

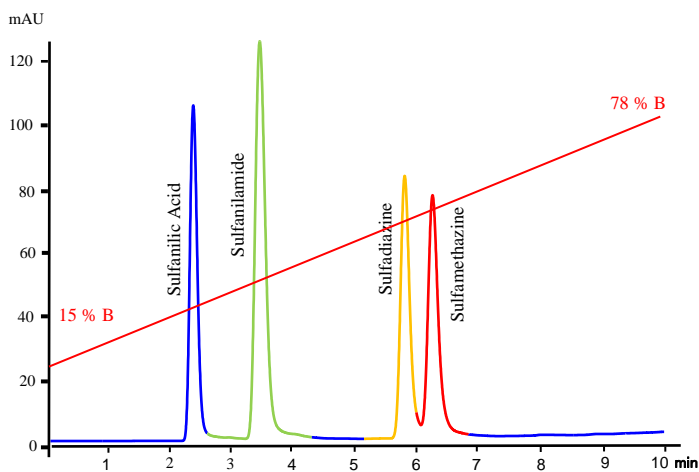
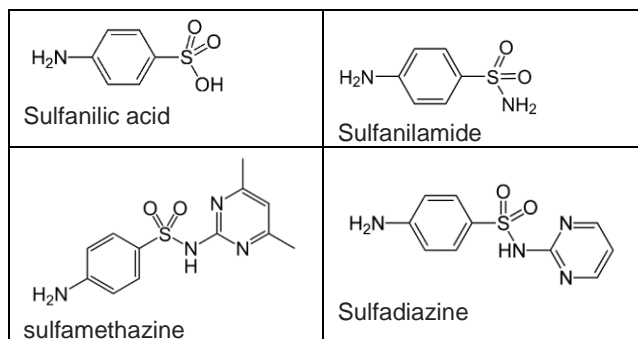
## APPLICATION NOTE

### STYROS® 2R Simulated-Monolith™ Polymeric Reversed Phase.

#### Use of Micro Bore column of 1 mm ID using high and low pH's to separate Sulfa drugs and precursor.

The use of polymeric at all pH's is an added advantage for the end user to explore better separations while keeping in mind the compatibility of the solvents' additives with mass spectrometers. The present series of application notes highlight such advantage using Sulfa drugs in acidic and basic pH's with smaller bore columns.

4 small molecules were separated on reversed phase Simulated-Monolith™ of 1 x 300 mm column:



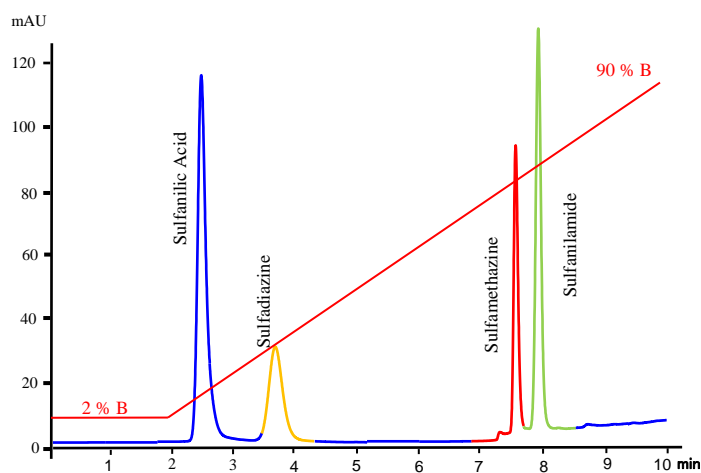
**Chromatogram 1**  
Separation of 4 Sulfa drug on **STYROS® 2R/MB**  
Flow Rate: 0.08 ml/min at acidic pH.

#### Operating parameters.

<b>HPLC System.</b>	Agilent 1290 with thermostatted column compartment.
<b>Columns</b>	<b>STYROS® 2R/MB 1 X 300 mm</b>
<b>Mobile phase.</b>	A: 0.075% TFA in H2O B: 0.075% TFA in ACN: H2O 95:5
<b>Flow rate</b>	0.08 ml/min.
<b>Gradient</b>	15 to 78 % B in 10 minutes (~4.5 cv)
<b>Temperature</b>	60°C
<b>Detection</b>	254 nm

<b>Injection volume</b>	0.5 µl
<b>Pressure Drop</b>	99 bar (~1,436 psi) at the start of gradient
<b>Sample:</b>	4 Sulfa drug and precursor acid

The elution changes at pH basic:



**Chromatogram 2**  
Separation of 4 Sulfa drug on **STYROS® 2R/MB**  
Flow Rate: 0.08 ml/min at basic pH.

#### Operating parameters.

<b>HPLC System.</b>	Agilent 1290 with thermostatted column compartment.
<b>Columns</b>	<b>STYROS® 2R/MB 1 X 300 mm</b>
<b>Mobile phase.</b>	A: DI H2O with 20 mM NH4OH, pH=12 B: ACN: A 95:5
<b>Flow rate</b>	0.08 ml/min.
<b>Gradient</b>	2 % B for 2 min, to 90 % B in 10 minutes (~4.5 cv)
<b>Temperature</b>	60°C
<b>Detection</b>	254 nm
<b>Injection volume</b>	0.2 µl
<b>Pressure Drop</b>	90 bar (~1,300 psi) at the start of gradient
<b>Sample:</b>	4 Sulfa drug and precursor acid

Note the high amount of organic needed for complete sample elution.

