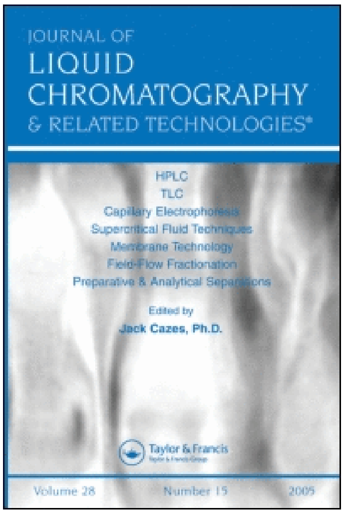

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Effects of Perchloric Acid on High Temperature Liquid Chromatography

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Abstract: The effects of the chaotropic agent perchloric acid on high temperature liquid chromatography (HTLC) with water as mobile phase have been investigated over a temperature range from 130°C to 200°C. Aniline, N-methylaniline, 3-ethylaniline and N,N-dimethylaniline were used as analytes. Similar to the effects of increasing the organic content of the mobile phase in regular reverse phase HPLC, the increase of column temperature in HTLC results in the decrease of capacity factors for all the aniline test compounds. By adding low concentrations of perchloric acid in the mobile phase, the capacity factors of the anilines also decrease which is equivalent to the increase of column temperature. Comparisons of $HClO_4$ gradient and temperature program for the separation of the four anilines in HTLC are presented.

Keywords: Anilines, Chaotropic additive, High temperature LC, Perchloric acid

INTRODUCTION

Water under ambient conditions is too polar to solvate most organic compounds. However, the polarity of liquid water can be changed over a wide range by controlling temperature under moderate pressures to

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maintain water in the liquid state. Increasing water temperature to 200–250°C leads to a similar change in water polarity as is achieved by the 100% acetonitrile. The use of high-temperature water (HTW) as a mobile phase in HPLC at temperatures above 100°C has been studied and used in different areas.[1–6] HTW is structurally different from ambient liquid water. Hydrogen bonding, which gives water unique properties, becomes weaker as the temperature rises. At the same time, the increase in temperature produces a decrease in density. Thus, unlike the infinite percolating network of hydrogen bonds found in ambient liquid water, the hydrogen bond network in HTW exists in the form of small clusters of hydrogen bonded water molecules.[7–10] With increasing temperature and decreasing density, the average cluster size decreases.[11–13] Changes in the extent of hydrogen bonding are accompanied by corresponding changes of the dielectric constant. A higher dielectric constant is typically associated with a greater ability to dissolve electrolytes or polar compounds. As temperature increases, the dielectric constant of water decreases. HTW therefore behaves like polar organic solvents, and therefore, small organic compounds are soluble in HTW.[14,15] A number of polar analytes have been separated using a pure water mobile phase.[16–22] The change in solvating power relative to temperature has also been proposed and demonstrated as a means of programming solvent strength.[16,19,23] So far, HTLC has been used for applications in pharmaceutical industry,[1–6,24,25] and other areas.[26–28]

Perchloric acid ($HClO_4$) has been demonstrated as a good chaotropic agent in reversed-phase chromatography.[29–32] In the present study, water and water with different concentrations of $HClO_4$ were used to elute anilines at different temperatures. The capacity factors of the anilines on an XBridge C-18 column under high temperatures were investigated to evaluate the effects of $HClO_4$ on the separation, as well as to understand chromatographic mechanisms.

EXPERIMENTAL

An Agilent HP1100 HPLC (Agilent Technologies, Wilmington, DE, USA) system with a diode array detector was employed. For all the experiments the column was placed in a Polaratherm Series 9000 total temperature controller (Selerity Technologies Inc., Salt Lake City, UT, USA). The precision of the column oven temperature is ±0.5°C. Chromatograms were acquired and processed using ChemStation (Agilent Technologies, Wilmington, DE, USA).

