Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1073 (2005) 269–275
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Evaluation of a monolithic silica column operated in the hydrophilic interaction chromatography mode with evaporative light scattering detection for the separation and detection of counter-ions

Brian W. Pack*, Donald S. Risley

Eli Lilly and Company, Pharmaceutical Product Research and Development, Lilly Research Laboratories, Indianapolis, IN 46285, USA

Available online 26 October 2004

Abstract

In this work a monolithic silica column operated in the hydrophilic interaction chromatography (HILIC) mode in conjunction with an evaporative light scattering detector (ELSD) was investigated. Lithium, sodium and potassium were used as the test counter-ions for this evaluation. Chromatographic properties of this column operated in the HILIC mode were determined by varying key mobile phase parameters, such as pH, flow rate, buffer strength, acid and organic modifier. As organic content was increased from 60 to 90% acetonitrile, retention time increased on average by a factor of seven for the test cations listed above. Buffer concentration and pH were also observed to have an effect, although not as significant as the HILIC effect that was observed by changing organic content. Flow rates up to 5 mL/min were utilized to perform counter-ion separations in less than 3 min. After examining the changes in retention, resolution, and peak shape an optimized method was established and then further evaluated for linearity, reproducibility, and limit of detection (LOD) for sodium. Linearity was acceptable with an R^2 value of 0.999 across the working-standard range and a LOD of 0.1 μg/mL was calculated. The reproducibility on the counter-ion determination from pharmaceutical sodium salts was 1.6% R.S.D. on average, and the accuracy of the counter-ion prediction was approximately 3% from theory when salt content was corrected for potency.

Keywords: Monolithic columns; Hydrophilic interaction chromatography; Evaporative light scattering detection; Counter-ion; Silica column

1. Introduction

Monolithic silica columns consist of macropores and mesopores that form a network of interconnected flow paths. Due to the porous nature of this packing material which is nearly 80% porosity as compared to 65% with packed columns, higher flow rates are possible without an excessive increase in back pressure. The macropores are approximately 2 μm in size and offer channels for the analytes to be transported rapidly to the active sites within the pores. Due to the high surface area within the column, a reduction in diffusion path length and flow resistance, as well as, an increase in mass transport is achieved; thus, a faster high efficiency separation is possible. The main advantage of reduced run time is realized when higher flow rates can be utilized with monolithic columns.

Tanaka et al. published reviews on the capabilities and possible limitations afforded by monolithic column technology [1,2]. The concept of monolithic (or single piece) columns is not new, as Kubin et al. introduced a size exclusion monolith in 1967, but the performance was not good based upon permeability considerations [3]. In the mid nineties a more robust manufacturing process was introduced offering the full advantages of monolithic columns that could now be realized [3].

Recently, monolithic octadecylsilyl-silica gel columns were utilized for the high-speed ion determination of rainwater acidity using conductimetric detection [4]. In this study, H^+ was separated from Na^+, K^+, Mg^{2+}, and Ca^{2+}. To accomplish this separation, the silica gel column was first

* Corresponding author. Tel.: +1 317 651 2838; fax: +1 317 277 2177.
E-mail address: packbw@lilly.com (B.W. Pack).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.09.061

pre-treated with lithium dodecylsulfate. For the separation of anions, a reversed-phase C18 (RP-18e 4.6 mm × 50 mm, Merck KGaA) column was permanently coated with didodecyldimethylammonium bromide, and used in conjunction with conductivity detection [5]. The separation of seven anions in less than 60 s was accomplished with this methodology. The main draw back in this work was indicated to be the limited lifetime of the column, which was approximately 12 h. The use of monolithic columns in the pharmaceutical industry was examined in relation to performance in a good manufacturing practices (GMP) environment [6]. This evaluation utilized a C18 reversed-phase monolithic column with a phosphate buffer mobile phase. When compared to a conventional method with a particle packed column (RP18, 5 μm, 250 mm × 4 mm), the monolithic column method reduced the analysis time by a factor of 6 while achieving equivalent resolution. However, there was a dramatic loss of theoretical plates as the flow rate was increased from 1 mL/min to 5 mL/min. Although, the work suggested that for smaller molecules higher optimum flow rates could be achieved. Additional findings of their work indicated that the monolithic column offers comparable repeatability, reproducibility, and stability when compared to a packed column.

