Agilent InfinityLab LC Method Development Solutions

User Guide

Notices

Document Information

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In This Guide...

This manual covers the InfinityLab LC Method Development Solutions, for example the 1260 Infinity III and 1290 Infinity III LC method development solutions. An LC method development solution consists of the corresponding hardware setup and the Method Scouting Wizard.

1 Prerequisites for the LC Method Development Solution

This chapter gives information on prerequisites for the LC Method Development Solution.

2 The Method Development System

This chapter gives information on hardware system components, column and solvent switching.

3 System Setup and Installation

This chapter provides information on system setup, installation of valve heads, heat exchangers and capillaries, and installation of solvent selection parts.

4 Configuring the System in ChemStation and Creating Methods

This chapter explains how to configure the system in the control software and how to create methods.

5 The Agilent ChemStation Method Scouting Wizard

This chapter provides information on installation, use and features of the software.

6 Method Development Strategy

This chapter provides information on method development strategy, concerning LC and LC/MS columns selection, pH and mobile phase.

7 Appendix

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

Contents

1 Prerequisites for the LC Method Development Solution 6

Prerequisites for the LC Method Development Solution 7

2 The Method Development System 9

Overview 10
The Agilent 1290 Infinity III Method Development System 15
The Agilent 1260 Infinity III Method Development System 21
The Classic LC Method Development System 26

3 System Setup and Installation 36

Documentation of the Individual Modules 37
System Setup 38
Configurations and Capillary Setup 41
Recommended Column Configurations 45
Installing the Solvent Selection Part 47

4 Configuring the System in ChemStation and Creating Methods 52

Clustering of Modules in ChemStation using the RC.NET Drivers 53
Settings for Column Compartments 62
Settings for Solvent Selection 67

5 The Agilent ChemStation Method Scouting Wizard 70

Overview of the Agilent Method Scouting Wizard 71
Software Installation 76
Defining the Campaign 77
Define Screening Campaign Base 79
Selecting the Columns 81
Selecting the Solvents 83
Specifying and Selecting Gradients 87

Defining and Selecting the Temperatures 92
Review the Selected Methods 94
Settings for System Volumes, Flushing, Equilibration, Column Storage and Advanced 96
Setting Up the Samples 111
Summary 113
Reporting 115

6 Method Development Strategy 117

Further Information 118

7 Appendix 119

General Safety Information 120
Agilent Technologies on Internet 126

1 Prerequisites for the LC Method Development Solution

Prerequisites for the LC Method Development Solution 7

This chapter gives information on prerequisites for the LC Method Development Solution.

Prerequisites for the LC Method Development Solution

High Pressure Liquid Chromatography (HPLC) allows the efficient separation of compounds and therefore is a key technology in pharmacology and chemistry. The interaction of analyte molecules with so-called stationary and mobile phases determines the compound separation. Developing the optimal method for different compounds can be quite demanding. There are almost endless possible combinations of crucial parameters like solvent type, column type, temperature, and gradient.

The InfinityLab LC Method Development Solutions (Meth Dev) allows simple generation of a sequence that varies all crucial method parameters systematically. The Meth Dev is based on a skilled soft- and hardware configuration.

Hardware Configuration

The system consists of a 1260 Infinity II/III or 1290 Infinity II/III LC with the following options:

- Single Multicolumn Thermostat (MCT) solution with one MCT module.
- A valve thermostat cluster (VTC) with multiple valve hosts (Multicolumn Thermostats, Valve Drives, Thermostatted Column Compartments) and temperature zones (Multicolumn Thermostats, Thermostatted Column Compartments, Integrated Column Compartments) (1290 Infinity II/III only).

(For a list of all supported modules see Table 2 on page 15.)

Functions of the hardware:

- Solvent delivery that is combined with external solvent selection:

  Variation of liquid phase and generation of gradients
- Column compartment:

  Determination of temperature
- Column selection:

  Variation of stationary phase

Software Configuration

The ChemStation Method Scouting Wizard A.02.10 Update 3 is an Add-on for OpenLab ChemStation.

The Method Scouting Wizard automatically generates all steps to flush the system with any required solvents, performs column equilibration procedures, and defines storage conditions for columns in predifined 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. The Method Scouting Wizard requires the following software components:

- OpenLab ChemStation LTS 01.11 Update 3

NOTE The Method Scouting Wizard compatibility matrix information can also be found in the readme file on the installation media.

NOTE Not supported: Secure Workstation and Distributed AIC

- LC & CE Drivers 3.7

NOTE Make sure that driver version 3.7 is installed:

- Check the installed driver version with your OpenLab ChemStation LTS 01.11 Update 3.
- Upgrade or downgrade if the driver version is different.

For details on the possible hardware configurations, see Table 2 on page 15.

For details on the software requirements, see "Overview of the Agilent Method Scouting Wizard" on page 71 and see Table 6 on page 75.

NOTE If you upgrade modules to this latest firmware release (firmware set), assure that *all* modules in the system are upgraded to latest version!

2 The Method Development System

Overview 10
Overview on Available Valves for the Method Development System 10
Solvent Delivery 11
External Solvent Selection 12
Solvent Switching 12
Sample Introduction 13
Column Compartments 13
Column Selection 13
Sample Detection and Analysis 14
Capillary Kits 14
The Agilent 1290 Infinity III Method Development System 15
Description of the Agilent 1290 Infinity III Method Development System 15
Solvent Switching 16
Column Selection 17
The Agilent 1260 Infinity III Method Development System 21
Description of the Agilent 1260 Infinity III Method Development System 21
Solvent Switching 22
Column Selection 23
The Classic LC Method Development System 26
Description of the Classic Method Development System 26
Solvent Switching 27
Column Selection 31

This chapter gives information on hardware system components, column and solvent switching.

Overview

Overview on Available Valves for the Method Development System

Table 1 Valve overview for the Method Development System

Valve type	Maximum pressure (bar)	Order number	G1316C	G7116A	G7116B	G1170A
2-Position/6-Port	600	G5631A	✓	✓	✓	✓
	800	G4231A	✓	✓	✓	✓
	1300	G4231C	✓		✓	✓
4-Position/10-Port	600	G5639A	✓	✓	✓	✓
	800	G4237A	✓	✓	✓	✓
6-Position/14-Port	800	G4234A	✓	✓	✓	✓
	1300	G4234C	✓		✓	✓
	1300	G5640A	✓		✓	✓
8-Position/18-Port	1300	G4239C	✓		✓	✓
8-Position/9-Port	600	G4230A[1]	✓		✓	✓
	1200	G4230B[1]	✓		✓	✓

[1] A cluster of two

Solvent Delivery

Solvent delivery

For increased solvent selection capability pumps can additionally be equipped with external solvent selection valves. This extends the number of available solvents on one pump channel to a maximum of 12. See Figure 1 on page 11 as an example.

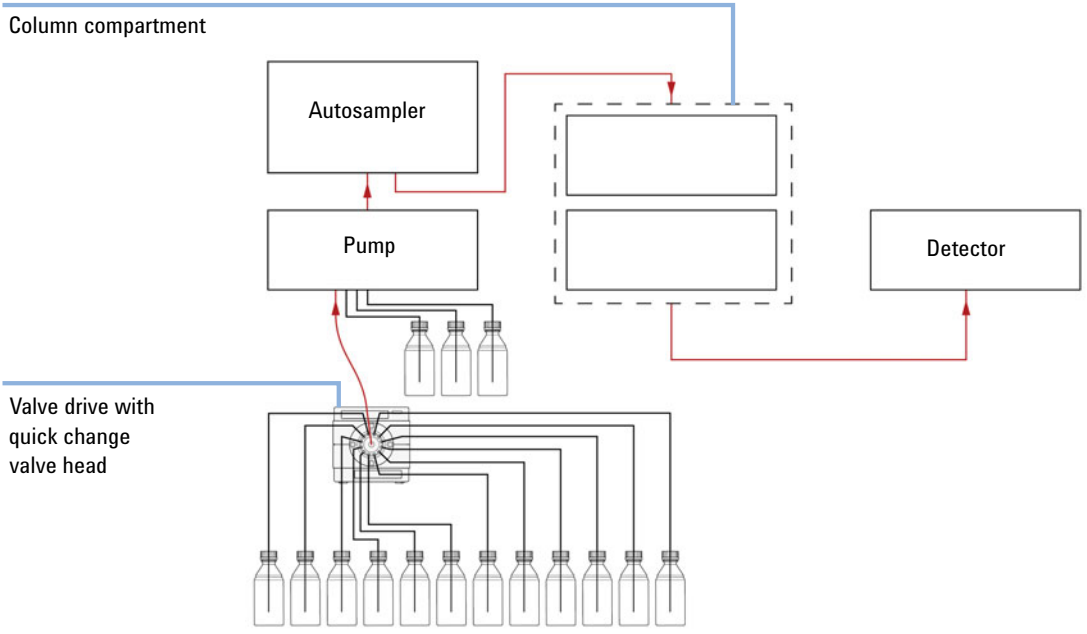


Figure 1 **Method development system principle with external solvent solution**

External Solvent Selection

External solvent selection

The external solvent selection valve consists of a 1290 Valve Drive (G1170A) in combination with a G4235A 12-Pos/13-Port Solvent Selection Valve Head.

NOTE

If two external solvent selection valves are configured, they must both be connected either before or after the degasser. Connection of one valve on either side of the degasser is not supported.

For further details and examples refer to chapter *System Setup and Installation*.

Solvent Switching

In addition to the two or four solvents directly supported by the pump, up to two ports of the pump can be connected to an external Valve Drive (G1170A) equipped with a 12-position/13-port solvent selection valve head (G4235A). This valve is able to switch between up to 12 solvents giving a total of up to 26 solvents on the Method Development System.

An overview of the components required for solvent selection depends on the system:

- Agilent InfinityLab LC Series Systems:
  - "Components for Solvent Selection (Agilent Infinity-II/III LC)" on page 16
- Classic Systems:
  - "Components for Solvent Selection (Agilent 1290 Infinity LC)" on page 27
  - "Components for Solvent Selection (Agilent 1260 Infinity LC)" on page 29

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 Agilent 1260 Infinity Binary Pumps the built-in solvent selection option is recommended.

For the selection of the degasser and the number of degassers in a system, refer to "Deciding on the Position of the Degasser" on page 48.

Sample Introduction

Sample introduction

Any of the Autosamplers supported by LC&CE Drivers 3.7 can be used (see Table 2 on page 15). Select your autosampler suitable to the required maximum pressure capability of the system.

Column Compartments

Column compartments

For the temperature zones there is a great variety of possibilities. Up to eight short (or four long or four short and two long...) columns can be used with one 1290 Infinity II/III Multicolumn Thermostat (G7116B) (single MCT). With a valve thermostat cluster (VTC) of up to four 1290 Infinity II/III Multicolumn Thermostat (G7116B) modules it is possible to use up to 32 columns. In addition to that, it is possible to combine a multicolumn thermostat with other components such as ICC (G7130A).

For further details and examples refer to chapter *System Setup and Installation*.

Column Selection

Column selection

Several valve hosts like G7116A (MCT), G7116B (MCT) or G1170A (1290 Valve Drive) can be used for column selection. For the compatible valves heads/valve kits see Table 2 on page 15.

Sample Detection and Analysis

Sample detection and analysis

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, for example chiral detectors, can be used by acquiring the signal via an analog to digital converter.

Capillary Kits

For use with MCT the following capillary kits are available:

- Capillary Kit, 0.12 mm, 8-column sel, short columns, PC-HEx (5067-4248) for 8-column selector with Quick-Connect heat exchangers (standard flow), see "Capillary Kit for 8 Columns in one 1290 Infinity II/III MCT" on page 17.
- Capillary Kit, 0.12 mm, 8-column sel, long columns, PC-HEx (5067-6697) to cluster two MCTs, if long columns are used (see "Capillary Kit for 8 Columns >150 mm in two 1290 Infinity II/III MCTs" on page 19).
- Cap kit 0.12 mm, 4-col, incl. QC-HE (5067-6596) for 4 columns in one 1260 Infinity II/III MCT (see "Capillary Kits for 4 Columns in one 1260 Infinity II/III MCT" on page 24).

For use with TCC the following capillary kits are available:

- Cap Kit 0.12 mm LDHE double 8/9 vlv short col (5067-6220), see "Low Dispersion Capillary Kit for Short Columns" on page 33
- Cap Kit 0.12 mm LDHE double 8/9 vlv long col (5067-4289), see "Low Dispersion Capillary Kit for Long Columns" on page 35
- Cap Kit 0.17 mm 8/9 multi purpose (5067-4290), see "General Purpose Capillary Kit" on page 34

The capillary kit that is used depends on the columns to be installed.

All kits, and all components of the kits, can be ordered separately as consumables.

NOTE For more details, refer to the technical information of the corresponding Agilent Valve Kit.

The Agilent 1290 Infinity III Method Development System

Description of the Agilent 1290 Infinity III Method Development System

The system components of the Agilent 1290 Infinity III Method Development System are listed in Table 2 on page 15.

Table 2 Agilent 1290 Infinity III Method Development System Components

Function	Module	Description	Comment
Solvent delivery	G7104A	1290 Flexible Pump 1300 bar	Equipped with built-in degasser
	G7131A	1290 Bio Flexible Pump 1300 bar	Equipped with built-in degasser
	G7120A	1290 High Speed Pump 1300 bar	Equipped with built-in degasser
	G7132A	1290 Bio High-Speed Pump 1300 bar	Equipped with built-in degasser
External solvent selection	G1170A	1290 Valve Drive	
	G4235A	12-Pos/13-Port Solvent Selection Valve Head 210 bar	Additional tubing kits required (Solvent selection tubing kit, 4 solvents (5067-4601))
Sample introduction	G7129B	1290 Vialsampler	
	G7167B	1290 Multisampler	Including dual needle option
	G7137A	1290 Bio Multisampler	
Column compartment	G7116B	1290 Multicolumn Thermostat	
	G7130A	1290 Integrated Column Compartment	Used with G7129B
Column selection	For suitable valves, see Table 1 on page 10.		

Solvent Switching

Components for Solvent Selection (Agilent Infinity-II/III LC)

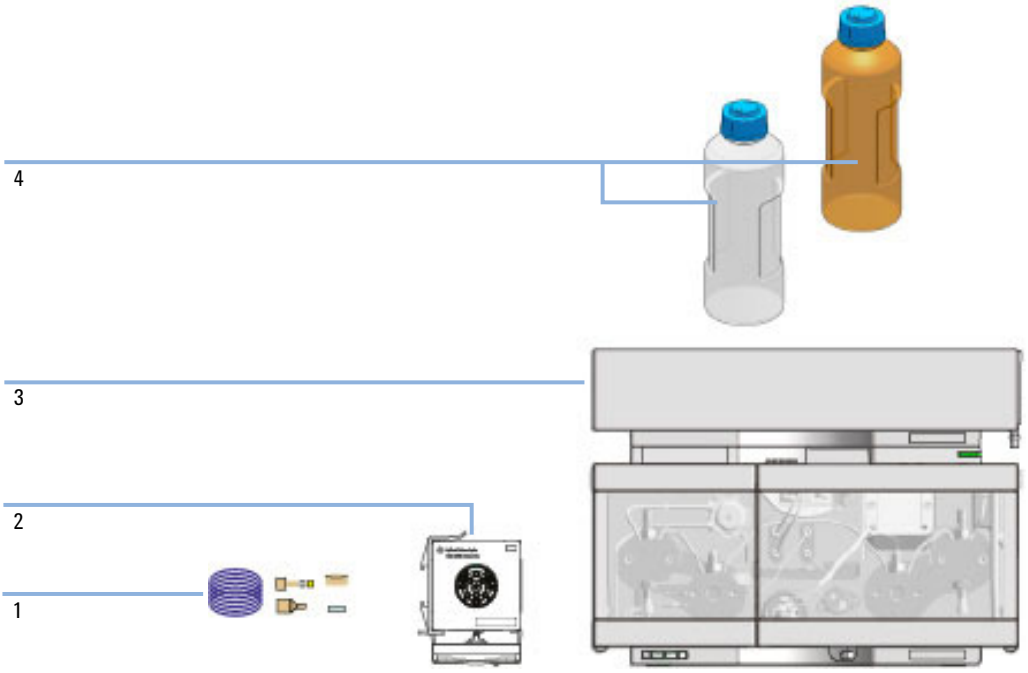


Figure 2 Components for Solvent Selection (Agilent Infinity-II/III LC)

Item	p/n	Description
1	5067-4601	Solvent selection tubing kit, 4 solvents
	G4235A	12-position/13-port solvent selection valve, bio-inert, 210 bar
2	G1170A	1290 Valve Drive in combination with 12pos/13port valve, bio-inert (G4235A)
3	5067-5760	InfinityLab LC Series Solvent Cabinet
4	9301-6526	Solvent bottle, amber, 1000 mL
4	9301-6524	Solvent bottle, clear, 1000 mL

NOTE

One solvent cabinet and bottles are included with the pump.

Column Selection

Column Selection with one G7116B (single MCT)

Column Selection with one G7116B (single MCT)

To perform automated column switching with the Method Development System, one 1290 Infinity II/III Multicolumn Thermostat equipped with a column selector valve head is required. For a 8 column selection valve (G4239C), 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 110 °C, depending on the flow rate.

Capillary Kit for 8 Columns in one 1290 Infinity II/III MCT

Capillary Kit, 0.12 mm, 8-column sel, short columns, PC-HEx (5067-4248) for 8-column selector with Quick-Connect heat exchangers (standard flow):

p/n	Description
G7116-60015	Quick Connect Heat Exchanger Standard
5500-1199	Capillary ST 0.12 mm x 130 mm M4-SL PS-PS
5500-1200	Quick Turn Capillary ST 0.12 mm x 130 mm SL/M
5500-1201	Capillary ST 0.12 mm x 105 mm SL
G1314-68703	Capillary fitting kit special
5500-1202	Capillary ST 0.12 mm x 500 mm M4-SL PS-PS
5500-1203	Capillary ST 0.12 mm x 280 mm M4-SL PS-PS
5500-1204	Capillary ST 0.12 mm x 150 mm M4-M4 PS-PS
G1375-87326	Waste tube, FEP, 1.6 mm od, 0.8 mm id
5023-2504	Hex driver SW-4 slitted
5043-0915	Fitting mounting tool
5067-6141	M4 Blank nut
5063-6591	PEEK Fittings 10/PK
G4239-90002	8 Column Selector Valve Kit Instructions
5067-6654	Number Kit 1-8 colored

Column Selection in a Valve Thermostat Cluster (VTC)

Column Selection in a Valve Thermostat Cluster (VTC)

A valve thermostat cluster (VTC) is a powerful solution to automatically switch between up to 32 columns in a system. Different topologies allow multiple combinations of valve hosts (Multicolumn Thermostats, Valve Drives, Thermostatted Column Compartments) and temperature zones (Multicolumn Thermostats, Thermostatted Column Compartments, Integrated Column Compartments). The recommended VTC topologies are described in the following.

NOTE Agilent 1260 Infinity II/III Multicolumn Thermostat (G7116A) does not support clustering!

A Valve Thermostat Cluster (VTC) for long columns

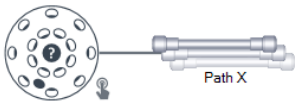


Figure 3 Topology 1: VC

This most simple VTC topology is needed for clustering one valve with a column host (one or two Column Compartments). With two MCT it is possible to use up to 8 long columns (or any combinations of long and short columns).

It is also possible to use this topology with a valve in an external valve drive and an ICC.

Capillary Kit for 8 Columns >150 mm in two 1290 Infinity II/III MCTs

Capillary Kit, 0.12 mm, 8-column sel, long columns, PC-HEx (5067-6697):

p/n	Description
5023-2504	Hex driver SW-4 slitted
5500-1201	Capillary ST 0.12 mm x 105 mm SL
G1314-68703	Capillary fitting kit special
5067-6141	M4 Blank nut
5500-1286	Capillary ST 0.12 mm x 340 mm SL/M 16
5500-1272	Capillary ST 0.12 mm x 800 mm M4-SL 1
5500-1203	Capillary ST 0.12 mm x 280 mm M4-SL PS-PS
5500-1204	Capillary ST 0.12 mm x 150 mm M4-M4 PS-PS
G7116-60015	Quick Connect Heat Exchanger Standard
G1375-87326	Waste tube, FEP, 1.6 mm od, 0.8 mm id
5062-8541	Fingertight fitting long, 10/pk
G4239-90002	8 Column Selector Valve Kit Instructions
5067-6654	Number Kit 1-8 colored

A Valve Thermostat Cluster (VTC) for up to 32 Columns

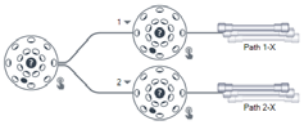


Figure 4 **Topology 2: V(VC)2**

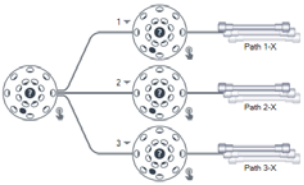


Figure 5 **Topology 3: V(VC)3**

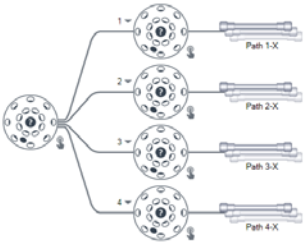


Figure 6 **Topology 4: V(VC)4**

In the valve thermostat cluster, the described VTC solution (see "A Valve Thermostat Cluster (VTC) for long columns" on page 19) can be multiplicated up to four times. These branched topologies are typically realized via an external valve and some MCT modules, each equipped with an 8-column selection valve.

NOTE

The number of branches is fix. If a topology with four branches is selected, four valves have to be configured in the branches - plus one branching valve (root valve).

The Agilent 1260 Infinity III Method Development System

Description of the Agilent 1260 Infinity III Method Development System

The system components of the Agilent 1260 Infinity III Method Development System are listed in

Table 3 Agilent 1260 Infinity III Method Development System Components

Function	Module	Description	Comment
Solvent delivery	G7111A	1260 Quaternary Pump VL 400 bar	
	G7111B	1260 Quaternary Pump 600 bar	Equipped with built-in degasser
	G7112B	1260 Binary Pump 600 bar	Equipped with built-in degasser
	G4782A	1260 Binary SFC Pump	Requires external degasser
	G5654A	1260 Bio-inert Pump	Equipped with built-in degasser
	G7104C	1260 Flexible Pump 800 bar	Equipped with built-in degasser
	G7131C	1260 Bio Flexible Pump 800 bar	Equipped with built-in degasser
External solvent selection	G1170A	1290 Valve Drive	
	G4235A	12-Pos/13-Port Solvent Selection Valve Head	Additional tubing kits required (e.g. 5067-4601 Solvent selection tubing kit, 4 solvents)
Sample introduction	G7129A	1260 Vialsampler	
	G7167A	1260 Multisampler	Including dual needle option
	G4767A	1260 SFC Multisampler	
	G5668A	1260 Bio-inert Multisampler	
Column compartment	G7116A	1260 Multicolumn Thermostat	Exchangeable valve heads up to 4 positions and up to 800 bar
	G7130A	1290 Integrated Column Compartment	Used with G7129A
Column selection	For suitable valves, see Table 1 on page 10.		

