Supporting information

Native reversed-phase liquid chromatography: a technique for LCMS of intact antibody-drug conjugates

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Contents.

Conditions for LC in Figure 2.

b) RPLC of the denatured model ADC after reduction with DTT, showing the expected mAb subunits resolved in six peaks, using a commercial RPLC column. Column: Thermo MabPac RP, 2.1 x 50 mm, 4 um, supermacroporous polymer particles, A: H_2O + 0.1% DFA, B: ACN + 0.1% DFA, Gradient: 27-42% B/15min, Q: 200 uL/min at 80 °C, Injection: 10 ug, Detection: 280 nm. **c**) RPLC of the model ADC without DTT denaturing, with assignments based on mass spectra. Column: Supelco Bioshell A400 Protein C4 column, 2.1 x 100 mm, 3.4 µm, 400 Å pores particles, A: H_2O + 0.1% FA and 0.015%TFA, B: ACN + 0.1% FA and 0.015%TFA, Gradient: 10% B/1min, 31-48% B/13min, Q: 300 uL/min at 70 °C, Injection: 0.5 ug, Detection: 280 nm. **d**) HIC of the intact ADC using Thermo-HIC butyl column for conventional HIC gradient of A: 50 mM Na_3PO_4, 1 M $(NH_4)_2SO_4$, pH 7, B: 50 mM Na_3PO_4, 30% IPA, pH 7, Gradient: 0-100 %B / 15 min, 100 %B /5 min, flow rate: 1,000 µL/min at 30 °C and 2 µg injected, in both cases.

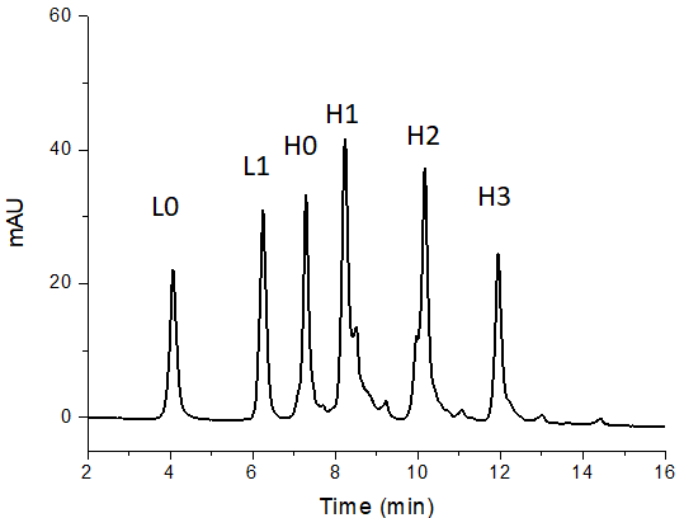

Figure S1. RPLC Chromatogram for AbbVie model ADC after reduction with DTT. Thermo MabPac RP; A: H2O + 0.1% DFA, B: ACN + 0.1% DFA; Gradient: 27-42% B/15min; Fc: 200 uLmin at 80 °C; Injection: 10 ug; Detection, 280 nm.

