Study on an Hydrophilic Interaction Electrochromatography Method for Separation of Sulfonamide Antibiotics

Sülfonamid Antibiyotiklerin Ayırımı için Hidrofilik Etkileşim Elektrokromatografi Yönteminin Kullanımı

Research Article

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ABSTRACT

This study describes the preparation of a hydrophilic monolithic column and its application to sulfonamide antibiotics. The column was prepared by single step in situ polymerization of 2-hydroxyethyl methacrylate (HEMA), ethylene dimethacrylate (EDMA) and methacrylic acid (MAA) in a binary porogenic solvent consisting of toluene and 1-dodecanol, inside a 100 μm-i.d. capillary. The resulting monolith was electrochromatographically characterized as well as SEM. The prepared column showed hydrophilic behaviour using thiourea and toluene as markers. The SEM images showed that the monolithic column composed of spherical particles of approximately 2 μm in diameter. Using this hydrophilic monolith as stationary phase, hydrophilic interaction electrochromatography of sulfonamide antibiotics as a new method was developed. The method was successfully used for the separation of sulfonamide antibiotics. Some parameters including acetonitrile (ACN) content, pH and ionic strength on the separation of the sulfonamides, namely sulfaprydine, sulfadiazine, sulfamethazine, sulfisoxazole and sulfadoxine were also investigated. A typical hydrophilic interaction separation mechanism was revealed at higher organic solvent content (ACN > 60%).

Key Words

Capillary electrochromatography; hydrophilic monolith; sulfonamide antibiotics, retention mechanism.

ÖZET

Bu çalışma hidrofilik monolitik kolonların hazırlanması ve sülfonamid antibiyotiklerin ayırımında kullanımını içermektedir. Kolon, 2-hidoksietil metakrilat (HEMA), etilen dimetakrilat (EDMA) ve metakrilik asitin (MAA) toluen ve 1-dodekanol çözücü çifti eşliğinde, 100 μm iç çaplı (i.d) kapiler kolon içinde tek basamaklı olarak sentezlenmesiyle hazırlandı. Monolitik kolon, hem elektrokromatografik olarak hemde taramalı elektron mikroskobu (SEM) ile karakterize edildi. Hazırlanan kolon tiyoüre ve toluen'in işaretçi olarak kullanıldığı kromatografi sisteminde hidrofilik davranış göstermektedir. SEM görüntüleri, monolitik kolonun yaklaşık 2 μm çapında küresel partiküller içerdiğini göstermektedir. Hazırlanan hidrofilik monolitin, sülfonamidlerin elektrokromatografik ayırımında sabit faz olarak kullanımı yeni bir metod olarak geliştirilerek ayırım işlemi başarılı bir şekilde gerçekleştirildi. ACN miktarı, pH, iyonik şiddet gibi bazı parametrelerin sülfopiridin, sülfodiazin, sülfometazin, sülfisoksazol ve sülfadoksin gibi antibiyotiklerin ayırımına etkisi de incelendi. Yüksek organik solvent (ACN>60%) içeriğinde tipik bir hidrofilik etkileşim mekanizması gözetlendi.

Anahtar Kelimeler

kapiler elektrokromatografi, hidrofilik monolit, sülfonamid antibiyotikler, alıkonma mekanizması

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INTRODUCTION

Hydrophilic interaction chromatography (HILIC) has been attracting researchers for over two decades and has become an interesting alternative for analysis of polar biomolecules. It is a complementary technique to reverse phase chromatography when complex sample mixtures are being analyzed. The development of HILIC stationary phases is of great significance. Polymer and silica-based monoliths have widely been prepared and applied for hydrophilic interaction electrochromatography (HI-CEC) [1-3]. Polymer based monoliths include polystyrene, polyacrylamide and polymethacrylate. Among these, polymethacrylate based monoliths (PMMCs) are used for different electro-liquid chromatographic applications [4-7]. Recently, hydrophilic PMMCs of different kinds including neutral, anionic, cationic, zwitterionic and amphoteric monoliths have been developed for HI-CEC. For example, Zhong and El Rassi prepared polar and neutral monolithic column for HI-CEC [8]. Lammerhofer et al. prepared a novel hydrophilic monolith using 2-dimethylaminoethyl methacrylate, HEMA and EDMA in the presence of porogenic mixture of dodecanol and cyclohexanol [9]. Jiang et al. reported PMMC based zwitterionic column for the separation of polar analytes [10]. The studies of HILIC monoliths have been continued with increasing attention [11-14].

