



ADC Analysis with LC-MS compatible mobile phases

Buffers and additives in mobile phase have to be volatile for LC-MS methods, as the solvents are evaporated within the ionisation process. Non-volatile additives would contaminate the mass spectrometer.

For small molecules measured at low pH values formic acid is commonly used due to its higher compatibility to MS than TFA. TFA can't be used in negative mode and remains in the MS.

Using formic acid as additive usually produces peak

broadening and low intensity for the analysis of proteins.

In this application YMC-Triart Bio C4 was used to analyse a reduced monoclonal antibody (MAB) and a reduced ADC mimic (see Figure 2) with formic acid or TFA as additives. The MAb and ADC mimic were reduced with 10 mM DDT for an hour at 30 °C. The analysis was performed using a YMC-Triart Bio C4 UHPLC column at 80 °C column temperature.

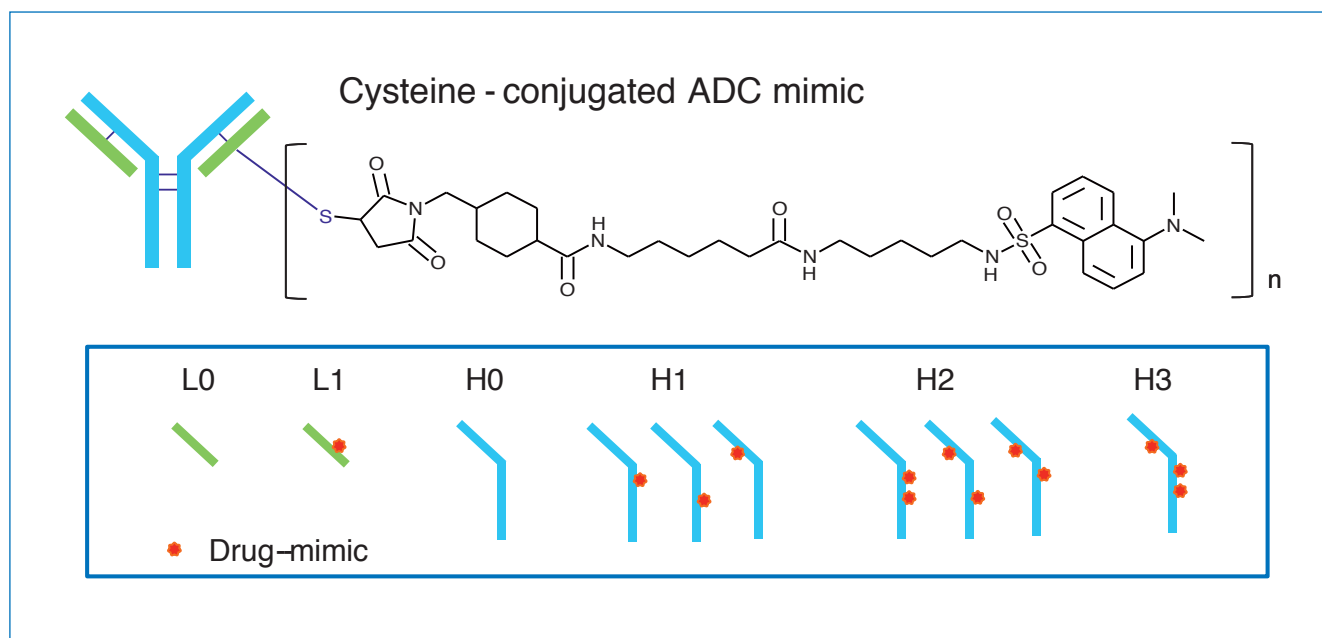
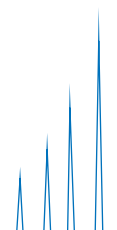


Figure 1: Cysteine-conjugated ADC mimic.

By using YMC-Triart Bio C4 and the addition of 0.1 % TFA excellent resolution and peak shapes are obtained. The separation of the reduced ADC mimic using 0.1 % formic acid was also suitable for structural analysis via LC-MS, even though slightly broader peaks and shorter retention times were observed.



