Analytical Characterization of Monoclonal Antibody IgG2

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INTRODUCTION

Monoclonal antibodies have been the fastest growing protein therapeutics. Due to the molecular complexity of monoclonal antibodies, the characterization remains a challenge and required step throughout the development and manufacturing process. In order to determine the efficacy of the molecules, aggregation, heterogeneity such as charge variants, C-terminal lysine processing, deamidation, glycosylation must be screened for their structural and biological changes. Analytical techniques are employed for these product and process characterizations at different stages of product cycles such as in process monitoring, lot release and product stability studies.

In this poster, we would like to present the IgG2 Vectibix (panitumumab) characterization in a few different chromatographic areas. Vectibix is a fully human mAb IgG2 specific to the epidermal growth factor receptor (EGFR). At first we apply size exclusion chromatography (SEC) with 1.8 µm particle size, 300 Å modified resin surface for Vectibix IgG2 aggregate, monomer and fragment analysis. With added MALS detector, molecular weight of different species can be determined in the same SEC separation. The second characterization method is the strong cation exchange chromatography. It provides the charge variants separation which may be due to IgG2's major disulfide-mediated structural isoforms. Fractions of the charge variants separation can be collected for further characterization. In the third chromatographic method, Proteomix HIC butyl provides an orthogonal analysis of Vectibix variants under the native running condition based upon the different species' hydrophobicity. Lastly Intact IgG2 and DTT reduced subunits can be analyzed with reversed phase chemistry. Polymer based Proteomix RP-1000 provides excellent evaluation of IgG2 subunit heterogeneity. In conclusion, these four chromatographic methods provide a comprehensive characterization of IgG2 Vectibix heterogeneity.

EXPERIMENTAL

Columns: Zenix® - C SEC-300-LS (3 µm, 7.8 x 300 mm)
Unix® - C SEC-80 (1.8 µm, 4.6 x 300 mm)
Proteomix® SCX NP5 (5 µm, 2.1 x 250 mm)
Proteomix® HIC Butyl-NP5 (5 µm, 4.6 x 50 mm)
Proteomix® RP-1000 (5 µm, 1000 Å, 2.1 x 100 mm)

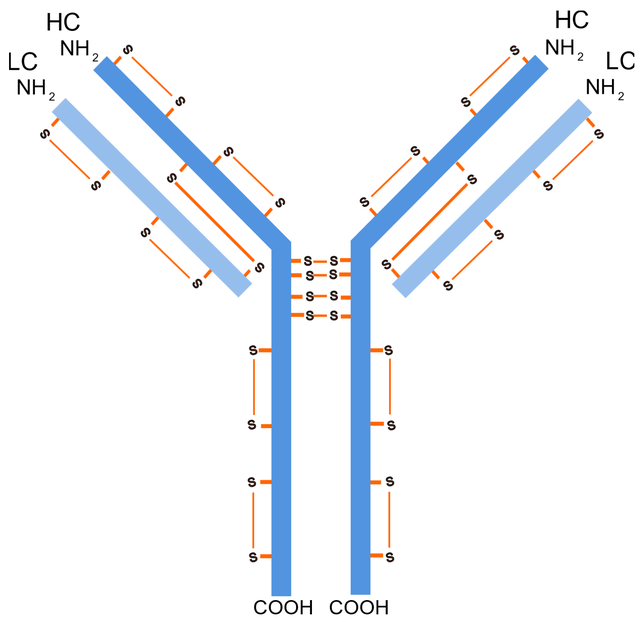
Samples: Monoclonal Antibody
Vectibix IgG2
Erbitux IgG1

