Method transfer for the purification of synthetic oligonucleotides

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Abstract

Purpose: Demonstrate the complete method transfer of dual-labelled 15mer oligonucleotides from a competitor LC and column workflow to a Thermo Fisher Scientific solution using HPLC to purify along with HPLC-HRMS quality control under Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS).

Methods: The workflow transfer of the oligonucleotide purification, quantification, and qualification was performed using TEAA-base RP-LC gradient conditions. The purification was run peak-based to isolate the components. A similar chromatography was used for the LC-UV QC and mass identification via LC-HRMS.

Results: The intended transfer of the complete workflow i.e. HPLC purification systems, LC-UV systems, and columns was successfully performed and verified. The versatility of methods provides the opportunity to switch to the complete Thermo workflow solution from competitor vendors.

Introduction

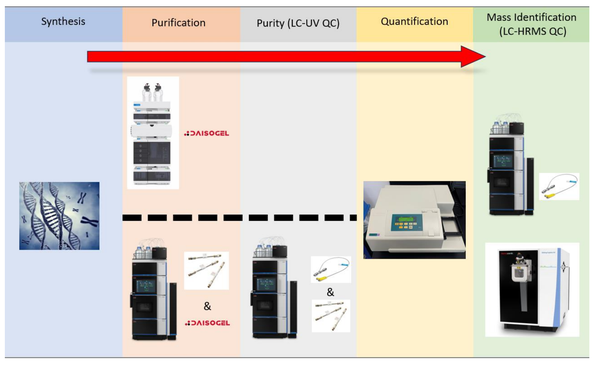
The process of oligonucleotide synthesis involves multiple reactions, leading to the accumulation of impurities, such as truncated nucleotide sequences , partial deprotection, and fluorophore/quencher degradation. Therefore, it is crucial to purify the desired oligonucleotides effectively and with high purity for downstream applications, such as quantitative polymerase chain reactions (qPCR), integrated human identification (HID) solutions for forensics, and gene transfer agents (GTA).

When developing methods to separate and purify oligonucleotides, it is essential to consider their unique characteristics. These characteristics include the length of the oligo, the specific sequence, fluorophore/quencher combination, and ability to form secondary structures. Other factors that influence an oligonucleotide's interaction with the stationary phase translating to retention time include buffer (pH and salt concentration), reversed-phase column, and LC system selection [2].

It is imperative that oligonucleotide-manufacturing laboratories investing in new HPLC technology understand the impact and importance of column selection, LC hardware/software, and automation on purity. Therefore, the ability to seamlessly transfer oligonucleotide purification methods from one vendor instrumentation and columns to another while meeting or exceeding the expected quality criteria is a valuable tool.

This work demonstrates the effective method transfer of the semi-preparative RP-HPLC purification of two different dual-labelled 15mer oligonucleotides from a source LC instrument and column to an alternate vendor column and HPLC resulting in passing QC standards for the yield and purity. By-products of the DNA synthesis are resolved and the target oligonucleotides are successfully isolated and collected. LC-HRMS and HPLC analysis were used to confirm oligonucleotide purity, suggesting a successful method transfer for the preparation of the high-purity oligonucleotides for downstream application [Figure 1].

Figure 1. The step-by-step validation for the method transfer from a competitor setup to a complete Thermo Fisher solution.



Materials and methods

Sample preparation

Oligonucleotide Name	Sequence	Monoisotopic Mass
ABY-MGB	[ABY]-TTGGTCTCTATCTGC-[MGB]	6357.2
JUN-MGB	[JUN]-TTGGTCTCTATCTGC-[MGB]	6437.3

- Samples were prepared separately.
- 500 µL Solvent A pipetted into crude dual-labelled oligonucleotide sample vessel. No dissolution initially observed.
- Add 300 µL Solvent B pipetted into tube. Dissolution observed.
- Vortex for 1 min.
- 5 min. sonication at 40° C
- Vortex for 1 min.
- Add 200 µL Solvent A pipetted into the sample tube.
- Vortex for 1 min.
- 5 min. sonication at 40° C
- Vortex for 1 min.

Test Method(s)

The exact purification methods were performed on the Agilent 1260 Infinity II Analytical-Scale LC Purification System & the Thermo Scientific™ Vanquish™ Analytical Purification LC System. The LC-UV was performed on a Thermo Scientific™ Vanquish™ Flex™ UHPLC System. The Thermo Scientific™ Hypersil GOLD™ Analytical and Semi-Preparatory columns' performance was 1:1 compared to that of the competitor options. Mass confirmation was performed on a Thermo Scientific™ Orbitrap Exploris™ 480 Mass Spectrometer.

Column comparison

Table 1. The columns used in the comparison.

Column	Description
Daisogel semi-preparative column	Daisogel SP-100 ODS-P; 5µm, 100Å 10x150mm
Waters analytical column	Waters™ ACQUITY UPLC BEH C18 Column, 130Å, 1.7 µm, 2.1x50mm
Thermo Scientific™ Hypersil GOLD™ C18 RP HPLC column – analytical	C18 RP HPLC column, 2.1 × 50 mm, 175 Å, 1.9 µm (P/N 25002-052130)
Thermo Scientific™ Hypersil GOLD™ C18 Prep HPLC column – semi-preparative	C18 RP HPLC column, 10 x 150 mm, 175 Å, 5 µm (P/N 25005-159070A)

Data Analysis

Chromeleon 7.3.2 CDS for purification & LC-UV QC. Thermo Scientific™ Xcalibur™ and FreeStyle software for mass confirmation.

