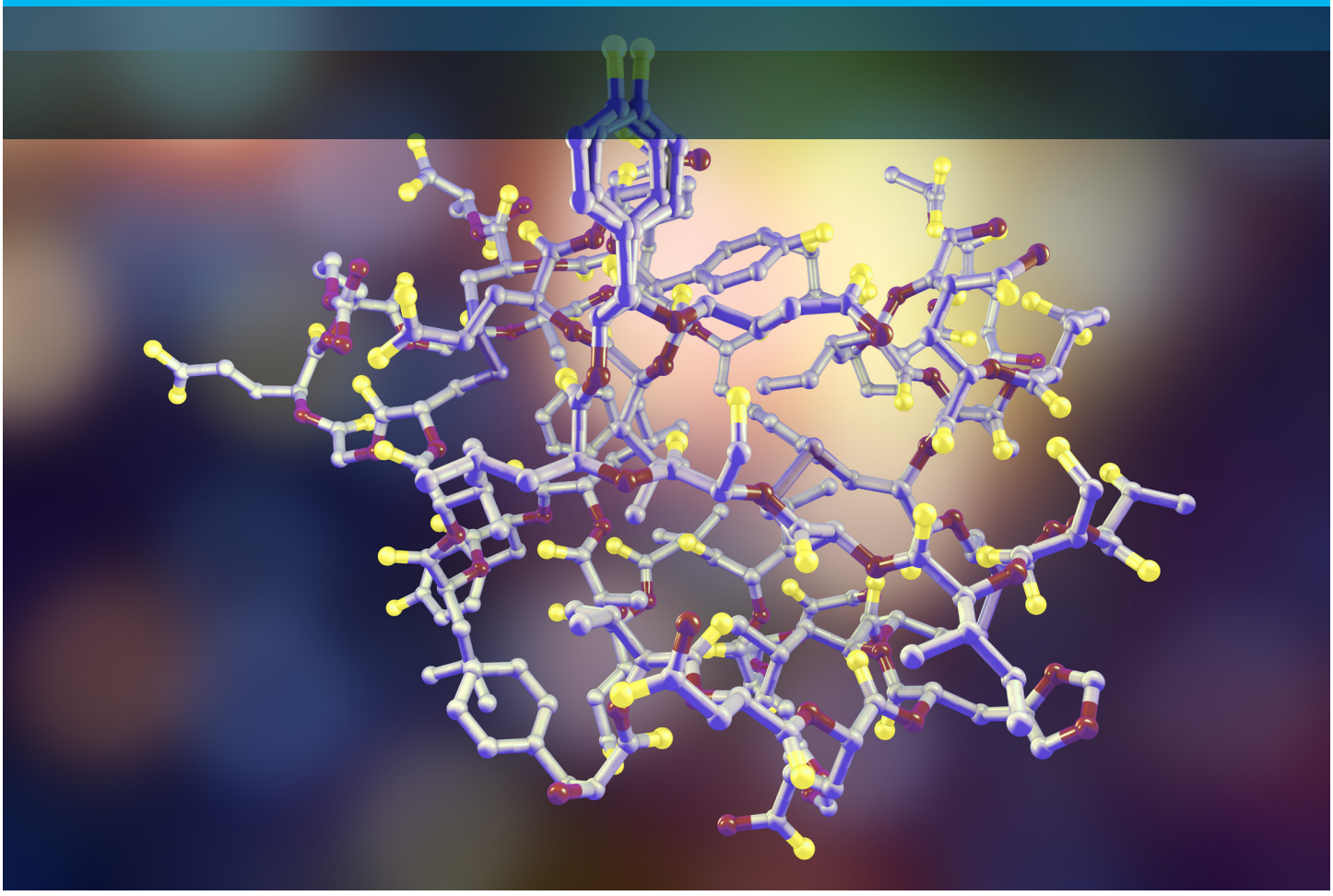
Agilent InfinityLab

Workflow Solutions for Peptide Therapeutics

Application Compendium



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Trusted Answers

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Introduction

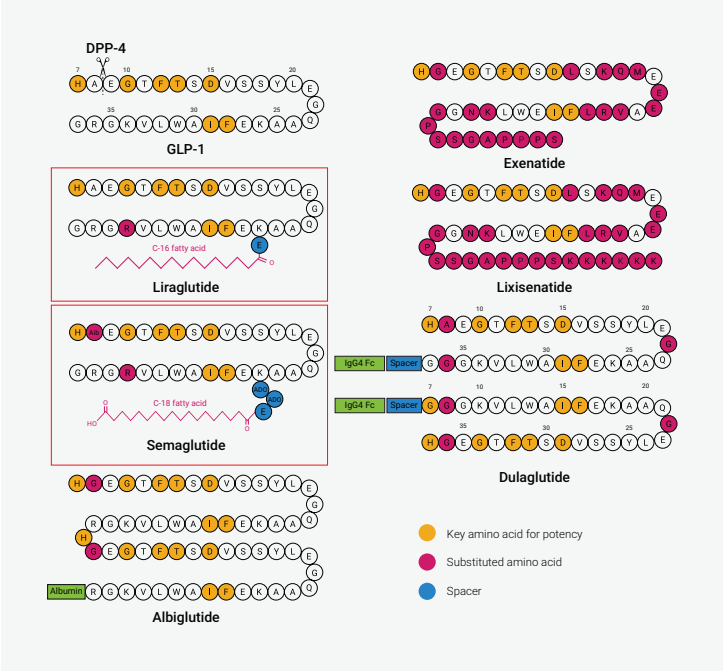
Peptides are short chains of amino acids, known as the “building blocks” of proteins, and play a critical role in various important biological processes like cell signaling, immune response, and hormone regulation. As a result, they are being increasingly developed as therapeutics against a wide range of disease conditions, due to their favorable safety, efficacy, and low toxicity, and their ability to specifically target certain cells or receptors. In recent years, research has significantly advanced the design and formulation of peptide therapeutics to overcome drug delivery limitations.

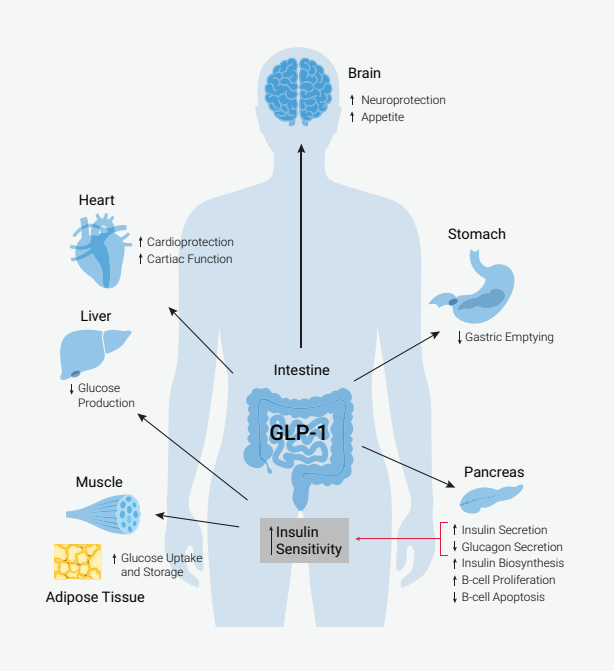
Agilent offers several solutions for the analysis and development of peptide therapeutics, from purification and purity/impurity analysis to characterization and identity and structure analysis. This application compendium presents several practical solutions to elevate your analysis and development of peptide therapeutics.

An emerging class of peptide therapeutics: GLP-1

A popular emerging class of peptide therapeutics are glucagon-like peptide-1 (GLP-1) agonists, which are approved by the U.S. FDA for the treatment of type 2 diabetes and obesity management.

The main action of GLP-1 occurs at the pancreas, where GLP-1 stimulates insulin secretion and inhibits glucagon secretion in a glucose-dependent manner. The following diagram[1] indicates the types of GLP-1 drugs (left) and the biological activities of GLP-1 (right).





Analytical advances in peptide therapeutics

Despite the differences in production methods for synthetic and recombinant peptides, the techniques for analytical characterization of these products are largely similar. However, the types of impurities that can arise during production (known as process-related impurities) can differ—and this distinction is crucial for ensuring product quality and safety.

With the rise of generic peptide drugs, regulatory guidance has become stricter to ensure that these products align with the efficacy and safety of Reference Listed Drugs. The enforcement of these quality standards has opened the opportunity for more advanced analytical tools, with new possibilities for innovation in peptide therapeutic development.

Key analyses for synthetic and recombinant peptide therapeutics

To ensure the quality, safety, and efficacy of peptide therapeutics, a comprehensive set of analytical tests is required, spanning identity, purity, structural integrity, and safety attributes:

- **Identification assays:** RP-LC-UV, LC/MS, or cell-based bioassays
- **Purity and impurity tests:** RP-LC-UV or RP-LC/MS
- **Pharmacokinetic (half-life) and degradation studies:** LC/MS
- **High molecular weight analysis:** Size-exclusion chromatography (SEC) or LC/MS
- **Structural analysis:** Collision cross section (CCS) or isomerization (IM/MS)
- **Raw material identification:** Warehouse-based analysis using handheld Raman spectroscopy, or lab-based analysis using RP-LC-UV or FTIR
- **Host-cell protein identification (recombinant products only):** LC/MS/MS
- **Residual solvents analysis:** GC or GC/MS
- **Trace elemental impurities analysis:** ICP-MS
- **Water content:** European Pharmacopoeia (Ph. Eur)
- **Bacterial endotoxins:** Ph. Eur.
- **Total aerobic microbial count (TAMC):** Ph. Eur.
- **Total combined yeasts and molds count (TCYMC):** Ph. Eur.

In addition to the Ph. Eur. Standards, the United States Pharmacopeia (USP) General Chapter <1503> provides a structured framework for the quality control (QC) of synthetic peptide drug substances. It outlines critical quality attributes (CQAs), impurity profiling strategies, and analytical method requirements tailored to the complexity of peptides. Referencing USP <1503> ensures that analytical approaches are not only scientifically robust but also regulatory compliant, particularly for the U.S. market.

Types of impurities in peptide therapeutics

A variety of impurities can be introduced during synthesis, expression, formulation, or storage of peptide therapeutics. These impurities can be grouped into the following three main categories:

Process-related impurities

- Starting materials (chemical purity and optical purity)
- Reagents, solvents, and catalysts
- Fermentation and cell culture media components (such as antibiotics and buffers)
- Residual DNA and proteins

Product-related impurities

- Starting materials, intermediates, and by-products
- Peptide variants
- Degradation products like β-elimination, truncation, and hydrolysis
- Reactants with excipients
- Aggregates, deamidated forms, and oxidized forms

Container closure system

- Glass, plastic, and rubber components
- Leachables and extractables
- Microbial contaminants

Agilent offerings for GLP-1 drug characterization

Agilent has developed several workflows that provide complete solutions for peptide drug development, from early research to manufacturing and QA/QC processes. Major workflows include raw material identification, peptide purity and impurity profile analysis, target peptide mass and peptide sequence confirmation, as well as preparative-scale peptide purification.

