

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

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Novel Aspect

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

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 (14-mer PS-ODN, 20-mer PS-ODN, and 22-mer ds-siRNA) 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 plasma sample will be extracted using a novel SPE method as described in another poster.

Instrumentation



RP-HPLC Conditions

System: Waters ACQUITY UPLC
Column: Thermo DNAPac C18 column (2x50 mm, 4 µm)

Column Temp.: 60 °C

Solvent A: 2% HFIP & 0.4% TEA in water

Solvent B: 2% HFIP & 0.4% TEA in MeOH

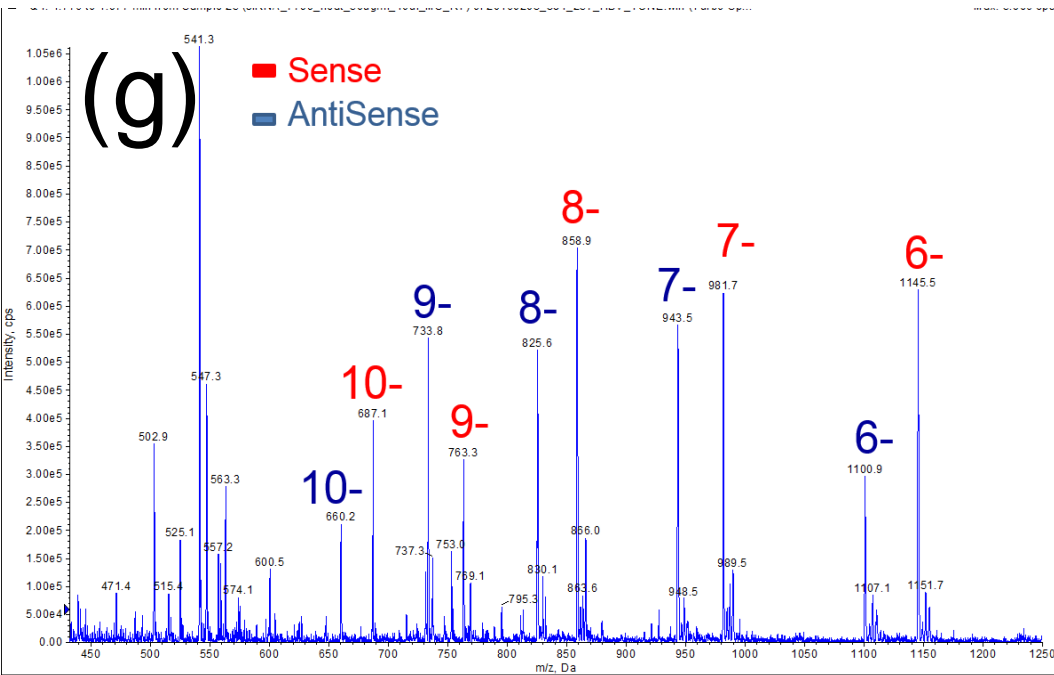
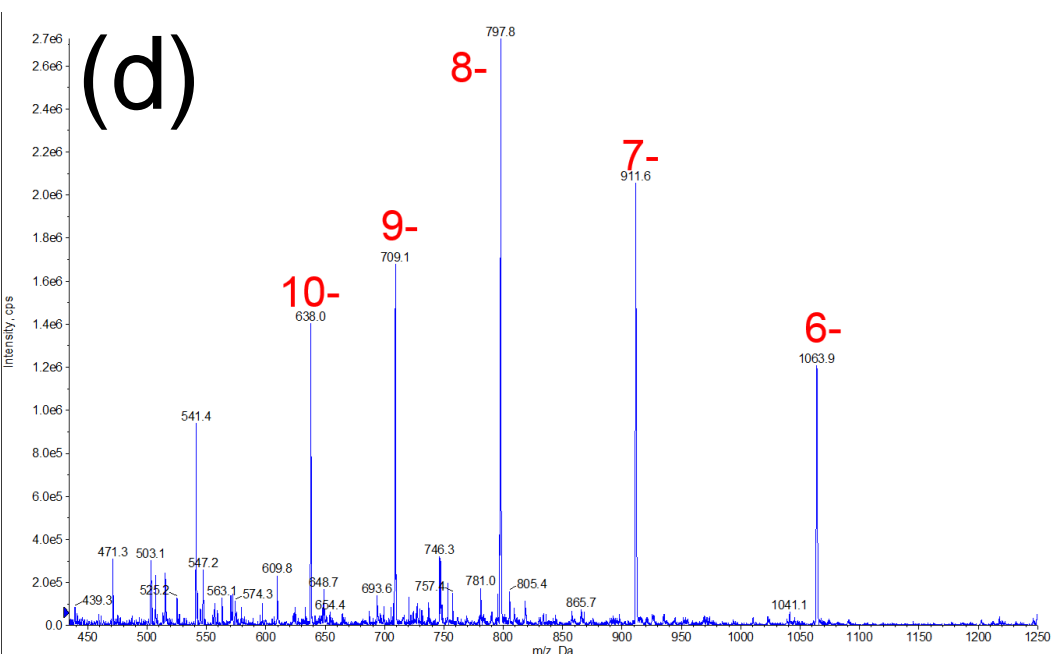
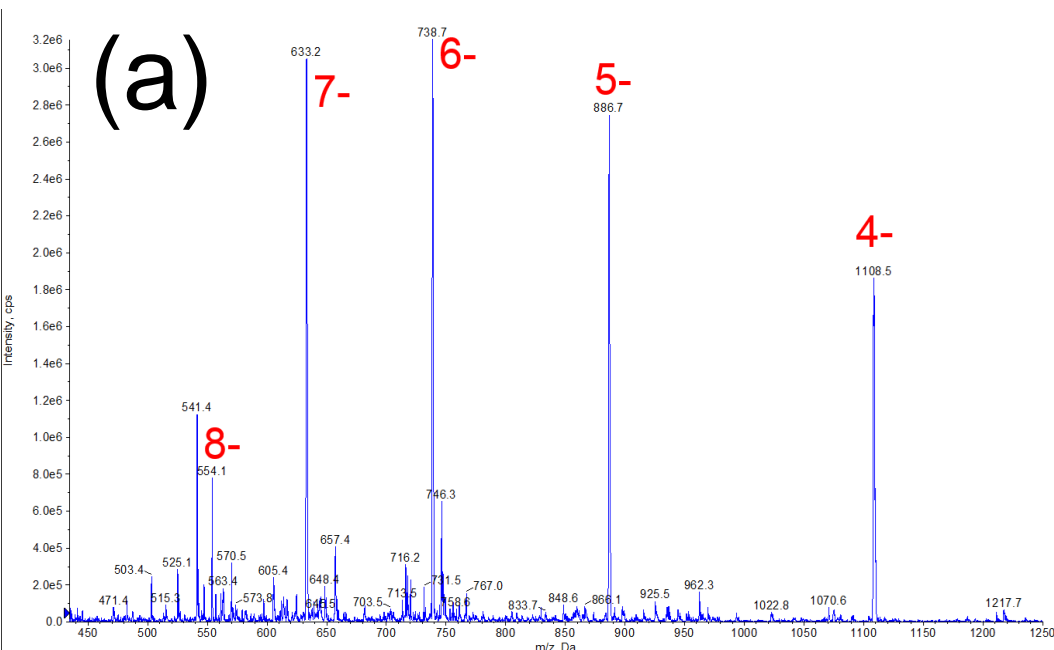
Gradient:

Time (min)	Flow Rate (mL/min)	%A	%B
0	0.35	90	10
0.2	0.35	90	10
2.2	0.35	55	45
2.7	0.35	25	75
3.7	0.35	25	75
3.8	0.35	10	90
4.5	0.35	10	90
4.6	0.35	90	10
5	0.35	90	10

Results

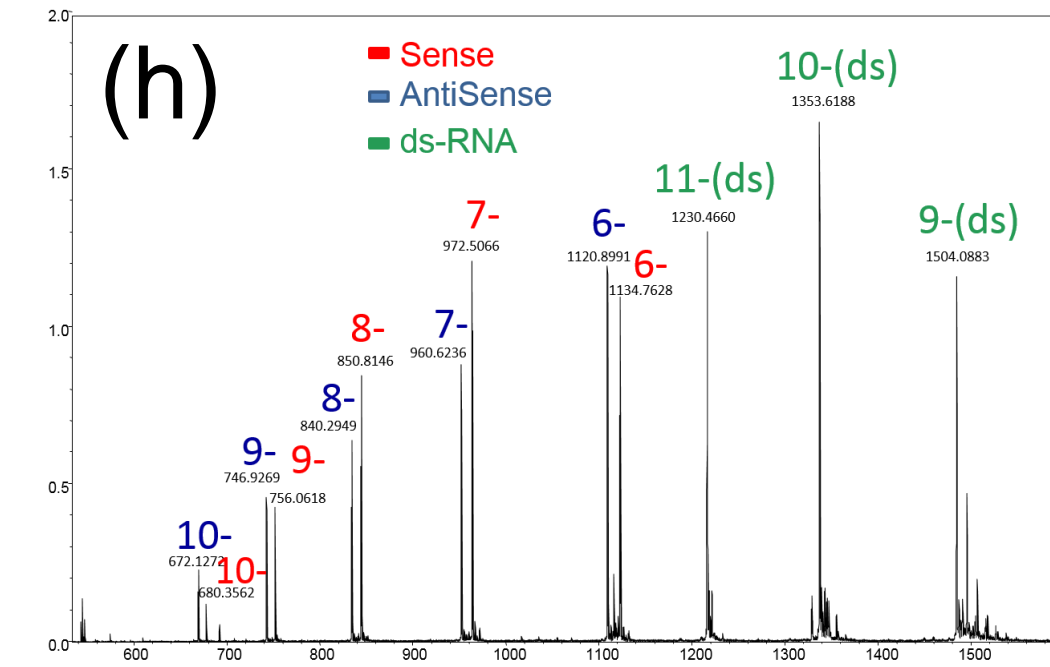
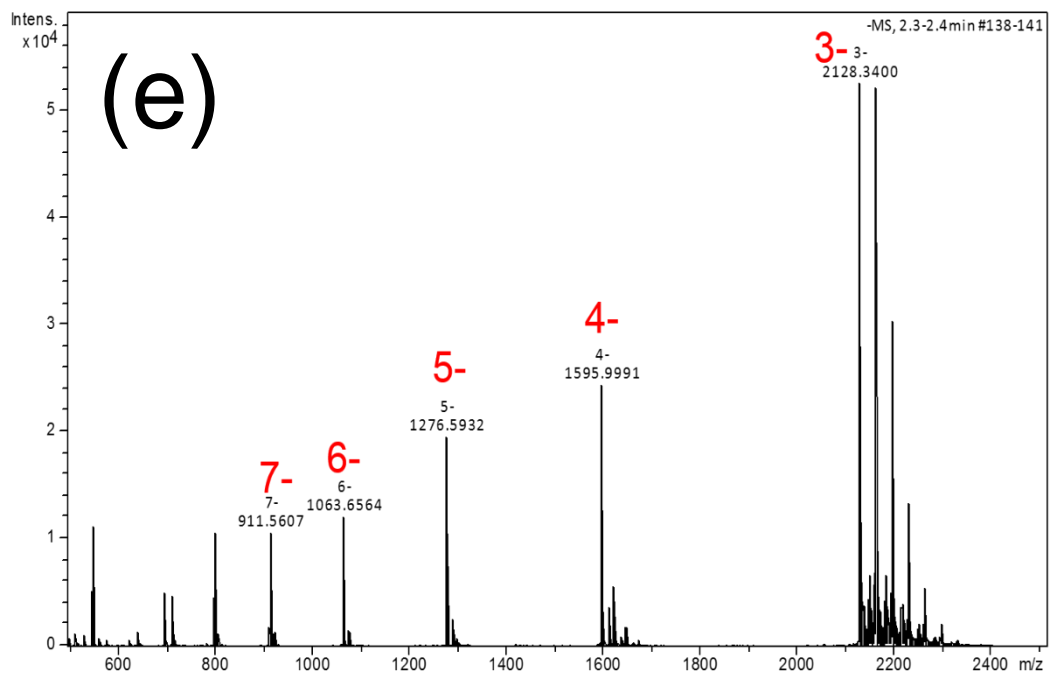
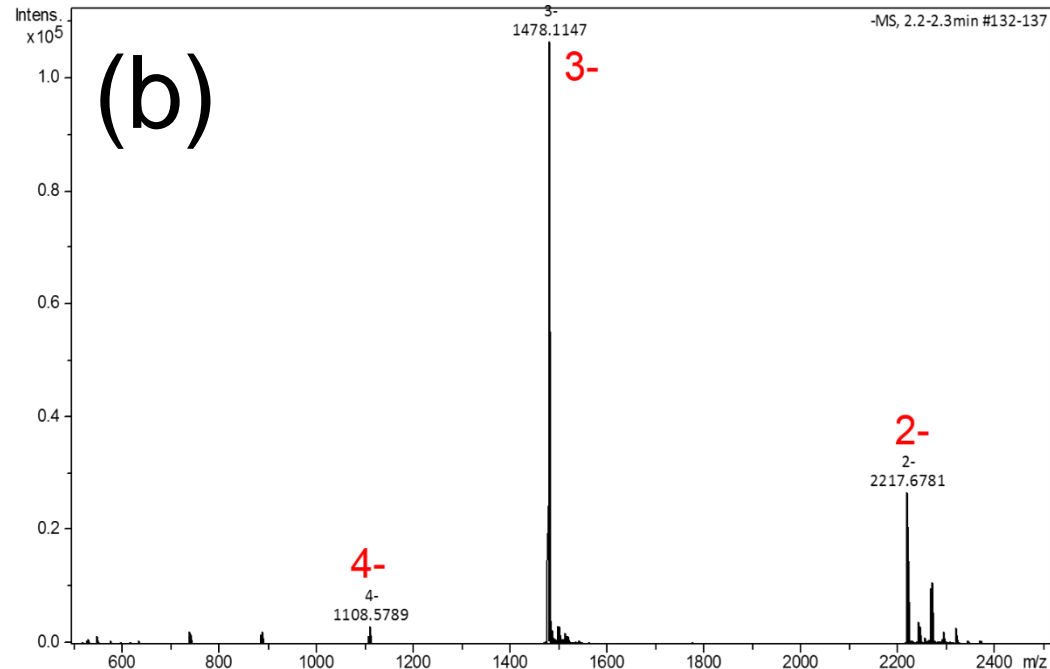
SCIEX API 5000

