

## Reversed-Phase vs. HILIC-Based Analysis of a Lys-C Digest of Trastuzumab

### LC Conditions:

LC system: ACQUITY UPLC H-Class Bio System  
Sample temp.: 10 °C  
Vials: Polypropylene 12 × 32 mm Screw Neck, 300 µL volume (p/n: 186002640)

### Reversed-Phase LC

Column: ACQUITY UPLC Peptide BEH C<sub>18</sub>, 300Å, 1.7 µm, 2.1 × 150 mm  
Part number: 186003687  
Column temp.: 60 °C  
Injection: RP injection volume: 24.2 µL (aqueous digest)  
Flow rate: 0.2 mL/min  
Mobile phase A: 0.1% (v/v) TFA, water  
Mobile phase B: 0.1% (v/v) TFA, acetonitrile  
Gradient:

Time	%A	%B	Curve
0.0	98.0	2.0	6
96.0	50.0	50.0	6

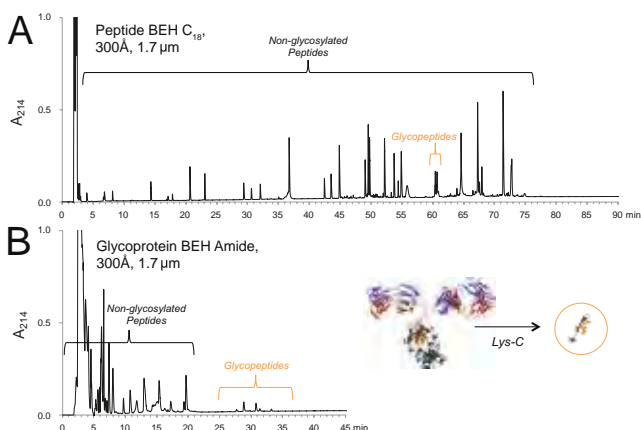
### HILIC LC Conditions:

Column: ACQUITY UPLC Glycoprotein BEH Amide, 300Å, 1.7 µm, 2.1 × 150 mm Column Kit (p/n: 176003702) that contains Glycoprotein Performance Test Standard (p/n: 186008010)  
Column temp.: 30 °C  
Injection volume: 100–250 µL (Aqueous digests were diluted with 4 parts acetonitrile and 0.1 part dimethylsulfoxide to obtain a miscible, HILIC compatible diluent).  
Flow rate: 0.2 mL/min  
Mobile phase A: 0.1% (v/v) TFA, water  
Mobile phase B: 0.1% (v/v) TFA, acetonitrile  
Gradient:

Time	%A	%B	Curve
0.0	20.0	80.0	6
60.0	50.0	50.0	6

### MS Conditions:

MS system: SYNAPT® G2-S HDMS<sup>+</sup>  
Ionization mode: ESI+  
Analyzer mode: Resolution (~20 K)  
Capillary voltage: 3.0 kV  
Cone voltage: 25 V  
Source temp.: 120 °C  
Desolvation temp.: 350 °C  
Desolvation gas flow: 800 L/Hr  
Acquisition: 50–2500 m/z, 0.1 sec scan rate  
Data management: MassLynx® Software v4.1/UNIFI® v1.7



A traditional reversed-phase separation of the Lys-C digest using an ACQUITY UPLC Peptide BEH C<sub>18</sub>, 300Å, 1.7 µm, 2.1 × 150 mm Column (top) vs. a HILIC separation of the Lys-C digest using an ACQUITY UPLC Glycoprotein BEH Amide, 300Å, 1.7 µm, 2.1 × 150 mm Column (bottom). In each analysis, 9.2 µg of the Lys-C digest was separated using the same gradient slope and injecting sample from a diluent comprised of either approximately 0.2% TFA in 80:20 acetonitrile/water (HILIC) or 100% water (reversed-phase). For more information, reference application note 720005409EN.

## Ordering Information

### ACQUITY UPLC Glycoprotein BEH Amide 300Å Columns and Kits (Includes the Glycoprotein Performance Test Standard)

	Dimension	P/N
Particle Size: 1.7 µm		
BEH Amide, 300Å	2.1 × 5 mm	176003699*
	2.1 × 50 mm	176003700
	2.1 × 100 mm	176003701
	2.1 × 150 mm	176003702

\*VanGuard Pre-column 3/pk.

### ACQUITY UPLC Glycoprotein BEH Amide 300Å Method Validation Kits\* (Includes the Glycoprotein Performance Test Standard)

	Dimension	P/N
Particle Size: 1.7 µm		
BEH Amide, 300Å	2.1 × 100 mm	176003703

\*Each Method Validation Kit contains 3 columns, each from a different batch.

## Standards

Description	P/N
Glycoprotein Performance Test Standard	186008010
Intact mAb Mass Check Standard	186006552