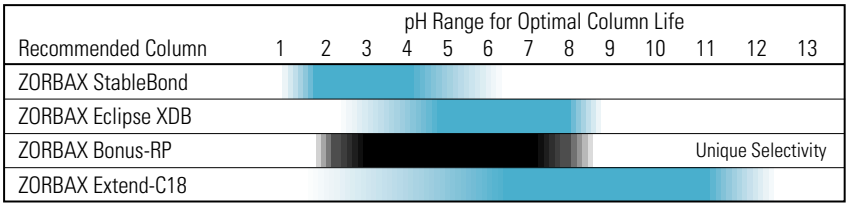
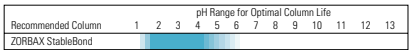
Agilent ZORBAX RP-HPLC Bonded Phases

Long Lifetime, Excellent Peak Shape, Superior Resolution from pH 1 - 12

Agilent ZORBAX reversed-phase bonded phases, based on Rx-SIL, provide the best peak shape and sample resolution as well as the longest column lifetimes for the low, mid and high pH regions. The retention of ionizable compounds changes with pH, therefore achieving optimum resolution for complex samples requires establishing methods at any pH from pH 1 - 12. Several types of bonded phases are required to cover this wide pH range and provide maximum column lifetime. The ZORBAX columns described below use unique bonding chemistry to increase the lifetime of the bonded phase and the silica support where needed, thereby providing "Resolution that Lasts" at every pH.

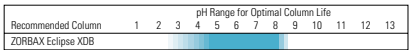


For longest lifetime using low pH mobile phase — Agilent ZORBAX StableBond

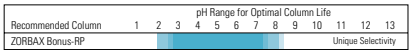


StableBond columns are made using bulky, patented, unique silanes that sterically protect the siloxane bond. Acid labile endcapping reagents are not used. The result is vastly improved column life and extraordinary chemical and temperature stability in the pH 1 - 6 range for a wide variety of phases (SB-C3, SB-CN, SB-Phenyl, SB-C8, and SB-C18). Excellent peak shape is expected for many basic compounds because of the ultra-pure Rx-SIL support.

For longest lifetime using mid-range pH mobile phase — Agilent ZORBAX Eclipse XDB or Bonus-RP

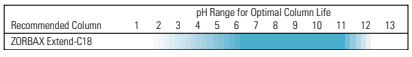


Eclipse XDB columns are best used in the pH range of 3 - 8 but can be used successfully over the extended pH range of 2 - 9. These columns provide excellent peak shape and high resolution for acidic, basic and neutral compounds. The densely-bonded, double-endcapped silica surface minimizes any silanol interactions and protects the silica support from dissolution.



Bonus-RP columns provide unique selectivity and applicability for mid pHs with an embedded amide linkage in a C14-alkyl chain. At pH 7 Bonus-RP provides excellent peak shape for even the most challenging basic compounds. Bonus-RP is protected from hydrolysis at low pH using the same sterically protecting bulky side chains as StableBond, and also is triple endcapped to minimize silanol interactions and improve stability at mid pH. Bonus-RP can be used without phase collapse in 100% aqueous mobile phases.

For longest lifetime using high pH mobile phase — Agilent ZORBAX Extend-C18



Extend-C18 columns are the newest addition to our product portfolio and incorporate a unique patented bidentate silane, combined with a double-endcapping process that protects the silica from dissolution at high pH — up to pH 11.5. Extend-C18 columns are best applied for separations of compounds that are either (1) basic and have little or no retention at low or intermediate pHs, (2) more stable or more soluble at high pH, or (3) basic and show poor peak shape at low or intermediate pH.

