

Therapeutic Peptides Workflow Resource Guide

End-To-End Workflow Solutions for Therapeutic Peptides

From research discovery to production QA/QC



Ensuring Quality in Therapeutic Peptide Development

Peptides are short chains of amino acids, which are the "building blocks" of proteins. Some peptide molecules can mimic the functions of proteins in the body. They play crucial roles in various biological processes, including cell signaling, immune response, and hormone regulation.

Peptide therapeutics normally refer to peptides composed of 40 or fewer amino acids. One of the main advantages of peptide therapeutics is their ability to target certain cells or receptors more specifically, which can lead to fewer side effects compared to traditional small-molecule drugs. However, they also face challenges such as metabolic instability and poor bioavailability. Peptide therapeutics have gained significant interest and rapid growth in recent years for the treatment of various metabolic diseases, due to their favorable safety and efficacy. Peptide therapeutics are generally less toxic than traditional small-molecule drugs and less immunogenic than biologics.

Over the past decade, significant advancements have been made in the design and formulation development of therapeutic peptides to overcome drug delivery limitations. Many peptide mimetics have been created, which are small molecules that mimic the biological activity of peptides but are more stable and easier to administer. An emerging class of peptide drugs are glucagon-like peptide-1 (GLP-1) agonists. GLP-1 agonists are approved by the U.S. Federal Drug Administration (FDA) for the treatment of type 2 diabetes and obesity management.

According to FDA guidance, studies are required to assess the active pharmaceutical ingredient (API) impurity profiles during the biotherapeutic development and manufacturing processes. Consequently, the purity test of a drug substance becomes a critical quality attribute in ensuring its quality, serving as a benchmark for evaluating and improving purification or synthetic schemes.

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.

Other common peptide drug workflow solutions include relative quantitative analysis of target peptide and specific impurities, amino acid analysis, trace elemental impurity analysis, and residual solvent analysis.

Raw material identification (warehouse- or lab-based solutions)

Raw material identification (RMID) or starting material identification is a critical quality assurance or safety control analysis that is performed for materials used in drug manufacturing. Confirming the identity of received starting materials helps to establish quality as well as prevent contaminated, counterfeit, and incorrectly labeled materials from proceeding to production.

Agilent has developed several workflow solutions for raw material identification using Raman spectroscopy, Fourier transform infrared (FTIR) spectroscopy, or high-performance liquid chromatography (HPLC). They are:

- A warehouse-based solution using the Agilent Vaya Raman raw material identity verification system
- Lab-based solutions using the Agilent Cary 630 FTIR spectrometer or the Agilent 1290 Infinity III bio LC

Handheld Raman: Through-barrier RMID for peptide synthesis

Vaya—a handheld Raman spectrometer—provides an efficient and simple solution for the identification or differentiation of biopharmaceutical materials, such as fluorenylmethoxycarbonyl (Fmoc)-protected amino acids used in peptide synthesis. The Vaya workflow protects the sterility of raw materials by enabling measurement through transparent and opaque containers, which can be performed directly in the warehouse in cGMP manufacturing environments.

Benchtop solutions: Versatile RMID for peptide synthesis

FTIR is also widely used for material identification studies. The Cary 630 FTIR spectrometer, along with Agilent MicroLab software, has a range of intuitive sampling interfaces that can be used to perform quick, easy, and reliable RMID of different sample types in QC laboratories. The optional Agilent MicroLab Pharma software provides secure data storage and user/access privilege management for users operating in GMP environments. Agilent has also developed an HPLC-based workflow solution using the 1290 Infinity III bio LC and Agilent OpenLab ChemStation software for excellent sample separation and sensitive detection for raw or starting material identification.

The Agilent workflow solutions for raw material identification are shown in Figure 1.

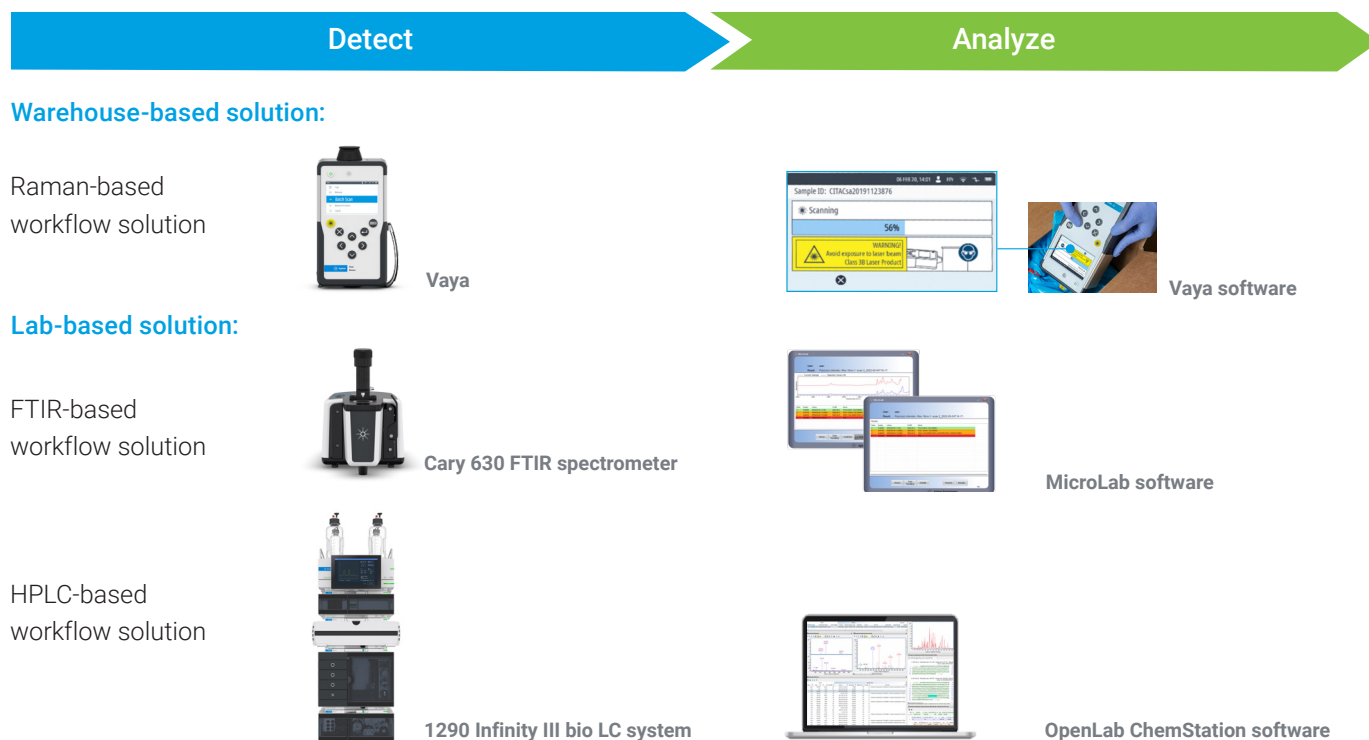


Figure 1: Raw material identification using warehouse-based (Raman spectroscopy) or lab-based (FTIR spectroscopy or HPLC) workflow solutions.