Pharmaceutical compounds are often polar. Sulfonamides are the first antimicrobial group of drugs used in medical treatment. These are anti-bacterial and anti-infective compounds used widely in veterinary practice. Some anlytical methods were developed for sulfonamides and pharmaceuticals using electrochromatography techniques [15-17]. These types of hydrophilic compounds are often insufficiently retained on reverse phase (RP) chromatographic columns.

In order to achieve adequate retention of polar pharmaceutical compounds such as sulfonamides highly aqueous mobile phases are often required, which can lead to a number of issues such as bubble formation in electrochromatography and interruption of the separation process and even the stationary phase collapse in RP column. An alternative to RP column is the use of hydrophilic stationary phase, which exhibit a different retention mechanism. However, few studies have investigated the application of polar pharmaceuticals using hydrophilic stationary phases in electrochromatography. To date, there has been no report of hydrophilic interaction electrochromatography of sulfonamide antibiotics. Therefore, this study first reported hydrophilic interaction application of the sulfonamide antibiotics using the hydrophilic monolithic capillary column.

The study on which we report here involved the preparation and characterization of a hydrophilic monolithic column with a relatively strong cathodic EOF. The monolithic column was prepared by single step in situ polymerization of 2-hydroxyethyl methacrylate (HEMA), ethylene dimethacrylate (EDMA) and methacrylic acid (MAA) in a binary porogenic solvent consisting of toluene and 1-dodecanol. The ratio of MAA and HEMA to EDMA and the composition of the pore forming solvent was systematically altered and optimized to obtain satisfactory hydrophilicty. The resultant monolithic column was characterized and applied to the separation of sulfonamide antibiotics

MATERIALS AND METHODS

Materials

HEMA, EDMA, 3-trimethoxysilylpropyl methacrylate (TMSPM), Sulfaprydine (SPRY), Sulfadiazine (SDZ), Sulfamethazine (SMZ), Sulfisoxazole (SXZ) and Sulfadoxine (SDX) were purchased from Sigma-Aldrich Chemical (Milwaukee, WI, USA). Fused-silica capillary (i.d.100 μm and, o.d. 375 μm) was supplied by Polymicro Technologies (Phoenix, AZ, USA). ACN and methanol were of HPLC grade and supplied by Merck A.G (Darmstadt, Germany). MAA was also obtained from Merck A.G. All the test compounds were of analytical grade.

Electrochromatography

The phosphate buffer solution (10 mM) was prepared and pH was adjusted to the desired value with 0.2 M HCl or 0.2 M NaOH. Mobile phases were prepared by mixing appropriate volumes of ACN and buffer solution, and were degassed in an

ultrasonic bath for 10 min before use. Before an experiment, the monolith was conditioned with the mobile phase for 30 min. The standard solutions for each analyte of sulfonamides were prepared in the range of 0.1-1.0 mg/mL in phosphate buffer and stored at -4°C. The capillary column of 27 cm in effective length and 36 cm in total length was used in the experiments. Prior to analysis in CEC system, the monolithic column was conditioned by mobile phase 65/35% (ACN/PB, 10 mM, pH 4.0) for 2 h.

Preparation of the hydrophilic column

In order to covalently anchor the polymer to the capillary wall, the capillary column was pretreated with a vinyl silanizing agent according to our previous study [3]. To synthesize hydrophilic monolithic column, MAA, HEMA, EDMA and AIBN were used as monomer, co-monomer, crosslinker and thermal initiator, respectively. The monomer solution was prepared by mixing MAA (41.3%, w/w), HEMA (20.5%, w/w), EDMA (38.2%, w/w), and AIBN (0.1%, w/w) in the porogen solution comprised of toluene (79.5%, w/w) and dodecanol (20.5%, w/w). The solution was filled by applying an external pressure of 0.75 bar in CEC. Then, the capillary was plugged at both ends with GC septa and submerged into a thermostatic bath at 60°C for 8 h. After polymerization, the capillary monolithic column was connected to μHPLC pump and washed with methanol for 1 h to remove unreacted reagents. A window section of length 1 cm centred 28.5 cm from the column input end was created by removing the polyimide coating on the fused silica capillary, using a microtorch.