The approach presented here uses a monolithic silica column operated in the hydrophilic interaction chromatography (HILIC) mode with evaporative light scattering detection (ELSD). Alpert coined the term hydrophilic interaction chromatography for the separation of proteins, peptides, and polar molecules [7], although this mechanism had been previously established for the separation of carbohydrates [8,9]. The HILIC mode employs polar stationary phases with mixed aqueous/organic mobile phases creating a stagnant enriched water layer around the polar stationary phase. This enriched layer allows analytes to partition between the two phases based on their polarity. In contrast to reversed-phase (RP) chromatography, where a non-polar stationary phase is employed and analyte elution is facilitated by the organic strength of the mobile phase, analyte elution is facilitated by the aqueous component of the mobile phase in HILIC mode. The separation mechanism and retention order in the HILIC mode is therefore opposite to that of the RP mode. Although the HILIC mode is more similar to the normal phase (NP) and polar organic modes, it is different in that the HILIC mobile phases contain a relatively high amount of water (5–40%), which can provide a significant solubility advantage for very hydrophilic samples. The HILIC mode can be generated by a variety of polar stationary phases, for example, the counter-ion piperazine has been determined utilizing the HILIC mode on a cyano column [10] and polar pharmaceutical analytes have been separated using both amino and silica columns [11]. The HILIC mode has also been employed for chiral separations using cyclodextrin and macrocyclic antibiotic based packings [12,13].

In HPLC, the evaporative light scattering detector has an extensive application base, but it is especially important when ultraviolet (UV) detection is not feasible. The concept and operation of commercially available evaporative light-scattering detectors as sensitive universal detectors has been thoroughly discussed in the literature [14]. The ELSD has been shown to successfully detect many substances, such as, phospholipids [15], triglycerides, fats and fatty acid esters [16], carbohydrates [17], synthetic polymers [18], steroids [19], and amino acids [20]. The HPLC–ELSD system has also been extremely useful for the determination of pharmaceutical impurities, raw materials, cleaning verification and small organic compounds [21–24]. A more recent niche for the ELSD in the pharmaceutical industry is for the detection and quantitation of counter-ions from pharmaceutical salt forms. Our laboratory first introduced the applicability of HPLC–ELSD for the detection and quantitation of inorganic ions, such as chloride and sodium [25,26]. A comparison of the HPLC–ELSD technique to ion chromatography, capillary electrophoresis, and titration for the determination of Cl^- in pharmaceutical drug substances has been statistically compared and it was determined that the four techniques were equivalent [26]. However, the ELSD is a cost effective detector that can be utilized with many other HPLC applications in addition to the analysis of counter-ions (e.g. assay and impurity determinations for compounds lacking a strong chromophore) which gives it a unique advantage over other techniques. The aim of this paper is to show the application of a new monolithic silica column operated in the HILIC mode for the detection and rapid quantitation of lithium, sodium and potassium using ELSD.

2. Experimental

2.1. Chemicals

Ammonium acetate, sodium chloride, lithium chloride, and potassium chloride were obtained from Fisher Scientific (Fairlawn, NJ). Acetonitrile was purchased from Burdick and Jackson (Muskegon, MI). Acetic acid, naproxen sodium, and warfarin sodium were obtained from Sigma–Aldrich Chemical Company (St. Louis, MO). A sodium standard solution was acquired from Fluka Chemika (Buchs, Switzerland). The pH 2.00 and 7.00 buffers were from Red Bird Service (Osgood, IN). Deionized water and nitrogen were from an in-house system. The new chemical entity sodium salt was synthesized at Eli Lilly and Company (Indianapolis, IN).

2.2. Equipment

The HPLC system consisted of a Hewlett-Packard 1050 pump and auto sampler (Wilmington, DE) integrated with an Alltech 800 evaporative light scattering detector from Alltech Associates (Deerfield, IL). The detector was operated at 50 °C, 3.8 bar nitrogen and a gain setting of eight throughout the experiments. A Chromolith Performance SI (100 mm × 4.6 mm) silica column from Merck KgaA was

used for the separation (Darmastadt, Germany). An Orion model 720A pH meter from Orion Research, Inc. was used to measure the pH of the mobile phase buffers (Beverly, MA).

2.3. Standard and sample preparation

Sodium standards were prepared from the Fluka sodium standard solution (1.000 g/L) by making serial dilutions with 80% acetonitrile/20% water. The standard curve for five calibration standards was calculated by least-squares regression analysis of peak area versus concentration. The samples were individually weighed and the weights were based on the theoretical content of sodium to result in a target concentration of approximately 0.1 mg sodium/mL (80% acetonitrile/20% water). The concentration of the sodium in the samples was determined by comparing the peak area to the standard curve.