Solvent Switching

Components for Solvent Selection (Agilent Infinity-II/III LC)

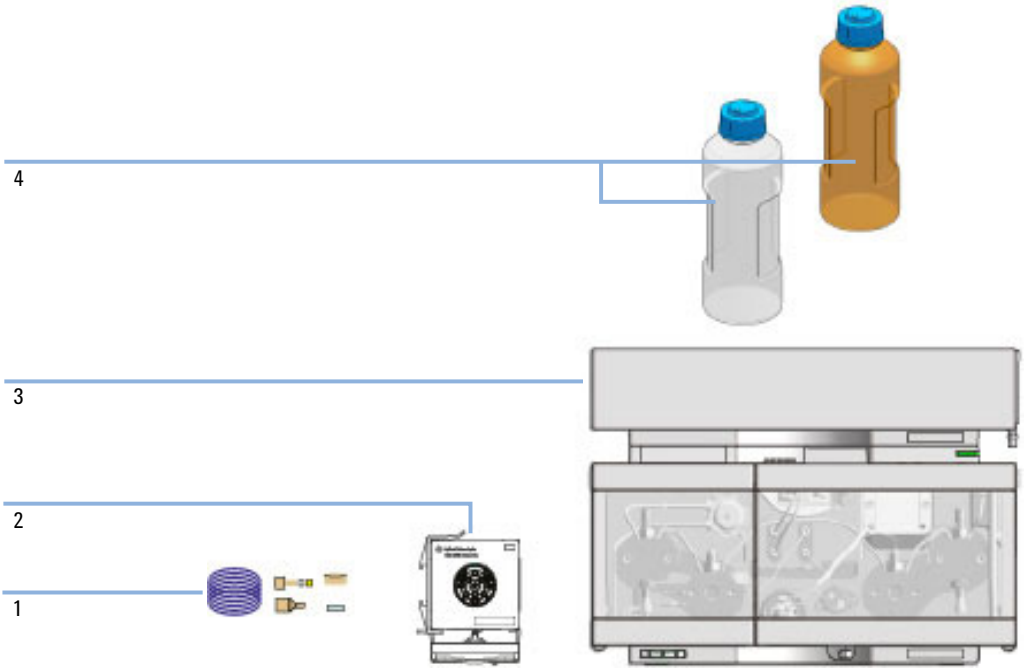


Figure 7 Components for Solvent Selection (Agilent Infinity-II/III LC)

Item	p/n	Description
1	5067-4601	Solvent selection tubing kit, 4 solvents
	G4235A	12-position/13-port solvent selection valve, bio-inert, 210 bar
2	G1170A	1290 Valve Drive in combination with 12pos/13port valve, bio-inert (G4235A)
3	5067-5760	InfinityLab LC Series Solvent Cabinet
4	9301-6526	Solvent bottle, amber, 1000 mL
4	9301-6524	Solvent bottle, clear, 1000 mL

NOTE One solvent cabinet and bottles are included with the pump.

Column Selection

Column Selection with one G7116A (single MCT)

To perform automated column switching Method Development System, one 1260 Infinity II/III Multicolumn Thermostat equipped with a 4-column selector valve head is required. A maximum of four positions are available to connect columns, bypass, or waste lines.

p/n	Description
G4237A	4 column selector valve head, 800 bar
G5639A	4 column selector valve kit, bio-inert, 600 bar

NOTE

Agilent 1260 Infinity II/III Multicolumn Thermostat (G7116A) does not support clustering!

Therefore no valve thermostat cluster (VTC) is possible.

Capillary Kits for 4 Columns in one 1260 Infinity II/III MCT

Cap kit 0.12 mm, 4-col, incl. QC-HE (5067-6596):

p/n	Description
G7116-60015	Quick Connect Heat Exchanger Standard
5500-1202	Capillary ST 0.12 mm x 500 mm M4-SL PS-PS
5500-1199	Capillary ST 0.12 mm x 130 mm M4-SL PS-PS
5500-1200	Quick Turn Capillary ST 0.12 mm x 130 mm SL/M
5063-6591	PEEK Fittings 10/PK
5500-1201	Capillary ST 0.12 mm x 105 mm SL
5500-1203	Capillary ST 0.12 mm x 280 mm M4-SL PS-PS
5500-1204	Capillary ST 0.12 mm x 150 mm M4-M4 PS-PS
G1314-68703	Capillary fitting kit special
5023-2504	Hex driver SW-4 slitted
5067-6141	M4 Blank nut
G1375-87326	Waste tube, FEP, 1.6 mm od, 0.8 mm id
G4237-90010	Valve Kit Tech Note ENG
5067-6654	Number Kit 1-8 colored

Cap kit 0.17 mm, 4-col, incl. QC-HE (5067-4300):

p/n	Description
G7116-60051	Quick Connect Heat Exchanger Large ID
5067-6188	Capillary ST 0.17 mm x 500 mm SL-M
5067-5109	Capillary ST 0.17 mm x 90 mm SL/M
5067-4746	Capillary ST 0.12 mm x 250 mm SLV/M
5500-1200	Quick Turn Capillary ST 0.12 mm x 130 mm SL/M
0100-1516	Fitting, PK, 1/16" x 10-32, SH, male nut, 2/pk
5500-1193	InfinityLab Quick Turn Capillary ST 0.17 mm x 105 mm, long socket
5500-1203	Capillary ST 0.12 mm x 280 mm M4-SL PS-PS
5500-1204	Capillary ST 0.12 mm x 150 mm M4-M4 PS-PS
5065-4454	Fitting screw long, front and back ferrules 10/pk
5023-2504	Hex driver SW-4 slitted
5067-6141	M4 Blank nut
G1375-87326	Waste tube, FEP, 1.6 mm od, 0.8 mm id
G4237-90010	Valve Kit Tech Note ENG
G1314-68703	Capillary fitting kit special
5067-6654	Number Kit 1-8 colored

The Classic LC Method Development System

Description of the Classic Method Development System

The system components of the Classic Method Development System are listed in

Function	Module	Description	Comment
Solvent delivery	G1311B	1260 Infinity Quaternary Pump, 600 bar	Equipped with built-in degasser
	G1312B	1260 Infinity Binary Pump, 600 bar	Requires external degasser
	G4204A	1290 Infinity Quaternary Pump, 1200 bar	Equipped with built-in degasser
	G4220A	1290 Infinity Binary Pump, 1200 bar	Equipped with built-in degasser
	G4220B	1290 Infinity Binary Pump VL	Equipped with built-in degasser
	G4302A	1260 Infinity SFC Binary Pump	Requires external degasser
	G5611A	1260 Infinity Bio-inert Quaternary Pump, 600 bar	Equipped with built-in degasser
External solvent selection	G1170A	1290 Valve Drive	
	G4235A	12-Pos/13-Port Solvent Selection Valve Head	Additional tubing kits required (Solvent selection tubing kit, 4 solvents (5067-4601))
Sample introduction Any of the Agilent Autosamplers supported by LC&CE Drivers 3.7 can be used. Select your autosampler suitable to the required maximum pressure capability of the system.			
Column compartment	G1316C	1290 Infinity Thermostatted Column Compartment	
	G1316A	1260 Infinity Thermostatted Column Compartment	No low dispersion heat exchangers possible Only as third Thermostatted Column Compartment for additional space
Column selection	For suitable valves, see Table 1 on page 10.		

Solvent Switching

Components for Solvent Selection (Agilent 1290 Infinity LC)

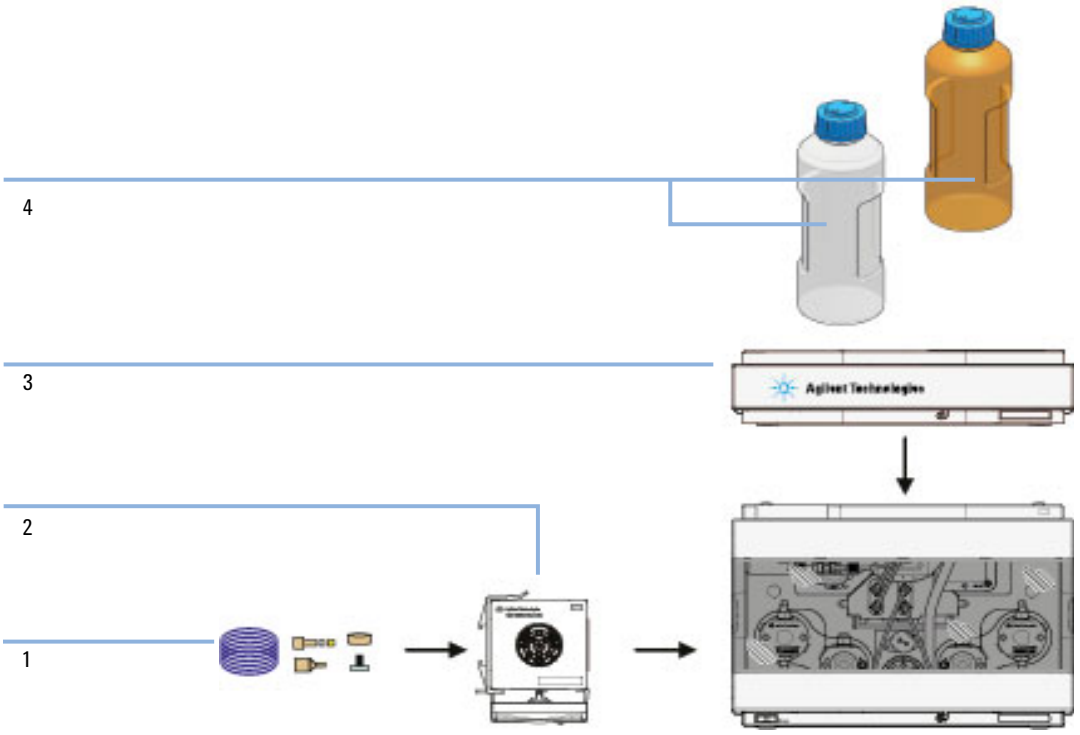


Figure 8 Components for solvent selection (Agilent 1290 Infinity LC)

Item	p/n	Description
1	5067-4601	Solvent selection tubing kit, 4 solvents
OR 1	5067-6697	Capillary Kit, 0.12 mm, 8-column sel, long columns, PC-HEx
2	G1170A	1290 Valve Drive in combination with 12ps/13pt valve, bio-inert (G4235A)
	5067-4634	Valve rail assembly
3	5065-9981	Solvent cabinet 1200 Infinity, including all plastic parts
4	9301-6526	Solvent bottle, amber, 1000 mL
OR 4	9301-6524	Solvent bottle, clear, 1000 mL

NOTE One solvent cabinet and bottles are included with the pump.

Components for Solvent Selection (Agilent 1260 Infinity LC)

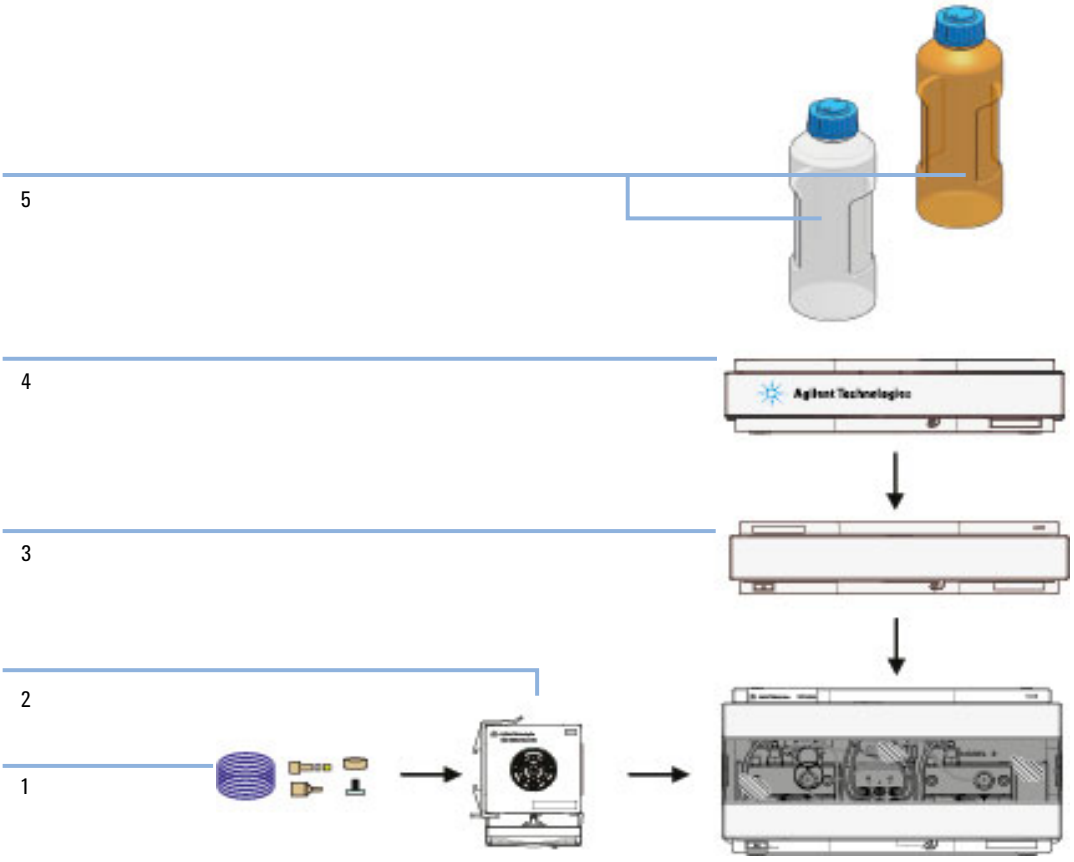


Figure 9 Components for solvent selection (Agilent 1260 Infinity LC)

NOTE The G1311B Quaternary Pump has a built-in degasser. An additional external degasser is required for G1312B Binary Pumps.

Item	p/n	Description
1	5067-4601	Solvent selection tubing kit, 4 solvents Up to 4 required
2	G1170A	1290 Valve Drive in combination with 12ps/13pt valve, bio-inert (G4235A)
	5067-4634	Valve Rail Kit
3	G4225A	High Performance Degasser Four solvent channels
4	5065-9981	Solvent cabinet 1200 Infinity, including all plastic parts
5	9301-6526	Solvent bottle, amber, 1000 mL
OR 5	9301-6524	Solvent bottle, clear, 1000 mL

NOTE One solvent cabinet and bottles are included with the pump.

Components of the Tubing Kit

The tubing kit (Solvent selection tubing kit, 4 solvents (5067-4601)) contains the following items:

#	p/n	Description
5	0100-2298	Adapter, PEEK int. 1/4-28 to ext. 10-32
1	5063-6598	Tefzel ferrules and SSL lock rings, 1/8 inch, 10/pck
1	5063-6599	PPS nuts, 1/8 inch, 1/4-28 thread, 10/pck
1	0890-1760	Tubing Flexible 1 ea / 1 meter
4	G7120-60007	Bottle Head Assembly
1	5042-9954	Tubing clip

Column Selection

Column Selection with two to three TCC

Figure 10 Topology 5: V-C-V

To perform automated column switching with the Agilent 1200 Infinity SeriesMethod Development System, two 1290 Infinity Thermostatted Column Compartments (G1316C), each equipped with an 8pos/9port valve, 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 (G1316C), depending on the flow rate. If six columns longer than 100 mm have to be installed and solvent pre-heating is required, either 3x G1316C or 2x G1316C and 1x G1316A/B are necessary.

For automated column switching, the following parts and modules have to be integrated:

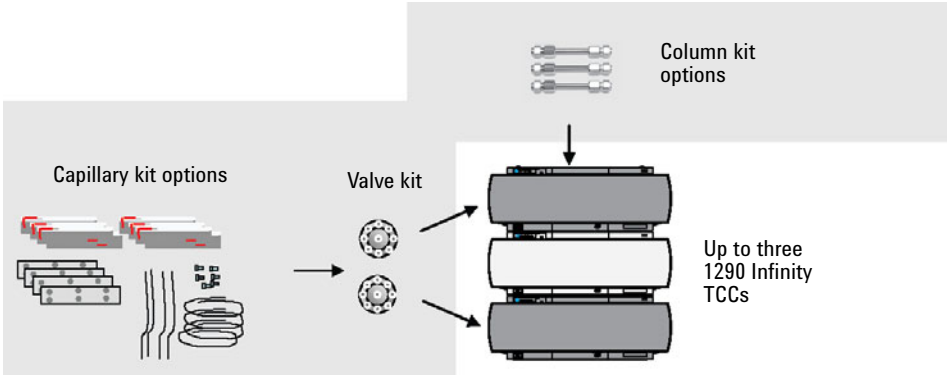


Figure 11 Modules and parts needed for column switching

Two different valve kits are available:

- The G4230A valve kit is required for Agilent LC systems with specified pressure range of up to 600 bar. The kit contains two 8-position/9-port valve heads (8 position/9 port valve head, 600 bar (5067-4107)) rated to a maximum pressure of 600 bar.
- The G4230B valve kit is required for an Agilent 1290 Infinity LC, and contains two different valve heads:
  - On the column inlet side, an ultra-high-pressure-rated 8-position/9-port valve head that is rated to a maximum pressure of 1200 bar (Valve Head 8 Position/9 Port, 1200 bar (5067-4121)) is necessary;
  - on the column outlet side, only little backpressure is given and a standard 600 bar selection valve (8 position/9 port valve head, 600 bar (5067-4107)) is sufficient.

One of three available capillary kits is delivered along with the valve kit.

NOTE

The kits listed above and their part numbers are no longer available for sale. As a reference for possible configurations of the Leagacy system, the required part numbers are still listed in the manual.

Infinity Capillary Kits

Low Dispersion Capillary Kit for Short Columns

The components of the low dispersion capillary kit for short columns (Cap Kit 0.12 mm LDHE double 8/9 vlv short col (5067-6220)) are listed below.

NOTE

The kit and part number listed above are no longer available for sale. As a reference for possible configurations of the Leagacy system, the required part numbers are still listed in the manual.

Valve 1 refers to the inlet valve and Valve 2 refers to the outlet valve.

#	p/n	Description
4	G1316-60005	Low Dispersion Heat Exchanger Double Assy
8	5500-1201	Capillary ST 0.12 mm x 105 mm SL Heatexchanger to column, SL fitting pre-swaged
1	G1316-90123	Technical Note (*Installation of the Low Dispersion Heat-Exchanger Double Assemblies in the 1290 Series Thermostatted Column Compartment (G1316C)*, ENG)
8	G7167-68703	Fitting Intermediate Kit
1	G1316-87319	Capillary ST 0.12 mm x 340 mm S/S Autosampler with thermostat to valve 1
1	5500-1157	Capillary ST 0.12 mm x 500 mm SL/S Autosampler to valve 1 in a dual stack configuration
8	5067-4604	Capillary ST 0.12 mm x 280 mm S/SX Valve 1 to heat-exchanger
8	5500-1191	InfinityLab Quick Turn Capillary ST 0.12 mm x 280 mm, long socket Column outlet to valve 2, capillary w.o. fittings, use PEEK fingertight
2	0890-1713	Waste tubing, 2 m
1	5042-9918	Column clip set, eight colors
1	5041-2115	Folding box
1	5063-6591	PEEK Fittings 10/PK Column outlet fitting
1	5062-8541	Fingertight fitting long, 10/pk Column outlet capillary valve 2 ports 4

General Purpose Capillary Kit

The components of the general purpose capillary kit (Cap Kit 0.17 mm 8/9 multi purpose (5067-4290)) are listed below.

NOTE The kit and part number listed above are no longer available for sale. As a reference for possible configurations of the Leagacy system, the required part numbers are still listed in the manual.

Valve 1 refers to the inlet valve and Valve 2 refers to the outlet valve.

NOTE The general purpose capillary kit is not recommended for 1290 Infinity LCs.

#	p/n	Description
2	5500-1196	Capillary ST 0.17 mm x 280 mm, long socket Autosampler to TCC heater, vlv-det
1	5500-1236	Capillary ST 0.17 mm x 400 mm, long socket ALS therm-TCC heater
1	5065-9933	Capillary ST 0.17 mm x 600 mm Autosampler to TCC heater in a dual stack configuration
12	5500-1235	Capillary ST 0.17 mm x 380 mm, long socket 6 x col - vlv, 6 x vlv - col
1	5063-6591	PEEK Fittings 10/PK Column outlet fitting, 12 x for cap. 5500-1235
1	5062-8541	Fingertight fitting long, 10/pk Column outlet capillary valve 2 ports
1	5065-4454	Fitting screw long, front and back ferrules 10/pk 6 x for cap. 5500-1235 valve to column, 2 x for cap. 5500-1236
2	5067-4607	Capillary ST 0.17 mm x 280 mm SX/SX Bypass line, TCC heater - vlv
2	0890-1713	Waste tubing, 2 m Waste-line
1	5067-1540	SST hex head nut with PEEK ferrule, 6/pk Steel fitting with PEEK nut, 6 x for cap. 5500-1235 valve to column (column side)
1	5042-9918	Column clip set, 8 colors
1	5041-2115	Folding box

Low Dispersion Capillary Kit for Long Columns

The components of the low dispersion capillary kit for long columns (Cap Kit 0.12 mm LDHE double 8/9 vlv long col (5067-4289)) are listed below.

NOTE

The kit and part number listed above are no longer available for sale. As a reference for possible configurations of the Leagacy system, the required part numbers are still listed in the manual.

Valve 1 refers to the inlet valve, and Valve 2 refers to the outlet valve.

#	p/n	Description
3	G1316-60005	Low Dispersion Heat Exchanger Double Assy
6	5500-1201	Capillary ST 0.12 mm x 105 mm SL Heat exchanger to column, SL fitting pre-swaged
6	G7167-68703	Fitting Intermediate Kit
1	G1316-90123	Technical Note (*Installation of the Low Dispersion Heat-Exchanger Double Assemblies in the 1290 Series Thermostatted Column Compartment (G1316C)*, ENG)
1	5500-1191	InfinityLab Quick Turn Capillary ST 0.12 mm x 280 mm, long socket Valve 2 to detector
1	5067-4669	Capillary ST 0.12 mm x 600 mm S/SL Autosampler to valve in dual stack configuration
1	5067-4647	Capillary ST 0.12 mm x 340 mm S/SX Therm. ALS to valve
6	5500-1251	Capillary ST 0.12 mm x 400 mm SL/SL Valve 1 to heat exchangers (PL29)
6	5500-1192	Capillary ST 0.12 mm x 500 mm, long socket Columns to valve 2
1	5067-4607	Capillary ST 0.17 mm x 280 mm SX/SX Bypass line
1	5063-6591	PEEK Fittings 10/PK Column outlet fitting 2
1	5062-8541	Fingertight fitting long, 10/pk Column outlet capillary valve 2 ports 4
1	5042-9918	Column clip set, eight colors
1	5041-2115	Folding box
2	0890-1713	Waste tubing, 2 m Waste-line

3 System Setup and Installation

Documentation of the Individual Modules 37
System Setup 38
Configurations and Capillary Setup 41
Configuration and Capillary Setup (G7116B) 41
Setup Examples 43
Recommended Column Configurations 45
Installing the Solvent Selection Part 47
Deciding on the Position of the Degasser 48
Installing the Solvent Tubing 50

This chapter provides information on system setup, installation of valve heads, heat exchangers and capillaries, and installation of solvent selection parts.

