Journal of Chromatography A, 1232 (2012) 190–195

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Comparison of reversed-phase/cation-exchange/anion-exchange trimodal stationary phases and their use in active pharmaceutical ingredient and counterion determinations

Xiaodong Liu*, Christopher A. Pohl

Thermo Fisher Scientific, 1228 Titan Way, Sunnyvale, CA 94088, USA

ARTICLE INFO

Article history:
Available online 9 December 2011

Keywords:
Mixed-mode chromatography
Trimodal stationary phases
Chromatography properties
Retention behavior
Active pharmaceutical ingredient (API)
Counterions

ABSTRACT

This study involved three commercial reversed-phase (RP)/anion-exchange (AEX)/cation-exchange (CEX) trimodal columns, namely Acclaim Trinity P1 (Thermo Fisher Scientific), Obelisc R (SIELC Technologies) and Scherzo SM-C18 (Imtakt). Their chromatographic properties were compared in details with respect to hydrophobicity, anion-exchange capacity, cation-exchange capacity, and selectivity, by studying retention behavior dependency on organic solvent, buffer concentration and pH. It was found that their remarkably different column chemistries resulted in distinctive chromatography properties. Trinity P1 exhibited strong anion-exchange and cation-exchange interactions but low RP retention while Scherzo SM-C18 showed strong reversed-phase retention with little cation-exchange and anion-exchange capacities. For Obelisc R, its reversed-phase capacity was weaker than Scherzo SM-C18 but slightly higher than Trinity P1, and its ion-exchange retentions were between Trinity P1 and Scherzo SM-C18. In addition, their difference in selectivity was demonstrated by examples of determining the active pharmaceutical ingredient (API) and counterion of drug products.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

While most HPLC applications involving small molecules are developed on reversed phase (RP) columns [1] such as C18, C8, polar-embedded phases, phenyl and others, the selectivity options are rather limited. For example, RP columns often fail to retain highly hydrophilic analytes, such as catecholamines and inorganic ions (e.g., Na^+ and Cl^-). Although ion-pairing liquid chromatography can improve the retention and selectivity of hydrophilic ionic or ionizable analytes, it often requires long equilibration times, a dedicated column, and usually a MS-incompatible mobile phase [1,2]. While ion chromatography (IC) provides a reliable, selective, and sensitive solution to the analysis of ionic analytes [3,4], it is unsuitable for determinations of neutral analytes. Hydrophilic interaction liquid chromatography (HILIC) complements the aforementioned techniques and is suitable for analyzing highly hydrophilic analytes [5,6], but its use is hindered by poor solubility of the analytes in organic solvent rich solution and by greater organic solvent consumption compared to other separation modes.

Mixed mode chromatography provides a viable solution to this selectivity challenge. Mixed mode chromatography combines both reversed-phase (RP) and ion-exchange (IEX) retention mechanisms, and has been in practice for more than twenty years [7]. The biggest benefit of mixed-mode columns is selectivity can be optimized by adjusting mobile phase ionic strength, pH and/or organic solvent [8–11]. As the result, not only are these columns complementary to RP columns, but also complementary to themselves under different conditions. The presence of both RP and IEX functionalities requires no ion-pairing agents in the mobile phase to separate highly hydrophilic charged analytes, which simplifies the mobile phase and makes the method 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 [12].

Salt formation is important in drug development to improve biopharmaceutical and physicochemical properties of the drug [13]. Approximately 50% of all drug molecules are administered as salts [14]. 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 instrument platforms. Similarly, in pharmaceutical analysis, an API and its counterion are also often determined 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

* Corresponding author. Tel.: +1 408 4814500; fax: +1 408 733 6245.
E-mail address: xiaodong.liu@thermofisher.com (X. Liu).

0021-9673/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2011.12.009

Table 1
Information on trimodal columns in this study.

Column name	Acclaim Trinity P1	Obelisc R	Scherzo SM-C18
Manufacturer	Thermo Fisher Scientific	SIELC Technologies	Imtakt
Column dimension	3 mm × 100 mm	3.2 mm × 100 mm	3 mm × 100 mm
Particle size (μm)	3	5	3
Pore size (Å)	300	100	130
Surface area (m^2/g)	100	Unknown	300
Column technology	Nanopolymer silica hybrid	Covalent bonding of a special silane consisting of RP, WCX and WAX functionalities on silica gel	Mixed bed with C18/WAX and C18/WCX functionalized silica particles
Reversed-phase	C10 alkyl moiety covalently attached to the silica surface	An alkyl group connecting both WAX and WCX at each end	C18 alkyl group
Anion-exchange	Terminus tertiary amine (WAX) at the top of C10 alkyl group	A WAX (pKa ~ 10) group incorporated in a special silane and in the proximity of silica surface	A WAX bearing silyl ligand co-bonded with the C18 silane
Cation-exchange	Fully sulfonated nano-sized polymer beads electro-statically attached to the outer surface of the RP/WAX bonded silica	A terminus WCX (pKa ~ 4) group incorporated in a special silane	A WCX bearing silyl ligand co-bonded with the C18 silane

determination of all APIs and counterions would be 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 would be highly desirable.

