Comparison of Binding Capacity of TOYOPEARL® AF-rProtein A HC-650F Affinity Resin at Varying Bed Heights and Constant Column Volume

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Introduction

- Protein A affinity chromatography most often serves as the initial capture step and is typically the most expensive step in the downstream production of mAb biotherapeutics.

- TOYOPEARL AF-rProtein A HC-650F affinity resin utilizes a recombinant, alkaline stable protein A ligand with increased dynamic binding capacity and excellent pressure-flow characteristics.

Introduction

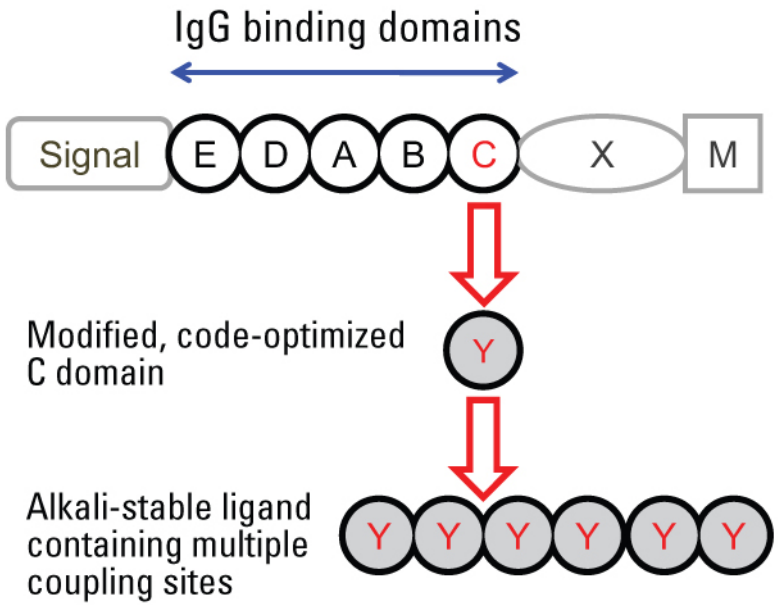
- A variety of column dimensions in terms of internal diameter and length are used by scientists when scaling up protein purification procedures to optimize speed, yield, and recovery to make the production as efficient and cost effective as possible.

- Here, we show a study on the effect of altering column dimensions to favor a shorter, wider column and its impact on the binding capacity of TOYOPEARL AF-rProtein A HC-650F affinity resin when compared with a longer, narrower column of the same total volume.

- The capabilities of TOYOPEARL AF-rProtein A HC-650F resin for removing host cell proteins is also briefly mentioned in this report.

Introduction

- TOYOPEARL AF-rProtein A HC-650F – high capacity Protein A resin
- An enhanced rProtein A ligand is bound to the TOYOPEARL HW-65F base bead via a multipoint attachment.



Properties of TOYOPEARL AF-rProtein A HC-650F

Pore size (mean):	100 nm
Particle size (mean):	45 µm (F-grade)
Pressure rating:	0.3 MPa
Shipping buffer:	20% ethanol
pH stability:	2-13
Shelf life (room temperature):	>1 year (ongoing)

- The 100 nm pore diameter of the TOYOPEARL AF-rProtein A HC-650F resin can accommodate large globular proteins up to 5×10^6 Da.
- This resin has a pressure rating of 0.3 MPa
- The resin is stable in the pH range 2-13.

Materials

- Resin: TOYOPEARL AF-rProtein A HC-650F
- Columns: Omnifit® Benchmark columns
  - 0.66 cm ID × 21.5 cm
  - 1.0 cm ID × 9.4 cm
  - 1.5 cm ID × 4.2 cm
- TSKgel® G3000SW$_{XL}$, 5 µm, 7.8 mm ID × 30 cm (column No. T00122 -05T)
- Sodium phosphate, monobasic (anhydrous) (Fisher S369-3)
- Sodium phosphate, dibasic (dodecahydrate) (Acros 206510025)
- Sodium chloride (Sigma S1679)
- Sodium citrate (Fisher L-13298)
- Sodium hydroxide (Fisher L-12647)
- Ethanol, 200 proof (Fisher S25309B)
- Deionized water - milli Q 18.2 ohm.cm^{-1}
- Instrumentation:
  - ÄKTA® avant 150
  - Agilent 1100 Series Chromatography Chemstation® (ver. B.04.02)
- Sample - monoclonal antibody (TBL-mAb-001)

