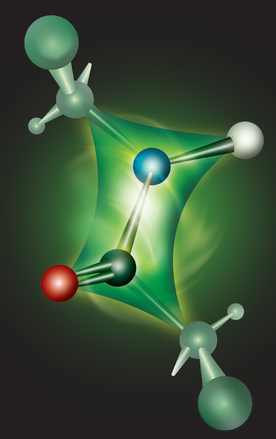
Unique New Columns for Peptide Separations

Waters Peptide Separation Technology offers a new unique column chemistry that meets the demanding requirements of peptide separations

- Improved chromatography of peptides compared to the use of conventional reversed-phase columns
- Narrow, symmetrical peaks for best resolution
- Successful separations of a wide range of peptides: large and small, acidic and basic, hydrophobic and hydrophilic
- Good peak shape and retention in formic acid and trifluoroacetic acid for optimal chromatography and detection
- Available in 130Å and 300Å pore sizes for varying sample requirements
- Wide range of particles sizes and column dimensions for consistent separations in applications from high sensitivity proteomics to high mass load purification
- Unique column chemistry combined with the benefits of sub-2 µm particles used with UPLC™ for the ultimate resolution



Tips for Improving Peptide Separations

Establish whether the goal is improved resolution, increased sensitivity, or reduced runtime. All three are important so priorities must be defined. An example of a typical objective is reducing run time without altering resolution or sensitivity.

Primary variables are gradient slope, flow rate, modifier and column length. Secondary variables include temperature, mobile phase and modifier concentration.

Gradient Slope

- Increased Gradient Slope (larger %/column volume)
  (Constant Flow Rate and Column Length)
  – Decreased Resolution
  – Increased Sensitivity
  – Decreased Run Time
- Decreased Gradient Slope (smaller %/column volume)
  (Constant Flow Rate and Column Length)
  – Increased Resolution
  – Decreased Sensitivity
  – Increased Run Time

Flow Rate

- Increased Flow Rate (200 µL/min)
  (Constant Gradient Slope and Column Length)
  – Decreased Resolution
  – Decreased Sensitivity
  – Decreased Run Time
- Decreased Flow Rate (100 µL/min)
  (Constant Gradient Slope and Column Length)
  – Increased Resolution
  – Increased Sensitivity
  – Increased Run Time

