IMPROVEMENTS IN HYBRID PARTICLE TECHNOLOGY: A NEW FAMILY OF HYBRID PACKING MATERIALS FOR REVERSED-PHASE HPLC

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INTRODUCTION

Method developers continue to seek efficient, robust ways to rapidly develop LC methods. To be successful, the analyst must rely on HPLC columns that are chromatographically efficient, robust over a wide range of conditions, and that provide a wide range of selectivity. Unlike commonly used bonded phases, the XBridge™ family of column technologies offers excellent stability at low *and* high pH.

In methods development, pH has long been recognized as a major parameter influencing selectivity. Along with pH, further selectivity enhancement occurs with mobile phase composition and column chemistry. Figure 1 outlines the synergy between these variables. Often diverse selectivity results when combinations of different elements of the selectivity triangle are used. Therefore, it is important to methodically test a variety of conditions to ensure success.

In this research, we will outline a method development strategy that uses the selectivity variables outlined in Figure 1. The presented data supports the use of several stationary phases across an expanded pH range and illustrates the important selectivity differences across the various stationary phases for rapid method deveopment.

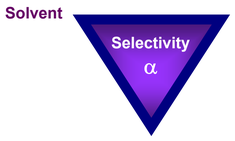


Figure 1. Selectivity variables. It is important to utilize all tools in method development; particularly the synergistic effects of solvent, pH and column chemistry.

Method Development Approach

Mobile Phase A1: 200 mM Ammonium Bicarbonate, pH 10
Mobile Phase A2: 200 mM Ammonium Formate, pH 3
Mobile Phase B1: Methanol
Mobile Phase B2: Acetonitrile
Mobile Phase C: Water
Flow Rate: 1.4 mL/min
Gradient:

Time (min)	%A	%B	%C
0	10	5	95
15	10	90	0

Temperature: 30°C
Instrument: Waters Alliance® 2695 with Six Column Selector
Detection: Waters 2996 PDA 210-400 nm; Waters ZQ®
Column Selection:
XBridge™ C_{18} 4.6 x 100 mm, 3.5 µm
XBridge™ Shield RP_{18} 4.6 x 100 mm, 3.5 µm
XBridge™ Phenyl 4.6 x 100 mm, 3.5 µm
SunFire™ C_{18} 4.6 x 100 mm, 3.5 µm
Atlantis® dC_{18} 4.6 x 100 mm, 3.5 µm

ACCELERATED HIGH-pH AGING

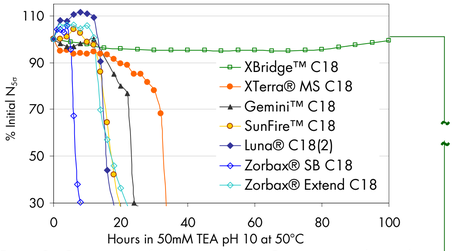


Figure 2. Comparison of efficiency loss for C_{18} stationary phases during exposure to **50 mM TEA pH 10** mobile phase at 50°C. Analyte: Acenaphthene. Test mobile phase: 65/35 methanol/20 mM K_2HPO_4, pH 7.0 (v/v) at 50°C.

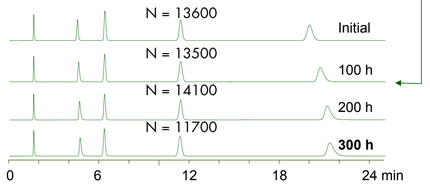


Figure 3. XBridge™ C_{18} after 0, 100, 200, and 300 hours of exposure to **50 mM TEA pH 10** mobile phase at 50°C. Analytes: (1) Uracil, (2) Propranolol, (3) Naphthalene, (4) Acenaphthene, and (5) Amitriptyline. Test mobile phase: 65/35 methanol/20 mM K_2HPO_4, pH 7.0 (v/v) at 50°C.

ACCELERATED LOW-pH AGING

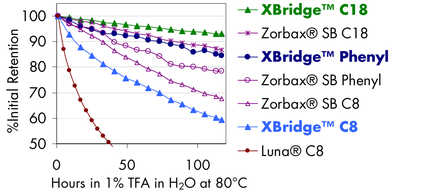


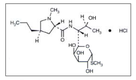
Figure 4: **1% trifluoroacetic acid (TFA) pH 1.0 stability** of the XBridge™ trifunctional stationary phases compared to several benchmark columns. Analyte: Benzene.

