

Is HILIC the Best Way for Determination of Active Pharmaceutical Ingredients and Counterions?

Xiaodong Liu and Christopher Pohl
Dionex Corporation, Sunnyvale, CA, USA

Now sold under the
Thermo Scientific brand

Thermo
SCIENTIFIC

INTRODUCTION

Determinations of active pharmaceutical ingredients (APIs) and counterions are important assays in pharmaceutical drug development. Due to the wide variety of ionizable substituents and hydrophobicities of these pharmaceutical-related molecules, it is challenging to simultaneously separate APIs and respective counterions on a reversed-phase column. This is because many counterions, such as sodium and chloride ions, cannot be retained on these columns without the use of ion-pair reagents.

Hydrophilic interaction liquid chromatography (HILIC) is a technique that is complementary to reversed-phase liquid chromatography (RPLC) with several benefits. These include retention of polar analytes that cannot be retained on an RP column, improved compatibility with electrospray LC/MS for good sensitivity, and simplification of sample preparation by eliminating the need for evaporation and reconstitution of a sample when dissolved in a nonaqueous solvent. Consequently, HILIC has been used for analyzing simultaneous determination of APIs and counterions. However, despite its suitability to separate highly polar molecules, HILIC fails to adequately retain more strongly hydrophobic APIs. Moreover, HILIC requires an organic solvent-rich mobile phase, which is undesirable from cost and environmental-sustainability standpoints. While HILIC is often useful for hydrophilicity-based class separation, its flexibility in method development is limited.

Here, the authors present an alternative to HILIC for API and counterion assays, which addresses aforementioned issues with HILIC. The proposed approach involves a mixed-mode chromatography column—the Acclaim[®] Trinity[™] P1, which provides cation-exchange, anion-exchange, and reversed-phase interactions. The new approach is suited to the simultaneous determination of APIs and counterions regardless of their hydrophobicity, provides better control of selectivity in method development, and does not require a high concentration of organic solvent in the mobile phase. In all pharmaceutical applications described here, a popular and heavily promoted HILIC column—ZIC[®]-HILIC—was used for comparison purpose.

EXPERIMENTAL

HPLC columns used in this study were Acclaim Trinity P1 columns in both 3 × 50 mm and 3 × 100 mm, 3 μm formats (Dionex, Sunnyvale, CA, USA) and a 4.6 × 150 mm, 5 μm, 200 Å, ZIC-HILIC column (SeQuant, Umeå, Sweden).

Separations were performed on a modular UltiMate[®] 3000 Rapid Separation LC (RSLC) system (Dionex) equipped with an LPG 3400RS

Quaternary RS Pump, WPS-3000TRS RS Thermostatted Autosampler, TCC-3000RS Thermostatted Column Compartment RS, and a DAD-3000RS RS Diode Array Detector. For detection of nonvolatile analytes, an NQAD detector (Quant Technologies, Blaine, Minnesota, USA) and a Corona[®] *ultra*[™] Charged Aerosol Detector (Dionex) were used. Chromeleon[®] 6.70 Chromatography Data System software (Dionex) was used for system control and data processing.

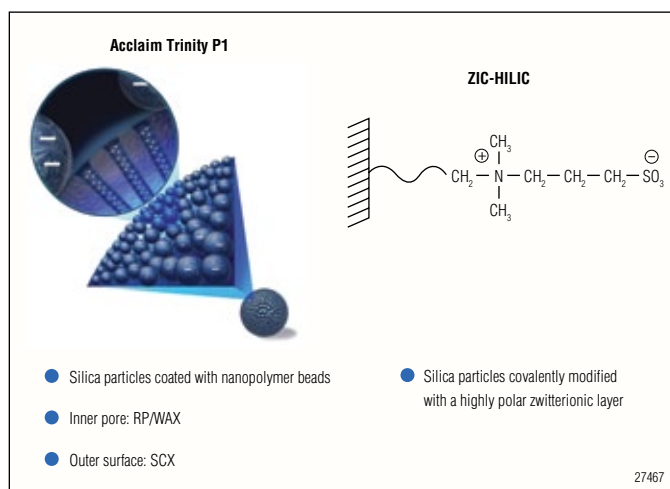


Figure 1. Column chemistry: Acclaim Trinity P1 vs ZIC-HILIC columns.

