Agilent 1290 Infinity 2D-LC-Solution

User Guide

Agilent Technologies

Notices

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In This Book

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

1 Product description

This chapter describes the product and the concepts of 2D-LC.

3 Standard Heart-Cutting 2D-LC

This chapter describes in detail the installation, configuration, method parameters, data analysis and checkout/familiarization of standard heart-cutting two dimensional liquid chromatography with the Agilent 1290 Infinity 2D-LC-Solution.

4 Multiple Heart-Cutting 2D-LC

This chapter describes in detail the installation, configuration, method parameters, data analysis and checkout/familiarization of multiple heart-cutting two dimensional liquid chromatography with the Agilent 1290 Infinity 2D-LC-Solution.

5 Full Comprehensive 2D-LC

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

6 Theoretical Background

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

7 Possible ways to install the System

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

8 Appendix

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

Contents

1
Product description

This chapter describes the product and the concepts of 2D-LC.

Introduction

The Agilent 1290 Infinity 2D-LC Solution is a combination of existing 1290 Infinity LC based hardware products with a new 2D-LC acquisition software add-on for OpenLAB CDS ChemStation edition and some new hardware products to set-up 2D-LC systems combining two orthogonal LC separations in a serial way to maximize separation efficiency in a single run.

It is designed to fulfill needs for highest separation power, for example in bio-pharma, pharma, proteomics, natural products, traditional chinese medicine, food matrices, polymers, and many more.

The Agilent 1290 Infinity 2D-LC Solution offers the following features and benefits:

- Easy-to-use for easiest 2D-LC system and method set-up
- Highest performance to achieve highest separation power
- Flexible hardware with upgrade possibilities of existing LC-systems (see "System Components" on page 258)
  - Different pumps, autosamplers and detectors supported - existing systems can be upgraded
  - Detectors at different positions
    - After 1st dimension column for peak triggered operation or to monitor first dimension separation directly
    - After 2nd dimension column

      Standard position for 2D-LC data acquisition

      At the waste-line to monitor the waste-line: In comprehensive 2D-LC no peak should appear here, in heart-cutting 2D-LC only the not-sampled peaks should appear at the waste line
  - Allows the Agilent 1290 Infinity Quaternary Pump, Agilent 1290 Infinity Binary Pump, Agilent 1260 Infinity Binary Pump and almost any Agilent autosampler in the 1st dimension but requires an Agilent 1290 Infinity Binary Pump for the 2nd dimension

- Supports almost any detectors for data acquisition in the second dimension and many UV-detectors after the first dimension column for peak-triggered operations
- Supports the new Agilent 2D-LC Quick-Change Valve (single valve with fully symmetric flow-paths and symmetric fill/flush-out behavior and countercurrent flush-out of both loops) (see "Valve Options" on page 38)
- Supports also many literature known valve configurations based on 2 Pos/10 Port or 2 Pos/6 Port valves (see "Valve Options" on page 38)
- Supports dual valve-head configurations with automatic synchronization of valve-drives

- Innovative features:
  - Hardware:
    - New special 2D-LC valve for fully symmetric flow-paths and counter-current fill/analyze direction of loops in comprehensive 2D-LC to reduce literature known artifacts and improve separation performance
  - Software:
    - Prepared for comprehensive 2D-LC (see "Comprehensive 2D-LC (LCxLC)" on page 159) and heart-cutting 2D-LC (see "Heart-Cutting 2D-LC (LC-LC)" on page 25)
    - Easy-to-use time segments for time-based or peak-triggered operation to save solvents and increase life-time of interfacing valve
    - *Shifted gradient* feature for improved separations in comprehensive 2D-LC – most complex 2D-gradients set-up by a graphical tool in a minimum time
    - Shows all modules in one dashboard
    - Standard repeating 2D-gradient with start- and end-time
    - *Shifted gradient* feature, offering: *Advancing isocratic* gradients
    - 2D-gradients with constantly shifted $\%B_{2D}$ from one gradient to the next
    - 2D-gradients with constantly shifted $\%B_{2D}$ and shifted $\Delta\%B_{2D}$ from one gradient to the next

- Allows combinations of the above listed gradient modes at different time during the first dimension separation like combination of iscocratic with advancing isocratic, or standard gradient with shifted $\%B_{2D}$ and/or shifted $\Delta\%B_{2D}$

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.

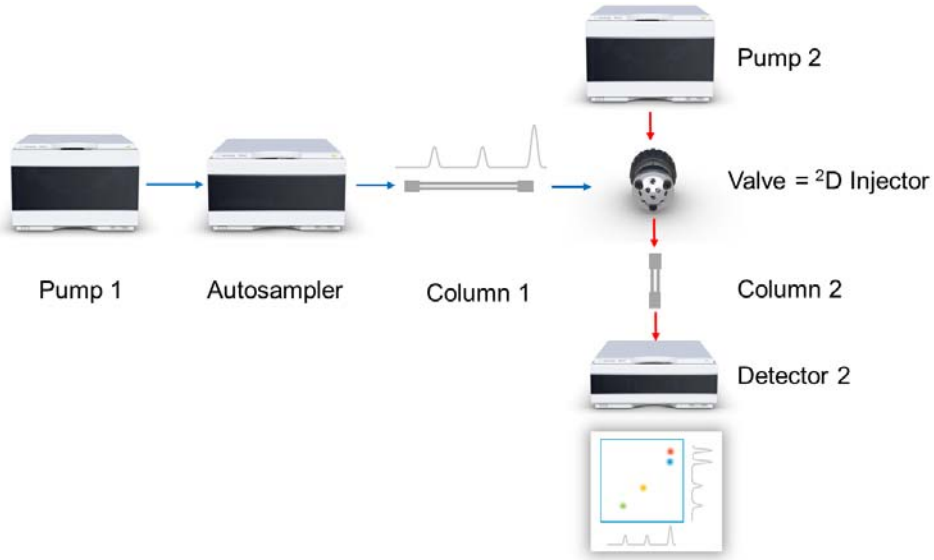
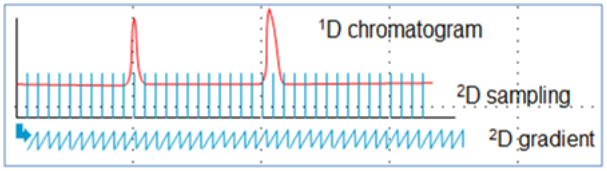


Figure 1 Concept of a 2D-LC-System

Comprehensive 2D-LC

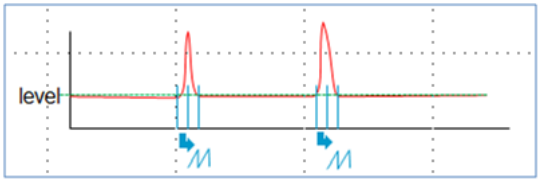
In comprehensive 2D-LC (also known as LC×LC), the total eluent from the first dimension is injected on to the column in the second dimension using two equal-sized sampling loops that are alternated by a switching valve. While the first loop is being filled in the first dimension, the contents of the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



Peak-triggered comprehensive 2D-LC

In peak-triggered comprehensive 2D-LC, only peaks that exceed a specified threshold level are sampled in the first dimension. As with normal comprehensive 2D-LC, the gradient analysis in the second dimension is less than or equal to the sampling time in the first dimension:



Depending on the peak width and the duration of the second-dimension gradient analysis, a peak may give rise to more than one sample.

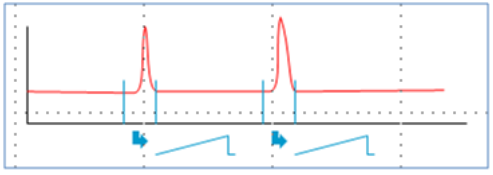
Heart-cutting 2D-LC

In heart-cutting 2D-LC (also known as LC-LC), only parts of the eluent from the first dimension are injected into the second dimension using a switching valve and at least one sample loop. The gradient analysis in the second dimension can be longer than the sampling time, but in the case of single-loop sampling must be shorter than the interval between first-dimension samples.

The volume of sample to be injected on to the second dimension is defined either by a peak trigger or by a time window. When heart cutting starts, the loop is switched into the flow path of the first dimension, and switched back eother when the peak ends or at the end of the time window. The second dimension analysis starts when sampling is complete.

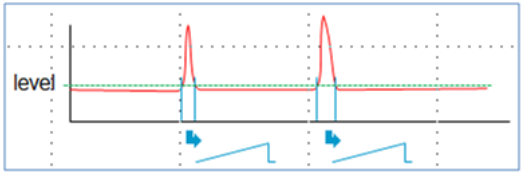
Time-based heart cutting

In time-based heart cutting, specific sampling time windows are set up; only the eluent from the first dimension in the specified time windows are injected and analyzed in the second dimension:



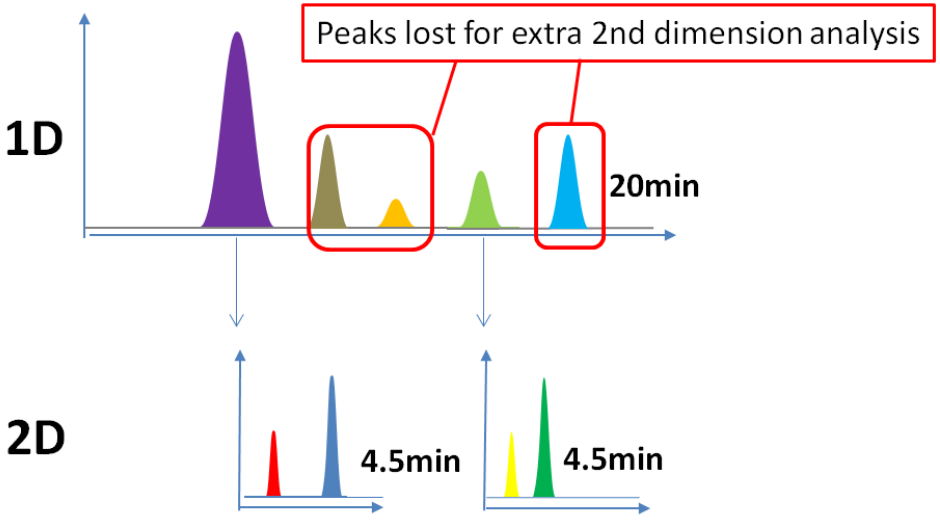
Peak-triggered heart cutting

In peak-triggered heart cutting, only peaks that exceed a specified threshold level are sampled in the first dimension and injected and analyzed in the second dimension:

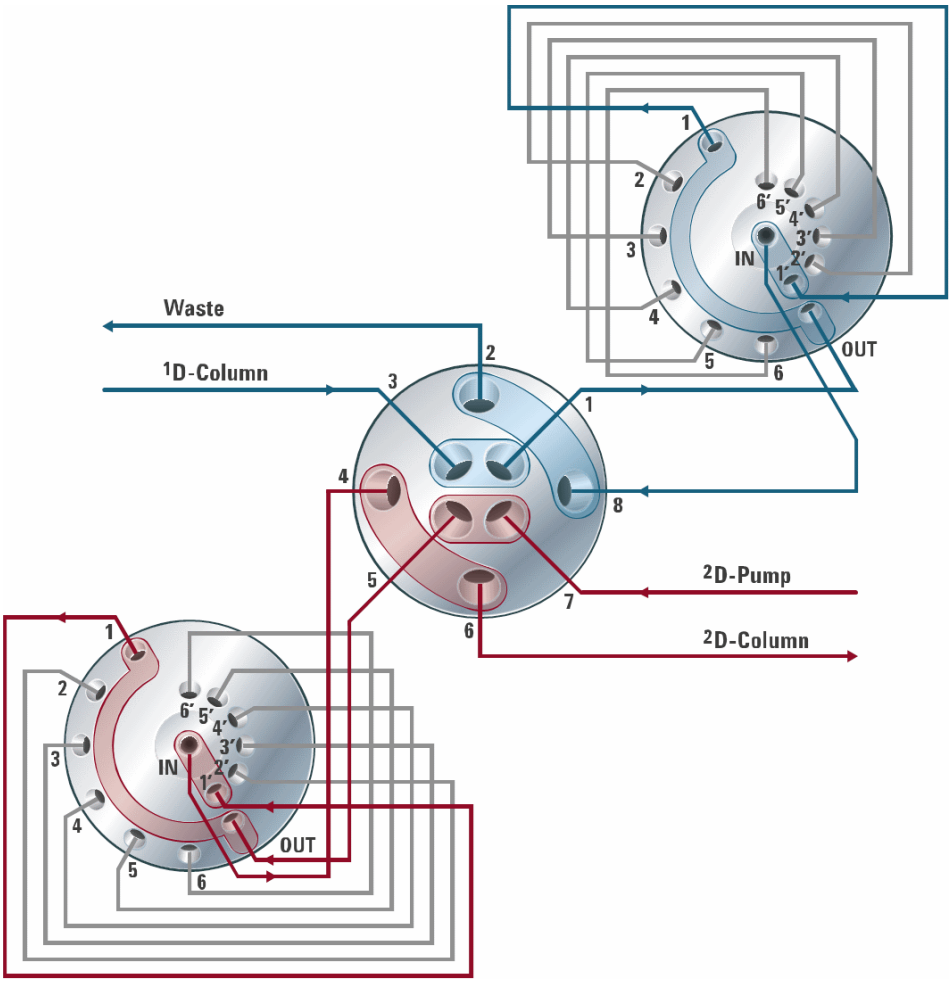


Multiple Heart-cutting

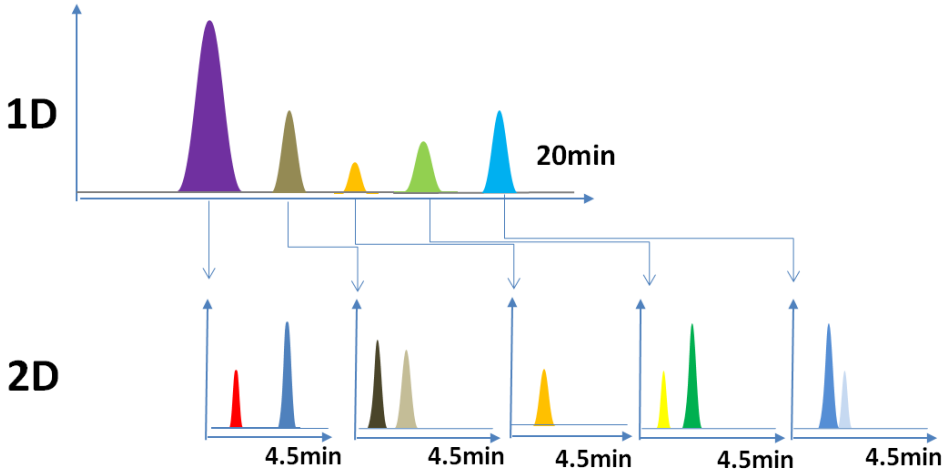
Typically, the gradient time in the second dimension is much longer for *heart-cutting* than with the *comprehensive* technique. The disadvantage of the standard heart-cutting techniques is that peaks cannot be sampled while a second dimension gradient is still running. In the examples shown here, the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

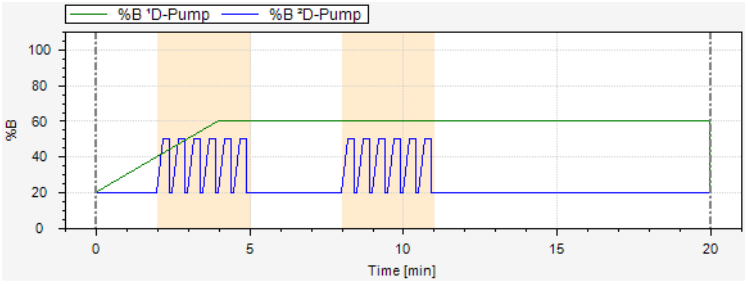


Peaks that are cut out and stored during a run are analyzed consecutively in the second dimension, even when the first dimension is still running. To avoid carry-over the peaks are analyzed in reverse order of storage in a single Multiple Heart-Cutting Valve.



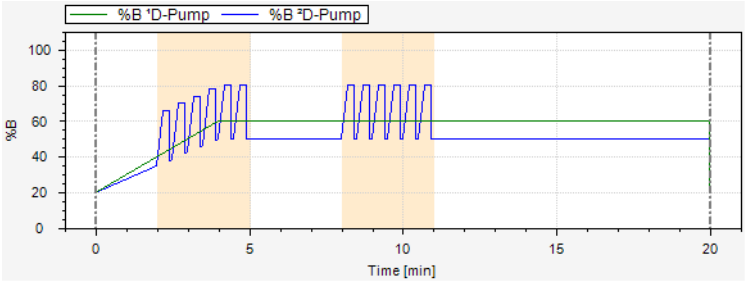
Gradient Shift

When the chromatographic separation in the first dimension requires a gradient elution, it may be necessary or desirable to change the solvent gradient in the second dimension in line with the changing solvent composition of the first dimension. This is handled by a gradient shift, which is set up in the second dimension gradient timetable.



In the upper case, the second dimension solvent gradient does not change, despite the changing composition of the solvent in the first dimension.

In the lower case, the gradient composition in the second dimension is shifted to match the changing solvent composition in the first dimension.



Reference Signal

You can can load a reference signal (a chromatographic signal from an LC detector) that is shown in the gradient preview, and becomes part of the 2D-LC method. This means that the 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 (and the display), or replaced by another signal.

The reference signal helps you to setup (time-based) heart cuts when the chromatogram of the sample is known in advance, and allows you to visualize the peaks that would be analyzed in the second dimension based on the current peak detector settings (threshold, slope).

2
Quick Installation Guide

Quick Installation of the Agilent 1290 Infinity 2D-LC-Solution

One of the strengths of the Agilent 1290 Infinity 2D-LC solution is it's hardware flexibility. Many different modules can be used and even existing systems can be upgraded. The only requirement is a 1290 Infinity Binary pump as second dimension pump and a unit containing an Agilent Quick Change valve drive plus a valve suitable for 2D-LC. Still the pre-defined system setups are highly recommended as only these setups are supported for the familiarization procedure and its methods.

The final stack configurations and necessary parts depend on the 2D-LC-Method.

- Standard Heart-cutting, see "Recommended fix Setups for Standard Heart-Cutting 2D-LC" on page 29
- Multiple Heart-cutting, see "Recommended fix Setups for Multiple Heart-Cutting 2D-LC" on page 97
- Comprehensive: "Recommended fix Setups for Comprehensive 2D-LC" on page 164

Prerequisites

Agilent 2D-LC software A.01.02 requires ChemStation C.01.07. The minimum firmware revision set is A.06.5x, B/C/D06.70, B06.71 (1290 Infinity pumps).

NOTE

Software Edition

Recommended: at least M8301AA OpenLAB CDS ChemStation Edition C.01.04.

Best choice: M8301AA OpenLAB CDS ChemStation Edition C.01.07 (provides the Heart-Cut-Viewer option, which is esential for the use of the multiple heartcutting technology).

For the very complex data sets produced with comprehensive 2D-LC Agilent recommends GC Image LCxLC edition Software from GC Image LLC, USA.

1 Set up the components.

NOTE In general, keep the connecting capillaries between the modules as short as possible. All modules come with connecting capillaries in their specific accessory kit. If additional or different capillaries are needed, Agilent offers a broad variety as can be found in the *Agilent LC Capillary Supplies Selection Guide (Publication Partnumber 5991-0121EN)*.

2 Interface the first and second dimension.

For interfacing opportunities, see "Interfacing the First and Second Dimension" on page 266

NOTE To reduce band spreading and for best performance keep the especially the capillaries after the columns as short as possible.

3 Physically connect hardware to computer with controller (OpenLAB CDS ChemStation edition C01.03 or higher).

4 Install the software.

For details, see "Installing the Software" on page 42.

5 Configure the system.

For details, see "Overview Menu Extensions" on page 42

6 Setup the method.

7 Run the system.

8 Analyse the Data, see "Overview" on page 207 , "Data Analysis for Heartcutting 2D-LC (LC-LC)" on page 68, or "Heart-Cutting Viewer" on page 69.

NOTE The Agilent 1290 Infinity 2D-LC Solution always produces one single data-file for the data acquired on the detector in the second dimension.

2 Quick Installation Guide

Quick Installation of the Agilent 1290 Infinity 2D-LC-Solution

3
Standard Heart-Cutting 2D-LC

This chapter describes in detail the installation, configuration, method parameters, data analysis and checkout/familiarization of standard heart-cutting two dimensional liquid chromatography with the Agilent 1290 Infinity 2D-LC-Solution.

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).

There are two modes of LC-LC:

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

Time-triggered LC-LC

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

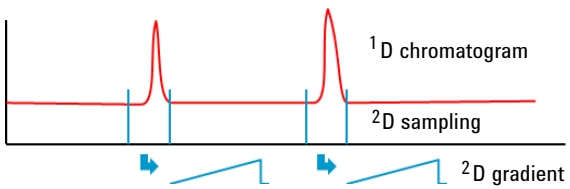


Figure 2 Principles of time-triggered LC-LC

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.

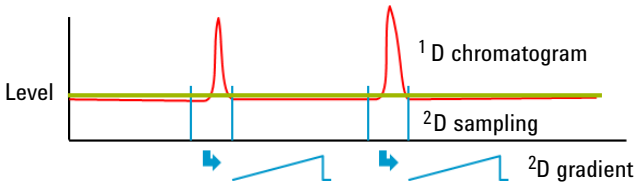


Figure 3 Principles of peak-triggered LC-LC

Concept of Peak Triggering

Triggering is done in advanced settings similar to integrator settings by threshold and/or slope, see Figure 4 on page 27.

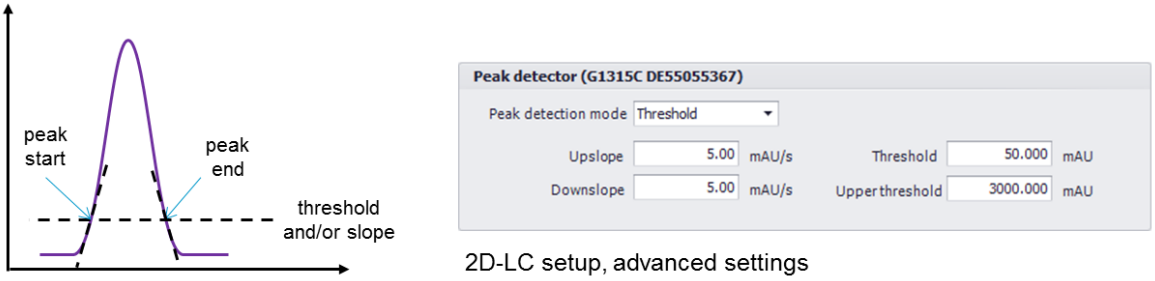


Figure 4 Peak triggering

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

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

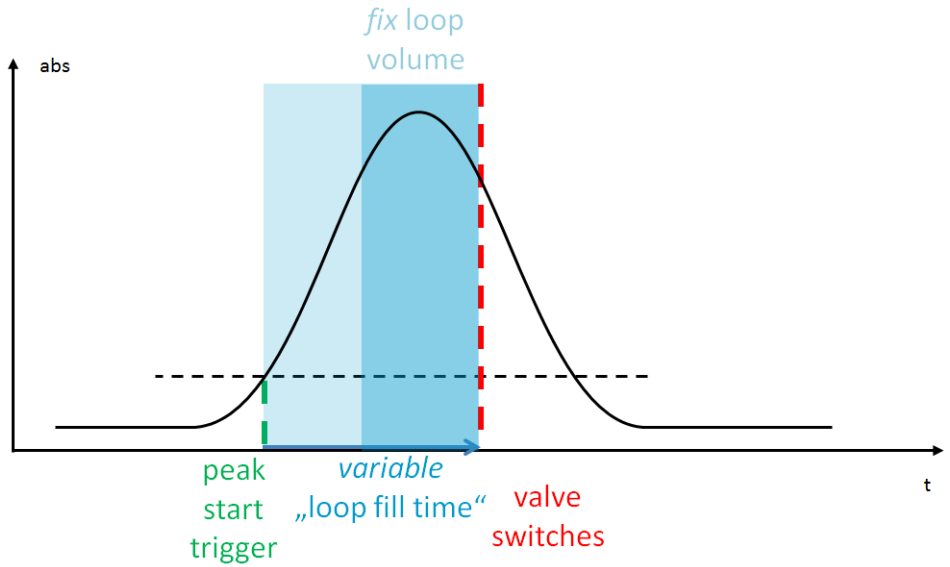


Figure 5 Peak triggering concept (elapsed sampling time)

- If the signal falls below threshold or slope.

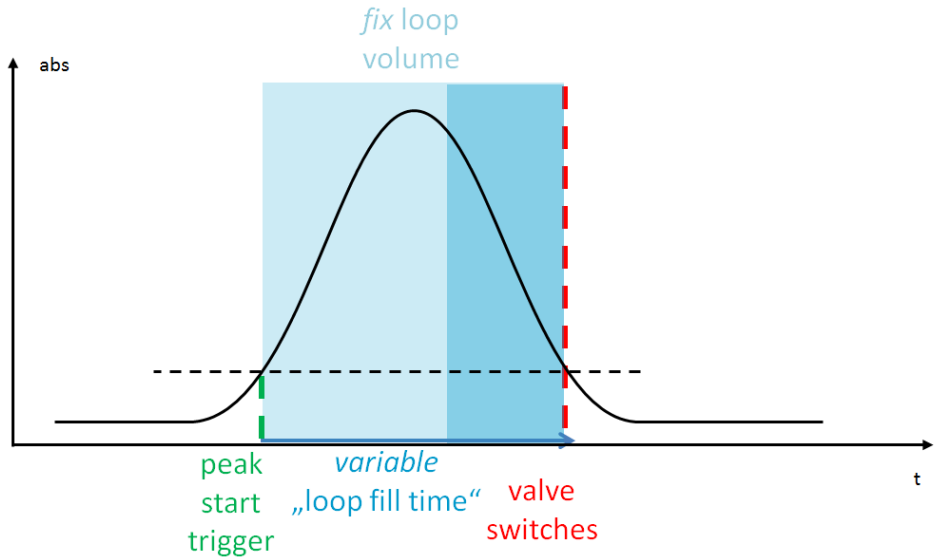


Figure 6 Peak triggering concept (signal falls below threshold or slope)

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.

Installation

Recommended fix Setups for Standard Heart-Cutting 2D-LC

Table 1 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 The optional available 1D/2D switch 2pos/6port Valve head 1200 bar (G4231B) may be installed in the Thermostatted Column Compartment. For details see "1D/2D-Switch" on page 41.

Table 2 1290 Infinity Quaternary LC in first dimension

	Partnumber	Description	Comment
1st Dim	G4204A	**1290 Infinity Quaternary 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 The optional available 1D/2D switch 2pos/6port Valve head 1200 bar (G4231B) may be installed in the Thermostatted Column Compartment. For details see "1D/2D-Switch" on page 41.

Table 3 1260 Infinity Binary LC in first dimension

	Partnumber	Description	Comment
1st Dim	G1312B	**1260 Infinity Binary Pump**	
	G1367E	1260 Infinity High Performance Autosampler	
	G1330B	1290 Thermostat	
	G1316A	1260 Thermostatted Column Compartment	
	G4212B	1260 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 The optional available 1D/2D switch 2pos/6port Valve head 600 bar (G4231A) (G1316A#055) should be installed in the Thermostatted Column Compartment. For details see "1D/2D-Switch" on page 41.

Possible stack configurations

The following configurations optimize the system flow path, ensuring minimum delay volume.

NOTE The capillary connections should be as short as possible, to ensure optimum performance of the system.

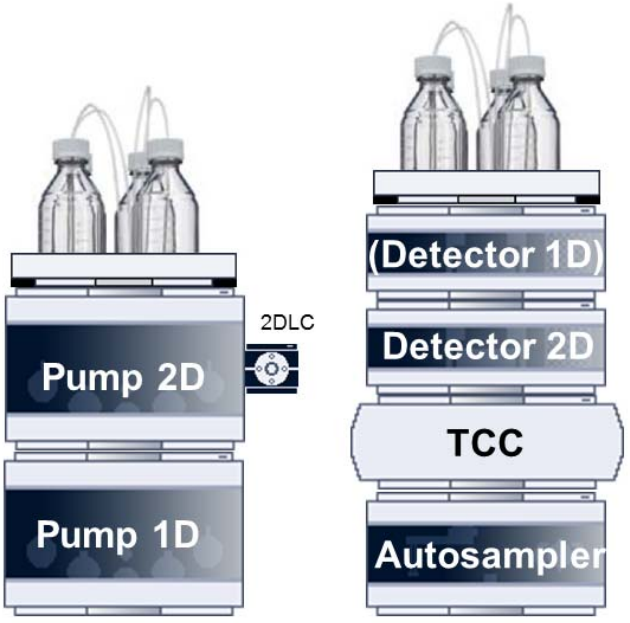


Figure 7 Stack configuration for Standard Heart-cutting 2D-LC

Table 4 1290 Infinity 2D-LC-System

Left Stack	Right Stack
	Solvent Cabinet
	^{1}D detector (DAD (G4212A or G7117A/B)) - (optional)
Solvent Cabinet	^{2}D detector (DAD (G4212A or G7117A/B))
^{2}D pump (G4220A) with Valve Drive (G1170A) attached	TCC (G1316C)
^{1}D pump (G4220A/G4204A)	Autosampler (G4226A)

Table 5 1260 Infinity 2D-LC-System

Left Stack	Right Stack
	Solvent Cabinet
	^{1}D detector (DAD (G4212A/B or G7117A/B)) - (optional)
Solvent Cabinet	^{2}D detector (DAD (G4212A/B or G7117A/B))
^{2}D pump (G4220A) with Valve Drive (G1170A) attached	TCC (G1316C)
^{1}D pump (G4220A or G4204A or G1312A/B/C or G1311A or G1376A)	Autosampler (G4226A or G1367E/F)

Capillary connections (kits)

After placing the modules of the first dimension and second dimension and making the electrical connections the flow paths must be build.

Capillary Kit for 2D-LC

Item	p/n	Description
1	5021-1820 (2x)	Flex capillary, 0.12 x 105 mm, no fittings
2	G1316-87321	Capillary column-heat exchanger 105 mm lg, 0.17 mm i.d.
3	5021-1822	Flexible tubing, 280 mm
4	5021-1823 (3x)	Capillary column – detector SST 400 mm lg, 0.12 mm i.d.
5	5021-1819	Capillary ST 0.17 mm x 400 mm S/S
6	5065-9964	Capillary ST 0.12 mm x 500 mm
7	5067-4609	Capillary ST 0.17 mm x 500 mm SX/-
8	5067-4669	Capillary ST 0.12 mm x 600 mm S/SL
9	01078-87305	Capillary, 0.17 mm x 80 cm, male fit
10	G1316-80022 (2x)	LDHE double kit for G1316C

Supported valves and valve hosts

Two valves are supported as modulation valve in the 1D-2D interface:

- G4236A 2,4 Duo valve (highly recommended!)
- G4232B 2,10 valve (possible, but not recommended)

NOTE The formerly support setup using a 2/6 valve in the interface is not supported anymore from SW version *A.01.02*.

The following valve hosts are available in Agilent´s portfolio:

- G1170A 1290 Infinity Valve Drive
  Highly recommended for 2D-LC valve
- G1316C 1290 Infinity Thermostatted Column Compartment
  not recommended/forbidden for G4236A, but allowed for 1D/2D-switch

Interfacing the First and Second Dimension

Thermostatted Column Compartment Setup

Different variants of thermostatted column compartment setup are supported with the optional 2D-LC capillary kit for the 2D-LC valve (G4236A#3):

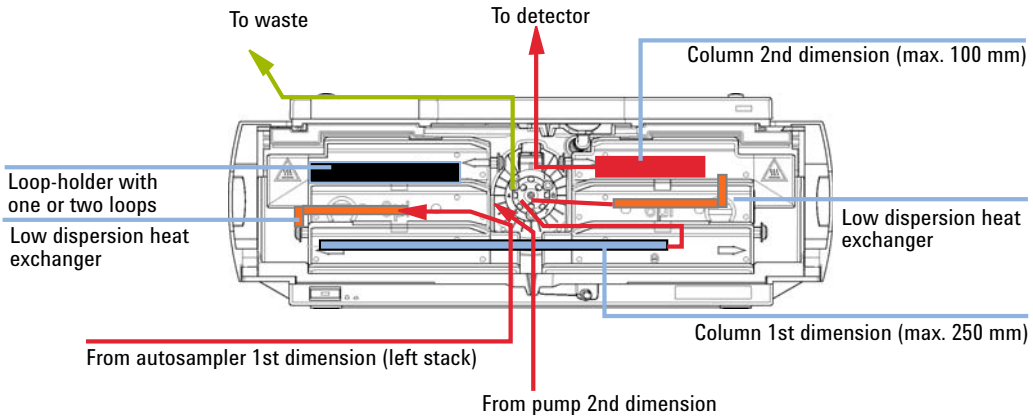


Figure 8 Columns at different temperatures,long 1st dimension column, no 1st dimension detector, for comprehensive 2D-LC booth loops can be placed in the loop holder.

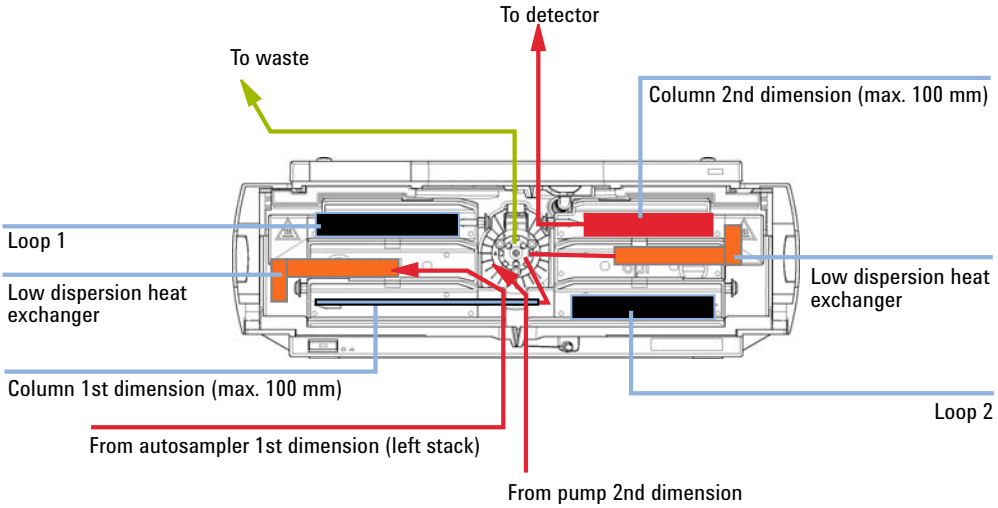


Figure 9 Columns at different temperatures, medium 1st dimension column, no 1st dimension detector

Valve Options

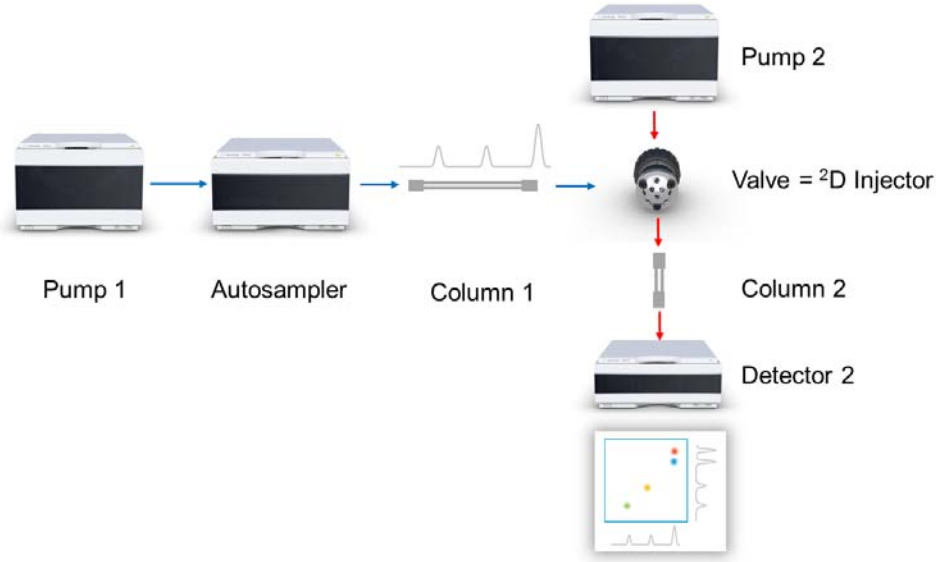


Figure 10 Concept of a 2D-LC-System

The Agilent 1290 Infinity 2D-LC Solution supports the following valve configurations:

- 2D-LC-Valve (2pos/4port-duo valve) (highly recommended)
- 2 Pos/10 Port Valve

2D-LC Quick-Change Valve

Advantages of the Agilent 2D-LC valve:

- Has fully symmetric flow paths (no additional bridging loops)
- Offers symmetric fill- and flush-out behavior and allows depending on plumbing either counter-current or co-current flush-out of both loops
- Due to its special design it delivers lowest pressure spikes to the columns. This lower stress guarantees a longer life time of the columns in the second dimension.

For details, see Table 6 on page 40 (standard heart-cutting) and Table 28 on page 176 (full comprehensive).

2pos/10port Valve

Support of 2pos/10port valve for comprehensive and heart-cutting 2D-LC allows easy transfer or existing 2D-LC methods. Both symmetric and asymmetric set-up supported in the software.

Interfacing First and Second Dimension for Heartcutting 2D-LC (LC-LC)

In a heartcutting experiments only a part of the first dimension is transferred to the second dimension and analyzed.

NOTE Heartcutting 2D-LC experiments usually are characterized by longer runtimes and shallower 2D gradients, compared to comprehensive 2D-LC (LCxLC) experiments.

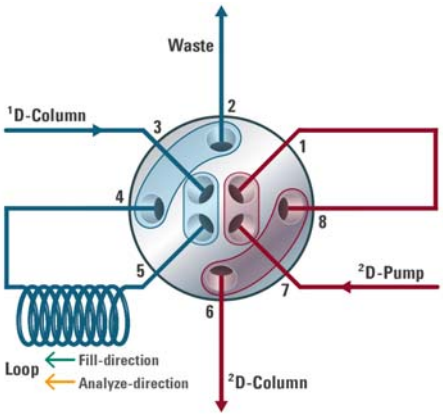
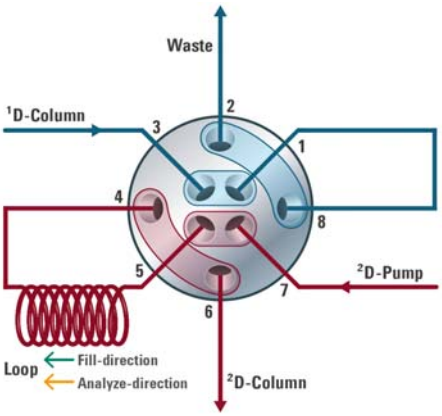
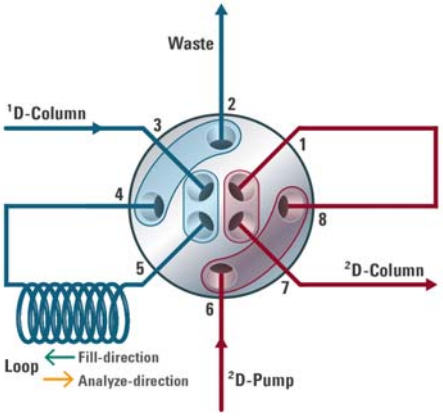
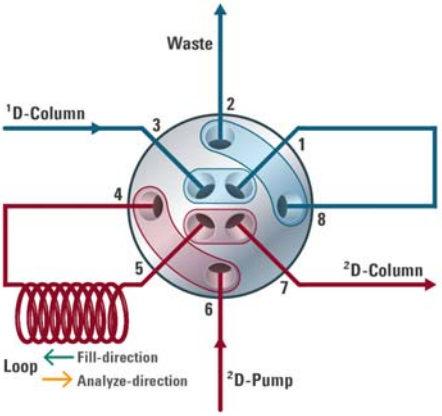
NOTE In general the valve set-up with two loops as used for comprehensive 2D-LC can be used as well for heart-cutting 2D-LC but one must keep in mind that additional solvent volumes are caught in the not used loop. Depending on the method this might cause artifacts.

Dual 2 Pos/4 Port Valve

In this case of heartcutting 2D-LC only one sampling loop is connected to the valve. The other position is connected by a short capillary. This enables the system to switch a clean capillary from the second dimension to the first dimension when loading is necessary and back for analysis after loading. The first dimension eluent in the short capillary can be neglected and does not contaminate the second dimension (Table 6 on page 40).

This type of plumbing for a heartcutting experiments can be done in different flow schemes:

Table 6 Plumbing for 2D-LC Valve Head, heart-cutting 2D-LC

	Filling	Analyzing
Cocurrent	 **Figure 11** 2D-LC Valve Head, position 1	 **Figure 12** 2D-LC Valve Head, position 2
Countercurrent	 **Figure 13** 2D-LC Valve Head, position 1	 **Figure 14** 2D-LC Valve Head, position 2

Supported modules/systems

1D/2D-Switch

The 1D/2D-Switch enables the user to simply switch between a 1D- and 2D-LC setup without replacing the capillary connections. For this setup (bypass of 2nd dimension) an additional 2/6 valve is needed. For details, see Automated Switching Between 1D-LC and Comprehensive 2D-LC Analysis (5991-4843EN).

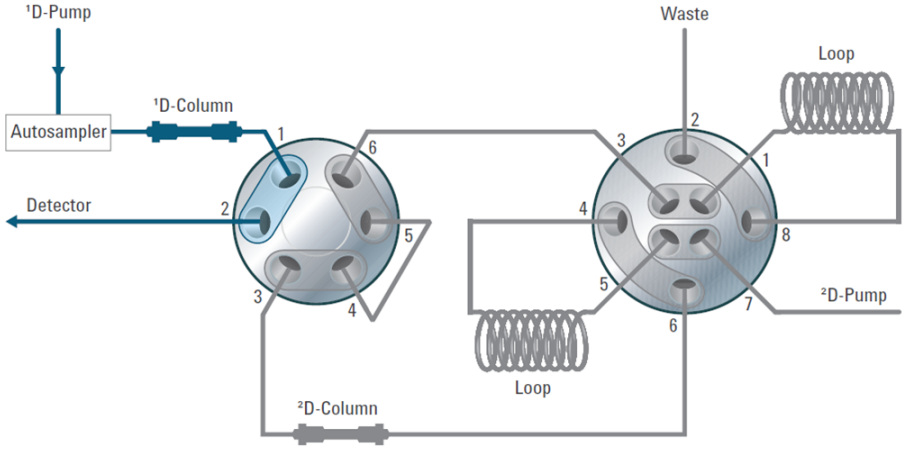


Figure 15 Setup for 1D-LC analysis

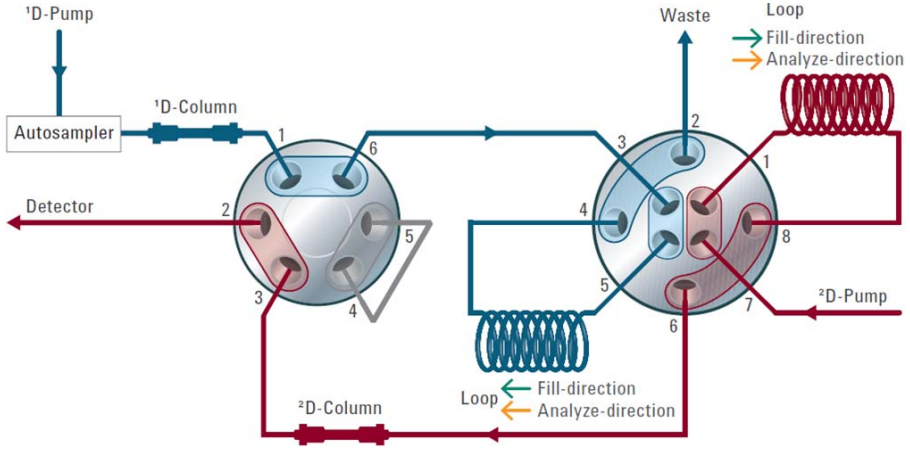


Figure 16 Setup for 2D-LC analysis

Installing the Software

Software required OpenLAB ChemStation Edition C.01.07 (or higher) installed

NOTE For installing the 2D-LC Software, please use the OpenLAB Additional Software and Drivers Deployment Wizard.

