A Critical Comparison of Different Reversed-Phase/ Cation-Exchange/Anion-Exchange Trimodal Stationary Phases

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ABSTRACT

Because of the complexity and variety of HPLC analytes in terms of hydrophilicity and ionization, a stationary phase capable of retaining and selecting between neutral and charged species at the same time would be highly desirable. This necessitates trimodal stationary phases that possess reversed-phase (RP), anion-exchange (AEX), and cation-exchange (CEX) interactions. The current study compares three commercially available RP/AEX/CEX trimodal columns with different chemistry designs. Detailed chromatography comparisons are made with respect to hydrophobicity, anion-exchange capacity, cation-exchange capacity, and selectivity. In addition, the retention behavior dependency on organic solvent, buffer concentration, and pH will be presented. The suitability for analysis of active pharmaceutical ingredients (APIs) and counterions will also be discussed.

INTRODUCTION

While most HPLC applications involving small molecules are developed on reversed-phase columns such as C18, C8, phenyl, polar-embedded phases, and others, the selectivity options are rather limited. Mixed-mode chromatography provides a viable solution to the selectivity challenge. Mixed-mode chromatography combines both reversed-phase (RP) and ion-exchange (IEX) retention mechanisms. The biggest benefit of mixed-mode columns is that the selectivity can be optimized by adjusting mobile phase ionic strength, pH, and/or organic solvent. As a result, not only are these columns complementary to RP columns, but also self complementary under different chromatographic conditions. The presence of both RP and IEX functionalities eliminates the need for ion-pairing agents in the mobile phase for separation of highly hydrophilic charged analytes, which simplifies the mobile phase, making it compatible with MS. With adjustable selectivity, it is also possible to separate analytes with dramatically different hydrophobicity and charge state, such as simultaneous separation of active pharmaceutical ingredient (API) and corresponding counterion, in a single analysis.

Salt formation is important in drug development to improve biopharmaceutical and physicochemical properties of the drug. Approximately 50% of all drug molecules are administered as salts. For the assay of counterions by liquid chromatography, anions and cations need to be analyzed separately using different methods, different separation columns, and very often, different instruments. Similarly, in pharmaceutical analysis, both API and counterion are often analyzed using different methods, different separation columns, and different instruments. Moreover, many medicines contain neutral drugs as well as acidic and basic ones with respective counterions. Thus, simultaneous determination of all APIs and counterions is even more challenging. Because of the complexity and variety of HPLC analytes in terms of hydrophilicity and ionization, a stationary phase capable of retaining and selecting between neutral and charged species at the same time is highly desirable.

This study compares three commercially available RP/AEX/CEX trimodal columns with different chemistry designs. The detailed chromatography comparisons are made with respects to hydrophobicity, anion-exchange capacity, cation-exchange capacity, selectivity, and other parameters. In addition, the retention behavior dependencies on organic solvent, buffer concentration, and pH are compared. Their suitability for analysis of active pharmaceutical ingredients (APIs) and corresponding counterions is also discussed.

EXPERIMENTAL

Separation Columns

Dionex Acclaim® Trinity™ P1, 3 µm, 3.0 × 100 mm (Thermo Scientific)
Obelisc R, 5 µm, 3.2 × 100 mm (SIELC Technologies)
Scherzo SM-C18, 3 µm, 3.0 × 100 mm (Imtakt Corporation)

Instrumentation

Dionex UltiMate® 3000 HPLC (Thermo Scientific) with a PDA in series with an ESA Corona® ultra™ detector (Thermo Scientific) or Sedex 85 ELS detector (Sedere)

Mobile Phases

Acetonitrile /ammonium acetate buffer system, pH adjusted with acetic acid

Test Probes (Figure 1)

Phenanthrene and dimethylphthalate (reversed-phase retention)
Uracil (HILIC retention)
NaCl (Na^+ for cation exchange and Cl^- for anion exchange)

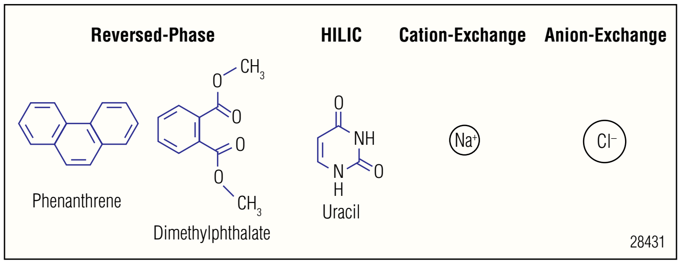


Figure 1. Test probes.