HPLC-grade acetonitrile was obtained from Burdick and Jackson (Muskegon, MI, USA) and Fisher Scientific (Pittsburgh, PA, USA), respectively. Deionized water (>18 MΩ-cm) was purified by a Milli-Q[®] water purification system (Millipore, Bedford, MA, USA). All chemicals and standards were purchased from Sigma-Aldrich (St. Louis, MO, USA).

RESULT AND DISCUSSION

Column Chemistry Comparison

The Acclaim Trinity P1 is a novel reversed-phase/anion-exchange/cation-exchange, trimodal, silica-based, stationary phase column developed using Nanopolymer Silica Hybrid (NSH) technology. The new material consists of high-purity porous spherical silica particles coated with nanopolymer beads using an electrostatically driven self-assembly process that results in a distinctive spatial separation of the anion-exchange and cation-exchange regions, and allows both retention mechanisms to function simultaneously and be controlled independently.

The ZIC-HILIC is a highly polar, zwitterionic, silica-based, stationary phase column, specially designed for separating polar analytes under HILIC conditions. Because of its zwitterionic nature, selectivity cannot be controlled by adjusting the pH, and it retains ions by salt exchange rather than ion exchange. Consequently, this phase fails to provide controllable selectivity for both anions and cations. Moreover, the low hydrophobicity prevents it from retaining less hydrophilic molecules.

Pharmaceutical Counterions

Figure 2A demonstrates that the Acclaim Trinity P1 column provides ideal selectivity for separating pharmaceutical counterions (including both cations and anions) using acetonitrile/ammonium acetate mobile phase and a Corona *ultra* detector. Baseline separation of five cations and five anions is achieved within a single run. The column selectivity is designed such that cations elute before anions. This is the first and only column available that separates both cations and anions easily and reliably.

The same application was tested using a ZIC-HILIC column. To obtain the best possible chromatographic condition, various mobile phase acetonitrile contents (20 to 80% in 10% intervals) and buffer concentrations (10, 15, and 20 mM) were tested. The optimal separation was achieved at 80% acetonitrile with 15 mM ammonium acetate (shown in Figure 2B). However, compared to the Trinity P1 column, the ZIC-HILIC column provides inferior separation, consumes more organic solvent, and requires longer analysis time. In addition, the ZIC-HILIC exhibited a level of column bleed that was too high to be compatible with the Corona *ultra* detector. Therefore, an NQAD detector was used for the remainder of this study to provide fair comparisons.

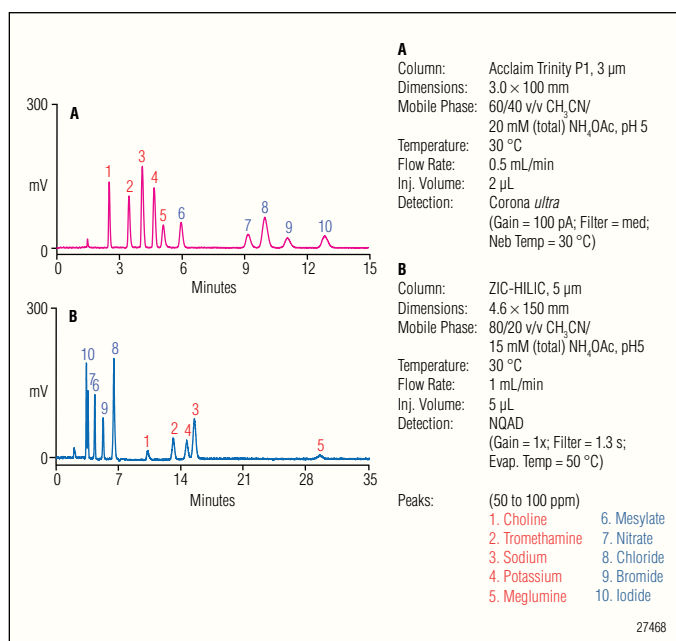


Figure 2. Simultaneous separation of pharmaceutical counterions.

