

APPLICATION NOTE

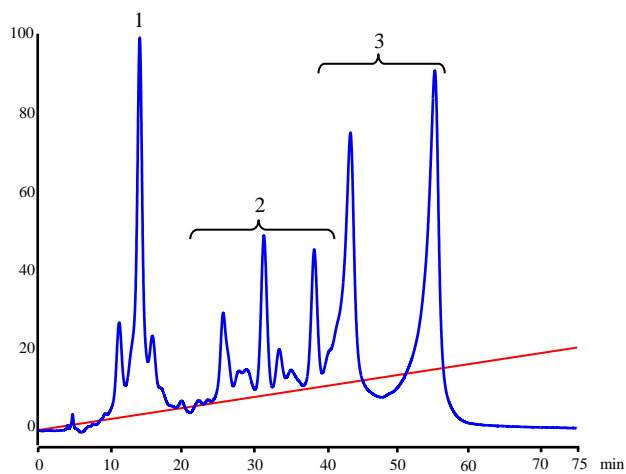
Quaternary Amino Ethyl (STYROST™ QAE) Anion Exchanger: Comparison with Quaternary Amino Methyl (STYROST™ HQ).

Anion exchanger chromatography with quaternary amine functions is a common mode of separation using media with permanently charged surface.

It is also called SAX or Strong Anion Exchanger chromatography indicating the constancy of charges independent of the pH.

In Application Note 59 **STYROST™ HQ Simulated Monolith™** was compared with Mono Q HR 16/10 using its function test under conditions suggested by the manufacturer.

In the present application note the performance of **STYROST™ QAE Simulated Monolith™** is tested with the same mixture of proteins as before.



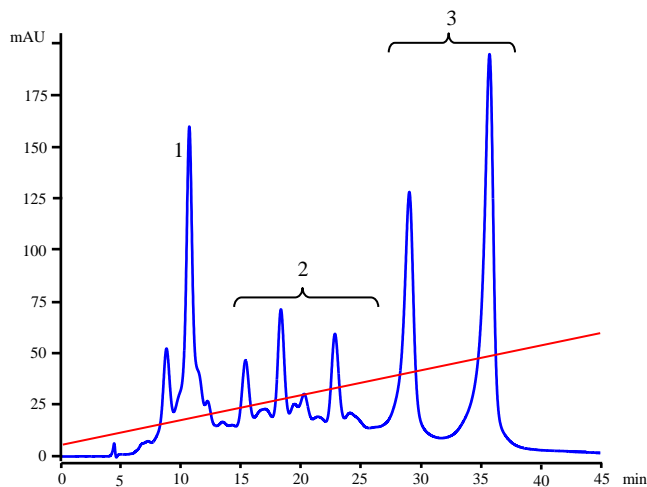
STYROST™ QAE Simulated Monolith™ 4.6 x 300 mm Stainless Steel.

Table 1. Operating parameters.

HPLC System.	Agilent 1100 with thermostatted column compartment.
Columns	STYROST™ QAE 4.6 X 300 mm Stainless Steel. 4.98 ml, column volume
Mobile phase.	A: 20 mM Piperazine, pH=6 B: A + 1 M NaCl, pH=6
Flow rate	0.8 ml/min (290 cm/hr of linear flow rate)
Gradient	0.5 to 20 % B in 75 min (12 cv)
Temperature	30°C
Detection	280 nm
Injection volume	100 µl
Sample:	1. Transferrin (human) 2 mg/ml, 2. Ovalbumin, 4 mg/ml, 3. b-Lactoglobulin, 4 mg/ml

The back pressure of the column is 16 bar (232 psi) at 0.8 ml/min. and 30 °C.

Under similar conditions, the back pressure of a **4.6 x 300 mm HQ** column is also 16 bar (232 psi) providing a different resolution for the separation.



STYROST™ HQ Simulated Monolith™ 4.6 x 300 mm Stainless Steel.

Table 2. Operating parameters.

HPLC System.	Agilent 1100 with thermostatted column compartment.
Columns	STYROST™ HQ 4.6 X 300 mm Stainless Steel. 4.98 ml, column volume
Mobile phase.	A: 20 mM Piperazine, pH=6 B: A + 1 M NaCl, pH=6
Flow rate	0.8 ml/min (290 cm/hr of linear flow rate)
Gradient	2 to 30 % B in 45 min (7 cv)
Temperature	30°C
Detection	280 nm
Injection volume	100 µl
Sample:	1. Transferrin (human) 2 mg/ml, 2. Ovalbumin, 4 mg/ml, 3. b-Lactoglobulin, 4 mg/ml

High dynamic capacity, high resolution and low back pressure of **STYROST™ HQ and QAE Simulated Monolith™** provide the appropriate columns for Simulated Moving Bed chromatography.

SMB is necessary in addressing the limitations of downstream processing as it presently stands.

Simulated Moving Bed chromatography can reduce chromatography media volume and buffer consumption per unit of productivity.

The use of multiple channels provides high cumulative flow rates.

While the use of monolith in Simulated Moving Bed chromatography is an improvement over the use of conventional media, **Simulated Monolith™** is the next step in this process as it addresses the capacity limitations of monolith while reducing the back pressures and preserving the resolution of high performance media.