This study compares three commercial RP/AEX/CEX trimodal columns with remarkably different chemistry designs. Detailed chromatography comparisons are made with respects to hydrophobicity, anion-exchange capacity, cation-exchange capacity, selectivity, etc. In addition, the retention behavior dependency on organic solvent, buffer concentration and pH is discussed. The suitability of these columns for the determination of active pharmaceutical ingredients (APIs) and corresponding counterions is also demonstrated.

2. Experimental

2.1. Separation columns

The following columns were used in this study: Acclaim Trinity P1 (3 μm, 3 mm × 100 mm, Thermo Fisher Scientific, Sunnyvale, CA, USA); Obelisc R (5 μm, 3.2 mm × 100 mm, SIELC, Prospect Heights, IL, USA); Scherzo SM-C18 (3 μm, 3 mm × 100 mm, Imtakt, USA, Philadelphia, PA, USA). More information for these columns is provided in Table 1.

2.2. Reagents and materials

HPLC grade acetonitrile was obtained from Burdick and Jackson (Muskegon, MI, USA). De-ionized water (>18 MΩ cm^{-1}) was purified by a Milli-Q water purification system (Millipore, Bedford, MA, USA). Ammonium acetate salt (99.99 + % pure) other reagents, and all standards were purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.3. Instrumentation

Separations were performed on a modular UltiMate™ 3000 HPLC System (Thermo Fisher Scientific) equipped with a LPG 3600 gradient pump, WPS-3000 Autosampler, TCC-3200 column oven, and PDA-3000 detector. A Corona ultra charged aerosol detector (Thermo Fisher Scientific) or a Sedex-85 evaporative light scattering detector (Sedere, Alfortville, France) was used to detect analytes with no or weak chromophore. Chromeleon® 6.80 Chromatography Management Software (Thermo Fisher Scientific) was used for system control and data processing.

2.4. Chromatographic conditions

Phenanthrene, dimethylphthalate, uracil and sodium chloride (NaCl) were used as test probes in this study. Both phenanthrene and dimethylphthalate are neutral and were used for hydrophobicity comparison. Uracil was used to study HILIC retention due to its hydrophilic nature. NaCl was used to compare cation-exchange (Na^+) and anion-exchange (Cl^-) properties among different phases. Their structures are shown in Fig. 1. The void time t_0 was determined by examining the disturbance in UV spectrum as a PDA detector was used. The retention factor (k) was defined as $(t_R - t_0)/t_0$. The detailed chromatographic conditions can be found in the figure captions.

In retention-organic solvent dependency study, mobile phases were generated by proportioning from three components: acetonitrile, ammonium acetate buffer (100 mmol L^{-1} at pH5), and D.I. water. While ammonium acetate buffer was kept constant at 10% (v/v), the contents of acetonitrile in mobile phase were controlled by various acetonitrile to D.I. water ratios. The retention factors were recorded at 10, 20, 30, 40, 50, 60, 70, 80 and 90% (v/v) acetonitrile.

In retention-buffer concentration dependency study, mobile phases were generated by proportioning from three components: acetonitrile, ammonium acetate buffer (100 mmol L^{-1} at pH5), and D.I. water. While acetonitrile was kept constant at 50% (v/v), the buffer concentrations of mobile phase were controlled by various ammonium acetate buffers to D.I. water ratios. The retention factors were recorded at 5, 10, 20, 30, 40 and 50 mmol L^{-1} ammonium acetate buffer.

In retention-pH dependency study, mobile phases were generated by proportioning from four components: acetonitrile, ammonium acetate salt solution (100 mmol L^{-1} at pH7), acetic acid solution (1000 mmol L^{-1}) and D.I. water. The buffer concentration was normalized to [NH_4^+]. While acetonitrile and ammonium acetate buffer were kept constant at 50% (v/v) and 10% (v/v), respectively, pH levels were controlled by acetic acid solution to D.I. water

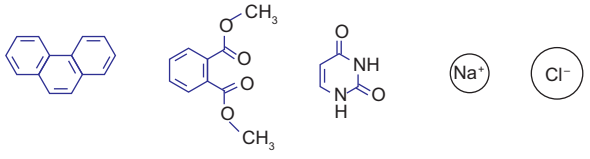

Fig. 1. Test probes for retention behavior study.

various ratios. The retention factors were recorded at pH 3.70, 3.85, 4.00, 4.30, 4.65, 4.95. 5.60 and 5.90.

