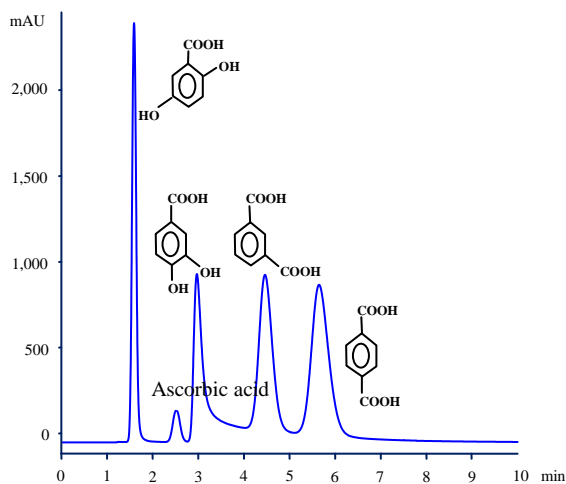


## APPLICATION NOTE

### STYROS™ HILIC Simulated Monolith Polymeric Normal Phase: Separation of Aliphatic and Aromatic Acids.

HILIC or Hydrophilic Interaction Chromatography is a variation of normal phase chromatography. It provides complementary selectivity compared to reversed phase chromatography. The following chromatogram shows the separation of 4 Aromatic acids on a **STYROS™ HILIC Simulated Monolith** column at 30°C.



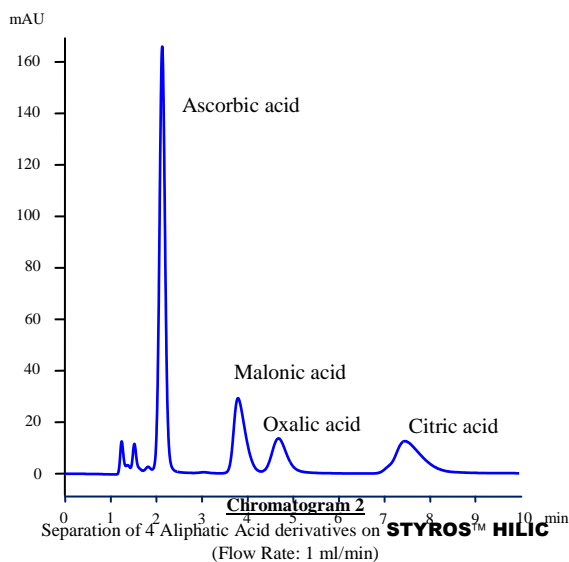
**Chromatogram 1**  
Separation on **STYROS™ HILIC**  
(Flow Rate: 1 ml/min)

**Table 1. Operating parameters.**

<b>HPLC System.</b>	Agilent 1100 with thermostatted column compartment.
<b>Columns</b>	<b>STYROS™ HILIC</b> 4.6 X 100 mm
<b>Mobile phase.</b>	A: DI H <sub>2</sub> O, B: ACN C: 100 mM CO <sub>3</sub> (NH <sub>4</sub> ) <sub>2</sub> , pH=9.6
<b>Flow rate</b>	1 ml/min (360 cm/hr of linear flow rate)
<b>Isocratic Gradient</b>	0 % A, 78%B, 22 % C (total ionic strength 22 mM)
<b>Temperature</b>	30°C
<b>Detection</b>	230 nm
<b>Injection volume</b>	5 µl
<b>Sample:</b>	2,5-Dihydroxybenzoic acid, Ascorbic acid (0.5 ug/ml), 3,4-Dihydroxybenzoic acid( 1.5 mg/ml), Isophthalic acid, Terephthalic acid ( 1mg/ml each in B:C 70:30)

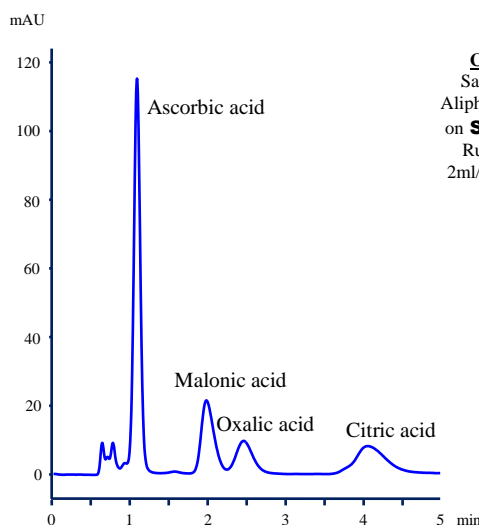
Ascorbic acid was added to stabilize the 3,4-Dihydroxybenzoic acid or Protocatechuic acid, a particularly sensitive compound found in plants and to allow the separation under the chromatographic conditions.

The same separation can be run using 4 aliphatic acids



**Table 1. Operating parameters.**

<b>HPLC System.</b>	Agilent 1100 with thermostatted column compartment.
<b>Columns</b>	<b>STYROS™ HILIC</b> 4.6 X 100 mm
<b>Mobile phase.</b>	A: DI H <sub>2</sub> O, B: ACN C: 100 mM CO <sub>3</sub> (NH <sub>4</sub> ) <sub>2</sub> , pH=9.6
<b>Flow rate</b>	1 ml/min (360 cm/hr of linear flow rate)
<b>Isocratic Gradient</b>	10 % A, 75 %B, 15 % C (total ionic strength 15 mM)
<b>Temperature</b>	30°C
<b>Detection</b>	230 nm
<b>Injection volume</b>	5 µl
<b>Sample:</b>	Ascorbic acid (0.5 mg/ml), Malonic acid, Oxalic acid, Citric acid 1 mg/ml each in B:C 70:30



**Chromatogram 3**  
Same separation of 4 Aliphatic Acid derivatives on **STYROS™ HILIC** Run at a flow rate of 2ml/min or 720 cm/hr of linear flow rate.

