



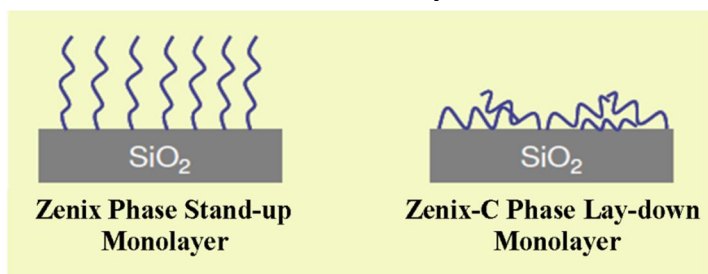
Antibody Drug Conjugates Solution Portfolio

Highlighted FACTS:

- ADC aggregate, monomer and fragment analysis can be achieved by using **Zenix®-C SEC-300** size exclusion chromatography. This analysis can be part of the ADC lot release and stability assays.
- Free small molecule drugs analysis after the conjugation reaction can be accomplished with **Zenix®-C SEC-80** (the smallest pore size 80 Å in the Sepax SEC product line).
- ADC charge variants can be analyzed and fraction collected off Sepax **Proteomix® SCX** cation exchange chromatography for further characterization.
- ADC Drug to Antibody (DAR) can be analyzed with **Proteomix® HICBu** hydrophobic interaction chromatography under native condition and **Proteomix® RP-1000** reverse phase chromatography with online mass spec determination capability.

SEC Analysis of MAb and ADC

Difference in Surface Chemistry



Zenix is ideal for mAb:

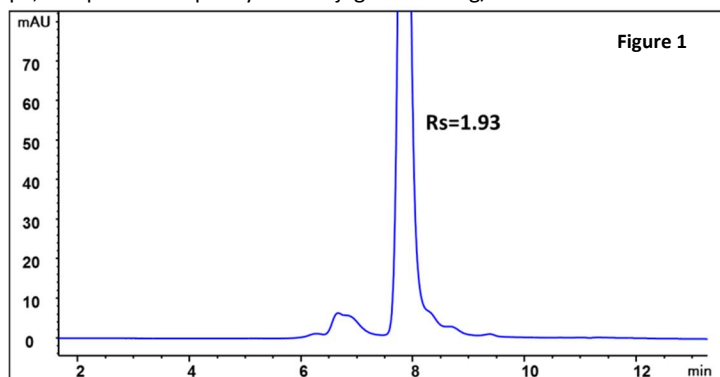
Various types of non-hydrophobic protein including **MAb** and etc.

Zenix-C is ideal for ADC:

ADC, Derivatized MAb, Pegylated Protein, Membrane Protein, "Super sticky protein", and etc.

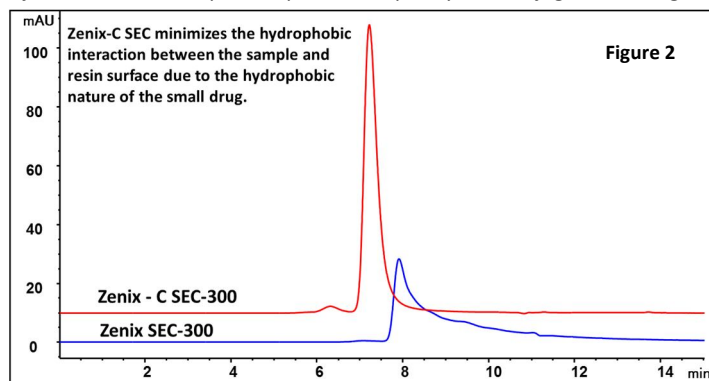
Herceptin Analysis on Zenix® SEC-300

Column: Zenix® SEC-300 (3 µm, 300 Å, 7.8 x 300 mm)
Mobile phase: 150 mM phosphate buffer; Flow rate: 1 mL/min;
Detector: UV 280 nm; Column temperature: 25 °C; Injection volume: 10 µL; Samples: Herceptin lysine conjugate 2.05 mg/mL



