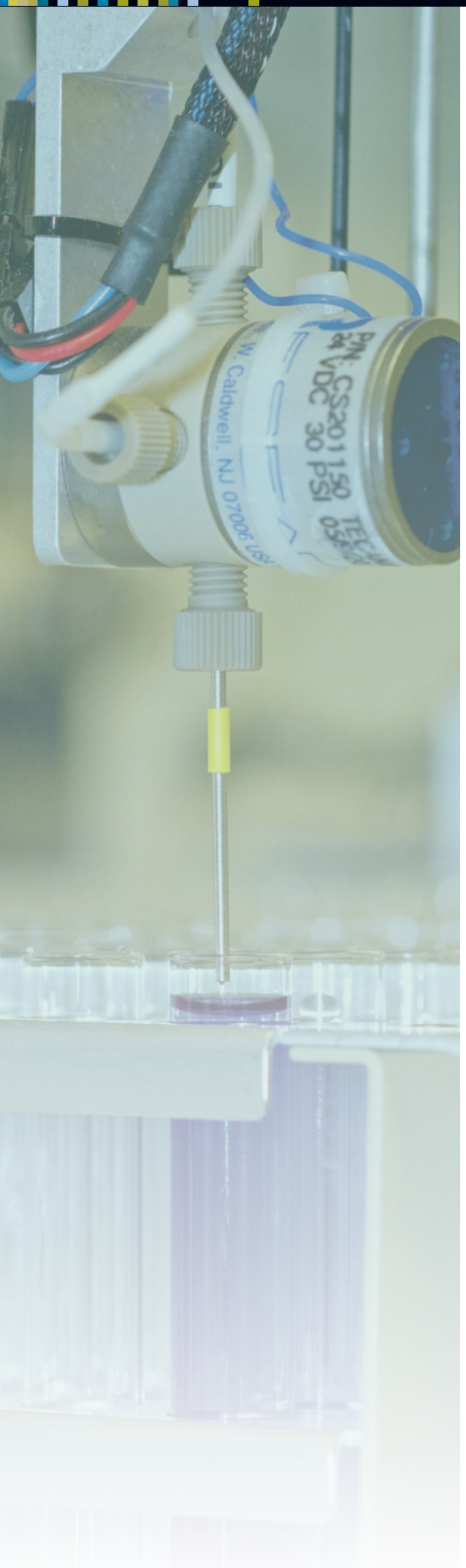
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



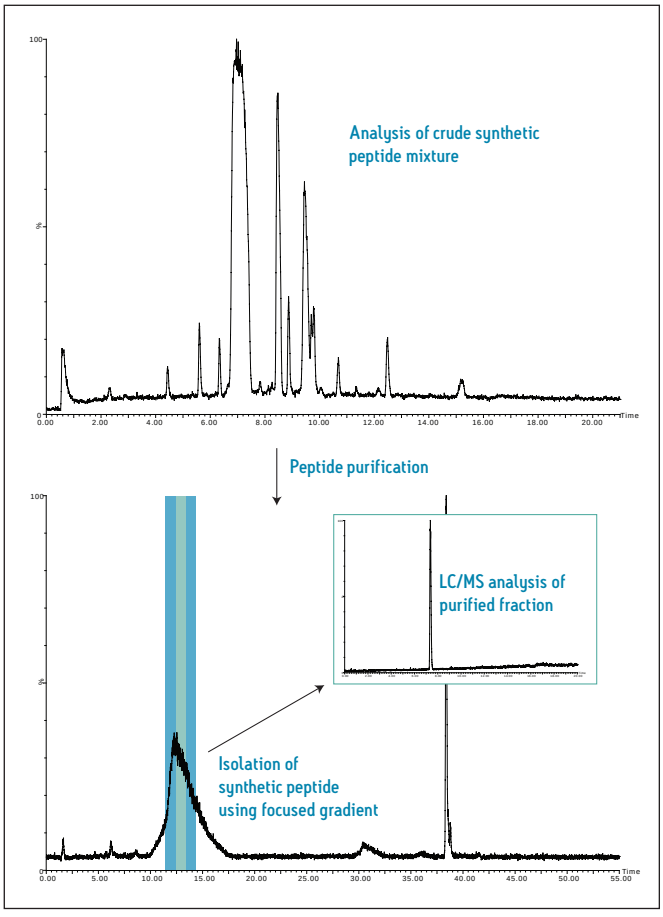
SYNTHETIC PEPTIDE PURIFICATION SOLUTIONS

