Analytical Characterization of Monoclonal Antibody IgG2

Vectibix (PanitumumAb)

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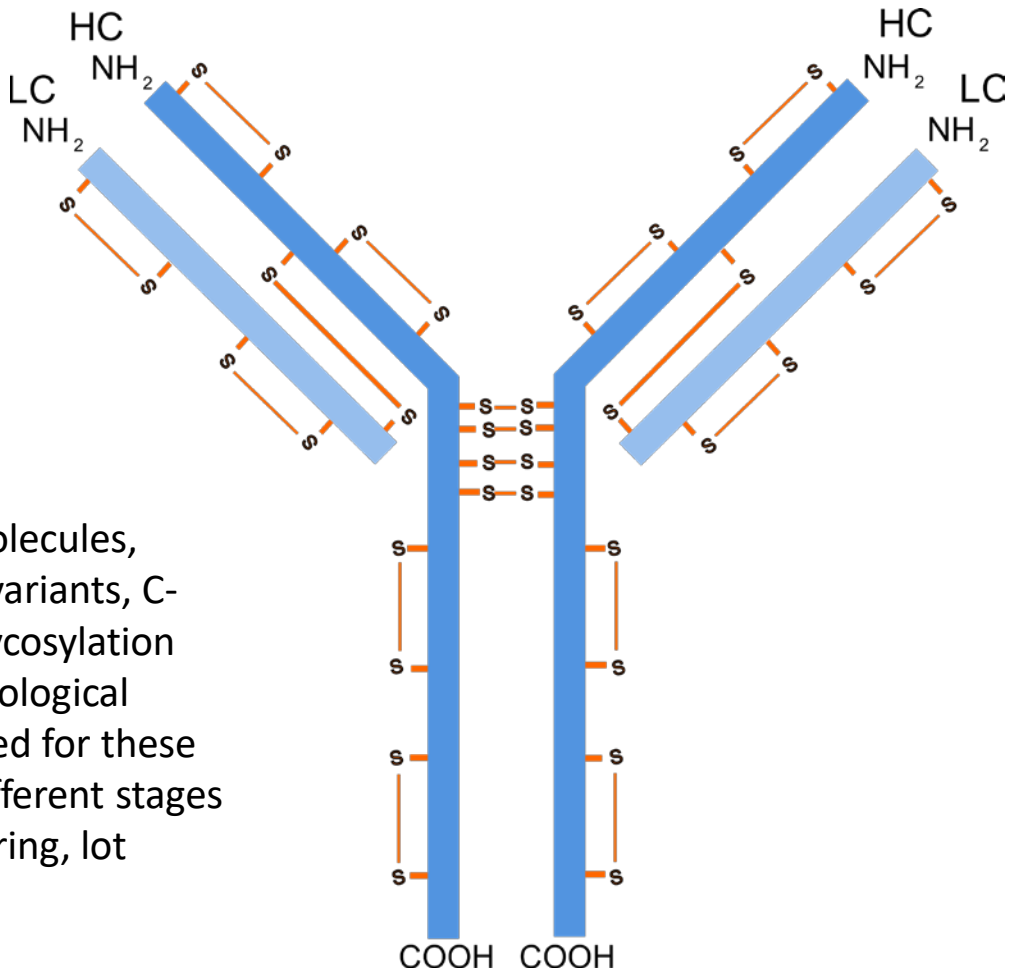
Monoclonal antibodies

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.



IgG2 Structure

Introduction

Vectibix is a fully human mAb IgG2 specific to the epidermal growth factor receptor (EGFR). In this application note, we would like to present the IgG2 Vectibix (panitumumab) characterization in a few different chromatographic areas:

- **SEC:** 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.

- **SCX:** 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.

- **HIC:** 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.

