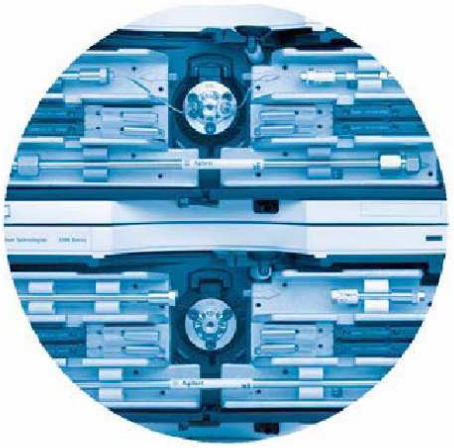

Agilent 1200 Series Method Development System

System Manual

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Notices

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Contents

A Appendix

1
Quick Installation Guide

Arrange the Modules

See Chapter 3, “System Setup,” starting on page 26 and Chapter 3, “Installing the Solvent Selection Part,” starting on page 43.

- **The connecting capillaries between the modules should be as short as possible.**
- The system can include **two or three column compartments (TCCs).**
- If you are using solvent selection, refer to step 11

Set Up with Two or Three TCCs

See Chapter 3, “System Setup,” starting on page 26.

- **Two G1316C TCCs with installed valve drive must be in close proximity**
- One additional G1316A/B or C **without** valve drive might be used in the system; if **low dispersion** heat exchanger needs to be used, the TCC must be **at least a G1316B**.
- Ensure **short connections** from the TCCs with integrated valve drives to the autosampler and to the detector.

Insert the Valve Heads

See Chapter 3, “Installation of the Valve Heads,” starting on page 28.

- Inlet valve close to the autosampler.
- Outlet valve close to the detector.
- Triple TCC setup: Inlet and outlet valve always in neighboring TCCs

The low pressure valve kit (**G4230A**) contains two identical low pressure valve heads (PN 5067-4108).

NOTE

The high pressure valve kit (G4230B) contains two different valve head types:

- low pressure valve head (PN 5067-4108) ⇒**outlet** side of the columns and before the detector
- high pressure valve head (PN 5067-4107) ⇒**inlet** side of the columns and after the autosampler

Install the Heat Exchanger

See Chapter 3, “Connecting Valves, Heat Exchanger and Columns,” starting on page 30.

- **Low dispersion** capillary kits (**5067-1595** or **5067-1597**):
  - place the low dispersion heat exchanger carrier (G1316-89200) at appropriate positions (typically in the middle positions) of the TCCs.
  - **short columns** (up to 100 mm): four carriers and eight heat exchangers to be placed in two TCCs (all middle positions).
  - **long columns**: three carriers and six heat exchangers to be placed at the left middle positions.
- **General purpose** capillary kit (**5067-1596**): Use the built-in 3 µL and 6 µL heat exchangers.

Insert Color Code Column Clip

See Chapter 3, “Connecting Valves, Heat Exchanger and Columns,” starting on page 30.

Place the differently colored column clips (part of PN 5042-9918) at the planned column positions. More than one clip can be used for a long column, but use the same color per column.

Identify and Color Code Capillaries

See Chapter 3, “Identifying the Capillaries,” starting on page 36.

Valve ports (excluding the central port) must be equipped with extra-long fittings. The capillaries required to connect the valves are pre-swaged (excluding the waste line and those connecting to the central port).

Install the Capillaries, Waste-Line and Columns

See Chapter 3, “Installing Capillaries,” starting on page 35.

Two types of fittings are available to **connect columns**:

- long SS fittings: **not** removable (always the same brand of columns in use)
- high-pressure-rated PEEK ferrules and SS nuts: removable (for different column brands)

The nuts of these two types of fittings are not interchangeable.

Use the same color code as the respective column clip for all interconnecting capillaries of matching valve ports.

The waste-line must be connected to the Inlet valve.

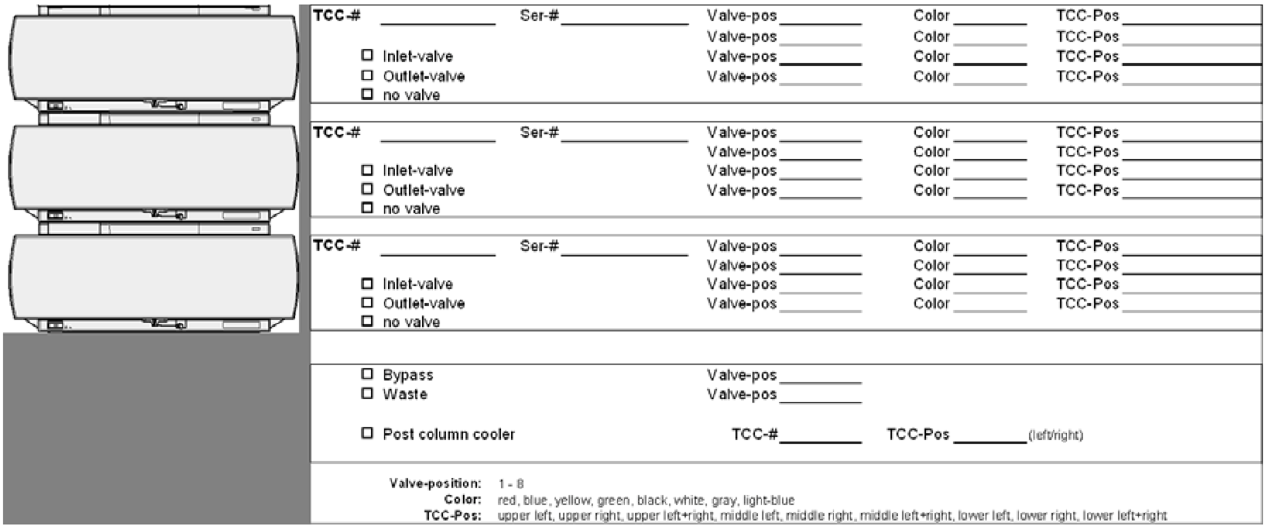
Ideally, mark all positions/color codes/valve ports in parallel in the ChemStation software or note these information in the provided systems diagram.

Set Up the Module Cluster in the ChemStation

See Chapter 4, “Clustering of Modules in ChemStation,” starting on page 49.

Make the required settings for the module cluster in the ChemStation. If your computer is remote from the instrument position, note all required information in the system diagram below.

When you have set up the cluster, restart the ChemStation.



Define Columns

See Chapter 4, “Settings for Clustered Modules,” starting on page 53.

- Edit the ChemStation **column data base** (instruments/columns).
- Assign the columns to the position in the TCC in the **More Column Thermostat Cluster** menu under **Configure Columns**.

Install the Solvent Selection Valve

See Chapter 4, “Clustering of Modules in ChemStation,” starting on page 49 and Chapter 4, “Settings for Solvent Selection,” starting on page 56.

- Replace the cover of your pump with an additional cover kit (5067-1567 for binary pumps or 5042-9912 for the quaternary pump) that carries a mounting rail for the G1160A valve.
- Slide the G1160A solvent selection valve onto the mounting rail.
- Make hydraulic connections using the Solvent Selection Tubing Kit 5067-4601.
  - Place all tubing adapters (0100-2298) into the ports of the G1160A-valve.
  - Connect solvent bottles, degasser and solvent selection valve with the 1.5 mm ID PTFE tubing using the enclosed fittings.

NOTE When degassing after the solvent selection valve, it might be necessary to take the bottle head assemblies apart and refit them with longer tubing.

- Set up the cluster containing the solvent selection valve and the pump in ChemStation. **Restart the ChemStation**. Assign the solvents in the solvent table.

1 Quick Installation Guide

2
The Agilent 1200 Series Method Development System

System Components

Description of the System

The Agilent 1200 Series Method Development System consists of an Agilent 1200 LC or RRLC system and two or three clustered G1316C column compartments. In a 400 bar LC system, a G1311A quaternary pump or a G1312A binary pump can be used; in a 600 bar LC system, a G1312B binary pump SL must be used. It is recommended to equip binary pumps with the internal solvent selection valve that allows up to two different solvents per pump channel. Additionally, an external G1160A solvent selection valve that can extend the number of available solvents on one pump channel to a maximum of 12 is fully supported (see Figure 1 on page 17).

One column compartment contains the valve that is connected to the autosampler and delivers the flow to the different columns. The other column compartment contains the valve that is connected to the detector. A maximum of eight columns, each up to 100 mm length can be installed in two clustered column compartments using the low dispersion heat exchangers. Using three clustered column compartments up to six columns longer than 100 mm (max. 300 mm) or alternatively four columns of up to 100 mm and four columns longer than 100 mm length (max. 300 mm) can be used.

A range of autosamplers is available, depending on the required maximum pressure capability of the system. A very broad range of detectors is supported, including light detectors (UV, DAD, FLD), mass selective detectors, evaporative light scattering detectors and refractive index detectors. Other detectors, e.g. chiral detectors, can be used by acquiring the signal via an analog to digital converter.

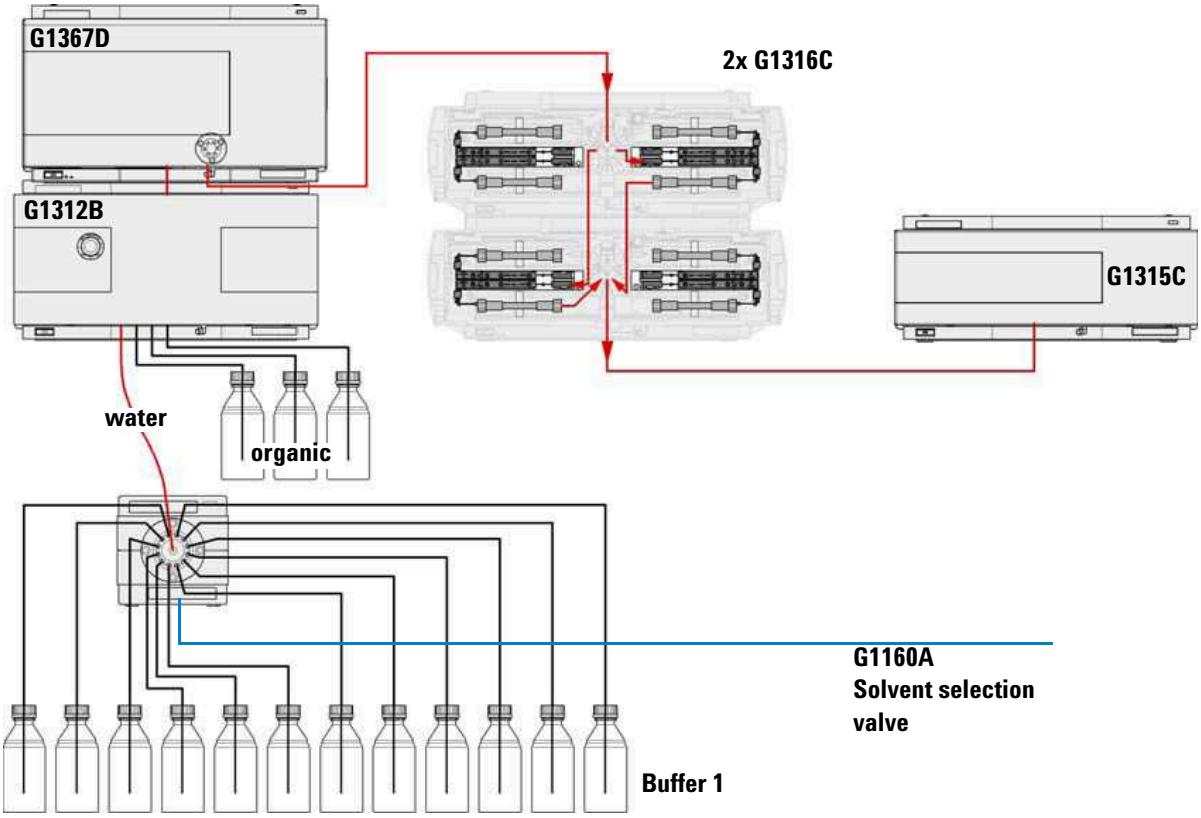


Figure 1 Method development system principle

NOTE ChemStation Version 4.01 or higher and Firmware revision A06.10 or higher for all modules are needed.

Column Switching

To perform automated column switching with the Agilent 1200 series method development solution a minimum of two G1316C Thermostatted Column Compartments SL plus equipped with column selection valves are required. A maximum of eight positions are available to connect columns, bypass or waste lines. By using the Agilent low dispersion heat exchanger, up to eight solvent streams can be pre-heated to a maximum of 100 °C, depending on the flow rate. If six columns longer than 100mm have to be installed, and solvent pre-heating is required, three column compartments are necessary. The third column compartment should ideally also be a G1316C, but versions G1316A and B are also supported. Note: if low dispersion heat exchangers need to be used, a G1316B or C is required. For automated column switching the following parts and modules have to be integrated, see Figure 2.

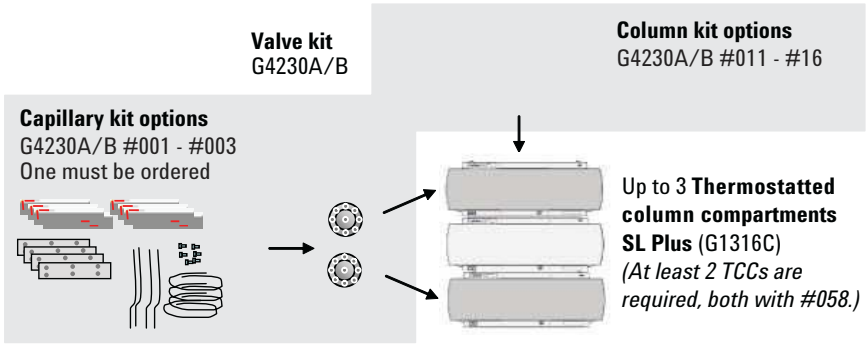


Figure 2 Modules and parts needed for column switching

Two different valve kits are available. The G4230A valve kit is required for standard Agilent 1200 LC systems and contains two 8-position/9-port valve heads rated to a maximum of 400 bar (PN 5067-4108). The G4230B valve kit is required for an Agilent 1200 Series RRLC system and contains two different valve heads. On the inlet side of the columns, a high-pressure-rated 8-position/9-port valve head tested to a maximum of 600 bar (PN 5067-4107) is necessary; on the outlet side of the columns, only little backpressure is given and a standard 400 bar selection valve (PN 5067-4108) is sufficient. One of three available capillary kits is delivered along with the valve kit.

The capillary kit that is used depends on the columns that will be installed; refer also to Table 6 on page 30. The components of the capillary kits are listed in Table 1 to Table 3 (where Valve1 refers to the inlet valve and Valve2 refers to the outlet valve , ps = pre-swaged, ns = non-swaged, lg = extra-long nut, sh = short nut).

Table 1 Components of the low dispersion capillary kit for short columns (PN 5067-1595)

Part Number	Description		Connection
5021-1822	Flexible tubing, 280 mm, 0.12 mm id.	2	Autosampler to Valve1 & Valve2 to detector
G1316-87319	SS Capillary 340 x 0.12 mm, m/m, n-s/n-s	1	Autosampler with Thermostat to Valve1
5065-9964	Flex capillary, 500 mm, 0.12 mm id, n-s/n-s	1	Autosampler to Valve1 in a dual stack configuration
5067-4604	SS Capillary 280 x 0.12 mm ps/ps 1lg 1sh nu	8	Valve1 to heat-exchanger
5067-4605	SS Capillary 280 x 0.12 mm ps/ns 2lg 1sh nu	8	Column to Valve2
G1316-89200	Carrier for low dispersion heat exchanger	4	
G1316-68706	Fitting Holder Assembly	4	
G1316-80002	Heat Exchanger Long-Up, 1.6 µL	4	
G1316-80003	Heat Exchanger Long-Down, 1.6 µL	4	
5067-4607	SS Capillary 280 x 0.17 mm ps/ps 2 long nut	1	Bypass-line
0890-1713	Flexible tubing	2 m	Waste-line
5065-4454	Long Fittings and Ferrules 10/pk	1 pk	
5021-9918	Clip set for color coding (8 colors/pk)	2 pk	

Table 2 Components of the general purpose capillary kit (PN 5067-1596)

Part Number	Description		Connection
5021-1818	Flex capillary, 0.17 x 280 mm, no fitting	2	Autosampler to Valve1 & Valve2 to detector
5021-1819	Flexible tubing, 400 mm, 0.17 mm id.	1	Autosampler with Thermostat to Valve1
5065-9933	Flex capillary, 0.17 x 600 mm, n-s/n-s	1	Autosampler to Valve1 in a dual stack configuration
5067-4608	SS Capillary 280 x 0.17 mm ps/ps 1lg 1sh nu	6	Valve1 to heat-exchanger
G1316-87300	Capillary, 0.17 x 90mm 1/16" male/male	6	Heat exchanger to column
5067-4609	SS Capillary 500 x 0.17 mm ps/ns 2lg 1sh nu	6	Column to Valve2
5067-4607	SS Capillary 280 x 0.17 mm ps/ps 2 long nut	1	Bypass line
0890-1713	Flexible tubing	2 m	Waste-line
5065-4454	Long fittings and ferrules, SS, 10/pk	2 pk	
5067-1540	SS Hex Head Nut with PEEK Ferrule,6/pk	1 pk	
0100-1259	Plastic fitting	8	
5042-9918	Clip set for color coding (8 colors/pk)	2 pk	

Table 3 Components of the low dispersion capillary kit for long columns (PN 5067-1597)

Part Number	Description		Connection
5021-1822	Flexible tubing, 280 mm, 0.12 mm id.	2	Autosampler to Valve1 & Valve2 to detector
G1316-87319	SS Capillary 340 x 0.12 mm, m/m, n-s/n-s	1	Autosampler with Thermostat to Valve1
5065-9964	Flex capillary, 500 mm, 0.12 mm id, n-s/n-s	1	Autosampler to Valve1 in a dual stack configuration
5067-4604	SS Capillary 280 x 0.12mm ps/ps 1lg 1sh nu	6	Valve1 to heat-exchanger
G1316-89200	Carrier for low dispersion heat exchanger	3	
G1316-68706	Fitting Holder Assembly	3	
G1316-80002	Heat Exchanger Long-Up, 1.6 µL	3	
G1316-80003	Heat Exchanger Long-Down, 1.6 µL	3	
5067-4606	SS Capillary 400 x 0.12 mm ps/ns 2lg 1sh nu	6	Column to Valve2
5067-4607	SS Capillary 280 x 0.17 mm ps/ps 2 long nut	1	Bypass line
0890-1713	Flexible tubing	2 m	Waste-line
5065-4454	Long Fittings and Ferrules 10/pk	1 pk	
5042-9918	Clip set for color coding (8 colors/pk)	2 pk	

All kits, and all components of the kits, are orderable separately as consumables.

Solvent Switching

In addition to the two or four solvents directly supported by the pump, **one** channel of the pump can be equipped with an additional **G1160A solvent selection valve**. This valve is able to switch between up to 12 solvents giving a total of up to 15 solvents on the Agilent 1200 Series LC Method Development Solution (see Table 4 and Table 5 and Figure 3 on page 22).

All Agilent pumps are delivered together with one solvent cabinet and as many solvent reservoirs as available solvent channels of the pump (two or four). **For binary pumps** the built-in solvent selection **option #031 is recommended**.

For the selection of the degasser and the number of degassers in a system, refer to Chapter 3, “Installing the Solvent Selection Part,” starting on page 43.

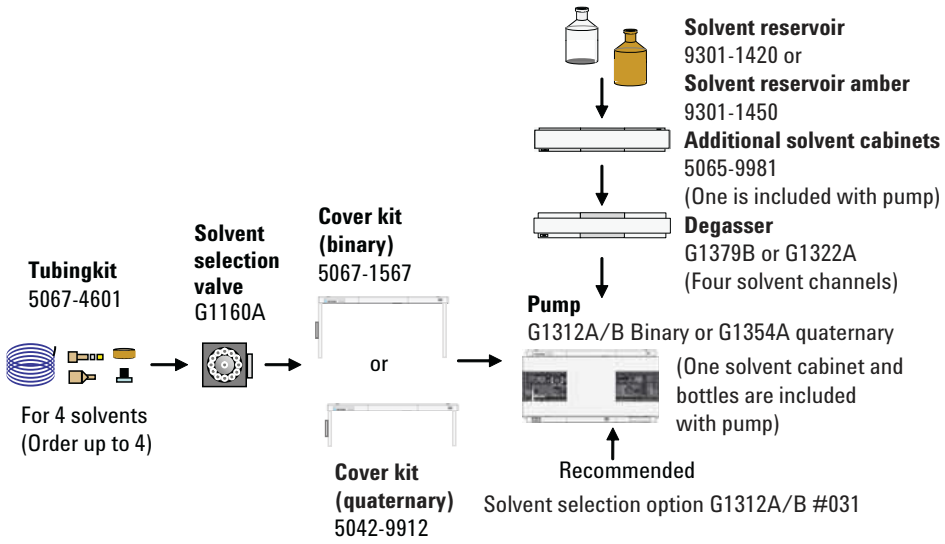


Figure 3 Components for solvent selection

Table 4 Components for solvent selection

Part Number	Description	Comment
G1160A	Solvent Selection Valve (12 pos/13 pt)	External CAN valve
5067-4601	Tubing kit for up to four additional solvents (incl. bottle head assemblies)	Up to 4 required
5067-1567	Cover kit for binary pumps	
5042-9912	Cover kit for quaternary pump	
G1379B	Micro-degasser for four solvents	see Installing the Solvent Selection Part 43
G1322A	Std. degasser for four solvents	see Installing the Solvent Selection Part 43
5065-9981	Solvent Cabinet	
9301-1420	Solvent Reservoir 1L	
9301-1450	Solvent Reservoir 1L amber	

Table 5 Components of the tubing kit 5067-4601

Part Number	Description	Comment
5062-2483	Solvent tubing, 5 m, 1.5 mm id, 3 mm od	2
0100-2298	Adapter, PEEK int. 1/4-28 to ext. 10-32	5
G1311-60003	Bottle Head Assembly	4
5063-6598	Tefzel ferrules/SS rings, 1/8 in 10/pk	1 pk
5063-6599	Nuts 10/pk	1 pk

2 The Agilent 1200 Series Method Development System

3
System Setup and Installation

System Setup

Either two or three column compartments must be installed, depending on the number and size of columns which are to be installed.