Documentation of the Individual Modules

The Method Development System works in lots of different hardware configurations. For details of the individual modules mentioned in this guide, refer to the Agilent Information Center (AIC).

System Setup

The Method Development System works in lots of different hardware configurations. Examples for different proved and tested method development system set-ups are shown in Figure 12 on page 38, Figure 13 on page 39, and Figure 14 on page 39.

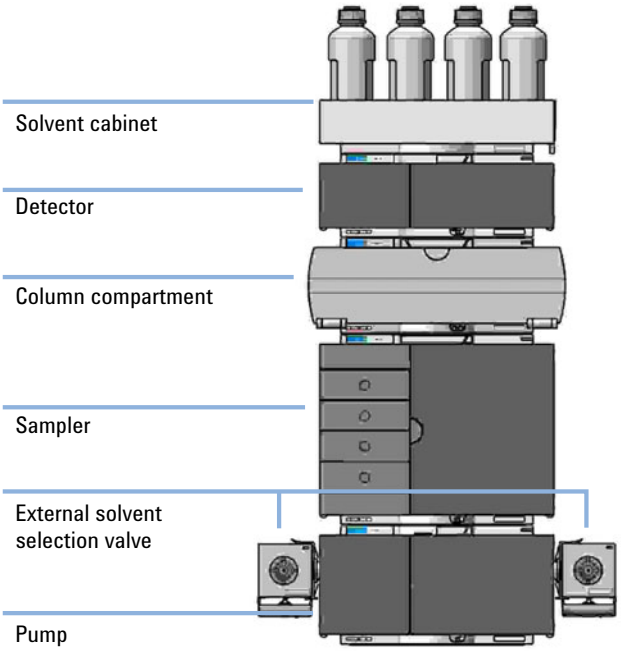


Figure 12 Example setup based on a 1290 Infinity III LC

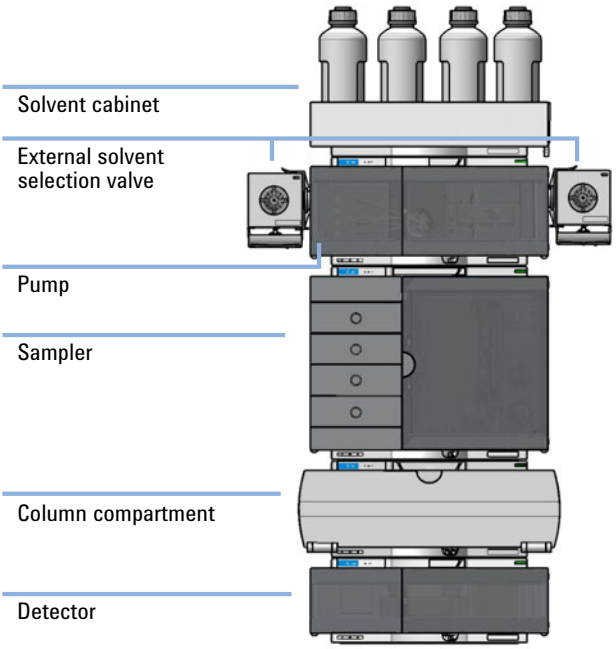


Figure 13 Example setup based on a 1260 Infinity III Multisampler with Quaternary Pump (G7111B)

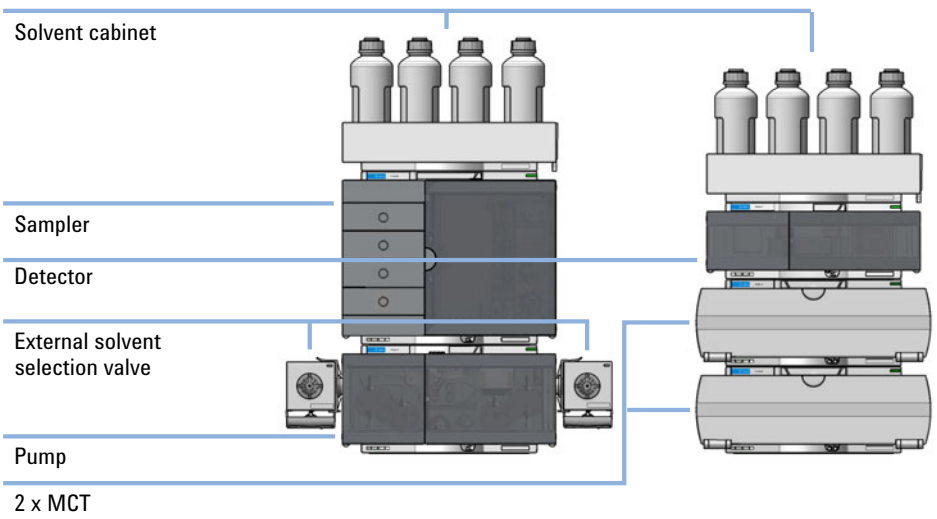


Figure 14 Example setup based on a 1290 Infinity III LC (with two MCT), two stack configuration

In principle, the connecting capillaries should be kept as short as possible to reduce extra-column band-broadening, and to keep the backpressure 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. Several set-ups taking these considerations into account are covered with the available capillary kits.

Configurations and Capillary Setup

Configuration and Capillary Setup (G7116B)

CAUTION

Damage to the rotor seal

Instant pressure release within the valve will lead to water jet effects that can harm internal parts of the valve. This pressure release typically happens if the valve gets switched under high pressure over unused or open channels.

✓ **Block all unused channels properly with the M4 blank nut.**

NOTE

To minimize valve movement over open connections it is recommended to plumb the column connected channels in one row.

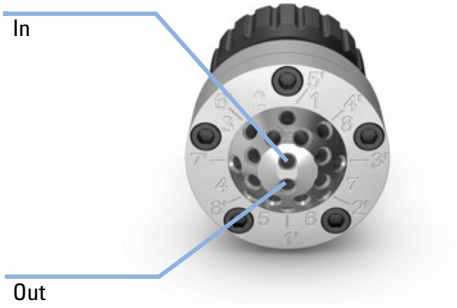
e.g. 8-Column Selection valve:

- channel 1 – column 1
- channel 2 – column 2
- channel 3 – column 3
- channel 4 – column 4
- channel 5 – blocked
- channel 6 – blocked
- channel 7 – waste
- channel 8 – bypass

NOTE

The blank nuts are only required for the ports on the inner circle that connect the valve with the column inlet.

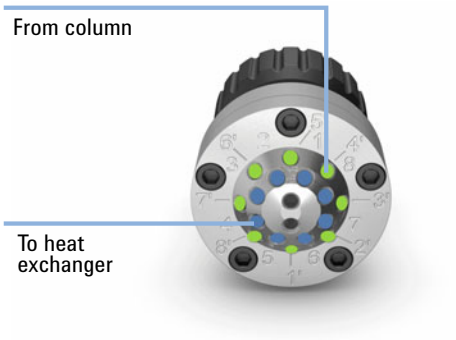
1 Install the in and out connectors.
   - from sampler to the valve (Capillary ST 0.12 mm x 500 mm M4-SL PS-PS (5500-1202))
   - from valve to the detector (Capillary ST 0.12 mm x 280 mm M4-SL PS-PS (5500-1203))



The *In* port is hydraulically connected to the column inlet ports 1-8 on the inner ring while the *Out* port connects to the column outlet ports 1`-8` on the outer ring.

2 Install the column inlet and outlet connections.

●	*ports 1-8* for connections from valve to the heat exchanger
●	*ports 1`-8`* for connections from column outlet to valve



Setup Examples

1 Eight column selection

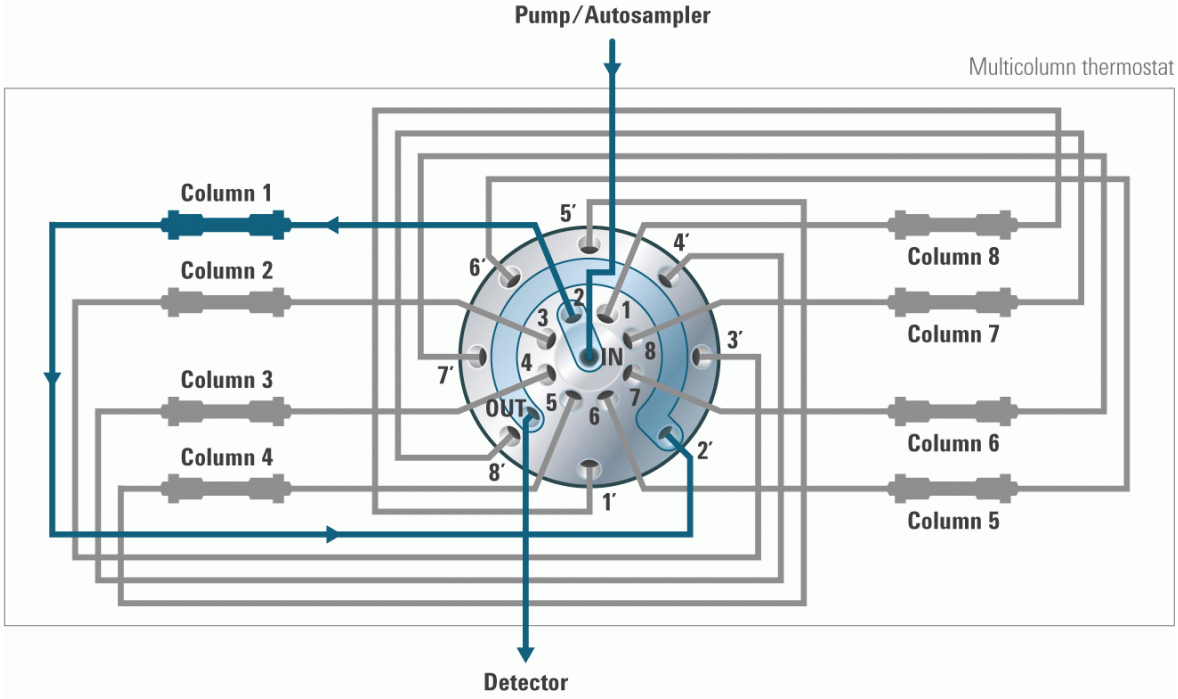


Figure 15 Hydraulic flow path schematics for an 8-column selection setup

2 Six column selection with purge line and valve- bypass

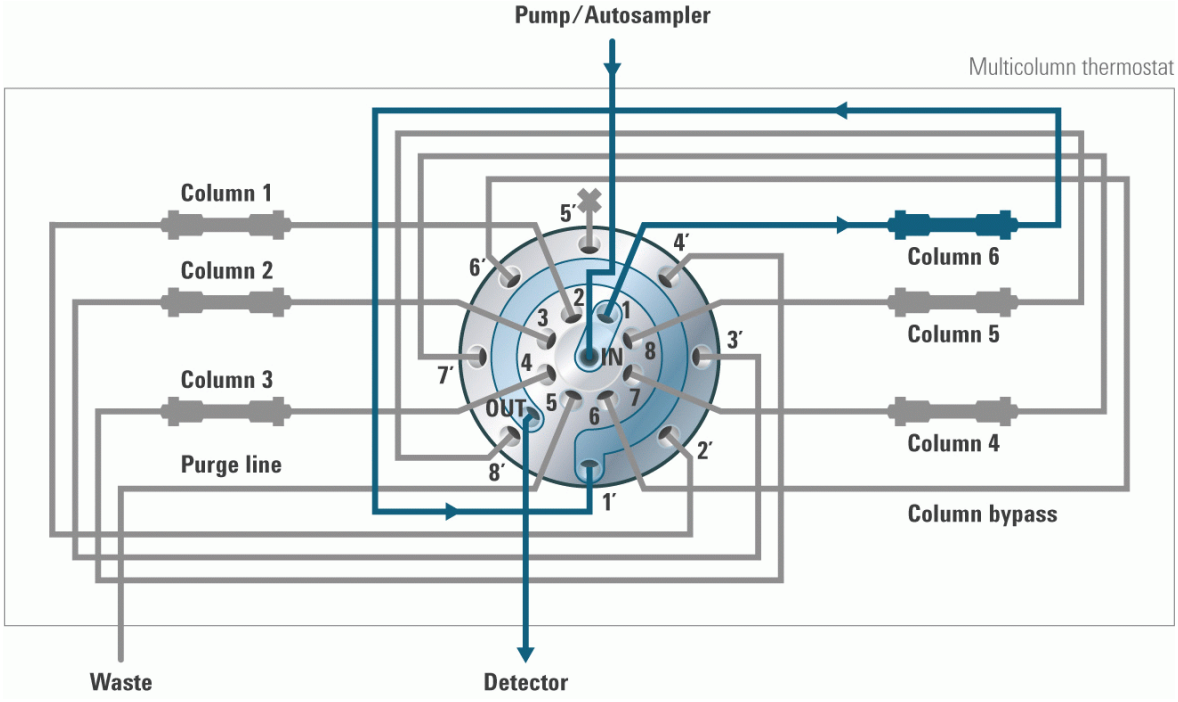


Figure 16 Hydraulic flow path schematics for 6-column selection setup with purge and valve bypass line.

Recommended Column Configurations

Column Configurations with one MCT

The column configurations with the MCT are very flexible and cover most of the major requirements for method development. MCT configurations with a Column Selector Valve and Quick Connect Heat Exchangers offer the following options:

- Quick change between up to eight (G7116B) respectively four (G7116A) different columns of 100 mm length
  - Different stationary phases for different applications, or
  - Identical stationary phases in columns with different dimensions for either faster run-times or higher resolutions, or
  - Different internal diameters for loading studies
- Two independent heating zones for four columns each (heating room divider strongly recommended)

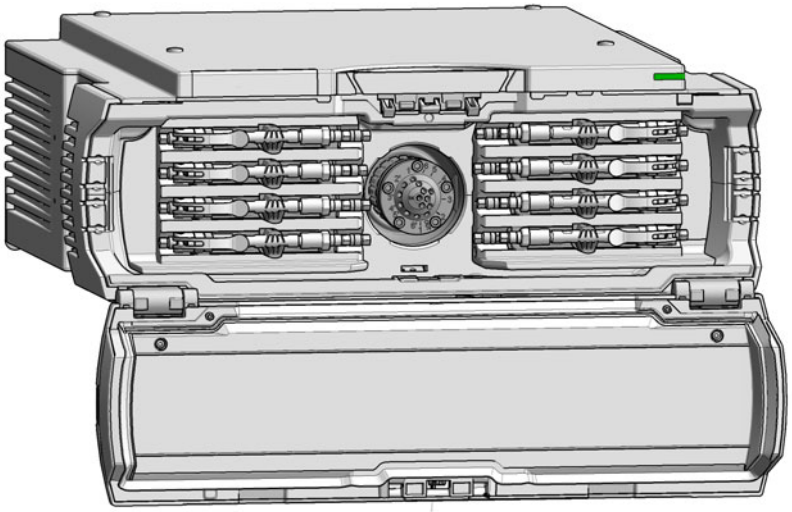


Figure 17 The G7116B Multicolumn Thermostat equipped with an InfinityLab Quick Change 8-column selector valve head

Column Configurations with two MCT (for long columns)

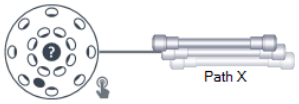


Figure 18 Topology 1: VC

The same situation as described in "Column Configurations with one MCT" on page 45 can be achieved using two MCT modules and one valve. In this configuration it is possible to use up to 8 long columns (or several combinations with short columns). If more columns are needed, see "A Valve Thermostat Cluster (VTC) for up to 32 Columns" on page 20.

NOTE

Agilent 1260 Infinity II/III Multicolumn Thermostat (G7116A) does not support clustering!

Installing the Solvent Selection Part

Especially for LC method development, but also for frequently changing application needs, 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 large influence on the separation. Therefore, the Agilent Method Development Systems offers the possibility to equip the pump with up to two additional external valve drives (G1170A), equipped with a 12-position/13-port solvent selection valve head (G4235A). This multiplexes a channel of the pump and provides up to 12 additional solvent channels. With the quaternary pump, this gives a maximum of 26 available solvents and allows the generation of up to 169 solvent combinations (for a binary gradient), or 288 unique solvent combinations (for ternary gradients). When the external solvent selection valves are combined with a binary pump with no internal solvent selection valve, 144 solvent combinations are possible. If the binary pump is fitted with an internal solvent selection valve, providing two different solvents per channel, up to 169 solvent combinations (for a binary gradient) are possible. For an overview, see Table 4 on page 47.

Table 4 Possible Solvents and Solvent Combinations in Different Configurations

Number of External Solvent Selection Valves:	0	1	2
Available Solvents:			
G1312B, G7112B	2	13	24
G4220AB, G7120A, G1312B, G7112B (SSV installed)	4	15	26
G1311B, G7111B, G4204AB, G7104A	4	15	26
Binary Solvent Combinations:			
G1312B, G7112B	1	12	144
G4220AB, G7120A, G1312B, G7112B (SSV installed)	4	26	169
G1311B, G7111B, G4204AB, G7104A	4	36	169
Ternary Solvent Combinations:			
G1311B, G7111B, G4204AB, G7104A	4	36	288

Deciding on the Position of the Degasser

It is recommended to use the built-in degassing unit, and to place it in the flow path behind the external solvent selection valve. The built-in degassing unit has a low delay volume and a very high degassing efficiency.

The Agilent 1260 Infinity I Binary Pump (G1312B) do not have a built-in degasser. For Agilent 1260 Infinity Binary LCs, you need to decide where in the flow path to position the external degassing unit. There are two options available: Solvent degassing can be done before or after the external solvent selection valve.

NOTE

If two external solvent selection valves are configured in the system, only degassing either before or after the external solvent selection valve is supported. A configuration with one valve before the degasser and one after it is not supported.

Degassing before the solvent selection valve

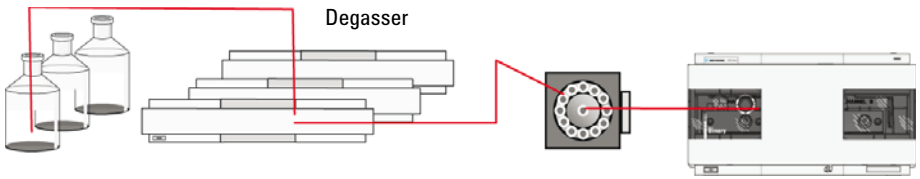


Figure 19 Solvent degassing before the solvent selection valve

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:

- the requisite number of G4225A degasser units (one degasser is for four solvents only)
- Solvent cabinet 1200 Infinity, including all plastic parts (5065-9981) (one is included to the pump)
- InfinityLab LC Series Solvent Cabinet (5067-5760) (one is included to the pump)
- Solvent bottle, amber, 1000 mL (9301-6526) or
- Solvent bottle, clear, 1000 mL (9301-6524)
- n x Solvent selection tubing kit, 4 solvents (5067-4601)

Degassing after the solvent selection valve

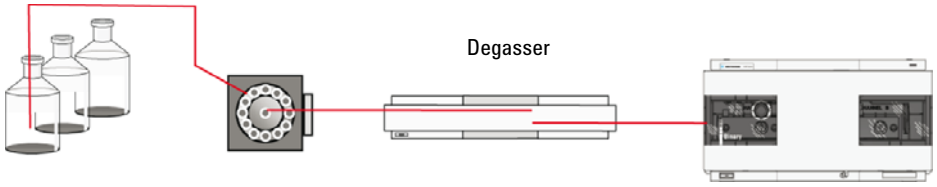


Figure 20 Solvent degassing after the solvent selection valve

Pro:	No risk of re-solution of air, only one degasser required
Con:	Considerably longer flushing times because after every solvent change, the degasser chamber (with the G4225A ca. 1.2 mL) needs to be flushed thoroughly, which means with at least five times the volume of the degasser chamber.

Required parts:

- 1x G4225A degasser (the setup above shows the concept of a build-in degasser in a pump, for example 1290 Binary Pump)
- Solvent cabinet 1200 Infinity, including all plastic parts (5065-9981) (one is included to the pump)
- InfinityLab LC Series Solvent Cabinet (5067-5760) (one is included to the pump)
- Solvent bottle, amber, 1000 mL (9301-6526) or
- Solvent bottle, clear, 1000 mL (9301-6524)
- n x Solvent selection tubing kit, 4 solvents (5067-4601)

NOTE

Normally, the Agilent 1260 Infinity High Performance Degasser (G4225A) should be sufficient for most applications. However, for high flow rates and for special solvents with low boiling point and high vapor pressure, the standard Agilent 1260 Infinity II/III Degasser (G7122A) can be used as an alternative.

Installing the Solvent Tubing

One solvent selection tubing kit (Solvent selection tubing kit, 4 solvents (5067-4601)) is delivered with all required parts to connect up to four solvent bottles to the 12-Position/13-Port valve (G4235A) on the G1170A External Valve Drive. If you need more solvents, additional kits need to be ordered.

Depending on whether degassing is done before or after solvent selection, there are two possible set-ups for the hydraulic connections, as described below.

Degassing with several degassers before the solvent selection valve:

1. Place the solvent bottles in additional solvent cabinets on top of the degasser.
2. Use the pre-installed bottle head assemblies to connect the bottles with the degasser inlet ports.
3. Cut appropriate lengths of the 2 x 5 m solvent tubing and fit them to the fittings.

NOTE

Use the prefitted bottle head assemblies as examples of how these are fitted together.

4. Attach the PEEK-adapters to the ports of the 12-position/13-port valve and connect the solvent tubing from the outlet port of the degasser to the peripheral ports of the valve.
5. Connect the central port of the 12-position/13-port valve to one port of the pump, or to one port of the optional internal solvent selection valve of the pump (binary pumps only).

Degassing after the solvent selection valve:

The pre-installed tubings need to be replaced with much longer ones that have to be cut to appropriate lengths from the 5 m tubing.

1. Disassemble the bottle head assemblies.
2. Cut appropriate lengths of the 2 x 5 m solvent tubing.
3. Refit the bottle head assemblies with the longer tubings.
4. Connect the tubings with the peripheral ports of the 12-position/13-port valve using the PEEK adapters.
5. Connect the central port of the 12-position/13-port valve with the inlet port of one degasser channel and the corresponding outlet port with one channel of the pump.