Chromatography Conditions

A prototype silica-silicon based ethyl-bridged hybrid C18, 3.5 μm (15 × 0.21 cm I.D.) column manufactured by Waters (Milford, MA, USA) was used for all studies. The chromatography separations were conducted under both isocratic and isothermal conditions with a flow rate of 1.0 mL/min. For the studies with perchloric acid, the mobile was prepared by pre-mixing perchloric acid with water. The analytes were dissolved in methanol at a concentration of 1 mg/mL and 1 μL was injected onto the HPLC system. Compounds were detected using a UV photodiode array detector set to a wavelength of 220 nm. A test mixture of aniline, N-methylaniline, 3-ethylaniline and N,N-dimethylaniline was used as a system suitability check before and after each experiment to ensure the stability of the column and system performance.

The surface area of the molecules was calculated with Hyperchem Professional version 7.5 (Hypercube, Inc., Gainesville, FL). All other chromatographic parameters were calculated with ChemStation Rev. B. 01 (Agilent Technologies, Wilmington, DE, USA).

Chemicals

Aniline, N-methylaniline, 3-ethylaniline and N,N-dimethylaniline were purchased from Aldrich (Milwaukee, WI, USA). HPLC grade water was purchased from EM Science (Gibbstown, NJ, USA). $HClO_4$ (redistilled 99.999% purity) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

RESULTS AND DISCUSSION

Influence of the Temperature on the Separation

To study the behavior of the aniline compounds on the XBridge column, a van't Hoff plots were generated. If the stationary phase undergoes a change in conformation at a certain temperature, the enthalpy and the entropy of the retention process will change, and the van't Hoff plot will show a change in slope and intercept at the transition temperature.[33,34] So the initial experiments were conducted under isothermal conditions with pure water as mobile phase. The column temperature was varied between 130°C and 200°C.

An example of van't Hoff plots for the four anilines is presented in Figure 1 and typical chromatograms are presented in Figure 2. For all the four anilines, the plot of $\ln k'$ vs $1/T$ shows a straight

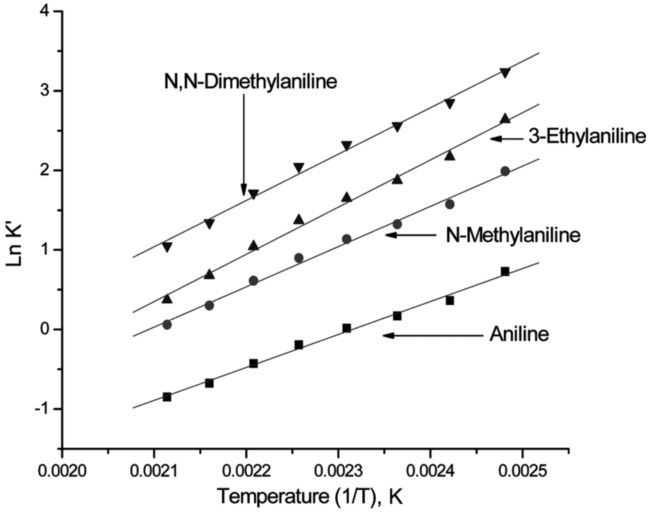

Figure 1. Effect of column temperature on $\ln k'$. Column: 15×0.21 cm I.D, 3.5 μm XBridge C18. Mobile phase: 100% water at a flow rate of 1.0 mL/min. Detection: UV 220 nm. Sample: aniline, N-methylaniline, 3-ethylaniline and N,N-dimethylaniline at 1mg/mL with a injection volume of 1 μL.

line with a $R^2 > 0.99$ which indicate that there is no conformation change in the temperature range studied. While the phase ratio could not be determined, the result shows the dependence of the enthalpies of transfer for the anilines from the mobile phase to the stationary phase (see Table 1). For the aniline and N-alkyl anilines, $\Delta H°$ becomes more negative as the number of alkyl groups increases, indicating a stronger interaction with the stationary phase. At the same time, the $\Delta H°$ becomes more negative as the surface area of the anilines increases from aniline to N,N-dimethylaniline, which is consistent with the hydrophobic interaction principles: the higher the surface area of the analyte, the stronger the interaction with the reversed-phase stationary phase.