NOTE Do not try installing the software by double-clicking the msi file, as this may result in an incomplete installation.

NOTE To install the Add on, the OpenLAB CDS Chemstation Software must be not active.

1 Start OpenLAB Additional Software and Driver Deployment Wizard by going to Windows **> Start > Agilent Technologies > OpenLAB > OpenLAB Additional Software and Drivers**

2 Follow steps described in the Wizard for installation or software upgrades.

Overview Menu Extensions

The installation of the Agilent 1290 Infinity 2D-LC Acquisition Software adds 2D-LC specific items to the Agilent OpenLAB CDS controller software.

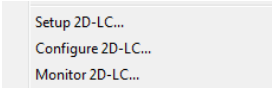


Figure 17 Agilent OpenLAB CDS controller software, 2D-LC specific items

The new menu items appear in the **Instrument Menu** of the **Method & Run Control View**. The following items are available:

- **Setup 2DLC...**

  Displays the 2DLC method dialog

- **Configure 2DLC...**

  Displays the 2DLC configuration dialog

- **Monitor 2DLC...**

  Displays the 2DLC status monitor

Configuration

Overview Configuration Dialog

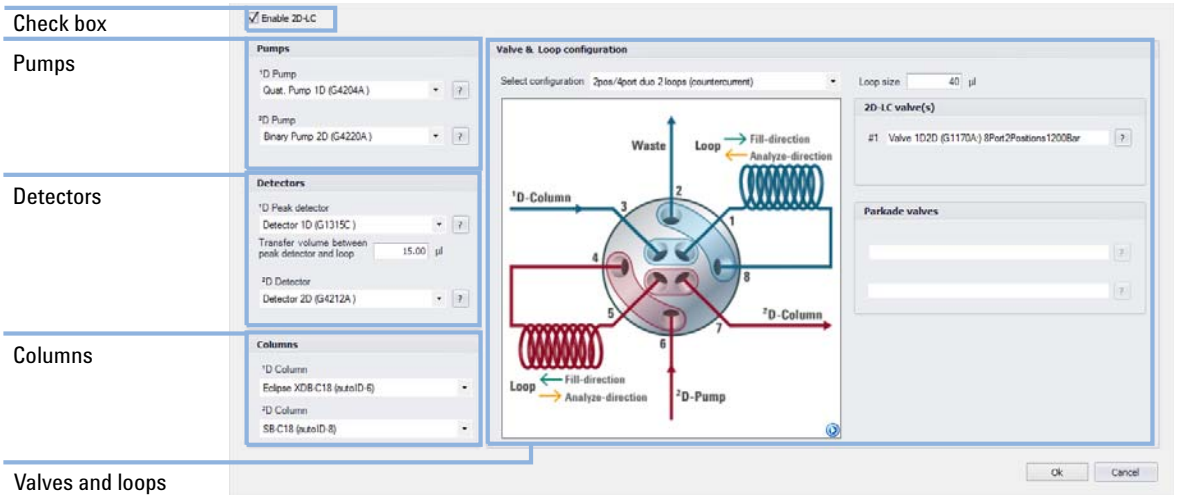


Figure 18 Overview 2D-LC configuration grafical user interface

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 & Loops**

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

Enable 2D-LC

1 Select check box **Enable 2D-LC**.

The 2D-LC functionality is enabled for the configured system.

NOTE If disabled, all all sections in the configuration user interface are disabled and the menu item **Setup 2D-LC method...** is grayed out such that the system uses normal parameters.

Configure Pumps

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

- 1st dimension:

  The drop-down list contains all configured pumps that can be used in the 1st dimension (binary, quaternary, capillary or nano pumps).

- 2nd dimension:

  Only 1290 Infinity Binary Pumps (G4220A/B) or 1290 Infinity II High Speed Pumps (G7120A) can be selected in the 2nd dimension.

The **Identify** button triggers the blinking of the status LED of the corresponding pump module. The button is only enabled in the Online version of the ChemStation

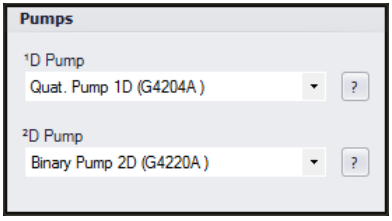


Figure 19 Configuration screen (for example if 1st dimension pump is an Agilent 1290 Infinity Binary Pump)

Preparations

- OpenLAB ChemStation Edition C.01.03 (or higher) installed (Multiple heart-cutting method requires OpenLAB ChemStation Edition C.01.07 (or higher))
- 1290 Infinity 2D-LC Acquisition Software installed
- Check box **Enable 2D-LC** selected.

1 Select the pump for the 1st dimension from the drop-down list **Pump (1D)**.

2 Select the pump for the 2nd dimension from the drop-down list **Pump (2D)**.

3 To save settings click **OK**.

Pumps are configured for 2D-LC.

Configure Detectors

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

- 1st dimension:

  The drop-down list contains all configured detectors and a **None** entry.

- 2nd dimension:

  The drop-down list contains all configured detectors that can be used as peak detector (DAD, MWD, VWD, FLD, RID), and a **None** entry.

The **Identify** button triggers the blinking of the status LED of the corresponding detector module. The button is only enabled in the Online version of the ChemStation

Transfer volume is the volume between the peak detector in the 1st dimension and the loop(s). *In standard setups this volume is 9 µL*. The field is only visible if a peak detector is configured.

For special setups or if the default setting needs to be changed, the following methods for determination of the delay volume exist:

- Use the following volumes:

Table 7 Volumina of capillaries

Capillary	Volume [µL]
Flex capillary, 0.12 x 105 mm, no fittings (5021-1820)	1.187
Flexible tubing, 280 mm (5021-1822)	3.167
Capillary column – detector SST 400 mm lg, 0.12 mm i.d. (5021-1823)	4.524
Capillary ST 0.12 mm x 500 mm (5065-9964)	5.655
Capillary ST 0.12 mm x 600 mm S/SL (5067-4669)	6.786
Capillary, 0.17 mm x 80 cm, male fit (01078-87305)	18.158

- The delay volume of the capillary connection between peak-detector and loop can be either calculated by the following formula:

  $V_D = L * \pi * d^2/4$ (L = length of capillary, d = diameter of capillary

- Alternatively, and with higher accuracy the volume can be measured by two ways:
  - First, by determining the increase in retention time of a narrow peak after adding the respective capillary to an existing HPLC system. A sample like one micro-liter of acetone can be injected to a system using a restriction capillary instead of a column to generate a very narrow peak. In a second measurement, add the capillary of interest directly in front of the detector by using a zero-dead volume union. The set flow rate multiplied with the detected time difference will result the capillary volume.
  - A second option to measure the volume of the capillary is to fill the dried capillary with pure water an weigh it. The weight divided by the density of water (1 mg/µL) will result in the volume. Attention must be given not to have any additional droplets of water being attached to the capillary.

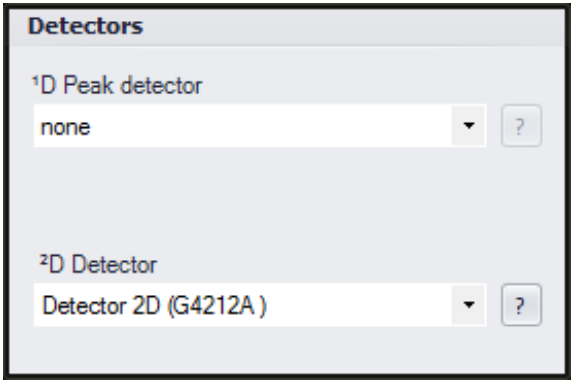


Figure 20 Configuration screen with no peak detector

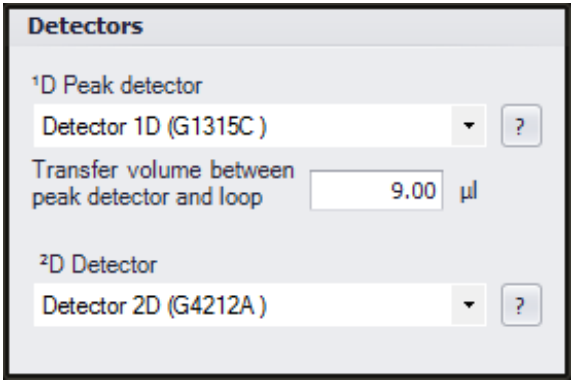


Figure 21 Configuration screen with peak detector

Preparations

- OpenLAB ChemStation Edition C.01.03 (or higher) installed (Multiple heart-cutting method requires OpenLAB ChemStation Edition C.01.07 (or higher))
- 1290 Infinity 2D-LC Acquisition Software installed
- Check box **Enable 2D-LC** selected.

1 Select the **Detector (2D)** from the drop-down list.

2 Select the **Peak detector (1D)** from the drop-down list.

3 To save settings click **OK**.

Detectors are configured for 2D-LC.

Configure Columns

The user can select columns for the 1st and 2nd dimension from the ChemStation column database. The columns are identified by the column description and serial number.

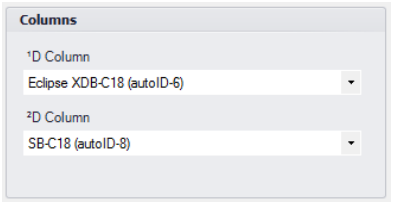


Figure 22 2D-LC column configuration

Preparations

- OpenLAB ChemStation Edition C.01.03 (or higher) installed
- 1290 Infinity 2D-LC Acquisition Software installed
- Check box **Enable 2D-LC** selected.

NOTE The software uses the column parameter to calculate the ratio of the injection volume to the column volume, that typically should not exceed ca. 10 % to avoid break-through of the compounds during the second dimension separation.

1 Select **Column (1D)** from the drop-down list.

2 Select **Column (2D)** from the drop-down list.

3 To save settings click **OK**.

Configure Valve and Loop

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

- 1st dimension:

  The **Valve 1** drop-down list contains all configured valves which can be used for 2DLC 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.

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

Loop size specifies the volume of the loop(s). In case of two loops, the software assumes that both loops have the same volume.

All possible loop configurations depending on the selected valves are listed separately and illustrated on screen (see, "Interfacing the First and Second Dimension" on page 266).

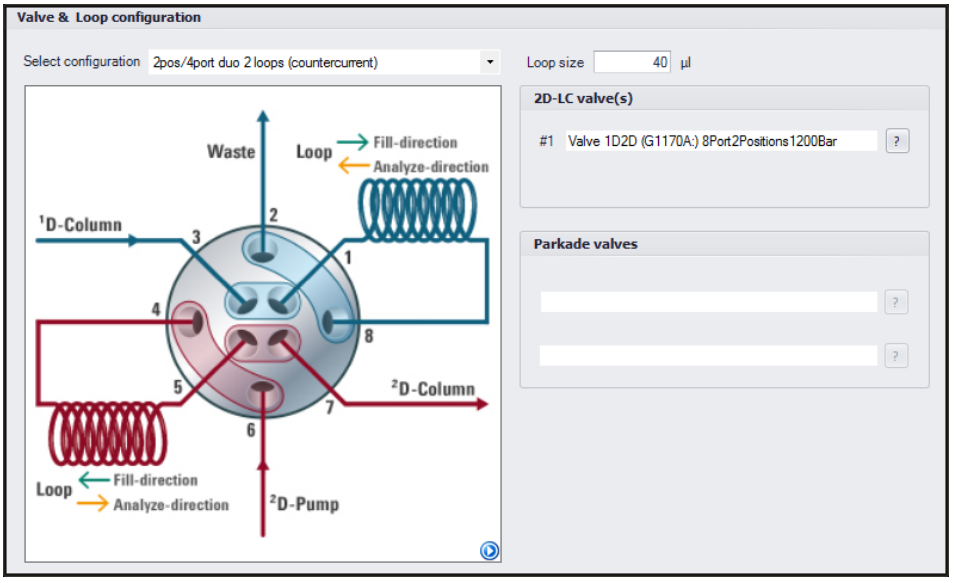


Figure 23 2D-LC valve and loop configuration

Preparations

- OpenLAB ChemStation Edition C.01.03 (or higher) installed (Multiple heart-cutting method requires OpenLAB ChemStation Edition C.01.07 (or higher))
- 1290 Infinity 2D-LC Acquisition Software installed
- Check box **Enable 2D-LC** selected.

NOTE Valves may be part of a 1290 Infinity Thermostatted Column Compartment (G1316C) or the 1290 Infinity Valve Drive (G1170A).

1 Select **Valve 1**.

2 Select **Valve 2**.

3 To save settings click **OK**.

Valves and loops are configured for 2D-LC.

Method parameters

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.

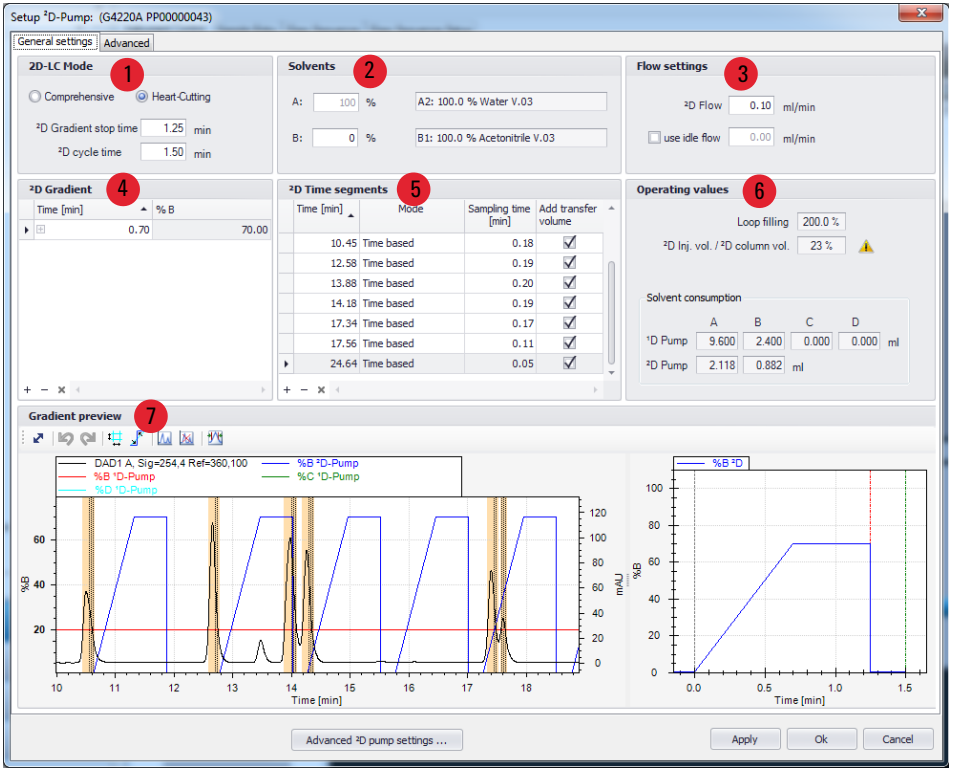


Figure 24 2D-LC method setup (**General settings**)

The setup of following method parameters is available:

1 **2D-LC Mode**, see "Set 2D-LC Mode" on page 53
2 **Solvents**, see "Set solvents" on page 60
3 **Flow settings**, see "Set flow" on page 61
4 **^{2}D Gradient**, see "Set Solvent Composition Gradient" on page 62
5 **^{2}D Time segments**, see "Set 2D Time Segments" on page 64
6 **Operating values**, see "Define Peak detector parameter" on page 66
7 **Gradient preview**, see "Setup Second Dimension Gradient with the Graphical User Interface" on page 202

Set 2D-LC Mode

Setting the mode has the following consequences (for details, see "Introduction" on page 8):

- **Heart cutting**:

  A relevant volume of the 1st dimension is cut off and injected onto the 2nd dimension column using the pump in the 2^{nd} dimension. The volume to be injected on the 2^{nd} 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 2^{nd} dimension starts running the gradient of the 2^{nd} 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 25 on page 55):

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 Table 8 on page 56. In Table 8 on page 56, Table 9 on page 57 and Table 10 on page 58 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 Table 9 on page 57.

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 10 on page 58.

The peak volume that will be sampled usually is larger than the loop volume. For details see "Concept of Peak Triggering" on page 27.

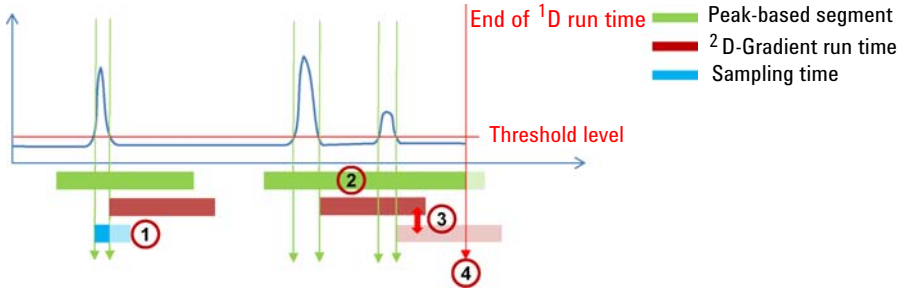


Figure 25 Heart cutting 2D-LC (general considerations) (delay times have been omitted for clarity, besides threshold also the peak-slope can be used for peak detection)

Table 8 Peak-based heart-cutting 2D-LC, Dual-Loop set-up

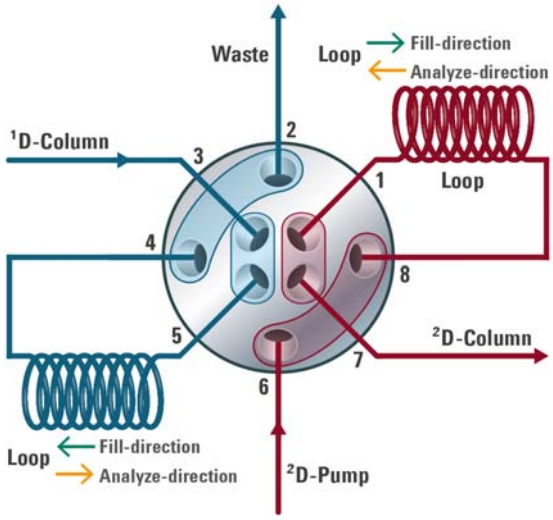


Figure 26 Valve and loop setup for heart-cutting 2D-LC with the 2D-LC Valve (dual-loop setup)

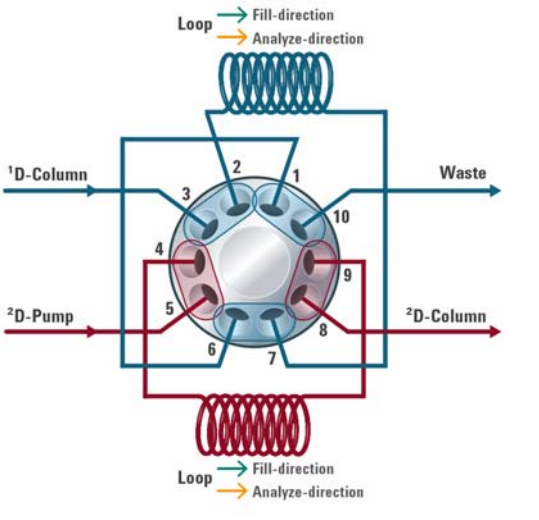
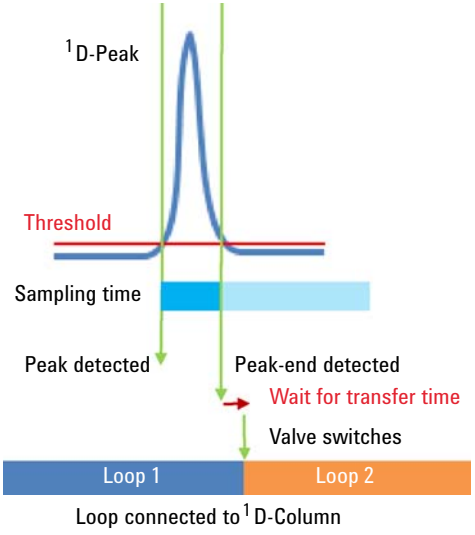


Figure 27 Valve and loop setup for heart-cutting 2D-LC with a 2/10 Valve (dual-loop setup) - not recommended



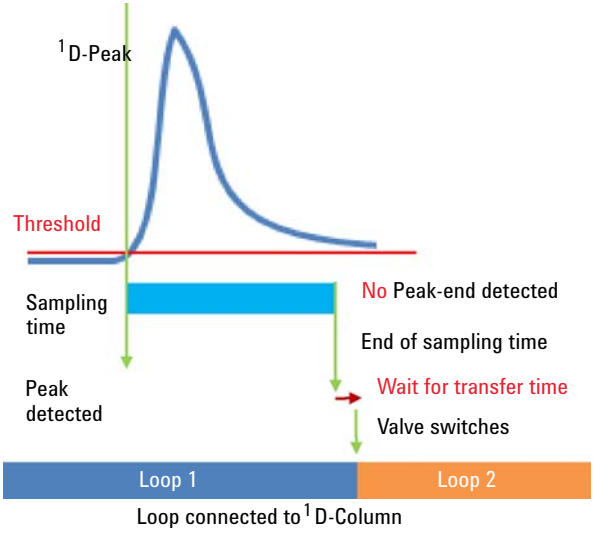


Table 9 Peak-based heart-cutting 2D-LC, Single-Loop set-up

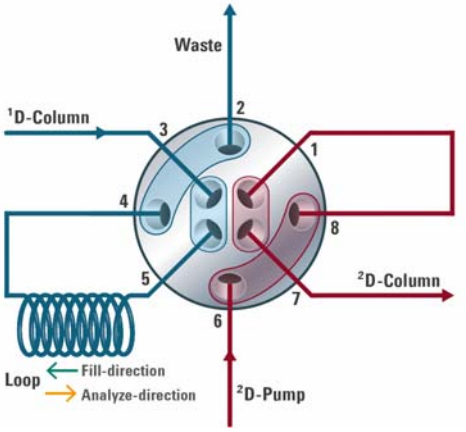


Figure 28 Valve and loop setup for heart-cutting 2D-LC with the 2D-LC Valve (single-loop setup)

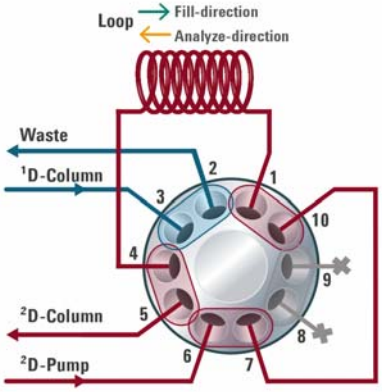
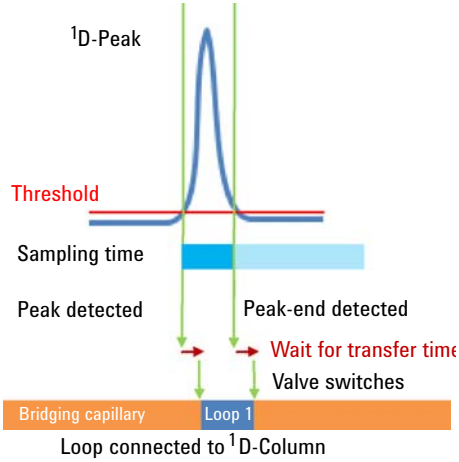


Figure 29 Valve and loop setup for heart-cutting 2D-LC with a 2/10 Valve (single-loop setup) - not recommended



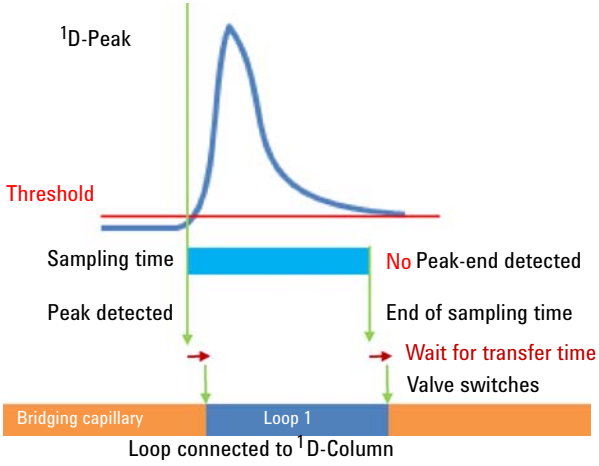


Table 10 Time-based heart-cutting 2D-LC

Dual-loop set-up

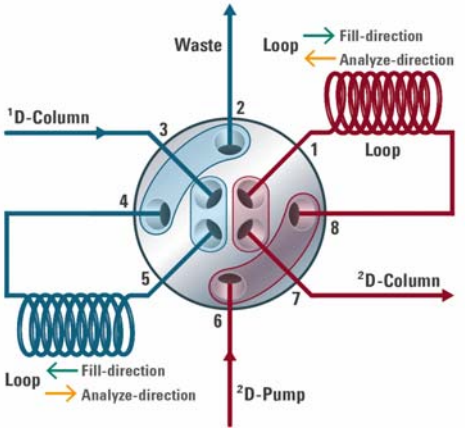
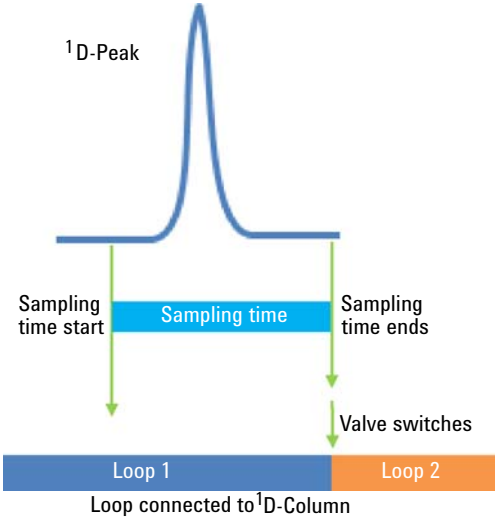


Figure 30 Valve and loop setup for heart-cutting 2D-LC with the 2D-LC Valve

(example valve)



Single-loop set-up

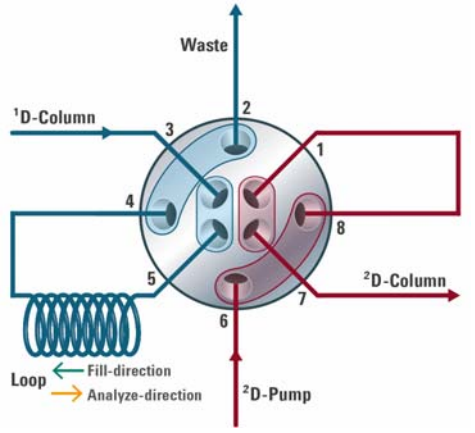
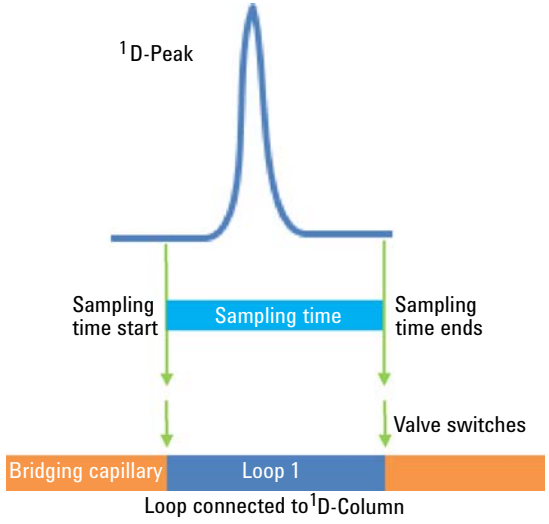


Figure 31 Valve and loop setup for heart-cutting 2D-LC with 2D-LC Valve

(example valve)



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

NOTE

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

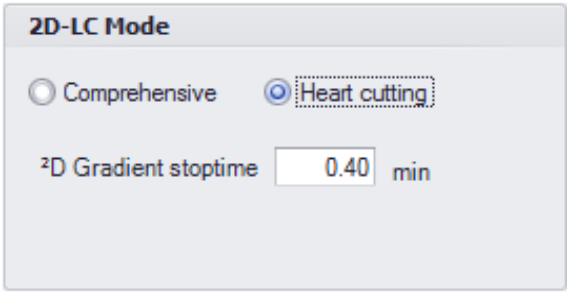


Figure 32 2D-LC **Heart cutting** mode

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 (in case of comprehensive 2D-LC) the **Modulation time** as a green vertical line.

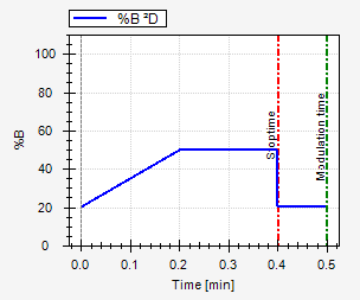


Figure 33 **Stop time** and **Modulation time**

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 2D 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 %.

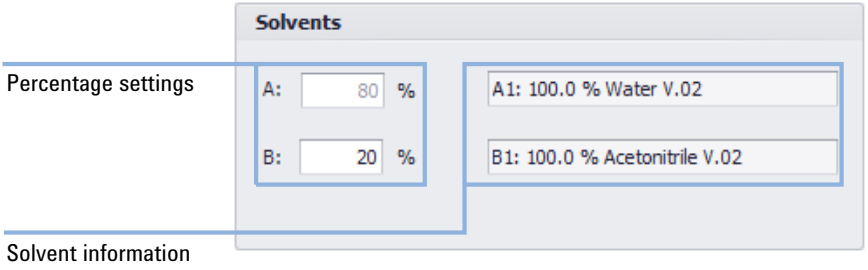


Figure 34 2D-LC solvent settings

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 "Overview Configuration Dialog" on page 43).

Set flow

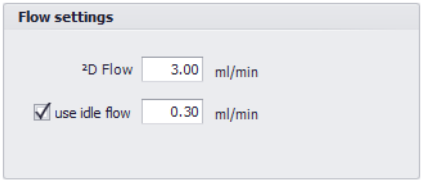


Figure 35 Flow settings

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 2D-Flow is also used while 2D-LC mode is OFF.

Set Solvent Composition Gradient

Set Solvent Composition Gradient

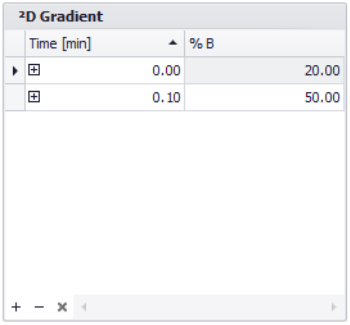
The timetable in the **2D Gradient** group allows changing the solvent composition.

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 2D** will be ignored.

Setup ^{2}D Gradient graphically

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

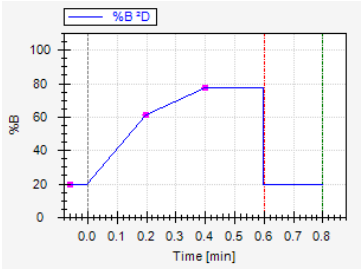


Figure 36 ^{2}D Gradient window in edit mode

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

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 11 Definitions 2D Time Segements

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 Heartcutting mode

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

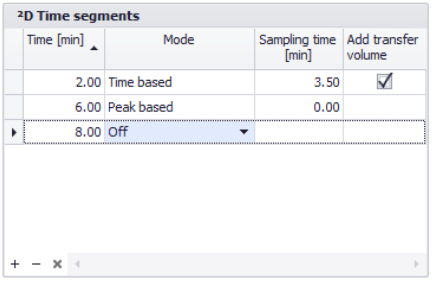
Specifies the point in time of the 1D runtime where the actual trigger mode gets active.

NOTE

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

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

Trigger 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 25 on page 55.

- **Peak based**

  The peak detector is enabled at the specified time. For details see Figure 25 on page 55.

- **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 **2D Time Segments** now are 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).

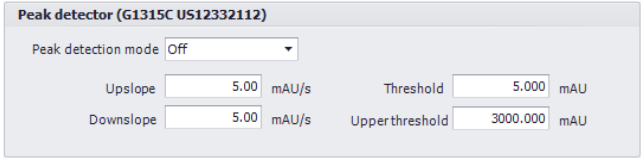
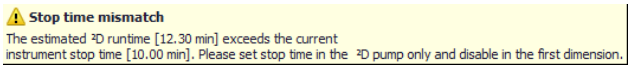


Figure 37 Overview on peak detector parameters

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 "Overview Configuration Dialog" on page 43) this section is disabled. The currently configured peak detector (name & serial number of the detector) is shown in the section header.

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.

Data analysis

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.

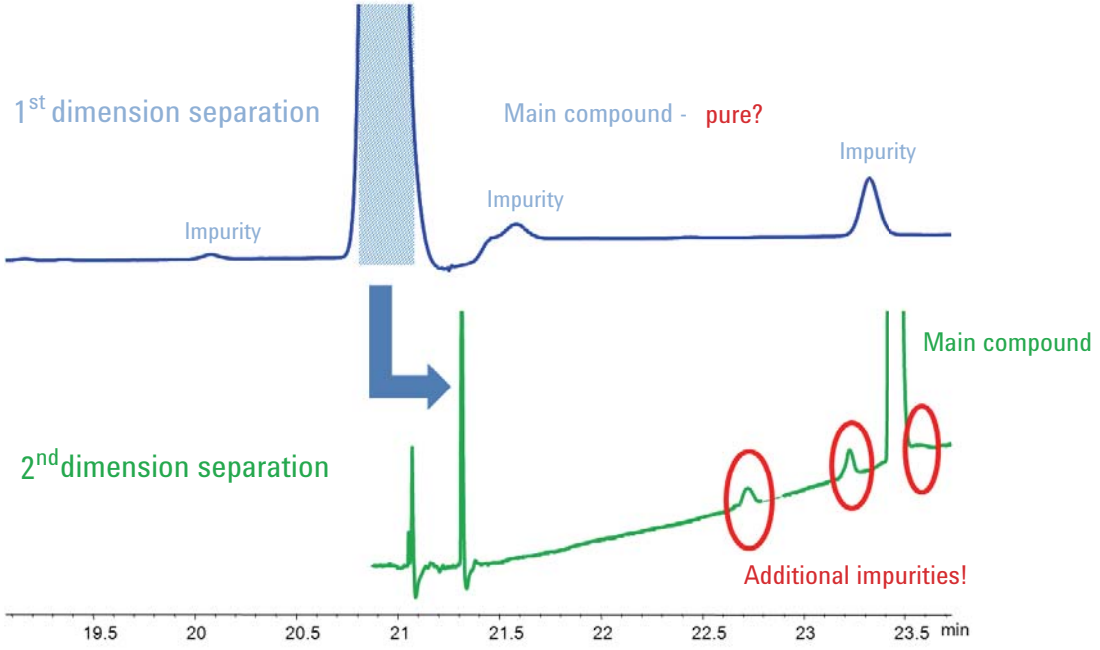


Figure 38 Example of a heartcutting 2D-LC experiment. Data analysis and display done by OpenLAB CDS ChemStation Edition, the data of a detector placed after the first dimension column (blue) and of the 2D data detector (green). The additional impurities (marked red) could not be detected by 1D-LC only.

Heart-Cutting Viewer

Using the Multiple Heart-Cutting Upgrade kit, the Agilent 1290 Infinity 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 2D gradients are possible without loss of 1D peaks. But it is quite difficult to review the 2D 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 Heart-Cut Viewer (available with 2D-LC software A.01.02, which requires OpenLAB C.01.07 or higher) 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 Heart-Cut Viewer

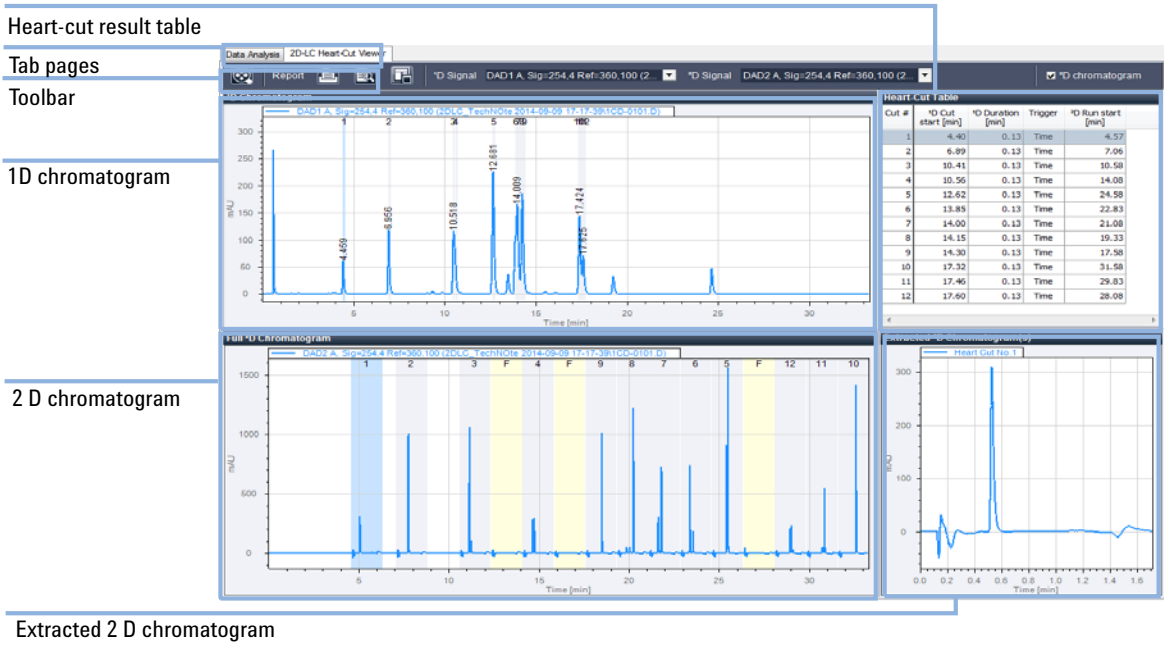


Figure 39 Overview of the 2D-LC Heart-Cut Viewer graphical user interface

The 2D-LC Heart-Cut Viewer provides the following functions:

- Tab pages enable the user to switch between
  - **2D-LC Heart-Cut 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.
- **Heart-Cut Results** table
- 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)

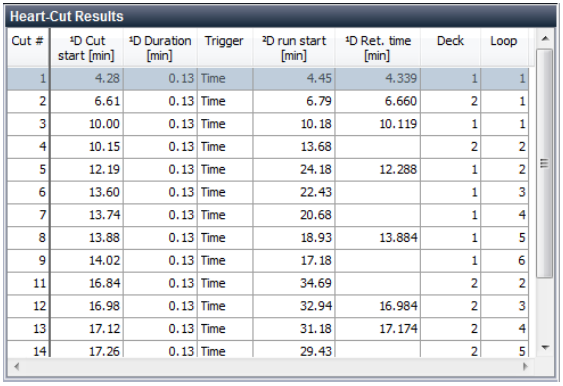
    Hide / unhide the full **^{2}D Chromatogram** window
  - **^{1}D Signal** list box

    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)
- **Extracted ^{2}D Chromatogram(s)**

Heart-Cut Results Table

The table lists all heart-cuts which have been analyzed in the 2nd dimension.



Heart-Cut Results

Cut #	1D Cut start [min]	1D Duration [min]	Trigger	2D run start [min]	1D Ret. time [min]	Deck	Loop
1	4.28	0.13	Time	4.45	4.339	1	1
2	6.61	0.13	Time	6.79	6.660	2	1
3	10.00	0.13	Time	10.18	10.119	1	1
4	10.15	0.13	Time	13.68		2	2
5	12.19	0.13	Time	24.18	12.288	1	2
6	13.60	0.13	Time	22.43		1	3
7	13.74	0.13	Time	20.68		1	4
8	13.88	0.13	Time	18.93	13.884	1	5
9	14.02	0.13	Time	17.18		1	6
11	16.84	0.13	Time	34.69		2	2
12	16.98	0.13	Time	32.94	16.984	2	3
13	17.12	0.13	Time	31.18	17.174	2	4
14	17.26	0.13	Time	29.43		2	5

Figure 40 **Heart-Cut Results** (example)

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

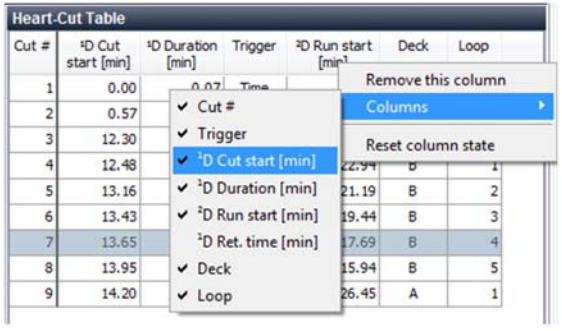


Table 12 Legend for Heart-Cut Results

PosNr	Description
Cut #	The current number of the heart-cut
1D Cut start [min]	Time when the heart-cut starts (peak begin or time value in trigger table)
1D Duration [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
2D run start [min]	Time when the analyses of this heart-cut in the 2nd dimension starts (gradient start)
1D 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)

1D Chromatogram

Selected heart-cut

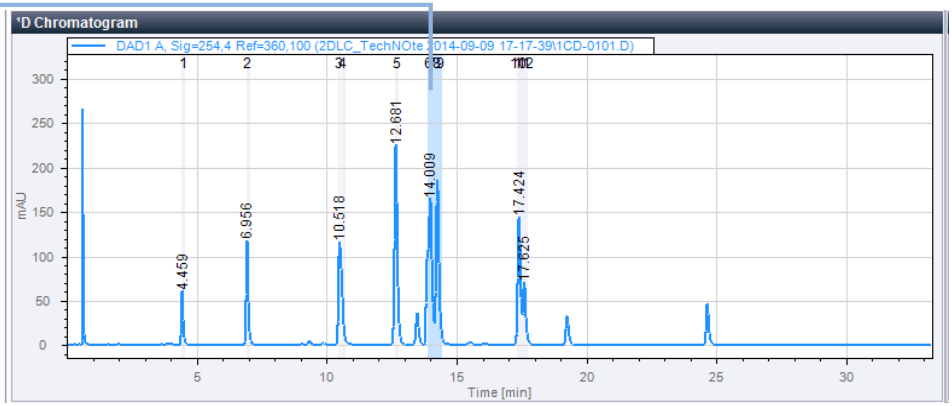


Figure 41 **1D Chromatogram** (example)

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 1D 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

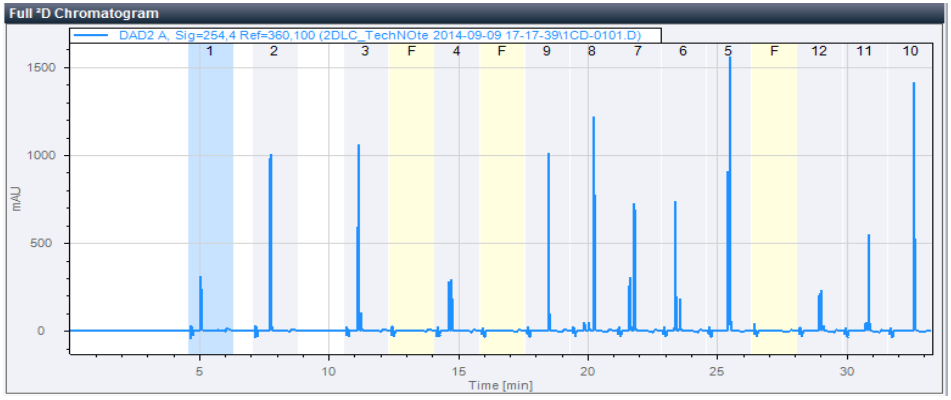


Figure 42 **^{2}D Chromatogram** (example - only visible, if **^{2}D chromatogram** (checkbox) is checked)

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 heart-cut table).
- **F** indicates a bypass (or flush) gradient, which was used to flush the transfer capillaries after switching the 2D-LC valve.

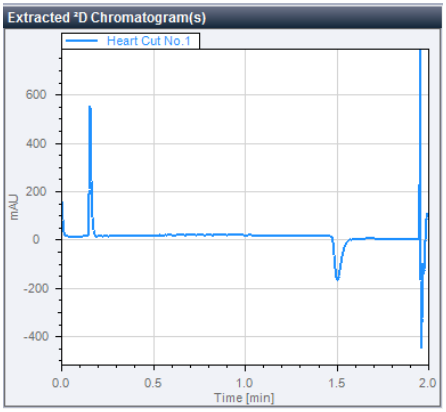


Figure 43 **Extracted ^{2}D Chromatogram** (example from **^{2}D Chromatogram** above, Heart Cut No. 1)

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.

