Characterization of a therapeutic protein by optimized Peptide Mapping, SEC and IEX

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Introduction

In depth physico-chemical characterization of therapeutic proteins is required during all phases of drug development to ensure drug safety and efficacy. Providing proof of protein drug identity and integrity is usually demonstrated by performing several (LC-based) assays such as peptide mapping, size exclusion (SEC) ion-exchange chromatography (IEX). Resolution and robustness is required for all techniques in order to separate and identify potential aberrations that might occur during the production process or during drug formulation in a reproducible manner.

In this study we present data for a 26 kDa recombinantly expressed NBE, protein P128 under development at GangaGen Biotechnologies for human therapy. Peptide mapping, SEC and ion-exchange chromatography are performed for this protein using the new Agilent Bio HPLC columns in combination with a new Agilent 1260 Infinity bio-inert quaternary LC instrument especially developed for the analysis of large bio-molecules.

Fig.1 New Agilent 1260 Infinity bio-inert LC with completely metal-free bio-inert sample flow path, wide pH range, 600 bar compatibility, bio-inert high sensitivity flow cell

Experimental

System

G5611A	1260 Bio-inert Quaternary pump
G5667A	1260 Bio-inert HiP-autosampler
G1330B	1200 Series Thermostat
G1316C	1260 Thermostatted column compartment with bio-inert click-in heating elements
G4212B	1290 DAD with bio-inert with 60 mm Max-Light flow cell or
G1316C	1260 DAD with 10 mm flow cell

System contains bio-inert high-pressure capillaries

Peptide Mapping:

Column: Eclipse Plus C18 3.0 X 100 mm, 1.8 µm.

Mobile phase A: 0.1% TFA;

Mobile phase B: 99.2% ACN, 0.08% TFA

Size Exclusion Chromatography:

Column 1: Agilent SEC 3, 7.8 X 300mm, 3 µm, 300Å

Column 2: Agilent SEC 5, 7.8 X 300mm, 5 µm, 300Å

Column 3: Competitor's column

Mobile phase: 150 mM Sodium Phosphate (pH 7) + 150 mM Sodium Chloride. Flow rates: 1 mL/min, UV detection: 220 nm

Experimental

Ion Exchange Chromatography:

Column 1: Agilent Bio MAb NP 5, PK, 4.6 X 250mm, 5 µm

Column 2: Agilent Bio MAb NP10, PK, 4.6 X 250mm, 10 µm

Column 3: Competitor's column

Mobile phase A: 20 mM Sodium Phosphate (pH 6.0)

Mobile phase B: 20 mM Sodium Phosphate (pH 6.0) + 1 M Sodium Chloride

Flow rates: 0.5 mL/min, UV detection: 220 nm.

Gradient conditions used :0 min 10% B; 35 min 35% B; 36 min 10% B; 45 min 10% B

Results and Discussion

PEPTIDE MAPPING

UV-based peptide maps are often generated in a QA/QC new biological entity (NBE) environment in order to detect any sequence abnormalities that are caused during the manufacturing or processing of a recombinant protein drug. Highest possible resolution is required for a complex mixture which often results in excessive long analysis times. Using STM particle technology in combination with UHPLC instrumentation allows for shortening analysis time while preserving resolution (Fig. 2). Bio-inert instrumentation has been beneficial especially for hydrophobic peptides and proteins that tend to stick to metal surfaces.