Purification

Multiple preparative-scale formats:

- Analytical, semipreparative, and preparative LC systems
- Scale-up fraction collection options

Multiple detector-based preparative formats:

- LC/UV
- LC/MS

Purity and impurities analysis

Purity assay:

- CE/MS
- LC
- LC/MS
- UV
- FTIR

Product-related impurities:

- LC
- LC/MS
- LC/MS/MS

In-process tests (for recombinant peptides):

- Cell-based test / microbial contamination
- Host-cell proteins (LC/MS/MS)

Process-related impurities:

- Residual solvents (GC)
- Elemental analysis (ICP/MS)

Raw material identification:

- Molecular spectroscopy
- Raman spectroscopy

Identity, structure, and characterization

Identity:

- Molecular weight and sequence confirmation by LC/MS/MS

Higher order structures:

- Isomerization analysis and collision cross section (CCS) by ion mobility (IM/Ms)

Agilent 1290 Infinity II LC purification systems

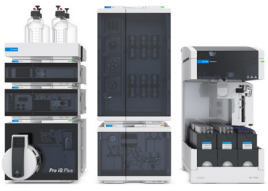
LC-based preparative systems

1290 Infinity II preparative LC system

1290 Infinity II autoscale preparative LC system

InfinityLab reversed-phase preparative columns

LC/MS-based preparative systems



1290 Infinity II preparative LC/MSD system

1290 Infinity II autoscale preparative LC/MSD system

Workflows:
Preparative-scale peptide purification

Spectroscopy-based tools

Vaya Raman system

Cary 630 FTIR spectrometer

RapID system

Cary 3500 UV-Vis

Workflows:
- Raw material identification and purity analysis
- Peptide purity and impurities analysis

Separation-based tools

AdvanceBio columns

1260/1290 Infinity III bio LC

1290 Infinity III 2D-LC

Workflows:
- Raw material identification and purity analysis
- Peptide purity and impurities analysis
- Peptide stability testing
- Peptide aggregation analysis
- Excipients analysis

Sample preparation tool

AssayMAP Bravo protein sample prep plarform and AssayMAP cartridges

Workflows:
- Peptide purity and impurities analysis
- Peptide quantitative analysis
- Peptide isomer characterization
- Peptide stability testing
- Peptide aggregation analysis
- Peptide DMPK / degradation analysis
- Residual solvent analysis
- Trace elemental impurity analysis

MS-based tools

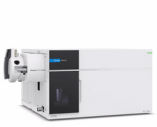
7900 ICP-MS

6545XT AdvanceBio LC/Q-TOF

6230 TOF LC/MSD

8890 GC / 5977 GC/MSD

8890 GC / 8697 headspace sampler



6495D LC/TQ

Spectroscopy-based tools

Cary 60 UV-Vis Spectrophotometer

Cary 3500 UV-Vis

Workflows:
- Intact molecular weight (full length and impurities) determination
- Peptide sequence and post-translational modification confirmation
- Peptide DMPK / degradation analysis
- Host-cell protein analysis

Separation-based tools

LC/UV

AdvanceBio Peptide Mapping columns

MS-based tools

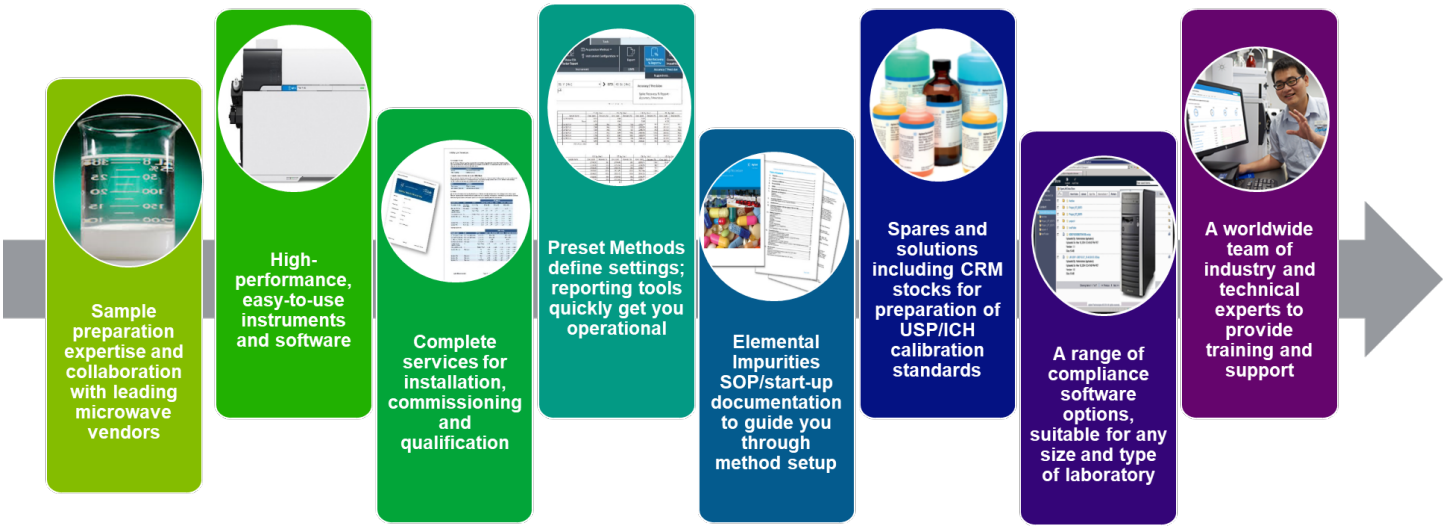
InfinityLab LC/ MSD iQ

LC/TOF

LC/Q-TOF

Everything your lab needs to set up and run current pharma USP/ICH methods

Agilent is your ideal partner to help you excel in therapeutic peptide development. The diagram below outlines our end-to-end solutions and expertise to support you at every stage of your journey.



Agilent workflow solutions for GLP-1 peptide therapeutics

Bioanalytical applications

Agilent's portfolio of bioanalytical solutions offers high-sensitivity and high-accuracy platforms for peptide drug development and QC, delivering outstanding performance and reproducibility for peptide-based therapeutic research.

Major CQAs

Understanding and monitoring the following CQAs is essential for ensuring the safety, efficacy, and consistency of peptide therapeutics from development to commercial production.

Application notes

Bioanalysis

Application note:

The full application note (5994-6087EN) is available here.

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Optimizing Analysis and Purification of a Synthetic Peptide Using PLRP-S Columns

Most peptide drugs are produced using solid-phase peptide synthesis. The synthesis is performed on a polymeric support or resin, which can easily be filtered from reactions. The synthetic route includes multiple deprotection, activation, and coupling steps. The final peptide sequence is separated from the resin using a cleavage cocktail containing scavengers and other components, resulting in a final crude product that is ready for purification.

This application note describes the analysis of a synthetic peptide and how to scale up methods using an Agilent PLRP-S analytical HPLC column. The gradient and loading information are applied directly to a larger preparative column packed with an identical material. The product and any closely eluting impurities could be easily identified by reanalyzing the appropriate fractions on the analytical columns (Figure 1).

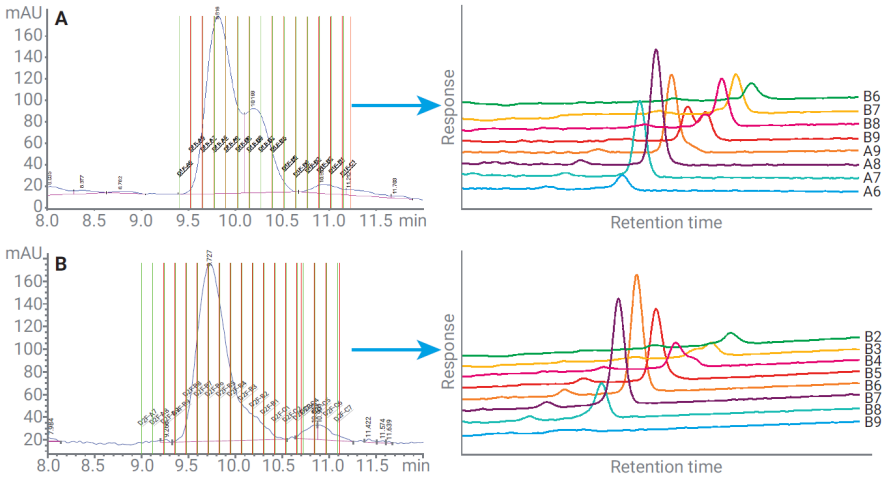

Figure 1. Two peptides (A) and (B) on an Agilent PLRP-S 100 Å column showing fraction reanalysis (right).

Comprehensive Characterization of Multiple GLP-1 Analogs

Using an Agilent 6545XT AdvanceBio LC/Q-TOF with electron capture dissociation and ExDViewer software

This application note showcases the in-depth sequence analysis and precise localization of modifications in three synthetic GLP-1 analogs using the Agilent 6545XT AdvanceBio LC/Q-TOF and ExDViewer software. Electron capture dissociation (ECD) enables clear differentiation of isomers like aspartate and isoaspartate without the need to optimize collision energy. ExDViewer enhances data interpretation with intuitive visualization tools, supporting both ECD and collision-induced dissociation (CID) workflows. This method offers a robust foundation for analyzing GLP-1 analogs and developing future derivatives and impurity profiling.

Figure 2 demonstrates how ExDViewer software automatically annotates side-chain evidence, simplifying the analysis of amino acid isomers. Hovering over the W_{23}^{2+} ion opens an informative tool tip that describes the percent hydrogen transfer, mass, side-chain loss formula, score, and number of matched isotopes.

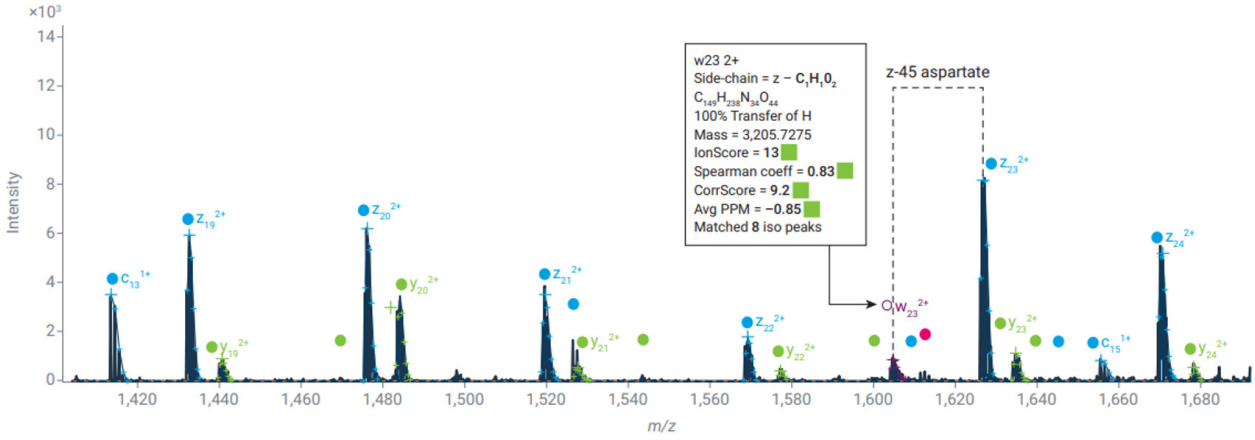

Figure 2. A representative fragmentation spectrum from liraglutide showing the automatically annotated fragment evidence for isomeric amino acids such as aspartate and isoaspartate. Aspartate is identified by the loss of a CHO2 group from the z23 2+ ion.

Application note:

The full application note (5994-7994EN) is available here.

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Figure 3 highlights several examples of modification containing ECD ions detected for tirzepatide, demonstrating the range of ion match quality that can be observed in a fragmentation spectrum.

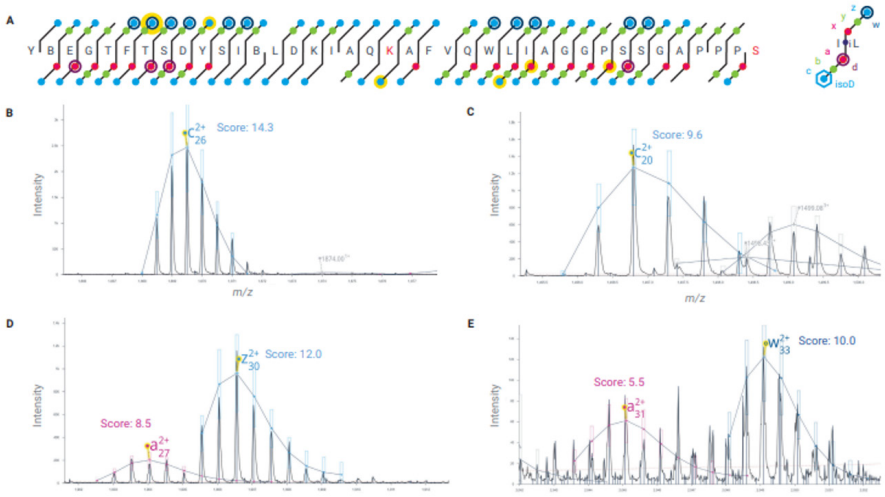


Figure 3. Representative tirzepatide fragments with various ion scores. Each ion is assigned a score based on the m/z and predicted intensity match, as well as the surrounding noise levels. (A) Tirzepatide sequence coverage map where only ions with a score of 5 or greater were considered. (B–E) Examples of modification containing fragments and their corresponding ion scores. The modified lysine is indicated in red text. The c20 2+ ion is a site-specific localizing fragment, while the other highlighted ions are modification-containing peptides fragmented at various positions in the peptide backbone.