With the many options for pumps, autosamplers and detectors, many different configurations can be created. For two examples, see Figure 4. In principle, the connecting capillaries should be kept as short as possible to reduce extra-column band-broadening, and to keep the backpressure caused by the capillaries small. It is very important to keep the distance to the detector as short as possible. The next important connection is from the autosampler to the column compartment cluster. Several set-ups taking these considerations into account are covered with the available capillary kits. Refer to Figure 5 on page 27 for possible configurations with two TCCs, and Figure 6 on page 27 for possible configurations with three TCCs. The column compartment with the high pressure valve (G4230B only) should always be installed close to the auto sampler. The column compartment with the low pressure valve should be installed close to the detector.

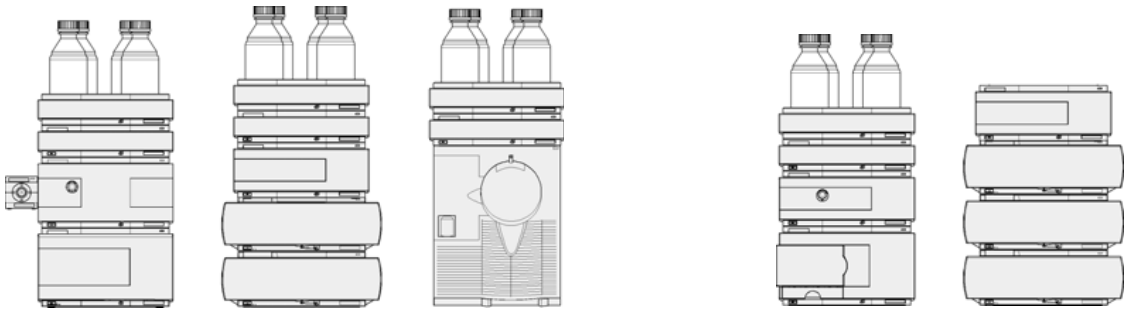


Figure 4 Two examples of different set-up possibilities for method development systems

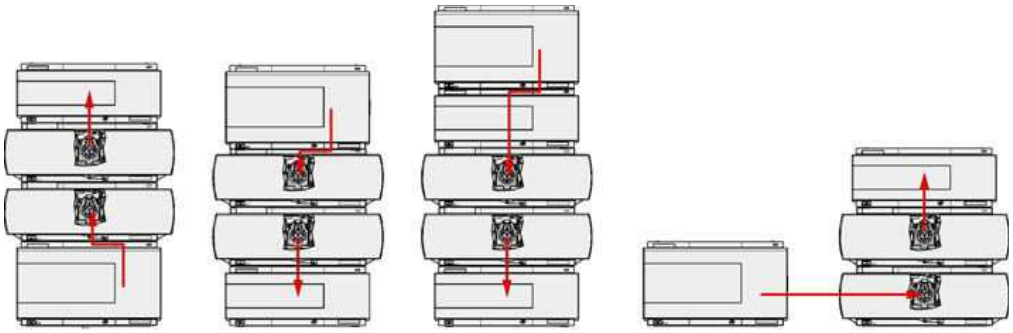


Figure 5 Different system set up possibilities with 2 column compartments. Only the autosampler (if necessary with thermostat) and a DAD as detector are shown. In the dual-stack setup note that the autosampler and the lower TCC are at the same level, also a distance of both stacks of 8 cm should be met.

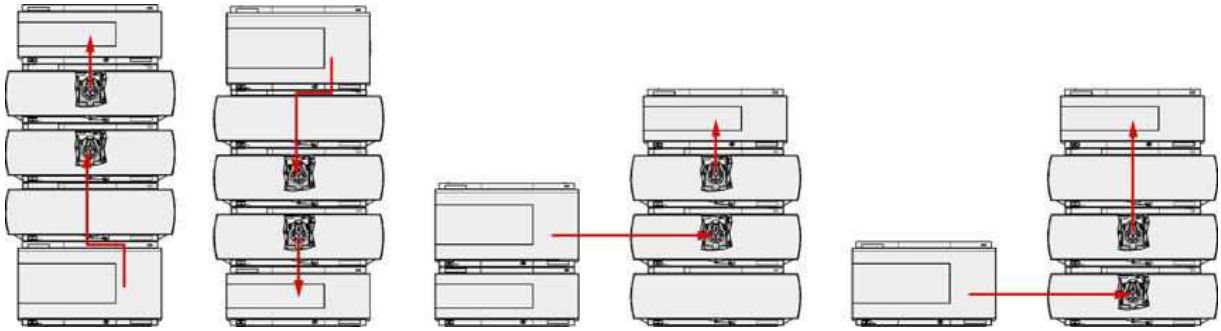


Figure 6 Different system set up possibilities with 3 column compartments. Only the autosampler (if necessary with thermostat) and a DAD as detector are shown. The TCCs carrying the valves are always close to each other. In the dual-stack a distance of both stacks of 8 cm should be met.

If three column compartments have to be installed, the column compartments with integrated valves should be installed close to each other.

Installation of the Valve Heads

The valve drives are factory-installed. The valve heads are interchangeable and can be easily mounted. At the first installation, the transportation lock and the dummy valve have to be removed, see Figure 7. The valve heads can be installed by mounting the valve heads onto the valve drives and fastening the nut manually (do not use any tools). Be sure that the guide pin snaps into the groove of the valve drive thread. The valves are mounted on pull-out rails to allow easy installation of capillaries. Push the valve gently into its housing until it snaps into the inner position, push it again and it slides out. If all capillaries are installed, push the valve back into its housing, see Figure 8 on page 29.

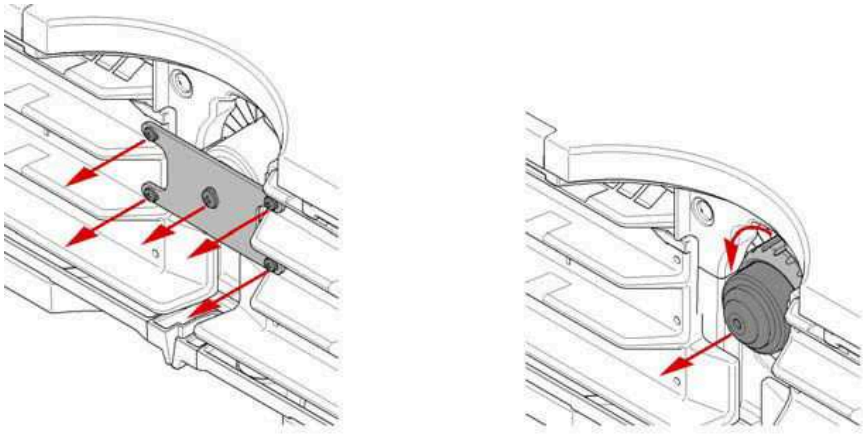


Figure 7 Removing the transportation lock and the valve dummy. When unscrewing the transportation lock, push it back until the last screw is removed – the valve rail is spring-loaded. To remove the valve dummy, loosen the nut manually.

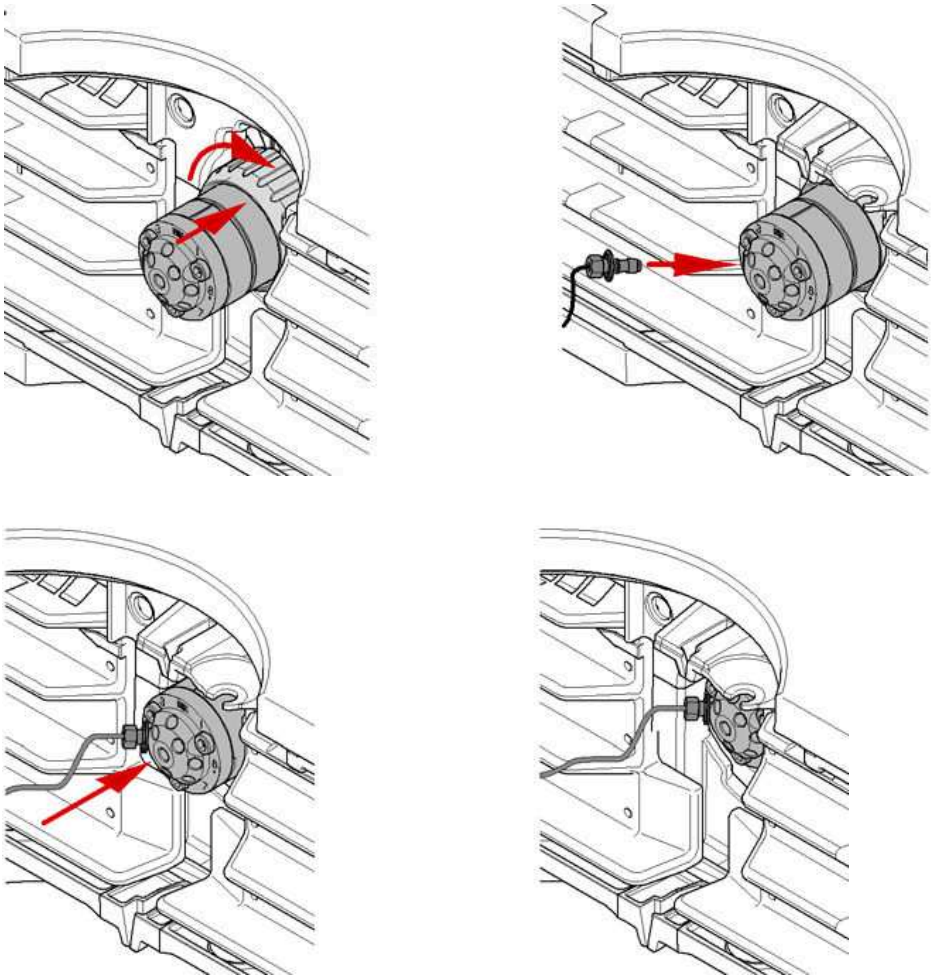


Figure 8 Installing the valve head and connecting capillaries. Place the valve head on the valve drive thread – be sure that the guiding pin of the valve head snaps into the groove of the thread. Fasten the nut manually. Install all capillaries and finally push the valve into its inner position.

Connecting Valves, Heat Exchanger and Columns

Available Capillary Kits

There are three kits available that cover all typical column dimensions as used in analytical LC (Table 6 on page 30). Along with your valve kit, you have received at least one of these, according to your order. All kits and components of the kits are also available as consumables (see Chapter 2, "Column Switching," starting on page 18). The capillary option PN 5067-1595 is the only one that allows the use of eight columns (max. length 100 mm) with solvent preheating. Note: restrictions on the maximum flow rate above 2.5 mL/min (especially at very high temperatures) and number of columns might apply.

Table 6 Selecting the right capillary kit. Note that with all low dispersion capillary kits, restrictions on the flow rate above 2.5 mL/min might apply, depending on the temperature and viscosity of the solvent.

Column ID	Column Length	Capillary kit 5067-1595 low dispersion, short columns	Capillary kit 5067-1596 general purpose	Capillary kit 5067-1597 low dispersion, long columns
2.1 mm ID	max. 100 mm	YES	Possible but not recommended	NO
	>100 - 250 mm	NO	Possible but not recommended	YES Max column length 150 mm
3.0 mm ID	max. 100 mm	YES	YES	NO
	>100 - 250 mm	NO	YES	YES Max column length 150 mm
4.6 mm ID	max. 100 mm	YES	YES	NO
	>100 - 250 mm	NO	YES	NO
Comment		0.12 mm ID capillaries, low dispersion heat exchanger included. **Only with this a maximum of eight columns is supported.**	0.17 mm ID capillaries, built-in heat exchange to be used. **Maximum of two columns per TCC with solvent pre-heating.**	0.12 mm ID capillaries, low dispersion heat exchanger included. **Maximum of two columns per TCC with solvent pre-heating.**

If columns longer than 250 mm or any special combinations of column lengths and internal diameters are to be used, additional capillaries might need to be ordered separately.

Installing the Low Dispersion Heat Exchangers

The carriers (PN G1316-89200) for the low-dispersion heat exchangers must be attached to the standard built-in heat exchangers of the TCCs (see Figure 9 on page 32), preferably in the middle. Remove the protective foil from the gray thermal conductive foil of the carrier and fasten the three screws. Mount the fitting holder assembly (PN G1316-68706) on this carrier. The fitting clips hold the capillary unions from the low dispersion heat exchangers and make plumbing of capillaries much easier. Finally, attach the low dispersion heat exchanger (PN G1316-80002 or PN G1316-80003). It is important to fix it tightly so that a good thermal conductivity is achieved. Figure 10 on page 33 show how to install the heat exchangers for columns up to 100 mm long in two column compartments. Figure 11 on page 34 shows how to install the heat exchangers for columns longer than100 mm in three column compartments. The columns are held by color-coded clips for more convenient installation. These colors are later also used to identify the columns in the software.

If a low-dispersion post-column cooler is required for maximum UV sensitivity at high flow rates and high temperatures, this can be ordered separately (PN G1316-80004) but needs to be installed alone on one side of the TCC – sacrificing up to two columns.

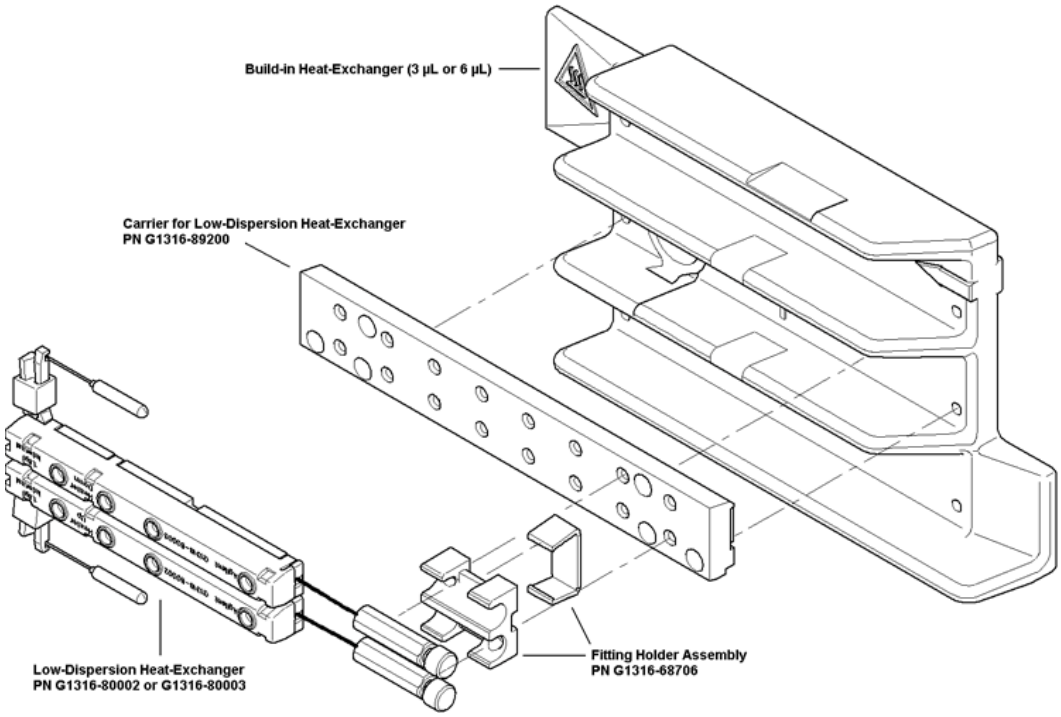


Figure 9 Installing the low dispersion heat exchangers

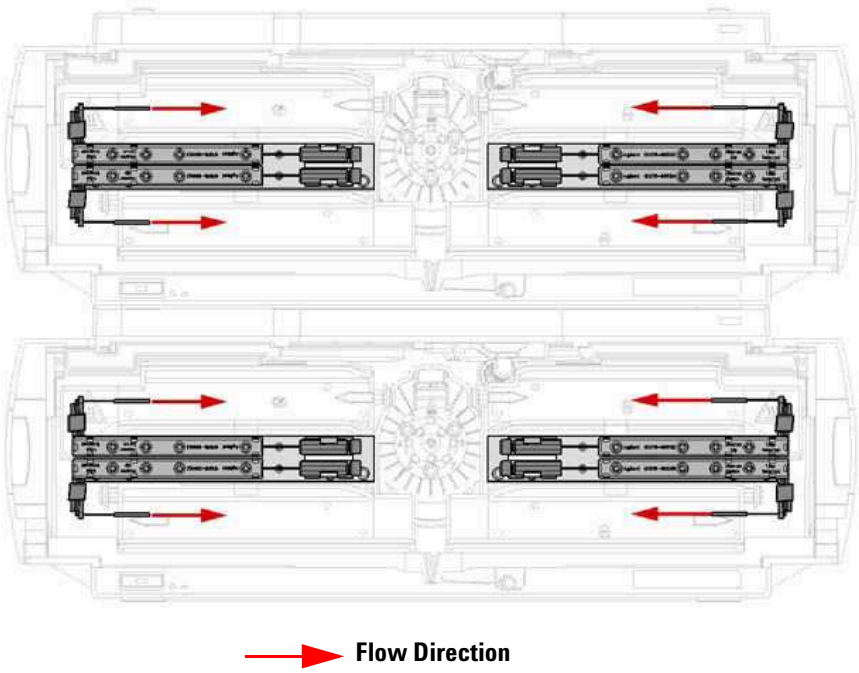


Figure 10 Positioning the low dispersion heat exchangers for 8 columns up to a length of 100mm in 2 column compartments

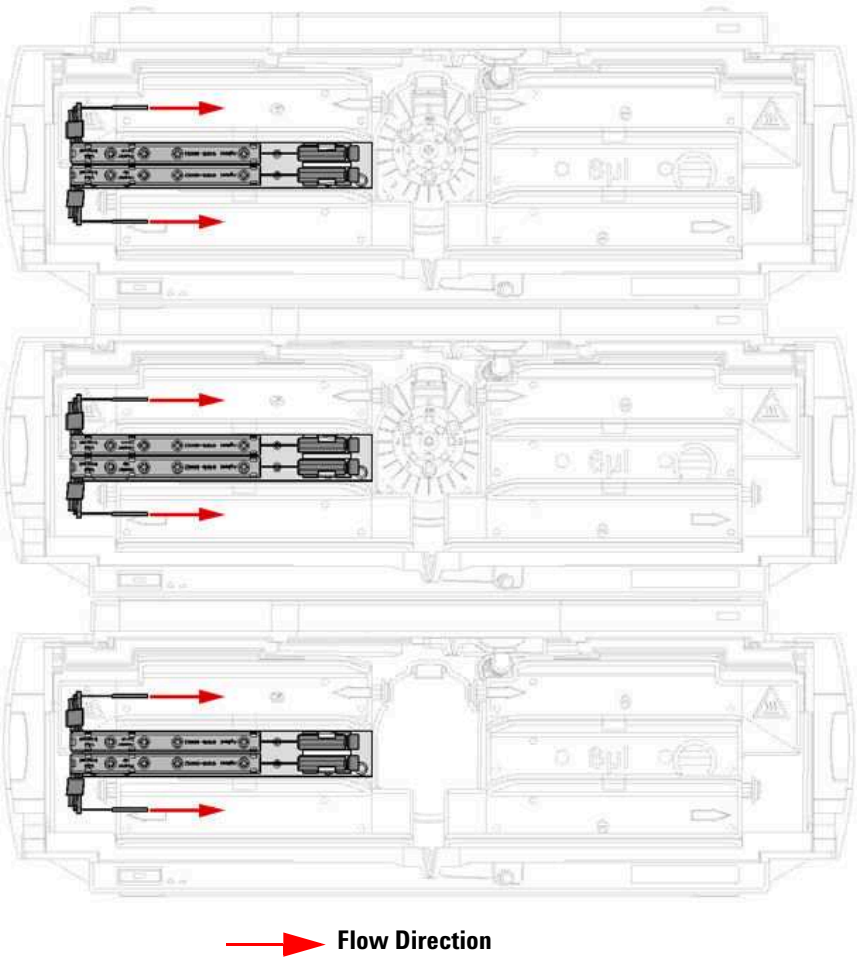


Figure 11 Positioning the low dispersion heat exchangers for 6 columns with a length >100mm in 3 column compartments

Installing Capillaries

See Table 7 for an optimized work flow to install the capillaries required for column selection.

Table 7 Optimized workflow for the installation of the capillaries of a TCC-cluster

Step	Task
1	color code all capillaries that are required to connect a column
2	install the capillaries to the central port of the valves, fasten them with a spanner
3	[optional] install the bypass-line (finger tight) mark the position in the clustering software or on the template in the Quick Installation Guide
4	[optional] install the waste-line at the inlet valve, plug the corresponding position at the outlet valve, mark the position
5	install the capillaries from the inlet valve to the heat exchanger (only finger-tight) – use the same color as the column clip at the corresponding position
6	[general purpose capillary kit only] install the capillaries from the built-in heat exchanger to the column
7	install the capillaries from the outlet valve to the column
8	[low dispersion kit only] place the unions of the low dispersion heat-exchanger into their clips
9	Tighten all fittings, starting at the inlet valve, then at the heat exchanger, finally at the outlet valve.
10	push the valves into the rear position
11	place capillaries between two TCCs into the capillary guides
12	install the columns, note their positions
13	stow any over-lengths of the capillaries
14	check for any leaks
15	close the front covers

Identifying the Capillaries

First identify the required capillaries in your capillary kits (Table 8 on page 36):

- All capillaries that connect to a peripheral position of the valves are pre-swaged with extra long fittings.
- Capillaries from the valve to a heat exchanger are pre-swaged on the second side with a standard fitting.
- All capillaries that connect to a column are non-swaged on one side but two types of fittings are included - a long non-removable three-part SS-fitting and a removable fitting consisting of a SS nut and a PEEK ferrule.
- All capillaries that connect to an autosampler or to a detector are non-swaged.
- The bypass capillary is pre-swaged with extra long SS fittings on both sides

Table 8 Identifying the capillaries required for the installation

Connection \ Capillary Kit	Low dispersion, short columns 5067-1595	General purpose 5067-1596	Low dispersion, long columns 5067-1597
Autosampler to valve	280 mm x 0.12 mm 5021-1822 or 340 mm x 0.12 mm G1316-87319 or 500 mm x 0.12 mm 5065-9964	280 mm x 0.17 mm 5021-1818 or 400 mm x 0.17 mm 5021-1819 or 600 mm x 0.17 mm 5065-9933	280 mm x 0.12 mm 5021-1822 or 340 mm x 0.12 mm G1316-87319 or 500 mm x 0.12 mm 5065-9964
Valve to heat excha	280 mm x 0.12 mm ps/ps 5067-4604	280 mm x 0.17 mm ps/ps 5067-4608	280 mm x 0.12 mm ps/ps 5067-4604
Heat exchanger to column	(use low dispersion heat exchanger G1316-80002 or G1316-80003)	90 mm x 0.17 mm G1316-87300	(use low dispersion heat exchanger G1316-80002 or G1316-80003)
Column to heat exchanger	280 mm x 0.12 mm ps/ns 5067-4605	500 mm x 0.17 mm ps/ps 5067-4609	400 mm x 0.12 mm ps/ns 5065-4606
Valve to detector	280 mm x 0.12 mm 5021-1822	280 mm x 0.17 mm 5021-1818	280 mm x 0.12 mm 5021-1822
Bypass (optional)	280 mm x 0.17 mm ps/ps 5067-4607	280 mm x 0.17 mm ps/ps 5067-4607	280 mm x 0.17 mm ps/ps 5067-4607
Waste (optional)	PTFE Tubing, flex 0890-1713	PTFE Tubing, flex 0890-1713	PTFE Tubing, flex 0890-1713

A flexible Teflon waste capillary is also included in the kits. The waste line always needs to be installed at the inlet-valve – use a plastic plug to close the corresponding position on the outlet-valve.

