

Evaluation of Positively Charged Surface Stationary Phases for Improved Chromatographic Separations of Basic Analytes in Small Molecules and Peptides

William Miles, Benjamin Libert, Conner McHale, Peter Pellegrinelli, [Harry J. Ritchie](#)
Advanced Materials Technology Inc., Wilmington, DE

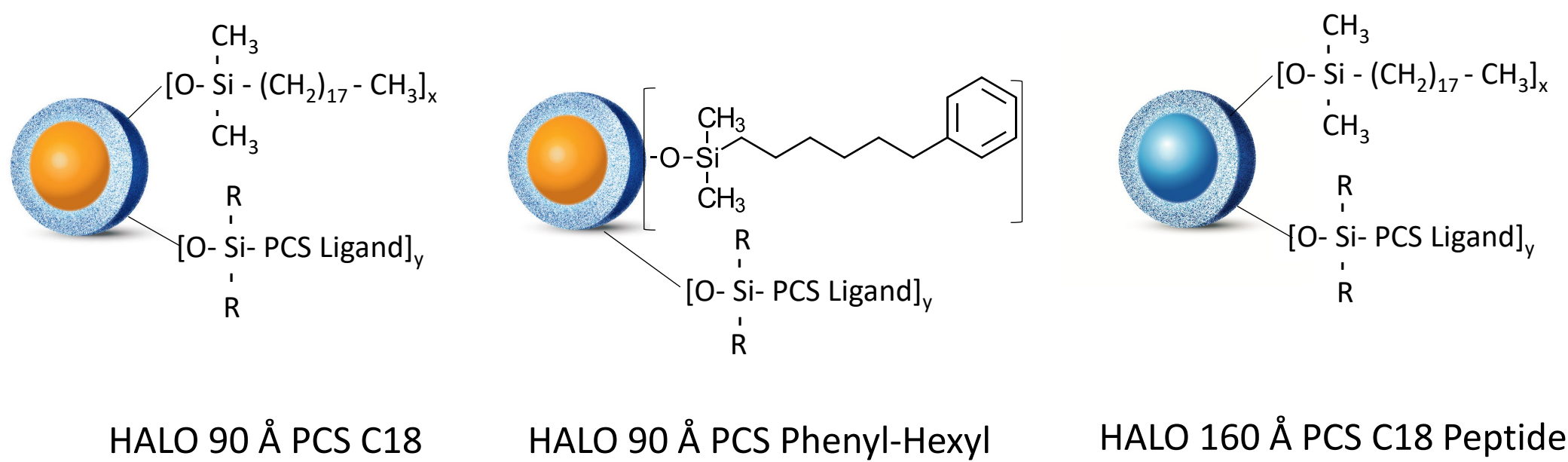
 advancedmaterialstechnology
ISC 2024
Liverpool, UK

INTRODUCTION

Problem: basic compounds become charged at low pH leading to tailed peak shape as sample load is increased under typical low ionic strength reversed-phase LC and LCMS conditions

Solutions: To improve peak shape, there are a few options such as adding an ion pair reagent or adding a buffer, but these options are not always 100% compatible with MS detection. Specifically, trifluoroacetic acid (TFA) reduces MS ionization efficiency and phosphate buffer is not MS compatible. Another solution is to use a stationary phase with a positive charged ligand. The HALO® PCS (positive charged surface) product family incorporates a positively charged ligand in addition to a traditional stationary phase on superficially porous silica particles. This stationary phase enables improved peak shape, sample loading, and better impurity analysis.