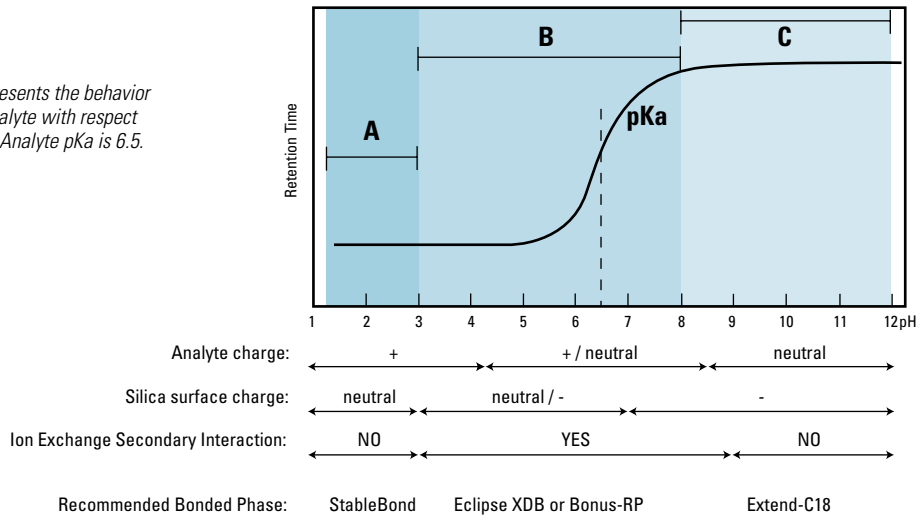
Method Development from pH 1 - 12

Chromatographic resolution between two or more peaks depends upon three factors — column efficiency, selectivity and retention. With ionizable analytes — bases and acids — all of these factors change dramatically with pH. For example retention can be improved by changing the separation pH, so that analytes are separated in their non-ionized form. Changes in mobile phase pH also improve column efficiency because the ionization of the analyte and the residual silanols can both be altered. This minimizes secondary interactions between analytes and the silica surface that cause poor peak shape. Achieving optimum resolution requires changing the mobile phase pH. The following method development strategy explains how this is done with superior column lifetime.

Low, mid and high pH are the three general regions for chromatographic separations as defined in Figure 1. This figure highlights the benefits of performing separations of ionizable analytes in each pH region. Method development proceeds by investigating chromatographic separations at low pH and then higher pH until optimum results are achieved. The ideal column is available for each pH region.

FIGURE 1
Three pH Regions for HPLC Separations of Basic Compounds

This figure represents the behavior of one basic analyte with respect to pKa and pH. Analyte pKa is 6.5.



Low pH 1-3 — Region A

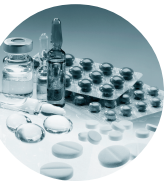
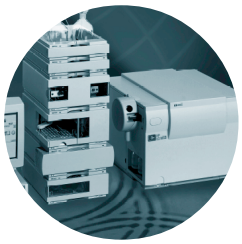
- Start method development at low pH, where silanols on a RP-HPLC column are protonated. This minimizes peak tailing by eliminating silanol/base interactions.
- At low pH basic compounds are positively charged, and their retention may be reduced.
- Acidic compounds may be protonated and have increased retention.
- Retention times are usually stable with small changes in pH, producing a robust method.
- Volatile mobile phase additives, such as formic acid or trifluoroacetic acid (TFA), are often used at low pH with LC/MS.

Mid pH 3 - 8 — Region B

- Develop methods at pH's at least 1 pH unit above or below the pKa to minimize changes in retention with small changes in pH .
- Some silica surface SiOH groups become SiO^- at pH 4 - 6, tailing interactions may be possible.
- Minimize interactions by selecting an endcapped column, using additives such as TEA (less desirable) or using "polar" bonded phases.
- As pH increases, silica dissolution is decreased by endcapping.
- Silica breakdown is prevented by innovative bonding chemistry and use of Rx-SIL.

High pH — Region C

- In this region, basic compounds may be in their free base form.
- Increased retention and resolution of basic compounds are likely.
- Retention changes little in this region - thus robust methods can be developed.
- Silica breakdown is prevented by innovative bidentate column chemistry, use of Rx-SIL and optimum mobile phase.
- Ammonium hydroxide is a volatile mobile phase buffer at high pH.


Method Development at Low pH is Preferred — StableBond Columns

StableBond — pH ≤ 3

With so many choices available, how do you select a column for method development? This is actually an easy decision. Most analytes today are acidic or basic - ionizable - and the best initial approach is to use a low pH mobile phase (Figure 1, Region A). Using a low pH mobile phase results in the best peak shape for basic compounds because ion-exchange interactions (BH^+ + SiOH ≠ secondary interaction) are eliminated, and many acidic compounds are protonated thereby maximizing analyte retention. Therefore, StableBond columns are a good first choice providing the longest lifetime and most robust separations at low pH.

