# Two Dimensions for Ultimate Separation Power

Agilent InfinityLab 2D-LC Solutions

Agilent InfinityLab

Agilent

Trusted Answers
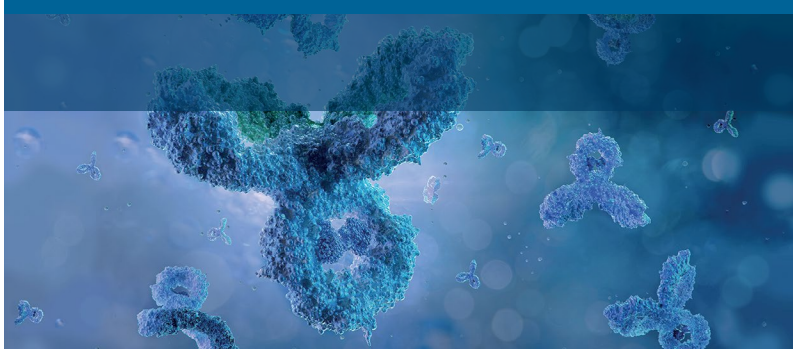
# Elevate Complex Separations with 2D-LC

Whenever an extra level of resolution and confidence is required, two-dimensional liquid chromatography (2D-LC) is the ideal solution. A wide range of applications from different industries like pharma, biopharma, chemical, energy, food, life sciences research, environmental and forensic toxicology benefit from this technology.
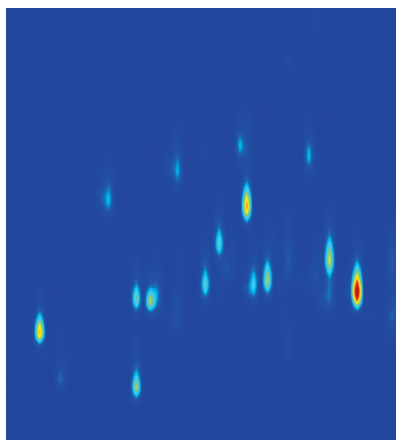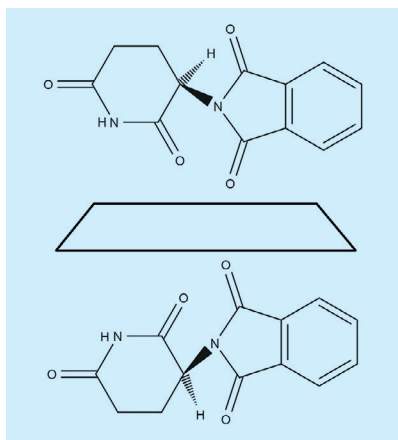






## Solve
### complex separations

Structurally similar compounds (e.g., isomers) are often difficult to separate, coeluting in conventional one-dimensional separations. By combining two orthogonal separations in one run, 2D-LC enables highest resolution.

## Analyze
### complex samples

For highly complex samples or sample matrices, 2D-LC is the optimal separation strategy. 2D-LC provides exceptionally high peak capacity, which allows you to resolve thousands of compounds in a single analysis.

## Simplify
### complex workflows

Manual procedures can make workflows needlessly complex. 2D-LC solutions achieve better results by simplifying workflows through elimination of manual steps or offline tasks such as sample preparation or desalting.

# One Solution for All Your Needs

For more than 10 years, Agilent has been a leading provider of 2D-LC technology. The Agilent InfinityLab 2D-LC Solution is based on the robust InfinityLab LC series and offers easy-to-use software. With this flexible solution, you can choose from a wide range of available Agilent LC modules. Configure your solution and expand it over time to meet your latest application requirements.

Ranked

**# 1**

## Powerful platform

Achieve ultrahigh performance for pressure ranges up to 1,300 bar and fast gradients. The InfinityLab 2D-LC Solution has been consistently cited as the best performing 2D-LC instrument since 2016, according to an LCGC user survey.

## BIO For bioanalysis and beyond

For biopharma applications and applications utilizing high salt and extreme pH conditions, use the Agilent InfinityLab Bio 2D-LC Solution. Its biocompatible solvent and sample flow path ensures full integrity of your biomolecules and keeps your system robust.

### Fast and reproducible analysis of mAbs

The Agilent InfinityLab 2D-LC ProtA-SEC Kit is designed to support titer and aggregation analysis of mAbs. Benefit from short analysis times and a fully automated, robust workflow for reproducible quantitative determination of CQAs of mAbs. Service is available for implementing this workflow.

