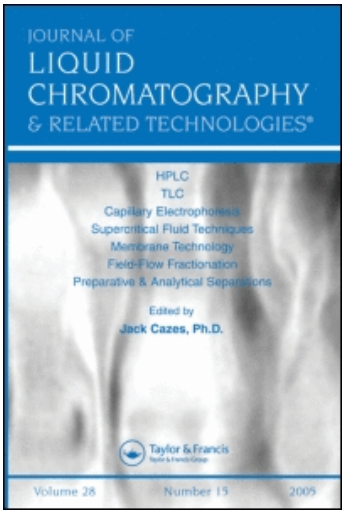

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Abstract: Hydrophilic interaction chromatography (HILIC) was explored for the separation of selected quaternary amine compounds of biological and environmental importance, including acetylcholine, choline, betaine, chlormequat, and mepiquat. The HILIC method was successful in separating the model quaternary amine compounds without the need for ion-pairing reagents. The present study indicates that the amide phase provides much stronger retention for the quaternary amine compounds than the aminopropyl phase, and also exhibits different selectivity toward the model compounds. Separation conditions including acetonitrile content, column temperature, buffer salt type, and concentration were found to have significant impact on the separation of the model compounds. In addition, the HILIC separation was coupled to a single quadruple mass spectrometer in this study, and an extremely low limit of detection of approximately 0.4 fmol for choline was achieved using selective ion monitoring. The liquid chromatography/mass spectrometry sensitivity of the hydrophilic interaction chromatography/mas spectrometry method was 75 times higher than that of the reversed-phase liquid chromatography/mass spectrometry method reported in the literature.

Keywords: HILIC, HILIC/MS, LOD, choline, acetylcholine

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INTRODUCTION

Small quaternary amine compounds such as acetylcholine, choline, chlormequat, and mepiquat are of great biomedical and environmental significance. Chromatographic methods are the primary means for the analysis of these compounds in various biological and environmental matrixes.[1–6] Gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) methods require conversion of the quaternary amine compounds into more volatile species by chemical derivatization.[1–3] This not only complicates analytical procedures, but also negatively affects method reproducibility and sensitivity. Liquid chromatography (LC) approaches used for the analysis of acetylcholine and choline include ion chromatography (IC) and reversed-phase liquid chromatography (RPLC) methods. However, the IC method has only found a few limited applications.[7–9] The more popular RPLC method faces two challenges in analyzing the quaternary amine compounds. First, these compounds are cationic in nature and difficult to retain on conventional reserved-phase columns (e.g., C_{18}) even with low-level organic solvent in the mobile phase. Therefore, ion-pairing reagents are often added to the mobile phase to obtain sufficient retention. The second challenge is the lack of chromophores in most quaternary amine compounds. Various detection schemes have been developed including refractive index,[10] conductivity,[11] chemiluminescence,[12] electrochemical,[13–17] and mass spectrometry (MS) detection.[18–22] Electrochemical detection provides the best sensitivity for acetylcholine and choline, but MS detection is more desirable for its high sensitivity, selectivity, and ability to provide positive structural confirmation.

Hydrophilic interaction chromatography (HILIC) offers an alternative approach to the separation of polar compounds. The term "HILIC" proposed by Alpert in 1990, refers to a mode of liquid chromatography where the separation occurs through "partitioning between the mobile phase and a layer of mobile phase enriched with water and partially immobilized on the stationary phase".[23] In contrast to RPLC, polar compounds are more strongly retained and water is a stronger solvent in HILIC. It has been mostly used in the area of carbohydrates,[24–26] nucleic acids,[23] and peptide analysis.[27–29] Recently, several papers have demonstrated the application of HILIC in the separation of polar compounds in food and pharmaceutical analysis.[30–34]

An extensive literature search indicates that there has been no published report on the application of HILIC to the separation of quaternary amine compounds. An objective of this study was to demonstrate the feasibility of separating these compounds by HILIC and to investigate their retention behavior under various separation conditions. To this end, acetylcholine, choline, and betaine were selected as the model compounds for their important neurological and biomedical functions. The model compounds also included two quaternary ammonium herbicides, that is, chlormequat and mepiquat, because of their importance in environmental monitoring. The selected model compounds

share a quaternary amine moiety in their structures, but differ in the functional group attached to the quaternary ammonium center, as shown in Figure 1.

EXPERIMENTAL

Reagents

High performance liquid chromatography (HPLC) grade acetonitrile (ACN) was purchased from EM Science (Hawthorne, NY). HPLC grade water was obtained from a Milli-Q Gradient water purification system (Millipore, Bedford, MA). Ammonium acetate and ammonium formate were of HPLC grade (Aldrich, Milwaukee, WI). Acetylcholine chloride, choline chloride, and betaine hydrochloride were purchased from Aldrich. Chlormequat chloride and mepiquat chloride were obtained from Chem Service (West Chester, PA). The YMC-Pack NH_2 (150 mm × 4.6 mm I.D., 3 μm particles, 120 Å pore size) column was purchased from Waters (Milford, MA) and the TSKgel Amide-80 column (250 mm × 4.6 mm I.D., 5 μm particles, 80 Å pore size) from Tosoh Bioscience (Montgomeryville, PA).