- **RP:** 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

Samples:

Monoclonal Antibody Vectibix IgG2 and Erbitux IgG1

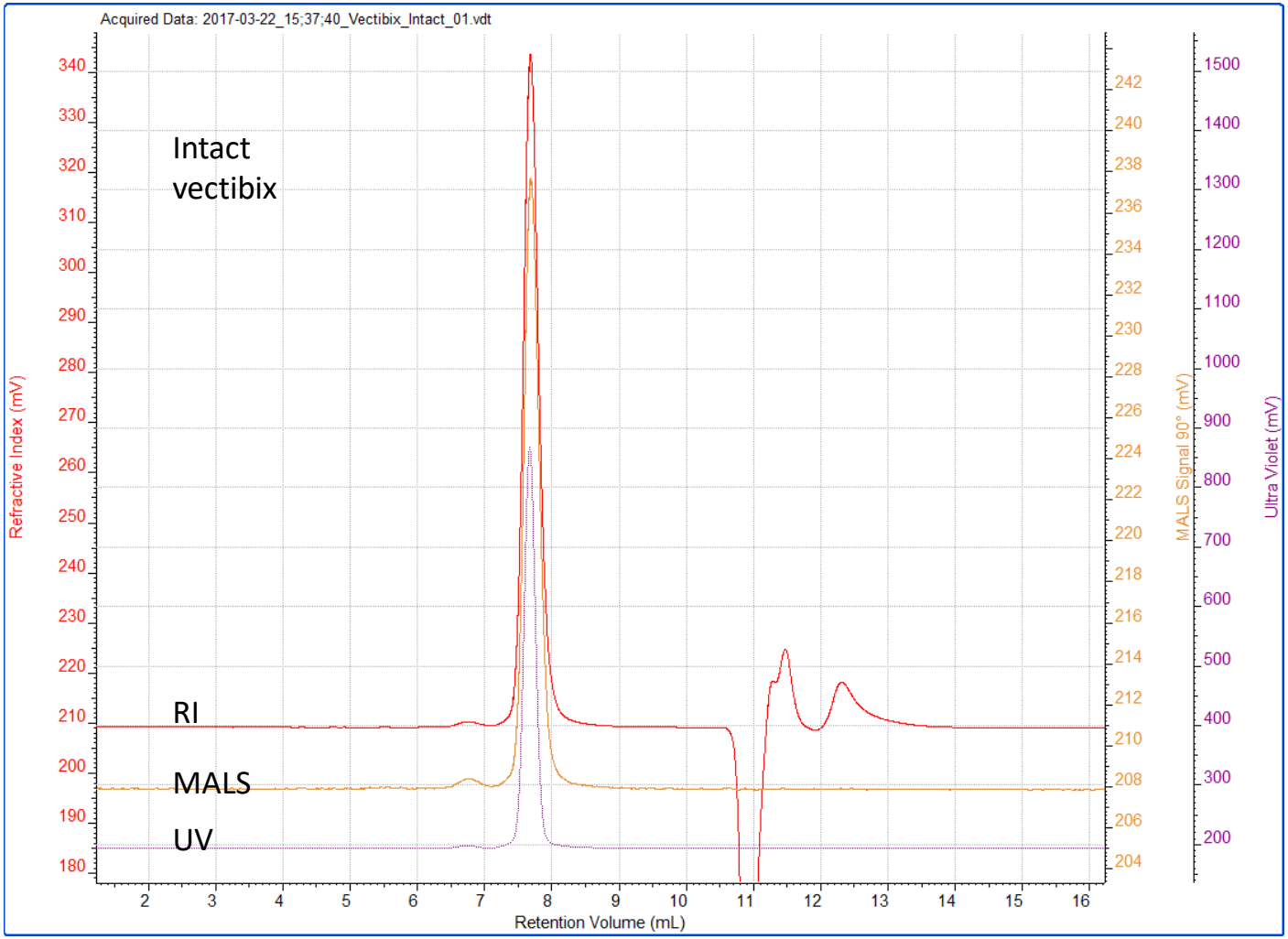
Columns:

- Zenix®-C SEC-300-LS (3 µm, 300 Å, 7.8 x 300 mm, PN: 233300LS-7830)
- Unix™-C SEC-300 (1.8 µm, 300 Å, 4.6 x 300 mm, PN: 231300-4630)
- Proteomix® SCX NP5 (5 µm, NP, 2.1 x 250 mm, PEEK, PN: 401NP5P-2125)
- Proteomix® HIC Butyl-NP5 (5 µm, NP, 4.6 x 50 mm, PN: 431NP5-4605)
- Proteomix® RP-1000 (5 µm, 1000 Å, 2.1 x 100 mm, PN: 465950-2110)