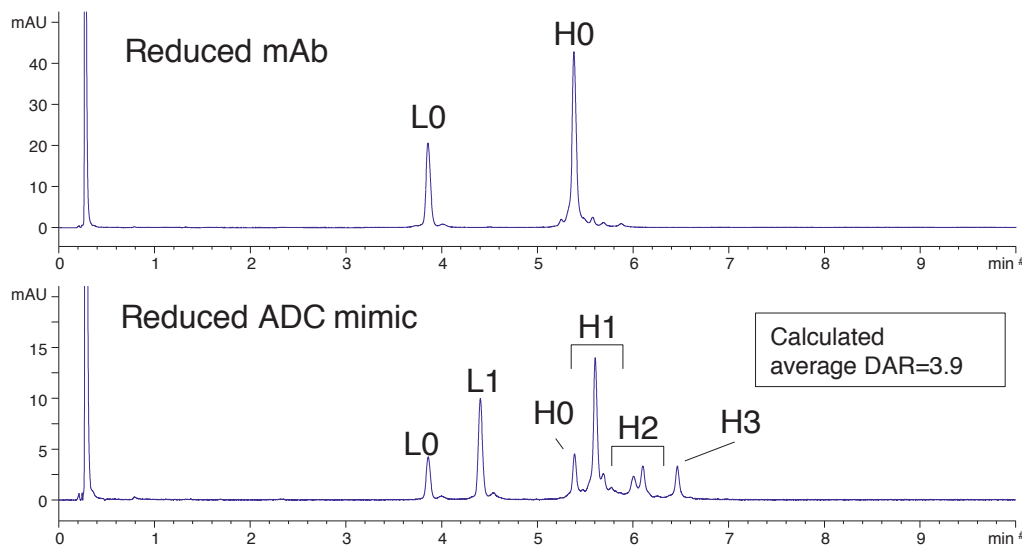
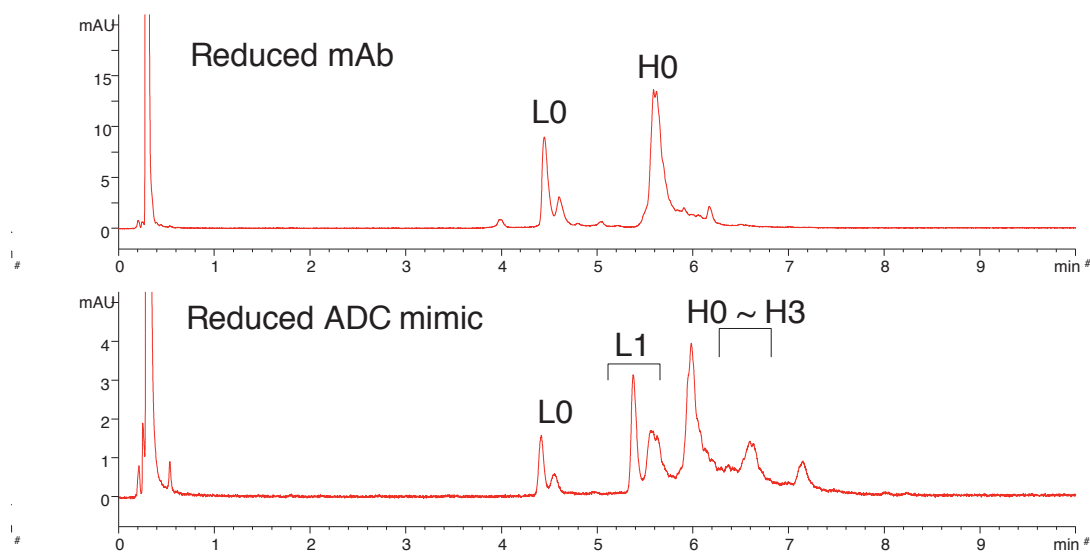
**Mobile phase containing 0.1% TFA****Mobile phase containing 0.1% formic acid**

Figure 2: Analysis of a reduced MAb and ADC mimic using 0.1% TFA (top) and 0.1% formic acid (bottom) as additive.

Table 1: Chromatographic conditions

Column:	YMC-Triart Bio C4 (1.9 μm , 300 \AA) 50 x 2.1 mm ID
Part No.:	TB30SP9-05Q1PT
Eluent [TFA]:	A) water/TFA (100/0.1) B) acetonitrile/TFA (100/0.1)
Gradient [TFA]:	27–42%B (0–10 min), 90%B (10–12.5 min)
Eluent [formic acid]:	A) water/formic acid (100/0.1) B) acetonitrile/formic acid (100/0.1)
Gradient [formic acid]:	22–37%B (0–10 min), 90%B (10–12.5 min)
Detection:	UV at 280 nm
Flow rate:	0.4 mL/min
Temperature:	80 $^{\circ}\text{C}$
Sample:	MAb and ADC mimic were reduced with 10 mM DTT at 30 $^{\circ}\text{C}$, 1 hr
Injection:	2 μL (1.0 mg/mL) for MAb 4 μL (0.625 mg/mL) for ADC mimic