Results

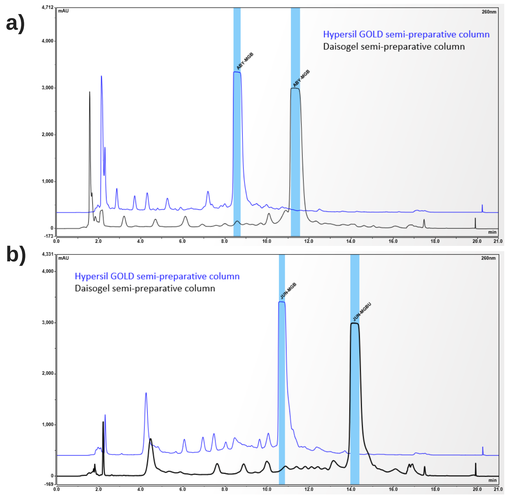
Step 1 – Purification

Table 2. The LC-UV chromatographic conditions for the purification of the oligonucleotides.

Mobile Phase	Solvent A: 100 mM TEAA Solvent B: 1:1 MeCN:MeOH	
Gradient		
Time (min)	A	B
-10	65	35
0.0	60	40
15	33	67
15.01	5	95
18.0	5	95
18.01	65	35
21	65	35
Flow Rate	4.5 mL/min.	

…Purification

Figure 2. Column performance comparison between the Daiso and Hypersil GOLD prep columns for the a) ABY-MGB crude oligo synthesis purification and the b) JUN-MGB.



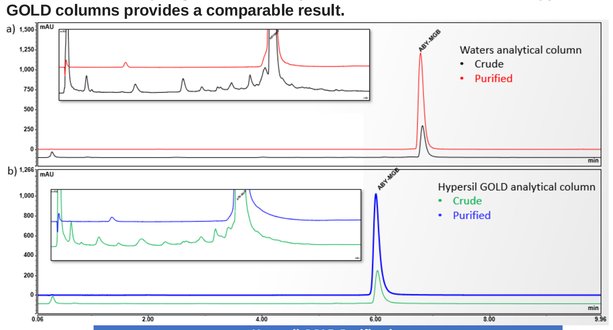
Step 2 – LC-UV QC

Sample purity comparison

Table 3. Column purity comparison between the Daiso and Hypersil GOLD prep columns

Sample	Daiso semi-preparative column	Hypersil GOLD semi-preparative column
ABY-MGB	98.41%	99.70%
JUN-MGB	98.76%	99.92%

Figure 3. Column comparison between the Waters and Hypersil GOLD analytical columns when analyzing the ABY-MGB probe. It can be concluded the Hypersil GOLD columns provides a comparable result.

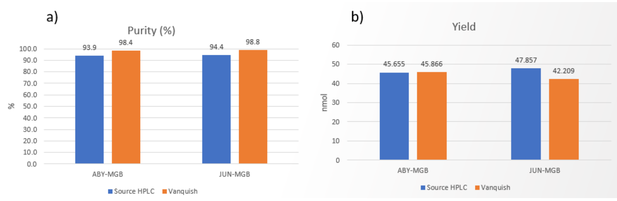


	Hypersil GOLD Purification			
	Waters LC-UV analytical column		Hypersil GOLD LC-UV analytical column	
	Crude	Purified	Crude	Purified
ABY-MGB	85.84%	98.62%	86.61%	99.70%
JUN-MGB	81.99%	99.68%	82.67%	99.92%

Step 3 – Mass identification and quantification

The mass spectrum of each sample was measured to confirm the presence of the full-length oligonucleotide product and identify any impurities. The expected monoisotopic mass was observed (+/- 0.2 Da) for each sample and no critical impurities were detected.

Figure 4. Using the source method semi-preparative and analytical columns, the LC purification systems were compared to show that the method can be transferred as-is from an Agilent Technologies 1260 Infinity II Preparative-Scale LC Purification System to the Vanquish Analytical Purification LC System where a) the purities of the oligonucleotide samples was compared as well as b) the yields were also comparable from the source LC instrument solution to the Vanquish LC instrument solution.



Conclusions

- A simple method transfer was successfully shown for the purification of the dual-labelled oligonucleotide samples from a non-Thermo Fisher Scientific LC purification system to the Vanquish Analytical Purification LC System.
- Vanquish purification yields quantitation/QC data comparable to Agilent purification for the ABY/JUN-MGB probes (Figure 5).
- The Hypersil GOLD semi-preparative column yields similar quantification and purification performance to that of the non-Thermo Scientific semi-preparative column used before, while isolating the short-chain dual-labelled oligonucleotide samples with the added benefit that the fraction volume is reduced making for less solvent to be evaporated from the purified products.
- For QC, the Hypersil GOLD analytical column performs successfully passed the criteria demonstrating to be a suitable substitute of the non-Thermo Scientific analytical column.

References

1. Roberts et al. (2020). Advances in oligonucleotide drug delivery. Nature Reviews Drug Discovery, 19, 673–694.
2. Zhang et al. (2016 Dec 18). Recent Methods for Purification and Structure Determination of Oligonucleotides. International Journal of Molecular Sciences, 17(12), 2134.

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