Influence of the Concentration of $HClO_4$ on the Separation

To study the influence of the chaotropic agent $HClO_4$ on the hybrid stationary phase, the composition of the mobile phase was varied between 0.1 mM to 0.9 mM $HClO_4$. Typical chromatograms of the mixture of analytes at 0.1 mM of $HClO_4$ at different temperatures are shown in Figure 3. Compared with the chromatogram employing water as mobile phase (Figure 2), the peak shape changes for the later eluting

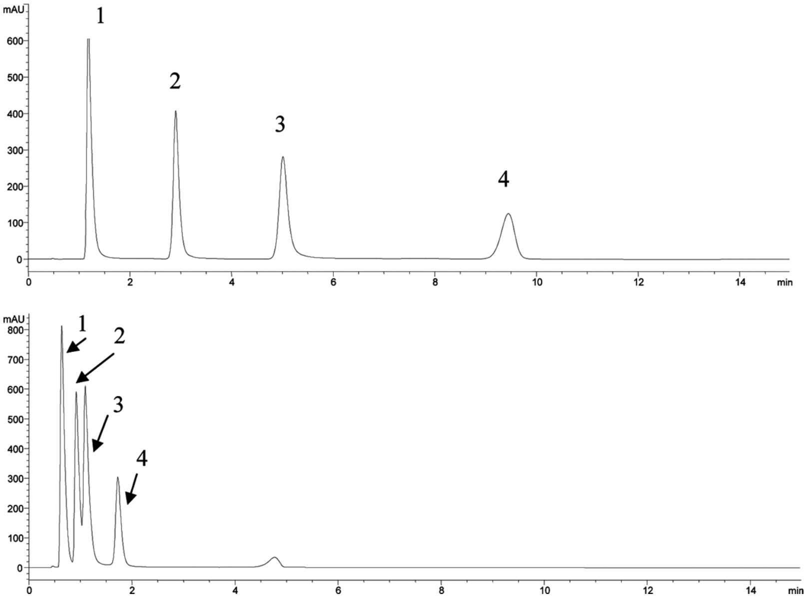

Figure 2. Chromatogram with water as mobile phase (top: column temperature 130°C bottom: column temperature 200°C). Column: 15 × 0.21 cm I.D, 3.5 μm XBridge C18. Mobile phase: 100% water at a flow rate of 1.0 mL/min. Detection: UV 220 nm. Peaks: 1 = aniline, 2 = N-methylaniline, 3 = 3-ethylaniline and 4 = N,N-dimethylaniline.

anilines, and especially for N,N-dimethylaniline which elutes last. This phenomenon is under investigation.

Equation (1) shows that the plot of $\ln k'$ vs. the volume fraction of the organic modifier in reversed-phase HPLC is a straight line with a slop of S and the intercept of $\ln k'_w$:[35–37]

$$\ln k' = \ln k'_w - S\varphi \qquad (1)$$

Table 1. Determined thermodynamic parameters

Analytes	Molecular surface area (A^2)	$\Delta H°$ (kcal/mol)
Aniline	256.90	−8.23
N-Methylaniline	286.07	−10.05
3-Ethylaniline	345.17	−11.78
N,N-Dimethylaniline	303.95	−11.58