PROVIDING EFFICIENT AND RELIABLE PEPTIDE PURIFICATION THAT IS CUSTOMIZED TO YOUR NEEDS

The purification of peptides from synthetic reaction mixtures or natural sources can be challenging. Peptide purification challenges arise from the chemical complexity of peptide molecules, and the need to isolate the desired peptide from a mixture of many closely-related impurities. An ideal purification process should be as simple as possible with a minimum number of steps. Waters Purification Solutions can be used to reliably isolate and purify microgram to multigram quantities of your peptide.

Waters provides complete solutions for synthetic peptide purification:

- Specialized Peptide Separation Technology Columns
- Full suite of instruments for analytical and preparative LC
- Detection options with UV or mass spectrometry
- Application-specific and easy-to-use software



In a typical purification procedure, the crude synthetic peptide mixture is first analyzed and then the bulk of the impurities are removed with a Waters Purification System. A fraction highly-enriched in the desired peptide is obtained.

BUILD A SYSTEM TO MATCH YOUR REQUIREMENTS, WORKLOAD, AND BUDGET

Waters provides a wide assortment of instrumentation for peptide purification. Selecting the best system for your laboratory depends on your application requirements. First, consider the scale of your synthesis and the number of samples you need to run. Next, take into account how often you need to develop new methods for a specific sample. Finally, think about sophisticated detection options that may be helpful in identifying the most pure fraction of the target peptide.

Waters' versatile purification systems are also simple to upgrade:

- Build a system that is rugged and easy to use
- Configure a system that can run multiple methods, columns, and run unattended samples around the clock
- Add MS to improve selectivity for fraction collection

Versatile purification system configurations

Basic Purification

Purify a few samples per day using the Waters 1525, 2535, or 2545 binary or quaternary gradient HPLC pumps with the 2489 UV/Vis Detector and Fraction Collector.

AutoPurification™ UV System

The Quaternary Gradient Module provides convenience and flexibility to address multiple methods. Using Auto•Blend™ Technology to auto-generate gradients, this configuration is particularly useful when many samples require unique methods. Run samples around the clock with the 2767 Sample Manager and add the System Fluidic Organizer so that small- and large-scale columns are available to check fraction purity or to develop modified methods for a particular sample.

AutoPurification MS System

All the characteristics of the UV system are supplemented with the information of MS by adding the 3100 Mass Detector, a compact, single quadrupole mass detector. This automated peptide purification system can monitor the elution of each targeted peptide, and is useful for triggering specific collection of a desired peptide.

Flexibility to match your sample load, column size, or flow rate

Sample Load	Column ID	Fluid Handling Unit	Max Flow Rate
mg	3.9 - 7.8 mm	1525 Binary HPLC Pump*	10 mL/min
mg - 10s mg	3.9 - 19 mm	1525 Binary HPLC Pump* and EF Kit	22.5 mL/min
mg - g	4.6 - 30 mm	2535 Quaternary Gradient Module	75 mL/min
mg - g	4.6 - 50 mm	2545 Binary Gradient Module*	150 mL/min
mg - g	4.6 - 50 mm	2545 Quaternary Gradient Module	150 mL/min
mg - 10s g	7.8 - 75 mm	2555 Quaternary Gradient Module	300 mL/min

* High pressure mixing

Choose a solvent delivery system based on the amount of material you will be loading on the column and the size of the column.

