Agilent

Agilent 1290 Infinity II 2D-LC Solution

User Guide

Notices

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In This Book

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

1 Introduction

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

2 Concepts of 2D-LC

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

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. 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 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 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 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.

16 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.

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 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 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 1290 Infinity Valve Drive
- 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.

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.

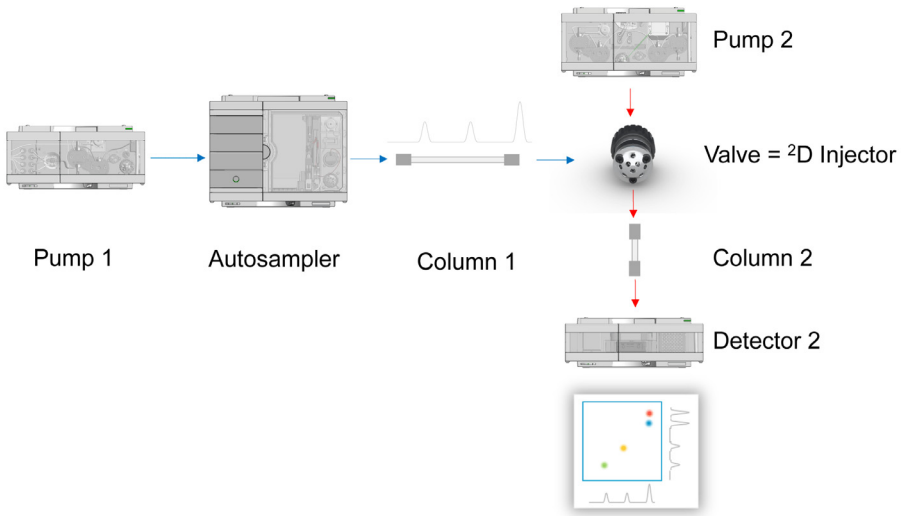


Figure 2 Concept of a 2D-LC-System

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).

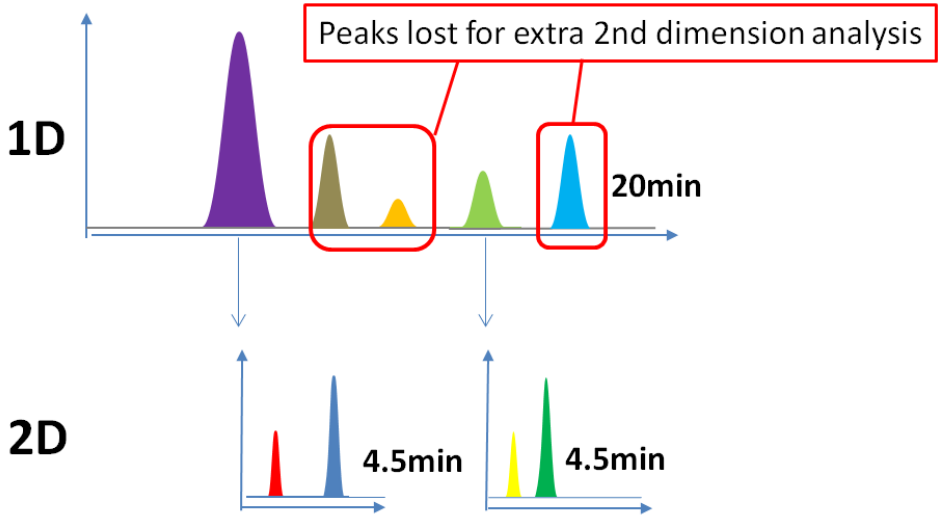
NOTE Multiple peaks eluted from the first dimension column can be sampled and analyzed in the second dimension but the run time of the second dimension must match the retention time between two first dimension peaks. *A started second dimension analysis will always be finished!* Thus, a second peak being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

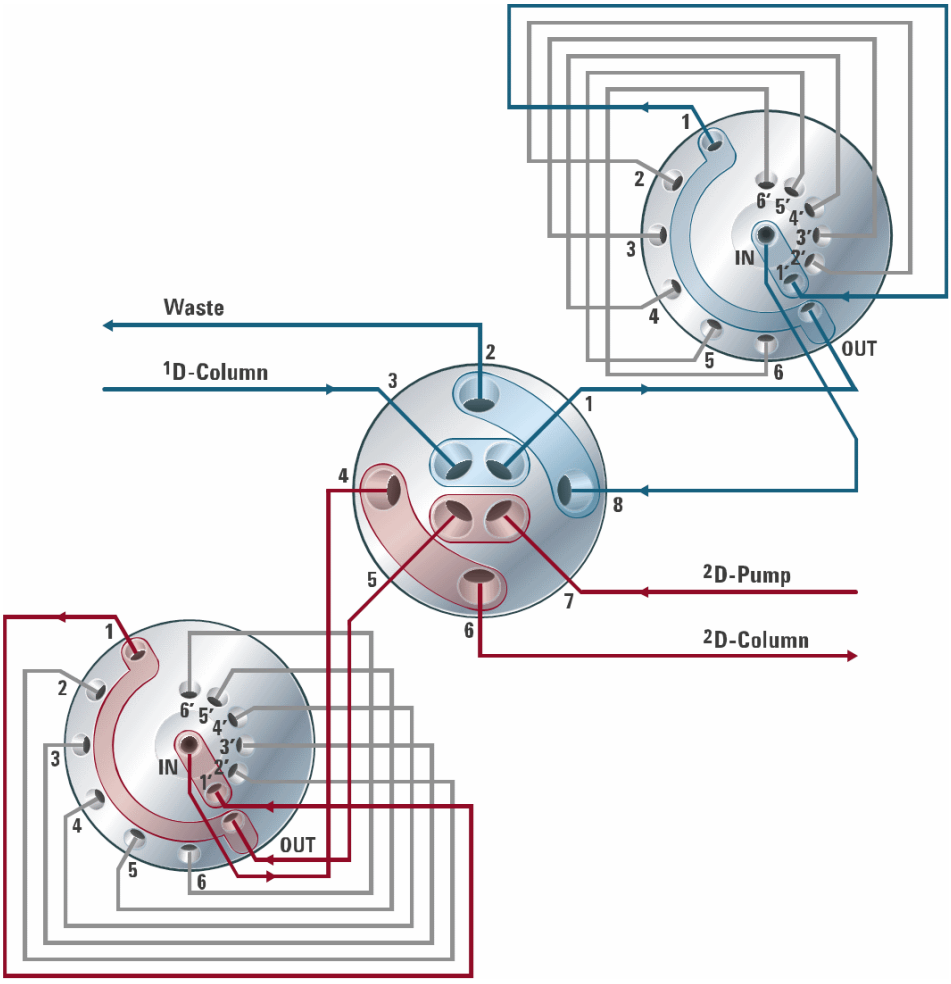
- Time-triggered LC-LC
- Peak-triggered LC-LC

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

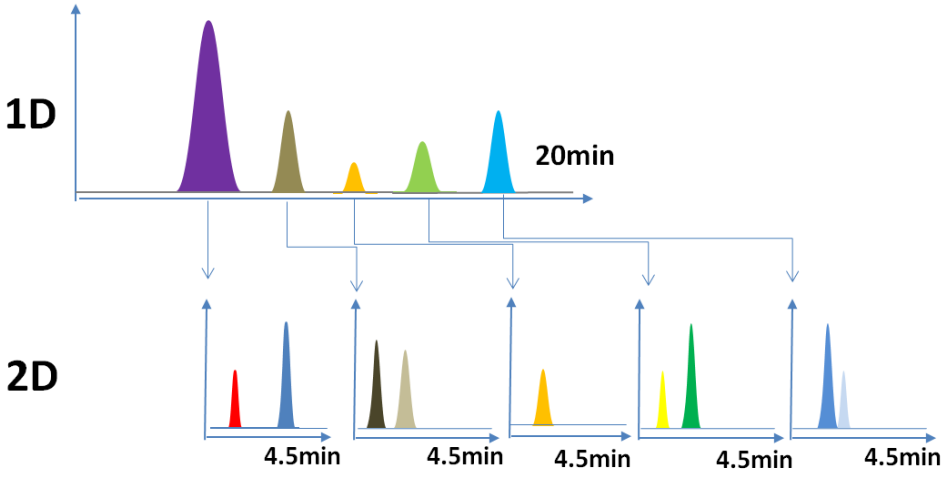
Typically, the gradient time in the second dimension is much longer for *heart-cutting* than with the *comprehensive* technique. The disadvantage of the standard heart-cutting techniques is that peaks cannot be sampled while a second dimension gradient is still running. In the examples shown here, the 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

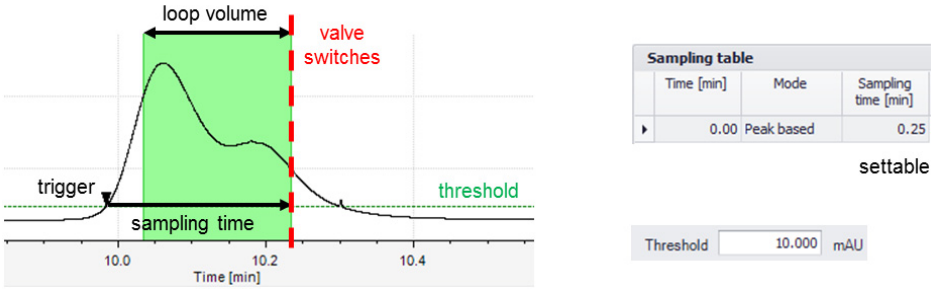


Figure 3 Peak-based mode

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.

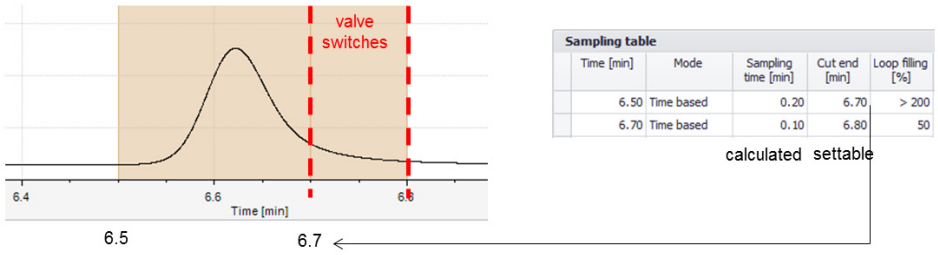


Figure 4 Time-based mode

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

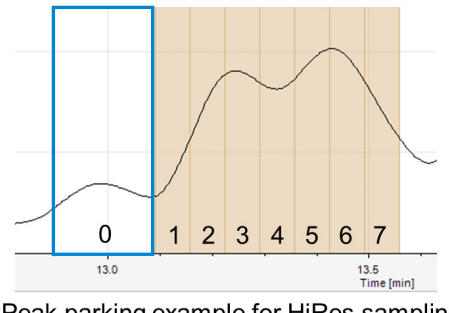
In the **HiRes sampling** mode, the multiple heart-cutting (MHC) valve is switched *before and after* parking the peak. This has the following consequences:

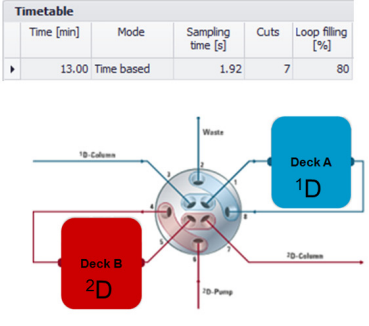
- Each loop for consecutive snips stores the same sample volume.
- First and last loop cannot be used for parking.
- Solvent transfer from ^{1}D to ^{2}D can be reduced.
- Cut number 5 cannot be parked entirely in the sample loop. Otherwise cut 6 would got partially to the transfer capillary and would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

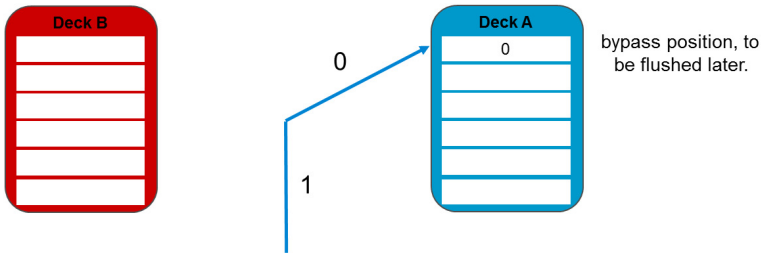
Peak parking example for HiRes sampling

- 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.

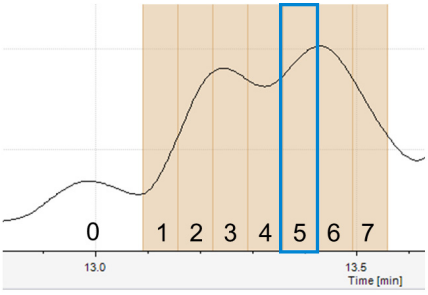


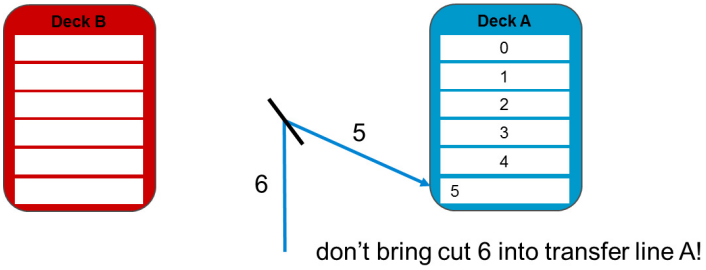


Peak parking example for HiRes sampling

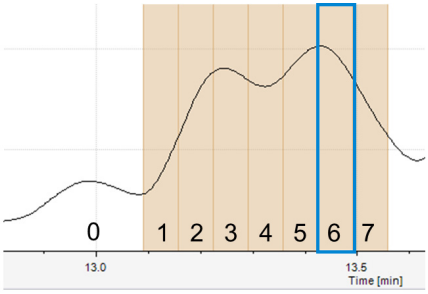


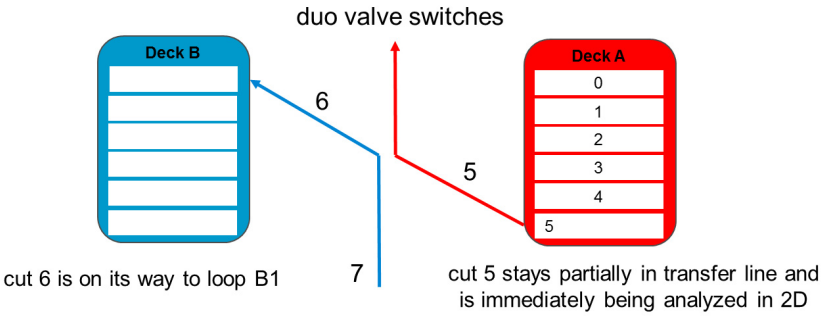
- MHC valve switches right before parking cut 1, 2, 3, 4, 5
- Cut number 5 cannot be parked entirely in the sample loop, otherwise cut 6 would go partially to the transfer capillary and would therefore be lost or spoil cut 5



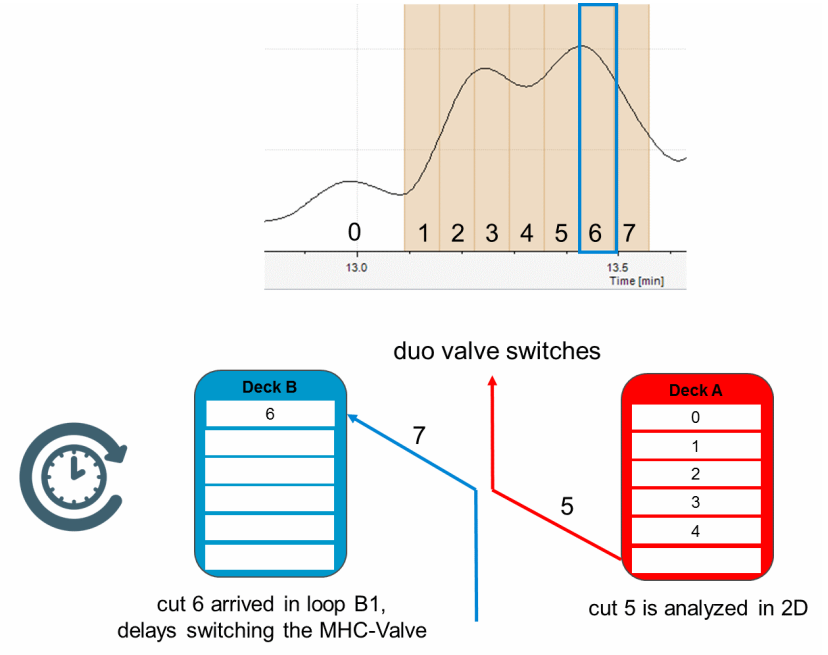


- Cut 5 stays partially in transfer line and is immediately analyzed in ^{2}D

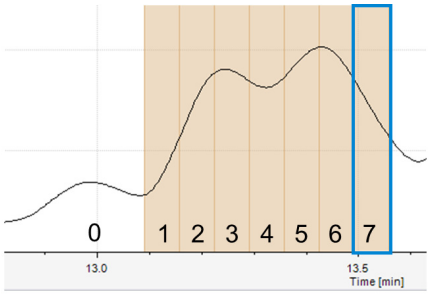


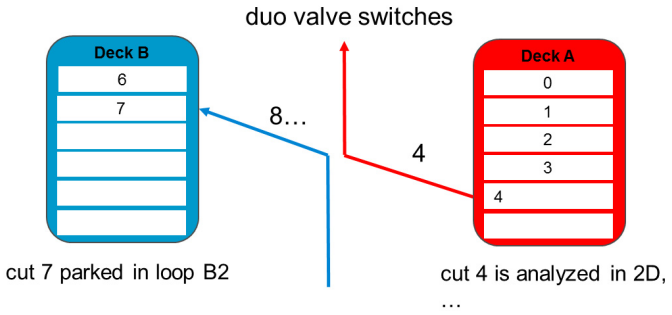


- For parking cut 6 into the sample loop, the cut first needs to be moved from the 2D-LC Valve to the deck valve.



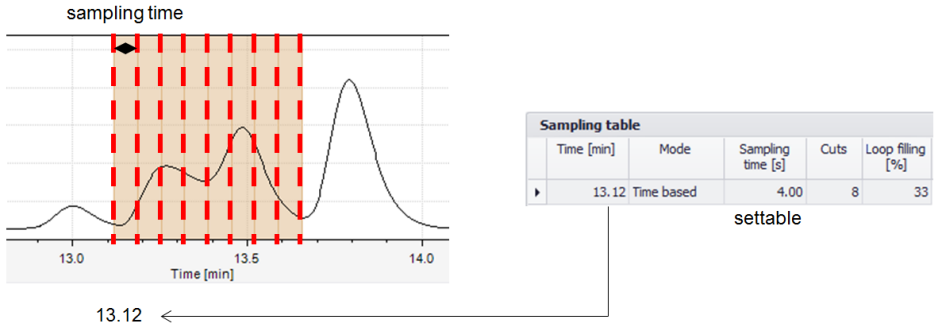
- Cut 7 will be parked in loop B2





- 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:

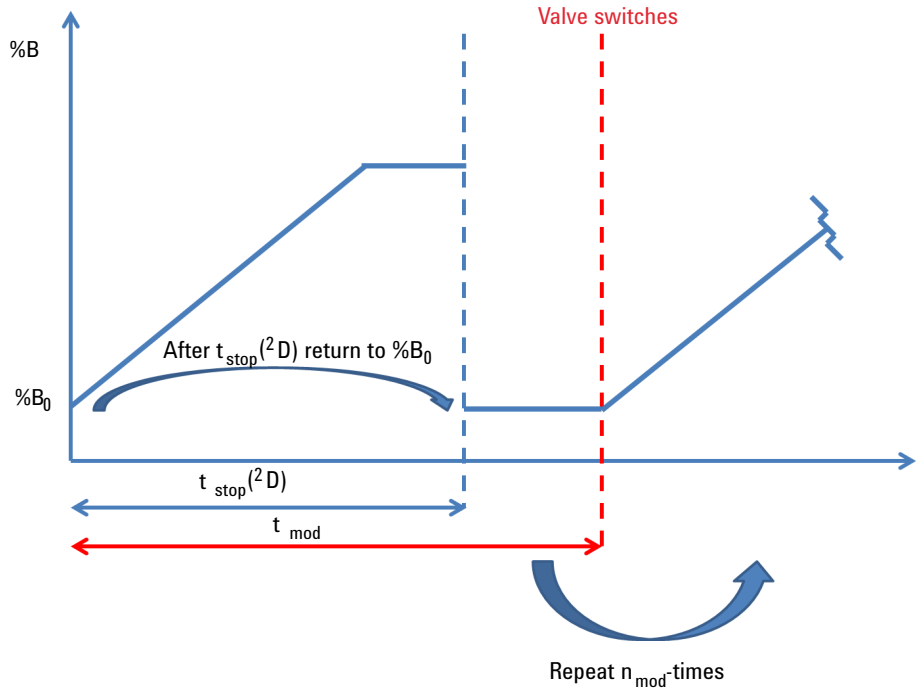


Figure 5 Characteristics of LCxLC

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.

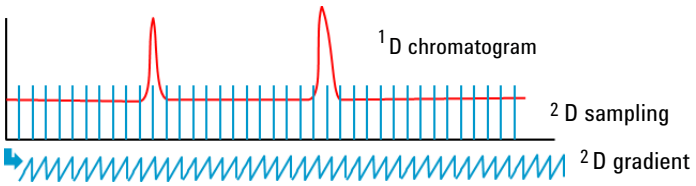


Figure 6 Principle of standard LCxLC

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.

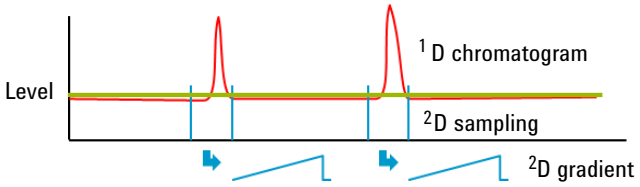


Figure 7 Principles of peak-triggered LC-LC

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).

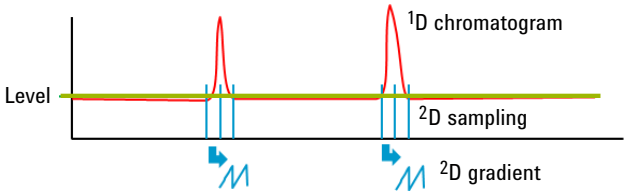


Figure 8 Principle of peak-triggered LCxLC

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.

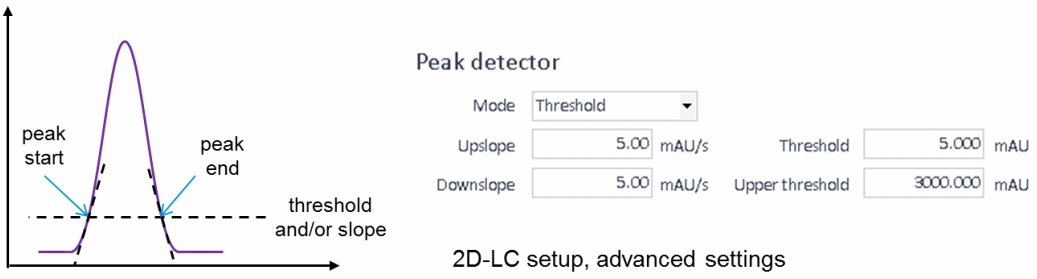


Figure 9 Peak triggering

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

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

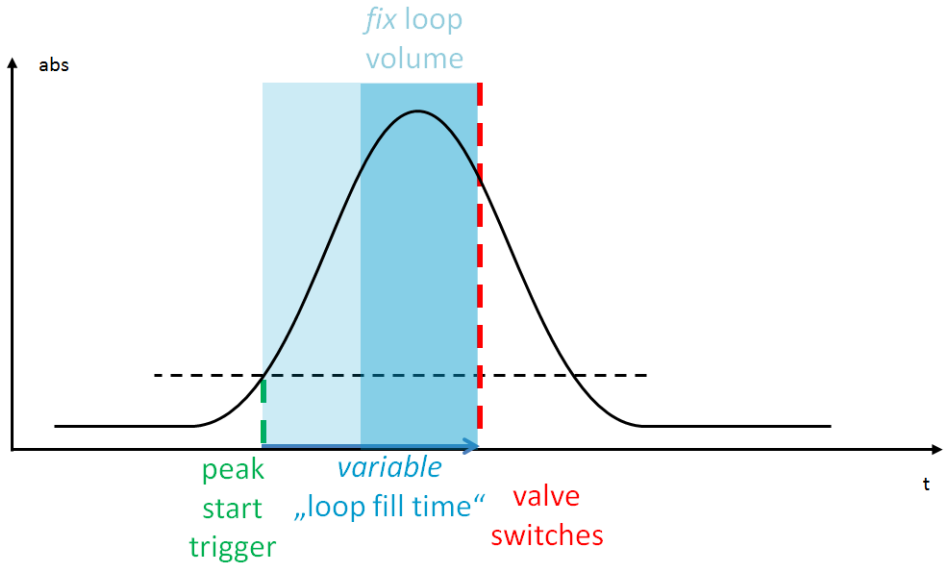


Figure 10 Peak triggering concept (elapsed sampling time)

- If the signal falls below threshold or slope.

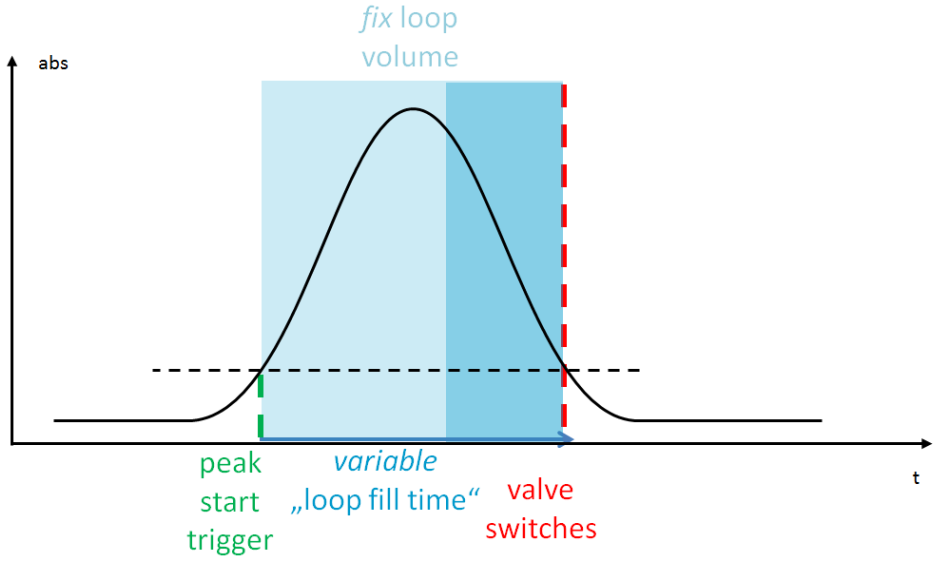


Figure 11 Peak triggering concept (signal falls below threshold or slope)

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.

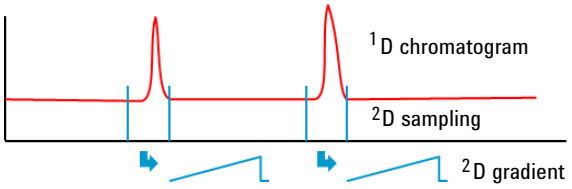


Figure 12 Principles of time-triggered LC-LC

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.

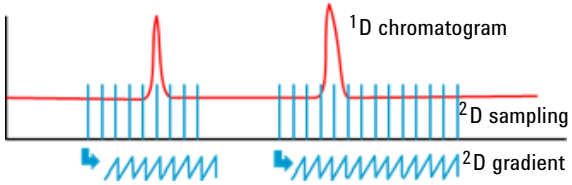


Figure 13 Principle of time-triggered LCxLC

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]

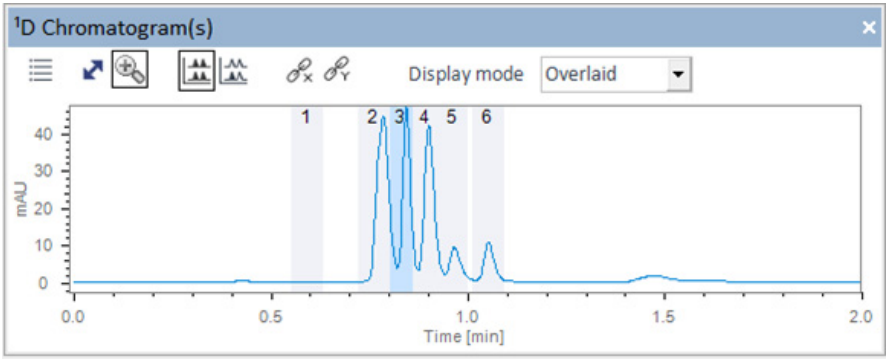


Figure 14Analysis of pesticides using a HILIC separation with high organic solvent composition in ^{1}D

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.

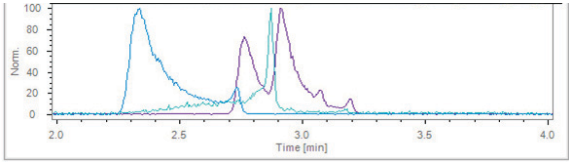


Figure 15 Conventional analysis of Cut#3 using a reversed phase separation in ^{2}D

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.

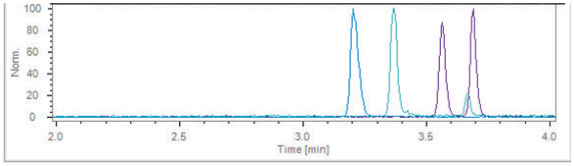


Figure 16 ASM analysis of Cut#3 using a reversed phase separation in ^{2}D

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

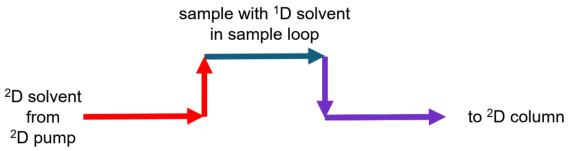


Figure 17 Operating principle with sample loop in flow path (schematic view)

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

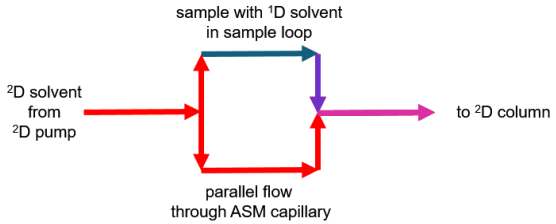


Figure 18 Operating principle with sample loop and ASM capillary in parallel flow path (schematic view)

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

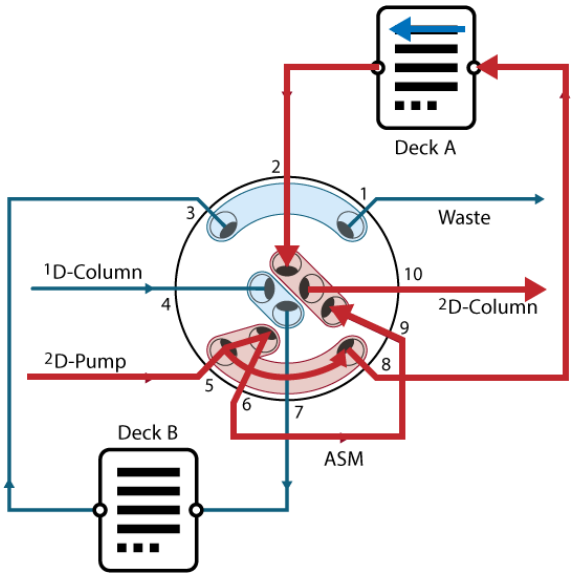


Figure 19 Operating principle with sample loop and ASM capillary in parallel flow path

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.

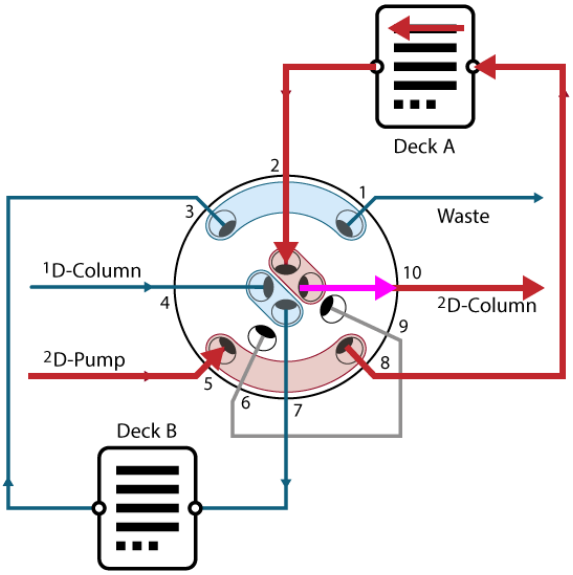


Figure 20 Operating principle with sample loop flow path

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.

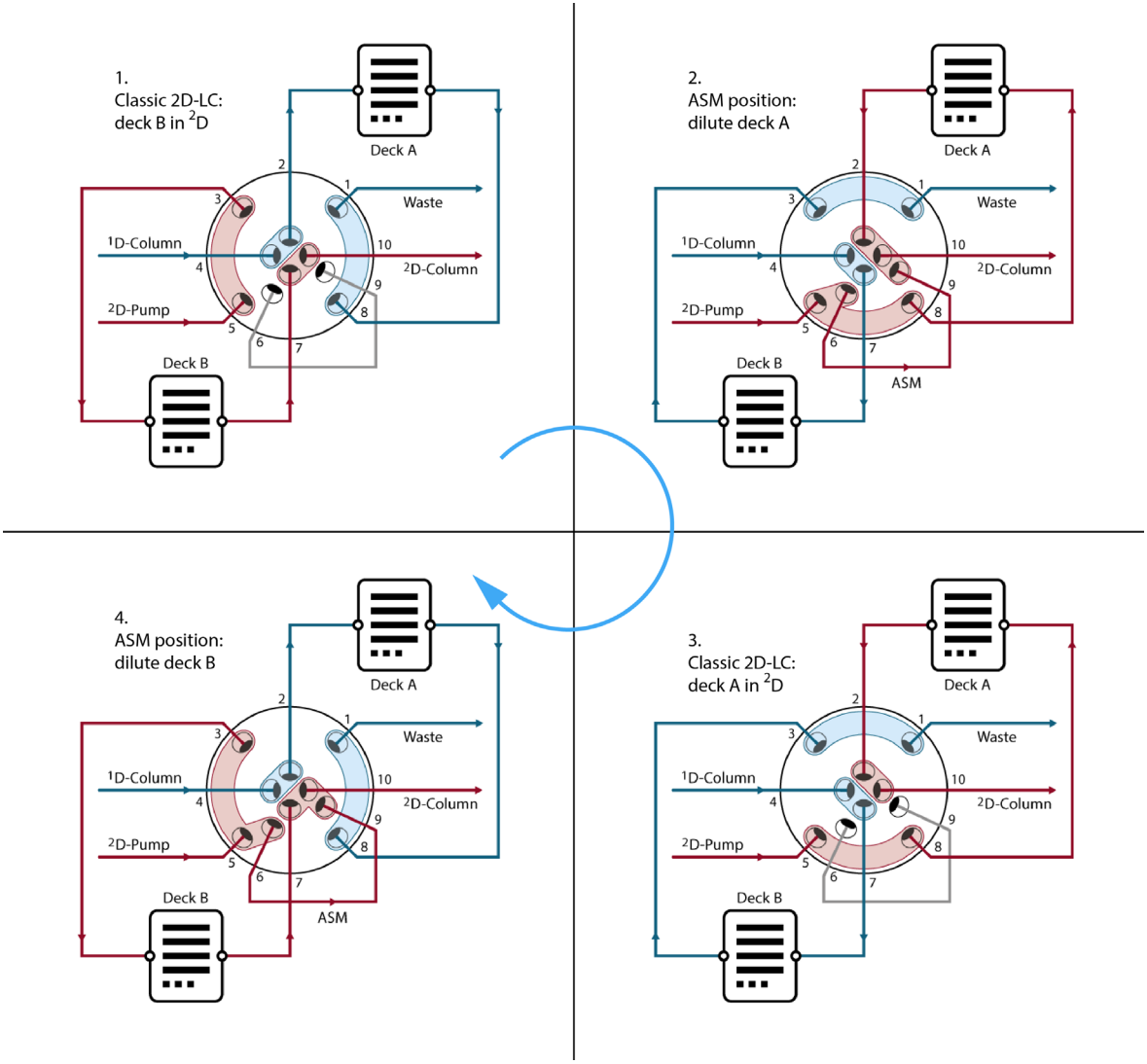


Figure 21 Switching cycle of the ASM valve (countercurrent mode)

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.

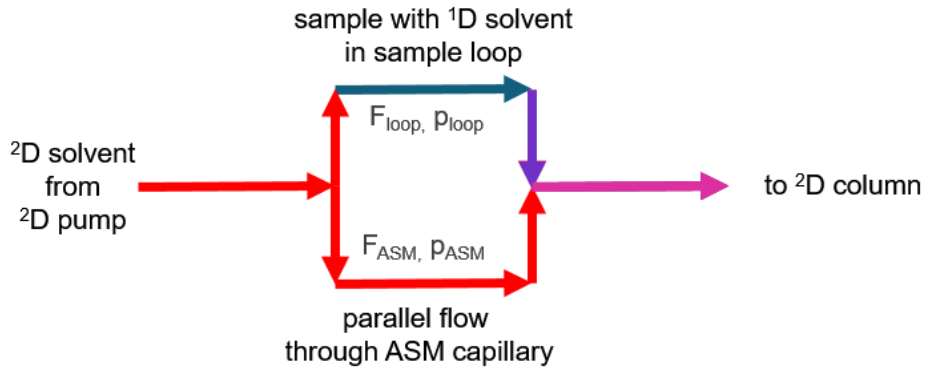


Figure 22 Principle of active solvent modulation (schematic view)

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.

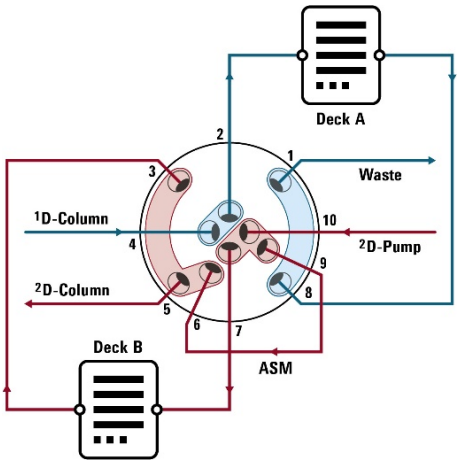


Figure 23 Backpressure of two flow paths in ASM

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 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.

Add on SW 2D-LC	ChemStation Version	LC & CE Driver Version
A.01.04 SR3	C.01.10 (or higher)	3.0/3.1 (or higher)
A.01.04 SR2/SR3	C.01.09/Update 1	A.02.19 SR3

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 OpenLab CDS 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.07.01
Agilent 1200 Series, 1200 Infinity and 1120 Compact LC	B.07.27
Agilent 1200 Infinity Hosted Modules	C.07.20
Agilent 1290 Infinity II Modules	D.07.27

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. 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.

Delivery checklist

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 mmX 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 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	

Recommendations for instrument setup

General Information

InfinityLab 2D-LC Solutions come in several flavors, still allowing flexible combination of InfinityLab Series and 1200 Series Infinity modules. For 2D-LC, a two-stack configuration is necessary. 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 detectors. When using multiple detectors at different positions of the system, it is recommended to use the same detector type. Depending on the number of solvents used, both stacks offer the possibility to place a solvent cabinet on top.

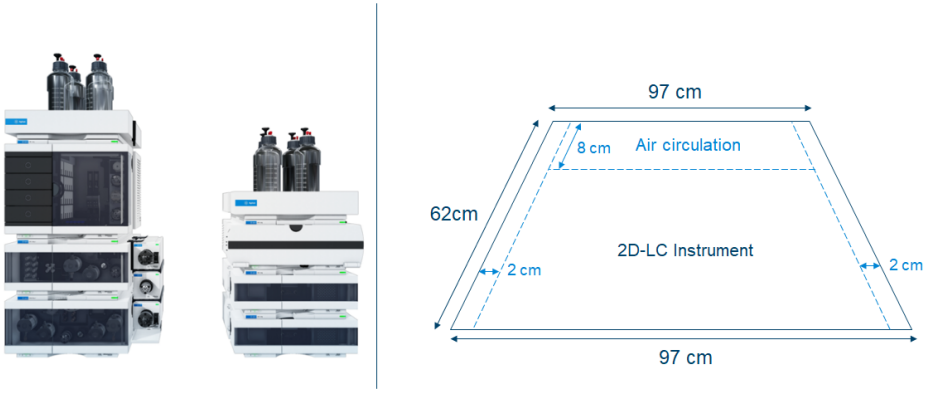


Figure 24 **Left: Recommended stack configuration for the 1290 Infinity II 2D-LC System. Right: Bench space requirements of the 1290 Infinity II 2D-LC System.**

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.

Agilent InfinityLab 2D-LC Solutions offer two general valve configurations that decide 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 2 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.