In the bypass position, the flow is guided through the valves directly to the detector. This position can be used to flush the system after a solvent change with, for example, non miscible solvents. This ensures that the flow path from pump up to the detector can be flushed with appropriate solvents, for example, for cleaning the system of buffers before changing to a 100% organic phase.

In the waste position, the flow is passing the inlet valve only and then going directly to waste. This position can be used to exchange solvents in the degasser and in the pump with maximum flow rates.

When low dispersion heat exchangers are used, two capillaries from and to the valves need to be connected per column (see Figure 12 on page 39). If the 3 µl and 6 µl heat exchangers are used, a third capillary from the heat exchanger to the column is needed per column, see Figure 13 on page 40.

There are several lengths for the capillary from the autosampler to the inlet valve available to match different set-ups (see Chapter 3, “System Setup,” starting on page 26).

Color Coding

After identifying the required capillaries, place the color-coded column clips (part of 5042-9918) between the fins of the TCCs at the positions where you want to place a column later. For short columns, one clip is sufficient, for long columns two clips of the same color can be used. Use the color-coded rings (part of 5042-9918) to code both nuts of the capillaries with the same color.

Installing the Capillaries

Now start installing the capillaries. Begin with the central ports of the valves that go to the autosampler or the detector; tighten this fitting immediately with a spanner. Install the bypass and waste line if required and also note the valve positions. Then install all remaining capillaries of the valves that connect to a heat-exchanger or column. Start with the inlet valve. Use the same colors as the column clips you have place already in the corresponding position. Ideally, you have installed the ChemStation software already and can make the required settings (valve positions, TCC-positions and corresponding color codes) directly into the software (see Chapter 4, “Clustering of Modules in ChemStation,” starting on page 49, Figure 20 on page 51). If not, use the template in the Quick Installation Guide to note these important settings. At first, finger-tighten all capillaries. If you are using a low dispersion heat exchanger, clip the unions into the corresponding clips. Now fasten all fittings with a spanner. Starting at the inlet valve from position one through eight, fasten the fittings on the heat exchanger. Finally, fasten all fittings of the outlet valve again, from position one through eight. All unused valve ports should be fitted with a plastic plug.

NOTE Use outmost care to avoid any void volumes caused by poor connections.

Push the valves into the rear positions, place the capillaries that go from one TCC to the other into the capillary guides to prevent squeezing them when closing the front cover (Figure 14 on page 41) and finally stow any excess lengths of the capillaries. Install the columns and note in parallel their positions (by the color codes) and do a final leak-check. When you close the front cover, the hardware installation for automated column switching is complete.

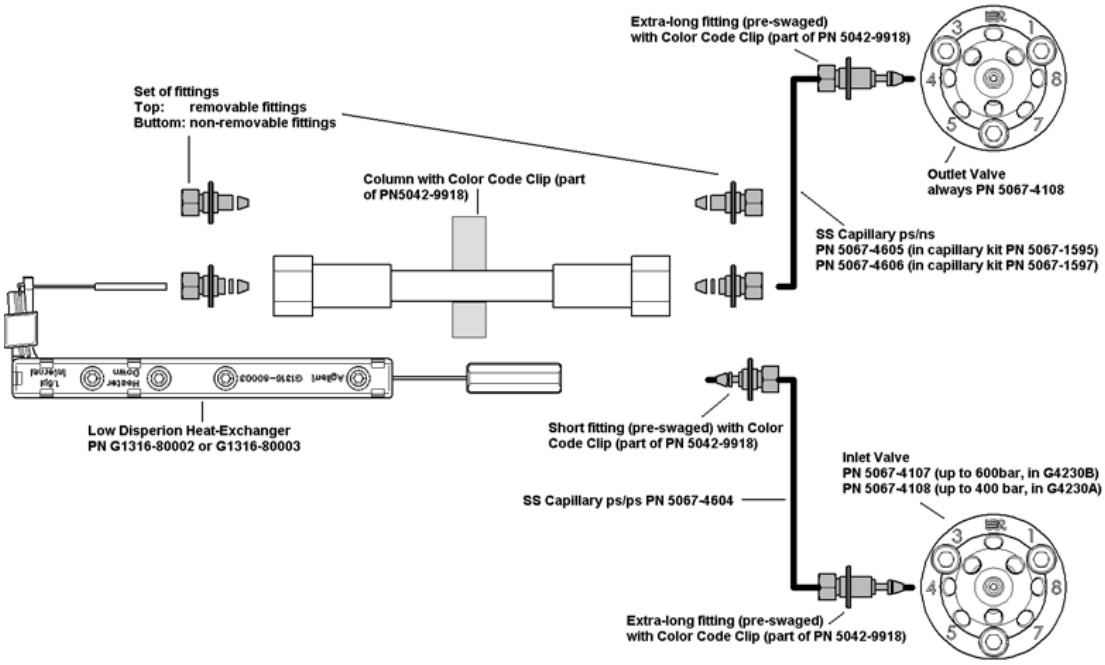


Figure 12 Plumbing, if small heat exchangers are used

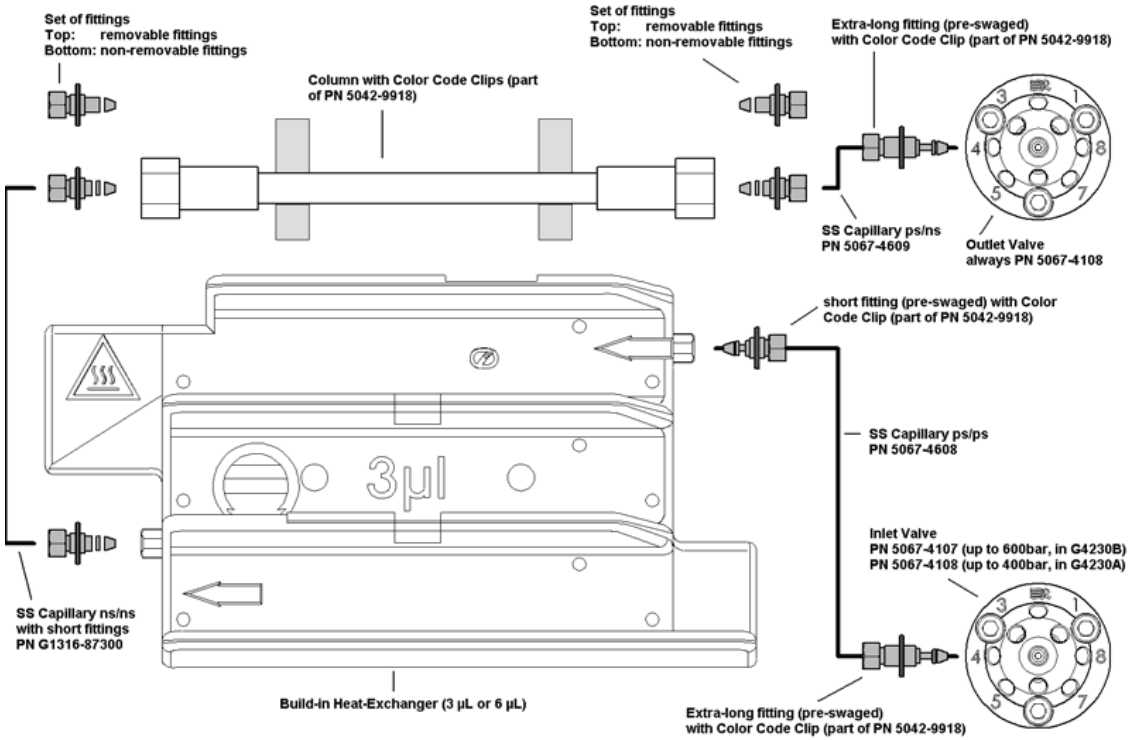


Figure 13 Plumbing, if internal heat exchangers are used

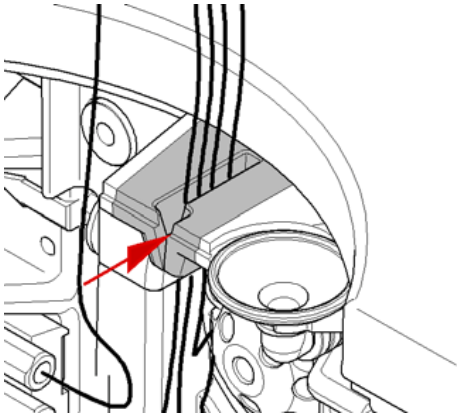


Figure 14 Capillary guides of the column compartments

Special Column Configurations

The Agilent 1200 Series Method Development solution can, of course, also be used with column configurations that include columns of different lengths and diameters. For example, one column thermostat could be equipped with for columns up to 100 mm length for fast screening and two additional column compartments could be equipped with another four longer columns for increased separation efficiency (see Figure 15 on page 42). The same setup, using the four short RRHT columns in the top TCC for method development and the same selectivity in 3.5 µm or 5 µm particle sizes with correspondingly scaled longer columns placed in the bottom TCCs, would provide a system suitable both for method development and scaling of methods to a typical QA/QC standard system. In this case, a low-dispersion capillary kit for short columns would be needed, plus some additional capillaries to connect the long columns. Other special column configurations could require maybe columns of the same length but with increasing internal diameters. In this case, starting with the low dispersion capillary kit for long columns plus additional capillaries and using the built-in heat exchanger as well might be a good solution.

The Agilent 1200 Series Method Development solution, with up to three clustered column compartments and different choices of capillary kits and heat exchangers, as well as up to six independently controlled temperature zones, gives you great flexibility for your method development tasks.

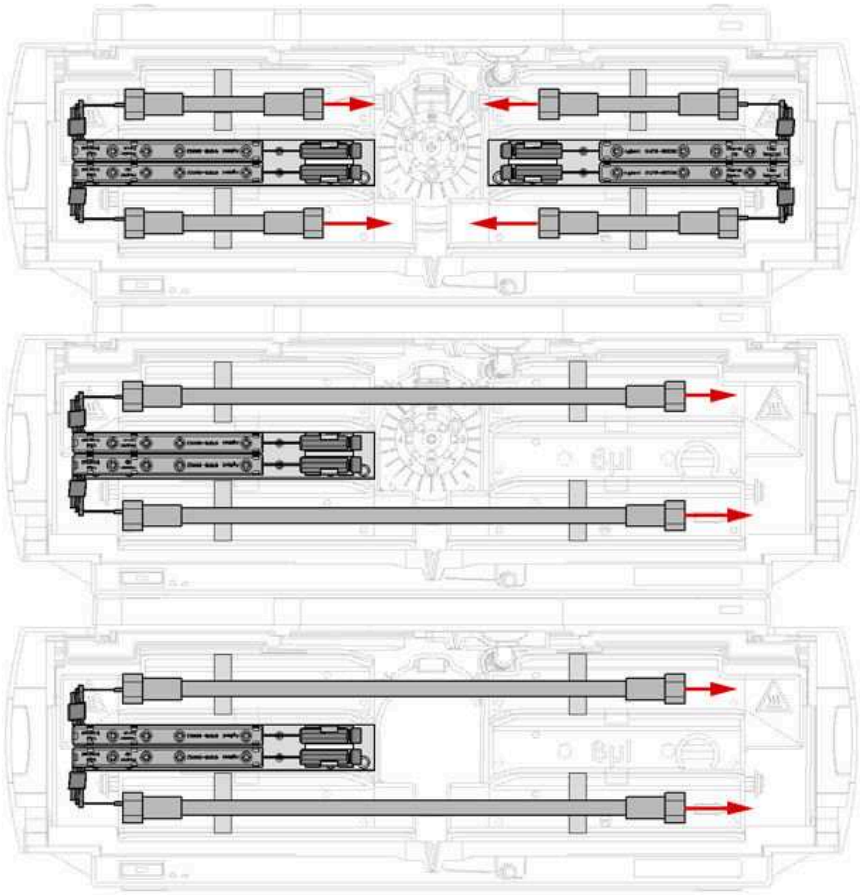


Figure 15 Example of a specialized system using a mix of short and long columns

Installing the Solvent Selection Part

For LC method development, it is very important to have a large choice of different mobile phases available, because the pH-value, the organic modifier and the ionic strength have a big influence on the separation. Therefore, the Agilent 1200 Series Method Development Systems offers the possibility to equip the pump with an additional external solvent selection valve (G1160A), which multiplexes one channel of the pump and provides up to 12 solvent channels. With the quaternary pump, this gives a maximum of 15 available solvents and allows the generation of up to 36 solvent combinations for a binary gradient. When the external solvent selection valve is combined with a binary pump with no internal solvent selection valve, 12 solvent combinations are possible. If the binary pump is fitted with an internal solvent selection valve, providing two different solvents per channel, up to 26 solvent combinations for a binary gradient are possible.

Deciding on the Position of the Degasser

First, you need to decide how the solvents should be degassed. There are two options available – refer also to Chapter 5, “Flushing Conditions,” starting on page 81 for more details:

Degassing before the G1160A Solvent Selection Valve, see Figure 16 on page 43.

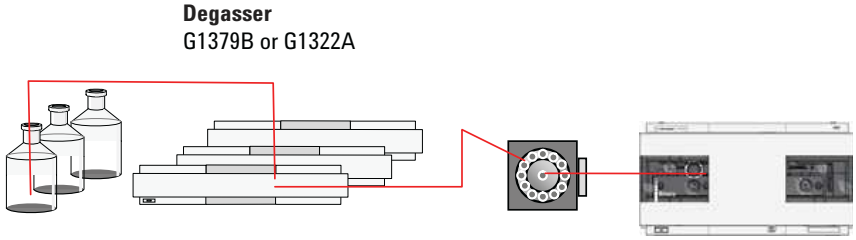


Figure 16 solvent degassing before the solvent selection valve

Pro: Short flush times (the degasser chamber does not need to be flushed)

Con: Potential re-solution of air in the longer tubing from the degasser to the pump, more than one degasser required

Required parts:

- up to 4x G1379B or G1322A degasser
- 5065-9981 Solvent Cabinets (one is included to the pump)
- 9301-1420 Solvent Reservoirs 1L or
- 9301-1450 Solvent Reservoirs 1L (amber bottles)
- n x 5067-4601 Tubing Kit (one kit is for 4 solvents only!)

Degassing after the G1160A Solvent Selection Valve, see Figure 17.

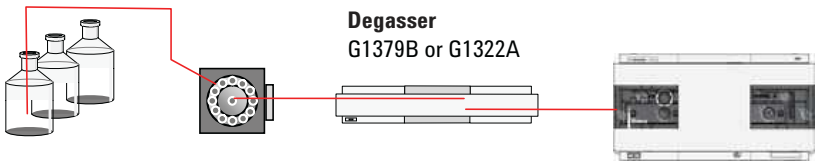


Figure 17 Degassing of solvents after the solvent selection valve

Pro: No risk of re-solution of air, only one degasser required

Con: Up to **50 times** longer flushing times because after every solvent change, the degasser chamber (with the G1322A ca. 10 mL) needs to be flushed thoroughly, which means with at least five times the volume of the degasser chamber.

Required parts:

- 1x G1379B or G1322A degasser
- 5065-9981 Solvent Cabinets (one is included to the pump)
- 9301-1420 Solvent Reservoirs 1L or
- 9301-1450 Solvent Reservoirs 1L (amber bottles)
- n x 5067-4601 Tubing Kit (one kit is for 4 solvents only!)

Mounting of the Solvent Selection Valve

To mount the solvent selection valve, special covers for the pumps are available. These covers have a guide rail on the left side onto which the valve can be slid. Choose between

- 5067-1567 cover kit for binary pumps (G1312A/B)
- 5042-9912 cover kit for quaternary pumps (G1311A)

These cover kits are delivered as three parts – the top cover and the two side-covers. It is of utmost importance to install the side panel with the guide rail for the solvent selection valve at the left side of the cover (the front of the top cover has the cut-out to place the solvent tubing). **Once the parts are clicked into each other, they can no longer be detached**. The new cover replaces the installed cover of the pump, see Figure 18. Remove the name plate from the old cover and click it onto the new one. The solvent selection valve is slid onto the guide rail.

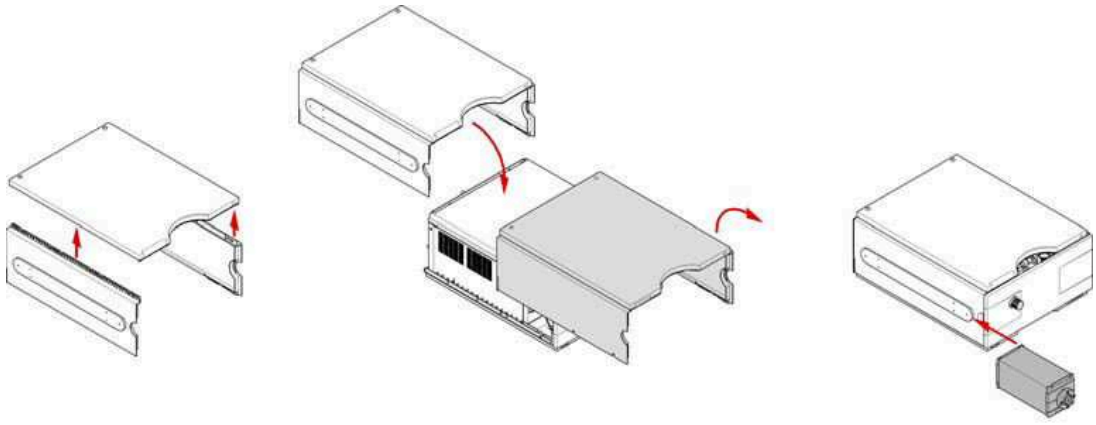


Figure 18 Installation of the cover kits and mounting the valve

Make the electrical connections of the G1160A solvent selection valve. The power line can be connected either to the autosampler or to the pump; the CAN-connection can be connected to any free CAN-port.

Installing the Solvent Tubing

One solvent selection tubing kit (PN 5067-4601) is delivered with all required parts to connect up to four solvent bottles to the G1160A solvent selection valve. If you need more solvents, additional kits need to be ordered.

Making the connections for a set-up with degassing using several degassers before the solvent selection valve is very straightforward. Place the solvent bottles in additional solvent cabinets on top of the degasser. Use the pre-installed bottle head assemblies to connect the bottles with the degasser inletports. Then cut appropriate lengths of the 2 x 5m solvent tubing and fit them to the fittings (use the prefitted bottle head assemblies as examples of how these are fitted together). Attach the PEEK-adapters to the ports of the solvent selection valve and connect the solvent tubing from the outlet port of the degasser to the peripheral ports of the solvent selection valve. Finally, connect the central port of the solvent selection valve to one port of the pump, or to one port of the optional internal solvent selection valve of the pump (binary pumps only).

If you prefer to use degassing after the solvent selection valve (accepting significantly increased flush times), the procedure is slightly more complicated. You need to take the bottle head assemblies apart, since you need to replace the pre-installed tubing with much longer ones that you cut to appropriate lengths from the 5m-tubing. After you have refitted the bottle head assemblies with the longer tubing, connect them with the peripheral ports of the solvent selection valve using the PEEK adapters. Finally, connect the central port of the solvent selection valve with the inlet port of one degasser channel and the corresponding outlet port with one channel of the pump.

In both cases, you need to make the corresponding settings in the software (entering the clustering information, and entering the names of the solvents into the solvent table) – see the next chapter.

4
Configuring the System in ChemStation and Creating Methods

1 For instrument control, ChemStation B04.01 or higher needs to be installed. Switch on the power of all modules of your system.

2 Establish a connection to your system and configure the LC or LCMS system correctly (refer to the corresponding ChemStation documentation).

3 Start the ChemStation and select all recognized and green highlighted modules as active modules.

Clustering of Modules in ChemStation

Configure Column Thermostat Cluster

To cluster and configure the column compartments, open the **Instruments** menu and select **Configure Column Thermostat Cluster**.

All available column compartments (G1316A/B/C) are shown in the left table. Select all column compartments you want to include in the cluster by moving them with the arrow-buttons to the right table. A maximum of three is allowed, and at least two G1316C modules that include a valve drive with an 8-position/9-port valve head are required.

Use the up/down arrow buttons to move the clustered column compartments up or down in the table. The position in the table should reflect the physical set-up of the system.

If two different pressure ranges of the valve heads are detected, the one with the higher pressure range is set as the inlet valve automatically. If two identical pressure ranges of the valve heads are available, you specify which one has to be used as the inlet valve and which as the outlet valve. Use the “Identify” button to blink the status LED of the corresponding column compartment, see Figure 19.

