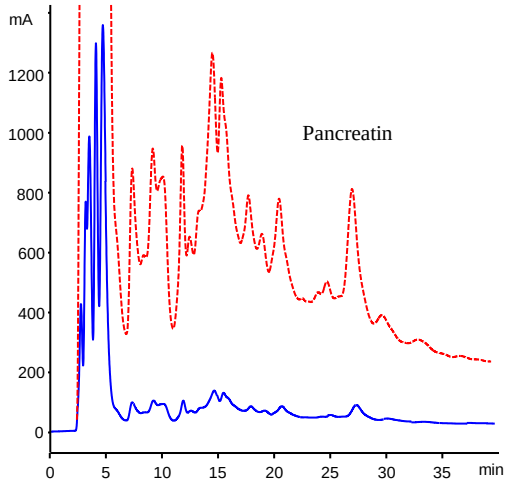
OraChrom, Inc.
The Vanguard of Liquid Chromatography.

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

APPLICATION NOTE

Separation of Pancreatin on STYROS™ HPA (High Capacity Weak Anion Exchanger): Column Length.

Pancreatin is a complex dynamic mixture. It contains enzymes such as Amylase, Trypsin, Lipase, Ribonuclease, and other proteases.
The following chromatograms show that the column length remains a major factor in identifying the majority of the components present in the sample.
Such feature requires the stationary phase to be amenable to longer columns as well as in its use on all chromatography systems including FPLC.
It is also a requirement that the same media offers the aptitude of scale up avoiding additional redundant work.
Although the separation was carried on a 100 cm column identifying 5 major peaks right at the start of the gradient, additional peaks can be detected with a similar phase using a column 3 times longer.

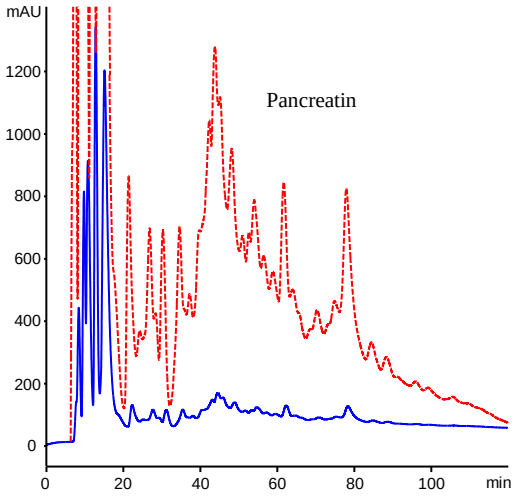


Chromatogram 1
Pancreatin Separation on **STYROS™ HPA** (4.6 x 100 mm)
High Capacity Weak Anion Exchanger.

Table 1. 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=8.15 B: A + 1 M NaCl, pH= 8.15
Flow rates	0.5 ml/min (180 cm/hr of linear velocities)
Gradient	1 to 3% B in 6 min to 80 % B in 40 min.
Temperature	30°C
Detection	214 nm
Injection volume	40µl
Pressure Drop	4 bar (58psi)
Sample:	Pancreatin 5mg/ml in buffer A.

As it shows most of the sharp peaks of the previous chromatogram have revealed new compounds adding to the initial ones.



Chromatogram 2
Pancreatin Separation on **STYROS™ HPA** (4.6 x 300 mm)
High Capacity 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 300 mm (4.98 ml)
Mobile phase.	A: 20 mM Tris, pH=8.15 B: A + 1 M NaCl, pH= 8.15
Flow rates	0.5 ml/min (180 cm/hr of linear velocity)
Gradient	1 to 3% B in 22 min to 80% B in 120 min.
Temperature	30°C
Detection	214 nm
Injection volume	100µl
Pressure Drop	10 bar (145 psi)
Sample:	Pancreatin 5mg/ml in buffer A.

The column is equilibrated in a short time running it at 4 to 5 ml/min (1,500 to 1,800 cm/hr).
Unlike soft gel it can be run at high flow rates.
All size columns are also available including 300 mm long columns.
These are some of the important features a stationary phase must have to be considered as “seamless” leading to process scale operations

Inc.