

APPLICATION NOTE

STYROS™ Simulated-Monolith™. Choice of the Right Column.

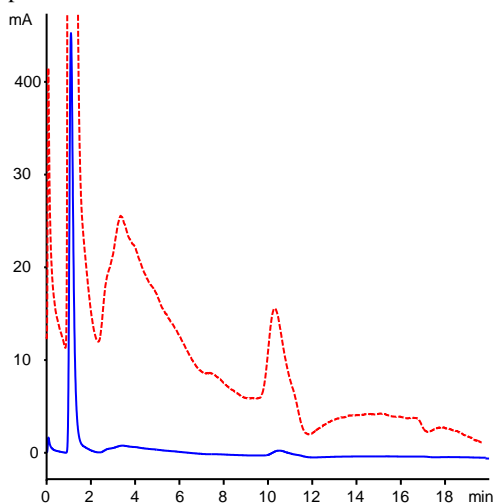
To highlight the importance of the choice of the right columns to run a sample and ultimately use that run in large scale for the processing, a weak cation exchanger (CM) was compared with a weak anion exchanger (HPA) in the separation of egg white components.

Both columns are **STYROS™ Simulated Monolith™** polymeric.

The main component, egg albumin has an isoelectric point of 4.5 to 4.9.

It elutes early on when using the CM at a pH of 6.8, whereas it is the last component to elute at pH 8.2 and using HPA.

These two chromatograms indicate clearly that the CM phase is not discriminatory enough to isolate Ovalbumin from other components.

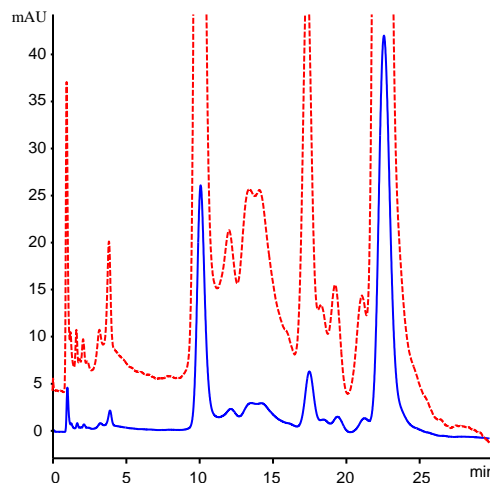


Chromatogram 1

Separation of Egg White on **STYROS™ CM** (4.6 x 100 mm)
Weak Cation Exchanger.

Table 1. Operating parameters.

HPLC System.	Agilent 1100 with thermostatted column compartment and quaternary pump.
Columns	STYROS™ CM/XH 4.6 X 100 mm (1.66 ml)
Mobile phase.	A: 20 mM Phosphate, pH=6.8 B: A + 1 M NaCl, pH= 6.8
Flow rates	1 ml/min (360 cm/hr of linear velocities)
Gradient	1 to 30 % B in 18 cv
Temperature	30°C
Detection	280 nm
Injection volume	10µl
Pressure Drop	8 bar (116psi)
Sample:	Fresh Egg White mixed 1:9 with buffer A



Chromatogram 2

Separation of Egg White on **STYROS™ HPA** (4.6 x 100 mm)
Weak Anion Exchanger.

Table 2. Operating parameters.

HPLC System.	Agilent 1100 with thermostatted column compartment and quaternary pump.
Columns	STYROS™ HPA/XH 4.6 X 100 mm (1.66 ml)
Mobile phase.	A: 20 mM Tris, pH=8.2 B: A + 1 M NaCl, pH= 8.2
Flow rates	1 ml/min (360 cm/hr of linear velocity)
Gradient	1 to 30 % B in 18 cv
Temperature	30°C
Detection	280 nm
Injection volume	10µl
Pressure Drop	10 bar (145 psi)
Sample:	Fresh Egg White mixed 1:9 with buffer A

In application Note 87 a sample of commercial OVA is displayed with a measured purity of 98 % using gel electrophoresis. One way to justify the number of peaks would be to show the existence of such variety of OVA from their sources.

