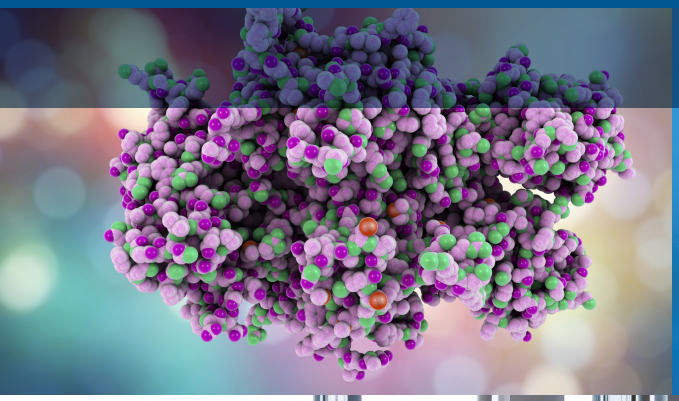
Agilent

Trusted Answers

Innovation That Drives Breakthroughs in Biopharma

Agilent 6545XT AdvanceBio LC/Q-TOF system



Ultimate Flexibility for More Informed Decisions

One instrument - Multiple workflows for biopharma

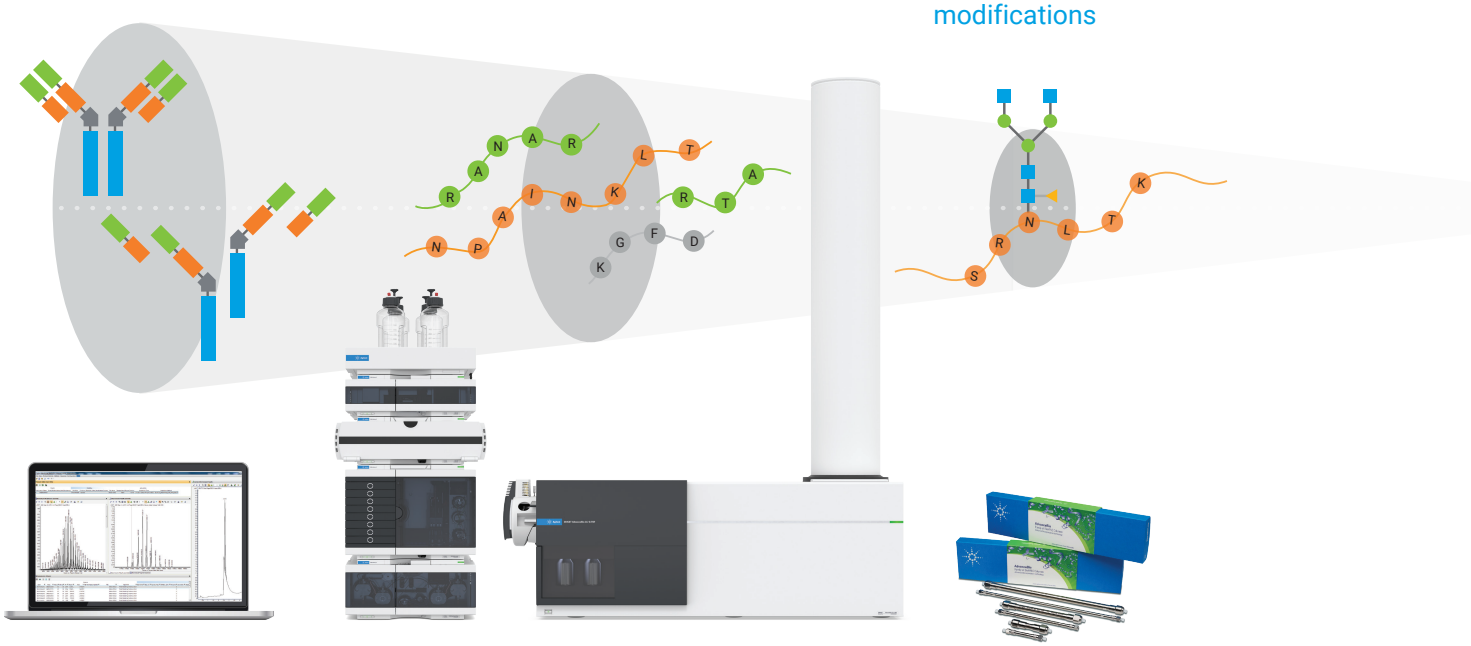
Each workflow presents unique challenges, but now you can tackle them with one instrument: the Agilent 6545XT AdvanceBio LC/Q-TOF system. In fact, Agilent has everything you need to prepare, separate, detect, and analyze biomolecules—with dedicated reports to share your results.

Full characterization means you need a workflow to analyze your protein at every level. Every bit of information is critical to push your project forward. Whether you are analyzing a single sample or freezer full of plates, the 6545XT provides methods for each level of analysis to get you up and running fast.

Intact protein analysis

Peptide mapping

Posttranslational modifications



Characterizing biotherapeutics requires multiple approaches to access multiple levels of information:

- Intact proteins (native or denatured)
- Antibody-drug ratio (ADR)
- Subunit analysis of mAbs
- Peptide mapping
- Posttranslational modifications
- Host cell protein (HCP) analysis
- Released glycans
- Therapeutic peptides
- Oligonucleotide analysis
- Top-down, middle-down, and bottom-up characterization with electron capture dissociation (ECD)

