BIO_28 - Streamlining workflow from characterization to monitoring of therapeutic oligonucleotides impurities across IPRP-LC-HRAM-MS platforms

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Introduction: In order to support fast growing therapeutic oligonucleotides programs, sensitive and robust analytical strategies are desired to efficiently characterize and monitor these novel modalities and their impurities during development, manufacturing and quality control.

Objectives: To developed a system performance evaluation test (SPET) that uses a 6-oligonucleotide mixture to monitor relevant metrics of LC-MS system based on a comprehensive set of acceptance criteria.

Methodology: Sample Preparation: For SPET, oligonucleotide mixture ranging from 10mer to 55mer was obtained from Life Technologies. For modified RNA characterization and monitoring experiments, desalted and HPLC purified modified single stranded RNA was obtained from Integrated DNA Technologies. Chromatography: 25pmol of the oligonucleotide mixture was injected onto a DNAPac RP column using Thermo Scientific Vanquish Horizon UHPLC system. 1µg of RNA sample was used for characterization and monitoring experiments. MS Conditions: For characterization experiments Orbitrap Exploris 240 mass spectrometer was used. Sample analysis was performed using data dependent MS/MS acquisition. For monitoring experiments, data was collected with an Orbitrap Exploris MX mass detector. Data Processing: BioPharma Finder using the oligonucleotide sequencing workflow. Enterprise compliance ready Chromeleon CDS was used for all instrument control, data acquisition, processing, and reporting.

Results: The BioPharma Finder software provides interactive report and automated tools for identification and mapping of the oligonucleotide sequences. The monoisotopic mass and the MS2 fragmentation pattern of the identified components are compared to the predicted oligonucleotide components. A confidence score is provided based on the evaluation of mass accuracy, isotopic distribution, charge state determination, and correlation between the predicted and measured fragmentation pattern. The software also calculates an average structural resolution (ASR) value, which in an ideal case, all bonds between each individual nucleotide residue has been broken and resulting fragment ions matched the predicted MS/MS spectra. The combination of high confidence score with low delta mass ppm deviation and a low ASR value (e.g., 1.0) gives strong confidence in the sequence being correctly matched.

Conclusion: A streamlining workflow from characterization to monitoring of therapeutic oligonucleotides impurities across IPRP-LC-HRAM-MS platforms is demonstrated.

Keywords: Mass spectrometry oligonucletotide biopharmaceuticals