

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 C₁₈, 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

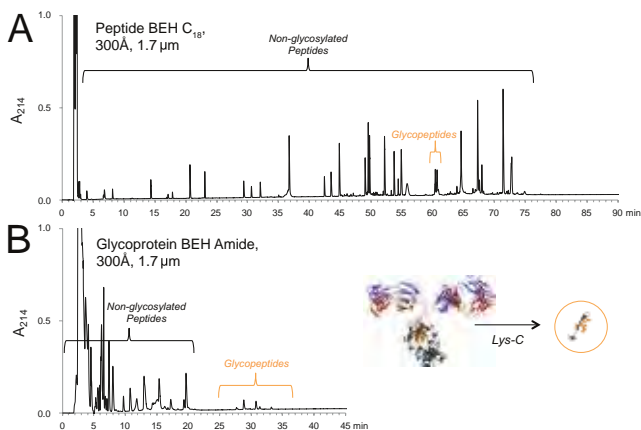
HILIC LC Conditions:

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⁺
 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 C₁₈, 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