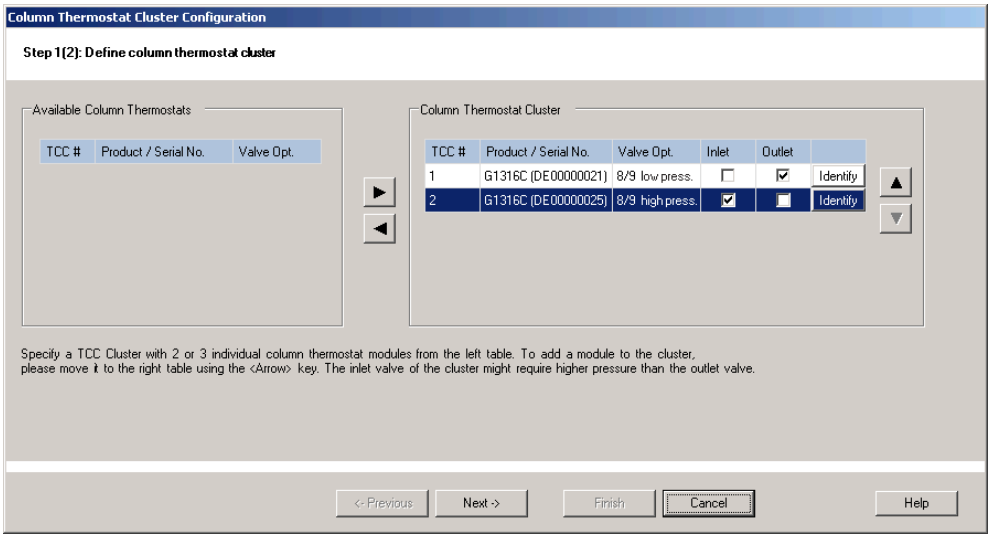


Figure 19 Selecting column compartments to be clustered

Clicking the “Next” button leads you to the second screen for the detailed setup of the column compartment cluster, see Figure 20 on page 51. In the table, the valve positions (1 – 8) are set. You can now assign the color codes you chose during the hardware set-up to these flow-lines, and specify whether the corresponding valve port is connected to a column, to the bypass line or to the waste-line. You also need to set the position of the column. First, specify in which column compartment it is located (you can use the “identify” buttons again to blink the status LEDs) and finally you specify the exact position of the column in the column compartment. The available selections refer to the column locations between the fins of the built-in heat exchanger (upper/middle/lower – left/right). If you are installing long columns, the selection upper/middle/lower – left **and** right) not only shows the columns in the user interface in the corresponding position but also **always couples the temperatures of both heat exchangers!**

If you need to use a post column cooler (for maximum UV-sensitivity at high temperatures and high flow rates), you need to mark the corresponding check box at the bottom of the screen and assign the position of the post-column cooler (TCC and heat exchanger).

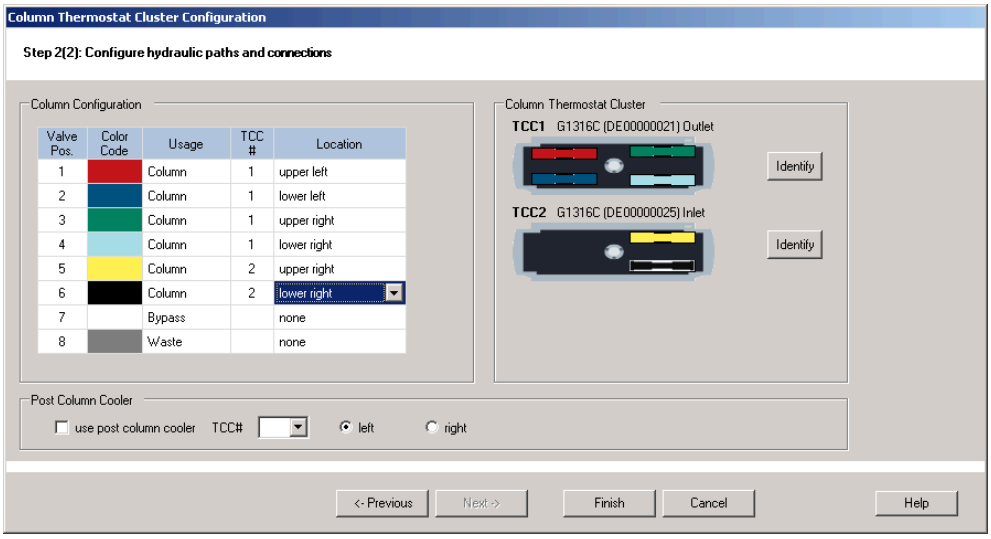


Figure 20 Assigning the usage of valve positions as well as locations and color codes of columns in the column compartment cluster.

Configure Solvent Delivery Cluster

If an external G1160A solvent selection valve is installed, it is possible to cluster it with a pump. After clustering, the G1160A valve does not appear with its own user interface, but the pump user interface allows the selection of the solvent of the corresponding channel of the G1160A valve. Open the **Instruments** menu and select **Configure Solvent Selection**, see Figure 21 on page 52. Select the pump and the G1160A valve by their serial numbers (use the “Identify” button to blink the status LED) and the pump channel to which the solvent selection valve is connected.

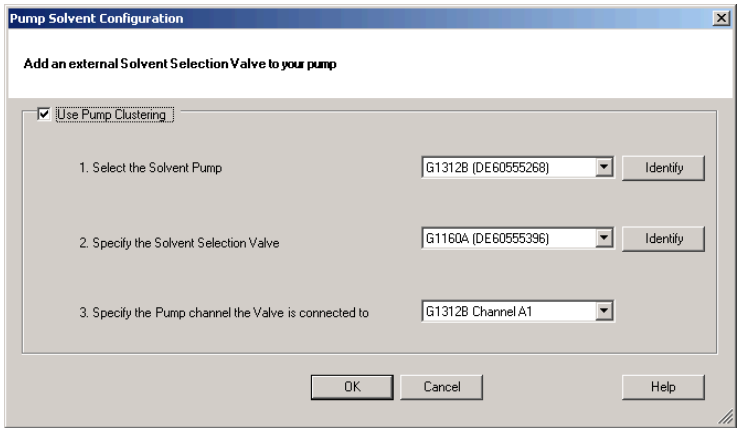


Figure 21 Clustering of an external solvent selection valve

NOTE When you have completed the configuration steps, close the ChemStation and open it again. Your changes become active, and you can continue with the next steps.

Settings for Clustered Modules

Settings for Column Thermostat Cluster

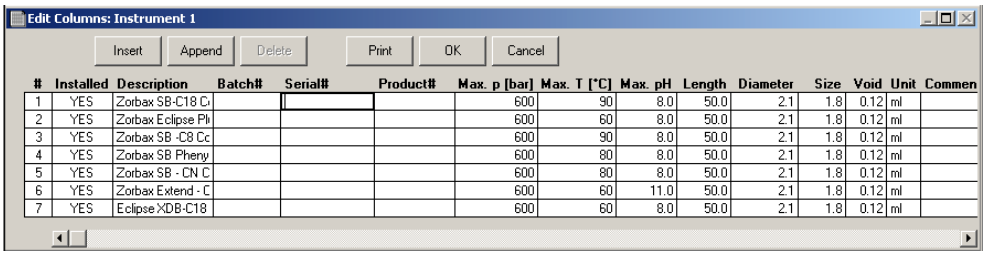
When the ChemStation has restarted, the columns that are to be used for the experiments can be specified.

Columns Definition

To specify the columns, open the **Instruments** menu and select **Columns**. Fill in the columns you regularly use in your lab, see Figure 22 on page 53. This table is needed to enable you to configure columns for the column positions, shown in Figure 23 on page 54. You should enter at least a Description, the physical dimensions and, it these values are identical for some columns, a serial number to allow correct identification later. If you are using the Agilent ChemStation Method Scouting Wizard, it is important that you set the column void volume correctly using [mL] as units, otherwise flushing and equilibration times will not be calculated correctly.

Alternatively, the total porosity can be entered by selecting [%] as units (e.g. 60 %). In this case the column dimensions in [mm] must be entered as well otherwise the column void volume cannot be calculated.

When you are using the Method Scouting wizard, it is also advisable to enter a maximum temperature and pH (this means the maximum basic pH-value). It is not required to enter the “installed” value (this entry is needed only for non-method-development systems). See in the User Contributed Libraries on the ChemStation DVD for a tool to import column tables.



#	Installed	Description	Batch#	Serial#	Product#	Max. p [bar]	Max. T [°C]	Max. pH	Length	Diameter	Size	Void	Unit	Commen
1	YES	Zorbax SB-C18 C				600	90	8.0	50.0	2.1	1.8	0.12	ml	
2	YES	Zorbax Eclipse Ph				600	60	8.0	50.0	2.1	1.8	0.12	ml	
3	YES	Zorbax SB -C8 Cc				600	90	8.0	50.0	2.1	1.8	0.12	ml	
4	YES	Zorbax SB Pheny				600	80	8.0	50.0	2.1	1.8	0.12	ml	
5	YES	Zorbax SB - CN C				600	80	8.0	50.0	2.1	1.8	0.12	ml	
6	YES	Zorbax Extend - C				600	60	11.0	50.0	2.1	1.8	0.12	ml	
7	YES	Eclipse XDB-C18				600	60	8.0	50.0	2.1	1.8	0.12	ml	

Figure 22 Table of columns which are used for method development experiments

NOTE The "installed"-value is not used in case of a column compartment cluster.

Defining Installed Columns

When you have completed the Columns table, you can specify the column positions. Open the **Instruments** menu, select **More Column Thermostat Cluster** and select **Configure Column** from the submenu. Select the column from a drop down menu and assign it by the color code you have specified in the hardware setup for a certain position. You can see the selected positions in the graphical representation on the right site of the screen, see Figure 23 on page 54.

You also specify the Standby-Temperature for the columns; this is the temperature that the columns are set to when they are not in use as specified in a method. Note that stand-by temperatures of some columns might be coupled, depending on the usage of two low dispersion heat exchangers mounted on a built-in heat exchanger, or when assigning positions to long columns (the corresponding coupled temperature of a column is updated but cannot be changed independently).

The temperature of a post column cooler, if installed, is also set here. This can be set to a fixed temperature, or can be dynamically adjusted to the temperature of the detector cell (recommended).

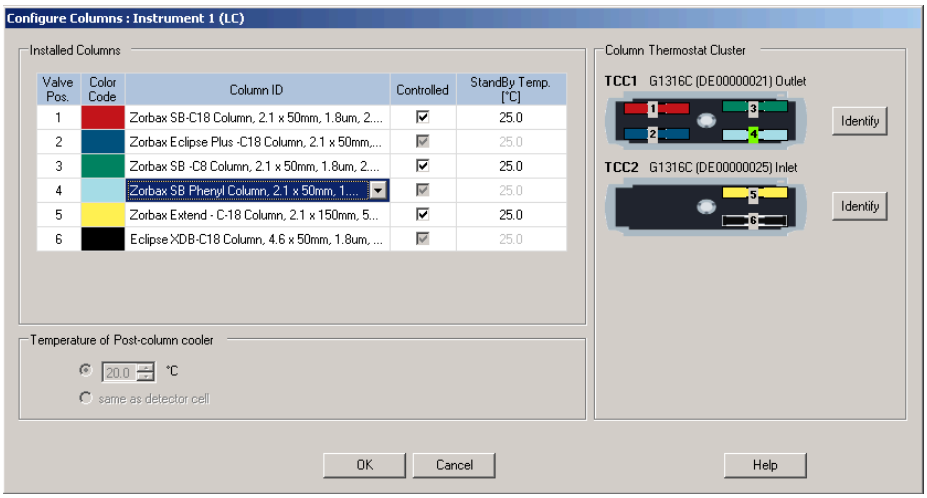


Figure 23 Assigning columns to positions and defining the standby temperature

Method Setup for Column Compartment Cluster

When you have configured all columns, open the **Instruments** menu and select **Set Up Column Thermostat Cluster** to select a column to be used in a method (the same screen appears at the appropriated time when using **Edit Entire Method** or when clicking on the column compartment cluster icon. Click on the column (or waste or bypass) you want to use in an analytical run, see Figure 24 on page 55. The position of the column in the column compartment cluster and the corresponding valve position are shown in the graphical representation. Together with the color code, this allows simplest identification of the flow path in the cluster.

In this screen, the temperature to be set for the column in the flow path can also be set.

A new feature of the G1316C column compartments is the recognition of the position of the front door (open or closed). By default, an analysis starts only when the door is closed (recommended); however, when the corresponding check box is marked, the analysis is enabled even with an open door.

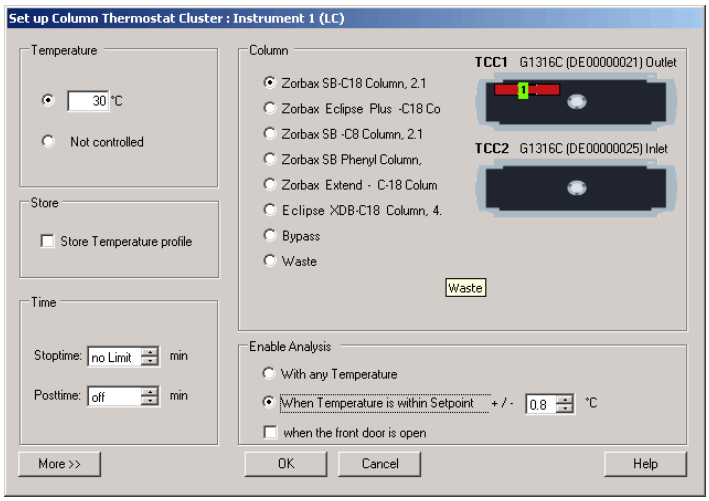


Figure 24 Selecting the columns and setting the temperatures for the analytical run

If you need to apply temperature gradients during a run, click the More button and enter the additional time programming of the temperature.

Settings for Solvent Selection

Configuring the Solvent table

If an external G1160A solvent selection valve is clustered as shown in Figure 21, solvents can be specified and named by opening the **Instruments** menu, selecting **More Pump** and selecting **Configure Solvent Table** from the submenu, see Figure 25.

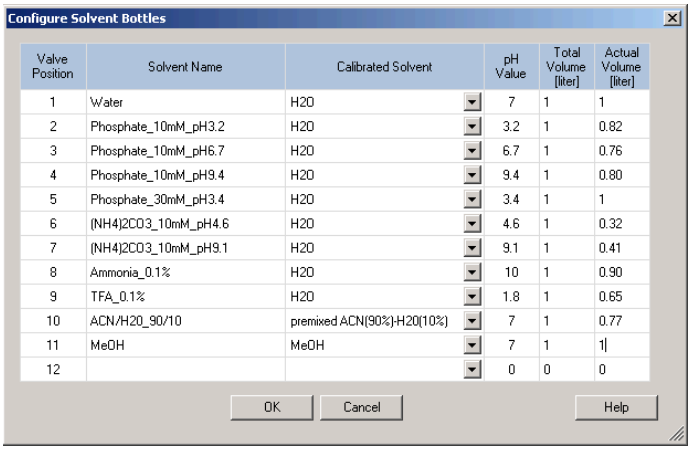


Figure 25 Configuring solvents attached to the external G1160A solvent selection valve

The left column shows the valve position. In the **Solvent Name** column, you can assign a name to the solvent attached to that valve position. If your system uses a pump that uses calibration tables for the compressibility and elasticity of the solvents (for example the G1312B binary pump SL), you also need to enter the calibration solvent here. The entry for the pH value is optional, but is recognized in the Agilent ChemStation Method Scouting Wizard to exclude incompatible combinations of solvents and columns. Finally, you enter the total volume of the corresponding solvent bottle as well as the actual volume that the bottle contains; this will be reduced automatically according to the usage. The bottle filling dialog box, accessible from the instrument graphics, is updated for the multiplexed channel of the pump accordingly. If the values in the bottle filling dialog box are changed for the multiplexed channel, the values in the solvent table are updated, but only for the currently selected channel on the external solvent selection valve.

Method Setup for Solvent Delivery Cluster

In the pump setup screen, the solvents for an experiment can be selected without the need to switch the solvent selection valve to the appropriate position in an additional screen, see Figure 26 on page 57. You simply select the solvent you want to use by its name. If you need to enter a calibrated solvent for the pump installed in your system, this is taken automatically from the solvent table (see above).

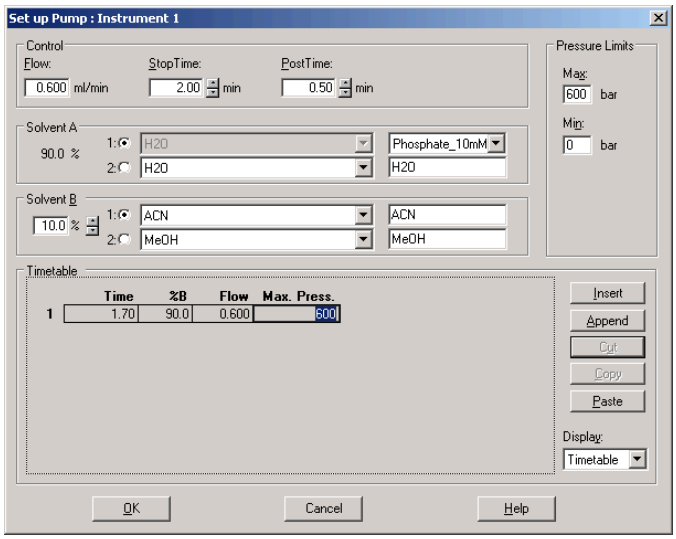


Figure 26 Selecting a mobile phase attached to the solvent selection valve

When you have configured and set up the complete system, you can set up the different methods for the different columns, set up the sequence and start it.

5 The Agilent ChemStation Method Scouting Wizard

Overview of the Agilent ChemStation Method Scouting Wizard

The optional Agilent ChemStation Method Scouting Wizard is an add-on to the ChemStation (version B04.01 or higher) that enables you to set up methods and sequences for easy and logical method scouting using an Agilent Method Development System. It allows you to create all methods and a sequence that have all possible combinations of available columns, solvents, a set of predefined gradients and a set of predefined temperatures. It does not do automatic method optimization.

The Method Scouting Wizard automatically generates all steps to flush the system with any required solvents, performs column equilibration procedures and can store columns in predefined storage solvents. In this respect, it uses waste and/or available bypass lines intelligently to allow fast flushing procedures. Flushing, equilibration and column storage procedures, and temperature changes are arranged in the workflow such that a minimal number of these steps need to be performed to save valuable time and solvents. In Figure 27 a part of a complete sequence is shown as an example of how the different steps are put together.

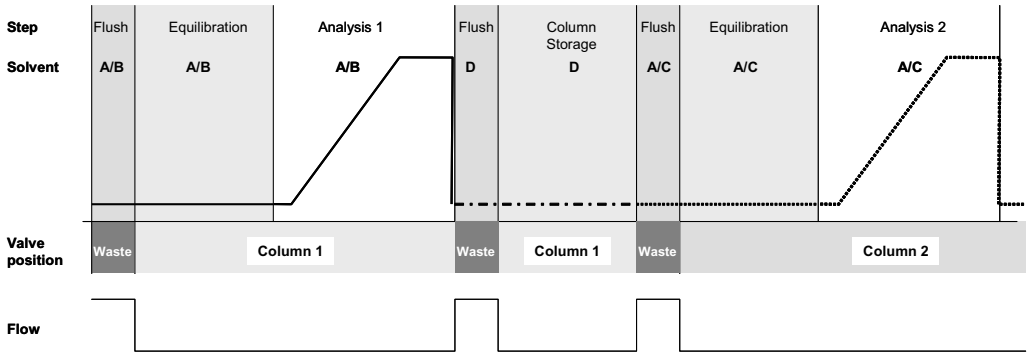


Figure 27 Different steps during a method scouting campaign as generated by the Agilent ChemStation Method Scouting Wizard

The analysis is performed on column1 using a solvent combination A/B, followed by post-run storage of column 1 in a special storage solvent D, and finally switching to column using a new solvent combination A/C. The system has a waste line available (see Chapter 5, “Specifying and Selecting Gradients” and Chapter 5, “Settings for System Volumes, Flushing, Equilibration and Column Storage,” starting on page 77).

Software Installation

Install the Agilent ChemStation Method Scouting Wizard after installing the ChemStation by double clicking the “MethodScouting.msi”-file on the CD.

Defining the Project

All methods, the sequence and the project file that contains all settings of a campaign are saved in a screening-campaign folder. You can create a new campaign or open an existing one. In the latter case, you can choose between overwriting the old one and saving it with a new name. **Create a new screening campaign** includes naming of the campaign and specifying the path where the campaign is saved. It is recommended to save the campaign in a separate folder, such as **Screening**. This screen is always the start for the setup of an experiment (see Figure 28).

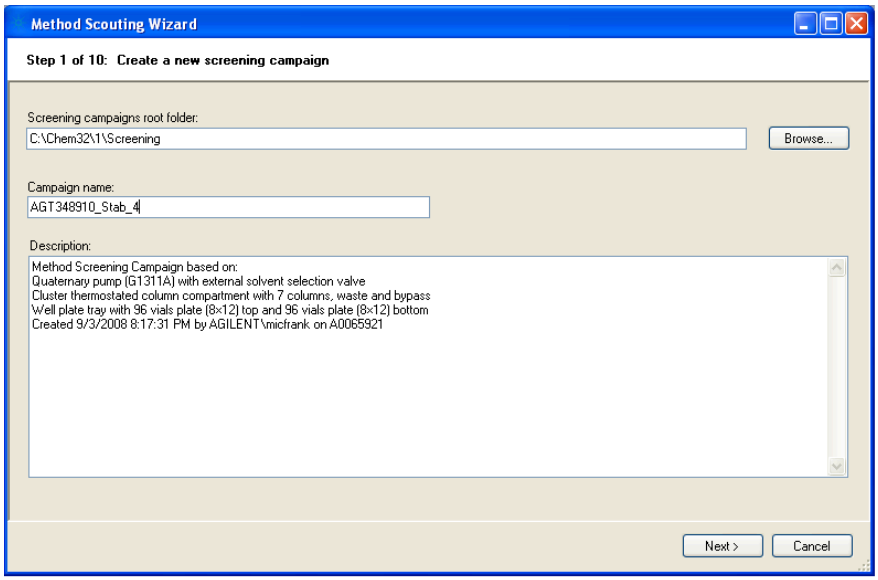


Figure 28 Start screen for a campaign

Define Screening Campaign Base

For a method scouting campaign, a base method needs to be set up (you do this as usual in the ChemStation - it is recommended to use Edit Entire Method). All parameters that are not changed are taken from this base method. These are typically the detector settings, autosampler settings, pump settings that are not altered, and data processing parameters (see Figure 29 on page 65).

In addition to selecting the base method, you also need to specify the scope of the screening campaign. That means the combinations of column screening, solvent screening, gradient screening and temperature screening that you would like to perform. Here, you specify the dimensions of the method scouting campaign; in the next screens, the ranges of the selected dimensions are specified.

NOTE

With a quaternary pump ambiguities could occur if "solvent screening" is selected and the gradient would be taken form the base method during solvent screening. To prevent this if no gradient screening is selected, the "gradient screening" will be checked automatically. If no no gradient screening is demanded by the user only one gradient has to be defined in the later gradient screening page.

