Using the High-pH Stability of ZORBAX Poroshell 300Extend-C18 to Increase Signal-to-Noise in LC/MS

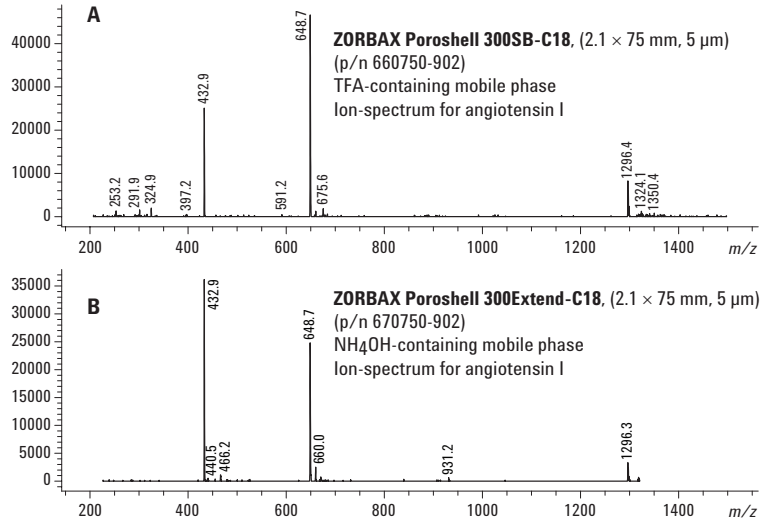
Application

Drug Manufacturing/QA/QC

Robert D. Ricker

ZORBAX Poroshell technology is designed to facilitate extremely rapid HPLC separations at high relative flow rates (high linear velocities). Poroshell particles consist of a solid silica core surrounded by a very thin (0.25 µm) porous silica crust. High linear velocities of mobile phase are tolerated because of the short diffusion distance for molecules moving into and out of this thin crust. Typical flow rates are five times that used for a comparable column packed with totally porous material.

ZORBAX Poroshell 300Extend-C18 is made using the same, reliable, Extend-C18 bonding used for ZORBAX totally porous packings; column lifetime at high pH is extended. Enhanced lifetime of the column at high pH makes the columns useful for atmospheric pressure ionization-electrospray (API-ES) mass spectrometric (MS) detection of peptides and small proteins using NH_4OH in the mobile phase. There are advantages in signal-to-noise (S/N) and in charge state distribution when using NH_4OH at high pH rather than trifluoroacetic acid (TFA) or formic acid at low pH. The following positive-mode spectra demonstrate the effect of the mobile-phase modifier on the MS signal.



Conditions

Column	See column info in figure (operation at ambient temperature)
Sample	330-ng Angiotensin I, 1-µL injection
Gradient	0%–100% B in 2 min, F = 0.5 mL/min (see mobile phase at right)
Detection	Agilent 1100 Series MSD, API-ES Pos Scan Mode, 200–1500 *m/z* , 0.52 s/cycle, Capillary = 4500V, Frag = 70V, Nebulizer 35 psi, Drying gas 12 L/min @325 °C

Highlights

- ZORBAX Poroshell technology facilitates very rapid HPLC separations at flow rates, up to 5x that used for totally porous packing.
- ZORBAX Poroshell 300Extend-C18 allows for MS detection of peptides and small proteins using NH_4OH in the mobile phase, which can lead to reduced background noise and a higher S/N ratio.
- Reliable use of NH_4OH with ZORBAX Poroshell 300Extend-C18 provides a means of altering charge-state distribution.

Mobile phase (pH 1.8)	
A:	0.1% TFA in H_2O
B:	0.085% TFA in ACN

Mobile phase (pH 10)	
A:	10 mM NH_4OH in H_2O
B:	10 mM NH_4OH in ACN

The figure demonstrates the difference in S/N in the MS ion spectrum from angiotensin I analyzed with TFA in the mobile phase (A) or with NH_4OH in the mobile phase (B). For speed of analysis, ZORBAX Poroshell packings were used in each case - the 300SB-C18 version for stability under low-pH operation and the 300Extend-C18 version for stability under high-pH operation. Relative to the spectrum obtained using TFA, the spectrum obtained using NH_4OH shows decreased background noise, especially within the 200–400 *m/z* range. The molecular ion appears at an *m/z* of 1296, with the +2 and +3 ions appearing at 648.7 and 432.9, respectively. There is a shift from +2 being the dominant ion in TFA, to the +3 being the dominant ion in NH_4OH. Note that the abundance is similar for these ion species at low pH with TFA and at high pH with NH_4OH even though the detector is set to positive mode. While the abundance is slightly less (5000 vs. 4000) this makes use of NH_4OH with ZORBAX Poroshell 300Extend-C18 a useful technique. Not only may background noise be reduced and the charge distribution shifted, but also the column can be expected to perform reliably at high pH to produce very rapid analyses. Please refer to the following Agilent application notes for relevant information: Comparison of ZORBAX Poroshell 300Extend-C18 to totally porous packing for rapid separations at high pH, 5989-0675EN [1]. Use of low and high pH to achieve unique selectivity, 5989-0645EN and 5989-0676EN [2, 3].

The angiotensin peptides are part of a system that controls hypertension and cardiovascular structure. Angiotensin II, the principal peptide, is derived from angiotensin I by cleavage of the C-terminal dipeptide his-leu. For reference, the sequence and molecular weights of angiotensins I, II, and III are shown in Table 1.

Table 1. The 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

References

1. R. Ricker, “Comparison of ZORBAX Poroshell 300Extend-C18 and totally porous packing in achieving very rapid, high-pH separation of peptides”, Agilent Technologies, publication 5989-0675EN www.agilent.com/chem.
2. R. Ricker, “Using ZORBAX Poroshell Column Selectivity for Ultra-Fast Analysis of Angiotensin I, II, III at Low and High pH”, Agilent Technologies, publication 5989-0645EN www.agilent.com/chem.
3. R. Ricker, “Using ZORBAX Poroshell 300Extend-C18 to Achieve Unique Selectivity at pH 2 and 10: Angiotensins”, Agilent Technologies, publication 5989-0676EN www.agilent.com/chem.

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, Applications Expert, is based at Agilent Technologies, Inc., Wilmington, Delaware.

Printed in the USA
March 1, 2004
5989-0683EN