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

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Innovative Mass Spectrometric Solutions

Overview

A high throughput SPE extraction method using a mechanism that is different from conventional reverse phase and ion exchange mechanism has been developed.

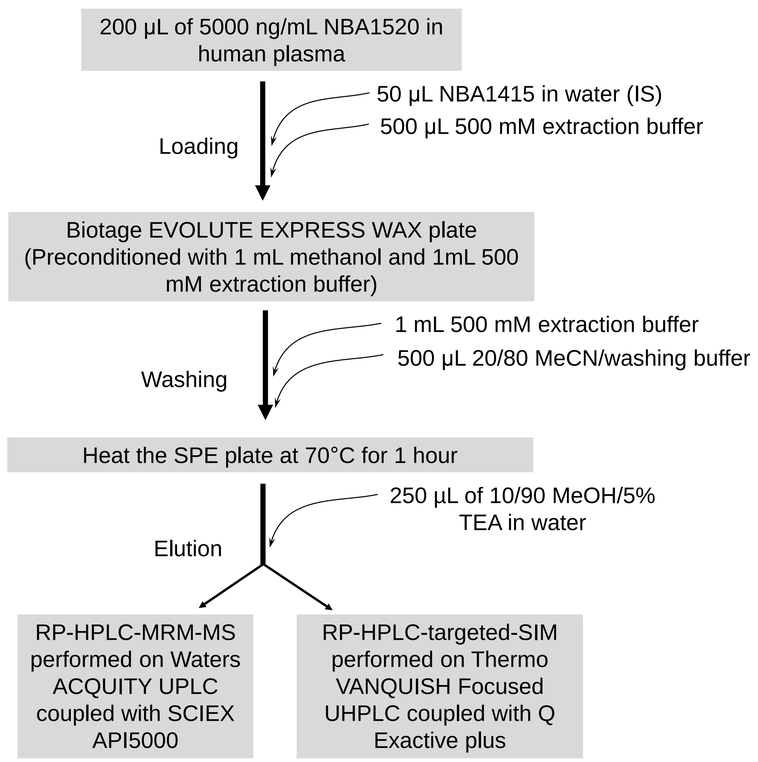
Introduction

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

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 SPE conditions, the loading buffer, washing buffer, and elution buffer were screened and optimized by extracting 200 µL of 5,000 ng/mL NBA1520 in human plasma using the Biotage EVOLUTE EXPRESS WAX 30 mg Fixed Well plate (Part No.: 604-0030-PX01). The eluents were analyzed directly on a Thermo DNApac C18 column (2x50 mm, 4 µm) with a gradient of HFIP/TEA buffered water and methanol. The standard curve (0.5 ng/mL-500 ng/ml) and QC samples were quantitatively analyzed by SCIEX API5000 (MRM-MS) and Thermo Q Exactive plus (targeted-SIM) under negative ion electrospray mode.

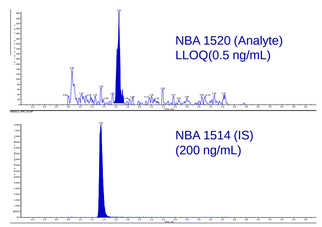
Experimental Workflow

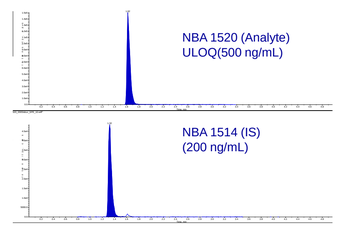


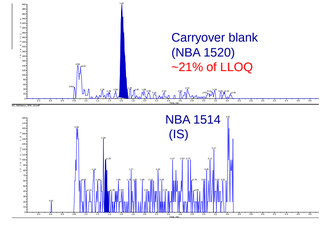
Conclusions

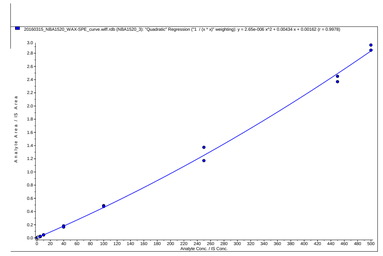
- A high throughput SPE extraction method have successfully developed to purify phosphorothioate DNA oligonucleotide from human plasma
- LC-HRAM on Q-Exactive plus can achieve comparable sensitivity as LC-MS/MS on triple quads for same analytes

