High Throughput and Robust Method of Analysis of a Variety of Immunoglobulin G

Derived from Multiple Sources and from Crude Feedstock using an Analytical Protein A Affinity Chromatography Column

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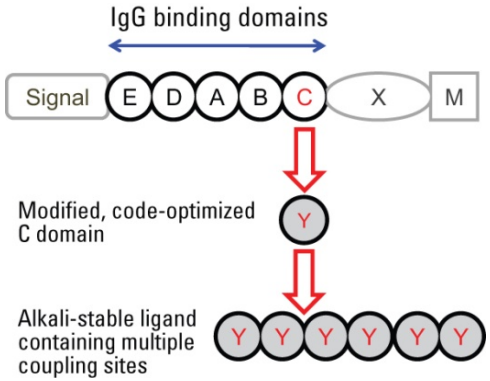
Introduction

- Early in mAb development there are many harvested cell supernatant samples which contain mAbs that are secreted by cell cultures.
- Most of the monoclonal antibody biotherapeutics on the market today are based on IgG_1. Interest in IgG_2 and IgG_4 is rapidly growing.
- These samples must be screened for mAb titer; affinity protein A columns are often employed for this purpose.
- Determination of the optimal time for harvesting mAbs from cell culture supernatant and screening for the best cell clones that express the most amount of mAb are also accomplished using protein A columns.
- With many samples to be screened for different purposes, a reliable and high throughput column is needed for this workflow.
- Determination of mAb concentration from harvested cell culture supernatant requires a column with good linearity over a wide dynamic range so that the concentrations of mAb can be accurately determined.

Introduction

- There are many Protein A affinity chromatography columns currently on the market.
- Many of these columns do not provide sufficient titer determination due to the lack of binding of the protein A ligand to IgG subclasses that are produced by different host cell sources.
- In this study we have used a TSKgel Protein A-5PW column, containing a recombinant protein A ligand which is a code-modified hexamer of the C domain.
    - *The column is packed with hydroxylated methacrylic polymer beads with a high degree of crosslinking which allows the use of high flow rates for high throughput analysis while still maintaining chromatographic efficiency, peak width and resolution.*
    - *The selective recombinant protein A ligand is designed for the capture of IgG derived from multiple subclasses and sources.*
    - *Robustness of the packing technology used in the TSKgel Protein A-5PW column is demonstrated with usage of crude feedstock.*

Pore size (mean):	100 nm
Particle size:	20 µm
pH stability:	2.5 - 7.5
Exclusion limit:	1,000 kDa
Ligand:	Recombinant protein A, hexamer of C domain



Introduction

Species	Subclass	Protein A ligand of Protein A-5PW	Native Protein A
Human	IgG_1	+++++	++++
	IgG_2	+++++	++++
	IgG_3	-	-
	IgG_4	+++++	++++
Mouse	IgG_1	++++	+
	IgG_{2a}	+++++	++++
	IgG_{2b}	+++++	+++
	IgG_3	++++	++
Rat	IgG_1	++++	-
	IgG_{2a}	-	-
	IgG_{2b}	+++	-
	IgG_{2c}	++++	-
Goat	IgG_s	++++	-
Chicken	IgY	-	-
Rabbit	IgG	+++++	++++

The selective recombinant protein A ligand of the TSKgel Protein A-5PW column is designed for the capture of IgG derived from multiple subclasses and sources.