Learn more: **agilent.com/chem/2dlc-prot-a-sec-kit**

# Unique valve technology

### Optimized 2D-LC Valve for highest reproducibility

Symmetrical flowpaths ensure highest precision of retention times and areas. This valve precisely transfers effluent to the second dimension. One valve is sufficient for comprehensive 2D-LC and single heart-cutting.

### Robust Multiple Heart-Cutting Valves for unmatched capacity

Achieve high flexibility with multiple heart-cutting and high-resolution sampling. This ready-to-use valve set offers high capacity to analyze more peaks of interest.

### ASM Valve minimizes solvent effects

Active Solvent Modulation Valve (ASM) minimizes the effect of first dimension solvent on second dimension separations through adjustable predilution, thereby increasing resolution and sensitivity.

# Multiple 2D-LC Modes in One Software to Meet Your Analysis Needs

Agilent InfinityLab 2D-LC Solutions 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.

## Heart-cutting 2D-LC 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.

Ideal to:

- Increase method development flexibility with multiple heart-cutting valves
- Analyze (multiple) selected spots in two dimensions
- Achieve high resolution in both dimensions
- Get reproducible quantitative results
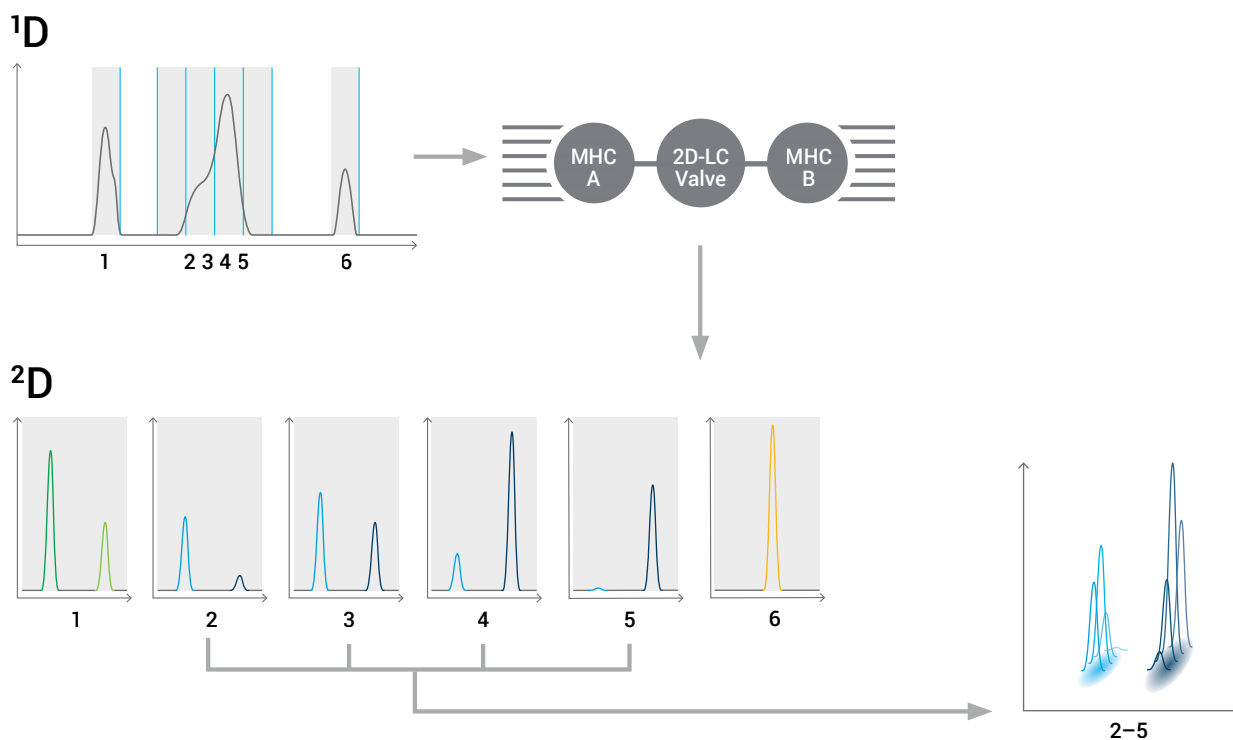
Schematic illustrating how heart-cutting 2D-LC works. Select cuts from the first dimension separation are re-analyzed in the second dimension to improve resolution.
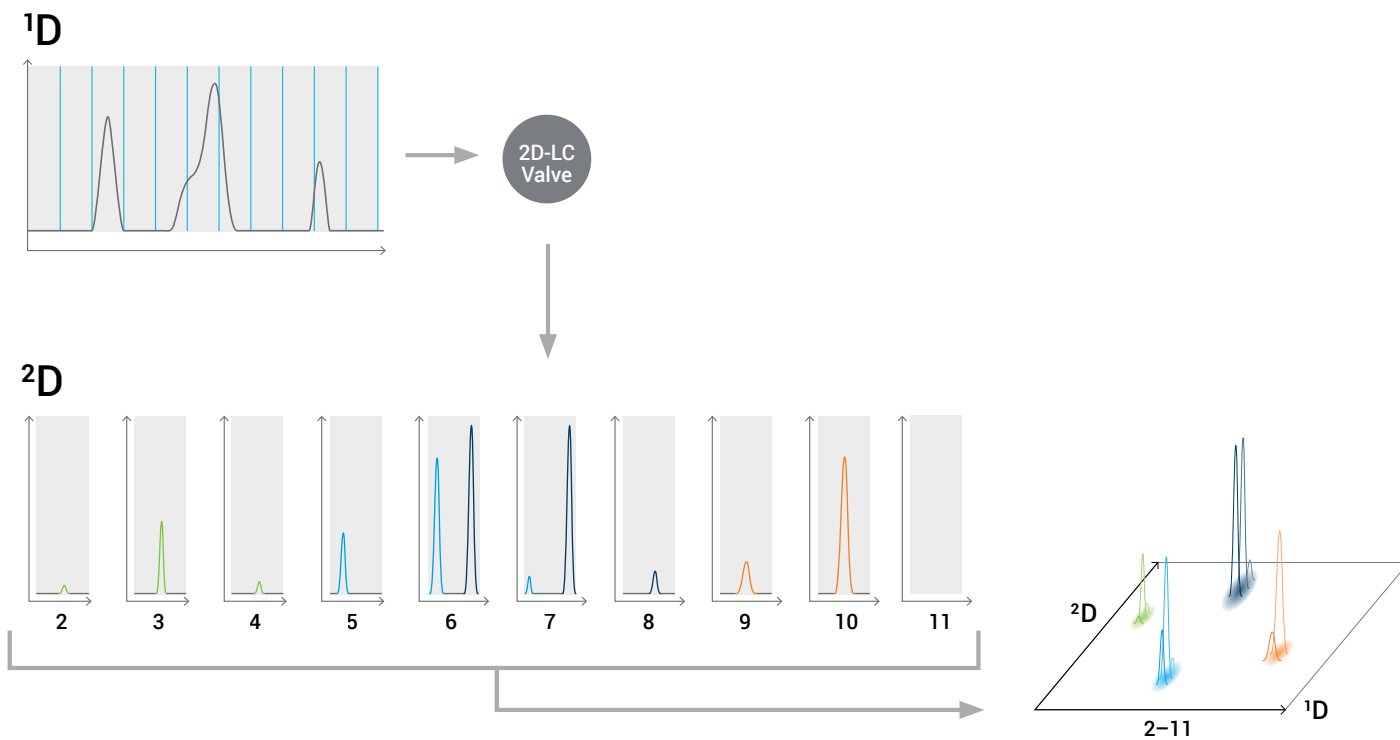
## Comprehensive 2D-LC
## for highest peak capacity

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

**Ideal to:**

– Achieve additional separation power for the entire chromatogram

– Chromatographically resolve complex samples or sample matrices and unknown samples

– Screen samples or perform identity control

**¹D**



2D-LC Valve

**²D**

| 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |

²D
2–11
¹D

Schematic illustrating how comprehensive 2D-LC works. All cuts from the first dimension separation are re-analyzed in the second dimension to improve resolution across the entire sample.

# Easy-to-Use, yet Powerful 2D-LC Software Accommodates Any Scientist or Application

Agilent 2D-LC Software offers features for new 2D-LC users and experienced users alike. New users can quickly and easily set up methods, visualize and analyze two-dimensional data, and report 2D-LC results. Experienced users can further improve 2D-LC efficiency with advanced features like multi-inject and shifted gradients.

