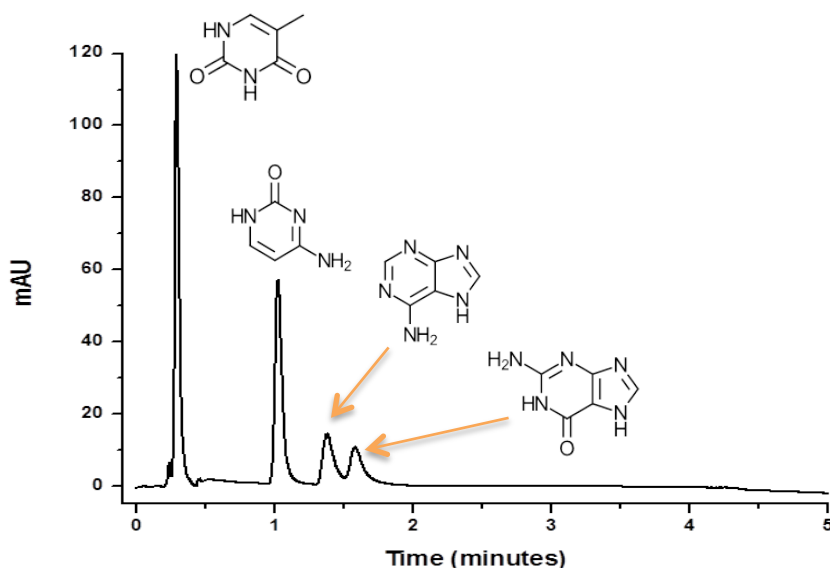


FLARE HILIC Column: Separation of Nucleobases

HPLC Conditions

Column Name:	FLARE HILIC (Amino-Diol)
Column Dimensions:	4.6 x 33mm, 3.6 μ m, 180 \AA (FH1090-1)
HPLC System:	Agilent 1290
Injection Volume:	0.5 μ l
Detection:	UV at 270nm
Flow Rate:	1.0ml/min
Mobile Phase:	A: 0.85% H ₃ PO ₄ B: 0.85% H ₃ PO ₄ in ACN; 95% to 80% B in 3mins
Temperature:	50 $^{\circ}$ C
Analytes:	1. Thymine 2. Cytosine 3. Adenine 4. Guanine



Notes

The four DNA nucleobases and their log P values considered above are as follows: Thymine (logP: -0.46), Cytosine (logP: -1.24), Adenine (logP: -0.53), and Guanine (logP: -1.15). The negative log P values confirm the highly polar nature of these compounds and the well-documented challenge of analyzing them on a traditional C18 reverse phase column. In this application note, using the FLARE HILIC column under low pH conditions, the bases are baseline resolved in less than 2 minutes. Further, the elution is not in order of increasing polarity as is often the case with commercially available HILIC phases. Hydrogen-bonding plays an important role in separating these compounds on the FLARE HILIC column.

References

1. www.chemicalize.org