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ORIGINAL RESEARCH

Investigation on liquid chromatographic separation of basic compounds using silica column with aqueous/organic mobile phase containing triethylamine and acetic acid

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ABSTRACT: A high-performance liquid chromatography (HPLC) method using silica column eluted with aqueous solvent mobile phase containing triethylamine (TEA) and acetic acid (ACH) at trace percentages was characterized for the analysis of basic compounds. The key mechanism of this system is ion-exchange accompanying interaction of silanol groups. The increase in the ACH concentration in the mobile phase minimizes the ionization of the silanol group, leading to reduced retention time. However, the greater extent of ionization of silanol caused by the increase of TEA concentration helps to retain basic compounds in the column. Further, the protonated TEA that is positively charged also competes for the ionized silanol group with basic compounds, resulting in the modification of retention time. On the other hand, the retention becomes longer with increasing proportion of either organic or aqueous solvent in mobile phase, and partial replacement of methanol with acetonitrile. Copyright © 2004 John Wiley & Sons, Ltd.

KEYWORDS: silica column; aqueous eluents; basic compounds; TH, tetrahydrozoline HC1; triethylamine; acetic acid

INTRODUCTION

Jane (1975) was the first researcher to conduct analysis of basic substances of abuse on pure silica pickings in methanol-rich aqueous ammonium nitrate at high pH. Several researchers have reported similar systems since then (Wheals, 1980; Bidlingmeyer *et al.*, 1982; Richardson and Bidlingmeyer, 1984). A more thorough study of the use of these aqueous eluents with silica for the chromatography of basic compounds at methanol compositions over 50% has been reported (Cox *et al.*, 1977). This group concluded that ion-exchange mechanisms were less important than other interactions between protonated species and silanol groups and ion-pair interactions. However, in order to study the phenomena involved in those interactions between bases and silanol groups, Cox and Stout (1987) have studied the performance characteristics of a set of nitrogenous bases on a number of silicas using aqueous organic mobile phases. They proposed that a combination of ionic-exchange and interaction with siloxane and silanol groups over the entire range of concentration of organic solvents was the underlying mechanism for controlling the retention mechanism. They also revealed that the differences in retention on silica were due largely to the differences in ion-exchange strength of the silanol groups and the surface concentration of the siloxane bridges.

These methods all followed the same conditions as originally reported by Jane, i.e. using silica columns with high-pH buffered eluents (ammonium nitrate) containing a high proportion of methanol, which was one of the most successful approaches in the separation of basic compounds. Smith and Rabuor (1989) have reported the use of methanol–aqueous ethylenediamine–ammonium nitrate as an eluent for the high-performance liquid chromatographic separation of basic drugs on silica stationary phases. These eluents were shown to be more reproducible than previously reported systems based on methanol–aqueous ammonium nitrate eluents.

Actually, the use of aqueous mobile phase on an unmodified silica gel in the determination of basic drug concentrations in plasma (Butterfield *et al.*, 1978; Dutcher and Strong, 1977; Pershing *et al.*, 1982; Peat and Jennison, 1978) dates back to 1977. However, since there are several potential risks in this approach, it has often been avoided. These potential risks include the dissolution of the silica gel with the use of aqueous

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Abbreviations used: AC, acetaminophen; ACH, acetic acid; CA, caffeine; CL, cloperastine HCl; CM, chlorpheniramine maleate; TEA, triethylamine.

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mobile phase, particularly at a pH above 8, and the consequent distortion of column efficiency. This occurs even with the use of a precolumn to saturate the mobile phase (Atwood *et al.*, 1979). Since, according to the proposed mechanism, only basic amines are retained in the silica gel column (Bidlingmeyer *et al.*, 1982), very clean chromatograms and a much improved signal-noise ratio can be obtained. The application of silica gel column on the assay of plasma concentration was further modified (Shi *et al.*, 1986, 1987, 1991) with the use of low ammonium phosphate concentration adjusted to pH 7.0 by adding concentrated phosphoric acid, with the ability to detect at low nanogram levels from deproteinized plasma.

