ELSEVIER

Journal of Chromatography A, 964 (2002) 91–98

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Organic modifiers for the separation of organic acids and bases by liquid chromatography

Shelly Li[1], James S. Fritz*

Ames Laboratory, US Department of Energy and Department of Chemistry, 322 Wilhelm Hall, Iowa State University, Ames, IA 50011, USA

Received 28 January 2002; received in revised form 2 May 2002; accepted 8 May 2002

Abstract

A straight-chain alcohol or diol additive in the mobile phase was used to modify and improve the HPLC separation of organic acids and bases. Incorporation of 2% 1-butanol, 1% 1,2-hexanediol, or 0.25% 1,2-octanediol into an aqueous mobile phase greatly improved the separation of alkane carboxylic acids on a silica C_{18} column, both in terms of separation time and peak shape. When 1.5% 1-hexanol, 0.09% 1-decanol or 0.01% 1-dodecanol was added to an acetonitrile–water (30:70) mobile phase, much sharper peaks and better resolution were obtained for aromatic bases separated on an underivatized polystyrene–divinylbenzene column. The mobile phase additive is believed to coat the stationary phase surface by a dynamic equilibrium. The coated surface is more hydrophilic and facilitates the efficient partitioning of analytes between the mobile and stationary phases.

Keywords: Stationary phases, LC; Mobile phase composition; Organic acids; Organic bases

1. Introduction

Relatively small, polar organic compounds are often separated by ion-exclusion chromatography (IEC). These separations are usually performed on columns of fairly large dimensions packed with a gel resin containing sulfonate or carboxylate groups for separation of acids or neutral analytes, or quaternary ammonium groups for separation of basic analytes [1,2]. The separation mechanism is considered to be the partitioning of the analytes between the predominantly aqueous mobile phase and the stagnant water inside the resin gel. However, several authors have proposed a mixed-mode mechanism in which partitioning also occurs between the sample solutes and the polymeric resin matrix [3,4].

Morris and Fritz [5] showed that the lower alkyl carboxylic acids could be efficiently separated on a porous but non-gel polystyrene–divinylbenzene (PS–DVB) packed column using water containing only a small percentage of *n*-butanol as the mobile phase. The separation involved a typical high-performance liquid chromatography (HPLC) partitioning mechanism, although this was complicated somewhat by an ion-exchange effect due to adsorbed carbonic acid. The *n*-butanol in the mobile phase was believed to coat the surface of the PS–DVB resin and establish a dynamic equilibrium between the mobile and station-

*Corresponding author. Tel.: +1-512-294-5987; fax: +1-512-294-3578.

E-mail address: kniss@ameslab.gov (J.S. Fritz).

[1]Present address: Pfizer, Eastern Point Road, MS 8257, Groton, CT 60340, USA.

0021-9673/02/$ – see front matter
PII: S0021-9673(02)00584-8

ary phases. This increased the hydrophilicity of the resin surface and reduced the hydrophobic attraction of the analytes for the resin phase.

Several authors have reported that the organic solvent used in conventional aqueous–organic eluents used in HPLC undergoes an equilibrium to create a ''third phase'' on the surface of the bonded-silica. This effect is often quite small with commonly used solvents such as methanol or acetonitrile, but it can have a significant effect on chromatographic behavior when other mobile phase additives are used. McCormick and Karger [6] found that as little as 2% (v/v) of an organic modifier of higher molecular mass added to a methanol–water or acetonitrile–water mobile phase can have a major effect on HPLC separations.

In a paper on the separation of ethanolamines with a PS–DVB resin containing quaternary ammonium groups, Tanaka et al. [7] obtained a broad, very tailed peak for triethanolamine when water was used as the mobile phase. However, an aqueous mobile phase containing 0.2 *M* xylitol, fructose, glucose or sorbitol gave a sharp, well-resolved peak for each of the four analytes. The improved behavior was shown to be due to the increased hydrophilicity of the surface resulting from adsorption of the sugar.

Addition of a relatively low concentration (usually 50 m*M*) of either an ionic or non-ionic surfactant to the mobile phase (acetonitrile–water, 60:40) gave a dramatic improvement in the separation of aromatic compounds on a standard C_{18} silica column [8,9]. Retention times were shorter and the peaks were sharper due to an interaction between the analytes and the surfactant in the mobile phase. The presence of organized micelles was doubtful because of the high acetonitrile content of the mobile phase.

