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Application News

USP-Compliant Analysis of Antibody Drugs Using Size-Exclusion Chromatography (SEC)

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User Benefits

- The United States Pharmacopeia (USP)-compliant system compatibility test for antibody drugs can be performed with good repeatability.
- The corrosion-resistant Nexera lite inert ensures stable data collection even employing mobile phases with high salt concentration.

■ Introduction

Proteins easily aggregate due to variances in temperature, pH, and concentration during manufacturing process and storage. Since aggregation of protein products such as monoclonal antibodies (mAbs) may affect safety and efficacy, the degree of aggregation of the products is confirmed by dedicated analytical procedures [1]. Size exclusion chromatography (SEC) is available as a method to confirm aggregation, and USP-NF 2022, Issue 2, General chapter <129> describes a procedure to analyze impurities in monoclonal antibodies using SEC [2]. Since this method employs high concentration of salt in the eluent, there are some concerns about instrument deterioration due to precipitation and corrosion. This article introduces USP-complied SEC analysis of monoclonal IgG antibodies using Nexera lite inert, high-performance liquid chromatograph (HPLC), which is more durable against salt and acid than ordinary HPLC using stainless steel for wetted parts.

Fig. 1 Nexera™ lite inert

■ System Suitability Test

Table 1 and Table 2 show the analytical conditions and the acceptance criteria for the system suitability test, respectively. Test samples were prepared to make concentration of 10 mg/mL by adding 200 µL of eluent to 2 mg of monoclonal IgG antibody standard (Monoclonal IgG System Suitability (2 mg), USP reference standard). The system suitability test requires two items: "Chromatogram Similarity" and "Quantitative Criteria".

Table 1 Analytical Conditions

Column	: TSKgel G3000 SWXL (300 mm × 7.8 mm I.D., 5 µm)
Mobile phase	: 200 mmol/L Potassium Phosphate buffer containing 250 mmol/L KCl (pH 6.2)
Flow rate	: 0.5 mL/min
Column temp.	: 25 °C
Injection vol.	: 20 µL
Vial	: SHIMADZU TORAST™-H Glass *1
Detection	: UV 280 nm (Inert cell)

*1 P/N: 370-04302-01

Table 2 Acceptance Criteria of System Suitability Test

Chromatogram Similarity	Obtained chromatographic profile is coincident with that shown in USP Certificate (Fig. 2). As follows specifically. ①All following peaks are confirmed • high-molecular-weight species (HMWS) • Monomer • low-molecular-weight species (LMWS) ②Resolutions are good enough ③Elution order is identical as that of Fig. 2 HMWS→Monomer→LMWS
Quantitative Criteria	Normalized peak areas are within following ranges. HMWS: 0.4-0.67 % Main Peak*2: 99.1-99.6 % LMWS: Not more than 0.2 %

*2 Main Peak = Monomer

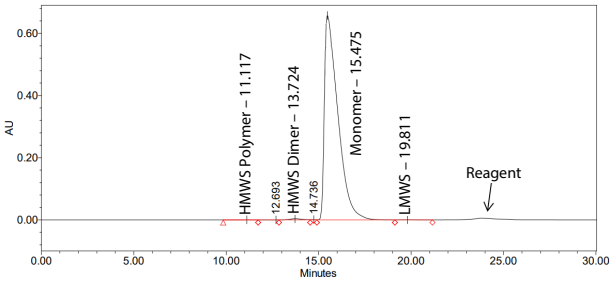

Fig. 2 Chromatogram Shown in USP Certificate [3]
(Excerpt from USP Certificate)

Fig. 3 shows the chromatogram (black) of the IgG standard solution (10 mg/mL) and calibration curve (blue). Four peaks listed in the USP Certificate were eluted in the elution order specified in the USP Certificate. Peak identifications were carried out based on the molecular weights obtained from the calibration curve created using protein standards of which the molecular weights ranged from 13.7 kDa to 500 kDa. For all peaks, the same degrees of resolutions were obtained as shown in Fig. 2. Table 3 shows the results of the system suitability test. The normalized respective peak areas were within the allowable range described in Quantitative Criteria. Table 4 shows the results of six consecutive analyses of the monoclonal IgG antibody USP standard (10 mg/mL). Obtained repeatability of %RSD = 0.01 for the normalized monomer peak area was good enough.

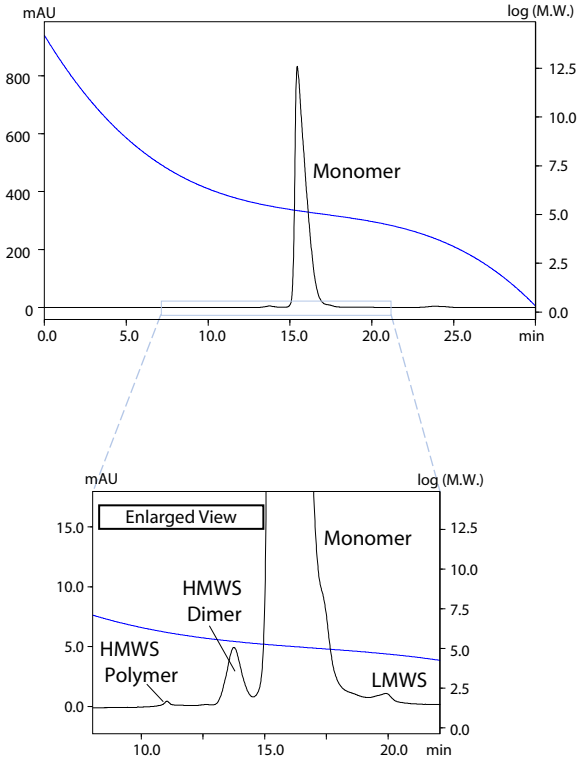

Fig. 3 Chromatogram (black) of the Monoclonal IgG Antibody Standard Solution (10 mg/mL) and Calibration Curve (Blue)

Table 3 Results of System Suitability Test

	Area (%)	Judgements
HMWS (Polymer+Dimer)	0.53	Passed
Monomer	99.4	Passed
LMWS	0.04	Passed

Table 4 Repeatabilities of Elution Time and Normalized Peak Area (n=6)

	Elution time (min)	Area (%)
1	15.448	99.425
2	15.457	99.417
3	15.460	99.428
4	15.454	99.426
5	15.460	99.431
6	15.459	99.434
Average	15.456	99.427
%RSD	0.03	0.01

■ Conclusion

SEC analysis of monoclonal IgG antibodies was performed in accordance with USP-NF 2022, Issue 2 General chapter <129>, and the analytical results were confirmed to be within the allowable ranges of the Quantitative Criteria. In addition, Nexera lite inert was able to provide good repeatability of analyses even employing mobile phase containing high concentrations of salt.

<References>

(1) Iyakushinhatsu (Pharmaceutical Affairs Bureau Notification) No. 571 corresponding to ICH Q6B "SPECIFICATIONS : TEST PROCEDURES AND ACCEPTANCE CRITERIA FOR BIOTECHNOLOGICAL /BIOLOGICAL PRODUCTS"

(2) USP-NF 2022, Issue 2 <129>Analytical Procedures for Recombinant Therapeutic Monoclonal Antibodies

(3) USP Certificate Typical Chromatogram
USP Monoclonal IgG System Suitability RS

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