Column lifetime study of a silica-based, diol-bonded size exclusion chromatography column for protein separations

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Objective:

- To study the column lifetime of TSKgel G3000SW$_{XL}$: a silica-based, diol-bonded size exclusion chromatography column widely used for the separation of monoclonal antibodies
- To study the lot-to-lot variation in peak parameters in the analysis of proteins and monoclonal antibodies

Introduction

- A column is at the heart of a good HPLC system.
- Column lifetime is an important issue, both financially and for accurate analyses.
- Over many years now, column to column reproducibility and column lifetime consistently remain as the top two factors - above price - to chromatographers when selecting an appropriate column (as shown in the survey in the next slide).
- Development of a reliable analytical HPLC method requires these qualities to be independent of the lot of base silica, as well as the bonding and packing procedures.

Introduction continued

Factors considered when selecting an HPLC column supplier			
Factor	**Respondents (Normalized %)**		
	2007	**2009**	**2011**
Column-to-column reproducibility	21	21	19
Column lifetime	16	17	15
	37%	**39%**	**34%**
Price	12	14	13
Reputation of company	14	12	9.6
Column plate number	8.7	9.1	9.1
Technical assistance	5.7	5.8	6.7
Variety of phases available	4.5	4.0	6.4
Tailing factor	6.3	5.4	5.0

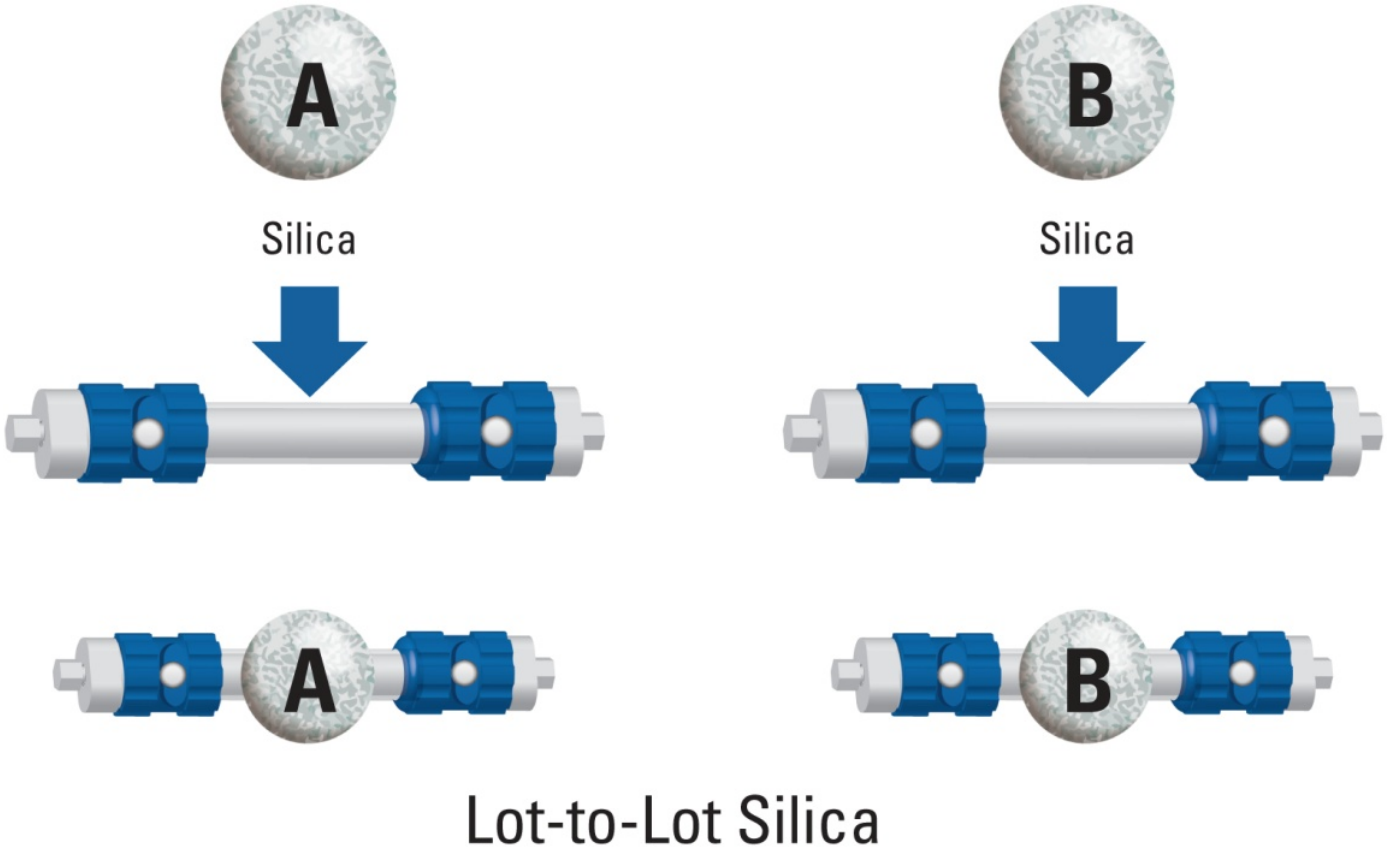
Ref: LCGC: Jan 1, 2012; Article: Current trends in HPLC column usage – By: Ron Majors

Introduction continued

- Symptoms of column failure are a loss of resolution, peak broadening, or significant tailing factors that may affect quantitation.
- One of the most important warning signs that a column may be on the verge of failing can be predicted by a gradual increase in backpressure.
- Retention time shift can sometimes be related to a loss of packing material or stationary phase.
- Other factors chromatographers consider before declaring the column dead include: failure of an established method specification, failure to pass QC test using a standard protein mixture, failure to pass system suitability requirements, a high %RSD (relative standard deviation) value over a number of consecutive injections.
- Although the use of a guard column to protect the analytical column is highly recommended, and ought to be part of a standard operating procedure, in practice not all users utilize guard columns.
- When comparing column performance, it is necessary that the extra-column effects at a manufacturing site matches with a user's equipment. This could cause a variation in quantitation as well.

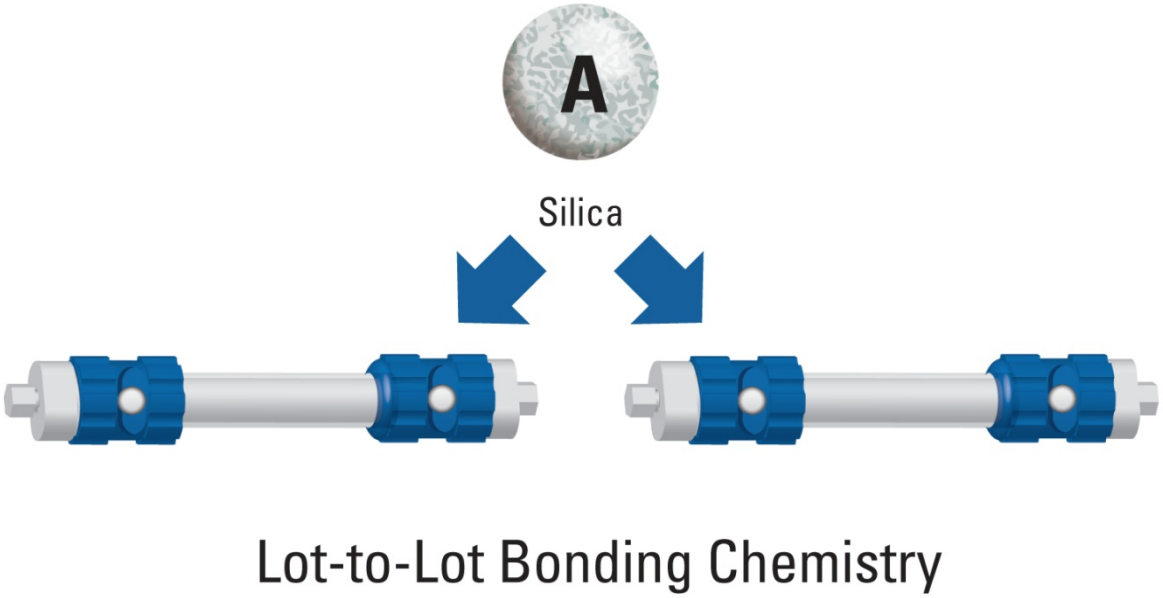
Introduction continued

Two different sources of silica can be a factor in lot-to-lot reproducibility.



