Agilent 1290 Infinity 2D-LC Solution

A new flexible and user-friendly 2D-LC solution for the most complex samples

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What is 2D-LC?

2D-LC: Injecting the efluent or a part of the effluent of one column to a second column, ideally with orthogonal separation behavior.

**Purpose:** increase total separation power.

Peak capacities **multiply** for orthogonal separation mechanisms!

Two different modes:

- **Comprehensive 2D-LC** (“LCxLC”)
- **Heart-cutting 2D-LC** (“LC-LC”)

2D-LC - Difference between comprehensive 2D-LC and heart-cutting 2D-LC

Comprehensive 2D-LC (LCxLC):

The complete effluent of the first column will be injected to the second column and will be analyzed with very fast gradients, a peak of the first dimension should be sampled at least 3 to 4 times. The run time of the 2nd dimension method matches the collection time of the 1st dimension effluent. Finally, the peaks will be re-constructed.
2D-LC - Difference between comprehensive 2D-LC and heart-cutting 2D-LC

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

Only *parts* of the effluent of the first column – the peaks eluted from the 1\textsuperscript{st} dimension column - will be injected to the second column. Typically a peak from the first dimension will be sampled as a whole and a gradient with a longer run time than the collection time will be used. Also longer columns with higher separation efficiency are being used in as 2\textsuperscript{nd} dimension column.

Care must be taken if peaks are eluting from the first dimension column when a gradient on the second dimension is still running – this peak will be lost.
What is the Agilent 1290 Infinity 2D-LC solution?

- A new Agilent OpenLAB CDS ChemStation edition **software add-on** and **new firmware features** to allow easy set-up of 2D-LC methods using Agilent standard hardware.

- For comprehensive 2D-LC and heart-cutting 2D-LC

- **Unique features** like automatically shifted gradients or peak-triggered operation

- Highest focus on **simplest** but still highly flexible 2D-LC method set-up

- Highest flexibility on hardware set-up
  - Different pumps, autosamplers and detectors supported, **existing systems can be upgraded!**
  - Detectors at different positions (after 1\(^{st}\) dimension column, after 2\(^{nd}\) dim. column, at waste-line)
  - Different valve set-up possibilities supported, **one special new 2D-LC QuickChange valve head**
  - High performance data analysis by partner-solution

- Available June 2012
Data-Analysis

For heart-cutting 2D-LC by Agilent’s OpenLAB CDS ChemStation edition.

For comprehensive 2D-LC Agilent recommends:

**GC Image LCxLC Edition Software** from GC Image LLC.

**Key features offered:**
- Direct Agilent datafile import
- Peak identification, integration, annotation
- Comparative Analysis and Visualization
- Compound-libraries
- MS-data handling
Hardware – Module-flexibility

1. Dimension

- 1290 Infinity Binary Pump
- 1290 Infinity Autosampler or 1260 HiP Autosampler
- 1260 Infinity Capillary Pump
- 1260 Infinity Binary or Quaternary Pump

2. Dimension

- One or two 1290 Infinity TCC
- 1290 Infinity Binary Pump
- Optional 1260/1290 Infinity Detector
- For 1st dimension chromatogram and peak-trIGGERing
- 1260/1290 Infinity detector
- Optional 1260/1290 Infinity Detector
- To monitor waste-line

Almost any Agilent pump or autosampler in the 1st dimension!
Almost any detectors are supported!
A 1290 Infinity Binary Pump for the 2nd dimension is required.
Hardware – Module-flexibility

1. Dimension

- 1290 Infinity Binary Pump
- 1290 Infinity Autosampler or 1260 High Performance Autosampler
- Optional 1260/1290 Infinity Detector

2. Dimension

- One or two 1290 Infinity TCC
- 1290 Infinity Binary Pump
- Optional 1260/1290 Infinity Detector

For 1st dimension chromatogram and peak-triggering

1260/1290 Infinity Detector

To monitor waste-line

Most literature known valve configurations based on 2pos-10port or 2pos-6port valves are supported.
Hardware – Valves, uniqueness and flexibility

1. Dimension

New Agilent-only special 2D-LC-QuickChange valve. Single valve with fully symmetric flow-paths and symmetric fill/flush-out behavior. Only valve that allows countercurrent flush-out of both loops.
Advantages of the new Agilent 2D-LC QuickChange valve head

1. All flow paths are equal (no additional bridging loops)
2. Symmetric countercurrent fill/analyze direction of loops (reducing band-spread)
3. All in one valve (no synchronization, costs)
Hardware – Valve-flexibility

Most other valve configurations for comprehensive and heart-cutting 2D-LC are supported as well. Easy transfer of existing 2D-LC methods!
Hardware – Valve-flexibility

1. Dimension

2. Dimension

2x 2pos/6port valves

Loop1

Loop2

waste

Also dual valve-head configurations supported with automatic synchronisation of valve-drives!
2D-LC Acquisition software
An add-on to Agilent’s OpenLAB CDS ChemStation edition

Dashboard:

All modules in one dashboard can be relabeled individually, e.g. „BinPump-1st-Dim“
Supported 2D-LC operation modes

**Comprehensive LCxLC**

- Standard
  - or
  - Time-/Peak-triggered

complex/unknown samples: bio-pharma, food, polymers....

**Heart-cutting LC-LC**

- Time-triggered
  - or
  - Peak-triggered

Known samples/improving confidence: pharma, meth.-dev..
Comprehensive 2D-LC – gradient modes

Special 2D-gradient modes to improve resolution

- standard repeating with start- and end-time
- constantly shifted %B_{2D}
- constantly shifted %B_{2D} and shifted Δ%B_{2D}
- Any combination
Time-segmenting in comprehensive 2D-LC

Reduction cost!