This interesting application of silica column not only offers a powerful and versatile approach to the analysis of a wide range of basic drugs and their metabolites with simple methanol–buffer eluents (Law, 1990), but also provides a hydrophilic interaction chromatography for separating hydrophilic peptides (Brons and Olieman, 1983; Yoshida and Okada, 1999), with the use of an acetonitrile–water mixed solution containing 0.1% trifluoroacetic acid. In contrast to ion-pair and reversed-phase systems, most compounds show excellent symmetry, with symmetry factors better than 1.2. Naidong *et al.* (2001) also reported that the use of silica stationary phase and mobile phase containing acetonitrile–water with added ammonium acetate (or formic acid, trifluoroacetic acid or acetic acid) could significantly enhance LC/MS/MS method sensitivity (Naidong *et al.*, 2001). McKeown *et al.* (2001) concluded that the use of silica allowed high volume fractions of acetonitrile to be used in the mobile phase that is highly applicable to the rapid LC/MS analysis of hydrophilic bases. This investigation confirmed that all the unbounded phases examined possessed a hydrophobic/adsorption and ion-exchange character. The applications of silica column were further simplified and improved on the assay of basic drug concentration in plasma (Huang *et al.*, 2001) or pharmaceutical preparations (Huang *et al.*, 2002) with the use of methanol or acetonitrile with water containing a combination of triethylamine (TEA) and acetic acid (ACH) at less than 0.1% each. The aim of the present study was to fully explore the influential factors on the resolution of basic compounds with the use of silica column eluted with the mobile phase consisting of only organic solvent, water and traces of TEA and ACH.

EXPERIMENTAL

Chemicals. Five model drugs, three basic compounds of tetrahydrozoline HCl (TH, pK_a 10.5), cloperastine HCl (CL) and chlorpheniramine maleate (CM, pK_a 9.0), and two non-basic compounds of acetaminophen (AC, pK_a 9.7) and caffeine (CA, pK_a 14), were reference standards supplied by National Laboratories of Foods and Drugs, Department of Health, Executive Yuan, ROC. Methanol and acetonitrile were LC grade and obtained from Lab-Scan Co. (Ireland). Triethylamine (pK_a 10.7) and acetic acid (pK_a 4.8) were both purchased from Merck Co. (Germany). All other reagents used were reagent grade or better.

Apparatus. A high-performance liquid chromatographic system equipped with a pump (Waters model 510 solvent delivery pump, USA), UV–vis detector (Waters 486 tunable absorbance detector, USA), and an injector (Waters U6K injector, USA) were used. A 250 × 4 mm (i.d.) silica column (LiChrospher Si-60, Merck, Germany) with a particle size of 5 μm was employed. The silica column was initially preserved in hexane. Before analysis, a serial recondition was conducted by eluting with solvent of gradually increasing polarity from ethyl acetate, dichloromethane, acetonitrile, methanol and finally water (the volume for each solvent was approximately 100 mL). Thereafter, this silica column could be eluted with the use of reversed-phase eluents. The mobile phase consisted of methanol or acetonitrile in varying proportions with different ratios of TEA and ACH (v/v) added. The flow rate was set at 1.0 mL/min. The eluent was detected with UV wavelength set at 254 nm.

Preliminary study. Chromatographic analysis of basic compounds with this system was first examined for feasibility. Comparisons of chromatographic analysis were made by eluting five model drugs with four different compositions of mobile phase on a LiChrospher silica column. These included methanol–water (70:30, v/v), methanol–water (70:30, v/v) containing ACH (0.05%, v/v), methanol–water (70:30, v/v) containing ACH (0.05%, v/v) and pH adjusted to 5.99 with 0.1 N NaOH, and methanol–water (70:30, v/v) containing TEA and ACH (both 0.05%, v/v). The capacity factor (k') for each of the five compounds was calculated as defined for comparison.

The influence of total amount of TEA/ACH on capacity factor. A series of ratios from 0.01/0.01 to 0.07/0.07% v/v for TEA/ACH was added to the mobile phase consisting of methanol and water at a volume ratio of 70:30. Capacity factors calculated as defined for these five basic compounds were compared on the same silica column as above.

The influence of added amount of ACH at a fixed percentage of TEA on capacity factor. A serial increase of ACH from 0.01 to 0.06% (v/v) at a fixed amount of TEA (0.03%, v/v) was added to the mobile phase consisting of methanol and water at a volume ratio of 70:30. The change in capacity factor calculated as defined for each of the five basic compounds was compared on the same silica column as above.

