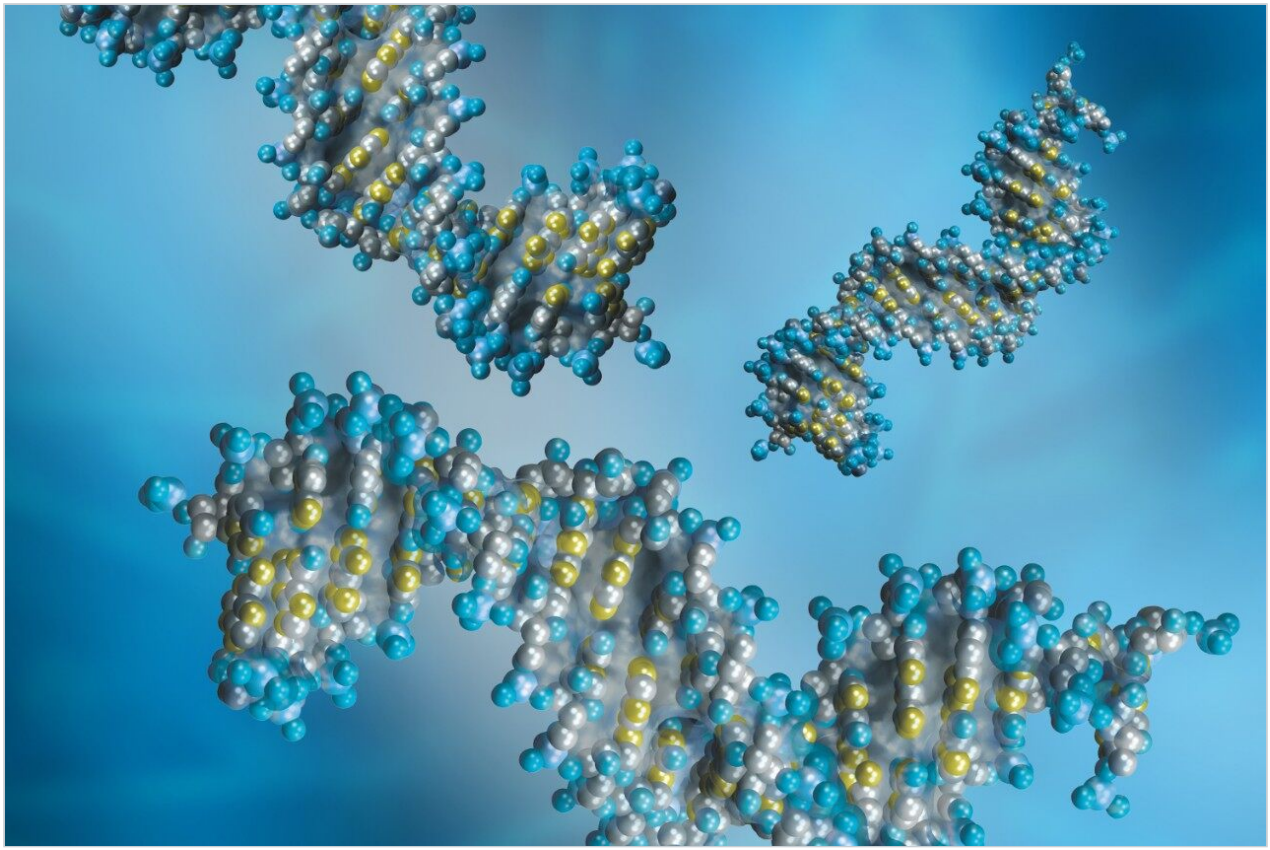
Waters™

Note d'application

LC-MS Analysis of Synthetic Oligonucleotides

Waters Corporation



Abstract

This application note demonstrates LC-MS analysis of synthetic oligonucleotides.

Introduction

LC-MS – The method of choice for characterization of oligonucleotides

Quality control and characterization is an important requirement for therapeutic oligonucleotides. Liquid chromatography with mass spectrometry detection (LC-MS) is the most suitable method for this analysis. Liquid chromatography using XTerra MS C_{18} Columns provides good oligonucleotide resolution up to 60mer using a mobile phase compatible with electrospray mass spectrometry (ESI-MS). The methods were developed for sensitive LC-MS analysis of native and modified oligonucleotides using a 1.0 x 50 mm XTerra MS C_{18} Column with a Capillary HPLC System and ESI (Tof) mass spectrometer.