Column Length

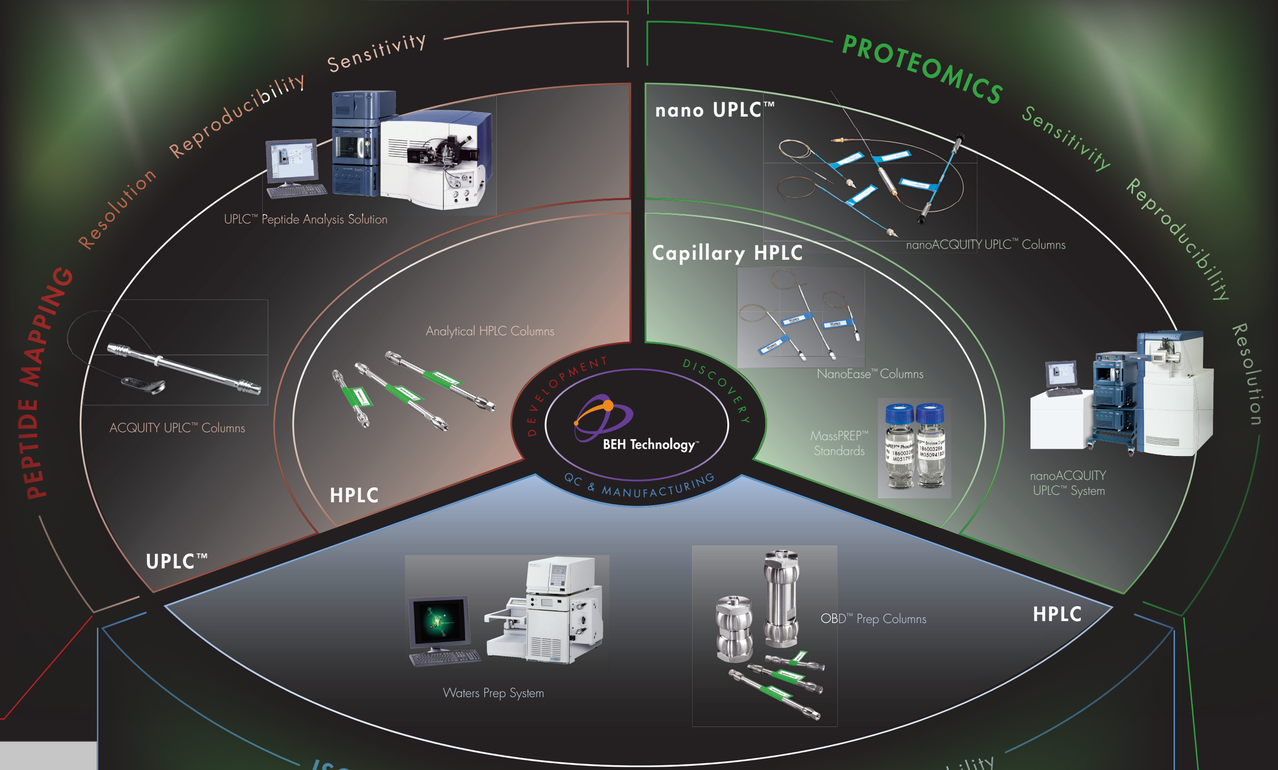
- Increased Column Length
  (Constant Gradient Slope and Flow Rate)
  – Increased Resolution
  – Decreased Sensitivity (with the same injection volume; same sensitivity if injection is increased proportional to column volume)
  – Increased Run Time
- Decreased Column Length
  (Constant Gradient Slope and Flow Rate)
  – Decreased Resolution
  – Increased Sensitivity (with the same injection volume; same sensitivity if injection is decreased proportional to column volume)
  – Decreased Run Time

Peptide Analytical Columns

ACQUITY UPLC™ Column, 1.7 µm 130Å and 300Å				
I.D. (mm)	Length (mm)	pmoles of Digest	Weight of Digest	Typical Flow Rate (µL/min)
2.1	50	0.5 - 500	50 ng - 50 µg	100 - 300
2.1	100	0.5 - 500	50 ng - 50 µg	100 - 300
2.1	150	0.5 - 500	50 ng - 50 µg	100 - 300

HPLC Columns, 3.5 µm and 5 µm 130Å and 300Å				
I.D. (mm)	Length (mm)	pmoles of Digest	Weight of Digest	Typical Flow Rate (µL/min)
1	50	0.5 - 100	50 ng - 10 µg	40 - 100
1	100	0.5 - 100	50 ng - 10 µg	40 - 100
1	150	0.5 - 100	50 ng - 10 µg	40 - 100
2.1	50	2.5 - 500	250 ng - 50 µg	150 - 400
2.1	100	2.5 - 500	250 ng - 50 µg	150 - 400
2.1	150	2.5 - 500	250 ng - 50 µg	150 - 400
2.1	250	2.5 - 500	250 ng - 50 µg	150 - 400
4.6	50	12.5 - 2,500	1.25 µg - 250 µg	800 - 2,000
4.6	100	12.5 - 2,500	1.25 µg - 250 µg	800 - 2,000
4.6	150	12.5 - 2,500	1.25 µg - 250 µg	800 - 2,000
4.6	250	12.5 - 2,500	1.25 µg - 250 µg	800 - 2,000