Instrument and Method

All experiments were conducted on an Agilent HPLC system (HP1100, Agilent Technologies, Palo Alto, CA) consisting of a quaternary pump

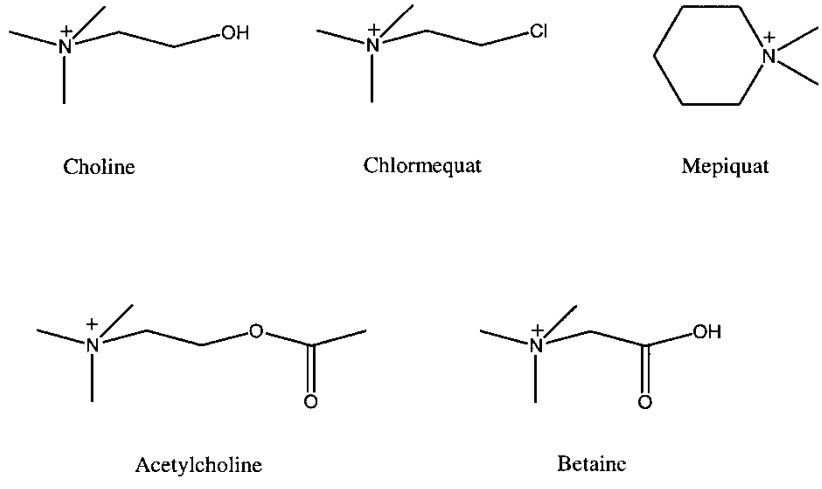

Figure 1. Structures of quaternary amine compounds.

(Model G1311A), a degasser (Model G1322A), a column heater (Model G1316A), and an auto-injector (Model G1313A). The HPLC system was coupled to a mass selective detector (MSD, Model SL G1619), which was a single quadruple mass spectrometer with an electrospray ionization (ESI) interface. Agilent ChemStation (LC/MSD) software (Rev. A.09.01) was used for data acquisition and analysis.

The MSD, operated in the positive ion mode, was used for the detection of the model compounds. Drying gas was nitrogen heated to 350°C with a flow rate of 12 L/min. The nebulizer was set at 55 psi, and the capillary voltage was at 3000 V. A narrow mass range (90–200 m/z) was used to increase sensitivity. For the sensitivity study on choline, the MSD was operated in the selected-ion monitoring (SIM) mode at 104 m/z.

The mobile phase was prepared by first dissolving the required amount of ammonium acetate or ammonium formate in water, then mixing with the desired volume of ACN. The salt concentration in the text and figure captions refers to the final concentration in the mobile phase. The pH of the salt solution was not adjusted. Stock standard solutions for acetylcholine, choline, betaine, chlormequat, and mepiquat (~0.1 mg/mL free base) were prepared in ACN/water (90 : 10). The stock standard solutions were mixed and diluted to the desired concentration. Standard solutions of choline in the linearity and sensitivity study were prepared by series dilution of choline stock solution.

RESULTS AND DISCUSSION

Retention of Quaternary Amine Compounds on Polar Stationary Phases

Polar stationary phases such as silica, aminopropyl, and amide phase are typically employed in the HILIC separation.[26–34] In this study, only amino and amide phases were tested for the model compounds. A YMC-Pack NH_2 column containing the aminopropyl phase was chosen over other amino columns because it was stored in an ACN and water mixture instead of typical normal phase solvents (e.g., hexane), thus avoiding time-consuming washing and equilibration. The amide phase was provided by a TSKgel Amide-80 column, which contained carbamoyl groups covalently bonded to the silica surface through a carbon chain.[35] Under the same mobile phase conditions (ACN/water, 85 : 15, 10 mM ammonium acetate), acetylcholine was barely retained ($k' \sim 0.1$) on the amino column, but had significant retention ($k' \sim 2.9$) on the amide column. The capacity factor for choline increased by over 10 times from 0.9 on the amino column to 9.2 on the amide column, and betaine also exhibited stronger retention on the amide column than on the amino column. The chromatograms in Figure 2 display the separation of five quaternary amine compounds on the amino and amide

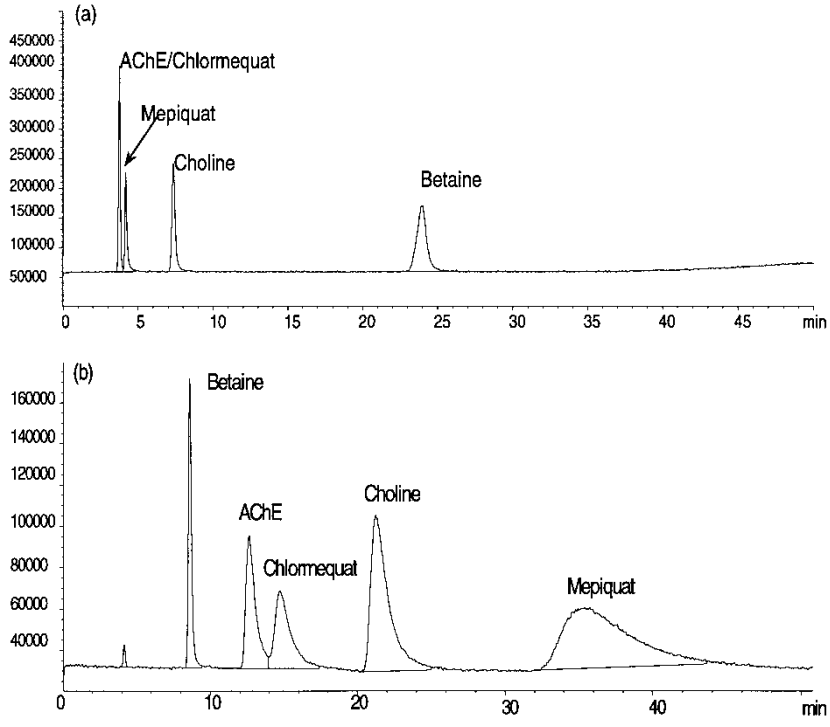

Figure 2. HILIC separation of quaternary amine compounds on the amino (a) and amide columns (b). Column temperature, 30°C; flow rate, 1 mL/min; injection volume, 5 μL. Mobile phase: (a) ACN/water (90 : 10, v/v) and (b) ACN/water (70 : 30, v/v) containing 10 mM ammonium acetate.