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



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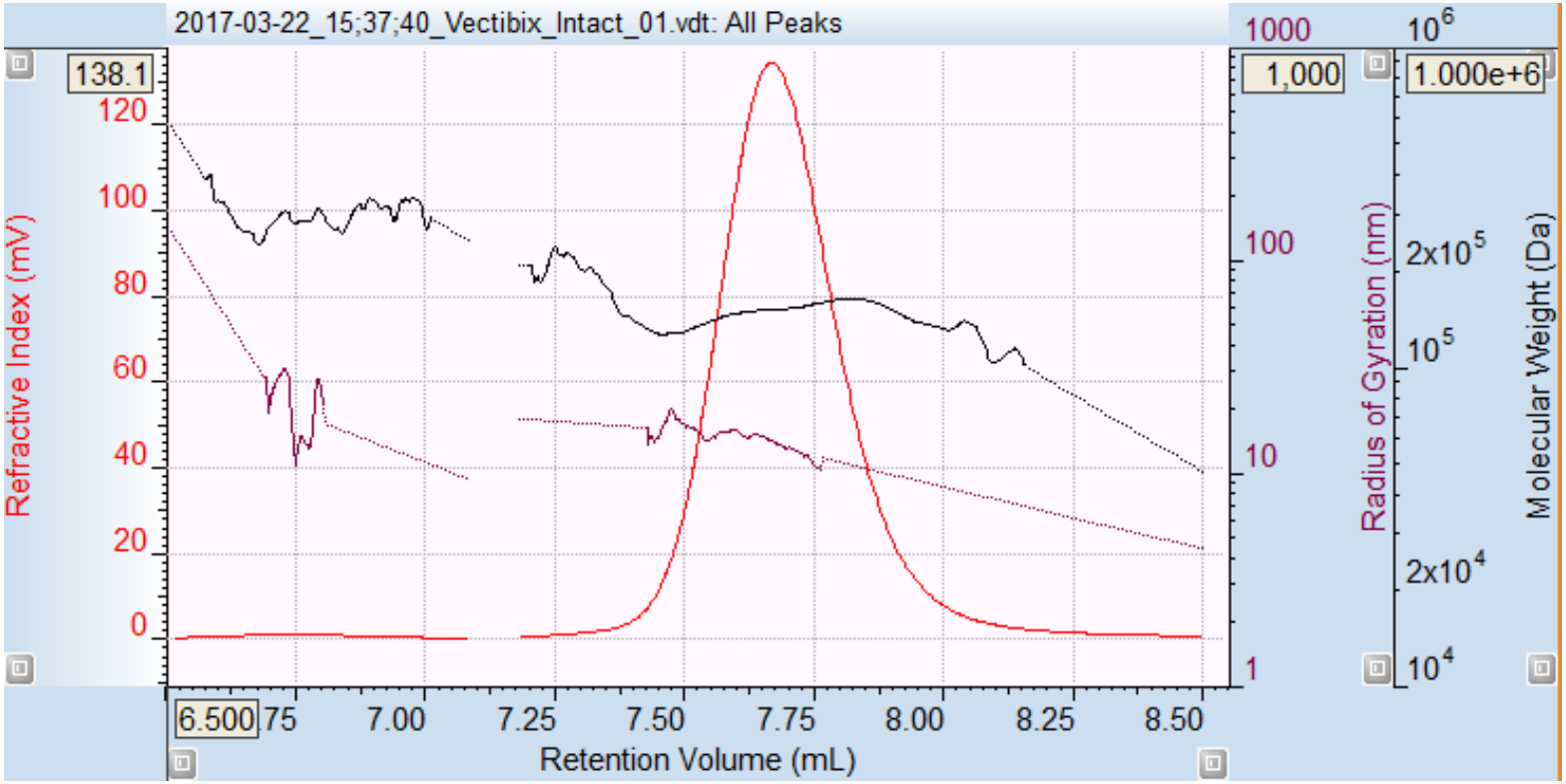
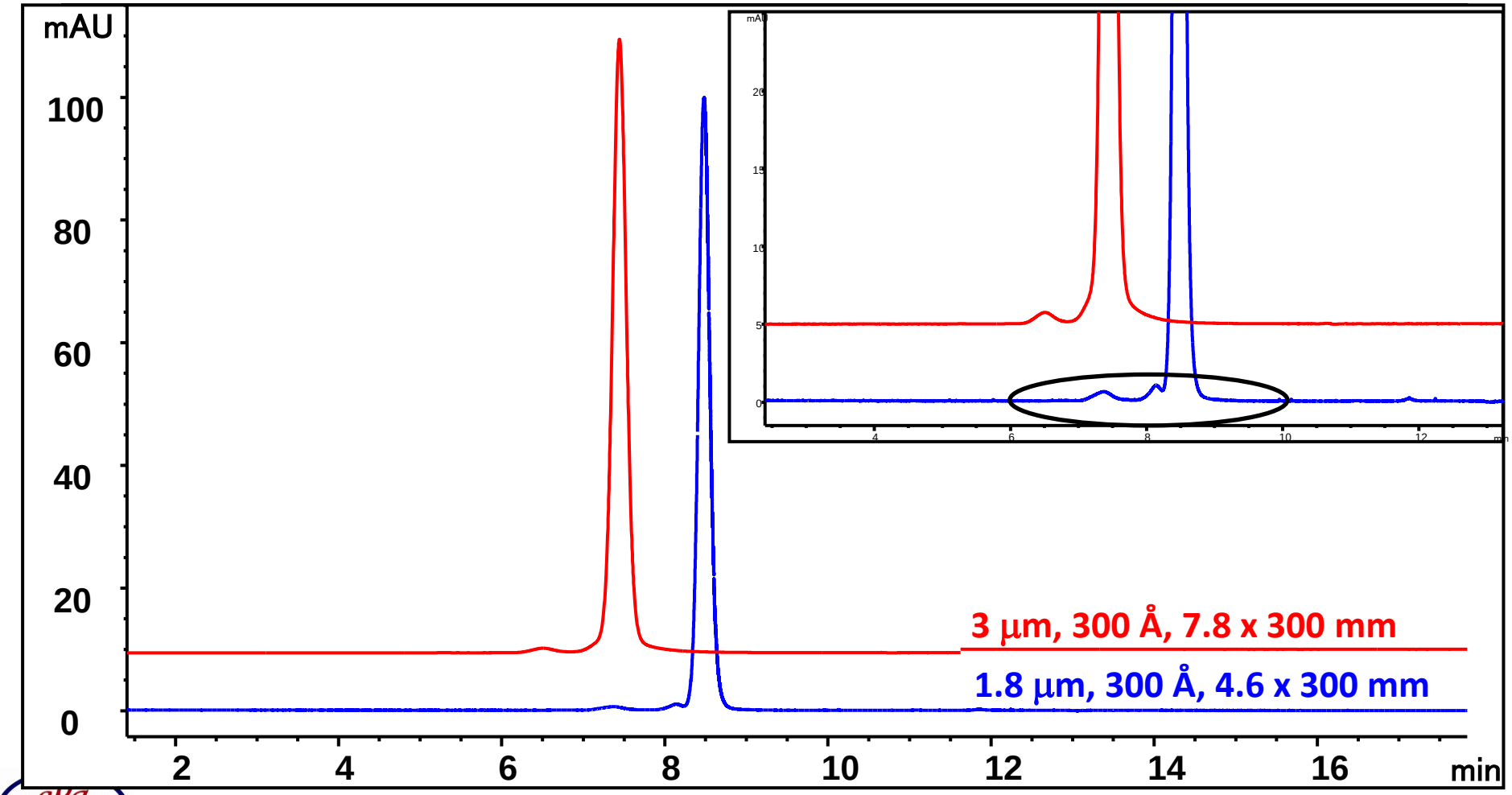


