Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

ACQUITY UPLC H-Class Bio System

Built for your biomolecular characterization

THE BIOCOMPATIBLE UPLC PLATFORM

The ACQUITY UPLC® H-Class Bio System – the latest extension to the family of ACQUITY UPLC Systems, brings the benefits of UPLCt Technology to the analysis of biological macromolecules. This system is designed and developed for the types of complex samples that often require multiple chromatographic techniques for the complete characterization of biomolecules.

UPLC Technology has already been proven to deliver improved chromatographic results with enhanced productivity to laboratories seeking robust characterization in bioseparations. The ACQUITY UPLC H-Class Bio System retains all of these benefits while bringing increased compatibility for biomolecule analysis.

With its flow-through-needle injector and quaternary solvent delivery system, the ACQUITY UPLC H-Class Bio System has been specifically engineered with an inert flow path and Auto•Blend Plus™ Technology to deliver the benefits of UPLC in a system optimized for the analysis of proteins, peptides, nucleic acids, and glycans.

The ACQUITY UPLC H-Class Bio System delivers the same high quality separations that you expect from UPLC, with the flexibility and ruggedness to run whatever mode of chromatography you require – ion exchange (IEX), size exclusion (SEC), hydrophilic interaction (HILIC), or reversed phase – all on the same system. You can perform the required specific assays for protein characterization including peptide mapping, intact protein analysis, and glycan analysis.

ACQUITY UPLC H-Class Bio System.

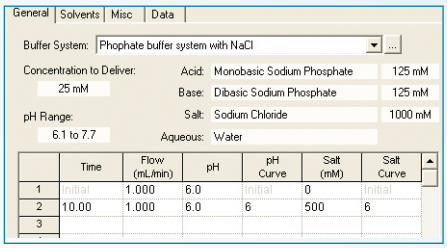
CHROMATOGRAPHIC FLEXIBILITY

- Reversed phase
- Ion exchange (IEX)
- Size exclusion (SEC)
- Hydrophilic interaction (HILIC)

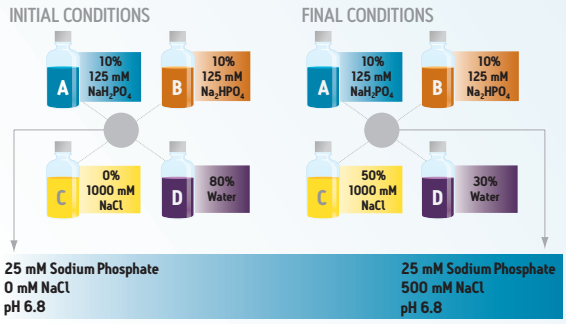
AUTO•BLEND PLUS TECHNOLOGY

Efficient gradient management, systematic method development, and reliable routine operation

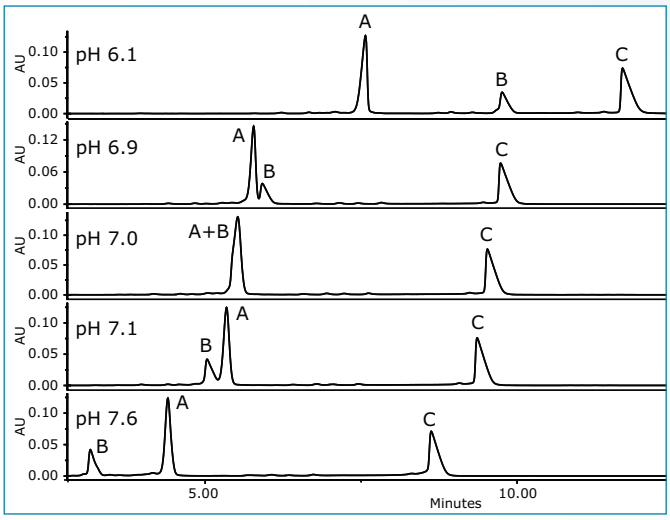
One of Waters' key technologies is Auto•Blend Plus, a software technique that maximizes separation selectivity by using pure solvents and concentrated buffer stocks with the ACQUITY UPLC H-Class Bio System's quaternary solvent manager. Desired mobile phase combinations and gradients are automatically blended on demand to match the optimum retention and selectivity requirements of the separation.



The user programs the desired pH and ionic strength gradient to be derived from the stored library of buffers.



Auto•Blend Plus calculates the percentage of flow taken from each of the four solvents at each point in the gradient time range.



A mixture of proteins – alpha-Chymotrypsinogen A (peak A), Ribonuclease A (peak B), and Cytochrome C (peak C) – was separated using cation exchange chromatography using the ACQUITY UPLC H-Class Bio System with a Protein-Pak™ Hi Res SP, 7 µm, 4.6 x 100 mm Column.

