Tips and tricks from our application experts:

LC-MS setup part II

The power of precision

Kaoru Karasawa, Professional Specialist, Application Support at SCIEX, Japan, shares her tips and tricks on oligonucleotides analysis with LC-MS

Tip 1: Sample handling

Oligonucleotides tend to bind to pipette tips and vials. In addition, oligonucleotides can be sensitive to the degradation by nucleases. I therefore recommend using low-binding LC vials and pipette tips and recommend wearing gloves and using nuclease-free water for sample preparation to minimize the risk of sample loss due to adsorption and/or degradation. Furthermore, the use of an internal standard is beneficial. It can help with minimizing the adsorption of the target analyte and can increase quantitative accuracy for quantitative studies.

Tip 2: Replace LC solvents

To facilitate chromatographic separation while enabling MS sensitivity, alkylamines and hexafluoro-2-propanol (HFIP) are commonly used for IP-RP-LC-MS analysis. However, the additives in the solvents can evaporate quickly. The change in concentration and pH can lead to changes in chromatographic performance and decreased MS signal intensities. I recommend checking in advance how long your solvent can be used and design experiments accordingly. If you notice a decrease in expected MS peak intensity, prepare fresh solvents.

Tip 3: Select suitable columns

In many cases, the separation of analytes from impurities or metabolites requires high column temperatures, and in some cases, can go up to 90°C. The analysis of double stranded analytes, such as siRNA, require high temperatures to separate the sense from the complementary antisense strand. In addition, the solvents used for IP-RP-LC are high in pH. I recommend carefully choosing a column that can withstand these conditions, such as the Phenomenex BioZen Oligo LC column.

Tip 4: Optimize MS methods

The MS signal for therapeutic oligonucleotides is usually distributed across multiple charge states—mainly -2 to -10—and the distribution varies based on LC and MS conditions, sequence composition and length. For quantitative analyses, I recommend optimizing the collision energy for several charge states, since the most intense charge state does not necessarily provide the most intense fragment ion with high specificity. For qualitative analyses, such as sequencing, it is beneficial to combine the MS/MS information from different charge states for best results.

Kaoru Karasawa has more than 25 years of mass spectrometry experience, primarily in high-resolution MS. She leverages from extensive hands-on experience in impurity analysis, MetID and quantitation of small molecules and oligonucleotides for pharmaceutical and omics applications. Prior to joining SCIEX, Kaoru worked as a natural product chemist for the Roche Group, Japan. Currently, she is participating in a project for oligonucleotide development in Japan and contributing to the development of analytical techniques. Kaoru holds a Master's Degree in engineering.

More questions?

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