For standard analytical work start method development with acetonitrile as the mobile phase organic modifier and 25 - 50 mM phosphate buffer (pH ≤ 3) as the aqueous component. This provides good pH control, necessary for the most reproducible analyses of ionizable compounds. StableBond SB-C8, or SB-C18 bonded-phases are usually chosen first because they effectively separate most compounds. Changes to the mobile phase (% organic, pH, ionic strength and organic modifier) and column temperature can be used to optimize the separation, as necessary. A unique feature of StableBond columns is the temperature stability. The temperature can be used as an additional tool to improve resolution.

StableBond Bonded-Phase Selectivity

The other StableBond phases — SB-CN, SB-C3, and SB-Phenyl — can be selected to improve sample resolution. All three of these bonded-phases are more polar than SB-C8 and SB-C18 and can change selectivity. Analysis time for non-polar compounds may also be reduced substantially while retention is maintained for polar compounds. In addition, the SB-CN bonded phase is an excellent choice when you have a mixture of polar and non-polar compounds; as it will reduce the retention of the non-polar compounds and possibly eliminate the need for a gradient separation. All of these short-chain bonded-phases provide the same exceptional stability as the SB-C8 and SB-C18 columns.

Method Development at Mid pH — Eclipse XDB and Bonus-RP Columns

Eclipse XDB — pH 7

If low pH is not desirable or acceptable for your separation due to poor analyte retention, solubility, selectivity or stability, then a mid-range pH, such as pH 7, should be investigated (Figure 1, Region B). This usually maximizes differences in retention and selectivity in comparison to low pH. The Eclipse XDB-C18 and C8 bonded-phases are normally the first choice for use at this pH because they provide high resolution and superior column lifetime. Phosphate buffer is normally the first choice for mobile phase modifier at pH 7 because its buffer range is pH 6.2 - 8.2. Phosphate buffers, although commonly used, should be used at temperatures lower than 40°C for maximum column life. A second choice for mid pH is acetate buffer since it buffers from pH 3.8 - 5.8 and is normally selected for LC/MS compatibility. Method development optimization can continue from here by changing the mobile phase, pH, and column temperature (up to 60°C) or by changing the bonded-phase to Eclipse XDB-Phenyl.

Bonus-RP — pH 7

For even more dramatic changes in selectivity or to improve the peak symmetry of basic compounds in the mid-pH range, try the Bonus-RP column. The Bonus-RP column exhibits a modified reversed-phase behavior. By choosing this column, the method development process will be the same as with the Eclipse XDB columns. You can select the same types of buffered mobile phases and use the same organic modifiers; you can even vary the temperature up to 60°C. In general the Bonus-RP is slightly less retentive than alkyl C8 columns because of the polar amine group embedded in the bonded-phase. The Bonus-RP column has enhanced stability down to pH 2, so it can also be selected to improve peak shape of difficult basic compounds that tail badly on many other alkyl type phases. Bonus-RP columns can be used with 100% aqueous mobile phases.