Light Scattering Results:

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

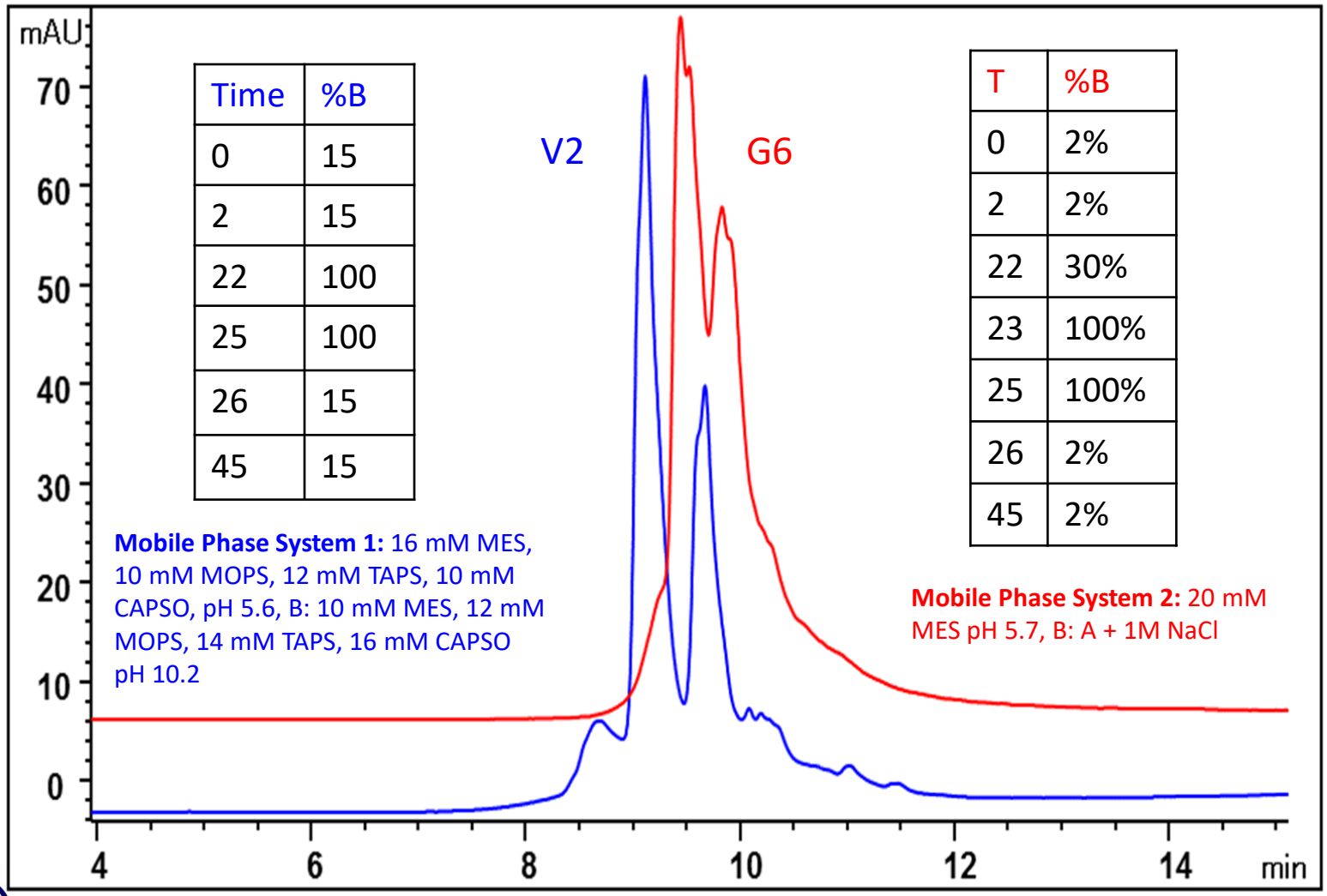
Unix-C 1.8 µm vs. Zenix-C 3 µm SEC-300 on Vectibix

Column: Unix™-C SEC-300 (1.8 µm, 300 Å, 4.6 x 300 mm) and Zenix-C SEC-300 (3 µm, 300 Å, 7.8 x 300 mm)
Mobile phase: 150 mM phosphate buffer, pH 7.0
Flow rate: 0.3 mL/min
Column temperature: RT
Samples: 20 mg/mL Vectibix 50 mM sodium acetate 100 mM NaCl, IgG2



Charge Variants Separation of IgG2 with Proteomix SCX

Column: Proteomix® SCX NP5 (5 µm, 2.1×250 mm, PK)
Temperature: 25°C
Flow Rate: 0.3 mL/min
Sample: Vectibix 5 mg/mL
Detection: UV 280 nm
Injection Volume: 2 µL



