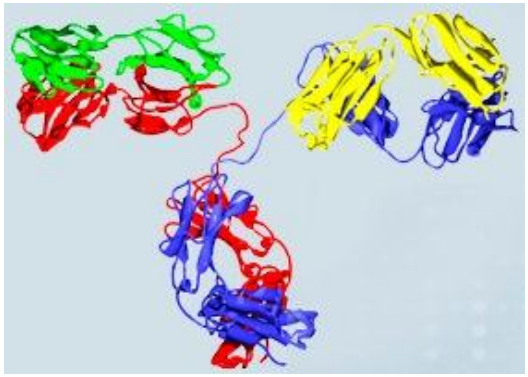
Antibody Solution Kit for Separation and Characterization of Monoclonal Antibodies



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Agenda

- Introduction to Antibody solution Kit (Zenix-300, Antibodix WCX and Bio-C8)
- Zenix-300 SEC
  - Intact monoclonal antibody (MAb) separation
  - Heavy light chain separation of reduced Mab
  - On-line SEC-MS for direct mass detection of heavy and light chains.
- Antibodix WCX for MAb variants study
- Bio-C8-alternative LC for MAb fragment charaterization

Zenix-300 (3 μm, 300 Å) for intact monoclonal antibody analysis

Technical specifications of Zenix™ 7.8x300 mm

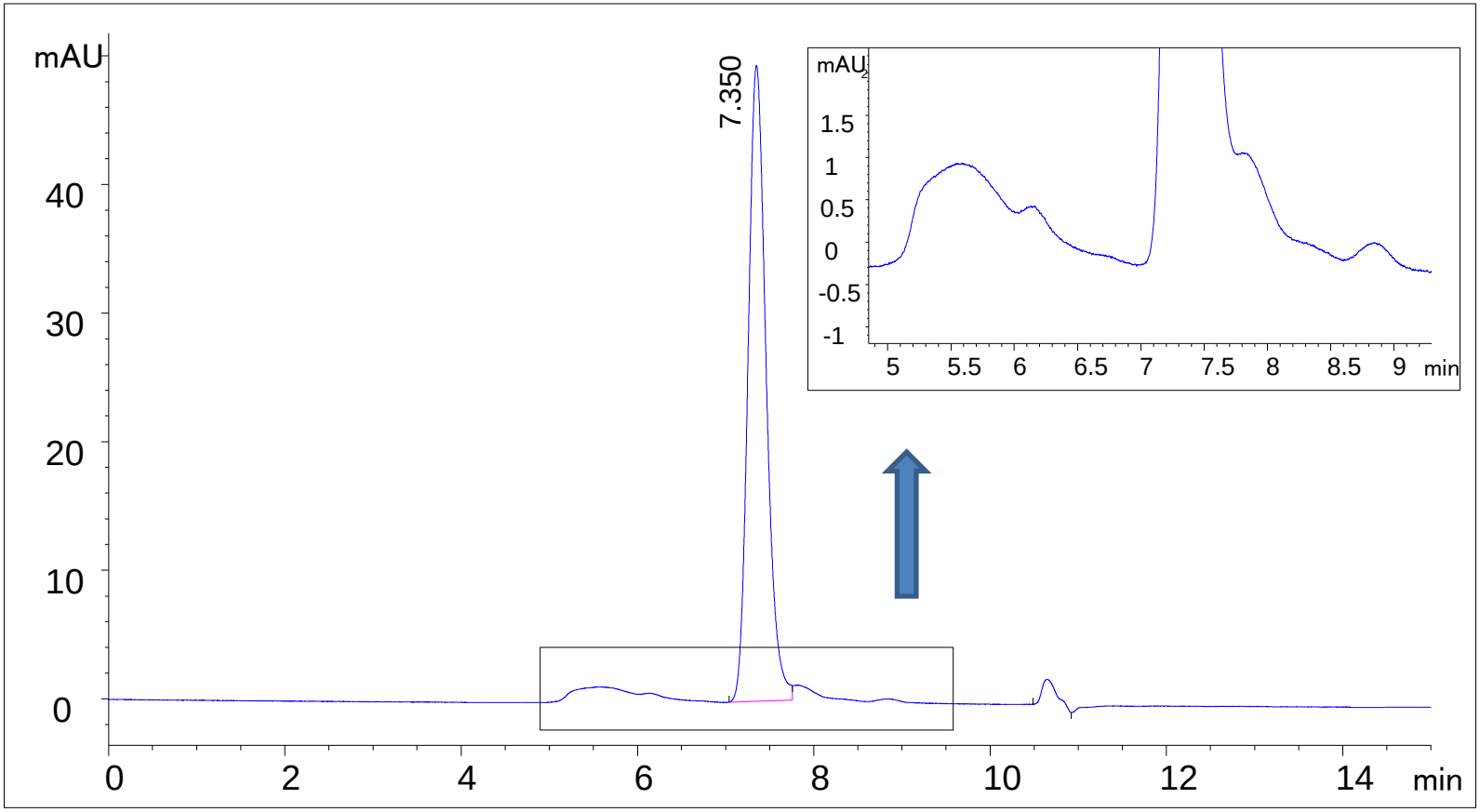
Phase	Zenix™ 300
Material	Neutral, hydrophilic film bonded silica
Particle size	3 µm
Pore size (Å)	~ 300
Protein MW range (native)	5,000 – 1,250,000
pH stability	2 – 8.5 (pH 8.5-9.5 can be tolerated temporarily)
Standard flow rate	1 mL/min
Backpressure for 7.8x300 mm (1.0 mL/min)	~ 1,100 psi
Maximum back pressure (psi)	~ 3,500
Salt concentration range	20 mM - 2.0 M
Maximum temperature (°C)	~ 80
Mobile phase compatibility	Aqueous and organic

MAb 321 separation on Zenix™-300

Column: Zenix SEC-300 (3µm, 7.8x300mm)