3. Results and discussion

3.1. Detection and retention of cations

Since evaporative light scattering detection is employed in this investigation, a volatile buffer is required for the detection of the ions. For example, ammonium acetate buffer is used here so that a particle of $Na^+CH_3COO^-$ will be formed during desolvation and subsequently detected by light scattering. In the case of Cl^-, which elutes very close to the solvent front (see Fig. 1) under these conditions, $NH_4{}^+Cl^-$ is formed and subsequently detected. The first important aspect of this work was to establish that the monolithic silica stationary phase could retain and separate group I cations. As can be seen in the bottom chromatographic trace in Fig. 1 (60% ACN), lithium (Li^+), sodium (Na^+), and potassium (K^+) are easily separated in approximately four minutes. This experiment was performed at pH 6.55, with an ammonium acetate buffer of 50 mM. As the organic content of the mobile phase is increased from 60 to 90% ACN, the retention times of the ions are substantially increased from 2.9, 3.4, and 4.1 to 17.0, 24.0, and 29.1 min for Li^+, Na^+, and K^+, respectively. In a typical reversed-phase interaction (not that group I cations are typically retained on a reversed-phase column), the retention time would have been observed to decrease (or elute in the solvent front) as the organic composition is increased. Therefore, this bare silica monolithic column is showing retention behavior that is characteristic of the HILIC mode for the separation of cations as evidenced by the observed increased retention-time effect with increased organic content in the mobile phase.

In addition, this column could exhibit ion-exchange characteristics as part of the retention mechanism; the retention of the cations should be affected by a change in buffer concentration and pH.

3.2. Effect of buffer concentration

In addition to allowing for the detection of these ions by ELSD, the buffer concentration also has a pronounced effect on the chromatographic separation and peak shape. As can be seen in Fig. 2a, when the buffer concentration is increased from 10 to 200 mM ammonium acetate, the retention times of the counter-ions are drastically affected. This experiment was performed with 70% acetonitrile/30% ammonium acetate at a pH of 6.65, and a flow rate of 1 mL/min. Since the main

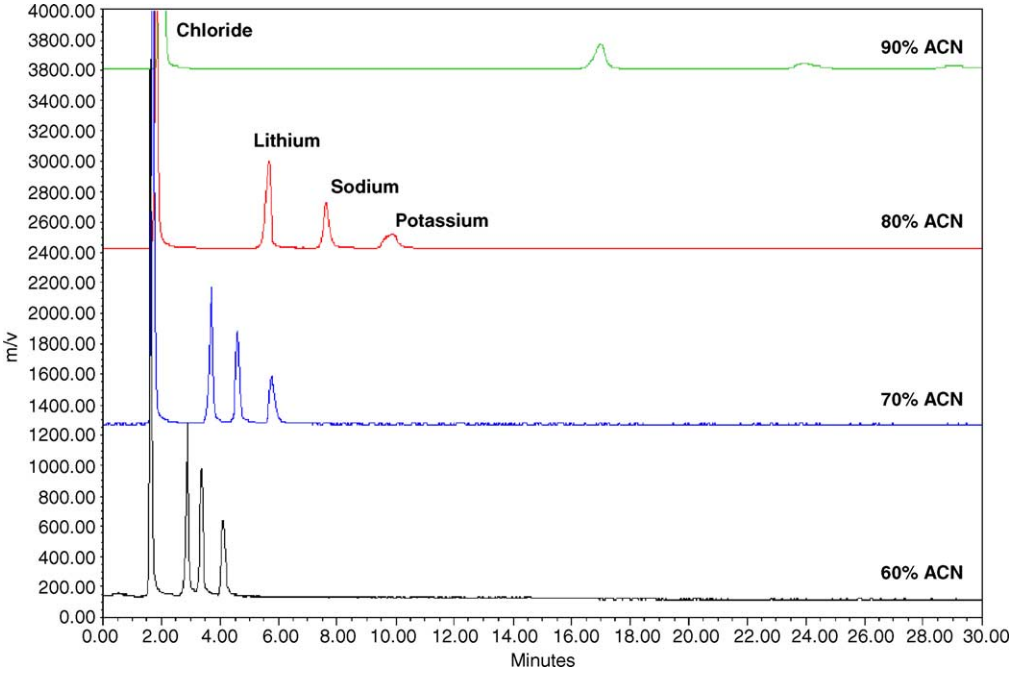

Fig. 1. HPLC–ELSD chromatograms illustrating the effect of increasing acetonitrile composition with 50 mM ammonium acetate, pH 6.55 buffer.