Experimental

Preparation of 16.3 mM TEA - 400 mM HFIP buffer

Dissolve 41.5 mL of HFIP in ~950 mL of water. While mixing vigorously add 2.3 mL of TEA. Adjust volume to 1L with water. The pH of solution should be close to 7.9.

Preparation of 8.6 mM TEA - 100 mM HFIP buffer

Dissolve 10.5 mL of HFIP in ~950 mL of water. While mixing vigorously add 1.2 mL of TEA. Adjust volume to 1L with water. The pH of solution should be close to 8.3.

Results and Discussion

XTerra Columns for Sensitive LC-MS analysis and HPLC conditions

XTerra MS C_{18} Columns are packed with porous 2.5 µm hybrid particles. The sorbent has extended stability at temperatures and pH's typically used for oligonucleotide separations (50–60 °C; pH 7–9). The 1.0 x 50 mm column is operated at the mobile phase flow rate compatible with direct sensitive MS detection. Mobile phases consist from aqueous triethylamine (TEA) and hexafluoroisopropanol (HFIP) solutions (ion-pairing buffer) and methanol. Oligonucleotide resolution achieved with this system was greater than with traditional

triethylammonium acetate (TEAA) ion-pairing buffer. Contrary to TEAA based mobile phases, little or no ion suppression was observed with TEA-HFIP buffers.

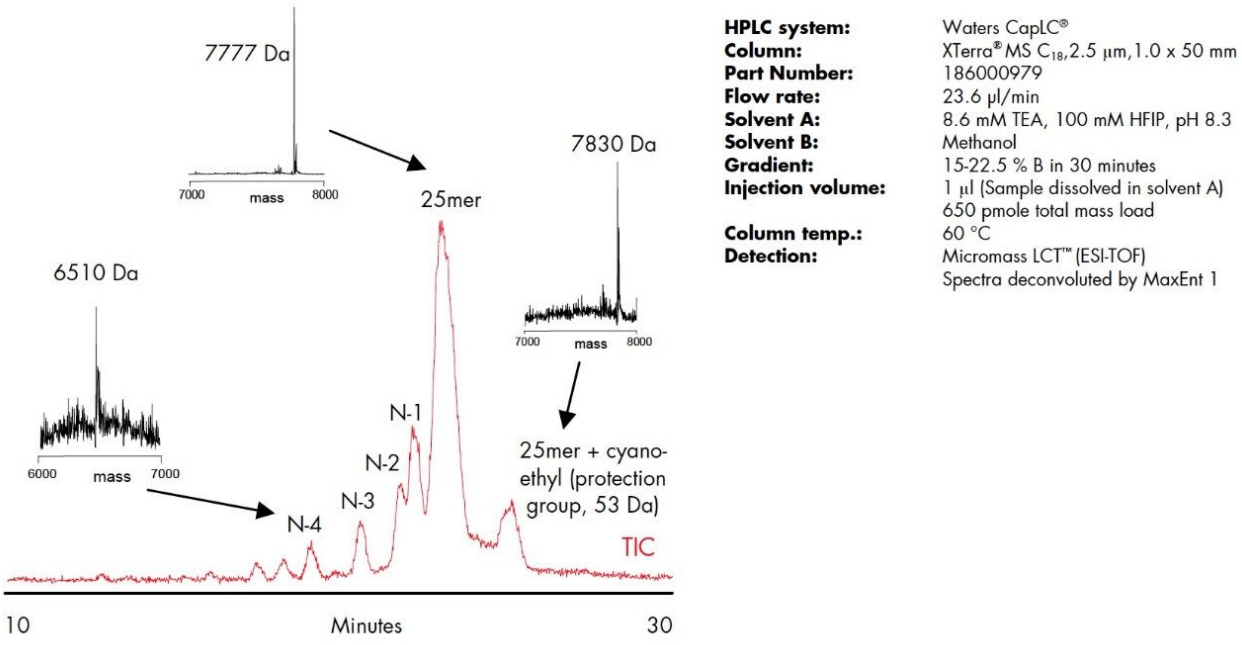
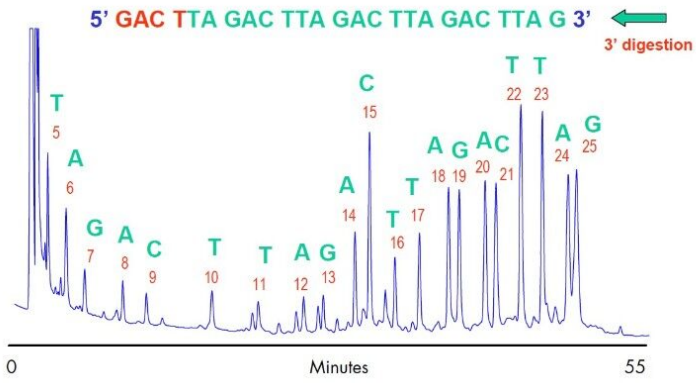


