



# Waters Application Library

Synthetic Oligonucleotides Separation  
**HPLC** Application Library

**Compound:** Synthetic Oligonucleotides  
**Type:** Deoxyribose  
**Matrix:** Tris Buffer  
**Secondary Matrix:**

## Conditions:

This Analysis Uses Gradient Conditions

**Column / Capillary:** Waters Gen-Pak FAX  
**Column / Capillary Dimensions:** 4.6 x 100mm  
**Column / Capillary Part Number:** 15490  
**Flow Rate / Voltage:** 0.75 ml/min  
**Temperature:** 60 C  
**Injection Volume / Type:** 40 uls  
**Injection Conditions:**  
**Sample Concentration:** 0.01 OD 260nm units / ul  
**Sample Preparation:** Microfuge or filter through Millex-HV 0.45um device  
**Run Time:** 30 min

**Mobile Phase / Electrolyte:** A= 25mM Tris/HCl, pH 8.0 with 10% Acetonitrile B= A + 1.0M NaCl

**Gradient Conditions:** 20 - 60%B in 30 min

**Detection (Primary):** 210 - 300nm on Millennium 996 PDA at 1.2nm resolution setting

**Detection (Secondary):**

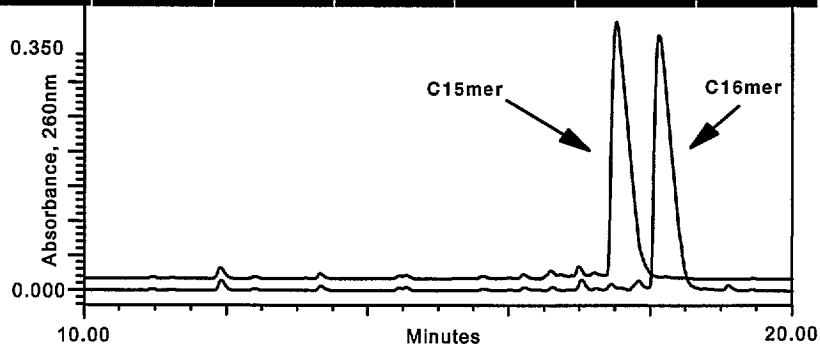
## Instrumentation /System:

Waters BioDiscovery System

# Chromatogram / Electropherogram:

## HPLC Separation of a 16mer and its "N-1" 15mer Oligonucleotide

Sequence Name	RT (Min)	Match #1 Angle	Match #1 Name	Match # 2 Angle	Match # 2 Name	Match #3 Angle	Match #3 Name
C15mer	17.522	0.028	C15mer	0.901	C16mer	1.958	W15mer
C16mer	18.115	0.025	C16mer	0.857	C15mer	1.342	Z16mer



Spectral Contrast Match Angle reproducibility for C16mer sequence = +/- 0.03 (N=12)

### Objectives:

Synthetic oligonucleotides can be successfully resolved and quantitated by high performance, anion exchange chromatography with single wavelength detection. Photodiode array detection in conjunction with the Millennium Spectral Contrast Technique provides additional qualitative information on HPLC separated samples. In combination, these separation and detection techniques provide information not obtainable in other single analytical method.

### Details:

Additional examples of this technology will be available shortly as a CPM module with script

### Ordering Information:

Part Number	Description	Quantity
15490 UNKNOWN	WATERS GEN-PAK FAX COLUMN WATERS BIO DISCOVERY SYSTEM	1 1

**References:**

<b>Reference 1</b>	Results from this synthetic DNA study will be submitted to BioTechniques for publication.
<b>Reference 2</b>	Also see: "Identifying Peptides through Mathematically Enhanced Spectral Analysis" by Young and Gorenstein in Genetic Engineering News. 1993. Vol.13(19). Page 25.
<b>Reference 3</b>	
<b>Reference 4</b>	
<b>Reference 5</b>	

**Journal Name:** To be published in BioTechniques

**Volume Number:** **Page#:**

**Author:**

**Ref. Number:**

**Obsolete:** ☐ Yes  
☒ No

**Date:**

**Year:**