RESULTS AND DISCUSSION

Column Chemistry

In this study, three commercially available columns that have anion-exchange, cation-exchange, and reversed-phase functional groups were evaluated (Figure 2). The first type (Scherzo SM-C18 column) uses uniformly blended packing material consisting of two types of porous silica particles: silica modified with C18 and cation-exchange silyl ligands, and silica modified with C18 and anion-exchange silyl ligands. The second type (Obelisc R column) is based on silica particles covalently functionalized with a silane containing reversed-phase, cationic, and anionic groups on the same ligand. The cationic group is close to the silica surface, separated from the anionic group by a hydrophobic chain such that each ligand forms a ring (contrary to what is indicated in Figure 2). The third trimodal phase, the Dionex Acclaim Trinity P1 column, prepared from an electrostatically driven self-assembly process, consists of high-purity porous spherical silica particles whose inner-pore area is covalently modified with silyl ligands containing both RP and anion-exchange moieties, while the outer surface is coated with negatively charged nano-polymer beads by electrostatic interactions. This chemistry design creates a distinctive spatial separation of the anion-exchange and cation-exchange regions, and allows reversed-phase, cation-exchange, and anion-exchange retention mechanisms to function simultaneously and be controlled independently.

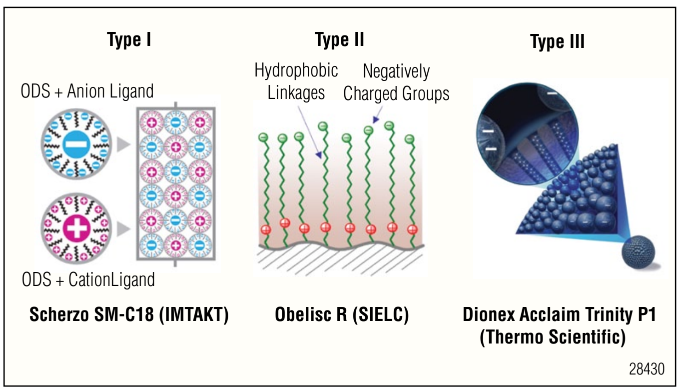


Figure 2. Different chemistry of trimode columns.

Chromatographic Evaluation

Organic Solvent Effect

As shown in Figure 3A, the neutral molecule (dimethylphthalate) is retained solely by RP mechanism—higher organic solvent resulting in lower retention—on all three phases. The Scherzo SM-C18 column provides significantly higher RP retention than the other two phases.

The data on uracil (Figure 3B) suggests that all three phases exhibit slight HILIC behavior at high organic solvent levels, and the Dionex Acclaim Trinity P1 column demonstrates somewhat higher HILIC characteristic. As for Na^+ ion (Figure 3C), from 10 to 70% acetonitrile, the Acclaim Trinity P1 column shows highest cation-exchange retention, the Scherzo SM-C18 exhibits virtually no retention, and the Obelisc R is somewhere in between. Above 70% solvent, retention of Na^+ ion increases with solvent content on all three phases, especially for Obelisc R on which the Na^+ retention rapidly surpasses that on the Acclaim Trinity P1 column. Figure 3D illustrates the solvent effect on retention of Cl^-. We can see that at any solvent level, the Acclaim Trinity P1 column has much higher anion-exchange interaction, while the ScherzoSM-C18 column shows very weak anion-exchange capacity. It is interesting to see that for the Acclaim Trinity P1 and Obelisc phases, retention of Cl^- decreases with solvent content in the range between 10% and 80%. Beyond 80% acetonitrile, retention of Cl^- instead increases with the solvent content.