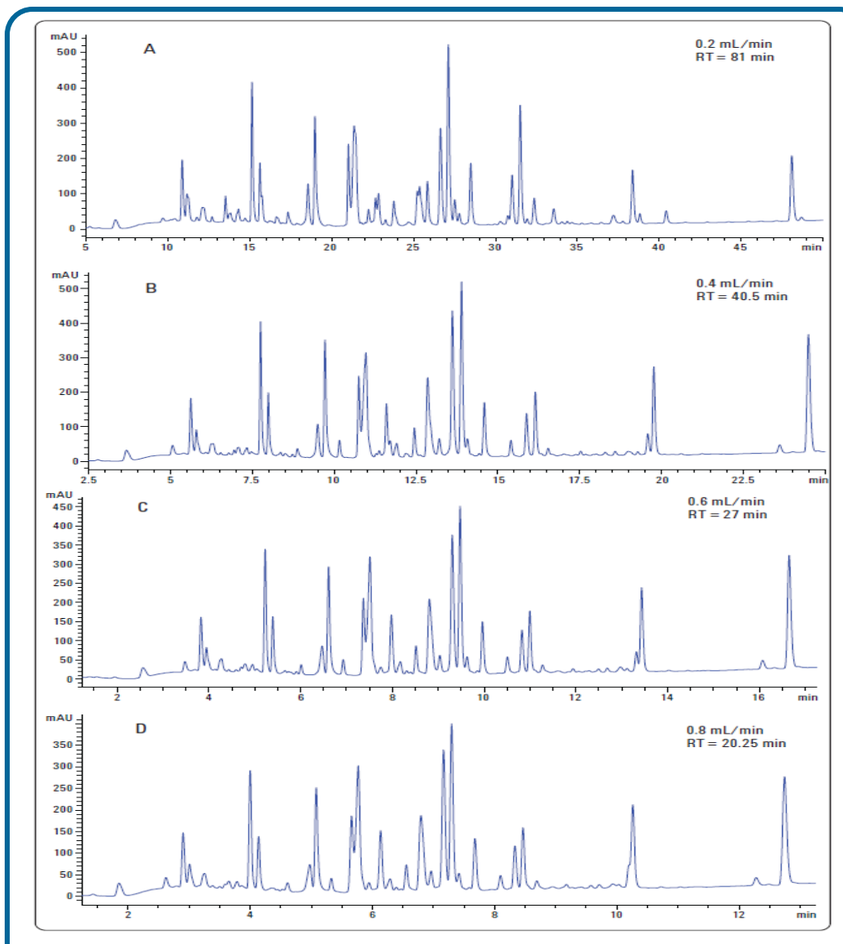

Fig.2. Analysis of a tryptic peptide map for P128 protein. The gradient slope was identical for all experiments.

Robustness and repeatability of analyses are of highest importance when characterizing therapeutic proteins in order to provide proof of integrity and identity of the respective NBE and to fulfill regulatory requirements. Five replicate injections on Eclipse Plus C18 3.0 x 100 cm, 1.8 µm particle with the Agilent 1260 Infinity Quaternary LC (Fig 3) demonstrate excellent overlay of all analyses.

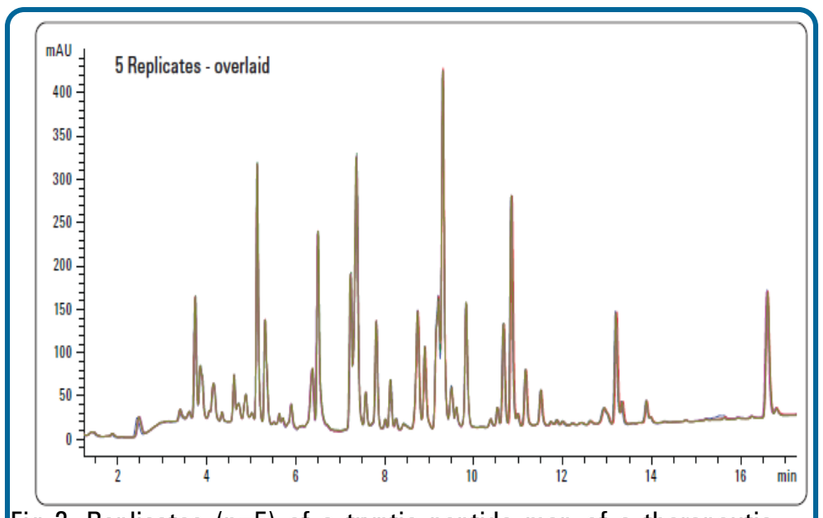

Fig 3. Replicates (n=5) of a tryptic peptide map of a therapeutic protein at 0.6 ml/min.

Results and Discussion

SIZE EXCLUSION CHROMATOGRAPHY

In order to demonstrate protein integrity or to prove absence of dimer or multimer formation in its native conformation, size exclusion chromatography (SEC) in combination with UV detection is a commonly applied analytical technique. A disadvantage of size exclusion is the lack of resolution. New small particle SEC columns provide superior resolution with minimal secondary interaction providing a powerful tool to detect impurities or multimer formation. The same protein used for peptide mapping was used to perform the SEC experiment. Results were compared for two 5 µm particle um particle columns and a 3 µm particle column. Only with the Agilent SEC 3 µm column the 2nd compound could be detected (Fig 4).