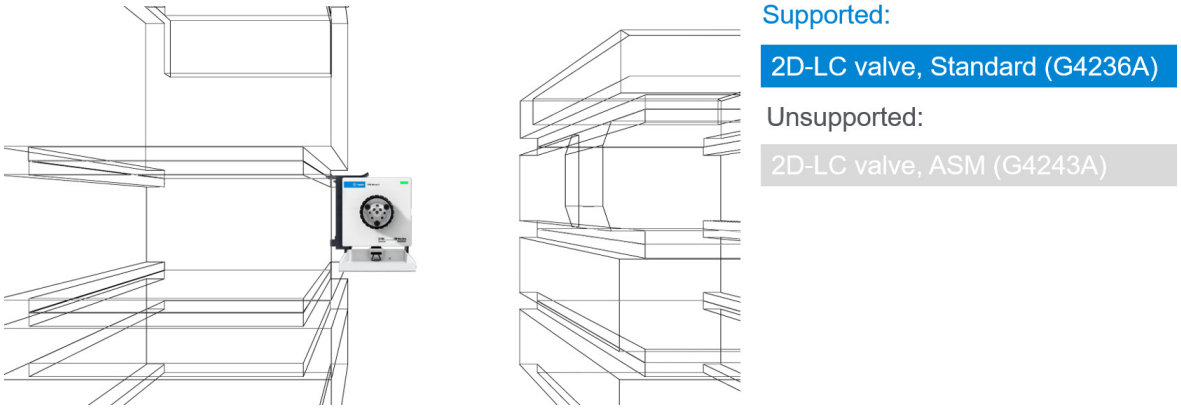


Figure 25 **Schematics of a Single Heart-Cutting (SHC) Configuration with supported valves. For technical reasons, the ASM valve (G4243A) is not supported in Single Heart-Cutting setups.**

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.

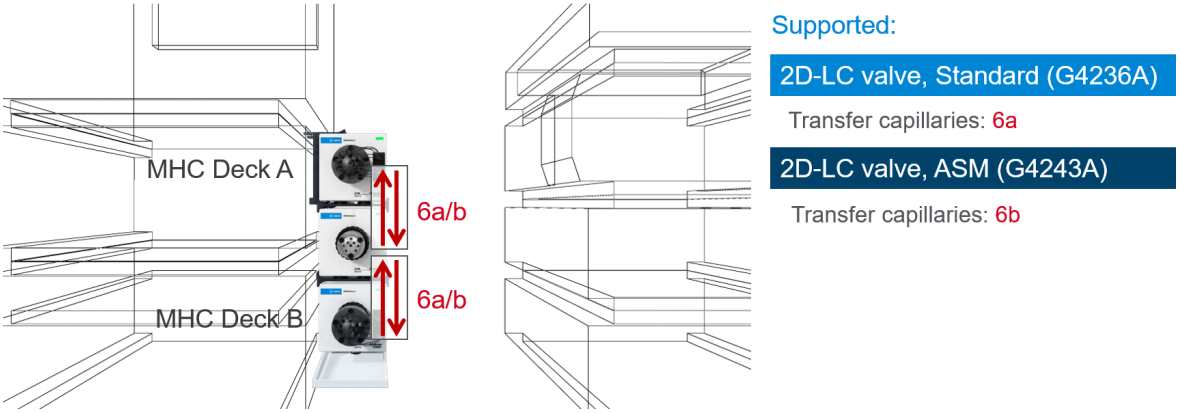


Figure 26 **Schematics of a Multiple Heart-Cutting (MHC) Configuration with supported valves and transfer capillaries.**

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 basic stack configurations are distinguished by the pump used for the first and second dimension. 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 3 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) 1290 Infinity II Flexible Pump (G7104A) 1290 Infinity II High-Speed Pump (G7120A)	**1290 Infinity / 1290 Infinity II** 1290 Infinity II High-Speed Pump (G7120A) 1290 Infinity Binary Pump (G4220A)
2	**1290 Infinity** 1290 Infinity Quaternary Pump (G4204A) 1290 Infinity Binary Pump (G4220A)	**1290 Infinity II** 1290 Infinity II High-Speed Pump (G7120A)
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)

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 concurrent direction for the Standard 2D-LC Valve (G4236A) and countercurrent for the ASM 2D-LC Valve (G4243A). To connect the 2D-LC Valve, Standard in countercurrent direction, please follow the schematics shown in the 2D-LC Software Configuration screen. For more information, please read the 2D-LC Software Configuration section below. Connection to valve port are mentioned in brackets, for example. ASM Valve (2) = ASM Valve, Port 2.

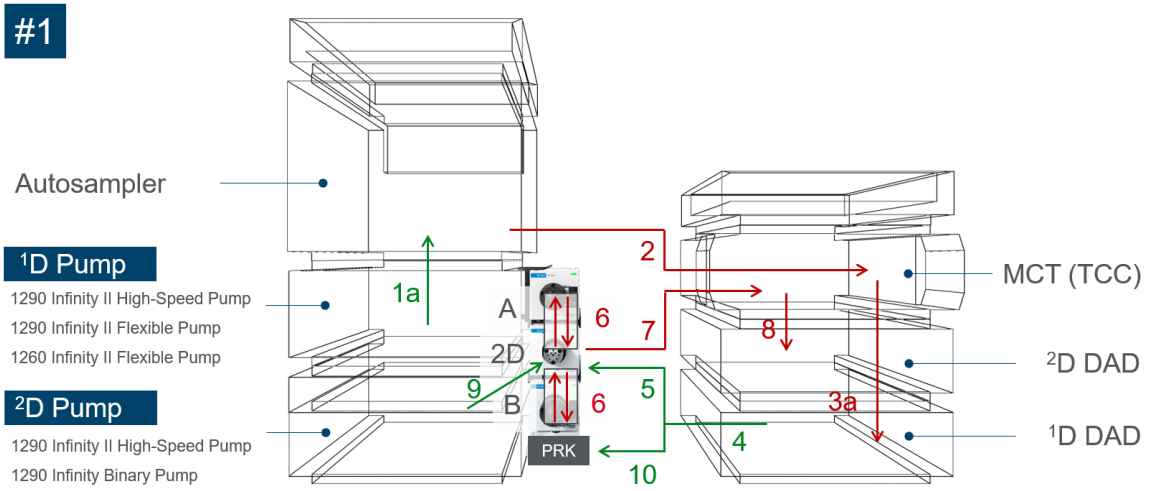


Figure 27 **Stack Setup #1. Recommended setup if both pumps are Infinity II modules or the 2D pump is a 1290 Infinity Binary pump.**

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

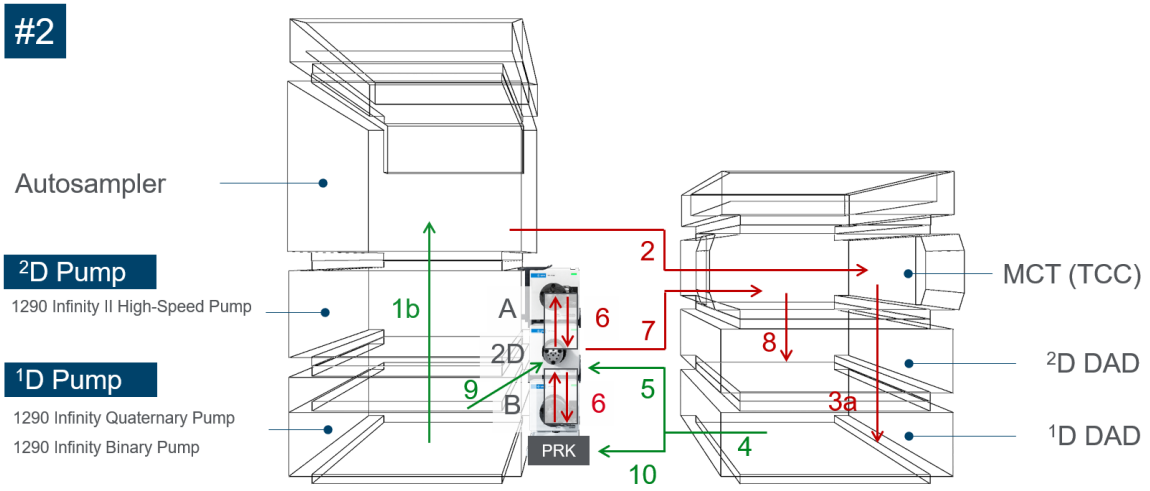


Figure 28 Stack Setup #2. Recommended setup if the 1D pump is a 1290 Infinity Binary Pump or a 1290 Infinity Quaternary Pump.

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

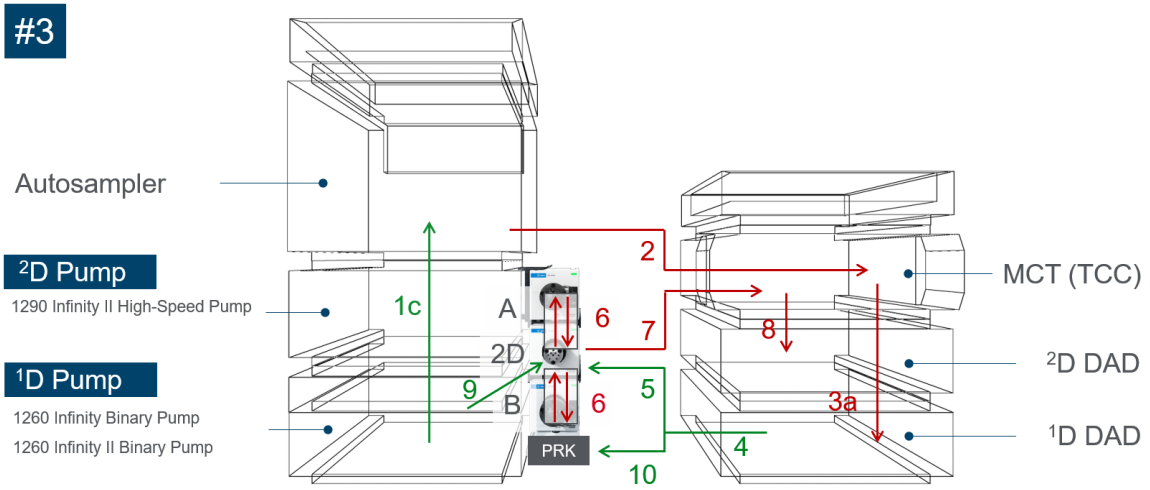


Figure 29 Stack Setup #3. Recommended setup if the 1D pump is a 1260 Infinity or 1260 Infinity II Binary Pump.

Number of Capillary	#	Connection	L x ID [mm]	P/N	Description
1c	1	1D pump (bottom) to sampler	900 x 0.17	5500-1217	Capillary ST 0.17x900 SI/SX
2	1	Autosampler to 1D column (in MCT)	600 x 0.12	5067-4669	Capillary ST 0.12x600 S/SL
3a	1	1D column to 1D DAD	400 x 0.12	5500-1251	Capillary ST 0.12x400 SL/SL
4	1	1D DAD to T-piece of PRK	400 x 0.17	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)	105 x 0.17	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)	170 x 0.12	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)	170 x 0.12	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)	400 x 0.12	5500-1251	Capillary ST 0.12x400 SL/SL
8	1	2D column (in MCT) to 2D DAD	280 x 0.12	5067-4651	Capillary ST 0.12x280 SL/SX
9	1	2D pump to 2D-LC Valve (7) / ASM Valve (5)	280 x 0.17	5067-4608	Capillary ST 0.17x280 SX/S
10	1	T-piece of PRK to damper capillary	150 x 0.17	5500-1227	Capillary ST 0.17x150 SL/SL
11	1	waste line	self-cut x 0.7	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 2 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 4 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 30 on page 67
B	Setups that contain separate ^{1}D and ^{2}D MCTs/TCCs		See Figure 31 on page 68
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 32 on page 69
D	Setup with a MS diverter valve		See Figure 33 on page 71
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 34 on page 73
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 35 on page 74

A

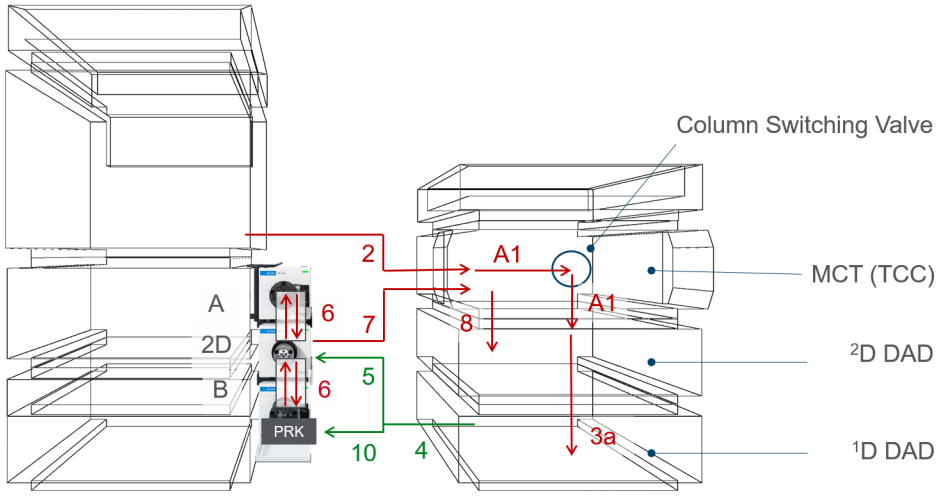


Figure 30 **Setup A. Recommended setup if a column switching valve (for example 6-position/14-port InfinityLab Quick-Change Valve) is used. For a InfinityLab 2-position/6-port Quick-Change Valve, adapters A1 are not necessary.**

Number of Capillary	#	Connection	L x ID [mm]	P/N	Description
A1	2	Adapter: capillary 2 to column switching valve, (Port IN) / Adapter column switching valve (Port OUT) to capillary 3a	75x0.12mm	G1316-87326	SST Capillary 75x0.12mm, f/m, ns 0.8 (must be purchased separately)

For all other capillaries / connections, please refer to Figure 27 on page 63, Figure 28 on page 64, and Figure 29 on page 65.

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

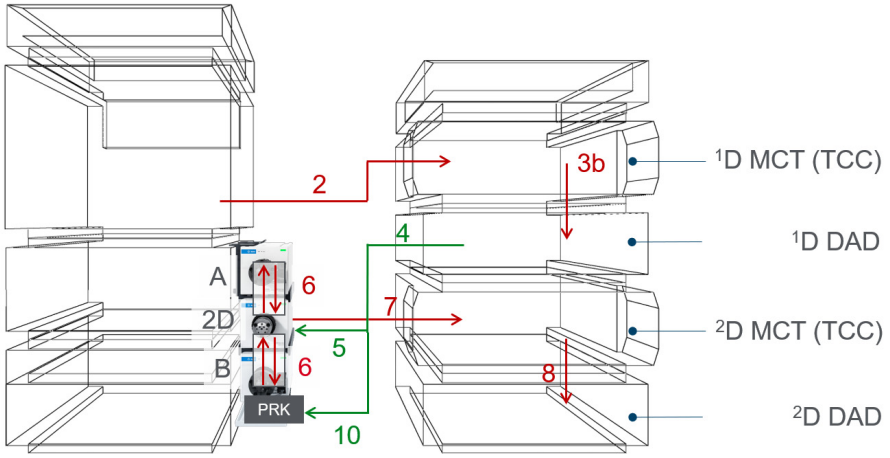


Figure 31 Setup B. Recommended setup if the instrument contains separate MCTs/ TCCs for ^{1}D and ^{2}D columns.

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

For all other capillaries / connections, please refer to Figure 27 on page 63, Figure 28 on page 64, and Figure 29 on page 65.

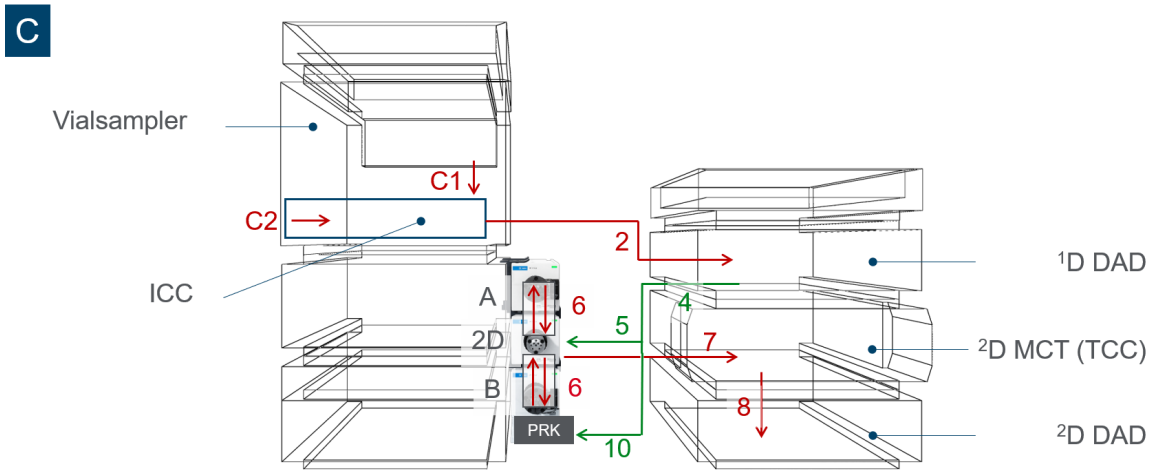


Figure 32 Setup C. Recommended setup if ^{1}D column is hosted in an Integrated Column Compartment (ICC).

Number of Capillary	#	Connection	L x ID [mm]	P/N	Description
C1	1	Injection Valve to ICC	0.12x105mm	5500-1238	Capillary ST 0.12x105 SL/SL (provided with ICC)
C2	1	Heat exchanger out to column (InfinityLab Quick Connect Fitting)	0.12x280mm	5500-1170	Capillary ST 0.12x280 (must be purchased separately)
8	1	^{2}D column (in ^{2}D MCT) to ^{2}D DAD	280 x 0.12	5067-4651	Capillary ST 0.12x280 SL/SX (part of 2D-LC capillary kit)
For all other capillaries / connections, please refer to Figure 27 on page 63, Figure 28 on page 64, and Figure 29 on page 65.					

The 2D-LC Software allows only certain valves to be configured as diverter valves. A list of supported valves can be found in Table 3 on page 62. More information is available in the Agilent Technical Note *Agilent InfinityLab 2D-LC Solution with mass spectrometric detection and diverter valve* (G4236-90100).

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

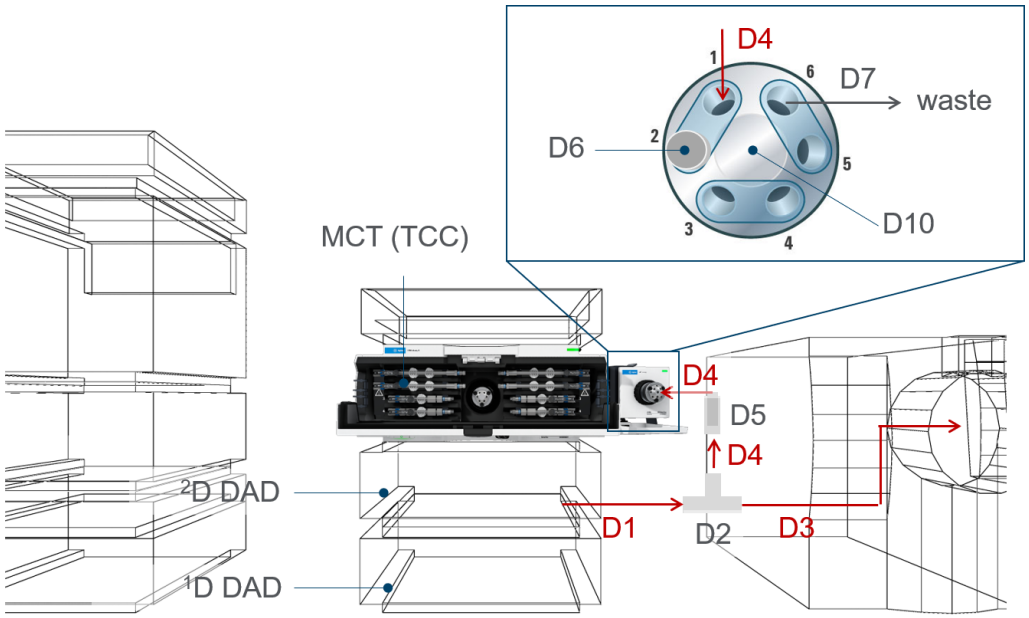


Figure 33 Setup D. Recommended setup of a MS diverter valve.

Number of Capillary	#	Connection	L x ID [mm]	P/N	Description
D1	1	Capillary from ^{2}D detector to T-piece	0.12 x 400	5067-4606	Capillary ST 400x0.12 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
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 27 on page 63, Figure 28 on page 64, and Figure 29 on page 65.					

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 in the 2D-LC Software Configuration Tab.

E

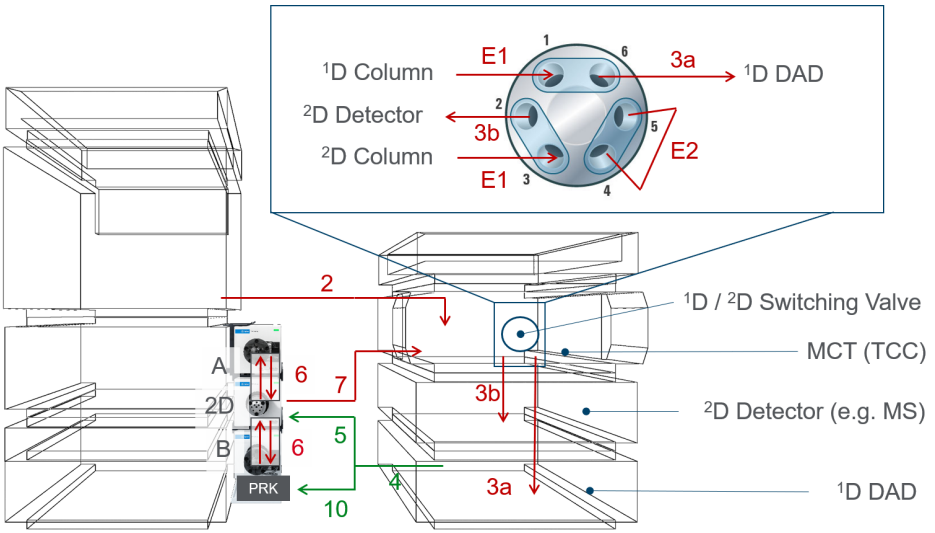


Figure 34 Setup E. Recommended setup for the ^{1}D/^{2}D switching valve.

Number of Capillary	#	Connection	L x ID [mm]	P/N	Description
3a	1	MCT / TCC to ^{1}D DAD	400 x 0.12	5500-1251	Capillary ST 0.12x400 SL/SL
8	1	^{1}D MCT / TCC to ^{1}D DAD	280 x 0.12	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)	120 x 0.12	5067-4652	Capillary ST 0.12x 120 SX/SX
E2	1	Connection capillary ^{1}D/^{2}D Switching Valve (4) to (5)	90 x 0.12	5067-4649	Capillary ST 0.12x90 SX/S
For all other capillaries / connections, please refer to Figure 27 on page 63, Figure 28 on page 64, and Figure 29 on page 65.					

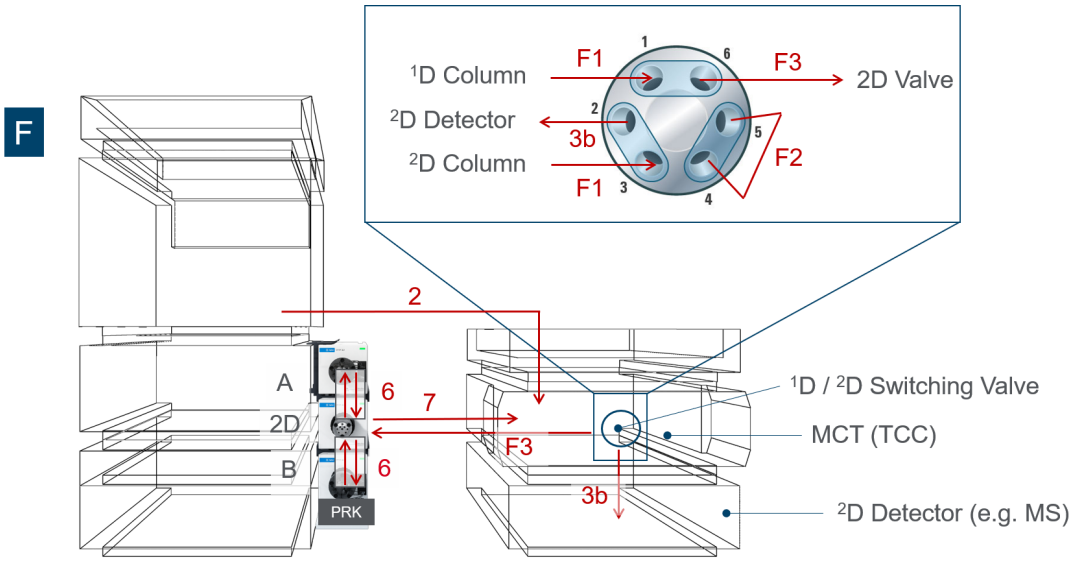


Figure 35 Setup F. Recommended setup for the ^{1}D/^{2}D switching valve without ^{1}D detector.

Number of Capillary	#	Connection	L x ID [mm]	P/N	Description
3b	1	^{1}D/^{2}D Switching Valve (2) to ^{2}D DAD	280 x 0.12	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)	120 x 0.12	5067-4652	Capillary ST 0.12x 120 SX/SX
F2	1	Connection ^{1}D/^{2}D switching valve ports (4) to (5)	90 x 0.12	5067-4649	Capillary ST 0.12x90 SX/S
F3	1	MCT/TCC to 2D-LC valve (6) / ASM valve (4)	500 x 0.12	5500-1157	Capillary ST 0.12x500 SL/S
For all other capillaries / connections, please refer to Figure 27 on page 63, Figure 28 on page 64, and Figure 29 on page 65.					

Installation of the 2D-Valves

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)	Mooting 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.

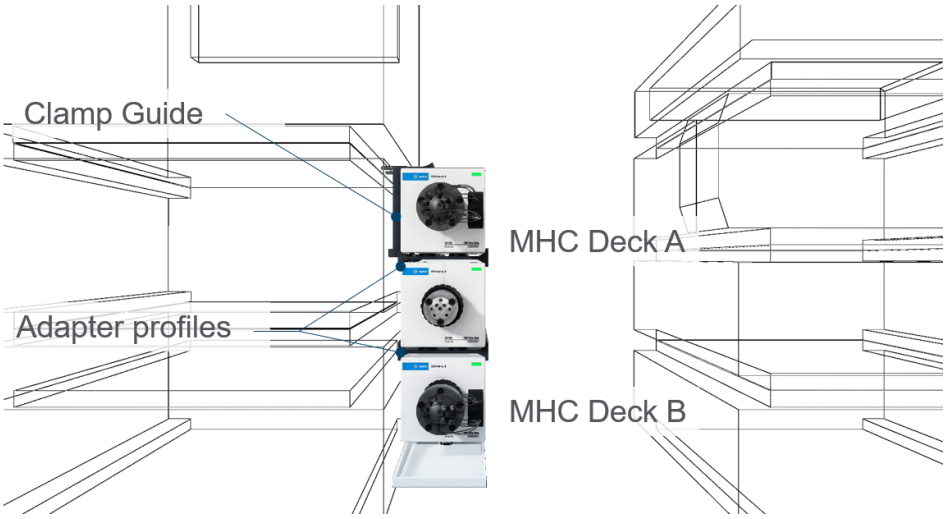


Figure 36 **Schematic of the installation and attachments of the 2D-LC valve and optionally the MHC decks.**

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 36 on page 76.
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 82.

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. For the countercurrent setup, please refer to Countercurrent configuration of the Standard 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.

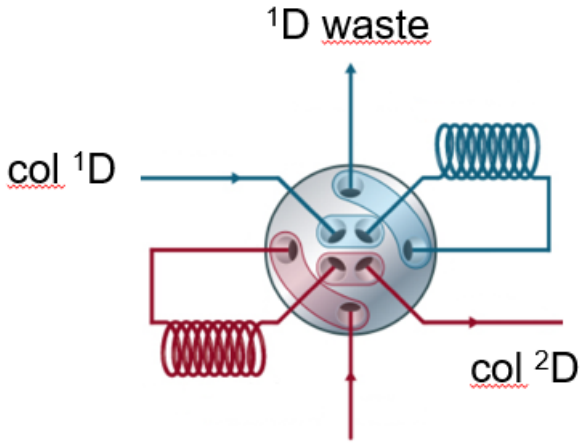


Figure 37 Schematic representation of the Standard 2D-LC Valve (G4236A) in concurrent flow.

Port	Number of Capillary	Connection	L x ID [mm]	P/N	Description
1	6a	transfer capillary to MHC Valve (OUT), deck A	170 x 0.12	5500-1270	Capillary ST 0.12x170 S/M
2	11	waste line	self-cut x 0.7	0890-1713	Tubing-flexible 0.8/1.61mm PTFE WT (delivered with UV detector)
3	5 F3	from pressure release kit; from 1D column, 1D detector	105 x 0.17 500 x 0.12	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	170 x 0.12	5500-1270	Capillary ST 0.12x170 S/M
5	6a	transfer capillary to MHC Valve (OUT), deck B	170 x 0.12	5500-1270	Capillary ST 0.12x170 S/M
6	7	to 2D column	400 x 0.12	5500-1251	Capillary ST 0.12x400 SL/SL
7	9	from 2D pump	280 x 0.17	5067-4608	Capillary ST 0.17x280 SX/S
8	6a	transfer capillary to MHC Valve (IN), deck A	170 x 0.12	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**:

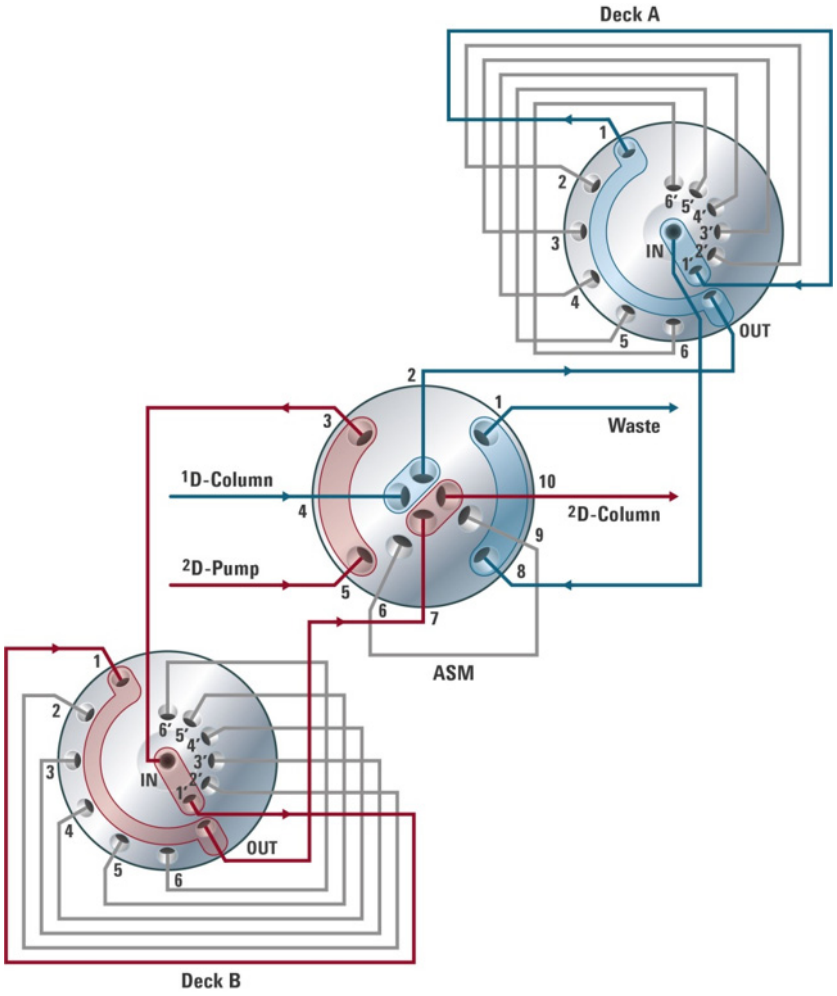


Figure 38 Schematic representation of the ASM 2D-LC Valve (G4243A) in countercurrent flow.

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

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

Installing the Pressure Release Kit

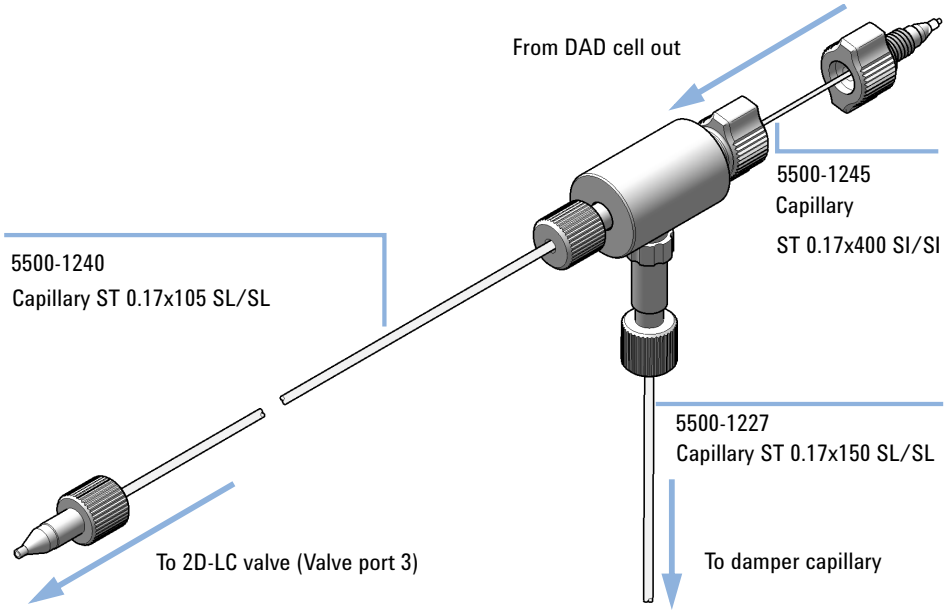
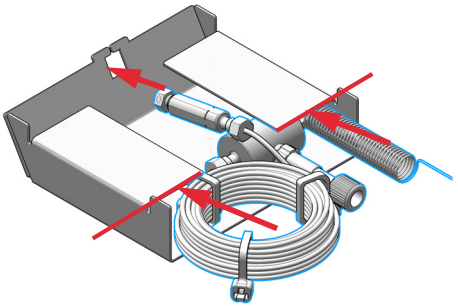


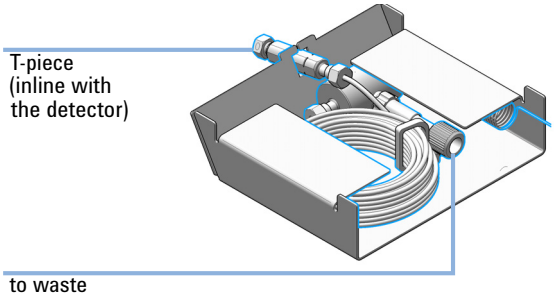
Figure 39 Connections to the pressure release kit

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

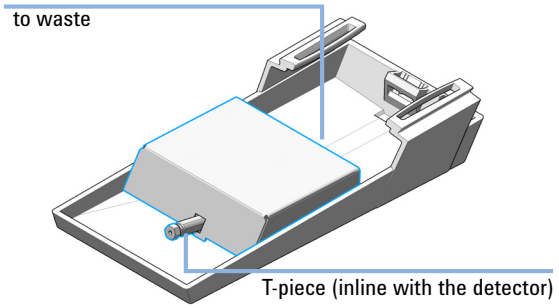
1 Push the pressure release valve assembly in the frame.



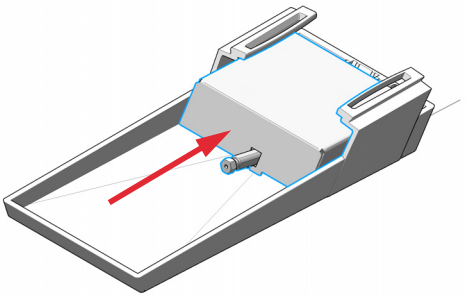
2 Take care for the correct orientation.



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



4 Push the pressure release assembly in the correct position.



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

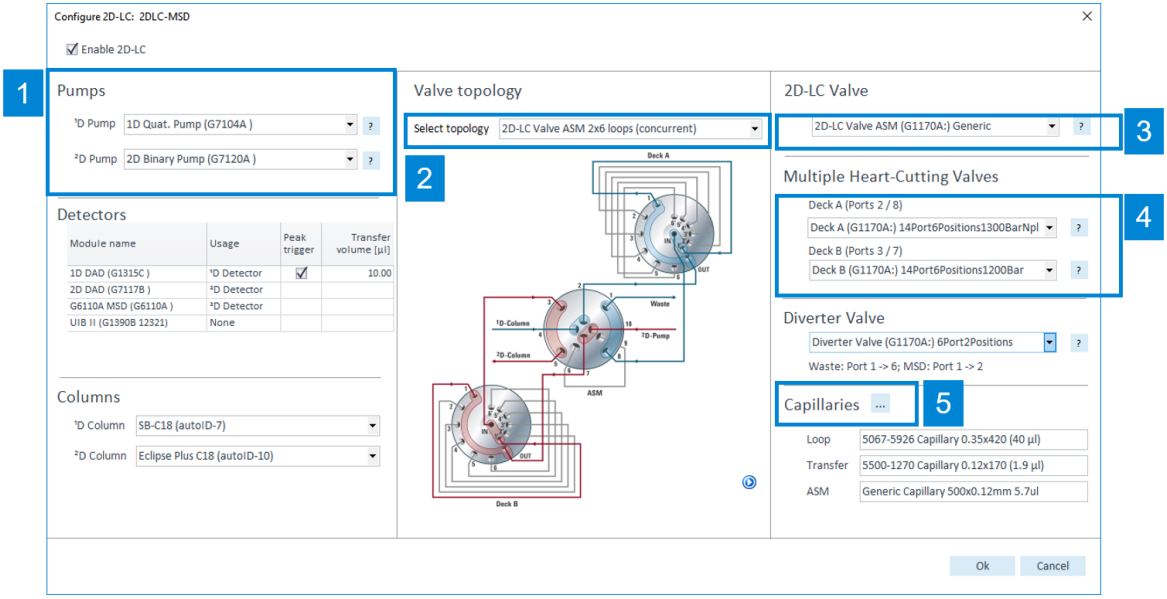


Figure 40 The Configure 2D-LC screen. All basic configurations of the 2D-LC systems, viz. ^{1}D and ^{2}D pump, valve topology, capillary connections as well as all 2D-LC valves are defined here.

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

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 on page 81).

   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**.

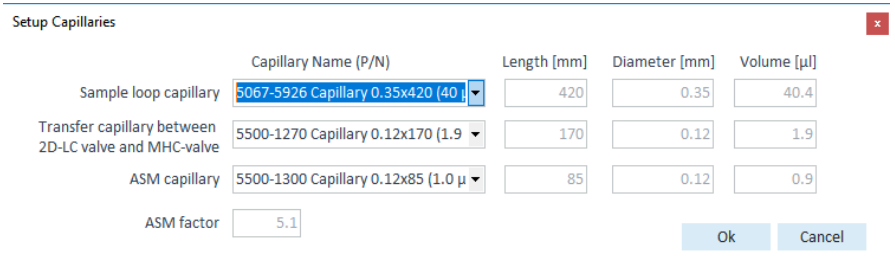


Figure 41 The Setup Capillaries screen. Here, you can define ASM and transfer capillaries as well as define other loop sizes.

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.

Installation of Active Solvent Modulation (ASM)

Delivery checklist

p/n	Description
G4243-90000	Agilent G4243A 2D-LC ASM Valve Guide Technical Note
5067-4266	2D-LC ASM Valve Head, 1300 bar
G4236-68000	2D-LC Easy Starter Kit Internal part, not orderable
G1680-63721	Network LAN Switch
5500-1300	Capillary ST 0.12x85M/M ASM
5500-1301	Capillary ST 0.12x170M/M ASM
5500-1302	Capillary ST 0.12x340M/M ASM
5500-1303	Capillary ST 0.12x680M/M ASM
5500-1376	Capillary ST 0.12x170M/M transfer
5067-6171	Capillary Kit 2D-LC, Infinity Classic (optional) Internal part, not orderable
5067-6585	Capillary Kit 2D-LC, 1290 Infinity II Internal part, not orderable

Install the valve head and connecting capillaries

For instructions on how to install the valve head and connecting capillaries, refer to “Replace Valve Heads (G1170A)” on page 269.

5 Method Parameters

This chapter provides background information on method parameters. It helps to optimize methods in Agilent 1290 Infinity II 2D-LC Solution 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.