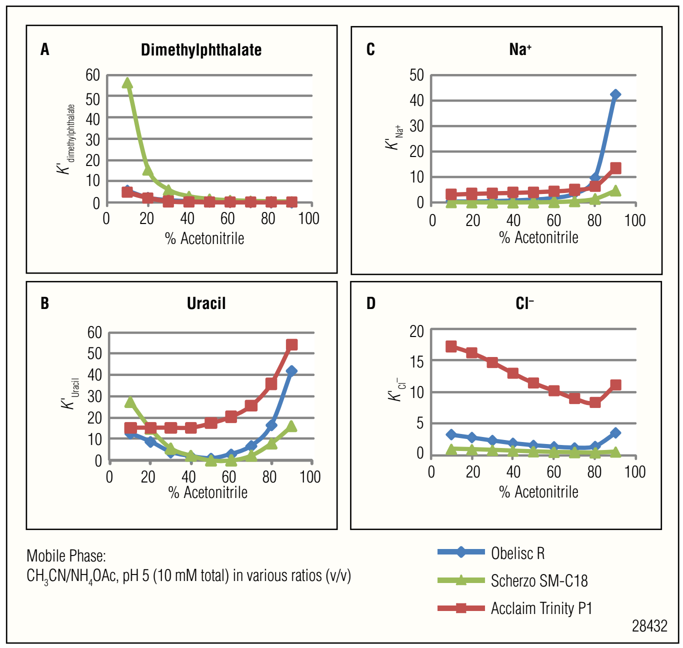


Figure 3. Retention vs organic solvent.

Buffer Concentration Effect

Figure 4 shows the retention dependency on buffer concentration. As expected, the neutral molecule is virtually unaffected by buffer concentration. Once again, Scherzo SM-C18 exhibits significantly higher RP retention than the other two phases. Obelisc R offers slightly higher RP retention compared to the Acclaim Trinity P1 column (Figure 4A). As for both Na^+ and Cl^-, retentions decrease as buffer concentration increases, typical of cation-exchange and anion-exchange mechanisms (Figures 4B and 4C). The Acclaim Trinity P1 column provides significantly higher ion-exchange retention while the Scherzo SM-C18 column shows virtually no ion-exchange capacity and the Obelisc R column quite weak ion-exchange capacity.

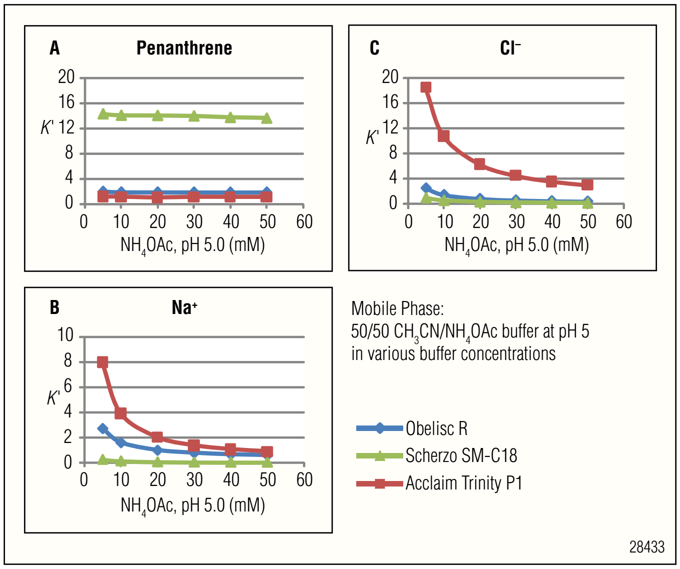


Figure 4. Retention vs buffer concentration.

pH Effect

When tested at various pH levels while the rest of the parameters were kept constant, all three columns show virtually no change in retention for the neutral molecule (phenanthrene) (Figure 5A). However, with increased pH, Na^+ retention remains constant on the Acclaim Trinity P1 column and increases on the Obelisc R column, while there is virtually no meaningful Na^+ retention on the Scherzo SM-C18 at any pH (Figure 5B). The reason is postulated as follows: the Acclaim Trinity P1 has a distinctive spatial separation between the anion-exchange and cation-exchange regions, thus cation-exchange retention is governed by nano-polymer beads coated on the outer-surface and is not affected by the surface silanol groups. Because the buffer concentration is normalized to $[NH_4^+]$ and remains virtually constant, the Na^+ retention is unaffected by the pH. The situation is different for the Obelisc R. Since both anion-exchange and cation-exchange functional groups are connected with a flexible alkyl chain, the bonded phase is zwitterionic and its net charge or ion-exchange capacity is pH dependent. In addition, the charge of surface silanol groups increase with pH, and they will neutralize the anion-exchange capacity or enhance cation-exchange capacity.