Powerful solution for comprehensive biomolecule analysis

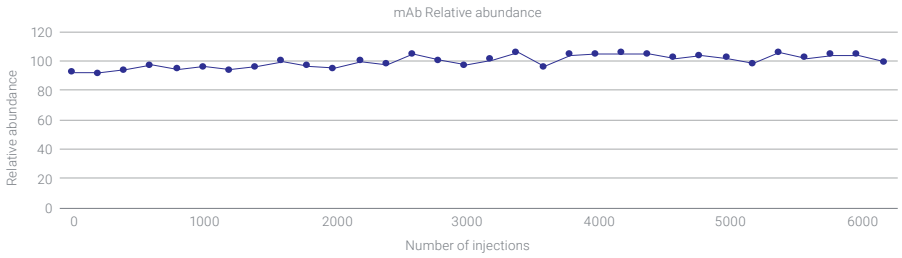
- Excellent protein spectral clarity from ultrahigh TOF vacuum 10^{-8} Torr
- One-click optimization of large molecule detection using Agilent SWARM autotune
- Capable of analyzing very large molecules, with a variable mass range up to *m/z* 30,000
- Ease of maintenance with vent-free capillary removal

Enhance your peptide and protein analyses with the **Agilent ExD cell**

- Performs ECD for peptides and proteins
- Next-generation design featuring improved efficiency and simplified operation
- Interpret the rich spectra with confidence using ExDViewer analysis software

Robust and reproducible

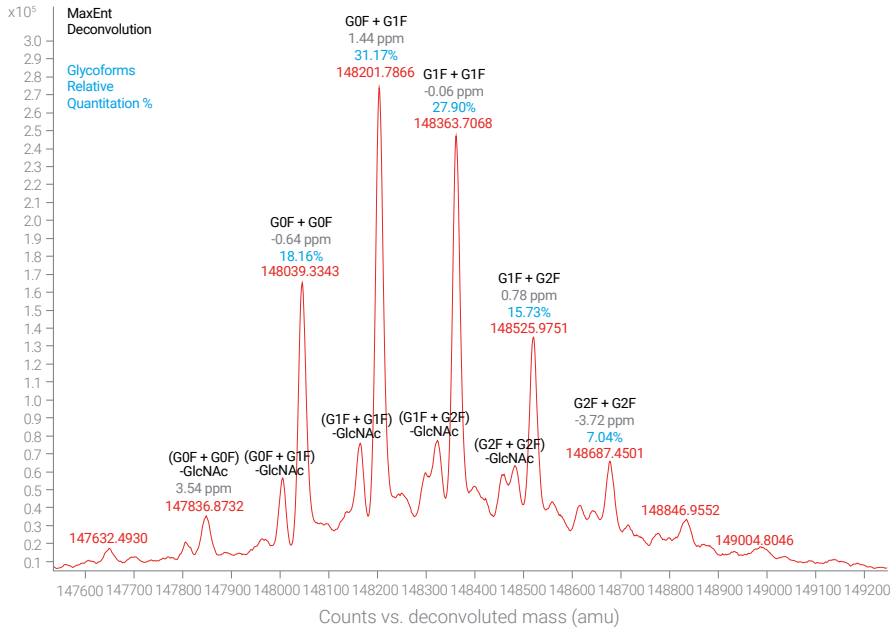
Every sample you have to re-analyze, every minute you have to spend maintaining your analytical equipment laboratory, is time you aren't moving forward on your lab's goals. With the 6545XT Q-TOF LC/MS, you can be sure that your instrument is going to be ready for your samples, and the data you report is accurate day in and day out.



Over 6,000 replicate injections of 1 µg trastuzumab showed no degradation in response.

Intact protein analysis

Designed specifically for large molecule analysis, the 6545XT LC/Q-TOF gives you accurate mass measurements down to low ppm levels. Excellent mass sensitivity and data processing that preserves fine detail allows you to detect and monitor low-level isoforms at the intact level. Need to analyze noncovalent protein complexes? The 6545XT LC/Q-TOF has a mass range that extends up to *m/z* 30,000. Optimizing your system for intact proteins is easy with SWARM autotune.



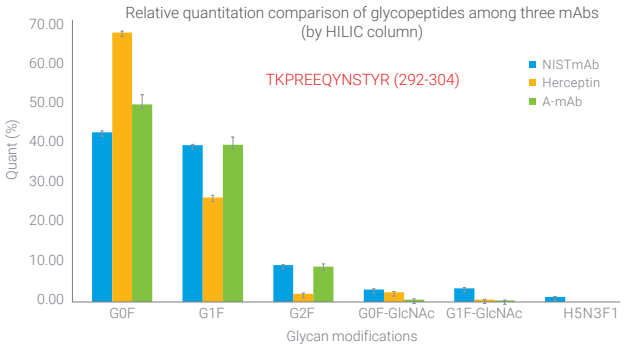
Agilent MassHunter BioConfirm: Full display of analysis details at a glance

Easy execution of workflows and full display of both overview and details make **Agilent MassHunter BioConfirm software** the ideal analysis software for biotherapeutics. Particularly for peptide mapping, where sequence information of peptides as well as relative abundances for modifications need to be displayed.

Whether you are performing routine sequence confirmation, or are faced with the challenge of hard-to-detect peptides, the 6545XT LC/Q-TOF along with Agilent MassHunter software, is ready to help. With Agilent MassHunter BioConfirm, you can quickly confirm sequence coverage, and with Agilent MassHunter Walkup, users of any experience level can get LC/MS protein sequence data. Useful acquisition tools such as iterative MS/MS and automatic sequence matching can help you pull out low-level peptides and improve identification for hard-to-detect peptides. Every molecule presents different challenges. The 6545XT gives you the flexibility to fit your approach to each challenge with the same set of software tools.