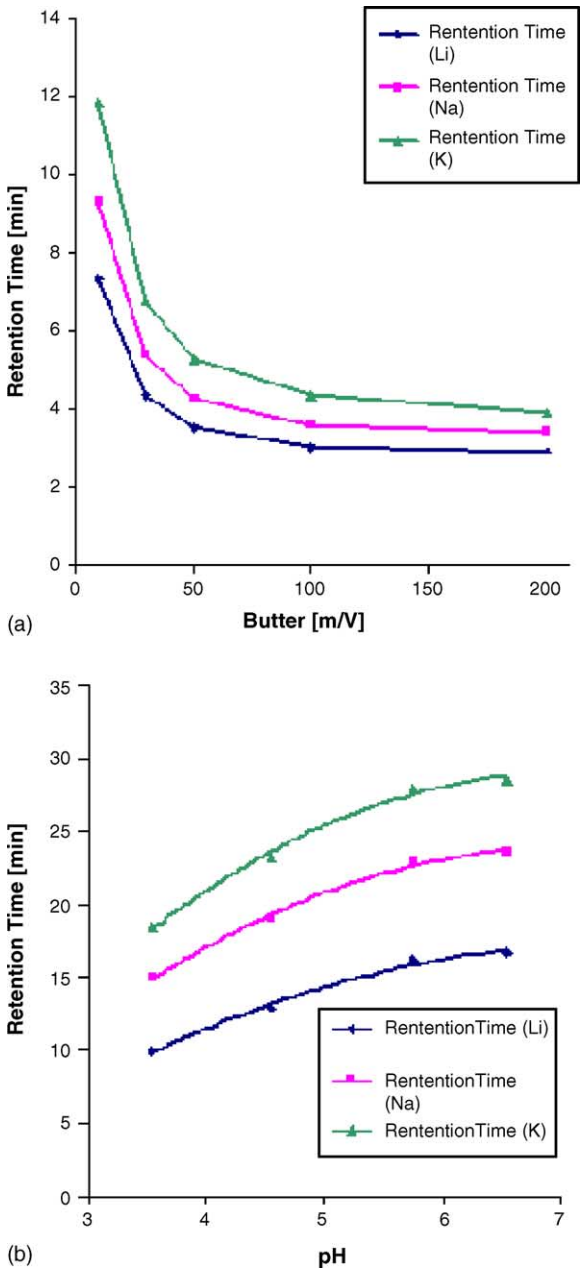

Fig. 2. Effect of increasing the (a) ammonium acetate buffer from 10 to 200 mM on the retention of Li^+, Na^+ and K^+; (b) pH of the buffer from the 3.5 to 6.5 on the retention of Li^+, Na^+ and K^+.

mechanism of interaction in the HILIC mode is based upon a partitioning of the ions into and out of the aqueous phase that forms a stagnant layer on the silica surface, the decrease in retention time may be best understood by a shift in equilibrium concentrations and ion exchange with active silanol sites. As the NH_4^+ concentration increases preferentially in the aqueous layer, there is less opportunity for the analyte counter-ions to partition into the aqueous layer based on the diffusion gradient. Increasing the buffer concentration competes with the active silanol sites, which decreases the ability of the positively charged analytes to interact with these sites. Thus, the ions are swept through the column (mainly in the organic layer) with less interaction with the column and the aqueous phase. In addition to retention-time effects, buffer concentration also impacts peak shape. With a very low buffer concentration (10 mM), the peak shapes exhibited fronting. As the buffer concentration was increased, the peak symmetry improved. This effect again supports that claim that ion exchange is part of the retention mechanism. An interesting side effect of buffer concentration was observed on the retention of the chloride ion that eluted very near the solvent front. As the buffer concentration was increased, the chloride retention increased; as was previously noted, the cation retention decreased. While the mechanism of the anion retention is not known at this time, this phenomenon could point to the feasibility of separating anions on this column at the same time as cations without the addition of modifiers.

3.3. Effect of pH

A similar, but not as marked, effect on retention is observed as the pH is lowered from 6.65 to 3.55 (Fig. 2b). The mobile phase in this case was 90% acetonitrile/10% 50 mM ammonium acetate (pH was adjusted with acetic acid) with a flow rate of 1 mL/min. The retention times of Li^+, Na^+, and K^+ were 16.7, 23.6, and 28.6 min, respectively, at a pH of 6.65. As the pH was lowered to pH 3.55, the retention times were reduced to 10.0, 15.0, 18.5 min, respectively, for Li^+, Na^+, and K^+. Although there was an impact on the retention of the analyte ions in relations to pH, the most notable effect of pH was the improvement in peak shape.

