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Retention Mechanisms of Modified Ribonucleosides on Hydrophilic Interaction Liquid Chromatography Columns

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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 Uridine (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

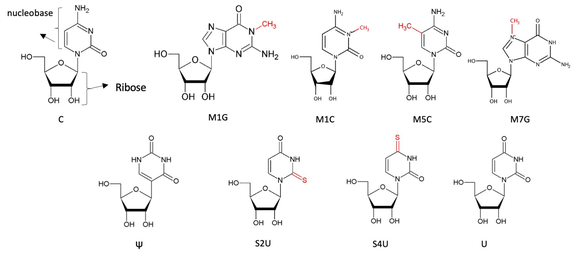

Figure 1: Chemical structures of ribonucleoside modifications used in the current study

Materials and Methods

Mobile Phase Preparation

The mobile phase that was used had a ratio of 85% acetonitrile and 15% aqueous 50mM 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.

Instrument Settings

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

Figure 2: Agilent 1260 Infinity II HPLC system

Column Information

Table 1: HILIC Columns with different stationary phases.

Manufacturer	Column Name	Column size	Bead size	Stationary Phase
Phenomenex	Luna HILIC	150 x 2 mm	3 µM	Silica diol
YMC	Ymc- Triart Diol-HILIC	150 x 2.1 mm	3 µM	Silica Diol
Waters	Xbridage@BEH amide	150 x 2.1 mm	2.5 µM	Silica Amide
Waters	Xbridge@BEH HILIC	150 x 2.1 mm	2.5 µM	Silica
Shodex	HILIC pak VN-50	150 x 2 mm	5 µM	Polymer Diol

Results

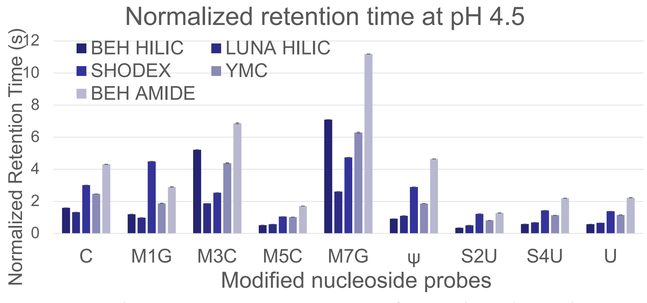

Figure 3: The average retention times for nucleoside probes on various columns at pH4.5.

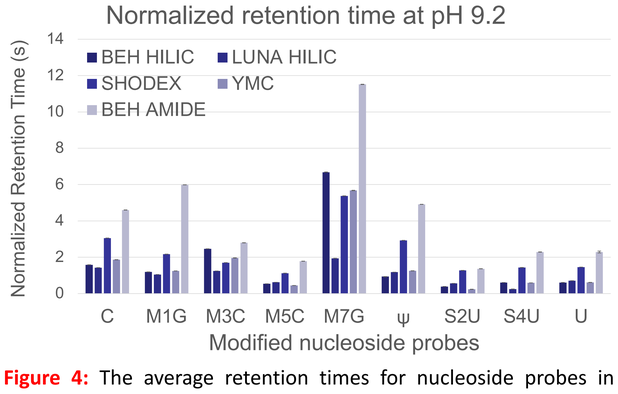

Figure 4: The average retention times for nucleoside probes in basic conditions for various columns.

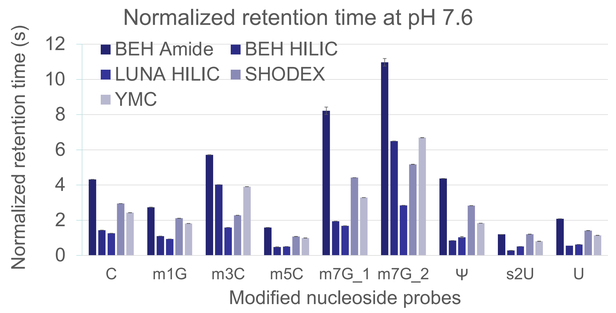

Figure 5: Average retention times under neutral pH conditions

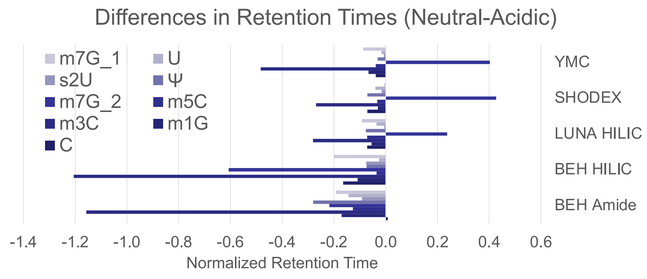

Figure 6: Differences in retention times between neutral and acidic conditions.

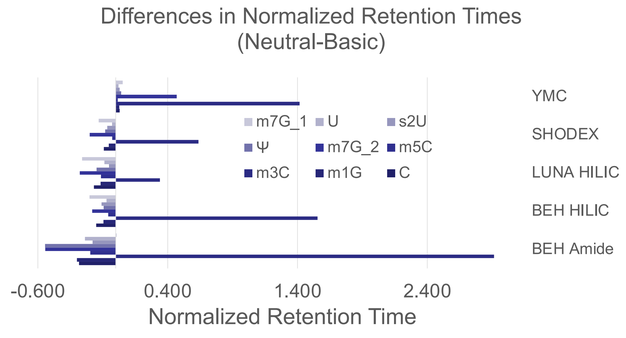

Figure 7: Differences in retention times between neutral and basic conditions.

Figures 3, 4 and 5 depicts the retention times of nucleoside probes under acidic, basic and neutral pH conditions. Three injections were made for each sample and the average retention time with standard devastation was plotted. Out of tested probes, 7-methylguanosine (m^7G) and YMC column exhibited longest retention time.

Discussion

The goal of this project was to determine the how nucleoside probe behave in multiple stationary phase with different pH conditions so that an optimal pH and stationary phase can be identified. As seen on **Figure 6 and 7** the least variant column is Luna HILIC by Phenomenex. While the while the most variant is the BEH HILIC and BEH Amide for both **Figure 6 and 7**. Almost all columns exhibited variance for all modifications at the three different pH levels tested **(Figures 4,5 & 6)** .

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**

Lobue, P. A., et al. (2019). J Chromatogr A **1595**: 39-48.

Acknowledgments

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