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Comparison of Q-Tof, Q-Exactive and Triple Quad for Quantitative Bioanalysis of Oligonucleotide Therapeutics

Introduction

With the advancement of formulation and delivery technologies, oligonucleotide and RNA based therapeutics have emerged to be a major class of biopharmaceuticals with more specific drug action mechanisms and more diverse range of drug targets. To support drug development and clinical diagnosis, LC-MS/MS and LC-HRAM methods have been developed for quantitative and qualitative bioanalysis of oligonucleotides as well as their metabolites. Currently there are three major instrument platforms (Q-Tof, Q-Exactive and Triple Quads) being used in bioanalytical laboratories. The advantages and disadvantages of each instrument platforms for each particular application will be compared with case studies.

Methods

Oligonucleotides were dissolved in de-ionized water at approximately 50.0 µg/mL. 10 µL of the solution was injected onto a Thermo DNApac C18 column (2x50 mm, 4 µm) and eluted with a gradient of HFIP/TEA buffered water and methanol. The eluate was delivered to a Sciex API5000 triple quad, Thermo Q-Exactive Plus, or Bruker microTOF-QII Q-Tof mass spectrometer. The mass spectrometers were operated under negative mode for acquisition of both Q1 scan and product ion scan mass spectrums. For quantitative analysis, target MRM transitions were monitored on API5000 triple quad while both full scan and targeted SIM scans were acquired on Q Exactive Plus. The human sample will be extracted using a novel SPE method as described in another poster.

Preliminary Data

The Q1 scan mass spectrums of single stranded phosphorothioate oligonucleotides (20-mer, 19-mer, 18-mer and 14-mer) and a double stranded phosphodiester siRNA (22-mer) were evaluated in negative electrospray ionization mode (ESI-) on a Sciex API5000, Thermo Q-Exactive Plus and Bruker microTOF-QII Q under the same LC gradient conditions (2.0% HFIP & 0.4% TEA in water and methanol). Multiple charge envelopes were observed for all tested oligonucleotides. However, the charge distribution envelops are significantly different for different oligonucleotides on different instrument platforms. For single strand phosphorothioate oligonucleotides, the predominant peak has 3 negative charges on Q-Tof and Q Exactive Plus high resolution mass spectrometers, while the most intense peaks carry 6-8 negative charges on API5000 triple quad. For the 22-mer double stranded siRNA, the most intense m/z peaks have 5 and 6 negative charges for the two individual RNA strands, respectively, on Q-Tof and Q Exactive Plus; while the most intense peaks carry 8 and 9 negative charges on API5000. With addition of 0.5% DMSO (supercharging reagent) into LC mobile phases, the most intense peaks shifted to higher charge states on all MS platforms. To compare the assay sensitivity and specificity for quantitative bioanalysis of oligonucleotides, the limit of detection (LOQ) of the LC-MS/MS or LC-

HRAM assays for a 20-mer phosphorothioate DNA oligonucleotide in human plasma will be compared on API5000 and Q Exactive Plus.

Novel Aspect

The first side-by-side comparison of triple quad, Q-Exactive and Q-Tof for quantitative and qualitative bioanalysis of oligonucleotides.