In both cases, make the corresponding settings in the software (entering the clustering information, and entering the names of the solvents into the solvent table).

4 Configuring the System in ChemStation and Creating Methods

Clustering of Modules in ChemStation using the RC.NET Drivers 53
Column Compartment Cluster Configuration (VTC) 54
Solvent Delivery Cluster Configuration 59
Settings for Column Compartments 62
Settings 63
Settings for Solvent Selection 67

This chapter explains how to configure the system in the control software and how to create methods.

Clustering of Modules in ChemStation using the RC.NET Drivers

During autoconfiguration, if the system detects that there are modules that can be clustered, a dialog box is displayed (Figure 21 on page 53) that allows you to select the modules via check boxes to be included in the cluster. You select the modules for the Column Compartment Cluster and the Pump/Valve Cluster separately.

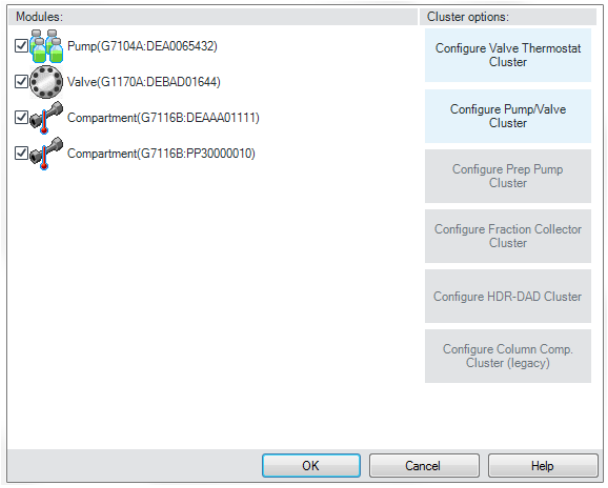


Figure 21 Screen Agilent LC Modules and Systems Auto Configuration, Selection of modules to be included in the cluster

To start the clustering, klick one of the available cluster option buttons.

Column Compartment Cluster Configuration (VTC)

When you click **Configure Valve Thermostat Cluster** in the cluster creation dialog box (Figure 21 on page 53), the VTC configuration dialog box is displayed that allows you to configure the column compartment cluster.

Select the topology with the drop-down list and the topology scheme will be shown beneath. Red caution icons indicate, that valves are not defined yet.

Example 1: Topology 1 VC for long columns

Topology 1 enables clustering of one valve with up to eight columns. And with two MCT modules it is possible to use up to 8 long columns.

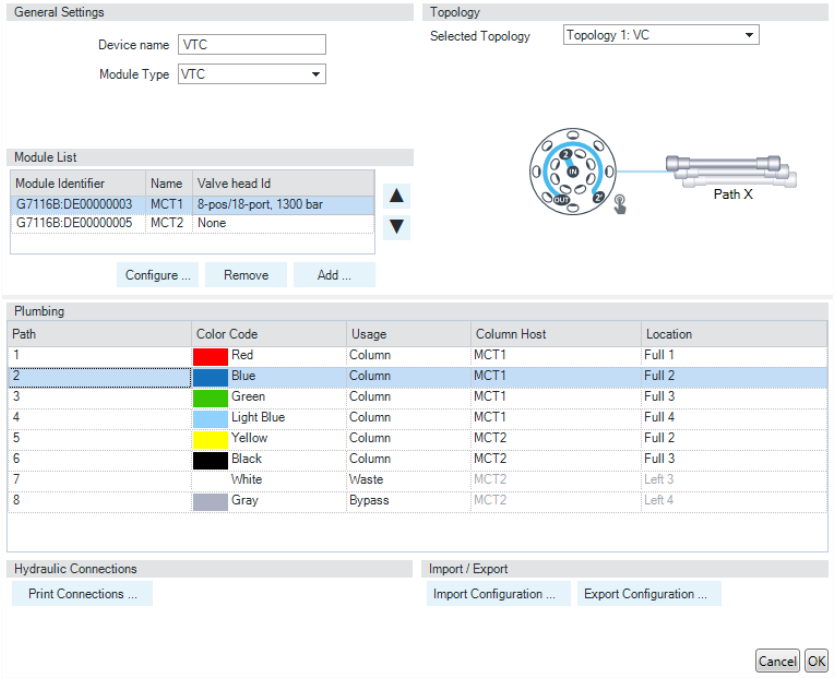


Figure 22 Screen Valve Thermostat Cluster Post Auto Configuration, Configuring Topology 1

First you have to update your module list by adding or removing modules. 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. The sequence you select here is displayed in all column compartment cluster images.

Click **Configure...** (when you add a module, you are automatically asked to configure it after having confirmed your module type) and select your valve version by a drop-down list with a click on **Not installed** (see Figure 23 on page 55).

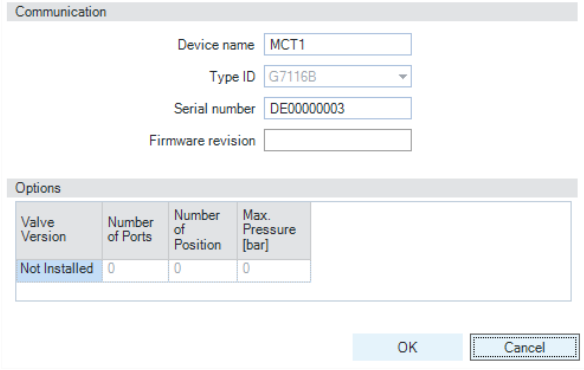


Figure 23 Screen Configure single device, installing valve versions

If this setting is completed, you have to assign the valve in the topology scheme with a click on the red caution icons or finger icons. A context menu will show available valves. This needs to be repeated for each valve in the topology.

The Plumbing section enables you to specify how the installed columns are connected to the column selection valve, and where they are located in the column compartment cluster. The valve positions are color-coded; the colors are reflected in the Column Assignment diagram (see Figure 30 on page 63).

Colors usages and locations are all specified using drop-down menus in the relevant cells. Column locations are specified by column compartments (**Column Host**) and position (**Location**). Note that if you specify a long column occupying both left and right positions in a column compartment, the temperatures of both heat exchangers are coupled and set to the same temperature. This has implications for any other columns in the same column compartment. Any conflict in the configuration is marked with a red warning sign, and a tooltip gives the reason for the error.

For Plumbing, select the **Color Code**, **Usage**, **Column Host** and **Location** from the drop-down list which appears by clicking on the corresponding table cell.

NOTE

With this flexible system, all selectable plumbings are possible. But to keep track of the system, a logical assignment is very helpful. You can see all paths in the topology scheme by clicking on the path number in the plumbing table. Finally you will have an overview of the set hydraulic connections by clicking **Print Connections...**.

Example 2: Topology 2 V(VC)$_2$

With branched topologies it is possible to increase the number of columns. In topology 2, the simplest form, typically an external root valve (first level) branches between two MCTs (second level).

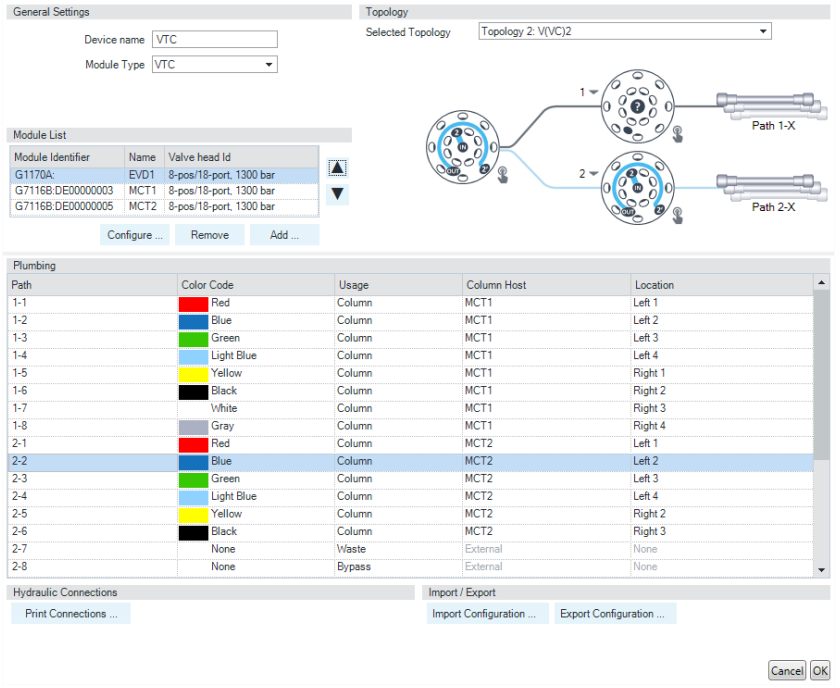


Figure 24 Screen Valve Thermostat Cluster Post Auto Configuration, Configuring Topology 2

When the valves are assigned (see Figure 22 on page 54), in branched topologies a default port of the host valve (level 1) is proposed (indicated by a number left to the valve). This is the port of the valve to the left (level 1 valve), where the valve to the right (level 2 valve) is connected to. In order to change this port (e.g. to achieve a shorter capillary connection), click on the number and select a different port number from the drop-down list (see Figure 25 on page 57).

NOTE

The selected number is the valve switching position and not the port (they may be equal).

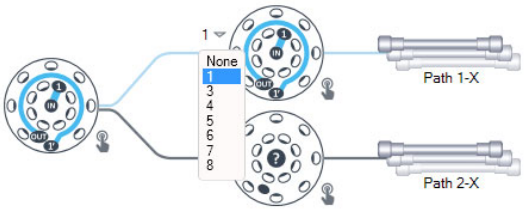


Figure 25 Valve switching positions

Topologies 3 and 4 are configured in the same way.

Example 3: Topology 5 V-C-V

This topology uses two bracketing valves: one for inlet and one for outlet. The left valve is the inlet valve in the high pressure flow path, the right one the outlet valve in the low pressure flow path downstream to columns. With this topology TCC-clustering is possible and can only be used with two 8-pos/9-port valves.

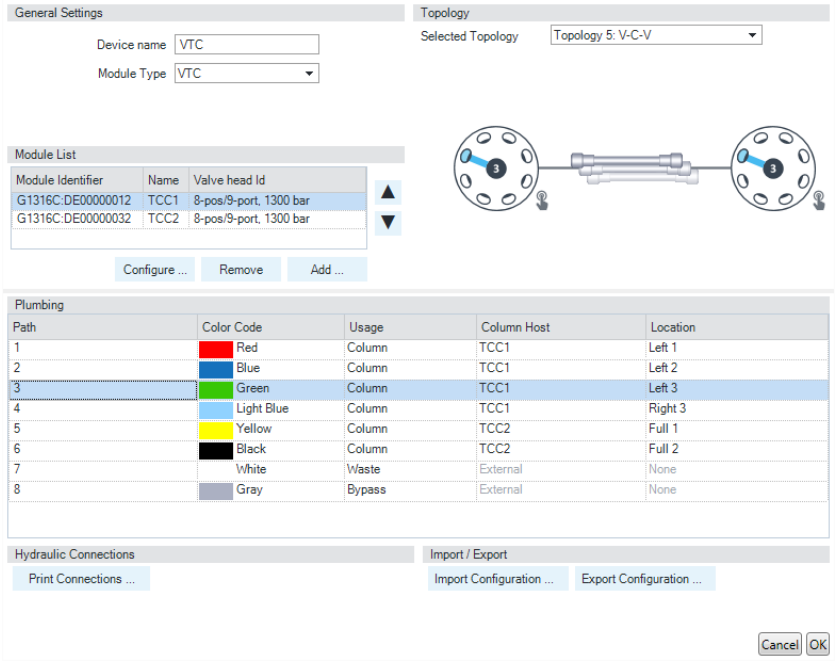


Figure 26 Screen Valve Thermostat Cluster Post Auto Configuration, Configuring Topology 5

Solvent Delivery Cluster Configuration

When you click **Configure Pump/Valve Cluster** in the cluster creation dialog box (Figure 21 on page 53), the **Pump Valve Cluster Configuration** dialog box is displayed that allows you to configure the solvent delivery cluster (see Figure 27 on page 59).

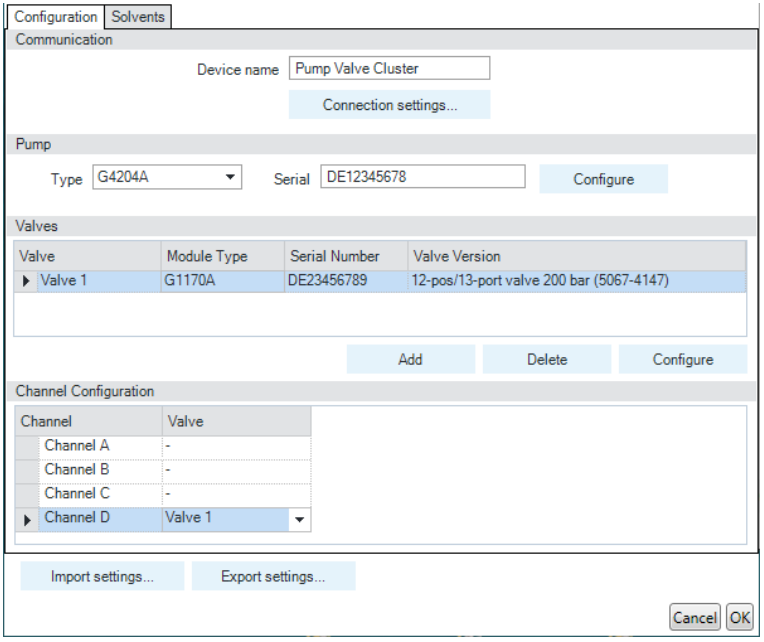


Figure 27 Screen Pump Valve Cluster Configuration, Tab Configuration

During autoconfiguration, the dialog box sections contain the pump and valve(s) that were selected in the cluster creation dialog box. To complete the configuration of the solvent delivery cluster, you specify the pump channels the valves are associated with by selecting from a drop-down list in the **Channel Configuration** table. The table lists all available channels, depending on the configured pump:

Quaternary pump	A, B, C, D
Binary pump	A, B
Binary pump with internal solvent selection valve	A1, A2, B1, B2

Solvent configuration

Use the **Solvents** tab, available in the post-autoconfiguration mode, to specify the solvent for each column. All available channels are listed; the number of available channels depends on the configured pump and the number of valves in the cluster.

In addition to specifying the solvent name and either specifying a compressibility value (for example, G7111B, G7104C) or selecting a solvent definition (for example, G7120A, G7132A), you can optionally specify other solvent characteristics (pH, viscosity and molarity).

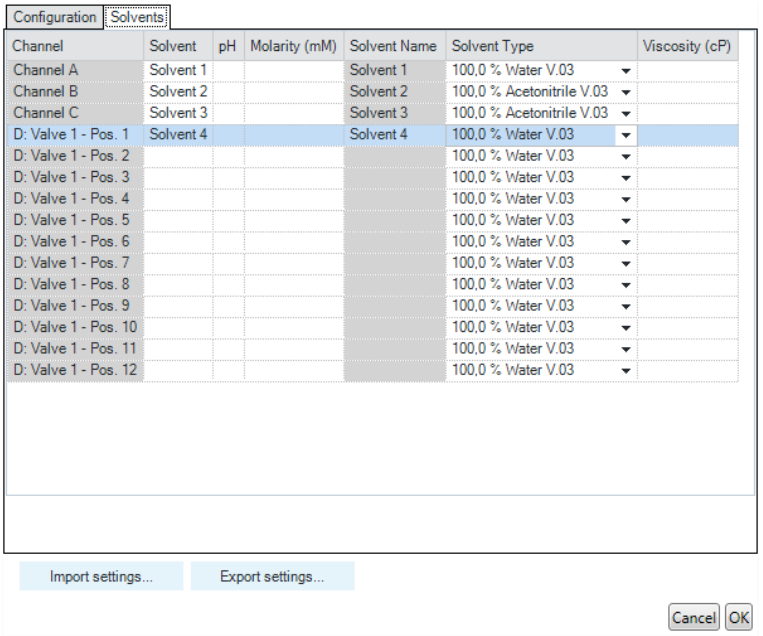


Figure 28 Screen Pump Valve Cluster Configuration, Tab Solvents

Configure Solvent Type Catalogs opens the **Solvent Type Catalogs**, which lists all available solvent types.

The solvent name that is reported is automatically concatenated from your entries in the columns **Solvent**, **pH** and **Molarity** (if you enter values for pH and/or molarity) as in the following example:

Column	Your entry
Solvent	Phosphate buffer
pH	4.5
Molarity	20

The **Solvent Name** automatically generated and used by the system will be: *Phosphatebuffer pH: 4.5 20mM"*

NOTE

The pH value is used by the Method Scouting Wizard to check for potential incompatibilities of columns and solvents. The viscosity column is for future use.

Bottle fillings

Bottle sizes and fillings are managed by the standard bottle filling interface. The system automatically assigns the values to the selected solvents of the external valves according to the valve positions.

Settings for Column Compartments

Columns definition

To specify the columns, select **Instrument >Columns**. Fill in the columns you regularly use in your lab, see Figure 29 on page 62. This information is required for configuring the column locations shown in Figure 30 on page 63. You should enter at least a **Description**, the physical dimensions and, if these values are identical for some columns, a serial number to allow correct identification later. If you are using the 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 maximum and minimum pH values. 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.

Insert | Append | Delete | Plumbing... | View | Move... | OK | Cancel | Help

#	Installed	Location	Dev. Serial#	Tag	Description	Col. Serial#	Batch#	Product#
1	YES	Left 1	PP30000010		SB-C18	autoID-21		827975-902
2	YES	Left 2	PP30000010		SB-C18	autoID-19		827700-902
3	YES	Right 1	PP30000010		Eclipse XDB-C18	autoID-8	1	927975-902
4	YES	Left 1	DEAAA01111		Poroshell 120 Bonus-RP	autoID-33		695768-901
5	YES	Left 2	DEAAA01111		ZORBAX Eclipse AAA	autoID-53	N/A	966400-902
6	YES	Left 3	DEAAA01111		Poroshell 300SB-C18	autoID-59	N/A	660750-902
7	YES	Left 4	DEAAA01111		Eclipse XDB-C18	autoID-23		993967-902

Figure 29 **Screen Edit Columns, Table of columns which are used for method development experiments**

NOTE

The **Installed** value is not used in case of a column compartment cluster.

Plumbing... leads you directly to the Column Assignment (see Figure 30 on page 63).

Settings

Plumbing (see Figure 30 on page 63) enables you to specify how the installed columns are connected to the column selection valve, and where they are located in the Thermostat.

The valve positions are color-coded; the colors are reflected in the **Column Tag Information** and shown in the **Visualization** scheme.

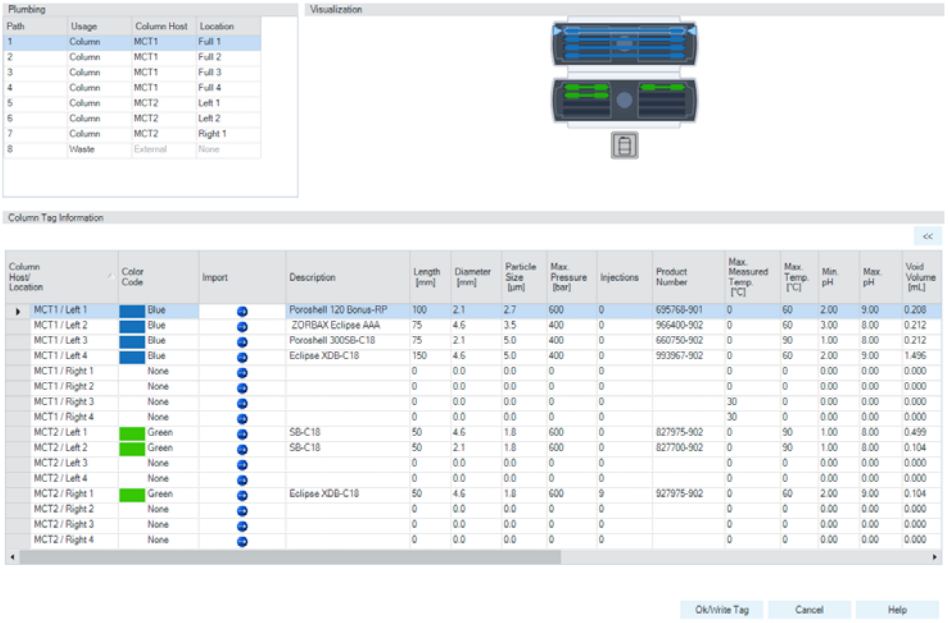


Figure 30 Screen Column Assignment

The **Column Tag Information** tab shows information about the types of columns used for analysis. With a click on the icons in the **Import** column, you can select the column from a list which can be imported with all parameters (see Figure 31 on page 64).

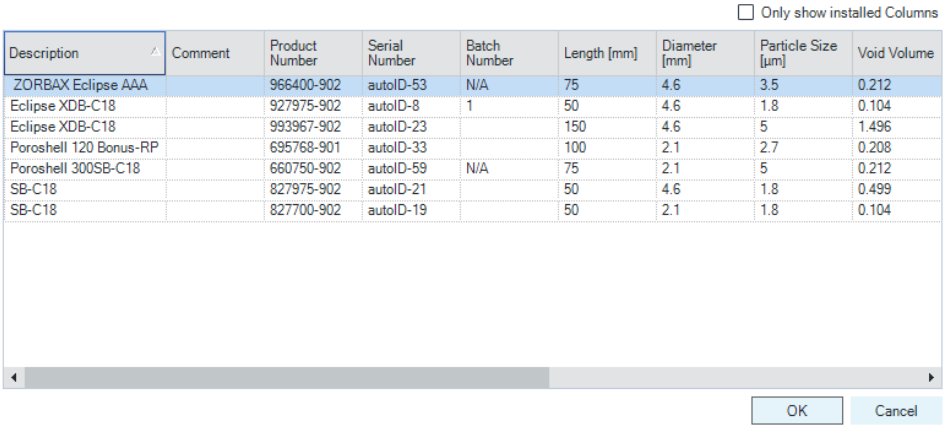


Figure 31 Screen Import Column From Column Database

Method setup for Multicolumn Thermostat

After configuration of all columns, select **Instruments >Set Up Column comp. (G7116B)** to setup the parameters for analysis.