Introduction continued

Bonding chemistry developed on the same silica at different times can also be a factor in lot-to-lot reproducibility.



Introduction continued

- In this presentation, we report the results of a column lifetime study of a silica-based TSKgel G3000SW$_{XL}$, 5 µm, 7.8 mm ID × 30 cm SEC column used for the separation of monoclonal antibodies.
- We also focus on lot-to-lot variations in the peak parameters.
- Altogether, 9 columns were used to study the effect of reproducibility arising from both silica and bonding chemistry, as explained below.
  - Lot 09R – 3 columns (S1261, S1262, S1263)
  - Lot 08R – 3 columns (S1237, S1238, S1239)

  } **Silica Lot A**

  - Lot 30P – 3 columns (S6210, S6211, S6212)

  } **Silica Lot B**

- Specification for TSKgel G3000SW$_{XL}$ column passing QC:
  - N [p-aminobenzoic acid (PABA)] >20,000 and AF = 0.7 – 1.6

Material and methods

Chromatographic Conditions

Instrument:	all analyses were carried out using an Agilent 1200 HPLC system run by Chemstation (ver B.04.02)
Mobile phase:	100 mmol/L KH_2PO_4/K_2HPO_4, pH 6.7, 100 mmol/L Na_2SO_4 + 0.05% NaN_3
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Temperature:	ambient
Injection vol.:	10 µL
Samples:	standard TSKgel SWXL test mixture: thyroglobulin (0.5 g/L), γ-globulin (1 g/L), ovalbumin (1 g/L), ribonuclease A (1.5 g/L), PABA (0.01 g/L) Monoclonal antibody: BI-mAb-2 from Boehringer-Ingelheim; concentration: 4.5 g/L in glycine/Na phosphate, pH 6.0 Bovine Serum Albumin (Sigma Aldrich A7906, Lot # 080M1251V); >98% purity, 10.2 mg/mL

Material and methods continued

In this study:

- sample and mobile phase were not filtered through a 0.45 micron syringe filter
- no guard column was used to provide extra stress on the column
- in one case, the mobile phase was recycled to provide additional stress to the column, basically to encourage column failure
- column was not cleaned throughout the experiment
- high purity HPLC grade Sigma Aldrich chemicals were used in this study
- high purity 18.2 m.Ohm-cm quality water was used to make buffer and samples

Figure 1: Analysis of protein standard mixture using a TSKgel G3000SW$_{XL}$, 5 µm, 7.8 mm ID × 30 cm column (S6212-30P)

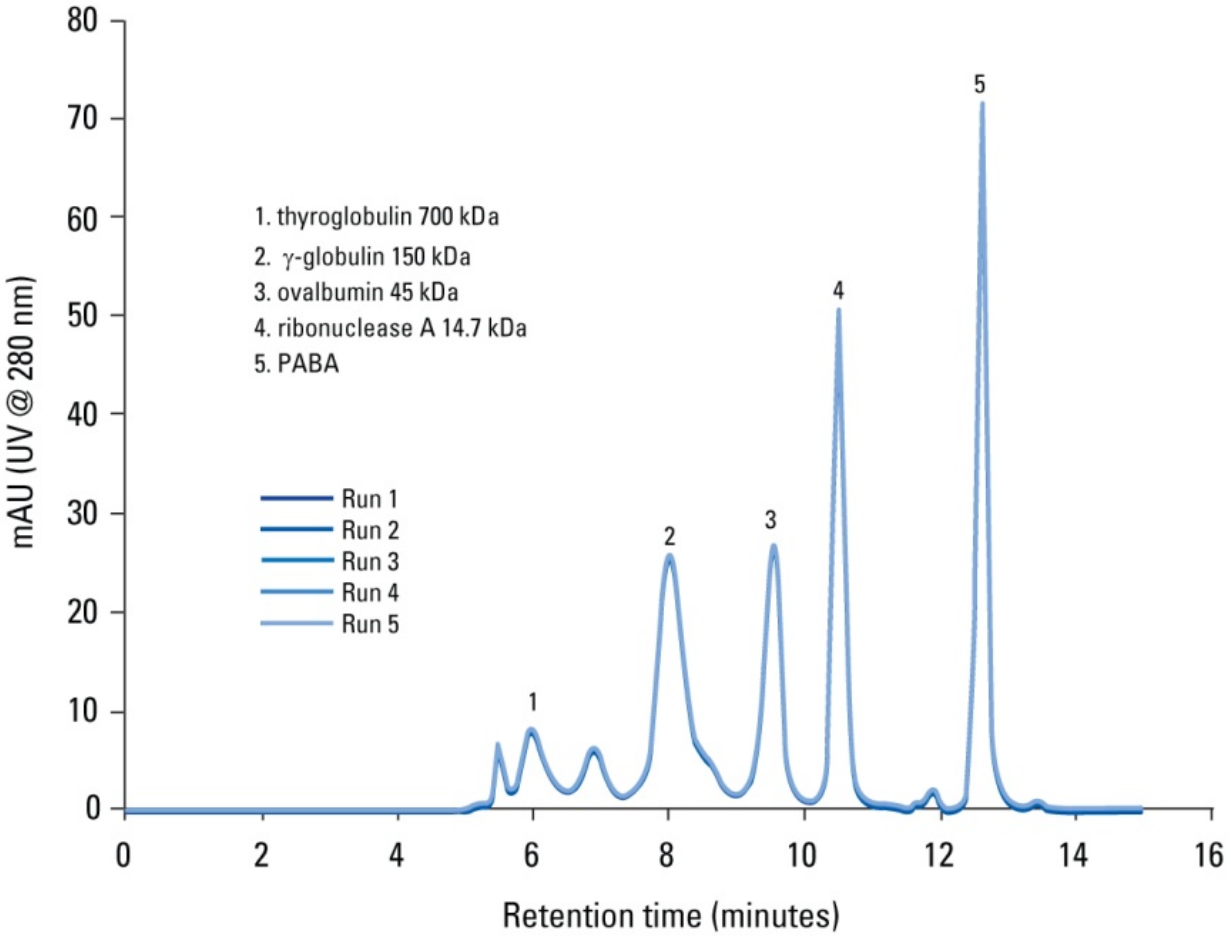


Figure 1: conclusions

- Five consecutive runs yielded an excellent reproducibility: very low %RSD value of all the peak parameters
- Similarly, all 9 columns from different silica and bonding lots yielded excellent reproducibility in 5 consecutive runs: N was always ~32,000 and other peak parameters also remained consistent
- The table in the next slide shows the change of retention time of protein peaks as a function of silica and bonding chemistry

Table 1: Change of retention time of protein peaks as a function of silica and bonding chemistry

Retention times								
Column #		Thy			γ-Glo	Ova	Ribo-A	PABA
		Peak 1	Peak 2	Peak 3				
S1237-08R		5.547	5.993	6.971	8.084	9.546	10.596	12.638
S1238-08R		5.571	6.072	6.994	8.110	9.636	10.620	12.740
S1239-08R		5.546	5.996	6.977	8.083	9.601	10.579	12.672
S1261-09R		5.472	5.922	6.895	8.000	9.528	10.506	12.639
S1262-09R		5.476	5.989	6.914	8.024	9.544	10.513	12.613
S1263-09R		5.469	5.993	6.924	8.038	9.552	10.518	12.577
S6210-30P		5.544	5.969	6.939	8.050	9.588	10.577	12.725
S6211-30P		5.499	5.993	6.924	8.039	9.572	10.539	12.613
S6212-30P		5.493	5.981	6.904	8.018	9.549	10.517	12.622
	Average	5.513	5.990	6.938	8.050	9.568	10.552	12.649
	STDEV	0.039	0.039	0.035	0.036	0.034	0.042	0.054
	%RSD	0.707	0.645	0.502	0.446	0.358	0.398	0.427

The percentage of relative standard deviations of retention times (RT) from lot-to-lot as a function of both silica and bonding chemistry was very low for all protein peaks.