Instrumentation

The experimental studies were performed using a Prince CEC-760 (Prince Technologies B.V. Cornelis Houtmanstraat, Netherlands) equipped with a photodiode array detector and a high voltage power supply (-30 kV and +30 kV). The Ultimate 3000 Chromatography System with ultimate 3000 pump (Dionex Technologies, Munich, Germany) was used to flush and condition the monolithic column.

RESULTS AND DISCUSSION

Synthesize and characterization of hydrophilic monolithic column

In order to synthesize a hydrophilic monolith, the incorporation of polar monomers into the monoliths is necessary. The functional monovinyl monomers such as MAA and HEMA were used for this purpose. MAA was used as hydrophilic weak cation exchange monomer, which can generate cathodic EOF in the column. HEMA provides hydrophilic character for monolith composition. Although these type of hydrophilic column is reported by the previous works [9,18], the preparation process of this column was quite simple. The another difference is that the preparation procedure of the hydrophilic monolith was different with respect to porogen ratios and polymerization temperature. This new synthesis method was confirmed by the morphological properties of the prepared column as well as SEM images. Because the mixture of toluene and dodecanol acted as a good solvent, toluene and dodecanol were used as a binary porogenic system. To investigate the influence of the monovinyl monomers on the preparation of the hydrophilic column, while binary porogenic system was kept constant at toluene and dodecanol 4:1 (w/w), the ratios of HEMA/MAA (w/w) was changed. The obtained results showed that the back pressure of monolithic column was increased with increasing HEMA content in monolithic structure and the column permeability decreased. This result could be explained that the increasing content of HEMAin polymeric mixture caused to the formation of microglobules, and thus led to high back pressure. Using the column with the molar ratio of HEMA:MAA 1:2 (v/v) respectively, the values of the back pressure and permeability of the column were optimized.

Several parameters can influence the porous structure of the monolithic column including reaction temperature, porogenic solvent and ratio of monomer to crosslinker. Briefly, monolithic column was prepared according to the procedure containing monomer to crosslinker ratio as 4:1. Total monomer ratio to porogen amount was estimated as 1:1.5 (v/v). AIBN (10 mg) was added to the mixture as initiator. The polymerization

temperature was performed at 60°C for 8 h.

In order to investigate the chromatography properties of the prepared monolith, toluene and thiourea were used as test compounds. When ACN content increased from 60 to 90 %, toluene, which is treated as a nonpolar compound, eluted first. By contrary, thiourea eluted after toluene (data not shown). This result demonstrates hydrophilic interaction mechanism at higher ACN content.

A very important characteristic of a column is its permeability K, which represents the resistance to mobile phase flow through the monolithic column. K can be determined by pumping different solvents through the column at different linear flow rates. The monolithic column permeability K, was measured by flowing methanol solution through the column. Permeability was calculated using Eq. (1) ;

$$K=(F\eta L)/(\pi r^2 \leftarrow P) \quad (1)$$

where, F is flow rate, η is viscosity, L is length of the column, r is internal radius of the column and ΔP is pressure drop.

The pressure drop across the columns was measured as a function of linear velocity using methanol, the results showed a good permeability for monolithic column. Pressure drop versus the velocity of the fluid shows a linear relationship, this indicates that permeability and mechanical stability of the prepared monolithic stationary phases are excellent. Pressure drop increased with increasing flow rate of from 1 μL/min to 5 μL/min of methanol as linearly (R^2=0.99).

