Development of a High Resolution SEC Column Suited for Analysis of Monoclonal Antibody Aggregates and Fragments

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Introduction

Antibody drugs are expected to enhance the effect of therapies without adverse side effects. During the manufacturing processes of immunoglobulin G (IgG), which is a main ingredient of antibody drugs, its aggregates and fragments could be generated as impurities. Size exclusion chromatography (SEC) analysis is known as a common method for determination of IgG characteristics as well as quality control in the production processes of IgG. YMC has developed a new SEC column with 2 µm particle size and Diol bonded phase. High resolution analysis of aggregates and fragments derived was performed with this new SEC column. Furthermore, smooth method transfer from conventional 5 µm SEC column to this new 2 µm SEC column could also be achieved while reducing analysis time by using a shorter column. In this poster, we will introduce these advantages of this 2 µm Diol SEC column through several examples of IgG aggregates and fragments analysis.

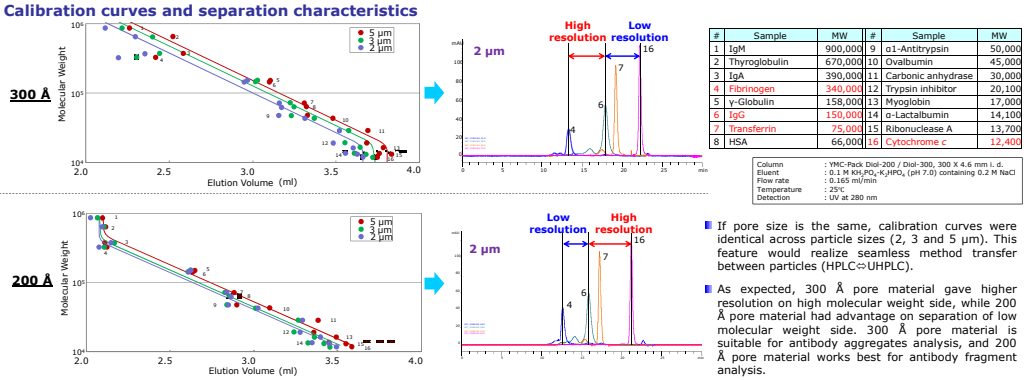
Basic characteristics

Specifications of novel Size Exclusion Diol column

Product name	Pore size (Å)	Particle size (µm)	Base material	Functional group	Pressure limit
Diol-200	200	2 3 5	Porous silica	Dihydroxy propyl	2 µm: 6530 psi (45 MPa) 3,5 µm: 2900 psi (20 MPa)
Diol-300	300				

- Silica gel based size exclusion chromatography (SEC) columns bonded with dihydroxypropyl group.
- High pressure tolerance that is sufficient to withstand operating pressure required for high throughput SEC analyses
- Very low nonspecific adsorption of biomacromolecules, including monoclonal antibodies

Calibration curves and separation characteristics



#	Sample	MW	#	Sample	MW
1	IgM	900,000	9	α1-Antitrypsin	50,000
2	Thyroglobulin	670,000	10	Ovalbumin	45,000
3	IgA	390,000	11	Carbonic anhydrase	30,000
4	Fibrinogen	340,000	12	Trypsin inhibitor	20,100
5	γ-Globulin	158,000	13	Myoglobin	17,000
6	IgG	150,000	14	α-Lactalbumin	14,100
7	Transferrin	75,000	15	Ribonuclease A	13,700
8	HSA	66,000	16	Cytochrome *c*	12,400