Figure 2: Analysis of protein standard mixture using a TSKgel G3000SWXL, 5 µm, 7.8 mm ID × 30 cm column (S6212-30P), Inj. #1000

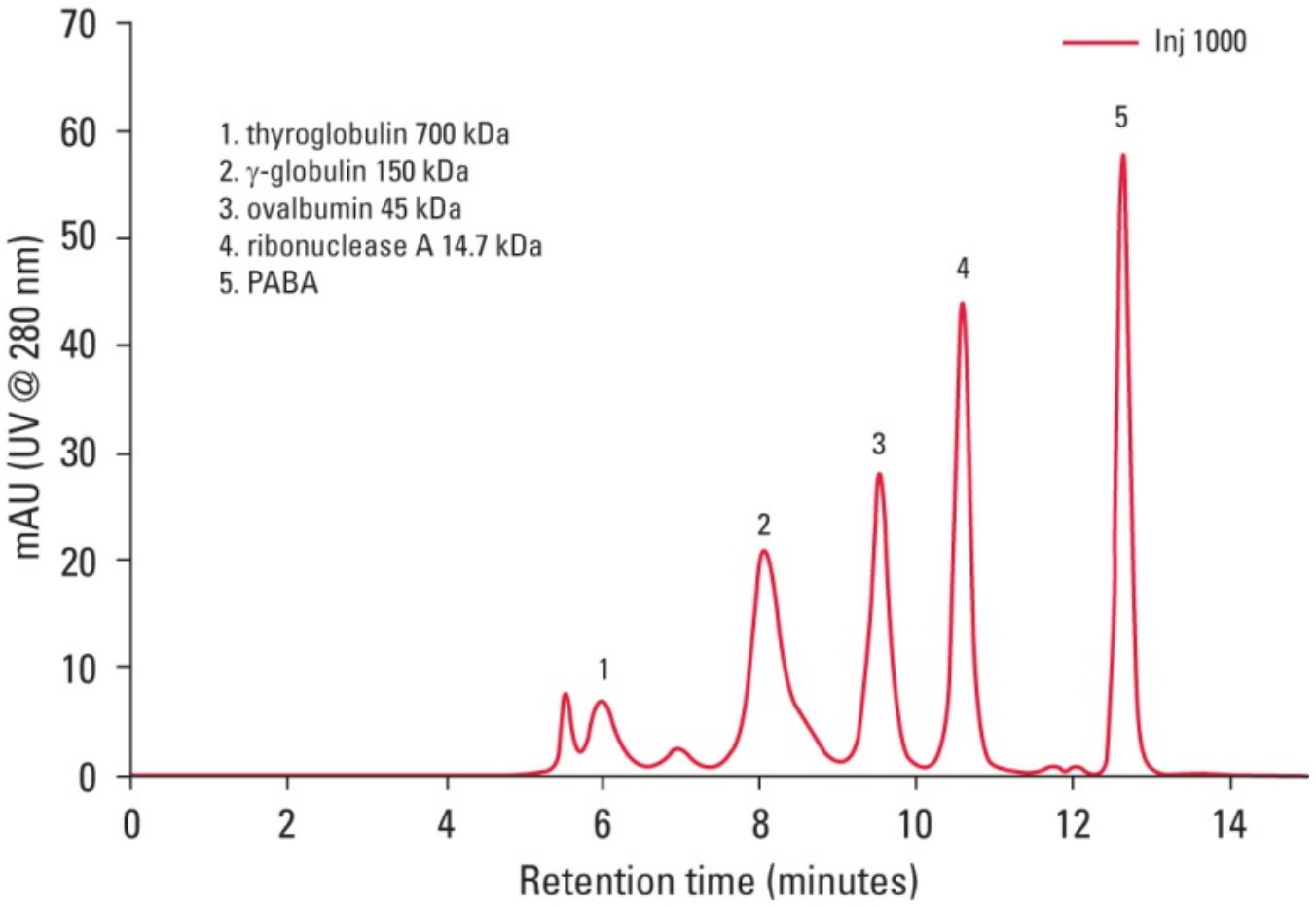


Table 2: Retention time, efficiency, and peak symmetry at the 1st, 500th and 1000th injections

	γ-globulin			PABA		
	Inj. 1	Inj. 500	Inj. 1000	Inj. 1	Inj. 500	Inj. 1000
t_R	8.065	8.027	8.065	12.635	12.647	12.616
N	2021	2002	1847	32,483	33,187	32,381
AF	1.47	1.47	1.49	1.19	1.21	1.28

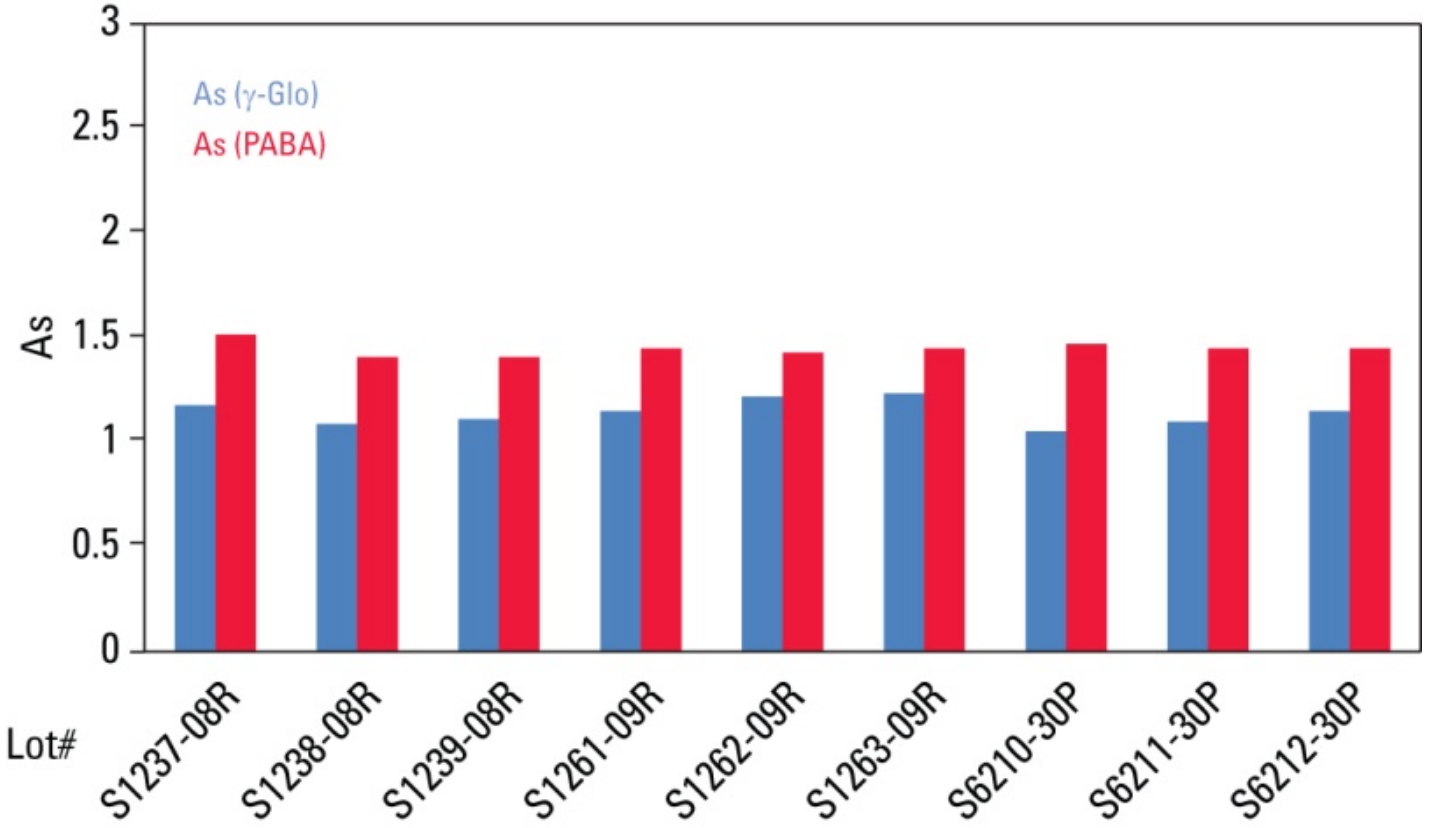
Table 3: %RSD of peak parameters for each 10th injection during the 1000 injection cycle (n = 100)