Figure 1 shows the backpressure values of the monolithic column. At a flow rate of 1 μL/min, the pressure drop of the column (id. 100 μm; effective length: 27 cm; total length: 36 cm) was 18.4 bar. The permeability was calculated as $5.6.10^{-12}$ m^2, means good permeability of the monolithic column. with this flow rate. The scanning electron

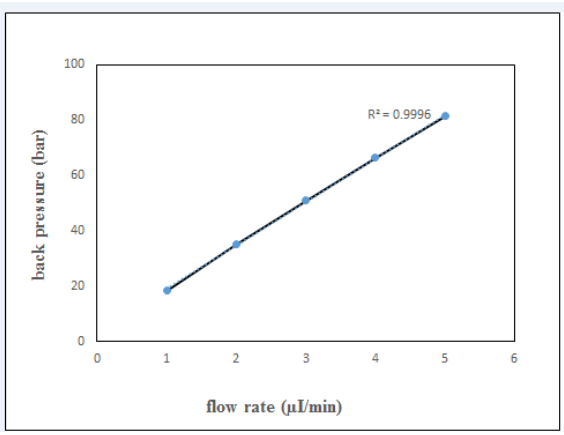

Figure 1. The effect of flow rate on the back pressure of the column; mobile phase: methanol; flow rate: 1-5 μL/min; T:25 °C.

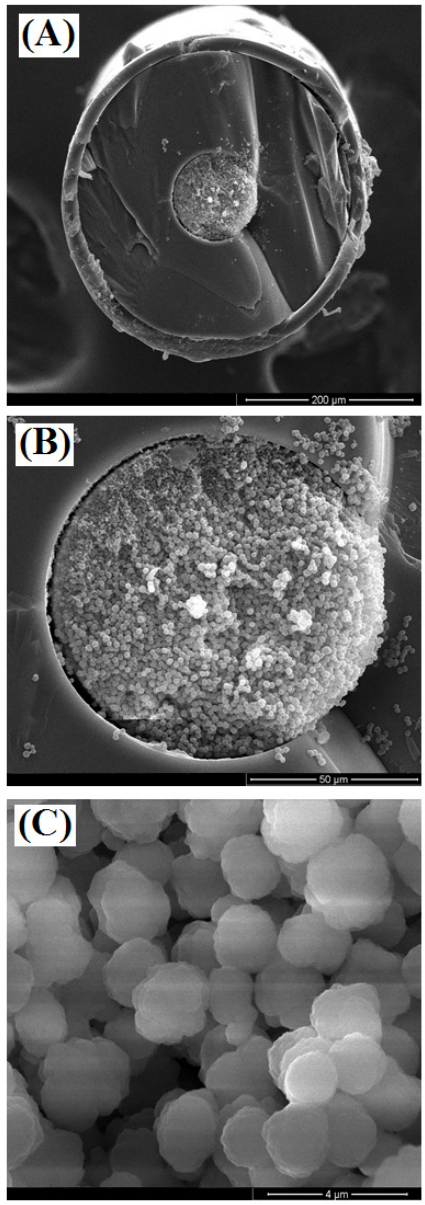

Figure 2. SEM images of the poly(MAA-HEMA-EDMA) monolithic column at magnification of A) 600x B) 2400x and C) 25000x.