Herceptin Lysine ADC Analysis on SEC-300

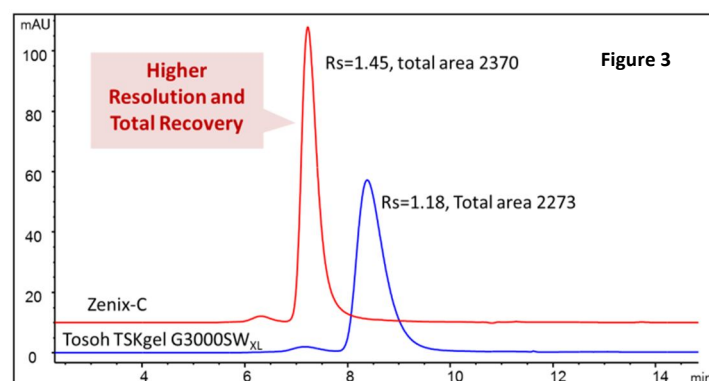
Column: Zenix® SEC-300, Zenix® - C SEC-300 (3 µm, 300 Å, 7.8 x 300 mm)
Mobile phase: 150 mM phosphate buffer; Flow rate: 1 mL/min; Detector: UV 280 nm; Column temperature: 25 °C;
Injection volume: 10 µL; Samples: Herceptin lysine conjugate 2.05 mg/mL



Herceptin-Lysine Drug Conjugate Analysis

Zenix-C SEC-300 vs. Tosoh TSKgel G3000SW_{XL}

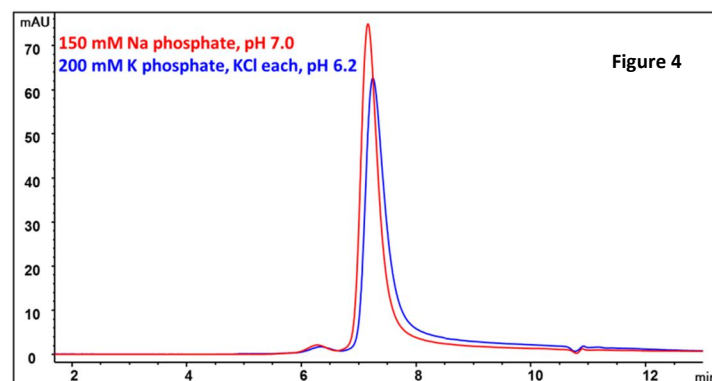
Column: Zenix® - C SEC-300 (3 µm, 300 Å, 7.8 x 300 mm),
Tosoh TSKgel G3000SW_{XL} (5 µm, 250 Å, 7.8 x 300 mm)
Mobile phase: 150 mM phosphate buffer, pH 7.0;
Flow rate: 1 mL/min; Detector: UV 280 nm; Column temperature: 25 °C;
Injection volume: 10 µL; Samples: Herceptin-lysine drug conjugate 2.05 mg/mL



Disclaimer: TSKgel and Tosoh Bioscience are registered trademarks of Tosoh Corporation; Comparative separations may not be representative of all applications.

Herceptin lysine ADC Analysis on SEC-300 - Salt Difference

Column: Zenix® - C SEC-300 (3 µm, 300 Å, 7.8 x 300 mm)
Mobile phase: as indicated; Flow rate: 1 mL/min; Detector: UV 214 nm;
Column temperature: 25 °C;
Injection volume: 10 µL; Samples: Herceptin lysine ADC 2.05 mg/mL

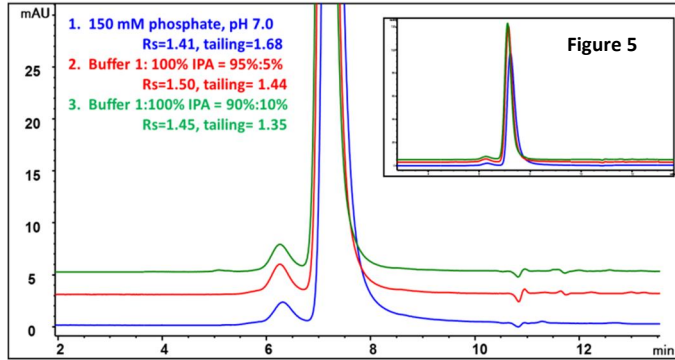




Antibody Drug Conjugates Solution Portfolio

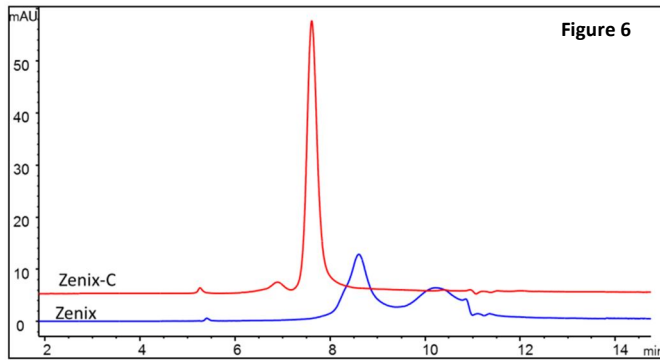
Herceptin Lysine ADC Analysis on SEC-300 - Organic Modifier