Peptide Separation Technology Applications



Peptide Nano and Capillary Columns

nanoACQUITY UPLC™ Columns, 1.7 µm 130Å and 300Å					
I.D. (µm)	Length (mm)	fmoles of Digest	Weight of Digest	Weight of Lysate	Typical Flow Rate (µL/min)
75	100	0.5 - 500	50 pg - 50 ng	0.5 µg - 2 µg	0.2 - 0.5
75	150	0.5 - 500	50 pg - 50 ng	0.5 µg - 2 µg	0.2 - 0.5
75	200	0.5 - 500	50 pg - 50 ng	0.5 µg - 2 µg	0.2 - 0.5
75	250	0.5 - 500	50 pg - 50 ng	0.5 µg - 2 µg	0.2 - 0.5
100	100	1 - 1,000	100 pg - 100 ng	1 µg - 4 µg	0.35 - 1
150	100	2 - 2,000	200 pg - 200 ng	2 µg - 8 µg	1 - 2.5

NanoEase Columns, 3.5 µm 130Å and 300Å					
I.D. (µm)	Length (mm)	fmoles of Digest	Weight of Digest	Weight of Lysate	Typical Flow Rate (µL/min)
75	50	0.5 - 500	50 pg - 50 ng	0.5 µg - 2 µg	0.2 - 0.5
75	100	0.5 - 500	50 pg - 50 ng	0.5 µg - 2 µg	0.2 - 0.5
75	150	0.5 - 500	50 pg - 50 ng	0.5 µg - 2 µg	0.2 - 0.5
100	50	1 - 1,000	100 pg - 100 ng	1 µg - 4 µg	0.35 - 1
100	100	1 - 1,000	100 pg - 100 ng	1 µg - 4 µg	0.35 - 1
100	150	1 - 1,000	100 pg - 100 ng	1 µg - 4 µg	0.35 - 1
150	50	2 - 2,000	200 pg - 200 ng	2 µg - 8 µg	1 - 2.5
150	100	2 - 2,000	200 pg - 200 ng	2 µg - 8 µg	1 - 2.5
150	150	2 - 2,000	200 pg - 200 ng	2 µg - 8 µg	1 - 2.5
300	50	8 - 8,000	800 pg - 800 ng	8 µg - 32 µg	4 - 10
300	100	8 - 8,000	800 pg - 800 ng	8 µg - 32 µg	4 - 10
300	150	8 - 8,000	800 pg - 800 ng	8 µg - 32 µg	4 - 10

Formulas for Scaling a Separation

$$M_2 = M_1 \times \frac{L_2}{L_1} \times \frac{d_2^2}{d_1^2} \qquad F_2 = F_1 \times \frac{d_2^2}{d_1^2}$$

$$IV_2 = IV_1 \times \frac{L_2}{L_1} \times \frac{d_2^2}{d_1^2} \qquad t_2 = t_1 \times \frac{L_2}{L_1} \times \frac{d_2^2}{d_1^2} \times \frac{F_1}{F_2}$$

*where: **M** mass • **F** Flow rate • **L** Length • **d** diameter • **t** gradient time • **IV** Injection Volume*

For more information regarding scaling your preparation, please go to www.waters.com/prepcalculator to request your copy of the calculator

Peptide Scaling Calculations

Dimensions	4.6 x 100 mm	19 x 100 mm	19 x 150 mm
Flow Rate	1.4 mL/min	23.9 mL/min	23.9 mL/min
Sample Injection Volume	20 µL	340 µL	510 µL
Sample Load	5 mg	85 mg	128 mg
Gradient Duration	11 min	11 min	16.5 min

Peptide Isolation & Purification Columns

OBD™ Prep Columns, 5 µm and 10 µm 130Å and 300Å				
I.D. (mm)	Length (mm)	µmoles of a Single Peptide	Weight of a Single Peptide (mg)	Typical Flow Rate (mL/min)
10	50	0.25 - 5	0.5 - 10	4.5 - 9
10	100	0.25 - 5	0.5 - 10	4.5 - 9
10	150	0.25 - 5	0.5 - 10	4.5 - 9
10	250	0.25 - 5	0.5 - 10	4.5 - 9
19	50	1 - 18	2.0 - 36	16 - 32
19	100	1 - 18	2.0 - 36	16 - 32
19	150	1 - 18	2.0 - 36	16 - 32
19	250	1 - 18	2.0 - 36	16 - 32