## Meet your 2D-LC applications needs with ease

Interactively set up and develop methods and take advantage of fully automated instrument control. Get complete support for qualitative workflows, including spectral capabilities and reproducible quantitative workflows. Avoid tedious and time-intensive manual tasks, such as time table programming for method optimization. And, experience fast and powerful data analysis with Agilent 2D-LC Software.

Two software platforms are available for your detector of choice. Use either OpenLab CDS for UV and single quadrupole MS detection or MassHunter for Q-TOF and triple quadrupole detection.

However challenging your application is, be confident that Agilent 2D-LC Software is ready to help you achieve your separation goals.
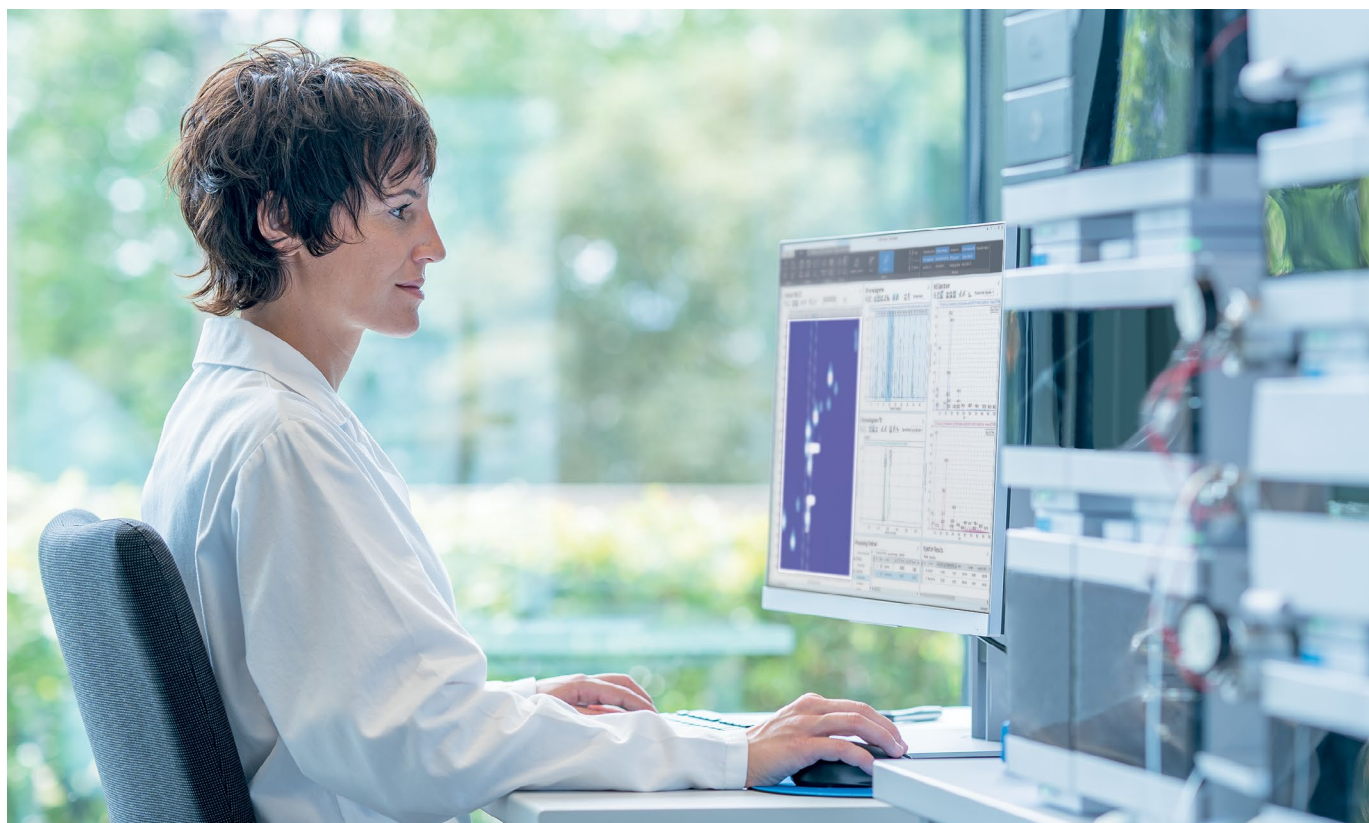
# Novice 2D-LC users can get results easily and quickly with intuitive 2D-LC software

### Quick method setup

Simply set up and develop methods. Schedule peaks for second dimension analysis with a mouse click in the 2D-LC Method Editor. The analysis of all cuts is fully automated.