Comparing ion scores of GLP-1 analogs analyzed with ECD or CID reveals that ECD fragmentation generates more high-quality, modification containing fragments than CID (Figure 4).

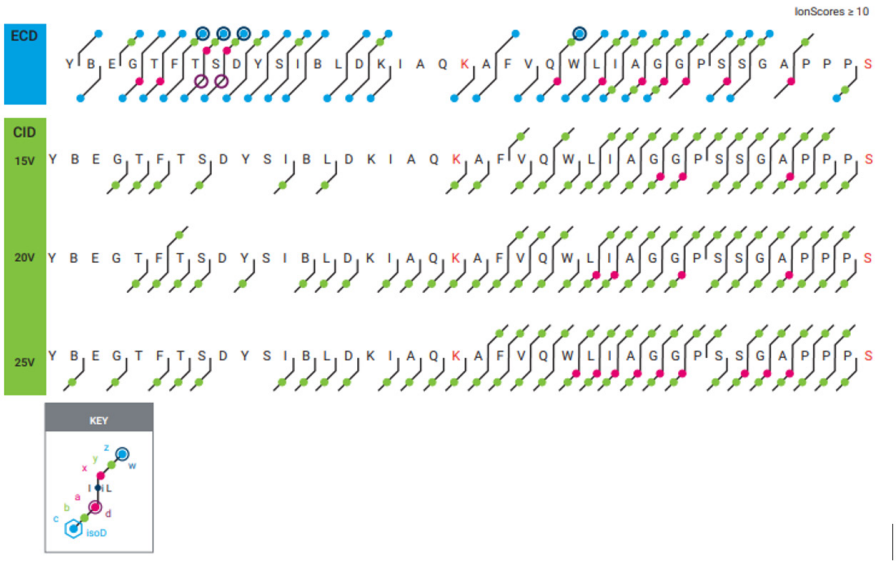


Figure 4. Tirzepatide fragments identified in ECD or CID experiments with ion scores of 10 or greater. ECD provided more high-quality modification-containing fragments, particularly on the N-terminal side of the modification. ECD yielded a more complete ion series with several complimentary ion types. The exception to this is the proline-rich region because ECD does not cleave on the N-terminal side of proline.

LC/MS Based Characterization Workflow of GLP-1 Therapeutic Peptide Liraglutide and its Impurities

This application note demonstrates the power of the Agilent 1290 Infinity II LC coupled with the Agilent 6545XT AdvanceBio LC/Q-TOF MS (Figure 5) for the identification and characterization of therapeutic peptides. This application is exemplified for the GLP-1 agonist liraglutide (Figure 6) and its impurities. The therapeutic peptide workflow within Agilent MassHunter BioConfirm software allows for accurate mass-based identification (Figure 7), sequence conformation, and identification of impurities, with extra or missing amino acid impurities (Figure 8) that can arise during solid phase synthesis. These impurities may pose a potential safety risk and eventually may impact the efficacy of the product.

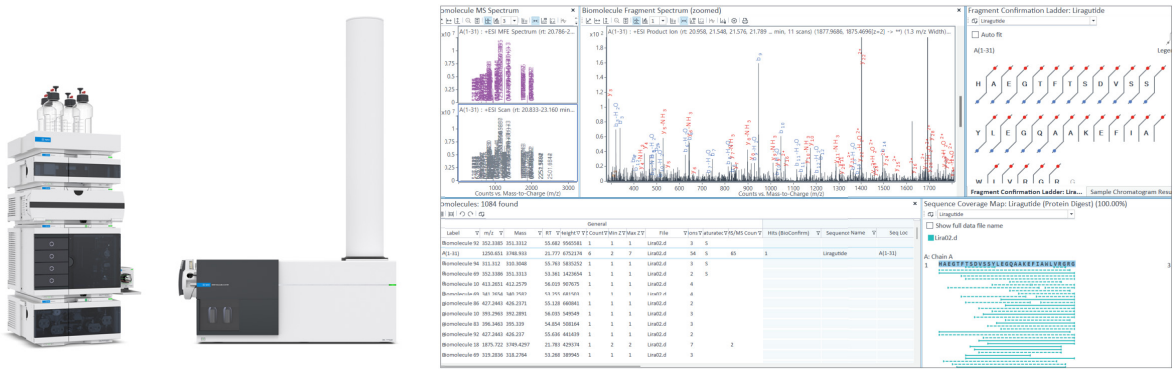
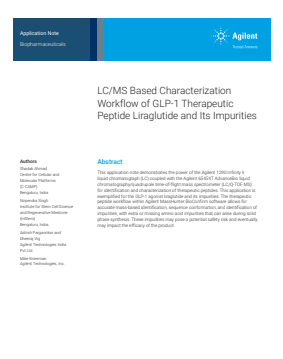


Figure 5. Agilent 1290 Infinity II LC coupled to an Agilent 6545XT AdvanceBio LC/Q-TOF and MassHunter BioConfirm software.

Figure 6. Structure of liraglutide.



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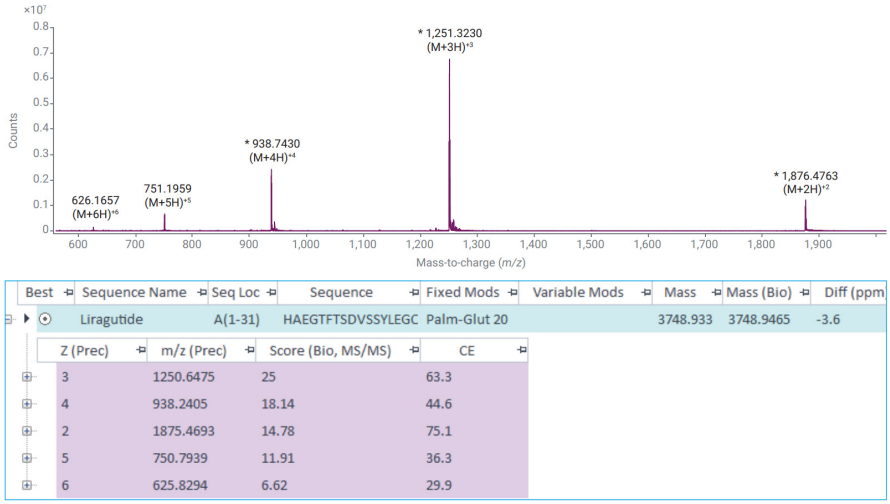


Best	Sequence Name	Seq Loc	Sequence	Fixed Mods	Variable Mods	Mass	Mass (Bio)	Diff (ppm
	Liragutide	A(1-31)	HAEGTFTSDVSSYLEGC	Palm-Glut 20		3748.933	3748.9465	-3.6

Z (Prec)	m/z (Prec)	Score (Bio, MS/MS)	CE
3	1250.6475	25	63.3
4	938.2405	18.14	44.6
2	1875.4693	14.78	75.1
5	750.7939	11.91	36.3
6	625.8294	6.62	29.9

Figure 7. The intact mass charge state distribution identified by Agilent MassHunter BioConfirm software for liraglutide.

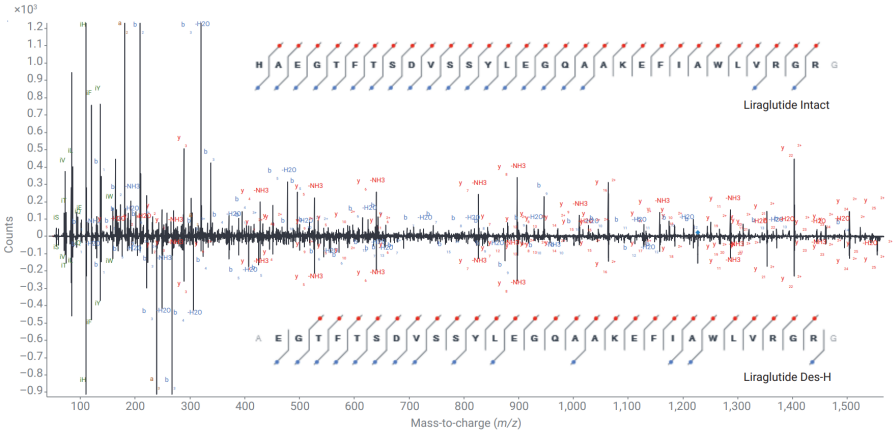
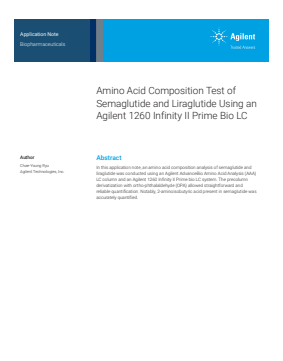


Figure 8. Missing H (1) impurity in liraglutide: Comparison of the y and b ions from the MS/MS spectrum for modified b1 ions shows an effective change over those ions in unmodified peptide, suggesting that histidine (H) is missing at position 1.



Application note:

The full application note (5994-7749EN) is available here.

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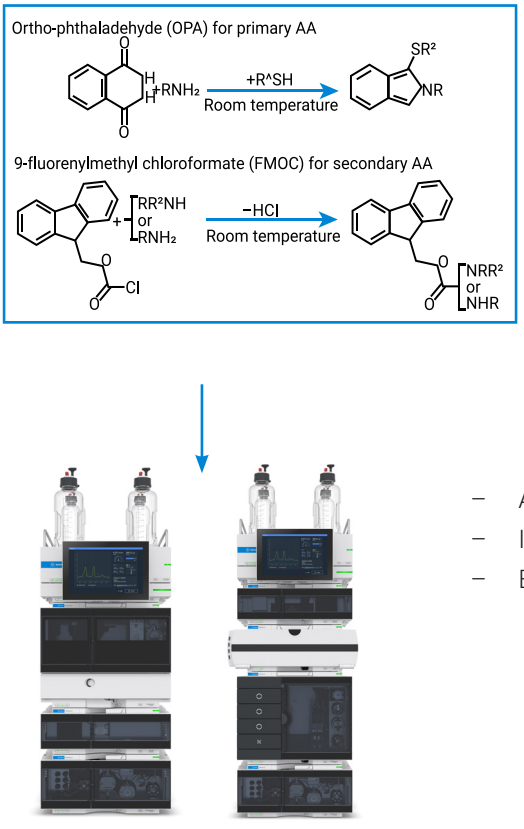
Amino Acid Composition Test of Semaglutide and Liraglutide Using an Agilent 1260 Infinity II Prime Bio LC

The Agilent solution for amino acid composition analysis using automated derivatization provides a powerful and reliable platform for analyzing semaglutide and liraglutide.

In this application note, amino acid profiling was conducted using the Agilent 1260 Infinity II Prime bio LC system and the AdvanceBio Amino Acid Analysis (AAA) LC column. Precolumn derivatization with ortho-phthalaldehyde (OPA) enabled accurate and straightforward quantification, notably allowing the precise measurement of 2-aminoisobutyric acid present in semaglutide. Automated derivatization in the autosampler eliminates human errors associated with manual sample preparation (Figure 9). Unlike conventional LC/MS/MS, which cannot distinguish isoleucine from leucine, the Agilent solution allows accurate identification and quantification of isoleucine, leucine, and modified amino acids (Figure 10). Additionally, chromatographic interferences from intentionally added amines were successfully resolved through method optimization (Figure 11).

Increase precision with Autosamler automation



- Automated reagent addition
- Increase precision
- Eliminates manual processes

Figure 9. Online derivatization of OPA and FMOC. Separation of polar amino acids on RP-phase and detection by UV and fluorescence.