Mobile phase: 150 Mm Sodium Phosphate, pH 7; Flow rate: 1.0 mL/min

Detection: 280 nm; T=Ambient; Sample: MAb (1 mg/mL); Injection: 10 µL



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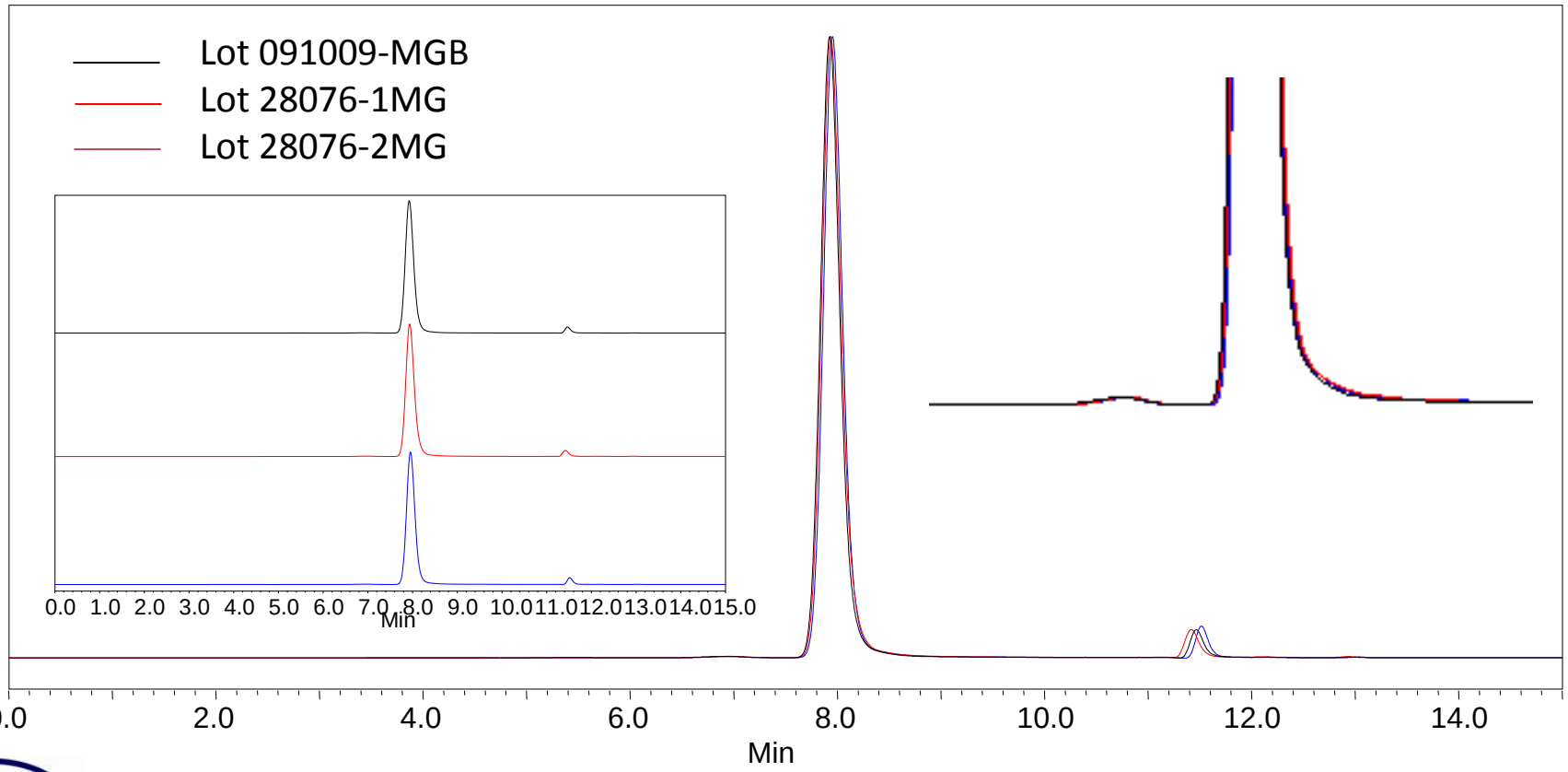
Zenix Column Lot-to-Lot Reproducibility for MAb

Column: Zenix SEC-300 (3μm, 7.8x300mm)

Mobile phase: 150 Mm Sodium Phosphate, pH 7; Flow rate: 1.0 mL/min

Detection: 214 nm; T=Ambient; Sample: MAb (2.5 mg/mL); Injection: 10 μL



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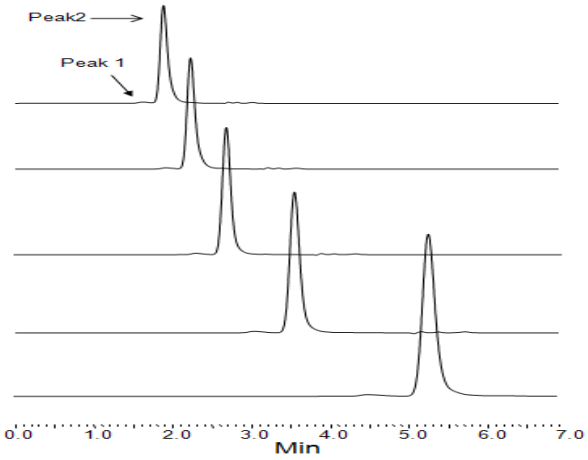
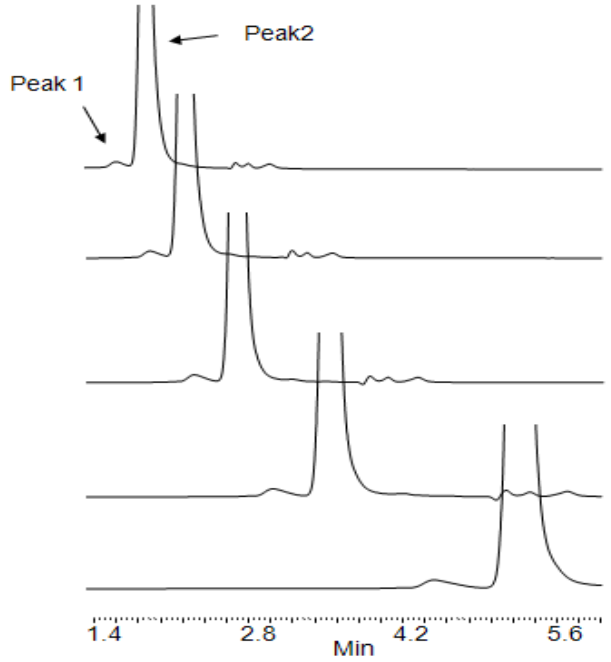
FAST Mab Separation – Zenix 300, 7.8x200mm





Test	Flow rate (mL/min)	Backpressure (psi)	Retention time (min)		% Area		USP Resolution
			Peak 1	Peak 2	Peak 1	Peak 2	
A	1.0	550	4.51	5.28	1.38	97.92	1.9
B	1.5	1020	3.04	3.55	1.36	97.84	1.6
C	2.0	1550	2.23	2.71	1.31	97.20	1.6
D	2.5	2050	1.92	2.23	1.38	97.02	1.5
E	3.0	2450	1.62	1.89	1.26	96.92	1.3