### Simple, powerful data analysis

Visualize and analyze $^2$D data as chromatograms, contour plots, or numeric results, and easily navigate between $^1$D and $^2$D results. Get qualitative data including UV and MS spectra, plus reproducible quantitative results.

### Easy results reporting

Report two-dimensional results with OpenLab CDS Advanced Reporting using templates or highly customizable report items. Export and share results.

# Expert users can further optimize 2D-LC measurements with advanced software features

### Peak-based and dynamic peak parking

Handle samples with unknown or shifting first dimension retention times using an optional first dimension detector.

### Multi-inject

Use the software for flexibly setting sampling volumes without making hardware modifications. Save time by analyzing a high-resolution series at once.

### Shifted gradients

Interactively set up gradients. By shifting second dimension gradients depending on the first dimension gradient, resolution can be maximized and run time minimized.

# Achieve Even More Separation Power by Combining 2D-LC and MS Detection

2D-LC provides the ultimate chromatographic separation power. Combining the 1290 Infinity II 2D-LC System with powerful MS detection adds additional selectivity and sensitivity to resolve the most complex samples.

## 2D-LC Software for MassHunter

The integrated Agilent 2D-LC Software for MassHunter provides all software functions for 2D-LC method setup and development, as well as instrument control and monitoring.

In addition to the first and second dimension chromatographic results, the spectral information of the two-dimensional results can be analyzed with MassHunter Qualitative Analysis, MassHunter Quantitative Analysis, and BioConfirm software.

## More in-depth analyses with 2D-LC/MS

The highest LC separation power of a 2D-LC/MS system complements the high selectivity and sensitivity of MS detection like Q-TOF, TQ, or a single quadrupole detector. Many applications in pharma, biopharma, and others benefit from that unique extra level of sample information, e.g. for isomers with the same mass, complex samples, or large molecules.

2D-LC can also be used for desalting LC effluents, making buffer-based LC separations MS compatible.

# A Robust, Compliant 2D-LC Solution for QA/QC

The 1290 Infinity II 2D-LC provides the reproducible quantitative results that QA/QC labs desire. Combined with 2D-LC Software for OpenLab CDS, the solution also delivers the compliance QA/QC labs need, meeting regulated requirements such as 21 CFR Part 11.

## 2D-LC in QA/QC labs

2D-LC has been implemented and integrated successfully into many research labs throughout various industries. With the advancement of 2D-LC to a mature commercial solution, it also plays a growing role in quality control facilities. Here, 2D-LC addresses the needs for maximum sample information and optimum resolution, e.g. for coeluting compounds in impurity analysis.

Quality control laboratories require robust instrumentation that is provided by the Agilent 1290 Infinity II 2D-LC System. Such labs are also often regulated under 21 CFR Part 11. 2D-LC Software for OpenLab CDS uses the infrastructure provided by the CDS for access control, audit trails, system suitability tests, and Advanced Reporting. And, the Agilent automated compliance engine (ACE) includes dedicated protocols for qualifying Agilent 1290 Infinity II 2D-LC Systems.
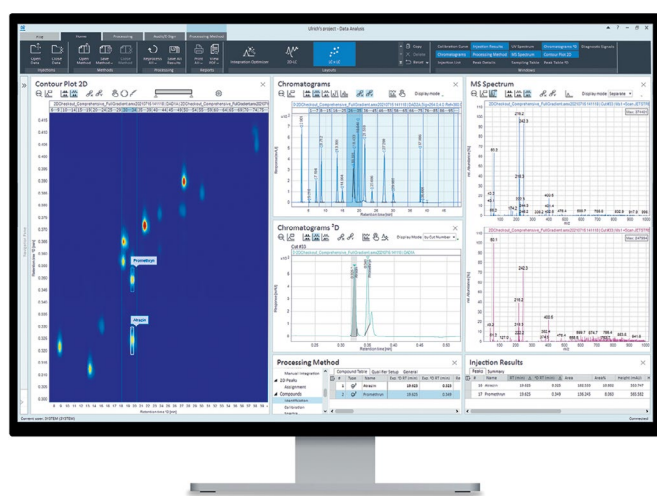


### Ranked #1

**Agilent 2D-LC instrumentation cited as most reliable**

Regular LCGC reader surveys since 2016 show Agilent 2D-LC instrumentation most frequently cited as most reliable (graph shows data from 2022 survey).

Agilent

Vendor A

Vendors B & C

# 2D-LC Versatility for Application Flexibility

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 analysis of the entire sample or cut out dedicated sections for further separation.