3. Results and discussion

3.1. Column chemistry

Three commercial reversed-phase/anion-exchange/cation-exchange trimodal columns in this study have distinctive chemistry designs. The Scherzo SM-C18 column is constructed by mixing two types of bonded silica particles: one modified with C18 silanes and silanes with weak cation-exchange functionality and the other with C18 silanes and silanes with weak anion-exchange functionality [15]. Therefore, it is a RP/WAX/WCX tromodal phase. The silica substrate used in this column was in 3 μm particle size and 130 Å pore diameter. The manufacturer recommends this column for separation of basic and acidic compounds under neutral pH conditions. However, a, earlier study suggests that the inherent drawback of this approach relates to undesirable selectivity drift due to the difference in hydrolytic stability between the RP ligand and the IEX ligand bonding sites [16].

The Obelisc R column was packed with silica particles covalently functionalized with a silane ligand containing a hydrophobic alkyl chain, a basic group and an acidic group in the same molecule. The silica substrate was 5 μm in particle size and 100 Å in pore diameter. According to the manufacturer, the basic group had a pKa of around 10, was in the proximity of silica surface, and was separated from the terminal acidic group (pKa ~ 4) by a hydrophobic alkyl chain [17]. Therefore, Obelisc R is a RP/WAX/WCX trimodal phase. The manufacturer also suggested that the pH range of 3–6 was most suitable for fully utilizing both anion-exchange and cation-exchange functionalities, and this column could operate in reversed-phase, cation-exchange and anion-exchange mode simultaneously, or one of the modes depending on mobile phase composition. On the other hand, it should be noted that although both WAX and WCX functionalities are present, they can neutralize one another due to their proximity promoted by the flexible alkyl linker when both functional groups are ionized. Consequently, it behaves as a salt-exchange material rather than an ion-exchanger in which both anion-exchange and cation-exchange mechanisms function without mutual interferences.

The Acclaim Trinity P1 is prepared by an electrostatically driven self-assembly process and consists of high-purity porous spherical silica particles whose inner-pore area is covalently modified with silyl ligands containing both reversed-phase and weak anion-exchange moieties while the outer surface is coated with fully sulfonated nano-polymer beads by electrostatic interactions [10,18]. The synthetic process was described in an earlier work [10]. First, spherical porous silica particles (d_p = 3 μm; pore size = 300 Å; surface area = 100 m^2/g) are covalently modified with a silane containing both a hydrophobic alkyl chain (for hydrophobic retention) and a terminal tertiary amine (for weak anion-exchange retention) in the same molecule to obtain the surface modified silica particles. Then nano-sized fully sulfonated polystyrene-divinylbenzene polymer beads are allowed to mix with above modified silica particles under proper conditions such that both components are ionized, to ensure an electro-statically driven self-assembly process. Because the size of nano-polymer beads is in the range of 1000–3000 Å, much larger than the pore size of silica particles (300 Å), these charged nano-sized polymer beads are selectively and permanently attached to the outer surface area by electrostatic attraction, but are excluded from the inner-pore area due to steric hindrance. This chemistry design creates a 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.

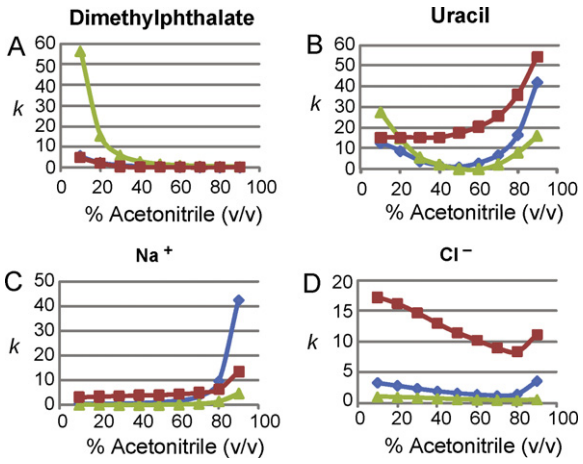

Fig. 2. Effect of organic solvent. Columns, Acclaim Trinity P1 (square), Obelisc R (diamond), and Scherzo SM-C18 (triangle); mobile phase, acetonitrile/ammonium acetate, pH 5.2 (10 mmol L^{-1} total concentration) in various ratios; flow rate, 0.45 mL min^{-1}; injection volume, 2 μL; temperature, 30 °C; and detection, UV at 230 nm and ELSD. Analytes: dimethylphthalete, uracil, and NaCl (0.5 mg mL^{-1} each).

