

Automated Magnetic Solid-Phase Extraction Method for Oligonucleotide Bioanalysis

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Introduction

Accurate quantification of oligonucleotides in biological matrices is essential for pharmacokinetic evaluation and therapeutic development. Oligonucleotide therapeutics, including antisense and siRNA modalities, have demonstrated significant clinical potential for treating previously intractable diseases, driven by their ability to modulate gene expression with high specificity and favorable safety profiles. Conventional sample preparation methods, such as solid-phase extraction (SPE) and ligand-based approaches, are widely used but can be labor-intensive, costly, and require complex conjugation steps. Mesoporous Magnetic solid-phase extraction (MSPE) using paramagnetic beads with weak anion exchange chemistry provides a simplified alternative. This conjugation-free approach enables efficient isolation of oligonucleotides and siRNA from plasma and tissue matrices while supporting automation and high-throughput LC-MS/MS analysis. The resulting workflow improves reproducibility, reduces cost, and streamlines bioanalytical operations.

Method

Experiment Condition / Reagents

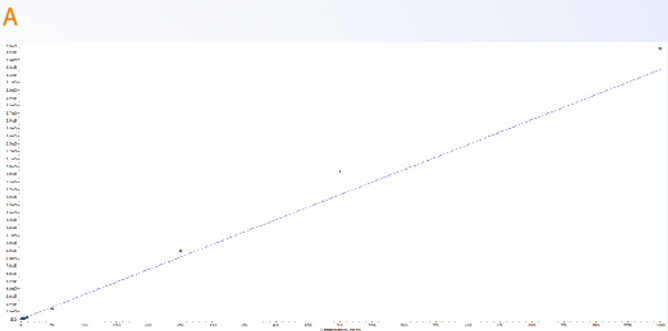
Sample	Oligonucleotides ASO/siRNA + plasma/naïve mouse liver
Digestion	200uL Sample, + 40uL GuHCl + 20uL TCEP + 100uL Proteinase K
MSPE	OligoPlus Beads (~2 mg/well)
Automation	Auto M96/Auto M32
Wash Step 1	50 mM ammonium acetate (pH 5.5)
Wash Step 2	30% methanol in water
Elution Buffer	100 mM triethylamine in 50% methanol + 0.3% ammonium hydroxide (pH 11.5)
Post Extraction	The eluate is dried under nitrogen and reconstituted in 150 µL of water containing 100 µM EDTA
LC /MS/MS System	Shimadzu LC-30AD/AB Sciex 5500 Triple Quad in negative MRM mode
Column	Waters XBridge® Premier Oligonucleotide BEH C18, 130Å, 2.5µm, 2.1x50mm

Results

Table 1. OligoPlus m-SPE extraction data for 20-mer Oligonucleotide

Oligonucleotide	Recoveries (n=3)	RSD (n=3)
20-mer ASO	99.7%	8.0%

Figure 1. A. Calibration curve of 20-mer ASO (MW 7060) in human plasma (2–1,000ng/mL). B. Representative chromatograms. This method can detect ASO oligo with the concentration as low as 2ng/mL



Standard Level (ng/mL)	2.00	5.00	10.0	50.0	250	500	1000
Calculated Conc. (ng/mL)	2.20	4.07	9.03	41.4	272	592	1080
Accuracy (%)	110	81.3	90.3	82.8	109	118	108