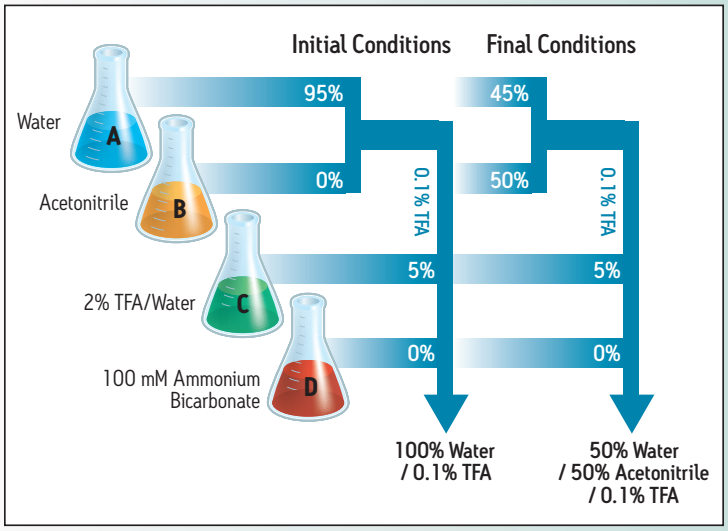
Software automates fraction collection and tracking

FractionLynx™ Application Manager, part of MassLynx™ Software, automates the collection of detected fractions, tracks samples and fractions, and then presents the data in an easy-to-read format. The software can be used with a variety of detector signals including UV/Vis and MS. It also provides full-cycle automation, from evaluation and setting collection thresholds, to purification, to fraction analysis. Fractions can be injected automatically if required, and the information can be exported.

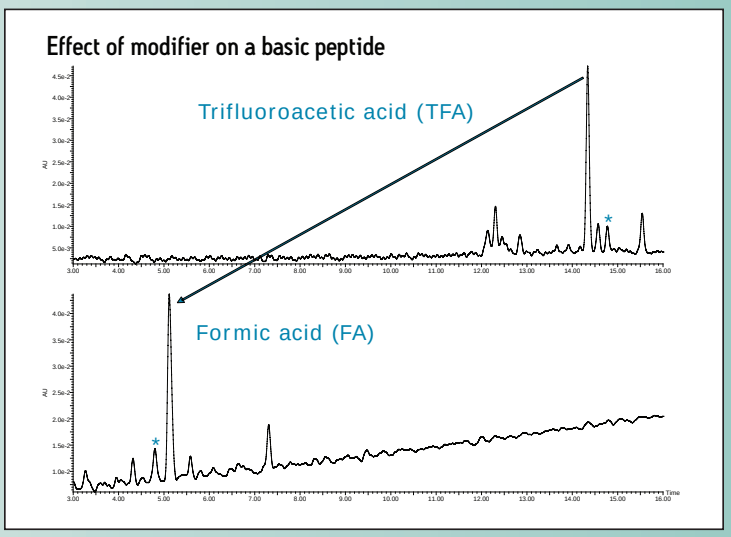
PURIFICATION SYSTEM FEATURES MAKE INSTRUMENTS EASY TO USE AND MAINTAIN

Auto•Blend programming technology and peptide purification

Auto•Blend Technology enables you to blend different solvents and modifiers at different concentrations, allowing you to take full advantage of Peptide Separation Technology Columns. Auto•Blend improves up-time by providing straightforward eluent management, increasing the flexibility and efficiency of your laboratory. Methods can be easily developed for difficult samples. Auto•Blend is also convenient for running multiple separation methods automatically.