Peptide purity analysis

Analyzing the purity of synthetic peptides is crucial for ensuring their quality and patient safety, especially in therapeutic applications. Commonly used methods for analyzing peptide purity are: HPLC, mass spectrometry (MS), nuclear magnetic resonance, capillary electrophoresis, and amino acid analysis.

For peptide purity analysis, as well as amino acid analysis, Agilent has developed a LC/UV technique with reversed-phase columns, and an LC/MS-based technique. These solutions have been designed for efficient peptide separation and purity confirmation.

The Agilent workflow solutions for peptide purity analysis are shown in Figure 2.

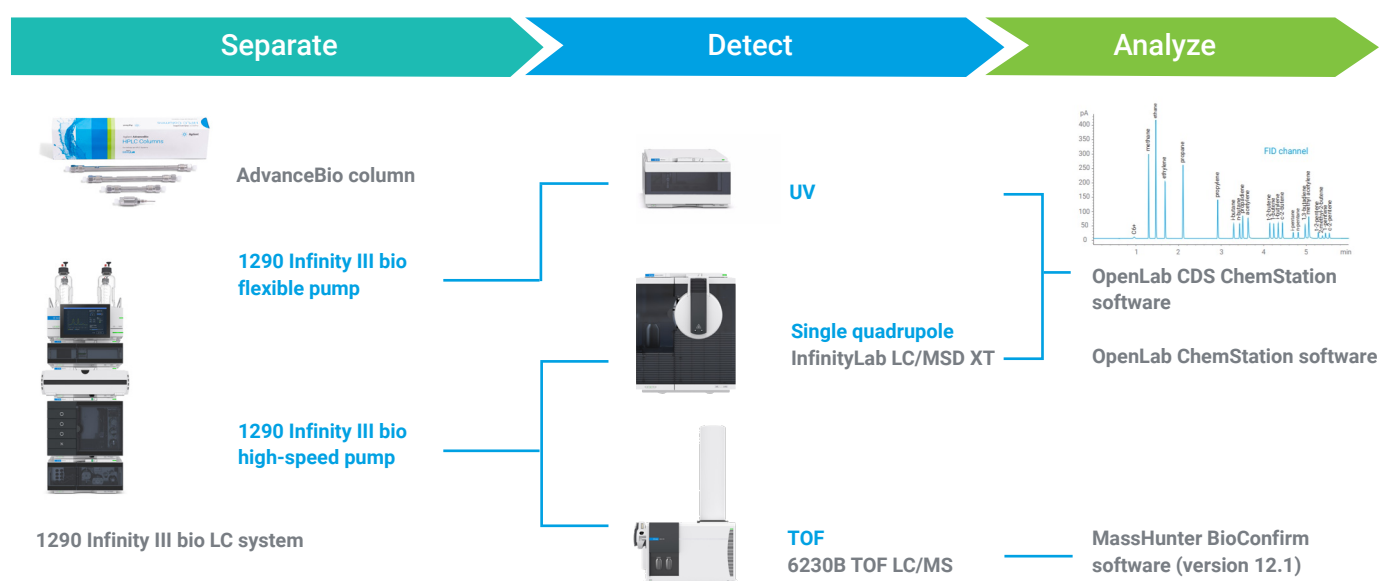


Figure 2: LC/UV- and LC/MS-based workflow solutions for peptide purity analysis.

Amino acid analysis

In synthetic peptide production, it is common to have various impurities (unintended insertions, deletions, or substitutions of amino acids) in the final product. Therefore, the ability to confirm the peptide sequence or its amino acid composition is crucial.

The Agilent AdvanceBio Amino Acid Analysis (AAA) end-to-end solution (including chemicals, standards, columns, and application support) provides fast, sensitive, and automated amino acid analysis. This complete solution combines the advantages of the Agilent Infinity III LC Series instrumentation and column technology with proven precolumn derivatization chemistry. The guidelines of the testing procedure are outlined in the Agilent Amino Acid Analysis 'How-to' Guide.

The Agilent workflow solution for amino acid analysis is shown in Figure 3.

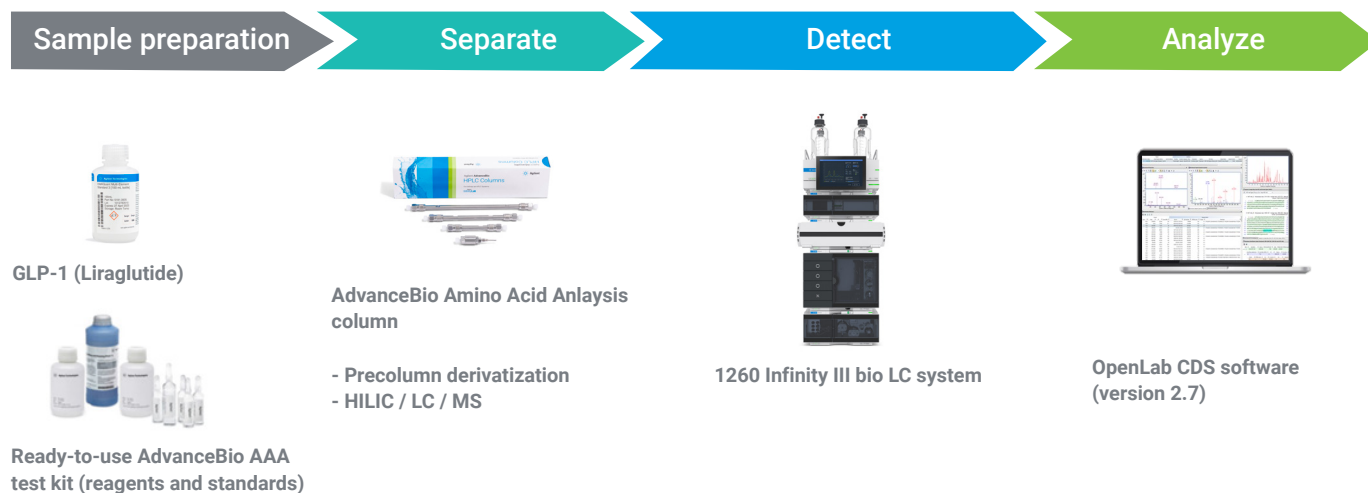


Figure 3: LC-based workflow solution for amino acid analysis.

