

APPLICATION NOTE

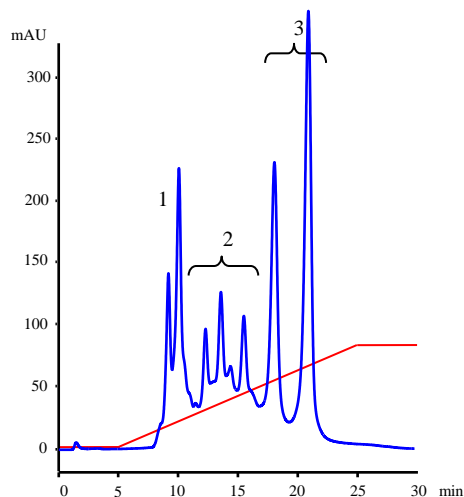
Quaternary Amino Methyl (HQ) Anion Exchanger: Comparison with Mono Q

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.

The following chromatogram shows the separation of three proteins on a **STYROS™ HQ Simulated Monolith™** column at 30°C.

The conditions used are similar to those used for the function test of Mono Q HR 16/10.



STYROS HQ™ Simulated Monolith™ 4.6 x 100 mm Stainless Steel.

Table 1. Operating parameters.

HPLC System.	Agilent 1100 with thermostatted column compartment.
Columns	STYROS™ HQ 4.6 X 100 mm Stainless Steel. 1.66 ml, column volume
Mobile phase.	A: 20 mM Piperazine, pH=6 B: A + 0.3 M NaCl, pH=6
Flow rate	0.8 ml/min (290 cm/hr of linear flow rate)
Gradient	0.5% B for 5 min, to 100% B in 25 min
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 next chromatogram is a depiction of the Function test of Mono Q HR 16/10.

As shown the sample is initially run for 2.5 column volume before starting the gradient.

The high dynamic capacity of STYROS™ HQ Simulated Monolith™ does not require such preloading. The run can therefore be reduced to half as seen on the opposite chromatogram.

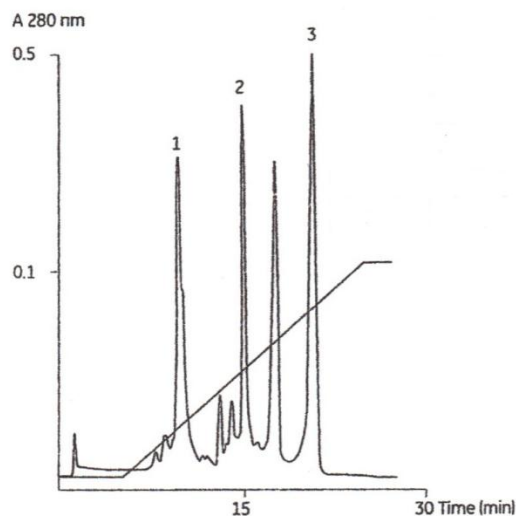


Table 2. Operating parameters.

Columns	Mono Q HR 16/10 16 X 100 mm, 20 ml column volume
Mobile phase.	A: 20 mM Piperazine, pH=6 C: A + 0.3 M NaCl, pH=6
Flow rate	10 ml/min (300 cm/hr of linear flow rate)
Gradient	0.5% B for 5 min, to 100% B in 25 min
Detection	280 nm
Injection volume	500 µl
Sample:	1. Transferrin, 2 mg/ml, 2. Ovalbumin, 4 mg/ml, 3. b-Lactoglobulin, 4 mg/ml
Chart speed	0.5 cm/min

STYROS HQ™ Simulated Monolith™ 4.6 x 100 mm Stainless Steel.

Same separation ran in half the time on the same column.

Gradient: 0.6 to 100% B in 15 minutes.

Application Note 60 takes advantage of the STYROS™ Simulated Monolith™ media stability to move beyond the resolving power of a 10 cm column without any back pressure restrictions.