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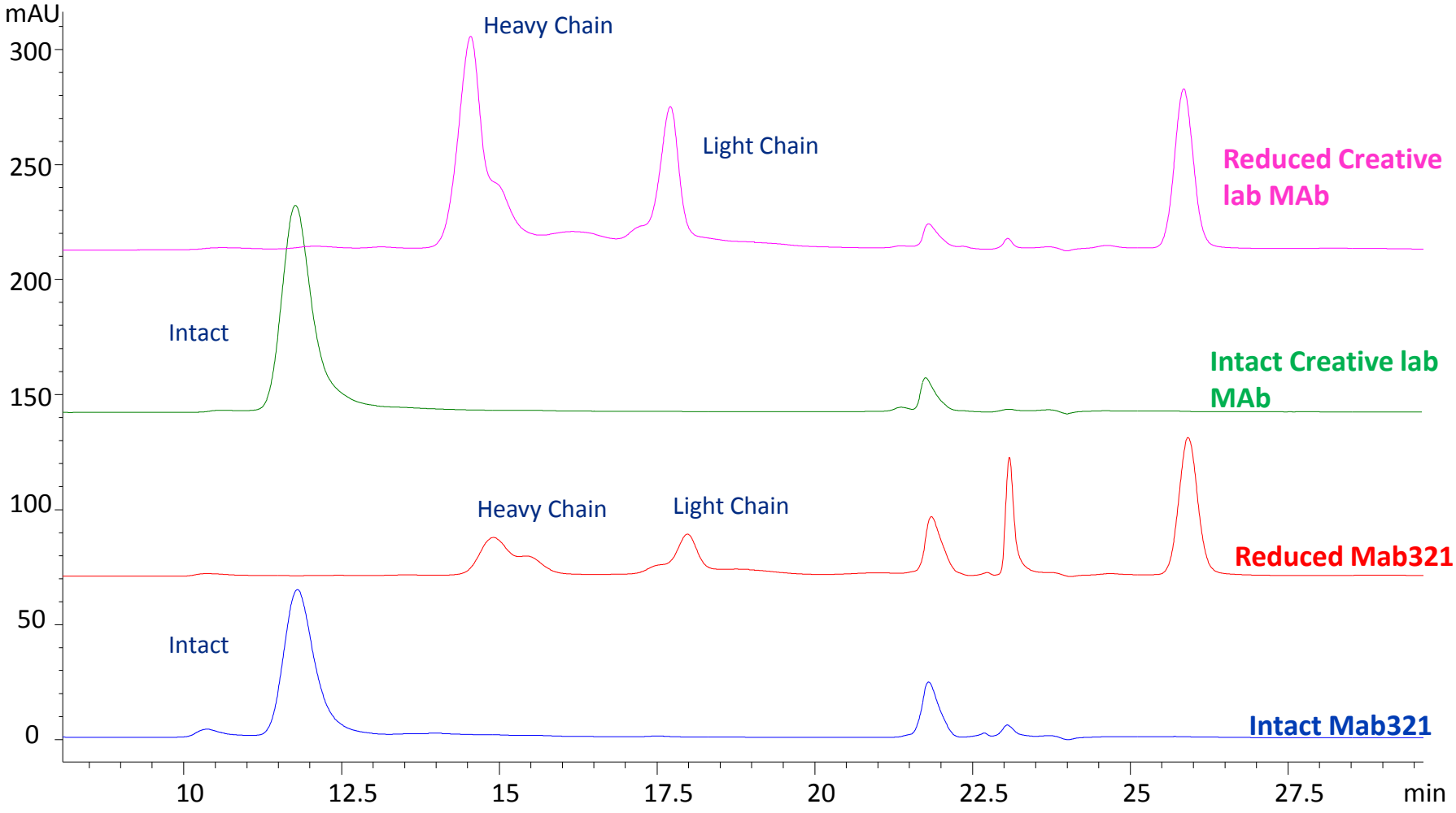
Zenix-300 (3 μm, 300 Å) for heavy light chain separation with direct mass spec detection

Light/Heavy chain MAb separation on Mab321 and Creative Lab MAb DTT reduced

- MAb321 (5 mg/mL) and Creative Lab MAb (10 mg/mL)
  - Dilute to 1mg/mL with 150mM PB, pH 7.0, add 0.5 M DTT to final 20 mM DTT
  - A control buffer (150mM PB and 20mM DTT)
  - Incubate DTT samples at 65 ^{o}C for 15 minutes
  - Inject 20µl each sample
  - HPLC running condition:
    - Column Zenix-300 (3 µm, 300Å 7.8x300 mm)
    - UV 280nm, Mobile phase: 0.1% TFA, 0.1% formic acid, 20% acetonitrile

