Agilent 1290 Infinity II 2D-LC Solution ChemStation

User Guide

Notices

Document Information

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In This Book

This manual covers the Agilent 1290 Infinity II 2D-LC Solution ChemStation and 2D-LC Acquisition software (G2198AA).

1 Introduction

This chapter describes the product of Agilent 1290 Infinity II 2D-LC Solution ChemStation.

2 Concepts of 2D-LC

This chapter describes the concepts of Agilent 1290 Infinity II 2D-LC Solution ChemStation.

3 Compatibility Matrix

The compatibility matrix provides information about installation and execution prerequisites with respect to hardware, firmware and the operating system. Agilent 2D-LC Software is an OpenLAB CDS ChemStation Edition plug-in.

4 Installation

This chapter describes the installation of the Agilent 1290 Infinity II 2D-LC Solution ChemStation. These installation instructions are valid for the modes standard heart-cutting, multiple heart-cutting, high resolution sampling and comprehensive 2D-LC except the connections to the 2D-LC valve. These connections depend on the method, see chapter "Run the System for detailed information.

5 Method Parameters

This chapter provides background information on method parameters. It helps to optimize methods in Agilent 1290 Infinity II 2D-LC Solution ChemStation in the modes standard heart-cutting, multiple heart-cutting, high resolution sampling and comprehensive 2D-LC.

6 Run the System

This chapter describes how to run the Agilent 1290 Infinity II 2D-LC Solution ChemStation in the modes standard heart-cutting, multiple heart-cutting, high resolution sampling and comprehensive 2D-LC.

7 Investigate the effects of using different gradients in the 2Dimension

This chapter describes, how shifted gradients in the second dimension can be used to enlarge the accessible two-dimensional separation space.

8 Data Analysis

This chapter descibes the analyzation of data in 2D-LC and is separated in a section heart-cutting 2D-LC and a section comprehensive 2D-LC.

9 Troubleshooting and Diagnostics

This chapter gives an overview about the troubleshooting and diagnostic features and the different user interfaces.

10 Error Information

This chapter describes the meaning of error messages, and provides information on probable causes and suggested actions how to recover from error conditions.

11 Maintenance

This chapter describes the maintenance of the 2D-LC Solution.

12 Parts for Maintenance

This chapter provides information on parts material required for the solution.

13 Alternative ways to install the System

This chapter describes alternative ways to install and setup the system.

14 Agilent 2D-LC Solution with the selectivity of mass selective detection (MSD)

This chapter describes the different options to use the Agilent 2D-LC Solution with mass selective detection (MSD).

15 Additional Notes

This chapter provides content from original release and technical notes for reference.

16 Theoretical Background

This chapter gives the theoratical background of 2D-LC and describes the system components (soft- and hardware) of the Agilent 1290 Infinity II 2D-LC Solution ChemStation.

17 Appendix

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

Contents

1 Introduction

This chapter describes the product of Agilent 1290 Infinity II 2D-LC Solution ChemStation.

Product Description

2D-LC offers great improvements in resolving power over conventional 1D-LC. The advantages are as follows:

- Further resolution of a complex mixture that cannot be separated on a single column
- Increased peak capacity
- Sample cleanup by removing matrix or interfering compounds
- Increase sample throughput (two separations going on at once)

Pre-configured systems and a dedicated 2D-LC training allow an easy start into 2D-LC.

The Agilent 1290 Infinity II 2D-LC Solution ChemStation can switch to 1D-UHPLC and all the different 2D-LC techniques easily. The easy-to-use software of the Agilent 1290 Infinity II 2D-LC Solution ChemStation is designed for fasted method setup in all available modes:

- Comprehensive
- Heart-cutting
- Multiple heart-cutting
- High resolution sampling

Figure 1 **Dual Stack Configuration**

Features

- Boosts performance through ultrahigh peak capacity in excess of 1000 – for unmatched power to separate most complex samples
- Saves time through trouble-free instrument setup – with easy starter kit to enable fastest familarization
- Reduces costs through single-vendor solution – higher returns on investment with one system for both 1D-LC and 2D-LC
- Supports comprehensive, heart-cutting and multiple heart-cutting 2D-LC – for any kind of 2D-LC operation("Comprehensive 2D-LC (LCxLC)" on page 29, "Heart-Cutting 2D-LC (LC-LC)" on page 16, "Multiple Heart-Cutting and High Resolution Sampling 2D-LC" on page 17)
- Utilizes the powerful 1290 Infinity Binary Pump in the second dimension – for highest speed, accuracy, precision and resolution
- Highly flexible in the first dimension – even older Agilent LC systems can be upgraded for a very cost-effective access to the power of 2D-LC
- Allows fully symmetric flow path for comprehensive 2D-LC and matched loops – using innovative and unique new 2D-LC QuickChange valve head for 1290 Infinity Valve Drive or 1290 Infinity Flexible Cube
- New Heart-Cut Viewer software – for unmatched usability in multiple heart-cutting data analysis
- Easy-to-use 2D-LC acquisition software - for fastest and most easy system and method set-up
- Powerful comprehensive 2D-LC data analysis - using LC Image Software from GC Image LLC, USA

2 Concepts of 2D-LC

This chapter describes the concepts of Agilent 1290 Infinity II 2D-LC Solution ChemStation.

Concepts of 2D-LC

In a 2D-LC-System, Pump 1 generates the first dimension gradient. An autosampler injects the sample and separates it by column 1. A 2D-LC Valve (Injector) connects the first dimension to the second dimension and stores sample peaks intermediately. These sample peaks are re-injected to the second dimension, separated by a second column and measured by Detector 2.

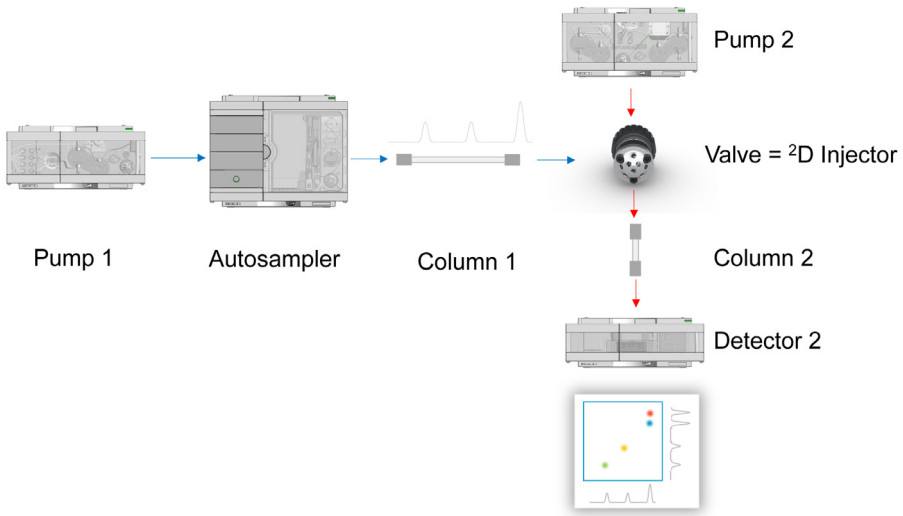


Figure 2 Concept of a 2D-LC-System

In 2D-LC the following concepts exist:

- Comprehensive 2D-LC (LC×LC)

  In LC×LC, the total eluent from the first dimension is injected on to the column in the second dimension.

- Heart-cutting 2D-LC (LC-LC)

  In LC-LC only parts of the eluent from the first dimension are injected on to the column in the second dimension.

Heart-Cutting 2D-LC (LC-LC)

The following items are characteristic for LC-LC:

- Only parts of the effluent of the first column - only the peaks of interest eluted from the 1st dimension column - are injected to the second column
- A peak from the 1st dimension is sampled as a whole and a method with a lower flow rate and a gradient typically with a longer run time than the collection time is used to improve separation efficiency
- Typically longer columns with higher separation efficiency are used in 2nd dimension column

NOTE Heart-Cutting 2D-LC (LC-LC) is the method of choice if the samples to analyze are known or to improve confidence of an experiment (pharma, method development and so on).

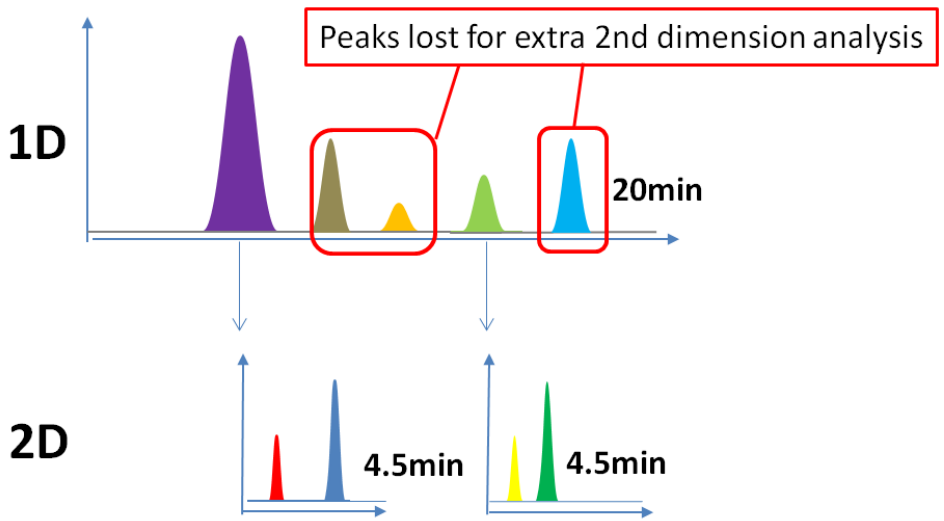
NOTE Multiple peaks eluted from the first dimension column can be sampled and analyzed in the second dimension but the run time of the second dimension must match the retention time between two first dimension peaks. *A started second dimension analysis will always be finished!* Thus, a second peak being eluted from the first dimension might be lost, if sampled while the second dimension analysis is still running.

There are two modes of LC-LC:

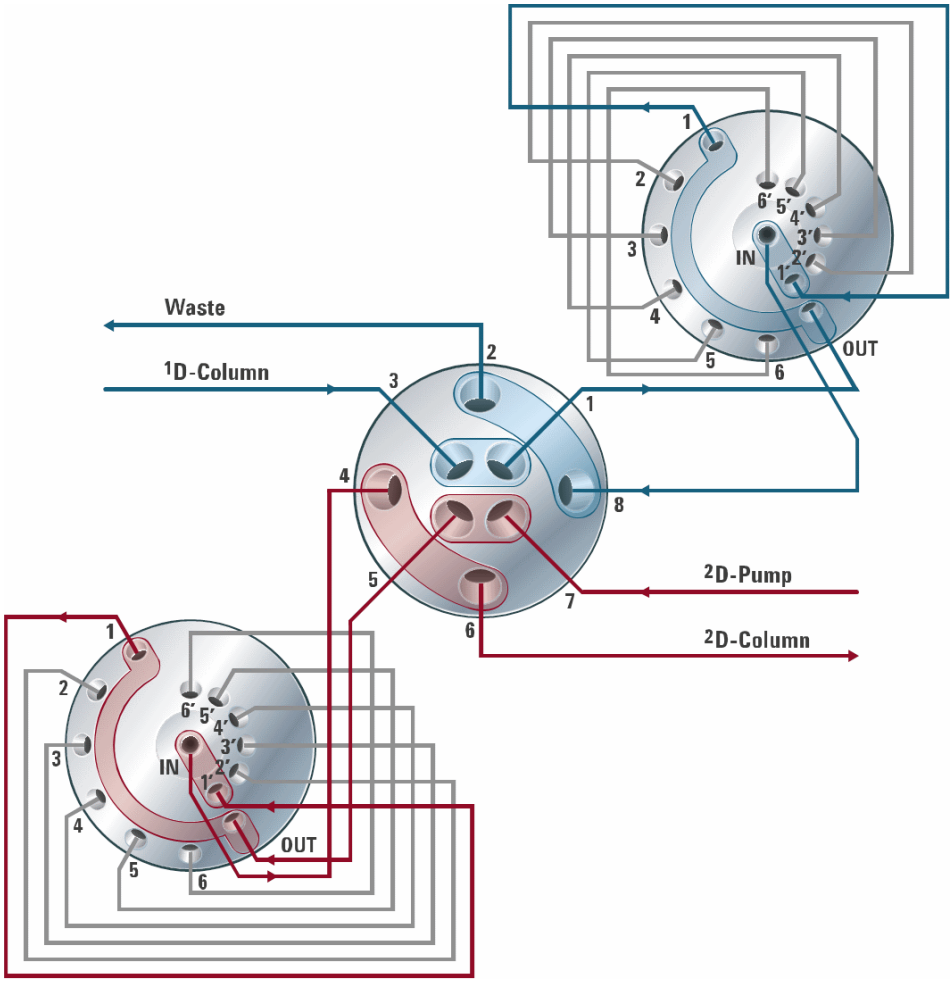
- Time-triggered LC-LC
- Peak-triggered LC-LC

Multiple Heart-Cutting and High Resolution Sampling 2D-LC

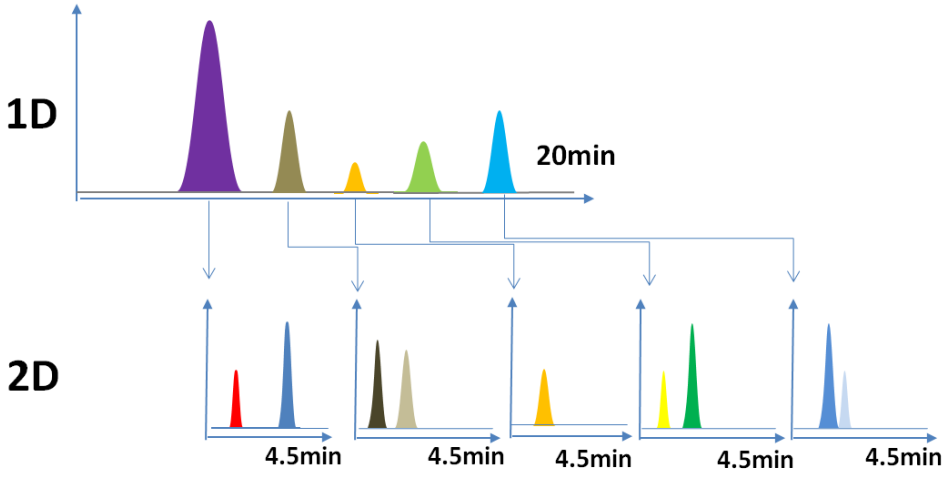
Typically, the gradient time in the second dimension is much longer for *heart-cutting* than with the *comprehensive* technique. The disadvantage of the standard heart-cutting techniques is that peaks cannot be sampled while a second dimension gradient is still running. In the examples shown here, the gradient from the second dimension is analyzing the first peak (purple), while the second and third peak (gray and yellow) elute from the first dimension column. The second dimension is ready when the 4th peak (green) elutes from the first dimension; this peak can be analyzed. As the second dimension is occupied again, the fifth peak (blue) cannot be analyzed.



This problem is addressed using a setup called *multiple heart-cutting 2D-LC*. Here, the sampling loops on the 2D-LC valve are exchanged with 6-position/14-port selection valves, which are equipped with six loops each. In this configuration, a peak can be cut out and stored, then analyzed as soon as the second dimension is free.



Peaks that are cut out and stored during a run are analyzed consecutively in the second dimension, even when the first dimension is still running. To avoid carry-over the peaks are analyzed in reverse order of storage in a single Multiple Heart-Cutting Valve.



Principles of Heart-cutting 2D-LC

Multiple Heart-Cutting - Principles

Multiple Heart-Cutting - Principles

Multiple Heart-Cutting 2D-LC is a complex workflow, working on a special algorithm for filling the sample loops and analyzing the stored cuts, based on different criteria. "Multiple Heart-Cutting - Principles" on page 20 illustrates the principles of the Multiple Heart-Cutting algorithm, following these principles:

- ^{2}D analysis is done as soon as possible. As long as the 2nd dimension is free, any next cut from the 1st dimension will be always directly transferred to the 2nd dimension and analysed. This means:
  - The first 1D cut will be always directly analysed in the 2nd dimension.
  - If the 2nd dimension is free, when the next ^{1}D cut is taken, it will also be directly analysed.
- If the 2nd dimension is occupied, the next ^{1}D cut will be stored in the next sample loop.
- If all sample loops in the 1st dimension are occupied, the peak is lost.
- A peak parking deck will always be completely analysed, before switching to the other parking deck.
- Before analyzing a new parking deck, a flush gradient is run to avoid contamination.
- Stored cuts are analysed in backwards order to avoid contamination.

Peak-based mode in multiple heart-cutting

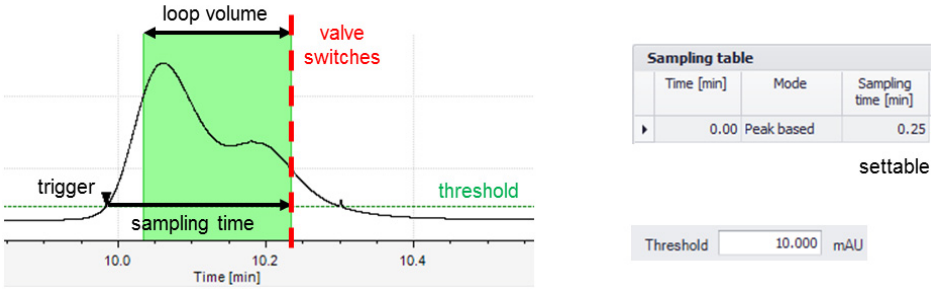


Figure 3 Peak-based mode

In peak based mode, three parameters determine how peaks are parked:

1. A trigger marked by a black triangle indicates, if a peak has been detected, e.g. because a reference signal (if available) exceeds the threshold or the slope as defined in advanced settings.
2. The cut is parked by switching the valve. This happens either if the peak end is detected (signal falls below threshold or slope) or if the settable sampling time has been exceeded, whatever comes first. The purpose of the sampling time is delaying the parking such that a defined part of the peak, typically its center, is parked.
3. The width t of the green area, which is used for parking a peak fix and calculated from the loop volume V and flow rate F in the first dimension by t = V/F.

NOTE

Please note that the peak parking may start even before the peak trigger if the sampling time is shorter than the time corresponding to the loop volume. In this case, the green area will start left to the trigger triangle.

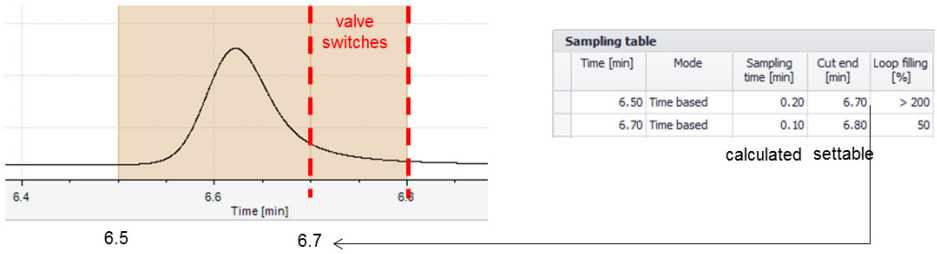


Figure 4 Time-based mode

In time-based mode, the time given in the sampling table corresponds to the beginning of the cut parking. The sampling time is usually fix in this case and is given by t = V/F. The cut is parked by switching the valve at the time “cut end”. Ultimately, only the cut end has relevance for the method and instrument control. The cut end is displayed in an optional column, which needs to be configured by a right-click on sampling table column headers choosing option "Columns".

For backward compatibility, adjacent cuts can be parked in the multiple heart-cutting mode. In such cases, the sampling time can be lower than a value corresponding to the normal loop fill time, which is also indicated by loop filling values below 100%. In multiple heart-cutting, loops should be overfilled (> 100%). For the second and later cuts in a series of adjacent cuts, short cuts can be created by editing the cut end (which is in an optional table column) or by moving the corresponding area in the preview window. High-Resolution Sampling should be preferred for adjacent cuts.

NOTE

Previous revisions of the 2D-LC software used same settings for peak-based versus time-based modes, which could result in start times before the beginning of peak parking in time-based mode. Loop fill time/sampling time could be set manually, which is practically not possible (see above).

If such methods are loaded to software A.01.03 and above, this will not change the behavior of such methods, as the valve switching times (= cut ends) are not changed. However the start times will be corrected automatically in order to reflect the physical reality, which may look as it would be a method change.

Please also note the sampling time is related to the flow rate. If the 1D flow rate is changed, valve switch times are kept constant and the peak start time changes. Please note that the reference signal becomes invalid for a changed flow rate.

High Resolution Sampling - Peak Parking Principles

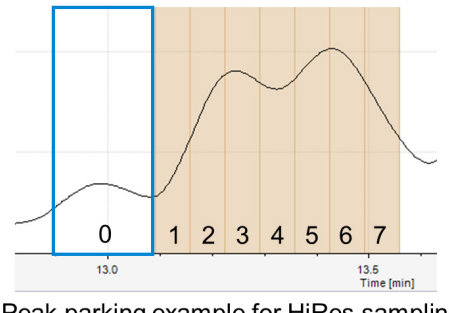
In the **HiRes sampling** mode, the multiple heart-cutting (MHC) valve is switched *before and after* parking the peak. This has the following consequences:

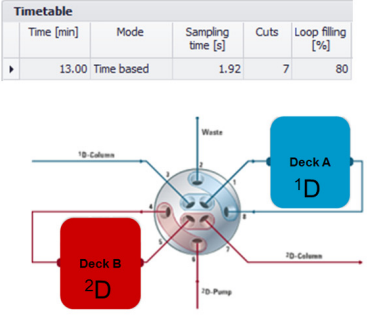
- Each loop for consecutive snips stores the same sample volume.
- First and last loop cannot be used for parking.
- Solvent transfer from ^{1}D to ^{2}D can be reduced.
- Cut number 5 cannot be parked entirely in the sample loop. Otherwise cut 6 would got partially to the transfer capillary and would therefore be lost or spoil cut 5.

  Cut 5 stays partially in the transfer line and is immediately being analyzed in ^{2}D.
- For parking cut 6 in the sample loop, the cut first needs to be moved from the 2D-LC valve to the deck valve.

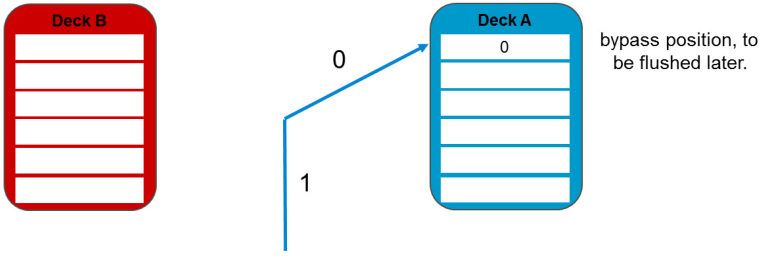
Peak parking example for HiRes sampling

- In High-Resolution sampling, the first loop is a bypass position. When switching to the second loop for the first cut, unknown content may be parked in the first loop, which must be flushed at the end of the unparking procedure.

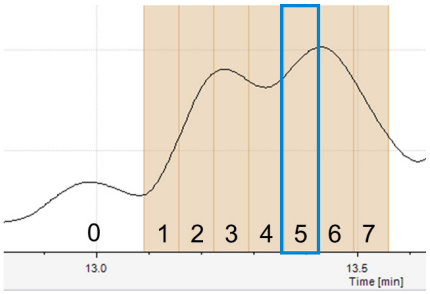


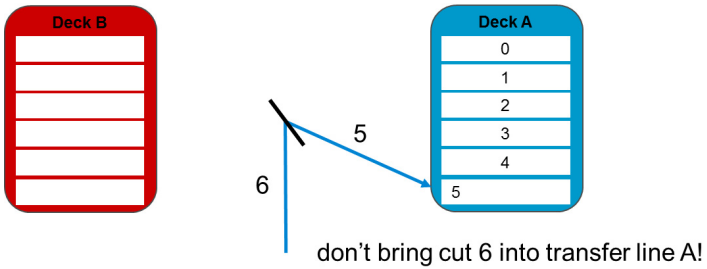


Peak parking example for HiRes sampling

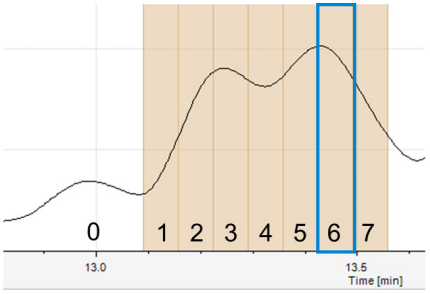


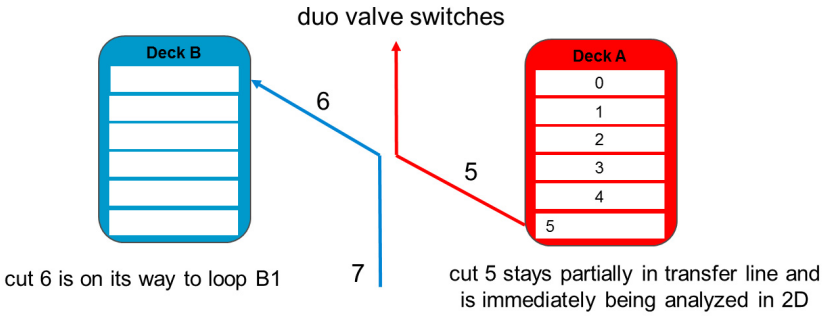
- MHC valve switches right before parking cut 1, 2, 3, 4, 5
- Cut number 5 cannot be parked entirely in the sample loop, otherwise cut 6 would go partially to the transfer capillary and would therefore be lost or spoil cut 5



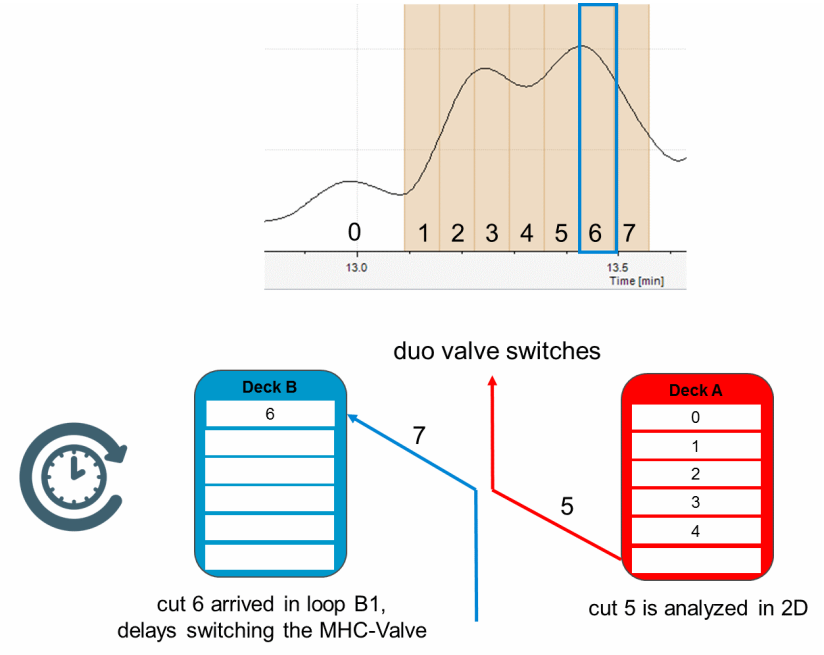


- Cut 5 stays partially in transfer line and is immediately analyzed in ^{2}D

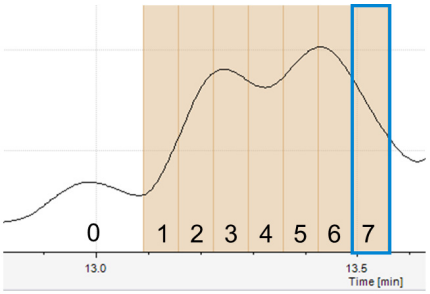


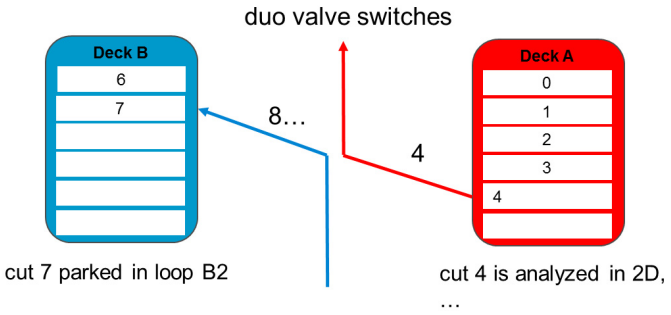


- For parking cut 6 into the sample loop, the cut first needs to be moved from the 2D-LC Valve to the deck valve.



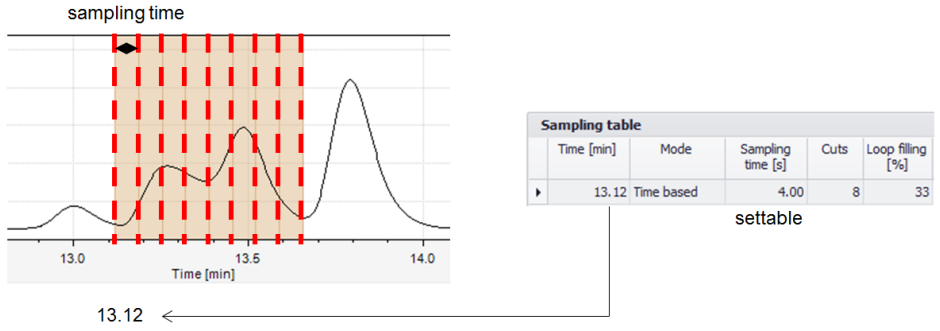
- Cut 7 will be parked in loop B2





- Last loop is required for flow-through while other deck runs analysis. During analysis, loops are filled with solvent of ^{2}D gradient base.

High-resolution sampling (time-based mode)



For high-resolution sampling, a (start) time can be set, the sampling time and the number of cuts for a peak or range. The sampling time should be less than the time which is needed for filling one sample loop corresponding to a loop filling below 80%. Because of the parabolic flow profile, a filling greater than 80% will cause samples going to waste.

The minimum sampling time/volume is given by the transfer volume between the 2D-LC valve and the deck valve. The last cut of a deck is stored in the transfer capillary such that switching to the second deck will bring that peak to the second dimension. If a volume smaller than that transfer volume would be chosen, two cuts would be in the same capillary resulting in a loss of resolution and reproducibility.

Comprehensive 2D-LC (LCxLC)

In comprehensive 2D-LC (also known as LC×LC), the total eluent from the first dimension is injected on to the column in the second dimension using two equal-sized sampling loops that are alternated by a switching valve. While the first loop is being filled in the first dimension, the contents of the second loop is analyzed in the second dimension; the switching valve then switches the second loop into the first dimension for sampling and the first loop into the second dimension for analysis.

The gradient analysis in the second dimension is less than or equal to the sampling time in the first dimension:

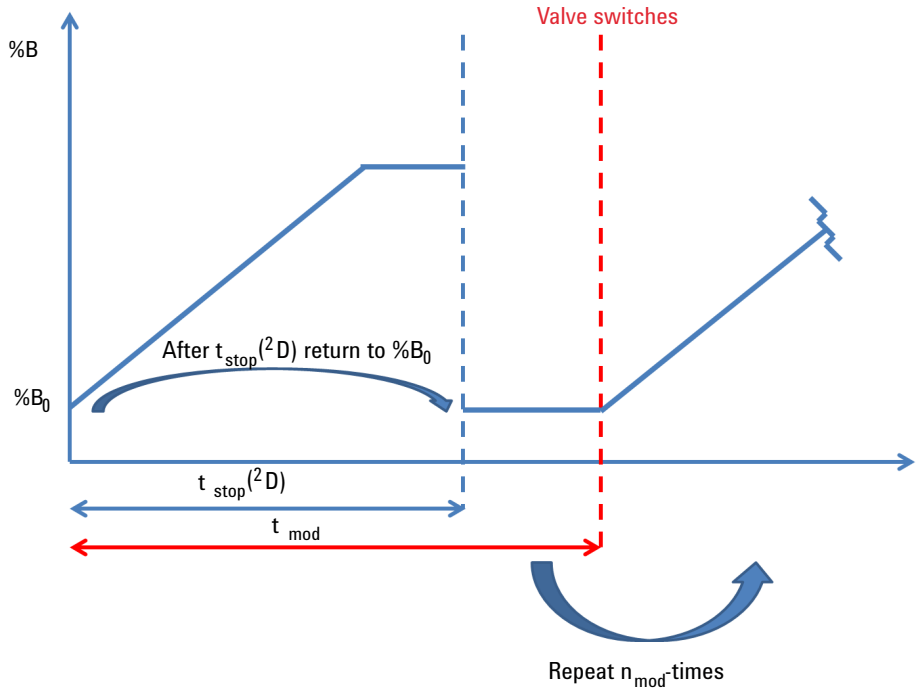


Figure 5 Characteristics of LCxLC

Standard LCxLC

In standard LCxLC the total eluent of the 1st dimension is injected onto the column in the 2nd dimension using two sampling loops alternatingly by switching a modulation valve. This will be repeated from the start to the end of the first dimension separation.

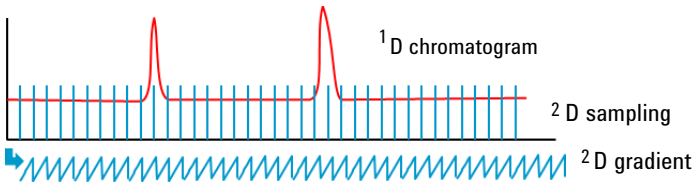


Figure 6 Principle of standard LCxLC

Triggering of 2D-LC

Concept of Peak Triggering

Peak-triggered LC-LC

One or more peaks of the 1st dimension exceeding a given level are injected onto the 2D-column. Further peaks eluted from the 1D-column during the 2nd dimension gradient time are ignored.

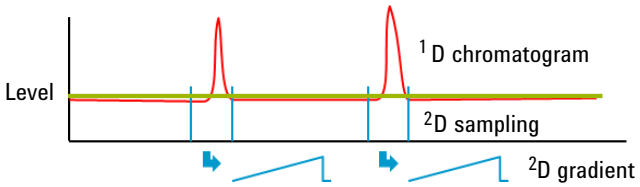


Figure 7 Principles of peak-triggered LC-LC

Peak-Triggered LCxLC

In peak-triggered LCxLC only peaks in the first dimension that exceed a given level at a peak detector placed between the first dimension column and the modulation valve will be injected onto the column in the 2nd dimension. At the beginning of a peak triggered segment the flow will be increased from an set idle flow rate value to the method flow rate. After the time resulting from the delay volume between the peak detector and the loop (to be specified in the configuration screen of the 2D-LC Acquisition software) the sampling and the second dimension analysis will start. As soon as the peak parameters are below the set limits, sampling and second dimension analysis will stop (again, under consideration of the delay volume). The flow rate will return to its idle flow rate, if set, at the end of a defined peak triggered segment. An increase in peak dispersion might occur depending on the cell characteristics, the first dimension set-up and the used second dimension separation mode (for example large flow cell volume, small first dimension peak volumes and isocratic second dimension separation).

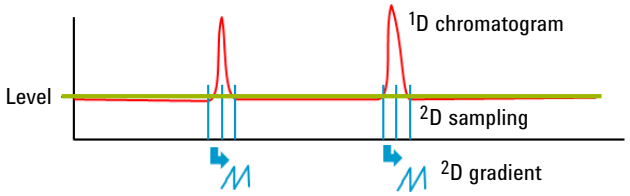


Figure 8 Principle of peak-triggered LCxLC

Relevant parameters for peak triggering

Concept of Peak Triggering

Triggering is done in advanced settings similar to integrator settings by threshold and/or slope, see Figure 9 on page 32.

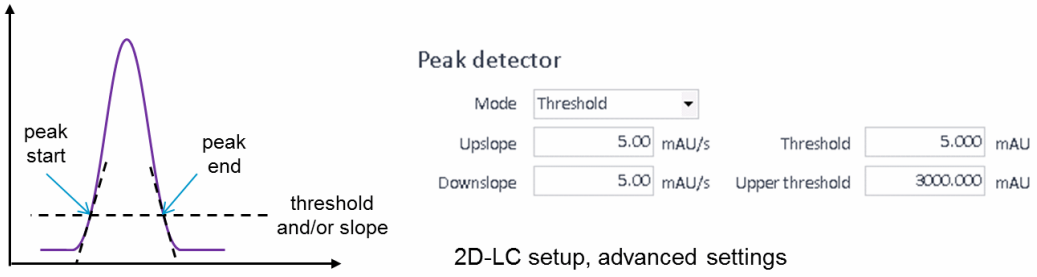


Figure 9 Peak triggering

The valve switches under the following conditions (whichever comes first):

- If the **Sampling time** has elapsed, or

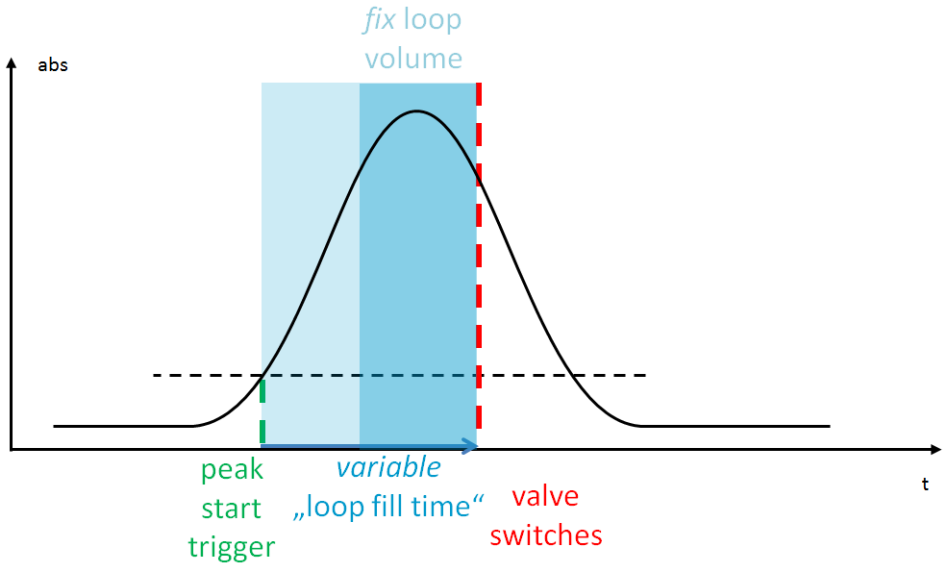


Figure 10 Peak triggering concept (elapsed sampling time)

- If the signal falls below threshold or slope.

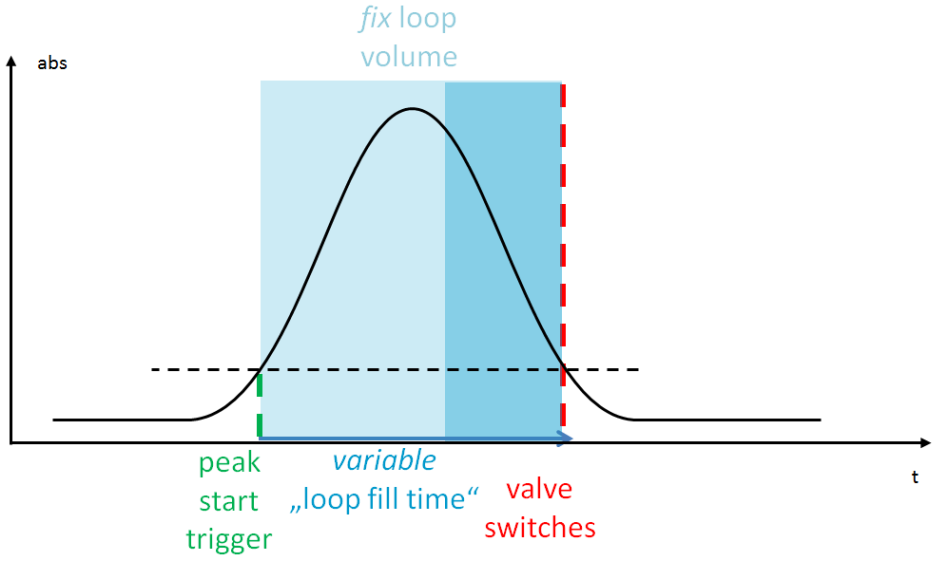


Figure 11 Peak triggering concept (signal falls below threshold or slope)

Concept of Time Triggering

Time-triggered LC-LC

One or more parts of the 1st dimension in given time frames are directly injected onto the 2D-column.

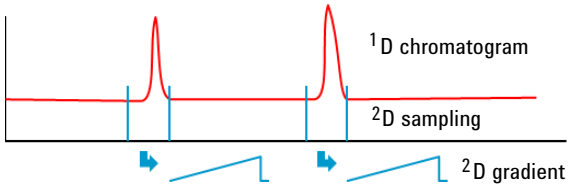


Figure 12 Principles of time-triggered LC-LC

Time triggered LCxLC

In time-triggered LCxLC the user can set start and end-times of second dimension sampling in the software, multiple time segments can be used. This can be used for example if the samples are known with known areas without peaks being eluted from the first dimension. Or, to save solvent and reduce valve wear for the time before the first peak is eluted from the first dimension column or during its wash-out or re-equilibration time. The software allows to set an idle flow rate for these times.

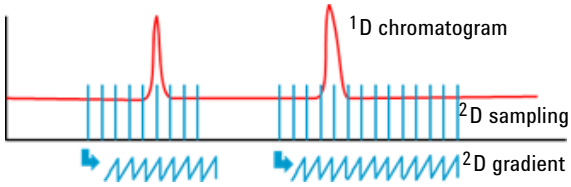


Figure 13 Principle of time-triggered LCxLC

Active solvent Modulation (ASM)

Introduction to Active Solvent Modulation (ASM)

In conventional 2D-LC, ^{1}D solvent in the sample loop is injected to the second dimension column. If the ^{1}D solvent has high elution strength in respect to the ^{2}D column, it impairs separation in the second dimension. This results in unretained elution, broad and distorted peaks, and loss of separation (see Figure 15 on page 36).

Active Solvent Modulation (ASM) dilutes the content of the sampling loop (sample and ^{1}D solvent) with weak ^{2}D solvent before it reaches the ^{2}D column and therefore improves the separation in the second dimension (see Figure 16 on page 36).

Different ASM capillaries allow optimizing the dilution for different applications (see "Understanding the ASM factor" on page 41).

The ASM solution is primarily designed for 2D-LC modes multiple heart-cutting and high-resolution sampling. The 2D-LC Valve ASM is backward compatible to the standard 2D-LC valve G4236A. If ASM is not needed or for use in comprehensive 2D-LC, the ASM functionality can be disabled.

ASM is based on the 2D-LC Valve ASM G4243A and requires the InfinityLab 2D-LC solution and 2D-LC Software A.01.04 or later.

Example: ASM with HILIC in ^{1}D and reversed phase in ^{2}D

In this example, a HILIC separation was run in the first dimension and a reversed phase separation in the second dimension. If sample cuts are transferred to the second dimension, 40 µL of high organic solvent are brought to a reversed phase column.[1]

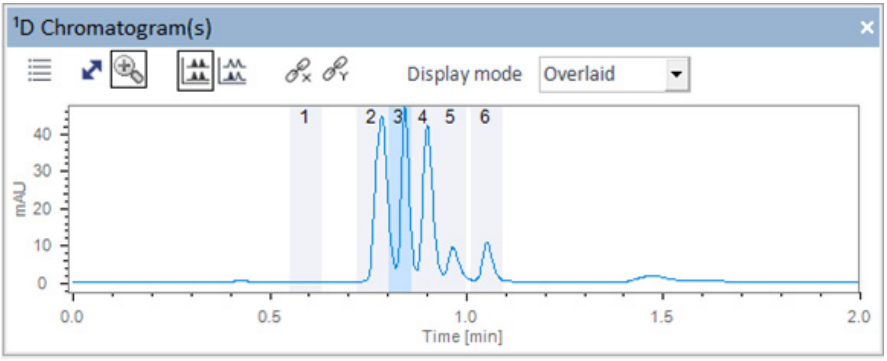


Figure 14Analysis of pesticides using a HILIC separation with high organic solvent composition in ^{1}D

2D resolution with conventional valve

The high elution strength of ^{1}D solvent causes bad separation with broad and distorted peaks in the left ^{2}D chromatogram.

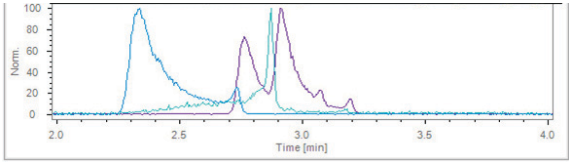


Figure 15 Conventional analysis of Cut#3 using a reversed phase separation in ^{2}D

2D resolution with ASM valve

In the right 2D chromatogram a 2D-LC Valve ASM was used instead of a conventional 2D-LC valve. Peaks are resolved and the sensitivity is increased.

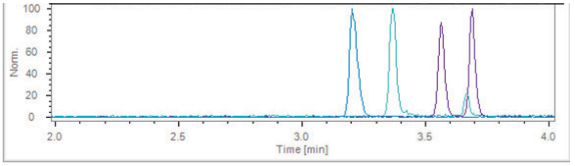


Figure 16 ASM analysis of Cut#3 using a reversed phase separation in ^{2}D

1 ^{1}D analysis of pesticides using: ^{1}D: Zorbax RX-SIL (150 x 2.1 mm ID, 5 µm), A = 10 mM NH_4Ac in H_2O; B = ACN, Gradient: 100 to 95% acetonitrile in 5 min, 500 µL/min. MHC with 40 µL loops. ^{2}D : Bonus RP (50 x 2.1 mm, 1.8 µm), H_2O/acetonitrile gradient (0.2% formic acid), weak solvent 3% acetonitrile, 400 µL/min, EICs from conventional 2D-LC (undiluted)

Operating Principle

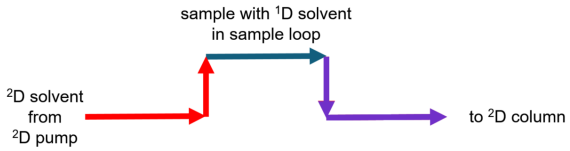


Figure 17 Operating principle with sample loop in flow path (schematic view)

¹D Solvent in the sample loop is partially diluted by ²D solvent from the ²D pump.*

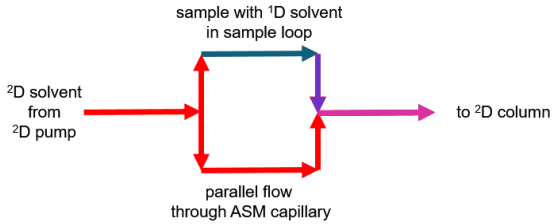


Figure 18 Operating principle with sample loop and ASM capillary in parallel flow path (schematic view)

Introducing a parallel flow through an ASM capillary strongly dilutes ¹D solvent with weaker ²D solvent. These solvent conditions focus the sample on the head of the ²D column and therefore enable a good separation.*

*red: ²D solvent from ²D pump, blue: sample with ¹D solvent in sample loop

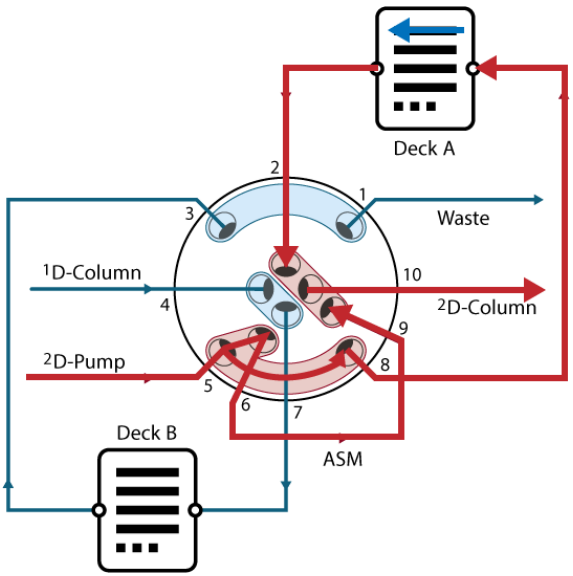


Figure 19 Operating principle with sample loop and ASM capillary in parallel flow path

This is how the same flow path looks inside the 2D-LC valve ASM. The flow coming from the ^{2}D pump splits up at valve port 10. One part goes through the sample loop in deck A and carries parked sample cuts and ^{1}D solvent. The other part of ^{2}D solvent goes through the ASM capillary between valve ports 9 and 6. Flows unite at port 5 and ^{1}D solvent is diluted before it arrives at the ^{2}D column head.

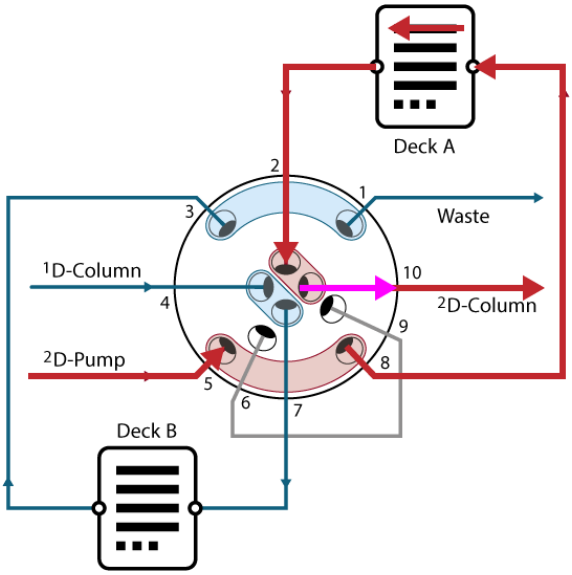


Figure 20 Operating principle with sample loop flow path

Once the ASM phase has finished, which is a settable method parameter, the analytical gradient starts. As opposed to a dilution with a permanent by-pass, the ASM capillary is no longer in the flow path, such that fast ^{2}D gradients are possible through the sample loop only.

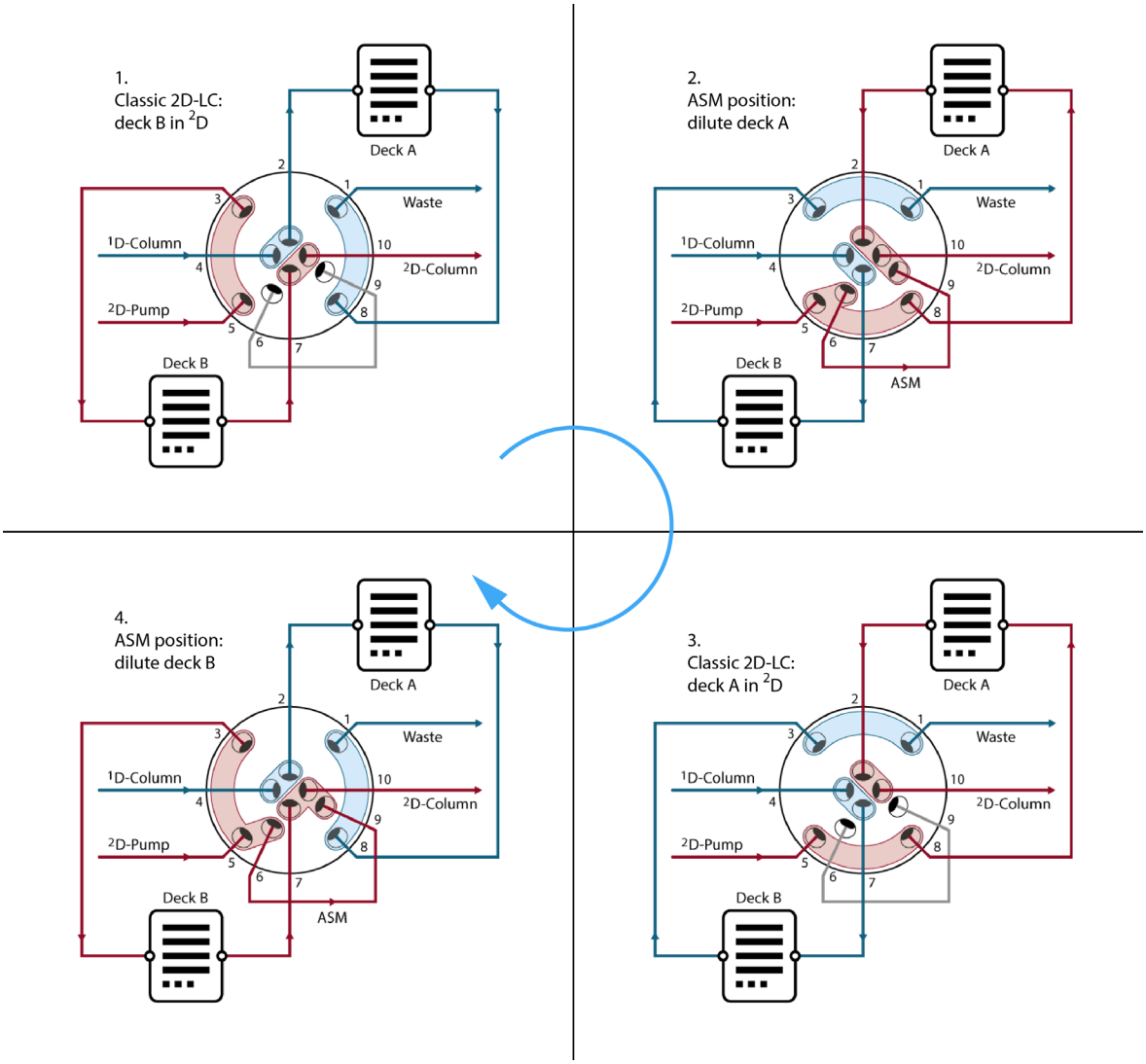


Figure 21 Switching cycle of the ASM valve (countercurrent mode)

1	Cuts are parked in deck A.
2	^{2}D solvent flows through deck A and the ASM capillary.
3	ASM capillary leaves flow path, normal analysis with flow passing deck A. Further cuts are meanwhile parked in deck B.
4	Cuts in deck B are analyzed with ASM.
5	= 1. Cuts in deck B are further analyzed without ASM, new cuts are parked in deck A.

A full switching cycle of the ASM valve has 4 positions. Positions 1 and 3 are the same as for the standard 2D-LC valve G4236A. The ASM valve has two additional positions in step 2 and 4. In both steps, the ASM capillary is in the second dimension and dilutes solvent in deck A and B, respectively.

Understanding the ASM factor

The principle of ASM is diluting ^{1}D sample loop solvent with ^{2}D solvent.

The ASM solution achieves this dilution by a parallel flow of solvents via sample loop and ASM capillary.

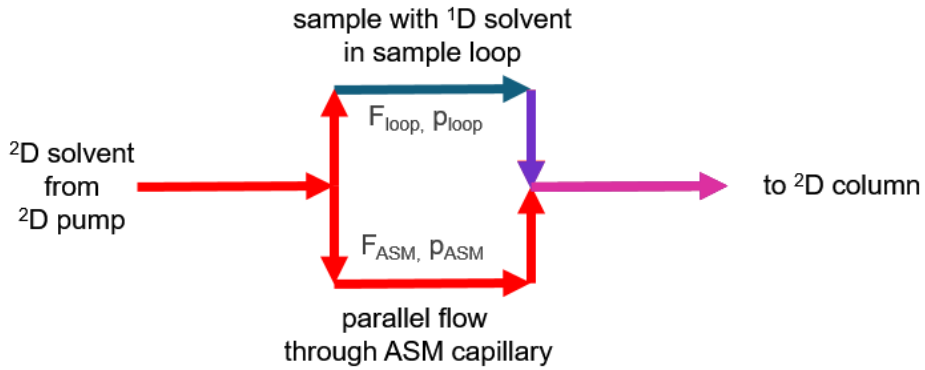


Figure 22 Principle of active solvent modulation (schematic view)

The flow rates *F* through these parallel capillaries depend on the different backpressures *p* of the capillaries in use. The backpressure of a capillary depends on the capillary length *l*, radius *r* to the power of 4, and the viscosity η of the solvent.

$$p = \frac{8\eta l F}{\pi r^4}$$ Hagen-Poiseuille equation

The Hagen-Poiseuille equation describes the relation of these parameters.

Different ASM capillary lengths have an effect on the following parameters:

- Capillary back pressure
- Dilution factor
- Optimum dilution for different applications

Example for calculation of split ratio and ASM factor.

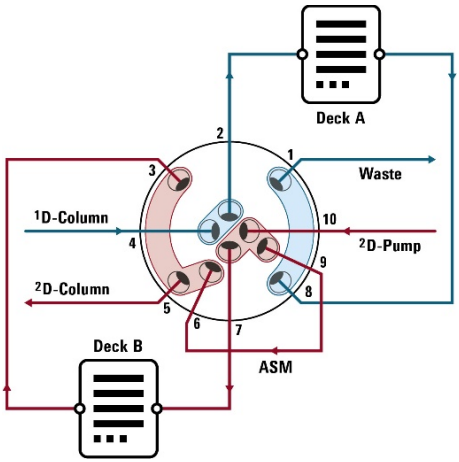


Figure 23 Backpressure of two flow paths in ASM

A longer capillary results in higher backpressure and therefore lower flow compared to a short capillary.

Example:

If the back pressure of the capillaries between ports 7 and 3 (2D-LC valve to sample loop and back) is twice as high as the back pressure of the ASM capillary between ports 9 and 6, twice as much solvent will run through the ASM capillary.

This will dilute 1D solvent in the sample loop by a factor of about 3, which is called the ASM factor.

NOTE

Usage of the ASM capillary kit results in the following situation:

- The capillaries in ASM branch and transfer branch have the same inner diameter.
- The two transfer capillaries are equally long.
- The difference between ID_{loop} = 0.35 mm and $ID_{capillaries}$ = 0.12 mm is large. Therefore the backpressure of the loops is negligible (this is, because the radius enters the Hagen-Poiseuille-Equation with the power of 4).
- Solvent composition and their viscosity in the parallel flowpaths are not predictable.

In the recommended configuration with the ASM capillary kit (see note above) one can simplify the formulae for the calculation of split ratio and ASM factor as follows:

$$Split\ ratio = \frac{l_{ASM}}{(2l_{tc1,2})}$$

l_{ASM} = Length of ASM capillary

$l_{tc1,2}$ = Length of transfer capillary 1 or 2

$$ASM\ factor = 1 + \left(\frac{1}{Split\ ratio}\right)$$

NOTE

The ASM factor calculated by the software should not be considered to be a fix number but as a guiding value which is subject to method development.

Comprehensive 2D-LC and Active Solvent Modulation

The ASM Valve can also be used for improving comprehensive 2D-LC measurements, but it is primarily optimized for multiple heart-cutting and high-resolution sampling measurements.

The ASM phase contributes to the modulation cycle. When keeping the modulation time constant, this reduces available time for the separation phase of the cycle. Otherwise, increasing the modulation time may require reducing the ^{1}D flow rate to fill the same sample loop volume. This would change ^{1}D chromatography.

The ASM solution requires back pressure from capillaries between the 2D-LC valve to multiple heart-cutting valves. Therefore, comprehensive 2D-LC sample loops cannot be installed directly at the ASM valve. In addition, comprehensive 2D-LC sample loops have standard fittings, which do not fit to the M4 ports of the ASM valve.

Please note that ASM valves require twice as many switches as a standard 2D-LC valve. Comprehensive 2D-LC uses many valve switches and in combination with ASM, this may reduce the maintenance interval of the valve.

3 Compatibility Matrix

The compatibility matrix provides information about installation and execution prerequisites with respect to hardware, firmware and the operating system. Agilent 2D-LC Software is an OpenLAB CDS ChemStation Edition plug-in.

Supported Chromatographic Data Systems

Following revision of OpenLab CDS ChemStation Edition is recommended:

- OpenLab CDS ChemStation Edition C.01.10 (or higher)

Using the Single Quadrupole functionality of the Agilent 1290 Infinity II 2D-LC Solution ChemStation requires a MS license for OpenLab CDS ChemStation Edition M8362AA, which further requires license M8360AA for spectral data evaluation.

The Secured File System feature in OpenLab CDS ChemStation Edition is not supported.

This software has been tested successfully with 12 LC modules. Please note that complex systems increase memory consumption in ChemStation, which may decrease system stability. In order to reduce the likelihood of issues, please

- Restart ChemStation from time to time, e.g. once per week or more often for complex systems
- Perform data analysis, reporting, online help reading in an off-line copy of the ChemStation instrument
- Save data before starting new tasks
- Avoid high levels of interactivity during runs by editing methods, changing signal plots settings, etc.

Supported Drivers

For software compatibility, see table below.

Agilent 2D-LC Software for ChemStation	ChemStation Version	LC & CE Driver Version
A.01.04 SR5 [43]	C.01.10 Update 6	3.4 (or higher)
A.01.04 SR4 [36]	C.01.10 Update 3	3.3

Supported Operating Systems

Supported operating systems are the same as for the corresponding CDS revision, which are

- Windows 7 SP1 (64 Bit)
- Windows 8.1 (64 Bit)
- Windows Server 2012 R2 (64 Bit)
- Windows 10 (64-Bit)

For details, please refer to the documentation of your Agilent ChemStation edition.

Supported Firmware

Please use the firmware which is included to the DVD with Agilent 2D-LC Software in folder Firmware.

Agilent 2D-LC Software has been tested with following firmware revisions:

Device	Firmware
Agilent 1100 Series, 1200 Series and 1200 Infinity	A.07.02
Agilent 1200 Series, 1200 Infinity and 1120 Compact LC	B.07.35
Agilent 1200 Infinity Hosted Modules	C.07.30
Agilent 1290 Infinity II Modules	D.07.35

NOTE

- Agilent releases LC firmware updates for so-called “firmware sets.”
- All Agilent LC instrument firmware sets have been designed and tested to be truly and strictly backwards compatible for the installed software base (CDS).
- The latest module firmware contained in each set is fully compatible and interoperable with all other module firmware of the same set.
- Agilent always recommends using the latest module firmware revision of a firmware set to avoid interoperatibility issues.
- Generally Agilent always recommends keeping the LC instrument firmware current.
- Do not mix firmware revisions between different sets. Agilent does not guarantee operation of mixed firmware revisions from older or newer sets.

Available Languages

Language settings: Agilent 2D-LC Software is available in English and has been tested with English versions of operating systems and CDSs.

It has also been tested successfully on a Chinese operating (Windows 7 64-Bit SP1) and chromatographic data system.

PC Requirements

See requirements for the OpenLAB CDS ChemStation edition. A minimum RAM of 8 GB is strongly recommended.

4 Installation

This chapter describes the installation of the Agilent 1290 Infinity II 2D-LC Solution ChemStation. These installation instructions are valid for the modes standard heart-cutting, multiple heart-cutting, high resolution sampling and comprehensive 2D-LC except the connections to the 2D-LC valve. These connections depend on the method, see chapter "Run the System for detailed information.

Hardware Installation of the 1290 Infinity II 2D-LC System

Delivery Checklist

Item	p/n	Description
1	G4243-90000	Agilent G4243A 2D-LC ASM Valve Guide Technical Note
2	5067-4266	2D-LC ASM Valve Head, 1300 bar
3	G4236-68000	2D-LC Easy Starter Kit (legacy) Internal part, not orderable
4	G4236-68100	2D-LC Easy Starter Kit for ESZ Service Internal part, not orderable
5	G1680-63721	Network LAN Switch
6	5500-1300	Capillary ST 0.12 mm x 85 mm M/M
7	5500-1301	Capillary ST 0.12 mm x 170 mm M/M
8	5500-1302	Capillary ST 0.12 mm x 340 mm M/M
9	5500-1303	Capillary ST 0.12 mm x 680 mm M/M
10	5500-1376	Capillary ST 0.12 mm x 170 mm M/M
11	5067-6171	Capillary Kit 2D-LC, Infinity Classic (optional) Internal part, not orderable
12	5067-6585	Capillary Kit 2D-LC, 1290 Infinity II Internal part, not orderable

The Capillary Kit 2D-LC, 1290 Infinity II contains the following parts:

#	p/n	Description
2	5043-0269	Adapter-profile for Agilent 1290 Valve Drive (G1170A)
1	5067-4608	Capillary ST 0.17 mm x 280 mm SX/S
2	5067-4651	Capillary ST 0.12 mm x 280 mm SL/SX
1	5067-4669	Capillary ST 0.12 mm x 600 mm S/SL
1	5067-4670	Capillary ST 0.17 mm ID 600 mm pre-swaged
1	5500-1217	Capillary, ST, 0.17 mm x 900 mm SI/SX
1	5500-1227	Capillary ST 0.17 mm x 150 mm SL-SL
1	5500-1240	Capillary ST 0.17 mm x 105 mm SL/SL
2	5500-1245	Capillary ST 0.17 mm x 400 mm SI/SI
2	5500-1251	Capillary ST 0.12 mm x 400 mm SL/SL

NOTE

Depending on the set up of you instrument, extra parts and capillaries might be required for instrument set up. Those parts are ordered separately or are shipped with other components, for example the 2D-LC or MHC valves. Their origin as well as their function is described in the instrument setup section below.

Options

NOTE

The Agilent 1290 Infinity II 2D-LC Solution ChemStation must contain an Agilent Infinity II High Speed Pump G7120A or Agilent 1290 Infinity Binary Pump G4220A as 2nd dimension pump.

This is necessary to achieve the following:

- Synchronize valve switches
- Run fast gradients on the 2nd dimension column

Table 1 Overview of recommended hardware configurations

Function in ^{2}D	Functional Element	Part Number	Module	Comment
1^{st} dimension	Pump	G7120A	1290 Infinity II High Speed Pump	
		G7112B	1260 Infinity II Binary Pump	
		G7111B	1290 Infinity II Quaternary Pump	
		G7104A	1290 Infinity II Flexible Pump	
		G7104C	1260 Infinity II Flexible Pump	
		G4220A/B	1290 Infinity Binary Pump	
		G4204A	1290 Infinity Quarternary Pump	
		G1312B	1260 Infinity Binary Pump	
	Sampler	G7129B	1290 Infinity II Vialsampler	
		G7167B	1290 Infinity II Multisampler	
	Column Compartment	G7116B	1290 Infinity II Multicolumn Thermostat	
		G1316C	1290 Infinity Thermostatted Column Compartment	
	Detector	G7117A/B/C	1260/1290 Infinity II Diode Array Detector	Recommended for multiple heart-cutting and high-resolution sampling as a peak trigger or for monitoring. Optional for comprehensive 2D-LC. ^{1}D flow cells require a minimum pressure stability of 60 bar (which excludes FLD and RID detectors).
		G7114A/B	1260/1290 Infinity II Variable Wavelength Detector	
		G7115A	1260 Infinity II Diode Array Detector WR	
		G7165A	1260 Infinity II Multiple Wavelength Detector	

Table 1 Overview of recommended hardware configurations

Function in ^{2}D	Functional Element	Part Number	Module	Comment
Interface	Valve drive	G1170A	1290 Infinity Valve Drive	1x for SHC, LCxLC 3x for MHC, HighRes Sampling
	2D-LC Valve	G4236A	2D-LC valve kit, Standard	Contains the 2D-LC valve head
		G4243A	2D-LC valve kit, ASM	Contains the 2D-LC valve head with Active Solvent Modulation (ASM) functionality
	MHC Valves	G4236A#007 G4243A#007	Multiple Heart-Cutting Kit	Contains two MHC valve heads
		G4242A	2D-LC Multiple Heart-Cutting Upgrade Kit	Kit to upgrade MHC valves to an existing 2D-LC system
	Pressure Relief Kit (PRK)	G4236-60010	Pressure Relief Kit	Mandatory if a ^{1}D detector is used. The kit prevents pressure pulses and protects detector flow cells!

Table 1 Overview of recommended hardware configurations

Function in ^{2}D	Functional Element	Part Number	Module	Comment
2^{nd} dimension	Pump	G7120A	1290 Infinity II High Speed Pump	1290 Infinity or Infinity II Binary Pump required.
		G4220A/B	Infinity 1290 Binary Pump	
	Column Compartment	G7116B	1290 Infinity II Multicolumn Thermostat	Optional: A second column compartment is optional for large temperature differences between 1^{st} and 2^{nd} dimension. Any of these are supported as well as others or older modules.
		G1316C	1290 Infinity Thermostatted Column Compartment	
	Detector	G7117A/B/C	1260/1290 Infinity II Diode Array Detector	
		G7114A/B	1260/1290 Infinity II Variable Wavelength Detector	
		G7115A	1260 Infinity II Diode Array Detector WR	
		G7165A	1260 Infinity II Multiple Wavelength Detector	
		G1321B	1260 Infinity FLD	
		G4260A	1260 Infinity ELSD	
		G6125B	Agilent Single Quadrupole Detector LC/MSD	
		G6135B	Agilent Single Quadrupole Detector LC/MSD XT	

Agilent LC/MS Single Quad 6100 Series

The following Agilent LC/MS instruments can be controlled with OpenLab CDS and ChemStation.

Table 2 Agilent LC/MS instruments that can be controlled with OpenLab CDS and ChemStation

Product Number	Description	Compatibility Statement
61xxA	LC/MS family	Only supported by ChemStation
G6160A	InfinityLab LC/MSD iQ	Only supported by OpenLab CDS
61xxB	LC/MS family	Supported by ChemStation For support by OpenLab CDS, the instrument requires a smart card update 61x0B to 61x0C via upgrade kit (G2735N) 61x5B to 61x5C via upgrade kit (G4934C)
G6150B	MS Module	Only supported by ChemStation
G6120C G6125C G6130C G6135C	MS Module LC/MSD MS Module LC/MSD XT	Only supported by OpenLab CDS ESI or AJS source required for tuning
Ion Sources		
G1947B G1971B	APCI APPI (photoionization)	
G1948B	ESI	
G1958B	Agilent Jet Stream for Single Quad	
G1978B	Multimode Source	

Recommendations for Instrument Setup

General Information

InfinityLab 2D-LC Solutions come in several flavors, still allowing flexible HPLC combination of InfinityLab Series and 1200 Series Infinity modules. In combination with the Agilent Mass Spectrometer the HPLC part of the 2D-LC solution requires a two-stack configuration. For 2D-LC, a two-stack configuration is always preferred. On the left stack, the order of the modules from bottom to top is: pumps for both dimensions, then Vial- or Multisampler.

The sampler must be placed on top of the pumps. The right stack consists of one or two column compartments and one or two standard UV detectors.

Depending on the number of solvents used, both stacks offer the possibility to place a solvent cabinet on top.

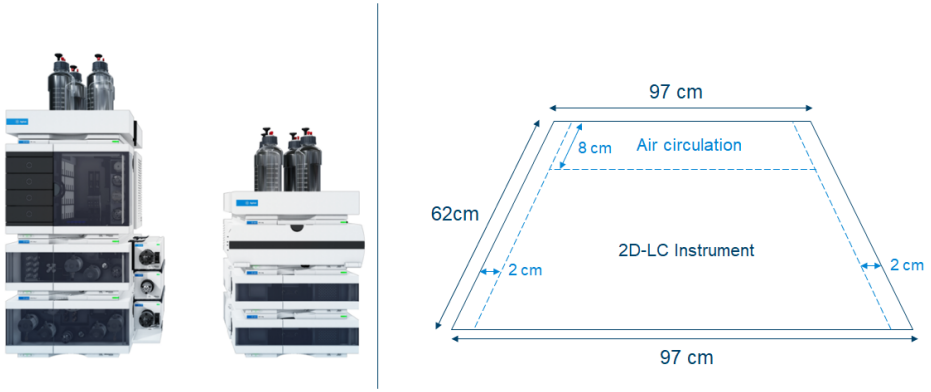


Figure 24 **Left: Recommended stack configuration for the 1290 Infinity II 2D-LC System. Right: Bench space requirements of the 1290 Infinity II 2D-LC System.**

NOTE

The dual stack configuration for 2D-LC requires at least 97 x 62 cm (24.4 x 38.2 inches) free, vertical bench space. 2.5 cm (1.0 inches) of space on either side and approximately 8 cm (3.1 inches) in the rear is reserved for air circulation and electric connections.

Installation of the 2D-LC Valve and optional MHC decks

Attaching the external valve drives

For InfinityLab 2D-LC instruments that comprise at least one 1260 Infinity II or 1290 Infinity II pump, valve drives are attached to this pump with the Valve Clamp Kit IF II (5067-5685), while the valve drives are interconnected by the Adapter profile (5043-0269). The 2D-LC valve and if selected the MHC decks are mounted on external valve drives (G1170A).

#	Holders / connectors	Connection	P/N
3	1290 Infinity Valve Drive (must be purchased separately)	Mounting of Valves	G1170A
1	Clamp Guide Kit IF II (delivered with G1170A)	Top valve to pump	5067-5685
2	Adapter-profile (delivered with MHC Decks)	between G1170A drives	5043-0269

For a SHC configuration, the 2D-LC valve (G4236A) is attached to the upper pump of the stack. In case of a MHC configuration, the upper MHC deck is attached to the upper pump.

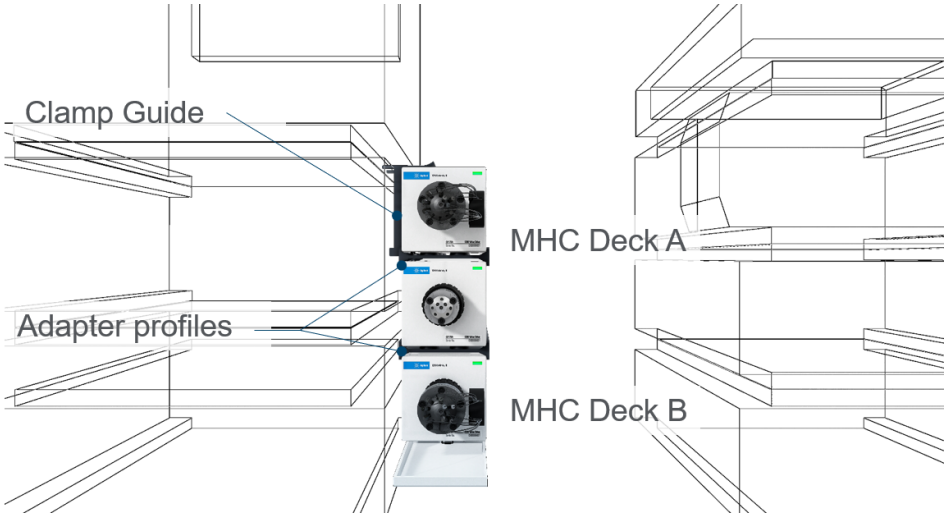


Figure 25 Schematic of the installation and attachments of the 2D-LC valve and optionally the MHC decks.

1. Mount the clamp guide on the right side of the Infinity II Pump: Markings in the form of round dips are on the body housing. Make a small hole with a peaked screw driver and tighten the clamp guide with the 3 self-cutting tapping screws.
2. Mount the valve heads on the G1170A external valve drives.
3. Clamp the first external valve drive with the MHC valve on top.
4. Attach the adapter-profile on each of the other external valve drives and mount them according to the positions shown in Figure 25 on page 61.
5. Mount the leak tray with sensor underneath the lowest external valve drive.
6. Install the Pressure release kit, see "Installing the Pressure Release Kit" on page 85.

Valve Configurations

Agilent InfinityLab 2D-LC Solutions offer two general valve configurations that decide which of the 2D-LC modes that can be used with the instrument. While the Single Heart-Cutting (SHC) configuration offers access to Single Heart-Cutting and Comprehensive 2D-LC, the Multiple Heart-Cutting (MHC) configurations additionally gives access to Multiple Heart-Cutting and High-Resolution Sampling 2D-LC. In addition, the Active Solvent Modulation valve (G4243A) is only available for the MHC configuration. An overview of all available 2D-LC modes can be found in Optional hardware configurations (Table 1 on page 55).

Stack setups of all other LC modules (reference) remain valid since those setups are independent of the valve configuration.

Table 3 Overview of 2D-LC modes dependent on valve configuration of the 2D-LC system

Valves	SHC Configuration	MHC Configuration
		
2D-LC Valve, Standard	✓	✓
2D-LC Valve, Active Solvent Modulation (ASM)	X	✓
Operation Modes	**SHC Configuration**	**MHC Configuration**
Comprehensive (LCxLC)	✓	✓
Single Heart-Cutting	✓	✓
Multiple Heart-Cutting	X	✓
High-Resolution Sampling	X	✓

Single Heart-Cutting Configuration

2D-LC instruments that are exclusively used for Single Heart-Cutting and Comprehensive 2D-LC experiments only require the standard 2D-LC valve (G4236A). The valve can be conveniently attached to any Infinity II pump that is installed. For a SHC configuration, transfer capillaries (6a/6b) are not necessary since MHC decks are not installed.

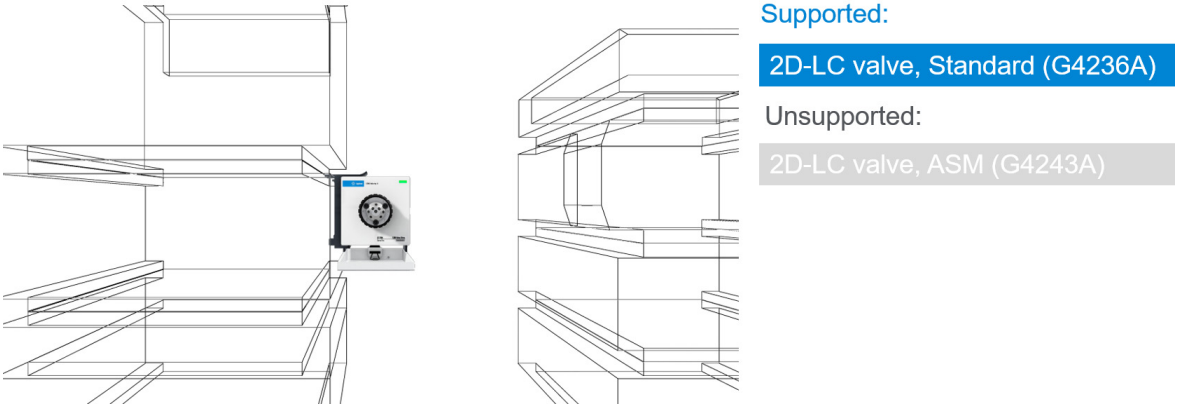


Figure 26 **Schematics of a Single Heart-Cutting (SHC) Configuration with supported valves. For technical reasons, the ASM valve (G4243A) is not supported in Single Heart-Cutting setups.**

Multiple Heart-Cutting Configuration

2D-LC instruments that are used for Multiple Heart-Cutting or High-Resolution Sampling 2D-LC require additional MHC decks. For MHC configurations, both the standard 2D-LC valve (G4236A) and the ASM valve head (G4243A) are supported. The valves can be conveniently attached to any Infinity II pump in the stack. Depending on the valve head that is used, different transfer capillaries (6a/6b) must be installed. For installation, please follow the guidance below.

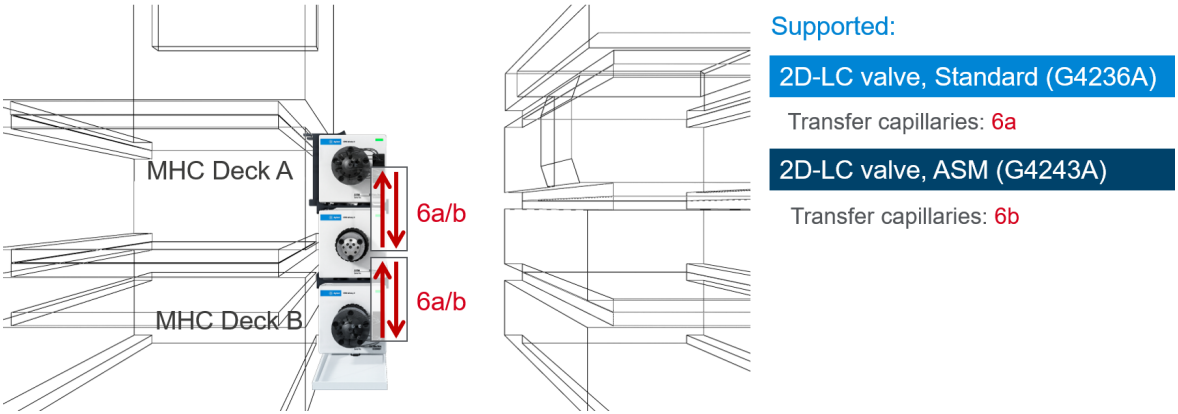


Figure 27 Schematics of a Multiple Heart-Cutting (MHC) Configuration with supported valves and transfer capillaries.

Recommended Stack Setups

InfinityLab 2D-LC Solutions allow three basic stack setups in three variations depending on the column compartment concept that is used. The pumps used for the first and second dimension distinguish the basic stack configurations. In the second dimension, a 1290 Infinity or 1290 Infinity II High-Speed Pump is mandatory. Agilent 1290 Infinity pumps are always based on the bottom. The capillary kit covers all recommended configurations. The following configurations optimize the system flow path, ensuring minimum delay and dispersion volumes:

Table 4 **Supported instrument configurations with a list of supported LC pumps. Numbers refer to the stack setup that is recommended.**

#	^{1}D pump	supported ^{2}D pumps
1	**1290 Infinity II / 1260 Infinity II Prime LC** 1260 Infinity II Flexible Pump (G7104C) Agilent 1260 Infinity II Bio Flexible Pump (G7131C) 1290 Infinity II Flexible Pump (G7104A) Agilent 1290 Infinity II Bio Flexible Pump (G7131A) 1290 Infinity II High-Speed Pump (G7120A) Agilent 1290 Infinity II Bio High-Speed Pump (G7132A)	**1290 Infinity / 1290 Infinity II** 1290 Infinity II High-Speed Pump (G7120A) Agilent 1290 Infinity II Bio High-Speed Pump (G7132A) 1290 Infinity Binary Pump (G4220A) See Figure 31 on page 72
2	**1290 Infinity** 1290 Infinity Quaternary Pump (G4204A) 1290 Infinity Binary Pump (G4220A)	**1290 Infinity II** 1290 Infinity II High-Speed Pump (G7120A) See Figure 32 on page 73
3	**1260 Infinity Binary / 1260 Infinity II Binary** 1260 Infinity II Binary Pump (G7112B) 1260 Infinity Binary Pump (G1312B)	**1290 Infinity II** 1290 Infinity II High-Speed Pump (G7120A) See Figure 33 on page 74

NOTE

This guide only covers setups that contain at least one Infinity II pump module! Setups that contain exclusively 1200 Infinity Series modules must be installed with the corresponding capillary kit.

Connections mentioned in this setup are the following:

- Concurrent direction for the Standard 2D-LC Valve (G4236A) with Single Heart Cut Configuration

  See Figure 28 on page 66.

- Countercurrent for the ASM 2D-LC Valve (G4243A) or Standard 2D-LC Valve (G4236A) with a Multiple Heart-Cutting Configuration

  See Figure 30 on page 70.

In the instruction table, the connections to valve port are mentioned in brackets, for example ASM Valve (2) = ASM Valve, Port 2.

If you want to connect the 2D-LC Valve in another direction than in these recommended 2D-LC setups, please follow the schematics shown under Valve Topology in the 2D-LC Software Online help.

Connecting the 2D-LC Valve, Standard (G4236A)

The capillary connections of the 2D-LC valves depend on whether a con- or countercurrent configuration achieved. For the standard 2D-LC Valve, both concurrent and countercurrent operation is possible. Schematics in this chapter will reflect a concurrent direction.

If you want to connect the 2D-LC Valve in a different direction, follow the schematics shown under Valve Topology in the 2D-LC Software Online help.

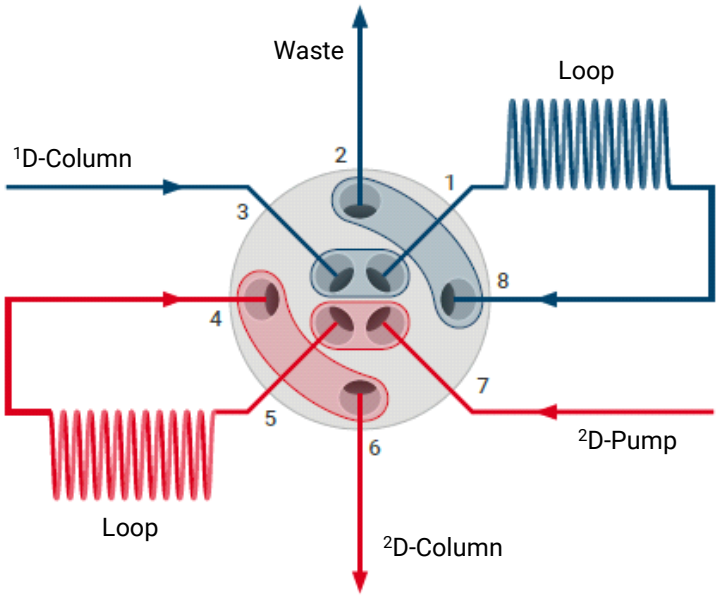


Figure 28 Schematic representation of the Standard 2D-LC Valve (G4236A) in concurrent flow.

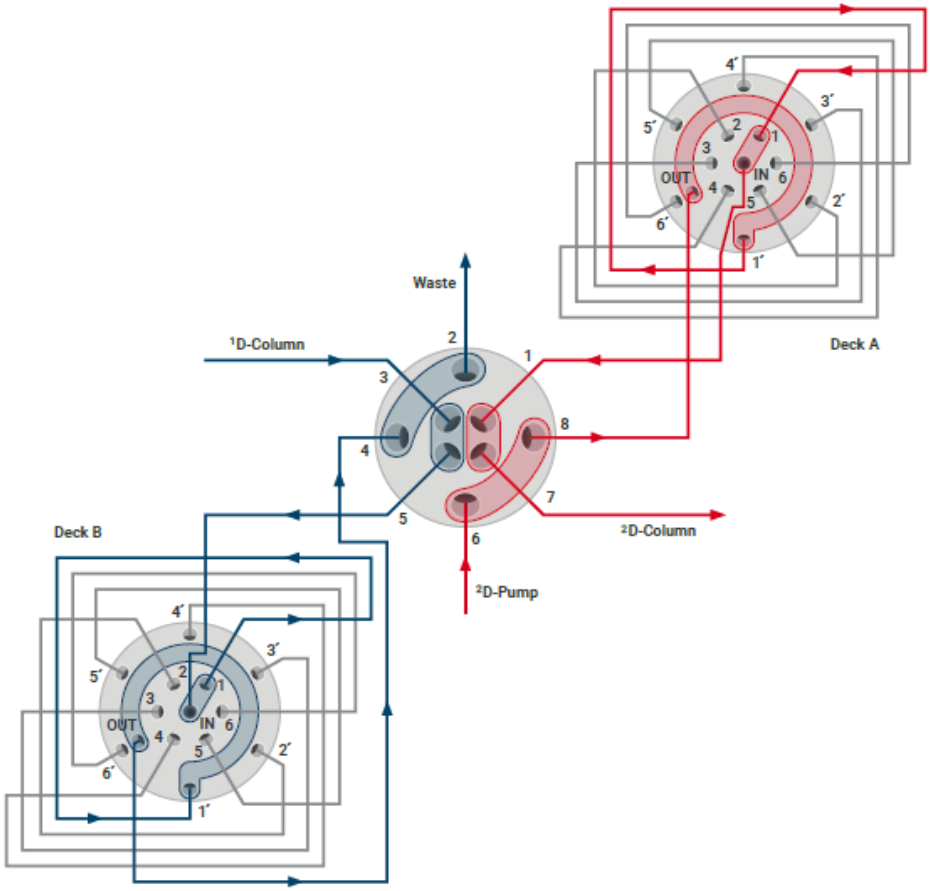


Figure 29 Standard 2D-LC valve (G4236A) with MHC 1300 bar (counter current)

Port	Number of Capillary	Connection	ID x L [mm]	P/N	Description
1	6a	transfer capillary to MHC Valve (OUT), deck A	0.12 x 170	5500-1270	Capillary ST 0.12x170 S/M
2	11	waste line	0.7 x self-cut	0890-1713	Tubing-flexible 0.8/1.61mm PTFE WT (delivered with UV detector)
3	5	from pressure release kit; from ^{1}D column, ^{1}D detector	0.17 x 105 0.12 x 500	5500-1240 5500-1157	Capillary ST 0.17x105 SL/SL Capillary ST 0.12x500 SL/S
4	6a	transfer capillary to MHC Valve (IN), deck B	0.12 x 170	5500-1270	Capillary ST 0.12x170 S/M
5	6a	transfer capillary to MHC Valve (OUT), deck B	0.12 x 170	5500-1270	Capillary ST 0.12x170 S/M
6	7	to ^{2}D column	0.12 x 400	5500-1251	Capillary ST 0.12x400 SL/SL
7	9	from ^{2}D pump	0.17 x 280	5067-4608	Capillary ST 0.17x280 SX/S
8	6a	transfer capillary to MHC Valve (IN), deck A	0.12 x 170	5500-1270	Capillary ST 0.12x170 S/M

Connecting the 2D-LC Valve, ASM (G4243A)

In contrast to the standard 2D-LC Valve (G4236A) Agilent recommends using a counter-current configuration for the ASM 2D-LC Valve (G4243A) when working in ASM mode. This section describes the setup for a counter-current configuration of the ASM Valve. For the concurrent setup, please refer to concurrent configuration of the ASM 2D-LC Valve in the 2D-LC Software. You find the **Valve topology** configuration screen in OpenLab CDS ChemStation Edition under **Instrument >2D-LC Configuration**.

