[CONSUMABLES GUIDE BOOK]

Quantifying Oligonucleotides

Sample Extraction and LC Tools for Reliable LC-MS Quantitation of Oligonucleotides

Waters™

A Changing Industry

There has been a resurgence in synthetic oligonucleotide therapeutics in the past decade, as seen with burgeoning pipelines across the industry. These new drug candidates operate at the level of gene transcription and translation and are providing opportunities to address not only inherited disorders but also historically difficult to treat ailments, such as cardiovascular disease.

GalNAc conjugated small interfering RNA (siRNA)

Lipid conjugated antisense oligonucleotide (ASO)

Oligonucleotide Extraction from Biological Matrices

A critical first step in carrying out quantitative LC-MS analysis of oligonucleotide therapeutics is being able to extract them from diverse biological matrices, including plasma, urine, cerebral spinal fluid (CSF), cells harvested from in-vitro cell-based assays, and tissue material collected from biological organisms. The success of quantitative analytical workflows largely depends on the ability to achieve high recoveries (>80%) across samples with consistency, and to effectively do that, the strong binding that naturally occurs between oligonucleotides and proteins in biological samples must first be disrupted via a sample pretreatment process.

Sample Pretreatment → **Solid Phase Extraction (SPE)** → **Quantitative LC-MS Analysis**

OligoWorks™ SPE

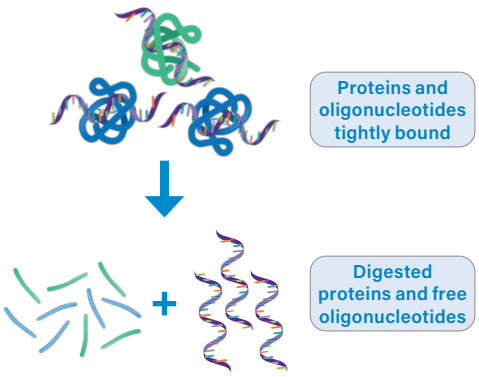
MAXPEAK™ PREMIER

Acquity™ PREMIER

XEVO™ TQ ABSOLUTE

RapiZyme Proteinase K to Disrupt Protein Binding

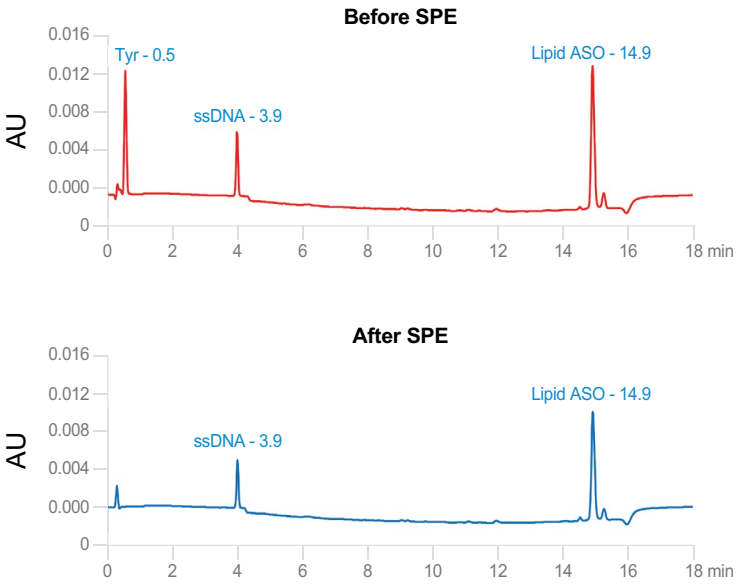
Lysis buffers containing detergent have traditionally been used to disrupt the binding of oligonucleotides to proteins, but there are drawbacks to this approach for both SPE capture efficiency and LC-MS analysis. Ionic and non-ionic interferences limit SPE capacity and increase variability. In addition, extra SPE wash steps and higher volumes are often required when surfactants are applied to minimize MS interferences.



To avoid these drawbacks Waters has developed a detergent-free sample pretreatment method that utilizes a highly active recombinant Proteinase K for protein digestion. In addition to the Proteinase K enzyme, our RapiZyme™ Digestion Module includes both a denaturant (Guanidine HCL, 6 M) and reductant (TCEP, 0.5 M) that further enhance digestion efficiency, improving robustness and repeatability.

OligoWorks SPE WAX Sorbent for Reliable Extraction

The OligoWorks™ SPE WAX Sorbent in every OligoWorks SPE Device is carefully selected from batches of Oasis™ WAX material that Waters™ has been producing for nearly 20 years. Both a 20-mer ssDNA and 16-mer lipid-conjugated, highly modified antisense oligonucleotide (ASO) are included in our QC test, and only batches that pass our high recovery thresholds (90% for ssDNA; 85% for lipid conjugated oligo) are qualified for use as OligoWorks SPE Sorbent. This is a key process by which we ensure consistent, repeatable performance from lot-to-lot.



