# STREAMLINING HPLC METHOD DEVELOPMENT

Waters

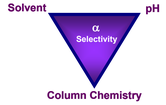
*Kevin Jenkins, Diane Diehl and Jeffrey Mazzeo*
*Waters Corporation*
*34 Maple Street, Milford, MA 01757 USA*

## OVERVIEW

Every scientist has a particular approach to HPLC method development. Underlying those approaches are several key principles that control the selectivity of the separation. In this presentation, we cover three main mechanisms that are available to the user to adjust the selectivity of a separation: column chemistry, organic solvent and mobile phase pH. We illustrate these mechanisms using a test mix of acids, bases and neutrals on a variety of stationary phases. Putting those selectivity tools into practice, we outline an approach to method development and use this approach to develop a stability indicating assay. In this assay, we use mass spectrometry with UV detection for peak tracking. We discuss how to evaluate the data, make choices about column chemistry, pH and mobile phase, and then how to optimize the method.

## INTRODUCTION

The selectivity of a separation is governed by the column chemistry, the organic solvent and mobile phase pH. Utilizing the conditions outlined below, we explored the importance of these selectivity tools to help outline a straightforward method development approach.




## METHODS

### LC Conditions

Columns: 4.6 x 100 mm, 3.5 µm
XBridge™ $C_{18}$, XBridge™ Shield $RP_{18}$, XBridge™ Phenyl
SunFire™ $C_{18}$, Atlantis® $dC_{18}$
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 Profile

| (min) | %A | %B | %C |
|---|---|---|---|
| 0.010 | | 5 | 85 |
| 15.010 | 90 | | 0 |

Temperature: 30 °C
Detection: UV @ 210-400 nm and mass spectrometry (scan 100-500 amu)
Instrument: Waters Alliance® 2695 with 2996 PDA and Micromass® Quattro micro™

Buffers for tight pH control

Constant column temperature

## Column Chemistry

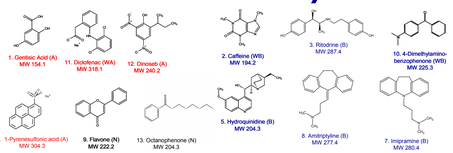



Figure 1. Structures of analytes used in the selectivity study. A = Acid; WA = Weak acid; B = Base; WB = Weak base; N = Neutral.

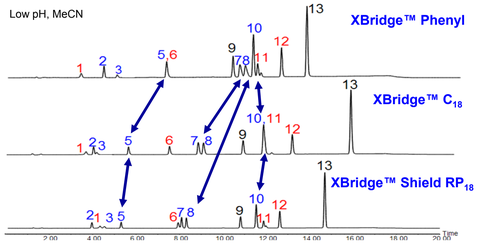



Figure 2. Selectivity shifts due to different ligands. Phenyl ligand has significantly more retention for the tricyclic antidepressants (peaks 7,8). This indicates the importance of using different column chemistries in method scouting.

## Organic Solvent

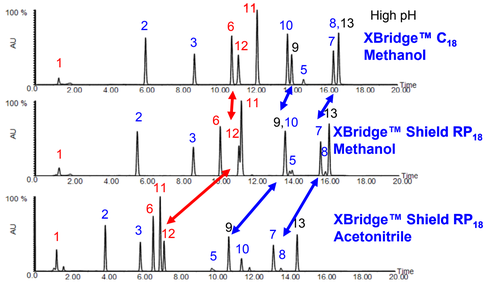



Figure 3. Selectivity shifts due to different solvents. Maximum selectivity benefits arise when comparing a C18 column run with MeOH and a Shield column (embedded polar group) run with MeCN (1).

## Mobile Phase pH

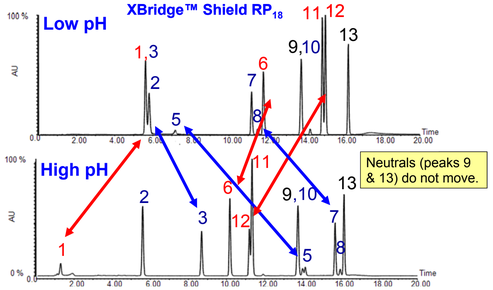



Figure 4. Selectivity shifts due to mobile phase pH. Neutral peaks 9 and 13 do not move. As the pH moves from low to high pH, acids (red) are less retained and bases (blue) are more retained.