Auto•Blend Plus Technology was used to automatically find the optimum concentration of mobile phase modifier in an unattended series of automated runs without the need to prepare several individual solvents. Note the small protein peak (peak B) that elutes in the center at pH 6.1, coelutes with the first peak at pH 7.0, and moves to elute as the first peak at pH 7.6.

In reversed phase separations, for example peptide maps or protein analysis, the method can be adjusted by changing the concentration of mobile phase modifier and the organic solvent. Instead of manually preparing many solvent mixtures, the ACQUITY UPLC H-Class Bio System makes blends from pure solvents and concentrated stocks on demand. This process ensures that the optimum method is identified and that the method can then be transferred to other laboratories and run routinely with less chance of variability.

Auto•Blend Plus provides flexibility to use common protein separation techniques with the ACQUITY UPLC H-Class Bio System. Both IEX and SEC are optimized with the careful adjustment of pH and ionic strength, typically a labor-intensive and painstakingly iterative method development and adjustment process. With Auto•Blend Plus, you can specify the pH and ionic strength required for the mobile phase and have the software calculate the proportions of buffer stocks required for the desired conditions. The automated computation can be based on known pK values or an empirical calibration table, making any possible buffer combination available. Users can also create their own library of unique buffer combinations.

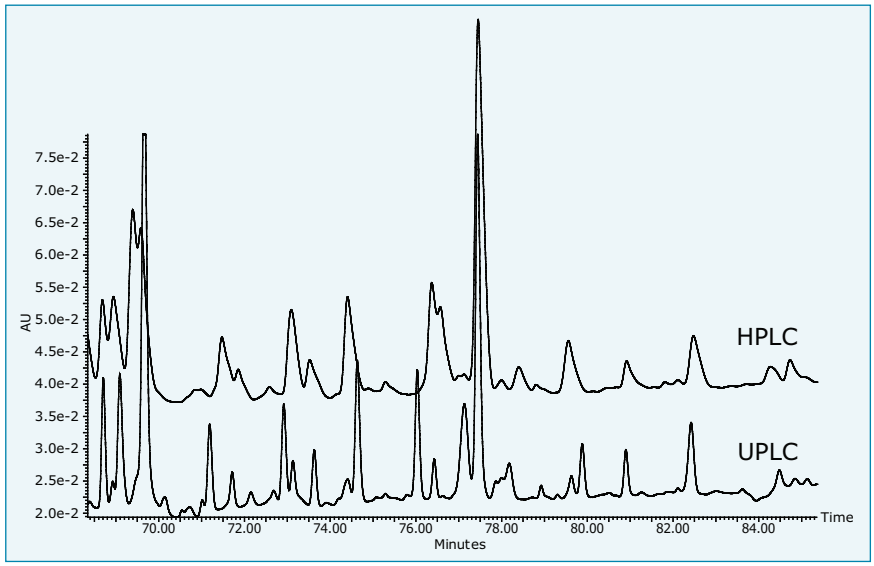
This proprietary software ensures the best separation conditions are identified and routinely used with the ACQUITY UPLC H-Class Bio System – maximizing laboratory productivity.

BENEFITS OF UPLC FOR BIOSEPARATIONS

UPLC delivers the sensitivity, resolution, and throughput required for biomolecule analyses, giving you the answers that you need more readily.

Increased resolution and sensitivity

Exceptional resolution is possible with UPLC because separations obtained using sub-2-µm columns can only be achieved with instrumentation that provides reduced system volumes and minimized band broadening to preserve the high efficiency of these separations. This reduced system dispersion is combined with exact control of flow and compositional accuracy to ensure complete control over retention and selectivity. Additionally, Waters offers a series of ACQUITY UPLC columns and chemistries specific for bioseparations. Each of these separation technologies is developed, proven, and quality-control tested with the intended application. This is the trademark of UPLC Technology – a holistic design philosophy that is founded on maximizing your chromatographic potential.

