Using pH-LC™ to Control Selectivity of Acidic and Basic Compounds on Gemini®-NX

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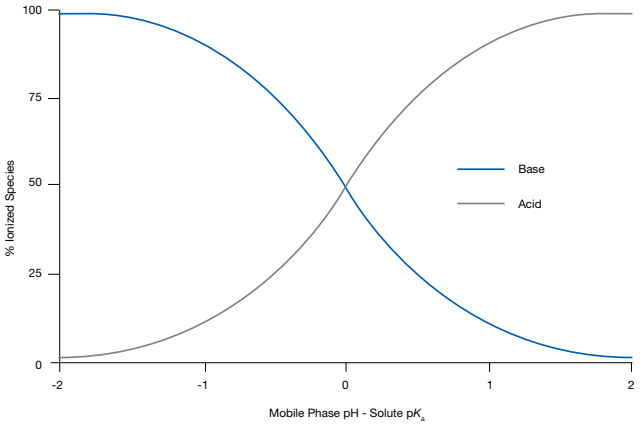
Introduction

The use of mobile phase pH to control analyte ionization states (pH-LC™) in reversed phase HPLC separations is a highly effective way to change selectivity. The ionized species of an analyte is shown to have higher polarity (less hydrophobicity) than the neutral species, which results in a loss of expected retention for that analyte. This can be attributed to less interaction with the hydrophobic stationary phase and greater affinity with the aqueous portion of the mobile phase. Ionized species also have ionic interactions with exposed and activated silanols, which impact peak shape and reproducibility.

Acidifying the mobile phase to neutralize silanol groups is a common approach to reduce secondary interactions. However, this does not resolve the issue of ionized analytes having less hydrophobic interaction with the stationary phase, especially basic compounds with a high pK_a. In addition, surface silanol groups may not share the same pK_a and therefore are not all neutralized at the same pH.

The relationship between mobile phase pH and analyte ionization states can be shown in Figure 1.

Figure 1. Relationship between mobile phase pH and solute ionization states



In this figure, the mobile phase pH in which there is partial dissociation (mixture of ionized and neutral species) of acids and bases, occurs within two pH units above and below the analyte pK_a. In order to have predominantly single ionization states in solution, the mobile phase pH must be at least two units above the analyte pK_a.

We investigated the effectiveness of using pH-LC™ techniques in the separation of a mixture of acidic (naproxen), basic (amitriptyline), and neutral (toluene) compounds using a Gemini®-NX 5 µm C18, 150 x 4.6 mm, which was designed to be very tolerant of changes in mobile phase pH without column degradation. The structures and pK_a are shown in Table 1.

Experimental Conditions

System

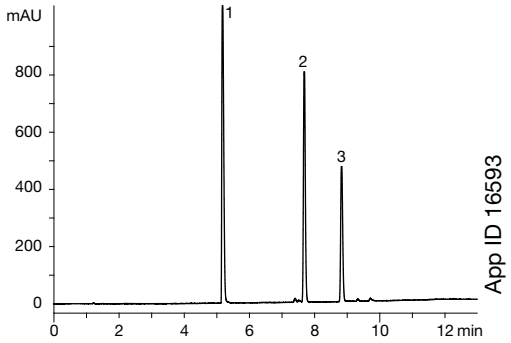
Agilent HP1100 HPLC system equipped with quaternary pump (G1311A), degasser (G1322A), autosampler (G1313A) and DAD detector (G1315B). A heating/cooling, 6-column selector (POWERSelector™) by Analytical Sales and Services, Inc was used. Data analysis using ChemStation Software (Rev A.10.02).

Materials

Formic acid, 98 % ACS Grade, by EMD (FX0440-11)
Ammonium acetate, HPLC grade, by Fluka (17836)
Potassium phosphate, monobasic, ACS grade, by Fluka (P285-3)
Ammonium bicarbonate, >99 %, by Fluka (09832)
Amitriptyline hydrochloride, >98 %, by Sigma (A8404)
Naproxen, 98 %, by Aldrich (284785)
Toluene, 99.5 %, by Sigma (179418)

Results and Discussion

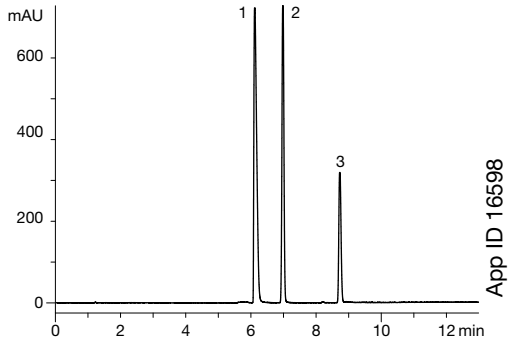
This work demonstrated the different effects of mobile phase pH using various buffers on a mixture of an acid, base, and neutral probes.