The influence of organic solvent/water volume ratio on capacity factor. At a fixed ratio of TEA–ACH (0.03:0.02%, v/v), the ratio change of organic solvent (methanol or acetonitrile) to water (from 10/90 to 90/10, v/v) on the capacity factors calculated as defined of five basic compounds was compared on the same silica column as above.

The influence of relative ratio change of methanol to acetonitrile in organic solvent on capacity factor. At a fixed ratio of TEA–ACH (0.03:0.02%, v/v), the influence of the relative ratio change of methanol to acetonitrile (70:0 to 0:70%, v/v) in organic solvent with a fixed ratio of organic solvent to water (70:30, v/v) on the capacity factors calculated as defined of five basic compounds were compared on the same silica column as above.

RESULTS AND DISCUSSION

The application of unmodified polar phases such as alumina or silica in combination with aqueous solvent has been recognized as a potential approach to correcting problems frequently encountered in reversed-phase liquid chromatographic analysis of basic compounds (Scott, 1980). The retention behavior of basic compounds in these phases was proposed to be dependent primarily on the ion-exchange mechanism and to be controlled predominately by the pH, and the nature and concentration of the organic modifier (Lingeman and Underberg, 1988). Therefore, due to the presence of acidic silanol (SiOH) functions on the surface of this material, silica can only be used as a cation-exchange material, and the higher the pH of the eluent the higher the ion-exchange capacity of this material. Likewise, silica exhibits cation-exchange properties at all pH values down to pH 2, but the ion-exchange capacity falls sharply with decreasing pH.

Different components with a particular function were needed in the mobile phase to achieve the chromatographic separation of basic compounds on the silica column. First of all, a buffer solution (e.g. phosphate, citrate) was added to control the pH of the eluent, because only positively charged molecules can be retained on these cation-exchange systems. Therefore, the pH of the eluent was chosen so that the solute ions were protonated and the materials possessed ion-exchange capacity. Furthermore, competing ions (e.g. tetramethyl or tetrabutylammonium bromide) were added to control the retention of the analyte cations by means of a competition between the different cations for the active sites on the sorbent. Finally, the addition of a modifier, methanol or acetonitrile, was necessary to improve the resolution of the system.

Buffers, such as phosphate and citrate, might be precipitated at higher contents of organic modifier, causing tubing blocking and residual problems. Column cleansing also takes time since it is necessary to elute with water first and then with organic solvent for storage. A longer time for equilibrium and cleansing with the addition of competing ions (usually quaternary compounds such as tetramethyl- or tetrabutylammonium bromide) is commonly encountered. In this study, the influence of a combination of TEA and ACH added to the mobile phase on the retention behavior of basic compounds on silica column was investigated. TEA and ACH was used to control the pH value of the mobile phase, and the protonated form of TEA functioned as a competing ion. Both are liquid in nature to minimize the problems encountered with a solid buffer.

The feasibility of using this combination of TEA and ACH in the mobile phase for the separation of basic compounds on silica column is demonstrated in Fig. 1. With the mobile phase consisting only of methanol and water at a volume ratio of 70:30, a short retention time for two non-basic compounds was observed and three basic compounds were retained in the silica column for longer than 30 min [Fig. 1(A)]. With the addition of ACH at 0.05% v/v in the mobile phase consisting of methanol and water at the same ratio as above, the results of elution were the same [Fig. 1(B)]. However, during elution with the mobile phase mentioned above with pH adjusted to 5.99 with 0.1 N NaOH, three basic compounds appeared as broad peaks [Fig. 1(C)], but still with a longer retention time. When the mobile phase containing the same ratio of TEA and ACH (0.05%, v/v, pH 5.99) was used for elution, sharp peaks for three basic compounds appeared within only 10 min and were also well separated.