Column: Zenix® - C SEC-300 (3 µm, 300 Å, 7.8 x 300 mm)
 Mobile phase: as indicated;
 Flow rate: 1 mL/min; Detector: UV 280 nm;
 Column temperature: 25 °C;
 Injection volume: 10 µL;
 Samples: Herceptin lysine conjugate 2.05 mg/mL



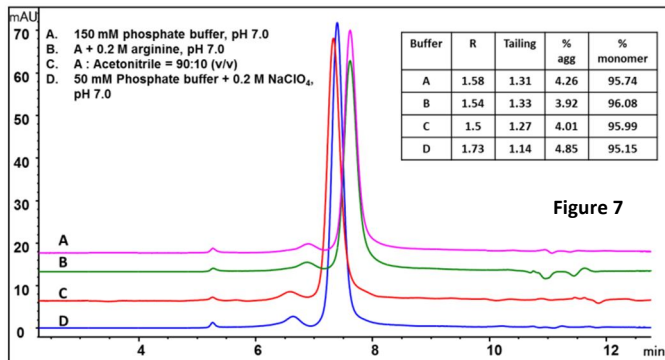
Cysteine ADC Analysis on SEC-300 Phase Comparison

Column: Zenix® SEC-300, Zenix®-C SEC-300 (3 µm, 300 Å, 7.8 x 300 mm)
 Mobile phase: 150 mM phosphate buffer;
 Flow rate: 1 mL/min;
 Detector: UV 280 nm;
 Column temperature: 25 °C;
 Injection volume: 20 µL; Samples: 1.68 mg/mL ADC



Cysteine ADC Analysis on Zenix®-C SEC-300 -Mobile Phase Difference

Column: Zenix®-C SEC-300 (3 µm, 300 Å, 7.8 x 300 mm)
 Mobile phase: As indicated; Flow rate: 1 mL/min; Detector: UV 280 nm;
 Column temperature: 25 °C; Injection volume: 20 µL;
 Samples: 1.68 mg/mL ADC

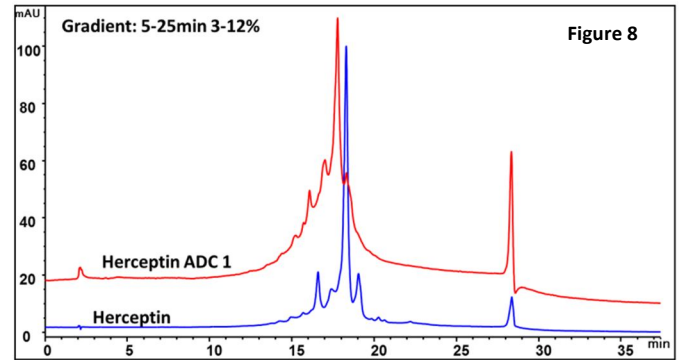


With 10% acetonitrile and 200 mM NaClO₄, total protein recovery, resolution and tailing factor of monomer peak are improved.

ADC Change Variants Separation on CEX

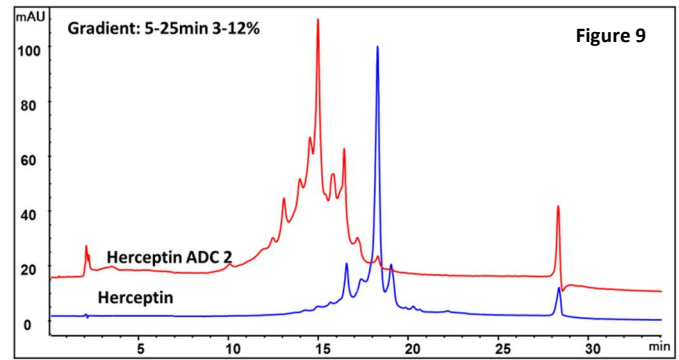
Herceptin and Cysteine ADC1 with Cleavable Linker-HEPES Gradient

