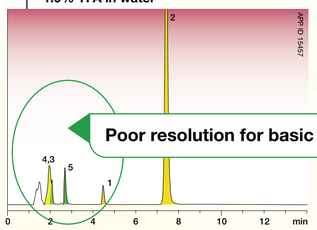
HPLC pH METHOD DEVELOPMENT

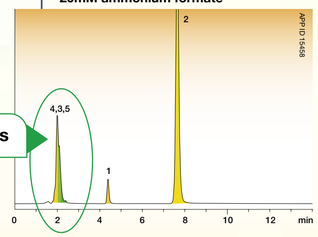
1 2 3 4 5 6 7 8 9 10 11 12

pH 1.5
1.0% TFA in water

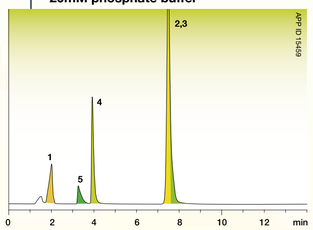


Poor resolution for basic compounds

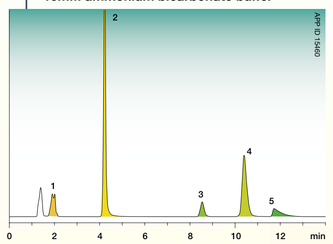
pH 4.0
20mM ammonium formate



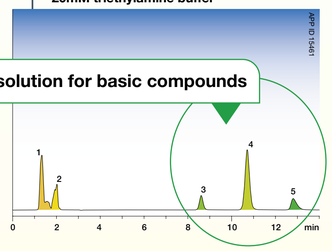
pH 7.0
20mM phosphate buffer



pH 10.0
10mM ammonium bicarbonate buffer



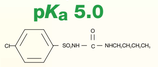
pH 12.0
20mM triethylamine buffer



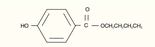
Good resolution for basic compounds

Column: Gemini 5µ C18
Dimensions: 150 x 4.6mm
Order No.: 00F-4435-E0
Mobile Phase: Acetonitrile/Buffers at various pH's (see chromatograms), (50:50)
Flow Rate: 1 mL/min
Temperature: 22°C
Detection: UV @ 254nm
Sample Analytes:

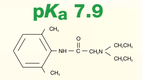
1. **Chlorpropamide** pK_a 5.0



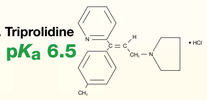
2. **Butylparaben**



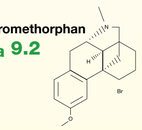
3. **Lidocaine** pK_a 7.9



4. **Triprolidine** pK_a 6.5



5. **Dextromethorphan** pK_a 9.2



Method Development Tips

Column
- Use ultra-pure spherical silica particles
- Wider pH range increases method development flexibility

Mobile Phase
- Use Methanol and Acetonitrile to vary selectivity
- Choose pH to be at least 1-2 units above or below pK_a value of compound

Buffer Selection For Method Development

Next to organic solvent, buffer selection is the most important variable in HPLC method development. There are several criteria in selecting the appropriate buffer. The first is choosing a buffer that has a pK_a near the desired working pH. Other criteria like ionic strength, ion-pairing properties, as well as mass spectrometer compatibility should be considered before selecting any mobile phase modifier.

Buffer†	pK_a	Buffer Range (pH)	MS Compatible
Trifluoracetic Acid	**< 2**	**< 2.5**	•‡
Phosphoric Acid (pK_1)	2.1	1.1 - 3.1	
Citric Acid (pK_1)	3.1	2.1 - 4.1	
Formic Acid	**3.8**	**2.8 - 4.8**	•
Citrate (pK_2)	4.7	3.7 - 5.7	
Acetic Acid	**4.8**	**3.8 - 5.8**	•
Citrate (pK_3)	5.4	4.4 - 6.4	
Carbonate (pK_1)	**6.4**	**5.4 - 7.4**	•
Phosphate (pK_2)	**7.2**	**6.2 - 8.2**	
Triethanolamine	7.8	6.8 - 8.8	•
TRIS	8.3	7.3 - 9.3	
Diethanolamine	8.9	7.9 - 9.9	•
Ammonia	**9.2**	**8.2 - 10.2**	•
Ethanolamine	9.5	8.5 - 10.5	•
Carbonate (pK_2)	**10.3**	**9.3 - 11.3**	•
Diethylamine	10.5	9.5 - 11.5	•
Triethylamine	**11.0**	**10.0 - 12.0**	•
Piperidine	11.1	10.1 - 12.1	
Phosphate (pK_3)	12.3	11.3 - 13.3	

Sources: Practical HPLC Method Development; L.R. Snyder, JJ Kirkland, and JL Glajch. Wiley Interscience, 1997, and *Introduction to Protein and Peptide HPLC*; TP Bradshaw, Phenomenex, 1998.

† Common buffers are in bold.

‡ TFA can be used at low concentrations for LC/MS applications but can affect MS sensitivity.

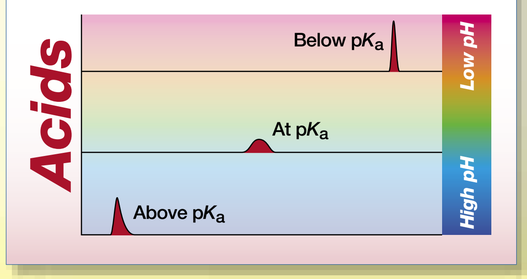
Retention of Different Functional Groups

The functionalities that analytes contain will affect retention on a reversed phase column. Whether a functionality is protonated or deprotonated will usually affect the chromatographic behavior of a compound; altering the pH of a separation will often change an elution profile. A general observation is that an acidic functionality will tend to have greater retention and efficiency at a pH below its pK_a value and less retention at a pH above its pK_a value. Basic compounds follow the opposite trend: basic functionalities are often less retained at pH's below their pK_a values and demonstrate greater retention and better peak shape at pH's above their pK_a values.

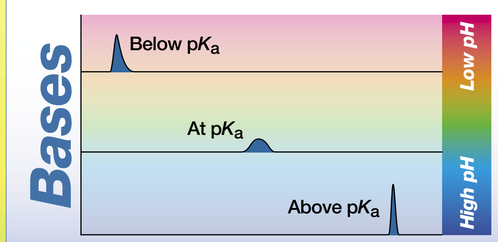
Protonated Functional Group	Deprotonated Functional Group	Approx. pK_a	
		1.5	Acids
		2.0	
		4.7	
		5.3	
		7.0	
		9.8	
		10.0	
		10.6	
		10.7	Bases

These values are approximate at best. Other nearby functionalities can DRAMATICALLY affect pK_a of functional groups.
Source: *Introduction to Organic Chemistry* - Streitwieser and Heathcock, MacMillan Publishing, 1981.

Effects of Mobile Phase pH on Acids



Effects of Mobile Phase pH on Bases



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