LC Column Running condition: See detail result section

CONCLUSION

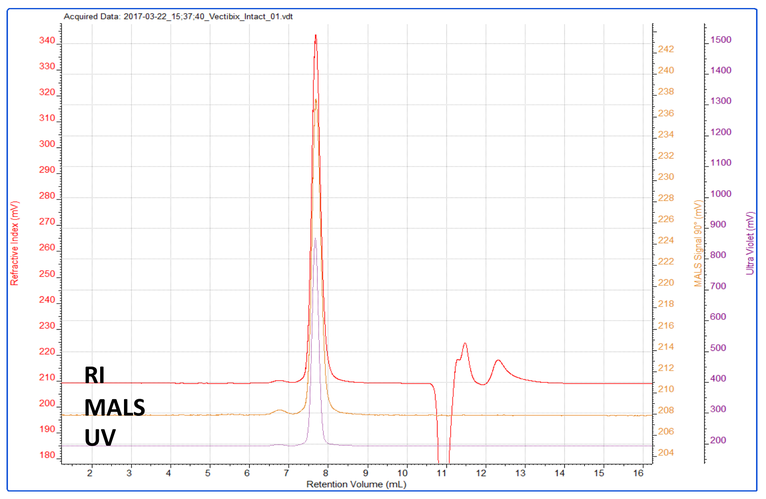
- Zenix-C SEC-300 size exclusion chromatography provides high resolution separation of Vectibix aggregates and monomers. With multi-angle light scattering detector, absolute molecular weight can be determined, aggregation behavior of the mAb can be monitored.
- Zenix-C LS column exhibits extreme low shedding with high SEC resolution.
- Sub 2µm Unix-C SEC offers higher resolution between aggregate, monomer and fragments of the biomolecule.
- Proteomix SCX provides excellent charge variants separation, further fraction collection and peptide mapping can yield information on disulfide shuffling.
- Two complete different charge variant profiles are generated with Proteomix SCX for IgG1 and IgG2 due to structure difference while targeting same EGFR.
- Hydrophobicity of mAbs can be evaluated using native LC conditions with Proteomix HIC. IgG2 Vectibix and IgG1 Erbitux have different HIC profiles.
- With large pore reversed phase chromatography, reduced Vectibix fragments can be separated with online mass spec analysis capability.
- High resolution SEC, cationic exchange, native HIC and large pore reversed phase offer a wide range of orthogonal analysis for monoclonal antibody heterogeneity.

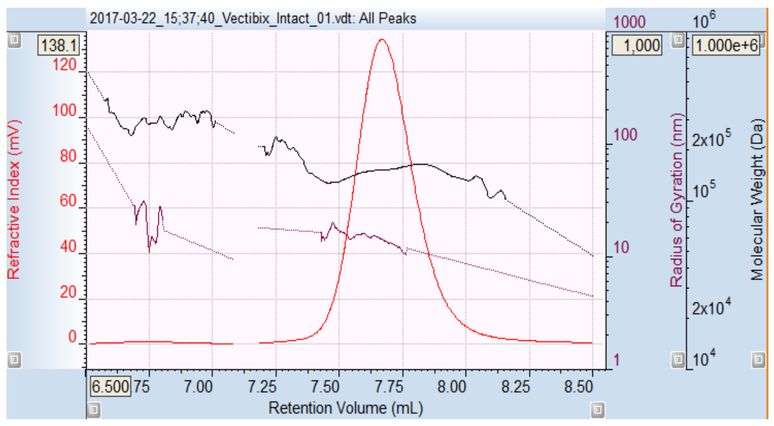
IGG2 STRUCTURE



SIZE EXCLUSION CHROMATOGRAPHY (SEC-MALS)

Column: Zenix® - C SEC-300 –LS (3 µm, 300 Å, 7.8 x 300 mm)
Mobile phase: 150 mM sodium phosphate, pH 7.0
Flow rate: 1 mL/min; Column temperature: 25 °C
Triple Detector: UV 280 nm; RI; MALS
Injection volume: 100 µg Vectibix 20 mg/mL



