



The Vanguard of Liquid Chromatography.

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APPLICATION NOTE

Reversed Phase Polymeric: Comparison of STYROS™ 2R with Commercial Non Porous Polymeric.

A non altered set of proteins found in egg white, were separated on a **STYROSTM** 2R/XH 4.6 x 50 mm column (volume 0.83 ml) at a linear flow rate of 1,450 cm/hr (4 ml/min volumetric flow) and compared with the performance of a non porous polymeric column of 4.1 mm x 30 mm (volume 0.4 ml) run at 900 cm/hr of linear flow rate (2 ml/min volumetric flow).

The results show a clear advantage with the **STYROS™** column both in performance and operating conditions.



Separation of egg white proteins on **STYROS™** 2R/XH

Table 1. Operating parameters.

HPLC System.	Agilent 1100 with thermostatted column compartment.
Columns	STYROS™ 2R/XH 4.6 X 50 mm
Mobile phase.	A: 0.075% TFA in H2O.
	B: 0.075% TFA in Acetonitrile: H2O (95:5).
Flow rate	4 ml/min (1,450 cm/hr)
Gradient	30 to 50 % B in 1 min. 50% B to 2 min.
Temperature	30°C
Detection	215 nm
Injection volume	10 µl
Sample:	Egg white in buffer A. Mixture of 1:9
	1-Lysozyme 2- Conalbumin 3- Ovalbumin

The three major proteins are separated at high flow rates in less than 2 minutes.



<u>Chromatogram 2</u> Egg white proteins separated on a commercial non porous polymeric media.

Table 2. Operating parameters.

Columns	Commercial non porous polymeric 4.1 x 30 mm
Mobile phase.	A: 0.1% TFA in H2O.
	B: 0.1% TFA in Acetonitrile
Flow rate	2 ml/min (900 cm/hr)
Gradient	0 to 60 % B in 20 min
Temperature	Room temperature
Detection	215 nm
Sample:	Egg white in buffer A. Mixture of 1:9

The two major peaks have been identified as Conalbumin and Ovalbumin.

This clearly shows the importance of the proper manufacture of porous polymeric media in order to have a narrow distribution of the throughpores. The stability of the media and the absence of fines and sub-micron particles is also important as they result in clogging of the throughpores that would operate as long diffusive pores.

