投 稿 ▶原著

バイオ医薬品の品質評価に用いられるサイズ排除クロマトグラフィーの多機関共同測定による分析性能評価と，試験法設定における留意点の考察

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Interlaboratory Evaluation of the Analytical Performance of Size Exclusion Chromatography for Small Aggregates in Biopharmaceuticals and Considerations on Development of Analytical Procedures

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Summary

Because protein aggregates may induce an immune response, the amount of aggregates in biopharmaceuticals is considered as a critical quality attribute. Protein aggregation could occur during manufacture and storage, so the amount of aggregates should be controlled appropriately. Many analytical techniques are used for the analysis of protein aggregates, depending on their size. Size exclusion chromatography (SEC) is often employed to evaluate small aggregates, such as dimers and multimers, due to its ease of use, high reproducibility and relatively high throughput. In this multi-laboratory study, a questionnaire survey of analytical conditions was conducted, and then analytical performance was evaluated using a therapeutic monoclonal antibody and forcibly degraded samples in order to clarify key points for consideration in developing SEC test procedures for small aggregates in biopharmaceuticals. The effects of operating parameters on analytical performance is discussed. Samples were analyzed using a TSKgel G3000SW$_{XL}$ column with a mobile phase of 0.3 mol/L sodium chloride in 100 mmol/L sodium phosphate buffer, pH 7.0. The results showed that the repeatability and reproducibility of percent area of high-molecular weight species were < 3 % and < 10 %, respectively. Furthermore, the values of percent area were consistent with results obtained by analytical

ultracentrifugation. When the concentration of sodium chloride in the mobile phase was decreased to 0.2 mol/L, the percentage of larger aggregates was decreased due to adsorption, while the percentage of dimer was not changed. Considering that SEC is also used for stability testing, it is necessary that SEC can adequately evaluate the levels of larger and adhesive aggregates, which may not be present in the drug substance or drug products at the time of release testing. Evaluation using forcibly degraded samples is important during analytical validation.

Key words

Size exclusion chromatography, Biopharmaceuticals, Protein aggregate, Multi-laboratory study, Therapeutic antibody