In the present work the effects of mobile phase additives on the HPLC separation of polar organic solutes are reported and rational mechanisms for the mechanism are proposed. In the first system studied, addition of a very low concentration of an alkane diol to the aqueous mobile phase was shown to provide a faster separation of alkyl carboxylic acids with sharper peaks of each on a silica C_{18} column. In the second system a low concentration of a C_6, C_{10} or C_{12} alkanol in an acetonitrile–water (30:70) mobile phase gave much sharper peaks and improved resolution of aromatic amines and pyridines on a PS–DVB column.

2. Experimental

2.1. Chromatographic system

The chromatographic system consisted of several components, including a LKB 2156 solvent conditioner (LKB, Bromma, Sweden). A Dionex DXP pump (Dionex, Sunnydale, CA, USA) was used to deliver a flow of 1 ml/min. A 7010 Rheodyne injector (Rheodyne, Berkeley, CA, USA) delivered 10 μl sample. The bases were detected by a Kratos Spectra flow 783 UV absorbance detector (Kratos Analytical Instrument, Ramsey, NJ, USA), and the carboxylic acids were detected by a Dionex CDM-3 conductivity detector. Separations were recorded by a Servogor 120 chart recorder (Abb Goerz Instrument, Vienna, Austria). The column used for the separation of the bases was prepared in our laboratory using 5-μm underivatized macroporous PS–DVB resin (Transgenomic, Santa Clara, CA, USA). A Supelcosil LC-18 column (150 mm×4.6 mm I.D.) (Supelco, Bellefonte, PA, USA) was used as the separation of carboxylic acids using straight-chain alcohols and diols as the modifiers. A Shandon HPLC packing pump (Shandon Southern, Sewickley, PA, USA) was used for column packing.

2.2. Reagents and chemicals

Reagents used for the sulfonation reactions were of reagent grade, and methanol and acetonitrile (ACN) were of HPLC grade. They were used as obtained from Fisher (Pittsburgh, PA, USA) and Allied Chemical (Morristown, NJ, USA). The mobile phase additives and analyte chemicals were of reagent grade and were all used as obtained from Aldrich (Milwaukee, WI, USA). All eluents were prepared daily. Stock solutions were used to prepare all sample solutions by diluting with mobile phase. A Barnstead Nanopure II system (Sybron Barnstead, Boston, MA, USA) was used to further deionize distilled water for all eluents and sample mixtures.

2.3. Chromatographic procedure

A flow-rate of 1 ml/min was selected for all the chromatographic separations. The separation column was equilibrated with mobile phase until the baseline was stabilized. Sample injections were made at this

point. The eluted bases were detected by a UV–Vis detector at 262 nm with an output range of 0.010 AUFS, and the eluted acids were detected by a conductivity detector with the output range of 3 μS, 10 μ or 30 μS.

Capacity factor, k', was calculated according to expression: $k'=(t_r-t_0)/t_0$. The system dead time, t_0, used to calculate capacity factor k', was measured by injecting nitrate solution into the system. An average of at least three replicates was used to do all the calculations.

3. Results and discussion

3.1. Separation of carboxylic acids on a silica C_{18} column

The separation of five alkane carboxylic acids, formic through valeric, was attempted on a silica C_{18} column with water alone as the eluent and with conductivity detection. Sharp, well resolved peaks were obtained for formic, acetic and propionic acids, but a broad late peak was obtained for butyric acid, and valeric acid failed to elute. Retention times are listed in Table 1. The strong retention of butyric and valeric acids was most likely due to strong interaction of their alkane chains with the hydrophobic C_{18} groups on the stationary phase. When 2% (v/v) *n*-butanol was added to the otherwise aqueous eluent, retention times of the analytes were all reduced, but to different degrees. An excellent separation was obtained for all five analytes, with the retention times listed in Table 1. All peaks were sharp and well-shaped except for valeric acid, which was somewhat fronted.

