A novel approach for multiple PQAs monitoring of mAbs from bioreactors using 2D-LC-MS

Maria Grübner [a], Christof Mitterer [b], Alexander Schwahn [c], Aleš Holfeld [c], Sara Carillo [e], Ken Cook [d], Jonathan Bones [e]
[a-d] Thermo Fisher Scientific ([a] Germering/Germany; [b] Langerwehe/Germany; [c] Reinach/Switzerland; [e] Hemel Hempstead/UK)
[e] NIBRT, Dublin/Ireland

Abstract

Purpose: Simultaneous titer determination and monoclonal antibody (mAb) purification for subsequent charge or size variant analysis.

Methods: Heart-cut two-dimensional liquid chromatography coupled to mass spectrometry (2D-LC-MS); protein A affinity chromatography (ProA); strong cation exchange chromatography (SCX); size exclusion chromatography (SEC)

Switchable 2D-LC-MS methods for ProA-SCX-MS and ProA-SCX-SEC-MS analysis of intact mAbs from bioreactor samples under native conditions.

Results: Straightforward monitoring of multiple product quality attributes (PQAs), including mAb titer, glycation pattern, PTMs, and aggregation level throughout the bioproduction process.

Introduction

The inherent microheterogeneity of biopharmaceutical products like mAbs and its impact on critical biochemical and biophysical drug properties, requires a thorough characterization and monitoring of PQAs e.g., post-translational modifications (PTMs), glycan patterns and aggregation levels throughout the manufacturing chain. In that, coupling of MS to LC separations serves the demand for deeper and more comprehensive analytical insights.[1]

Materials and methods

Material

- Thermo Scientific™ Vanquish™ Flex Simple Switch™ 2D-LC system for loop heart-cutting consisting of Quaternary Pump F (VF-P20-A), Binary Pump F (VF-P10-A-01), Dual Split Sampler FT (VF-A40-A-02), 2x Column Compartment H (VH-C10-A-03), and Diode Array Detector FG (VF-D11-A-01)
- Thermo Scientific™ Orbitrap Exploris™ MX Mass Detector (BRE725536) with BioPharma option (BRE725539)
- Thermo Scientific™ MAbPac™ Protein A Antibody Analysis and Purification HPLC Column, 12 µm, 4 x 35 mm (PN 082539)
- Thermo Scientific™ ProPac™ 3R SCX, 2 x 50 mm, 3 µm (PN 43103-052068)
- Thermo Scientific™ MAbPac™ SEC-1, 5 µm, 4 x 300 mm (PN 074696)

Samples

Harvested cell culture (HCC) samples of a Chinese ovary hamster (CHO) cell culture expressing a NISTmAb analogue (cNISTmAb, from NIST™ research grade test material 10197), were collected from a lab-scale bioreactor over 10 days.

Test Method

A switchable multi-method 2D-LC platform coupled to high-resolution accurate mass MS was set up (**Figure 1B**). The first dimension (^{1}D) employed ProA for mAb isolation from cell culture matrix and titer determination. The purified mAb was subsequently transferred via a heart-cut loop to a short, high-efficiency SCX column in the second dimension (^{2}D). The SCX column was either eluted by a pH gradient for charge variant analysis or used as a trap column to facilitate size variant analysis by switching the SCX column in-line with the SEC column and transfer of the mAb by pulse-elution. All LC methods were developed with MS compatible mobile phases to facilitate direct MS hyphenation. The study design is depicted in **Figure 1A**.

Data Analysis

Thermo Scientific™ Chromeleon™ 7.3.2 Chromatography Data System (CDS) and Thermo Scientific™ BioPharma Finder™ 5.2 software

Results

The **^{1}D affinity separation** of the mAb from host cell material (**Figure 2**) was accomplished by a step gradient from pH 7 to pH 2.5. HCC sample mAb titer results increased from 7 µg/mL on day 1 to 52 µg/mL on day 10 (**Figure 3**).