Checkout/FamiliarizationProcedure

Checkout runs - 1290 Infinity Binary or Quaternary LC in 1D

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 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

See Table 4 on page 33

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

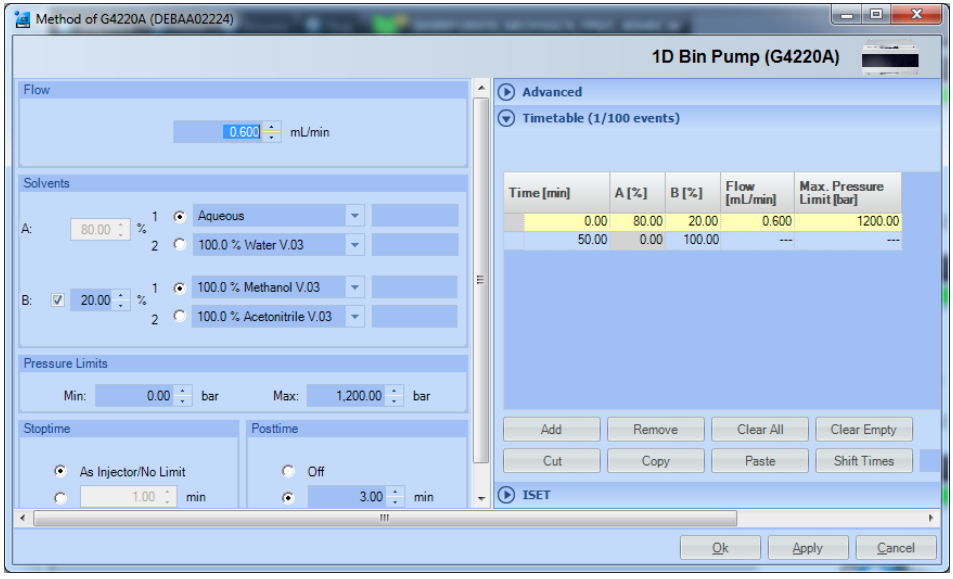
Preparations:

1. Prepare dilution solvent (20 MeOH in mobile phase A): Add 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 xxxx.yyyyy from the CD.

1 Apply the following method parameters for ^{1}D:

- Column: RRHD SB-C18, 2.1x 100 mm, 1.8 µm, 1200 bar (858700-902)
- Solvent:
  - A: H_2O + 0.2 % formic acid
  - B: Methanol
- Gradient:
  - 0.0 min – 20 % B
  - 50 min – 100 % B
  - Stoptime: 40 min
  - Posttime: 10 min
- Flow rate: 0.300 mL/min
- Temperature: 40 °C
- Post Time: 6 min

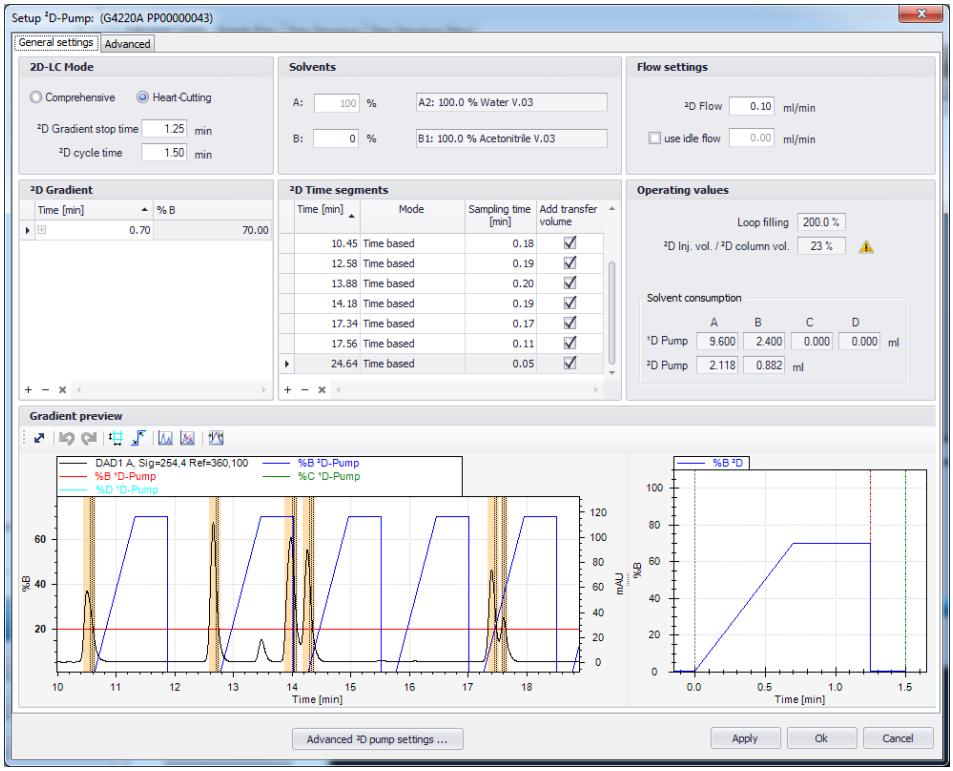
The 1D method should be set up as displayed in the screen:



2 Apply the following method parameters for ^{2}D:

- Column: RRHD Bonus-RP, 2.1x 50 mm, 1.8 µm, 1200 bar (857768-901)
- Solvent:
  - A: H_2O + 0.2 % formic acid
  - B: Acetonitrile
- Gradient:
  - 0.0 min – 10 % B
  - 1.25 min – 60 % B
  - 2D Gradient stoptime: 1.25 min
  - 2D Cycle Time: 1.75 min
- Stop Time: 40 min (will be automatically prolonged, if peaks in 2nd dimension are not worked off)
- Gradient shift: 0→20 min from 10→30 %B (only downslope)
- Flow rate: 1.0 mL/min
- Temperature: 40 °C

The 2D method should be set up as displayed in the screen:



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	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

4 Detection:

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

Acquisition rate: 5 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

Checkout runs - 1260 Infinity Binary in 1D

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 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

See Table 5 on page 33

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

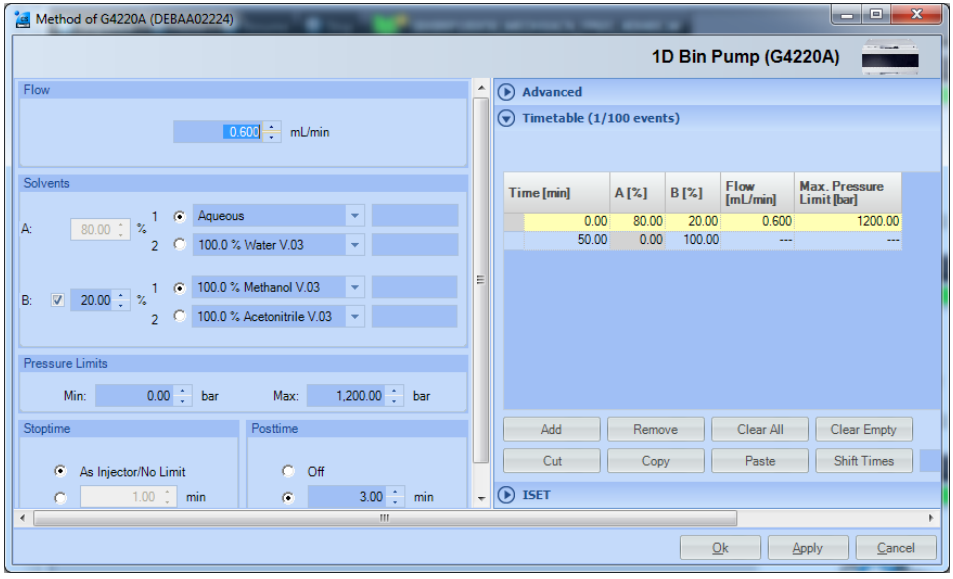
Preparations:

1. Prepare dilution solvent (20 MeOH in mobile phase A): Add 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 xxxx.yyyyy from the CD.

1 Apply the following method parameters for ^{1}D:

- Column: RRHD SB-C18, 2.1x 100 mm, 1.8 µm, 1200 bar (858700-902)
- Solvent:
  - A: H_2O + 0.2 % formic acid
  - B: Methanol
- Gradient:
  - 0.0 min – 20 % B
  - 50 min – 100 % B
  - Stoptime: 40 min
  - Posttime: 10·e min
- Flow rate: 0.300 mL/min
- Temperature: 40 °C
- Post Time: 6 min

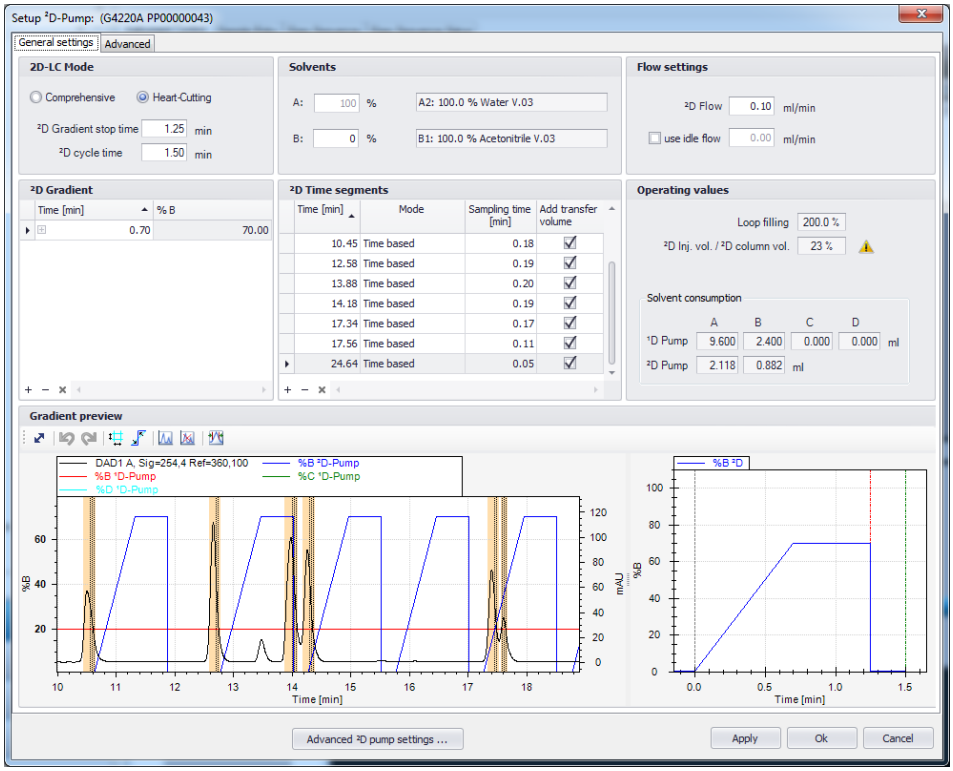
The 1D method should be set up as displayed in the screen:



2 Apply the following method parameters for ^{2}D:

- Column: RRHD Bonus-RP, 2.1x 50 mm, 1.8 µm, 1200 bar (857768-901)
- Solvent:
  - A: H_2O + 0.2 % formic acid
  - B: Acetonitrile
- Gradient:
  - 0.0 min – 10 % B
  - 1.25 min – 60 % B
  - 2D Gradient stoptime: 1.25 min
  - 2D Cycle Time: 1.75 min
- Stop Time: 40 min (will be automatically prolonged, if peaks in 2nd dimension are not worked off)
- Gradient shift: 0→20 min from 10→30 %B (only downslope)
- Flow rate: 1.0 mL/min
- Temperature: 40 °C

The 2D method should be set up as displayed in the screen:



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

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

4 Detection:

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

Acquisition rate: 5 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

4
Multiple Heart-Cutting 2D-LC

This chapter describes in detail the installation, configuration, method parameters, data analysis and checkout/familiarization of multiple heart-cutting two dimensional liquid chromatography with the Agilent 1290 Infinity 2D-LC-Solution.

Multiple Heart-Cutting 2D-LC

NOTE

While in Comprehensive 2D-LC the modulation time describes both, the sampling time and the full 2D gradient time inclusive re-equilibration, these values are not aligned in Heart-Cutting 2D-LC. In Heart-Cutting the 2D gradient is much longer than the sampling time. While the 2D gradient is still running, no additional 1D cut can be transferred to the occupied 2nd dimension.

The Agilent 1290 Infinity 2D-LC Solution addresses this task with a dedicated valve, which is equipped with 6 loops to store cuts, eluting from the 1st dimension run. Using this Multiple Heart-Cutting Valve it is possible to consecutively analyze the cuts in the 2nd dimension (see "Multiple Heart-cutting" on page 14). The whole valve setup is shown in Figure 44 on page 89.

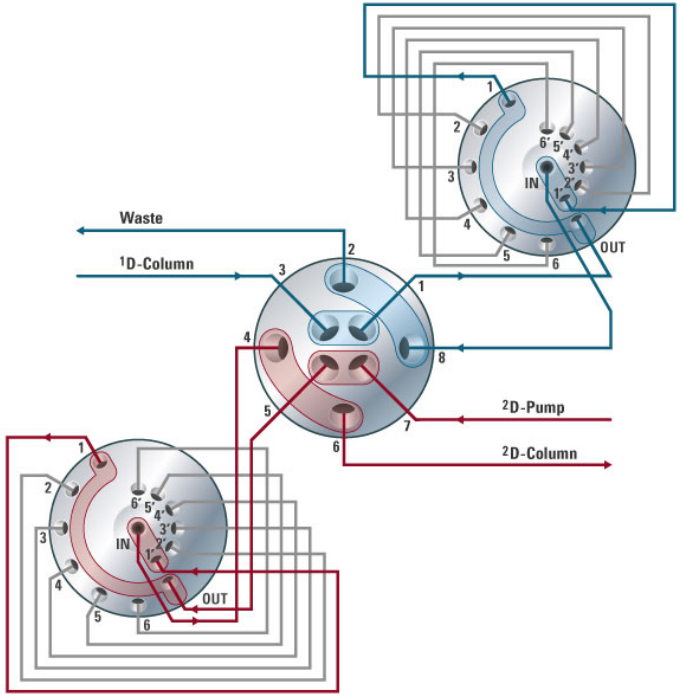


Figure 44 Valve setup with two 6/14 valves for peak parking

The following items are characteristic for LC-LC:

- Only parts of the effluent of the 1st dimension column are injected to the 2nd dimension column. The peaks of interest are selected and injected onto the 2nd dimension.
- A peak from the 1st dimension is sampled as a whole and a gradient typically with a longer run time than the collection time is used to improve separation efficiency. For details about the peak sampling and collection time, see "Concept of Peak Triggering" on page 93.
- Typically longer columns with higher separation efficiency are used in 2nd dimension column

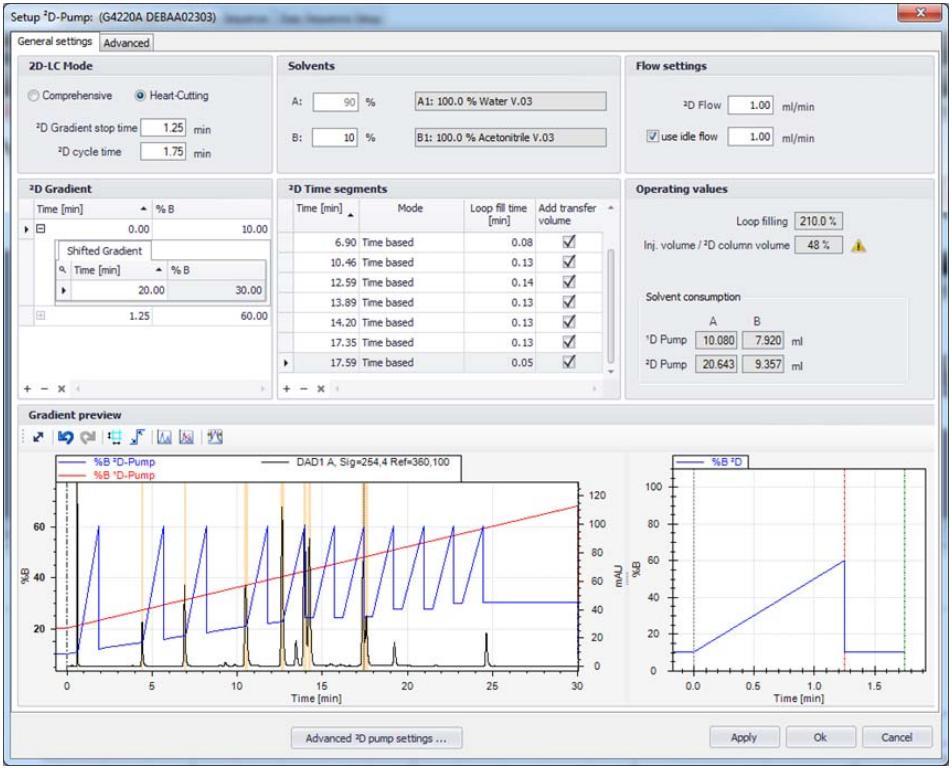
NOTE Heartcutting 2D-LC (LC-LC) is the method of choice if the samples to analyze are known or to improve confidence of an experiment (pharma, method development and so on).

There are two modes of LC-LC:

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

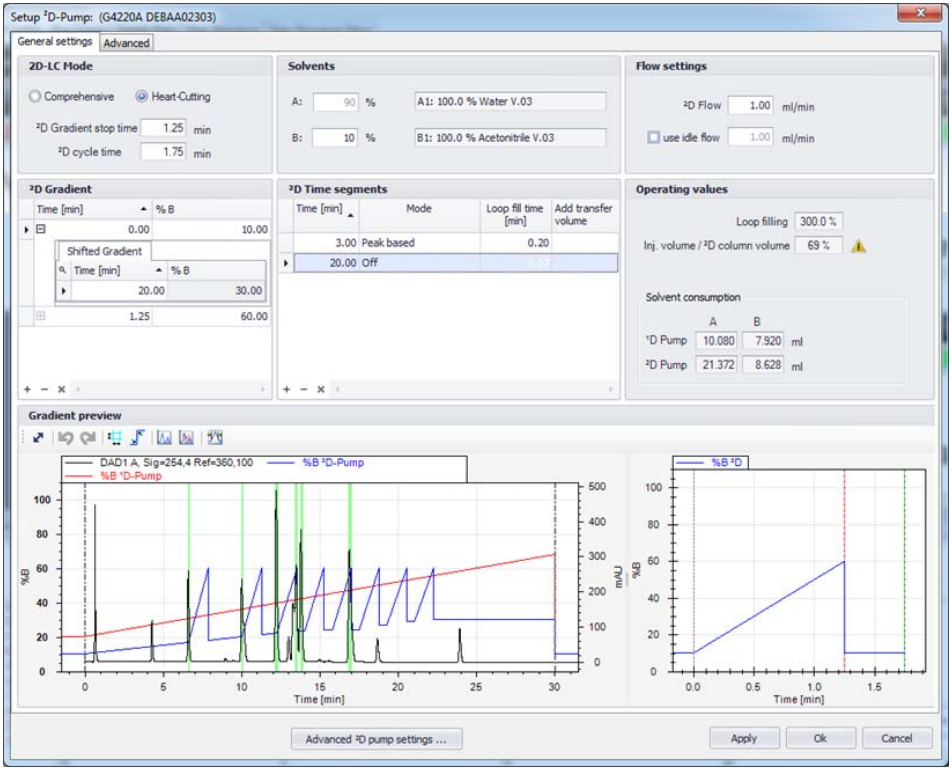
Time-triggered LC-LC

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



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.



Concept of Peak Triggering

Triggering is done in advanced settings similar to integrator settings by threshold and/or slope, see Figure 45 on page 93.

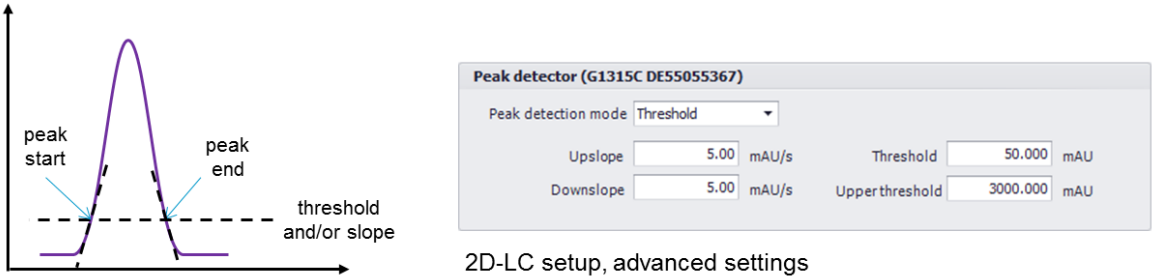


Figure 45 Peak triggering

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

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

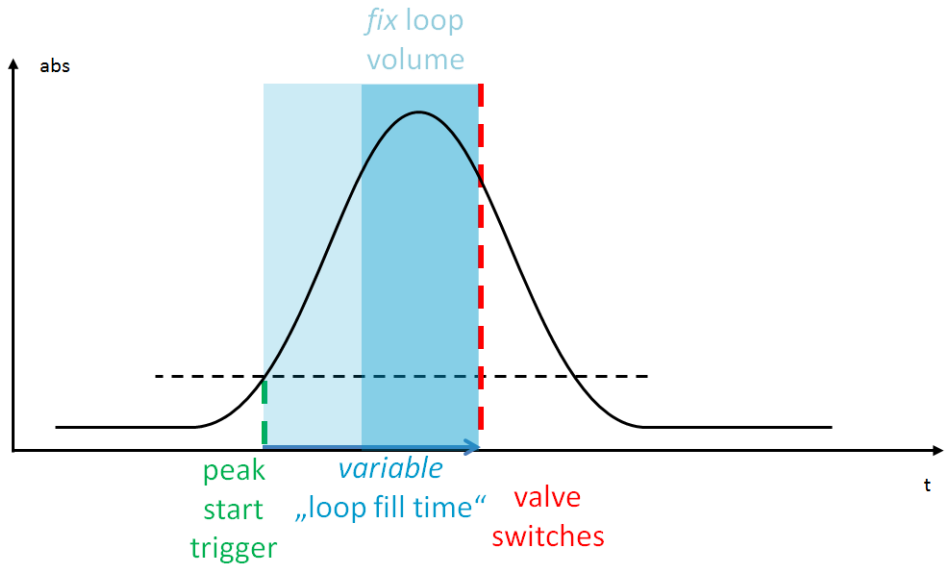


Figure 46 Peak triggering concept (elapsed sampling time)

- If the signal falls below threshold or slope.

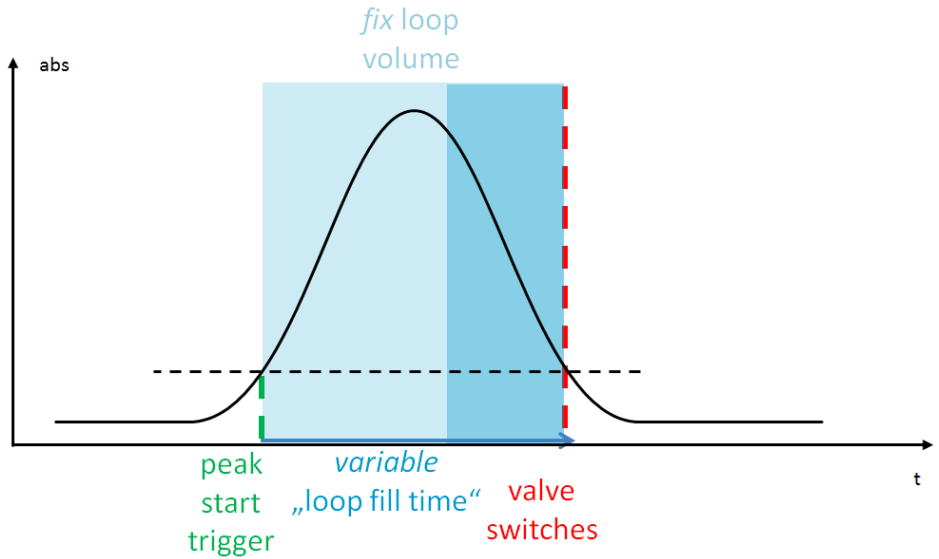


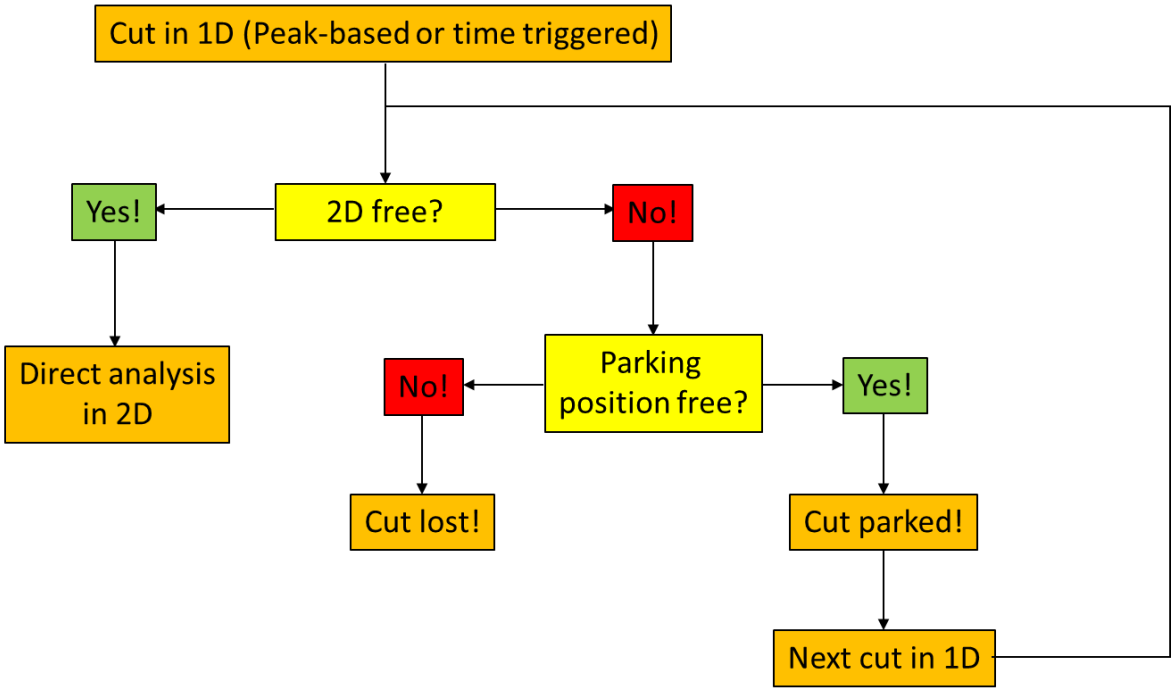
Figure 47 Peak triggering concept (signal falls below threshold or slope)

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. Fig. XX illustrates the principles of the Multiple Heart-Cutting algorithm, following these principles:

- 2D 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 1D cut is taken, it will also be directly analysed.
- If the 2nd dimension is occupied, the next 1D 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!

The algorithms is shortly explained with the attached flow chart. For a detailed explanation, please refer to Agilent´s Technical Notes and Application Notes, describing the algorithm in more detail.


Cut in 1D (Peak-based or time triggered)
2D free?
Yes!
No!
Direct analysis in 2D
Parking position free?
No!
Yes!
Cut lost!
Cut parked!
Next cut in 1D

Installation

Recommended fix Setups for Multiple Heart-Cutting 2D-LC

Table 13 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 (3x)	1290 Infinity Valve Drive	1x for 2D-LC valve 2x for MHC valves
	G4236A	2D-LC Valve Kit, 1200 bar	
	G4212A	1290 Infinity Diode-Array Detector	

NOTE For the optional available 1D/2D switch 2pos/6port Valve head 1200 bar (G4231B), see "1D/2D-Switch" on page 110

Table 14 1290 Infinity Quaternary LC in first dimension

	Partnumber	Description	Comment
1st Dim	G4204A	**1290 Infinity Quaternary 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 (3x)	1290 Infinity Valve Drive	1x for 2D-LC valve 2x for MHC valves
	G4236A	2D-LC Valve Kit, 1200 bar	
	G4212A	1290 Infinity Diode-Array Detector	

NOTE For the optional available 1D/2D switch 2pos/6port Valve head 1200 bar (G4231B), see "1D/2D-Switch" on page 110

Table 15 1260 Infinity Binary LC in first dimension

	Partnumber	Description	Comment
1st Dim	G1312B	**1260 Infinity Binary Pump**	
	G1367E	1260 Infinity High Performance Autosampler	
	G1330B	1290 Thermostat	
	G1316A	1260 Thermostatted Column Compartment	
	G4212B	1260 Infinity Diode-Array Detector	
2nd Dim	G2198AA	2D-LC Acquisition Software	
	G4220A	1290 Infinity Binary Pump	
	G1170A (3x)	1290 Infinity Valve Drive	1x for 2D-LC valve 2x for MHC valves
	G4236A	2D-LC Valve Kit, 1200 bar	
	G4212A	1290 Infinity Diode-Array Detector	

NOTE For the optional available 1D/2D switch 2pos/6port Valve head 600 bar (G4231A), see "1D/2D-Switch" on page 110

Possible stack configurations

The following configurations optimize the system flow path, ensuring minimum delay volume.

NOTE The capillary connections should be as short as possible, to ensure optimum performance of the system.

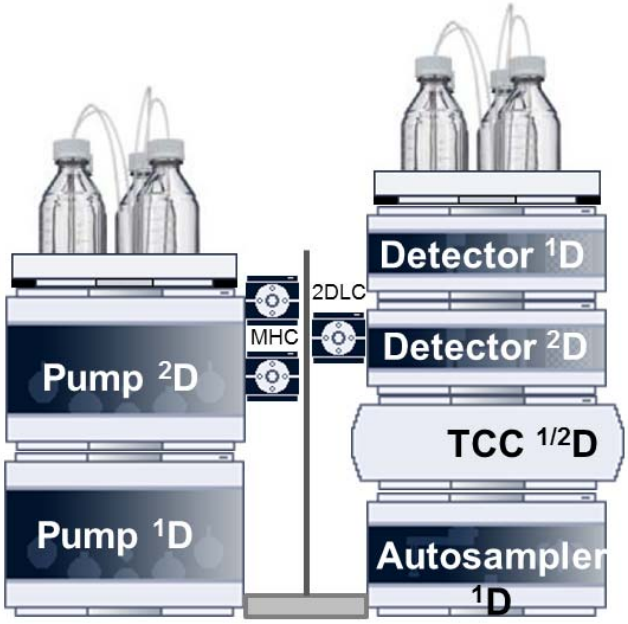


Figure 48 Stack configuration for Multiple Heart-cutting 2D-LC

Table 16 1290 Infinity 2D-LC-System MHC

Left Stack	Column Organizer	Right Stack
		Solvent Cabinet
		^{1}D detector (DAD (G4212A or G7117A/B)) - (optional)
Solvent Cabinet		^{2}D detector (DAD (G4212A or G7117A/B))
^{2}D pump (G4220A)	3x Valve Drive (G1170A)	TCC (G1316C)
^{1}D pump (G4220A/G4204A)		Autosampler (G4226A)

Table 17 1260 Infinity 2D-LC-System MHC

Left Stack	Column Organizer	Right Stack
		Solvent Cabinet
		^{1}D detector (DAD (G4212A/B or G7117A/B)) - (optional)
Solvent Cabinet		^{2}D detector (DAD (G4212A/B or G7117A/B))
^{2}D pump (G4220A)	3x Valve Drive (G1170A)	TCC (G1316C)
^{1}D pump (G4220A or G4204A or G1312A/B/C or G1311A or G1376A)		Autosampler (G4226A or G1367E/F)

Capillary connections (kits)

After placing the modules of the first dimension and second dimension and making the electrical connections the flow paths must be build.

Capillary Kit for 2D-LC

Item	p/n	Description
1	5021-1820 (2x)	Flex capillary, 0.12 x 105 mm, no fittings
2	G1316-87321	Capillary column-heat exchanger 105 mm lg, 0.17 mm i.d.
3	5021-1822	Flexible tubing, 280 mm
4	5021-1823 (3x)	Capillary column – detector SST 400 mm lg, 0.12 mm i.d.
5	5021-1819	Capillary ST 0.17 mm x 400 mm S/S
6	5065-9964	Capillary ST 0.12 mm x 500 mm
7	5067-4609	Capillary ST 0.17 mm x 500 mm SX/-
8	5067-4669	Capillary ST 0.12 mm x 600 mm S/SL
9	01078-87305	Capillary, 0.17 mm x 80 cm, male fit
10	G1316-80022 (2x)	LDHE double kit for G1316C

Supported valves and valve hosts

Two valves are supported as modulation valve in the 1D-2D interface:

- G4236A 2,4 Duo valve (highly recommended!)
- G4232B 2,10 valve (possible, but not recommended)

NOTE The formerly support setup using a 2/6 valve in the interface is not supported anymore from SW version *A.01.02*.

The following valve hosts are available in Agilent´s portfolio:

- G1170A 1290 Infinity Valve Drive
  Highly recommended for 2D-LC valve
- G1316C 1290 Infinity Thermostatted Column Compartment
  not recommended/forbidden for G4236A, but allowed for 1D/2D-switch

Interfacing the First and Second Dimension

Thermostatted Column Compartment Setup

Different variants of thermostatted column compartment setup are supported with the optional 2D-LC capillary kit for the 2D-LC valve (G4236A#3):

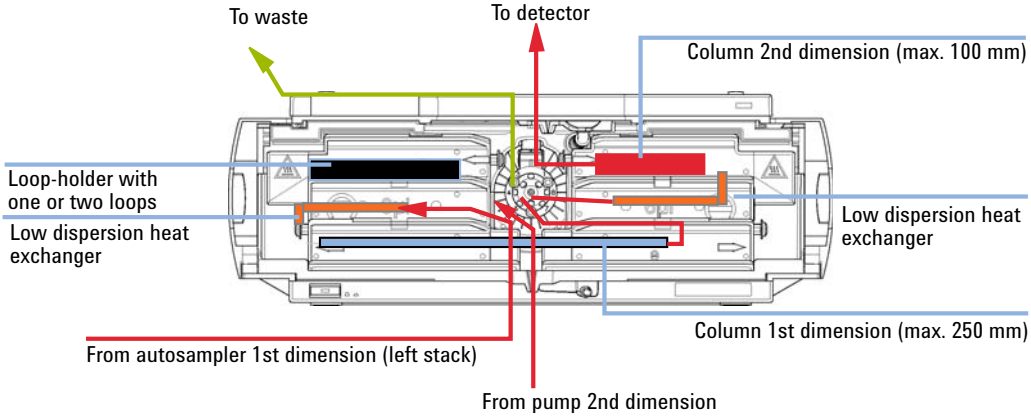


Figure 49 Columns at different temperatures,long 1st dimension column, no 1st dimension detector, for comprehensive 2D-LC booth loops can be placed in the loop holder.

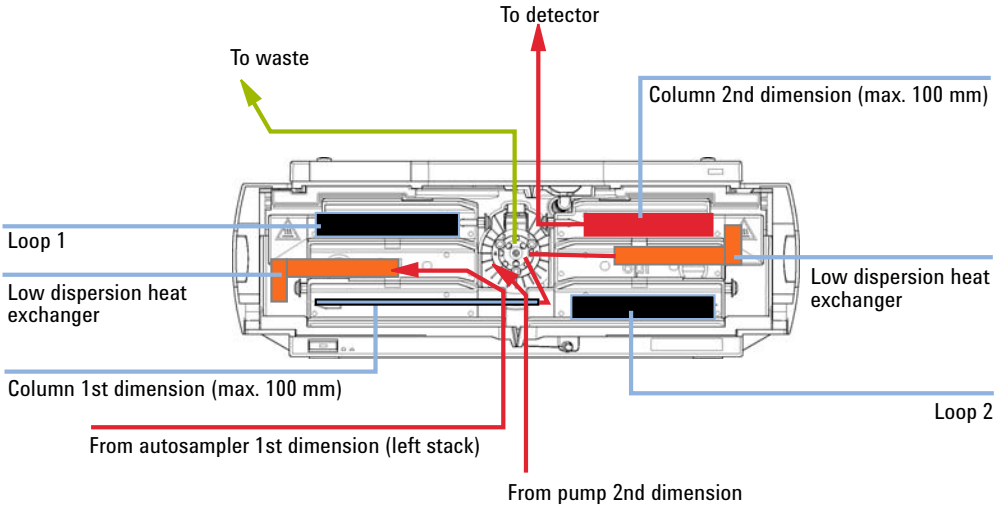


Figure 50 Columns at different temperatures, medium 1st dimension column, no 1st dimension detector

Valve Options

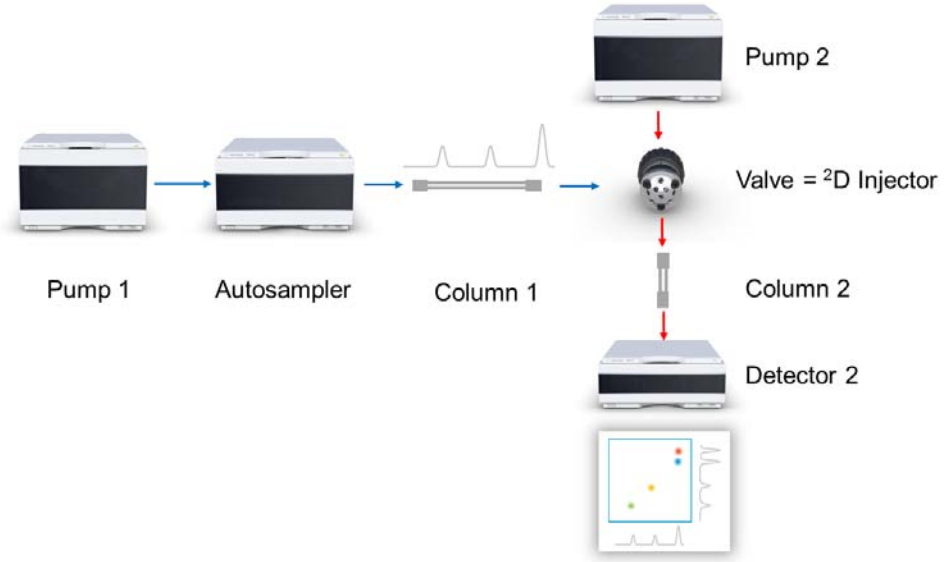


Figure 51 Concept of a 2D-LC-System

The Agilent 1290 Infinity 2D-LC Solution supports the 2D-LC Valve (2pos/4port-duo valve) in combination with 2 x 6/14 Column Selection Valve for multiple heart-cutting LC.

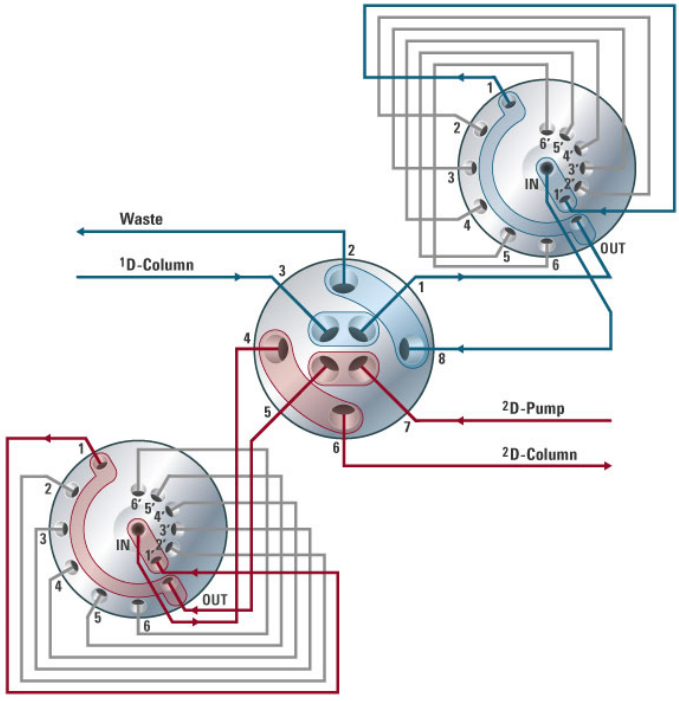


Figure 52 Valve setup with two 6/14 valves for peak parking

Interfacing First and Second Dimension for Heartcutting 2D-LC (LC-LC)

In MHC experiments only selected parts of the first dimension are transferred to the second dimension and anazlyzed.

NOTE Heartcutting 2D-LC experiments usually are characterized by longer runtimes and shallower gradients, compared to comprehensive 2D-LC (LCxLC) experiments.

Table 18 Plumbing for 2D-LC Multiple Heart-Cutting 2D-LC - Single Storage

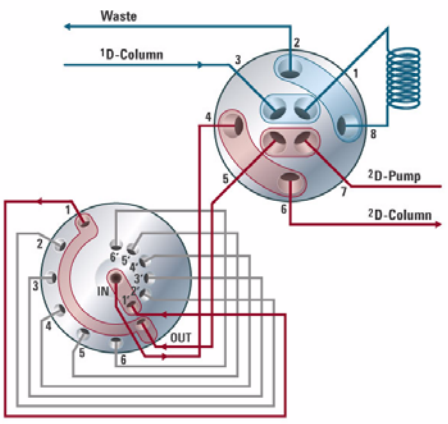
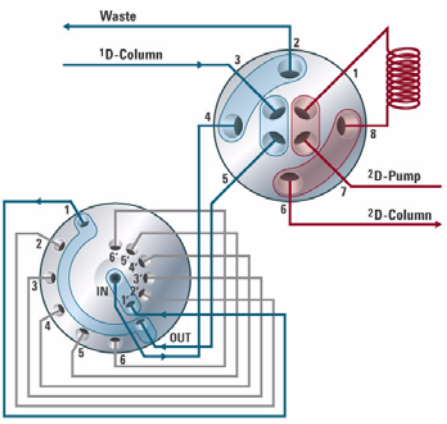
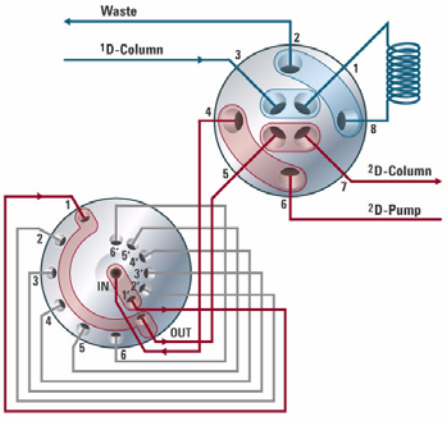
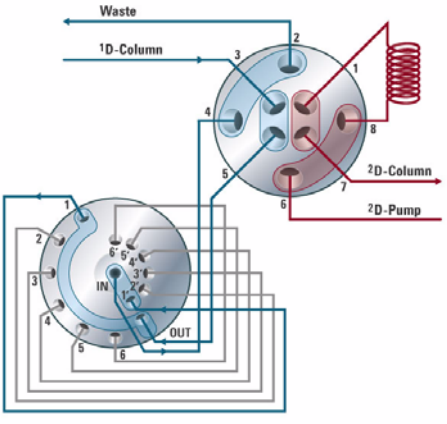
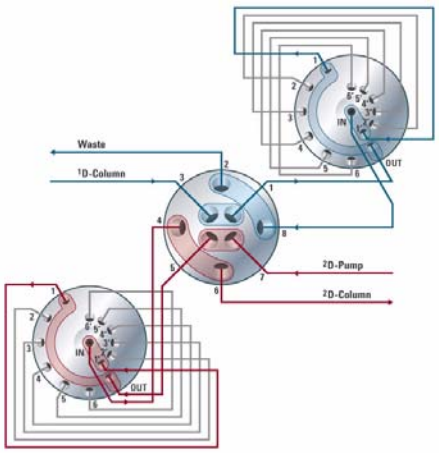
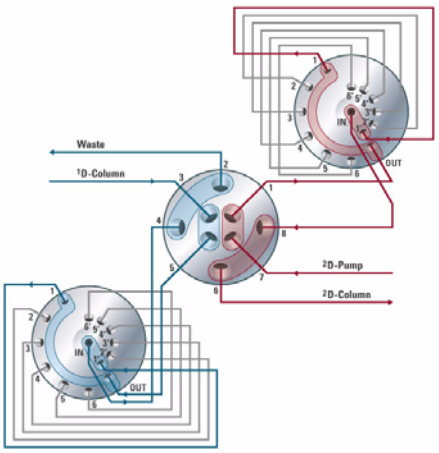
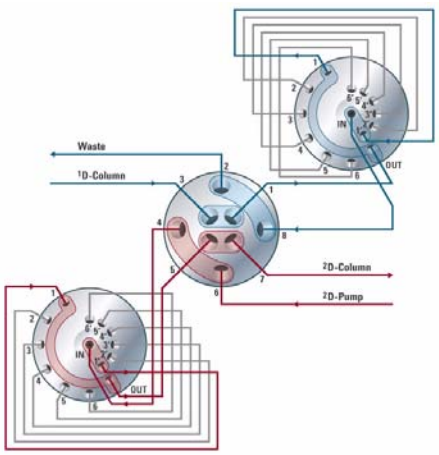
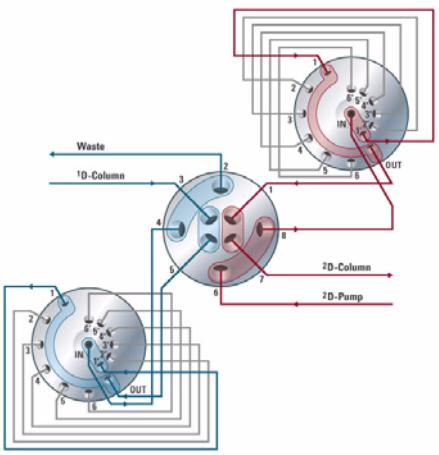
	Filling	Analyzing
Cocurrent		
Countercurrent		

Table 19 Plumbing for 2D-LC Multiple Heart-Cutting 2D-LC - Double Storage

	Filling	Analyzing
Cocurrent		
Countercurrent		

Supported modules/systems

1D/2D-Switch

The 1D/2D-Switch enables the user to simply switch between a 1D- and 2D-LC setup without replacing the capillary connections. For this setup (bypass of 2nd dimension) an additional 2/6 valve is needed. For details, see Automated Switching Between 1D-LC and Comprehensive 2D-LC Analysis (5991-4843EN).