Overlays for Mab321 and Creative lab MAb on Zenix-300



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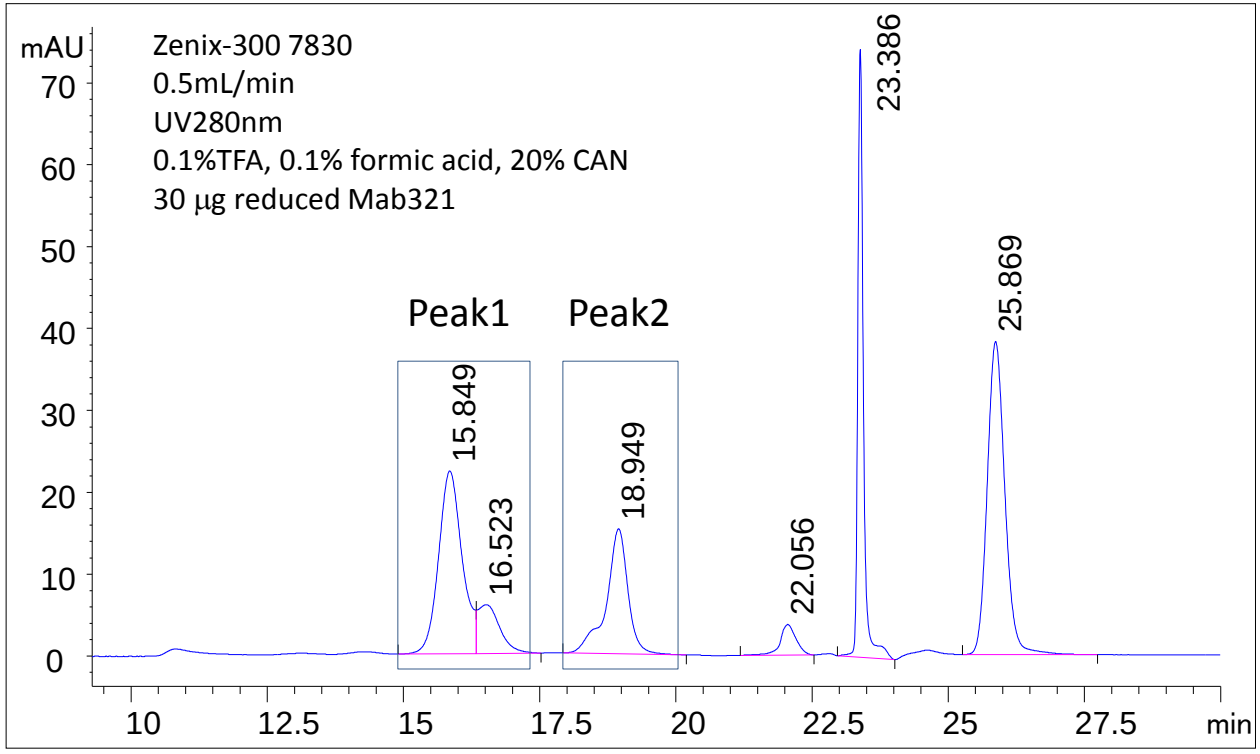
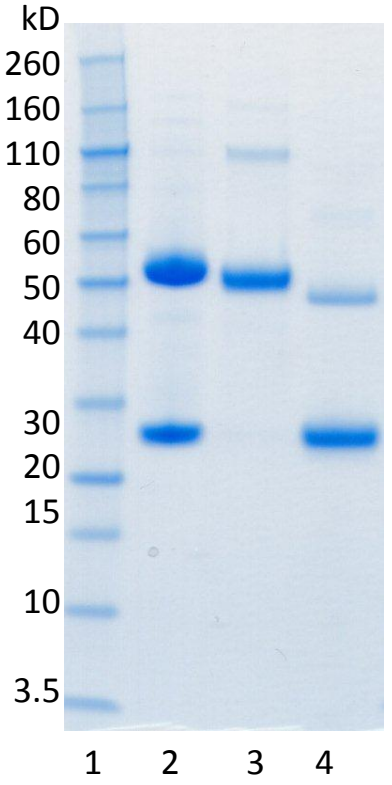
Heavy and light chain of Mab321 on Zenix-300

1. Peak 1 and Peak 2 were collected and dried with speed vac.
2. Re-dissolve in SDS-PAGE gel sample buffer and run gel.



Heavy chain 50 kD, light chain 25 kD



1. Protein marker
2. Reduced Mab321
3. Peak 1 heavy chain
4. Peak 2 light chain

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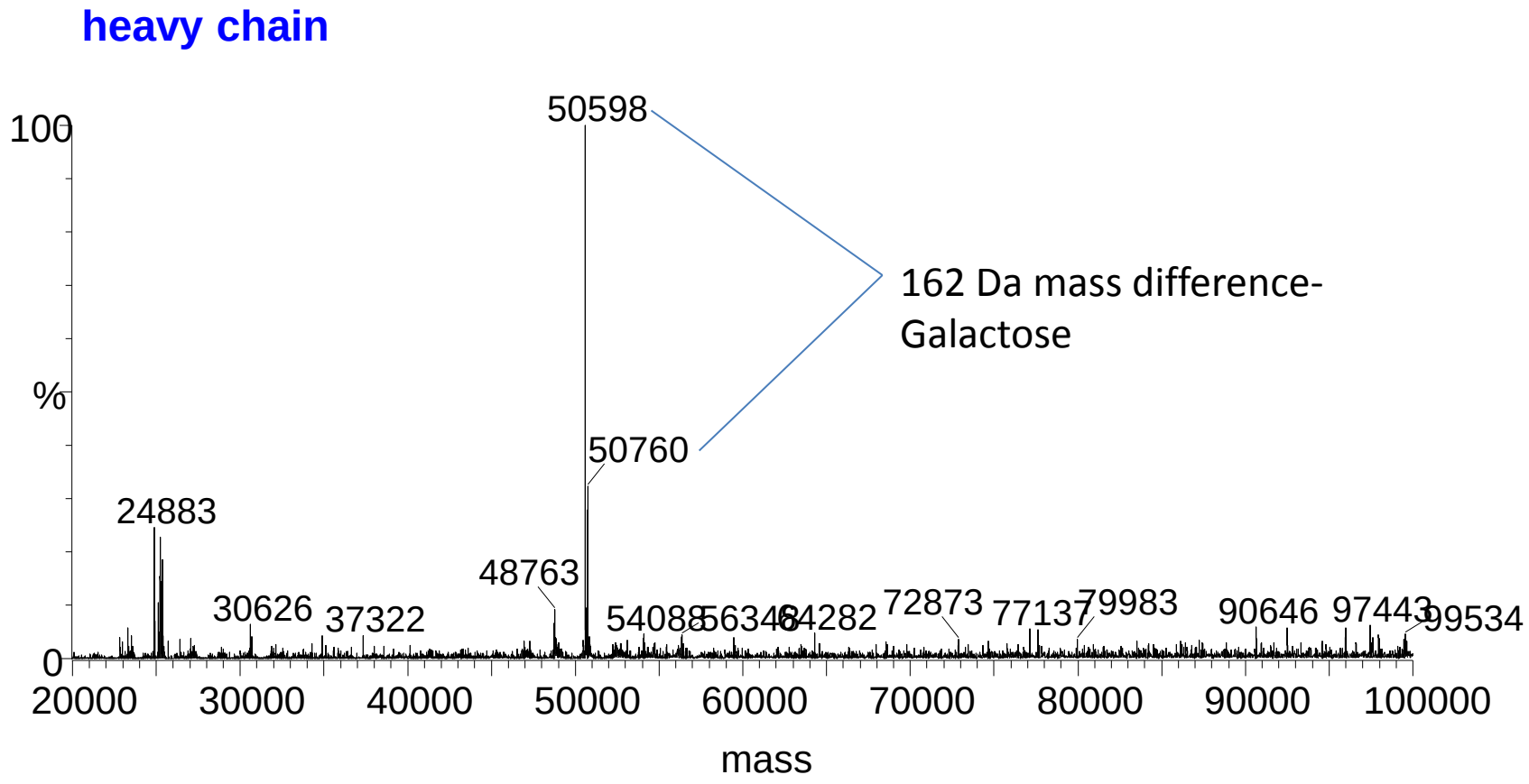
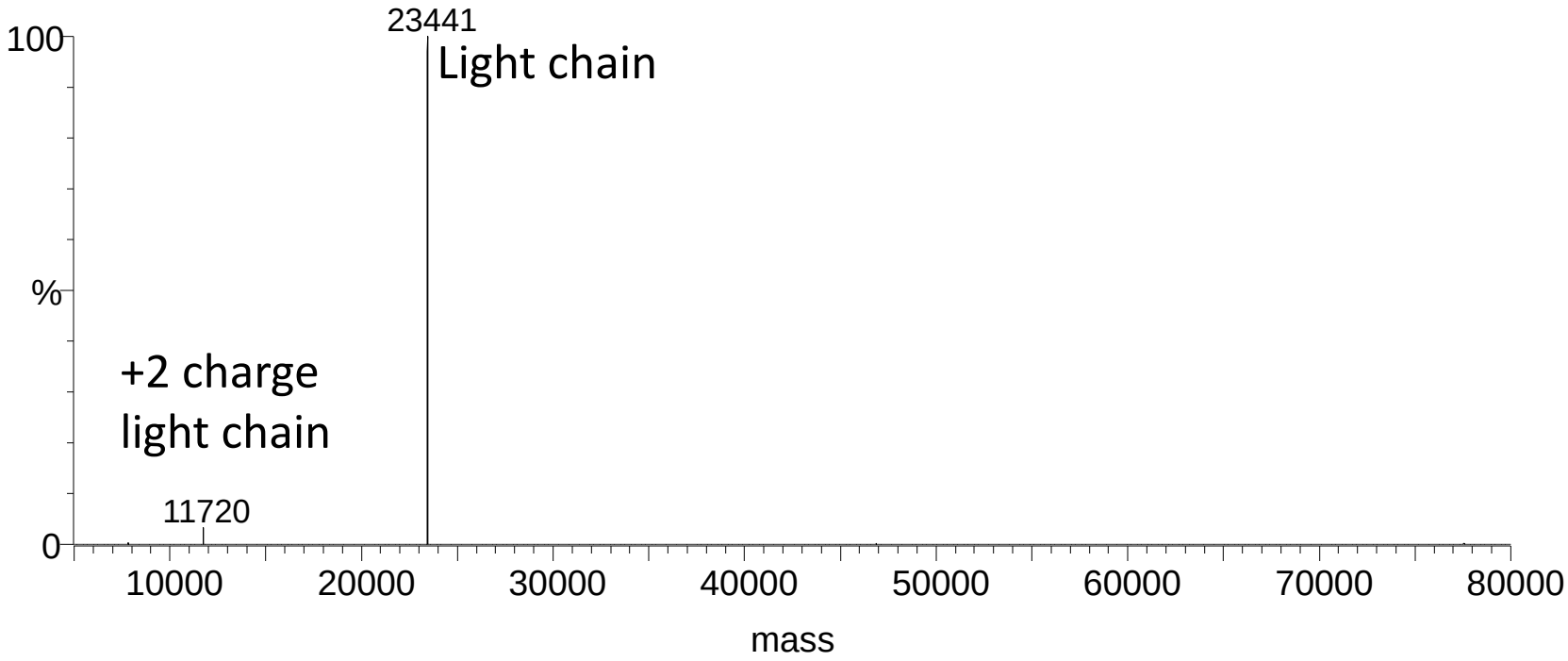
Peak 1 Heavy chain mass spec



