Symmetry® Columns

- **Tightest specifications**
- **Consistent results**
- **Year to year reproducibility**
- **SymmetryShield™ columns provide improved peak shape**
- **SymmetryShield™ columns complementary selectivity to Symmetry® columns**
- **Symmetry300™ columns demonstrate the highest column peak capacity**
- **SymmetryPrep™ columns provide direct scale up while maintaining resolution**

SYMMETRY300™: COLUMNS THE FIRST COLUMNS SPECIFICALLY ENGINEERED FOR THE DISCOVERY AND DEVELOPMENT OF NEW BIOPHARMACEUTICALS

As a scientist, you know that tremendous time and resources are required when developing HPLC assays for well-characterized biopharmaceuticals. You also know that column-to-column variability is the "Achilles heel" of this demanding process.

In the past you've had no choice but to deal with variability by finding costly and time-consuming ways around this problem. The regulatory guidelines being adopted worldwide as a result of the International Conference on Harmonization (ICH) and the U.S. Food and Drug Administration's specified biological products initiatives are placing increasingly stringent demands on this process. Full analytical characterization is becoming more important for regulatory filing, making column variability an unacceptable risk.

Now, regardless of whether you're developing HPLC assays for purity, stability or identity, you can have confidence in the long-term compliance of your methods, batch-to-batch, column-after-column, year-after-year. That's because Waters has developed Symmetry300™ columns, columns specifically engineered for the discovery and development of new bio-pharmaceuticals.

Symmetry300™ columns are a 300Å reversed-phase addition to the existing Symmetry® family of columns. They have been specifically designed to provide maximum reproducibility and material recovery of protein and peptide assays. Symmetry300™ columns are offered in two particle sizes: 3.5 and 5 µm, and in two chemistries: C_4 for large peptides and proteins, and C_{18} for smaller peptides.

HIGH RECOVERIES OF PEPTIDES AND PROTEINS

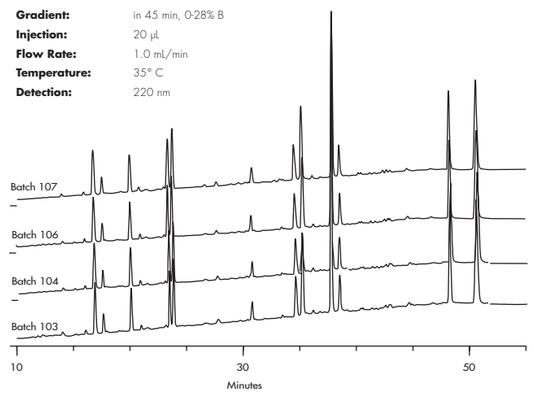
The heart of the column is high purity based deactivated silica. Waters' dedicated chromatography chemistry manufacturing plant operates under the stringent standards of cGMP supplemented with FDA registration for Class 1 medical devices and ISO 9002. The silica used in Symmetry300™ columns is synthesized using ultrapure organic reagents, resulting in high purity with very low silanol activity.

The high purity silica combined with the high surface coverage of the bonded phase results in higher recoveries and increased productivity.

There are several factors to assess when developing a reversed-phase HPLC method for peptides. Batch-to-batch reproducibility of the column is an important consideration when developing a validated and transferable method. Symmetry300™ columns have unsurpassed batch-to-batch and column-to-column reproducibility.

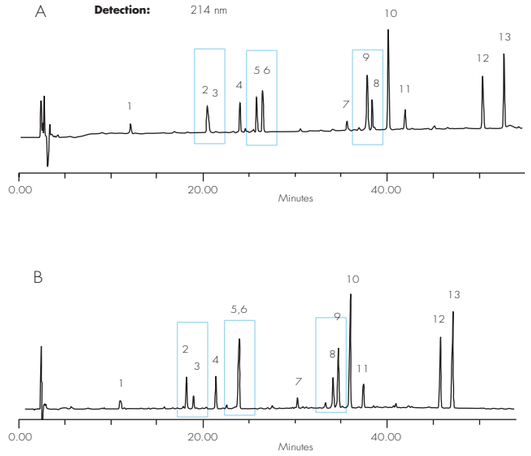
SYMMETRY300™ BATCH-TO-BATCH REPRODUCIBILITY

Column: Symmetry300™ C_{18}, 5 µm, 3.9 mm x 150 mm
Sample: Tryptic digests of cytochrome c (bovine)
Mobile Phase: Solvent A: 0.05% TFA in water; Solvent B: 0.1% TFA in acetonitrile
Gradient: in 45 min, 0-28% B
Injection: 20 µL
Flow Rate: 1.0 mL/min
Temperature: 35° C
Detection: 220 nm



PORE SIZE EFFECTS ON RESOLUTION—SYMMETRY® 100Å AND SYMMETRY300™

Column: A. Symmetry® C_{18}, 5 µm, 100Å, 4.6 mm x 150 mm; B. Symmetry300™ C_{18}, 5 µm, 300Å, 4.6 mm x 150 mm
Sample: Tryptic digests of cytochrome c (bovine)
Mobile Phase: Solvent A: 0.1% TFA in water; Solvent B: 0.1% TFA in acetonitrile
Gradient: 0-50 min, 0-30% B
Injection: 20 µL
Flow Rate: 0.75 mL/min
Temperature: 35° C
Detection: 214 nm



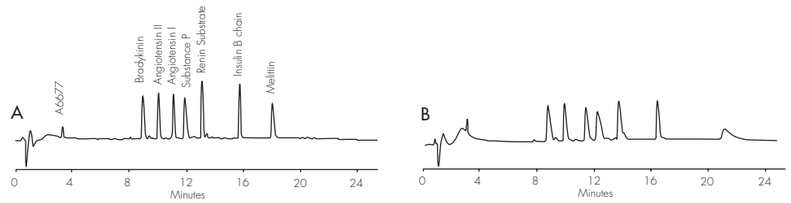
Different pore sizes change selectivity. Pore size can have an effect on selectivity. The key to a successful separation is the selection of a column that gives the highest chemistry resolution with maximum peak capacity and recovery.

