OraChrom, Inc.
The Vanguard of Liquid Chromatography.

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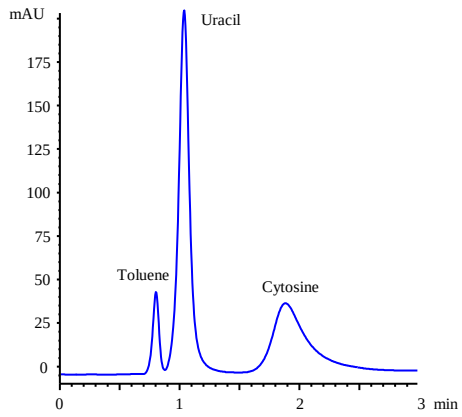
APPLICATION NOTE

STYROS™ Amino HILIC Simulated Monolith Polymeric Normal Phase: Standard Separations.

HILIC or Hydrophilic Interaction Chromatography is a variation of normal phase chromatography with the major difference of providing the possibility of using solvents that are not miscible with water.
It also provides complementary selectivity compared to reversed phase chromatography. Highly polar analytes that are not retained in reversed phase mode would be retained on HILIC mode.



Chromatogram 1
Separation on **STYROS™ Amino HILIC**
(Flow Rate: 1 ml/min)

Table 1. Operating parameters.

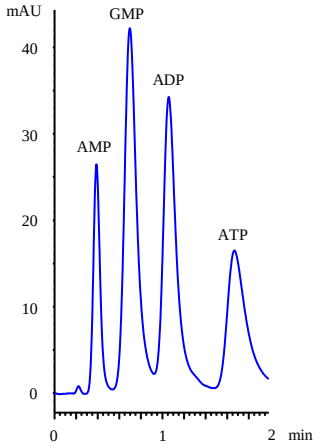
HPLC System.	Agilent 1100 with thermostatted column compartment.
Columns	**STYROS™ Amino-HILIC** 4.6 X 50 mm
Mobile phase.	A: ACN B: 200 mM HCOONH4, pH=8.7
Flow rate	1 ml/min (360 cm/hr of linear flow rate)
Gradient	Isocratic 94 % A, 6 % B
Temperature	30°C
Detection	254 nm
Injection volume	5 µl
Sample:	Toluene, Uracil, Cytosine (50-300 ug/ml each in ACN:H2O 50:50)

Uracil, a common void volume marker on reversed phase shows retention during separation on HILIC.
Toluene on the other hand is not retained on HILIC mode whereas it shows high retention on reversed phase due to its hydrophobic property.

During a typical SPE or liquid/liquid extraction, an organic solvent such as acetonitrile or isopropanol is used in the final stage. The sample can be injected on reversed phase only after evaporating the organic solvent followed by reconstitution in the aqueous starting buffer.
The use of Hydrophilic Interaction Chromatography shortens considerably the process by eliminating the evaporation-reconstitution step. The final extract can be directly injected as an organic eluent.
HILIC is also used to enhance sensitivity in mass spectrometry. Indeed the use of high concentration of organic in the mobile phase (>80-90%) enhances ESI-MS response.

The stationary phases found on the market are typically made of a polar material such as silica or functionalized silica with diol, cyano, and alike most of which bleed due to the solubility of the matrix.

STYROS™ Amino-HILIC Simulated Monolith on the other hand, are made of gigaporous hard gel polymeric that are stable at all pH's and offer similar mechanical stability compared to silica.



Chromatogram 2
Separation of 4 Nucleotides on **STYROS™ Amino HILIC**
(Flow Rate: 4 ml/min)

Table 1. Operating parameters.

HPLC System.	Agilent 1100 with thermostatted column compartment.
Columns	**STYROS™ Amino-HILIC** 4.6 X 50 mm
Mobile phase.	A: ACN B: 200 mM HCOONH4, pH=8.7
Flow rate	4 ml/min (1,450 cm/hr of linear flow rate)
Gradient	50 to 10 % A, 30 to 90 % B in 1.5 min
Temperature	30°C
Detection	254 nm
Injection volume	5 µl
Sample:	1. Adenosine-5'-monophosphate, 2. Guanosine-5'-monophosphate, 3. Adenosine-5'-diphosphate, 4. Adenosine-5'-triphosphate, (70-140 ug/ml each in ACN:H2O 50:50)

The typical salts or buffers used are ammonium formate or acetate. Phosphates should be avoided due to their low solubility in organic solvents. Ion pairing agents such as TFA should be avoided as well.

STYROS™ Amino-HILIC Simulated Monolith is stable in the full pH range.

Unlike Monolith **STYROS™ Simulated Monolith** columns are available in many sizes for additional resolving capabilities.