OraChrom, Inc.
The Vanguard of Liquid Chromatography.

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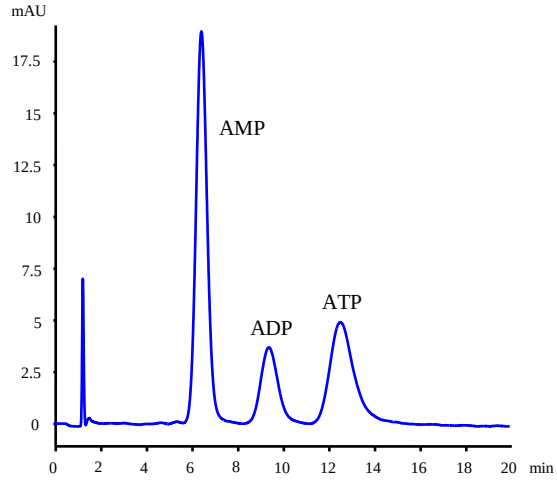
APPLICATION NOTE

STYROS™ HILIC Simulated Monolith™ Polymeric Normal Phase: Separation of Nucleotides.

HILIC or Hydrophilic Interaction Chromatography is a variation of normal phase chromatography. It provides complementary selectivity compared to reversed phase chromatography.
The following chromatograms show the separation of Adenosine and Guanosine Phosphates on a **STYROS™ HILIC Simulated Monolith™** column at 30˚C.

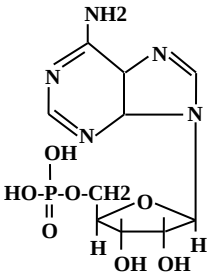


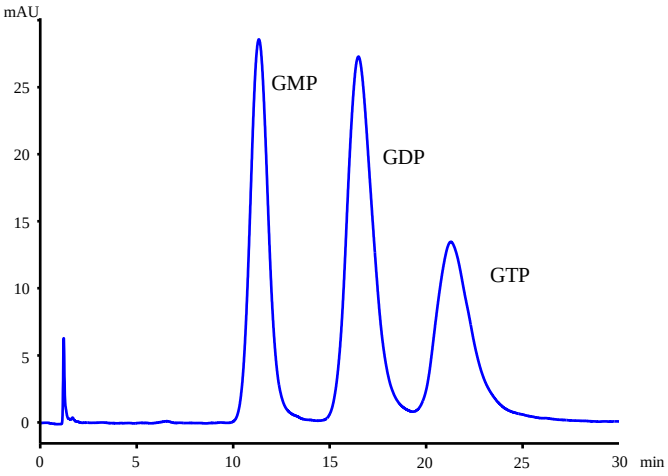
Chromatogram 1
Separation of 3 Adenosine derivatives on **STYROS™ HILIC**
(Flow Rate: 1 ml/min)

Table 1. Operating parameters.

HPLC System.	Agilent 1100 with thermostatted column compartment.
Columns	**STYROS™ HILIC** 4.6 X 100 mm
Mobile phase.	A: DI H2O, B: ACN C: 100 mM CO3(NH4)2, pH=9.6
Flow rate	1 ml/min (360 cm/hr of linear flow rate)
Isocratic Gradient	15 % A, 75%B, 10 % C (total ionic strength 10 mM)
Temperature	30°C
Detection	254 nm
Injection volume	5 µl
Sample:	Adenosine-5'-monophosphate, Adenosine-5'-diphosphate, Adenosine-5'-triphosphate (330 ug/ml each in B:C 50:50)

Adenosine-5'-monophosphate and the similar structures di- and triphosphate derivatives are baseline separated.



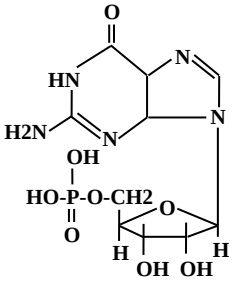


Chromatogram 2
Separation of 3 Guanosine derivatives on **STYROS™ HILIC**
(Flow Rate: 1 ml/min)

Table 1. Operating parameters.

HPLC System.	Agilent 1100 with thermostatted column compartment.
Columns	**STYROS™ HILIC** 4.6 X 100 mm
Mobile phase.	A: DI H2O, B: ACN C: 100 mM CO3(NH4)2, pH=9.6
Flow rate	1 ml/min (360 cm/hr of linear flow rate)
Isocratic Gradient	15 % A, 75%B, 10 % C (total ionic strength 10 mM)
Temperature	30°C
Detection	254 nm
Injection volume	5 µl
Sample:	Guanosine-5'-monophosphate, Guanosine-5'-diphosphate, Guanosine-5'-triphosphate (167 ug/ml each in B:C 50:50)

Similar separation occurs with Guanosine-5'-monophosphate and its derivatives.



To be noted is the total ionic strength of 10 mM of Ammonium Carbonate required for the elution.