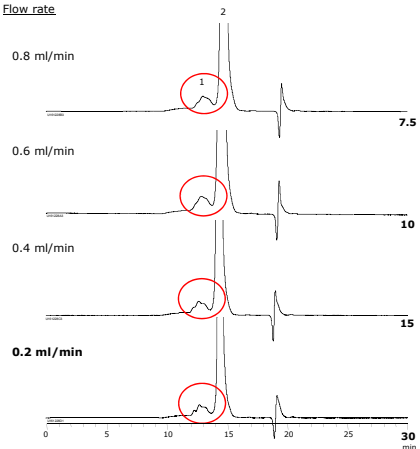
Column : YMC-Pack Diol-200 / Diol-300, 300 X 4.6 mm i. d.
Eluent : 0.1 M KH_2PO_4-K_2HPO_4 (pH 7.0) containing 0.2 M NaCl
Flow rate : 0.165 ml/min
Temperature : 25°C
Detection : UV at 280 nm

- If pore size is the same, calibration curves were identical across particle sizes (2, 3 and 5 µm). This feature would realize seamless method transfer between particles (HPLC⇔UHPLC).
- As expected, 300 Å pore material gave higher resolution on high molecular weight side, while 200 Å pore material had advantage on separation of low molecular weight side. 300 Å pore material is suitable for antibody aggregates analysis, and 200 Å pore material works best for antibody fragment analysis.

Analysis of monoclonal antibody and its aggregates

Influence of flow rate on antibody analysis

Diol-300 , 2 µm, 300 X 4.6 mm i. d.

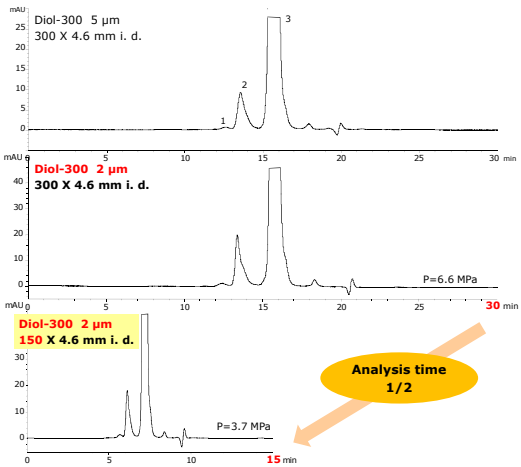


Flow rate (ml/min)	N (2)	P (MPa)
0.8	7,300	28.3
0.6	8,900	20.9
0.4	9,400	14.4
0.2	15,200	7.2

Column : YMC-Pack Diol-300, 2 µm, 300 X 4.6 mm i. d.
Eluent : 0.1 M KH_2PO_4-K_2HPO_4 (pH 7.0) containing 0.2 M NaCl
Detection : UV at 280 nm
Temperature : ambient
Sample : Humanized monoclonal IgG1

- Theoretical plate count and resolution of monoclonal antibody increased as flow rate was decreased. This suggests that lower flow rate is preferable if scope is high resolution separation analysis. On the other hand, higher flow rate is suitable for increasing analysis throughput, especially when resolution is sufficient.
- The backpressure at 0.8 ml/min with 300 mm length by 4.6 mm i. d. column was within the pressure limit.

Improvement of resolution / analysis throughput by using the 2 µm column

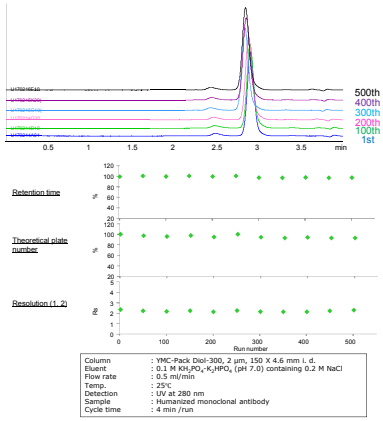


Particle size (µm)	Column size length X i. d. (mm)	N (3)	Rs (1,2)	Rs (2,3)
5	300 X 4.6	8,500	0.88	2.67
2	300 X 4.6	16,200	1.17	4.15
2	**150 X 4.6**	8,700	0.85	2.75

Column : YMC-Pack Diol-300
Eluent : 0.1 M KH_2PO_4-K_2HPO_4 (pH 7.0) containing 0.2 M NaCl
Flow rate : 0.2 ml/min
Detection : UV at 280 nm
Temperature : ambient
Sample : Humanized monoclonal IgG1