By comparison, Scherzo SM-C18 doesn't provide any meaningful cation-exchange interaction. As shown in Figure 5C, the retention of Cl^- is pH dependent on all three phases: Cl^- retentions decrease with pH increase. This is because anion-exchange retention is provided by the silyl ligand bonded to the close proximity of the silica surface, thus heavily influenced by the charge state of silanols which is determined by the local pH.

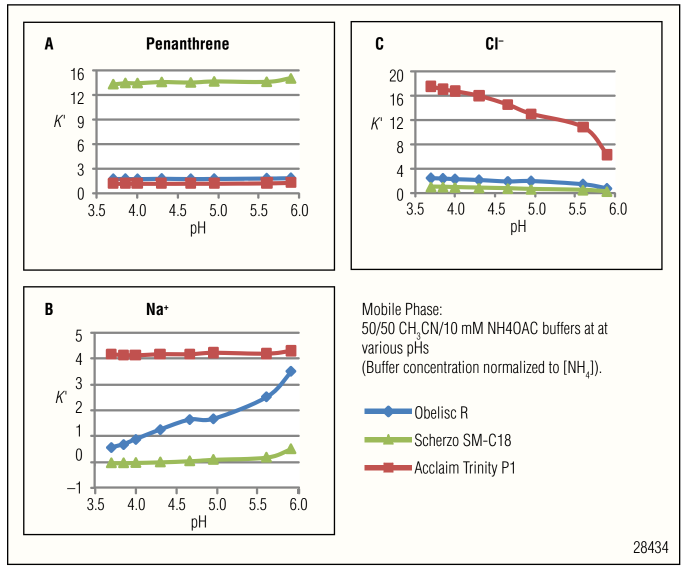


Figure 5. Retention vs pH.

Comments on Selectivity

Figure 6 illustrates the comparison among three different trimode phases with respect to reversed-phase, cation-exchange, and anion-exchange retention. Scherzo SM-C18 exhibits the highest RP retention (expressed in blue bar)—more than 7 times and 12 times higher compared to the Obelisc R and the Acclaim Trinity P1, respectively. Meanwhile, both cation-exchange capacity (represented in red) and anion-exchange capacity (represented in green) follow the order of Acclaim Trinity P1 > Obelisc R > Scherzo SM-C18. Different combinations of RP, CEX, and IEX capacities result in different selectivity.

