G1390B Universal Interface Box for electrical connections (not shown, part of G1968F)
- One 5067-6175 Capillary Kit for Mass-based Systems

NOTE

Supported modules (for analytical method transfer):

- G4220B Binary Pump as solvent delivery module
- G1312B Binary Pump as solvent delivery module
- G1311B Quaternary Pump as solvent delivery module (supported is only a configuration where %B represents the organic solvent of the eluent
- G4226A Autosampler
- G1329B Standard Autosampler
- G4212A Diode Array Detector
- G1315C/D Diode Array Detector
- G6120/30/50 Single Quadrupole LC/MS System Bundle

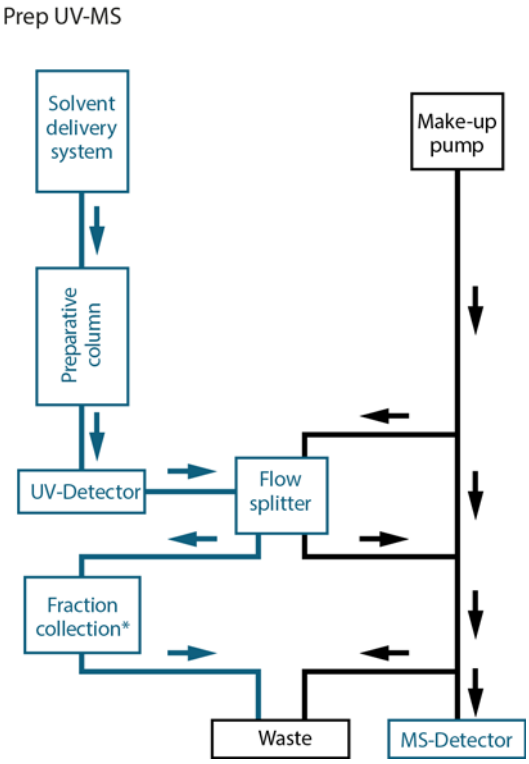

Figure 5 Schematic overview for a UV-MS detection based preparative scale system

* Up to three fraction collectors (FC) as cluster with G1170A Valve drive/G4731A Valve kit

Preparative-Scale LC with UV-Detection

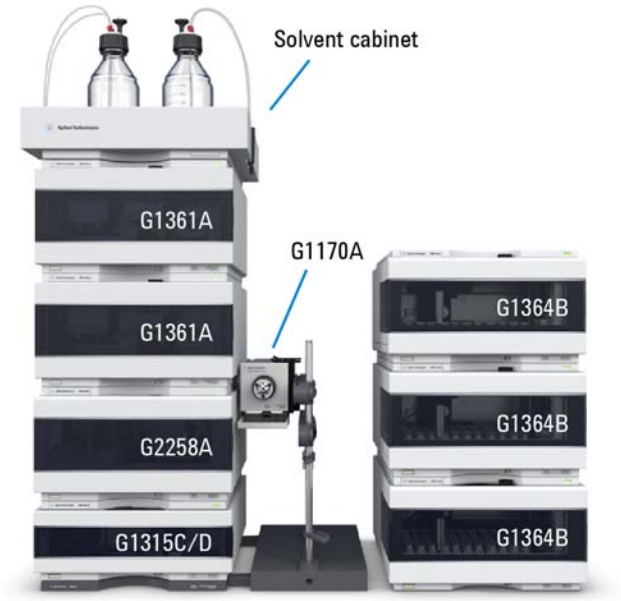

Figure 6 Stack configuration for Agilent 1260 Infinity Purification Solution with UV detections

The preparative-scale LC instrument with UV-detection consists of the following modules:

- Two G1361A Preparative Pumps and G1391 Accessory kit as gradient system
- One G2258A Dual-loop Autosampler for sample injection
- One G1315C/D Diode Array Detector or G1365C/D Multiple Wavelength Detector for UV-Detection

- G1364B Fraction Collector (FC) for sample collection, in the following configurations:
  - One FC, or
  - Up to three FCs as cluster with G1170A Valve Drive/G4731A Valve Kit (option shown in Figure 6 on page 21)
- G1170-68705 Accessory Kit to configure the G1170A Valve Drive for plumbing the combined system (not shown)
- One 5067-6175 Capillary Kit for UV-based Systems

NOTE

Supported modules (for analytical method transfer):

- G4220B Binary Pump as solvent delivery module
- G1312B Binary Pump as solvent delivery module
- G1311B Quaternary Pump as solvent delivery module (supported is only a configuration where %B represents the organic solvent of the eluent
- G4226A Autosampler
- G1329B Standard Autosampler
- G4212A Diode Array Detector
- G1315C/D Diode Array Detector
- G6120/30/50 Single Quadrupole LC/MS System Bundle

Prep UV

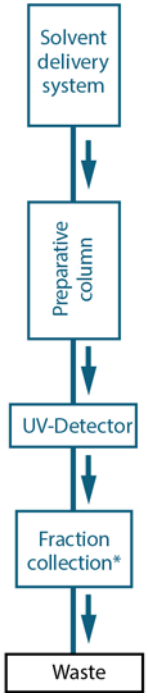

Figure 7 Schematic overview for a UV detection based separate preparative to preparative scale system

* Up to three fraction collectors (FC) as cluster with G1170A Valve drive/G4731A Valve kit

Specifications

Physical Specifications

Table 1 Physical Specifications (Agilent 1200 Infinity Purfication System Modules)

Type	Specification	Comments
Line voltage	100 – 240 VAC, ± 10 % 200 – 240 VAC, ± 5 %	Wide-ranging capability For the LC-MSD
Line frequency	50 or 60 Hz, ± 5 %	
Power consumption (apparent power)	200 – 300 VA 1500 VA (max.)	Maximum for each module For the LC-MSD
Ambient operating temperature	4 – 55 °C (39 – 131 °F)[1] 15 – 35 °C (59 – 95 °F	For the LC-MSD
Ambient non-operating temperature	-40 – 70 °C (-40 – 158 °F)	
Humidity	< 95 % r.h. at 40 °C (104 °F)	Non-condensing
Operating altitude	Up to 2000 m (6562 ft)	
Non-operating altitude	Up to 4600 m (15091 ft)	For storing the module
Safety standards: IEC, CSA, UL	Installation category II, Pollution degree 2	For indoor use only.

[1] This temperature range represents the technical specifications for this instrument. The temperatures mentioned may not be suitable for all applications and all types of solvent.

Table 2 Dimension and Weight Specifications - for Agilent 1260 Infinity Purification Solution

Module	Specification (height × width × depth)	Weight
G1310B	180 × 345 × 435 mm	11 kg
G1311B	180 × 345 × 435 mm	14.5 kg
G1361A/G1391A	200 × 345 × 440 mm	15 kg
G2258A	200 × 345 × 440 mm	14 kg
G1383A	560 × 585 × 420 mm	2.6 kg
G1315C/D, G1365C/D	140 × 345 × 435 mm	11.5 kg
G1364B	200 × 345 × 440 mm	13.5 kg
G6120B - G6150B[1] (including foreline pump[2])	575 × 730 × 690 mm	approximately 100 kg

[1] For more details, refer to the 6100 Series Single Quad LCMS Site Preparation Guide.

[2] Foreline pump requires extra space below the bench

Performance Specifications

For performance specifications of the individual modules of your system, refer to the *Agilent 1200 Infinity Series - Specifications Compendium* or the User Manuals.

Leak and Waste Handling

The Agilent 1260 Infinity Purification Solution has been designed for safe leak and waste handling. It is important that all security concepts are understood and instructions are carefully followed. For the details, refer to the *Agilent 1260 Infinity Purification Solution - Installation Checklist*. All conditions that are listed there, have to be met to run the system.

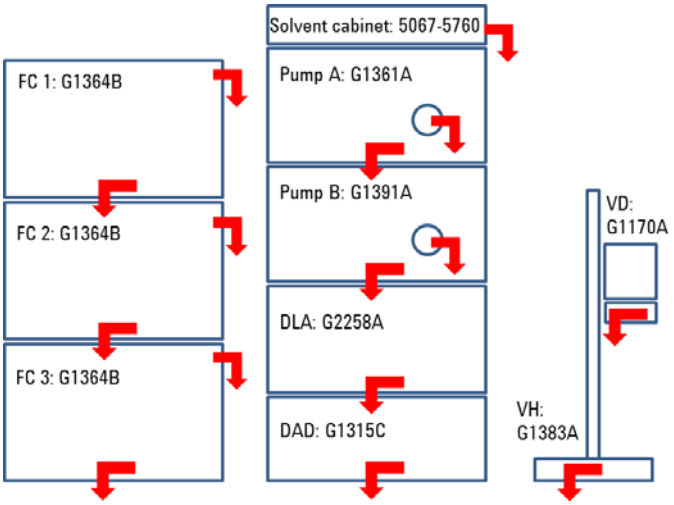

Figure 8 Schema of leak and waste handling in a stack configuration for UV-detection with clustered fraction collectors

