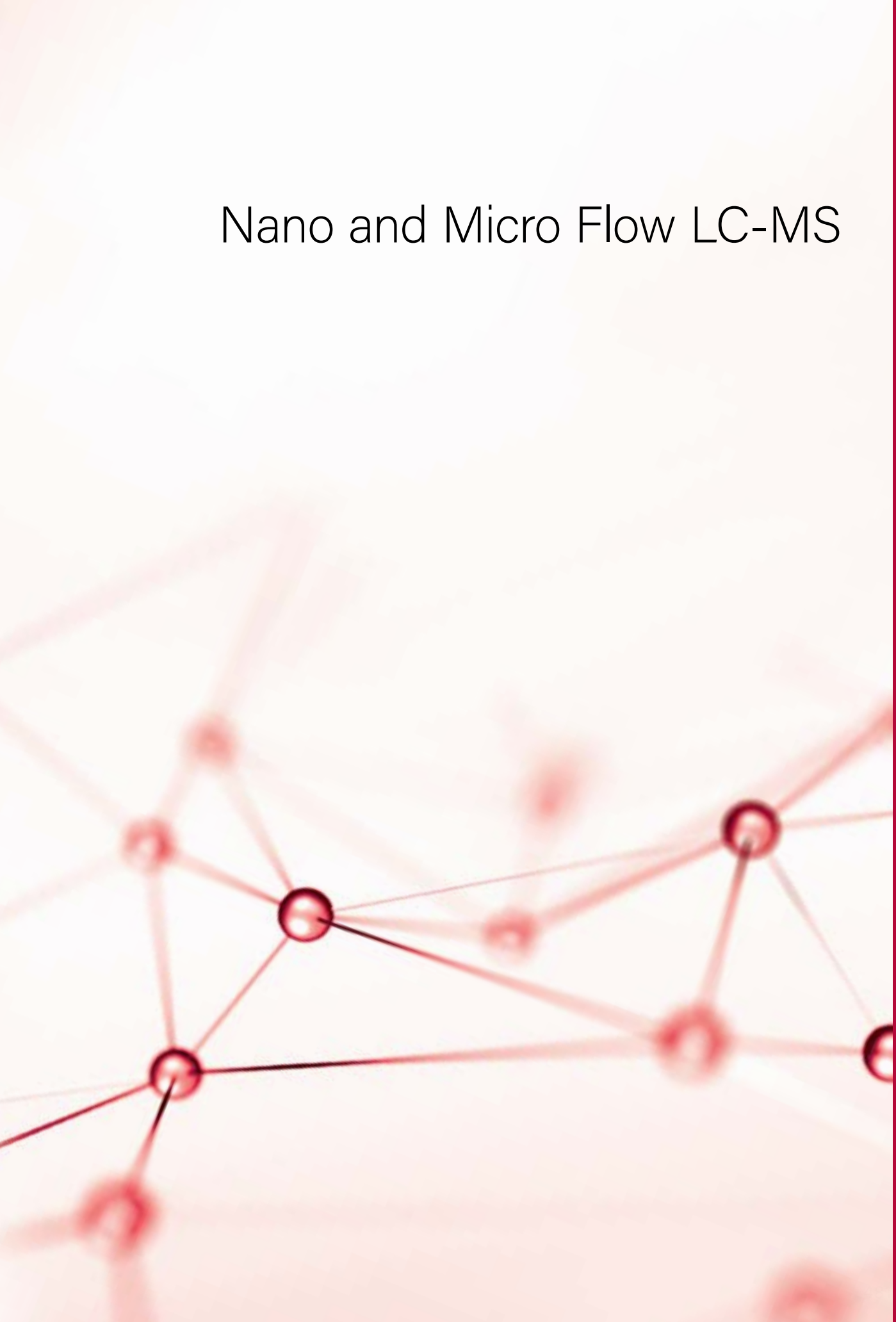
Nano and Micro Flow LC-MS



Nano and Micro Flow LC-MS

Contents

Nano- and Micro-Flow LC-MS

Our nano- and micro-flow LC Columns fully exploit the separation power of small, sub-2-µm particles to deliver superior chromatographic resolution.