	thyroglobulin	γ-globulin	ovalbumin	rib. A	PABA
t_R	0.16	0.21	1.05	0.20	0.28
N	5.48	5.27	2.40	2.87	1.70
AF	4.32	1.38	2.24	2.56	2.66

- This study shows that the column remained stable and precise over 1,000 injections.
- The results shown in tables 1 and 2 show that the following 3 important chromatographic peak parameters remained consistent over 1000 runs with low %RSD:

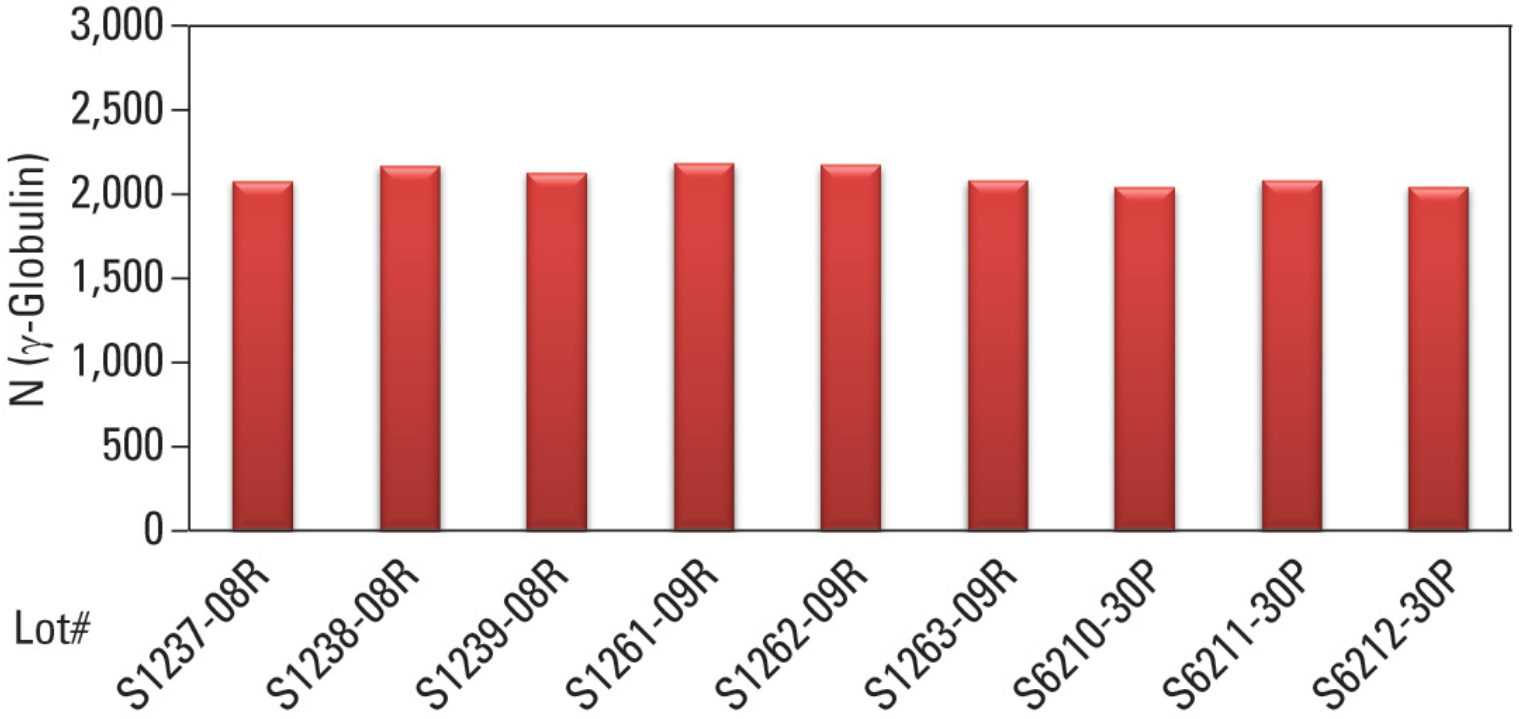
  1. retention time (t_R) 2. theoretical plate count (N) 3. asymmetry factor (AF)

Figure 3: Change of peak symmetry as a function of silica and bonding chemistry in the analysis of protein standard mixture using TSKgel G3000SW$_{XL}$, 5 µm, 7.8 mm ID × 30 cm columns



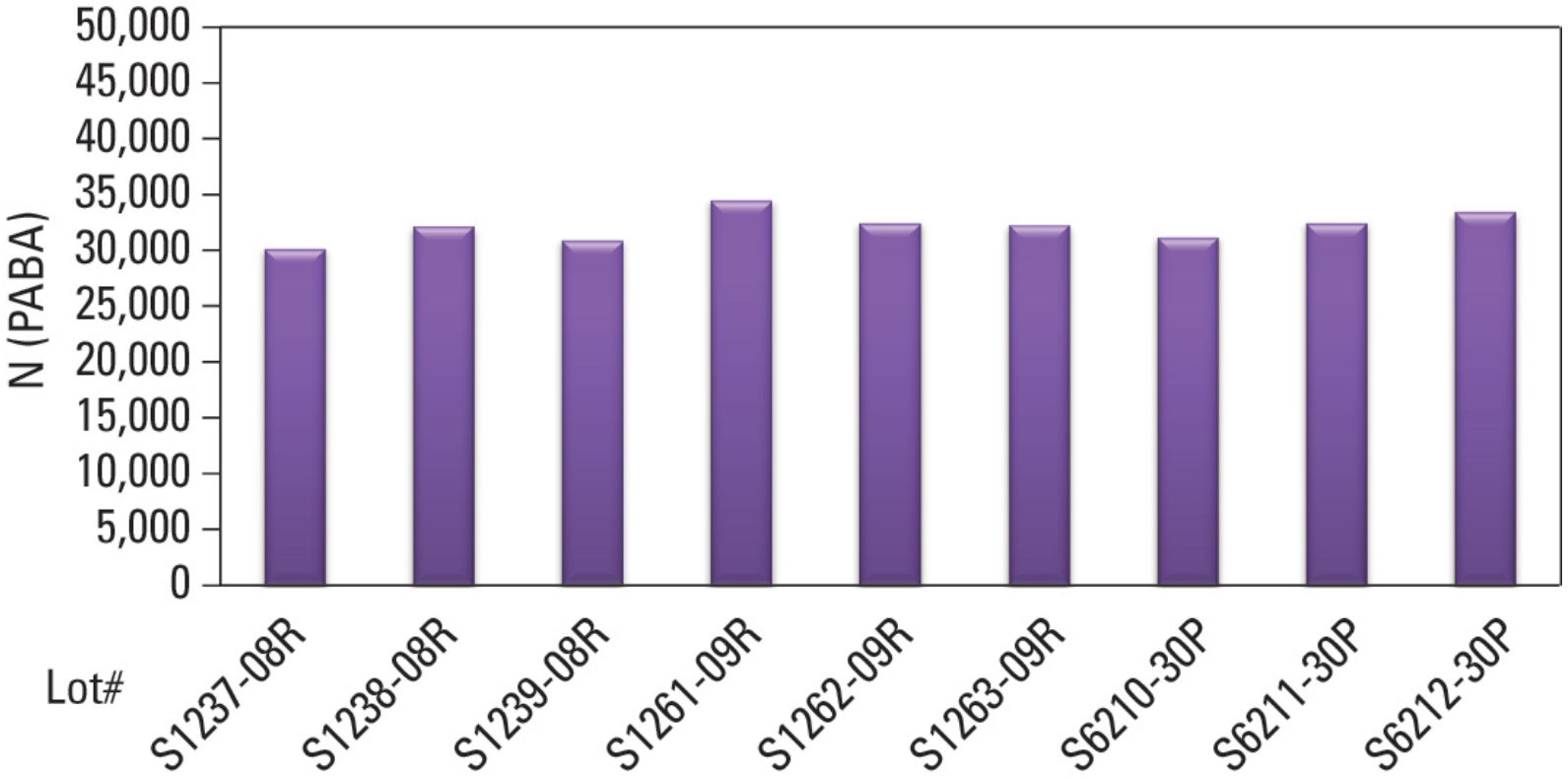
The peak symmetry was consistent over 9 different columns from 3 different lots (including silica lot-to-lot and bonding chemistry lot).