In a simple mobile phase only containing methanol and water, a strong interaction between the salt form of basic compounds and the polar surface of the silica material might exist, as theoretically predicted, leading to retention of these three basic drugs in the silica column for a longer time. This interaction was not observed for non-basic compounds, such as AC and CA. It has been reported that the pH (uncorrected for changes in electrode performance with changes in methanol concentration) increased with added methanol, reaching a value of 6.4 at 75% (Cox and Stout, 1987). This means that the silica surface was increasingly ionized at the high methanol concentration as used in this mobile phase, and this was probably the cause of the increased retention of the three basic compounds. Although the addition of ACH could minimize such ionization by decreasing the pH of the system, there existed a small proportion of silanol group that was strongly acidic in nature and still ionized even at low pH. This further indicates that three basic compounds were still possibly retained by this interaction in the silica column with the use of this mobile phase.

The number of ionized silanol groups for interaction should be increased when the pH value of the mobile phase was adjusted to 5.99 with 0.1 N NaOH, predictably leading to an even longer retention time for three basic compounds than that eluted with the mobile phase without adjusting the pH to the same value. However, this seems not to be the case, as shown by Fig. 1(C), which shows that three basic compounds appeared as a broad peak with less retention. Since sodium ion was also recognized as a competing ion with

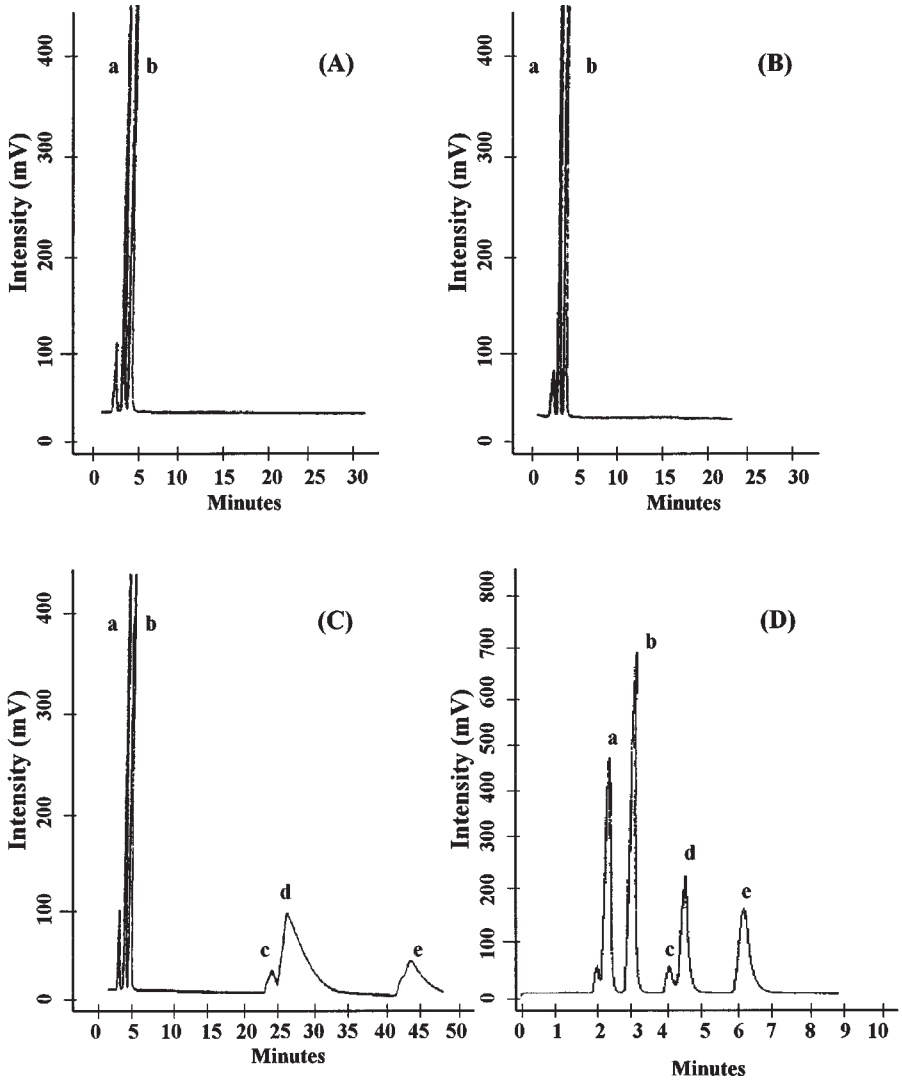

Figure 1. HPLC Chromatograms of AC (a), CA (b), TH (c), CL (d) and CM (e) eluted with the mobile phase containing different solvent compositions: (A) methanol–water, 70:30 (v/v); (B) methanol–water, 70:30 with addition of 0.05% (v/v) ACH; (C) methanol–water, 70:30 with addition of 0.05% (v/v) ACH and pH adjusted to 5.99 with 0.1 N NaOH; (D) methanol–water, 70:30 with addition of 0.05% (v/v) ACH and TEA.