Peptide impurity profile and aggregation analysis

The presence of impurities can play an important role in the efficacy and safety aspects of biotherapeutics. The impurities associated with the product constitute a significant risk associated with adverse immunological reactions. Impurities generated during the synthetic process—such as diastereoisomers, protected sequences, amino acid insertions or deletions, side-chain reactions, oxidation, and reduction—can present substantial challenges. Therefore, it is crucial to identify and quantify these impurities to ensure that the therapeutic proteins perform as intended without causing adverse effects.

To address these challenges, Agilent has developed several workflow solutions for peptide impurity and aggregation analysis. The workflow solution for peptide impurity analysis (shown in Figure 4) is composed of the Agilent 1290 Infinity III bio LC or 1290 Infinity III 2D-LC and the Agilent 6230B time-of-flight (TOF) LC/MS or 6545XT AdvanceBio quadrupole TOF LC/MS (LC/Q-TOF). The workflow solution for peptide aggregation analysis (shown in Figure 5) is composed of the Agilent 1290 Infinity III 2D-LC system and the Agilent InfinityLab LC/MSD XT or 6230B TOF LC/MS. The Agilent workflow solutions for peptide impurity profile and aggregation analysis are shown in Figures 4 and 5.

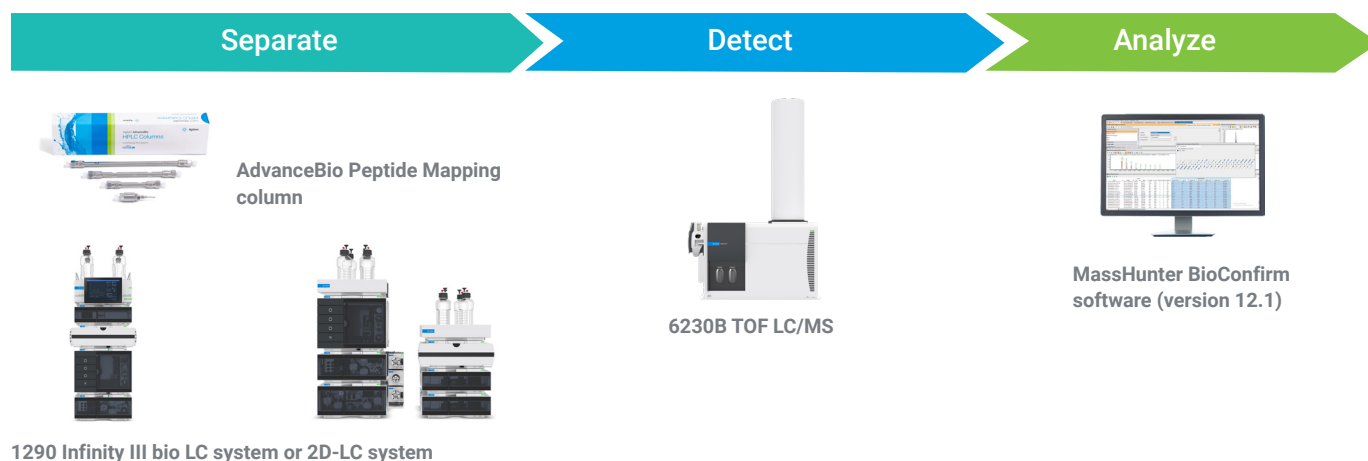


Figure 4: Workflow solution for peptide impurity analysis by LC/MS or 2D-LC/MS.

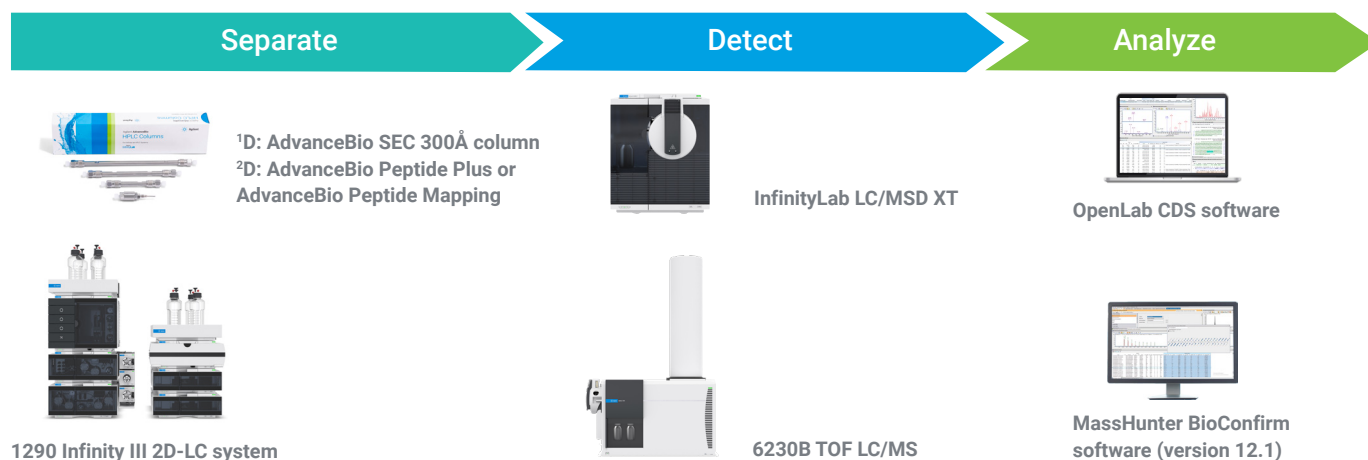


Figure 5: Workflow solution for peptide aggregation analysis by 2D-LC/MS.