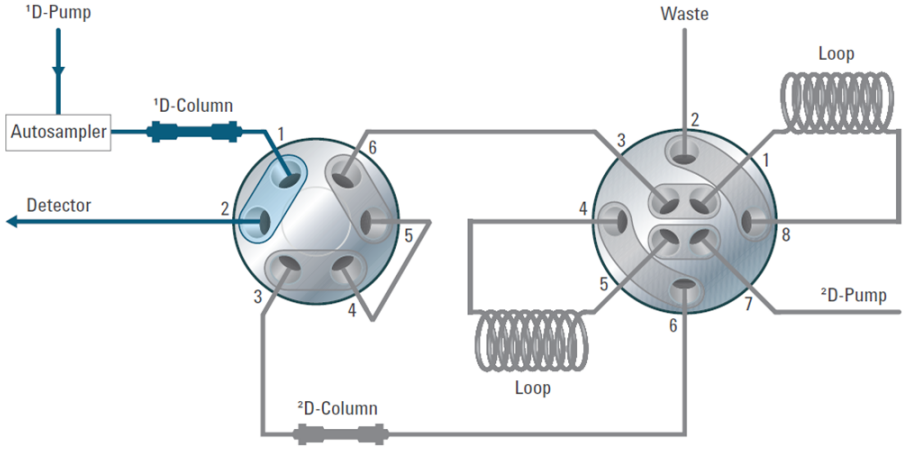


Figure 53 Setup for 1D-LC analysis

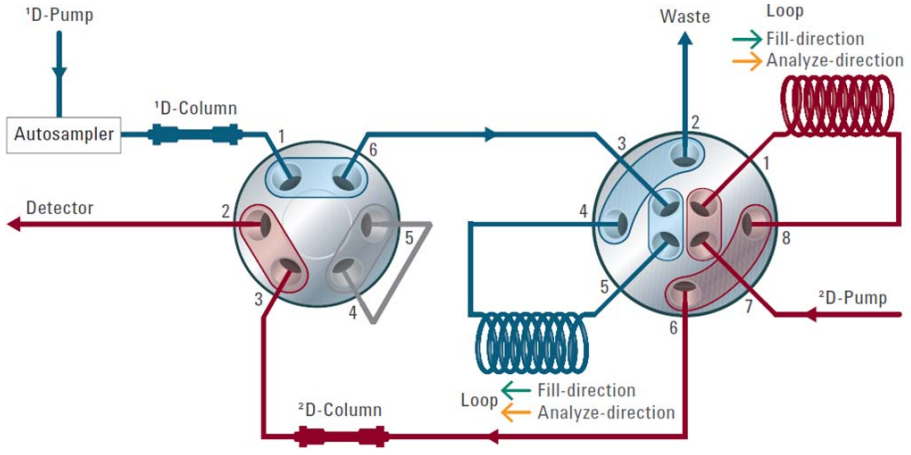


Figure 54 Setup for 2D-LC analysis

Installing the Software

Software required OpenLAB ChemStation Edition C.01.07 (or higher) installed

NOTE For installing the 2D-LC Software, please use the OpenLAB Additional Software and Drivers Deployment Wizard.

NOTE Do not try installing the software by double-clicking the msi file, as this may result in an incomplete installation.

NOTE To install the Add on, the OpenLAB CDS Chemstation Software must be not active.

1 Start OpenLAB Additional Software and Driver Deployment Wizard by going to Windows **> Start > Agilent Technologies > OpenLAB > OpenLAB Additional Software and Drivers**

2 Follow steps described in the Wizard for installation or software upgrades.

Overview Menu Extensions

The installation of the Agilent 1290 Infinity 2D-LC Acquisition Software adds 2D-LC specific items to the Agilent OpenLAB CDS controller software.

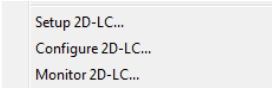


Figure 55 Agilent OpenLAB CDS controller software, 2D-LC specific items

The new menu items appear in the **Instrument Menu** of the **Method & Run Control View**. The following items are available:

- **Setup 2DLC...**

  Displays the 2DLC method dialog

- **Configure 2DLC...**

  Displays the 2DLC configuration dialog

- **Monitor 2DLC...**

  Displays the 2DLC status monitor

Configuration

Overview Configuration Dialog

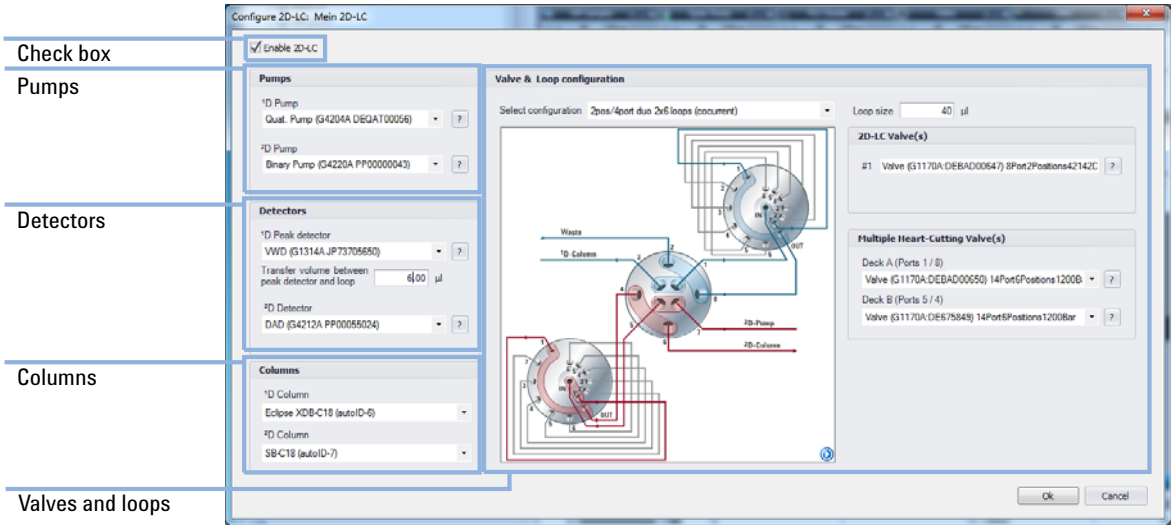


Figure 56 Overview 2D-LC configuration grafical user interface

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 & Loops**

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

Enable 2D-LC

1 Select check box **Enable 2D-LC**.

The 2D-LC functionality is enabled for the configured system.

NOTE If disabled, all all sections in the configuration user interface are disabled and the menu item **Setup 2D-LC method...** is grayed out such that the system uses normal parameters.

Configure Pumps

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

- 1st dimension:

  The drop-down list contains all configured pumps that can be used in the 1st dimension (binary, quaternary, capillary or nano pumps).

- 2nd dimension:

  Only 1290 Infinity Binary Pumps (G4220A/B) or 1290 Infinity II High Speed Pumps (G7120A) can be selected in the 2nd dimension.

The **Identify** button triggers the blinking of the status LED of the corresponding pump module. The button is only enabled in the Online version of the ChemStation

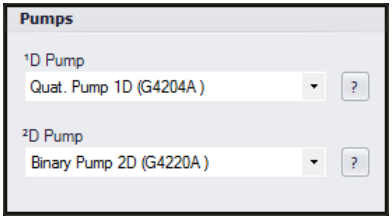


Figure 57 Configuration screen (for example if 1st dimension pump is an Agilent 1290 Infinity Binary Pump)

Preparations

- OpenLAB ChemStation Edition C.01.03 (or higher) installed (Multiple heart-cutting method requires OpenLAB ChemStation Edition C.01.07 (or higher))
- 1290 Infinity 2D-LC Acquisition Software installed
- Check box **Enable 2D-LC** selected.

1 Select the pump for the 1st dimension from the drop-down list **Pump (1D)**.

2 Select the pump for the 2nd dimension from the drop-down list **Pump (2D)**.

3 To save settings click **OK**.

Pumps are configured for 2D-LC.

Configure Detectors

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

- 1st dimension:

  The drop-down list contains all configured detectors and a **None** entry.

- 2nd dimension:

  The drop-down list contains all configured detectors that can be used as peak detector (DAD, MWD, VWD, FLD, RID), and a **None** entry.

The **Identify** button triggers the blinking of the status LED of the corresponding detector module. The button is only enabled in the Online version of the ChemStation

Transfer volume is the volume between the peak detector in the 1st dimension and the loop(s). *In standard setups this volume is 9 µL*. The field is only visible if a peak detector is configured.

For special setups or if the default setting needs to be changed, the following methods for determination of the delay volume exist:

- Use the following volumes:

Table 20 Volumina of capillaries

Capillary	Volume [µL]
Flex capillary, 0.12 x 105 mm, no fittings (5021-1820)	1.187
Flexible tubing, 280 mm (5021-1822)	3.167
Capillary column – detector SST 400 mm lg, 0.12 mm i.d. (5021-1823)	4.524
Capillary ST 0.12 mm x 500 mm (5065-9964)	5.655
Capillary ST 0.12 mm x 600 mm S/SL (5067-4669)	6.786
Capillary, 0.17 mm x 80 cm, male fit (01078-87305)	18.158

- The delay volume of the capillary connection between peak-detector and loop can be either calculated by the following formula:

  $V_D = L * \pi * d^2/4$ (L = length of capillary, d = diameter of capillary

- Alternatively, and with higher accuracy the volume can be measured by two ways:
  - First, by determining the increase in retention time of a narrow peak after adding the respective capillary to an existing HPLC system. A sample like one micro-liter of acetone can be injected to a system using a restriction capillary instead of a column to generate a very narrow peak. In a second measurement, add the capillary of interest directly in front of the detector by using a zero-dead volume union. The set flow rate multiplied with the detected time difference will result the capillary volume.
  - A second option to measure the volume of the capillary is to fill the dried capillary with pure water an weigh it. The weight divided by the density of water (1 mg/µL) will result in the volume. Attention must be given not to have any additional droplets of water being attached to the capillary.

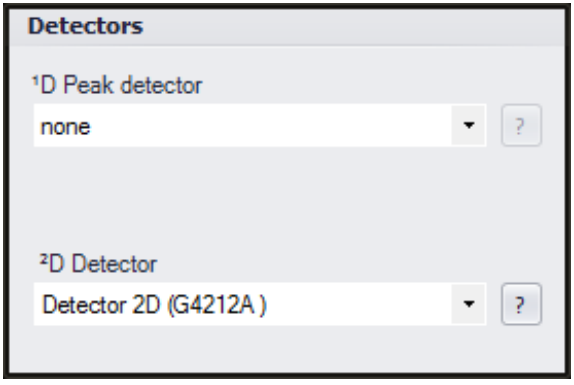


Figure 58 Configuration screen with no peak detector

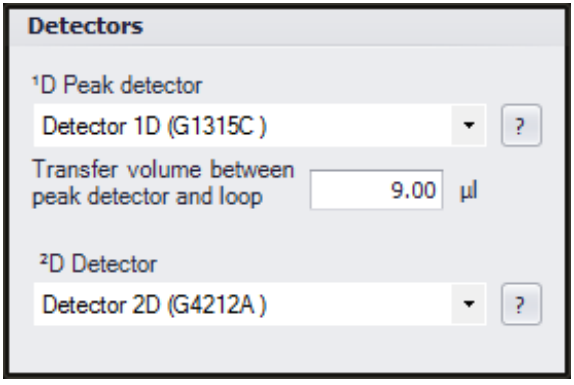


Figure 59 Configuration screen with peak detector

Preparations

- OpenLAB ChemStation Edition C.01.03 (or higher) installed (Multiple heart-cutting method requires OpenLAB ChemStation Edition C.01.07 (or higher))
- 1290 Infinity 2D-LC Acquisition Software installed
- Check box **Enable 2D-LC** selected.

1 Select the **Detector (2D)** from the drop-down list.

2 Select the **Peak detector (1D)** from the drop-down list.

3 To save settings click **OK**.

Detectors are configured for 2D-LC.

Configure Columns

The user can select columns for the 1st and 2nd dimension from the ChemStation column database. The columns are identified by the column description and serial number.

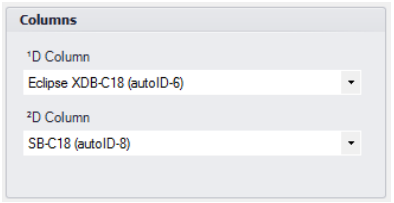


Figure 60 2D-LC column configuration

Preparations

- OpenLAB ChemStation Edition C.01.03 (or higher) installed (Multiple heart-cutting method requires OpenLAB ChemStation Edition C.01.07 (or higher))
- 1290 Infinity 2D-LC Acquisition Software installed
- Check box **Enable 2D-LC** selected.

NOTE The software uses the column parameter to calculate the ratio of the injection volume to the column volume, that typically should not exceed ca. 10 % to avoid break-through of the compounds during the second dimension separation.

1 Select **Column (1D)** from the drop-down list.

2 Select **Column (2D)** from the drop-down list.

3 To save settings click **OK**.

Configure Valve and Loop

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

- **Select configuration**:

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

- **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-LV 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-LV valve.

    Shows the 2^{nd} valve to be used for the injection on the 2^{nd} 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).

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

- **Loop size** specifies the volume of the loop(s). In case of two loops, the software assumes that both loops have the same volume.

All possible loop configurations depending on the selected valves are listed separately and illustrated on screen (see, "Interfacing the First and Second Dimension" on page 104).

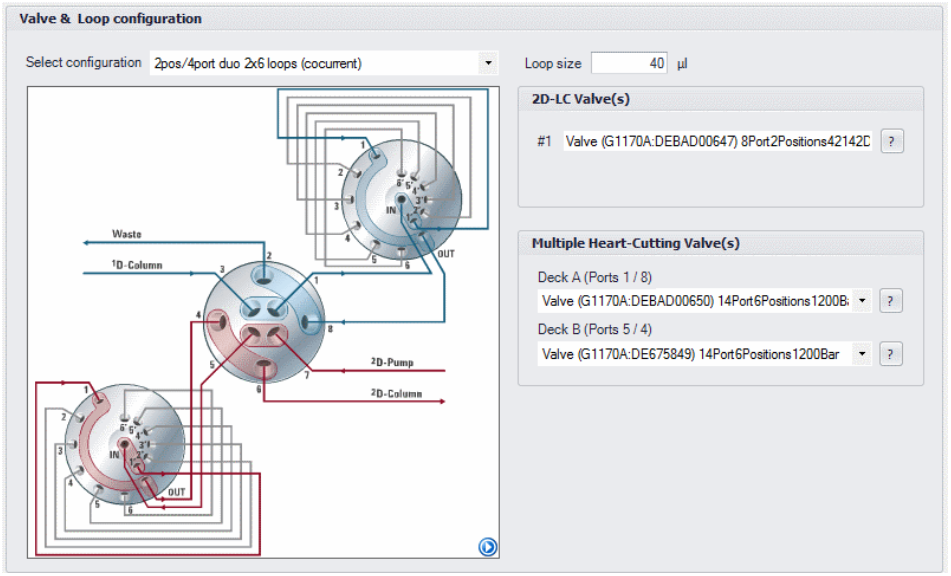


Figure 61 2D-LC valve and loop configuration

Preparations

- OpenLAB ChemStation Edition C.01.03 (or higher) installed (Multiple heart-cutting method requires OpenLAB ChemStation Edition C.01.07 (or higher))
- 1290 Infinity 2D-LC Acquisition Software installed
- 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 configuration**).

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 To save settings click **OK**.

Method parameters

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.

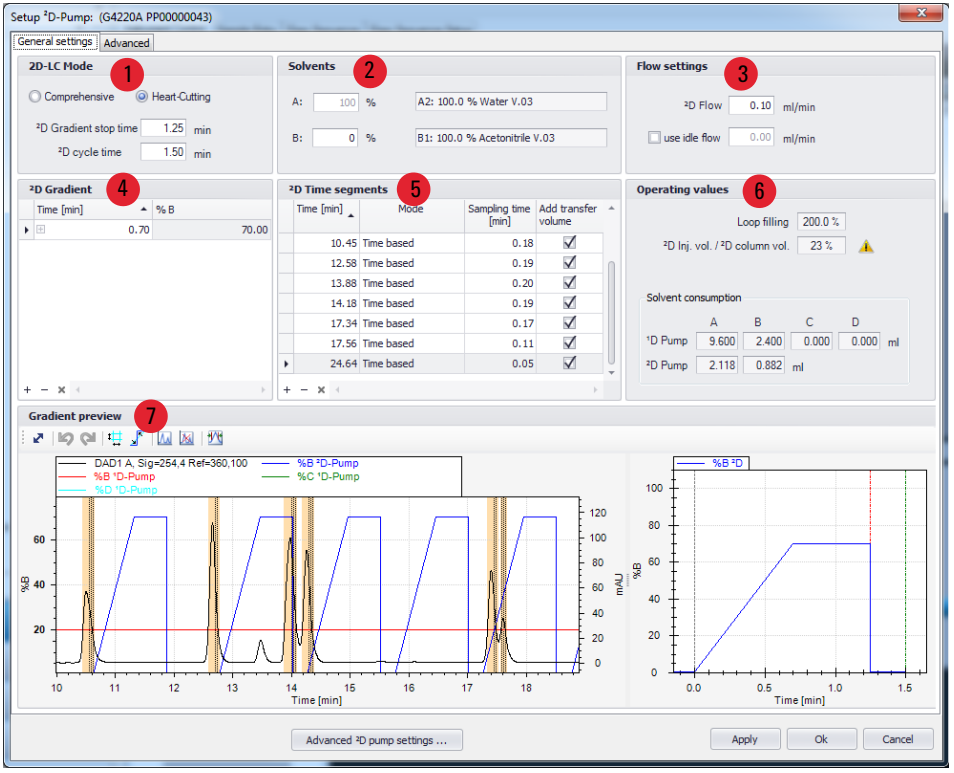


Figure 62 2D-LC method setup (**General settings**)

The setup of following method parameters is available:

1 **2D-LC Mode**, see “Set 2D-LC Mode” on page 122

2 **Solvents**, see “Set solvents” on page 125

3 **Flow settings**, see “Set flow” on page 126

4 **^{2}D Gradient**, see “Set Solvent Composition Gradient” on page 127

5 **^{2}D Time segments**, see “Set 2D Time Segments” on page 129

6 **Operating values**, see “Define Peak detector parameter” on page 132

7 **Gradient preview** with toolbar, see “Gradient Preview Functionality” on page 133.

Set 2D-LC Mode

Setting the mode has the following consequences (for details, see "Introduction" on page 8):

- **Heart cutting**:

  A relevant volume of the 1st dimension is cut off and injected onto the 2^{nd} dimension column using the pump in the 2^{nd} dimension. The volume to be injected on the 2^{nd} 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 2^{nd} dimension starts running the gradient of the 2^{nd} 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 25 on page 55):

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 2D-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 1D run-time will always finish any 2D-operations.

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

1 Select the **Heart cutting** 2D-LC Mode (correct for standard heart cutting and multiple heart cutting setup).

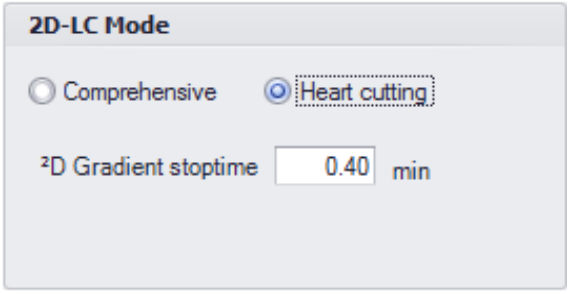


Figure 63 2D-LC **Heart cutting** mode

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 2D 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 %.

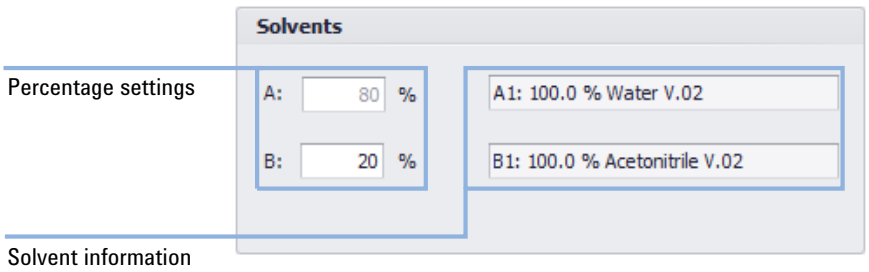


Figure 64 2D-LC solvent settings

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 "Overview Configuration Dialog" on page 43).

Set flow

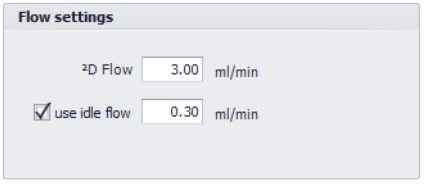


Figure 65 Flow settings

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 2D-Flow is also used while 2D-LC mode is OFF.

Set Solvent Composition Gradient

Set Solvent Composition Gradient

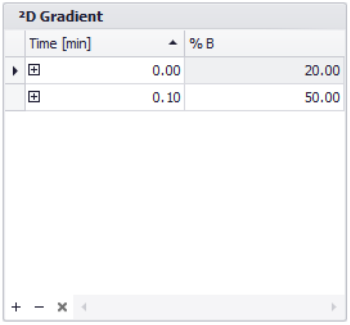
The timetable in the **2D Gradient** group allows changing the solvent composition.

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 2D** will be ignored.

Setup ^{2}D Gradient graphically

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

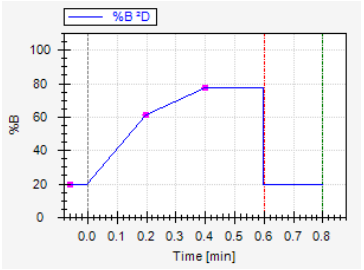


Figure 66 ^{2}D Gradient window in edit mode

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

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 21 Definitions 2D Time Segements

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 Heartcutting mode

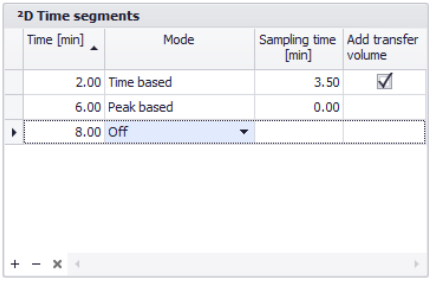
1 To specify, when the actual trigger mode gets active, fill the **Time** column.

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 **2D-stop time**.

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

Trigger 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 25 on page 55.

- **Peak based**

  The peak detector is enabled at the specified time. For details see Figure 25 on page 55.

- **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 **^{2}D Time Segments** now are 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).

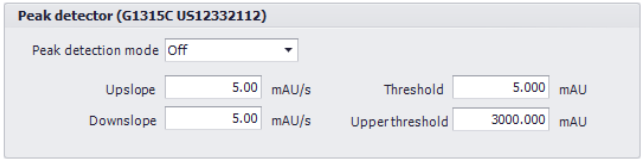


Figure 67 Overview on peak detector parameters

NOTE If no peak detector is configured (see "Overview Configuration Dialog" on page 43) this section is disabled. The currently configured peak detector (name & serial number of the detector) is shown in the section header.

1 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.

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

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

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

5 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.

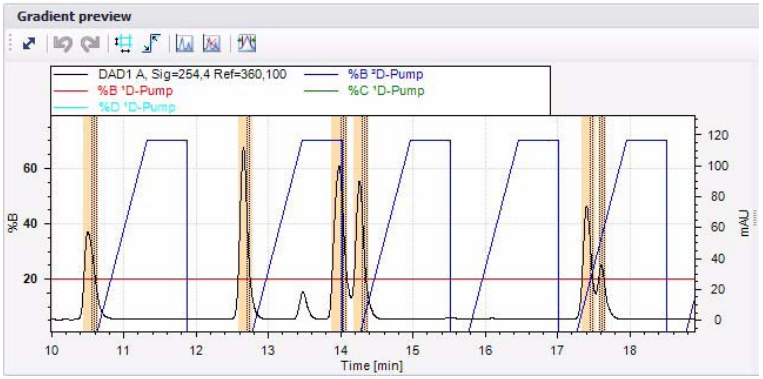


Figure 68 Gradient preview

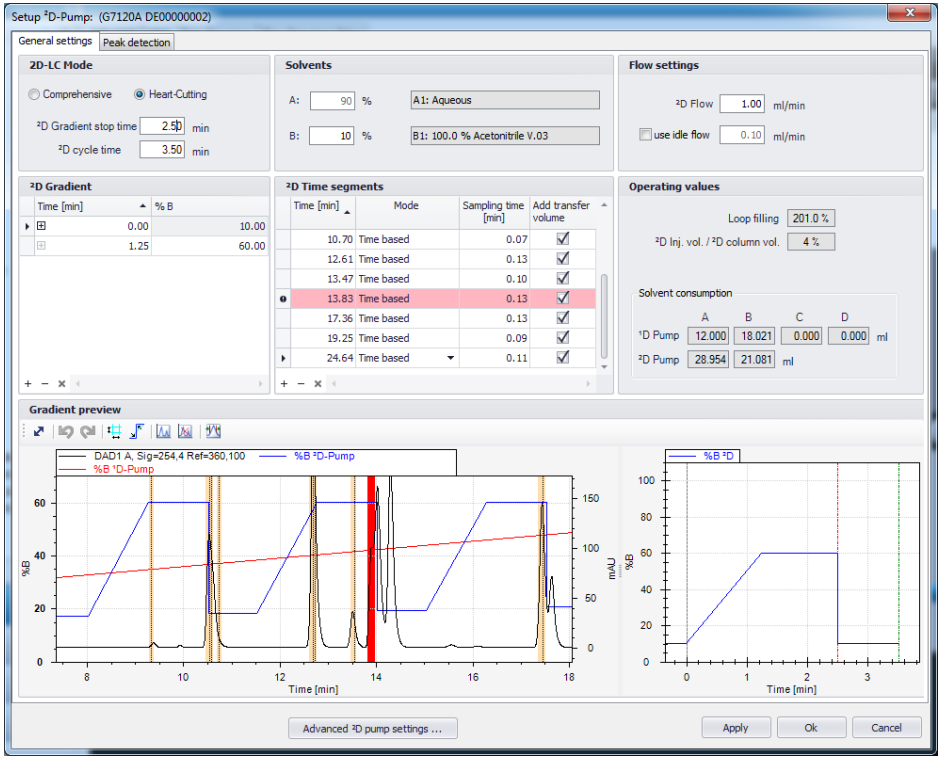
The reference signal offers the following:

- 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)

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 132)
- 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



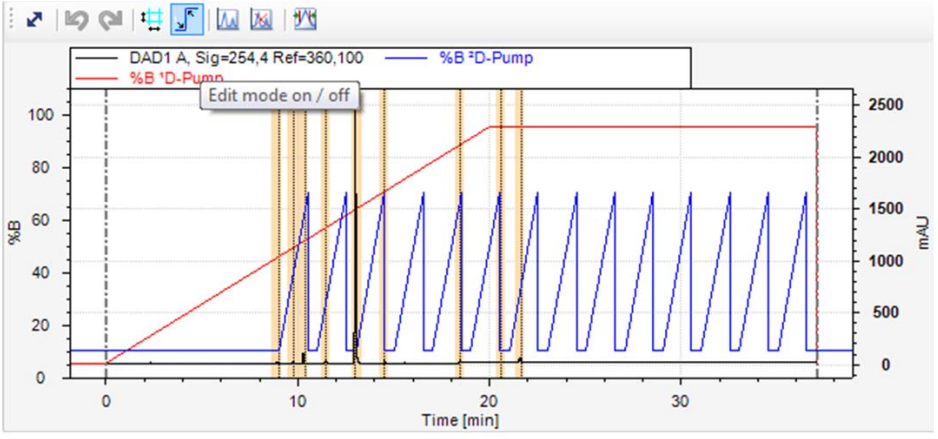
Graphical explanation

- **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 93
- 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 127

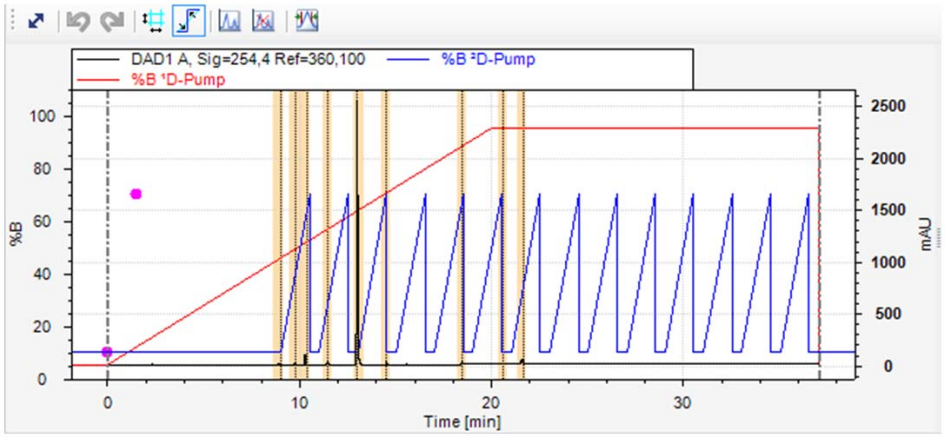
Setup Second Dimension Gradient with the Graphical User Interface

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

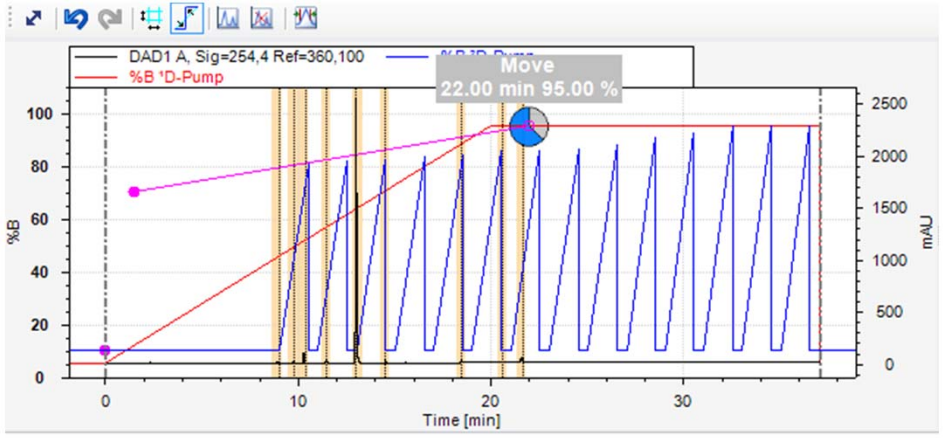
1 To enter the editing mode, use the context menu.



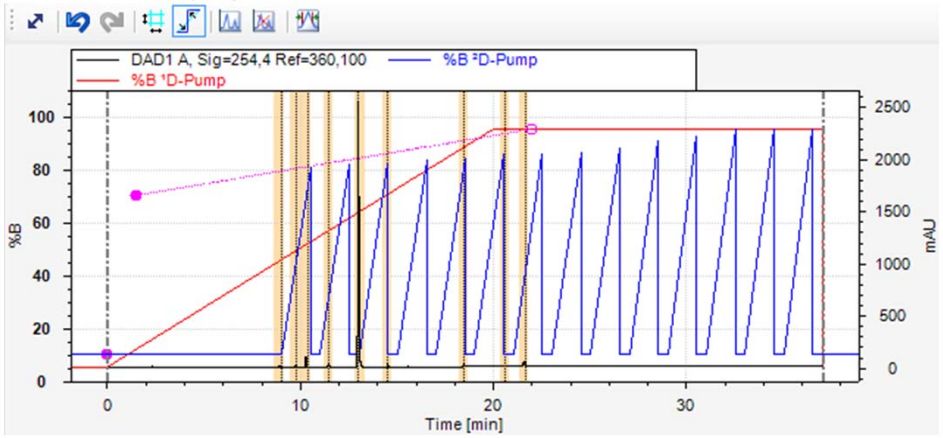
2 Click into the menu. Timetable entries are marked with circles.



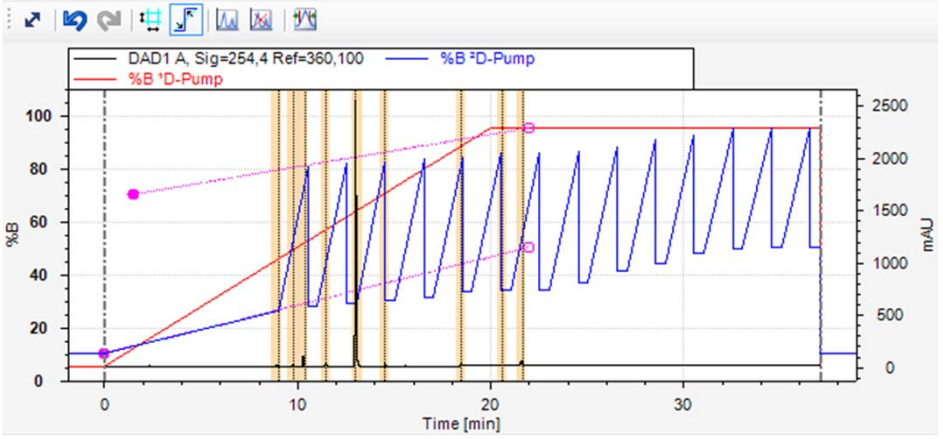
3 Drag the mouse to a new %B value at a specified runtime of the 1st dimension. This draws a straight line.



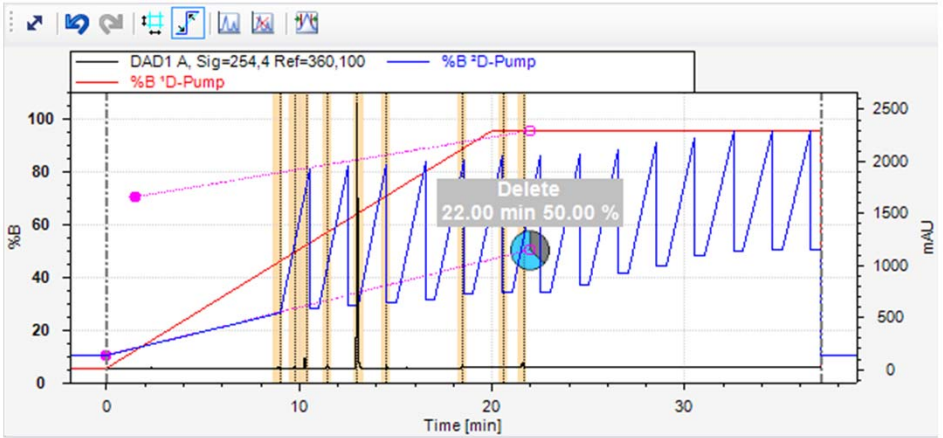
4 When releasing the mouse, a new timetable entry is made and the gradient rollout is automatically updated.



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



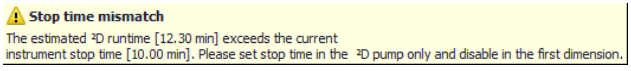
6 Move the mouse cursor near to a shift line to change menu context and insert or delete shift points as needed.



The stop time for a 2D-LC measurement must be set for the 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 extends this run time automatically, 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, 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.



Data analysis

Heart-Cutting Viewer

Using the Multiple Heart-Cutting Upgrade kit, the Agilent 1290 Infinity 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 2D gradients are possible without loss of 1D peaks. But it is quite difficult to review the 2D 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 Heart-Cut Viewer (available with 2D-LC software A.01.02, which requires OpenLAB C.01.07 or higher) 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 Heart-Cut Viewer

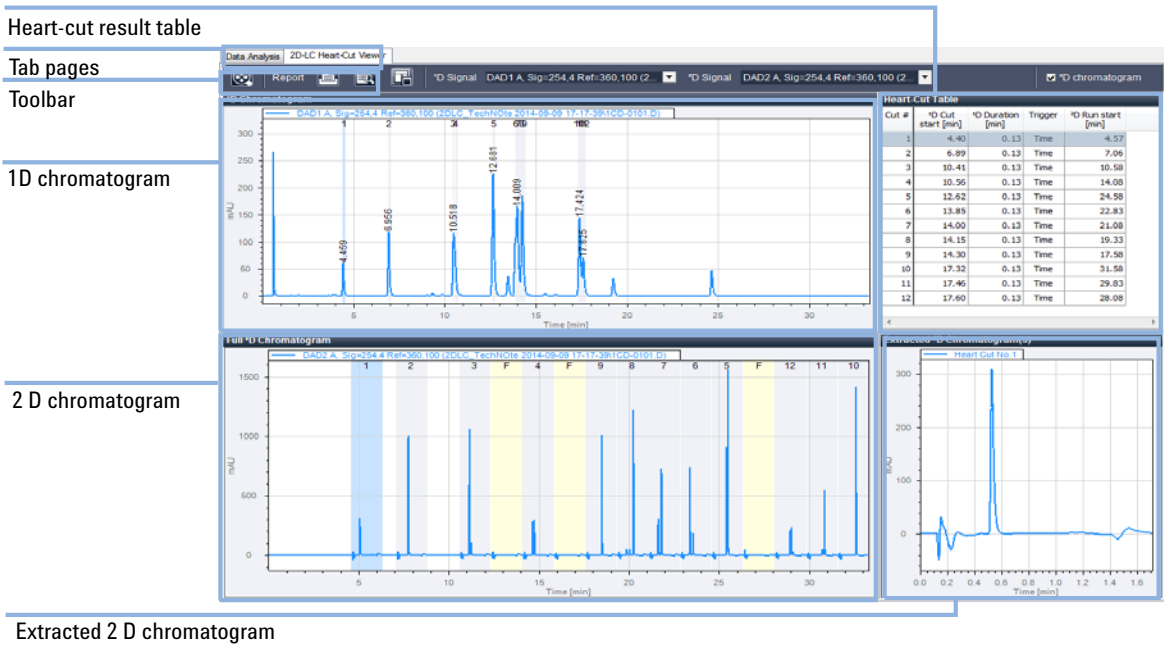


Figure 69 Overview of the 2D-LC Heart-Cut Viewer graphical user interface

The 2D-LC Heart-Cut Viewer provides the following functions:

- Tab pages enable the user to switch between
  - **2D-LC Heart-Cut 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.
- **Heart-Cut Results** table
- 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 Chromatogram** 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)
- **Extracted ^{2}D Chromatogram(s)**

Heart-Cut Results Table

The table lists all heart-cuts which have been analyzed in the 2nd dimension.

Heart-Cut Results

Cut #	¹D Cut start [min]	¹D Duration [min]	Trigger	²D run start [min]	¹D Ret. time [min]	Deck	Loop
1	4.28	0.13	Time	4.45	4.339	1	1
2	6.61	0.13	Time	6.79	6.660	2	1
3	10.00	0.13	Time	10.18	10.119	1	1
4	10.15	0.13	Time	13.68		2	2
5	12.19	0.13	Time	24.18	12.288	1	2
6	13.60	0.13	Time	22.43		1	3
7	13.74	0.13	Time	20.68		1	4
8	13.88	0.13	Time	18.93	13.884	1	5
9	14.02	0.13	Time	17.18		1	6
11	16.84	0.13	Time	34.69		2	2
12	16.98	0.13	Time	32.94	16.984	2	3
13	17.12	0.13	Time	31.18	17.174	2	4
14	17.26	0.13	Time	29.43		2	5

Figure 70 **Heart-Cut Results** (example)

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

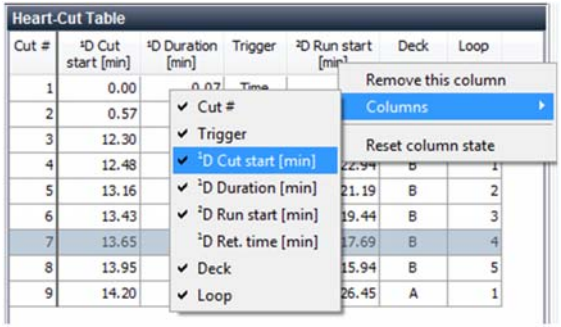


Table 22 Legend for Heart-Cut Results

PosNr	Description
Cut #	The current number of the heart-cut
1D Cut start [min]	Time when the heart-cut starts (peak begin or time value in trigger table)
1D Duration [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
2D run start [min]	Time when the analyses of this heart-cut in the 2nd dimension starts (gradient start)
1D 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

Selected heart-cut

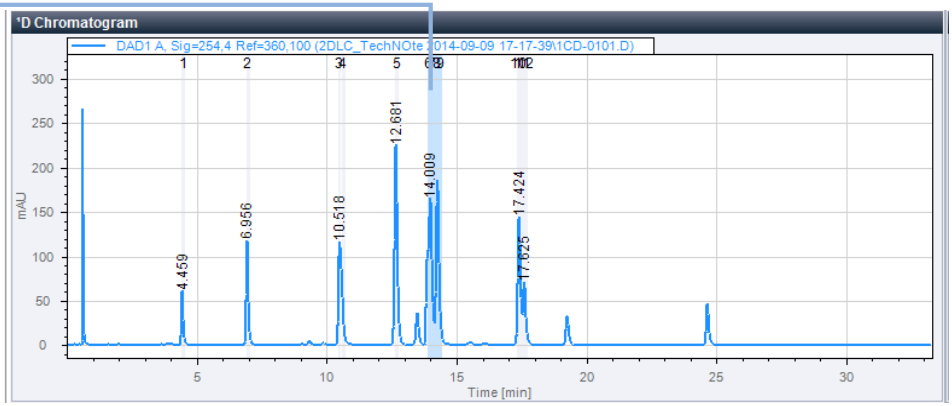


Figure 71 **^{1}D Chromatogram** (example)

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

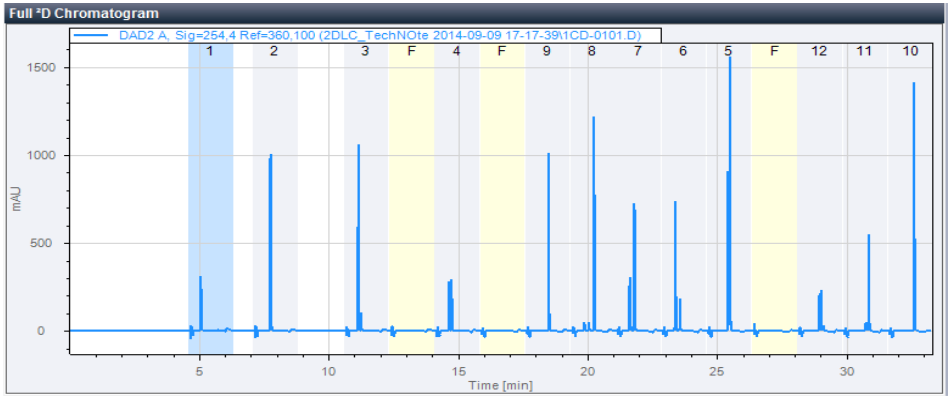


Figure 72 **^{2}D Chromatogram** (example - only visible, if **^{2}D chromatogram** (checkbox) is checked)

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 heart-cut table).
- **F** indicates a bypass (or flush) gradient, which was used to flush the transfer capillaries after switching the 2D-LC valve.