solute for ionized silanol groups, it follows the predictions of ion-exchange theory (Kraak, 1982) that for a pure ion-exchange mechanism the retention of basic compounds decreased with increasing competing-ion concentration. Nevertheless, broad chromatographic peaks of basic compounds with the use of a strong basic sodium ion as competing ion suggested that a base less basic than NaOH might be a better choice for competing ion.

TEA, with pK_a 10.75, was selected in conjunction with ACH to control the pH value of the mobile phase. A sharp peak with proper retention time for basic compounds, as shown in Fig. 1(D), was obtained when the elution with this mobile phase was performed on a silica column. The feasibility of using a simple combination of TEA and ACH in liquid form at very low concentration in the mobile phase for the separation of basic compounds on silica column was concluded. The influence of the composition variables relating to this mobile phase system on the retention of basic compounds on a silica column was examined, as follows.

Figure 2 demonstrates the effect of changing total percentage of TEA and ACH (both at the same ratio) on the capacity factors of these five compounds with the mobile phase consisting of the same volume ratio of methanol to water (70:30) as above. Apparently, the capacity factors of three basic compounds gradually decrease with increasing total percentage of TEA and ACH, while the capacity factors of the two non-basic compounds affected by this change were insignificant. The pH value of these seven compositions of the

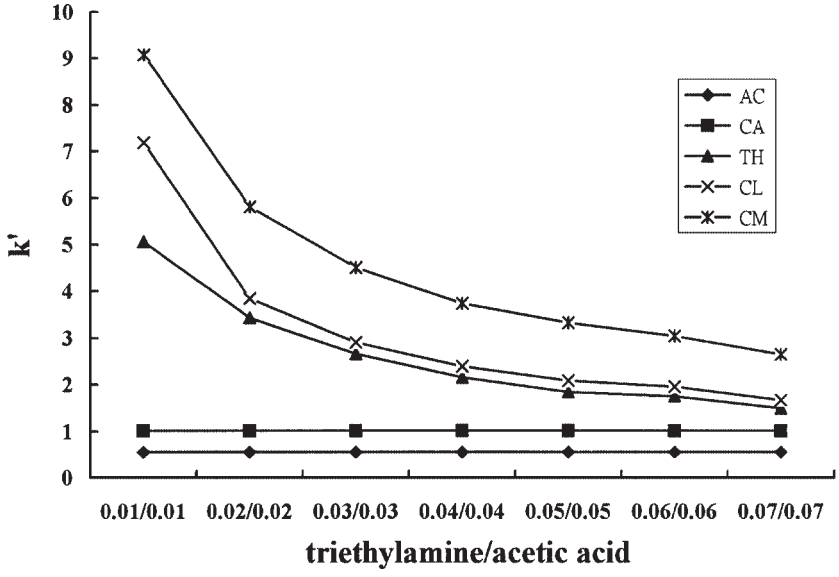

Figure 2. Effect of the total concentration of TEA to ACH at the same ratio on the capacity factor (k') eluted with the mobile phase consisting of methanol and water, 70:30 v/v. For key refer to Fig. 1.

mobile phase was measured as within the range 5.85–6.07. At this similar ionization condition for silanol groups on silica surface, the retention of these basic compounds would be expected to be dependent on the concentration of the competing ion, TEA in this case. Therefore, the influence of increasing TEA concentration in the mobile phase on the capacity factors of basic compound was consistent with that predicted by ion-exchange theory.

The effect of different ratio of ACH to TEA in the mobile phase consisting of methanol and water at a 70:30 volume ratio on the capacity factors is shown in Fig. 3. Similarly, the capacity factors of three basic compounds decreased with increasing the amount of ACH added, while this influence was still minimal on the capacity factors of two non-basic compounds. At the same TEA concentration as the competing ion, increasing ACH concentration would be expected to decrease the number of ionized silanol groups interacting with basic compounds, leading to the decrease of their capacity factors. This is also predictable by ion-exchange theory.