Figure 2. ^{1}D ProA UV chromatogram of day 5 sample with heart-cut indicated

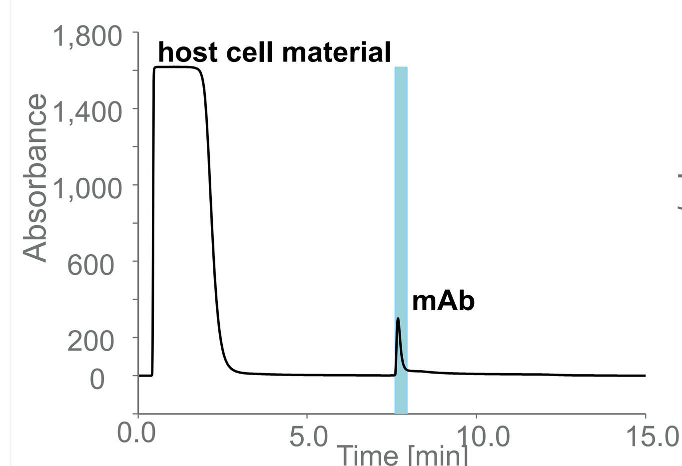
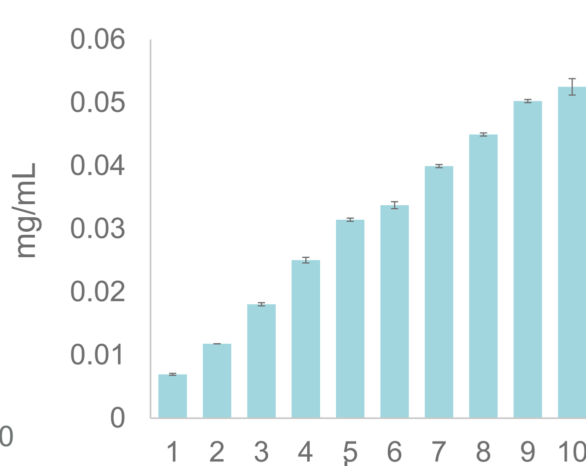


Figure 3. mAb titer of HCC samples over 10 days from ProA-SCX runs



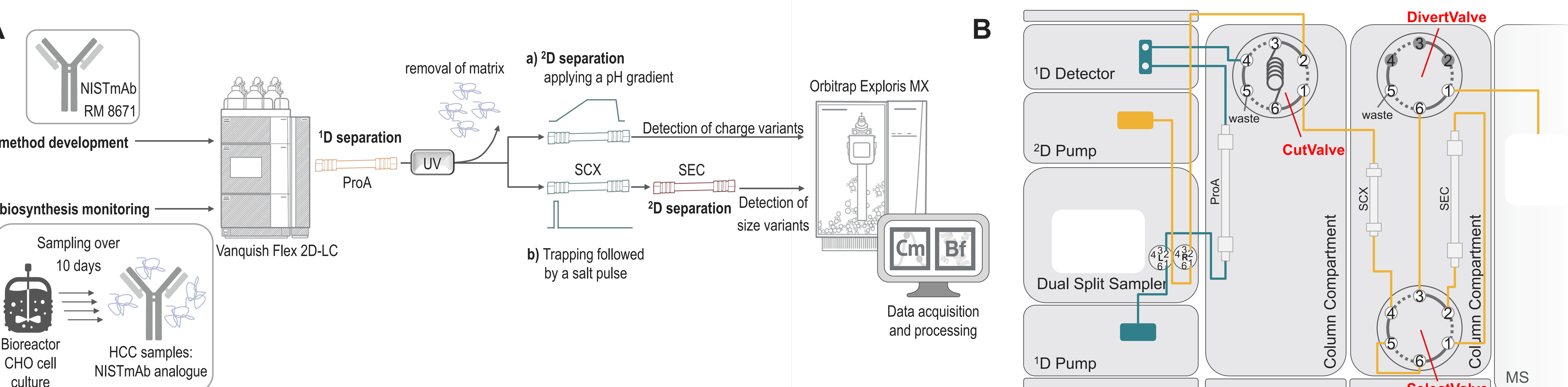
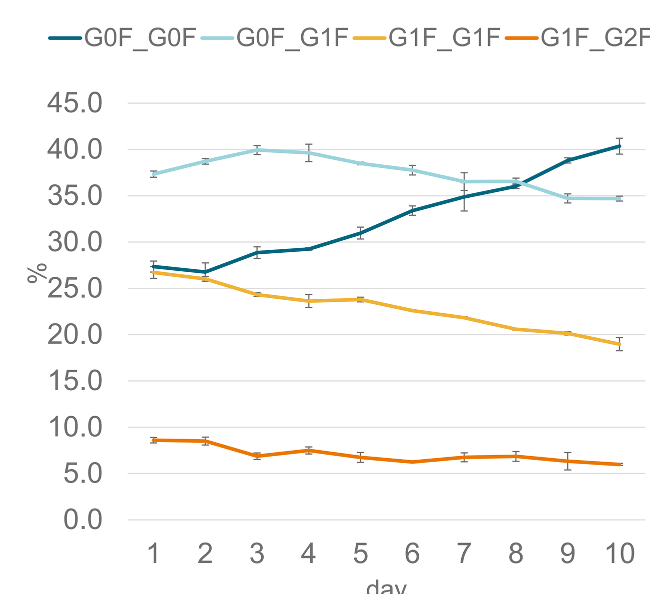


