Reversed phase HPLC analyses are predominantly performed on C18 columns, which, in many cases, are suitable. There are, however, situations in which a conventional C18 column produces less than optimal chromatography. For example, C18 columns have little retention for hydrophilic compounds, basic compounds often exhibit peak tailing, and highly aqueous conditions can cause inconsistent retention or even phase collapse.

One way in which column manufacturers attempt to address these issues, and yet maintain the favorable hydrophobic interaction of a C18 column, is to impart polar functionality into an alkyl phase. The Ultra IBD column is an example of such a polar embedded column. Compared to a C18 column, this column offers enhanced retention and selectivity towards a wider range of compounds, orthogonal separations, improved base-deactivation, and compatibility with entirely aqueous mobile phases.

**Degree of Polarity**
The Ultra IBD column exhibits a high degree of polarity relative to conventional and aqueous C18 phases. Because the Ultra IBD column possesses both nonpolar and highly polar characteristics, it can be used in both normal phase mode and reversed phase mode. The bonding chemistry used in the Ultra IBD column makes it a very adaptable column capable of unique separations.

**Base-Deactivation**
The Ultra IBD column bonding chemistry alleviates one of the common problems associated with alkyl phases—peak tailing of basic analytes. Comparing the analysis of amitriptyline on a conventional C18 column and an Ultra IBD column demonstrates the effectiveness of this bonding chemistry. Amitriptyline is a highly basic, tricyclic antidepressant that commonly tails on silica-based alkyl phases. Even at a neutral pH and, importantly, with no modifiers, the Ultra IBD column exhibits excellent peak shape for amitriptyline (Figure 1). This is advantageous because it provides needed flexibility for method development, especially for analytes that are labile under acidic conditions. In applications where Gaussian peak shape is needed for accurate integrations, such as potency assays, or when tighter system suitability criteria are required, an intrinsically base-deactivated stationary phase offers a benefit that a conventional C18 column cannot—exceptional peak shape with a simplified mobile phase.

**Retention and Selectivity**
In contrast to conventional C18 columns, the Ultra IBD has a polar functional group embedded within the alkyl chain. Retention, therefore, is attributed not only to hydrophobic interactions (the major retention mechanism of an alkyl (or C18) phase, but also to polar attraction between the analyte and stationary phase. This mixed-mode mechanism results in high retention for hydrophilic compounds or compounds with polar moieties, such as purines (Figure 2).

Orthogonal separations also can be achieved through the Ultra IBD phase chemistry. For example, a small group of hydroxybenzoic acids was also assayed on a C18 and IBD column under identical conditions. The elution order of the analytes differed and dihydroxybenzoic acid was more retained on the Ultra IBD column (Figure 3). Additionally, the unique phase chemistry of the Ultra IBD column makes it suitable for a simultaneous analysis of a wide range of compounds—acidic through basic, as well as zwitterions (Figure 4).
Figure 2 The Ultra IBD column exhibits high retention for hydrophilic compounds or compounds with polar moieties, and is compatible with up to 100% aqueous mobile phases ruggedness in aqueous mobile phases.

Figure 3 The Ultra IBD column gives needed flexibility for polar compounds; it increases retention, enhances resolution, and creates alternate selectivity.

Conventional C18

Peak List
1. 4-hydroxybenzoic acid
2. 2,5-dihydroxybenzoic acid
3. 3-hydroxybenzoic acid
* unknown peak

Sample:
Inj.: 20µL
Solvent: 20mM ammonium acetate, pH 5.8

Column: Ultra IBD
Cat.#: 9175565
Dimensions: 150mm x 4.6mm
Particle size: 5µm
Pore size: 100Å

Conditions:
Mobile phase: 20mM ammonium acetate, pH 5.8: methanol (97.5:2.5)
Flow: 1.0mL/min.
Temp.: 35°C
Det.: UV @ 260nm

Figure 4 The versatility of the Ultra IBD makes it well-suited for analyzing a wide range of compounds.

Sample List:
1. ATP 258
2. ADP 320
3. AMP 274
4. adenine 84
5. adenosine 254

Sample:
Inj.: 20µL
Solvent: 20mM ammonium acetate, pH 5.8

Column: Ultra IBD
Cat.#: 9175565
Dimensions: 150mm x 4.6mm
Particle size: 5µm
Pore size: 100Å

Conditions:
Mobile phase: 20mM ammonium acetate, pH 5.8: methanol (97.5:2.5)
Flow: 1.0mL/min.
Temp.: 35°C
Det.: UV @ 260nm

Conclusion
The Ultra IBD, through unique bonding chemistry, is an extremely versatile HPLC column. It offers alternate selectivity, and a high degree of both polar and nonpolar retention, making it a powerful tool for analyzing a wide range of compounds. The Ultra IBD also addresses the inherent problems attributed to linear alkyl phases, providing excellent peak shape for basic compounds and heightened retention of hydrophilic compounds. The versatility of the Ultra IBD makes it an excellent tool for the practicing method developer.