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The Vanguard of Liquid Chromatography.

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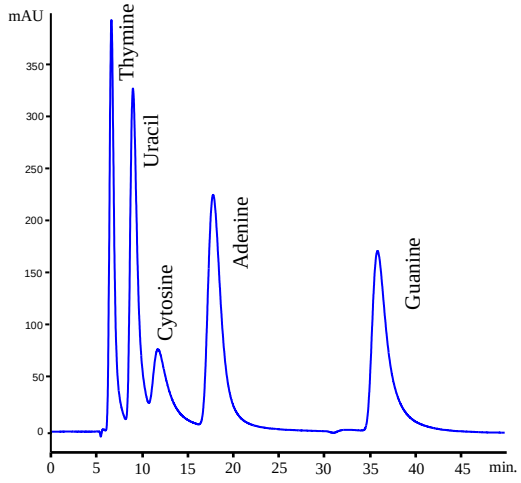
APPLICATION NOTE

STYROS™ Amino-HILIC Simulated Monolith™ Polymeric Normal Phase: Separation of Purines and Pyrimidines on Narrow Bore Column Intended for MS. No Bleed.

The use of HILIC enhances sensitivity in mass spectrometry. Indeed high concentration of organic in the mobile phase (>80-90%) increases ESI-MS response.

Retention is typically from least to most polar that is the opposite of Reversed Phase chromatography.
H2O is considered the strongest solvent in the following order: THF>ACN>i-PrOH>EtOH>MeOH>H2O.

The following chromatogram shows the separation of Purines and Pyrimidines on a **STYROS™ Amino-HILIC/NB Simulated Monolith™** column at 30˚ C with 5 times less sample.



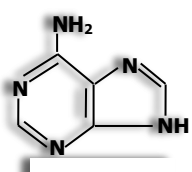
Chromatogram 1
Separation of Purines and Pyrimidines on **STYROS™ Amino-HILIC/NB**
(Flow Rate: 0.2 ml/min, 350 cm/hr)

Table 1. Operating parameters.

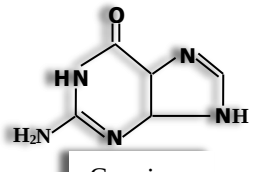
HPLC System.	Agilent 1100 with thermostatted column compartment.
Columns	**STYROS™ Amino-HILIC/NB** 2.1 X 300 mm
Mobile phase.	A: H2O, B:ACN C: 100 mM CO3(NH4)2, pH=9.6
Flow rate	0.2 ml/min (350 cm/hr of linear velocity)
Step Gradient	6 % A, 93 % B, 1% C (total ionic strength 1 mM) for 20 minutes to 11 % A, 88 % B, 1 % C for 50 minutes .
Temperature	30°C
Detection	254 nm
Injection volume	1 µl
Pressure Drop	4 bar (58 psi)
Sample:	Thymine, Uracil, Cytosine, Adenine, Guanine (1 mg/ml each in 0.4 M NaOH)

The low back pressure of the column (4 bars for a 2.1 x 300 mm at 0.2 ml/min) is typical of a Simulated Monolith™ column.
The symmetrical peak shapes allows the quantitation of each entity in the mixture.

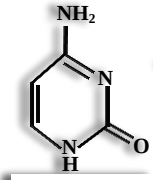
Up to 93 % of organic (ACN) is being used for this isocratic separation with the total ionic strength of only 1 mM salt that is ideal for mass spectrometry.



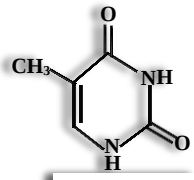
Adenine



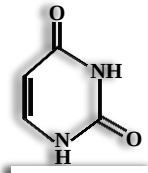
Guanine



Cytosine



Thymine



Uracil

It is important to note that the base matrix of polystyrene-divinylbenzene is not biodegradable and therefore one should not be concerned about leaching especially when dealing with the high sensitivity of mass spectroscopy.

Also of note is the low back pressure of these columns lending itself to all instruments including FPLC.