Detection of Protein Heterogeneity by HPLC

TSKgel
APPLICATION NOTE

Introduction

Protein heterogeneity is generated by post-translational modification, decomposition, and a variety of other processes, including chemical modification, denaturation, and aggregation. Since antibodies and recombinant proteins are now widely used for therapeutic treatment, it is essential to evaluate their heterogeneity during development, stability testing, and in the quality control of the final product. Analysis of the aggregates and denatured proteins is also important because they might increase the risk of anaphylaxis or immunoreaction.

The detection and separation of protein heterogeneity by HPLC can be performed using four different modes of TSKgel chromatography columns: Size Exclusion (SEC), Reversed Phase (RPC), Ion Exchange (IEX) and Hydrophobic Interaction (HIC). In this application note, the use of a TSKgel Protein C_4-300 reversed phase column is shown to be applicable for the evaluation of protein heterogeneity. The silica-based, wide pore 300 Å TSKgel Protein C_4-300 column packed with 3 µm particles is optimized for the separation of large biomolecules such as proteins.

Experimental Conditions

Conditions for SEC

Column:	TSKgel G3000SW$_{XL}$, 7.8 mm ID x 30 cm x 2
Mobile phase:	20 mmol/L phosphate buffer, pH 7.0 + 0.3 mol/L NaCl
Flow rate:	1.0 mL/min
Temperature:	25° C
Sample:	monoclonal antibody (human IgG_1)

Conditions for RPC

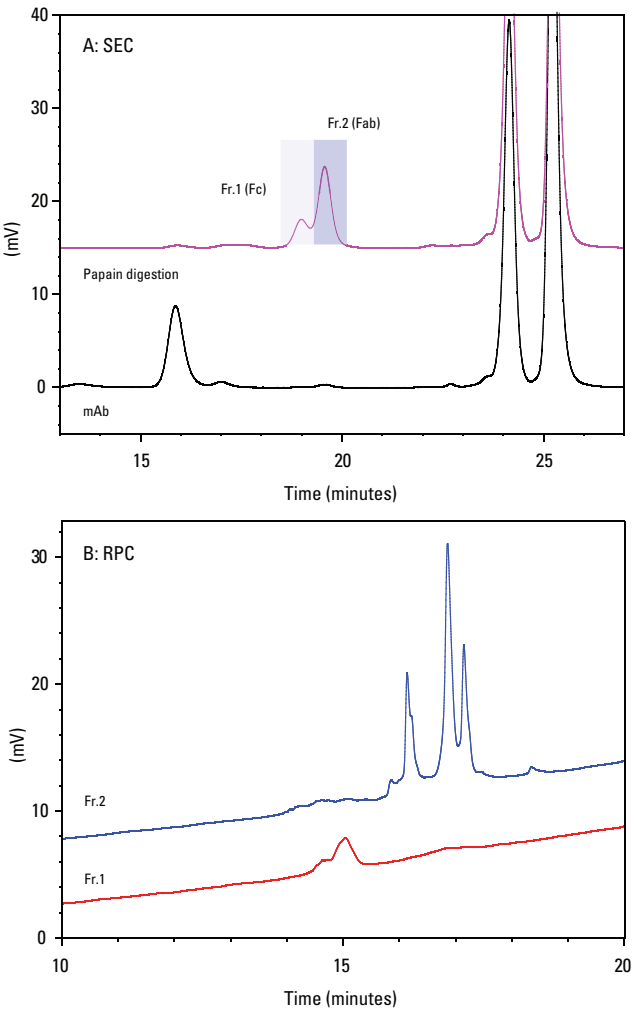
Column:	TSKgel Protein C_4-300, 4.6 mm ID x 15 cm
Mobile phase:	A: 0.05% TFA in water
	B: 0.05% TFA in acetonitrile
Gradient:	0 min (5% B), 20 min (50% B)
Flow rate:	1.0 mL/min
Temperature:	70° C
Sample:	monoclonal antibody (human IgG_1)

Results and Discussion

Figures A & B show the analysis of antibody fragments. The monoclonal antibody human IgG_1 was first papain digested and separated using a TSKgel G3000SW$_{XL}$ SEC column *(Figure A)*. The intact form of the antibody, partially digested fragments, and completely digested fragments were separated on the basis of molecular size.

Two fractions were obtained from the SEC analysis and each fraction was analyzed with the TSKgel Protein C_4-300 reversed phase column, as shown in *Figure B*. Several peaks were observed in each chromatogram of the analysis of Fc (fragment 1) and Fab (fragment 2), indicating that the antibody used in this study was heterogeneous in hydrophobicity.

Figures A & B. Analysis of antibody fragments



For figure conditions, see experimental conditions section.

Conclusions

TSKgel columns of IEC, SEC, HIC, and RPC modes are excellent choices to determine protein heterogeneity. The TSKgel Protein C_4-300 column, which has a large pore size of 300 Å, is suitable for highly-efficient, reversed phase separations of proteins such as recombinant proteins, antibody fragments or PEGylated proteins.

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