3.2. Chromatographic evaluation

3.2.1. Organic solvent effect

Fig. 2 shows the retention dependency versus organic solvent using dimethylphthalate, uracil, Na^+ and Cl^- as RP, HILIC, cation-exchange and anion-exchange probes, respectively. As shown in Fig. 2A, retentions of dimethylphthalate decreased with mobile phase acetonitrile increase on all three columns, but were affected by neither buffer concentration nor pH (not shown), suggesting a sole RP retention mechanism. It is noted that under the same mobile phase condition Scherzo SM-C18 provided significantly higher RP retention than the other two phases, suggesting a stronger hydrophobicity. Fig. 2B exhibits "U" shaped retention versus acetonitrile curves for uracil on all three columns, which is an indication of bimodal retention behavior – a RP mechanism in highly aqueous condition and a HILIC mechanism in high acetonitrile condition. However, all three columns demonstrated rather weak HILIC characteristics (low uracil retention) with Trinity P1 showing slightly higher retention of uracil at 90% acetonitrile compared to the other two columns. More discussion on hydrophobicity and hydrophilicity comparisons can be found in Section 3.2.4.

Trinity P1 showed highest cation-exchange retention, Scherzo SM-C18 exhibited virtually no retention, and Obelisc R was somewhere in between. As for Na^+ ion (Fig. 2C), from 10 to 70% acetonitrile, its retention on all three columns increased with solvent content but at a slow rate. Above 70% acetonitrile, Na^+ retention increased more rapidly with solvent content on all three columns, especially for Obelisc R on which the Na^+ retention rapidly surpassed that on Trinity P1 beyond 80% acetonitrile. Fig. 2D illustrates that the Cl^- retention (k) decreased continuously with acetonitrile increase from 10% to 80% acetonitrile levels. On Trinity P1, it dropped from 17 at 10% acetonitrile down to 8 at 80% acetonitrile. Further increase in acetonitrile resulted in a rapid retention increase to a k value of 11 at 90% acetonitrile. Compared to Trinity P1, Obelisc R exhibited a similar trend but with lower retentions while Scherzo SM-C18 showed little anion-exchange capacity. Cl^- ion showed significantly higher retention on Trinity P1 at all solvent levels.

The retention-solvent dependency on a mixed-mode stationary is a complex matter. While retentions of neutral hydrophobic and

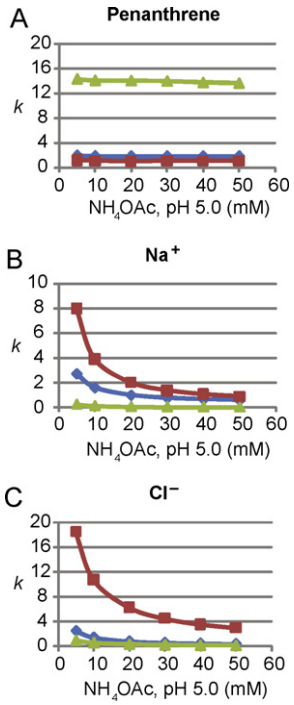

Fig. 3. Effect of buffer concentration. Columns, Acclaim Trinity P1 (square), Obelisc R (diamond), and Scherzo SM-C18 (triangle); mobile phase, acetonitrile/5–50 mmol L^{-1} ammonium acetate, pH 5.2 (50:50, v/v) at various ammonium acetate concentrations; flow rate, 0.45 mL min^{-1}; injection volume, 2 μL; temperature, 30 °C; and detection, UV at 230 nm and ELSD. Analytes: phenanthrene and NaCl (0.5 mg mL^{-1} each).