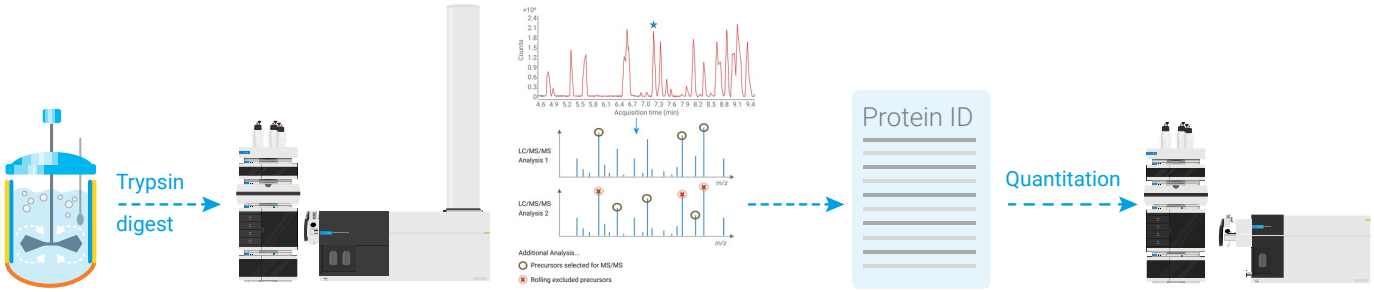
Posttranslational modifications

To fully understand the precise composition of your protein, you need to see more than just the backbone structure. You need to know what modifications and variations are occurring during expression. Whether that means characterizing levels of oxidation, localizing deamidation sites, or profiling glycan structures, the 6545XT LC/Q-TOF was designed to help. Autotune unlocks the full potential of the system by flexibly optimizing for large proteins or small labile structures such as glycans.



In-depth host-cell protein (HCP) analysis

Host-cell protein analysis is one of the most challenging analysis methods, as low levels of peptides from the expression system need to be detected next to high abundant peptides from the enriched and purified mAb protein after digestion. For this, a high in-spectra dynamic range is an ultimate requirement. The 6545XT Q-TOF LC/MS has proven to be an ideal system for this analysis. For lowest level identification, iterative MS/MS is used, where after each run all selected precursors will be excluded in the subsequent run, leading to lower levels of analyte analysis for each additional run. Once host cell proteins are identified, they can either be monitored by the 6545XT LC/Q-TOF, or transferred to an Agilent triple quadrupole LC/MS for targeted monitoring.



A schematic view of the HCP-AIMS workflow.

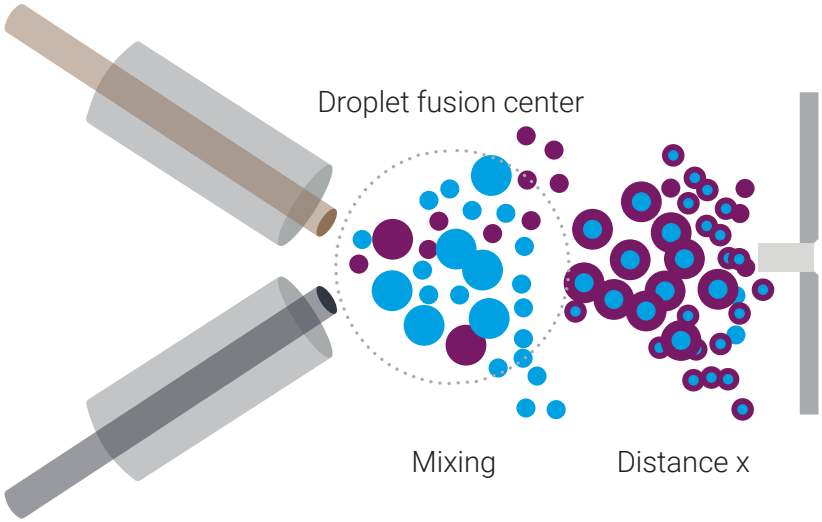
Huang, Y.; Molden, R.; Hu, M.; Qiu, H.; Li, N. Toward unbiased identification and comparative quantification of host cell protein impurities by automated iterative LC–MS/MS (HCP-AIMS) for therapeutic protein development. *JPBA.* **2021**, 200, 114069.

Sook, Y.E.; Hu, Y.; Molden, R.; Qiu, H.; Li, N. Identification and quantification of a problematic host cell protein to support therapeutic protein development. *J. Pharm. Sci.* **2023**, 112(3), 673-679.

Flash characterization for high-throughput analysis

Traditionally, antibody characterization with IdeS enzyme digestion, reduction, and enzymatic deglycosylation require extended incubation time (minimum 30 minutes) in bulk solution. Recently, attention has been drawn to the use of microdroplet reactions for antibody analysis.[1] The microdroplet reactions are attractive due to the rapid reaction rate (microseconds) and high reaction yield achieved in the ESI spray chamber. In addition to the time savings, the cost of analysis is dramatically lowered due to the reduction in enzyme and antibody consumption for characterization.

The Agilent unique ion source design, using dual sprayers, offers the ability to perform microdroplet reactions by simply using the reference nebulizer as introduction sprayer for the reactants, and the analytical sprayer for mAb introduction.



