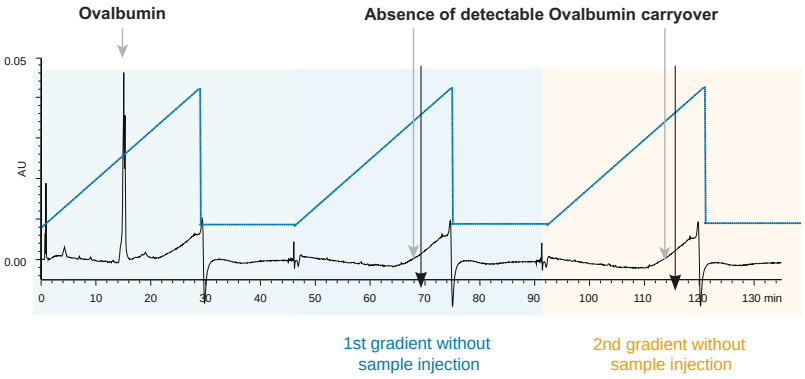
Minimal Protein Carryover

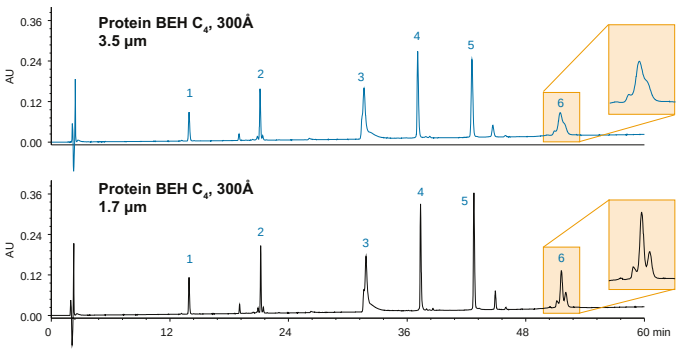


Conditions:

Columns:	ACQUITY UPLC Protein BEH C_4, 300Å, 1.7 µm, 2.1 mm × 50 mm
Part number:	186004495
Sample:	1.3 µg Ovalbumin injected
Eluent A:	0.1% trifluoroacetic acid in 100% HPLC-grade water
Eluent B:	0.075% trifluoroacetic acid in 71.4% acetonitrile
Flow rate:	0.2 mL/min
Gradient:	28–100% B in 25 min, hold at 100 %B for 2 min then re-equilibrate column at 28% B for 18 min, gradient restarted at 45 min and at 90 min
Column temp.:	40 °C
Detection:	UV @ 220 nm

Column carryover was tested by running multiple gradients following a single injection. Protein peaks observed during the first gradient are not found in subsequent gradients.

Improved Protein Resolution with UPLC Technology



Conditions:

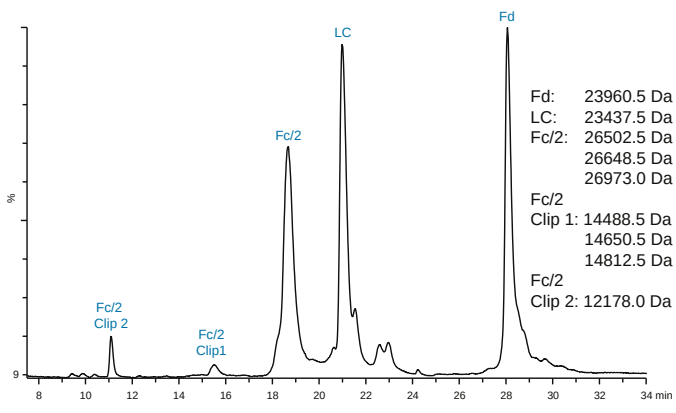
Columns:	ACQUITY UPLC Protein BEH C_4, 300Å, 1.7 µm (top), 3.5 µm (bottom), 2.1 × 150 mm
Part numbers:	186004497 (1.7 µm); 186004500 (3.5 µm)
Sample:	MassPREP Protein Standard, 0.1% TFA in 5% acetonitrile
Part number:	186004900
Mobile phase A:	0.1% trifluoroacetic acid in water
Mobile phase B:	0.1% trifluoroacetic acid in 100% acetonitrile
Flow rate:	0.2 mL/min
Gradient:	20–55% B in 60 min
Column temp.:	80 °C
Injection:	3.3 µL
Detection:	UV @ 220 nm

Compounds:

1. Ribonuclease A	0.04 mg/mL
2. Cytochrome *c*	0.06 mg/mL
3. Bovine Serum Albumin	0.20 mg/mL
4. Myoglobin	0.13 mg/mL
5. Enolase	0.22 mg/mL
6. Phosphorylase b	0.59 mg/mL

Two separations were performed, traditional and UPLC. The traditional separation used a Protein BEH C_4, 300Å Column packed with 3.5 µm particles. The UPLC separation used a Protein BEH C_4, 300Å Column packed with 1.7 µm particles. The UPLC separation evidences sharper peaks for all proteins in the test mixture. The multiple peaks around phosphorylase, at approximately 50 minutes, attest to this improved resolution. The comparison was performed fitting both columns onto a UPLC system, to preserve the minimized band broadening. The benefits of the small-particle UPLC BEH C_4 300Å Column would be lost without the optimized ACQUITY UPLC System.

Protein BEH C_4, 300Å Columns for Protein Characterization with UPLC-MS



Conditions:

Column:	ACQUITY UPLC Protein BEH C_4, 300Å, 1.7 µm, 2.1 × 50 mm
Part number:	186004495
Sample:	Lys-C digested (limited) reduced monoclonal antibody
Eluent A:	0.1% formic acid in 100% HPLC grade water
Eluent B:	0.1% formic acid in 100% acetonitrile
Flow rate:	0.2 mL/min
Gradient:	5% B for 5 min then 5–21% B in 0.1 min, then 21–29% B in 30 min
Column temp.:	80 °C

Waters LCT™ Premier XE MS Detection

Capillary voltage:	3200 V
Cone voltage:	45 V
Source temp.:	150 °C
Desolvation temp.:	350 °C
Desolvation gas:	000 L/h

The large fragments obtained through LysC digestion of a monoclonal antibody can be separated on the ACQUITY UPLC Protein BEH C_4, 300Å Column coupled directly to ESI/Tof MS for identification of the individual peptide products.

Note: ACQUITY UPLC Protein BEH C_4, 300Å, 1.7 µm Columns are designed for use with the ACQUITY UPLC System. The benefits of the small-particle packing in ACQUITY UPLC Protein BEH C_4, 300Å, 1.7 µm Columns are realized only with the low system volume and low detector dispersion of an ACQUITY UPLC System.