For example, at a pH of 6.65, Li^+ was observed to have a peak front with a USP tailing of 0.89; at pH 4.55 a peak with a USP tailing factor of 1.5 was observed; and at pH 3.55 Li^+ had good peak shape with a USP tailing factor 1.17. Both of these observations can be attributed again to active silanol sites and ion exchange. At a pH of 6.65 there are more silanol active sites present than at pH 3.55. The presence of active sites promotes the interaction of analyte cations with deprotonated silanols which is supported by the data that indicate improved tailing factors and decreased retention times at the lower pH. As can be seen in Figs. 1 and 2, organic content and buffer strength can be used in conjunction with pH to have a significant effect on retention time and peak shape, but the resolution between the peaks remains sufficient to perform the separation.

Through manipulation of these parameters, the separation of these ions alone could easily be accomplished in less than 1 min, perhaps less than 30 s. However, a counter-ion determination is desired which requires time for the typically non-polar compound to elute ahead of the counter-ion.

3.4. Effect of flow rate

In previous investigations with monolithic HPLC columns, it has been noted that there is a substantial loss in resolving power as the flow rate is increased. This phenomenon was observed to a greater degree as the molecular

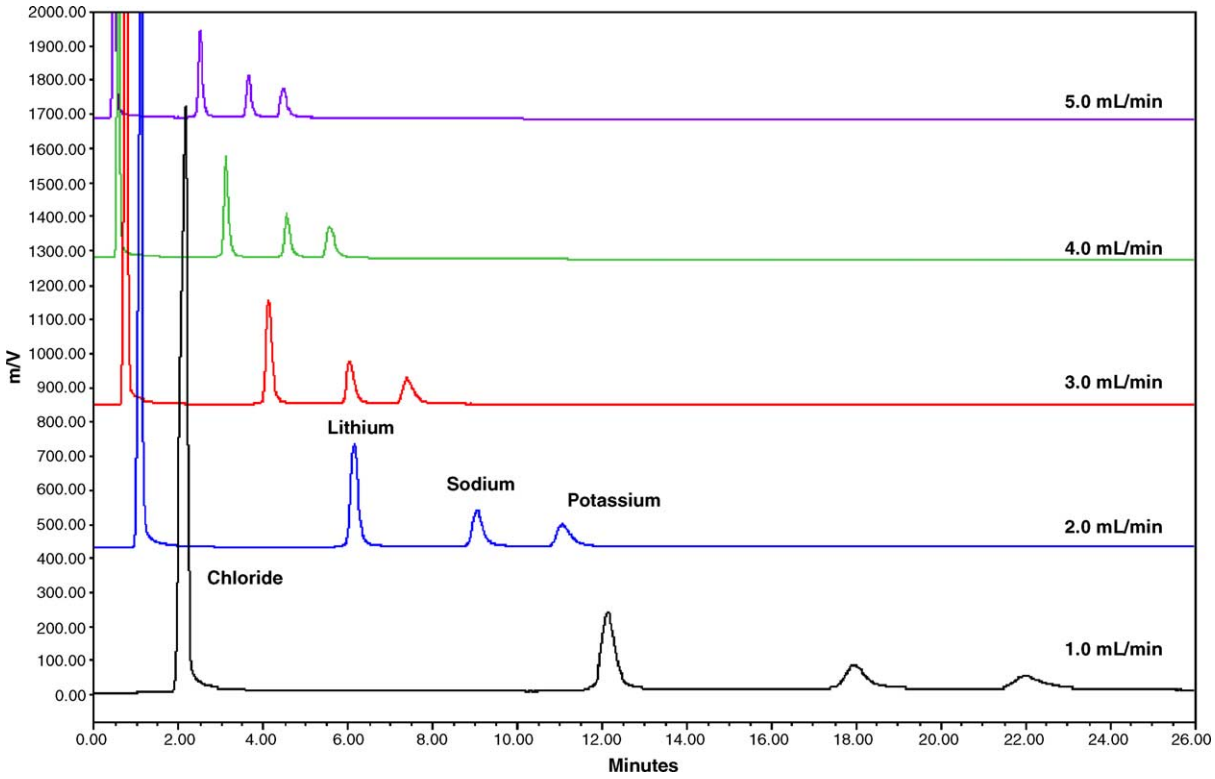

Fig. 3. HPLC–ELSD chromatograms illustrating the effect of flow rate from 1 to 5 mL/min on retention of Li^+, Na^+ and K^+.

weight of the compound increased. In the work by Gerber et al., 90% of the theoretical plates were lost at 5 mL/min [6]. In this investigation, where ions are detected, that loss in resolution should not be of significant concern since mass transport should be very rapid. In order to evaluate the effect of flow rate on the separation of these ions, 90% acetonitrile/10% 50 mM ammonium acetate adjusted to a pH 4.45 with acetic acid was utilized. These conditions allowed for significant change in retention time to be realized, while offering some of the benefits of good peak shape of a moderate pH and buffer strength. The flow rate was increased from 1 to 5 mL/min in increments of 1 mL/min.