pH 2.7

Mobile Phase: A: 0.1 % Formic Acid in Water
B: 0.1 % Formic Acid in Acetonitrile
Gradient: A/B (95:5) to (5:95) in 10 min, Hold for 2 min
Flow Rate: 1.5 mL/min
Temperature: Ambient
Detection: UV @ 254 nm
Sample: 1. Amitriptyline
2. Naproxen
3. Toluene

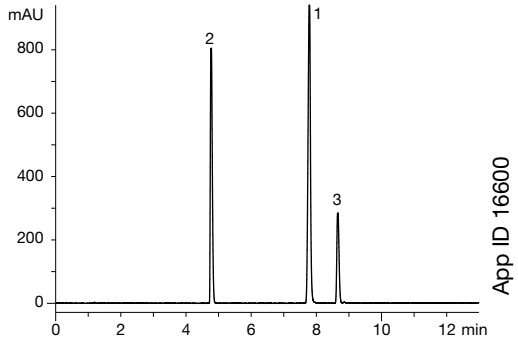
At mobile phase pH 2.7, the base amitriptyline (pK_a 9.4) is ionized and exhibits retention at 5.2 minutes. The acid naproxen (pK_a 4.5) is neutral and exhibits retention at 7.7 minutes. The neutral probe, toluene, will show relatively unchanged retention (8.7 min) throughout the pH range.



pH 4.8

Mobile Phase: A: 10 mM Ammonium Acetate pH 4.8
B: Acetonitrile
Gradient: A/B (95:5) to (5:95) in 10 min, Hold for 2 min
Flow Rate: 1.5 mL/min
Temperature: Ambient
Detection: UV @ 254 nm
Sample: 1. Amitriptyline
2. Naproxen
3. Toluene

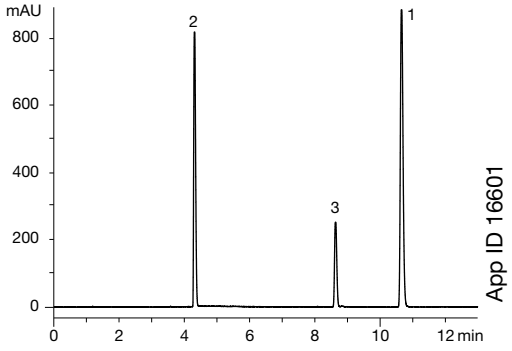
At mobile phase pH 4.8 the base amitriptyline shows increased retention (6.1 ≤min), which may be attributed to ionic interaction with activated silanols. The acid naproxen is partially dissociated (ionized and neutral) and shows a loss of retention (7.0 min). The retention of the neutral compound toluene remains unchanged.



pH 7.0

Mobile Phase: A: 20 mM Potassium Phosphate pH 7.0
B: Acetonitrile
Gradient: A/B (95:5) to (5:95) in 10 min, Hold for 2 min
Flow Rate: 1.5 mL/min
Temperature: Ambient
Detection: UV @ 254 nm
Sample: 1. Amitriptyline
2. Naproxen
3. Toluene

At mobile phase pH 7.0, the base amitriptyline shows a slight increase in retention (7.8 min) while the acid naproxen, which is approaching a predominantly ionized species, drops in retention (4.8 min).



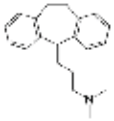
pH 10.5

Mobile Phase: A: 10 mM Ammonium Bicarbonate pH 10.5
B: Acetonitrile
Gradient: A/B (95:5) to (5:95) in 10 min, Hold for 2 min
Flow Rate: 1.5 mL/min
Temperature: Ambient
Detection: UV @ 254 nm
Sample: 1. Amitriptyline
2. Naproxen
3. Toluene

At mobile phase pH 10.5, the base amitriptyline is predominantly neutral and exhibits the most retention (10.6 min), almost double compared to retention at pH 2.7. The acid naproxen shows minimal decrease in retention (4.3 min) as it remains in a predominantly ionized state. Toluene has remained unchanged.

The structures and pK_a values of each analyte are listed below.

Table 1. Basic, Acidic, and Neutral Probes and Corresponding pK_a Values

Probe	Analyte	pK_a
Basic	Amitriptyline 	9.5
Acidic	Naproxen 	4.5
Neutral	Toluene 	n/a

Conclusion

With the use of mobile phase pH in conjunction with the pH-stable column, Gemini-NX C18, powerful selectivity manipulation can be achieved when working with ionizable compounds in reversed phase HPLC. Increased retention for most basic compounds can be achieved by increasing the mobile phase pH above their pK_a values. The same benefits can be achieved for most acidic compounds by decreasing the mobile phase pH below their pK_a values.

The method flexibility provided and improved selectivity with these techniques make Gemini-NX ideal for new method development where the columns may be exposed to a variety of pH conditions before optimized compound-dependent methods are established.

ORDERING INFORMATION

Part No.	Description
00F-4454-E0-TN	Gemini-NX 5 µm C18, 150 x 4.6 mm
00G-4454-E0-TN	Gemini-NX 5 µm C18, 250 x 4.6 mm

Technique: HPLC

Application Note: TN-1042

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