1	Tubing to waste

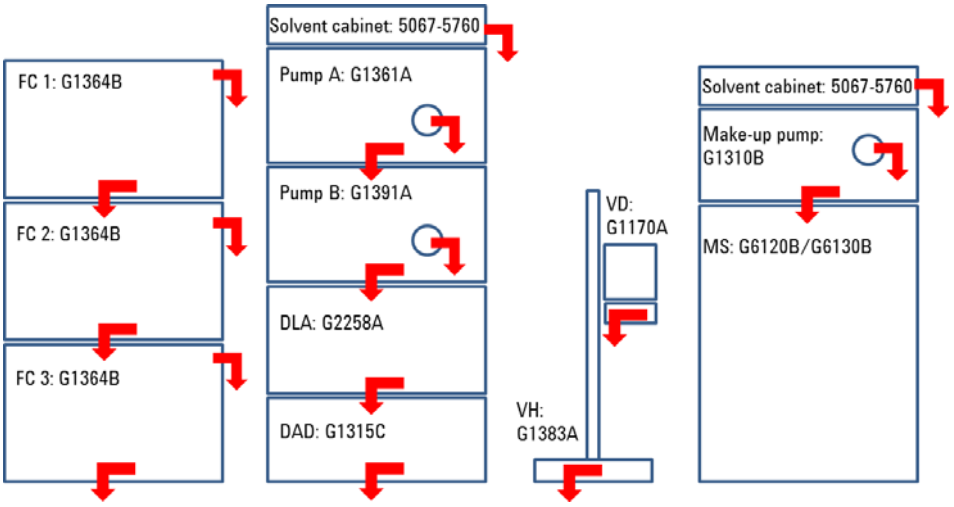

Figure 9 Schema of leak and waste handling in a stack configuration for UVMS-detection with clustered fraction collectors

1	Tubing to waste

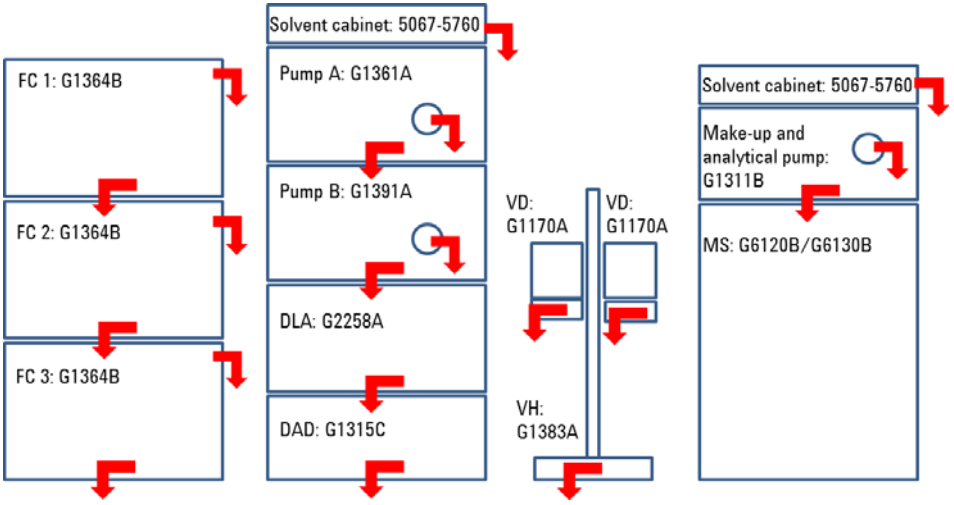

Figure 10 Schema of leak and waste handling in a stack configuration of a combined system with clustered fraction collectors

1	Tubing to waste

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

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

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

The condense drain outlet of the autosampler cooler guides condensates to waste.

The waste tube of the purge valve guides solvents to waste.

The waste tube that is connected to the leak pan outlet on each of the modules guides the solvent to a suitable waste container.

The waste tube of the fraction collector guides needles fractions to waste. For the details, on how to properly connect the fraction collector, refer to the chapter *Installation* in the *Agilent 1260 Infinity Analytical- and Preparative-scale Fraction Collectors - User Manual.*

2
Operating Instructions

This chapter provides information on how to use and operate the Agilent 1260 Infinity Purification Solution.

Automated Purification Software Operation

The Agilent Automated Purification Software provides two levels of user access: *Expert* mode and *Easy Prep* mode. The two user modes are set up by an administrator using user authentication.

Expert Mode

Expert mode is designed for Method Developers. In *Expert* mode, the Method Developer has full access to all purification menu items, and all content of the purification task user interface. The Method Developer is responsible for setting up the analytical and preparative systems parameters, preparing the necessary OpenLAB CDS ChemStation Edition methods, and creating the master tasks for all system combinations. The master tasks include the fine-tuning of the parameters to which the Operator, working in *Easy Prep* mode, has no access. Master tasks serve as templates to allow Operators to create their own tasks.

NOTE

Since the Operator has no access to OpenLAB CDS ChemStation Edition, analytical and preparative methods should not include screen reports.

If it is necessary for the Operator to review the analytical results before starting the purification run, this must be set up by the Method Developer.

In addition, *Expert* mode allows further interaction with OpenLAB CDS ChemStation Edition, for example, to view and modify instrument status and curves, and other actions. However, full access to OpenLAB CDS ChemStation Edition also creates risks, for example, when automatically generated purification methods are modified while the purification sequence is running. The Method Developer should be an experienced OpenLAB CDS ChemStation Edition user, who recognizes the risks and acts suitably.

For full details of the operation of the Automated Purification Software in *Expert* mode, see the *Method Developer's Quick Reference Guide*.

Easy Prep Mode

The *Easy Prep* mode provides restricted access to the purification software for routine operation. Operators, working in *Easy Prep* mode, can view the analytical or preparative systems parameters but cannot make any changes, and cannot edit any of the purification parameters. The Operator uses a master task to set up a new purification task; the new task is a clone of the master task, and the Operator only has to add minimal information before starting the task. Once started, the task runs automatically; if configured by the Method Developer, the analytical run results are displayed for review before the purification run can be started. The Operator has no access to OpenLAB CDS ChemStation Edition from the Automated Purification Software.

For full details of the operation of the Automated Purification Software in *Easy Prep* mode, see the *Operator's Quick Reference Guide*.

Software Administration

When user authentication is enabled in the OpenLAB Control Panel, the Automated Purification Software supports both Method Developers, working in *Expert* mode, and Operators, working in *Easy Prep* mode. When user authentication is not enabled, the Automated Purification Software operates in *Expert* mode. The two types of users are associated with OpenLAB CDS ChemStation Edition privilege to edit methods (ChemStation: Method: Modify Instrument Method). An OpenLAB CDS ChemStation Edition user with method edit rights is a Method Developer; otherwise, the user is an Operator.

The user type defines

- the menu items that are enabled in the Automated Purification Software
- the user interface items that are available
- access to OpenLAB CDS ChemStation Edition from the Automated Purification Software

Administration tasks for the Automated Purification Software include:

- enable user authentication
- set up users
- assign roles to users
- check or edit the privileges of the user roles

For full details of the software administration tasks, see the *Administrator's Quick Reference Guide*.

Tray Handling for Fraction Collection

Recommended Plates and Closing Mats

Warnings

WARNING

Explosive gas mixtures

There is a risk of explosive gas mixtures in the instrument if flammable solvents are used.

➔ Cover the plates.

➔ Remove the plates from the fraction collector after turning it OFF.

➔ Only use solvents with a flash point higher than 200 °C.

CAUTION

Contamination with adhesives

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

➔ 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.

List of Recommended Plates and Closing Mats

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

p/n	Description
5042-1386	96 well plate 0.5 ml, PP (pack of 10)
5042-1385	96 well plate 0.5 ml, PP (pack of 120)
5042-6454	96DeepAgilent31mm
5065-4402	96CappedAgilent
5188-5321	Glass inserts, 0.35 ml, 1000/Pack
5042-1388	384Agilent
5042-8502	96Agilent conical
G2255-68700	Vial plate for 54 x 2 mL vials (6/pk)
5022-6539	Vial plate for 15 x 6 mL vials (1/pk)
5022-6538	Vial plate for 27 Eppendorf tubes (1/pk)
5042-1389	Closing mat for all 96 Agilent plates

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

Installing a Fraction Collector Tray

Installing the Fraction Collector Trays

WARNING

Explosive gas mixtures

There is a risk of explosive gas mixtures in the instrument if flammable solvents are used.

➔ Cover the plates.

➔ Remove the plates from the fraction collector after turning it OFF.

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CAUTION

Contamination with adhesives

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

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

NOTE

Installed trays are automatically detected and identified.

6 Press the front of the fraction collector tray down to secure the tray in the fraction collector.

7 Close the front door.

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

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

Numbering of Vial, Test Tube and Well-plate Positions

With the 4 plates full tray

- Plate in the left front position: P1
- Plate in the left back position: P2
- Plate in the right front position: P3
- Plate in the right back position: P4
- Vessel: A1; A2;... B1; B2;...

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

- Plate in the front position: P1
- Plate in the back position: P2
- Vessel: A1; A2;... B1; B2;...
- Vials / funnels: 1 - 10

With the 100 vials std. tray

- Vial: 1 - 100

With the half-trays

- Left-hand 40-position tray: 1 - 40
- Center 40-position tray: 101-140
- Right-hand 40-position tray: 201 - 240

or

- Left-hand 15-position tray: 1 - 15
- Center 15-position tray: 101-115
- Right-hand 15-position tray: 201 - 215

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.