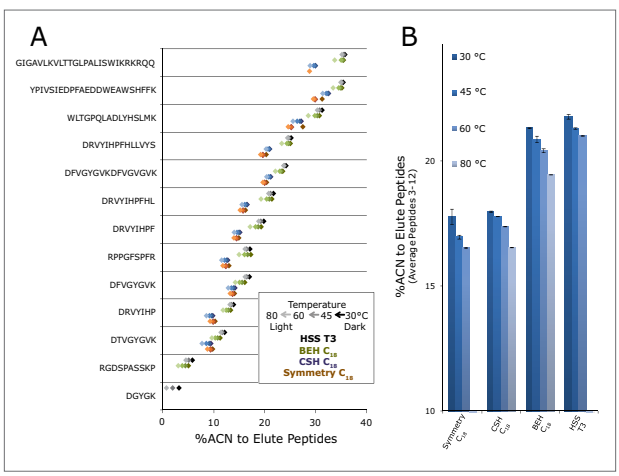
The selected stationary phases for nano-LC columns facilitate the efficiency and selectivity required for separations of complex peptide and protein separations as well as other sample-limited analyses.

Hybrid Particles			Silica-based Particles
			
BEH Technology™		CSH™ Technology	HSS HIGH STRENGTH SILICA
130 Å	300 Å	130 Å	100 Å
1.7 µm	1.7 µm	1.7 µm	1.8 µm
C_{18}	C_{18}, C_4	C_{18}	T3

Peptide Separation Technology stationary phases are specifically QC tested with tryptic digests of cytochrome *c* to ensure consistent performance for peptide separations.

Protein Separation Technology stationary phases are specifically designed for the high resolution analysis of proteins of various sizes, hydrophobicities, and isoelectric points. Particles are QC tested using a protein standard mix.

Trap Elute Peptide Separation



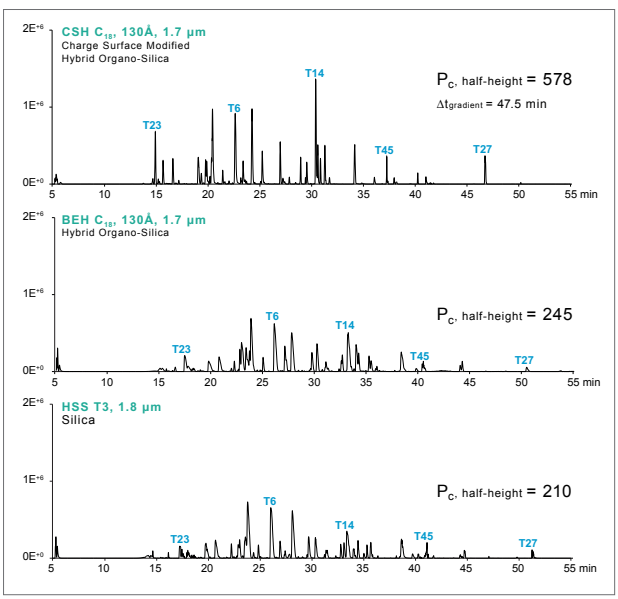
Peptide retentivity comparison of different stationary phases, including Symmetry Silica (the lower retention of Symmetry is used in trap-elute separations).

For more information on Waters Particle Technology, please refer to page 85.

In nano- and micro-flow LC-MS, analyzing large-volume samples using a single column can be impractical. In such cases, you can trap analytes at higher flow rates. It is preferable to perform online trapping of analytes at microscale flow rates and to subsequently elute and separate those analytes across an analytical column, wherein a significantly lower nanoscale flow rate is employed.

To be effective, the trapping column's retentivity must be lower than that of the analytical column. This relationship between trapping and analytical columns ensures refocusing of analytes on the analytical column after elution from the trap at the start of the gradient, delivering high peak capacity separations.

Peak Capacity and Retentivity



Comparison of a base peak ion chromatogram of MassPREP Enolase Digestion Standard, 1 µg, direct injection on a 75 µm (I.D.) column.

Nano- and micro-flow LC-MS is commonplace in areas of bio-separation such as peptide bioanalysis, intact antibody analysis, proteomics, lipidomics and metabolomics. This technique addresses limited sample availability and the need for high sensitivity and the requirement for low limits of detection or quantification.