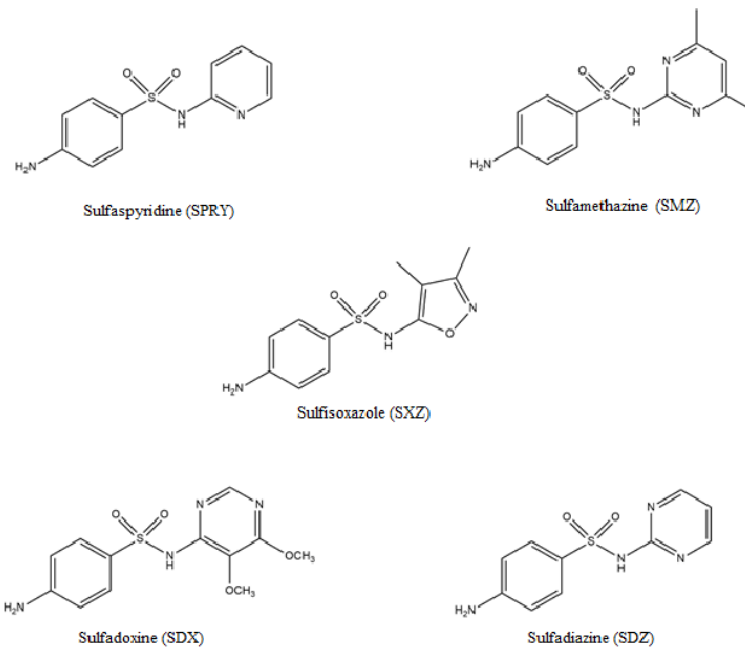

Figure 3. The structures of sulfonamide antibiotics used in this study.

microscope (SEM) images of the poly(HEMA-EDMA- MAA) monolithic column (Figure 2A-C) show the copolymerized monolith composed of spherical microglobules (2 μm) agglomerated into larger clusters interdispersed by large-pore channels, which are characteristic structure of monolithic columns.

Hydrophilic interaction electrochromatography of sulfonamide antibiotics

Five sulfonamides, the structure of which were given in Figure 3 were selected as test compounds. Depending on their pKa values and the pH of the electrolyte solution, they can be separated as either positively charged, negatively charged or neutral components. Sulfonamides can be converted into anions and cations depending on the solution pH.

At low pH values few carboxyl groups in MAA structure were ionized and consequently low electroosmotic flow (EOF) was observed. The retention time of the sulfonamides increased with increasing pH value, however sulfonamide compounds could not be separated well at pH values higher than 4. Also some of them did not elute at higher pH values. All sulfonamides are almost neutral at pH 4.0 (SPRY pKa1: 2.72 pKa2: 8.40; SDZ pKa1: 2.10 pKa2: 6.72; SMZ: pKa1: 2.28 pKa2: 7.42; SXZ pKa1: 2.4 pKa2: 5.0; SDX pKa1: 2.38 pKa2: 5.77) and they were expected to be separated mainly by means of hydrophilic interaction using the hydrophilic column. Table 1 shows the retention times of sulfonamides at different pH values (10 mM Phosphate buffer, 80% ACN). As can be seen that some sulfonamides were not eluted at weak acidic and neutral pH values. These results can be explained on the bases of EOF and charge of sulfonamides. The carboxylic acid groups on the surface afford to generate cathodic EOF and provide cation-exchanger interaction sites simultaneously. The

Table 1. The retention times (in min) of sulfonamide antibiotics at different pH. The conditions were: mobile phase 80:20 % ACN/Phosphate buffer (10 mM), electrokinetic injection: 12 kV, 5 s applied voltage: 20 kV, detection wavelength: 200 nm.

pH	SPRY	SDZ	SMZ	SXZ	SDX	ACN%
3	1.9	4.25	7.0	8.2	10.45	80
4	1.9	4.35	7.9	8.4	10.65	80
5	-	4.75	8.6	9.8	-	80
6	-	-	9.2	-	-	80
7	-	-	10.40	-	-	80

cathodic EOF was greater at pH 7.0 as more carboxylic acid groups of MAA were ionised (pKa 4.35). But, most of the compunds were negatively charged at this pH, they would be migrating in an opposite direction to the EOF. Some sulfonamides couldn't be eluted with the increasing pH in spite of greater EOF. The longest retention times were observed at pH 7.0.

These results should be explained that when the buffer pH incresed to pH 7.0, sulfonamides became deprotonated and therefore negatively charged and more hydrophilic, thus leading to stronger hydrophilic interaction and potential electrostatic repulsion, as well as the effect of electrophoretic mobility which was opposite to the direction of EOF, and thus higher retention.

Low EOF was observed due to ionization suppression of MAA associated with acidic conditions. Retention time of sulfonamides decreased also at low pH values. The unexpected behaviour can be attributed was mainly on hydrophilic interaction mechanism or no repulsion effect between sulfonamides and MAA. On the other hand, we observed that the sulfonamides with high degree of ionisation couldn't be injected electrophoretically to the prepared column. The results were similar reported by Dube et al. [16].

