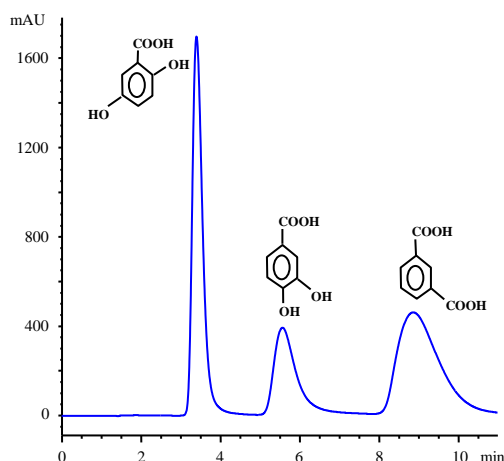


## APPLICATION NOTE

### STYROS™ Amino-HILIC Simulated Monolith™ Polymeric: Effect of Ionic Strength and Temperature on the Separation of 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 3 organic acids on a **STYROS™ Amino-HILIC Simulated Monolith** column at 25°C.



**Chromatogram 1**  
Separation of 3 organic acids on  
**STYROS™ Amino HILIC**

**Table 1. Operating parameters.**

<b>HPLC System.</b>	Agilent 1100 with thermostatted column compartment.
<b>Columns</b>	<b>STYROS™ Amino-HILIC</b> 4.6 X 100 mm
<b>Mobile phase.</b>	A: ACN B: 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>Gradient</b>	Isocratic 75 % A, 25 % B
<b>Temperature</b>	25°C
<b>Detection</b>	230 nm
<b>Injection volume</b>	5 µl
<b>Sample:</b>	1- 2,5-Dihydroxybenzoic acid, 2- 3,4-Dihydroxybenzoic acid, 3- Isophthalic acid (2 mg/ml each) in A:B 75:25.

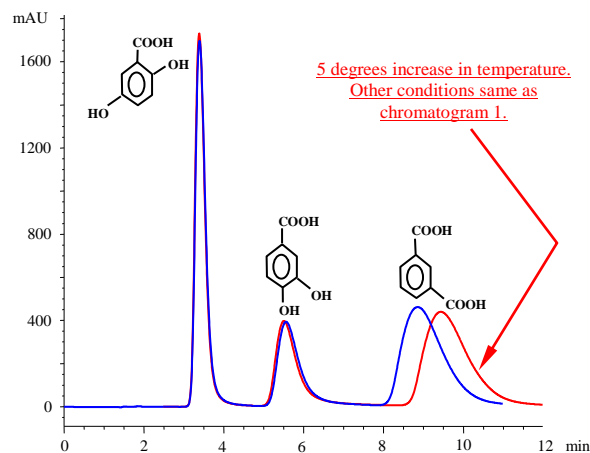
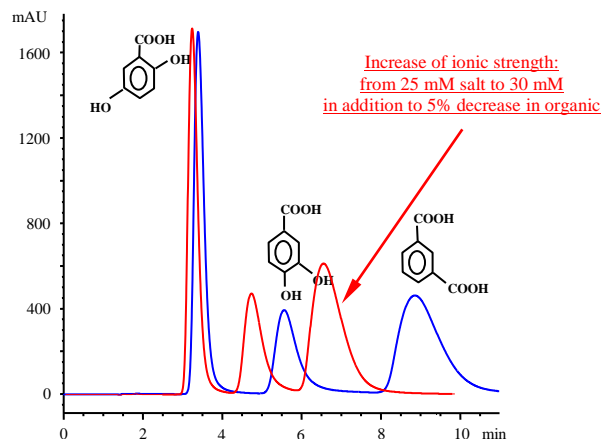
The same separation can be run under different conditions in order to either shorten the run time or further isolate a targeted fraction.

3,4-Dihydroxybenzoic acid or Protocatechuic acid which can be found in plants is particularly sensitive to the salt used in the separation.

We have used Ammonium carbonate at high pH to run baseline separation.

In the next two chromatograms the previous separation is compared with alternative ones of the same compounds and the same salt.

**STYROS™ Amino-HILIC Simulated Monolith** columns are stable in the full pH range and high temperatures.



Unlike Monolith, **STYROS™ Simulated Monolith** columns are available in many sizes for additional resolving capabilities.