Column: Proteomix® SCX NP5 (5 µm, 4.6 x 250 mm PEEK)
 Mobile phase A: 20 mM HEPES, pH 7.2, B: A+1M NaCl, pH 7.2;
 Flow rate: 0.8 mL/min; Detector: UV 280 nm;
 Column temperature: 25 °C; Injection: 25 µg;
 Sample: 4.13 mg/mL Herceptin-cysteine ADC 1 with cleavable linker and 2.43 mg/mL Herceptin



Herceptin and cysteine ADC2 with Non-cleavable Linker-HEPES Gradient

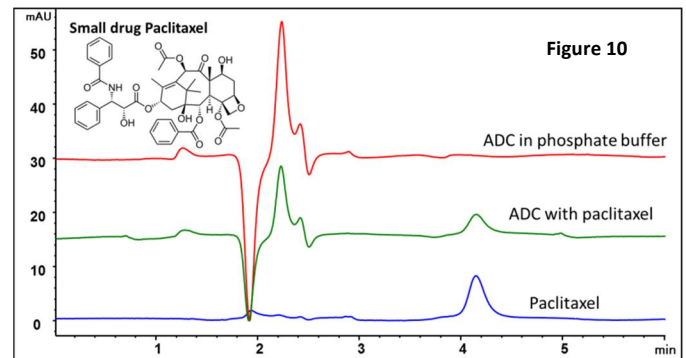
Column: Proteomix® SCX NP5 (5 µm, 4.6 x 250 mm PEEK)
 Mobile phase A: 20 mM HEPES, pH 7.2, B: A+1M NaCl, pH 7.2;
 Flow rate: 0.8 mL/min; Detector: UV 280 nm;
 Column temperature: 25 °C; Injection: 25 µg;
 Sample: 7.52 mg/mL Herceptin-cysteine ADC 2 with non-cleavable linker and 2.43 mg/mL Herceptin



Antibody Drug Conjugate and Free Drug Analysis

ADC and Free Drug Paclitaxel Analysis on Zenix® - C SEC-80

Column: Zenix®-C SEC-80 (3 µm, 80 Å, 4.6 x 50 mm)
 Mobile phase: 50 mM NH₄Ac : ACN = 80 : 20 (v/v); Flow rate: 0.3 mL/min;
 Detector: UV 228 nm, Column temperature: 25 °C; Injection volume: 2 µL;
 Samples: See below; Pressure: 21 bar



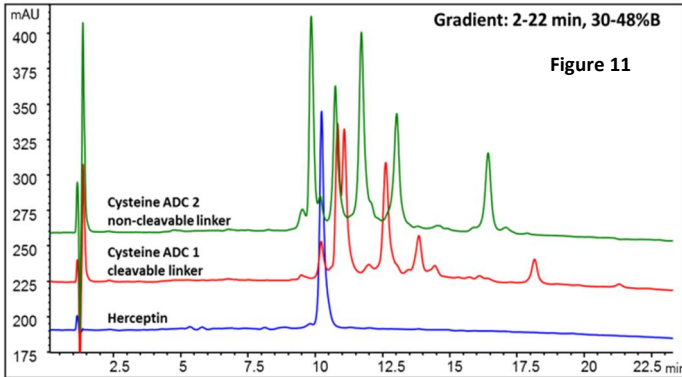


Antibody Drug Conjugates Solution Portfolio

Intact and Reduced MAb/ADC, MAb Fragment Separation on Reverse Phase Proteomix RP

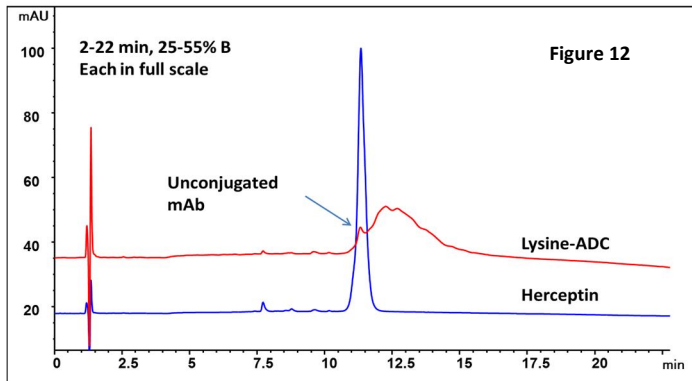
MAb Herceptin and its ADCs Separation on Proteomix® RP-1000