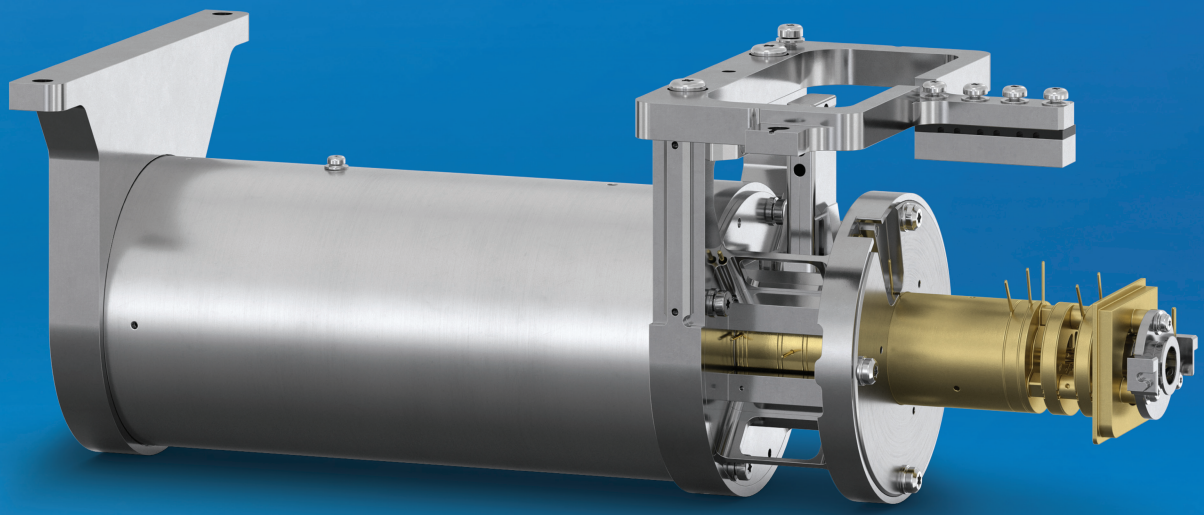
Under normal reference sprayer conditions where reference masses are introduced at 6 psi, no interaction between reference ions and ions introduced by the analytical sprayer occur, but for microdroplet reactions a pressure of 60 psi is applied.

Gunawardena, H.P.; Ai, Y.; Gao, J.; Zare, R.N.; Chen, H. Rapid characterization of antibodies via automated flow injection coupled with online microdroplet reactions and native-pH mass spectrometry. *Anal. Chem.* **2023**, 95(6), 3340-3348.

Flash Characterization of Antibodies via Microdroplet Reactions in an Unmodified Jet Stream Source. *Agilent Technologies application note*, publication number 5994-6752EN, **2023**.

Gain deeper insights with the Agilent ExD cell

The Agilent ExD cell is a field-installable add-on to the 6545XT Q-TOF LC/MS which enables electron capture dissociation (ECD) as an alternative to collision induced dissociation (CID) for enhancing the speed and depth of peptide and protein characterization.



Key features

Make faster, more-informed decisions

Complement CID data with ECD to characterize fragile modifications and amino acid isomers, and perform top-down protein characterization.

High ECD efficiency over-comes constraints

High ECD efficiency means greater applicability for lower-charge ($>2^+$) peptides and less time spent fine-tuning in the pursuit of optimal performance.

Flexible fragmentation

ECD and CID are independently controlled and can be employed separately or together.

Removing barriers to entry

The simple design of the ExD cell brings the advantages and possibilities of ECD within reach, bridging the gap between specialized high-end instrumentation and more widely available LC/MS technology.

Perform top- and middle-down analysis. Save time, minimize chemical artifacts, and simplify data analysis compared to workflows relying on enzymatic digestion and peptide mapping.

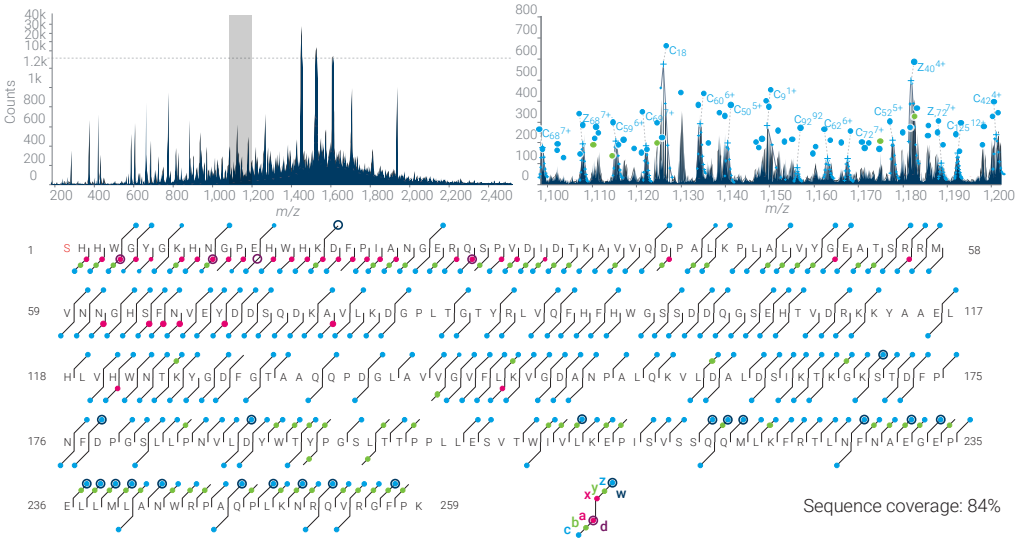


Fig 1. Top-down ECD of 29 kDa carbonic anhydrase, infused. Left: The 20^+ precursor (*m/z* 1452.2) was isolated and subjected to ECD, yielding a rich fragment ion spectrum. Right: There are over 100 fragment ions in a *m/z* 100 window of the spectrum, but the signal-to-noise and isotope ratios are robust enough for the Agilent ExDViewer software to match with high confidence. Bottom: Amino acid sequence coverage from top-down ECD was 84%. The entire experiment took less than 10 minutes.

Map and characterize fragile modifications. Preserve fragile modifications while generating sequence-informative fragment ions.