Replacing a Fraction Collector Tray

When you replace a fraction collector tray, the behavior of the Purification software depends on the tray type:

- If you replace the tray with a different tray type, the Purification software automatically resets the fraction volumes and starts fraction collection on the affected collector(s) from the first position.
- If you replace the tray with the same tray type, however, the Purification software does not automatically reset the fraction volumes and the fraction positions.

  This feature allows you to interrupt the collection for inspecting the tray.

CAUTION Mixing or contamination of fractions, or partial loss of a fraction after an overflow.

→ Use only empty and clean replacement trays.

NOTE Reset the fraction volumes manually after you replaced a tray with a new tray of the same tray type. Failure to comply with this rule will prevent the collector from using the tray's full capacity.

NOTE If the tray is replaced when the fraction collector is switched off, then always reset vial fill volumes.

1 Open the front door.

2 Remove the tray.

3 Load the fraction collector tray with fraction collector vials as required.

   a From within the purification task screen in the top tool bar click **View fraction collector**.

      The fraction collector screen launches and the current tray configuration and fraction fill volume state is visible.

   b Reset the fraction fill volumes (only required if the new tray has the same tray type).

4 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.

5 Press the front of the fraction collector tray down to secure the tray in the fraction collector.

6 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.

Inspecting a Fraction Collector Tray

The Purification software allows you to inspect your fraction collector tray. You can safely continue to process your fraction collection, *as long you do not close the door when no tray is inserted.*

1 Open the front door.

2 Inpect the tray. If you have to remove the tray for inspection, make sure the door remains open until the tray is inserted again. *Do not close the door when the tray is not inserted.*

CAUTION

Closing the door when no tray is loaded resets the fraction positions

Failure to comply with this instruction might lead to mixing of fractions, or to a partial loss of a fraction after an overflow.

➔ Do not continue to process your tray whenever the fraction volumes and positions have been reset. Instead, *reset the vial fill volumes* and continue with a clean and empty tray.

3 Insert the tray again (if appropriate), otherwise replace the tray with an empty and clean tray and reset the vial fill volumes.

4 Close the front door.

5 Continue to process your fraction collection.

Leak and Waste Handling

WARNING

Toxic, flammable and hazardous solvents, samples and reagents

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

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

WARNING

Harmful vapors

The Agilent 6120/30 Single Quadrupole LC/MS is not connected to the CAN bus and is not integrated in the leak shutdown concept of the LC stack.

The flow splitter in front of the ESI nebulizer (simple T-Piece) enables a split ratio of approximately 1:3. The flow into the source is approximately 500 µL/min.

➔ Transfer the exhaust from the ion source and from the roughing pump to a fume hood.

➔ Avoid higher flow rates than 800 µL/min directly without splitting into the source.

➔ Ensure that there is spray and that the T-piece splitter is not blocked.

➔ Never use non volatile buffers into the ion source.

WARNING

Potential risk of electrostatic discharge in fraction collectors

Ignition in the system, especially in the fraction collector with pure organic solvents (Heptane, Hexane, Acetonitrile) at low humidity and flow rates > 25 mL/min.

➔ Careful grounding of all solvent reservoirs.

➔ Usage of conductive capillaries and tubing. Do not replace any stainless steel capillary in the system with PEEK tubing or other non-conductive material.

NOTE

Recommendations for Solvent Cabinet

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

For details on correct installation, see *Agilent 1260 Infinity Purification Solution - Installation Guide*.

3
Required Practice

This chapter provides practical hints for optimizing the purification, avoiding common problems and optimizing the workflow.

Optimizing Recovery Results with the Dual-Loop Autosampler

NOTE

Important information

Read before using the module.

The injection principle of the dual-loop autosampler PS is different to that of other Agilent 1260 Infinity autosamplers. It is a fixed-loop push-through design, see Figure 11 on page 42. Since the sample has to be drawn into a buffer loop, and then transferred into the injection loop, several items have to be considered to achieve best recovery results.

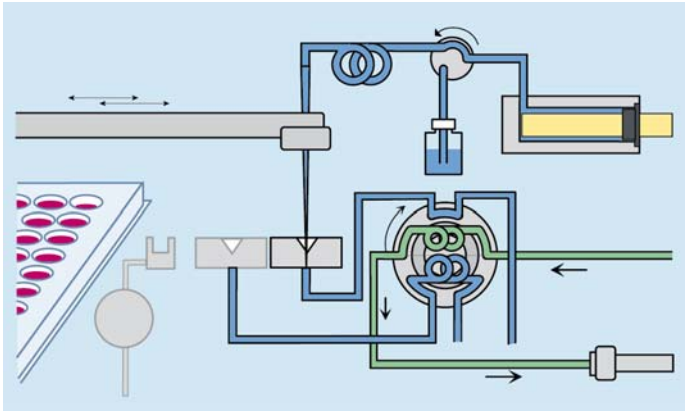

Figure 11 Injection Principle Dual-Loop Autosampler

Rinse Solvent

NOTE The rinse solvent is used to rinse the buffer loop, injection port, the seat capillary, and the injection valve after sample injection.

- Degas the rinse solvent before use.
- To avoid precipitation problems, the sample solvent should dissolve all compounds well. At the same time, to achieve good chromatographic performance, it should contain as little organic solvent as possible. For details, refer to "Sample Draw and Eject Speed" on page 46).
- Use the Rinse function after each injection. The rinse volume, which is calculated from the injection volume and a user-defined factor, should be at least 300 µL.
- Before the first run of a sequence, the syringe should be purged at least 5x. Purge, using the **Purge Syringe** command from the **Instrument/More Injector** menu.

Flush Upper Loop Before Rirst Run

Flush upper loop with starting composition of a new analytical run when the instrument was not used for some time or when the pumps were purged.

NOTE The upper loop can contain large amounts of organic solvent. They could go to the column during the injection step and would modify the elution behavior of the sample compounds. Thus the analytical data would not be a valid input for the Agilent Automated Purification Software.

Enable Needle Wash Step

To minimize contamination of the dual-loop autosampler seat, enable Needle wash in dual-loop autosampler method and set mode **Flush port** and **Time**15 s.

Vial/Well Bottom Sensing

Bottom sensing decreases the amount of sample that cannot be reached by the dual-loop autosampler needle.

NOTE Use bottom sensing only with flat bottom glass vials. Other shapes and materials (such as conical plastic wells) can result into situation that the needle hits the bottom in high velocity. This may cause needle damage and/or pierce the vessel wall. In both cases the injected amount will be incorrect.

Draw Plug Before and After the Sample

- Use **Draw Plug before and after the sample** feature (dual-loop autosampler method) for analytical injections with partial loop filling.
- Set **Draw Plug from** to a vial location and use maximum volume of the plug (= [loop volume - injected volume]/2).
- As a plug solvent use starting composition of the analytical gradient or simply just water, but consider the solubility of the sample.
- Replace the plug vial solvent frequently to avoid cross-contamination and algae growth.

NOTE The **Draw Plug before and after the sample** feature removes the **Rinse solvent** from the analytical injection loop, which is there due to Rinse purge of the seat capillary.

In total approximately 27 µL of the **Rinse solvent** gets into the lower loop and needs to be pushed out by the **Plug solvent**. The **Rinse solvent** typically containins high amounts of organic solvent, which can alter results of the analytical run.

Minimum Partial Loop Filling Injection Volume 10 µL

Do not inject less than 10 µL using partial loop filling injection mode. Injected amount becomes gradually very imprecise below this limit.

Flush Solvent

The flush solvent is used to flush the needle's exterior before injection of the sample. Therefore a solvent should be used, in which the sample is readily soluble. Typically this can be the same solvent as the rinse solvent (for example acetonitril/water 80/20). For a list of solvents compatible with the tubing of the peristaltic pump see Table 3 on page 45.

Table 3 Solvents Compatible with Tubings

Solvent	PharMed[1]	Silicone[2]
Acetic acid > 5 %	A	B
Acetone	D	D
Acetonitrile	A	-
Hexane, Heptane	C	-
Ammonium acetate	C	-
Ethanol	C	B
Formic acid	A	C
Methanol	D	A
Propanol	C	A
Trichloroacetic acid	D	D
Water	A	A

1 Pre-installed

2 Can be ordered (5042-8507)

Table 4 Legend

A	Fully compatible
B	Minor reaction, e.g. slight corrosion or discoloration
C	Not recommended for continuous use. Swelling/shrinkage, loss of strength.
D	Severe reaction, not recommended for use

Sample Draw and Eject Speed

Due to the push-through design of the dual-loop autosampler, the sample draw and eject speed influence the recovery. Increasing the draw speed has only a minor effect, however the influence of the eject speed has a much higher impact, as shown in Figure 12 on page 46.

