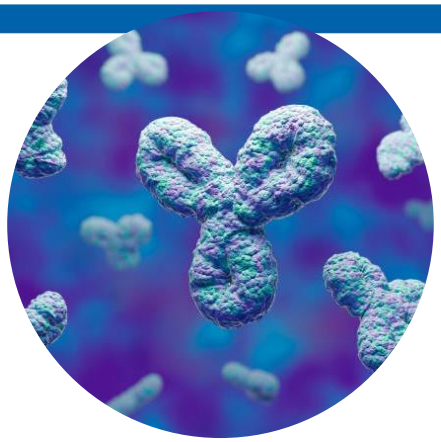

HPLC as a Complimentary Technique to Quantify Bevacizumab

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Overview

Bevacizumab is a monoclonal antibody used as an immunotherapy for several types of cancers. One common technique to quantify monoclonal antibodies (mAb) is the use of Enzyme-Linked Immunosorbent Assay (ELISA). ELISA offers some advantages, including a high selectivity and sensitivity and the possibility to perform analysis without complicated sample pre-treatment. However, it is very labor intensive and its elevated cost and relatively short expiration date due to antibody instability limits its use in routine analytical quantification. There is also a high potential for false positive/negative results which leads to variability and low confidence in those results. In many instances there is inadequate inhibition of immunogenic antigens due to cross reactivity, leading to weak (or low) signal intensity.

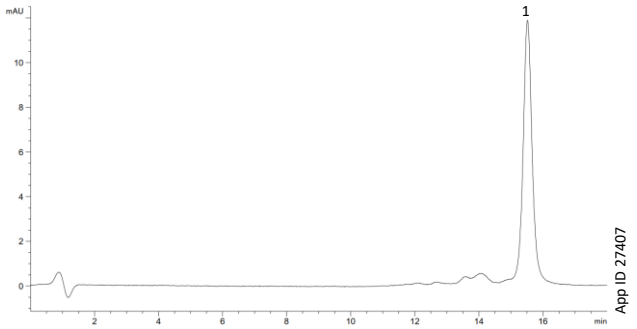
High-performance Liquid Chromatography (HPLC) alleviates many of the common challenges associated with ELISA. It is a simpler technique that typically has a shorter total time for analysis. Compared to ELISA, HPLC is low-cost, more reproducible, and more sensitive, providing more reliable results. As such, HPLC is a more suitable tool in discovery, development, and manufacturing environments.

Employing an HPLC method allowed us to evaluate the capability of albumin-based nanoparticles to encapsulate Bevacizumab, to study its release from these nanoparticles in simulated physiological media and show adequate separation between peaks from Human Serum Albumin (HSA) (65 kDa) and Bevacizumab (150 kDa). In this application note, we provide a simple, rapid, and low-cost method for the quantification of Bevacizumab formulated in HSA-based nanoparticles using a Biozen 3 µm dSEC-2 HPLC Column. The bioinert hardware and hydrophilic surface chemistry of the Biozen dSEC-2 column offers sufficient resolution to reliably separate and quantify both Bevacizumab and HSA reproducibly.

LC Conditions

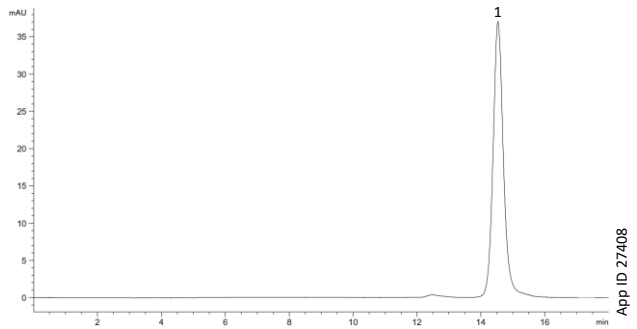
Columns:	Biozen™ 3 µm dSEC-2
Dimensions:	300 x 4.6 mm
Part No.:	00H-4788-E0
Mobile Phase:	35 mM Phosphate Buffer + 300 mM NaCl, pH 6.8
Flow Rate:	0.2 mL/min (Isocratic)
Injection Volume:	10 µL
Temperature:	25 °C
Detector:	PDA @ 280 nm
LC System:	Agilent® 1200 Series

Figure 1. 200 µg/mL HSA in an Aqueous Solution on Biozen™ 3 µm dSEC-2 Column.



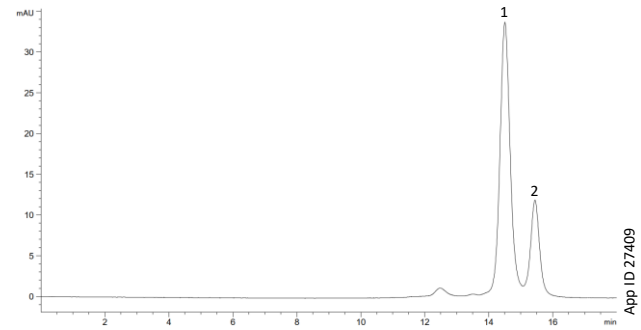
Peak No.	Analyte	Retention Time (min)	Width	Area	Height
1	HSA	15.518	0.291	224.74	11.65

Figure 2. 200 µg/mL Bevacizumab in an Aqueous Solution on Biozen 3 µm dSEC-2 Column.



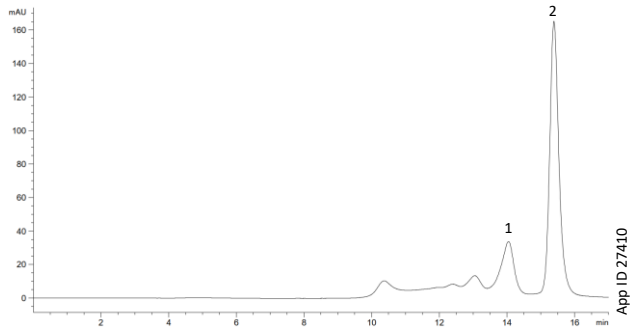
Peak No.	Analyte	Retention Time (min)	Width	Area	Height
1	Bevacizumab	14.534	0.345	846.44	37.01

Figure 3. 200 µg/mL HSA and 200 µg/mL Bevacizumab in an Aqueous Solution on Biozen 3 µm dSEC-2 Column.



Peak No.	Analyte	Retention Time (min)	Width	Area	Height
1	Bevacizumab	14.504	0.329	704.86	32.83
2	Human Serum Albumin	15.445	0.279	199.00	11.01

Figure 4. Bevacizumab Encapsulated in HSA Nanoparticles on Biozen 3 µm dSEC-2 Column.



Peak No.	Analyte	Retention Time (min)	Width	Area	Height
1	Bevacizumab	14.045	0.384	805.76	30.49
2	Human Serum Albumin	15.387	0.288	3113.16	163.40

Table 1. Comparison Between the Quantification of Bevacizumab by ELISA versus HPLC.

	ELISA	HPLC
Lowest limit of quantification	30 ng/mL	12.5 µg/mL
Total time for analysis (min)	70	17
Sample numbers	96	Depends on the lifetime of the column (~1000 injections)
Bevacizumab loading (µg Bevacizumab/mg formulation)*	26 ± 4	28 ± 2

*Data expressed as Mean ± SD, n=10.

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