Figure 1. LC-MS analysis of 25mer phosphorothioate oligonucleotides.

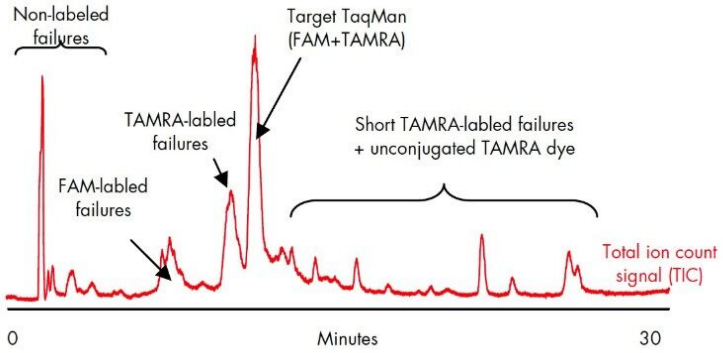
Principles of Oligonucleotide Identification by MS

The comparison of theoretical and measured mass was used for oligonucleotide identification. The Tof mass spectrometer routinely achieves mass accuracy ±1Da for oligonucleotides <50mer or even longer. Figure 2 shows oligonucleotides generated by digesting 25mer with 3′ exonuclease. They were positively identified by their molecular mass. The difference in mass for 24/25mer pair was 329.2 Da, indicating the loss of G mononucleotide. Similarly, the difference in mass for 23/24, 22/23, and 20/21 shows a loss of A, T and C mononucleotide, respectively (313.2, 304.2 304.2, and 289.2 Da). This method can be used for sequence verification and failure products identification of therapeutic and diagnostic oligonucleotides (Gilar, *Anal. Biochem*. 298 (2001) 196–206). Figure 3 shows analysis of TaqMan oligonucleotide. Accurate mass measurement was used for identification of singly-labeled failure products from “one-pot” synthesis. First elute nonlabeled oligonucleotides, followed with 5′FAM products, 3′TAMRA labeled oligonucleotides and the dually-labeled target product. Later eluting peaks are 1-4mer TAMRA labeled products and un-conjugated dye.



HPLC system:	Waters CapLC®
Column:	XTerra® MS C_{18}, 2.5 µm,1.0 x 50 mm
Part Number:	186000979
Flow rate	23.6 µl/min
Solvent A	16.3 mM TEA, 400 mM HFIP, pH 7.9
Solvent B	Methanol
Gradient:	11-20 % B in 30 minutes, 20-24.5 % B from 30 to 60 min
Injection volume:	1 µl (sample dissolved in solvent A)
Column temp.:	50 °C
Detection:	PDA and MS. Micromass™ LCT (ESI-TOF) Spectra were deconvoluted by MaxEnt 1.

Figure 2. LC-MS identification of oligonucleotides.



HPLC system:	Waters CapLC®
Column:	XTerra® MS C_{18}, 2.5 µm, 1.0 x 50 mm
Part Number:	186000386
Flow rate:	23.6 µl/min
Solvent A	8.6 mM TEA, 100 mM HFIP, pH 8.3
Solvent B:	Methanol
Gradient:	15-22.5 % B in 30 minutes
Injection volume:	1 µl (Sample dissolved in solvent A), 650 pmole total mass load
Column temp.:	60 °C
Detection:	Micromass LCT (ESI-TOF) Spectra deconvoluted by MaxEnt 1

Figure 3. LC-MS identification of 21mer TaqMan and failure by-products generated by one-pot Probe synthesis.

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