Tips and tricks from our application experts:

LC-MS setup part I

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DilipKumar Reddy Kandula (PhD), Staff Applications Scientist at SCIEX, US, shares his tips and tricks on how to prepare your system for oligonucleotide LC-MS analysis.

Dr. Dilip Reddy has 10 years of experience as a research scientist in different pharma and biopharma companies, where he focused on using MS for the analysis of biologics and small molecules. His work included the characterization of complex molecules, bioanalysis and MetID studies with triple quad and high-resolution LC-MS instrumentation. For the past 7 years, he applied his extensive knowledge within various functions at SCIEX. Dilip holds a PhD in protein characterization using mass spectrometry from the Shri JJT University in Rajasthan, India.

The background on formation of metal adducts

It is very common for oligonucleotides to form adducts with alkali metal ions during LC-MS analysis. Adduct formation occurs due to the electrostatic attraction between the negatively charged phosphate backbone of the oligonucleotide and the positively charged alkali metal ions. The formation of alkali metal adducts can negatively impact the data quality. Signal intensities of analytes are reduced due to spreading the signal across different adducts. Additionally, identification and accurate quantitation of species is further complicated. I summarized my tips below to help improve your oligonucleotide LC-MS analysis.

Tip 1: Choose the right consumables

Glass bottles tend to leach sodium ions, hence plastic bottles are recommended as containers for mobile phases. Before the first use, it is recommended to soak the plastic bottles overnight in isopropanol (IPA) containing 10% acetic acid and rinse them 10–15 times with milliQ water. Using high-quality LC-MS grade additives (acetic acid, ion pairing agents) and solvents can help minimize adduct formation. Ensure that a set of tubing and columns is set aside for oligonucleotide analysis usage only, such as the Phenomenex BioZen Oligo LC column.

Tip 2: Prepare your LC system

Before starting analysis, ensure your LC system is prepared for oligonucleotide analysis with ion pairing reversed phase liquid chromatography (IP-RP-LC). My recommendation is to flush the solvent and autosampler lines with LC-MS grade IPA for 10 to 15 minutes. Include your tubing and electrode for all flushing steps but use a connector piece instead of your column. Then, switch to 10% acetic acid for 1-2 hours. Doing "dummy" injections of a 10 % acetic acid solution is beneficial for cleaning the injection parts. As a next step, switch to LC-MS grade or MilliQ water for 1-2 hours to remove the acetic acid. Then install your oligonucleotide column and equilibrate system with mobile phases for analysis.

Tip 3: Determine an MS cleaning schedule

It is important to clean the ion source regularly to prevent contamination, reduce the adduct formation and maintain optimal sensitivity. Follow the SCIEX guidelines for cleaning procedures and recommended cleaning solutions and keep a specific cleaning schedule. A regular cleaning schedule of your source and MS front-end is recommended to help preventing any contamination travelling further into the system, especially when working with ion pairing agents for IP-RP-LC analysis.

More questions?

Contact us

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