Peak 2 Light chain mass spec

light chain

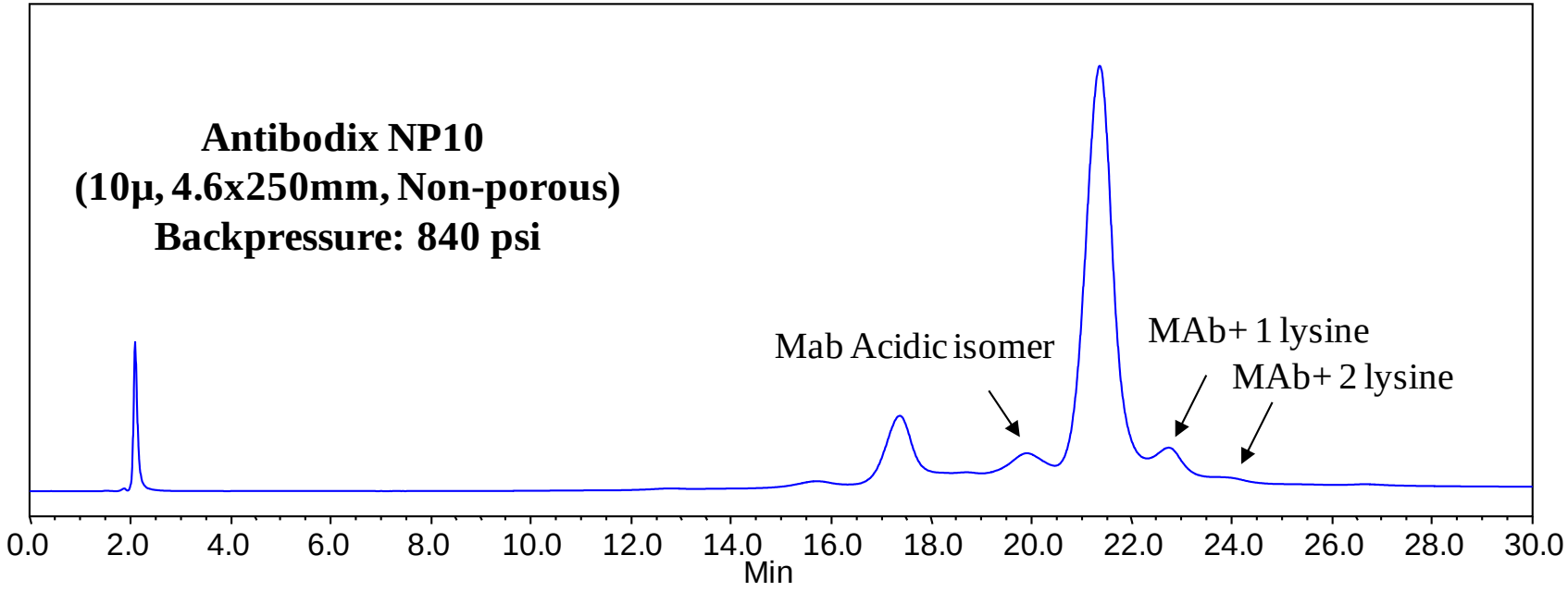


Antibodix WCX NP10 for intact MAb variants separation

Technical specifications of Antibodix™ WCX NP10 4.6 x 250 mm

Phase	Weak cation exchanger with carboxylate functional groups chemically bonded and hydrophilic
Material	Highly cross-linked non-porous PS/DVB particle
Particle size	10 µm
Pore size (Å)	non-porous
pH stability	2 – 12
Standard flow rate	0.8 mL/min
Backpressure for 4.6 x 250 mm (0.8 mL/min)	~ 950
Maximum back pressure (psi)	~ 4,000
Maximum temperature (°C)	~ 80
Mobile phase compatibility	Aqueous and organic