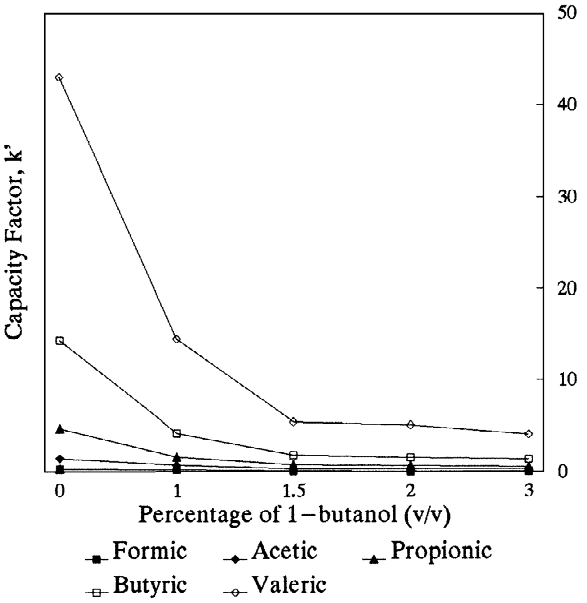

Fig. 1. Effect of 1-butanol in aqueous mobile phase on the retention of carboxylic acids (all at low ppm concentrations). Supercosil LC-18 column (150×4.6 mm), flow-rate 1 ml/min, conductivity detection.

The dramatic effect of *n*-butanol in improving peak sharpness and in reducing retention times was similar to that previously reported for columns packed with PS–DVB resins [5] except that an even lower *n*-butanol concentration could be used in the present system. The effect of *n*-butanol concentration on retention time (or capacity factor) is plotted in Fig. 1. The major reductions in capacity factor were between 0 and 1.5% *n*-butanol.

With polymeric resin columns [5], *n*-butanol was found to be more effective than the lower alcoholic

Table 1
Retention times for carboxylic acids on a Supercosil LC-18 column (150×4.6 min) with pure water or water containing 2% (v/v) *n*-butanol as the mobile phase. Conductivity detection was used

Acid	Retention time (min)			
	Water only	2% BuOH	1% Hexanediol	0.25% Octanediol
Formic	2.0	0.9	1.4	1.1
Acetic	3.3	1.2	1.7	1.5
Propionic	6.3	1.7	2.2	1.7
Butyric	20.1	2.9	3.7	2.6
Valeric	–	7.4 (fronted)	9.6	6.4

t_0=0.7 min.

eluent additives in improving peak sharpness. Because *n*-alcohols greater than four carbon atoms have limited water miscibility, we performed several experiments in which a 1,2-alkane diol was incorporated into the aqueous mobile phase. All five carboxylic acids were separated in <10 min with 1% 1,2-hexanediol but a baseline dip was observed after each of the first two analyte peaks. This could have been caused by increased uptake of an eluent component by an analyte zone followed by a vacancy when the zone has passed [10]. An aqueous mobile phase containing only 0.25% of 1,2-octanediol gave the fastest separation with the sharpest peaks (Fig. 2). The early baseline dips may have occurred because no diol was added to the sample. Retention times for each of these diols are listed in Table 1. 1,2-Decanediol was insufficiently soluble in water to be used.

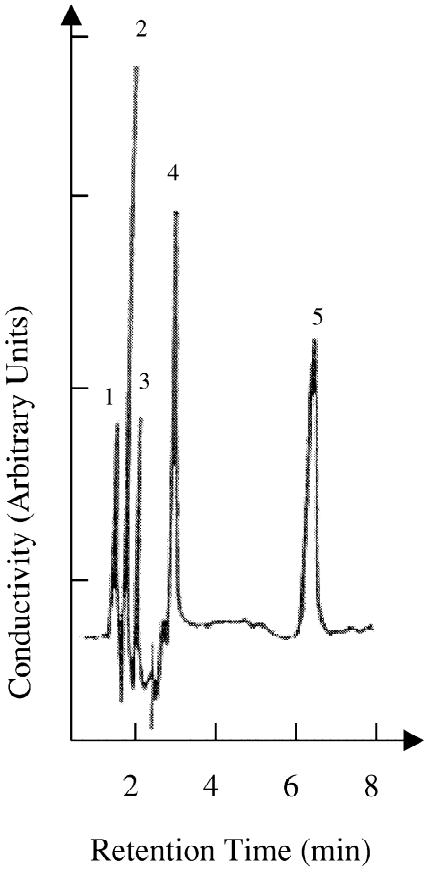

Fig. 2. Separation of carboxylic acids (low ppm concentrations) with an aqueous mobile phase containing 0.25% 1,2-octanediol. Peak identification: 1=formic acid, 2=acetic acid, 3=propionic acid, 4=butyric acid, 5=valeric acid.