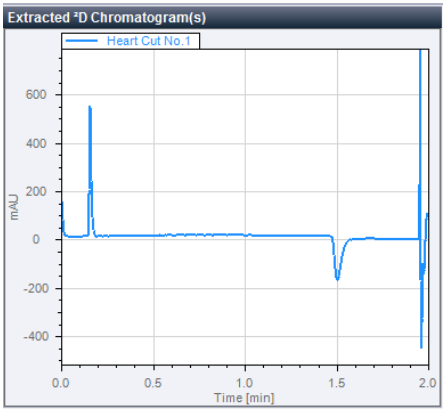


Figure 73 **Extracted ^{2}D Chromatogram** (example from **^{2}D Chromatogram** above, Heart Cut No. 1)

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.

Checkout/FamiliarizationProcedure

Checkout runs - 1290 Infinity Binary or Quaternary LC in 1D

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 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

See Table 16 on page 101

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

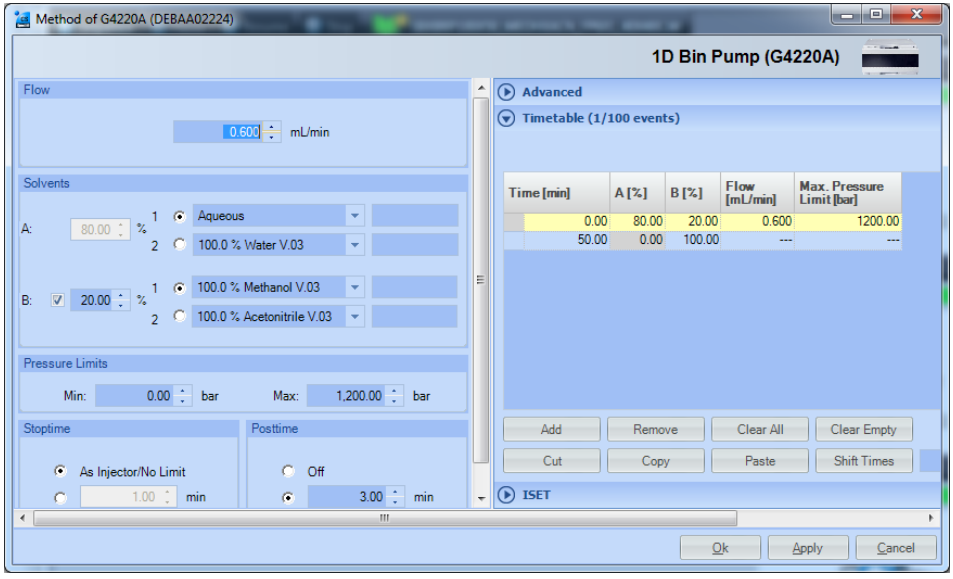
Preparations:

1. Prepare dilution solvent (20 MeOH in mobile phase A): Add 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 xxxx.yyyyy from the CD.

1 Apply the following method parameters for ^{1}D:

- Column: RRHD SB-C18, 2.1x 100 mm, 1.8 µm, 1200 bar (858700-902)
- Solvent:
  - A: H_2O + 0.2 % formic acid
  - B: Methanol
- Gradient:
  - 0.0 min – 20 % B
  - 50 min – 100 % B
  - Stoptime: 40 min
  - Posttime: 10 min
- Flow rate: 0.300 mL/min
- Temperature: 40 °C
- Post Time: 6 min

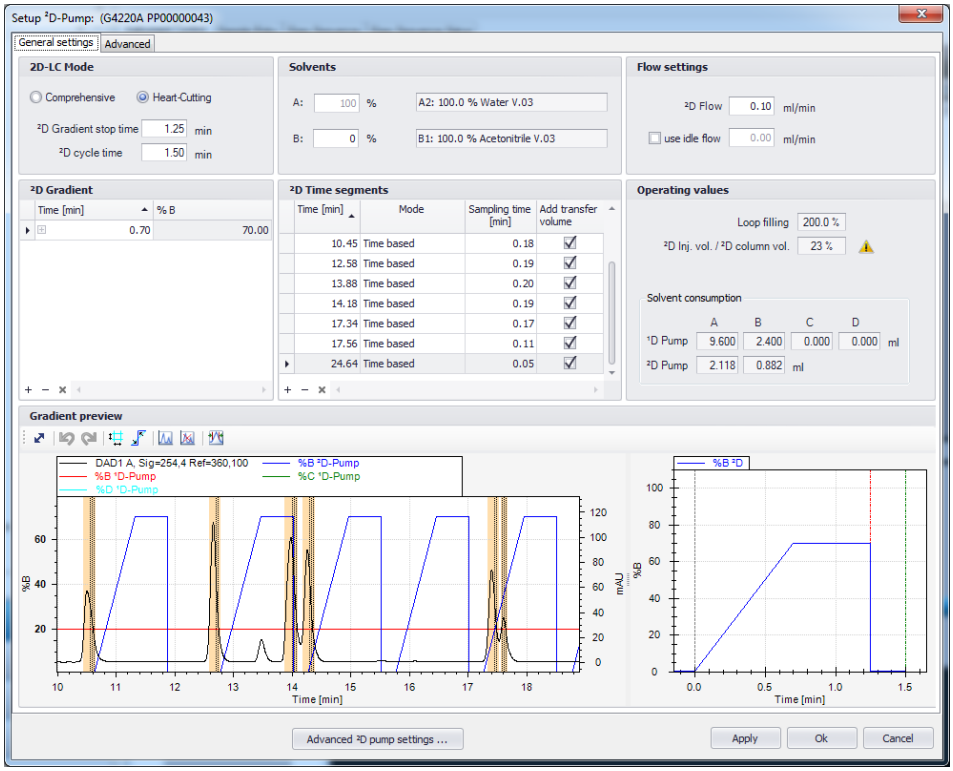
The 1D method should be set up as displayed in the screen:



2 Apply the following method parameters for ^{2}D:

- Column: RRHD Bonus-RP, 2.1x 50 mm, 1.8 µm, 1200 bar (857768-901)
- Solvent:
  - A: H_2O + 0.2 % formic acid
  - B: Acetonitrile
- Gradient:
  - 0.0 min – 10 % B
  - 1.25 min – 60 % B
  - 2D Gradient stoptime: 1.25 min
  - 2D Cycle Time: 1.75 min
- Stop Time: 40 min (will be automatically prolonged, if peaks in 2nd dimension are not worked off)
- Gradient shift: 0→20 min from 10→30 %B (only downslope)
- Flow rate: 1.0 mL/min
- Temperature: 40 °C

The 2D method should be set up as displayed in the screen:


Setup ²D-Pump: (G4220A PP00000043)
General settings
Advanced
2D-LC Mode
Comprehensive
Heart-Cutting
²D Gradient stop time 1.25 min
²D cycle time 1.50 min
Solvents
A: 100 % A2: 100.0 % Water V.03
B: 0 % B1: 100.0 % Acetonitrile V.03
Flow settings
²D Flow 0.10 ml/min
use idle flow 0.00 ml/min
²D Gradient
Time [min] % B
0.70 70.00
²D Time segments
Time [min] Mode Sampling time [min] Add transfer volume
10.45 Time based 0.18
12.58 Time based 0.19
13.88 Time based 0.20
14.18 Time based 0.19
17.34 Time based 0.17
17.56 Time based 0.11
24.64 Time based 0.05
Operating values
Loop filling 200.0 %
²D Inj. vol. / ²D column vol. 23 %
Solvent consumption
A B C D
¹D Pump 9.600 2.400 0.000 0.000 ml
²D Pump 2.118 0.882 ml
Gradient preview
DAD1 A, Sig=254,4 Ref=360,100
%B ¹D-Pump
%B ²D-Pump
%D ¹D-Pump
%C ²D-Pump
%B
mAU
Time [min]
%B ²D
Advanced ²D pump settings ...
Apply
Ok
Cancel

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	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

4 Detection:

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

Acquisition rate: 5 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

Checkout runs - 1260 Infinity Binary in 1D

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 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

See Table 17 on page 101

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

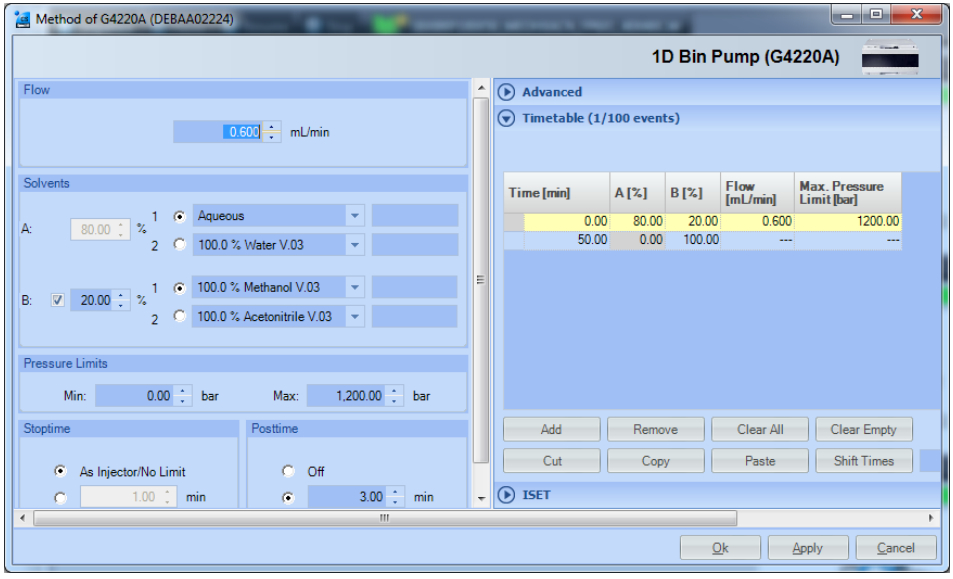
Preparations:

1. Prepare dilution solvent (20 MeOH in mobile phase A): Add 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 xxxx.yyyyy from the CD.

1 Apply the following method parameters for ^{1}D:

- Column: RRHD SB-C18, 2.1x 100 mm, 1.8 µm, 1200 bar (858700-902)
- Solvent:
  - A: H_2O + 0.2 % formic acid
  - B: Methanol
- Gradient:
  - 0.0 min – 20 % B
  - 50 min – 100 % B
  - Stoptime: 40 min
  - Posttime: 10·e min
- Flow rate: 0.300 mL/min
- Temperature: 40 °C
- Post Time: 6 min

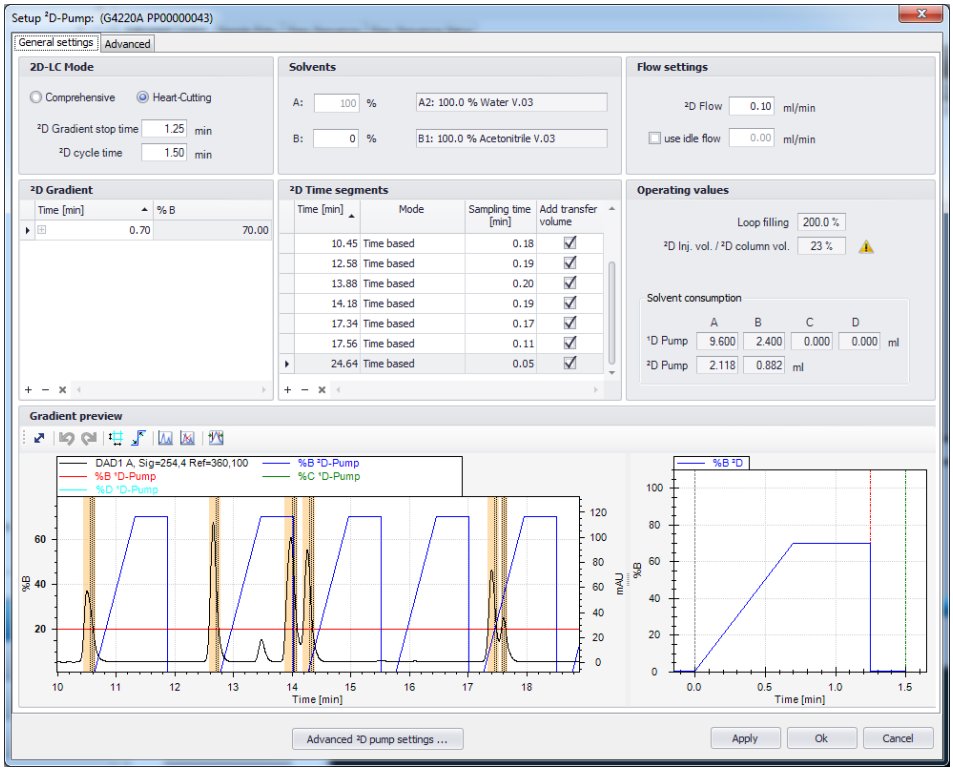
The 1D method should be set up as displayed in the screen:



2 Apply the following method parameters for ^{2}D:

- Column: RRHD Bonus-RP, 2.1x 50 mm, 1.8 µm, 1200 bar (857768-901)
- Solvent:
  - A: H_2O + 0.2 % formic acid
  - B: Acetonitrile
- Gradient:
  - 0.0 min – 10 % B
  - 1.25 min – 60 % B
  - 2D Gradient stoptime: 1.25 min
  - 2D Cycle Time: 1.75 min
- Stop Time: 40 min (will be automatically prolonged, if peaks in 2nd dimension are not worked off)
- Gradient shift: 0→20 min from 10→30 %B (only downslope)
- Flow rate: 1.0 mL/min
- Temperature: 40 °C

The 2D method should be set up as displayed in the screen:


Setup ²D-Pump: (G4220A PP00000043)
General settings
Advanced
2D-LC Mode
Comprehensive
Heart-Cutting
²D Gradient stop time 1.25 min
²D cycle time 1.50 min
Solvents
A: 100 % A2: 100.0 % Water V.03
B: 0 % B1: 100.0 % Acetonitrile V.03
Flow settings
²D Flow 0.10 ml/min
use idle flow 0.00 ml/min
²D Gradient
Time [min] % B
0.70 70.00
²D Time segments
Time [min] Mode Sampling time [min] Add transfer volume
10.45 Time based 0.18
12.58 Time based 0.19
13.88 Time based 0.20
14.18 Time based 0.19
17.34 Time based 0.17
17.56 Time based 0.11
24.64 Time based 0.05
Operating values
Loop filling 200.0 %
²D Inj. vol. / ²D column vol. 23 %
Solvent consumption
A B C D
¹D Pump 9.600 2.400 0.000 0.000 ml
²D Pump 2.118 0.882 ml
Gradient preview
DAD1 A, Sig=254,4 Ref=360,100
%B ¹D-Pump
%B ²D-Pump
%D ¹D-Pump
%C ¹D-Pump
%B
mAU
Time [min]
%B ²D
Advanced ²D pump settings ...
Apply
Ok
Cancel

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

Cut-#	Cut-Time [min] 1260 Binary LC
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

4 Detection:

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

Acquisition rate: 5 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

5
Full Comprehensive 2D-LC

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

Comprehensive 2D-LC (LCxLC)

The following items are characteristic for LCxLC:

- The complete effluent of the first column is injected to the second column. Two identical loops are used in an alternating way. With the G4236A 2D-LC valve kit special loop pairs with matched volumes are optionally available. While one loop is filled in the 1^{st} dimension, the volume of the other loop is separated with very fast gradients on the second column. After the so called modulation time the valve will switch and filling and analyzing of the loop content will switch between both loops. The stop-time of the 2^{nd} dimension gradient should be equal or shorter than the modulation time. If it is shorter than the modulation time the %B of the gradient will return to its initial value. The modulation time should be (equal or) shorter than half of the first dimension stop time. If it is equal to the half of the first dimension stop time only two samplings will be made, this usually does not lead to satisfying results, and typically the modulation time is significantly shorter.

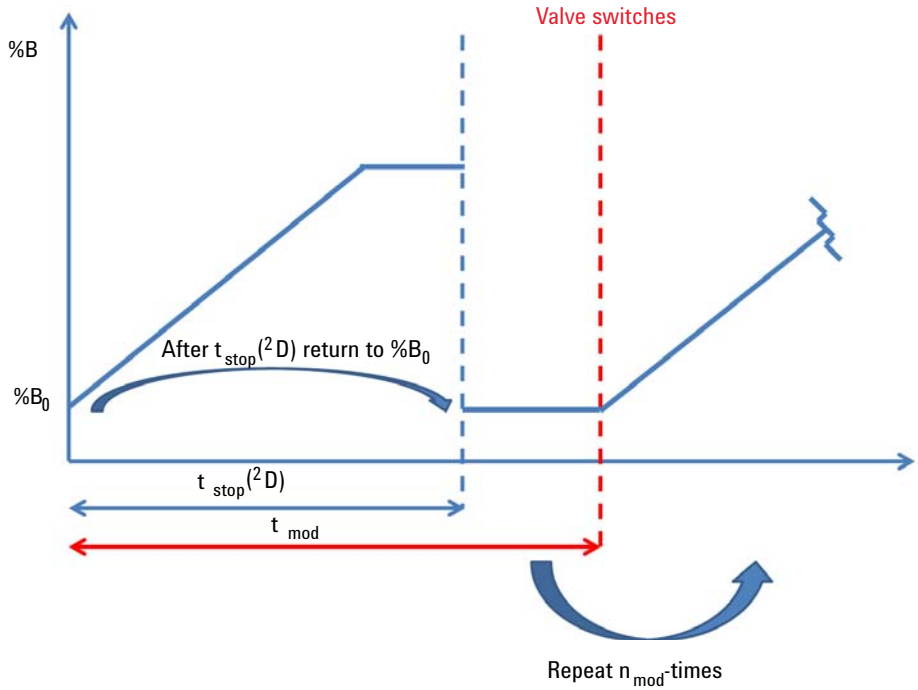


Figure 74 Characteristics of LCxLC

$t_{stop}(^1D)$ = ^1D-Gradient Stoptime; $0 < t_{stop}(^2D) \leq t_{mod} \leq (t_{stop}(^1D)/2)$

$t_{stop}(^2D)$ = ^2D-Gradient Stoptime

t_{mod} = Modulation-time

n_{mod} = Modulation; $n_{mod} = t_{stop}(^2D)/t_{mod}$

- The run time of the 2nd dimension method matches the collection time of the 1st dimension effluent.

- By doing so, short snapshots of the first dimension peak elution order will be achieved. To see the elution order of the peaks from the first dimension, the chromatograms acquired in the second dimension column will be stacked by suitable software packages like GC Image LCxLC edition one after the other to re-constructed the first dimension chromatogram. It is also possible with the 1290 Infinity 2D-LC solution to place a detector directly after the first dimension column to acquire the 1st dim chromatogram directly. This set-up is also required for any peak triggered operations but some additional bandspreading should be taken into consideration.

NOTE Comprehensive 2D-LC (LCxLC) is the method of choice if the samples to analyze are unknown or complex (biopharma, food, polymers and so on).

There are three modes of comprehensive 2D-LC (LCxLC) supported with the 1290 Infinity 2D-LC solution:

- Standard LCxLC
- Time-triggered LCxLC
- Peak-triggered 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.

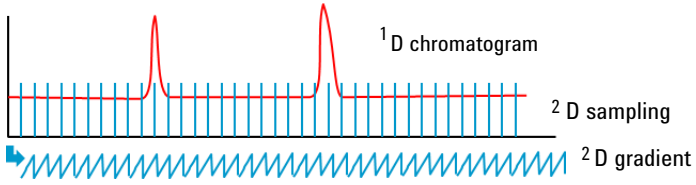


Figure 75 Principle of standard LCxLC

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.

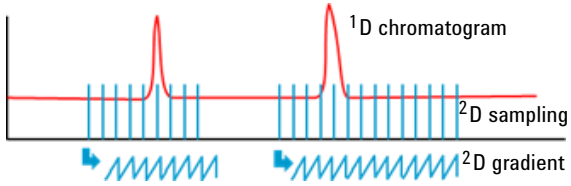


Figure 76 Principle of time-triggered LCxLC

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).

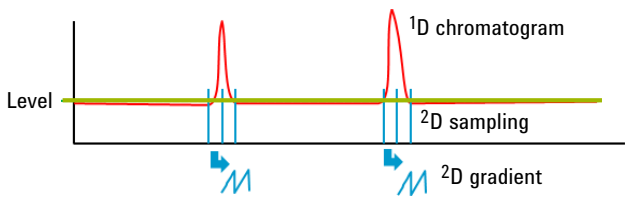


Figure 77 Principle of peak-triggered LCxLC

Installation

Recommended fix Setups for Comprehensive 2D-LC

Table 23 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 For the optional available 1D/2D switch 2pos/6port Valve head 1200 bar (G4231B), see "1D/2D-Switch" on page 177.

Table 24 1290 Infinity Quaternary LC in first dimension

	Partnumber	Description	Comment
1st Dim	G4204A	**1290 Infinity Quaternary 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 For the optional available 1D/2D switch 2pos/6port Valve head 1200 bar (G4231B), see "1D/2D-Switch" on page 177.

Table 25 1260 Infinity Binary LC in first dimension

	Partnumber	Description	Comment
1st Dim	G1312B	**1260 Infinity Binary Pump**	
	G1367E	1260 Infinity High Performance Autosampler	
	G1330B	1290 Thermostat	
	G1316A	1260 Thermostatted Column Compartment	
	G4212B	1260 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 For the optional available 1D/2D switch 2pos/6port Valve head 600 bar (G4231A), see "1D/2D-Switch" on page 177.

Possible stack configurations

The following configurations optimize the system flow path, ensuring minimum delay volume.

NOTE The capillary connections should be as short as possible, to ensure optimum performance of the system.

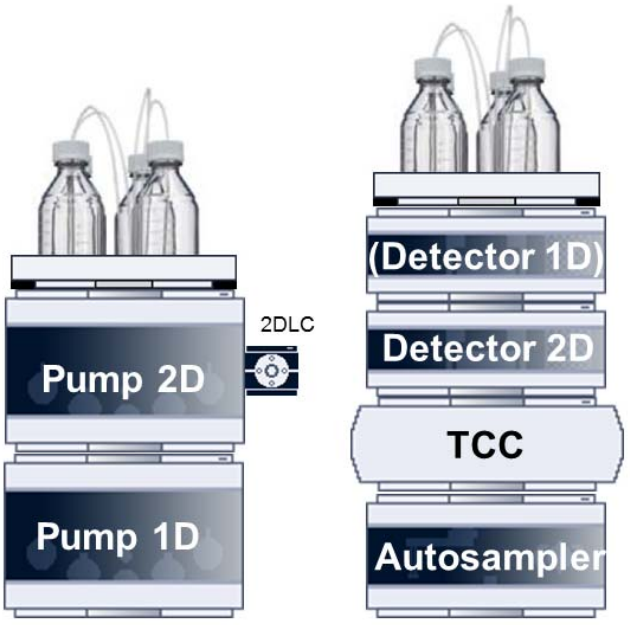


Figure 78 Stack configuration for Standard Heart-cutting 2D-LC

Table 26 1290 Infinity 2D-LC-System

Left Stack	Right Stack
	Solvent Cabinet
	^{1}D detector (DAD (G4212A or G7117A/B)) - (optional)
Solvent Cabinet	^{2}D detector (DAD (G4212A or G7117A/B))
^{2}D pump (G4220A) with Valve Drive (G1170A) attached	TCC (G1316C)
^{1}D pump (G4220A/G4204A)	Autosampler (G4226A)

Table 27 1260 Infinity 2D-LC-System

Left Stack	Right Stack
	Solvent Cabinet
	^{1}D detector (DAD (G4212A/B or G7117A/B)) - (optional)
Solvent Cabinet	^{2}D detector (DAD (G4212A/B or G7117A/B))
^{2}D pump (G4220A) with Valve Drive (G1170A) attached	TCC (G1316C)
^{1}D pump (G4220A or G4204A or G1312A/B/C or G1311A or G1376A)	Autosampler (G4226A or G1367E/F)

Capillary connections (kits)

After placing the modules of the first dimension and second dimension and making the electrical connections the flow paths must be build.

Capillary Kit for 2D-LC

Item	p/n	Description
1	5021-1820 (2x)	Flex capillary, 0.12 x 105 mm, no fittings
2	G1316-87321	Capillary column-heat exchanger 105 mm lg, 0.17 mm i.d.
3	5021-1822	Flexible tubing, 280 mm
4	5021-1823 (3x)	Capillary column – detector SST 400 mm lg, 0.12 mm i.d.
5	5021-1819	Capillary ST 0.17 mm x 400 mm S/S
6	5065-9964	Capillary ST 0.12 mm x 500 mm
7	5067-4609	Capillary ST 0.17 mm x 500 mm SX/-
8	5067-4669	Capillary ST 0.12 mm x 600 mm S/SL
9	01078-87305	Capillary, 0.17 mm x 80 cm, male fit
10	G1316-80022 (2x)	LDHE double kit for G1316C

Supported valves and valve hosts

Two valves are supported as modulation valve in the 1D-2D interface:

- G4236A 2,4 Duo valve (highly recommended!)
- G4232B 2,10 valve (possible, but not recommended)

NOTE The formerly support setup using a 2/6 valve in the interface is not supported anymore from SW version *A.01.02*.

The following valve hosts are available in Agilent´s portfolio:

- G1170A 1290 Infinity Valve Drive
  Highly recommended for 2D-LC valve
- G1316C 1290 Infinity Thermostatted Column Compartment
  not recommended/forbidden for G4236A, but allowed for 1D/2D-switch

Interfacing the First and Second Dimension

Thermostatted Column Compartment Setup

Different variants of thermostatted column compartment setup are supported with the optional 2D-LC capillary kit for the 2D-LC valve (G4236A#3):

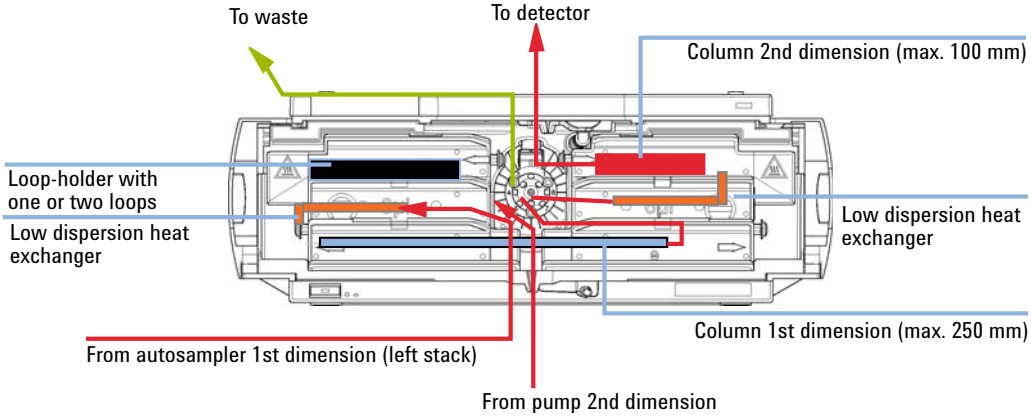


Figure 79 Columns at different temperatures,long 1st dimension column, no 1st dimension detector, for comprehensive 2D-LC booth loops can be placed in the loop holder.

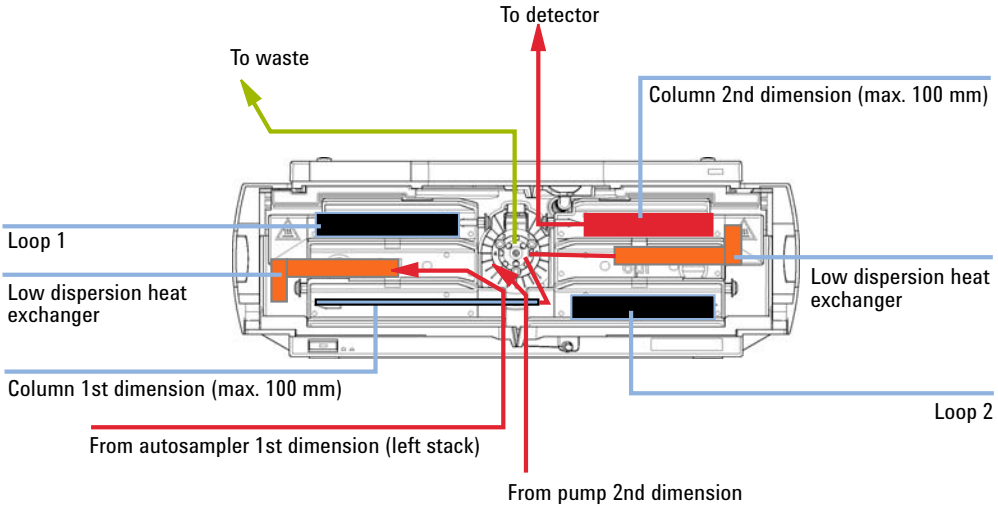


Figure 80 Columns at different temperatures, medium 1st dimension column, no 1st dimension detector

Valve Options

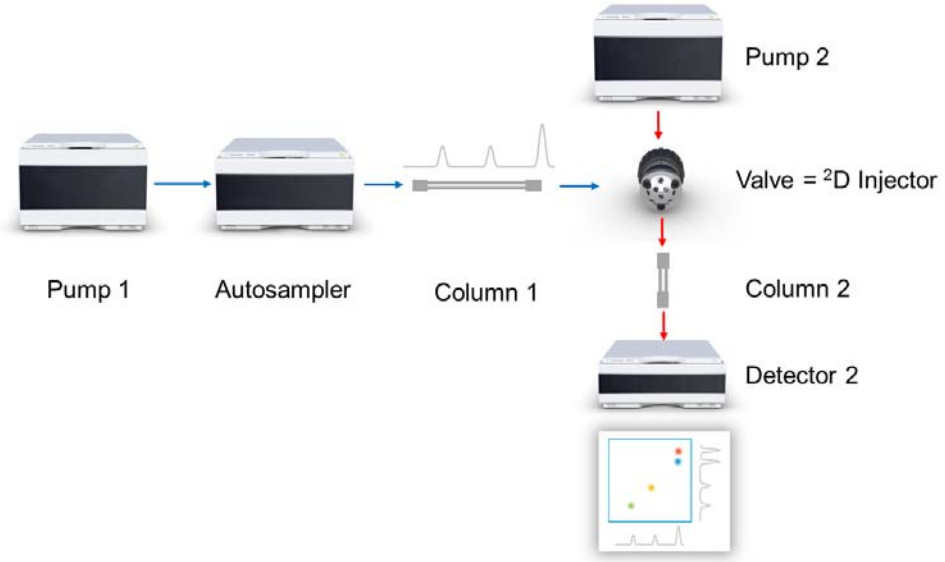


Figure 81 Concept of a 2D-LC-System

The Agilent 1290 Infinity 2D-LC Solution supports the following valve configurations:

- 2D-LC-Valve (2pos/4port-duo valve) (highly recommended)
- 2 Pos/10 Port Valve

2D-LC Quick-Change Valve

Advantages of the Agilent 2D-LC valve:

- Has fully symmetric flow paths (no additional bridging loops)
- Offers symmetric fill- and flush-out behavior and allows depending on plumbing either counter-current or co-current flush-out of both loops
- Due to its special design it delivers lowest pressure spikes to the columns. This lower stress guarantees a longer life time of the columns in the second dimension.

For details, see Table 6 on page 40 (standard heart-cutting) and Table 28 on page 176 (full comprehensive).

2pos/10port Valve

Support of 2pos/10port valve for comprehensive and heart-cutting 2D-LC allows easy transfer or existing 2D-LC methods. Both symmetric and asymmetric set-up supported in the software.

Interfacing First and Second Dimension for Heartcutting 2D-LC (LC-LC)

To interface first and second dimension for LCxLC, the following opportunities exist:

- 2 Pos/4 Port Duo Valve
- 2 Pos/10 Port Valve

2 Pos/4 Port Duo Valve

The 2 Pos/4 Port Duo Valve is especially constructed for its use in 2D-LC applications. The main advantage is that the flow stream through the loop capillaries can be guided in a cocurrent or countercurrent manner by means of the respective plumbing.

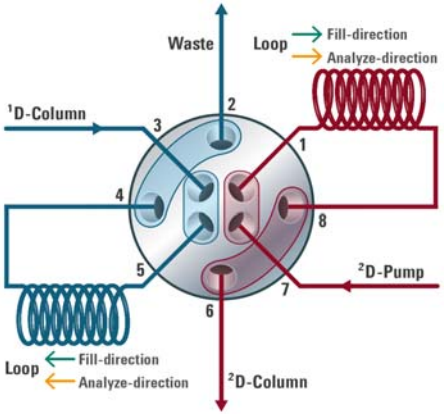
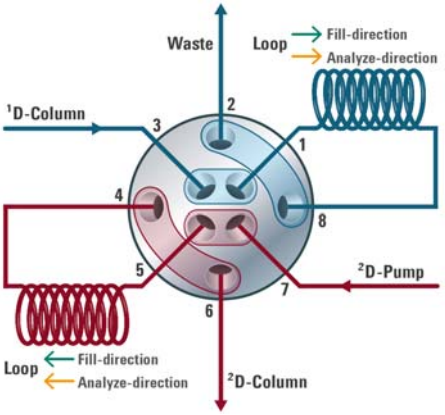
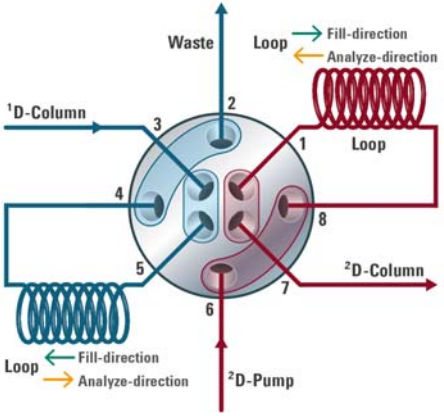
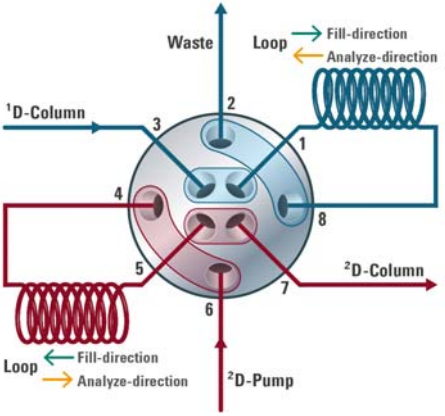
- Cocurrent:

  The flow for filling and eluting (analyze direction) the loops is entering the loop from the same side for the cocurrent plumbing.

- Countercurrent:

  The flow entering the loops for filling and analyzing is opposite for the countercurrent plumbing.

Table 28 Plumbing for 2D-LC Valve Head, comprehensive 2D-LC

	Filling	Analyzing
Cocurrent		
	Figure 82 2D-LC Valve Head, position 1	**Figure 83** 2D-LC Valve Head, position 2
Countercurrent		
	Figure 84 2D-LC Valve Head, position 1	**Figure 85** 2D-LC Valve Head, position 2

Supported modules/systems

1D/2D-Switch

The 1D/2D-Switch enables the user to simply switch between a 1D- and 2D-LC setup without replacing the capillary connections. For this setup (bypass of 2nd dimension) an additional 2/6 valve is needed. For details, see Automated Switching Between 1D-LC and Comprehensive 2D-LC Analysis (5991-4843EN).

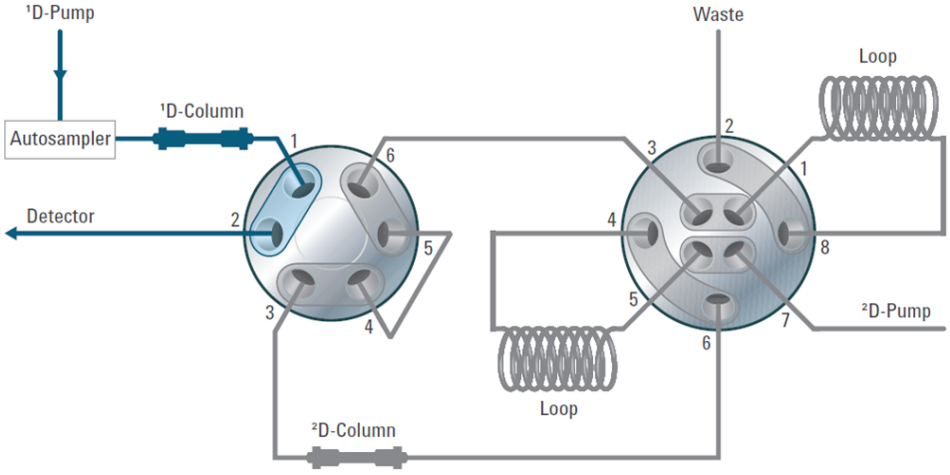


Figure 86 Setup for 1D-LC analysis

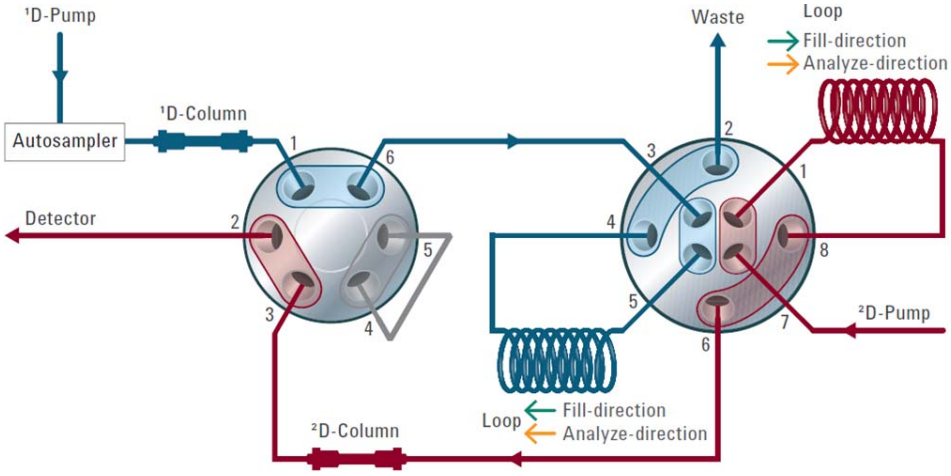


Figure 87 Setup for 2D-LC analysis

Installing the Software

Software required OpenLAB ChemStation Edition C.01.07 (or higher) installed

NOTE For installing the 2D-LC Software, please use the OpenLAB Additional Software and Drivers Deployment Wizard.

NOTE Do not try installing the software by double-clicking the msi file, as this may result in an incomplete installation.

NOTE To install the Add on, the OpenLAB CDS Chemstation Software must be not active.

1 Start OpenLAB Additional Software and Driver Deployment Wizard by going to Windows **> Start > Agilent Technologies > OpenLAB > OpenLAB Additional Software and Drivers**

2 Follow steps described in the Wizard for installation or software upgrades.

Overview Menu Extensions

The installation of the Agilent 1290 Infinity 2D-LC Acquisition Software adds 2D-LC specific items to the Agilent OpenLAB CDS controller software.

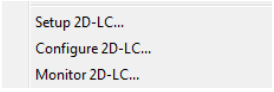


Figure 88 Agilent OpenLAB CDS controller software, 2D-LC specific items

The menu items appear in the **Instrument Menu** of the **Method & Run Control View**. The following items are available:

- **Setup 2DLC...**

  Displays the 2DLC method dialog

- **Configure 2DLC...**

  Displays the 2DLC configuration dialog

- **Monitor 2DLC...**

  Displays the 2DLC status monitor

Configuration

Overview Configuration Dialog

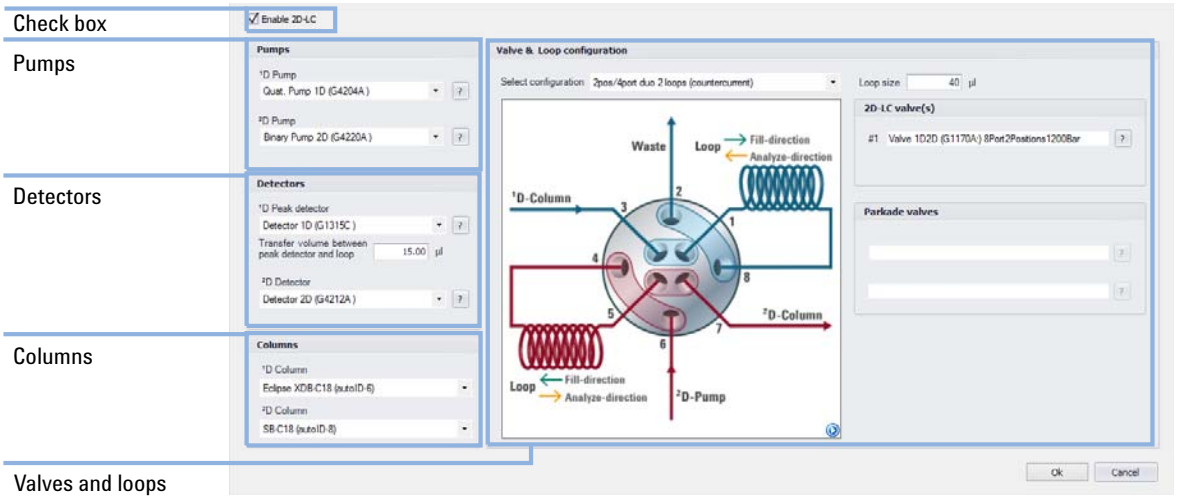


Figure 89 Overview 2D-LC configuration grafical user interface

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 & Loops**

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

Enable 2D-LC

1 Select check box **Enable 2D-LC**.

The 2D-LC functionality is enabled for the configured system.

NOTE If disabled, all all sections in the configuration user interface are disabled and the menu item **Setup 2D-LC method...** is grayed out such that the system uses normal parameters.

Configure Pumps

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

- 1st dimension:

  The drop-down list contains all configured pumps that can be used in the 1st dimension (binary, quaternary, capillary or nano pumps).

- 2nd dimension:

  Only 1290 Infinity Binary Pumps (G4220A/B) or 1290 Infinity II High Speed Pumps (G7120A) can be selected in the 2nd dimension.

The **Identify** button triggers the blinking of the status LED of the corresponding pump module. The button is only enabled in the Online version of the ChemStation

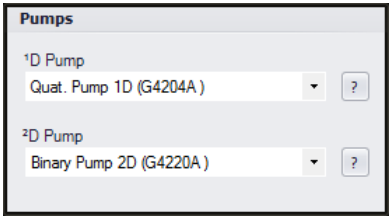


Figure 90 Configuration screen (for example if 1st dimension pump is an Agilent 1290 Infinity Binary Pump)

Preparations

- OpenLAB ChemStation Edition C.01.03 (or higher) installed (Multiple heart-cutting method requires OpenLAB ChemStation Edition C.01.07 (or higher))
- 1290 Infinity 2D-LC Acquisition Software installed
- Check box **Enable 2D-LC** selected.

1 Select the pump for the 1st dimension from the drop-down list **Pump (1D)**.

2 Select the pump for the 2nd dimension from the drop-down list **Pump (2D)**.

3 To save settings click **OK**.

Pumps are configured for 2D-LC.

Configure Detectors

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

- 1st dimension:

  The drop-down list contains all configured detectors and a **None** entry.

- 2nd dimension:

  The drop-down list contains all configured detectors that can be used as peak detector (DAD, MWD, VWD, FLD, RID), and a **None** entry.

The **Identify** button triggers the blinking of the status LED of the corresponding detector module. The button is only enabled in the Online version of the ChemStation

Transfer volume is the volume between the peak detector in the 1st dimension and the loop(s). *In standard setups this volume is 9 µL*. The field is only visible if a peak detector is configured.