Table 2. The retention times (in min) of sulfonamide antibiotics with different ACN ratio at pH 4.0. The conditions were: mobile phase 60-80 % ACN/Phosphate buffer pH 4.0 (10 mM), electrokinetic injection: 12 kV, 5 s applied voltage: 20 kV, detection wavelength: 200 nm.

pH	SPRY	SDZ	SMZ	SXZ	SDX	ACN%
4.0	1.0	3.50	6.90	7.30	9.80	60
4.0	1.3	3.70	7.25	7.75	10.20	70
4.0	1.9	4.35	7.90	8.40	10.65	80

Figure 4 shows the electrochromatogram of the sulfonamides at pH 4.0. A typical hydrophilic separation mechanism of the sulfonamide antibiotics was observed at a high percent of organic mobile phase (ACN>60%) on the prepared hydrophilic column, the retention time of the compounds also increased with increasing ACN content in mobile phase. The separation of the solutes was succesfully achieved when ACN ratio increased from 60% to 80% in the mobile phase can be seen in Table 2 (80:20 ACN/phosphate buffer pH 4.0, 10 mM). Retention mechanism was governed primarily by hydrophilic interaction between the solutes and the stationary phase due to lower EOF at this pH. Baseline separation was obtained at ACN concentration higher than 80%.

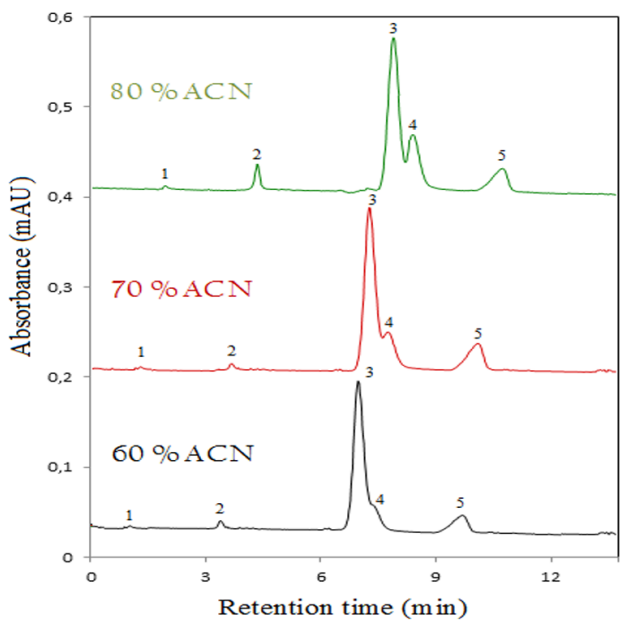

Figure 4. The electrochromatograms of the sulfonamide antibiotics. The conditions were: mobile phase 60-80 % ACN/ Phosphate buffer (pH 4.0, 10 mM); electrokinetic injection: 12 kV, 5 s; applied voltage: 20 kV; detection wavelength : 200 nm; Peaks: (1) SPRY (2) SDZ (3) SMZ (4) SXZ (5) SDX.