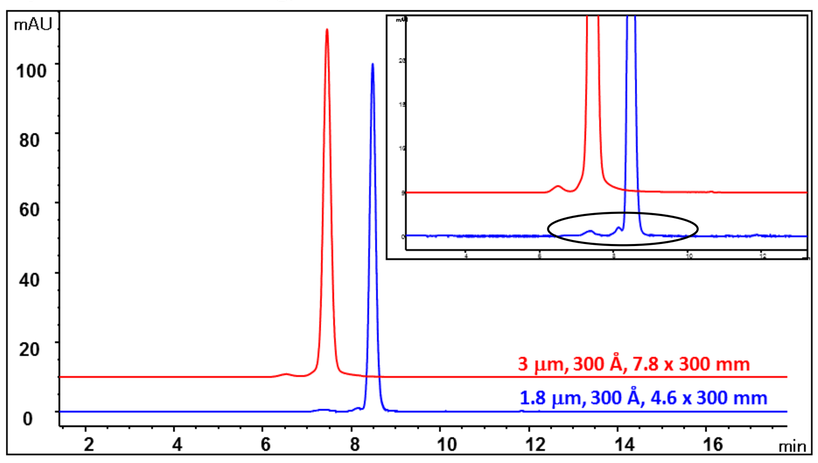


Light Scattering Results

Peak	1	2
Ret Vol (mL)	6.735	7.663
Mw (Da)	302,951	148,948
Mw/Mn	1.020	1.012
Rg(w) (nm)	29.06	13.23
Wt Fr (Peak)	0.0114	0.9886
MALS Area (90°)	0.18	8.32

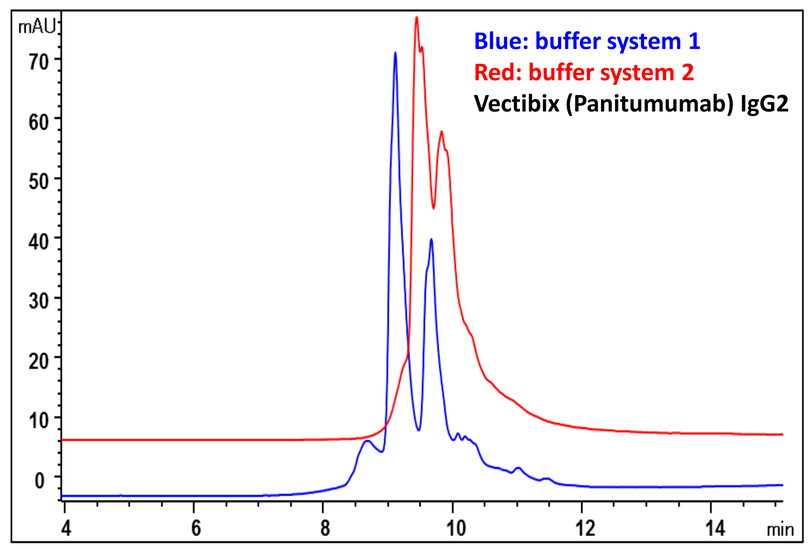
SUB 2 µM SIZE EXCLUSION CHROMATOGRAPHY (SEC)