Analysis of MAb with Charge Variance on Antibodix™ NP10 (10 µm, 4.6x250 mm)



Mobile Phases: A, 10 mM phosphate buffer, pH 7.5; B, A + 100 mM NaCl
Gradient: 15-55% B (30 min)
Flow Rate: 0.8 mL/min
Detection: UV 214 nm
Temperature: Ambient
Sample Concentration: 5 mg/mL
Injection Volume: 5 µL
Sample: Monoclonal antibody

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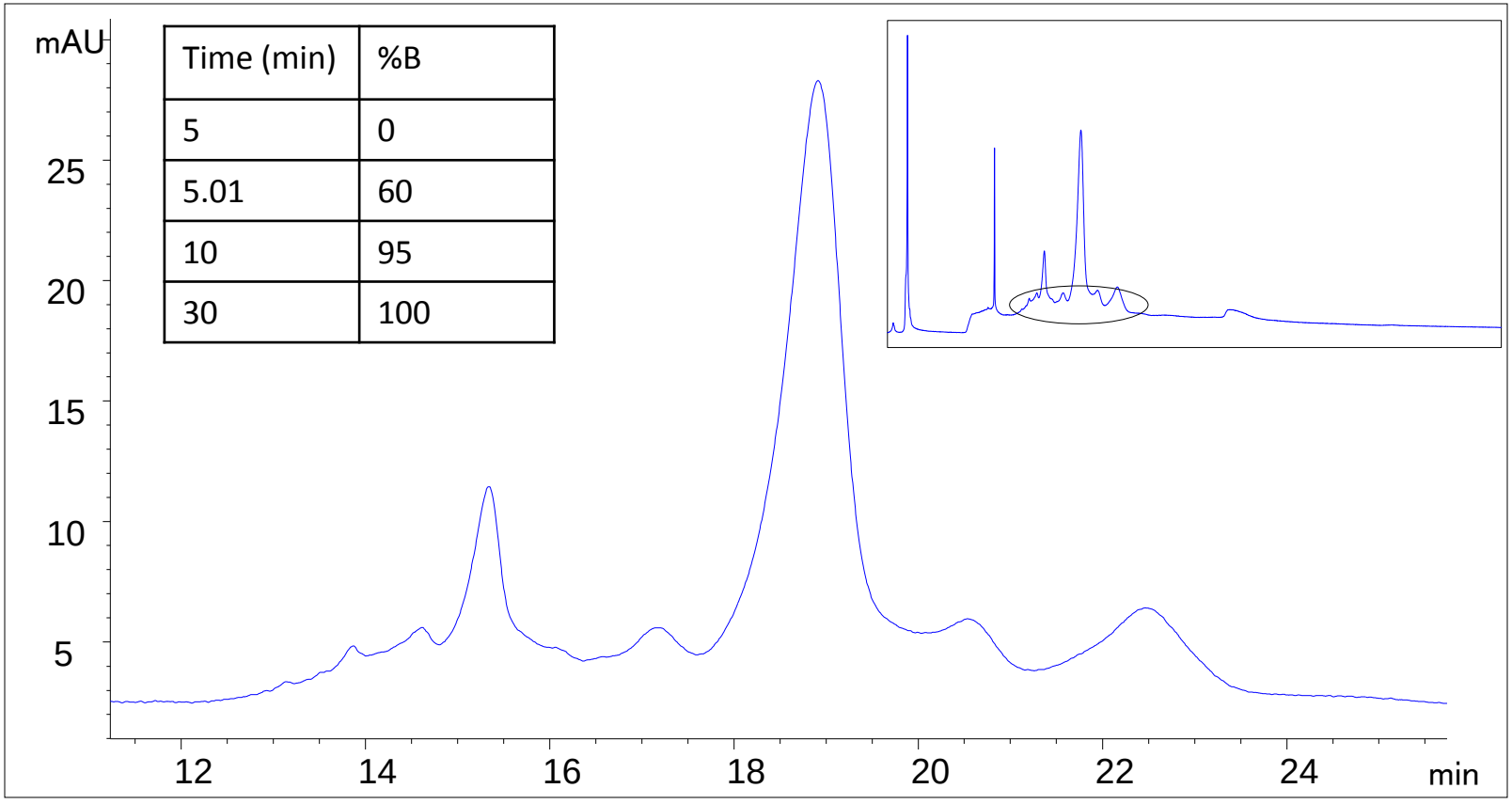
Mab 321 separation on Antibodix WCX-NP10 4.6 x 250 mm