QC Analytes:

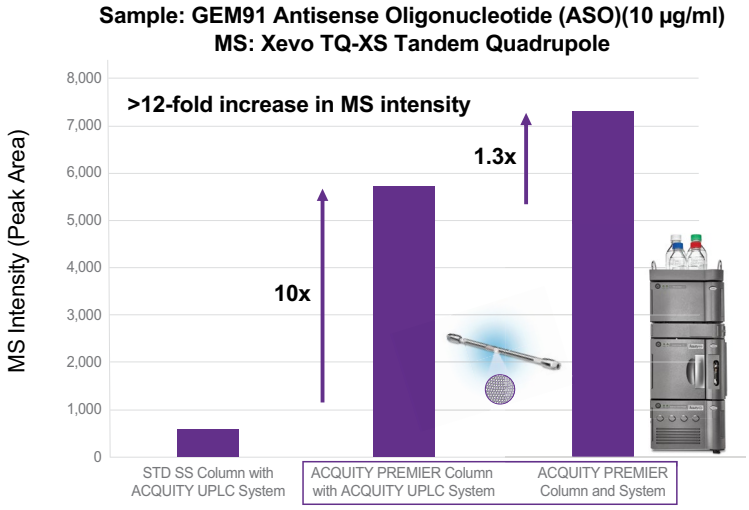
- Tyrosine (negative control)
- 20-mer ssDNA (unmodified)
- 16-mer Lipid-conjugated, highly modified ASO

Figure 2. Elements of robust SPE-based oligonucleotide extraction, Araya et.al.

Enhanced Sensitivity and Repeatability with MaxPeak Premier Columns

Bioanalysis methods aim to quantify often trace-level amounts of drug substance present in biofluids or tissues, so tools that enhance sensitivity and enable lower limits of detection and quantification are highly desired. Oligonucleotides in particular - being highly negatively charged - readily bind to metal surfaces and benefit greatly from the use of our MaxPeak™ Premier High-performance Surface (HPS) Technology, which dramatically reduces non-specific adsorption and enhances sensitivity, repeatability and productivity.

Enhanced Oligonucleotide Sensitivity with MaxPeak Premier



Application Note 720007019EN: Improved Oligonucleotide SPE-LC-MS Analysis Using MaxPeak High Performance Technology, Brennan et.al.

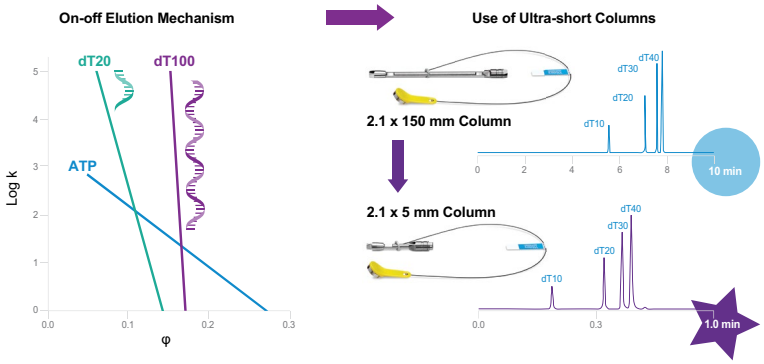
Rapid LC Separations for Fast LC-MS Quantitation

Bioanalytical labs need analytical solutions that are robust, scalable and fast to maximize performance and productivity.

As demonstrated in a 2023 publication by Honorine Lardeux *et.al.**, oligonucleotides exhibit a strong on/off elution mechanism, making them amenable to fast separations using ultrashort columns.

Reversed-phase columns with a 50 mm length are commonly used today, but to further accelerate separations, increase throughput and reduce costs, new ACQUITY™ Premier Oligonucleotide BEH™ C_{18} Ultrashort Columns with a 20 mm length are now available from Waters.

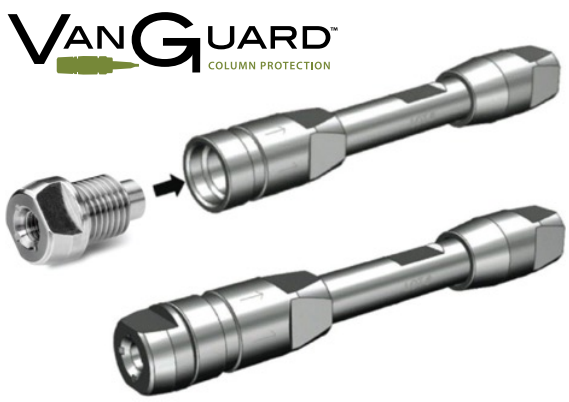
Ultra-Fast Separations of Oliglonucleotides



**High-Throughput Chromatographic Separation of Oligonucleotides: A Proof of Concept Using Ultra-Short Columns by Honorine Lardeux et.al. (Analytical Chemistry 2023, 95, 27, 10448-10456).*

Extending Column Life with the VanGuard FIT Column Format

Extending column life is another way bioanalytical labs can reduce costs and enhance overall productivity. As injections onto an LC column increase, so to does the potential for column fouling, which occurs when particulates, aggregates and other sample contaminants accumulate and begin to clog the head of the column. The VanGuard™ FIT Column format-unique to Waters-includes a fully integrated 5 mm pre-column cartridge packed with the same sorbent as the analytical column, and it acts as a guard preventing particulates from reaching the analytical column.