columns. Different mobile phase conditions had to be used for each column to accommodate their difference in relatively. A weaker mobile phase (90% ACN) was necessary to obtain sufficient retention for early eluting compounds on the amino column (Figure 2a), but a stronger mobile phase (70% ACN) helped to elute the model compounds in reasonable time on the amide column (Figure 2b). On the amino column, acetylcholine and chlormequat co-eluted and betaine was the most retained compound (Figure 2a). In contrast, betaine was least retained on the amide column due to low ACN content in the mobile phase, as further discussed in the next section. Acetylcholine and chlormequat were nearly baseline separated and mepiquat was the last compound to elute from the amide column as shown in Figure 2b. This reflected selectivity differences between the two phases. In addition, all peaks showed tailing on the amide column, possibly due to secondary interactions between the positively charged amine compounds and negatively charged surface silanol groups.

Effect of ACN Content on Retention

HILIC often employs simple ACN and water mixtures as the mobile phase. As in RPLC, organic solvent content has been shown to have significant impact on the retention of polar compounds in HILIC.[23,31,34] The effect of ACN content on retention was investigated by changing the ACN content from 67% to 94% (v/v) while keeping ammonium acetate concentration unchanged at 10 mM. The plot in the upper panel in Figure 3 illustrates the

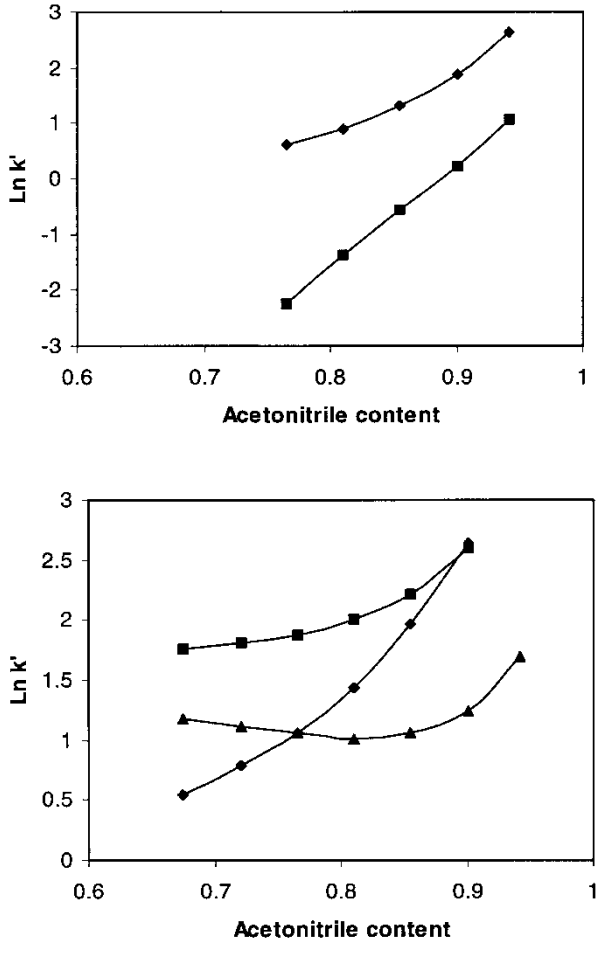

Figure 3. Plots of $\ln k'$ vs. ACN content in the mobile phase for acetylcholine (▲), betaine (◆), and choline (■) on the amino (top) and amide columns (bottom). Column temperature, 30°C; flow rate, 1 mL/min. Mobile phase, ACN/water containing 10 mM ammonium acetate.

relationship between $\ln k'$ and ACN content for betaine and choline on the amino column. Acetylcholine was not included due to its very small capacity factor. Both betaine and choline exhibited typical HILIC behavior of increasing retention as the ACN content increased. The $\ln k'$ values for choline increased with the ACN content almost in a linear fashion; however, betaine slightly deviated from the linear behavior possibly due to secondary interactions (e.g., electrostatic repulsion) that may have contributed to retention.

The effect of the ACN content on retention was also investigated for the amide column. The plot in the bottom panel in Figure 3 shows a more complicated relationship between $\ln k'$ and ACN content. Betaine exhibited very strong retention at high ACN content, but its retention dropped sharply with decreasing ACN content. In contrast, only a moderate decrease in retention was observed for choline as the ACN content decreased. Interestingly, the retention for acetylcholine decreased initially with increasing ACN content over the range of 67–81%, and then started to increase from 81% to 90%. This unusual behavior of acetylcholine could be the result of complex interactions between the analyte and the stationary phase, such as hydrophilic interaction, hydrophobic interaction with the aliphatic chain of the stationary phase, and ionic interaction with the silica surface.