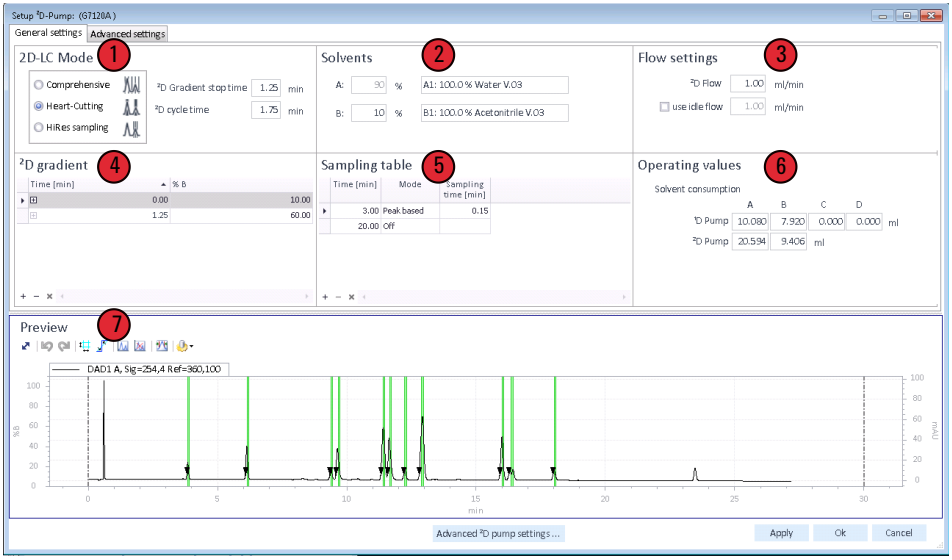


Figure 42 2D-LC method setup (General settings shown in Heart-Cutting mode as an example)

The setup of following method parameters is available:

1 **2D-LC Mode**, see "Set 2D-LC Mode" on page 89
2 **Solvents**, see "Set solvents" on page 96
3 **Flow settings**, see "Set flow" on page 97
4 **²D Gradient**, see "Set Solvent Composition Gradient" on page 98
5 **Sampling table**, see "Sampling table" on page 100
6 **Operating values**, see "Define Peak Detector Parameter" on page 102
7 **Preview**, see "Setup Second Dimension Gradient with the Graphical User Interface" on page 138

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 43 on page 91):

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 44 on page 92. In Figure 44 on page 92, Figure 45 on page 93 and Table 5 on page 94 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 45 on page 93.

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 5 on page 94.

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

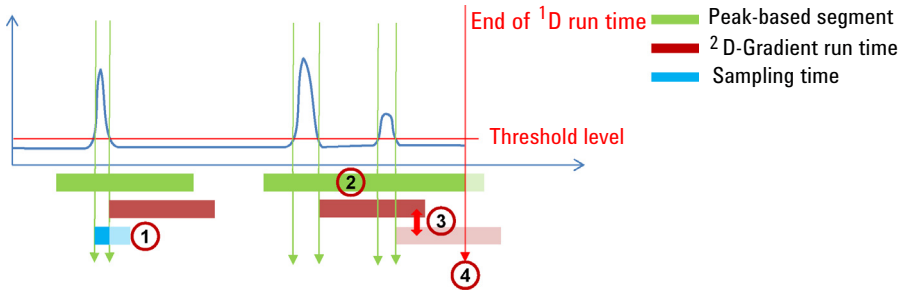


Figure 43 Heart-cutting 2D-LC (general considerations) (delay times have been omitted for clarity, besides threshold also the peak-slope can be used for peak detection)

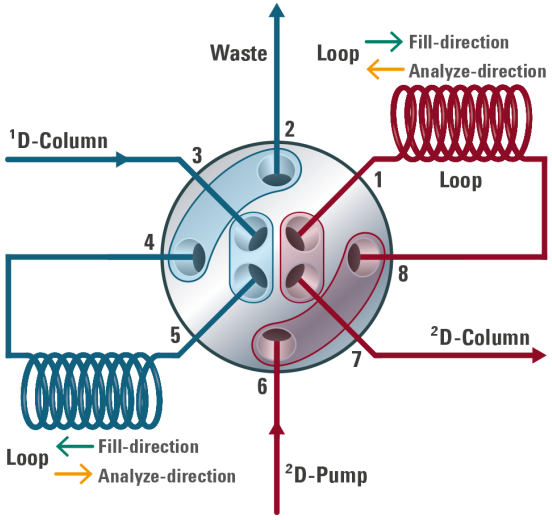
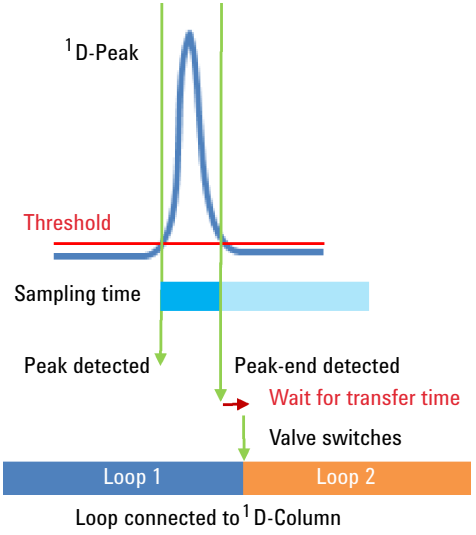
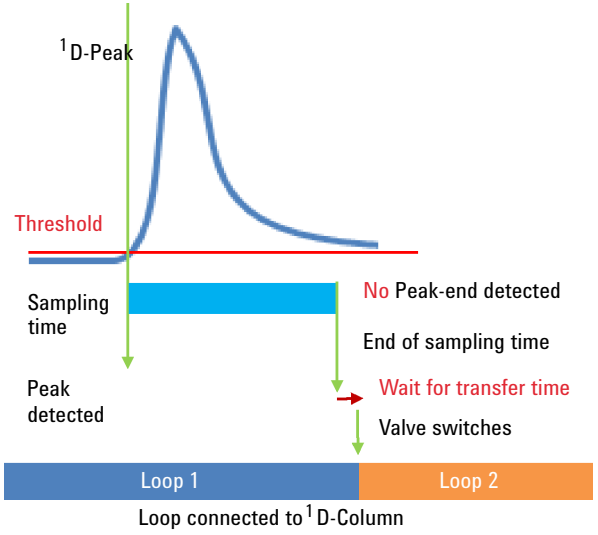


Figure 44 Valve and loop setup for heart-cutting 2D-LC with the 2D-LC Valve (dual-loop setup)





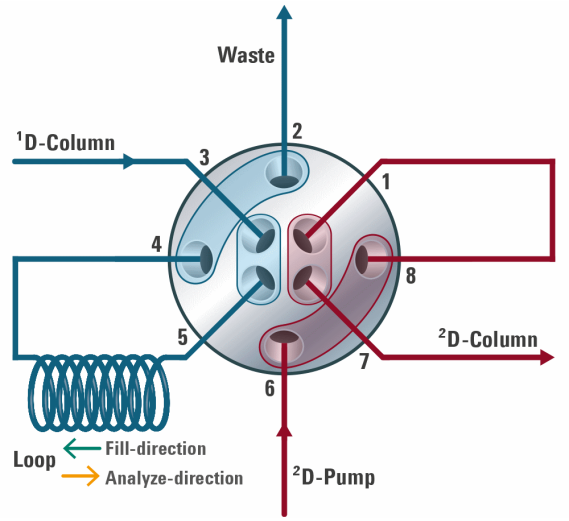
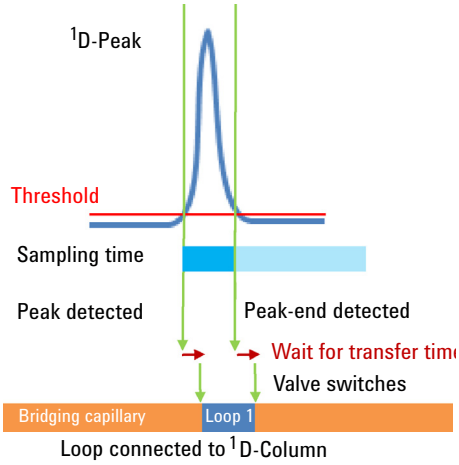


Figure 45 Valve and loop setup for heart-cutting 2D-LC with the 2D-LC Valve (single-loop setup)



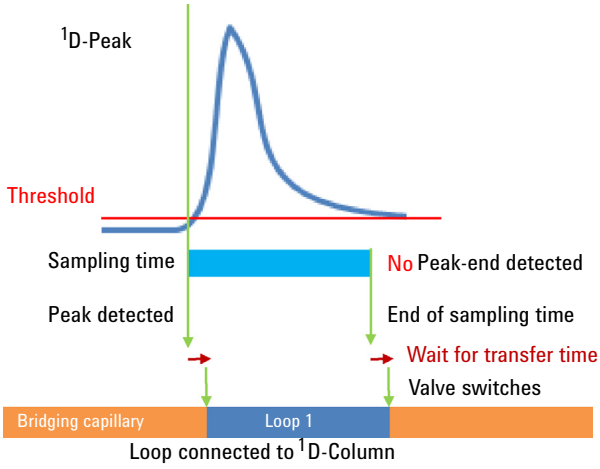


Table 5 Time-based heart-cutting 2D-LC

Dual-loop set-up	Single-loop set-up

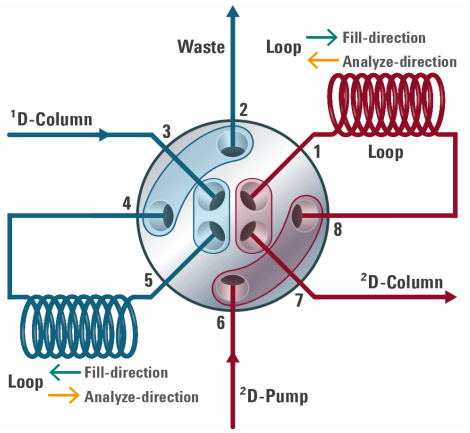
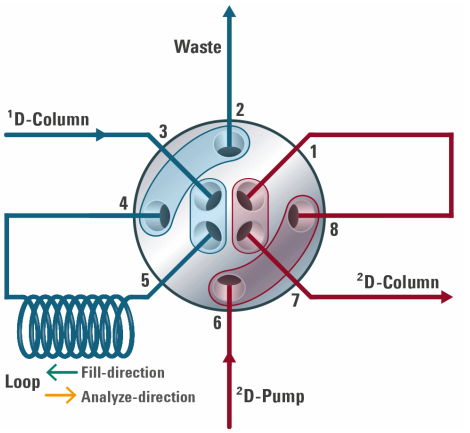
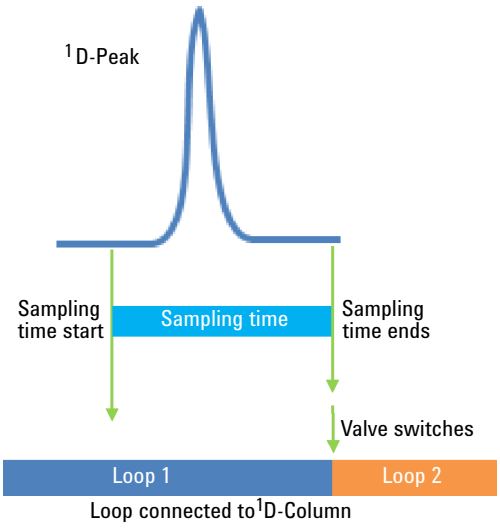
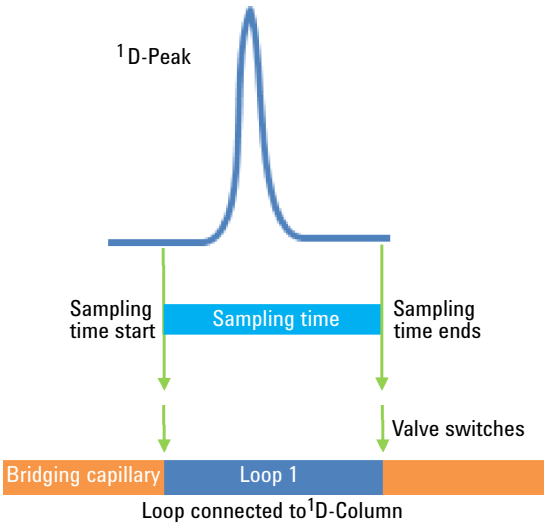

	

Figure 46 Valve and loop setup for heart-cutting 2D-LC with the 2D-LC Valve	**Figure 47** Valve and loop setup for heart-cutting 2D-LC with 2D-LC Valve
(example valve)	(example valve)
	

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.

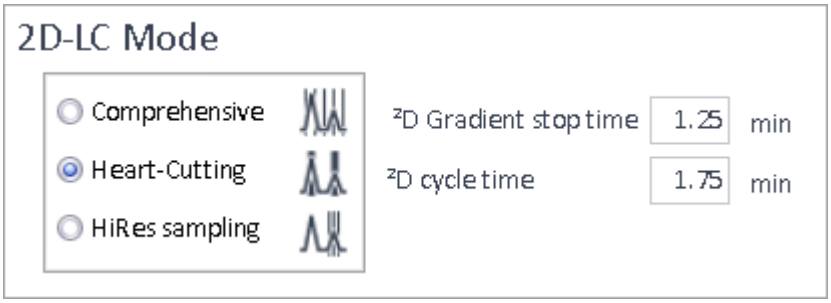


Figure 48 2D-LC ModeHeart-Cutting

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.

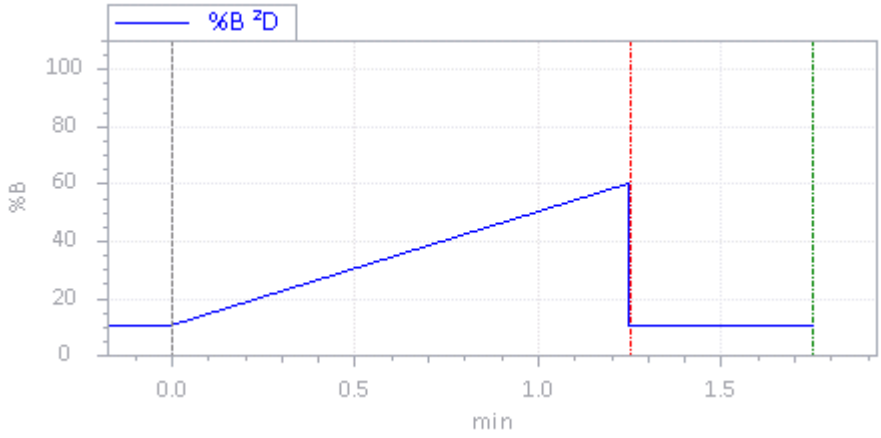


Figure 49 Stop time and Modulation time

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 50 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 148).

Set flow

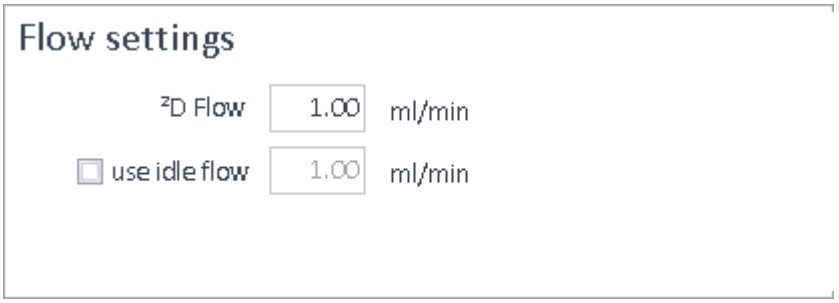


Figure 51 Flow settings

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.

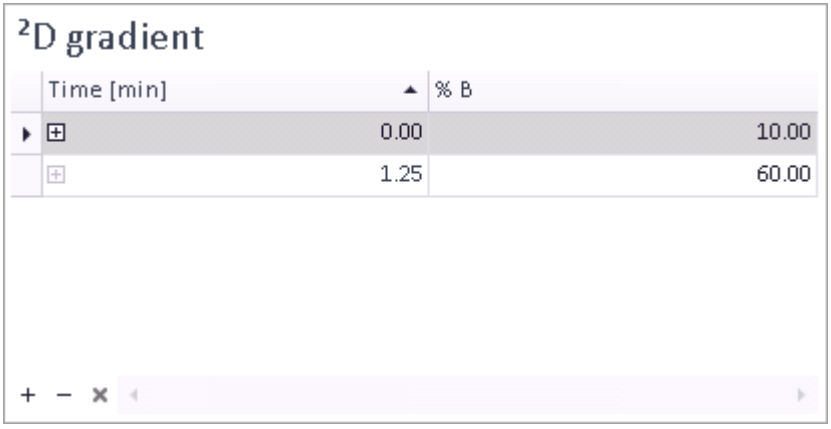
Percent B ranges from 0 – 100 %.

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.

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.

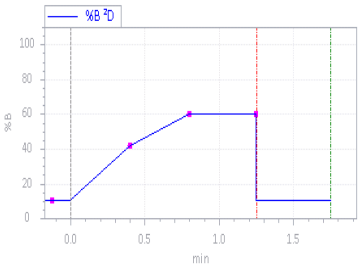


Figure 52 ^{2}D Gradient window in edit mode

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 6 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 43 on page 91.

- **Peak based**

  The peak detector is enabled at the specified time. For details see Figure 43 on page 91.

- **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).

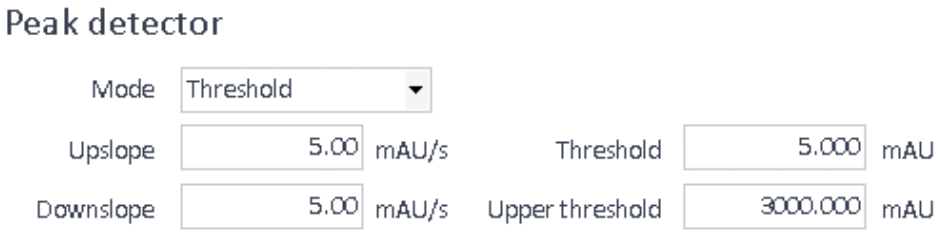
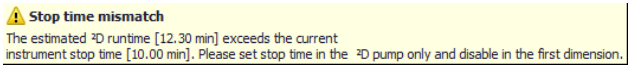


Figure 53 Overview on Peak detector parameters

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 148) 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.

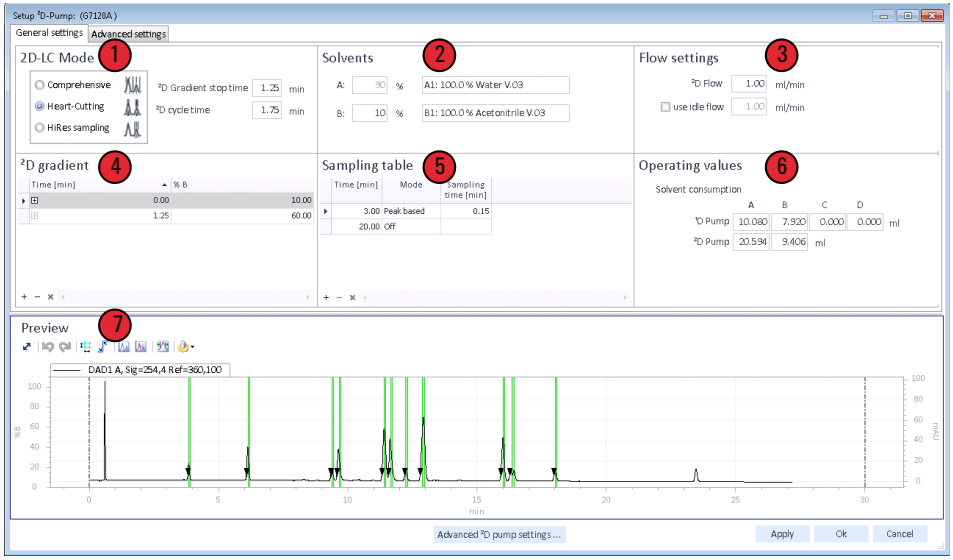


Figure 54 2D-LC method setup (General settings shown in Heart-Cutting mode as an example)

The setup of following method parameters is available:

1 **2D-LC Mode**, see "Set 2D-LC Mode Multiple Heart-Cutting" on page 105
2 **Solvents**, see "Set solvents" on page 109
3 **Flow settings**, see "Set flow" on page 110
4 **2D Gradient**, see "Set Solvent Composition Gradient" on page 111
5 **2D Time segments**, see "Set 2D Time Segments (Multiple Heart-Cutting Only)" on page 113
6 **Operating values**, see "Define Peak Detector Parameter" on page 118
7 **Gradient preview** with toolbar, see "Gradient Preview Functionality" on page 120.

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 43 on page 91):

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).

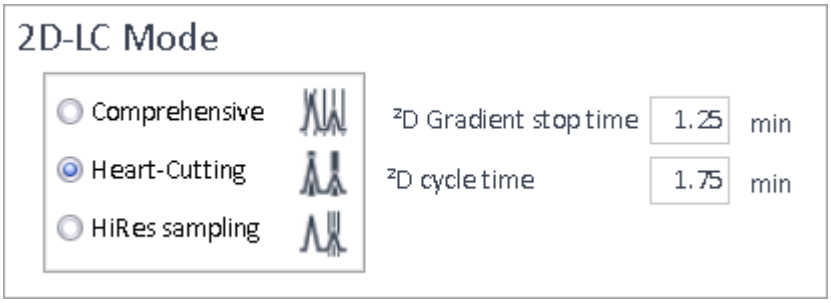


Figure 55 2D-LC ModeHeart-Cutting

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

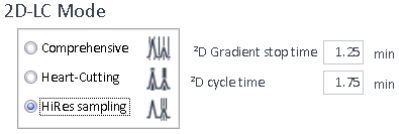
Considerations for high-resolution sampling 2D-LC

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 56 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 148).

Set flow

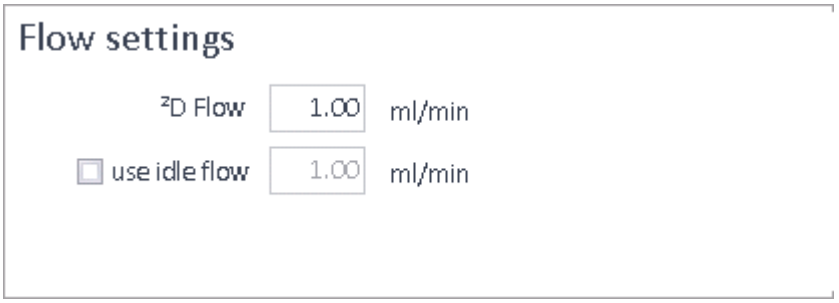


Figure 57 Flow settings

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.

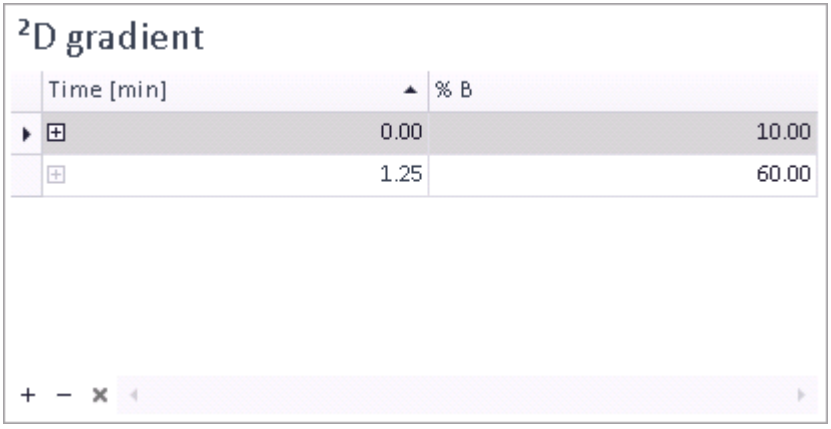
Percent B ranges from 0 – 100 %.

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.

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.

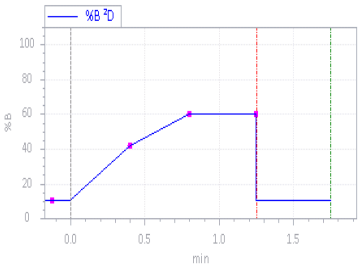


Figure 58 ^{2}D Gradient window in edit mode

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 7 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 43 on page 91.

- **Peak based**

  The peak detector is enabled at the specified time. For details see Figure 43 on page 91.

- **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 $\frac{5\ \mu L}{500\ \mu L/\ min} = 0.6\ s$

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:

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

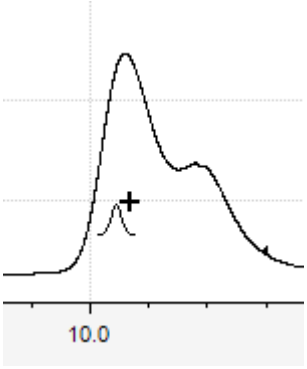
NOTE

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.

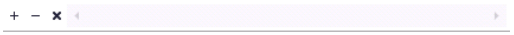


OR

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

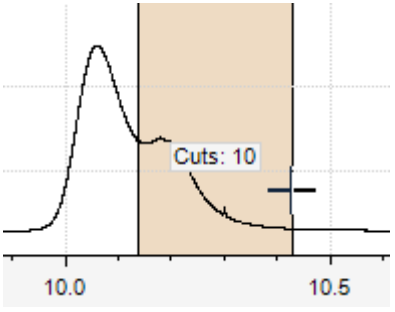
Sampling table

Time [min]	Mode	Sampling time [s]	Cuts	Loop filling
11.15	Time based	2.50	10	62

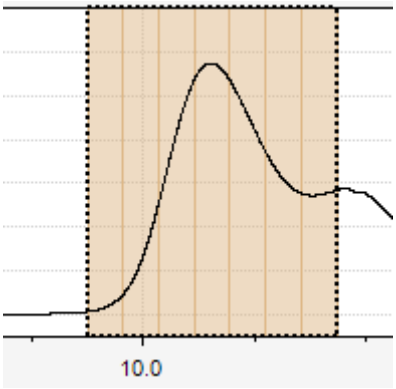


OR

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).

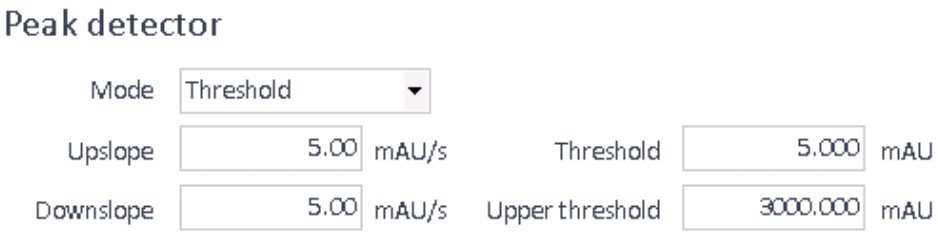
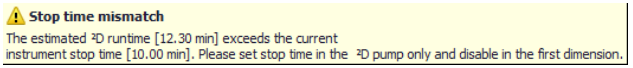


Figure 59 Overview on Peak detector parameters

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 148) 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.

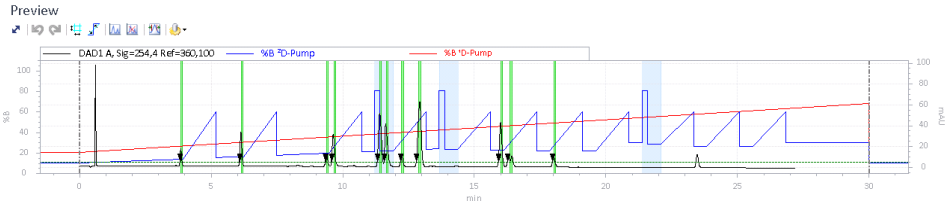


Figure 60 Gradient preview

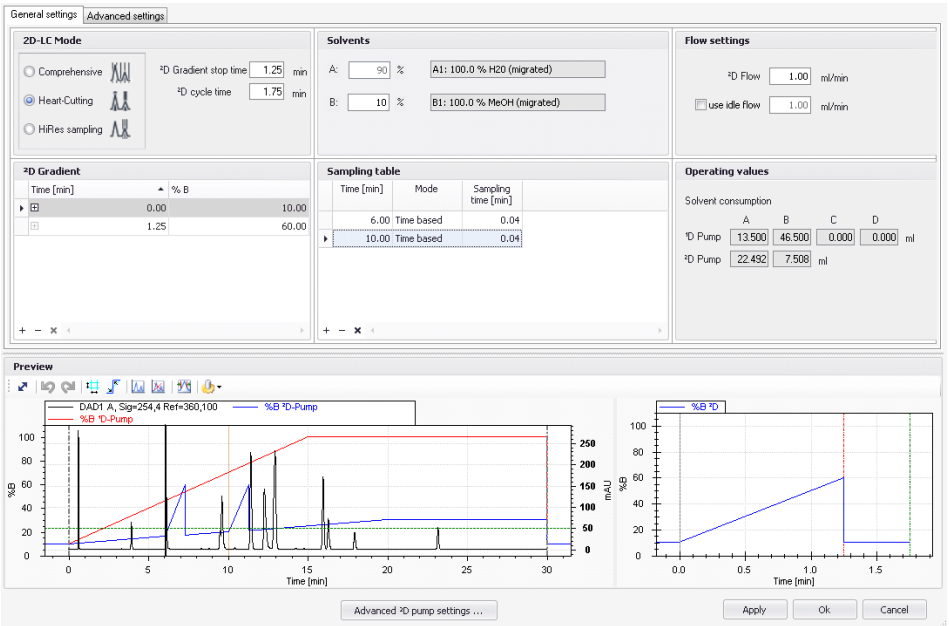
The reference signal offers the following:

- 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 118)
- 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

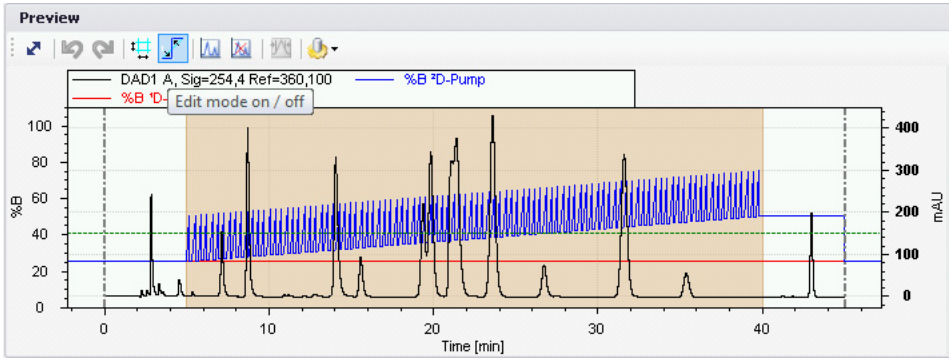
- **Time based** triggering displays peaks in yellow. Sampling time is generated automatically by the thresholds of the peaks.

- **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 111

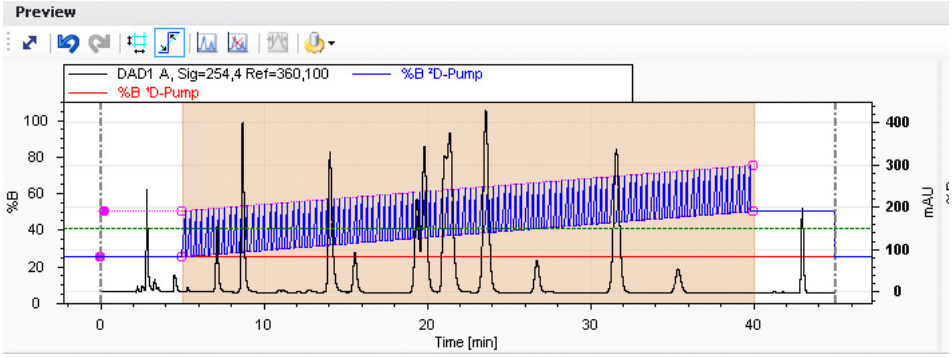
Setup Second Dimension Gradient with the Graphical User Interface

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

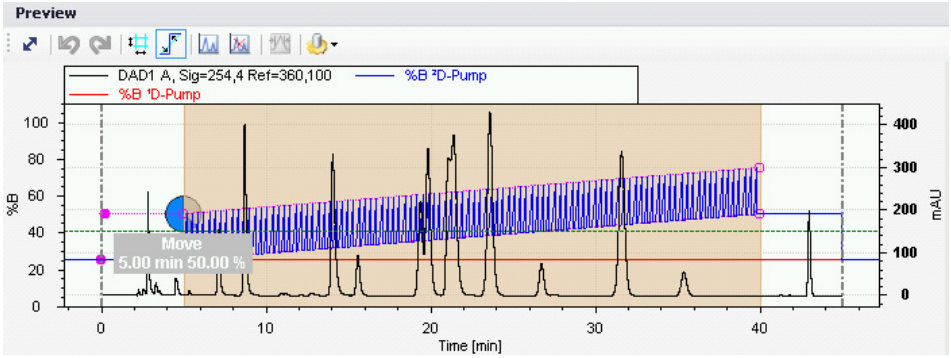
1 To enter the editing mode, use the context menu.



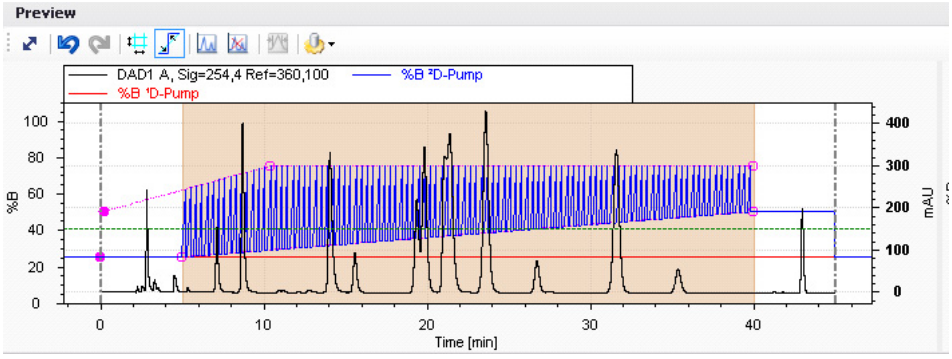
2 Click into the menu. Timetable entries are marked with circles.



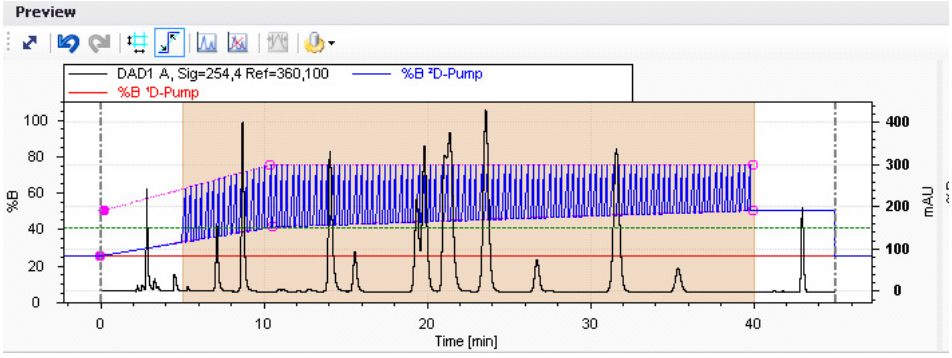
3 Drag the mouse to a new %B value at a specified runtime of the 1st dimension. This draws a straight line.



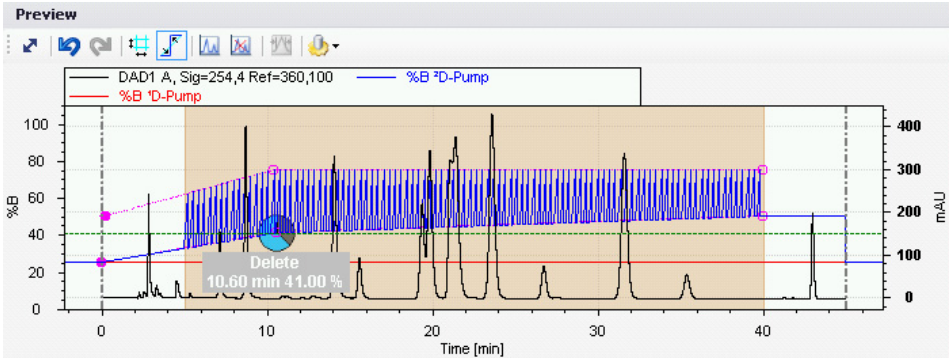
4 When releasing the mouse, a new timetable entry is made and the gradient rollout is automatically updated.



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



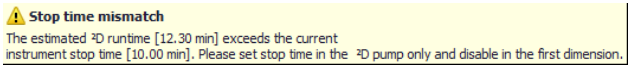
6 Move the mouse cursor near to a shift line to change menu context and insert or delete shift points as needed.



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.

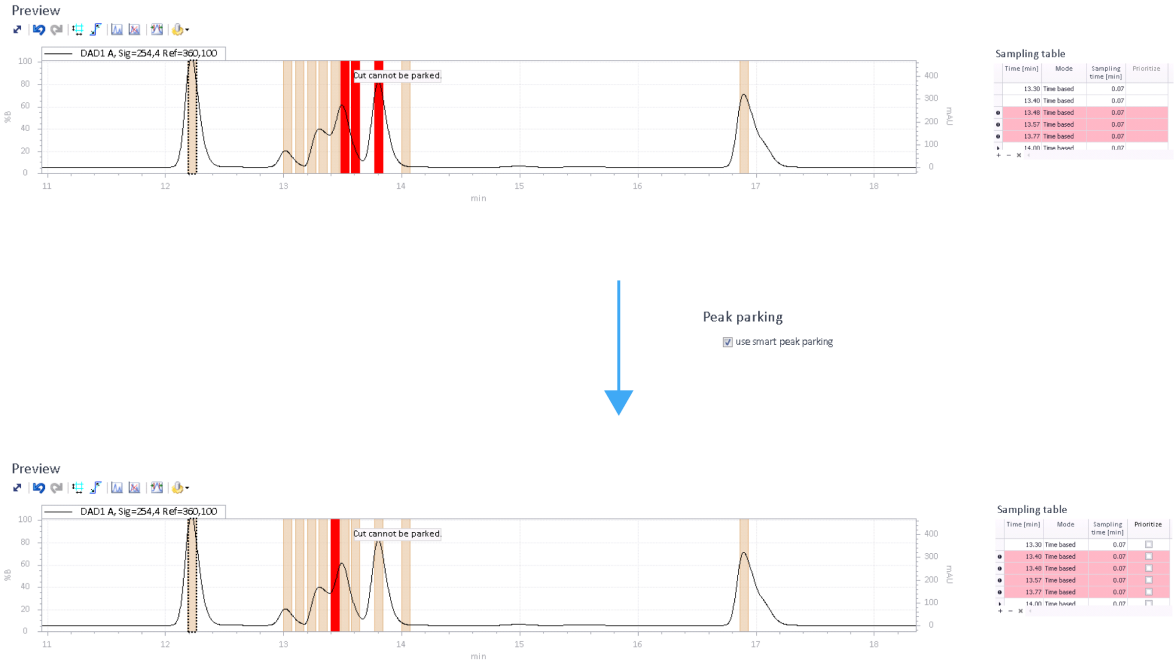
Multiple Heart-Cutting extends this run time automatically, as analyzing parked peaks takes usually longer than the 1st dimension run only.

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

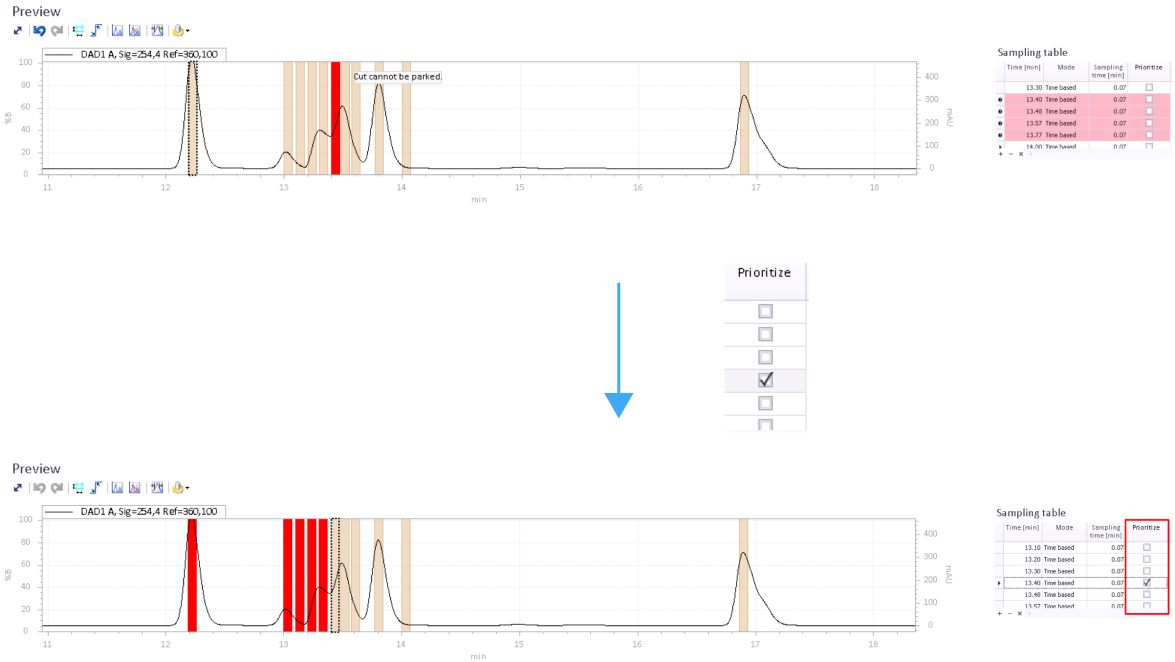
Smart peak parking optimizes parking for all time-based peaks in a reference signal.



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.