The installation of a 2D-LC system depends on which modules you are using for which 2D-LC mode and is described above. The connection scheme is displayed in the graphical user interface of the 2D-LC Configuration as **Valve Topology**:

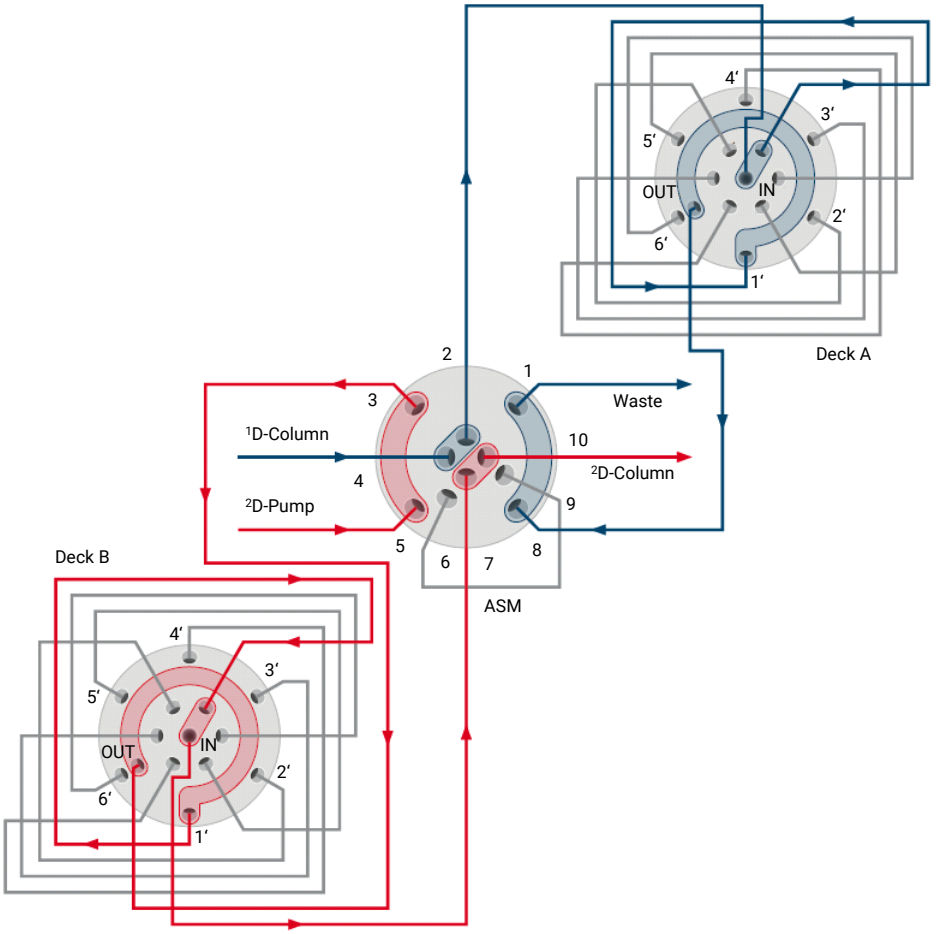


Figure 30 Schematic representation of the ASM 2D-LC Valve (G4243A) in countercurrent flow.

NOTE

Against the example shown in the figure above, for 1200 bar MHC Valves that have a different symmetry, the connection is OUT/IN.

Port	Number of Capillary	Connection	ID x L [mm]	P/N	Description
1	11	waste line	0.7 x self-cut	0890-1713	Tubing-flexible 0.8/1.61mm PTFE WT (delivered with UV detector)
2	6b	transfer capillary to MHC Valve (IN), deck A	0.12 x 170	5500-1376	Capillary ST 0.12x170 M/M
3	6b	transfer capillary from MHC Valve (OUT), deck B	0.12 x 170	5500-1376	Capillary ST 0.12x170 M/M
4	5 F3	from pressure release kit; from ^{1}D column, ^{1}D detector	0.17 x 105 0.12 x 500	5500-1240 5500-1157	Capillary ST 0.17x105 SL/SL Capillary ST 0.12x500 SL/S
5	9	from ^{2}D pump	0.17 x 280	5067-4608	Capillary ST 0.17x280 SX/S
6	ASM1-4	outlet to ASM capillary	0.12 x L		see list below
7	6b	transfer capillary to MHC Valve (IN), deck B	0.12 x 170	5500-1376	Capillary ST 0.12x170 M/M
8	6b	transfer capillary from MHC Valve (OUT), deck A	0.12 x 170	5500-1376	Capillary ST 0.12x170 M/M
9	ASM1-4	inlet from ASM capillary	0.12 x L		see list below
10	7	to ^{2}D column	0.12 x 400	5500-1251	Capillary ST 0.12x400 SL/SL

Which ASM capillary shall be used depends on the ASM factor, which is optimum for your application. You may choose from following capillaries:

Table 5 Available ASM Capillaries and properties

Capillary p/n	Length (mm)	Inner diameter (mm)	Volume (µl)	ASM factor	Split ratio (loop:ASM)
5500-1300	85	0.12	0.96	5	1:4
5500-1301	170	0.12	1.9	3	1:2
5500-1302	340	0.12	3.8	2	1:1
5500-1303	680	0.12	7.7	1.5	1:0.5

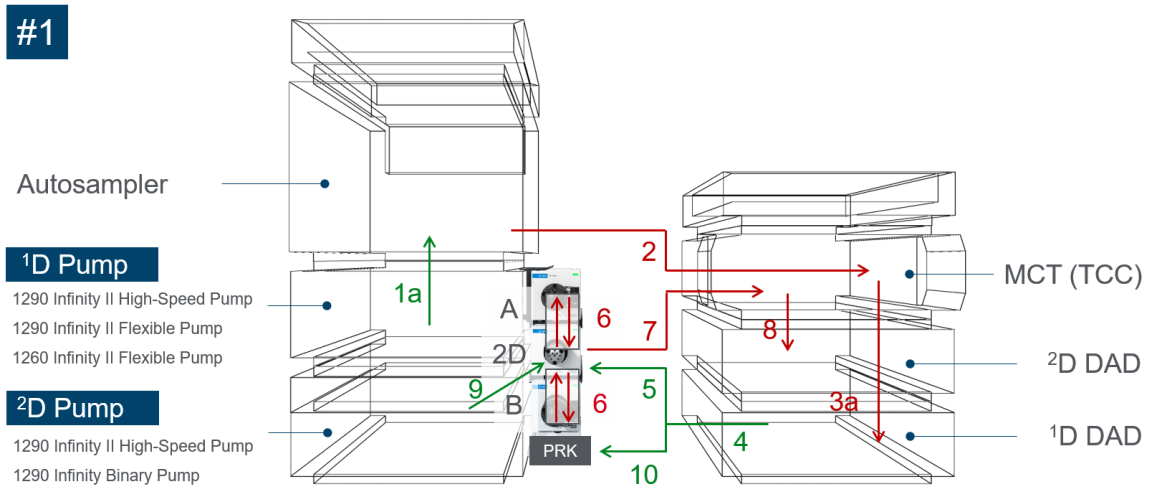


Figure 31 **Stack Setup #1. Recommended setup if both pumps are Infinity II modules or the 2D pump is a 1290 Infinity Binary pump.**

Number of Capillary	#	Connection	ID x L [mm]	P/N	Description
1a	1	1D pump (top) to autosampler	0.17 x 400	5500-1245	Capillary ST 0.17x400 SI/SI
2	1	Autosampler to 1D column (in MCT)	0.12 x 600	5067-4669	Capillary ST 0.12x600 S/SL
3a	1	1D column to 1D DAD	0.12 x 400	5500-1251	Capillary ST 0.12x400 SL/SL
4	1	1D DAD to T-piece of PRK	0.17 x 400	5500-1245	Capillary ST 0.17x400 SI/SI
5	1	T-piece of PRK to Standard 2D-LC Valve (Port 3) / ASM Valve (Port 4)	0.17 x 105	5500-1240	Capillary ST 0.17x105 SL/SL
6a	4	2D-LC Valve (1) - Deck (IN) – Deck (Out) - 2D-LC Valve (8) 2D-LC Valve (5) - Deck (IN) – Deck (Out) - 2D-LC Valve (4)	0.12 x 170	5500-1270	Capillary ST 0.12x170 S/M
6b	4	ASM Valve (7) - Deck (IN) – Deck (Out) - ASM Valve (3) ASM Valve (2) - Deck (IN) – Deck (Out) - ASM Valve (8)	0.12 x 170	5500-1376	Capillary ST 0.12x170 M/M (delivered with 2D-LC Valve Kit, ASM)
7	1	2D-LC valve (6) / ASM valve (10) to 2D column (in MCT)	0.12 x 400	5500-1251	Capillary ST 0.12x400 SL/SL
8	1	2D column (in MCT) to 2D DAD	0.12 x 280	5067-4651	Capillary ST 0.12x280 SL/SX
9	1	2D pump to 2D-LC Valve (7) / ASM Valve (5)	0.17 x 280	5067-4608	Capillary ST 0.17x280 SX/S
10	1	T-piece of PRK to damper capillary	0.17 x 150	5500-1227	Capillary ST 0.17x150 SL/SL
11	1	waste line	0.7 x self-cut	0890-1713	Tubing-flexible 0.8/1.61mm PTFE WT (delivered with UV detector)

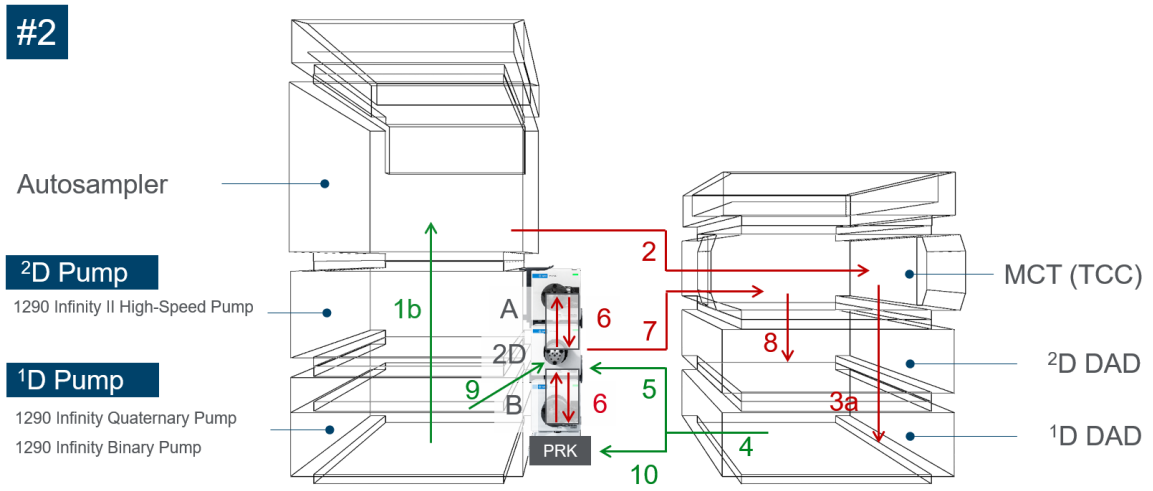


Figure 32 Stack Setup #2. Recommended setup if the ^{1}D pump is a 1290 Infinity Binary Pump or a 1290 Infinity Quaternary Pump.

Number of Capillary	#	Connection	ID x L [mm]	P/N	Description
1b	1	^{1}D pump (bottom) to sampler	0.17 x 600	5067-4670	Capillary ST 0.17x600 S/SH
2	1	Autosampler to ^{1}D column (in MCT)	0.12 x 600	5067-4669	Capillary ST 0.12x600 S/SL
3a	1	^{1}D column to ^{1}D DAD	0.12 x 400	5500-1251	Capillary ST 0.12x400 SL/SL
4	1	^{1}D DAD to T-piece of PRK	0.17 x 400	5500-1245	Capillary ST 0.17x400 SI/SI
5	1	T-piece of PRK to Standard 2D-LC Valve (Port 3) / ASM Valve (Port 4)	0.17 x 105	5500-1240	Capillary ST 0.17x105 SL/SL
6a	4	2D-LC Valve (1) - Deck (IN) – Deck (Out) - 2D-LC Valve (8) 2D-LC Valve (5) - Deck (IN) – Deck (Out) - 2D-LC Valve (4)	0.12 x 170	5500-1270	Capillary ST 0.12x170 S/M
6b	4	ASM Valve (7) - Deck (IN) – Deck (Out) - ASM Valve (3) ASM Valve (2) - Deck (IN) – Deck (Out) - ASM Valve (8)	0.12 x 170	5500-1376	Capillary ST 0.12x170 M/M (delivered with 2D-LC Valve Kit, ASM)
7	1	2D-LC valve (6) / ASM valve (10) to ^{2}D column (in MCT)	0.12 x 400	5500-1251	Capillary ST 0.12x400 SL/SL
8	1	^{2}D column (in MCT) to ^{2}D DAD	0.12 x 280	5067-4651	Capillary ST 0.12x280 SL/SX
9	1	^{2}D pump to 2D-LC Valve (7) / ASM Valve (5)	0.17 x 280	5067-4608	Capillary ST 0.17x280 SX/S
10	1	T-piece of PRK to damper capillary	0.17 x 150	5500-1227	Capillary ST 0.17x150 SL/SL
11	1	waste line	0.7 x self-cut	0890-1713	Tubing-flexible 0.8/1.61mm PTFE WT (delivered with UV detector)

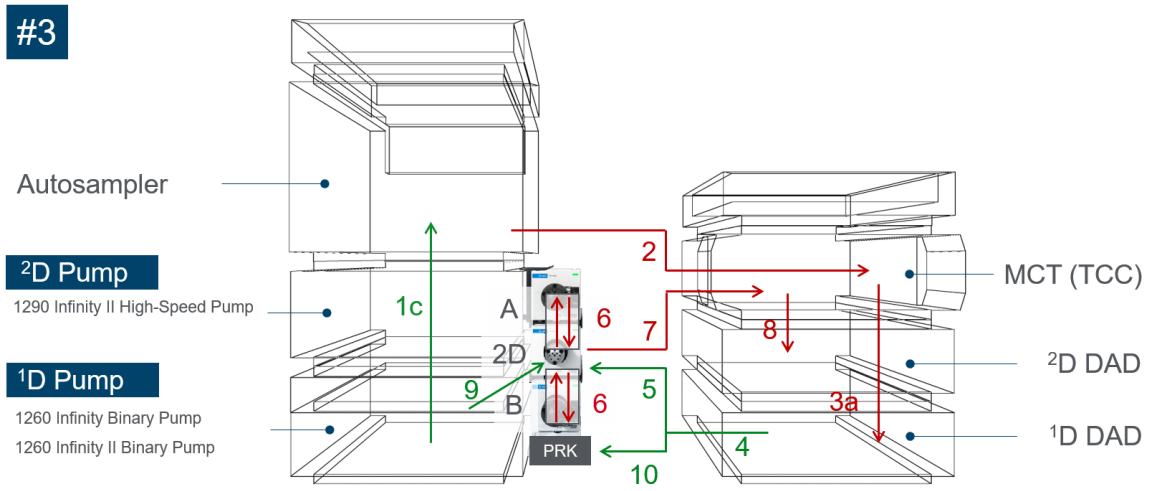


Figure 33 Stack Setup #3. Recommended setup if the 1D pump is a 1260 Infinity or 1260 Infinity II Binary Pump.

Number of Capillary	#	Connection	ID x L [mm]	P/N	Description
1c	1	1D pump (bottom) to sampler	0.17 x 900	5500-1217	Capillary ST 0.17x900 SI/SX
2	1	Autosampler to 1D column (in MCT)	0.12 x 600	5067-4669	Capillary ST 0.12x600 S/SL
3a	1	1D column to 1D DAD	0.12 x 400	5500-1251	Capillary ST 0.12x400 SL/SL
4	1	1D DAD to T-piece of PRK	0.17 x 400	5500-1245	Capillary ST 0.17x400 SI/SI
5	1	T-piece of PRK to Standard 2D-LC Valve (Port 3) / ASM Valve (Port 4)	0.17 x 105	5500-1240	Capillary ST 0.17x105 SL/SL
6a	4	2D-LC Valve (1) - Deck (IN) – Deck (Out) - 2D-LC Valve (8) 2D-LC Valve (5) - Deck (IN) – Deck (Out) - 2D-LC Valve (4)	0.12 x 170	5500-1270	Capillary ST 0.12x170 S/M
6b	4	ASM Valve (7) - Deck (IN) – Deck (Out) - ASM Valve (3) ASM Valve (2) - Deck (IN) – Deck (Out) - ASM Valve (8)	0.12 x 170	5500-1376	Capillary ST 0.12x170 M/M (delivered with 2D-LC Valve Kit, ASM)
7	1	2D-LC valve (6) / ASM valve (10) to 2D column (in MCT)	0.12 x 400	5500-1251	Capillary ST 0.12x400 SL/SL
8	1	2D column (in MCT) to 2D DAD	0.12 x 280	5067-4651	Capillary ST 0.12x280 SL/SX
9	1	2D pump to 2D-LC Valve (7) / ASM Valve (5)	0.17 x 280	5067-4608	Capillary ST 0.17x280 SX/S
10	1	T-piece of PRK to damper capillary	0.17 x 150	5500-1227	Capillary ST 0.17x150 SL/SL
11	1	waste line	0.7 x self-cut	0890-1713	Tubing-flexible 0.8/1.61mm PTFE WT (delivered with UV detector)

Alternative instrument setups for additional functionality

The standard stack setups can be upgraded with additional valves to add additional functionality. Table 4 on page 65 gives an overview of all supported modifications of a standard 2D-LC instrument. At a time, only one modification is recommended to ensure correct operation of the instrument. The standard stack setup uses one column compartment that hosts both the ^{1}D and ^{2}D column.

Table 6 List up supported modifications of a standard 2D-LC instrument configuration.

Alternative column compartment concepts		Comment	Page
A	^{1}D MCT/TCC hosts column switching valve	If a 6-position/14-port or 8-position/18-port InfinityLab Quick Change Valve is used, additional two adapters necessary (2xG1316-87326, must be purchased separately)	See Figure 34 on page 76
B	Setups that contain separate ^{1}D and ^{2}D MCTs/TCCs		See Figure 35 on page 77
C	Setups in which the ^{1}D column is hosted in an Integrated Column Compartment (ICC)	Longer capillary (5500-1170) for Quick Connect Fitting at column inlet or new 0.12x280mm Quick Connect Fitting assembly (5067-5960) necessary (must be purchased separately).	See Figure 36 on page 78
D	Setup with a MS diverter valve		See Figure 37 on page 80
E	Setup of a ^{1}D/^{2}D Switching Valve	If a ^{1}D and ^{2}D detector is used; not supported with modifications A-C	See Figure 38 on page 82
F	^{1}D/^{2}D Switching Valve w/o ^{1}D detector	For setups that do not have a ^{1}D detector, e.g. for certain LCxLC setups or setups with a QQQ mass spectrometer as a ^{2}D detector; not supported with modifications A-C	See Figure 39 on page 83
G	Single Heart-Cutting Configuration as Single Sample Loop Setup	For this setup port 4 and port 5 of the 2D-LC Standard must be used to connect the single loop while the bypass capillary is installed at the other position (Port 1 and 8) (for instance see application G4245A ProtA-SEC Kit).	See Figure 39 on page 83

A

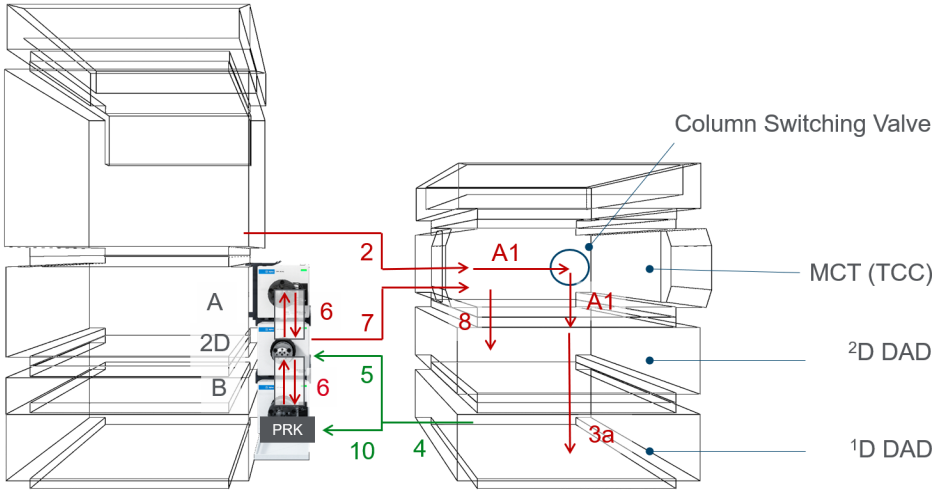


Figure 34 **Setup A. Recommended setup if a column switching valve (for example 6-position/14-port InfinityLab Quick-Change Valve) is used. For a InfinityLab 2-position/6-port Quick-Change Valve, adapters A1 are not necessary.**

Number of Capillary	#	Connection	ID x L [mm]	P/N	Description
A1	2	Adapter: capillary 2 to column switching valve, (Port IN) / Adapter column switching valve (Port OUT) to capillary 3a	0.12 x 75	G1316-87326	SST Capillary 0.12x75mm, f/m, ns 0.8 (must be purchased separately)

For all other capillaries / connections, please refer to Figure 31 on page 72, Figure 32 on page 73, and Figure 33 on page 74.

NOTE Adapters to and from the column switching valve are only necessary if a 6-position/14-port InfinityLab Quick-Change Valve or a for example 8-position/18-port InfinityLab Quick-Change Valve is used.

B

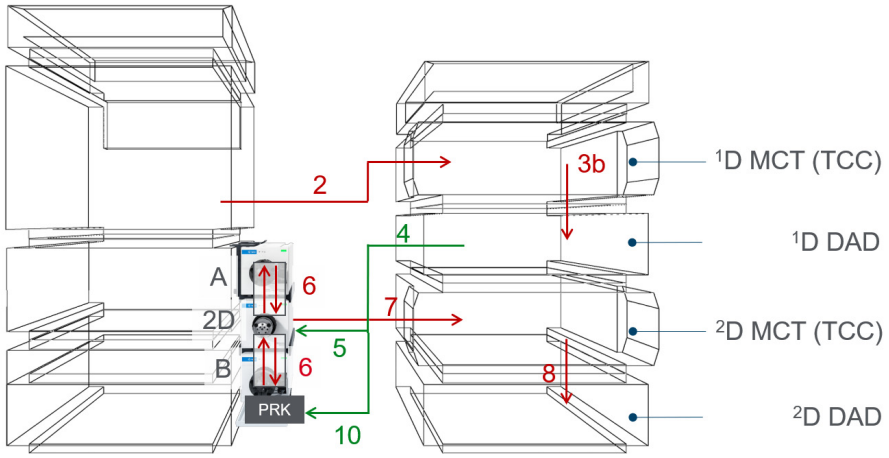


Figure 35 Setup B. Recommended setup if the instrument contains separate MCTs/ TCCs for ^{1}D and ^{2}D columns.

Number of Capillary	#	Connection	ID x L [mm]	P/N	Description
3b	1	^{1}D column to ^{1}D DAD	0.12 x 280	5067-4651	Capillary ST 0.12x280 SL/SX
8	1	^{2}D column (in ^{2}D MCT) to ^{2}D DAD	0.12 x 280	5067-4651	Capillary ST 0.12x280 SL/SX (part of 2D-LC capillary kit)

For all other capillaries / connections, please refer to Figure 31 on page 72, Figure 32 on page 73, and Figure 33 on page 74.

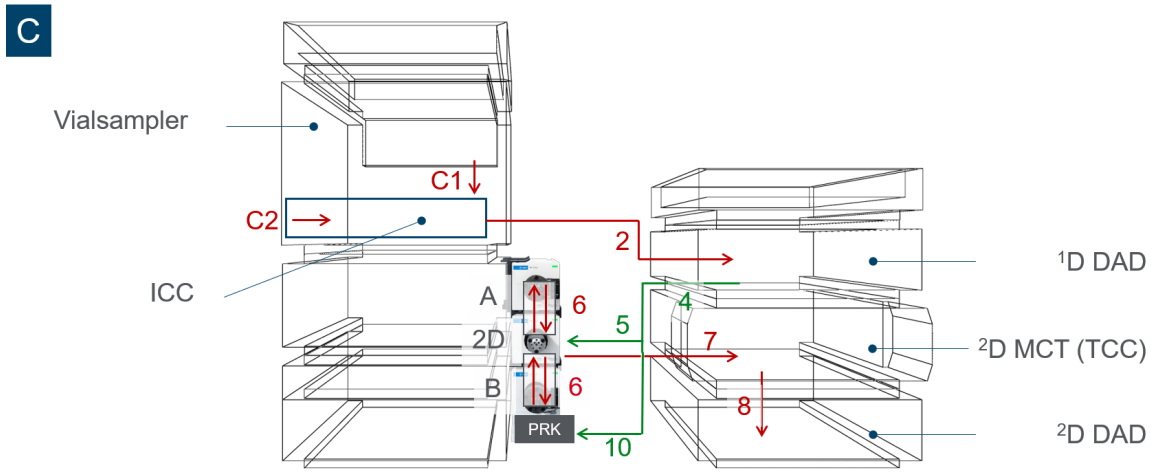


Figure 36 Setup C. Recommended setup if [1]D column is hosted in an Integrated Column Compartment (ICC).

Number of Capillary	#	Connection	ID x L [mm]	P/N	Description
C1	1	Injection Valve to ICC	0.12 x 105	5500-1238	Capillary ST 0.12x105 SL/SL (provided with ICC)
C2	1	Heat exchanger out to column (InfinityLab Quick Connect Fitting)	0.12 x 280	5500-1170	Capillary ST 0.12x280 (must be purchased separately)
8	1	[2]D column (in [2]D MCT) to [2]D DAD	0.12 x 280	5067-4651	Capillary ST 0.12x280 SL/SX (part of 2D-LC capillary kit)
For all other capillaries / connections, please refer to Figure 31 on page 72, Figure 32 on page 73, and Figure 33 on page 74.					

The driver-based 2D-LC Solution allows only certain valves to be configured as diverter valves which can be used for example as an effective desalting tool.

A list of supported valves can be found in Table 4 on page 65

More information is available in the following sections:

- "Method Parameters" on page 117
- "Run the System" on page 177

p/n	Description
G4231A	2pos/6port valve head, 800 bar
G4231C	2pos/6port valve head, 1300 bar
G4232C	2pos/10port valve head, 800 bar
G4232D	2pos/10port valve head, 1300 bar

D

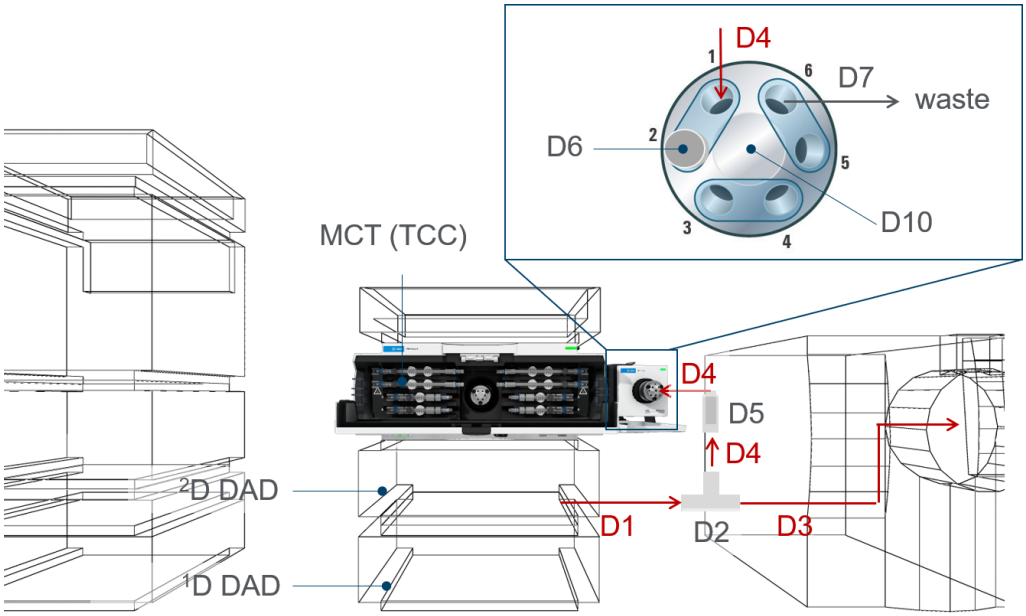


Figure 37 Setup D. Recommended setup of a MS diverter valve.

Number of Capillary	#	Connection	ID x L [mm]	P/N	Description
D1	1	Capillary from ^{2}D detector to T-piece	0.12 x 400	5067-4606	Capillary ST 0.12x400 S/SH
D2	1	T-piece		0100-0969	1/16in Tee, SST, Low Dead Volume
D3	1	Capillary from MS to T-piece (self cut)	0.12 x 400	0890-1915	Capillary PEEK, 0.12x1250
D4	2	T-piece to pressure relief valve; pressure relief valve to diverter valve	0.3 x 80	5500-1228	Capillary ST 0.3x80 SL/SL
D5	1	Pressure relief valve		G4212-60022	Pressure relief valve
D6	1	blank nut		01080-83202	Blanking Nut 1/16 in SST
D7	1	diverter valve to waste		5062-2462	Tubing PTFE 0.7 mm x 5m
D8	1	peak fittings		5063-6591	Fitting-Fingertight PEEK for 1/16-in
D9	1	Valve holder for Valve drive to attach to MCT		5067-6138	Valve Holder Kit Right-IF-II-G

Number of Capillary	#	Connection	ID x L [mm]	P/N	Description
D10	1	Diverter Valve		G4231A G4231C G4232A G4232C	2pos/6port, 800bar 2pos/6port, 1300bar 2pos/10port, 800bar 2pos/10port, 1300bar
For all other capillaries / connections, please refer to Figure 31 on page 72, Figure 32 on page 73, and Figure 33 on page 74.					

The ^{1}D/^{2}D switching valve offers the possibility to exclude the ^{2}D flow path of the instrument to run both ^{1}D and ^{2}D experiments which is useful for example if one mass spectrometer is used for both ^{1}D and ^{2}D experiments. Two basic setups are supported (setup E and F). The recommended setups for a ^{1}D/^{2}D Switching valves do not support the use of ICC column compartments, column switching valves or the use of separate ^{1}D and ^{2}D MCTs/TCCs! To run 1D experiments, the ^{2}D mode must be disabled.

E

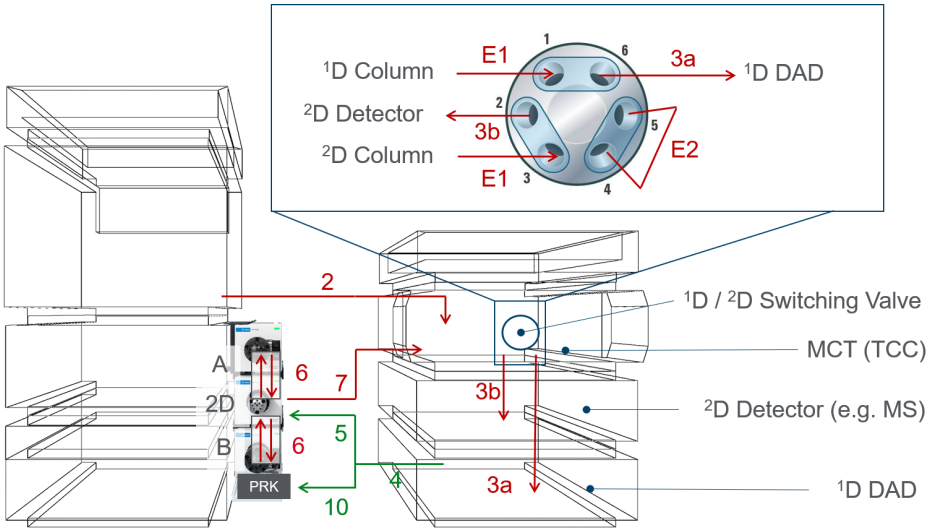


Figure 38 Setup E. Recommended setup for the ^{1}D/^{2}D switching valve.

Number of Capillary	#	Connection	ID x L [mm]	P/N	Description
3a	1	MCT / TCC to ^{1}D DAD	0.12 x 400	5500-1251	Capillary ST 0.12x400 SL/SL
8	1	^{1}D MCT / TCC to ^{1}D DAD	0.12 x 280	5067-4651	Capillary ST 0.12x280 SL/SX
E1	2	^{1}D column to ^{1}D/^{2}D Switching Valve (1); ^{2}D column to ^{1}D/^{2}D Switching Valve (3)	0.12 x 120	5067-4652	Capillary ST 0.12x120 SX/SX
E2	1	Connection capillary ^{1}D/^{2}D Switching Valve (4) to (5)	0.12 x 90	5067-4649	Capillary ST 0.12x90 SX/S
For all other capillaries / connections, please refer to Figure 31 on page 72, Figure 32 on page 73, and Figure 33 on page 74.					

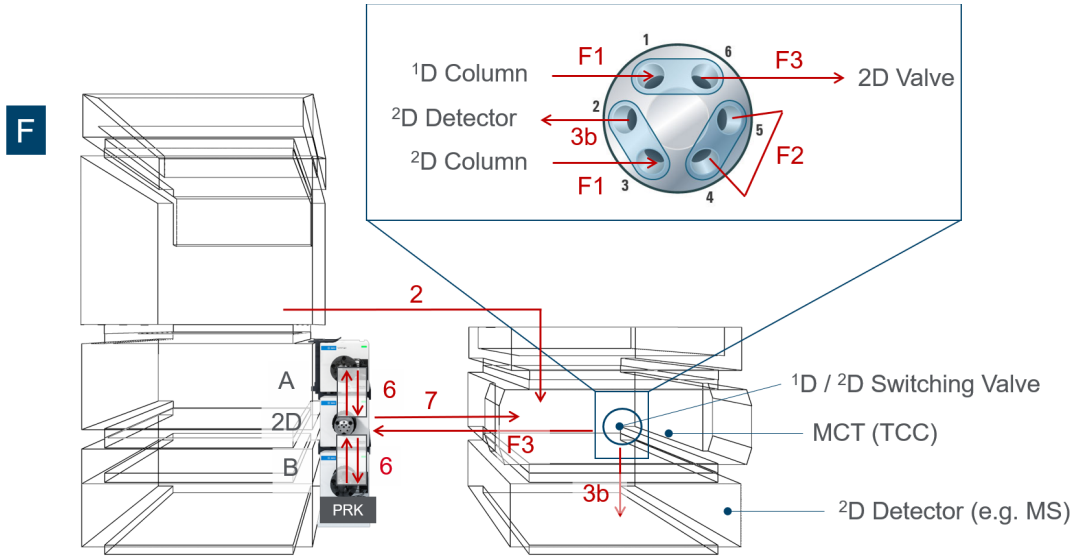


Figure 39 Setup F. Recommended setup for the ^{1}D/^{2}D switching valve without ^{1}D detector.

Number of Capillary	#	Connection	ID x L [mm]	P/N	Description
3b	1	^{1}D/^{2}D Switching Valve (2) to ^{2}D DAD	0.12 x 280	5067-4651	Capillary ST 0.12x280 SL/SX
F1	2	^{1}D column to ^{1}D/^{2}D Switching Valve (1); ^{2}D column to ^{1}D/^{2}D switching valve (3)	0.12 x 120	5067-4652	Capillary ST 0.12x120 SX/SX
F2	1	Connection ^{1}D/^{2}D switching valve ports (4) to (5)	0.12 x 90	5067-4649	Capillary ST 0.12x90 SX/S
F3	1	MCT/TCC to 2D-LC valve (6) / ASM valve (4)	0.12 x 500	5500-1157	Capillary ST 0.12x500 SL/S
For all other capillaries / connections, please refer to Figure 31 on page 72, Figure 32 on page 73, and Figure 33 on page 74.					

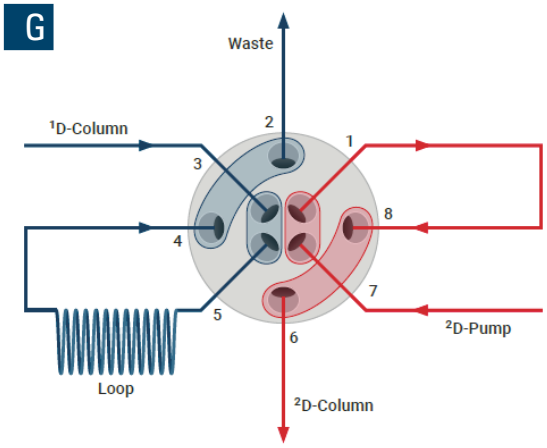


Figure 40 Setup G. Single Heart-Cutting Configuration as Single Sample Loop Setup

Number of Capillary	#	Connection	ID x L [mm]	P/N	Description
1	1	Bypass capillary (OUT)	0.12 x 105	5500-1238	Capillary, ST 0.12x105 SL/SL
2	1	Waste line	0.7 x self-cut	0890-1713	Tubing-flexible 0.8/1.61mm PTFE WT (delivered with UV detector)
3	1	From pressure release kit; from ^{1}D column, ^{1}D detector	 0.17 x 105 0.12 x 500	 5500-1240 5500-1157	 Capillary ST 0.17x105 SL/SL Capillary ST 0.12x500 SL/S
4		Sample Loop (IN)		5004-0036	180 µL Loop 2D-LC as an example
5		Sample Loop (OUT)		5004-0036	180 µL Loop 2D-LC as an example
6	1	To ^{2}D column	0.12 x 400	5500-1251	Capillary ST 0.12x400 SL/SL
7	1	From ^{2}D pump	0.17 x 280	5067-4608	Capillary ST 0.17x280 SX/S
8		Bypass capillary (IN)	0.12 x 105	5500-1238	Capillary, ST 0.12x105 SL/SL

For all other capillaries / connections, see Figure 31 on page 72, Figure 32 on page 73, and Figure 33 on page 74.

Installing the Pressure Release Kit

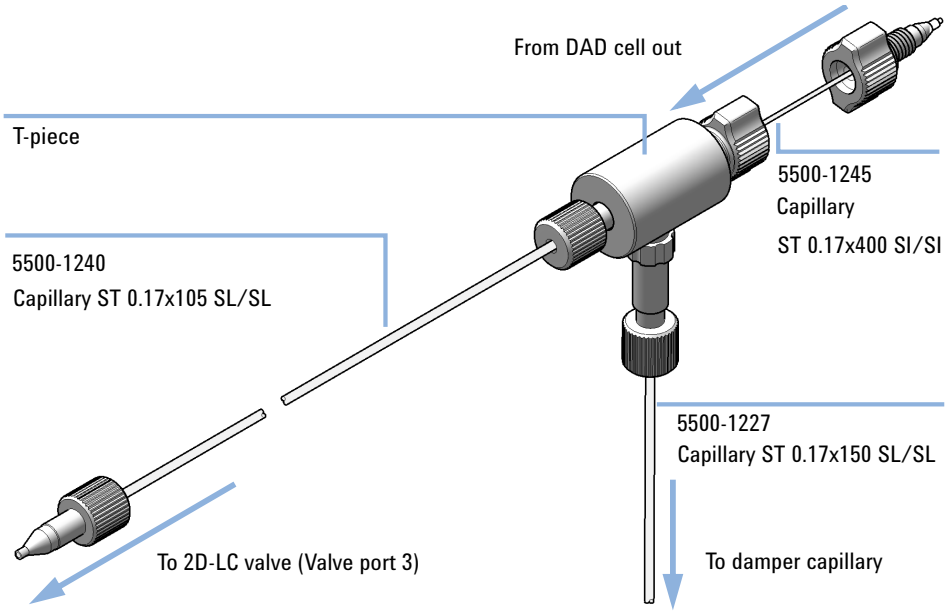
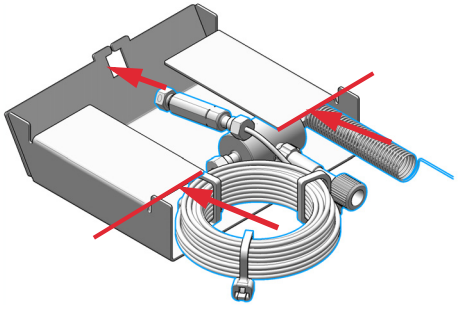


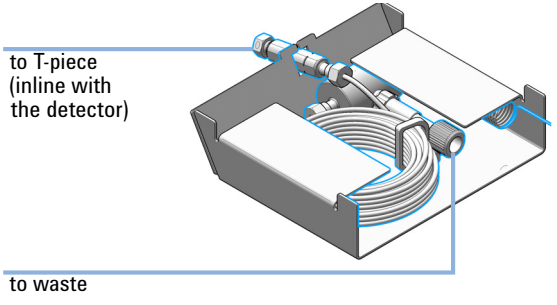
Figure 41 Connections to the pressure release kit

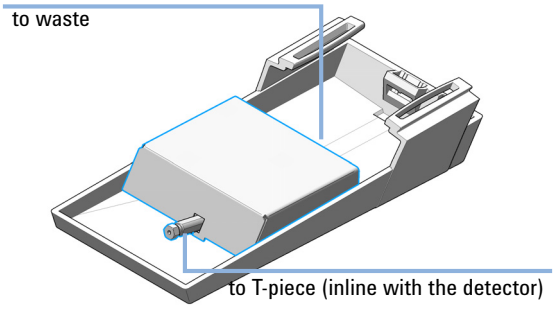
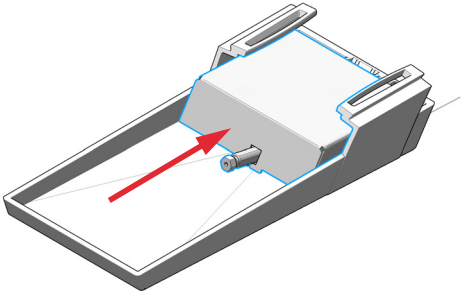
Parts required	#	p/n	Description
	1	G4236-60010	2D-LC Pressure Release Kit

1 Push the pressure release valve assembly in the frame.



2 Take care for the correct orientation.



3 Insert the pressure release assembly to the leak tray, orientation as shown.  	**4** Push the pressure release assembly in the correct position. 
5 Connect with the T-piece, see Figure 41 on page 85.	

Install the Valve Head and Connecting Capillaries

For instructions on how to install the valve head and connecting capillaries, see "Replace Valve Heads (G1170A)" on page 301.

Hardware Installation of the 1290 Infinity II Bio 2D-LC System

Delivery Checklist

For 1290 Infinity II Bio LC modules, use bio / biocompatible parts only.
Do not mix parts between 1260 Infinity II Bio-Inert LC modules and 1290 Infinity II Bio LC modules.

The InfinityLab Bio 2D-LC ASM Valve kit (G5643B) contains the following parts:

#	p/n	Description
1	5005-0078	Agilent InfinityLab Bio 2D-LC ASM Valve
1	5190-6895	2D-LC starter sample, 1 x 2 mL
2	G5642-64000	Bio Compatible MHC Loop Assembly SST
1	699968-301	Poroshell 120 Bonus-RP, 3.0 x 50 mm, 2.7 µm
1	G4236-64000	2D-LC Easy Start USB Media Kit
1	5005-0077	InfinityLab Bio 2D-LC Capillary Kit
1	G2453-85060	Formic Acid-Reagent Grade 5 mL (5 cc)
1	685775-902	Poroshell SB-C18, 2.1 x 100 mm, 2.7 µm
1	G1680-63721	Network LAN Switch
1		Regional power cord

The InfinityLab Bio 2D-LC Capillary Kit (5005-0077) contains the following parts:

#	p/n	Description
3	5500-1603	Quick Turn Capillary MP35N 0.17 mm x 400 mm
1	5004-0031	Capillary MP35N 0.12 mm x 600 mm
2	G7116-60071	Quick Connect Bio Heat Exchanger Standard Flow
2	5500-1578	Quick Connect Capillary MP35N 0.12 mm x 105 mm
2	5500-1597	Quick Turn Capillary MP35N 0.12 mm x 400 mm
1	5500-1599	Quick Turn Capillary MP35N 0.17 mm x 105 mm
1	5500-1600	Quick Turn Capillary MP35N 0.17 mm x 150 mm
1	5500-1596	Quick Turn Capillary MP35N 0.12 mm x 280 mm
2	5067-5965	InfinityLab Quick Connect LC fitting
20	5067-5966	InfinityLab Quick Turn Fitting
1	0890-1713	Tubing, PTFE, ID/OD 0.8/1.6 mm
1	5063-6591	PEEK Fittings 10/PK

The Bio Compatible MHC Loop Assembly SST (G5642-64000) contains the following parts:

p/n	Description
5043-0269	Adapter-profile for G1170A
5067-4273	6-column selector valve head, 1300 bar
5004-0027	Capillary MP35N 0.35 mm x 420 mm M/M 40 µL (6x) Pre-installed on 6 column selector

NOTE

Depending on the set up of you instrument, extra parts and capillaries might be required for installation. Those parts are ordered separately or are shipped with other components. Their origin as well as their function is described in the instrument setup section below or in the 2D-LC User manual or in the Bio LC device manuals.

Bio Materials

For the 1290 Infinity II Bio LC System, Agilent Technologies uses highest-quality materials in the flow path (also referred to as wetted parts). Life scientists prefer these materials, as they are known for optimum inertness to biological samples and ensure best compatibility with common samples and solvents over a wide pH range. To enable chromatography at very high pressures, while maintaining inertness the metal alloy MP35N is used instead of stainless steel throughout the system.

The MP35N is a nonmagnetic, nickel-cobalt-chromium-molybdenum alloy with an excellent resistance to sulfation, oxidation, saline solutions, and most mineral acids. Its superior properties ensure reliable performance, even under UHPLC conditions.

Bio Part Identification

CAUTION

Bio-inert parts are made of PEEK or other low pressure rated materials and cannot withstand high pressure above 600 bar.

Bio-inert parts are *not compatible* with 1290 Infinity II Bio LC modules.

- ✓ **For 1290 Infinity II Bio LC modules, use bio/biocompatible parts only.**
- ✓ **For bio-inert modules, use bio-inert parts only.**
- ✓ **Do not mix parts between 1260 Infinity II Bio-Inert LC modules and 1290 Infinity II Bio LC modules.**

NOTE

The installation of stainless steel-cladded PEEK capillaries (bio-inert) requires a special handling. Please read the Technical Note Installation of Stainless Steel Cladded PEEK Capillaries. (G5611-90120) for further and detailed description.

Important Hints for the Use of Bio Capillaries in a 1290 Infinity II Bio LC System

CAUTION

HNO_3 based procedures, and/or stainless steel in the flow path.

Damage of parts.

Metal ions may be introduced to the originally iron-free flow path.

- ✓ **Do not us HNO_3-based procedures for the 1290 Infinity II Bio LC System.**
- ✓ **Do not install mixed systems including biocompatible and regular stainless steel modules, parts, or capillaries.**

NOTE

The Technote *Best Practices for Using an Agilent LC System* contains recommendations for 1290 Infinity II Bio modules like installation, operation, and maintenance procedures.

Maintenance intervals of the bio valve may vary depending on the operation mode and the different solvents used, such as solvents with high buffer concentrations.

NOTE

To ensure optimum biocompatibility of your Agilent 1290 Infinity II Bio LC System:

- Do not include non-Bio standard modules or parts to the flow path
- Do not use any parts that are not labeled as Agilent *Bio*

For solvent compatibility of bio, biocompatible, and bio-inert materials, see *General Information about Solvent/Material Compatibility* in the Bio LC user manuals.

NOTE

Do not use stainless steel capillaries in the 1290 Infinity II Bio LC System. Watch out for orange stripe on the PTFE tubing of the capillary.

To avoid salt precipitation and blockages:

- Do not exceed or approach the solubility limit of buffer salt when prepare solvents
- Do not use > 50 mM buffer salt with high (> 60 %) acetonitrile concentrations

Important Notice on Fittings

Poroshell and AdvanceBio PEEK-lined columns

- Care must be taken to avoid damage to PEEK-lined columns during installation. Combined compression and rotation may cause internal damage. Fittings without ferrules (such as PEEK finger-tight fittings) are not recommended.
- Either use Agilent stainless steel cladded PEEK capillaries (1260 bio-inert solution) or MP35N capillaries with Quick Turn or Quick Connect fittings (1290 biocompatible solution).
- To choose the best fitting and capillary for bio-inert instrument setup

  www.agilent.com/chem/bioinertfittings
- To choose the best fitting and capillary for stainless steel system

  www.agilent.com/chem/fittings

Options

NOTE

The 1290 Infinity II Bio 2D-LC System must contain an Agilent Infinity II Bio High-Speed Pump (G7132A) as ^{2}D pump.

This is necessary to achieve the following:

- Enable 2D-LC functionality
- Run fast gradients on the ^{2}D column

Table 7 Overview of recommended bio hardware configurations

Function	Functional Element	Part Number	Module	Comment
^{1}D	Pump	G7131A	1290 Infinity II Bio Flexible Pump	
		G7131C	1260 Infinity II Bio Flexible Pump	
		G7132A	1290 Infinity II Bio High-Speed Pump	
		G5654A	1260 Infinity II Bio-inert Quaternary Pump	
	Sampler	G7137A	1290 Infinity II Bio Multisampler	
		G5668A	1260 Infinity II Bio-inert Multisampler	
	Thermostat	G7116A	1260 Infinity II Multicolumn Thermostat	Column compartments need biocompatible parts in the flow path. The G7116A is limited to use only valves up to 800 bar.
		G7116B	1290 Infinity II Multicolumn Thermostat	
	Detector	G7165A	1260 Infinity II Multiple Wavelength Detector	Detectors need biocompatible parts in the flow path. Adjust the ^{1}D flow rate to the flow cell pressure specifications. See also the comment on the Pressure Release Kit.
		G7115A	1260 Infinity II Diode Array Detector WR	
		G7114A	1260 Infinity II Variable Wavelength Detector	
		G7114B	1290 Infinity II Variable Wavelength Detector	
		G7117A	1290 Infinity II Diode Array Detector FS	
		G7117B	1290 Infinity II Diode Array Detector	
Interface	Valve drive	G1170A	1290 Infinity II Valve Drive	
	Bio 2D-LC Valve	G5643B	InfinityLab Bio 2D-LC ASM Valve Kit	For the flow path refer to the *Agilent 1290 Infinity II 2D-LC Solution OpenLab CDS and MassHunter Acquisition for TOF and Q-TOF User Guide*.
	MHC Valves		InfinityLab Bio Multiple Heart-Cutting Valve	These valves are included in G5643B. Stainless steel valves and biocompatible capillaries.
	Pressure Release Kit (PRK)	G4236-60010	Pressure Release Kit	Mandatory if a ^{1}D detector is used. The kit prevents pressure pulses and protects detector flow cells!

Table 7 Overview of recommended bio hardware configurations

Function	Functional Element	Part Number	Module	Comment
^{2}D	Pump	G7132A	1290 Infinity II Bio High-Speed Pump	1290 Infinity II Bio High-Speed Pump required.
	Column Compartment	G7116A	1260 Infinity II Multicolumn Thermostat	The second column compartment in the Bio 2D-LC System is recommended for large temperature differences between ^{1}D and ^{2}D. Any of these are supported as well as others or older bio modules. Need biocompatible parts in the flow path. The G7116A is limited to use only valves up to 800 bar.
		G7116B	1290 Infinity II Multicolumn Thermostat	
	Detector	G7117A	1290 Infinity II Diode Array Detector FS	Need biocompatible parts in the flow path.
		G7117B	1290 Infinity II Diode Array Detector	
		G7117C	1260 Infinity II Diode Array Detector HS	
		G7114A	1260 Infinity II Variable Wavelength Detector	
		G7114B	1290 Infinity II Variable Wavelength Detector	
		G7115A	1260 Infinity II Diode Array Detector WR	
		G7165A	1260 Infinity II Multiple Wavelength Detector	
		G7121B	1260 Infinity II Fluorescence Detector Spectra	
			Agilent Single Quadrupole Detector LC/MSD	
			High End Masspectrometer 6200 Series TOF and 6500 Series QTOF LC/MSD	

NOTE

For an overview of compatible mass spectrometers, see section *Agilent LC/MS Single Quad 6100 Series*. in the *1290 Infinity II 2D-LC Solution OpenLab CDS and MassHunter Acquisition for TOF and Q-TOF User Guide*.

NOTE

It is possible to connect third party detectors via UIB2 G1390A analog digital converter. But these third party modules have limited features in the CDS.

NOTE

Due to potential tailing, G7117A/B and G4212A/B Flow cells are not recommended for WCX and low salt SEC.

NOTE

To analyze photosensitive samples with UV-detectors (e.g. VWD, DAD WR, or LSS), prefer suitable flow cells and low light intensities. This is especially important for detectors in the first dimension.

Recommendations for Bio 2D-LC System

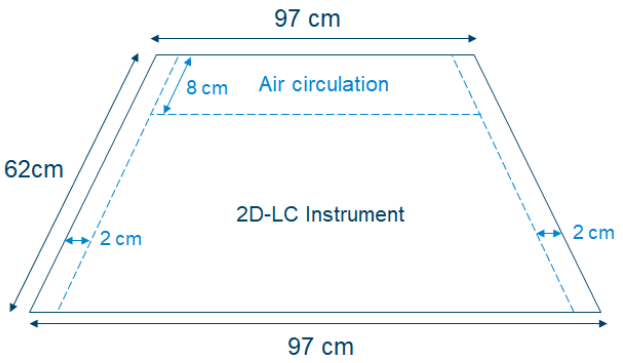
General Information

1290 Infinity II Bio 2D-LC Systems come in several flavors, still allowing flexible HPLC combination of the Agilent 1290/1260 Infinity II Bio LC System and Agilent 1260 Infinity Bio-inert LC. For a biocompatible 2D-LC system, a two-stack configuration is necessary. On the left stack, the order of the modules from bottom to top is: bio pumps for both dimensions, then bio autosampler.

The sampler must be placed on top of the pumps. The recommendation for the right stack consists of two column compartments to be more flexibly in respect to large temperature differences and column sizes and one or two standard UV detectors.

Both stacks offer the possibility to place a solvent cabinet on top.

Table 8 Recommended stack configuration and required bench space

Recommended stack configuration for the 1290 Infinity II Bio 2D-LC System	Bench space requirements of the 1290 Infinity II Bio 2D-LC System
	

NOTE

The dual stack configuration for Bio 2D-LC requires at least 97 x 62 cm (24.4 x 38.2 inches) free, vertical bench space. 2.5 cm (1.0 inches) of space on either side and approximately 8 cm (3.1 inches) in the rear is reserved for air circulation and electric connections.

Installation of the Bio 2D-LC ASM Valve and Optional MHC Decks

Attaching the external valve drives

For 2D-LC instruments that comprise at least one bio pump from the 1260 Infinity II or 1290 Infinity II series, valve drives are attached to this pump with Clamp Guide Kit-IF-II (5067-5685), while the valve drives are interconnected by Adapter-profile (5043-0269). The Bio 2D-LC valve and the MHC decks are mounted on external valve drives (G1170A).

#	Holders/connectors	Connection	P/N
3	1290 Infinity Valve Drive (must be purchased separately)	Mounting of Valves	G1170A
1	Clamp Guide Kit IF II (delivered with G1170A)	Top valve to pump	5067-5685
2	Adapter-profile (delivered with MHC Decks)	between G1170A drives	5043-0269

For an SHC configuration, the Bio 2D-LC ASM valve (G5643B) is attached to the upper pump of the stack. In an MHC configuration, the upper MHC deck is attached to the upper pump.

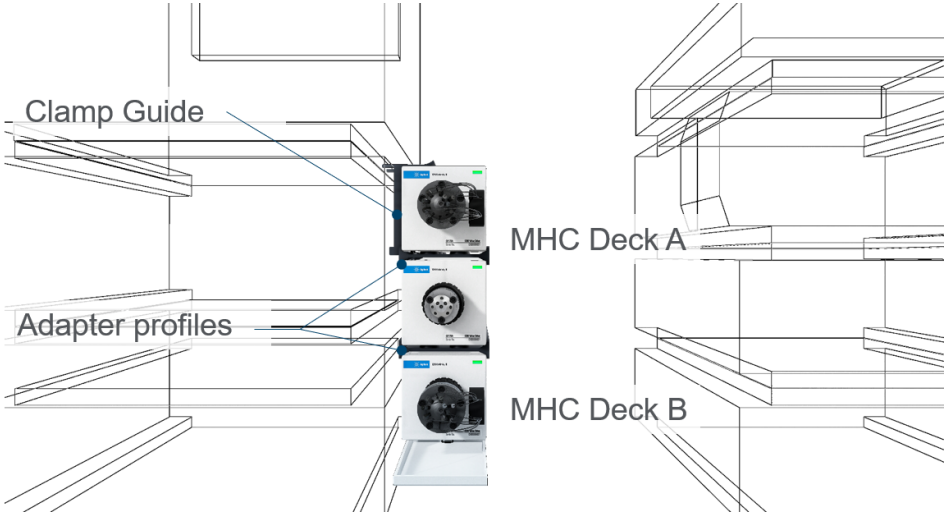


Figure 42 Schematic of the installation and attachments of the Bio 2D-LC valve and optionally the MHC decks

1. Mount the clamp guide on the right side of the Infinity II Pump: Markings in the form of round dips are on the body housing. Make a small hole with a peaked screw driver and tighten the clamp guide with the three self-cutting tapping screws.
2. Mount the valve heads on the G1170A external valve drives.
3. Clamp the first external valve drive with the MHC valve on top.
4. Attach the adapter-profile on each of the other external valve drives and mount them according to the positions shown in Figure 42 on page 95.
5. Mount the leak tray with sensor underneath the lowest external valve drive.
6. Install the pressure release kit, see "Installing the Pressure Release Kit" on page 111.

Valve Configurations

For 1290 Infinity II Bio LC modules, use bio / biocompatible parts only.
Do not mix parts between 1260 Infinity II Bio-Inert LC modules and 1290 Infinity II Bio LC modules.

Agilent 1290 Infinity II Bio LC Systems offer two general valve configurations that decide which of the 2D-LC modes that can be used with the instrument. While the Single Heart-Cutting (SHC) configuration offers access to Single Heart-Cutting and Comprehensive 2D-LC, the Multiple Heart-Cutting (MHC) configurations also give access to Multiple Heart-Cutting and High-Resolution Sampling 2D-LC. The Active Solvent Modulation valve is available for the SHC and MHC configuration. An overview of the recommended Bio 2D-LC mode can be found in the hardware configuration ("Recommended Bio Stack Setups" on page 100).

Stack setups of all other LC modules (reference) remain valid since those setups are independent of the valve configuration.

Table 9 Overview of 2D-LC modes dependent on valve configuration of the Bio 2D-LC system

Valves	SHC Configuration with ASM Valve	MHC Configuration
		
Bio 2D-LC Valve, Active Solvent Modulation (ASM)	✓	✓
Operation Modes	**SHC Configuration with ASM Valve**	**MHC Configuration**
Comprehensive (LCxLC)	✓	✓
Single Heart-Cutting	✓	✓
Multiple Heart-Cutting	X	✓
High-Resolution Sampling	X	✓

Single Heart-Cutting Configuration

BIO

For 1290 Infinity II Bio LC modules, use bio / biocompatible parts only.
Do not mix parts between 1260 Infinity II Bio-Inert LC modules and 1290 Infinity II Bio LC modules.

Biocompatible 2D-LC systems that are exclusively used for Single Heart-Cutting and Comprehensive 2D-LC experiments require the 2D-LC ASM valve. The valve can be conveniently attached to any Infinity II pump that is installed. For an SHC configuration, transfer capillaries are not necessary since MHC decks are not installed.

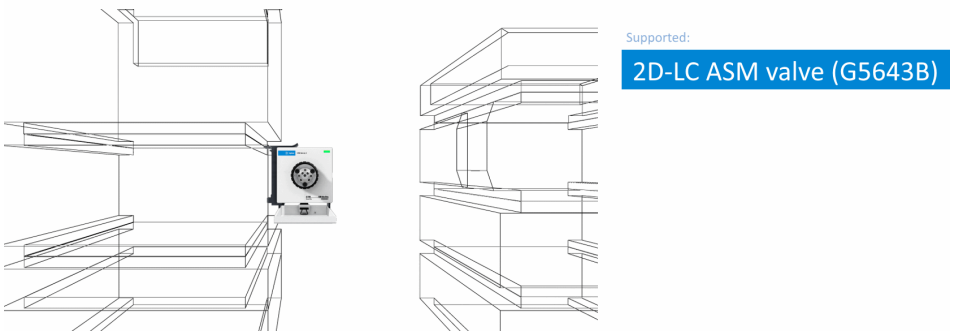


Figure 43 Schematics of a Single Heart-Cutting (SHC) Configuration with supported valves

NOTE

For the Bio 2D-LC setup (Single Heart-Cutting (SHC) with ASM Valve), LC driver 3.5 is required.

NOTE

Due to the increased wear, ASM functionality is not recommended for comprehensive runs in SHC or MHC configuration.

Multiple Heart-Cutting Configuration

BIO

For 1290 Infinity II Bio LC modules, use bio / biocompatible parts only.
Do not mix parts between 1260 Infinity II Bio-Inert LC modules and 1290 Infinity II Bio LC modules.

Biocompatible 2D-LC Systems that are used for Multiple Heart-Cutting or High-Resolution Sampling 2D-LC require extra Bio MHC decks. For MHC configurations, the Bio ASM valve head is supported. The valve can be conveniently attached to any bio pump in the stack. For the installation on the valve head, the transfer bio capillaries must be installed as follows.

NOTE

The Bio MHC Valve SST (G5642-64000) uses sample loops which have a biocompatible coating on the internal side of the stator and a PEEK rotor for protecting sensitive bio samples.

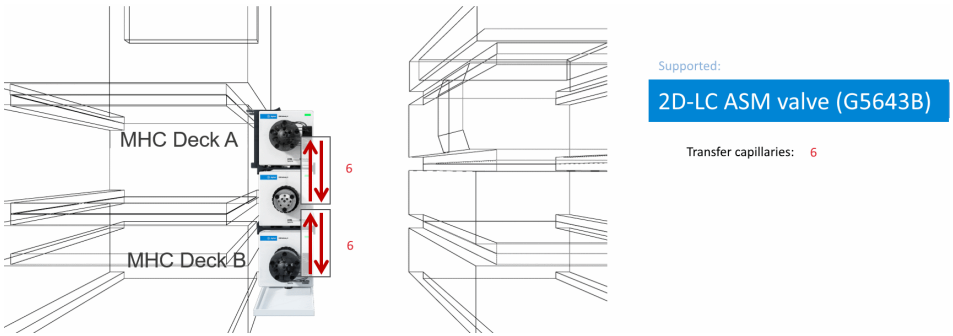


Figure 44 Schematics of a Multiple Heart-Cutting (MHC) Configuration with supported bio valves and bio transfer capillaries

Recommended Bio Stack Setups

1290 Infinity II Bio 2D-LC Systems allow two basic stack setups. The pumps used for the first and second dimension distinguish the basic stack configurations. In the second dimension, a 1290 Infinity II Bio High-Speed Pump is mandatory. The pumps are always based on the bottom. Other variations depend on the column compartment concept that is used. The bio capillary kit covers all recommended configurations. The following configurations ensure minimum delay and dispersion volumes and therefore optimize the system flow path:

Table 10 Supported instrument configurations with a list of supported Bio LC pumps. Numbers refer to the recommended bio stack setup

#	^{1}D pump	Supported ^{2}D pumps
1	**1290 Infinity II / 1260 Infinity II Prime LC** Agilent 1260 Infinity II Bio Flexible Pump (G7131C) Agilent 1290 Infinity II Bio Flexible Pump (G7131A) Agilent 1290 Infinity II Bio High-Speed Pump (G7132A)	**1290 Infinity II** Agilent 1290 Infinity II Bio High-Speed Pump (G7132A)
2	**1260 Infinity II Binary** Agilent 1260 Infinity II Bio-Inert Quat Pump (G5654A)	**1290 Infinity II** Agilent 1290 Infinity II Bio High-Speed Pump (G7132A)

NOTE

This guide only covers setups with bio pumps of the Agilent 1290 Infinity II series. Setups with other bio modules of the 1200 Infinity Series can require extra bio capillaries.

Connections mentioned in this setup are the following:

- Concurrent direction for the Bio 2D-LC ASM Valve with Single Heart Cut Configuration

  See Figure 45 on page 101.

- Countercurrent for the Bio 2D-LC ASM Valve with a Multiple Heart-Cutting Configuration

  See Figure 46 on page 103.

If you want to connect the Bio 2D-LC Valve in another direction than in these recommended 2D-LC setups, please follow the schematics shown under *Valve Topology* in the *2D-LC Software Online help*.

Connecting the Bio 2D-LC ASM Valve without MHC

BIO

For 1290 Infinity II Bio LC modules, use bio / biocompatible parts only.
Do not mix parts between 1260 Infinity II Bio-Inert LC modules and 1290 Infinity II Bio LC modules.

The capillary connections of the 2D-LC valves depend on whether a con- or countercurrent configuration is used. For the Bio ASM Valve, both concurrent and countercurrent operation are possible. Schematics in this chapter will reflect a concurrent direction.

If you want to connect the Bio ASM Valve in a different direction, follow the schematics shown under Valve Topology in the 2D-LC Software Online help.

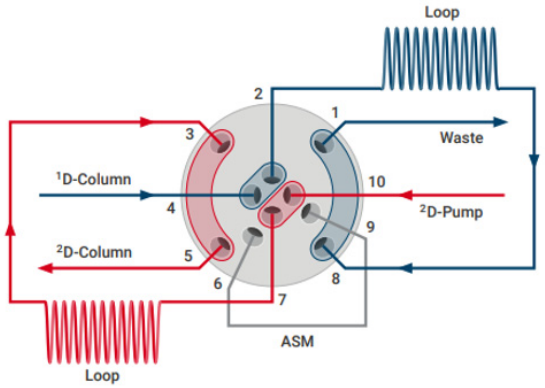


Figure 45 Schematic representation of the Bio 2D-LC ASM Valve without MHC in concurrent flow

NOTE

For the ASM functionality of the Single Loop Set up, the installation of transfer capillaries is recommended.

NOTE

Bio 2D-LC ASM Valve without MHC requires LC drivers 3.5.

Port	Number of Capillary	Connection	ID x L [mm]	P/N	Description
1		Waste line	0.7 x self-cut	0890-1713	Tubing-flexible 0.8/1.61 mm PTFE WT (delivered with UV detector)
2		Sample Loop (blue) (IN)	0.35 x 831	5004-0028	Capillary MP35N 0.35x831 M/M 80 µl see port 8 (This is an example and can be replaced by any other sample loop)
3		Sample Loop (red) (OUT)	0.35 x 831	5004-0028	Capillary MP35N 0.35x831 M/M 80 µl see port 7 (This is an example and can be replaced by any other sample loop)
4		from pressure release kit; from ^{1}D column,^{1}D detector	0.12 x 170	5500-1603	Quick Turn Capillary MP35N 0.17x400 M/M
5		to ^{2}D column (Heat exchanger)	0.12 x 170	5500-1597	Quick Turn Capillary MP35N 0.12x400 M/M
6		ASM Capillary e.g. ASM f-3	0.12 x 170	5004-0022	Capillary MP35N 0.12x170 M/M See port 9
7		Sample Loop (red) (IN)	0.35 x 831	5004-0028	Capillary MP35N 0.35x831 M/M 80 µl see port 3 (This is an example and can be replaced by any other sample loop)
8		Sample Loop (blue) (OUT)	0.35 x 831	5004-0028	Capillary MP35N 0.35x831 M/M 80 µl see port 2 (This is an example and can be replaced by any other sample loop)
9		ASM Capillary e.g. ASM f-3	0.12 x 170	5004-0022	Capillary MP35N 0.12x170 M/M See port 6
10		from ^{2}D pump	0.17 x 400	5500-1603	Quick Turn Capillary MP35N 0.17x400

Connecting the Bio 2D-LC Valve, ASM with MHC

For 1290 Infinity II Bio LC modules, use bio / biocompatible parts only.
Do not mix parts between 1260 Infinity II Bio-Inert LC modules and 1290 Infinity II Bio LC modules.

In contrast to the Bio 2D-LC ASM Valve in SHC configuration Agilent recommends using a counter-current setup for the Bio 2D-LC ASM Valve in MHC

configuration. This section describes the setup for a counter-current configuration of the Bio 2D-LC ASM Valve. For the concurrent setup, please refer to concurrent configuration of the ASM 2D-LC Valve in the 2D-LC Software. You find the **Valve topology** configuration screen in OpenLab ChemStation under **Instrument >2D-LC Configuration** or in OpenLab CDS 2.6 under **Valve Topology** in the 2D-LC Software Online help.

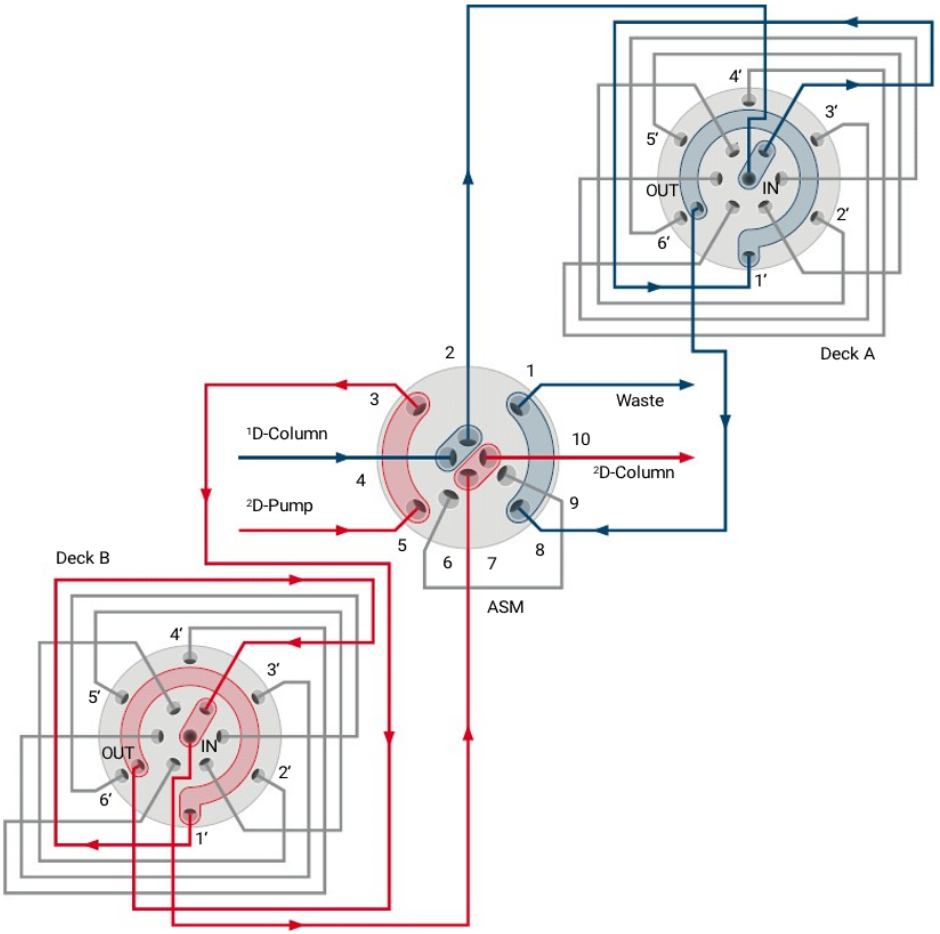


Figure 46 Schematic representation of the Bio 2D-LC ASM Valve in countercurrent flow

NOTE

Against the example shown in the figure above, for 1200 bar MHC Valves that have a different symmetry, the connection is OUT/IN.

Port	Number of Capillary	Connection	ID x L [mm]	P/N	Description
1	11	waste line	0.7 x self-cut	0890-1713	Tubing-flexible 0.8/1.61 mm PTFE WT (delivered with UV detector)
2	6	Bio transfer capillary to MHC Valve (IN), deck A	0.12 x 170	5004-0020	Capillary MP35N 0.12x170 M/M
3	6	Bio transfer capillary from MHC Valve (OUT), deck B	0.12 x 170	5004-0020	Capillary MP35N 0.12x170 M/M
4	5 F3	from pressure release kit; from ^{1}D column, ^{1}D detector	0.17 x 400	5500-1603	Quick Turn Capillary MP35N 0.17x400 M/M
5	9	from ^{2}D pump	017 x 400	5500-1603	Quick Turn Capillary MP35N 0.17x400 M/M
6	ASM1-4	outlet to Bio ASM capillary	0.12 x L		see list below
7	6	Bio transfer capillary to MHC Valve (IN), deck B	0.12 x 170	5004-0020	Capillary MP35N 0.12x170 M/M
8	6	Bio transfer capillary from MHC Valve (OUT), deck A	0.12 x 170	5004-0020	Capillary MP35N 0.12x170 M/M
9	ASM1-4	inlet from Bio ASM capillary	0.12 x L		see list below
10	7	to ^{2}D column	0.12 x 400	5500-1597	Quick Turn Capillary MP35N 0.12x400 M/M

Which Bio ASM capillary (MP35N) shall be used depends on the ASM factor, which is optimum for your application. You may choose from following capillaries:

Table 11 Available ASM Capillaries and properties

Bio Capillary p/n	Length (mm)	Inner diameter (mm)	Volume (µL)	ASM factor	Split ratio (loop:ASM)
5004-0021	85	0.12	0.96	5	1:4
5004-0022	170	0.12	1.9	3	1:2
5004-0023	340	0.12	3.8	2	1:1
5004-0024	680	0.12	7.7	1.5	1:0.5

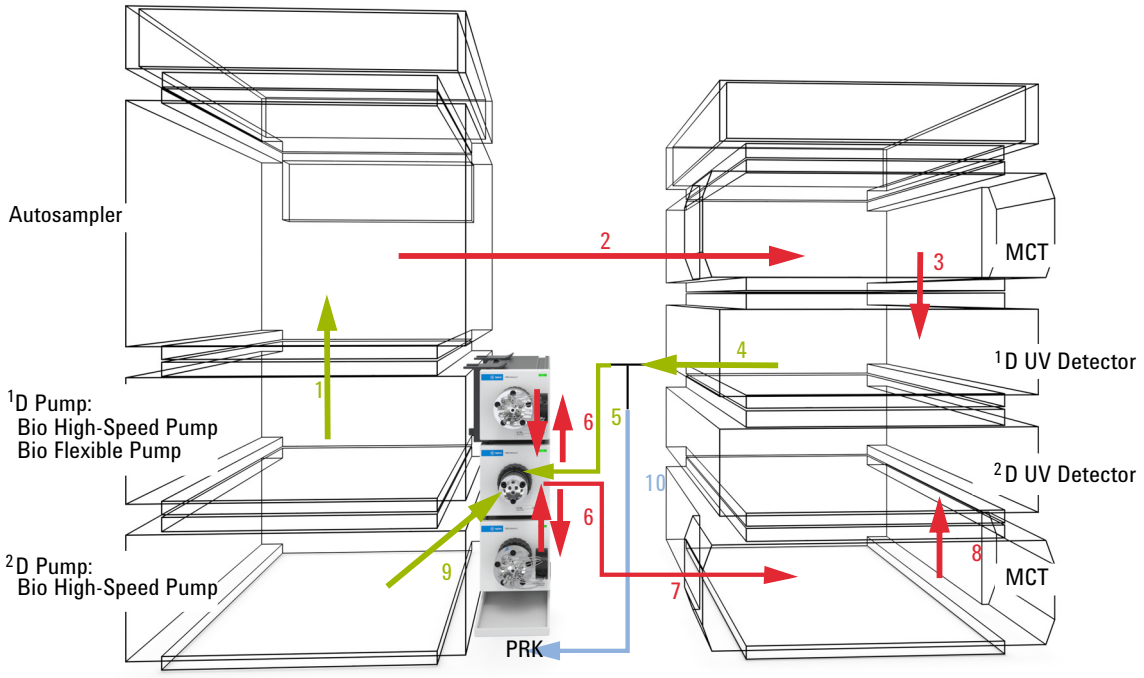


Figure 47 Recommended setup if both bio pumps are Infinity II modules or the [2]D pump is a 1290 Infinity Bio High-Speed Pump

Number of Capillary	#	Connection	ID x L [mm]	P/N	Description
1	1	^{1}D pump (top) to autosampler	0.17 x 400	5500-1603	Quick Turn Capillary MP35N 0.17 x 400 M/M
2	1	Autosampler to Bio Quick-Connect Heat Exchanger Standard Flow (MCT1)	0.12 x 600	5004-0031	Capillary MP35N 0.12 x 600
	1	Bio Quick-Connect Heat Exchanger Standard Flow to ^{1}D column (in MCT1)	0.12 x 105	5500-1578	Quick-Connect Capillary MP35N 0.12x105 M/M
3	1	^{1}D column to ^{1}D detector	0.12 x 400	5500-1597	Quick Turn Capillary MP35N 0.12 x 400 M/M
4	1	^{1}D detector to T-piece of PRK	0.17 x 105	5500-1599	Quick Turn Capillary MP35N 0.17 x 105 M/M
5	1	T-piece of PRK to Bio 2D-LC ASM Valve (Port 4)	0.17 x 400	5500-1603	Quick Turn Capillary MP35N 0.17 x 400 M/M
6	4	Bio 2D-LC ASM Valve (Port 7) - Deck (IN), Deck (Out) - Bio 2D-LC ASM Valve (Port 3) Bio 2D-LC ASM Valve (Port 2) - Deck (IN), Deck (Out) - Bio 2D-LC ASM Valve (Port 8)	0.12 x 170	5500-1376	Capillary ST 0.12 x 170 M/M (delivered with 2D-LC Valve Kit, ASM)
7	1	Bio 2D-LC ASM valve (Port 10) to Bio Quick-Connect Heat Exchanger Standard Flow (MCT1 or 2)	0.12 x 400	5500-1597	Quick Turn Capillary MP35N 0.12 x 400 M/M
	1	Bio Quick-Connect Heat Exchanger Standard Flow to 2D column (in MCT1 or 2)	0.12 x 105	5500-1578	Quick-Connect Capillary MP35N 0.12 x 105 M/M
8	1	^{2}D column (in MCT 1or 2) to ^{2}D detector	0.12 x 280	5500-1596	Quick Turn Capillary MP35N 0.12 x 280 M/M
9	1	^{2}D pump to Bio 2D-LC ASM Valve (Port 5)	0.17 x 400	5500-1603	Quick Turn Capillary MP35N 0.17 x 400 M/M
10	1	T-piece of PRK to damper capillary	0.17 x 150	5500-1600	Quick Turn Capillary MP35N 0.17 x 150
	1	Bio 2D-LC ASM Valve (Port 1) to Waste (not shown)	0.7 x self-cut	0890-1713	Tubing-flexible 0.8/1.61 mm

NOTE InfinityLab Quick Turn fittings require the capillaries specified in this table.

Alternative Instrument Setups for Additional Functionality

For 1290 Infinity II Bio LC modules, use bio / biocompatible parts only.
Do not mix parts between 1260 Infinity II Bio-Inert LC modules and 1290 Infinity II Bio LC modules.

The driver-based Bio 2D-LC Solution allows only certain valves to be configured as bio diverter valves which can be used for example as an effective desalting tool.

More information is available in the following sections:

- "Method Parameters" on page 117
- "Run the System" on page 177

Table 12 Supported valves

Description	P/N
2-position/6-port valve head, 600 bar, bio-inert	5067-4148
2-position/10-port valve, bio 1300 bar, PEEK, MP35N	5067-6682

D

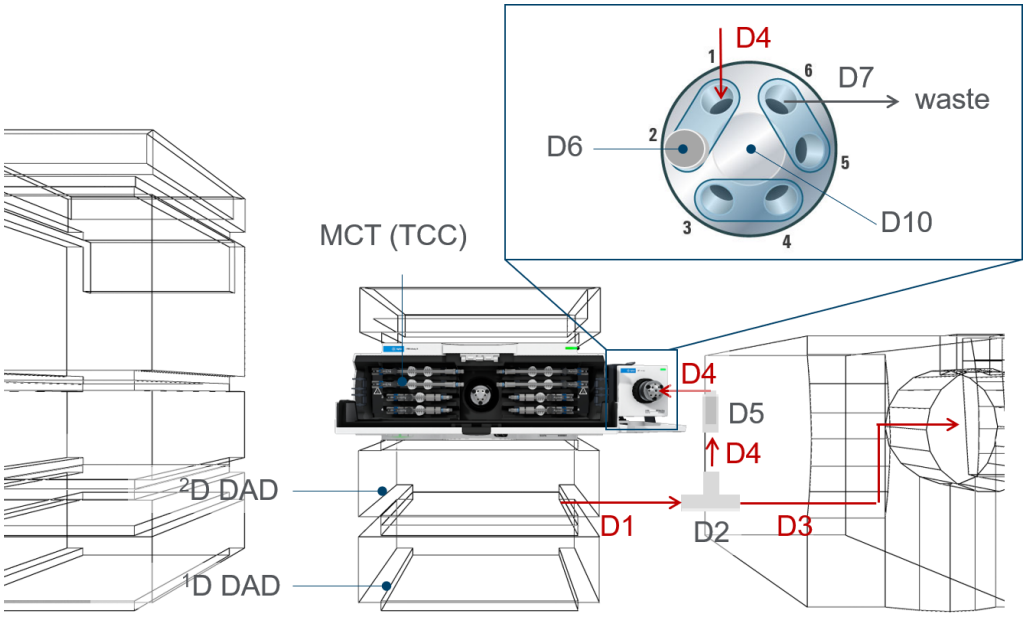


Figure 48 Recommended setup of a MS diverter valve

For all other capillaries / connections, see:

- Figure 31 on page 72,
- Figure 32 on page 73 , and
- Figure 33 on page 74.

Table 13 Available capillaries

Number of Capillary	#	Connection	ID x L [mm]	P/N	Description
D1	1	Capillary from 2D detector to T-piece	0.12 x 400	5500-1597	Quick Turn Capillary MP35N 0.12x400
D2	1	T-piece (PEEK includes fittings)		5022-2144	1/16in Tee, SST, Low Dead Volume
D3	1	Capillary from MS to T-piece (self cut)	0.12 x 400	0890-1915	Capillary PEEK, 0.12x1250
D4	2	T-piece to pressure relief valve; pressure relief valve to diverter valve	0.3 x 80	5500-1473	Capillary MP35N 0.3x80 SL/SL
D5	1	Pressure relief valve		G4212-60022	Pressure relief valve
D6	1	Blank nut		5043-0277	Blanking Nut long 10-32
D7	1	Diverter valve to waste (Waste line)		0890-1713	Tubing-flexible 0.8/1.61mm PTFE WT
D8	1	Peak fittings		5063-6591	Fitting-Fingertight PEEK for 1/16-in
D9	1	Valve holder for Valve drive to attach to MCT		5067-6138	Valve Holder Kit Right-IF-II-G
D10	1	Diverter Valve		G5631A	2-position/6-port valve head, 600 bar, bio-inert
				G5641A	2-position/10-port valve, bio 1300 bar PEEK, MP35N

NOTE To be recognized as a diverter valve in the driver-based 2D-LC solution, the diverter valve must be installed in an external valve drive (G1170A).

Installing the Pressure Release Kit

BIO

For 1290 Infinity II Bio LC modules, use bio / biocompatible parts only.
Do not mix parts between 1260 Infinity II Bio-Inert LC modules and 1290 Infinity II Bio LC modules.

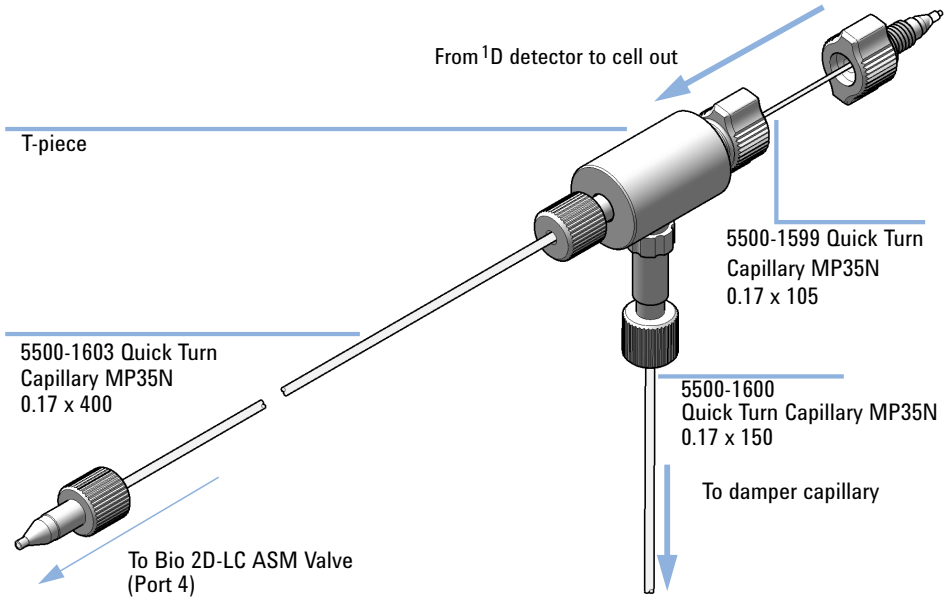
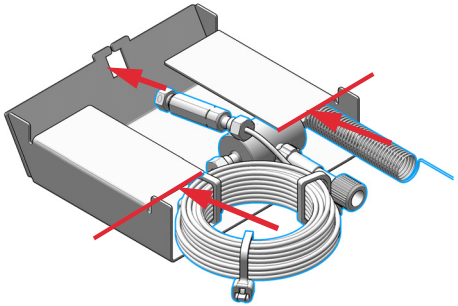


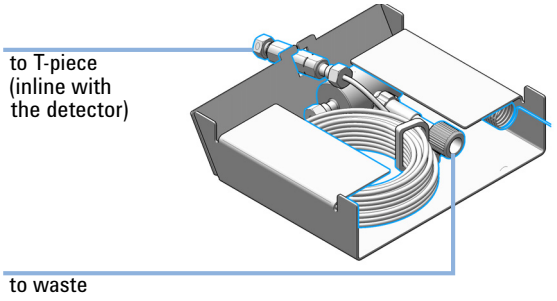
Figure 49 Connections to the pressure release kit

Parts required	#	p/n	Description
	1	G4236-60010	2D-LC Pressure Release Kit

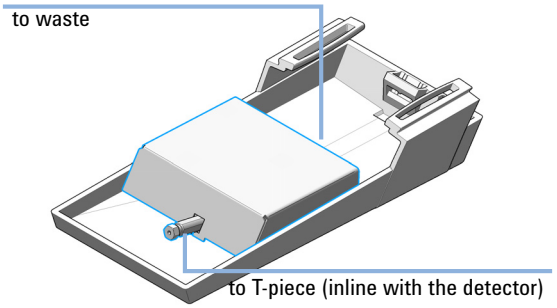
1 Push the pressure release valve assembly in the frame.



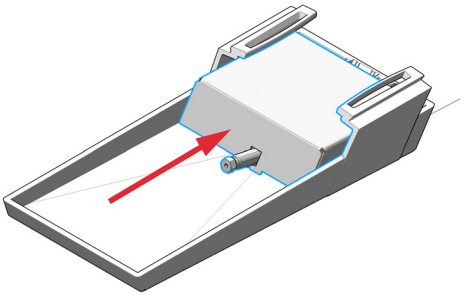
2 Take care for the correct orientation.



3 Insert the pressure release assembly to the leak tray, orientation as shown.



4 Push the pressure release assembly in the correct position.



5 Connect with the T-piece, see Figure 49 on page 111.

Install the Valve Head and Connecting Capillaries

For instructions on how to install the valve head and connecting capillaries, see the user manual.

NOTE

For alternative instrument setups with extra functionality, please see the 2D-LC User Manual or the standard quick installation guide, which gives an overview.

Recommendations for Biocompatible and Bio-Inert Systems

- Make sure all supplies (fittings, capillaries, inline filters, columns, etc.) are bio-inert or biocompatible.
  - Be aware that even columns recommended for bio applications may have a stainless steel case and can introduce iron and other metal ions in the flow path. This material in the flow path may lead to adsorption of susceptible samples like phosphorylated nucleotides. In this case, use PEEK-lined columns.
- After using the system with solvents or samples containing salts, flush it extensively with water to prevent blockages caused by salt crystals.
- If pressure falls below 20 bar, reliable operation of 1290 pumps during analysis cannot be guaranteed. For optimal results, pressure should be at least 50 bar continuously. Therefore, when using columns that create low backpressure (<50 bar, such as SEC columns with 1290 LC systems), install a restriction capillary between the pump and the sampler, to achieve at least 50 bar.
- Perform daily flush of the Multisampler with water if the Multiwash Option is installed (see *Best Practices for Using an Agilent LC System Technical Note*)

CAUTION

Agilent Bio-inert and Bio LC systems should not be subject to passivation or similar procedures

This can cause irreversible damage to the system's internal surfaces

✓ **Do not perform passivation or similar procedures on bio-inert and biocompatible systems.**

Flushing Procedure

- ✓ Perform this procedure regularly, when salt-containing mobile phases are used. To remove salt deposits from the flow path and surfaces in contact with the solvents, repeat the procedure regularly. Repeat the procedure at least once a week, or prior a long standby or off time. How to prepare the system for shutting down, see section *Shut Down the System* in the Bio LC user manual.
- ✓ The procedure is mandatory for switching from salt-containing mobile phase to reversed phase applications (or any applications running with high organics), where the precipitation of salt can occur.
- Flush the column with recommended storage solvent, be sure that this solvent is compatible with current mobile phase and cannot cause precipitation.
- Replace the column with a union, replace the salt-containing solvent bottle with a new bottle of HPLC-grade water at room temperature.
- Clean the bottle head assembly using lint-free wipes to minimize carry over of remaining salt solution into the new water bottle.
- Autosampler: Perform at least 15 min purge with water. This measure removes salt residues from all lines, both needle wash and seat backflush for Multiwash Option. Visually control needle/seat/washport for salt residues, if necessary manually clean needle/seat/washport.
- Purge each pump channel that has pumped buffer separately, for at least 10 min at 5 mL/min.
- Flush the entire system flow path with water for at least 10 min at 2 mL/min. During this step, switch the injection valve and the column selection valve (if installed) position every 1 min. Repeat this step until every position has been selected for at least five times.
- To minimize salt carry over, replace water with fresh solvent bottles.