Methods

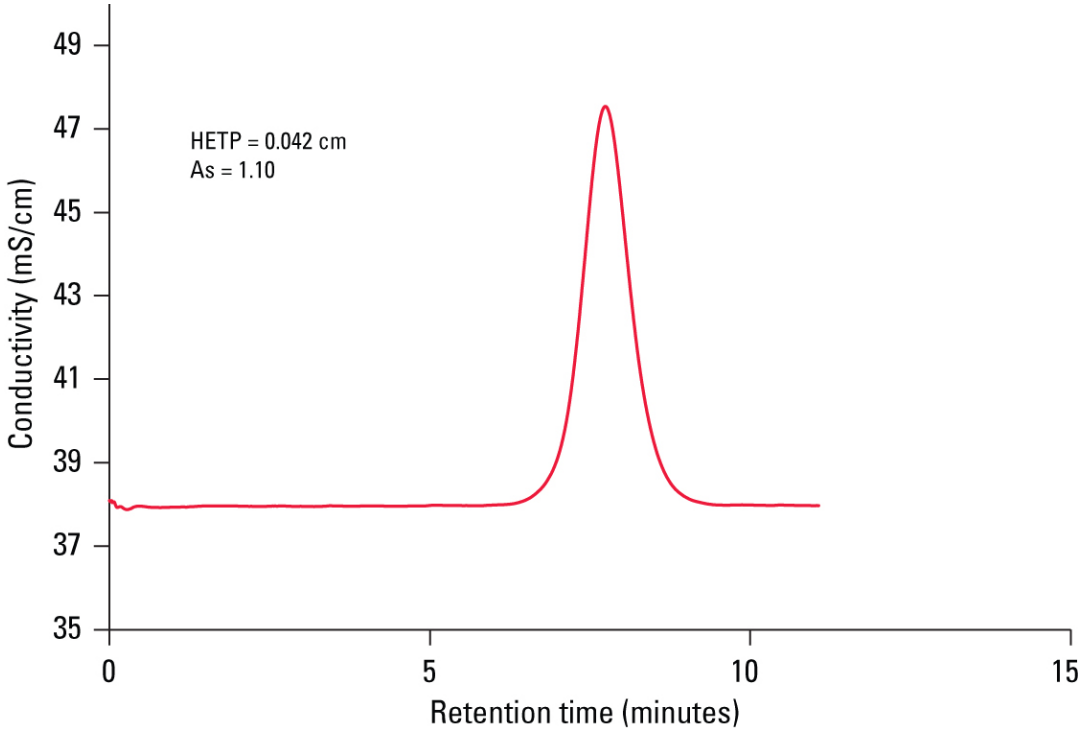
- Columns were prepared using TOYOPEARL AF-rProtein A HC-650F resin.
- Unused resin was measured out and allowed to settle to a final volume of 9.5 mL.
  - The initial suspension, shipped in in 20% ethanol, was buffer exchanged and defined 4 times in 0.4 mol/L sodium chloride packing buffer.
    - This process was completed by buffer exchanging three times with packing buffer following a 60 minute settling period.
      - Once the resin had settled, the supernatant was decanted and fresh packing buffer was added.
    - Following the third buffer exchange, the resin was allowed to settle overnight at 4 ºC to verify settled resin volume.
      - The supernatant was then discarded, and a final buffer exchange occurred.