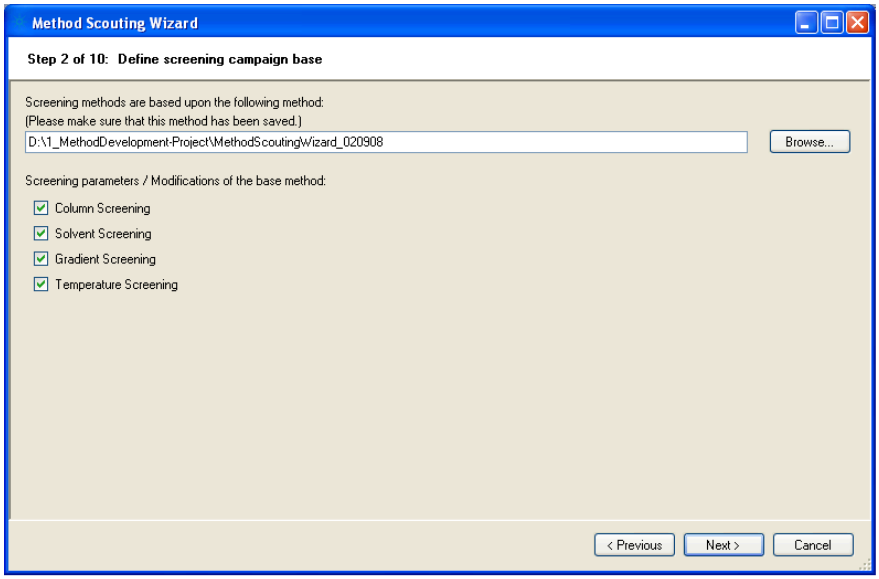


Figure 29 Screen for defining the campaign range and selection of the master method

Selecting the Columns

In this screen, you select the columns to be evaluated (see Figure 30). You can select only the columns that are installed in the system and have been specified previously in the **Configuring Columns** user interface screen under **Instruments**. The installed columns as defined will be shown in the Method Scouting Wizard; important variables such as the position, the specified void volume, the maximum temperature and pH-value are shown, but are not editable. You select the columns you want to use in the method scouting campaign by check the **Use** check boxes.

If you do not select column screening as a dimension of the method scouting campaign, the column specified in the base method is used.

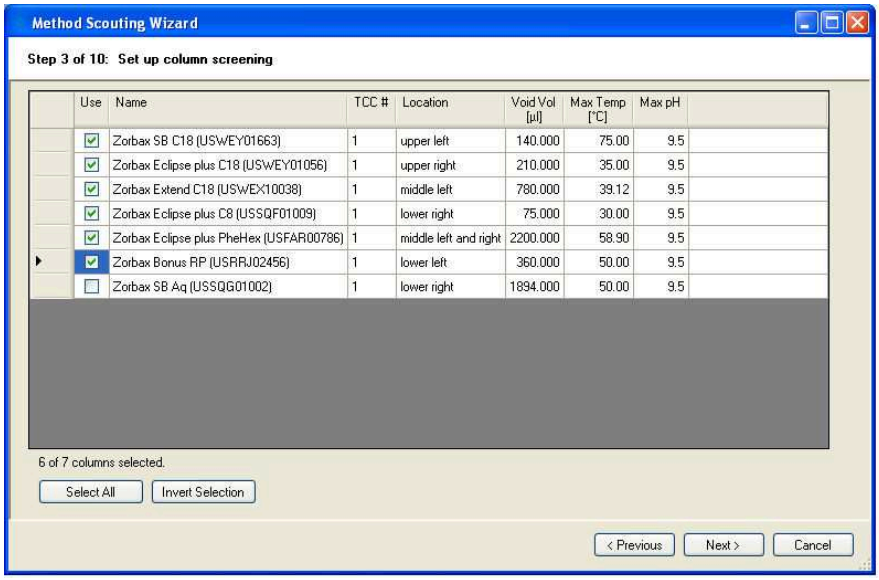


Figure 30 Selection of columns

Selecting the Solvents

If solvent screening is selected, the solvents to be varied are specified in the following screen (see Figure 31). The Method Scouting Wizard automatically detects the system configuration and shows the appropriate screen. For a binary pump without any additional solvent selection valves, no choices are available. If an additional internal solvent selection valve is installed, the solvents to be used can be selected. If an additional external G1160A solvent selection valve is installed, all solvents that are available and specified in the solvent table are shown as choices for the corresponding channel where the valve is installed. You mark the corresponding check boxes of the solvents that are used to create binary gradients.

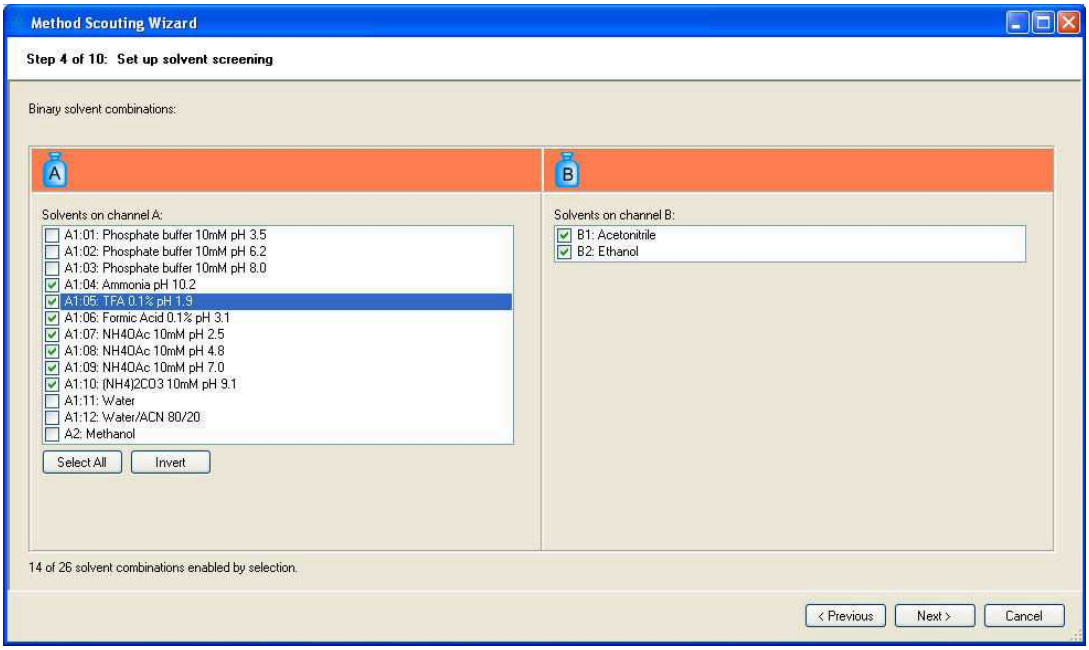


Figure 31 Selecting the solvent with a binary pump

If a quaternary pump is installed, the **Set Up Solvent Screening screen** looks slightly different, see Figure 32 on page 68. Again, the position of an optionally installed external solvent selection valve is detected. Additionally, you choose to create binary, ternary or quaternary gradients. Besides, for quaternary gradients that do not allow further choices you specify here which variations should be created, that is for a binary gradient on a system with an external solvent selection valve attached to channel A, for example, combinations of those solvents on A versus the B-, C- and D-channel (A_01-B, A_01-C, A-01-D, A_02-B, A_02-C, A_02-D, etc). Other combinations might be solvents on the A-channel as well as the B-channel versus the C- and D-channel (A_01-C, A-01-D, A_02-C, A_02-D, B-C, B-D, etc).

The number of selected solvent combinations and the maximum possible number of combinations with the given system are indicated at the bottom of this screen.

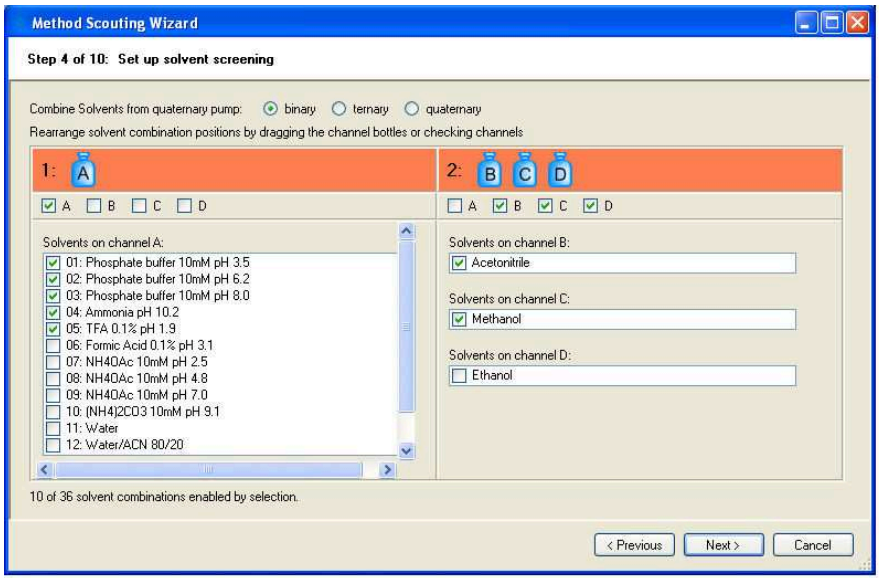


Figure 32 Set up solvents screening using a quaternary pump, a setup for a binary gradient of channel A (with an attached external solvent selection valve) versus the channels B, C and D is shown.

Specifying and Selecting Gradients

If the gradient is also to be varied, the following screen is displayed, where you can specify gradients, their initial composition, run time, post-time and the flow rate to be used. You have the possibility to enter the gradients based on a table (time vs. percentage of solvent, see Figure 33) or graphically (see Figure 34 on page 70). With the graphical tool, you can set gridlines and specify that the cursor snaps onto the gridlines, with selectable snapping ranges. Finally, you can also overlay the selected gradients.

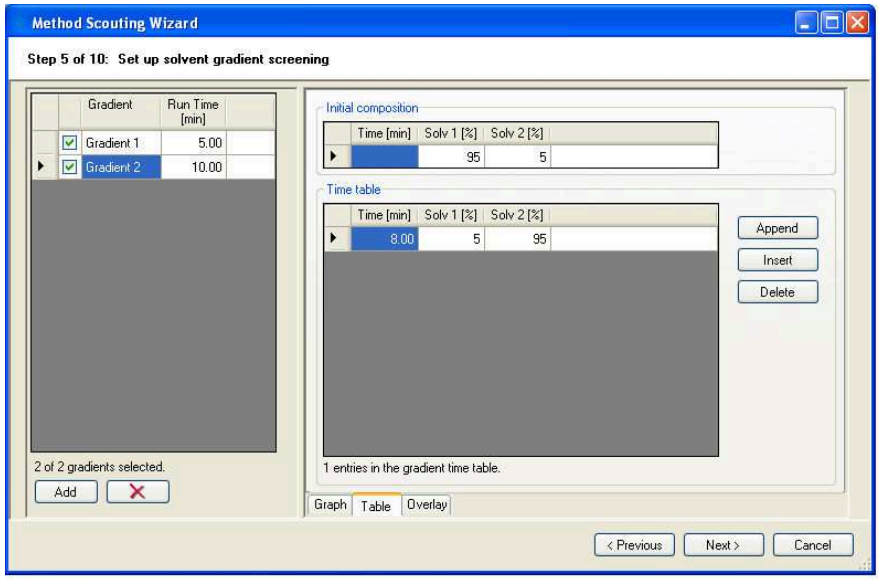


Figure 33 Defining and selecting different gradients – gradient table

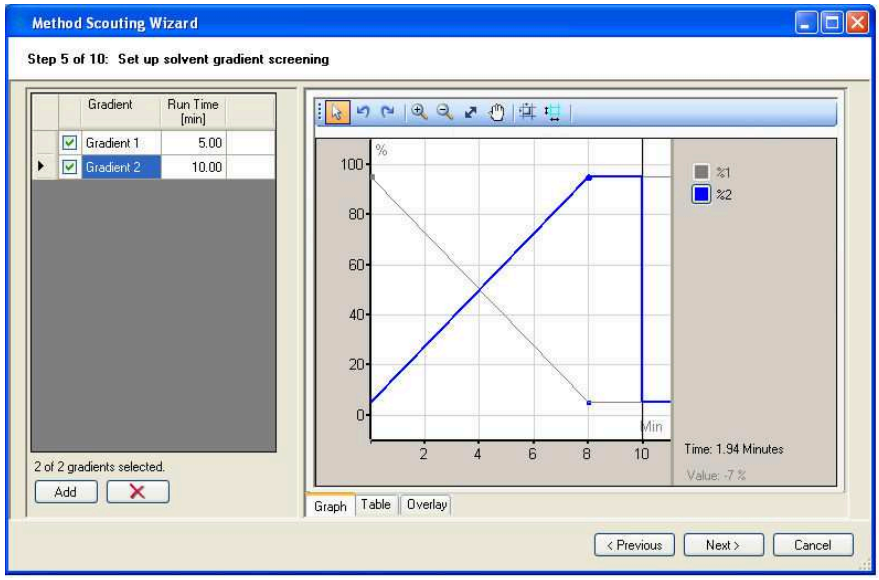


Figure 34 Defining and selecting different gradients – graphical interface

It is extremely important to set the initial composition correctly. This is the composition that the pump generates after the method is loaded but the injection has not yet occurred, and the composition that is used after the run time (data acquisition) has elapsed and until the next method is loaded (for example, during the post-time or system overhead time). If you do not explicitly change the initial composition the one from the base method is used.

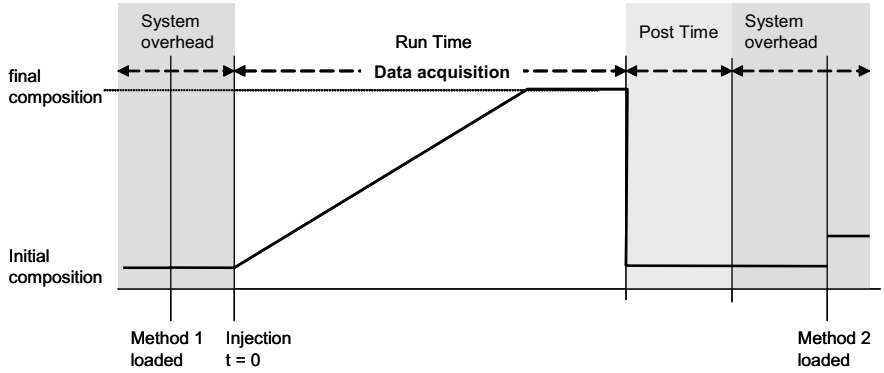
The initial composition determines the settings for a flushing or equilibration method that is executed immediately before the gradient method is applied to a sample, or a subsequent column storage method that does not use a dedicated storage solvent.

A simple linear gradient consists of one line in the table – the final composition that has to be reached. If the time value of the final composition is lower than the run time, the final composition is held until the run time is reached. If you have set a post-time, the initial composition is applied from the “run time” until the “post-time” (see Figure 35 on page 71). To correctly specify gradients, you also need to consider how the Method Scouting Wizard applies the Equilibration procedures (see Chapter 5, “Settings for System Volumes, Flushing, Equilibration and Column Storage,” starting on page 77). Take care when setting time values at 0 min or close to 0 min; unless this value

matches the initial composition, you will have a situation with an improperly equilibrated column and maybe nonreproducible results, see Figure 37 on page 72).

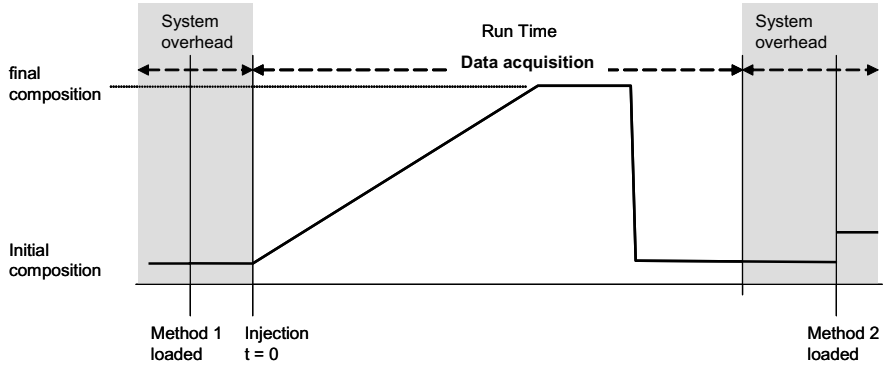
NOTE

Be sure to use either the post-time or to have a suitable time point set in your gradient method to ensure column equilibration after repeated use of the analytical method (e.g. multiple injections or multiple samples) See Chapter 5, "Settings for System Volumes, Flushing, Equilibration and Column Storage," starting on page 77!



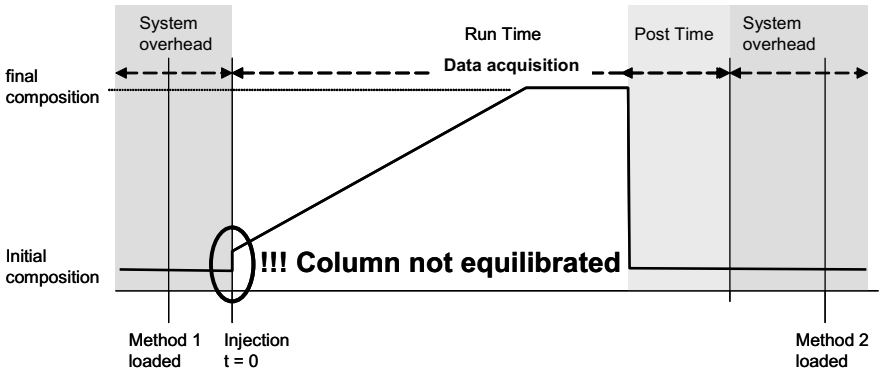
Post time base method	15 min	
Run time Meth. 1	10 min	
Initial composition	90 %A	10 %B
Gradient table		
8 min	10 %A	90 %B

Figure 35 Typical gradient setting with post-time. Equilibration occurs during the post-time without data acquisition during that time.



Post time base method	off	
Run time Meth. 1	15 min	
Initial composition	90 %A	10 %B
Gradient table		
8 min	10 %A	90 %B
10 min	10 %A	90 %B
10.01 min	90 %A	10 %B

Figure 36 Typical gradient setting without post-time but an added equilibration period in the gradient table. Data acquisition occurs during the equilibration phase of the column.



Post time base method	15 min	
Run time Meth. 1	10 min	
Initial composition	**95 %A**	**5 %B**
Gradient table		
0 min	**90 %A**	**10 %B**
8 min	10 %A	90 %B

Figure 37 Avoid composition settings at 0 min or close to 0 minutes in the gradient table that do not match the composition of the initial composition as this leads to improper equilibration.

Defining and Selecting the Temperatures

If you also selected temperature screening, you can enter the temperatures you want to test (see Figure 38 on page 74). You can enter all values that are available for the given system.

NOTE If you are using a mixed system containing a G1316C and a G1316A column compartment, the maximum temperature is defined by the G1316A (80°C instead of 100°C). The lowest temperature that can be entered is -5°C.

The G1316-series column compartments are able to cool to 10°C below ambient, but since the ambient temperature can change during the course of the analysis series, you should consider having some safety margin. If your lab has a rather high ambient temperature, you might want to set the lowest screening temperature to ca. 20-25°C. If this temperature cannot be reached, the column compartments stays in a not-ready state because the Method Scouting Wizard enforces the enablement of an analysis only when the column compartment has reached the set-point in the allowed range as specified in the base method (**Set Up Column Thermostat Cluster > Enable Analysis**). For flushing or column storage methods, the method starts without reaching the set point.

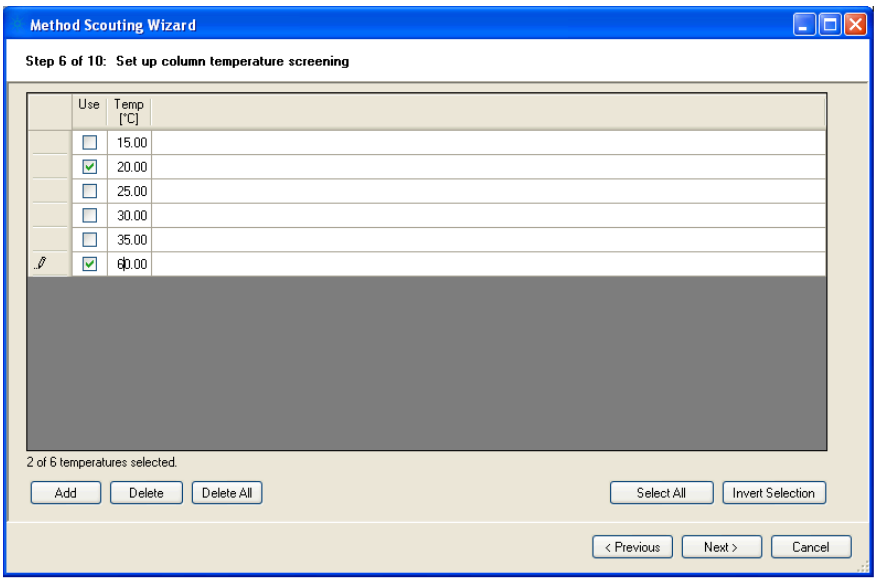


Figure 38 Temperature screening

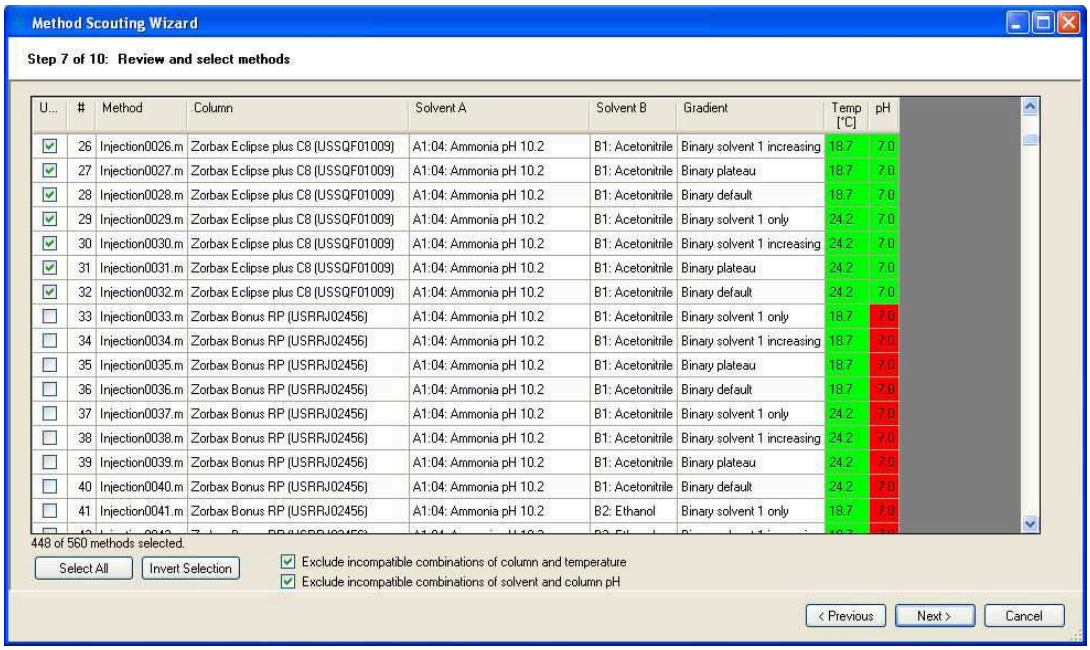
Review the Selected Methods

This screen shows you an overview of all combinations of columns, solvents, gradients and temperatures (see Figure 39 on page 76). Additionally, two check boxes at the bottom allow you to automatically deselect incompatible combinations of columns with solvent-pH and temperatures. This option requires that the values for “max temp.” and “max pH” are set for the columns in the ChemStation column database, and that the solvent pH is set correctly in the solvent table of the pump if an external G1160A solvent selection valve is clustered to the system. The maximum allowed pH and temperature of the column is shown in the table and marked either with a green or red color to indicate compatible and incompatible values.