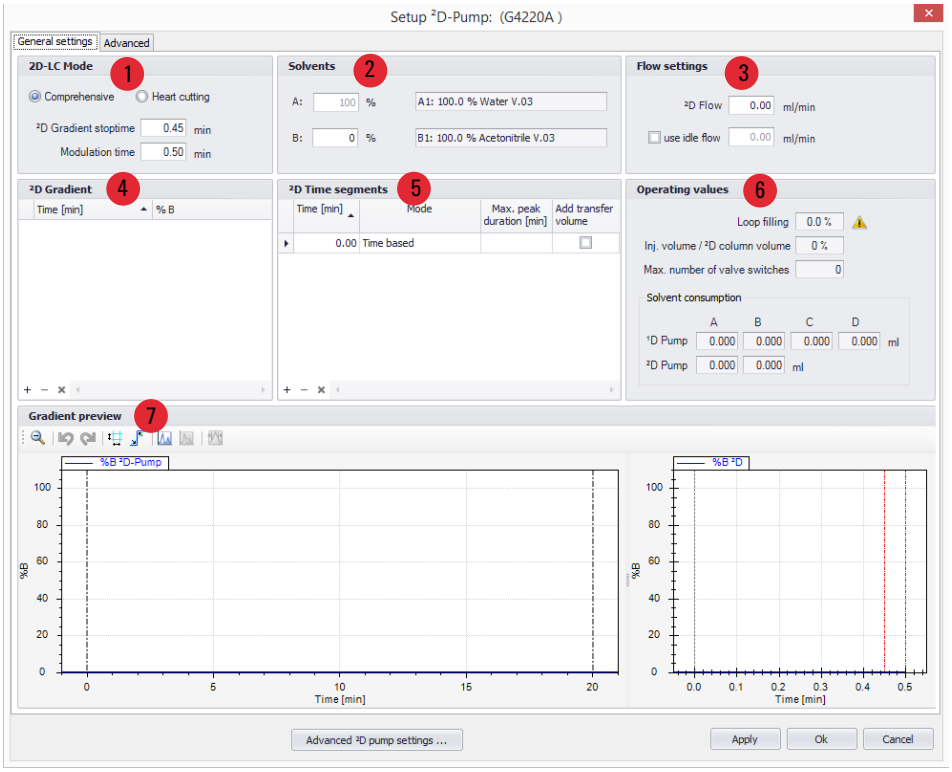


Figure 61 2D-LC method setup (General settings)

The setup of following method parameters is available:

1 **2D-LC Mode**, see "Set 2D-LC Mode" on page 89
2 **Solvents**, see "Set solvents" on page 129
3 **Flow settings**, see "Set flow" on page 130
4 **²D Gradient**, see "Set Solvent Composition Gradient" on page 131
5 **²D Time segments**, see "Set 2D Time Segments" on page 134
6 **Operating values**, see "Define Peak Detector Parameter" on page 136
7 **Gradient preview**, see "Gradient Preview Functionality" on page 137

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.

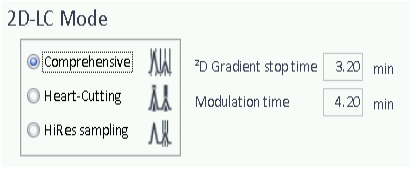


Figure 62 2D-LC Comprehensive mode

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.

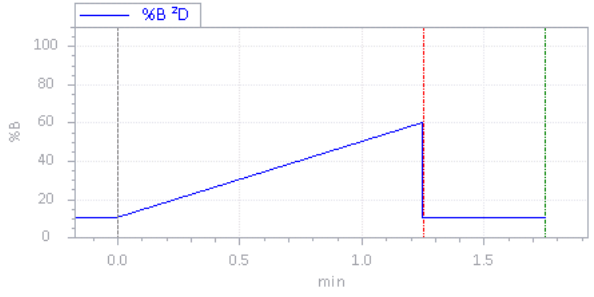


Figure 63 Stop time and Modulation time

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 64 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 148).

Set flow

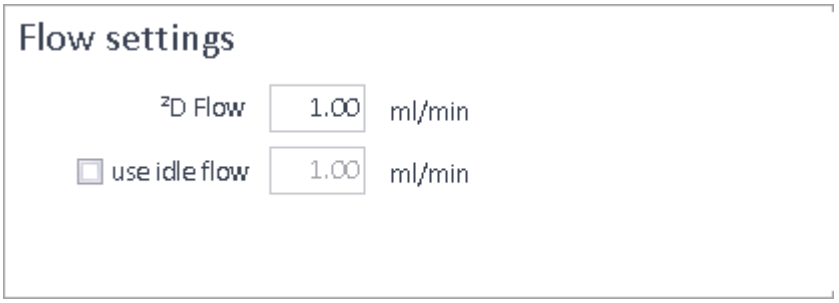


Figure 65 Flow settings

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.

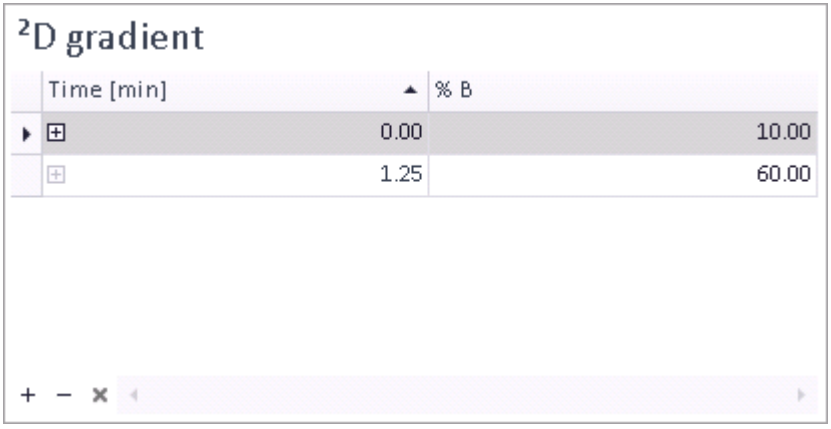
Percent B ranges from 0 – 100 %.

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

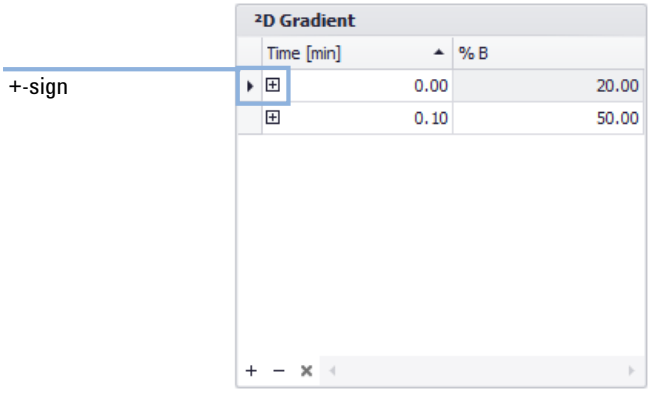
Different start conditions in the first row may cause step gradients and RI-effects (density differences of the different liquid phases may cause different DAD detection through baseline disturbances).



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.



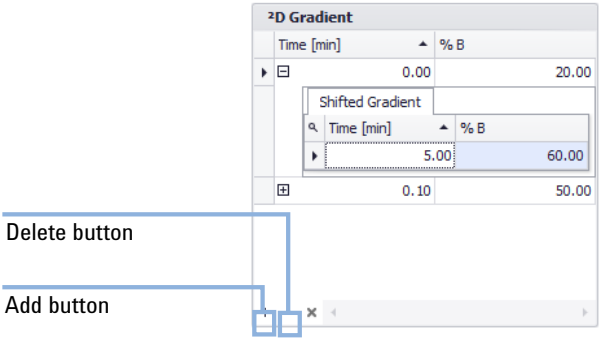
NOTE

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.

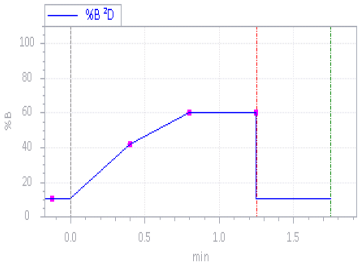


Figure 66 2D Gradient window in edit mode

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 8 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

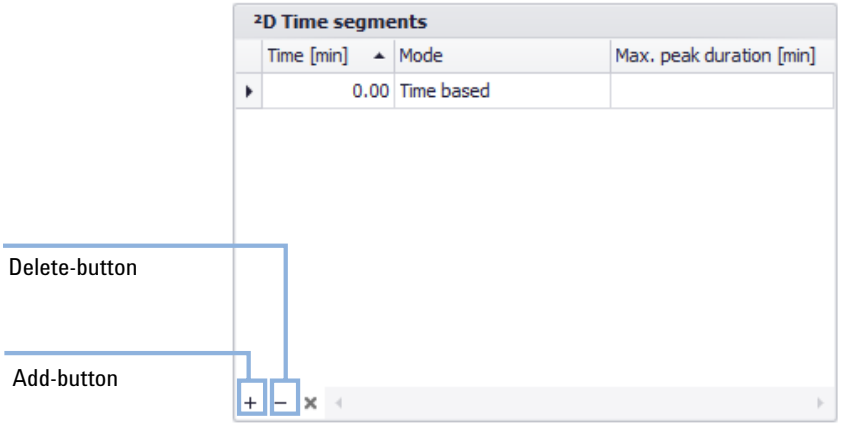
If the **^{2}D Time Segments** table is empty, no 2D-LC operation will be executed at all.

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).

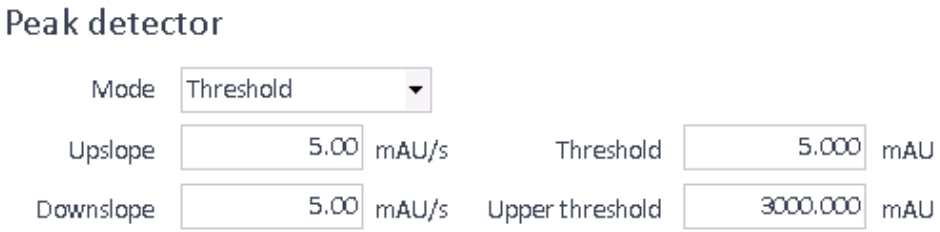
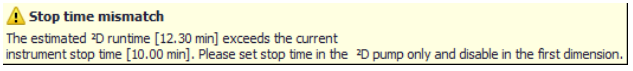


Figure 67 Overview on Peak detector parameters

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 148) 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.

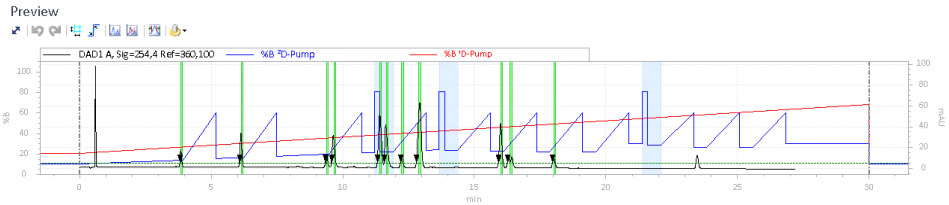


Figure 68 Gradient preview

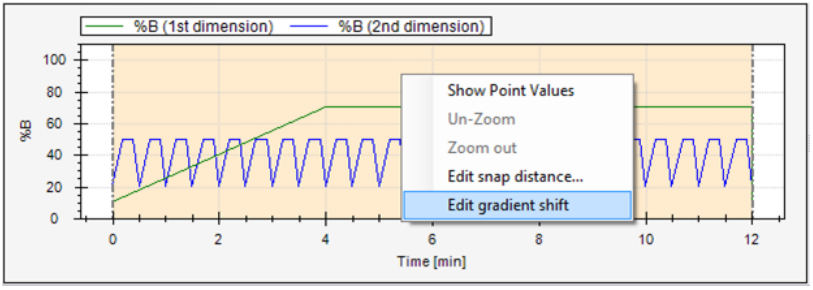
The reference signal offers the following:

- 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)

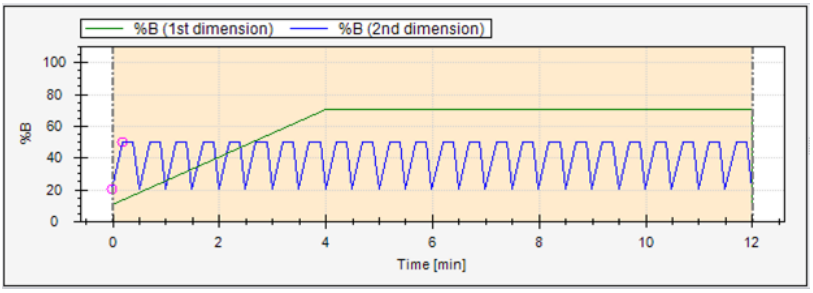
Setup Second Dimension Gradient with the Graphical User Interface

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

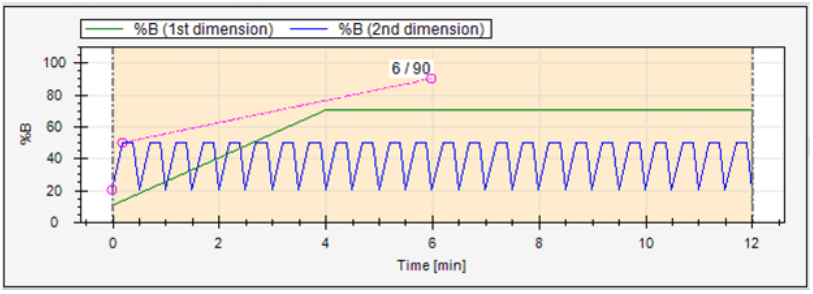
1 To enter the editing mode, use the context menu.



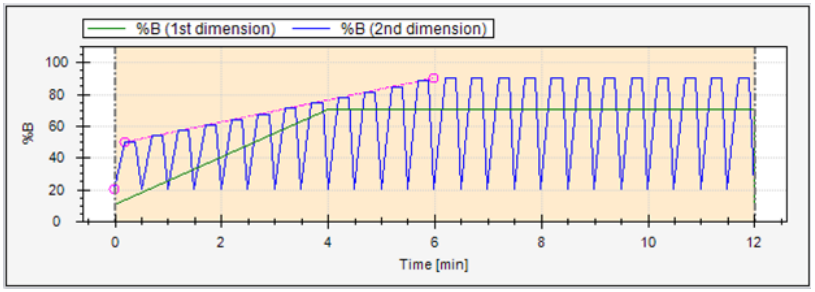
Timetable entries are marked with circles.



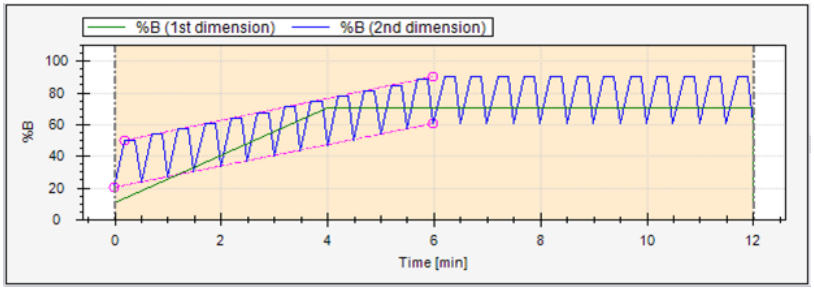
2 Drag the mouse to a new %B value at a specified runtime of the 1st dimension.



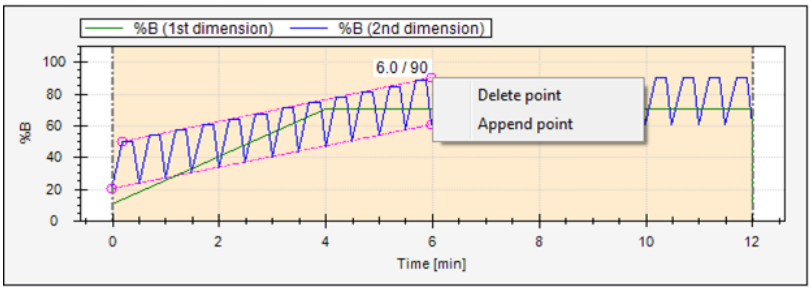
This draws a straight line. When releasing the mouse, a new timetable entry is made and the gradient rollout is automatically updated.



3 Repeat step 2 on page 139 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 142), execute the steps in the following order:

1. "Optimizing the dilution by using ASM capillaries" on page 143
2. "Optimizing the sample loop flush" on page 143
3. "Including the ASM phase to the 2D gradient" on page 144
4. "Optimizing dilution through method settings" on page 145

Method parameters

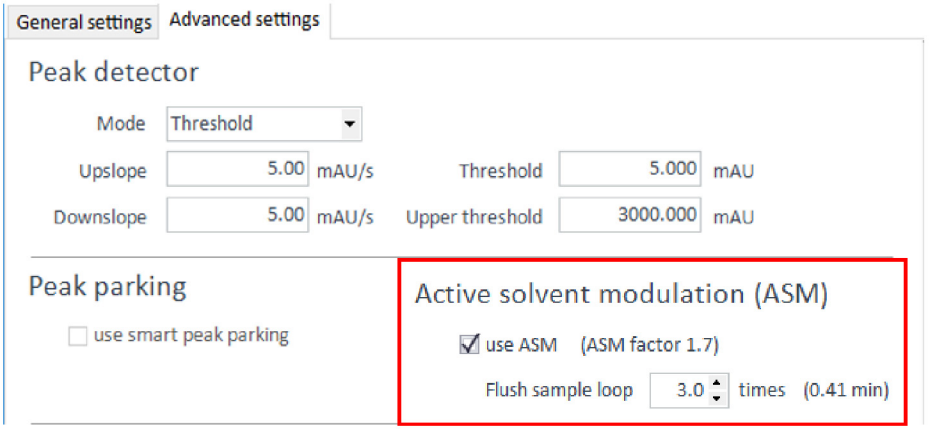


Figure 69 Method parameters for the ASM Valve (example)

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 190) 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.

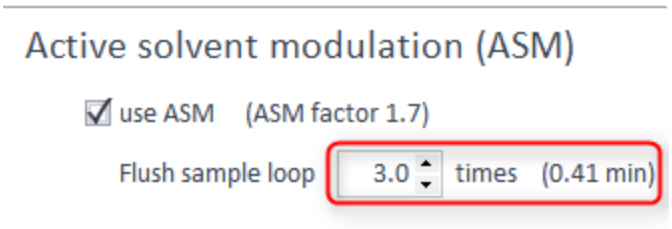


Figure 70 Set Flush sample loop (example)

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

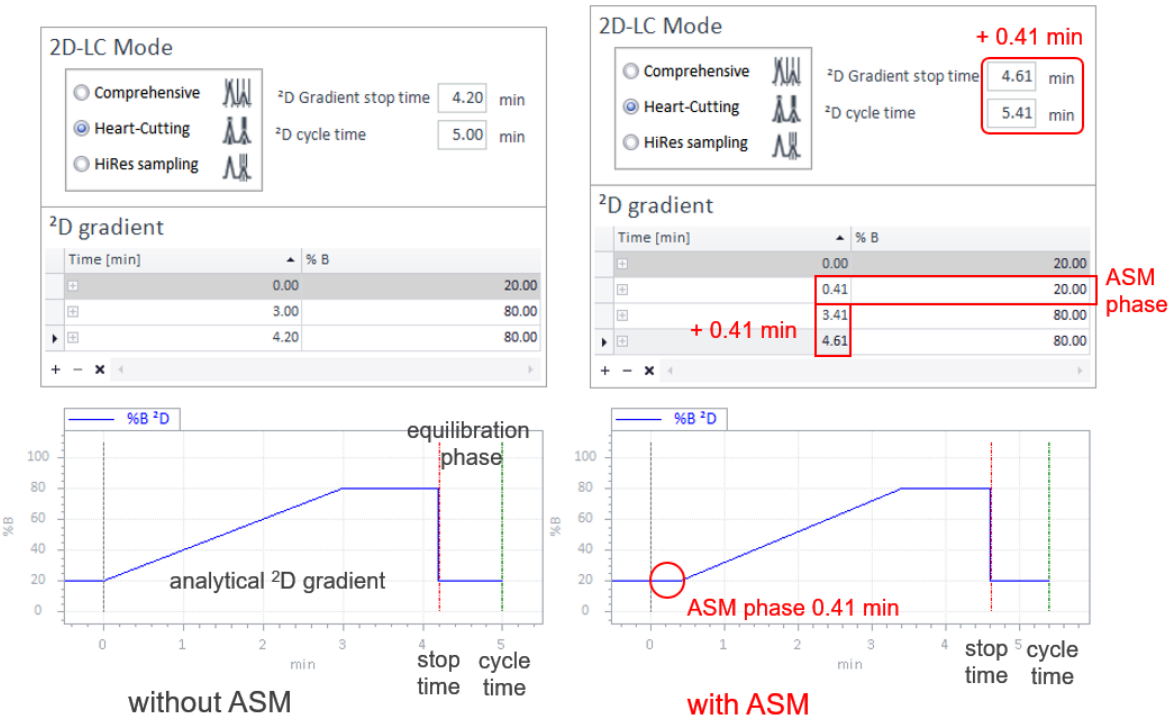


Figure 71 Programming the ^{2}D gradient table (example)

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

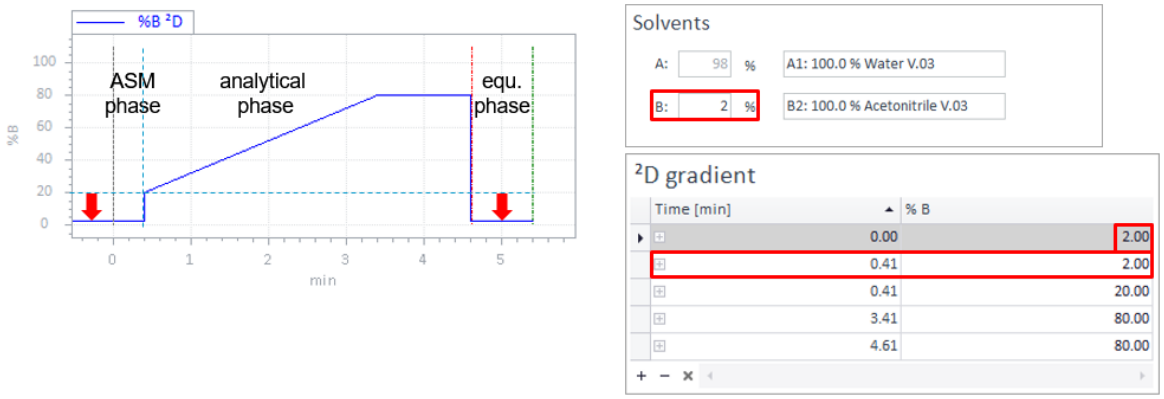


Figure 72 Optimizing separation by using a lower percentage of B for the ASM and column equilibration phase (example)

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 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 149
- Multiple heart-cutting

  "Configure Valve and Loop" on page 161
- High resolution sampling
- "Configure Valve and Loop" on page 161
- Comprehensive

  "Configure Valve and Loop" on page 149

Standard Heart-Cutting 2D-LC

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

Configuration

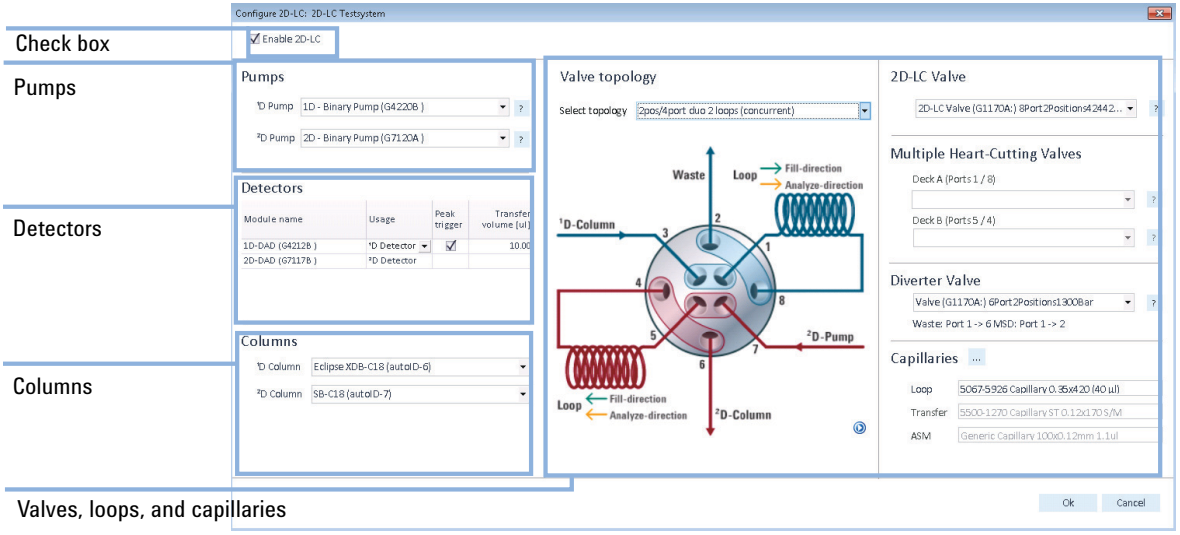


Figure 73 Overview 2D-LC configuration grafical user interface

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.

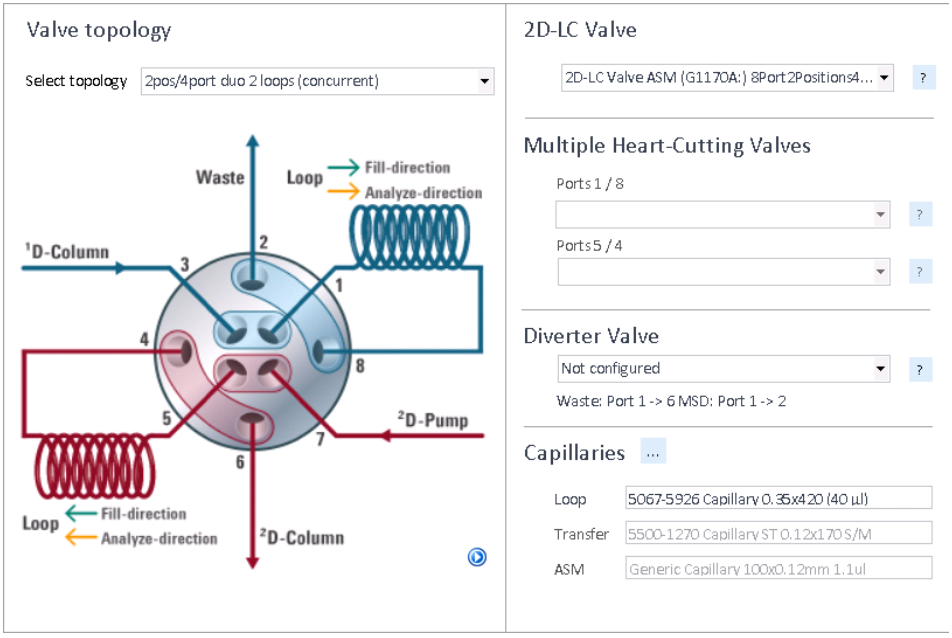


Figure 74 2D-LC valve and loop configuration (concurrent)

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.

Checkout Familiarization Procedure

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 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 60
- 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 9 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 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 60
- 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 10 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 in multiple heart-cutting and high resolution sampling 2D-LC.

Configuration

Overview Configuration Dialog

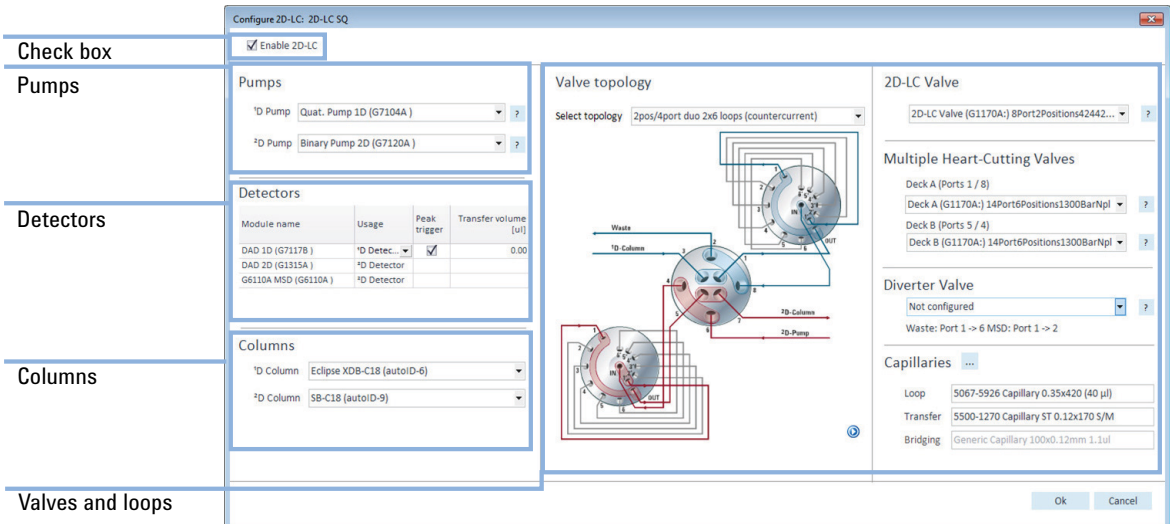


Figure 75 Overview 2D-LC configuration graphical user interface

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.

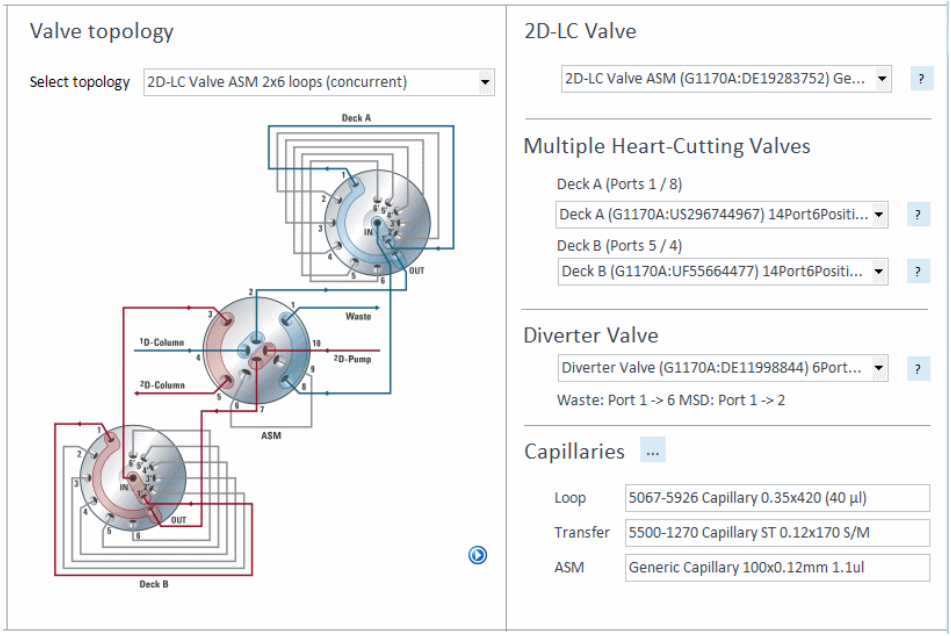


Figure 76 2D-LC valve and loop configuration

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**.

Checkout/Familiarization Procedure

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 61

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 11 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 61

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 12 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 61

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 13 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.

Configuration

Overview Configuration Dialog

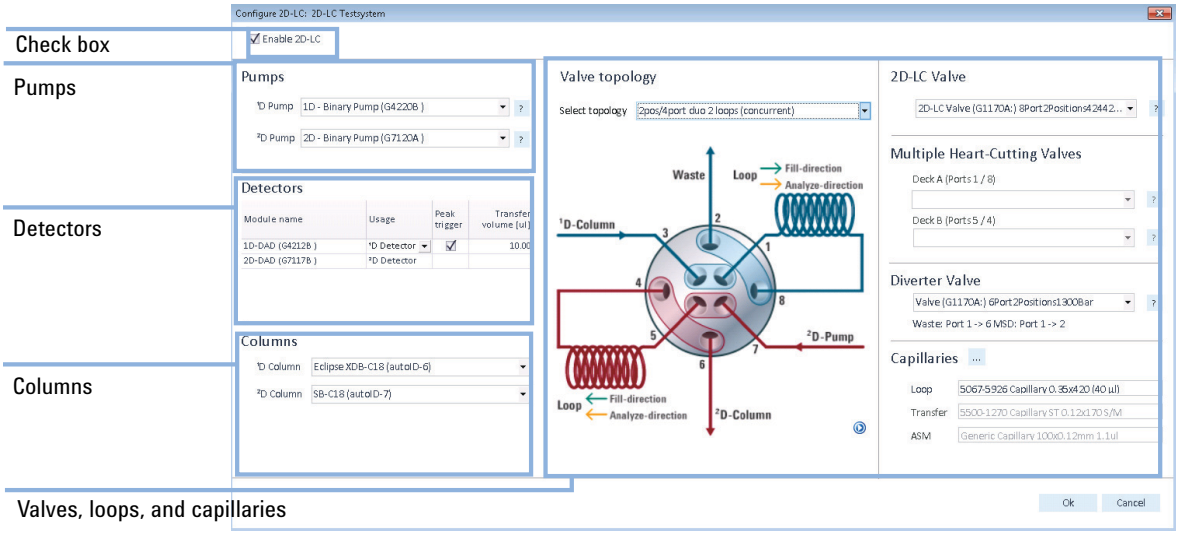


Figure 77 Overview 2D-LC configuration grafical user interface

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.

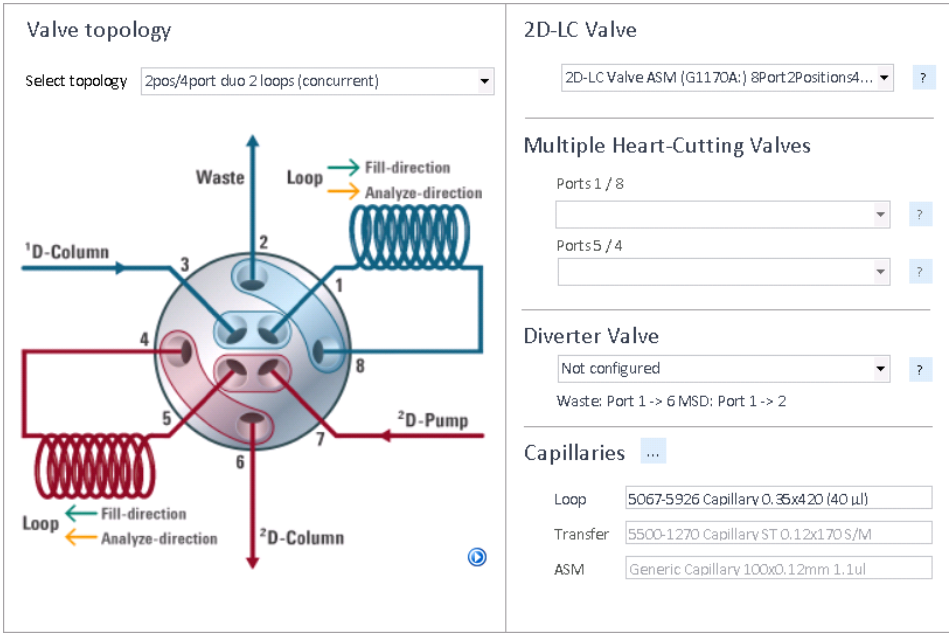


Figure 78 2D-LC valve and loop configuration (concurrent)

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.

Checkout/Familiarization Procedure

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 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

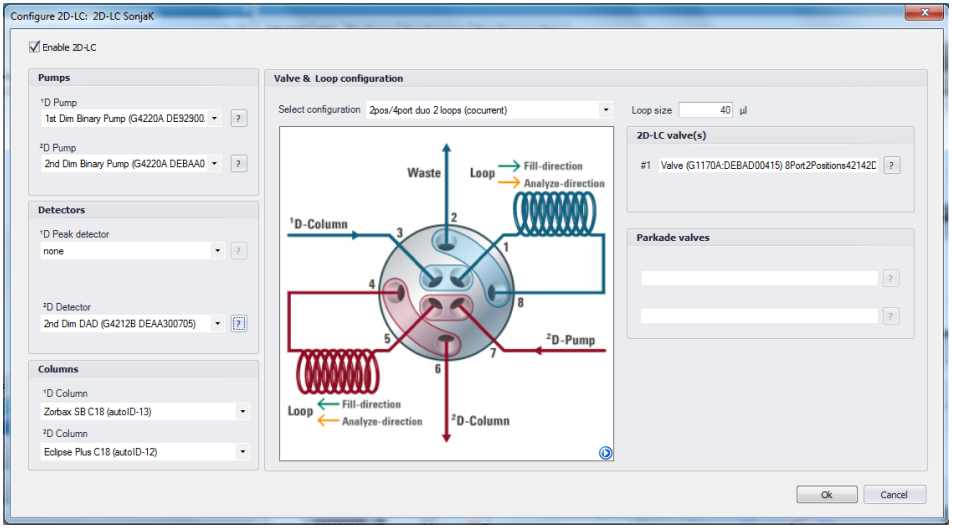
- Sample:

  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.

