Waters
Lab Highlights
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Rapid Compositional Confirmation of Synthetically Prepared Oligodeoxyribonucleotides by LC

Over the past two to three years, instruments capable of synthesizing short DNA molecules called oligodeoxyribonucleotides (oligomers) have become available. It is assumed, however, the sequence obtained is correct based upon the step by step synthesis itself, since there is no convenient procedure to verify the sequence of the small oligomers. The two popular methods of sequence determination for larger DNA, the Sanger and Maxam-Gilbert methods, are difficult to perform on small synthetic DNA without adaptation. In addition, they employ laborious and time consuming electrophoretic techniques, as well as additional reagents and radioisotope measurement techniques. In order to circumvent these problems, we have recently developed a *fast and convenient LC method*[1] *that verifies the composition of these types of compounds*, providing valuable information in lieu of the complex and time consuming electrophoretic sequencing procedures.

The procedure subjects the synthetic oligonucleotides, which in this case were prepared and isolated according to standard methods using a MilliGen AutoGen™ 6500, to an acid hydrolysis step, followed by compositional confirmation as base ratios with the aid of reverse-phase (μBondapak C_{18}) LC (see reference 1). The results of the chromatographic methods development are shown in Figure 1A, where all four of the bases are baseline resolved with good peak shape in under 6 minutes.

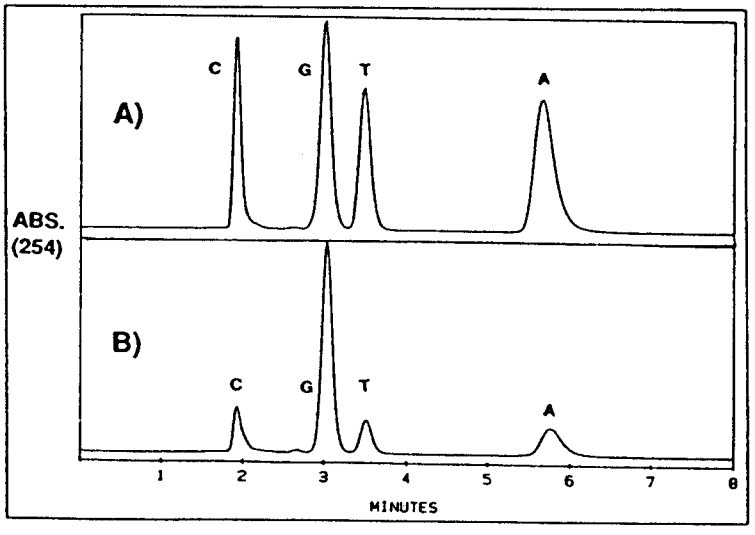

Figure 1. Reverse Phase LC Analysis of Nucleotide Base Standards (1A) and 14 MER Hydrolysis Product (1B). Peaks are cytosine (C), guanine (G), thymine (T) and adenine (A). Reverse phase LC was carried out on a μBondapak™ C18 column, (3.9 x 150 mm). The isocratic mobile phase was 90% 0.025 M triethylammonium acetate, pH 7.0, 10% methanol (v/v); the flow rate was 1.0 mL/min. Mobile phase and samples were prepared with Milli-Q® water. The chromatographic system consisted of a Model 510 Solvent Delivery System, Model 710B WISP™ Autosampler, Model 490 Programmable Multiwavelength Detector (operated at 254 nm) and a Model 840 Data and Chromatography Control Station (Waters). Nucleotide base standards were prepared in 6 M ammonium hydroxide, and diluted with water prior to use. Final concentration of each base standard was 1.25 ng/μl. An injection volume of 15 μl was used.

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To test the hydrolysis and chromatographic procedure, several samples were subjected to the acid hydrolysis conditions described previously. The calculated empirical formulae are compared with the known compositions in Table 1. In addition, a representative chromatogram of the 14 MER hydrolysis product is illustrated in Figure 1B. It is evident from the data presented in Table 1 that these preliminary results show promise for small oligomers, and experimentation continues to extend this method to larger, more complex molecules.

Table 1. Compositional Analysis of Various Synthetic Oligodeoxyribonucleotides Following Hydrolysis and LC

	Compound		
	4MER	8MER	14MER
Cytosine			
Observed	0.88	1.94	1.94
Actual	1	2	2
Guanine			
Observed	0.92	1.72	7.98
Actual	1	2	8
Thymine			
Observed	1.29	2.40	2.14
Actual	1	2	2
Adenine			
Observed	0.91	1.94	1.94
Actual	1	2	2

1. Swartz, M., *Bio Chromatography,* **2** (2), 98, 1987. Reprints are available upon request.

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