HPLC Separations with Micro-Bore Columns Using High-Temperature Water and Flame Ionization Detection

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Abstract

Previous work demonstrates that a flame ionization detector (FID) may be used as a detector for high-temperature water separation. However, the relatively high flow rate of the eluent required by standard high-performance liquid chromatography columns causes instability of the FID. In this work, micro-bore columns are packed with poly(styrene-divinylbenzene) (PRP-1) or Daiso gel octadecylsilane-bonded phase particles. Because micro columns require low volume flow rates, the eluent used in high-temperature water chromatography does not cause instability of the FID. Separation of carbohydrates, amino acids, and other organic acids and bases is performed on two micro-bore columns using a homemade high-temperature water chromatograph with FID. Both isothermal and programmed temperatures are used in this work. The limit of detection and the linear range are also determined for amino acids tested.

Introduction

As is known, reversed-phase liquid chromatography (RPLC) is a very popular separation and analytical technique. However, RPLC requires a mixture of organic solvent and water (e.g., methanol–water or acetonitrile–water) as the mobile phase. Another limitation of RPLC is the lack of a sensitive and universal detector, such as the flame ionization detector (FID) used in gas chromatography (GC). Chromatographers investigated the use of flame ionization or flame photometric detection in LC separations in which organic solvents were involved in the mobile phase (1–7). However, because organic solvents present in the mobile phase caused significantly high FID background, such LC–FID systems were limited.

A possible solution for overcoming the RPLC limitations is to perform the separation using high-temperature water as the mobile phase and FID as the detector because FID does not respond to water. Recently, the application of FID in LC separations using only water as the mobile phase has been reported (8–14). Either an interface between the LC column and FID was used or the FID was modified in some of these LC–FID systems. Although the direct coupling of LC with nonmodified FID was also reported (8,12), the FID was found to be unstable at water flow rates greater than 0.2 mL/min. In order to solve this problem, a split subcritical water chromatography–FID system was constructed and tested (12). Although this split approach works fine for analytes with adequate concentrations, it is not appropriate for analytes with low concentrations because only a very small fraction of solutes is detected by FID (12).

In this work, micro columns have been used for high-temperature water separation with FID detection. There are at least a couple of advantages for using micro-bore columns. First, because the micro-bore column has a smaller internal diameter compared with traditional, standard high-performance liquid chromatography (HPLC) columns, the optimized volume flow rate is much slower than that for standard HPLC columns. This low flow rate in turn will not cause instability of FID when water is used as the mobile phase. Because no split of the eluate is required before FID detection, it is possible to detect solutes with low concentrations using the system reported in this work. Secondly, faster thermal equilibration can be established within the micro-bore column compared to the traditional HPLC columns (e.g., 4.6-mm i.d.) when a high temperature is used. Therefore, temperature programming can be employed to achieve better separation.

Experimental

Reagents

L-Proline, L-leucine, D-phenylalanine, L-tryptophan, α-D-glucose, D-maltose monohydrate, sucrose, 5-aminophenol, 3-aminophenol, 2-aminophenol, resorcinol, catechol, benza-

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mide, aniline, and pyridine were purchased from Sigma-Aldrich (Milwaukee, WI). Deionized water (18MΩ cm) was prepared in our laboratory using a Sybron/Barnstead system (Sybron/Barnstead, Boston, MA) and used as a mobile phase in this work. HPLC-grade methanol was obtained from Fisher Scientific (Fair Lawn, NJ) and employed as slurry and pressurized solvents for packing micro-bore columns. HPLC-grade acetone (purchased from Fisher Scientific) was used for cleaning the apparatus of the column packer. The solutions of analytes were prepared in pure water.

Column preparation

The columns used in this study were prepared in our laboratory. Daiso gel octadecylsilane-bonded phase (ODS-BP) packing (100-Å pore size, 5-µm particle size) (Daiso Co. Ltd., Osaka, Japan) was used for separation of sugars while PRP-1 resins (120-Å pore size, 3-µm particle size) (Hamilton Company, Reno, NV) were used for separation of other analytes. Empty micro-bore columns (250- × 0.5-mm i.d.) were purchased from SEG Incorporated (Austin, TX,).

Slurry of the Daiso gel ODS-BP or PRP-1 particles was prepared in methanol with approximately 0.1% (w/v, particles/methanol) and sonicated for 30 min before filling the slurry reservoir (¼-inch inlet, ¼-inch outlet, 40 mL) (Alltech, Deerfield, IL). Before packing, the micro-bore column and the slurry reservoir were rinsed with acetone and dried.