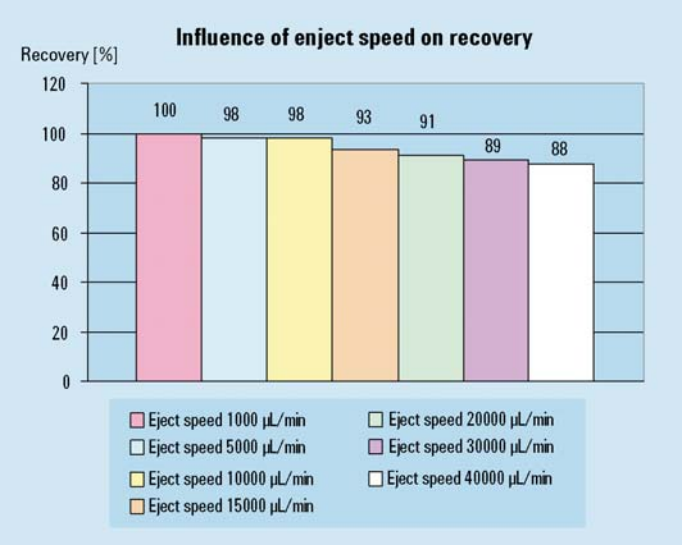

Figure 12 Influence of Eject Speed on Recovery (Draw Speed 20000 µL/min

- Keep speeds for preparative run such that injection lasts for approximately 5 – 10 s but below 20000 µL/min for draw and 10000 µL/min for eject speeds.
- Use 1000 µL/min draw and eject speeds for analytical injections.
- For best recovery results the sample draw speed should not exceed 20000 µL/min.
- Lower eject speeds yield better recoveries. The default eject speed is 10000 µL/min, however, for best recovery results this value should be lowered further.
- Highly viscous rinse solvents like DMSO, for example, yield lower recoveries even with low eject speeds. Therefore, we recommend using a mixture of DMSO/acetonitrile 50:50 v/v instead.

Sample Loop Overfill Factor for Complete Loop Filling

Depending on the size of the used sample loop, the loop overfill factor should range between at least 3 – 5 times the volume of the used loop in order to achieve best possible reproducibility of the results with minimum deviations.

The overfill factor should be closer to 3 times for large samples and closer to 5 for small samples.

Sample Loop Fill Factor for Partial Loop Fill

Figure 13 on page 47 shows the result of several injections with various injection volumes using the same sample loop (1000 µL). The peak area increases linearly until the loop is filled approximately up to 50 % (fill factor 0.5), which is represented by the red line. This means that in order to maximize the sample recovery the maximum injection volume should not exceed 50 % of the sample loop volume.

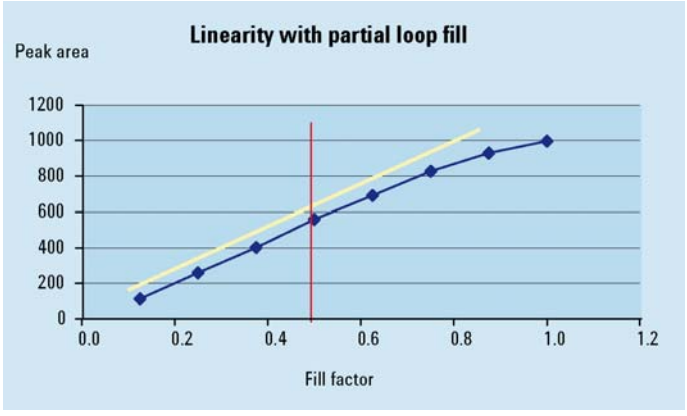

Figure 13 Linearity of Peak Area for Partial Loop Fill

Successful Use of the Preparative Pump

1. Flush the pump extensively, when changing to a new solvent, or when the instrument was not used for some time.
2. The system pressure must be higher than 20 bar at the pump outlet for optimum performance of the pump.
3. Place the solvent cabinet with the solvent bottles always on top of the preparative pump (or at a higher level).
4. Prevent blocking of solvent inlet filters (never use the pump without solvent inlet filters). Growth of algae should be avoided (see "Prevent Blocking of Solvent Filters" on page 49).
5. Regularly clean the filter cup and the filter frit installed in the multi assembly. A back pressure greater than 10 bar, when purging pumps with pure HPLC grade water at a flow rate of 50 mL/min while the electromagnetic purge valve (EMPV) is open, indicates that one of the two filters is blocked or that the EMPV does not switch (open) properly. Make sure to follow the correct procedures for cleaning filters (see *Simple Repairs in the pump manual*) and the EMPV (see *Prep Pump EMPV Cleaning Description in the pump manual*). Always clean the pump's filters, after exchanging seals.
6. Confirm that the pump and the rest of the system are completely leak tight by performing the Leak-test (see *Prep Pump Leak Test Description in the pump manual*) and Pressure-test (see *Prep Pump Pressure Test Description in the pump manual*) regularly.
7. When using buffer solutions, flush the system with plenty of water to remove all buffer solution from the entire system before switching it OFF or before changing to an organic solvent.
8. Always use the seal wash function.
9. Check the pump seals. Scratched pistons will lead to micro leaks and will decrease the lifetime of the seal. Check pistons for scratches when changing the seals and exchange them, if scratched.
10. For the generation of gradients in systems with multiple pump setups, make sure that the pump cluster delivers more than 5 mL/min at any time during the gradient run, in order to achieve best performance. For the creation of scouting runs, at lower flow rates, an analytical pump is used also on combined systems.

Prevent Blocking of Solvent Filters

NOTE Never use the system without solvent filter installed.

1. Use a sterile, if possible amber, solvent bottle to slow down algae growth.
2. Filter solvents through filters or membranes that remove algae.
3. Exchange solvents every two days or refilter.
4. If the application permits add 0.01 – 0.1 % sodium azide to the solvent.
5. Place a layer of argon on top of your solvent.
6. Avoid exposure of the solvent bottle to direct sunlight.
7. Filter HPLC grade (dry) Acetonitrile before use. The dryer the Acetornitrile, the stronger the tendency to form polymers and therefore block the system. Refilter at least every two days.

Solvent Information

Observe the following recommendations on the use of solvents.

- Follow recommendations for avoiding the growth of algae, see pump manuals.
- Small particles can permanently block capillaries and valves. Therefore, always filter solvents through 0.22 µm filters.
- Avoid or minimize the use of solvents that may corrode parts in the flow path. Consider specifications for the pH range given for different materials like flow cells, valve materials etc. and recommendations in subsequent sections.

Solvent compatibility for stainless steel in standard LC systems

Stainless steel is inert against many common solvents. It is stable in the presence of acids and bases in the pH range specified for standard HPLC (pH 1 – 12.5). It can be corroded by acids below pH 2.3. In general following solvents may cause corrosion and should be avoided with stainless steel:

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

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

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

Algae Growth in HPLC Systems

The presence of algae in HPLC systems can cause a variety of problems that may be incorrectly diagnosed as instrument or application problems. Algae grow in aqueous media, preferably in a pH range of 4-8. Their growth is accelerated by buffers, for example phosphate or acetate. Since algae grow through photosynthesis, light will also stimulate their growth. Even in distilled water small-sized algae grow after some time.

Instrumental Problems Associated With Algae

Algae deposit and grow everywhere within the HPLC system causing:

- Blocked solvent filters or deposits on inlet or outlet valves resulting in unstable flow, composition or gradient problems or a complete failure of the pump.
- Small pore high pressure solvent filters, usually placed before the injector to plug resulting in high system pressure.
- PTFE frits blockage leading to increased system pressure.
- Column filters to plug giving high system pressure.
- Flow cell windows of detectors to become dirty resulting in higher noise levels (since the detector is the last module in the flow path, this problem is less common).

Symptoms Observed with the Agilent 1260 Infinity HPLC

The presence of algae in the Agilent 1260 Infinity can cause the following to occur:

- Increased system pressure caused by clogging of the inline filter. Algae deposits are barely visible on the stainless steel filter frit. Replace the frit if the backpressure of the pump in purge mode (water, 50 mL/min) exceeds 1 bar.
- Short lifetime of solvent filters (bottle head assembly). A blocked solvent filter in the bottle, especially when only partly blocked, is more difficult to identify and may show up as gradient performance problems, intermittent pressure fluctuations etc.
- Algae growth may also be the possible source for failures of the ball valves and other components in the flow path.

Regular System Cleaning and Preventive Maintenance

NOTE To achieve maximum performance of the system, keep the system clean and perform preventive maintenance. Follow the recommendations, as described in the module manuals.

Choosing a Preparative Flow Cell

To achieve optimal results, it is important to choose the correct flow cell for each system configuration.

- UV in front of splitter (or no splitter at all):
  - Usually: 0.06 mm flow cell for any flow rate.

NOTE Use backpressure regulator at maximum 7 bar (100 psi).

  - For diluted samples or samples with very low UV absorbance: 0.3 mm flow cell
- UV behind the splitter:

  3 or 10 mm flow cell, depending on the split factor for the MSD while UV signal is still acceptable.

NOTE Use a backpressure regulator when preparative cell types (0.3 and 0.06 mm) are used in combination with flow rates below 10 mL/min. Outgassing air bubbles in the cell because of a missing backpressure will cause a high baseline noise.

For details on flow cells, see *Technical Note for Preparative Flow Cells*.

Calibration Procedures

For details on calibration procedures, see the *Automated Purification Software Online Help* or the *Agilent 1260 Infinity Purification Solution Method Developer's Quick Start Guide*.

4
Troubleshooting

Overview about troubleshooting.

