

Complementary Selectivities of Synergi™ Polar-Functionalized HPLC Columns for Nucleobases and Nucleosides

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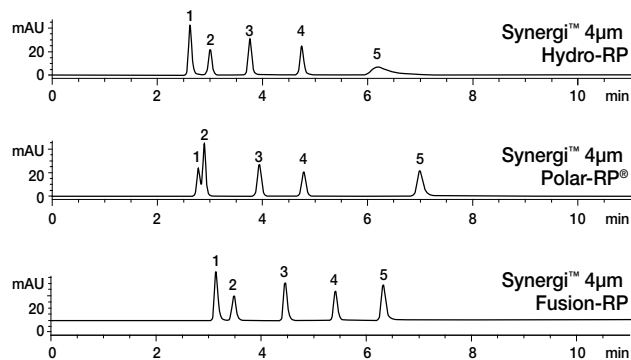
Introduction

A variety of HPLC methods have been developed for the analysis of pyrimidine/purine bases and the corresponding 2'-deoxynucleosides, the basic building blocks of nucleic acids. These include reversed phase (RP-HPLC), ion exchange (IEX) and normal phase chromatography^{1,2}. These molecules are highly polar and hence their retention on traditional C18 and other alkylsilyl phases under RP-HPLC conditions is problematic. Ion pairing reagents are commonly used to enhance retention for highly polar compounds; however, the use of ion-pairing reagents adds a complexity to the mobile phase and can result in lengthy column equilibration. In this note, the retention characteristics of these biologically important probes on three polar, reversed-phase columns: Synergi™ Hydro-RP (polar endcapped C18), Synergi™ Polar-RP® (ether functionalized phenyl, polar endcapped) and Synergi™ Fusion-RP (a polar embedded C18 phase) are investigated in the absence of ion pairing reagents.

Experimental

Nucleobases, nucleosides, and solvents were obtained from Sigma (St. Louis, MO, USA). All HPLC runs were performed on Agilent 1100 (Palo Alto, CA, USA) instrument equipped with a quaternary pump, in-line degasser, DAD detector, autosampler and data processed by HP Chemstation software. The Synergi series of columns were procured from Phenomenex (Torrance, CA, USA). HPLC conditions are included with the chromatograms shown in Figures 1 and 2.

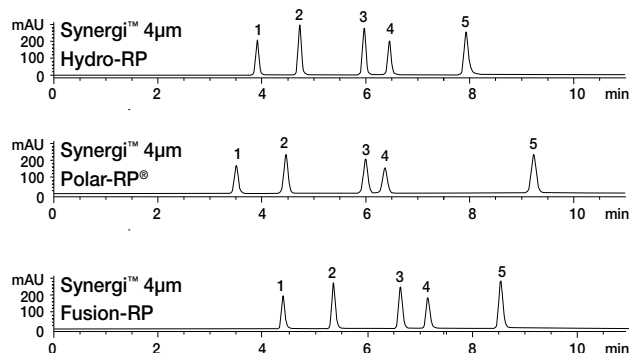
Figure 1: HPLC Chromatograms of DNA Nucleosides on Different Synergi™ Reversed Phase Columns



Conditions the same for all columns

Dimensions: 150 x 4.6 mm
Mobile Phase: A: 20 mM Ammonium Acetate
 B: Acetonitrile:20 mM Ammonium Acetate (95:5), pH 4.5
Gradient: From 5% to 40% B in 10 minutes
Temperature: Ambient
Flow Rate: 1.0 mL/min
Detection: 260 nm
Sample: 1. Uracil 4. Thymine
 2. Cytosine 5. Adenine
 3. Guanine

Figure 2: HPLC Chromatograms of RNA Nucleosides on Different Synergi™ Reversed Phase Columns



Conditions the same for all columns

Dimensions: 150 x 4.6 mm
Mobile Phase: A: 20 mM Ammonium Acetate
 B: Acetonitrile:20 mM Ammonium Acetate (95:5), pH 4.5
Gradient: From 5% to 40% B in 10 minutes
Temperature: Ambient
Flow Rate: 1.0 mL/min
Detection: 260 nm
Sample: 1. Cytidine 4. Thymidine
 2. Uridine 5. Adenosine
 3. Guanosine

“Synergi™ Fusion-RP is optimum in its retention and selectivity for nucleobases and 2'-deoxynucleosides even in the absence of an ion pairing agent.”

Results and Discussion

The chromatographic data in Figure 1 shows that Synergi Fusion-RP exhibits the maximum retention for all the nucleobases compared to other polar selective columns, Synergi Hydro-RP and Synergi Polar-RP (except for adenine). This enhanced polar retention of Synergi Fusion-RP is attributable to strong hydrogen bonding and dipole-dipole interactions of the nucleobases with the polar-embedded functional group. On the other hand, Synergi Polar-RP displays a unique selectivity for adenine, owing to strong π - π stacking interactions. At the same time, Synergi Hydro-RP exhibits the best resolution between uracil and cytosine among the Synergi family of columns possibly due to stronger dipole-induced dipole interactions.



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The introduction of a ribose moiety on to the nucleobase skeleton seems to enhance hydrophobicity as can be seen from the uniform increase in retention with all columns (see **Figure 2**). However, Synergi Polar-RP still shows strong retention characteristics for adenosine and the least retention for cytidine and uridine. Synergi Fusion-RP continues to demonstrate the overall most retention for nucleotides as a group.