2D-LC Software Configuration

All 2D-LC specific configurations are defined in the “Configure 2D-LC…” screen. This screen allows you to:

- Select your ^{1}D and ^{2}D pump
- Valve topology
- 2D-LC valve head (if multiple Valve heads are available)
- Select the connections of MHC decks (if installed)
- Capillary connections

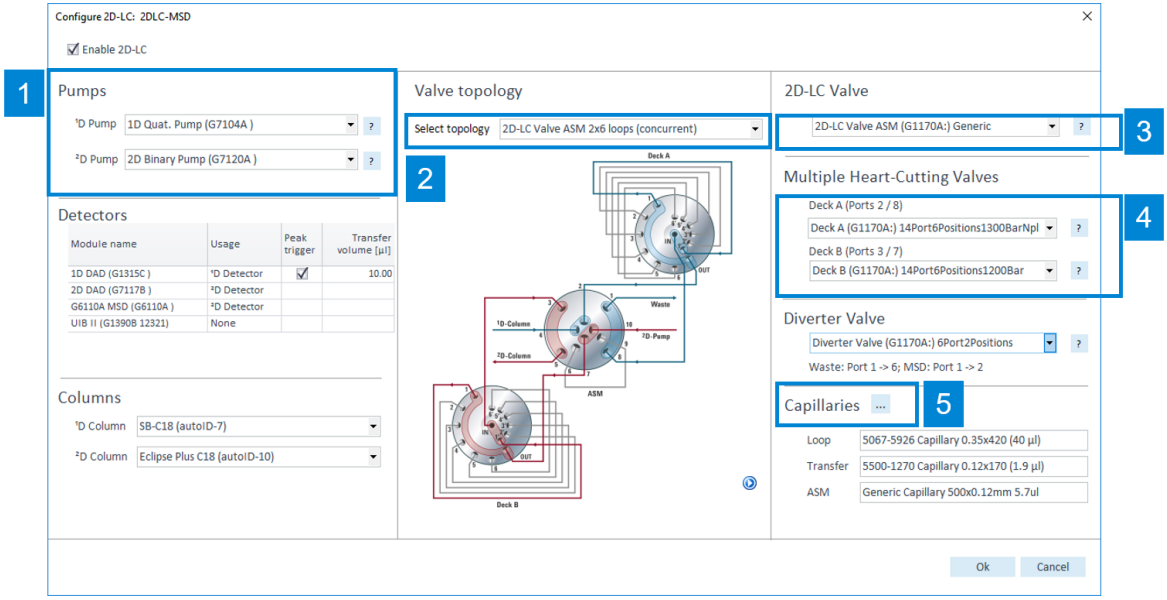


Figure 50 The Configure 2D-LC screen. All basic configurations of the 2D-LC systems, viz. ^{1}D and ^{2}D pump, valve topology, capillary connections as well as all 2D-LC valves are defined here.

1	Pump configuration
2	Valve topology
3	2D-LC Valve
4	Multiple Heart-Cutting Valves
5	Capillaries

NOTE With LC Driver 3.5 and C.01.10 Update 6, the biocompatible modules are supported.

1 In OpenLab ChemStation under **Method and Run Control**, click on **Instrument**, then **2D-LC Configuration...**

2 Select your your ^{1}D and your ^{2}D pump. Please note that this will not rename your pumps. A descriptive naming should be also entered during initial instrument setup in the instrument configuration.

3 Select your valve topology. Depending on the 2D-LC valve that you have installed, viz. the Standard 2D-LC (G4236A) or the ASM 2D-LC Valve (G4243A), your valve will automatically appear here. Please specify whether you connected your valve in concurrent or countercurrent mode

4 Optional: if the system contains multiple 2D-LC valves, please specify which valve head is to be used.

5 Optional: if your system contains Multiple Heart-Cutting decks, specify which valve head is corresponds to Deck A or B respectively.

6 Select your installed capillaries. Check for correct loop size and correct length of the transfer capillaries. If an ASM 2D-LC valve is used, define the ASM capillary which defines your split ratio (see Table 5 on page 71).

   a To define a ASM capillary, click on **Capillaries...**.

   b Choose a pre-defined ASM capillary

   OR

   Define a generic capillary by choosing **Generic Capillary**.

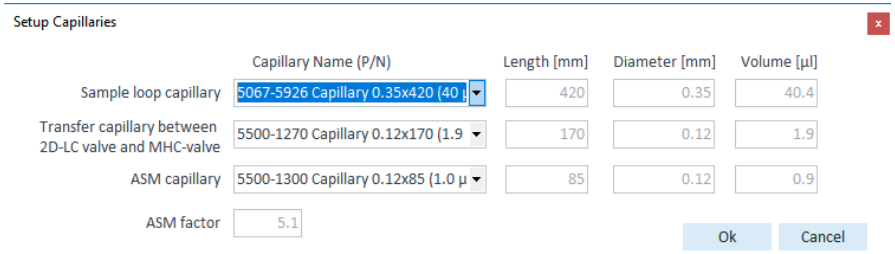


Figure 51 The Setup Capillaries screen. Here, you can define ASM and transfer capillaries as well as define other loop sizes.

The settings for the transfer volumes are 13.4 µL between ^{1}D detector and sample loop, and 1.9 µL between 2D-LC valve and MHC valve for a set-up with a MaxLight Cartridge Cell.

5 Method Parameters

This chapter provides background information on method parameters. It helps to optimize methods in Agilent 1290 Infinity II 2D-LC Solution ChemStation in the modes standard heart-cutting, multiple heart-cutting, high resolution sampling and comprehensive 2D-LC.

Method Parameters Standard Heart-Cutting 2D-LC

Software Method Setup

The method setup dialog is used to edit the 2D-LC specific method parameters of the 2nd dimension pump that were not part of the standard method user interface of the pump.

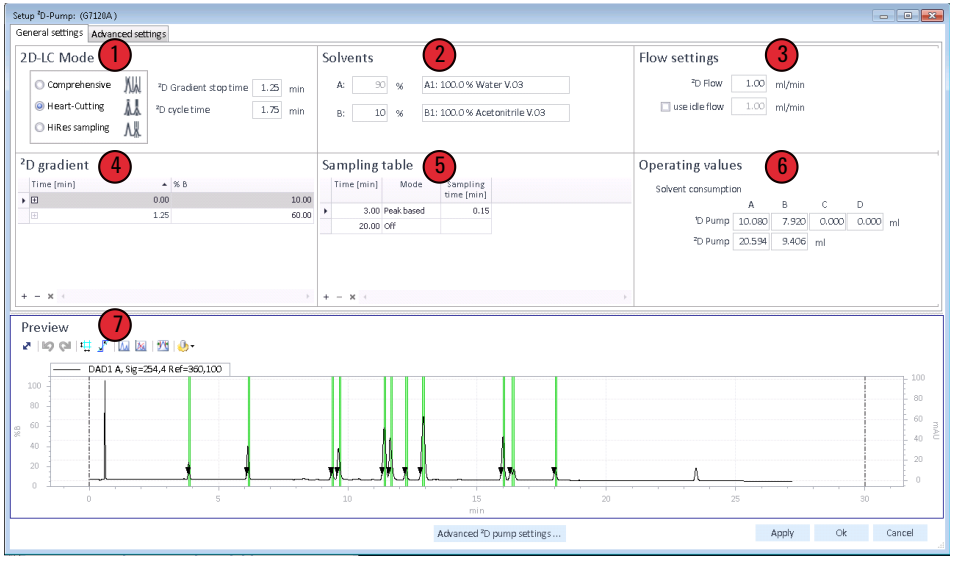


Figure 52 2D-LC method setup (General settings shown in Heart-Cutting mode as an example)

The setup of following method parameters is available:

1 **2D-LC Mode**, see "Set 2D-LC Mode" on page 120
2 **Solvents**, see "Set solvents" on page 127
3 **Flow settings**, see "Set flow" on page 128
4 **2D Gradient**, see "Set Solvent Composition Gradient" on page 129
5 **Sampling table**, see "Sampling table" on page 131
6 **Operating values**, see "Define Peak Detector Parameter" on page 133
7 **Preview**, see "Setup Second Dimension Gradient with the Graphical User Interface" on page 169

HINT

For details, see Online-Help of the software.

Set 2D-LC Mode

Setting the mode has the following consequences:

- **Heart-Cutting**:

  A relevant volume of the 1st dimension is cut off and injected onto the 2nd dimension column using the pump in the 2nd dimension. The volume to be injected on the 2nd column is either defined by a peak trigger or by a time window. When heart-cutting starts, a loop is filled with the peak of interest. Then the injection on the 2nd dimension starts running the gradient of the 2nd dimension pump.

NOTE

General considerations for heart-cutting 2D-LC

In heart-cutting 2D-LC keep the following general considerations in mind, when setting up the experiments (see Figure 53 on page 122):

1 The peak-end detection always overrules any loop-fill times.

The loop fill time represents the maximum time in case no peak end can be detected, for example with strong tailing peaks.

2 In Peak-based segments more than one peak can be detected and handled, but take in account the following points:

- A once started 2D-run will be finished even if a second peak will be detected.

  This second peak could be lost! In doubt, shorten the 2D-run time.
- The end of the 1D run-time will always finish any 2D-operations.

  In doubt, add a complete 2D-gradient run time to the 1D-run time.

There is also a different valve switching behavior depending if the modulation valve is equipped with either one or two loops.

- Two loops:

  With two loops the valve switches only when the end of the peak is detected or if the loop fill time is reached (in case no peak end is detected). If a transfer volume was entered for the peak detector the system will take account for the resulting transfer time, see Figure 54 on page 123. In Figure 54 on page 123, Figure 55 on page 124 and Table 14 on page 125 the valves are always shown in the position a peak would be sampled.

- One loop:

  For a set-up with one loop and a short bridging capillary in case of a 2-position/4port-duo valve or a 2-position/10-port valve or just with the valve groove in case of an 2pos/6-port valve, the system will switch the loop into the 1D-flow-path to collect the peak after the peak was detected (again respecting a given delay-time). At the end of the peak or after the **Sampling time**, in case no peak end could be detected, the loop will be switched into the 2D-flow path to let the content of the loop be analyzed in the second dimension, see Figure 55 on page 124.

This behavior is similar for time-triggered operation but with the difference that usually the transfer volume will be taken in account. That means, the valve will switch exactly at the given time-points, see Table 14 on page 125.

The peak volume that will be sampled usually is larger than the loop volume. For details see "Concept of Peak Triggering" on page 32.

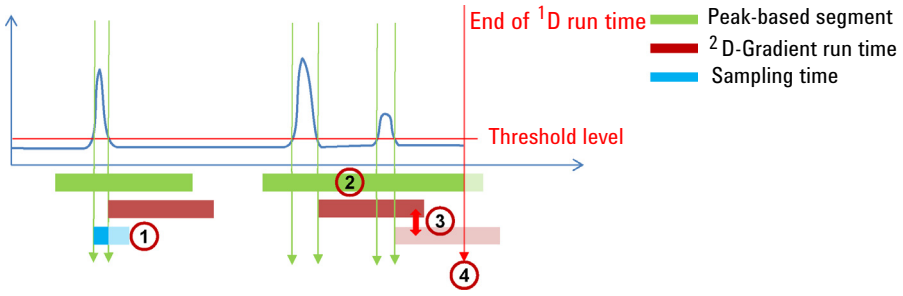


Figure 53 Heart-cutting 2D-LC (general considerations) (delay times have been omitted for clarity, besides threshold also the peak-slope can be used for peak detection)

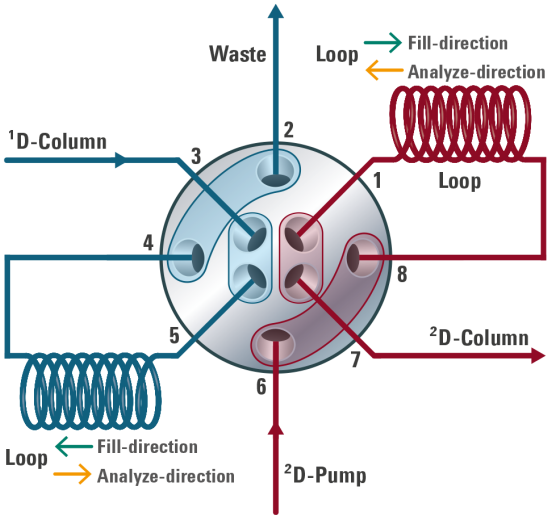
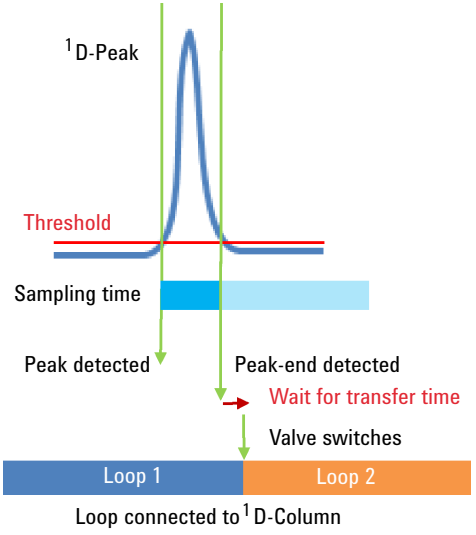
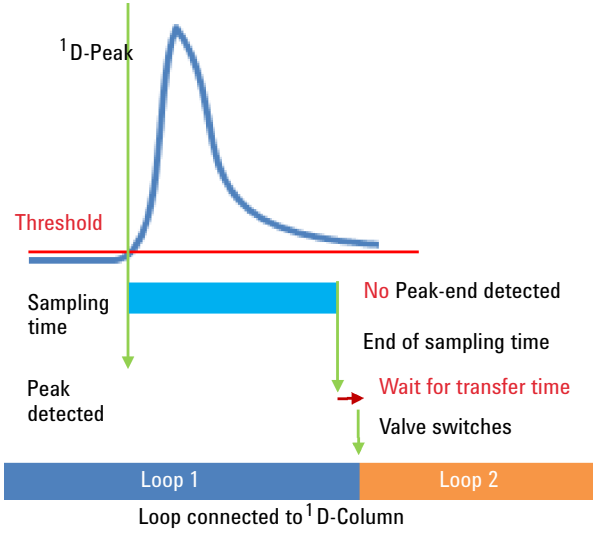


Figure 54 Valve and loop setup for heart-cutting 2D-LC with the 2D-LC Valve (dual-loop setup)





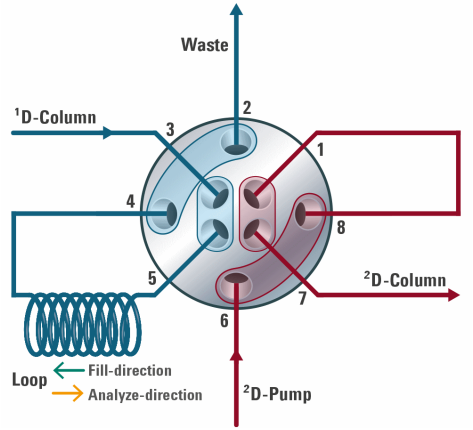


Figure 55 Valve and loop setup for heart-cutting 2D-LC with the 2D-LC Valve (single-loop setup)

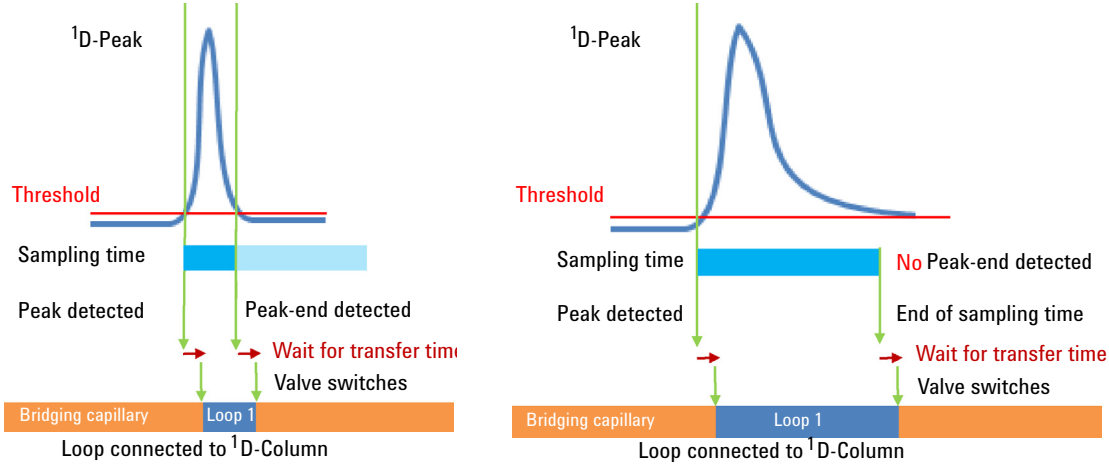


Table 14 Time-based heart-cutting 2D-LC

Dual-loop set-up

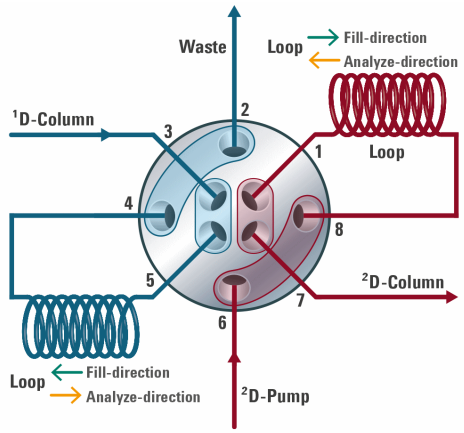
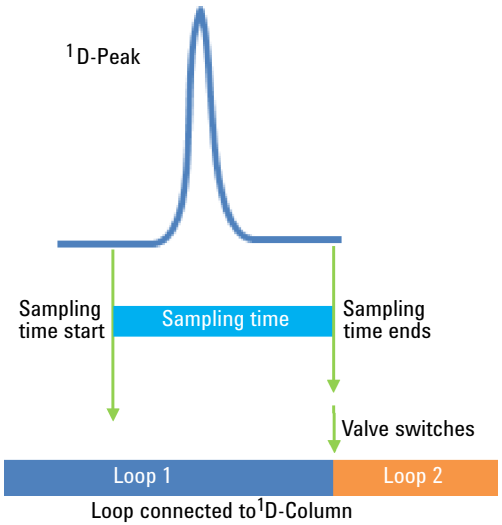


Figure 56 Valve and loop setup for heart-cutting 2D-LC with the 2D-LC Valve

(example valve)



Single-loop set-up

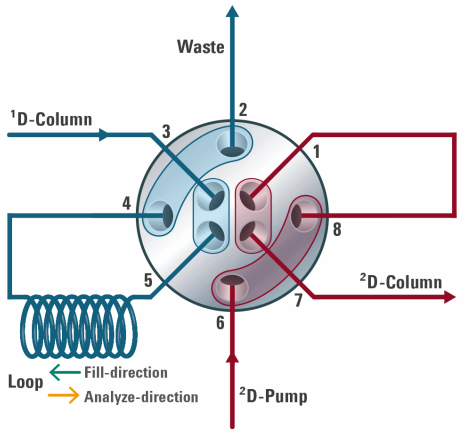
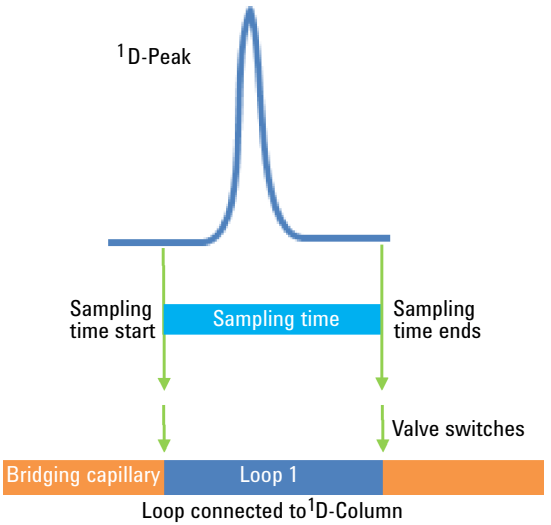


Figure 57 Valve and loop setup for heart-cutting 2D-LC with 2D-LC Valve

(example valve)



1 Select **Heart cutting** in **2D-LC Mode**.

NOTE

The **^{2}D Gradient Stoptime** reflects the maximal duration of the gradient in the 2nd dimension; the smallest value is 0.01 min. After that time, the Percent B value before the gradient (or the timetable entry at time = 0.0) is restored. In case of the Comprehensive 2D-LC mode the gradient stops latest when the **Cycle time** is reached.

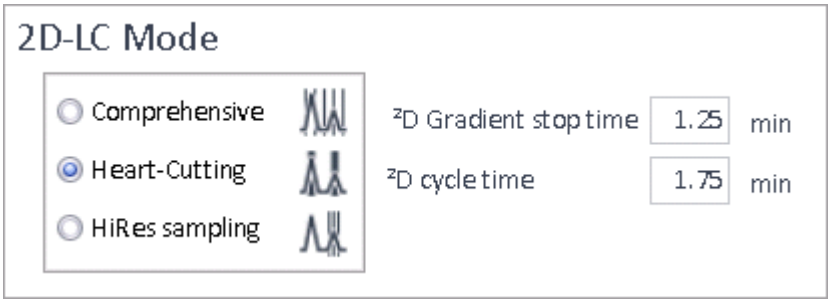


Figure 58 2D-LC ModeHeart-Cutting

The gradient of the 2nd dimension is graphically displayed in a window in the lower right part of the method screen showing also the **Stop time** (as a red vertical line) and (in case of comprehensive 2D-LC) the **Modulation time** as a green vertical line.

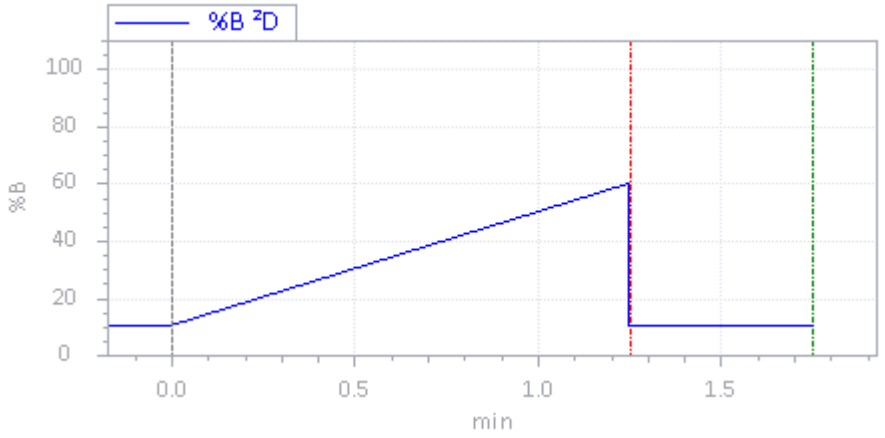


Figure 59 Stop time and Modulation time

Set solvents

NOTE

The selection of the solvents itself must be done in the standard pump method user interface.

- Open the pump method dialog using the button **Advanced ^{2}D pump settings...** and change the selection of the solvents there.
- After closing the dialog, the solvent settings should be updated immediately.

1 Set the percentage of solvent B to any value from 0 – 100 % in steps of 0.01 %.

Figure 60 2D-LC solvent settings

Solvent A always delivers the remaining percentage of volume. If the rate of solvent B is for example set to 20 %, solvent A, following the calculation %A = 100 - %B, automatically is set to 80 %.

The name of the selected solvents and their solvent channels (**A1:...** or **A2:...** and **B1:...** or **B2:...**) are shown in the corresponding text fields.

NOTE

The corresponding Percent B value in the Standard Pump user interface will be ignored as long as the 2D-LC functionality is enabled (see "Configuration" on page 179).

Set flow

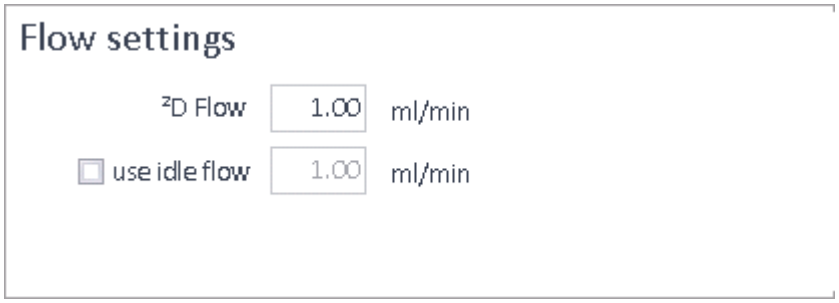


Figure 61 Flow settings

1 Set the **^{2}D Flow** (range 0 – 5.0 mL/min).

   This defines the flow in the 2nd dimension being used while 2D-LC is active (within 2D time segments where mode is not equal to OFF)

2 To set and use **Idle Flow** select check box **use idle flow**.

   This defines the flow in the 2nd dimension that is used while the 2D-LC mode is OFF (range 0 – 5.0 mL/min).

NOTE If **use idle flow** is not selected, the **^{2}D Flow** is also used while 2D-LC mode is OFF.

NOTE The recommendation for the maximum ^{1}D flow rate is 1 mL/min.

Set Solvent Composition Gradient

Set Solvent Composition Gradient

The timetable in the **^{2}D Gradient** group allows changing the solvent composition.

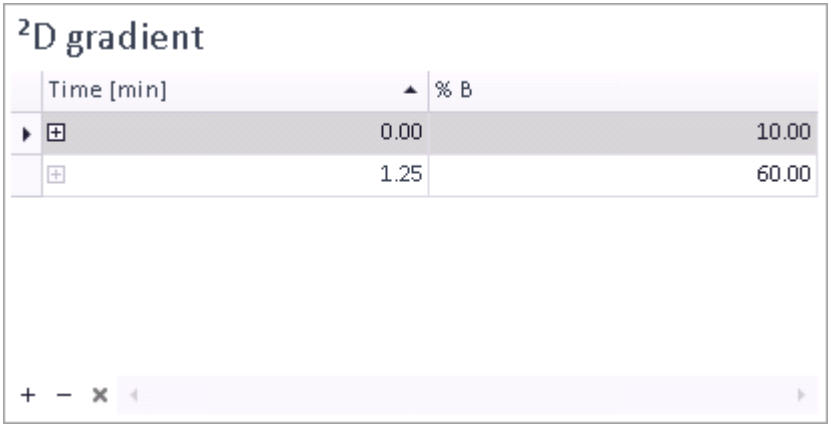
Percent B ranges from 0 – 100 %.

Change the solvent composition at a specified time

1 To change the solvent composition (%B) at the specified time apply a percent B range from 0 – 100 %

NOTE

Different start conditions in the first row may cause step gradients and RI-effects (density differences of the different liquid phases may cause different DAD detection through baseline disturbances).



The time axis relates to the Stoptime of the 2nd dimension pump. **Time [min]** = **0.00** marks the start of the maybe repetitive gradient cycles, a time greater than **Stoptime ^{2}D** will be ignored.

Setup ^{2}D Gradient graphically

The user can graphically setup the ^{2}D gradient including the initial composition (%B) value, the ^{2}D-stoptime and the modulation (repetition) time.

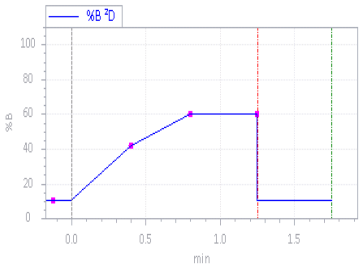


Figure 62 ^{2}D Gradient window in edit mode

1. Click to enable the graphical editing capabilities.
2. To add a new gradient point, move the cursor within the drawing area close to a new gradient point until the cursor changes to and click.
3. To delete a gradient point, move the cursor close to the gradient pointto be deleted until the cursor changes to , select the right segment and click.
4. To move a gradient point, move the cursor close to the gradient point to be moved until the cursor changes to , select the left segment and drag.
5. To change the stop time, move the cursor close to the red dotted vertical line until the cursor changes to ↔ and drag.
6. To change the modulation time, move the cursor close to the green dotted vertical line until the cursor changes to ↔ and drag.
7. To change the initial composition, move the cursor close to the filled circle most left near the y-axis until the cursor changes to ↕ and drag the point.

Sampling table

The content of the **Sampling table** specifies when (within the runtime of the 1st dimension) the selected 2D-LC mode is active.

Table 15 Definitions 2D Time Segments

Column name	Description
Time	Specifies when a new segment starts (or ends)
Mode	Following options exist: • Time based The specified time defines the beginning of a time segment. • Peak based The peak detector is enabled at the specified time. • Off The time segments ends at the specified time.
Maximum peak duration (**Comprehensive** mode only)	Only valid in case of trigger mode = peak-based. After that time the 2D-gradient repetition ends regardless of the peak detector state.
Sampling time (**Heartcutting** mode only)	Set the time the loop remains in the flow path of the 1st dimension.
Add transfer volume	• Checked: Valve is switched at the specified time plus the time to deliver the delay volume • Unchecked Valve is switched at the specified time (This check box is available only for Time based mode)

NOTE

If the **Sampling table** is empty, no 2D-LC operation will be executed at all.

Set Sampling table for Heartcutting mode

1 To specify, when the actual trigger mode gets active, fill the **Time** column.

Specifies the point in time of the 1D runtime where the actual trigger mode gets active.

NOTE

Time segments must not overlap. **Time** of a segment must be always set longer than **Time** of previous segment plus **Sampling time** plus ^{2}**D-stop time**.

Otherwise a warning icon is shown in the respective time column of the table.

Sampling table (Heartcutting)

2 To specify the mode and time, select **Time based**, **Peak based** or **Off** from the drop-down list in the **Mode** column fill the **Time** field.

- **Time based**

  The specified time defines the beginning of a heartcut segment. For details see Figure 53 on page 122.

- **Peak based**

  The peak detector is enabled at the specified time. For details see Figure 53 on page 122.

- **Off**

  The time segments ends at the specified time.

3 Set the **Sampling time**.

This defines the time the loop remains in the flow path of the 1st dimension.

NOTE

In Peak-triggered mode the **Sampling time** specifies the maximum sampling time in case no peak end is detected by the peak detector.

4 To add or delete table rows, use the **+** and **-** icons below the table.

The **Sampling table** now is defined for **Comprehensive** or **Heartcutting** mode.

Define Peak Detector Parameter

This section allows parameterizing the peak detector to be used for peak-triggered 2D-LC operation (comprehensive or heart cutting).

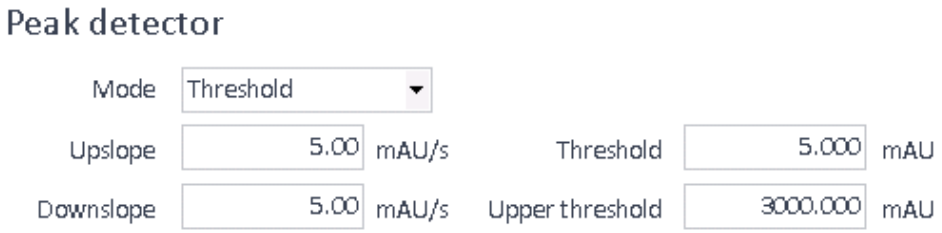
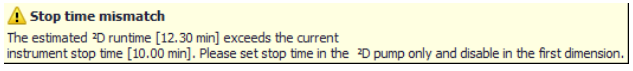


Figure 63 Overview on Peak detector parameters

The *stop time for a 2D-LC measurement must be set for the 2D pump*, which can be accessed through the **advanced** settings. It must be at least the 1D run time and applies to the entire measurement, not to partial 2D-only runs/gradients for parked peaks.

Multiple Heart-Cutting *automatically extends this run time*, if required, as analyzing parked peaks takes usually longer than the 1st dimension run only.

If you define a 1D stop time, it will be applied unchanged, for example the analysis will stop after that time without processing any parked peaks. This is not recommended and will lead to a warning in the gradient preview.



NOTE If no peak detector is configured (see "Configuration" on page 179) this section is disabled. The currently configured peak detector (name & serial number of the detector) is shown in the section header.

NOTE To facilitate the determination of parameters, it is possible to preview **Threshold** and **Slope** in the reference chromatogram.

1. Go to **Instrument >Setup 2DLC** and tab **Advanced**.
2. Select **Peak detection mode** from the drop-down list.

   The following options are available:

   - **Off**

     The peak detector is not used.
   - **Threshold only**

     Detects peaks based on threshold values only.
   - **Threshold/Slope** values

     Detects peaks based on both - threshold and slope.
   - **Slope only**

     Detects peaks based on slope values only.
3. To define **Upslope** (slope of the rising peak), add the required values to the corresponding field.
4. To define **Downslope** (slope of the falling peak), add the required values to the corresponding field.
5. To define **Threshold** (height of the peak that triggers collection), add the required values to the corresponding field.
6. To define **Upper threshold** (height of the peak that ensures that collection is not switched off even for a saturated signal that might be expected to do so), add the required values to the corresponding field.

Method Parameters Multiple Heart-Cutting and High Resolution Sampling 2D-LC

Software Method Setup

The method setup dialog is used to edit the 2D-LC specific method parameters of the 2nd dimension pump that were not part of the standard method user interface of the pump.

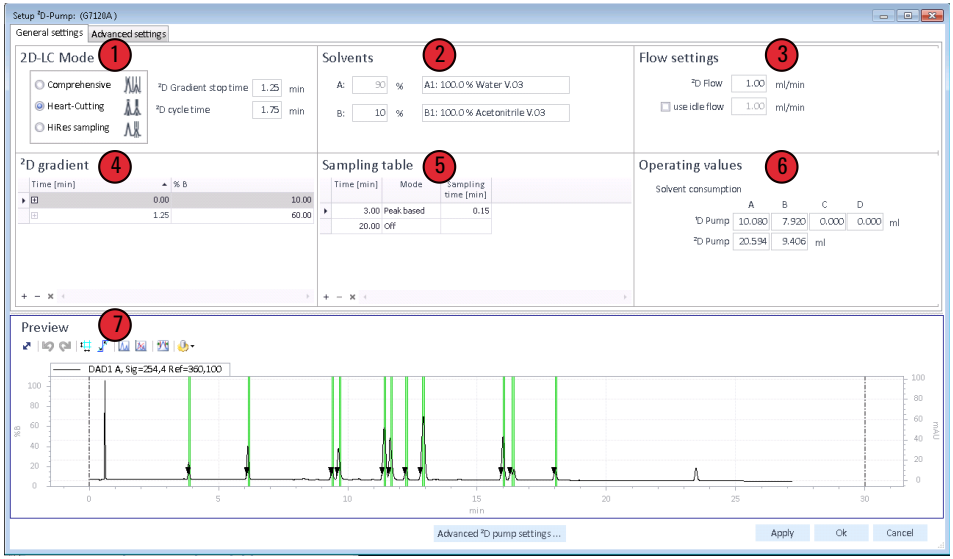


Figure 64 2D-LC method setup (General settings shown in Heart-Cutting mode as an example)

The setup of following method parameters is available:

1 **2D-LC Mode**, see "Set 2D-LC Mode Multiple Heart-Cutting" on page 136

2 **Solvents**, see "Set solvents" on page 140

3 **Flow settings**, see "Set flow" on page 141

4 **²D Gradient**, see "Set Solvent Composition Gradient" on page 142

5 **²D Time segments**, see "Set 2D Time Segments (Multiple Heart-Cutting Only)" on page 144

6 **Operating values**, see "Define Peak Detector Parameter" on page 149

7 **Gradient preview** with toolbar, see "Gradient Preview Functionality" on page 151.

Set 2D-LC Mode Multiple Heart-Cutting

Setting the mode has the following consequences:

- **Heart-Cutting**:

  A relevant volume of the 1st dimension is cut off and injected onto the 2nd dimension column using the pump in the 2nd dimension. The volume to be injected on the 2nd column is either defined by a peak trigger or by a time window. When heart cutting starts, a loop is filled with the peak of interest. Then the injection on the 2nd dimension starts running the gradient of the 2nd dimension pump.

NOTE

Considerations for multiple heart cutting 2D-LC

In multiple heart-cutting 2D-LC keep the following general considerations in mind, when setting up the experiments (see Figure 53 on page 122):

1. The peak-end detection always overrules any **Sampling Time**. The **Sampling Time** represents the time, how long the loop is flown through. Only the last part of the cut (exactly the volume of the built-in loop) is transferred to the 2nd dimension.
2. In Peak-based segments more than one peak can be detected and handled, but take in account the following points:
   - A once started ^{2}D-run will be finished even if further peaks will be detected.

     In order to over-come the limitation of potentially lost peaks, the multiple heart cutting solution allows the storage of up to 10 peaks.

     As for single heart-cutting, the same valve connects the first and second dimension. Additionally, there are two **Multiple Heart-Cutting Valves** (G4242-64000), which select one out of six sample loops, which can store sample peaks intermediately in sample decks.
     - Analysis is done as soon as possible
     - Deck is flushed before unparking
     - Once started, a deck content is analyzed completely
     - Unparking is done in reverse order (avoids carry-over)
     - Peaks get lost if ^{2}D deck is full and no ^{1}D deck postion is available
   - The end of the ^{1}D run-time will always finish any ^{2}D-operations.

     In doubt, add a complete ^{2}D-gradient run time to the ^{1}D-run time, or define the run-time in the ^{2}D pump only (the run-time will then be increased if necessary).

1 Select the **Heart-Cutting2D-LC Mode** (correct for standard heart-cutting and multiple heart-cutting setup).

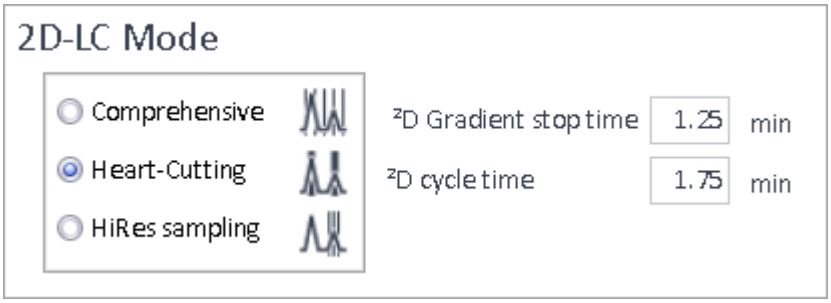


Figure 65 2D-LC ModeHeart-Cutting

Set 2D-LC Mode HiRes Sampling

Setting the mode has the following consequences (for details, see "High Resolution Sampling - Peak Parking Principles" on page 23):

- **HiRes sampling**:

  In contrast to **Heart-Cutting**, which uses the continuous flow-through principle, the MHC valve is switched before and after parking the peak.

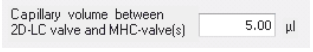
NOTE

Considerations for high-resolution sampling 2D-LC

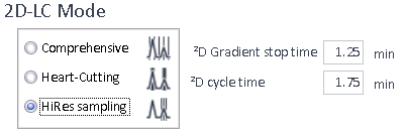
In high-resolution sampling 2D-LC keep the following general considerations in mind, when setting up the experiments (see "High Resolution Sampling - Peak Parking Principles" on page 23):

1. Each loop for consecutive snips stores the same sample volume.
2. First and last loop cannot be used for parking.
3. Solvent transfer from ^{1}D to ^{2}D can be reduced.
4. Cut number 5 cannot be parked entirely in the sample loop. Otherwise cut 6 would got partially to the transfer capillary and would therefore be lost or spoil cut 5.

   Cut 5 stays partially in the transfer line and is immediately being analyzed in ^{2}D.
5. For parking cut 6 in the sample loop, the cut first needs to be moved from the 2D-LC valve to the deck valve. This new volume must be defined in the configuration of the 2D-LC system.



1. Select the **HiRes sampling** Mode.



Set solvents

NOTE

The selection of the solvents itself must be done in the standard pump method user interface.

- Open the pump method dialog using the button **Advanced ^{2}D pump settings...** and change the selection of the solvents there.
- After closing the dialog, the solvent settings should be updated immediately.

1 Set the percentage of solvent B to any value from 0 – 100 % in steps of 0.01 %.

Figure 66 2D-LC solvent settings

Solvent A always delivers the remaining percentage of volume. If the rate of solvent B is for example set to 20 %, solvent A, following the calculation %A = 100 - %B, automatically is set to 80 %.

The name of the selected solvents and their solvent channels (**A1:...** or **A2:...** and **B1:...** or **B2:...**) are shown in the corresponding text fields.

NOTE

The corresponding Percent B value in the Standard Pump user interface will be ignored as long as the 2D-LC functionality is enabled (see "Configuration" on page 179).

Set flow

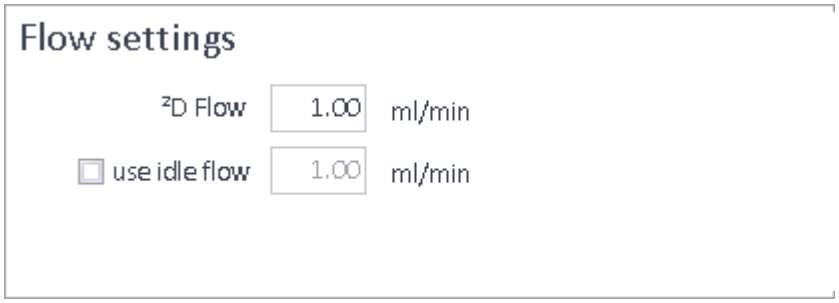


Figure 67 Flow settings

1 Set the **2D Flow** (range 0 – 5.0 mL/min).

   This defines the flow in the 2nd dimension being used while 2D-LC is active (within 2D time segments where mode is not equal to OFF)

2 To set and use **Idle Flow** select check box **use idle flow**.

   This defines the flow in the 2nd dimension that is used while the 2D-LC mode is OFF (range 0 – 5.0 mL/min).

NOTE

If **use idle flow** is not selected, the **2D Flow** is also used while 2D-LC mode is OFF.

Set Solvent Composition Gradient

Set Solvent Composition Gradient

The timetable in the **^{2}D Gradient** group allows changing the solvent composition.

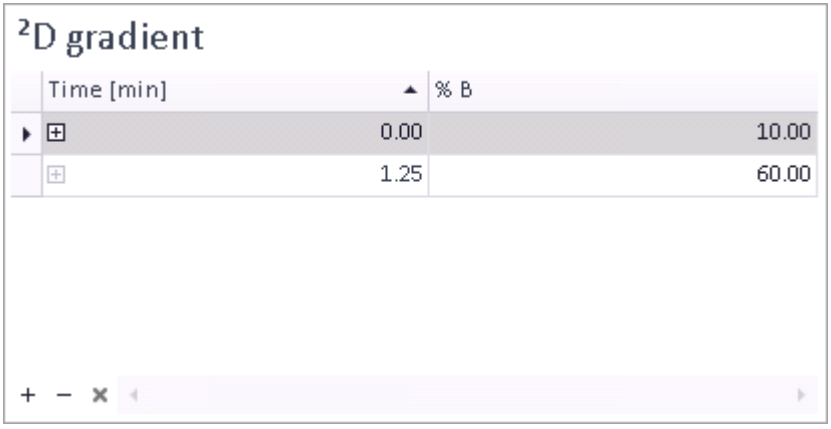
Percent B ranges from 0 – 100 %.

Change the solvent composition at a specified time

1 To change the solvent composition (%B) at the specified time apply a percent B range from 0 – 100 %

NOTE

Different start conditions in the first row may cause step gradients and RI-effects (density differences of the different liquid phases may cause different DAD detection through baseline disturbances).



The time axis relates to the Stoptime of the 2nd dimension pump. **Time [min]** = **0.00** marks the start of the maybe repetitive gradient cycles, a time greater than **Stoptime ^{2}D** will be ignored.

Setup ^{2}D Gradient graphically

The user can graphically setup the ^{2}D gradient including the initial composition (%B) value, the ^{2}D-stoptime and the modulation (repetition) time.

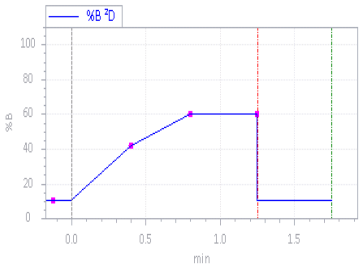


Figure 68 ^{2}D Gradient window in edit mode

1. Click to enable the graphical editing capabilities.
2. To add a new gradient point, move the cursor within the drawing area close to a new gradient point until the cursor changes to and click.
3. To delete a gradient point, move the cursor close to the gradient pointto be deleted until the cursor changes to , select the right segment and click.
4. To move a gradient point, move the cursor close to the gradient point to be moved until the cursor changes to , select the left segment and drag.
5. To change the stop time, move the cursor close to the red dotted vertical line until the cursor changes to ↔ and drag.
6. To change the modulation time, move the cursor close to the green dotted vertical line until the cursor changes to ↔ and drag.
7. To change the initial composition, move the cursor close to the filled circle most left near the y-axis until the cursor changes to ↕ and drag the point.

Set ^{2}D Time Segments (Multiple Heart-Cutting Only)

The content of the **Sampling table** specifies when (within the runtime of the 1st dimension) the selected 2D-LC mode is active.

Table 16 Definitions ^{2}D Time Segments

Column name	Description
Time	Specifies when a new segment starts (or ends)
Mode	Following options exist: • Time based The specified time defines the beginning of a time segment. • Peak based The peak detector is enabled at the specified time. • Off The time segments ends at the specified time.
Maximum peak duration (**Comprehensive** mode only)	Only valid in case of trigger mode = peak-based. After that time the 2D-gradient repetition ends regardless of the peak detector state.
Sampling time (**Heartcutting** mode only)	Set the time the loop remains in the flow path of the 1st dimension.
Add transfer volume	• Checked: Valve is switched at the specified time plus the time to deliver the delay volume • Unchecked Valve is switched at the specified time (This check box is available only for Time based mode)

NOTE

If the **Sampling table** is empty, no 2D-LC operation will be executed at all.

Set Sampling table for Heartcutting mode

1 To specify, when the actual trigger mode gets active, fill the **Time** column.

Specifies the point in time of the ^{1}D runtime where the actual trigger mode gets active.

NOTE

Time segments must not overlap. **Time** of a segment must be always set longer than **Time** of previous segment plus **Sampling time** plus **^{2}D-stop time**.

Otherwise a warning icon is shown in the respective time column of the table.

Sampling table (Heartcutting)

2 To specify the mode and time, select **Time based**, **Peak based** or **Off** from the drop-down list in the **Mode** column fill the **Time** field.

- **Time based**

  The specified time defines the beginning of a heartcut segment. For details see Figure 53 on page 122.

- **Peak based**

  The peak detector is enabled at the specified time. For details see Figure 53 on page 122.

- **Off**

  The time segments ends at the specified time.

3 Set the **Sampling time**.

This defines the time the loop remains in the flow path of the 1st dimension.

NOTE

In Peak-triggered mode the **Sampling time** specifies the maximum sampling time in case no peak end is detected by the peak detector.

4 To add or delete table rows, use the **+** and **-** icons below the table.

The **Sampling table** now is defined for **Comprehensive** or **Heartcutting** mode.

Set cuts in the software (HiRes Sampling only)

NOTE

Peak parking steps for high-resolution sampling

- Flow goes through loop 1 in deck A.
- Cut 1 detected. Valve in deck A switches at beginning of
- cut 1 to loop A2 and is parked there,
- cut 2 to loop A3
- cut 3 to loop A4
- cut 4 to loop A5
- Cut 5 is on its way (in the transfer capillary) to loop A6 – the last loop in deck A. After switching the 2D-LC valve, the cut will be analyzed immediately.
- Cut 6 goes to loop 1 in deck B, transfer requires time, which needs to be configured:
- Cut 7 goes to loop B2,...,
- cut 10 goes to loop B5.
- Last loop is required for flow-through while other deck runs analysis.
- During analysis, loops are filled with solvent of ^{2}D gradient base.

NOTE

Transfer volume and sampling time

The volume of the capillary between 2D-LC valve and each deck valve determines the minimum sampling time.

$t = \frac{V}{F}$; for example $\frac{5\ mL}{500\ pL/\ min} = 0.6\ s$

This is the time t, which is needed for filling the transfer volume V at a given ^{1}D flow rate F.

If the sampling time would be less than that, two cuts could end up in the same transfer capillary.

NOTE

By default, sample loops are filled by up to 80 %, which reflects the parabolic flow profile in capillaries.

Practically, loops can't be filled to 100 %, which would partially send samples to waste. A lower loop filling/shorter cuts can be achieved by reducing the sampling time. The default sampling time t is calculated from the loop volume V, flow rate F and default loop filling 80 % by the following equation:

$$t \leq \frac{V \cdot 80\%}{F}$$

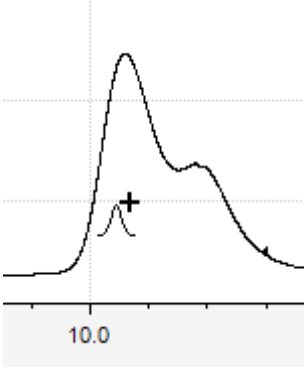
NOTE

High-resolution sampling works only in time based mode. Peak based mode would be possible and may be added for future versions but creating an unpredictable number of cuts from an unpredictable number of peaks easily exceeds the storage capacity of MHC valves.

Currently, only one series of cuts can be stored in one MHC deck for the same reason. If one deck is half full, it is unlikely to be sufficient for another series.

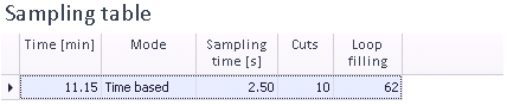
As soon as a deck is empty, it may be used for further series.

1 Use a left-click for sampling peaks.



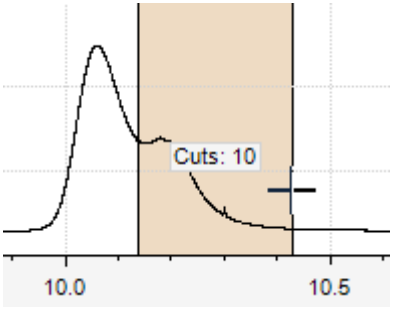
OR

enter values to the table (sampling time, number of cuts)

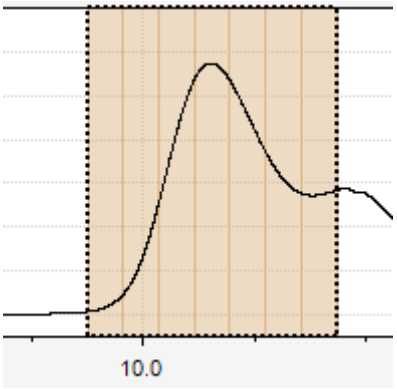


OR

drag the mouse over the desired range



2 Select a series of cuts and drag it to a better position (if necessary).



Define Peak Detector Parameter

This section allows parameterizing the peak detector to be used for peak-triggered 2D-LC operation (comprehensive or heart cutting).

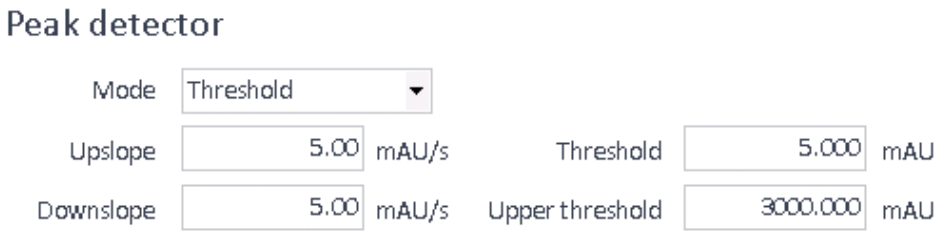
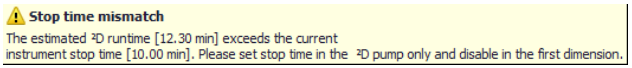


Figure 69 Overview on Peak detector parameters

The *stop time for a 2D-LC measurement must be set for the 2D pump*, which can be accessed through the **advanced** settings. It must be at least the 1D run time and applies to the entire measurement, not to partial 2D-only runs/gradients for parked peaks.

Multiple Heart-Cutting *automatically extends this run time*, if required, as analyzing parked peaks takes usually longer than the 1st dimension run only.

If you define a 1D stop time, it will be applied unchanged, for example the analysis will stop after that time without processing any parked peaks. This is not recommended and will lead to a warning in the gradient preview.



NOTE

If no peak detector is configured (see "Configuration" on page 179) this section is disabled. The currently configured peak detector (name & serial number of the detector) is shown in the section header.

NOTE

To facilitate the determination of parameters, it is possible to preview **Threshold** and **Slope** in the reference chromatogram.

1 Go to **Instrument >Setup 2DLC** and tab **Advanced**.

2 Select **Peak detection mode** from the drop-down list.

The following options are available:

- **Off**

  The peak detector is not used.

- **Threshold only**

  Detects peaks based on threshold values only.

- **Threshold/Slope** values

  Detects peaks based on both - threshold and slope.

- **Slope only**

  Detects peaks based on slope values only.

3 To define **Upslope** (slope of the rising peak), add the required values to the corresponding field.

4 To define **Downslope** (slope of the falling peak), add the required values to the corresponding field.

5 To define **Threshold** (height of the peak that triggers collection), add the required values to the corresponding field.

6 To define **Upper threshold** (height of the peak that ensures that collection is not switched off even for a saturated signal that might be expected to do so), add the required values to the corresponding field.

Gradient Preview Functionality

The gradient preview provides the following functions:

- Displays the gradient (%B) of the 1st dimension pump
- Displays the gradient (%B) of the 2nd dimension during the runtime of the first dimension, depending on the selected 2D-LC mode (comprehensive OR heart-cutting)
- Allows to graphically edit the gradient shifting
- Displays a reference signal by which a user can easily setup the trigger table or optimize his peak detector settings

Reference Signal

The user can load a chromatographic signal from an LC detector - a so-called reference signal. The signal will be shown in the gradient preview. This signal is automatically shown in the gradient preview of the setup dialog as long as the reference signal is part of the method. The signal can also be removed from the method or replaced by another signal.

Loading / removing a reference signal is triggered by toolbar buttons (or the context menu) of the gradient preview.

When a reference signal is loaded, a reference signal related y-axis is shown on the right side of the gradient preview window. The grid of the graphic window is either adjusted to the absorbance axis (right axis) or the %B axis (left) and can be changed by clicking on the corresponding axis. The signal name (e.g. DAD1 A, Sig= 280, 190 Ref=550,100) is shown in the legend of the graphic window.

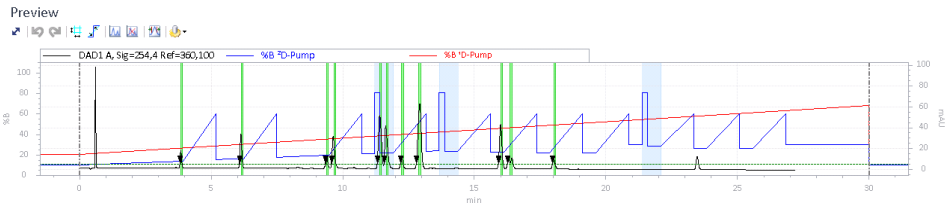


Figure 70 Gradient preview

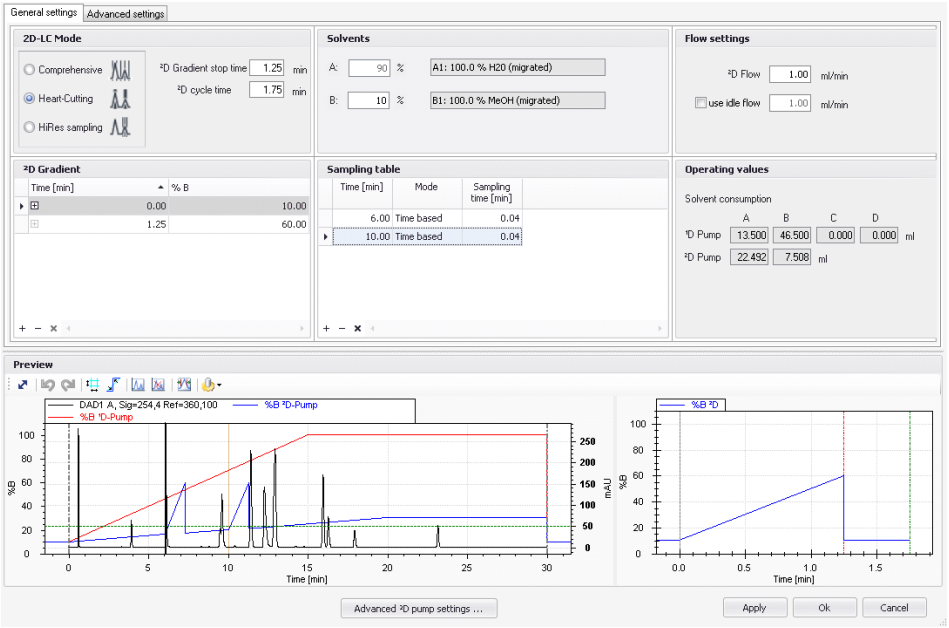
The reference signal offers the following:

- Simplified set-up of (time-based) heart cuts in case the chromatogram of the sample is known in advance
- Preview of peaks, which would be analyzed in the 2nd dimension that is based on the current peak detector settings (threshold, slope)

Gradient Preview and Toolbar

The method screen allows you to set up and modify the 2D-LC method graphically. The different orders from the toolbar are:

- Edit mode on/off: Enables to shift gradients as in comprehensive 2D-LC method setup
- load/remove reference signal: Uploads a reference signal into the method screen. This is very helpful to illustrate, which at which positions of the chromatogram cuts will be taken.
- Generate time segments: Creates a time table based on the reference signal and peak detector settings in advanced parameters ("Define Peak Detector Parameter" on page 149)
- Autoscale: Zooms to scale of highest signal
- Edit Snap Distance: Allows you to specify the precision to which a gradient point can be placed interactively in the Gradient Preview



Graphical explanation

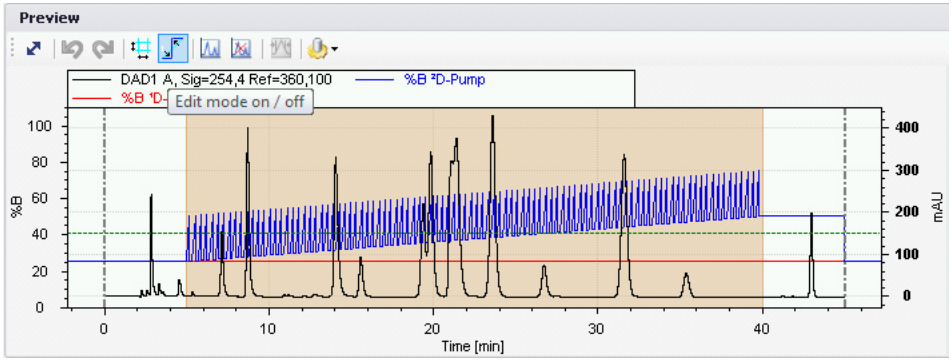
- **Time based** triggering displays peaks in yellow. Sampling time is generated automatically by the thresholds of the peaks.

- **Peak based** triggering displays peaks in green. All peaks, which can be cut, are displayed. Sampling time is put in by user and is determined by the shape of peak.
- Loop content is displayed by shaded areas (dark yellow/green), see "Concept of Peak Triggering" on page 32
- Missed cuts are displayed in red
- Missed peaks are marked by exclamation mark icon
- Gradient can be adjusted such that it matches the reference signal, see "Set Solvent Composition Gradient" on page 142

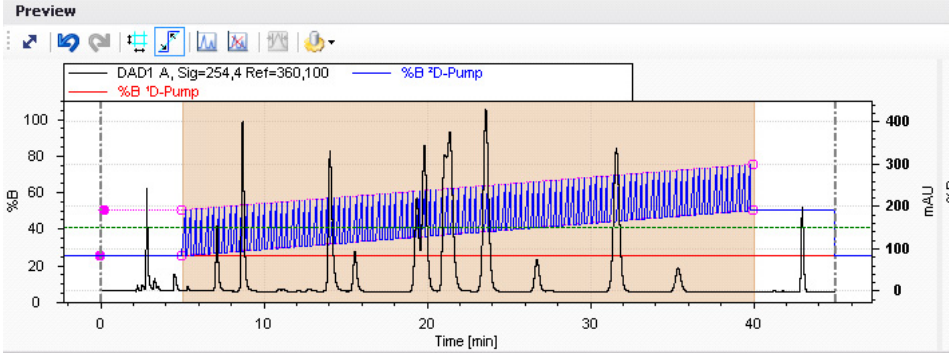
Setup Second Dimension Gradient with the Graphical User Interface

The gradient preview allows to edit gradient shifting graphically, see below. This replaces the editing of large timetables by a few mouse operations.

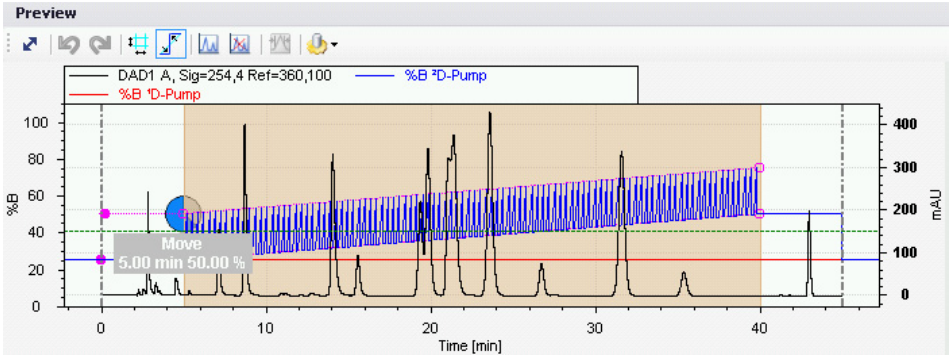
1 To enter the editing mode, use the context menu.



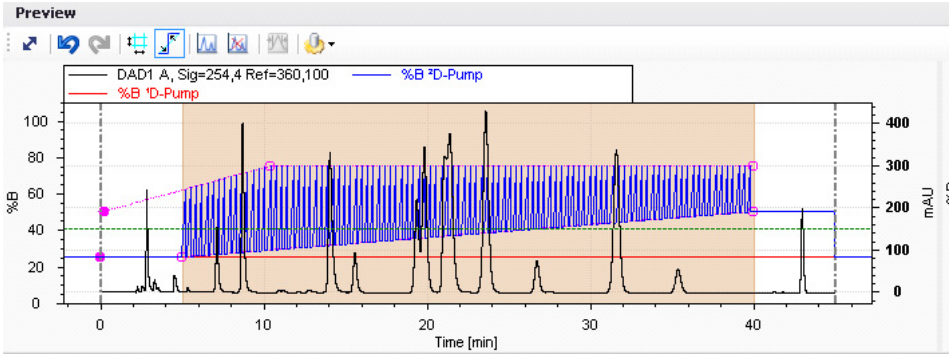
2 Click into the menu. Timetable entries are marked with circles.



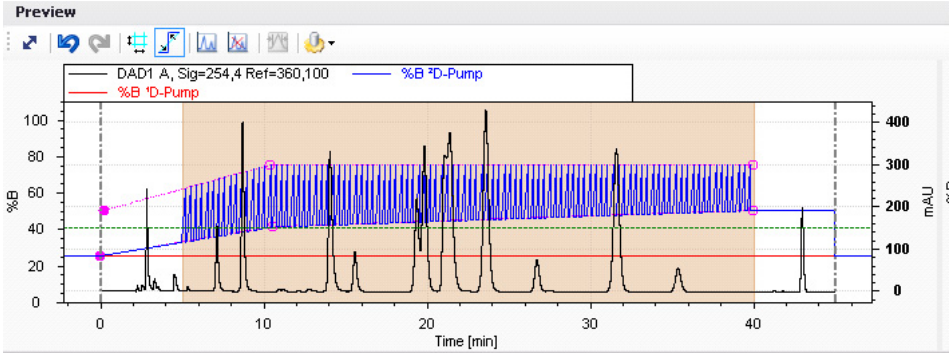
3 Drag the mouse to a new %B value at a specified runtime of the 1st dimension. This draws a straight line.



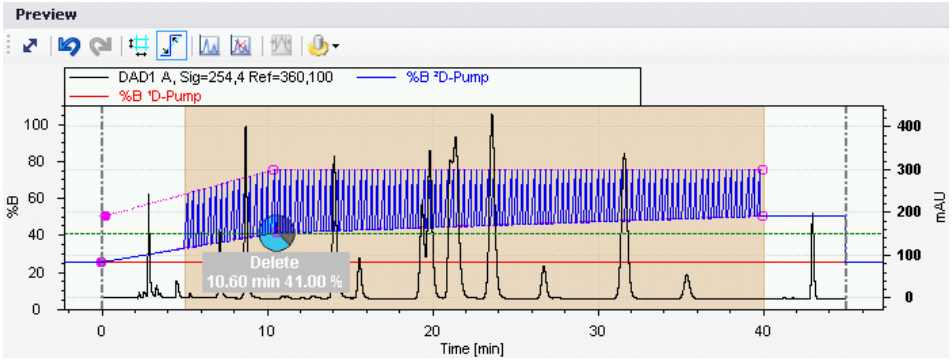
4 When releasing the mouse, a new timetable entry is made and the gradient rollout is automatically updated.



5 Repeat step 2 with other timetable entries at the bottom of the gradients.



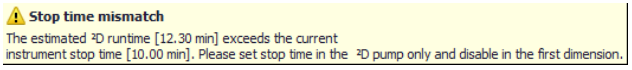
6 Move the mouse cursor near to a shift line to change menu context and insert or delete shift points as needed.



The stop time for a 2D-LC measurement must be set for the ^{2}D pump, which can be accessed through the *advanced* settings. It must be at least the ^{1}D run time and applies to the entire measurement, not to partial ^{2}D-only runs/gradients for parked peaks.

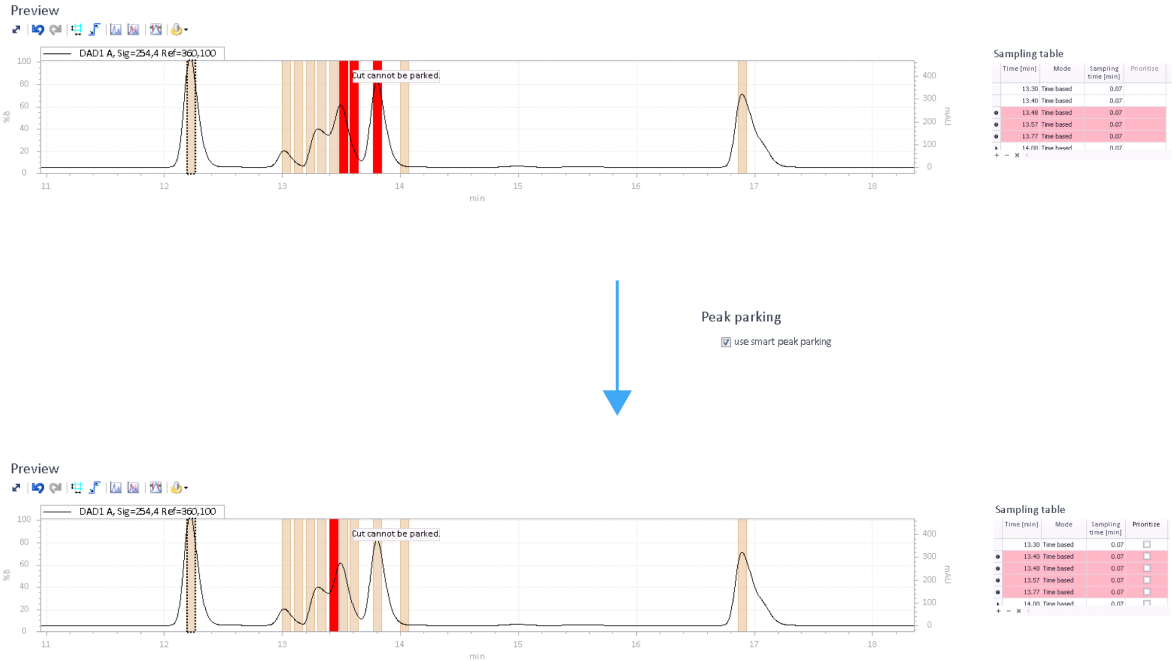
Multiple Heart-Cutting extends this run time automatically, as analyzing parked peaks takes usually longer than the 1st dimension run only.

If you define a ^{1}D stop time, it will be applied unchanged, i.e. the analysis will stop after that time without processing any parked peaks. This is not recommended and will lead to a warning in the gradient preview.



Smart Peak Parking

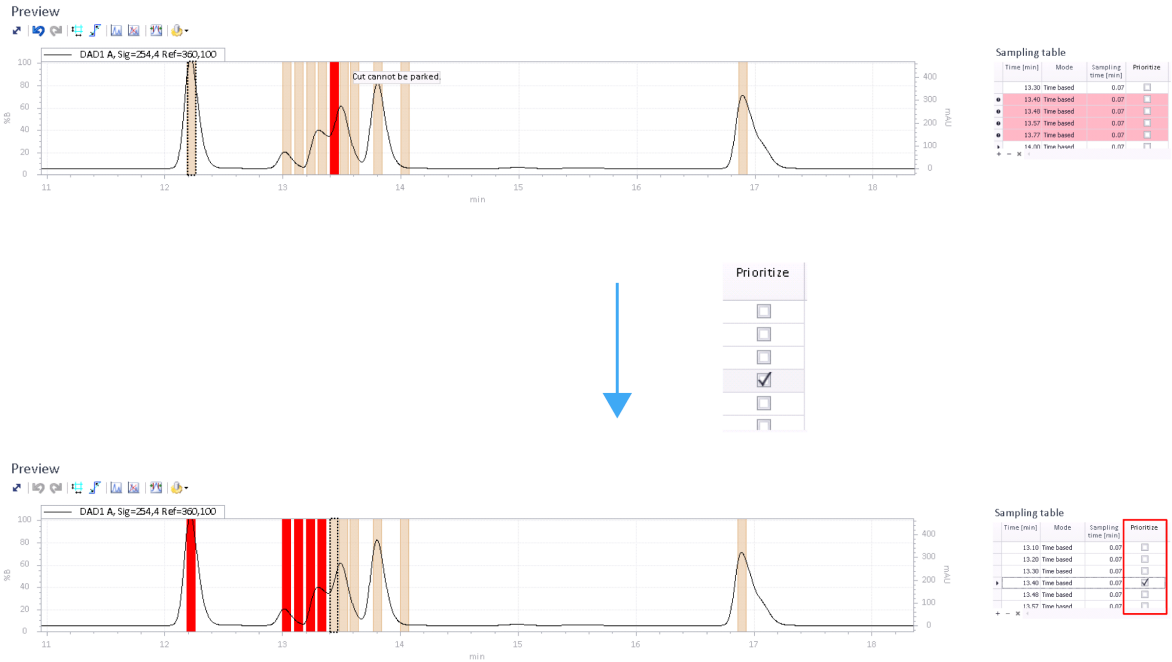
Smart peak parking optimizes parking for all time-based peaks in a reference signal.



Goals:

- Capture as many peaks as possible.
- Analyze them as fast as possible.

If still some peaks cannot be parked, user can define important peaks (**Prioritize**).



NOTE By default, smart peak parking is active. For backward compatibility, smart peak parking can be disabled.

NOTE The software displays a preview of what can be parked. This is a prediction only. The firmware decides in real-time, if a cut can be parked or not. In some cases, cuts may still be missed.

Method Parameters Comprehensive 2D-LC

Software Method Setup

The method setup dialog is used to edit the 2D-LC specific method parameters of the 2nd dimension pump that were not part of the standard method user interface of the pump.

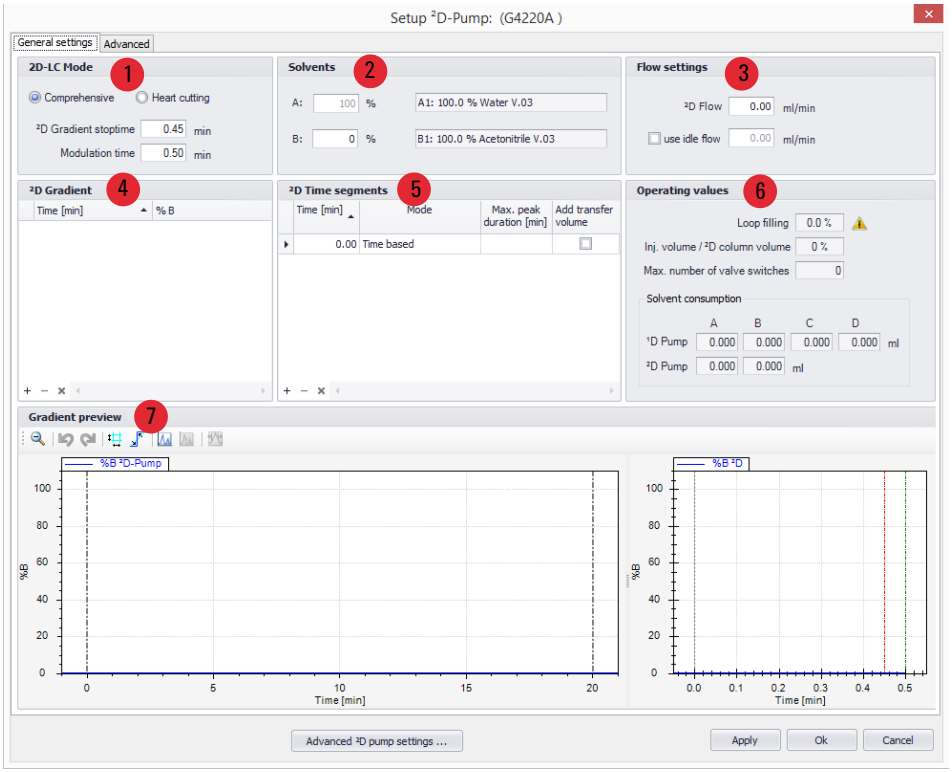


Figure 71 2D-LC method setup (General settings)

The setup of following method parameters is available:

1 **2D-LC Mode**, see "Set 2D-LC Mode" on page 120
2 **Solvents**, see "Set solvents" on page 160
3 **Flow settings**, see "Set flow" on page 161
4 **²D Gradient**, see "Set Solvent Composition Gradient" on page 162
5 **²D Time segments**, see "Set 2D Time Segments" on page 165
6 **Operating values**, see "Define Peak Detector Parameter" on page 167
7 **Gradient preview**, see "Gradient Preview Functionality" on page 168

Set 2D-LC Mode

Setting the mode has the following consequences:

- **Comprehensive** 2D-LC:

  The entire volume of the 1st dimension will be injected (using the pump in the 2nd dimension) onto the 2nd column. Two identical loops are used alternating, while one loops is filled in the 1st dimension, the volume of the other loop is separated with the 2nd column.

1 Select **Comprehensive** in **2D-LC Mode**.

NOTE

The **Modulation time** reflects the duration of one injection cycle in the 2nd dimension. After that time, the solvent composition gradient will be repeated. The parameter **Modulation time** is only used in the **Comprehensive** mode.

The **^{2}D Gradient Stoptime** reflects the maximal duration of the gradient in the 2nd dimension; the smallest value is 0.01 min. After that time, the Percent B value before the gradient (or the timetable entry at time = 0.0) is restored. In the Comprehensive 2D-LC mode the gradient stops latest when the **Modulation time** is reached.

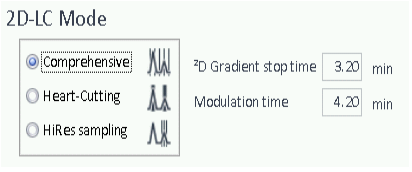


Figure 72 2D-LC Comprehensive mode

The gradient of the 2nd dimension is graphically displayed in a window in the lower right part of the method screen showing also the **Stop time** (as a red vertical line) and the **Modulation time** as a green vertical line.

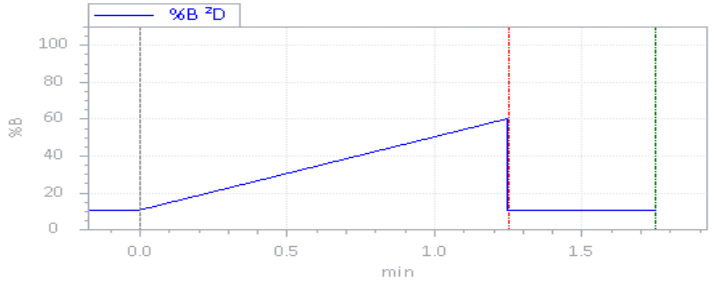


Figure 73 Stop time and Modulation time

Set solvents

NOTE

The selection of the solvents itself must be done in the standard pump method user interface.

- Open the pump method dialog using the button **Advanced ^{2}D pump settings...** and change the selection of the solvents there.
- After closing the dialog, the solvent settings should be updated immediately.

1 Set the percentage of solvent B to any value from 0 – 100 % in steps of 0.01 %.

Figure 74 2D-LC solvent settings

Solvent A always delivers the remaining percentage of volume. If the rate of solvent B is for example set to 20 %, solvent A, following the calculation %A = 100 - %B, automatically is set to 80 %.

The name of the selected solvents and their solvent channels (**A1:...** or **A2:...** and **B1:...** or **B2:...**) are shown in the corresponding text fields.

NOTE

The corresponding Percent B value in the Standard Pump user interface will be ignored as long as the 2D-LC functionality is enabled (see "Configuration" on page 179).

Set flow

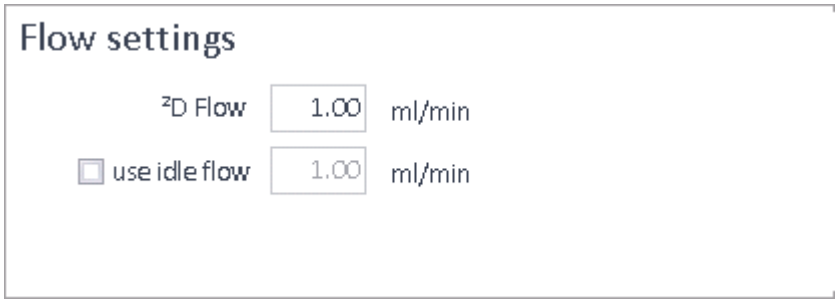


Figure 75 Flow settings

1 Set the **^{2}D Flow** (range 0 – 5.0 mL/min).

   This defines the flow in the 2nd dimension being used while 2D-LC is active (within 2D time segments where mode is not equal to OFF)

2 To set and use **Idle Flow** select check box **use idle flow**.

   This defines the flow in the 2nd dimension that is used while the 2D-LC mode is OFF (range 0 – 5.0 mL/min).

NOTE

If **use idle flow** is not selected, the **^{2}D Flow** is also used while 2D-LC mode is OFF.

Set Solvent Composition Gradient

Set Solvent Composition Gradient

The timetable in the **^{2}D Gradient** group allows changing the solvent composition.

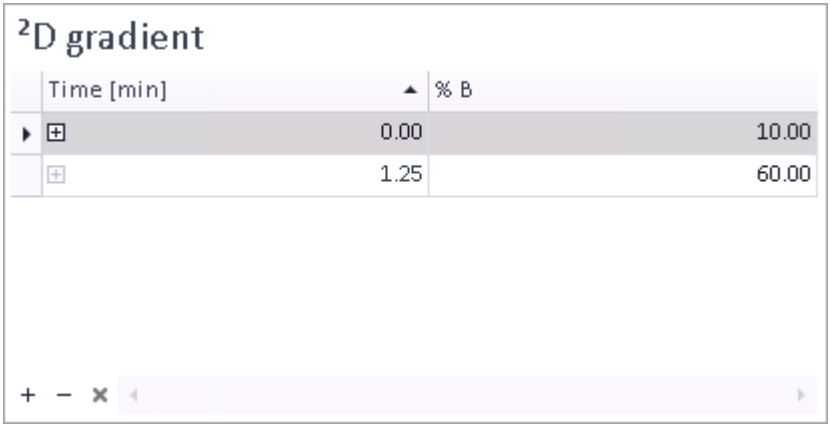
Percent B ranges from 0 – 100 %.

Change the solvent composition at a specified time

1 To change the solvent composition (%B) at the specified time apply a percent B range from 0 – 100 %

NOTE

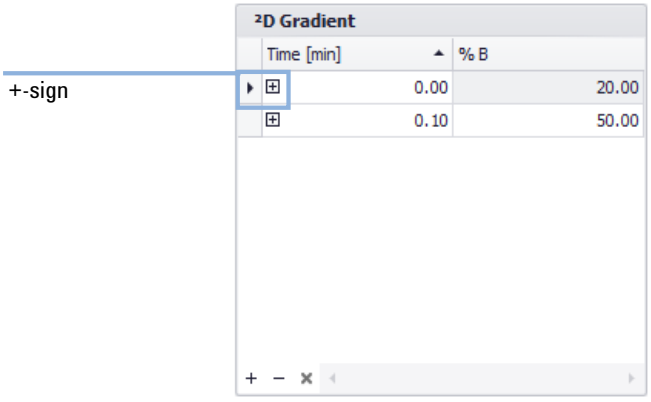
Different start conditions in the first row may cause step gradients and RI-effects (density differences of the different liquid phases may cause different DAD detection through baseline disturbances).



The time axis relates to the Stoptime of the 2nd dimension pump. **Time [min]** = **0.00** marks the start of the maybe repetitive gradient cycles, a time greater than **Stoptime ^{2}D** will be ignored.

Define shifted gradients

1 To modify an entry in the timetable over the runtime of the 1st dimension (shifted gradient), click **+**-sign at the beginning of the line and add one or more lines.



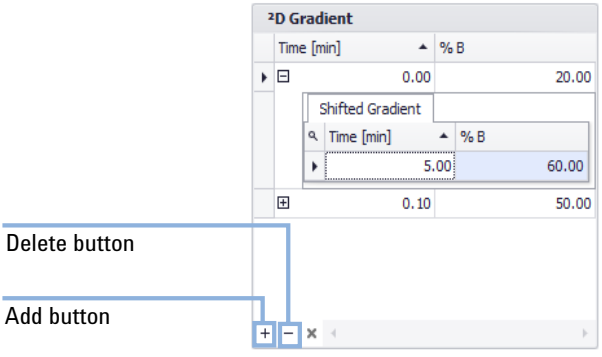
NOTE

A gray colored **+** indicates that the associated nested table has no entries, otherwise the **+** is black.

a To add a new entry, use the add button (**+** sign at the table bottom) in the timetable or in the shifted gradient table depending on the current focus. A new empty line is added at the end of the table, after editing the new line the table will be sorted automatically ascending by time.

OR

To delete the currently selected entry, use the delete button (**-** at the table bottom).



The time column specifies time values relative to the runtime of the 1st dimension. In the example above, the original timetable entry 20 %B at time = 0.0 min will be changed to 42 %B at time = 4.0 min doing linear interpolation in between. Between 4.0 min and 8.0 min, the value will change from 42 %B to 25 %B.

Setup 2D Gradient graphically

The user can graphically setup the 2D gradient including the initial composition (%B) value, the 2D-stoptime and the modulation (repetition) time.

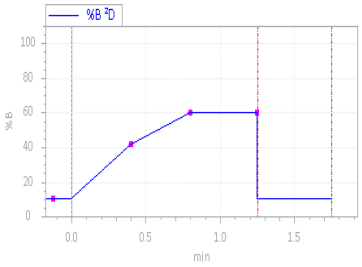


Figure 76 2D Gradient window in edit mode

1. Click to enable the graphical editing capabilities.
2. To add a new gradient point, move the cursor within the drawing area close to a new gradient point until the cursor changes to and click.
3. To delete a gradient point, move the cursor close to the gradient pointto be deleted until the cursor changes to , select the right segment and click.
4. To move a gradient point, move the cursor close to the gradient point to be moved until the cursor changes to , select the left segment and drag.
5. To change the stop time, move the cursor close to the red dotted vertical line until the cursor changes to ↔ and drag.
6. To change the modulation time, move the cursor close to the green dotted vertical line until the cursor changes to ↔ and drag.
7. To change the initial composition, move the cursor close to the filled circle most left near the y-axis until the cursor changes to ↕ and drag the point.

Set ^{2}D Time Segments

The content of the **^{2}D Time Segments** table specifies when (within the runtime of the 1st dimension) the selected 2D-LC mode is active.

Table 17 Definitions ^{2}D Time Segments

Column name	Description
Time	Specifies when a new segment starts (or ends)
Mode	Following options exist: • Time based The specified time defines the beginning of a time segment. • Peak based The peak detector is enabled at the specified time. • Off The time segments ends at the specified time.
Maximum peak duration (**Comprehensive** mode only)	Only valid in case of trigger mode = peak-based. After that time the 2D-gradient repetition ends regardless of the peak detector state.
Sampling time (**Heartcutting** mode only)	Set the time the loop remains in the flow path of the 1st dimension.
Add transfer volume	• Checked: Valve is switched at the specified time plus the time to deliver the delay volume • Unchecked Valve is switched at the specified time (This check box is available only for Time based mode)

NOTE

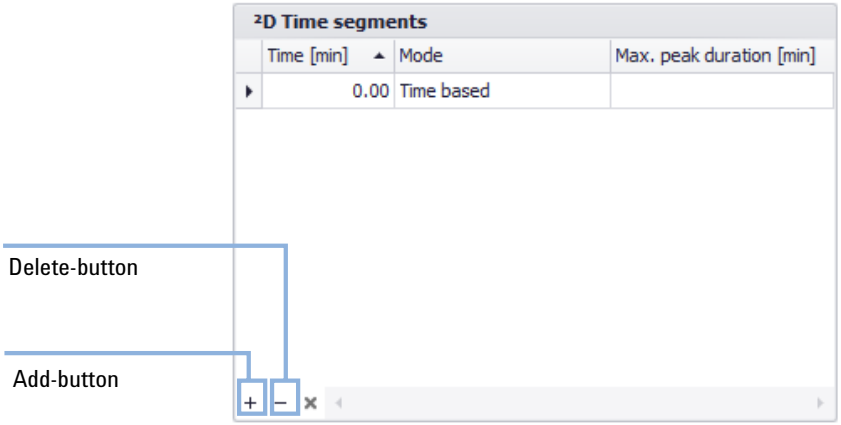
If the **^{2}D Time Segments** table is empty, no 2D-LC operation will be executed at all.

Set ^{2}D Time Segments for Comprehensive mode

1 To specify when a new segment starts, fill in required time in the time column.

After the time defined, the 2D-gradient repetition ends regardless of the peak detector state.

Trigger table (Comprehensive)



2 To specify the mode and time, select **Time based**, **Peak based** or **Off** from the drop-down list in the **Mode** column fill the **Time** field.

- **Time based**

  The specified time defines the beginning of a time segment where comprehensive 2D-LC is active. The ^{2}D-gradient repetition starts immediately and ends when the ^{1}D-Stoptime is reached or at the time specified in the next timetable entry. The actual gradient cycle is always completed except the ^{1}D stoptime is reached

- **Peak based**

  The peak detector is enabled at the specified time. The ^{2}D-gradient repetition is started when a begin peak is detected and ends either with peak-end or when max. peak duration is reached. The time segment ends when the ^{1}D-Stoptime is reached or at the time specified in the next timetable entry. It is possible to collect multiple peaks within one time segment.

- **Off**

  The time segments ends at the specified time.

3 In case of trigger-mode, define the the time in the **Max. peak duration** column.

4 To add or delete table rows, use the **+** and **-** icons below the table.

The **^{2}D Time Segments** now are defined.

Define Peak Detector Parameter

This section allows parameterizing the peak detector to be used for peak-triggered 2D-LC operation (comprehensive or heart cutting).

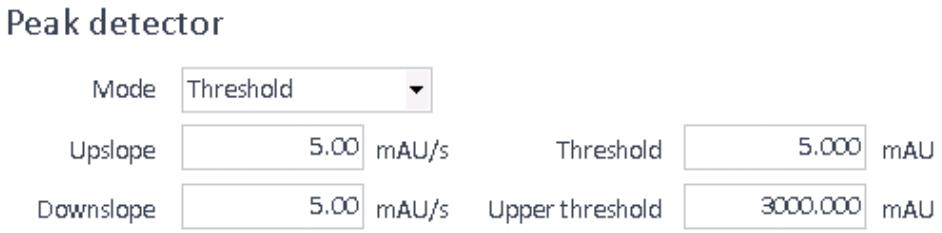
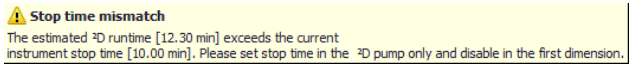


Figure 77 Overview on Peak detector parameters

The *stop time for a 2D-LC measurement must be set for the 2D pump*, which can be accessed through the **advanced** settings. It must be at least the 1D run time and applies to the entire measurement, not to partial 2D-only runs/gradients for parked peaks.

Multiple Heart-Cutting *automatically extends this run time*, if required, as analyzing parked peaks takes usually longer than the 1st dimension run only.

If you define a 1D stop time, it will be applied unchanged, for example the analysis will stop after that time without processing any parked peaks. This is not recommended and will lead to a warning in the gradient preview.



NOTE If no peak detector is configured (see "Configuration" on page 179) this section is disabled. The currently configured peak detector (name & serial number of the detector) is shown in the section header.

NOTE To facilitate the determination of parameters, it is possible to preview **Threshold** and **Slope** in the reference chromatogram.

1 Go to **Instrument >Setup 2DLC** and tab **Advanced**.

2 Select **Peak detection mode** from the drop-down list.

The following options are available:

- **Off**

  The peak detector is not used.

- **Threshold only**

  Detects peaks based on threshold values only.
- **Threshold/Slope** values

  Detects peaks based on both - threshold and slope.
- **Slope only**

  Detects peaks based on slope values only.

3 To define **Upslope** (slope of the rising peak), add the required values to the corresponding field.

4 To define **Downslope** (slope of the falling peak), add the required values to the corresponding field.

5 To define **Threshold** (height of the peak that triggers collection), add the required values to the corresponding field.

6 To define **Upper threshold** (height of the peak that ensures that collection is not switched off even for a saturated signal that might be expected to do so), add the required values to the corresponding field.

Gradient Preview Functionality

The gradient preview provides the following functions:

- Displays the gradient (%B) of the 1st dimension pump
- Displays the gradient (%B) of the 2nd dimension during the runtime of the first dimension, depending on the selected 2D-LC mode (comprehensive OR heart-cutting)
- Allows to graphically edit the gradient shifting
- Displays a reference signal by which a user can easily setup the trigger table or optimize his peak detector settings

Reference Signal

The user can load a chromatographic signal from an LC detector - a so-called reference signal. The signal will be shown in the gradient preview. This signal is automatically shown in the gradient preview of the setup dialog as long as the reference signal is part of the method. The signal can also be removed from the method or replaced by another signal.

Loading / removing a reference signal is triggered by toolbar buttons (or the context menu) of the gradient preview.

When a reference signal is loaded, a reference signal related y-axis is shown on the right side of the gradient preview window. The grid of the graphic window is either adjusted to the absorbance axis (right axis) or the %B axis (left) and can be changed by clicking on the corresponding axis. The signal name (e.g. DAD1 A, Sig= 280, 190 Ref=550,100) is shown in the legend of the graphic window.

