Improved intact antibody characterization with a new ion source design

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Introduction

- Reversed Phase LC-MS is a powerful tool for characterization of next generation biotherapeutics and has become a routine process for intact antibodies within the biopharmaceutical industry.
- Signal intensity along with a robust source setup is crucial using LC-MS as high-throughput approach for identity confirmation.
- In this study we compared an established ESI ion source setup with a new heated ESI design coupled to a QTOF mass spectrometer.
- A well-characterized system suitability test (SST) sample, routinely measured to ensure the accurate and reproducible performance of our intact LC-MS setup, was used for this comparison.

Methods

- The SST Samples consisted of three different antibody (Ab) formats (a standard monoclonal antibody –mAb-, a 1+1 bispecific antibody –bsAb-, and a 2+1 bsAb, fig. 1).
- The sample concentration for intact Ab analysis was adjusted to 0.5 mg/ml.
- After Ab reduction, the subunit concentration was adjusted to 0.25 mg/ml for additional sample characterization.
- The samples were measured in triplicate using an Acquity UPLC H-Class system (Waters) equipped with a PLRP-S column (Agilent) and coupled to a maXis II ETD mass spectrometer (Bruker Daltonics) with settings tuned for intact and subunit Ab analysis.
- First set of measurements was performed with the Apollo II source, followed by exchange of the ion source to the VIP-HESI source.
- Roche In-House software was used for data processing.

Results

- Signal intensity for all three intact Ab molecules from triplicate measurements was highly reproducible with both ion sources (Fig. 2).
- Data acquired with the new VIP-HESI source showed an increase in signal intensity by a factor ≥2 for all three intact Ab molecules (Fig. 2 and Fig. 3) compared to the data acquired with the Apollo source.
- Comparing the peaks of selected charge states, an improved peak width was observed for signals acquired with the new ion source (Fig. 3).
- The data acquired with the VIP-HESI source provided spectra with better separation from method-induced adduct signals and a reduced baseline caused by the better desolvation process of this source.
- The measurements of Ab subunits showed a significant gain a signal intensity (Fig. 2).

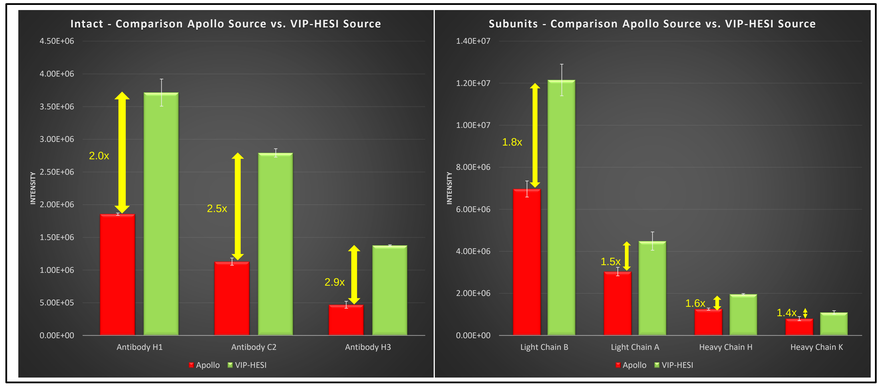

Fig. 2: Average signal intensity from triplicate injections of three different intact Ab formats. Signal intensity obtained with the new VIP-HESI source (green) was increased by a factor ≥2 compared to the Apollo source (red), (left diagram).
Average signal intensity from triplicate injections of different Light Chain and Heavy Chain sequences. Also here, a significant gain by acquisition with the VIP-HESI source (green) was observed compared to the data obtained with the Apollo source (red), (right diagram).

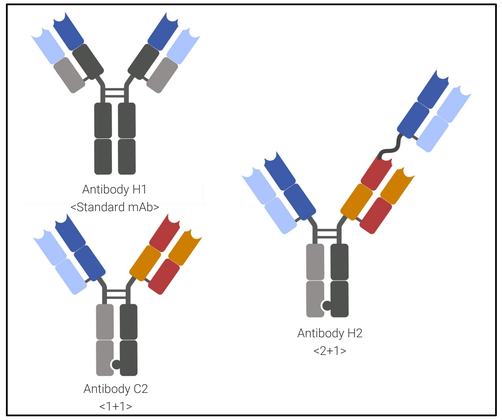

Fig. 1: Structure f the three different antibodies. Standard mAb H1 (top left), 1+1 antibody C2 (bottom left), and 2+1 antibody H3 (right).

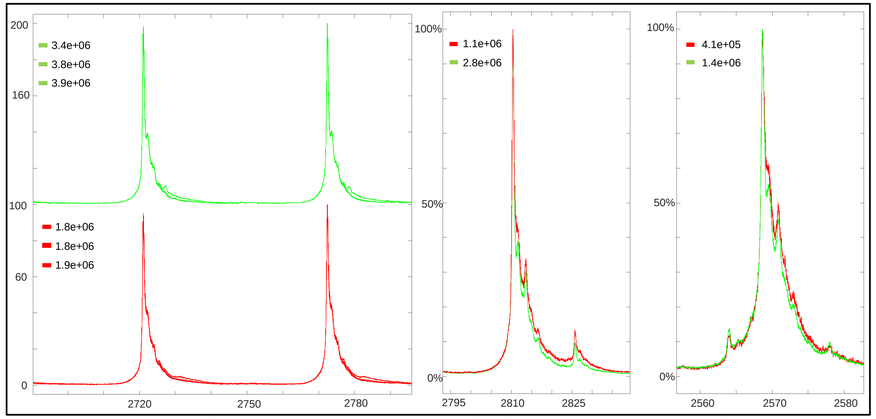

Fig. 3: Comparison of charge state intensities of Ab H1, overlay of three injections per source (left, VIP-HESI green, Apollo red). Selected charge states of Ab C2 (middle) and Ab H3 (right), overlay of signals from the different two sources showing improved peak width.

Summary

- The new design of the VIP-HESI ion source is providing improved spectral data quality for the LC-MS based characterization process of next generation biotherapeutics.
- More robust setup for high-throughput RP-MS provided by the new ion source.
- The signal intensity is improved significantly for antibody subunits as well as for the three different intact Ab structure types, with an improvement by a factor ≥2 for all three intact molecules.
- More straightforward identification of possible side products and/or adducts enabled.
- Superior data quality obtained, led to implementation of the new VIP-HESI source in the standard workflow for LC-MS based characterization of biopharmaceuticals.

Conclusion

- New HESI source in combination with high resolution QTOF mass spectrometer provides improved intact antibody quality.
- Gain in signal intensity provided is crucial for improved biotherapeutics identity confirmation on intact protein level as well as for antibody subunits.
- New peak shape quality enables higher accuracy for mass determination and less error-prone automated data evaluation.
- Further investigation of probe gas temperature and flow rate to evaluate additional improvement capabilities for intact and subunit antibody characterization.

Antibodies