OraChrom, Inc.
The Vanguard of Liquid Chromatography.

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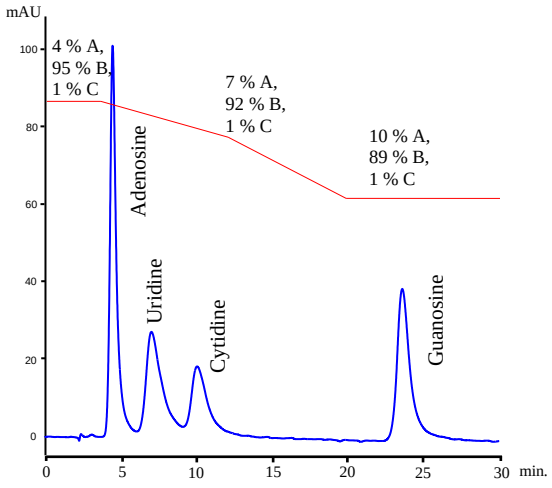
APPLICATION NOTE

STYROS™ Amino-HILIC Simulated Monolith™ Polymeric Normal Phase: Separation of Nucleosides 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 4 nucleosides on a **STYROS™ Amino-HILIC/XH Simulated Monolith™** column at 30˚C.



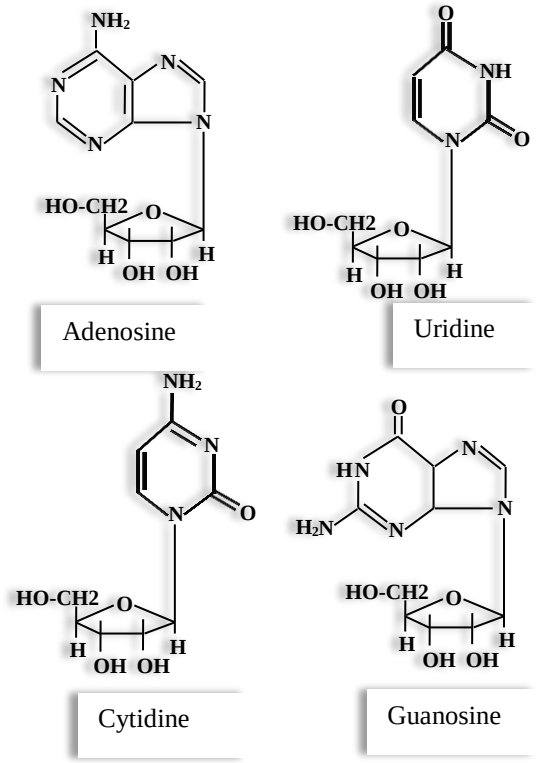
Chromatogram 1
Separation of Nucleosides on **STYROS™ Amino-HILIC/XH**
(Flow Rate: 1 ml/min, 360 cm/hr)

Table 1. Operating parameters.

HPLC System.	Agilent 1100 with thermostatted column compartment.
Columns	**STYROS™ Amino-HILIC/XH** 4.6 X 150 mm
Mobile phase.	A: H2O, B:ACN C: 100 mM CO3(NH4)2, pH=9.6
Flow rate	1 ml/min (360 cm/hr of linear velocity)
Step Gradient	Starting with 4 % A, 95 % B, 1% C (total ionic strength 1 mM) as shown in Chromatogram 1.
Temperature	30°C
Detection	254 nm
Injection volume	1 µl
Pressure Drop	4 bar (58 psi)
Sample:	Adenosine, Uridine, Cytidine, , Guanosine (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 95 % of organic (ACN) is being used for this step gradient separation with the total ionic strength of only 1 mM salt that is ideal for mass spectrometry.



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.