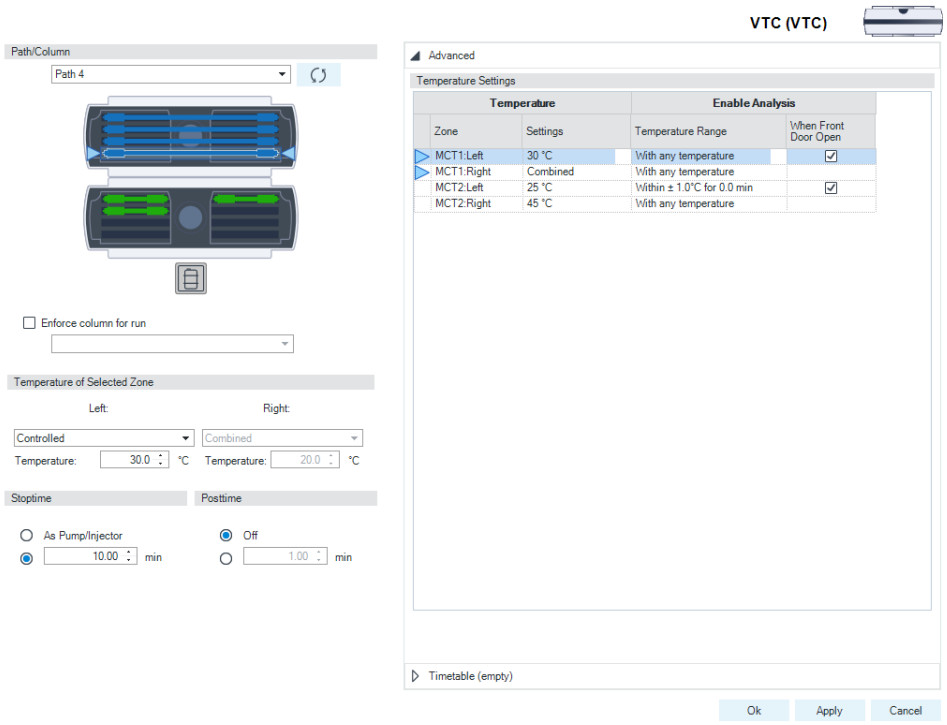


Figure 32 Screen Method of VTC, Setting parameters for the analytical run (MCT)

The user interface allows to set the following parameters:

- **Path/Column**

  The position of the column in the MCT and the corresponding valve position are shown in the graphical representation. Together with the color code, this allows the identification of the flow path.

  The column used for the individual method can be selected in the **Method** window of the 1290 Infinity II/III Multicolumn Thermostat. The appropriate column can be selected either by the drop-down menu, which shows all assigned columns, or by just clicking the column with the correct color code in the image of the 1290 Infinity II/III Multicolumn Thermostat. The current valve position, which automatically connects to the chosen column, is shown. For quick information, the valve position, the color code of the chosen column, and its product number are shown in Agilent ChemStation.
- **Temperature of Selected Zone**
- **Stoptime**
- **Posttime**
- **Advanced**

  In **Temperature Settings**, a list of the temperature zones is given and the corresponding options for enabling analysis. Selected columns in the schema are indicated in the list with a blue arrow.

  The software recognizes the position of the front door (open or closed) of the MCT. 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.
- **Timetable**

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

Settings for Solvent Selection

Configuring the solvent table

If an external G4235A solvent selection valve is clustered, solvents can be specified and named by selecting **Instrument >More Pump Valve Cluster >Pump Valve Cluster Configuration**, see Figure 33 on page 67.

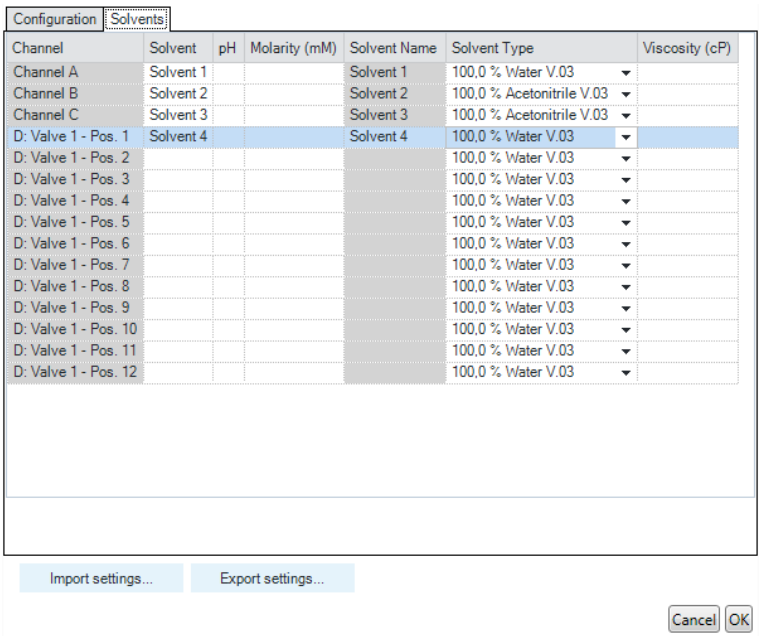


Figure 33 Screen Pump Valve Cluster Configuration, Tab Solvents

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. For pumps using calibration tables for the compressibility and elasticity of the solvents (for example the G1312B Binary Pump), you also need to enter the **Calibrated Solvent** here. The entry for the **pH Value** is optional, but is recognized in the Method Scouting Wizard to exclude incompatible combinations of solvents and columns.

Actual Volume and **Total Volume** are set in the **Bottle Fillings** dialog (also for the solvents attached to the solvent selection valve), see Figure 34 on page 68.

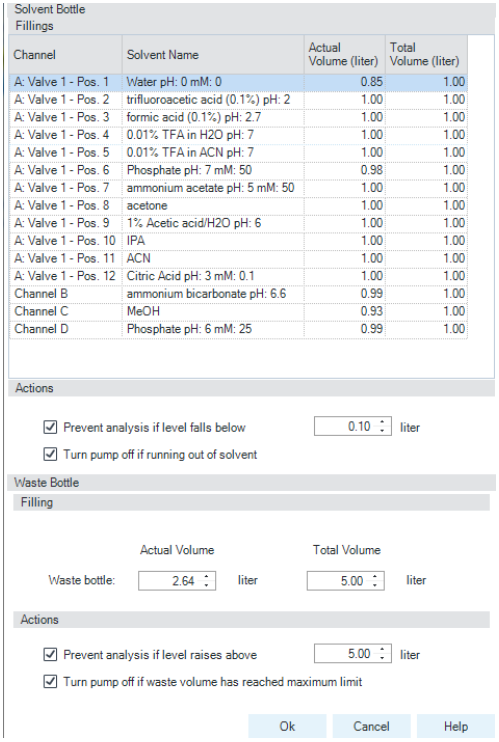


Figure 34 Screen Bottle Fillings

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 35 on page 69. You simply select the solvent that you want to use by its name. If you need to enter a calibrated solvent for the pump that is installed in your system, it is taken automatically from the solvent table (see Figure 33 on page 67).

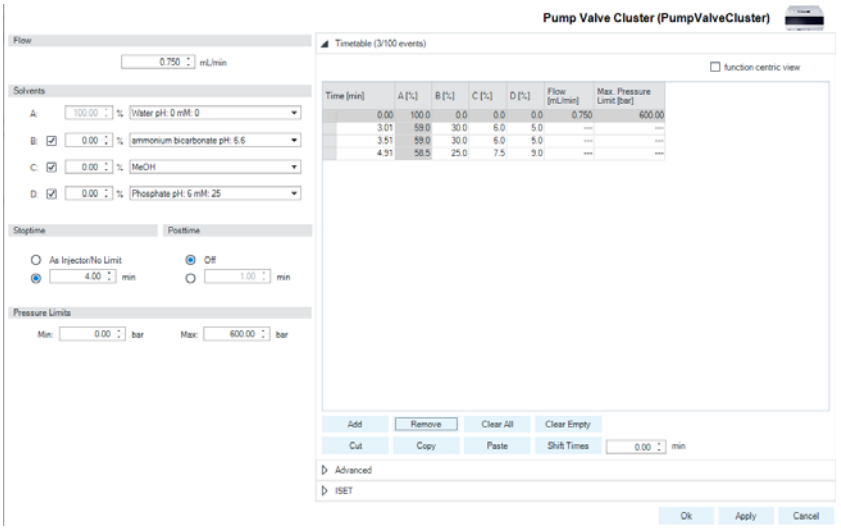


Figure 35 Screen Method of PumpValveCluster, Selecting a mobile phase attached to the solvent selecting valve

After configuring and setting 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 Method Scouting Wizard 71
Features 72
Software Compatibility 75
Software Installation 76
Defining the Campaign 77
Define Screening Campaign Base 79
Selecting the Columns 81
Selecting the Solvents 83
Specifying and Selecting Gradients 87
Defining and Selecting the Temperatures 92
Review the Selected Methods 94
Settings for System Volumes, Flushing, Equilibration, Column Storage and Advanced 96
Defining System Volumes 97
Flushing Conditions 102
Column Equilibration Procedure 104
Column Storage Procedures 107
Blank-Run Data File Handling 108
Advanced (Transition Method) 109
Setting Up the Samples 111
Summary 113
Reporting 115

This chapter provides information on installation, use and features of the software.

Overview of the Agilent Method Scouting Wizard

The Agilent ChemStation Method Scouting Wizard is a software add-on to the OpenLab ChemStation that enables you to scout LC parameters like solvent, column, temperature and gradient applying an Agilent Method Development System. The software automatically generated a logical sequence of the different methods including flushing, equilibration and column storage runs.

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 36 on page 71, a part of a complete sequence is shown as an example of how the different steps are put together.

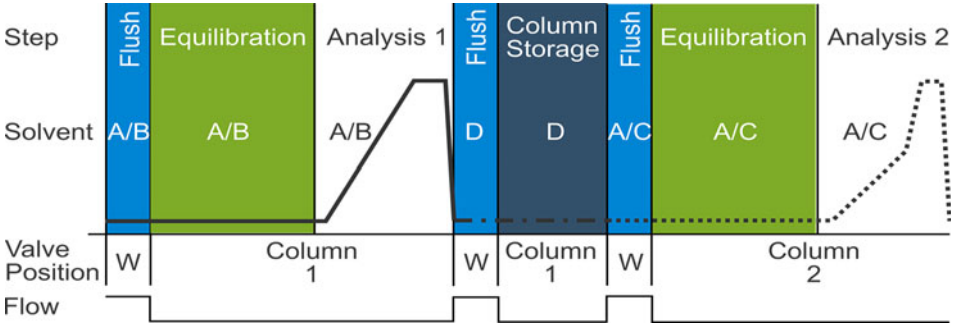


Figure 36 Different steps during a method scouting campaign as generated by the Agilent ChemStation Method Scouting Wizard

The analysis is performed on column 1 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 2 using a new solvent combination A/C. The system has a waste line available (see "Specifying and Selecting Gradients" on page 87 and "Settings for System Volumes, Flushing, Equilibration, Column Storage and Advanced" on page 96).

Features

The Agilent Method Scouting Wizard provides customer benefit in LC method development. For an overview on features of the software, see Table 5 on page 72.

Table 5 Features of Agilent Method Scouting Wizard

Feature	Description
Version	MSW A.02.10 Update 3
Sample introduction	Any of the Agilent 1200 Infinity Series Autosamplers supported by LC&CE Drivers 3.7 can be used. Select your autosampler suitable to the required maximum pressure capability of the system. In particular, the following samplers are supported: • 1260 Infinity II/III SFC MultiSampler (G4767A) • 1260 Infinity II/III Standard Autosampler (G7129C)
Supported screening pumps	• 1290 Infinity Binary Pump (G4220A) • 1290 Infinity Binary Pump VL (G4220B) • 1290 Infinity Quaternary Pump(G4204A) • 1260 Infinity Quaternary Pump (G1311B) • 1260 Infinity Binary Pump (G1312B) • 1260 Infinity Bio-inert Quaternary Pump (G5611A) • 1260 Infinity SFC Binary Pump (G4302A) • 1290 Infinity II/III High Speed Pump (G7120A) • 1290 Infinity II/III Bio High Speed Pump (G7132A) • 1290 Infinity II/III Flexible Pump (G7104A) • 1290 Infinity II/III Bio Flexible Pump (G7131A) • 1260 Infinity II/III Flexible Pump (G7104C) • 1260 Infinity II/III Bio Flexible Pump (G7131C) • 1260 Infinity II/III Quaternary Pump (G7111B) • 1260 Infinity II/III Quaternary VL Pump (G7111A) • 1260 Infinity II/III Binary Pump (G7112A) • 1260 Infinity II/III Binary SFC Pump (G4782A)

Support of pump clusters	• Pump Valve Cluster with all pumps above, and with
	  ◦ G1170A
	  ◦ G4235A
	• 1260 Infinity Preparative Pump Cluster (2x G1361A)

Support of multiple screening pumps, as e.g. in SFC hybrid system	If more than one pump is configured, as e.g. with the G4302A plus LC pump, the user can select which pump will be used in the screening campaign.

Table 5 Features of Agilent Method Scouting Wizard

Feature	Description
Pump specific configuration parameter	The default parameter used for flushing, equilibration, and column storage can be stored separately for each pump.

Supported column screening configuration	• 1260 Infinity II/III Multicolumn Thermostat (G7116A) with 4/10 valve
	• Single 1290 Infinity II/III Multicolumn Thermostat (G7116B) with 4/10, 6/14 or 8/18 valve
	• Valve Thermostat Cluster solution for using up to 32 columns per system.
	  ◦ Multiple valve hosts
	  (Flexible Cubes (G4227A) are not yet supported!)
	  ◦ 1290 Infinity II/III Multicolumn Thermostat (G7116B)
	  ◦ 1290 Infinity Valve Drive (G1170A)
	  ◦ 1290 Infinity Thermostatted Column compartment (G1316C)
	  ◦ Temperature zones
	  ◦ 1290 Infinity II/III Multicolumn Thermostat (G7116B)
	  ◦ 1290 Infinity II/III Integrated Column Compartment (G7130A)
	  ◦ 1290 Infinity Thermostatted Column compartment (G1316C)
	  ◦ 1260 Infinity Thermostatted Column compartment (G1316A)
	• Column compartment cluster of 2 or 3 1290 Infinity Thermostatted Column Compartments (G1316C)

Report templates – Intelligent Reporting – Easy Method Filter	• Several report templates are provided to support identification of most interesting chromatograms. • Special report template available dedicated for export to a spread sheet program, e.g. MS EXCEL. • “Interactive” Report templates where you can set custom criteria interactively to filter your result set. Filter are provided for criteria like Number of Peaks, Minimum Resolution etc.
Automatic scheduling of scouting sequences	The system will automatically schedule a screening sequence (OpenLab ChemStation).

Table 5 Features of Agilent Method Scouting Wizard

Feature	Description
Scouting Sequence Optimization	MSW decides on the optimal sequence run order now based on calculating *several* different run orders with varying combination priorities of solvent, column and temperature changes. These single results, by itself optimal in their run structure, are finally compared to determine the overall optimal sequence with the shortest total run time and best solvent usage under given setup conditions.
Support Intelligent System Emulation Technology (ISET)	MSW supports ISET functionality with solvent screening when using a pump method with enabled emulation mode (ISET)

Software Compatibility

To take full advantage of InfinityLab LC Method Development Solutions, Agilent Method Scouting Wizard (MSW) software is recommended.

To run MSW A.02.10 Update 3 with all its features, it is required to have OpenLab ChemStation LTS 01.11 Update 3 installed.

This MSW version is not backwards compatible and cannot be installed on a lower ChemStation revision.

Please be aware, that neither MassHunter nor EZChrom support MSW.

Instrument Driver

The MSW only supports LC RC.Net drivers; the classic driver architecture is NOT supported!

The following minimal LC RC.Net driver is required for revision MSW A.02.10 Update 3:

- Agilent LC/CE Driver Package 3.7

NOTE

Make sure that driver version 3.7 is installed:

- Check the installed driver version with your OpenLab ChemStation LTS 01.11 Update 3.
- Upgrade or downgrade if the driver version is different.

Table 6 Software Compatibility Matrix

CDS	MSW Version SW needed	Minimal Driver needed
OpenLab ChemStation LTS 01.11 Update 3	MSW A.02.10 Update 3	LC/CE Driver Package 3.7
OpenLab CDS ChemStationEdition C.01.10	MSW A.02.10	LC and CE Driver Package 3.0
OpenLab CDS ChemStationEdition C.01.09	MSW A.02.09	LC and CE Driver Package A.02.19 SR1
OpenLab CDS ChemStationEdition C.01.08	MSW A.02.08	LC and CE Driver Package A.02.18
OpenLab CDS ChemStationEdition C.01.07 SR3	MSW A.02.07	LC and CE Driver Package A.02.16

NOTE

The Method Scouting Wizard compatibility matrix information can also be found in the readme file on the installation media.

Software Installation

Prerequisites

If there is a previous version of the Method Scouting Wizard on your computer, open the Windows **Control Panel**, select **Add or Remove Programs** and select to remove the previous version.

1 To install the Agilent Method Scouting Wizard, follow the instructions given in the Install Instructions.pdf located on the Method Scouting Wizard disk.

Defining the Campaign

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 37 on page 77).

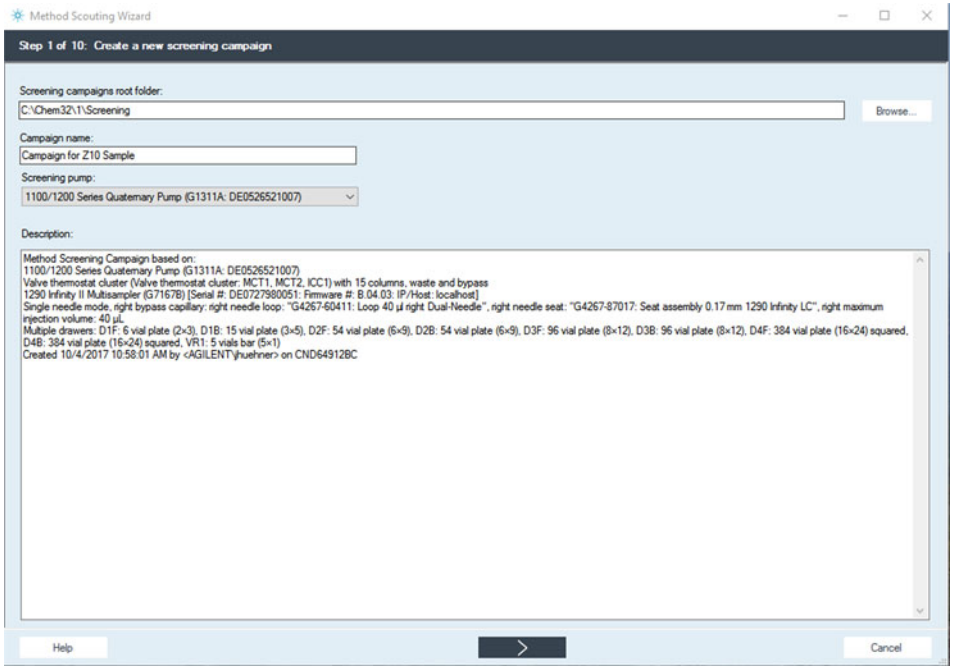


Figure 37 Start screen for a campaign

If the column screening comprises columns with different internal diameters (IDs) and different column lengths, the flow and the injection volume settings in the base method need to be adapted for the column with the highest ID, and the gradient length for the longest column used in the campaign. The same applies for every gradient used in the screening campaign for a correct calculation of flow, gradient length and injection volume, performed by the Method Scouting Wizard. The stop time of the base method must be set in the pump.

NOTE You can open a screening campaign from the previous revision of Method Scouting Wizard. If the current instrument configuration does not match the instrument configuration of the old campaign, the campaign will be adjusted accordingly. When you save the campaign, it is automatically converted into the format of the current revision.

NOTE The last method that was used with the current instrument configuration is selected by default as base method.

You can also view an existing screening campaign; all the information for the campaign is displayed in the sequence of screens, but you cannot make any modifications, and all buttons that allow modification are hidden. The compatibility of the current instrument configuration is not checked, and the base method is not required to be available. This allows you to review the content of any existing campaign without modifying it.

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 38 on page 80).

NOTE The stop time in the base method must be set in the pump.

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 gradient screening is demanded by the user only one gradient has to be defined in the later gradient screening page.

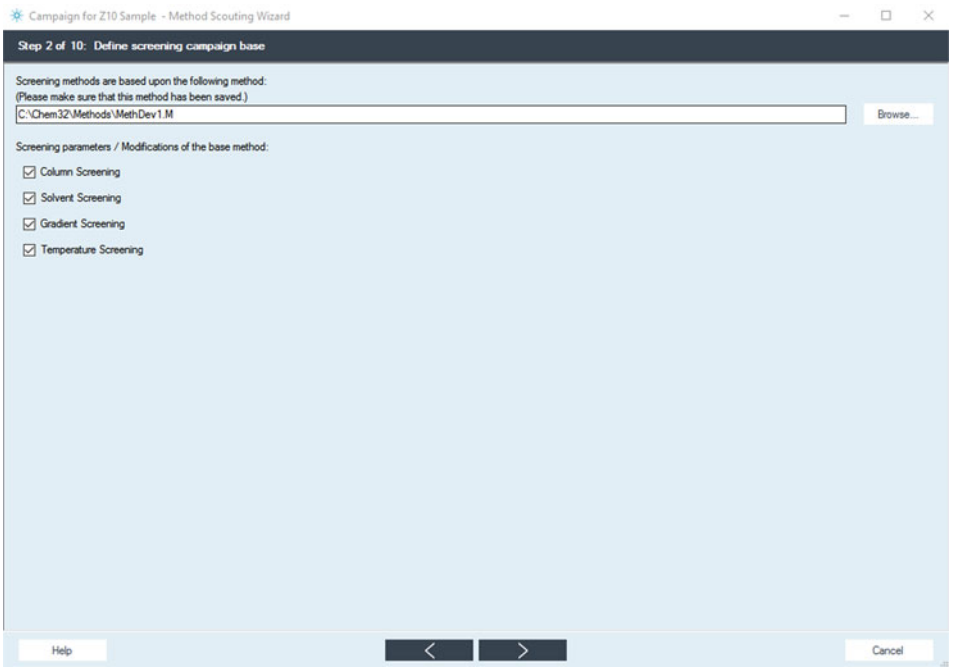


Figure 38 Screen for defining the campaign range and selection of the master method

Column Screening Retrieves a list of all configured columns with some of their properties, for example, column name, column dimension, etc.
For more details, see "Selecting the Columns" on page 81.

Solvent Screening Screens your solvents and allows you to define a solvent combination. The solvent combination possibilities depend on the configured pump, whereas the Method Scouting Wizard automatically detects the system configuration and shows the appropriate possibilities.
For more details, see "Selecting the Solvents" on page 83.

Gradient Screening Displays the composition cardinality according to your selected solvent combination type.
For more details, see "Specifying and Selecting Gradients" on page 87.