Materials

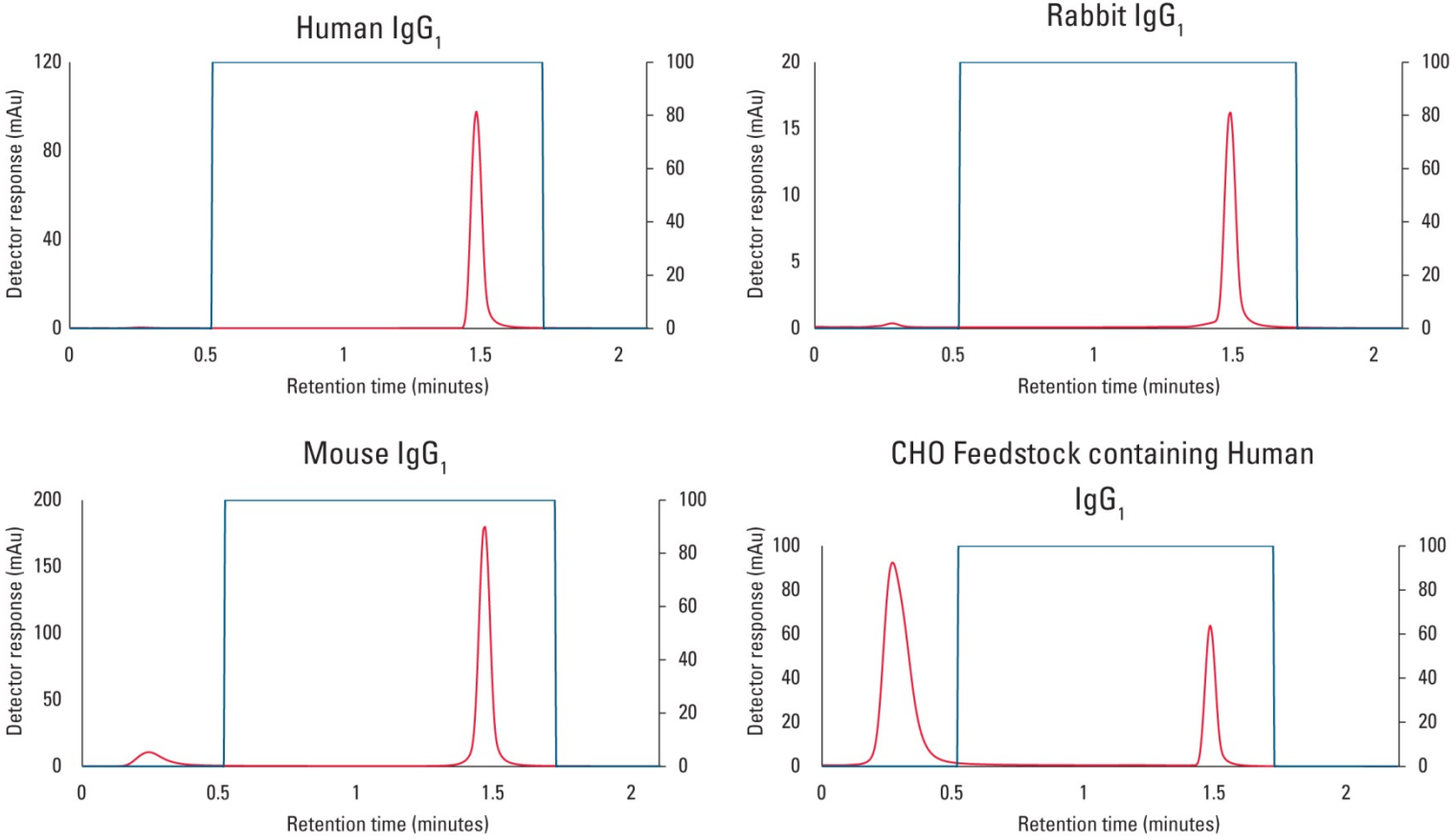
	Conc. (mg/mL)	Injection vol.
Human IgG_1	5 mg/mL	5 µL
Rabbit IgG_1	1.0 mg/mL	5 µL
Mouse IgG_1	1.0 mg/mL	5 µL
IgG_1 in CHO feedstock	2.95 mg/mL	5 µL
IgG_2	0.82 mg/mL	5 µL
IgG_4	1.0 mg/mL	5 µL

	pKa	Conc. used
HCl	-7	12 mmol/L
Citric acid	3.13, 4.76 & 6.40	100 mmol/L
Glycine	2.64 & 9.69	100 mmol/L
Phosphate	2.148, 7.198 & 12.319	20 mmol/L
Acetic acid	4.76	5 %

