

# OLIGONUCLEOTIDE SOLID PHASE EXTRACTION AND ANALYSIS COMPARING A MANUAL EXTRACTION APPARATUS TO A NOVEL SEMI-AUTOMATED EXTRACTION MANIFOLD

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## INTRODUCTION

Solid phase extraction (SPE) is a common technique for sample preparation in oligonucleotide analysis and it often requires a large amount of time and focus by experienced scientists. Oligonucleotides are large and complex in nature, and can easily become lost during SPE by many mechanisms. One such mechanism is using inadequate flow of the sample through a sorbent bed. Additionally, scientists that manually control the flow of oligonucleotides through the sorbent with the use of a vacuum or manual positive pressure manifold increase the risk of losses during this process due to the variability in pressure being applied.

The work described herein compares the recoveries of a variety of oligonucleotides when using a manual vacuum apparatus versus the Otto SPEcialist, a new semi-automated positive pressure manifold, with an aim to reduce the risk of manual error during the SPE workflow while also achieving optimal precision and accuracy.

## METHODS

### Sample solution preparation

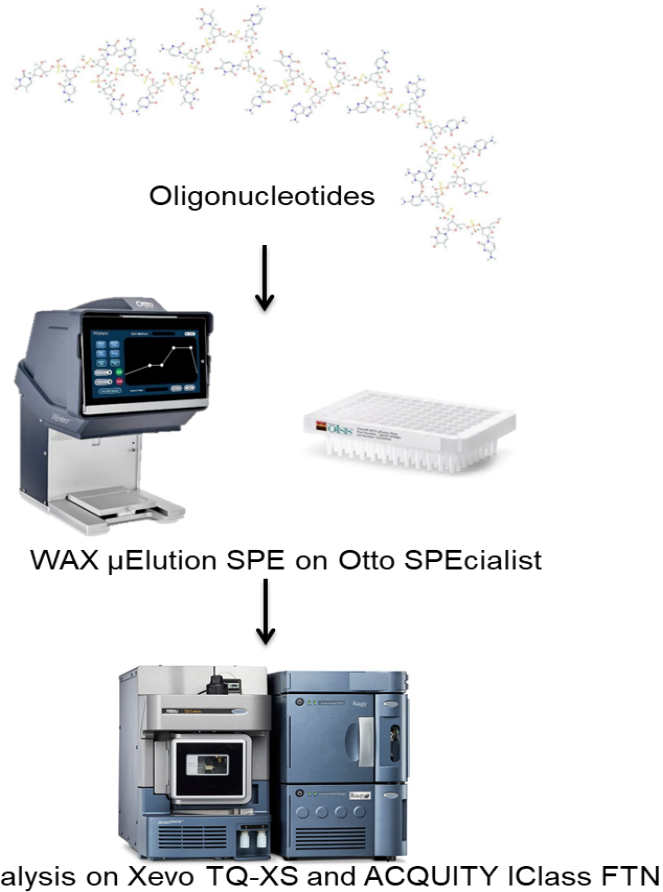
All Oligonucleotides were obtained from Integrated DNA Technologies (Coralville, IA) . Stock solutions of deoxythymidines (15-35T) and phosphorothioated (Gem 91) oligonucleotides were prepared in RNase free water at 100 µg/mL and 2.50 mg/mL, respectively. Gem 132 was used as an internal standard (IS) prepared at 16 mg/mL. Neat samples were prepared from the stock solutions in 50 mM NH<sub>4</sub>OAC buffer, pH 5.5 for 15-35T and Gem 91. IS solution was added to each sample of Gem 91 with a final concentration of 25 µg/mL.

### SPE Sample Extraction

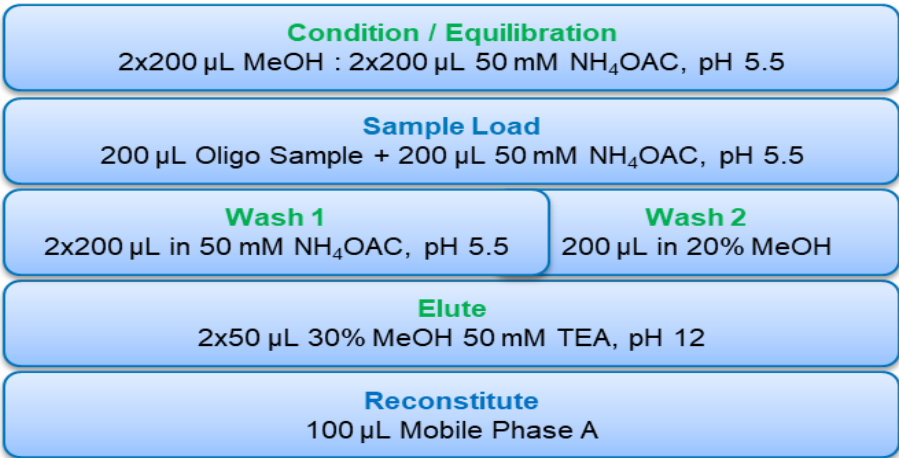
This bioanalytical sample extraction and LC-MS workflow is highlighted in Figure 1. For this work, 200 µL of the prepared oligonucleotide samples in neat solution were extracted manually and with the Otto SPEcialist using an Oasis WAX SPE 96-well plate in the µElution format according to the protocol highlighted in Figure 2.