Methods

- The columns were packed at a final flow rate of 800 cm/hr.
- Each column was packed using the same initial 9.5 mL resin sample.
  - Each column was packed, used, unpacked, and then repacked in the next progressively larger column.
- As each column was packed, column performance was tested by injecting 1% column volume of 3 mol/L sodium chloride with a mobile phase of 0.40 mol/L sodium chloride at a flow rate of 60 cm/hr.
- Each column was found to be acceptable for use in the study (a representative chromatographic profile is shown in the following slide).

Column Performance Test

- Column performance was tested by injecting 1% column volume of 3 mol/L sodium chloride with a mobile phase of 0.40 mol/L sodium chloride at a flow rate of 60 cm/hr.
- Each column was found to be acceptable for use in this study.



Methods: Preparation of Partially Purified mAb

Resin:	TOYOPEARL AF-rProtein A HC-650F
Instrument:	ÄKTA avant 150
Binding buffer:	0.1 mol/L sodium phosphate, 0.15 mol/L sodium chloride, pH 7.4
Elution buffer:	0.1 mol/L sodium citrate, pH 3.0
Flow velocity:	varied, all flow rates chosen to establish a 5 minute residence time
Detection:	UV @ 280 nm
Temperature:	ambient
Injection vol.:	150 mL
Sample:	TBL-mAb-001 (2.998 g/L)
Sample load:	~63 g/L resin (at approximately 90% dynamic binding capacity, as explained below)

Methods: Preparation of Partially Purified mAb

Column was equilibrated with 6 CV binding buffer.

Sample was loaded, and column was washed with 10 CV of binding buffer.

Protein was eluted with elution buffer, with peak fractionation starting when A280 reached 100 mAu, and stopping when A280 fell to 75 mAu.

Column was cleaned-in-place with 3 CV of 0.1 mol/L NaOH in upflow, and re-equilibrated with 3 CV of binding buffer.

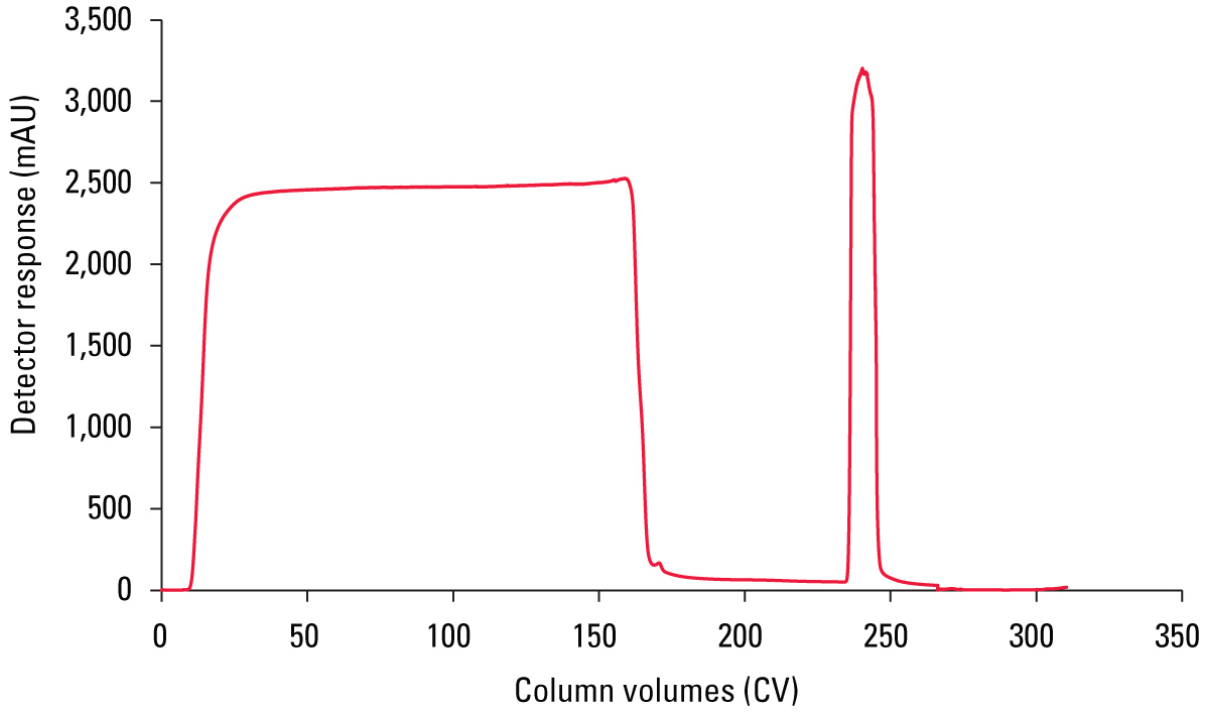
Purification of TBL-mAb-001 using TOYOPEARL AF-rProtein A HC-650F Resin