The micro-bore columns were packed by using a high-capacity slurry packer (95551U, Alltech) and air compressor (PC 350E, Werther International, Inc., Houston, TX). The pump line was rinsed by circulating methanol. An empty micro-bore column was connected to the outlet of the slurry reservoir via a micro column packing adapter (obtained from SEG Inc.). After the slurry of the stationary phase was filled into the slurry reservoir and the connection between the inlet of slurry reservoir and the outlet of the packing pump was made, the micro-bore column was ready to be packed. The inlet pressure of the packer was set at 82 psi to obtain an outlet pressure of 10,000 psi. The packing process was started by opening the inlet air valve of the slurry packer. During the preparation of the micro column, the pressure at the outlet of the packing pump was maintained at 10,000 psi. After collecting the same amount of methanol as the volume of the slurry reservoir (40 mL), the packing of the micro-bore column was completed.

After turning off the slurry packer, the packed micro column was depressurized. Excess packing particles were scraped away using a razor, and then the column outlet was sealed with a frit and an end fitting (the inlet of the column was sealed before packing). The micro column was flushed using pure water for 1 h before use for subcritical water separation.

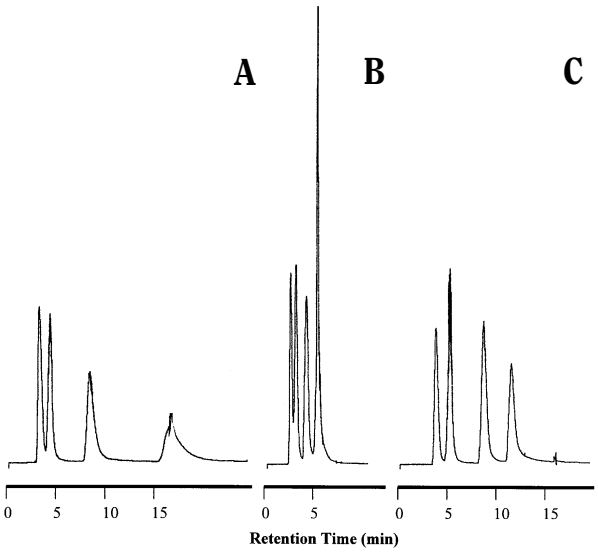

Figure 1. Chromatogram of amino acids obtained on the PRP-1 column: flow rate, 20 µL/min; 50°C (A); 100°C (B); and programmed temperature as described in text (C). Peak order: L-proline, L-leucine, D-phenylalanine, and L-tryptophan.

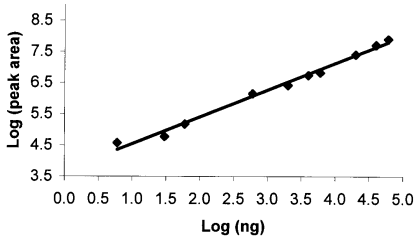

Figure 2. Linear range for L-proline.

Subcritical water chromatography–FID system

An LDC pump (constaMetric 3200, LDC Analytical, Riviera Beach, FL) was employed to deliver the mobile phase. Either a Valco six-port injector with a 2-µL sample loop (purchased from Keystone Scientific, Bellefonte, PA) or a four-port micro-injector with 0.1-µL sample holder (Keystone Scientific) was connected to the outlet of the LDC pump using stainless steel tubing (100-cm × 127-µm i.d.) (Supelco, Bellefonte, PA) and placed outside the GC oven (HP 5890 Series 2, Hewlett Packard, Avondale, PA). The packed micro-bore column was connected to the outlet of the injector with a piece of stainless steel tubing (50-cm × 127-µm i.d.) and a Valco stainless steel union (1/16 to 1/16 inch) (Varian Associates, Sugar Land, TX). Another Valco stainless steel union was used for connecting the outlet tubing of the micro-column and a piece of stainless steel capillary (80-cm × 56-µm i.d.) (Isco Inc., Lincoln, NE) that

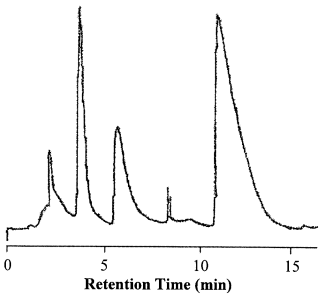

Figure 3. Chromatogram of aminophenols and pyridine obtained on the PRP-1 column: flow rate, 30 µL/min; and separation temperature, 100°C. Peak order: 5-aminophenol, 3-aminophenol, 2-aminophenol, and pyridine.

was inserted into the FID tube. This stainless steel capillary was required to provide the backpressure so that water was kept in the liquid state inside the separation column at elevated temperature. Graphite ferrules (Alltech) were used to connect the capillary to the outlet of the column and FID. The end of the capillary was positioned at 4 cm below the tip of the FID jet. The FID temperature was set at 400°C for all separations. The hydrogen and air flow rates were 31 mL/min and 310 mL/min, respectively. The mobile phase flow rate ranged from 20 to 30 µL/min.

