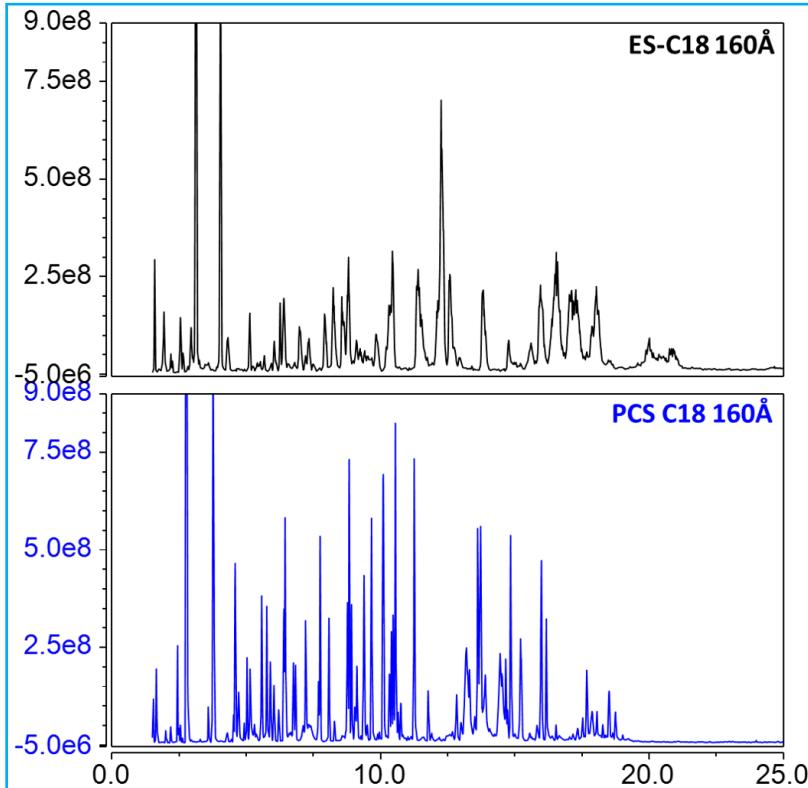


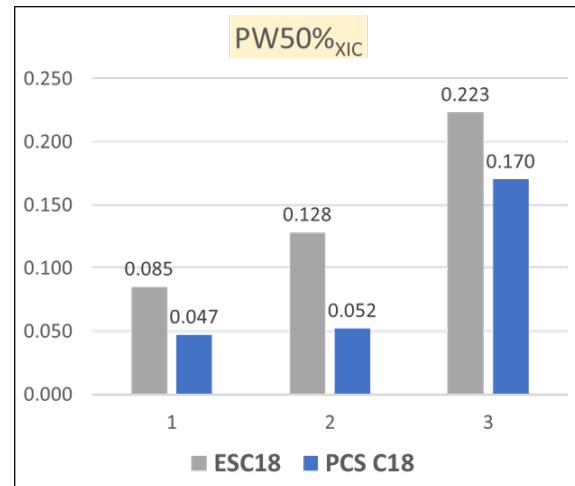


Increased Peak Capacity of Trastuzumab Tryptic Digest on PCS C18

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#	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 ammonium 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 sample prior 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 conditions 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.





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

