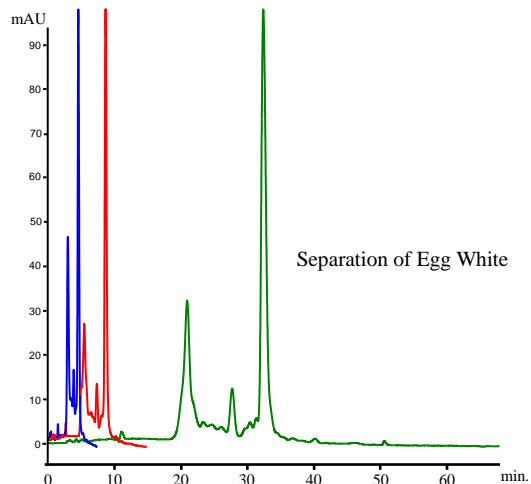


APPLICATION NOTE

STYROS™ HQ Simulated Monolith™. Importance of Column Length.

The superimposed chromatograms shown here highlight the practical rhythm of scouting for the optimal conditions of a separation on **STYROS™ Simulated Monolith™** columns as a general rule.

Part of this exercise is the choice of the appropriate length to make the separation possible. It is therefore crucial for the end user to have at his disposal various lengths with the appropriate practical pressure drop that can be used.



Chromatogram 1

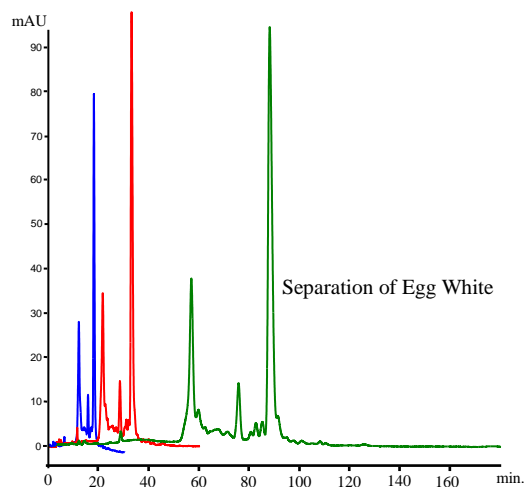
Separation of Egg White on **STYROS™ HQ 4.6 x 50 mm**,
4.6 x 100 mm and **4 x 300 mm**.

Table 1. Operating parameters.

HPLC System.	Agilent 1100 with thermostatted column compartment and quaternary pump.
Columns	STYROS™ HQ/XH 4.6 X 50 mm (0.83 ml) STYROS™ HQ/XH 4.6 X 100 mm (1.66 ml) and STYROS™ HQ/XH 4 X 300 mm (3.77 ml)
Mobile phase.	A: 20 mM Tris, pH=8.2 B: A + 1 M NaCl, pH= 8.2
Flow rates	2 ml/min (720 and 478 cm/hr of linear velocities)
Gradient	1 to 30 % B in 18 cv
Temperature	30°C
Detection	280 nm
Injection volume	5µl, 10µl and 15 µl
Pressure Drop	6 bar (87psi), 13 bar (189 psi), and 20 bar (290 psi)
Sample:	Fresh Egg White mixed 1:9 with buffer A

Such sequence allows one to quickly reach the optimal conditions of the separation and then use a longer column for added resolution.

Note that the back pressure of the 300 mm column is similar to a 50 mm Monolith column. The resolution however is markedly higher.



Chromatogram 2

Separation of Egg White on **STYROS™ HQ 4.6 x 50 mm**,
4.6 x 100 mm and **4.6 x 300 mm**.

Table 2. Operating parameters.

HPLC System.	Agilent 1100 with thermostatted column compartment and quaternary pump.
Columns	STYROS™ HQ/XH 4.6 X 50 mm (0.83 ml) STYROS™ HQ/XH 4.6 X 100 mm (1.66 ml) and STYROS™ HQ/XH 4.6 X 300 mm (4.98 ml)
Mobile phase.	A: 20 mM Tris, pH=8.2 B: A + 1 M NaCl, pH= 8.2
Flow rates	0.5 ml/min (180 cm/hr of linear velocity)
Gradient	1 to 30 % B in 18 cv
Temperature	30°C
Detection	280 nm
Injection volume	5µl, 10µl and 15 µl
Pressure Drop	1 bar (15psi), 2 bar (29 psi), and 8 bar (116 psi)
Sample:	Fresh Egg White mixed 1:9 with buffer A