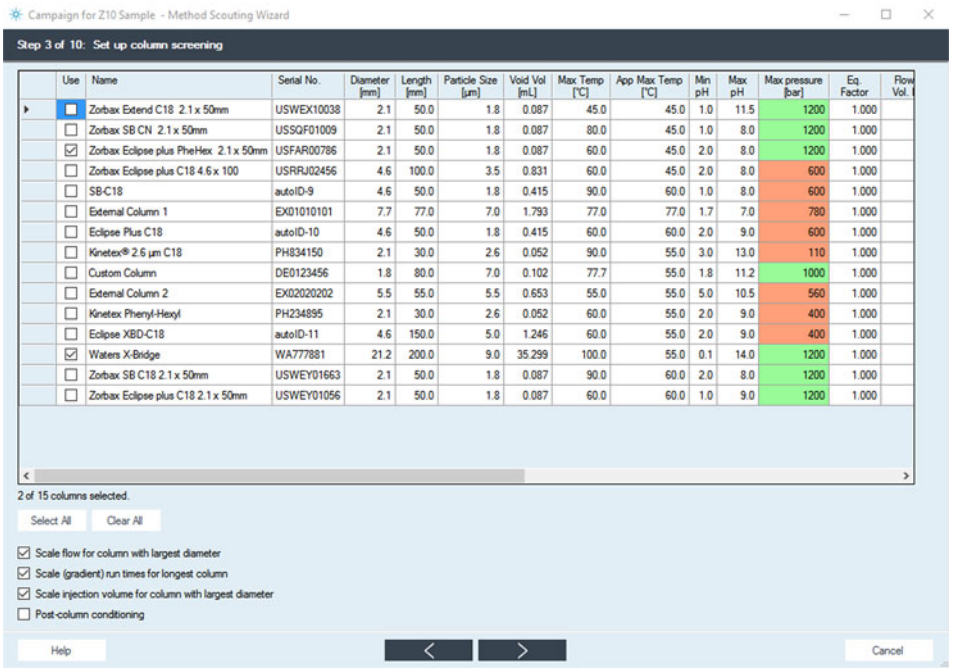
Temperature Screening Set up to screen for multiple temperatures.
For more details, see "Defining and Selecting the Temperatures" on page 92.

NOTE

The option **Solvent Screening** is mandatory when ISET emulation is enabled in the base method of a quaternary pump. Thus, the solvent combination type, the emulation mode and the type of gradient curve are defined.

Selecting the Columns

In this screen, you select the columns to be evaluated (see Figure 39 on page 81). 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. Column properties are read from the Columns table of the ChemStation. You select the columns you want to use in the method scouting campaign by checking the **Use** check boxes.



Use	Name	Serial No.	Diameter [mm]	Length [mm]	Particle Size [µm]	Void Vol [mL]	Max Temp [°C]	App Max Temp [°C]	Min pH	Max pH	Max pressure [bar]	Eq. Factor	Flow Vol.
☐	Zorbax Extend C18 2.1 x 50mm	USWEX10038	2.1	50.0	1.8	0.087	45.0	45.0	1.0	11.5	1200	1.000	
☐	Zorbax SB CN 2.1 x 50mm	USSQF01009	2.1	50.0	1.8	0.087	80.0	45.0	1.0	8.0	1200	1.000	
☑	Zorbax Eclipse plus PheHex 2.1 x 50mm	USFAR00786	2.1	50.0	1.8	0.087	60.0	45.0	2.0	8.0	1200	1.000	
☐	Zorbax Eclipse plus C18 4.6 x 100	USRRJ02456	4.6	100.0	3.5	0.831	60.0	45.0	2.0	8.0	600	1.000	
☐	SB-C18	autoID-9	4.6	50.0	1.8	0.415	90.0	60.0	1.0	8.0	600	1.000	
☐	External Column 1	EX01010101	7.7	77.0	7.0	1.793	77.0	77.0	1.7	7.0	780	1.000	
☐	Eclipse Plus C18	autoID-10	4.6	50.0	1.8	0.415	60.0	60.0	2.0	9.0	600	1.000	
☐	Kinetex® 2.6 µm C18	PH834150	2.1	30.0	2.6	0.052	90.0	55.0	3.0	13.0	110	1.000	
☐	Custom Column	DE0123456	1.8	80.0	7.0	0.102	77.7	55.0	1.8	11.2	1000	1.000	
☐	External Column 2	EX02020202	5.5	55.0	5.5	0.653	55.0	55.0	5.0	10.5	560	1.000	
☐	Kinetex Phenyl-Hexyl	PH234895	2.1	30.0	2.6	0.052	60.0	55.0	2.0	9.0	400	1.000	
☐	Eclipse XBD-C18	autoID-11	4.6	150.0	5.0	1.246	60.0	55.0	2.0	9.0	400	1.000	
☑	Waters X-Bridge	WA777881	21.2	200.0	9.0	35.299	100.0	55.0	0.1	14.0	1200	1.000	
☐	Zorbax SB C18 2.1 x 50mm	USWEY01663	2.1	50.0	1.8	0.087	90.0	60.0	2.0	8.0	1200	1.000	
☐	Zorbax Eclipse plus C18 2.1 x 50mm	USWEY01056	2.1	50.0	1.8	0.087	60.0	60.0	1.0	9.0	1200	1.000	

Figure 39 Selection of columns

The check boxes below the table allow you to define post-column conditioning, and to scale flow rates, gradients, injection volume for columns with different dimensions.

Scale flow for column with largest diameter	Ensures that the velocity of the eluent is maintained irrespective of the diameter of the column. Flow rates of narrower columns are reduced relative to those of wider columns in the ratio of the squares of the column internal diameters.
Scale (gradient) run times for longest column	Ensures that the full gradient will run on all columns, irrespective of their length. All gradient time points, the run time and the posttime for shorter columns are reduced relative to those of longer columns in the ratio of the column lengths.
Scale injection volume for column with largest diameter	Ensures that the injection volume is adjusted to the column volume in order to always apply the same scaling factor.
Post-column conditioning	To set a controlled temperature at the specified thermostat for all methods; it also includes flush, equilibration, injection, and column storage runs. You can select only thermostats that are not used for selected **Column Screening**. Other thermostats that are not (or no longer) used for column screening and preheating are not controlled for temperature. Selecting and deleting columns from the screening that occupy or release a thermostat zone affects the list of available thermostats for post-column conditioning. The applicable temperature range depends on the selected thermostat and the columns connected to it. Post-column conditioning is available if the instrument has at least one thermostat with at least two temperature zones that can be controlled independently in the method. If left and right temperature zones are connected by at least one long (full length) column, then the zones are not independent; they are treated as one combined zone.

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

Selecting the Solvents

If solvent screening is selected, the solvents to be varied are specified in the following screen (see Figure 40 on page 84). The Method Scouting Wizard automatically detects the system configuration and shows the appropriate screen.

Setup Solvents with a Binary Pump

Allows the solvents from channel A to be combined with solvents from channel B, see Figure 40 on page 84 .

The solvents can also be accessed via an internal or external solvent selection valve; in this case, the solvent names are prefixed accordingly. If an additional internal solvent selection valve is installed, the solvents to be used can be selected. If an additional external G4235A 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.

Select the check box of each solvent channel you want to use to create binary gradients. If all check boxes are selected, all solvent combinations will be used. If your selection is error-free, the number of solvent combinations (i.e. the number of screening states) is displayed in the status line, calculated as: no. of solvent combinations = no. of selected solvents on channel A × no. of selected solvents on channel B

Fill in the ratio of channel A to channel B (in %).

For a binary pump without any additional solvent selection valves, no solvent choices are available.

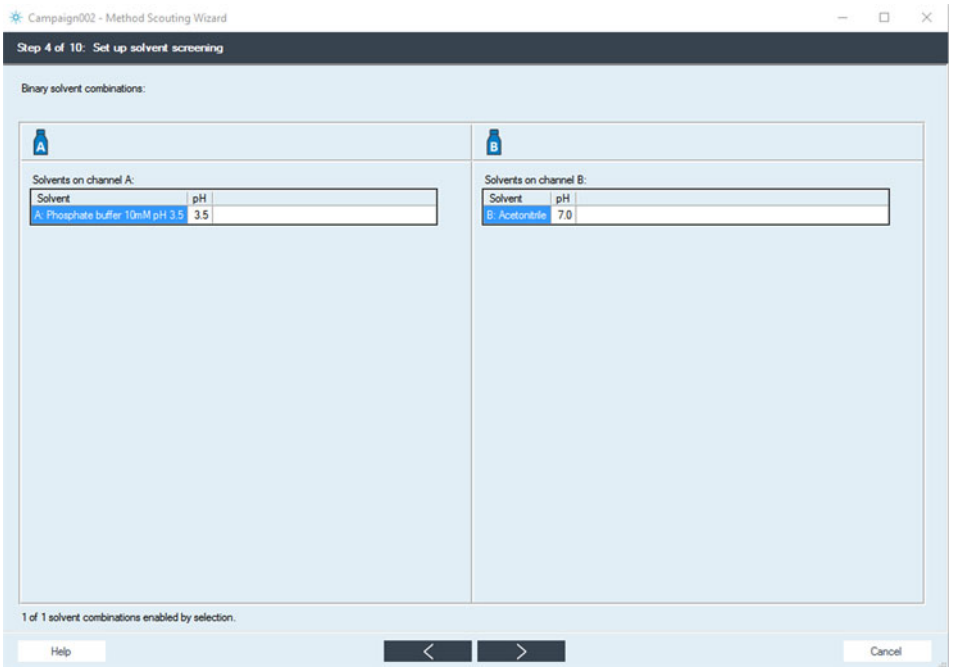


Figure 40 Selecting the solvent with a binary pump

Setup Solvents with a Quaternary Pump

If a quaternary pump is installed, the **Set up solvent screening** screen looks slightly different, see Figure 41 on page 85. Again, the position of an optionally installed external solvent selection valve is detected. Additionally, you choose to create binary, ternary or quaternary gradients.

The number of possible solvent combinations depends on the presence of a solvent selection valve, and the number of solvents selected. The available solvents for each channel are listed, and the position of each channel in the combination is shown. For the quaternary combination, the positions are fixed; for binary and ternary combinations, change the position of each channel in the combination by selecting its check box. Or, drag the channel bottle and drop it in the field where you want to use it. If there is no error, the total number of solvent combinations is displayed in the status line. The number is calculated as the product of the number of selected solvents for each combination.

Three different ways of combining the available channels:

- **binary**: Use any combination of two of the four channels; the unused channels are turned off. This results in either three or four possible combinations of channels.
- **ternary**: Use any combination of three of the four channels; the unused channels are turned off. This results in two possible combinations of channels.
- **quaternary**: Use all four channels. This results in only one combination of channels. 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).

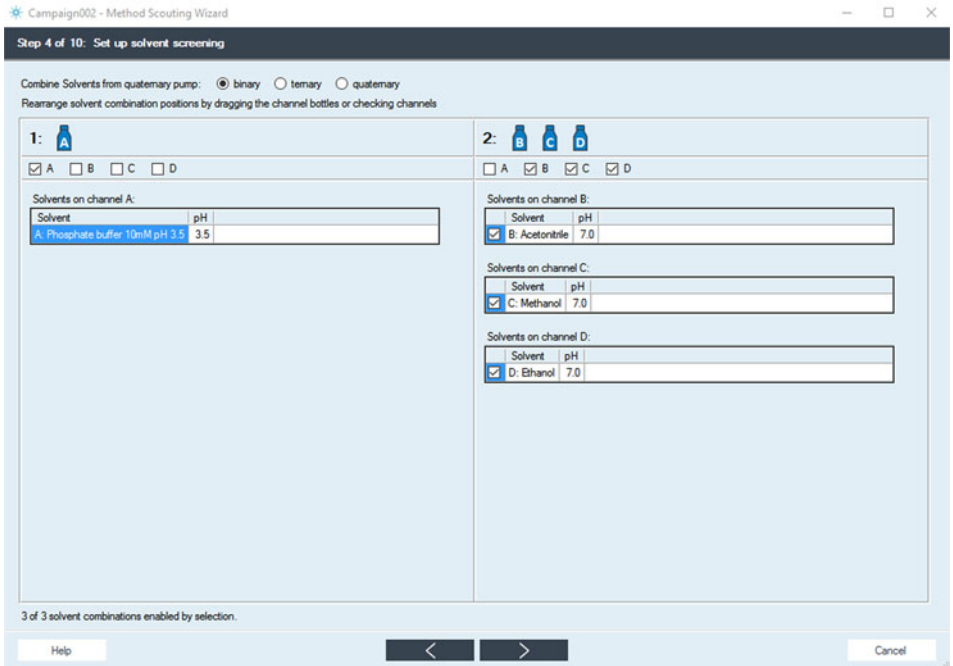


Figure 41 **Set up solvents screening using a quaternary pump, a setup for a binary gradient of channel A (with an attached external solvent selection valve) versus the channels B, C and D is shown.**

NOTE

If you have configured a quaternary pump, you can only use the **Solvent Screening** option in combination with the **Gradient Screening** option. This guarantees that the solvent concentrations are clearly defined for a specific solvent combination.

NOTE

The option **Solvent Screening** is mandatory when ISET emulation is enabled in the base method of a quaternary pump. Thus, the solvent combination type, the emulation mode and the type of gradient curve are defined.

If no Pump Valve Cluster is set-up and you use a single pump, the entry of the pH value is not supported at the driver level. In this case, the Agilent ChemStation Method Scouting Wizard allows you to enter the pH-values of the solvents at this point.

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 42 on page 87) in the **Table** tab or graphically (see Figure 43 on page 88) in the **Graph** tab. 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 in the **Overlay** tab, see Figure 44 on page 89.

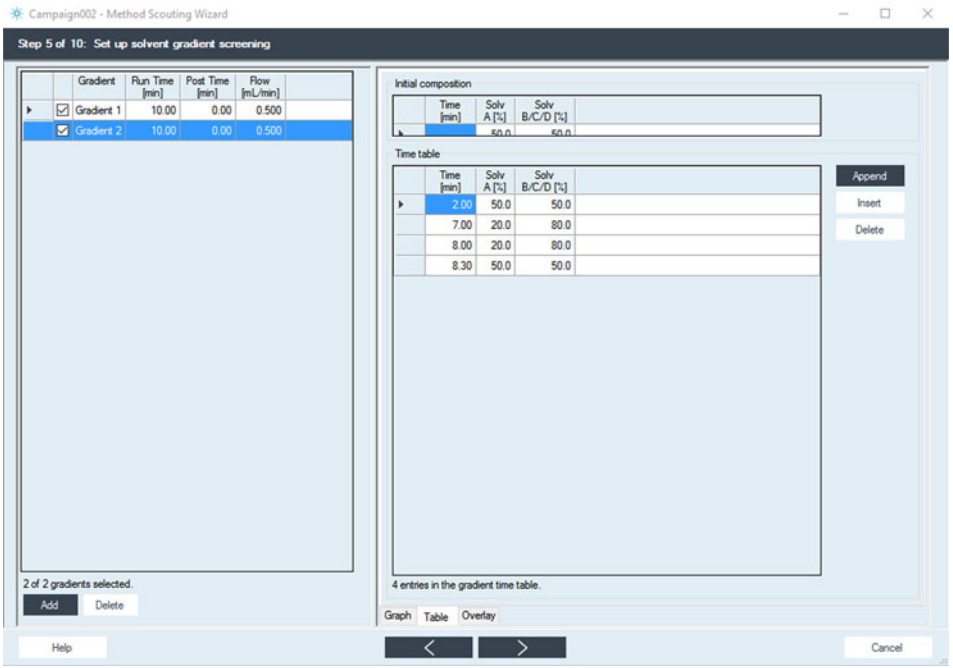


Figure 42 Defining and selecting different gradients - gradient table

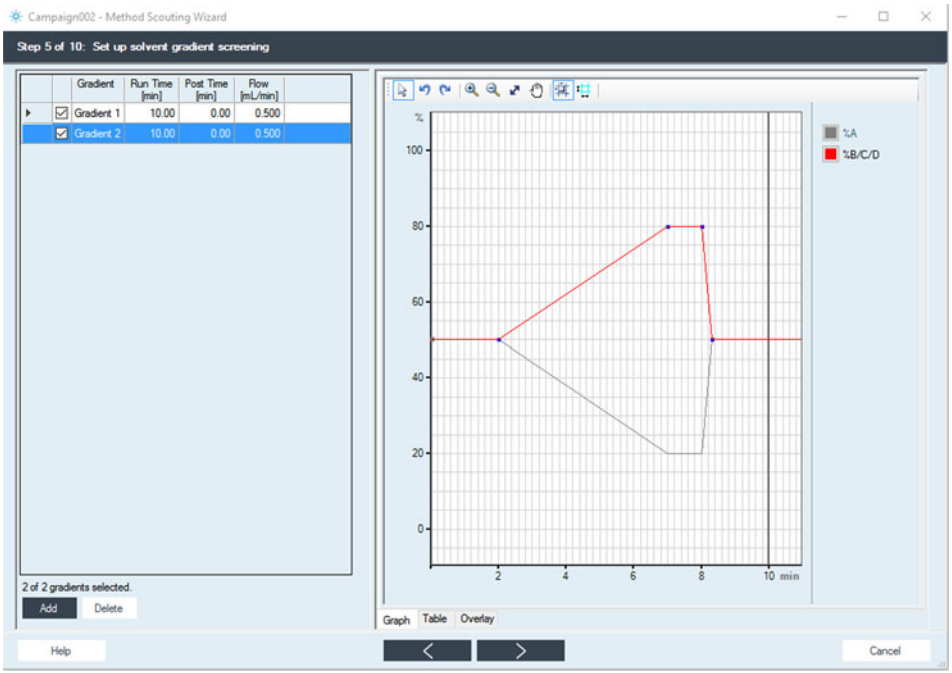


Figure 43 Defining and selecting different gradients – graphical interface

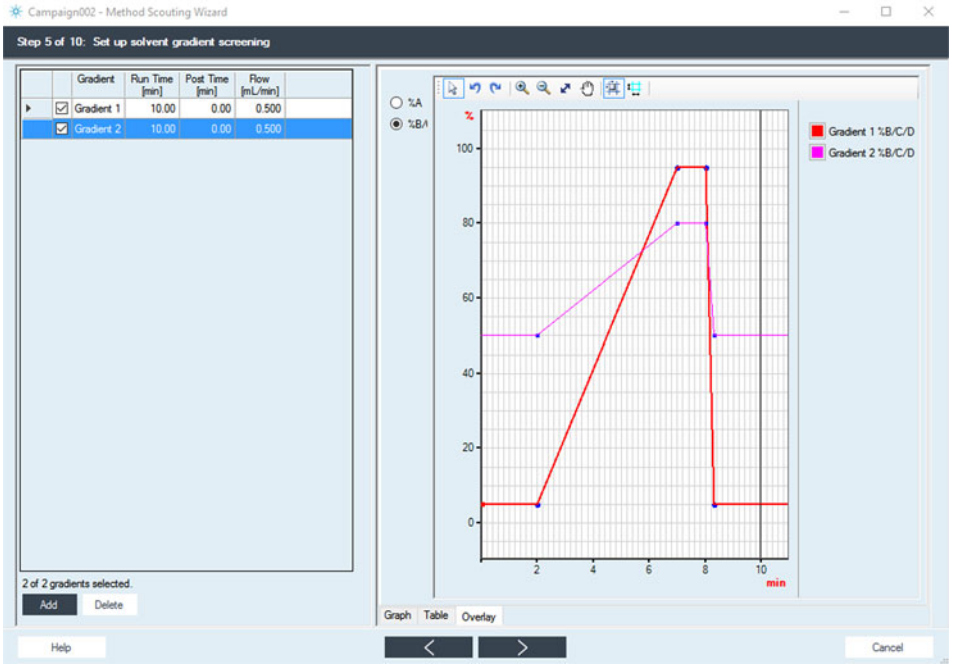


Figure 44 Defining and selecting different gradients – overlay interface

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 45 on page 90). To correctly specify gradients, you also need to consider how the Method Scouting Wizard applies the Equilibration procedures (see "Settings for System Volumes, Flushing, Equilibration, Column Storage and Advanced" on page 96). 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 non-reproducible results, see Figure 47 on page 91).

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 "Settings for System Volumes, Flushing, Equilibration, Column Storage and Advanced" on page 96.

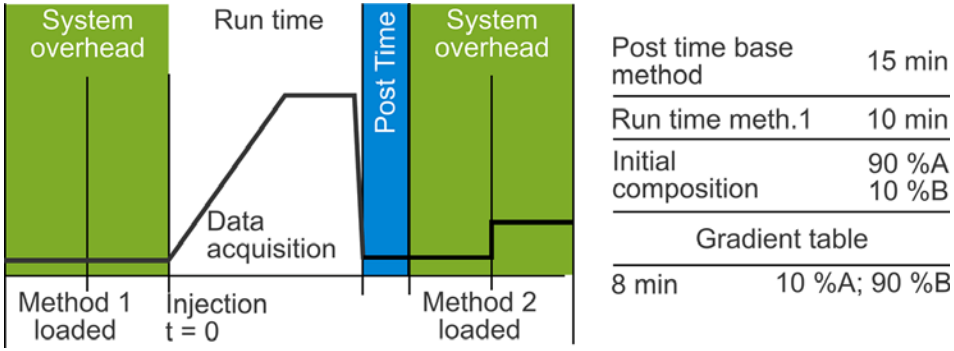
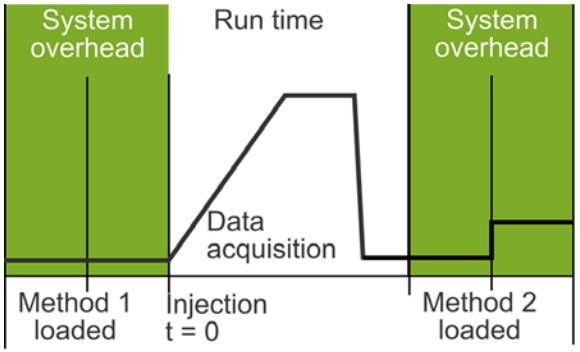


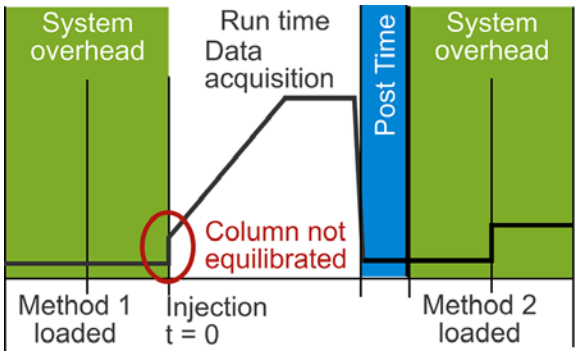
Figure 45 Typical gradient setting with post-time. Equilibration occurs during the post-time without data acquisition during that time.



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 46 **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 15 %B

Gradient table	
0 min	90 %A; 10 %B
8 min	10 %A; 90 %B

Figure 47 **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.**

NOTE

The pump flow rates apply at least for the methods for columns with the largest internal diameter. If the **Scale flow for column with largest diameter** check box is marked on the **Set up column screening** page, then the flow rates will be adjusted relative to the squares of the column IDs.