The fully integrated design has no impact on sample resolution and should fouling occur the cartridge can be easily replaced, thus extending the life of the analytical column.

Automating OligoWorks SPE Sample Prep with Andrew +

OligoWorks SPE Kits and Components are automation friendly and the sample pre-treatment and SPE protocols are easily automated on most liquid handling platforms, including the Andrew+™ Pipetting Robot from Waters, as highlighted in our app note: *An Automated, Standardized, Kit-Based Sample Preparation Workflow for Bioanalytical Quantification of Therapeutic Oligonucleotides* (Lit code: 720008068EN).

Pre-scripted methods for the pretreatment and SPE protocols are available as free downloads from our OneLab™ Library, and all the deck and pipetting accessories needed to run these methods, such as the Extraction+ Device and Microplate Gripper Tool have been combined into bundled part numbers for easy ordering.

Application Notes

Waters application notes are being published everyday. Join us on the Resource tab of our **waters.com/OligoWorks** landing page to find your next helpful INSIGHT about oligo BioA.

Ordering Information

The columns, standards and reagents that can help you quantify oligonucleotides are provided below.

SAMPLE EXTRACTION AND LC TOOLS FOR RELIABLE LC-MS QUANTITATION OF OLIGONUCLEOTIDES

OligoWorks Kits

	Part Number
OligoWorks SPE Microplate Kit: 2 mg/well	186010614
OligoWorks SPE Macroplate Kit: 30 mg/well	186010615
OligoWorks SPE 3cc Cartridge Kit: 60 mg, x 50	186010616

Oligonucleotide Standards

	Part Number
MassPREP OST Standard: 15–35-mer OligodT Ladder	186004135
Lipid conjugated ASO LC-MS Standard	186010747
ssDNA 10 to 60-mer Ladder	186009449
ssDNA 20 to 100-mer Ladder	186009448
ssDNA 20-mer LC-MS Standard	186009451

OligoWorks Standalone Components

	Part Number
RapiZyme Proteinase K Digest Module	186010601
OligoWorks SPE Eluent: 25 mL	186010610
OligoWorks SPE Eluent: 125 mL	186010611
TCEP HCl Reagent: 1.5 mL (0.5 mol/L)	186010612
OligoWorks SPE Microplate: 2 mg/well	186010618
OligoWorks SPE Macroplate: 10 mg/well	186010752
OligoWorks SPE Macroplate: 30 mg/well	186010619
OligoWorks SPE Macroplate: 60 mg/well	186010749
OligoWorks SPE 1 cc Cartridge: 10 mg, x50	186010750
OligoWorks SPE 3 cc Cartridge: 60 mg, x50	186010620
OligoWorks SPE 6 cc Cartridge: 150 mg, x30	186010753

OLIGO RPLC

ACQUITY Premier Oligonucleotide BEH C_{18} 1.7 µm Columns

Format	Diameter	20 mm	50 mm	100 mm	150 mm	20 mm	50 mm	100 mm	150 mm
		130 Å				300 Å			
Standard Column	2.1 mm	186011015	186009484	186009485	186009486	186011021	186010539	186010540	186010541
VanGuard FIT Column	2.1 mm	–	186010685	186010686	186010687	–	186010754	186010755	186010756

XBridge™ Premier Oligonucleotide BEH C_{18} 2.5 µm Columns

Format	Diameter	50 mm	100 mm	150 mm	50 mm	100 mm	150 mm
		130 Å			300 Å		
Standard Column	2.1 mm	186009836	186009837	186009838	186010542	186010543	186010544
	4.6 mm	186009901	186009902	186009903	186010545	186010546	186010547
VanGuard FIT Column	2.1 mm	186010688	186010689	186010690	186010757	186010758	186010759
	4.6 mm	186010691	186010692	186010693	186010760	186010761	186010762

HILIC

ACQUITY Premier BEH Amide 1.7 µm Columns

Format	Diameter	50 mm	100 mm	150 mm
		130 Å		
Standard Column	2.1 mm	186009504	186009505	186009506
VanGuard FIT Column	2.1 mm	186009507	186009508	186009509

XBridge Premier BEH Amide 2.5 µm Columns

Format	Diameter	50 mm	100 mm	150 mm
		130 Å		
Standard Column	2.1 mm	186009928	186009929	186009930
	4.6 mm	186009935	186009936	186009937
VanGuard FIT Column	2.1 mm	186009931	186009932	186009933
	4.6 mm	186009938	186009939	186009940

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