

## TN-1313

# Guard Column Lifetime and Capacity to Protect the Biozen™ dSEC-2 Column

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### Introduction

The capacity of the Biozen dSEC-2 Guard Column will prolong the efficiency of your analytical dSEC-2 column throughout hundreds of injections and incidental sample overloading. An ideal sample will prolong the life of an SEC column when prepared at a suitable concentration with the mobile phase as the diluent. Real-world samples often entail mixtures of proteins at different concentrations, and in some scenarios prepared in a diluent that does not entirely match the mobile phase. Such non-idealized samples present the challenge of overloading the column, introducing contamination, and subjecting the column to a solvent front from the diluent. The mechanical strength of the particle used for the dSEC-2 media affords robustness towards such challenges from a sample. The inclusion of the 3 µm dSEC-2 media within a formal guard will enhance performance by offering additional protection to the analytical column.

In this technical note, we evaluate the robustness of the guard and attached analytical column towards the efficiency of the Trastuzumab biosimilar Kanjinti® at suitable loading mass upon repeatedly injecting high loading masses of bovine serum albumin (BSA). The robustness of the guard alone is also reaffirmed upon performing repeated injections of Uridine dissolved in water.

### LC Conditions - Kanjinti

**Column:** Biozen 3 µm dSEC-2

**Dimensions:** 300 x 7.8 mm

**Part No.:** [00H-4788-KO](#)

**Guard Column:** Biozen dSEC-2 Guard Column

**Guard Dimensions:** 40 x 7.8 mm

**Part No.:** [03Q-4788-KO](#)

**Mobile phase:** 200 mM Potassium Phosphate  
+ 250 mM Potassium Chloride, pH 6.2

**Flow Rate:** 1 mL/min

**Detector:** UV @ 280 nm

**Temperature:** Ambient

**Sample:** See Table 1

### LC Conditions - Uridine

**Guard Column:** Biozen dSEC-2 Guard Column

**Guard Dimensions:** 40 x 7.8 mm  
30 x 4.6 mm

**Part Nos.:** [03Q-4788-KO](#) (7.8 mm)  
[03A-4788-E0](#) (4.6 mm)

**Mobile phase:** 100 mM Potassium Phosphate, pH 6.8  
+ 0.025 % (w/v) Sodium Azide

**Flow Rate:** 1 mL/min (7.8 mm)  
0.35 mL/min (4.6 mm)

**Detector:** UV @ 280 nm

**Temperature:** Ambient

**Sample:** Uridine, 0.05 mg/mL in water

**Table 1.** Sample Sequence

Line	Sample	Injection Volume	Loading Mass	Injection No.
1	Mobile-phase Blank	4 µL	--	2
2	Kanjinti (10 mg/mL)	4 µL	40 µg	5
3	Mobile-phase Blank	4 µL	--	2
4	BSA (20 mg/mL)	20 µL	400 µg	2

*NOTE: This sequence was repeated on a continuous cycle*



## Results and Discussion

The efficiency of an optimal 40 µg loading mass of Kanjinti® was demonstrated across injections that repeatedly followed high 400 µg loading masses of BSA, as shown in **Table 1**. The sample sequence continuously cycled 72 times over the span of 106 hours, during which the efficiency of a 40 µg loading mass of Kanjinti was monitored on the Biozen™ dSEC-2 guard column (40 x 7.8 mm) coupled to a Biozen dSEC-2 analytical column (300 x 7.8 mm).