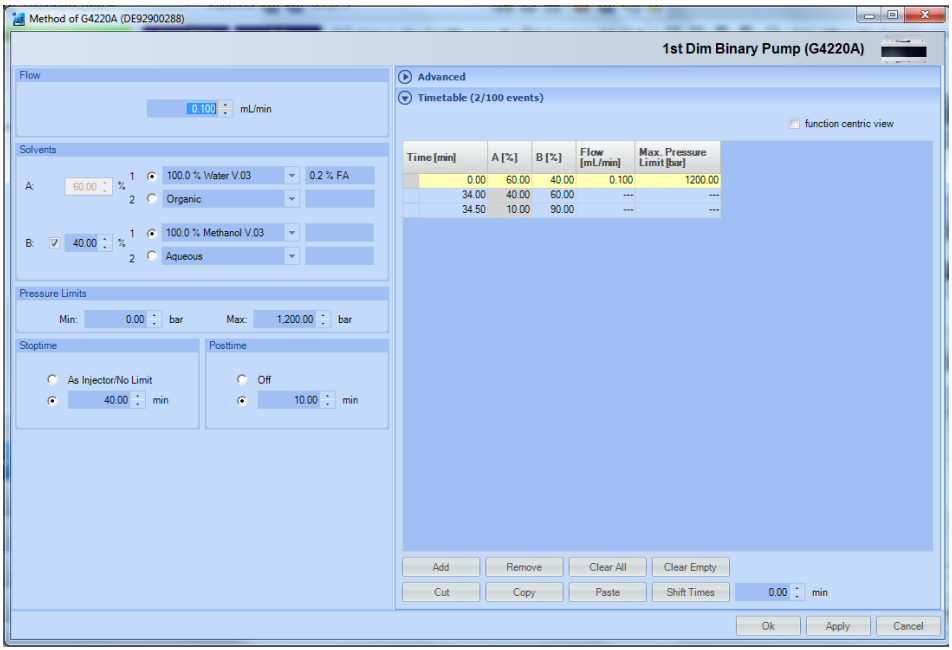
- Injection volume:

  2 µL

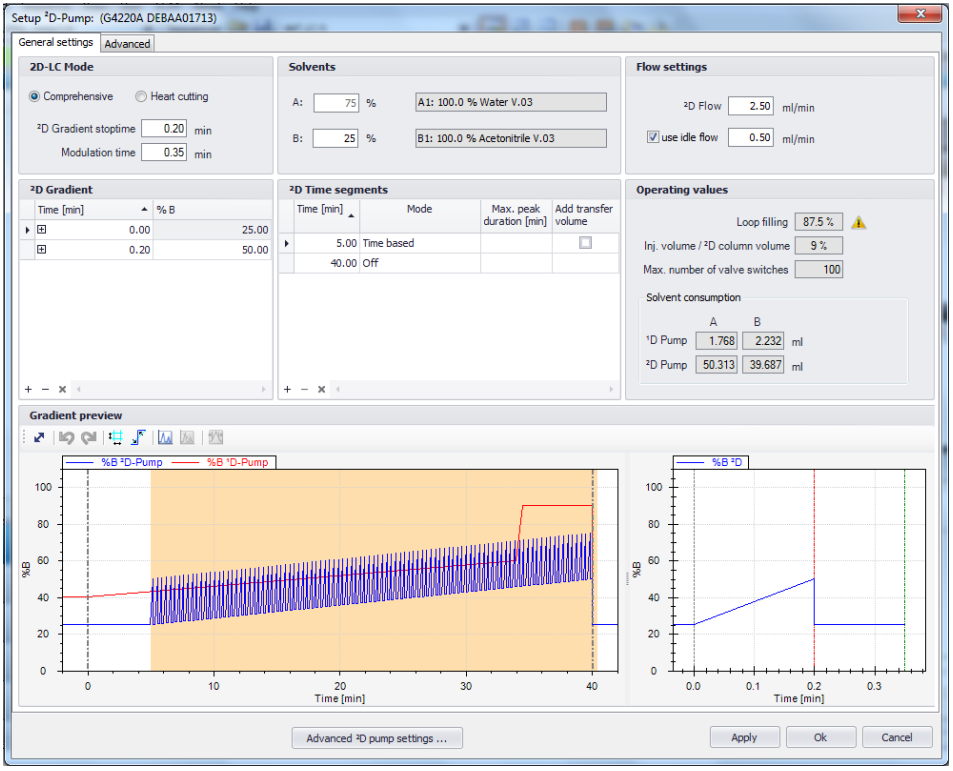
2 Verify that the Valve **&** Loop configuration is set up as shown below:



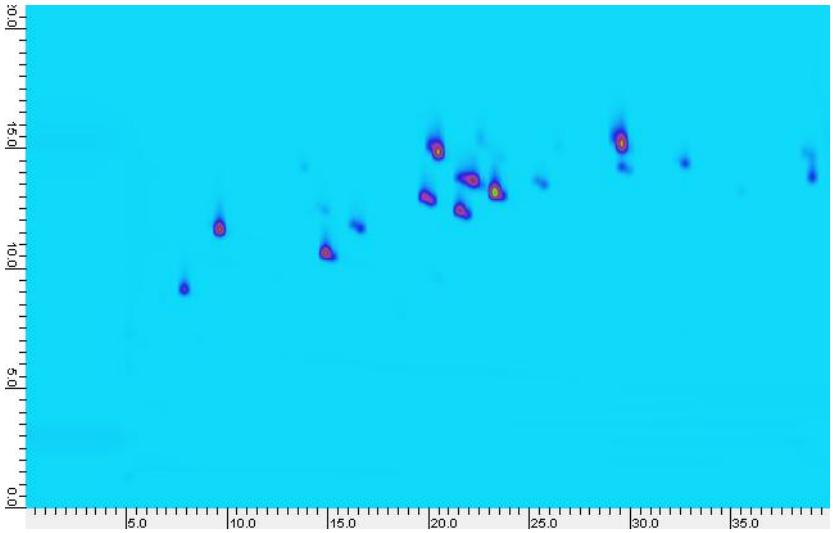
3 Verify the ^{1}D pump method is set up as shown below:



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 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:

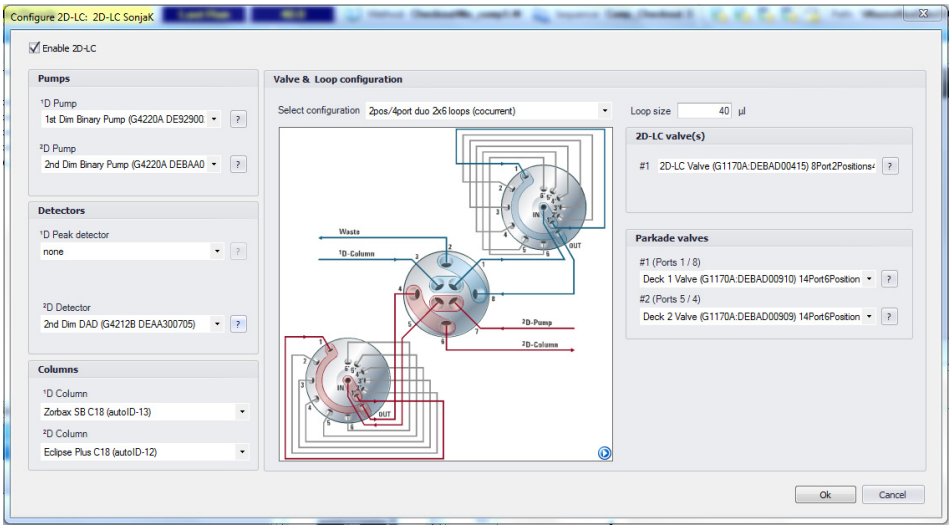
- 1D: mobile phase A = water with 0.2 % Formic Acid-Reagent Grade 5 mL (5 cc) (G2453-85060), B = methanol
- 2D: mobile phase A = water with 0.2 % Formic Acid-Reagent Grade 5 mL (5 cc) (G2453-85060), B = acetonitrile

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

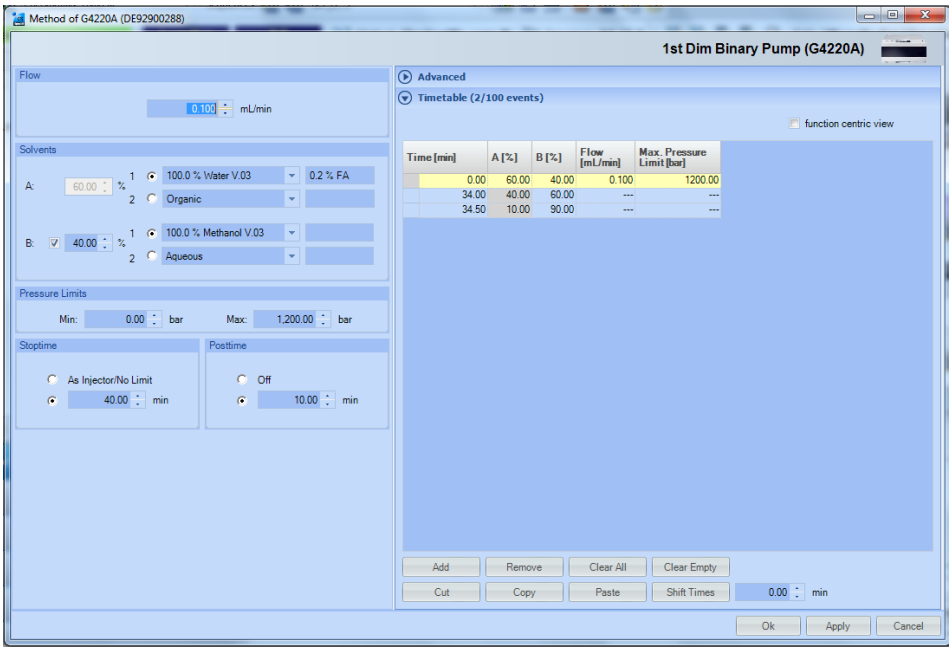
- Sample:

  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.

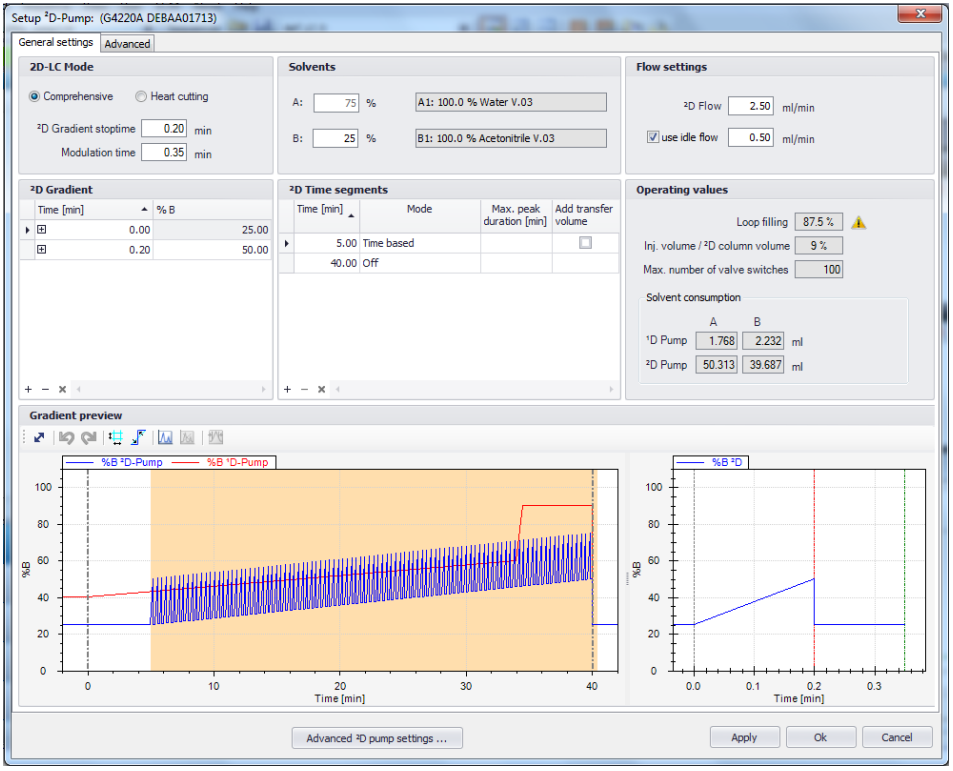
- Injection volume:

  2 µL

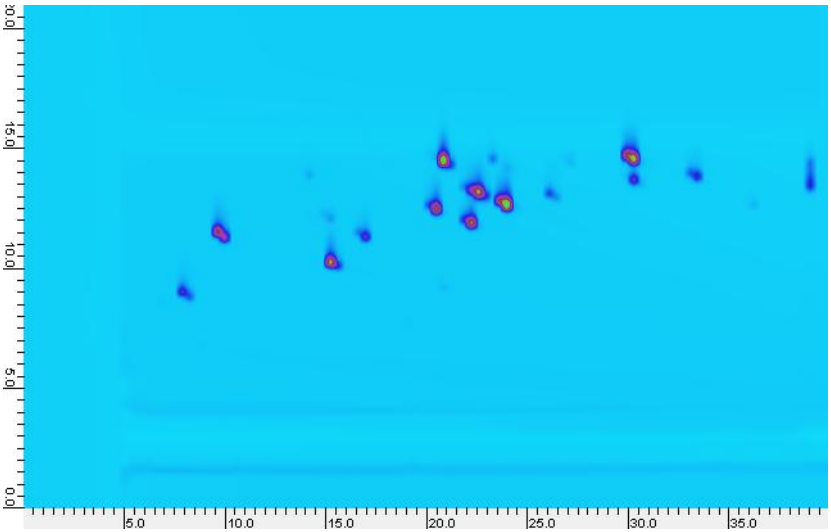
3 Verify the ^{1}D pump method is set up as shown below:



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

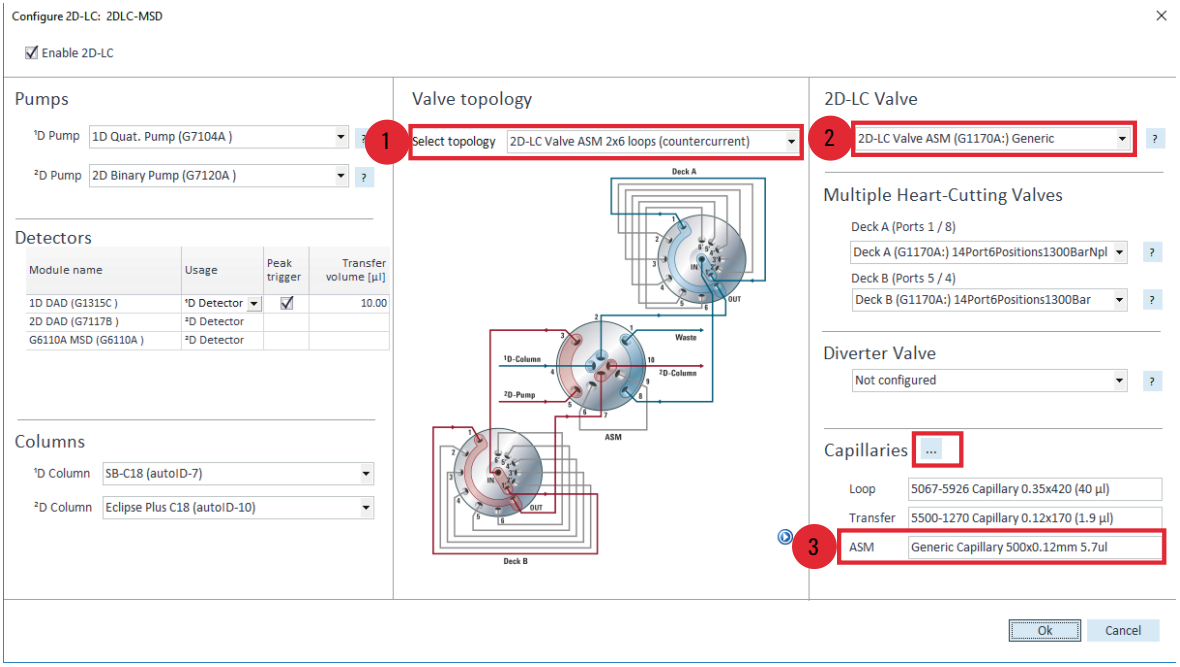


Figure 79 ASM Valve configuration (overview)

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 79 on page 190).

b Select any of the pre-defined ASM capillaries.

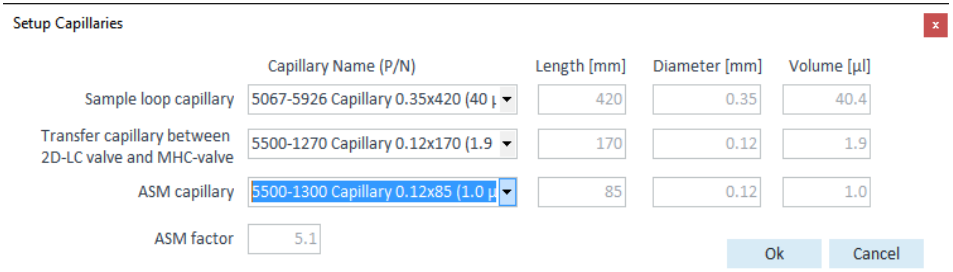


Figure 80 Configuration of the ASM valve with predefined capillaries

OR

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

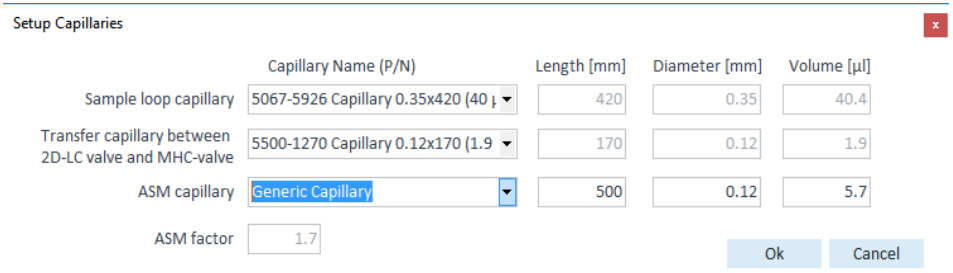


Figure 81 ASM valve configuration (overview)

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 79 on page 190.

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

NOTE Please note that port positions given in the MHC valve configuration in 2D-LC Software A.01.04 refer to standard 2D-LC valves, not ASM. Please use correct ports displayed figure **Valve topology**. This will be corrected in 2D-LC Software A.01.04 SR1.

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

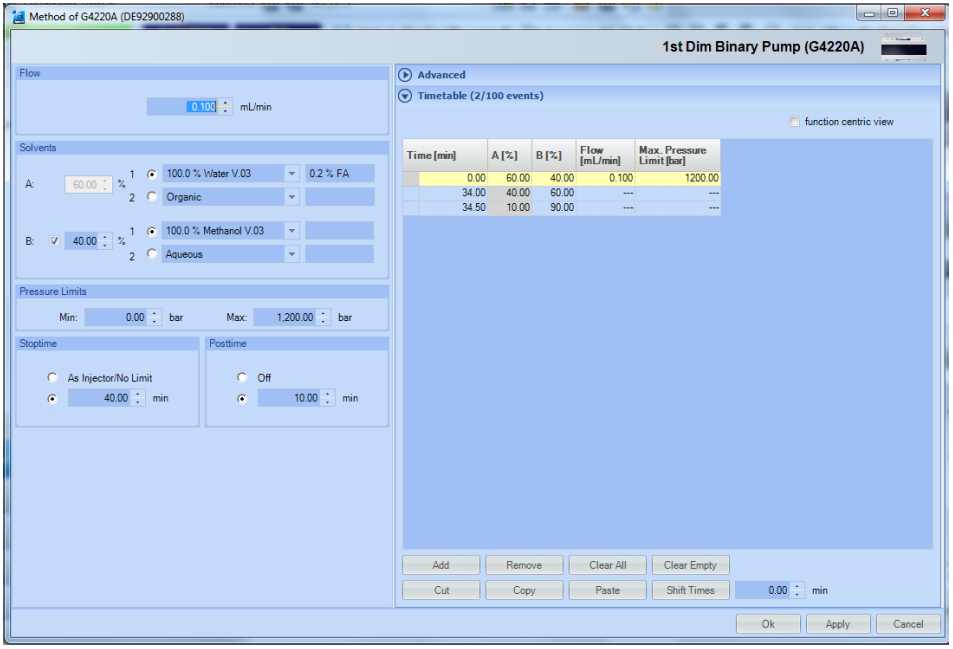
This chapter describes, how shifted gradients in the second dimension can be used to enlarge the accessible two-dimensional separation space.

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

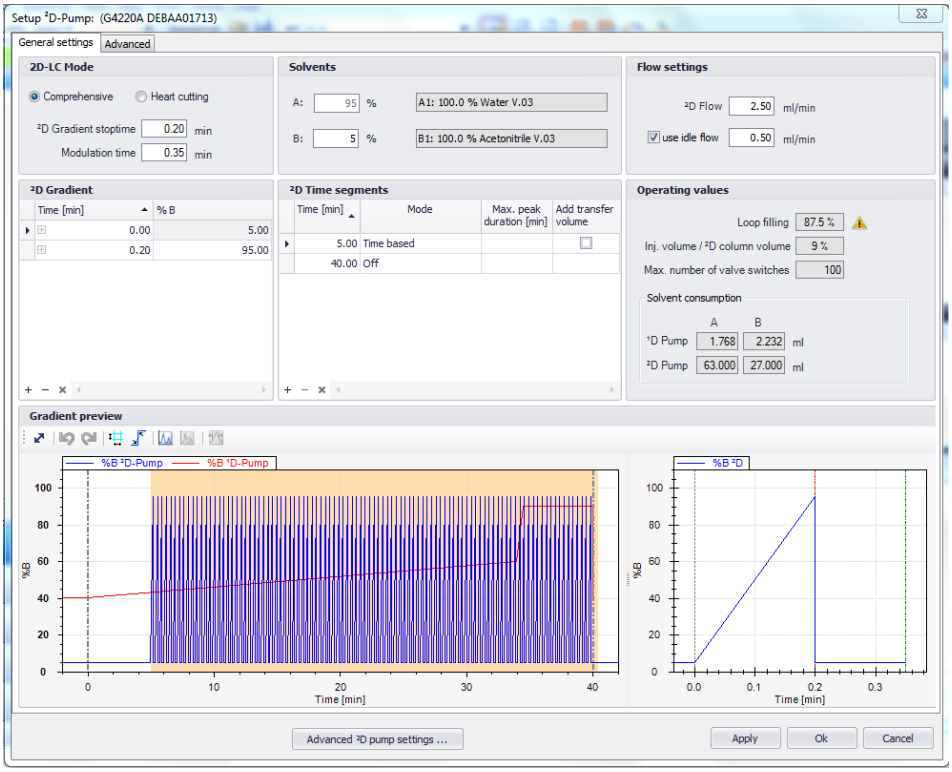
When combining separation systems with related separation mechanisms in the first and second dimension (as in RPxRP), orthogonality is limited. As a result, only a part of the available two-dimensional separation space will be occupied. In such a case, shifted gradients in the second dimension can be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

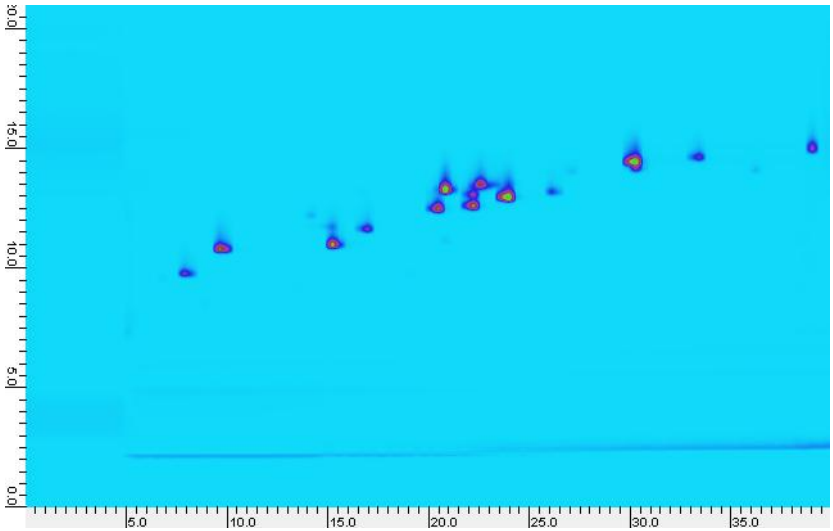
The 1D pump method should be set up as during the checkout runs (see below):



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



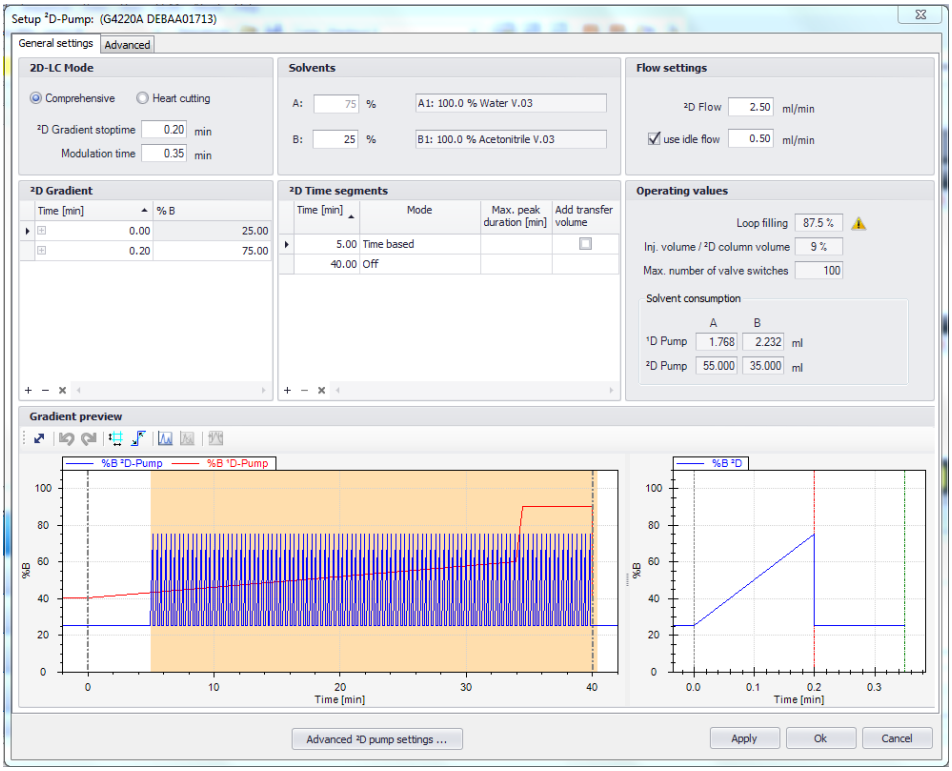
3 Run the comprehensive 2D-LC analysis. The resulting separation should look similar to the one shown below:



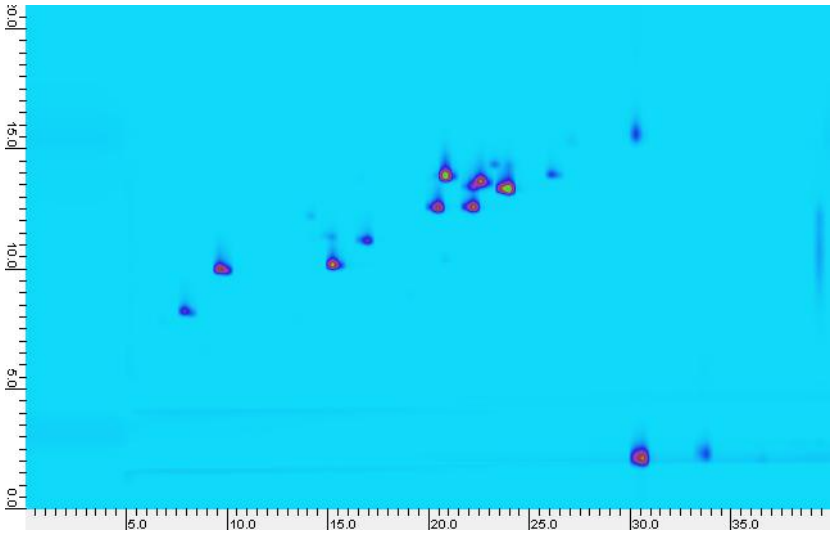
NOTE

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).

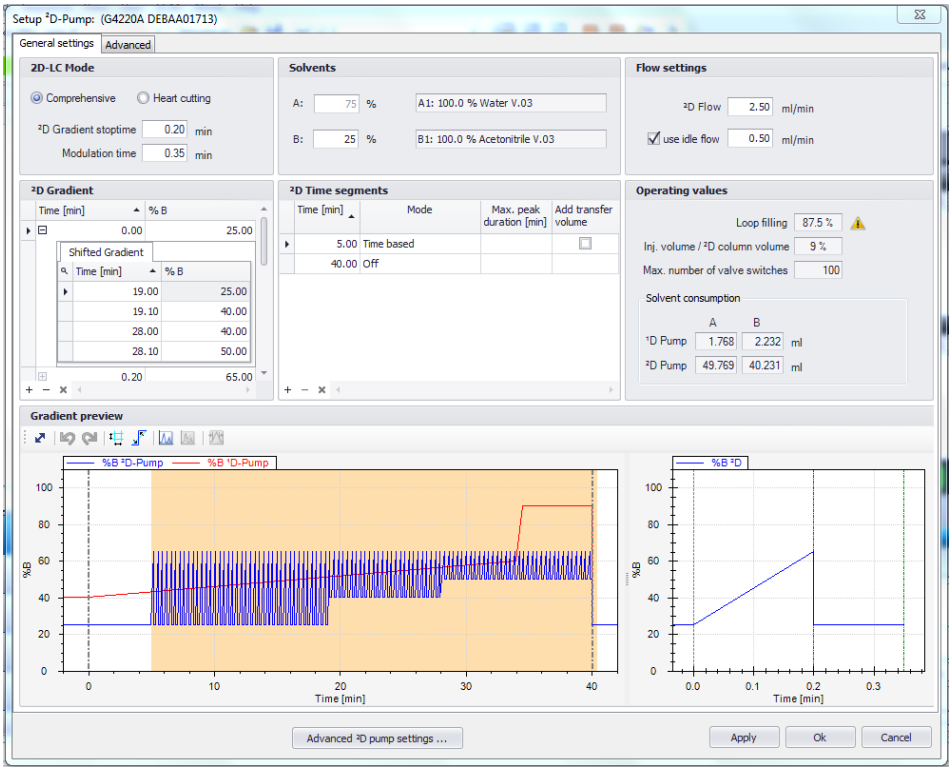
To be able to use even shallower gradients in the second dimension to further improve the separation and to also avoid the occurrence of wrap-around, continuously shifted gradients can be used in the second dimension (as was done during the checkout runs).

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

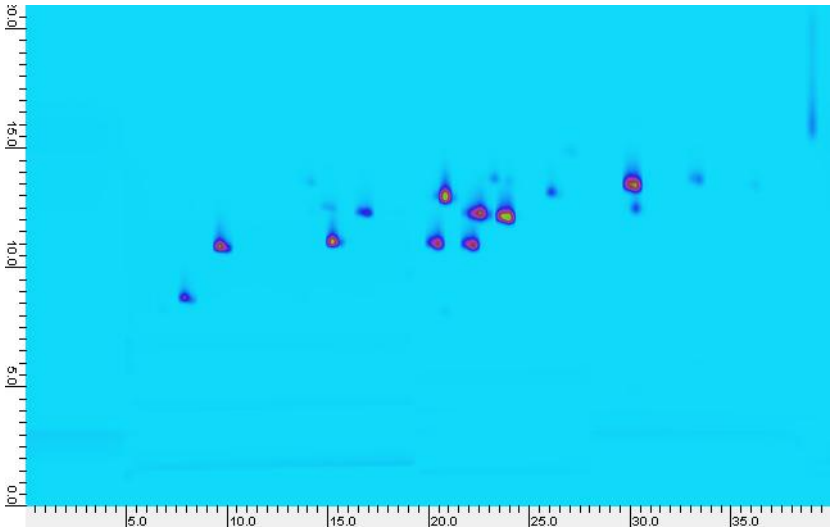
Notice how the peaks are spread more widely across the two-dimensional separation space (the accessible two-dimensional separation space is enlarged) when shifted gradients are used. Also, notice the effect that using continuously shifted second dimension gradients has on the second dimension retention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

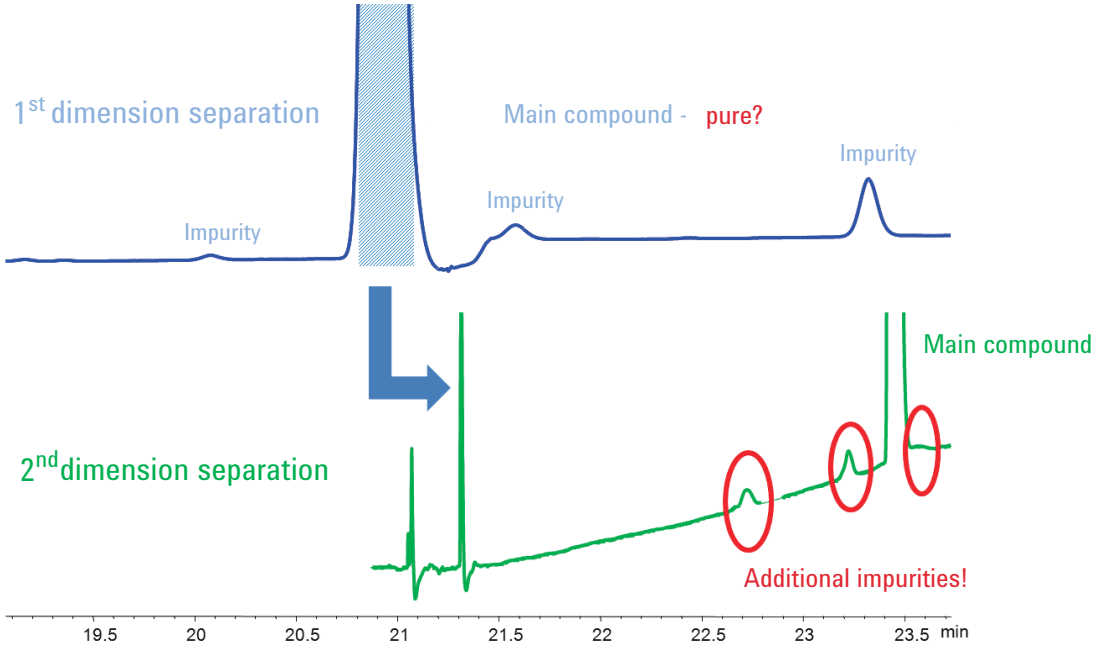


Figure 82 **Example of a heartcutting 2D-LC experiment. Data analysis and display done by OpenLAB CDS ChemStation Edition, the data of a detector placed after the first dimension column (blue) and of the 2D data detector (green). The additional impurities (marked red) could not be detected by 1D-LC only.**

2D-LC Viewer

Using the Multiple Heart-Cutting Upgrade kit, the Agilent 1290 Infinity II 2D-LC Solution 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 83 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.

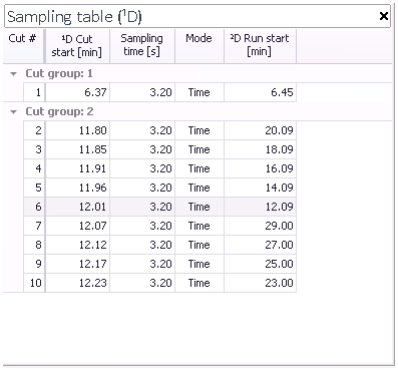


Figure 84 Sampling table (^{1}D) (example)

The different columns can be selected or deselected by right mouseclicking the headline of the table.

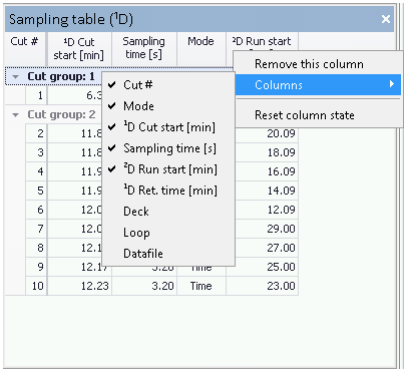


Table 14 Legend for Sampling Table

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

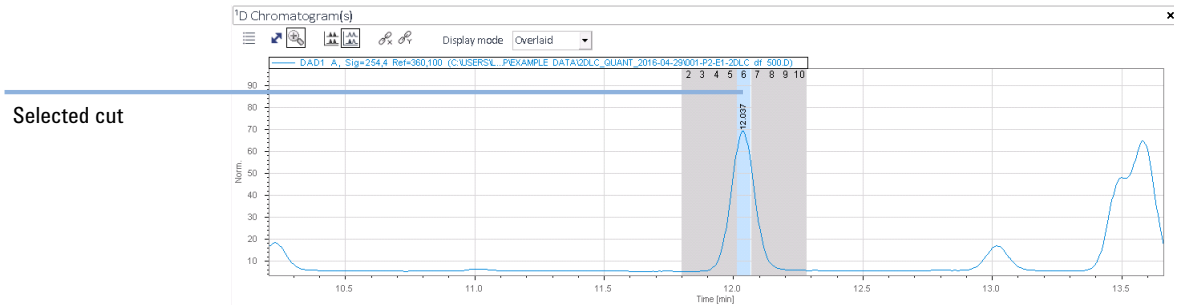


Figure 85 ^{1}D Chromatogram (example)

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

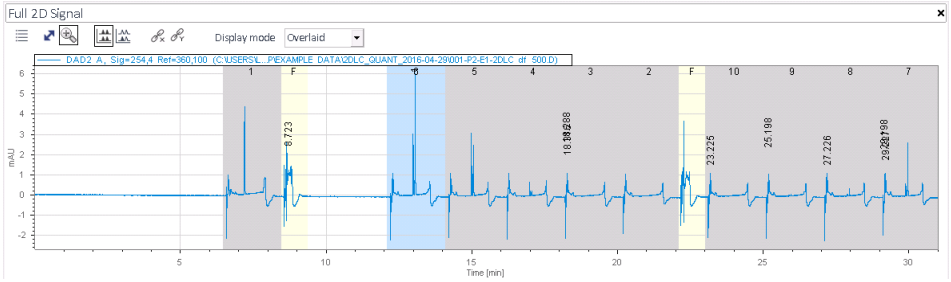


Figure 86 Full ^{2}D Signal (example - only visible, if Full ^{2}D Signal (checkbox) is checked)

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.

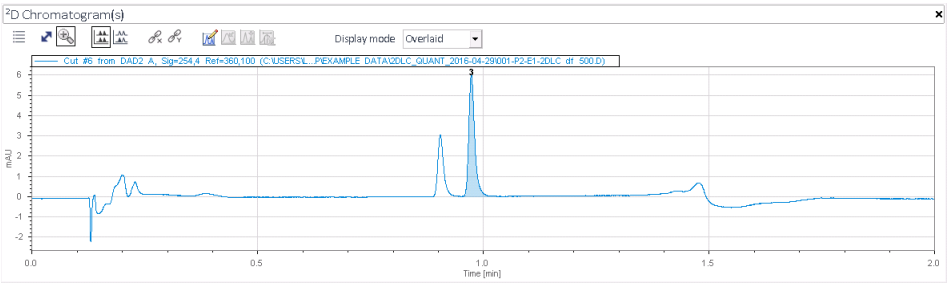


Figure 87 ^{2}D Chromatogram (example from ^{2}D Chromatogram above)

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 88 Results peak group 1

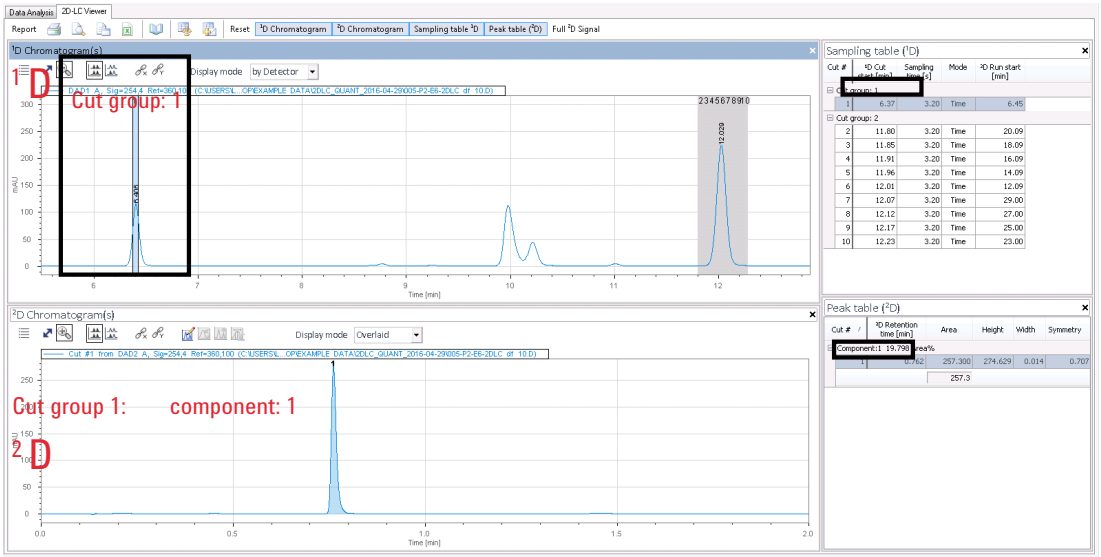


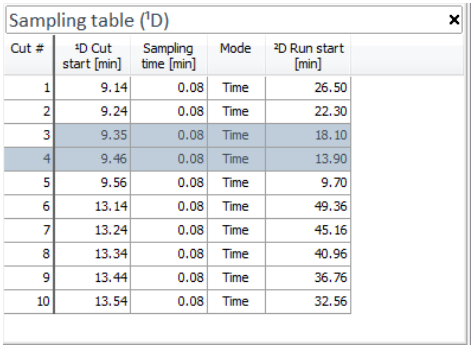
Figure 89 Results peak group 2

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 90 Sampling table

1	First level: All signals for all runs
2	Second level: Peak groups
3	Third level: Cuts numbered continuously per signal

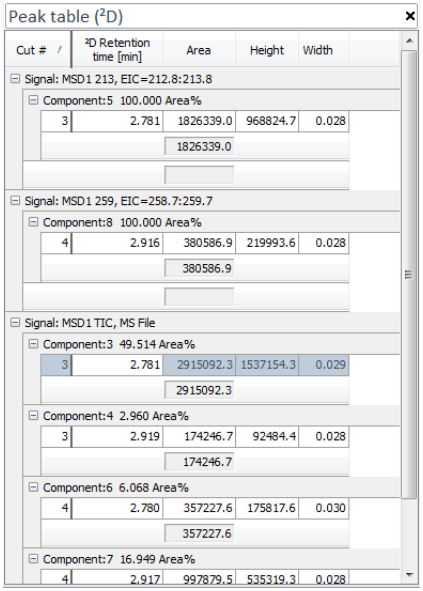


Figure 91 Peak Table

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.

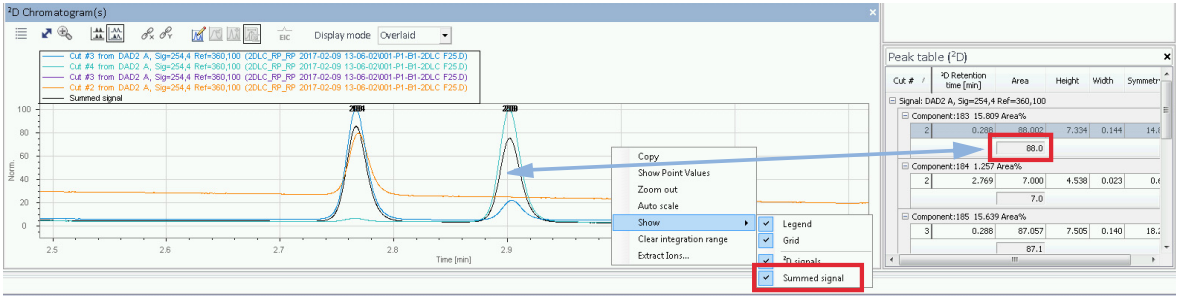


Figure 92 Summed signal

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.

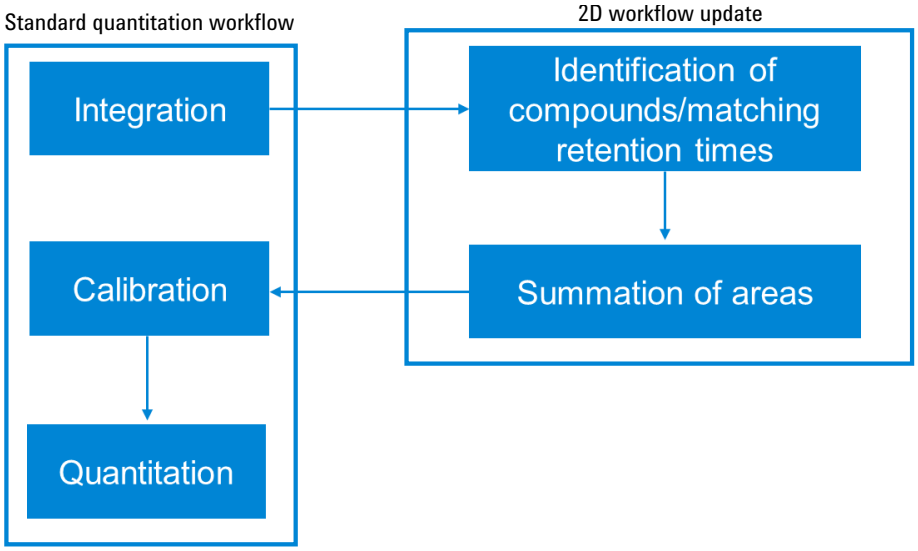
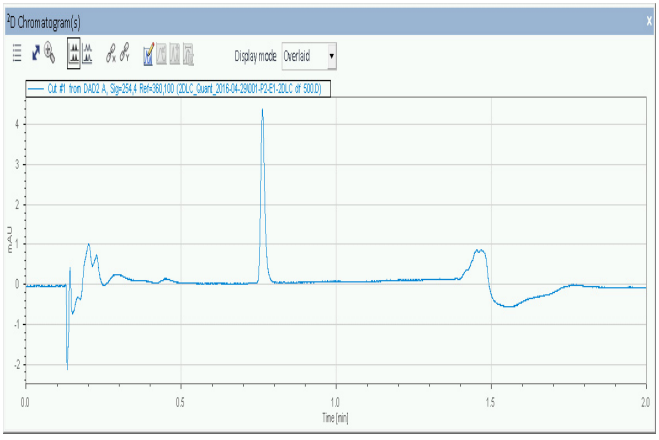


Figure 93 Necessary steps for quantitation in 2D-LC

Identify compounds

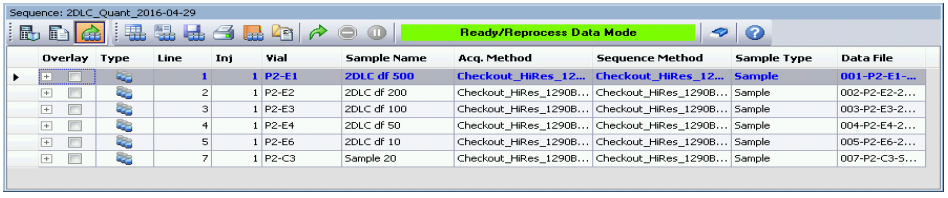
This procedure exemplarily shows a quantitative measurement of the 2D-LC checkout sample using a 3-level calibration of two compounds at two wavelengths.