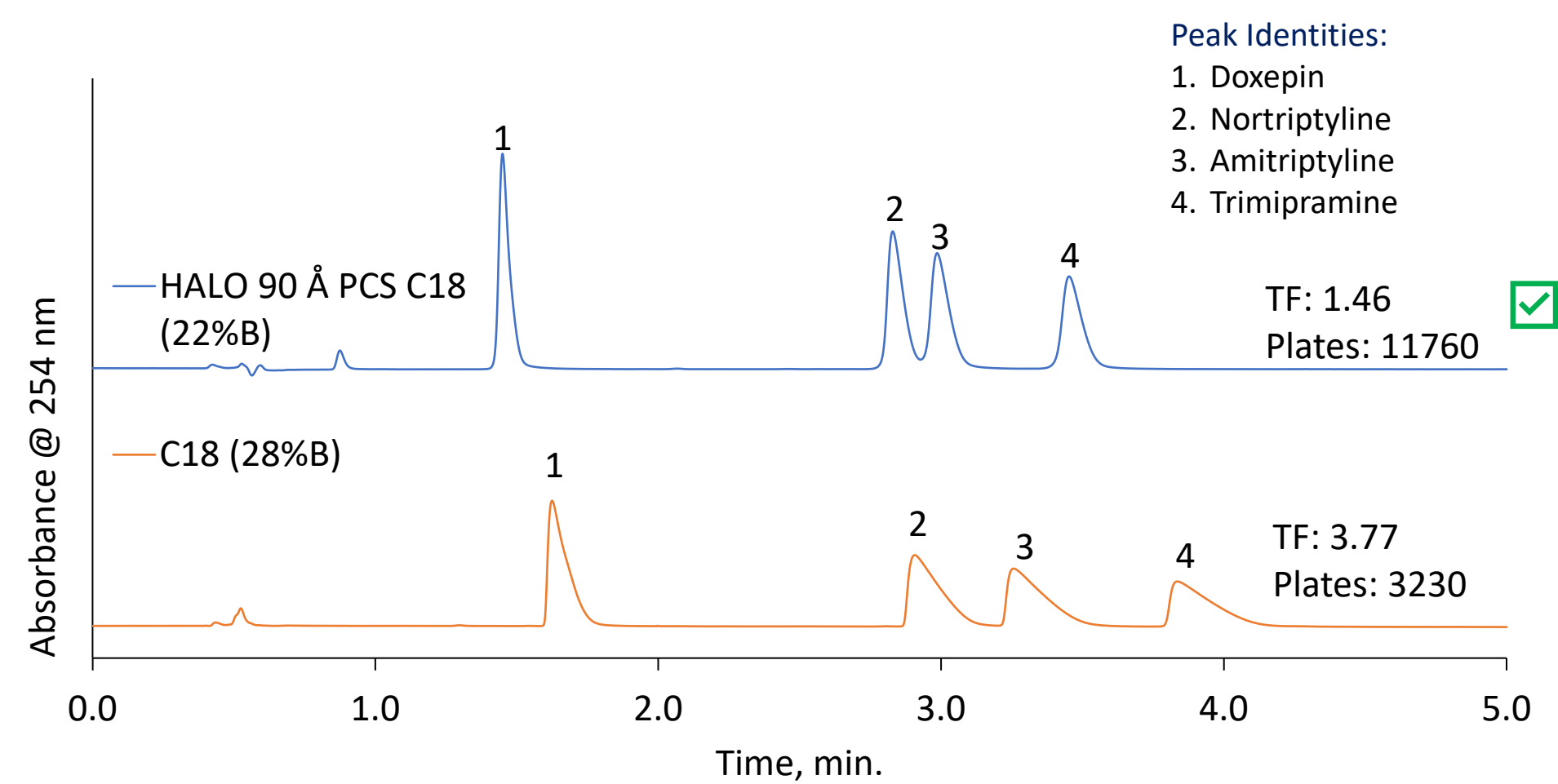
HALO® PCS STATIONARY PHASES



- 2.7 µm particle size with 0.5 µm thick shell
- 90 Å pore size for small molecules and 160 Å for peptides and tryptic fragments
- Excellent peak shape and increased loading capacity for basic compounds
- 100% aqueous compatible
- UHPLC and LCMS compatible

IMPROVEMENTS TO LC OF BASIC MOLECULES

2.1 x 100 mm, A: Water, 0.1% Formic Acid; B: Acetonitrile, 0.1% Formic Acid; Flow Rate: 0.4 mL/min; Back Pressure: 242 bar; Temperature: 30 °C; Injection: 0.5 µL (31 µg)
Sample Solvent: 75/25 Water/ACN; Wavelength: PDA, 254 nm, LC System: Shimadzu Nexera X2

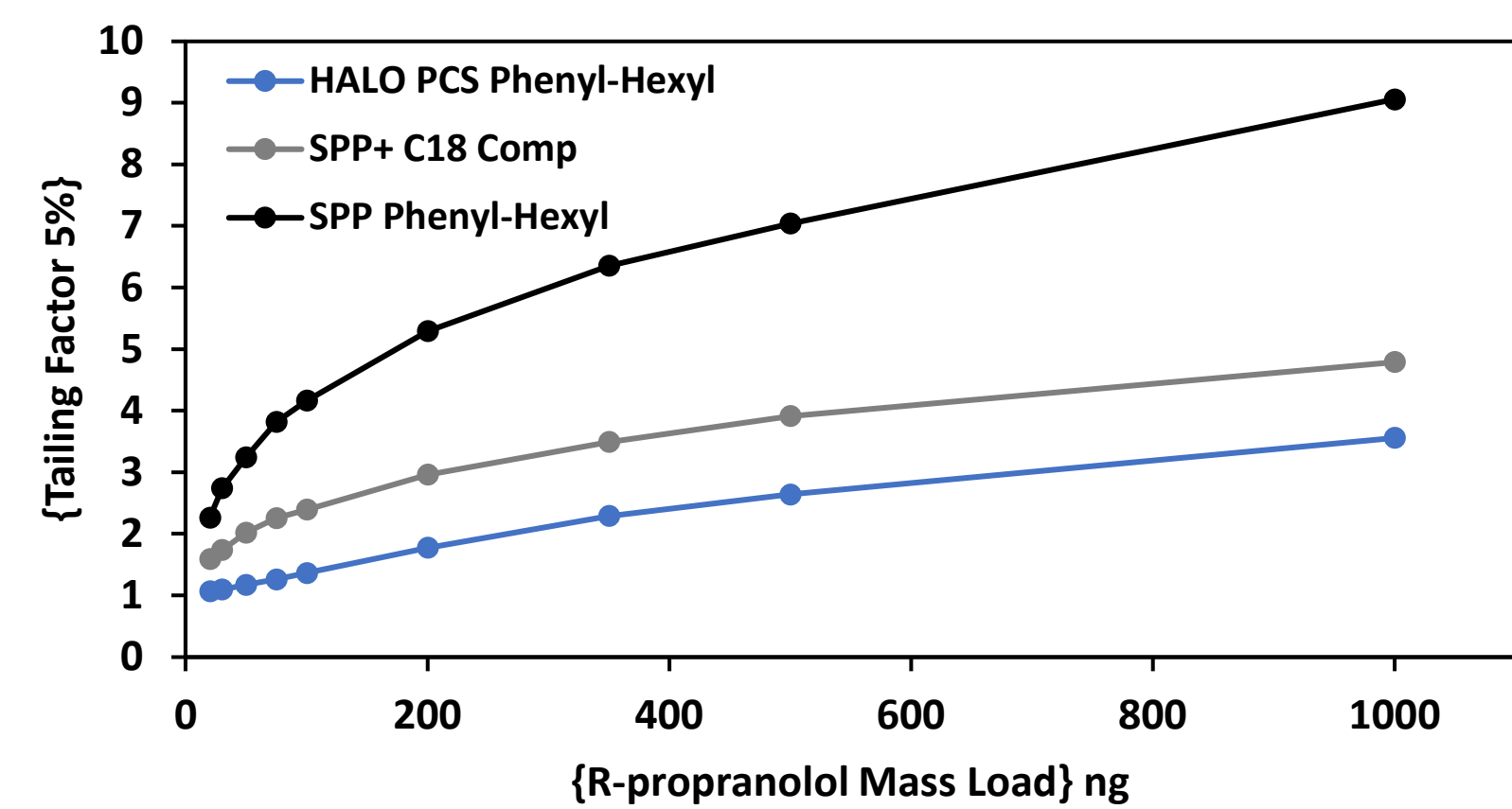
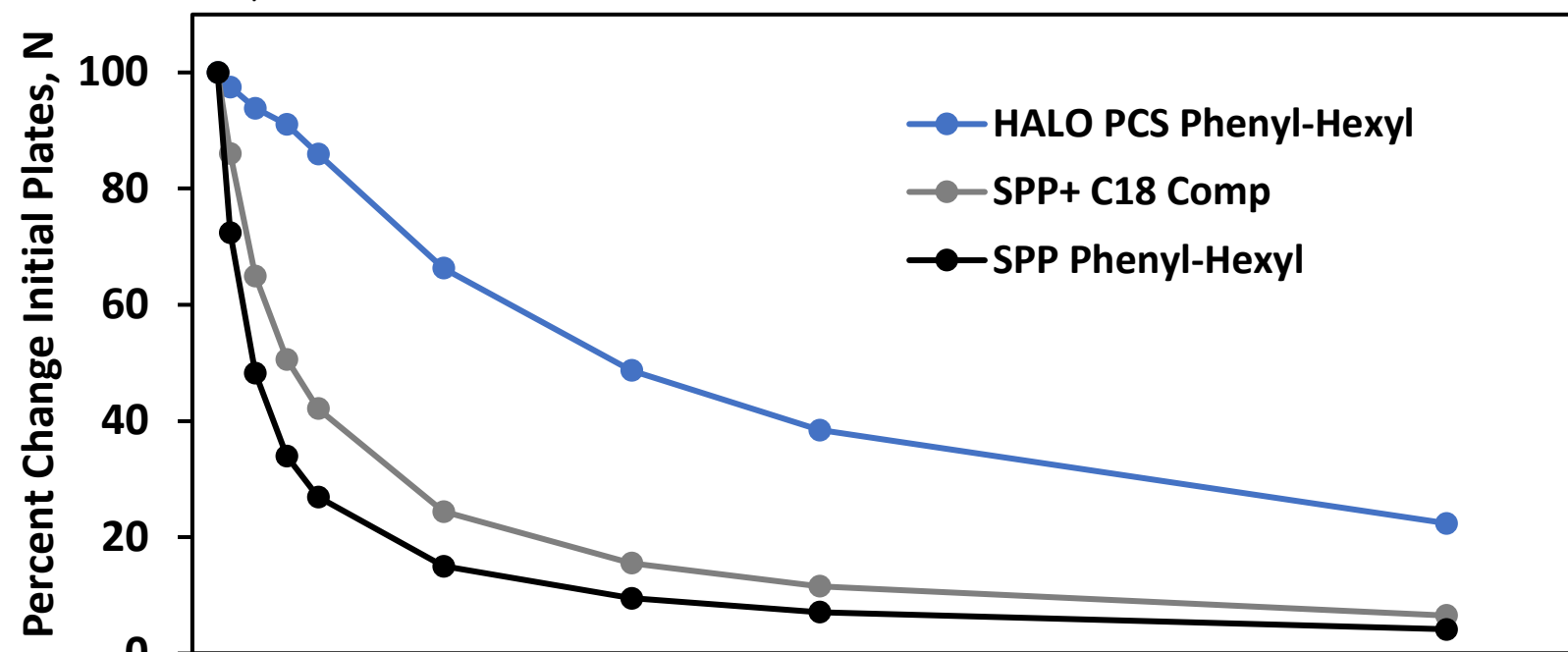