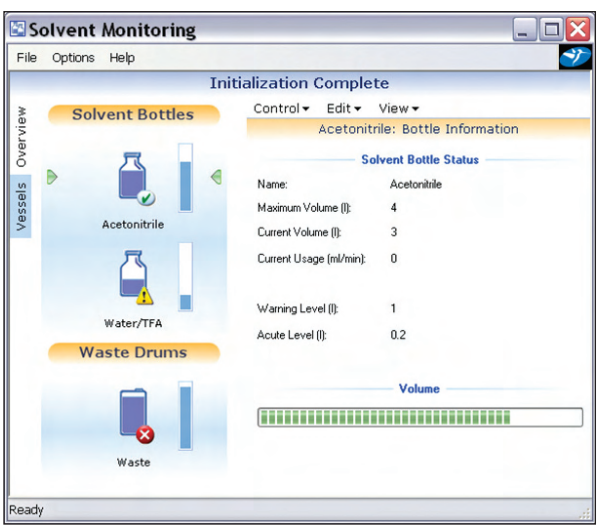
With Auto•Blend, gradients can be generated automatically. Here, methods can be switched from low pH to high pH by selecting modifiers C or D.



Selectivity can be altered to improve yield and purity by changing the modifier; in this example, good peak shape is maintained with both modifiers with a Peptide Separation Technology Column.

Solvent monitoring

Included in the FractionLynx Application Manager is the ability to monitor solvent reservoirs. Each solvent reservoir and waste container is uniquely identified in the solvent monitor console. Email notification is available when a warning level has been reached.



Each solvent reservoir and waste container clearly shows its own status level; initial and acute warning level parameters can be entered by the user in the vessel status page. Here, the current volume of solvent in the acetonitrile reservoir is 3 L, with an initial warning level of 1 L and an acute level of 0.25 L.

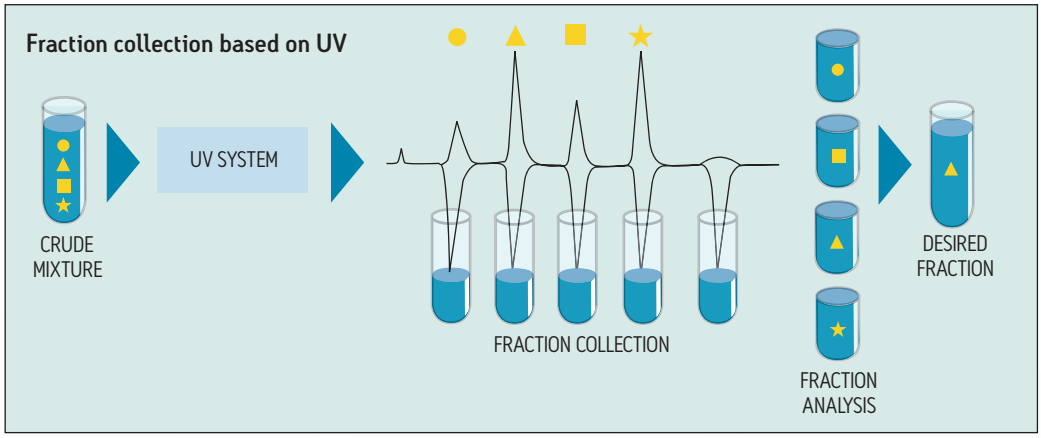
Remote status monitoring

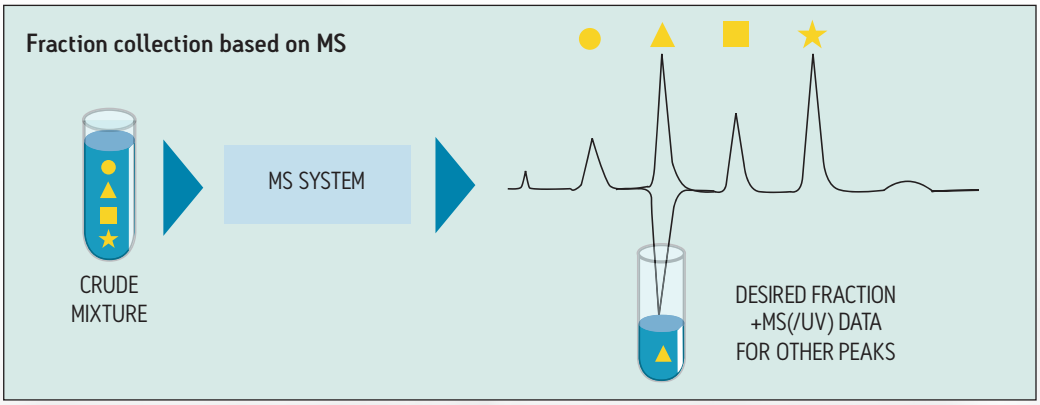
Waters' easy-to-use systems and software allow samples to be run with unattended system operation using the remote status monitor:

- Track the sample queue, instrument, and solvent and waste container status online from any PC on the network
- Achieve greater productivity since the user does not have to return to the instrument to check on its status; results can be delivered to their desktop

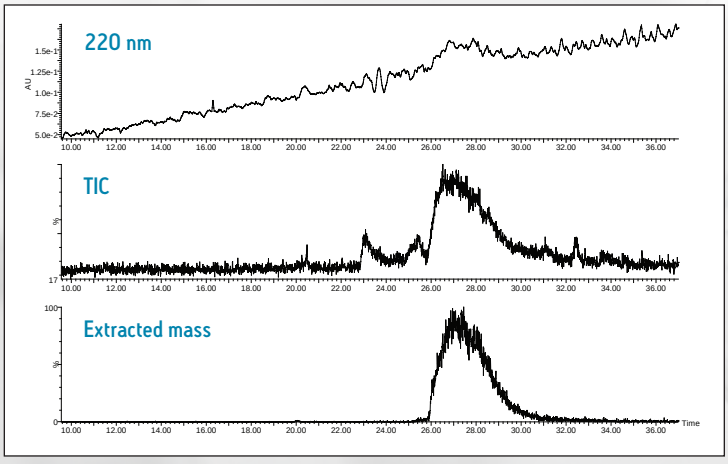
PEPTIDE FRACTION COLLECTION USING UV AND MS

When fraction collection is based on UV absorbance, it is often necessary to collect multiple fractions and to assay those fractions to identify the desired peak. By using the AutoPurification System with the 3100 Mass Detector, MS can be used with purification to identify the target peptide. Fraction collection can also be triggered by mass detection, intelligently collecting only the peak containing the mass of interest.





An MS detector can simplify the isolation process by tracking the product peptide. In the analytical run shown, the need to trigger on mass is evident since there is no definitive peak in the UV trace. This particular peptide has no strong chromophores.