Fig. 3 clearly shows the effect of flow rate on retention time. No attempts were made to optimize the ELSD for sensitivity at each flow rate. This experiment was intended solely to demonstrate the benefits of the monolithic column operated in the HILIC mode. If a low-level determination was required, the detector could be optimized for gas-flow pressure and desolvation temperature. As predicted, the decrease in retention time is directly proportional to the linear velocity of the mobile phase. However, there was a decrease in resolution and height equivalent theoretical plates as the flow rate was increased (Table 1). It is still possible that even for the analysis of small ions mass transfer is not sufficiently rapid at higher flow rates to maintain the same separation efficiency. For Li^+, there was approximately a 50% drop in theoretical plates. However, the resolving power at 5 mL/min is sufficient to do most counter-ion assays and the shortened run time can offer the advantage of high throughput. The resolution between the Li^+ and Na^+ was calculated to have a value of 8 at 1 mL/min and a value of 6 at 5 mL/min. The Na^+ and K^+ selectivity was calculated to be 4 at 1 mL/min and 3.5 at 5 mL/min. Values greater than 2 are generally considered acceptable. An important consideration at this point is the natural band broadening that occurs in any sample introduction system that employs a nebulizer and spray chamber design. The peak width, in this case, does decrease as the flow rate increases. However, there is a fundamental limit on peak sharpness that is dictated by the wash-out time of the spray chamber. There is a Gaussian contribution to peak

Table 1
Summary of chromatographic properties as the flow rate is increased from 1 to 5 mL/min

Flow rate (mL/min)	Back pressure (bar)	Li^+ theoretical plates	Na^+ theoretical plates	K^+ theoretical plates	Li^+–Na^+ resolution	Na^+–K^+ resolution
1.0	18	6647	7041	5184	8.12	3.99
2.0	34	5801	7093	4241	7.63	3.86
3.0	52	4211	6621	4622	6.96	3.65
4.0	72	3829	5090	4683	6.26	3.35
5.0	93	2798	4498	4270	5.77	3.31

shape from the chromatographic input into the nebulizer, but there is also an exponential decay function that is dictated by spray chamber volume and flow rate of the nebulizing gas that ultimately dictates peak width [27]. A better prediction of the effect of flow rate on resolution and theoretical plates would be made in a detection scheme where this inherent band broadening does not persist. One important characteristic of the monolithic column is that the pressure drop (ΔP) across the column increased from 18 to 93 bar when going from 1 to 5 mL/min, respectively (Table 1). Based upon ΔP considerations and resolution requirements, higher flow rates would be feasible. One inherent advantage of the monolithic column that was discovered to be of great benefit is the short equilibration time. Since the equilibration time is a function of the number of column volumes that are passed through the column, high flow rates can expedite this process. Even if a moderate flow rate of 2 mL/min is used during method development a much higher flow rate could be utilized for equilibration. Thus, more experiments can be performed in a shorter amount of time, which ultimately can improve lab efficiency.

3.5. Counter-ion determination

This work was concluded by determining the Na^+ concentration in several pharmaceutically relevant salts by using the monolithic silica column operated in the HILIC mode in conjunction with ELSD. Naproxen, warfarin, and a new chemical entity from Eli Lilly and Company were chosen as representative salts. Optimized HPLC–ELSD conditions using a mobile phase of 80% acetonitrile/20% 50 mM ammonium acetate (pH 3.75 with acetic acid) were chosen based upon previous data. Since a counter-ion determination was performed, a slightly longer (2.5 min) run was desired for adequate separation of the compound and its salt. The linearity of sodium standards was first evaluated. A coefficient of determination of 0.994 was observed over the range of 0.0004–0.9 mg/mL Na^+ with slight non-linearities occurring at both the low and the high end of the curve. These non-linearities were expected and are solely a function of the detector. However, there is usually no need to have an extended range such as this when performing counter-ion determinations. The concentration of the free acid can be manipulated to place the counter-ion in the linear working range of the detector. For this study, a range of 0.05–0.15 mg/mL Na^+ was utilized and the coefficient of determination was 0.999. The target Na^+ concentration of the counter-ion was 0.1 mg/mL. A limit of detection (LOD) using 3σ was calculated at 0.1 μg/mL for Na^+.