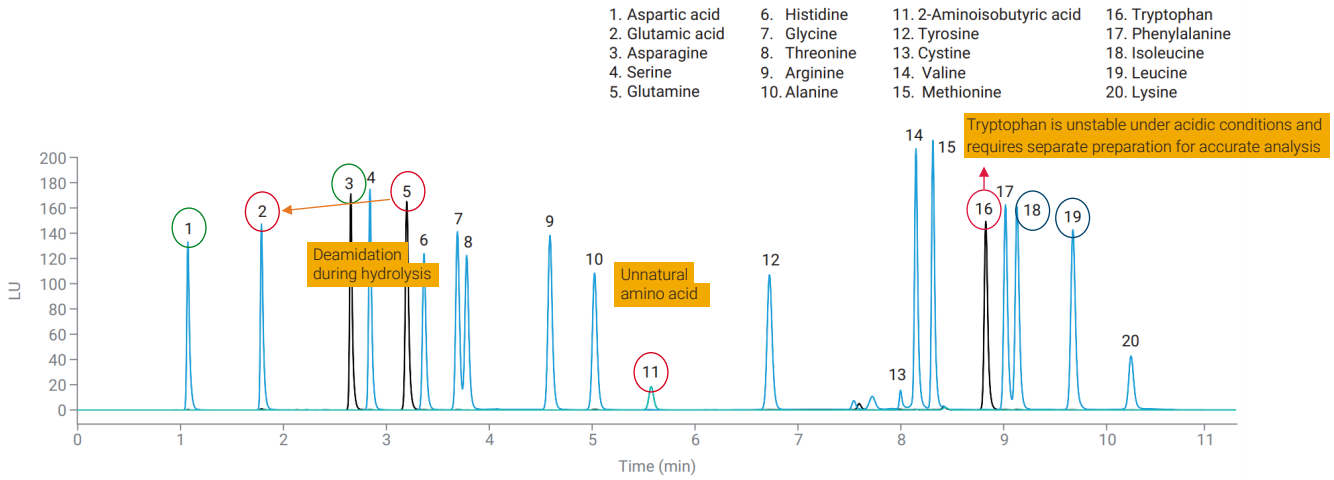


Figure 10. FLD profile of 100 µM standard solution of 20 amino acids.

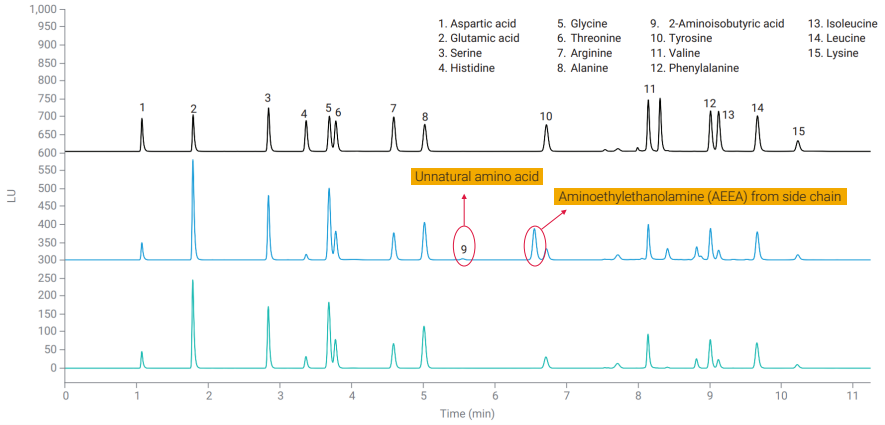


Figure 11. FLD chromatograms of hydrolyzed semaglutide and liraglutide solutions derivatized with OPA using the Agilent AdvanceBio AAA column (black = amino acid standard 100 µM; blue = semaglutide; green = liraglutide).

Comprehensive Aggregate Profiling of Liraglutide and Semaglutide Using an Agilent 1290 Infinity II Bio 2D-LC and Agilent InfinityLab LC/MSD XT

In this application note, the Agilent 1290 Infinity II bio 2D-LC and InfinityLab LC/MSD XT systems offer a high-resolution, MS-compatible solution for accurate analysis of peptide aggregates.

Traditional SEC methods used for protein analysis are not directly applicable to peptide aggregates due to stronger interactions between the analyte and the column. To address this challenge, peptide aggregates of semaglutide and liraglutide were separated using an Agilent AdvanceBio SEC 300 Å column with organic solvents as the mobile phase. However, these SEC conditions are not directly compatible with MS. To enable MS-based analysis, a 1290 Infinity II bio 2D-LC system was coupled with the InfinityLab LC/MSD XT, allowing aggregate separation and detection under MS-friendly conditions. Covalently bonded aggregates were characterized by their SEC retention times.

Using advanced 2D-LC capabilities—such as multi-inject for high-resolution sampling and active solvent modulation—molecular weight information was successfully obtained via deconvolution using Agilent OpenLab CDS software, version 2.8.

The configuration of the 2D-LC valve and its loop configuration during the different modes are illustrated in Figure 12.

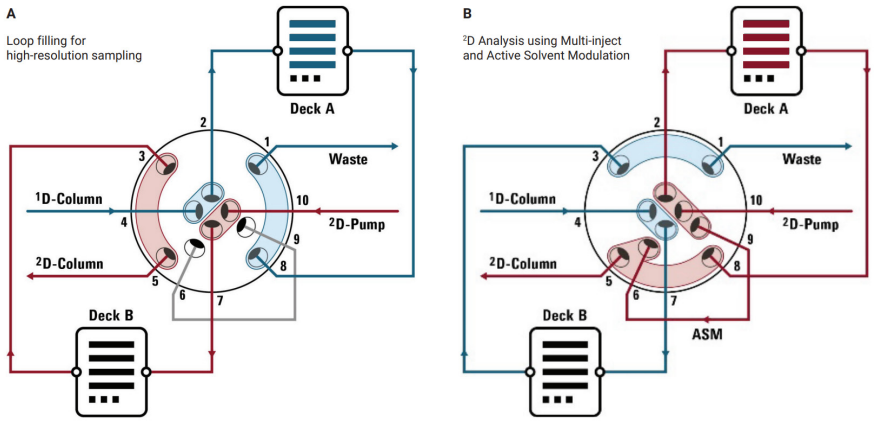
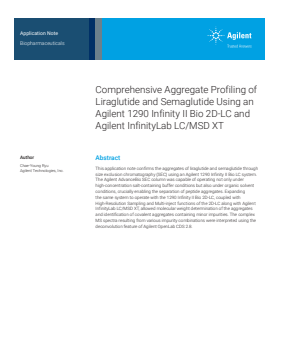


Figure 12. Diagram of the flow path through the Agilent 1290 Infinity II bio 2D-LC ASM valve during (A) loop filling for high-resolution sampling and (B) 2D analysis using multi-inject and active solvent modulation.



Application note:

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Figure 8 illustrates the 2D analysis results of Ozempic.

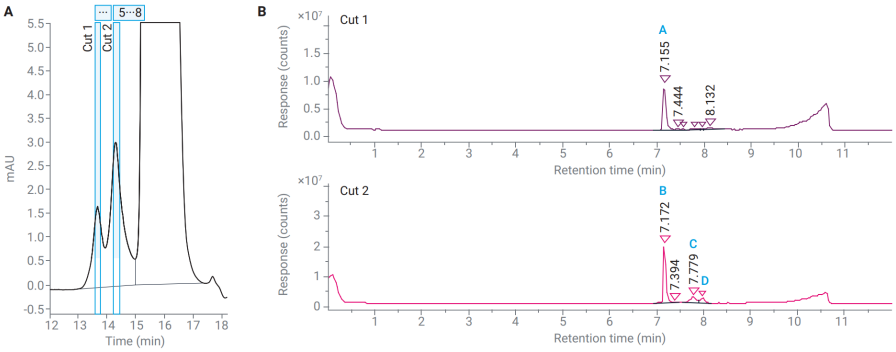


Figure 13. (A) ¹D cut annotation and (B) the total ion chromatogram (TIC) of LC/MSD XT analyzing the cut in ²D.

Peaks C and D were identified as impurities with molecular weights approximately twice that of semaglutide (Figure 14).

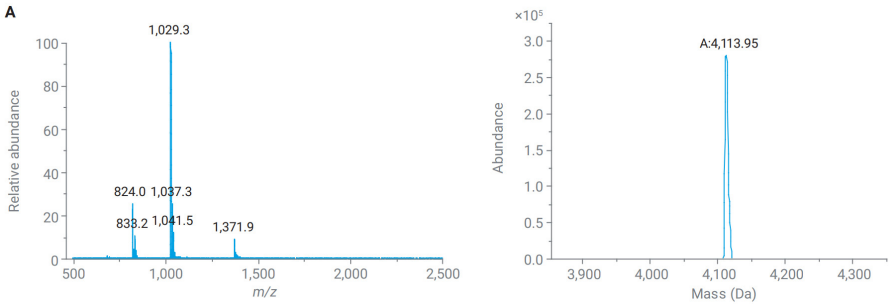


Figure 14. Raw spectrum (left) and deconvoluted spectrum (right) of the peak obtained from the 2D TIC in Figure 13.

Quantification of Glucagon-Like Peptide-1 Agonist Tirzepatide Using an Agilent 6495D Triple Quadrupole LC/MS

Synthetic peptide-related impurities generated during manufacturing and storage may impact the safety and efficacy of therapeutic peptides. Among them, GLP-1 receptor agonists represent one of the most promising classes. Tirzepatide, a GLP-1 agonist, regulates blood glucose levels and reduces body weight.

This application note highlights a highly sensitive and accurate quantification method for both native and mono-oxidized tirzepatide using the Agilent 6495D triple quadrupole LC/MS system coupled with the Agilent 1290 Infinity II bio LC system.

A multiple reaction monitoring (MRM)-based method was developed, achieving a limit of quantitation (LOQ) of 0.025 ng/mL for tirzepatide. The method demonstrated a wide dynamic range, covering four orders of magnitude for native tirzepatide and three for mono-oxidized forms, offering high accuracy and precision for impurity profiling.

Figure 15 shows the MRM chromatograms for unmodified/native tirzepatide and mono-oxidized tirzepatide. These MRMs verify the peptide identity and demonstrate the use of MRM to monitor post-translational modifications such as oxidation. As expected, the oxidized form elutes early (at 3.9 minutes) due to reduced hydrophobicity compared to the native peptide, which elutes later (at 4.0 minutes). The *m/z* 1,208.1 and 396.3 MRM transition shows another peak at 4.3 minutes, corresponding to an unknown signal that was also present in the unmodified/native tirzepatide sample.

The correlation coefficients (R2) were 0.997 and 0.996 for the native and mono-oxidized forms, respectively. Precision and accuracy were excellent at all levels, with percent relative standard deviation (%RSD) < 6% and accuracy ranging from 81 to 115% (Table 1).

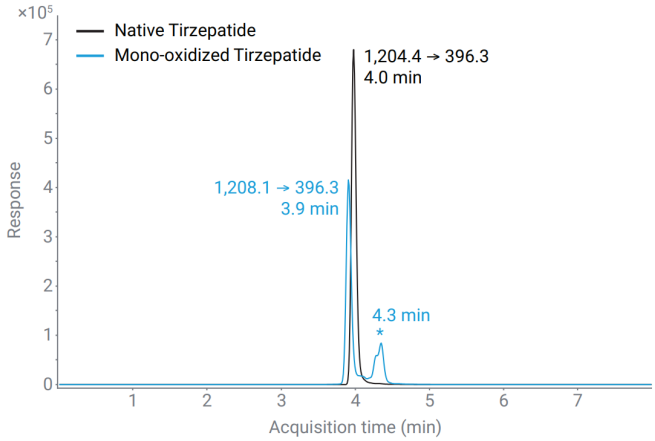
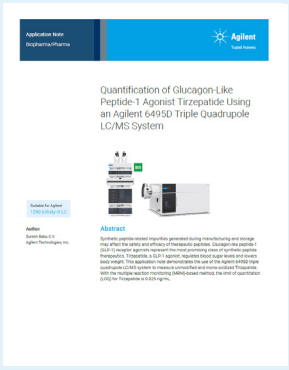


Figure 15. MRM chromatograms of native tirzepatide (standard, black) and mono-oxidized tirzepatide (2% H_2O_2 treated, blue). The peak for the native/unmodified peptide transition (*m/z* 1,204.4 and 396.3) occurs at 4.0 minutes, and the peak for the mono-oxidized peptide transition (*m/z* 1,208.1 and 396.3) occurs at 3.9 minutes. An asterisk (*) marks the unknown peak.



