投稿 ▶原著

複数種の液体クロマトグラフ質量分析計を用いたモデル核酸医薬品の分析データの比較

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Comparison of Analysis Data of Model Oligonucleotide Therapeutics Obtained with Different Types of Liquid Chromatograph–Mass Spectrometers

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Summary

In evaluating the quality of oligonucleotide therapeutics, it is necessary to pay close attention to the presence of oligonucleotide impurities. As an analysis method, LC/MS, combining liquid chromatography (LC) and mass spectrometry (MS), is generally used for quality assessment. However, since the physicochemical properties of active pharmaceutical ingredient (API)–derived impurities are likely to be similar to those of the API, there are technical limitations on their separation and purification. Therefore, it is important to be aware of such limitations and to understand the capabilities of the analytical techniques used in evaluating the quality of oligonucleotide therapeutics with due consideration of the characteristics of the oligonucleotides and their manufacturing processes.

In order to achieve this, we analyzed model oligonucleotides using several different types of commercial liquid chromatograph–mass spectrometers (LC–MS). We investigated the LC separation of impurities from the parent oligonucleotide, the relative quantification of impurities, the characterization of the parent oligonucleotide, and the identification of impurities. In the LC separation of the impurities from the parent oligonucleotide, none of the LC–MS analyses achieved complete separation (Rs > 1.5) of all the major impurities. However, almost complete separations were observed between the parent oligonucleotide and three or more nucleotide–deleted oligonucleotide impurities. The relative quantification of impurities was not consistent among the LC–MS instruments tested here, and inconsistent ion suppressions or enhancements were observed. For the characterization of the parent oligonucleotide, the accuracy of deconvoluted mass was demonstrated to be within 3 ppm for all LC–MS analyses, and MS/MS sequence analysis showed 100% coverage in more than half of the cases. For the structural estimation of impurities, the mass accuracy was within 2 ppm for all LC–MS analyses when impurities were spiked at 0.1% or more, and the MS/MS sequence coverage was 76% or more when the spiked amounts of impurities were 1% or more. These results indicate that generally used LC/MS methods could provide reliable information for estimating the composition of impurities present at a level of 1% or more.

Key words

Oligonucleotide therapeutics, Quality evaluation, Impurities, Liquid chromatography, Mass spectrometry