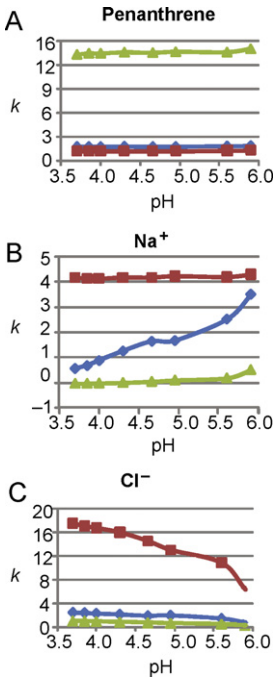

Fig. 4. Effect of pH. Columns, Acclaim Trinity P1 (square), Obelisc R (diamond), and Scherzo SM-C18 (triangle); mobile phase, acetonitrile/10 mmol L^{-1} ammonium acetate buffer at various pH levels (50:50, v/v); flow rate, 0.45 mL min^{-1}; injection volume, 2 μL; temperature, 30 °C; and detection, UV at 230 nm and ELSD. Analytes: phenanthrene and NaCl (0.5 mg mL^{-1} each).

neutral hydrophilic molecules are governed by RP and HILIC mechanisms, respectively, retentions of ionic analytes such as Na^+ and Cl^- depend heavily upon ionization of WCX and WAX functionalities on the stationary phase and of buffer salt (ammonium acetate) in the mobile phase. As solvent content increases, the ionization of stationary phase WCX and WAX functionalities decreases, resulting in less affinity of ionic analytes (e.g. Na^+ and Cl^-) towards opposite charges on the stationary phase or lower retentions. However, since the ionizations of mobile phase ions, ammonium and acetate, also decrease at the same time, making mobile phase ionic strength weaker thus longer retention times for Na^+ and Cl^- ions. Therefore, the ion-exchange process is a relative phenomenon. The solvent effect is an indication of the effect of solvent on the selectivity of the ion-exchange site for the competing ion in the mobile phase relative to the selectivity of the ion-exchange site for the analyte. Higher retention with increased solvent could mean lower affinity for the competing ion in the mobile phase or higher affinity for the analyte.

3.2.2. Buffer concentration effect

Fig. 3 shows the retention dependency on mobile phase buffer concentration. As expected, the retention of phenanthrene (neutral molecule) was virtually unaffected by buffer concentration. Scherzo SM-C18 exhibited significantly higher phenanthrene retention than Obelisc R and Trinity P1 (Fig. 3A). Meanwhile, retentions of Na^+ and Cl^- decreased with buffer concentration increase, suggesting typical cation-exchange and anion-exchange mechanisms (Fig. 3B and C). It is clear that at any given buffer concentrations, Trinity P1 provided significantly higher ion-exchange retention while Obelisc R had weaker ion-exchange capacity and the Scherzo SM-C18 column exhibited virtually no ion-exchange capacity.

3.2.3. pH effect

To study the pH effect, phenanthrene and NaCl samples were injected onto the columns using mobile phases containing 50% acetonitrile (v/v) and 10 mmol L^{-1} ammonium buffer at various pH levels (from 3.7 to 5.8). Phenanthrene retention was unaffected by the pH (Fig. 4A), but retentions of Na^+ and Cl^- were sensitive to the pH change. Once again, Scherzo SM-C18 showed the highest hydrophobicity among all three columns. As shown in Fig. 4B and C, Trinity P1 provided highest retentions for both Na^+ and Cl^- ions compared to the other two columns. Na^+ retention was constant at all pH levels while Cl^- retention decreased with pH. By comparison, on Obelisc R, the retention of Na^+ ion increased with pH while that of Cl^- gradually decreased with pH. Although exhibiting the similar trend to Obelisc R, Scherzo SM-C18 showed insignificant anion-exchange and cation-exchange capacities. The reason for these observations can be postulated as follows: Trinity P1 has a distinctive spatial separation between the WAX region (in inner-pore area) and SCX region (on the outer surface). Therefore, the cation-exchange retention is governed by fully sulfonated nano-polymer beads on the outer surface with ionization unaffected by pH change. Because the buffer concentration was normalized to [NH_4] and remained constant, the Na^+ retention was virtually independent on the pH. The situation was different for Obelisc R: although under testing condition WAX groups (pKa ~ 10) were fully charged, the ionization of WCX groups (pKa ~ 4) was dependent on pH. As both WAX and WCX functional groups were connected with a flexible alkyl chain, the bonded phase was zwitterionic and its "net charge" or ion-exchange capacity was pH dependent. In addition, the charge of surface silanol groups increased with pH, and the negatively charged silanols neutralized the positively charged WAX groups located in close proximity to the silica surface, thus enhancing cation-exchange retention. The situation was similar to Scherzo SM-C18 except that its cation-exchange capacity was much lower as the result of the column chemistry. Strong pH dependency for the retention of Cl^- was observed on all three columns with the

same trend: Cl^- retention decreased with pH increase. While WAX groups (pKa ~ 10) were fully charged under the experimental conditions, the ionization of acetate ions in the mobile phase increased with pH, leading to a faster elution of Cl^-. In addition, ionization of underivatized silanols on the silica surface also increased with pH, resulting in increased electrostatic repulsion between Cl^- and the stationary phase, or lower Cl^- retention.