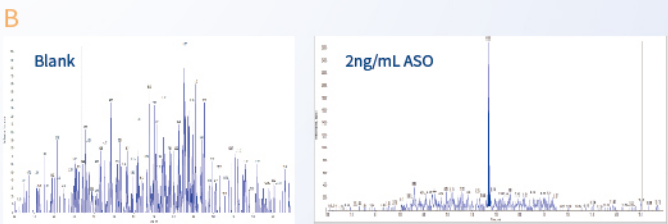
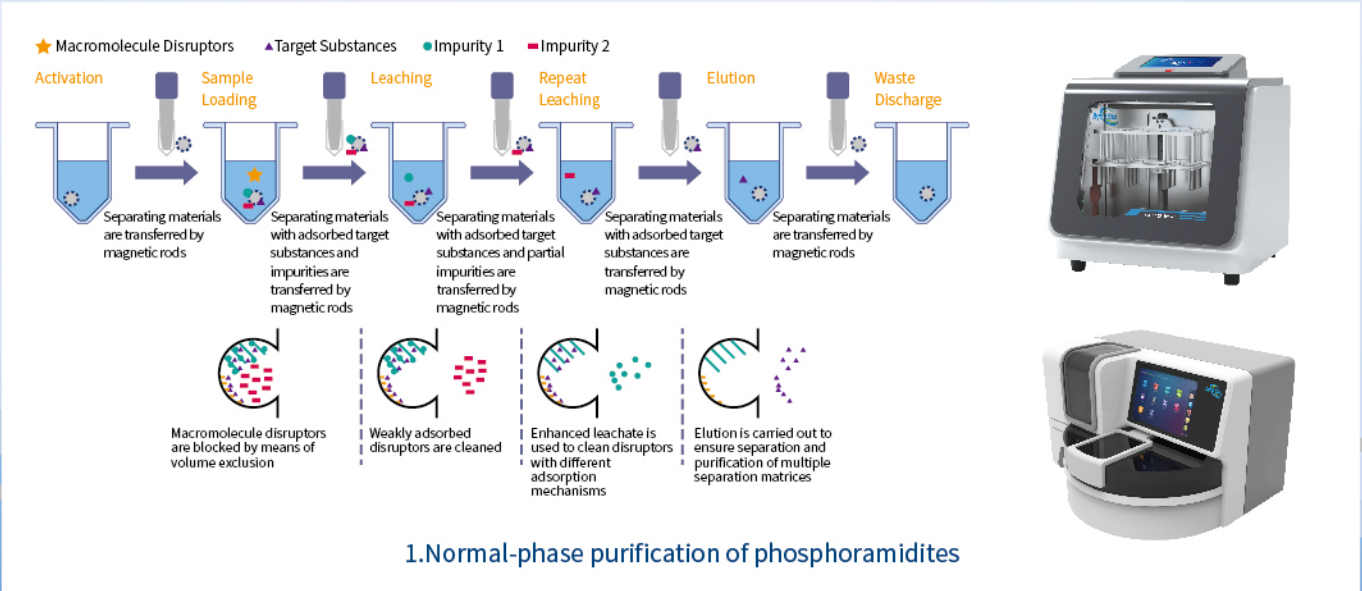


Table 2. Comparison of recoveries for Trecovirsen, Fomivirsen, and Mipomersen using MSPE and a competitor 96-Well SPE plate

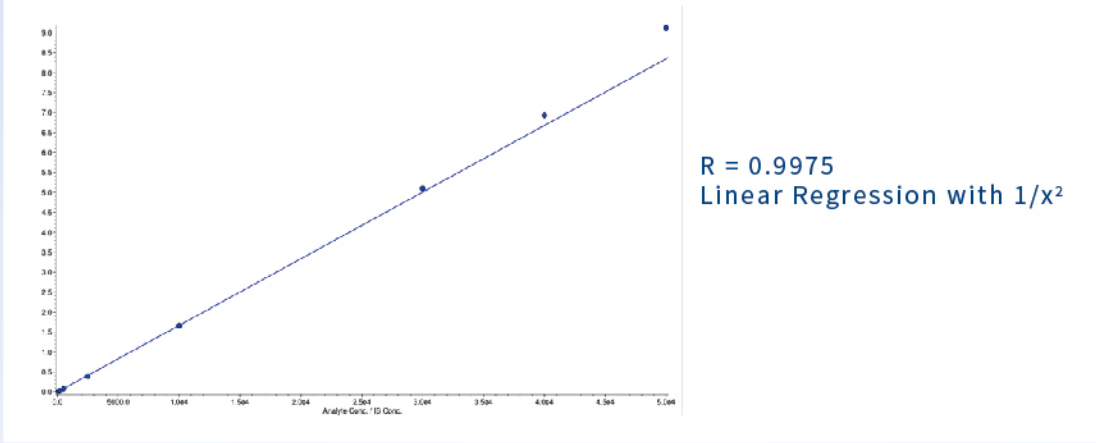
Oligonucleotide	MW	Recovery (%)	
		AgileBio MSPE	96-well SPE plate from a competitor
Trecovirsen	7776.31	97.79	99.82
Fomivirsen	6682.35	91.08	86.74
Mipomersen	7594.76	72.03	66.42

Illustration of MSPE Extraction Process



1.Normal-phase purification of phosphoramidites

Figure 2. Calibration curve of siRNA-LNP (100–50000ng/mL).



Standard Level (ng/mL)	100	200	500	2500	10000	30000	40000	50000
Calculated Conc. (ng/mL)	106	182	476	2340	9970	30600	41500	54600
Accuracy (%)	105.7	91.0	95.2	93.4	99.7	101.9	103.8	109.2

Table 2. QC statics

siRNA analyte	Analyte Concentration(ng/mL)	Calculated Concentration (ng/mL)	Accuracy (%)
QC300-1	300	290	96.8
QC300-2	300	309	103.1
QC15000-1	15000	17000	113.3
QC15000-2	15000	16900	112.7
QC37500-1	37500	40300	107.5
QC37500-2	37500	39300	104.9

Figure 3 Representative QC chromatograms for siRNA-LNP