Conditions

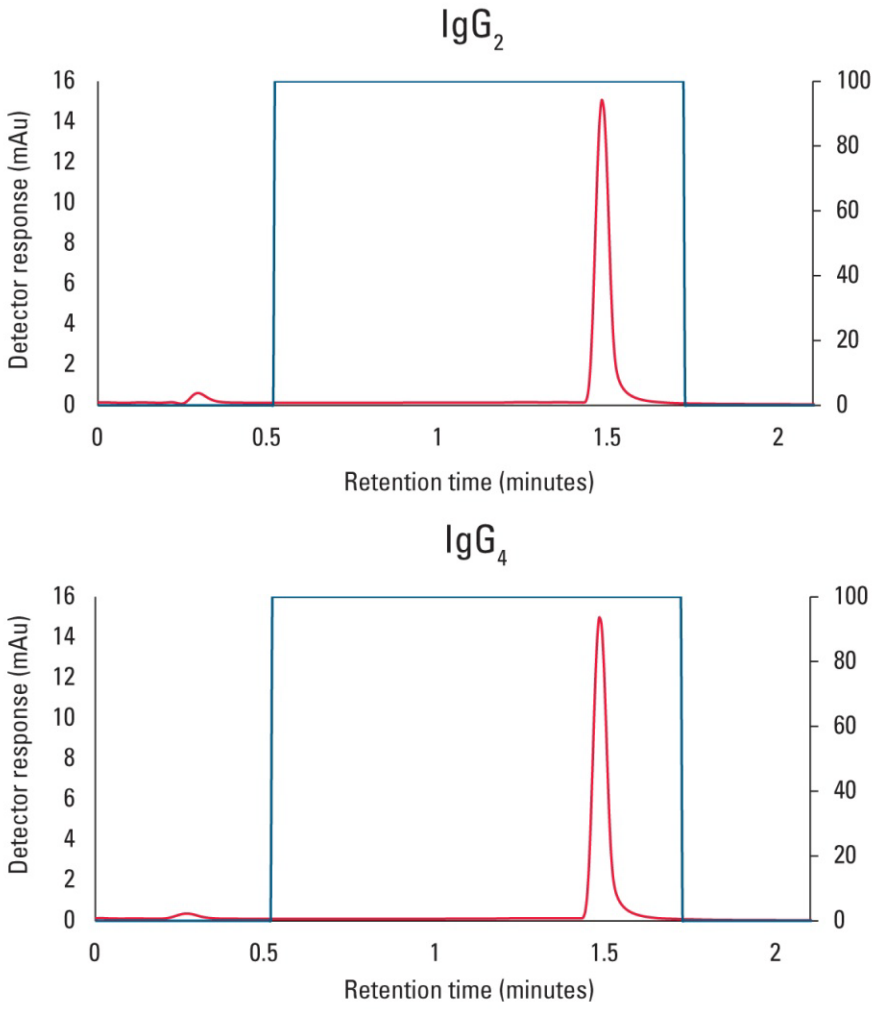
Column:	TSKgel Protein A-5PW, 20 µm, 4.6mm ID x 3.5 cm
Binding buffer:	20 mmol/L Sodium Phosphate, pH 7.4
Elution buffer:	12 mmol/L HCl
Step gradient:	0.0-0.5 min: Binding buffer
	0.51-1.7 min: Elution buffer
	1.71-2.2 min: Binding buffer
Flow rate:	2 mL/min
Detection:	UV @ 280
Samples:	IgG_1, IgG_2, IgG_4