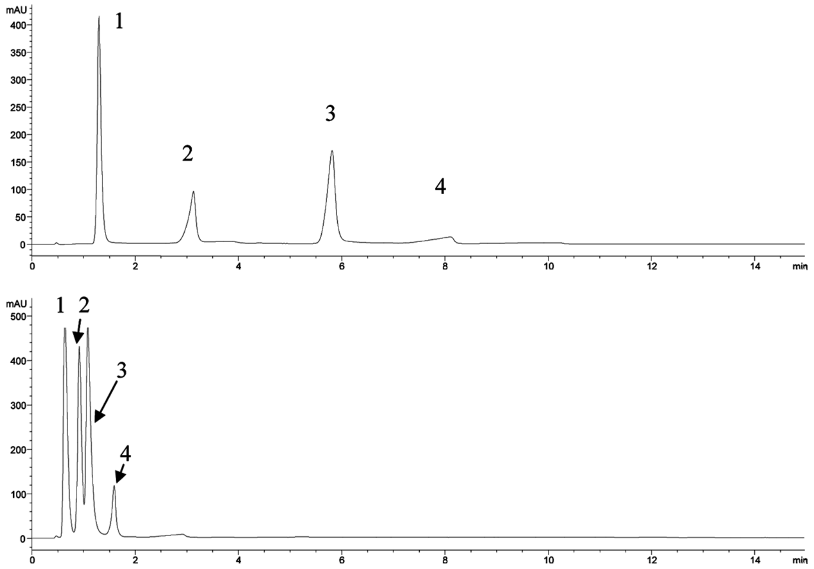

Figure 3. Chromatograms of anilines with mobile phase additive of $HClO_4$ at different temperatures (top: 130°C with 0.1 mM $HClO_4$, bottom, 200°C with 0.1 mM $HClO_4$). Peaks: 1 = aniline, 2 = N-methylaniline, 3 = 3-ethylaniline, 4 = N,N-dimethylaniline.

where k' is the solute capacity factor at a specific mobile phase composition (φ) and k'_w is the extrapolated k' for pure water ($\varphi = 0$). S is proportional to the free energy of solute transfer from water to pure organic mobile phase and correlates with the molecular size. In our case, a graph of $\ln k'$ vs. the volume fraction of $HClO_4$ led to a straight line. Figure 4 shows a graph of $\ln k'$ vs. the mM concentration of $HClO_4$ in the mobile phase for anilines at 130°C. A linear correlation was obtained with an $R^2 > 0.99$ for all the anilines except N,N-dimethylaniline for which the retention time is shifted because of the peak shape change. With the increase of the concentration of $HClO_4$ in the mobile phase, the $\ln k'$ decreases for all the compounds investigated which is consistent with Eq. (1) when phase composition (φ) is replaced by the concentration of $HClO_4$. In this case, the role of $HClO_4$ is similar to the role of organic phase in regular HPLC. Table 2 lists the S values and the molecular surface area for all the four anilines at 130°C. For the N-alkylanilines, the S value increases with the increase of surface area. The retention of the analytes decreases as the volume fraction of $HClO_4$ increases.[29–31,38] At high temperatures, the structure of water was disturbed and the weakened hydrogen bonds were unable to sustain the percolated structure of water, leading to clusters which approached the

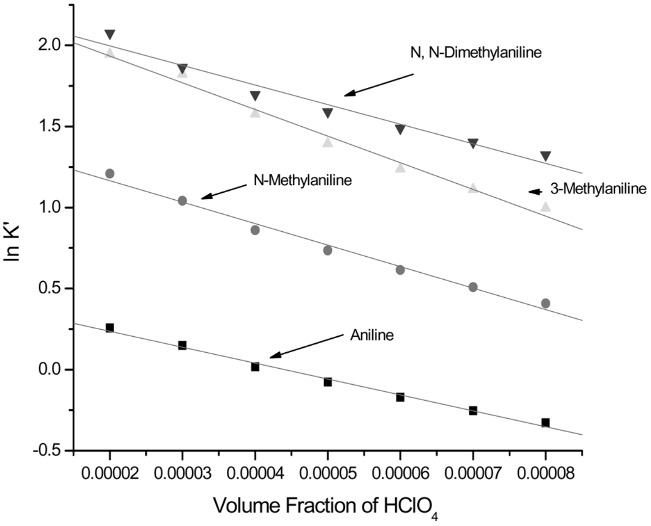

Figure 4. Effect of the volume fraction of $HClO_4$ on $\ln k'$ at 130°C. Column: 15 × 0.21 cm I.D, 3.5 μm XBridge C18. Mobile phase: pre-mixed $HClO_4$ with water at a flow rate of 1.0 mL/min. Detection: UV 220 nm. Sample: aniline, N-methylaniline, 3-ethylaniline and N,N-dimethylaniline at 1 mg/mL with an injection volume of 1 μL.

