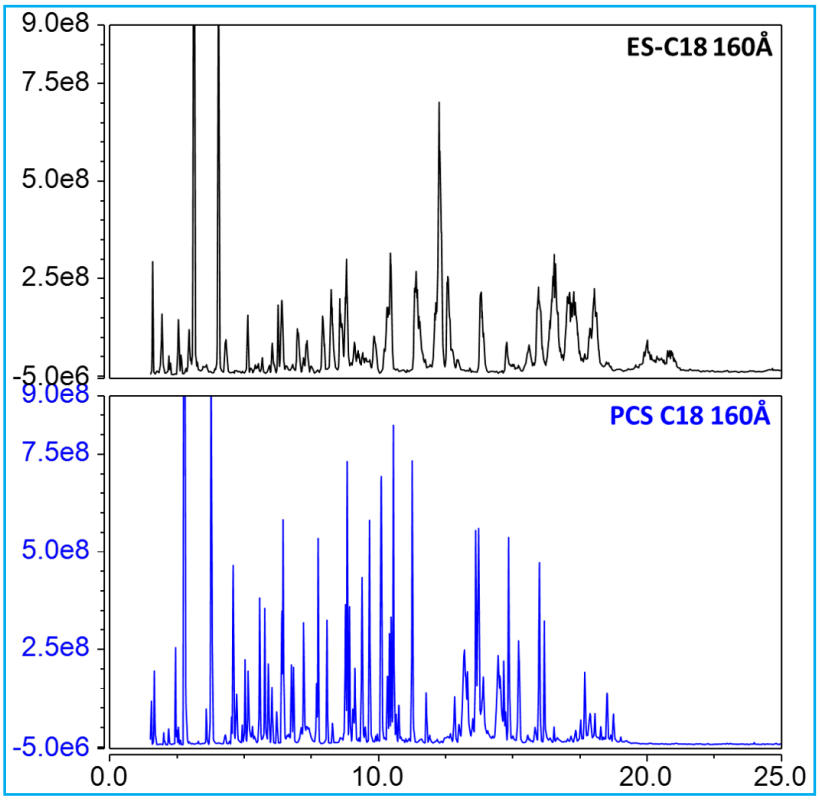
HALO®

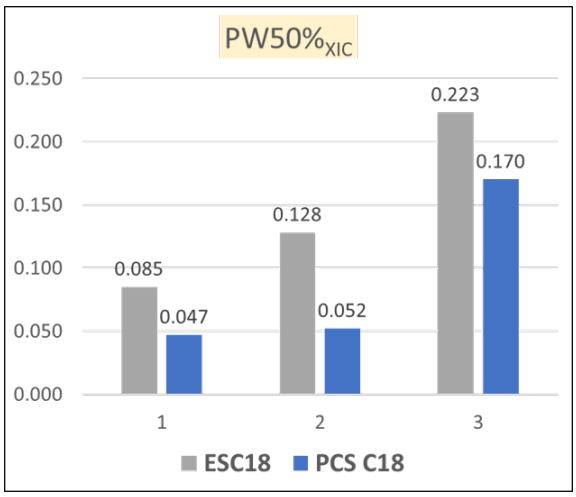
BIOPHARMACEUTICALS

Increased Peak Capacity of Trastuzumab Tryptic Digest on PCS C18

357



#	Tryptic Peptide	XIC	$t_{R\ (min)}$
1	AEDTAVYYC(Carbamidomethyl)SR	667.7877 Z=2	ES-C18: 6.41 PCS C18: 4.60
2	TPEVTC(Carbamidomethyl)VVVDVSHEDPEVK	713.6807 Z=3	ES-C18: 12.28 PCS C18: 10.11
3	TVAAPSVFIFPPSDEQLK	973.5171 Z=2	ES-C18: 17.12 PCS C18: 14.47



DIGESTION PROCEDURE:

Standard digest conditions were used for an overnight digestion of trastuzumab drug product (stock concentration 21mg/mL) at 37 °C with shaking (final concentration 1.25µg mAb/µL). The sample buffer was 50mM ammnonium bicarbonate. The sample was diluted with 50mM ABC to 1.5M Guanidine prior to trypsin digestion. The next day, the digest was adjusted to 0.5% formic acid prior to LCMS analysis. 2% ACN was added to the samplprior to analysis to aid solubility. The injected sample consisted of 1.5M guanidine HCl, 2% ACN, 0.5%Formic Acid, ~50mM Ammonium Bicarbonate, 1.25µg/µL digested mAb, 0.06µg/µL trypsin.

A separation of Trastuzumab tryptic digest is performed on two 160 Å HALO columns, the ES-C18 and PCS C18 phases. On the MS system a formic acid mobile phase is used in order to maintain high ionization efficiencies. Because of the use of a low ionic mobile phase additive (formic acid) separation of the digested mAb is difficult for the standard ES-C18 phase. By using a positively charged stationary phase (PCS C18) with low ionic condtions allows for an alternative selectivity and better separation of the peptides. By measuring peak width @ 50% of 3 distinct peptides it can be seen how the effect of the PCS C18 phase can significantly help peptide separations that require low ionic mobile phases such as formic acid.

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TEST CONDITIONS:

Column: HALO 160 Å ES-C18 , 2.7 µm, 2.1 x 150 mm
Part Number: 92122-702
Column: HALO 160 Å PCS-C18 , 2.7 µm, 2.1 x 150 mm
Part Number: 92112-717
Mobile Phase A: Water + 0.1% Formic Acid
Mobile Phase B: Acetonitrile + 0.1% Formic Acid
Gradient:

Time	%B
0.0	3
30.0	50
30.1	95
33.0	95
33.1	3
37.0	3

Flow Rate: 0.4 mL/min
Pressure: 465 bar
Temperature: 60 °C
Injection Volume: 1 µL
Sample: Trastuzumab Tryptic Digest (1.25 µg/µL)
Sample Solvent: Refer to Digestion Procedure
LC System: Shimadzu Nexera X2

MS CONDITIONS:

System: QExactive HF
ESI positive polarity
300-2000 m/z
Source voltage: 3.2kV
Sheath Gas: 40
Aux Gas: 20
Aus Gas Temp: 275°C
Capillary Temp: 320°C
µscans: 1
Max Injection Time: 200 msec
S-Lens RF: 50

Tubing Optimization:

Column outlet to Diverter Valve: AMT MarvelXACT™ PEEKsil™ 50 µm ID x 350 mm
Part Number: PS7050350
Diverter Valve to Ground: AMT MarvelXACT™ PEEKsil™ 50 µm ID x 350 mm
Part Number: PS7050350
Ground to Source: AMT MarvelXACT™ PEEKsil™ 50 µm ID x 150 mm
Part Number: PS7050150

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