- Improved tailing factor and efficiency are obtained with HALO® PCS C18 when compared to a traditional uncharged C18 stationary phase for this mix of 4 tricyclic antidepressants

Improved Sample Loading of Basic Compounds

Test Conditions:
Columns: 2.1 x100 mm
90 Å HALO PCS Phenyl-Hexyl 2.7 µm (14 %B)
90 Å Competitor SPP Charged C18, 2.7µm (19 %B)
90 Å SPP Uncharged Phenyl-Hexyl 2.7 µm (25 %B)
Flow Rate: 0.50 mL/min

MP A: Water/0.1% FA
MP B: ACN/0.1% FA
Injection: 1.0 µL
Temperature: 35 °C
Absorbance: PDA, 280 nm
Instrument: Shimadzu Nexera

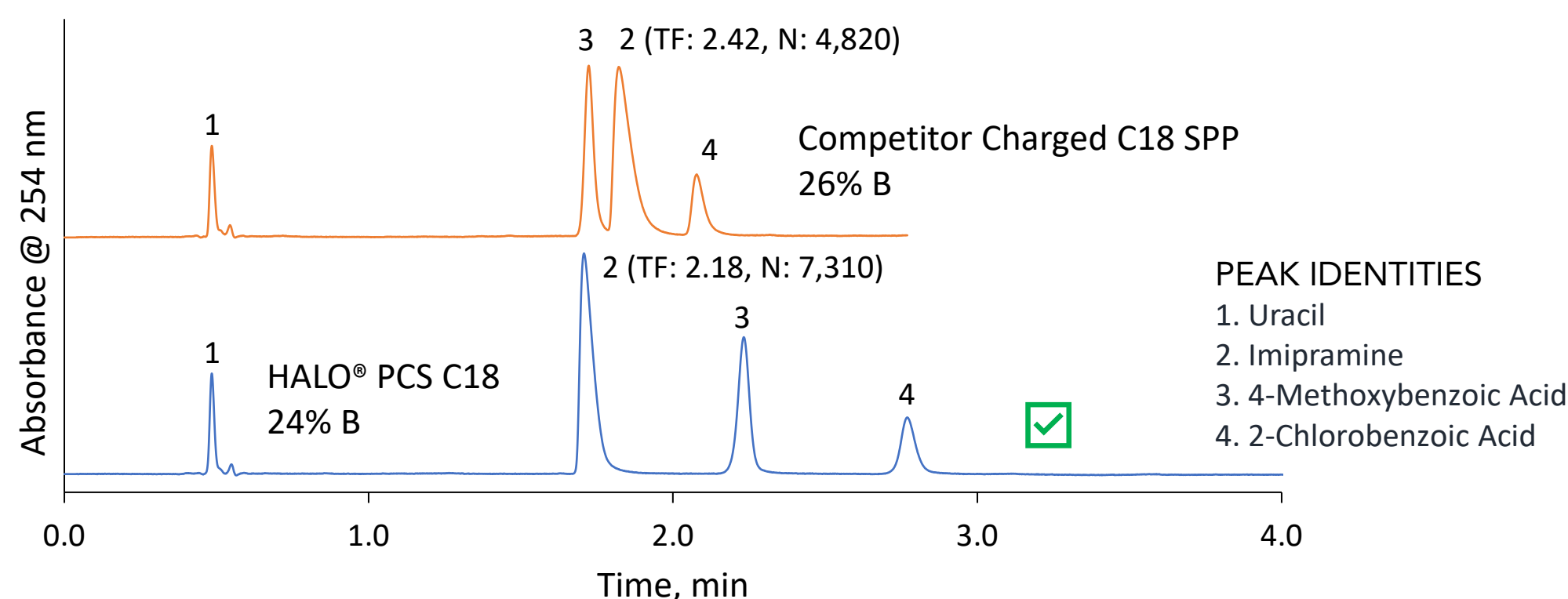


Loading Results			
20 ng R-propranolol			
Column	k'	TF 5%	Plates, N
HALO PCS Phenyl-Hexyl	3.18	1.06	12357
Competitor SPP Charged C18	2.99	1.59	10713
SPP Uncharged Phenyl-Hexyl	3.27	2.26	6317