Effect of Column Temperature on Retention

Column temperature is another important parameter that affects the retention of polar compounds in HILIC.[23,34] The effect of column temperature on the retention of some quaternary amine compounds was investigated on the amino and amide columns in the temperature range of 15–55°C. The impact of column temperature on retention was studied using the Van't Hoff equation:

$$\ln k' = -\frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R} + \ln \phi$$

where ΔH^0 and ΔS^0 are the retention enthalpy and entropy, R is the gas constant, and ϕ is the phase ratio. The top panel in Figure 4 shows Van't Hoff plots for choline and betaine on the amino column. Acetylcholine was not included due to its very small capacity factors in the mobile phase of ACN/water (90 : 10, v/v) containing 10 mM ammonium acetate at higher temperatures. The Van't Hoff plots for acetylcholine, choline, and betaine on the amide column are displayed in the bottom panel in Figure 4. The ACN content in the mobile phase was reduced to 81% in order to elute choline in a reasonable time frame from the amide column. All Van't Hoff

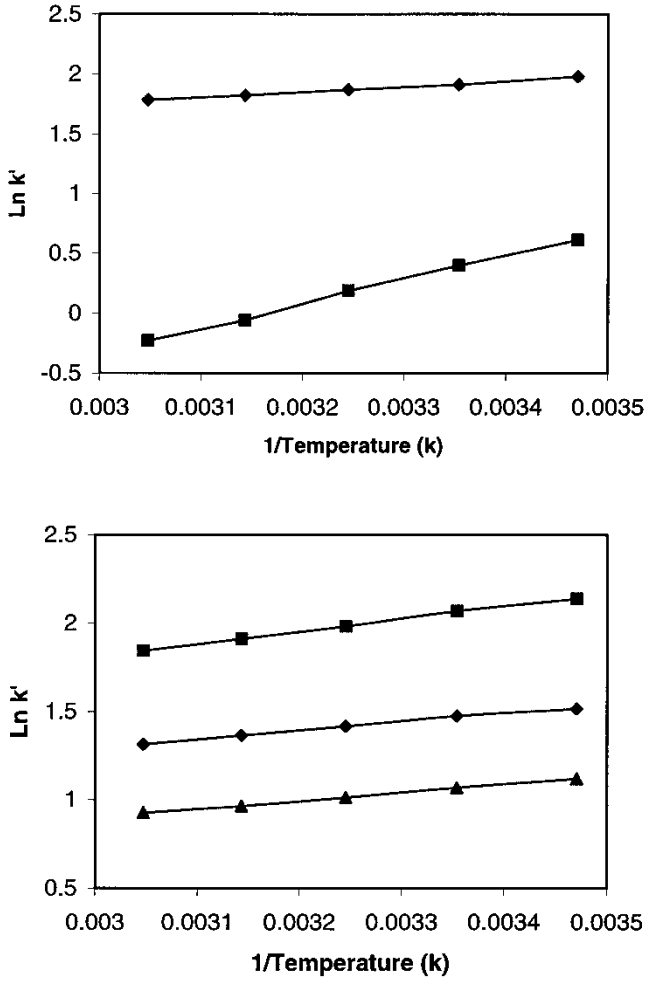

Figure 4. Van't Hoff plots for acetylcholine (▲), betaine (◆), and choline (■) on the amino (top) and amide columns (bottom). Mobile phase, ACN/water (90 : 10, v/v) for the amino column, ACN/water (81 : 19, v/v) for the amide column containing 10 mM ammonium acetate.

plots for the model compounds were linear with positive slopes on both columns, which implied that the transition of the solutes from the mobile to the stationary phase was an energetically favorable exothermic process. Furthermore, it was observed that choline was more strongly affected by the column temperature than betaine on the amino column, and than acetylcholine and betaine on the amide column, as indicated by the larger slope of the Van't Hoff plots.

Effect of Salt Type and Concentration

Low concentrations of buffer salts in mobile phase have been shown to improve peak shape and separation efficiency in HILIC.[31] The buffer salts used for the HILIC separation are required to have good solubility at high organic contents and need to be volatile if a mass spectrometer is used for detection. Ammonium acetate and formate salts are commonly used in HILIC separation due to their good solubility and volatility. In this study, ammonium acetate or formate salts were tested at 10 mM for the separation of three model compounds, namely, acetylcholine, choline, and betaine on both amino and amide columns. As shown in Figure 5, changing buffer salt from ammonium acetate to ammonium formate caused a small decrease in retention time for the model compounds, but led to improved peak shape especially for betaine on the amino column. A similar decrease in retention time was also observed on the amide column when ammonium acetate was replaced with ammonium formate at the same salt concentration.

The effect of salt concentration on retention was investigated by varying the concentration of ammonium acetate from 5 mM to 20 mM in the mobile phase of ACN/water (85 : 15, v/v) for the amino column, and from 5 mM to 40 mM in the mobile phase of ACN/water (75 : 25, v/v) for the amide column. Different ACN contents were used to accommodate large retentivity differences between the amino and amide columns. The salt concentration could not be further increased due to solubility limitations of the salt in the mobile phase. The effect of salt concentration on retention of three model compounds is illustrated in the bar charts in Figure 6. On the amino column, the retention time for the model compounds increased when the salt concentration increased from 5 mM to 20 mM. On the amide column, however, the retention time decreased significantly when the salt concentration increased from 5 mM to 40 mM, except for acetylcholine. The decrease in retention time for choline and betaine on the amide columns could be attributed to the ion-exchange effect of surface silanol groups.[23] However, the opposite behavior on the amino column was more perplexing and possibly reflected complex factors influencing the retention behavior of the model compounds, such as ion-exclusion effects or modification of silica surface by salt ions.