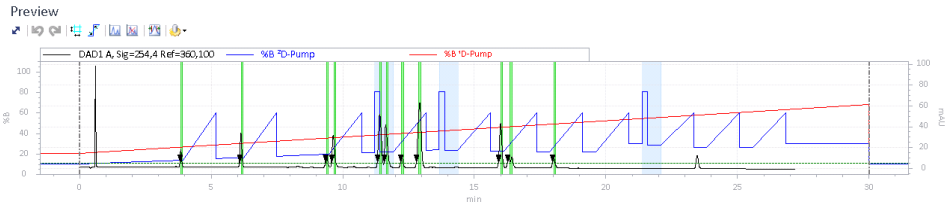


Figure 78 Gradient preview

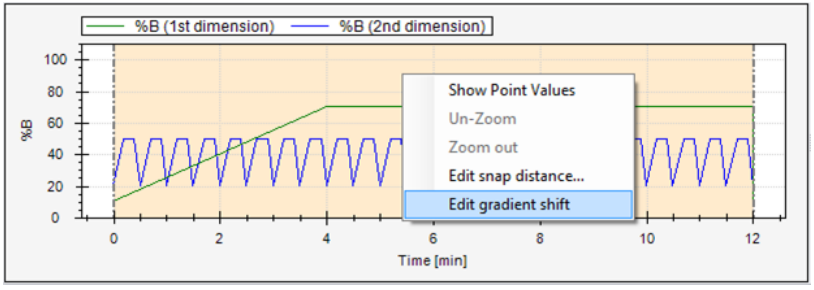
The reference signal offers the following:

- Simplified set-up of (time-based) heart cuts in case the chromatogram of the sample is known in advance
- Preview of peaks, which would be analyzed in the 2nd dimension that is based on the current peak detector settings (threshold, slope)

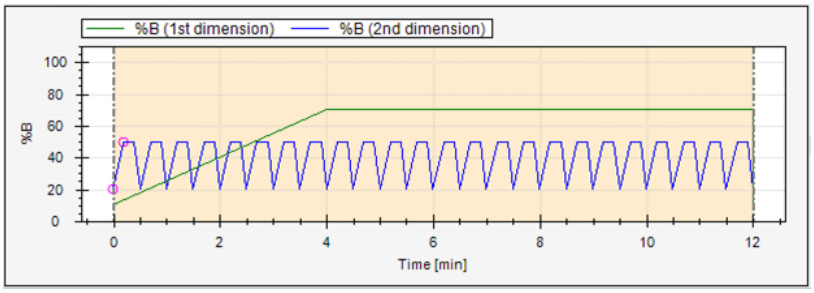
Setup Second Dimension Gradient with the Graphical User Interface

The gradient preview allows to edit gradient shifting graphically. This replaces the editing of large timetables by a few mouse operations.

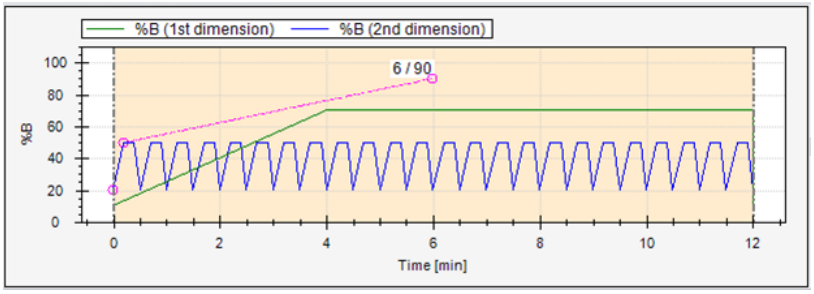
1 To enter the editing mode, use the context menu.



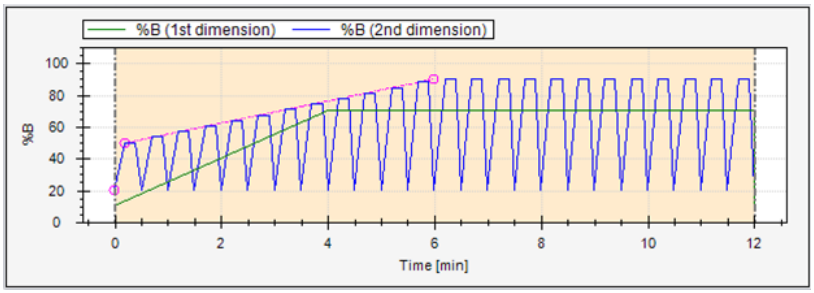
Timetable entries are marked with circles.



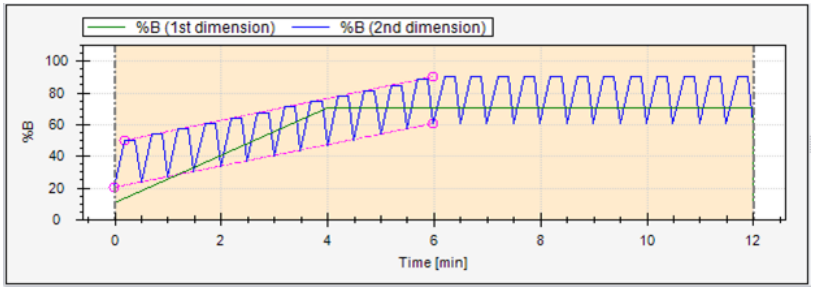
2 Drag the mouse to a new %B value at a specified runtime of the 1st dimension.



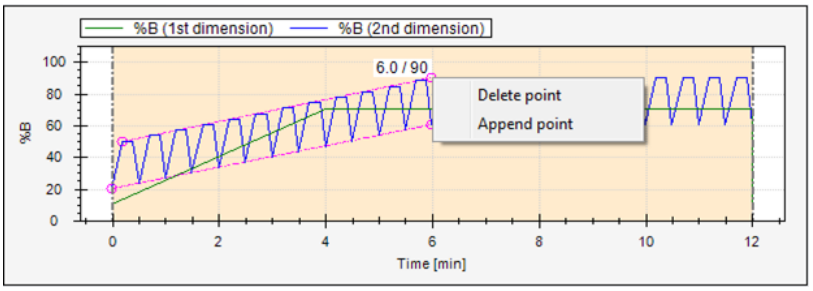
This draws a straight line. When releasing the mouse, a new timetable entry is made and the gradient rollout is automatically updated.



3 Repeat step 2 on page 170 with other timetable entries.



4 Move the mouse cursor near to a shift line to change menu context and insert or delete shift points as needed.



Method Development of Active Solvent Modulation (ASM)

ASM method development helps finding the optimal dilution of ^{1}D solvents in the sample loop for best ^{2}D resolution at lowest cycle time.

After switching on the ASM functionality (see "Method parameters" on page 173), execute the steps in the following order:

1. "Optimizing the dilution by using ASM capillaries" on page 174
2. "Optimizing the sample loop flush" on page 174
3. "Including the ASM phase to the 2D gradient" on page 175
4. "Optimizing dilution through method settings" on page 176

Method parameters

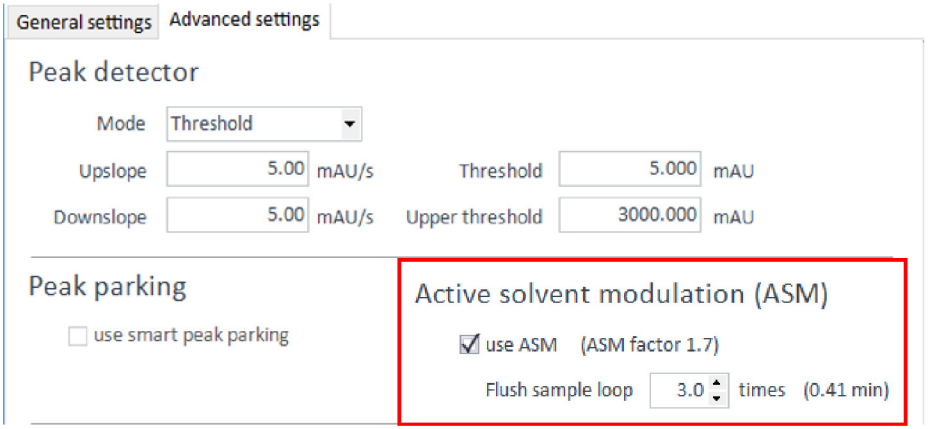


Figure 79 Method parameters for the ASM Valve (example)

Advanced settings of 2D-LC method parameters allow switching on and off the use of the ASM functionality.

- If this option is off, it works as a standard 2D-LC valve without dilution.
- If this option is on, the user can set how often he wants to flush the sample loop during the ASM phase.

Optimizing the dilution by using ASM capillaries

A choice of four different ASM capillaries is available for achieving best results.

Longer capillaries reduce, shorter capillaries increase the dilution of ^{1}D solvent in the sample loop.

Install and configure different ASM capillaries (see "Configure the ASM Valve" on page 221) for optimizing the results.

Capillary p/n	Length (mm)	Inner diameter (mm)	Volume (µl)	ASM factor	Split ratio (loop:ASM)
5500-1300	85	0.12	0.96	5	1:4
5500-1301	170	0.12	1.9	3	1:2
5500-1302	340	0.12	3.8	2	1:1
5500-1303	680	0.12	7.7	1.5	1:0.5

Optimizing the sample loop flush

Activate ASM in the software and set Flush sample loop to 3.0 times.

NOTE

Flushing the sample loop 3 times is typically enough and the recommended default. Less time may be sufficient and can be verified during optimization. The user interface displays how long this will take.

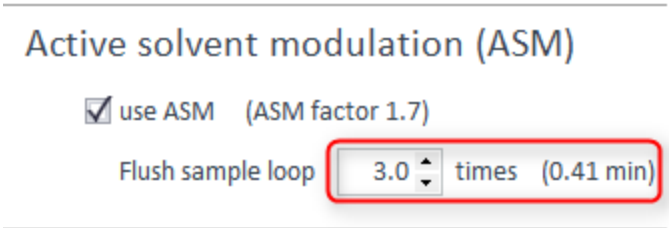


Figure 80 Set Flush sample loop (example)

Including the ASM phase to the ^{2}D gradient

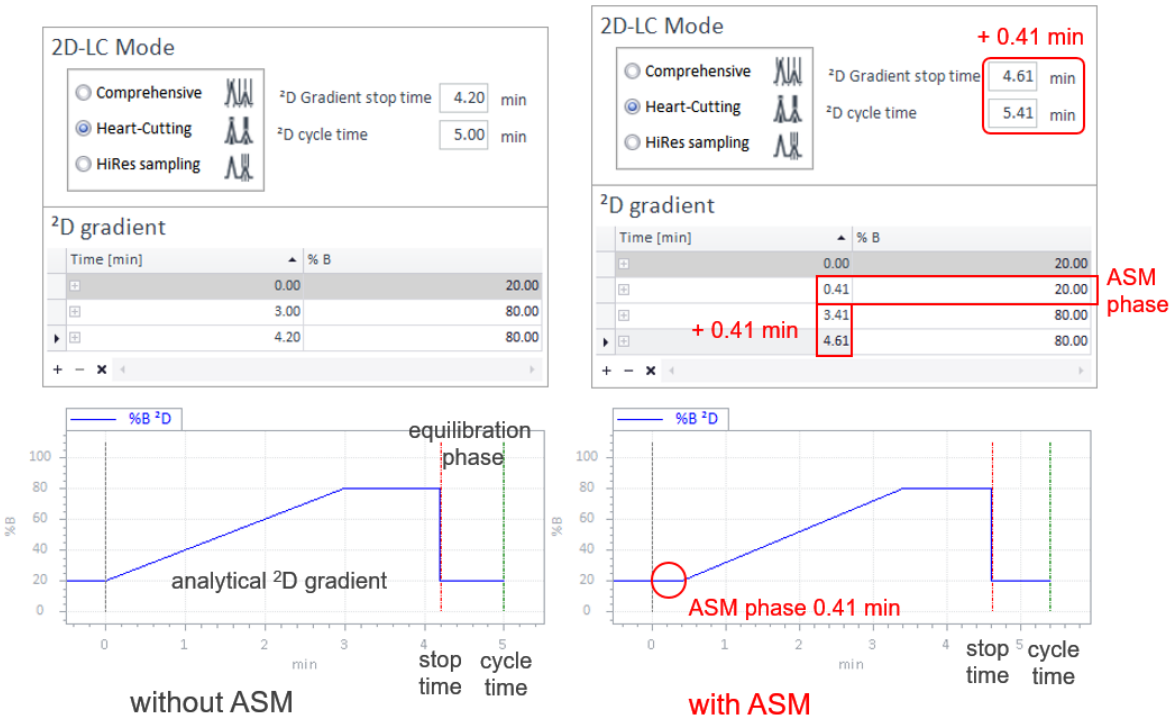


Figure 81 Programming the ^{2}D gradient table (example)

Gradients that were programmed for the second dimension originally without ASM Valve must be shifted by the delay caused by this dilution during the ASM phase such that the analytical gradient starts after the ASM phase.

If the ASM phase takes for example 0.41 min (based on selected ASM capillary, flush factor and ^{2}D flow rate), all times are shifted compared to a ^{2}D gradient without ASM.

- Gradient ends later and the gradient stop time is increased by 0.41 min
- ^{2}D Cycle time is increased accordingly
- One line is added to the gradient table for the ASM phase
- All times for the analytical gradient are shifted by 0.41 min.

  This is true for shifted gradient steps as well (if applicable).

Optimizing dilution through method settings

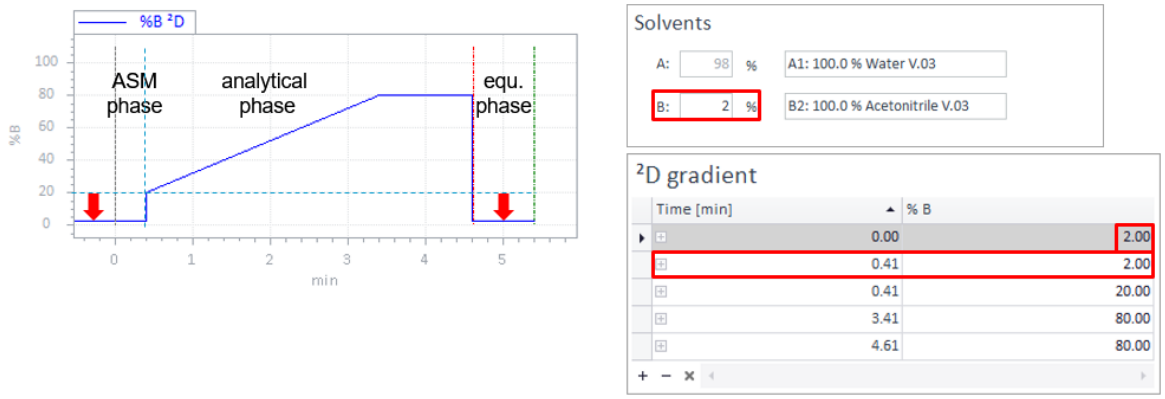


Figure 82 Optimizing separation by using a lower percentage of B for the ASM and column equilibration phase (example)

For optimizing separation, you may use a lower percentage of B for the ASM phase and column equilibration phase compared to the original gradient for increasing dilution before the ^{2}D column.

If for example the original analytical gradient started at 20 % B, you may use an ASM phase of for example 2 % B for diluting ^{1}D solvent more strongly during the ASM phase by changing the gradient start condition and adding a line to the ^{2}D gradient table for the ASM phase. The starting point for the analytical gradient does not change. The solvent composition of the equilibration phase is automatically reduced to the start condition.

Apply high-resolution sampling with small cut sizes. Small cut sizes reduce the transfer of solvent volume from ^{1}D to ^{2}D, which can further improve solvent compatibility and ^{2}D resolution.

6 Run the System

This chapter describes how to run the Agilent 1290 Infinity II 2D-LC Solution ChemStation in the modes standard heart-cutting, multiple heart-cutting, high resolution sampling and comprehensive 2D-LC.

Connect the capillaries to the 2D-LC valve

NOTE

Plumbing of 2D-LC valve

The correct plumbing of the 2D-LC valve differs between comprehensive versus heart-cutting mode and cocurrent versus countercurrent mode.

Use the 2D-LC software to find out the correct plumbing of the 2D-LC valve ports.

1 Install all capillaries to the 2D-LC valve.

Use the 2D-LC software to find out the correct plumbing of the 2D-LC valve ports:

- Standard heart-cutting:
  "Configure Valve and Loop" on page 180
- Multiple heart-cutting
  "Configure Valve and Loop" on page 192
- High resolution sampling
- "Configure Valve and Loop" on page 192
- Comprehensive
  "Configure Valve and Loop" on page 180

Standard Heart-Cutting 2D-LC

This section describes how to run the Agilent 1290 Infinity II 2D-LC Solution ChemStation in standard heart-cutting 2D-LC.

Configuration

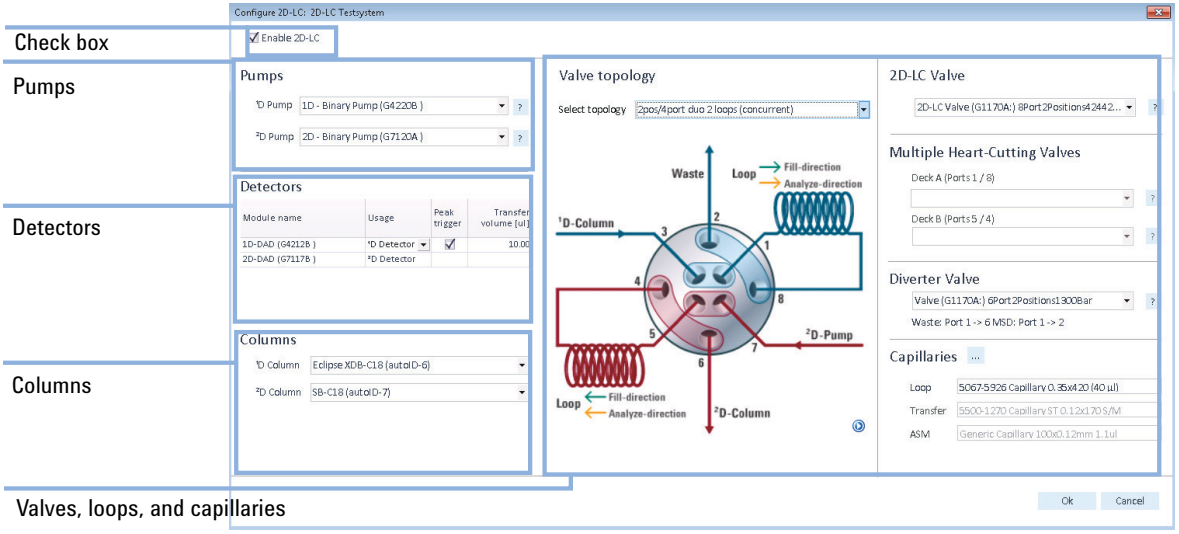


Figure 83 Overview 2D-LC configuration grafical user interface

The configuration of the 2D-LC-system is done via the configuration dialog in the software. The order of configuration is mandatory. The following configuration parameters are available:

- **Pumps**

  Section to define which pump is in the first and which one in the second dimension.
- **Detectors**

  Section to define which detector is in the second dimension and which detector should be used for peak detection (optional).
- **Columns**

  Section to define the columns being used in 1st and 2nd dimension.
- **Valve topology**, **2D-LC Valve**, **Multiple Heart-Cutting Valves**, **Diverter Valve**, **Capillaries**

  Section to identify the modulation valve(s) used for toggling the loop(s) and section to define the volume of the sampling loop(s).

Configure Valve and Loop

To run 2D-LC, it must be defined, which valve is used for 1st and 2nd dimension.

- 1st dimension:

  The **2D-LC Valve** drop-down list contains all configured valves which can be used for 2D-LC functionality.

- 2nd dimension(only relevant for multiple heart-cutting 2D-LC):

  If more than one valve matches the current valve/loop configuration, the user can select from a drop-down list, which valve is used to connect 1st and 2nd dimension.

- **Diverter Valve**

  If more than one valve matches the **Diverter Valve** configuration, a list-box is shown where the user can select the diverter valve.

The **Identify** button triggers the blinking of the status LED of the corresponding column compartment or valve drive. The button is only enabled in the Online version of the ChemStation.

All possible loop configurations depending on the selected valves are listed separately and illustrated on screen.

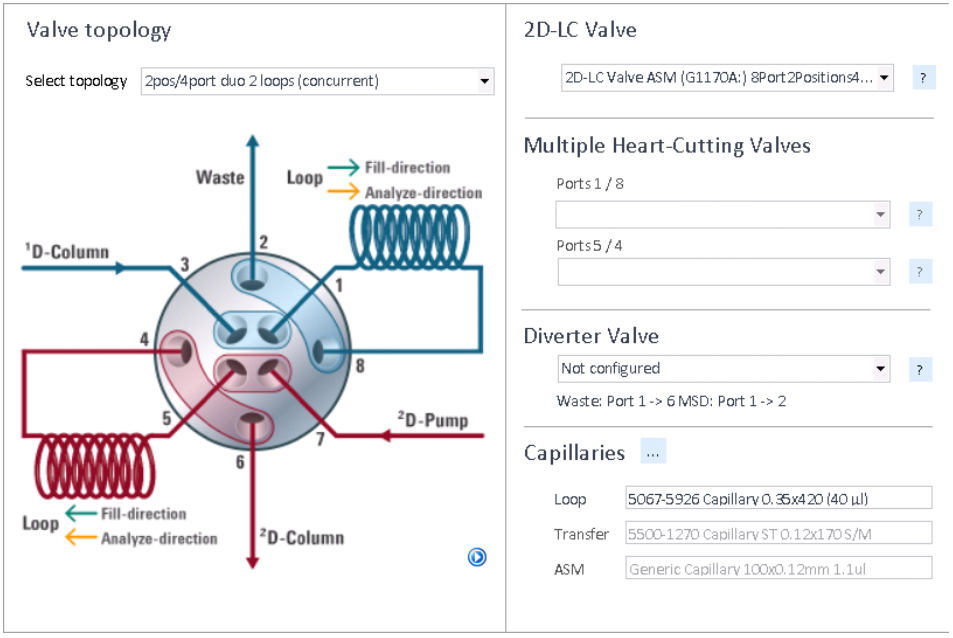


Figure 84 2D-LC valve and loop configuration (concurrent)

Software required 1290 Infinity 2D-LC Acquisition Software

Preparations Check box **Enable 2D-LC** selected

NOTE

Valves may be part of a column compartment (G7116A/B, G1316C) or a valve drive (G1170A).

1 Select **2D-LC Valve**.
2 **Select topology** under **Valve topology**.
3 To save settings click **OK**.

Valves and loops are configured for 2D-LC.

Legacy Checkout Familiarization Procedure

NOTE

For ESZ checkout procedure, please see the 2D-LC Installation Checklist or the *Agilent InfinityLab LC Series 1290 Infinity II 2D-LC Solution OpenLab CDS and MassHunter Acquisition for TOF and Q-TOF User Guide*.

Checkout runs - heart-cutting: 1290 Infinity Binary or Quaternary LC in ^{1}D

The familiarization procedure illustrates the system´s 2D-LC capabilities and supports the user to start the method for a specific analytical task. The familarization procedure will guide the user through the most important setups and analysis function.

The sample provided with the familiarization procedure can be determined with a UV-detector and a mass spectrometer. The methods to analyze the starter sample are delivered together with the full package to ensure a smooth familarization and checkout procedure. With the given method, peaks will overlap in the first dimension and will be separated in the second dimension.

The Agilent 1290 Infinity II 2D-LC Solution ChemStation is delivered together with all required parts for a complete familiarization procedure for (multiple) heart-cutting and comprehensive 2D-LC.

Parts required

p/n	Description
5190-6895	2D-LC starter sample, 1 x 2 mL Includes 2 mL
858700-902	RRHD SB-C18, 2.1x100 mm, 1.8 µm, 1200 bar ^{1}D
857768-901	RRHD Bonus-RP, 2.1x50 mm, 1.8 µm, 1200 bar ^{2}D, Heart-cutting
G2453-85060	Formic Acid-Reagent Grade 5 mL (5 cc)

Hardware required

- "Single Heart-Cutting Configuration" on page 63
- Capillary ST 0.35 x 420 mm M/M 40 µl (5067-5926)

Software required CD

Preparations

Solvents needed:

- ^{1}D: mobile phase A = water with 0.2 % Formic Acid-Reagent Grade 5 mL (5 cc) (G2453-85060), B = methanol
- ^{2}D: mobile phase A = water with 0.2 % Formic Acid-Reagent Grade 5 mL (5 cc) (G2453-85060), B = acetonitrile

Preparations:

1. Prepare dilution solvent (20 MeOH in mobile phase A): Add 300 µL MeOH to 1200 µL Mobile Phase A.
2. Prepare 400 µL sample: Add 40 µL 2D-LC starter sample to 360 µL dilution solvent.
3. Load method Checkout_MHC_Comp_1290BinX1290Bin.M from the 2D-LC Addon SW CD and modify the settings for your single heart or multiple heart cutting configuration.

1 Apply method parameters for ^{1}D

Table 18 Checkout method parameter settings ^{1}D

Module	Menu Path	Parameter	Value
Column			RRHD SB-C18, 2.1x 100 mm, 1.8 µm, 1200 bar (858700-902)
^{1}D Pump	**Set up Instrument Method... >Setup Method**`1D Pump`	Solvent **A**	H_2O + 0.2 % formic acid
		Solvent **B**	Methanol
		Timetable [1/100 events] (Gradient)	**Time [min]:**0.0 min, **B[%]**20
			Time [min]:50 min, **B[%]**100
			Stoptime: 40 min
			Posttime: 10 min
		Flow rate	0.300 mL/min
		Posttime	6 min
^{1}D Column Compartment	**Instrument >Setup Method**`1D Column Compartment`	**Temperature**	40 °C
^{1}D Detector	**Instrument >Setup Method**`1D Detector`	**Signal A**	**Wavelength**: 254 nm **Bandwidth**: 4 nm **Reference Wavelength**: 360 nm **Reference Bandwidth**: 100 nm
		Peakwidth:	5 Hz
^{1}D Sampler	**Instrument >Setup Method**`1D Sampler`	**Injection volume**:	2.0 µL

2 Apply method parameters for ^{2}D.

Module	Menu Path	Parameter	Value
Column			RRHD Bonus-RP, 2.1x 50 mm, 1.8 µm, 1200 bar (857768-901)
^{2}D Pump	Setup ^{2}D-Pump	Solvent **A**	H_2O + 0.2 % formic acid
		Solvent **B**	Acetonitrile
		^{2}D gradient	**Time [min]:**0.0 min, **B[%]**10
			Time [min]:1.25 min, **B[%]**60
		^{2}D Gradient stoptime:	1.25 min
		^{2}D cycletime	1.75 min
		^{2}D Flow	1.0 mL/min
	Setup ^{2}D-Pump **PreviewEdit mode**	Gradient shift:	0->20 min from 10->30 % B (only downslope)
	Setup ^{2}D-Pump **Advanced ^{2}D pump settings...**	Stoptime	40 min (will be automatically prolonged, if peaks in ^{2}D are not worked off)
^{1}D Column Compartment		**Temperature**	40 °C
^{2}D Detector	**Instrument >Setup Method**2D Detector	**Signal A**	**Wavelength**: 254 nm **Bandwidth**: 4 nm **Reference Wavelength**: 360 nm **Reference Bandwidth**: 100 nm
		Peakwidth:	≥40 Hz

3 Program and/or find the following cuts in the predifined method:

Cut-#	Cut-Time [min] 1290 Binary LC	Cut-Time [min] 1290 Quaternary LC[1]
1	4.25	4.35
2	6.58	6.86
3	10.05	10.4
4	13.3	13.7
5	16.8	17.15
6	23.9	24.6

[1] The Cut-Time can vary slightly depending on the configuration

4 Run the method with the 2D-LC starter sample, 1 x 2 mL (5190-6895), 1:10 diluted with Methanol/Water (20/80; v/v) with 0.1 % formic acid.

Checkout runs - heart-cutting: 1260 Infinity Binary in ^{1}D

The familiarization procedure illustrates the system´s 2D-LC capabilities and supports the user to start the method for a specific analytical task. The familarization procedure will guide the user through the most important setups and analysis function.

The sample provided with the familiarization procedure can be determined with a UV-detector and a mass spectrometer. The methods to analyze the starter sample are delivered together with the full package to ensure a smooth familarization and checkout procedure. With the given method, peaks will overlap in the first dimension and will be separated in the second dimension.

The Agilent 1290 Infinity II 2D-LC Solution ChemStation is delivered together with all required parts for a complete familiarization procedure for (multiple) heart-cutting and comprehensive 2D-LC.

Parts required

p/n	Description
5190-6895	2D-LC starter sample, 1 x 2 mL Includes 2 mL
858700-902	RRHD SB-C18, 2.1x100 mm, 1.8 µm, 1200 bar ^{1}D
857768-901	RRHD Bonus-RP, 2.1x50 mm, 1.8 µm, 1200 bar ^{2}D, Heart-cutting
G2453-85060	Formic Acid-Reagent Grade 5 mL (5 cc)

Hardware required

- "Single Heart-Cutting Configuration" on page 63
- Capillary ST 0.35 x 420 mm M/M 40 µl (5067-5926)

Software required

CD

Preparations

Solvents needed:

- ^{1}D: mobile phase A = water with 0.2 % Formic Acid-Reagent Grade 5 mL (5 cc) (G2453-85060), B = methanol
- ^{2}D: mobile phase A = water with 0.2 % Formic Acid-Reagent Grade 5 mL (5 cc) (G2453-85060), B = acetonitrile

Preparations:

1. Prepare dilution solvent (20 MeOH in mobile phase A): Add 300 µL MeOH to 1200 µL Mobile Phase A.
2. Prepare 400 µL sample: Add 40 µL 2D-LC starter sample to 360 µL dilution solvent.
3. Load method Checkout_MHC_Comp_1290BinX1290Bin.M from the 2D-LC Addon SW CD and modify the settings for your single heart or multiple heart cutting configuration.

1 Apply method parameters for ^{1}D

Table 19 Checkout method parameter settings ^{1}D

Module	Menu Path	Parameter	Value
Column			RRHD SB-C18, 2.1x 100 mm, 1.8 µm, 1200 bar (858700-902)
^{1}D Pump	**Set up Instrument Method... >Setup Method**`1D Pump`	Solvent **A**	H_2O + 0.2 % formic acid
		Solvent **B**	Methanol
		Timetable [1/100 events] (Gradient)	**Time [min]:**0.0 min, **B[%]**20
			Time [min]:50 min, **B[%]**100
			Stoptime: 40 min
			Posttime: 10 min
		Flow rate	0.300 mL/min
		Posttime	6 min
^{1}D Column Compartment	**Instrument >Setup Method**`1D Column Compartment`	**Temperature**	40 °C
^{1}D Detector	**Instrument >Setup Method**`1D Detector`	**Signal A**	**Wavelength**: 254 nm **Bandwidth**: 4 nm **Reference Wavelength**: 360 nm **Reference Bandwidth**: 100 nm
		Peakwidth:	5 Hz
^{1}D Sampler	**Instrument >Setup Method**`1D Sampler`	**Injection volume**:	2.0 µL

2 Apply method parameters for ^{2}D.

Module	Menu Path	Parameter	Value
Column			RRHD Bonus-RP, 2.1x 50 mm, 1.8 µm, 1200 bar (857768-901)
^{2}D Pump	Setup ^{2}D-Pump	Solvent **A**	H_2O + 0.2 % formic acid
		Solvent **B**	Acetonitrile
		^{2}D gradient	**Time [min]:**0.0 min, **B[%]**10
			Time [min]:1.25 min, **B[%]**60
		^{2}D Gradient stoptime:	1.25 min
		^{2}D cycletime	1.75 min
		^{2}D Flow	1.0 mL/min
	Setup ^{2}D-Pump **PreviewEdit mode**	Gradient shift:	0->20 min from 10->30 % B (only downslope)
	Setup ^{2}D-Pump **Advanced ^{2}D pump settings...**	Stoptime	40 min (will be automatically prolonged, if peaks in ^{2}D are not worked off)
^{1}D Column Compartment		**Temperature**	40 °C
^{2}D Detector	**Instrument >Setup Method**2D Detector	**Signal A**	**Wavelength**: 254 nm **Bandwidth**: 4 nm **Reference Wavelength**: 360 nm **Reference Bandwidth**: 100 nm
		Peakwidth:	≥40 Hz

3 Program and/or find the following cuts in the predifined method:

Cut-#	Cut-Time [min] 1260 Binary LC[1]
1	9.5
2	13.13
3	17.6
4	21.2
5	25.25
6	31.55

[1] The Cut-Time can vary slightly depending on the configuration

4 Run the method with the 2D-LC starter sample, 1 x 2 mL (5190-6895), 1:10 diluted with Methanol/Water (20/80; v/v) with 0.1 % formic acid.

Multiple Heart-Cutting and High Resolution Sampling 2D-LC

This section describes how to run the Agilent 1290 Infinity II 2D-LC Solution ChemStation in multiple heart-cutting and high resolution sampling 2D-LC.

Configuration

Overview Configuration Dialog

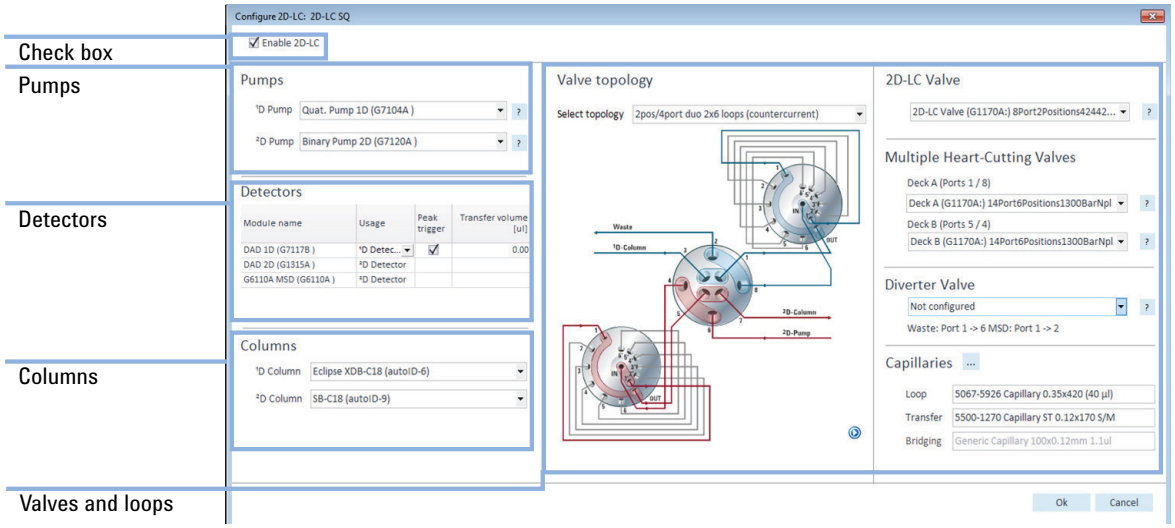


Figure 85 Overview 2D-LC configuration graphical user interface

The configuration of the 2D-LC-system is done via the configuration dialog in the software. The order of configuration is mandatory. The following configuration parameters are available:

- **Pumps**

  Section to define which pump is in the first and which one in the second dimension.

- **Detectors**

  Section to define which detector is in the second dimension and which detector should be used for peak detection (optional).

- **Columns**

  Section to define the columns being used in 1st and 2nd dimension.

- **Valve topology**, **2D-LC Valve**, **Multiple Heart-Cutting Valves**, **Diverter Valve**, **Capillaries**

  Section to identify the modulation valve(s) used for toggling the loop(s) and section to define the volume of the sampling loop(s).

Configure Valve and Loop

To run Multiple Heart-cutting/High-resolution sampling 2D-LC, the following parameters must be defined:

- **Select topology**:

  The drop-down list contains all possible valve/loop topologies.

- **2D-LC valve(s)**:

  - **#1**:

    If more than one valve matches the current valve / loop configuration, a list-box is shown where the user can select the 2D-LC valve.

  - **#2**:

    If more than one valve matches the current valve / loop configuration, a list-box is shown where the user can select the 2D-LC valve.

    Shows the 2nd valve to be used for the injection on the 2nd dimension. This field is only visible if a **Dual 2pos/6port** configuration is selected.

- **Multiple Heart-Cutting Valve(s)**

  - **Deck A (Ports 1 / 8)**:

    Shows the 6port/14port valve used for the parking deck 1. The field is only enabled in case of a valve / loop configuration with one or two Multiple Heart-Cutting Valves (6 or 12 loops). If more than one 6/14 valve is available, a list-box is shown where the user can select the appropriate valve.

  - **Deck B (Ports 5 / 4)**:

    Select the 6port/14port valve used for the parking deck 2. The list-box is only enabled in case of a valve / loop configuration with two Multiple Heart-Cutting Valves (6 or 12 loops).

- **Diverter Valve**

  If more than one valve matches the **Diverter Valve** configuration, a list-box is shown where the user can select the diverter valve.

- **Capillaries**

  A list-box is shown where the user can select the capillaries.

- The **Identify** button triggers the blinking of the status LED of the corresponding valve or column compartment. The button is only enabled in the Online version of the ChemStation.

All possible loop configurations depending on the selected valves are listed separately and illustrated on screen.

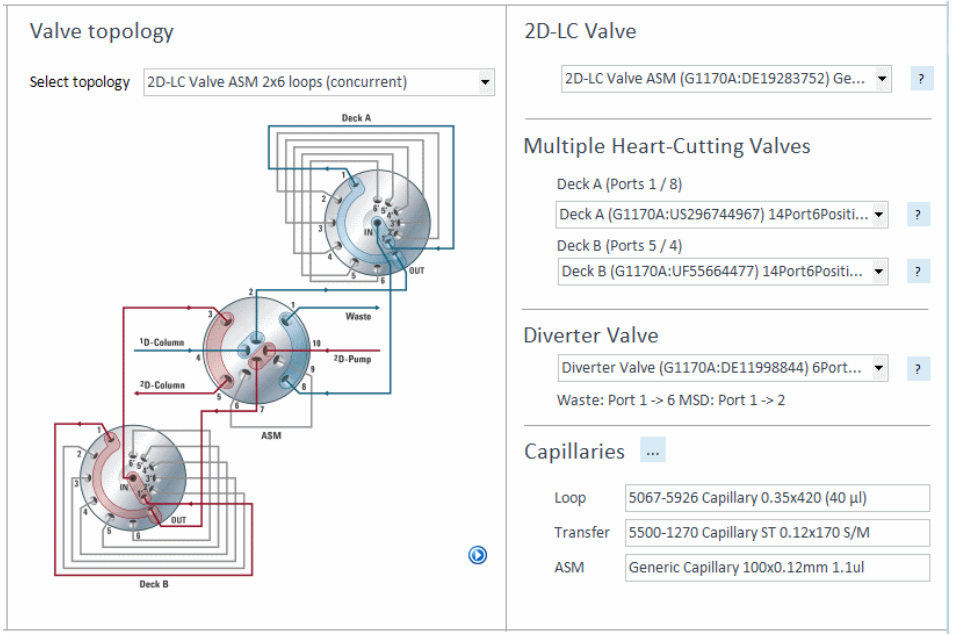


Figure 86 2D-LC valve and loop configuration

Preparations

- OpenLAB ChemStation Edition C.01.10 (or higher)
- 1290 Infinity 2D-LC Acquisition Software
- Check box **Enable 2D-LC** selected

NOTE

Valves must be part of the 1290 Infinity Valve Drive (G1170A).

1 Select the valve / loop combination for the injection on the 1st dimension (**Select topology**).

2 Specifiy the volume of the loop (**Loop size**).

3 Select the valve for the injection on the 2nd dimension (**2D-LC valve(s)**).

4 Select the valve for parking peaks (**Multiple Heart-Cutting Valve(s)**).

5 Select the capillaries used in your system.

6 To save settings click **OK**.

Legacy Checkout/Familiarization Procedure

NOTE

For ESZ checkout procedure, please see the 2D-LC Installation Checklist or the *Agilent InfinityLab LC Series 1290 Infinity II 2D-LC Solution OpenLab CDS and MassHunter Acquisition for TOF and Q-TOF User Guide*.

Checkout runs - MHC: 1290 Infinity Binary or Quaternary LC in ^{1}D

Parts required

p/n	Description
5190-6895	2D-LC starter sample, 1 x 2 mL Includes 2 mL
858700-902	RRHD SB-C18, 2.1x100 mm, 1.8 µm, 1200 bar ^{1}D
857768-901	RRHD Bonus-RP, 2.1x50 mm, 1.8 µm, 1200 bar ^{2}D, Heart-cutting
G2453-85060	Formic Acid-Reagent Grade 5 mL (5 cc)

Hardware required "Multiple Heart-Cutting Configuration" on page 64

Software required CD

Preparations

Solvents needed:

- ^{1}D: mobile phase A = water with 0.2 % Formic Acid-Reagent Grade 5 mL (5 cc) (G2453-85060), B = methanol
- ^{2}D: mobile phase A = water with 0.2 % Formic Acid-Reagent Grade 5 mL (5 cc) (G2453-85060), B = acetonitrile

Preparations:

1. Prepare dilution solvent (20 MeOH in mobile phase A): Add 300 µL MeOH to 1200 µL Mobile Phase A.
2. Prepare 400 µL sample: Add 40 µL 2D-LC starter sample to 360 µL dilution solvent.
3. Load method Checkout_MHC_Comp_1290BinX1290Bin.M from the 2D-LC Addon SW CD and modify the settings for your single heart or multiple heart cutting configuration.

1 Apply method parameters for ^{1}D

Table 20 Checkout method parameter settings ^{1}D

Module	Menu Path	Parameter	Value
Column			RRHD SB-C18, 2.1x 100 mm, 1.8 µm, 1200 bar (858700-902)
^{1}D Pump	**Set up Instrument Method... >Setup Method**`1D Pump`	Solvent **A**	H_2O + 0.2 % formic acid
		Solvent **B**	Methanol
		Timetable [1/100 events] (Gradient)	**Time [min]:**0.0 min, **B[%]**20
			Time [min]:50 min, **B[%]**100
			Stoptime: 40 min
			Posttime: 10 min
		Flow rate	0.300 mL/min
		Posttime	6 min
^{1}D Column Compartment	**Instrument >Setup Method**`1D Column Compartment`	**Temperature**	40 °C
^{1}D Detector	**Instrument >Setup Method**`1D Detector`	**Signal A**	**Wavelength**: 254 nm **Bandwidth**: 4 nm **Reference Wavelength**: 360 nm **Reference Bandwidth**: 100 nm
		Peakwidth:	5 Hz
^{1}D Sampler	**Instrument >Setup Method**`1D Sampler`	**Injection volume**:	2.0 µL

2 Apply method parameters for ^{2}D.

Module	Menu Path	Parameter	Value
Instrument	**Instrument >Setup 2D-LC > >**		Verify that the 2D-LC mode is set to **Multiple Heart-Cutting**
Column			RRHD Bonus-RP, 2.1x 50 mm, 1.8 µm, 1200 bar (857768-901)
^{2}D Pump	Setup ^{2}D-Pump	Solvent **A**	H_2O + 0.2 % formic acid
		Solvent **B**	Acetonitrile
		^{2}D gradient	**Time [min]:**0.0 min, **B[%]**10
			Time [min]:1.25 min, **B[%]**60
		^{2}D Gradient stoptime:	1.25 min
		^{2}D cycletime	1.75 min
		^{2}D Flow	1.0 mL/min
	Setup ^{2}D-Pump **PreviewEdit mode**	Gradient shift:	0->20 min from 10->30 % B (only downslope)
	Setup ^{2}D-Pump **Advanced ^{2}D pump settings...**	Stoptime	40 min (will be automatically prolonged, if peaks in ^{2}D are not worked off)
^{1}D Column Compartment		**Temperature**	40 °C
^{2}D Detector	**Instrument >Setup Method**`2D Detector`	**Signal A**	**Wavelength**: 254 nm **Bandwidth**: 4 nm **Reference Wavelength**: 360 nm **Reference Bandwidth**: 100 nm
		Peakwidth:	≥40 Hz

3 Program and/or find the following cuts in the predifined method:

Cut-#	Cut-Time [min] 1290 Binary LC	Cut-Time [min] 1290 Quaternary LC[1]
1	4.25	4.35
2	6.58	6.86
3	10.05	10.4
4	12.19	12.58
5	13.3	13.7
6	13.44	13.85
7	13.58	14
8	13.72	14.15
9	13.86	14.3
10	16.8	17.15
11	16.94	17.3
12	17.08	17.45
13	17.22	17.6
14	17.36	17.75
15	23.9	24.6

[1] The Cut-Time can vary slightly depending on the configuration

4 Run the method with the 2D-LC starter sample, 1 x 2 mL (5190-6895), 1:10 diluted with Methanol/Water (20/80; v/v) with 0.1 % formic acid.

Checkout runs - MHC: 1260 Infinity Binary in ^{1}D

Parts required

p/n	Description
5190-6895	2D-LC starter sample, 1 x 2 mL Includes 2 mL
858700-902	RRHD SB-C18, 2.1x100 mm, 1.8 µm, 1200 bar ^{1}D
857768-901	RRHD Bonus-RP, 2.1x50 mm, 1.8 µm, 1200 bar ^{2}D, Heart-cutting
G2453-85060	Formic Acid-Reagent Grade 5 mL (5 cc)

Hardware required "Multiple Heart-Cutting Configuration" on page 64

Software required CD

Preparations

Solvents needed:

- ^{1}D: mobile phase A = water with 0.2 % Formic Acid-Reagent Grade 5 mL (5 cc) (G2453-85060), B = methanol
- ^{2}D: mobile phase A = water with 0.2 % Formic Acid-Reagent Grade 5 mL (5 cc) (G2453-85060), B = acetonitrile

Preparations:

1. Prepare dilution solvent (20 MeOH in mobile phase A): Add 300 µL MeOH to 1200 µL Mobile Phase A.
2. Prepare 400 µL sample: Add 40 µL 2D-LC starter sample to 360 µL dilution solvent.
3. Load method Checkout_MHC_Comp_1290BinX1290Bin.M from the 2D-LC Addon SW CD and modify the settings for your single heart or multiple heart cutting configuration.

1 Apply method parameters for ^{1}D

Table 21 Checkout method parameter settings ^{1}D

Module	Menu Path	Parameter	Value
Column			RRHD SB-C18, 2.1x 100 mm, 1.8 µm, 1200 bar (858700-902)
^{1}D Pump	**Set up Instrument Method... >Setup Method**`1D Pump`	Solvent **A**	H_2O + 0.2 % formic acid
		Solvent **B**	Methanol
		Timetable [1/100 events] (Gradient)	**Time [min]:**0.0 min, **B[%]**20
			Time [min]:50 min, **B[%]**100
			Stoptime: 40 min
			Posttime: 10 min
		Flow rate	0.300 mL/min
		Posttime	6 min
^{1}D Column Compartment	**Instrument >Setup Method**`1D Column Compartment`	**Temperature**	40 °C
^{1}D Detector	**Instrument >Setup Method**`1D Detector`	**Signal A**	**Wavelength**: 254 nm **Bandwidth**: 4 nm **Reference Wavelength**: 360 nm **Reference Bandwidth**: 100 nm
		Peakwidth:	5 Hz
^{1}D Sampler	**Instrument >Setup Method**`1D Sampler`	**Injection volume**:	2.0 µL

2 Apply method parameters for ^{2}D.

Module	Menu Path	Parameter	Value
Instrument	**Instrument >Setup 2D-LC > >**		Verify that the 2D-LC mode is set to **Multiple Heart-Cutting**
Column			RRHD Bonus-RP, 2.1x 50 mm, 1.8 µm, 1200 bar (857768-901)
^{2}D Pump	Setup ^{2}D-Pump	Solvent **A**	H_2O + 0.2 % formic acid
		Solvent **B**	Acetonitrile
		^{2}D gradient	**Time [min]:**0.0 min, **B[%]**10
			Time [min]:1.25 min, **B[%]**60
		^{2}D Gradient stoptime:	1.25 min
		^{2}D cycletime	1.75 min
		^{2}D Flow	1.0 mL/min
	Setup ^{2}D-Pump **PreviewEdit mode**	Gradient shift:	0->20 min from 10->30 % B (only downslope)
	Setup ^{2}D-Pump **Advanced ^{2}D pump settings...**	Stoptime	40 min (will be automatically prolonged, if peaks in ^{2}D are not worked off)
^{1}D Column Compartment		**Temperature**	40 °C
^{2}D Detector	**Instrument >Setup Method**`2D Detector`	**Signal A**	**Wavelength**: 254 nm **Bandwidth**: 4 nm **Reference Wavelength**: 360 nm **Reference Bandwidth**: 100 nm
		Peakwidth:	≥40 Hz

3 Program and/or find the following cuts in the predifined method:

Cut-#	Cut-Time [min] 1260 Binary LC[1]
1	9.5
2	13.13
3	17.6
4	20.2
5	20.45
6	20.7
7	21.2
8	21.45
9	21.7
10	22.2
11	24.6
12	25.25
13	25.5
14	25.75
15	27

[1] The Cut-Time can vary slightly depending on the configuration

4 Run the method with the 2D-LC starter sample, 1 x 2 mL (5190-6895), 1:10 diluted with Methanol/Water (20/80; v/v) with 0.1 % formic acid.

Checkout run - high-resolution sampling

Parts required

p/n	Description
5190-6895	2D-LC starter sample, 1 x 2 mL Includes 2 mL
858700-902	RRHD SB-C18, 2.1x100 mm, 1.8 µm, 1200 bar ^{1}D
857768-901	RRHD Bonus-RP, 2.1x50 mm, 1.8 µm, 1200 bar ^{2}D, Heart-cutting
G2453-85060	Formic Acid-Reagent Grade 5 mL (5 cc)

Hardware required "Multiple Heart-Cutting Configuration" on page 64

Software required CD

Preparations

Solvents needed:

- ^{1}D: mobile phase A = water with 0.2 % Formic Acid-Reagent Grade 5 mL (5 cc) (G2453-85060), B = methanol
- ^{2}D: mobile phase A = water with 0.2 % Formic Acid-Reagent Grade 5 mL (5 cc) (G2453-85060), B = acetonitrile

Preparations:

1. Prepare dilution solvent (20 MeOH in mobile phase A): Add 300 µL MeOH to 1200 µL Mobile Phase A.
2. Prepare 400 µL sample: Add 40 µL 2D-LC starter sample to 360 µL dilution solvent.
3. Load method Checkout_MHC_Comp_1290BinX1290Bin.M from the 2D-LC Addon SW CD and modify the settings for your single heart or multiple heart cutting configuration.

1 Apply method parameters for ^{1}D.

Table 22 Checkout method parameter settings ^{1}D

Module	Tool	Parameter	Value
Column			RRHD SB-C18, 2.1x 100 mm, 1.8 µm, 1200 bar (858700-902)
^{1}D Pump	**Instrument >Setup Method**`1D Pump`	Solvent **A**	H_2O + 0.2 % formic acid
		Solvent **B**	Methanol
		Timetable [1/100 events] (Gradient)	**Time [min]:**0.0 min, **B[%]**20
			Time [min]:20 min, **B[%]**55
			Time [min]:21 min, **B[%]**95
			Stoptime: select **As Injector/No Limit**
			Posttime: select **Off**
		Flow rate	0.600 mL/min
		Posttime	**Off**
^{1}D Column Compartment	**Instrument >Setup Method**`1D Column Compartment`	**Temperature**	40 °C
^{1}D Detector	**Instrument >Setup Method**`1D Detector`	**Signal A**	**Wavelength**: 254 nm **Bandwidth**: 4 nm **Reference Wavelength**: 360 nm **Reference Bandwidth**: 100 nm
		Signal B	**Wavelength**: 260 nm **Bandwidth**: 4 nm **Reference Wavelength**: 360 nm **Reference Bandwidth**: 100 nm
		Peakwidth:	5 Hz
^{1}D Sampler	**Instrument >Setup Method**`1D Sampler`	**Injection volume**:	1.0 µL , 2.0 µL , 5.0 µL , and 3.0 µL

2 Apply method parameters for ^{2}D.

Module	Tool	Parameter	Value
Column			RRHD Bonus-RP, 2.1x 50 mm, 1.8 µm, 1200 bar (857768-901)
^{2}D Pump	Setup ^{2}D-Pump	Solvent **A**	H_2O + 0.2 % formic acid
		Solvent **B**	Acetonitrile
		^{2}D gradient	**Time [min]:**0.0 min, **B[%]**10
			Time [min]:1.25 min, **B[%]**60
		^{2}D Gradient stoptime:	1.25 min
		^{2}D cycletime	1.75 min
		^{2}D Flow	1.0 mL/min
	Setup ^{2}D-Pump **PreviewEdit mode**	Gradient shift:	0->20 min from 10->30 % B (only downslope)
	Setup ^{2}D-Pump **Advanced ^{2}D pump settings...**	Stoptime	23 min
		Posttime	3 min
^{1}D Column Compartment		**Temperature**	40 °C
^{2}D Detector	**Instrument >Setup Method**2D Detector	**Signal A**	**Wavelength**: 254 nm **Bandwidth**: 4 nm **Reference Wavelength**: 360 nm **Reference Bandwidth**: 100 nm
		Signal B	**Wavelength**: 260 nm **Bandwidth**: 4 nm **Reference Wavelength**: 360 nm **Reference Bandwidth**: 100 nm
		Peakwidth:	≥40 Hz

3 In **2D-LC Mode** select **HiRes sampling**.

4 Run a survey run with the 2D-LC starter sample, 1 x 2 mL (5190-6895), 1:10 diluted with Methanol/Water (20/80; v/v) with 0.1 % formic acid of the first dimension to find out the exact retention time of the peak that elutes at around 11.7 min.

5 Run HiRes sampling with the sampling time adjusted to the ^{1}D retention time of the peak of interest in your system.

Settings for High-Resolution sampling:

- Sampling time: 3 s
- Number of cuts: 10
- Injection volume: 1, 2, 5, and 3 µL,

Comprehensive 2D-LC

This section describes in detail the installation, configuration, method parameters, data analysis and checkout/familiarization of comprehensive two dimensional liquid chromatography with the Agilent 1290 Infinity II 2D-LC Solution ChemStation.

Configuration

Overview Configuration Dialog

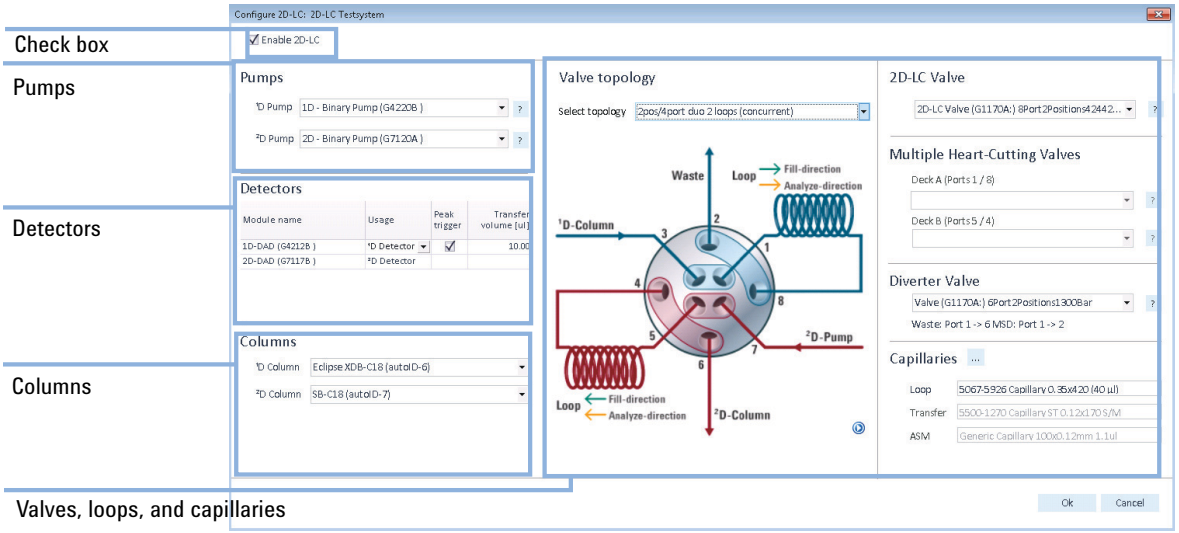


Figure 87 Overview 2D-LC configuration grafical user interface

The configuration of the 2D-LC-system is done via the configuration dialog in the software. The order of configuration is mandatory. The following configuration parameters are available:

- **Pumps**

  Section to define which pump is in the first and which one in the second dimension.

- **Detectors**

  Section to define which detector is in the second dimension and which detector should be used for peak detection (optional).

- **Columns**

  Section to define the columns being used in 1st and 2nd dimension.

- **Valve topology**, **2D-LC Valve**, **Multiple Heart-Cutting Valves**, **Diverter Valve**, **Capillaries**

  Section to identify the modulation valve(s) used for toggling the loop(s) and section to define the volume of the sampling loop(s).

Configure Valve and Loop

To run 2D-LC, it must be defined, which valve is used for 1st and 2nd dimension.

- 1st dimension:

  The **2D-LC Valve** drop-down list contains all configured valves which can be used for 2D-LC functionality.

- 2nd dimension(only relevant for multiple heart-cutting 2D-LC):

  If more than one valve matches the current valve/loop configuration, the user can select from a drop-down list, which valve is used to connect 1st and 2nd dimension.

- **Diverter Valve**

  If more than one valve matches the **Diverter Valve** configuration, a list-box is shown where the user can select the diverter valve.

The **Identify** button triggers the blinking of the status LED of the corresponding column compartment or valve drive. The button is only enabled in the Online version of the ChemStation.

All possible loop configurations depending on the selected valves are listed separately and illustrated on screen.

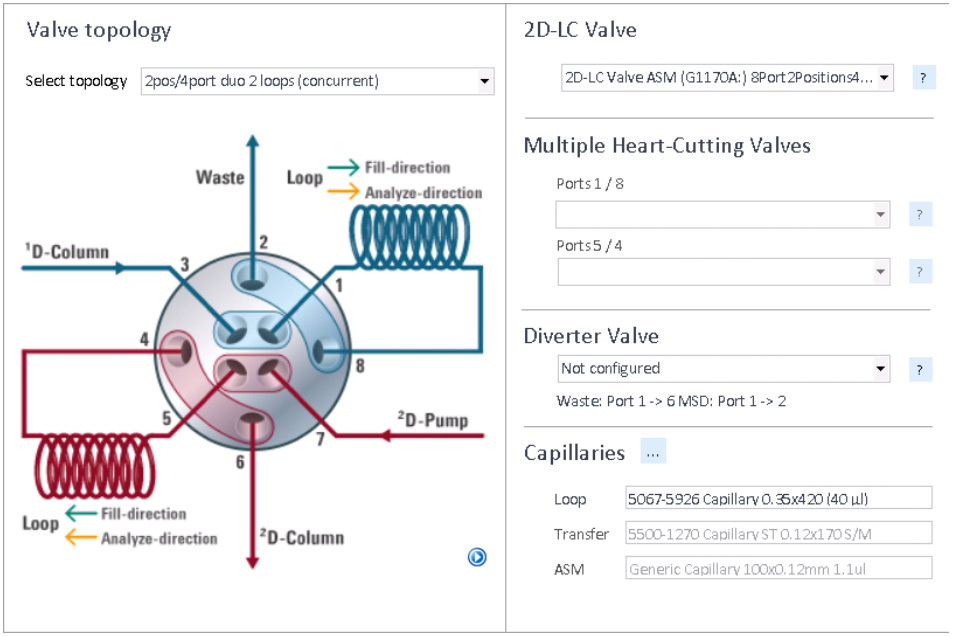


Figure 88 2D-LC valve and loop configuration (concurrent)

Software required 1290 Infinity 2D-LC Acquisition Software

Preparations Check box **Enable 2D-LC** selected

NOTE Valves may be part of a column compartment (G7116A/B, G1316C) or a valve drive (G1170A).

1 Select **2D-LC Valve**.
2 **Select topology** under **Valve topology**.
3 To save settings click **OK**.

Valves and loops are configured for 2D-LC.

Legacy Checkout/Familiarization Procedure

NOTE

For ESZ checkout procedure, please see the 2D-LC Installation Checklist or the *Agilent InfinityLab LC Series 1290 Infinity II 2D-LC Solution OpenLab CDS and MassHunter Acquisition for TOF and Q-TOF User Guide*.

Checkout run - comprehensive (standard setup)

The familiarization procedure illustrates the system´s 2D-LC capabilities and supports the user to start the method for a specific analytical task. The familarization procedure will guide the user through the most important setups and analysis function, described in the chapters before.

The sample provided with the familiarization procedure can be determined with a UV-detector and a mass spectrometer. The methods to analyze the starter sample are delivered together with the full package to ensure a smooth familarization and checkout procedure. With the given method, peaks will overlap in the first dimension and will be separated in the second dimension.

The Agilent 1290 Infinity II 2D-LC Solution ChemStation is delivered together with all required parts for a complete familiarization procedure for (multiple) heart-cutting and comprehensive 2D-LC.

Parts required

p/n	Description
5190-6895	2D-LC starter sample, 1 x 2 mL
858700-902	RRHD SB-C18, 2.1x100 mm, 1.8 µm, 1200 bar, ^{1}D
959757-302	RRHD Eclipse Plus C18, 3.0x50 mm, 1.8 µm
G2453-85060	Formic Acid-Reagent Grade 5 mL (5 cc)

Software required CD

Preparations

Solvents needed:

- 1D: mobile phase A = water with 0.2 % Formic Acid-Reagent Grade 5 mL (5 cc) (G2453-85060), B = methanol
- 2D: mobile phase A = water with 0.2 % Formic Acid-Reagent Grade 5 mL (5 cc) (G2453-85060), B = acetonitrile

Preparations:

1. Prepare dilution solvent (20 % MeOH in mobile phase A): Add 500 µL MeOH to 2000 µL Mobile Phase A.
2. Prepare 400 µL sample: Add 40 µL 2D LC starter sample to 360 µL dilution solvent.
3. Load method Checkout_MHC_FullComp_1290BinX1290Bin.MCheckout_MHC_FullComp_1290QuatX1290Bin.M, or Checkout_MHC_FullComp_1260BinX1290Bin.M(depends on your pump setup) from the CD.

1 Run the full comprehensive checkout run using single storage loops. The method for the checkout run is available on the 2D-LC Addon SW DVD under checkout methods.

- Detection:

  UV Detection at 254 nm, BW 4 nm; reference at 360 nm, BW 100 nm

- Acquisition rate:

  20 Hz

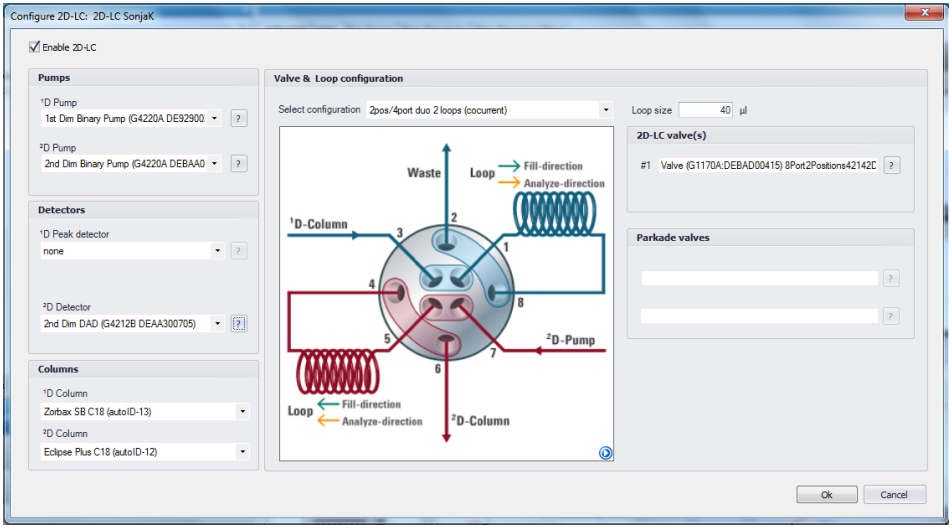
- Sample:

  2D-LC starter sample, 1 x 2 mL (5190-6895), 1:10 diluted with Methanol/Water (20/80; v/v) with 0.1 % formic acid.

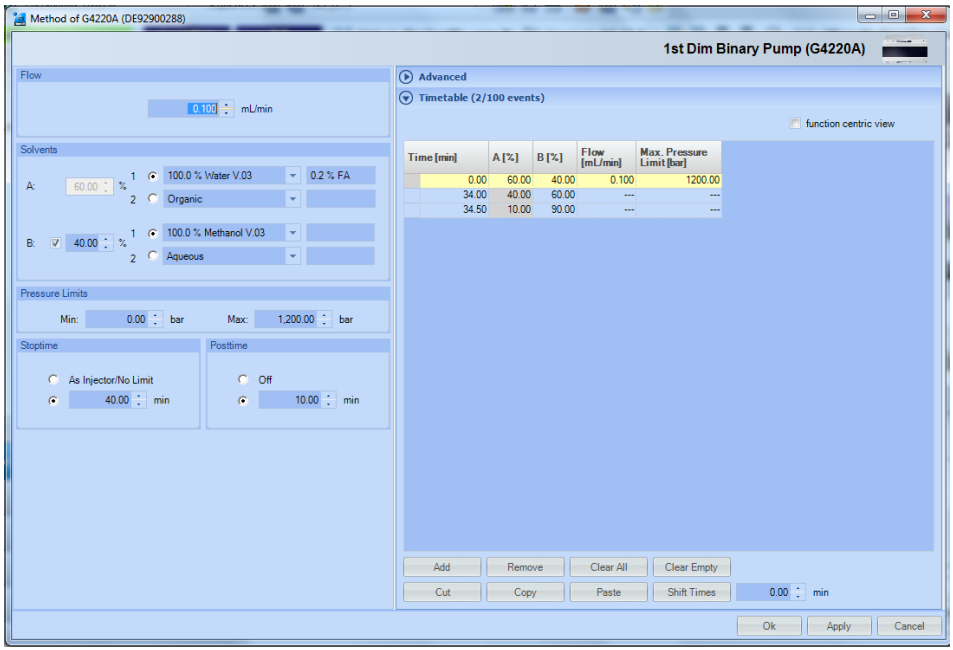
- Injection volume:

  2 µL

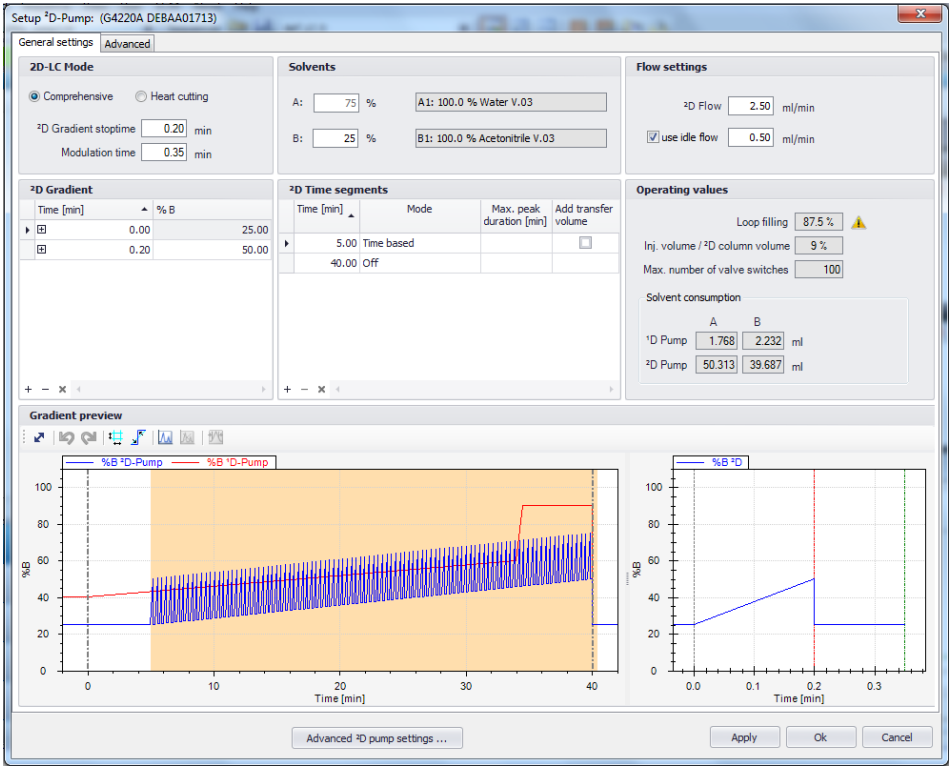
2 Verify that the Valve & Loop configuration is set up as shown below:



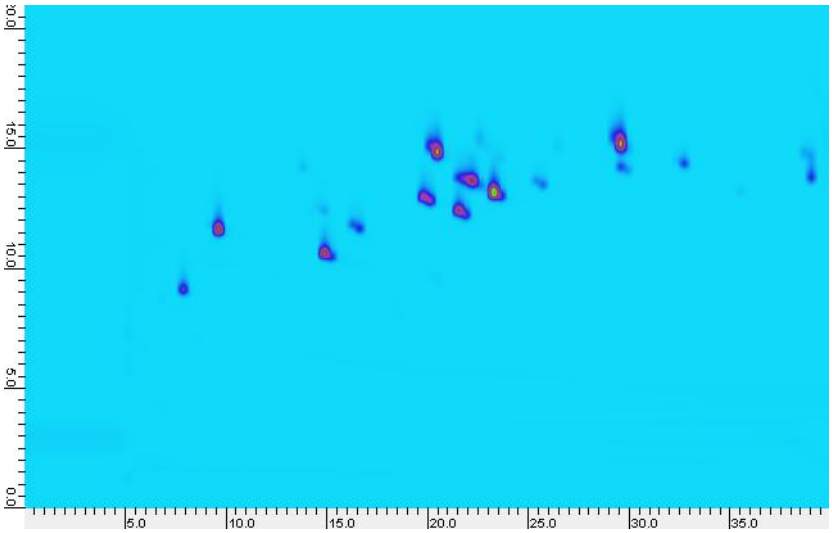
3 Verify the [1]D pump method is set up as shown below:



4 In **Instrument >Setup 2D-LC**, verify that the 2D-LC mode is set to **Comprehensive** and that the 2D pump and modulation method are set up as shown below:



5 Run the full comprehensive checkout run using single storage loops and review the obtained data with the GC Image software. The resulting separation should look similar to the one shown below:



Checkout run - comprehensive (multiple heart-cutting setup)

The familiarization procedure illustrates the system´s 2D-LC capabilities and supports the user to start the method for a specific analytical task. The familarization procedure will guide the user through the most important setups and analysis function, described in the chapters before.

The sample provided with the familiarization procedure can be determined with a UV-detector and a mass spectrometer. The methods to analyze the starter sample are delivered together with the full package to ensure a smooth familarization and checkout procedure. With the given method, peaks will overlap in the first dimension and will be separated in the second dimension.

The Agilent 1290 Infinity II 2D-LC Solution ChemStation is delivered together with all required parts for a complete familiarization procedure for (multiple) heart-cutting and comprehensive 2D-LC.

Parts required

p/n	Description
5190-6895	2D-LC starter sample, 1 x 2 mL
858700-902	RRHD SB-C18, 2.1x100 mm, 1.8 µm, 1200 bar ^{1}D
959757-302	RRHD Eclipse Plus C18, 3.0x50 mm, 1.8 µm
G2453-85060	Formic Acid-Reagent Grade 5 mL (5 cc)

Software required CD

Preparations

Solvents needed:

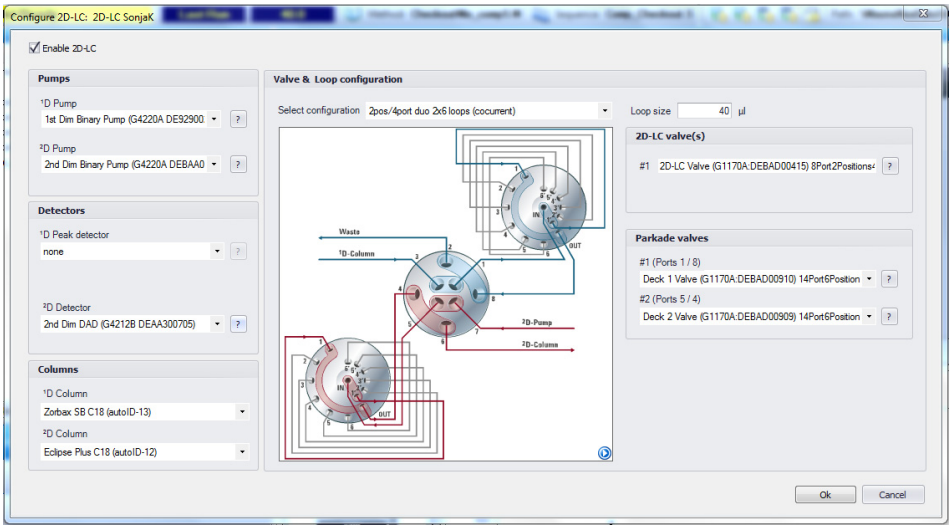
- 1D: mobile phase A = water with 0.2 % Formic Acid-Reagent Grade 5 mL (5 cc) (G2453-85060), B = methanol
- 2D: mobile phase A = water with 0.2 % Formic Acid-Reagent Grade 5 mL (5 cc) (G2453-85060), B = acetonitrile

Preparations:

1. Prepare dilution solvent (20 % MeOH in mobile phase A): Add 500 µL MeOH to 2000 µL Mobile Phase A.
2. Prepare 400 µL sample: Add 40 µL 2D LC starter sample to 360 µL dilution solvent.
3. Load method Checkout_MHC_FullComp_1290BinX1290Bin.MCheckout_MHC_FullComp_1290QuatX1290Bin.M, or Checkout_MHC_FullComp_1260BinX1290Bin.M(depends on your pump setup) from the CD.

1 Repeat the full comprehensive checkout run using MHC valves instead of single storage loops. For this purpose, disconnect the transfer capillaries from the 2D-LC valve to the storage loops and install MHC valves between ports 4 and 5, respectively ports 1 and 8, of the 2D-LC valve (cocurrent configuration).

2 In **Instrument >Configure 2D-LC**, change the Valve & Loop configuration to 40 µL storage loops as shown below:



- Detection:

  UV Detection at 254 nm, BW 4 nm; reference at 360 nm, BW 100 nm

- Acquisition rate:

  20 Hz

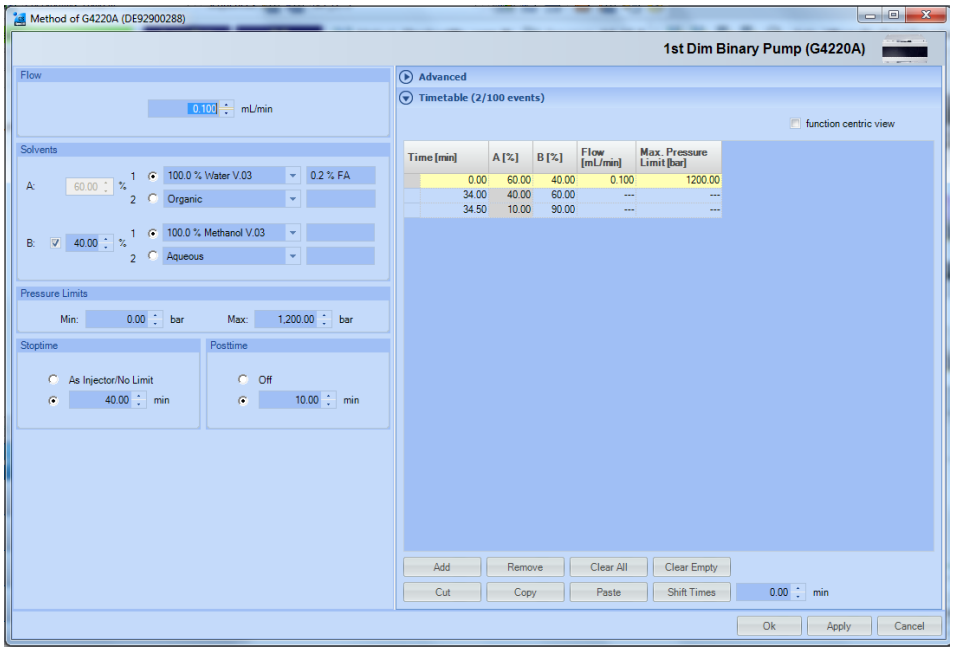
- Sample:

  2D-LC starter sample, 1 x 2 mL (5190-6895), 1:10 diluted with Methanol/Water (20/80; v/v) with 0.1 % formic acid.

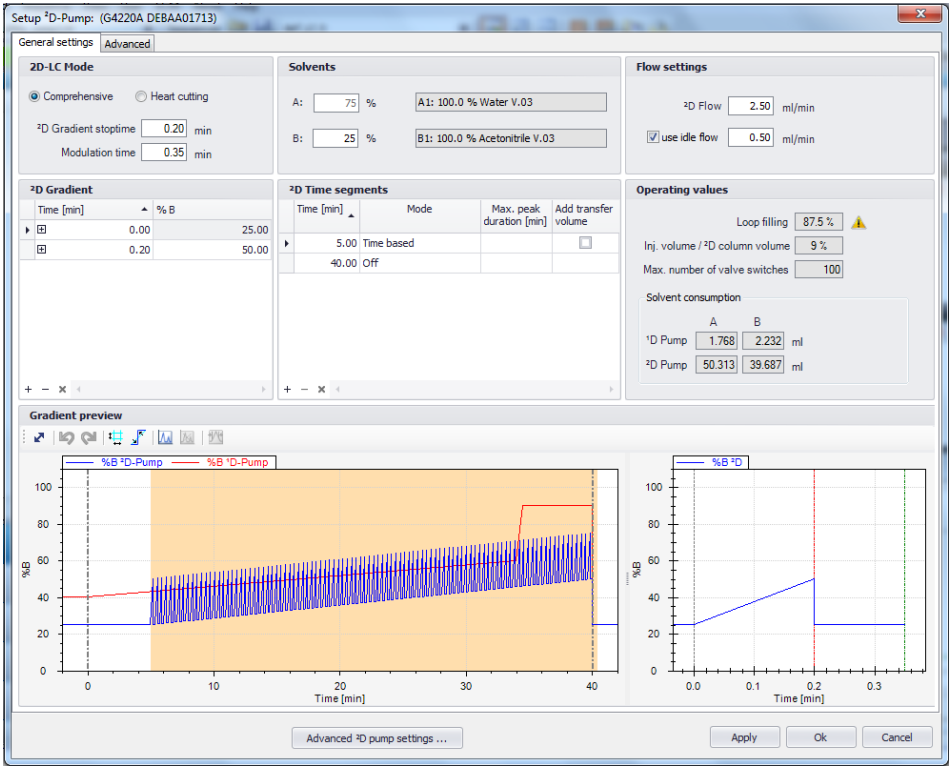
- Injection volume:

  2 µL

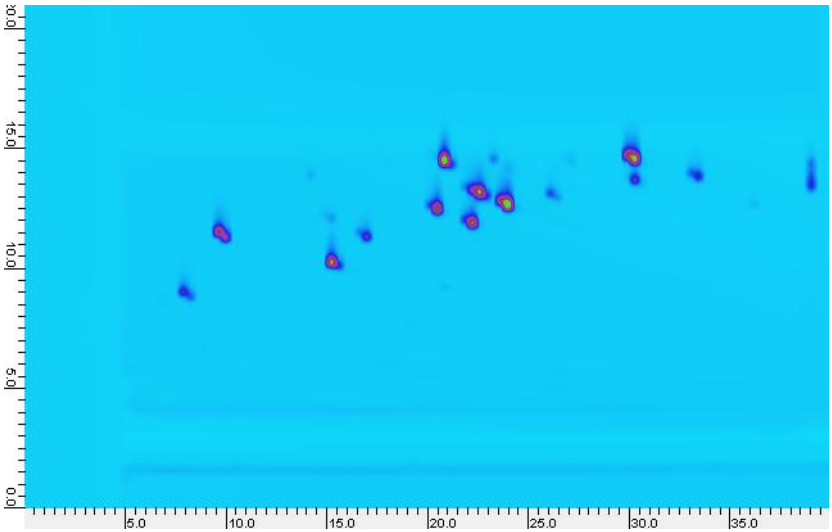
3 Verify the [1]D pump method is set up as shown below:



4 In **Instrument >Setup 2D-LC**, verify that the 2D-LC mode is set to **Comprehensive** and that the 2D pump and modulation method are set up as shown below:



5 Run the full comprehensive checkout run using single storage loops. The resulting separation should look similar to the one shown below:



6 Compare the result to that obtained using the MHC valves.

Active Solvent Modulation (ASM)

Configuration

Adjusting the split ratio

Different ASM capillaries are available for adjusting the split ratio and therefore the dilution.

The method can therefore be optimized either for optimum resolution (strong dilution) or lowest cycle time (weak dilution).

Configure the ASM Valve

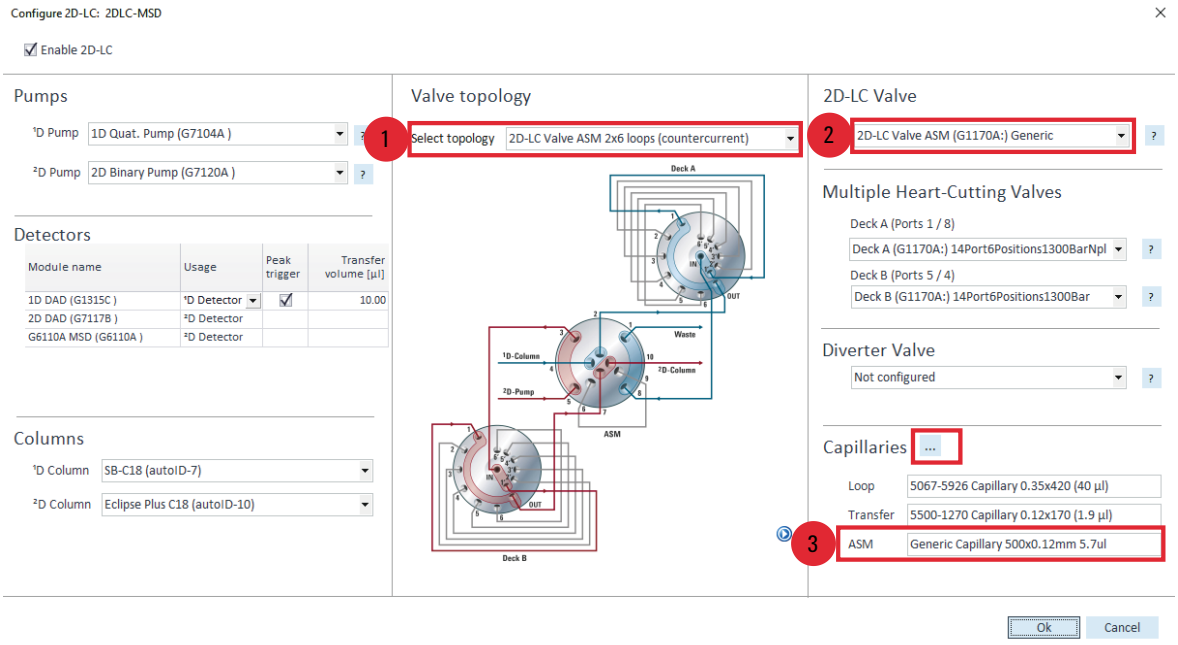


Figure 89 ASM Valve configuration (overview)

1	Select a topology for using the ASM Valve.
2	Choose the ASM Valve as 2D-LC Valve. This is usually done automatically based on installed valves.
3	Choose an ASM capillary. This defines the split ratio.

Preparations All modules including the ASM Valve are configured in OpenLAB CDS ChemStation Edition.

1 Select a valve topology using an ASM Valve.

HINT For minimum carry-over, please use counter-current installation for the ASM Valve.

2 Select the ASM Valve as 2D-LC Valve (which is usually pre-selected).

3 Define the ASM capillary.

a To configure capillaries, click on **Capillaries...** (see Figure 89 on page 221).

b Select any of the pre-defined ASM capillaries.

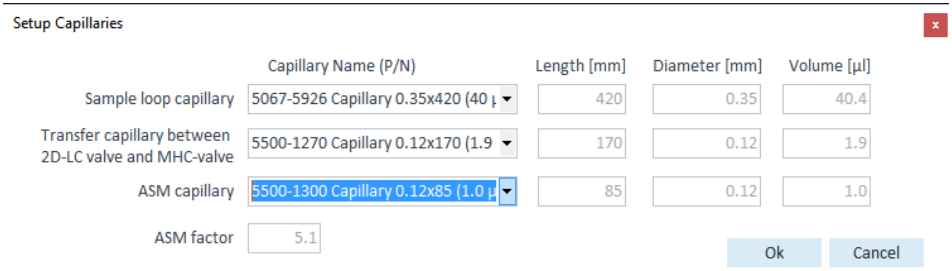


Figure 90 Configuration of the ASM valve with predefined capillaries

OR

If you are using a different capillary, you can choose **Generic Capillary**

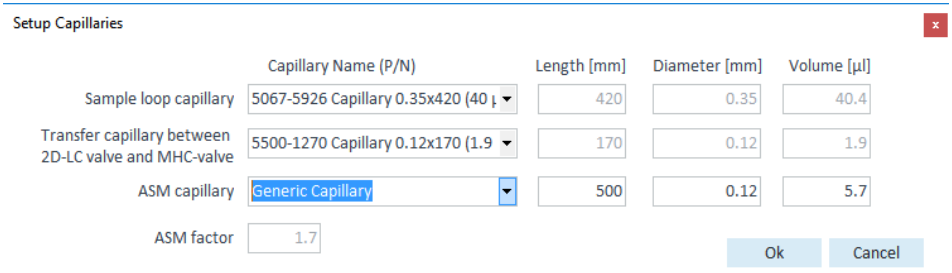


Figure 91 ASM valve configuration (overview)

In this case, you need to enter two of following three parameters: length, diameter or volume. These parameters are required for calculating the flush volume and back pressure, see "Understanding the ASM factor" on page 41

The ASM factor is calculated and displayed based on selected capillaries.

4 Install capillary connections as displayed in figure **Valve topology** in the UI, see Figure 89 on page 221.

NOTE

Please note that ASM capillaries are labeled with ASM (in contrast to transfer and other capillaries).

7 Investigate the effects of using different gradients in the ^{2}Dimension

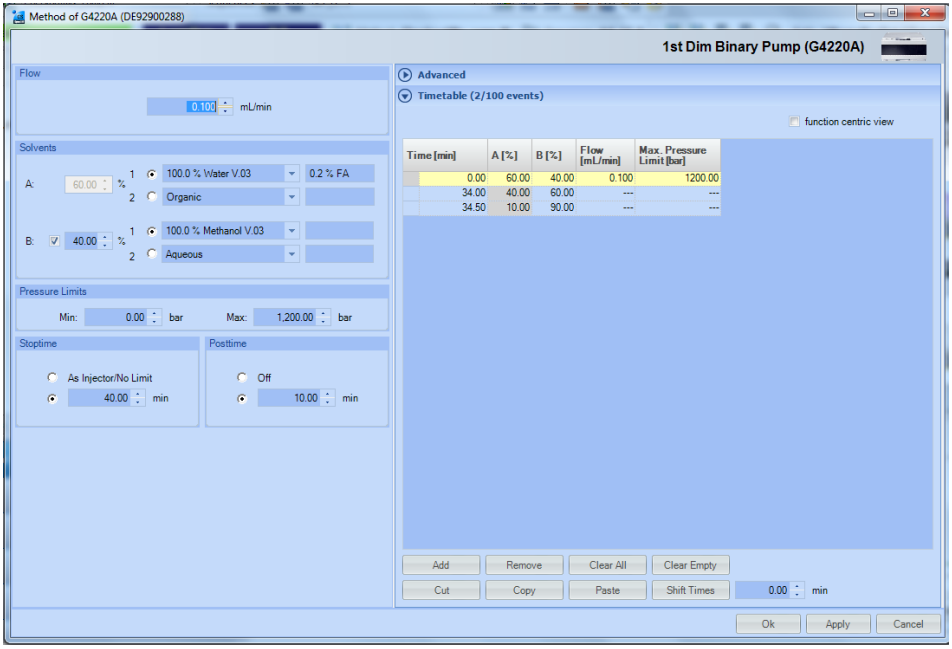
This chapter describes, how shifted gradients in the second dimension can be used to enlarge the accessible two-dimensional separation space.

Investigate the effects of using different gradients in the ^{2}Dimension

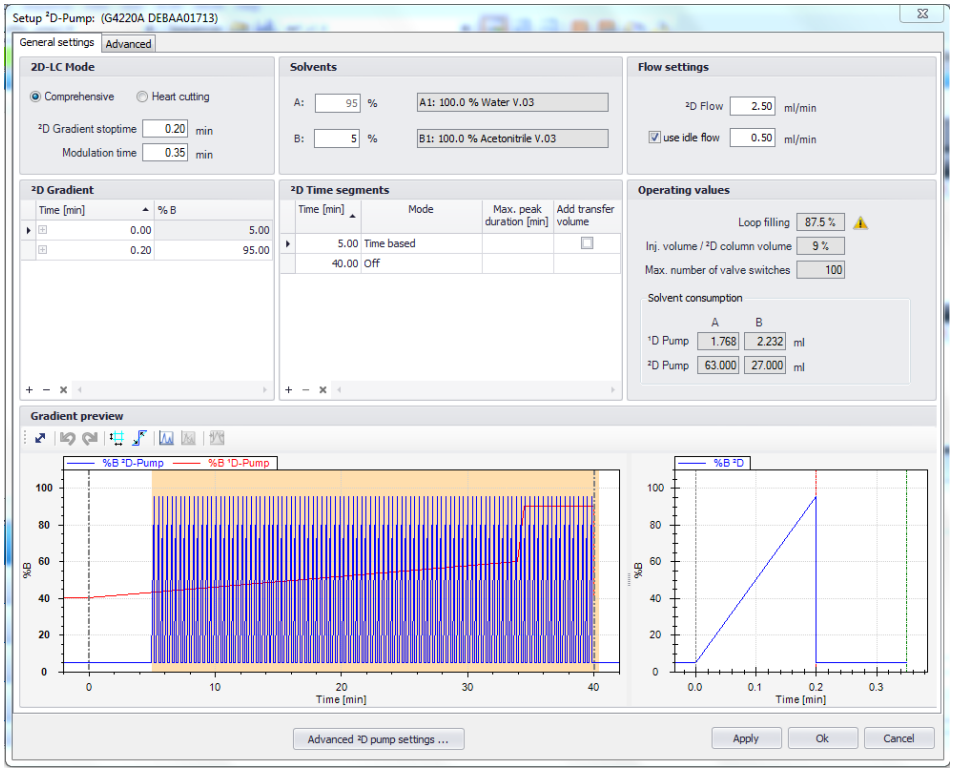
When combining separation systems with related separation mechanisms in the first and second dimension (as in RPxRP), orthogonality is limited. As a result, only a part of the available two-dimensional separation space will be occupied. In such a case, shifted gradients in the second dimension can be used to enlarge the accessible two-dimensional separation space.

1 To investigate the effects of using different gradients in the second dimension, firstly run a comprehensive 2D-LC separation with the same second dimension gradient from 5-95 % B repeated during the whole run.