Hydrophilic Acidic API and Counterion

Penicillin G is an antibiotic compound belonging to the β -lactam antibiotic family; it is often formulated as a potassium salt. Because of the highly hydrophilic nature of both the API and the counterion, it is impossible to assay both components within the same analysis on any RP column. The Trinity P1 column provides baseline separation of both penicillin G and K⁺ ions in either RP/IEX mode or HILIC mode, with excellent peak shape and adequate retention (Figures 3A and 3B). Note that elution order can be changed by adjusting acetonitrile content in the mobile phase. By comparison, the ZIC-HILIC column fails to provide adequate retention (k' = 0.5) for this drug molecule under HILIC conditions, while the retention of the counterion (K⁺) is excessively long (k' = 15.0), as shown in Figure 3C

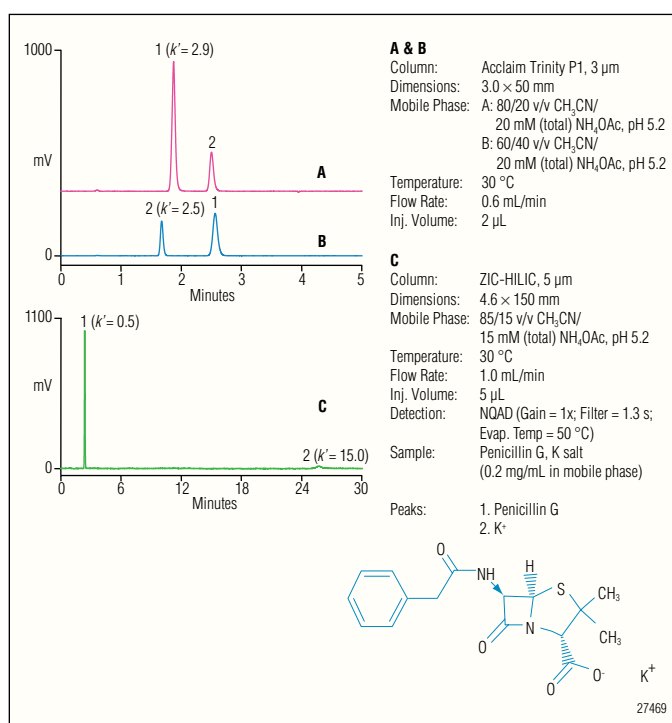


Figure 3. Hydrophilic acidic API and counterion (penicillin G potassium).

2 Is HILIC the Best Way for Determination of Active Pharmaceutical Ingredients and Counterions?

Hydrophilic Basic API and Counterion

1,1-Dimethylbiguanide hydrochloride (metformin), a highly hydrophilic basic drug formulated in chloride salt, is an antidiabetic agent that reduces blood glucose levels and improves insulin sensitivity. Figure 4A illustrates separations of the API and its counterion using the Trinity P1 column with a concentration of 30% acetonitrile in the mobile phase. Due to both the hydrophilic nature of the analytes and the multiple retention mechanisms provided by the column, baseline separations can be achieved in both RP/IEC mode and HILIC mode (not shown) with good peak shape and adequate retention. As shown in Figure 4B, the ZIC-HILIC column also performs well for this application under HILIC conditions, but not under RPLC conditions (not shown).

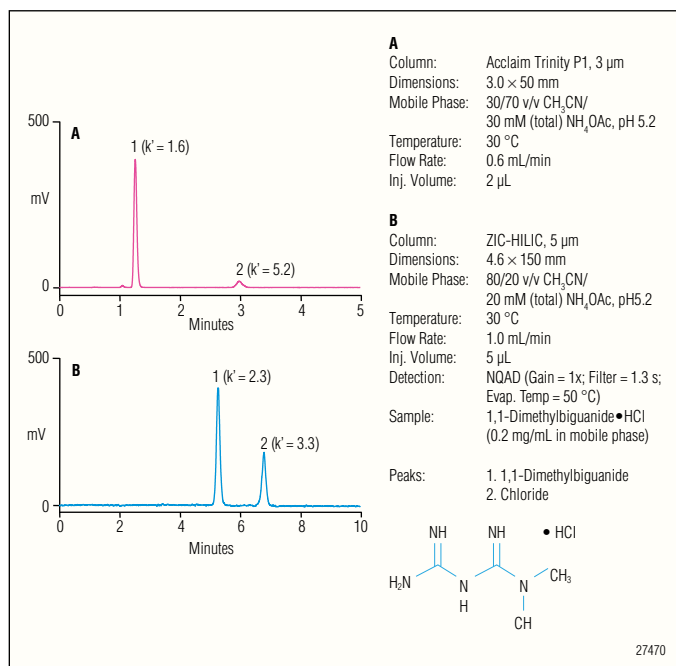


Figure 4. Hydrophilic basic API and counterion (metformin).