3.1.1. Mechanism

Although addition of a low concentration of an alcohol or diol to the mobile phase may shift the stationary–mobile phase equilibrium somewhat by stronger solvation of the analytes in the mobile phase, it is difficult to explain the effects noted by solvation alone because the mobile phase was at least 98% water in all cases. A study by Scott and Simpson [11] showed that short-chain aliphatic moderators such as alcohols, carboxylic acids and aldehydes can be adsorbed onto the surface of a C_{18} silica and that a Langmuir-type adsorption is followed. A mono-layer surface coverage of the silica was observed that reached a plateau at about 1.5% *n*-butanol. In our system this coating would modify the surface properties of the stationary phase and reduce the hydrophobic attraction of the carboxylic acid analytes for the silica phase.

A spectroscopic study of the molecular basis of wetting a C_{18} surface by long-chain *n*-alcohols provides a more detailed picture of the mechanism [12]. This study showed that short-chain alcohols such as methanol and propanol wet the water–C_{18} interface but do not sufficiently affect the underlying C_{18} chains. However, the experimental results reveal that longer-chain alcohols adsorb in a submonolayer, interpenetrating with the chains of the C_{18} monolayer.

This adsorption causes the C_{18} chains to tilt toward the surface normal and become more ordered. It was suggested that this could alter chromatographic selectivity, particularly shape selectivity [12]. It seems logical to assume that adsorption of 1,2-diols would alter the surface properties in a similar manner to *n*-alcohol mobile phase additives.

A comprehensive study by Tan and Carr [13] provides a clear indication that the chemical properties of the stationary phase greatly depend on the mobile phase composition. In reversed-phase liquid chromatography (RPLC) complex mobile phase mixtures are sorbed into the bonded phase and significantly modify the chemical nature of the stationary phase. Water, as well as the organic modifier, is sorbed into the bonded phase. At a particular organic

modifier concentration in the mobile phase the amount of water entering the stationary phase is in the order: tetrahydrofuran (THF)>MeOH>ACN.

The diol mobile-phase additives used in our system have longer apolar carbon chains than the more conventional additives such as methanol, acetonitrile and tetrahydrofuran. A fairly substantial amount of a longer-chain diol may be adsorbed even when diol concentration in the mobile phase is quite low. It is likely that additional water as well as diol is also adsorbed. Thus the hydrophobicity of the stationary phase is greatly reduced.

3.2. Separation of organic bases on an organic polymer column

After demonstrating the effectiveness of *n*-alcohols and 1,2-diols as mobile phase additives for HPLC separations with predominately aqueous mobile phase, we wanted to test this concept for chromatographic separations with a more conventional eluent such as methanol-water or acetonitrile–water. Fig. 3A shows a separation of seven basic compounds using acetonitrile–water (30:70) as the mobile phase. Although the separation was complete except for peaks 4 and 5, the peaks were quite broad. This could be partly due to the somewhat broad particle size range of the polymeric packing material. However, several of the analytes could exist as a mixture of their protonated and molecular forms and thereby give significantly broader peaks. Addition of 0.1 *M* methylamine to the eluent reduced the widths of the sample peaks and gave good resolution, but several were tailed (Fig. 3B). The average theoretical plate numbers of peaks 5, 6 and 7 were $N=1060$ in Fig. 3A and $N=1700$ in Fig. 3B (see Table 2).