Peptide sequence confirmation and isomeric characterization

To better characterize synthetic peptides, Agilent has also developed a sequence confirmation workflow comprising the 1290 Infinity III bio LC, AdvanceBio Peptide Mapping column, 6545XT AdvanceBio LC/Q-TOF system (equipped with an Agilent ExD cell for electron capture dissociation), and MassHunter BioConfirm software (version 12.1). This workflow enables fast, in-depth characterization of peptide sequences, modifications, and impurities. Also, by combining ExD hardware and ExDViewer software tools, peptides with isomeric characteristics, such as aspartate and isoaspartate, can be easily identified.

Moreover, Agilent has developed an ion mobility mass spectrometry (IM-MS)-based workflow solution using the Agilent 6560C ion mobility LC/Q-TOF system for peptide isomer analysis. IM-MS is a separation technique that separates ions by their size and shape as a function of the ion's collision cross section (CCS). Due to their slight differences in structure (shape), GLP-1 peptide and its isomers will post different drift times and CCS values under IM-MS analysis.

The Agilent solution for peptide sequence confirmation is shown in Figure 6, and the solution for peptide impurity and isomeric analysis is shown in Figure 7.



Figure 6: Workflow for peptide sequence confirmation.

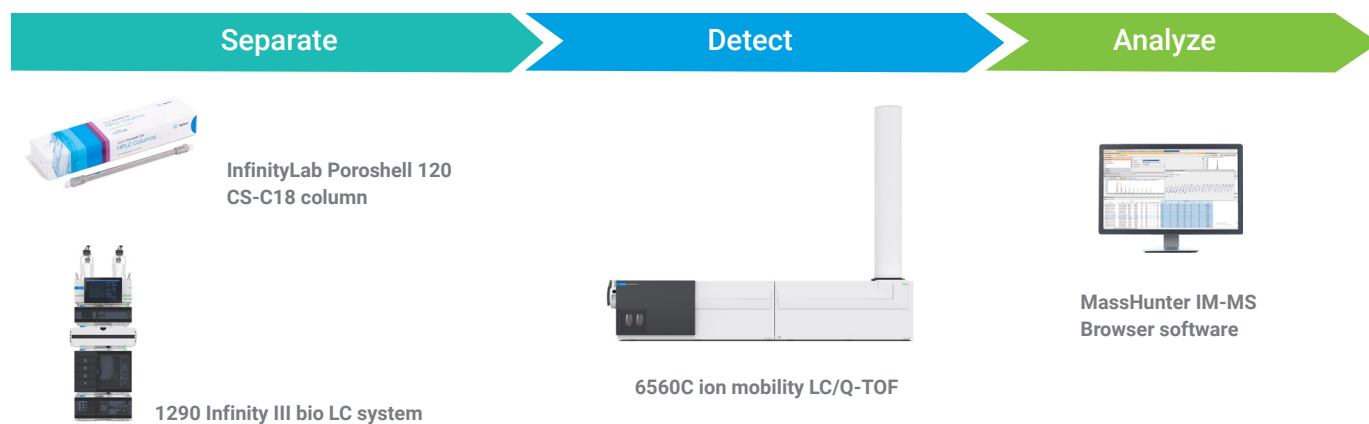


Figure 7: Workflow solution for peptide impurity and isomeric analysis by ion mobility LC/Q-TOF.

Preparative-scale peptide purification solutions

Agilent offers the most comprehensive portfolio of flexible and reliable workflow solutions for sample purification by LC. No matter what scale of LC purification you are working at, Agilent has high-performance instrumentation, columns, software, and services that ensure highest purity and maximum recovery. With reversed-phase preparative LC columns, you will achieve the same robustness, reliability, quality, and separation power as Agilent analytical columns.

Agilent 1290 Infinity III LC preparative-scale peptide purification systems are displayed in Figure 8.

A comparison of analyte quantities and flow rates for Agilent systems operating at different scales is shown in Figure 9.

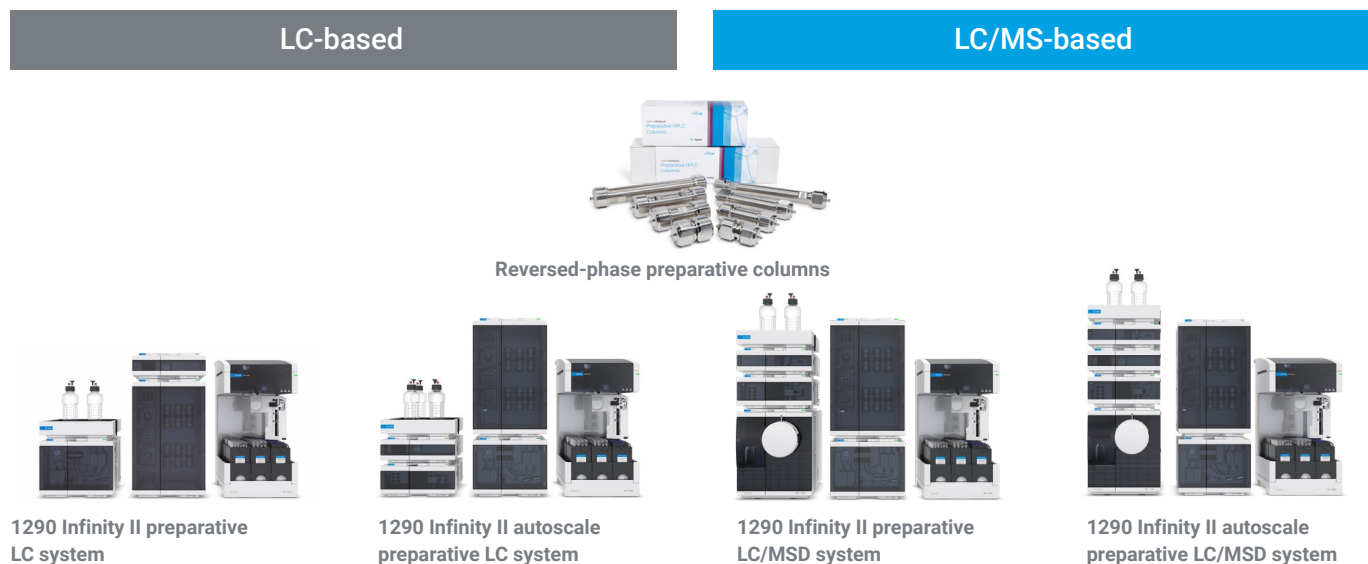


Figure 8: Preparative-scale peptide purification solutions.