Time	%B
0	15
2	15
22	100
25	100
26	15
45	15

Mobile Phase System 1: 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

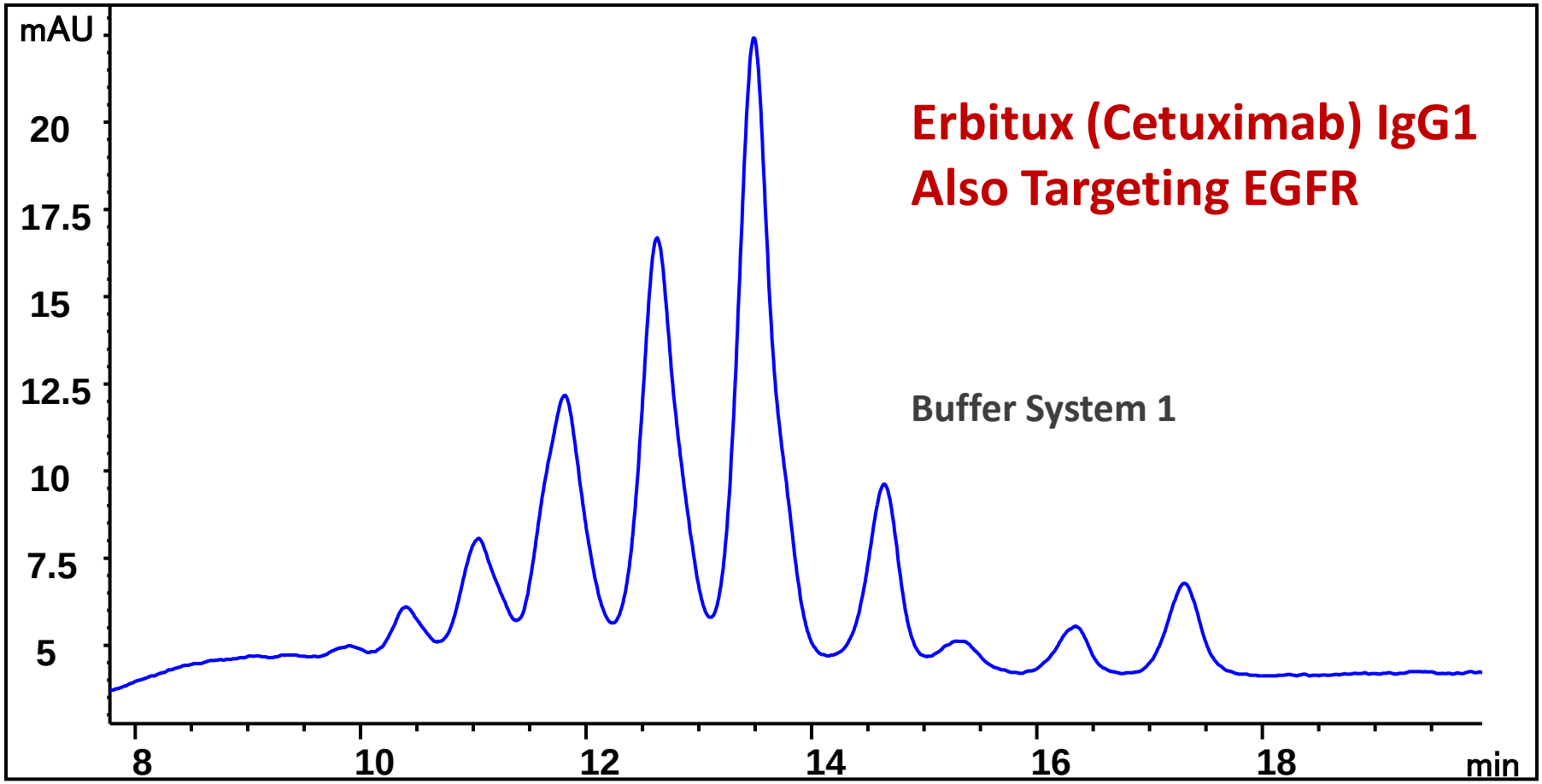
T	%B
0	2%
2	2%
22	30%
23	100%
25	100%
26	2%
45	2%

Mobile Phase System 2: 20 mM MES pH 5.7, B: A + 1M NaCl

Charge Variants Separation of IgG1 with Proteomix SCX

EM1007

Column: Proteomix® SCX NP5 (5 µm, 2.1×250 mm, PK)
Temperature: 25°C
Flow Rate: 0.3 mL/min
Sample: Vectibix 5 mg/mL
Detection: UV 280 nm
Injection Volume: 2 µL



Hydrophobic Interaction Chromatography
Erbitux IgG1 vs. Vectibix IgG2

EM1007

Column: Proteomix® HIC Butyl-NP5 (4.6 x 50 mm)