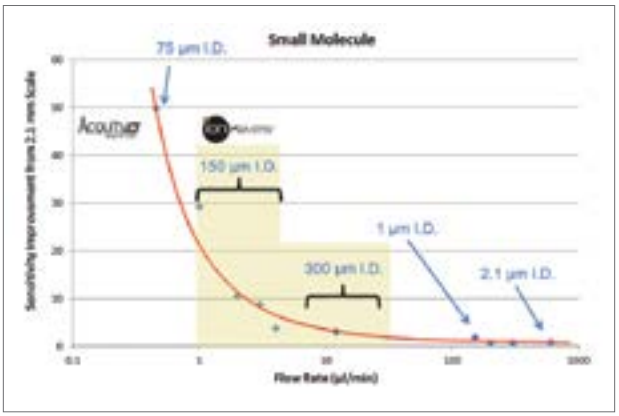
In micro-flow LC-MS, the inner diameter of the separation column, and thus the flow rate of the mobile phase can dramatically alter the sensitivity of the mass spectrometry as follows:

- By increasing sampling efficiency
- By increasing ionization efficiency
- By reducing matrix effects

Nano- LC-MS provides a higher sensitivity increase, compared with 2.1 mm UPLC Columns. Micro-flow separations, which use larger-diameter columns, increase sample throughput dramatically while continuing to deliver excellent sensitivity for many complex biomolecular analyses.

We offer solutions that satisfy the most demanding requirements for assays that rely on nano- and micro-flow LC-MS technology—solutions that ensure the assays' successful performance.

Gaining Sensitivity by Reducing Column Diameter and Flow Rate



Sensitivity enhancement for a series of small molecules relative to a 2.1 mm I.D. separation performed on an ACQUITY UPLC System. The volume and concentration of sample injected on each column format was identical.

Nano- and Micro-flow LC-MS Consumables

ionKey/MS™

- Includes a 150 µm I.D. separation channel, for highest sensitivity, and a 300 µm I.D. channel, for high-throughput analysis
- Greatly simplified micro-flow LC-MS, with fitting-free connections
- The 150 µm I.D. iKey™ Separation Device demonstrates as much as 40 times the sensitivity of the 2.1 mm I.D. UPLC column
- The 300 µm I.D. iKey, during high-throughput UPLC-cycle times, delivers as much as six times the sensitivity of a 2.1 mm I.D. UPLC column
- Easy post-column addition of MS-modifier solvents

nanoEase M/Z Columns

zenFiT™ Connection Technology

- nanoEase M/Z Columns with easy-to-use ZenFit™ Connection Technology
- Column inner diameters range from 75 to 300 µm
- Column lengths range from 50 to 250 mm
- Trapping columns range from 180 to 300 µm I.D.

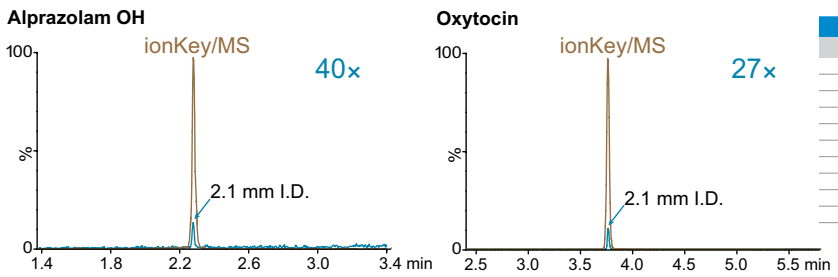
ionKey/MS

SIMPLIFIED MICRO-FLOW LC-MS WITH ENHANCED SENSITIVITY

The ionKey/MS System integrates the micro-flow UPLC separation into the source of the mass spectrometer. This delivers LC-MS system performance and sensitivity that cannot be achieved any other way. ionKey/MS Systems are enabled by the iKey Separation Device, which replaces the need for traditional fittings and columns and simplifies the user experience. The "plug and play" design of the iKey Separation Device eliminates operator variability common in traditional micro-flow LC-MS analyses.

The ionKey MS System with the ACQUITY UPLC M-Class System and Xevo TQ-S Mass Spectrometer.

150 µm I.D. iKey: Up to 40× Increase in Sensitivity Compared to 2.1 mm UPLC LC-MS Applications

