Using protein-specific retention behavior to improve the characterization of therapeutic antibodies

Bastiaan Duivelshof[1,2], Jean-Luc Veuthey[1,2], Davy Guillarme[1,2]

[1]*Institute of Pharmaceutical Sciences of Western Switzerland (ISPSO), University of Geneva, CMU-Rue Michel Servet 1, 1211 Geneva 4, Switzerland*

[2] *School of Pharmaceutical Sciences, University of Geneva, CMU-Rue Michel Servet 1, 1211 Geneva 4, Switzerland*

bastiaan.duivelshof@unige.ch

Therapeutic monoclonal antibodies (mAbs) have grown significantly in popularity over the last 30 years as a class of human therapeutics, leading to an interesting alternative to small molecule drugs in the pharmaceutical industry. The characterization of mAbs is complex but of utmost importance, due to the occurrence of post-translational modifications (PTMs) that could hamper the drug safety and efficacy. Liquid chromatography has emerged as a key technique in the characterization of size, charge, hydrophobic, and hydrophilic protein variants. Coupled to mass spectrometry, chromatographic techniques provide well-established strategies to characterize the aforementioned structural protein variants. Moreover, the functional characterization of therapeutic antibodies using affinity liquid chromatography has become increasingly important to relate the observed PTMs to distinct pharmacodynamic and pharmacokinetic (PK/PD) properties *in vivo*. Together, this provide vital information on the critical quality attributes (CQAs) of therapeutic antibodies.

In this work, we took a closer look into how recently discovered protein-specific retention behavior can improve the current characterization techniques for therapeutic antibodies. We focused specifically on the use of ultra-short column formats (i.e., 10-15 mm), retention modelling software[1] and the use of special multi-isocratic and negative gradient types in affinity chromatography[2]. This could help to further improve the speed and/or selectivity of these state-of-the-art characterization techniques and prepare them for the next-generation of antibody-based products such as, antibody-drug conjugates (ADCs), fusion proteins, and bi-specific antibodies.

[1] B.L. Duivelshof, A. Zöldhegyi, D. Guillarme, M.A. Lauber, S. Fekete; J Pharm Biomed Anal., 221 (2022); https://doi.org/10.1016/j.jpba.2022.115039

[2] T. Bouvarel, B.L. Duivelshof, J. Camperi, T. Schlothauer, A. Knaupp, C. Stella, D. Guillarme; J Chromatogr A, 1682 (2022); https://doi.org/10.1016/j.chroma.2022.463518