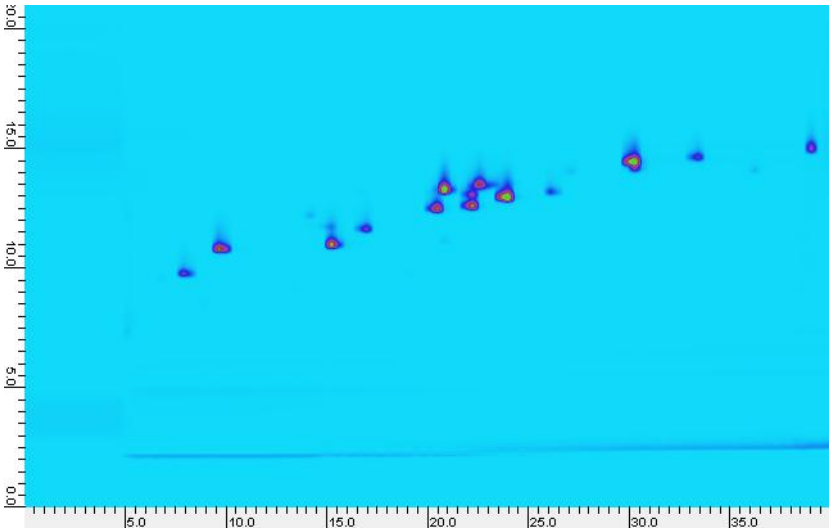
The 1D pump method should be set up as during the checkout runs (see below):



2 In **Instrument >Setup 2D-LC**, set up a 2D pump and modulation method with repeating gradients from 5 – 95 % B as shown below:



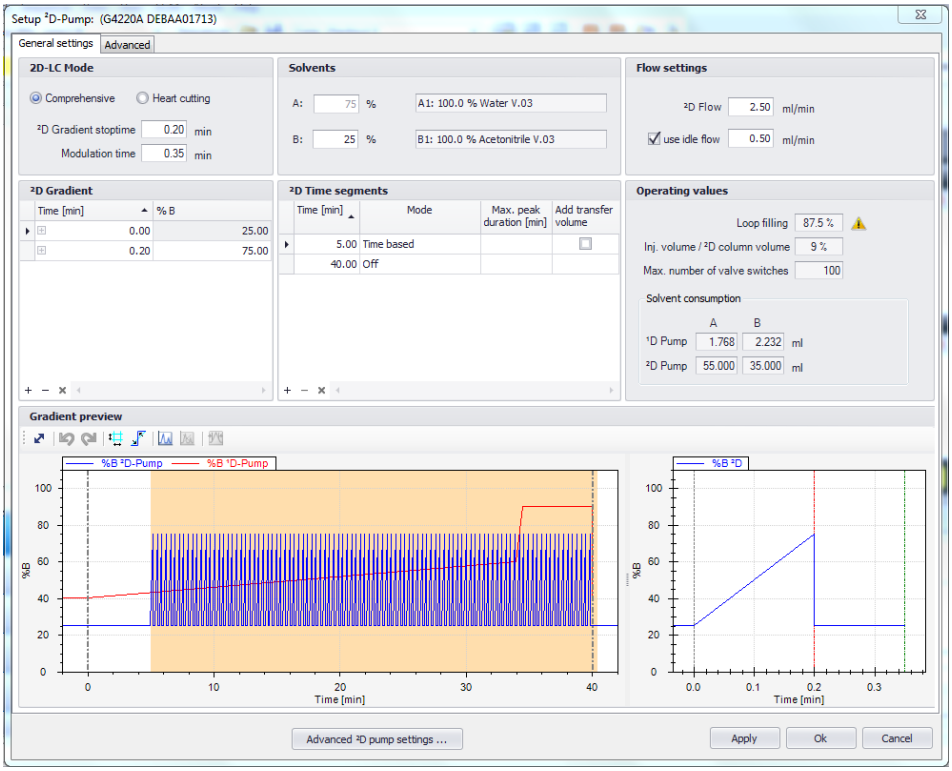
3 Run the comprehensive 2D-LC analysis. The resulting separation should look similar to the one shown below:



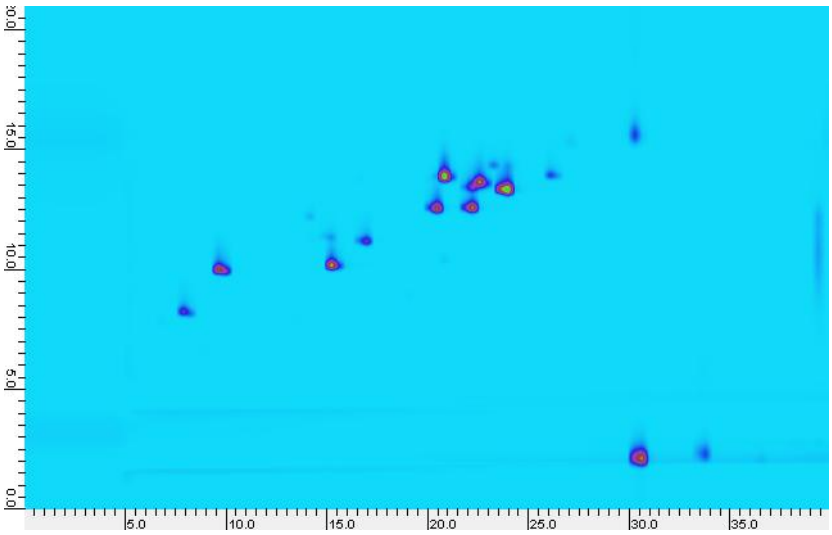
NOTE

Notice how the peaks are distributed around a diagonal line, indicating related separation mechanisms in the first and second dimension.

4 To improve the separation in the second dimension, a shallower second dimension gradient (e.g. from 25 – 75 % B) could be used. The setup of this 2D method is shown below (this is just shown for explanation purpose; you do not need to run this method!):



The separation resulting from using repeating gradients from 25 – 75 % B in the second dimension is shown below:



NOTE

Notice how the peaks are slightly further separated in the second dimension compared to using repeating gradients from 5 – 95 % B. Also notice that the last peaks eluting from the first dimension column are not eluted in one modulation cycle from the second dimension column (wrap-around; see marked area).

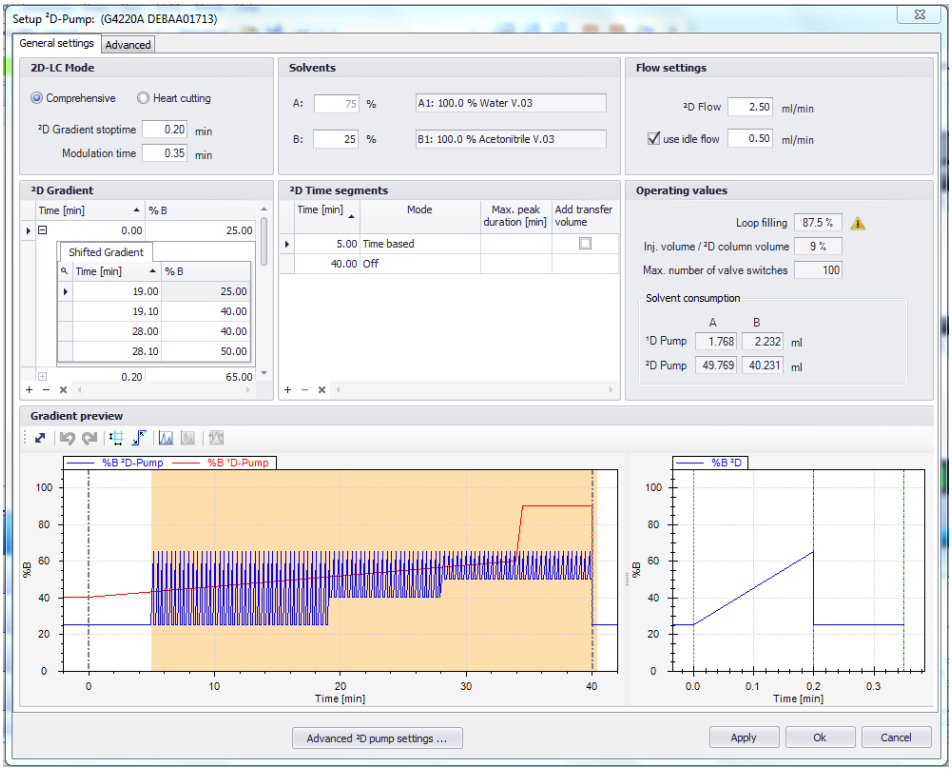
To be able to use even shallower gradients in the second dimension to further improve the separation and to also avoid the occurrence of wrap-around, continuously shifted gradients can be used in the second dimension (as was done during the checkout runs).

5 Compare the separations resulting from using the same second dimension gradient (from 5 – 95 % B and also from 25 – 75 % B) repeating during the whole run to the separation obtained using continuously shifted second dimension gradients in the checkout run.

NOTE

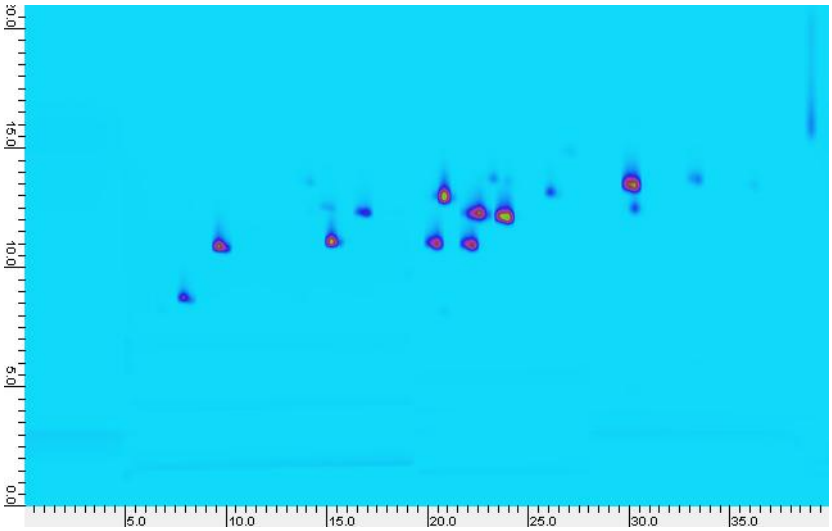
Notice how the peaks are spread more widely across the two-dimensional separation space (the accessible two-dimensional separation space is enlarged) when shifted gradients are used. Also, notice the effect that using continuously shifted second dimension gradients has on the second dimension retention times of consecutive fractions of the same first dimension peak.

6 Apart from using continuously shifted gradients in the second dimension, as was done during the checkout runs, it is also possible to stepwise shift the second dimension gradients. For this purpose, keep the valve & loop configuration as well as the 1D pump method the same. In **Instrument >Setup 2D-LC**, set up a 2D pump and modulation method with stepwise shifted gradients as shown below:



7 Run the comprehensive 2D-LC analysis with stepwise shifted gradients in the second dimension.

The resulting separation should look similar to the one shown below:



NOTE

Notice how consecutive fractions of the same first dimension peak have exactly the same retention time in the second dimension, as they experienced exactly the same second dimension gradient (in contrast to using continuously shifted gradients in the second dimension, which leads to consecutive fractions of one first dimension peak experiencing slightly different second dimension gradients). But be careful! This is only true if the stepwise shifting of the second dimension gradients is performed at times, when no peaks are eluting from the first dimension column.

In case your resulting separation looks different from the one shown above: Your peaks might show a different first dimension retention time due to the use of another first dimension pump (in the separation shown above, a 1290Bin Pump was used in the first dimension). Check whether the stepwise shifting of the second dimension gradients was performed at times when peaks eluted from the first dimension column in your separation and understand the effect this can have on the second dimension retention times of consecutive fractions of the same first dimension peak!

8 Data Analysis

This chapter descibes the analyzation of data in 2D-LC and is separated in a section heart-cutting 2D-LC and a section comprehensive 2D-LC.

Data Analysis for Heartcutting 2D-LC (LC-LC)

For data-analysis of heart-cutting 2D-LC data OpenLAB CDS ChemStation edition is usually fully sufficient.

Again, the data will be stored in one data-file. If more than one peak was analyzed in the second dimension they will simply follow one after the other in a distance of the second dimension run-time.

If a detector right after the first dimension column was used, e.g. as peak detector for peak triggered operation, these data will be available as a second data-trace.

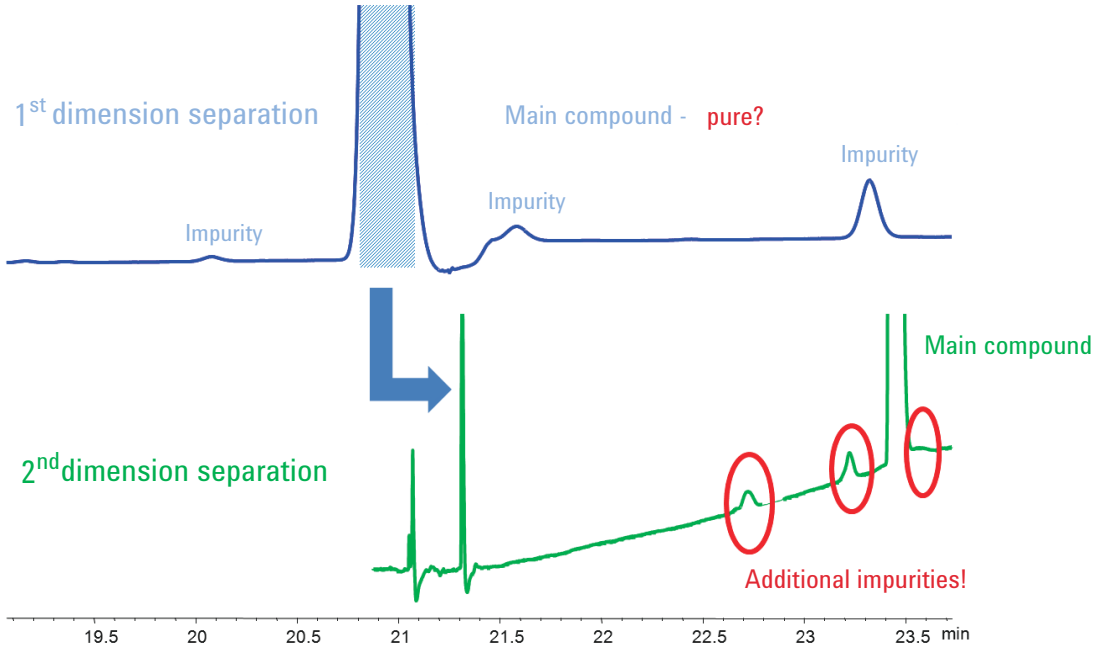


Figure 92 Example of a heartcutting 2D-LC experiment. Data analysis and display done by OpenLAB CDS ChemStation Edition, the data of a detector placed after the first dimension column (blue) and of the 2D data detector (green). The additional impurities (marked red) could not be detected by 1D-LC only.

2D-LC Viewer

Using the Multiple Heart-Cutting Upgrade kit, the Agilent 1290 Infinity II 2D-LC Solution ChemStation offers the possibility to store multiple peaks in several sample loops. These stored samples are then injected to the second dimension one by one.

Thus long ^{2}D gradients are possible without loss of ^{1}D peaks. But it is quite difficult to review the ^{2}D results using the standard ChemStation Data Analysis. Especially as parked peaks are analyzed in a different order as they have been parked in (this is necessary to avoid carry-over).

The **2D-LC Viewer** offers the opportunity to view and analyze second dimension chromatograms comfortably. The viewer can also be used for the analysis of standard heart-cutting 2D-LC data.

Overview **2D-LC Viewer**

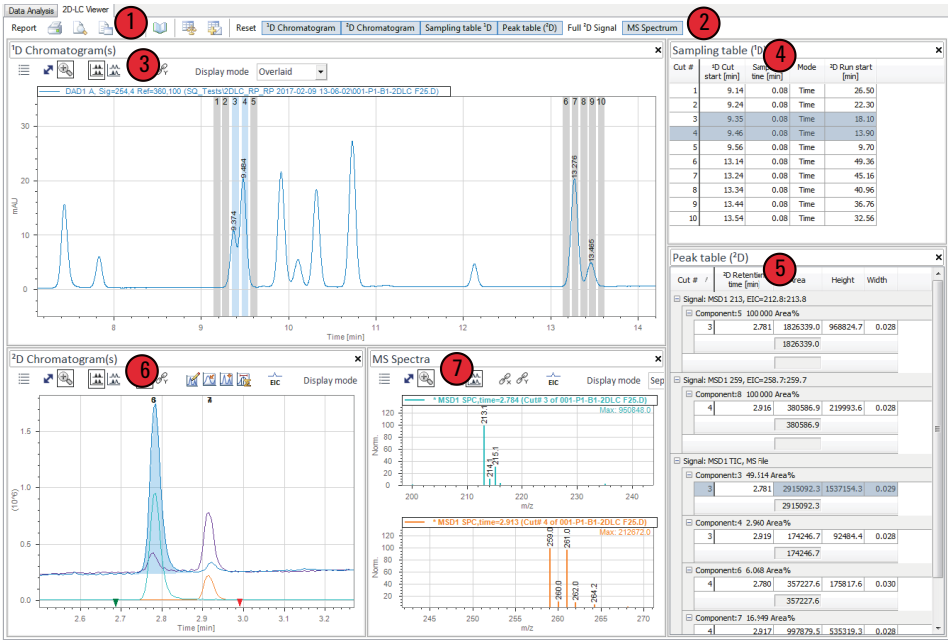


Figure 93 Overview of the 2D-LC Viewer graphical user interface

1	Tab **2D-LC Viewer**
2	Separate displays tabs
3	**^{1}D Chromatogram(s)**
4	**Sampling table (^{1}D)**
5	**Peak table (^{2}D)**
6	**^{2}D Chromatogram(s)**
7	**MS Spectra**

The **2D-LC Viewer** provides the following functions:

- Tab pages enable the user to switch between
  - **2D-LC Viewer**, and
  - **Data Analysis**
- All panes are connected. Highlighting a cut or a chromatogram in one of the fields, will automatically highlight it in the other fields.
- **Sampling table (^{1}D)**
- **Peak table (^{2}D)**
- Toolbar with the elements:
  - Print to printer

    Prints the report according to the options set in the **Report Options** dialog using the standard print dialog.
  - Print preview

    Shows the rendered report in a preview window. It is possible to print the report directly from the preview window.
  - Report options

    Shows up the Report Options Dialog
  - Auto scale

    Resets all chromatogram windows to their default scaling
  - **^{2}D chromatogram** (checkbox)

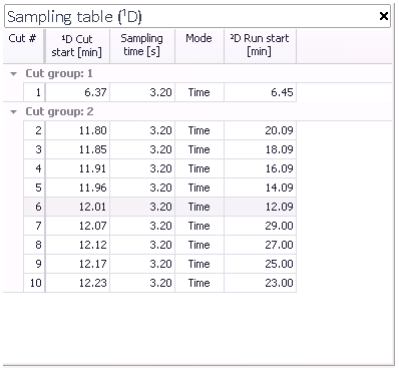
    Hide / unhide the full **^{2}D Signal** window
  - **^{1}D Signal** list box

    Used to select a signal from the ^{1}D detector
  - **^{2}D Signal** list box

    Used to select a signal from the ^{2}D detector
- **^{1}D Chromatogram**
- **^{2}D Chromatogram** (hidden, if **^{2}D chromatogram** checkbox unchecked)
- **^{2}D Chromatogram(s)**
- **MS Spectrum**

Sampling table (^{1}D)

The table lists all heart-cuts which have been analyzed in the 2nd dimension.


Sampling table (1D)

Cut #	1D Cut start [min]	Sampling time [s]	Mode	2D Run start [min]
Cut group: 1				
1	6.37	3.20	Time	6.45
Cut group: 2				
2	11.80	3.20	Time	20.09
3	11.85	3.20	Time	18.09
4	11.91	3.20	Time	16.09
5	11.96	3.20	Time	14.09
6	12.01	3.20	Time	12.09
7	12.07	3.20	Time	29.00
8	12.12	3.20	Time	27.00
9	12.17	3.20	Time	25.00
10	12.23	3.20	Time	23.00

Figure 94 Sampling table (^{1}D) (example)

The different columns can be selected or deselected by right mouseclicking the headline of the table.

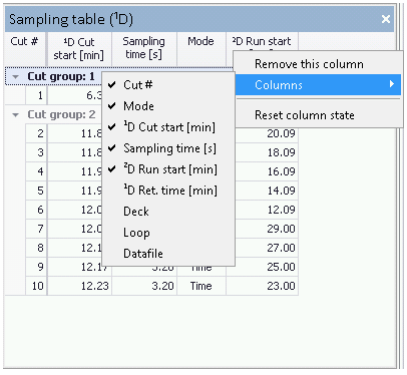


Table 23 Legend for Sampling Table

PosNr	Description
Cut #	The current number of the heart-cut
^{1}D Cut start [min]	Time when the heart-cut starts (peak begin or time value in trigger table)
^{1}D Sampling time [min]	The duration (in minutes) of the heart-cut in the 1st dimension. The duration is determined either by the loop fill time, the end-of-peak detection or the max peak duration
Trigger	Indicates whether heart-cut was taken based on a peak-trigger (Peak) or based on a time given in the trigger table
^{2}D run start [min]	Time when the analyses of this heart-cut in the 2nd dimension starts (gradient start)
^{1}D Ret. Time [min]	The retention time (as given by the integrator) of the highest peak in the 1st dim. signal within heart-cut time range. The table cell is empty if no peak found or the signal isn't integrated. (Column not visible by default)
Deck	Number of the deck (1 or 2) where the cut (peak) has been parked / analyzed (Column not visible by default)
Loop	Number of the loop (1... 6) where the cut (peak) has been parked / analyzed (Column not visible by default)

^{1}D Chromatogram

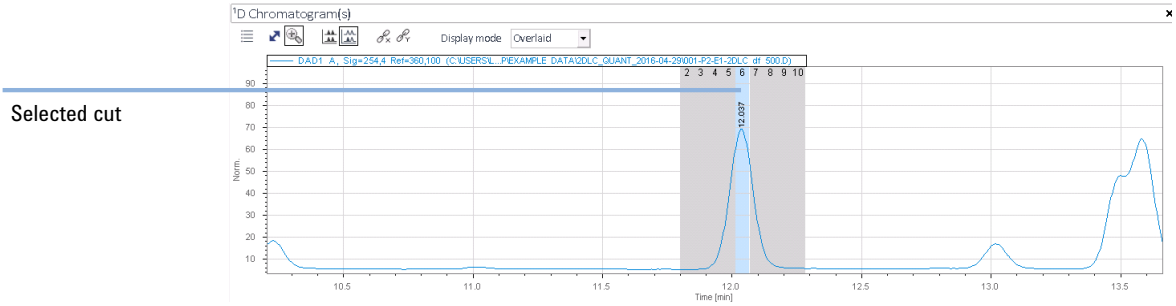


Figure 95 ^{1}D Chromatogram (example)

Heart-cuts can be selected using left mouse button, multiple selection using **Ctrl-key** + left mouse button is also supported.

The selected signal (see toolbar) from the ^{1}D detector is shown.

- Heart cuts are indicated by a grey rectangle area
- Selected heart-cut(s) is (are) marked in a blue rectangle
- Heart-cuts are annotated using the retention time if available.

  Otherwise the heart-cuts are annotated using their current number.
- Peaks (cuts) that couldn't be taken during acquisition will be marked by a warning icon on the x-axis at the time the heart-cut should have been taken.
- A tooltip provides more information about time and reason why the peak couldn't be cut.

^{2}D Chromatogram

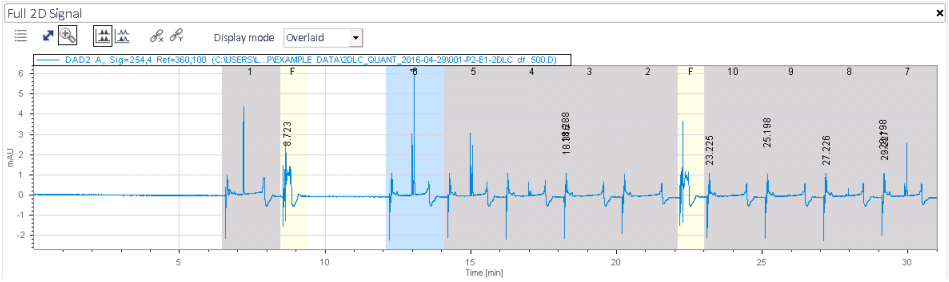


Figure 96 Full ^{2}D Signal (example - only visible, if Full ^{2}D Signal (checkbox) is checked)

This window shows the selected signal of the ^{2}D detector containing the individual analyses of the heart-cuts.

- The selected heart-cut(s) is marked as a blue area.
- The area of a heart-cut is marked with a gray rectangle when hovering with the mouse over the chromatogram window.
- A heart-cut can be selected by clicking in such a rectangle.
- Multiple selections are supported using **Ctrl**-click.
- All heart-cuts are annotated using the heart-cut number (see also **Sampling table**).
- **F** indicates a bypass (or flush) gradient, which was used to flush the transfer capillaries after switching the 2D-LC valve.

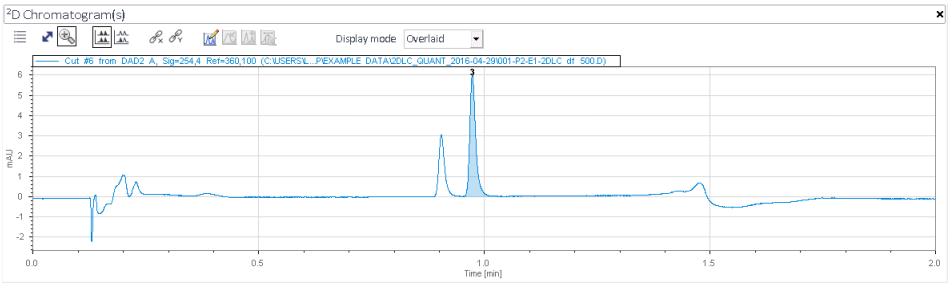


Figure 97 ^{2}D Chromatogram (example from ^{2}D Chromatogram above)

The ^{2}D chromatogram of the selected heart-cut is shown as an individual run (x-axis starting at time 0). Chromatograms are overlaid if multiple heart-cuts are selected

For further details, refer to the online help.

High-resolution sampling - Results

Figure 98 Results peak group 1

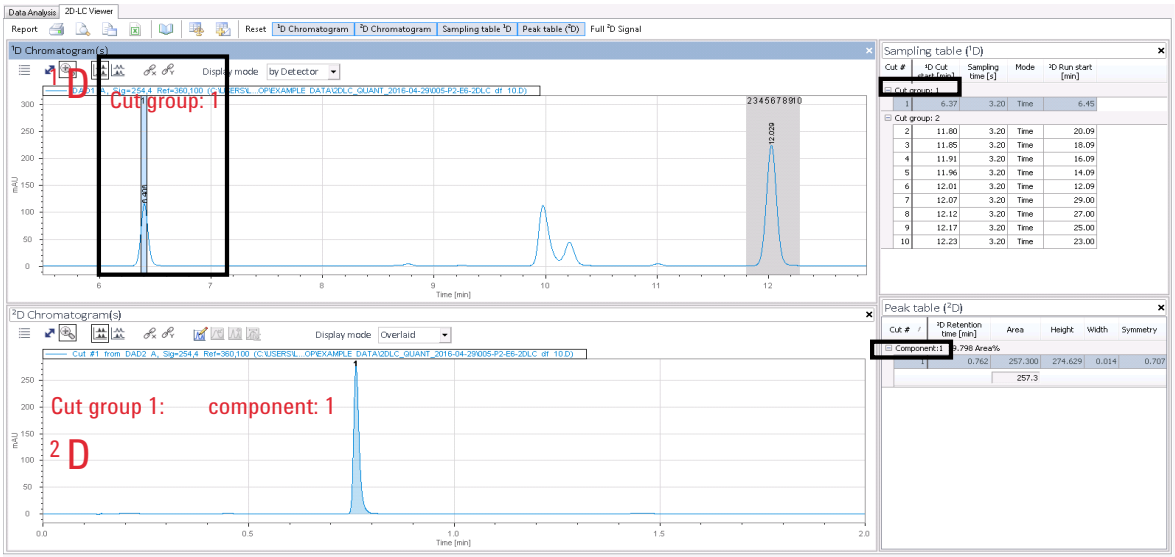


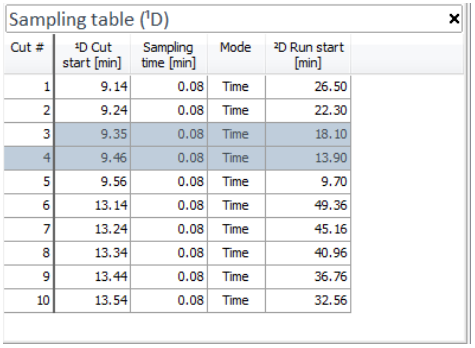
Figure 99 Results peak group 2

The **^{1}D chromatogram** shows 2 groups of peaks, which have been defined in the setup of a High-Resolution measurement. This information of compounds in ^{1}D belonging together will later be used for data analysis. The heart-cut table lists all cuts created in the first dimension, structured by signals and peak groups.

The **^{2}D Chromatogram(s)** shows an overlay of all cuts, which have been selected in the heart-cut table. The Peak Table lists all peaks (peak areas), which have been found in the second dimension by applying ChemStation integrator settings.

Areas of peaks which belong together are summed up automatically.

Structure of Sampling and Peak Tables



Sampling table (¹D)

Cut #	¹D Cut start [min]	Sampling time [min]	Mode	²D Run start [min]
1	9.14	0.08	Time	26.50
2	9.24	0.08	Time	22.30
3	9.35	0.08	Time	18.10
4	9.46	0.08	Time	13.90
5	9.56	0.08	Time	9.70
6	13.14	0.08	Time	49.36
7	13.24	0.08	Time	45.16
8	13.34	0.08	Time	40.96
9	13.44	0.08	Time	36.76
10	13.54	0.08	Time	32.56

Figure 100 Sampling table

1	First level: All signals for all runs
2	Second level: Peak groups
3	Third level: Cuts numbered continuously per signal

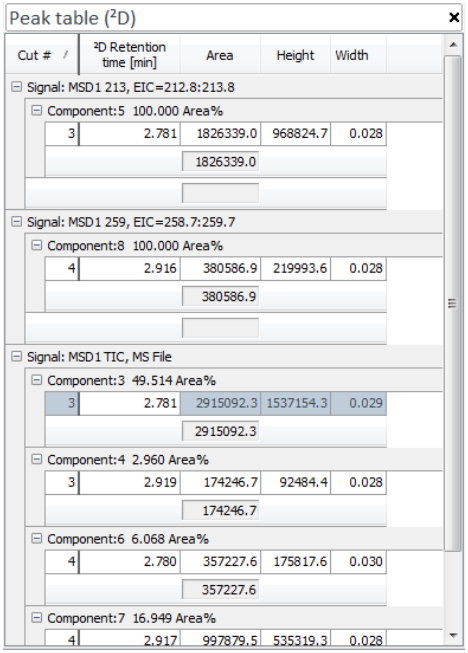


Figure 101 Peak Table

1	First level: All signals for all runs
2	Second level: Components with matching 2D retention times
3	Third level: Cuts and area sum per compound

Summed signal

The optional summed signal (black line) shows the sum of all selected cuts.

This allows easy navigation: one area of interest in ^{1}D corresponds to one summed 2D chromatogram (for trying, choose option **Show summed signal** only).

For a single wavelength, this corresponds to the entire ^{2}D area used for quantitation.

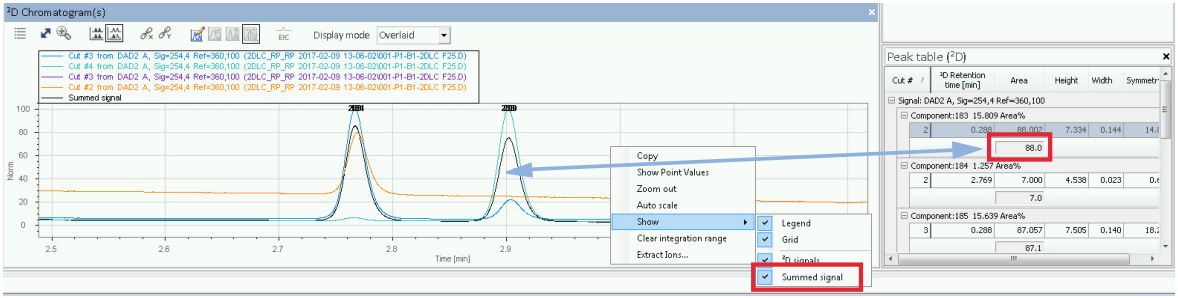


Figure 102 Summed signal

Quantitation

Compared to a standard quantitation, a 2D-LC quantitation requires 2 additional steps (identification of compounds, summation of areas) carried out by the 2D-LC software.

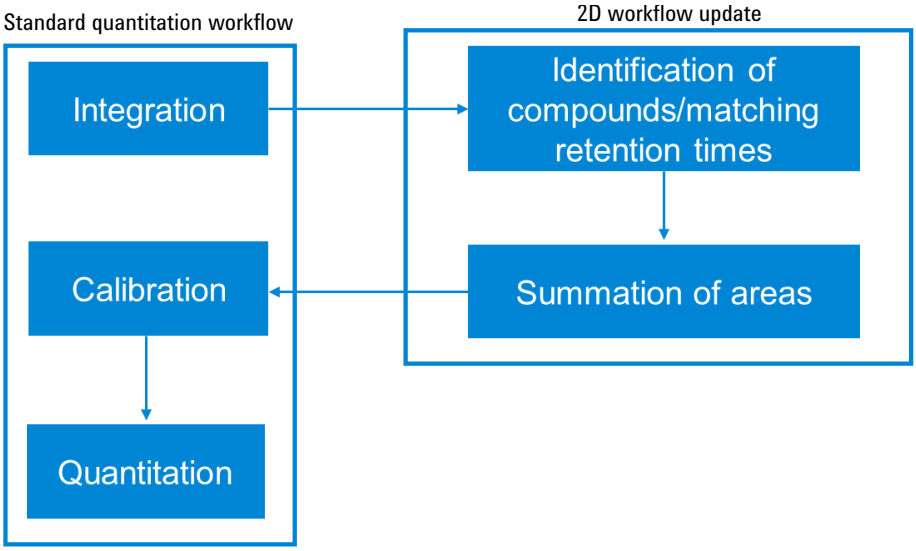
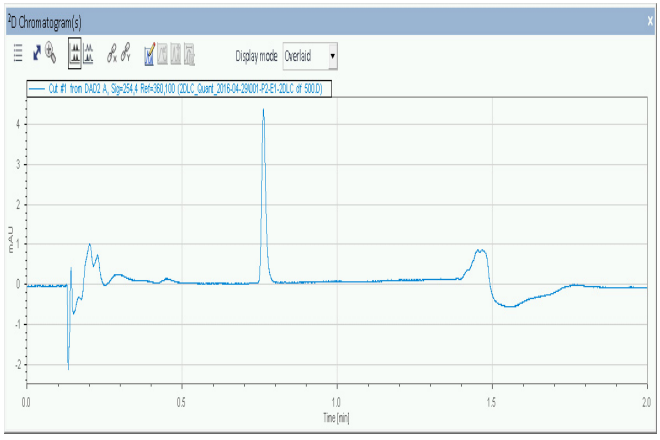


Figure 103 Necessary steps for quantitation in 2D-LC

Identify compounds

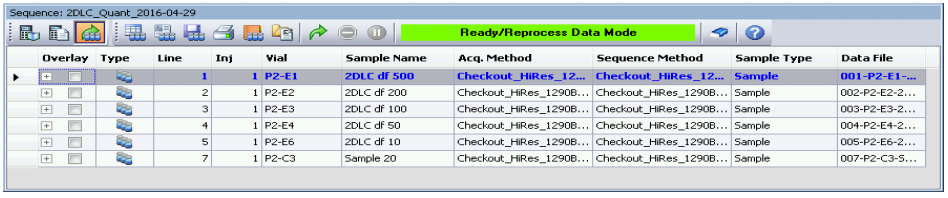
This procedure exemplarily shows a quantitative measurement of the 2D-LC checkout sample using a 3-level calibration of two compounds at two wavelengths.



1 Select signal.

NOTE

Use a signal with a low concentration.



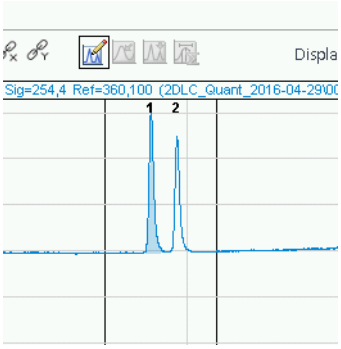
Overlay	Type	Line	Inj	Vial	Sample Name	Acq. Method	Sequence Method	Sample Type	Data File
		1	1	P2-E1	2DLC df 500	Checkout_HiRes_12...	Checkout_HiRes_12...	Sample	001-P2-E1-...
		2	1	P2-E2	2DLC df 200	Checkout_HiRes_1290B...	Checkout_HiRes_1290B...	Sample	002-P2-E2-2...
		3	1	P2-E3	2DLC df 100	Checkout_HiRes_1290B...	Checkout_HiRes_1290B...	Sample	003-P2-E3-2...
		4	1	P2-E4	2DLC df 50	Checkout_HiRes_1290B...	Checkout_HiRes_1290B...	Sample	004-P2-E4-2...
		5	1	P2-E6	2DLC df 10	Checkout_HiRes_1290B...	Checkout_HiRes_1290B...	Sample	005-P2-E6-2...
		7	1	P2-C3	Sample 20	Checkout_HiRes_1290B...	Checkout_HiRes_1290B...	Sample	007-P2-C3-S...

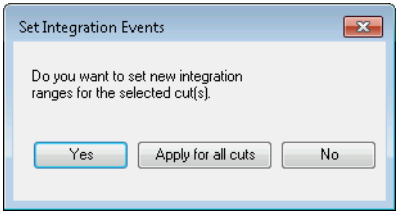
2 Integrate the cuts.

NOTE

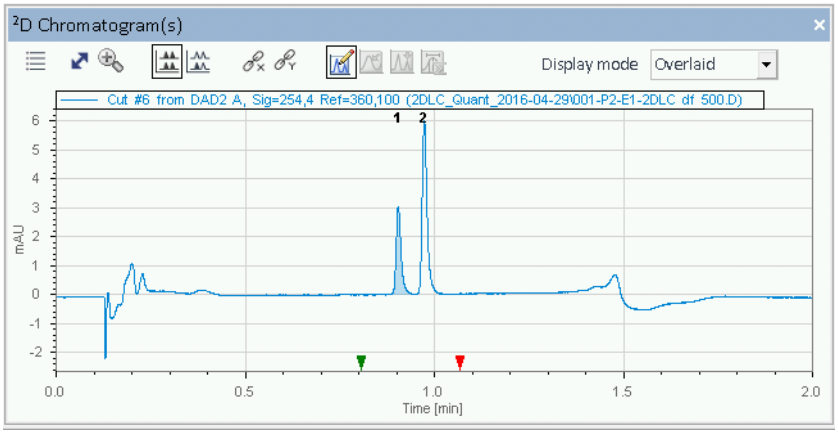
Integration is done using the ChemStation integrator.

a Click on  to **Set Integration Range**.





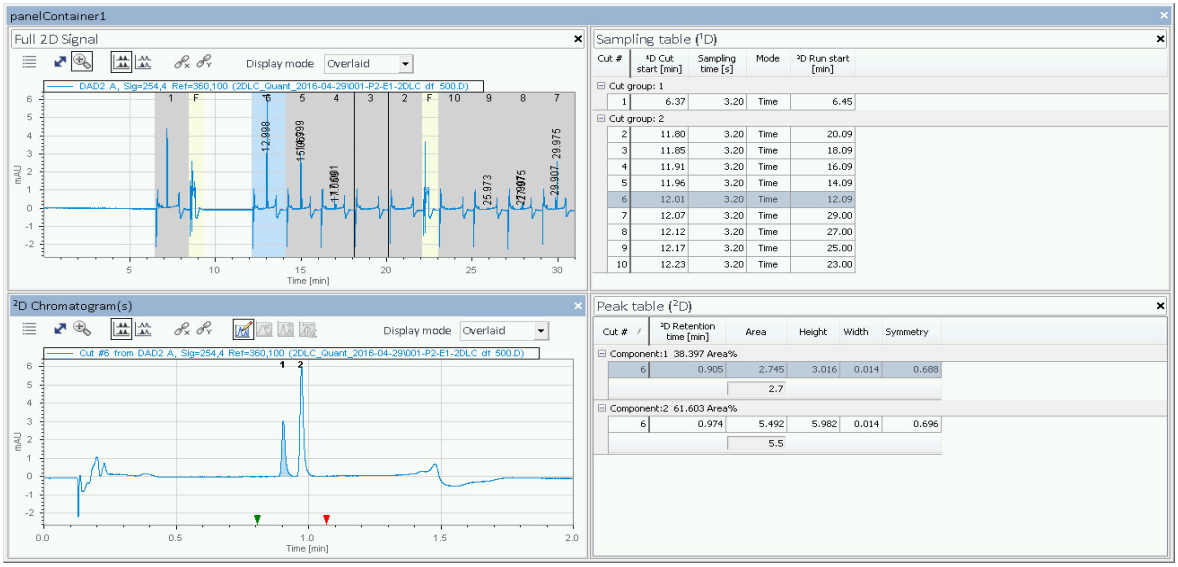
The 2D-LC Viewer offers simplified access for changing the integration of one or multiple limits.



3 Verify small peaks in ^{2}D. Use Full ^{2}D Signal or ^{2}D run start time for navigation.

NOTE

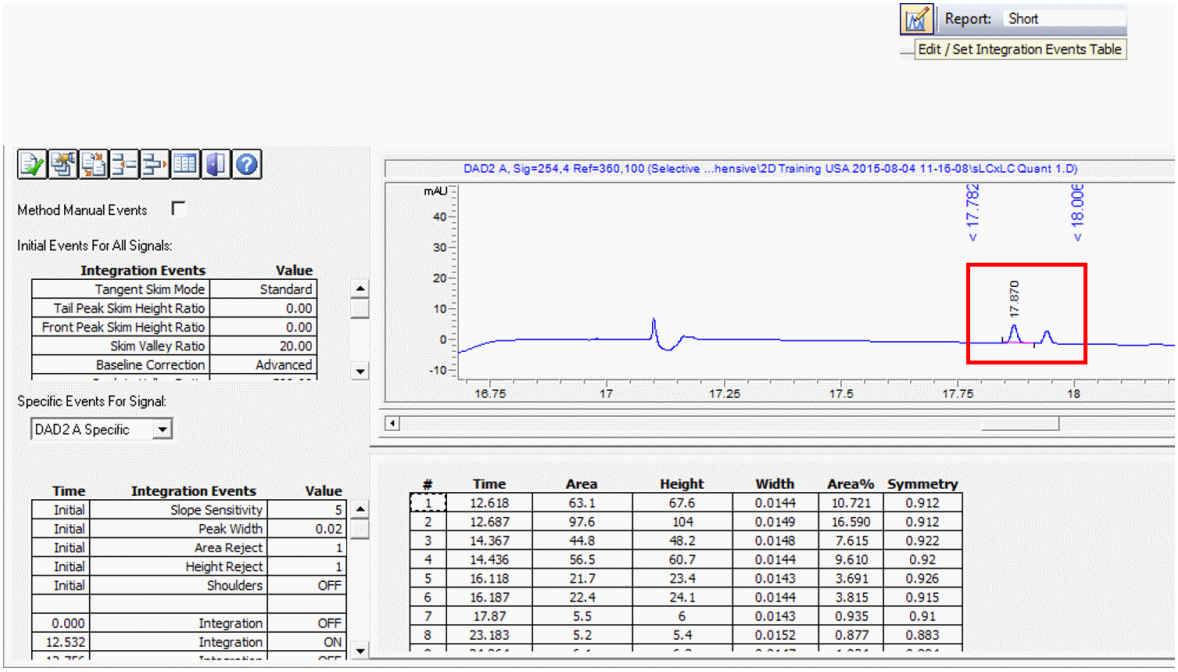
Peak integration is based on ChemStation integrator settings. Please verify, if all peaks of interest have been found and integrated. If not, ChemStation settings need to be adjusted. For finding the corresponding peak in the ^{2}D signal, use the peak table and heart-cut table.



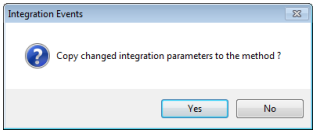
4 Adjust integrator settings in ChemStation.

NOTE

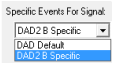
ChemStation has no information about cuts and individual ^{2}D chromatograms. So it is important to know, where to look at. In the example above, Cut #2 starts at 16.97 minutes. At 17.939 minutes is compound 2 of cut #2, which is not integrated. In this example, decreasing the area reject parameter from 5 to 1 enables integrating the peak.



5 Apply the adjusted integrator settings to other signals.



NOTE

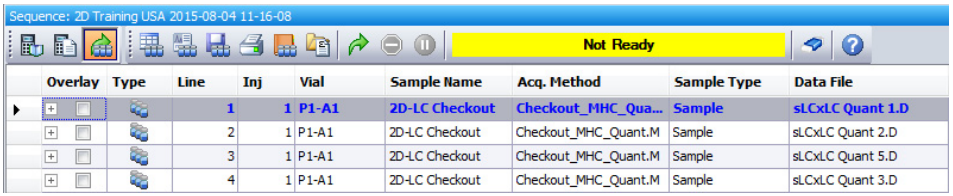


In integration events, change to a different wavelength for applying settings here.

Consider using same limits for other signals, apply to sequence method.

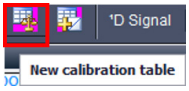
Calibration

Create a calibration table

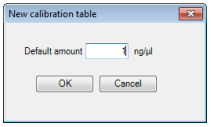


Overlay	Type	Line	Inj	Vial	Sample Name	Acq. Method	Sample Type	Data File
		1	1	P1-A1	2D-LC Checkout	Checkout_MHC_Qua...	Sample	sLCxLC Quant 1.D
		2	1	P1-A1	2D-LC Checkout	Checkout_MHC_Quant.M	Sample	sLCxLC Quant 2.D
		3	1	P1-A1	2D-LC Checkout	Checkout_MHC_Quant.M	Sample	sLCxLC Quant 5.D
		4	1	P1-A1	2D-LC Checkout	Checkout_MHC_Quant.M	Sample	sLCxLC Quant 3.D

1 Create a new calibration table from the MHC Viewer.



2 Enter amount/concentration.

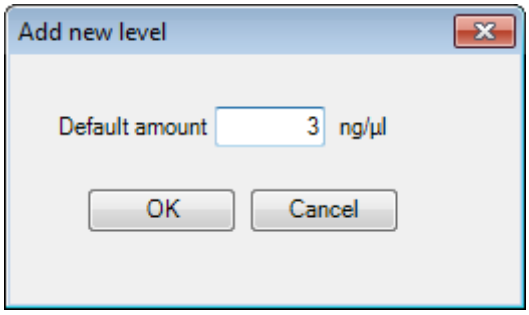


Add levels

Overlay	Type	Line	Inj	Vial	Sample Name	Acq. Method	Sample Type	Data File
		1	1	P1-A1	2D-LC Checkout	Checkout_MHC_Quant.M	Sample	sLCxLC Quant 1.D
		2	1	P1-A1	2D-LC Checkout	Checkout_MHC_Qua...	Sample	sLCxLC Quant 2.D
		3	1	P1-A1	2D-LC Checkout	Checkout_MHC_Quant.M	Sample	sLCxLC Quant 5.D
		4	1	P1-A1	2D-LC Checkout	Checkout_MHC_Quant.M	Sample	sLCxLC Quant 3.D

1 Select and add more signals and levels as needed.

2 Set concentration for each level.



Calibration table

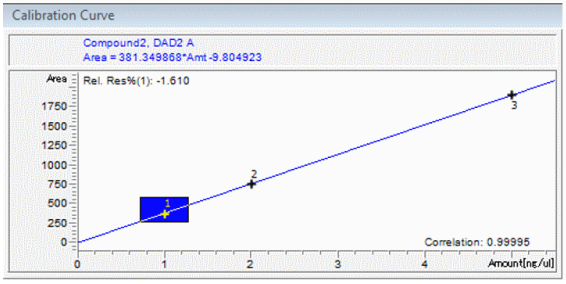
Calibration Table

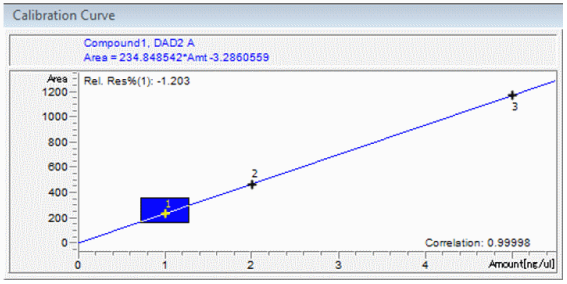
Enter | Delete | Insert... | Print | OK | Help

#	RT	Signal	Compound	Lvl	Amt[ng/ul]	Area
1	12.617	DAD2 A	Compound1	1	1.000	228.780
				2	2.000	464.650
				3	5.000	1172.200
	12.617	DAD2 B		1	1.000	69.095
				2	2.000	141.200
				3	5.000	355.910
2	12.686	DAD2 A	Compound2	1	1.000	365.560
				2	2.000	744.530
				3	5.000	1901.500
	12.686	DAD2 B		1	1.000	206.250
				2	2.000	419.610
				3	5.000	1065.300

Peak Table

Cut #	2D Retention time (rel.) [min]	Area	Height	Width	Symmetry
5	0.901	320.121	336.944	0.015	0.906
6	0.901	210.633	226.007	0.014	0.907
7	0.900	138.931	147.254	0.015	0.897
8	0.897	67.435	71.419	0.015	0.902
9	0.896	28.679	30.527	0.015	0.902
10	0.896	11.465	12.105	0.015	0.890
		1172.219			





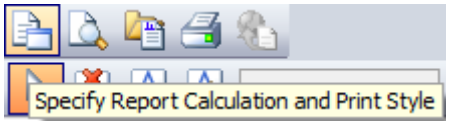
Quantification

Quantifying samples with unknown concentration based on the calibration table can be done using ChemStation reporting as usual.

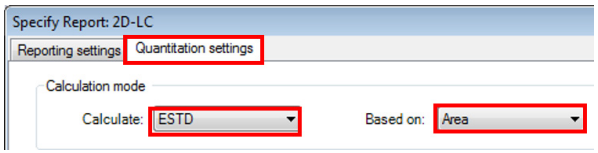
Quantification

	Overlay	Type	Line	Inj	Vial	Sample Name	Acq. Method	Sample Type	Data File
			1	1	P1-A1	2D-LC Checkout	Checkout_MHC_Quant.M	Sample	sLCxLC Quant 1.D
			2	1	P1-A1	2D-LC Checkout	Checkout_MHC_Quant.M	Sample	sLCxLC Quant 2.D
			3	1	P1-A1	2D-LC Checkout	Checkout_MHC_Quant.M	Sample	sLCxLC Quant 5.D
▶			4	1	P1-A1	2D-LC Checkout	Checkout_MHC_Qua...	Sample	sLCxLC Quant 3.D

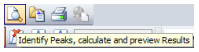
1



2



3




```
Signal 1: DAD2 A, Sig=254,4 Ref=360,100

RetTime  Type     Area      Amt/Area    Amount   Grp   Name
 [min]           [mAU*s]               [ng/ul]
-------|------|----------|----------|----------|--|-----------------
 12.617 BB      705.28630 4.27790e-3    3.01715    Compound1
 12.686 BBA    1134.78678 2.64492e-3    3.00142    Compound2

Totals :                                6.01857
```

Data Analysis for Comprehensive 2D-LC (LCxLC)

Typically very complex samples are analyzed by comprehensive 2-dimensional liquid chromatography. The compounds which are often co-eluting form the first dimension are further separated in the second dimension. With the Agilent 1290 Infinity II 2D-LC Solution ChemStation always one large data-file spanning the run-time of the two-dimensional analysis will be acquired. As an example, a 2-dimensional analysis of a mixture of 26 polyphenolic standard compounds is shown in a one dimensional data analysis display (Figure 104 on page 252). Theoretically, the data can be analyzed with OpenLAB CDS ChemStation edition software.

But for easier data-analysis and better visualization of the comprehensive 2D-LC data special software is recommended. Agilent recommends GC Image LCxLC edition Software from GC Image LLC, Nebraska, USA. A trial download can be found on **www.GCImage.com** as well as an online manual. Agilent 2D-LC data files also including UV spectra and mass spectra data can be directly imported. This software, with the information of the modulation time, is capable to extract the data and isolate each second dimension run. Data will be reconstructed in a two-dimensional display of the retention times. This can be displayed as a colored 2-dimensional map of compound peaks (Figure 105 on page 252). After baseline correction the peaks can be automatically detected by a peak detection algorithm inherent in the 2D-LC data analysis software (Figure 106 on page 253). Since the third dimension is the intensity of the peaks a 3-dimensional plot of the data is possible (Figure 107 on page 253). With the given data set further qualitative and quantitative data analysis is possible.

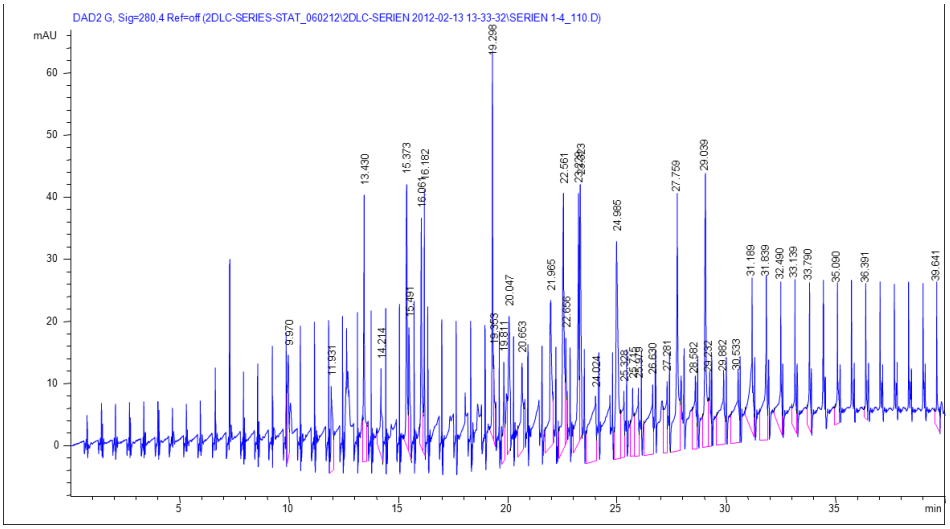


Figure 104 Display of two-dimensional LC data with a one-dimensional data analysis software

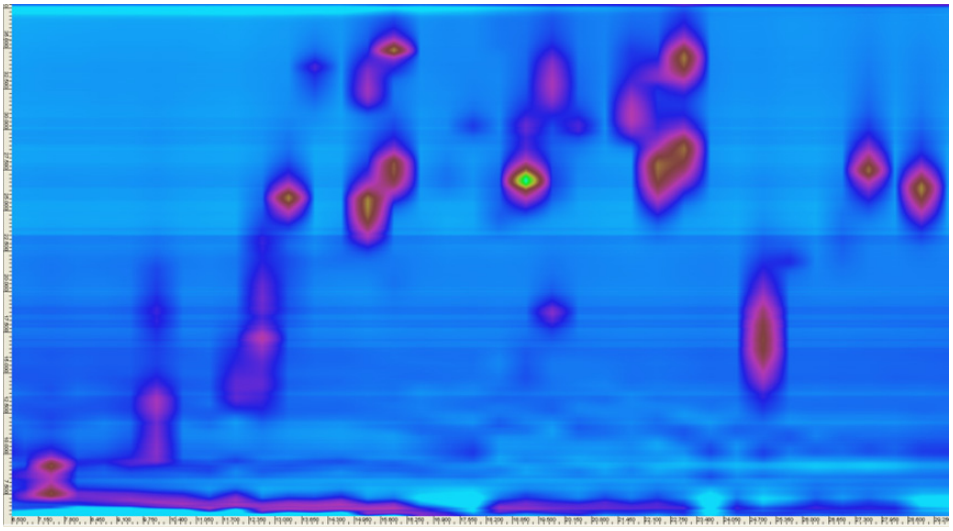


Figure 105 2D-LC plot of the optimized separation of 26 polyphenolic compounds

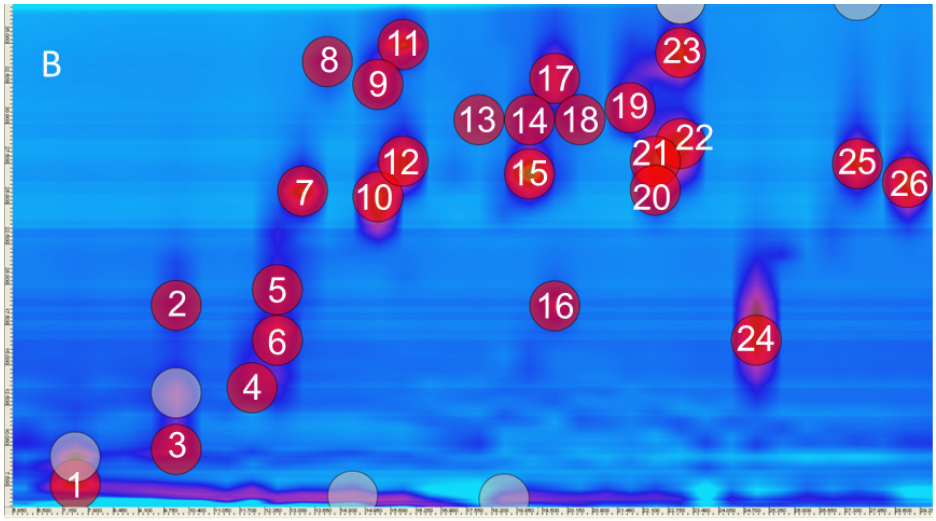


Figure 106 2DLC plot after baseline correction and with software detected peak annotation

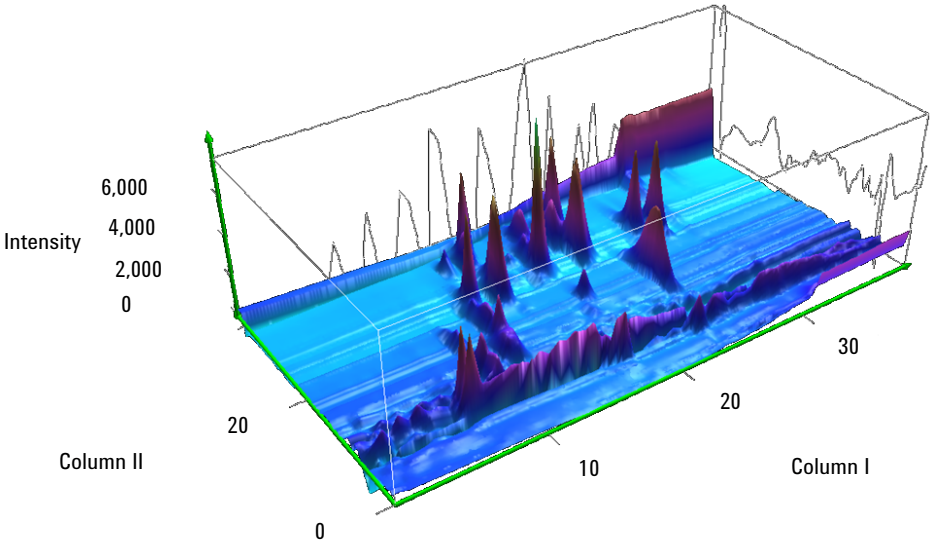


Figure 107 3-Dimensional display of the separation of the 26 compound standard mixture. The first dimension separation takes 40 minutes and each second dimension separation takes 39 seconds. The back side shows a generated first dimension chromatogram and gives the impression which peaks are coeluting and separated in the second dimensions.

Overview

GC Image LC x LC Edition (short GC Image) is a software for for visualization and data analysis of full comprehensive two-dimensional liquid chromatograms:

- M8700AA GC Image LCxLC Edition for UV and Single Quad measurements
- M8710AA GC Image LCxLC-HRMS Edition for UV and/or High Resolution MS measurements (Q-TOF)

Installation

Parts required	Description
	Description
	CD with software
	License dongle (Wibu Key)
	Activation code

1. The CD contains two executables: LCxLC2.9-MPr3-64bit.exe (or higher), LCxLC2.9-MPr3-HRMS-64bit.exe (or higher). Choose the appropriate version for your operating system. Corresponding versions are available for the UV only detection.
2. Double-click the chosen executable and follow the instructions on the screen.
3. Activate the software with the USB key. Insert the USB dongle and wait. The driver will install automatically.
4. Activate R2.9 (or higher) in the Windows Start Menu.
5. Enter the activation code, which is shipped with the software.

Use GCImage Software

GCImage is a powerful expert software with many sophisticated features for display, data analysis, compound identification, library search, workflow automation, reporting etc.

The basic knowledges to successfully use the software are the following:

- Import 2D ChemStation data files
- Setting the modulation period
- Choosing a color mapping
- Navigate in the display
- Navigate in the display
- Detect peaks (Blobs)

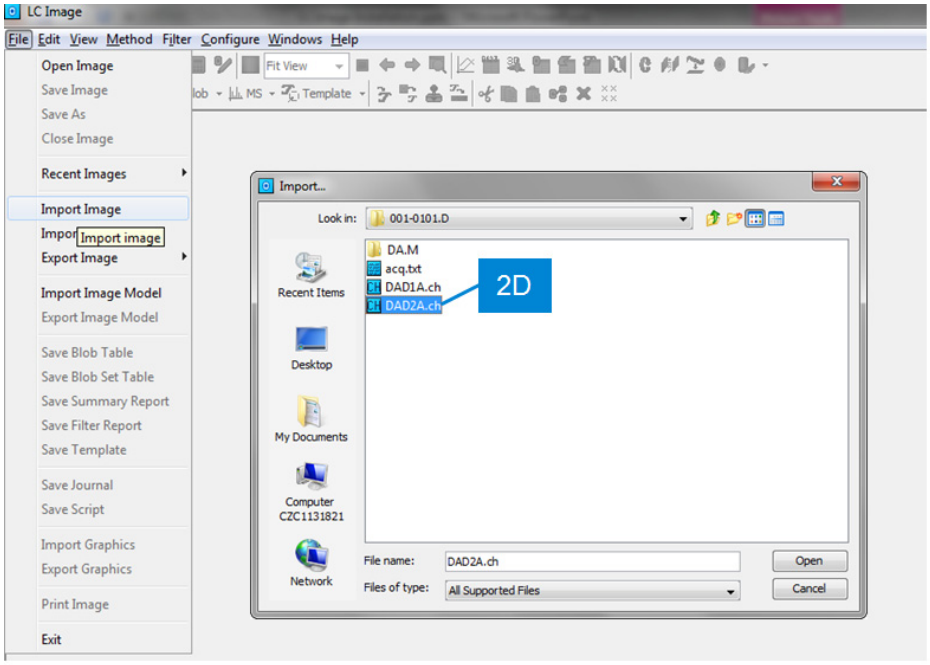
Preparations The USB dongle needs always to be inserted when working with GCImage software. If not, you will be asked to insert it.

Basic knowledges

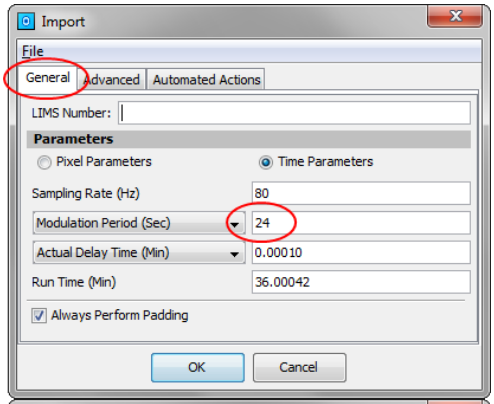
1 Start up LCImage

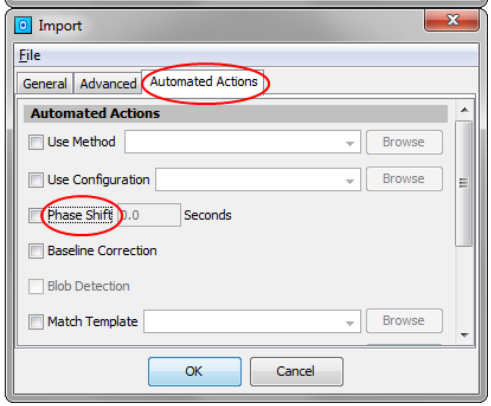
LCImage offers optionally a password protected user management system. If you don't need it, simply click „Login with system", which is based on Windows user account.

2 Import the UV signal from the second dimension detector.

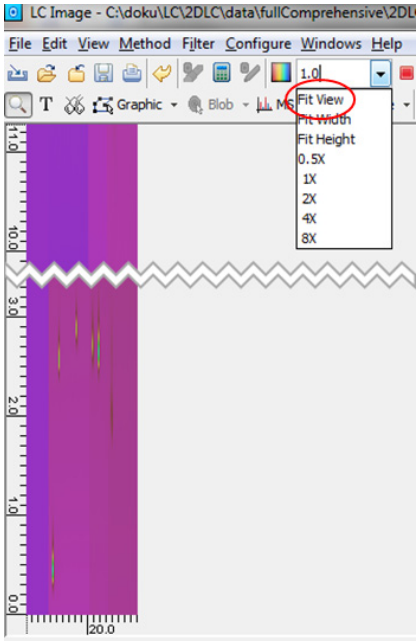


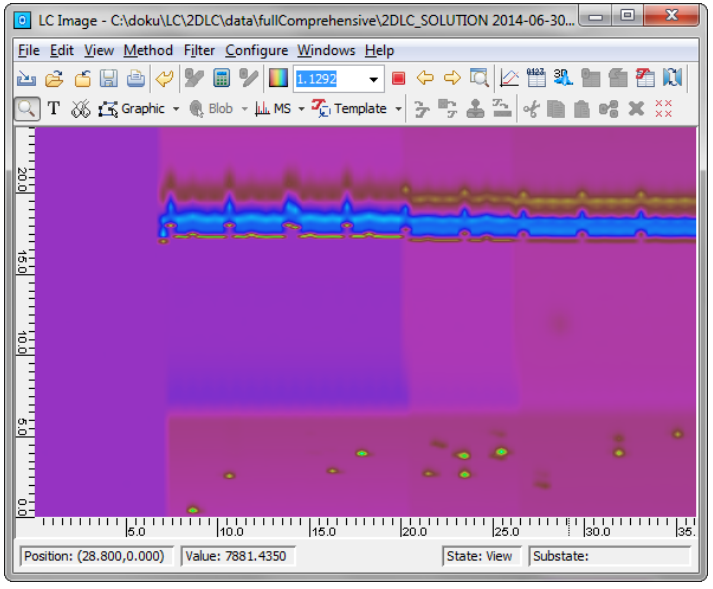
3 Import parameters



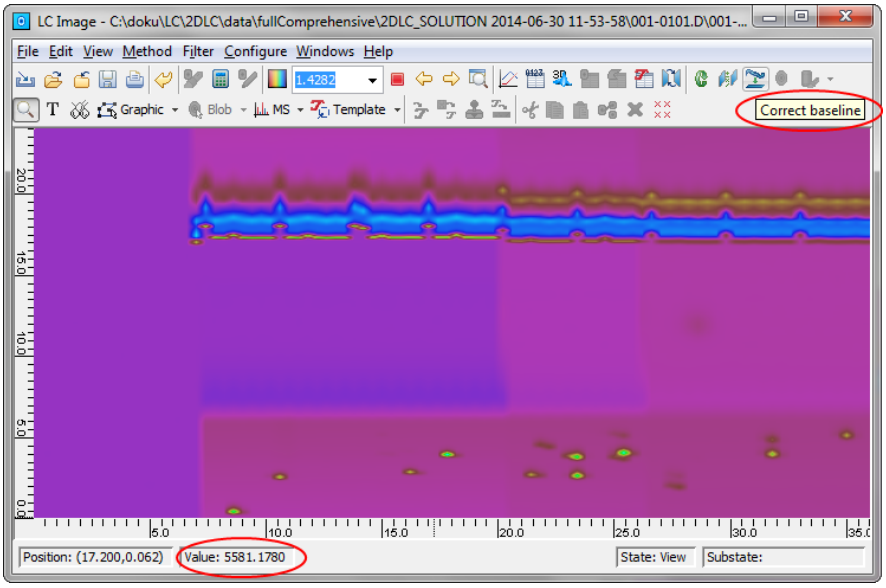


4 Fit view

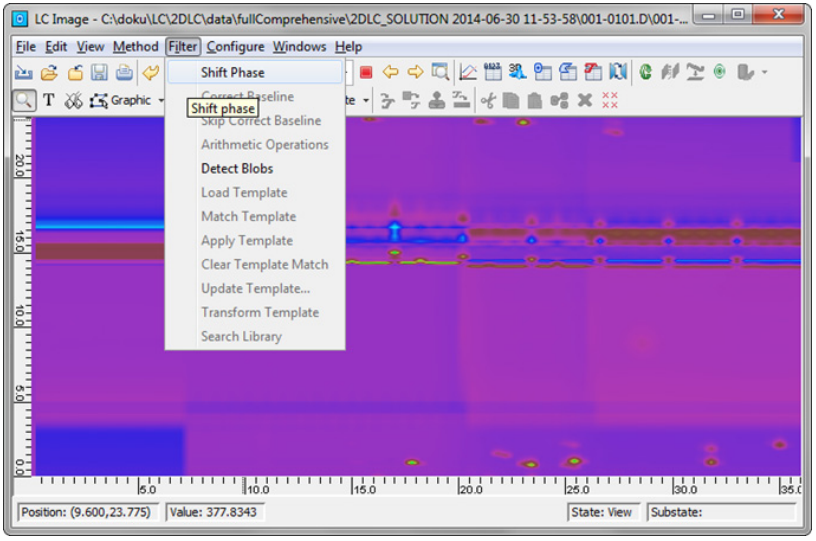

LC Image - C:\doku\LC\2DLC\data\fullComprehensive\2DL
File Edit View Method Filter Configure Windows Help
1.0
Graphic
Blob
Fit View
Fit Width
Fit Height
0.5X
1X
2X
4X
8X
20.0


LC Image - C:\doku\LC\2DLC\data\fullComprehensive\2DLC_SOLUTION 2014-06-30...
File Edit View Method Filter Configure Windows Help
1.1292
Graphic
Blob
MS
Template
20.0
15.0
10.0
5.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.
Position: (28.800,0.000)
Value: 7881.4350
State: View
Substate:

5 Correct Baseline

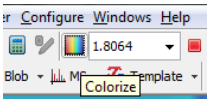


6 Shift phase

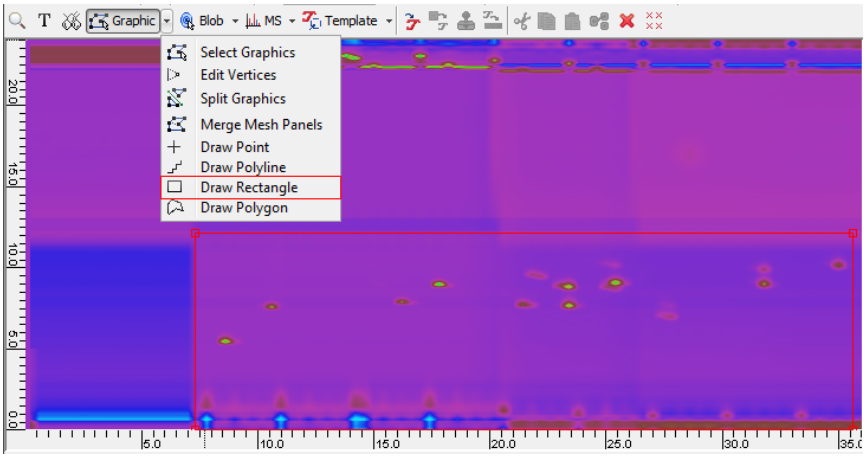


7 Zoom into an interesting region by using the right mouse button and dragging over the display

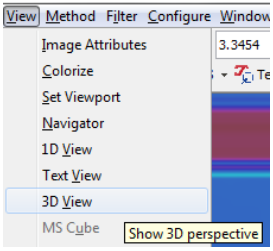
8 Adjust colors: LC Image offers refined possibilities for optimizing the color scales. Play around with settings for improving the contrast.



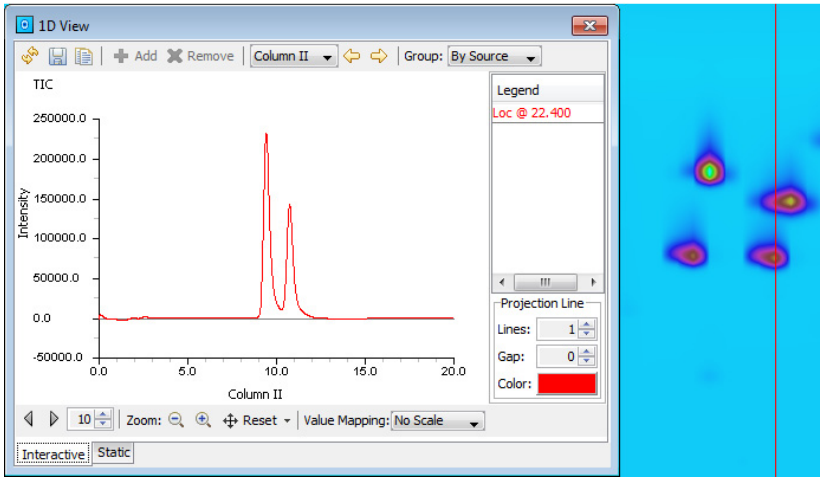
9 Select a data range.



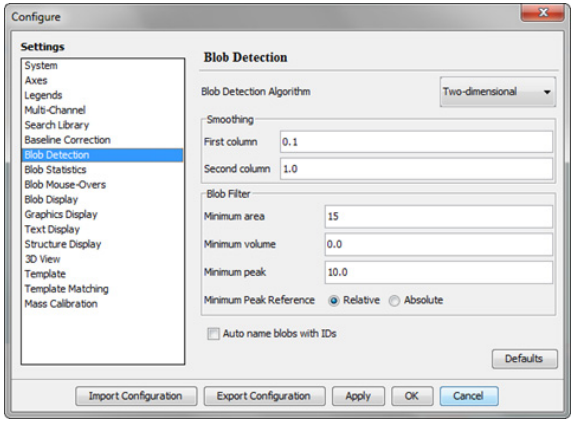
10 By clicking the „Show 3D perspective“ button or the corresponding menu item, you can easily create a customizable 3D plot.



11 View single 2D chromatograms

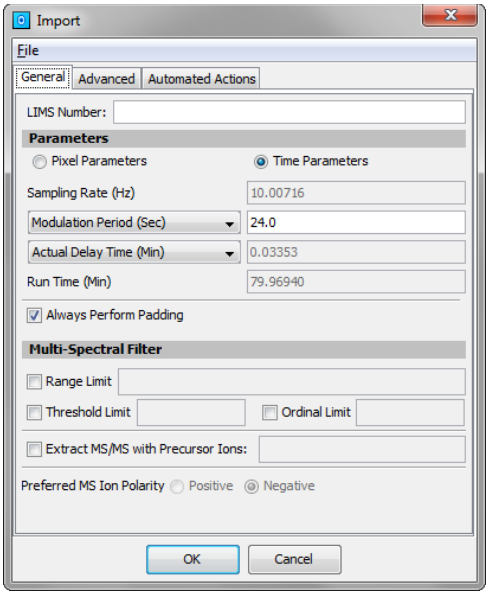


12 Select blobs



MS Data

1 Import MS data: The import functionality of MS data is very similar to those of UV measurements. Additionally, you can for example filter to a certain mass range („range limit"), that you are interested in.



2 By clicking on „Show 1D view", you can display the TIC for that 2D slice.

3 By clicking on data points or blobs in the 2D view, you can display MS spectra of corresponding plots.

9 Troubleshooting and Diagnostics

This chapter gives an overview about the troubleshooting and diagnostic features and the different user interfaces.

Overview of the Module’s Indicators and Test Functions

For an overview of the module's indicators and test functions, refer to the manuals of the modules installed in your system.

User Interfaces

- Depending on the user interface, the available tests and the screens/reports may vary.
- Preferred tool should be Agilent Lab Advisor Software, see "Agilent Lab Advisor Software" on page 266.
- The Agilent OpenLAB ChemStation C.01.03 and above do not include any maintenance/test functions.
- Screenshots used within these procedures are based on the Agilent Lab Advisor Software.

Agilent Lab Advisor Software

The Agilent Lab Advisor Software (basic license, shipped with an Agilent LC pump) is a standalone product that can be used with or without a chromatographic data system. Agilent Lab Advisor helps to manage the lab for high-quality chromatographic results by providing a detailed system overview of all connected analytical instruments with instrument status, Early Maintenance Feedback counters (EMF), instrument configuration information, and diagnostic tests. By the push of a button, a detailed diagnostic report can be generated. Upon request, the user can send this report to Agilent for a significantly improved troubleshooting and repair process.

The Agilent Lab Advisor software is available in two versions:

- Lab Advisor Basic
- Lab Advisor Advanced

Lab Advisor Basic is included with every Agilent 1200 Infinity Series and Agilent InfinityLab LC Series instrument.

The Lab Advisor Advanced features can be unlocked by purchasing a license key, and include real-time monitoring of instrument actuals, all various instrument signals, and state machines. In addition, all diagnostic test results, calibration results, and acquired signal data can be uploaded to a shared network folder. The Review Client included in Lab Advisor Advanced allows to load and examine the uploaded data no matter on which instrument it was generated. This makes Data Sharing an ideal tool for internal support groups and users who want to track the instrument history of their analytical systems.

The optional Agilent Maintenance Wizard Add-on provides an easy-to-use, step-by-step multimedia guide for performing preventive maintenance on Agilent 1200 Infinity and Agilent InfinityLab LC Series instrument.

The tests and diagnostic features that are provided by the Agilent Lab Advisor software may differ from the descriptions in this manual. For details, refer to the Agilent Lab Advisor software help files.

The Basic Principle of Troubleshooting

Troubleshooting key Concept – Divide and Conquer

The following troubleshooting concept, shows exemplarily how to approach problems in 2D-LC chromatography.

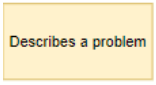
Most of the following explanations can also be used to isolate and detect standard LC issues.

The basic principle of troubleshooting should always be a step by step approach to the 2D-LC problem. As a first step, find out whether the cause of the error is either:

- The application method, or
- The 2D-LC instrument

For a recommended approach to isolate the cause of the issue, see the graphic below. All examples use symbols as described in the following table.

Table 24 Description for symbols as used in troubleshooting decision trees

Symbol	Description
	Shows and describes a problem in the 2D-LC system. Indicates the starting point for a series of actions and decisions leading to a solution for the problem.
	Illustrates, that the user must identify what an observation means. Then the user must take a decision, which further way of troubleshooting to follow.
	Shows, the user must act to proceed and come to the next decision or solution.

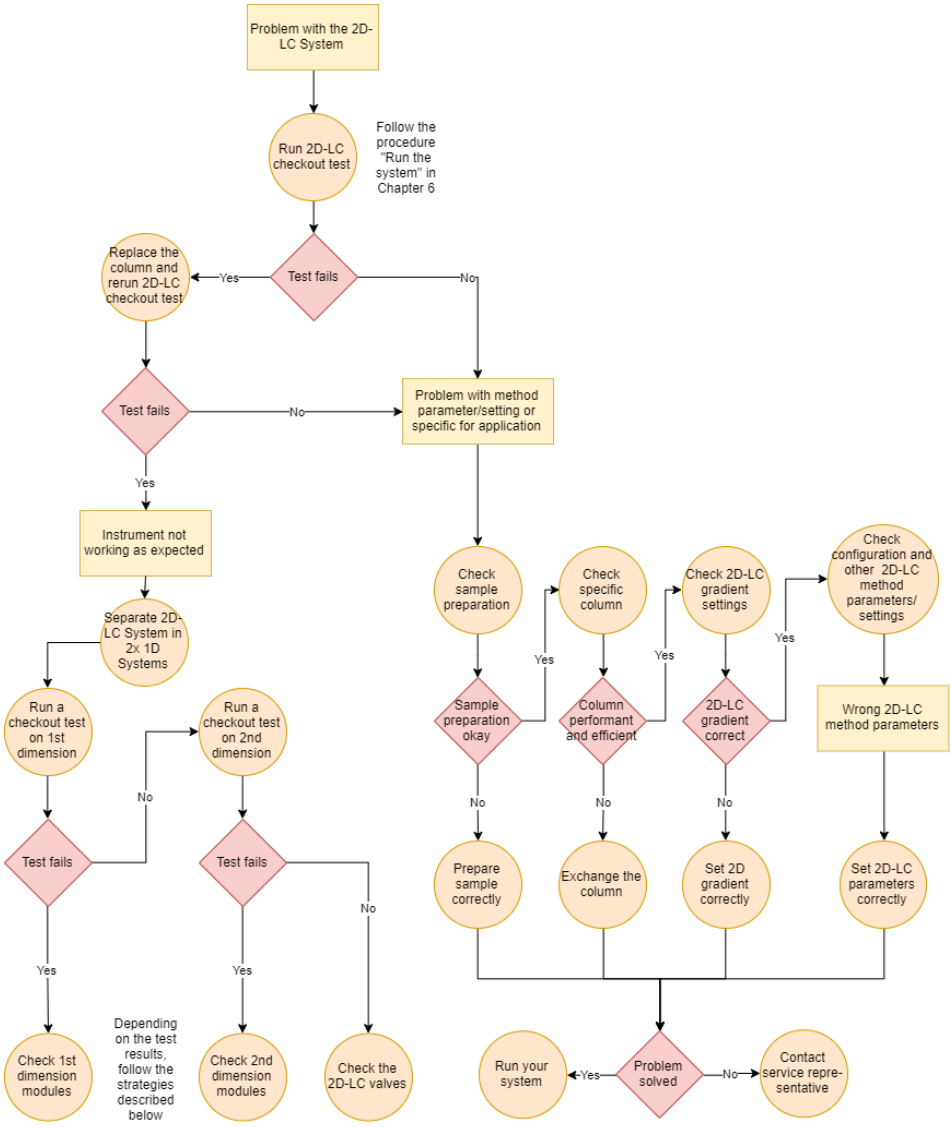


Figure 108 Example for a strategy to identify the application method or instrument as root cause for issues in 2D-LC chromatography

After ruling out the application method as the cause of the issue, one can start to search for the problem's root cause within the 2D-LC Instrument hardware.

Common HPLC hardware issues, along with the location of each problem's respective troubleshooting procedure are listed below:

Pressure too high

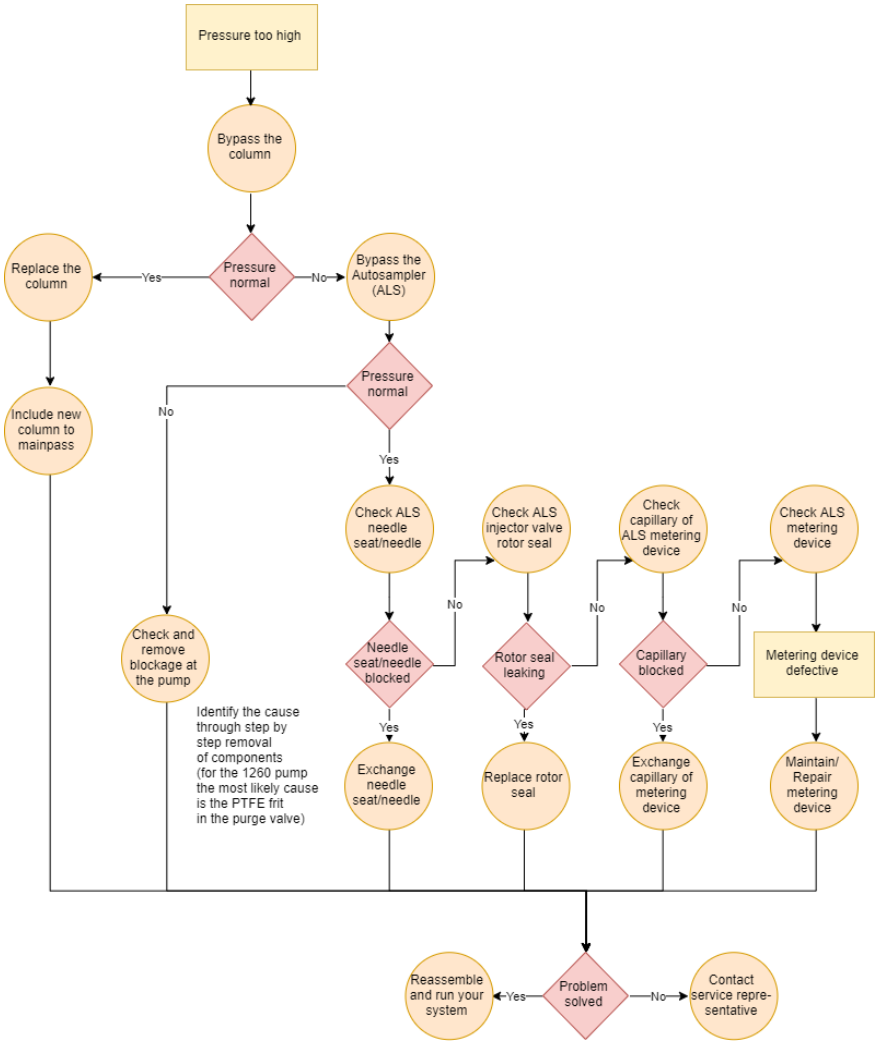


Figure 109 Example for a strategy to eliminate issues related to too high pressure in 2D-LC instruments

Pressure too low

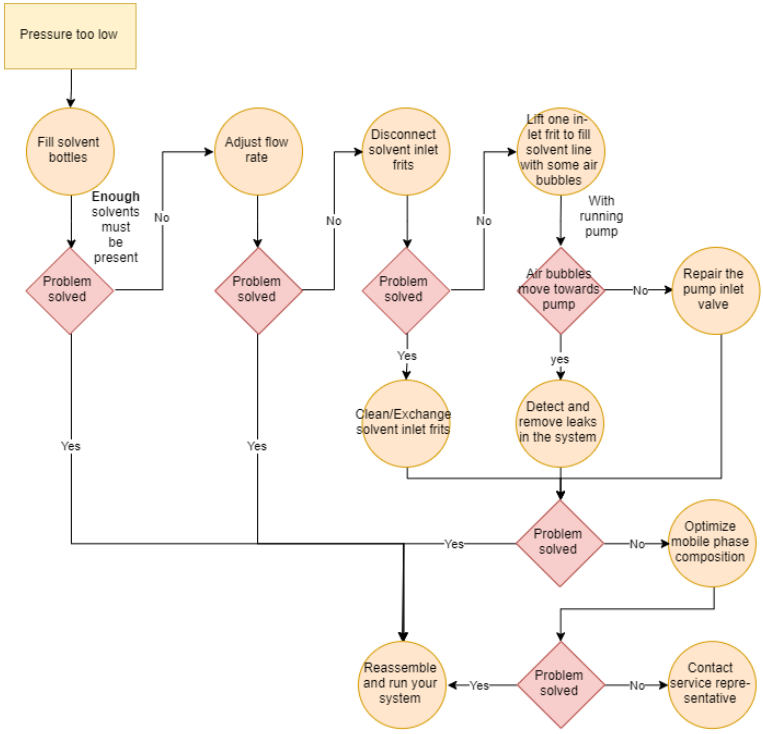


Figure 110 Example for a strategy to eliminate issues related to too low pressure in 2D-LC instruments

Peak area and peak height related

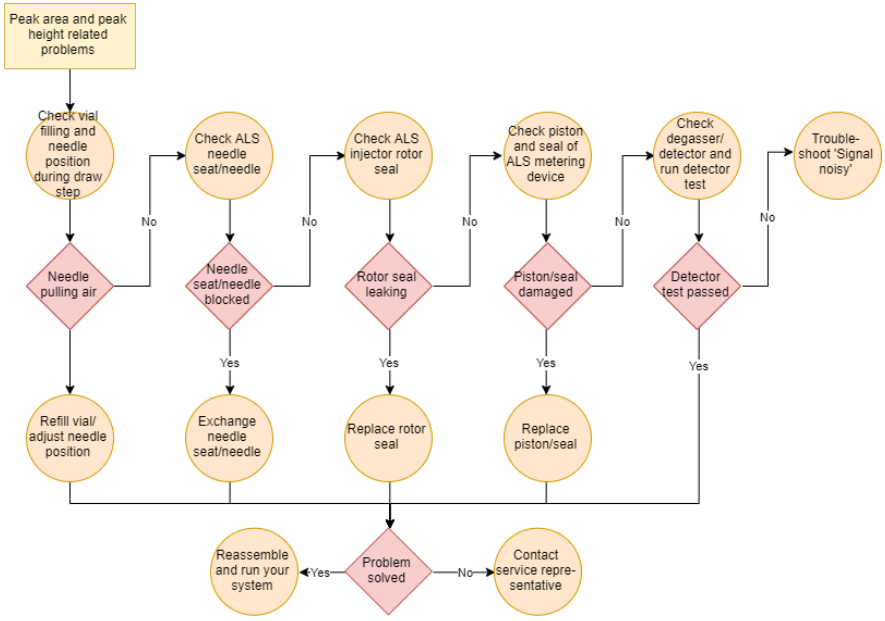


Figure 111 Example for a strategy to eliminate issues related to peak problems in 2D-LC instruments

Retention time related

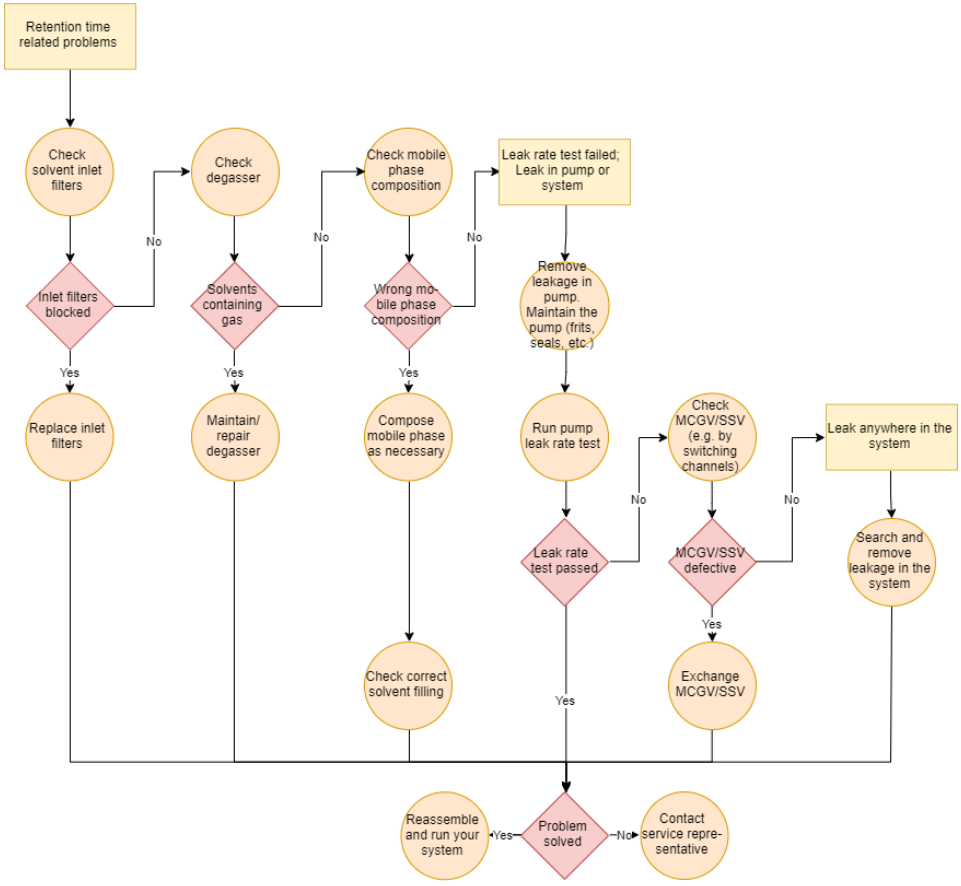


Figure 112 Example for a strategy to eliminate issues related to retention time in 2D-LC instruments

Missing signal linearity

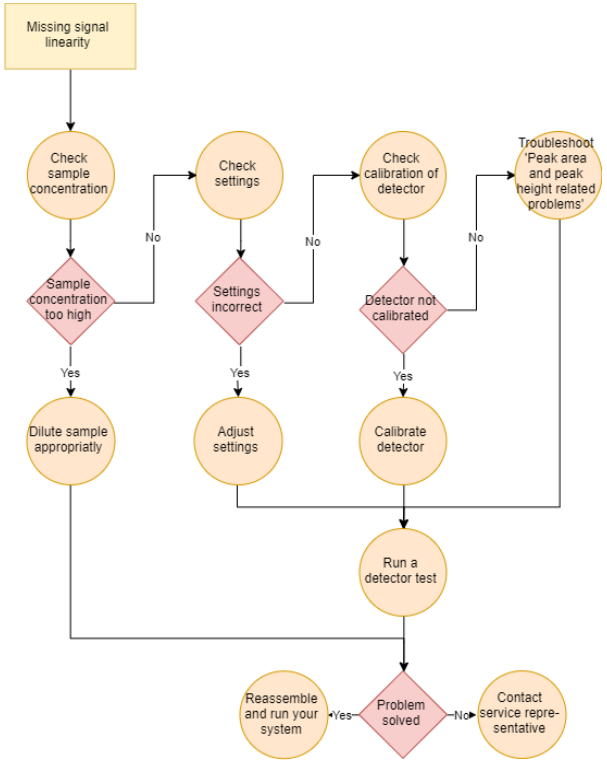


Figure 113 Example for a strategy to eliminate issues related to missing signal linearity in 2D-LC instruments

Drifting signal

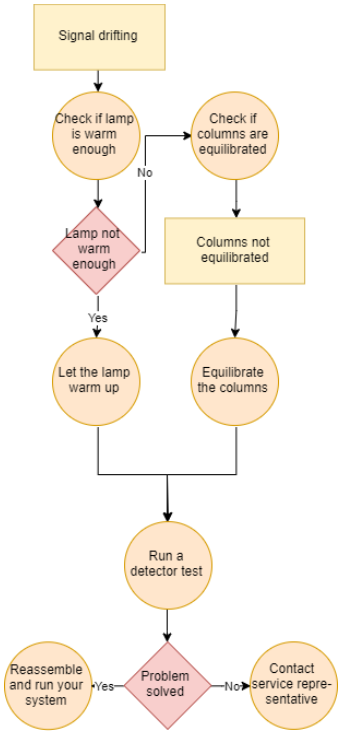


Figure 114 Example for a strategy to eliminate issues related to drifting signal in 2D-LC instruments

Signal noisy

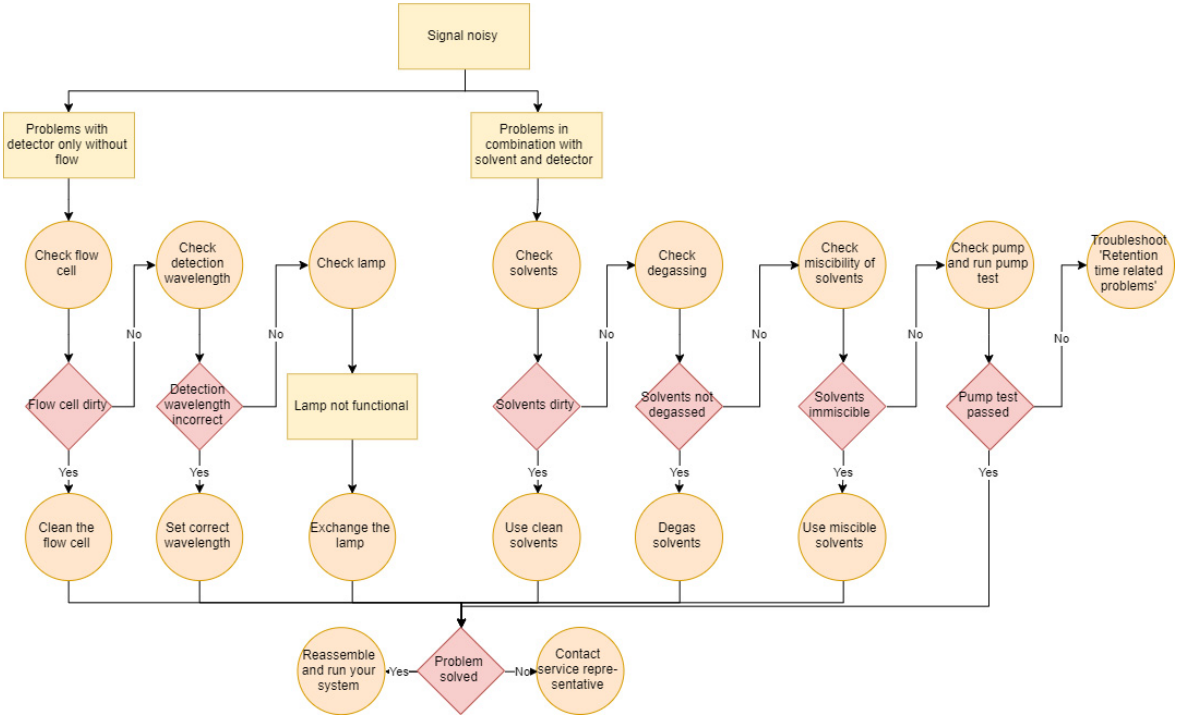


Figure 115 Example for a strategy to eliminate issues related to signal noise in 2D-LC instruments

Recommended Tests to Conclude Troubleshooting

The following table shows the most important tests to conclude troubleshooting.