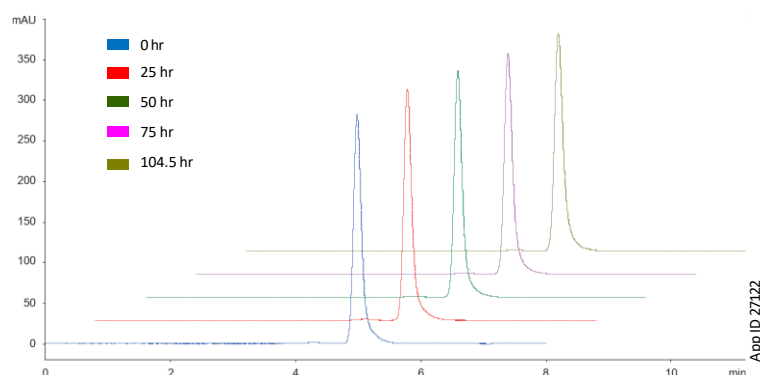
A total of 144 injections of BSA at high loading mass were injected on to the column during the 72 cycles of the experiment. Kanjinti incurred a 1.4 % loss of efficiency over 375 injections during the initial 50 hours, while a 6 % loss of efficiency was evident during the 562<sup>nd</sup> injection after 75 hours (**Figures 1 and 2**). Ultimately, Kanjinti exhibited a 10.8 % loss of efficiency during the 795<sup>th</sup> injection after 106 hours of repeating the sample sequence.

The guard column demonstrated protection of the analytical column and integrity of the analysis during the first 560 sample injections within the sequence, despite the persistent subjection of the column to injections of BSA at a high mass loading (400 µg). The gradual drop-off between injections (560 to 795) was likely due to the eventual breakthrough of the repeated injections of BSA.

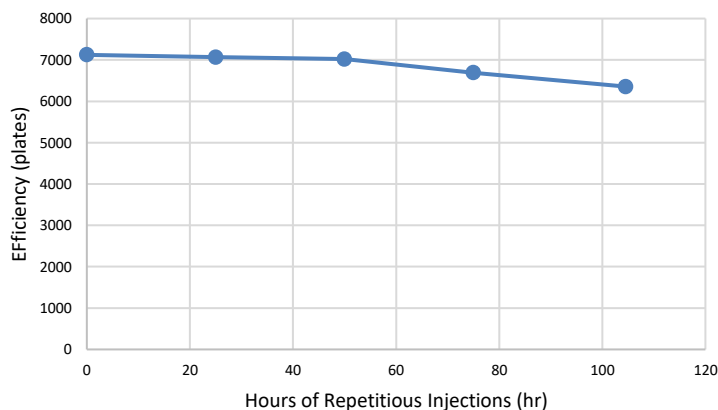
The lifetime of the guard column alone was demonstrated with thousands of repetitious injections of Uridine (**Figure 3**), for which the peak efficiency was monitored. Uridine was prepared at 0.05 mg/mL in water and evaluated repeatedly with a 2-minute analysis runtime. Uridine acts as a “fully included” analyte that speaks to the integrity of the guard column packing, as measured by the efficiency of the Uridine peak. Approximately 3300 injections over 121 continuous hours were needed before the guard column (40 x 7.8 mm) demonstrated a loss of 22 % efficiency, as shown in **Figure 4**. Uridine efficiency remained consistent with only a 2.8 % loss between the 17 hour and 101 hour timepoints, during which 2240 injections were made. The 30 x 4.6 mm guard column was also evaluated and demonstrated an efficiency loss of only 2.9 % from the first to last injection of Uridine over the course of 5000 injections.

The guard column did exhibit an initial efficiency drop of 11.7 % for Uridine within the first 17 hours and over 450 injections. This initial drop will not impact the performance of the analytical run when paired with a column, as demonstrated by the experiment with repetitious injections of both Kanjinti at optimal loading mass and BSA at high loading mass.