## Biopharma

### Integrated solution of Bio 2D-LC/Q-TOF to analyze peptide-related impurities

2D-LC/Q-TOF is a superior combination to analyze complex biopharmaceuticals. Using forced-degraded insulin as an example, new concepts of 2D-LC Software for MassHunter are demonstrated. The software allows for flexible $^1$D sampling volumes, time savings in $^2$D analysis, compensating for retention shifts of $^1$D targets, and discrimination of related isomeric impurities.

Download Technical Overview from agilent.com, search for 5994-4743EN.
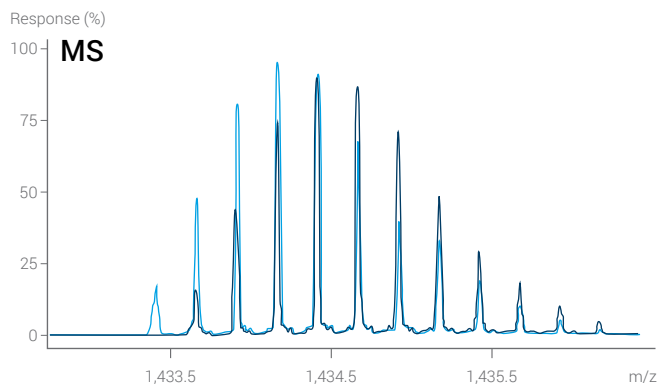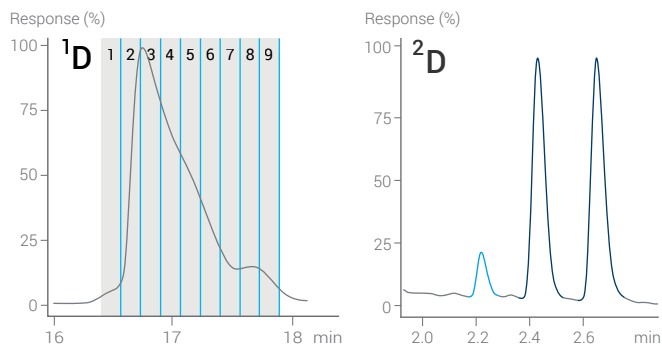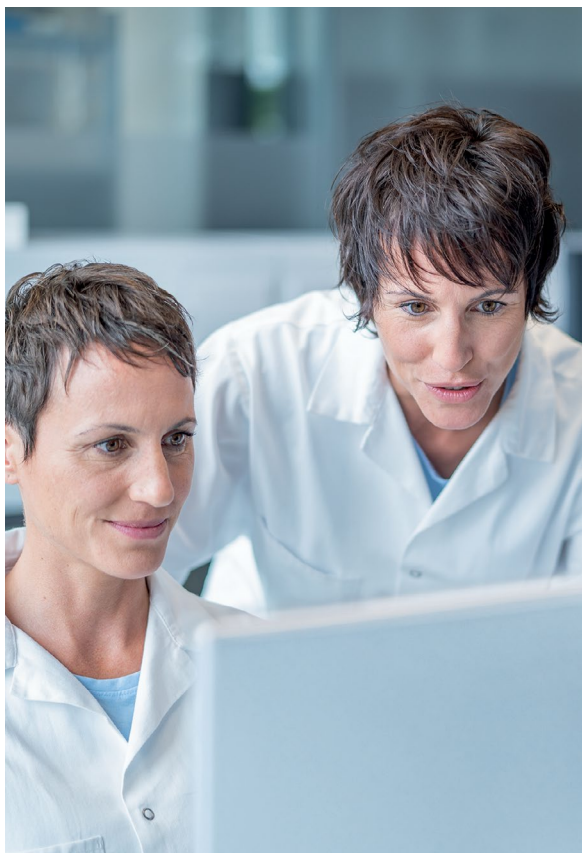