Column: Proteomix® RP-1000 (5 µm, 1000 Å, 4.6 x 100 mm);
Mobile phase: A: 0.1% TFA in water; B: 0.1% TFA in 100% ACN;
Flow rate: 1.0 mL/min; Detector: UV 210 nm; Column temperature: 80 °C;
Sample: Herceptin and ADCs 1 mg/mL diluted in water;
Injection volume: 10 µL



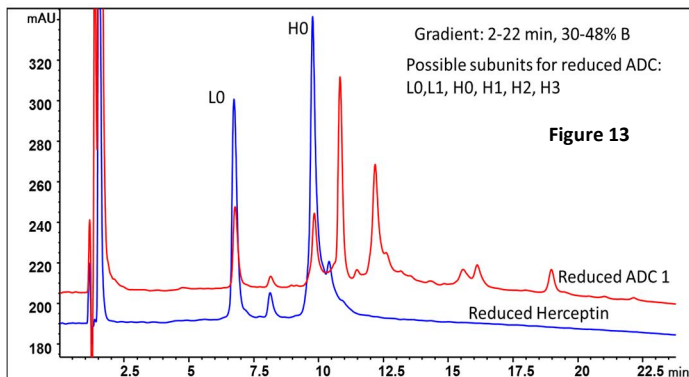
Herceptin and lysine-ADC Separation on Proteomix® RP-1000

Column: Proteomix® RP-1000 (5 µm, 1000 Å, 4.6 x 100 mm);
Mobile phase: A: 0.1% TFA in water; B: 0.1% TFA in 100% ACN;
Flow rate: 1.0 mL/min; Detector: UV 210 nm; Column temperature: 80 °C;
Sample: Herceptin and lysine ADC 1 mg/mL diluted in 0.1% TFA;
Injection volume: 10 µL



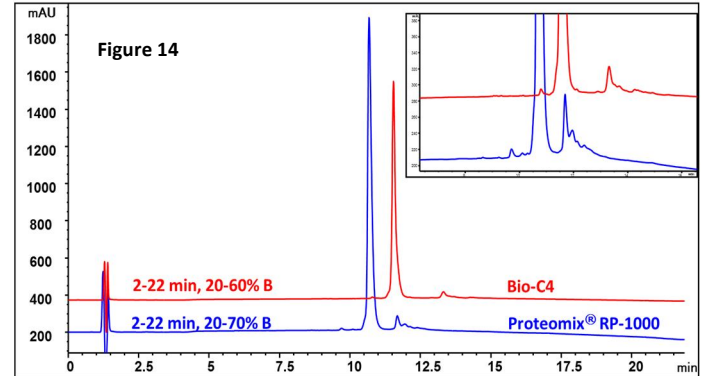
MAb/ADC Fragments: Reduced mAb Herceptin and Cysteine ADC 1

Column: Proteomix® RP-1000 (5 µm, 1000 Å, 4.6 x 100 mm);
Mobile phase: A: 0.1% TFA in water; B: 0.1% TFA in 100% ACN;
Flow rate: 1.0 mL/min; Detector: UV 210 nm; Column temperature: 80 °C;
Sample: Herceptin and ADC1 2 mg/mL reduced with 20 mM DTT, incubated at 65 °C for 20 minute;
Injection volume: Reduced herceptin 2 µL, Reduced herceptin ADC 1 5 µL



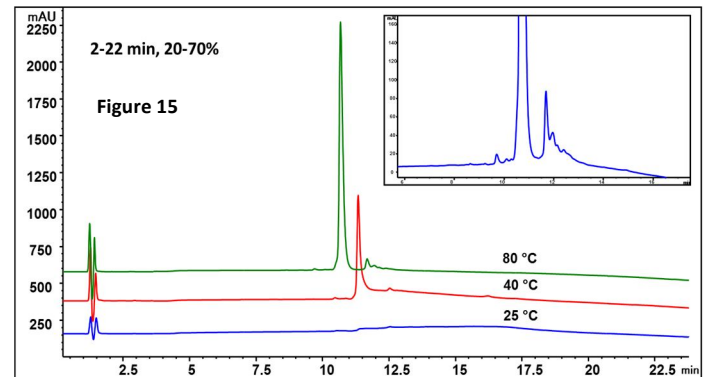
mAb 321 Separation

Column: Proteomix® RP-1000 (5 µm, 1000 Å, 4.6 x 100 mm);
Bio-C4 (5 µm, 300 Å, 4.6 x 100 mm)
Mobile phase: A: 0.1% TFA in water; B: 0.1% TFA in 100% ACN;
Flow rate: 1.0 mL/min; Detector: UV 210 nm; Column temperature: 80 °C;
Sample: mAb 321 1 mg/mL diluted in 0.1% TFA; Injection volume: 20 µL