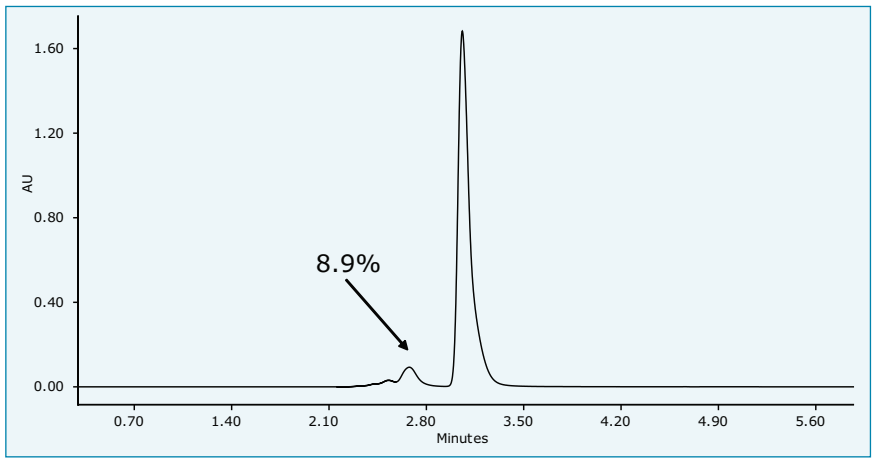


Comparing a section of a peptide map of Phosphorylase b from 68 to 85 min, the improvement in resolution from HPLC to UPLC is clearly observed. With UPLC, more peaks are resolved and the peaks are sharper – making it much easier to detect and quantitate the small peaks that represent trace amount of modified or damaged protein.

Increased productivity

UPLC is more than just fast LC. With fast LC, separation parameters are modified to reduce the time of the separation, but compromises to resolution, sensitivity, or robustness are commonly required. UPLC provides increased throughput that is made possible by better sensitivity and chromatographic resolution; this means less frequent need to reanalyze samples that have marginal or unexpected results.

This translates into measureable business benefits including lower cost of analysis, faster decisions, and reduced time-to-market – giving you the competitive advantage. However, when fast is what's needed, measureable improvements in run time can be made by optimizing your separation using smaller particles, or decreasing your column length.



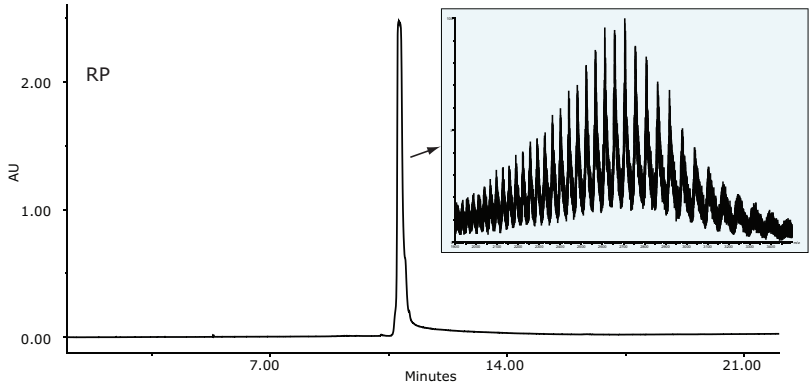
The ACQUITY UPLC H-Class Bio System, and proprietary ACQUITY UPLC BEH200 SEC 1.7-µm column chemistry, can be used to measure aggregates relative to the amount of monomeric IgG. This SEC analysis of a humanized monoclonal antibody delivers improved resolution and sensitivity in under 3.7 min.

BUILT FOR RELIABLE OPERATION UNDER DEMANDING CONDITIONS

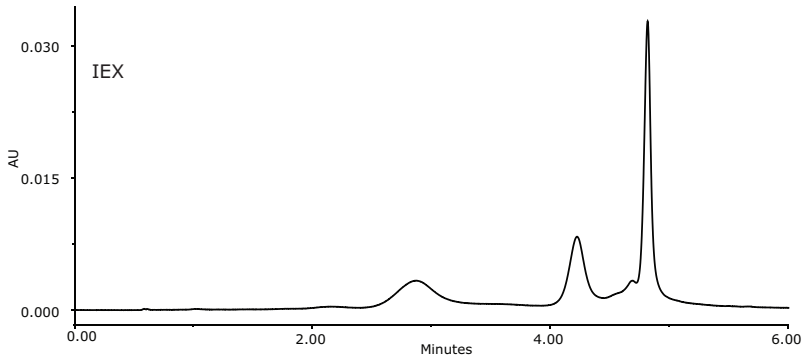
Working together with our users – scientists dedicated to supporting research and businesses focused on biopharmaceutical products – Waters developed the ACQUITY UPLC H-Class Bio System specifically to make chromatography of biological macromolecules easier and more robust. Constructed with materials that were selected as the ultimate in corrosion resistance, the system is uniquely suited for the high ionic strength aqueous conditions commonly used for protein separations. Additionally, the system flow path has been tested to ensure that it minimizes undesirable protein interactions and leaching of material into the mobile phase. The result: a system that is ideally suited for routine, trouble-free operation with the maximum recovery of biomolecules without inadvertent modification during analysis.