- Although the low pH stability of the XBridge™ C_8 is not as good as that of the XBridge™ C_{18} or Phenyl, its stability is substantially better than most of the leading brands of C_8 stationary phases.
- The XBridge™ C_{18} and Phenyl stationary phases have the best low pH stability of any of the respective class of tested phases.

Example 1: Importance of pH

OVERVIEW

Chromatographically, lincomycin is a challenging analyte (Figure 5) . The lack of a good UV chromophore limits the choices in mobile phase additives. Many common buffers such as formate, citrate, and acetate absorb strongly at UV wavelengths below 215 nm rendering them useless for this analysis. Phosphate however is UV transparent at the low wavelength required for this analysis. This application demonstrates the utility of phosphate buffers for method development at pH 2, 7, and 12.



pK_a = 7.6

Figure 5. Lincomycin molecular structure.

RESULTS

CHROMATOGRAPHIC CONDITIONS

Column: XBridge™ C_{18} 3.0 x 100 mm, 3.5 µm
Mobile Phase A1: 100 mM Potassium Phosphate pH 2
Mobile Phase A2: 100 mM Potassium Phosphate pH 7
Mobile Phase A3: 100 mM Potassium Phosphate pH 12
Mobile Phase B: Acetonitrile
Mobile Phase C: Water
Flow Rate: 0.6 mL/min
Gradient:

Time (min)	%A	%B	%C
0.0	20	5	75
15.0	20	50	30

Temperature: 30 °C
Injection Volume: 20 µL @ 30 mg/mL in water
Detection: Waters 2996 @ 215 nm
Instrument: Waters Alliance® 2695

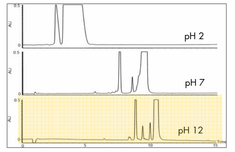


Figure 6: Effects of pH on the resolution and peak shape.

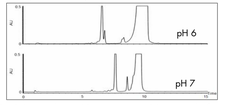


Figure 7. Near the pK_a, a one pH unit shift dramatically changes the chromatography. Therefore, working 2 pH units away from the pKa of a molecule insures more robust methods.

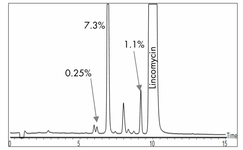


Figure 8. An optimized method for lincomycin.

Conditions
Column: XBridge™ C_{18} 3.0 x 100 mm, 3.5 µm
Mobile Phase A: 100 mM Potassium Phosphate pH 12
Mobile Phase B: Acetonitrile
Mobile Phase C: Water
Gradient:

Time (min)	%A	%B	%C
0.0	20	15	65
15.0	20	35	45

Flow Rate: 0.6 mL/min
Injection Volume 20 µL @ 30 mg/mL in water
Temperature: 30 °C
Detection: Waters 2996 @ 215 nm
Instrument: Waters Alliance® 2695

METHOD SUMMARY

From Figure 6, the best conditions to begin method optimization are at pH 12. Traditionally, this pH was unusable due to column instability outside a nominal pH range of 2 to 8 pH units. With second generation hybrid particles, pH stability using phosphate buffers increases dramatically allowing the method developer to fully utilize the pH range of phosphate buffers. At a mobile phase pH near the analyte pKa (7.6) Figure 7 shows the effect of a small change in mobile phase pH.

Figure 8 shows the results of an optimized method for lincomycin analysis. With minor changes to the scouting protocol the optimized method results in good peak shape and full resolution from the process impurities.

Example 2: Stability Indicating Assay

OVERVIEW

This application demonstrates the effectiveness of a method development strategy that utilizes HPLC coupled with mass spectrometry for peak tracking and purity. The simple Method Development Approach outlined here uses column chemistry, organic modifier and mobile phase pH to rapidly develop a HPLC method to identify the known structures for ranitidine illustrated in Figure 9.