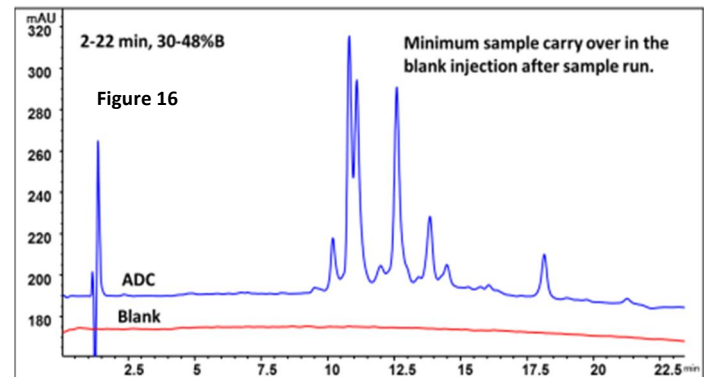
Temperature Effect: MAb Separation with 25°C / 40°C / 80°C

Column: Proteomix® RP-1000 (5 µm, 1000 Å, 4.6 x 100 mm);
Mobile phase: A: 0.1% TFA in water; B: 0.1% TFA in 100% ACN
Flow rate: 1.0 mL/min; Detector: UV 210 nm;
Sample: mAb 1mg/mL;
Injection volume: 20 µL



Minimum Sample Carry Over

Column: Proteomix® RP-1000 (5 µm, 1000 Å, 4.6 x 100 mm);
Mobile phase: A: 0.1% TFA in water; B: 0.1% TFA in 100% ACN;
Flow rate: 1.0 mL/min; Detector: UV 210 nm;
Column temperature: 80 °C;
Sample: Cysteine ADC 1 1 mg/mL diluted in water;
Injection volume: 8 µL



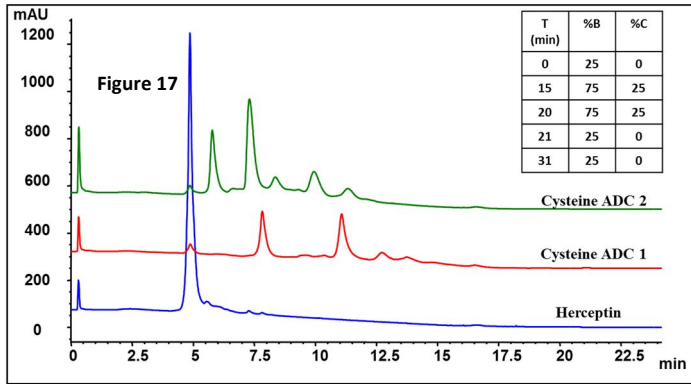


Antibody Drug Conjugates Solution Portfolio

ADCs Separation on Proteomix® HICBu

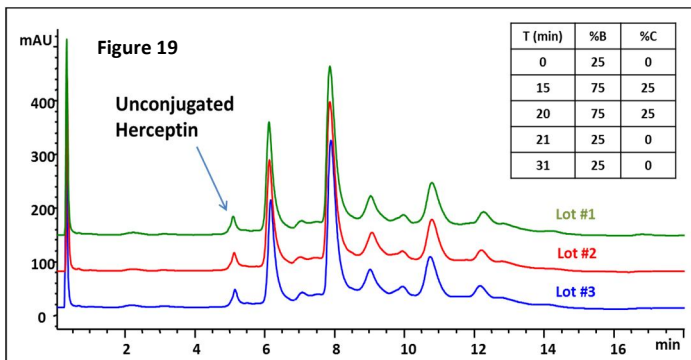
Herceptin and Its ADCs Separation on Proteomix® HIC