Method Development at High pH

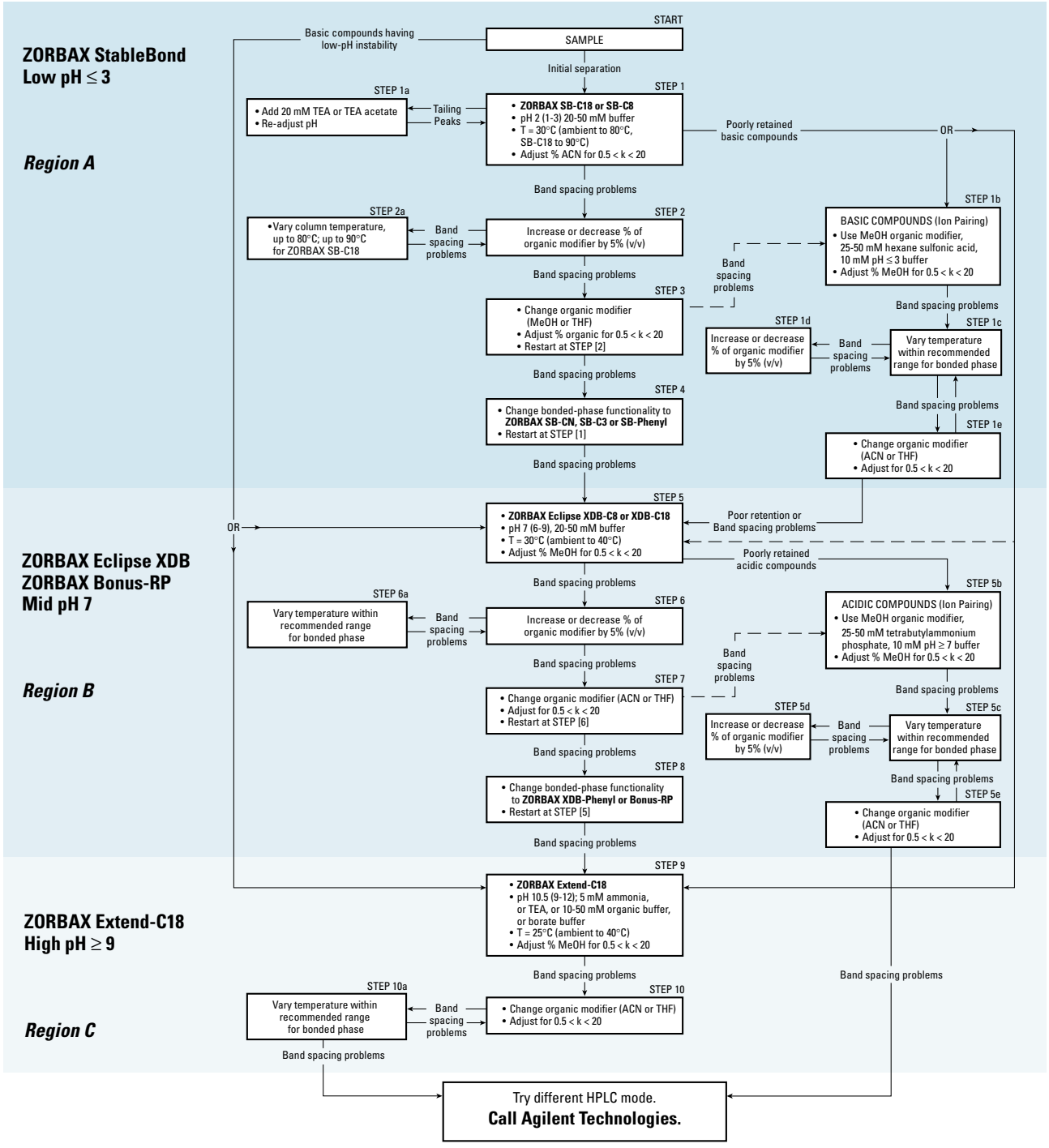
Extend-C18 Columns

At low or mid pHs some separations of basic compounds may still not have enough retention or the desired selectivity. For these samples high pH separations may be appropriate (Figure 1, Region C). At high pH, it may be possible to separate bases in their free base form - where they are not charged. Retention increases and the chance of obtaining the desired selectivity improves. The Extend-C18 column performs well with high pH mobile phases — up to pH 11.5. For many basic compounds with a pKa of 9 - 10, this extended pH range allows the separation to take place at least 1 pH unit above the analyte pKa, where they will be in their free base form. Method development using the Extend-C18 column is the same as on other columns — choose a buffered mobile phase appropriate for the pH range needed and an organic modifier. However, when using the Extend-C18 column organic buffers such as triethylamine, ammonium hydroxide, and pyrrolidine are needed for maximum column lifetime; phosphate buffer is not recommended. Methanol is the preferred organic modifier.

ZORBAX Columns Provide Resolution that Lasts

ZORBAX columns have been carefully designed for optimum sample resolution and longest column lifetimes for the low, mid, and high pH ranges. Following the approach described below simplifies method development using ZORBAX columns. You never have to take a column and make it work — your sample and mobile phases define the best column — so you can always achieve "Resolution that Lasts".

A Method Development Strategy that Optimizes Resolution and Column Life



Adapted and updated by R.D. Ricker, B.A. Bidlingmeyer and J.J. Kirkland from J.J. Kirkland, LC/GC, 14 (1996) 486.