As can be seen from Fig. 4, the Li^+ and K^+ counter-ions would also be separated under these conditions. The data in Table 2 is a summary of the Na^+ determination for the salts. The column labeled individual preparations shows the assay precision and accuracy when five replicates of the sodium salt are individually weighed out. The column labeled instrument precision represents one solution preparation that has been reinjected five times. There is very close agreement between these numbers. The number in parenthesis represents the expected Na^+ value when the compound weight is corrected for potency. For warfarin, the theoretical Na^+ concentration was 6.275%. With the individual preparation scheme, an R.S.D. of 1.7% was observed, while an instrument pre-

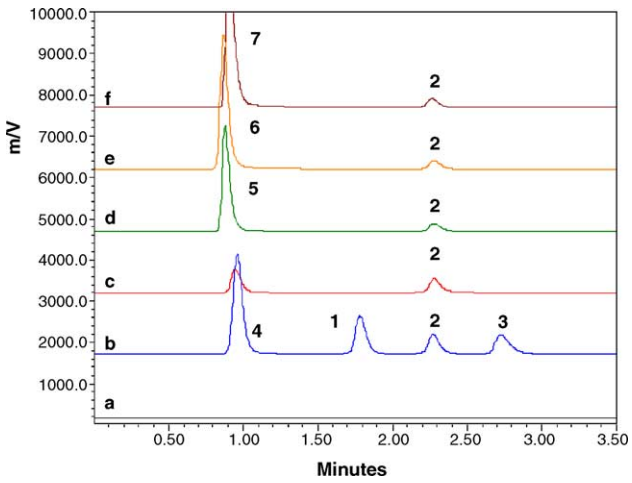

Fig. 4. HPLC–ELSD chromatograms of (a) sample solvent (blank); (b) counter-ion mixture; (c) sodium chloride standard; (d) naproxen sample; (e) warfarin sample; (f) new chemical entity sample. Peak identification: (1) Li^+; (2) Na^+; (3) K^+; (4) Cl^-; (5) naproxen; (6) warfarin; (7) new chemical entity. Other condition described in the text.

Table 2
Percentage of sodium determined by HPLC–ELSD

Sample	Sodium (%) individual preparations	Sodium (%) instrument precision	Theoretical value (%)
Warfarin	6.11	6.44	6.8 (6.11)[a]
	6.32	6.32	
	6.23	6.39	
	6.14	6.37	
	6.05	6.48	
Average	6.17	6.40	
R.S.D. (%)	1.72	0.97	
% Error	0.98	4.75	
Naproxen	8.75	8.50	9.12[b]
	8.39	8.40	
	8.59	8.39	
	8.59	8.42	
	8.53	8.52	
Average	8.57	8.45	
R.S.D. (%)	1.51	0.71	
% Error	−6.03	−7.39	
New chemical entity (NCE)	4.91	4.76	5.1 (4.96)[a]
	4.70	4.73	
	4.80	4.65	
	4.86	4.70	
	4.87	4.85	
Average	4.83	4.74	
R.S.D. (%)	1.69	1.58	
% Error	−2.66	−4.48	

[a] Corrected for volatiles and purity.
[b] Not corrected for volatiles and purity.

cision of 0.97% R.S.D. was obtained. A precision of 1–2% is typical for this HPLC–ELSD system. The percent error between the experimentally determined Na^+ concentration (individual preparations) and theory was found to be 1.7%. For the new chemical entity from Eli Lilly and Company, a precision of 1.69% was obtained and an accuracy of determination was a −2.66% error. For naproxen, the exact potency for the sodium salt was not available. Based on a 100% purity value, the Na^+ concentration would be 9.12%. Based on this value, the Na^+ determination in this case produced an error of −6.03% and a precision of 1.51% R.S.D. As can be seen from the data in this table, the experimentally determined salt values agree very well with theory. In general, the calculated Na^+ composition was within 3% of theory. Thus, this separation and detection scheme is appropriate for quantitation of the Na^+ counter-ion from a pharmaceutical salt form.