- Lowest tailing factor and highest plates found with HALO® PCS Phenyl-Hexyl compared to a charged C18 phase
- Significant improvements are observed when compared to an uncharged Phenyl-Hexyl phase

HALO® PCS C18 Compared to Competitor Charged C18

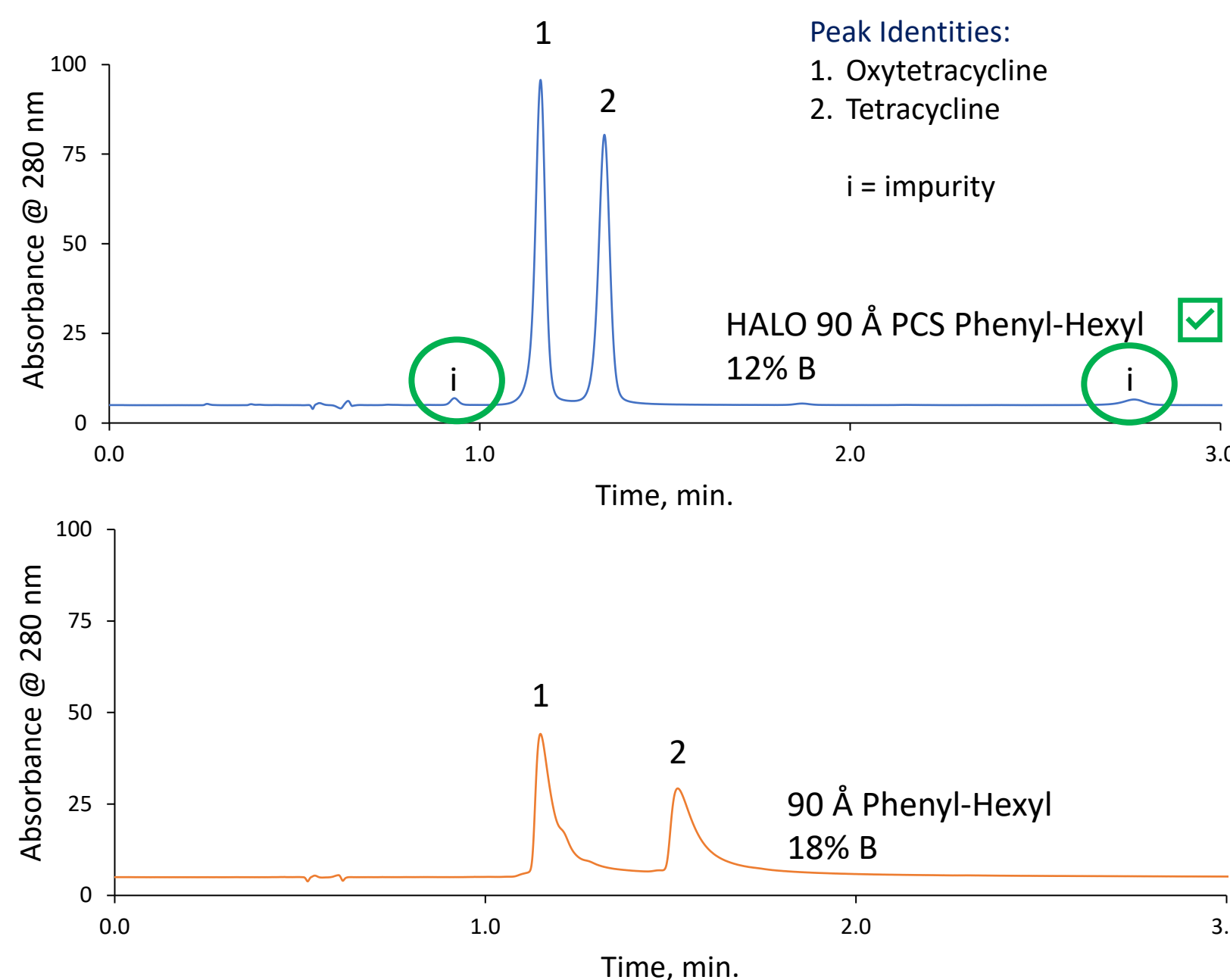
2.1 x 100 mm, A: Water, 0.1% Formic Acid; B: Acetonitrile, 0.1% Formic Acid; Isocratic as listed; Flow Rate: 0.4 mL/min; Back Pressure: 238 bar; Temperature: 35 °C; Injection: 1.0 µL
Sample Solvent: 70/30 Water/ACN; Wavelength: PDA, 254 nm, LC System: Shimadzu Nexera X2



- Better tailing and higher efficiency is observed with HALO® PCS C18 for the basic compound (peak 2)
- Peaks 3 & 4 (acids) show symmetrical peak shape with HALO® PCS C18