Overview about Troubleshooting

If you observe a too low intensity of UV- and MS-detection in the preparative run, the following causes are probable:

- Air bubbles in the syringe of the metering device
- Autosampler sucks air (tubing or metering device defect)
- Wrong vials (use vials with slit septa)
- Preparative column is defect
- Active flow splitter not working correctly
- Make-up pump not working correctly
- Clogged autosampler waste tube
- Needle incorrectly postioned
- Clogged needle or needle seat capillary
- Low quality of make-up solvent
- In cases, where UV-signal is ok and MS-signal is very low:
  - No acid added to the make-up solvent so ionization is difficult (only MS-signal low)
  - Plugged electrospray nebulizer needle
  - Electrospray chamber is too dirty
  - Incorrect MSD settings

Troubleshooting depends on the configuration of the system:

- Combined Analytical-Scale and Preparative-Scale LC Instrument With UV- and MS-Detection - UV and MSD in Split Flow
  - Analytical:
    - "Troubleshooting Low or no Signal on UV- and MS-Detector in Analytical Mode" on page 59
    - "Troubleshooting Low or no Signal on MS-Detector and Normal Signal on UV-Detector in Analytical Mode" on page 63
  - Preparative:
    - "Troubleshooting Low or no Signal on UV- and MS-Detector in Preparative Mode" on page 64
    - "Troubleshooting Low or no Signal on MS-Detector and Normal Signal on UV-Detector in Preparative Mode" on page 69
- Preparative-Scale LC Instrument With UV-Detection before Active Flow Splitter
  - "Troubleshooting a Preparative-Scale LC (UV-Detection before Flow Split)" on page 71
- Preparative System with UV Based Triggering
  - "Troubleshooting a Preparative-Scale LC (UV Based Triggering)" on page 74

To verify a successfull troubleshooting, refer to "Recovery Check" on page 76.

Troubleshooting a Combined Analytical-/Preparative-Scale LC

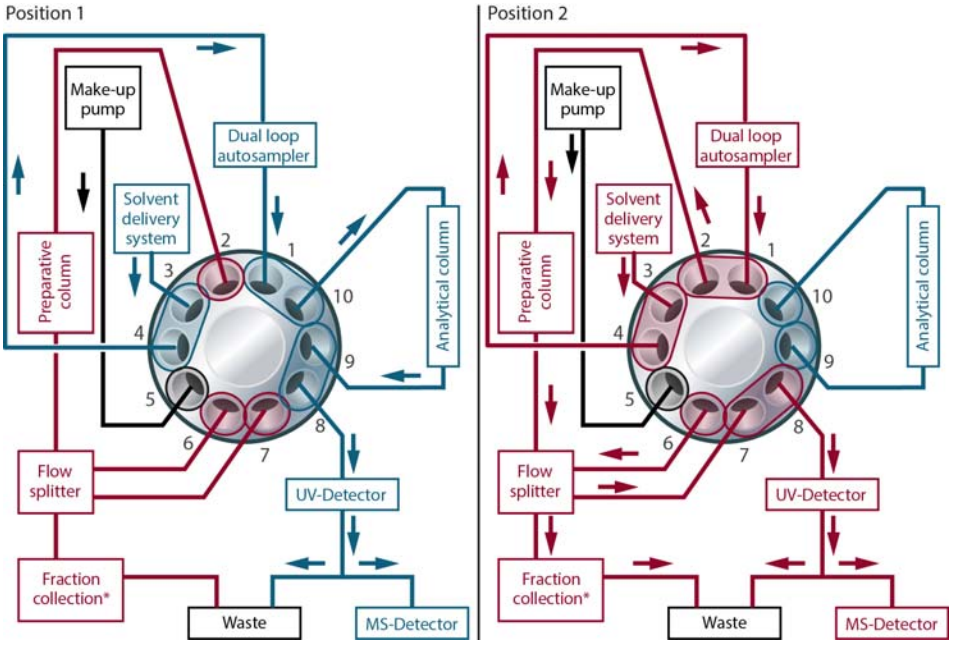

Figure 14 Schematic overview for a UV-MS detection based combined analytical to preparative scale system

* Fraction collector (FC) options:
One FC with one G1170A Valve drive/G4730 Valve kit, or
Up to three FCs as cluster with one G1170A Valve drive/G4730A Valve kit and one G1170A Valve drive/G4731A Valve kit

Troubleshooting in Analytical Mode

Troubleshooting Low or no Signal on UV- and MS-Detector in Analytical Mode

Parts required	#	p/n	Description
	1	5190-6886	Agilent Standard #1 (Spec out solution for 1260 LC-MS)
	1	G1361-25202	Valve Adapter long out (OPTIONAL)
	1	G1361-25203	Valve Adapter long in (OPTIONAL)
	1	G1361-60052	Valve Assy Double seat (OPTIONAL)

NOTE Use only vials with pre-slit septa and flat bottom glass.

---

1 Functional check of the Solvent Delivery System:

a Set flow to 2 mL/min at min 20 bar back pressure and observe %ripple in dashboard

- Solvent A: 100 % ripple should not exceed 10 %
- Solvent B: 100 % ripple should not exceed 10 %
- Solvent A and B: each 50 % ripple should not exceed 15 %

b If Ripple Test failed, check solvent delivery system for correct function.

- Check solvent reservoirs and refill if necessary.
- Prime the pumps to remove air bubbles (at least 75 mL each channel for 1 min) and check function.
- Check and if necessary replace valves of the pump (parts:Valve Adapter long out (G1361-25202), Valve Adapter long in (G1361-25203), and Valve Assy Double seat (G1361-60052)).

OR

Ripple test passed and solvent delivery system is delivering the right flow composition:

- Check if gradient profile has been ramped up after a sample has been injected. Usually this indicates to an error in the method. Correct method if necessary.

2 Functional check Autosampler:

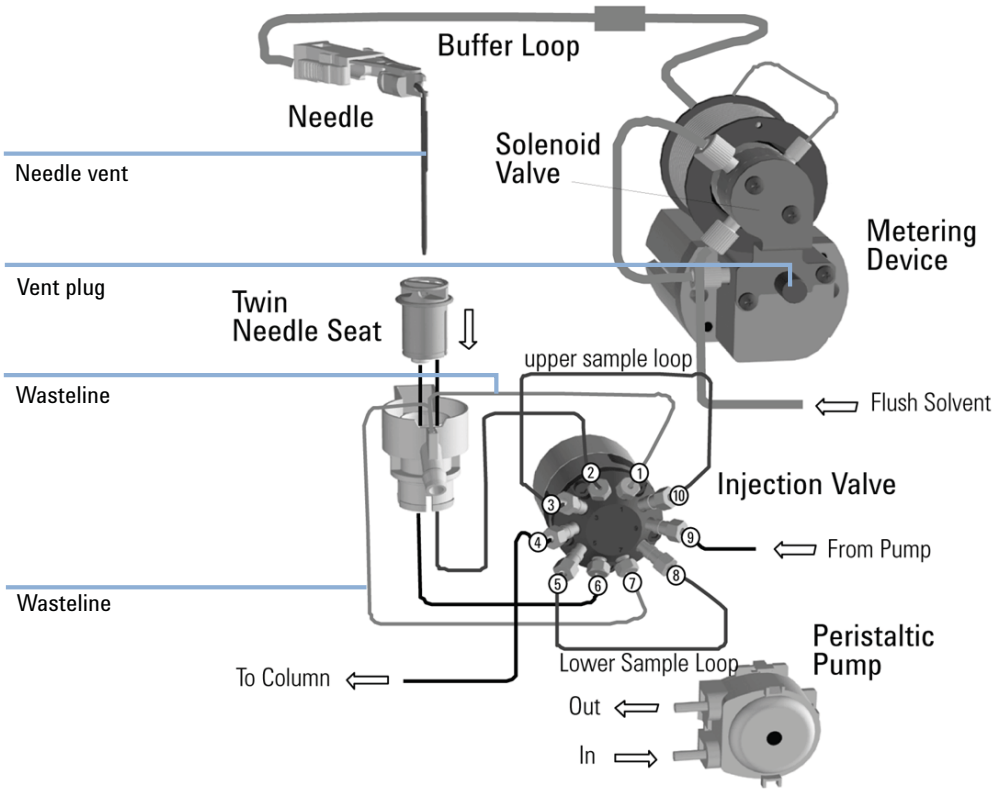

Figure 15 Hydraulic flow path of the Dual-Loop Autosampler (G2258A)

a Check for air bubbles in auxiliary solvent lines (after solvents have been refilled)

- If yes:
  - Purge syringe of the dual loop autosampler 10 x: To remove all air bubbles, open the purge port and use a syringe and inject at least 10 mL of water through the buffer loop. Check again.

OR

If no:

- Purge syringe of dual-loop autosampler 5x.
- Use lower (analytical) loop and start a run.
- Fill 1000 µL water in a vial (pre slitted septa or no septum, flat bottom), measure, and note its weight.
- Place the vial in the dual-loop autosampler. Use lower (analytical) loop. Use full loop injection mode (overfill factor 3) and inject 50 µL.
- Remove the waste tubing lower loop and check if the solvent from the loop goes into the waste line.

NOTE Waste lines could be blocked after large concentrated samples dried down in the dual-loop autosampler.

- Measure the weight of the vial after the injection. The weight should be reduced by approximately 150 mg (±10 %).
- Repeat the test with 10 µL injection volume in partial loop mode.
  - If this works: Autosampler is injecting correctly, or
  - If this does not work - neither without septum, nor with split septum: Exchange capillary injection needle and check again.

3 Functional check of the Column Selection Valve:

a Ensure that the position written in the method is correct (analytical).

b Ensure that you have flow through your column (remove the capillary to the UV detector and check if the flow is there).

4 Functional Check of the Analytical Column:

a Inject 10 µL of Agilent Standard #1 (5190-6886).

b Check peak shapes.