Addition of 1.5% (v/v) 1-hexanol, 0.09% (v/v) 1-decanol or 0.01% (v/v) 1-dodencanol to the metha-

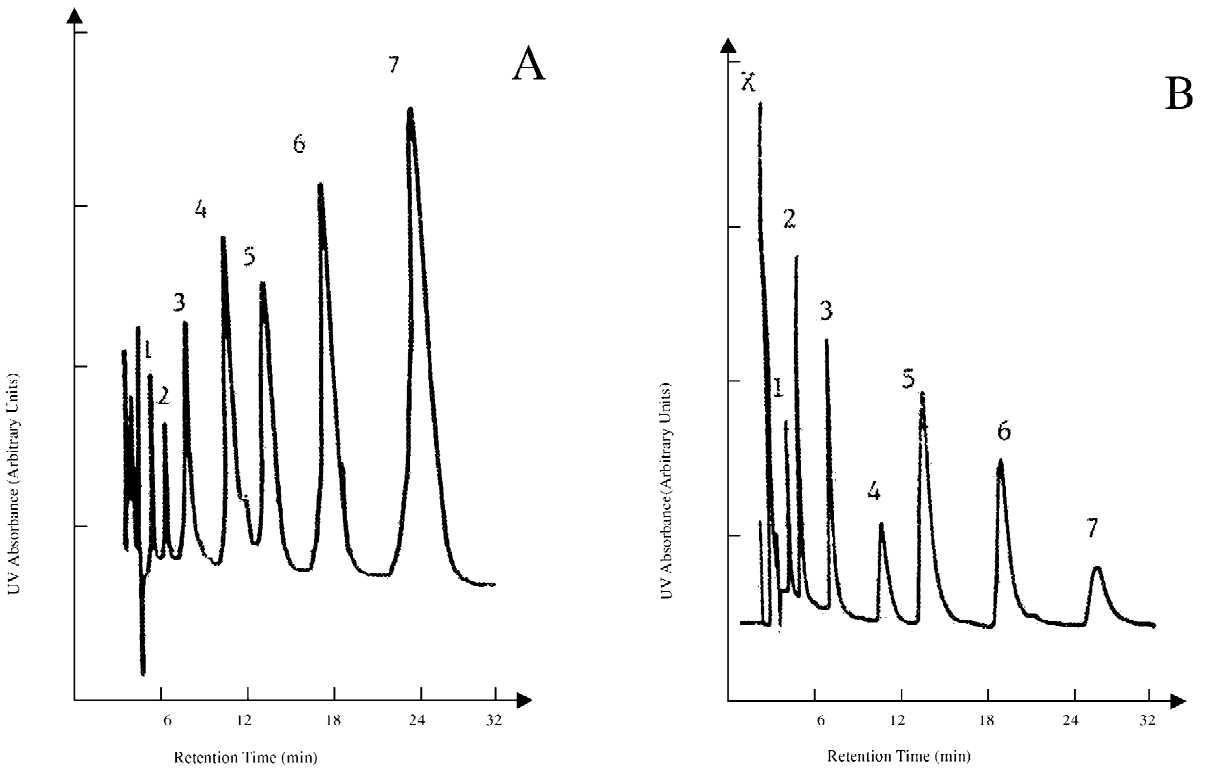

Fig. 3. Chromatographic separations on an underivatized polystyrene–divinylbenzene column (100 mm×4.6 mm). Eluent: (A) acetonitrile–water (30:70), (B) acetonitrile–water (30:70) containing 0.1 *M* methylamine. Flow-rate: 1 ml/min. Detection: UV at 262 nm. Peak identification: 1=pyridine, 2=picoline, 3=aniline, 4=quinoline, 5=quinaldine, 6=*N*-methylaniline, 7=4-benzylpyridine.

Table 2
Theoretical plate numbers (N) for selected peaks with various mobile phase additives

Peak	Mobile phase additive				
	None	Methylamine	1-Hexanol	1-Decanol	1-Dodecanol
Quinaldine	725	775	4300	925	1700
N-Methylaniline	1240	2200	6400	1800	4200
4-Benzylpyridine	1220	2100	2800	3150	2170
Average	1060	1700	4500	1960	2840

nol–aqueous eluent (30:70) gave a much greater improvement in peak sharpness, as shown in Fig. 4. The average theoretical plate numbers for peaks 5, 6 and 7 were $N=4500$ for 1-hexanol, $N=1960$ for 1-decanol and $N=2840$ for 1-dodecanol (Table 2). Resolution of the seven analytes was almost complete with 1-hexanol, but peaks 1 and 2 and peaks 4 and 5 were poorly resolved with 1-decanol. All seven analytes plus an additional analyte (4-ethylpyridine) could be resolved with 1-dodecanol as the additive (Fig. 4C), although the entire separation took 22 min compared to 18 min with 1-hexanol.