Column internal diameter	Analytical	Semipreparative and preparative			
2.1 mm	0.4 - 0.6 mL/min				
3.0 mm	0.5 - 1 mL/min				
4.6 mm	1 - 2 mL/min				
9.4 or 10 mm		4 - 10 mL/min			
21.2 mm			18 - 42 mL/min		
30 mm				34 - 85 mL/min	
50 mm					94 - 236 mL/min
Purification range (mg)	1 - 15	7 - 70	30 - 300	64 - 640	180 - 1800
Instrumentation	1220/1260/1290 Infinity III analytical-scale LC purification systems 1260 Infinity II preparative LC systems 1290 Infinity II preparative LC systems				

Flow range extensions made possible by exchangeable pump heads

InfinityLab preparative LC columns portfolio

Figure 9: Comparison of analyte quantities and flow rates for Agilent systems operating at different scales.

Peptide quantitation in biological matrices

Peptide therapeutics like GLP-1s are highly potent, and require only low dosages for effective administration. Therefore, sensitive quantitative measurements are essential when examining the pharmacokinetic and pharmacodynamic profiles of peptide therapeutics. However, the unique structures of these peptides result in poor MS ionization and fragmentation, making the development of sensitive and robust LC/MS method for GLP-1 quantification challenging.

Agilent has developed a highly sensitive LC/MS workflow for quantitation of the GLP-1 analogs in diverse matrices using the Agilent AssayMAP Bravo protein sample prep platform, the 1290 Infinity III bio LC system with AdvanceBio Peptide Mapping column, and an ultrasensitive, highly reproducible triple quadrupole LC/MS (LC/TQ).

The Agilent workflow solution for peptide quantitative analysis is shown in Figure 10.

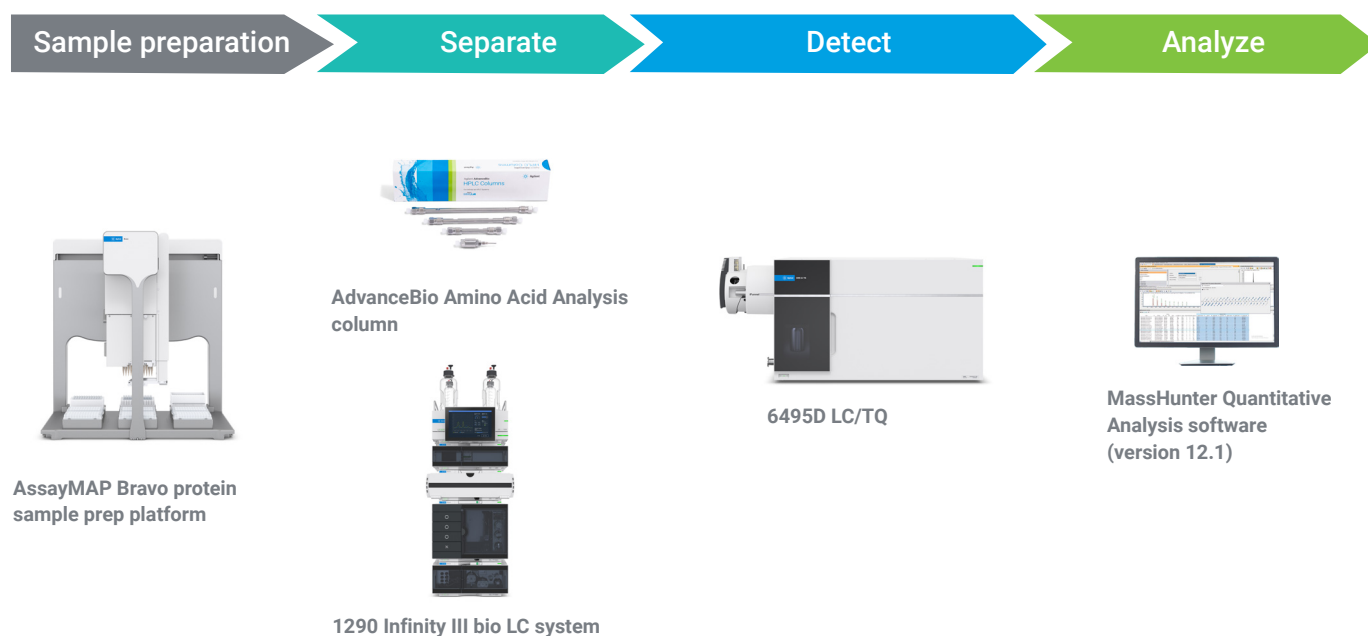


Figure 10: Workflow solution for peptide quantitative analysis by LC/MS.

Finished product quality control

UV-Vis absorption spectroscopy is commonly used with peptides for determining concentration and quality control (QC). The concentration of peptides can be easily estimated by measuring the UV absorbance at 210 to 220 nm and the established absorption coefficient. QC processes in the biopharmaceutical industry rely on UV-Vis spectroscopy, which is a mature and well-established technique.

The Agilent Cary 3500 Multicell UV-Vis spectrophotometer measures samples, standards, and controls simultaneously and with high accuracy, eliminating analytical variables. The Cary 3500 Multicell UV-Vis is designed for measuring up to seven samples and a reference (or other combinations in the eight cell positions). It is available in either an ambient, temperature-controlled, or multiple-temperature-zone (multizone) configuration.

The Agilent workflow solution for finished product QC by UV-Vis analysis is shown in Figure 11.



Figure 11: Workflow solution for finished product QC by UV-Vis analysis.

Trace elemental impurity analysis

Trace elemental impurities in pharmaceutical drug products may be toxic, affect drug stability or shelf life, and may cause unwanted side effects. Therefore, the current USP and ICH chapters require more elements to be monitored at lower levels than previously, and recommend modern instrumental analytical procedures to determine the concentration of elemental impurities. Manufacturers must be able to demonstrate that their biological drugs comply with the limits for the listed elements in their final drug formulation.

Figure 12 presents an Agilent workflow for trace elemental impurity analysis by ICP-MS. The Agilent 7850 ICP-MS is proven to be ideal for the determination of trace elemental impurities in pharmaceutical ingredients, producing excellent results in terms of sensitivity, stability, robustness, recovery, and detection limits for all the required elements.

