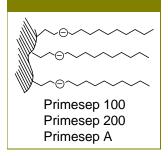
## Is There a Mystery in HPLC Separations?

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Figure 2: Simplified structure of Primesep® stationary phases. The ligand is attached to the silica support.



It is often difficult to analyze samples containing weak acidic, basic, and zwitterionic compounds in a single HPLC analysis. Charged functionalities act as an extreme case of polarity and therefore charged analytes are un-retained by hydrophobic interactions. Ionizable compounds are much more hydrophobic in their neutral state than their ionized form and can often be retained by reversed-phase in this state. This concept is frequently employed to retain carboxylic acids by reversedphase under acidic conditions (pH below 3) where they are neutral. However, for bases, the mobile phase pH must be above 11 for the compounds to be neutral and therefore hydrophobic enough for retention under reversed-phase conditions. Unfortunately, operation under these conditions was not practical until recent advances with pH stable hybrid particle technologies were made.

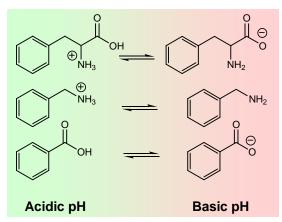
Although an excellent advance in column pH stability, hybrid particles have yet to solve the mystery of retaining polar acidic, basic, and zwitterionic compounds in a single analysis. In order to be retained by hydrophobic interaction, ionizable species must be in their neutral state. This can only occur at low pH for acids, and high pH for bases. There is no pH at which both acids and bases would be neutral. The zwitterions always have at least one charged group which makes them extremely polar (Figure 1). Therefore, as illustrated in Figure 3a, at

high pH, basic compounds (benzylamine) are retained by reversed-phase, while acidic compounds (benzoic acid) will be retained only at low pH (Figure 3b). The zwitterions will not be retained at any condition.

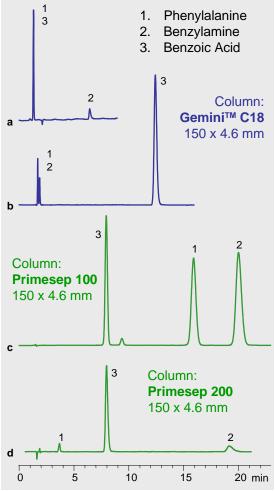
Our Primesep, mixed-mode technology incorporates both cation exchange and reversed-phase properties into a single ligand, as illustrated in Figure 2. Under acidic mobile phase (typical reversed-phase) conditions, acids will be neutral and retained as well. At this reversed-phase condition, bases and zwitterions will bear a (+) charge and can be efficiently retained by a cation-exchange mechanism (Figure 3cd). Table 1 summarizes the results of retention of polar compounds at various mobile phase pHs.

| <b>Table 1:</b> Retention of polar compounds at different pH |
|--|
| using special RP columns.                                    |

| Polar<br>compounds | Primesep® | High pH stable columns<br>( <b>Gemini™, Xterra</b> ®) |           |
|--------------------|-----------|---|-----------|
|                    | Acidic pH | Acidic pH   | Basic pH  |
| Basic              | Good      | No  | Good      |
|                    | retention | retention   | retention |
| Acidic             | Good      | Good  | No        |
|                    | retention | retention   | retention |
| Zwitterionic       | Good      | No  | No        |
|                    | retention | retention   | retention |



**Figure 1:** Charged states of different classes of compounds at acidic pH 2.0 and basic pH 12.0 conditions.



**Figure 3:** Separation of an acid, a base, and an amino acid at different pH with columns designed to retain polar compounds. Conditions: Flow rate: 1.0 mL/min, mobile phase: **a**. MeCN – 20%, TEA 20 mM, pH 12; **b,c** MeCN – 20%, H<sub>3</sub>PO<sub>4</sub> – 0.3%, pH 2; **d** MeCN – 20%, AmFm – 20 mM, pH 3.

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