1 Select signal.

NOTE

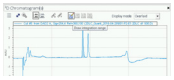
Use a signal with a low concentration.

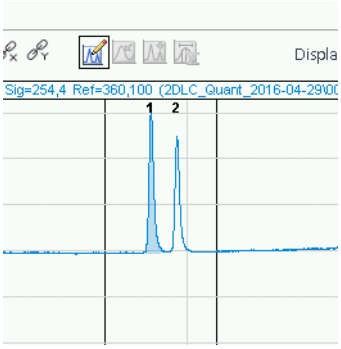


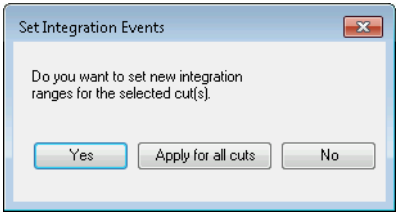
2 Integrate the cuts.

NOTE

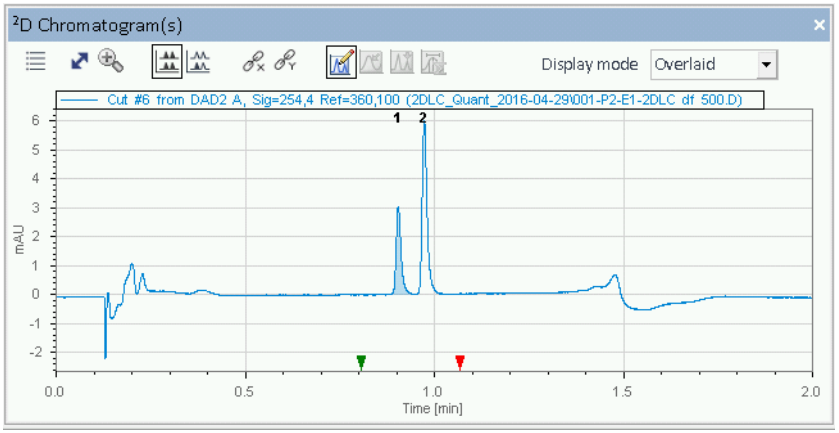
Integration is done using the ChemStation integrator.

a Click on  to **Set Integration Range**.





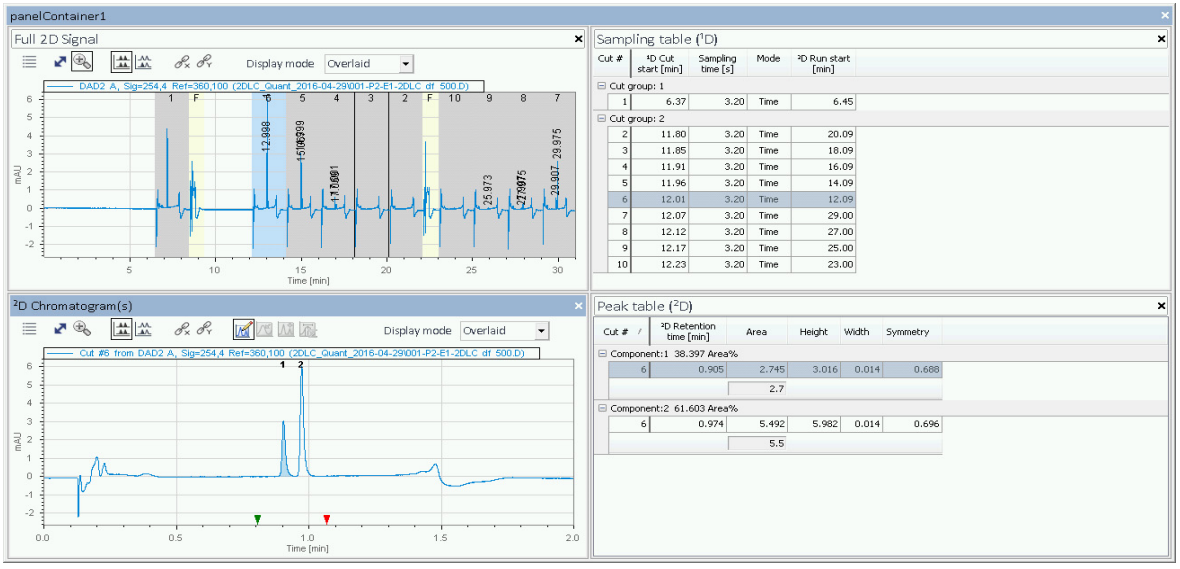
The 2D-LC Viewer offers simplified access for changing the integration of one or multiple limits.



3 Verify small peaks in ^{2}D. Use Full ^{2}D Signal or ^{2}D run start time for navigation.

NOTE

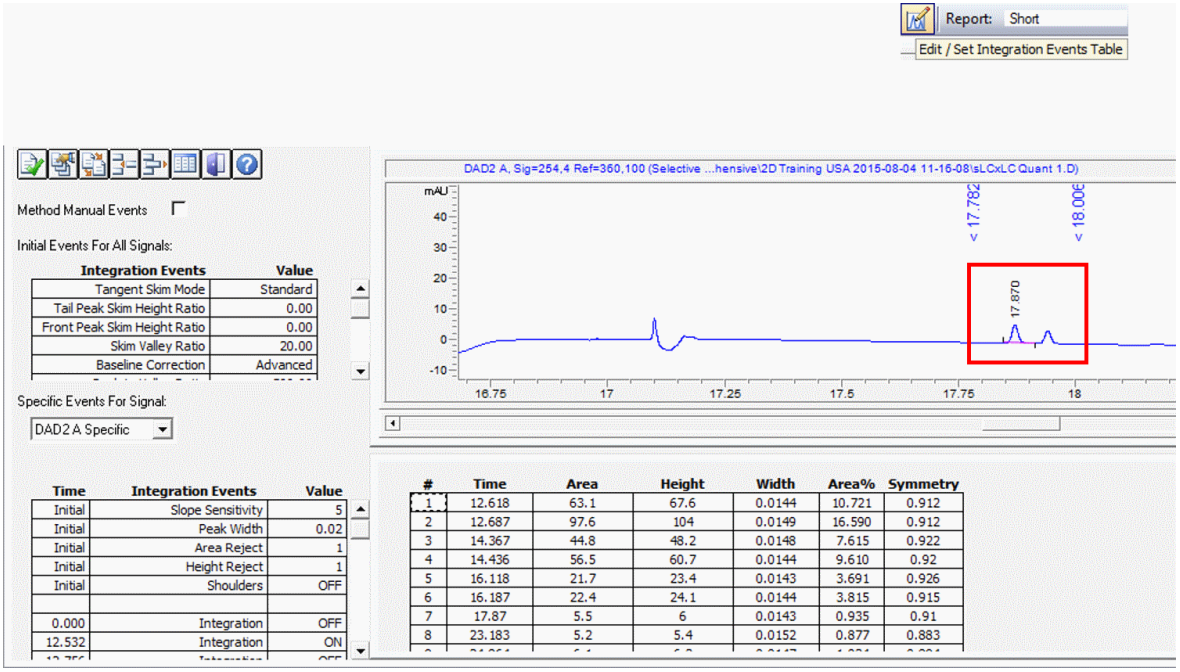
Peak integration is based on ChemStation integrator settings. Please verify, if all peaks of interest have been found and integrated. If not, ChemStation settings need to be adjusted. For finding the corresponding peak in the ^{2}D signal, use the peak table and heart-cut table.



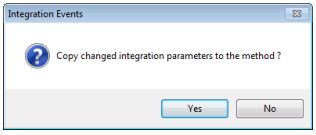
4 Adjust integrator settings in ChemStation.

NOTE

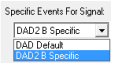
ChemStation has no information about cuts and individual ^{2}D chromatograms. So it is important to know, where to look at. In the example above, Cut #2 starts at 16.97 minutes. At 17.939 minutes is compound 2 of cut #2, which is not integrated. In this example, decreasing the area reject parameter from 5 to 1 enables integrating the peak.



5 Apply the adjusted integrator settings to other signals.



NOTE

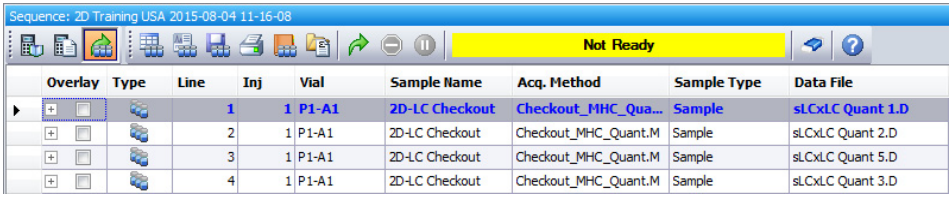


In integration events, change to a different wavelength for applying settings here.

Consider using same limits for other signals, apply to sequence method.

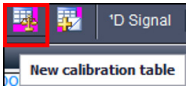
Calibration

Create a calibration table

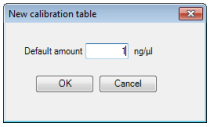


Overlay	Type	Line	Inj	Vial	Sample Name	Acq. Method	Sample Type	Data File
		1	1	P1-A1	2D-LC Checkout	Checkout_MHC_Qua...	Sample	sLCxLC Quant 1.D
		2	1	P1-A1	2D-LC Checkout	Checkout_MHC_Quant.M	Sample	sLCxLC Quant 2.D
		3	1	P1-A1	2D-LC Checkout	Checkout_MHC_Quant.M	Sample	sLCxLC Quant 5.D
		4	1	P1-A1	2D-LC Checkout	Checkout_MHC_Quant.M	Sample	sLCxLC Quant 3.D

1 Create a new calibration table from the MHC Viewer.



2 Enter amount/concentration.

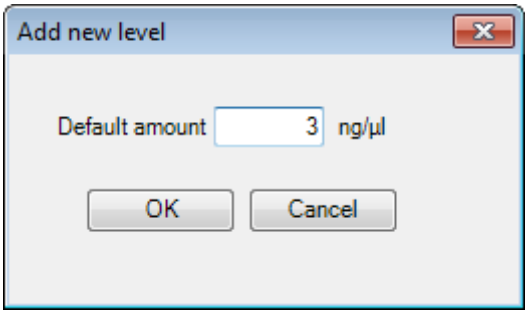


Add levels

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

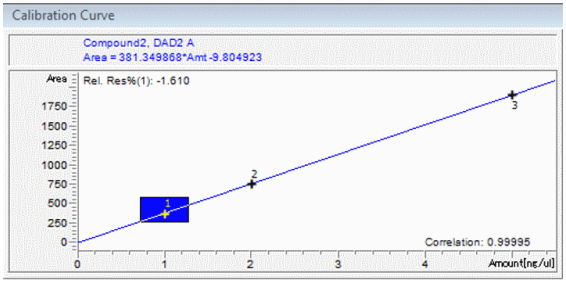
Calibration Table

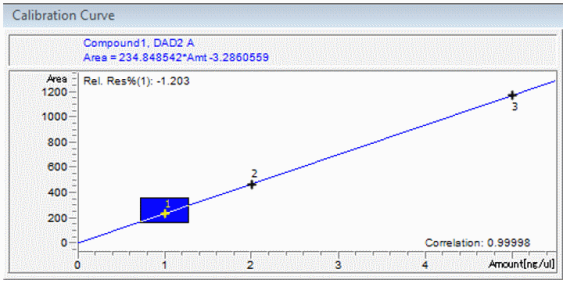
Enter | Delete | Insert... | Print | OK | Help

#	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			





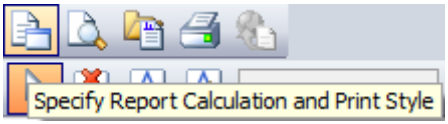
Quantification

Quantifying samples with unknown concentration based on the calibration table can be done using ChemStation reporting as usual.

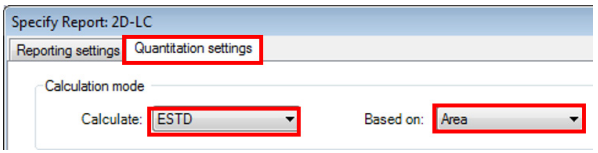
Quantification

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

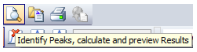
1



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 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 94 on page 220). 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 95 on page 220). After baseline correction the peaks can be automatically detected by a peak detection algorithm inherent in the 2D-LC data analysis software (Figure 96 on page 221). Since the third dimension is the intensity of the peaks a 3-dimensional plot of the data is possible (Figure 97 on page 221). With the given data set further qualitative and quantitative data analysis is possible.

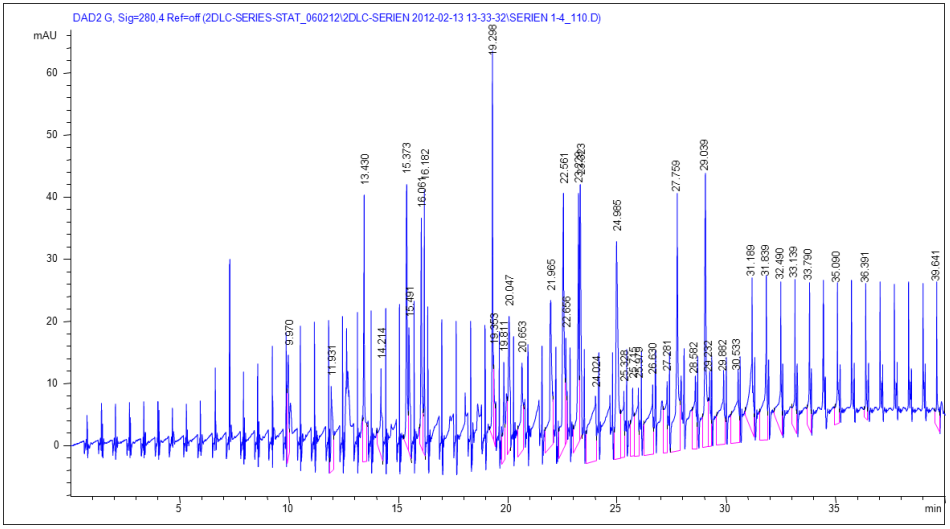


Figure 94 Display of two-dimensional LC data with a one-dimensional data analysis software

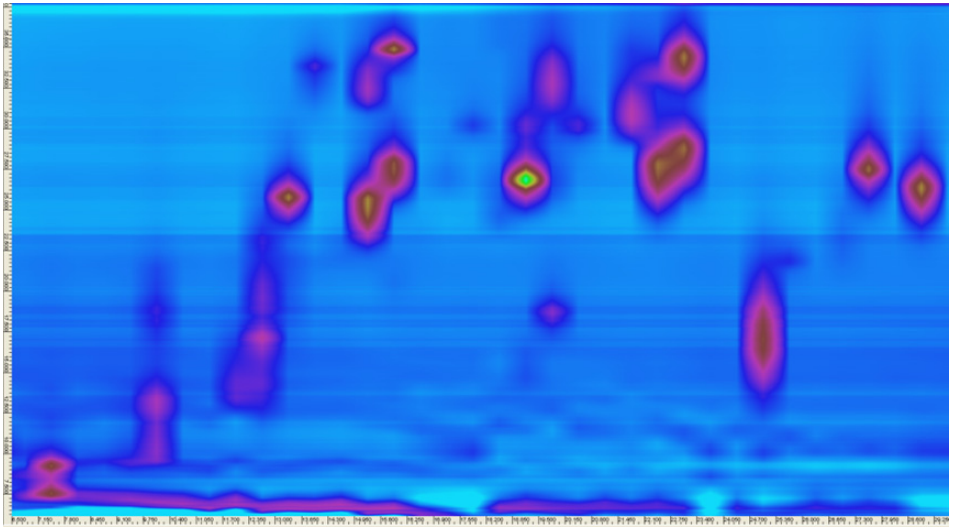


Figure 95 2D-LC plot of the optimized separation of 26 polyphenolic compounds

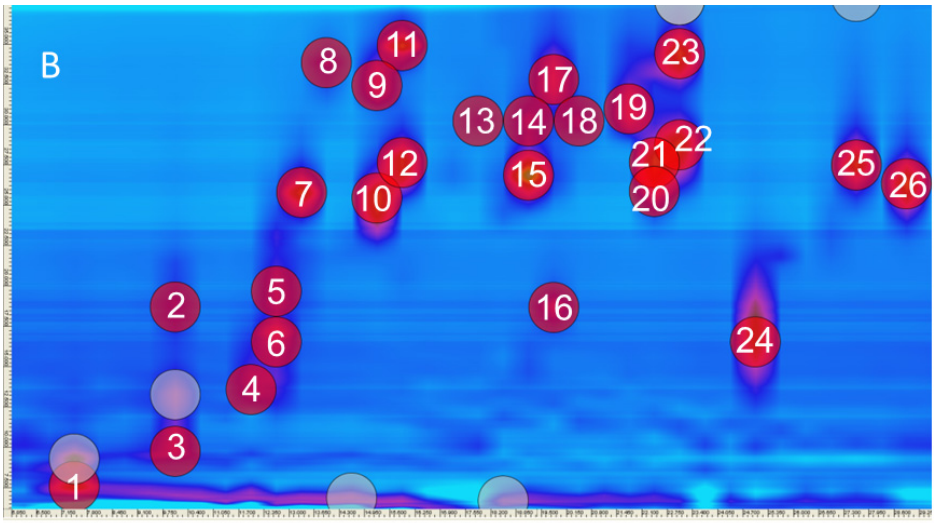


Figure 96 2DLC plot after baseline correction and with software detected peak annotation

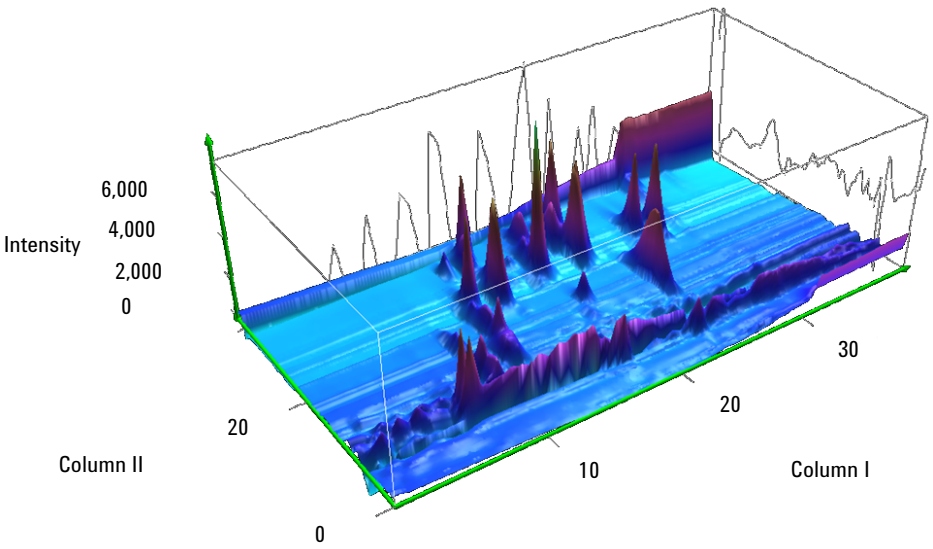


Figure 97 3-Dimensional display of the separation of the 26 compound standard mixture. The first dimension separation takes 40 minutes and each second dimension separation takes 39 seconds. The back side shows a generated first dimension chromatogram and gives the impression which peaks are coeluting 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
	CD with software
	License dongle (Wibu Key)
	Activation code

1. The CD contains two executables: LCxLC2.8r3-HRMS.exe (or higher), LCxLC2.8r3-HRMS-64.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 R2.8 (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)

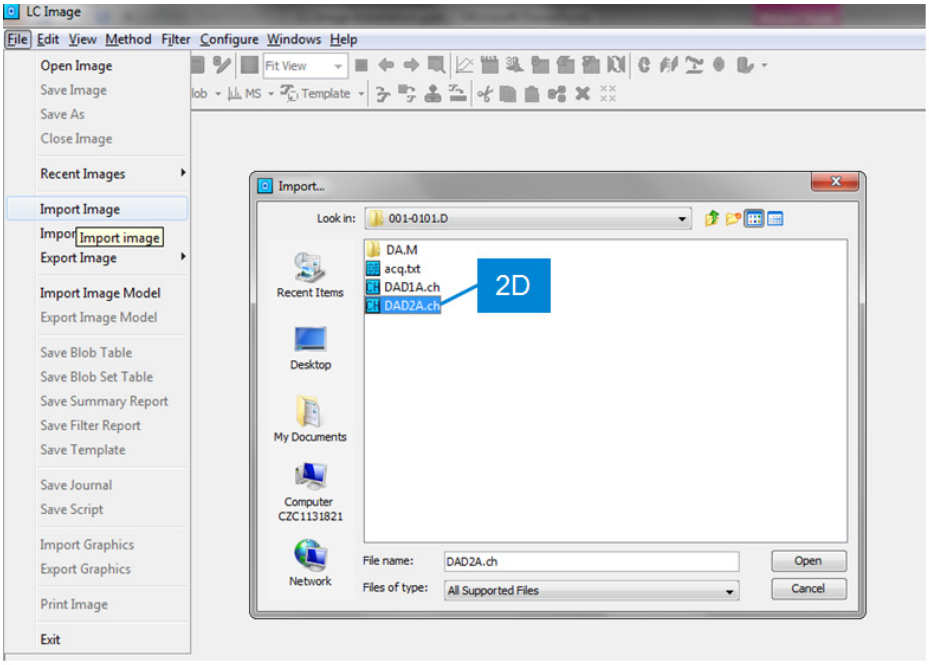
Preparations The USB dongle needs always to be inserted when working with GCImage software. If not, you will be asked to insert it.

Basic knowledges

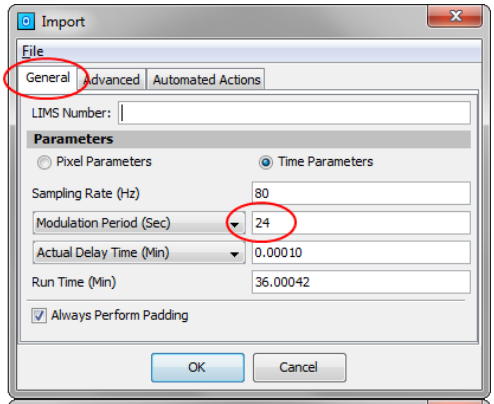
1 Start up LCImage

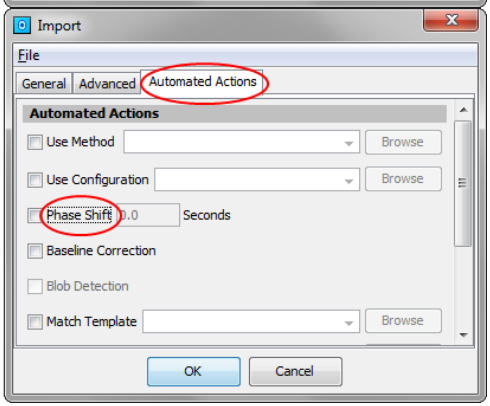
LCImage offers optionally a password protected user management system. If you don't need it, simply click „Login with system", which is based on Windows user account.

2 Import the UV signal from the second dimension detector.

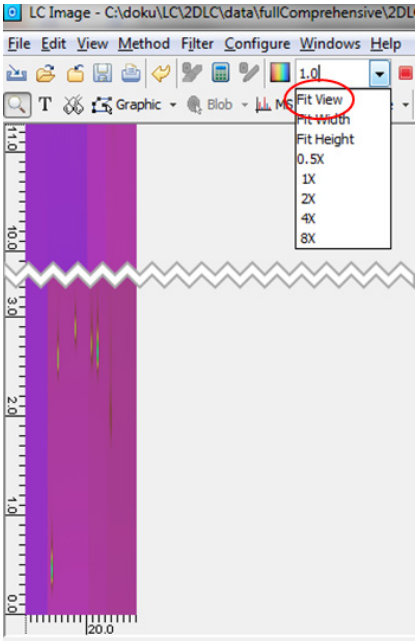


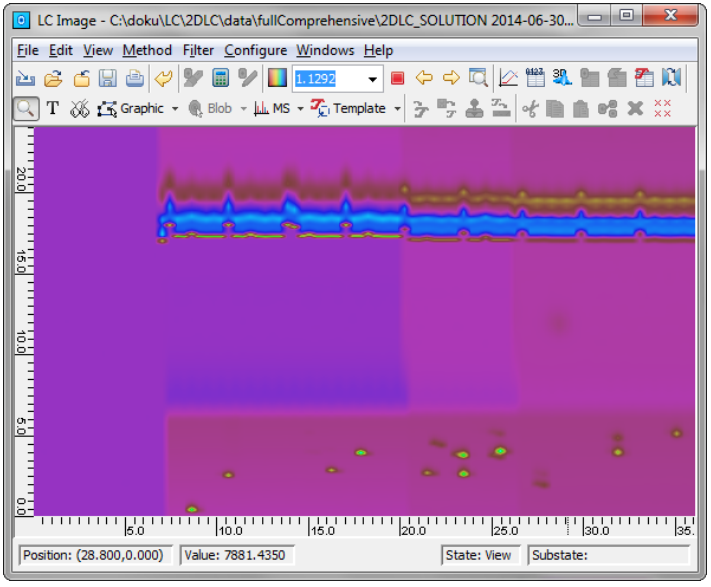
3 Import parameters



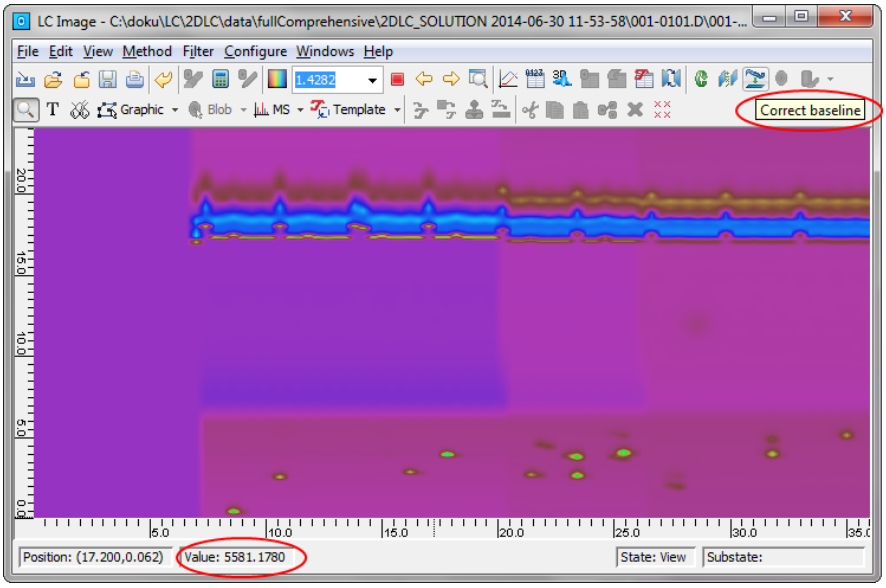


4 Fit view

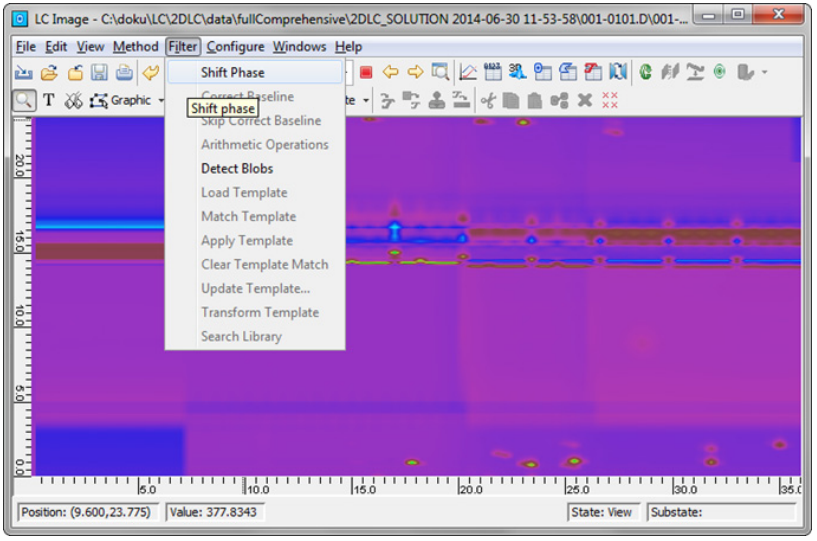

LC Image - C:\doku\LC\2DLC\data\fullComprehensive\2DL
File Edit View Method Filter Configure Windows Help
1.0
Graphic
Blob
Fit View
Fit Width
Fit Height
0.5X
1X
2X
4X
8X
20.0


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
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

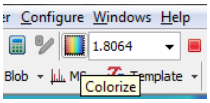


6 Shift phase

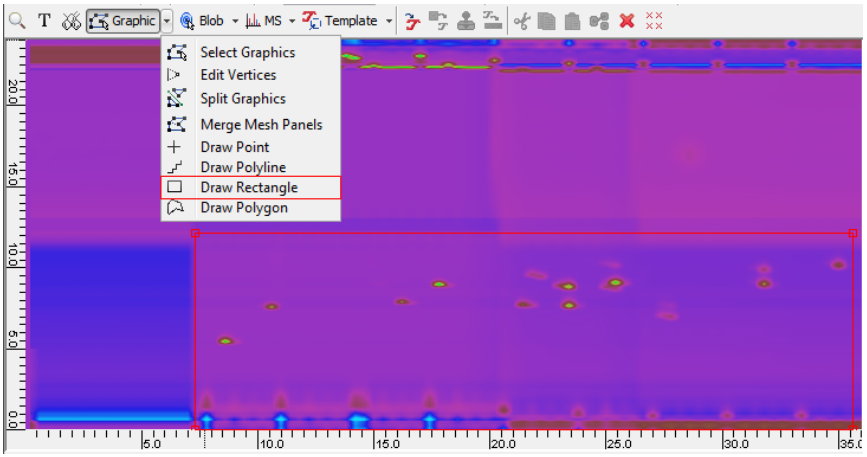


7 Zoom into an interesting region by using the right mouse button and dragging over the display

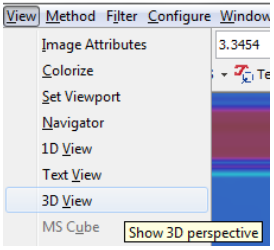
8 Adjust colors: LC Image offers refined possibilities for optimizing the color scales. Play around with settings for improving the contrast.



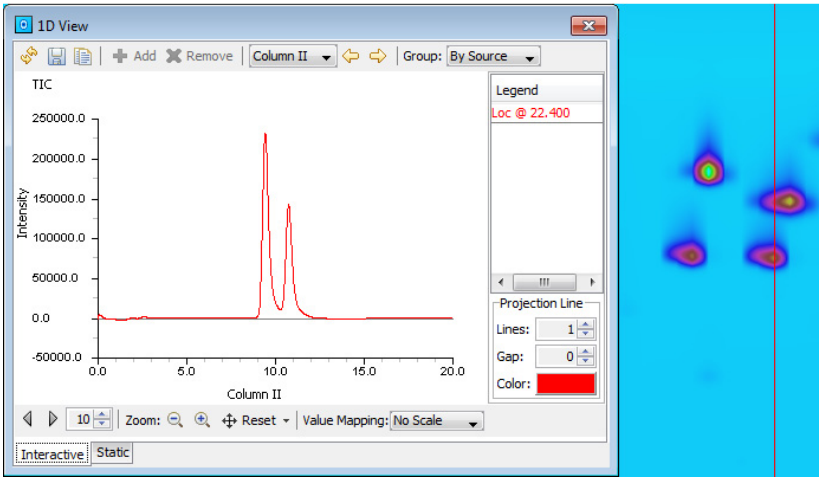
9 Select a data range.



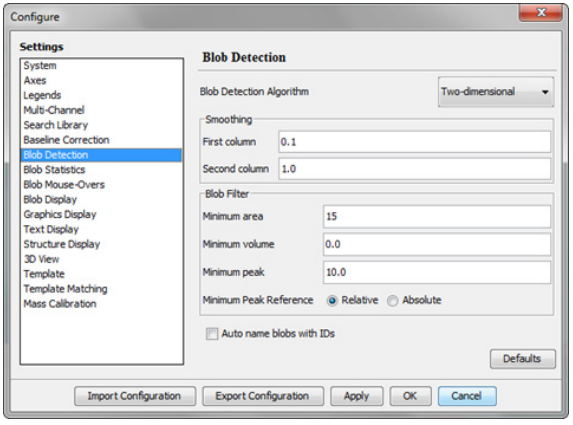
10 By clicking the „Show 3D perspective“ button or the corresponding menu item, you can easily create a customizable 3D plot.



11 View single 2D chromatograms

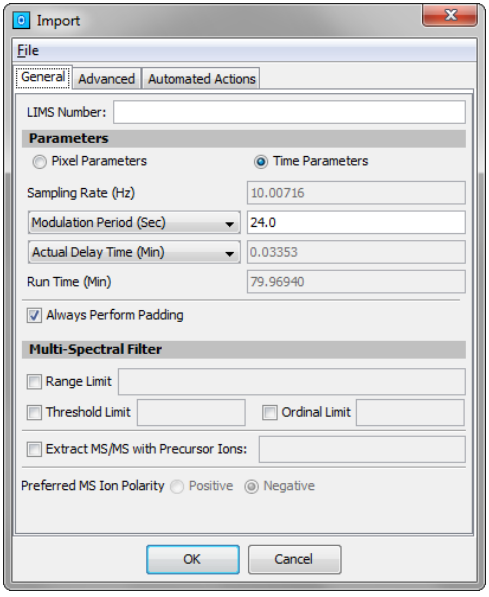


12 Select blobs



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 234.
- 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.

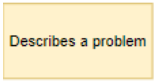
Most of the following explanations can also be used to isolate and detect standard LC issues.

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 15 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.