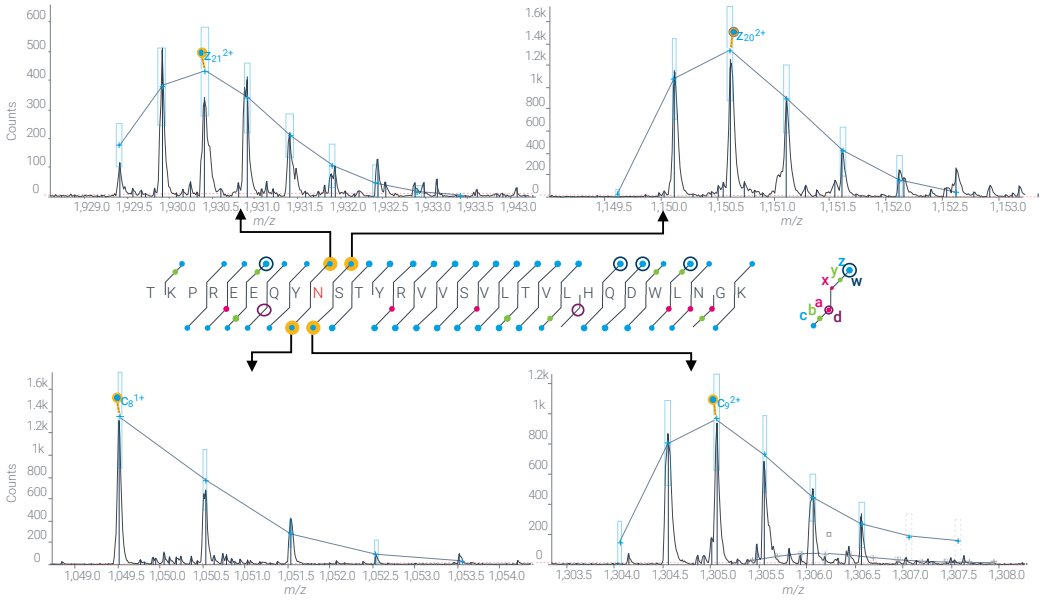


Fig 2. Select ECD fragment ions from NISTmAb tryptic glycopeptide, 5^+ precursor (*m/z* 982.2). ECD fragment ions z_{21}^{2+}, z_{20}^{2+}, c_8^{1+}, and c_9^{2+} flank the glycan modification site to confirm its location and identity as G0F, without fragmenting the fragile modification itself.

Differentiate isobars and isomers. Fragment amino acid side chains differentiate isobars such as aspartate/isoaspartate and leucine/isoleucine.

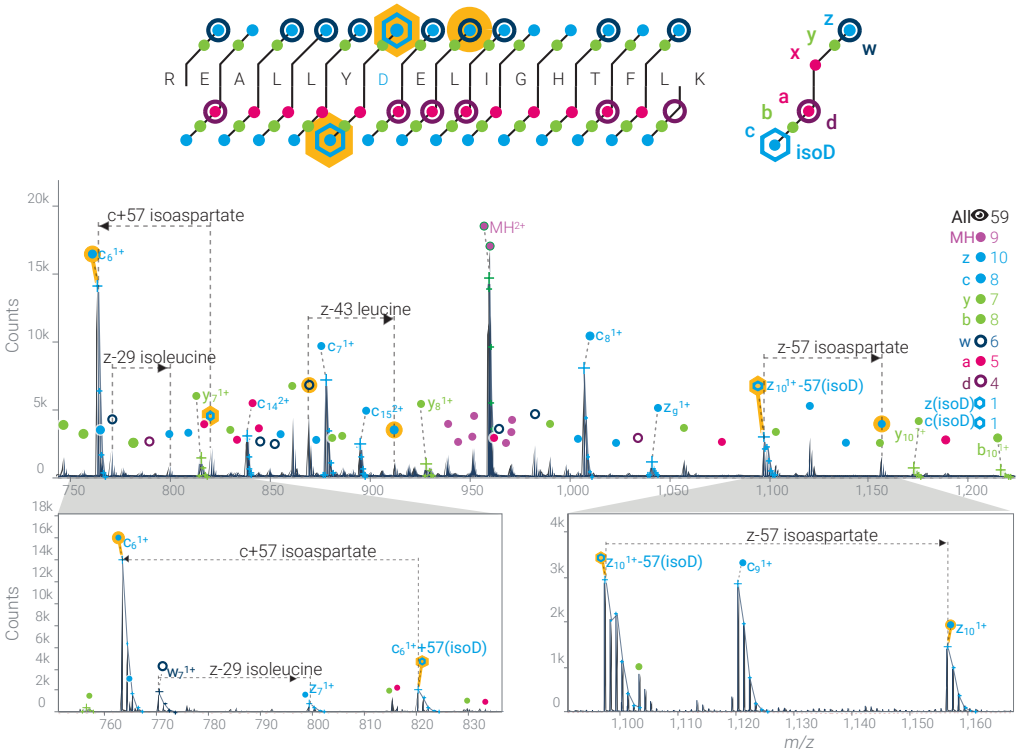


Fig 3. Top: A peptide with sequence REALLYDELIGHTFLK has one I and four L residues, such that 32 different isomers could produce the same CID spectrum. Aspartate isomerization expands the search space to 64 isomeric peptides. Middle: ECD produces side chain fragments (dark blue rings) that directly differentiate I from L residues. Bottom: ECD also produces fragment ions (light blue hexagons) that can distinguish isoaspartate from aspartate residues.

Generate complementary structural information. High-efficiency ECD even for low-charge peptides yields fragment ions complementary to those generated via CID, especially useful for de novo peptide sequencing and modification characterization.