Symmetry® Columns

Many applications including peptide mapping and protein identification require MS detection. In order to achieve a sensitive LC-MS analysis, the use of MS friendly mobile phases is desirable. The examples below demonstrate the high performance of Symmetry300™ C_{18} in low tri-fluoroacetic acid (TFA) or formic acid conditions. As you can see in the chromatograms, Symmetry300™ C_{18} columns maintain excellent peak shape and peak capacity when using 0.02% TFA or 0.1% formic acid.

PEPTIDE SEPARATION OF 18 µg TOTAL MASS LOAD IN FORMIC ACID (0.1%)

Column: A. Symmetry300™ C_{18}, 5 µm, 300Å, 4.6 mm x 50 mm; B. Leading competitive column, 5 µm, 300Å, 4.6 mm x 50 mm
Sample: Standard peptide test mixture
Mobile Phase: Solvent A: 0.1% Formic Acid in water; Solvent B: 0.065% Formic Acid in acetonitrile
Gradient: 0-25 min, 0-50% B
Injection: 20 µL
Flow Rate: 0.75 mL/min
Temperature: 40° C
Detection: 220 nm



		SYMMETRY® COLUMNS		SYMMETRYSHIELD™ COLUMNS		SYMMETRY300™ COLUMNS	
Dimensions	**Particle Size**	**C_{18}**	**C_8**	**RP_{18}**	**RP_8**	**C_{18}**	**C_4**
1.0 x 50mm	3.5 µm	WAT106056	WAT106052	186000175	WAT106060		
1.0 x 150mm	3.5 µm	WAT248059	WAT248072	186000176	WAT106048	186000185	186000276
2.1 x 30mm	3.5 µm	WAT058973	WAT058977	186000171	WAT106001		
2.1 x 50mm	3.5 µm	WAT200650	WAT200624	186000172	WAT094257	186000187	186000277
2.1 x 100mm	3.5 µm	WAT058965	WAT058961	186000173	WAT058969	186000188	186000278
2.1 x 150mm	3.5 µm	WAT106005	WAT106011	186000174	WAT106008	186000200	186000279
3.0 x 100mm	3.5 µm	186000696	186000698	186000700	186000703		
3.0 x 150mm	3.5 µm	186000695	186000697	186000699	186000702		
4.6 x 30mm	3.5 µm	186000271	186000270				
4.6 x 50mm	3.5 µm	WAT200625	WAT200620	186000177	WAT094260	186000201	186000280
4.6 x 75mm	3.5 µm	WAT066224	WAT066200	186000178	WAT094263	186000189	186000281
4.6 x 100mm	3.5 µm	WAT066220	WAT066204	186000179	WAT094266	186000190	186000282
4.6 x 150mm	3.5 µm	WAT200632	WAT200630	186000180	WAT094269	186000197	186000283
2.1 x 50mm	5 µm	186000206	186000212	186000217	186000223		
2.1 x 150mm	5 µm	WAT056975	WAT056955	186000111	WAT094245	WAT106172	186000285
3.0 x 150mm	5 µm	WAT054200	WAT054230	186000692	WAT094243		
3.0 x 250mm	5 µm	186000690	186000691	186000693	186000694		
3.9 x 150mm	5 µm	WAT046980	WAT046970	186000108	WAT200655	WAT106154	186000286
4.6 x 50mm	5 µm	186000207	186000213	186000218	186000224	WAT106209	186000287
4.6 x 150mm	5 µm	WAT045905	WAT045995	186000109	WAT200662	WAT106157	186000288
4.6 x 250mm	5 µm	WAT054275	WAT054270	186000112	WAT200670	WAT106151	186000289
7.8 x 10mm	5 µm	186000711	186000712				
7.8 x 50mm	5 µm	186000208	186000214				
7.8 x 100mm	5 µm	186000209	186000215				
19 x 10mm	5 µm	186000715	186000716	186001835	186001841	186001847	
19 x 50mm	5 µm	186000210	186000216	186001836	186001842	186001848	
19 x 100mm	5 µm	186000211	186000229	186001837	186001843	186001849	
19 X 150mm	5 µm		186001838	186001844	186001850		
30 x 50mm	5 µm	186000235	186000237				
30 x 100mm	5 µm	186000236	186000238				
7.8 x 10mm	7 µm	186000713	186000714				
7.8 x 150mm	7 µm	WAT066288	WAT066285				
7.8 x 300mm	7 µm	WAT066235	WAT066225				
19 x 10mm	7 µm	186000717	186000718				
19 x 150mm	7 µm	WAT066240	WAT066228	186001839	186001845		
19 x 300mm	7 µm	WAT066245	WAT066230	186001840	186001846		