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Live Webinar Q&A Sheet:

Leveraging Advanced Analytical Technologies to Expedite the Development of Antibody Drug Conjugates

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The recorded webinar may be viewed from the Biotherapeutics webinars page. These questions were submitted by live viewers. Additional information on SEC-MALS and DLS may be found on the Wyatt web Library under Webinars, Application Notes, Featured Publications and Bibliography, as well as on the corresponding Product page and Solutions page of our web site.

Please contact info@wyatt.com with any additional questions.

Questions & Answers

GENERAL MALS QUESTIONS

Q: *What are some considerations when performing non- SEC-MALS runs like anion exchange or reverse phase chromatography?*

A: Different fractionation techniques like AEX and RP are compatible with the MALS detector. The concern is that the dRI detector won't be able to provide the most accurate quantitative data due to the slope of the baseline because an RI detector is sensitive to the different solvent gradients. For these techniques, we recommend a TUV or PDA. Many people run their system with both the TUV or PDA and a RI detector.

Q: *For IEX [fractionation] of an ADC if we do need UV-MALS, do we need standards to find out UV Extinct coefficient?*

A: Many people use a program like ProtParam to get the molar extinction coefficient of their protein using the primary sequence. There isn't a standard value like there is for the RI of proteins, which is 0.185 mL/g. As a user you will need to input the unique molar extinction coefficient into the software for accurate protein-conjugate analysis.

Q: *What is the wavelength to detect the payload (unconjugated drug)? Could you give us a few examples?*

A: Most people commonly use 280 nm and 260 nm. It really depends on your sample and where your molecule will absorb.

Q: *At any time did you lose the tertiary structure during these analytical processes?*

A: We were able to use both SEC-Optical, SEC-MALS and DLS to analyze the amount of aggregation, which implies a loss of tertiary structure. We observed aggregation only after external stresses were applied to the samples, for case study one, that used DLS, this was observed after thermal stress was applied and for case study three, <2% of the sample aggregated following a 30-day hold at 4 °C.

Q: *How narrow of a range can MALS differentiate between an IgG and ADC? for example 100-200 Da?*

A: SEC-MALS has a molecular weight (MW) of 5% or better. Under optimal conditions members of the analytical sciences team have reported precision of MW measurement at ~1%. This being said, the difference of 150.2 kDa vs 150.1 kDa as you mentioned above is only a 0.1% difference. We wouldn't be able to tell the difference between these two hypothetical differences. However, the drug conjugates that we investigated in our studies were closer to 960 Da and when considering a DAR of 8 vs 0 or smaller differences, the instrument can detect this change. For more information please refer to the following application note: AN1609: Drug-Antibody ratio analysis in ADCs.

Q: *Did you see any disulfide bond scrambling, during conjugation??*

A: We didn't identify any disulfide scrambling. For more details, please read our application note on this subject: H. Shion, et al. Development of Integrated Informatics Workflows for the Automated Assessment of Comparability of Antibody Drug Conjugates (ADCs) Using LV-UV and LV-UV/MS. *Waters Application Note*. 720005366EN.