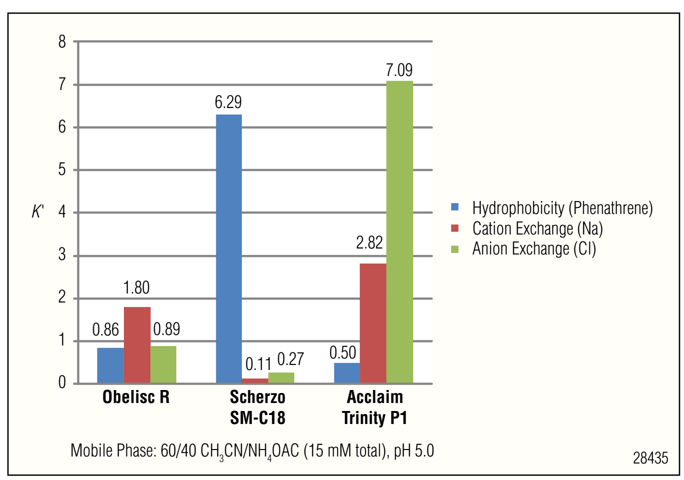


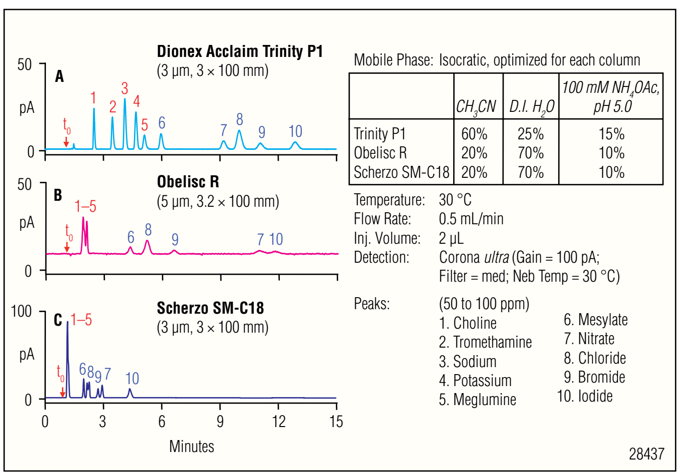
Figure 6. Reversed-phase and ion-exchange comparison.

The Acclaim Trinity P1 provides strong anion-exchange, substantial cation-exchange, and rather weak RP retention. Thus it can be characterized as an RP-modified cation- and anion-exchange phase. Scherzo SM-C18 shows strong RP retention, but very little cation-exchange and anion-exchange capacity. Thus it can be viewed as an ion-exchange-modified RP material. The RP and IEX retention of Obelisc R is somewhere between the Acclaim Trinity P1 and Scherzo SM-C18, clearly a different type of trimode phase.

Applications

Simultaneous Separation of Pharmaceutical Counterions

Salt formation is a critical step in drug development because it provides improved biopharmaceutical and physicochemical properties, ease of purification and handling. A broad selection of inorganic and organic ions can be used as pharmaceutical counterions. The most commonly used counterions are sodium and chloride ions. No reversed-phase columns can retain or separate either sodium or chloride. It is highly desirable to separate both pharmaceutically important anions and cations within the same analysis and in a reasonable amount of time. As shown in Figure 7A, the Acclaim Trinity P1 column provides baseline separation of 10 commonly used pharmaceutical counterions (five cations and five anions) in a single analysis. Under optimized conditions, Obelisc R can separate five anions, but all monovalent cations co-elute close to the void (Figure 7B). By comparison, Scherzo SM-C18 is clearly unsuitable for this application, most likely limited by its column chemistry (Figure 7C).



Mobile Phase: Isocratic, optimized for each column

	CH_3CN	D.I. H_2O	100 mM NH_4OAc, pH 5.0
Trinity P1	60%	25%	15%
Obelisc R	20%	70%	10%
Scherzo SM-C18	20%	70%	10%

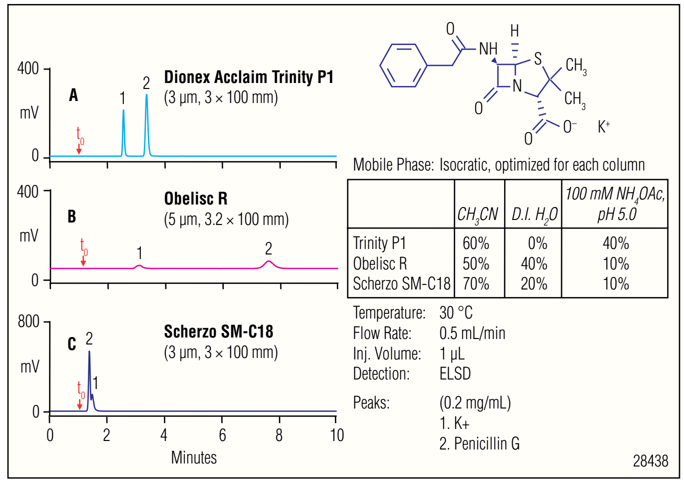
Temperature: 30 °C
Flow Rate: 0.5 mL/min
Inj. Volume: 2 µL
Detection: Corona *ultra* (Gain = 100 pA; Filter = med; Neb Temp = 30 °C)

Peaks: (50 to 100 ppm)
1. Choline
2. Tromethamine
3. Sodium
4. Potassium
5. Meglumine
6. Mesylate
7. Nitrate
8. Chloride
9. Bromide
10. Iodide

Figure 7. Pharmaceutical counter cations and anions.