4. Conclusion

A monolithic silica column operated in the hydrophilic interaction chromatography mode in conjunction with an evaporative light scattering detector was evaluated. Lithium, sodium and potassium were the test counter-ions for this evaluation. Chromatographic properties of this column were determined by varying key mobile phase parameters, such as pH, flow rate, buffer strength, and organic concentration. It was found that all of these optimization parameters had a pronounced effect on retention, but also had an effect on peak shape. The mechanism of retention is believed to be a combination of factors. The HILIC effect was observed by an increase in retention with an increase in organic concentration. However, there was an ion-exchange mode of separation that was observed when pH and buffer concentration were altered. Therefore, it is important to consider these three important parameters before the flow rate is optimized for speed of analysis. After examining the changes in retention and resolution, a somewhat optimized method was established and then further evaluated for linearity, reproducibility, and limit of detection. Three pharmaceutically relevant salts were evaluated for Na^+ concentration that was within 3% of theory when the compound was corrected for purity. This work demonstrated that monolithic silica column technology has several advantages when operated in the HILIC mode for the separation of counter-ions with the most prevalent advantage being reduced analysis time.

References

[1] N. Tanaka, H. Kobayashi, N. Ishizuka, H. Minakuchi, K. Nakanishi, K. Hosoya, T. Ikegami, J. Chromatogr. A 965 (2002) 35.
[2] N. Tanaka, H. Kobayashi, K. Nakanishi, H. Minakuchi, N. Ishizuka, Anal. Chem. 73 (15) (2001) 420A.
[3] H. Zou, X. Huang, M. Ye, Q. Luo, J. Chromatogr. A 954 (2002) 5.
[4] Q. Xu, K. Tanaka, M. Mori, M.I.H. Helaleh, W. Hu, K. Hasebe, H. Toada, J. Chromatogr. A 997 (2003) 183.
[5] P. Hatsis, C.A. Lucy, Anal. Chem. 75 (2003) 995.
[6] F. Gerber, M. Krummen, H. Potgeter, A. Roth, C. Siffrin, C. Spoendlin, J. Chromatogr. A 1036 (2004) 127.
[7] A.J. Alpert, J. Chromatogr. 499 (1990) 177.
[8] L.A.Th. Verhaar, B.F.M. Kuster, J. Chromatogr. 234 (1) (1982) 57.
[9] P. Orth, H. Englehardt, Chromatographia 15 (2) (1982) 91.
[10] C. McClintic, D.M. Remick, J.A. Peterson, D.S. Risley, J. Liq. Chromatogr. Rel. Technol. 26 (18) (2003) 3093.
[11] B.A. Olsen, J. Chromatogr. A 913 (1–2) (2001) 113.
[12] S.C. Churms, J. Chromatogr. A 720 (1–2) (1996) 75.
[13] D.S. Risley, M.A. Strege, Anal. Chem. 72 (8) (2000) 1736.
[14] A. Stolyhwo, H. Colin, G. Guiochon, J. Chromatogr. 265 (1) (1983) 1.
[15] W.S. Letter, J. Liq. Chromatogr. 15 (2) (1992) 253.
[16] A. Stolyhwo, M. Martin, G. Guiochon, J. Liq. Chromatogr. 10 (6) (1987) 1237.
[17] R. Macrae, L.C. Trugo, J. Dick, Chromatographia 15 (7) (1982) 476.
[18] P.D. Green, H. Meng, J.E. Seely, Polymer Preprints (American Chemical Society Division of Polymer Chemistry) 38 (1) (1997) 608.
[19] P.A. Asmus, J.B. Landis, J. Chromatogr. 316 (1984) 461.
[20] J.A. Peterson, L.J. Lorenz, D.S. Risley, B.J. Sandmann, J. Liq. Chromatogr. Rel. Technol. 22 (7) (1999) 1009.
[21] D.S. Risley, J.A. Peterson, J. Liq. Chromatogr. 18 (15) (1995) 3035.
[22] M. Rajevic, P. Betto, J. Liq. Chromatogr. Rel. Technol. 21 (18) (1998) 2821.
[23] D.S. Risley, K.F. Hostettler, J.A. Peterson, LC-GC 16 (6) (1998) 562.
[24] C.E. Kibbey, Molecular Diversity 1 (4) (1995) 247.
[25] J.A. Peterson, D.S. Risley, J. Liq. Chromatogr. 18 (2) (1995) 331.
[26] D.S. Risley, J.A. Peterson, K.L. Griffiths, S. McCarthy, LC-GC 14 (12) (1996) 1040.
[27] B.W. Pack, S.J. Ray, R.A. Potyrailo, G.M. Hieftje, Appl. Spectrosc. 52 (12) (1998) 1515.