NOTE Currently, only the pH-values of solvents attached to the external solvent selection valve are taken into consideration.

By clearing the **Use** check boxes in the left column of the table, you can eliminate unwanted combinations of the full matrix. This is very useful, because it allows you to save and re-use such special scouting campaigns with other samples.

The total number of methods is shown at the bottom, and gives a quick overview on the complexity of the specified scouting campaign. Detailed information is available at the end after flushing, column storage and equilibration procedures have been specified and added to the campaign.



U...	#	Method	Column	Solvent A	Solvent B	Gradient	Temp [°C]	pH
☑	26	Injection0026.m	Zorbax Eclipse plus C8 (USSQF01009)	A1:04: Ammonia pH 10.2	B1: Acetonitrile	Binary solvent 1 increasing	18.7	7.0
☑	27	Injection0027.m	Zorbax Eclipse plus C8 (USSQF01009)	A1:04: Ammonia pH 10.2	B1: Acetonitrile	Binary plateau	18.7	7.0
☑	28	Injection0028.m	Zorbax Eclipse plus C8 (USSQF01009)	A1:04: Ammonia pH 10.2	B1: Acetonitrile	Binary default	18.7	7.0
☑	29	Injection0029.m	Zorbax Eclipse plus C8 (USSQF01009)	A1:04: Ammonia pH 10.2	B1: Acetonitrile	Binary solvent 1 only	24.2	7.0
☑	30	Injection0030.m	Zorbax Eclipse plus C8 (USSQF01009)	A1:04: Ammonia pH 10.2	B1: Acetonitrile	Binary solvent 1 increasing	24.2	7.0
☑	31	Injection0031.m	Zorbax Eclipse plus C8 (USSQF01009)	A1:04: Ammonia pH 10.2	B1: Acetonitrile	Binary plateau	24.2	7.0
☑	32	Injection0032.m	Zorbax Eclipse plus C8 (USSQF01009)	A1:04: Ammonia pH 10.2	B1: Acetonitrile	Binary default	24.2	7.0
☐	33	Injection0033.m	Zorbax Bonus RP (USRRJ02456)	A1:04: Ammonia pH 10.2	B1: Acetonitrile	Binary solvent 1 only	18.7	7.0
☐	34	Injection0034.m	Zorbax Bonus RP (USRRJ02456)	A1:04: Ammonia pH 10.2	B1: Acetonitrile	Binary solvent 1 increasing	18.7	7.0
☐	35	Injection0035.m	Zorbax Bonus RP (USRRJ02456)	A1:04: Ammonia pH 10.2	B1: Acetonitrile	Binary plateau	18.7	7.0
☐	36	Injection0036.m	Zorbax Bonus RP (USRRJ02456)	A1:04: Ammonia pH 10.2	B1: Acetonitrile	Binary default	18.7	7.0
☐	37	Injection0037.m	Zorbax Bonus RP (USRRJ02456)	A1:04: Ammonia pH 10.2	B1: Acetonitrile	Binary solvent 1 only	24.2	7.0
☐	38	Injection0038.m	Zorbax Bonus RP (USRRJ02456)	A1:04: Ammonia pH 10.2	B1: Acetonitrile	Binary solvent 1 increasing	24.2	7.0
☐	39	Injection0039.m	Zorbax Bonus RP (USRRJ02456)	A1:04: Ammonia pH 10.2	B1: Acetonitrile	Binary plateau	24.2	7.0
☐	40	Injection0040.m	Zorbax Bonus RP (USRRJ02456)	A1:04: Ammonia pH 10.2	B1: Acetonitrile	Binary default	24.2	7.0
☐	41	Injection0041.m	Zorbax Bonus RP (USRRJ02456)	A1:04: Ammonia pH 10.2	B2: Ethanol	Binary solvent 1 only	18.7	7.0

Figure 39 Review and select analytical runs to be performed

Settings for System Volumes, Flushing, Equilibration and Column Storage

This important screen allows you to specify the following settings (see Figure 40 on page 78 and Figure 42 on page 84):

- how solvent lines are flushed after a solvent change has occurred
- how a column is taken care of after it has been used
- how columns are equilibrated
- how the data files created during any of the above procedures are handled

NOTE

If the number of sequence lines required for analytical runs as specified by the campaign plus additional blank runs for flushing, column care and equilibration exceeds 1999, a warning is displayed, and you cannot proceed until either the range or dimensions of the screening campaign is reduced. This can be accomplished, for example, by deselecting a dimension such as temperature screening, or reducing the range of a dimension such as fewer gradients or temperatures. Alternatively, but usually not recommended, you can also deselect flushing, column storage or column equilibration. This takes only one sample into account, If you plan to have more than one sample, you might get another warning during sample set up and need to further reduce the number of sequence entries.

Defining System Volumes

First, the volumes of the system that have to be flushed with solvents have to be defined (see Figure 40 on page 78). These settings have to be only entered once and will be stored for following screening campaigns as user defined settings, but they can be altered any time. In case an external solvent selection valve is connected to a solvent channel of the pump the position of the degasser has to be selected. Two choices are available - before the solvent selection valve or after the solvent selection valve. In the later case it is necessary that the volume of the degasser chamber will be flushed. This volume can be in case of a G1322A degasser significant.

Depending on the availability of an external solvent selection system and the selected position of the degasser different system diagrams will be shown and different entries have to be made. Move with the mouse over the entry boxes and the according volumes in the system diagram will be highlighted.

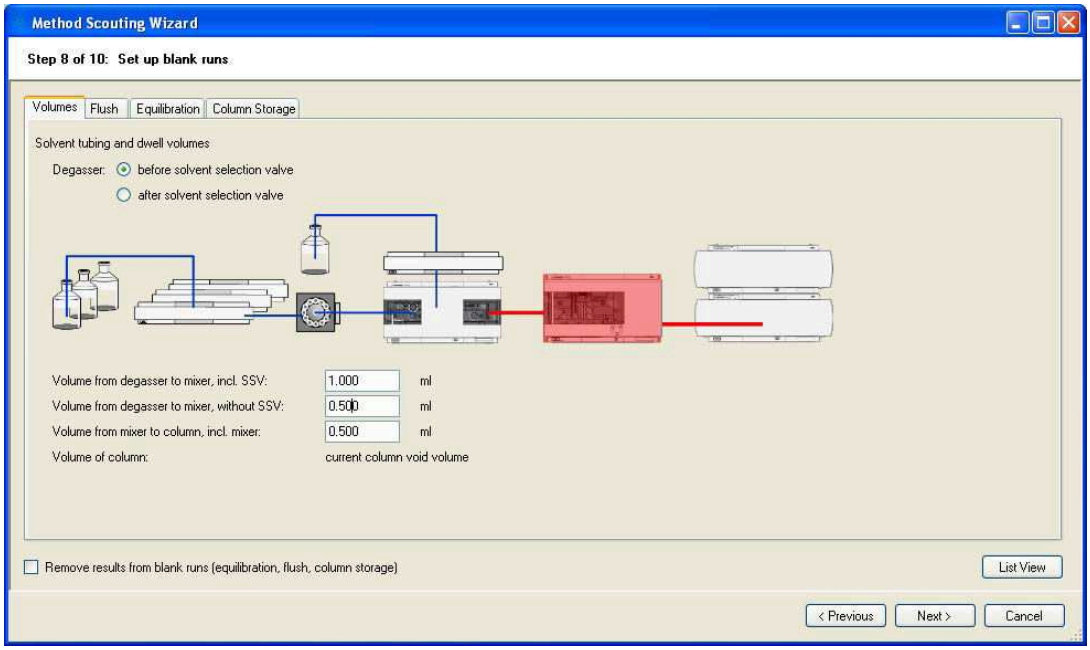


Figure 40 Setting of the volumes in the system

The following volumes are required:

- volume from degasser to mixing point of the pump (excluding the degasser chamber) - this volume will be flushed with 100% of the according solvent
- volume from the mixing point of the pump to the column inlet - this volume will be flushed with the composition of the following analytical method
- volume from solvent selection valve to degasser (including the degasser chamber) - this volume can be flushed with 100% of the according solvent

Table 9 gives you some guidelines for the volumes of different parts of a system:

Table 9 Typical internal volumes of parts that need to be flushed for a solvent change (note: all volumes are for low pressures such as when flushing into waste)

Part	Internal Volume (geometric)	Comment
G1322A degasser	12.0 mL	per chamber
G1379B micro degasser	1.0 mL	per chamber
Solvent tubing 1.5 mm ID	1.8 mL per meter	
G1312A binary pump	0.6 mL	
G1312B binary pump SL	0.12 mL	at low delay configuration
G1312B binary pump SL	0.32 mL	at low delay config. + small mixer
G1312B binary pump SL	0.6 mL	at std. delay configuration
G1311A quaternary pump	0.8 mL	ca. 0.2 mL before the mixing point, ca. 0.6 mL after the mixing point
Capillary 0.17mm ID	0.02 mL per meter	
Capillary 0.12mm ID	0.01 mL per meter	
G1329A autosampler	0.3 mL	in mainpass
G1367B/C autosampler	0.3 mL	in mainpass
G1367D autosampler	0.14 mL	in mainpass
G1367B/C/D autosampler	0.01 mL	in bypass

Example 1:

Degassing in front of the solvent selection valve, system with a binary pump SL in low delay volume configuration and G1367D autosampler in mainpass:

Volume A:	ca. 20 cm solvent tubing	=	**0.36 mL**
Volume B:	pump	=	0.12 mL
	Autosampler	=	0.14 mL
	Capillaries ca. 1.2 m 0.12mmID	=	0.013 mL
			0.27 mL

Example 2:

Degassing after the solvent selection valve with a G1322A degasser, system with a quaternary pump and a G1329A autosampler:

Volume A:	ca. 50 cm solvent tubing	=	0.9 mL
	Degasser chamber	=	12.0 mL
	Quat. pump before mixing point	=	0.2 mL
			13.1 mL
Volume B:	Quat. pump after mixing point		0.6 mL
	Autosampler		0.3 mL
	Capillaries ca. 1.2 m 0.17mmID	=	0.3 mL
			0.93 mL

It is obvious that the flushing times can vary significantly depending on the instrument set-up. In the first example, the flushing times for the volumes A and B are very similar, whereas in the second case they are totally different. In the second case, the overall volume is more than 20 times the volume in the first case. This means a 20-fold flushing time and a 20-fold solvent consumption.

In case more then one solvent line needs to be flushed, for example at initial solvent line flush at the very beginning of a campaign, the pump will be set to a composition that reflects the volume ratios of the different channels (e.g.

high percentage for a solvent that is delivered through an external solvent selection valve and a degasser after the SSV and low percentage for the remaining solvents that are directly attached to the degasser).

The void volume is usually taken from the column data base. In few special cases it can be necessary to enter the void volume of the column as well.

Flushing Conditions

If you have included solvent screening, or you want to store your columns with a storage solvent at the end of their usage, solvents have to be exchanged in the system. **It is highly recommended not to deselect the flushing option, as this can led to irreproducible results**. However, in a few cases, it might be appropriate to deselect this option; for example, when the volumes to be flushed are very low, high flow rates and equilibration conditions in the method would cover the times needed to flush the solvent delivery system.

First, it is necessary to select the flushing solvent (see Figure 41 on page 82). Usually, this is the solvent of the next method, but in some cases, it might be necessary to flush the system first with a neutral solvent followed by the solvent of the next method. Cases that require an intermediate neutral solvent might be immiscible solvents or any solvent combinations that might cause precipitation of buffers. If you fear such problems, you can select one of the available solvent from your system, and the complete system (including the column) is flushed first with this solvent and then with the solvent of the following analytical run or a solvent that was selected for a column care procedure (see Chapter 5, “Column Storage Procedures,” starting on page 86).

NOTE Note that additional flushing with a neutral solvent doubles the required flushing time.

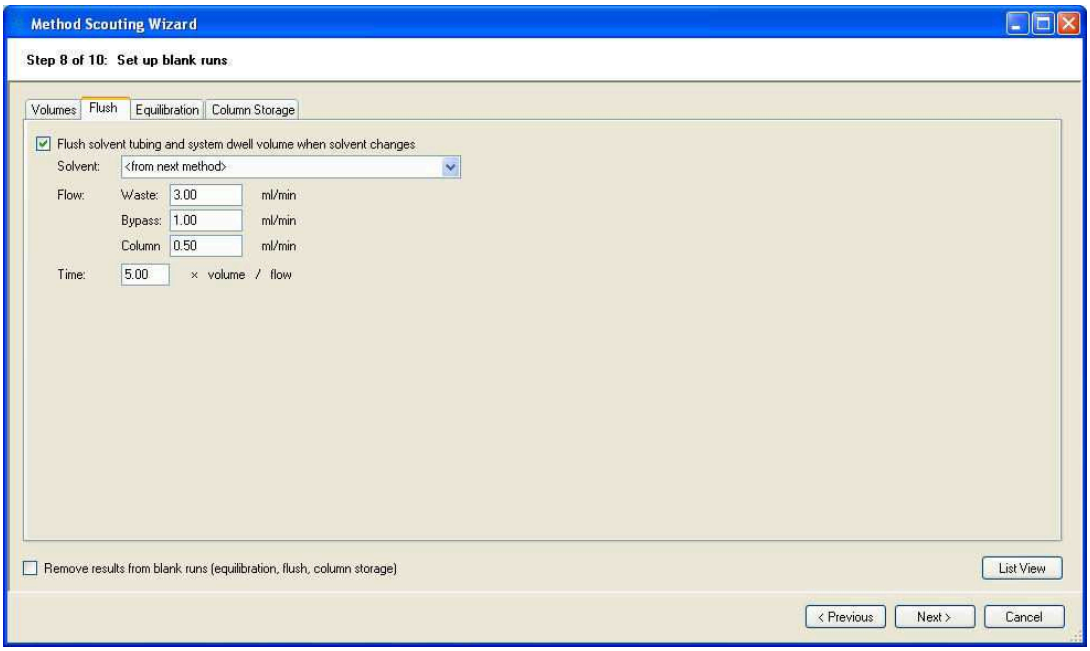


Figure 41 Column settings for flushing conditions

The settings for the flushing conditions depend on the availability of a waste and/or bypass line in the system. With a waste line, a much higher flow can be applied giving reduced flush time.

If only a bypass line is available, a lower flow rate as with a waste line might be considered, depending on the detector in use. For example, the backpressure generated at high flow rates by the flow cell might be too high for an FLD-detector, and some detectors such as mass spectrometers or ELSD detectors typically have maximum allowed flow rates in the range of 1 – 2 mL/min.

If no waste or bypass line is available the flow needs to go through the column. You must set an appropriate flow rate, taking into account **different viscosities and possible immiscibility of the new solvent with the solvent residing in the column**.

Finally, the number of flush volumes (n) needs to be entered. The calculated flush time is (n) times the flush volume divided by the flow rate. A value of at least 5 is advisable to achieve a thorough flushing.

If flushing is selected, the solvent lines used in the first analysis are flushed at the beginning of a sequence generated by the Agilent Method Scouting Wizard to ensure proper starting conditions.

Column Equilibration Procedure

NOTE The column equilibration procedure is applied after the change of a column, the change of a solvent, after the change of the temperature or the change of a gradient. It is not applied inbetween multiple uses of the same method for multiple injections or multiple samples or both.

If equilibration is selected, a column is treated with the conditions of the following analytical run for the specified time. The equilibration time can be set to a fixed value or can be calculated depending on the column void volumes. The column volume used for some of the calculations is taken from the column data base in ChemStation, and correct settings are assumed. To calculate the void volume of a column use the following equation:

$$V_m = \pi\left(\frac{d_c}{2}\right)^2 \times L_c \times \frac{\varepsilon_t}{1000}$$

V_m = column volume [mL]
d_c = internal diameter [mm]
L_c = column length in [mm]
ε_t = total porosity

Typically, the total porosity, which is the fraction of the column that is not taken up by the stationary phase and accessible to the mobile phase, is in the range of 0.6 – 0.8; for example, an Agilent Zorbax reversed phase column has a total porosity of ca 0.6. In this case, a minimum of 5 column void volumes should be exchanged to ensure a proper equilibration, but much higher values might be appropriate, depending on the type of columns used. Note: the value set here is used for all columns. If a certain column needs significantly longer equilibration times, you could deliberately set the value in the column data base to a higher value than the physical value to achieve a longer flushing time for this column.

NOTE The use of the column equilibration procedure is highly recommended.

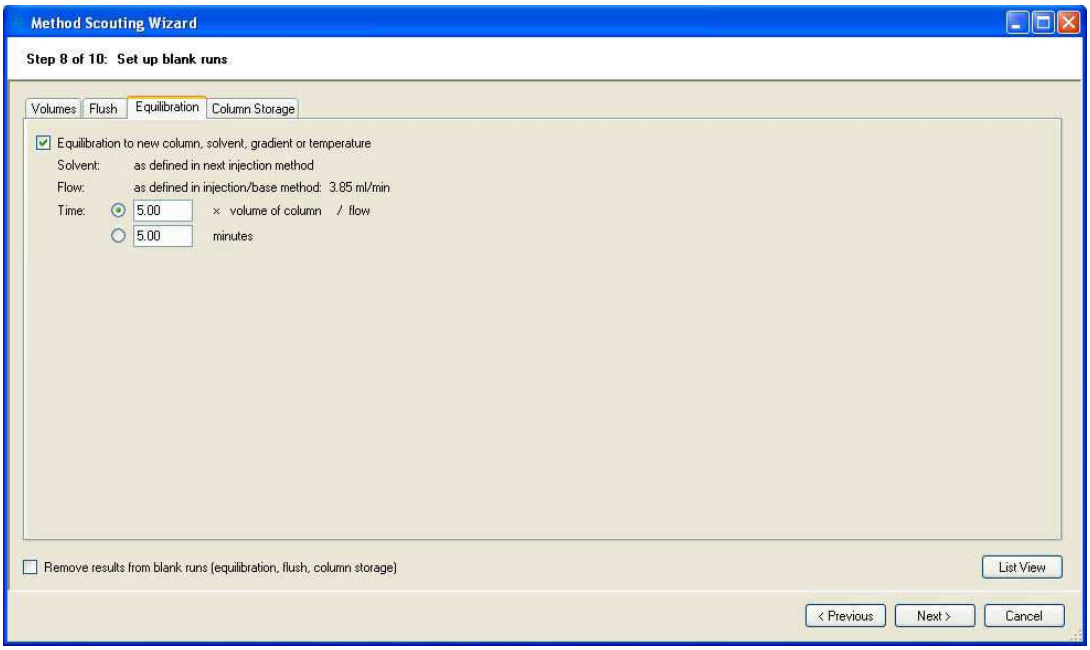


Figure 42 Settings for equilibration conditions

If column equilibration is not selected, it is recommended that the gradients used in the screening campaign contain an adequate equilibration time, and that the sequence starts with a blank sample. Otherwise, the first run will not have reproducible results. Also, if multiple injections are planned with the same method, either the method must contain a post-time or the gradient must include a programmed time for equilibration.

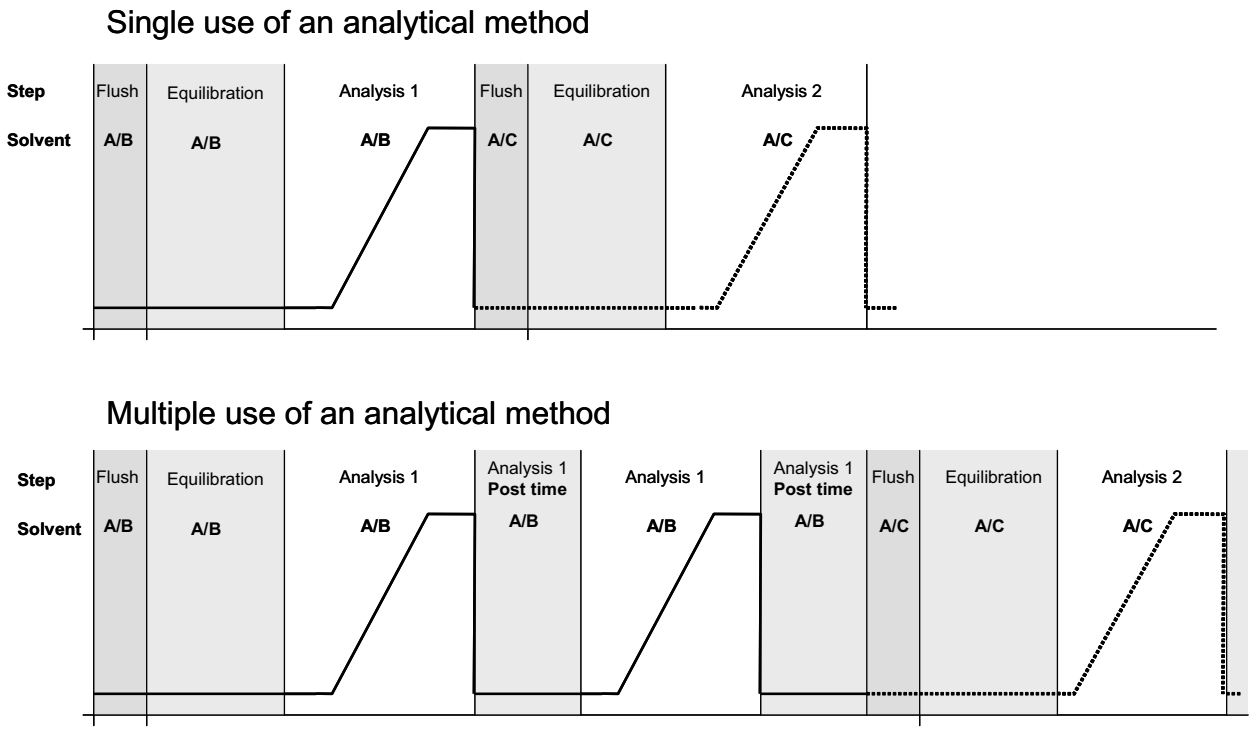


Figure 43 **Top:** an analytical method is used only once, then new conditions are applied. The Equilibration procedure ensures proper column equilibration under the new conditions.
Bottom: multiple injections or multiple samples are planned. Since the Equilibration procedure is applied only after a change of column, solvents, temperatures or gradient, the method must contain a post-time (or the gradient must include a programmed equilibration period) to ensure proper equilibration in-between the multiple analysis.