The gradient times apply at least for the methods for the longest columns. If the **Scale (gradient) run times for largest column** check box is marked on the **Set up column screening** page, then the gradient times will be adjusted relative to the column lengths.

Defining and Selecting the Temperatures

If you also selected temperature screening, you can enter the temperatures you want to test (see Figure 48 on page 93). You can enter all values that are available for the given system.

NOTE

If you are using a mixed system containing column compartments of the G1316-series and G7116-series (column compartment G1316C or G7116B and column compartment G1316A or G7116A), the maximum temperature is defined by the G1316A or G7116A (80 °C/85 °C instead of 100 °C/110 °C). The lowest temperature that can be entered is 4 °C.

The G7116B column compartment, for example, is able to cool to 20 °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 approximately 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 analysis to be enabled 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.

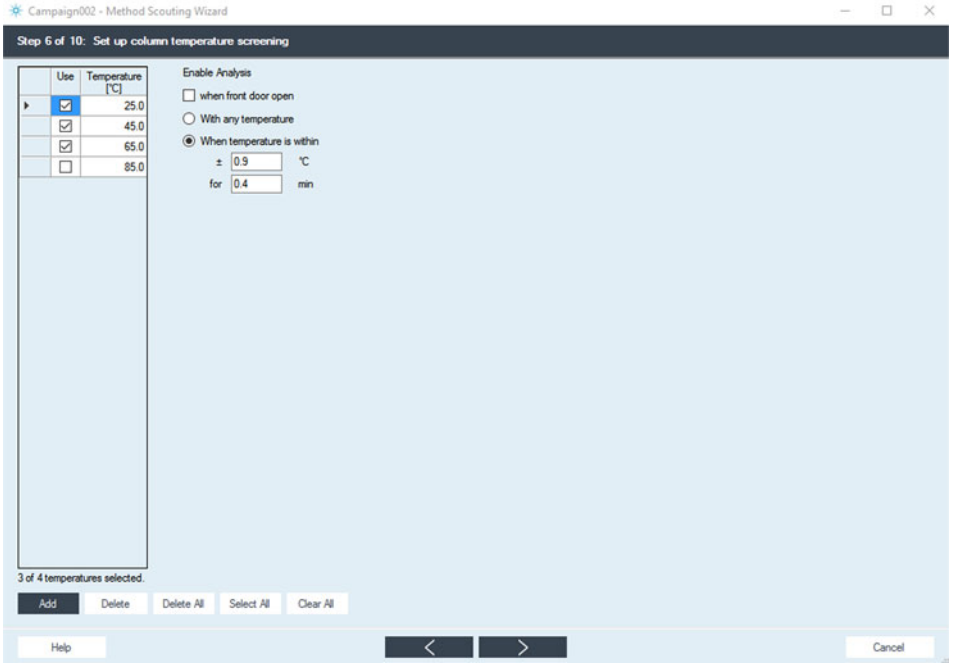


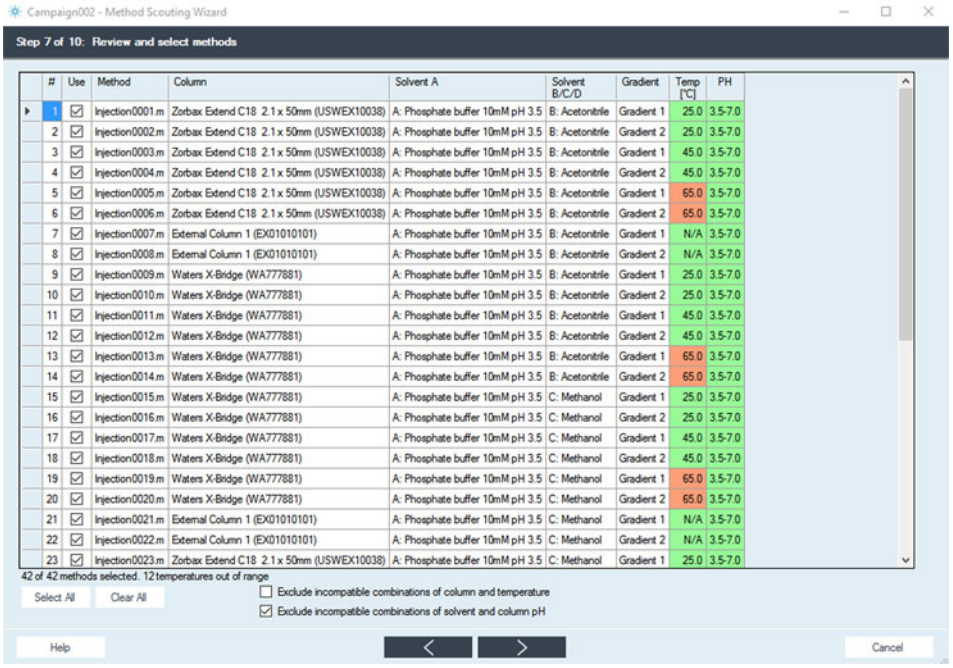
Figure 48 Temperature screening window

Review the Selected Methods

This screen shows you an overview of all combinations of columns, solvents, gradients and temperatures (see Figure 49 on page 95). 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/min 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 G4235A solvent selection valve is clustered to the system or in the solvent screening interface. The allowed pH range 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. If the maximum temperature value for a column is not available, you cannot exclude columns on the basis of temperature; if the pH values for a column are not available, you cannot exclude columns on the basis of pH.

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.



#	Use	Method	Column	Solvent A	Solvent B/C/D	Gradient	Temp [°C]	PH
1	☑	Injection0001.m	Zorbax Extend C18 2.1 x 50mm (USWEX10038)	A: Phosphate buffer 10mM pH 3.5	B: Acetonitrile	Gradient 1	25.0	3.5-7.0
2	☑	Injection0002.m	Zorbax Extend C18 2.1 x 50mm (USWEX10038)	A: Phosphate buffer 10mM pH 3.5	B: Acetonitrile	Gradient 2	25.0	3.5-7.0
3	☑	Injection0003.m	Zorbax Extend C18 2.1 x 50mm (USWEX10038)	A: Phosphate buffer 10mM pH 3.5	B: Acetonitrile	Gradient 1	45.0	3.5-7.0
4	☑	Injection0004.m	Zorbax Extend C18 2.1 x 50mm (USWEX10038)	A: Phosphate buffer 10mM pH 3.5	B: Acetonitrile	Gradient 2	45.0	3.5-7.0
5	☑	Injection0005.m	Zorbax Extend C18 2.1 x 50mm (USWEX10038)	A: Phosphate buffer 10mM pH 3.5	B: Acetonitrile	Gradient 1	65.0	3.5-7.0
6	☑	Injection0006.m	Zorbax Extend C18 2.1 x 50mm (USWEX10038)	A: Phosphate buffer 10mM pH 3.5	B: Acetonitrile	Gradient 2	65.0	3.5-7.0
7	☑	Injection0007.m	External Column 1 (EX01010101)	A: Phosphate buffer 10mM pH 3.5	B: Acetonitrile	Gradient 1	N/A	3.5-7.0
8	☑	Injection0008.m	External Column 1 (EX01010101)	A: Phosphate buffer 10mM pH 3.5	B: Acetonitrile	Gradient 2	N/A	3.5-7.0
9	☑	Injection0009.m	Waters X-Bridge (WA777881)	A: Phosphate buffer 10mM pH 3.5	B: Acetonitrile	Gradient 1	25.0	3.5-7.0
10	☑	Injection0010.m	Waters X-Bridge (WA777881)	A: Phosphate buffer 10mM pH 3.5	B: Acetonitrile	Gradient 2	25.0	3.5-7.0
11	☑	Injection0011.m	Waters X-Bridge (WA777881)	A: Phosphate buffer 10mM pH 3.5	B: Acetonitrile	Gradient 1	45.0	3.5-7.0
12	☑	Injection0012.m	Waters X-Bridge (WA777881)	A: Phosphate buffer 10mM pH 3.5	B: Acetonitrile	Gradient 2	45.0	3.5-7.0
13	☑	Injection0013.m	Waters X-Bridge (WA777881)	A: Phosphate buffer 10mM pH 3.5	B: Acetonitrile	Gradient 1	65.0	3.5-7.0
14	☑	Injection0014.m	Waters X-Bridge (WA777881)	A: Phosphate buffer 10mM pH 3.5	B: Acetonitrile	Gradient 2	65.0	3.5-7.0
15	☑	Injection0015.m	Waters X-Bridge (WA777881)	A: Phosphate buffer 10mM pH 3.5	C: Methanol	Gradient 1	25.0	3.5-7.0
16	☑	Injection0016.m	Waters X-Bridge (WA777881)	A: Phosphate buffer 10mM pH 3.5	C: Methanol	Gradient 2	25.0	3.5-7.0
17	☑	Injection0017.m	Waters X-Bridge (WA777881)	A: Phosphate buffer 10mM pH 3.5	C: Methanol	Gradient 1	45.0	3.5-7.0
18	☑	Injection0018.m	Waters X-Bridge (WA777881)	A: Phosphate buffer 10mM pH 3.5	C: Methanol	Gradient 2	45.0	3.5-7.0
19	☑	Injection0019.m	Waters X-Bridge (WA777881)	A: Phosphate buffer 10mM pH 3.5	C: Methanol	Gradient 1	65.0	3.5-7.0
20	☑	Injection0020.m	Waters X-Bridge (WA777881)	A: Phosphate buffer 10mM pH 3.5	C: Methanol	Gradient 2	65.0	3.5-7.0
21	☑	Injection0021.m	External Column 1 (EX01010101)	A: Phosphate buffer 10mM pH 3.5	C: Methanol	Gradient 1	N/A	3.5-7.0
22	☑	Injection0022.m	External Column 1 (EX01010101)	A: Phosphate buffer 10mM pH 3.5	C: Methanol	Gradient 2	N/A	3.5-7.0
23	☑	Injection0023.m	Zorbax Extend C18 2.1 x 50mm (USWEX10038)	A: Phosphate buffer 10mM pH 3.5	C: Methanol	Gradient 1	25.0	3.5-7.0

Figure 49 Review and select analytical runs to be performed

Settings for System Volumes, Flushing, Equilibration, Column Storage and Advanced

This important screen allows you to specify the following settings (see Figure 50 on page 98 and Figure 52 on page 105):

- 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
- how the flow is reduced to avoid pressure spikes when switching the column switching valve

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 50 on page 98). 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. If 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. For the G1322A degasser, this volume can be significant.

NOTE

If two external solvent selection valves are configured in the system, only degassing either before or after the external solvent selection valves is supported. A configuration with one valve before the degasser and one after it is not supported.

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 corresponding volumes in the system diagram will be highlighted.

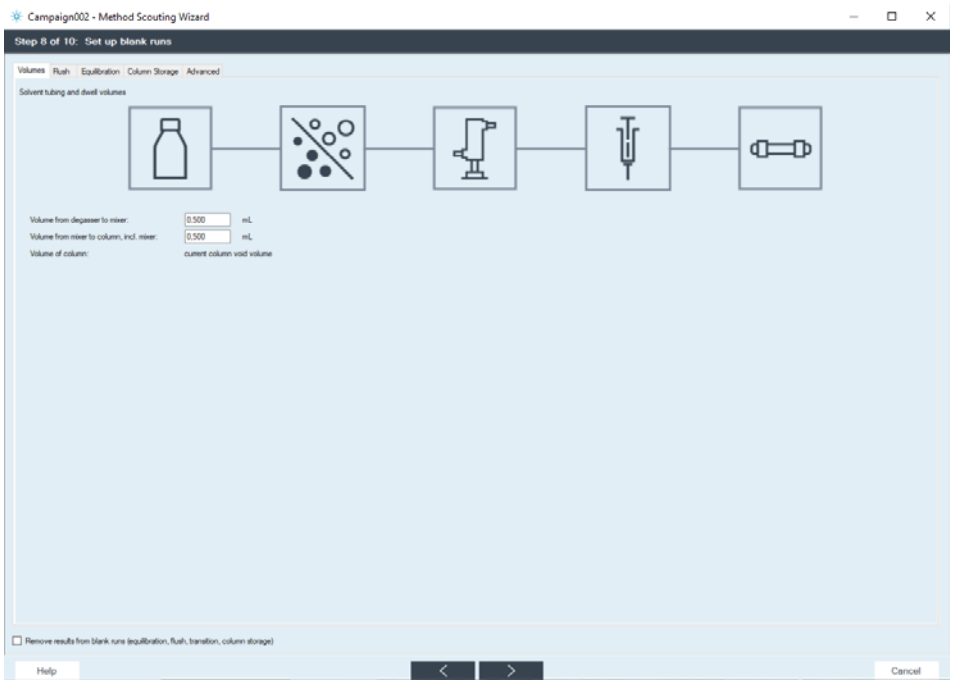


Figure 50 Setting of the volumes in the system

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 corresponding solvent
- volume from solvent selection valve to degasser (including the degasser chamber) — this volume can be flushed with 100 % of the corresponding 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

Table 7 on page 99 and Table 8 on page 100 gives you some guidelines for the volumes of different parts of a system.

Table 7 Typical internal volumes of parts that need to be flushed for a solvent change (for pumps)

Pump	Mixer	Damper	Internal Volume	Comment
G1311A	yes	yes	850 µL	at 200 bar
G1311B	yes	yes	850 µL	at 300 bar
G1312A	yes	yes	850 µL	at 200 bar
G1312B	yes no	yes no	860 µL 220 µL	at 300 bar at 300 bar
G7111A	yes	yes	850 µL	at 200 bar
G7111B	yes	yes	850 µL	at 300 bar
G7112B	yes	yes	850 µL	at 300 bar
G4220A/B	no 35 µL 100 µL 380 µL	no no no no	33 µL 75 µL 160 µL 390 µL	
G7120A	no 35 µL 100 µL 380 µL	no no no no	33 µL 75 µL 160 µL 390 µL	
G7132A	no 35 µL 100 µL 380 µL	no no no no	33 µL 75 µL 160 µL 390 µL	
G4204A	no 35 µL 100 µL 380 µL	no no no no	33 µL 75 µL 160 µL 390 µL	
G7104A/C	no 35 µL 100 µL 380 µL	no no no no	33 µL 75 µL 160 µL 390 µL	
G7131A/C	no 35 µL 100 µL 380 µL	no no no no	33 µL 75 µL 160 µL 390 µL	

Table 8 Typical internal volumes of parts that need to be flushed for a solvent change

Part	Internal Volume (geometric)	Comment
Built-in degassing unit (G4220A/B, G7120A, G4204A, G1311B, G7111B pumps)	1.5 mL	
G4225A Degasser	0.45 mL	per channel
Solvent tubing 1.5 mm ID	1.8 mL/m	
Capillary 0.17 mm ID	0.02 mL/m	
Capillary 0.12 mm ID	0.01 mL/m	
Sampler (hydraulic volume without seat):		
G1329A/B, G1313A, G1367A/B/C/D/E, G5667A, G4226A, G1120 LC System Sampler, G1220 LC/VL System Sampler	0.3 mL	Sample loop 100 µL
G1367D, G1377A	0.142 mL	Sample loop 40 µL
G1367E	0.118 mL	Sample loop 40 µL
G4226A	0.08 mL	Sample loop 20 µL
G4226A	0.118 mL	Sample loop 40 µL
G5668A	0.118 mL	Sample loop 40 µL
G7167A/B, G7129A/B/C	0.04 mL	Sample loop 40 µL
G7167A/B, G7129A/B/C	0.0615 mL	Sample loop 100 µL
G7167A/B, G7129A/C	0.1772 mL	Sample loop 900 µL

NOTE

Sampler: The seat volume has to be added as configured by the customer.

Example:

Degasser *after solvent selection valve*, system with a binary pump G7120A / 100 µL mixer and the G7167B autosampler with 100 µL Syringe:

Volume from SSV to degasser, incl. degasser:

Ca. 30 cm tubing	0.54 mL
Built-in degaasing unit	1.5 mL
	2.04 mL

Volume from degasser to mixer:

20 cm tubing	0.36 mL
Pump head	0.06 mL
	0.42 mL

Volume from mixer to column, incl. mixer:

Mixer	0.10 mL
Capilliaries ca. 1.2 m 0.12 ID	0.013 mL
Autosampler	0.0615 mL
	0.1745 mL

In case more than 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 non-reproducible results.

However it might be appropriate to deselect this option, as for example in the following cases:

- The volumes to be flushed are very low, or
- high flow rates and equilibration conditions cover the times needed to flush the solvent delivery system.

First, it is necessary to select the flushing solvent (see Figure 51 on page 103). 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 "Column Storage Procedures" on page 107).

NOTE

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

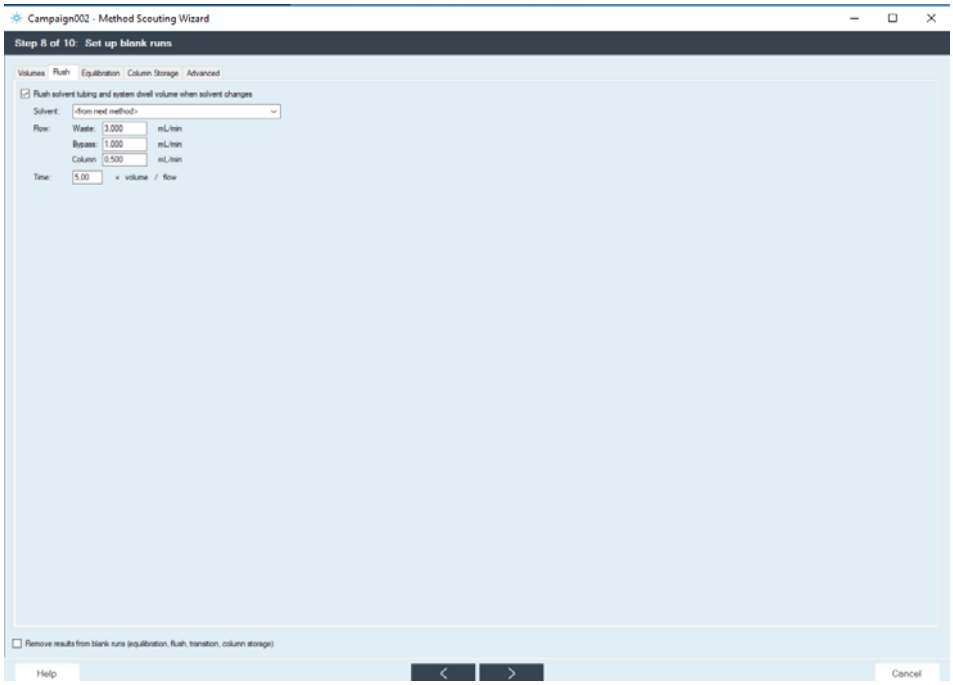


Figure 51 Column settings for flushing conditions

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 between 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:

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

where

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

You can also calculate the void volume of the column with the HPLC Advisor App.

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.

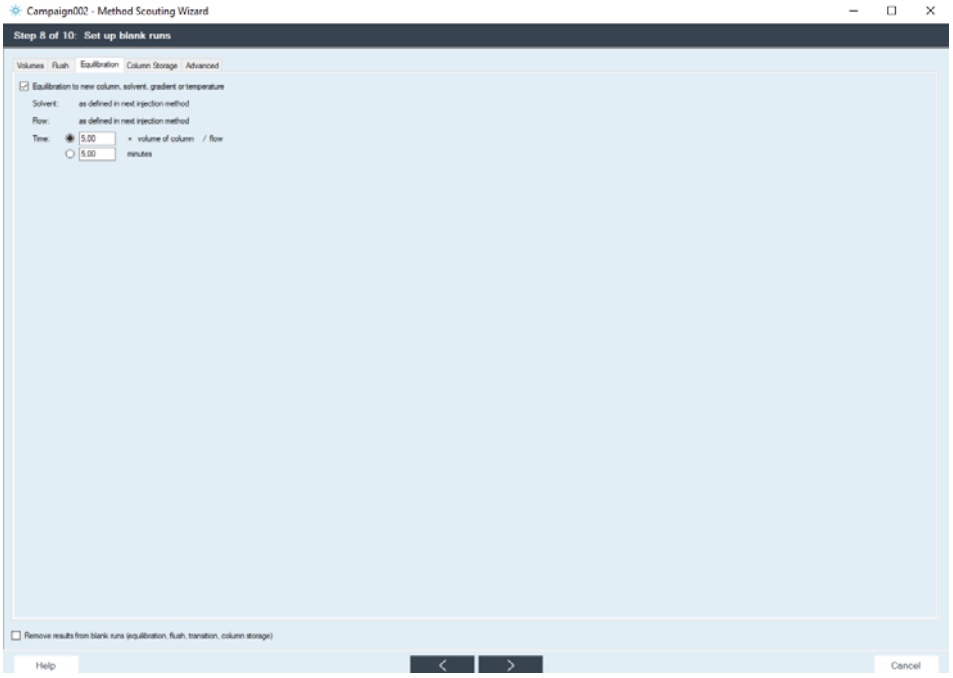


Figure 52 Settings for equilibration conditions

If the equilibration time has been specified directly in minutes, and not calculated from the column void volume, and time scaling has been defined on the **Set up column screening** page, the respective column length ratio, r, is applied to scale the time as given in the user interface.

The equilibration time specified here refers to columns without extended equilibration factor.

The actual equilibration time is a multiple of the time specified here and the column equilibration factor. The actual equilibration time is displayed in the Sequence tab on the **Summary** page.

The equilibration factor f_e applies to the equilibration time t_e only. If a flush tubing volume flow time t_{flush} has been added to the equilibration time t_e, the extended equilibration time t_{ext} computes to:

$$t_{ext} = f_e * t_e + t_{flush}$$

When the column length ratio, r, is also applied, the equation becomes:

$$t_{ext} = r * f_e * t_e + t_{flush}$$

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.