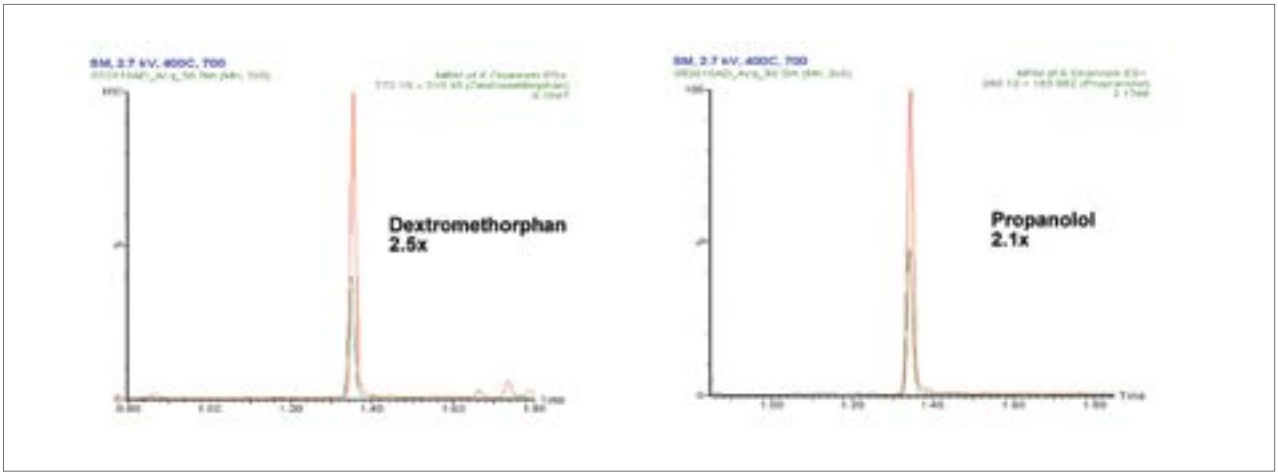


UPLC/MRM comparison of ionKey/MS relative to 2.1 mm I.D. chromatography

Compound Name	Relative Peak Area	Relative S/N
Small Molecule		
Lidocaine	3	3
Fluconazole	15	39
Propranolol	2	2
Alprazolam OH	14	40
Alprazolam	7	12
Nefazodone	3	6
Tolbutamide	5	6
Warfarin	2	4
Clopidogrel	2	2
Mean value	6	13

Sensitivity comparison between ionKey/MS™ and 2.1 mm I.D. chromatography; 1 µL injection of equal sample load on each.

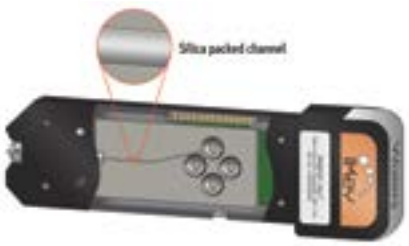
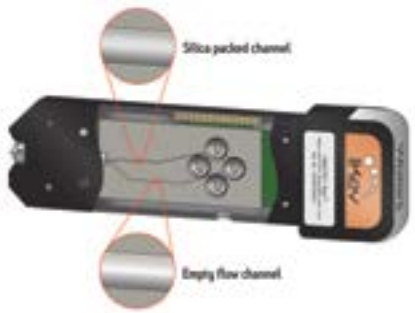
300 µm I.D. iKey HT: Increased LC-MS Sensitivity with UPLC Throughput



Sensitivity gains using (300 µm × 50 mm) iKey HT BEH C_{18} Separation Device (red) compared to (2.1 mm × 50 mm) UPLC BEH C_{18} Column (green) under identical injection volume and gradient conditions.

iKey Separation Device

In an ionKey/MS System, the iKey Separation Device contains the fluid connections, electronics, ESI interface, column heater, eCord, and chemistry needed to perform UPLC separations. As such, it replaces the need for traditional fittings and columns, simplifying the user experience. The "plug and play" design of the iKey eliminates user-dependent variation in results that often occurs in traditional micro-flow LC-MS analyses, regardless of users' skill level.

iKey Separation Device	iKey Separation Device with Post-Column Addition (PCA)
	
The major component of the ionKey/MS System, the iKey Separation Device performs sub-2-µm UPLC separations, resulting in highly sensitive, efficient, micro-flow LC-MS analyses. The iKey Separation device is available with two inner diameters: 150 µm I.D. which provides the highest level of sensitivity, and the 300 µm I.D. iKey HT for higher throughput separations.	The PCA iKey incorporates a separation channel as well as a post-column addition (PCA) channel. The design allows for mixing the mobile phase post separation with a desired solvent. Both effluents are merged and collected at the inlet of the emitter. Post-column addition of solvents can enhance the electrospray process and increase sensitivity without adversely affecting the separation.