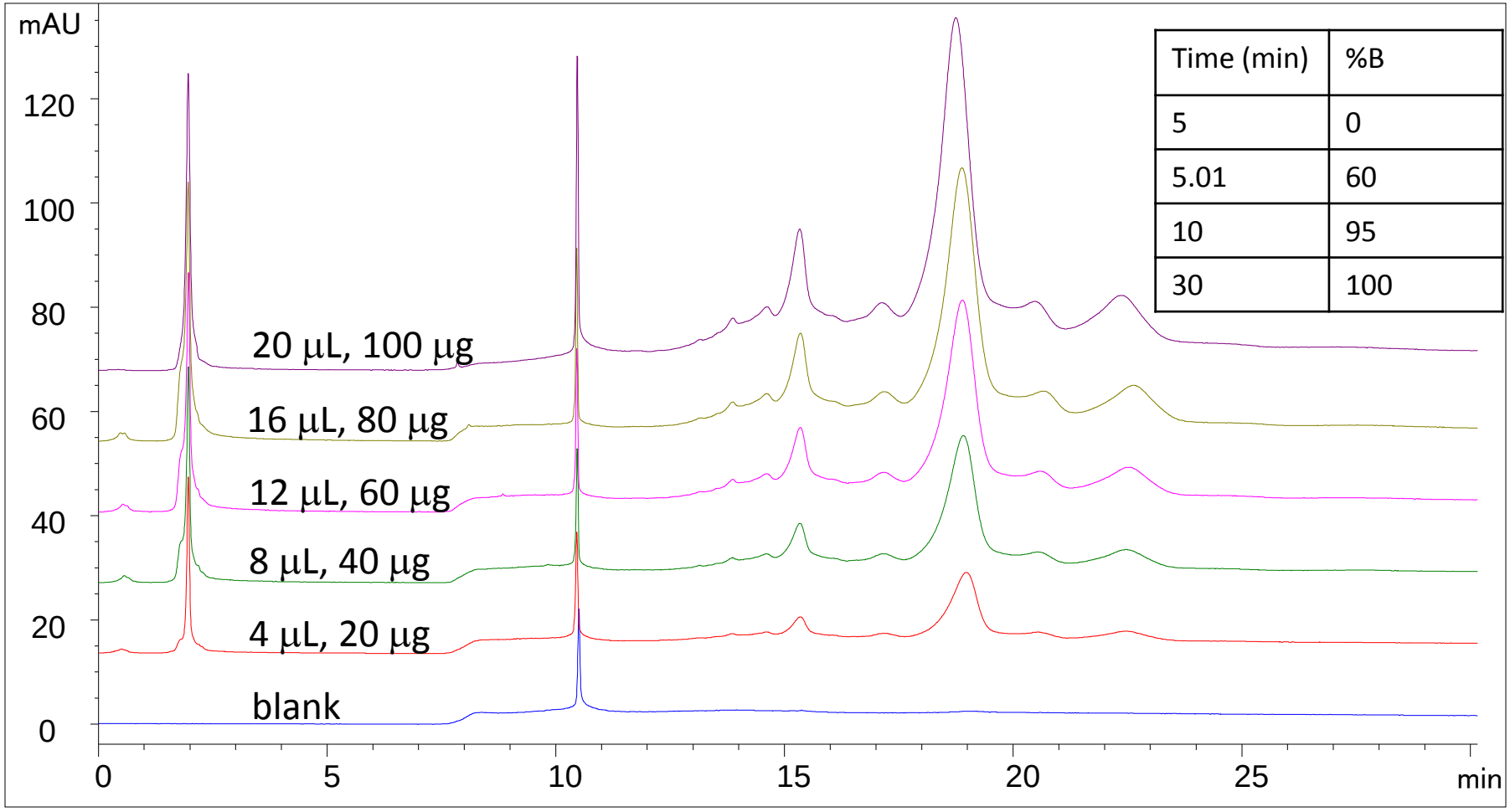
Mobile phase A: 20 mM phosphate buffer, pH 5; B: 20 mM phosphate buffer + 10 mM NaCl, pH 7.5
UV 280 nm,
Sample: Mab 321 5 mg/mL in tris buffer ; Injection: 12 µL, 60µg
Flow rate: 0.8 mL/min

Time (min)	%B
5	0
5.01	60
10	95
30	100



Different Mab321 loading on Antibodix WCX-NP10 4625

Mobile phase A: 20 mM phosphate buffer, pH 5; B: 20 mM phosphate buffer + 10 mM NaCl, pH 7.5
UV 280 nm, Sample: MAb 321 5 mg/mL in tris buffer
Flow rate: 0.8 mL/min



