Comparison of SEC-MS Robustness Against UV Under Challenging Mobile Phase Conditions

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Introduction

Size exclusion chromatography (SEC) is a routinely used method to determine the monomeric purity of a monoclonal antibody (mAb) as a QC release test. Coupling this separation method with mass spectrometry detection provides an additional dimension of data that can be used to identify the monomeric product, aggregates and fragment species.

An important feature of QC release testing is robustness of the method and instrument. Drug substance specifications are dependent on both process capability and the performance of analytical methods used for batch release testing.

To demonstrate instrument robustness, a high salt mobile phase method was used to probe for quantitation robustness of the monomeric peak area integration by comparing mass spectrometry against UV.

Methods

For this study four different intact mAb samples (Rituximab, Trastuzumab, Bevacizumab, cB72.3) were measured with an Ultimate UHPLC (Thermo) coupled to a benchtop UHR-QTOF mass spectrometer (impact II, Bruker Daltonics). 4µg of each protein were injected onto a BEH200 SEC column (Waters, 4.6 x 300 mm, 1.7 µm) using 100 mM ammonium acetate as mobile phase utilizing a 30 minutes isocratic run. Samples were injected in triplicate and data evaluation was based on integration of obtained UV (280 nm) and EIC peaks.

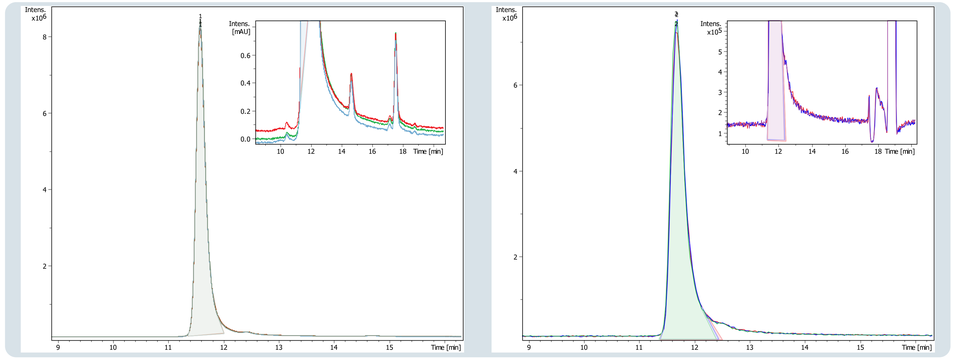

Fig. 1 UV Traces (left) and EIC Traces (m/z 500-6500, right) in overlay mode for triplicate analysis of the mAb sample Rituximab. Inserts show zoom for baseline.

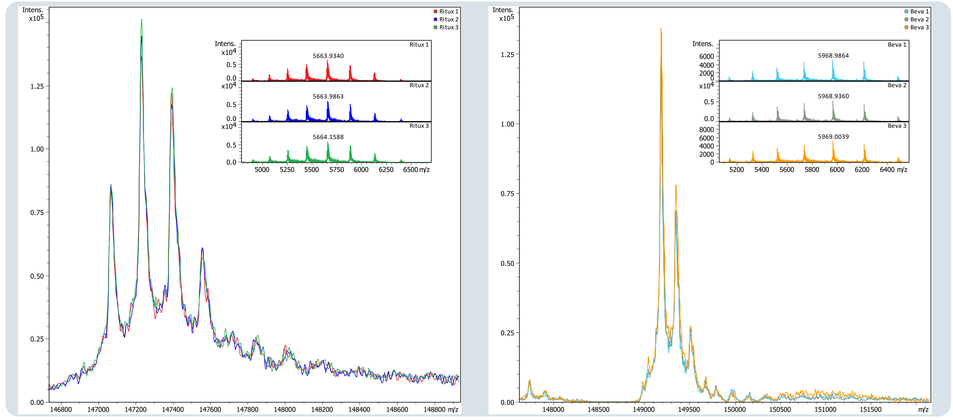

Fig. 2 MaxEnt deconvoluted spectra in overlay mode for triplicate injections of Rituximab, insert shows raw data spectra (left). On the right, same data are shown for sample Bevacizumab.

Molecule	Mass Error (ppm)	UV Area (%RSD)	EIC area (%RSD)	Retention time (%RSD)
Bevacizumab	21.7	1.39	1.06	0.080
cB72.3	11.3	0.40	0.58	0.072
Rituximab	11.2	0.99	0.52	0.022
Trastuzumab	19.2	0.72	2.25	0.038

Tab. 1 Relative standard deviation (RSD) and ppm values from triplicate injections of all evaluation criteria for four different injected mAb samples.

Results

- Data evaluation was based on the following criteria: main peak retention time, UV peak area and TIC peak area reproducibility, expressed as % RSD (Tab. 1). The fourth criteria is the mass error (Tab. 1) of the calculated Mr values based on MaxEnt deconvolution of the MS1 data (Fig. 2), expressed as average ppm error of measurement from the triplicate injections.
- RSD values for both UV and EIC peak area were <2.5% across triplication injections. Average mass error for all molecules was <25 ppm.
- Aggregate levels measured by UV were <1.0% and it was determined that the MS could not reach this level of detection by native ionization under this intentionally challenging mobile phase system.

Summary

Native SEC-MS shows applicability for QC release testing as the MS response is demonstrated to be in line with the reproducibility of the UV detector and mass errors were shown to be appropriate for identification of the monomeric molecules.

During the course of the measurements (7.5 hours + blanks) the MS response did not decrease nor did the ion source require any cleaning.

Instrument up-time between cleaning is a key requirement for a QC application.

Conclusions

- Quantitation by MS was demonstrated to be as robust as by UV within this dataset, which highlights the applicability of this method as a QC application.
- Despite the challenging chromatographic conditions such as using high molarity buffer known to affect MS sensitivity and robustness, the instrument performance remained unaffected over the course of the 8h experiment.
- Sensitivity and Resolution of UHR-OTOF mass spectrometers together with an efficient ionization process allows highly reproducible detection of mAb samples in native state

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