### LC-MS/MS Conditions

LC-MS/MS quantification of the various analytes was performed using a Waters Xevo® TQ-XS tandem quadrupole MS (ESI-). Chromatographic separation was achieved using an ACQUITY I-Class PLUS UPLC® system and ACQUITY PREMIER Oligonucleotide BEH C<sub>18</sub>, 1.7 µm, 2.1 x 100 mm column. Mobile phases A and B consisted of 1% HFIP, 0.1% DIPEA in water, and 0.75% HFIP, 0.0375% DIPEA in 65% acetonitrile, respectively. A shallow gradient from 5-25% B over 3.50 minutes was used at a flow rate of 0.6 ml/min.



**Figure 1. Standardized and semi-automated sample preparation and LC-MS/MS bioanalytical workflow using Oasis WAX SPE 96-well plate in the µElution format with the Otto SPEcialist positive pressure manifold to streamline the sample preparation process, maximize productivity, reduce errors, and improve analytical method performance.**



**Figure 2. OASIS WAX SPE protocol for the extraction of various Oligonucleotides performed by the Otto SPEcialist positive pressure manifold. SPE extraction time <20 minutes.**



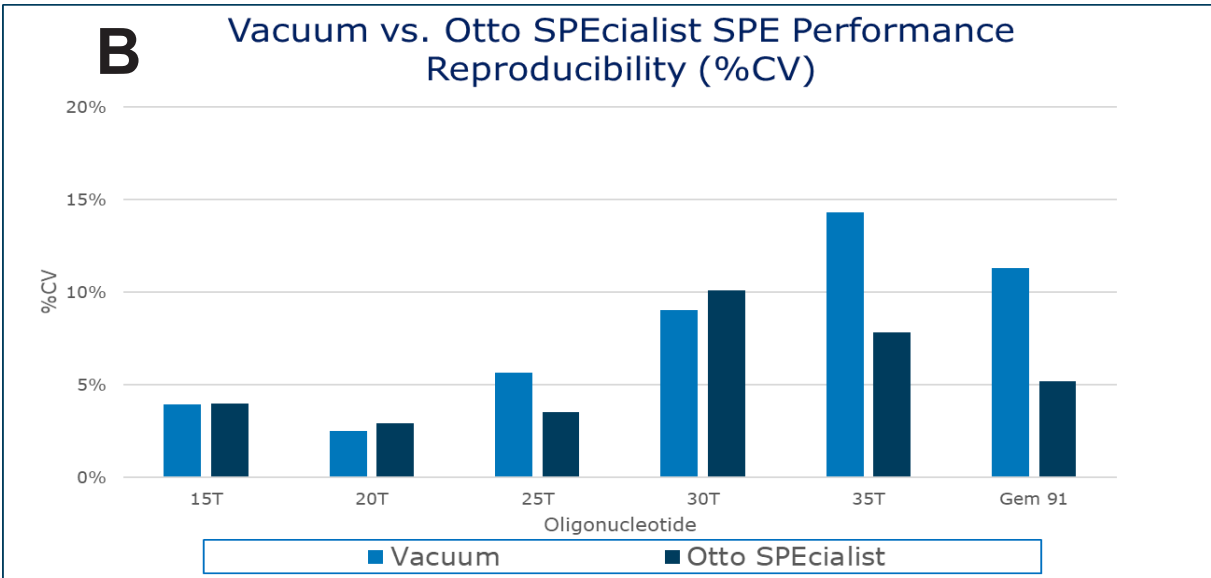
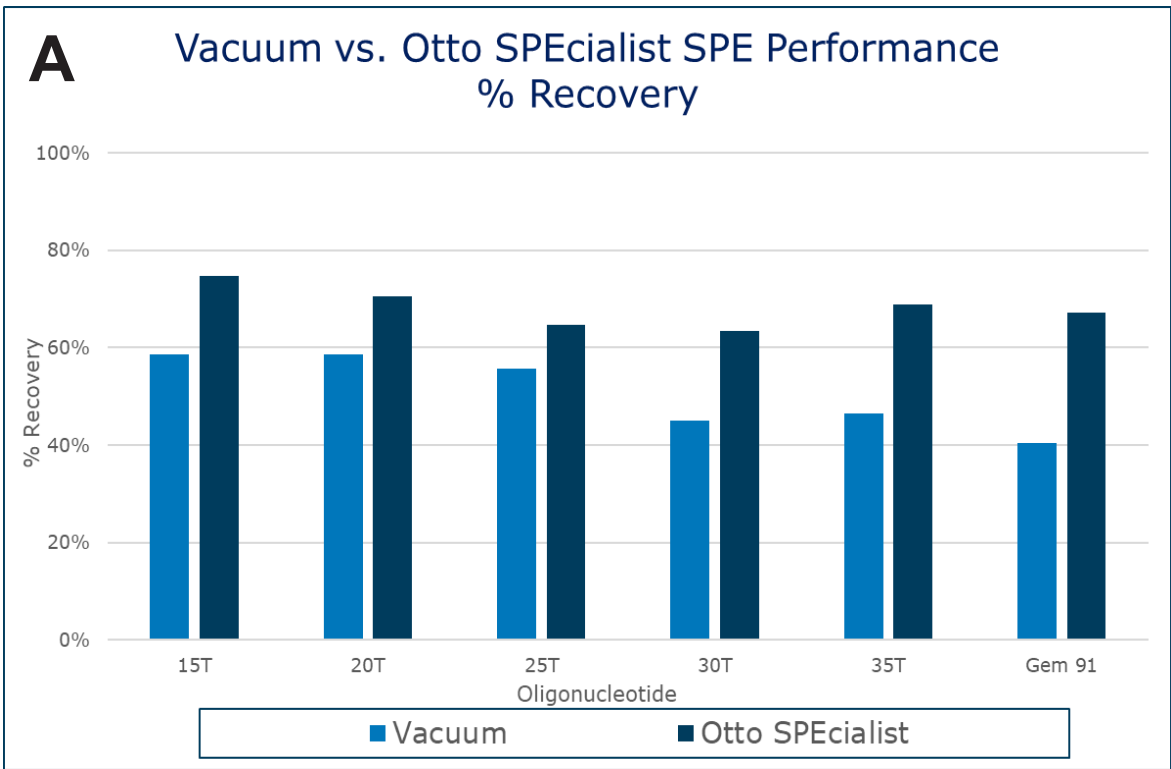
**Figure 3. Visual representation of a pressure profile run on the Otto Software. The Otto SPEcialist will use a pressure profile to autonomously apply positive pressure to the SPE sorbent bed.**