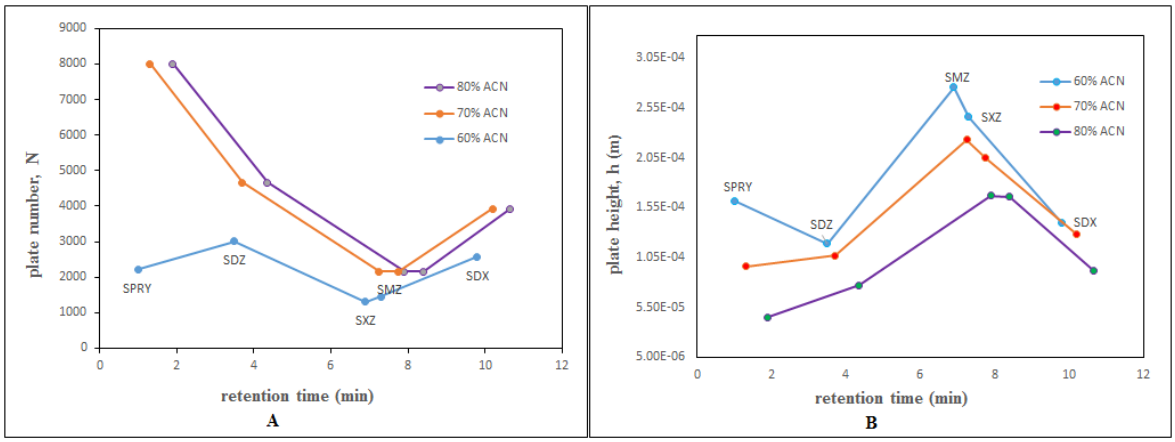

Figure 5. Column efficiency; A) Theoretical plate number B) Theoretical plate height . The conditions were: mobile phase 60-80 % ACN/Phosphate buffer (pH 4.0, 10 mM); compounds: SPRY, SDZ, SMZ, SXZ, SDX.

The ionic strength effect of the sulfonamides was also investigated. However, the separation mechanism showed no ion exchange mechanism in the range of 10-40 mM (data not shown). The appropriate concentration was chosen as 10 mM. The behaviour of this type of the hydrophilic monolithic column can be explained as a typical HILIC mode. The elution order of the compounds were SPRY, SDZ, SMZ, SXZ and SDX, respectively.

At high percentage of ACN (80:20 ACN/ phosphate buffer pH 4.0, 10mM) the theoretical plate number N, increased with the decreased theoretical plate height (h) for each compound, (from 60% to 80%ACN). Volume fraction of mobile phase affects the ionization of MAA. So the less protonation degree of MAA at high ACN content results longer retention times. Maximum and minumum plate numbers were estimated as a 8000 and 2161 for SPRY and for SMZ respectively at high ACN content in the mobile phase.
When the ratio of ACN in mobile phase increased from 60% to 80%, plate number increased for each compound. Signals was narrower at the high ratio of ACN resulted in increment of theoretical plate number. Because of low plate number for SMZ and SXZ (2161, 2164), the column efficiency was poor to separate these sulfonamides from each other. Relatively low plate number compared to electrochromatography indicates that the separation results primarily from the hydrophilic interactions, while electrophoretic migration contributes only slightly. But the separation of the other sulfonamides from each other was successfully achieved.

The theoretical plate number (N) was calculated using Eq. (2) for a column with a length of L. In Eq. (2), t_r and $w_{0.5}$ are the retention time and peak width at half-height, respectively. The plate height (h) was found by using Eq. (3). These calculations were performed for each component included in the sample mixture of sulfonamides. Figure 5 shows the plate number and height for each compound.

$$N = 5.54(t_r/w_{0.5})^2 \quad (2)$$

$$h = L/N \quad (3)$$

The reproducibilty of the poly (MAA-HEMA-EDMA) monolith was assessed through the percent relative standard deviation (RSD) for the retention factor of all five sulfonamide antibiotics. The run-to-run reproducibility (n=10) of SPRY, SDZ, SMZ, SXZ and SDX are 0.13, 0.28, 0.43, 0.51 and 0.67 respectively. The day-to-day reproducibilities (n=3) for SPRY, SDZ, SMZ, SXZ and SDX are 1.20, 1.52, 1.47, 1.62 and 1.34 respectively. This confirms the robustness of the monolithic column since its separation ability does not destroy either with time or number of injections. In order to evaluate column to column reproducibilty, we prepared five monolithic columns under same polymerization conditions. Column-to-column (n=5) reproducibility measurements gave RSD values for retention factor (k) of all sulfonamide antibiotics less than 3% and for peak area less

than 5%. The results reveal that the prepared HILIC column is highly robust and reliable.

CONCLUSIONS

A hydrophilic monolithic column was synthesized and its application to the sulfonamide antibiotics were successfully performed. The prepared hydrophilic monolith was composed of porous spherical microglobules with average diameter of 2 μm and showed good permeability. The hydrophilic interaction of sulfonamide antibiotics with the monolithic column was demonstrated and the good column efficiency at high ACN content of the mobile phase was obtained. The reproducibility studies of the hydrophilic monolith showed good efficiency and stability.