- For further detailed information, see:
  - Maintenance information in the specific manual of each module.
  - *Troubleshooting Guide* poster 5994-0709EN.
  - *Best Practice for Using an Agilent LC System* 01200-90090.
- For additional help, contact your local Agilent Technologies service representative.

Table 25 Recommended Tests for 2D-LC System Troubleshooting

Pump	Column Compartment	Autosampler	Valve	Detector	2D-LC Instrument
Pressure Test Leak Test	Thermostat Test Pressure Test (if column valve is present)	Pressure Test Inject standards or inject different volumina or blanks	Switching valve position/Check pressure reading Pressure Test	Lamp Intentity Test Wavelength calibration In addition there are detector specific tests.	Run Checkout For 2D-LC Instruments • Pressure test of the 1D-LC Part • Pressure Test of the 2D-LC Part
Pump characteristic • Pump Ripple (1260 Pump) • Tuning (1290 pump)					

10 Error Information

This chapter describes the meaning of error messages, and provides information on probable causes and suggested actions how to recover from error conditions.

What Are Error Messages

Error messages are displayed in the user interface when an electronic, mechanical, or hydraulic (flow path) failure occurs which requires attention before the analysis can be continued (for example, repair, or exchange of consumables is necessary). In the event of such a failure, the red status indicator at the front of the module is switched on, and an entry is written into the module logbook.

If an error occurs outside a method run, other modules will not be informed about this error. If it occurs within a method run, all connected modules will get a notification, all LEDs get red and the run will be stopped. Depending on the module type, this stop is implemented differently. For example, for a pump the flow will be stopped for safety reasons. For a detector, the lamp will stay on in order to avoid equilibration time. Depending on the error type, the next run can only be started, if the error has been resolved, for example liquid from a leak has been dried. Errors for presumably single time events can be recovered by switching on the system in the user interface.

Special handling is done in case of a leak. As a leak is a potential safety issue and may have occurred at a different module from where it has been observed, a leak always causes a shutdown of all modules, even outside a method run.

In all cases, error propagation is done via the CAN bus or via an APG/ERI remote cable (see documentation for the APG/ERI interface).

General Error Messages

General error messages are generic to all Agilent series HPLC modules and may show up on other modules as well.

Timeout

Error ID: 0062

The timeout threshold was exceeded.

Probable cause	Suggested actions
1 The analysis was completed successfully, and the timeout function switched off the module as requested.	Check the logbook for the occurrence and source of a not-ready condition. Restart the analysis where required.
2 A not-ready condition was present during a sequence or multiple-injection run for a period longer than the timeout threshold.	Check the logbook for the occurrence and source of a not-ready condition. Restart the analysis where required.

Shutdown

Error ID: 0063

An external instrument has generated a shutdown signal on the remote line.

The module continually monitors the remote input connectors for status signals. A LOW signal input on pin 4 of the remote connector generates the error message.

Probable cause	Suggested actions
1 Leak detected in another module with a CAN connection to the system.	Fix the leak in the external instrument before restarting the module.
2 Leak detected in an external instrument with a remote connection to the system.	Fix the leak in the external instrument before restarting the module.
3 Shut-down in an external instrument with a remote connection to the system.	Check external instruments for a shut-down condition.
4 The degasser failed to generate sufficient vacuum for solvent degassing.	Check the vacuum degasser for an error condition. Refer to the *Service Manual* for the degasser or the pump that has the degasser built-in.

Remote Timeout

Error ID: 0070

A not-ready condition is still present on the remote input. When an analysis is started, the system expects all not-ready conditions (for example, a not-ready condition during detector balance) to switch to run conditions within one minute of starting the analysis. If a not-ready condition is still present on the remote line after one minute the error message is generated.

Probable cause	Suggested actions
1 Not-ready condition in one of the instruments connected to the remote line.	Ensure the instrument showing the not-ready condition is installed correctly, and is set up correctly for analysis.
2 Defective remote cable.	Exchange the remote cable.
3 Defective components in the instrument showing the not-ready condition.	Check the instrument for defects (refer to the instrument's documentation).

Lost CAN Partner

Error ID: 0071

During an analysis, the internal synchronization or communication between one or more of the modules in the system has failed.

The system processors continually monitor the system configuration. If one or more of the modules is no longer recognized as being connected to the system, the error message is generated.

Probable cause	Suggested actions
1 CAN cable disconnected.	• Ensure all the CAN cables are connected correctly. • Ensure all CAN cables are installed correctly.
2 Defective CAN cable.	Exchange the CAN cable.
3 Defective mainboard in another module.	Switch off the system. Restart the system, and determine which module or modules are not recognized by the system.

Leak Sensor Short

Error ID: 0082

The leak sensor in the module has failed (short circuit).

The current through the leak sensor is dependent on temperature. A leak is detected when solvent cools the leak sensor, causing the leak sensor current to change within defined limits. If the current increases above the upper limit, the error message is generated.

Probable cause	Suggested actions
1 Defective leak sensor.	Please contact your Agilent service representative.
2 Leak sensor incorrectly routed, being pinched by a metal component.	Please contact your Agilent service representative.

Leak Sensor Open

Error ID: 0083

The leak sensor in the module has failed (open circuit).

The current through the leak sensor is dependent on temperature. A leak is detected when solvent cools the leak sensor, causing the leak-sensor current to change within defined limits. If the current falls outside the lower limit, the error message is generated.

Probable cause	Suggested actions
1 Leak sensor not connected to the main board.	Please contact your Agilent service representative.
2 Defective leak sensor.	Please contact your Agilent service representative.
3 Leak sensor incorrectly routed, being pinched by a metal component.	Please contact your Agilent service representative.

Compensation Sensor Open

Error ID: 0081

The ambient-compensation sensor (NTC) on the main board in the module has failed (open circuit).

The resistance across the temperature compensation sensor (NTC) on the main board is dependent on ambient temperature. The change in resistance is used by the leak circuit to compensate for ambient temperature changes. If the resistance across the sensor increases above the upper limit, the error message is generated.

Probable cause	Suggested actions
1 Defective main board.	Please contact your Agilent service representative.

Compensation Sensor Short

Error ID: 0080

The ambient-compensation sensor (NTC) on the main board in the module has failed (open circuit).

The resistance across the temperature compensation sensor (NTC) on the main board is dependent on ambient temperature. The change in resistance is used by the leak circuit to compensate for ambient temperature changes. If the resistance across the sensor falls below the lower limit, the error message is generated.

Probable cause	Suggested actions
1 Defective main board.	Please contact your Agilent service representative.

Fan Failed

Error ID: 0068

The cooling fan in the module has failed.

The hall sensor on the fan shaft is used by the main board to monitor the fan speed. If the fan speed falls below a certain limit for a certain length of time, the error message is generated.

Depending on the module, assemblies (e.g. the lamp in the detector) are turned off to assure that the module does not overheat inside.

Probable cause	Suggested actions
1 Fan cable disconnected.	Please contact your Agilent service representative.
2 Defective fan.	Please contact your Agilent service representative.
3 Defective main board.	Please contact your Agilent service representative.

Leak

Error ID: 0064

A leak was detected in the module.

The signals from the two temperature sensors (leak sensor and board-mounted temperature-compensation sensor) are used by the leak algorithm to determine whether a leak is present. When a leak occurs, the leak sensor is cooled by the solvent. This changes the resistance of the leak sensor which is sensed by the leak-sensor circuit on the mainboard.

Probable cause	Suggested actions
1 Loose fittings.	Ensure all fittings are tight.
2 Broken capillary.	Exchange defective capillaries.

Module-Specific Error Messages

For further module-specific errors, please see the manual of the module in question.

Initialization of Valve Failed

Error ID: 24000

During the initialization process the motor of the valve drive moves to some special positions depending on the installed valve head. A failure in this process means either that the movement couldn't be performed properly or it was not noticed correctly by the sensor.

Probable cause	Suggested actions
1 Mechanical problems. Friction too high or blockages on the valve drive's motor or on the valve head.	• Check valve head for correct installation • Try to identify the source of trouble by installing a different valve head if possible. • Contact your Agilent Service representative.
2 Defect Sensor on the Valve Drive Motor	• Check valve head for correct installation • Try to identify the source of trouble by installing a different valve head if possible. • Contact your Agilent Service representative.

Valve Switching Failed

Error ID: 24001

The valve drive was not able to operate the valve head correctly. Either due to mechanical reasons or the movement couldn't be detected correctly.

Probable cause	Suggested actions
1 Mechanical problems. Friction too high or blockages on the valve drive's motor or on the valve head.	• Check valve head for correct installation • Try to identify the source of trouble by installing a different valve head if possible. • Contact your Agilent Service representative.
2 Defect Sensor on the Valve Drive Motor	• Check valve head for correct installation • Try to identify the source of trouble by installing a different valve head if possible. • Contact your Agilent Service representative.

Valve Tag Violation

Error ID: 24006

The valve drive identified a different valve head than it had identified during the last initialization.

Probable cause	Suggested actions
1 A valve head has been exchanged (hot-plugged) while the valve drive was still powered on.	Change the valve head. It is important to have the valve switched off for at least 10 s after or before a new valve head has been installed.

NOTE

Soft power-down power supply of the valve drive.

Whenever you want to power cycle the valve drive for a re-boot, it needs to be powered off for at least 10 seconds.

Pressure Cluster Partner Missing

The connection from the valve drive to a defined pressure cluster partner is lost.

Probable cause	Suggested actions
1 Communication issues	Check the CAN cable connections of the modules.
2 Configuration mismatch	Check and correct if necessary the valve configuration and presence of defined pressure cluster partner.

Position Cluster Partner Missing

Probable cause	Suggested actions
1 Communication issues	Check the CAN cable connections of the modules.
2 Configuration mismatch	Check and correct if necessary the valve configuration and presence of defined position cluster partner.

External Valve falls into resident mode

Error ID: Flashing status indicator

The valve drive was not able to operate correctly

Probable cause	Suggested actions
1 Communication issues	• Check the CAN cable connections of the modules. • Check if the hosted module is present.
2 Configuration mismatch	• Check if the firmware on the entire stack is out of the same firmware set. • Check if the limit of 3 hosted modules for each host module is not exceeded. • Check if the dipswitch settings are correct. • Check if the firmware on the entire stack has to be the latest version.

11 Maintenance

This chapter describes the maintenance of the 2D-LC Solution.

Introduction to Maintenance

The 2D-LC solution is designed for easy maintenance. The most frequent maintenance can be done from the front with the modules in place in the system stack. Examples are maintenance of the needle, needle seats, rotor seals, valve heads, or replacing heat exchangers.

Warnings and Cautions

WARNING

Personal injury or damage to the product

Agilent is not responsible for any damages caused, in whole or in part, by improper use of the products, unauthorized alterations, adjustments or modifications to the products, failure to comply with procedures in Agilent product user guides, or use of the products in violation of applicable laws, rules or regulations.

- ✓ **Use your Agilent products only in the manner described in the Agilent product user guides.**

WARNING

Electrical shock

Repair work at the module can lead to personal injuries, e.g. shock hazard, when the cover is opened.

- ✓ **Do not remove the cover of the module.**
- ✓ **Only certified persons are authorized to carry out repairs inside the module.**

WARNING

Sharp metal edges

Sharp-edged parts of the equipment may cause injuries.

- ✓ **To prevent personal injury, be careful when getting in contact with sharp metal areas.**

WARNING

Toxic, flammable and hazardous solvents, samples and reagents

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

- ✓ **When working with these substances observe appropriate safety procedures (for example by wearing goggles, safety gloves and protective clothing) as described in the material handling and safety data sheet supplied by the vendor, and follow good laboratory practice.**
- ✓ **The volume of substances should be reduced to the minimum required for the analysis.**
- ✓ **Do not operate the instrument in an explosive atmosphere.**

CAUTION

Hot heat exchangers 

The column compartment has two heat exchanger assemblies that might be hot.

- ✓ **Allow them to cool down before starting repairs.**

CAUTION

Safety standards for external equipment

- ✓ **If you connect external equipment to the instrument, make sure that you only use accessory units tested and approved according to the safety standards appropriate for the type of external equipment.**

Overview of Maintenance

The following pages describe maintenance procedures (simple repairs) that can be done without opening the main cover.

Table 26 Maintenance procedures

Procedure	Typical Frequency	Notes
Cleaning the Module	If required	
Correcting Leaks	If a leak has occured	Check for leaks
Maintain the Column Switching Valve	If valve leaks	
Replace Valve Heads	If the valve performance shows indication of leakage or wear	
Replacing Parts of the Valve Head	If leak sensor is defective	
Replacing the Fuses of the Infinity Valve Drive	When a fuse is defective	
Replace the Module Firmware	If required	

Cleaning the Module

To keep the module case clean, use a soft cloth slightly dampened with water, or a solution of water and mild detergent.

WARNING

Liquid dripping into the electronic compartment of your module can cause shock hazard and damage the module

- ✓ **Do not use an excessively damp cloth during cleaning.**
- ✓ **Drain all solvent lines before opening any connections in the flow path.**

Correcting Leaks

Correcting Leaks (G7116B)

When

If a leakage has occurred at the heat exchanger or at the capillary connections or at the column switching valve.

Tools required

Description

Tissue

Pipette

Wrench, 1/4 – 5/16 inch
(for capillary connections)

1. Remove the door.
2. Use a pipette and tissue to dry the leak sensor area.
3. Observe the capillary connections and the column switching valve for leaks and correct, if required.
4. Re-install the door.

Correcting Leaks (G1170A)

When

If leakage has occured at the capillary connections or at the valve.

Tools required

Description

Tissue

Pipette

Wrench, 1/4 – 5/16 inch
(for capillary connections)

1. Use a pipette and tissue to dry the leak sensor area.
2. Observe the capillary connections and the valve for leaks and correct, if required.

Replace Valve Heads

Replace Valve Heads (G7116B)

Several optional valve heads are available, which can be installed and exchanged easily.

Parts required	Description
	Any Agilent Quick Change Valve Head.

CAUTION

The valve actuator contains sensitive optical parts, which need to be protected from dust and other pollution. Pollution of these parts can impair the accurate selection of valve ports and therefore bias measurement results.

- ✓ **Always install a valve head for operation and storage. For protecting the actuator, a dummy valve head (part of Transportation Lock Kit (G1316-67001)) can be used instead of a functional valve. Do not touch parts inside the actuator.**

CAUTION

Column Damage or Bias Measurement Results

Switching the valve to a wrong position can damage the column or bias measurement results.

- ✓ **Fit the lobe to the groove to make sure the valve is switched to the correct position.**

CAUTION

Valve Damage

Using a low pressure valve on the high pressure side can damage the valve.

- ✓ **When using multiple column compartments as part of a method development solution, make sure that the high pressure valve head is connected to the autosampler and the low pressure valve head is connected to the detector.**

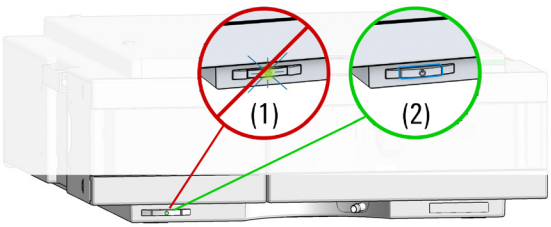
WARNING

Toxic, flammable and hazardous solvents, samples and reagents

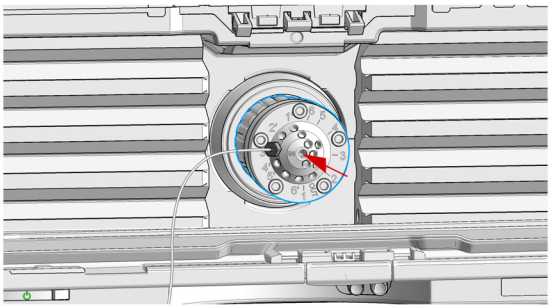
The handling of solvents, samples and reagents can hold health and safety risks.

- ✓ **Be sure that no solvent can drop out of the solvent connections when removing them from your valve head.**
- ✓ **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.**

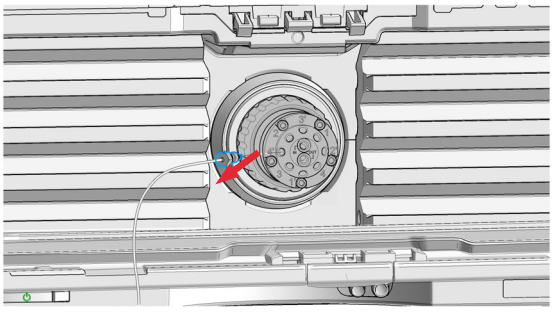
1 Switch off the module.



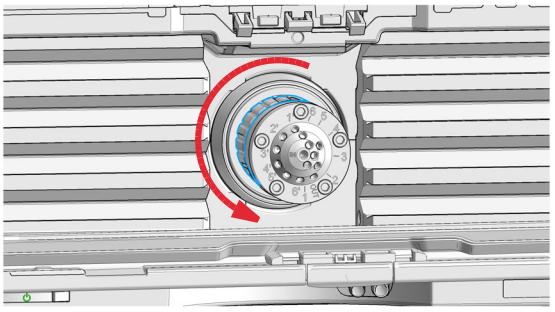
2 Push the valve head to bring it to its outer position.



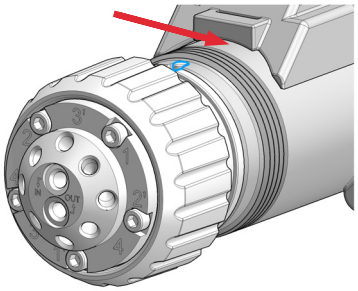
3 Remove all capillary connections from the valve head.



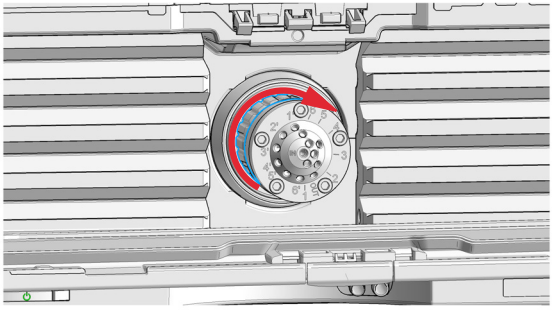
4 Unscrew the valve head.



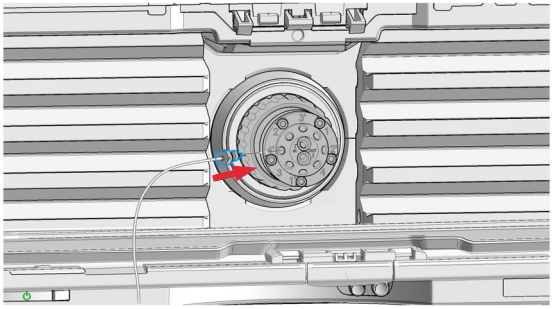
5 Put the new valve head onto the valve drive such that the lobe fits to the groove.



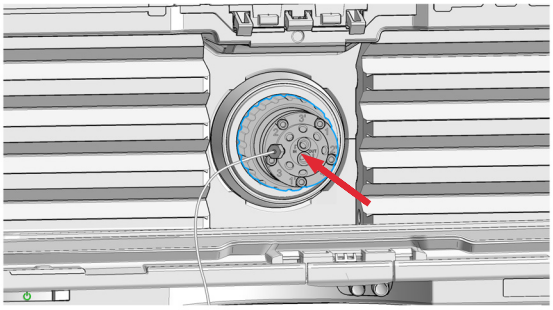
6 Screw the valve head onto the valve drive using the union nut.



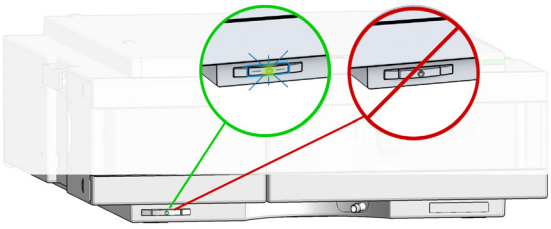
7 Install all required capillary connections to the valve.



8 Push the valve head until it snaps in and stays in the rear position.



9 Switch on the module.



Replace Valve Heads (G1170A)

The following procedure shows installation only. To remove the valve, follow the instructions in reverse order.

NOTE The following procedure exemplarily shows a valve head installation. For correct capillary connections see **Valve topology** in the GUI.

CAUTION **The valve actuator contains sensitive optical parts, which need to be protected from dust and other pollution. Pollution of these parts can impair the accurate selection of valve ports and therefore bias measurement results.**

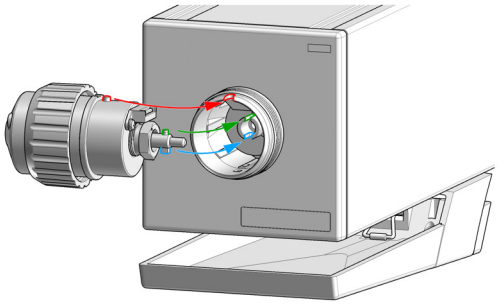
✓ **Always install a valve head for operation and storage. For protecting the actuator, a dummy valve head can be used instead of a functional valve. Do not touch parts inside the actuator.**

NOTE For a correct installation of the valve head, the outside pin (red) must completely fit into the outside groove on the valve drive's shaft (red). A correct installation is only possible if the two pins (green and blue) on the valve head fit into their corresponding grooves on the valve drive's actuator axis. Their match depends on the diameter of the pin and groove.

NOTE The tag reader reads the valve head properties from the valve head RFID tag during initialization of the module. Valve properties will not be updated, if the valve head is replaced while the module is on. Selection of valve port positions can fail, if the instrument does not know the properties of the installed valve.

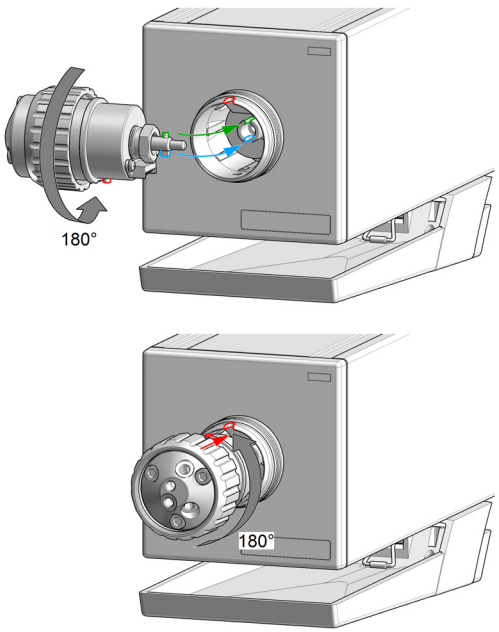
NOTE To allow correct valve identification, power off the module for at least 10 s.

1 Insert the valve head into the valve shaft.

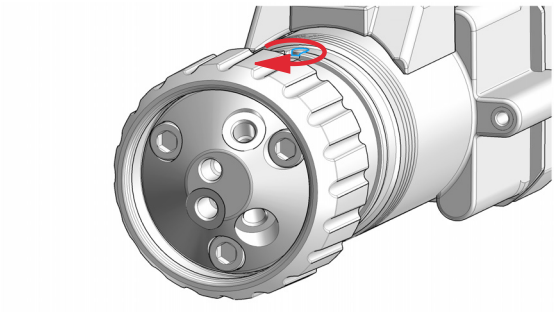


OR

If the outside pin does not fit into the outside groove, you have to turn the valve head until you feel that the two pins snap into the grooves. Now you should feel additional resistance from the valve drive while continuously turning the valve head until the pin fits into the groove.

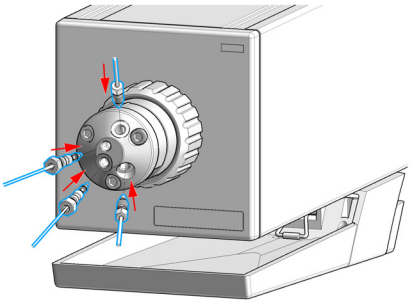


2 When the outer pin is locked into the groove, manually screw the nut onto the valve head.



NOTE

Fasten the nut with the 5043-1767 Valve Removal tool.

3 Install all required capillary connections to the valve. 	**4** Power on or power-cycle your module, so the valve head gets recognized during module initialization.

Replacing Parts of the Valve Head

When If valve leaks.

Tools required	**Description**
	Wrench, 1/4 inch
OR	Hexagonal key, 9/64 inch

1 Remove capillaries from ports.
2 Loosen each fixing stator screw two turns at a time. Remove bolts from head.
3 Remove the stator head (and stator face if applicable).
4 Remove the stator ring.
5 Remove the rotor seal (and isolation seal if damaged or contaminated).
6 Install the new isolation seal (if required). Ensure the metal spring inside the ring faces towards the valve body.
7 Install the new rotor seal.
8 Replace the stator ring. Ensure the stator ring is flush with the valve body.
9 Place the new (if required) stator face in place on the stator head. Reinstall the stator head.
10 Insert the stator screws in the stator head. Tighten the screws alternately two turns at a time until the stator head is secure.
11 Reconnect the pump capillaries to the valve ports.

CAUTION

Wrong use of Pressure Test may damage valve.

The current implementation of the Pressure Test automatically uses the maximum pressure generated by the pump used in the system.

✓ **Do not use the test for modules having a lower maximum pressure than the pump as this will damage the valve. For example do not use 400 bar valve in a TCC or Flexible Cube in combination with a 600 bar pump.**

12 Perform a **Pressure Test** to ensure the valve is pressure tight.

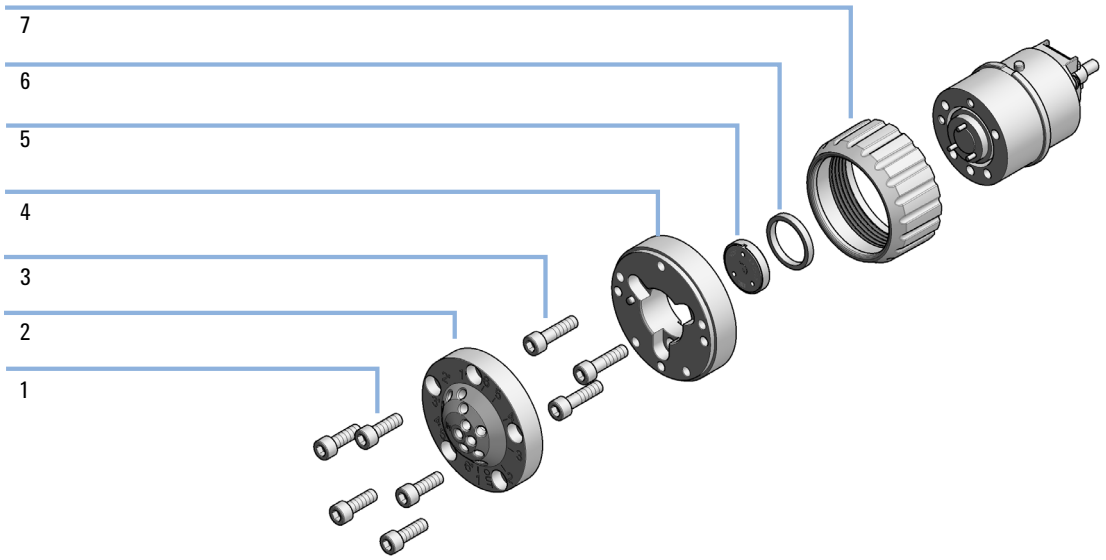


Figure 116 Valve Head Parts (example)

1	Stator screws
2	Stator head assembly
3	Stator ring screws (not available)
4	Stator ring (available for service only)
5	Rotor seal
6	Bearing ring
7	Spanner nut (available for service only)

NOTE

Figure 116 on page 305 illustrates replacement parts for the valve heads, with the 6-column Selector valve as an example. The valves can vary in their appearance and do not necessarily include all of the illustrated parts. Neither, every spare part is available for each flavor of the valve.

Replacing the Fuses of the Infinity Valve Drive

When If the flow module shows no reaction.

Tools required

Description
Screwdriver

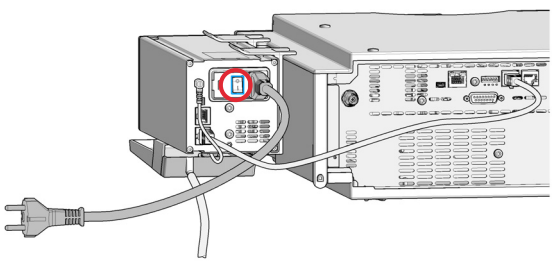
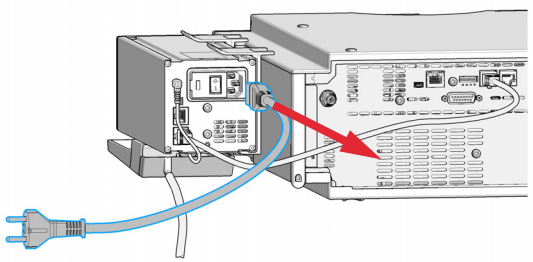
Parts required

#	p/n	Description
2	2110-1486	Fuse 2 AT250 V

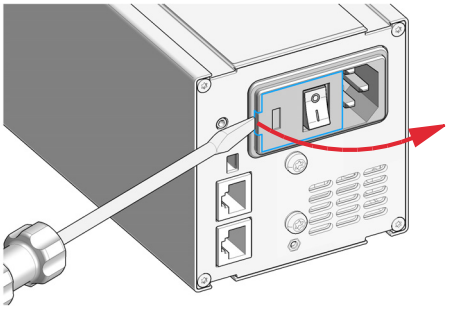
WARNING

Electrical shock

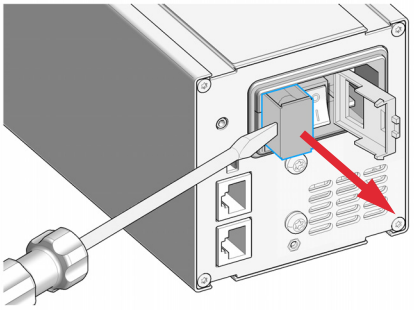
- ✓ **Disconnect the module from line power before changing a fuse or trying to open the hatch of the power input socket.**
- ✓ **Never reconnect the line power before havingthe power input socket closed.**

1 Switch off the instrument. The line switch is located at the rear of the module. 	**2** Disconnect the power cable from the power input socket. 

3 To access the fuse drawer, gently lift the outer plastic housing of the power inlet socket using a flat screwdriver.

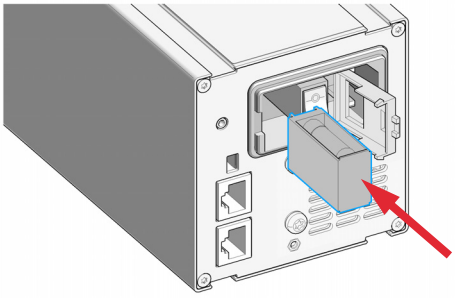


4 Pull out the fuse drawer as shown.

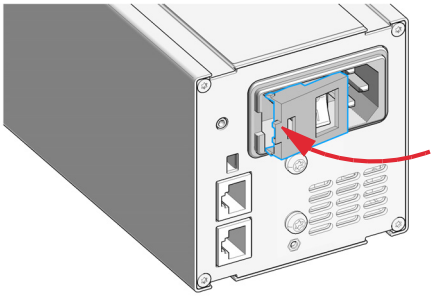


5 Replace the defect fuse(s).

6 Slide in the fuse drawer and push till it fits tightly.



7 Finally, close the fuse drawer housing, reconnect the instrument to the power line and switch it on.



Replace the Module Firmware

When

The installation of newer firmware might be necessary
- if a newer version solves problems of older versions or
- to keep all systems on the same (validated) revision.

The installation of older firmware might be necessary
- to keep all systems on the same (validated) revision or
- if a new module with newer firmware is added to a system or
- if third party control software requires a special version.

Tools required

Description
Agilent Lab Advisor software

Parts required

#	Description
1	Firmware, tools and documentation from Agilent web site

Preparations

Read update documentation provided with the Firmware Update Tool.

To upgrade/downgrade the module's firmware carry out the following steps:

1. Download the required module firmware, the latest FW Update Tool and the documentation from the Agilent web.
   http://www.agilent.com/en-us/firmwareDownload?whid=69761
2. For loading the firmware into the module follow the instructions in the documentation.

Module Specific Information

There is no specific information for this module.

12 Parts for Maintenance

This chapter provides information on parts material required for the solution.

Parts for the 1290 Infinity II 2D-LC System

2D-LC Loops

2D-LC Loops for Standard 2D-LC

p/n	Description
5067-5440	Calibrated loop kit for 2D-LC Internal part number, not orderable
5067-5446	Loop housing kit
5067-5424	20 µL Loop 2D-LC
5067-5425	40 µL Loop 2D-LC
5067-5437	60 µL Loop 2D-LC
5067-5426	80 µL Loop 2D-LC
5004-0036	180 µL Loop 2D-LC
5500-1238	Capillary ST 0.12 mm x 105 mm SL/SL (Bypass Capillary)

2D-LC Loops for MHC valve Fitting M4

p/n	Description
5067-6643	Capillary ST 0.35x104 mm, M/M, 10
5067-6644	Capillary ST 0.35x208 mm, M/M, 20 µL
5067-5926	Capillary ST 0.35x 420 mm M/M 40 µl
5067-6645	Capillary ST 0.35x831 mm, M/M, 80 µL
5067-6646	Capillary ST 0.35x1247 mm, M/M, 120 µL
5067-6647	Capillary ST 0.35x1870 mm, M/M, 180 µL
5067-6141	M4 Blank nut
5023-2504	Hex driver SW-4 slitted

2D-LC Capillaries

1200 Infinity Series 2D-LC Capillary Kit

p/n	Description
5021-1820	Flex capillary, 0.12 mm x 105 mm, no fittings
G1316-87321	Capillary column-heat exchanger 105 mm lg, 0.17 mm i.d.
5021-1822	Capillary, 0.12 mm x 280 mm
5021-1823	Capillary column – detector SST 400 mm lg, 0.12 mm i.d.
5021-1819	Capillary ST 0.17 mm x 400 mm S/S
5065-9964	Capillary ST 0.12 mm x 500 mm
5067-4609	Capillary ST 0.17 mm x 500 mm SX/-
5067-4669	Capillary ST 0.12 mm x 600 mm S/SL
01078-87305	Capillary, 0.17 mm x 80 cm, male fit
5065-4454	Fitting screw long, front and back ferrules 10/pk
G1316-60005	Low Dispersion Heat Exchanger Double Assy
G7116-60015	Quick Connect Heat Exchanger Standard
5500-1188	Quick Turn Capillary ST 0.12 mm x 105 mm, long socket

InfinityLab 2D-LC Capillary Kit legacy

p/n	Description
5067-4651	Capillary ST 0.12 mm x 280 mm SL/SX
5067-4669	Capillary ST 0.12 mm x 600 mm S/SL
5500-1245	Capillary ST 0.17 mm x 400 mm SI/SI
5500-1251	Capillary ST 0.12 mm x 400 mm SL/SL
5500-1240	Capillary ST 0.17 mm x 105 mm SL/SL
5500-1227	Capillary ST 0.17 mm x 150 mm SL-SL
5500-1217	Capillary, ST, 0.17 mm x 900 mm SI/SX
5067-4608	Capillary ST 0.17 mm x 280 mm SX/S
5067-4670	Capillary ST 0.17 mm ID 600 mm pre-swaged

ASM Capillaries

ASM Valve Capillary Replacement Kit

p/n	Description
5500-1300	Capillary ST 0.12 mm x 85 mm M/M
5500-1301	Capillary ST 0.12 mm x 170 mm M/M
5500-1302	Capillary ST 0.12 mm x 340 mm M/M
5500-1303	Capillary ST 0.12 mm x 680 mm M/M
5500-1376	Capillary ST 0.12 mm x 170 mm M/M

Pressure Release Kit

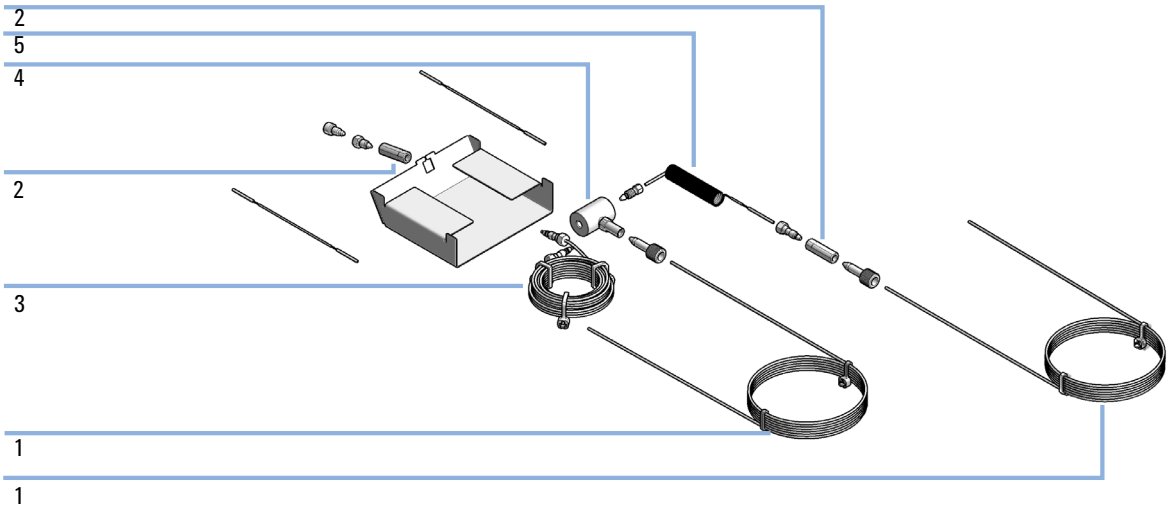


Figure 117 Pressure release kit, parts

Item	p/n	Description
	G4236-60010	2D-LC Pressure Release Kit
	0100-0969	TEE, ST, 1/16 inch, Low Dead Volume Not shown
1	5021-1816	Capillary i.d. 0.17 mm, 105 mm lg
2	5022-2184	Union, stand LC flow, no fitting
3	G7167-87307	500 µL Loop extension
4	G4212-60022	Pressure Relief Valve
5	5067-5939	Splitter-Capillary 0.05-ID L-1000 mm

2D-LC Easy Starter Kit

2D-LC Easy Starter Kit for ESZ Service (G4236-68100) not orderable internal part number

Item	p/n	Description
1	5190-6895	2D-LC starter sample, 1 x 2 mL
2	G2453-85060	Formic Acid-Reagent Grade 5 mL (5 cc)
3	685775-902	Poroshell SB-C18, 2.1 x 100 mm, 2.7 µm
4	699968-301	Poroshell 120 Bonus-RP, 3.0 x 50 mm, 2.7 µm

2D-LC Easy Starter Kit (legacy) (G4236-68000) not orderable internal part number

p/n	Description
5190-6895	2D-LC starter sample, 1 x 2 mL
G2453-85060	Formic Acid-Reagent Grade 5 mL (5 cc)
858700-902	RRHD SB-C18, 2.1x100 mm, 1.8 µm, 1200 bar
857768-901	RRHD Bonus-RP, 2.1x50 mm, 1.8 µm, 1200 bar
959757-302	RRHD Eclipse Plus C18, 3.0x50 mm, 1.8 µm

Valve Drive Parts

Item	p/n	Description
1	5043-0275	Clamp guide For attaching the valve to a rail assembly
2	5067-4792	Leak sensor assembly External leak sensor
3	5043-0271	Holder leak plane
4	5043-0270	Leak plane
5	5068-0106	Spanner nut
	2110-1486	Fuse 2 AT250 V
6	5067-4634	Valve rail assembly
7	5067-1510	Rail assy for column organizer

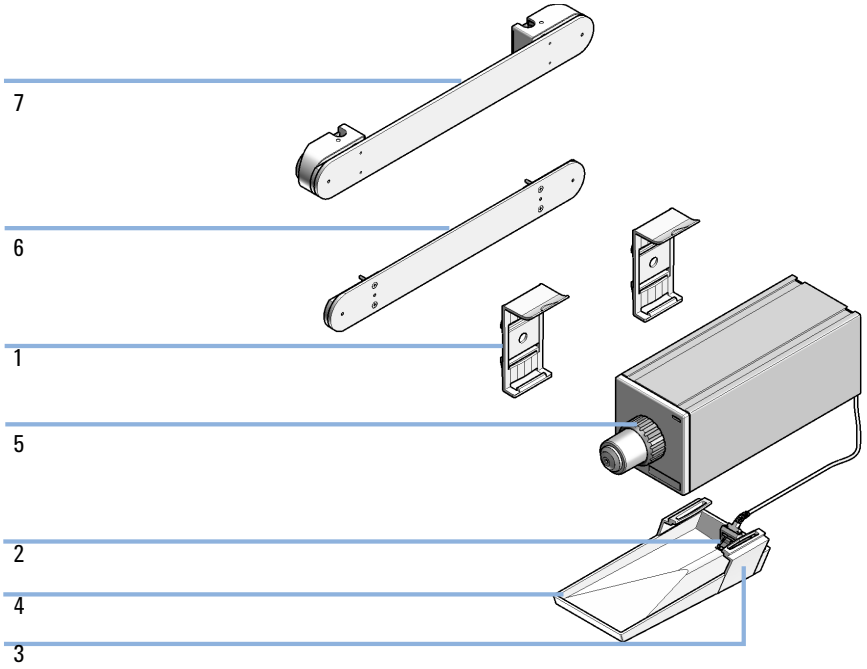


Figure 118 Parts for 1290 Infinity Valve Drive

Valve Driver Parts Infinity II

Item	p/n	Description
1	5067-6138	Valve Holder Kit Right-IF-II-G, For G7116A/B
	5067-6139	Valve Holder Kit Left-IF-II-G, For G7116A/B (Not shown)
2	5067-5685	Clamp Guide Kit-IF-II
3	5067-4792	Leak sensor assembly, External leak plane
4	5043-0271	Holder leak plane
5	5043-0270	Leak plane
6	2110-1486	Fuse 2 AT250 V
7	5063-6527	Tubing, Silicon Rubber, 1.2 m, ID/OD 6/9 mm
8	5181-1519	CAN cable, Agilent module to module, 1 m
9	5500-1156	T-Tube Connector ID6.4
10	5043-0269	Adapter-profile, For G1170A (Multiple valve drives can be connnected with adapter profiles)

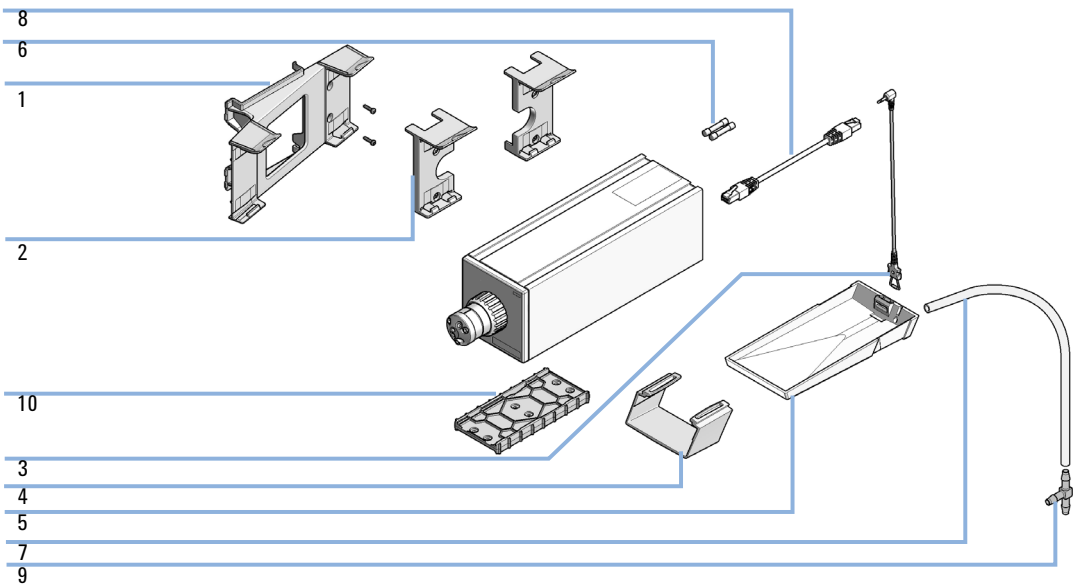


Figure 119 Parts for 1290 Infinity II Valve Drive

Valve Head Parts

NOTE

The figure below illustrates replacement parts for the valve heads, with the 12-position/13-port Selector valve as an example. The valves can vary in their appearance and do not necessarily include all of the illustrated parts. Neither, every spare part is available for each flavor of the valve.

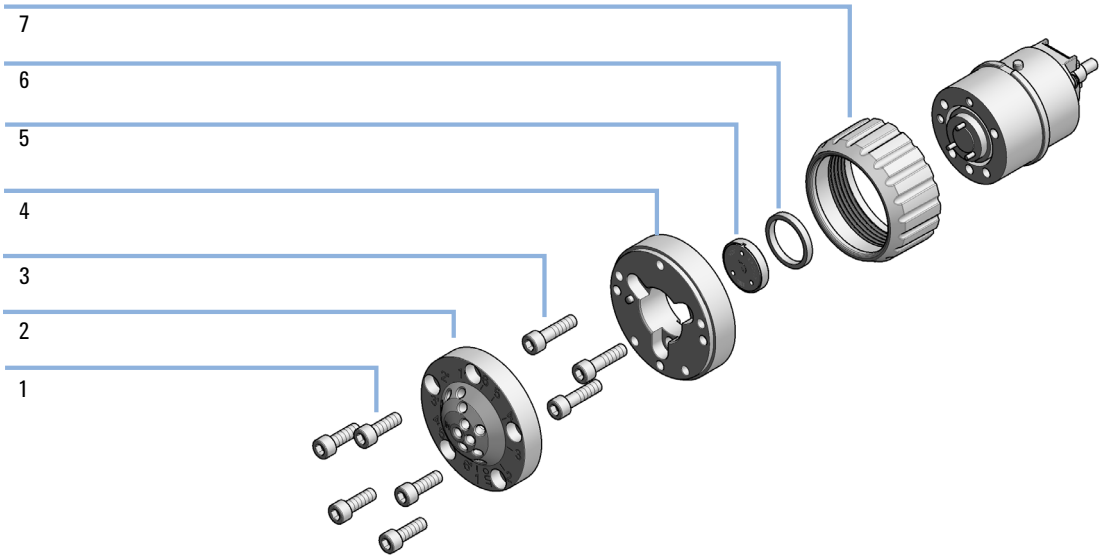


Figure 120 Valve Head Parts (example)

1	Stator screws
2	Stator head assembly
3	Stator ring screws (not available)
4	Stator ring (available for service only)
5	Rotor seal
6	Bearing ring
7	Spanner nut (available for service only)

Technical specifications

Table 27 Technical specifications

Max. Pressure:	1300 bar
Liquid Contacts:	Stainless Steel, PEEK
Connections:	Accepts 10-32 male threaded and M4 fittings

Tools

Tool for extra fittings

p/n	Description
8710-2462	Hex Key Driver 3/32 inch
5023-2504	Hex driver SW-4 slitted For M4 fittings
5067-6141	M4 Blank nut For plugging unused valve ports
5067-6127	Blank Nut SL

Valve Options Overview (for 2D-LC)

The 1300 bar InfinitLab Quick Change Valves are backward compatible to the 1200 bar Valves.

NOTE

The service life of a stator depends on the stress to which the 2D-LC valve is subjected. Therefore, a visual inspection of the surface during maintenance is very important. If scratches or heavy wear is visible during the inspection, the stator must be replaced.

G4136A 2D-LC Valve Kit, Standard

#	p/n	Description
1	5067-4244	2D-LC Valve Head, 1300 bar
1	5067-5440	Calibrated loop kit for 2D-LC
1	5067-6171	Capillary Kit 2D-LC, Infinity Classic (optional) Internal part, not orderable
1	G4236-68100	2D-LC Easy Starter Kit for ESZ Service Internal part, not orderable
2	G4242-64000	Multiple Heart-Cutting Valve
1	5067-6585	Capillary Kit 2D-LC, 1290 Infinity II Internal part, not orderable
1	G1680-63721	Network LAN Switch

5067-4244 2D-LC Valve Head, 1300 bar

#	p/n	Description
3	1535-4857	Stator screws, 10/pk
1	1535-4045	Bearing ring
1	5068-0214	Rotor Seal (VHP)
1	5068-0120	Stator ring
1	5068-0115	Stator

G4243A 2D-LC Valve Kit, ASM

#	p/n	Description
1	5067-4266	2D-LC ASM Valve Head, 1300 bar
1	G4236-68100	2D-LC Easy Starter Kit for ESZ Service Internal part, not orderable
1	G1680-63721	Network LAN Switch
1	5500-1300	Capillary ST 0.12 mm x 85 mm M/M
1	5500-1301	Capillary ST 0.12 mm x 170 mm M/M
1	5500-1302	Capillary ST 0.12 mm x 340 mm M/M
1	5500-1303	Capillary ST 0.12 mm x 680 mm M/M
4	5500-1376	Capillary ST 0.12 mm x 170 mm M/M (Transfer Capillary)

5067-4266 2D-LC ASM Valve Head, 1300 bar

p/n	Description
5068-0019	Stator screws
5068-0257	Bearing Ring
5068-0240	Rotor Seal (VHP)
5068-0239	Stator

G4242-64000 Multiple Heart-Cutting Valve

#	p/n	Description
1	5067-4273	6-column selector valve head, 1300 bar
6	5067-5926	Capillary ST 0.35x 420 mm M/M 40 µl
2	5500-1270	Capillary ST 0.12 mm x 170 mm S/M (Transfer Capillary)
1	5043-0269	Adapter-profile

5067-4273 6-column selector valve head, 1300 bar

#	p/n	Description
5	5068-0089	Stator screws
1	1535-4045	Bearing ring
1	5068-0242	Rotor Seal (PEEK)
1	5068-0241	Stator Head

Obsolete Valve Heads

The following 1200 bar valve heads are no longer orderable:

5067-4214 2D-LC Valve 1200 bar legacy

p/n	Description
5068-0186	Rotor Seal (Vespel)
5068-0115	Stator
1535-4857	Stator screws, 10/pk
1535-4045	Bearing ring

Multiple Heart-Cutting Valve legacy

#	p/n	Description
1	5067-4142	Valve head 6 column selector (1200 bar)
6	5067-5926	Capillary ST 0.35x 420 mm M/M 40 µl
1	5974-0197	Capillary Cover Label
2	5067-5113	Capillary ST 0.17 mm x 250 mm SL/M
2	5067-6188	Capillary ST 0.17 mm x 500 mm SL-M

5067-4142 6-Column selector valve 1200 bar legacy

p/n	Description
5068-0077	Stator Head
5068-0067	Rotor Seal (Vespel)
5068-0089	Stator screws
1535-4045	Bearing ring

MS Diverter Valve

G4231A 2-position/6-port valve head, 800 bar

p/n	Description
5067-4282	2-position/6-port valve head, 800 bar
5067-4730	2/10 Cap kit 0.17 mm
5067-4249	2/6 Cap Kit 0.12 mm, incl. QC-HEx
5067-4250	2/6 Cap Kit 0.12 mm, incl. LD-HEx
5067-6597	2/6 Cap Kit 0.17 mm, incl. QC-HEx

Alternative diverter valves (2 position / 6 port, PEEK Rotor Seal)

p/n	Description
5067-4137	2-postion/6-port valve head, 600 bar
5067-4282	2-position/6-port valve head, 800 bar
0101-1409	Rotor Seal (PEEK)

Alternative diverter valves (2-position/10-port, PEEK Rotor Seal)

p/n	Description
5067-4145	2-position/10-port valve head, 600 bar
5067-4283	2-position/10-port valve head, 800 bar
0101-1415	Rotor Seal (PEEK)

Alternative diverter valves (2-position/6-port, Vespel Rotor Seal)

p/n	Description
5067-4117	2-position/6-port ultra high pressure valve head, 1200 bar
5068-0008	Rotor Seal (Vespel)

Alternative diverter valves (2-position/10-port, Vespel Rotor Seal)

p/n	Description
5067-4118	2-position/10-port ultra high pressure valve head, 1200 bar
5068-0012	Rotor Seal (Vespel)

Additional Parts for the MS Diverter Valve Setup

p/n	Description
G4212-60022	Pressure Relief Valve
5067-4606	Capillary ST 0.12 mm x 400 mm SX/-
0890-1915	Capillary PK 0.13 mm x 150 cm
5500-1228	Capillary ST 0.3 mm x 80 mm SL-SL
5063-6591	PEEK Fittings 10/PK
0100-0969	TEE, ST, 1/16 inch, Low Dead Volume
5067-6127	Blank Nut SL
5062-2462	Tube PTFE 0.7 mm x 5 m, 1.6 mm od

Valve Options Overview (for G7116B)

Valve Options Overview (G7116B)

Table 28 Replacement parts standard valve heads for G7116B

Valve Head	Rotor Seal	Stator Head	Stator Screws	Stator Ring
5067-4233 8-Position/18-Port Valve 1300 bar	5068-0200 (PEEK)	5068-0199	5068-0089	n.a.
5067-4241 2-Position/6-Port Valve 1300 bar	5068-0207 (PEEK)	5068-0006	1535-4857	5068-0120
5067-4240 2-Position/10-Port Valve 1300 bar	5068-0205 (PEEK)	5068-0011	5068-0019	n.a.
5067-4273 6-Position/14-Port Valve 1300 bar	5068-0242 (PEEK)	5068-0241	5068-0089	n.a.
5067-4284 6-Position/14-Port Valve 800 bar	5068-0298 (PEEK)	5068-0241	5068-0089	n.a.
5067-6682 2-Position/10-Port Valve Bio 1300 bar	5068-0205 (PEEK)	5068-0286	5068-0019	n.a.
5067-4237 8-Position/9-Port Valve 1300 bar	5068-0202 (PEEK)	5068-0001	1535-4857	5068-0120

Obsolete Valve Heads

The following 1200 bar valve heads are no longer orderable:

Table 29 Replacement parts obsolete valve heads for G7116B

Valve Head	Rotor Seal	Stator Head	Stator Screws	Stator Ring
5067-4121 8-Position/9-Port Valve 1200 bar	5068-0002 (Vespel)	5068-0001	1535-4857	5068-0127
5067-4117 2-Position/6-Port Valve 1200 bar	5068-0008 (Vespel)	5068-0006	1535-4857	5068-0127
5067-4118 2-Position/10-Port Valve 1200 bar	5068-0012 (Vespel)	5068-0011	5068-0019	n.a.
5067-4142 6-Position/14-Port Valve 1200 bar	5068-0067 (Vespel)	5068-0077	5068-0089	n.a.

Additional Heater Devices

Table 30 Heat Exchanger Overview

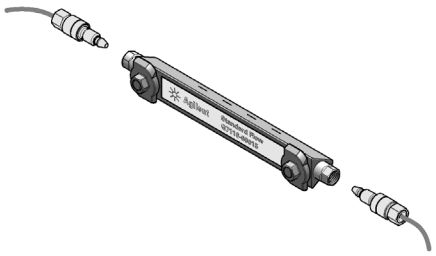
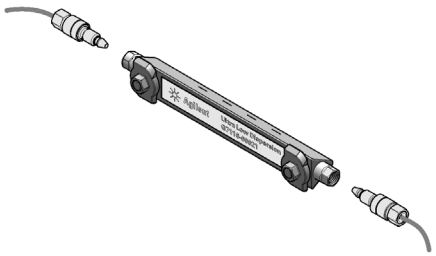
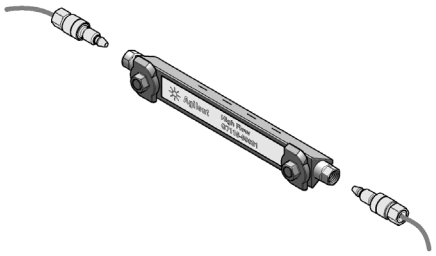
Flow rate	0.075 mm i.d. capillary	0.12 mm i.d. capillary
< 2 mL/min	*Ultra-low Dispersion* G7116-60021 (Internal volume: 1.0 µL)	*Standard Flow* G7116-60015 (Internal volume: 1.6 µL)
> 2 mL/min		*High Flow* G7116-60031 (Internal volume: 3.0 µL)

For details, see Table 31 on page 328.

Additional Heater Devices (for G7116B)

Blank heater assemblies without capillaries and fittings:

Table 31 InfinityLab Quick Connect Heat Exchanger

Item	Description
	Quick Connect Heat Exchanger Standard (G7116-60015)
	Quick Connect Heat Exchanger Ultra Low Dispersion (G7116-60021) **NOTE** Use InfinityLab Quick Turn Fittings to connect to the Quick Connect Heat Exchanger Ultra Low Dispersion.
	Quick Connect Heat Exchanger High Flow (G7116-60031)

Accessories and Consumables (for G7116B)

G7116-68705 Accessory Kit (for G7116B)

The Accessory Kit (for G7116B) contains accessories and tools needed for the installation and maintenance.

p/n	Description
5181-1516	CAN cable, Agilent module to module, 0.5 m
5063-6527	Tubing, Silicon Rubber, 1.2 m, ID/OD 6/9 mm
5500-1191	InfinityLab Quick Turn Capillary ST 0.12 mm x 280 mm, long socket
5067-5966	InfinityLab Quick Turn Fitting
5067-5957	InfinityLab Quick Connect Assy ST 0.12 mm x 105 mm
G7116-60015	Quick Connect Heat Exchanger Standard
G7116-68003	Column Holder Lamella, 2/pk (delivered as a part of G7116-60015)
5043-0915	Fitting mounting tool
G7116-60006	Divider Assembly MCT
5022-2184	Union, stand LC flow, no fitting
	Double Drain Connector

Available Consumables (for G7116B)

p/n	Description
G7116-68003	Column Holder Lamella, 2/pk
G7116-68004	Column Holder Clamp, 2/pk
5500-1191	InfinityLab Quick Turn Capillary ST 0.12 mm x 280 mm, long socket Capillary from column outlet to DAD, no fittings.
G7116-60006	Divider Assembly MCT For separating different temperature zones between left and right heater elements.
5067-5917	InfinityLab Column Identification Tag Blank column ID tag (column ID tag reader kit is required)
G7116-60013	InfinityLab Thermal Equilibration Device

Number Kit

p/n	Description
5067-6654	Number Kit 1-8 colored Column Info in red, blue, green, cyan, yellow, black, white, and gray

InfinityLab Quick Connect and Quick Turn Fittings

For further info check either the consumables catalog or “Important Customer Web Links” on page 365.

InfinityLab Quick Connect Fittings

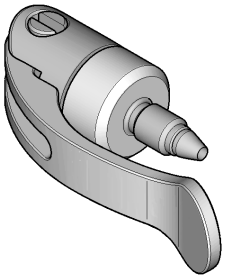


Figure 121 InfinityLab Quick Connect Fitting

p/n	Description
5067-5965	InfinityLab Quick Connect LC fitting (fitting without preinstalled capillary)
5043-0924	Front Ferrule for Quick Connect/Turn Fitting
5067-5961	InfinityLab Quick Connect Assy ST 0.075 mm x 105 mm
5067-6163	InfinityLab Quick Connect Assy ST 0.075 mm x 150 mm
5067-6164	InfinityLab Quick Connect Assy ST 0.075 mm x 220 mm
5067-6165	InfinityLab Quick Connect Assy ST 0.075 mm x 280 mm
5067-5957	InfinityLab Quick Connect Assy ST 0.12 mm x 105 mm
5067-5958	InfinityLab Quick Connect Assy ST 0.12 mm x 150 mm
5067-5959	InfinityLab Quick Connect Assy ST 0.12 mm x 220 mm
5067-5960	InfinityLab Quick Connect Assy ST 0.12 mm x 280 mm
5067-6166	InfinityLab Quick Connect Assy ST 0.17 mm x 105 mm
5067-6167	InfinityLab Quick Connect Assy ST 0.17 mm x 150 mm
5067-6168	InfinityLab Quick Connect Assy ST 0.17 mm x 220 mm
5067-6169	InfinityLab Quick Connect Assy ST 0.17 mm x 280 mm

InfinityLab Quick Connect Fitting Replacement Capillaries

p/n	Description
5500-1174	InfinityLab Capillary ST 0.075 mm x 105 mm
5500-1175	InfinityLab Capillary ST 0.075 mm x 150 mm
5500-1176	InfinityLab Capillary ST 0.075 mm x 220 mm
5500-1177	InfinityLab Capillary ST 0.075 mm x 250 mm
5500-1178	InfinityLab Capillary ST 0.075 mm x 280 mm
5500-1173	InfinityLab Capillary ST 0.12 mm x 105 mm
5500-1172	InfinityLab Capillary ST 0.12 mm x 150 mm
5500-1171	InfinityLab Capillary ST 0.12 mm x 220 mm
5500-1170	InfinityLab Capillary ST 0.12 mm x 280 mm
5500-1179	InfinityLab Capillary ST 0.12 mm x 400 mm
5500-1180	InfinityLab Capillary ST 0.12 mm x 500 mm
5500-1181	InfinityLab Capillary ST 0.17 mm x 105 mm
5500-1182	InfinityLab Capillary ST 0.17 mm x 150 mm
5500-1183	InfinityLab Capillary ST 0.17 mm x 220 mm
5500-1230	InfinityLab Capillary ST 0.17 mm x 280 mm
5500-1231	InfinityLab Capillary ST 0.17 mm x 500 mm
5500-1259	InfinityLab Capillary ST 0.25 mm x 150 mm
5500-1260	InfinityLab Capillary ST 0.25 mm x 400 mm

InfinityLab Quick Turn Fitting

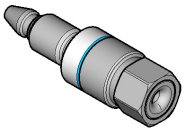


Figure 122 InfinityLab Quick Turn Fitting

p/n	Description
5067-5966	InfinityLab Quick Turn Fitting
5043-0924	Front Ferrule for Quick Connect/Turn Fitting

Capillary Kits

NOTE

Further capillary kits can be found in the *Agilent 1290 Infinity Valve Drive and Valve Heads User Manual* or on the webpage.

Table 32 Common capillary kit

Part Number	Connection	Amount
Capillary ST 0.12 mm x 340 mm S/SX (5067-4647)	Autosampler to valve	1
Capillary ST 0.17 mm x 700 mm S/SX (5067-4648)	^{2}D pump to valve	1
Capillary ST 0.12 mm x 90 mm S/SX (5067-4649)	Valve to heat exchanger	2
Capillary ST 0.12 mm x 150 mm SL/SX (5067-4650)	Short column to valve	2
Capillary ST 0.12 mm x 280 mm SL/SX (5067-4651)	Long column to valve	2
Capillary ST 0.12 mm x 120 mm SX/SX (5067-4652)	Valve to valve	1
Capillary ST 0.12 mm x 200 mm S/SX (5067-4653)	Valve to detector	1
Waste tubing, 2 m (0890-1713)	Valve to waste	2 m
Waste tube (G1375-87326) (includes M4 PEEK fitting)	Valve to waste	1
Plastic fitting (0100-1259)		4
Bag - plastics (9222-0518)		1

Parts for the 1290 Infinity II Bio 2D-LC System

Distinctive Features of the Biocompatible Capillaries

BIO

For 1290 Infinity II Bio LC modules, use bio / biocompatible parts only.
Do not mix parts between 1260 Infinity II Bio-Inert LC modules and 1290 Infinity II Bio LC modules.

Identification of the biocompatible capillaries:

- Biocompatible capillaries are made of MP35N material
- Capillaries look similar to standard stainless steel capillaries
- MP35N capillaries are marked with an orange stripe on the PTFE tube
- The other color of the PTFE tube codes the inner diameter

Figure 123 Color code for biocompatible capillaries

For correct installation of capillary connections it's important to choose the correct fittings, see "Syntax for Capillary Description" on page 461.

CAUTION

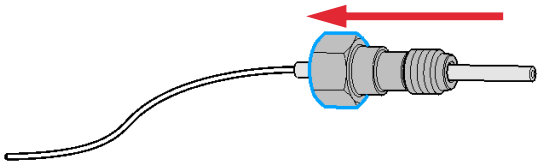
MP35N is harder than stainless steel.

Damage to the gold-plated ferrule.

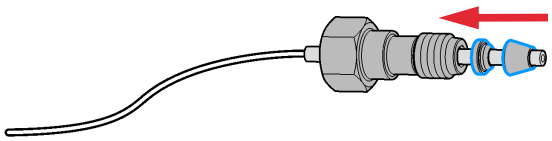
✓ **Do not overtighten the capillaries (finger tight + first resistance with the key + ¼ of a turn with the key).**

1 Select a nut that is long enough for the fitting you'll be using.

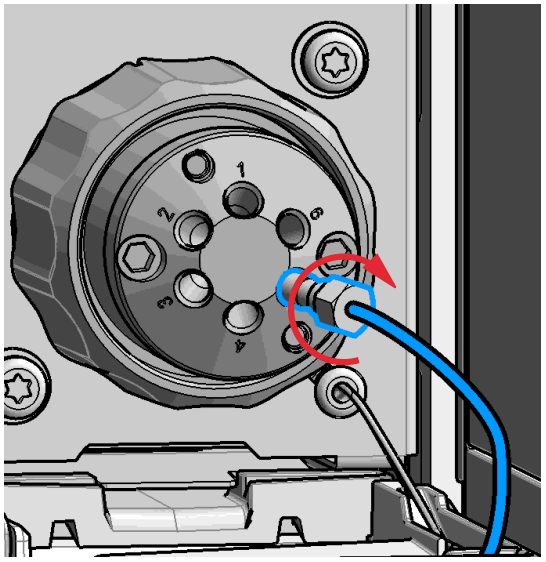
2 Slide the nut over the end of the tubing or capillary.



3 Carefully slide the ferrule components on after the nut and then finger-tighten the assembly while ensuring that the tubing is completely seated in the bottom of the end fitting.

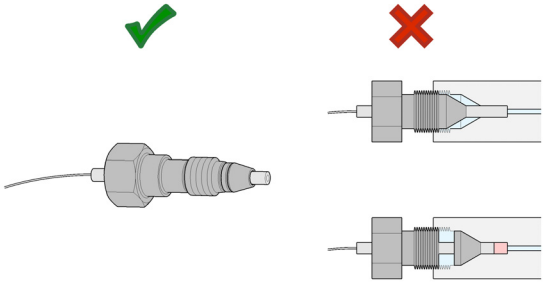


4 Use a column or injection valve to gently tighten the fitting which forces the ferrule to seat onto the tubing or capillary.



NOTE

Don't overtighten. Overtightening will shorten the lifetime of the fitting.

5 Loosen the nut and verify that the ferrule is correctly positioned on the tubing or capillary. 	

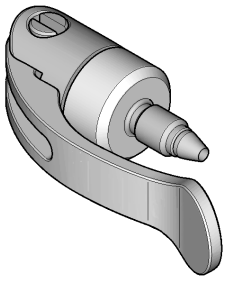
NOTE

The first time that the swagelock fitting is used on a column or an injection valve, the position of the ferrule is permanently set. If changing from a column or an injection valve to another, the fitting may leak or decrease the quality of the separation by contributing to band broadening.

Fittings

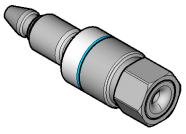
NOTE

InfinityLab Quick Connect fittings



InfinityLab Quick Connect fittings are truly "finger-tight," reusable fittings for UHPLC, leak-free to 1300 bar. (No tools required) Simply use your fingers to close the lever for a perfect connection every time. This fitting is perfect for the column inlet (closing the lever is equivalent to 1 complete turn of a wrench).

InfinityLab Quick Turn fittings



With InfinityLab Quick Turn fittings, you will get either a finger-tight connection (leak-free to 400 bar), or a UHPLC connection (leak-free to 800 bar with mounting tool p/n 5043-0915, and 1300 bar after a quarter turn of a wrench). The spring-loaded design guarantees zero-dead-volume and makes it ideal for connections at the column outlet and detector.

For details, see
Agilent InfinityLab: Making Great Connections – Less stress, more reliable fittings.

Bio 2D-LC Loops

For 1290 Infinity II Bio LC modules, use bio / biocompatible parts only.
Do not mix parts between 1260 Infinity II Bio-Inert LC modules and 1290 Infinity II Bio LC modules.

Bio Loops for SHC and MHC valve Fitting M4

p/n	Description
5004-0025	Capillary MP35N 0.35 mm x 104 mm M/M 10 µL
5004-0026	Capillary MP35N 0.35 mm x 208 mm M/M 20 µL
5004-0027	Capillary MP35N 0.35 mm x 420 mm M/M 40 µL
5004-0028	Capillary MP35N 0.35 mm x 831 mm M/M 80 µL
5004-0029	Capillary MP35N 0.35 mm x 1247 mm M/M 120 µL
5004-0030	Capillary MP35N 0.35 mm x 1870 mm M/M 180 µL

Bio 2D-LC Capillaries

For 1290 Infinity II Bio LC modules, use bio / biocompatible parts only.
Do not mix parts between 1260 Infinity II Bio-Inert LC modules and 1290 Infinity II Bio LC modules.

InfinityLab Bio 2D-LC Capillary Kit (5005-0077)

#	p/n	Description
3	5500-1603	Quick Turn Capillary MP35N 0.17 mm x 400 mm
1	5004-0031	Capillary MP35N 0.12 mm x 600 mm
2	G7116-60071	Quick Connect Bio Heat Exchanger Standard Flow
2	5500-1578	Quick Connect Capillary MP35N 0.12 mm x 105 mm
2	5500-1597	Quick Turn Capillary MP35N 0.12 mm x 400 mm
1	5500-1599	Quick Turn Capillary MP35N 0.17 mm x 105 mm
1	5500-1600	Quick Turn Capillary MP35N 0.17 mm x 150 mm
1	5500-1596	Quick Turn Capillary MP35N 0.12 mm x 280 mm
2	5067-5965	InfinityLab Quick Connect LC fitting
20	5067-5966	InfinityLab Quick Turn Fitting
1	0890-1713	Tubing, PTFE, ID/OD 0.8/1.6 mm
1	5063-6591	PEEK Fittings 10/PK

NOTE

InfinityLab Quick Connect fittings are truly "finger-tight," reusable fittings for UHPLC, leak-free to 1300 bar.

No tools required. Simply use your fingers to close the lever for a perfect connection every time. This fitting is perfect for the column inlet (Remember: closing the lever is equivalent to 1 complete turn of a wrench).

With InfinityLab Quick Turn fittings, you will get either a finger-tight connection (leak-free to 400 bar), or a UHPLC connection (leak-free to 800 bar with Fitting mounting tool (5043-0915), and 1300 bar after a quarter turn of a wrench). The spring-loaded design guarantees zero-dead-volume and makes it ideal for connections at the column outlet and detector.

Additional Biocompatible Capillaries

For 1290 Infinity II Bio LC modules, use bio / biocompatible parts only.
Do not mix parts between 1260 Infinity II Bio-Inert LC modules and 1290 Infinity II Bio LC modules.

p/n	Description
5500-1596	Quick Turn Capillary MP35N 0.12 mm x 280 mm for short columns
5500-1598	Quick Turn Capillary MP35N 0.12 mm x 500 mm for long columns
5500-1597	Quick Turn Capillary MP35N 0.12 mm x 400 mm
5500-1599	Quick Turn Capillary MP35N 0.17 mm x 105 mm
5500-1603	Quick Turn Capillary MP35N 0.17 mm x 400 mm
5500-1578	Quick Connect Capillary MP35N 0.12 mm x 105 mm
5500-1279	Capillary MP35N 0.12 mm x 500 mm SI/SI
5500-1419	Capillary MP35N 0.17 mm x 500 mm, SI/SI
5004-0031	Capillary MP35N 0.12 mm x 600 mm
5500-1376	Capillary ST 0.12 mm x 170 mm M/M
5500-1227	Capillary ST 0.17 mm x 150 mm SL-SL
5500-1283	Capillary MP35N 0.25 mm x 80 mm Pressure Sensor to Outlet Filter, to pump head, and to Multipurpose valve
5500-1284	Capillary MP35N 0.17 mm x 120 mm SI/SX
5004-0041	Capillary MP35N 0.17 x 130 mm SI/SX
5005-0046	Capillary MP35N 0.12 mm x 2 m

p/n	Description
5500-1593	Quick Turn Capillary MP35N 0.12 mm x 105 mm
5067-5966	InfinityLab Quick Turn Fitting
5043-0277	Blank nut long 10-32, PEEK

NOTE InfinityLab Quick Turn fittings require the capillaries specified in this table.

NOTE InfinityLab Quick Connect fittings are truly "finger-tight," reusable fittings for UHPLC, leak-free to 1300 bar.

No tools required. Simply use your fingers to close the lever for a perfect connection every time. This fitting is perfect for the column inlet (Remember: closing the lever is equivalent to 1 complete turn of a wrench).

With InfinityLab Quick Turn fittings, you will get either a finger-tight connection (leak-free to 400 bar), or a UHPLC connection (leak-free to 800 bar with Mounting tool for fitting (5043-0915), and 1300 bar after a quarter turn of a wrench). The spring-loaded design guarantees zero-dead-volume and makes it ideal for connections at the column outlet and detector.

Pressure Release Kit

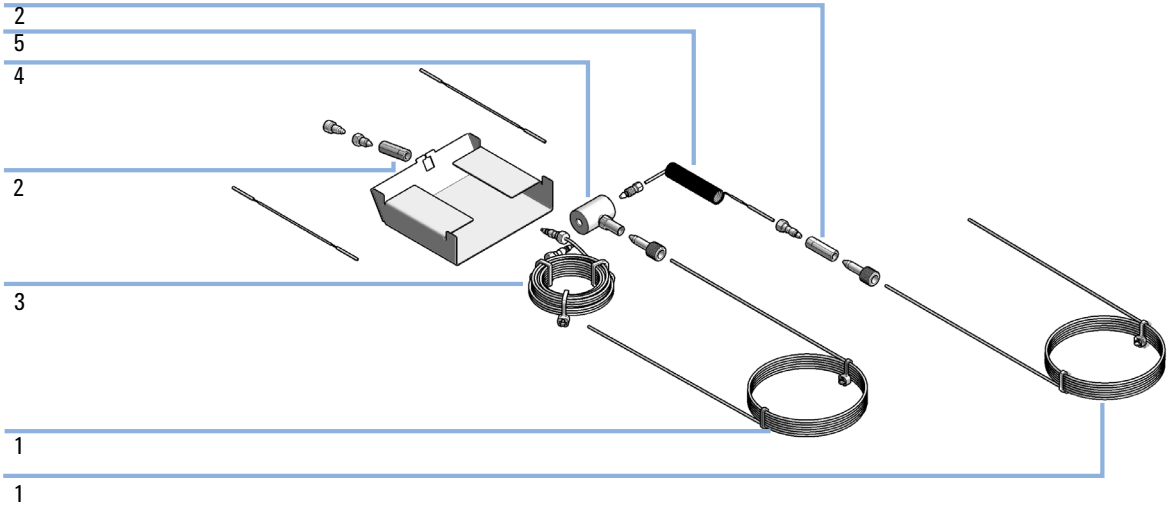


Figure 124 Pressure release kit, parts

Item	p/n	Description
	G4236-60010	2D-LC Pressure Release Kit
	0100-0969	TEE, ST, 1/16 inch, Low Dead Volume Not shown
1	5021-1816	Capillary i.d. 0.17 mm, 105 mm lg
2	5022-2184	Union, stand LC flow, no fitting
3	G7167-87307	500 µL Loop extension
4	G4212-60022	Pressure Relief Valve
5	5067-5939	Splitter-Capillary 0.05-ID L-1000 mm

Valve Options Overview (for Bio 2D-LC)

Bio 2D-LC Valve Kit ASM

For 1290 Infinity II Bio LC modules, use bio / biocompatible parts only.
Do not mix parts between 1260 Infinity II Bio-Inert LC modules and 1290 Infinity II Bio LC modules.

InfinityLab Bio 2D-LC ASM Valve Kit (G5643B)

p/n	Description
5005-0078	Agilent InfinityLab Bio 2D-LC ASM Valve
5190-6895	2D-LC starter sample, 1 x 2 mL
G5642-64000	Bio Compatible MHC Loop Assembly SST
699968-301	Poroshell 120 Bonus-RP, 3.0 x 50 mm, 2.7 µm
G4236-64000	2D-LC Easy Start USB Media Kit
5005-0077	InfinityLab Bio 2D-LC Capillary Kit
G2453-85060	Formic Acid-Reagent Grade 5 mL (5 cc)
685775-902	Poroshell SB-C18, 2.1 x 100 mm, 2.7 µm
G1680-63721	Network LAN Switch
	Regional power cord

InfinityLab Bio 2D-LC ASM Valve Kit (G5643A)

p/n	Description
5005-0085	Agilent InfinityLab Bio 2D-LC ASM Valve
5190-6895	2D-LC starter sample, 1 x 2 mL
G5642-64000	Bio Compatible MHC Loop Assembly SST
699968-301	Poroshell 120 Bonus-RP, 3.0 x 50 mm, 2.7 µm
G4236-64000	2D-LC Easy Start USB Media Kit
5005-0077	InfinityLab Bio 2D-LC Capillary Kit
G2453-85060	Formic Acid-Reagent Grade 5 mL (5 cc)
685775-902	Poroshell SB-C18, 2.1 x 100 mm, 2.7 µm
G1680-63721	Network LAN Switch
	Regional power cord

Bio 2D-LC ASM Valve Head (1300 bar)

For 1290 Infinity II Bio LC modules, use bio / biocompatible parts only.
Do not mix parts between 1260 Infinity II Bio-Inert LC modules and 1290 Infinity II Bio LC modules.

Agilent InfinityLab Bio 2D-LC ASM Valve (5005-0078)

p/n	Description
5320-0017	Bio 2D-LC ASM Valve Head, 1300 bar
5004-0021	Capillary ST 0.12 mm x 85 mm M/M
5004-0022	Capillary ST 0.12 mm x 170 mm M/M
5004-0023	Capillary ST 0.12 mm x 340 mm M/M
5004-0024	Capillary ST 0.12 mm x 680 mm M/M
5004-0020	Capillary ST 0.12 mm x 170 mm M/M
0890-1713	Tubing, PTFE, ID/OD 0.8/1.6 mm

Agilent InfinityLab Bio 2D-LC ASM Valve (5005-0085)

p/n	Description
5067-4266	2D-LC ASM Valve Head, 1300 bar
5004-0021	Capillary ST 0.12 mm x 85 mm M/M
5004-0022	Capillary ST 0.12 mm x 170 mm M/M
5004-0023	Capillary ST 0.12 mm x 340 mm M/M
5004-0024	Capillary ST 0.12 mm x 680 mm M/M
5004-0020	Capillary ST 0.12 mm x 170 mm M/M
0890-1713	Tubing, PTFE, ID/OD 0.8/1.6 mm

ASM-Valve-Head Bio

For 1290 Infinity II Bio LC modules, use bio / biocompatible parts only.
Do not mix parts between 1260 Infinity II Bio-Inert LC modules and 1290 Infinity II Bio LC modules.

#	p/n	Description
1	5068-0257	Bearing Ring
1	5068-0240	Rotor Seal (VHP)
5	5068-0019	Stator screws
1	5299-0005	Stator 5-10 PD CF 1300 bar BIO

Multiple Heart-Cutting Valve

For 1290 Infinity II Bio LC modules, use bio / biocompatible parts only.
Do not mix parts between 1260 Infinity II Bio-Inert LC modules and 1290 Infinity II Bio LC modules.

Multiple Heart-Cutting Valve (G5642-64000)

#	p/n	Description
1	5067-4273	6-column selector valve head, 1300 bar
6	5004-0027	Capillary MP35N 0.35 mm x 420 mm M/M 40 µL Transfer Capillary
1	5043-0269	Adapter-profile

NOTE The current version of this MHC valve uses biocompatible sample loops and a biocompatible valve head.

2-Position/10-Port valve Bio (1300 bar)

For 1290 Infinity II Bio LC modules, use bio / biocompatible parts only.
Do not mix parts between 1260 Infinity II Bio-Inert LC modules and 1290 Infinity II Bio LC modules.

2-position/10-port valve, bio 1300 bar (G5641A) PEEK, MP35N

p/n	Description
5067-6682	2-position/10-port bio valve head, 1300 bar
5068-0286	Stator MP35N
5068-0205	Rotor Seal (PEEK)
5068-0019	Stator screws
5013-0002	Bio 2/10 Capillary Kit 1300 bar (separately orderable)

12-Position/13-Port Selector Valve Head Bio-Inert (210 bar)

For bio-inert modules use bio-inert parts only!

12 position/13 port selector valve head, 210 bar, bio-inert (5067-4159)

#	p/n	Description
4	5068-0059	Stator screws
1	1535-4045	Bearing ring
1	0101-1288	Rebuild kit (rotor seal and stator face)
1	5068-0097	Bio-inert stator head

2-Position/6-Port Valve Bio-Inert (600 bar)

For bio-inert modules use bio-inert parts only!

2 position/6 port valve head, 600 bar, bio-inert (5067-4148)

p/n	Description
5068-0060	Bio-inert stator head
0101-1409	Rotor Seal (PEEK)
0100-1851	Stator face, ceramic
1535-4045	Bearing ring
5068-0020	Stator Screws, 10/pack

4-Column Selector Valve Bio-Inert (600 bar)

For bio-inert modules use bio-inert parts only!

4-column selector valve head, 600 bar, bio-inert (5067-4134)

#	p/n	Description
1	5068-0045	Bio-inert rotor seal, PEEK
1	5068-0044	Bio-inert stator head
1	5068-0093	Stator face assy
5	5068-0059	Stator screws
1	1534-4045	Bearing ring

Overview of Other Biocompatible Spare Parts of Various Bio-LC Modules

1290 Infinity II Bio High-Speed Pump (G7132A) Biocompatible Parts

For 1290 Infinity II Bio LC modules, use bio / biocompatible parts only.
Do not mix parts between 1260 Infinity II Bio-Inert LC modules and 1290 Infinity II Bio LC modules.

1290 Infinity II Bio High-Speed Pump (G7132A) Biocompatible Parts

p/n	Description
G7132-60002	Biocompatible capillary MP35N 0.17 mm x 300 mm Purge Valve to Jet Weaver
5500-1421	Biocompatible capillary MP35N 0.25 mm x 130 mm Purge Valve to Pressure Sensor
5500-1420	Biocompatible capillary MP35N 0.25 mm x 250 mm Purge Valve to Pump Head Assemblies channel A and B
5500-1419	Capillary MP35N 0.17 mm x 500 mm, SI/SI Jet Weaver to Multisampler (Standard Bio-LC Setup)

For further bio pump parts, refer to the user manuals.

1290 Infinity II Bio Flexible Pump (G7131A/C) Biocompatible Parts

For 1290 Infinity II Bio LC modules, use bio / biocompatible parts only.
Do not mix parts between 1260 Infinity II Bio-Inert LC modules and 1290 Infinity II Bio LC modules.