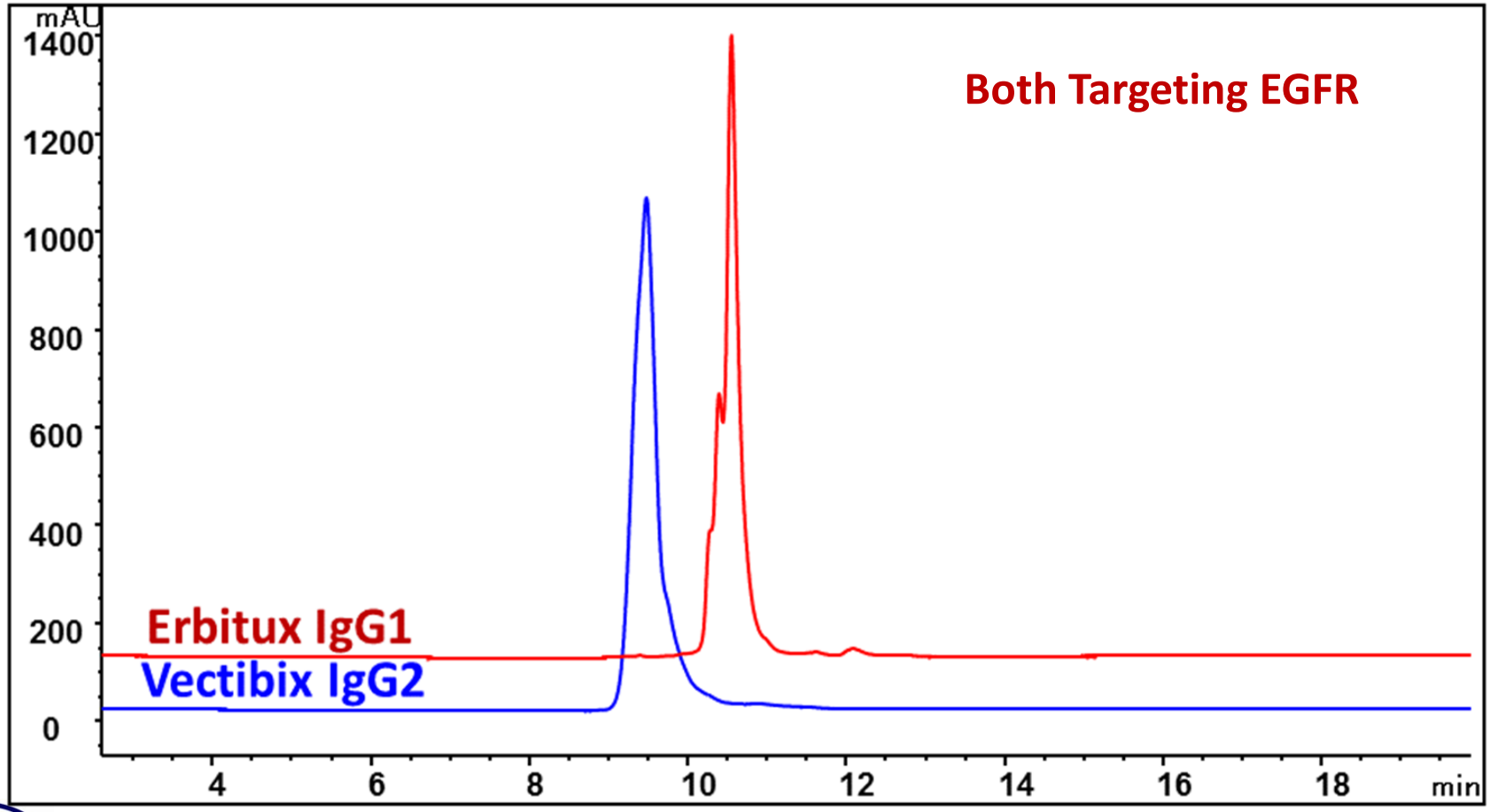
Flow rate: 1 mL/min

Detector: UV 214 nm

Column temperature: 25 ℃

Mobile phase: A: 2.0 M ammonium sulfate in 100 mM sodium phosphate, pH 7.0, B: 100 mM sodium phosphate pH 7.0,

Injection:10 µg mAb



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Reversed phase Proteomix RP-1000 separation after IDES proteolysis and DTT reduction