Capture of IgG_1 from Different Sources using 12 mmol/L HCl

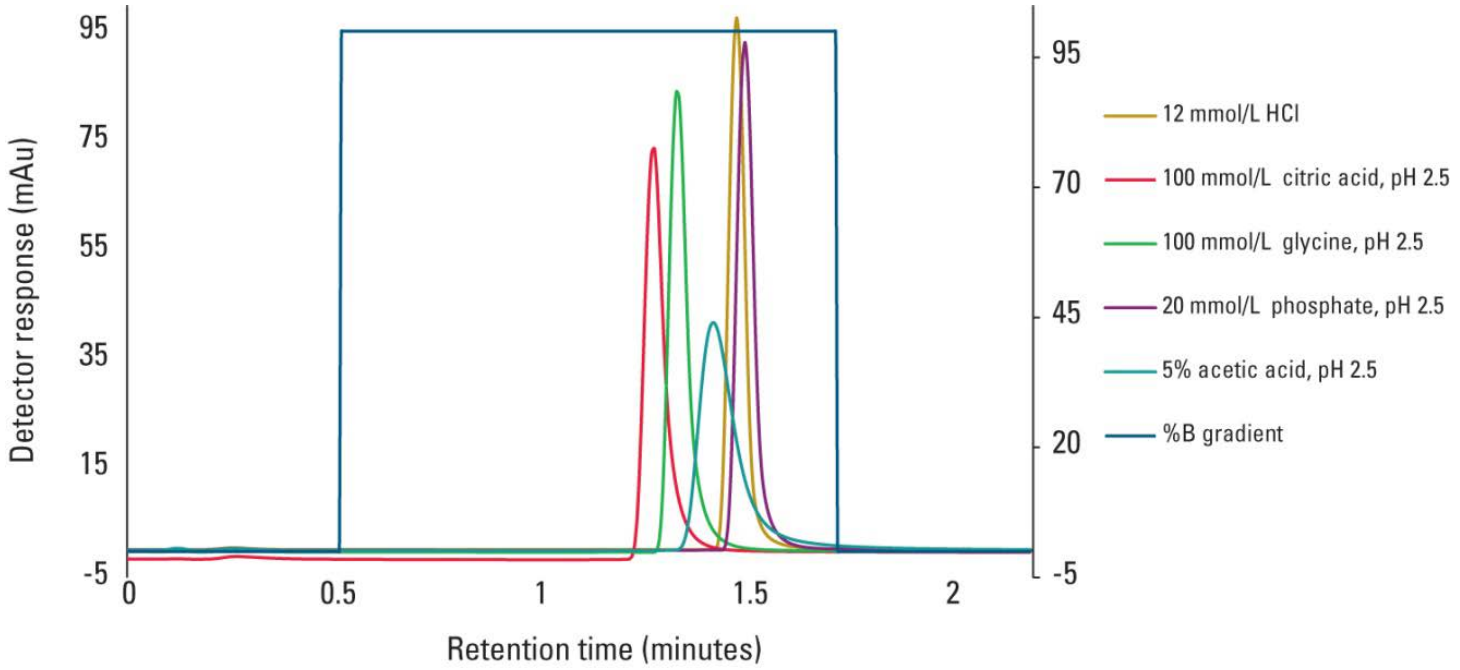


The data demonstrates the affinity of the TSKgel Protein A-5PW column for a variety of IgG subclasses.

Capture of IgG_2 and IgG_4 using 12 mmol/L HCl

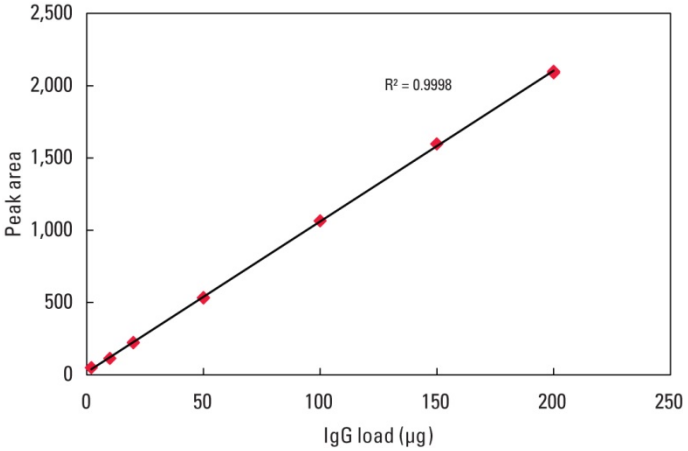


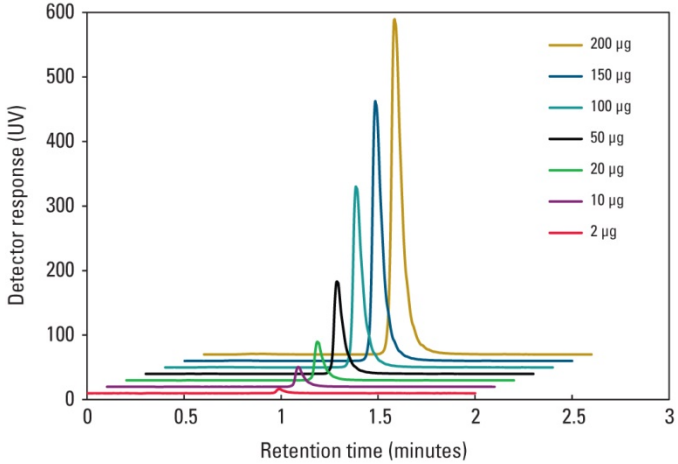
Compatibility with Multiple Elution Buffers



The TSKgel Protein A-5PW column is compatible with various elution buffers.