dielectric constant of an organic solvent. The mechanism of action of the low concentration of $HClO_4$ in HTLC is that the perchlorate anion enhances the disturbance of the weakened hydrogen bonds and makes the water much less polar.[39–41] van't Hoff plots of the four anilines with 0.23 mM of $HClO_4$ mobile phase additive is presented in Figure 5. The plots also are linear with $R^2 > 0.99$ for all the anilines studied, which indicates that there are no conformation changes in this temperature range with 0.23 mM $HClO_4$. Table 3 lists the enthalpies of the four anilines at the above conditions. The comparison of the data of Table 1 with Table 3 shows that the addition of $HClO_4$ decreases the interaction of the analytes with stationary phase.

Table 2. Calculated S value at 130°C

Compounds	S value	Molecular surface area (A^2)
Aniline	0.843	256.90
N-Methylaniline	1.139	286.07
3-Ethylaniline	1.414	345.17
N,N-Dimethylaniline	1.039	303.95

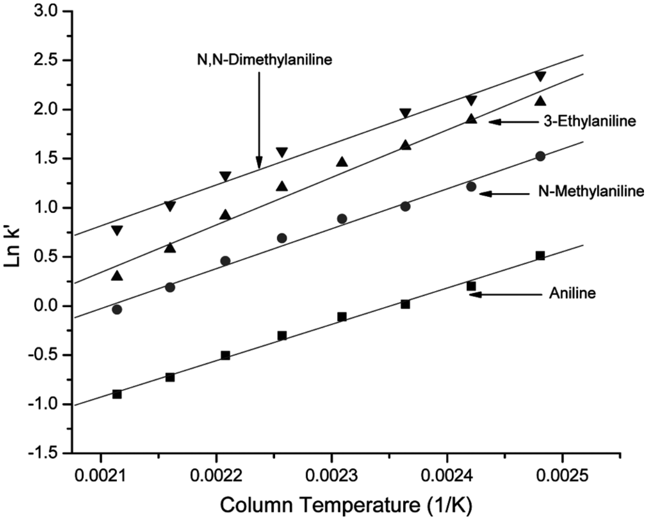

Figure 5. Temperature effect on $\ln k'$ with a mobile phase additive of $HClO_4$ at 0.23 mM. Column: 15 × 0.21 cm I.D, 3.5 μm XBridge C18. Mobile phase: premixed $HClO_4$ with water at a flow rate of 1.0 mL/min. Detection: UV 220 nm. Sample: aniline, N-methylaniline, 3-ethylaniline and N,N-dimethylaniline at 1 mg/mL with a injection volume of 1 μL.

Separation of Anilines by $HClO_4$ Gradient and Temperature Program

To demonstrate the effect of perchloric acid on the separation, and the applicability of the effect in the separation of the anilines, a $HClO_4$ concentration gradient was performed at a column temperature of 130°C. The gradient starts with 0 mM $HClO_4$ and proceeds to 0.9 mM at a rate of 0.16 mM/min of $HClO_4$. Figure 6 shows the resulting chromatogram. Aniline, N-methylaniline and 3-ethylaniline show good

Table 3. Determined thermodynamic parameters at 0.23 mM $HClO_4$

Analytes	$\Delta H°$ (kcal/mol)/0.23 mM $HClO_4$
Aniline	−7.34
N-Methylaniline	−8.07
3-Ethylaniline	−9.61
N,N-Dimethylaniline	−8.28