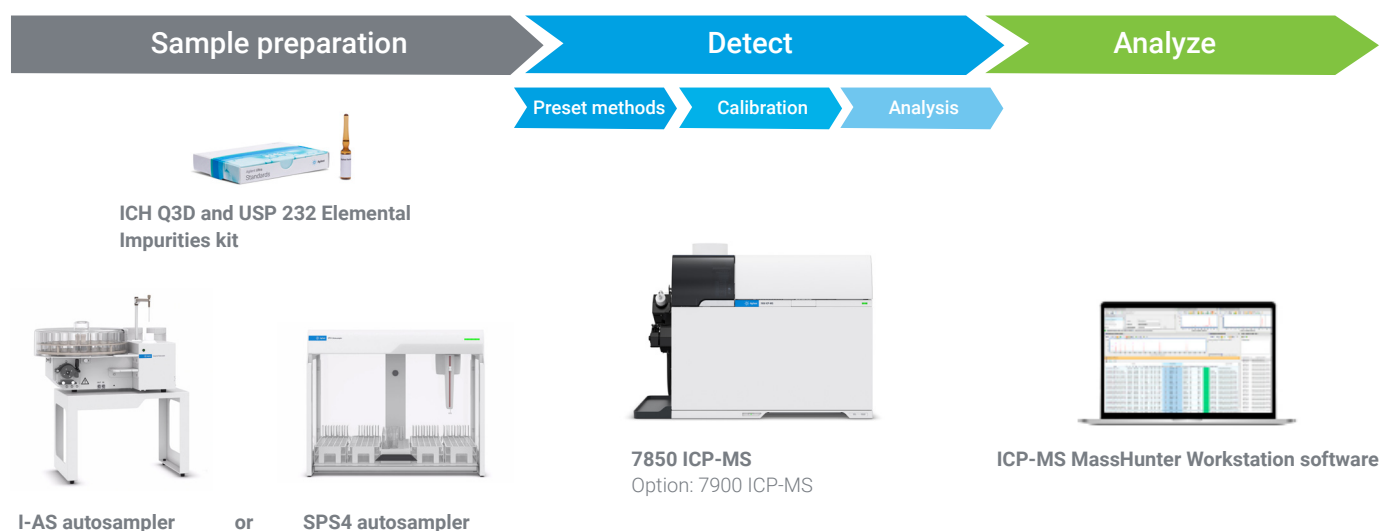


Figure 12: Workflow solution for trace elemental impurity analysis by ICP-MS.

Residual solvent analysis

Similarly, residual solvents in pharmaceutical drug products do not enhance product efficacy; therefore, they must be removed to meet product specification and GMPs.

Agilent offers the most comprehensive portfolio of flexible and reliable solutions for residual solvent analysis by GC or GC/MS. No matter what kind of residual solvent analysis (routine, high throughput, or unknown) you are working on, the Agilent 8890 GC system, equipped with an Agilent 8697 headspace sampler (HS) and inert tee, provides an excellent method for separating, identifying, and quantifying all the relevant residual solvents outlined by USP <467>.

The Agilent workflow solution for residual solvent analysis is shown in Figure 13.

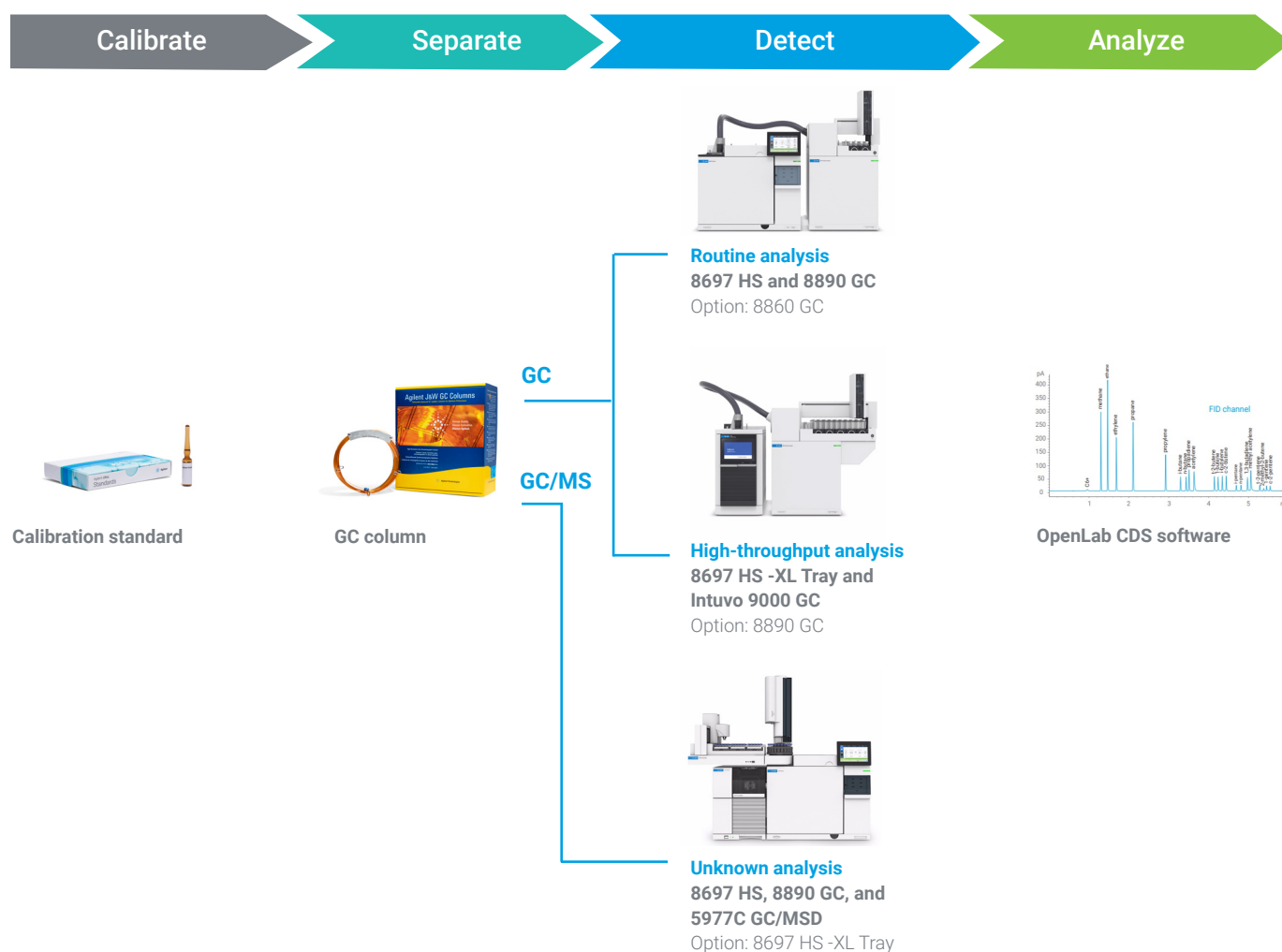


Figure 13: Workflow solution for residual solvent analysis by GC or GC/MS.

