OraChrom, Inc.
The Vanguard of Liquid Chromatography.

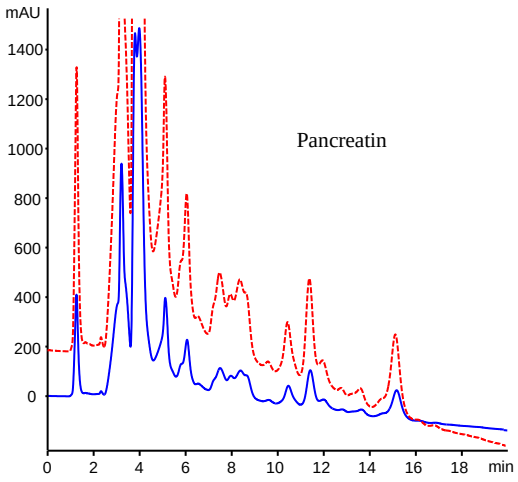
10-B Henshaw Street Woburn, MA 01801 USA
Phone (781) 932 0151 Fax (781) 932 0787
E-mail: info@orachrom.com www.orachrom.com

APPLICATION NOTE

Separation of Pancreatin on STYROS™ QAE (Strong Anion Exchanger): Simulated-Monolith™ and 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 and ability of scale up avoiding additional redundant work.
The separation here reveals major peaks right at the start of the gradient using a 100 cm long column, additional peaks can be detected with a similar phase using a column 3 times longer.

f

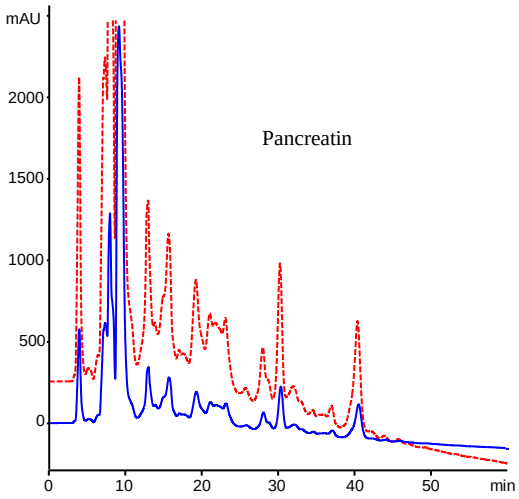


Chromatogram 1
Pancreatin Separation on **STYROS™ QAE** (4.6 x 100 mm).
Strong Anion Exchanger (Quaternary Amino Ethyl).

Table 1. Operating parameters.

HPLC System.	Agilent 1100 with thermostatted column compartment and quaternary pump.
Columns	**STYROS™ QAE/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	1 ml/min (360 cm/hr of linear velocities)
Gradient	0 to 40 % B in 20 min.
Temperature	30°C
Detection	214 nm
Injection volume	30µl
Pressure Drop	10 bar (145 psi)
Sample:	Pancreatin 10 mg/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™ HQ** (4.6 x 300 mm)
Stong Anion Exchanger.

Table 2. Operating parameters.

HPLC System.	Agilent 1100 with thermostatted column compartment and quaternary pump.
Columns	**STYROS™ QAE/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	1 ml/min (360 cm/hr of linear velocity)
Gradient	0 to 40 % B in 60 min.
Temperature	30°C
Detection	214 nm
Injection volume	100µl
Pressure Drop	14 bar (203 psi)
Sample:	Pancreatin 10 mg/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” in order to be used in the following step of process scale operations.

Inc.