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A Universal Method for the Quantitation of Multiple Oligonucleotides from Plasma by MFLC-MS/MS

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Purpose

Bioanalysis of oligonucleotides by HPLC-MS/MS in support of regulatory submission has not been widely adopted. Several obstacles such as lack of sensitivity, adduct formation and analytical instrument dedication are barriers that many laboratories are unwilling to challenge. Traditional ELISA and qPCR bioanalysis of oligonucleotides may not have the selectivity to accurately quantify the therapeutic oligonucleotide from complicated biological matrix. HPLC-FL methods require a probe design that increases the method development time and cost of the assay. Additionally, the probe is not suitable for oligonucleotides smaller than 20 nucleotides. HPLC-MS/MS analysis is an alternative approach that provides better selectivity, increased dynamic range and the flexibility to analyze a vast array of oligonucleotides from biological matrix. In order to increase the sensitivity of the method, analysis was conducted using microflow HPLC (MFLC) conditioned for oligonucleotide analysis thus reducing adduct formation.

Methods

Four oligonucleotides were chosen for the analysis. Each oligonucleotide had at least 20 nucleotides with phosphorothioate linkage and ranged from 6667-6682 amu molecular weights. The MRM analysis was performed using an API-5500 mass spectrometer operating in negative ESI mode and was fitted with an Optiflow source. The MFLC system was a Waters M Class operating with binary gradient method and a flowrate of 20 µL/min. Separation was achieved using a Phenomenex Gemini C18 column (5 cm x 0.3 mm, 3 µm). In order to increase the sensitivity and retention of the oligonucleotides 100 mM HFIP and 10 mM DIEA were added to the mobile phases (Water and Acetonitrile). The oligonucleotides were extracted from the plasma using Clarity OTX SPE (Phenomenex). In order to reduce the formation of adducts the instrument was cleaned to Q0 and the microflow cart that is dedicated to oligo analysis was used for the MFLC.

Results

The developed method resulted in a sensitive and selective assay that can be quickly transferred to any instrument. The oligonucleotides were well retained on the column and a detection limit of 1.0 ng/mL was established. A full scan of the compounds indicated that no significant adduct formation had occurred.

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Conclusion

A universal MFLC and solid phase extraction (SPE) method was developed to extract and analyze four oligonucleotides from human plasma. The microflow system is on a portable cart, allowing the scientist the ability to transport the pumps to any mass spectrometer available without allocating an entire LC-MS/MS system for oligonucleotide analysis.