Application NOTE

Waters

# EVALUATION OF DNA SYNTHESIS QUALITY USING ION-PAIR REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (IP-RP-HPLC)

## Quality Control of Oligonucleotides by IP-RP-HPLC

Current methods of DNA synthesis can routinely produce high quality oligonucleotides, provided that automated synthesizers are in optimal operating condition. While suboptimal synthesis can still yield acceptable and usable oligonucleotides, diagnostic and therapeutic applications require high purity oligonucleotide probes.

A method utilizing ion-pair reversed-phase high performance liquid chromatography (IP-RP-HPLC) with ultraviolet (UV) detection has been developed for the routine quality control (QC) of synthetic homo– and heterooligonucleotides up to 30mer in length. The 5-minute per sample duty cycle allows for fast diagnosis of the performance of DNA synthesizers.

## Separation of Synthetic Oligonucleotides on XTerra® Intelligent Speed (*IS*™) Columns

Separation was performed on a 4.6 × 20 mm XTerra® MS $C_{18}$, 2.5 µm *IS*™ column. Mobile phases were comprised of 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) and triethylamine (TEA) with a concave methanol (MeOH) gradient. Since XTerra® columns are packed with hybrid particles, they can be operated at the elevated pH and temperature levels needed for DNA separation (pH 7—9; 60°C). The short column length (20 mm) allows for separation times under 5 minutes. *Figure 1* shows the IP-RP-HPLC separation of four different homooligonucleotides ($dG_{20}$, $dC_{20}$, $dA_{20}$, and $dT_{20}$). Baseline separation of closely eluting impurities (i.e. N—1, 2, 3, etc.) from the target oligonucleotide was routinely achieved.

| | |
|---|---|
| **HPLC system**: | Waters Alliance® HT Separations Module |
| **Column**: | XTerra® MS $C_{18}$, 2.5 µm, 4.6 x 20 mm *IS*™ Column |
| **Part Number**: | 186001889 |
| **Flow rate**: | 1.0 mL/minute |
| **Mobile phase A**: | 5 % MeOH in TEA/HFIP (16.3 mM/400 mM), pH 7.9 |
| **Mobile phase B**: | 30 % MeOH in TEA/HFIP (16.3 mM/400 mM), pH 7.9 |
| **Gradient**: | 0—60 % B in 4.25 min.; gradient profile 4 (concave) |
| **Injection volume**: | 2 µL (dissolved in mobile phase A)<br>200 pmole total mass load |
| **Column temp.**: | 60°C |
| **Detection**: | Waters 2487 Dual λ Absorbance Detector; 254 nm |
| **Buffer preparation**: | Dissolve 41.5 mL of HFIP in ~ 950 mL of water. While mixing vigorously, add 2.3 mL of TEA. Adjust final volume to 1 L with water. The pH of the solution should be about 7.9 |