When observing incorrect peak shapes (fronting, tailing, peak splitting), replace the column.

5 Functional check of the UV-Detector:

a In the method, set the wavelength of 254 (4 nm bandwidth).

NOTE This is necessary, when using Agilent Standard #1 (5190-6886).

b Ensure that there is an appropriate cell in use:

- Analytical injection volumes: 3 mm or 10 mm cell
- Preparative injection volumes: 0.3 mm or 0.06 mm cell

c Set bandwidth to 4 nm.

NOTE Large bandwidths (200 nm and higher) will decrease the sensitivity.

6 Functional check of the MS-Detector

a Remove the sprayer and ensure that there is a spray flow rate between 100 – 500 µL/min (measure the flow into the waste container and calculate the flow into the MS as a difference).

b Ensure that the right method parameters have been set (source parameters, acquisition parameters).

NOTE Most likely (if UV and MS signals are missing or have a low intensity)

c Check for wrong flow path (method error), autosampler malfunction, solvent delivery malfunction.

7 Check the recovery of the Purification Solution System, see “Recovery Check” on page 76.

Troubleshooting Low or no Signal on MS-Detector and Normal Signal on UV-Detector in Analytical Mode

NOTE Use only vials with pre-slit septa and flat bottom glass.

1 Functional check of the MS-Detector

   a Remove the sprayer and ensure that there is a spray flow rate between 100 – 500 µL/min (measure the flow into the waste container and calculate the flow into the MS as a difference).

   b Ensure that correct method parameters have been set (source temperatures, ionization modes, acquisition parameters).

   c If spray flow and method parameters are correct: Connect the capillary from the tuning solution reservoir directly to the source sprayer and run autotune function.

NOTE If autotune passes successful, the MSD works correctly.

- If autotune does not pass: Check the solvent quality and its composition and correct if necessary:
  - If the formic acid buffer is missing, add formic acid in the appropriate concentration.
  - Use only appropriate buffers below 10 mM.
  - Use only LC-MS grade solvents.

NOTE Low quality solvents (LC-MS grades solvents needed) contain a high level of contaminants which cause ion suppression.

OR

If solvent quality and its composition is correct: Check whether the column is appropriate and change if necessary.

2 Check the recovery of the Purification Solution System, see "Recovery Check" on page 76.

Troubleshooting in Preparative Mode

Troubleshooting Low or no Signal on UV- and MS-Detector in Preparative Mode

Parts required	#	p/n	Description
	1	5190-6886	Agilent Standard #1 (Spec out solution for 1260 LC-MS)
	1	G1361-25202	Valve Adapter long out (OPTIONAL)
	1	G1361-25203	Valve Adapter long in (OPTIONAL)
	1	G1361-60052	Valve Assy Double seat (OPTIONAL)

NOTE Use only vials with pre-slit septa and flat bottom glass.

NOTE Only ever troubleshoot one pump at a time.

1 First check, if the system works correct in analytical mode. To do this, inject 10 µL of Agilent Standard #1 (5190-6886).

   If the system works well with analytical flow path, it is likely that something with your preparative column or your active flow splitter or your make up solvent flow is not working correct.

2 Functional check of the Solvent Delivery System

   a Perform ripple test with appropriate flow rates to your column size (for example 20 mL/min or 30 mL/min).

   - Solvent A: 100 % ripple should not exceed 7 %
   - Solvent B:: 100 % ripple should not exceed 7 %
   - Solvent A and B: each 50 % ripple should not exceed 12 %
   - Check solvent reservoirs and refill if necessary.
   - Prime the pumps to remove air bubbles (at least 75 mL each channel for 1 min) and check function.

- Check and if necessary replace valves of the pump (parts:Valve Adapter long out (G1361-25202), Valve Adapter long in (G1361-25203), and Valve Assy Double seat (G1361-60052)).
- Check if gradient profile has been ramped up after a sample has been injected. Usually this indicates to an error in the method. Correct method if necessary.

3 Functional check Autosampler

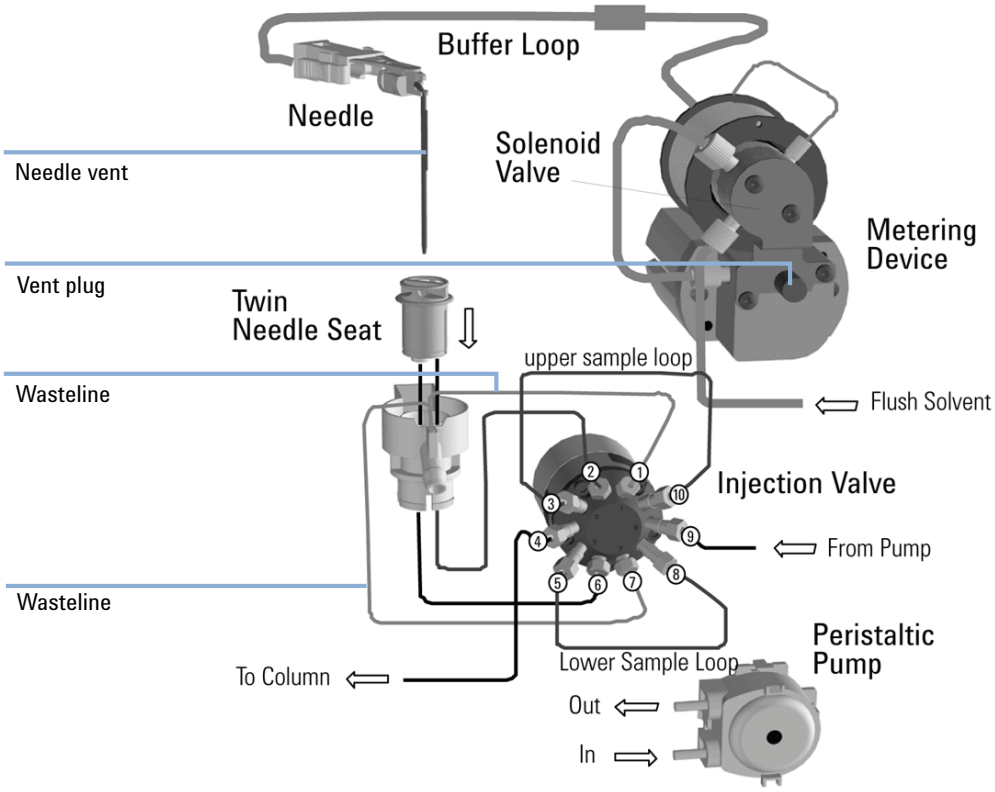

Figure 16 Hydraulic flow path of the Dual-Loop Autosampler (G2258A)

a Check for air bubbles in auxiliary solvent lines (after solvents have been refilled)

b If yes:

- Purge syringe of the dual loop autosampler 10 x: To remove all air bubbles, open the purge port and use a syringe and inject at least 10 mL of water through the buffer loop. Check again.

OR

If no:

- Purge syringe of dual-loop autosampler 5x.
- Use the upper loop, start a run.
- Fill 1000 µL water in a vial (pre slitted septa or no septum, flat bottom), measure, and note its weight.
- Place the vial in the dual-loop autosampler. Use upper (preparative) loop. Use partial loop injection mode and inject 250 µL.
- Remove the waste tubing lower loop and check if the solvent from the loop goes into the waste line.

NOTE Waste lines could be blocked after large concentrated samples dried down in the dual-loop autosampler.

- Measure the weight of the vial after the injection. The weight should be reduced by approximately 250 mg (± 10 %).
  - If this works: Autosampler is injecting correctly, or
  - If this does not work - neither without septum, nor with split septum: Clean or if necessary exchange venting capillary injection needle and check again.

4 Functional test of the column selection valve

CAUTION Overpressure in the system

➔ Make sure, the valve is in preparative position.

a Inject 250 µL of Agilent Standard #1 (5190-6886).

b Check peak shapes.

When observing incorrect peak shapes (fronting, tailing, peak splitting), replace the column.

5 Functional check of the active flow splitter:

a Set correct split ratio (1:1000), see *MRA Operating Manual* (G1968F)

b Synchronize the splitter with the method to ensure it will start to run after the sample has been injected, see *MRA Operating Manual*.

6 Functional check of the make-up pump

a Ensure that the make-up flow is between 1 and 2 mL/min)

b Ensure that the purge valve of the make up pump is closed and that no air bubbles are inside the make-up pump lines.

c Ensure that the make up solvent has an LC-MS supported composition (for example 90 % Acetonitrile / 9.9 % Water / 0.1 % Formic Acid - all solvents LC-MS grade quality).

7 Functional check of the UV-Detector:

a In the method, set the wavelength of 254 (4 nm bandwidth).

NOTE This is necessary, when using Agilent Standard #1 (5190-6886).

b Ensure that there is an appropriate cell in use:

- Analytical injection volumes: 3 mm or 10 mm cell
- Preparative injection volumes: 0.3 mm or 0.06 mm cell

c Set bandwidth to 4 nm.

NOTE Large bandwidths (200 nm and higher) will decrease the sensitivity.

8 Functional check of the MS-detector.

a Remove the sprayer and ensure that there is a spray flow rate between 100 – 500 µL/min (measure the flow into the waste container and calculate the flow into the MS as a difference).

b Ensure that the right method parameters have been set (source parameters, acquisition parameters)

NOTE If the system works well with analytical flow path, it is likely that something with your preparative column or your active flow splitter or your make-up solvent flow is not working correct.

