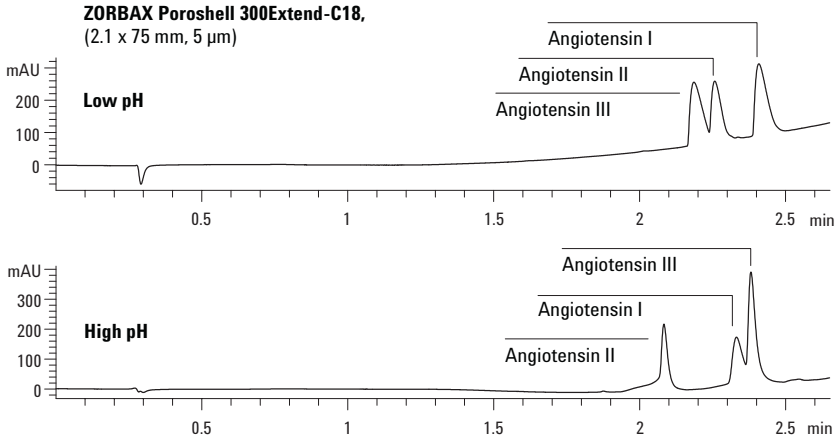
Using ZORBAX Poroshell 300Extend-C18 to Achieve Unique Selectivity at pH 2 and 10: Angiotensins

Application

Biotechnology/QA/QC/Basic R&D

Robert D. Ricker

Selectivity (α factor) is the most powerful chromatographic parameter for obtaining resolution. In reversed-phase HPLC, change in mobile-phase pH can effectively achieve resolution where other chromatographic changes cannot. This is especially true for polar and charged analytes such as biological macromolecules. In this type of analysis, altering pH can shift the charge on the analytes as well as that on the packing surface. ZORBAX Poroshell 300Extend-C18 is made using the same, reliable, Extend-C18 bonding used for ZORBAX totally porous packings; both low-pH and high-pH mobile phases are tolerated, with extended column performance. Enhanced lifetime of the column over a broad pH range makes the material ideal for method development of peptides and small proteins. The following chromatograms demonstrate the use of mobile-phase pH to achieve selectivity differences.



Conditions

Column	**ZORBAX Poroshall 300Extend-C18** (2.1 × 75 mm, 5 µm) (p/n 670750-902)
Sample	330 ng each peptide
Gradient	0%–100% B in 2 min (see mobile phase at right).
Detection	UV (215 nm)
Temperature	Ambient
Injection	1 µL
Flow rate	0.5 mL/min

Highlights

- ZORBAX Poroshell 300Extend-C18 columns provide wide range of selectivity for fast analysis of angiotensins at low and high pH.
- ZORBAX Poroshell is also available in SB-C18, SB-C8, and SB-C3 bonding for selectivity options at low pH and high temperature.
- Use of appropriate column and mobile phase permits selective isolation of angiotensin types.

Mobile phase (pH 2.4)

A: 0.1% Formic acid in H_2O

B: 0.1% Formic acid in ACN

Mobile phase (pH 10)

A: 10 mM NH_4OH in H_2O

B: 10 mM NH_4OH in ACN

Angiotensins I, II, and III are part of a group of peptides that are integral to the control of hypertension. In these experiments, they also serve to show how selectivity between peptides (differing only slightly in polarity and length) may be manipulated through a change in mobile-phase pH. The three related forms of angiotensin were separated on a ZORBAX Poroshell 300Extend-C18 column at low pH (0.1% formic acid, lower chromatogram) or high pH (10-mM NH_4OH, upper chromatogram), as indicated. The flow rate, gradient time, organic percentages, temperature, and column were kept constant for the two experiments. Comparing peptide elution times from top (pH 2.4) to bottom (pH 10) chromatogram reveals a significant increase in retention for angiotensin III (from 2.2 to 2.4 min) while angiotensins II and I move toward earlier elution. From a practical standpoint, formic acid allows angiotensin I to be well resolved, while at high pH, angiotensin II is well resolved. To better understand the causes of these changes in selectivity, amino acid sequences for these angiotensin peptides are shown in Table 1. Toward lower pH, positive charges occur at the amino-terminus (NH_2-terminal) and histidine (his) residues. The carboxy-terminus (C-terminal) and aspartic acid (asp) residues will be uncharged. When pH is raised, protons will be lost, first from the C-terminal and then from the asp. Charge on the peptides will shift towards neutral and then towards overall negative charge as the NH_2-terminal and his lose a proton. Selectivity is changed with pH because of these charge changes induced in the peptides and the overall hydrophobic contribution of the amino acids making up the peptide. One other factor to consider is shift in the charge of the packing surface as any remaining silanols lose their proteins and take on a negative charge. This increases retention of positively charged species, beyond that of the hydrophobic contribution to retention.

Table 1. Amino Acid Sequence and MW for Angiotensins I, II, and III

Peptide	Peptide sequence	MW
Angiotensin I	asp-arg-val-tyr-ile-his-pro-phe-his-leu	1296.5
Angiotensin II	asp-arg-val-tyr-ile-his-pro-phe	1046.2
Angiotensin III	arg-val-tyr-ile-his-pro-phe	931.1

For More Information

For more information on our products and services, visit our Web site at www.agilent.com/chem.

The author, Robert D. Ricker, is an Applications Scientist based at Agilent Technologies, Wilmington, Delaware.

Printed in the USA
February 20, 2004
5989-0676EN

Agilent Technologies