A high-resolution sampling series across a broad first dimension peak resolves three compounds in $^2$D including two isomeric impurities.
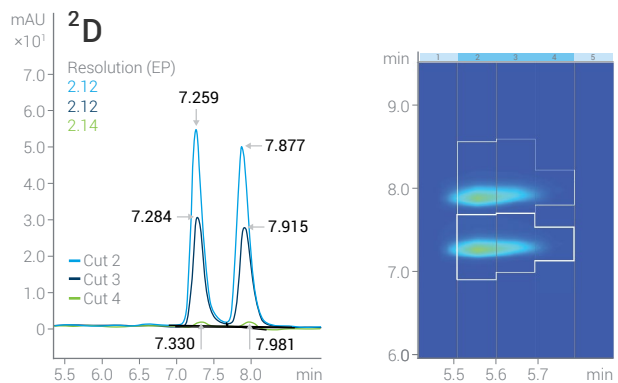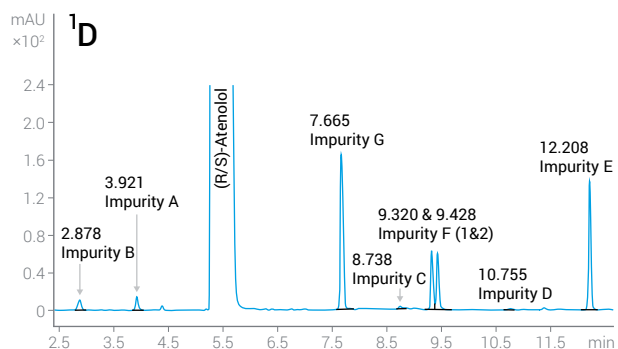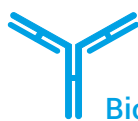
## Small Molecule Pharma

### Simultaneous impurity analysis and enantioseparation of atenolol

Enantiomeric variants of APIs can be separated with chiral columns. However, these columns often do not have sufficient selectivity for their impurities. In this application, separation from impurities, quantitation of API at or below 0.25% area RSD, and measurement of the enantiomeric excess ratio was accomplished in one run.

Download application note from agilent.com, search for 5994-4441EN.

## 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 agilent.com, search for 5991-8437EN.



Enantiomers are baseline separated in ²D chromatograms.
²D peak areas are equal for the racemic mixture (ee 0%).
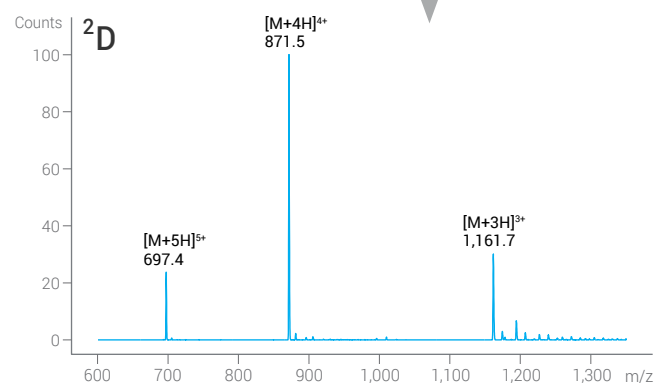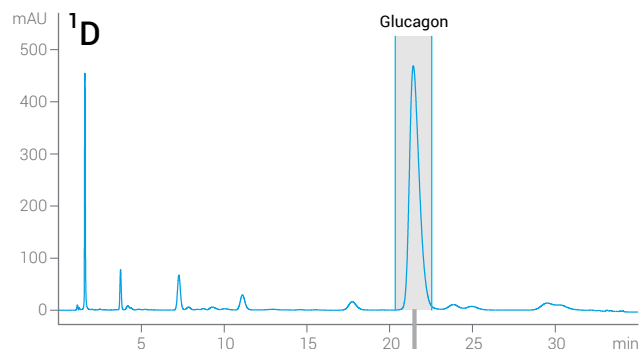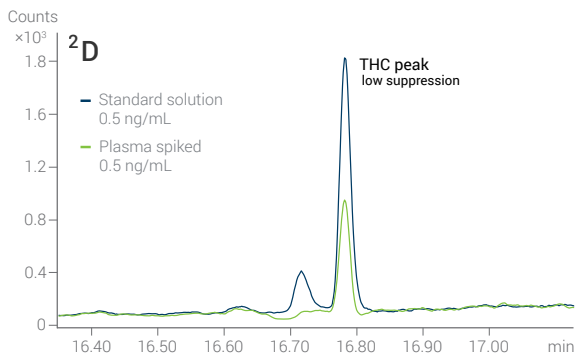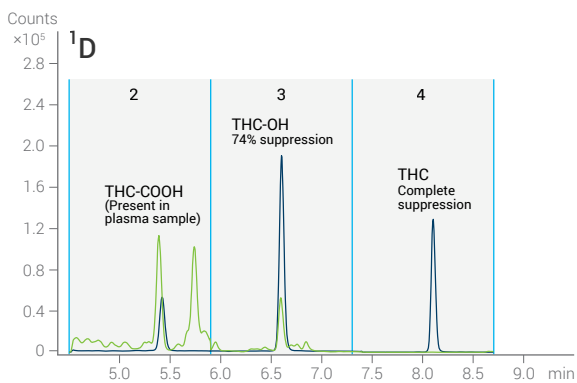


Chromatogram of ¹D separation using phosphate buffer.
MS spectrum obtained after desalting in the second dimension.