Figure 4: Change of column efficiency as a function of silica and bonding chemistry in the analysis of protein standard mixture using TSKgel G3000SW$_{XL}$, 5 µm, 7.8 mm ID × 30 cm columns



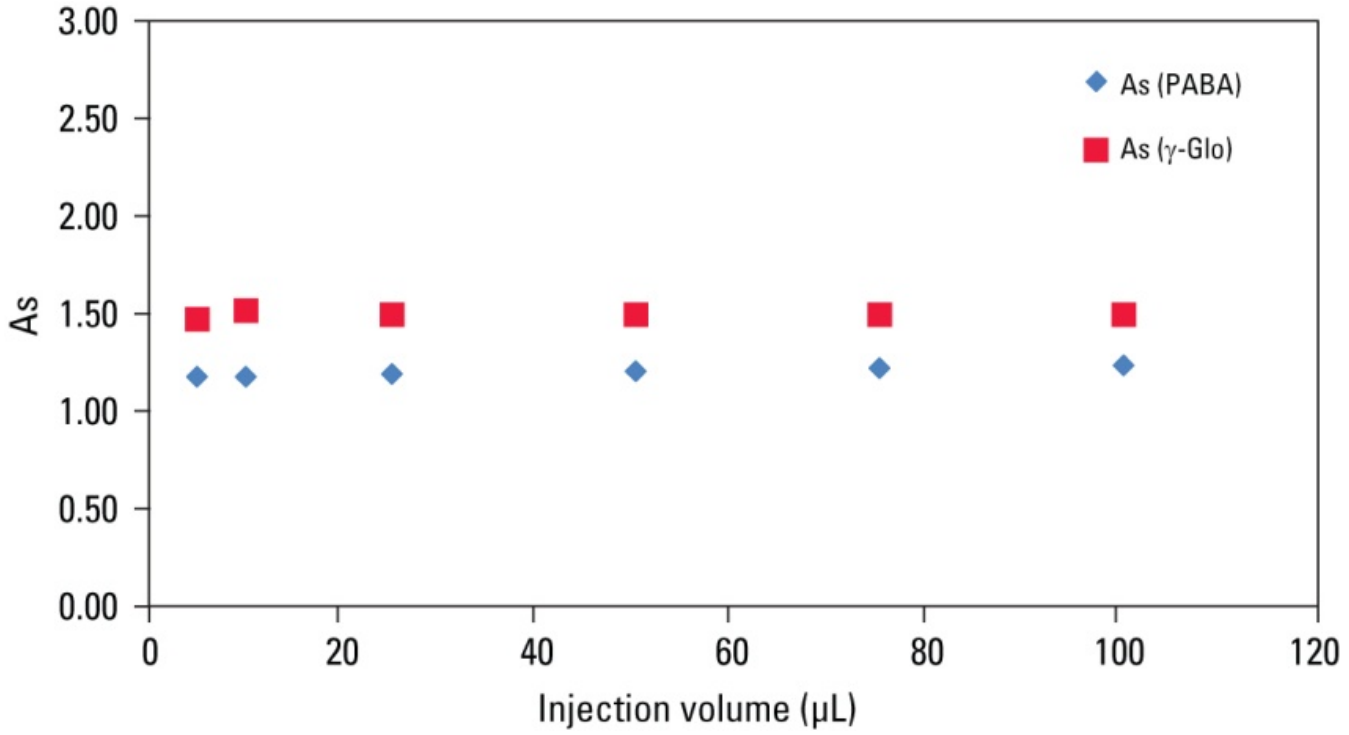
The number of theoretical plates (γ-globulin) was consistent over 9 different columns from 3 different lots (including silica lot-to-lot and bonding chemistry lot) with an average value of 2,107.

Figure 5: Change of column efficiency as a function of silica and bonding chemistry in the analysis of protein standard mixture using TSKgel G3000SW$_{XL}$, 5 µm, 7.8 mm ID × 30 cm columns



The number of theoretical plates (PABA) was consistent over 9 different columns from 3 different lots (including silica lot-to-lot and bonding chemistry lot) with an average value of 32,087.

Figure 6: Effect of injection volume on the peak symmetry in the analysis of protein standard mixture using a TSKgel G3000SW$_{XL}$, 5 µm, 7.8 mm ID × 30 cm column (S1237-08R)