3.2.1. Mechanism

The basic analytes are more strongly solvated in 30% acetonitrile than in water alone so their hydrophobic attraction for the stationary phase is weaker than with the C_4 and C_5 carboxylic acids in the previous section. The use of 30% acetonitrile also permits the use of higher-molecular-mass additives than was possible in water alone. It is remarkable that as little as 0.01% (0.5 m*M*) 1-decanol had a profound effect on the chromatograms obtained.

The mechanism most likely involves coating of the stationary phase surface with a thin layer of the mobile phase additive or additive plus water. The use of 30% acetonitrile rather than water alone shifts the additive partition equilibrium more toward the mobile phase, but this is countered by the use of additives of higher molecular mass. The modified surface appears to provide better interfacial compatibility between the mobile and stationary phases and facilitates the rapid equilibration of analytes between the two phases. Sharper analyte peaks and improved peak resolution were the major benefits noted. The mobile phase additives caused a relatively small change in analyte retention times and no change in elution order.

4. Conclusions

A straight-chain alcohol or 1,2-diol (C_4 to C_{12}) was used as a mobile phase modifier to obtain improved HPLC separations of organic acids and bases. Incorporation of a very low concentration of an additive into a conventional mobile phase can alter the stationary phase surface and improve the partition characteristics of the analytes. The magnitude of the effect can be modulated by varying both the type and concentration of the additive. In some cases, solvation of the analytes in the mobile phase may be altered by the use of mobile phase additives. The validity of the general principle was confirmed by the use of alcohols and diols as additives in both the aqueous and aqueous–organic eluents. However, other types of additives should also be effective.

The simple concept of a mobile phase additive should have far-reaching possibilities in HPLC, ion chromatography, and possibly in solid-phase extraction. It is simpler than using a variety of chemically derivatized stationary phases. Chemical additives can be used to alter the properties of the stationary phase; fine tuning is feasible by varying the chemical nature of the modifier.

Acknowledgements

The authors wish to thank D.T. Gjerde of Transgenomic, Inc., for providing the polymeric resin used

