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日本薬局方糖鎖試験法の国際調和に関する研究 —日局参考情報「単糖分析及びオリゴ糖分析／糖鎖プロファイル法」への標準的な糖鎖試験の手順の追加に関する考察*3 —

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A Study of Standard Glycan Profiling Procedures for an Additional Supplement to JP General Information, Monosaccharide Analysis and Oligosaccharide Analysis/Oligosaccharide Profiling

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Summary

Glycoprotein biopharmaceuticals have heterogeneity in glycosylation, and changes of heterogeneity may have great impacts on safety and efficacy by modulating various properties, including clearance from the circulation, immunogenicity and functions. The glycan structures and heterogeneity of biopharmaceuticals may be influenced by subtle changes of culture conditions and purification procedures. Recently, a variety of control strategies and analytical methods have come to be used to regulate and monitor glycosylation heterogeneity based on quality-by-design strategies and advances in analytical technology and glycobiology. In order to clarify the necessary points to evaluate and control the glycosylation heterogeneity of biopharmaceuticals, Japanese pharmacopoeia (JP) general test <2.64> Glycosylation analysis of glycoprotein and general information monosaccharide analysis and oligosaccharide analysis/oligosaccharide profiling are included in the 17th edition, which became official from April 1st, 2016. This provides methods and general requirements, and useful information to develop a glycan analysis test. The United States Pharmacopeia (USP) and Europe Pharmacopoeia (EP) incorporated Glycan Analysis in 2011. Although there are some differences in the glycan analysis chapters of USP, EP and JP, they are essentially the same in terms of quality assurance of glycoprotein biopharmaceuticals. The next issue is whether representative procedures of oligosaccharide profiling should be added to the general information. Because glycan analysis is product-specific, analytical procedures have not been included. However, adding representative procedures to JP general information would be convenient for users who are going to develop oligosaccharide profiling methods, even though the procedures must be optimized for a specific product and then validated. We previously reported a standardized glycan profiling method for antibody therapeutics, which have mainly fucosylated neutral biantennary oligosaccharides. In this study, we optimized sample preparation procedures and mixed-mode hydrophilic interaction and anion-exchange chromatography of 2-aminobenzamide-labeled glycans. α1 Acid glycoprotein is used as a model for acidic glycans, and bovine ribonuclease B and two monoclonal antibodies derived from CHO and NS0 are used as models for neutral glycans. Furthermore, we considered the contents, description format, and useful analysis methods and techniques depending on the characteristics of glycan structures in order to add representative procedures to the general information.

Key words

Glycan analysis, Oligosaccharide profiling, Japanese Pharmacopoeia, Hydrophilic interaction chromatography