Supelco®
Analytical Products

MilliporeSigma

Rapid and Robust Protein Chromatography

Chromolith® WP 300 RP-18 2 mm I.D. HPLC Columns

The most hydrophobic of the Chromolith® WP 300 line, the RP-18 column is useful for the resolution of peptides and smaller proteins. One critical quality attribute (CQA) required by regulatory bodies is the peptide map of a biotherapeutic. Peptide maps generated by RP-HPLC provide valuable information about protein structure, stability, and purity. To be effective, the RP-HPLC column must be able to resolve a high percentage of the peptides in the sample. The more peptides, the better the information. The Chromolith® WP 300 RP-18 column gives unsurpassed RP-HPLC resolution of peptide maps from enzymatic digests. The improvements in silica and bonded-phase chemistry incorporated into the Chromolith® WP 300 line improve resolution by increasing efficiency and reducing peak tailing.

Key Benefits:

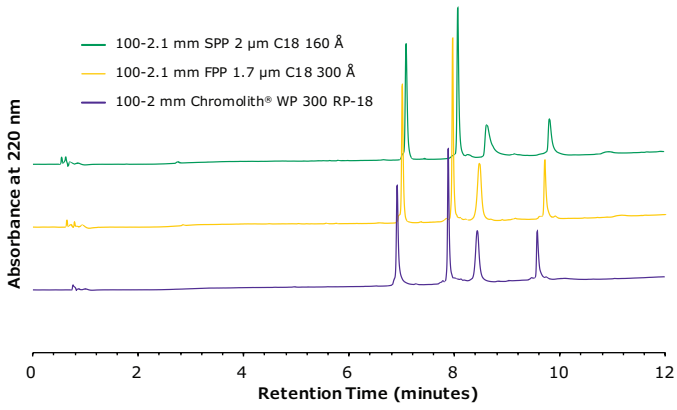
- Monolithic skeleton permits high flow rates to maximize throughput
- 300 Å mesopores permit large molecules to enter without fear of size-exclusion effects
- Matrix-loaded samples can be injected onto the column with little to no sample prep.

Protein Analysis on Chromolith® WP 300 RP-18

For large molecule separations, high efficiency separations are necessary in order to achieve resolution and good peak shape. Moving to sub-2 µm FPP-packed columns or 2.0 µm SPP-packed columns can deliver that desire; however, this comes at the cost of elevated backpressure. Chromolith® WP 300 RP-18, 2 mm I.D. columns provide UHPLC efficiencies, but at nearly 1/10th the backpressure.

Chromatographic conditions:

Columns:	Chromolith® WP 300 RP-18 100-2 mm (1.52370.0001) SPP, C18, 160 Å, 2.0 µm, 100-2.1 mm FPP, C18, 300 Å, 1.7 µm, 100-2.1 mm
Mobile phase:	A: water (0.1% TFA) B: acetonitrile (0.08% TFA)
Gradient:	4% B to 60% B in 10 minutes
Flow rate:	0.38 mL/min
Detection:	UV, 220 nm
Column temperature:	30 °C
Injection volume:	0.5 µL
Sample:	HPLC Protein Mix 1 mg/mL, water 1) Ribonuclease 2) Cytochrome C 3) Holo-Transferrin 4) Apomyoglobin

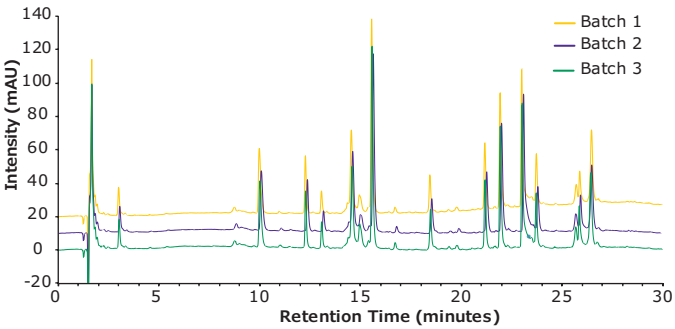


Excellent Lot-to-Lot Reproducibility

Chromolith® WP 300 RP-18 columns exhibit excellent batch-to-batch reproducibility, as demonstrated below with the same peptide map generated for Cytochrome C using three different Chromolith® WP 300 RP-18 columns across three different batches.