Improved Impurity Analysis with HALO® PCS Phenyl-Hexyl

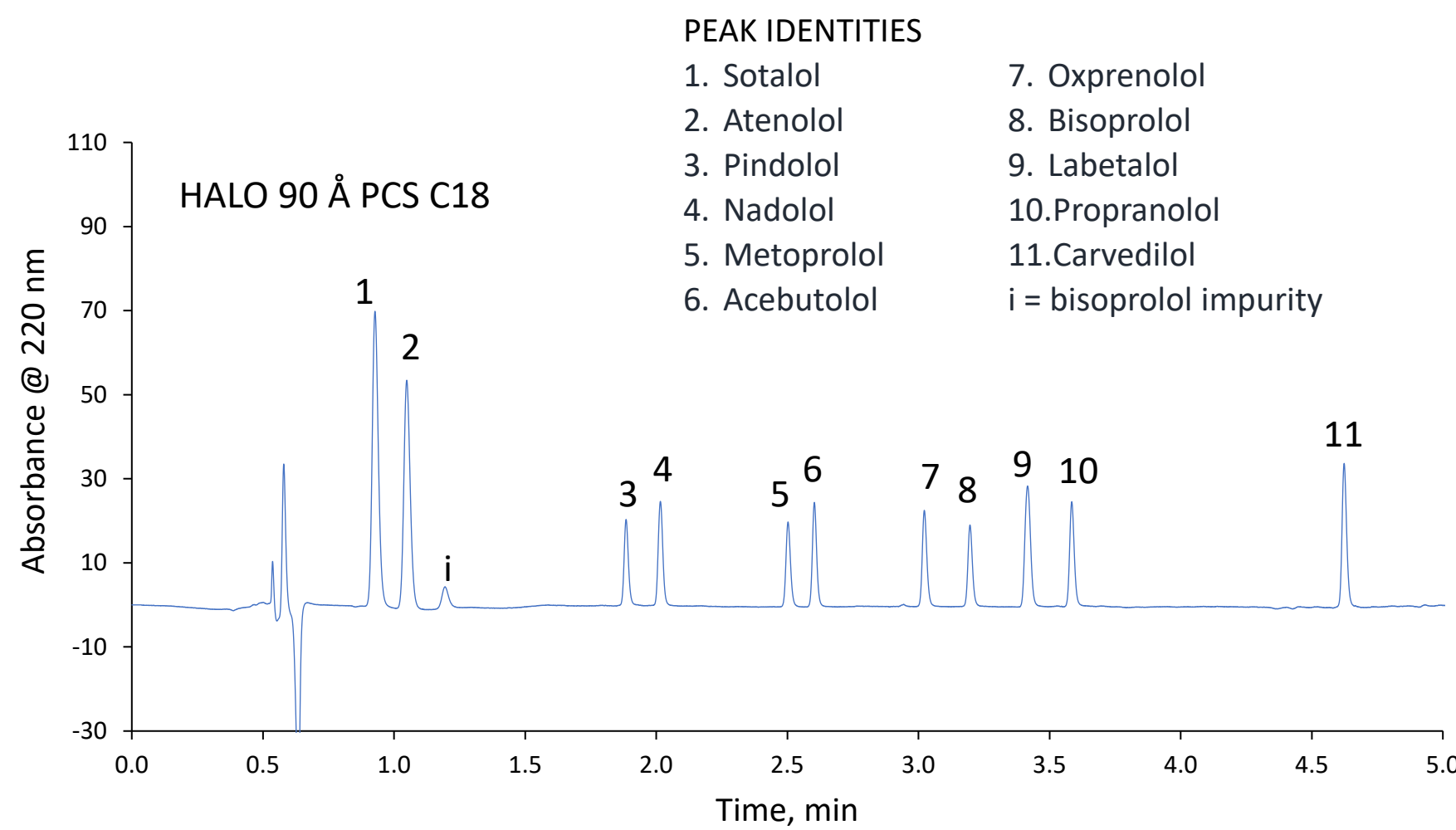
2.1 x 100 mm, A: Water, 0.1% Formic Acid; B: Acetonitrile, 0.1% Formic Acid; Isocratic as listed; Flow Rate: 0.4 mL/min; Back Pressure: 206 bar; Temperature: 35 °C; Injection: 1.0 µL
Sample Solvent: 90/10 Water/ACN; Wavelength: PDA, 280 nm, LC System: Shimadzu Nexera X2



- Peak widths are 50% smaller with HALO® PCS Phenyl-Hexyl
- Impurity peaks are clearly visible with HALO® PCS Phenyl-Hexyl since the peak shapes are so sharp, but are not visible at all on the uncharged Phenyl-Hexyl column

Fast Separation of β-Blockers

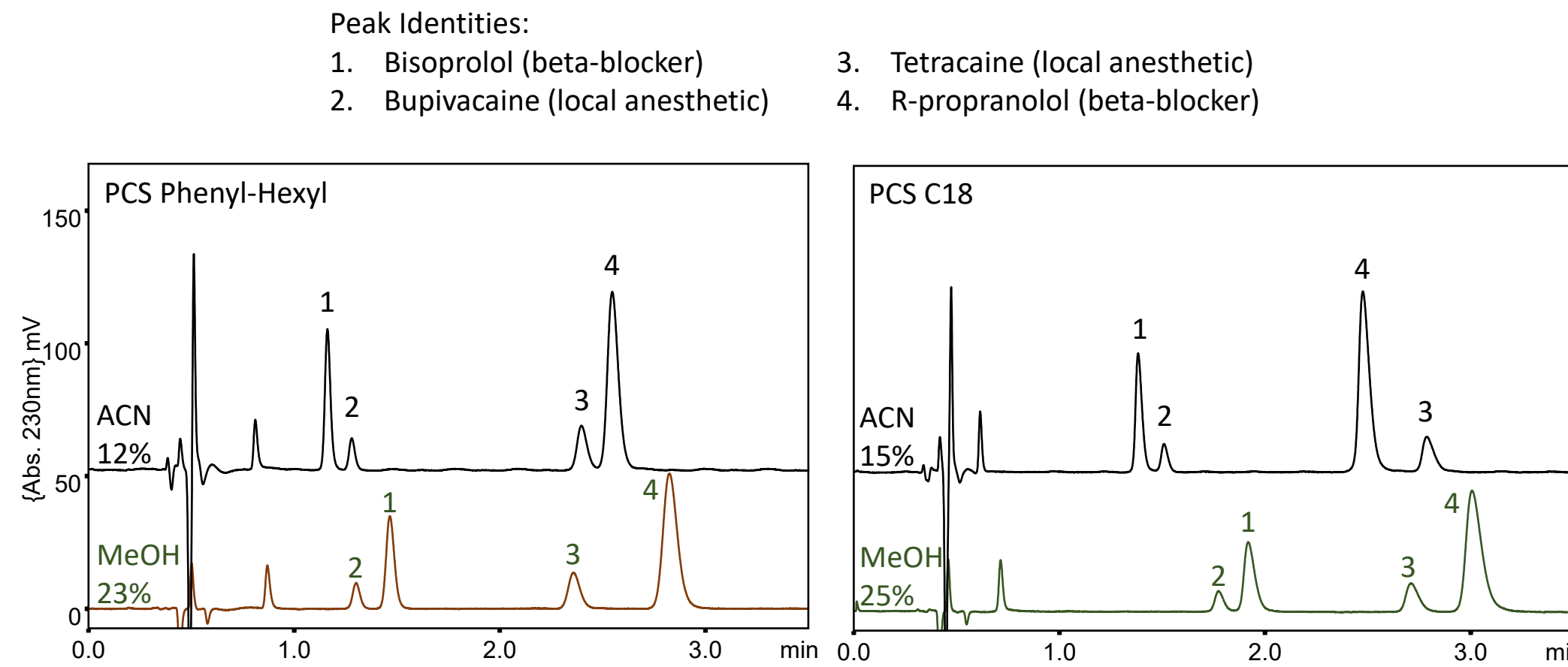
2.1 x 100 mm, A: Water, 0.1% Formic Acid; B: Acetonitrile, 0.1% Formic Acid; Gradient: 3-36% B in 5 min; Flow Rate: 0.4 mL/min; Back Pressure: 281 bar; Temperature: 30 °C; Injection: 1.0 µL
Sample Solvent: 93/7 Water/ACN; Wavelength: PDA, 220 nm, LC System: Shimadzu Nexera X2



