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Novel SPE Extraction Method for Sensitive and High Throughput Quantitative Analysis of Phosphorothioate Oligonucleotides in Human Plasma Using LC-MS/MS

Introduction

Oligonucleotides and RNA are gaining renewed confidence as a new class of drugs. Many bioanalytical methods have been developed to support drug research and development of oligonucleotide therapeutics. LC-MS/MS is one of the most popular techniques due to its unprecedented specificity; however, the sensitivity for most LC-MS/MS assays is still not as good as qPCR and ligand binding assays for some large oligonucleotides. To improve sensitivity and throughput of the LC-MS/MS assays, we have successfully developed a novel SPE extraction method which produces high quality extracts from human plasma samples and requires no drying down step.

Methods

200 µL of plasma sample, 50.0 µL of internal standard and 500 µL of 200 mM extraction buffer were aliquoted into corresponding wells of a 96-well plate. After mixing, the samples were transferred onto a Biotage WAX 96-Well Plate preconditioned with methanol and 200 mM extraction buffer. The samples were washed with 200 mM extraction buffer and 20/80 MeCN/(washing buffer). The sample plate was incubated at 70°C for 1 hour, then eluted with 500 µL of 10/90 MeOH/(5% TEA in water). Samples were directly analyzed on a Thermo DNApac C18 column (2x50 mm, 4 µm) and eluted with a gradient of HFIP/TEA buffered water and methanol. An API5000 was used to monitor the MRM transitions under negative ion electrospray mode.

Preliminary Data

A 20-mer phosphorothioate DNA oligonucleotide (NBA1520, Mw = 6387.1 Daltons) and a 14-mer analog oligonucleotide (NBA1514, Mw = 4437.6 Daltons) were used as a model analyte and internal standard, respectively. To optimize the extraction conditions, the loading buffer, washing buffer and elution buffer were screened and optimized by extracting 100 µL of 5,000 ng/mL NBA1520 in human plasma using the Biotage Evolute Express WAX 96-Well Plate. Loading buffers with different concentrations (from 50 mM to 200 mM) and different pH (from 3.0 to 8.5) were evaluated. It was found that 200 mM extraction buffer (pH=8) treated samples could result in quantitative analyte loading. For plate washing, no detectable amount of analyte was eluted out using washing buffer containing up to 20% MeCN. For sample elution, we surprisingly found that the recovery increased significantly with the increase of the duration time between the last washing step and the addition of elution buffer. Greater than 90% recovery was achieved when the sample plate (after being washed) was incubated at 70°C for 1 hours before eluting with 500 µL of 10/90 MeOH/(5% TEA water). The eluate can be directly injected onto LC-MS/MS system for analysis under optimized conditions. The MRM transitions for analyte and IS were 637.7→ 95.0 and 632.9→ 95.0, respectively. Very good linearity was demonstrated for NBA1520 with a calibration curve range of 0.2-200 ng/mL by extracting 200 µL of human plasma samples. The assay selectivity, specificity and accuracy/precision will be evaluated.

Novel Aspect

This high throughput SPE extraction method uses a mechanism that is different from conventional reverse phase and ion exchange mechanism.