Application note:

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Table 1. Precision (area %RSD) and accuracy for the standard curve analysis of unmodified/native (standard) tirzepatide and tryptophan mono-oxidized (2% H_2O_2 treated) tirzepatide (n = 4).

Samples	Tirzepatide Standard		2% H_2O_2-Treated Tirzepatide	
Concentration (ng/mL)	Area %RSD	Accuracy (%)	Area %RSD	Accuracy (%)
0.025	2.52	81.37	–	–
0.05	4.28	82.1	–	–
0.1	5.20	81.8	–	–
0,25	4.98	113.7	5.72	117.92
0.5	4.01	101.1	5.97	108.07
1.0	2.06	118.2	5.18	103.05
2.5	3.00	106.3	2.54	90.67
5	1.15	112.7	11.41	97.4
10	1.41	97.6	4.52	89.8
25	0.51	112.9	2.23	82.32
50	1.29	100.2	1.73	99.6
100	1.11	95.6	2.29	107.15
250	0.94	100.4	0.52	99.27

Peptide Drug Stability Analysis Using Agilent InfinityLab LC/MSD and OpenLab CDS Deconvolution

The FDA's ANDA guidelines recommend managing peptide-related impurities due to their immunogenic potential. This application note highlights the analysis of degradation impurities from liraglutide and Semaglutide under acidic, basic, and oxidative conditions using the Agilent 1260 Infinity II Prime bio LC, InfinityLab LC/MSD iQ and XT, and InfinityLab Poroshell 120 EC-C18 columns.

OpenLab CDS 2.8 software with deconvolution enabled neutral mass determination and clear identification of major impurities.

Combined with the AdvanceBio Peptide Mapping and InfinityLab Poroshell 120 columns, the system allows straightforward separation, impurity profiling, and comparison with RLDs—offering a robust and user-friendly solution for late-stage development and QC.

In the analysis of Liraglutide at 1 mg/mL, impurity peaks in both the UV and MS trace were visible. The MS spectrum of intact Liraglutide was derived from the main peak in the TIC, as shown in Figure 16.

For illustrative purposes, the MS spectrum and the molecular weight of the major peaks were extracted from the MS TIC of the sample heated at 60 °C for two days under acidic conditions, using the deconvolution feature of OpenLab CDS 2.8. The deconvolution results for the six representative peaks are shown in Figure 17 and Table 2.

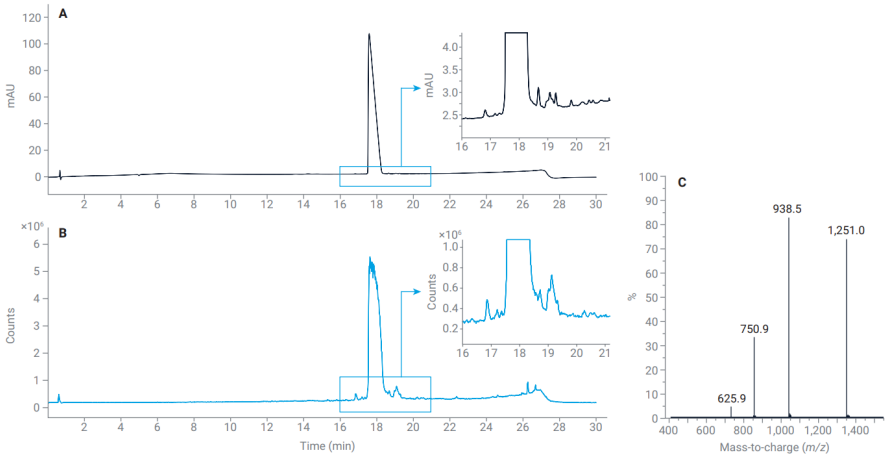
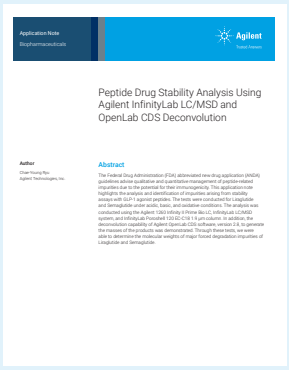


Figure 16. (A) UV chromatogram. (B) MS TIC of liraglutide 1 mg/mL. (C) MS spectrum of liraglutide.



Application note:

The full application note (5994-7500EN) is available here.

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Peak	Spectrum RT (min)	Component	Mass (Da)	Absolut Abundance	Mass Difference (Da)
A	54.372	A	3,516.37	159,683	–234
	54.372	B	3,577.72	78,012	–173
B	91.635	A	3,754.39	875,778	4
	91.635	B	3,816.13	453,879	66
C	95.557	A	3,750.56	9,934,175	(Liraglutide)
D	99.057	A	2,808.54	777,637	–942
	99.057	B	2,836.56	370,460	–914
	99.057	C	2,871.36	259,511	–879
E	103.088	A	3,359.83	876,644	–391
	103.088	B	3,421.03	462,119	–330
F	111.134	A	3,541.24	279,455	–209
	111.134	B	3,569.02	156,874	–182

Table 2. The deconvolution results from the mass spectrum of the liraglutide sample heated for two days under acidic conditions and the mass difference compared to liraglutide using an Agilent InfinityLab LC/MSD iQ and OpenLab CDS 2.8 deconvolution.

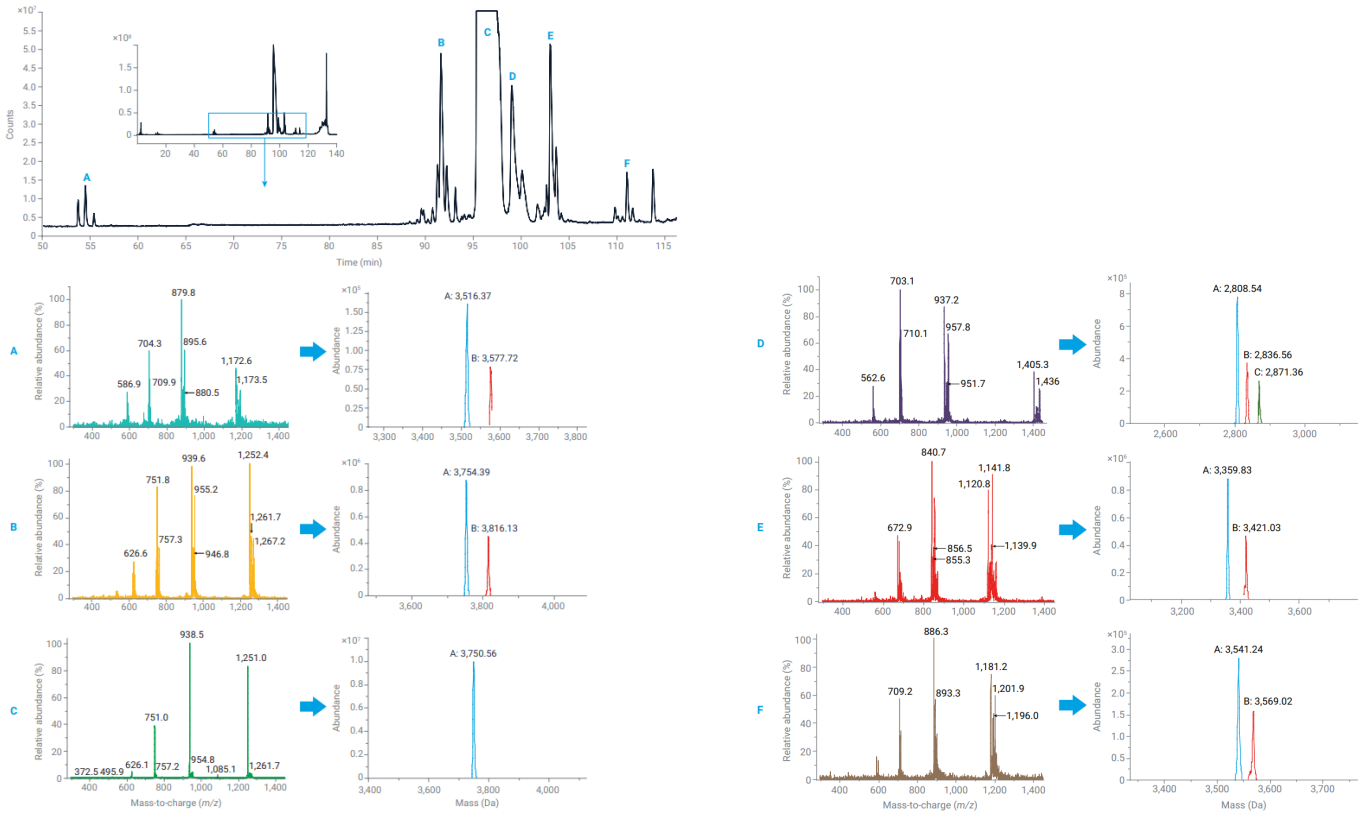


Figure 17. TIC of Liraglutide sample heated for two days under acidic conditions (top) using an Agilent InfinityLab LC/MSD iQ, and the MS raw spectrum (A to F, left) and the deconvoluted spectrum (A to F, right) for each of the six peaks.

Rapid Confirmation of GLP-1 Analog (Liraglutide) Using Agilent InfinityLab LC/MSD iQ

This application note highlights the analysis of liraglutide, a GLP-1 analog, using the Agilent InfinityLab LC/MSD iQ system. The system enables high-sensitivity mass scanning up to 1,450 m/z, making it ideal for rapid molecular weight confirmation of synthetic peptides.

The deconvolution feature in OpenLab CDS 2.8 was used to confirm the intact mass of liraglutide. Rapid method development was supported by the Agilent InfinityLab Quick Change valve and Agilent 1260 Infinity II flexible pump, which allowed efficient screening of reversed-phase columns and acidic modifiers.

Among the evaluated conditions, the AdvanceBio Peptide Mapping column with TFA showed excellent UV detection performance, while the same column with DFA provided high MS specificity and sensitivity. This setup offers a streamlined solution for both method development and intact mass confirmation of GLP-1 analogs like liraglutide (Figures 18 and 19).

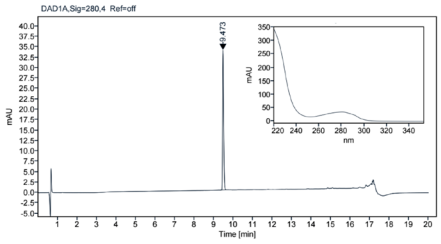


Figure 18. Chromatogram and UV spectrum of liraglutide 100 µg/mL under TFA conditions

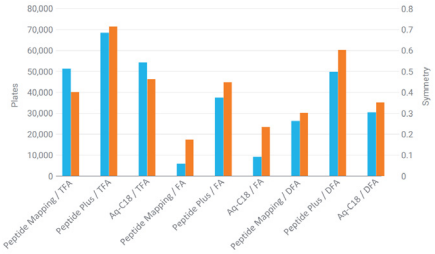


Figure 19. Results of plates and symmetry for each condition (left axis, blue: plates; right axis, orange: symmetry). The results of different column chemistries and modifiers were summarized to access the performance based on separation capabilities.