References

Peptide purity analysis

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- 2) Ryu, C.Y. Rapid Confirmation of GLP-1 Analog (Liraglutide) Using Agilent InfinityLab LC/MSD iQ. Agilent Technologies application note, publication number [5994-7415EN](#), 2024.
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- 3) Ryu, C.Y. Peptide Drug Stability Analysis Using Agilent InfinityLab LC/MSD and OpenLab CDS Deconvolution. Agilent Technologies application note, publication number [5994-7500EN](#), 2024.

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Analytical- and preparative-scale peptide purification

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- 3) Rieck, F. Efficient Purification of Synthetic Peptides at High and Low pH. Agilent Technologies application note, publication number [5994-5311EN](#), 2022.

Additional peptide analysis workflows

- 1) Ryu, C.Y. Amino Acid Composition Test of Semaglutide and Liraglutide Using an Agilent 1260 Infinity II Prime Bio LC. Agilent Technologies application note, publication number [5994-7749EN](#), 2024.
- 2) Welsby, C., Grise S. and Prulliere, F. Verification of Raw Materials for Synthetic Peptide Production with the Agilent Vaya Raman System. Agilent Technologies application note, publication number [5994-7610EN](#), 2024.
- 3) Amino Acid Analysis – ‘How-To’ Guide. Agilent Technologies application note, publication number [5991-7694EN](#), 2021.

Trace elemental impurity analysis by ICP-MS

- 1) Data Integrity Options for GxP facilities: For Agilent ICP-MS and ICP-QQQ spectrometers. Agilent Technologies flyer, publication number [5994-4746EN](#), 2022.
- 2) Sanderson, J.; Whitecotton, L. Analysis of Artificial Tear Eye Drops for Elemental Impurities. Agilent Technologies application note, publication number [5994-1561EN](#), 2022.
- 3) Whitecotton, L.; McCurdy, E.; Jones, C.; Liba, A. Validating performance of an Agilent ICP-MS for USP <232>/<233> & ICH Q3D(R2)/Q2(R1). Agilent Technologies application note, publication number [5991-8335EN](#), 2022.
- 4) USP <232>/<233> and ICH Q3D Elemental Impurities Analysis: The Agilent ICP-MS Solution. Agilent Technologies white paper, publication number [5991-8149EN](#), 2021.
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Residual solvent analysis by GC or GC/MS

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- 2) Zhang, Y.; Na, S. Analysis of USP <467> Residual Solvents of Class 1, Class 2, and Class 3 Using the Agilent 8890 GC/ FID /5977B MSD System. Agilent Technologies application note, publication number [5994-1488EN](#), 2019.
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Optimized workflows: Configuration and ordering information

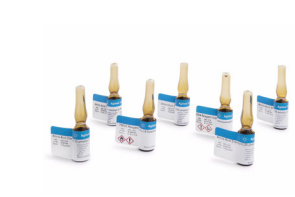
Raw material identification (warehouse- or lab-based solutions)

Bundle	Vaya (Warehouse based)	FTIR (Lab based)	LC/UV (Lab based)
Device	 Vaya p/n G6915A	 Cary 630 FTIR spectrometer p/n G8043AA or G8044AA (with PC) Recommended: #200 and #320 PIKE-162-5450	 1290 Infinity III bio LC p/n G7131A or G7132A, G7137A, and G7116B, G7114B (VWD) or G7117B (DAD)
Software	 Vaya software	 MicroLab MicroLab Pharma p/n G4984AA	 OpenLab ChemStation p/n M8301AA p/n M8510AA (instrument driver for Agilent LC) Optional: p/n M8360AA - 3D UV (PDA) add-on

Peptide purity analysis

Bundle	LC/UV	LC/MSD XT	TOF LC/MS
Column		AdvanceBio Peptide Plus p/n 695775-949 or AdvanceBio Peptide Mapping p/n 653750-902	
LC		1290 Infinity III bio LC Required: p/n G7131A or G7132A; G7137A; and G7116B Either: p/n G7114B (VWD) or G7117B (DAD)	
MS		 InfinityLab LC/ MSD XT p/n G6135BA (with OpenLab ChemStation software) or p/n G6135CA (with OpenLab CDS software)	 6230B TOF LC/MS p/n G6230BA
Software	 In case customer has third-party CDS, if UV only, below software is optional OpenLab CDS p/n M8414AA or OpenLab ChemStation p/n M8380AA and M8510AA	 OpenLab ChemStation p/n G6135BA or OpenLab CDS p/n G6135CA is included. Add Deconvolution p/n M8363AA	 BioConfirm (version 12.1) p/n M6025AA Single workstation: p/n M6026AA or M6027AA For network workstation: p/n M6035AA or M6036AA or M6037AA

Amino acid analysis

Bundle	LC/UV or LC/FLD
Sample preparation	 AdvanceBio Amino Acid Reagents Kit (10 standards and reagents) p/n 5190-9426
Column	 AdvanceBio Amino Acid Analysis columns p/n 655950-802
LC	 1290 Infinity III bio LC Required: p/n G7131A or G7132A; G7137A; and G7116B Either: p/n G7114B (VWD), G7117B (DAD) or G7121B (FLD)
Software	 OpenLab CDS p/n G6135CA

