OraChrom, Inc.
The Vanguard of Liquid Chromatography.

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APPLICATION NOTE

STYROS™ HILIC Simulated Monolith™ Polymeric Normal Phase: Separation of Nucleotides Intended for MS. No Bleed.

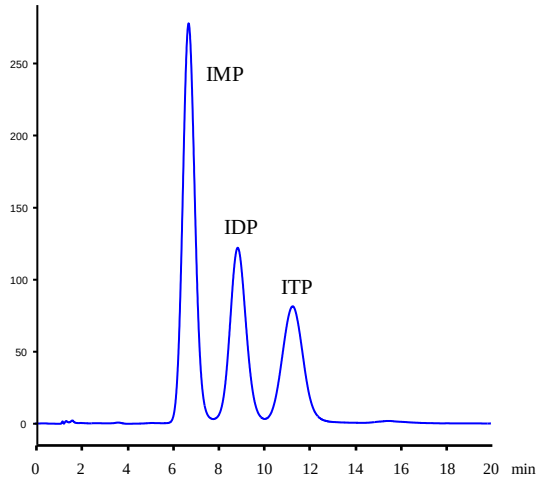
HILIC or Hydrophilic Interaction Chromatography is a variation of normal phase chromatography. It provides complementary selectivity compared to reversed phase chromatography.

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 following chromatogram shows the separation of Ionosine Phosphates on a **STYROS™ HILIC Simulated Monolith™** column at 30˚C.

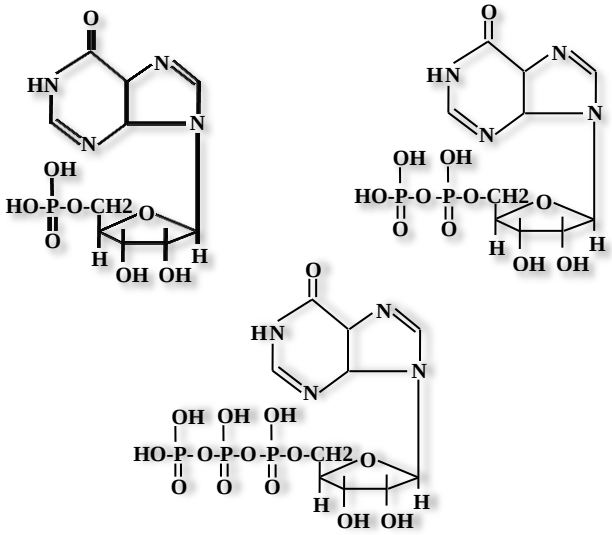


Chromatogram 1
Separation of 3 Ionosine derivatives on **STYROS™ HILIC**
(Flow Rate: 1 ml/min, 360 cm/hr)

Table 1. Operating parameters.

HPLC System.	Agilent 1100 with thermostatted column compartment.
Columns	**STYROS™ HILIC** 4.6 X 100 mm
Mobile phase.	A: ACN B: 100 mM CO3(NH4)2, pH=9.6
Flow rate	1 ml/min (360 cm/hr of linear flow rate)
Isocratic	75% A, 25 % B (total ionic strength 25 mM)
Temperature	30°C
Detection	254 nm
Injection volume	5 µl
Pressure Drop	3 bar (43.5 psi)
Sample:	Ionosine-5'-monophosphate, Ionosine-5'-diphosphate, Ionosine-5'-triphosphate (1 mg/ml each in B:C 50:50)

Ionosine-5'-monophosphate and the similar structures di- and triphosphate derivatives are baseline separated.
The symmetrical peak shapes allows the quantitation of each entity in the mixture.



Similar separation occurs with Guanosine-5'-monophosphate and its derivatives as well as Adenosine and its phosphates.

To be noted is the total ionic strength of 25 mM of Ammonium Carbonate required for the elution.

It is important to note that the base matrix of polystyrene-divinylbenzene is not biodegradable and therefore one should not be concerned about leaching specially 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.