Robust, Reproducible, and Reliable

1 user X 1 system X 1 iKey	Peptide from P00924, yeast enolase		5 users X 5 systems X 18 iKeys
	6.84 min	Retention time	6.69 min
	0.01 min	SD of retention time	0.11 min
	3.01 sec	Average peak width FWHM	2.72 sec
0.15% retention time RSD			**1.64%** retention time RSD
QC reproducibility for one iKey with over 500 injections of protein precipitated plasma.			*QC reproducibility across multiple users, ionKey/MS Systems, and iKeys.*

The iKey Separation Device is LC-MS tested to ensure consistent performance not only for a particular iKey but from one iKey to another. The device also exhibits robust performance—performance that achieves high-quality results, even after hundreds of injections.

Ordering Information

iKey Separation Devices

	Particle Size: 1.7 µm	
	Dimension	P/N (1/pk)
BEH C_{18}, 130 Å	150 µm × 50 mm	186007256
	150 µm × 50 mm (PCA)	186007580
	150 µm × 100 mm	186007258
CSH C_{18}, 130 Å	150 µm × 50 mm	186007244
	150 µm × 100 mm	186007245
HSS T3, 100 Å	150 µm × 50 mm	186007260
	150 µm × 100 mm	186007261
	300 µm × 50 mm	186008727

iKey Peptide Separation Devices

	Particle Size: 1.7 µm	
	Dimension	P/N (1/pk)
BEH C_{18}, 130 Å	150 µm × 50 mm	186006764
	150 µm × 50 mm (PCA)	186007557
	150 µm × 100 mm	186006766
CSH C_{18}, 130 Å	150 µm × 50 mm	186007257
	150 µm × 100 mm	186007259
BEH C_{18}, 300 Å	150 µm × 50 mm	186006969
	150 µm × 100 mm	186006970

iKey Protein Separation Devices

	Particle Size: 1.7 µm	
	Dimension	P/N (1/pk)
BEH C_4, 300 Å	150 µm × 50 mm	186006765
	150 µm × 100 mm	186006968

iKey Utility Devices

	Dimension	P/N (1/pk)
iKey Infusion Device	85 µm × 50 mm	186007049
iKey Flow Injection Analysis Device	85 µm × 50 mm	186007051
iKey Diagnostic Device V3	n/a	186008450

For additional information, please go to waters.com/iKey

Nano- and Micro-Flow Columns and Trapping Columns

Waters Columns for nano-to-microscale LC-MS analyses are designed for low-dispersion nano-UPLC Systems. Our rigorous quality-control measures ensure that the columns achieve their full potential for sensitivity, resolution, and reproducibility for biomarker discovery and also for identifying and characterizing peptides and proteins.

SEPARATION COLUMNS

These columns enable nano- and microscale separations with MS detection under UPLC conditions at 15,000 psi. They take full advantage of the separation power of sub-2-µm particle technology. Columns between 75 and 300 µm I.D. provide chromatographic separations with flow rates between 200 nL/min and 100 µL/min, covering a 170-fold range of sample amounts. The varying characteristics of available particle technologies provide alternate selectivity, retentivity, and loadability, and thus the flexibility to achieve the most suitable separation for complex LC-MS analyses.

TRAPPING COLUMNS

Trapping columns are used to desalt and enrich the sample before eluting onto the analytical column for the final separation with MS detection. For fast loading of the trap column and to reduce the cycle time, trap columns are packed with larger 5 µm particles.

nanoEase M/Z Columns with ZenFit Connection Technology

Waters ZenFit Connection Technology introduces easy-to-use, re-usable, fingertight, liquid-line connectors to the family of nanoEase M/Z Columns. These columns are capable of withstanding pressures as high as 15,000 psi and eliminating dead volume, a frequent source of variability associated with regular fittings. ZenFit Connection Technology does not require user training or any further special attention.

*To use nanoEase M/Z Columns on the ACQUITY UPLC M-Class System, equip systems with the appropriate upgrade kit. The 300 µm I.D. ACQUITY UPLC M-Class Columns and Traps are compatible with ZenFit Connections.