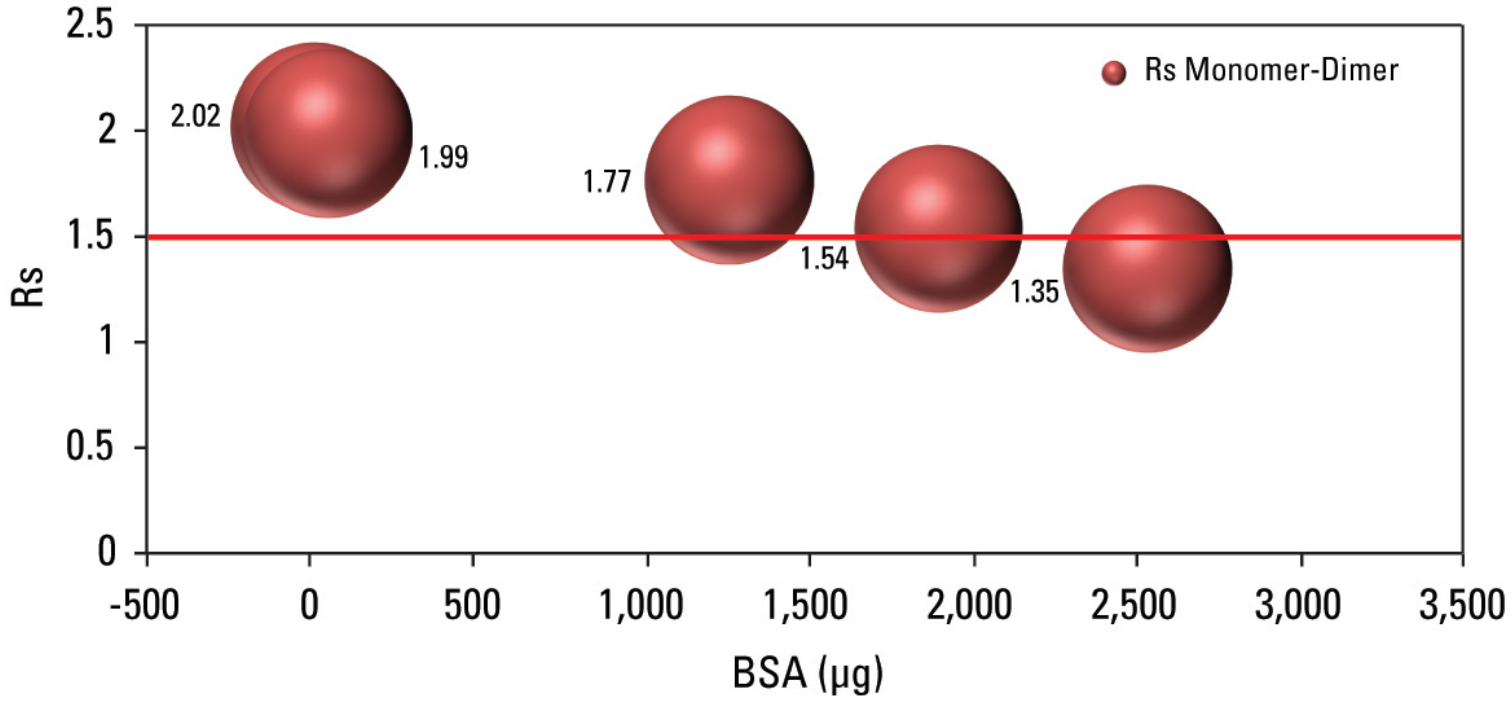
- Peak symmetry is important to ensure no loss of resolution.
- In analytical separations by SEC where optimum resolution is required, the total load volume should not exceed 1-2% of the total column volume – this study is within that range.
- The column maintained low %RSD of 1.9% (γ-globulin) and 0.9% (PABA) over the range of this study.

Table 4: Analysis of loading capacity of BSA using a TSKgel G3000SW$_{XL}$, 5 µm, 7.8 mm ID × 30 cm column (1262-09R)

- A TSKgel G3000SW$_{XL}$ column (lot # 1262-09R) was previously used for a column lifetime study for 1,255 injections of protein standard mixture.
- The TSKgel G3000SW$_{XL}$ column was very stable even after 1,255 injections [N (PABA) >30000].
- The following data is the results of a loading capacity study using this column.

BSA (µg)	RT (min) Dimer	RT (min) Monomer
10.2	7.972	9.013
51	7.985	9.015
1272	8.007	9.069
1908	8.035	9.103
2544	8.044	9.106
Average	8.009	9.061
STDEV	0.03	0.05
%RSD	**0.39**	**0.50**

Figure 7: Analysis of BSA using a TSKgel G3000SWXL, 5 µm, 7.8 mm ID × 30 cm column (1262-09R) – effect of loading on resolution between monomer and dimer peak

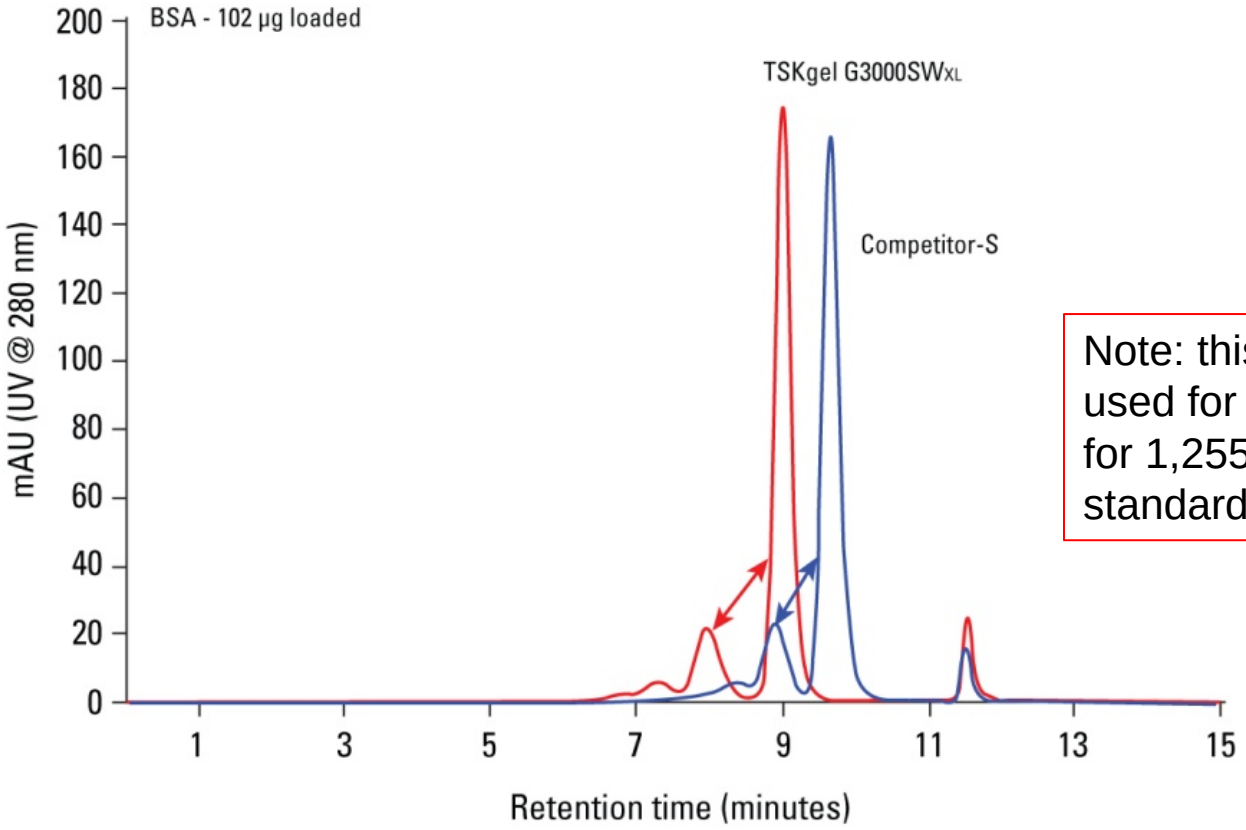


- The red line represents the acceptable resolution of 1.5 between the monomer and dimer peaks.
- The resolution between monomer and dimer remained above 1.5 until 1.9 mg BSA load.

Figure 7 and Table 4: conclusions

- Baseline separation of the monomer from the dimer and aggregates was achieved.
- No shift in retention time of monomer and dimer peak was noticed.
- Even with 1.9 mg load of BSA, the dimer and monomer peak was separated to the baseline.
- The resolution between the monomer and dimer peak was >1.5 up to a load of 1.9 mg of BSA.
- The monomer peak did not split with the higher load of 1.9 mg of BSA.
- With a load higher than 1.9 mg of BSA, the monomer peak developed a hump and the resolution between the monomer and dimer peak became 1.35 (lower than an acceptable value of 1.5)
- %RSD of the retention times of monomer and dimer was very low over the range of this loading capacity study

Figure 8: Analysis of BSA using a TSKgel G3000SWXL, 5 µm, 7.8 mm ID × 30 cm column (1262-09R) and Competitor-S, 5 µm, 7.8 mm ID × 30 cm column



Note: this column was previously used for a column lifetime study for 1,255 injections of protein standard mixture

Compared to a competitive column, the TSKgel G3000SWXL column has much higher resolution of monomer and dimer peaks.