3.2.4. Comments on hydrophobicity, hydrophilicity, ion-exchange property and selectivity

In a previous study [11], uracil and phenanthrene were used as testing probes to compare hydrophilicity and hydrophobicity among different mixed-mode columns including Trinity P1. In this study, hydrophilicity and hydrophobicity of the stationary phase were defined as retention factor (k) of uracil in 90% acetonitrile (v/v), and retention factor (k) of phenanthrene in 40% acetonitrile (v/v), respectively. Applying the same protocol to the current work, hydrophilicity was found in the order of Trinity P1 (0.52) > Obelisc R (0.40) > Scherzo SM-C18 (0.15), and hydrophobicity in the order of Scherzo SM-C18 (37.00) > Obelisc R (4.91) > Trinity P1 (3.12). Accordingly, hydrophilicity index, defined as "(k of uracil/k of phenanthrene) × 100", was in the order of Trinity P1 (16.7) > Obelisc R (8.1) > Scherzo SM-C18 (0.4). It was clear that Scherzo SM-C18 provided significantly higher hydrophobic retention compared to Obelisc R and Trinity P1. Meanwhile, Scherzo SM-C18 showed the least ion-exchange capacity among all three columns. Both cation-exchange capacity and anion-exchange capacity followed the order of Trinity P1 > Obelisc R > Scherzo SM-C18. The different combinations of RP, CEX and IEX capacities result in different selectivity. Trinity P1 provides high anion-exchange and high cation-exchange capacity with relatively low hydrophobicity. Thus it can be characterized as a "RP modified cation and anion-exchange phase." Scherzo SM-C18 exhibits strong RP retention but weak cation-exchange and anion-exchange capacities, thus can be regarded as an "ion-exchange modified RP material." The RP and IEX retentions of Obelisc R are somewhere between Trinity P1 and Scherzo SM-C18, clearly a different type of trimode phase.

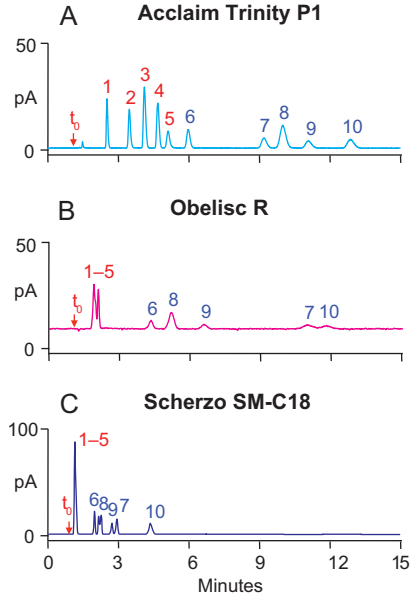

Fig. 5. Pharmaceutical counter cations and anions. Columns, Acclaim Trinity P1 (A), Obelisc R (B), and Scherzo SM-C18 (C); mobile phase, acetonitrile/D.I. water/100 mmol L^{-1} ammonium acetate buffer, pH 5 (60:25:15, v/v/v for Acclaim Trinity P1; 20:70:10, v/v/v for Obelisc R and Scherzo SM-C18); flow rate, 0.5 mL min^{-1}; injection volume, 2 μL; temperature, 30 °C; and detection, Corona ultra (gain = 100 pA; filter = med; nebulizer temperature = 30 °C). Sample: 0.05–0.1 mg mL^{-1}. Peaks: (1) choline, or N,N,N-trimethylethanolammonium; (2) tromethamine, or tris(hydroxymethyl)aminomethane; (3) sodium; (4) potassium; (5) meglumine, or N-methyl glucamine; (6) mesylate, or methanesulfonate; (7) nitrate; (8) chloride; (9) bromide; (10) iodide.

3.3. Applications

3.3.1. Pharmaceutical counterions

Salt formation is a critical step in drug development because it provides improved biopharmaceutical and physicochemical properties as well as ease of purification and handling [13,19]. A broad selection of inorganic and organic ions can be used as pharmaceutical counterions [14,20]. The most commonly used counterions are sodium and chloride ions which cannot be retained on any reversed-phase columns. 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 Fig. 5A, the Trinity P1 provided baseline separation of 10 commonly used pharmaceutical counterions (five cations and five anions) in a single analysis. Even under its own optimized condition, Obelisc R only separated five anions while all cations co-eluted close to the void (Fig. 5B). By comparison, Scherzo SM-C18 was clearly unsuitable for this application: at its best, five anions were partially separated with five cations co-eluting in the void (Fig. 5C).