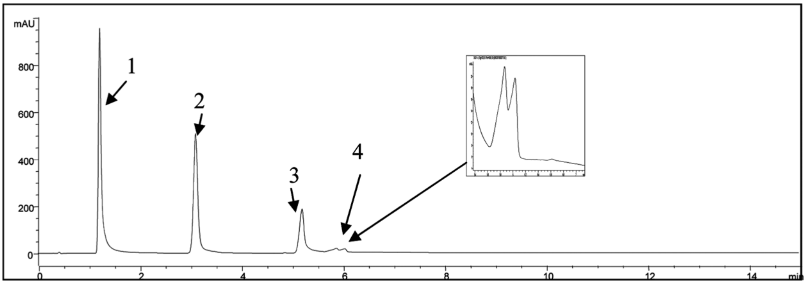

Figure 6. $HClO_4$ gradient for the separation of four anilines. Mobile phase A = water, mobile phase B = 5.8 mM $HClO_4$ in water. Gradient: 0% B to 16% B in 6 minutes, hold 16% B for 9 minutes. Peaks: 1 = aniline, 2 = N-methylaniline, 3 = 3-ethylaniline, 4 = N,N-dimethylaniline.

peak shapes and the elution times were decreased due to the effect of the $HClO_4$. However, the N,N-dimethylaniline peak was distorted and split which could result from partial ionization of the molecule. An expanded chromatogram for N,N-dimethylaniline is inserted in the chromatogram. The split peak shape is consistent with the peak shape described by Lloyd, et al.[42] for a partially ionized compound. To compare the separation with a temperature program using water as mobile phase, the analytes were also separated using a temperature program from 130°C to 180°C at a rate of 5°C/min (see Figure 7). All four anilines were well separated and maintained good peak shapes. Table 4 lists the resolutions for the 4 anilines under $HClO_4$ gradient and temperature program. For all the aniline pairs, the $HClO_4$ gradient shows better resolution than that in temperature program, except the pair 3-ethylaniline and

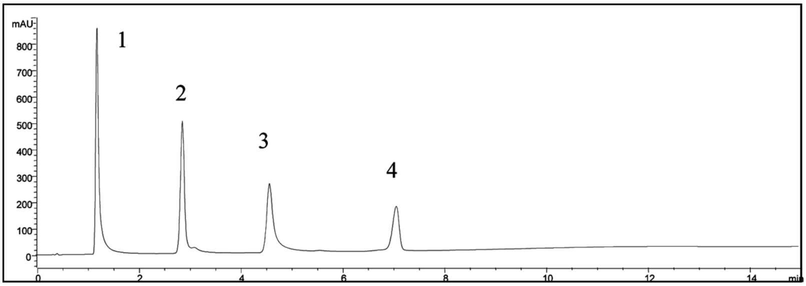

Figure 7. Temperature program for the separation of four anilines. 130°C, hold 1 minute, then temperature program at 6°C/minute to 180°C, hold 4 min. Peaks: 1 = aniline, 2 = N-methylaniline, 3 = 3-ethylaniline, 4 = N,N-dimethylaniline.

Table 4. Comparison of the separation between $HClO_4$ gradient and temperature program

Analytes	R^* with $HClO_4$	R^* with pure water
Aniline	N/A	N/A
N-Methylaniline	15.0	13.5
3-Ethylaniline	12.8	9.2
N,N-Dimethylaniline	2.8	10.6

$^*R$ = Resolution.

N,N-dimethylaniline, which was caused by peak shape change of N,N-dimethylaniline at the condition described.

CONCLUSIONS

Separation of substituted anilines was demonstrated using HTW and HTW with $HClO_4$ chaotropic agent. Low concentrations of $HClO_4$ had a significant effect on the capacity factor of investigated anilines and can be used as a mobile phase additive to adjust analyte retentions. The increase in the concentration of $HClO_4$ shows an equivalent effect to the increase in temperature in HTLC. Instead of temperature program in HTLC, $HClO_4$ gradient can be used to adjust analyte retention.

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