For special setups or if the default setting needs to be changed, the following methods for determination of the delay volume exist:

- Use the following volumes:

Table 29 Volumina of capillaries

Capillary	Volume [µL]
Flex capillary, 0.12 x 105 mm, no fittings (5021-1820)	1.187
Flexible tubing, 280 mm (5021-1822)	3.167
Capillary column – detector SST 400 mm lg, 0.12 mm i.d. (5021-1823)	4.524
Capillary ST 0.12 mm x 500 mm (5065-9964)	5.655
Capillary ST 0.12 mm x 600 mm S/SL (5067-4669)	6.786
Capillary, 0.17 mm x 80 cm, male fit (01078-87305)	18.158

- The delay volume of the capillary connection between peak-detector and loop can be either calculated by the following formula:

  $V_D = L * \pi * d^2/4$ (L = length of capillary, d = diameter of capillary

- Alternatively, and with higher accuracy the volume can be measured by two ways:
  - First, by determining the increase in retention time of a narrow peak after adding the respective capillary to an existing HPLC system. A sample like one micro-liter of acetone can be injected to a system using a restriction capillary instead of a column to generate a very narrow peak. In a second measurement, add the capillary of interest directly in front of the detector by using a zero-dead volume union. The set flow rate multiplied with the detected time difference will result the capillary volume.
  - A second option to measure the volume of the capillary is to fill the dried capillary with pure water an weigh it. The weight divided by the density of water (1 mg/µL) will result in the volume. Attention must be given not to have any additional droplets of water being attached to the capillary.

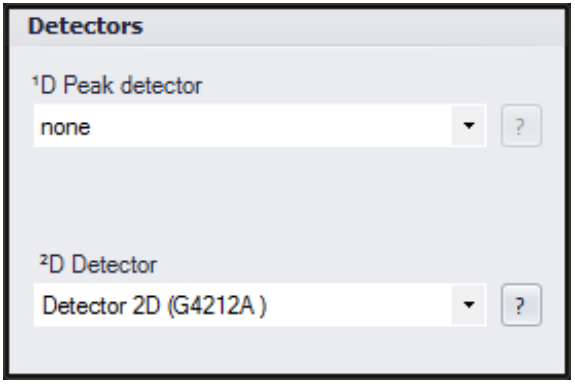


Figure 91 Configuration screen with no peak detector

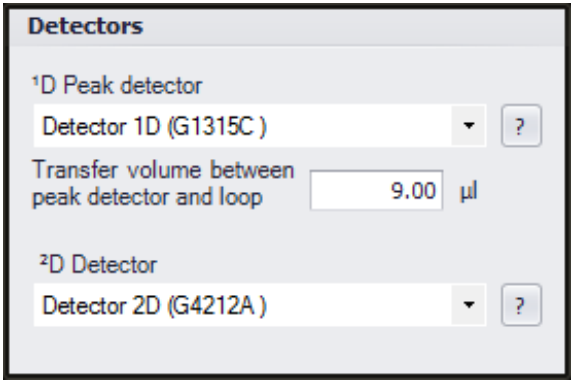


Figure 92 Configuration screen with peak detector

Preparations

- OpenLAB ChemStation Edition C.01.03 (or higher) installed (Multiple heart-cutting method requires OpenLAB ChemStation Edition C.01.07 (or higher))
- 1290 Infinity 2D-LC Acquisition Software installed
- Check box **Enable 2D-LC** selected.

1 Select the **Detector (2D)** from the drop-down list.

2 Select the **Peak detector (1D)** from the drop-down list.

3 To save settings click **OK**.

Detectors are configured for 2D-LC.

Configure Columns

The user can select columns for the 1st and 2nd dimension from the ChemStation column database. The columns are identified by the column description and serial number.

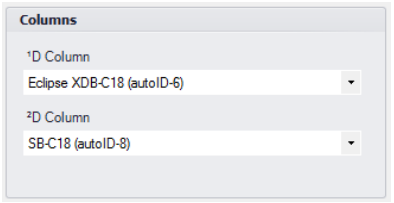


Figure 93 2D-LC column configuration

Preparations

- OpenLAB ChemStation Edition C.01.03 (or higher) installed (Multiple heart-cutting method requires OpenLAB ChemStation Edition C.01.07 (or higher))
- 1290 Infinity 2D-LC Acquisition Software installed
- Check box **Enable 2D-LC** selected.

NOTE The software uses the column parameter to calculate the ratio of the injection volume to the column volume, that typically should not exceed ca. 10 % to avoid break-through of the compounds during the second dimension separation.

1 Select **Column (1D)** from the drop-down list.

2 Select **Column (2D)** from the drop-down list.

3 To save settings click **OK**.

Configure Valve and Loop

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

- 1st dimension:

  The **Valve 1** drop-down list contains all configured valves which can be used for 2DLC 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.

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

Loop size specifies the volume of the loop(s). In case of two loops, the software assumes that both loops have the same volume.

All possible loop configurations depending on the selected valves are listed separately and illustrated on screen (see, "Interfacing the First and Second Dimension" on page 266).

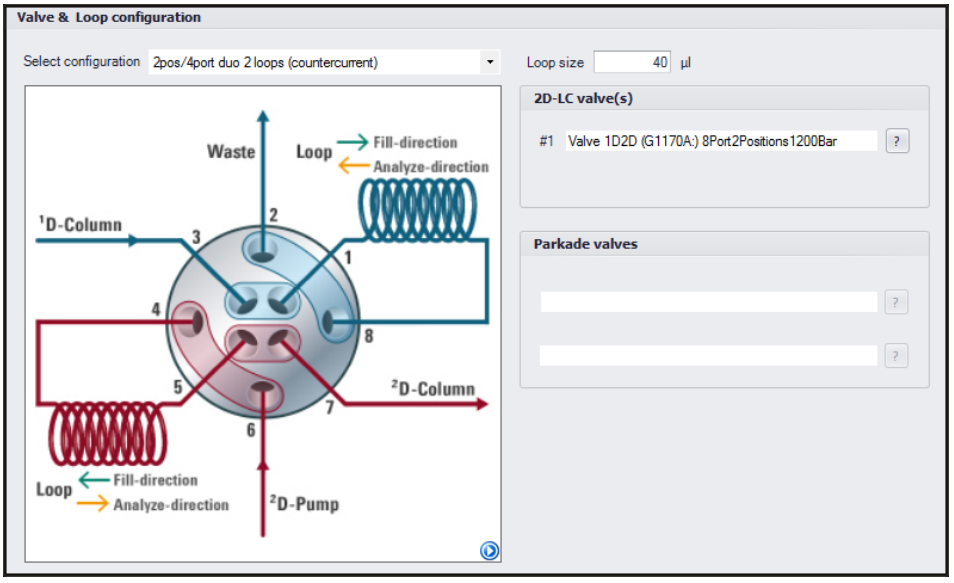


Figure 94 2D-LC valve and loop configuration

Preparations

- OpenLAB ChemStation Edition C.01.03 (or higher) installed (Multiple heart-cutting method requires OpenLAB ChemStation Edition C.01.07 (or higher))
- 1290 Infinity 2D-LC Acquisition Software installed
- Check box **Enable 2D-LC** selected.

NOTE Valves may be part of a 1290 Infinity Thermostatted Column Compartment (G1316C) or the 1290 Infinity Valve Drive (G1170A).

1 Select **Valve 1**.

2 Select **Valve 2**.

3 To save settings click **OK**.

Valves and loops are configured for 2D-LC.

Method parameters

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.

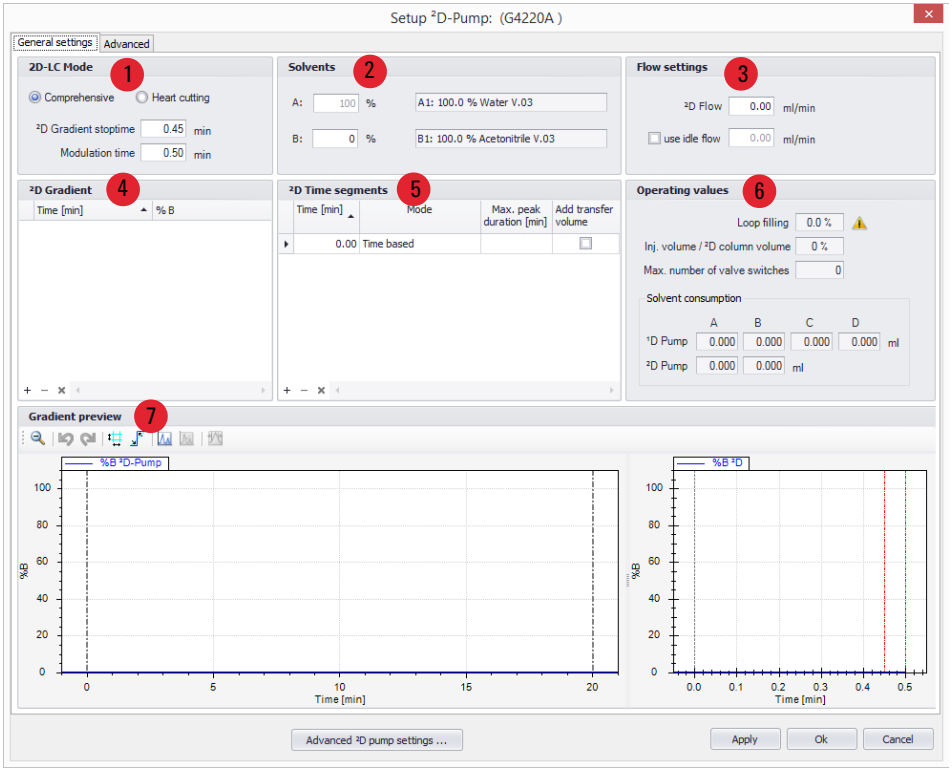


Figure 95 2D-LC method setup (**General settings**)

The setup of following method parameters is available:

1 **2D-LC Mode**, see “Set 2D-LC Mode” on page 53
2 **Solvents**, see “Set solvents” on page 191
3 **Flow settings**, see “Set flow” on page 192
4 **^{2}D Gradient**, see “Set Solvent Composition Gradient” on page 193
5 **^{2}D Time segments**, see “Set 2D Time Segments” on page 197
6 **Operating values**, see “Define Peak detector parameter” on page 199
7 **Gradient preview**, see “Gradient Preview Functionality” on page 200

Set 2D-LC Mode

Setting the mode has the following consequences (for details, see "Introduction" on page 8):

- **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.

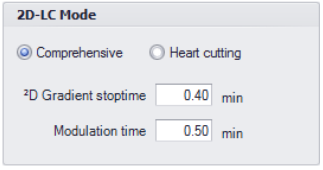


Figure 96 2D-LC **Comprehensive** mode

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.

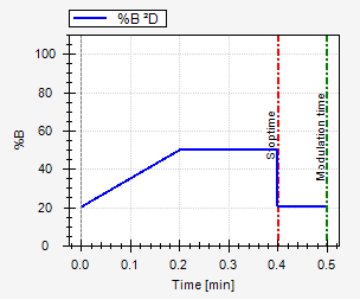


Figure 97 **Stop time** and **Modulation time**

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 2D 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 %.

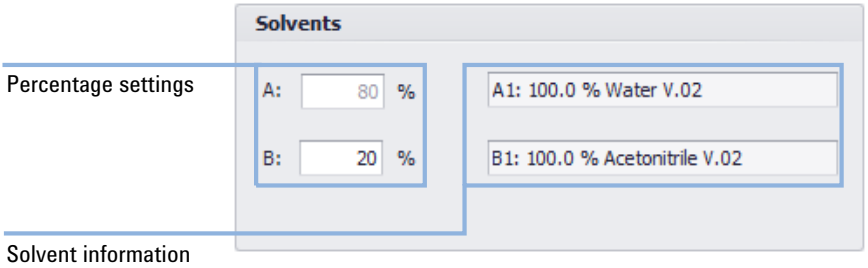


Figure 98 2D-LC solvent settings

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 "Overview Configuration Dialog" on page 180).

Set flow

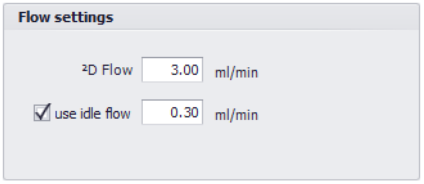


Figure 99 Flow settings

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 2D-Flow is also used while 2D-LC mode is OFF.

Set Solvent Composition Gradient

Set Solvent Composition Gradient

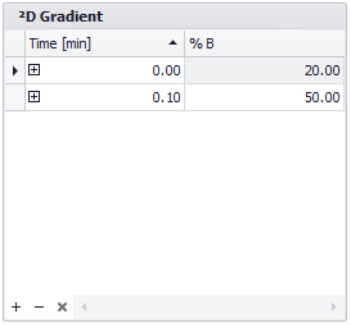
The timetable in the **2D Gradient** group allows changing the solvent composition.

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 %

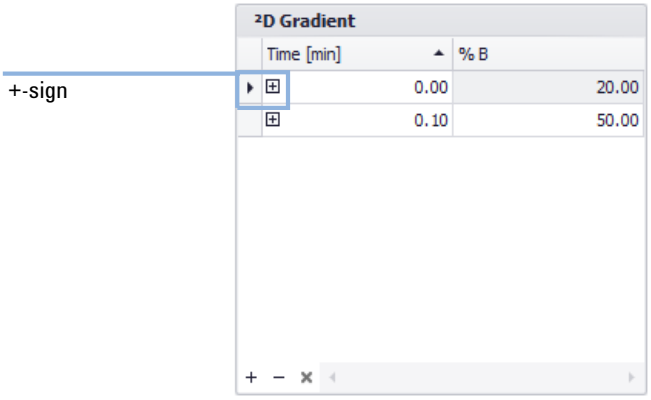
NOTE Different start conditions in the first row may cause step gradients and RI-effects (density differences of the different liquid phases may cause different DAD detection through baseline disturbances).



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 2D** 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.



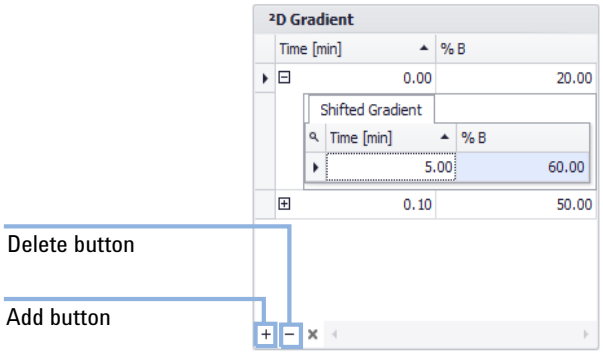
NOTE A gray colored **+** indicates that the associated nested table has no entries, otherwise the **+** is black.

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 ^{2}D Gradient graphically

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

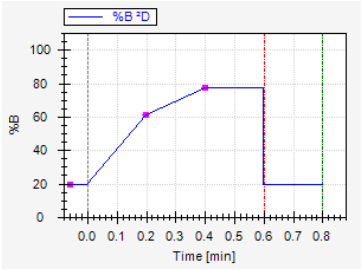


Figure 100 ^{2}D Gradient window in edit mode

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

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 30 Definitions 2D Time Segements

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)

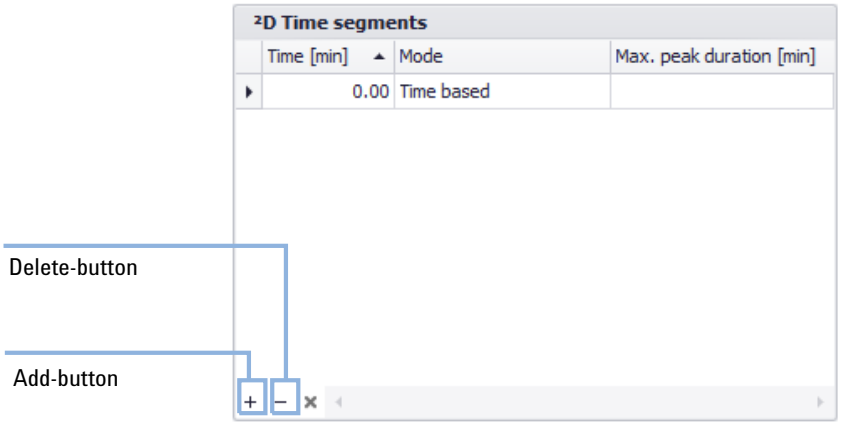
NOTE If the **^{2}D Time Segments** table is empty, no 2D-LC operation will be executed at all.

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 2D-gradient repetition starts immediately and ends when the 1D-Stoptime is reached or at the time specified in the next timetable entry. The actual gradient cycle is always completed except the 1D stoptime is reached
- **Peak based**
  The peak detector is enabled at the specified time. The 2D-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 1D-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).

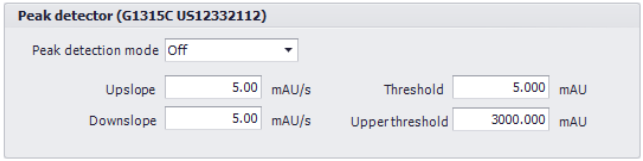
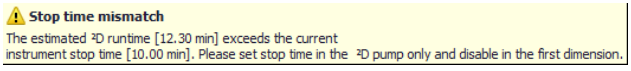


Figure 101 Overview on peak detector parameters

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 "Overview Configuration Dialog" on page 180) this section is disabled. The currently configured peak detector (name & serial number of the detector) is shown in the section header.

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.

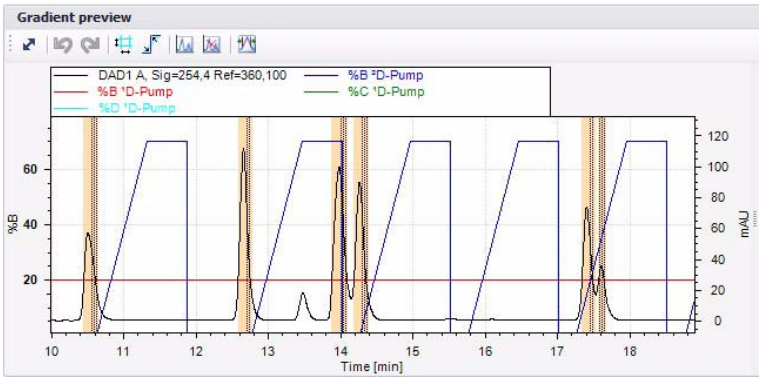


Figure 102 Gradient preview

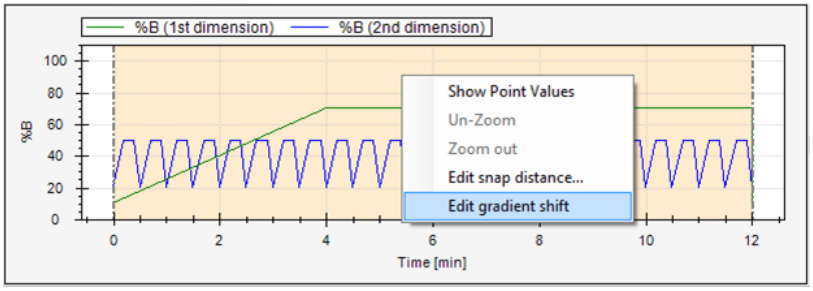
The reference signal offers the following:

- 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)

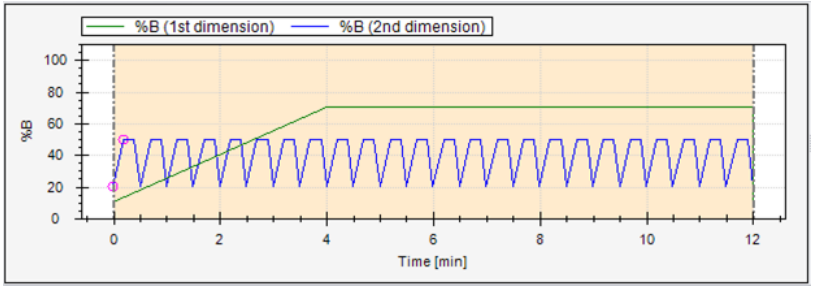
Setup Second Dimension Gradient with the Graphical User Interface

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

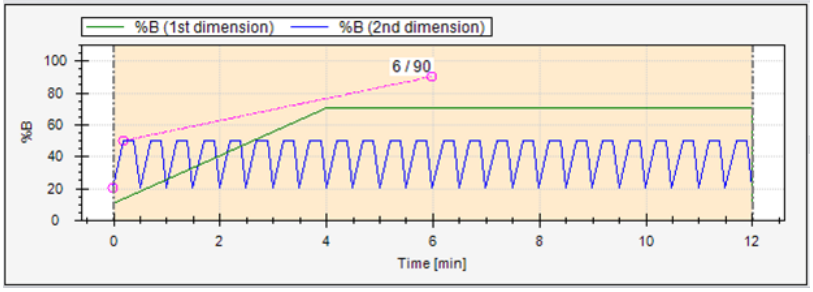
1 To enter the editing mode, use the context menu.



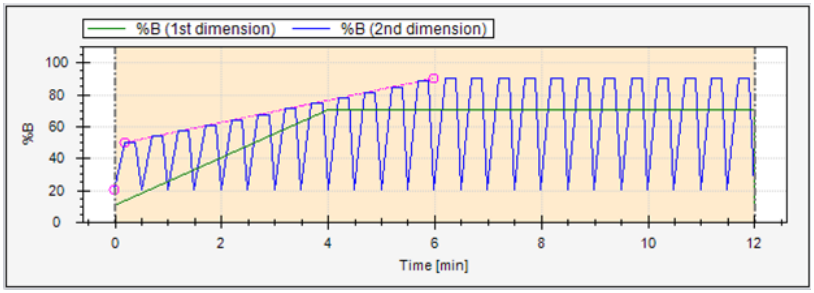
Timetable entries are marked with circles.



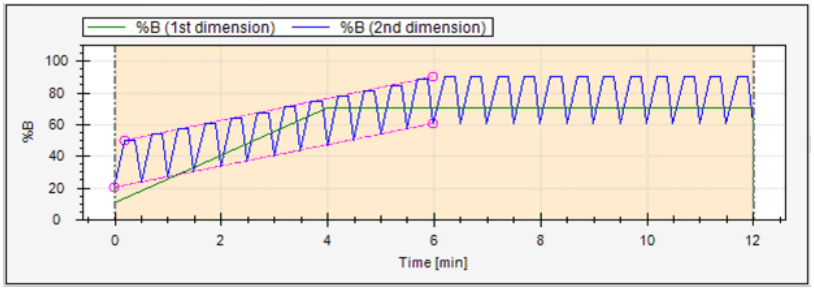
2 Drag the mouse to a new %B value at a specified runtime of the 1st dimension.



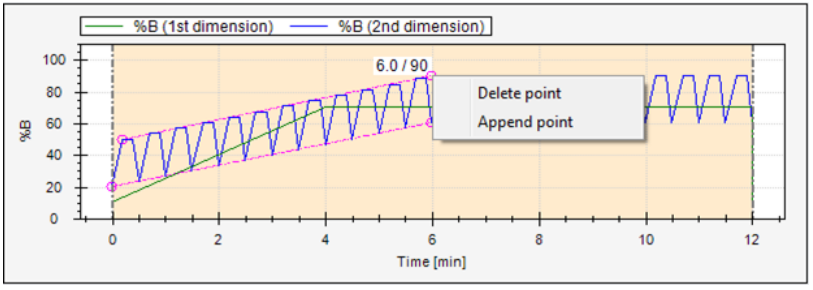
This draws a straight line. When releasing the mouse, a new timetable entry is made and the gradient rollout is automatically updated.



3 Repeat step 2 on page 202 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.



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 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 103 on page 205). 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 104 on page 205). After baseline correction the peaks can be automatically detected by a peak detection algorithm inherent in the 2D-LC data analysis software (Figure 105 on page 206). Since the third dimension is the intensity of the peaks a 3-dimensional plot of the data is possible (Figure 106 on page 206). With the given data set further qualitative and quantitative data analysis is possible.

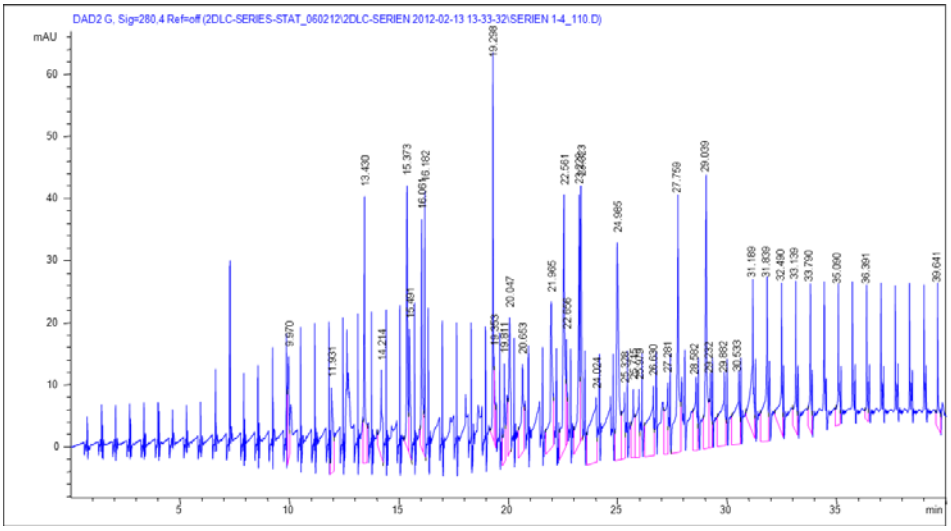


Figure 103 Display of two-dimensional LC data with a one-dimensional data analysis software

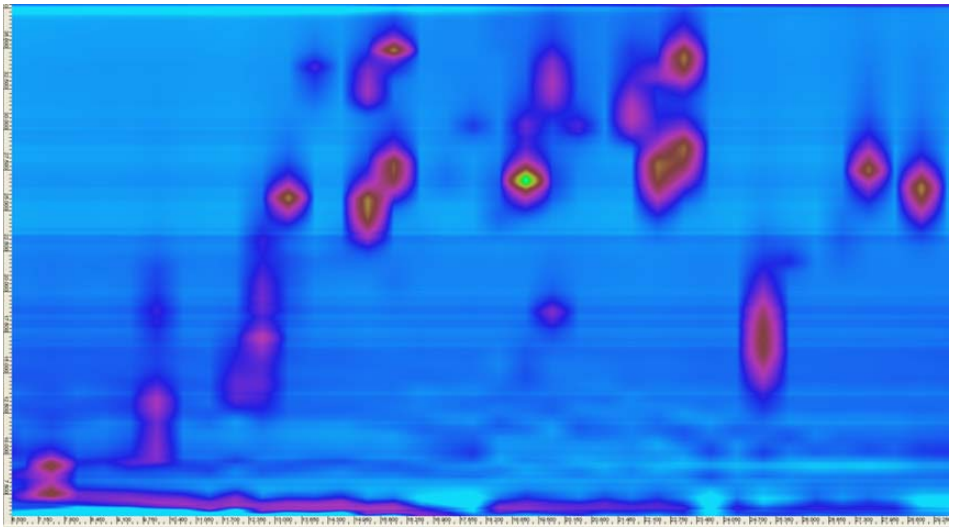


Figure 104 2D-LC plot of the optimized separation of 26 polyphenolic compounds

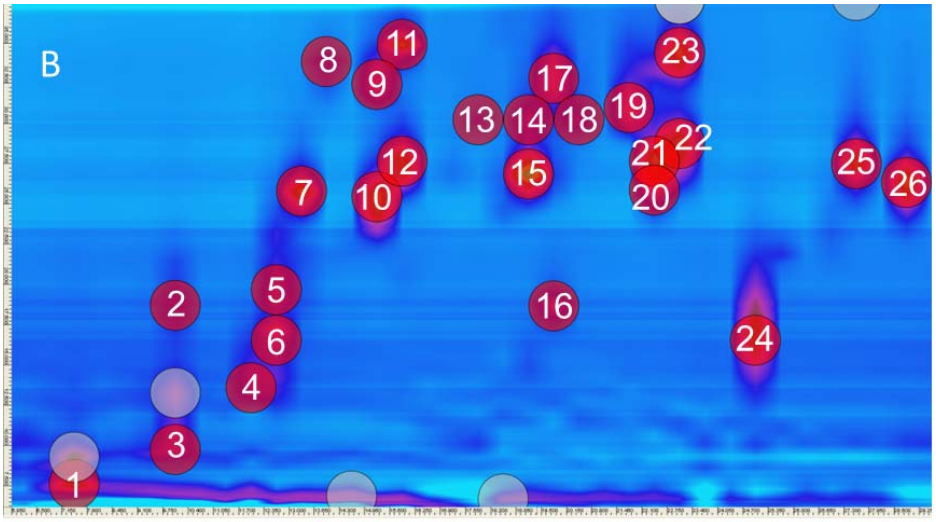


Figure 105 2DLC plot after baseline correction and with software detected peak annotation

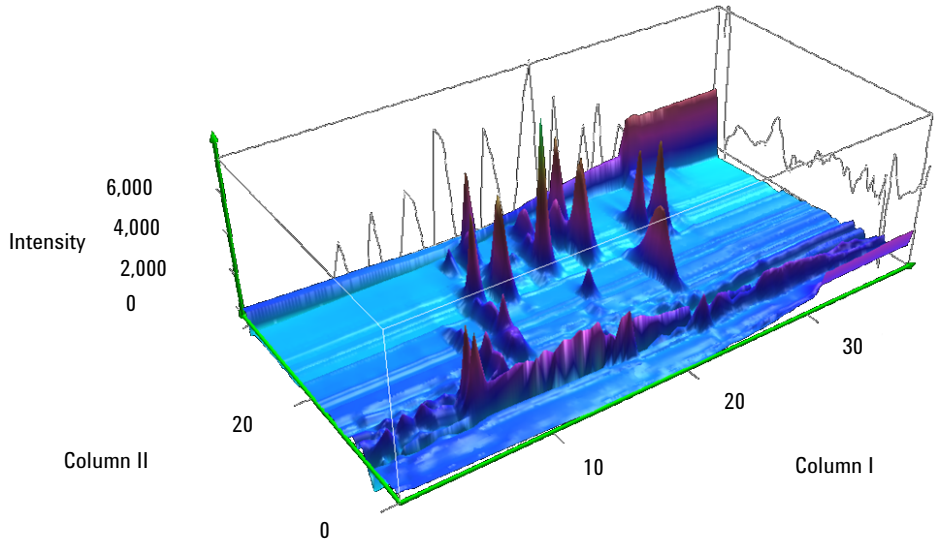


Figure 106 3-Dimensional display of the separation of the 26 compound standard mixture. The first dimension separation takes 40 minutes and each second dimension separation takes 39 seconds. The back side shows a generated first dimension chromatogram and gives the impression which peaks are coeluting 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: LCxLC2.4b2-HRMS.exe, LCxLC2.4b2-HRMS-64bit.exe. 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 R2.4 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

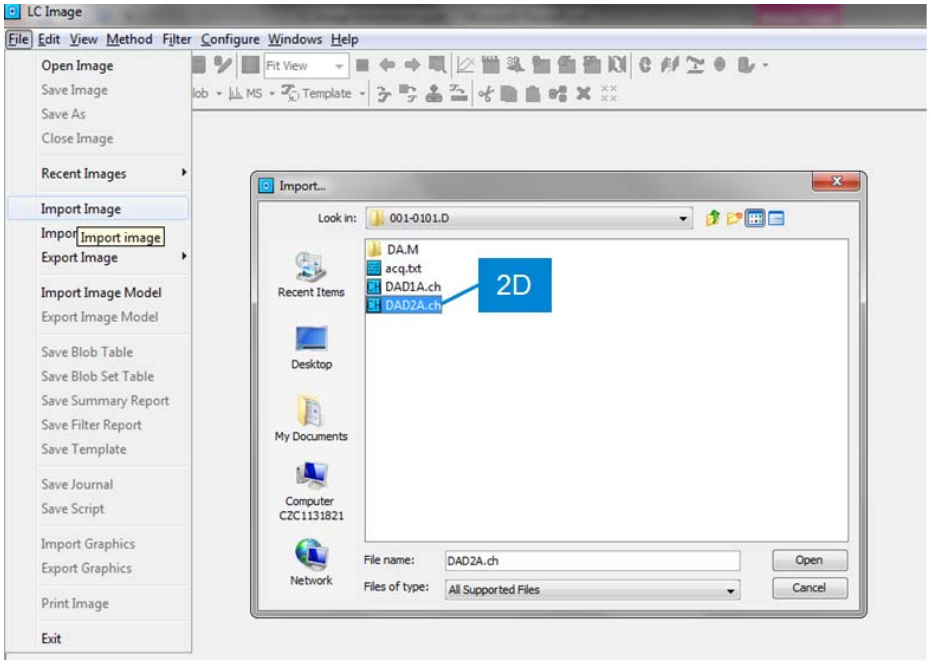
The USB dongle needs always to be inserted when working with GCImage software. If not, you will be asked to insert it.

Basic knowledges

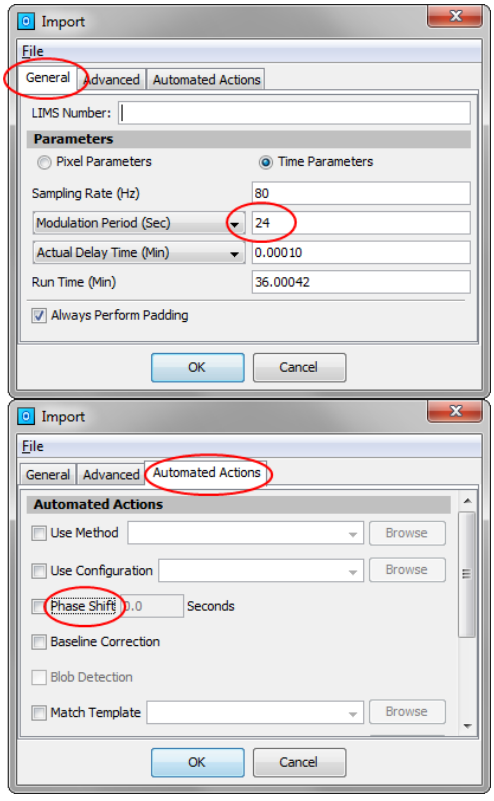
1 Start up LCImage

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.

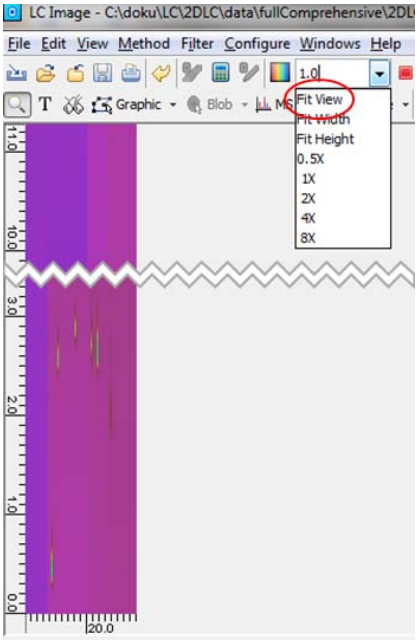
2 Import the UV signal from the second dimension detector.

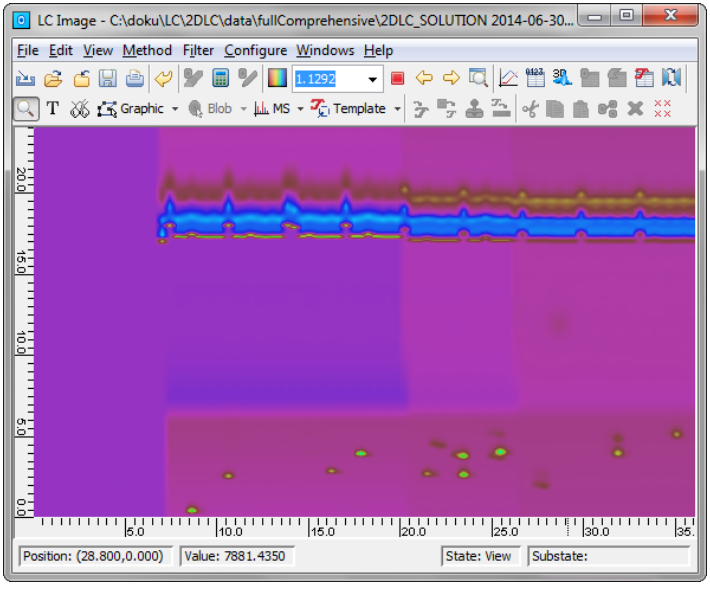


3 Import parameters

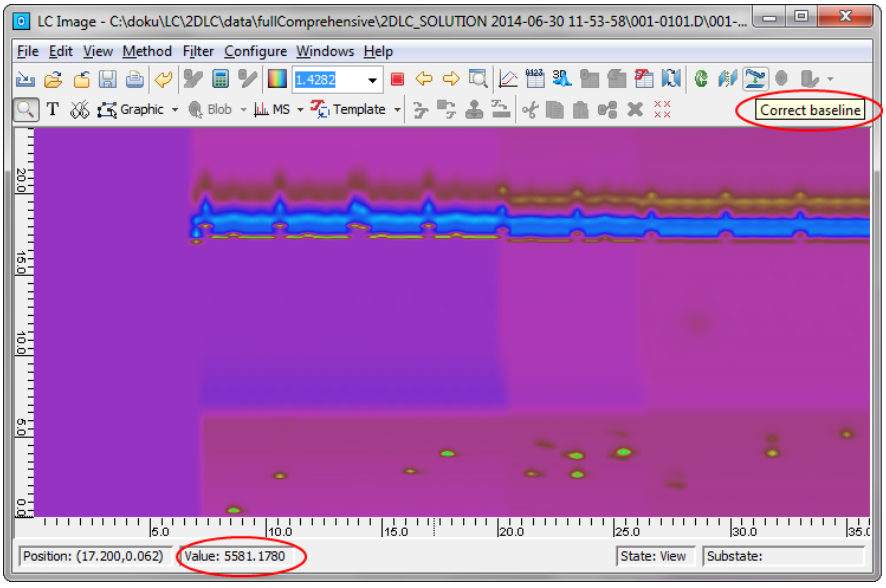


4 Fit view

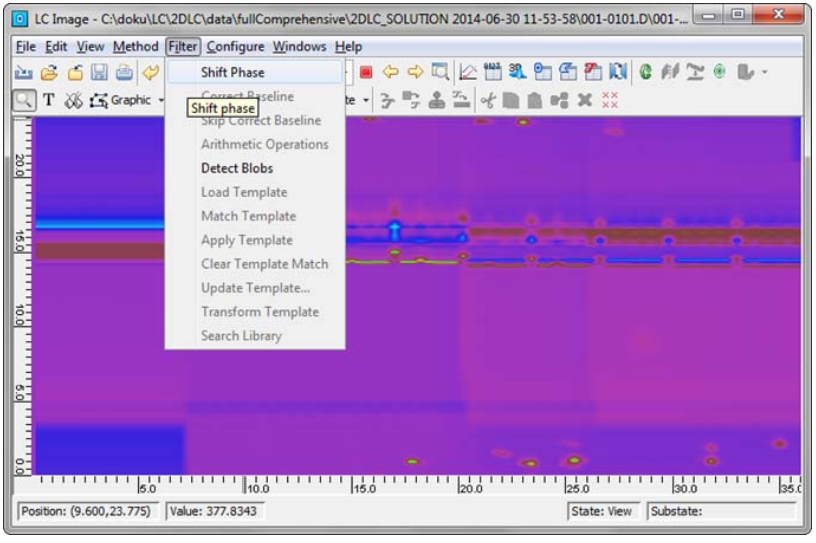

LC Image - C:\doku\LC\2DLC\data\fullComprehensive\2DL
File Edit View Method Filter Configure Windows Help
1.0
Graphic
Blob
Fit View
Fit Width
Fit Height
0.5X
1X
2X
4X
8X
20.0


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
Position: (28.800,0.000)
Value: 7881.4350
State: View
Substate:

5 Correct Baseline

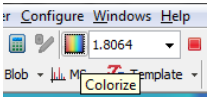


6 Shift phase

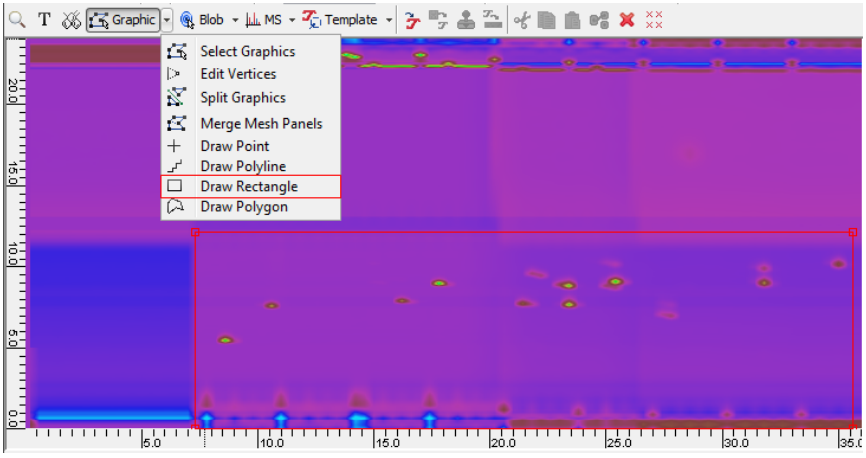


7 Zoom into an interesting region by using the right mouse button and dragging over the display

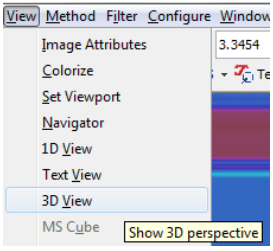
8 Adjust colors: LC Image offers refined possibilities for optimizing the color scales. Play around with settings for improving the contrast.



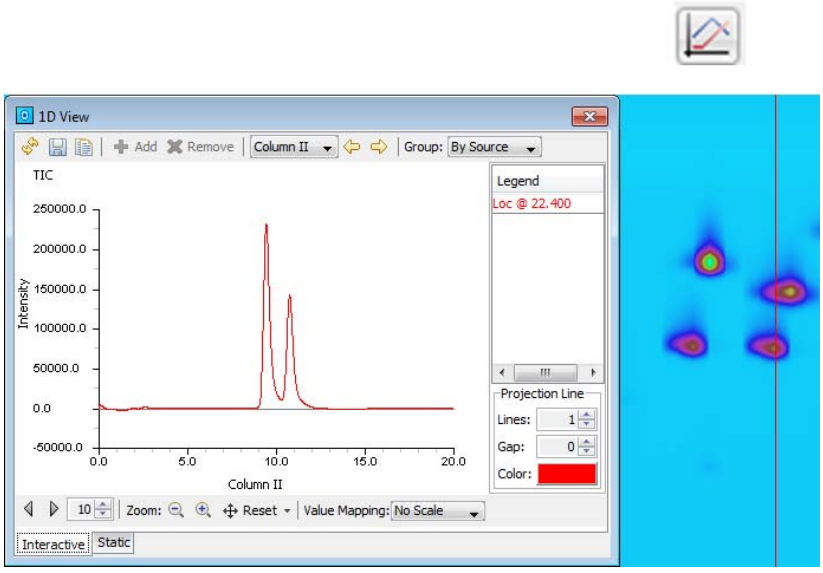
9 Select a data range.



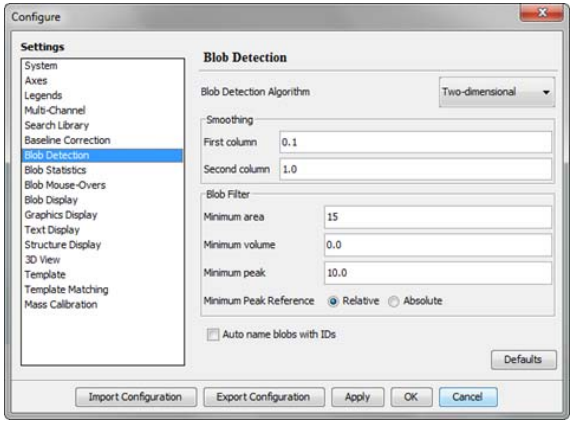
10 By clicking the „Show 3D perspective“ button or the corresponding menu item, you can easily create a customizable 3D plot.