- Beta blockers are used for the treatment and/or prevention of heart and circulatory conditions, such as arrhythmias, heart attack, and high blood pressure
- Eleven different beta blockers are separated in under 5 minutes using a HALO® PCS C18 column with UV detection and a mobile phase that is MS compatible

Effect of Organic Solvent on HALO® PCS Selectivity

2.1 x 100 mm, A: Water, 0.1% Formic Acid; B: Specified Solvent, 0.1% Formic Acid; Isocratic at specified % B; Flow Rate: 0.5 mL/min; Temperature: 30 °C; Injection: 1.0 µL
Wavelength: PDA, 230 nm, LC System: Shimadzu Nexera X2



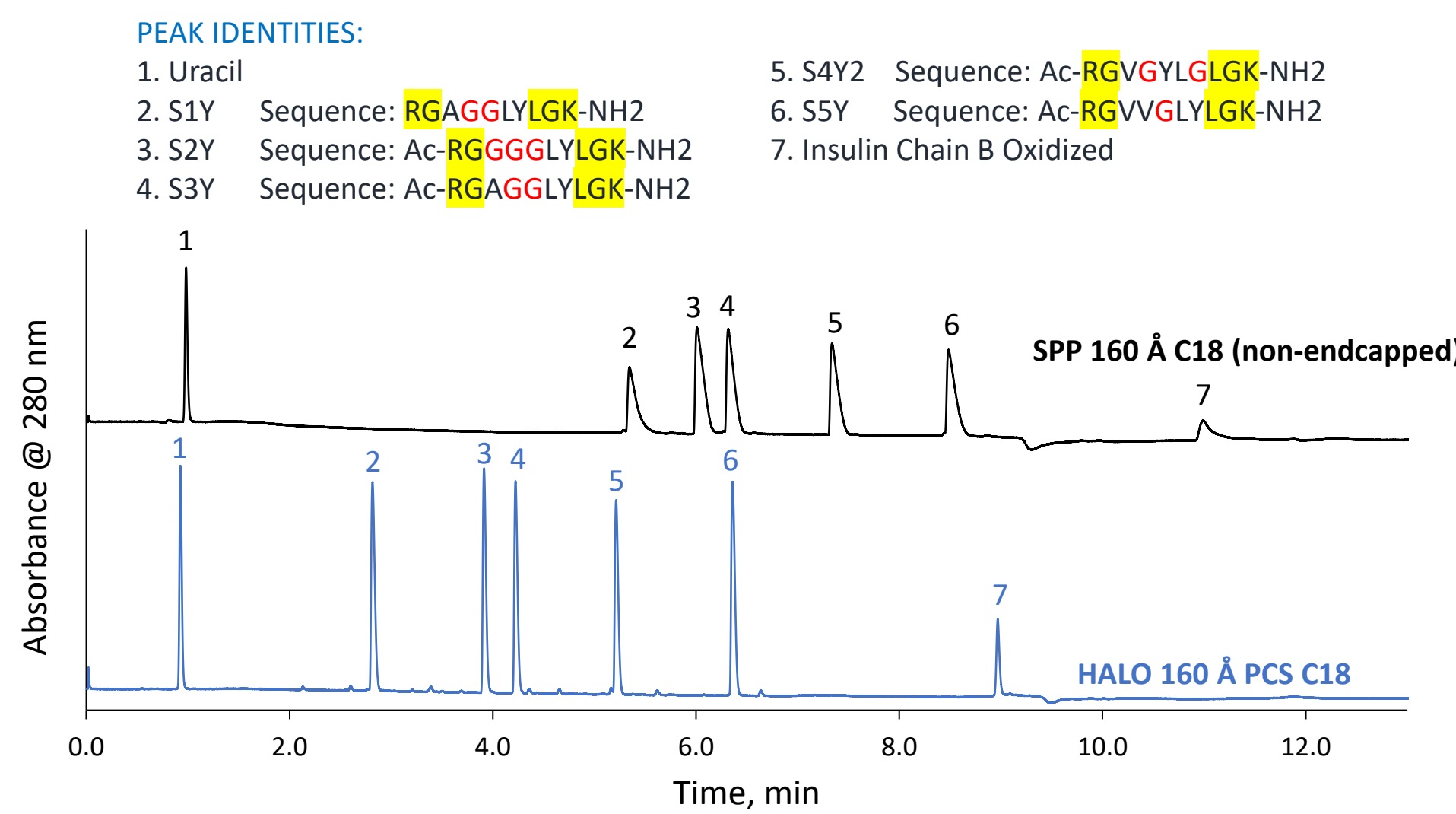
Acetonitrile Mobile Phase				
Bonded Phase	Peaks 1 and 2		Peaks 3 and 4	
	Selectivity	Rs	Selectivity	Rs
PCS Phenyl-Hexyl	1.17	2.22	1.08	1.56
PCS C18	1.13	2.08	1.15	3.07

Methanol Mobile Phase				
Bonded Phase	Peaks 1 and 2		Peaks 3 and 4	
	Selectivity	Rs	Selectivity	Rs
PCS Phenyl-Hexyl	1.20	2.29	1.25	3.95
PCS C18	1.11	1.61	1.13	2.30

- Changing from acetonitrile to methanol gives elution order changes for both PCS C18 and PCS Phenyl-Hexyl
- Increased selectivity and resolution are observed with PCS Phenyl-Hexyl compared to PCS C18 when run with methanol