3.3.2. 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

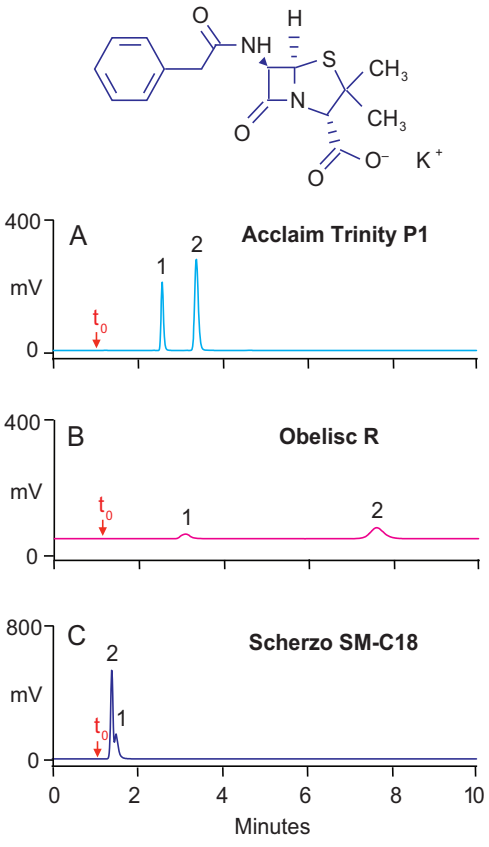

Fig. 6. Acidic API and counterion – penicillin G potassium. Columns, Acclaim Trinity P1 (A), Obelisc R (B), and Scherzo SM-C18 (C); mobile phase, acetonitrile/D.I. water/100 mmol L^{-1} ammonium acetate buffer, pH 5 (60:0:40, v/v/v for Acclaim Trinity P1; 50:40:10, v/v/v for Obelisc R; 90:0:10, v/v/v and Scherzo SM-C18); flow rate, 0.5 mL min^{-1}; injection volume, 1 μL; temperature, 30 °C; and detection, ELSD. Sample: penicillin G potassium (0.2 mg mL^{-1}). Peaks: (1) K^+; (2) penicillin G.

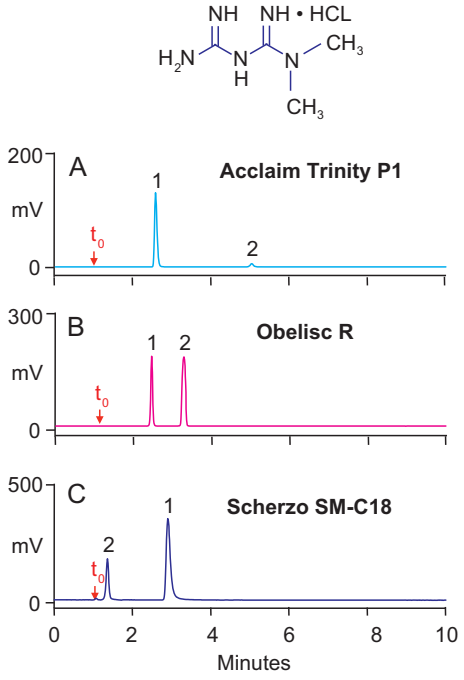

Fig. 7. Basic API and counterion – metforminHCl. Columns, Acclaim Trinity P1 (A), Obelisc R (B), and Scherzo SM-C18 (C); mobile phase, acetonitrile/D.I. water/100 mmol L^{-1} ammonium acetate buffer, pH 5 (20:30:50, v/v/v for Acclaim Trinity P1; 40:50:10, v/v/v for Obelisc R; 70:20:10, v/v/v and Scherzo SM-C18); flow rate, 0.5 mL min^{-1}; injection volume, 1 μL; temperature, 30 °C; and detection, ELSD. Sample: metformin hydrogen chloride (0.2 mg mL^{-1}). Peaks: (1) metformin; (2) Cl^-.

within the same analysis on any RP column. This drug molecule and its counterion were previously determined on the Trinity P1 column [11]. As shown in Fig. 6, both Trinity P1 and Obelisc R were capable of providing adequate retention and resolution, but Trinity P1 was more suited to this application because of its excellent resolution, sharper peak and faster analysis. By comparison, Scherzo SM-C18 failed to provide either adequate retention or resolution.