11 View single 2D chromatograms

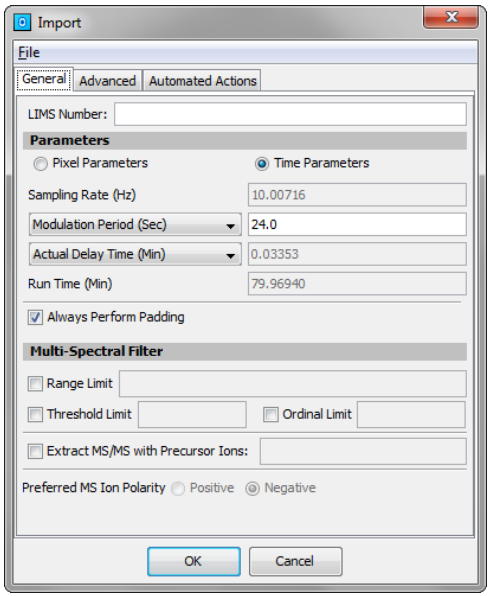


12 Select blobs



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.

Checkout/Familiarization Procedure

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 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)

Hardware required

- For 1290 Infinity, see:Table 26 on page 168
- For 1260 Infinity, see:Table 27 on page 168

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 The method provides the following method parameters for ^{1}D:

- Column: RRHD SB-C18, 2.1x 100 mm, 1.8 µm, 1200 bar (858700-902)
- Solvent:
  - A: H_2O + 0.2 % formic acid
  - B: Methanol
- Gradient:
  - 0.0 min – 40 % B
  - 34.0 min – 60 % B
  - 34.5 min – 90 % B
  - Stoptime: 40 min
  - Posttime: 10 min
- Flow rate: 0.100 mL/min
- Temperature: 40 °C

2 The method provides the following method parameters for ^{2}D:

- Column: RRHD Bonus-RP, 2.1x 50 mm, 1.8 µm, 1200 bar (857768-901)
- Solvent:
  - A: H_2O + 0.2 % formic acid
  - B: Acetonitrile
- Gradient:
  - 0.0 min – 25 % B
  - 0.20 min – 50 % B
  - 2D Gradient stoptime: 0.20 min
  - Modulation Time: 0.35·e min
- Gradient shift:

  0.00 min - 25 % B to 5.00 min - 25 % B to 40.00 min - 50 % B

  0.20 min - 50 % B to 5.00 min - 50 % B to 40.00 min - 75 % B
- Flow rate: 2.500 mL/min
- Temperature: 50 °C

3 Modulation:

- Loop size: 40 µL
- Configuration: cocurrent
- Modulation time: 0.35 min (21 s)
- Modulation on: 5 – 40 min

4 Detection:

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

Acquisition rate: 5 Hz

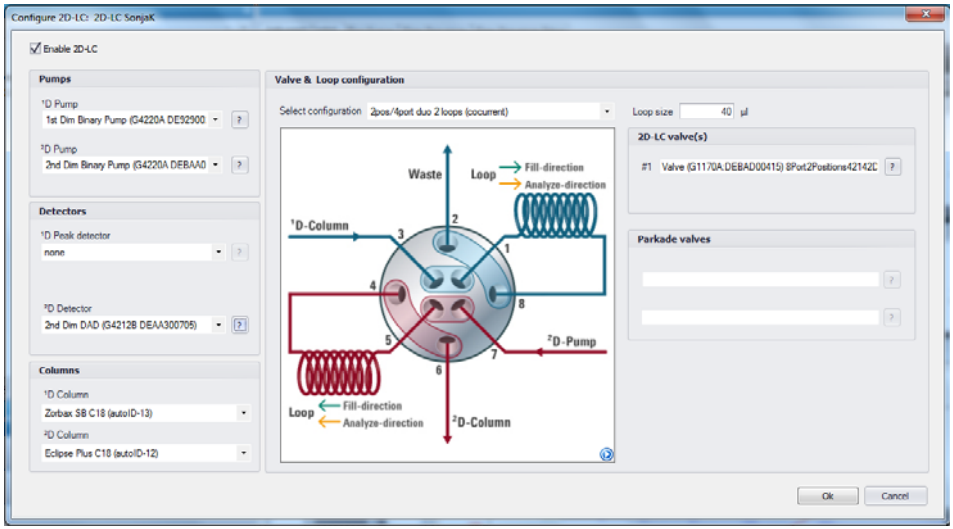
Sample: 2D-LC starter sample, 1 x 2 mL (5190-6895), 1:10 diluted with Methanol/Water (20/80; v/v) with 0.1 % formic acid.

Injection volume: 2 µL

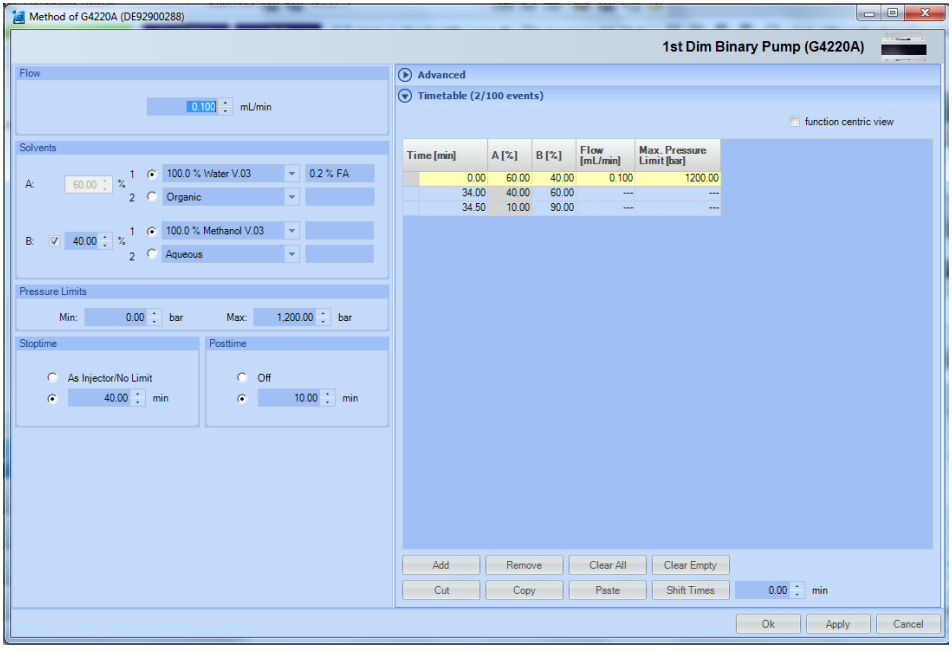
Checkout run using standard setup

1 Run the full comprehensive checkout run using single storage loops. The method for the checkout run is available on the DVD under **A.01.02 > Checkout methods**.

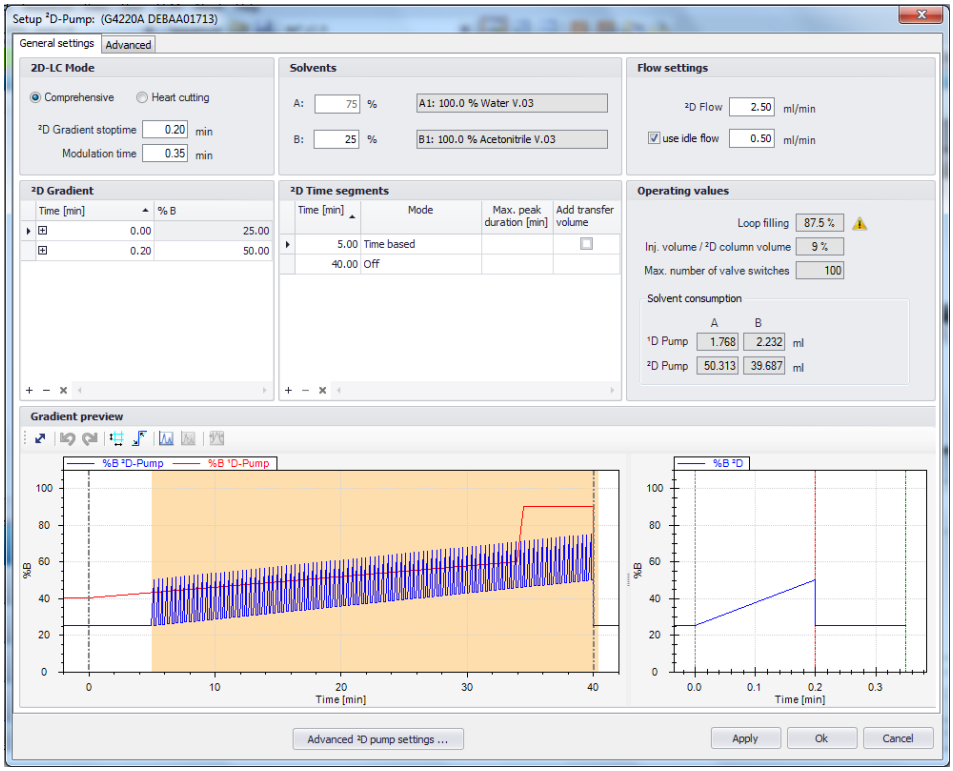
2 Verify that the Valve & Loop configuration is set up as shown below:



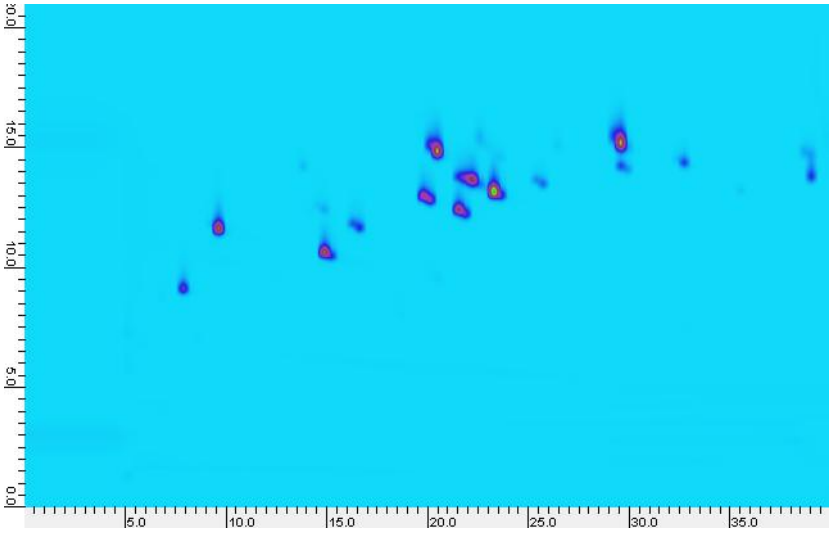
3 Verify the ^{1}D pump method is set up as shown below:



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:



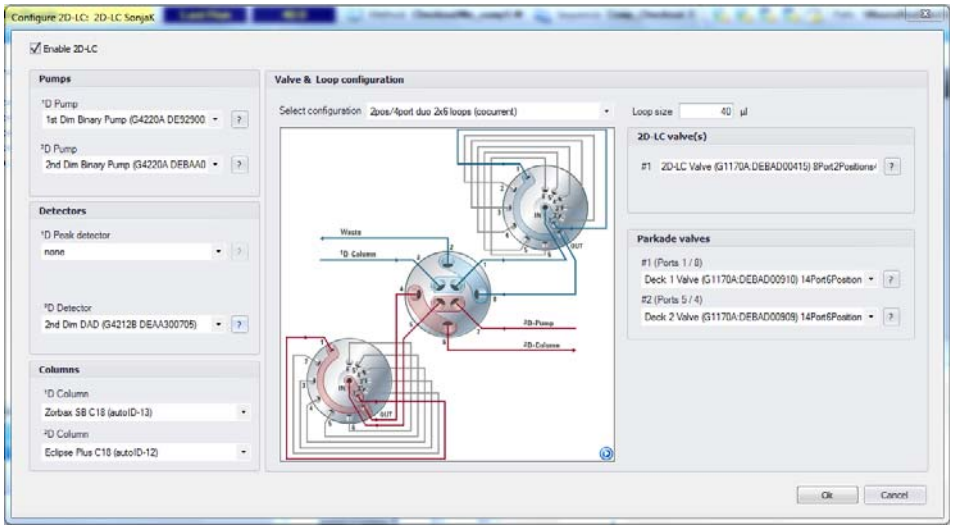
5 Run the full comprehensive checkout run using single storage loops and review the obtained data with the GC Image software. The resulting separation should look similar to the one shown below:



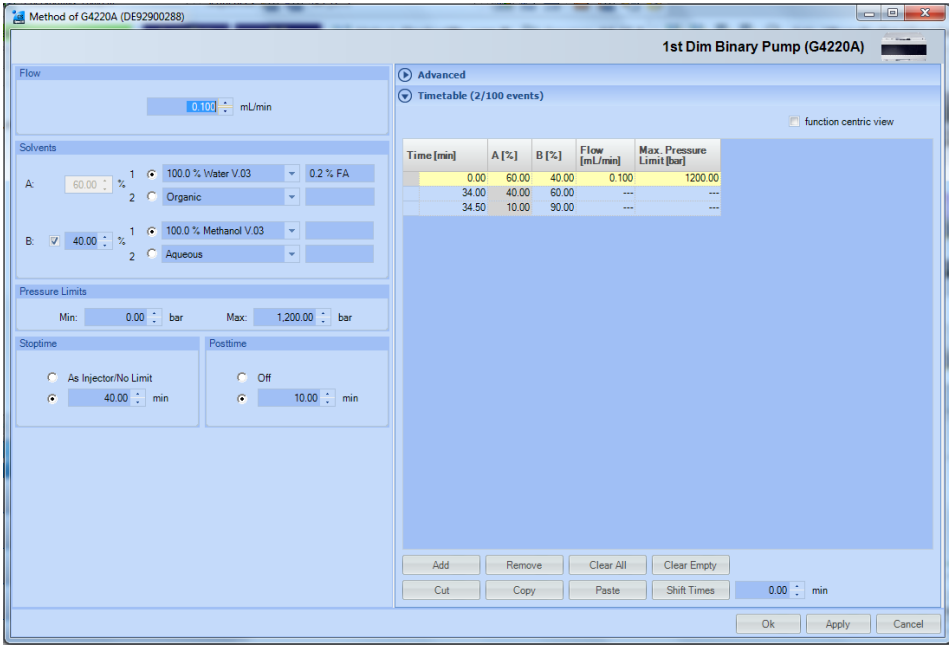
Checkout run using Multiple Heart-Cutting Valves

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).

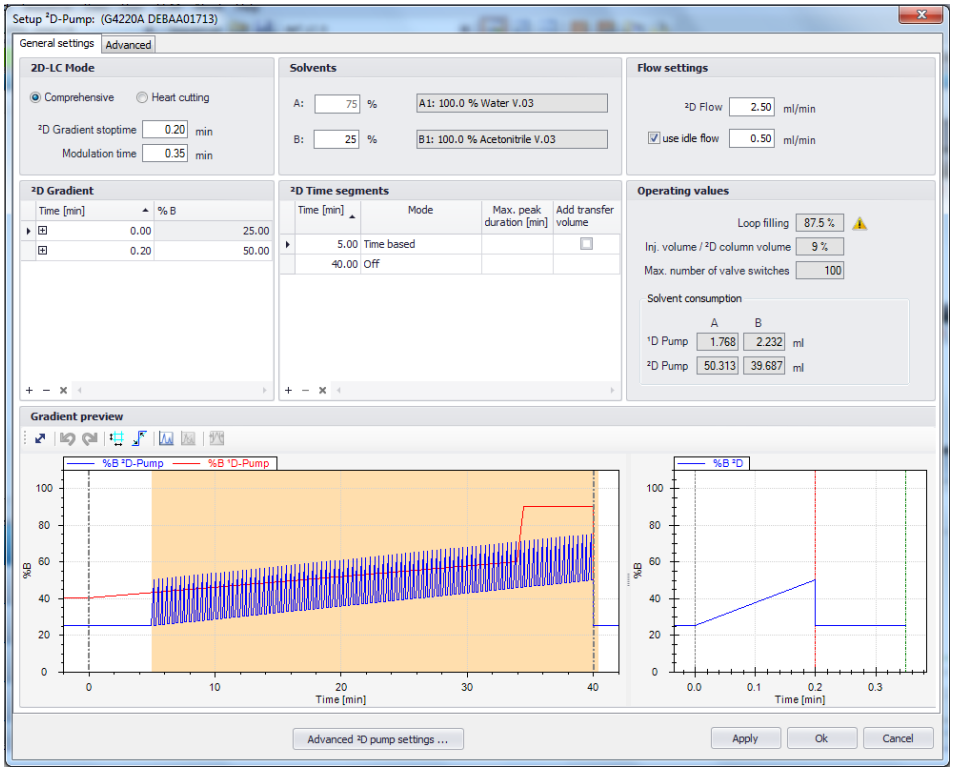
2 In **Instrument > Configure 2D-LC**, change the Valve & Loop configuration to 40 µL storage loops as shown below:



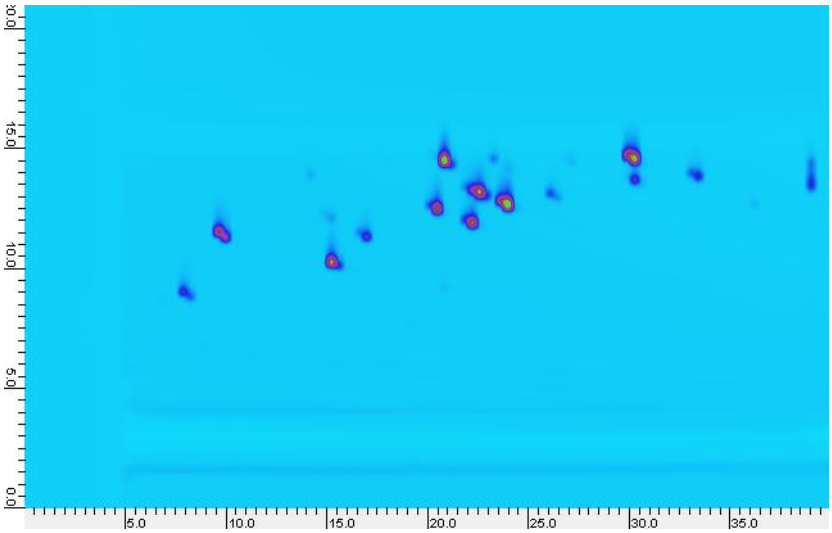
3 Verify the ^{1}D pump method is set up as shown below:



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:



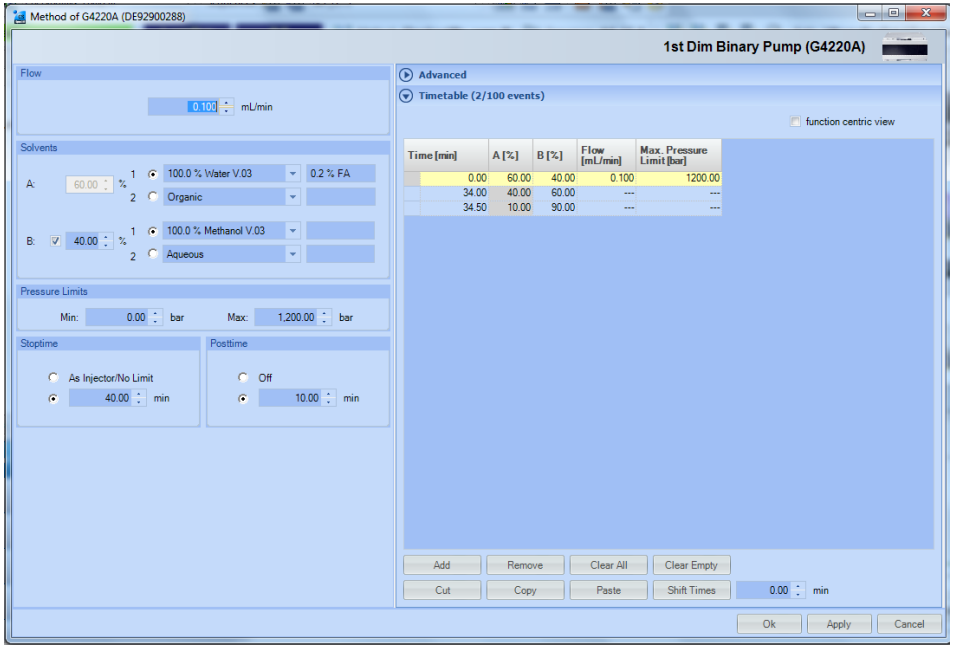
6 Compare the result to that obtained using the MHC valves.

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

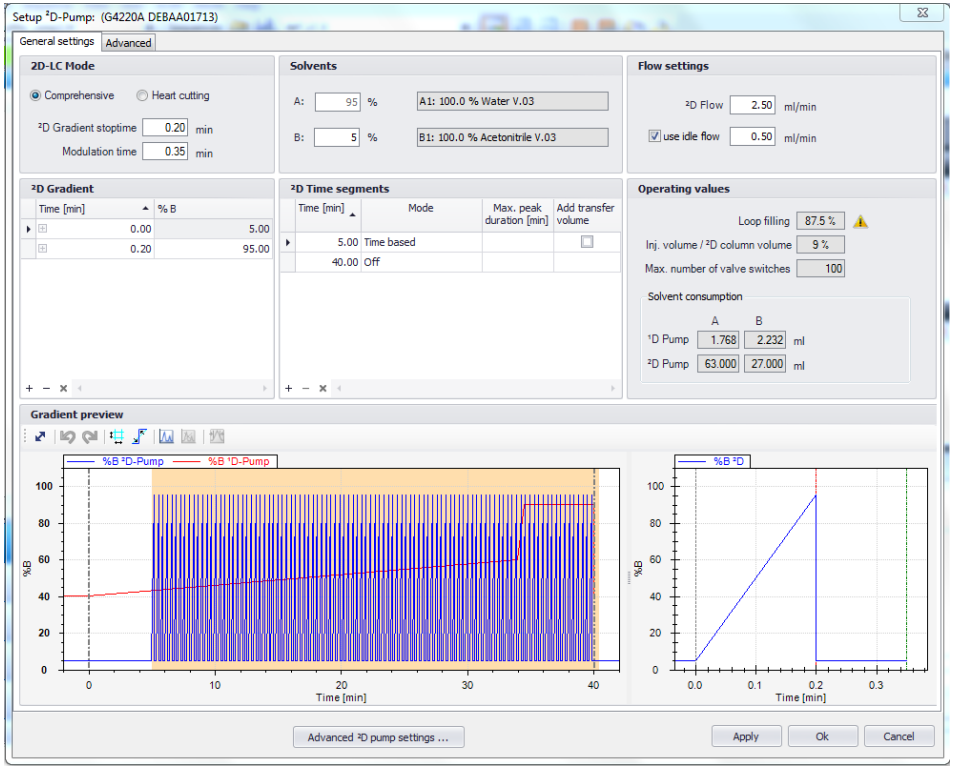
When combining separation systems with related separation mechanisms in the first and second dimension (as in RPxRP), orthogonality is limited. As a result, only a part of the available two-dimensional separation space will be occupied. In such a case, shifted gradients in the second dimension can be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

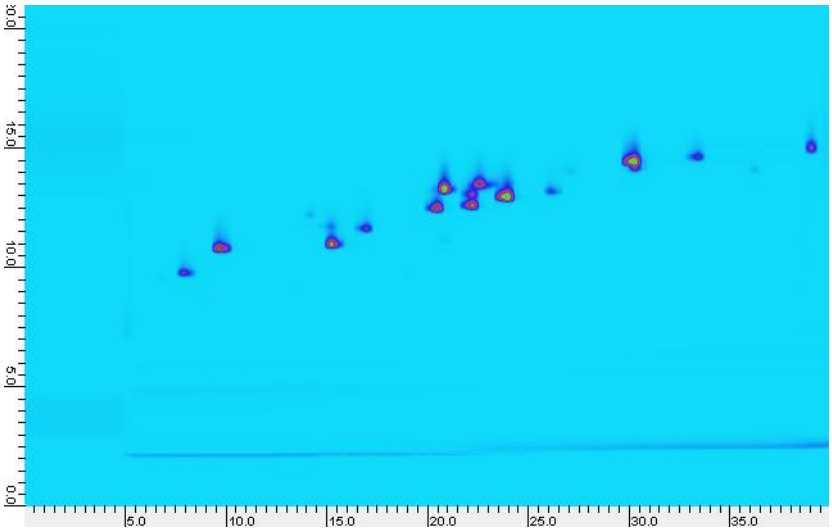
The 1D pump method should be set up as during the checkout runs (see below):



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

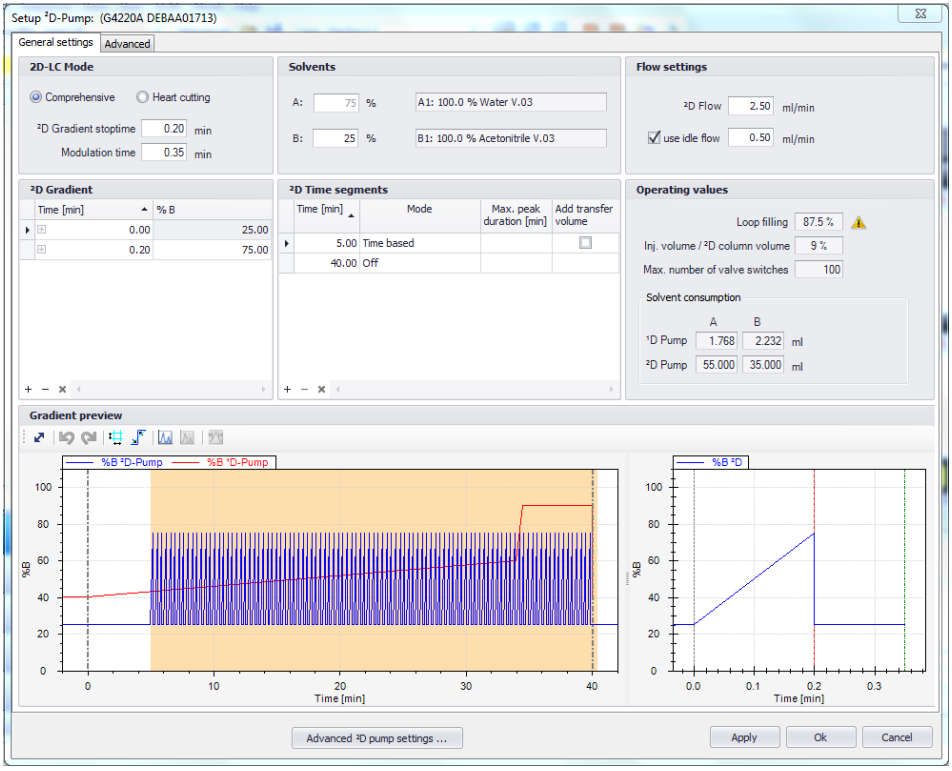


3 Run the comprehensive 2D-LC analysis. The resulting separation should look similar to the one shown below:

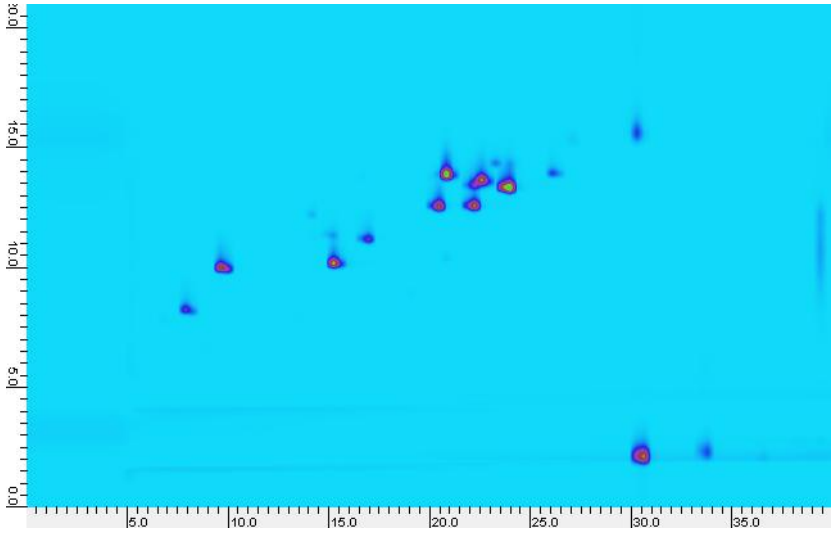


NOTE Notice how the peaks are distributed around a diagonal line, indicating related separation mechanisms in the first and second dimension.

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).

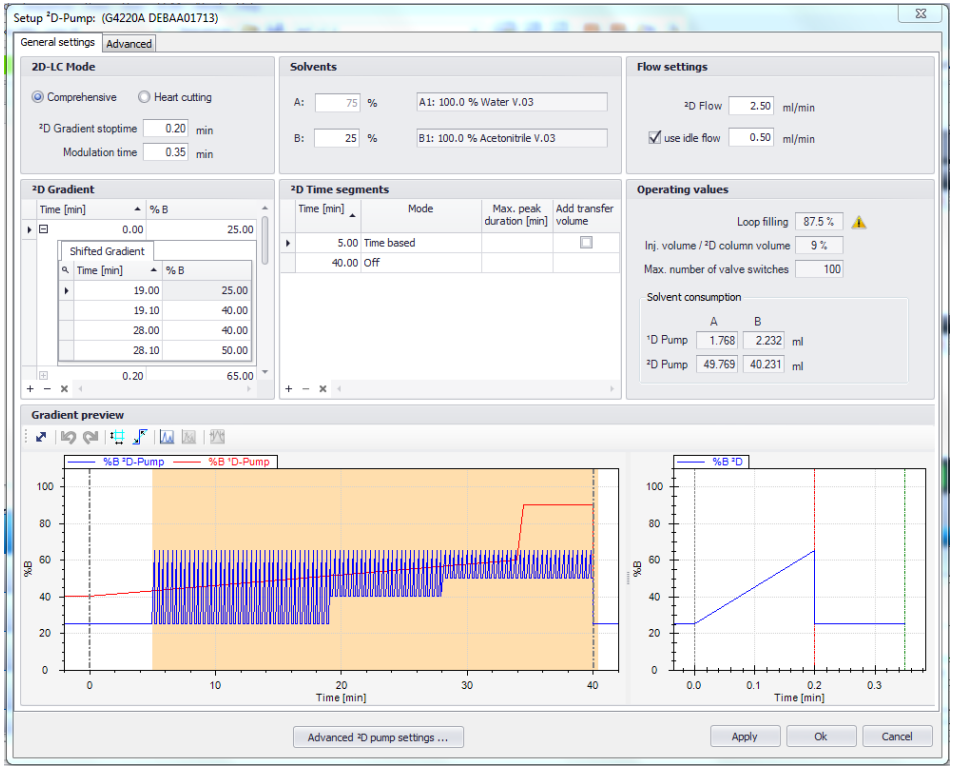
To be able to use even shallower gradients in the second dimension to further improve the separation and to also avoid the occurrence of wrap-around, continuously shifted gradients can be used in the second dimension (as was done during the checkout runs).

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

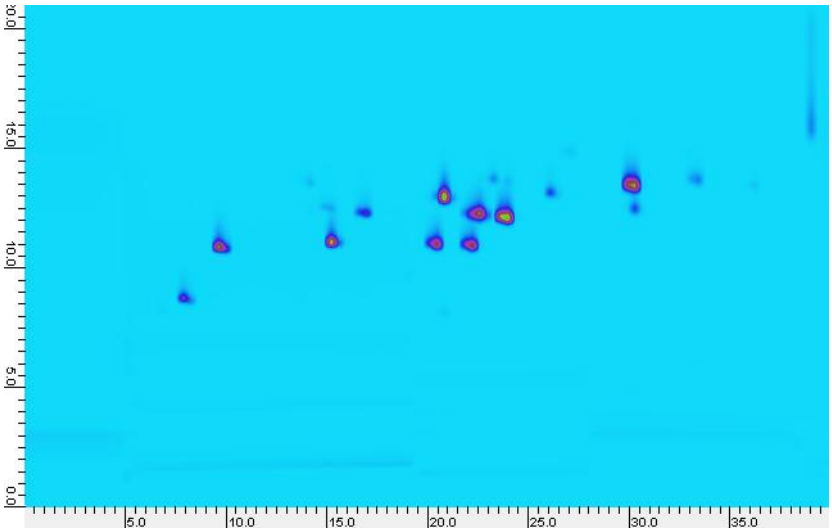
Notice how the peaks are spread more widely across the two-dimensional separation space (the accessible two-dimensional separation space is enlarged) when shifted gradients are used. Also, notice the effect that using continuously shifted second dimension gradients has on the second dimension retention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



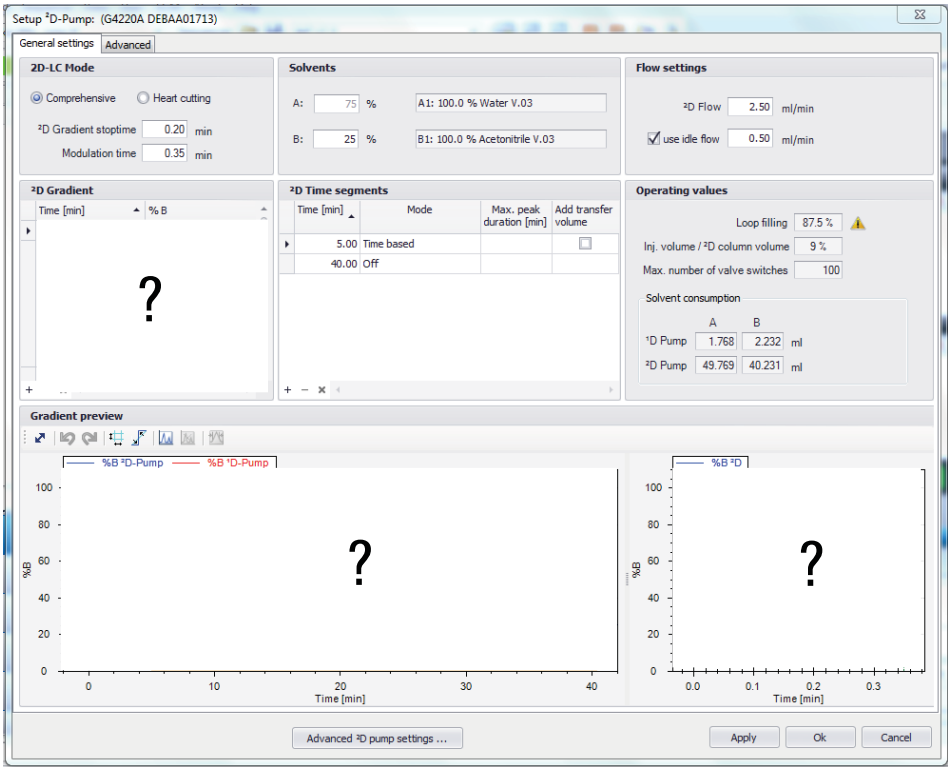
NOTE

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!

Optimize the ^{2}D method

1 Try to further optimize the ^{2}D method (further enlarge the accessible two-dimensional separation space) using shifted gradients in the second dimension. For this purpose, keep the valve & loop configuration as well as the ^{1}D pump method the same. In **Instrument > Setup 2D-LC**, set up your own ^{2}D pump and modulation method:



6
Theoretical Background

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

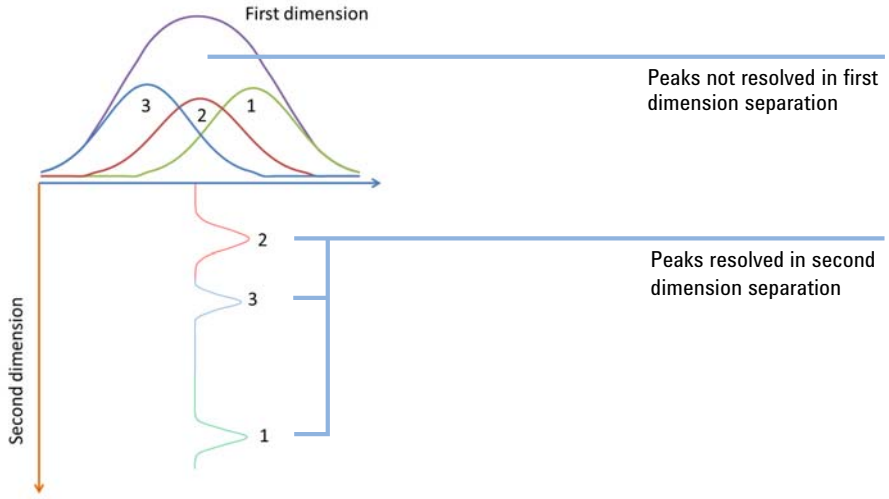


Figure 107 Peak capacity relationship between peak capacities of orthogonal first and second dimension

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 239,
- "Resolution" on page 239, and
- "Peak Capacity" on page 243

The following different methods of 2D-LC exist:

- Heartcutting (LC-LC)

  Only interesting portion of the first dimension effluent transferred to the second dimension (see "Heart-Cutting 2D-LC (LC-LC)" on page 25)

- Comprehensive (LCxLC)

  Entirety of first dimension effluent sequentially transferred to the second dimension (see "Comprehensive 2D-LC (LCxLC)" on page 159)

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 32 on page 248 and Table 33 on page 253.

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 108 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.

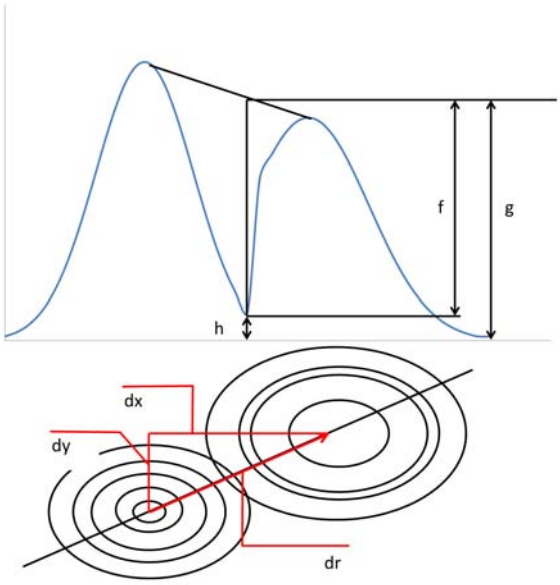


Figure 109 Diagram of 2D resolution measurement: Slice for resolution (top) and 2-dimensional contour plot (bottom)

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 110 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 111 2D-Resolution (peak to valley ratio relation)

Table 31 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:

$$n_c = PN^{1/2}$$

(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 110 on page 242)

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 246). 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)

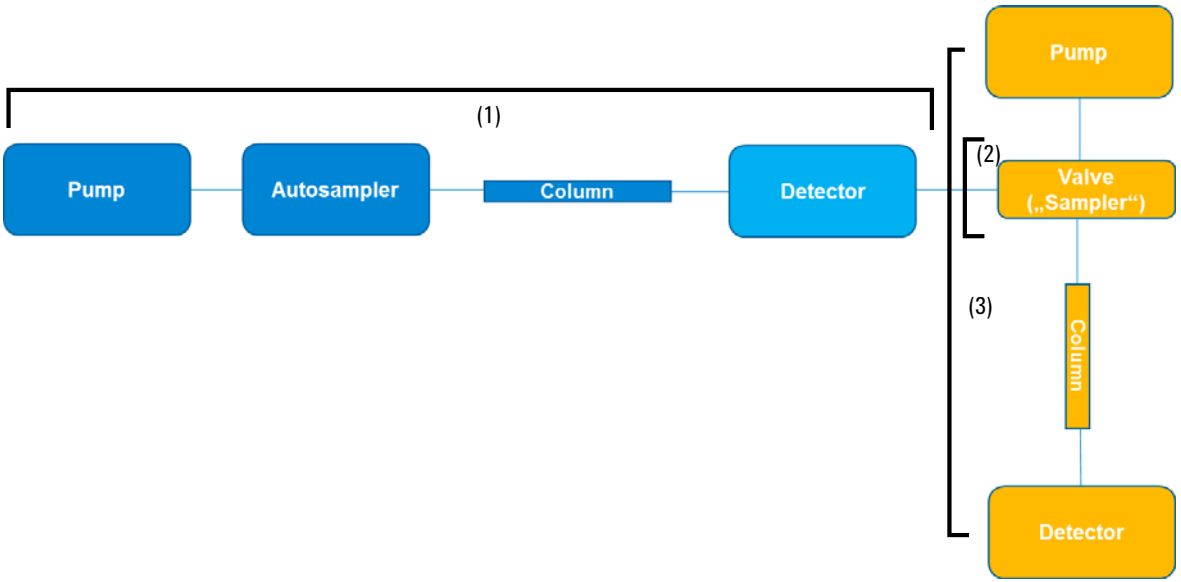


Figure 112 Diagram of instrumentation for 2D-LC

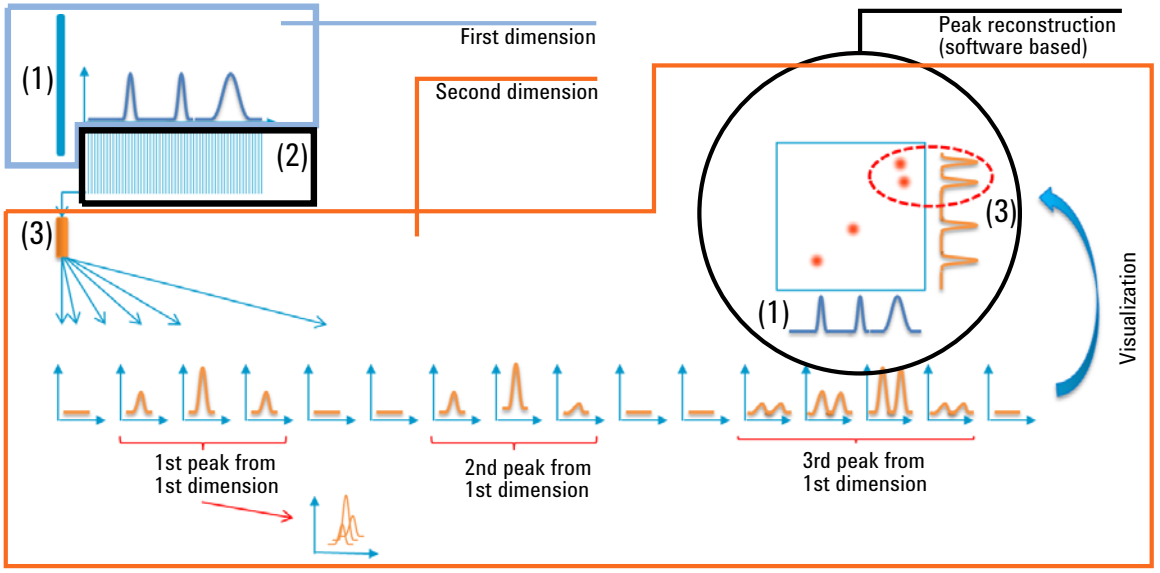


Figure 113 Principle of 2D-LC (example for LCxLC): Effluent of first column (1) is sampled (2) and injected to second column (3). Peaks of second column separation are detected and reconstructed.

(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 32 on page 248 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 32 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 33 on page 253 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

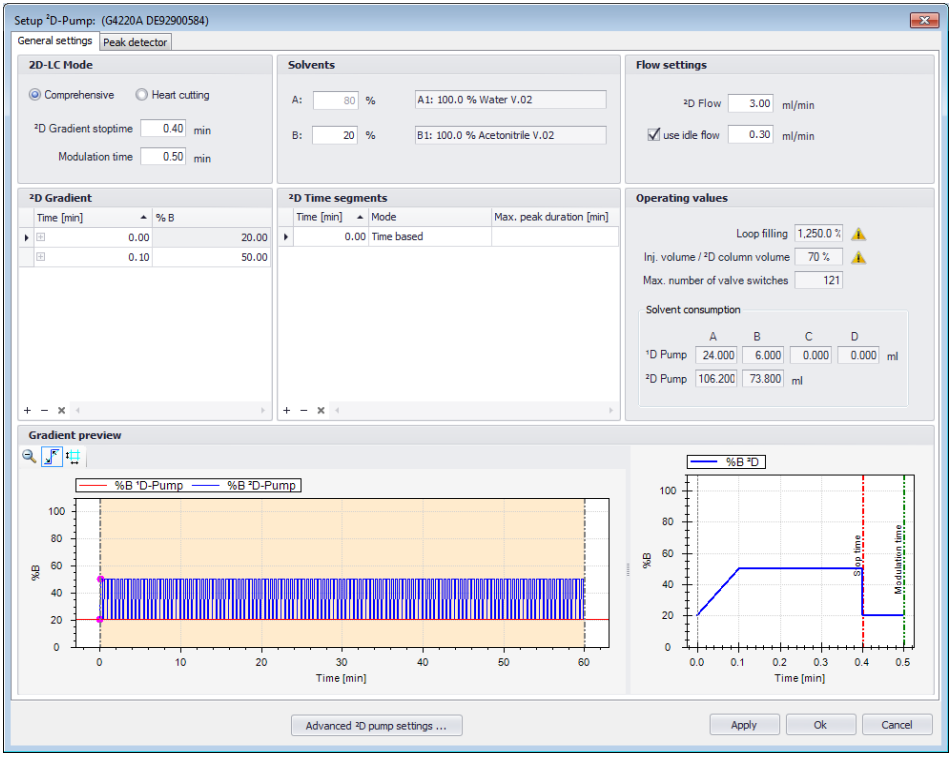


Figure 114 Standard gradient mode

- 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.