Hydrophobic Acidic API and Counterion

Naproxen, often formulated in the sodium form, is a nonsteroidal antiinflammatory drug (NSAID) commonly used for the reduction of moderate to severe pain, fever, and inflammation. While the API is a highly hydrophobic acidic molecule, the counterion Na⁺ cannot be retained on any RP columns. Because of the coexistence of anion-exchange and cation-exchange properties on the Trinity P1 column, both hydrophobic API and hydrophilic Na⁺ ion can be retained and separated using a 50 mm Trinity P1 column with excellent resolution, peak shape, and retention ($5 > k' > 2$) in merely 3 min (Figure 5A). The ZIC-HILIC column fails to provide adequate retention for naproxen under either HILIC (70 to 90% acetonitrile, results using 85% acetonitrile concentration, as shown in Figure 5B) or RPLC (10% acetonitrile, not shown).

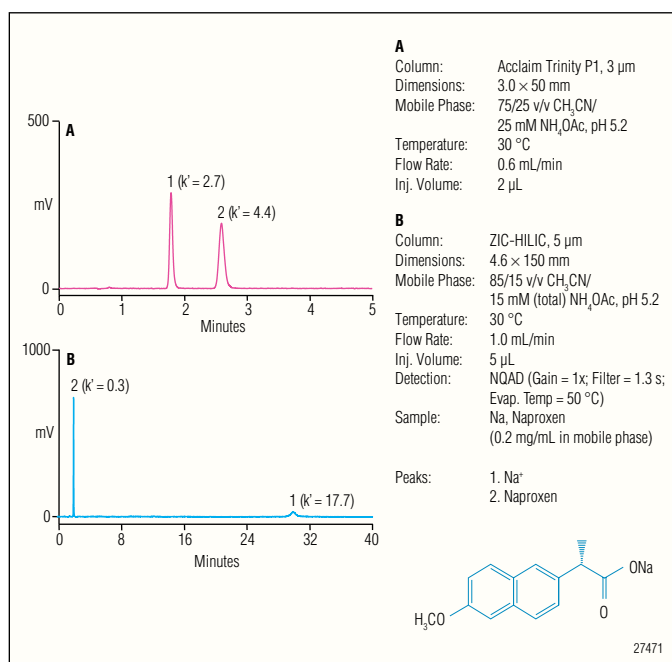


Figure 5. Hydrophobic acidic API and counterion (naproxen sodium salt).

Table 1. Comparison of Acclaim Trinity P1 and ZIC-HILIC for API and Counterion Analysis

Applications	Acclaim Trinity P1	ZIC-HILIC	Comments
Pharmaceutical counterions	✓	?	Acclaim Trinity P1 provides better selectivity (Figure 2)
Hydrophilic acidic API & counterion	✓	?	ZIC-HILIC fails to give adequate retention for the API (Figure 3)
Hydrophilic basic API & counterion	✓	✓	Both columns perform well (Figure 4)
Hydrophobic acidic API & counterion	✓	x	ZIC-HILIC fails to give adequate retention for the API (Figure 5)
Hydrophobic basic API & counterion	✓	x	ZIC-HILIC fails to give adequate retention for the API (Figure 6)

✓ - Suitable; ? - Marginal; x - Not suitable

Hydrophobic Basic API and Counterion

Trimipramine is a tricyclic antidepressant (TCA) often formulated as a maleate salt. It has antidepressant, anxiolytic, antipsychotic, sedative, and analgesic effects. While trimipramine is a highly hydrophobic, basic compound, its counterion maleate is very hydrophilic. The ZIC-HILIC column cannot provide adequate retention for the API under either HILIC (Figures 6B and 6C) or RPLC (not shown) conditions. By comparison, the Trinity P1 column demonstrates excellent separation capability, providing baseline resolution and fast analysis (less than 3 min run time) at 70% acetonitrile level (Figure 6A).