Dynamic Range and Linearity



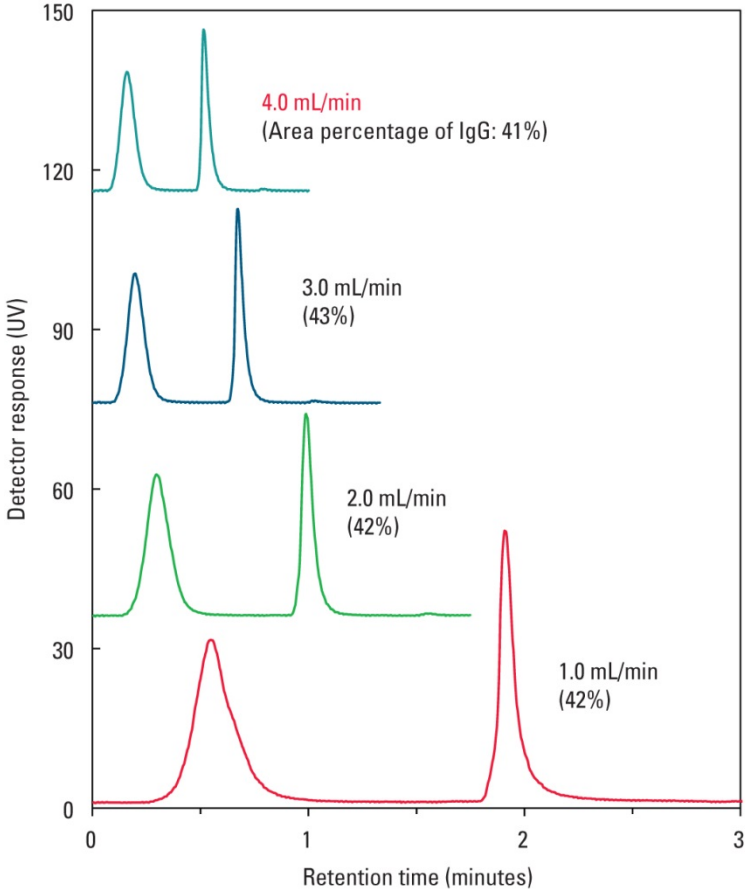


Sample:purified polyclonal IgG

Sample load (µg)	RT (min)
2	0.988
10	0.988
20	0.988
50	0.988
100	0.985
150	0.985
200	0.985

TSKgel Protein A-5PW column shows a wide dynamic range from 0.1 - 10 g/L (2 - 200 µg) with good linearity (R^2 > 0.999) for IgG.

High Flow Rates for High Throughput Analysis



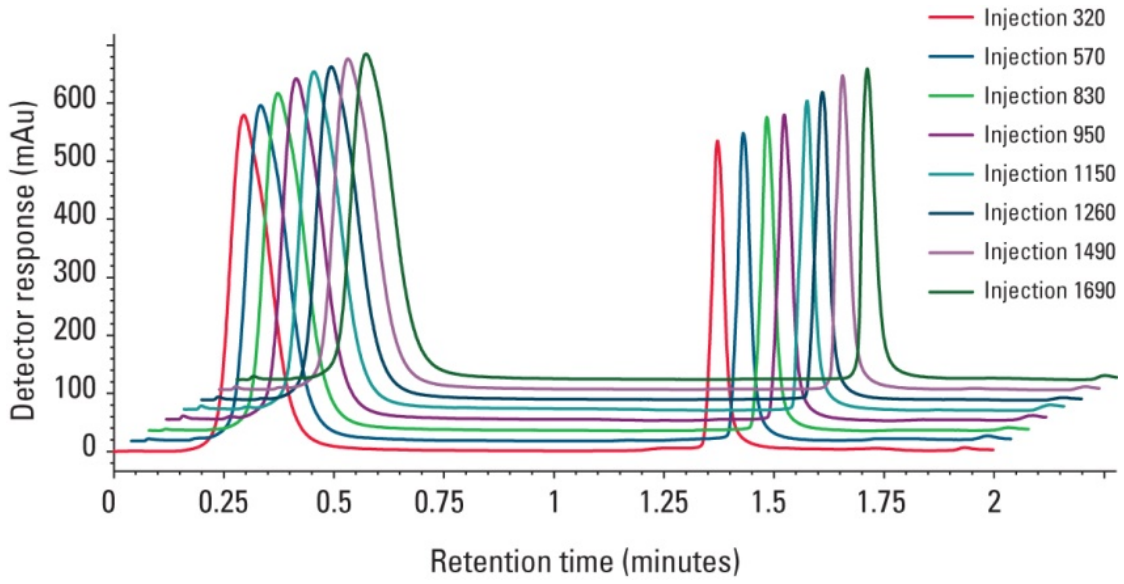
20 µL of CHO cell supernatant spiked with polyclonal antibody (0.5 mg/mL)