OBD™ Prep Columns, 10 µm 130Å and 300Å				
I.D. (mm)	Length (mm)	µmoles of a Single Peptide	Weight of a Single Peptide (mg)	Typical Flow Rate (mL/min)
30	50	2.5 - 25	5 - 100	40 - 80
30	100	2.5 - 25	5 - 100	40 - 80
30	150	2.5 - 25	5 - 100	40 - 80
30	250	2.5 - 25	5 - 100	40 - 80

Waters

Properties of Amino Acids

Symbols		Name and Composition		Monoisotopic Mass	Average Mass
Ala	A	Alanine	C_3H_5NO	71.03711	71.0788
Arg	R	Arginine	$C_6H_{12}N_4O$	156.10111	156.1876
Asn	N	Asparagine	$C_4H_6N_2O_2$	114.04293	114.1039
Asp	D	Aspartic Acid	$C_4H_5NO_3$	115.02694	115.0886
Cys	C	Cysteine	C_3H_5NOS	103.00919	103.1448
Gln	Q	Glutamine	$C_5H_8N_2O_2$	128.05858	128.1308
Glu	E	Glutamic Acid	$C_5H_7NO_3$	129.04259	129.1155
Gly	G	Glycine	C_2H_3NO	57.02146	57.0520
His	H	Histidine	$C_6H_7N_3O$	137.05891	137.1412
Ile	I	Isoleucine	$C_6H_{11}NO$	113.08406	113.1595
Leu	L	Leucine	$C_6H_{11}NO$	113.08406	113.1595
Lys	K	Lysine	$C_6H_{12}N_2O$	128.09496	128.1742
Met	M	Methionine	C_5H_9NOS	131.04049	131.1986
Phe	F	Phenylalanine	C_9H_9NO	147.06841	147.1766
Pro	P	Proline	C_5H_7NO	97.05276	97.1167
Ser	S	Serine	$C_3H_5NO_2$	87.03203	87.0782
Thr	T	Threonine	$C_4H_7NO_2$	101.04768	101.1051
Trp	W	Tryptophan	$C_{11}H_{10}N_2O$	186.07931	186.2133
Tyr	Y	Tyrosine	$C_9H_9NO_2$	163.06333	163.1760
Val	V	Valine	C_5H_9NO	99.06841	99.1326

www.waters.com

Austria and European Export (Central South Eastern Europe, CIS and Middle East) 43 1 877 18 07, **Australia** 61 2 9933 1777, **Belgium** 32 2 726 1000, **Brazil** 55 11 5094 3788, **Canada** 1 800 252 4752 x2205 **China** 8621 6495 6999, **CIS/Russia** 7 095 336 7000, **Czech Republic** 420 2 617 1 1384, **Denmark** 45 46 59 8080, **Finland** 358 9 5659 6288, **France** 33 1 30 48 72 00, **Germany** 49 6196 400600, **Hong Kong** 852 29 64 1800, **Hungary** 36 1 350 5086, **India and India Subcontinent** 91 80 2837 1900, **Ireland** 353 1 448 1500, **Italy** 39 02 27 421 1, **Japan** 81 3 3471 7191, **Korea** 82 2 820 2700, **Mexico** 52 55 5200 1860, **The Netherlands** 31 76 508 7200, **Norway** 47 6 384 60 50, **Poland** 48 22 833 4400, **Puerto Rico** 1 787 747 8445, **Singapore** 65 6273 1221, **Spain** 34 93 600 9300, **Sweden** 46 8 555 11 500, **Switzerland** 41 62 889 2030, **Taiwan** 886 2 2543 1898, **United Kingdom** 44 208 238 6100 **All other countries: Waters Corporation U.S.A.** 1 508 478 2000/1 800 252 4752

For more information visit www.waters.com/pst