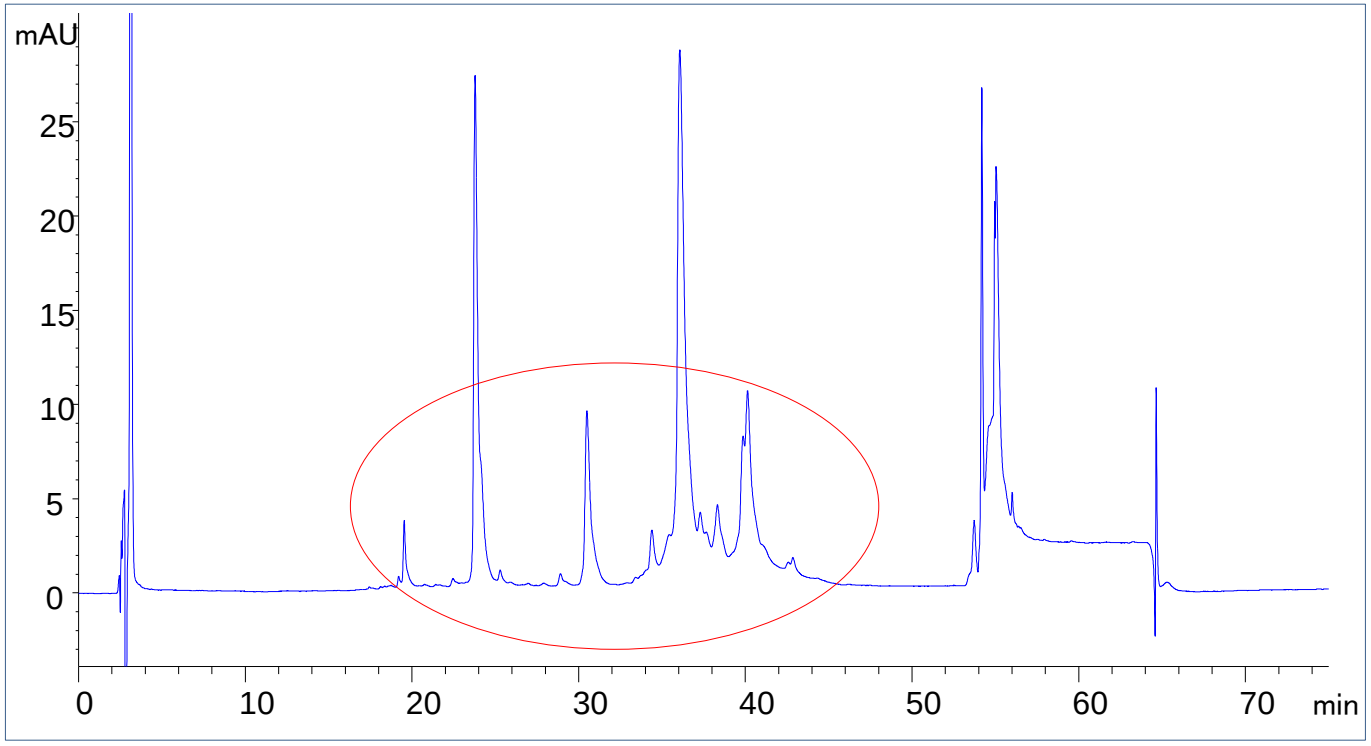
Time (min)	%B
5	0
5.01	60
10	95
30	100

Bio-C8 LC separation of heavy light chains of MAb

Mab321 heavy light chains separation on Bio-C8 4625 300Å

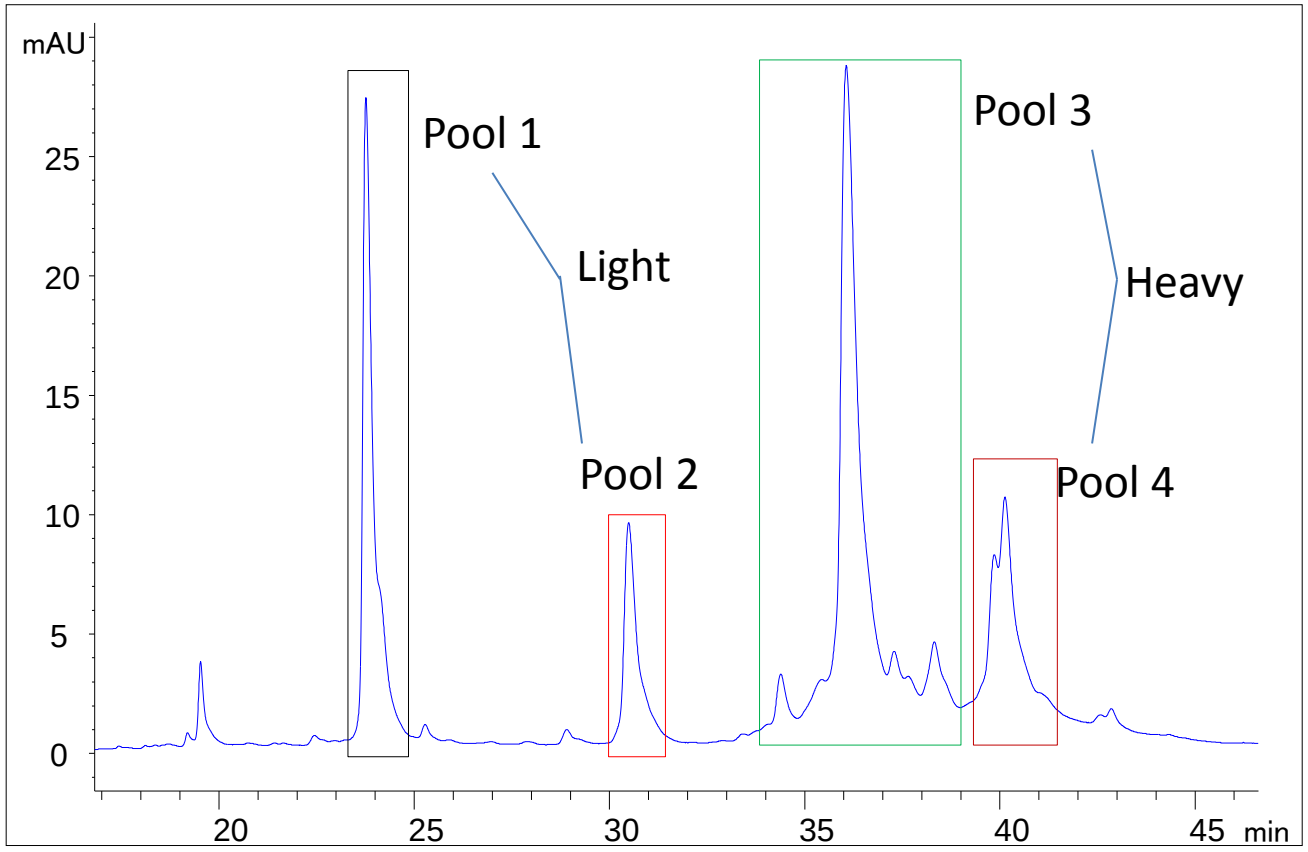
Time	Flow(ml/min)	%A	%B
0	0.5	75	25
5	0.5	75	25
15	0.5	68	32
50	0.5	62	38
50.1	0.5	5	95
60	0.5	5	95
60.1	0.5	75	25
75	0.5	75	25

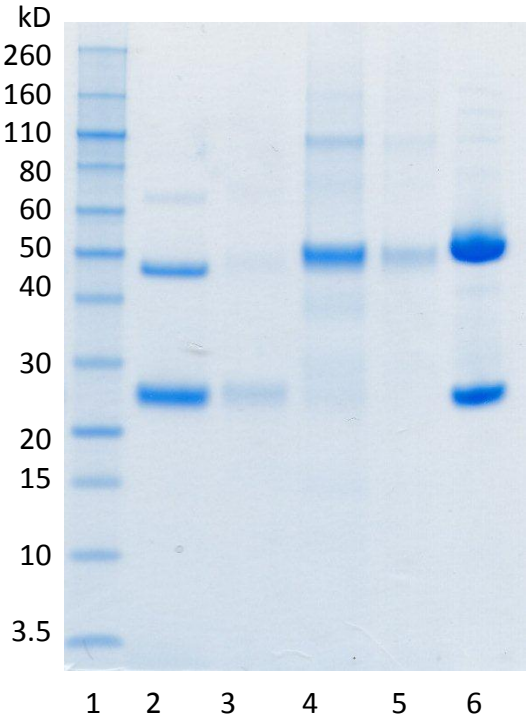
Column: Bio C8 4.6x100 (3µm, 300**Å**, 4.6x100mm)
Mobile Phase A: 0.11% TFA in water
Mobile Phase B: 0.09% TFA in ACN
Flow: 0.5 mL/min
Column Temperature: 75degrees
Detection: UV 280nm
Sample: 30 µg MAb 321



Zoom view next page

Zoom view from Bio-C8 run





1. Protein marker
2. Pool 1 (light chain)
3. Pool2 (light chain)
4. Pool 3(heavy chain)
5. Pool 4 (heavy chain)
6. Reduced Mab 321

Heavy chain 50 kD, light chain 25 kD

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Conclusion

- Sepax size exclusion column Zenix-300 (3 μm) provides a high resolution separation of intact monoclonal antibody monomer, aggregates and fragments under native conditions.
- Antibodix NP10 weak cation exchange column is suitable for MAb charge heterogeneity analysis using optimized salt or pH gradient.
- Sepax size exclusion chromatography Zenix-300 (3 μm) successfully separated reduced monoclonal antibody into heavy and light chains according to their molecular weights using volatile buffer 0.1% TFA, 0.1% formic acid and 20% acetonitrile.
- With reduced flow rate, on-line SEC (Zenix-300)-MS generated accurate masses for heavy and light chains.
- On-line SEC-MS with volatile buffers can be applied to general protein separation and mass detection, a complementary work flow to RP-HPLC-MS.

Acknowledgement

Sepax Technologies:

Haiying Chen

Katherine McGlaughlin

Dr. Ke Yang

Na Shao

University of Delaware:

Dr. Stephen Chan