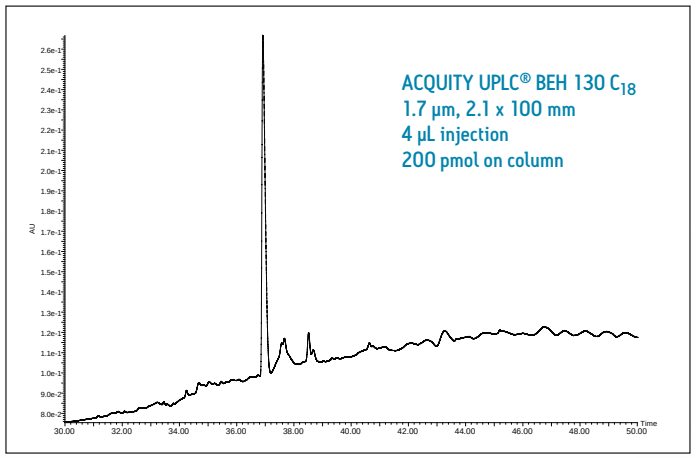


Fraction analysis and scaling from analytical UPLC to preparative purification

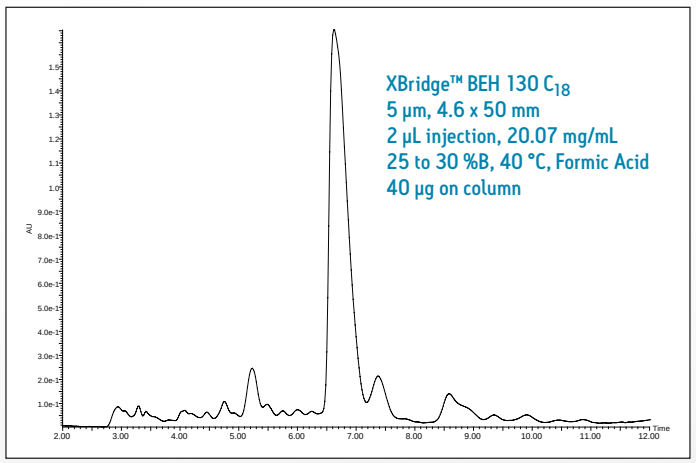
Waters UltraPerformance LC® (UPLC®) Technology improves resolution, sensitivity, and speed compared to HPLC. UPLC technology enhances your ability to perform sample screening to evaluate samples prior to purification, and provides you with a tool for the rapid assessment of fraction identity and purity. UPLC analytical methods can also suggest appropriate conditions for isolation. Purity of the final product can then be confirmed.

Additionally, scaling up from analytical UPLC to preparative HPLC is possible and more convenient with the use of focused gradients. The quality of UPLC chromatographic resolution can be carried through to purification, offering a significant increase in throughput and productivity.

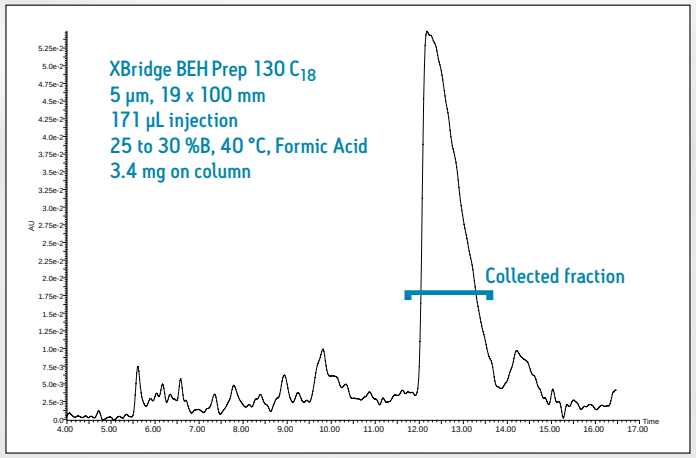
Scaling from screening to isolation



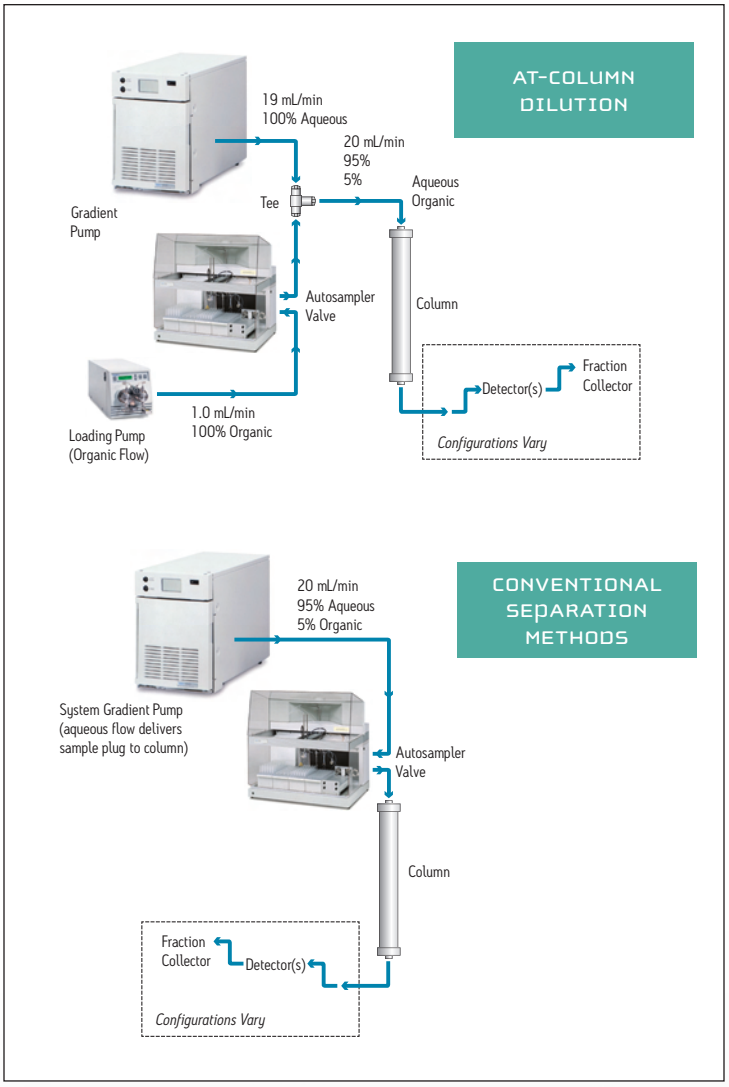
UPLC is used for pilot-scale analysis because it is best able to chromatographically resolve the peak of interest, a 1500 Da synthetic peptide, from its related impurities.