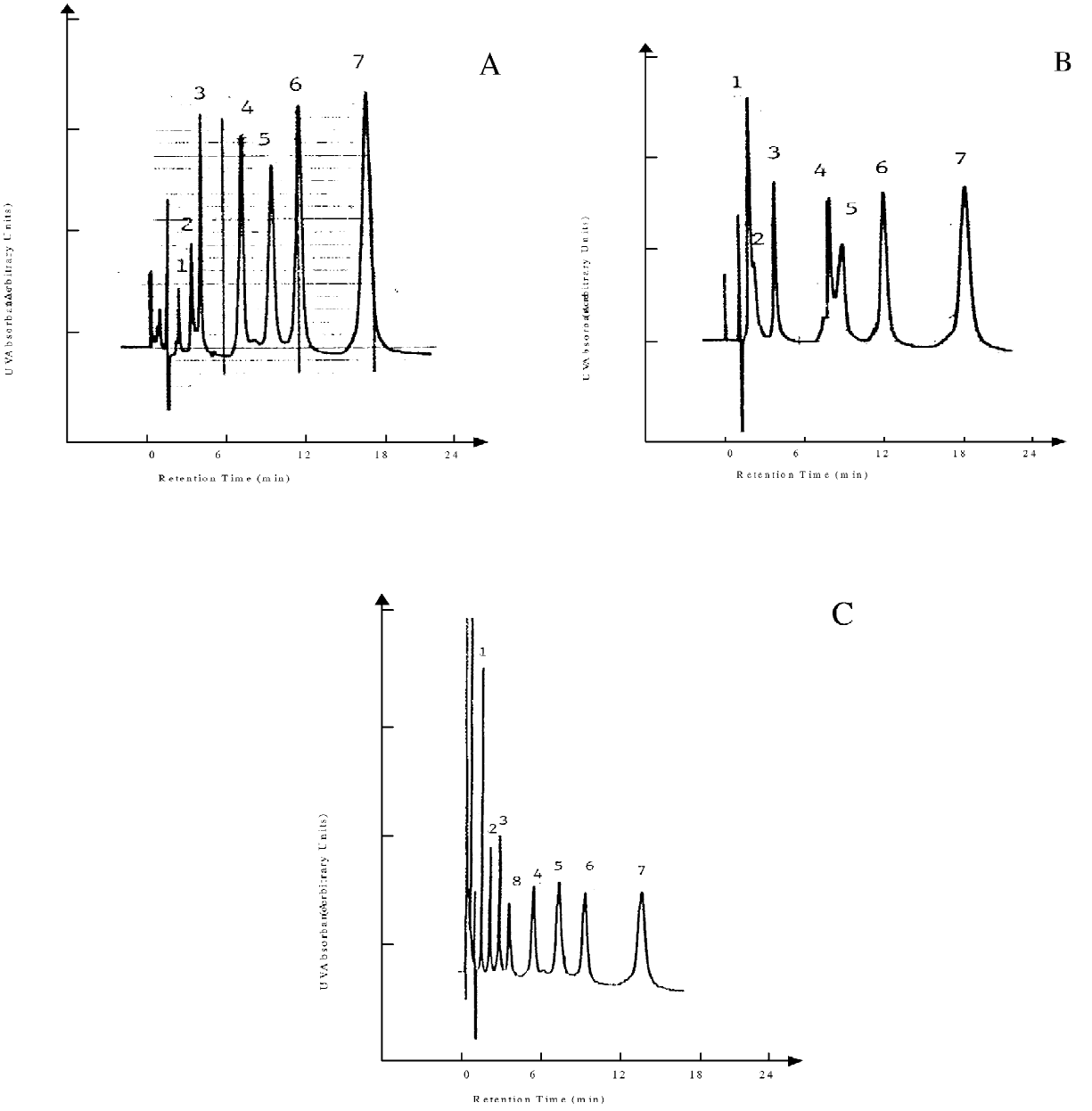

Fig. 4. Chromatographic separations on an underivatized polystyrene–divinylbenzene column (100 mm×4.6 mm). Eluent: acetonitrile–water (30:70) containing (A) 1.5% 1-hexanol, (B) 0.09% 1-decanol and (C) 0.01% 1-dodecanol. Flow-rate: 1 ml/min. Detection: UV at 262 nm. Peak identification: 1=pyridine, 2=4-picoline, 3=aniline, 4=quinoline, 5=quinaldine, 6=*N*-methylaniline, 7=4-benzylpyridine, 8=4-ethylpyridine.

in this work. This work was performed in the Ames Laboratory, which is operated for the US Department of Energy under contract W-7405-Eng-82.

References

[1] P.R. Haddad, P. Jackson, in: Ion Chromatography, Elsevier, Amsterdam, 1990, p. 195.
[2] J.S. Fritz, D.T. Gjerde, in: Ion Chromatography, 3rd ed., Wiley–VCH, Weinheim, 2000, Chapter 8.
[3] D.P. Lee, A.D. Lord, LC·GC 4 (1987) 261.
[4] P.R. Haddad, F. Hao, B.K. Glod, J. Chromatogr. A 671 (1994) 3.
[5] J. Morris, J.S. Fritz, Anal. Chem. 66 (1994) 2390.
[6] R.M. McCormick, B.L. Karger, J. Chromatogr. 199 (1980) 259.
[7] K. Tanaka, K. Ohta, J.S. Fritz, J. Chromatogr. A 739 (1996) 317.
[8] X. Li, J.S. Fritz, J. Chromatogr. A 728 (1996) 235.
[9] X. Li, J.S. Fritz, Anal. Chem. 68 (1996) 4481.
[10] J.J. Stranahan, S.N. Deming, Anal. Chem. 54 (1962) 1540.
[11] R.P.W. Scott, C.F. Simpson, Faraday Symp. Chem. Soc. 15 (1980) 69.
[12] M.E. Montgomery, M.J. Wirth, Anal. Chem. 66 (1994) 680.
[13] L.C. Tan, P.W. Carr, J. Chromatogr. A 799 (1998) 1.