Using Auto•Blend Plus with the ACQUITY UPLC H-Class Bio System for multi-mode structural characterization of a chimeric monoclonal antibody

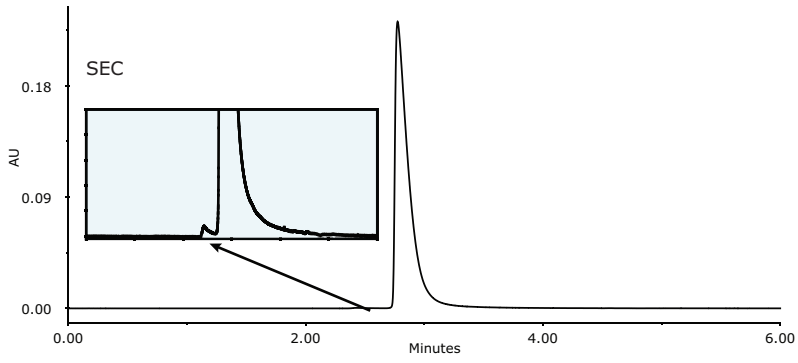
Reversed phase LC/MS used with the ACQUITY UPLC H-Class Bio System to measure glycosylation variation, structural changes, and post-translational modifications.



Cation exchange chromatography used with the ACQUITY UPLC H-Class Bio System to measure charge variants.



Size exclusion chromatography used with the ACQUITY UPLC H-Class Bio System to measure aggregates and other variations in size and shape.



SYSTEM HALLMARKS

Multi-solvent blending

The ACQUITY UPLC H-Class Bio System provides laboratories with the ultimate in flexibility with its multi-solvent blending capability, allowing for binary, ternary, or quaternary gradient operation. The addition of an optional, six-port solvent select valve greatly expands your solvent choices allowing you to optimize method conditions.

Sample introduction that's reliably quantitative

The system's flow-through-needle sample manager is accurate and precise with excellent sample recovery and virtually no carryover, resulting in more reliable and reproducible results.

Detection optimized for UPLC separations

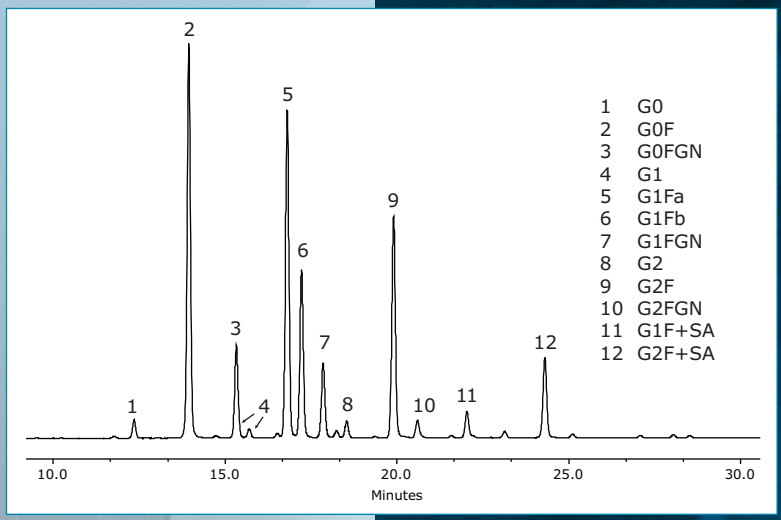
A UPLC separation is not complete without innovative, ultra-low-dispersion detectors designed to maintain peak integrity. Waters offers the widest range of UPLC-optimized detectors – from optical to mass spectrometry – enabling you to meet multiple detection requirements for biological applications.

Accurate column temperature control

A variety of column heaters and multi-column managers (with column switching) offers the lowest possible dispersion and exact temperature management for the best control of chromatographic selectivity and the highest retention time precision. Accurate results are ensured using methods that can be seamlessly transferred to other laboratories – down the hall or around the world.

APPLICATIONS FLEXIBILITY

The ACQUITY UPLC H-Class Bio System is suited for a variety of biochemical characterization applications. Here, the system is used for the analysis of glycans of human IgG using HILIC and fluorescence detection.



Pooled human IgG was digested with PNGase F, and the released glycans were labeled with 2-aminobenzamide. The separation was performed using the ACQUITY UPLC H-Class Bio System equipped with a fluorescence detector and an ACQUITY UPLC BEH Glycan, 1.7-µm, 2.1 x 150 mm Column.

THE COMPREHENSIVE SOLUTION FOR BIOPHARMACEUTICAL APPLICATIONS

ACQUITY UPLC H-Class Bio System

- Quaternary Solvent Manager
- Sample Manager
- Column heaters and multi-column managers

UPLC optimized detection capabilities

- Photodiode Array
- UV/Visible
- Fluorescence
- Evaporative Light Scattering
- Single and tandem quadrupole mass detectors
- Time-of-Flight mass spectrometers

Software and informatics

- Empower™ and MassLynx™ software
- BiopharmaLynx™ Application Manager

Full range of applications

- Peptide mapping
- Intact protein analysis
  - Ion exchange
  - Size exclusion
  - Reversed phase
- Glycan analysis
- Oligonucleotide analysis

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