The influence of changing methanol concentration in the mobile phase on the capacity factors of basic compounds is shown in Fig. 4. There was only a small increase in the capacity factor for CA with increasing methanol concentration, but this influence was minimal for AC. In contrast, the initial increase in methanol concentration from 10 to 50%, resulting in the decrease of capacity factors, was more obvious only for CM, whereas the increase of capacity factors with increasing methanol concentration from 50 to 90% was apparent for all three basic compounds. Since the pH increased

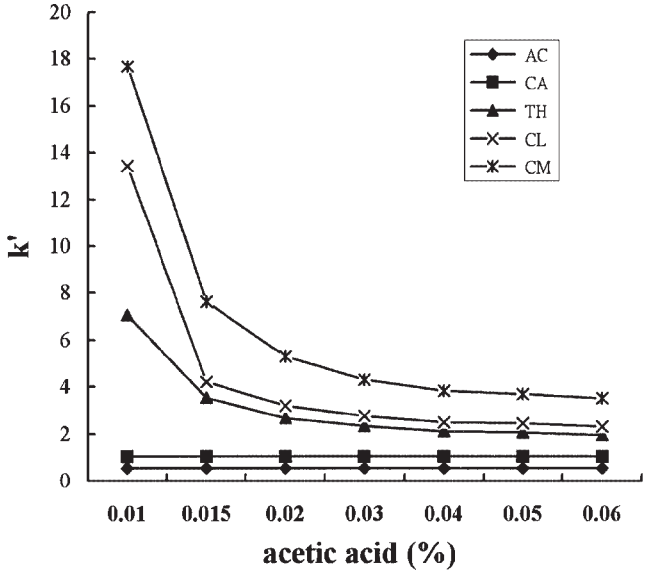

Figure 3. Effect of the increase in ACH concentration at the same TEA concentration on the capacity factor (k') eluted with a mobile phase consisting of methanol and water, 70:30 v/v. For key refer to Fig. 1.

with increasing added methanol, reaching a value of 6.4 at 75%, the silica surface was increasingly ionized at high methanol concentration. This probably explains why increasing methanol concentration from 50 to 90% increased capacity factors of three basic compounds. However, the pH of the mobile phase slowly increased with increasing methanol concentration, the pH of the mobile phase at low methanol concentration was not appreciably affected by the addition of methanol and it is reasonable to assume the number

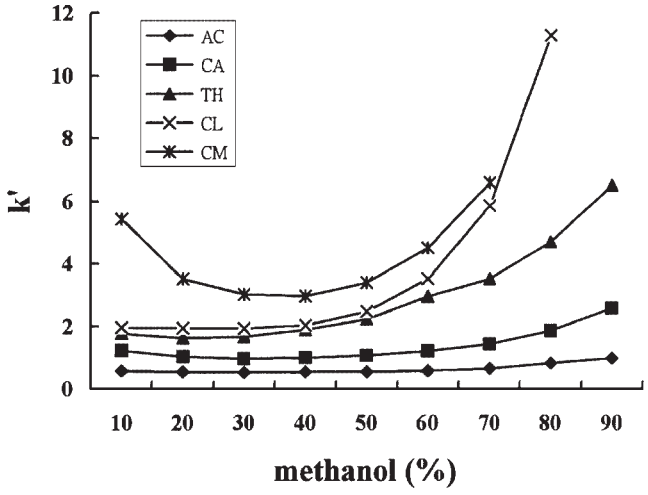

Figure 4. Effect of the change in the relative ratio of methanol to water on the capacity factor (k') eluted with a mobile phase consisting of methanol and water with addition of 0.03% v/v TEA and 0.02% v/v ACH. For key refer to Fig. 1.