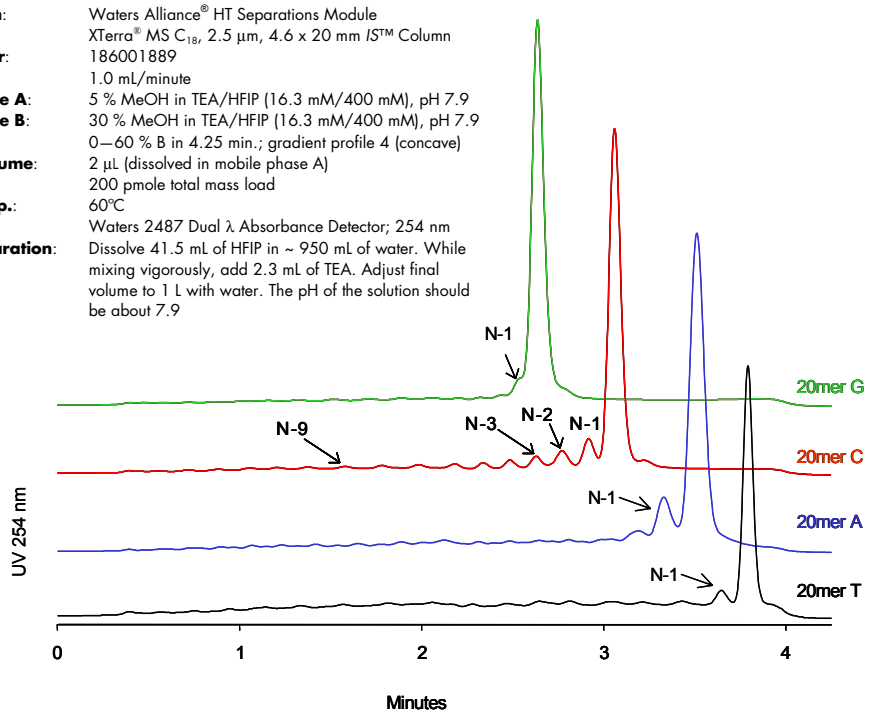


*Figure 1. IP-RP-HPLC separation of 20mer homooligonucleotides*

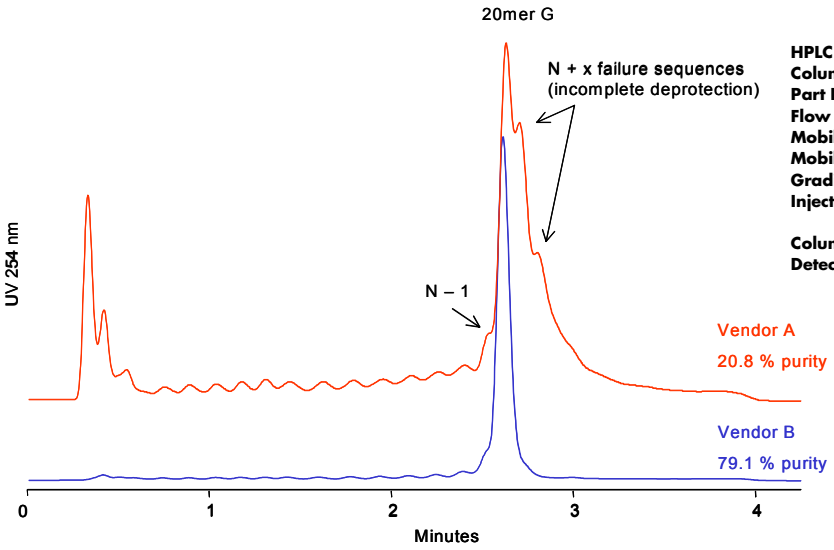


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*Figure 2. IP-RP-HPLC analysis of 20mer oligodeoxyguanosines ($dG_{20}$) from 2 different vendors*

## Fast Diagnosis of DNA Synthesizers

The quality of each homooligonucleotide is indicative of a particular problem with DNA synthesizer performance (coupling efficiency, incomplete deprotection, depurination, etc.). Two sets of four homooligonucleotides (same as in *Figure 1*) from five different vendors were analyzed in order to determine the variability of synthesis quality. *Figure 2* shows the fast HPLC analysis of a 20mer G homooligonucleotide from two of the vendors. Overall, a wide range of synthesis quality was found between vendors, which can be attributed to varying DNA synthesizer parameters. Since detection is performed by UV, the absolute amount of impurities in each synthesis can be quantitated. The IP-RP-HPLC method is capable of routine detection of 0.7 % (~ 7 pmoles) of failed products in the target oligonucleotide (*Figure 3*).

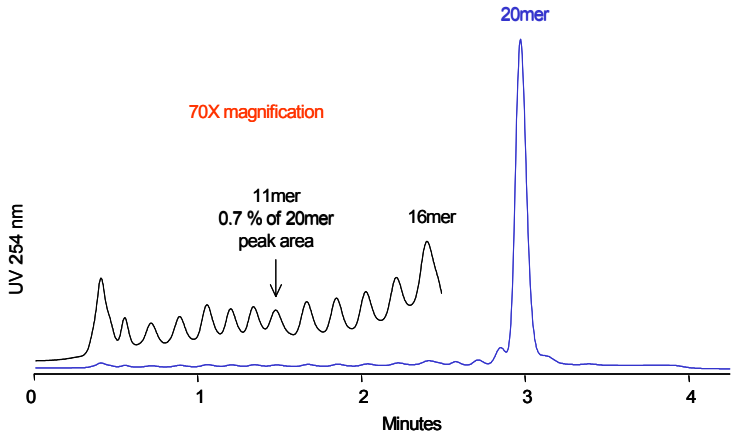


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*Figure 3. Quantitation of impurities in a 20mer oligodeoxycytidine ($dC_{20}$) synthesis*

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