Using a focused gradient, the UPLC separation is transferred to an HPLC preparative system to expand the separation.

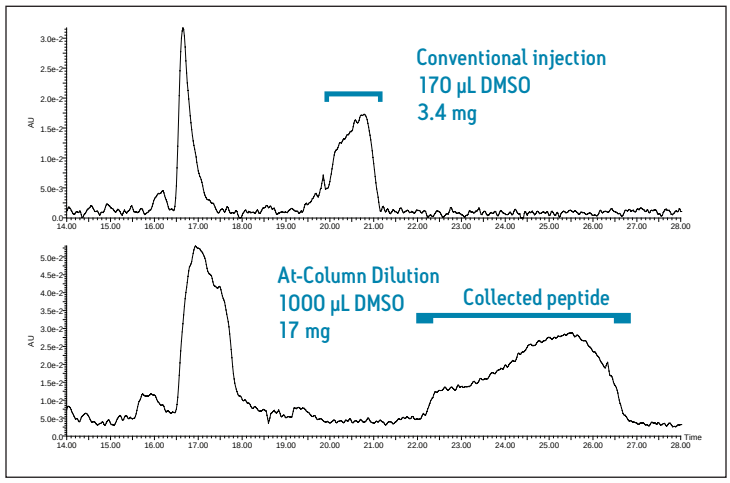


The separation is then scaled to a larger HPLC column for peptide isolation and fraction collection.



At-Column Dilution Technology for peptides

This patented technique allows increases in the mass load and injection volumes for peptide samples. At-Column Dilution was developed for injecting large volumes of relatively strong sample diluents. Such injections may distort the chromatography in a conventional system and limit the loading capacity for a peptide sample. At-Column Dilution eliminates the sample solvent effect, enabling significantly higher sample loading and improved mass capacity. Additionally, At-Column Dilution often increases system ruggedness and column lifetime by preventing precipitation in the sample loop or column.



At-Column Dilution can allow five-fold or greater increase in the amount of peptide that can be isolated in a single injection.