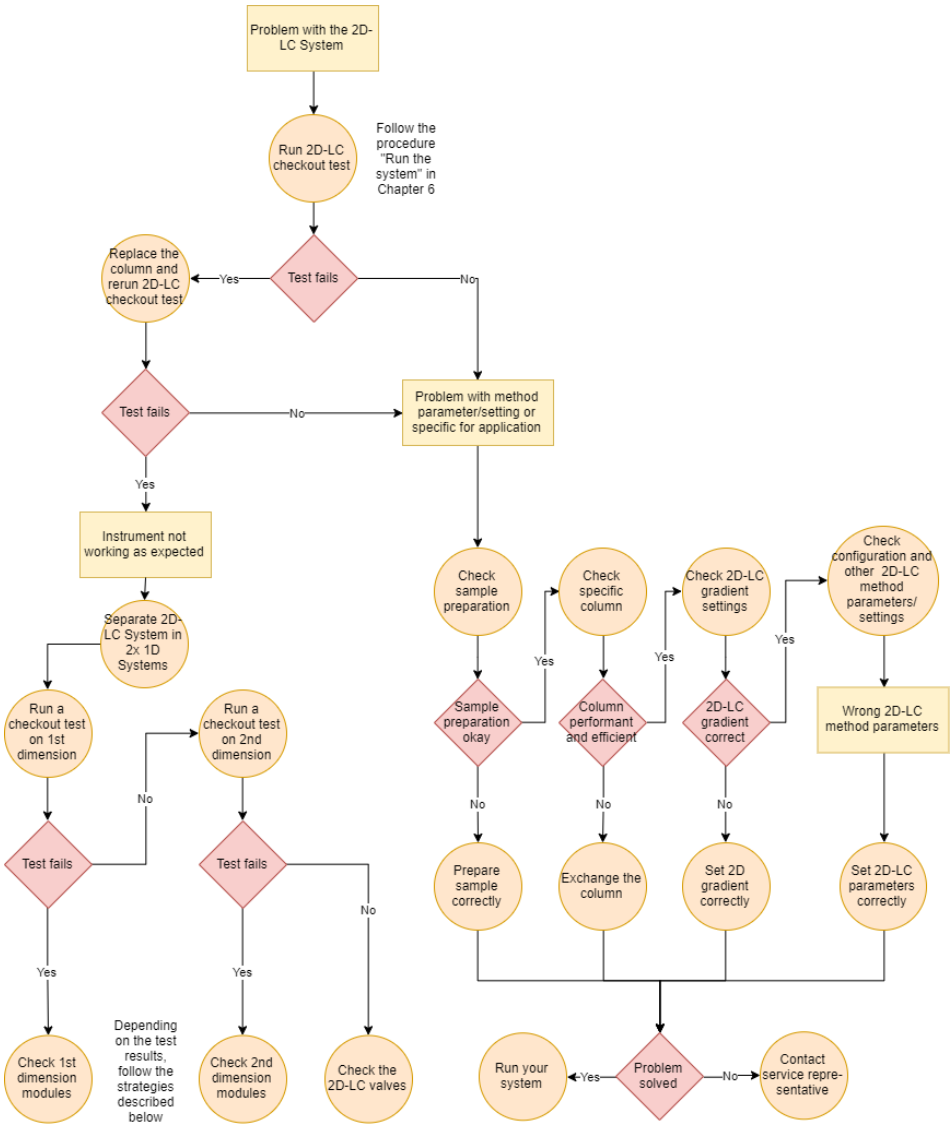


Figure 98 Example for a strategy to identify the application method or instrument as root cause for issues in 2D-LC chromatography

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" on page 238
- "Pressure too low" on page 239
- "Peak area and peak height related" on page 240
- "Retention time related" on page 241
- "Missing signal linearity" on page 242
- "Drifting signal" on page 243
- "Signal noisy" on page 244

Pressure too high

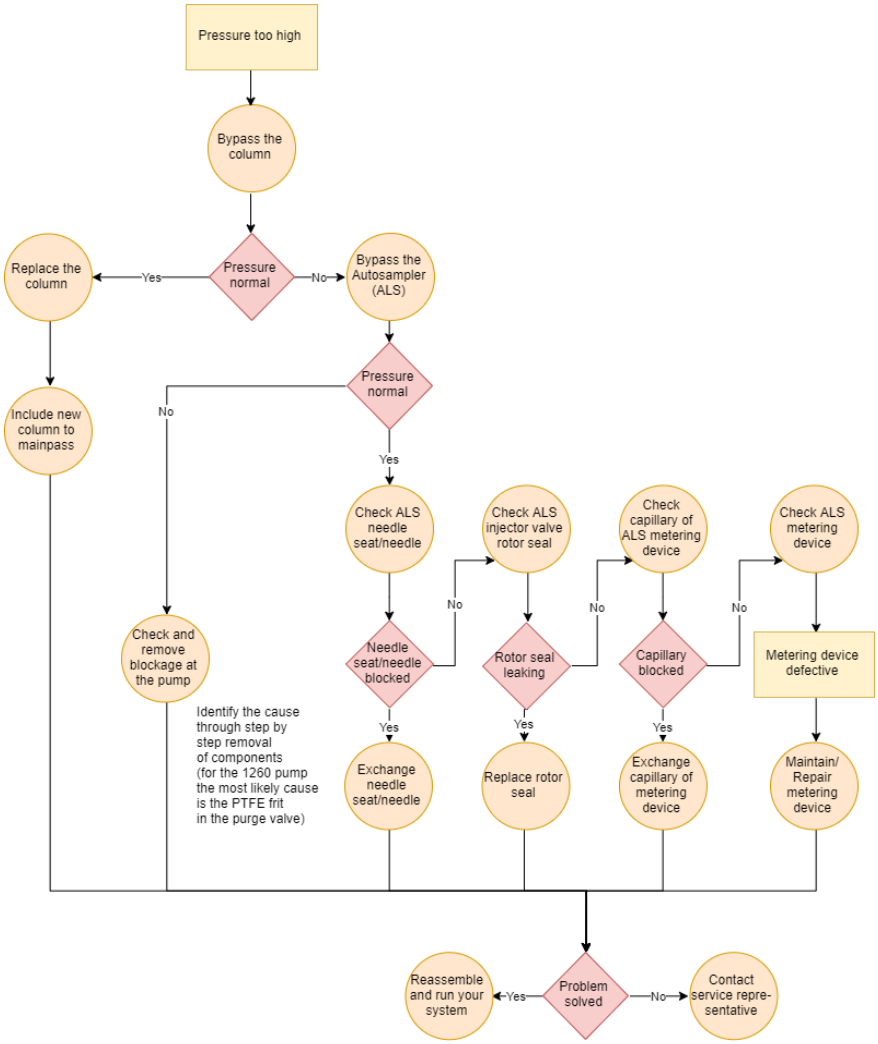


Figure 99 Example for a strategy to eliminate issues related to too high pressure in 2D-LC instruments

Pressure too low

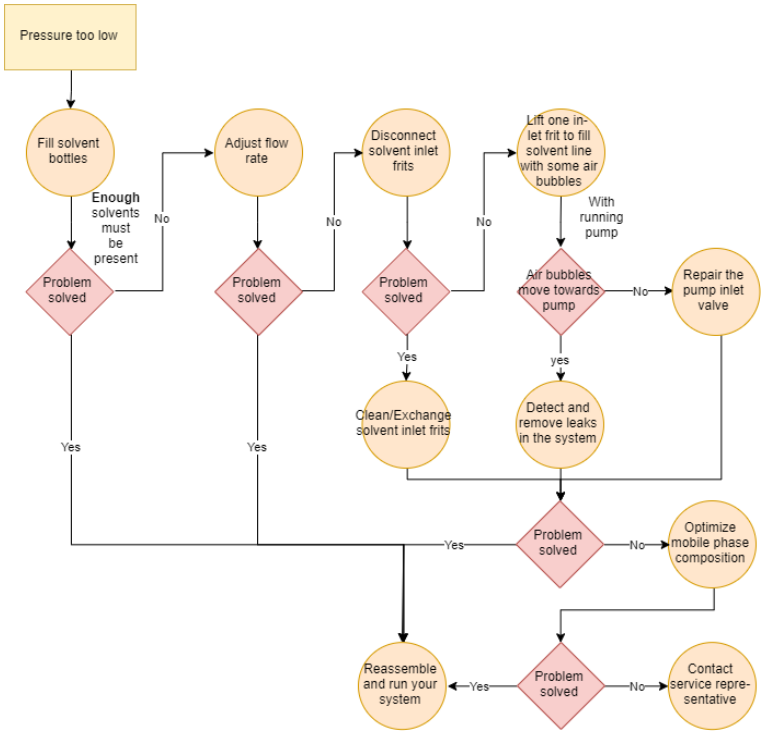


Figure 100 Example for a strategy to eliminate issues related to too low pressure in 2D-LC instruments

Peak area and peak height related

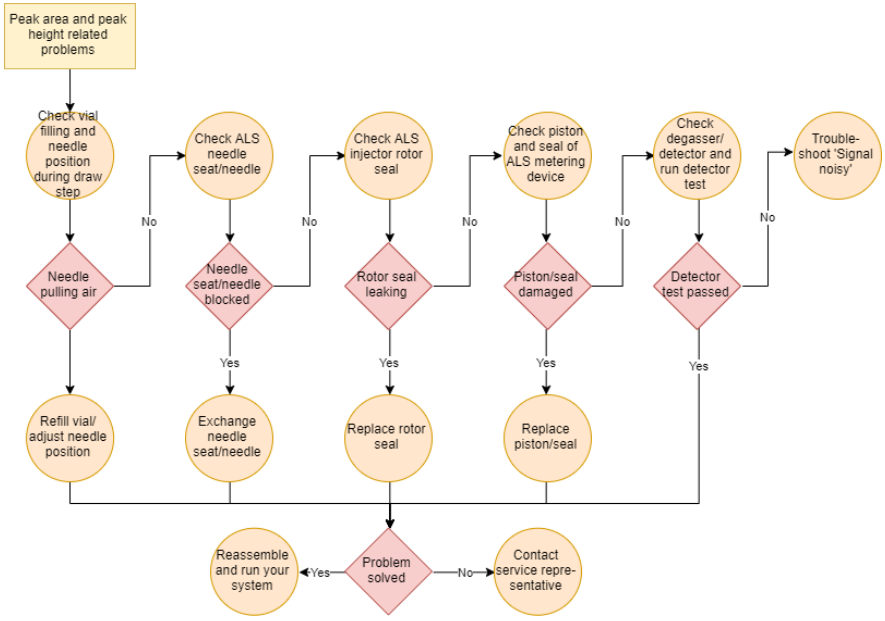


Figure 101 **Example for a strategy to eliminate issues related to peak problems in 2D-LC instruments**

Retention time related

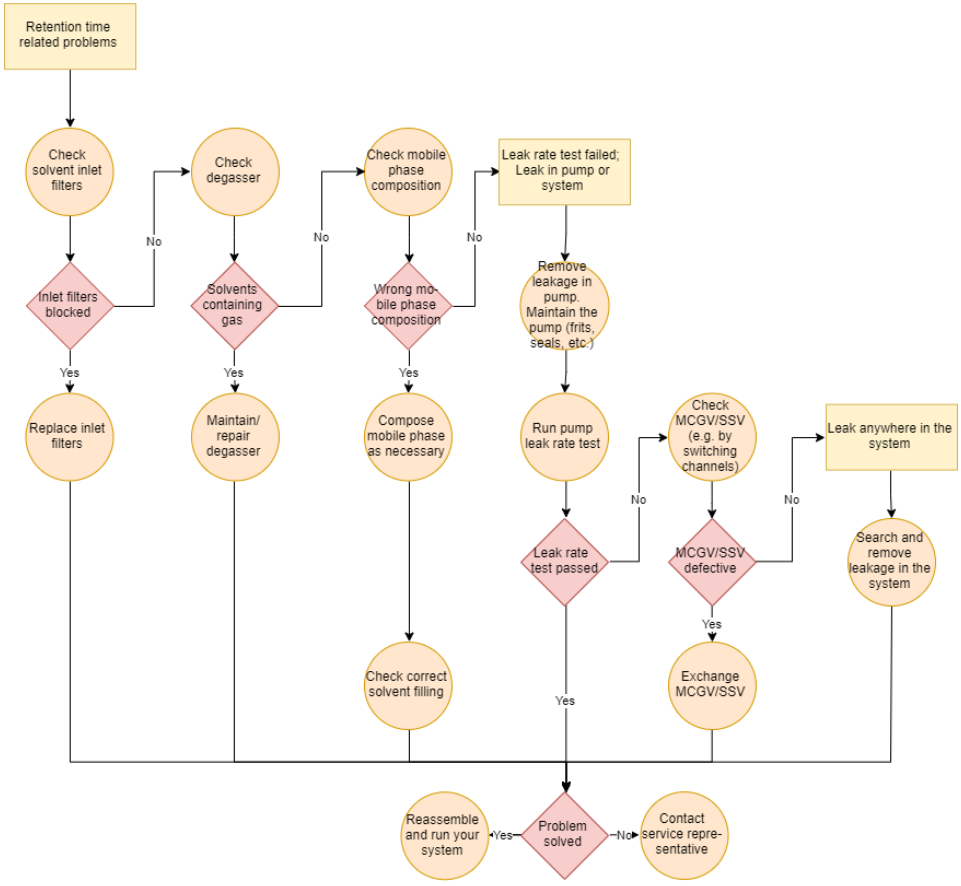


Figure 102 Example for a strategy to eliminate issues related to retention time in 2D-LC instruments

Missing signal linearity

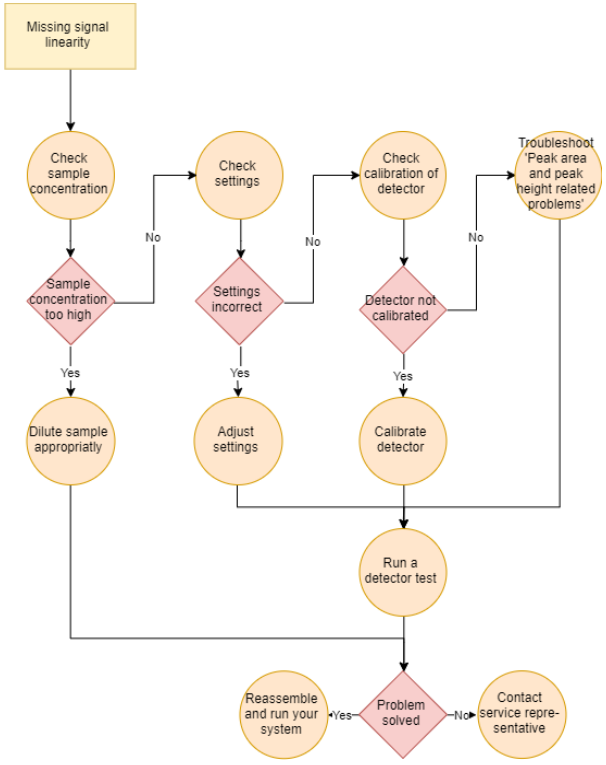


Figure 103 Example for a strategy to eliminate issues related to missing signal linearity in 2D-LC instruments

Drifting signal

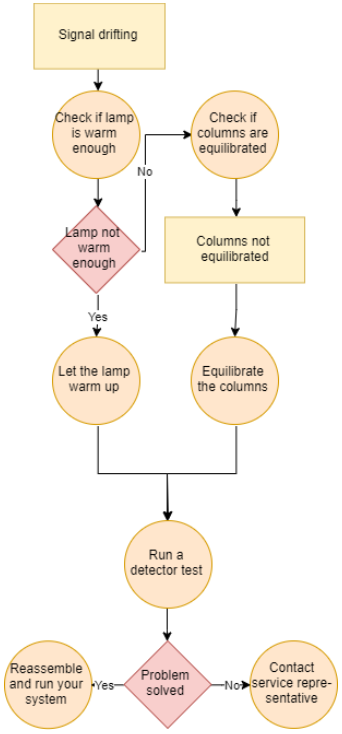


Figure 104 Example for a strategy to eliminate issues related to drifting signal in 2D-LC instruments

Signal noisy

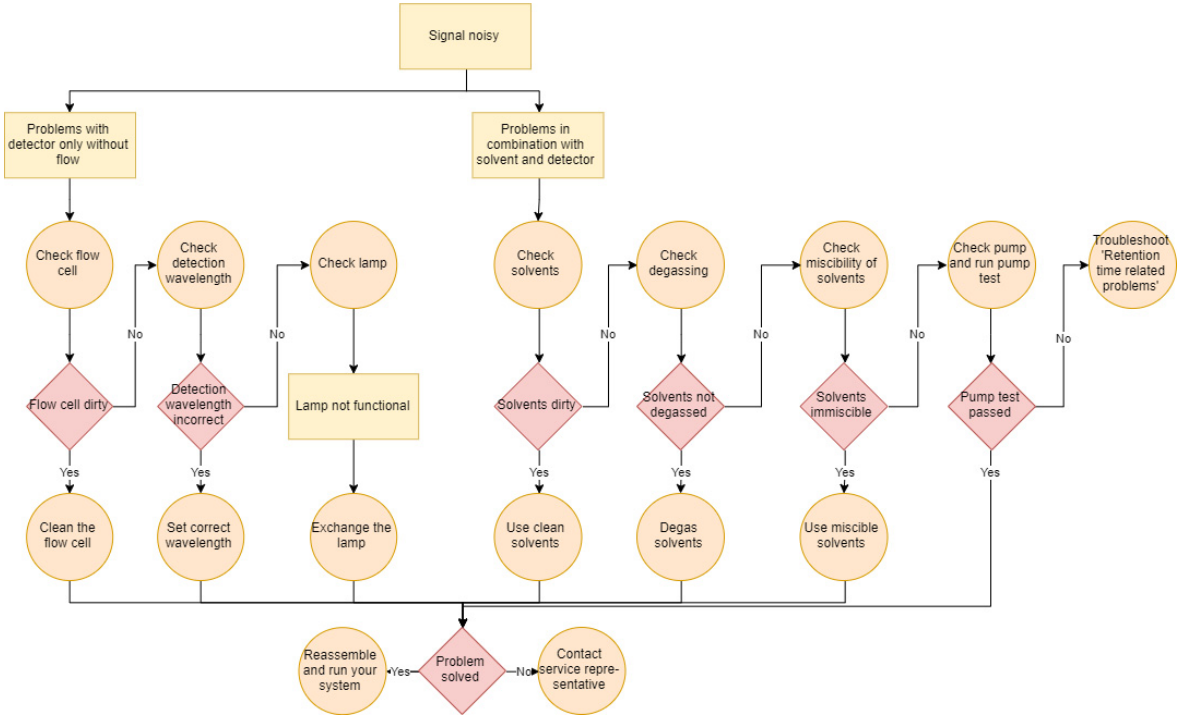


Figure 105 Example for a strategy to eliminate issues related to signal noise in 2D-LC instruments

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 16 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 17 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.**

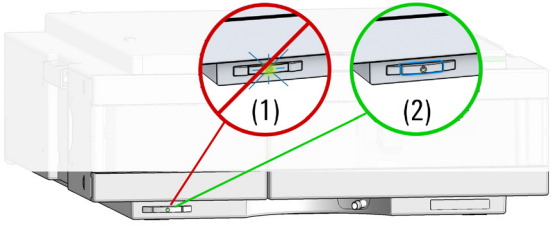
WARNING

Toxic, flammable and hazardous solvents, samples and reagents

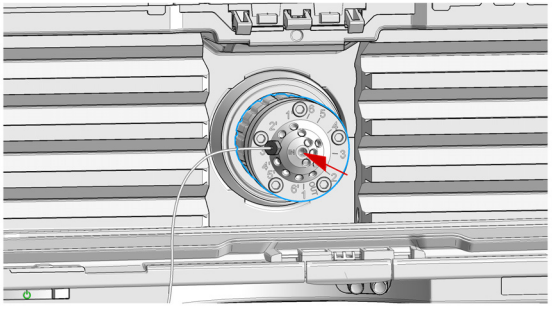
The handling of solvents, samples and reagents can hold health and safety risks.

- ✓ **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.**

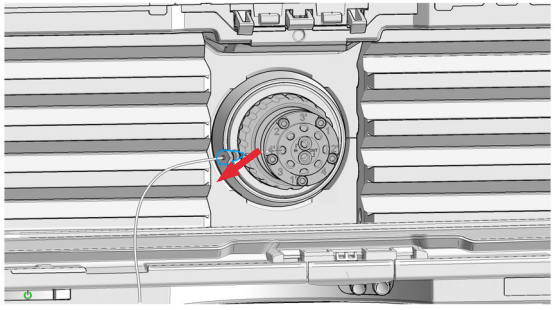
1 Switch off the module.



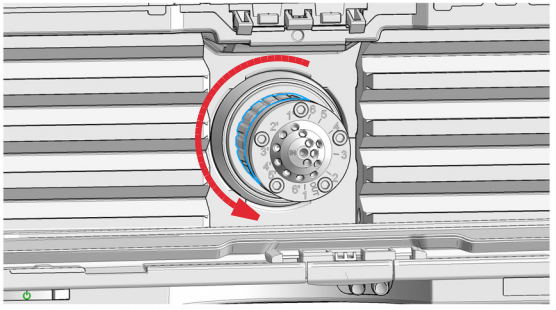
2 Push the valve head to bring it to its outer position.



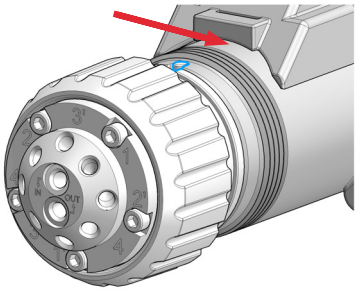
3 Remove all capillary connections from the valve head.



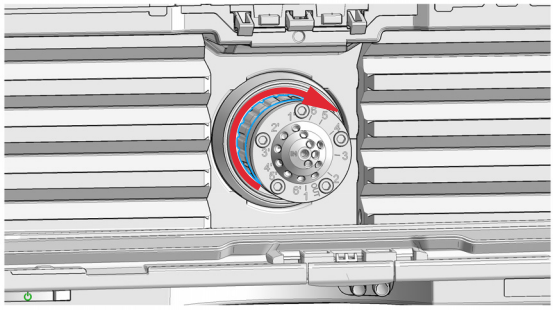
4 Unscrew the valve head.



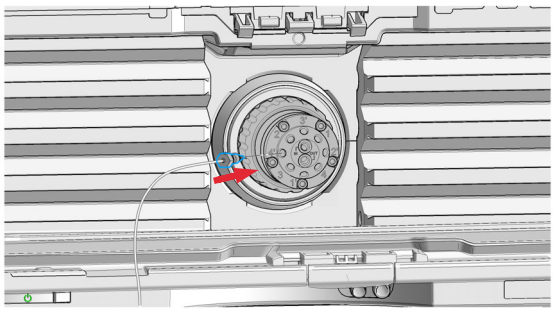
5 Put the new valve head onto the valve drive such that the lobe fits to the groove.



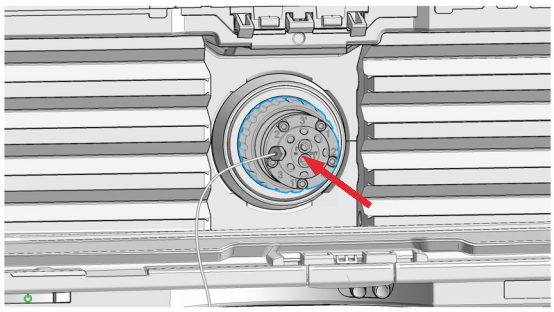
6 Screw the valve head onto the valve drive using the union nut.



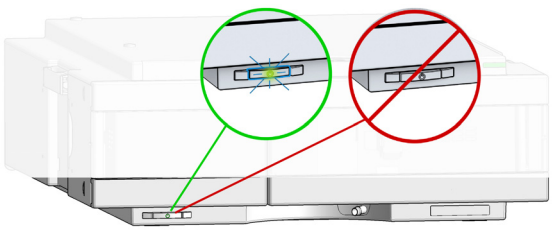
7 Install all required capillary connections to the valve.



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.**

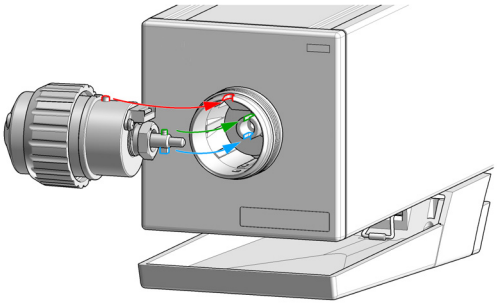
✓ **Always install a valve head for operation and storage. For protecting the actuator, a dummy valve head can be used instead of a functional valve. Do not touch parts inside the actuator.**

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.

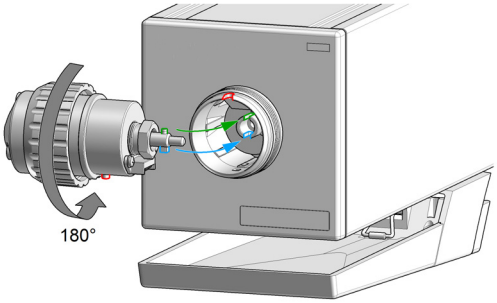
NOTE To allow correct valve identification, power off the module for at least 10 s.

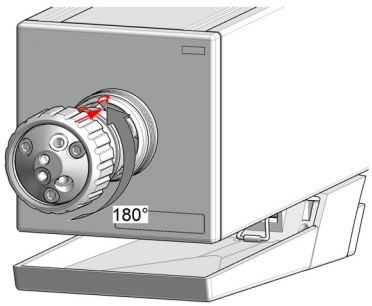
1 Insert the valve head into the valve shaft.



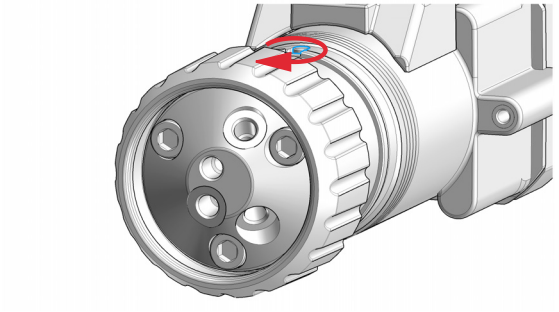
OR

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.



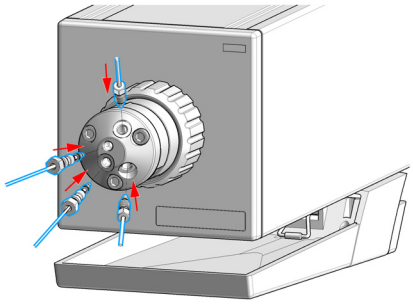


2 When the outer pin is locked into the groove, manually screw the nut onto the valve head.



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.

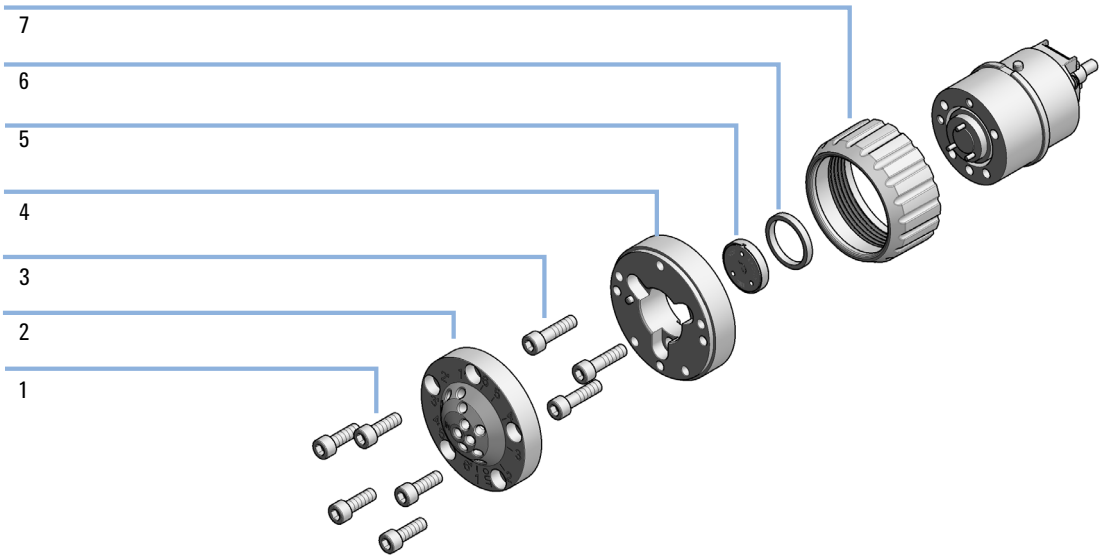


Figure 106 Valve Head Parts (example)

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 106 on page 273 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

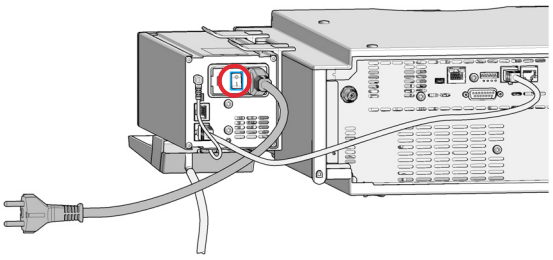
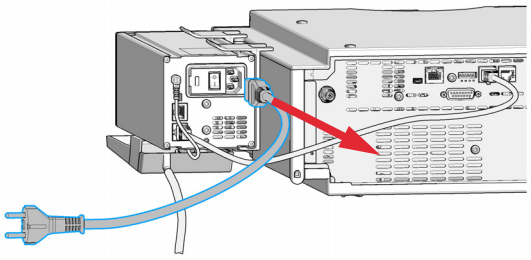
Parts required

#	p/n	Description
2	2110-1486	Fuse 2 AT250 V

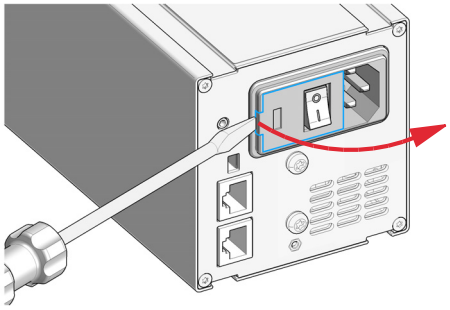
WARNING

Electrical shock

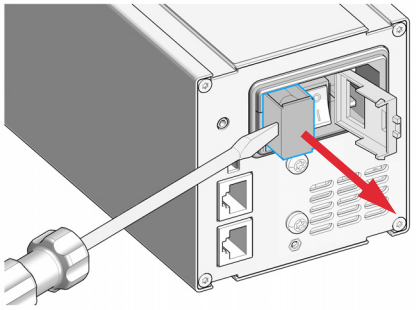
- ✓ **Disconnect the module from line power before changing a fuse or trying to open the hatch of the power input socket.**
- ✓ **Never reconnect the line power before havingthe power input socket closed.**

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 screw-driver.

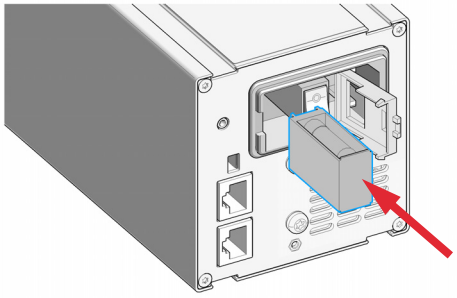


4 Pull out the fuse drawer as shown.

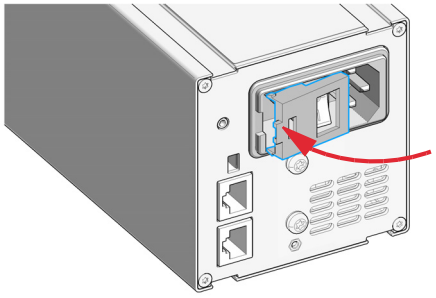


5 Replace the defect fuse(s).

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.

2D-LC Loops

2D-LC Loops for Standard 2D-LC

p/n	Description
5067-5440	Calibrated loop kit for 2D-LC
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

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 mmX 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 0.12x85M/M ASM
5500-1301	Capillary ST 0.12x170M/M ASM
5500-1302	Capillary ST 0.12x340M/M ASM
5500-1303	Capillary ST 0.12x680M/M ASM
5500-1376	Capillary ST 0.12x170M/M transfer

Pressure Release Kit

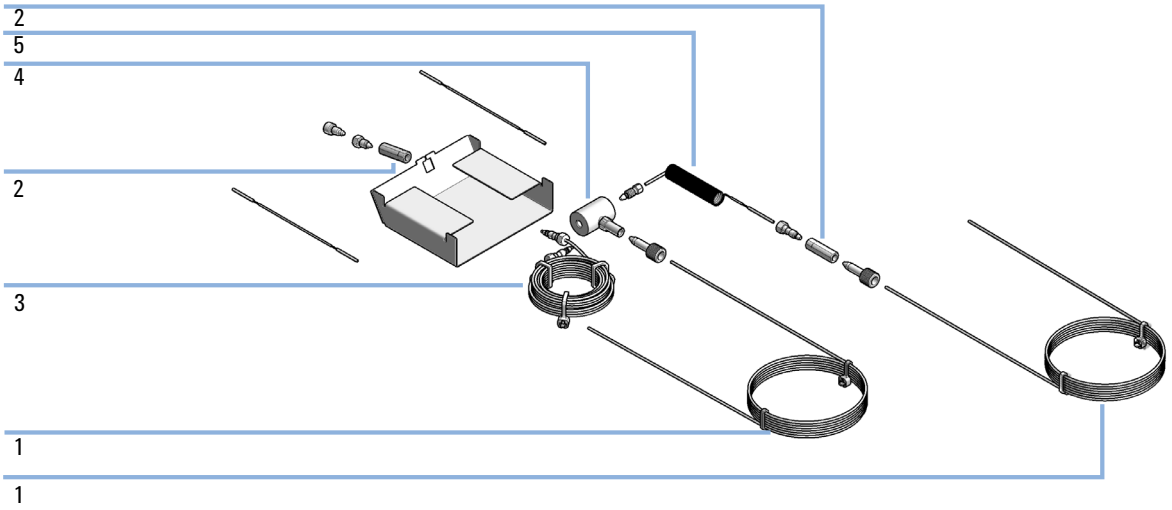


Figure 107 Pressure release kit, parts

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

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

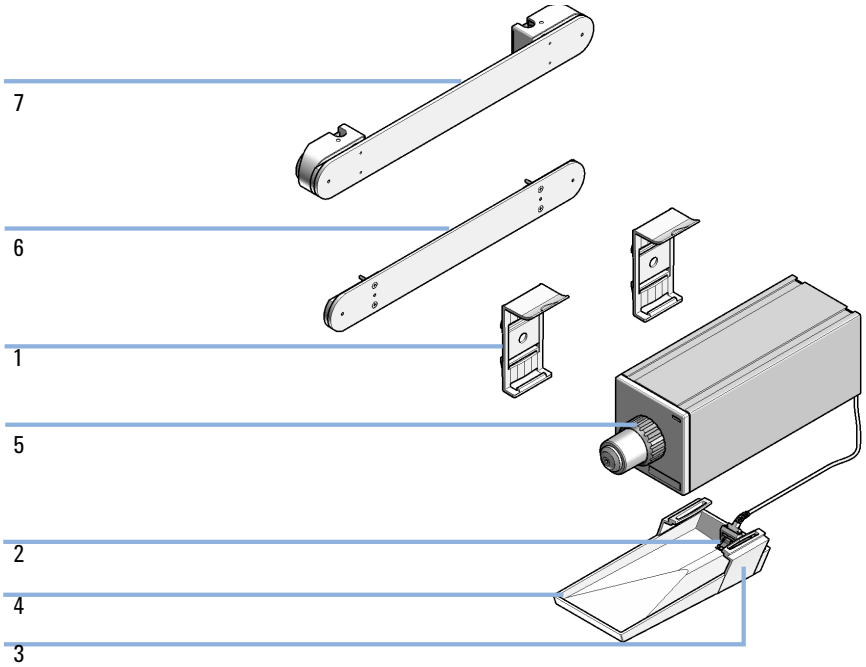


Figure 108 Valve Drive Parts

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)

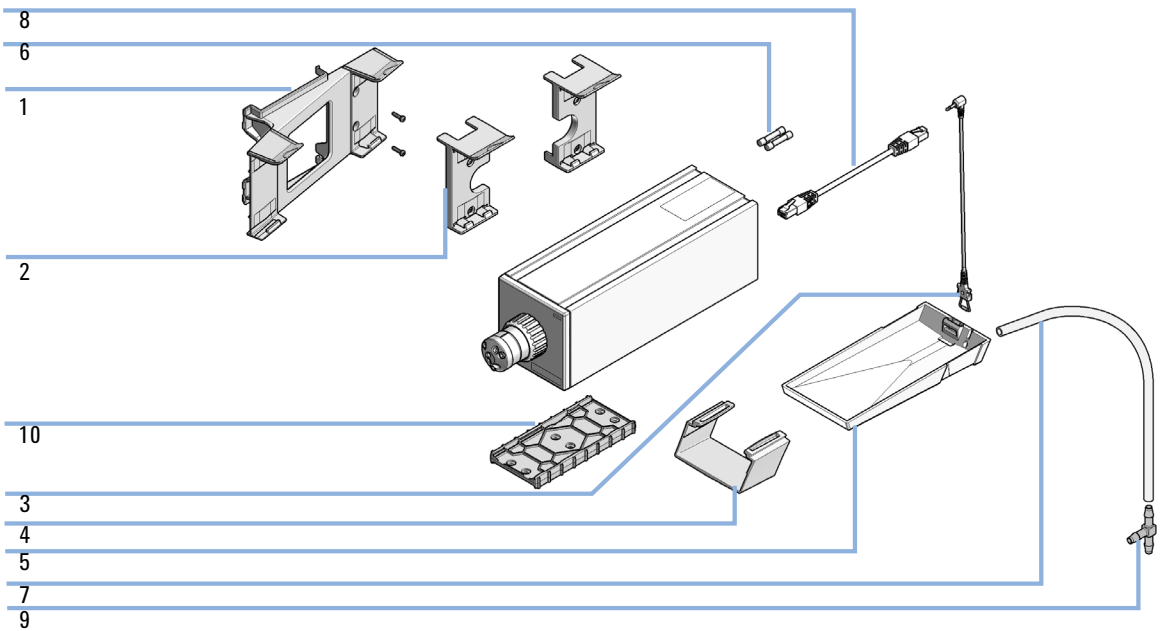


Figure 109 Parts for 1260/1290 Infinity II Valve Drive

Valve Head Parts

NOTE

The figure below illustrates replacement parts for the valve heads, with the 12ps/13pt 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.

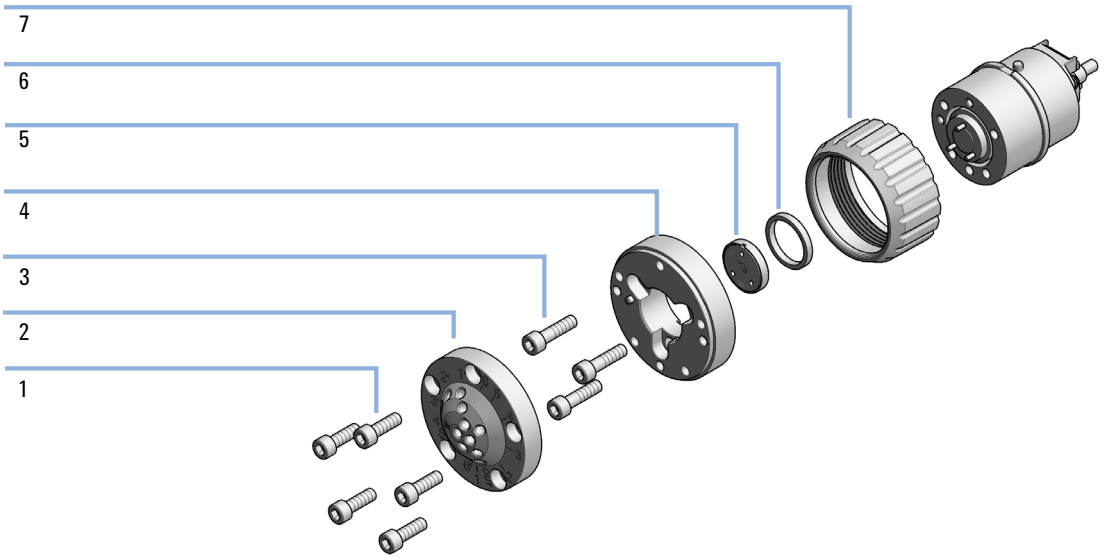


Figure 110 Valve Head Parts (example)

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 18 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

Vale 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 or the valve has passed 30000 switches, 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	G4236-68000	2D-LC Easy Starter Kit 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	G4236-68000	2D-LC Easy Starter Kit Internal part, not orderable
1	G1680-63721	Network LAN Switch
1	5500-1300	Capillary ST 0.12x85M/M ASM
1	5500-1301	Capillary ST 0.12x170M/M ASM
1	5500-1302	Capillary ST 0.12x340M/M ASM
1	5500-1303	Capillary ST 0.12x680M/M ASM
4	5500-1376	Capillary ST 0.12x170M/M transfer

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
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	Valve Head 2 Postion / 6 Port, 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	2ps/10pt valve head, 600 bar
5067-4283	2ps/10pt valve head, 800 bar
0101-1415	Rotor Seal (PEEK)

Alternative diverter valves (2 position / 6 port, Vespel Rotor Seal)

p/n	Description
5067-4117	2ps/6pt 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	2ps/10pt 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

NOTE

The 1300 bar InfinitLab Quick Change Valves are backward compatible to the 1200 bar Valves.

Table 19 Replacement parts standard valve heads for G7116B

Valve Head	Rotor Seal	Stator Head	Stator Screws	Stator Ring
5067-4233 8 Column Selector 1300 bar	5068-0200 (PEEK)	5068-0199	5068-0089	n.a.
5067-4241 2ps/6pt Valve 1300 bar	5068-0207 (PEEK)	5068-0006	1535-4857	n.a.
5067-4240 2ps/10pt Valve 1300 bar	5068-0205 (PEEK)	5068-0011	5068-0019	n.a.
5067-4273 6 Column Selector 1300 bar	5068-0242 (PEEK)	5068-0241	5068-0089	n.a.
5067-4284 6 Column Selector 800 bar	5068-0298	5068-0241	5068-0089	n.a.

Obsolete Valve Heads

The following 1200 bar valve heads are no longer orderable:

Table 20 Replacement parts obsolete valve heads for G7116B

Valve Head	Rotor Seal	Stator Head	Stator Screws	Stator Ring
5067-4121 8ps/9pt, 1200 bar	5068-0002 (Vespel)	5068-0001	1535-4857	5068-0127
5067-4117 2ps/6pt, 1200 bar	5068-0008 (Vespel)	5068-0006	1535-4857	5068-0127
5067-4118 2ps/10pt, 1200 bar	5068-0012 (Vespel)	5068-0011	5068-0019	n.a
5067-4142 6 Column Selector, 1200 bar	5068-0067 (Vespel)	5067-0077	5068-0089	n.a

Additional Heater Devices

Table 21 Heat Exchanger Overview

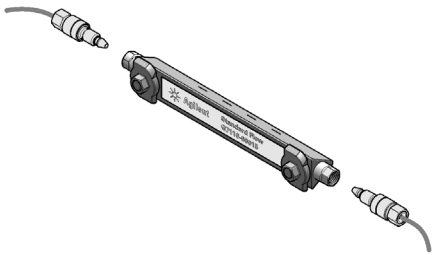
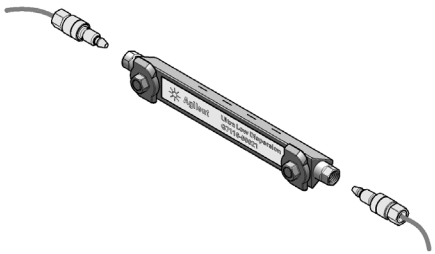
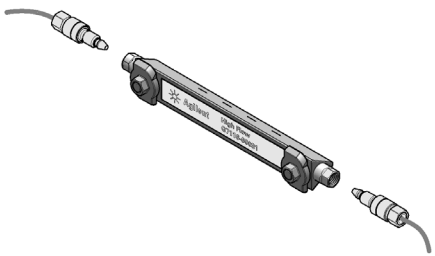
Flow rate	0.075 mm i.d. capillary	0.12 mm i.d. capillary
< 2 mL/min	*Ultra-low Dispersion* G7116-60021 (Internal volume: 1.0 µL)	*Standard Flow* G7116-60015 (Internal volume: 1.6 µL)
> 2 mL/min		*High Flow* G7116-60031 (Internal volume: 3.0 µL)

For details, see Table 22 on page 296.

Additional Heater Devices (for G7116B)

Blank heater assemblies without capillaries and fittings:

Table 22 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)

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	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
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	Capillary ST 0.12 mm x 280 mm, long socket (Capillary from column outlet to detector, 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)

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 304.

InfinityLab Quick Connect Fittings

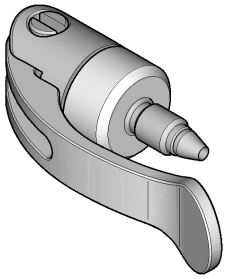


Figure 111 InfinityLab Quick Connect Fitting

p/n	Description
5067-5965	InfinityLab Quick Connect LC fitting (fitting without pre-installed 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

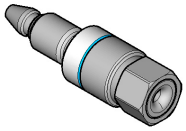


Figure 112 InfinityLab Quick Turn Fitting

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 23 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)	2nd 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

Important Customer Web Links

- Videos about specific preparation requirements for your instrument can be found by searching the *Agilent YouTube* channel at **https://www.youtube.com/user/agilent**
- To access *Agilent University*, visit **http://www.agilent.com/crosslab/university/** to learn about training options, which include online, classroom and onsite delivery.

  A training specialist can work directly with you to help determine your best options.
- A useful *Agilent Resource Center* web page is available, which includes short videos on maintenance, quick lists of consumables for new instruments, and other valuable information.
- Need technical support, FAQs, supplies? – visit our *Support Home page* at **http://www.agilent.com/search/support**
- Get answers. Share insights. Build connections:

  Join the *Agilent Community* at **https://community.agilent.com/welcome**

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.