1D Gradient/
1D Chromatogram

2D Time-segments
set in software

2D Pump flow rate
Solvent saving!!!

2D Pump flow rate

Valve toggling
Increase life time!

1D Method run time

1D Method post-run time

idle
Time-triggered
idle
Peak-triggered
idle

Idle flow rate

%B (1D)

F (2D)

%B (2D)

2D Gradients

1D Method run time

1D Method post-run time
Most easiest 2D-LC System Configuration

“One screen for the entire system”

- Define 1D / 2D pump
- Define detector in the second dimension
- Define peak detector (optional)

Select the valve(s) to be used for 2D-LC injection

Select a possible valve / loop configuration

Graphical representation of the selected valve / loop configuration:
- Flow path 1D & 2D
- Animated valve switching
Most easiest set-up of complex 2D-LC methods

2D-LC specific parameters of the 2D-pump

Select the 2D-LC mode: comprehensive / heart-cutting

Define repetition of 2nd dimension gradient (Modulation time)

Define the gradient of the 2nd dimension

Show rollout of gradient in the 2nd dim over the runtime of the 1st dimension

Graphical editing of gradient shift

Solvent & Flow-Settings

Define time window(s) where the selected 2DLC mode is active

Operation values, warnings

Solvent consumption calculation

Close-up of 2D-gradient

Access to standard method UI of the pump

Graphical editing of gradient shift
Example: graphical editing of a gradient shift – in less than a minute!

Replace editing of large timetables by a few mouse operations

1. Use context menu to enter the editing mode.

2. Timetable entries are marked with circles.

3. Draw a straight line by dragging the mouse to a new %B value at a specified runtime of the 1st dimension.

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

5. Repeat step 3 + 4 with other TT entries.

6. Insert / Delete shift points (mouse cursor and context menu changes near to a shift line).
Application examples

Advantage of shifted gradient features

RPLC x RPLC
Easy method set-up but only little orthogonality

Current state-of-the-art 2D-LC – narrow spread of peaks
Application examples

Advantage of shifted gradient features

Resolution optimized!

Use of shifted gradient feature

Imagine to program this gradient manually!

With the Agilent 2D-LC Acquisition software a matter of a minute!
Some Performance data

Testmix of 20 compounds – 2D-Precision

**2D-Retention Time Precision RSD (%)**

- **15 compounds** <1% RSD, all compounds <2% RSD

**2D-Peak Volume* Precision RSD (%)**

- **8 compounds** <1% RSD, 16 compounds <2% RSD

*) not a peak area is measured but a three dimensional peak volume (intensity x 1D-time x 2D-time)!

- **1st Dimension:**
  - Eclipse Plus RRHD, C18, 150 x 2.1 mm, 1.8 µm.
  - Flow rate: 0.1 mL/min.
  - Gradient: 0 min 5% B – 30 min 95% B, 40min stop-time.

- **2nd Dimension:**
  - Eclipse Plus RRHD, Phenyl Hexyl, 50 x 3.0 mm, 1.8 µm
  - Flow rate: 3 mL/min.
  - Initial Gradient: 0 min - 5% B, 0.5 min - 15% B, 0.51 min - 5% B, 0.65 min - 5% B.
### Applications examples

**Polyphenols from food matrix**

<table>
<thead>
<tr>
<th>Compound</th>
<th>RT 1st dim (min)</th>
<th>RT 2nd dim (sec)</th>
<th>Peak Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esculin</td>
<td>mean</td>
<td>9.75</td>
<td>177,383</td>
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<td></td>
<td>s.d.</td>
<td>nd</td>
<td>4,713</td>
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<tr>
<td></td>
<td>RSD (%)</td>
<td>nd</td>
<td>2.7</td>
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<tr>
<td>Rutin</td>
<td>mean</td>
<td>13.65</td>
<td>72,375</td>
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<td></td>
<td>s.d.</td>
<td>0.07</td>
<td>853</td>
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<td></td>
<td>RSD (%)</td>
<td>nd</td>
<td>1.2</td>
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<tr>
<td>Coumaric acid</td>
<td>mean</td>
<td>13.00</td>
<td>660,541</td>
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<td></td>
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<td>nd</td>
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<td></td>
<td>RSD (%)</td>
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<td>Resveratrol</td>
<td>mean</td>
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<td></td>
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<td></td>
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<td>nd</td>
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<td>Salicylic acid</td>
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<td></td>
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<td>Luteolin</td>
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<td>19.50</td>
<td>695,601</td>
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<td></td>
<td>RSD (%)</td>
<td>nd</td>
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<tr>
<td>7-Hydroxy-Flavone</td>
<td>mean</td>
<td>22.75</td>
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<td>RSD (%)</td>
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<td>Pinoslyvin</td>
<td>mean</td>
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<td>Chrysin</td>
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<tr>
<td></td>
<td>RSD (%)</td>
<td>nd</td>
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</table>

Peak volume: all compounds <3.6% RSD
Application Examples
Normal and stressed MAb, detection by ToF-MS
The Agilent 1290 Infinity 2D-LC solution brings the power of two-dimensional LC-separation to you at a never before experienced ease-of-use!

Highest separation power by 2D-LC combined with outstanding performance of the 1290 Infinity LC – the ideal tool for complex samples form biological origin, polymers, food-extracs, and many more.

Innovative new hardware and software features for ease-of-use, reduced operation costs and highest performance.

Upgradability or re-use of existing Agilent LC equipment.