WATERS COLUMNS FOR PEPTIDE ANALYSIS AND PURIFICATION

Peptide Separation Technology Columns provide a stable surface chemistry that can be used for most peptides. These unique columns meet the demanding requirements of peptide separations:

- Improved chromatography
  - Narrow, symmetrical peaks for improved resolution
  - Good peak shape and retention in formic acid and TFA for optimal chromatography and detection
  - Available in 130Å and 300Å pore sizes for varying sample requirements
- BEH Technology™ particles
  - Hybrid structure
  - Chemical and physical stability
  - Minimum secondary interactions
- Wide range of particle sizes and column dimensions for consistent separations in applications from analytical UPLC to high mass load purification

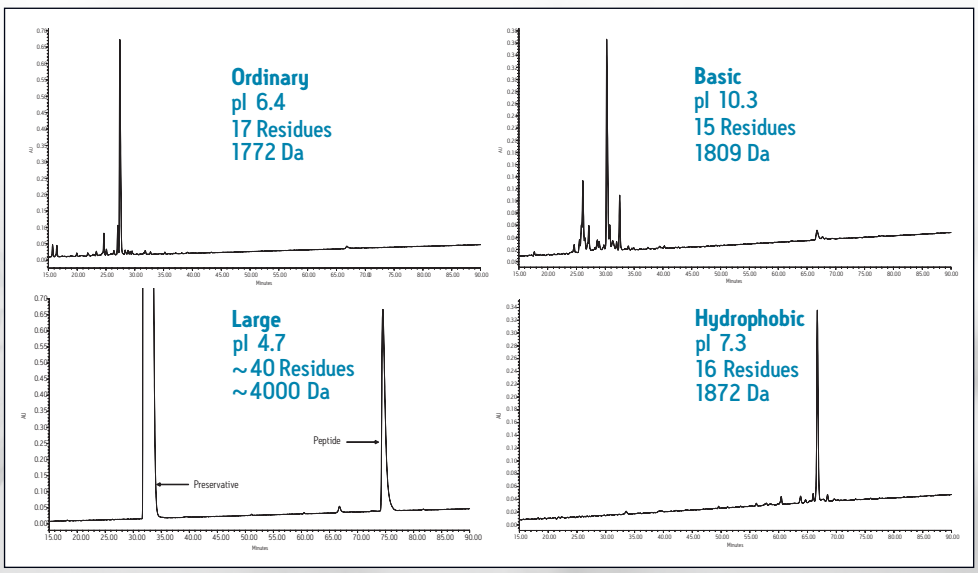
Waters' preparative columns with Optimum Bed Density (OBD™) design deliver the most reliable and consistent performance. OBD Technology combines hardware, particle characteristics, and packed bed densities in columns that provide excellent stability, superior reproducibility, high loadability, and extremely high efficiencies. When this column design is coupled with the stable surface chemistry of Peptide Separation Technology Columns, the process of peptide isolation and purification becomes very efficient and overall costs are reduced.

Utility and suitability for a wide range of peptides

Peptide Separation Technology Columns are suitable for a wide variety of peptides, including acidic, basic, long and short, hydrophilic and hydrophobic, and modified sequences. There is little need for screening columns to match a particular sample, or for maintaining an inventory of different column chemistries. Peptide Separation Technology Columns are additionally compatible with alternative mobile phases for flexibility in developing purification methods. Good peak shapes and separations can be obtained with both TFA and short-chain organic acids as mobile phase modifiers, and the columns can be used with both acetonitrile and alcohols. Good purification and yield can be obtained with biocompatible solvents so that the isolated peptide can be used in bioassays.

Column lifetime

Peptide Separation Technology Columns are designed to extend column lifetime by minimizing both physical and chemical failures. BEH Technology incorporates proprietary procedures for bonding and endcapping that reduce hydrolysis of the bonded phase, the predominant cause of short column lifetime in low-pH mobile phases. The physical stability of BEH Technology particles is ensured by OBD design, which was developed to produce the most stable packed beds. To achieve the maximum column lifetime benefits of these technologies, we offer matching guard columns.



Versatility for peptide isolation and purification

Peptide Separation Technology Columns are compatible with a broad range of peptides. A single column can be used to purify the numerous sample types generated in a peptide synthesis laboratory. There is no need to switch to different columns for separating peptides of extreme size, isoelectric point, or hydrophobicity.

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France 33 1 30 48 72 00

Germany 49 6196 400 600

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Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 508 478 2000
F: 508 872 1990
www.waters.com

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