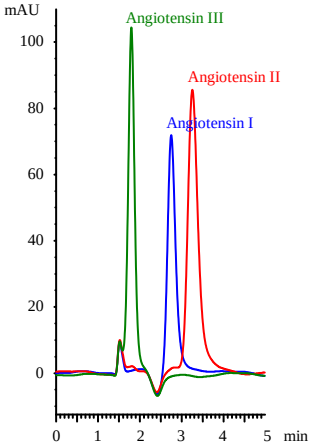
OraChrom, Inc.
The Vanguard of Liquid Chromatography.

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

STYROS™ Amino HILIC Simulated Monolith Polymeric: Separation of Angiotensins I, II and III

HILIC or Hydrophilic Interaction Chromatography is a variation of normal phase chromatography. It provides complementary selectivity compared to reversed phase chromatography.
The following chromatogram shows the separation of 3 closely related peptides on a **STYROS™ Amino-HILIC Simulated Monolith** column at 30˚C.



Chromatogram 1
Separation of 3 Angiotensins at 360 cm/hr linear flow rate
on **STYROS™ Amino HILIC**

Table 1. Operating parameters.

HPLC System.	Agilent 1100 with thermostatted column compartment.
Columns	**STYROS™ Amino-HILIC** 4.6 X 100 mm
Mobile phase.	A: ACN B: 50 mM CH3COONH4, pH=6.8
Flow rate	1 ml/min (360 cm/hr of linear flow rate)
Gradient	Isocratic 90 % A, 10 % B
Temperature	30°C
Detection	230 nm
Injection volume	2 µl of each
Sample:	1 mg/ml of each Angiotensin in DI H2O

Angiotensins I, II and III are oligopeptides found in the blood.

Angiotensin I is derived from the precursor molecule angiotensinogen, a serum globulin produced in the liver. It is a decapeptide made of the following amino acids sequence:

Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu

Angiotensin II is an octapeptide with the following amino acid composition:

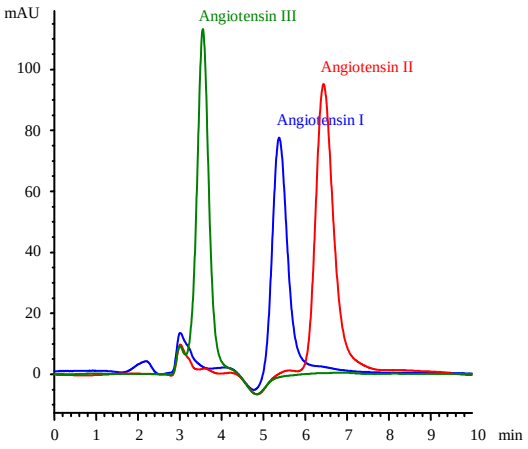
Asp-Arg-Val-Tyr-Ile-His-Pro-Phe where the **His-Leu** terminal of Angiotensin I have been cleaved.

Angiotensin III is a heptapeptide that derives from Angiotensin II it is made of the following sequence of amino acids:

Arg-Val-Tyr-Ile-His-Pro-Phe

These peptides are usually found together in the blood stream.

The present Amino HILIC separation procedure provides the proper LC method in hyphenation with mass spectroscopy to analyze these compounds.



Chromatogram 2
Separation of 3 Angiotensins at 180 cm/hr linear flow rate
on **STYROS™ Amino HILIC**

STYROS™ Amino-HILIC Simulated Monolith columns are stable in the full pH range and high temperatures.

Unlike Monolith, **STYROS™ Simulated Monolith** columns are available in many sizes for additional resolving capabilities.

Inc.