Introduction

Antibody Drug Conjugates (ADCs) are monoclonal antibodies to which cytotoxic drugs are covalently attached (Fig 1). The antibody brings specificity to drug delivery by binding to epitopes on the surface of cells responsible for a disease state (e.g. cancer). Once the ADC binds to its target, the conjugated drug is released creating a high local concentration of cytotoxic drug. The released cytotoxic drug can now act more effectively without affecting other non-cancerous cells. Hence ADCs are gaining market share due to their potential for improved efficacy and reduced side effects [1, 2]. Due to the nature of the conjugation chemistry, ADCs are a mixture of antibodies modified by different number of drugs. DAR can significantly alter the efficacy of the ADC; low drug loading reduces efficacy and reduced side effects [1, 2]. Due to the nature of the conjugation chemistry, ADCs are a mixture of antibodies modified by different number of drugs. DAR can significantly alter the efficacy of the ADC; low drug loading reduces efficacy and reduced side effects [1, 2].

Results and Discussion

Sample preparation

Lyophilized ADCs were reconstituted in deionized (DI) water to 5 mg/mL, aliquoted, and stored at -80 °C until used. For the intact ADC analysis, the reconstituted ADCs were diluted in 0.1% formic acid in water to 1 mg/mL, just before the LC/MS analysis. Deglycosylation of the intact mAb was performed by adding 1 µl of 50 unit/µL PNGase F (Sigma-Aldrich) in 20 mM Tris-HCl buffer (pH = 7.5) to 100 µg of ADC (100 µg) and then incubated overnight at 37 °C. Five microliters of intact ADC were subjected to LC/MS analysis. For reduced ADC analysis, ADCs were reduced by adding 10 µl of freshly dissolved dithiothreitol (DTT) (35 mM), 20 µl of 10 mM Tris buffer (pH = 7.5) to a 5 µl (5 µg) aliquot of ADCs followed by incubation at 50 °C for 10 minutes. For the reduced deglycosylated ADCs, ADCs were deglycosylated by adding 10 µl of PNGase F (Sigma-Aldrich) in 20 mM Tris-HCl buffer (pH = 7.5) to a 5 µl (5 µg) aliquot of ADCs followed by incubation at 37 °C for 10 minutes. One microliter of each sample was subjected to LC/MS for analysis.

LC/MS Analysis

LC/MS system - Agilent 1290 Infinity LC system including: • Agilent 1290 Infinity Binary Pump G4220A • Agilent 1290 Infinity TCC G1316C • Agilent 1290 Infinity Diode Array Detector G1315D with Agilent MassHunter BioConfirm Software and DAR calculator can be used to characterize both intact and reduced ADCs. The Agilent DAR calculator, which rapidly and easily integrates all DAR peaks with minimal user input and reports final DAR values.

Conclusions

- The reversed phase analysis of an ADC using an Agilent 1290 Infinity LC system coupled to an Agilent 6550 Q-Tof LC/MS can be extensively used to reproducibly generate accurate DAR values on both intact and reduced ADCs with excellent performance.
- Agilent MassHunter BioConfirm Software and DAR calculator can be used together to provide efficient data extraction, deconvolution, and an easy and intuitive approach to calculate and report DAR values for both intact and reduced ADCs.

References