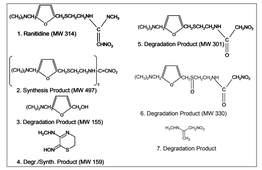


Figure 9. Ranitidine and related structures.[1,2]

EXPERIMENTAL

Sample Preparation:
A 10 mg/mL solution of ranitidine was prepared in water. The solution was placed in an 85 °C oven for 72 hours.

REFERENCES
1. M. Kelly, K. Altria, C. Grace, B. Clark, *J. Chrom. A.* 198 (1998) 297-306.
2. M. Evans, P. Haywood, D. Johnson, M. Martin-Smith, G. Munro, J. Wahlich, *J. Pharm. & Biomed. Anal.* 7(1989) 1-22.

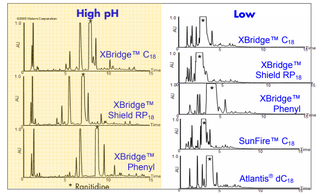


Figure 10. Screen using pH. High pH gives the best retention and best peak shape for further optimization.

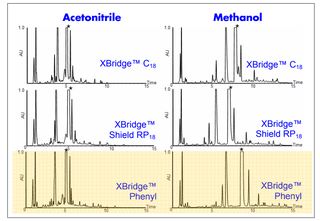


Figure 11. Screen using column chemistry and solvent (high pH). For this example the XBridge™ Phenyl column gives the best scouting results.

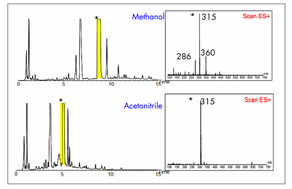


Figure 12. Screen using column chemistry and mass spectrometry for peak purity.

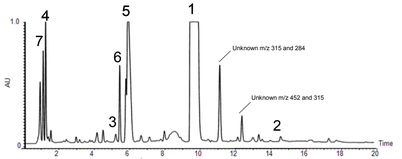


Figure 13. Optimized Method.

Optimized Conditions:
Column: XBridge™ Phenyl 4.6 x 100 mm, 3.5 µm
Mobile Phase A: 100 mM ammonium bicarbonate, pH 10
Mobile Phase B: Acetonitrile
Mobile Phase C: Water
Gradient:

Time (min)	%A	%B	%C
0.0	10	5	85
20.0	10	40	50

Flow Rate: 1.4 mL/min
Temperature: 30 °C
Detection: UV @ 254 nm
Injection Volume: 50 µL @ 10 mg/mL in water

METHOD SUMMARY

Figure 10 shows the effects of mobile phase pH on the chromatographic separation. From Figure 10, the high pH mobile phase shows consistently better peak shapes independent from the column chemistry. Also, it should be noted that the increased peak capacity at pH 10 will make remaining method development more straightforward.

Figure 11 shows the results when organic modifier and stationary phase are compared at pH 10. Now, the decision is not as straightforward as it was for the pH results. Trends can still be delineated, however, after careful scrutiny. At first glance, the XBridge™ Phenyl column with methanol appears to be the best solution. Ranitidine is a resolved peak and is well retained. However, MS analysis (Figure 12) revealed that there was a co-elution in this peak (MS scan highlighted yellow). Out of the six chromatograms, only the XBridge™ Phenyl column at pH 10 with acetonitrile cleanly resolved ranitidine from the degradents. Clearly in this case, visual inspection of the chromatograms is insufficient alone to determine the best optimization conditions. From the data presented, we selected the XBridge™ Phenyl column with acetonitrile at pH 10 for further optimization. Figure 13 shows the results of this optimization.

CONCLUSIONS

- The use of trifunctional ligands in combination with our proprietary end-capping technology provides the best stability at both high *and* low pH (Figures 2, 3 and 4). This improved stability allows the use of broader pH ranges in methods development.
- The XBridge™ Phenyl is the most stable phenyl stationary phase that we have tested.
- The XBridge™ C_8 outperforms silica-based C_{18} stationary phases at high pH and is among the most stable C_8 phases we have tested at low pH.
- The synergy between stationary phase, organic modifier, and mobile phase pH can be streamlined into an efficient method development procedure.

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