Column Storage Procedures

After a column has been used in an analysis, it might be advisable to flush the column with another solvent than the one used for the analysis. A typical example might be after analysis with high buffer concentrations Figure 44 on page 86. Different choices of flush solvents are available:

- the starting conditions of the current method, which might be of use if the gradient ends with a high concentration of organic solvent, for example,
- an additional care solvent provided on a separate channel of the solvent delivery system, which is probably the regular case

NOTE The flushing option should not be deselected.

You can make different settings for the flow rates and the flush times to ensure that a proper solvent exchange inside the column has occurred.

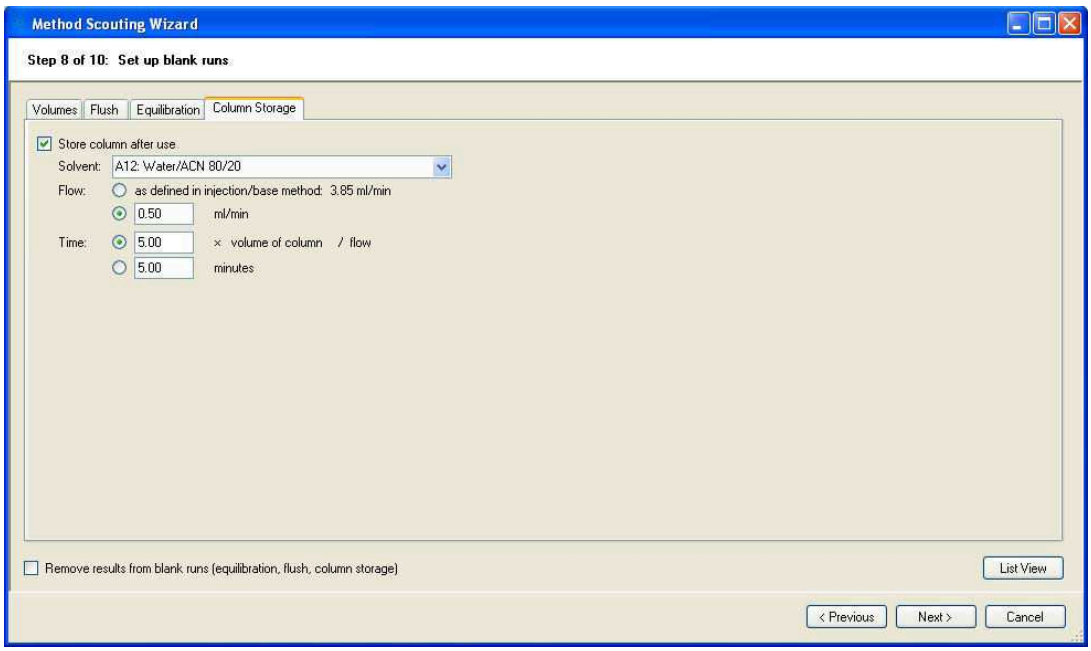


Figure 44 Settings for column storage conditions

Blank-Run Data File Handling

When this check box is marked, data files generated during flushing, column care or column equilibration runs are deleted automatically. The deletion of these data files is recommended in order to keep the amount of data generated low, but the files might be helpful for problem solving (for example, if compounds are eluted after the end of the analytical run because of improper gradient conditions).

Setting Up the Samples

The samples to be analyzed under the different method conditions are specified in this screen (see Figure 45 on page 89). At the top, you specify the total volume [µl] of a single vial (or well of a microtiter plate).

NOTE Only one sort of sample vial must be used.

In the central table, you enter a **sample name**, select the **vial-positions** in the graphical representation of the autosampler plates, and specify the individual **injection volume** and the **number of injections per sample** and number of repetitions. The total injection volume per sample and condition is calculated (repetitions x injection volume), the total sample volume for the campaign is also calculated (scouting conditions x repetitions x injection volume), and finally the number of required vials is calculated (scouting conditions x repetitions x injection volume / volume of a single vial).

NOTE For the number of required vials a safety margin of 10% of the required volume is taken into account.

If either the specified vial positions do not match the amount of vials required, or the vial positions of different samples overlap, a warning is displayed, and you cannot proceed until the faulty condition is cleared. You might also get a warning if the total number of sequence lines including all flushing, column storage and column equilibration methods exceeds 1999 lines.

More sample lines can be added by clicking on the **Add** button.

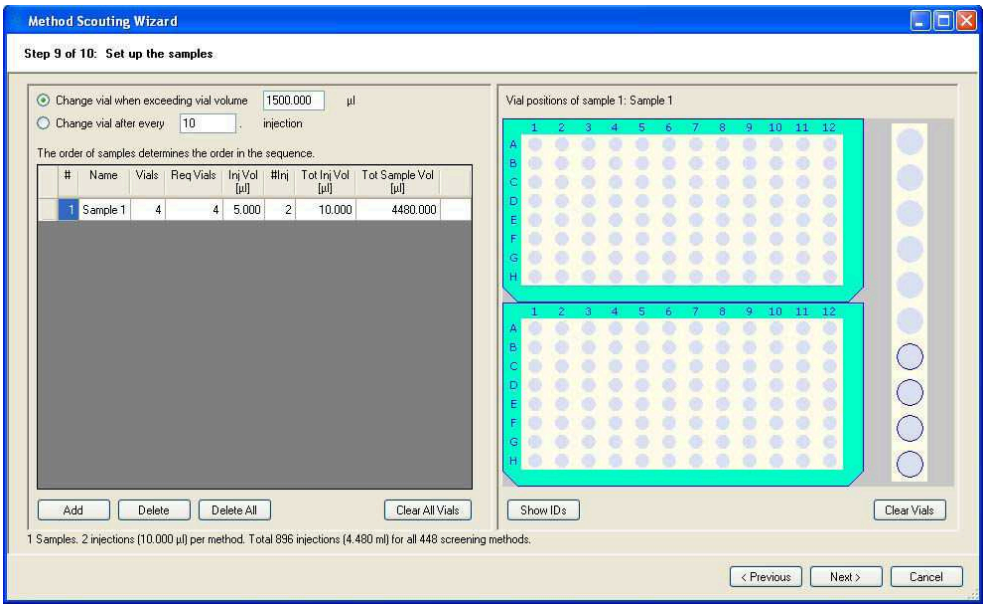


Figure 45 Set up the samples

Summary

The last screen of the wizard gives you detailed information on the specified method scouting campaign (see Figure 46 on page 91).

The **Description** tab gives general information:

- number of columns, solvents, gradients and temperatures
- name and path of the selected base method
- column storage and equilibration as well as flushing procedure
- sample names and required total sample volumes
- name and path of the sequence created

The **Sequence** tab shows details of the sequence that will be created.

- the complete sequence is shown including all flush, equilibration and column care lines (these arecolor coded)
- net run time (calculated without system overhead times)
- estimated run time (calculated with a general overhead factor plus a temperature-dependent factor that takes heating and cooling periods into account)
- number of equilibration, column storage and flush sequence lines
- number of column and solvent changes

The **Solvent Usage** tab gives an estimate of the required solvent volumes based on the type of solvent, the estimated run time for the specific solvent and the flow rate of the different methods using the specific solvent.

When you click the **Finish** button, the sequence is automatically set up according to the inputs given in the previous screens.

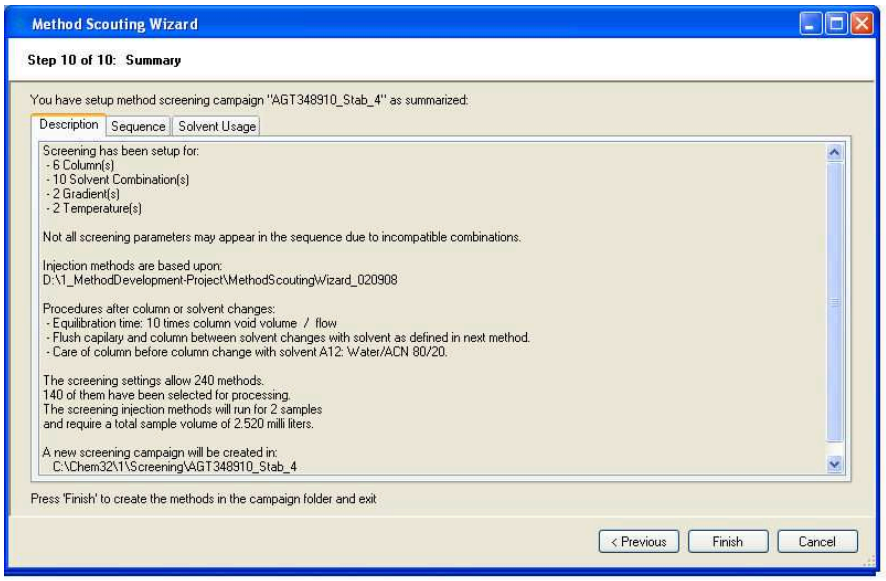


Figure 46 Summary screen

When you have completed the campaign set up, you can load and start the sequence. Open the Sequence menu, select **Load Sequence** and browse to the specified path for the campaigns. Highlight the appropriate campaign and press **OK**. The campaign is now loaded as a standard ChemStation sequence. You can specify the data directory (for example, as sub-directory in the campaign directory) by opening the **Sequence** menu and selecting **Sequence Parameter**; data-file naming conventions and sequence shutdown parameters can also be specified.

NOTE

To avoid negative effects on the results (for example caused by improper flushing or equilibration), take the utmost care when making changes to the sequence (for example, deleting sequence lines). It is advisable to make such changes in the Method Scouting Wizard.

Reporting

The Method Scouting Wizard automatically enters a detailed sample information field giving information about the column, the solvent combination, the gradient and the temperature used for the specific data file. All other standard report fields for sample information, method details etc. are available as usual.

6
Method Development Strategy

LC and LC/MS Columns Selection

Different classes of compounds require different separation mechanisms. The column selection guide in Figure 46 on page 95 allows you to find a suitable separation mechanism for a very broad range of chemical and biochemical compounds. To use the column selection guide, follow the path for your analyte and mobile phase.

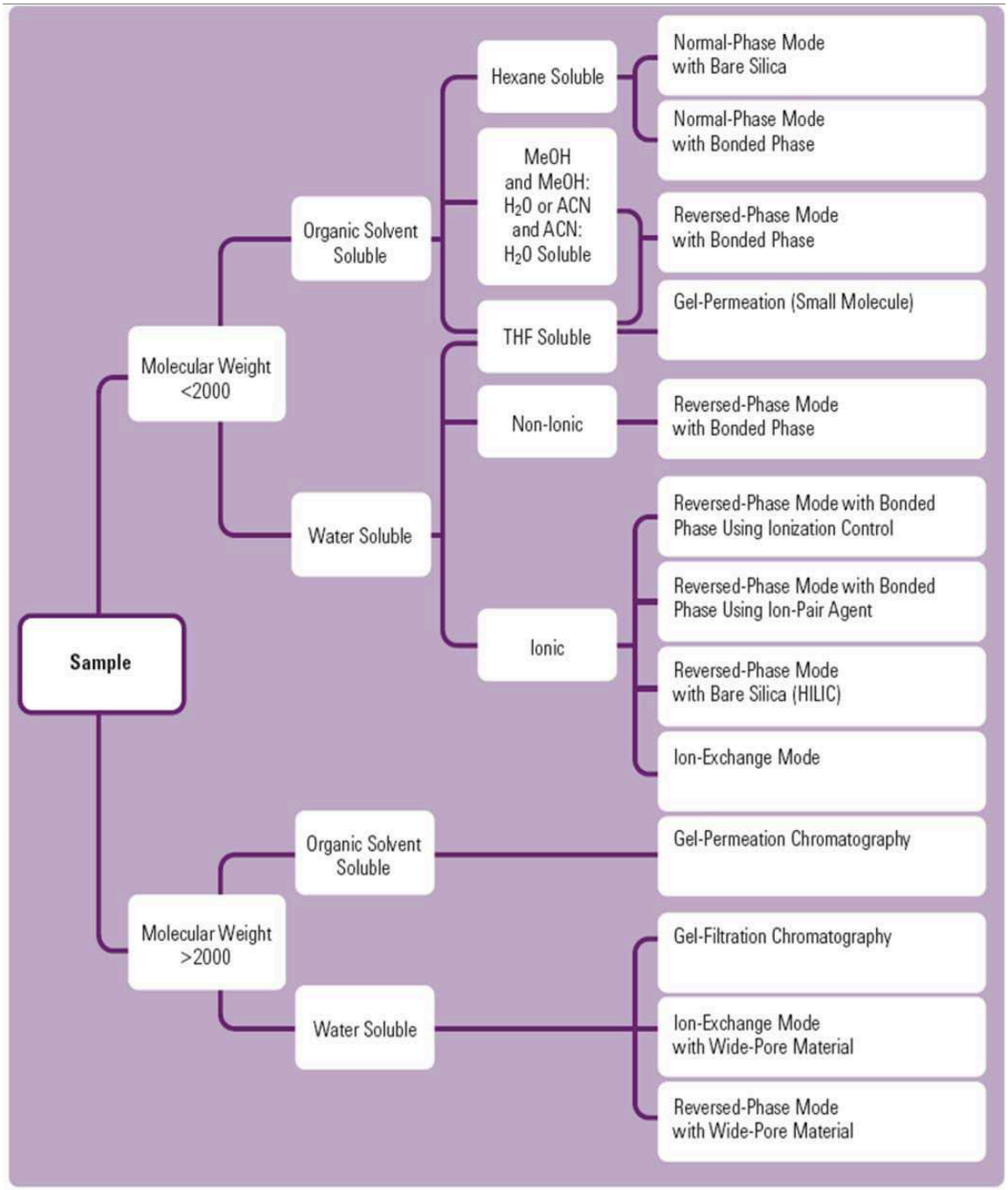


Figure 46 Find the right separation mechanism for a new sample.

HPLC columns consist of two parts, the column chemistry and the hardware. Both parts have a significant influence on the outcome of the final method, see Figure 47. Table 10 on page 96 gives you an idea for the optimal column internal diameter for your application. Note that the Agilent 1200 Series Method Development Solution offers optimized capillary kits for all application ranges.

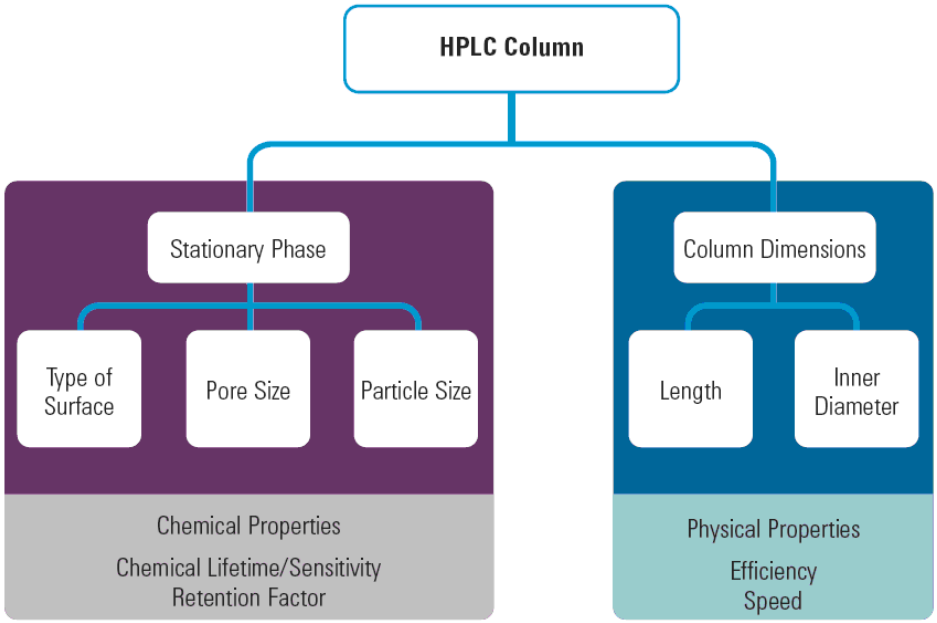


Figure 47 How column characteristics influence the separation

Table 10 Column choice relative to application objective

Application Objective	Column Diameter (mm)
Save solvent; special low-volume instrumentation is available	2.1
Special detectors, e.g., mass spec	2.1
High sensitivity, limited sample	2.1
Save solvent; standard HPLC equipment available, LC/MS	3.0
Standard separations	4.6

Pore Size Selection

If the solute molecular weight is less than about 5000 Da, choose a column packing with small pore size (60-120Å). Otherwise, use column packing with 300Å pore size.

Particle Size Selection

The standard particle size for HPLC columns is still 5 µm, with 3.5 µm now dominant for method development. If high-speed analyses or higher resolution analyses are required, packings with 1.8 µm and 3.5 µm particles are recommended. Shorter columns with these particles can produce faster highresolution separations, with the 1.8 µm particle size in Rapid Resolution HT columns providing the highest efficiency. The 3.5 µm particle size operates at a routine operating pressure and can be used on all LCs. Columns with 1.8 µm particle size can be used on optimized standard LCs if they are short (50 mm and below) and/or have internal diameters of at least 3.0 mm, while the longer and narrower columns usually require a higher pressure LC (one supporting pressures greater than 400 bar) such as the Agilent 1200 Series Rapid Resolution LC.

Silica Type and Bonded Phase

Agilent ZORBAX reversed phase columns use three different types of porous silica microspheres, the original ZORBAX SIL, ZORBAX Rx-SIL and modified ZORBAX Rx-SIL. ZORBAX Rx-SIL and modified ZORBAX Rx-SIL are highly purified and less acidic than the original ZORBAX SIL. Less acidic silica means less potential for interaction between the analyte and silanol groups on the silica surface, especially if the solutes are basic, and contributes to improved peak shape. For new method development, we strongly recommend using reversed-phase products based on modified ZORBAX Rx-SIL (Eclipse Plus) and ZORBAX Rx-SIL (Eclipse, StableBond etc.).

Bonded Phase

A good first choice for bonded phase is C18 or C8. If the sample solutes of interest are not adequately separated on these columns, CN and Phenyl columns may offer significant differences in selectivity from the straight-chain alkyl phases to effect the separation. In general, larger solutes, such as proteins, are best separated on short-chain reversed-phase columns (C3, CN) and peptides and small molecules are separated on longer-chain columns (C8, C18). There are many cases, however, where this conventional wisdom does not apply. For example, peptides can also be effectively separated using short-chain columns, and hydrophobic peptides can show better recovery on longer-chain phases. Therefore, it is best to initially select a phase in the middle of the hydrophobic spectrum (e.g., C8), then change to a more hydrophobic phase or more hydrophilic phase depending on initial results and solubility properties of your sample.

Table 11 Quick Guide to ZORBAX Reversed-Phase Bonded Phases - recommended uses and applications. Gray-shaded stationary phases are used in the method development column kits optionally available with an Agilent 1200 Series Method Development System.

Eclipse Plus	• Excellent first choice for method development • Different selectivity choices for flexible method development • Long life from pH 2-9 for reliable separations of basic, acidic and neutral compounds • Superior peak shape with basic compounds • Rigorous QA/QC testing for greater long-term reproducibility
Eclipse XDB	• Four selectivity choices for flexible method development • High performance over a wide pH range, pH 2-9 • Good peak shape for acids, bases and neutrals • Long lifetime with extra dense bonding and double endcapping
StableBond (SB)	• Basic, acidic, neutral compounds • Exceptional stability at low pH • Use of high temperature (up to 90°C for C18, 80°C for C8, C3, Phenyl, CN, and Aq) and • low pH as an added selectivity tool • Widest selection of bonded phases for different selectivity (C18, C8, C3, CN, Phenyl, Aq) • Uses mobile phases for LC/MS with formic acid, acetic acid, or TFA • Uses mobile phases with TFA for peptide and protein separation
ZORBAX Rx	• General separation of basic, acidic and neutral compounds at low pH with different selectivity than SB columns • Rx-C8 is the same as SB-C8
Bonus-RP	• Separating basic compounds in higher aqueous mobile phases • General separation of basic, neutral, acidic compounds at mid-range pH or low pH; • especially stable at low pH • Separating peptides for different selectivity
Extend-C18	• Separating basic compounds above their pKa in free base form; separation of basic, acidic, neutral compounds at high pH; up to pH 11.5 • Uses ammonium hydroxide as mobile phase additive with LC/MS with small molecules or peptides • Separating at high, mid-range and low pH for selectivity changes

pH and Mobile Phase

The choice of mobile phase for a reversed-phase system starts with the selection of the organic modifier. Selectivity differences and sample retention vary significantly among mobile phases containing acetonitrile, methanol, and tetrahydrofuran (THF). Sample solubility is likely to differ in such solvents and dictates the use of a specific solvent or solvents. UV detection at certain wavelengths is not possible with certain modifiers (e.g., methanol at 200 nm). Both pH and ionic strength of the aqueous portion of mobile phases are important parameters in developing rugged methods that are not sensitive to small variations in conditions. With ionic compounds, retention of typical species shows significant changes with pH. It is very important to control pH in such reversed-phase systems to stabilize retention and band spacing. A pH set between 2 and 4 generally provides the most stable conditions for retention vs. small changes in pH and this pH is recommended for starting method development for most samples, including basic compounds and typical weak acids.

Method Development from pH 1-12

Chromatographic resolution between two or more peaks depends upon three factors: column efficiency, selectivity, and retention. With ionizable analytes – bases and acids – all of these factors change dramatically with pH. For example, retention can be improved by changing the separation pH, so that analytes are separated in their non-ionized form. Changes in mobile phase pH also improve column efficiency because the ionization of the analyte and the residual silanols can both be altered. This minimizes secondary interactions between analytes and the silica surface that cause poor peak shape. Achieving optimum resolution can also require changing the mobile phase pH. The following method development strategy explains how this is done with superior column lifetime.