- 2 µm YMC-Pack Diol in 300 X 4.6 mm i. d. column greatly improved resolution between aggregates and monomer peak compared to 5 µm YMC-Pack Diol in the same dimension.
- On 2 µm, 150 mm length column, resolution between antibody monomer and aggregates was the same as the 5 µm, 300 mm length column. This would suggest that analysis time can be reduced by half just by changing the column from 5 µm, 300 mm length column to 2 µm, 150 mm length column.
- Operating pressure was low enough to run with conventional HPLC system. **Special care is needed in terms of minimizing system volume in order to avoid extra-column band spreading when using at low flow rate.*

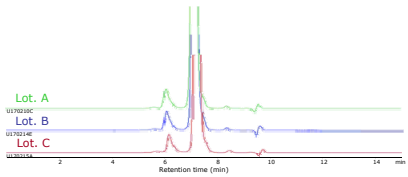
Evaluation of column durability



Column : YMC-Pack Diol-300, 2 µm, 150 X 4.6 mm i. d.
Eluent : 0.1 M KH_2PO_4-K_2HPO_4 (pH 7.0) containing 0.2 M NaCl
Flow rate : 0.5 ml/min
Temp. : 25°C
Detection : UV at 280 nm
Sample : Humanized monoclonal antibody
Cycle time : 4 min /run

- Stability of 2 µm YMC-Pack Diol column on monoclonal antibody analysis was evaluated. Performance of the column was maintained for 500 injections, and expected to last much longer.

Lot-to-Lot reproducibility



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Eluent : 0.1 M KH_2PO_4-K_2HPO_4 (pH 7.0) containing 0.2 M NaCl
Flow rate : 0.2 ml/min
Temp. : 25°C
Detection : UV at 280 nm
Sample : Humanized monoclonal antibody

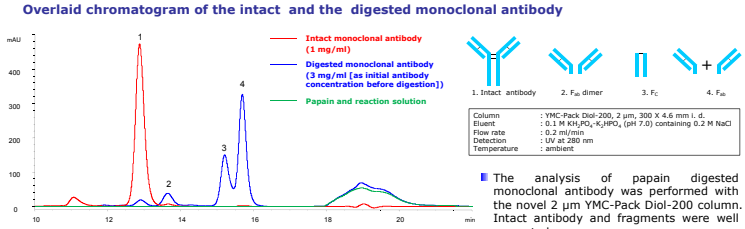
- Separation reproducibility of 2 µm YMC-Pack Diol column between manufacturing lots was evaluated. Separation was identical between lots. This feature would contribute to robust method development.

Analysis of monoclonal antibody fragments

Method of Papain digestion

1) 50 mM KH_2PO_4-K_2HPO_4 (pH 7.5) buffer was prepared.
2) Papain and other components were added to the buffer at the following concentrations.
   - Papain 0.1 g/l
   - NaCl 0.15 mol/l
   - L-cystein 0.01 mol/l
   - EDTA 0.01 mol/l
3) A 3 mg/mL sample of monoclonal antibody was made using solution 2 as the diluent.
4) The solution was stored for 17.5 hours at 25 °C.

Overlaid chromatogram of the intact and the digested monoclonal antibody



Column : YMC-Pack Diol-200, 2 µm, 300 X 4.6 mm i. d.
Eluent : 0.1 M KH_2PO_4-K_2HPO_4 (pH 7.0) containing 0.2 M NaCl
Flow rate : 0.2 ml/min
Detection : UV at 280 nm
Temperature : ambient

- The analysis of papain digested monoclonal antibody was performed with the novel 2 µm YMC-Pack Diol-200 column. Intact antibody and fragments were well separated.

Conclusions

The new 2 µm Diol SEC columns exhibited improved resolution and allowed higher throughput of monoclonal antibody analyses.

This feature would offer significant advantages for antibody analyses focused on aggregate and fragment control in research as well as quality control of pharmaceutical products.