Figure 1. Experimental setup; A: Overview of the study design; B: Fluidic scheme of the 2D-LC-MS setup for switchable ProA-SCX-MS and ProA-SCX-SEC-MS analysis

Figure 4. Development of main glycoform ratio of cNISTmAb bioreactor samples over 10 days



Within the main peak of the ^{2}D **charge variant analysis** the major glycoforms G0F_G0F, G0F_G1F, G1F_G1F, and G1F_G2F of the mAb were identified. The G0F_G1F was the most abundant form on days 1-8, (**Figure 4**). G0F_G0F and G1F_G1F forms started at equivalent levels (~27%). G1F_G1F decreased down to 19%. The abundance of G0F_G0F increased to 40%, emerging as the most abundant isoform on day 9 and 10. G1F_G2F went from 9 to 6%.

Several additional charge variants were detected in the deconvoluted mass spectra (**Figure 5**) and in parts were tentatively identified according to mass deltas (Δ) and retention (**Table 1**). Abundance trends relative to G0F_G1F are plotted in **Figure 6**.

For **size variant analysis** the mAb was trapped at the SCX column and eluted with a salt pulse in a narrow band to the SEC column. As with SEC sample bands cannot be re-focused at the column head, low band dispersion is extremely critical for adequate separation. The trap/elute procedure, with the SCX column in front of the SEC column (ProA-SCX-SEC), significantly improved peak shape compared to a setup with a direct ProA-SEC fraction transfer (**Figure 7**) and provided good separation of mAb monomer and high molecular weight (HMW) fraction (**Figure 8**).

Figure 5. Deconvoluted spectrum of ProA-SCX analysis of HCC sample after day 6 (18-26 min, 192 scans).