## Reducing ion suppression in the analysis of cannabinoids

In forensic toxicology, cannabinoids are frequently analyzed in biological matrices, which 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.

Download application note from agilent.com, search for 5991-7859EN.

## 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 agilent.com, search for 5991-5521EN.
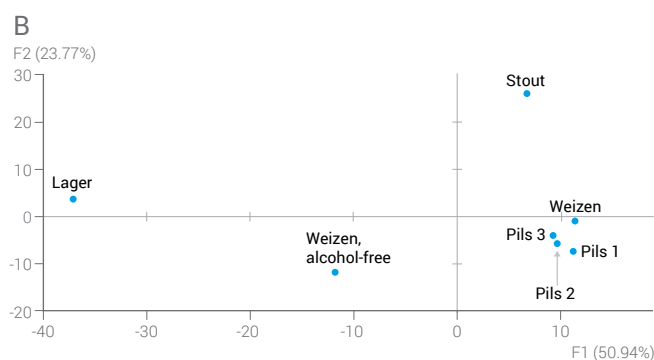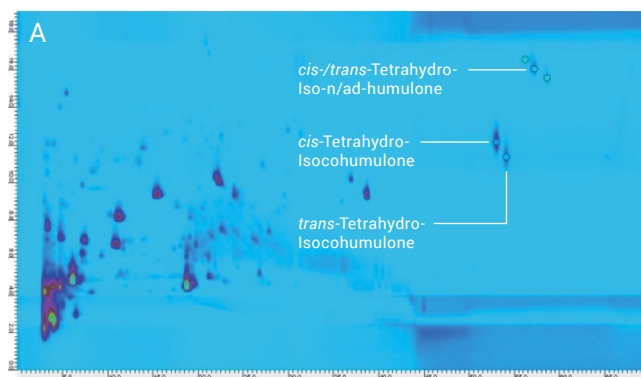




For Forensic Use.

Chromatogram of $^1$D separation showing 74% suppression of hydroxyl-THC and complete suppression of THC. High-resolution sampling separated THC from matrix compounds in plasma and enabled reproducible measurement with 1.78 area% RSD.





2D-LC analysis of an American lager beer (A). The tetrahydro-isohumulones found in the American beer (A) are prohibited under the German Beer Purity Law (Deutsches Reinheitsgebot). Principal component analysis separating the American lager beer from German beers (B).

# Services & Support for Your Lab's Success

Maximize instrument performance with Agilent CrossLab services and get the optimum outcome from your InfinityLab 2D-LC Solutions. Our industry-leading services—tailored to meet your needs—can help your lab extend uptime, produce reliable data, stay compliant, and have predictable service costs. And because a skilled team is a key driver of lab success, we also offer comprehensive learning opportunities from beginner to expert.

Learn more about CrossLab capabilities: **agilent.com/chem/crosslab-services**

### Set up your lab for success

Get installation, a short introduction, and First Run Assist for your 2D-LC system and software with a sample measurement.

### Develop, maintain, and optimize methods

Get 2D-LC Easy Start Service to learn method setup and development plus data analysis through hands-on, in-lab training.

Our application experts can offer extra assistance. For example, the InfinityLab 2D-LC ProtA-SEC Service shows how to use 2D-LC for titer and aggregation analysis of mAbs.

### Assure data integrity

Minimize your audit risk with the #1 ranked trusted compliance services provider by the regulated industry. Get full compliance with the most stringent quality standards.

### Protect your investment from the start

Choose a service plan with annual preventive maintenance for up to 24% less instrument downtime, saving you countless hours and thousands of dollars each year.

## Reliable, efficient, always innovating for your best result

You can rely on Agilent InfinityLab LC instruments, columns, and supplies to deliver rugged quality and robust analytical results. But our promise to you does not stop there. Every component of the Agilent InfinityLab family is designed to work together to help you improve your workflow, increasing efficiency and reducing operational costs.

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