Ordering Information

nanoEase M/Z Peptide Columns

	Particle Size: 1.7 µm	
	Dimension	**P/N (1/pk)**
BEH C_{18}, 130 Å	75 µm × 100 mm	186008792
	75 µm × 150 mm	186008793
	75 µm × 200 mm	186008794
	75 µm × 250 mm	186008795
	100 µm × 100 mm	186008796
	150 µm × 100 mm	186008797
BEH C_{18}, 300 Å	75 µm × 100 mm	186008798
	75 µm × 150 mm	186008799
	75 µm × 200 mm	186008800
	75 µm × 250 mm	186008801
	100 µm × 100 mm	186008802
	150 µm × 100 mm	186008803
CSH C_{18}, 130 Å	75 µm × 100 mm	186008807
	75 µm × 150 mm	186008808
	75 µm × 200 mm	186008809
	75 µm × 250 mm	186008810
	100 µm × 100 mm	186008811
	150 µm × 50 mm	186008812
	150 µm × 100 mm	186008813
	150 µm × 150 mm	186008814

nanoEase M/Z Protein Columns

	Dimension	**P/N (1/pk)**
	Particle Size: 1.7 µm	
BEH C_4, 300 Å	75 µm × 100 mm	186008804
	100 µm × 100 mm	186008805
	150 µm × 100 mm	186008806

nanoEase M/Z HSS Columns

	Dimension	**P/N (1/pk)**
	Particle Size: 1.8 µm	
HSS T3, 100 Å	75 µm × 100 mm	186008815
	75 µm × 150 mm	186008816
	75 µm × 200 mm	186008817
	75 µm × 250 mm	186008818
	100 µm × 100 mm	186008819
	150 µm × 100 mm	186008820

nanoEase M/Z Columns and ACQUITY UPLC M-Class Columns are preferred for use with the ACQUITY UPLC M-Class and nanoACQUITY UPLC Systems.

nanoEase M/Z Trap Columns*

	Particle Size: 5 µm	
	Dimension	P/N (1/pk)
Symmetry C_{18}, 100 Å	180 µm × 20 mm	186008821

*For 300 µm I.D. traps please refer to M-Class Trap Columns.

ACQUITY UPLC M-Class Columns

	Particle Size: 1.8 µm	
	Dimension	P/N (1/pk)
HSS T3, 100 Å	75 µm × 100 mm	186008006
	75 µm × 150 mm	186007473
	75 µm × 200 mm	186008007
	75 µm × 250 mm	186007474
	100 µm × 100 mm	186008008
	150 µm × 100 mm	186008009
	300 µm × 50 mm	186007559
	300 µm × 100 mm	186007560
	300 µm × 150 mm	186007472

ACQUITY UPLC M-Class Trap Columns

	Particle Size: 5 µm	
	Dimension	P/N (1/pk)
Symmetry C_{18}, 100 Å	180 µm × 20 mm	186007496[4]
	180 µm × 20 mm	186007497[5]
	180 µm × 20 mm	186007500[6]
	180 µm × 20 mm	186007592[7]
Symmetry C_{18}, 100 Å	300 µm × 25 mm	186007499[3]
	300 µm × 50 mm	186007498
Peptide BEH C_{18}, 130 Å	300 µm × 50 mm	186007471
BEH C_4, 300 Å	300 µm × 50 mm	186008470
HSS T3, 100 Å	300 µm × 50 mm	186008029

[3]Configuration HCP (2D).
[4]Configuration: 2G, V/M.
[5]Configuration: 2D, V/M.
[6]Configuration: 2G, V/V.
[7]Configuration: 2D, V/V.

ACQUITY UPLC M-Class Peptide Columns

	Particle Size: 1.7 µm	
	Dimension	P/N (1/pk)
BEH C_{18}, 130 Å	75 µm × 100 mm	186007481
	75 µm × 150 mm	186007482
	75 µm × 200 mm	186007483
	75 µm × 250 mm	186007484
	100 µm × 100 mm	186007485
	150 µm × 100 mm	186007486
	300 µm × 50 mm	186007564
	300 µm × 100 mm	186007565
	300 µm × 150 mm	186007566
BEH C_{18}, 300 Å	75 µm × 100 mm	186007487
	75 µm × 150 mm	186007490
	75 µm × 200 mm	186007491
	75 µm × 250 mm	186007492
	100 µm × 100 mm	186007488
	150 µm × 100 mm	186007489
	300 µm × 50 mm	186007570
	300 µm × 100 mm	186007571
	300 µm × 150 mm	186007572
CSH C_{18}, 130 Å	75 µm × 100 mm	186007475
	75 µm × 150 mm	186007476
	75 µm × 200 mm	186007477
	75 µm × 250 mm	186007478
	100 µm × 100 mm	186007479
	150 µm × 50 mm	186007513
	150 µm × 100 mm	186007480
	150 µm × 150 mm	186007514
	300 µm × 50 mm	186007561
	300 µm × 100 mm	186007562
	300 µm × 150 mm	186007563

ACQUITY UPLC M-Class Protein Columns

	Particle Size: 1.7 µm	
	Dimension	P/N (1/pk)
BEH C_4, 300 Å	75 µm × 100 mm	186007493
	100 µm × 100 mm	186007494
	150 µm × 100 mm	186007495
	300 µm × 50 mm	186007567
	300 µm × 100 mm	186007568
	300 µm × 150 mm	186007569

ACQUITY UPLC M-Class with HDX Technology

ACQUITY UPLC M-Class System.