Improved Sensitivity of HILIC/MS Method for Choline

Highly sensitive analytical methods are often required to determine acetylcholine and choline at very low levels in biological samples.[1] The conventional electrochemical detection method provides good sensitivity (20 fmol) toward acetylcholine and choline,[14] but requires the use of a post-column immobilized enzyme reactor (IMER) to convert acetylcholine to choline,

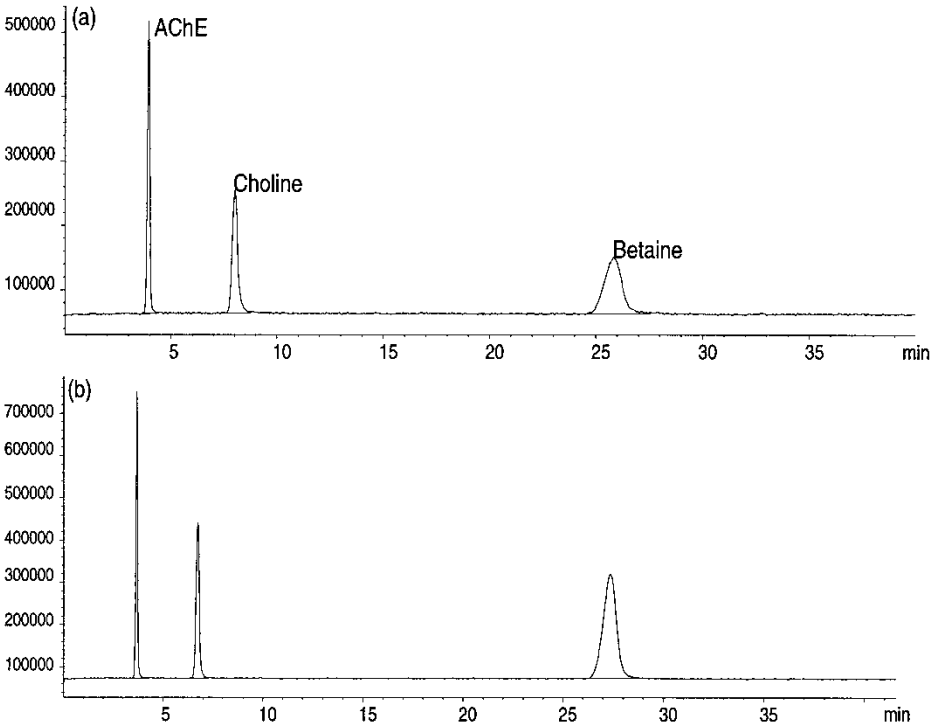

Figure 5. The effect of buffer salt on the retention time of quaternary amine compounds on the amino column. Column temperature, 25°C; flow rate, 1 mL/min. Mobile phase: ACN/water (90 : 10) containing (a) 10 mM ammonium acetate and (b) 10 mM ammonium formate.