- During the purification of TBL-mAb-001, a clear baseline is achieved prior to sample application and a clean elution profile can be seen.
- TBL-mAb-001 was applied as a function of resin volume at a rate of approximately 90% dynamic binding capacity, or 61 g/L of resin.
  - This was equal to 154.64 mL of sample applied to each column, however, due to quantity limitations, only 150 mL of sample was applied to each column.
- The change in pressure over each column ranged from 0.25 MPa on the 0.66 cm ID column to 0.02 MPa on the 1.0 cm ID column and < 0.01 MPa on the 1.5 cm ID column – within the max pressure limit of 0.3 MPa.

Purification of TBL-mAb-001 using TOYOPEARL AF-rProtein A HC-650F Resin

- Once each purification was completed, SEC-HPLC analysis was completed on each elution (as shown in the following figures).
- The citrate peak (solvent peak) indicated on each chromatogram was not included in purity analysis.
- Each of the SEC analyses yielded greater than 95% purity of mAb monomer when peak areas were compared. Individual chromatograms are shown in the following slides.

Purification of TBL-mAb-001 using TOYOPEARL AF-rProtein A HC-650F Resin

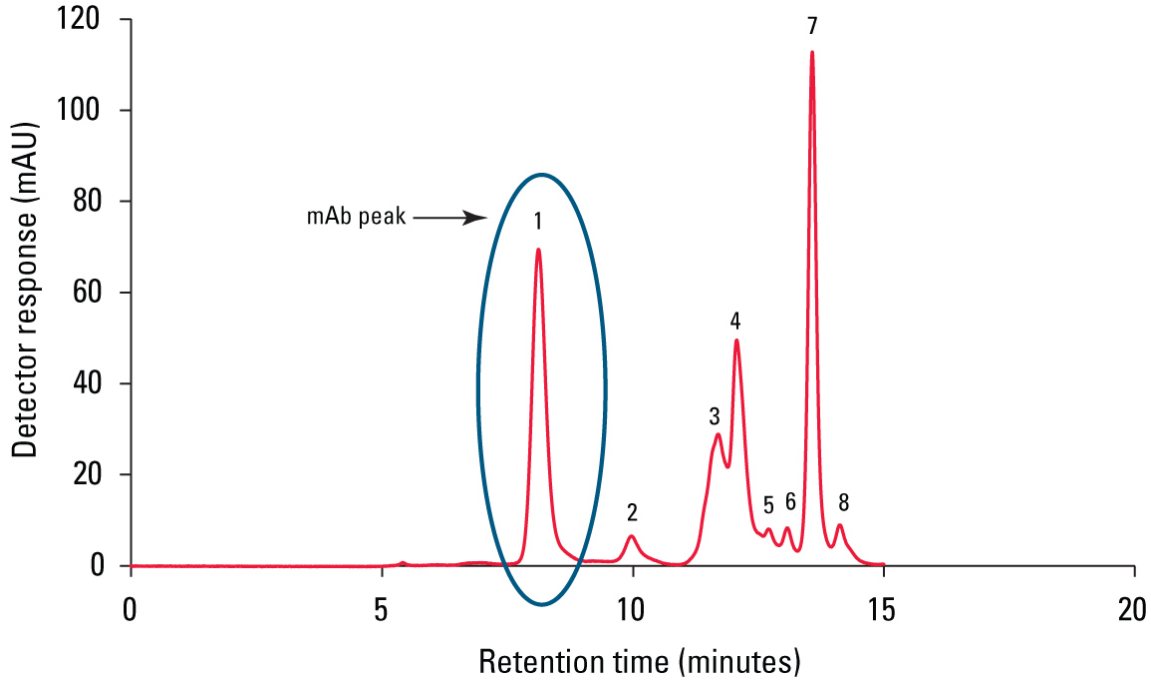


A representative chromatogram using a 0.66 cm ID × 21.5 cm column packed with TOYOPEARL AF-rProtein A HC-650F resin

Methods: SEC - HPLC Analysis of Protein Fractions

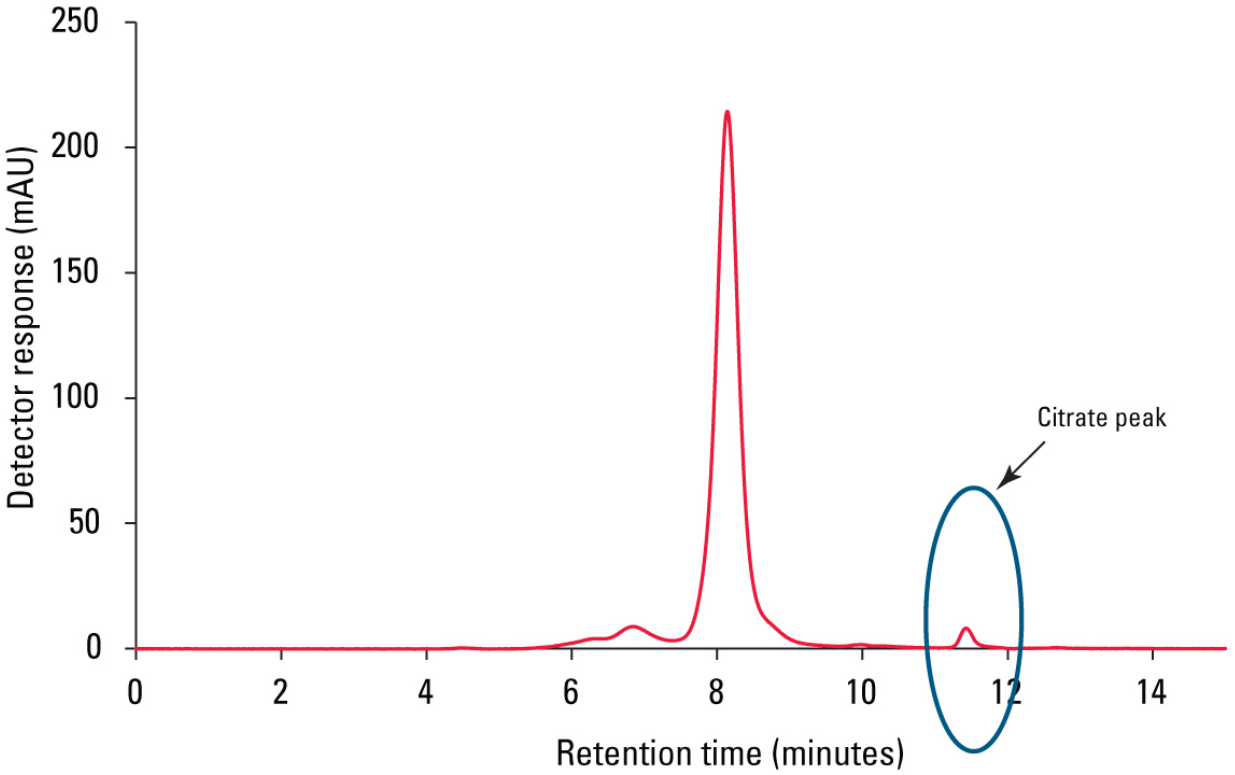
Column:	TSKgel G3000SW$_{XL}$
Mobile phase:	0.1 mol/L sodium phosphate, 0.1 mol/L sodium sulfate, 0.05% sodium azide, pH 6.7
Gradient:	isocratic
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Temperature:	25 ºC
Injection vol.:	10 µL