IMPROVEMENTS TO LC AND LCMS OF PEPTIDES

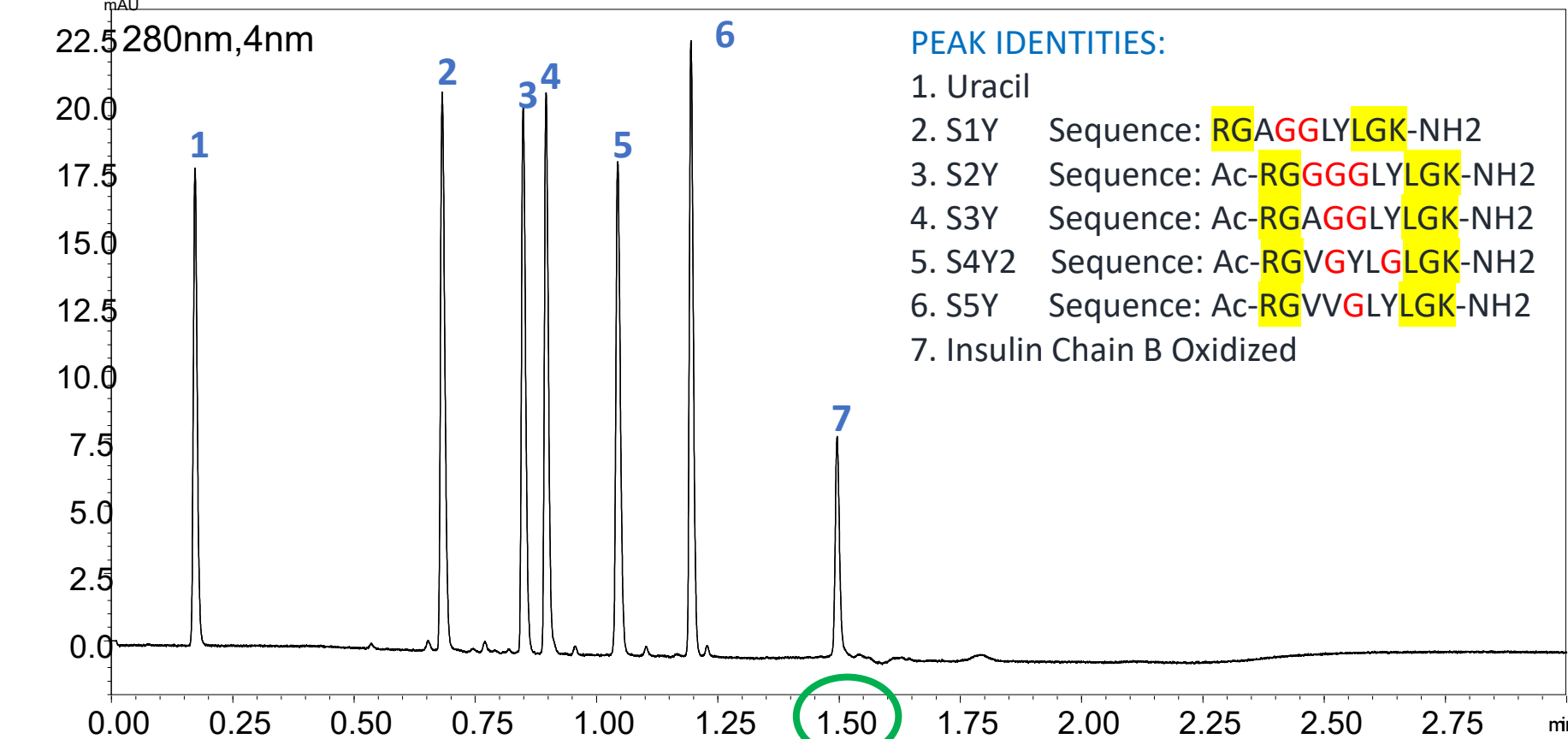
2.1 x 100 mm, A: Water, 0.1% Formic Acid; B: Acetonitrile, 0.1% Formic Acid; Gradient: 2-35 %B in 10 min.; Flow Rate: 0.3 mL/min; Temperature: 30 °C; Injection: 1.0 µL; Wavelength: PDA, 280 nm



- Gradient separation of 5 variant synthetic peptides + insulin B_{ox}
- Reduced retention time and increased resolution for HALO® PCS C18 Peptide compared to uncharged Peptide C18
- Improved peak widths and reduced tailing in formic acid

High Speed Peptide Analysis

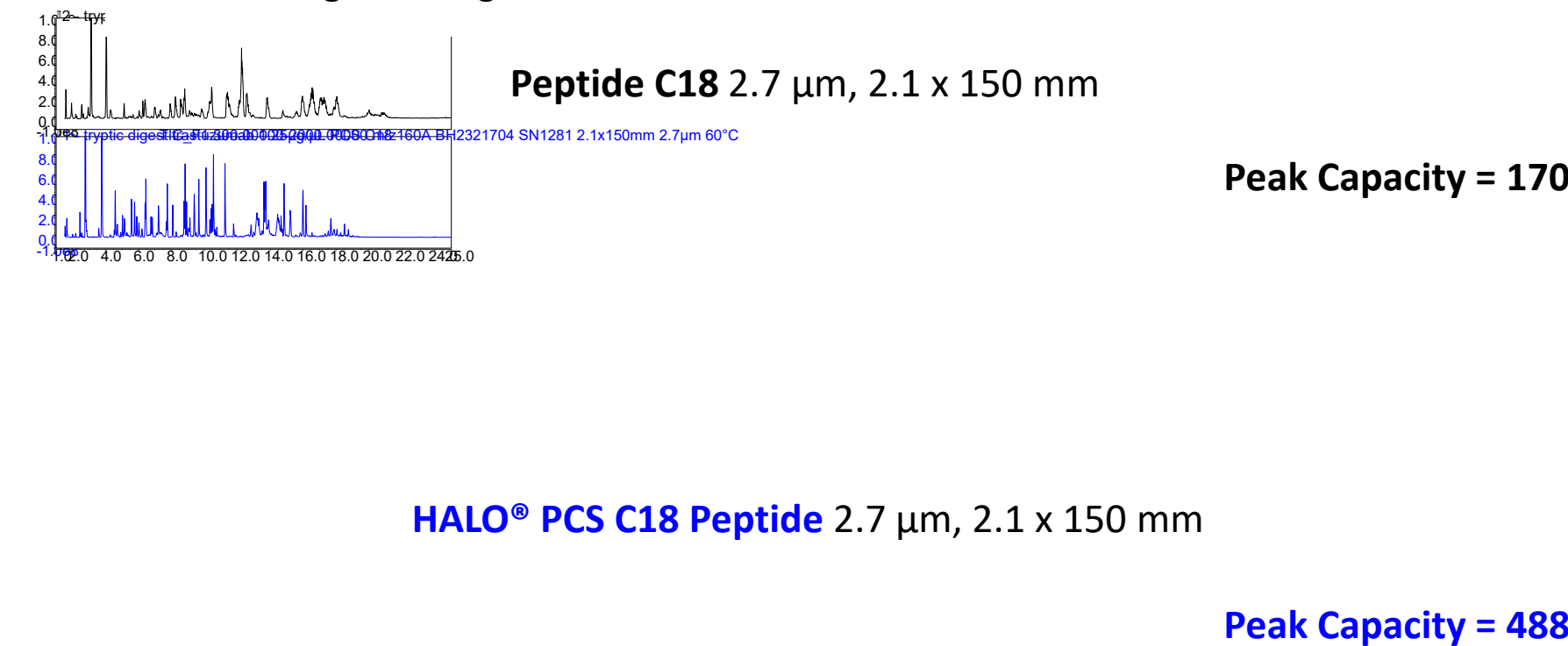
HALO 160 Å PCS C18, 2.1 x 50 mm, A: Water, 0.1% Formic Acid; B: Acetonitrile, 0.1% Formic Acid; Gradient: 0-35 %B in 1.5 min.; Flow Rate: 1.0 mL/min; Temperature: 30 °C; Injection: 1.0 µL; Wavelength: PDA, 280 nm