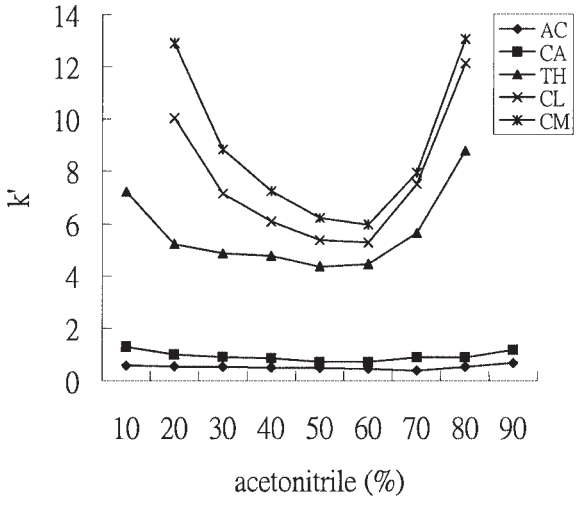

Figure 5. Effect of the change in the relative ratio of acetonitrile to water on the capacity factor (k') eluted with a mobile phase consisting of methanol and water with addition of 0.03% v/v TEA and 0.02% v/v ACH. For key refer to Fig. 1.

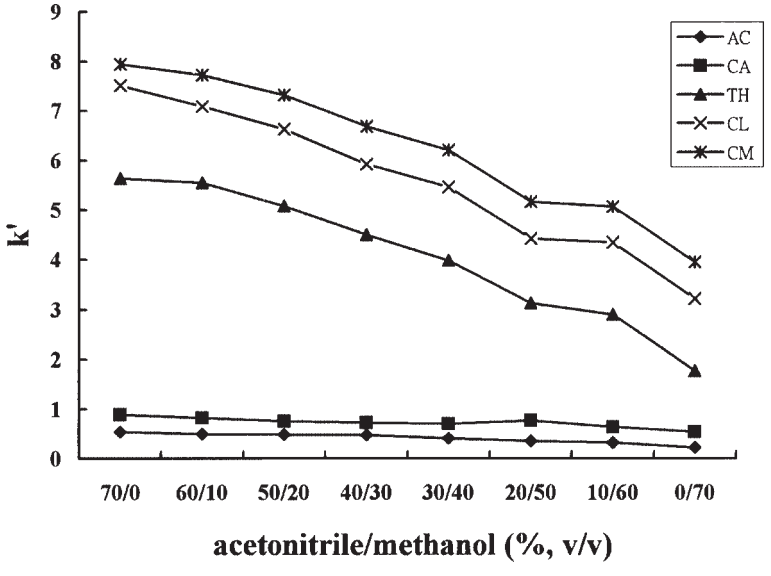

Figure 6. Effect of the change in the relative ratio of methanol to acetonitrile on the capacity factor (k') eluted with a mobile phase consisting of organic solvent and water, 70:30 v/v, with addition of 0.03% v/v TEA and 0.02% v/v ACH. For key refer to Fig. 1.

of ionized silanol groups on the silica surface did not appreciably increase. Thus, the influence of increasing methanol concentration at a low concentration range on the capacity factors was not significant and only to some extent for CM.

The influence of using acetonitrile as organic solvent in the mobile phase on the capacity factors of basic compounds is illustrated in Fig. 5. The similar tendency of the change in acetonitrile concentration to that of methanol was observed but to a greater extent. The same mechanism as discussed above for the effect of the change in methanol concentration on the capacity factors is applicable to explain this similar observation. The difference in the retention capacity between acetonitrile and methanol could be ascribed to the difference in the capacity for inducing ionization of silanol groups on the silica surface.

At the fixed ratio of organic solvent to water (70:30, v/v), the decrease of acetonitrile concentration relative to methanol (from 70:0 to 0:70) in organic solvent decreased the capacity factors of three basic compounds, as shown in Fig. 6. Since the retention capacity

of acetonitrile was greater than that of methanol, as discussed above, the decrease in capacity factors with decreasing acetonitrile concentration was expected. Similarly, this influence on the capacity factor of two non-basic compounds was minimal due to different retention mechanism.

CONCLUSION

In conclusion, the use of silica column to elute basic compounds with a simple mobile phase containing organic solvent and water with the addition of only trace concentrations of TEA and ACH was achievable. Systemic parameters, such as total concentration of TEA and ACH, the change in organic solvent concentration, and the kind of organic solvent, could be utilized to adjust the desired retention time to have better resolution for either single or multiple components. Both TEA and ACH were in liquid form and only trace amounts was used, leading to the easiness in the preparation of mobile phase, the reduction in the equilibrium time and hence total analysis time, and less burden in the cleansing of column and equipment tubing.

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