SEC Analysis of TBL-mAb-001 Prior to Purification

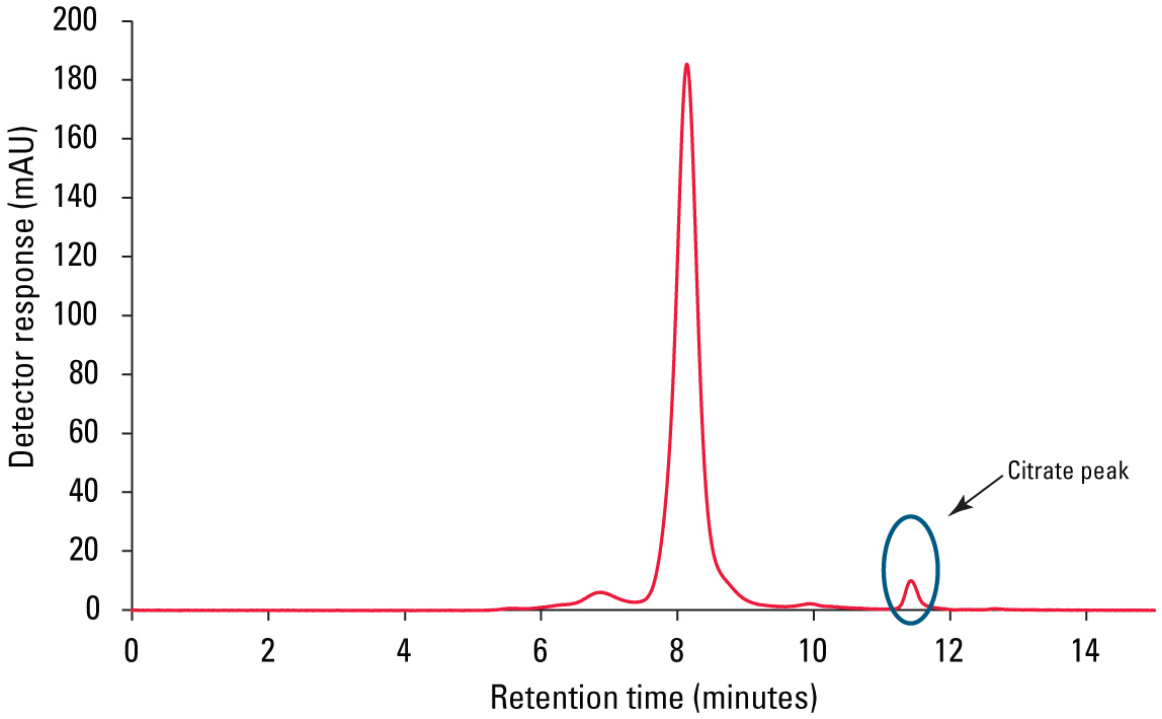


- SEC analysis prior to the purification yielded 8 peaks.
- Analysis of this data indicates that approximately 28.3% of the all the eluted material is the mAb of interest in this sample.

