LC-MS Analysis of Therapeutic and Diagnostic Oligonucleotides

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Overview
- LC-MS method was developed for characterization of oligonucleotides.
- Method was applied for analysis of therapeutic and diagnostic oligonucleotides.
- Excellent LC separation was achieved.
- Mobile phase is compatible with MS detection.

Introduction
Synthetic oligonucleotides are utilized for diagnostic as well therapeutic purposes (antisense drugs). Synthesis of chemically modified oligonucleotides is challenging and often results in a product of limited purity [1,2]. An LC-MS method has been developed for the characterization of oligonucleotide-based drugs and diagnostic probes [3]. The method was utilized for analysis of phosphorothioate oligonucleotides, dually labeled probes and native oligonucleotides.

LC-MS is useful for nucleic acids analysis [4,5]. We used an optimized LC-MS system (see Methods) for analysis of synthetic oligonucleotides and their failure products.

Phosphorothioate as well as guanidin-rich antisense oligonucleotide drugs are known to be particularly difficult to analyze [6]. Due to the chaotic properties of triethylamine-hexafluoroisopropanol (TEA-HFIP) buffers, we were able to successfully separate < 60mer antisense oligonucleotides [3] and identify the failure oligonucleotides in a drug form.

Guidelines for column selection and optimization of mobile phase composition are discussed. The ion-paired reversed-phase HPLC separation benefits from the use of 25 µm sorbent and elevated temperature.

The separation performance was equivalent or better than ion-exchange HPLC and in some cases rivaled capillary gel electrophoresis separation. Using a 50 x 1 mm Xterra column, we were able to obtain molecular weight confirmation for ~1-10 pmol of oligonucleotides injected.

Methods

- HPLC system: CapLC® Waters, equipped with a photodiode array detector
- Column: 50 x 1 mm Xterra® M S C18, 2.5 µm
- Column temperature: 50-60 °C
- Mobile phase flow rate: 23.6 µl per minute.
- HPLC conditions: see figure captions

- Ion pairing buffers: Triethylamine ion-pairing agent (8.6 mM) was buffered with 100 mM hexafluoroisopropanol (weak acid). TEA-HFIP buffer pH was 8.3.
- Alternately, 16.3 mM - 400 mM TEA-HFIP buffer, pH 7.9 was used. Oligonucleotides were eluted from the column with a methanol gradient.

- MS instrument: ESI-TOF mass spectrometer; LCT, M iThem Mass
- MS conditions: capillary 2000 V cone 18 V desolv. temperature 120 °C MCP 2700 V spectra were deconvoluted with MaxEnt1 software

Results
- Figure 1: RP-HPLC offers a similar performance for the separation of oligonucleotides as capillary gel electrophoresis.
- Figure 2: TEA-HFIP ion-pairing buffers allow for more sensitive oligonucleotide MS detection than triethylammonium acetate mobile phases.
- Figure 3: LC-UVMS analysis of 25mer phosphorothioate drug digestion. Metabolites are separated from parent drug and identified by MS.

Conclusions
- Present LC-MS method is simple, robust, and reproducible.
- It allows for characterization of therapeutic and diagnostic oligonucleotides.
- We successfully analyzed phosphorothioate oligonucleotides - G-rich oligonucleotide sequences - singly and dually labeled diagnostic oligonucleotides - locked nucleic acids.
- We achieved excellent LC separation with MS friendly mobile phases.
- The LC-MS method is capable of detecting ~1-10 pmol of oligonucleotides.

References