- The highly efficient 160 Å pore superficially porous particle permits very high throughput analysis
- The example shows separation conducted in less than 2 minutes, with modest backpressure, even at moderate temperature

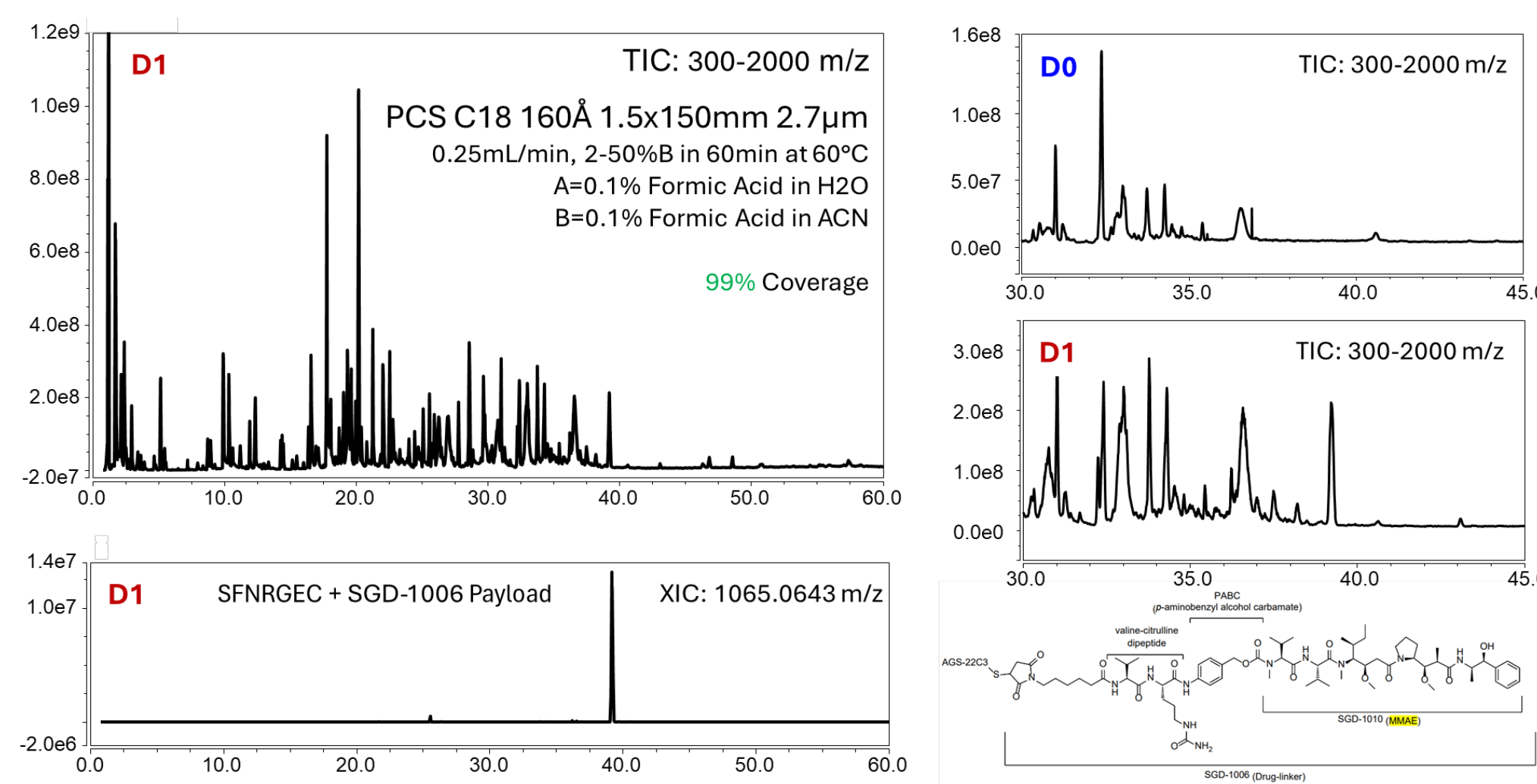
Improved Peak Capacity with HALO® PCS C18 Peptide

A: Water, 0.1% Formic Acid; B: Acetonitrile, 0.1% Formic Acid; Gradient: 3-50 %B in 30 min.; Flow Rate: 0.4 mL/min; Temperature: 60 °C; ; Shimadzu NexeraX2 -> diverter valve -> QExactive HF (res=240,000)
MarvelIXACT Post-Column Plumbing:
50 µm x 350 mm from column to diverter valve
50 µm x 350 mm from diverter valve to union
50 µm x 150 mm from grounding union to HESI I



- Peak capacities (n_{PC}) measured with modest load (2 µg) of trastuzumab tryptic digest on a 2.1 mm ID column
- n_{PC} based on 12 ID peptides measured using extracted ions (XICs) $PW_{1/2}$, t_R and Δt_G for this specific sample set
- Decreased peak widths effect notable increase in peak capacity

Tryptic digest LCMS analyses to identify payload site for isolated DAR2 ADC



- HIC isolated mAb with 2 vedotin-ejfv payload conjugated to enfortumab was digested with trypsin
- A single L-chain cystine site was occupied by the payload, with retention identified in the XIC, verified by MS/MS

CONCLUSIONS

- HALO® PCS phases improve load tolerance in formic acid vs. traditional uncharged stationary phases making PCS phases useful for analysis of less abundant impurities.
- HALO 160 Å PCS C18 exhibits favorable peak shape for peptides in weakly acidic mobile phase thus expanding the choice of mobile phase for effective LC/MS.
- HALO® PCS phases show symmetrical peak shape for basic, neutral, and acidic analytes.
- All HALO® PCS phases exhibit the speed and resolution advantages of Fused-Core® superficially porous particles.