Mode: Negative
Curtain GAS: 30
Ion Source Gas 1&2: 50
Spray Voltage: -4000
Temp.: 450
Decluster Potential: -100
Entrance Potential: -10
Collision Cell Exit Potential: -30



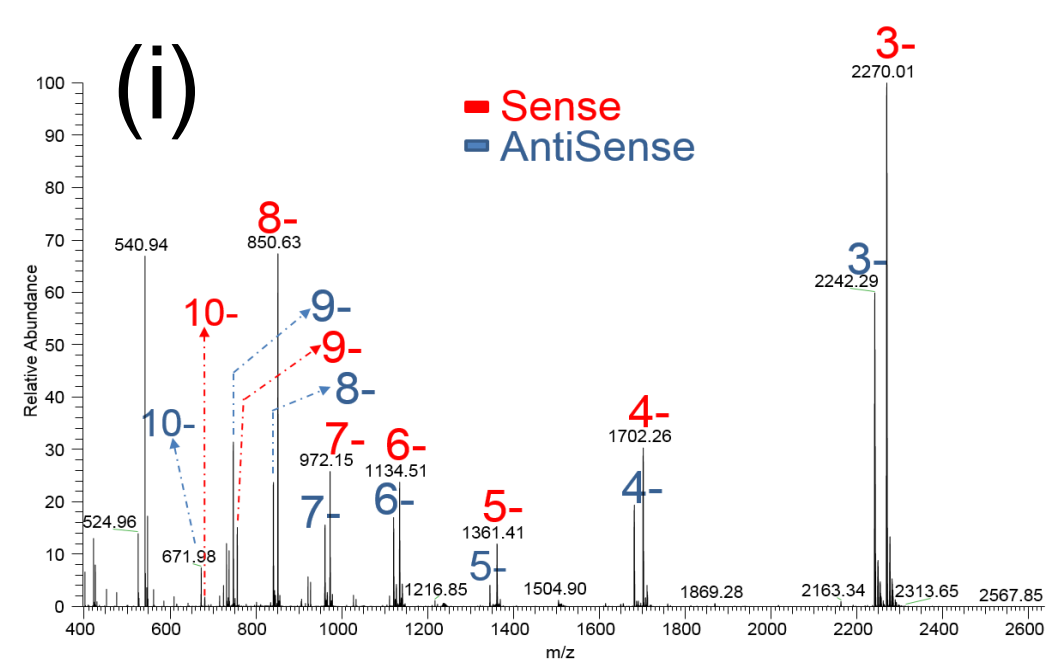
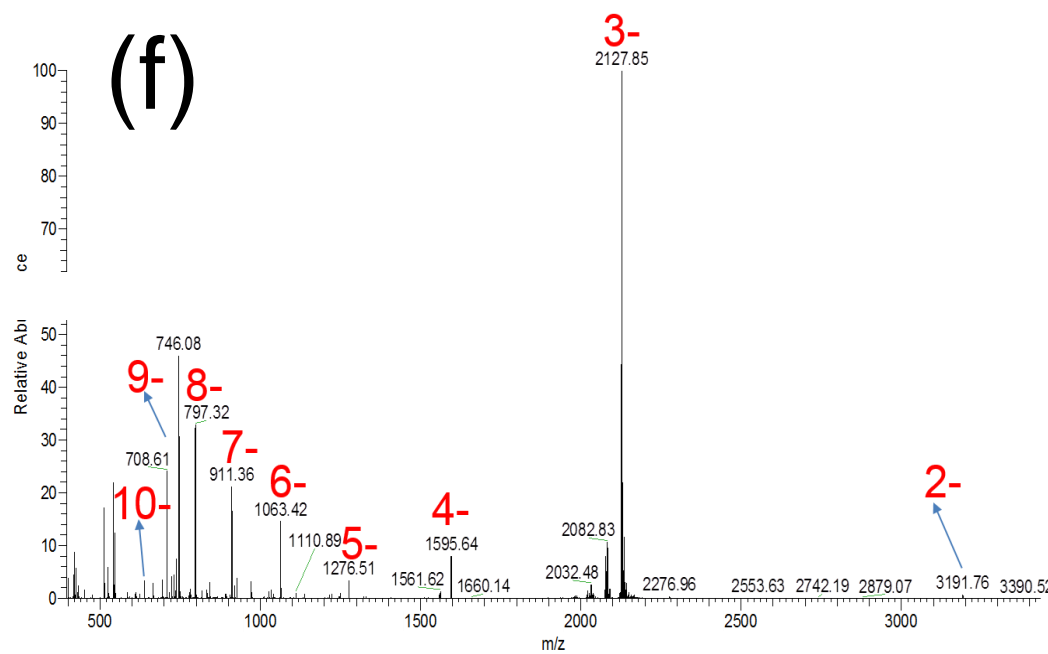
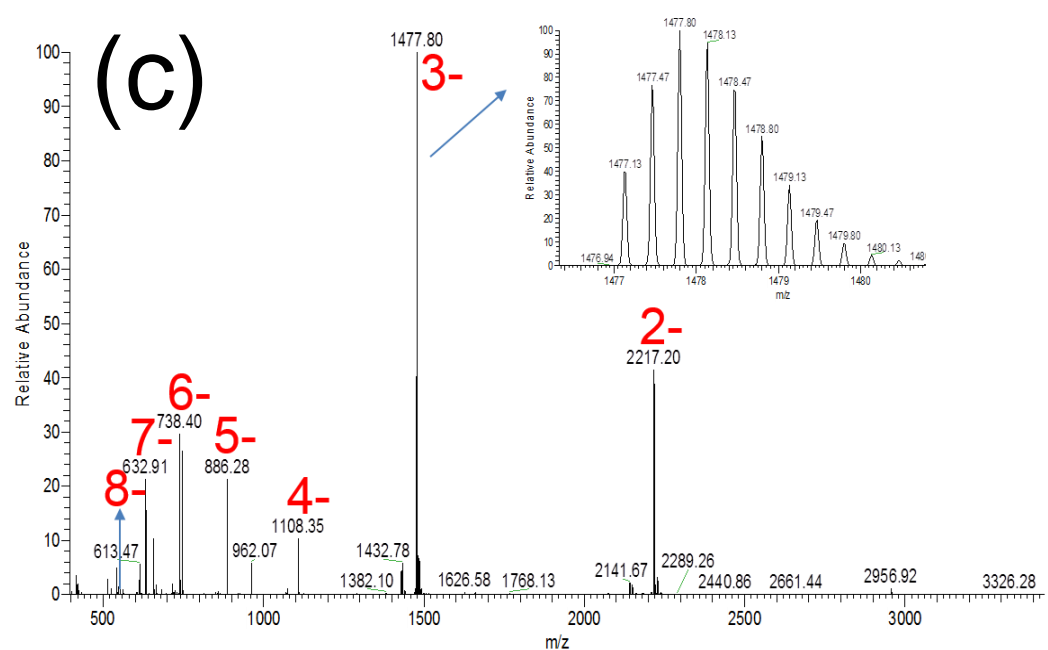
Bruker microTOF-QII

Mode: Negative
Capillary Voltage: 3000
Nebulizer Gas: 2.5
Dry Gas: 8.0
Dry Temp.: 180
Hexapole RF: 400
Collision RF: 680
Transfer Time: 120



Thermo Q-Exactive Plus

Mode: Negative
Spray Voltage: 2800
Capillary Temp.: 320
Sheath Gas: 35
Aux Gas: 10
Spare Gas: 2
Probe Heater Temp.: 310
S-Lens RF Level: 55



ESI Mass spectra of 14-mer PS-ODN (Figure a, b, and c), 20-mer PS-ODN (Figure d, e, and f), and 22-mer ds-siRNA (Figure g, h, and i) obtained from SCIEX API5000, Bruker microTOF-QII, and Thermo Q-Exactive Plus, respectively.