## Method Development Scouting

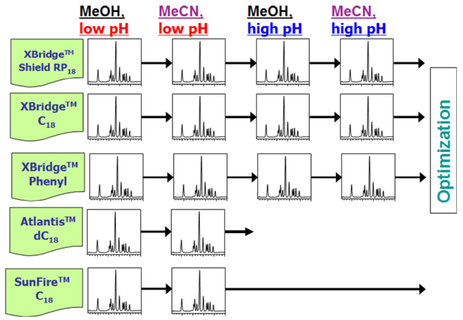
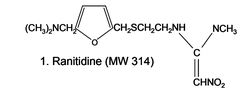


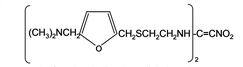

Figure 5. Method development scouting protocol. By incorporating five different column chemistries, two pH values and two organic solvents, an efficient approach can be outlined for selecting a set of conditions to optimize:

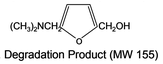
- Step 1: Select the pH
- Step 2: Select the organic solvent and column
- Step 3: Optimize/fine-tune separation

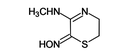
## An Example of Method Development Scouting

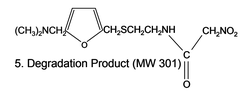
Zantac® (ranitidine – pKa's of 8.2 and 2.7)
–Used to treat and prevent ulcers in the stomach and intestines as well as treat gastroesophageal reflux disease (GERD)
Method Goals
–Develop LC/MS method for forced degradation sample, utilizing MS for peak tracking and peak purity
–Separate API from all other degradants
–Confirm presence of known degradants (Ref. 2, 3)

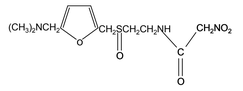

1. Ranitidine (MW 314)

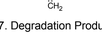

2. Synthesis Product (MW 497)


3. Degradation Product (MW 155)

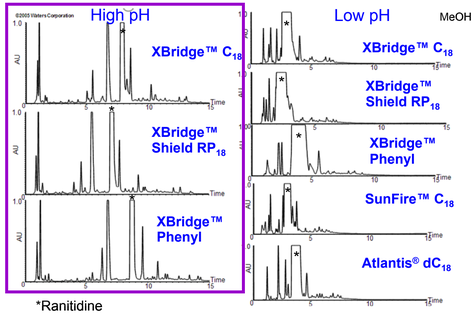

4. Degr./Synth. Product (MW 159)


5. Degradation Product (MW 301)


6. Degradation Product (MW 330)


7. Degradation Product

### Step 1: Select the pH




*Ranitidine

Figure 6. Since the first pKa is basic, high pH results in the best retention, loading, peak shape, resolution and peak capacity. We select high pH for the application.

### Step 2: Select the organic solvent and column.

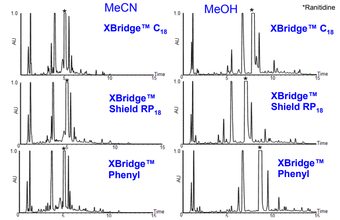



Figure 7. All six chromatograms look like they could work. We took a look at the mass spectrometry data to help make our selection.

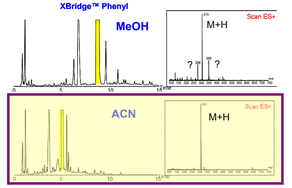
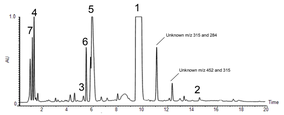



Figure 8. The ESI+ data for XBridge™ Phenyl run with MeOH contains the M+H for the parent, as well as two other significant fragments not related to the parent, indicating a co-elution. The ESI+ for XBridge™ Phenyl run with ACN do not show this co-elution, and we selected this set of conditions to optimize.

Figure 9. The optimized ranitidine method on the XBridge™ Phenyl column.




Mobile Phase A: 200 mM ammonium bicarbonate pH 10
Mobile Phase B: Acetonitrile
Mobile Phase C: Water
Gradient: Time Profile

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

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

1. Snyder, L. R., Dolan, J.W., Carr, P.W. J. Chromatogr. A 1060 (2004) 77-116.
2. M. Kelly, K. Altria, C. Grace, B. Clark, J. Chrom. A.198 (1998) 297-306.
3. M. Evans, P. Haywood, D. Johnson, M. Martin-Smith, G. Munro, J. Wahlich, J. Pharm. & Biomed. Anal. 7 (1989) 1-22.



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