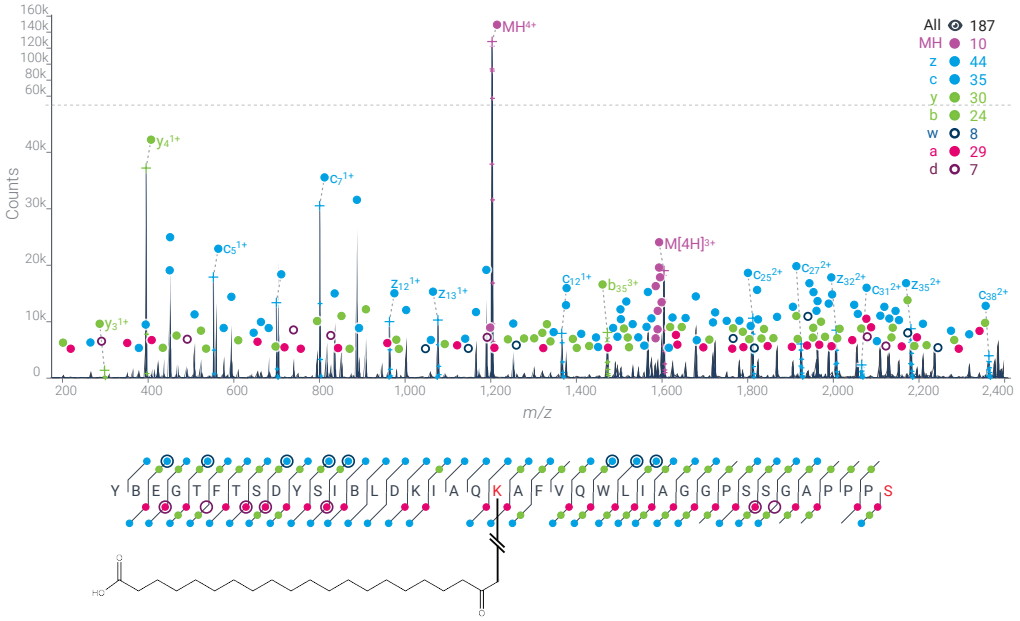


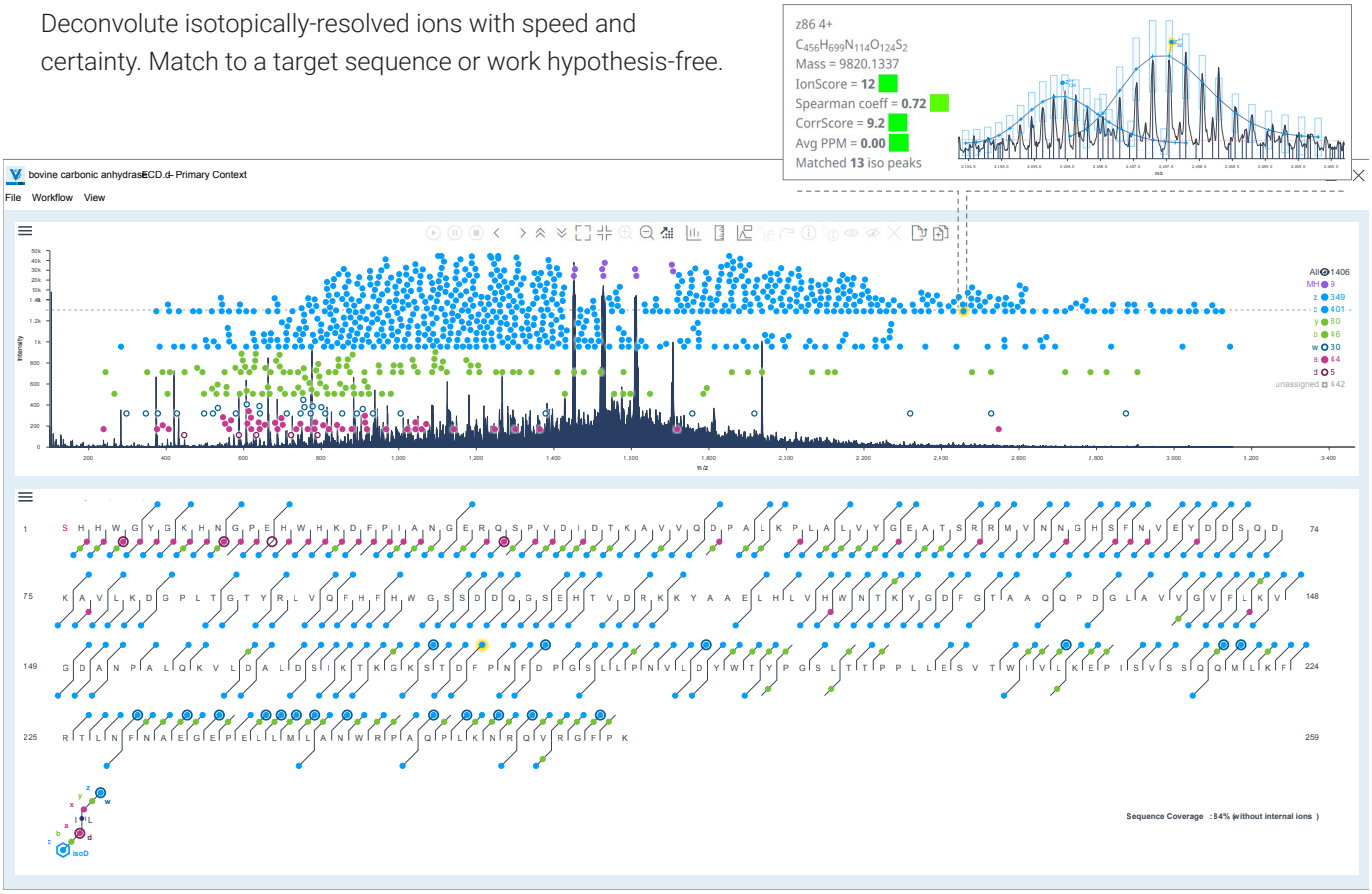
Fig 4. Tirzepatide ECD spectrum. ECD fragment ion intensities with signal-to-noise ratios over 1000 are observed after performing ECD on the isolated 4+ precursor of the synthetic peptide tirzepatide, which features a 20C fatty acid chain attached via linker to K20. ECD ions complement CID ions to confirm the sequence and modification.

Accelerate top-down analysis with ExDViewer

Agilent ExDViewer analysis software allows you to interpret information-rich top-down MS/MS spectra quickly, accurately, and intuitively, improving confidence in conclusions and the ease with which they are communicated.