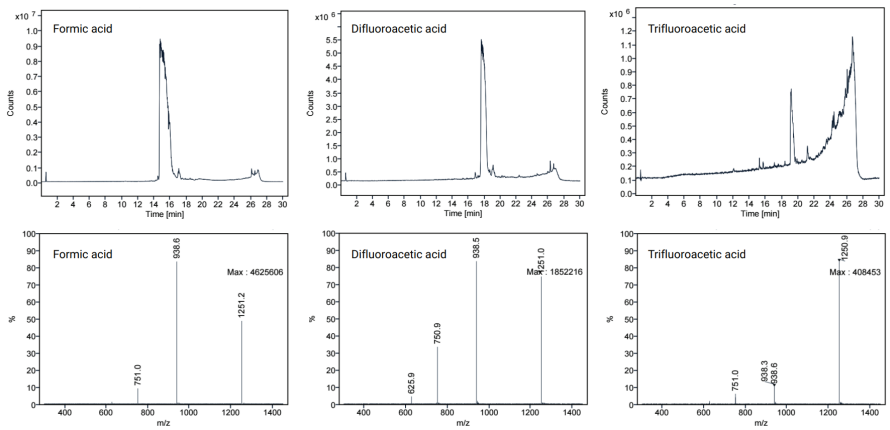
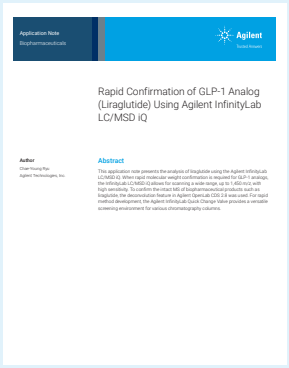


Figure 20. TICs and MS spectra of liraglutide (1 mg/mL) using various acidic modifiers with an Agilent AdvanceBio Peptide Mapping column and an Agilent InfinityLab LC/MSD iQ.



Application note:

The full application note (5994-7415EN) is available here.

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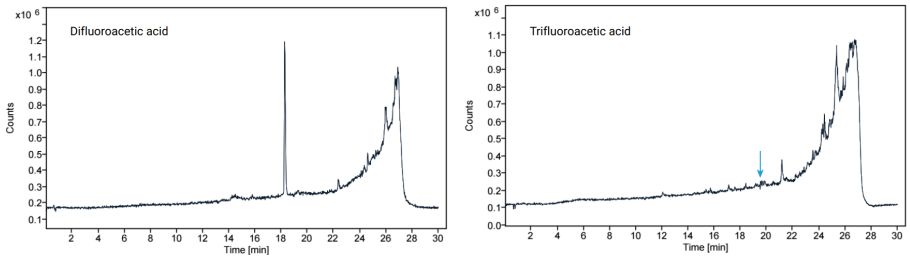


Figure 21. Comparison of TICs between DFA and TFA for liraglutide at 10 µg/mL.

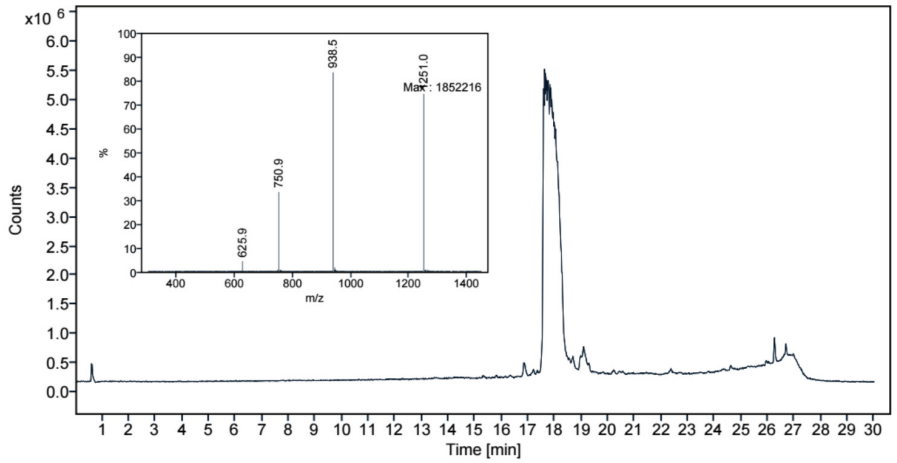


Figure 22. TIC and MS spectrum of liraglutide at 1 mg/mL.

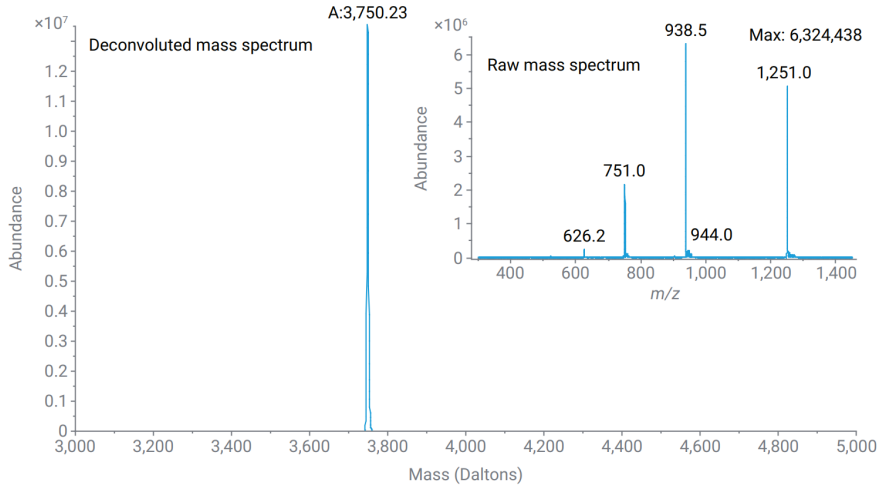


Figure 23. Deconvolution results for liraglutide.

Raw material identity verification

Verification of Raw Materials for Synthetic Peptide Production with the Agilent Vaya Raman System

This application note showcases the Agilent Vaya Raman raw material identity verification system's unique and efficient through-container identification of raw materials, as well as differentiation of Fmoc-protected amino acids—which are key building blocks for synthetic peptide manufacturing (Table 3, and Figures 24 and 25).

Table 3. Outline of raw materials.

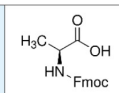
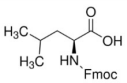
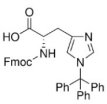
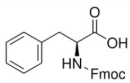
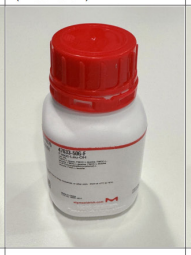
Structure of Raw Material				
Name of Raw Material	Fmoc alanine (Fmoc-Ala-OH)	Fmoc leucine (Fmoc-Leu-OH)	Fmoc histidine TRT (Fmoc-HIS(Trt)-OH)	Fmoc phenylalanine (Fmoc-Phe-OH)
Picture of Raw Material in Native Container				
Container Type Selected on the Device for Method Development	Glass	Thick plastic	Thick plastic	Thick plastic

Figure 24. The Agilent Vaya Raman system being used to identify Fmoc-protected amino acids.

Application note:

The full application note (5994-7610EN) is available here.

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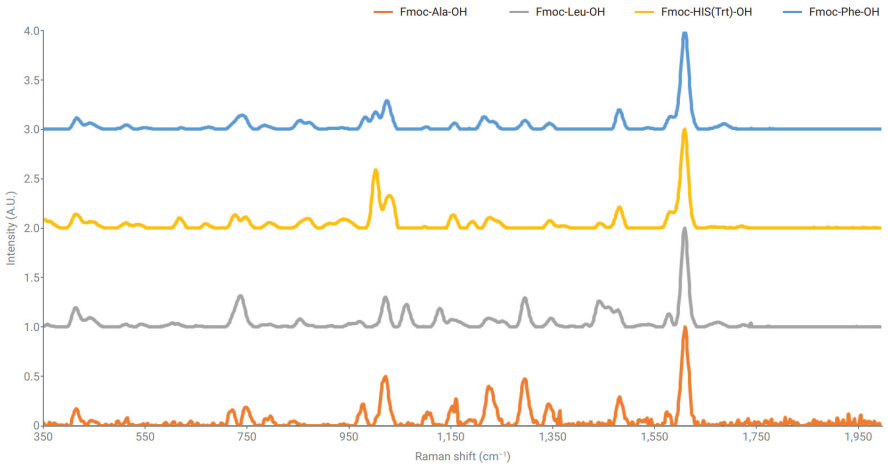


Figure 25. Spectra of Fmoc-protected amino acids.

Bioanalysis, beyond expectations

InfinityLab LC and bio LC systems

Agilent offers four innovative LC configurations designed to meet all your bioanalysis needs, from routine testing to high-throughput and precision workflows. Featuring advanced InfinityLab technology and metal-free bio-inert flow paths, these systems are ideal for analyzing even the most challenging biologics. By eliminating metal interaction, these systems ensure accurate and reliable results for sensitive biomolecule analysis.

1290 Infinity III LC and bio LC systems

Unmatched performance for complex bioanalysis

Bio LC

Metal-free components protect biomolecules and ensure accurate and reliable analysis across diverse sample types and conditions.

1300 bar

High-pressure capability (up to 1300 bar) enables high-throughput analysis with excellent resolution and faster results.

ISET

Emulates other LC systems for seamless method transfer and compatibility with existing HPLC methods.

BLEND ASSIST

Simplifies mobile phase blending and preparation through guided workflows.

Buffer Advisor

Recommends optimal buffer conditions for stability and performance.

Multiwash

Reduces carryover with multiple solvent wash paths, ensuring cleaner analysis.

1260 Infinity III Prime LC and Prime bio LC systems

Everyday performance with exceptional flexibility

Bio LC
Metal-free components protect biomolecules and ensure accurate and reliable analysis across diverse sample types and conditions.

800 bar
Everyday performance with exceptional flexibility

PAT/Online LC
Seamless integration into process analytical technology (PAT) environments enables real-time monitoring and quality control.

ISET
Emulates other LC systems for seamless method transfer and compatibility with existing HPLC methods.

BLEND ASSIST
Simplifies mobile phase blending and preparation through guided workflows.

Buffer Adviser
Recommends optimal buffer conditions for stability and performance.

1260 Infinity III bio-inert LC system

Ideal for complete bio-inert applications

Bio-inert
Completely metal-free flow path minimizes sample interaction, ensuring the highest recovery and stability for sensitive biomolecules.

600 bar
Operates up to 600 bar with high accuracy and long-term durability, ideal for bioanalysis requiring consistent performance.

Buffer Adviser
Recommends optimal buffer conditions for stability and performance.

Multiwash
Reduces carryover with multiple solvent wash paths, ensuring cleaner analysis.

Versatile solutions for diverse workflows

Workflow solutions

Agilent InfinityLab LC and bio LC solutions cover a wide range of analytical techniques and approaches applied across biopharmaceutical workflows. Choose from designated solutions for 2D-LC, online LC, LC/MS, LC/Q-TOF, SEC, sample preparation, and more to meet your various application needs.

1260 Infinity II GPC/SEC and Bio-SEC systems

Comprehensive protein characterization with SEC

Bio-inert
Uses metal-free PEEK materials throughout the sample flow path for maximum biocompatibility.

Biospecific light scattering system
Accurately determines molecular weights without relying on conventional calibration curves.

High sensitivity
Small 10 mm flow cell minimizes peak dispersion and enhances detection of large aggregates.

Accurate and reproducible data
Provides reliable information for protein sizing and aggregate analysis

Absolute molecular weight and size
Measures absolute values of biomolecules using dual-detection mechanisms.

1290 Infinity III 2D-LC / bio 2D-LC systems

Simplifying and accelerating complex separations

Bio LC
Uses biocompatible materials to ensure excellent peak shape and robustness across a wide pH and salt range.

Comprehensive 2D-LC
Combines orthogonal separation techniques for improved resolution and reduced analysis time.

Multiple heart-cutting
Enhances sensitivity and specificity by isolating and analyzing targeted fractions from 1D separations.

Compliance capabilities
Supports 21 CFR Part 11 and Annex 11 regulatory requirements.

2D-LC OpenLab software
Offers intuitive and flexible control over unique 2D-LC valve settings and analysis workflows.

6545XT AdvanceBio LC/Q-TOF

Extending LC/MS analytical capabilities

Ultralow vacuum
Provides ultrahigh vacuum (10^{-8} torr) to resolve complex intact protein spectra with clarity.

Compliance capabilities
Supports regulatory requirements including 21 CFR Part 11 and Annex 11.

Iterative MS/MS
Improves sequence coverage and enables sensitive PTM analysis, even for low-abundance peptides.

SWARM Autotune
Automatically tunes for large biomolecules, delivering high-quality data effortlessly.

AssayMAP Bravo protein sample prep platform

Automated protein sample preparation using validated protocols

High throughput
Processes up to 96 samples simultaneously for unmatched efficiency.

High reproducibility
Reduces variability through automated sample preparation, enhancing workflow consistency.

High recovery
Achieves maximum recovery of target analytes, even from limited sample volumes.