**Figure 4. Otto SPEcialist Manifold Layout for oligonucleotide sample extraction using Oasis WAX µElution SPE. The use of this semi-automated device will allow for even pressure applied across every well of the plate.**

## RESULTS

### I. EXTRACTION PERFORMANCE COMPARING SPE ON VACUUM VS. OTTO SPECIALIST



**Figure 5. Comparable SPE performance of manual vacuum apparatus to Otto SPEcialist using the Oasis WAX 96-well µElution SPE plate and LC-MS/MS analysis of various oligonucleotides. Panel A: Comparison of % recovery and Panel B: Reproducibility (%CV) comparison. These results demonstrate improved analyte recovery and improved CVs as compared to manual SPE extraction.**

### II. ACCURATE AND REPRODUCIBLE QUANTIFICATION USING SPE SAMPLE EXTRACTION PERFORMED WITH THE OTTO SPECIALIST

#### Standard Curve Performance

Oligonucleotide	Standard Curve Range (ng/mL)	Weighting	Linear Fit (r <sup>2</sup> )	Mean % Accuracy Range
OST 15 T	5-1,000	1/x	0.995	87-116
Gem 91			0.997	93-108

**Table 1. Representative standard curves for select oligonucleotides using the OASIS WAX 96-well µElution plate on the Otto SPEcialist. Quantification performance was excellent with dynamic ranges from 5-1,000 ng/mL, linear fit, with R<sup>2</sup> values ≥ 0.99, and accuracy ranges within 15% meeting standard bioanalytical performance criteria for method validation.**

#### Quality Control Performance

Inter-Day Precision and Accuracy of QC Samples (2 Days)						
QC Sample	Semi-Automated on Otto SPEcialist					
	LOQC		MQC		HQC	
	Conc. (ng/mL)		7.5		750	
	%CV	% Accuracy	%CV	% Accuracy	%CV	% Accuracy
OST 15T	5.9	83.9	8.6	106	4.1	105
Gem 91	7.5	103	6.2	94.5	2.7	96.4

**Table 2. Inter-day QC sample statistics for select oligonucleotides using the OASIS WAX 96-well µElution plate on the Otto SPEcialist. Inter-day mean QC sample % accuracies ranged from 84-106 %, with CVs < 10 %, demonstrating a highly accurate and reproducible sample preparation and LC-MS analytical method.**

## STUDY HIGHLIGHTS

- A total sample preparation workflow (Figure 1), which is a robust, flexible, and scalable solution, addresses the increasing demand for accurate and reproducible quantification
- Rapid, simplified sample preparation of oligonucleotides using the Otto SPEcialist and standardized SPE protocol, which streamlines the extraction process, maximizing productivity, reducing errors, ensuring analytical method performance (Figure 5) and frees up the analysts time to perform other tasks.
- This semi-automated approach yields excellent quantitative performance (Tables 1 and 2) with standard curve and QC accuracies between 84-116 % and mean CVs <10 % for the select oligonucleotides extracted from the Oasis WAX 96-well µElution plate.

## CONCLUSION

The Otto SPEcialist positive pressure manifold is an easy to use, reliable, and effective semi-automated solid phase extraction device. It gives excellent and reproducible quantitative performance for the extraction of oligonucleotides, while minimizing human intervention. It is a simple and easy semi-automated approach to SPE.