

Retention behavior of oligonucleotides isomers studied by means of hydrophilic interaction chromatography

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Abstract

Short oligonucleotides and its isomers are deeply studied for better understanding of nucleic acid properties and interactions. High Performance Liquid Chromatography (HPLC) provides suitable tool for their characterization. Our interest in this study was focused on the separation of pentamers, consisted of adenine, guanine and thymine by means of hydrophilic liquid chromatography mode (HILIC). The influence of experimental parameters such as mobile phase pH and/or buffer concentration, percentage of organic solvent in mobile phase and flow rate was evaluated and the most acceptable conditions were selected for further analysis. Finally, obtained results were compared with commonly applied Reverse Phase High Performance Liquid Chromatography (RP-HPLC).

Keywords: Hydrophilic interaction chromatography, oligonucleotides

Introduction

Liquid chromatography (LC) belongs to the most applied separation analytical methods in bioanalysis. Based on its general principles, HILIC mode affords alternative information to RP-HPLC separation (Gilar et al. 2002; Wang et al. 2005). The main goal of this work was the evaluation of relationship between oligonucleotide sequence and retention time in HILIC under various experimental conditions, consequential selection of the most suitable conditions for HILIC separation and comparison of results with RP-HPLC analysis.

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Materials and methods

All experiments were carried out on Shimadzu 10 AVP HPLC system with diode-array detector and ZIC-pHILIC (100 x 4.6mm, 5 µm) and Kinetex C18 (100 x 3 mm, 2.6 µm) chromatographic columns were used. Acetonitrile/ammonium carbonate mobile phase in HILIC mode and methanol/ammonium acetate mobile phase in RP mode were tested.

Results and Discussion

Equal base composition but unequal polynucleotide sequence results in various affinity to chromatographic column stationary phases and thus the analysis in different chromatographic systems leads to comprehensive results acquirement. Retention behavior of selected pentamers was studied under different conditions by HILIC and it was confirmed that analytes separated by use of HILIC elute in different order in comparison to RP-HPLC (Fig 1).

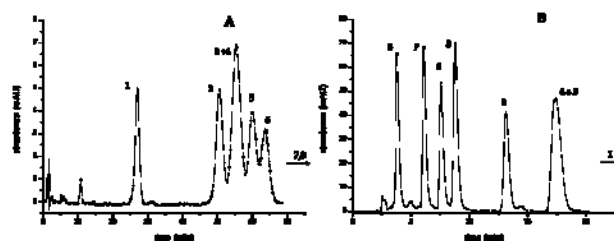


Figure 1: Separation of pentamer mixture using **A**, HILIC and **B**, RP-HPLC. 1 – ATTTT, 2 – ATGTT, 3 – ATGAT, 4 – AGTTT, 5 – AGTTA, 6 – AGATT, 7 – AGTAG, 8 – AGAGT

Conclusion

It was shown that both mentioned LC modes can be applied in short oligonucleotides separation. The most suitable conditions for HILIC separation were compared with RP-HPLC. However, RP-HPLC gives more sensitive results in shorter times and thus is more suitable to be used in this field of study.

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