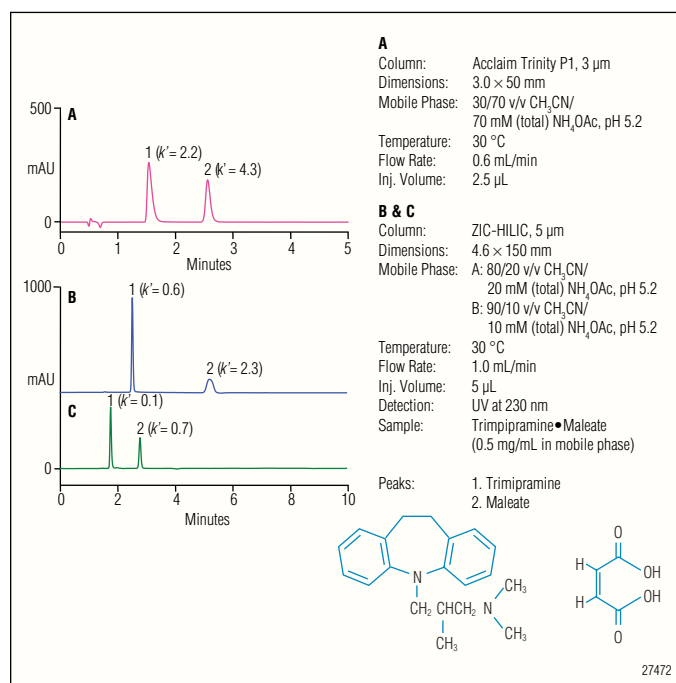


Figure 6. Hydrophobic basic API and counterion (trimipramine maleate).

CONCLUDING REMARKS

The Acclaim Trinity P1 column demonstrates superior separation to the ZIC-HILIC column for the analysis of APIs and their respective counterions (Table 1). Because of its novel column chemistry and unique selectivity, the Acclaim Trinity P1 allows excellent separation, faster analysis, and less organic solvent consumption (greener chromatographic methods).

REFERENCE

1. Dionex Acclaim Trinity P1 LC Column. <http://www.dionex.com/en-us/products/columns/lc/specialty/acclaim-trinity/lp-81754.html> (accessed March 21, 2011).
2. Dionex Acclaim Trinity P1 LC Column data sheet. <http://www.dionex.com/en-us/webdocs/70761-DS-Acclaim-Trinity-12Feb2010-LPN2239-02.pdf> (accessed March 21, 2011).
3. Liu, X.; Pohl, C. HILIC Behavior of a Reversed-Phase/Cation-Exchange/Anion-Exchange Trimode Column. *J. Sep. Sci.* **2010**, *33*, 779–786.
4. Zhang, K.; Dai, L.; Chetwyn, N. Simultaneous Determination of Positive and Negative Pharmaceutical Counterions Using Mixed-Mode Chromatography Coupled with Charged Aerosol Detector. *J. Chromatogr., A* **2010**, *1217*, 5776–5784.

Acclaim, Chromleon, Corona, and UltiMate are registered trademarks, and *ultra* and Trinity are trademarks of Dionex Corporation. All third party trademarks are the property of their respective owners.

Passion. Power. Productivity.



Dionex Corporation

1228 Titan Way
 P.O. Box 3603
 Sunnyvale, CA
 94088-3603
 (408) 737-0700

North America

U.S. / Canada (847) 295-7500

South America

Brazil (55) 11 3731 5140

Europe

Austria (43) 1 616 51 25 Benelux (31) 20 683 9768; (32) 3 353 4294
 Denmark (45) 36 36 90 90 France (33) 1 39 30 01 10 Germany (49) 6126 991 0
 Ireland (353) 1 644 0064 Italy (39) 02 51 62 1267 Sweden (46) 8 473 3380
 Switzerland (41) 62 205 9966 United Kingdom (44) 1276 691722

Asia Pacific

Australia (61) 2 9420 5233 China (852) 2428 3282 India (91) 22 2764 2735
 Japan (81) 6 6885 1213 Korea (82) 2 2653 2580 Singapore (65) 6289 1190
 Taiwan (886) 2 8751 6655

www.dionex.com



LPN 2731-01 3/11
 ©2011 Dionex Corporation