Figure 9: Backpressure of TSKgel G3000SWXL, 5 µm, 7.8 mm ID × 30 cm column

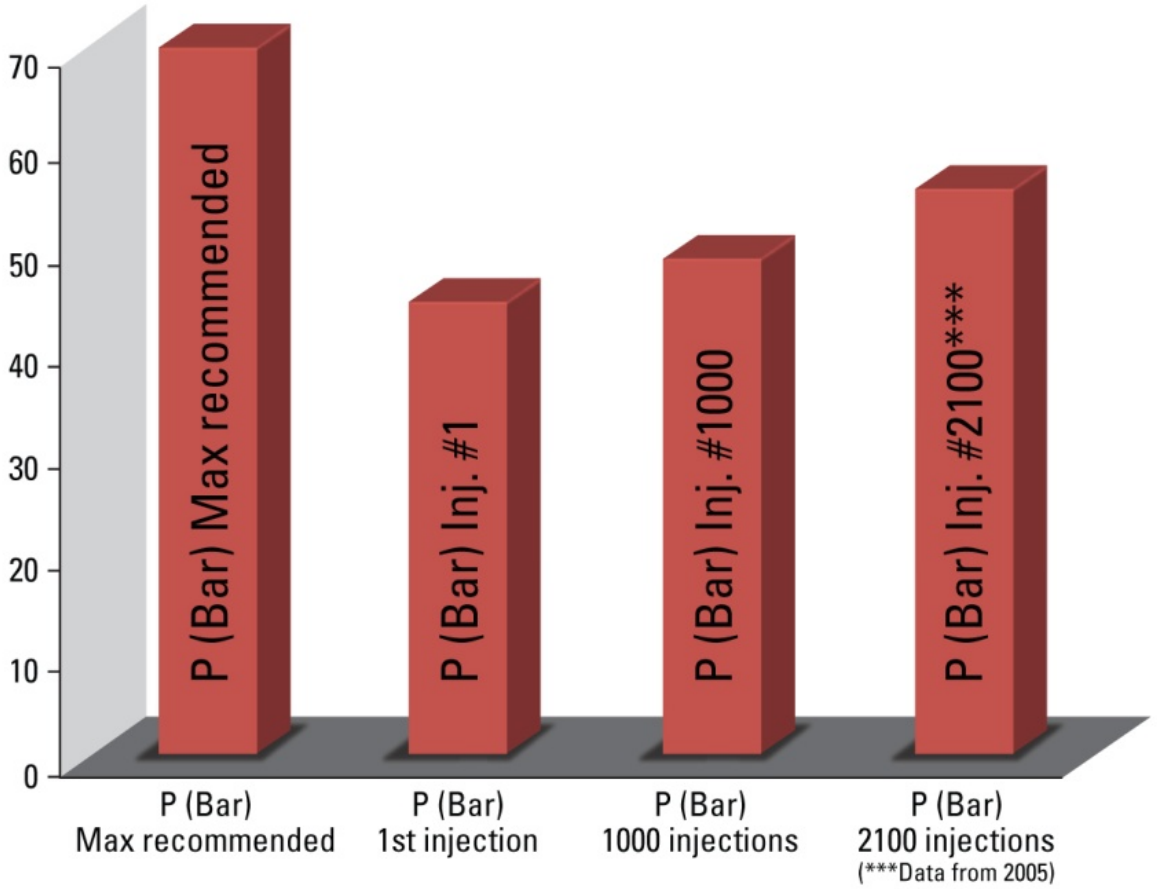
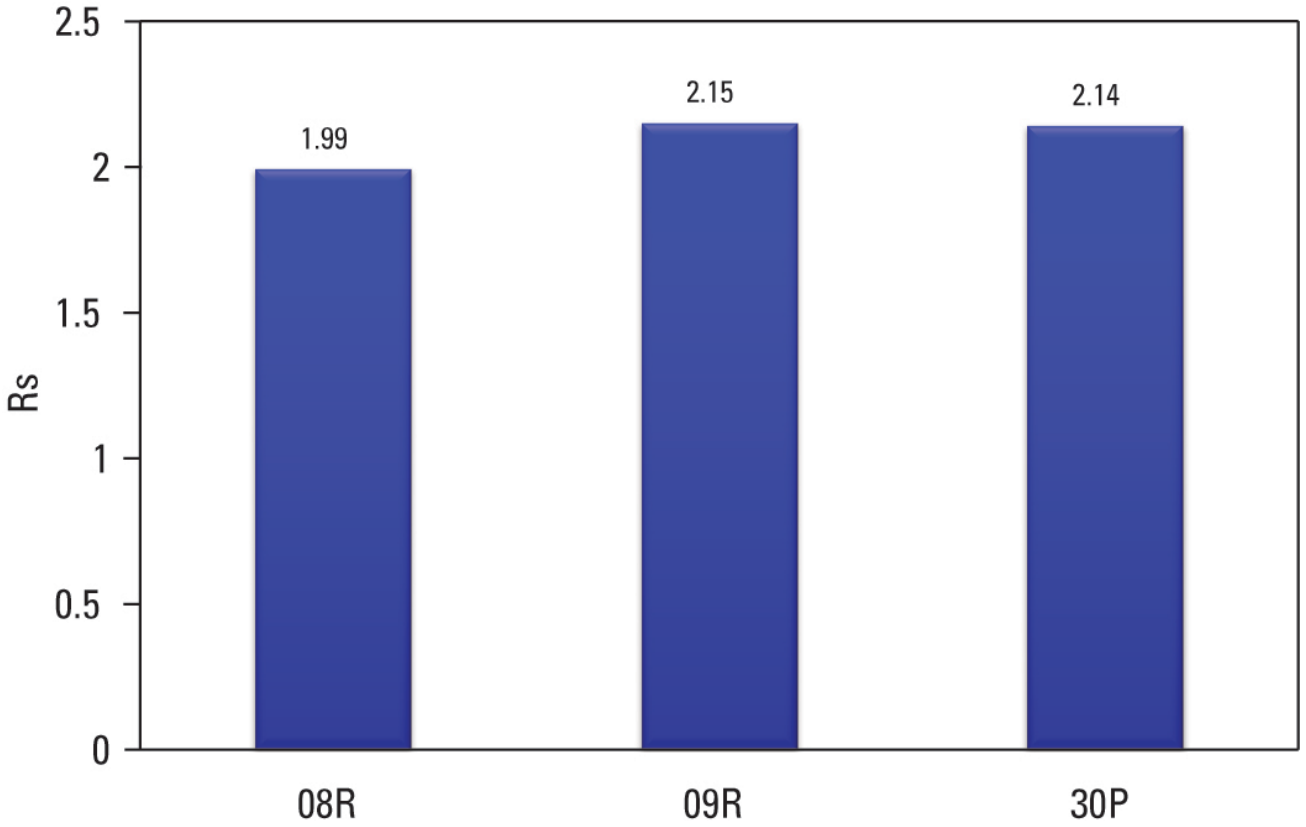