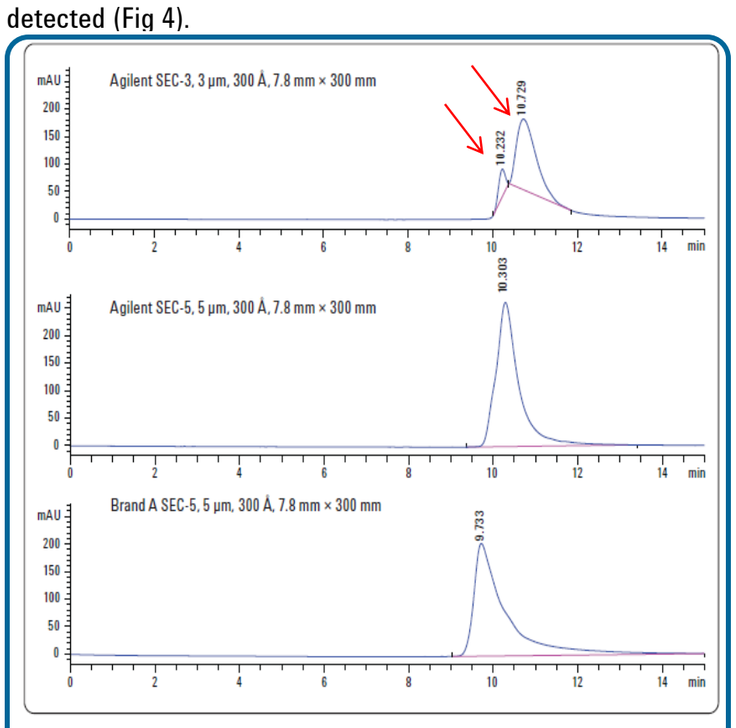

Fig 4. Size exclusion of a P128 therapeutic protein sample performed on the Agilent 1260 Infinity bio-inert LC using different columns. Arrows indicate that the two compounds present in the sample could only to be distinguished when using a 3 µm column.

ION EXCHANGE CHROMATOGRAPHY

A sample of P128 protein preparation was separated on the new Agilent 1260 Infinity bio-inert and corrosion resistant LC using the Agilent MAb 5 and MAb 10 column and commercially available column (Brand B) of similar dimensions. Superior peak shape and highest efficiency was achieved on the on the Agilent Mab 5 NP column (Fig 5).

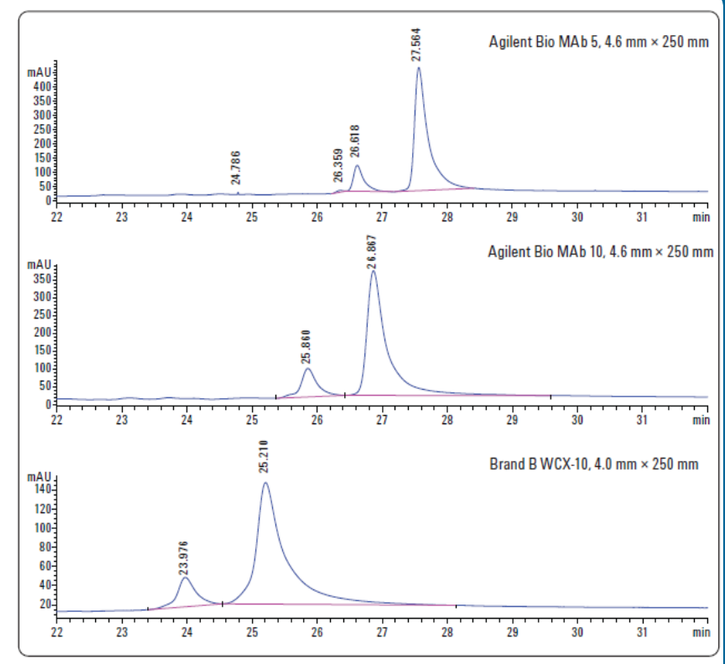

Fig 6. Weak cation exchange chromatography for a P128 therapeutic protein sample on the Agilent 1260 bio-inert LC-system.

Conclusions

The new Agilent 1260 Infinity bio-inert quaternary LC solution in combination with the Agilent Bio HPLC column portfolio provide a powerful and versatile tool to characterize the physico-chemical properties of a therapeutic protein drug by combining

- bio-inertness,
- superior resolution,
- corrosion resistance,
- high sensitivity and
- fast separation speed

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