

## Simplify Complex Separations. Fast.

Agilent InfinityLab 2D-LC Solutions





### Solve Complex Separation Challenges – Quickly and Simply

If complex separations are impeding your lab's ability to achieve highest productivity, take a closer look at the InfinityLab 2D-LC Solutions. These innovative solutions from Agilent are universally applicable to a wide variety of challenging separations, delivering the results you need – fast and efficiently.



#### Solve complex separations

Many compounds such as isomers are notoriously difficult to separate, coeluting on traditional one-dimensional separation systems. InfinityLab 2D-LC Solutions provide an extra degree of orthogonality, which enables separation with highest resolution.



### Analyze complex samples

Accurate determination of compounds of interest is always hampered by the sheer complexity of both the sample and the matrix. InfinityLab 2D-LC Solutions achieve highest peak capacity, combining the determination of all components in a single analysis.



### Simplify complex workflows

Manual procedures make many crucial workflows unnecessarily complex. InfinityLab 2D-LC Solutions can help you to achieve better results by simplifying your workflows through elimination of manual steps or offline tasks such as sample preparation or eluent desalting.

### Agilent InfinityLab - a perfect match

**Intuitive OpenLab** 

- Easy method setup

- Flexible 2D-LC modes

- Based on OpenLab CDS

ChemStation Edition

- Straightforward data analysis

Agilent

From Insight to Outcome

2D-LC software

The Agilent InfinityLab family of instruments, columns and supplies are matched to work seamlessly together, alongside Agilent OpenLab software and Agilent CrossLab services, to provide highest efficiency in laboratory workflows.



- Reproducible performance
- Robust hardware
- Tailored valve technology
- Sensitive UV and MS detection

### **Dedicated applications and CrossLab services**

- Wide range of published applications as your starting point
- Training and services to get up to speed fast

- Diverse column portfolio for a wide range of applications
- Maximized resolution through orthogonal separations
- Perfectly matched supplies

### Flexible Choice of 2D Mode to Match Your Application

Agilent InfinityLab 2D-LC Solutions combine flexible software and switching valves to facilitate multiple 2D separation modes, including heart-cutting and multiple heart-cutting, high-resolution sampling, and comprehensive 2D-LC. Easy access to any mode allows you to match separation power to your application needs.

### Simplicity for highest analytical efficiency

Agilent 1290 Infinity II High Speed Pumps deliver flow of the highest accuracy and precision, ensuring highest speed and performance in both dimensions. Lowest delay volumes enable fastest gradients and highest 2D-gradient repeatability. Meeting the needs for precision in two-dimensional liquid chromatography, a 2D-LC valve unique to Agilent couples the first and second dimensions for easy setup and straightforward operation. Combining the 2D-LC valve with multiple heart-cutting 2D-LC valves, also unique to Agilent, provides for unmatched capabilities to achieve highest resolution.



Typical setup for comprehensive and single heart-cutting 2D-LC, showing the symmetrical flowpaths for the first and second dimensions. Expand your capacity through straightforward installation of multiple heart-cutting 2D-LC valves. An optional <sup>1</sup>D detector allows you to sample in peak-based mode and simultaneously collect <sup>1</sup>D and <sup>2</sup>D information.

### Multiple heart-cutting for confident and quantitative peak-purity determination

Multiple heart-cutting allows you to combine orthogonal separation and detection systems, providing additional information about your sample in dedicated areas of your chromatogram – for an extra level of confidence. Use high-resolution sampling for quantitative results and the analysis of broad peaks such as in biopharmaceutical applications.



 Unmatched peak storage in ready-to-use valves and sample loops

- Ideal for accurate impurity analysis or desalting before MS detection
- Optimum resolution in both dimensions
- Fully automated two-dimensional analysis of multiple peaks – available only from Agilent

Heart-cutting and multiple heart-cutting 2D-LC allow you to focus on one or more peaks for utmost confidence in your purity determinations.

### Comprehensive 2D-LC for highest peak capacity

complex sample, showing overlapping peaks. Separation of all compounds is

not possible.

Comprehensive 2D-LC (LCxLC) achieves maximum separation power with highest overall peak capacity to give you a complete overview of the entire sample and determine every component.



Comprehensive 2D-LC separation of the same sample. Highest separation power delivers the highest level of information about the sample.

- Additional separation for the entire chromatogram
- Ideal for complex samples or matrices, and unknown samples
- Ideal for sample screening or identity control

# Innovative Valve Technology and Intuitive Software for Ultimate 2D-LC Flexibility

Whatever your separation challenge, Agilent 2D-LC Solutions with innovative switching valves and intuitive software will fit your workflow. Typical applications include qualitative or quantitative analysis, quality control of known samples, research of unknown samples, workflow simplification, or MS method compatibility.



### Dedicated 2D-LC valve for highest reproducibility

- Symmetrical flowpaths ensure highest precision of retention times and areas
- Special design minimizes pressure spikes, increasing the lifetime of the second-dimension column

### Robust multiple heart-cutting valve for unmatched capacity

- Ready-to-use two-valve set has high capacity to analyze more peaks of interest
- Proven robustness for durable performance





### Novel active solvent modulation technology minimizes solvent effects

For utmost flexibility in the combination of mobile phases, the Agilent Active Solvent Modulation (ASM) valve dilutes first-dimension solvent from the sample loop with seconddimension mobile phase – thereby minimizing <sup>1</sup>D solvent effects on the <sup>2</sup>D separation through sample focusing. Increased sample focusing on the <sup>2</sup>D column yields improved resolution and sensitivity for multiple heart-cutting and highresolution sampling applications.