Time saving
Optimized protocols shorten preparation time and enable faster data generation.

Explore More Agilent LC Systems and Related Resources

Gain confidence in CQA monitoring

AdvanceBio LC columns

Agilent AdvanceBio LC columns are designed and manufactured to provide reliable results for analyzing highly complex biotherapeutic molecules and monitoring their purity, potency, and other key quality attributes.

The following chart provides an overview of the specific chemistries. AdvanceBio LC columns are designed and produced to provide confident results when analyzing highly complex biotherapeutic molecules and monitoring their purity, potency, and other CQAs.

Peptide analysis		Aggregate analysis	Amino acid analysis	Synthesis peptide purification	Solid-phase peptide synthesis	Enantiomers
Reversed phase	Reversed phase	SEC	Derivatization reversed phase	Preparative scale, reversed phase < 300 Å	Polymeric supports	Chiral
Formic acid and TFA as ion pair reagent	TFA as ion pair reagent	AdvanceBio SEC 130 Å 2.7 µm	AdvanceBio Amino Acid Analysis reagent kit (precolumn derivatization)	PLRP-S 100 Å 8 µm, 10 µm	PL-Wang (75-150 µm) 0.4, 0.6, 0.9, 1.1 mmol/g	Poroshell Chiral-T LC column
AdvanceBio Peptide Plus 2.7 µm (charge surface column)	AdvanceBio Peptide Mapping 2.7 µm	AdvanceBio SEC 300 Å 2.7 µm	AdvanceBio Amino Acid Analysis column 2.7 µm	PLRP-S 300 Å 8 µm, 10 µm	PL-Rink (75-150 µm) 0.3, 0.7 mmol/g	CP-Chirasil Val GC/MS column
	InfinityLab Poroshell EC-C18 1.9 µm	AdvanceBio SEC 120 Å 1.9 µm		PLRP-S 100 Å bulk media 8 µm, 10 µm, 50 µm		
	AdvanceBio EC-C18, PEEK-lined 2.7 µm	AdvanceBio SEC 200 Å 1.9 µm		PLRP-S 300 Å bulk media 8 µm, 10 µm, 50 µm		
		AdvanceBio SEC 120 Å 1.9 µm				
		AdvanceBio SEC 200 Å 1.9 µm			PEEK-lined bio-inert column hardware	

AdvanceBio Peptide Plus columns achieve excellent and reproducible peak shape for target peptides and impurities, while using the common formic acid (FA) mobile phase additive for simplicity and transferability across multiple system platforms. It features a hybrid end-capped C-18 stationary phase on a 100 Å pore size, 2.7 µm particle that's modified to have a charged surface. This innovative charged surface provides superior performance under the MS-friendly FA mobile phase additive with better peak shape, alternate selectivity, and improved resolution.

AdvanceBio Peptide Plus

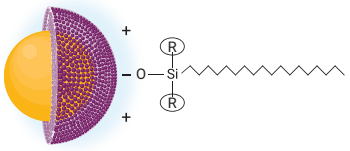
Poroshell 120, 2.7 µm — UHPLC performance at lower pressure

+

HPH chemistry — Provides pH stability from 1 to 11

+

Charged surface C18 — Good peak shape with formic acid Alternate selectivity



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AdvanceBio Peptide Plus: Sensitivity and resolution for accurate identification of synthetic peptide impurities when using formic acid as the mobile-phase additive

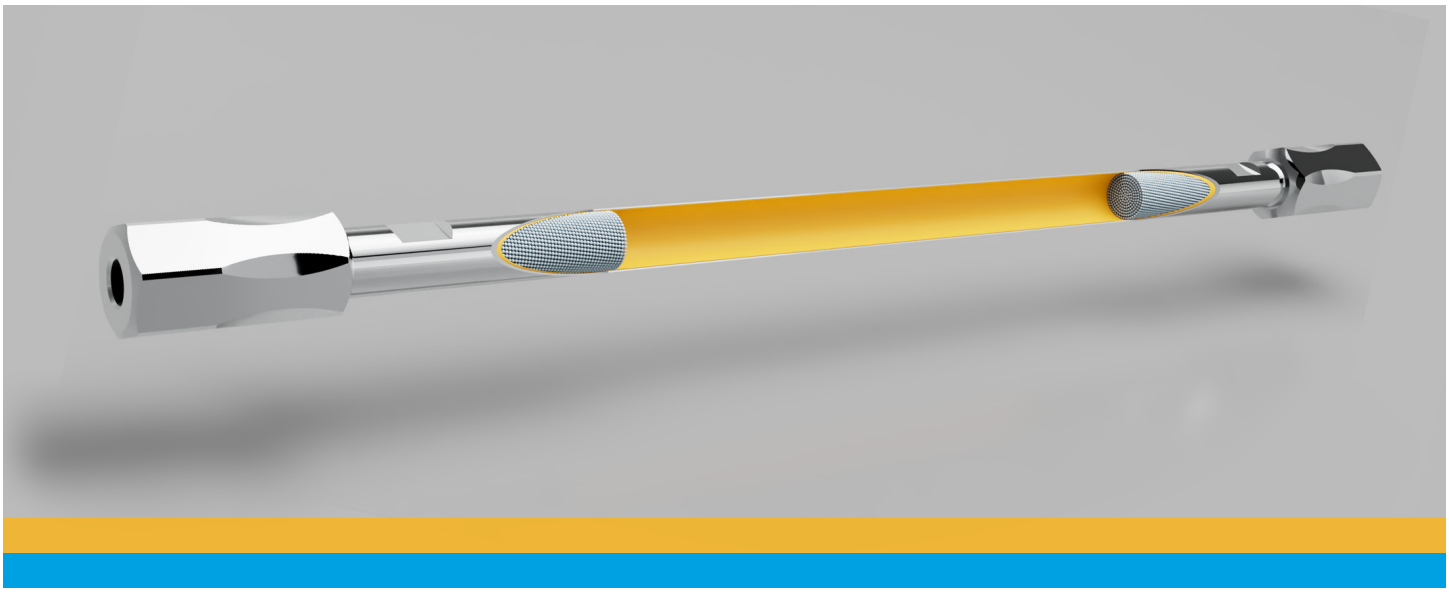
Unlock the true potential of HPLC with Ultra Inert technology

Experience the difference with Agilent Altura HPLC columns. Our Ultra Inert technology sets a new standard in liquid chromatographic performance, providing the reliability and efficiency you need for your most demanding applications. Choose Altura columns and elevate your analytical results to new heights.

Meet the Altura family of HPLC columns

Altura columns feature our innovative Ultra Inert technology. This advanced coating blocks active metal sites, ensuring an inert flow path while maintaining the strength, pressure tolerance, and consistency of a traditional stainless steel HPLC column.

The result? Altura columns unlock the true separation potential of the stationary phase. Experience superior chromatographic performance, faster equilibration, reduced carryover, and enhanced sensitivity for your most challenging metal-sensitive analytes.



Enhance your separations with Ultra Inert technology

Ultra Inert technology, featured in Altura HPLC columns, minimizes nonspecific interactions and metal adsorption to enhance the accuracy and reproducibility of analytical measurements. Delivering exceptional inertness across the entire sample path, Agilent inert LCs and Altura HPLC columns together ensure reliable results even with highly active or trace-level compounds. Backed by over a decade of expertise, innovation, and trusted performance, Ultra Inert components from Agilent have helped laboratories worldwide achieve lower detection limits, greater sensitivity, and more consistent data quality across a broad range of analytical applications.

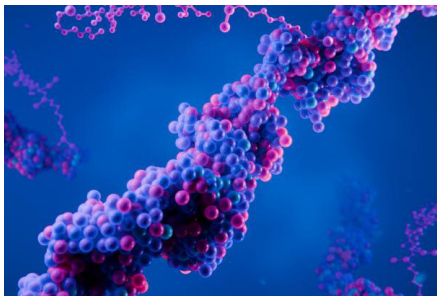
Altura HPLC columns with Ultra Inert technology offer:

- **Reduced nonspecific binding** for pure selectivity
- **Better peak shape** with less tailing
- **Enhanced sensitivity** due to improved sample recovery
- **Long lifetime** due to robust coating and column packing
- **Rapid equilibration** to get high-quality data faster
- **Versatile use** with various chromatographic techniques
- **Readiness for evolving regulatory needs** with lower limits of detection

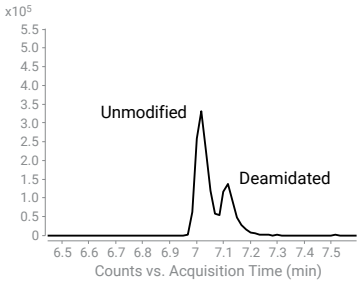
Get more confident results for your most demanding bio LC applications

Application note 5994-8055EN illustrates the benefit of an inert flow path combining an Agilent 1290 Infinity II bio LC with an Agilent Altura Oligo HPH-C18 column, yielding higher analyte recovery and narrow peaks. With parallel RNase 4 and RNase T1 cutters, 94.7% of the mRNA sequences could be covered.

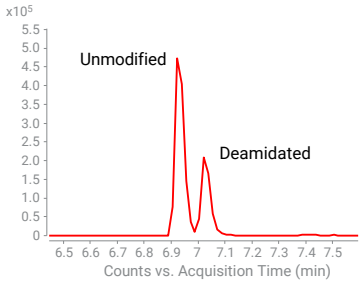
Application note 5994-8308EN shows the improved recovery and peak shape for GLP-1 peptides and related excipients using an inert Agilent 1290 Infinity III bio LC with an Altura Poroshell HILIC-Z column.



Stainless steel column



Altura Ultra Inert column



Altura ZORBAX Eclipse Plus C18 columns with Ultra Inert technology demonstrate decreased tailing and improved recovery for peptides, increasing reliability of PTM monitoring and quantitation.

High-quality standards and sample preparation for successful analysis

Standards, kits, and consumables

Complement your instruments with high-quality Agilent columns to consistently ensure high standards in all aspects of peptide analysis.

AdvanceBio columns

Rely on Agilent AdvanceBio columns for CQA analysis of your GLP-1 peptides. Whether you're performing peptide purity or impurity characterization, or aggregate analysis, we've got you covered with a complete suite of AdvanceBio columns.

Learn more: Advancebio Peptide Plus | AdvanceBio Peptide Mapping | AdvanceBio EC-C18 PEEK-lined | AdvanceBio SEC

AdvanceBio SEC and peptide quality control standards

Our protein mix consisting of five carefully selected proteins (ovalbumin, myoglobin, aprotinin, neurotensin, and angiotensin II) designed to calibrate the 130 Å Agilent AdvanceBio SEC columns.

Use the Agilent 10-peptide QC standard—the same standard Agilent uses to QC its columns—to evaluate your column performance over its lifetime. It can be used for HPLC or LC/MS.

Learn more: AdvanceBio SEC Standards | Agilent Peptide Standards

Automated amino acid analysis

The Agilent AdvanceBio Amino Acid Analysis (AAA) end-to-end solution consists of complete AdvanceBio AAA reagent kit and AdvanceBio AAA column. Along with Agilent autosamplers, you can automate your precolumn derivatization process thus giving you an accurate, sensitive, and reproducible way of quantifying amino acids.

Learn more: Amino Acid Analysis: How-To Guide

StratoSpheres: polymeric supports for solid-phase peptide synthesis

Agilent StratoSpheres are high-performance, high-quality polymeric resin supports that enable you to make the highest-quality peptide APIs.

These include resins for Boc and Fmoc chemistries and include chloromethylstyrene (CMS), aminomethylstyrene (AMS), Rink, Wang, and AmphiSpheres (PEG-modified polystyrene).

Learn more: StratoSpheres for Peptide Synthesis | Agilent

Innovative Solutions for Cell Analysis

Agilent offers a wide range of solutions that provide deep, reliable insights throughout the development and efficacy evaluation of biopharmaceuticals. From real-time live-cell monitoring to phenotypic analysis, cytotoxicity studies, and flow cytometry, Agilent enables researchers to uncover cellular mechanisms and identify novel therapeutic targets.

Agilent Seahorse Extracellular Flux (XF) Flex and Pro Analyzers

Optimized for 3D model analysis and flexible for a wide range of biological models.