Hydrogen-deuterium exchange mass spectrometry (HDS-MS) is used to study a protein's structural dynamics and conformational changes, a component of understanding its higher-order structure. Information about protein conformation from an HDX MS study can serve to compare a control compound with an analyte by measuring the relative amount of deuteration uptake. HDX-MS can monitor domain interaction, localized-protein breathing, and folding or unfolding in the solution phase. The ACQUITY UPLC M-Class System can quantify small changes in protein conformation by extending its pressure range to effect a higher-efficiency separation. An integral part of the ACQUITY UPLC M-Class HDX System is the Waters Enzymate™ BEH Pepsin Column, which performs online protein digestion.

The technology offers these benefits:

- True UPLC separations for peptide and protein HDX
- Reproducible, robust, and rapid separations (nano-to-micro-scale at 0 °C and pressure to 15,000 psi)

ENZYMATE PEPSIN ONLINE DIGESTION COLUMN

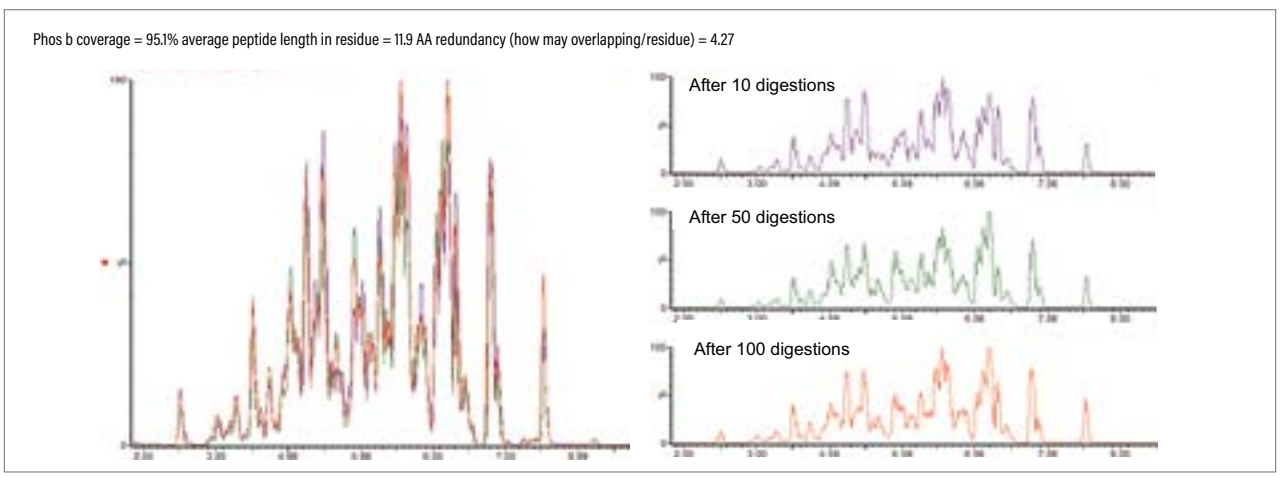
Enzymate Pepsin Online Digestion Column.

Waters Enzymate Pepsin Online Digestion Column digests intact proteins into peptides. The peptic peptides are then retained on a trapping column. Peptides eluting from the trapping column are refocused onto a sub-2-µm ACQUITY UPLC Column and then eluted into a high-resolution mass spectrometer.

