

# LC-MS Analysis of Synthetic Oligonucleotides

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## Abstract

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

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## 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<sub>18</sub> 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<sub>18</sub> Column with a Capillary HPLC System and ESI (Tof) mass spectrometer.

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## 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.

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## Results and Discussion

### XTerra Columns for Sensitive LC-MS analysis and HPLC conditions

XTerra MS C<sub>18</sub> 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.

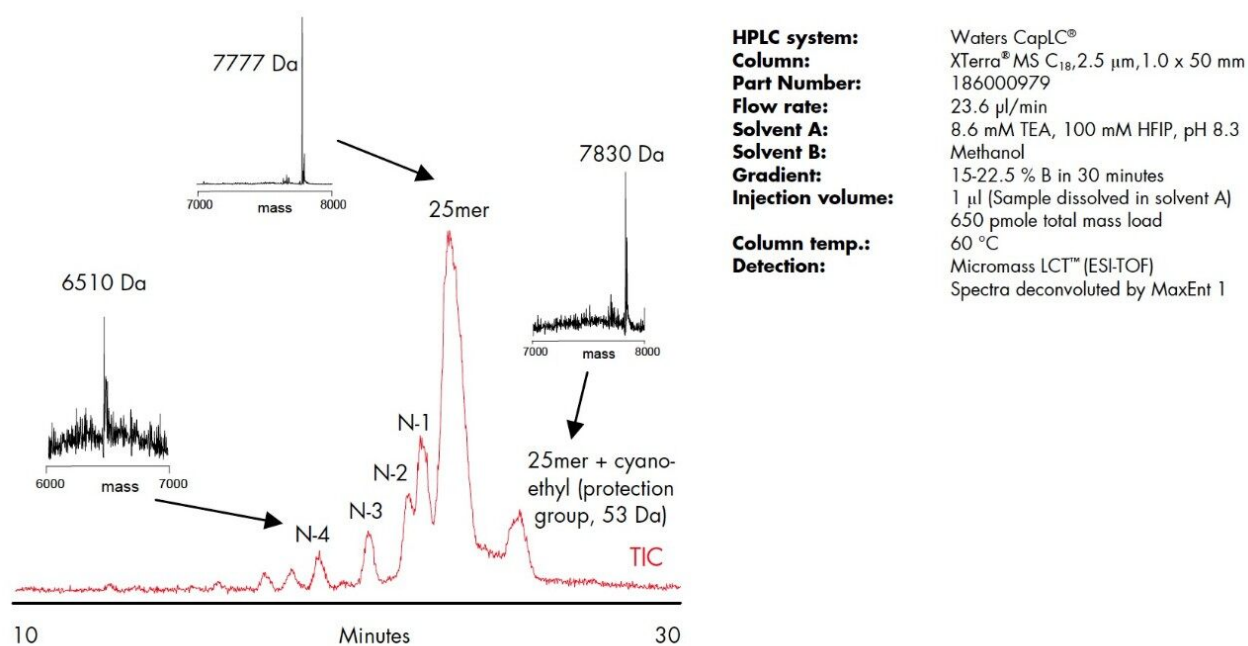


Figure 1. LC-MS analysis of 25mer phosphorothioate oligonucleotides.

## 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  $\pm 1$  Da 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.

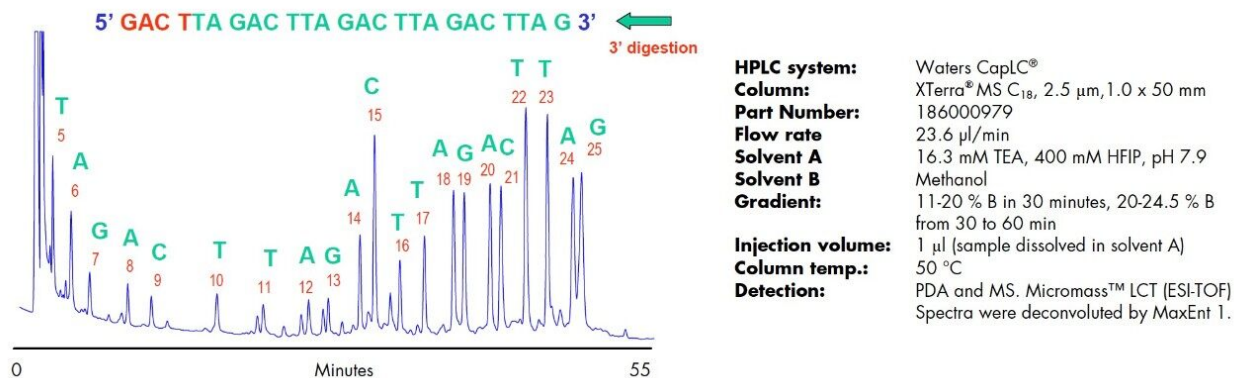


Figure 2. LC-MS identification of oligonucleotides.

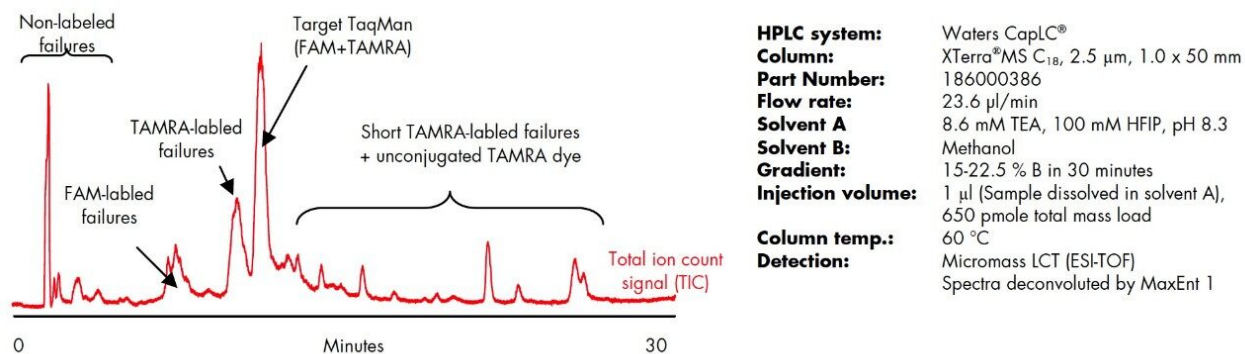


Figure 3. LC-MS identification of 21mer TaqMan and failure by-products generated by one-pot Probe synthesis.

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