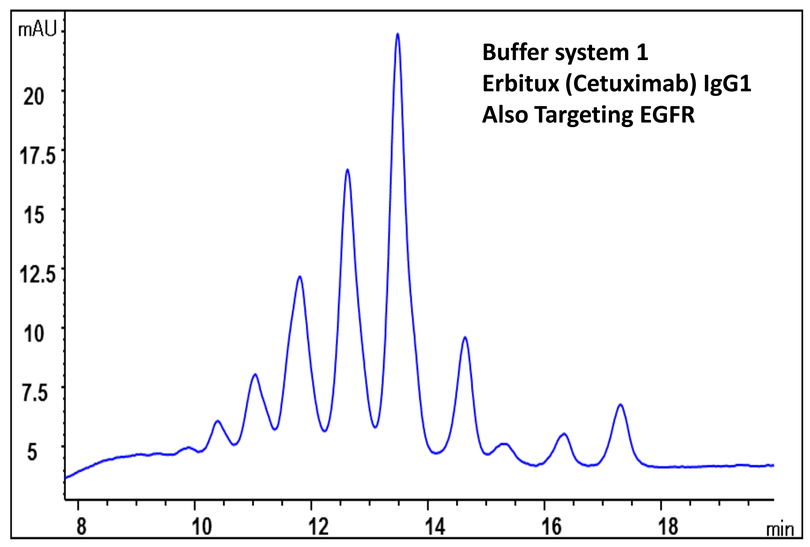
Column: Unix® - C SEC-300 (1.8 µm, 4.6 x 300 mm)
Mobile phase: 150 mM Phosphate buffer, pH 7.0
Flow rate: 0.3 mL/min; Detector: UV 280 nm;
Column temperature: Room temperature;
Injection: Vectibix 20 mg/mL



CHARGE VARIANTS SEPARATION WITH PROTEOMIX SCX

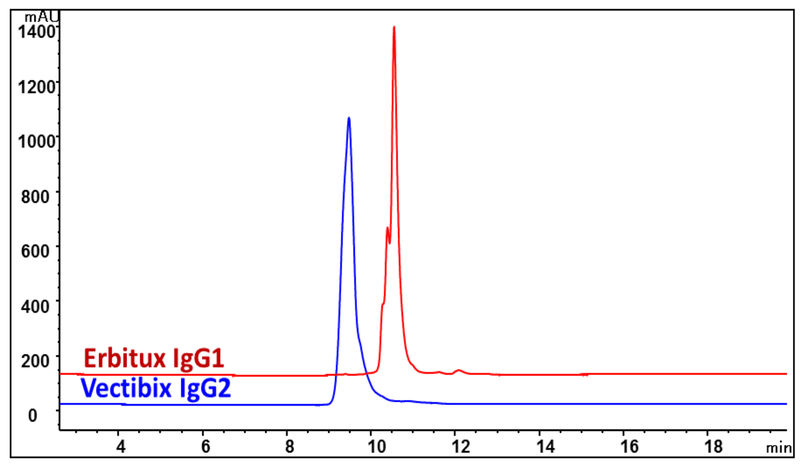
Column: Proteomix SCX NP5 (5 µm, 2.1×250 mm)
Flow Rate: 0.3 mL/min; Detection: UV 280 nm; Temperature:25°C;
Sample: Vectibix 5 mg/mL; Injection Volume: 2 µL
Mobile phase system 1:
A: 16 mM MES, 10 mM MOPS, 12 mM TAPS, 10 mM CAPSO, pH 5.6,
B: 10 mM MES, 12 mM MOPS, 14 mM TAPS, 16 mM CAPSO pH 10.2
Mobile phase system 2:
A: 20 mM MES pH 5.7, B: A + 1M NaCl





HYDROPHOBIC INTERACTION CHROMATOGRAPHY

Column: Proteomix ® HIC Butyl-NP5 (4.6 x 50 mm)
Flow rate: 1 mL/min; Detector: UV 214 nm;
Mobile phase: A: 2.0 M ammonium sulfate in 100 mM sodium phosphate, pH 7.0, B: 100 mM sodium phosphate pH 7.0,
Column temperature: 25 °C;
Injection:10 µg mAb



REVERSED PHASE PROTEOMIX RP-1000 SEPARATION AFTER IDES PROTEOLYSIS AND DTT REDUCTION

Column: Proteomix® RP-1000 (5 µm, 1000 Å, 2.1 x 100 mm)
Mobile phase: A: 0.1% TFA in water; B: 0.1% TFA in 100% ACN;
Flow rate: 0.3 mL/min; Detector: UV 214 nm;
Column temperature: 78 ˚C; Gradient: 2-22 min 30%-45% B;
Injection: 5 µg intact and DTT reduced Vectibix