Gradient conditions

Flow rate (mL/min)	Binding buffer (min)	Elution buffer (min)	Binding buffer (min)
4.0	0-0.25	0.25-0.55	0.55-1.00
3.0	0-0.33	0.33-0.73	0.73-1.33
2.0	0-0.50	0.50-1.10	1.10-2.00
1.0	0-1.00	1.00-2.20	2.20-4.00

- **TSKgel Protein A-5PW column shows similar recovery of IgG up to 4.0 mL/min.**
- **Less than 1 minute analysis was available at 4.0 mL/min with similar peak profile.**

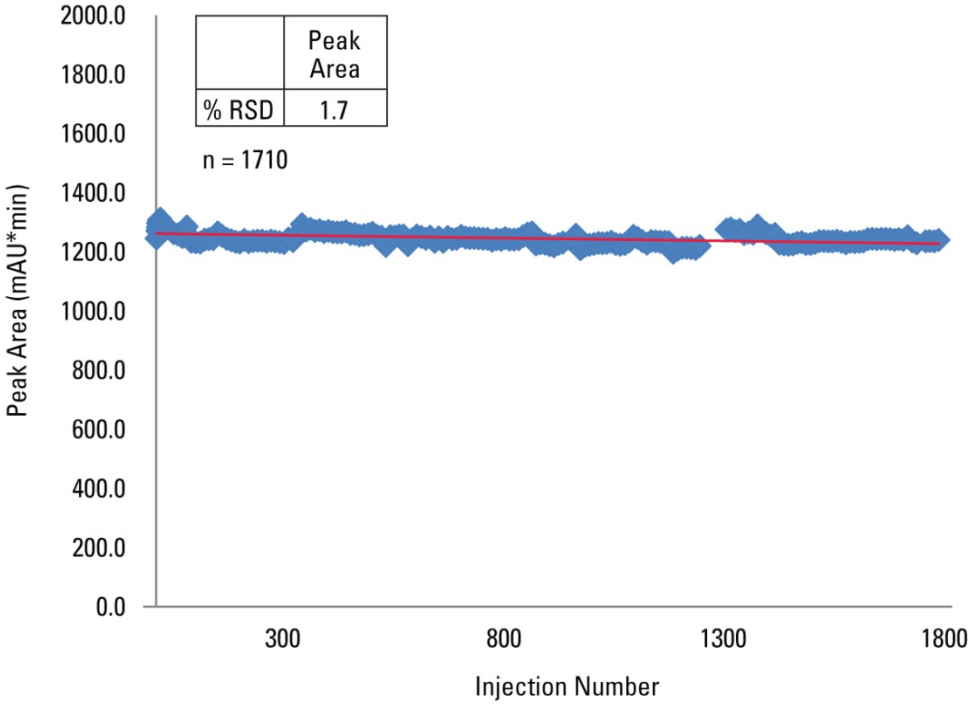
Durability study using CHO crude Feedstock containing IgG_1



- Injection vol.: 5 µL
- Sample: CHO crude feedstock containing IgG_1 (3.2 mg/mL)

With thousands of injections of crude feedstock, the TSKgel Protein A-5PW column did not show any significant change of peak profile for crude feedstock.

Durability study using CHO crude Feedstock containing IgG_1



Column cleaning conditions:

reversed flow at 0.5 mL/min for 20 CV:
a. 0.1 mol/L NaOH
b. DI Water
c. 1 mol/L acetic acid

normal flow for 20 CV:
a. DI water
b. 0.5 mol/L sodium phosphate, pH 6.5

50 CV: 20mm sodium phosphate pH 7.4

- **The column can be used with high flow rate while still maintaining peak area consistency with RSD of 1.7%.**
- **The column was cleaned after 1230 injections using a stepwise cleaning protocol.**

Conclusions

- The TSKgel Protein A-5PW column can capture and accurately quantitate monoclonal antibody from harvested cell culture media in less than 2 minutes.
- The wide range loading capacity of this column allows the titer of mAb at various stages of development to be determined.
- This study shows that the TSKgel Protein A-5PW column quantitatively purified IgG1 from crude feedstock at high flow rates; therefore, this column can be used for high throughput analysis.
- Even with the challenging chromatographic separation of crude feedstock, the TSKgel Protein A-5PW column proved to be robust.
- The column is useful for the high throughput analysis of IgG_1 from a variety of subclasses and sources.
- The TSKgel Protein A-5PW column is compatible with several different elution buffers, offering flexibility in study design.