References

1. T. Ikegami, K. Tomomatsu, H. Takubo, K. Horie, N. Tanaka, Separation efficiencies in hydrophilic interaction chromatography, J Chrom A, 1184 (2010) 474.
2. Que A.H, Konse T, Baker A.G, Novotny, M.V Analysis of bile acids and their conjugates by capillary electrochromatography/electrospray ion trap mass spectrometry, Anal Chem., 72 (2000) 2703.
3. M.L. Ye, H.F. Zou, R.N. Wu, H.Q. Fu, Z.D. Lei, Modeling and optimization for separation of ionic solutes in pressurized flow Capillary electrochromatograph, J Sep Sci., 25 (2002) 416.
4. C. Aydoğan, A. Denizli, Electrochromatographic Enantioseparation of Amino Acids Using Polybutylmethacrylate-based Chiral Monolithic Column by Capillary Electrochromatography, Chirality, 24 (2012) 606.
5. C. Aydoğan, A. Denizli, Preparation and electrochromatographic characterization of methacrylate-based weak cation exchange columns for capillary electrochromatography, Analyst, 138 (2013) 2118.
6. C. Aydoğan, A. Tuncel, A. Denizli, Polymethacrylate-based monolithic capillary column with weak cation exchange functionalities for capillary electrochromatography, J Sep Sci., 35 (2012) 1010.
7. C. Aydoğan, F. Yılmaz, A. Denizli A, Cation exchange/hydrophobic interaction monolithic chromatography of small molecules and proteins by nano liquid chromatography, J Sep Sci., 36 (2013)1685.
8. H. Zhong, Z. El Rassi, Neutral polar methacrylate-based monoliths for normal phase nano-LC and CEC of polar species including N-glycans, J Sep Sci., 32 (2009)10.
9. M. Lammerhofer, F. Svec, J.M.J. Frechet, W. Lindner, Capillary electrochromatography in anion exchange and normal-phase mode using monolithic stationary phases, J Chrom A, 925 (2001) 265.
10. Z. Jiang, N.W. Smith, P.D. Ferguson, M.R. Taylor, Hydrophilic interaction chromatography using methacrylate-based monolithic capillary column for the separation of polar analytes, Anal Chem., 79 (2006) 1243.
11. C. Viklund, K. Irgum, Synthesis of Porous Zwitterionic Sulfobetaine Monoliths and Characterization of Their Interaction with Proteins, Macromolecules, 33 (2000) 2539.
12. Y. Lv, Z. Lin, F. Svec, Hypercrosslinked large surface area porous polymer monoliths for hydrophilic interaction liquid chromatography of small molecules featuring zwitterionic functionalities attached to gold nanoparticles held in layered structure, Anal Chem., 84 (2012) 8457.
13. D.N. Gunasena, Z. El Rassi, Organic monoliths for hydrophilic interaction electrochromatography/chromatography and immunoaffinity chromatography, Electrophoresis, 33 (2012) 251.
14. J. Urban, P. Jandera, Recent advances in the design of organic polymer monoliths for reversed-phase and hydrophilic interaction chromatography separations of small molecules, Anal Bioanal Chem., 405 (2013) 2123.
15. Y.J. Cheng, S.H. Huang, B. Singco, H.Y. Huang, Simultaneous determination of thirteen polycyclic aromatic hydrocarbons and twelve aldehydes in cooked food by an automated on-line solid phase extraction ultra high performance liquid chromatography tandem mass spectrometry, J Chrom A, 1218 (2011) 7640.
16. S. Dube, R.M. Smith, Separation of Sulfonamides by Capillary Electrochromatography, Chromatographia, 53 (2001) 51.
17. M.C. Puyana, A.L. Crego, M.L. Marina, Recent advances in the analysis of antibiotics by CE and CEC, Electrophoresis, 31 (2010) 229.
18. X. Wang, H. Lü, X. Lin, Z.J. Xie, Electrochromatographic characterization of methacrylate-based monolith with mixed mode of hydrophilic and weak electrostatic interaction by pressurized capillary electrochromatography, J Chrom A, 1190 (2008) 365.