1290 Infinity II Bio Flexible Pump (G7131A/C) Biocompatible Parts

p/n	Description
G7131-20009	Seal biocompatible
G7131-60004	Outlet Filter Flex Bio-Compatible
5500-1283	Capillary MP35N 0.25 mm x 80 mm Pressure Sensor to Outlet Filter, to pump head, and to Multipurpose valve e.g. Pump Head to Pressure Sensor
5500-1419	Capillary MP35N 0.17 mm x 500 mm, SI/SI Purge Valve/Jet Weaver to Multisampler
5500-1284	Capillary MP35N 0.17 mm x 120 mm SI/SX Multipurpose Valve internal connections
5004-0041	Capillary MP35N 0.17 x 130 mm SI/SX To/from Jet Weaver
0905-1731	Bio-Inert Wash Seal
5320-0048	Frit for pump outlet filter Bio-Compatible 2/pk
5065-4445	Peristaltic pump with PharMed tubing
5720-0020	1290 Infinity II Bio Inline Filter Kit

For further bio pump parts, refer to the user manuals.

1290 Infinity II Bio Multisampler (G7137A) Biocompatible Parts

For 1290 Infinity II Bio LC modules, use bio / biocompatible parts only.
Do not mix parts between 1260 Infinity II Bio-Inert LC modules and 1290 Infinity II Bio LC modules.

1290 Infinity II Bio Multisampler (G7137A) Biocompatible Parts

p/n	Description
G7137-87201	Needle Biocompatible
G7137-87012	High pressure seat assembly 0.12 mm Biocompatible
5320-0010	Rotor Seal 1300 bar (PEEK)
G7137-20003	Metering seal 1290 Bio 2 mm piston, 40 µL
5065-4445	Peristaltic pump cartridge
5067-6739	2-position/6-port injection valve Bio 1300 bar
5068-0281	Stator face, MP35N
G7137-60300	Sample Loop MP35N 20 µL, right (red/orange coded)
G7137-60400	Sample Loop MP35N 40 µL, right (green/orange coded)
G7137-60500	Sample Loop MP35N 100 µL, right (blue/orange coded)

Standard

p/n	Description
5500-1278	Capillary MP35N 0.17 mm x 100 mm SL/SL Analytical Head to Injection Valve
5500-1279	Capillary MP35N 0.12 mm x 500 mm SI/SI Injection Valve to Quick Connect Heat Exchanger in MCT
5500-1419	Capillary MP35N 0.17 mm x 500 mm, SI/SI Jet Weaver to Multisampler

Multiwash

p/n	Description
5500-1278	Capillary MP35N 0.17 mm x 100 mm SL/SL Analytical Head to Injection Valve
5500-1280	Capillary MP35N 0.17 mm x 250 mm SL-SL Flush Head to Injection Valve
5500-1279	Capillary MP35N 0.12 mm x 500 mm SI/SI Injection Valve to Quick Connect Heat Exchanger in MCT (Standard Bio-LC Setup)
5500-1419	Capillary MP35N 0.17 mm x 500 mm, SI/SI Jet Weaver to Multisampler (Standard Bio-LC Setup)

For further sampler parts, refer to the user manuals.

1260 Infinity II Bio Multisampler (G5668A) Bio-Inert Parts

For bio-inert modules use bio-inert parts only!

1260 Infinity II Bio Multisampler (G5668A) Bio-Inert Parts

p/n	Description
G5668-87200	Needle Bio-Sampler
5068-0209	Rotor Seal (PEEK)
G5668-87017	Bio Seat ID 0.17
G5668-60500	Bio-inert Sample Loop 100 µL
5067-4263	2-position/6-port Injection Valve Bio-inert 600 bar
5068-0060	Bio-inert stator head
G5611-60500	Capillary 400 x 0.17 mm, titanium (Bio-inert) Pump to Injector (Standard Bio-LC Setup)
G5611-60502	Capillary Ti 0.17 mm x 900 mm, L (Bio-inert) Pump to Thermostatted Autosampler (Standard Bio-LC Setup)
5043-0277	Blank nut long 10-32, PEEK

NOTE

Be careful with installation of stainless steel-cladded PEEK capillaries (Bio-Inert). The capillaries require special attention and different handling compared to usual LC capillaries. See the Technical Note *Installation of stainless steel cladded PEEK capillaries (G5611-90120)* for detailed description

Standard

p/n	Description
5500-1278	Capillary MP35N 0.17 mm x 100 mm SL/SL Analytical Head to Injection Valve
5500-1256	Capillary Ti 0.17 mm x 100 mm SL/SL
5500-1279	Capillary MP35N 0.12 mm x 500 mm SI/SI Injection Valve to Quick Connect Heat Exchanger in MCT
5500-1419	Capillary MP35N 0.17 mm x 500 mm, SI/SI Jet Weaver to Multisampler

Multiwash

p/n	Description
5500-1278	Capillary MP35N 0.17 mm x 100 mm SL/SL Analytical Head to Injection Valve
5500-1280	Capillary MP35N 0.17 mm x 250 mm SL-SL Flush Head to Injection Valve
5500-1279	Capillary MP35N 0.12 mm x 500 mm SI/SI Injection Valve to Quick Connect Heat Exchanger in MCT (Standard Bio-LC Setup)
5500-1419	Capillary MP35N 0.17 mm x 500 mm, SI/SI Jet Weaver to Multisampler (Standard Bio-LC Setup)
5500-1257	Capillary Ti 0.17 mm x 250 mm SL/SL Injection Valve to Flushpump-head
5500-1256	Capillary Ti 0.17 mm x 100 mm SL/SL

For further sampler parts, refer to the user manuals.

1260/1290 Infinity II MCT (G7116A/B) Biocompatible Parts

For 1290 Infinity II Bio LC modules, use bio / biocompatible parts only.
Do not mix parts between 1260 Infinity II Bio-Inert LC modules and 1290 Infinity II Bio LC modules.

1260/1290 Infinity II MCT (G7116A/B) Biocompatible Parts

p/n	Description
G7116-60071	Quick Connect Bio Heat Exchanger Standard Flow 1.6 µL
G7116-60081	Quick Connect Bio Heat Exchanger High Flow 3.0 µL
G7116-60091	Quick Connect Bio Heat Exchanger Ultra Low Dispersion 1.0 µL

For further bio MCT parts, refer to the user manuals.

1260/1290 Infinity II MCT (G7116A) Bio-Inert Parts

For bio-inert modules use bio-inert parts only!

1260/1290 Infinity II MCT (G7116A) Bio-Inert Parts

p/n	Description
G7116-60009	Quick-Connect Heat Exchanger Bio-inert Standard Flow

For further bio MCT parts, refer to the user manuals.

1260/1290 Infinity II DAD (G7117A/B) Biocompatible Parts

For 1290 Infinity II Bio LC modules, use bio / biocompatible parts only.
Do not mix parts between 1260 Infinity II Bio-Inert LC modules and 1290 Infinity II Bio LC modules.

1260/1290 Infinity II DAD (G7117A/B) Biocompatible Parts

p/n	Description
G7117-60020	Max-Light Cartridge Cell LSS (10 mm, V(σ) 1.0 µL) MP35N, PEEK, fused silica
G7117-60101	Aperture

NOTE

Aperture is not compatible with other Max-Light Cartridges.

The Aperture should be installed for analysis of *light-sensitive samples*, which are likely to undergo photodegradation. For further details, check the *Agilent InfinityLab LC Series Diode Array Detectors User Manual*.

1260/1290 Infinity II DAD (G7117A/B) Bio-Inert Parts

For bio-inert modules use bio-inert parts only!

1260/1290 Infinity II DAD (G7117A/B) Bio-Inert Parts

p/n	Description
G5615-60018	Max-Light Cartridge Cell Bio-inert (10 mm, V(σ) 1.0 µL) includes Peek Capillary 1.5 m i.d. 0.18 mm (0890-1763) and PEEK Fittings 10/PK (5063-6591)

For further detector parts, refer to the user manuals.

1260 Infinity II DAD (G7115A) / 1260 Infinity II MWD (G7165A) Bio-Inert Parts

For bio-inert modules use bio-inert parts only!

1260 Infinity II DAD (G7115A) / 1260 Infinity II MWD (G7165A) Bio-Inert Parts

p/n	Description
G5615-60022	Standard flow cell bio-inert, 10 mm, 13 µL, 120 bar (12 MPa) for MWD/DAD, includes 0890-1763 – 0.18 x 1500 mm PEEK capillary and 5063-6591 – PEEK fittings

For further detector parts, refer to the user manuals.

1260/1290 Infinity II VWD (G7114A/B) Biocompatible Parts

For 1290 Infinity II Bio LC modules, use bio / biocompatible parts only.
Do not mix parts between 1260 Infinity II Bio-Inert LC modules and 1290 Infinity II Bio LC modules.

1260/1290 Infinity II VWD (G7114A/B) Biocompatible Parts

p/n	Description
G1314-60189	Bio micro flow cell VWD, 3 mm, Cell Vol. 2 µl, Sapphire, MP35N Sapphire, MP35N
G1314-60188	Bio standard flow cell VWD, 10 mm, Cell Vol. 14 µl, Sapphire, MP35N Sapphire, MP35N

For further detector parts, refer to the user manuals.

1290 Infinity II FLD (G7121A) Bio-Inert Parts

For bio-inert modules use bio-inert parts only!

1290 Infinity II FLD (G7121A) Bio-Inert Parts

p/n	Description
G5615-60005	Bio-inert flow cell, 8 µL, 20 bar (pH 1–12), includes Capillary Kit Flow Cells BIO (G5615-68755) and PEEK fittings

For further detector parts, refer to the user manuals.

Selection of 1290 Infinity II Bio LC Columns

For 1290 Infinity II Bio LC modules, use bio / biocompatible parts only.
Do not mix parts between 1260 Infinity II Bio-Inert LC modules and 1290 Infinity II Bio LC modules.

p/n	Description
653750-902	AdvanceBio Peptide Mapping 120 Å, 2.1 mm x 150 mm, 2.7 µm Peptide mapping (reversed-phase chromatography).
PL1912-1502	PLRP-S 1000 Å, 2.1 mm x 50 mm, 5 µm Analytical prep separations of peptides, proteins, and protein complexes (reversed-phase chromatography)
PL1980-3201PK	AdvanceBio SEC 200 Å, 2.1 mm x 150 mm, 1.9 µm, PEEK Aggregation and fragment analysis (size exclusion chromatography)

Additional information:

- 653750-902 (AdvanceBio Peptide Mapping, 2.1 x 150 mm) is a regular stainless steel column that is used for high resolution Peptide Mapping. It was used as an example in the following 2D-LC application *Fully Automated Characterization of Monoclonal Antibody Charge Variants Using 4D-LC/MS*.
- PL1912-1502 (PLRP-S 1000Å, 2.1 x 50 mm) is also a regular stainless steel column but there is also a PEEK lined version available (PL1912-1502PK). It was used as an example in the following 2D-LC application *Characterization of Antibody-Drug Conjugates (ADCs) Using 2D-LC and Native MS*

For further application details please check the application finder for 2D-LC Applications

https://www.agilent.com/en/promotions/applicationfinder

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13 Alternative ways to install the System

This chapter describes alternative ways to install and setup the system.

Legacy Stack Configuration

The following configurations optimize the system flow path, ensuring minimum delay volume in an Agilent Infinity I configuration.

NOTE The capillary connections should be as short as possible, to ensure optimum performance of the system.

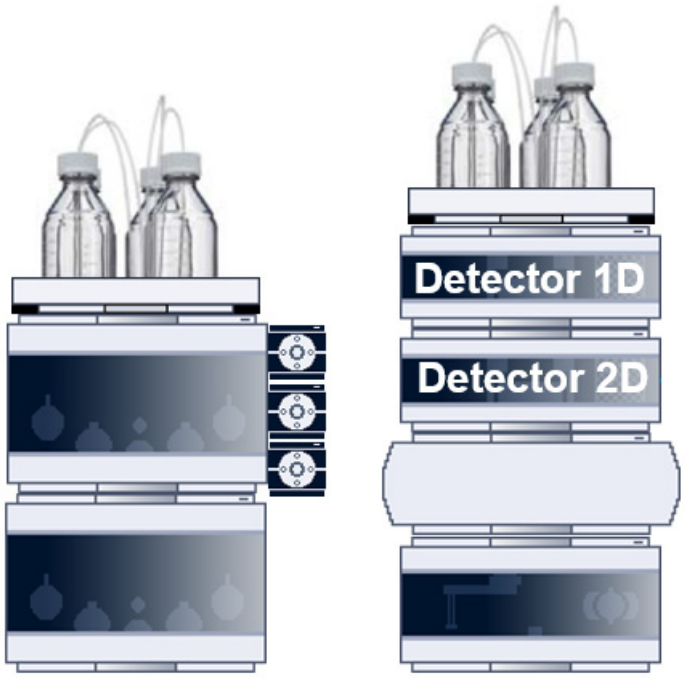


Figure 125 Legacy stack configuration for Multiple Heart-cutting 2D-LC

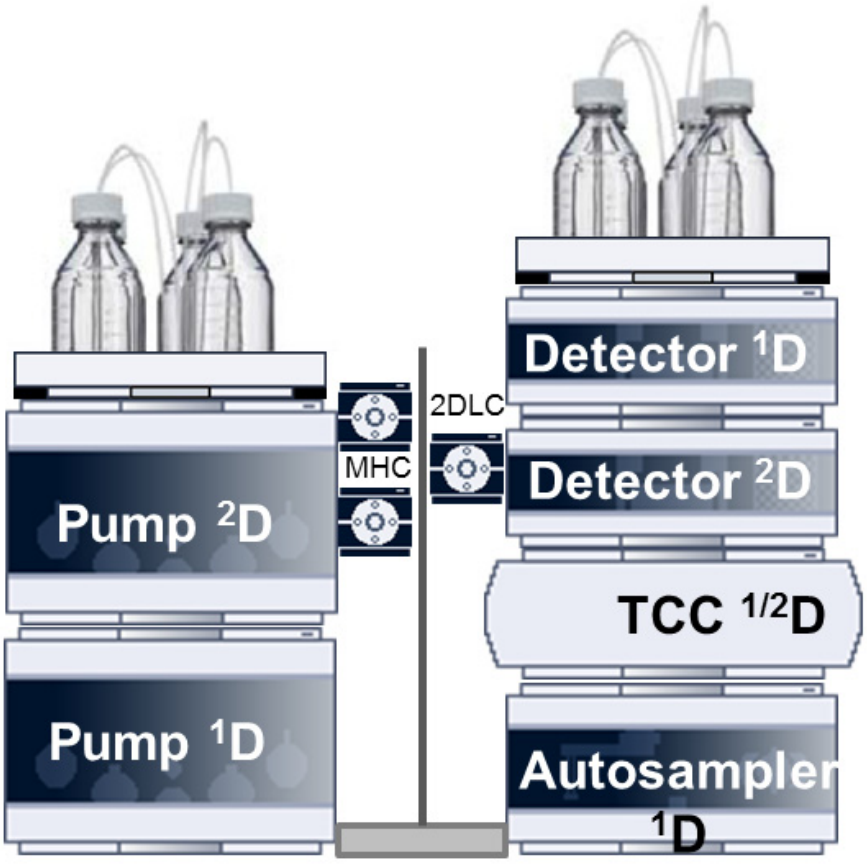


Figure 126 Previous stack configuration for Multiple Heart-cutting 2D-LC based on the column organized/valve holder

Table 33 1290 Infinity Binary LC in first dimension

	Partnumber	Description	Comment
1st Dim	G4220A	**1290 Infinity Binary Pump**	
	G4226A	1290 Infinity Autosampler	
	G1330B	1290 Thermostat	
	G1316C	1290 Thermostatted Column Compartment	
	G4212A	1290 Infinity Diode-Array Detector	
2nd Dim	G2198AA	2D-LC Acquisition Software	
	G4220A	1290 Infinity Binary Pump	
	G1170A	1290 Infinity Valve Drive	For 2D-LC valve
	G4236A	2D-LC Valve Kit, 1200 bar	
	G4212A	1290 Infinity Diode-Array Detector	

NOTE

There are a few other configurations when using multiple detectors at different positions. Examples are:

- After first dimension column, or
- At the waste line in addition to the standard 2D-LC detector after the second dimension columns.

If quantitative information is required, it is recommended to use same detector types and flow-cells.

NOTE

^{1}D flow cells require a minimum pressure stability of 60 bar (~870 psi) (which excludes FLD and RID detectors).

NOTE

^{2}D detectors should be connected to CAN bus for automatic run time extension. To be considered for ELSD or 3rd party detectors.

NOTE

Avoid high data rates in the first dimension for peak-based sampling. Otherwise, RI effects may be recognized as peaks, which can cause unwanted sampling. 5 Hz give enough data points for correct peak detection of long ^{1}D runs.

1D/2D Switching

Usually, it is necessary to reconnect capillaries to switch between performing 1D-LC methods and comprehensive 2D-LC methods using the same LC system. The 1D/2D switching setup is a flexible Agilent 1290 Infinity 2D-LC Solution with one detector. It contains an additional 2-position/6 port valve to allow switching between 1D-LC and comprehensive 2D-LC analysis with a few mouse clicks, and without any hardware change. For details, see Automated Switching Between 1D-LC and Comprehensive 2D-LC Analysis (5991-4843EN).

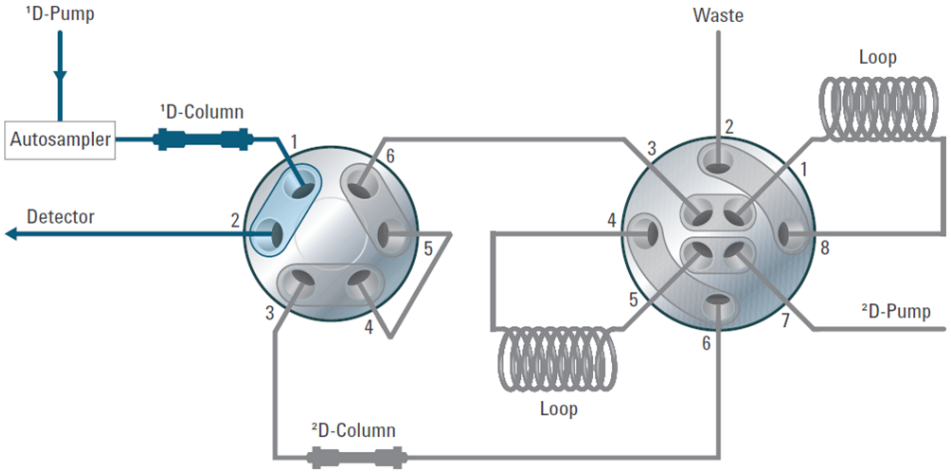


Figure 127 Setup of the Agilent 1290 Infinity 2D-LC Solution with an additional 2-position/6-port valve positioned for 1D-LC analysis.

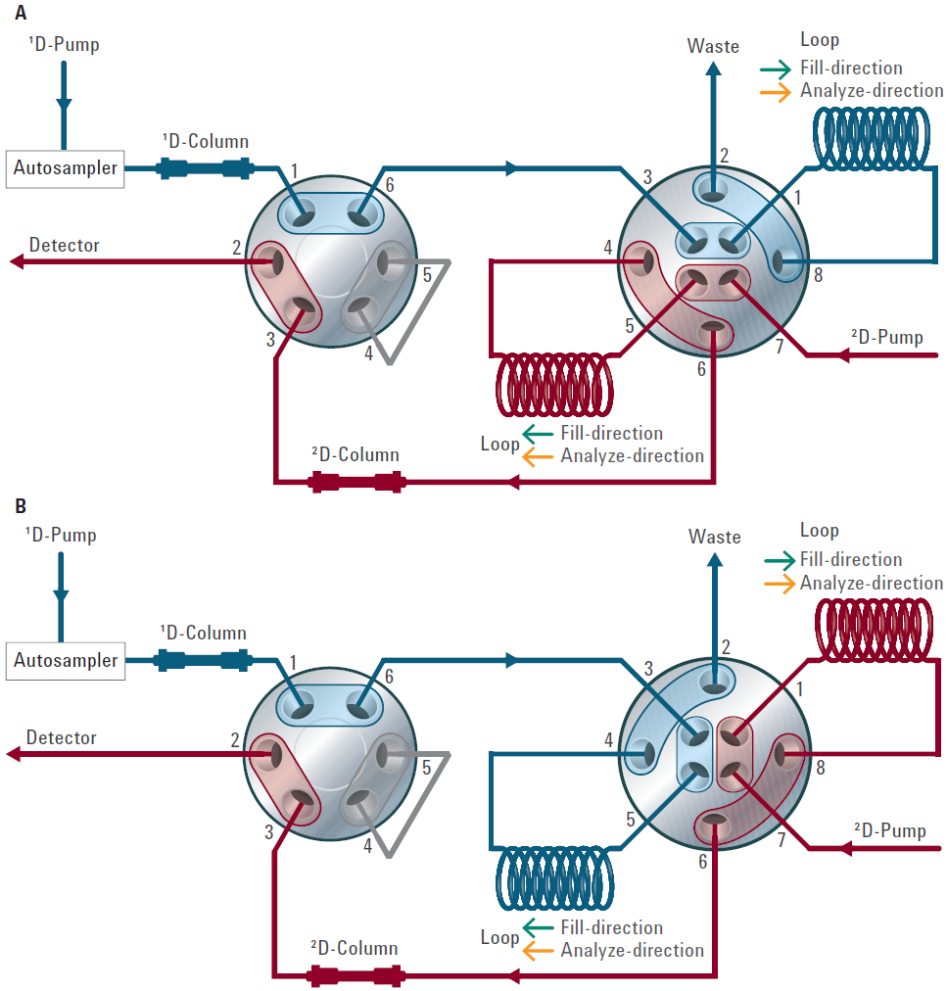


Figure 128 Setup of the Agilent 1290 Infinity 2D-LC Solution with an additional 2-position/6-port valve positioned for comprehensive 2D-LC analysis; (A) Collection of the effluent from the first dimension column in loop 1 (between ports 1 and 8); (B) Collection of the effluent from the first dimension column in loop 2 (between ports 4 and 5), analysis of the content of loop 1 on the second dimension column.

14 Agilent 2D-LC Solution with the selectivity of mass selective detection (MSD)

This chapter describes the different options to use the Agilent 2D-LC Solution with mass selective detection (MSD).

Today the 2D-LC software is an OpenLab CDS ChemStation Edition plug in, which comprises three functions: system control, data acquisition, and data analysis/reporting. The current 2D-LC software requires configuring at least one UV detector in either or both dimensions. A single quadrupole detector may instead be configured for the second dimension. Only UV detectors may be configured in 1D as peak triggering detectors. MS detectors can be used in the second dimension for acquiring 2D-LC data either in OpenLab CDS ChemStation Edition (single quadrupole) or MassHunter workstation (QQQ/Q-TOF).

Agilent 2D-LC Solution with an Agilent Single Quadrupole Detector

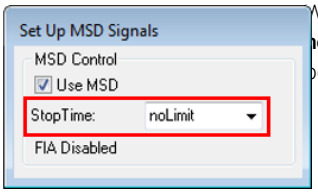
Agilent OpenLab 2D-LC Software is a fully integrated solution from method setup to data analysis for 2D-LC/MS within OpenLab CDS ChemStation Edition. It combines the separation power of two-dimensional chromatography with the selectivity of mass selective detection (MSD) in the second dimension. It can be used with all Agilent Single Quadrupole Detectors supported by ChemStation including latest LC/MSD (G6125B) and LC/MSD XT (G6135B). Only UV detectors may be configured in ^{1}D as peak triggering detectors.

NOTE

The 2D-LC system has been optimized for detectors connected to the CAN bus interface. For non-CAN detectors (e.g. third party detectors), suitable run time settings need to be conisdered in order to get complete signal data, see KPR 25 in the software status bulletin for details.

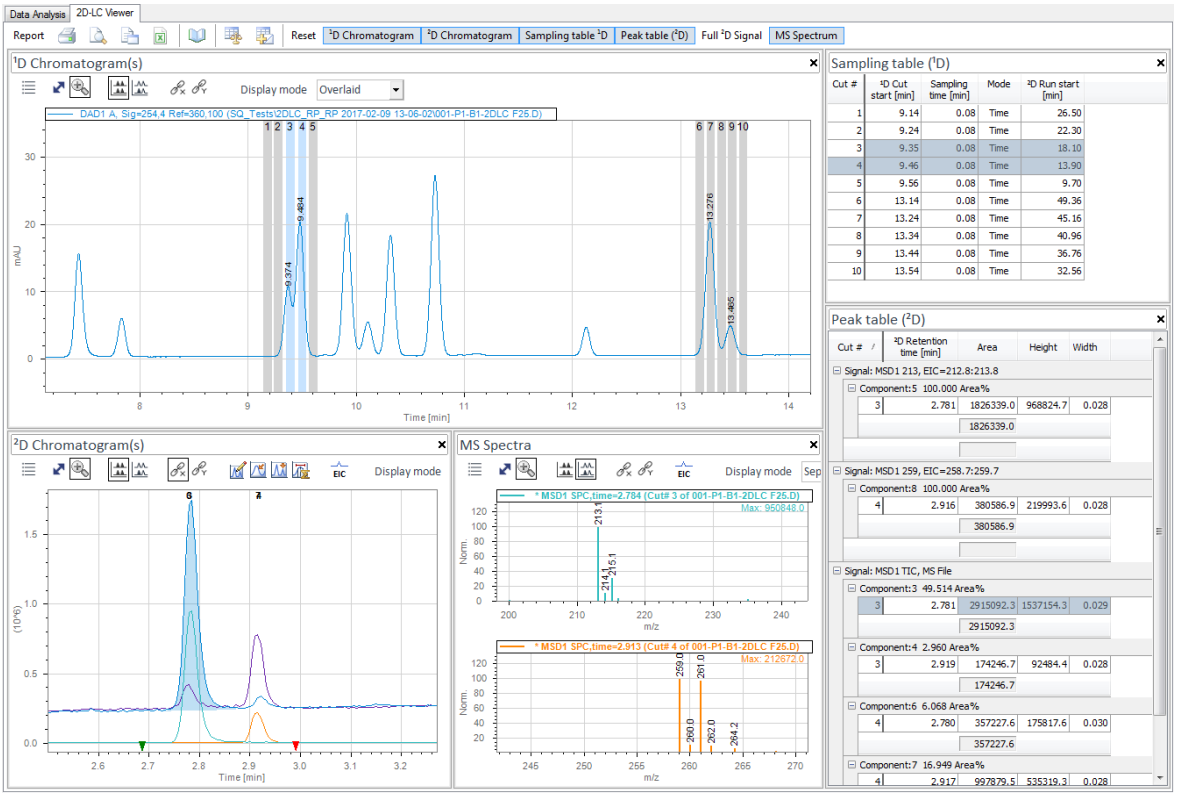
Using the Single Quadrupole Detector with 2D-LC

As a destructive detector, the single quadrupole detector is usually installed as a ^{2}D detector. It can be configured as described in section "Detector configuration".



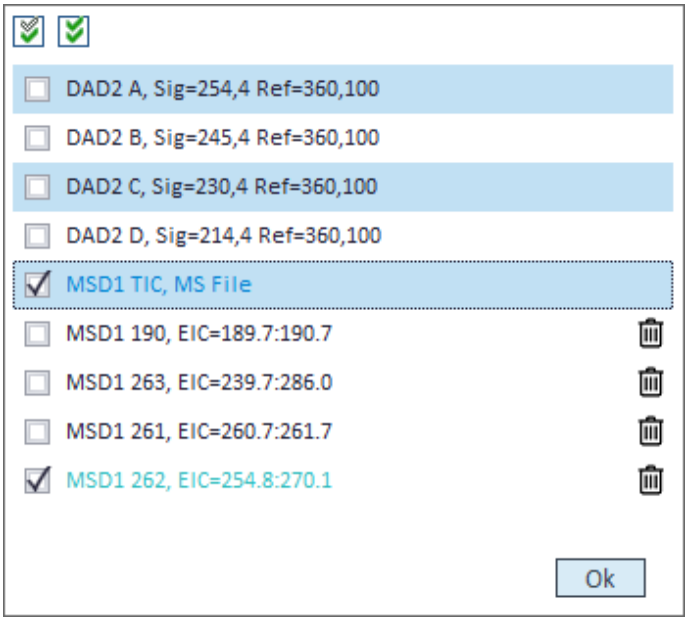
When setting up the MSD (**Instrument >Set up MSD signals >...**), set **noLimit** as the **Stop Time**. This is required as the run time needs to be extended for unparking cuts.

2D-LC Viewer for Single Quadrupole Data



The 2D-LC Viewer displays now also MS and UV spectra and extracted ion chromatograms.

Select signals

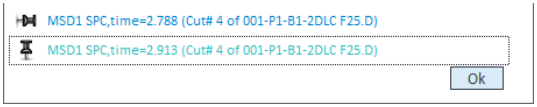


Multiple signals can be managed using the signal selector. Use checkmarks for choosing signals to be displayed.

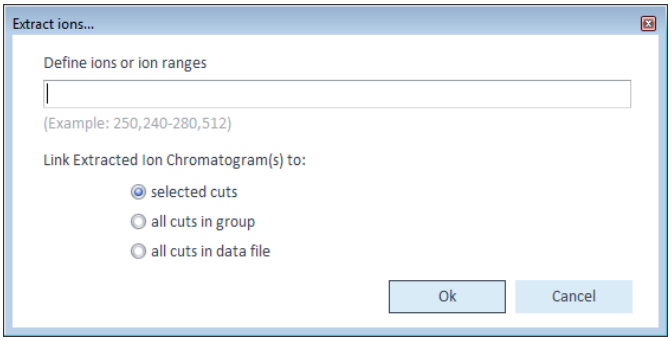
Select highlighted/all items
As a shortcut, multiple signals can quickly be highlighted by control- or shift-clicking signal names for the ^{2}D chromatogram panel and clicking the selection buttons.

time/apex/average per range
Spectra can be displayed for a time or a peak apex by clicking the ^{2}D chromatogram or dragging the mouse for an average spectrum per time range.

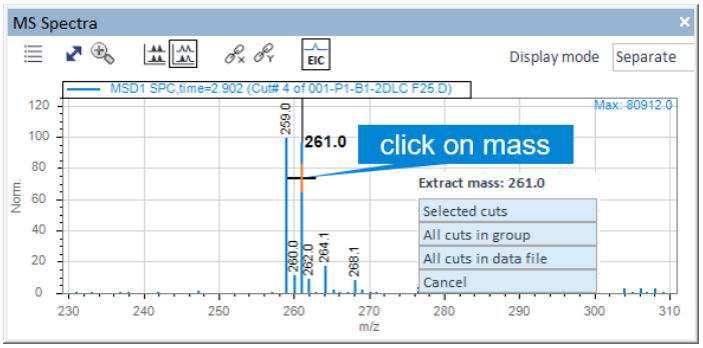
Pin spectra
Spectra can be pinned for storing them per associated cut for display and reporting:



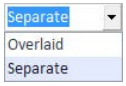
Extract ion chromatogram



Ion chromatograms can be extracted for a mass or mass range selected in the spectrum or entered for the ^{2}D chromatogram(s).



Ion chromatograms can also be extracted from spectra by clicking the EIC button and then selecting a mass.

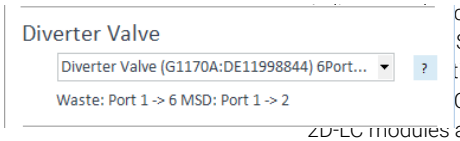


Separarate versus overlaid mode
Multiple (extracted ion) chromatograms or spectra can be displayed in the same window (overlay) or in separate windows.

same scale/full scale
Diagrams can be displayed using the same scale or the full scale for each diagram.

linked x and y axes
For zooming, the axes can optionally be linked for all displays. In case of linked axes, all displays will be zoomed to the same range.

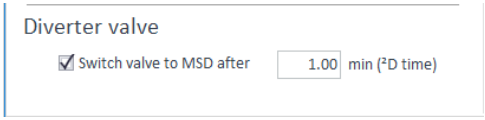
Diverter Valve Configuration



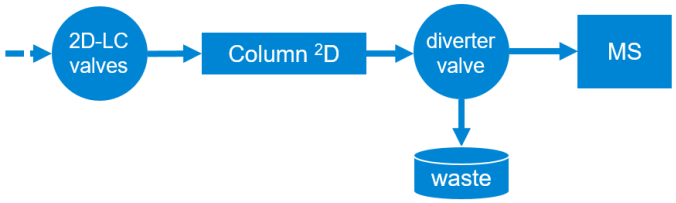
can be configured e.g. for removing buffers before S detector. Any 2-position LC valve can be chosen tion. Valves should be installed to external valve Control is done via the CAN connection between 2D-LC modules and the diverter valve, which is not yet available for diverter valves in MS detectors. The user interface shows, which valve outlet ports go to waste vs. detector.

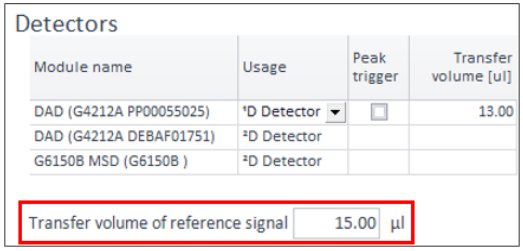
For details, see "Diverter Valve Configuration" on page 429.

Diverter Valve Method Parameters

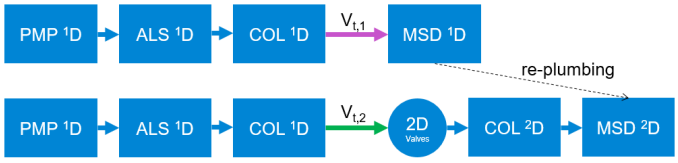


A two-position LC valve (e.g. a 2-pos/6-port valve) can be configured in the 2D-LC Configuration for diverting ^{2}D eluent with buffers before they enter an MS detector. As a method parameter, a ^{2}D time can be set, after which the flow path is directed to the detector.





A previously acquired ^{1}D MSD signal can be used as a reference signal after configuring an MS detector in ^{2}D without configuring a ^{1}D peak trigger. In this case, the transfer volume of the reference signal can be entered for that previously installed ^{1}D detector:



- Reference signal is acquired with MSD in first dimension.
- Capillary connections are changed and MSD is moved to ^{2}D. Then, there is no peakdetector in ^{1}D. The transfer volume V_t needs to be calculated as difference $V_{t,2}$-$V_{t,1}$ and configured.
- Reference signal is used for defining cuts in sampling table.
- MSD is used for detection in second dimension.

Agilent 2D-LC Solution with high-end MS detection

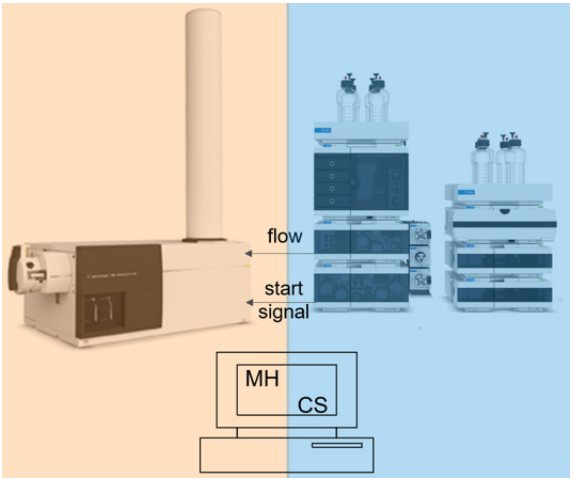


Figure 129 Typical system setup

NOTE

OpenLab CDS ChemStation and MassHunter have passed the test successfully for co-execution regarding parallel data acquisition. For further information please check the recommended conditions in the technical note *2D Chromatogram Creator for MassHunter*.

The LC part is an LC system as described in the previous pages of this 2D-LC system manual. A typical system uses a UV detector in the first dimension and an optional UV detector in the second dimension. The 2D-LC instrument control and UV signal acquisition is still done with OpenLab CDS ChemStation (CS) Edition. Therefoer the 2D-LC software requires configuring at least one UV detector.

The MS detector, a TOF, Q-TOF, or Triple Quadrupole, is used in the second dimension. It is controlled by MassHunter (MH) Acquisition software, which acquires the MS signal.

For details, see "2D Chromatogram Creator for MassHunter" on page 390.

For synchronizing signals of LC and MS, an APG remote cable connects both parts of the system, which communicates start and stop signals.

NOTE

For synchronizing run times (that is starting and stopping measurements simultaneously), please use **External start** as a run parameter in the Masshunter Acquisition software. You will find the information in section **Additional information**, add parameter **run type** and choose option **external run**. Set stop time **no limit** in MassHunter.

NOTE

Only UV detectors can be configured in ^{1}D as peak triggering detectors.

For comprehensive 2D-LC MS measurements, we recommend using the GC Image LCxLC-HRMS Edition software. This software can display and analyze the 2D-LC MS data. For further information please check the following:

- Chapter "Data Analysis for Comprehensive 2D-LC (LCxLC)" on page 251,
- LC Video Tutorial on the GC Image DVD,
- Or in the help file of the GC Image SW.

Table 34 Software for using Agilent 2D-LC Solution with high-end MS detection

Type of 2D-LC Mode	Instrument Control	Data Acquisition	Data Analysis
Multiple Heart-Cutting[1], High Resolution Sampling	ChemStation plugin	ChemStation plugin MassHunter	LC Image[1] or MassHunter in combination with 2D Chromatogram Creator for MassHunter software
Comprehensive	ChemStation plugin	ChemStation plugin MassHunter	LC Image

[1] MHC data analysis can be displayed as a false result because peaks from cuts that not belong together can be integrated as one bulb.

But the story is different if you are using Multiple Heart-Cutting (MHC) or High-Resolution to get the correct cut and identify the mass correctly with high-end MS detection. MassHunter has no knowledge about the size, order, and position of cuts created by OpenLab CDS ChemStation Edition. The raw MS TIC signal continuously measures MS data of the 2D eluent. Therefore, you need a tool that aligns the different cut operations from the OpenLab SW with the MS data of the eluent. For this purpose, you must use the 2D Chromatogram Creator for MassHunter.

User Interface/Features

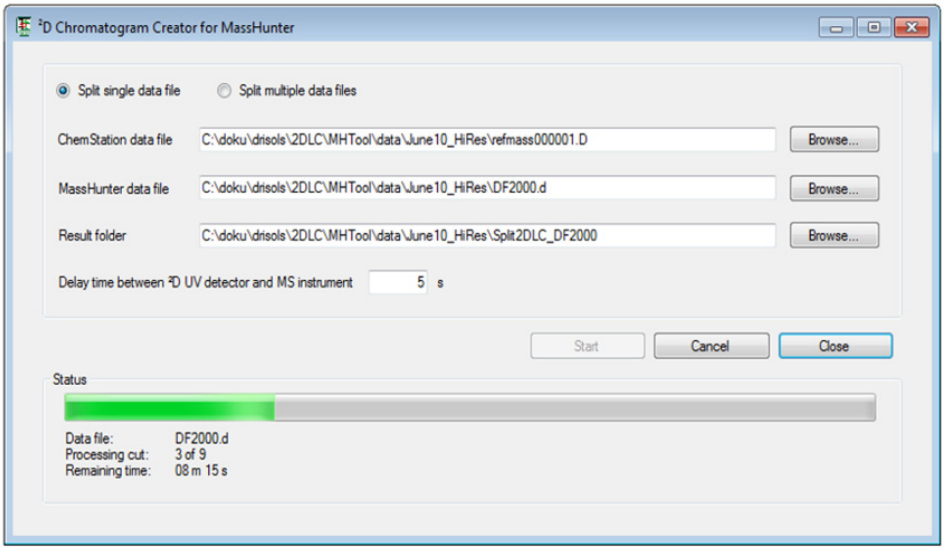


Figure 130 Use single data or sequences/worklists and set delay between optional 2D UV detector and MS detector for aligning signals

^{2}D Chromatogram Creator for MassHunter provides an easy access to 2D-LC measurements for Agilent MassHunter users. The^{2}D Chromatogram Creator for MassHunter is used for combining UV signals and metadata of cuts in OpenLab CDS ChemStation Edition with the MS signal acquired by MassHunter.

As output, it creates following MassHunter chromatograms:

- A series of ^{2}D chromatograms, one per cut including MS and optional UV signals
- If applicable, a ^{1}D UV chromatogram (for a typical but optional ^{1}D UV detector) in MassHunter data format including cut data
- An optional raw ^{2}D UV signal, and a
- ^{2}D MS raw signal

You will find the 2D Chromatogram Creator for MassHunter on the 2D-LC add-on DVD.

NOTE

More information about Installation, Example Data, and Workflow is available in the Agilent technical note *^{2}D Chromatogram Creator for MassHunter* (G2198-90101).

NOTE

Further information about the 2D-LC Diverter Valve Solution can be found in the technical note *Agilent InfinityLab 2D-LC Solution with mass spectrometric detection and diverter valve* (G4236-90100).

15 Additional Notes

This chapter provides content from original release and technical notes for reference.

2D-LC Diverter Valve Guide

Purpose of using a diverter valve

In two-dimensional liquid chromatography (2D-LC), the second dimension can be used as an effective desalting tool to allow online coupling of chromatographic methods using MS-incompatible mobile phases to MS detection. A diverter valve can be used to automatically divert salt or buffers coming from the first-dimension (^{1}D) mobile phase to waste at the beginning of every second-dimension (^{2}D) analysis. This is shown in Figure 131 on page 384[1].

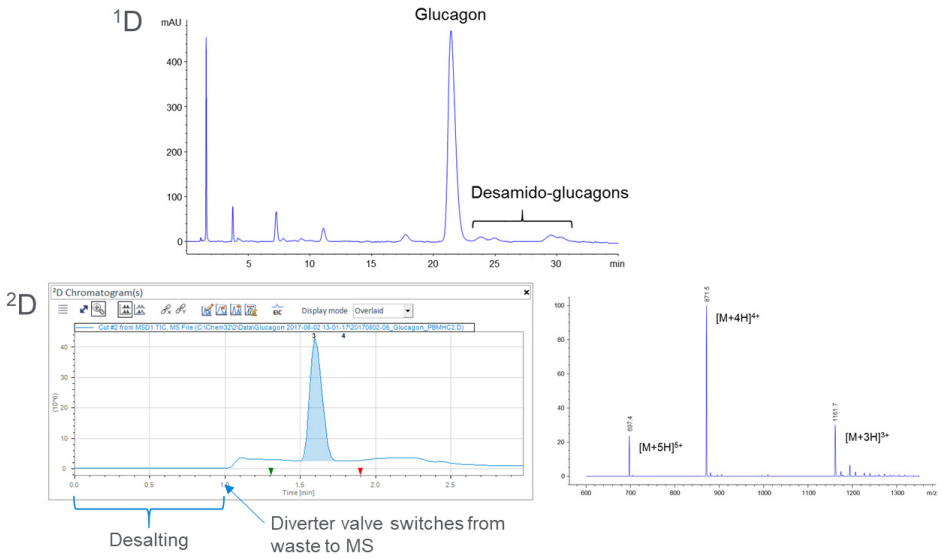


Figure 131 Peak-based multiple heart-cutting 2D-LC analysis of the peptide glucagon.

[1] Data from Agilent Application Note 5991-8437EN: 2D-LC as an Automated Desalting Tool for MSD Analysis - Direct Mass Selective Detection of a Pharmaceutical Peptide from an MS-Incompatible USP Method. Chromatographic conditions: ^{1}D: Column: Agilent ZORBAX Eclipse Plus C18, 3.0 × 150 mm, 3.5 µm, Solvent: A) 16.3 g KH2PO4 in 800 mL water adjusted to pH 2.7/200 mL acetonitrile, B) Water/acetonitrile (60/40), Flow rate: 0.5 mL/min, Temperature: 45 °C, Detection: VWD, 214 nm, Heart-cutting of the glucagon peak; ^{2}D: Column: Agilent AdvanceBio Desalting-RP, 2.1 × 12.5 mm, Solvent: A) Water + 0.1 % formic acid, B) Acetonitrile + 0.1 % formic acid, 1 min desalting time at 5% B, Flow rate: 0.4 mL/min, Detection: MSD.

Diverter valve solution for a combination of UV and MS detection

CAUTION

Switching a (diverter) valve while the flow is on generates pressure pulses.

When using a diverter valve downstream to the flow cell of a UV detector (^{2}D UV detector), this may damage the flow cell.

This is no specific 2D-LC issue but the valve switches more frequently for 2D-LC, once per cut analyzed in ^{2}D.

✓ **Install the diverter valve as recommended in this Technical Note.**

To avoid flow cells being damaged by pressure pulses, the installation of the diverter valve as shown in Figure 132 on page 386 for a 2-position/6-port valve is used. Coming from the ^{2}D detector, a T-piece is installed with connection to the MS and to the diverter valve.

The capillaries connecting the T-piece to the diverter valve and from diverter valve to waste have a large internal diameter and generate very little restriction compared to the restriction generated by the capillary between T-piece and MS and the MS sprayer. Therefore, most of the flow coming from the 2D detector goes to waste in diverter valve switching position 1. Depending on the back pressure ratio at the T-piece, a small flow will still go to the MS. In case of very high salt concentrations this should be considered. This diverter valve switching position is illustrated in Figure 132 on page 386 section A.

In switching position 2 of the diverter valve shown in Figure 132 on page 386 section B, the flow is blocked at the diverter valve by a blank nut such that the flow from the ^{2}D detector is let via the T-piece towards the MS. A pressure relieve valve is installed between the T-piece and the diverter valve, which protects the ^{2}D UV detector flow cell in case a blockage of the MS nebulizer occurs and the diverter valve is in position 2 (Figure 132 on page 386 section B).

The blank nut blocking the flow at port 2 of the diverter valve may be replaced by red PEEK tubing to generate a split between the MS and this tubing used as a (second) waste line. The ratio of the back-pressures generated in this flow path compared to the flow path towards the MS will determine the split ratio. This can be adjusted through cutting the length of the red PEEK tubing to a suitable length (Figure 132 on page 386 section C).

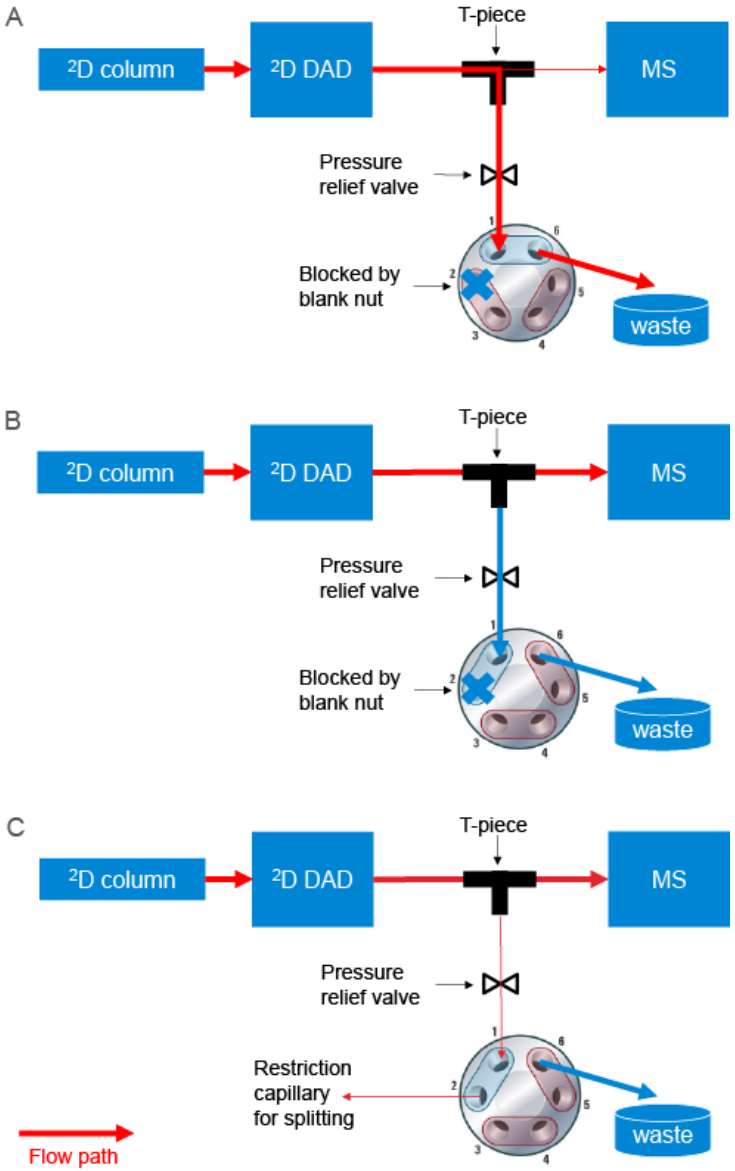


Figure 132 Diverter valve solution for a combination of UV and MS detection. (A) Position 1 during desalting, flow almost entirely goes to waste. (B) Position 2 during the analysis, flow goes to the MS. (C) The blank nut blocking the flow at port 2 of the diverter valve may be replaced by red PEEK tubing to generate a split between the MS and a waste line connected at this tubing. The ratio of the back-pressures generated in this flow path compared to the flow path towards the MS will determine the split ratio. This can be adjusted through cutting the length of the red PEEK tubing to the right length.

Installation and configuration of the diverter valve solution

A CAN-based 2-position switching valve can be used as a diverter valve. The 2-position switching valves listed below are recommended examples.

Parts required	#	p/n	Description
	1	5067-4282	2-position/6-port valve head, 800 bar
OR	1	5067-4117	2-position/6-port ultra high pressure valve head, 1200 bar
OR	1	5067-4283	2-position/10-port valve head, 800 bar
OR	1	5067-4118	2-position/10-port ultra high pressure valve head, 1200 bar

Hardware required The following valve hosts can be used:

- G1170A 1290 Infinity Valve Drive
- G7116B 1290 Infinity II Multicolumn Thermostat with valve drive installed
- G7116A 1260 Infinity II Multicolumn Thermostat with valve drive installed

Software required Agilent OpenLAB 2D-LC Software A.01.04 or higher

1 Install the capillaries (see Table 35 on page 387).

Table 35 Parts required for installation of the diverter valve

Description	Required part	Part number
Diverter valve	e.g. 2ps/6pt Valve head, 800 bar	5067-4282
T-piece	Tee, 1/16 in, 316 SST, low dead volume	0100-0969
Pressure relief valve	Pressure relief valve	G4212-60022
Blank nut	1/16 in stainless steel blanking nut	01080-83202
PEEK fittings	Finger-tight PEEK fittings, 1/16 in (10/pk)	5063-6591
Capillary from 2D detector to T-piece	Stainless steel connecting capillary, 400 mm long, 0.12 mm id	5067-4606
Capillary from T-piece to MS	Tubing, PEEK, 1.6 mm od, 0.12 mm id, 1.5 m, e.g. cut to 400 mm length	0890-1915
Capillary from T-piece to pressure relief valve	Capillary ST 0.3x80 mm SL-SL	5500-1228
Capillary from pressure relief valve to diverter valve	Capillary ST 0.3x80 mm SL-SL	5500-1228

2 Configure the diverter valve in OpenLab CDS ChemStation Edition Instrument Configuration (Figure 133 on page 388, section A).

3 Select the diverter valve in the 2D-LC Configuration (Figure 133 on page 388, section B).

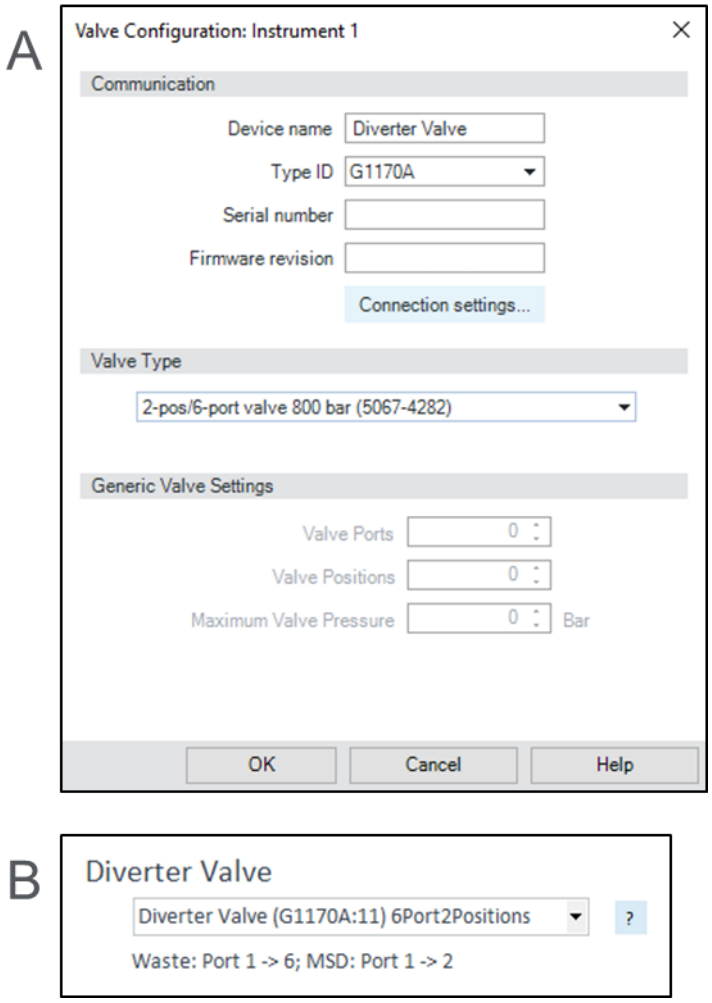


Figure 133 Configuration of the diverter valve in the ChemStation Instrument Configuration (A) and the 2D-LC Configuration (B).

Using the diverter valve

The diverter valve can be used to automatically divert salt or buffers coming from the ^{1}D mobile phase to waste at the beginning of every ^{2}D run. In the 2D-LC method, the ^{2}D time is defined after which the diverter valve switches to the MS (Figure 134 on page 389 section A). With the method setup shown in Figure 134 on page 389 section A, the diverter valve will automatically switch to waste for the first 1.00 minutes of every ^{2}D run. After 1.00 minutes, the diverter valve switches the flow to the MS.

The ^{2}D gradient needs to be programmed to allow trapping of the analytes on the ^{2}D column or desalting cartridge while salt or buffers from the ^{1}D mobile phase are eluted to waste. After the isocratic desalting phase, the actual ^{2}D gradient starts and trapped analytes are eluted to the MS. An example of a ^{2}D gradient used with a desalting cartridge is shown in Figure 134 on page 389 section B.

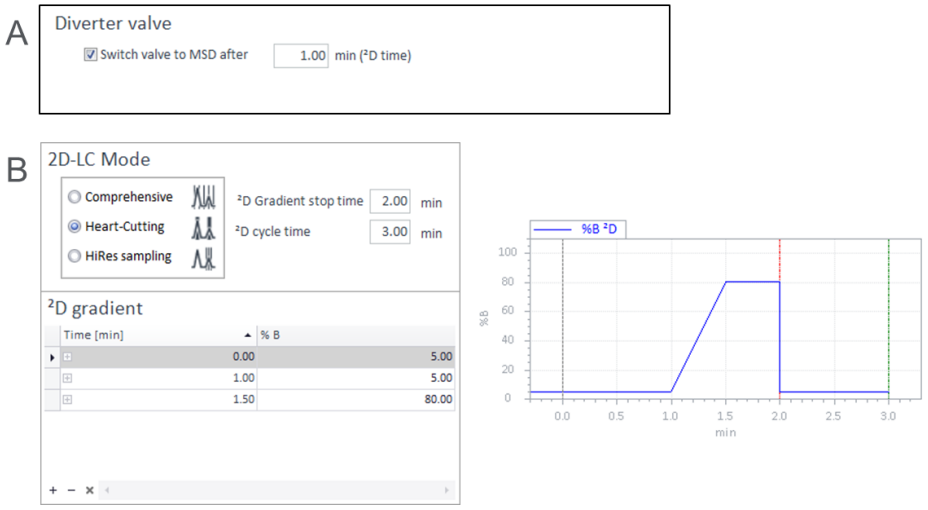


Figure 134 2D-LC method setup for diverting salt or buffers originating from the ^{1}D mobile phase to waste at the beginning of every ^{2}D run, (A) diverter valve setup in the Advanced settings tab of the 2D-LC method, (B) ^{2}D gradient setup in the General settings tab of the 2D-LC method.

2D Chromatogram Creator for MassHunter

Introduction

2D Chromatogram Creator for MassHunter provides an easy access to 2D-LC measurements for Agilent MassHunter users. Similar to the 2D-LC Viewer, it generates 2D chromatograms (abundancy over time, usually the TIC) for cuts created in **Multiple Heart-Cutting or High-Resolution Sampling 2D-LC** for display and data analysis in MassHunter.

Comprehensive 2D-LC measurements can be displayed and analyzed with GC Image LCxLC Edition Software, which is described in the 2D-LC system manual, that can be found in folder documentation of the 2D-LC Software.

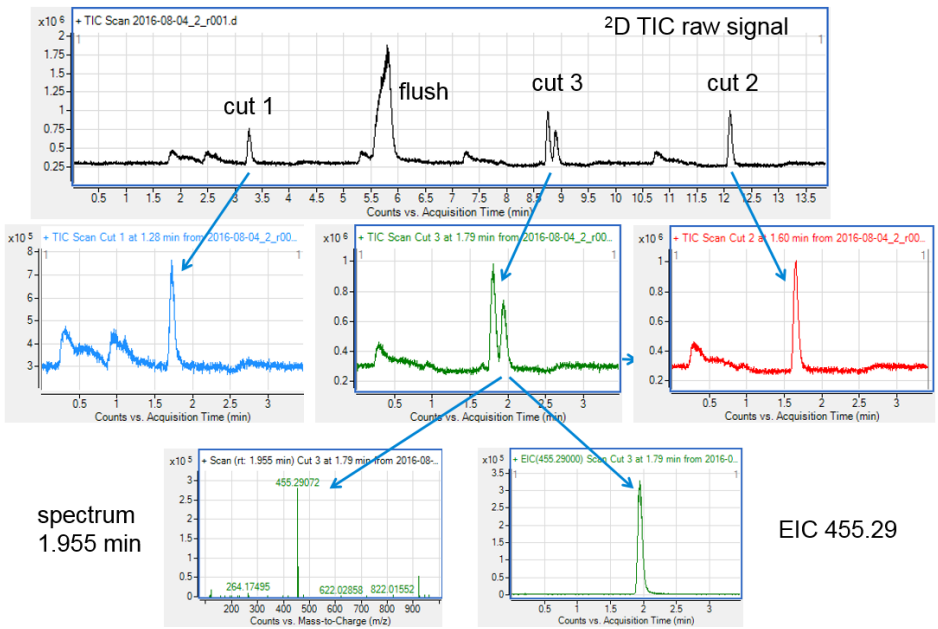


Figure 135 Splitting the raw data file into 2D chromatograms

The raw MS TIC signal continuously measures MS data of the 2D eluent. 2D Chromatogram Creator for MassHunter creates one 2D chromatogram per cut made in the first dimension of a multiple heart-cutting or high-resolution sampling measurement. These chromatograms can be used for further data analysis in MassHunter.

Function

For running 2D-LC/MS measurements, this is a typical system:

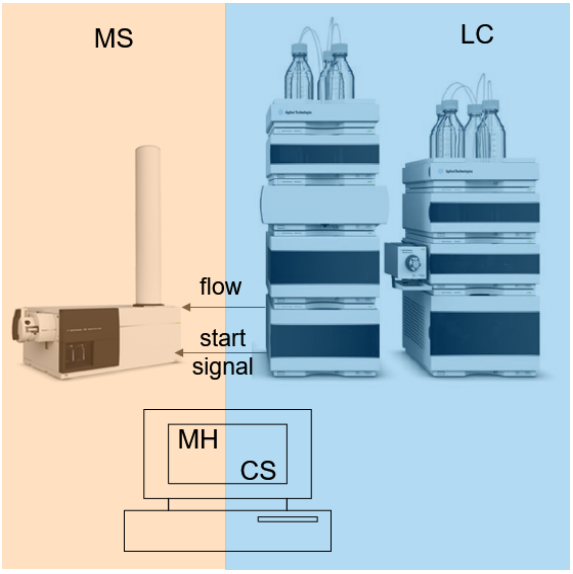
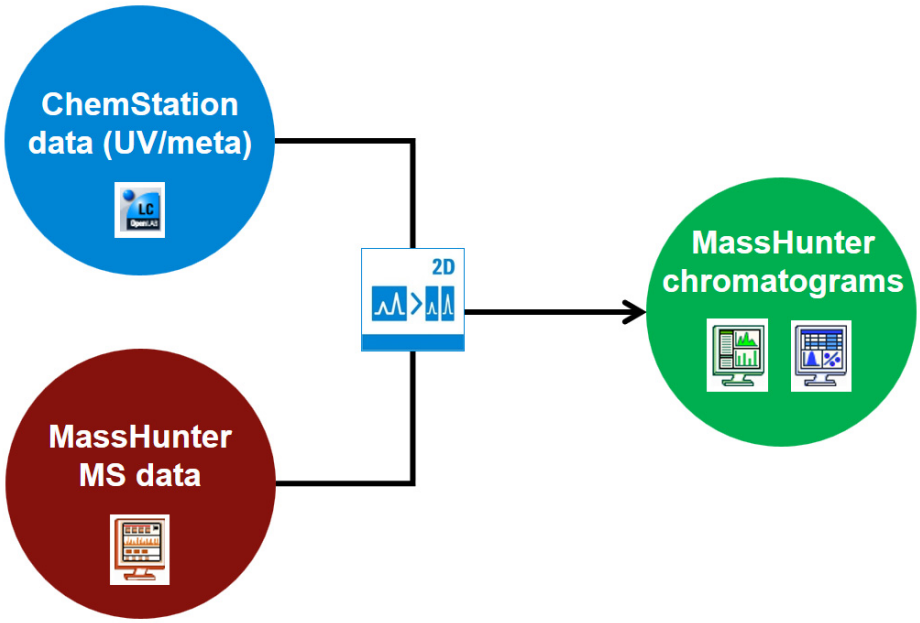


Figure 136 Typical system setup

The LC part is an LC system as described in the 2D-LC system manual. A typical system uses a UV detector in the first dimension and an optional UV detector in the second dimension. The 2D-LC instrument control and UV signal acquisition is done with OpenLAB CDS ChemStation (CS) Edition.

The MS detector, a TOF, Q-TOF or Triple Quadrupole, is used in the second dimension. It is controlled by MassHunter (MH) Acquisition Software, which acquires the MS signal.

For synchronizing signals of LC and MS, an APG remote cable connects both parts of the system, which communicates start and stop signals.



MassHunter has no knowledge about the size, order and position of cuts created by OpenLAB CDS ChemStation Edition. Therefore, ^{2}D Chromatogram Creator for MassHunter is used for combining UV signals and meta data of cuts in OpenLAB CDS ChemStation Edition with the MS signal acquired by MassHunter.

As output, it creates following MassHunter chromatograms:

- A series of ^{2}D chromatograms, one per cut including MS and optional UV signals
- If applicable, a ^{1}D UV chromatogram (for a typical but optional ^{1}D UV detector) in MassHunter data format including cut data
- An optional raw ^{2}D UV signal and a
- ^{2}D MS raw signal

Compatibility

NOTE

MassHunter LC/MS Data Acquisition Version 10.0 requires 2D Chromatogram Creator rev. 1.1.20.

^{2}D Chromatogram Creator for MassHunter, OpenLAB CDS ChemStation Edition and MassHunter have been tested for co-execution (i.e. installed and running on same PC) with respect to parallel data acquisition in following configuration:

- ^{2}D Chromatogram Creator 1.1.20
- OpenLAB CDS ChemStation Edition C.01.07 SR3 (Workstation)
- Agilent OpenLAB 2D-LC Software A.01.04
- MassHunter Q-TOF B.08.00
- MassHunter LC/QQQ B.08.02
- MassHunter Data Analysis B.08.00
- Operating system: Windows 10 Professional 64-Bit

Modules:

- 1290 Infinity II modules supported with LC Driver A.02.16
- QQQ or Q-TOF

PC Hardware:

- MassHunter bundle PC HP Z440 or equivalent

^{2}D Chromatogram Creator for MassHunter has also been tested with OpenLAB CDS ChemStation Edition C.01.07 SR2, MassHunter Acquisition for TOF and QTOF B.06.01 SP1, MassHunter Acquisition for QQQ B.08.00 SP1, MassHunter Qualitative Software B.07.00 SP1 and SP2 and MassHunter Quantitative Software B.07.01. No co-execution of OpenLAB CDS ChemStation Edition and MassHunter was tested in this configuration.

MassHunter Qualitative B.07.00 is known to display misleading signal names.

Installation

Install the 2D-LC/MS system as outlined above. The 2D-LC system installation is described in the 2D-LC system manual. The installation of the MS instrument and its hydraulic and electronic connection to an LC system is described in the MS manual. Installations of OpenLAB CDS ChemStation Edition and MassHunter software packages are described in respective user manuals.

Install ^{2}D Chromatogram Creator for MassHunter by double-clicking setup.exe in folder software of the 2D-LC Software DVD. For simple file handling, ^{2}D Chromatogram Creator for MassHunter should preferably be installed on the PC with MassHunter data analysis software.

Example Data

Folder "example Data\2D Chromatogram" on the 2D-LC software CD contains two subfolders MassHunter and ChemStation with example data which can be used for trying out this software. It uses a sequence/worklist with 3 measurements of 3 compounds:

Table 36 Example Data

#	Compound	m/z	^{1}D retention time
1	Imipramine	280.407	1.33 min
2	Protriptyline	263.37	1.66 min
3a	Nortriptyline	263.38	1.84 min
3b	Verapramile	454.6	1.84 min

I.e. compounds 3a and 3b are co-eluting in ^{1}D and are separated in ^{2}D. Compounds 2 and 3a have the same mass but are separated in the ^{1}D chromatogram. See also figures 1 and 13.

Workflow for a Single Measurement

For a 2D-LC/MS measurement, methods need to be set up in both OpenLAB CDS ChemStation Edition and MassHunter. Set up the 2D-LC method as described in the 2D-LC system manual. Set the stop time in the ^{2}D pump. Then create a method in MassHunter for the MS.

For synchronizing run times (i.e. starting and stopping measurements simultaneously), please use “External start” as a run parameter:

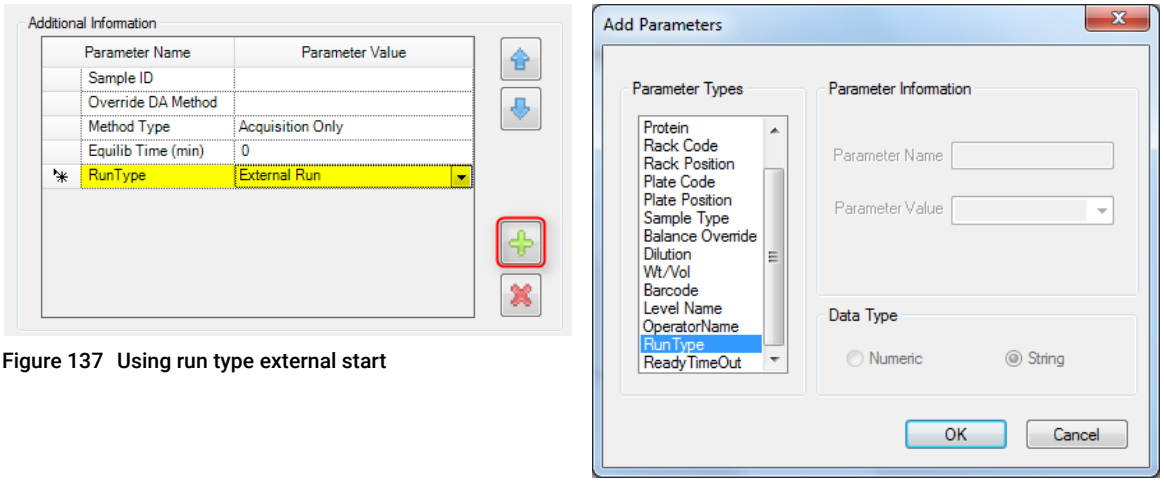


Figure 137 Using run type external start

In section “Additional information”, add parameter “run type” and choose option “external run”. Set stop time “no limit” in MassHunter.

Then start the run in OpenLAB CDS ChemStation Edition, which will automatically start the MassHunter run. Both chromatographic data systems will create data files, which will be used for combined data analysis. Make OpenLAB CDS ChemStation Edition and MassHunter data files available through a network share or copy data to the PC with ^{2}D Chromatogram Creator for MassHunter. Having both files on one PC is faster, more secure and recommended.

Start ^{2}D Chromatogram Creator for MassHunter.

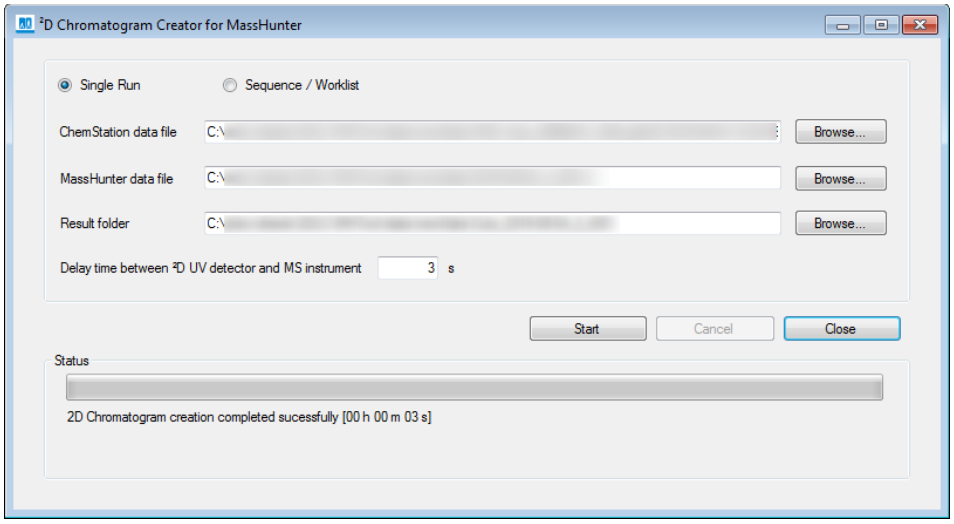


Figure 138 User interface for single data files

Use mode “Single Run”.

Provide data files for OpenLAB CDS ChemStation Edition and MassHunter. A result folder is proposed and can be changed as needed.

If an optional UV detector is used in ^{2}D, a delay time can be set between this detector and the MS detector. This value is used for aligning MS versus UV data by compensating the time offset.

Click the start button for starting the file processing. This can take some time depending on the file size.

Workflow for a Sequence/Worklist

Create methods in both OpenLAB CDS ChemStation Edition and MassHunter.

Set up a sequence in OpenLAB CDS ChemStation Edition.

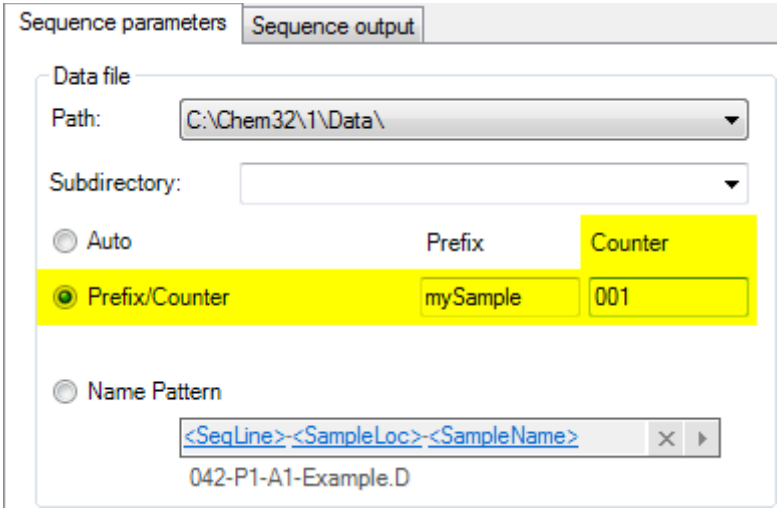


Figure 139 Using counters for data files in OpenLAB CDS ChemStation Edition

In sequence parameters, use an incremental counter for the data file names. This counter is the recommended way for later linking OpenLAB CDS ChemStation Edition data files to MassHunter data files.

Similarly set up a worklist in MassHunter and use an incremental counter. There are various possibilities for applying a counter, e.g. inserting multiple samples using a counter.

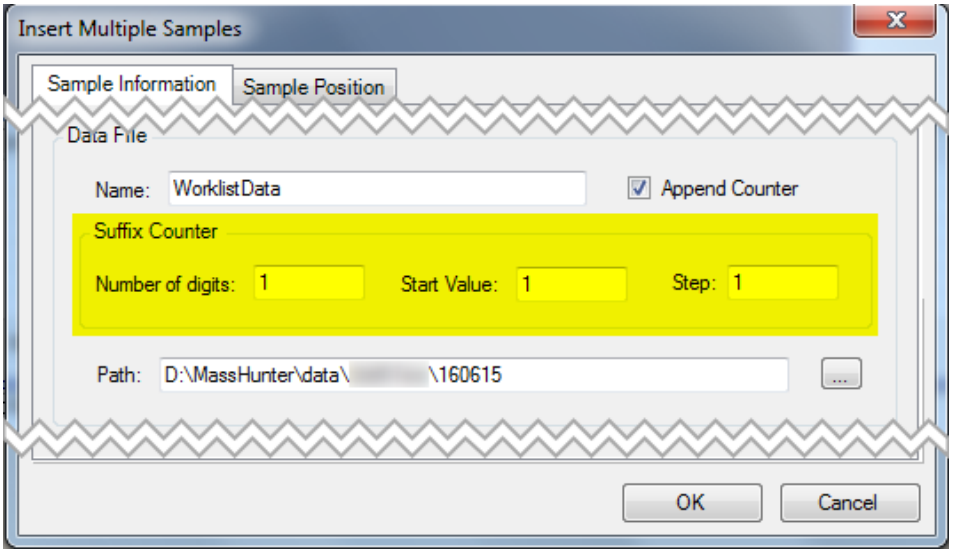
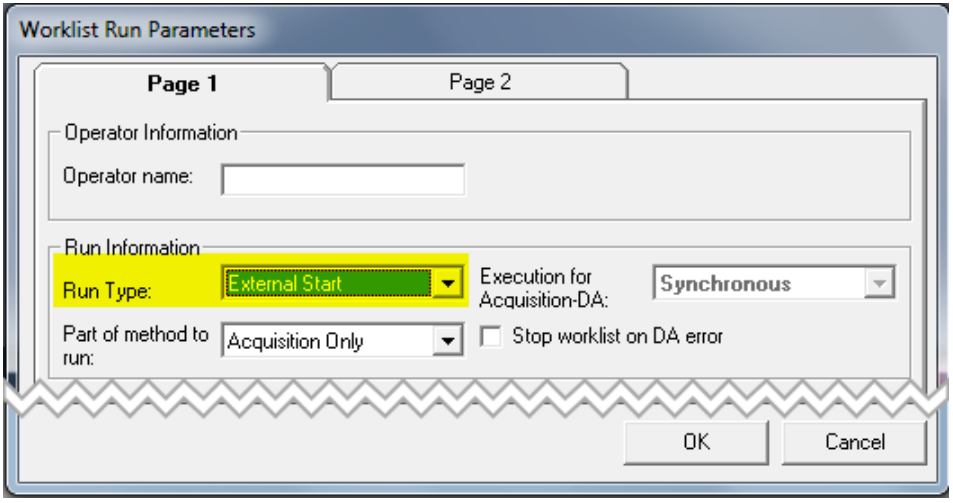


Figure 140 Using counters for data files in MassHunter Worklists

Set “External Start” as run type in “Worklist Run Parameters” for synchronizing runs.



Start ^{2}D Chromatogram Creator for MassHunter with option “Split multiple data files”:

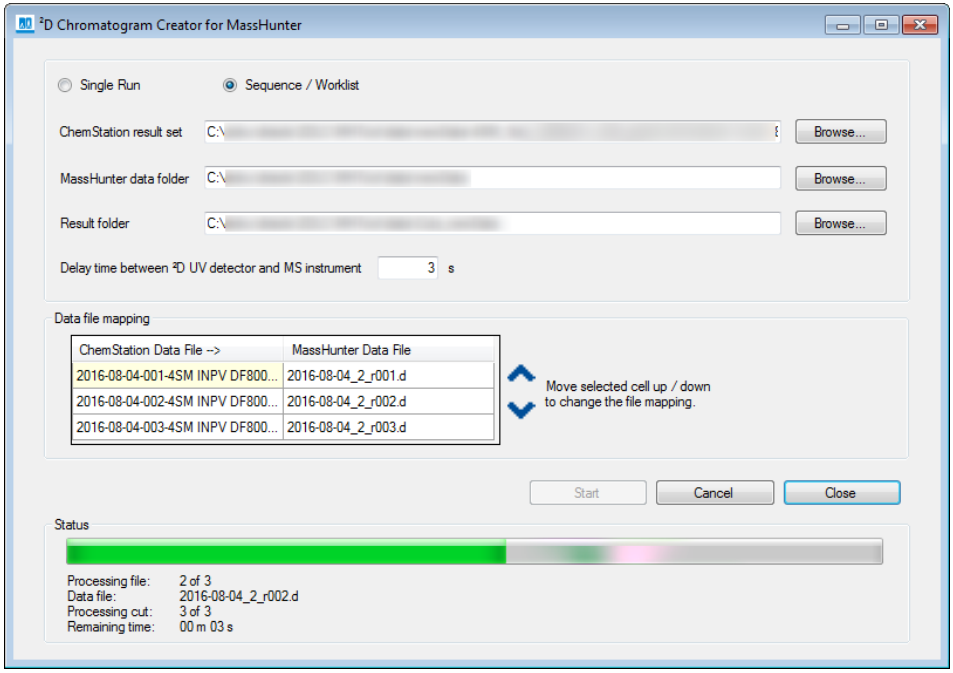


Figure 141 Data file mapping

In table “Data file mapping” OpenLAB CDS ChemStation Edition versus MassHunter data files are matched. Each line lists the OpenLAB CDS ChemStation Edition data file and the corresponding MassHunter data file. Please verify if the default linkage is correct, which sorts data files for both software editions by their creation date (same chronologic order is applied). However, this date may change when copying files. Sorting can be adjusted by right-clicking on the headers of the data file mapping table. Different sorting options are available for both OpenLAB CDS ChemStation Edition and MassHunter:

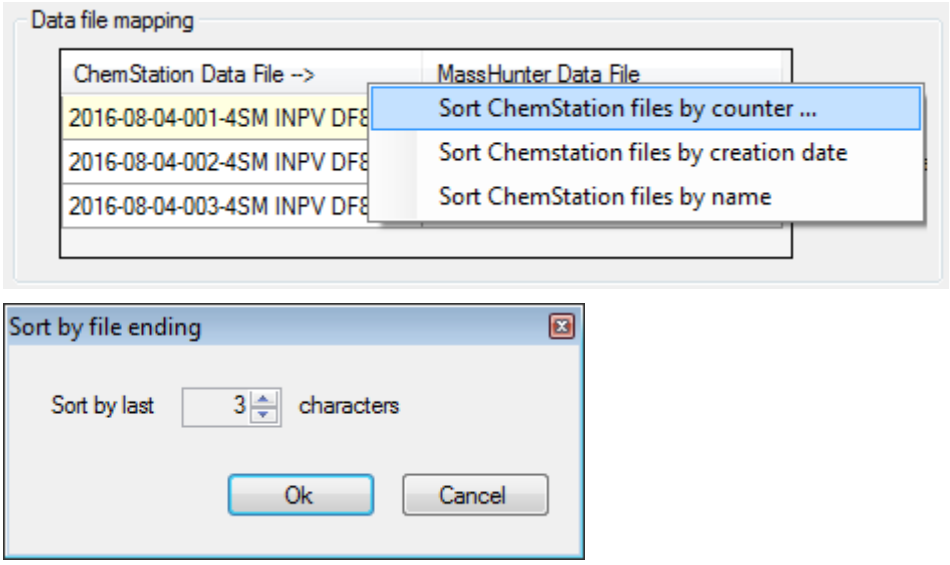


Figure 142 Sorting options

Sorting by file ending using the counter defined previously is the best user-defined way for linking data files.

Extracting and Displaying Result Data

Result files are stored to the result folder set above. Data for cuts can be imported as single files or by loading all data files at once. Each cut contains data for the ^{2}D signals (MS, optional UV) and the ^{1}D UV signal. By default, the ^{2}D TIC is displayed:

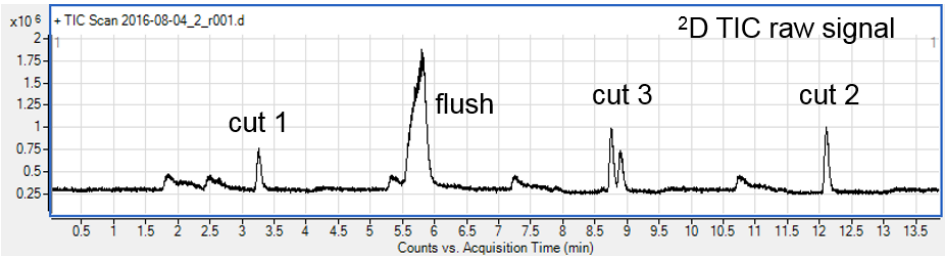


Figure 143 Raw ^{2}D MS signal (TIC)

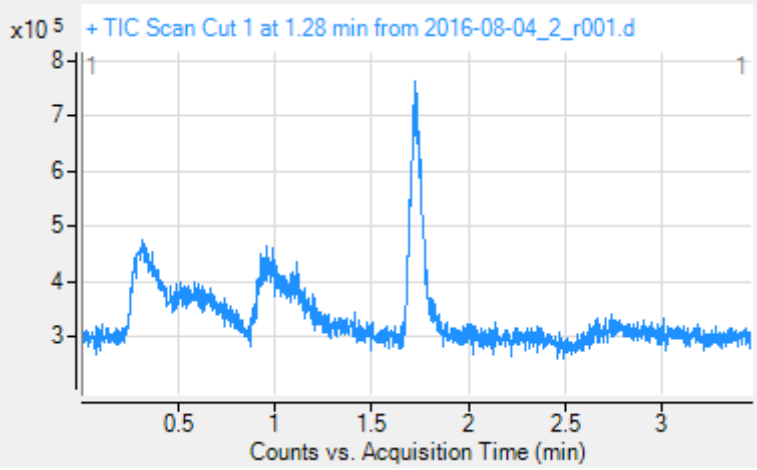


Figure 144 TIC for cut 1 at 1.28 minutes in ^{1}D

Figure 10 shows an example for cut 1. ^{2}D Chromatograms are named by their cut number, the first dimension retention time and the original MassHunter data file. This example uses a ^{2}D gradient time of 3.5 minutes corresponding to the length of that ^{2}D chromatogram.

In case of high-resolution sampling measurements, series of adjacent cuts can be defined. Then, cuts are numbered by the series and an incremental counter, i.e. cut 1-3 is the third cut of the first series.

The optional ^{2}D UV chromatogram can be extracted by right-clicking the TIC scan and using function “Extract Chromatograms…”, see Figure 11.

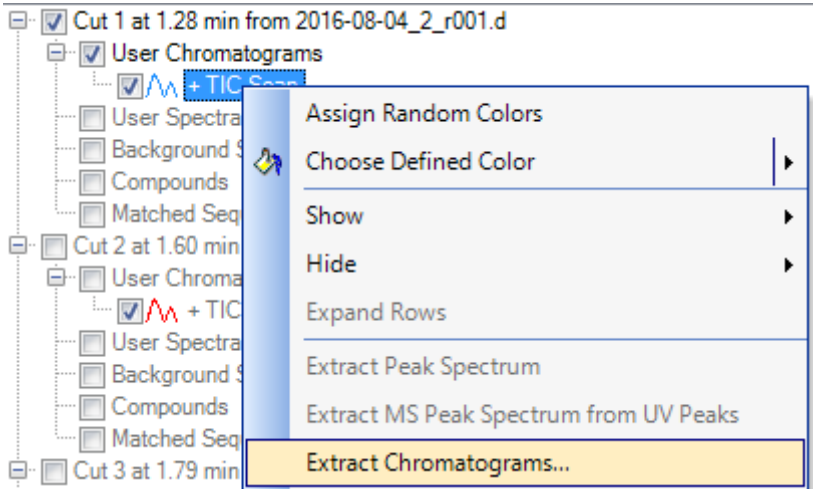


Figure 145 Extracting chromatograms

Use Type Other Chromatograms and choose the ^{2}D detector (in this example DAD2) and the signal with the wavelength of interest, see Figure 12.

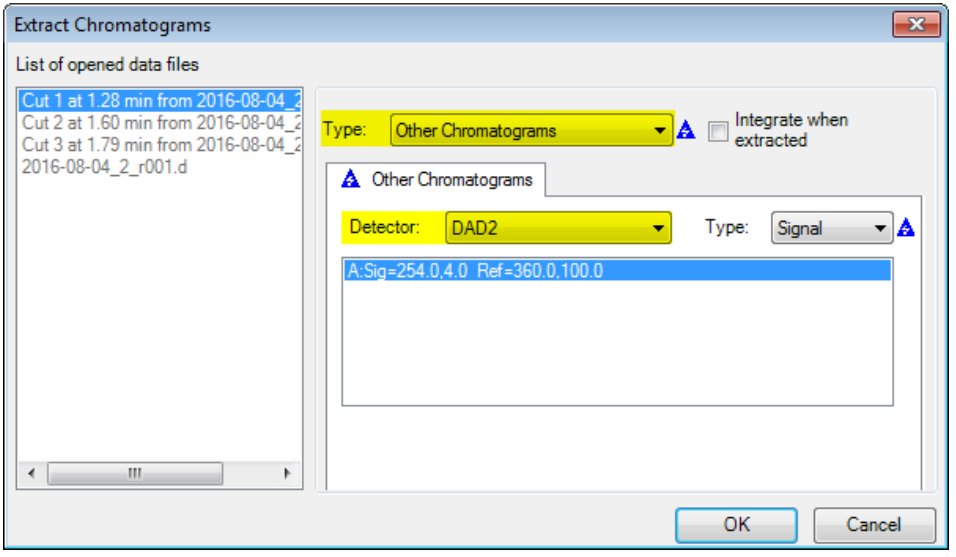


Figure 146 Available chromatograms

Similarly, chromatograms can be extracted from the original ^{2}D MS data file, which has been enriched by 2D-LC and UV data. Available signals are

- ^{1}D UV signal
- ^{2}D UV signal
- Cuts in ^{1}D

Similar to the 2D-LC Software, these data files can be used for displaying the ^{1}D measurement with cuts (here in red), see Figure 13.

All MS Chromatograms can then be processed further in MassHunter as usual, e.g. for extracting ion chromatograms, spectra and data analysis.

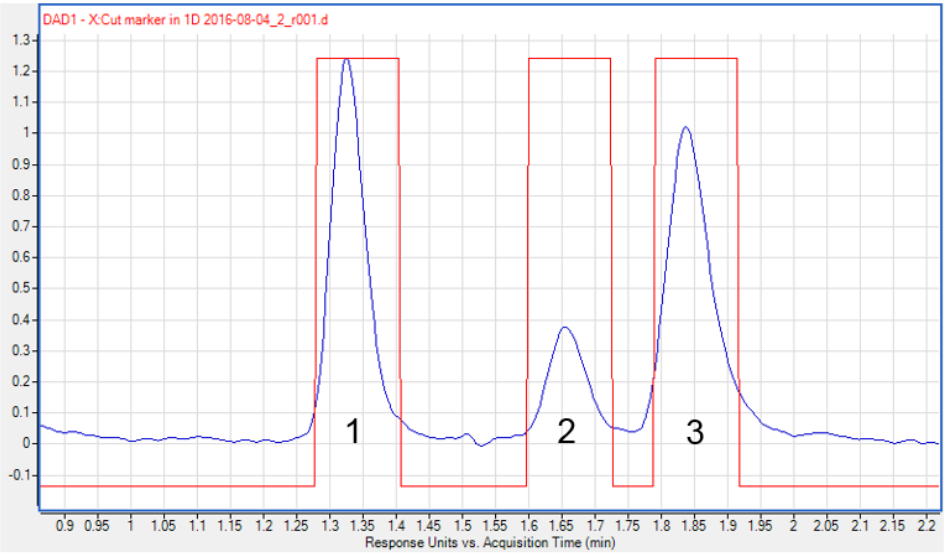


Figure 147 ^{1}D UV Chromatogram with cuts

Agilent OpenLab 2D-LC Software

Introduction and Overview

Agilent OpenLab 2D-LC Software A.01.04 introduces a fully integrated solution from method setup to data analysis for 2D-LC/MS within OpenLab CDS ChemStation Edition (herein also shortly called "ChemStation"). It combines the separation power of two-dimensional chromatography with the selectivity of

mass selective detection (MSD) in the second dimension. It can be used with all Agilent Single Quadrupole Detectors supported by ChemStation including latest LC/MSD (G6125B) and LC/MSD XT (G6135B).

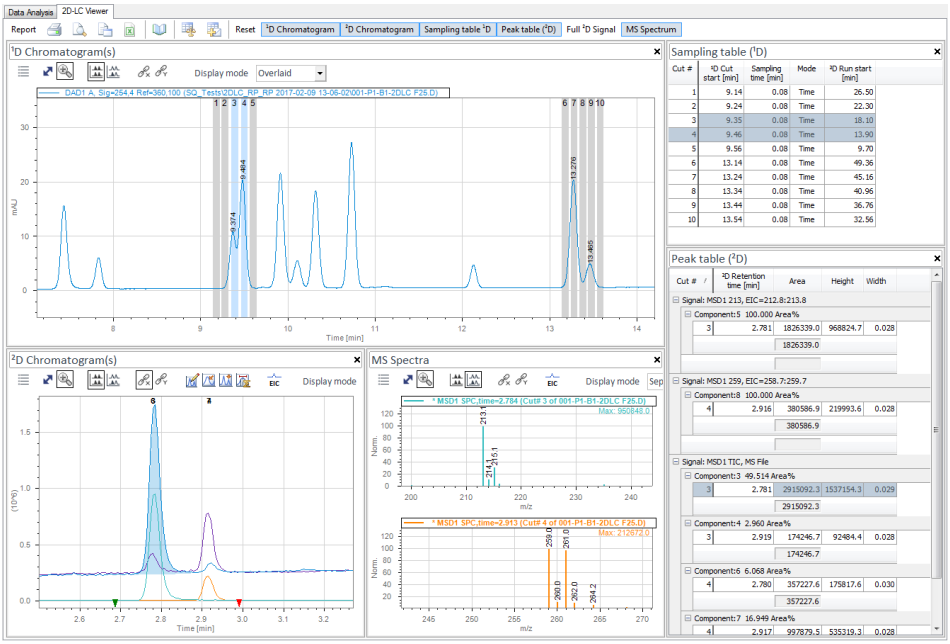
The popular 2D-LC viewer has been enhanced to display and analyze single quadrupole signals including extraction of ion chromatograms and spectra. An LC diverter valve (typically installed to an external valve drive G1170A) can be controlled automatically by the software, which helps using buffers in the first dimension and diverting them to waste in the second dimension before they may enter an MS detector.