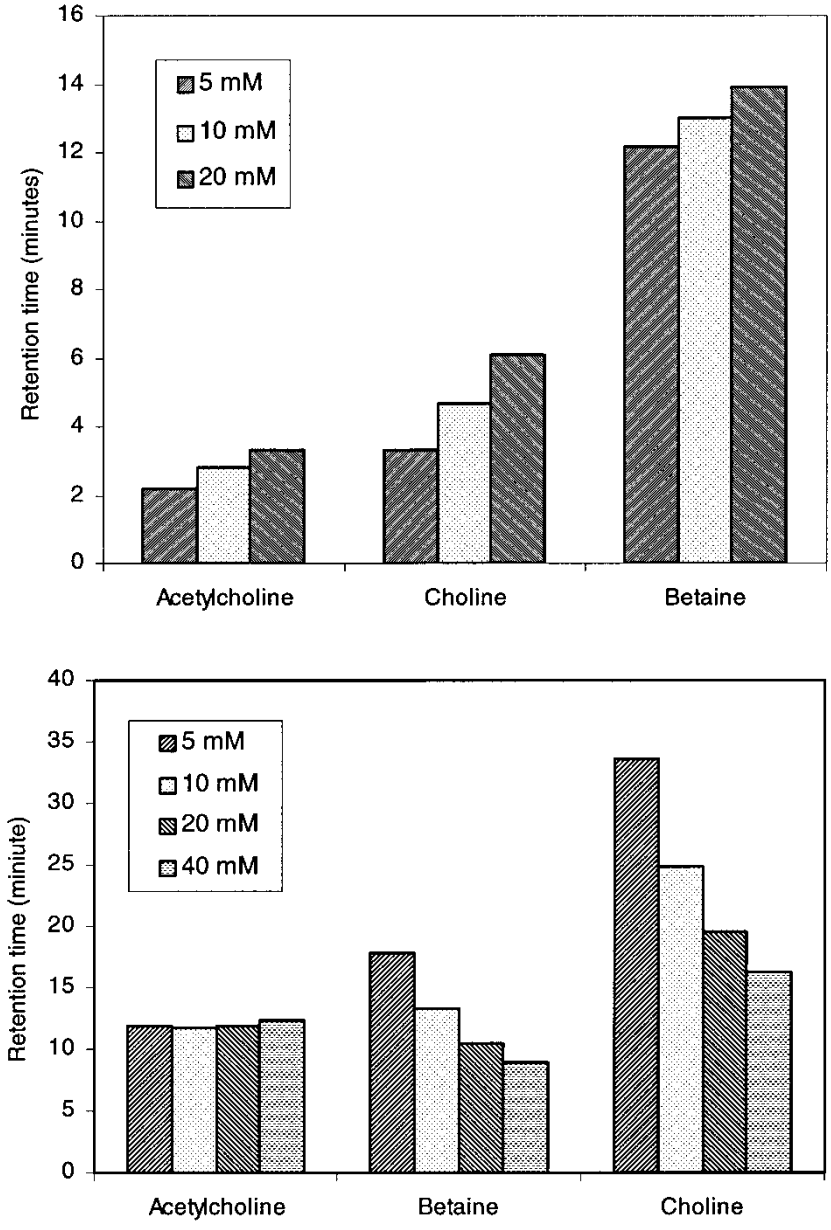

Figure 6. Effect of ammonium acetate concentration on the retention time of quaternary amine compounds on amino (top) and amide columns (bottom). Column temperature, 25°C; flow rate, 1 mL/min. Mobile phase: ACN/water (85 : 15, v/v) for the amino column and ACN/water (75 : 25, v/v) for the amide column.

which is subsequently converted to electrochemically active species to be detected on a Pt electrode. Moreover, the response of Pt electrodes often decreases rapidly due to electrode fouling over time.[1] MS methods have also been utilized to detect acetylcholine and choline.[18–22] Coupling RPLC to MS offers both selectivity and sensitivity for choline analysis. Dunphy and Burinsky recently reported a detection limit of 30 fmol for choline using ESI-MS coupled with the RPLC separation.[21] It should be pointed out that the RPLC method needed an MS compatible ion-pairing reagent to obtain sufficient retention for choline; however, the presence of the ion-pairing reagent might compromise the sensitivity of the MS detector.

In comparison, the HILIC method does not require any ion-pairing reagents or special mobile phase additives to achieve retention. Therefore, it is perfectly compatible with MS detection. In this study, a single quadruple mass spectrometer (Agilent MSD) with an electrospray interface was coupled to the HPLC instrument to explore the sensitivity for choline. Following the HILIC separation performed on the YMC-Pack NH_2 column, choline was detected by MSD in the selective ion-monitoring (SIM) mode at 104 m/z. The amide column was not used in this study, because choline exhibited tailing on this column (Figure 2b) and more water would be needed to elute choline quickly from the amide column. These two factors would have some negative impact on the MS sensitivity for choline. Figure 7a represents a typical chromatogram for choline with approximately 53 fmol on-column loading. A short analysis time (<5 minutes) was achieved by increasing the water content in the mobile phase to 19%. The HILIC/MS method had a linear response to choline ($R^2 > 0.999$) over a range of two orders of magnitudes (1.0–210 fmol). In addition, a signal-to-noise ratio of 4 was obtained for choline at the level, as low as 0.4 fmol, as shown in Figure 7b. The limit of detection (LOD) achieved for choline in this study was about 75 times lower than that of the RPLC/MS method (30 fmol),[21] and was also lower than that of the electrochemical detection method.[1,14] Two major factors contributed to the superior sensitivity of the HILIC/MS method. First, the presence of ion-pairing reagents has been recognized in the literature to have a negative effect on the MS response.[21] In contrast to RPLC, the HILIC separation did not require any ion-pairing reagents, and only a small amount of ammonium salt was used in the mobile phase to facilitate analyte ionization. Secondly, the conventional RPLC separation required low levels of organic solvents to maintain sufficient retention, even with the addition of the ion-pairing reagents. The HILIC separation, on the other hand, used a high content of organic solvents in the mobile phase to retain polar compounds. Previous research by Kebarle and coworkers demonstrated that solution surface tension had a great impact on the formation of gas-phase ions in the ESI process.[36] The release of ions from sprayed droplets was more efficient when the surface tension of the eluent was low. Therefore, the ion formation was far more efficient in the electro-spray

