

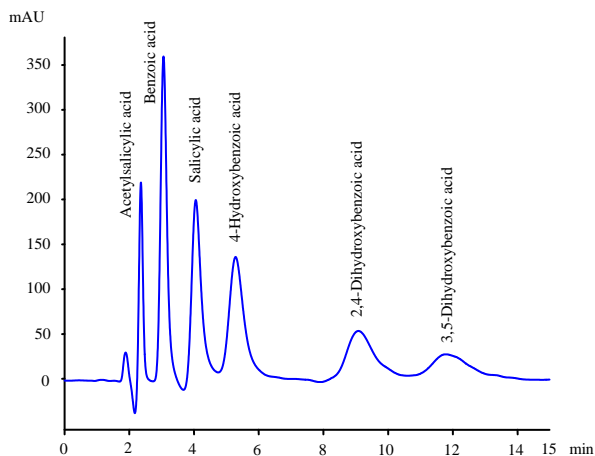
APPLICATION NOTE

STYROS™ Amino HILIC Simulated Monolith Polymeric: Separation of Benzoic Acid and Derivatives

HILIC or Hydrophilic Interaction Chromatography is a variation of normal phase chromatography with the major difference of providing the possibility of using solvents that are not miscible with water.

It also provides complementary selectivity compared to reversed phase chromatography.

The following chromatogram shows the separation of benzoic acid and its derivatives on a STYROS™ Amino-HILIC Simulated Monolith column at 30° C.



Chromatogram 1
 Separation of Benzoic acid and its derivatives on
 STYROS™ Amino HILIC

Table 1. Operating parameters.

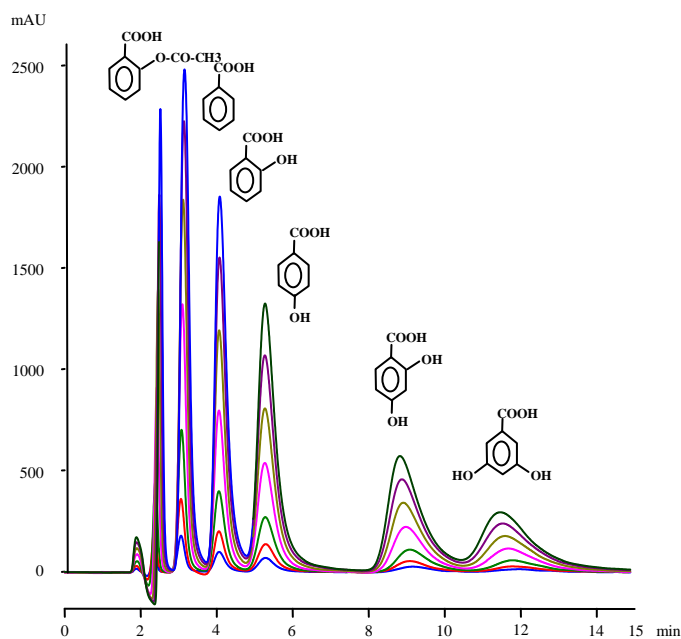
HPLC System.	Agilent 1100 with thermostatted column compartment.
Columns	STYROS™ Amino-HILIC 4.6 X 150 mm
Mobile phase.	A: ACN B: 200 mM HCOONH ₄ , pH=8.6
Flow rate	1 ml/min (360 cm/hr of linear flow rate)
Gradient	Isocratic 72 % A, 28 % B
Temperature	30°C
Detection	230 nm
Injection volume	10 µl
Sample:	1- Acetylsalicylic acid, 2- Benzoic acid, 3- Salicylic acid, 4- 4-Hydroxybenzoic acid, 5- 2,4-Dihydroxybenzoic acid, 6- 3,5-Dihydroxybenzoic acid (170 µg/ml each) in ACN:H ₂ O 50:50.

Ammonium formate is used in the present case at a concentration of 200 mM. The actual concentration is 56 mM when used with 72 % of ACN.

Phosphates should be avoided due to their low solubility in organic solvents. Ion pairing agents such as TFA should be avoided as well.

No tailing is observed at the present concentration even with the most retained eluent at 11.8 min.

The column bed remains stable at 30° C and the pressure remains low as a result of lower viscosity.



Chromatogram 2
 Loadability study on STYROS™ Amino HILIC

Table 2. Operating parameters.

HPLC System.	Agilent 1100 with thermostatted column compartment.
Columns	STYROS™ Amino-HILIC 4.6 X 150 mm
Mobile phase.	A: ACN B: 200 mM HCOONH ₄ , pH=8.6
Flow rate	1 ml/min (360 cm/hr of linear flow rate)
Gradient	Isocratic 72 % A, 28 % B
Temperature	30°C
Detection	230 nm
Injection volume	5 to 100 µl
Sample:	1- Acetylsalicylic acid, 2- Benzoic acid, 3- Salicylic acid, 4- 4-Hydroxybenzoic acid, 5- 2,4-Dihydroxybenzoic acid, 6- 3,5-Dihydroxybenzoic acid (170 µg/ml each) in CAN:H ₂ O 50:50.

All the components of the mixture remain baseline separated. There is no indication of column saturation seen at the void volume.

STYROS™ Amino-HILIC Simulated Monolith is stable in the full pH range and high temperature.

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