OpenLab 2D-LC Software A.01.04 SR1 and SR2 add several important bug fixes, while A01.04 SR3 is necessary to support Chemstation C.01.10. The current OpenLab 2D-LC Software A.01.04 SR4 fixes some minor software bugs and support the current OpenLab ChemStation C.01.10. Update 3.

TThe OpenLab 2D-LC Software A.01.04 SR4 USB Stick includes the next software 2D Chromatogram Creator for MassHunter rev. 1.20, which is necessary for processing MassHunter Data Acquisition Version 10.0 such that individual 2D chromatograms can be displayed in MassHunter.

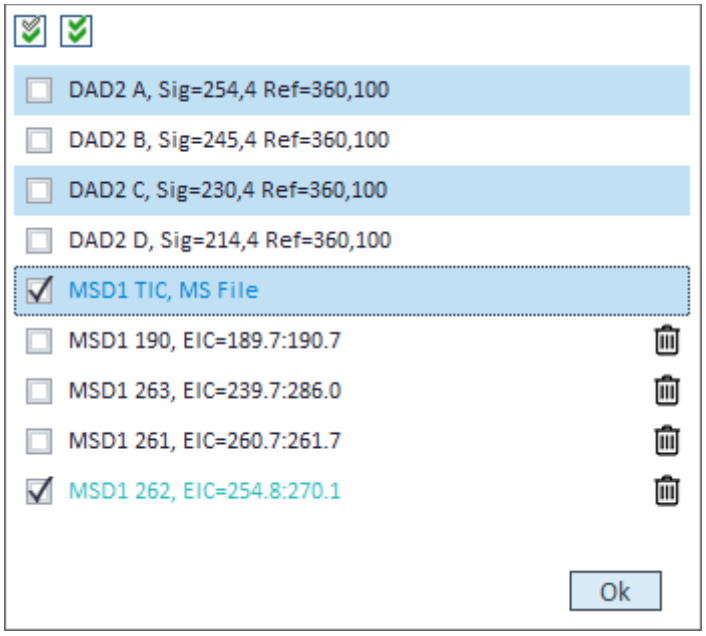
List of New Features

2D-LC Viewer for Single Quadrupole Data



The 2D-LC Viewer displays now also MS and UV spectra and extracted ion chromatograms.

select signals



Multiple signals can be managed using the signal selector. Use checkmarks for choosing signals to be displayed.

 select highlighted/all items

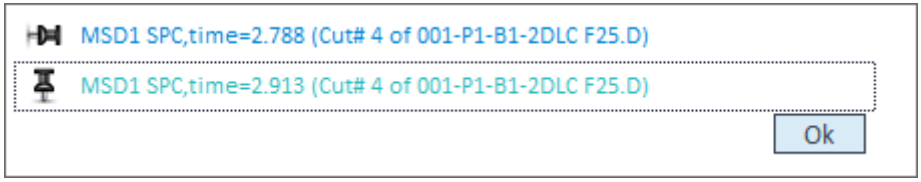
As a shortcut, multiple signals can quickly be highlighted by control- or shift-clicking signal names for the ^{2}D chromatogram panel and clicking the selection buttons.

 time/apex/average per range

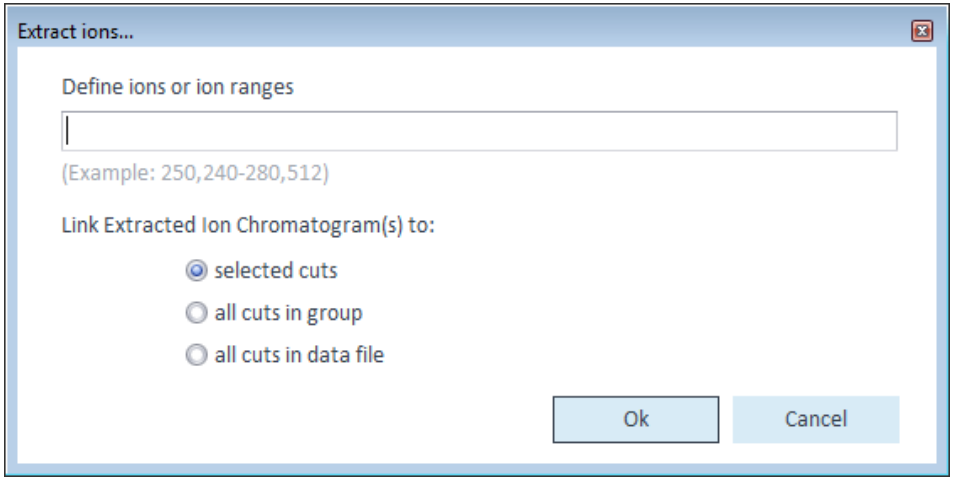
Spectra can be displayed for a time or a peak apex by clicking the ^{2}D chromatogram or dragging the mouse for an average spectrum per time range.

 pin spectra

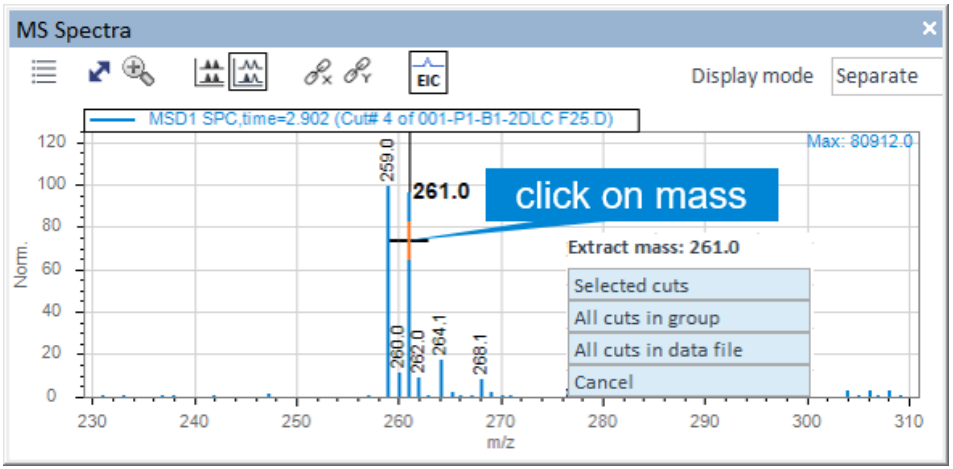
Spectra can be pinned for storing them per associated cut for display and reporting:



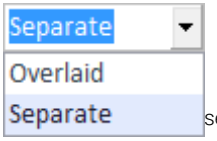
extract ion chromatogram



Ion chromatograms can be extracted for a mass or mass range selected in the spectrum or entered for the 2D chromatogram(s).



Ion chromatograms can also be extracted from spectra by clicking the EIC button and then selecting a mass.

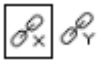


separate vs. overlaid mode

Multiple (extracted ion) chromatograms or spectra can be displayed in the same window (overlay) or in separate windows.

same scale/full scale

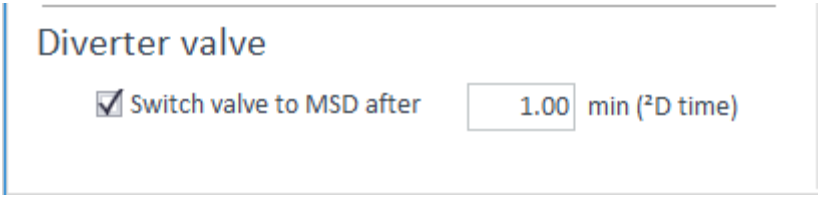
Diagrams can be displayed using the same scale or the full scale for each diagram.



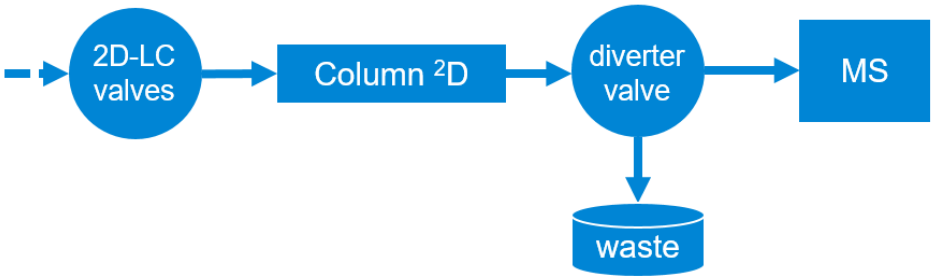
linked x and y axes

For zooming, the axes can optionally be linked for all displays. In case of linked axes, all displays will be zoomed to the same range.

Diverter Valve

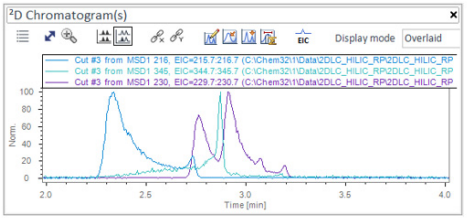


A two-position LC valve (e.g. a 2-pos/6-port valve) can be configured in the 2D-LC Configuration for diverting ^{2}D eluent with buffers before they enter an MS detector. As a method parameter, a ^{2}D time can be set, after which the flow path is directed to the detector.

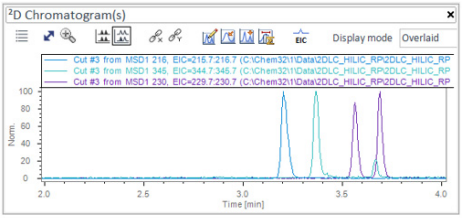


Active Solvent Modulation (ASM)

Active solvent modulation is supported by this software revision.



Classic 2D-LC



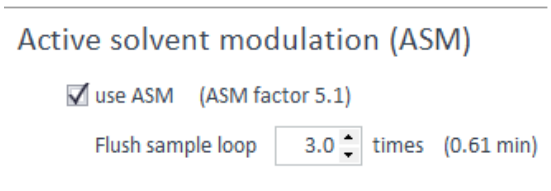
ASM: Improved resolution and sensitivity

Active Solvent Modulation (ASM) is a solution for improving solvent compatibility between the first and the second dimension.

^{1}D solvent in the sample loop may impair separation if it has a high elution strength in respect to the second dimension column. This may result in unretained elution, broad and distorted peaks, often with a loss of separation. ASM modulates the content of the sampling loop by dilution with weak solvent before it reaches the ^{2}D column. In this way the analytes in the sample focus on the column head. After enrichment of the analytes the valve switches to its final through-loop-position. This workflow is enabled by the 2D-LC Valve ASM G4243A. Different ASM capillaries can be installed for adjusting the dilution of ^{1}D solvent with ^{2}D solvent.

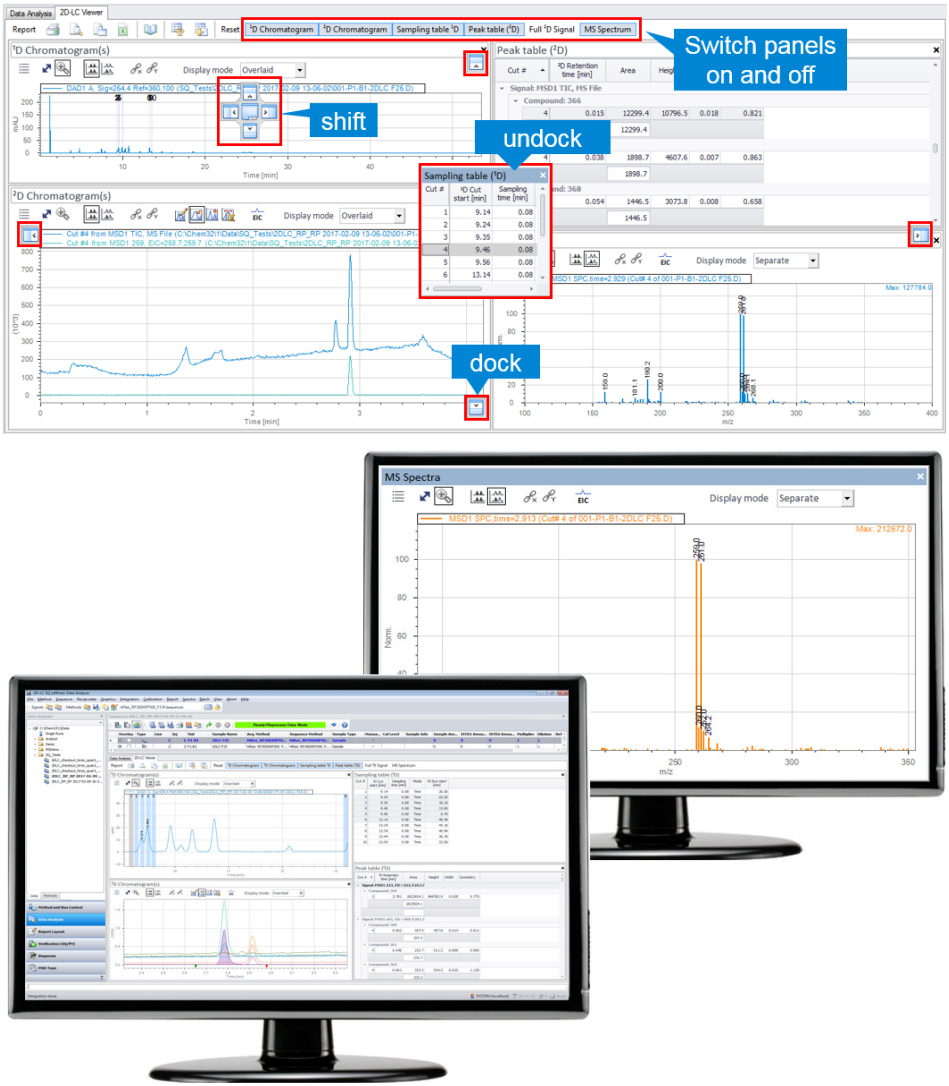
This software allows configuration (see "Appendix B: Installation and Configuration" on page 423) and use of the ASM valve and capillary. ASM can be switched on or off. Please input, how many times the sample loop shall be flushed before the ASM valve switches to the flow path without ASM bypass. A good starting point for optimizations is 3x.

Program your analytical gradient such that it starts after this time. In this example gradient start conditions should be kept for 0.61 min.



For details please refer to the Agilent G4243A 2D-LC ASM Valve Guide G4243-90000, which is available in folder documentation or from the Agilent web site.

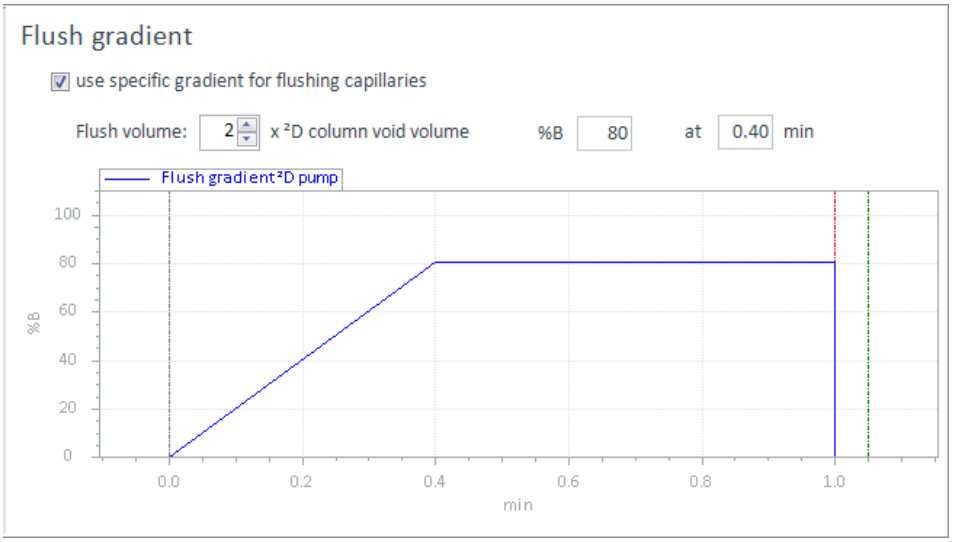
Flexible User interface



The flexibility of the user interface has been improved in order to freely arrange different panels. The can be switched on or off, shifted to new positions, docked or undocked and even use multiple monitors.

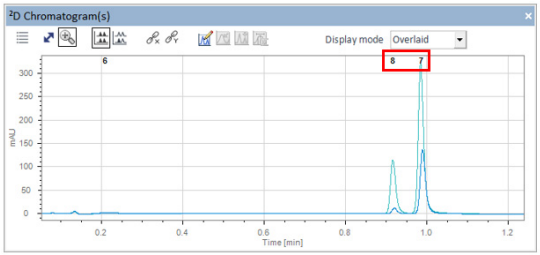
More Features

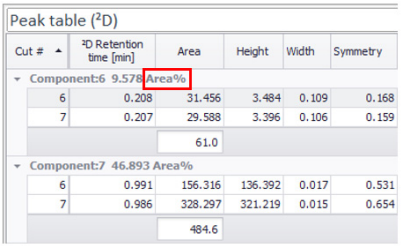
Flush gradients can now be programmed more flexibly by defining gradient ramps:



Flush gradients may be used instead of analytical gradients for faster flushing capillaries during the measurement.

Reporting has been improved to optionally include pinned spectra and extracted ion chromatograms. Graphic quality of diagrams has been improved.





Component labels Area%

Components identified in ^{2}D chromatograms are now labelled for better relation to the ^{2}D peak table. The peak table has been enhanced to include area%, which is the percentage of an area of that component compared to the sum of all integrated areas. A component table is included to the report.

Service Release 1 2D-LC Software A.01.04 SR1 adds important bug fixes and is highly recommended, see Table 37 on page 417 and "Appendix C: Improved heart-cutting" on page 430.

Compatibility Matrix

The compatibility matrix provides information about installation and execution prerequisites with respect to hardware, firmware and the operating system.

Agilent OpenLab 2D-LC Software is an OpenLAB CDS ChemStation Edition add-on.

Supported Chromatographic Data Systems

Following revision of OpenLab CDS ChemStation Edition is supported:

- OpenLab CDS ChemStation Edition C.01.10 Update 3 [Build 287].

Using the Single Quadrupole functionality of the Agilent OpenLab 2D-LC Software requires a MS license for OpenLab CDS ChemStation Edition M8362AA, which further requires license M8360AA for spectral data evaluation.

The Secured File System feature in OpenLab CDS ChemStation Edition is not supported.

This software has been tested successfully with 12 LC modules. Please note that complex systems increase memory consumption in ChemStation, which may decrease system stability. In order to reduce the likelihood of issues, please

- restart ChemStation from time to time, e.g. once per week or more often for complex systems
- Perform data analysis, reporting, online help reading in an off-line copy of the ChemStation instrument
- Save data before starting new tasks
- Avoid high levels of interactivity during runs by editing methods, changing signalplots settings, etc.

Supported Drivers

Agilent OpenLab 2D-LC Software A.01.04 SR4 [Build 035] requires LC & CE drivers 3.2 [023] for ChemStation C.01.10 Update 3 [Build 287]. The previous Agilent OpenLab 2D-LC Software A.01.04 SR3 [Build 033] requires LC & CE drivers 3.0 [Build 32] for Chemstation C.01.10 [Build 201].

Please use driver versions recommended for the selected CDS version.

Supported Operating Systems

Supported operating systems are the same as for the corresponding CDS revision, which are

- Windows 7 SP1 (64 Bit)
- Windows 8.1 (64 Bit)
- Windows Server 2012 R2 (64 Bit)
- Windows 10 (64-Bit)

For details, please refer to the documentation of your Agilent ChemStation edition.

Supported Firmware

Please use the firmware which is included on the Agilent OpenLab 2D-LC Software USB device. The data structure on the USB device contains a firmware folder. This firmware has been tested with Agilent OpenLab 2D-LC Software.

Following are the minimum firmware revisions required:

Device	Firmware
Agilent 1100 Series, 1200 Series and 1200 Infinity	A.07.02
Agilent 1200 Series, 1200 Infinity and 1120 Compact LC	B.07.35
Agilent 1200 Infinity Hosted Modules	C.07.30
Agilent 1290 Infinity II Modules	D.07.35

Available Languages

Language settings: Agilent 2D-LC Software is available in English and has been tested with English versions of operating systems and CDSs.

It has also been tested successfully on a Chinese operating (Windows 7 64-Bit SP1) and chromatographic data system.

PC Requirements

See requirements for the OpenLAB CDS ChemStation edition. A minimum RAM of 8 GB is strongly recommended.

Impact Analysis

Agilent recommends the following activities after installation of the release specified in this document:

Following table lists changes of the driver software that may cause a different behavior or performance with existing methods or change the system behavior in other ways that may require attention. Methods using related features should be reviewed in such cases in order to avoid unexpected or unwanted changes. For changes of interest, please look up the KPR# in the Software Release Bulletin (SRB) or Software Status Bulletin (SSB), see also section "Other Documents" on page 418.

Table 37 Changes of the OpenLab 2D-LC software

KPR#	SSB/SRB Problem Description Title	Fix Version
22	Wrong description for transfer volume from detector. Please read "Appendix B: Installation and Configuration" on page 423 for improvements.	A.01.04 SR1
23	Improved precision for multiple heart-cutting. Please read "Appendix C: Improved heart-cutting" on page 430.	A.01.04 SR1
32	Wrong default transfer capillary volume has been corrected. Please read SSB entry 32 for details.	A.01.04 SR2
407456	Configuration of the single UV Detector transfer volume is missing	A.01.04 SR4

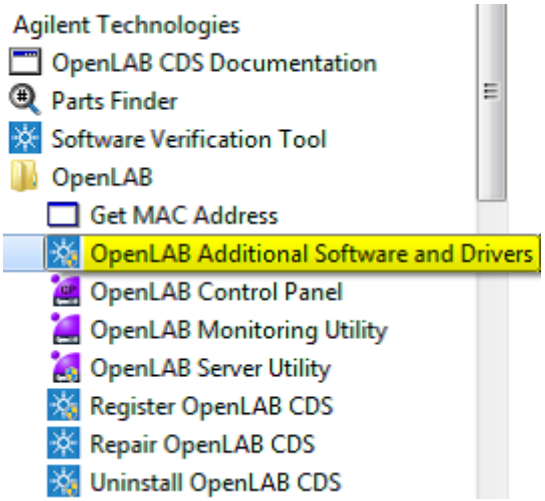
NOTE

The 2D-LC Software software A.01.04 SR4 does not contain any changes to the previous A.01.04 SR3 Add-on software, but allows the use with OpenLab CDS Chemstation C.01.10 Update 3.

Installation

Agilent OpenLab 2D-LC Software is installed by the Agilent OpenLab Master Installer. Installation prerequisites like CPU, memory and hard drive space are also mainly determined by the underlying CDS. Please refer to documentation of the CDS installer for installation, updates and uninstallation.

Please use "OpenLAB Additional Software and Drivers" for installing the driver from the Windows Start Menu.



Updates

Agilent continuously improves its drivers, firmware and software and recommends using latest updates. If applicable, any updates or bug fix releases for this software are available from Subscribenet at **https://agilent.subscribenet.com**.

Other Documents

Folder documents on the USB device includes more documents and videos with further information:

Agilent 2D-LC Software Video Tutorial. This video tutorial demonstrates the use of Agilent 2D-LC Software for ChemStation step-by-step. Nine videos cover the setup of methods in 2D-LC modes multiple heart-cutting measurements including high-resolution sampling and comprehensive 2D-LC. Active Solvent

Modulation is featured for improving resolution with challenging solvent combinations. Qualitative and quantitative data analysis of heart-cutting measurements are explained for UV and single quadrupole data. Configuration of a 2D-LC system is described and a commented demo run explains a 2D-LC system in action.

The system manual G2198-90001_2DLC_USR_EN.pdf gives an introduction for installation and use of the 1290 Infinity II 2D-LC System.

Document Primer 2D-LC 5991-2359EN.pdf gives an introduction to principles, practical implementation and applications for Two-Dimensional Liquid Chromatography.

Technical Note G2198-90101 in folder documentation of the Agilent OpenLab 2D-LC Software CD describes software 2D Chromatogram Creator for MassHunter.

Software Release Bulletin (SRB): The Software Release Bulletin bulletin is an excerpt from the SSB which lists issues which have been fixed with this revision.

SSB and SRB are included to the driver CD and can be found in folder documentation.

The SSB is updated regularly. Please visit our Website for the latest version at **http://www.agilent.com/cs/library/Support/Patches/SSBs/2D-LC.html**.

Firmware and firmware documentation are available for download from **http://www.chem.agilent.com/_layouts/agilent/downloadFirmware.aspx?whid=69761**.

Press **F1** in the software user interface for the Online Help with more information on specific software functions.

For more information about Agilent hardware, software and applications, please visit the Agilent web site at **http://www.agilent.com**.

Appendix A: Understanding Peak Parking

Peak-based mode in multiple heart-cutting

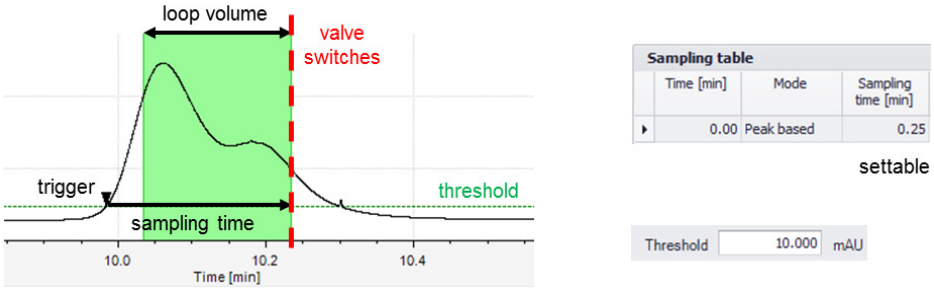


Figure 148 Peak-based mode

In peak based mode, three parameters determine how peaks are parked:

1. A trigger marked by a black triangle indicates, if a peak has been detected, e.g. because a reference signal (if available) exceeds the threshold or the slope as defined in advanced settings.
2. The cut is parked by switching the valve. This happens either if the peak end is detected (signal falls below threshold or slope) or if the settable sampling time has been exceeded, whatever comes first. The purpose of the sampling time is delaying the parking such that a defined part of the peak, typically its center, is parked.
3. The width t of the green area, which is used for parking a peak fix and calculated from the loop volume V and flow rate F in the first dimension by t = V/F.

NOTE

The peak parking may start even before the peak trigger if the sampling time is shorter than the time corresponding to the loop volume. In this case, the green area will start left to the trigger triangle.

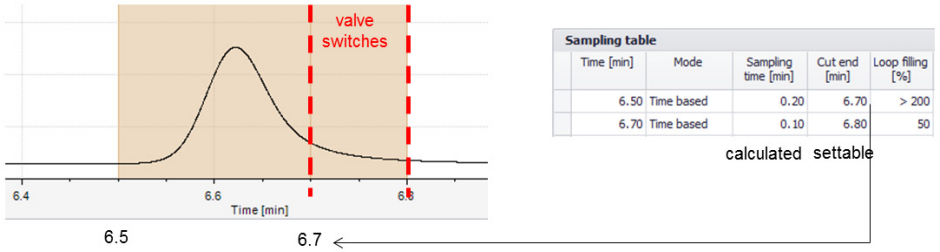


Figure 149 Time-based mode

In time-based mode, the time given in the sampling table corresponds to the beginning of the cut parking. The sampling time is usually fix in this case and is given by t = V/F. The cut is parked by switching the valve at the time “cut end”. Ultimately, only the cut end has relevance for the method and instrument control. The cut end is displayed in an optional column, which needs to be configured by a right-click on sampling table column headers choosing option "Columns".

For backward compatibility, adjacent cuts can be parked in the multiple heart-cutting mode. In such cases, the sampling time can be lower than a value corresponding to the normal loop fill time, which is also indicated by loop filling values below 100%. In multiple heart-cutting, loops should be overfilled (> 100%). For the second and later cuts in a series of adjacent cuts, short cuts can be created by editing the cut end (which is in an optional table column) or by moving the corresponding area in the preview window. High-Resolution Sampling should be preferred for adjacent cuts.

NOTE

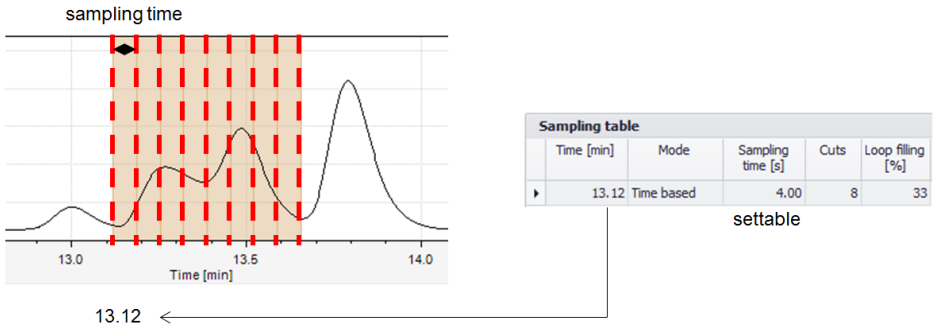
Previous revisions of the Agilent OpenLab 2D-LC Software used same settings for peak-based versus time-based modes, which could result in start times before the beginning of peak parking in time-based mode. Loop fill time/sampling time could be set manually, which is not possible in practice (see above).

If such methods are loaded to software A.01.03 and above, this will not change the behavior of such methods, as the valve switching times (= cut ends) are not changed. However the start times will be corrected automatically in order to reflect the physical reality, which may look as it would be a method change.

Please also note the sampling time is related to the flow rate. If the 1D flow rate is changed, valve switch times are kept constant and the peak start time changes. Please note that the reference signal becomes invalid for a changed flow rate.

High-resolution sampling (time-based mode)

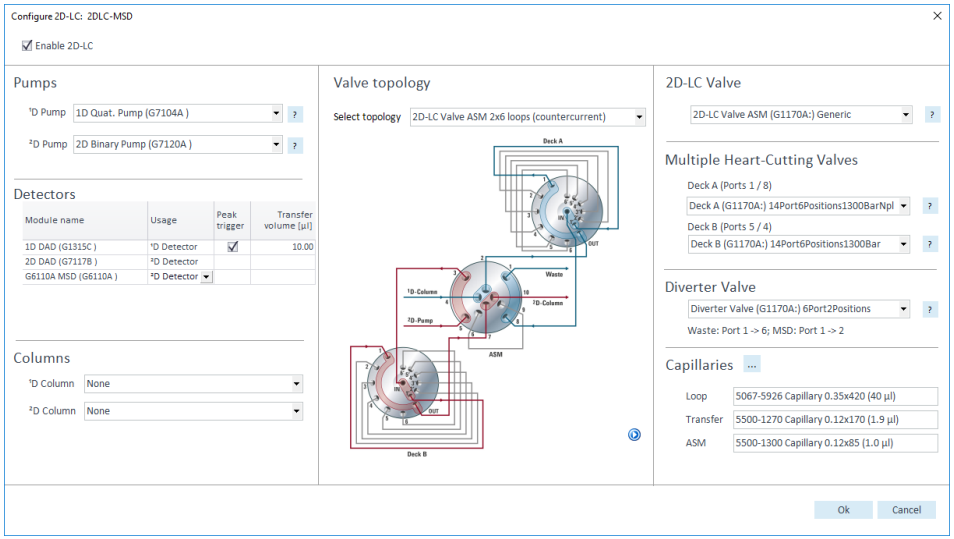
High resolution sampling is a solution as well for creating short cuts as parking larger peaks in a series of cuts for optimum resolution in both dimensions. High-resolution sampling uses significantly improved parking algorithms compared to multiple heart-cutting. It should be preferred for quantitative measurements and precise peak parking. Creating short cuts can also be used reducing the solvent transfer from the first to the second dimension for improving separation in the second dimension (see also explanation of active solvent modulation). For details, please refer to section “High resolution sampling” in Release Note A.01.03 SP1 in folder documentation.



For high-resolution sampling, a (start) time can be set, the sampling time and the number of cuts for a peak or range. The sampling time should be less than the time which is needed for filling one sample loop corresponding to a loop filling below 80%. Because of the parabolic flow profile, a filling greater than 80% will cause samples going to waste.

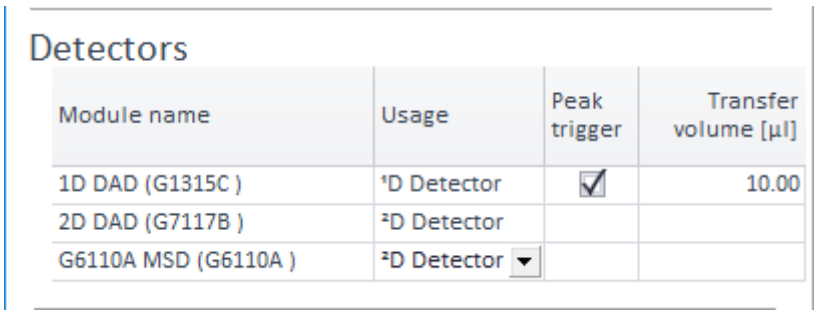
The minimum sampling time/volume is given by the transfer volume between the 2D-LC valve and the deck valve. The last cut of a deck is stored in the transfer capillary such that switching to the second deck will bring that peak to the second dimension. If a volume smaller than that transfer volume would be chosen, two cuts would be in the same capillary resulting in a loss of resolution and reproducibility.

Appendix B: Installation and Configuration



Agilent OpenLab 2D-LC Software A.01.04 SR4 provides an improved configuration user interface.

Detector Configuration



Multiple detectors can be used for the first and second dimension. One ^{1}D detector can be used as peak trigger for peak based sampling.

A *transfer volume* can be set for each ^{1}D detector, which is the volume between the ^{1}D flow cell and the 2D-LC Valve (not: sample loop!). To calculate the transfer volume, add half the volume of the detector flow cell plus the volume between the detector flow cell and the 2D-LC Valve. Only the transfer volume of the peak trigger detector is applied. If no ^{1}D detector is installed, it is now possible to

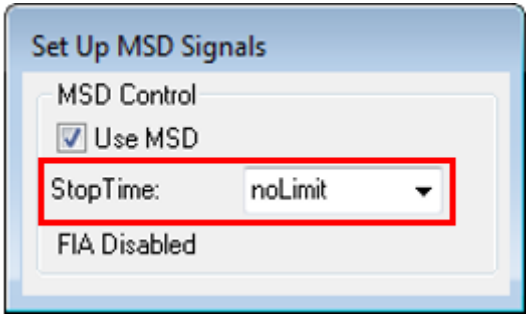
configure the transfer volume for 2D detector in Agilent OpenLab 2D-LC Software A.01.04 SR4. This corresponds to the use of the transfer volume in "Using the Single Quadrupole Detector with 2D-LC" on page 424.

NOTE This definition has been changed compared to previous software versions.

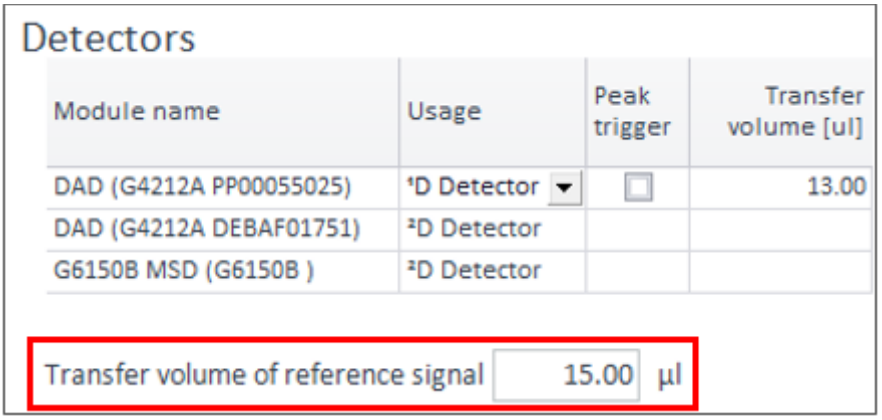
NOTE The 2D-LC system has been optimized for detectors connected to the CAN bus interface. For non-CAN detectors (e.g. third party detectors), suitable run time settings need to be considered in order to get complete signal data, see KPR 25 in the software status bulletin for details.

Using the Single Quadrupole Detector with 2D-LC

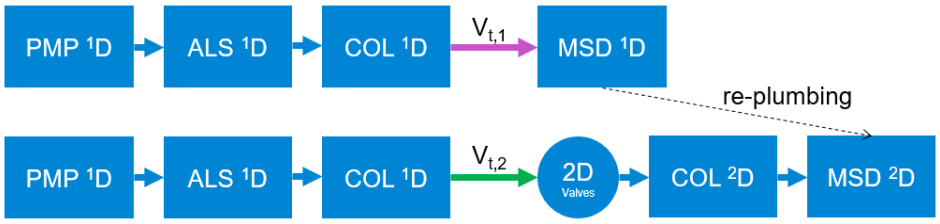
As a destructive detector, the single quadrupole detector is usually installed as a 2D detector. It can be configured as described in "Detector Configuration" on page 423.



When setting up the MSD (Instrument > Set up MSD signals...), set "noLimit" as the stop time. This is required as the run time needs to be extended for unparking cuts.

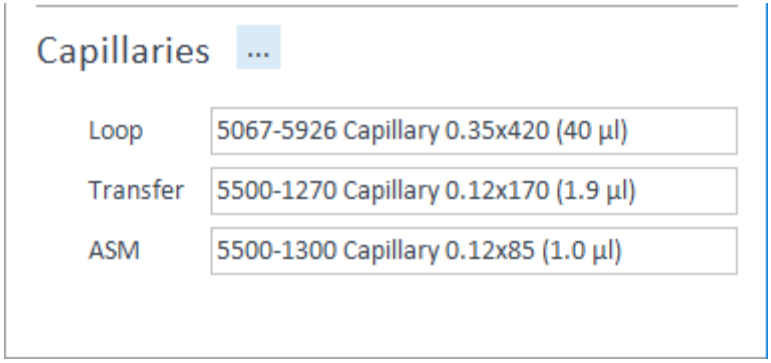


A previously acquired ^{1}D MSD signal can be used as a reference signal after configuring an MS detector in ^{2}D without configuring a ^{1}D peak trigger. In this case, the transfer volume of the reference signal can be entered for that previously installed ^{1}D detector:

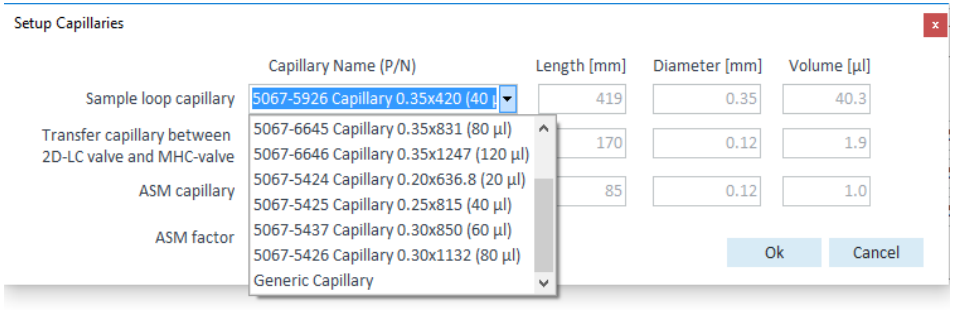


- Reference signal is acquired with MSD in first dimension
- Capillary connections are changed and MSD is moved to ^{2}D. Then, there is no peak detector in ^{1}D. The transfer volume V_t needs to be calculated as difference $V_{t,2}$-$V_{t,1}$ and configured.
- Reference signal is used for defining cuts in sampling table.
- MSD is used for detection in second dimension

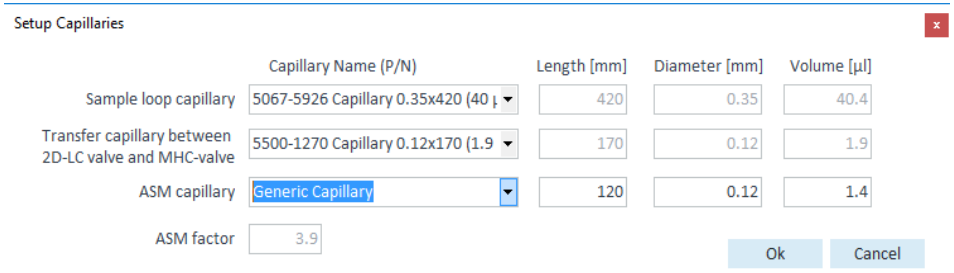
Capillary Configurations



Capillary configuration has been simplified. In the 2D-LC Configuration screen, click "..." for configuring capillaries.

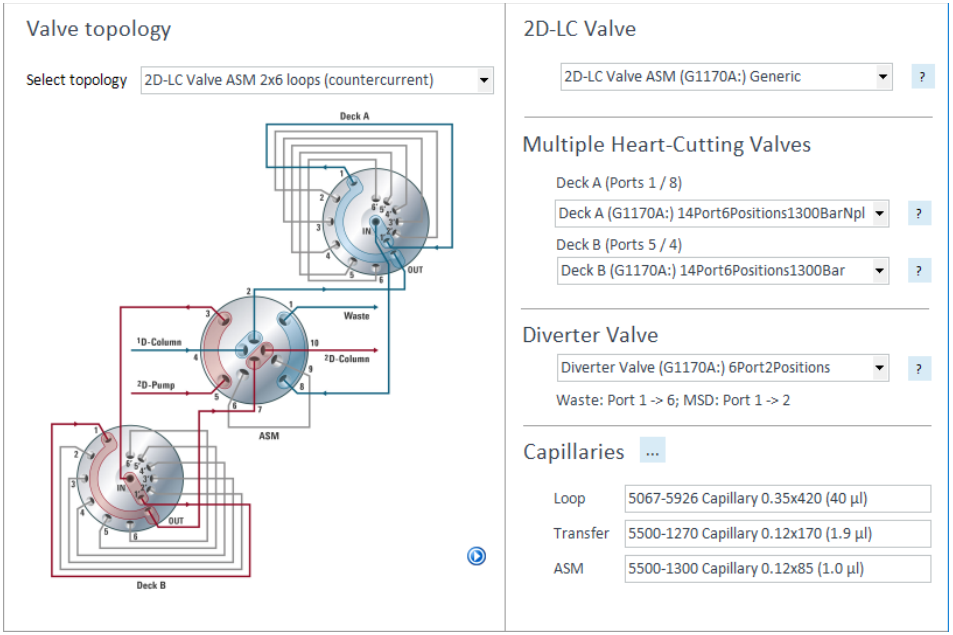


Select capillaries for different parts of the 2D-LC flow path. The software lists available capillaries with recommended capillaries as default.

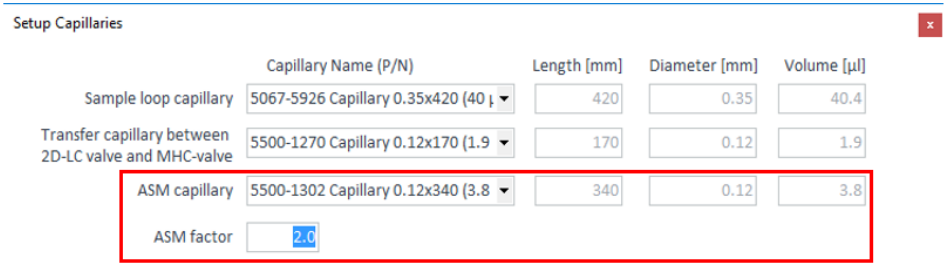


If you want to use a capillary which is not pre-defined in the software, please choose "Generic capillary" and enter length, diameter and/or volume.

ASM Valve Configuration



The 2D-LC ASM Valve can be selected as 2D-LC valve. It can be used in different topologies.



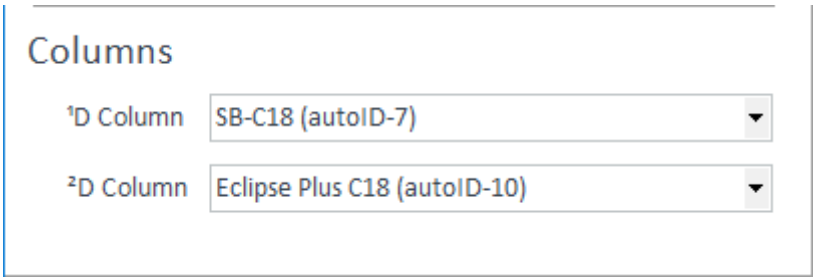
Different capillaries are available for the valve, which can be configured in the capillary setup.

Capillary	Length (mm)	ID (mm)	Volume (µl)	ASM factor	Split ratio (loop:ASM)
5500-1300	85	0.12	0.96	5	1:4
5500-1301	170	0.12	1.9	3	1:2
5500-1302	340	0.12	3.8	2	1:1
5500-1303	680	0.12	7.7	1.5	1:0.5

These capillaries have different lengths and therefore back pressures. The back pressure ratio of both flow paths (capillaries between 2D-LC valve and MHC valves and sample loop vs. ASM capillary) defines the relative flow in both paths. If for example the back pressure of the flow path with the sample loop is twice as high as of the flow path with the ASM capillary, twice as much solvent flows through the ASM capillary, i.e. the flow splits up loop:ASM = 1:2. This yields a dilution of the ^{1}D solvent in the sample loop of about 3, which is called the *ASM factor*. Factors listed in the table above are calculated based on recommended transfer capillaries (0.12 mm ID, 340 mm length). The ASM factor for configured capillaries is calculated and displayed by the software.

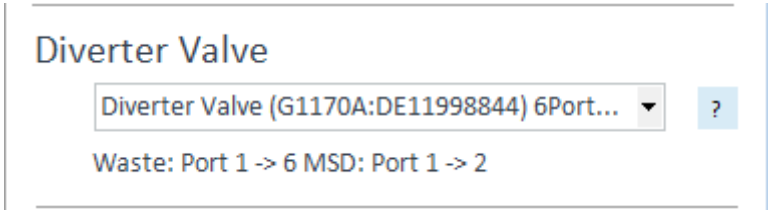
The optimum ASM factor depends on solvents in both dimensions.

Column Configuration

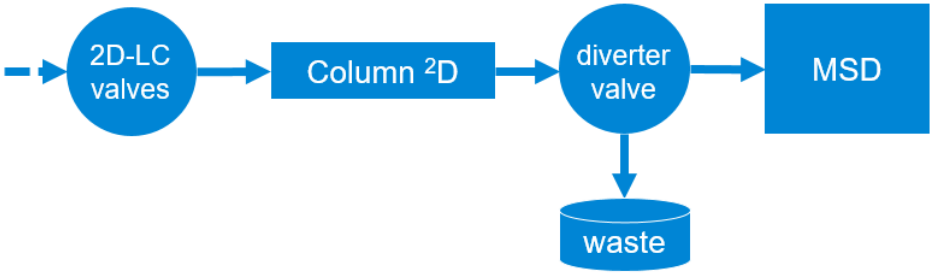


Columns can be configured for both dimensions. This information is used for calculations like the ^{2}D column injection ratio or the volume calculated for the flush gradient. Please note that this calculation is no longer valid, if different columns are used for different methods e.g. in the same sequence.

Diverter Valve Configuration



A diverter valve can be configured e.g. for removing buffers before they reach an MS detector. Any 2-position LC valve can be chosen in the configuration. Valves should be installed to external valve drives G1170A. Control is done via the CAN connection between 2D-LC modules and the diverter valve, which is not yet available for diverter valves in MS detectors. The user interface shows, which valve outlet ports go to waste vs. detector.



CAUTION

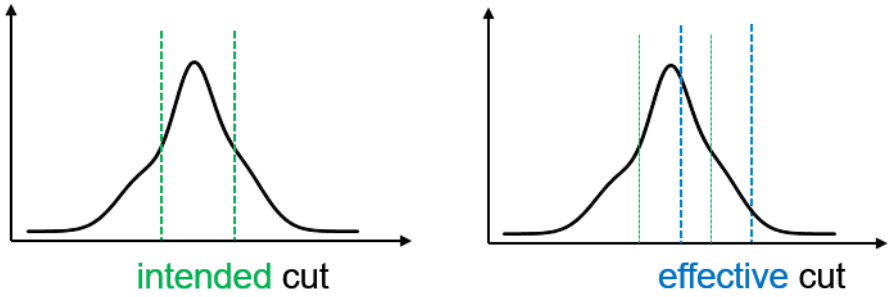
Risk of breaking flow cells

✓ **Follow the recommendation given here and in referenced technical note for protecting flow cells.**

1 When using the diverter valve in combination with a ^{2}D UV detector, which is typically installed upstream to the diverter valve, measures must be taken to protect the ^{2}D flow cell from pressure pulses generated by switching the valve under flow/pressure. Very short blockages can cause pulses of several hundred bars depending on the flow rate, which may damage UV detector flow cells (e.g. MaxLight Cartridge Cells specified for a maximum pressure of 60 bar).

The recommended solution is described in Technical Note G4236-90100: 2D-LC Diverter Valve Guide which can be found in folder documentation.

Appendix C: Improved heart-cutting



Because of a firmware issue, each cut created by switching the 2D-LC valve is parked with a small delay of about 2 s. This changes the eluent transferred to 2D and may reduce the area of the cut peak for (multiple) heart-cutting measurements. The extent depends on the 1D flow rate and position of the area of interest.

High-resolution sampling and comprehensive measurements are not affected. We recommend parking broad peaks entirely using HiRes sampling.

Firmware set A/B/C/D.07.21 fixes this issue for time-based cut parking. In peak-based mode, the sampling time should be set smaller than the expected peak width.

Depending on installed software, following upgrade scenarios are possible and are covered by Agilent warranty:

Table 38 Upgrade Scenarios

Supported ChemStation rev.	OpenLab 2D-LC Software revision	Latest supported LC driver revision
C.01.10 Update 3	A.01.04 SR4	3.2
C.01.10	A.01.04 SR3	3.0
C.01.09 Update 1	A.01.04 SR3	A.02.19 SR1
C.01.09	A.01.04 SR3	A.02.19 SR1

Whenever possible, a free upgrade to the latest version of OpenLab 2D-LC Software A.01.04 SR3 or SR4 is recommended. This may require an update of OpenLab CDS ChemStation Edition and LC drivers. If it is not covered by warranty or by Agilent Software Maintenance Agreements, such CDS upgrades may need to be purchased.

16 Theoretical Background

This chapter gives the theoratical background of 2D-LC and describes the system components (soft- and hardware) of the Agilent 1290 Infinity II 2D-LC Solution ChemStation.

Theoretical basis of 2D-LC

In 2D-LC, fractions from a chromatografic system (1st dimension) are transferred to a second chromatographic separation system (2nd dimension). So 2D-LC bases on the application of two independent liquid phase separation systems to a sample. 2D-LC is mainly used to improve resolution and sensitivity or to decrease analysis time.

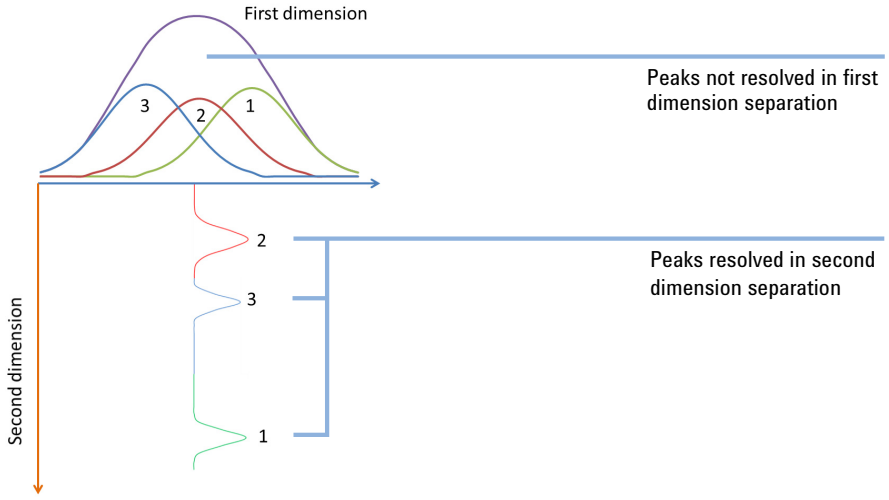


Figure 150 Peak capacity relationship between peak capacities of orthogonal first and second dimension

The most importand benefit of 2D-LC over 1D-LC is the increase of resolving power, which is especially important if dealing with complex samples.

For an overview on the main differences between 1D- and 2D-LC, refer to the following topics:

- "Orthogonality" on page 434,
- "Resolution" on page 434, and
- "Peak Capacity" on page 437

The following different methods of 2D-LC exist:

- Heartcutting (LC-LC)

  Only interesting portion of the first dimension effluent transferred to the second dimension.
- Comprehensive (LCxLC)

  Entirety of first dimension effluent sequentially transferred to the second dimension.

Orthogonality

The 2D-LC separation power depends the fact that the two selectivity mechanisms in the different separation stages must be as different as possible. If the mechanisms are completely different and independent the two separations are called *orthogonal*.

Any correlation between the selectivity mechanisms degrades orthogonality and reduces the efficiency of the 2D-LC system.

For strategies to achieve maximum orthogonality, refer to Table 40 on page 442 and Table 41 on page 447.

Resolution

A chromatographic separation can be optimized based on physical parameters of the HPLC column such as particle size, pore size, morphology of the particles, the length and diameter of the column, the solvent velocity, and the temperature. In addition, the thermodynamics of a separation can be considered and the properties of the solute and the stationary and mobile phases (percentage of organic solvent, ion strength, and pH) can be manipulated to achieve the shortest possible retention and highest selectivity.

1D-LC Resolution (R_S) can be described as a function of three parameters:

- Column efficiency or theoretical plates (N),
- Selectivity (α),
- Retention factor (k).

$$R_s = \frac{\sqrt{N}}{4}\left[\frac{\alpha - 1}{\alpha}\right]\left[\frac{k'_2}{k'_2 + 1}\right]$$

Figure 151 Resolution equation

This means that the selection of appropriate mobile and stationary phase properties and temperature is critical in achieving a successful separation.

Resolution in a one-dimensional separation usually is measured with:

$$R = \frac{\Delta t}{4\sigma}$$

R = Resolution
Δt = Difference in retention time maxima of two components
σ = Average standard deviation of two Gaussian peaks

Following results of this formula are important in practice:

- R > 1.5

  Peaks are completely baseline resolved
- R > 1

  Difference in retention time is larger than peak broadening, and therefore peak spacing is adequate to observe distinct component zones
- R < 0.5

  Peaks are completely fused

2D-LC In 2D-LC the separation behaviour is more complex and described below.

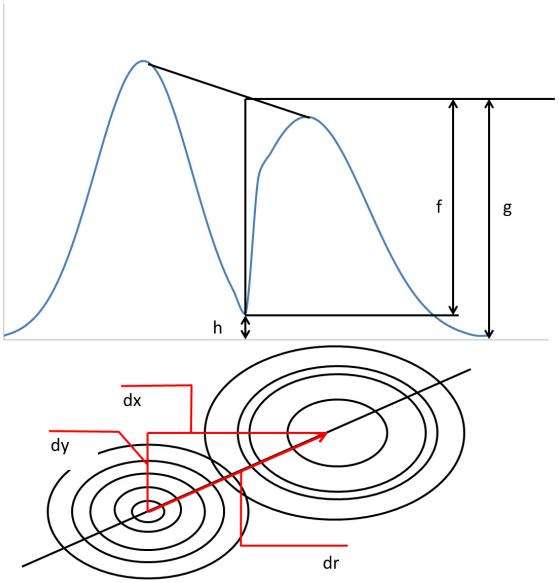


Figure 152 Diagram of 2D resolution measurement: Slice for resolution (top) and 2-dimensional contour plot (bottom)

The distance between two spots in the contour plot may be calculated by the Pythagorean expression:

$$dr = \sqrt{dx^2 + dy^2}$$

For the resolution along the axis of each dimension applies:

$$R_1 = \frac{dy}{4\sigma_1}$$

and

$$R_2 = \frac{dy}{4\sigma_2}$$

So for two dimensions the resolution may be calculated as follows:

$$R_{2D} = \frac{dr}{4\sigma} = \sqrt{\left(\frac{dx}{4\sigma}\right)^2 + \left(\frac{dy}{4\sigma}\right)^2}$$

Figure 153 2D-Resolution (Pythagorean relation)

or, σ approximated by the average of σ_1 and σ_2, using the easy to measure peak to valley ratio (P = f/g) and assuming that peaks are Gaussian:

$$Rs = \sqrt{-\frac{1}{2} ln\left(\frac{1-P}{2}\right)}$$

Figure 154 2D-Resolution (peak to valley ratio relation)

Table 39 Definitions

Symbol	Denotation
R	Resolution
Δt	Difference in retention time maxima of two components
σ	Average standard deviation of Gaussian peaks
dr	Distance between two spots in a plane
P	Peak to valley ratio
f	Difference between amplitude at the valley, h, and g
h	Valley
g	Average peak maximum

Peak Capacity

Peak capacity may be differently defined:

- As the maximum number of peaks that can be resolved in the available separation space (*Geometrical Definition*), or
- As the ratio of the total area of the chromatogram to the area required for the resolution of any zone (*General Definition*)

Geometrical Definition

The peak capacity may be defined as the maximum number of peaks that can be resolved in the available separation space. So peak capacity n_c is related to the number of theoretical plates N:

$$n_c = \mathrm{P}N^{1/2}$$

(P depends on the retention time range)

In practice peaks are usually not distributed randomly over the chromatogram and often overlap. Or in other words: In practice peaks don't fill the available separation space evenly. This is the reason, why the number of detectable components of a sample in 1D-LC is relatively small.

2D-LC separation offers an alternative possibility for increasing n_c: Orthogonal retention mechanisms generate a separation plane. Thus, the peak capacity in 2D-LC is the product of the peak capacities of the individual columns. Due to peak broadening in 1st and 2nd dimension, components in 2D-LC are present as two-dimensional ellipses on the retention plane.

How to calculate n_c depends on the method:

- For comprehensive 2D-LC:

$$n_c = \frac{L_1 L_2}{ab} = n_{c1} n_{c2}$$

L = Separation space for dimension
ab = Area for rectangle circumscribing the ellipse on the separation plane

- For heart-cutting 2D-LC:

$$n_c = \sum_{i=i}^{k} n_{ci}$$

General Definition

Alternatively peak capacity may be defined as the ratio of the total area A of the chromatogram to the area A_0 required for the resolution of any zone:

$$n_{c,alternat} = \frac{A}{A_0}$$

n_c defined that way is related to the geometrical definition by a factor:

$$n_c = \frac{\pi}{4} n_{c,alternat} \approx 0.79 n_{c,alternat}$$

Limits of Peak Capacity in 2D-LC

Under ideal circumstances (*orthogonality*), the overall peak capacity ($n_{c,2D}$) should be equal to the product of the individual peak capacities of the first and second dimension separations (^1n_c and ^2n_c)

$$n_{c,2D} = {}^1n_c \times {}^2n_c$$

In practice the increase in peak capacity is not directly proportional to increase in ability to resolve peaks.

Probable reason for this:

- In 1D-LC, with a baseline width of a single component peak $x_0 = 6\sigma$, x_0 units of component free space on both sides of the maxima is necessary to ensure baseline resolved peaks.
- In 2D-LC the single component zone is $A_0 = 2\pi r^2$ and an area of component free space of $\pi(2r)^2$.
- As a result: For every two component free widths in one dimension, four component free areas are required in two dimensions.

Conclusions for 2D-LC

1D-LC is inadequate for the separation of complex mixtures, as the number of observable peaks compared to number of peaks to observe is too low. One theoretical model (Statistical Model of Overlap = SMO), that correlates well with real world observations, predicts, that the maximal fraction of the total peak capacity that can be seen as chromatographic peaks is 37 % and even only 18 % as single peaks. This implicates that extremely high peak capacities are needed to separate complex samples with lots of components which is extremely difficult to achieve.

Compared to 1D-LC separations, it's complicated to predict the number of observable peaks in 2D-LC. For example, at a given peak capacity and a given number of components, the aspect ratio in the two axes of separation has impact on how effective the two separation are.

From the pracitcal point of view the performance between 1D- and 2D-LC should be compared, considering the following aspects:

- Peak capacity
- Number of peaks observed in experimental chromatograms

Ideal 2D Peak Capacity

One major problem in 2D-LC is loss of 1^{st} dimension resolution due to 2^{nd} dimension sampling process. The determining factors are:

- Gradient time of the 2^{nd} dimension separation cannot exceed the sampling interval of the 1^{st} dimension separation
- Resolution of a pair of peaks in the two-dimensional space is related to the resolution on the first and second dimensions as the Pythagorean average (see Figure 153 on page 436)

A 2D chromatogram is only a way of displaying a lengthy series of sequential chromatograms obtained on the second column and the second column and detector are just a unique type of chemically selective detector of what comes out of the first column (see, "2nd dimension as detector" on page 440). The peak width observed on the second column is independent of the sampling time used in the 1^{st} dimension.

This leads to two extreme scenarios, on how mixtures of components may behave:

- Unresolved mixture is injected into second column and second column separates analytes perfectly

  $R_{s,2D}$ is independent of 1^{st} dimension sampling rate
- Partially resolved mixture is injected into second column and analytes co-elute on the second column

  $R_{s,2D}$ strongly depends on first dimension sampling rate.

  This indicates, that it's very important to respect, how often the 1^{st} dimension effluent must be sampled to avoid loss of resolution.

NOTE

The theoretical limits for ideal 2D peak capacity are defined by the Murphy-Schure-Foley Criterion (M-S-F sampling criterion). According to this criterion, the effluent must be sampled at least 3 – 4 times over 8σ width of the first dimension peak.

2nd dimension as detector

Functionally the second dimension of 2D-LC operates like a chemically sensitive detector for the peaks that elute from the first dimension column. Thus, 2D-LC may be understood as a three step process:

- 1st dimension separation (1)
- Sampling of the 1st dimension (2)
- 2nd dimension separation and detection (3)

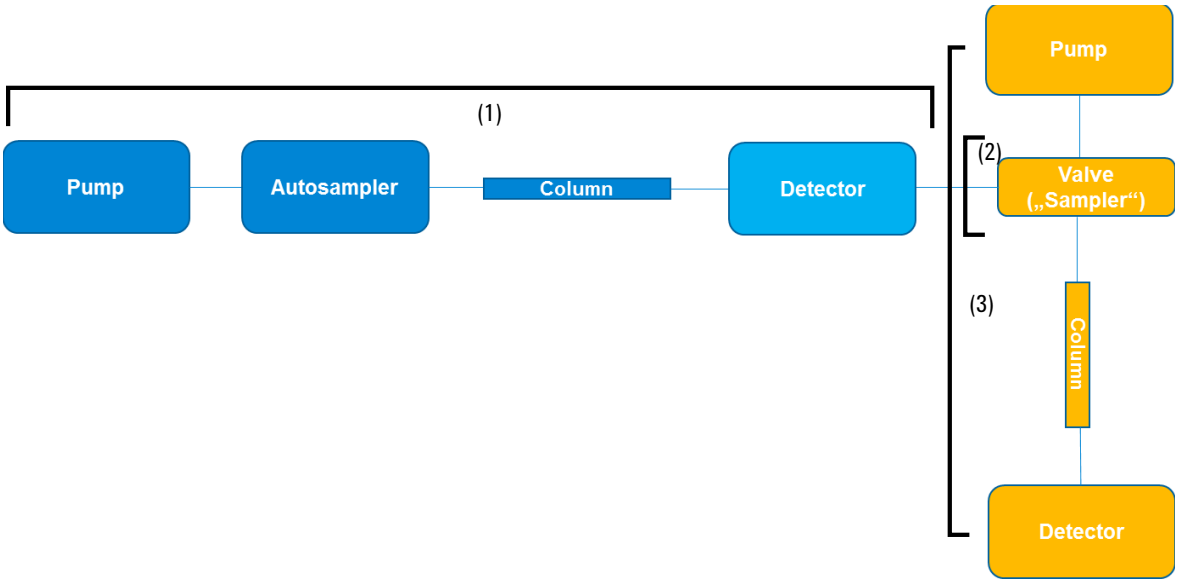


Figure 155 Diagram of instrumentation for 2D-LC

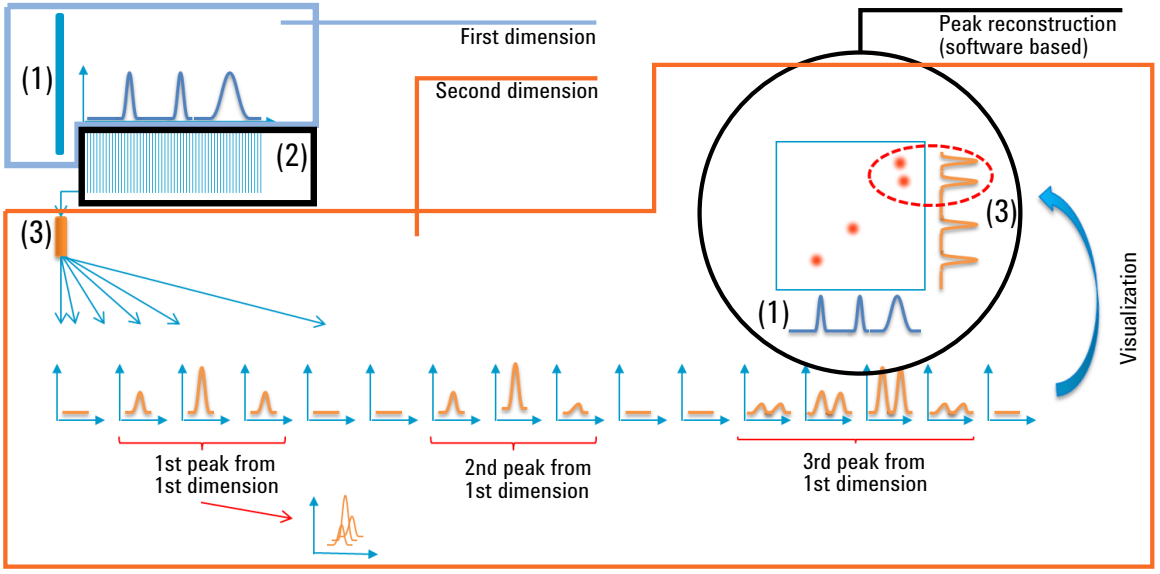


Figure 156 Principle of 2D-LC (example for LCxLC): Effluent of first column (1) is sampled (2) and injected to second column (3). Peaks of second column separation are detected and reconstructed.

(1)	First dimension separation
(2)	Sampling of the first dimension
(3)	Second dimension separation

Successful Mode Combinations

2D-LC separations are the more effective, the more the selectivity mechanisms involved in the two stages differ. Completely different and independ mechanisms are said to be orthogonal. Any correlation between the selectivity mechanisms degrades orthogonality and reduces the efficiency of the 2D-LC system.

Thus, selecting the best combination of stationary and mobile phase is the major issue to improve 2D-LC methods. Table 40 on page 442 summarizes the advantages and disadvantages of combinations of normal phase (NP), reverse phase (RP), ionexchange (IEC) and size exclusion chromatography (SEC) for 2D-LC operation.

Table 40 Mode combinations in 2D-LC (LCxLC)

Combination	Orthogonality	Peak capacity	Application	Comment
RP x RP	1	++[2]	Peptidomics, metabolomics, pharmaceuticals, foods, cosmetics	Miscible solvents, broadest application, fast speed, gradient elution on both dimensions
IEC and RP	+[3]	-	Proteomics, peptidomics	
SEC and RP	+	-[4]	Polymers, proteomics	
NP and RP	+		Polymers, pharmaceuticals, oils	Solvent incompatibility, limited application
Affinity and RP	+	-	Proteomics	
SEC and NP	+	-	Polymers	
SEC and IEC	+	-	Proteomics	

1 Orthogonality,depends on the column choice or mobile phase choice

2 very good

3 good

4 not so good

Solvent Elution Modes

Table 41 on page 447 focuses on the effects of elution modes for second dimension separation.

The following elution modes for second dimension separation are commonly used:

- Gradient

  A standard gradient of solvent A vs. solvent B for the second dimension separation will be repeated during the complete first dimension separation

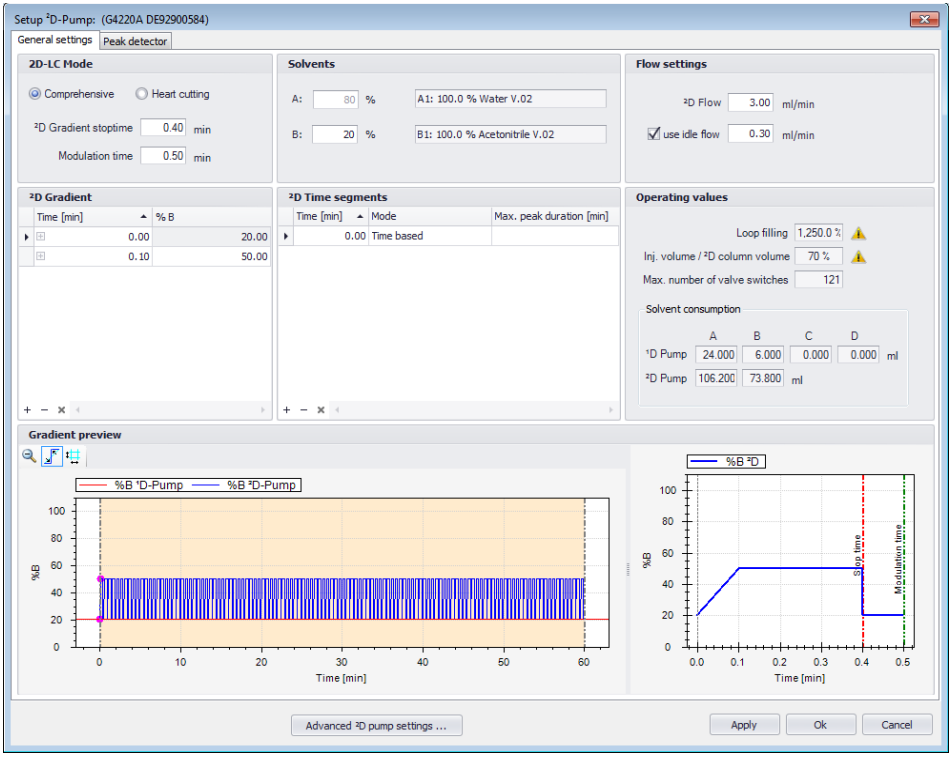


Figure 157 Standard gradient mode

- Shifted Gradient

  From each second dimension separation to the next the start-%B and end-%B values of the individual second dimension gradients will be increased in a defined way. Additionally, the gradient span can be increased from each second dimension gradient to the next.

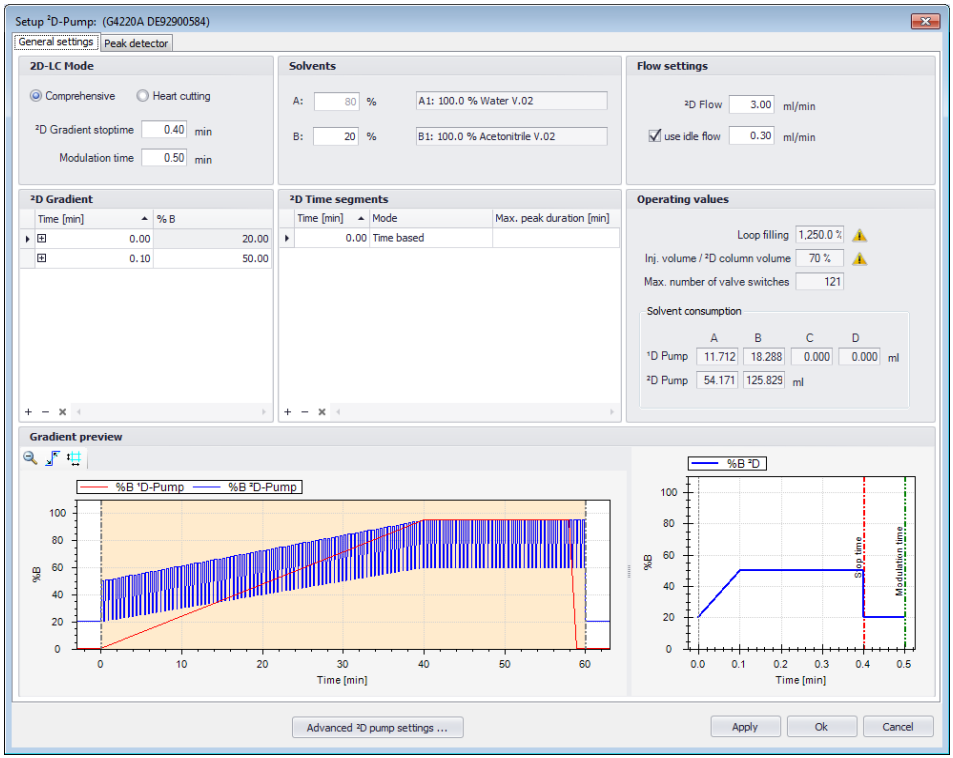


Figure 158 Shifted gradient mode with increase of start-%B

- Isocratic

  All second dimension separations will be carried out in an isocratic mode.

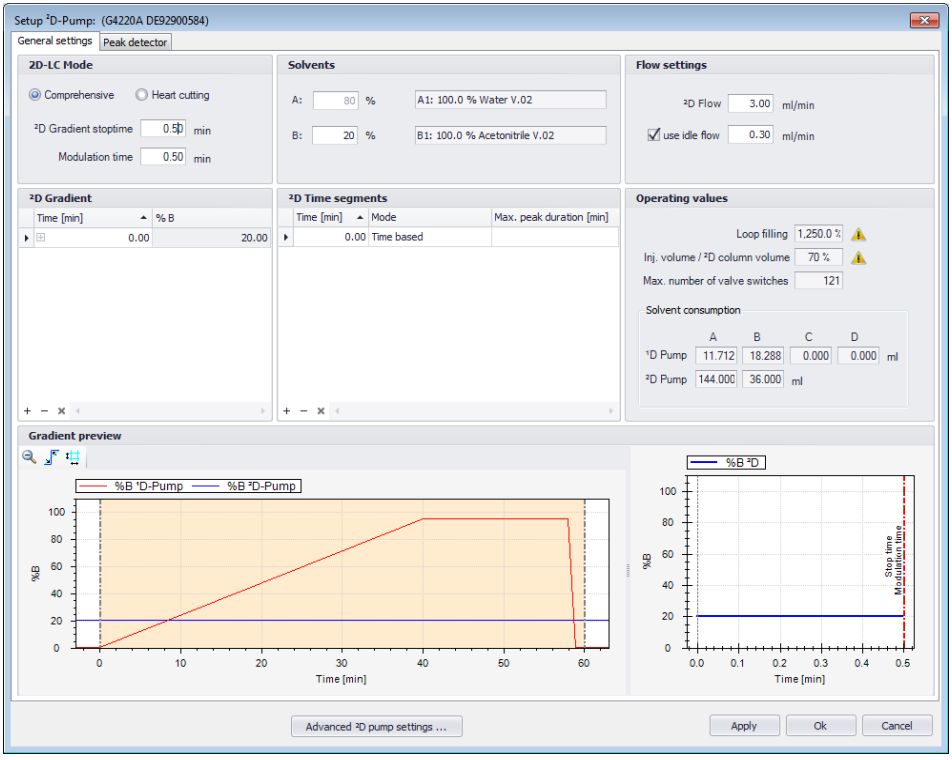


Figure 159 Isocratic mode

- Advancing isocratic

  Nearly isocratic conditions are used in each second dimension separation, with slightly increasing solvent strength in each successive run.

  The second dimension pumping system is fed with a shallow gradient in eluent composition over the course of the 2D-separation.

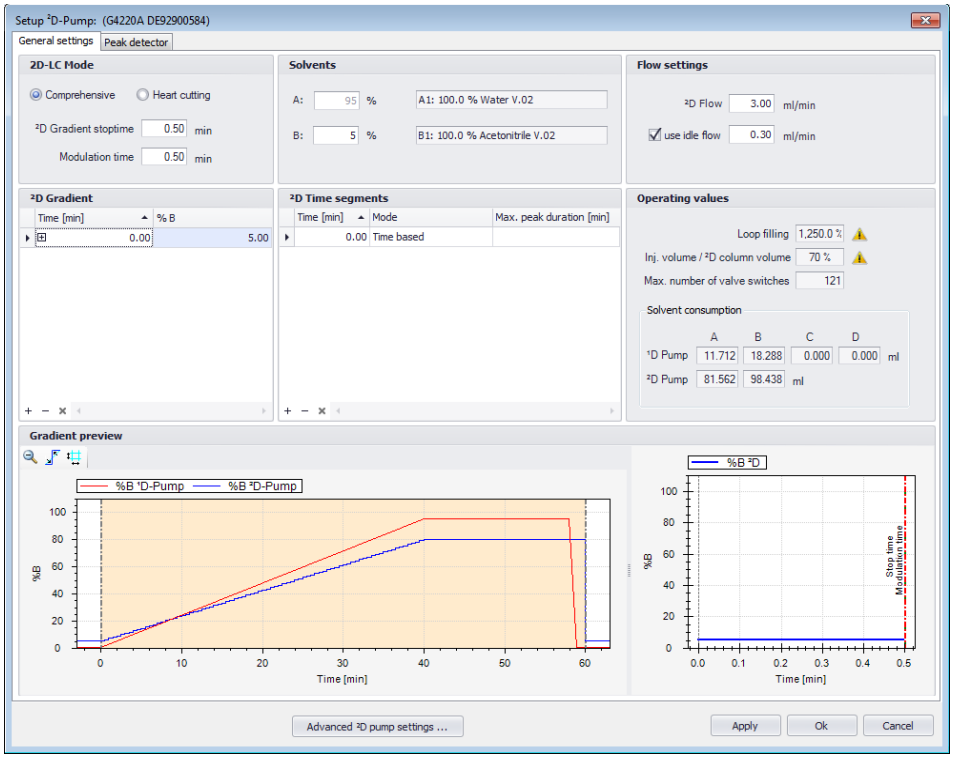


Figure 160 Advancing isocratic mode

Table 41 Different elution modes in the 2nd dimension (pros and conts)

Criterion	Gradient/Shifted gradient	Isocratic/Advancing isocratic
Peak capacity	Superior	Inferior
Diversity of samples (complex samples)	Superior	Inferior
Baseline performance (sensitivity	Inferior (baseline drift caused by solvent gradient)	Superior
Pressure stress (column lifetime!)	Inferior (large changes within every 2nd dimension gradient	Superior (no pressure changes with isocratic, gradually changing with advancing isocratic)

All modes are easily available with the Agilent 2D-LC Acquisition software.

Each mode has advantages and disadvantages. No single mode is superior in all applications of 2D-LC.

Effect of shifted gradient elution mode in the 2nd dimension

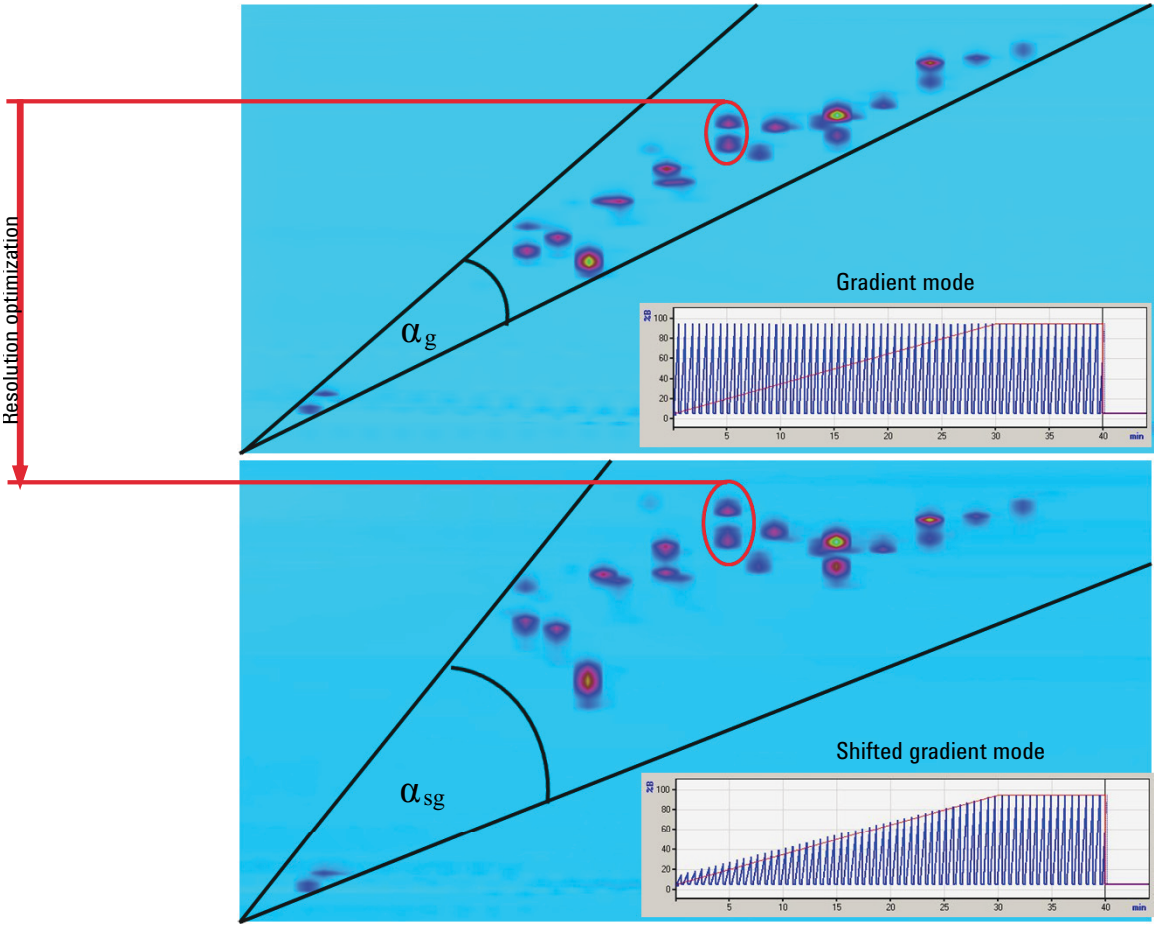


Figure 161 2nd dimension gradient mode compared to isocratic mode and its effect on resolution

α_{sg} as achieved in shifted gradient mode is larger than α_g achieved in standard gradient elution mode. This can lead to an improved peak detection and improved separation.

See *D. Li and O. J. Schmitz "Use of Shift Gradient in the Second Dimension to Improve the Separation Space in Comprehensive Twodimensional Liquid Chromatography" Anal. Bioanal. Chem. 405, 6511-6517 (2013)*

Practical Issues

The table below gives an overview, which practical issues have to be considered in 2D-LC.

Table 42 Practical issues in 2D-LC

Issue	Theoretical base	Comment
Choice of first dimension column diameter	Has impact on trade off between optimum first dimension flow rate and amount of sample injected into the second dimension column for each second column run	True gradient elution in the second dimension separation provides better peak capacity than in isocratic elution. Gradient elution is the best available mechanism for achieving peak focusing.
Ratio of column diameter in the two dimensions	Causes significant analyte dilution effects	
Goals of the analytical method	Chosen parameter depend on what is important in analysis: • separate as many constituents as possible or • focused on resolution and quantitation of a specific constituent	
Selection of the stationary phases and column formats	For RPLC in both dimensions the retentivity of the second dimension column must be much higher than that of the first dimension column required because: • a relatively large volume of the sample will be collected and injected into the second column • to minimize peak broadening the sample should be focused at the inlet of the second column	

Based on theory, in most cases following approaches to achieve best possible 2D-LC should be respected:

- Methodology

  As in Comprehensive 2D-LC is no direct need[1] for UV-detection in the first dimension, other eluents than acetornitril or methanol are possible. This implies the possibility to use unconventional organic solvents in the first dimension.

NOTE

Take care when using any unconventional organic solvents that these are still compatible with the used instrumentation. In doubt, refer to the module documentation or call Agilent.

- Instrumentation

  It is important to use very low delay-volume-gradient pumping systems that are able to produce high flow rates to achieve fast second dimension gradients with only little gradient delay - like the Agilent 1290 Infinity LC.

- Columns

  Total orthagonality is difficult to achieve, as there are relatively few combinations sufficiently phase selective.

- Detection methods

  Compared to mass spectrometry DAD based UV detection is faster, cheaper and offers higher reproducibility, thus mass spectrometry offers additional increase in peak capacity by expanding the separation space into the MS-domain. A high sensitivity UV-detector is recommended since a dilution of the first dimension peaks occurs in the second dimension separation – an Agilent 1260 or 1290 Infinity Diode-Array-Detector with 60 mm flow cell is ideal as second dimension detector.

- Data analysis

  2D-LC-data are complex. Use of special software is advisable.

[1] In case the peak and time triggered operation of the second dimension separation, which is optionally available with the Agilent 1290 Infinity 2D-LC solution, an UV-detector is required between the first dimension column and the modulation valve.

17 Appendix

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

General Safety Information

General Safety Information

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

WARNING

Ensure the proper usage of the equipment.

The protection provided by the equipment may be impaired.

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

Safety Standards

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

General

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

Before Applying Power

WARNING

Wrong voltage range, frequency or cabling

Personal injury or damage to the instrument

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

NOTE

Note the instrument's external markings described under "Safety Symbols" on page 456.

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 K 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 K 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 43 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 43 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.**

Waste Electrical and Electronic Equipment (WEEE) Directive

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

NOTE

Do not dispose of in domestic household waste

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

Radio Interference

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

Test and Measurement

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

Sound Emission

Manufacturer's Declaration

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

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

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

Capillary Coding Guide

Syntax for Capillary Description

The tables below are your guide to identifying the proper specifications for your capillary. On all capillaries, dimensions are noted in id (mm), length (mm) and, where applicable, volume (µL). When you receive your capillary, these abbreviations are printed on the packaging.

Using the guide: This fitting is coded as *SPF*, for Swagelok, PEEK, Fingertight.

Table 44 Capillary coding guide

Type **The type gives some indication on the primary function, like a loop or a connection capillary.**		**Material** **The material indicates which raw material is used.**		**Fitting left/fitting right** **The fitting left/right indicate which fitting is used on both ends of the capillary.**	
Key	**Description**	**Key**	**Description**	**Key**	**Description**
Capillary	Connection capillaries	ST	Stainless steel	W	Swagelok + 0.8 mm Port id
Loop	Loop capillaries	Ti	Titanium	S	Swagelok + 1.6 mm Port id
Seat	Autosampler needle seats	PK	PEEK	M	Metric M4 + 0.8 mm Port id
Tube	Tubing	FS/PK	PEEK-coated fused silica[1]	E	Metric M3 + 1.6 mm Port id
Heat exchanger	Heat exchanger	PK/ST	Stainless steel-coated PEEK[2]	U	Swagelok union
		PFFE	PTFE	L	Long
		FS	Fused silica	X	Extra long
		MP35N	Nickel-cobalt-chromium-molybdenium alloy	H	Long head
				G	Small head SW 4
				N	Small head SW 5
				F	Finger-tight
				V	1200 bar
				B	Bio
				P	PEEK
				I	Intermediate

1 Fused silica in contact with solvent

2 Stainless steel-coated PEEK

At-a-glance color-coding keys

The color of your capillary will help you quickly identify the capillary id.

Table 45 Color-coding key for Agilent capillary tubing

Internal diameter in mm		Color code
0.015		Orange
0.025		Yellow
0.05		Beige
0.075		Black
0.075	MP35N	Black with orange stripe
0.1		Purple
0.12		Red
0.12	MP35N	Red with orange stripe
0.17		Green
0.17	MP35N	Green with orange stripe
0.20/0.25		Blue
0.20/0.25	MP35N	Blue with orange stripe
0.3		Grey
0.50		Bone White

HINT

As you move to smaller-volume, high efficiency columns, you'll want to use narrow id tubing, as opposed to the wider id tubing used for conventional HPLC instruments

Solvent Information

Observe the following recommendations on the use of solvents.

- Brown glass ware can avoid growth of algae.
- Avoid the use of the following steel-corrosive solvents:
  - solutions of alkali halides and their respective acids (for example, lithium iodide, potassium chloride, and so on),
  - high concentrations of inorganic acids like sulfuric acid and nitric acid, especially at higher temperatures (if your chromatography method allows, replace by phosphoric acid or phosphate buffer which are less corrosive against stainless steel),
  - halogenated solvents or mixtures which form radicals and/or acids, for example:

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

    This reaction, in which stainless steel probably acts as a catalyst, occurs quickly with dried chloroform if the drying process removes the stabilizing alcohol,
  - chromatographic grade ethers, which can contain peroxides (for example, THF, dioxane, diisopropyl ether) should be filtered through dry aluminium oxide which adsorbs the peroxides,
  - solvents containing strong complexing agents (e.g. EDTA),
  - mixtures of carbon tetrachloride with 2-propanol or THF.
- Avoid the use of dimethyl formamide (DMF). Polyvinylidene fluoride (PVDF), which is used in leak sensors, is not resistant to DMF.

Further Information

Further information is available:

- Folder Documents on the software DVD:
  - Document Primer 2D-LC 5991-2359EN.pdf gives an introduction to principles, practical implementation and applications for Two-Dimensional Liquid Chromatography.
- Folder Documentation of the Agilent OpenLab 2D-LC Software CD:
  - Technical Note G2198-90101 describes software ^{2}D Chromatogram Creator for MassHunter.
- Folder Documents on the Driver CD:
  - Software Status Bulletin (SSB)

    The SSB is updated regularly. Please visit our Website for the latest version at **http://www.agilent.com/cs/library/Support/Patches/SSBs/2D-LC.html**.
  - Software Release Bulletin (SRB)

    The SRB is an excerpt from the SSB which lists issues which have been fixed with this revision.
- Firmware and firmware documentation are available for download from **http://www.chem.agilent.com/_layouts/agilent/downloadFirmware.aspx?whid=69761** .
- Press **F1** in the software user interface for the Online Help with more information on specific software functions.
- For more information about applications, please visite InfinityLab Application Finder **https://www.agilent.com/en/promotions/applicationfinder?s=learnmore**.
- For more information about Agilent hardware, and software, please visit the Agilent web site at **http://www.agilent.com**.

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In This Book

The manual describes the following:

- introduction,
- installing,
- configuring,
- using,
- data analysis,
- safety and related information.

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