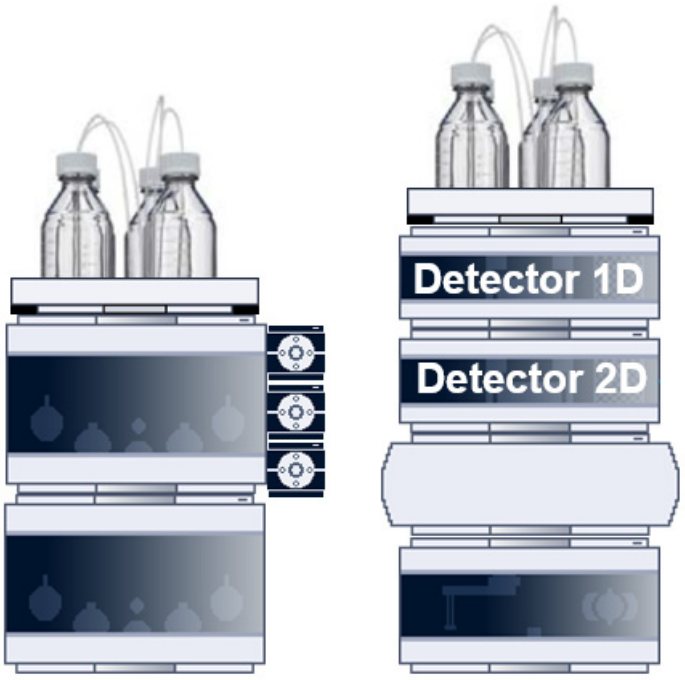


Figure 113 Legacy stack configuration for Multiple Heart-cutting 2D-LC

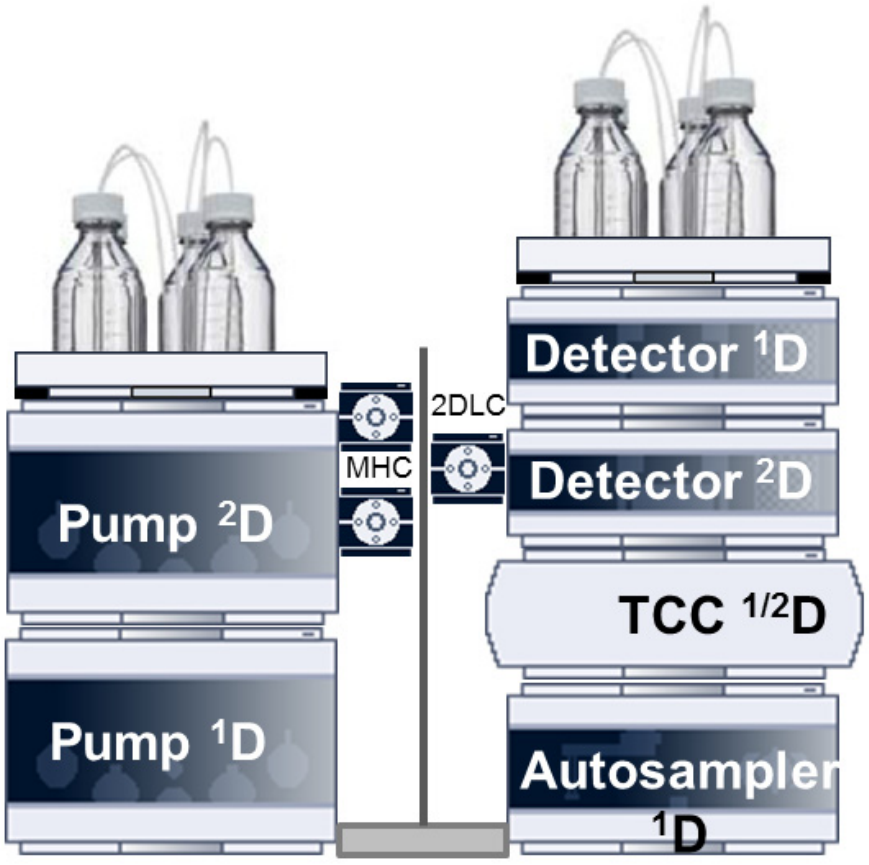


Figure 114 Previous stack configuration for Multiple Heart-cutting 2D-LC based on the column organized/valve holder

Table 24 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).

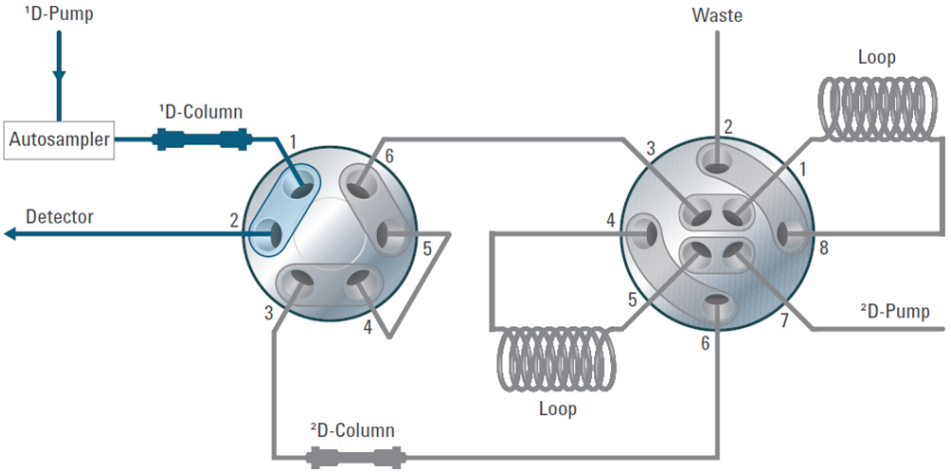


Figure 115 Setup of the Agilent 1290 Infinity 2D-LC Solution with an additional 2-position/6-port valve positioned for 1D-LC analysis.

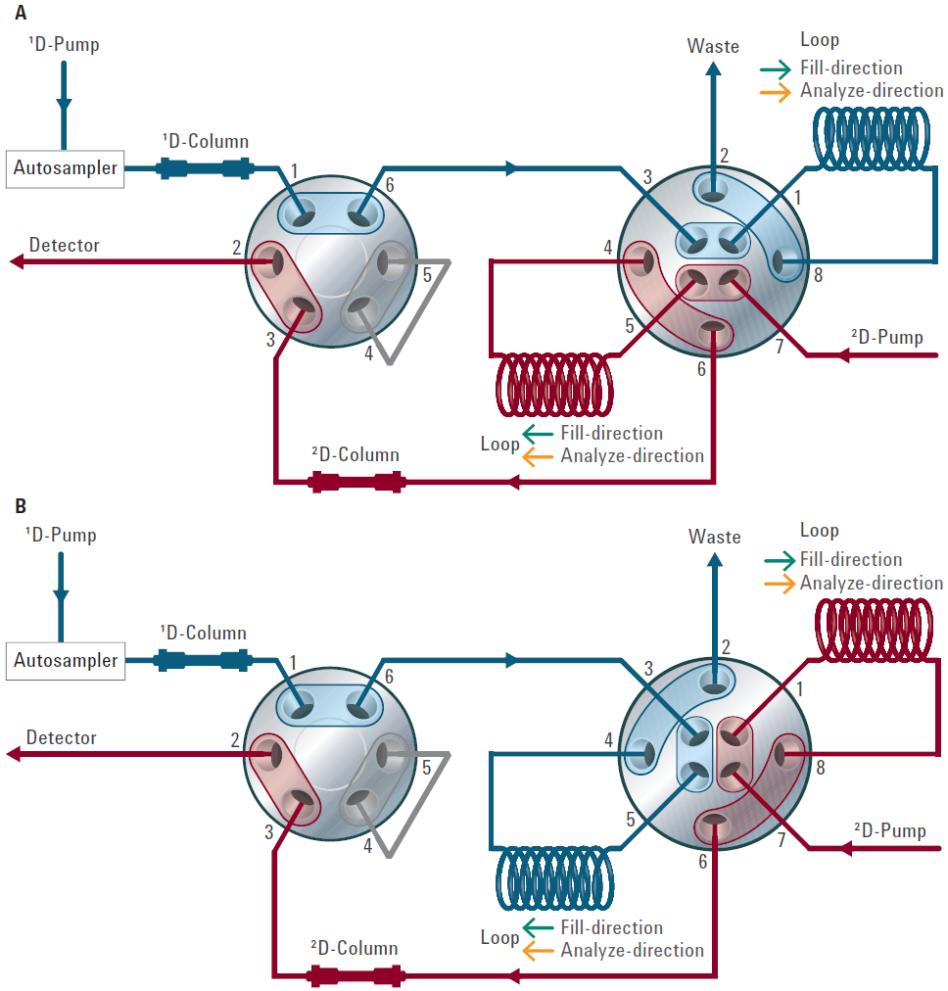


Figure 116 Setup of the Agilent 1290 Infinity 2D-LC Solution with an additional 2-position/6-port valve positioned for comprehensive 2D-LC analysis; (A) Collection of the effluent from the first dimension column in loop 1 (between ports 1 and 8); (B) Collection of the effluent from the first dimension 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

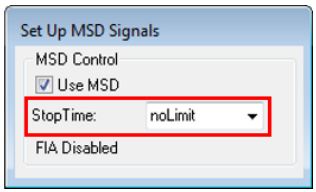
Agilent OpenLab 2D-LC Software is a fully integrated solution from method setup to data analysis for 2D-LC/MS within OpenLab CDS ChemStation Edition. It combines the separation power of two-dimensional chromatography with the selectivity of mass selective detection (MSD) in the second dimension. It can 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.

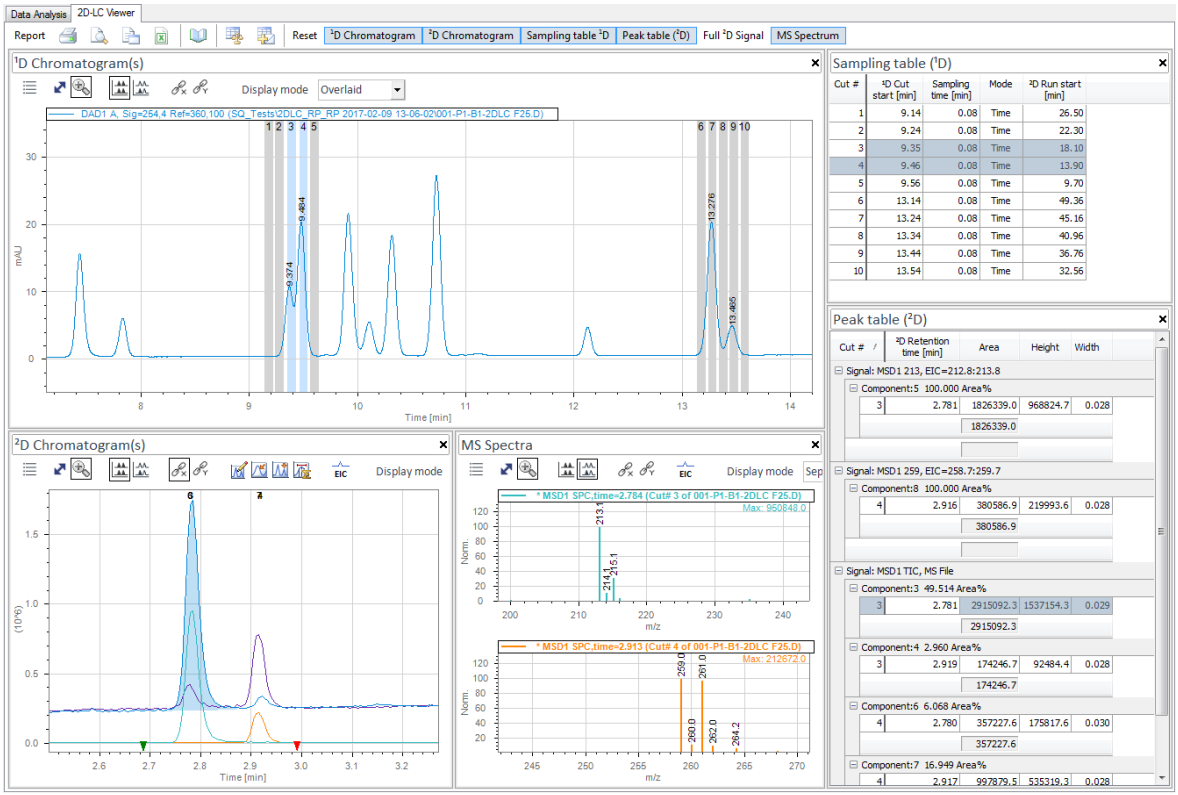
Using the Single Quadrupole Detector with 2D-LC

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".



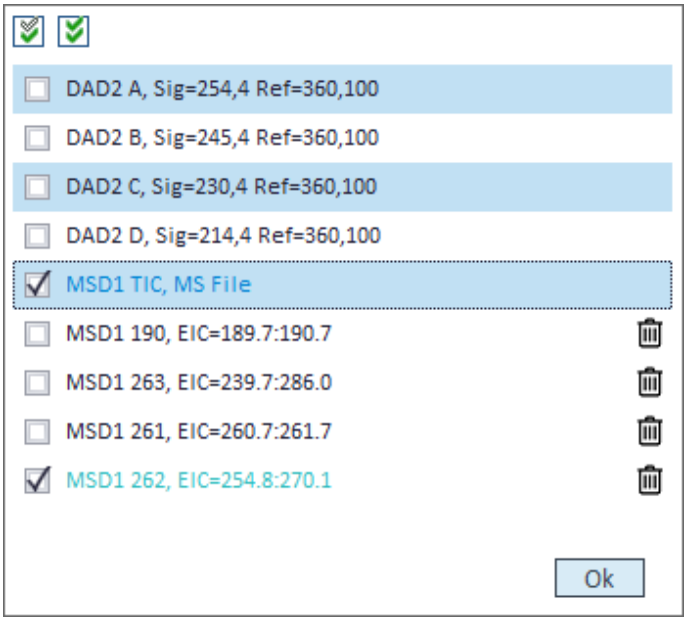
When setting up the MSD (**Instrument> Set up MSD signals> ...**), set **noLimit** as the **Stop Time**. This is required as the run time needs to be extended for unparking cuts.

2D-LC Viewer for Single Quadrupole Data



The 2D-LC Viewer displays now also MS and UV spectra and extracted ion chromatograms.

Select signals

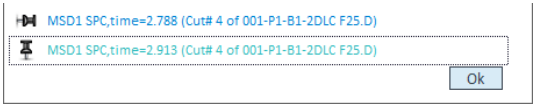


Multiple signals can be managed using the signal selector. Use checkmarks for choosing signals to be displayed.

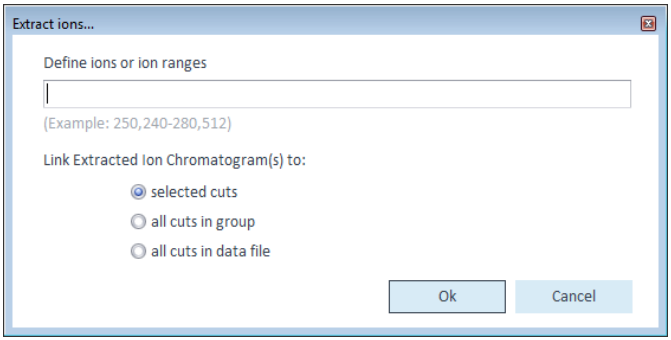
Select highlighted/all items
As a shortcut, multiple signals can quickly be highlighted by control- or shift-clicking signal names for the ^{2}D chromatogram panel and clicking the selection buttons.

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.

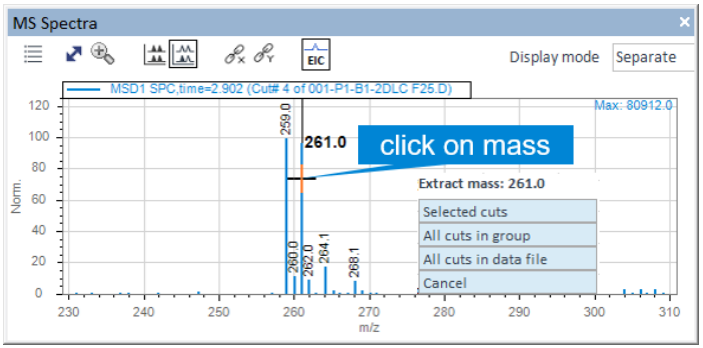
Pin spectra
Spectra can be pinned for storing them per associated cut for display and reporting:



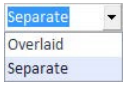
Extract ion chromatogram



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.

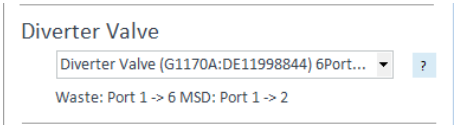


Separarate versus overlaid mode
Multiple (extracted ion) chromatograms or spectra can be displayed in the same window (overlay) or in separate windows.

same scale/full scale
Diagrams can be displayed using the same scale or the full scale for each diagram.

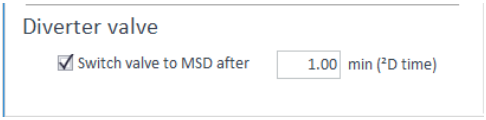
linked x and y axes
For zooming, the axes can optionally be linked for all displays. In case of linked axes, all displays will be zoomed to the same range.

Diverter Valve Configuration

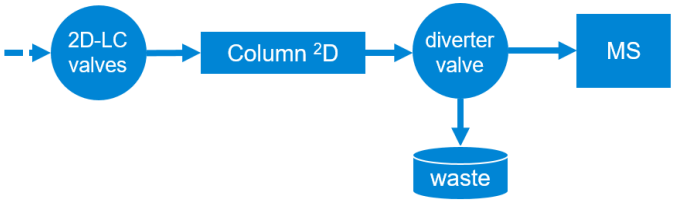


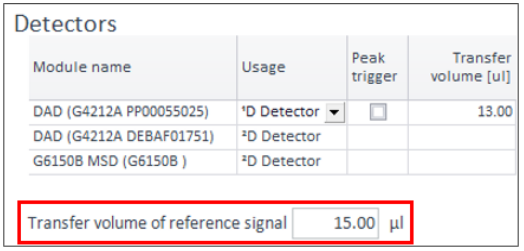
A diverter valve can be configured e.g. for removing buffers before they reach an MS detector. Any 2-position LC valve can be chosen in the configuration. Valves should be installed to external valve drives G1170A. Control is done via the CAN connection between 2D-LC modules and the diverter valve, which is not yet available for diverter valves in MS detectors. The user interface shows, which valve outlet ports go to waste vs. detector.

Diverter Valve Method Parameters

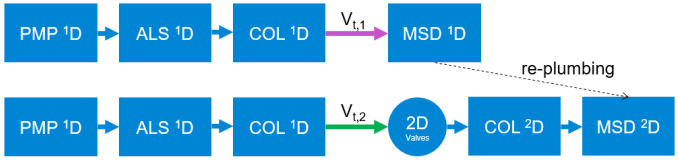


A two-position LC valve (e.g. a 2-pos/6-port valve) can be configured in the 2D-LC Configuration for diverting ^{2}D eluent with buffers before they enter an MS detector. As a method parameter, a ^{2}D time can be set, after which the flow path is directed to the detector.





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

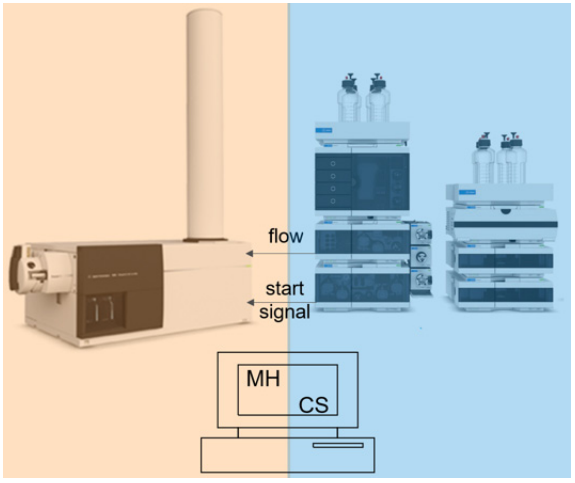


Figure 117 Typical system setup

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 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 219,
- LC Video Tutorial on the GC Image DVD,
- Or in the help file of the GC Image SW.

Table 25 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

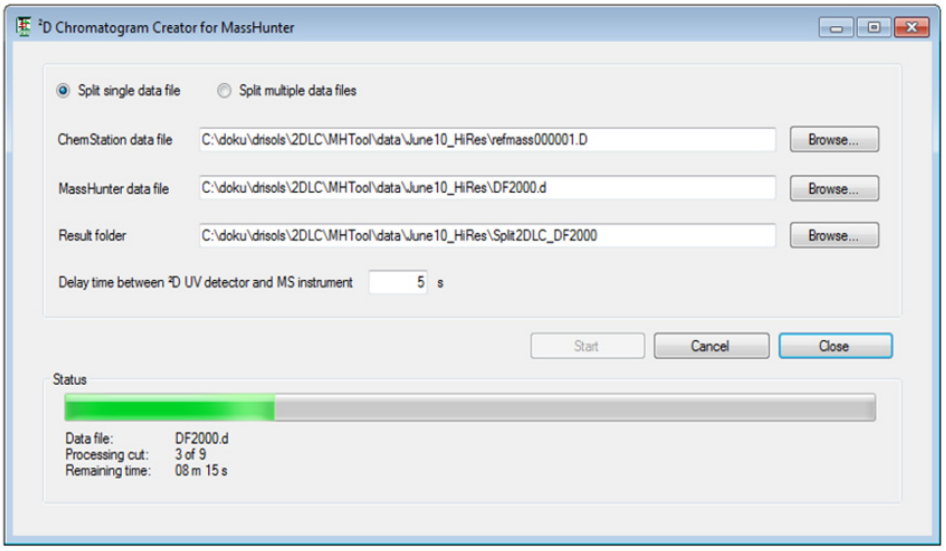


Figure 118 Use single data or sequences/worklists and set delay between optional 2D UV detector and MS detector for aligning signals

^{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 *2D 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 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.

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.

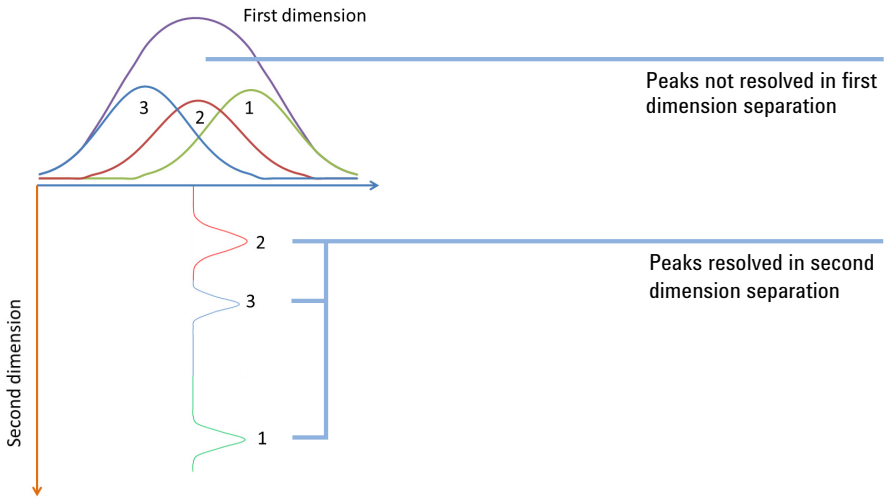


Figure 119 Peak capacity relationship between peak capacities of orthogonal first and second dimension

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 325,
- "Resolution" on page 325, and
- "Peak Capacity" on page 328

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 27 on page 333 and Table 28 on page 338.

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 120 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.

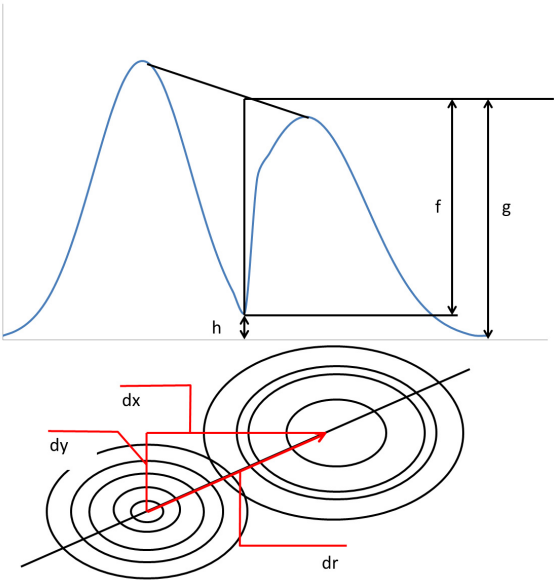


Figure 121 Diagram of 2D resolution measurement: Slice for resolution (top) and 2-dimensional contour plot (bottom)

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 122 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 123 2D-Resolution (peak to valley ratio relation)

Table 26 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 122 on page 327)

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 331). 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)

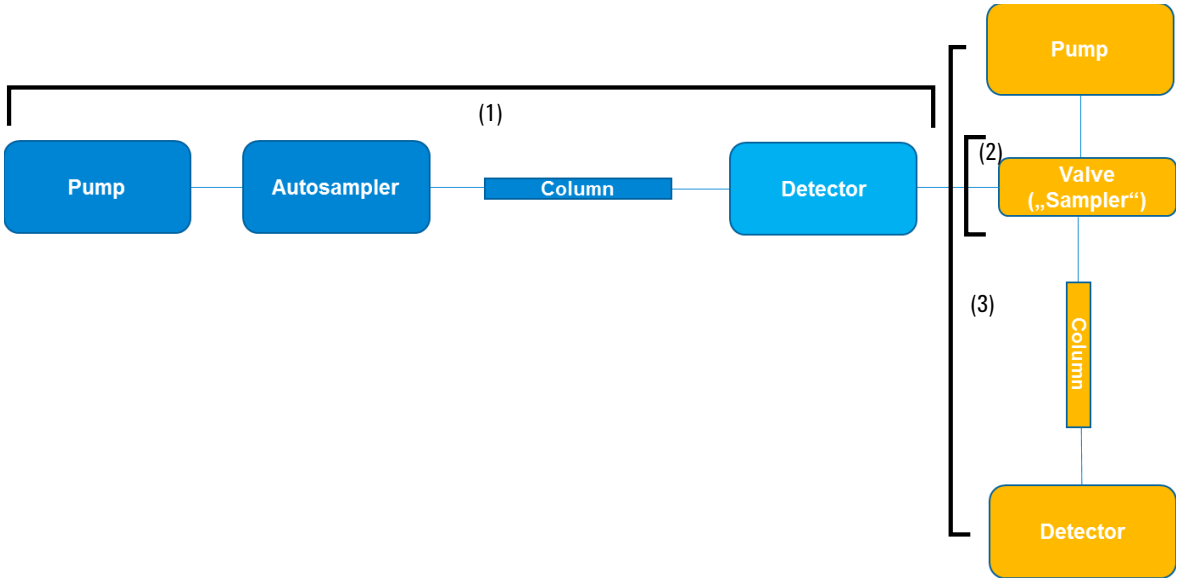


Figure 124 Diagram of instrumentation for 2D-LC

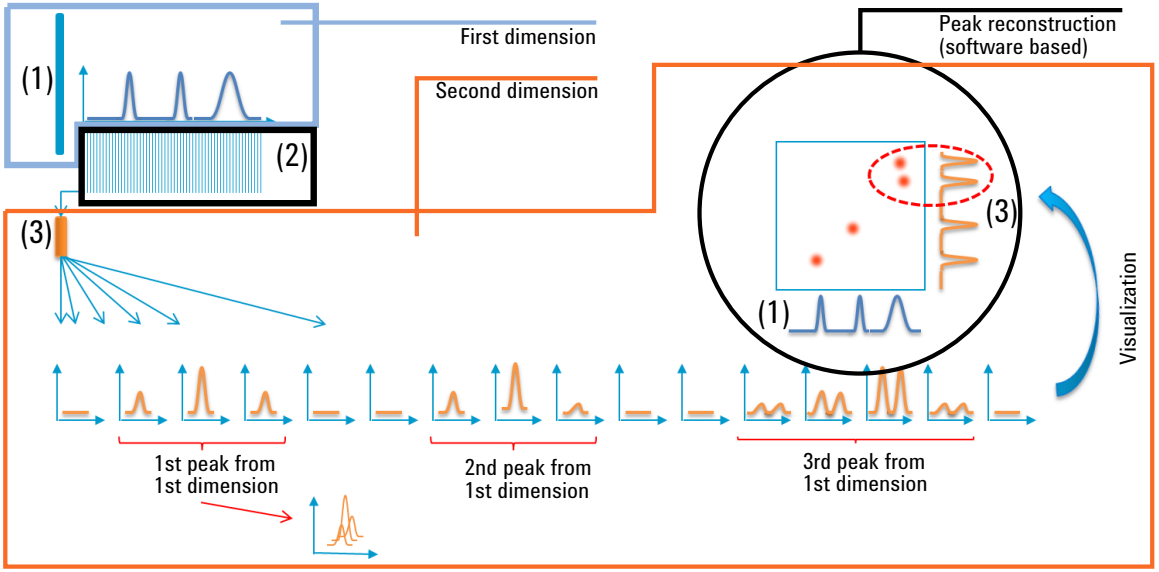


Figure 125 Principle of 2D-LC (example for LCxLC): Effluent of first column (1) is sampled (2) and injected to second column (3). Peaks of second column separation are detected and reconstructed.

(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 27 on page 333 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 27 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 28 on page 338 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

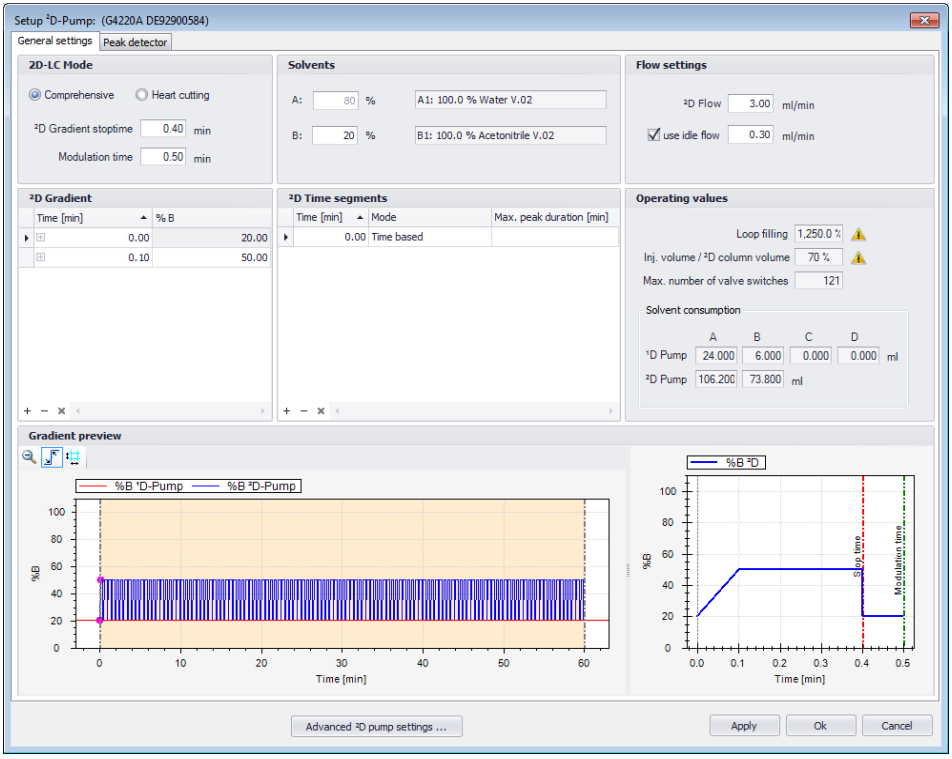


Figure 126 Standard gradient mode

- 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.

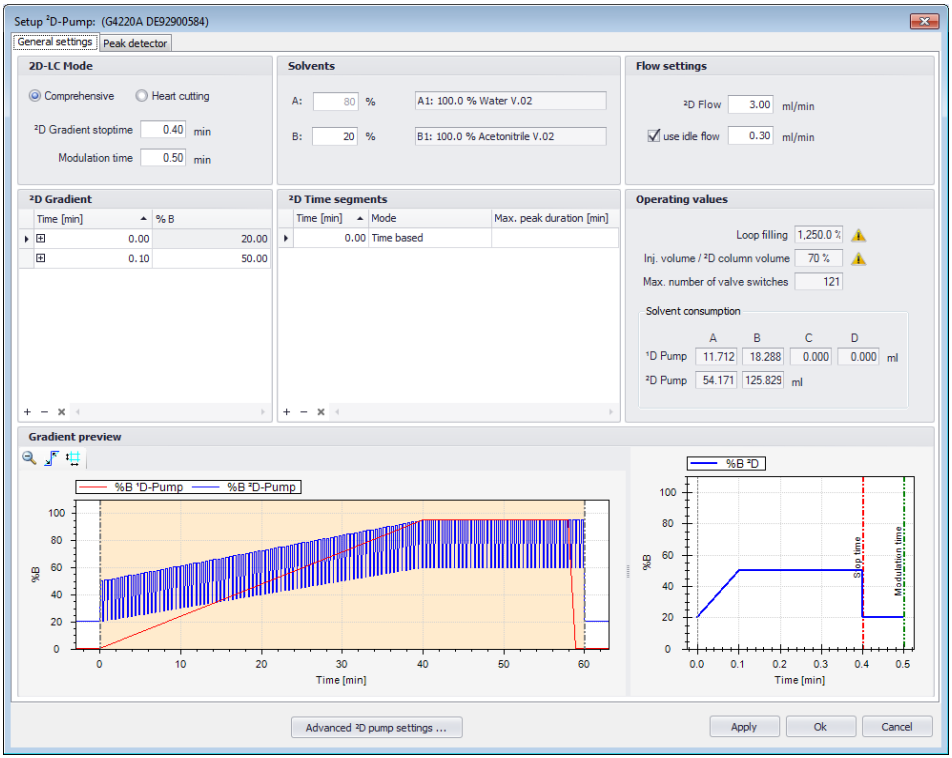


Figure 127 Shifted gradient mode with increase of start-%B

- Isocratic

  All second dimension separations will be carried out in an isocratic mode.

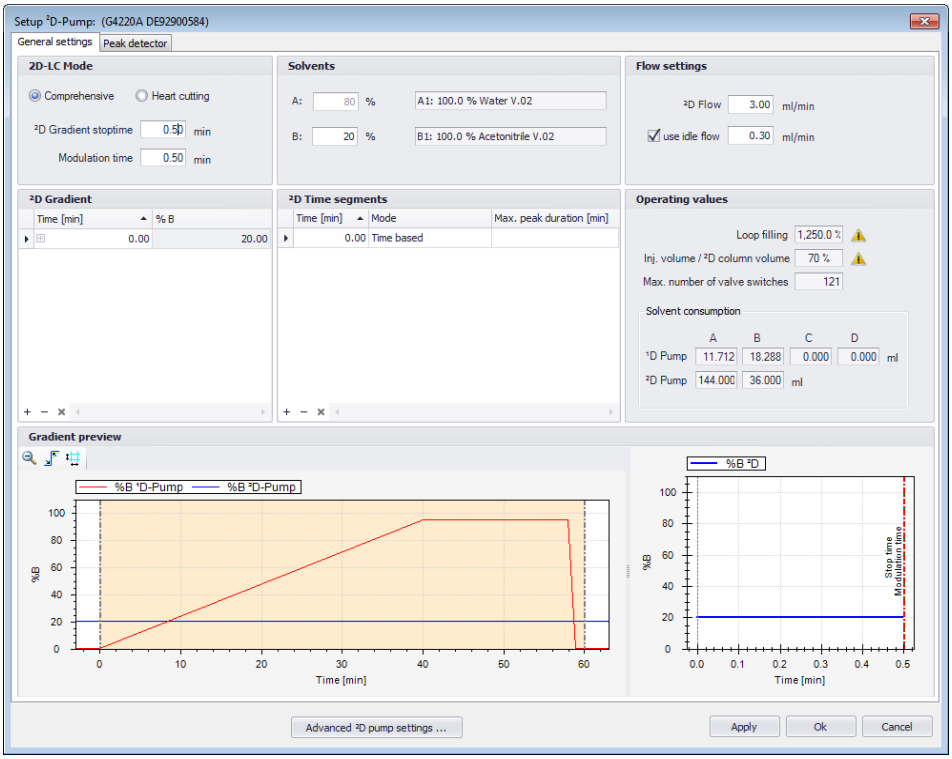


Figure 128 Isocratic mode

- 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.

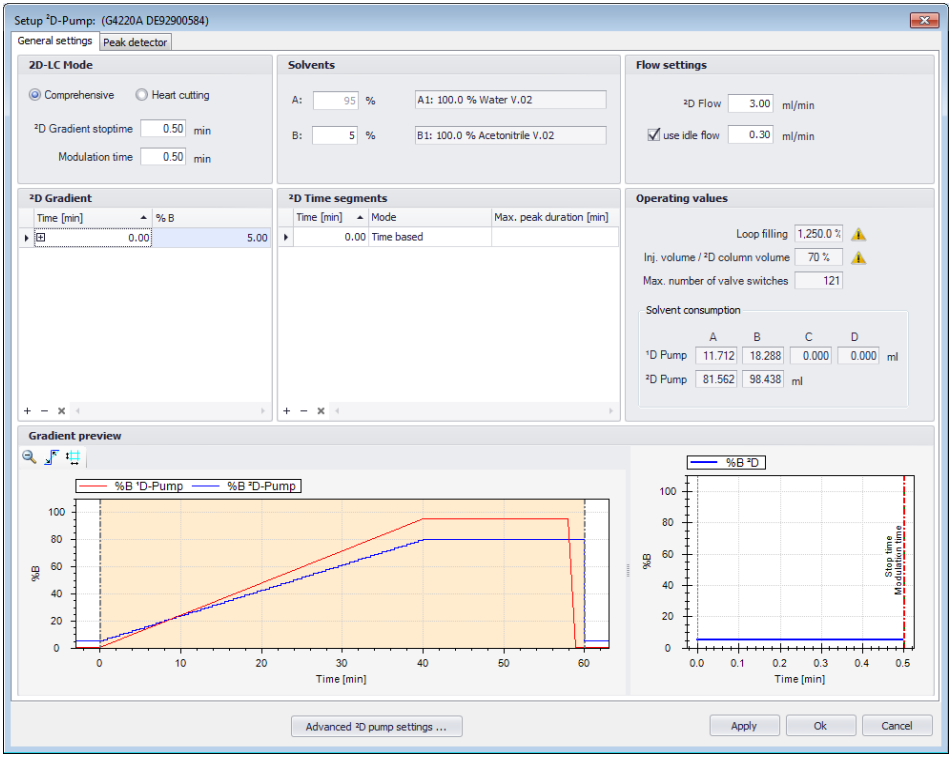


Figure 129 Advancing isocratic mode

Table 28 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

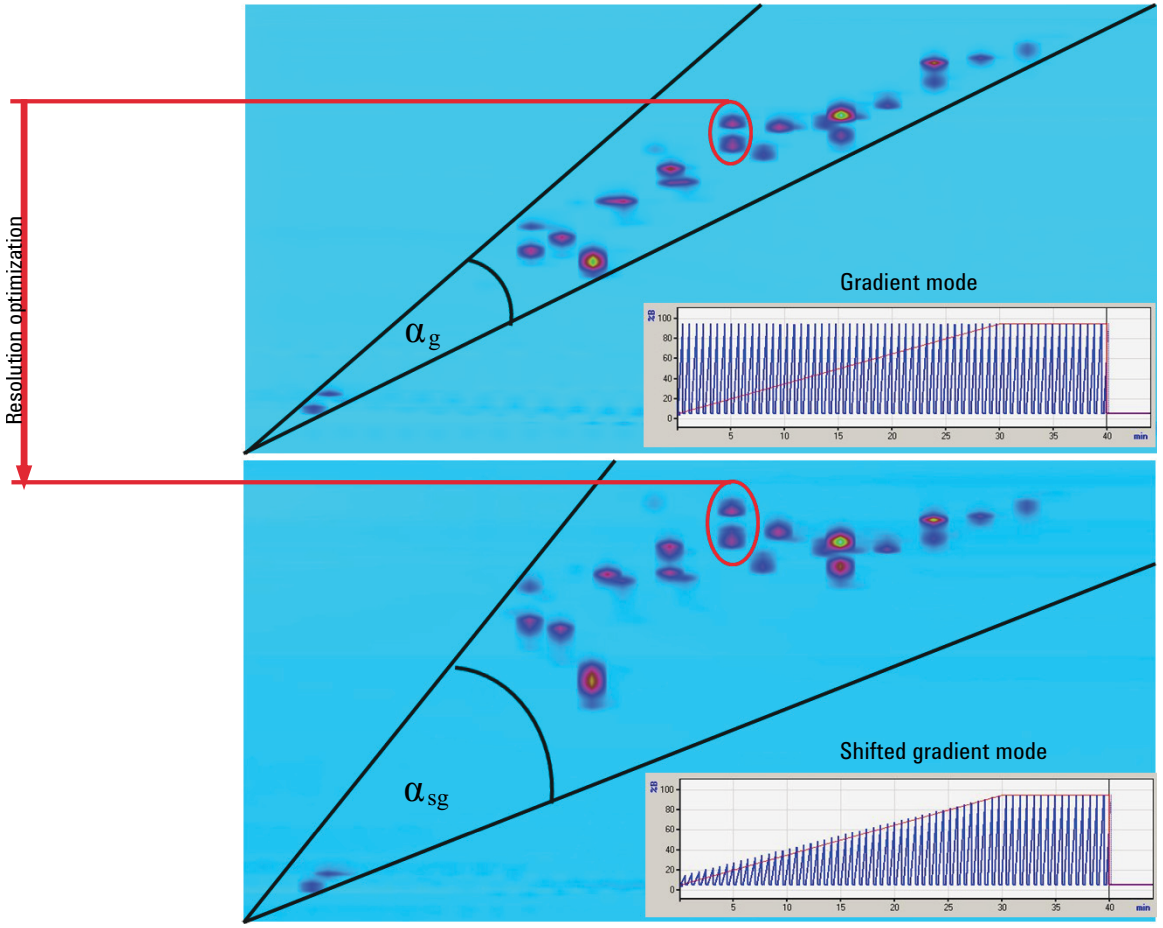


Figure 130 2nd dimension gradient mode compared to isocratic mode and its effect on resolution

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 29 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.

16 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 348.

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 30 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 30 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)

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**.

Agilent Technologies on Internet

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

http://www.agilent.com

In This Book

The manual describes the following:

- introduction,
- installing,
- configuring,
- using,
- data analysis,
- safety and related information.

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