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

High Speed Resolution: efficient use of long, narrow-bore columns

Rapid resolution of limited amounts of closely related compounds can often be achieved efficiently through the use of long, narrow-bore HPLC columns, typically in the 15 to 25 cm range.

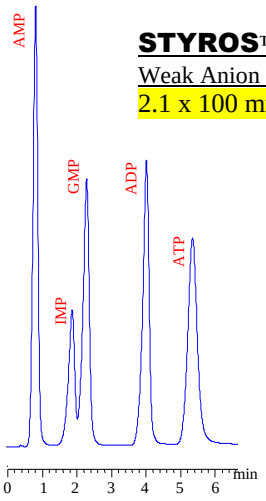
Back pressure rises rapidly in such columns, however, especially at desirable rates of flow, and the tolerance of chromatographic media to high pressures is critical. In other words, the availability of stable, pressure-tolerant fully pervious hard gel polymeric media becomes the limiting factor in designing HPLC applications.

The utility of increasing column length is illustrated below in the separation of 5 closely similar nucleotides. The two runs are identical with the exception of an additional 5 cm length in the second column achieving baseline separations in an additional 2 mins.

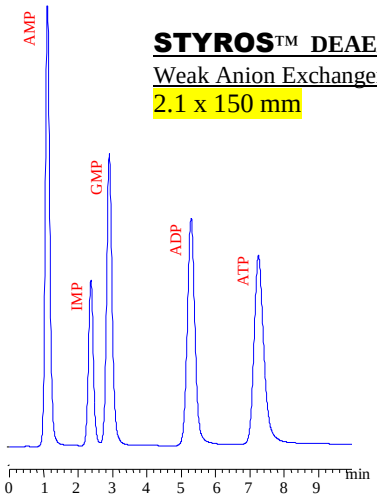
Table 1. Operating Parameters.

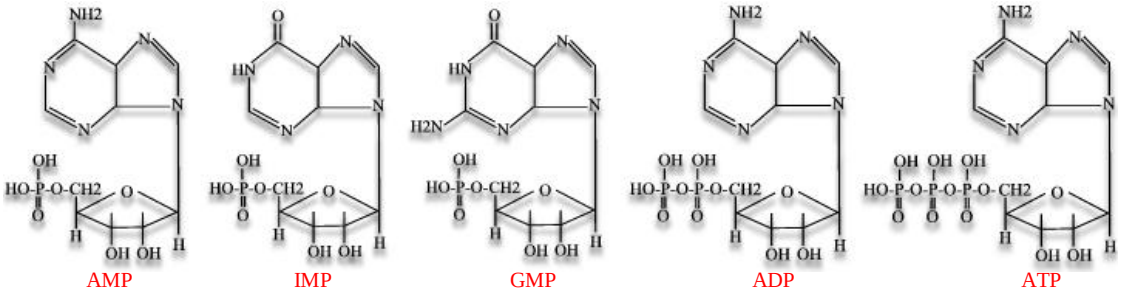
HPLC System.	HP 1100
Columns	**STYROS™** DEAE/NB 2.1 x 100 mm and 2.1 x 150 mm
Mobile Phase	A: ACN in 15 mM Phosphate, pH = 3, 20:80 B: ACN in 0.5 M Phosphate, pH = 3, 20:80
Flow rate	1 ml/min (1,800 cm/hr)
Gradient	0 to 30 % B in 7.2 cv, to 100 % B in 8.7 cv.
Temperature	37°C
Detection	260 nm
Injection volume	10 µl
Samples (5 Nucleotides)	1 mg/ml of AMP, IMP, GMP, ADP and ATP in buffer A.

STYROS™ DEAE/NB
Weak Anion Exchanger.
2.1 x 100 mm



STYROS™ DEAE/NB
Weak Anion Exchanger.
2.1 x 150 mm





Notice the linear velocity of 1,700 cm/hr (based on an empty column), far exceeds those used with either soft gel or non pervious media.