### Three easy steps for simple method setup

Whether you require comprehensive, heart-cutting, multiple heart-cutting, or Agilent's unique high-resolution sampling 2D-LC, Agilent 2D-LC OpenLab software makes it a fast and simple task to set up any 2D-LC method to solve the most challenging two-dimensional separations, easily.



#### Analyze your data with ease

The 2D-LC Viewer allows you to browse through your seconddimension chromatograms, revealing increased resolution and better separation for the selected areas of the first-dimension chromatogram.

- Identify compounds using UV and MS spectra
- Use quantitation workflows
- Compare and report your analysis results



View, compare, and report your analysis results with the 2D-LC Viewer.

### 2D-LC Versatility for Application Flexibility

Solve your most complex application challenges – fast and efficiently



Agilent InfinityLab 2D-LC Solutions offer the versatility you need to address a wide variety of applications. In a single run, you can use the maximum separation power for a complete overview of the entire sample, or cut out dedicated sections for further separation.

### Pharma: Impurity analysis of pharmaceuticals

The identification and quantification of impurities is one of the most important tasks in the analysis of pharmaceuticals. Targeted analysis using multiple heart-cutting 2D-LC enables determination of multiple hidden impurities at crucial places in the first-dimension separation – all in a single run.





A total of 11 heart cuts were taken from <sup>1</sup>D separation (A). Two of the cuts revealed coeluting impurities when separated in the second dimension (B).

### Biopharma: Automated desalting for mass selective detection

MS detection is highly beneficial for impurity analysis and characterization of therapeutic peptides such as glucagon. However, the USP 39 assay prescribes the use of eluent buffers that are incompatible with MS. Heart-cutting 2D-LC with automated desalting in the second dimension eliminates offline tasks and enables full characterization with MS detection in a single analysis.

Download Application Note from www.agilent.com, search for 5991-8437EN.

#### A) First dimension B) Second dimension [M+4H]4 871 5 Glucagon mAU 80 400 60 300 40 200 [M+5H]54 697.4 100 20

Chromatogram of 1D separation using phosphate buffer (A). MS spectrum obtained after desalting in the second dimension (B).

min

### Forensic Toxicology: Reducing ion suppression in the analysis of cannabinoids

The analysis of cannabinoids in biological matrices is of utmost importance in forensic toxicology but typically involves extensive sample preparation to remove proteins, lipids, and other interferences. Heart-cutting 2D-LC with triple quadrupole MS detection enables accurate quantification of cannabinoids, eliminating ion suppression and requiring minimal sample pretreatment.

600

800

900

1,000

Mass-to-charge (m/z)

Download Application Note from www.agilent.com, search for 5991-7859EN.



Chromatogram of <sup>1</sup>D separation showing 75 % suppression of hydroxyl-THC and complete suppression of THC (A). High-resolution sampling separated THC from matrix compounds in plasma and enabled reproducible measurement with 1.78 area% RSD (B).

[M+3H]34

1,161.7

1,300

### Food: Fingerprint profiling of different beer types

Fingerprint profiling is an important step in the determination of the level of bitterness and hence the classification of different beer types. Comprehensive 2D-LC achieves high peak capacity and is ideally suited for fingerprint profiling of beverage samples such as beer with highly complex compositions. 2D-LC can be complemented with quadrupole time-of-flight MS detection for compound identification.

Download Application Note from www.agilent.com, search for 5991-5521EN



Comprehensive 2D-LC chromatograms of a German pils beer (A) and an American lager beer (B). The tetrahydro-isohumulones found in the American beer are prohibited under the German Beer Purity Law (*Deutsches Reinheitsgebot*)

### Pharma: Analysis of drugs and metabolites in pharmacokinetic studies

This application of multiple heart-cutting 2D-LC replaces six one-dimensional methods and reduces overall measurement time by 75 %. Data quality is improved through automation compared to the previous workflow. Sample consumption is reduced while increasing sensitivity.



Download Application Note from www.agilent.com, search for 5991-8839EN

One-dimensional LC method showing incomplete separation and the risk of ion suppression (A). Successful separation of metabolite M1 using heart-cutting 2D-LC with MS/MS detection (B).

### **Biopharma: Characterization of monoclonal antibodies**

Characterization of biopharmaceuticals is vital to ensure their efficacy and safety. However, full characterization can be an extensive, time-consuming, and cost-intensive process. With high-resolution sampling 2D-LC, two of the most important quality attributes, aggregation and charge variant profiling, can be determined in a single analysis.

Download Application Note from www.agilent.com, search for 5991-6906EN.



Full characterization with aggregate analysis by size exclusion chromatography in the first dimension (A) followed by charge variant profiling with ion exchange chromatography in the second dimension (B).

### Analysis of synthetic oligonucleotides with online desalting

Before synthetically purified oligonucleotides can be analyzed, the sample fractions need to be desalted, which is typically performed manually using centrifugal filters. Heart-cutting 2D-LC with desalting in the first dimension enables direct analysis of synthetic oligonucleotides, saving time and improving reproducibility while avoiding losses during offline sample preparation.

Download Application Note from www.agilent.com, search for 5991-9490EN.



Direct analysis of a sample with high salt content yields a split peak (A, red trace). Good separation can be achieved but requires tedious manual desalting (A, green trace). Heart-cutting 2D-LC enables successful separation with automatic desalting in the first dimension (B).

### Applications, Training Courses, and More

We have compiled a comprehensive collection of 2D-LC applications, including examples of chemical and food analysis, lipidomics and metabolomics, herbal medicine analysis, and the combination of achiral and chiral separations. We also offer dedicated 2D-LC training courses for beginners and experienced chromatographers.

Visit our Application Finder and download the publications relevant to your field of work at www.agilent.com/en/promotions/applicationfinder

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