Low signals on both detectors UV and MS can indicate a malfunction of the active flow splitter. For maintenance of the active flow splitter, see *MRA Operating Manual* (G1968F).

- If spray flow and method parameters are correct: Connect the capillary from the tuning solution reservoir directly to the source sprayer and run autotune function.

NOTE If autotune passes successful, the MSD works correctly.

- If autotune does not pass: Check the solvent quality and its composition and correct if necessary:
  - If the formic acid buffer is missing, add formic acid in the appropriate concentration.
  - Use only appropriate buffers below 10 mM.
  - Use only LC-MS grade solvents.

NOTE Low quality solvents (LC-MS grades solvents needed) contain a high level of contaminants which cause ion suppression.

OR

If solvent quality and its composition is correct: Check whether the column is appropriate and change if necessary.

9 Check the recovery of the Purification Solution System, see "Recovery Check" on page 76.

Troubleshooting Low or no Signal on MS-Detector and Normal Signal on UV-Detector in Preparative Mode

1 Functional check of the MS-detector

   a Remove the sprayer and ensure that there is a spray flow rate between 100 – 500 µL/min (measure the flow into the waste container and calculate the flow into the MS as a difference).

   b Ensure that the right method parameters have been set (source parameters, acquisition parameters)

NOTE If the system works well with analytical flow path, it is likely that something with your preparative column or your active flow splitter or your make-up solvent flow is not working correct.

Low signals on both detectors UV and MS can indicate a malfunction of the active flow splitter. For maintenance of the active flow splitter, see *MRA Operating Manual* (G1968F).

- If spray flow and method parameters are correct: Connect the capillary from the tuning solution reservoir directly to the source sprayer and run autotune function.

NOTE If autotune passes successful, the MSD works correctly.

- If autotune does not pass: Check the solvent quality and its composition and correct if necessary:
  - If the formic acid buffer is missing, add formic acid in the appropriate concentration.
  - Use only appropriate buffers below 10 mM.
  - Use only LC-MS grade solvents.

NOTE Low quality solvents (LC-MS grades solvents needed) contain a high level of contaminants which cause ion suppression.

OR

If solvent quality and its composition is correct: Check whether the column is appropriate and change if necessary.

2 Check the recovery of the Purification Solution System, see “Recovery Check” on page 76.

Other issues

1 Check whether the make-up pump causes the isssue.

NOTE This is probable if the flow rate at detector is < 1.5 mL/min.

a To remove air-bubbles, purge the make-up pump and check flow rate at detector (should be 1.5 mL/min).

b If this didn't solve the problem, continue with step 3 on page 70.

2 Check whether the splitter causes the issue.

a Check split rate (20 equals a ratio of 1/1000). If split rate *correct*, continue with step b on page 70

OR

Check split rate (20 equals a ratio of 1/1000). If split rate *not correct*, set correct split rate and check the preparative peak in a new run.

b Bypass the splitter and inject 5, 10, and 20 µL of Agilent Standard #2 (5190-6887) to the analytical column (10 µL). Note the result.

- If the injection results in about 2000 mAU signal on the analytical column, check whether the splitter looses liquid.

  OR

  If the injection results in less than 2000 mAU signal on the analytical column, continue with step 1 on page 70.

- Reintegrate the splitter to the system.

c If the splitter looses liquid, change the gasket of the splitter or the splitter itself and check the peak of the preparative column in a new run.

If this solved the problem, finish procedure.

3 Check for other probable causes.

a Inject blue Agilent Standard #2 (5190-6887).

b Check the two PTFE-Tubes at the 10port valve. They must guide the blue liquid to waste. If tubes are clogged, clean tubes.

c If the two PTFE-Tubes at the 10port valve are clean and there are visible leaks: Change needle and needle seat of the dual-loop autosampler.

Troubleshooting a Preparative-Scale LC (UV-Detection before Flow Split)

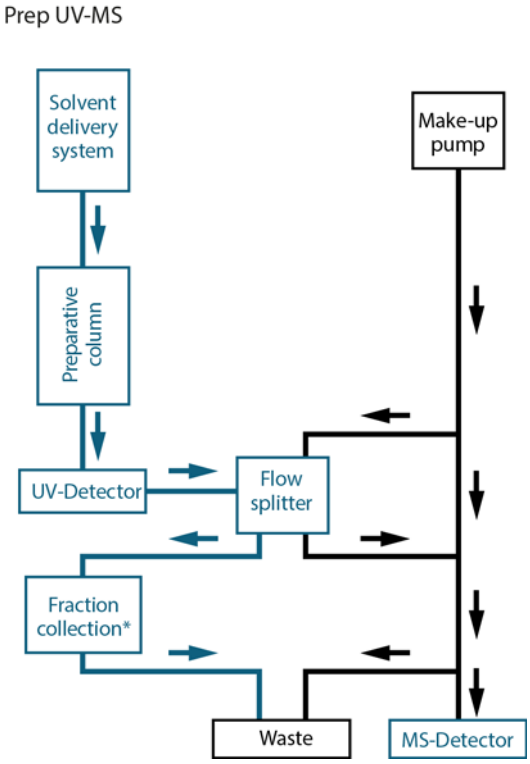

Figure 17 Schematic overview for a UV-MS detection based preparative scale system

* Up to three fraction collectors (FC) as cluster with G1170A Valve drive/G4731A Valve kit

Probable causes for low detector signals are the following:

- IF the signal intensity is low on both detectors (UV and MS) it is likely that there is an autosampler, a column or a solvent delivery malfunction.

For troubleshooting refer to step 2 on page 64, step 3 on page 65and step 4 on page 66

- IF the signal intensity is low only on the MSD, it is likely that there is a malfunction with the active flow splitter, the make up solvent or the MSD.

  For troubleshooting refer to step 5 on page 67, step 6 on page 67 and step 8 on page 67 .

Other issues

1 Check whether the make-up pump causes the isssue.

NOTE This is probable if the flow rate at detector is < 1.5 mL/min.

---

a To remove air-bubbles, purge the make-up pump and check flow rate at detector (should be 1.5 mL/min).

b If this didn't solve the problem, continue with step 3 on page 73.

2 Check whether the splitter causes the issue.

a Check split rate (20 equals a ratio of 1/1000). If split rate *correct*, continue with step b on page 72

OR

Check split rate (20 equals a ratio of 1/1000). If split rate *not correct*, set correct split rate and check the preparative peak in a new run.

b Bypass the splitter and inject 5, 10, and 20 µL of Agilent Standard #2 (5190-6887) to the analytical column (10 µL). Note the result.

- If the injection results in about 2000 mAU signal on the analytical column, check whether the splitter looses liquid.

  OR

  If the injection results in less than 2000 mAU signal on the analytical column, continue with step 1 on page 72.

- Reintegrate the splitter to the system.

c If the splitter looses liquid, change the gasket of the splitter or the splitter itself and check the peak of the preparative column in a new run.

If this solved the problem, finish procedure.

3 Check for other probable causes.

a Inject blue Agilent Standard #2 (5190-6887).

b Check the two PTFE-Tubes at the 10port valve. They must guide the blue liquid to waste. If tubes are clogged, clean tubes.

c If the two PTFE-Tubes at the 10port valve are clean and there are visible leaks: Change needle and needle seat of the dual-loop autosampler.

Troubleshooting a Preparative-Scale LC (UV Based Triggering)

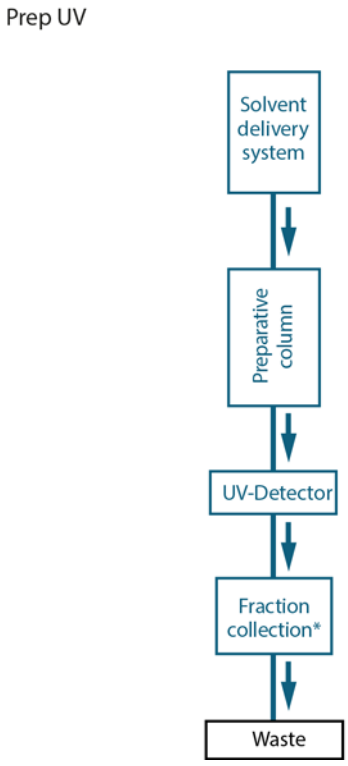

Figure 18 Schematic overview for a UV detection based separate preparative to preparative scale system

* Up to three fraction collectors (FC) as cluster with G1170A Valve drive/G4731A Valve kit

If the signal intensity on the UV-detector is low and an appropriate cell is used, it is likely that there is an autosampler (see step 3 on page 65), a column (see step 4 on page 66) or a solvent delivery (see step 2 on page 64) malfunction.

Parts required	#	p/n	Description
	1	5190-6886	Agilent Standard #1 (Spec out solution for 1260 LC-MS)
	1	G1361-25202	Valve Adapter long out (OPTIONAL)
	1	G1361-25203	Valve Adapter long in (OPTIONAL)
	1	G1361-60052	Valve Assy Double seat (OPTIONAL)

1 In the method, set the wavelength of 254 (4 nm bandwidth).

NOTE This is necessary, when using Agilent Standard #1 (5190-6886).

2 Ensure that there is an appropriate cell in use:

- Analytical injection volumes: 3 mm or 10 mm cell
- Preparative injection volumes: 0.3 mm or 0.06 mm cell

NOTE Use 0.3 mm or 0.06 mm flow cells in combination with 21.2 mm column diameters. For smaller column diameters the concentrations are too low to generate a proper response.