Peptide impurity and aggregation analysis

Bundle	LC/MSD XT	LC/TOF	2D-LC/Q-TOF
Column		AdvanceBio Peptide Plus p/n 695775-949 or AdvanceBio Peptide Mapping p/n 653750-902	 First dimension AdvanceBio SEC 300 Å, 2.7 µm p/n PL1180-5301 Second dimension Poroshell 120 CS-C18 p/n 695775-942
LC		1290 Infinity III bio LC Required: p/n G7131A or G7132A; G7137A; and G7116B Optional: p/n G7114B (VWD) or G7117B (DAD)	 1290 Infinity III 2D-LC Required: p/n G7120A, G7167B, G1170A, and G7116B Optional: p/n G7114B (VWD) or G7117B (DAD)
MS	 InfinityLab LC/MSD XT p/n G6135BA (with OpenLab ChemStation software) or p/n G6135CA (with OpenLab CDS software)		6230B TOF LC/MS p/n G6230BA or 6545XT AdvanceBio LC/Q-TOF p/n G6549AA
Software	 OpenLab ChemStation p/n G6135BA or OpenLab CDS p/n G6135CA is included. Add Deconvolution p/n M8363AA		BioConfirm (version 12.1) p/n M6025AA Single workstation: p/n M6026AA or M6027AA For network workstation: p/n M6035AA or M6036AA or M6037AA

Peptide sequence confirmation

Bundle	LC/Q-TOF	
Column		AdvanceBio Peptide Mapping p/n 653750-902
LC		1290 Infinity III bio LC Required: p/n G7131A or G7132A; G7137A; and G7116B Optional: p/n G7114B (VWD) or G7117B (DAD)
MS		6545XT AdvanceBio LC/Q-TOF with ExD cell p/n G6549AA and G1997AA
Software		BioConfirm (version 12.1) p/n M6025AA Single workstation: p/n M6026AA or M6027AA For network workstation: p/n M6035AA or M6036AA or M6037AA ExDViewer (version 4.6.12)

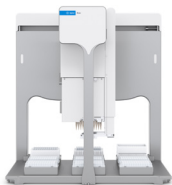
Peptide impurity and isometric characterization

Bundle	Ion Mobility LC/Q-TOF	
Column		InfinityLab Poroshell 120 CS-C18 p/n 679775-942
LC		1290 Infinity III bio LC Required: p/n G7131A or G7132A; G7137A; and G7116B Optional: p/n G7114B (VWD) or G7117B (DAD)
MS		6560C ion mobility LC/Q-TOF p/n G6560CA
Software		MassHunter IM-MS Browser

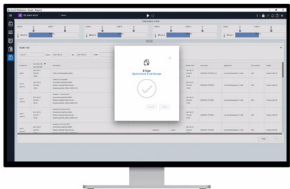
Peptide analytical- and preparative-scale purification

Bundle	Analytical LC/UV	Preparative LC/UV	Preparative LC/MSD XT
Column			
	AdvanceBio Peptide Mapping p/n 653750-902		
LC	 1260 Infinity III bio-inert analytical-scale LC purification system with fraction collector p/n G5654 and G5664B	 1290 Infinity II preparative LC p/n G7161B, G7158B, G7114A, and G7163B Optional: p/n G7166A	 1290 Infinity II preparative LC/MSD XT p/n G7161B, G7111B, G7158B, G7114A, G7115A, G7170B, G9324A, G1170A, G4738A, and G7163B Optional: p/n G7166A
MS			 InfinityLab LC/MSD XT p/n G6135BA (with OpenLab ChemStation software)
Software	 OpenLab ChemStation p/n M8380AA and M8510AA	 Automated Purification Software for OpenLab ChemStation p/n M8368AA	

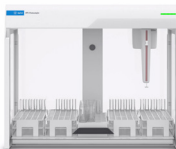
Peptide quantitative analysis

Bundle	LC/TQ	
Sample preparation		AssayMAP Bravo protein sample prep platform p/n G5571AA
Column		AdvanceBio Peptide Mapping p/n 653750-902
LC		1290 Infinity III bio LC Required: p/n G7131A or G7132A; G7137A; and G7116B Optional: p/n G7114B (VWD) or G7117B (DAD)
MS		6495D LC/TQ p/n G6495DA
Software		MassHunter Quantitative Analysis (version 12.1)

Finished product QC

Bundle		UV/Vis
Device		Cary 3500 Multicell UV-Vis spectrophotometer p/n G9874A #001 Option: p/n G9874A #002: Purge kit
Software		Cary UV Workstation Cary UV Workstation Plus without PC p/n G5194AA or Cary UV Workstation Plus with PC p/n G5195AA or Cary UV Networked Workstation software without PC p/n G6894AA or Cary UV Networked Workstation software with PC p/n G6896AA

Trace elemental impurity analysis

Bundle	ICP-MS	
Sample preparation		I-AS autosampler p/n G3160C
		SPS 4 autosampler p/n G8415A
Standard		USP standard (ICH Q3D and USP 232) Elemental Impurities kit p/n 5191-4553 (Five standards: p/n 5190-9766, 5190-9767, 5190-9768, 5190-9769, 5190-9770)
ICP-MS		7850 ICP-MS p/n G8422AA Option: 7900 ICP-MS p/n G8403AA
Software		ICP-MS MassHunter p/n G7201D

Residual solvent analysis

Bundle	GC (Routine analysis)	GC (High-throughput analysis)	GC/MS (Unknowns analysis)
Calibrate		USP 467 Class 1 : p/n 5190-0490 USP 467 Class 2A : p/n 5190-0492 USP 467 Class 2C : p/n 5190-0493	
GC column		Headspace injection: J&W DB-624 (60 m × 0.25 mm, 1.4 µm) p/n 122-1364 Liquid injection: J&W DB-WAX UI (30 m × 0.25 mm, 0.25 µm) p/n 122-7032UI	
GC	 8697 HS and 8890 GC 8697 HS p/n G4511A 8890 GC p/n G3540A Option: 8860 GC p/n G2790A	 8697 -XL Tray p/n G4512A Intuvo 9000 GC p/n G3950A Option: 8890 GC p/n G3540A	 8697 HS p/n G4511A 8890 GC p/n G3540A 5977C GC/MSD Option: 8697 -XL Tray p/n G4512A
MS			 5977C GC/MSD Inert Plus system p/n G7077CA
Software		OpenLab CDS Workstation Plus p/n M8410AA or OpenLab CDS AIC p/n M8420AA OpenLab CDS Instrument Connection p/n M8431AA	

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