Results



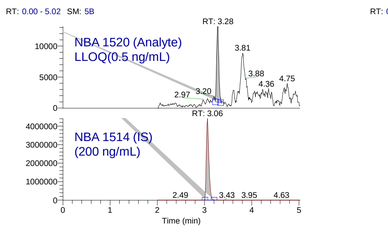


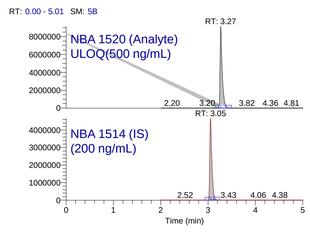


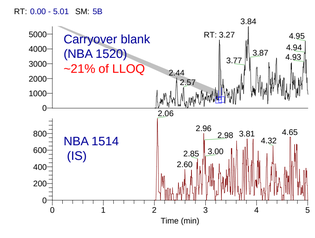


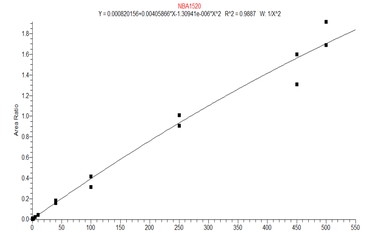
STD Name	Expected C (ng/mL)	Average C (ng/mL)	Used	%CV	Accuracy
STD01	0.5	0.48	1 of 2	N/A	95.91
STD02	1	1.07	2 of 2	9.28	107.09
STD03	2	1.76	1 of 2	N/A	88.12
STD04	5	4.99	1 of 2	N/A	99.83
STD05	10	10.03	2 of 2	2.39	100.27
STD06	40	38.58	2 of 2	7.39	96.44
STD07	100	104.06	2 of 2	1.75	104.06
STD08	250	253.29	2 of 2	9.90	101.32
STD09	450	437.64	2 of 2	1.98	97.25
STD10	500	507.11	2 of 2	1.55	101.42

QC name	Replicates	Expected C (ng/mL)	Average C (ng/mL)	CV%	Accuracy
Low QC	4 of 6	1.5	1.36	5.98	90.92
Low-Med QC	6 of 6	15	14.77	3.45	98.46
Medium QC	6 of 6	200	193.74	5.57	96.87
High QC	6 of 6	400	400.91	4.71	100.23









STD Name	Expected C (ng/mL)	Average C (ng/mL)	Used	%CV	Accuracy
STD01	0.5	0.48	1 of 2	N/A	96.09
STD02	1	1.14	1 of 2	N/A	113.64
STD03	2	1.87	2 of 2	15.192	93.62
STD04	5	5.02	2 of 2	3.2232	100.33
STD05	10	10.15	2 of 2	1.3263	101.52
STD06	40	42.11	2 of 2	9.6842	105.27
STD07	100	92.43	2 of 2	19.94	92.43
STD08	250	257.81	2 of 2	8.3526	103.13
STD09	450	414.62	2 of 2	16.721	92.14
STD10	500	538.22	2 of 2	11.255	107.64

QC name	Replicates	Expected C (ng/mL)	Average C (ng/mL)	CV%	Accuracy
Low QC	4 of 6	1.5	1.39	6.42	92.60
Low-Med QC	6 of 6	15	14.26	7.10	95.06
Medium QC	6 of 6	200	193.12	5.29	96.56
High QC	6 of 6	400	395.33	4.51	98.83