Chromatographic conditions:

Column	Chromolith® WP 300 RP-18 100-2 mm (1.52370.0001)
Mobile Phase	A: acetonitrile 0.08% (v/v) TFA B: water 0.1% (v/v) TFA
Gradient:	Time %A %B 0 5 95 25.0 30 70 30.0 30 70
Flow rate:	0.190 mL/min
Pressure:	18 bar
Detection:	Vanquish DAD 20 Hz, UV, 214 nm
Detector cell:	LightPipe 10 mm
Temperature:	30 °C
Injection volume:	0.2 µL
Sample:	Rapid Trypsin Digestion with SOLu-Trypsin Rapid Digestion Kit 2.5 mg Cytochrome C was added in a PCR vial and dissolved in 320 µL Rapid Trypsin Digestion Buffer. In the solution was 80 µL SOLu-Trypsin added and incubated at 60 °C for 1 hour in a Thermomixer. The digestion was quenched by adding 12 µL of hydrochloric acid 32 %.

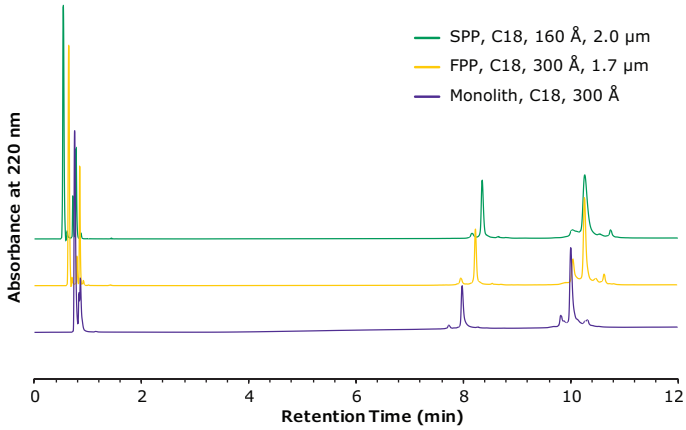


Antibody Fragment Analysis

Fragment analysis of a monoclonal antibody (mAb), also called middle-up analysis, is a useful technique in characterizing mAb domains without the inherent complexity of a peptide map. High efficiency is needed here to resolve subtle, structural variants of the mAb domains. The Chromolith® WP 300 RP-18 column is able to achieve the same separation efficiency and sensitivity as sub-2 µm FPP and 2.0 µm SPP-packed columns but at only 20% of the backpressure of those columns.

Chromatographic conditions:

Column	Chromolith® WP 300 RP-18, 2 mm I.D. (1.52370.0001) SPP, C18, 160 Å, 2.0 µm, 100-2.1 mm FPP, C18, 300 Å, 1.7 µm, 100-2.1 mm
Mobile Phase	A: Water (0.1% (v/v) TFA) B: Acetonitrile (0.08% (v/v) TFA)
Gradient:	Time (Min) %B 0 20 1 20 9 45
Flow rate:	380 µL/min
Detection:	UV, 220 nm
Temperature:	80 °C
Injection volume:	1.0 µL
Sample:	SigmaMAb, 2 mg/mL (SiLu™ Lite Universal Antibody)
DTT digest:	60 µL of 40 mM Dithiothreitol (DTT) solution was added in a PCR vial, 40 µL mAb was added and incubated at 37 °C for 30 minutes creating light chain (LC) and heavy chain (HC) parts of the antibody.



Ordering Information

Cat. No.	Description	Length (mm)	I.D. (mm)
11-010-277	Chromolith® WP 300 RP-18 Column	100	2
11-010-278	Chromolith® WP 300 RP-18 Column	50	2
11-010-279	Chromolith® WP 300 RP-18 Guard Columns (3 units)	5	2

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