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*

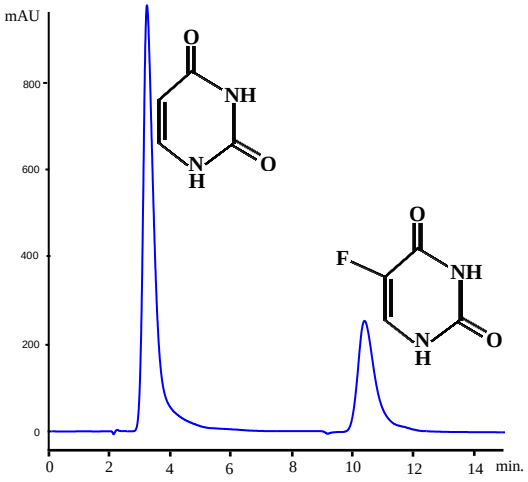
APPLICATION NOTE

STYROS™ Amino-HILIC Simulated Monolith™ Polymeric Normal Phase: Separation of Uracil from 5-Fluorouracil. Narrow Bore and Normal Bore Columns.

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 Uracil and 5-Fluorouracil on a **STYROS™ Amino-HILIC/XH Simulated Monolith™** column at 30˚C using 5 µl sample.



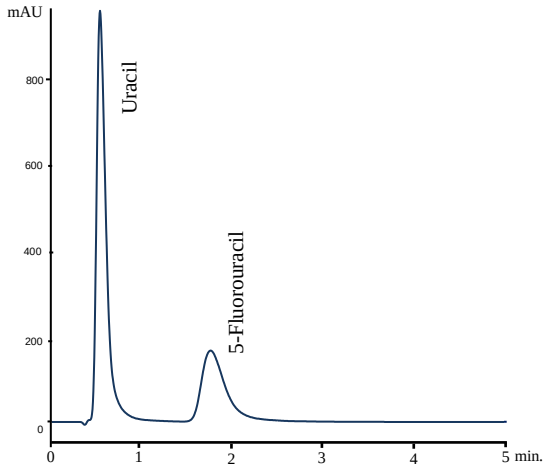
Chromatogram 1
Separation of Uracil and 5-Fluorouracil on **STYROS™ Amino-HILIC/XH**
(Flow Rate: 1 ml/min, 360 cm/hr)

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	8 % A, 91 % B, 1% C for 6 min to 11% A, 82 % B, 1 % C for 15 min. (total ionic strength: 1 mM)
Temperature	30°C
Detection	254 nm
Injection volume	5 µl
Pressure Drop	2 bar (30 psi)
Sample:	Uracil, 5-Flluorouracil (1 mg/ml each in 0.4 M NaOH)

The low back pressure of the column (2 bars for a 4.6 x 150 mm at 1 ml/min) is typical of a Simulated Monolith™ column.
The symmetrical peak shapes and the high resolution allow the quantitation of each entity in the mixture.
Up to 91 % of organic (ACN) is being used for this separation with the total ionic strength of only 1 mM salt that is ideal for mass spectrometry.

The same separation can be run on a narrow bore column at higher flow rates, in shorter time and with smaller sample:



Chromatogram 2
Separation of Uracil and 5-Flurouracil on **STYROS™ Amino-HILIC/NB**
(Flow Rate: 1 ml/min, 1,700 cm/hr)

Table 1. Operating parameters.

HPLC System.	Agilent 1100 with thermostatted column compartment.
Columns	**STYROS™ Amino-HILIC/NB** 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 (1,700cm/hr of linear velocity)
Step Gradient	8 % A, 91 % B, 1% C for 1 min to 11% A, 82 % B, 7 % C for 5 min. (total ionic strength: up to 7 mM)
Temperature	30°C
Detection	254 nm
Injection volume	1 µl
Pressure Drop	12 bar (174 psi)
Sample:	Uracil, 5-Flluorouracil (1 mg/ml each in 0.4 M NaOH)

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.

5-Fluorouracil (FU) continues to be studied for its mechanism of cytotoxicity as a neoplastic agent.