EM1007

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

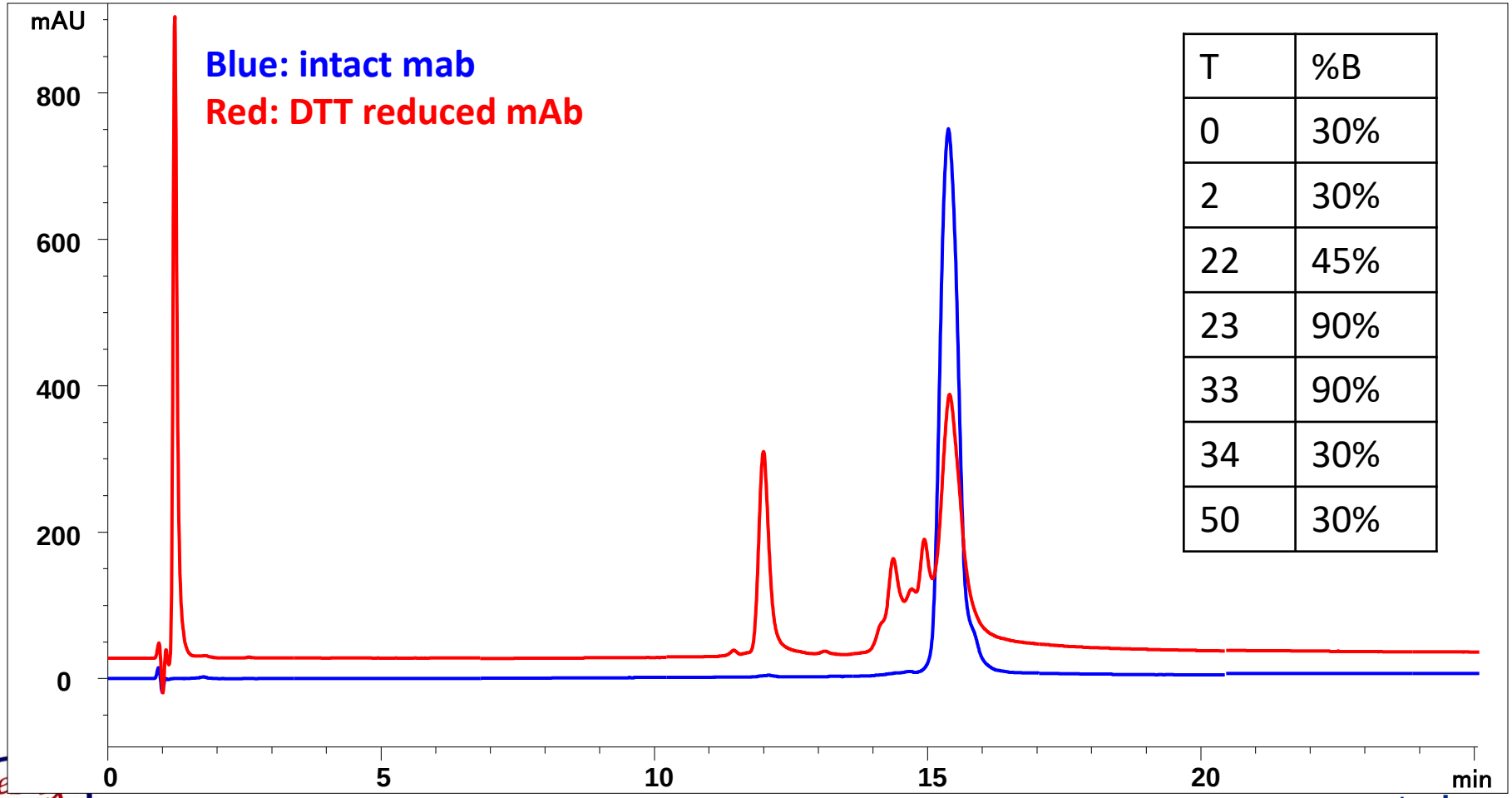
Flow rate: 0.3 mL/min

Detector: UV 214 nm

Column temperature: 78 ˚C

Gradient: 2-22 min 30%-45% B

Injection: 5 mg intact and DTT reduced Vectibix



T	%B
0	30%
2	30%
22	45%
23	90%
33	90%
34	30%
50	30%

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. It also exhibits extreme low shedding with high SEC resolution.
- Unix-C Sub 2 µm 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 by Proteomix RP-1000 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.