# Sensitive drug distribution measurements of an antibody drug conjugate with non-denaturing capillary SEC

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## Introduction

As protein drug modalities get increasingly complex, new methods need to be developed to characterize them. This has led to an increased interest in non-denaturing separation modes that allow measuring the mass of complex biologics and their heterogeneities. In the case of cysteine-based ADCs, SEC-MS can be used to accurately measure the distribution of linker drugs on the mAb scaffold. It is desirable to improve the sensitivity of this assay to make this method applicable to the analysis of dilute samples, for example purified after in-vivo exposure. Here we further optimize the method presented at the ASMS 2019 conference (1) to enable sensitive heterogeneity measurements with non-denaturing capillary SEC-MS coupled to a multi-nozzle sprayer.

## Methods

The method was developed on a prototype stage for mutinozzle emitter (10 µm M3 emitter, Newomics) and a maXis II UHR-QTOF (Bruker), SEC separation (5 µL/min) on a PolyHYDROXYETHYL A capillary column (150x0.3 mm, 300 Å , PolyLC). Data were processed in Data Analysis (Bruker).

## Ion Transfer Optimization

The impact of ESI voltage, temperature, ISCID energy, funnel pressure and collision cell energy were comprehensively evaluated for NISTmAb (SRM8671) and a mimic cysteine-based ADC (MSQC8, Sigma), diluted to 1mg/mL in 200 mM ammonium acetate

Optimum between sensitivity and protein integrity was found to be 4500V ESI voltage, 200°C, 180 eV ISCID, 4 mBar funnel pressure and 20 eV collision cell energy (Fig. 1).

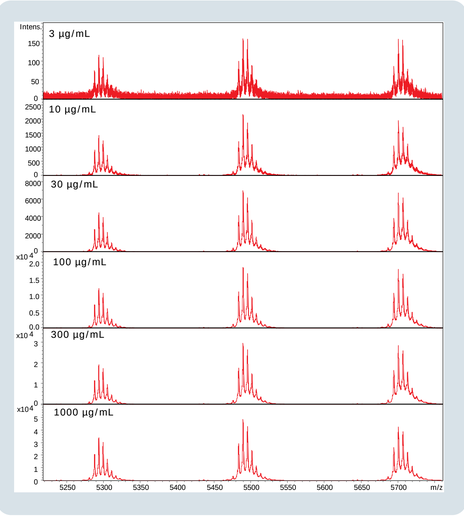


Fig. 2 Dilution series for 0.5 µL injection of NISTmAb from 3 to 1000 µg/mL analyzed by non-denaturing SEC

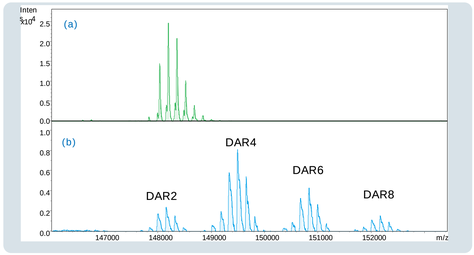


Fig. 1 Deconvoluted spectrum for optimized method, (a) NISTmAb and (b) MSQC8

## Results

Deconvolution of the NISTmAb glycoforms reveals the expected heterogeneities, including the presence of residual C-Terminal lysine residues. Peak symmetry suggests low level of adducts and indicates the SEC method is effective at desalting the sample.

The measured drug to antibody ratio for MSQC8 is 4.6, consistent with prior published results. Wider than expected peaks after deconvolution suggest the presence of additional heterogeneity such as partial hydrolysis of the maleimide linker (2). In addition some amount of free light chain eluting shortly after the ADC was observed.

The refined method allows the detection of all main NISTmAb glycoforms (Fig. 2) at concentrations of 3 µg/mL and higher (1.5 ng on column) and an LOD of 1 µg/mL despite the high ionic strength buffer. Consistent with previous results an extension of the working concentration range of more than 10X is observed with this setup.

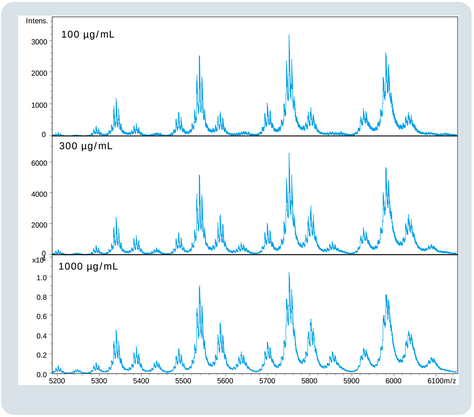


Fig. 3 Dilution series for 0.5 µL injection of MSQC8 from 100 to 1000 µg/mL analyzed by non-denaturing SEC

In the case of MSQC8, excellent spectra are still observed at 100 µg/mL concentrations (Fig.3). However, the ratio of DAR6 and DAR8 appears to drop with the concentration (DAR 4.43 at 300 µg/mL and DAR 3.95 at 100 µg/mL, Fig.4). In contrast the ratio of DAR2 and DAR4 appear unchanged indicative of a recovery issue for high DAR species at lower concentrations. Further investigation is required to reduce possible losses on e.g. HPLC vials.

## Summary

Enhanced MS settings allowed increasing the concentration range of this non-denaturing capillary SEC method. This offers the opportunity to characterize lower concentration samples incompatible with denaturing methods.

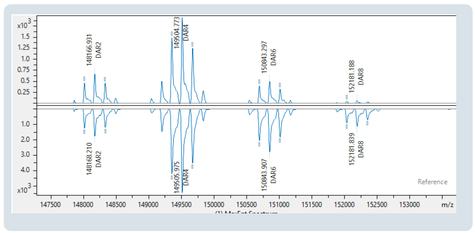


Fig. 4 Comparison of MSQC8 deconvoluted spectra for concentration of 100 (up) and 1000 µg/mL

### References

(1) MP677, ASMS annual conference 2019
(2) Application note LCMS-94 (Bruker)

## Conclusions

- maXis II can be tuned for high sensitivity analysis of proteins under non-denaturing conditions
- M3 multinozzle emitters improve sensitivity for proteins sprayed under non-denaturing conditions
- Capillary flow non-denaturing SEC is a useful tool to desalt and analyze solution with low concentration of mAbs or ADCs.

UHR-QTOF

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