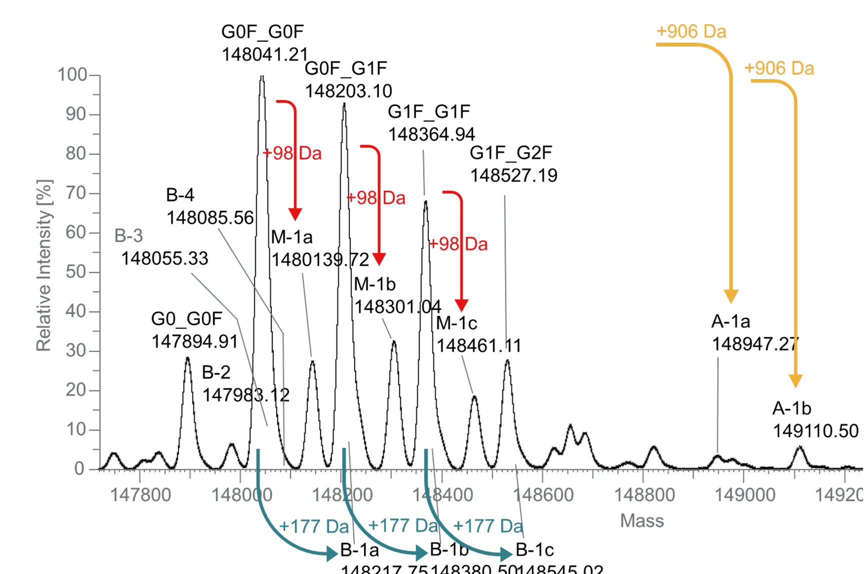


Table 1. Detected charge variants

variant	mass delta (Δ)	tentative identification
G0_G0F	G0F_G0F - 146 Da	G0_G0F
M-1a	G0F_G0F + 98 Da	unknown
M-1b	G0F_G1F + 98 Da	unknown
M-1c	G1F_G1F + 98 Da	unknown
B-1a	G0F_G0F + 177 Da	unknown
B-1b	G0F_G1F + 177 Da	unknown
B-1c	G1F_G1F + 177 Da	unknown
B-2	G0F_G0F - 58 Da	1x glycine loss and proline amidation
B-3		unknown
B-4	G0F_G1F - 116 Da	2x glycine loss and proline amidation
A-1a	G0F_G0F + 906 Da	2x sialic acid
A-1b	G0F_G1F + 906 Da	2x sialic acid
A-2	G0F_G0F + 1813 Da	4x sialic acid

Figure 6. Abundance trends of (unknown) charge variants over 10 days cNISTmAb production relative to the G0F_G1F glycoform