3 Set bandwidth to 4 nm.

NOTE Large bandwidths (200 nm and higher) will decrease the sensitivity.

4 Check the recovery of the Purification Solution System, see "Recovery Check" on page 76.

Recovery Check

Parts required	p/n	Description
	5190-6887	Agilent Standard #2 (Delay time calibration solution for 1260 LC-MS)

Preparations

Finish all troubleshooting procedures, that are suitable for the configuration of your system:

- Combined analytical-scale and preparative-scale LC instrument with UV- and MS-detection:
  - "Troubleshooting Low or no Signal on UV- and MS-Detector in Analytical Mode" on page 59
  - "Troubleshooting Low or no Signal on MS-Detector and Normal Signal on UV-Detector in Analytical Mode" on page 63
  - "Troubleshooting Low or no Signal on UV- and MS-Detector in Preparative Mode" on page 64
  - "Troubleshooting Low or no Signal on MS-Detector and Normal Signal on UV-Detector in Preparative Mode" on page 69
- Preparative-scale LC instrument with UV-detection before active flow splitter:
  - "Troubleshooting a Preparative-Scale LC (UV-Detection before Flow Split)" on page 71
- Preparative system with UV-based triggering:
  - "Troubleshooting a Preparative-Scale LC (UV Based Triggering)" on page 74

1 Run the delay time calibration procedure.

NOTE For details, see the *Automated Purification Software Online Help* or the *Agilent 1260 Infinity Purification Solution Method Developer's Quick Start Guide*.

2 Ensure that all delays have been set correctly.

NOTE UV detector and MSD must work synchronized to trigger fractions.

3 Set the flow rate to the flow range, to be used for operation mode.

4 Set the wavelength to 590 nm (bandwith 10 nm) at the UV detector

5 Use UV triggering only. Use threshold 10 mAU, upslope of 1 and a downslope of 1.

6 Inject 200 µL of Agilent Standard #2 (5190-6887) and start the method.

7 Observe the inlet and the waste tube of the fraction collector.

Does the fraction collector open its diverter valve to the fraction vessels when the blue dye arrived at the collector?

8 If no:

- The trigger is too early clear solvent will be collected first before it will turn into deep purple.
- The trigger is too late, blue dye will be already in the waste line before the diverter valve will switch.

a In this case: ensure that the delay volume has been set correctly, ensure that the solvent delivery pump A has been linked with the fraction collector.

b Repeat the delay time calibration.

c Repeat this test.

OR

If yes (with a tolerance of 0.5 – 1 s

d Use a combined mass and UV trigger.

e Keep the settings for UV like they have been used in the test before.

f Use all fraction triggers in the fraction collector method.

g Enter mass 227 in the sequence table.

h Ensure that the delay time between MSD and fraction collector has been set correctly into the method.

i Inject 200 µL of Agilent Standard #2 (5190-6887) and repeat the visual test.

Does the fraction collector open its diverter valve to the fraction vessels exactly when the blue dye arrived at the collector?

- IF no: The delay time or the threshold settings for the MSD are incorrect. Repeat the test with the correct settings.
- IF yes: The system will trigger correctly.

5
Maintenance, Repair and Parts

For detailed information on maintenance, repair and parts, see the individual module manuals.

Overview on Maintenance, Repair and Parts

NOTE The Agilent 1260 Infinity Purification Solution works in different configurations and with different modules. To get an overview on maintenance, repair and parts, refer to the manuals of the individual modules.

The documentation for these modules is available on the *Agilent Purification & Preparative LC User Documentation DVD*.

NOTE The Agilent 1260 Infinity Purification Solution is very flexible and offers several configurations. The kits that are listed below are designed to support all configurations.

Capillary Kit

	p/n	Description
	5067-6175	Capillary Kit for UV-based Systems
OR	5067-6176	Capillary Kit for Mass-based Systems
	846975-902	ZORBAX SB-C18, 4.6 x 50 mm, 5 µm
	870050-902	PrepHT,Zorbax, SB-C18, 21.2 x 50 mm, 5u Crt
	820400-901	PrepHT, Hardware, 21.2 mm Crt.End Ftg 2/PK
	5190-6886	Spec out sol for 1260 LC/MS 3 x 5 mL (standard #1)
	5190-6887	delaytime calib sol for 1260LC/MS 3 x 5 mL (standard #2)

Options

The following parts are available as options:

p/n	Description
G1361-68707	Gradient kit Shipped with every additional pump in a gradient system
G1968F	Mass-Based Purification Kit for 6100 Contains Delay Coil 5000 µL, Capillary PK 0.1 mm x 1250 mm SX/SH, Capillary PK 0.25 mm, G1390B Universal Interface Box II

6 Appendix

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

General Safety Information

General Safety Information

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

WARNING

Ensure the proper usage of the equipment.

The protection provided by the equipment may be impaired.

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

Safety Standards

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

General

Do not use this product in any manner not specified by the manufacturer. The protective features of this product may be impaired if it is used in a manner not specified in the operation instructions.

Before Applying Power

WARNING

Wrong voltage range, frequency or cabling

Personal injury or damage to the instrument

- ➔ Verify that the voltage range and frequency of your power distribution matches to the power specification of the individual instrument.
- ➔ Never use cables other than the ones supplied by Agilent Technologies to ensure proper functionality and compliance with safety or EMC regulations.
- ➔ Make all connections to the unit before applying power.

NOTE

Note the instrument's external markings described under "Safety Symbols" on page 86.

Ground the Instrument

WARNING

Missing electrical ground

Electrical shock

- ➔ If your product is provided with a grounding type power plug, the instrument chassis and cover must be connected to an electrical ground to minimize shock hazard.
- ➔ The ground pin must be firmly connected to an electrical ground (safety ground) terminal at the power outlet. Any interruption of the protective (grounding) conductor or disconnection of the protective earth terminal will cause a potential shock hazard that could result in personal injury.

Do Not Operate in an Explosive Atmosphere

WARNING

Presence of flammable gases or fumes

Explosion hazard

➔ Do not operate the instrument in the presence of flammable gases or fumes.

Do Not Remove the Instrument Cover

WARNING

Instrument covers removed

Electrical shock

➔ Do Not Remove the Instrument Cover

➔ Only Agilent authorized personnel are allowed to remove instrument covers. Always disconnect the power cables and any external circuits before removing the instrument cover.

Do Not Modify the Instrument

Do not install substitute parts or perform any unauthorized modification to the product. Return the product to an Agilent Sales and Service Office for service and repair to ensure that safety features are maintained.

In Case of Damage

WARNING

Damage to the module

Personal injury (for example electrical shock, intoxication)

➔ Instruments that appear damaged or defective should be made inoperative and secured against unintended operation until they can be repaired by qualified service personnel.

Solvents

WARNING

Toxic, flammable and hazardous solvents, samples and reagents

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

- ➔ When working with these substances observe appropriate safety procedures (for example by wearing goggles, safety gloves and protective clothing) as described in the material handling and safety data sheet supplied by the vendor, and follow good laboratory practice.
- ➔ Do not use solvents with an auto-ignition temperature below 200 °C (392 °F). Do not use solvents with a boiling point below 56 °C (133 °F).
- ➔ Avoid high vapor concentrations. Always keep the temperature in the sample compartment at least 25 K below the boiling point of the solvent used.
- ➔ Do not operate the instrument in an explosive atmosphere.
- ➔ Reduce the volume of substances to the minimum required for the analysis.
- ➔ Never exceed the maximum permissible volume of solvents (8 L) in the solvent cabinet. Do not use bottles that exceed the maximum permissible volume as specified in the usage guideline for solvent cabinet.
- ➔ Ground the waste container.
- ➔ Regularly check the filling level of the waste container. The residual free volume in the waste container must be large enough to collect the waste liquid.
- ➔ To achieve maximal safety, regularly check the tubing for correct installation.

NOTE

For details, see the usage guideline for the solvent cabinet. A printed copy of the guideline has been shipped with the solvent cabinet, electronic copies are available in the Agilent Information Center or via the Internet.

Safety Symbols

Table 5 Safety Symbols

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

WARNING

A WARNING

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

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

CAUTION

A CAUTION

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

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

Waste Electrical and Electronic Equipment Directive

Abstract

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

NOTE

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

Product Category:

With reference to the equipment types in the WEEE Directive Annex I, this product is classed as a Monitoring and Control Instrumentation product.

NOTE

Do not dispose of in domestic household waste

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

Batteries Information

WARNING

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

Danger of explosion if battery is incorrectly replaced.

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

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

WARNING

Lithiumbatteri - Eksplosionsfare ved fejlagtig håndtering.

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

➔ Lever det brugte batteri tilbage til leverandøren.

WARNING

Lithiumbatteri - Eksplosionsfare.

Ved udskiftning benyttes kun batteri som anbefalt av apparatfabrikanten.

➔ Brukt batteri returneres appararleverandoren.

NOTE

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

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)

Agilent Technologies on Internet

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http://www.agilent.com

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In This Book

This manual contains information about the Agilent 1260 Infinity Purification Solution.

The manual describes the following:

- Product description
- Installation instructions
- Operating instructions
- Best practice
- Maintenance and repair
- Parts
- Safety

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