

# Effect of Different Column Lengths in SEC using Biozen™ dSEC-2 Columns

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## Overview

Column length can be a powerful tool with which to improve chromatographic resolution, particularly when working with Size Exclusion Chromatography (SEC). The impact of column length was demonstrated here with a sample of Cetuximab on two Biozen dSEC-2 LC columns using the same method conditions, instruments, particle size, and internal diameters.

The 150 mm length column (**Figure 1**) provides adequate separation of the dimer and monomer but delivers subpar separation of fragment 1. As fragment 1 elutes on the tail of the monomer, integration and an accurate % area are hard to reliably obtain. When the length of the column is increased to 300 mm, the % area of fragment 1 drastically increases, as summarized in **Table 1**. The longer column allows for increased separation of the dimer and fragments from the monomer, as well as sharper peak shape to facilitate accurate and reproducible integration of the peaks.

The downside of using a longer column can be the increase in method time and solvent consumption. **Figure 3** illustrates how a 6-minute method on the 150 mm column now becomes a 12-minute method on the 300 mm. Method time, solvent consumption, and accuracy of method should be balanced when deciding between a shorter or longer column when working with SEC.

## LC-UV Conditions

**Column:** Biozen 1.8  $\mu$ m dSEC-2

**Dimensions:** 150 x 4.6 mm

300 x 4.6 mm

**Part Nos.:** [00F-4787-E0](#) (150 mm)

[00H-4787-E0](#) (300 mm)

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

**Flow Rate:** 0.35 mL/min

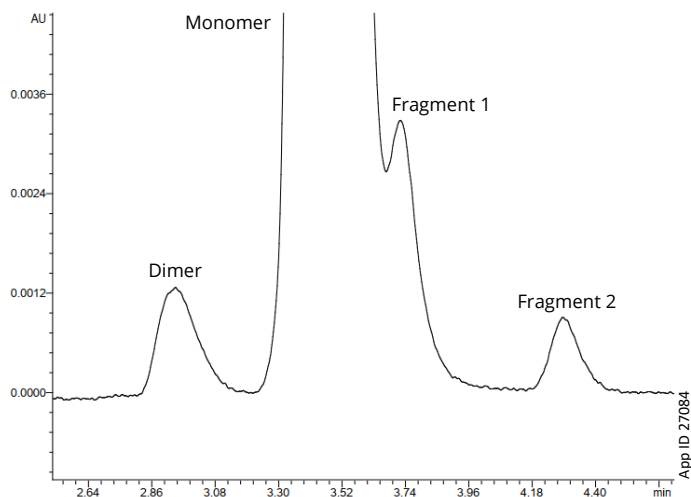
**Injection Volume:** 10  $\mu$ L

**Temperature:** 25  $^{\circ}$ C

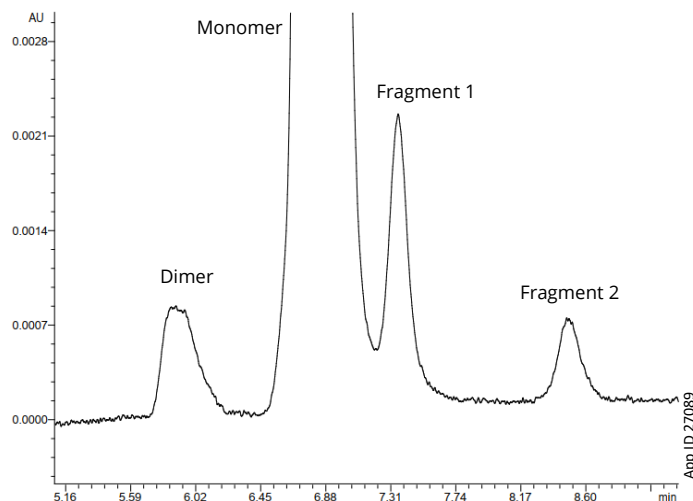
**Detection:** UV @ 280 nm

**Sample:** Cetuximab (2 mg/mL)

**Figure 1.** 150 mm Length Column



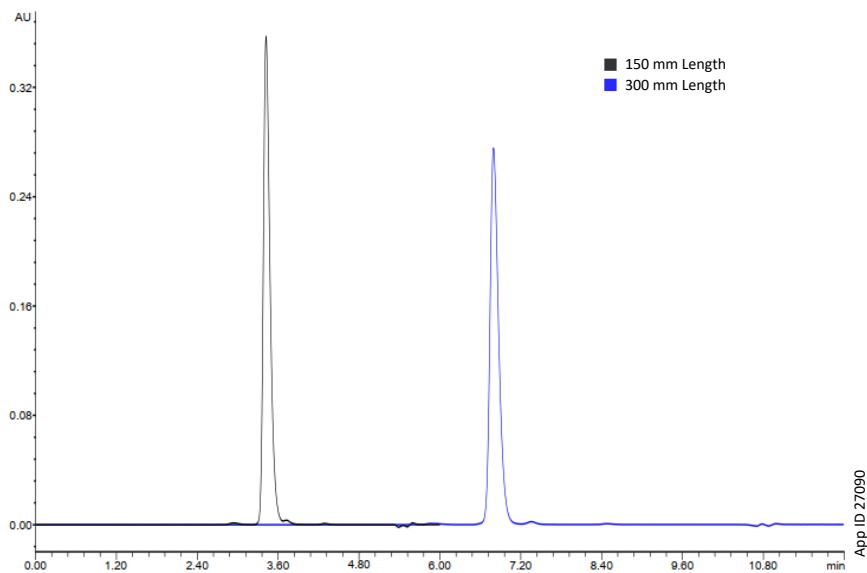
**Figure 2.** 300 mm Length Column



**Table 1.** Comparison of 150 mm to 300 mm Column Length

	Name	Tim (min)	Area	% Area	USP Rs
150 x 4.6 mm	Dimer	2.942	12377	0.49	-
	Monomer	3.421	2489604	98.98	2.15
	Fragment 1	3.768	6675	0.27	-
	Fragment 2	4.286	6587	0.26	-
300 x 4.6 mm	Dimer	5.858	10322	0.41	-
	Monomer	6.796	2458705	98.65	3.12
	Fragment 1	7.359	18816	0.75	2.32
	Fragment 2	8.473	4627	0.19	4.74

**Figure 3.** Overlay of Two Column Lengths



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