Enzymate Pepsin Online Digestion Columns, an integral part of the ACQUITY UPLC M-Class HDX System, offer these benefits:

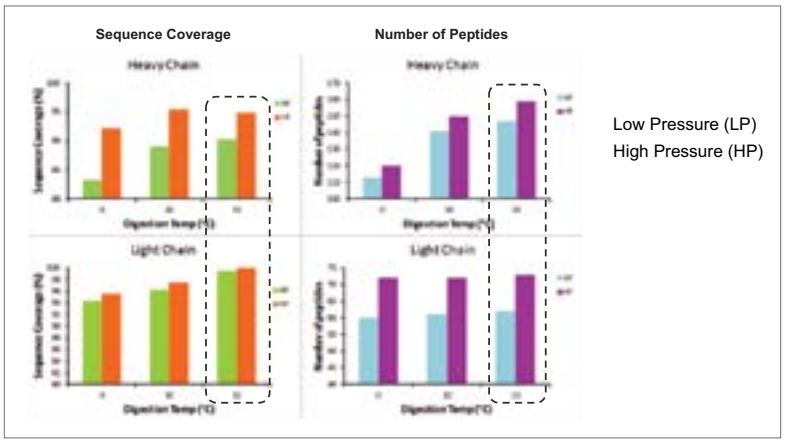
- Fast, reproducible, and efficient online protein digestion, typically within 30 seconds
- Shortened preparation time (overall) for protein samples
- Ability to optimize the efficiency of protein digestion by changing temperature, flow rate, or both

Overlay of Three Phos B Digestions within a 130-Injection HDX MS Study



Reproducible online pepsin digestions of phosphorylase b. A total of 130 digestions were performed using an Enzymate Pepsin Column. The 10,th 50,th and 100th digestions are shown. The sequence coverage is shown on the right.

Comparisons of Low- and High-Pressure Digestion Efficiencies



The Waters Enzymate BEH Pepsin Column was used for digestion of IgG2, at 1000 psi (NP), and 13,000 psi (HP). Results show high-pressure digestion increases protein-sequence coverage and spatial resolution of IgG2, compared with low-pressure digestion.

Ordering Information

Enzymate Pepsin Online Digestion Column

	Particle Size: 5 µm	
Description	**Dimension**	**P/N (1/pk)**
Enzymate Pepsin Online Digestion Column	2.1 × 30 mm	186007233

LC-MS Accessories

TRUVIEW LCMS CERTIFIED VIALS

TruView LCMS Certified Vials include stringent dimensional tolerances plus UV and MS cleanliness testing. The additional product attribute of TruView Vials is low polar analyte adsorption. The vials are manufactured by a process that limits the concentration of free ions on the surface of glass; ionic sites can cause analyte adsorption. Waters TruView LCMS Certified Vials are tested for high recovery of analyte at 1 ng/mL concentration using UPLC-MS/MS (MRM) and yield little adsorption. These vials exhibit the lowest adsorption of autosampler vials in the market.

Ordering Information

TruView LCMS Certified Vials

Description	Clear Glass	Amber Glass	Max Recovery	Total Recovery	Amber Max Recovery
TruView LCMS Certified Vials, 100/pk with cap and pre-slit silicone/PTFE septa	186005666CV	186005661CV	186005662CV	186005663CV	186005670CV
TruView LCMS Certified Vials, 100/pk with cap and silicone/PTFE septa	186005660CV	186005667CV	186005668CV	186005669CV	186005664CV

WATERS CERTIFIED CONTAINERS

Waters Certified Containers are uniquely processed, treated, and certified in the same unique manner as our highly regarded low TOC vials.

Ultra-clean containers can be used on any LC system, including UPLC, LC/UV, and LC-MS, among others Manufactured to stringent standards, they prevent extraneous peaks and baseline noise stemming from high TOC. To help assist with contamination prevention and facilitate recommended care and use, each container carries the Waters certified mark for easy differentiation in operational use.

Ordering Information

Certified Containers

Description	P/N
Certified Container Kit Kit contains: four certified 1 L bottles, three certified 500 mL bottles, one clean container cap kit	186007088
Low Volume Certified Container Kit Kit contains: five certified 250 mL clear bottles, one certified 500 mL clear bottle, one clean container cap kit	186007278
Certified Container, 1 L	186007089
Certified Container, 500 mL	186007090
Clean Container Cap Kit	205000642

pH BUFFERS

These pH Buffers are directly traceable to NIST SRMs, mercury free, guaranteed stable for at least one year after your receipt, and are supplied with a full certificate of analysis.

Ordering Information

pH Buffers

Description	Volume	P/N
pH 4 Liter pH 4 Buffer	1 L	129
pH 7 Liter pH 7 Buffer	1 L	133
pH 10 Liter pH 10 Buffer	1 L	137
pH 4 Pint pH 4 Buffer	1 pint	127
pH 7 Pint pH 7 Buffer	1 pint	131
pH 10 Pint pH 10 Buffer	1 pint	135