Results and Discussion

Separations of amino acids

A mixture of amino acids was separated using the PRP-1 micro column. Because the mobile phase composition in high-temperature water separation is not tunable, the separation temperature is the main factor that controls the elution strength of high-temperature water.

First, isothermal separation of amino acids was performed at 50°C and 100°C. The flow rate used was 20 µL/min. Although all four amino acids were separated at 50°C, the peaks of D-phenylalanine and L-tryptophan were broad as shown in Figure 1A. The shape of the L-tryptophan peak is especially poor. When the separation temperature was raised to 100°C, the last two peaks became much narrower as depicted in Figure 1B. However, the first two peaks were not base-line resolved. After the results of isothermal separation were obtained, temperature programmed separation was performed. The initial oven temperature of 50°C was held for 4 min, and then the oven temperature was raised to 100°C at a rate of 10°C/min. As shown in Figure 1C, all four amino acids were well separated.

Detection limits and linear ranges of amino acids

The limit of detection and the linear range of these amino acids were determined. Injection volumes of 0.1 and 2 µL were used. The programmed oven temperature described previously was employed in this experiment. The results revealed a detection limit of 0.3 ng for L-proline and 3 ng for the other three amino acids. The linear range was 6–6,000 ng for L-proline and D-phenylalanine, and 30–6,000 ng for L-leucine and L-tryptophan. Figure 2 demonstrates the linear relationship between log (mass of L-proline injected) and log (corresponding peak area). The correlation coefficient was 0.9898 in this case.

Separation of other organic acids and bases

The PRP-1 micro-bore column was also used for separation of aminophenols, polyhydroxylbenzenes, benzamide, pyridine, and aniline. The separation of aminophenols and pyridine was performed at 100°C with a water flow rate of 30 µL/min. The chromatogram of this mixture is shown in Figure 3. Programmed temperature was used for the separation of resorcinol, catechol, benzamide, and aniline. The initial oven temperature was 80°C. This initial temperature was increased to 150°C at a rate of 8°C/min. The flow rate of water was 30 µL/min. As demonstrated in Figure 4, all four solutes were efficiently separated.

Separations of carbohydrates

Separation of a carbohydrate mixture was performed on a micro-bore column packed with Daiso gel ODS-BP particles. The separation temperature was 36°C, and the flow rate of water was 20 µL/min. The chromatogram of α-D-glucose, D-maltose monohydrate, and sucrose is shown in Figure 5. All three solutes were well separated.

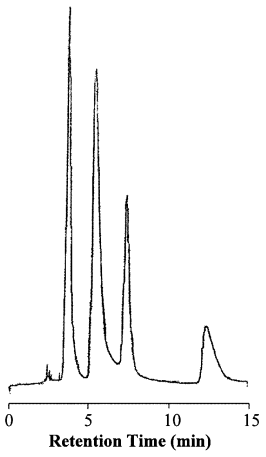

Figure 4. Chromatogram of other organic acids and bases obtained on the PRP-1 column: flow rate, 30 µL/min; and separation temperature, programmed temperature as described in text. Peak order: resorcinol, catechol, benzamide, and aniline.

Figure 5. Chromatogram of carbohydrates obtained on the Daiso gel ODS-BP column: flow rate, 20 µL/min; and separation temperature, 36°C. Peak order: α-D-glucose, D-maltose monohydrate, and sucrose.

Conclusion

Micro-bore columns were packed with either PRP-1 or Daiso gel ODS-BP particles in our laboratory. The PRP-1 column was used for the separation of amino acids, phenols, and organic bases using high-temperature water as the eluent and FID detection. A very low volume flow rate of the eluent was required because of the small internal diameter of the micro-bore column. This low flow rate of water did not cause any instability of FID. Good separation of amino acids and phenols was achieved using programmed temperature. The limit of detection for amino acids ranged from 0.3 ng to 3 ng. The linear range was 6–6,000 ng for L-proline and D-phenylalanine, and 30–6,000 ng for L-leucine and L-tryptophan. Separation of a mixture of carbohydrates was achieved on a micro-bore column packed with Daiso gel ODS-BP material.

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