Overview
The goal of this work is to understand the retention behavior of nucleoside probes on different stationary phases and pH conditions during hydrophilic interaction liquid chromatography (HILIC). We employed silica diol, amide, and polymer diol stationary phases, and three different pH conditions.

Introduction
Ribonucleic acid (RNA) is a biological polymer essential for living cells. Nucleosides are the building blocks of RNA consisting of ribose and nucleobase. These nucleosides are arranged in a chain through phosphodiester linkages. RNA contains post-transcriptional modifications on four canonical nucleosides: Adenosine (A), Cytosine (C), Guanosine (G), and Uracil (U) (Fig. 1). Smaller pieces of RNA, referred to as oligonucleotides, are analyzed by ion-pairing reversed phase chromatography. Recently, Hydrophilic Interaction Liquid Chromatography (HILIC) has emerged as an alternative separation technique. A comparative study between traditional ion-pair and HILIC on a Shodex VN-50 polymer-based diol column was recently done (Lobue 2019). A similar comparative study was done with a Waters BEH amide column (Demelenne 2019). The long-term goal of the current study is to understand which of the fundamental parameters play a significant role during oligonucleotide chromatography under HILIC conditions.

Materials and Methods

**Mobile Phase Preparation**

The mobile phase that was used had a ratio of 85% acetonitrile and 15% aqueous 50 mM ammonium acetate. For basic pH of 9.2 by adding ammonium hydroxide was added. For acidic pH of 4.5 acetic acid was added. Unadjusted ammonium acetate solution exhibited a pH of 7.6.

**Sample Preparation**

The samples were prepared by diluting standards to 0.15μg/μL with mobile phase.

**Results**

The Agilent 1260 Infinity II HPLC was employed (Fig. 2). 2 μL of sample was injected. Isocratic chromatography was done at a flow rate of 200μL/min, while maintaining the column 60°C.

**Table 1: HILIC Columns with different stationary phases.**

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Column Name</th>
<th>Column size</th>
<th>Bead size</th>
<th>Stationary Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenomenex</td>
<td>Luna HILIC</td>
<td>150 x 2 mm</td>
<td>3 µm</td>
<td>Silica diol</td>
</tr>
<tr>
<td>YMC</td>
<td>YMC-Triart Diol HILIC</td>
<td>150 x 2.1 mm</td>
<td>3 µm</td>
<td>Silica Diol</td>
</tr>
<tr>
<td>Waters</td>
<td>XBridge@B E H amide</td>
<td>150 x 2.1 mm</td>
<td>2.5 µm</td>
<td>Silica Amide</td>
</tr>
<tr>
<td>Waters</td>
<td>XBridge@B EH HILIC</td>
<td>150 x 2.1 mm</td>
<td>2.5 µm</td>
<td>Silica Amide</td>
</tr>
<tr>
<td>Shodex</td>
<td>HILIC pak VN-50</td>
<td>150 x 2 mm</td>
<td>5 µm</td>
<td>Polymer Diol</td>
</tr>
</tbody>
</table>

**Conclusions**

- The silica diol stationary phase in the Luna HILIC (Phenomenex) exhibited the least variance in the retention time when compared across the different pH conditions and stationary phases.
- The next best stationary phase was found to be polymer diol which was the Shodex HILIC pak VN-50 column.
- Variance in retention time was observed for all modifications with respect to the stationary phase.

**Future Work**

- Effect of temperature.
- Behavior of oligonucleotides at different pH and temperature conditions.
- Use of ammonium formate instead of ammonium acetate when making the HILIC aqueous phase. This will allow for testing of a broader pH ranges and different ionic strengths.

**References**

Demelenne, Alice et al. (2019). J Chromatogr A 1614

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