Low, mid, and high pH are the three general regions for chromatographic separations as shown in Figure 48 on page 101. This figure highlights the benefits of performing separations of ionizable analytes in each pH region. Method development proceeds by investigating chromatographic separations first at low pH and then at higher pH until optimum results are achieved. The ideal column is available for each pH region.

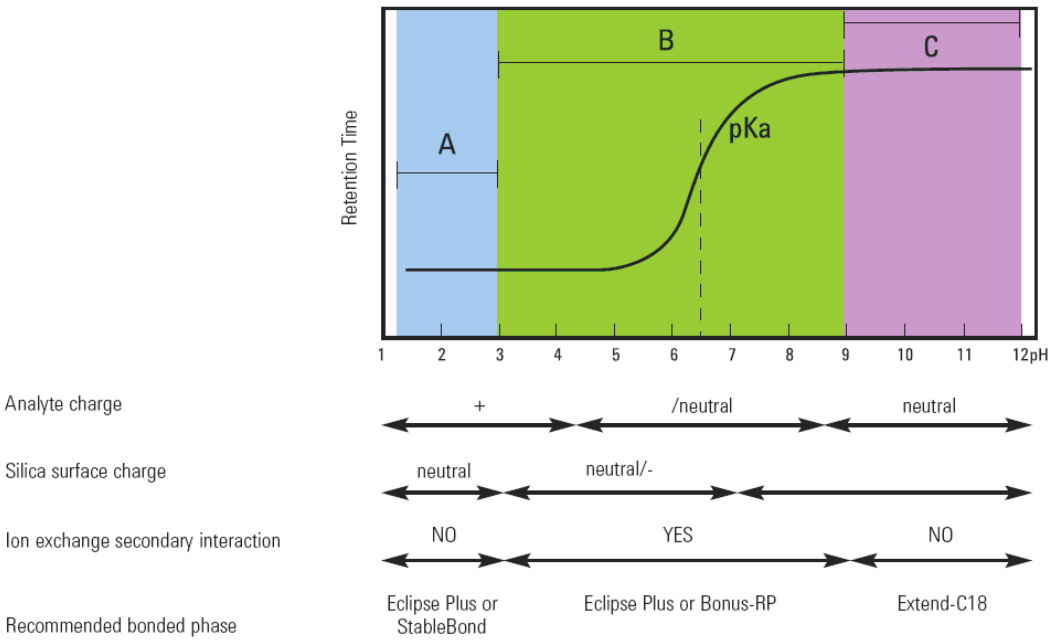


Figure 48 Three pH Regions for HPLC Separations of Basic Compounds. This figure represents the retention behavior of one basic analyte with respect to pKa and pH. Analyte pKa is 6.5.

Table 12 Characteristics of the three pH-regions

Low pH < 3 – Region A	Mid pH 7 – Region B	High pH > 9 – Region C
• Start method development at low pH, where silanols on a RPHPLC column are protonated. This minimizes peak tailing by eliminating silanol/base interactions. • At low pH, basic compounds are positively charged and their retention may be reduced. • Acidic compounds may be protonated and have increased retention. • Retention times are usually stable with small changes in pH, producing a robust method. • Volatile mobile phase additives, such as formic acid or trifluoroacetic acid (TFA), are often used at low pH with LC/MS.	• Develop methods at pHs at least 1 pH unit above or below the pKa to minimize changes in retention with small changes in pH. • Some silica surface SiOH groups become SiO^- above pH 4 to 5; tailing interactions may be possible. • Minimize interactions by selecting a well-designed endcapped column, using additives such as TEA (triethylamine) (less desirable) or using "polar-linked" bonded phases. • Silica breakdown is prevented by innovative bonding chemistry, heavy endcapping, and use of Rx-SIL.	• In this region, basic compounds may be in their free base form. • Increased retention and resolution of basic compounds are likely. • Retention changes little in this region, thus robust methods can be developed. • Silica breakdown is prevented by innovative bidentate column chemistry, heavy endcapping, use of Rx-SIL, and optimum mobile phase. • Ammonium hydroxide is an excellent volatile mobile phase modifier at high pH.

Start Method Development at low pH (pH 2-3)

With so many column choices available, how do you know where to start your method development? The recommended starting point for method development is to use a buffered low pH mobile phase – around pH 2-3. Using a low pH mobile phase most often results in the best peak shape for basic compounds on silica-based columns. At low pH, the silanols on the silica are fully protonated so positively charged basic compounds do not interact strongly. The result is good peak shape. Many acidic compounds are noncharged, maximizing their retention at low pH. These observations are key advantages to method development at low pH. For standard analytical work, start method development with acetonitrile as the mobile phase organic modifier and 20-50 mM phosphate buffer (pH 2-3) as the aqueous component for non-LC/MS applications. These conditions provide good pH control, necessary for the most reproducible analyses of ionizable compounds. For LC/MS applications formic acid or TFA are good mobile phase additives for low pH.

Choose Agilent ZORBAX Eclipse Plus First for Best Peak Shape

Select ZORBAX Eclipse Plus C18 or C8 columns first for method development at low pH. Eclipse Plus columns are the newest addition to the Eclipse family and use improved silica and bonding technologies to provide good peak shape for basic compounds. Eclipse Plus columns can be used from pH 2-9, providing flexibility for method development. They are stable down to pH 2 making them an ideal choice for initial method development.

Optimize Solvents and Bonded Phases at low pH

The initial method development steps may lead very quickly to a satisfactory separation. But if more optimization is needed, acetonitrile can be replaced by methanol or tetrahydrofuran and the separation reoptimized. This step may lead to a satisfactory solution, but if further optimization of selectivity is needed, the column bonded phase can be changed.

At low pH there are many bonded phase choices available for optimization. These include the Eclipse Plus phases as well as the Eclipse XDB family with C18, C8, Phenyl and CN. Alternate choices include six different StableBond bonded phases: SB-C18, SB-C8, SB-Phenyl, SB-CN, SB-C3, and SB-Aq. It may be necessary at low pH to improve the retention of acidic compounds. For these situations, lower the pH even further, down to pH 1-2, and use StableBond columns. These columns provide the greatest stability at very low pH and provide many selectivity options for achieving the highest resolution separations.

Method Development at mid pH (4-9) - Agilent ZORBAX Eclipse Plus

There are some samples that may not be resolved at low pH or may have better solubility and stability at mid pH. The Eclipse Plus C18 column can be used for method development in the mid pH range. The Eclipse Plus column is stable up to pH 9 so it is equally reliable at mid pH. These double endcapped columns have two key advantages: good peak shape at low and mid pH, and sufficient bonded phase density to protect the column from silica degradation from pH 6-9.

At mid pH, basic compounds (e.g., amines) may still have a positive charge, and the silanols on the silica surface may have a negative charge. Therefore, the best peak shape at mid pH is achieved when as many silanols as possible are covered. This makes the Eclipse Plus C18 the best starting choice for a column at mid pH. Phosphate buffer is usually the first choice for mobile phase modifier at pH 7 because its buffer range is pH 6.1-8.1. A second choice for mid pH is acetate buffer, since it buffers from pH 3.8-5.8 and its volatility makes it a good choice for LC/MS compatibility.

Alternate Selectivities – Agilent ZORBAX Eclipse Plus Phenyl-Hexyl, Eclipse XDB-Phenyl, CN and Bonus-RP

The method development process at mid pH mimics the process at low pH with optimization of the organic modifier and selecting an alternate bonded phase if resolution is not achieved after that step. The alternate bonded phases at mid pH are the Eclipse Plus Phenyl-Hexyl, Eclipse XDB-Phenyl, Eclipse XDB-CN and Bonus-RP. They provide very different selectivities for many samples and the method development process is followed again. The Bonus-RP column has a polar embedded amide group that provides different selectivity for many samples, provides good peak shape for basic compounds and allows the column to be used with up to 100% aqueous mobile phases.

Method Development at high pH (pH 9-12) - choose Agilent ZORBAX Extend-C18 Columns

At low or mid pH, some separations of basic compounds may still not have enough retention or the desired selectivity. For these samples, high pH separations may be appropriate. Until recently, high pH separations on silica-based columns were avoided because of short column lifetimes, due to dissolution of the underlying silica gel. Newer column technologies, such as the ZORBAX Extend-C18, can protect the silica from dissolution, so that a reasonable column lifetime can be achieved and the selectivity advantages of high pH can be explored.

The mobile phase buffer choices at high pH with the Extend-C18 column are organic buffers such as triethylamine and ammonium hydroxide. These buffers are best used with methanol as the organic modifier to extend the column lifetime at high pH.

Available Column Kits

As a starting point for method development six different column kits, each with three columns, are available as options to the G4230A or B Valve Kits (for the low pressure valve kit G4230A only four column kits are available). You have the choice between RRHT and RR columns, different internal diameters and different application focuses.

Method Development Column Kits Based on Changing Selectivity

Columns included are: Eclipse Plus **C18**, Eclipse Plus **Phenyl-Hexyl** and **Bonus-RP (polar alkyl amide)** These three columns provide dramatic differences in selectivity, and can alter the retention order and overall retention of acidic and basic compounds as well as aromatic and non-aromatic compounds, that is, the typical sample.

Method Development Column Kits Based on pH Variation

Columns included are: **Eclipse Plus C18, SB-C18** and **Extend-C18. These three C18** columns provide differences in selectivity and follow the Agilent recommended method development process for screening different pH-values. The recommended starting point is pH 2-3 with an **Eclipse Plus C18** column. This covers pH 2-9. The StableBond-**C18** is ideal for going below pH 2 and using a non-endcapped column for alternate selectivity whereas the **Extend-C18** is ideal for evaluating method selectivity above pH 9.

Table 13 Available method development column kits for the Agilent 1200 Series Method Development Solution

Part Number	Kit and column description
5190-1431	**RRHT Selectivity Met Dev Kit,2.1mm ID x 50 mm, 1.8 µm**
959741-902	• Eclipse Plus C18,2.1 x 50 mm,1.8 µm,600 bar
959741-912	• Eclipse Plus Ph-Hex, 2.1 x 50 µm,1.8 µm,600 bar
827768-901	• Bonus-RP, 2.1 x 50 mm, 1.8 µm, 600 bar
5190-1432	**RRHT pH Meth Dev Kit,2.1 mm ID x 50 mm, 1.8 µm**
959741-902	• Eclipse Plus C18,2.1 x 50 mm,1.8 µm,600 bar
827700-902	• SB-C18, 2.1 x 50 mm,1.8 µm,600 bar
727700-902	• Extend-C18, 2.1 x 50 mm,1.8 µm,600 bar
5190-1433	**RRHT Selectivity Met Dev Kit,4.6mm ID x 50 mm, 1.8 µm**
959941-902	• Eclipse Plus C18,4.6 x 50 mm, 1.8 µm, 600 bar
959941-912	• Eclipse Plus Ph-Hex, 4.6 x 50 mm, 1.8 µm, 600 bar
827668-901	• Bonus-RP, 4.6 x 50 mm, 1.8 µm, 600 bar
5190-1434	**RRHT pH Meth Dev Kit,4.6mm ID x 50 mm, 1.8 µm**
959941-902	• Eclipse Plus C18, 4.6 x 50mm, 1.8 µm,600 bar
827975-902	• SB-C18, 4.6 x 50 mm, 1.8 µm, 600 bar
727975-902	• Extend-C18,4.6 x 50mm, 1.8 µm, 600 bar
5190-1435	**Rapid Resolution Selectivity Met Dev Kit, 4.6mm ID x 100mm, 3.5 µm**
959961-902	• Eclipse Plus C18, 4.6 x 100 mm, 3.5 µm
959961-912	• Eclipse Plus Phenyl-Hexyl, 4.6 x 100 mm, 3.5 µm
864668-901	• Zorbax Bonus-RP, 3.5 µm, 4.6 x 100 mm
5190-1436	**Rapid Resolution pH Meth Dev Kit, 4.6mm ID x 100mm, 3.5 µm**
959961-902	• Eclipse Plus C18, 4.6 x 100mm, 3.5 µm
861953-902	• SB-C18 Rapid Res, 4.6 x 100mm, 3.5 µm
764953-902	• Zorbax Extend C18, 3.5 µm, 4.6 x 100 mm

In Figure 49 the above said is summarized in an easy to follow workflow.

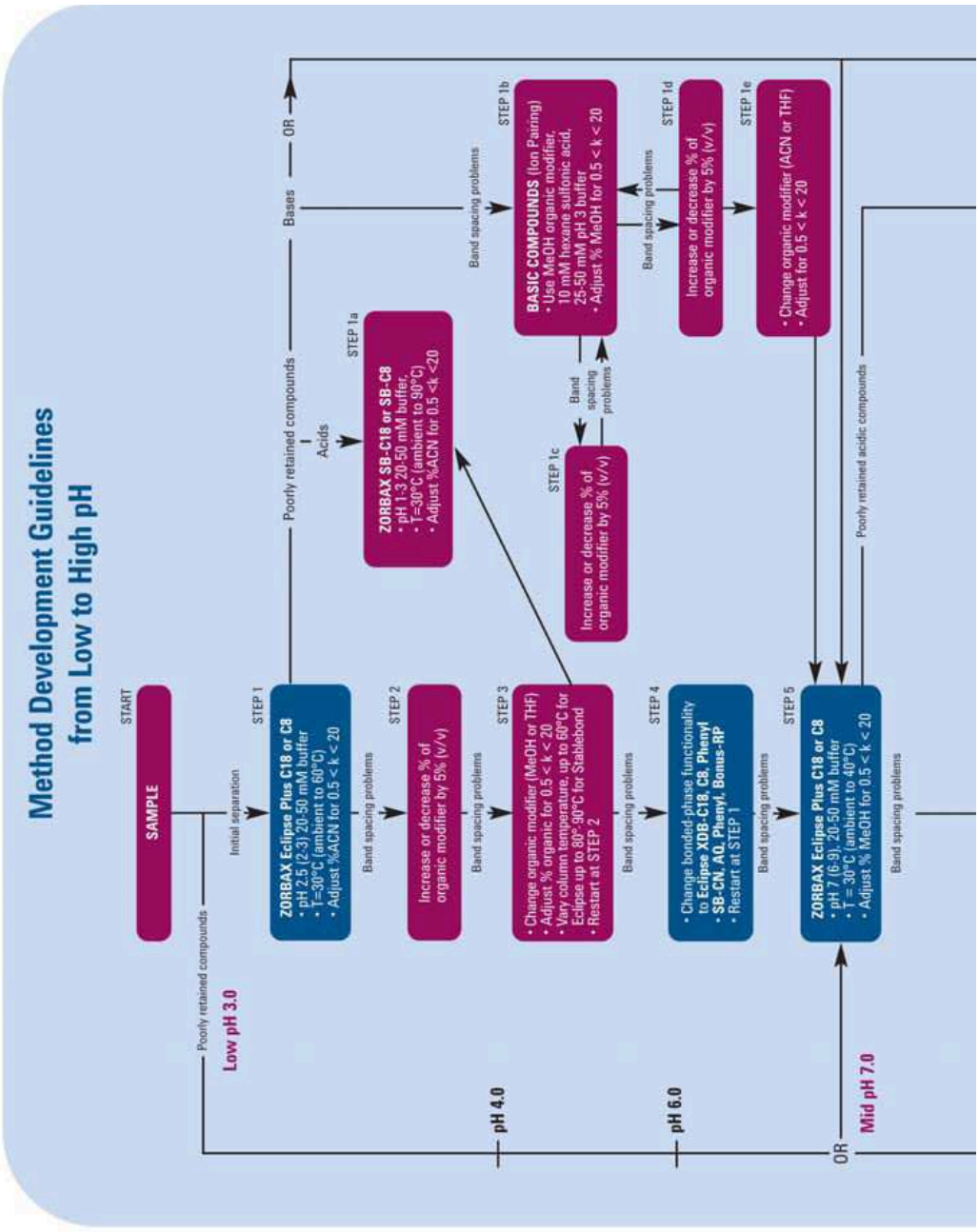
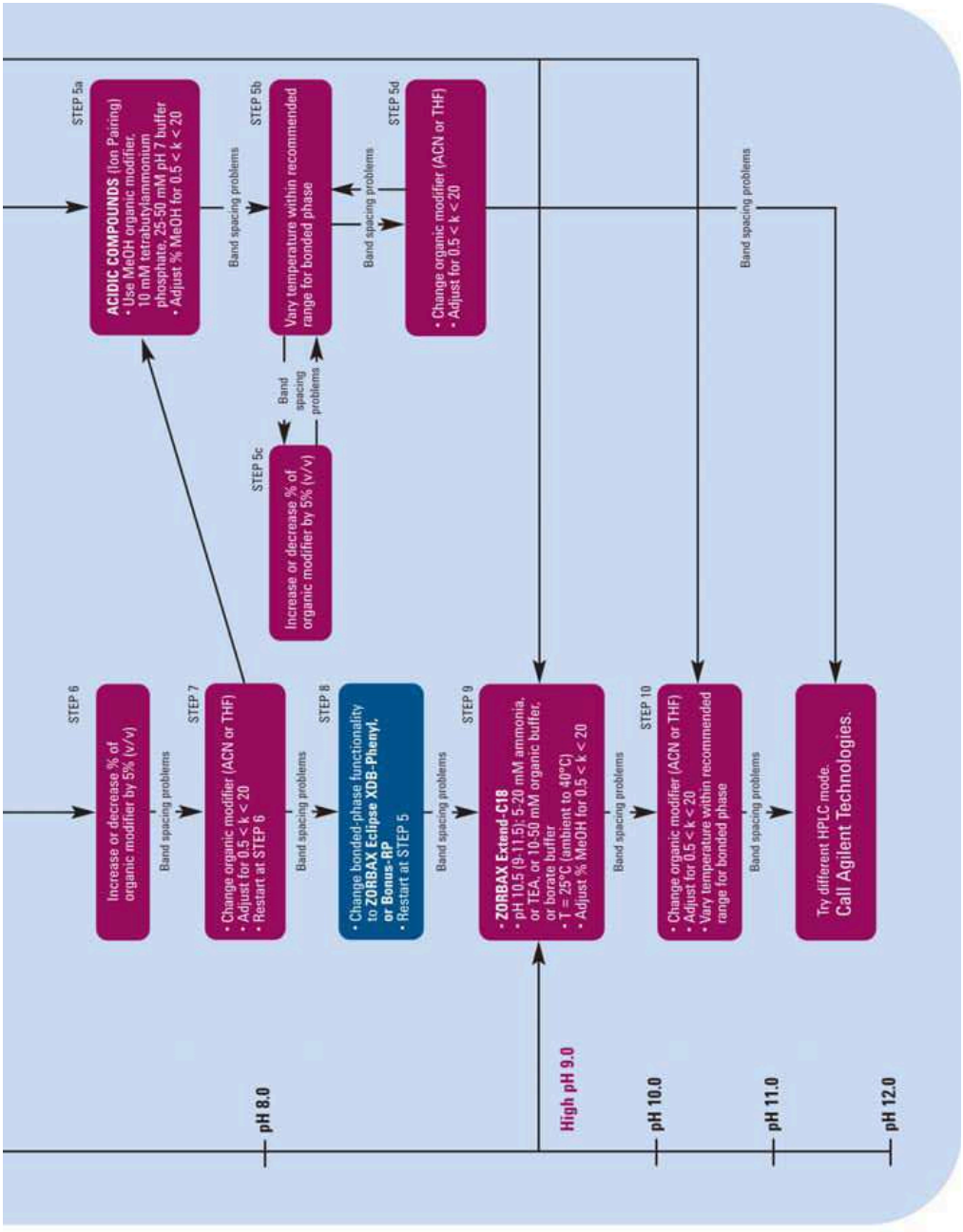


Figure 49 Recommended method development workflow


STEP 5a
ACIDIC COMPOUNDS (Ion Pairing)
• Use MeOH organic modifier.
10 mM tetrabutylammonium phosphate, 25-50 mM pH 7 buffer
• Adjust % MeOH for 0.5 < k < 20
Band spacing problems
STEP 5b
Vary temperature within recommended range for bonded phase
Band spacing problems
STEP 5d
• Change organic modifier (ACN or THF)
• Adjust for 0.5 < k < 20
Band spacing problems
Band spacing problems
STEP 5c
Increase or decrease % of organic modifier by 5% (v/v)
STEP 6
Increase or decrease % of organic modifier by 5% (v/v)
Band spacing problems
STEP 7
• Change organic modifier (ACN or THF)
• Adjust for 0.5 < k < 20
• Restart at STEP 6
Band spacing problems
STEP 8
• Change bonded-phase functionality to ZORBAX Eclipse XDB-Phenyl, or Bonus-RP
• Restart at STEP 5
Band spacing problems
STEP 9
• ZORBAX Extend-C18
• pH 10.5 (9-11.5); 5-20 mM ammonia, or TEA, or 10-50 mM organic buffer, or borate buffer
• T = 25°C (ambient to 40°C)
• Adjust % MeOH for 0.5 < k < 20
Band spacing problems
STEP 10
• Change organic modifier (ACN or THF)
• Adjust for 0.5 < k < 20
• Vary temperature within recommended range for bonded phase
Band spacing problems
Try different HPLC mode.
Call Agilent Technologies.
pH 8.0
High pH 9.0
pH 10.0
pH 11.0
pH 12.0

6 Method Development Strategy

A
Appendix

A Appendix

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.

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.

The Agilent 1200 Series modules are designed and certified as a general purpose laboratory instrument for research and routine application only. It is not certified for in-vitro or medical applications.

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.

CAUTION

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.

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

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

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

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

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

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

Safety Symbols

Table 14 shows safety symbols used on the instrument and in the manuals.

Table 14 Safety Symbols

Symbol	Description
	The apparatus is marked with this symbol when the user should refer to the instruction manual in order to protect the apparatus against damage.
	Indicates dangerous voltages.
	Indicates a protected ground terminal.

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.

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

Abstract

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

NOTE

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

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

Do not dispose off in domestic household waste

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

Lithium Batteries Information

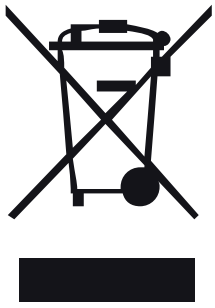
WARNING **Danger of explosion if battery is incorrectly replaced. Replace only with the same or equivalent type recommended by the equipment manufacturer. Lithium batteries may not be disposed-off into the domestic waste.**

Transportation of discharged Lithium batteries through carriers regulated by IATA/ICAO, ADR, RID, IMDG is not allowed. Discharged Lithium batteries shall be disposed off locally according to national waste disposal regulations for batteries.

WARNING **Lithiumbatteri - Eksplosionsfare ved fejlagtig 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)

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It will also provide the latest firmware of the Agilent 1200 Series modules for download.

A Appendix

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