– Simplify spectra with rapid deconvolution

Deconvolute isotopically-resolved ions with speed and certainty. Match to a target sequence or work hypothesis-free.



Explore interactive visualizations to speed up validation of ion assignments. Here, the z86 4⁺ ion of bovine carbonic anhydrase is highlighted in the spectrum window and the sequence coverage map. Customize the display to generate publication-quality figures.

– Interpret ECD, CID, and more

Assign protein backbone and satellite ion types — b, y, c, z, a, x, d, w.

– Identify structure from spectra

Define protein sequences, fixed and variable modifications, and amino acid building blocks. Look for evidence of variants with variable modification search.

– Collaborate with ease

Sharing impactful results has never been faster. Send your labmates a URL link to view your results.

Get started now

Entry barriers to analysis can prevent labs from realizing the advantages of top- and middle-down mass spectrometry for protein characterization. Get free access to ExDViewer today.
Visit **exdviewer.agilent.com**

End-to-end workflow solutions for synthetic oligonucleotide characterization

– Oligonucleotide Target Plus Impurities (TPI) analysis

Advanced analytical methods, such as LC/MS analysis, are indispensable for the characterization of target oligonucleotides and their impurities. Advanced techniques are needed because impurities are often numerous, present at very low abundances, and found in combination with one another. Because characterization of impurities can be challenging, software that supports and automates impurity profiling is valuable.

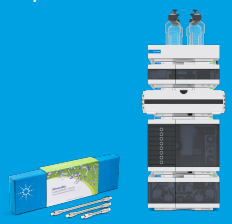
Sample preparation

Purification cartridge

TOP-DNA/TOP-RNA cartridges

TOP-DNA plates

Separate

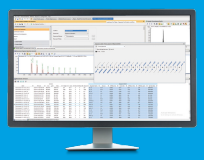


1290 Infinity II Bio LC system and AdvanceBio oligonucleotide columns

Detect

6545XT AdvanceBio LC/Q-TOF

Analyze



MassHunter BioConfirm

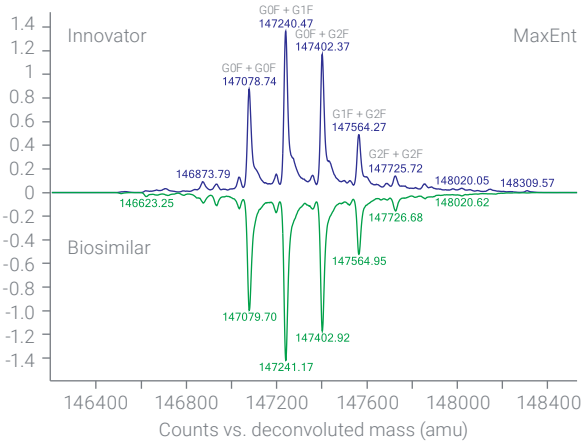
– Oligonucleotide sequence confirmation

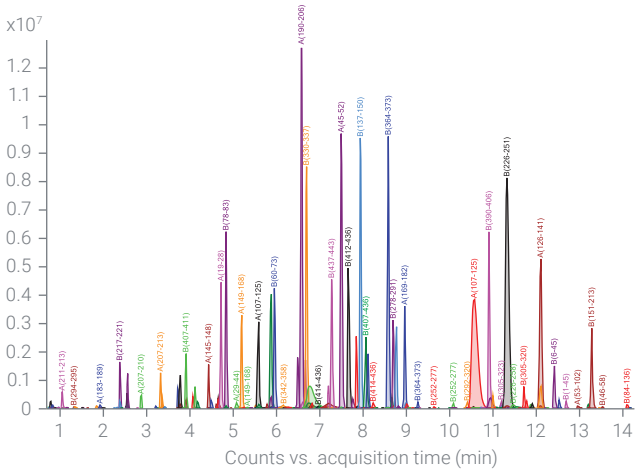
The sequence confirmation workflow uses an Agilent 1290 Infinity II Bio LC system coupled to the Agilent 6545XT AdvanceBio LC/Q-TOF. The workflow uses fragment confirmation at the MS2 level by matching isotope patterns against expectations that are calculated from the oligo sequence. This matching capability is a new feature of the BioConfirm software. It demonstrates the power of coupling a high-resolution accurate mass system with targeted MS/MS data. In this approach, oligonucleotides are structurally characterized by confirming heavily modified sequences and determining the positions of specific chemical groups.

BioHPLC columns

Agilent AdvanceBio LC columns are designed and produced to provide results you can be sure of when analyzing highly complex biotherapeutic molecules and monitoring their purity, potency, and other critical quality attributes. The columns provide the flexibility to use mobile phases compatible with mass spectrometry as well as other detection platforms.

Learn more about **biocolumns and bioconsumables for biopharma HPLC analysis**, along with associated tools for column selection and related resources.



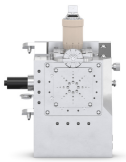


Reverse phase columns for biomolecule separations.