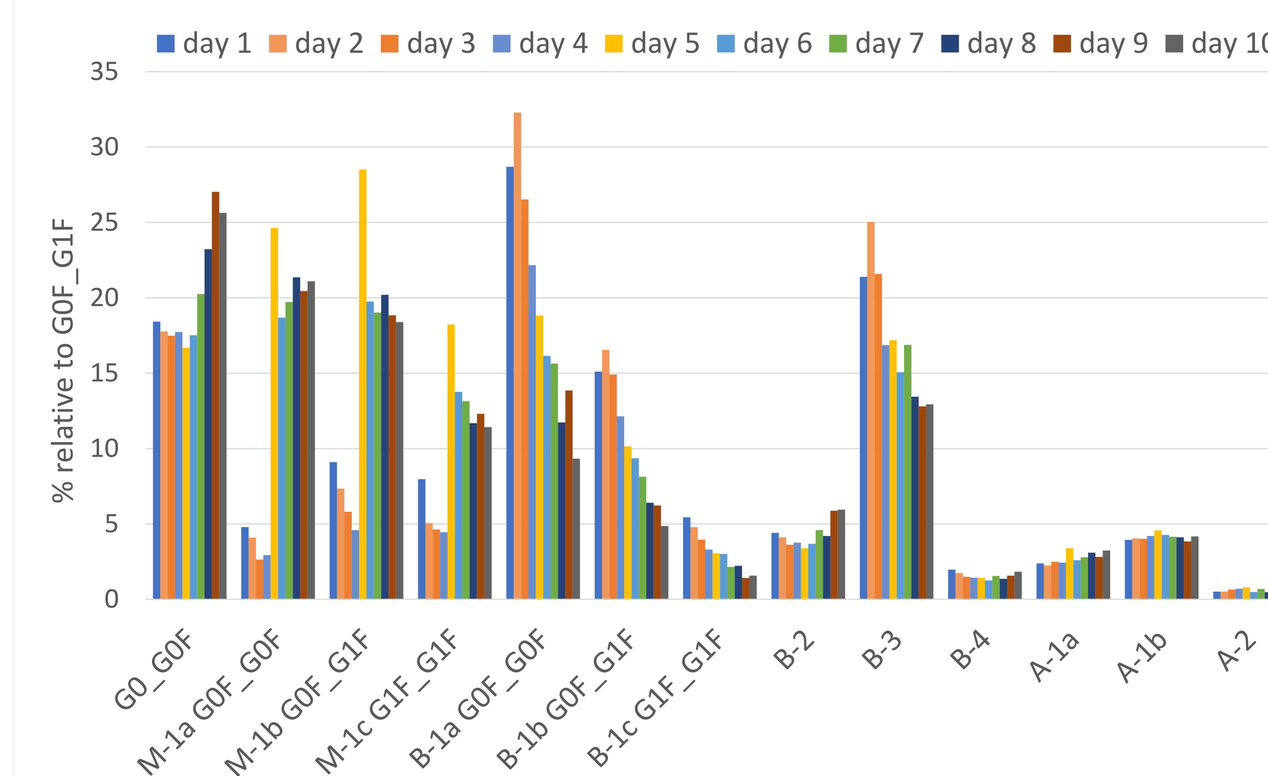


Figure 7. significant ^{2}D peak shape improvement by the ProA-SCX-SEC procedure compared to ProA-SEC (UV)

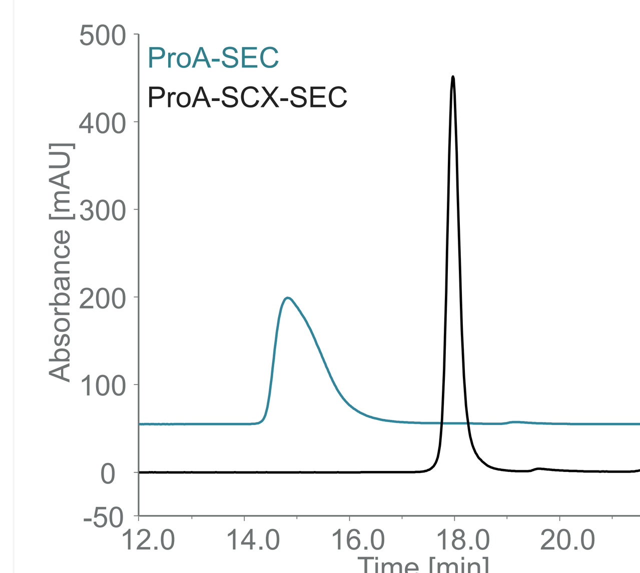
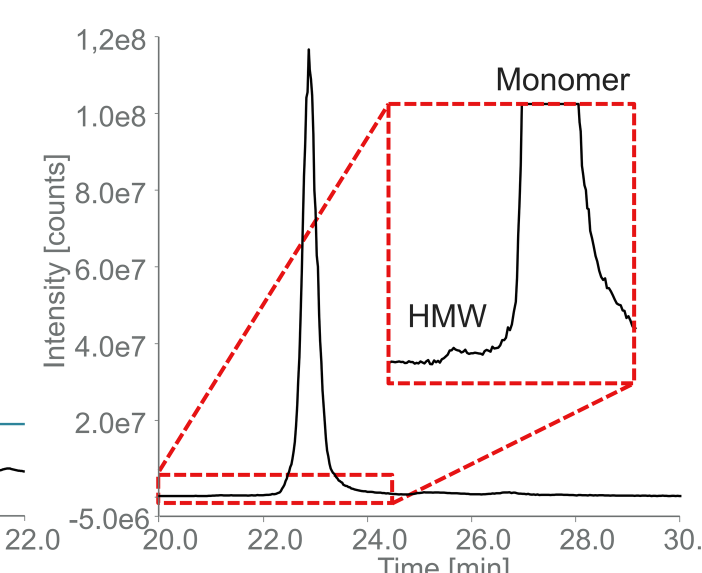


Figure 8. ^{2}D TIC of ProA-SCX-SEC analysis of day 9 HCC sample



Conclusions

- The switchable multi-method heart-cut 2D-LC-MS approach facilitated the monitoring of multiple PQAs from HCC samples: mAb titer determination; abundance monitoring of major glycoforms; monitoring of several unknown charge variants; tentative charge variant identifications; aggregation level monitoring
- The approach is well suited for the close process monitoring of new modalities, as well as for multi-attribute monitoring at the intact protein level (iMAM)[1] for well-known mAb products. Within around one hour a set of titer, charge and size variant information can be drawn from bioreactor samples.

References

1. Carillo *et al.*, Eur J Pharm Biopharm, (2022), 241–248, 177

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