Figure 9: conclusions

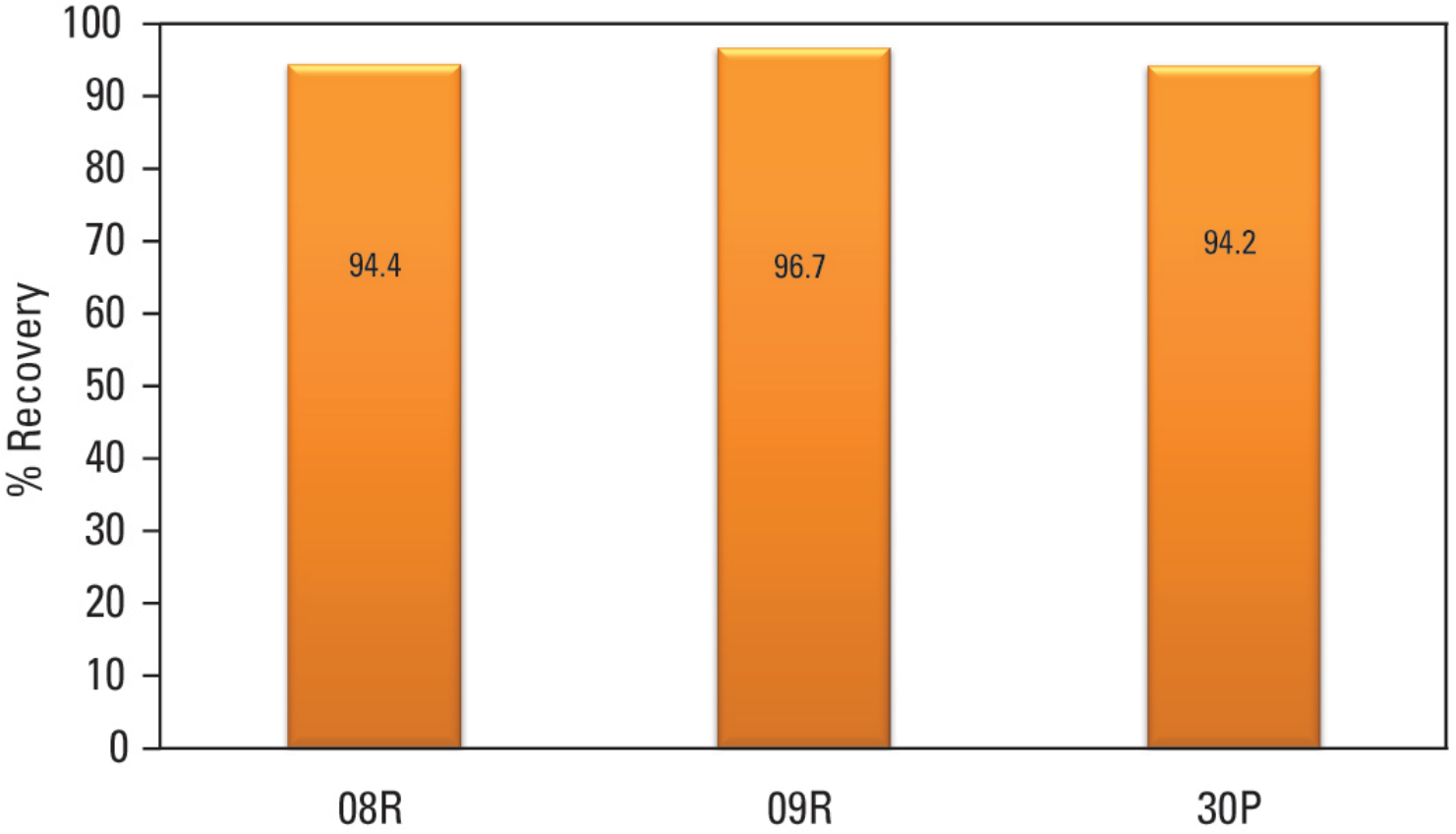
- In the 2005 study (marked by *** in the chart), the mobile phase was recycled to encourage column failure.
- The column maintained consistency in RT, AF and N over ~2,900 injections of protein standard mixture, after that the %RSD was greater than 5%.
- The columns never reached or exceeded pressure limit in this study.
- No significant lot-to-lot difference in back pressure was noticed.
- This study shows that the column maintained its consistency in terms of packing and bonding chemistry over the years.

Figure 10: Lot-to-lot variation in resolution between monomer and dimer peaks of BI-mAb-02 using a TSKgel G3000SWXL, 5 µm, 7.8 mm ID × 30 cm column



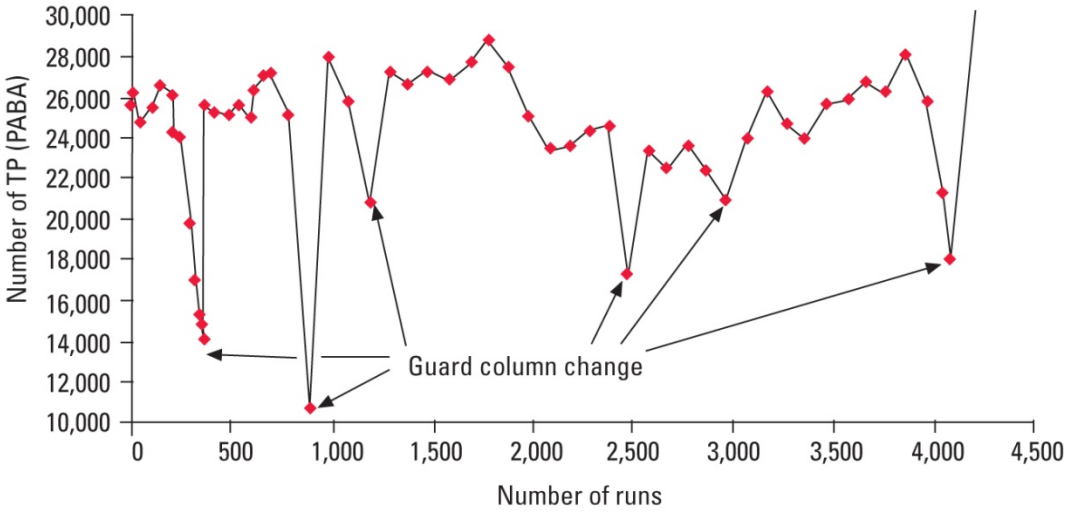
No significant lot-to-lot variation in resolution between monomer and dimer peaks of BI-mAb-02 was noticed.

Figure 11: Lot-to-lot variation in %recovery of BI-mAb-02 using a TSKgel G3000SW$_{XL}$, 5 µm, 7.8 mm ID × 30 cm column



%recovery was good and no significant lot-to-lot variation was noticed.

Figure 12: Guard column and column lifetime



Column:	TSKgel SuperSW3000, 4µm, 7.8mm ID x 30cm + guard column
Mobile phase:	0.1mol/L phosphate buffer + 0.1mol/L Na_2SO_4 + 0.05% NaN_3
Flow rate:	0.35mL/min
Detection:	UV@280nm, cell volume 1µL, response time 0.2sec.
Temperature:	ambient
Samples:	γ-globulin (156kDa), 1mg/mL mobile phase ovalbumin (43kDa), 1mg/mL mobile phase cytochrome C (12.4kDa), 0.5mg/mL mobile phase PABA (137kDa), 0.01mg/mL mobile phase

In systems consisting of analytical and guard columns, a loss in performance is often related mainly to the degradation of the guard column. Hence, separation performance can often be retained after exchanging the guard column only.

Conclusions

- A high degree of reproducibility of retention time, peak symmetry, and column efficiency over a large number of injections was achieved.
- These quality characteristics are independent of the lot of base silica as well as the bonding and packing procedures, as evident from this study using 9 different columns.
- There was very low percent relative standard deviation (%RSD) of <1% (n = 100) within the same lot and between the lots.
- Baseline separation of the monomer peak from the dimer peak of BSA was achieved with no shift in retention time over a wide loading range and without any splitting of the monomer peak.
- An overly used (>1,255 injections) TSKgel G3000SW$_{XL}$ column yielded better resolution of the monomer and dimer peak compared to an equivalent competitor column.

Conclusions continued

- No significant lot-to-lot variation in resolution between monomer and dimer peaks of monoclonal antibody BI-mAb-02 was evident.
- No significant difference in lot-to-lot variation of %recovery of BI-mAb-02 was obtained.
- Silica-based, diol-bonded size exclusion chromatography column TSKgel G3000SW$_{XL}$ is a reliable analytical HPLC column for protein separations.
- Since columns were studied without guard column and filtration of the sample, mobile phase, etc., lifetime of the column can further be improved by using these features to further protect the column.
- Please refer to the TosohTalk blog on the Tosoh Bioscience LLC website: “How long does a column last?” – a discussion on how to take care of columns to increase their lifetime at: www.tosohtalk.com