Column: Proteomix® HICBu-NP5 (5 mm, 4.6 x 35 mm)
 Mobile phase: A: 2 M ammonium sulfate in 0.025 M sodium phosphate, pH 7.0, B: 0.025 M sodium phosphate pH 7.0, C: 100% IPA; Flow rate: 0.8 mL/min; Detector: UV 214 nm; Column temperature: 25 °C; Injection: 10 µL
 Sample: Herceptin/ADC1/ADC2, 1 mg/mL in 25 mM sodium phosphate;



Proteomix® HICBu-NP5 for Herceptin-cysteine ADC separation- Three resin lot to lot consistency testing

Column: Proteomix® HICBu-NP5 (5 µm, 4.6 x 35 mm);
 Flow rate: 0.8 mL/min, Detector: UV 214 nm, Column temperature: 25 °C
 Mobile phase: A: 2 M ammonium sulfate in 0.025M sodium phosphate, pH 7.0, B: 0.025 M sodium phosphate pH 7.0, C: 100% IPA;
 Sample: ADC, 1mg/mL in 1M ammonium sulfate; Injection: 10 µL



Order Information:

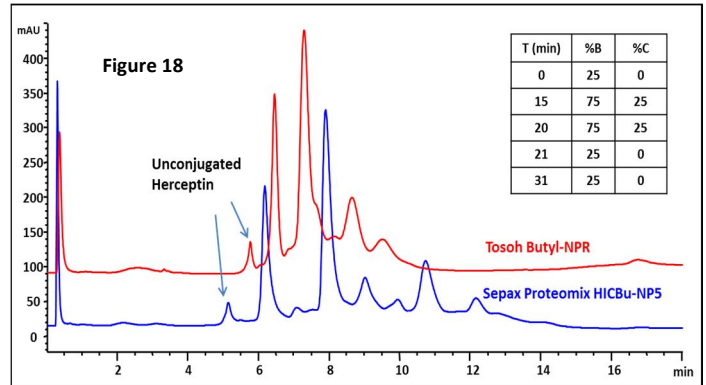
PN#	Description
233300-7830	Zenix-C SEC-300, 3 µm, 7.8 x 300 mm
233080-7805	Zenix-C SEC-80, 3 µm, 7.8 x 50 mm
401NP5P-4625	Proteomix SCX-NP5, 4.6 x 250 mm PEEK
465950-4610	Proteomix RP-1000, 5µm, 1000 A, 4.6x100 mm
431NP5-4603	Proteomix HICBu, NP5, 4.6 x 35 mm
313300-4610	BioHIC-300, 3µm, 4.6x100mm

*More selections of pore size, particle size and column dimension are available upon request

Herceptin-cysteine ADC separation

Sepax proteomix® HICBu-NP5 vs. TSKgel butyl-NPR

Column: Proteomix® HICBu-NP5 (5 µm, 4.6 x 35 mm); Flow rate: 0.8 mL/min, Detector: UV 214 nm, Column temperature: 25 °C
 Mobile phase: A: 2 M ammonium sulfate in 0.025M sodium phosphate, pH 7.0, B: 0.025 M sodium phosphate pH 7.0, C: 100% IPA;
 Sample: ADC, 1mg/mL in 1M ammonium sulfate; Injection: 10 µL



Disclaimer: TSKgel is registered trademarks of Tosoh Corporations; Comparative separations may not be representative of all applications.

Conclusion:

- **Zenix®-C 300A** SEC phase has better recovery and separation for antibody drug conjugate, which has secondary interaction with traditional resin surface due to the hydrophobic property from the conjugated small drugs.
- Different mobile phase additives such as organics, chaotropic agent can improve the sample recovery and separation resolution depending on individual ADCs.
- **Zenix®-C 80A** SEC with smaller pore size is proven to be beneficial in free drug analysis, which can be in line with mass spectrometry with volatile mobile phases.
- **Proteomix® SCX** can provide charge variants study for antibody drug conjugates. Conjugated and free mAb can be separated due to the different protein surface charges. Further characterization on the collect fractions of individual peaks is needed to identify the nature of those charge variants.
- **Proteomix® RP-1000** reversed phase can successfully separate ADCs with minimum sample carry over, and it also provides ideal chromatography for sample with online mass spec determination capability.
- **Proteomix® HIC Butyl** can successfully accomplish the analysis of both drug-linked species distribution and the average DAR determination of intact ADC under native condition.