Analytical Characterization of Antibody Drug Conjugates

Highlighted FACTS:

- ADC aggregate, monomer and fragment analysis 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 achieved 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 cation exchange chromatography for further characterization.

Experimental:

Columns: Zenix™ – C SEC-300 (3 µm, 7.8 x 300 mm)
Zenix™ – C SEC-80 (3 µm, 4.6 x 50 mm)
Proteomix™ SCX (5 µm, 4.6 x 250 mm PEEK)

Samples: monoclonal antibody, antibody drug conjugates, small molecule drug

Running condition: see detail result section

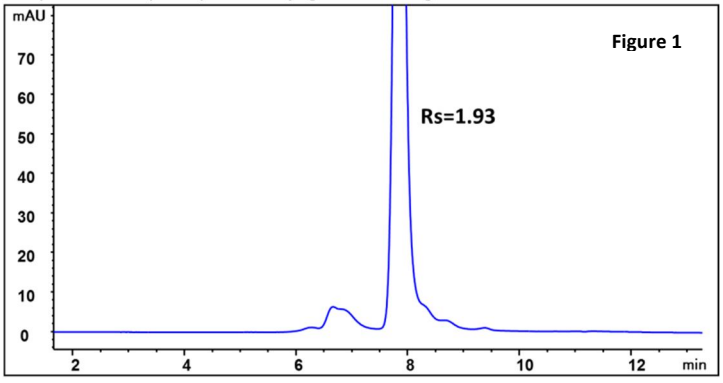
Reference:

Wakankar A., Chen Y., Gokarn Y. and Jacobson F.
Analytical methods for physicochemical characterization of antibody drug conjugates
mAbs 3:2, 161-172; March/April 2011

SEC Analysis of MAb and ADC

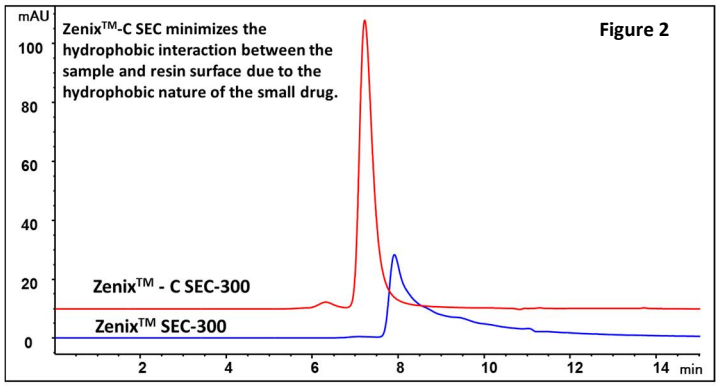
Herceptin Analysis on Zenix™ SEC-300

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



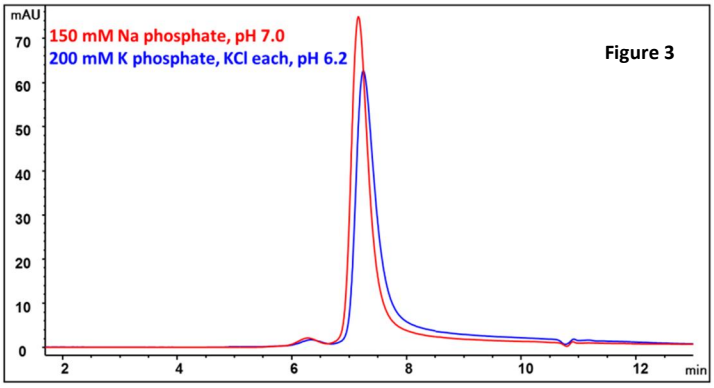
Herceptin Lysine ADC Analysis on SEC-300

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



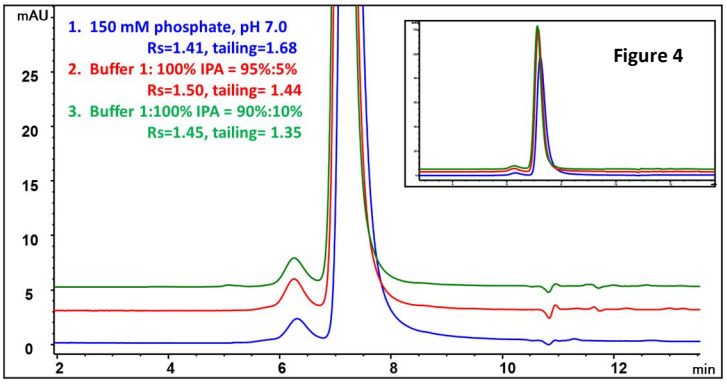
Herceptin lysine ADC Analysis on SEC-300 - salt difference

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



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



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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