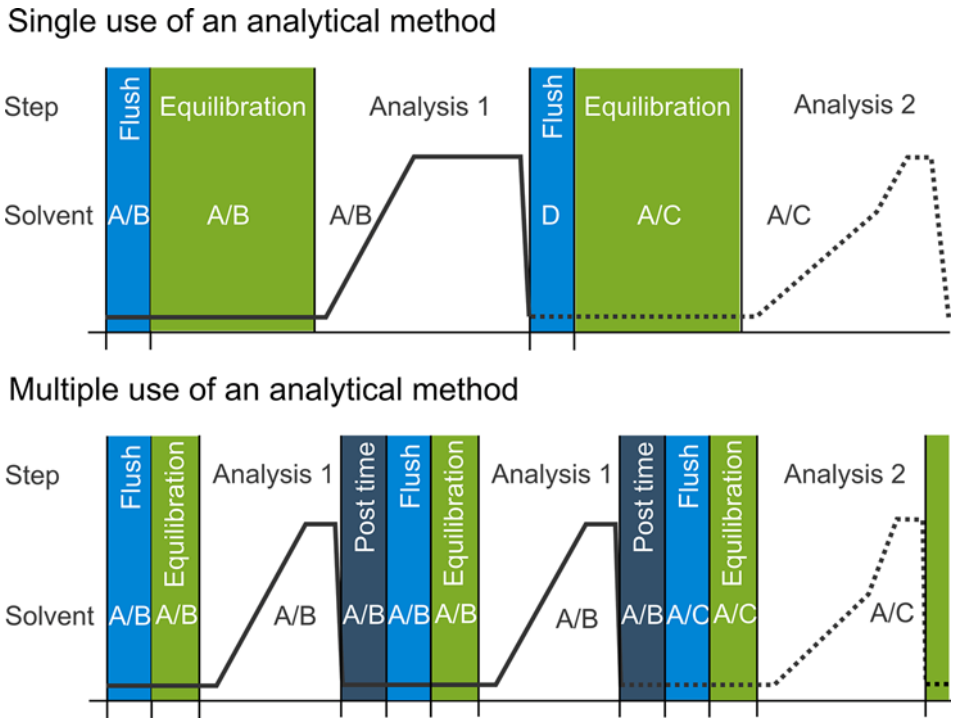


Figure 53 Top: an analytical method is used only once, then new conditions are applied. The Equilibration procedure ensures proper column equilibration under the new conditions. Bottom: multiple injections or multiple samples are planned. Since the Equilibration procedure is applied only after a change 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, see Figure 54 on page 107. 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.

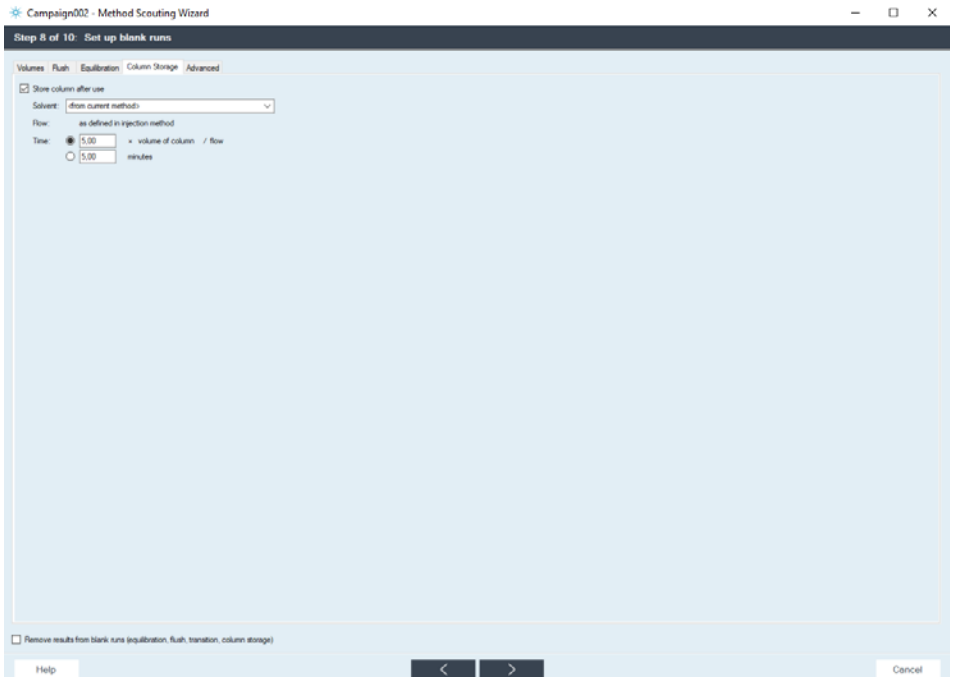


Figure 54 Settings for column storage conditions

If an extra column storage solvent is used and the flow has been defined directly, and not by the injection/base method, and flow scaling has been defined on the

Set up column screening page, the respective column squared-diameter ratio is applied to scale the flow as given in the user interface.

If the time has been specified directly in minutes, and not by column void volume, and time scaling has been defined on the **Set up column screening** page, the respective column length ratio, r, is applied to scale the time as given in the user interface.

The column storage time as specified here refers to columns without extended storage factor.

The actual column storage time is a multiple of the time as specified on this screen and the column storage factor as specified on the **Set up column screening** page. The actual column storage time is displayed in the **Sequence** tab on the **Summary** page.

The equilibration factor f_e applies to the column storage time t_{cs}only. If a flush tubing volume flow time t_{flush} has been added to the column storage time t_{cs}, then the extended column storage time t_{ext} computes to:

$$t_{ext} = f_e * t_{cs} + t_{flush}$$

When the column length ratio, r, is also applied, the equation becomes:

$$t_{ext} = r * f_e * t_{cs} + t_{flush}$$

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).

Advanced (Transition Method)

The tab allows you to:

- enable or disable a transition method to reduce flow when switching the column valve to avoid pressure spikes. By default, the transition method is enabled
- adjust the flow ramp (maximum flow gradient) for each method type. By default, either your last setting or 10 mL/min² is used

Such a transition method reduces the flow rate to 5 % of the analytical flow of the following method. The time of the transition method is calculated from its ramp-down speed and flow difference to the preceding method. Therefore, the column valve switch happens under low flow and pressure, protecting sensitive columns. A transition method can precede an equilibration method (if activated, otherwise the injection method). It can precede a flush column method, if it follows a flush waste or bypass using an extra flush solvent. Or it can precede a column storage method, if an extra column storage solvent is used that requires flush to waste or bypass.

Pumps with an extended flow ramp range (G4220, G4204, G7104, G7120, G7132) support a separate maximum flow ramp value for the ramp up and ramp down. The flow ramp settings of the base method are displayed read-only. The flow ramp settings of every other applicable method type (flush, transition, equilibration, injection, column storage) are displayed for editing. If transition methods are activated, the flow ramp down speed of the transition method is used to calculate the run time of the transition method, considering the flow difference to the preceding method. In this case, the time taken to ramp-up the transition method flow to the flow of the following method is calculated using the flow ramp up speed of the equilibration, flush or column storage method, whichever applies. This flow ramp-up time is added to the respective blank run method.

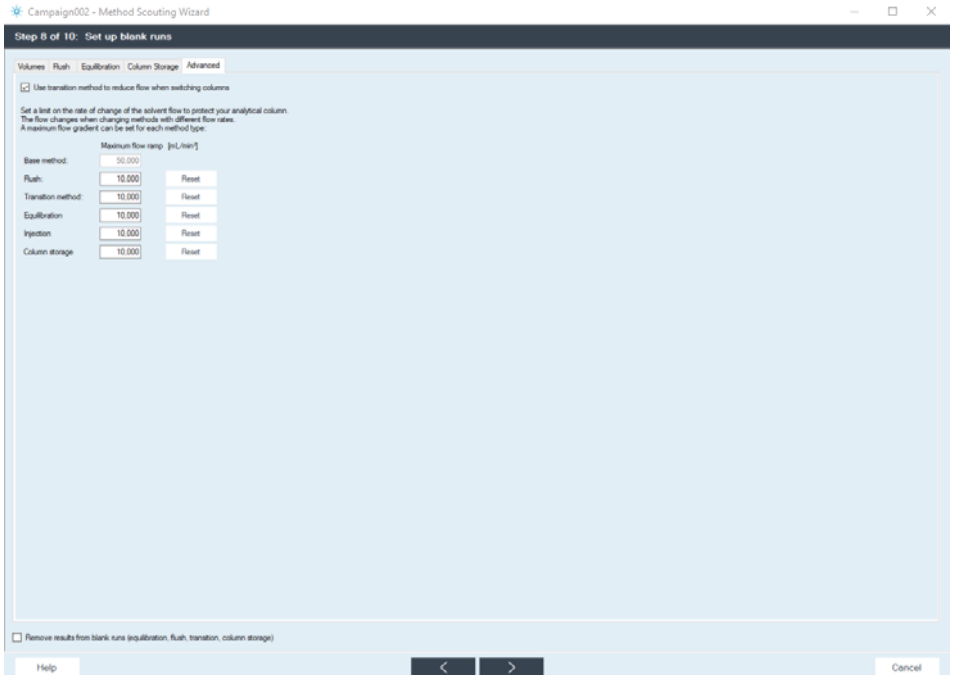


Figure 55 Advanced Settings

Setting Up the Samples

The samples to be analyzed under the different method conditions are specified in this screen (see Figure 56 on page 112). 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.

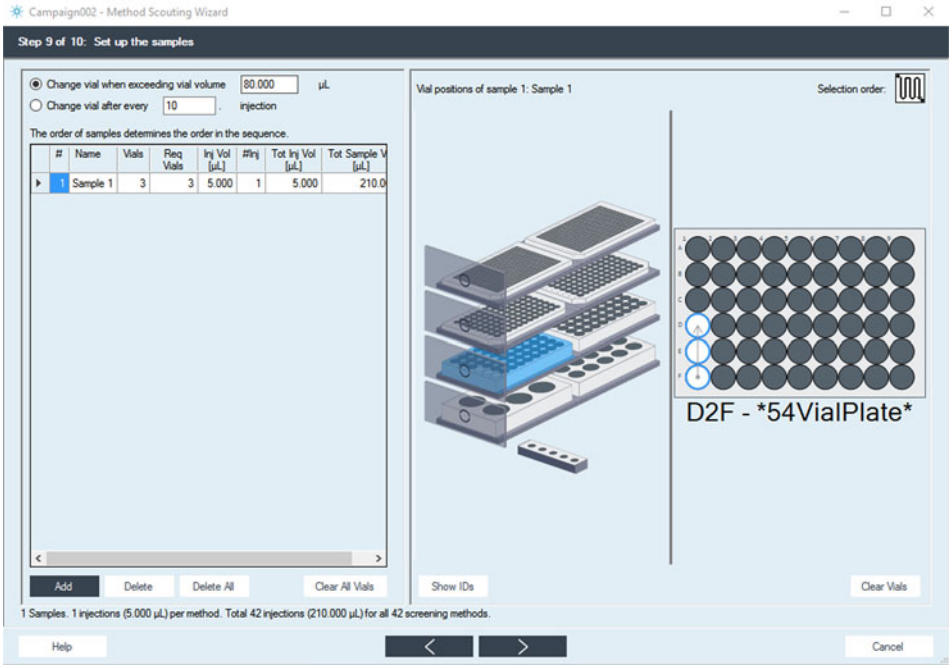


Figure 56 Set up the samples

Summary

The last screen of the wizard gives you detailed information on the specified method scouting campaign (see Figure 57 on page 114).

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

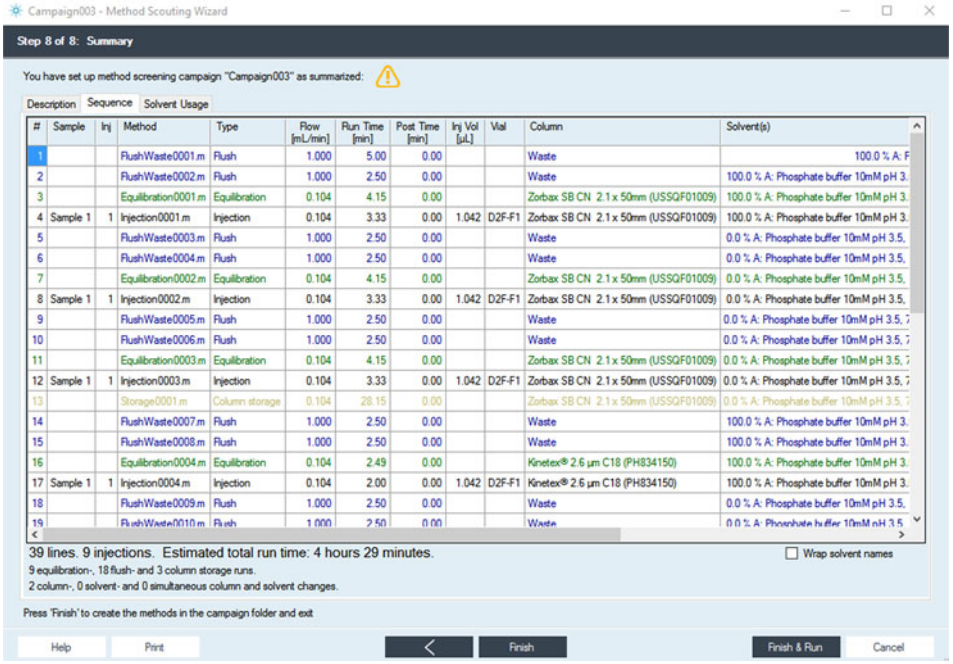
The **Sequence** tab shows details of the sequence that will be created:

- the complete sequence is shown including all flush, equilibration and column care lines (these are color 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.

All estimated solvent volume is summed to give a total waste volume, which is shown in the last row of the table. If your pump supports a waste bottle and your system is online, the computed waste volume is compared with the waste bottle capacity and is color-coded accordingly.

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



#	Sample	Inj	Method	Type	Flow [mL/min]	Run Time [min]	Post Time [min]	Inj Vol [µL]	Vial	Column	Solvent(s)
1			FlushWaste0001.m	Flush	1.000	5.00	0.00			Waste	100.0 % A: F
2			FlushWaste0002.m	Flush	1.000	2.50	0.00			Waste	100.0 % A: Phosphate buffer 10mM pH 3.
3			Equilibration0001.m	Equilibration	0.104	4.15	0.00			Zorbax SB CN 2.1 x 50mm (USSQF01009)	100.0 % A: Phosphate buffer 10mM pH 3.
4	Sample 1	1	Injection0001.m	Injection	0.104	3.33	0.00	1.042	D2F-F1	Zorbax SB CN 2.1 x 50mm (USSQF01009)	100.0 % A: Phosphate buffer 10mM pH 3.
5			FlushWaste0003.m	Flush	1.000	2.50	0.00			Waste	0.0 % A: Phosphate buffer 10mM pH 3.5,
6			FlushWaste0004.m	Flush	1.000	2.50	0.00			Waste	0.0 % A: Phosphate buffer 10mM pH 3.5,
7			Equilibration0002.m	Equilibration	0.104	4.15	0.00			Zorbax SB CN 2.1 x 50mm (USSQF01009)	0.0 % A: Phosphate buffer 10mM pH 3.5,
8	Sample 1	1	Injection0002.m	Injection	0.104	3.33	0.00	1.042	D2F-F1	Zorbax SB CN 2.1 x 50mm (USSQF01009)	0.0 % A: Phosphate buffer 10mM pH 3.5,
9			FlushWaste0005.m	Flush	1.000	2.50	0.00			Waste	0.0 % A: Phosphate buffer 10mM pH 3.5, 7
10			FlushWaste0006.m	Flush	1.000	2.50	0.00			Waste	0.0 % A: Phosphate buffer 10mM pH 3.5, 7
11			Equilibration0003.m	Equilibration	0.104	4.15	0.00			Zorbax SB CN 2.1 x 50mm (USSQF01009)	0.0 % A: Phosphate buffer 10mM pH 3.5, 7
12	Sample 1	1	Injection0003.m	Injection	0.104	3.33	0.00	1.042	D2F-F1	Zorbax SB CN 2.1 x 50mm (USSQF01009)	0.0 % A: Phosphate buffer 10mM pH 3.5, 7
13			Storage0001.m	Column storage	0.104	28.15	0.00			Zorbax SB CN 2.1 x 50mm (USSQF01009)	0.0 % A: Phosphate buffer 10mM pH 3.5, 7
14			FlushWaste0007.m	Flush	1.000	2.50	0.00			Waste	100.0 % A: Phosphate buffer 10mM pH 3.
15			FlushWaste0008.m	Flush	1.000	2.50	0.00			Waste	100.0 % A: Phosphate buffer 10mM pH 3.
16			Equilibration0004.m	Equilibration	0.104	2.49	0.00			Kinetex® 2.6 µm C18 (PH834150)	100.0 % A: Phosphate buffer 10mM pH 3.
17	Sample 1	1	Injection0004.m	Injection	0.104	2.00	0.00	1.042	D2F-F1	Kinetex® 2.6 µm C18 (PH834150)	100.0 % A: Phosphate buffer 10mM pH 3.
18			FlushWaste0009.m	Flush	1.000	2.50	0.00			Waste	0.0 % A: Phosphate buffer 10mM pH 3.5,
19			FlushWaste0010.m	Flush	1.000	2.50	0.00			Waste	0.0 % A: Phosphate buffer 10mM pH 3.5,

Figure 57 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.

For a fast data evaluation Method Scouting Wizard comes with interactive reporting including the easy method filter. This filter (see Figure 58 on page 115) allows you to set your criteria like number of peaks, minimum resolution and minimum symmetry. After refreshing, the report only contains the methods which fits to your criteria. Moreover the report contains two bubble plots for easy comparison (e.g. the runtime vs. the resolution) of the remaining methods.

Figure 58 Easy Method Filter

NOTE

All provided report templates use a filter to display only peaks and chromatograms of detector signal A by default. You must change this signal in the report template to report peaks from another signal.

NOTE

When updating ChemStation or a software Add-on, the templates of the old software version are not overwritten. This avoids the loss of customized report templates. You must use **Deploy current build in templates** option to use the templates of the latest software version.

For even more advanced and automated method optimization based on chromatographic data and considering aspects of quality by design (QbD), Agilent offers additional solutions from our partners. Please contact your local Agilent representative for more details.

6 Method Development Strategy

Further Information 118

This chapter provides information on method development strategy, concerning LC and LC/MS columns selection, pH and mobile phase.

Further Information

Fur further information on consumables and columns for method development, refer to the following sources:

- LC Handbook:

  https://www.agilent.com/cs/library/primers/Public/LC-Handbook-Complete-2.pdf
- Method development kits:

  http://www.agilent.com/en-us/products/liquid-chromatography/lc-columns/kits/method-development-kits
- Column selection guide:

  http://navigator.chem.agilent.com/
- Agilent InfinityLab HPLC Advisor App:

  https://www.agilent.com/en/product/liquid-chromatography/hplc-advisor

7 Appendix

General Safety Information 120
General Safety Information 120
Safety Standards 120
General 120
Before Applying Power 121
Ground the Instrument 121
Do Not Operate in an Explosive Atmosphere 122
Do Not Remove the Instrument Cover 122
Do Not Modify the Instrument 122
In Case of Damage 122
Solvents 123
Safety Symbols 124
Agilent Technologies on Internet 126

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

General Safety Information

General Safety Information

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

WARNING

Ensure the proper usage of the equipment.

The protection provided by the equipment may be impaired.

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

Safety Standards

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

General

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

Before Applying Power

WARNING

Wrong voltage range, frequency or cabling

Personal injury or damage to the instrument

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

NOTE

Note the instrument's external markings described under "Safety Symbols" on page 124.

Ground the Instrument

WARNING

Missing electrical ground

Electrical shock

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

Do Not Operate in an Explosive Atmosphere

WARNING

Presence of flammable gases or fumes

Explosion hazard

- ✓ **Do not operate the instrument in the presence of flammable gases or fumes.**

Do Not Remove the Instrument Cover

WARNING

Instrument covers removed

Electrical shock

- ✓ **Do Not Remove the Instrument Cover**
- ✓ **Only Agilent authorized personnel are allowed to remove instrument covers. Always disconnect the power cables and any external circuits before removing the instrument cover.**

Do Not Modify the Instrument

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

In Case of Damage

WARNING

Damage to the module

Personal injury (for example electrical shock, intoxication)

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

Solvents

WARNING

Toxic, flammable and hazardous solvents, samples and reagents

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

- ✓ **When working with these substances observe appropriate safety procedures (for example by wearing goggles, safety gloves and protective clothing) as described in the material handling and safety data sheet supplied by the vendor, and follow good laboratory practice.**
- ✓ **Do not use solvents with an auto-ignition temperature below 200 °C (392 °F). Do not use solvents with a boiling point below 56 °C (133 °F).**
- ✓ **Avoid high vapor concentrations. Keep the solvent temperature at least 40 °C (72 °F) below the boiling point of the solvent used. This includes the solvent temperature in the sample compartment. For the solvents methanol and ethanol keep the solvent temperature at least 25 °C (45 °F) below the boiling point.**
- ✓ **Do not operate the instrument in an explosive atmosphere.**
- ✓ **Do not use solvents of ignition Class IIC according IEC 60079-20-1 (for example, carbon disulfide).**
- ✓ **Reduce the volume of substances to the minimum required for the analysis.**
- ✓ **Never exceed the maximum permissible volume of solvents (8 L) in the solvent cabinet. Do not use bottles that exceed the maximum permissible volume as specified in the usage guideline for solvent cabinet.**
- ✓ **Ground the waste container.**
- ✓ **Regularly check the filling level of the waste container. The residual free volume in the waste container must be large enough to collect the waste liquid.**
- ✓ **To achieve maximal safety, regularly check the tubing for correct installation.**

NOTE

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

Safety Symbols

Table 9 Symbols

	The apparatus is marked with this symbol when the user shall refer to the instruction manual in order to protect risk of harm to the operator and to protect the apparatus against damage.
	Indicates dangerous voltages.
	Indicates a protected ground terminal.
	The apparatus is marked with this symbol when hot surfaces are available and the user should not touch it when heated up.
	Sample Cooler unit is designed as vapor-compression refrigeration system. Contains fluorinated greenhouse gas (refrigerant) according to the Kyoto protocol. For specifications of refrigerant, charge capacity, carbon dioxide equivalent (CDE), and global warming potential (GWP) see instrument label.
	Flammable Material For Sample Thermostat which uses flammable refrigerant consult Agilent Information Center / User Manual before attempting to install or service this equipment. All safety precautions must be followed.
	Confirms that a manufactured product complies with all applicable European Community directives. The European Declaration of Conformity is available at: **http://regulations.corporate.agilent.com/DoC/search.htm**
	Manufacturing date.
	Power symbol indicates On/Off. The apparatus is not completely disconnected from the mains supply when the power switch is in the Off position
	Pacemaker Magnets could affect the functioning of pacemakers and implanted heart defibrillators. A pacemaker could switch into test mode and cause illness. A heart defibrillator may stop working. If you wear these devices keep at least 55 mm distance to magnets. Warn others who wear these devices from getting too close to magnets.

Table 9 Symbols

	Magnetic field Magnets produce a far-reaching, strong magnetic field. They could damage TVs and laptops, computer hard drives, credit and ATM cards, data storage media, mechanical watches, hearing aids and speakers. Keep magnets at least 25 mm away from devices and objects that could be damaged by strong magnetic fields.
	Indicates a pinching or crushing hazard
	Indicates a piercing or cutting hazard.

WARNING

A WARNING

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

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

CAUTION

A CAUTION

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

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

Agilent Technologies on Internet

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

https://www.agilent.com

In This Book

This manual contains information on the InfinityLab LC Method Development Solutions.

The manual describes the following:

- Introduction,
- System setup and installation,
- configuration of the system using ChemStation,
- information on the Method Scouting Wizard,
- method development strategies.

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