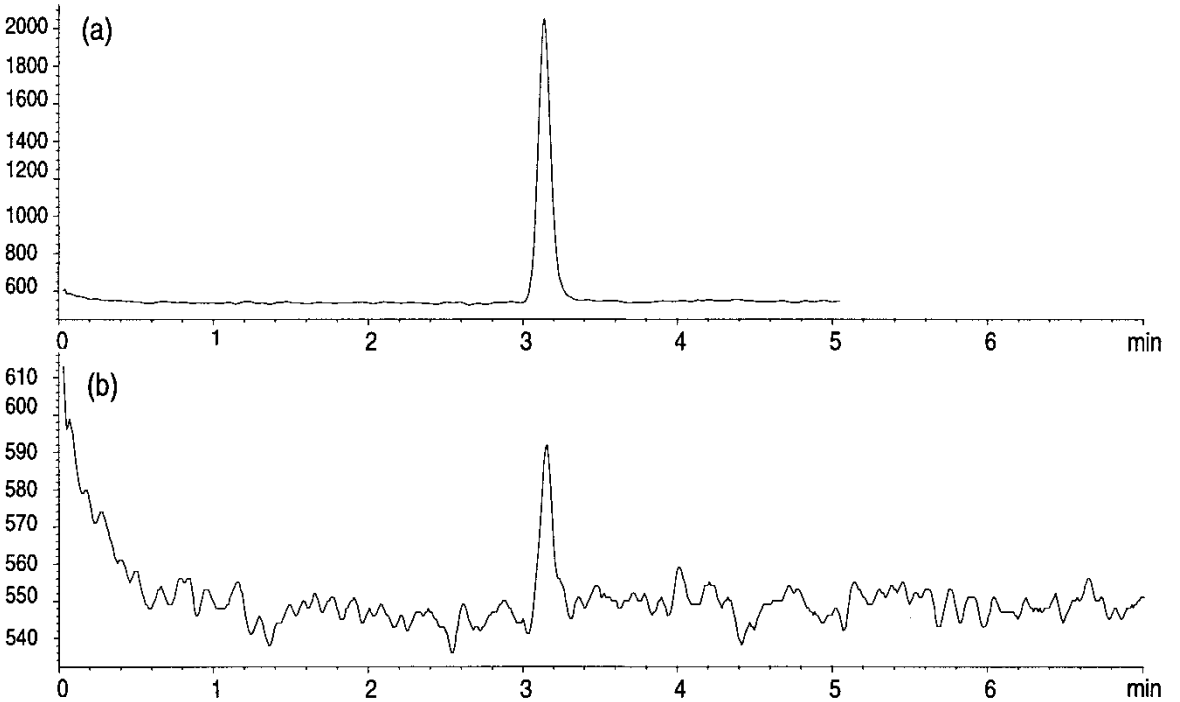

Figure 7. Ion chromatograms of choline from SIM acquisition (m/z: 104) for (a) 53 fmol and (b) 0.4 fmol. HILIC/MS conditions: column temperature, 30°C; flow rate, 1 mL/min. Mobile phase: ACN/water (81 : 19) containing 10 mM ammonium acetate.

process using a mobile phase containing higher levels of organic solvents. The absence of ion-pairing reagents and presence of high ACN content in the mobile phase provided much favorable conditions to improve the MS sensitivity of the HILIC/MS method.

CONCLUSION

The present study demonstrated the feasibility of separating quaternary amine compounds by HILIC. The model compounds were well retained on both the amino and amide columns using a simple ACN/water mobile phase containing ammonium acetate. The experimental results indicated that both the ACN content and salt concentration had significant impact on the retention of the quaternary amine compounds. In addition, the conditions required for the HILIC separation were favorable to MS detection. Not only was the issue associated with the detection of quaternary amine compounds successfully resolved by coupling MS detection with the HILIC separation, the method sensitivity for quaternary amine compounds was also significantly improved. An LOD of 0.4 fmol was achieved for choline by the HILIC/MS method. However, it should be pointed out that the excellent linear response and high sensitivity of the HILIC/MS method were based on matrix-free standards. The impact of biological matrices on the analytical performance needs to be evaluated for particular sample types. Nonetheless, the HILIC/MS approach still holds great potential for analysis of low-level choline or other quaternary amine compounds in biological or environmental samples.

REFERENCES

1. Tsai, T.J. Separation methods used in the determination of choline and acetylcholine. Chromatogr. B **2000**, *747*, 111.
2. Pomfret, E.A.; daCosta, K.A.; Schurman, L.L.; Zeisel, S.H. Measurement of choline and choline metabolite concentrations using high-pressure liquid chromatography and gas chromatography–mass spectrometry. Anal. Biochem. **1989**, *180*, 85.
3. Singh, A.K.; Drewes, L.R. Improved analysis of acetylcholine and choline in canine brain and blood samples by capillary gas-chromatography–mass spectrometry. J. Chromatogr. **1985**, *339*, 170.
4. Patterson, T.A.; Kosh, J.W. Simultaneous quantitation of arecoline, acetylcholine, and choline in tissue using gas chromatography/electron impact mass spectrometry. Biol. Mass Spectrom. **1992**, *21*, 299.
5. Damsma, G.; Flentge, F. Liquid chromatography with electrochemical detection for the determination of choline and acetylcholine in plasma and red blood cells, failure to detect acetylcholine in blood of humans and mice. J. Chromatogr. **1988**, *428*, 1.
6. Meek, J.L.; Eva, C. Enzymes adsorbed on an ion exchanger as a post-column reactor: application to acetylcholine measurement. J. Chromatogr. **1984**, *317*, 343.