Conclusion

As a group, nucleotides are poorly retained and resolved by traditional reversed phase columns. However, Synergi™ Fusion-RP is optimum in its retention and selectivity for nucleobases and 2'-deoxynucleosides even in the absence of an ion-pairing agent. Enhanced retention is a great benefit for those wishing to separate different nucleotides. The polar selectivity that Synergi™ Fusion-RP provides allows for separation of challenging nucleotide analogs for a majority of nucleotide bases. On the other hand, Synergi™ Polar-RP shows significantly increased retention characteristics for adenine and adenosine. This unique selectivity of Synergi™ Polar-RP for adenine-based nucleotides provides a complementary solution for those applications when Synergi™ Fusion-RP does not provide complete resolution. In summary, the harmonizing selectivities of the Synergi family of polar, reversed phase columns allows one to choose the ideal column for one's particular separation.

References

1. Lai-Sheng Li, Min Liu, Shi-Lu Da and Yu-Qi Feng, *Talanta*, 63 (2004), 433-441
2. P.J.M. van Haastert, *J. Chromatogr.*, 210, 229-240.

Product Information

Synergi™	Description	Particle Sizes (µm)	pH range	100% aqueous stable
Fusion-RP	Polar-Embedded C18	2, 4, 10	1.5 - 10	•
Hydro-RP	Polar-Endcapped C18	2, 4, 10	1.5 - 7.5	•
Polar-RP®	Ether-linked Phenyl	2, 4, 10	1.5 - 7.5	•
Max-RP	C12 with TMS endcapping	2, 4, 10	1.5 - 10	•

Ordering Information (Selected Dimensions, mm)*

Phase	Dimensions	Order No.
2µm Synergi™ MercuryMS™ Columns		
Synergi™ Fusion-RP	20 x 2.0	00M-4423-B0-TN
Synergi™ Hydro-RP	20 x 2.0	00M-4387-B0-TN
Synergi™ Polar-RP	20 x 2.0	00M-4371-B0-TN
Synergi™ Max-RP	20 x 2.0	00M-4372-B0-TN
Synergi™ Fusion-RP	30 x 2.0	00A-4423-B0-TN
Synergi™ Hydro-RP	30 x 2.0	00A-4387-B0-TN
Synergi™ Polar-RP	30 x 2.0	00A-4371-B0-TN
Synergi™ Max-RP	30 x 2.0	00A-4372-B0-TN
Synergi™ Fusion-RP	50 x 2.0	00B-4423-B0-TN
Synergi™ Hydro-RP	50 x 2.0	00B-4387-B0-TN
Synergi™ Polar-RP	50 x 2.0	00B-4371-B0-TN
Synergi™ Max-RP	50 x 2.0	00B-4372-B0-TN
Synergi™ Fusion-RP	50 x 4.6	00B-4423-E0-TN
Synergi™ Hydro-RP	50 x 4.6	00B-4387-E0-TN
Synergi™ Polar-RP	50 x 4.6	00B-4371-E0-TN
Synergi™ Max-RP	50 x 4.6	00B-4372-E0-TN
4µm Synergi™ Columns		
Synergi™ Fusion-RP	50 x 2.0	00B-4424-B0-TN
Synergi™ Hydro-RP	50 x 2.0	00B-4375-B0-TN
Synergi™ Polar-RP	50 x 2.0	00B-4336-B0-TN
Synergi™ Max-RP	50 x 2.0	00B-4337-B0-TN
Synergi™ Fusion-RP	50 x 3.0	00B-4424-Y0-TN
Synergi™ Hydro-RP	50 x 3.0	00B-4375-Y0-TN
Synergi™ Polar-RP	50 x 3.0	00B-4336-Y0-TN
Synergi™ Max-RP	50 x 3.0	00B-4337-Y0-TN
Synergi™ Fusion-RP	50 x 4.6	00B-4424-E0-TN
Synergi™ Hydro-RP	50 x 4.6	00B-4375-E0-TN
Synergi™ Polar-RP	50 x 4.6	00B-4336-E0-TN
Synergi™ Max-RP	50 x 4.6	00B-4337-E0-TN
Synergi™ Fusion-RP	100 x 2.0	00D-4424-B0-TN
Synergi™ Hydro-RP	100 x 2.0	00D-4375-B0-TN
Synergi™ Polar-RP	100 x 2.0	00D-4336-B0-TN
Synergi™ Max-RP	100 x 2.0	00D-4337-B0-TN
Synergi™ Fusion-RP	100 x 3.0	00D-4424-Y0-TN
Synergi™ Hydro-RP	100 x 3.0	00D-4375-Y0-TN
Synergi™ Polar-RP	100 x 3.0	00D-4336-Y0-TN
Synergi™ Max-RP	100 x 3.0	00D-4337-Y0-TN
Synergi™ Fusion-RP	100 x 4.6	00D-4424-E0-TN
Synergi™ Hydro-RP	100 x 4.6	00D-4375-E0-TN
Synergi™ Polar-RP	100 x 4.6	00D-4336-E0-TN
Synergi™ Max-RP	100 x 4.6	00D-4337-E0-TN
Synergi™ Fusion-RP	150 x 4.6	00F-4424-E0-TN
Synergi™ Hydro-RP	150 x 4.6	00F-4375-E0-TN
Synergi™ Polar-RP	150 x 4.6	00F-4336-E0-TN
Synergi™ Max-RP	150 x 4.6	00F-4337-E0-TN
Synergi™ Fusion-RP	250 x 4.6	00G-4424-E0-TN
Synergi™ Hydro-RP	250 x 4.6	00G-4375-E0-TN
Synergi™ Polar-RP	250 x 4.6	00G-4336-E0-TN
Synergi™ Max-RP	250 x 4.6	00G-4337-E0-TN

*Other Dimensions available, please contact your Phenomenex representative for more information.