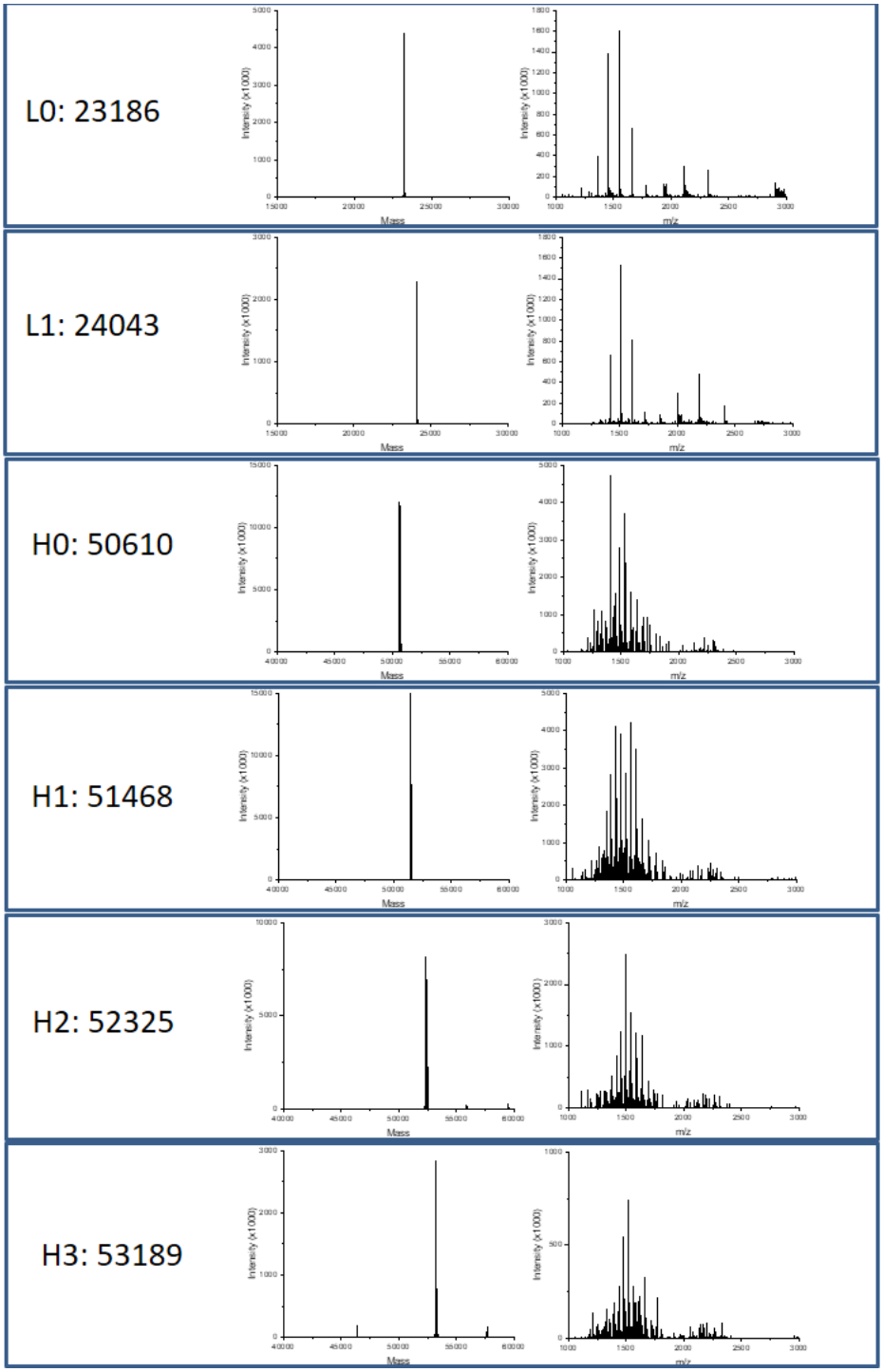

Figure S1 b) Raw mass spectra (right) and deconvoluted mass spectra (left) for each peak in the chromatograms of part a.

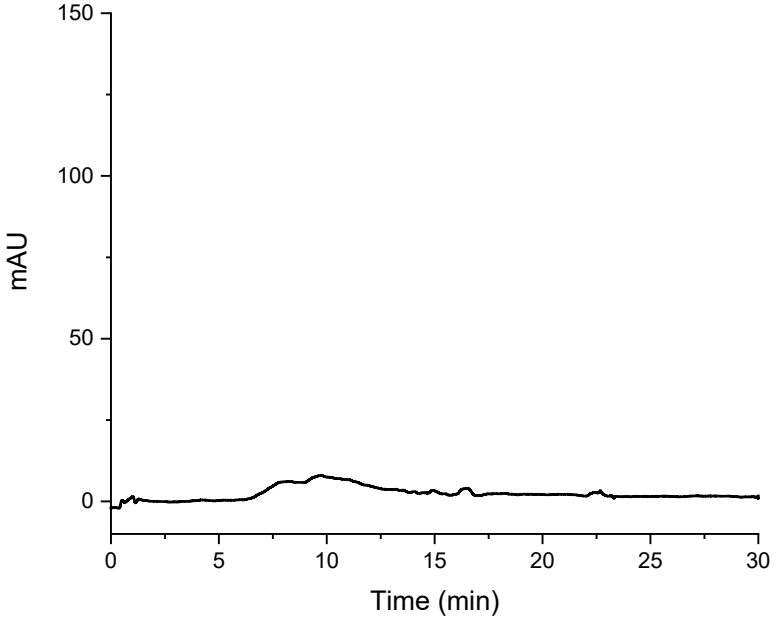

Figure S2. ADC is not fully eluted with a commercial HIC column under nRPLC conditions. Column: Thermo HIC butyl, model ADC. Gradient: A: 50 mM **NH_4OAc**, pH 7, B: 50 mM **NH_4OAc**, 50% IPA, pH 7; Gradient: 0-100 %B/15 min, followed by 100 %B/5 min; Flow rate: 1,000 µL/min, 30 ^{o}C. Wavelength: 280 nm.