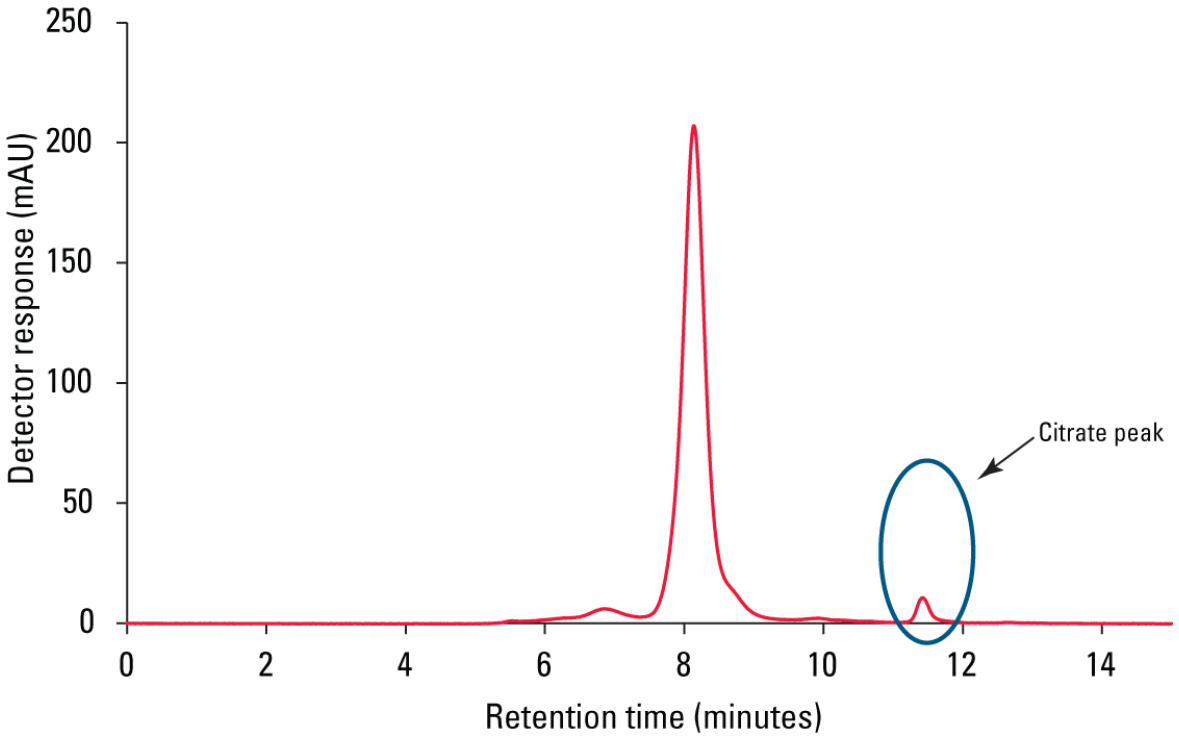
SEC Analysis of 0.66 cm ID × 21.5 cm Eluate



SEC Analysis of 1.0 cm ID × 9.4 cm Eluate



SEC Analysis of 1.5 cm ID × 4.2 cm Eluate



Recovery

Column dimensions (cm ID × cm)	Sample loaded (mg)	mAb Concentration 1:20 dilution (g/L)	mAb Concentration 1:50 dilution (g/L)	Mean concentration (g/L)	Eluate volume (mL)	mAb recovered (mg)	Yield (%)	Purity (%)
0.66 × 21.5	449.7	12.02	12.33	12.175	36	438.3	97.46	96
1.0 × 9.3	449.7	11.11	11.73	11.42	39	445.38	99.04	96.1
1.5 × 4.2	449.7	11.98	12.02	12.00	36	432.00	96.06	94.9

- The purification of protein using columns with 3 different dimensions yielded >96 % recovery.
- The column dimensions did not have any impact on the purification capabilities of TOYOPEARL AF-rProtein HC-650F resin.
- Preliminary reports demonstrated the successful use of TOYOPEARL AF-rProtein A HC-650F for host cell protein removal (Ref: Tosoh application note AN61). Further studies on HCP and DNA removal is in progress and will be published elsewhere. Preliminary data suggests consistent HCP removal between columns with > 2 log reduction (LRV) clearance.

Conclusions

- TOYOPEARL AF-rProtein HC-650F resin yielded > 96% recovery irrespective of the 3 different column dimensions studied in this report.
- The column dimension did not have any impact on the purification capabilities of TOYOPEARL AF-rProtein HC-650F resin when the sample was loaded at approximately 90% dynamic binding capacity.
- This study also shows that altering column dimensions to favor a shorter, wider column maintained equal binding capacities when compared with a longer, narrower column of the same total volume.
    - This will help when scaling-up in process development and purification.