**Figure 1.** Chromatogram Overlay of Kanjinti on a 7.8 mm ID Biozen dSEC-2 Guard Column and Analytical Column



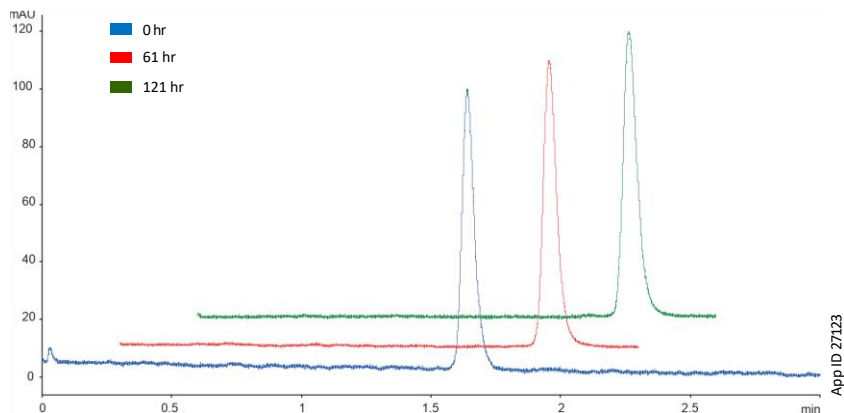
**Figure 2.** Efficiency for Kanjinti During Repeated Injections on a 7.8 mm ID Biozen dSEC-2 Guard Column and Analytical Column



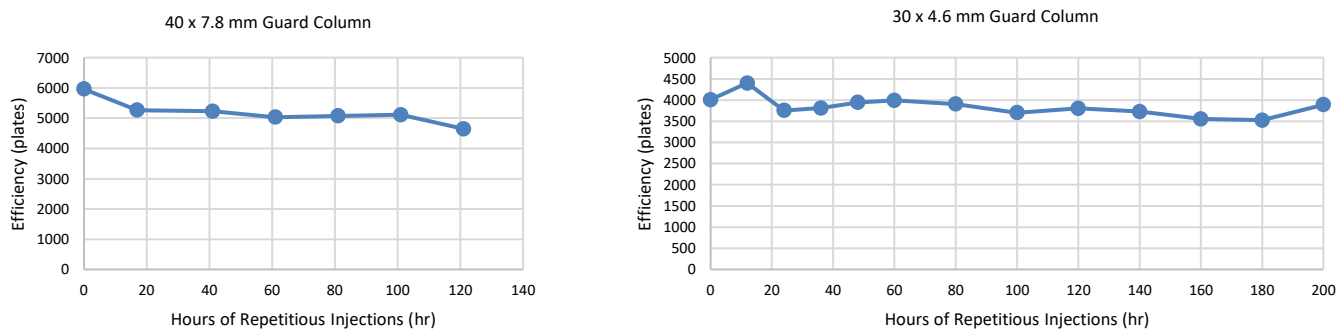
Hours	Efficiency	Percent Decrease
0 hr	7123 plates	--
25 hr	7067 plates	0.8 %
50 hr	7021 plates	1.4 %
75 hr	6693 plates	6.0 %
104.5 hr	6354 plates	10.8 %



**Figure 3.** Chromatogram Overlay of Uridine on a 40 x 7.8 mm Biozen™ Guard Column



**Figure 4.** Efficiency for Uridine During Repeated Injections on Biozen Guard Columns Only



## Conclusions

The Biozen dSEC-2 guard column has been demonstrated to protect your analytical dSEC-2 column from excessive loading masses from concentrated samples. The guard column has the capacity to mitigate the effects of large loading masses towards the protein analyte of interest. The analytical column will also be protected from any discrepant diluents in which a sample is prepared.



## Biozen™ dSEC Ordering Information

Biozen Columns						Biocompatible Guard Cartridges (mm)	
Phases	50 x 1.0	150 x 1.0	30 x 2.0	50 x 2.0	75 x 2.0	3/pk	ea
Biozen 1.8 µm dSEC-2	<a href="#">00B-4787-AN</a>	<a href="#">00F-4787-AN</a>	<a href="#">00F-4787-E0</a>	<a href="#">00H-4787-AN</a>	—	<a href="#">AJ0-9851</a>	<a href="#">AJ0-9000</a>
Biozen 3 µm dSEC-2	—	—	<a href="#">00F-4788-E0</a>	<a href="#">00H-4788-E0</a>	<a href="#">00F-4788-K0</a>	<a href="#">AJ0-9850</a>	<a href="#">AJ0-9000</a>
						For 4.6 mm	Holder

Biozen Guard Columns		
Phases	30 X 4.6	40 x 7.8
Biozen dSEC-2	<a href="#">03Q-4788-K0</a>	<a href="#">03A-4788-E0</a>



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