**INTRODUCTION**

Phenyl-type columns provide strong pi-pi interaction and offer selectivity complementary to other reversed-phase columns (e.g., C18). These columns have been successfully used to separate positional isomers, tocopherols, flavonoids, polynuclear aromatics and nitroaromatic compounds, active pharmaceutical ingredients (APIs) and related compounds. Early phenyl columns contained short alkyl phenyl ligands covalently bound to the silica surface. Later, diphenyl phases were developed to enhance pi-pi interaction. Due to the short alkyl link, these columns typically lack hydrophobic retention and exhibit low hydrolytic stability. To improve these features, phenyl columns with longer alkyl links were developed. By increasing the length of the alkyl spacer, steric selectivity and aromatic selectivity can be enhanced. Recently, a biphenyl phase was commercialized that exhibited both hydrophobic retention and pi-pi interaction compared to other commercially available phenyl columns. Here, the authors describe a newly developed phenyl column that features strong pi-pi interaction, high hydrophobicity, 100% aqueous compatibility, and unique selectivity compared to C18 and other phenyl-type columns in the market. Chromatographic evaluations and separations of aromatic hydrocarbons, steroids, fat-soluble vitamins, and phospholipids on this column are shown.

**DIONEX ACCLAIM PHENYL-1 OVERVIEW**

The Thermo Scientific Dionex Acclaim® Phenyl-1 column is based on covalent modification of high-purity, spherical, porous silica particles with a specially designed silane ligand bearing proprietary alkyl aromatic functionality. This novel column chemistry provides the following benefits.

- High aromatic selectivity
- High hydrophobic retention
- Unique and complementary selectivity
- Compatibility with highly aqueous mobile phase
- High efficiency and rugged packing

**CHROMATOGRAPHIC EVALUATIONS**

**High Aromatic Selectivity**

Triphenylene and o-terphenyl contain the same number of carbon atoms, but they are arranged in different shapes—the former planar, the latter propeller shaped. The retention factor ratio between triphenylene (T) and o-terphenyl (O), $\alpha_{(T/O)} = k'_{T}/k'_{O}$, is often used as a descriptor to measure column steric selectivity. However, with phenyl-type columns, this descriptor measures the aromatic selectivity rather than steric selectivity. Enhanced aromatic selectivity is beneficial for applications in drug development and testing where compounds commonly contain rings, conjugation byproducts, and ring substituents. In Figure 1, the Dionex Acclaim Phenyl-1 column demonstrates the highest $\alpha_{(T/O)}$ value compared to other commercial phenyl-type phases including biphenyl, diphenyl, C6-phenyl, and C3-phenyl phases.

![Figure 1. Aromatic selectivity comparison.](image-url)
High Hydrophobic Retention

Due to the incorporation of a long alkyl chain on the stationary phase, the Dionex Acclaim Phenyl-1 exhibits high hydrophobicity compared to other commercial phenyl-type phases, including biphenyl, diphenyl, C6-phenyl and C3-phenyl phases (Figure 2). This feature is desirable for retaining a wide range of analytes.

APPLICATIONS

Fullerenes

The increased usage of nanomaterials has triggered growing concern regarding their environmental behavior in aquatic environments. The ability to detect and quantify nanomaterials in complex water matrices has become an important issue. C60 fullerene is of special interest because of the widespread application of nanocarbon technology. Figure 5 shows separations of fullerenes in fullerene soot under isocratic conditions with heptane/isopropanol mobile phase using both a C18 column and the Dionex Acclaim Phenyl-1. Compared to the C18 column, the Dionex Acclaim Phenyl-1 exhibits different selectivity and provides better resolution for minor components.

Fully Compatible with Highly Aqueous Mobile Phase

Full compatibility with highly aqueous mobile phases is a desirable feature for HPLC columns. Figure 4 demonstrates that the Dionex Acclaim Phenyl-1 performs consistently well under 100% aqueous conditions.
Fat-Soluble Vitamins

Fat-soluble vitamins (A, D, E, and K) are essential nutrients with small amounts required for various roles in the human body. Fat-soluble vitamins are stored in the liver and fatty tissues, and are eliminated at a much slower rate. As a result, they can pose a greater risk for toxicity than water-soluble vitamins when consumed in excess. Conversely, some health problems may decrease the absorption of fat, and in turn, decrease the absorption of vitamins A, D, E, and K. Analyses of fat-soluble vitamins are important and challenging assays for various products like pharmaceuticals, foods, and nutritional supplements. As shown in Figure 8, the Dionex Acclaim Phenyl-1 column provides excellent selectivity for separating vitamins A, D2, D3, K1, K2, as well as E, E acetate and related substances, δ- and γ-tocopherols.

Figure 8. Separation of fat-soluble vitamins.
**Estrogens**

Estrogens are a group of steroid compounds that function as the primary female sex hormone. Three major naturally occurring estrogens in women are estrone (E1), estradiol (E2), and estriol (E3). Estradiol (E2) is the predominant form in nonpregnant females, estrone is produced during menopause, and estriol is the primary estrogen of pregnancy. Ethynylestradiol, a derivative of estradiol, is an orally bioactive estrogen used in almost all modern formulations of combined oral contraceptive pills. While the standard C18 column fails to separate these four compounds, the Dionex Acclaim Phenyl-1 column can resolve them to baseline isocratically (Figure 9).

**Glucocorticosteroids**

Glucocorticosteroids are a group of naturally occurring and synthetic hormones that moderate inflammation and other stress responses.\(^1\) All glucocorticosteroids are on the World Anti-Doping Agency's 2005 list of substances prohibited in competition when administered orally, rectally, intravenously, or intramuscularly.\(^2\) Separation of these substances can be difficult. The unique chemistry of the Dionex Acclaim Phenyl-1 provides superior selectivity to conventional C18 columns for the separation of glucocorticosteroids. As shown in Figure 10, eight glucocorticosteroid compounds are resolved to baseline on a 3 × 250 mm Dionex Acclaim Phenyl column using a methanol/water mobile phase.

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**CONCLUDING REMARKS**

The Acclaim Phenyl-1 column provides unique selectivity compared to C18 and other phenyl-type reversed-phase columns. The combination of high aromatic selectivity, high hydrophobicity, 100% aqueous compatibility and robust column packing makes it useful for a wide range of applications, such as fullerenes, lipids, fat-soluble vitamins, steroids, and many more.

**REFERENCES**


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