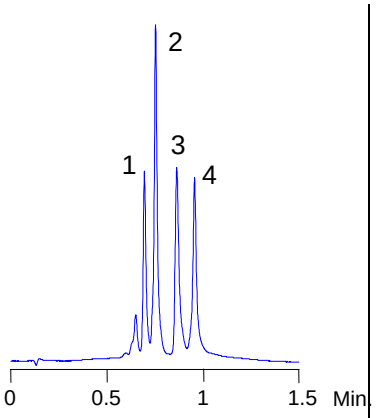
OraChrom, Inc.
The Vanguard of Liquid Chromatography.

10-B Henshaw Street Woburn, MA 01801 USA
Phone (781) 932 0151 Fax (781) 932 0787
E-mail: *info@orachrom.com* *orachrom.com*

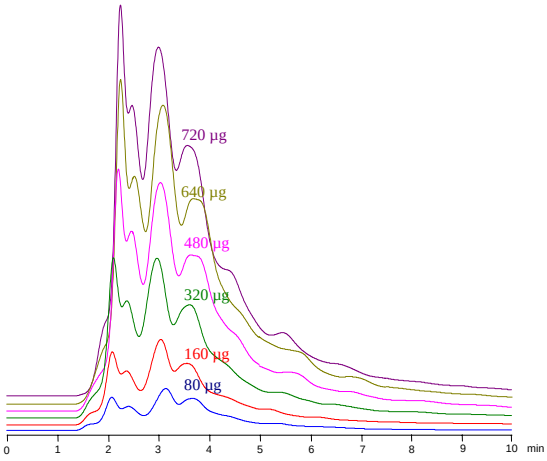
APPLICATION NOTE

Low Salt Separations on Shielded Weak Anion Exchangers: STYROS™ SWAX.

Although reversed phase chromatography provides well separated peaks, it cannot be used to identify small variations within the same biomolecule.
The chromatogram on the right depicts the separation of Ovalbumin (peak # 4) from 3 other proteins on STYROS™ 2R reversed phase polymeric, in less than one minute.
It is a limited separation of components each made of many variants.



Unlike reversed phase, ion exchange chromatography has the ability to differentiate the sub species that were left undetected in the single peak of Ovalbumin.
The following set of overlaid chromatograms show the same well resolved single peak Ovalbumin now separated on STYROS™ SWAX, Shielded Weak Anion Exchanger Simulated Monolith.

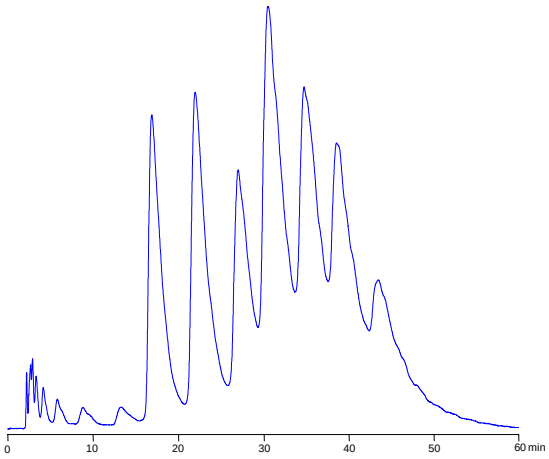


Chromatogram 1
Separation of phosphorylation variants of Ovalbumin on STYROS™ SWAX

Table 1. Operating parameters.

HPLC System	Agilent 1100 with thermostatted column compartment.
Columns	STYROS™ SWAX 4.6 X 150 mm
Mobile phase	A: 10 mM TRIS, pH=7 B: A + 1M NaCl, pH=7
Flow rate	1.5 ml/min (540 cm/hr)
Gradient	*2.2 to 5.2 % B* in 10 min. (6 cv)
Temperature	30°C
Detection	280 nm
Injection volume	10 to 90 µl
Sample	Albumin, chicken egg grade VI. 8mg/ml in buffer A.

Less than 50 mM of salt is needed to elute the different components of Ovalbumin. Although shielded, STYROS™ SWAX has the adequate capacity to maintain the resolution during high loads.
The shielding of charges becomes more important when the mixture is made up of highly charged species such as Oligonucleotides.
The following chromatogram shows the separation of a mixture of Oligothymidylic acid d (pT) 12-18mer, during an 8 % salt gradient from 11 to 19%.



Chromatogram 1
Separation of Oligothymidylic Acid d(pT) 12-18mer on STYROS™ SWAX

Table 2. Operating parameters.

HPLC System	Agilent 1100 with thermostatted column compartment.
Columns	STYROS™ SWAX 4.6 X 150 mm
Mobile phase	A: 10 mM TRIS, pH=7 B: A + 1M NaCl, pH=7
Flow rate	1 ml/min (360 cm/hr)
Gradient	*11 to 19 % B* in 60 min. (24 cv)
Temperature	30°C
Detection	260 nm
Injection volume	30 µl
Sample	5 units in 1 ml of buffer A.

The present Shielded Weak Anion Exchanger is the first in a series of such media intended to run separations at low salt.
Unlike Monolith, Simulated Monolith columns can be provided in many formats, from Nano (175 µm) to large bore of 10 mm and beyond.

Inc.