Quantification and Qualification of Oligonucleotides by High Resolution/Accurate Mass Orbitrap MS

Hongxia (Jessica) Wang1, Kevin Cook1, Patrick Bennett1
Thermo Fisher Scientific, San Jose, CA

Overview

Purpose: Identification and quantitation of oligonucleotides by UHPLC-high resolution Orbitrap MS.

Methods: Oligonucleotides were first qualified in full scan MS mode at 70,000 resolution, then quantified with both selected ion monitoring (SIM) at 70,000 resolution and targeted MS/MS methods at 15,000 resolution with a 3-gram gradient on Thermo Hypersil Gold column.

Results: Samples prepared in a human plasma based solution to reduce non-specific binding indicated good linearity with a calibration range of 1 - 50 nM. Performing SIM (70,000 resolution) shows 5-fold more sensitivity than targeted MS/MS method (1,500 resolution). With faster scan speed, there are at least 20 scans across a 5-sec UPLC peak around LOQ at 70,000 resolution.

Introduction

Qualitative and quantitative analysis of oligonucleotides in biological matrices are important aspects of the drug development process. There is a need to overcome the use of high resolution mass spectrometry (HRMS) as an alternative to overcome the limitations of nominal resolution provided by triple quadrupole MS. A high throughput generic UHPLC-HRMS assay is demonstrated for oligonucleotide analysis using Q Exactive OrbiTrap MS. The qualitative information provided during quantitation of analytes within one injection is a crucial advantage to accelerate drug development process by reducing instrument time and sample consumption.

Methods

Sample Preparation

Oligonucleotides (ODNs) at 1 mm and 20 µM bought from Invitrogen. Bio/Tectnimex was dissolved in water. The concentration of stock solutions were quantified by Thermo NanoDrop UV spectrometer. A 20-mer Phosphodiester ODN (5'-ATT CAG TTC ACT TAT CGT AT-3') was diluted in 3 different carriers [1]Solution A (2% HFIP + 0.4% TEA in Water) [2] 0.1% Human Plasma in Solution A [3] 0.1% Human Plasma + 0.1%TFA + 10% MeOH. The most abundant ions, [MX9H] were used for quantitation (spectrum not shown).

Oligonucleotides Charge State Distribution and SIM Quantitation

Analyte and internal standard ODNs were analyzed by LC-MS at full scan mode from m/z 400 to 5,000 at 70,000 resolution. The charge state distributions of two ODNs are shown in Figures 1 and 2, respectively. For 20-mer ODN, the most abundant ion is [MX9H]. Four isotopes, m/z 968.0706, 833.0189, 708.9672 and 583.8254 were used for quantitation (Figure 3). For 15-mer ODN, [MX3H] was the most abundant ion, three isotopes, m/z 1579.1551, 1579.4875 and 1579.8208 were used for quantitation (Figure 4).

Conclusion

1. Oligonucleotides were identified by high resolution accurate mass data with charge distribution. Their sequences were confirmed by MS/MS accurate mass spectrum. A 20-mer synthetic ODN was used as a model compound and was quantified by both SIM and targeted MS/MS methods.
2. Samples with human plasma as a carrier indicated good linearity with a calibration range of 1 - 50 nmO for SIM method (R²=0.9812, Linear 1/X with CV=10%).
3. SIM method (70,000 resolution) shows 5 fold more sensitive than targeted MS/MS method (1,500 resolution) for the synthetic ODN.
4. There are at least 20 scans across a 5-sec UPLC peak around LOQ at 70,000 resolution.
5. SIM at 160,000 resolution and targeted MS/MS provide better selectivity for the assay with complex matrices.

Reference


All trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries. This document is not to be reproduced in any manner that might infringe the intellectual property rights of others.