Ion sources for biopharma

Jet Stream Technology Ion source

Delivers highest sensitivity across a variety of sample types



Nanospray source

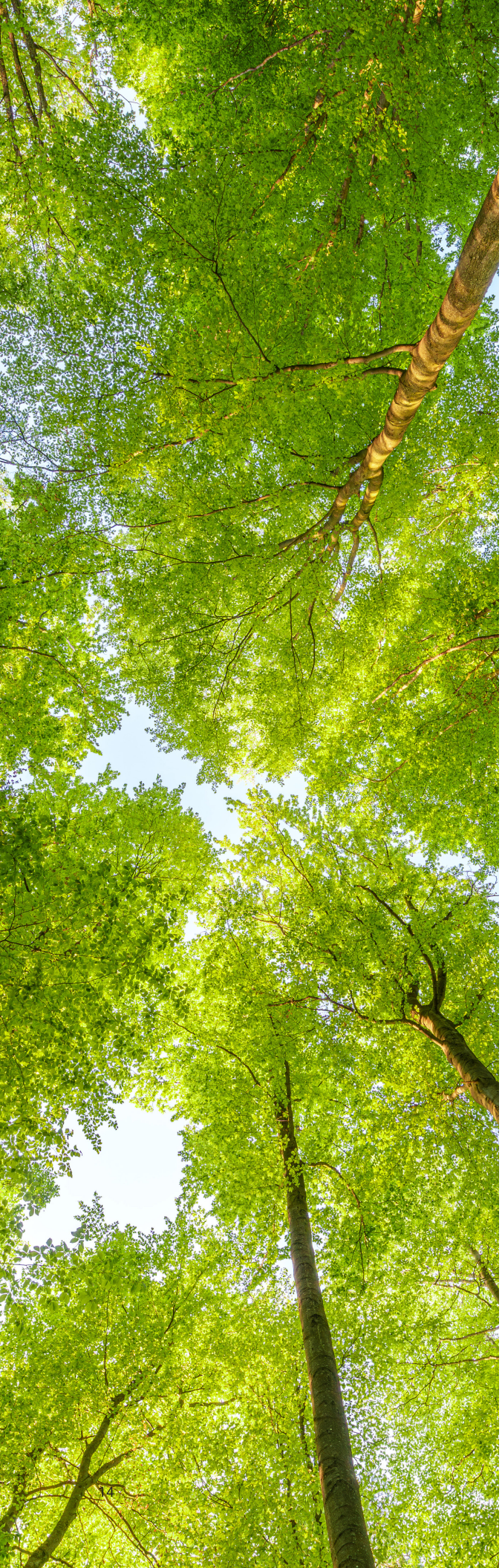
Offers the utmost sensitivity for small sample quantities analyzed in low-flow LC/MS applications

Electrospray source

Delivers excellent robustness and reliability for large and small polar compounds

Capillary Electrospray source

Interface for capillary separation techniques with mass spectrometry



Partnering for sustainability and business success

Sustainable thinking is transforming the way researchers, scientists, and manufacturers approach their products, processes, and supply chains. However, it can be a challenge for labs to lower their environmental impact while continuing to optimize workflows and lower costs.

At Agilent, we believe that efficiency, productivity, and sustainability are interlinked.

Working toward sustainability is an integral part of how we conduct business and respond to our customers' challenges. Together, we can help your lab achieve its sustainability goals-while increasing output, maintaining accuracy, and staying competitive.

my green lab.

Partnership with My Green Lab

Agilent has partnered with My Green Lab to have our instruments independently audited for their Accountability, Consistency, and Transparency (ACT) label. ACT labels provide information about the environmental impact of manufacturing, use, and disposal of a product and its packaging, so purchasers can make informed, sustainable choices. The Agilent 6545XT AdvanceBio LC/Q-TOF – along with Agilent 6230 time-of-flight (TOF) LC/MS, Agilent 6546 LC/Q-TOF and Agilent Revident LC/Q-TOF – have been extensively evaluated and have earned the ACT label.

Learn more about My Green Lab

The Agilent net-zero committment

Since our founding, Agilent has worked to reduce our energy, waste, water, and CO_2 emissions. Now we're taking it a step further. We're proud to announce that we will achieve net-zero greenhouse gas emissions by 2050. Our comprehensive approach to net zero includes Paris Agreement climate targets, clearly defined interim goals, and a commitment to the Science-Based Targets Initiative.

Read more

Comprehensive solutions

Automated protein sample preparation

With the Agilent AssayMAP Bravo automated liquid handling platform, you are just one click away from your protein sample preparation with workflows that include:

- Affinity purification
- Enzymatic digestion
- Reversed-phase cleanup
- Phosphopeptide enrichment
- Peptide fractionation

Agilent AssayMAP Bravo platform

Agilent 1290 Infinity II Bio LC system

The **1290 Infinity II Bio LC system** is a UHPLC, binary or quaternary, consisting of biocompatible material for use in biopharma and other applications utilizing high salt and extreme pH conditions. Biocompatibility ensures the integrity of your biomolecules and robustness of the system. The 1290 Infinity II Bio LC System provides the highest resolution and lowest dispersion at pressures up to 1300 bar for your biochromatography.

Agilent 1290 Infinity II Bio LC system

Maximize your LC/MS efficiency with Agilent InfinityLab

Agilent InfinityLab LC instruments, columns, and supplies are designed to work together to provide efficiency gains that help you get more done and reduce operation costs.

InfinityLab LC instruments

From routine analysis to cutting-edge research, the Agilent InfinityLab LC series offers a choice of HPLC and UHPLC systems to suit your application and budget.

InfinityLab LC columns

With three particle sizes and 20 chemistries, Agilent InfinityLab Poroshell 120 LC columns provide a range of selectivity, making your method development fast and easy.

InfinityLab LC supplies

The small parts of your workflow can make a big difference in the quality of your results. Agilent InfinityLab supplies are designed to improve the efficiency of daily tasks.

Agilent CrossLab

From Insight to Outcome

Supporting your success

CrossLab is an Agilent capability that integrates services and consumables to support workflow success, improve productivity, and enhance operational efficiency. In every interaction, we strive to provide insight that helps you achieve your goals.

Learn more about CrossLab at **www.agilent.com/crosslab**

Want to minimize errors and improve consistency between analysts?

Agilent University offers flexible, cost-effective training options to help you plan, prioritize, and manage lab resources. Your team will also gain insights into boosting efficiency and minimizing downtime. Plus, you can choose the training format that suits you best—including in person, virtual, and online.

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