7. Salamoun, J.; Nguyen, P.T.; Remien, J. Cation-exchange chromatography of choline and acetylcholine on free shielded silanols of silica-based reserved-phase stationary phases. J. Chromatogr. **1992**, *596*, 43.
8. Haen, E.; Hagenmaier, H.; Remien, J. Detection of choline and acetylcholine by high performance liquid chromatography: Limitations, pitfalls, sample preparation. J. Chromatogr. **1991**, *537*, 514.
9. Fujimori, K.; Yamamoto, K. Determination of acetylcholine and choline in perchlorate extracts of brain tissue using liquid chromatography-electrochemistry with an immonbilized-enzyme reactor. J. Chromatogr. **1987**, *414*, 167.
10. The 1990 United States Pharmacopeia XXII and National Formulary XVII. U.S. Pharmacopeia Convention, Inc.; 1989.
11. Dionex application note AN70. Dionex Corporation; 1991.
12. Honda, K.; Miyaguchi, K.; Nishino, H.; Tanaka, H.; Yao, T.; Imai, K. High performance liquid chromatography followed by peroxyoxalate chemiluminescence detection of acetylcholine and choline utilizing immobilized enzymes. Anal. Biochem. **1986**, *50*, 153.
13. Potter, P.E.; Meek, J.L.; Neff, N.H. Acetylcholine and choline in neuronal tissue measured by HPLC with electrochemical detection. J. Neurochem. **1983**, *41*, 188.
14. Greaney, M.D.; Marshall, D.L.; Bailey, B.A.; Acworth, I.N. Improved method for the routine analysis of acetylcholine release *in vivo*: quantitation in the presence and absence of esterase inhibitor. J. Chromatogr. **1993**, *622*, 125.
15. Flentge, F.; Venema, K.; Koch, T.; Korf, J. An enzyme-reactor for electrochemical monitoring of choline and acetylcholine: applications in high-performance liquid chromatography, brain tissue, microdialysis and cerebrospinal fluid. Anal. Biochem. **1992**, *204*, 305.
16. Kato, T.; Liu, J.K.; Yamamoto, K.; Osborne, P.G.; Niwa, O. Detection of basal acetylcholine release in the microdialysis of rat frontal cortex by high performance liquid chromatography using a horseradish peroxidase-osmium redox polymer electrode with pre-enzyme reactor. J. Chromatogr. B **1996**, *682*, 162.
17. Dong, Y.; Wang, L.; Shangguan, D.; Zhao, R.; Liu, G. Improved method for the routine determination of acetylcholine and choline in brain microdialysate using a horseradish peroxidase column as the immobilized enzyme reactor. J. Chromatogr. B **2003**, *788*, 193.
18. Liberato, D.J.; Yergey, A.L.; Weintraub, S.T. Separation and quantitation of choline and acetylcholine by thermospray liquid chromatography/mass spectrometry. Biomed. Environ. Mass Spectrom. **1986**, *13*, 171.
19. Ishimaru, H.; Ikarashi, Y.; Maruyuam, Y. Use of high performance liquid chromatography continuous-flow fast atom bombardment mass spectrometry for simultaneous determination of choline and acetylcholine in rodent brain regions. Bio. Mass Spectrom. **1993**, *22*, 681.
20. Acevedo, L.D.; Xu, Y.; Zhang, X.; Xin, R.J.; Pearce, A.; Yergey, A.L. Quantitation of acetylcholine in cell culture systems by semi-micro high performance liquid chromatography and electrospray ionization mass spectrometry. J. Mass Spectrom. **1996**, *31*, 1399.
21. Dunphy, R.; Burinsky, D.J. Detection of choline and acetylcholine in a pharmaceutical preparation using high performance liquid chromatography/electrospray ionization mass spectrometry. J. Pharm. Biomed. Anal. **2003**, *31*, 905.
22. Reubaset, J.L.E.; Ahlsen, E.; Haneborg, K.G.; Ringvold, A. Sample preparation and determination of acetylcholine in corneal epithelium cells using liquid chromatography-tandem mass spectrometry. J. Chromatogr. Sci. **2003**, *41*, 151.

23. Alpert, A.J. Hydrophilic interaction chromatography for the separation of peptides, nucleic acids, and other polar compounds. J. Chromatogr. **1990**, *499*, 177.
24. Churms, S.C. Recent progress in carbohydrate separation by high performance liquid chromatography based on hydrophilic interaction. J. Chromatogr. A **1996**, *720*, 75.
25. Alpert, A.J.; Shukla, M.; Shukla, A.K.; Zieske, L.R.; Yuen, S.W.; Ferguson, M.A.J.; Mehlert, A.; Pauly, M.; Orlando, R. Hydrophilic-interaction chromatography of complex carbohydrates. J. Chromatogr. A **1994**, *676*, 191.
26. Guile, G.R.; Rudd, P.M.; Wing, D.R.; Prime, S.B.; Dwek, R.A. A rapid high-resolution high performance liquid chromatographic method for separating glycan mixtures and analyzing oligosaccharide profiles. Anal. Biochem. **1996**, *240*, 210.
27. Oyler, A.R.; Armstrong, B.L.; Cha, J.Y.; Zhou, M.X.; Yang, Q.; Robinson, R.I.; Dunphy, R.; Burinsky, D.J. Hydrophilic interaction chromatography on amino-silica phases complements reversed-phase high performance liquid chromatography and capillary electrophoresis for peptide analysis. J. Chromatogr. A **1996**, *724*, 378.
28. Yoshida, T. Peptide separation in normal phase liquid chromatography. Anal. Chem. **1997**, *69*, 3038.
29. Mant, C.T.; Kondejewski, L.H.; Hodges, R.S. Hydrophilic interaction/cation-exchange chromatography for separation of cyclic peptides. J. Chromatogr. A **1998**, *816*, 79.
30. Olsen, B.A. Hydrophilic interaction chromatography using amino and silica columns for the determination of polar pharmaceuticals and impurities. J. Chromatogr. A **2001**, *913*, 113.
31. Strege, M.A. Hydrophilic interaction chromatography-electrospray mass spectrometry analysis of polar compounds for natural product drug discovery. Anal. Chem. **1998**, *70*, 2439.
32. Tolstikov, V.V.; Feihn, O. Analysis of highly polar compounds of plant origin: combination of hydrophilic interaction chromatography and electrospray ion trap mass spectrometry. Anal. Biochem. **2002**, *301*, 298.
33. Dallet, Ph.; Labat, L.; Kummer, E.; Dubost, J.P. Determination of urea, allantoin, and lysine pyroglutamate in cosmetic samples by hydrophilic interaction chromatography. J. Chromatogr. B **2000**, *742*, 447.
34. Guo, Y.; Huang, A. A HILIC method for the analysis of tromethamine as the counter ion in an investigational pharmaceutical salt. J. Pharm. Biomed. Anal. **2003**, *31*, 1191.
35. Normal Phase Chromatography: TSKgel Amide-80, Tosoh BioScience 2003 Laboratory Products Catalogue, www.tosohbiosep.com (accessed Mar. 2004).
36. Ikonomou, M.G.; Blades, A.T.; Kebarle, P. Electrospray-ion spray: a comparison of mechanism and performance. Anal. Chem. **1991**, *63*, 1989.

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