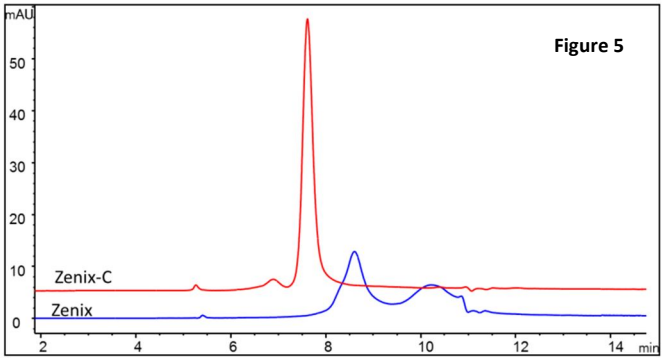
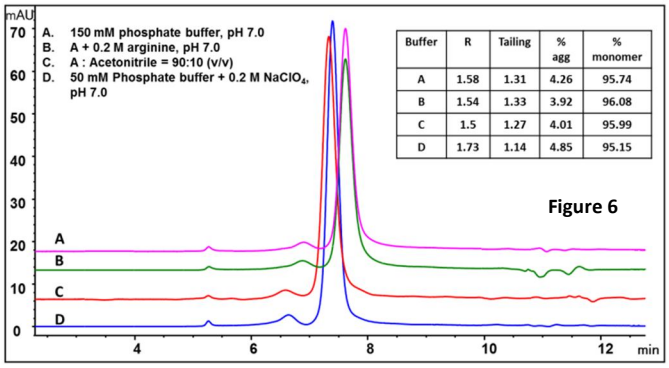


Figure 5

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



Buffer	R	Tailing	% agg	% monomer
A	1.58	1.31	4.26	95.74
B	1.54	1.33	3.92	96.08
C	1.5	1.27	4.01	95.99
D	1.73	1.14	4.85	95.15

Figure 6

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)
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

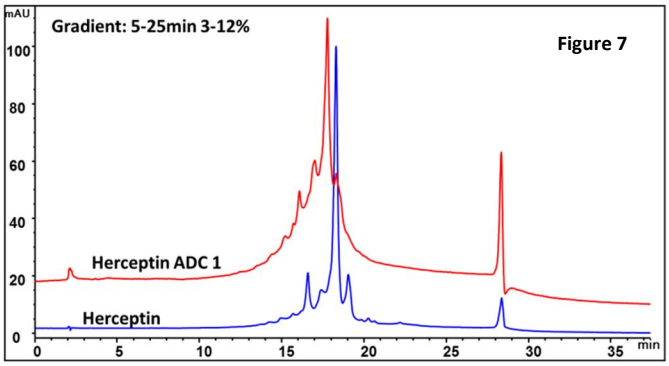


Figure 7

Herceptin and cysteine ADC2 with non-cleavable linker-HEPES gradient

Column: Proteomix™ SCX NP5 (5 µm, 4.6 x 250 mm)
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

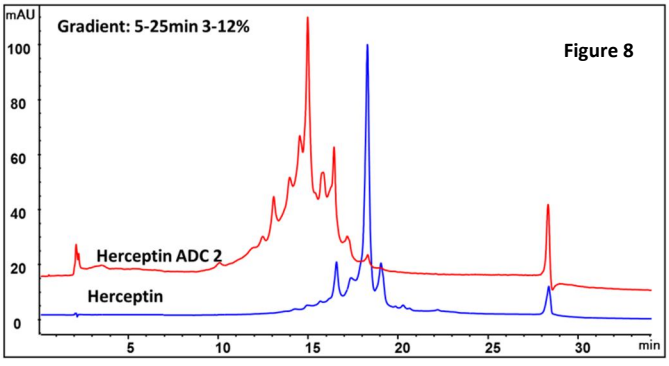


Figure 8

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

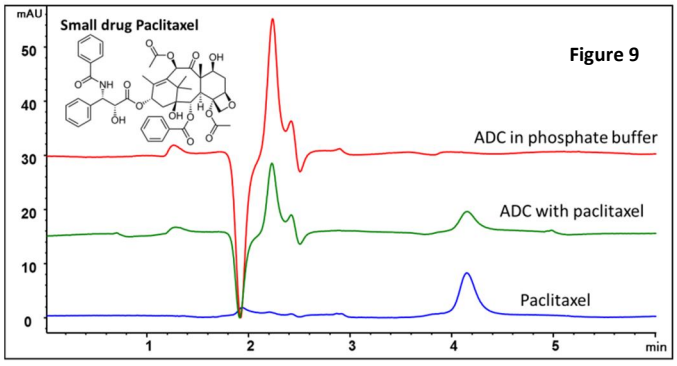


Figure 9

Conclusion

- Zenix™-C 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.
- Smaller pore size Zenix™-C SEC 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.