OraChrom, Inc.
The Vanguard of Liquid Chromatography.

10-B Henshaw Street Woburn, MA 01801 USA
Phone (781) 932 0151 Fax (781) 932 0787
E-mail: info@orachrom.com orachrom.com

APPLICATION NOTE

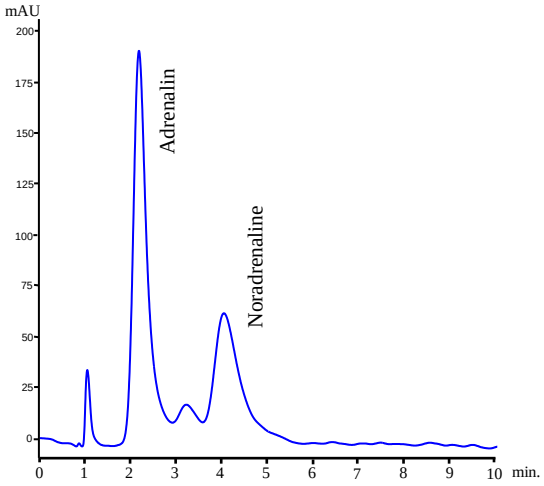
STYROS™ HILIC Simulated Monolith™ Polymeric Normal Phase: Separation of Adrenaline and Noradrenaline Intended for MS. No Bleed.

The use of HILIC in mass spectrometry is now well established.

Unlike in reversed phase it is possible with HILIC to retain and separate small polar molecules as well as many additional properties essential to the application of separation and detection with high level of sensitivity.

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>Me-CO-Me>ACN>i-PrOH>EtOH>MeOH>H2O.

The following chromatogram shows the separation of Adrenaline and Noradrenaline on a **STYROS™ HILIC/XH Simulated Monolith™** column at 30˚C.



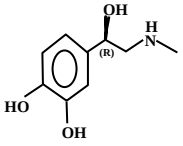
Chromatogram 1
Separation of Catecholamines on **STYROS™ HILIC/XH**
(Flow Rate: 1.5 ml/min, 540 cm/hr)

Table 1. Operating parameters.

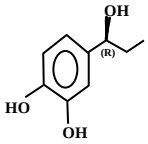
HPLC System.	Agilent 1100 with thermostatted column compartment. With quaternary pump.
Columns	**STYROS™ HILIC/XH** 4.6 X 100 mm
Mobile phase.	A: H2O, B:ACN C: 200 mM HCOONH4, pH= 3.4
Flow rate	1.5 ml/min (540 cm/hr of linear velocity)
Isocratic	5 % A, 91 % B, 4% C (total ionic strength 8 mM).
Temperature	30°C
Detection	230 nm
Injection volume	4 µl
Pressure Drop	4 bar (58 psi)
Sample:	Adrenaline, Noradrenaline (1 mg/ml each in buffer C)

The low back pressure of the column (4 bars for a 4.6 x 100 mm at 1.5 ml/min) is typical of a Simulated Monolith™ column.

The symmetrical peak shapes allows the quantitation of each entity in the mixture.
Up to 91 % of organic (ACN) is being used for this isocratic separation with the total ionic strength of only 8 mM ammonium formate that is ideal for mass spectrometry.



Adrenaline



Noradrenaline

Adrenaline and Noradrenaline are hormones and neurotransmitters found in biological fluids both in blood serum and urine. They are also found in some drugs. In the present case the retention is based on HILIC mode only.

This method can also detect small amounts of impurity that contaminate the products.

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.