Dedicated consumable for 3D studies
Optimized 3D workflows, and customized assay kit

xCELLigence RTCA eSight

The Agilent xCELLigence RTCA eSight system enables label-free, real-time cell analysis under physiological conditions.

2D and 3D imaging
Combine impedance with Brightfield and RGB fluorescence, all in one platform

Intuitive software
Enables real-time data acquisition

Cellular impedance
Measures cell health, morphology, and adhesion without labels

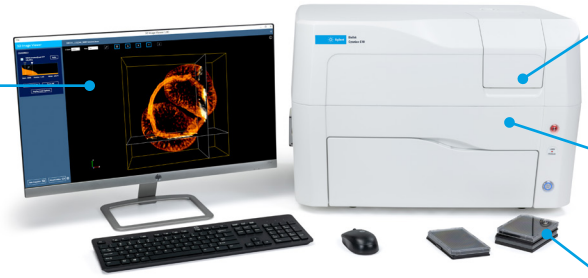
Real-time data
Continuous and noninvasive monitoring of cellular responses

Cytation C10 Confocal Imaging Reader

Automated confocal imaging system for high-resolution live-cell analysis

Confocal/widefield imaging and multimode plate reader in one
Combines high-quality confocal and widefield microscopy with a multimode plate reader

Powerful image analysis
Delivers advanced and quantitative image-based insights



Environmental controls for live-cell imaging
Provides precise temperature and gas control for real-time live-cell imaging

Automated confocal/widefield imaging
Automates 2D and 3D imaging of cells cultered in microplates

NovoCyte flow cytometers

The Agilent NovoCyte portfolio consists of conventional and full-spectrum flow cytometers that deliver advanced insights and enhance productivity.

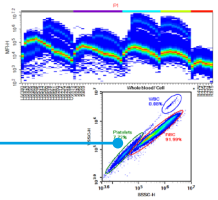
Advanced SiMP detector
High-sensitivity optics and multible detection channels

Expanded flexibility
Modular system adapts to various research needs

Automation
Supports easy, walkaway sample processing

Fiber-optic free
To maximize signal collection rovides precise temperature and gas control for real-time live-cell imaging.

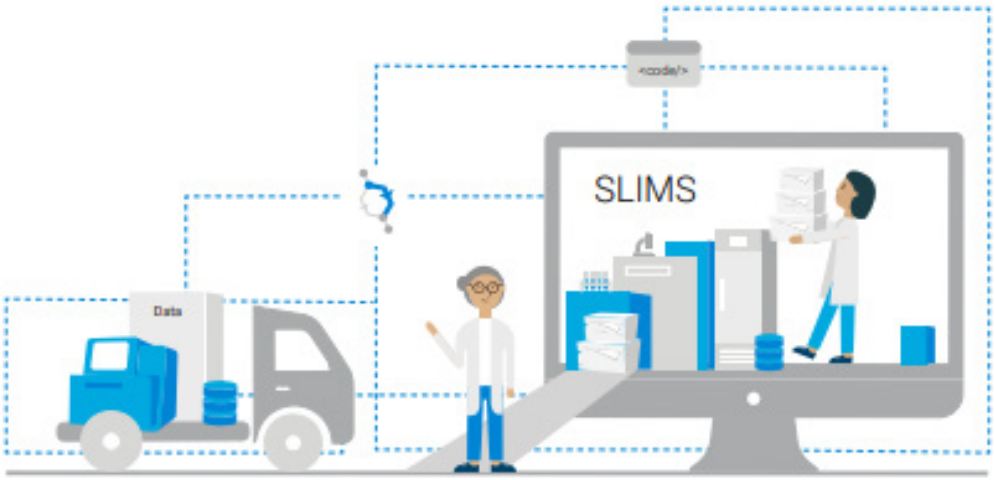
Industry-leading NovoExpress software
Streamline your sample acquisition, data analysis, and reporting, with exceptional user experience



Manage your data in one place

Agilent SLIMS

Agilent SLIMS combines a laboratory information management system (LIMS) and electronic laboratory notebook (ELN) in a single system, enabling comprehensive workflow management. SLIMS is flexible and configurable, helping you increase your lab's productivity. The system is designed to support the requirements of ISO17025, 21 CFR Part 11, HIPAA, and CLIA.



Maximize instrument performance and build a lab for success

Agilent CrossLab services

Agilent CrossLab
From Insight to Outcome

Through proactive services tailored to your needs, Agilent helps extend instrument uptime, generate reliable data, maintain compliance, and reduce service costs with greater predictability. With highly skilled teams, Agilent supports key success factors in the lab—from operational efficiency to regulatory readiness—offering comprehensive learning opportunities for users at every level, from beginners to experts.

Agilent Financial Solutions

Stay up to date with the latest technologies through seamless installment plans with no upfront payment.

Regulatory compliance services

Leverage Agilent's Network Distributed ACE platform to simplify system qualification, ensure data integrity, and streamline documentation with paperless reports and electronic signatures.

CrossLab service plans

Maintain peak performance with CrossLab service plans, which offer flexible warranty extensions and preventive maintenance—helping you avoid unexpected downtime.

Agilent University

Enhance productivity and minimize downtime with integrated training solutions, including on-demand, virtual, and customized online services.

CrossLab Start-Up

Ensure a smooth start with onsite setup, team training, and first-run support for optimal accuracy and usability from day one.

Analytical and application services

Partner with global application experts to overcome analytical challenges and accelerate time to results by establishing new workflows.

Agilent CrossLab Services overview

Lab-wide instrument services	Digital	Compliance

Lab-wide instrument services

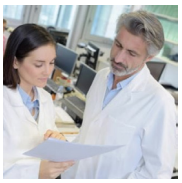
Agilent SLIMS combines a laboratory information management system.

CrossLab Connect

Agilent SLIMS combines a laboratory information management system.

Instrument qualification

Agilent SLIMS combines a laboratory information management system.

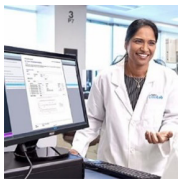


Asset management programs

A program designed for building and acquiring the tools needed for best-in-class lab operations.

Expert guidance

A dedicated customer success manager to help you find insights and data to optimize your lab operations.



Verification services

Verification services delivers cost-effective proof of verification for a range of analytical instruments.

Agilent Knowledge Portal

Agilent Community

Relocations

Lab relocation services

Agilent SLIMS combines a laboratory information management system.

Instrument relocation services

Agilent can move any piece of your equipment, regardless of manufacturer and destination.

Method services

Workflows

End-to-end workflows implementation and optimization, and staff training.

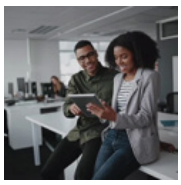
Method and application services

Maintain, optimize, implement, or develop new or existing methods in your lab.

Education services

Training

Building confidence in your operators and maximize the uptime of your chromatography instruments.



A diversified training offer

Our Agilent training experts can deliver training on-site, in an Agilent classroom or even virtually.

Agilent University

Speak to Specialist

Key application notes associated with peptide therapeutics analysis

Analytical Workflow	Application Note	Agilent Instrumentation
Raw Material Identification	Differentiating Biopharmaceutical Raw Materials Using Spatially Offset Raman Spectroscopy	Vaya Raman raw material identity verification system
	Verification of Raw Materials for Synthetic Peptide Production with the Agilent Vaya Raman System	
Peptide Purity and Impurities Analysis	Analysis of a Synthetic Peptide and its Impurities	1290 Infinity II LC and 6545XT AdvanceBio LC/Q-TOF
	Separation of Deamidated Peptides with an Agilent AdvanceBio Peptide Plus Column	1290 Infinity II LC and 6545XT AdvanceBio LC/Q-TOF
	Confirmation of Peptide-Related Impurity Intact Mass Using Agilent 1290 Infinity II Bio 2D-LC and InfinityLab LC/MSD XT	1290 Infinity II bio 2D-LC and InfinityLab LC/MSD XT
	Characterization of Forced Degradation Impurities of Glucagon-Like Peptide-1 Agonists by LC/Q-TOF Mass Spectrometry	1290 Infinity II bio LC and 6545XT AdvanceBio LC/Q-TOF
	Comprehensive Aggregate Profiling of Liraglutide and Semaglutide Using an Agilent 1290 Infinity II Bio 2D-LC and Agilent InfinityLab LC/MSD XT	1290 Infinity II bio 2D-LC and InfinityLab LC/MSD XT
Peptide Sequence Confirmation	LC/MS Based Characterization Workflow of GLP-1 Therapeutic Peptide Liraglutide and Its Impurities	1290 Infinity II LC and 6545XT AdvanceBio LC/Q-TOF
	Identification of Amino Acid Isomers Using Electron Capture Dissociation in the Agilent 6545XT AdvanceBio LC/Q-TOF System	6545XT AdvanceBio LC/Q-TOF with ExD cell
	Molecular Weight Confirmation of a Peptide Using MS Spectral Deconvolution for OpenLab CDS and the Agilent InfinityLab LC/MSD XT System	1290 Infinity II LC and InfinityLab LC/MSD XT
	Comprehensive Characterization of Multiple GLP-1 Analogs Using an Agilent 6545XT AdvanceBio LC/Q-TOF with electron capture dissociation and ExDViewer software	1290 Infinity II bio LC and 6545XT AdvanceBio LC/Q-TOF with ExD cell
Peptide Purification Solutions	Optimizing Analysis and Purification of a Synthetic Peptide Using PLRP-S Columns	1290 Infinity II LC and 6545XT AdvanceBio LC/Q-TOF and 1290 Infinity II preparative LC
	Efficient Purification of Synthetic Peptides at High and Low pH	1290 Infinity II autoscale preparative LC/MSD
Peptide Quantitative Analysis	Quantification of Glucagon-Like Peptide-1 Agonist Tirzepatide Using an Agilent 6495D Triple Quadrupole LC/MS System	1290 Infinity II bio LC and 6495D triple quadrupole LC/MS
	Quantification of Therapeutic Peptide Exenatide in Rat Plasma	
Peptide Drug Stability Analysis	Peptide Drug Stability Analysis Using Agilent InfinityLab LC/MSD and OpenLab CDS Deconvolution	1260 Infinity II Prime bio LC, InfinityLab LC/MSD iQ and InfinityLab LC/MSD XT
Residual Solvent and Trace Elemental Impurities	Analysis of USP <467> Residual Solvents of Class 1, Class 2, and Class 3 Using the Agilent 8890 GC/ FID /5977B MSD System	7697A headspace sampler, 8890 GC, and 5977B GC/MSD
	Determining Elemental Impurities in Pharmaceutical Ingredients Using ICP-MS	7800 ICP-MS
More Application Notes	An In-Depth Analysis of Semaglutide, a Glucagon-Like Peptide-1 Receptor Agonist	1290 Infinity II LC and 6545XT AdvanceBio LC/Q-TOF
	Amino Acid Composition Test of Semaglutide and Liraglutide Using an Agilent 1260 Infinity II Prime Bio LC	1260 Infinity II Prime bio LC
	Rapid Confirmation of GLP-1 Analog (Liraglutide) Using Agilent InfinityLab LC/MSD iQ	1260 Infinity II Prime bio LC and InfinityLab LC/MSD iQ
	Efficient Method Optimization of Semaglutide Analysis Using an Agilent 1260 Infinity II Bio Prime LC System and Blend Assist	1260 Infinity II Prime bio LC

Additional resources

Make column and consumables selection easy

- Consumables workflow ordering guide: Analysis and Purification of Synthetic Peptides by Liquid Chromatography
- Consumables workflow ordering guide: Agilent AdvanceBio Peptide Plus 2.7 µm Column for Peptide Characterization
- Selector tool: Biopharma HPLC Columns
- Brochure: Agilent AdvanceBio Peptide Plus columns

Reliable, efficient, and always innovating for the best results

Agilent InfinityLab LC instruments, columns, and supplies deliver high-quality, dependable analytical results. But Agilent's commitment doesn't stop there. Every component in the InfinityLab product family is designed to work in perfect harmony, helping you streamline workflows, boost efficiency, and reduce operational costs.

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community.agilent.com

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