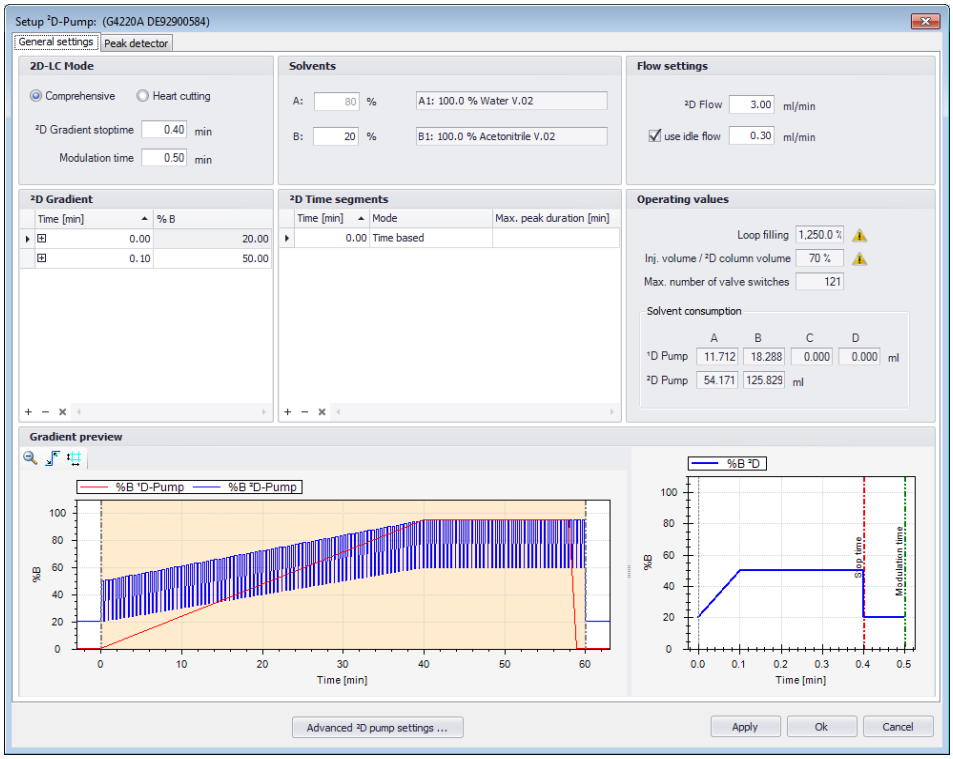


Figure 115 Shifted gradient mode with increase of start-%B

- Isocratic

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

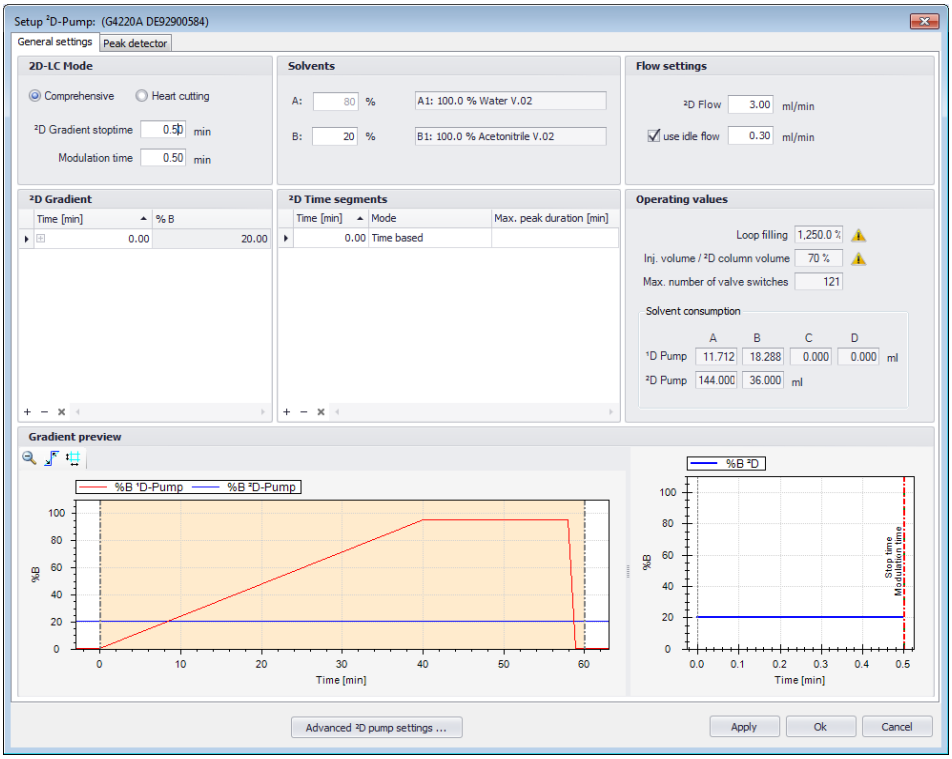


Figure 116 Isocratic mode

- 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.

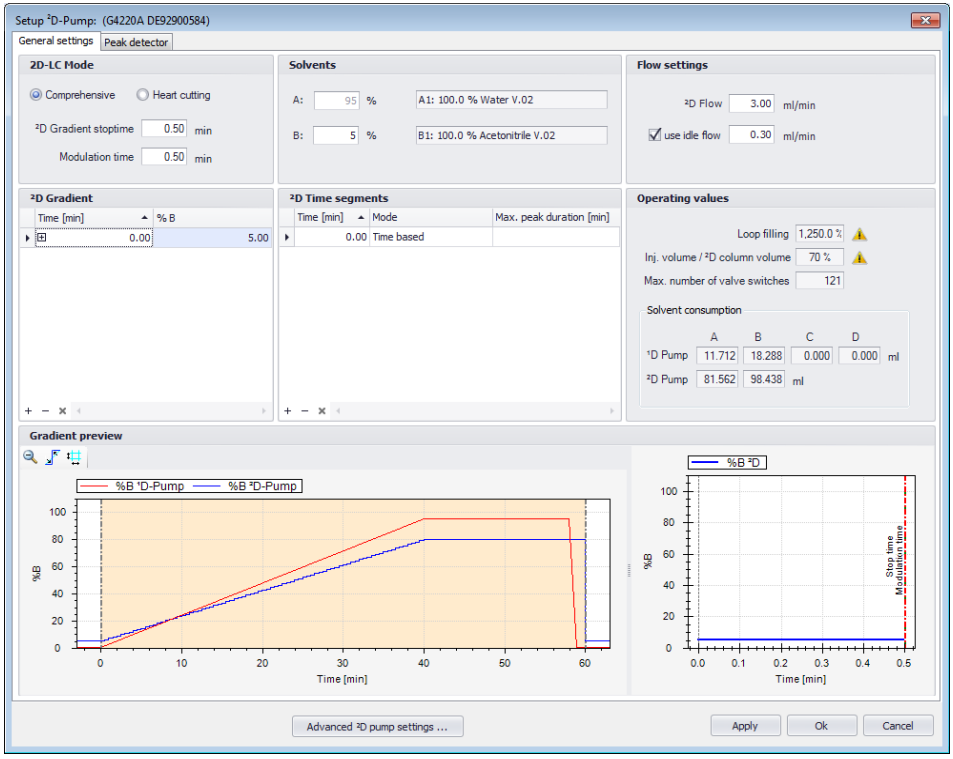


Figure 117 Advancing isocratic mode

Table 33 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

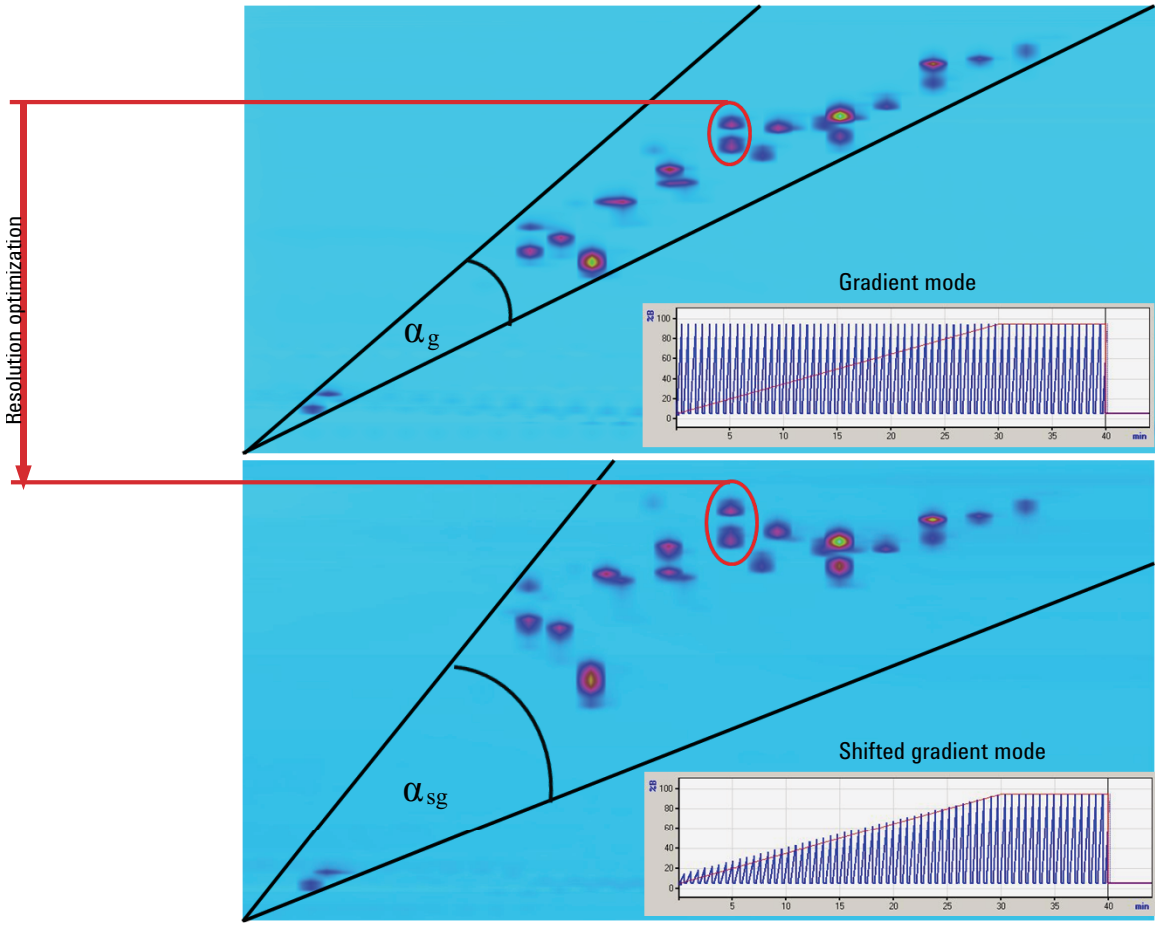


Figure 118 2nd dimension gradient mode compared to isocratic mode and its effect on resolution

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 34 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.

7
Possible ways to install the System

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

System Components

The Agilent 1290 Infinity 2D-LC-Solution is very flexible and may be adjusted to individual needs. For possible configurations, refer to Table 35 on page 259.

A complete high-end Agilent 1290 Infinity solution is possible, to obtain maximum flexibility and best results (see Table 36 on page 262).

NOTE The Agilent 1290 Infinity 2D-LC-Solution must contain an Agilent 1290 Infinity Binary Pump (G4220A) or a 1290 Infinity Binary Pump VL (G4220B) as 2nd dimension pump to synchronize valve switches with the 2nd dimension gradient repetition and to run the second dimension to deliver fast gradients to the 2nd dimension column.

NOTE When using multiple detectors at different positions like after first dimension column, at the waste line in addition to the standard 2D-LC detector after the second dimension columns, it is recommended to use same detector types and flow-cells if quantitive information are required.

Table 35 Overview of possible configurations of the Agilent 1290 Infinity 2D-LC-Solution

Function in 2D-LC	Functional Element	Part Number	Module	Comment
1st dimension	Pump	G4220A/B	1290 Infinity Binary Pump (VL) (1200 bar)	Any of these are supported as well as others or older modules.
	G4204A	1290 Infinity Quaternary Pump (1200 bar)		
	G1312B	1260 Infinity Binary Pump		
	G1382A	1260 Infinity Capillary Pump		
	Autosampler	G4226A	1290 Infinity Autosampler	Any of these are supported as well as others or older modules.
		G1367E	1260 Infinity HiP Autosampler	
		G1329B	1260 Infinity Autosampler	
		G1377A	1260 Infinity Micro Autosampler	
	Detector	G4212A/B	1290/1260 Infinity DAD	**Optional** To directly monitor 1st dimension or for peak-triggered operation. Any of these are supported as well as others or older modules. FLD not supported for peak-triggering
		G1315C/D	1260 Infinity DAD VL+/VL	
		G1365C/D	1260 Infinity MWD	
		G1314E/F	1260/1290 Infinity VWD	
		G1321B	1260 Infinity FLD	

Table 35 Overview of possible configurations of the Agilent 1290 Infinity 2D-LC-Solution

Function in 2D-LC	Functional Element	Part Number	Module	Comment
Interface	Valve drive	G1170A	1290 Infinity Valve Drive	Possible and supported
	Valve head	G4236A	2D-LC valve kit, 1200 bar	**Recommended** For comprehensive or heart-cutting 2D-LC. Dedicated capillary and loop-kits optionally available.
		G4232A/B	2pos/10port valve kit, 600/1200 bar	For comprehensive or heart-cutting 2D-LC. No dedicated capillary kit for 2D-LC.
2nd dimension	Pump	G4220A/B	1290 Infinity Binary Pump (VL) (1200 bar)	*Required!*
	Detector	G4212A/B	1290/1260 Infinity DAD	Recommended as DAD, 60 mm flow cell recommended.
		G1315C/D	1260 Infinity DAD VL+/VL	Any of these are supported as well as others or older modules
		G1365C/D	1260 Infinity MWD	
		G1314E/F	1260/1290 Infinity VWD	
		G1321B	1260 Infinity FLD	
		G4260A	1260 Infinity ELSD	
		G61xx	61xx Single Quad MS	

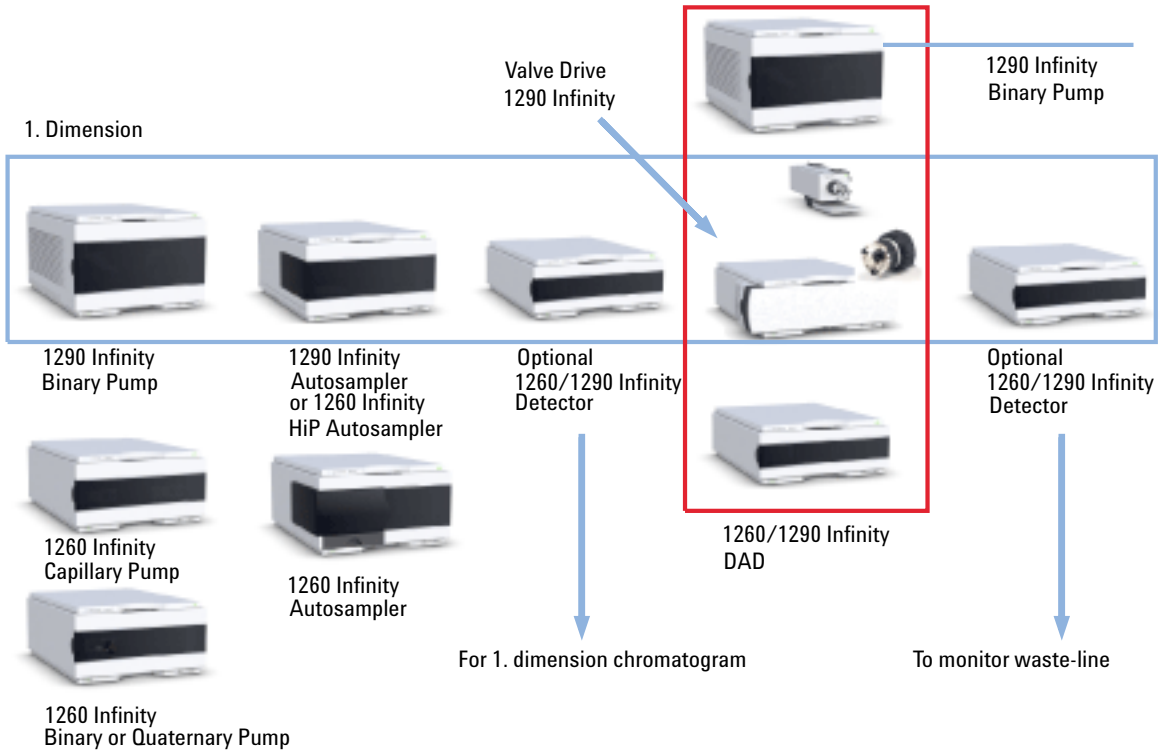


Figure 119 Illustrated overview of possible hardware in an Agilent 1290 Infinity 2D-LC-Solution

1st dimension components (in blue box)
2nd dimension components (in red box)
Modules outside the blue box are optional as 1st dimension components

High-end Agilent 1290 Infinity configuration

Table 36 Example for configuration of a high-end, complete 1290 Infinity 2D-LC system

Function in 2D-LC	Functional Element	Part Number	Module	Comment
1st dimension	Pump	**G4220A**	1290 Infinity Binary Pump	
		G4204A	1290 Infinity Quaternary Pump	
	Autosampler	**G4226A**	1290 Infinity Autosampler	
	Detector	**G4212A**	1290 Infinity DAD	**Optional** To directly monitor 1st dimension or for peak-triggered operation
Interface	Valve drive	**G1316C**	1290 Infinity TCC	**Recommended** For column thermostatting. For more flexibility also a second TCC might be used.
	Valve drive	**G1170A**	1290 Infinity Universal Valve Drive	
	Valve head	**G4236A**	2D-LC valve kit, 1200 bar	**Recommended** For comprehensive or heart-cutting 2D-LC. Dedicated capillary and loop-kits optionally available.
2nd dimension	Pump	**G4220A**	1290 Infinity Binary pump	**Required (G4220B - 1290 Infinity Binary Pump VL also supported)**
	Detector	**G4212A**	1290 Infinity DAD	60 mm flow cell recommended

Physical Setup of the Components

The following configurations optimize the system flow path, ensuring minimum delay volume.

NOTE The capillary connections should be as short as possible, to ensure optimum performance of the system.

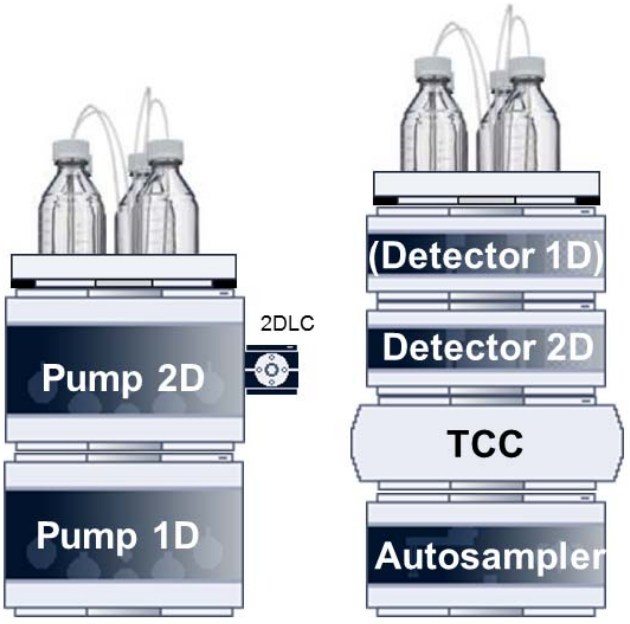


Figure 120 Stack configuration for Standard Heart-cutting 2D-LC

Table 37 1290 Infinity 2D-LC-System

Left Stack	Right Stack
	Solvent Cabinet
	[1]D detector (DAD (G4212A or G7117A/B)) - (optional)
Solvent Cabinet	[2]D detector (DAD (G4212A or G7117A/B))
[2]D pump (G4220A) with Valve Drive (G1170A) attached	TCC (G1316C)
[1]D pump (G4220A/G4204A)	Autosampler (G4226A)

Table 38 1260 Infinity 2D-LC-System

Left Stack	Right Stack
	Solvent Cabinet
	[1]D detector (DAD (G4212A/B or G7117A/B)) - (optional)
Solvent Cabinet	[2]D detector (DAD (G4212A/B or G7117A/B))
[2]D pump (G4220A) with Valve Drive (G1170A) attached	TCC (G1316C)
[1]D pump (G4220A or G4204A or G1312A/B/C or G1311A or G1376A)	Autosampler (G4226A or G1367E/F)

Fluidic connection between the modules

After placing the modules of the first dimension and second dimension and making the electrical connections the flow paths must be build.

Capillary Kit for 2D-LC

Item	p/n	Description
1	5021-1820 (2x)	Flex capillary, 0.12 x 105 mm, no fittings
2	G1316-87321	Capillary column-heat exchanger 105 mm lg, 0.17 mm i.d.
3	5021-1822	Flexible tubing, 280 mm
4	5021-1823 (3x)	Capillary column – detector SST 400 mm lg, 0.12 mm i.d.
5	5021-1819	Capillary ST 0.17 mm x 400 mm S/S
6	5065-9964	Capillary ST 0.12 mm x 500 mm
7	5067-4609	Capillary ST 0.17 mm x 500 mm SX/-
8	5067-4669	Capillary ST 0.12 mm x 600 mm S/SL
9	01078-87305	Capillary, 0.17 mm x 80 cm, male fit
10	G1316-80022 (2x)	LDHE double kit for G1316C

Interfacing the First and Second Dimension

The interface between first and second dimension is located at the valve in the MCT (see Figure 119 on page 261).

NOTE Also a 1290 Infinity Valve Drive (G1170A) can be used as valve drive but this would lack the thermostatting capability for the columns which is not recommended but supported.

For details on thermostatted column compartment set-up, see "Thermostatted Column Compartment Setup" on page 267.

Use of valves and plumbing of capillaries and loops depends on the LC-method you choose to get optimal results:

- Comprehensive 2D-LC (LCxLC), see "Interfacing First and Second Dimension for Comprehensive 2D-LC (LCxLC)" on page 270
- Heart cutting 2D-LC (LC-LC), see "Interfacing First and Second Dimension for Heartcutting 2D-LC (LC-LC)" on page 273

Thermostatted Column Compartment Setup

Different variants of thermostatted column compartment setup are supported with the optional 2D-LC capillary kit for the 2D-LC valve (G4236A#3):

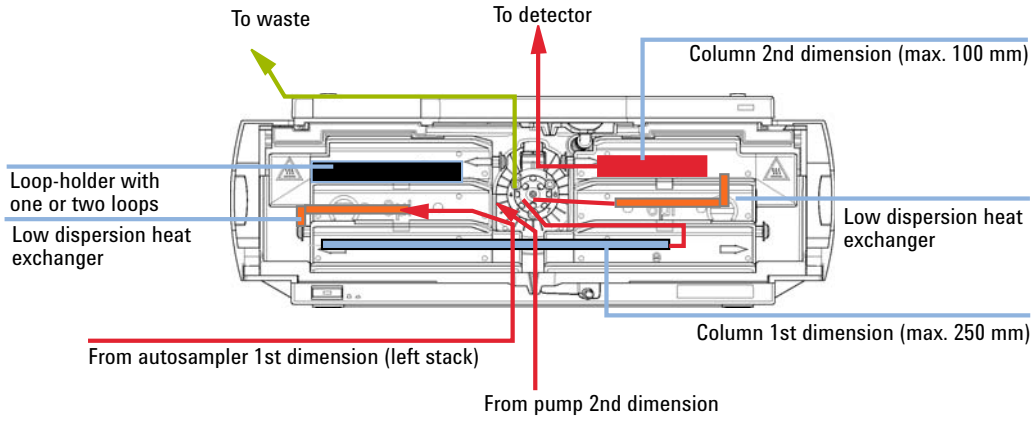


Figure 121 Columns at different temperatures,long 1st dimension column, no 1st dimension detector, for comprehensive 2D-LC booth loops can be placed in the loop holder.

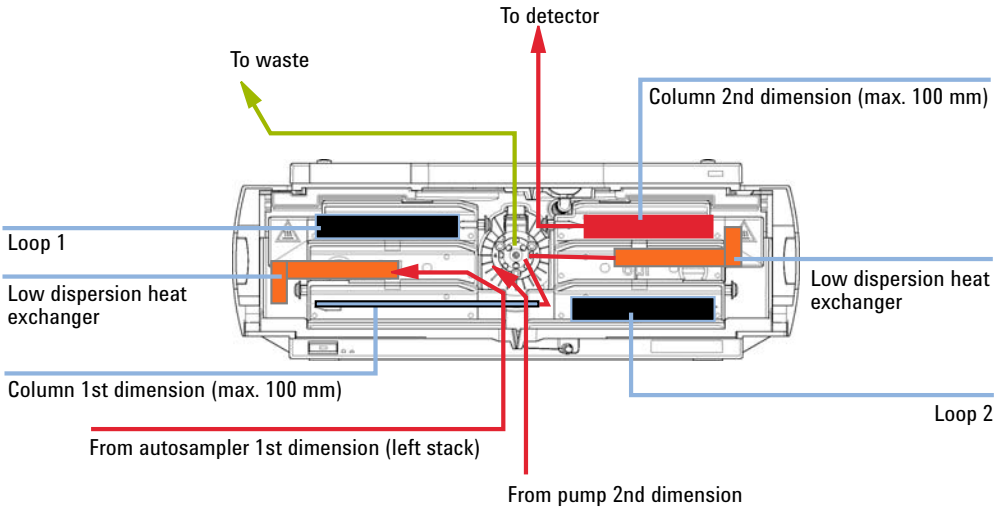


Figure 122 Columns at different temperatures, medium 1st dimension column, no 1st dimension detector

Valve Options

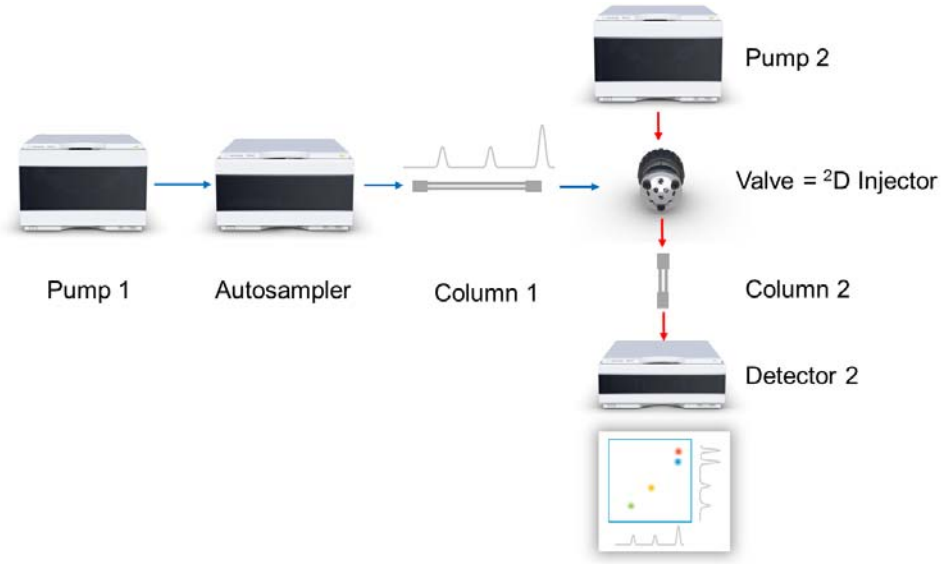


Figure 123 Concept of a 2D-LC-System

The Agilent 1290 Infinity 2D-LC Solution supports the following valve configurations:

- 2D-LC-Valve (2pos/4port-duo valve) (highly recommended)
- 2 Pos/10 Port Valve

2D-LC Quick-Change Valve

Advantages of the Agilent 2D-LC valve:

- Has fully symmetric flow paths (no additional bridging loops)
- Offers symmetric fill- and flush-out behavior and allows depending on plumbing either counter-current or co-current flush-out of both loops
- Due to its special design it delivers lowest pressure spikes to the columns. This lower stress guarantees a longer life time of the columns in the second dimension.

For details, see Table 41 on page 274 (standard heart-cutting) and Table 39 on page 271 (full comprehensive).

2pos/10port Valve

Support of 2pos/10port valve for comprehensive and heart-cutting 2D-LC allows easy transfer or existing 2D-LC methods. Both symmetric and asymmetric set-up supported in the software.

Interfacing First and Second Dimension for Comprehensive 2D-LC (LCxLC)

To interface first and second dimension for LCxLC, the following opportunities exist:

- 2 Pos/4 Port Duo Valve
- 2 Pos/10 Port Valve

2 Pos/4 Port Duo Valve

The 2 Pos/4 Port Duo Valve is especially constructed for its use in 2D-LC applications. The main advantage is that the flow stream through the loop capillaries can be guided in a cocurrent or countercurrent manner by means of the respective plumbing.

- Cocurrent:

  The flow for filling and eluting (analyze direction) the loops is entering the loop from the same side for the cocurrent plumbing.

- Countercurrent:

  The flow entering the loops for filling and analyzing is opposite for the countercurrent plumbing.

Table 39 Plumbing for 2D-LC Valve Head, comprehensive 2D-LC

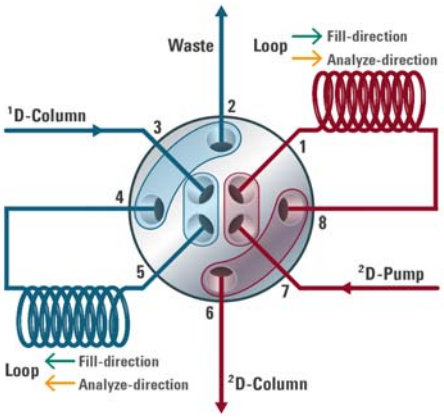
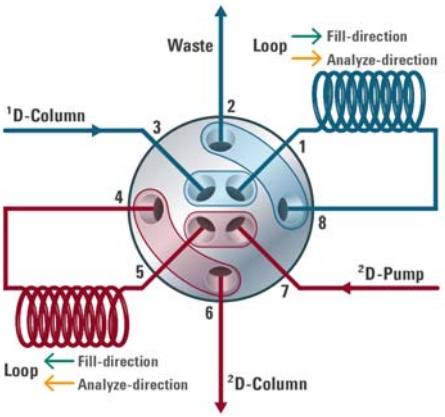
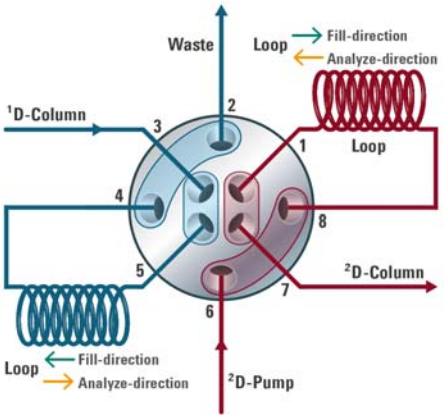
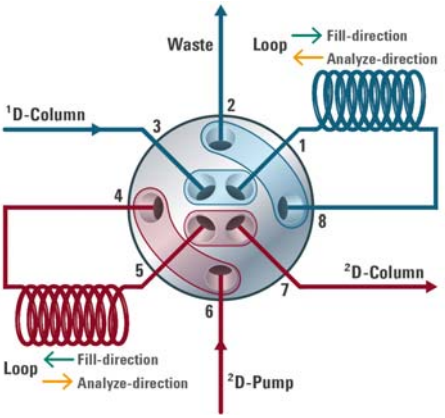
	Filling	Analyzing
Cocurrent		
	Figure 124 2D-LC Valve Head, position 1	**Figure 125** 2D-LC Valve Head, position 2
Countercurrent		
	Figure 126 2D-LC Valve Head, position 1	**Figure 127** 2D-LC Valve Head, position 2

Table 40 Plumbing for 2 Pos/10 Port Valve, comprehensive 2D-LC (not recommended)

Assymmetric
One loop is filled and eluted - cocurrent and the other one countercurrent. This is known to produce more artifacts than the symmetric set-up.

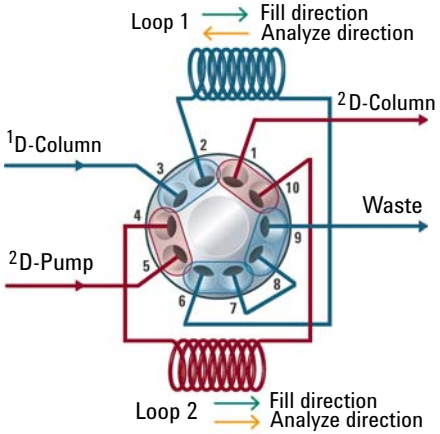


Figure 128 2 Pos/10 Port Valve, position 1

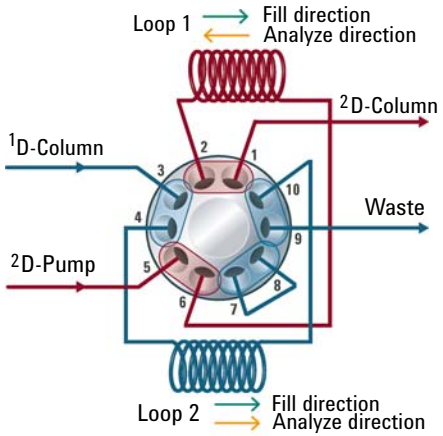


Figure 129 2 Pos/10 Port Valve, position 2

Symmetric
The loops are eluted both in a cocurrent manner. But the required bridging capillary is still known to produce artifacts.

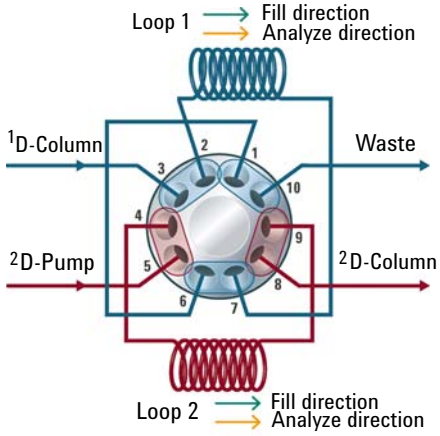


Figure 130 2 Pos/10 Port Valve, position 1

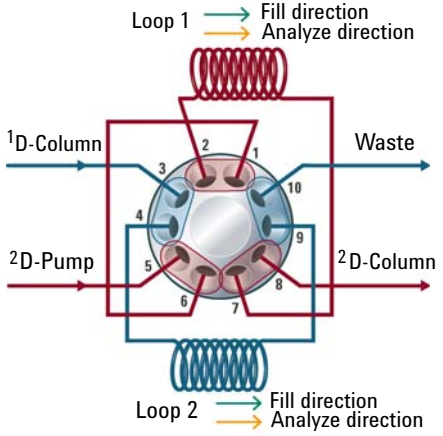


Figure 131 2 Pos/10 Port Valve, position 2

Interfacing First and Second Dimension for Heartcutting 2D-LC (LC-LC)

In a heartcutting experiments only a part of the first dimension is transferred to the second dimension and analyzed.

NOTE Heartcutting 2D-LC experiments usually are characterized by longer runtimes and shallower 2D gradients, compared to comprehensive 2D-LC (LCxLC) experiments.

NOTE In general the valve set-up with two loops as used for comprehensive 2D-LC can be used as well for heart-cutting 2D-LC but one must keep in mind that additional solvent volumes are caught in the not used loop. Depending on the method this might cause artifacts.

Dual 2 Pos/4 Port Valve

In this case of heartcutting 2D-LC only one sampling loop is connected to the valve. The other position is connected by a short capillary. This enables the system to switch a clean capillary from the second dimension to the first dimension when loading is necessary and back for analysis after loading. The first dimension eluent in the short capillary can be neglected and does not contaminate the second dimension (Table 41 on page 274).

This type of plumbing for a heartcutting experiments can be done in different flow schemes:

Table 41 Plumbing for 2D-LC Valve Head, heart-cutting 2D-LC

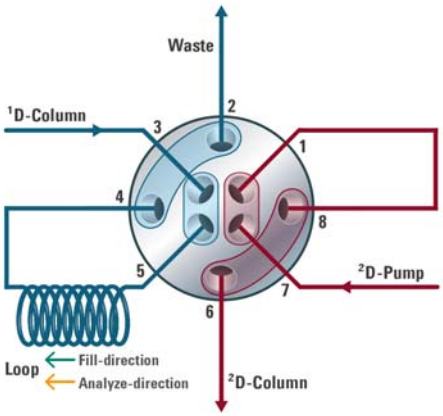
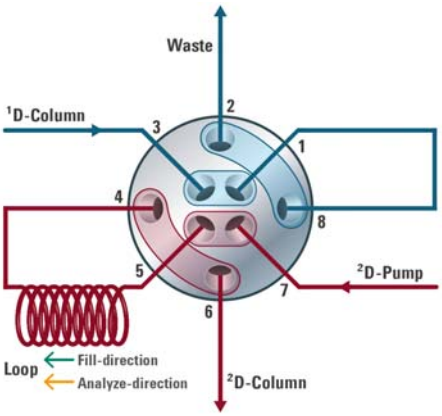
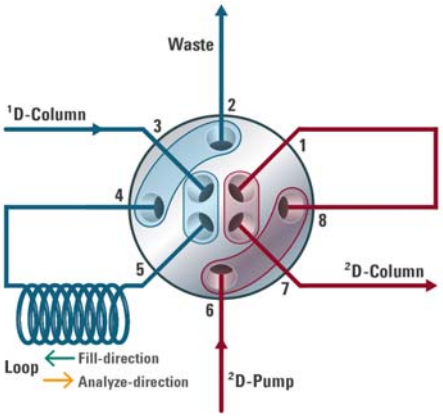
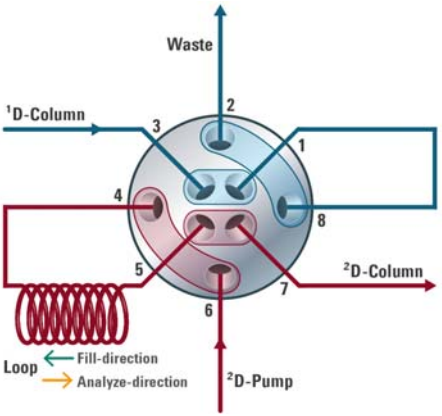
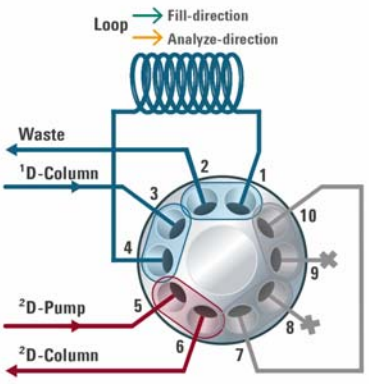
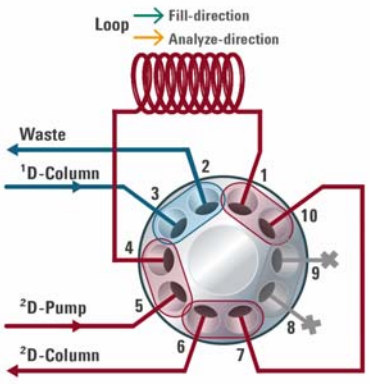
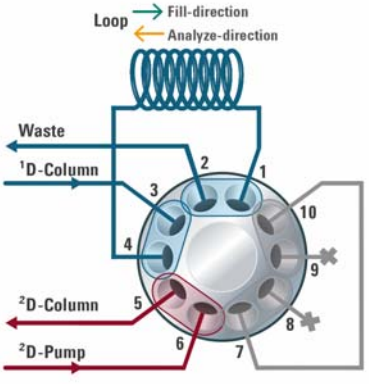
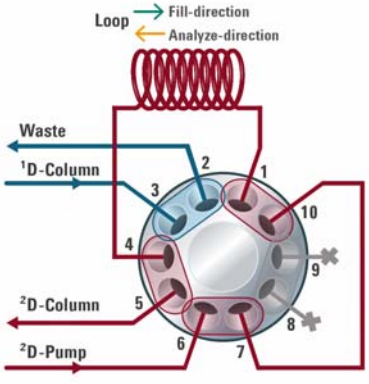
	Filling	Analyzing
Cocurrent	 **Figure 132** 2D-LC Valve Head, position 1	 **Figure 133** 2D-LC Valve Head, position 2
Countercurrent	 **Figure 134** 2D-LC Valve Head, position 1	 **Figure 135** 2D-LC Valve Head, position 2

Table 42 Plumbing for 2 Pos/10 Port Valve, heartcutting 2D-LC (not recommended)

	Filling	Analyzing
Cocurrent	 **Figure 136** 2 Pos/10 Port Valve, position 1	 **Figure 137** 2 Pos/10 Port Valve, position 2
Countercurrent	 **Figure 138** 2 Pos/10 Port Valve, position 1	 **Figure 139** 2 Pos/10 Port Valve, position 2

Installing the Software

Software required OpenLAB ChemStation Edition C.01.07 (or higher) installed

NOTE For installing the 2D-LC Software, please use the OpenLAB Additional Software and Drivers Deployment Wizard.

NOTE Do not try installing the software by double-clicking the msi file, as this may result in an incomplete installation.

NOTE To install the Add on, the OpenLAB CDS Chemstation Software must be not active.

1 Start OpenLAB Additional Software and Driver Deployment Wizard by going to Windows **> Start > Agilent Technologies > OpenLAB > OpenLAB Additional Software and Drivers**

2 Follow steps described in the Wizard for installation or software upgrades.

8 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 282.

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.
- ➔ The volume of substances should be reduced to the minimum required for the analysis.
- ➔ Do not operate the instrument in an explosive atmosphere.
- ➔ Never exceed the maximal permissible volume of solvents (6 L) in the solvent cabinet.
- ➔ Do not use bottles that exceed the maximum permissible volume as specified in the usage guideline for the Agilent 1200 Infinity Series Solvent Cabinets.
- ➔ Arrange the bottles as specified in the usage guideline for the solvent cabinet.
- ➔ A printed copy of the guideline has been shipped with the solvent cabinet, electronic copies are available on the Internet.
- ➔ Ground the waste container.
- ➔ The residual free volume in the appropriate waste container must be large enough to collect the waste liquid.
- ➔ Check the filling level of the waste container regularly.
- ➔ To achieve maximal safety, check the correct installation regularly.
- ➔ Do not use solvents with an auto-ignition temperature below 200 °C (392 °F).

Safety Symbols

Table 43 Symbols

	The apparatus is marked with this symbol when the user should refer to the instruction manual in order to protect risk of harm to the operator and to protect the apparatus against damage.
	Indicates dangerous voltages.
	Indicates a protected ground terminal.
	The apparatus is marked with this symbol when hot surfaces are available and the user should not touch it when heated up.
	Cooling 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.
	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

Table 43 Symbols

	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.
	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 Directive

Abstract

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

NOTE

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

Product Category:

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

NOTE

Do not dispose of in domestic household waste

To return unwanted products, contact your local Agilent office, or see 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, di-isopropyl ether) such ethers 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.

Agilent Technologies on Internet

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http://www.agilent.com

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In This Book

The manual describes the following:

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

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