3.3.3. *Basic API and counterion*

1,1-Dimethylbiguanide hydrogen chloride (metformin) is a highly hydrophilic basic drug often formulated in its chloride salt. Simultaneous determination of both API and counterion is not possible on any RP columns. As shown in Fig. 7, while both Trinity P1 and Obelisc R could retain and separate both API and the counterion, the Obelisc R provided faster analysis and better peak response. On the other hand, although Scherzo SM-C18 retained the API adequately, Cl^- ion only eluted next to the void due to its inadequate anion-exchange retention.

4. Concluding remarks

Acclaim Trinity P1, Obelisc R and Scherzo SM-C18 all contain reversed-phase, cation-exchange and anion-exchange functional groups, but they are different in column chemistry design, chromatographic characteristics, and targeted applications. The Trinity P1 provides high anion-exchange and cation-exchange capacity with weak RP retention. It is designed to provide optimal selectivity for separating charged analytes with a broad range of hydrophobicity, such as pharmaceutical counterions and drug substances. Thus it can be characterized as a "reversed-phase modified cation and anion-exchange phase." Scherzo SM-C18 shows strong reversed-phase retention with very little cation-exchange and anion-exchange capacities, thus is largely a general-purpose reversed-phase column slightly modified by ion-exchange properties. For the Obelisc R column, its reversed-phase capacity is weaker than Scherzo SM-C18 but slightly higher than the Trinity P1. In addition, its ion-exchange retentions are between Trinity P1 and Scherzo SM-C18, clearly suggesting a different type of trimode phase. While the availability of various trimodal columns offers greater potentials for a broad range of applications, understanding their column chemistry, retention behavior and differences will help chromatographers select suitable columns and develop rugged methods for the challenging applications unmet by reversed-phase columns.

Acknowledgments

The authors are thankful to Dr. Jeff Rohrer for reviewing this manuscript and providing constructive suggestions, and to Mr. Scott Lefferts and Ms. Emma Ramirez for preparing the figures.

References

[1] U.D. Neue, HPLC Columns: Theory Technology and Practice, Wiley-VCH, New York, 1997.
[2] J.G. Chen, S.G. Weber, L.L. Glavina, F.F. Cantwell, J. Chromatogr. A 656 (1993) 549.
[3] H. Small, Ion Chromatography, Plenum Press, New York, 1989.
[4] T. Weiss, J. Weiss, Handbook of Ion Chromatography, Wiley-VCH, Weinheim, 2005.
[5] A.J. Alpert, J. Chromatogr. 499 (1990) 177.
[6] P. Hemström, K.J. Irgum, Sep. Sci. 29 (2006) 1784, and references herein.
[7] L.W. McLaughlin, Chem. Rev. 89 (1989) 309.
[8] M. Lämmerhofer, M. Richter, J. Wu, R. Nogueira, W. Bicker, W. Lindner, J. Sep. Sci. 31 (2008) 2572.
[9] E. Apfelthaler, W. Bicker, M. Lämmerhofer, M. Sulyok, R. Krska, W. Lindner, R. Schuhmacher, J. Chromatogr. A 1191 (2008) 171.
[10] X. Liu, C. Pohl, A. Woodruff, J. Chen, J. Chromatogr. A 1218 (2011) 3407.
[11] X. Liu, C. Pohl, J. Sep. Sci. 33 (2010) 779.
[12] K. Zhang, L. Dai, N.P. Chetwyn, J. Chromatogr. A 1217 (2010) 5776.
[13] P.H. Stahl, C.G. Wermuth, Handbook of Pharmaceutical Salts; Properties, Selection, and Use, VHCA and Wiley-VCH, Zurich, Switzerland, and Weinheim, Germany, 2008.
[14] U.S. Food and Drug Administration Orange Book, http://www.fda.gov/cder/ob/.
[15] Imtaktusa Website: http://www.imtaktusa.com/site media/files/phase brochures/leaflet.pdf.
[16] Y. Bergqvist, C. Hopstadiua, J. Chromatogr. B 741 (2000) 189.
[17] SIELC Website: http://www.sielc.com/Products Obelisc.html.
[18] Acclaim Trinity P1 Datasheet: http://www.dionex.com/en-us/webdocs/70761-DS-Acclaim-Trinity-12Feb2010-LPN2239-02.pdf.
[19] A.T.M. Serajuddin, Adv. Drug Deliv. Rev. 59 (2007) 603.
[20] G.S. Paulekuhn, J.B. Dressman, C. Saal, J. Med. Chem. 50 (2007) 6665.