

APPLICATION NOTE

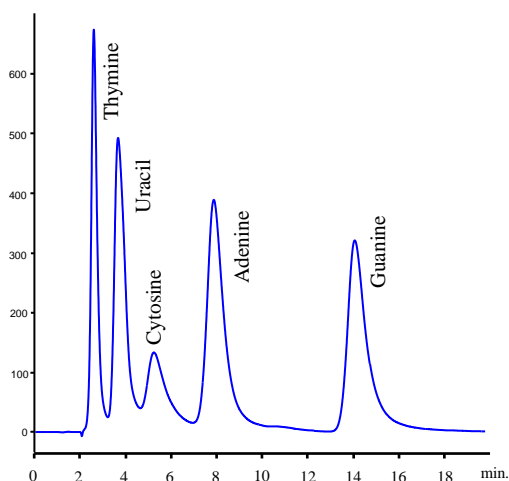
STYROS™ Amino-HILIC Simulated Monolith™ Polymeric Normal Phase: Separation of Purines and Pyrimidines Intended for MS. No Bleed.

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 Simulated Monolith™** column at 30°C.



Chromatogram 1

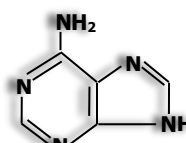
Separation of Purines and Pyrimidines on **STYROS™ Amino-HILIC**
(Flow Rate: 1 ml/min, 360 cm/hr)

Table 1. Operating parameters.

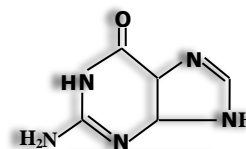
HPLC System.	Agilent 1100 with thermostatted column compartment.
Columns	STYROS™ Amino-HILIC 4.6 X 150 mm
Mobile phase.	A: H ₂ O, B:ACN C: 100 mM CO ₃ (NH ₄) ₂ , pH=9.6
Flow rate	1 ml/min (360 cm/hr of linear flow rate)
Step Gradient	6 % A, 93 % B, 1 % C (total ionic strength 1 mM) for 8 minutes to 11 % A, 88 % B, 1 % C for 20 minutes.
Temperature	30°C
Detection	254 nm
Injection volume	5 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 4.6 x 150 mm at 1 ml/min) is typical of a Simulated Monolith™ column. The symmetrical peak shapes allows the quantitation of each entity in the mixture.

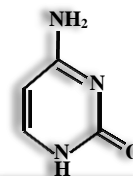
Up to 93 % of organic (ACN) is being used for this isocratic separation with the total ionic strength of only 1 mM salt that is appropriate for mass spectrometer.



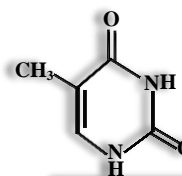
Adenine



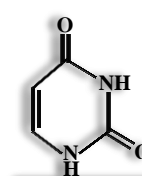
Guanine



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.