Acidic API and Counterion

Penicillin G is an antibiotic compound and is often formulated in the potassium salt form. Because of the highly hydrophilic nature of both API and counterion, it is impossible to assay both components within the same analysis on any RP column. As shown in Figure 8, both the Acclaim Trinity P1 and Obelisc R columns are capable of providing adequate retention and resolution, but the Scherzo SM-C18 fails to provide either adequate retention or resolution.



Mobile Phase: Isocratic, optimized for each column

	CH_3CN	D.I. H_2O	100 mM NH_4OAc, pH 5.0
Trinity P1	60%	0%	40%
Obelisc R	50%	40%	10%
Scherzo SM-C18	70%	20%	10%

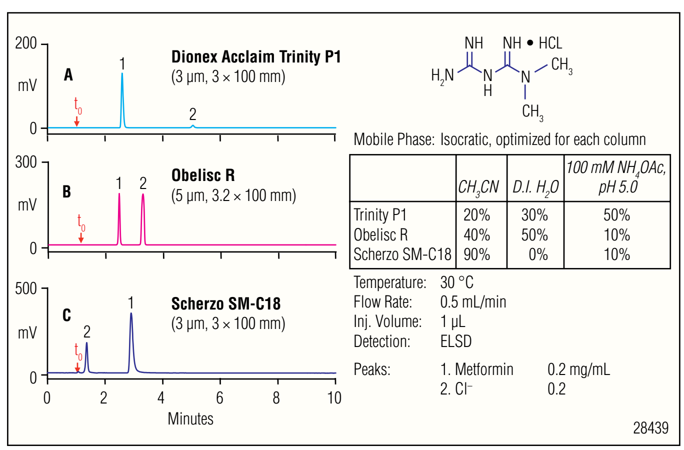
Temperature: 30 °C
Flow Rate: 0.5 mL/min
Inj. Volume: 1 µL
Detection: ELSD

Peaks: (0.2 mg/mL)
1. K+
2. Penicillin G

Figure 8. Acidic API and counterion—penicillin G potassium.

Basic API and Counterion

1,1-Dimethylbiguanide hydrochloride (metformin) is a highly hydrophilic basic drug formulated in its chloride salt. The simultaneous determination of both API and counterion is not possible on any RP columns. As shown in Figure 9, both the Acclaim Trinity P1 and Obelisc R columns can do the job, although the Obelisc R provides faster analysis. On the other hand, while Scherzo SM-C18 retains the API adequately, Cl^- elutes next to the void due to its inadequate anion-exchange retention.



Mobile Phase: Isocratic, optimized for each column

	CH_3CN	D.I. H_2O	100 mM NH_4OAc, pH 5.0
Trinity P1	20%	30%	50%
Obelisc R	40%	50%	10%
Scherzo SM-C18	90%	0%	10%

Temperature: 30 °C
Flow Rate: 0.5 mL/min
Inj. Volume: 1 µL
Detection: ELSD

Peaks:
1. Metformin 0.2 mg/mL
2. Cl^- 0.2

Figure 9. Basic API and counterion—metformin • HCl.

CONCLUSION

Although Acclaim Trinity P1, Obelisc R, and Scherzo SM-C18 columns have reversed-phase, cation-exchange, and anion-exchange functional groups, they are different in column chemistry design, chromatographic characteristics, and targeted applications.

The Dionex Acclaim Trinity P1 column provides strong anion-exchange, substantial cation-exchange, and rather weak RP retention. Thus it can be characterized as an RP modified cation- and anion-exchange phase and is positioned as an application-specific column for pharmaceutical applications. Scherzo SM-C18 shows strong RP retention, but very little cation- and anion-exchange capacities. Thus it can be viewed as an ion-exchange modified RP material. The RP and IEX retention of Obelisc R is somewhere between the Acclaim Trinity P1 and Scherzo SM-C18 columns, clearly a different type of trimode phase. Both Obelisc R and Scherzo SM-C18 columns are positioned as general-purpose columns to replace reversed-phase columns (e.g., C18).

The Dionex Acclaim Trinity P1 column is designed for separating pharmaceutical counterions and drug substances, and provides the most desirable selectivity for this application.

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