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Flame ionization detection after splitting the water effluent in subcritical water chromatography

Yu Yang*, Aaryn D. Jones, John A. Mathis, Melissa A. Francis

Department of Chemistry, East Carolina University, Greenville, NC 27858, USA

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Abstract

The coupling of subcritical water separation with flame ionization detection (FID) in the split mode has been investigated in this study. In order to keep the FID system stable during subcritical water separation, a Tee union was connected between the separation column and the FID system to split the water flow. The ratio of the water flow to the FID system over the flow-rate to a waste bottle varied depending on the dimension of capillary tubings and the total water flow-rate used. Separations of several carbohydrates, carboxylic acids, and amino acids were performed on commercially available columns using a laboratory-made subcritical water chromatography–FID system. The FID system was very stable in this split mode even at total flow-rate as high as 1.24 ml/min. The linear dynamic range was up to three orders of magnitude and the limit of detection (LOD) ranged from 38 to 111 ng (306–925 ng/μl injected) with split ratios of ~1:10 to ~1:17 (FID/waste bottle) for several analytes studied. However, the LOD can be significantly lowered by adjusting the dimensions of the restrictors to allow a higher percentage of the total flow to the FID system.

Keywords: Subcritical water chromatography; Detection, subcritical water chromatography; Flame ionization detection; Superheated water; Carbohydrates; Amino acids; Carboxylic acids

1. Introduction

While subcritical (high-temperature) water has been successfully used to quantitatively extract both polar and nonpolar organic species [1–10], liquid chromatographic (LC) separations with high-temperature water have also been reported [11–21]. Among the studies on subcritical water separation, UV detectors were used mostly. Since water does not respond to flame ionization detection (FID), FID can be used as a detection method in liquid chromatography with pure water as the mobile phase. The use of FID in subcritical water chromatography can overcome some drawbacks of UV detection methods previously used in subcritical water chromatography. First, FID can detect species with no chromophore. Second, the UV detector is located outside the oven in a high-temperature water separation system. The temperature of the water eluent drops when it exits the oven on the way to the UV detector. Thus, a temperature gradient is established in the outlet tubing between the column and the UV detector. This temperature drop may potentially cause solute deposition in the pathway to the UV detector and result in peak broadening. Since the connection tubing between the separation column and the FID

*Corresponding author. Tel.: +1-252-328-1647; fax: +1-252-328-6210.

E-mail address: yangy@mail.ecu.edu (Y. Yang).

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system is located inside the oven, no potential solute deposition can occur in the pathway to the FID system. Thus, no peak broadening can result from this point of view.

Some investigators studied the use of flame ionization or flame photometric detection in LC separations in which organic solvents were involved in the mobile phase [22–28]. Kientz et al. coupled a flame photometric detector to a narrow bore (0.32 mm I.D.) LC column. The eluent was directly introduced into the flame via a 100-μm I.D. fused-silica capillary [24]. Since organic solvents presented in the mobile phase, quenching caused by the eluent was observed. Malcomle-Lawes and Moss developed a novel transport detector that allows close control of solvent removal and permits the detector to be used for relatively volatile analytes [25]. The use of aerosol alkali FID for LC was evaluated by Conte and Barry [26]. They used a heated interface to vaporize the organic solvent prior to detection. Bernard et al. constructed a total-consumption flame photometric detector for sulfur- and phosphorus-containing compounds [27]. Moret et al. developed an automated on-line method that involved two LC systems and one gas chromatography (GC)–FID system [28]. After separation on a large column of the first LC system, a 6-ml fraction of the effluent was vaporized in an on-line evaporator. Then a second LC separation was performed using a different mobile phase and the components of interest were transferred to the GC–FID system through an in-line vaporizer/overflow interface [28].

Recently, several reports described the potential application of FID in LC separations using only water as the mobile phase. Miller and Hawthorne [11] reported the use of FID in subcritical water separation. Polar organic analytes were separated by using pure water at elevated temperatures and detected by FID. The FID sensitivity was optimized by varying the water flow-rate [11]. Ingelse et al. also investigated the feasibility of coupling subcritical water separation with flame ionization detection [12]. Hooijschuur et. al. coupled FID with microcolumn LC via an eluent–jet interface. Pure water was used as the eluent in their work [13]. Bruckner et al. used FID to detect volatile compounds that were separated on a column with low phase volume ratio (volume of stationary phase to mobile phase) using pure ambient water as the eluent [14]. A drop headspace cell was used as the interface between the liquid chromatograph and the FID system. Inside this drop headspace cell, a helium stream sampled the vapor of volatile components from individual drops of the eluent, and the vapor-enriched gas stream was sent to the FID system.

Since FID was not stable at flow-rates greater than 0.2 ml/min in the splitless mode [11], we constructed and tested a split subcritical water chromatography–FID system in this work. To the best of our knowledge, subcritical water separation of carbohydrates and carboxylic acids has not been reported yet. Therefore, separation of several carboxylic acids, carbohydrates, and amino acids was performed on two commercially available columns using the laboratory-made system. The performance of FID in this split mode was evaluated. The linear dynamic range and detection limit of the test analytes were also determined.

2. Experimental

2.1. Subcritical water chromatography–FID system and its performance

An LDC pump (Consta Metric 3200, Riviera Beach, FL, USA) was used to deliver the water mobile phase. A Valco six-port injector fitted with a 2-μl sample loop (Keystone Scientific, Bellefonte, PA, USA) was connected to the outlet of the pump. This injector was placed just outside the Perkin-Elmer GC oven (Auto System, Perkin-Elmer, Norwalk, CT, USA). A separation column was connected with the injector and placed inside the oven. A Tee union (1/16 to 1/16 in., Supelco, Bellefonte, PA, USA) was used to connect the separation column and the FID system (1 in.=2.54 cm). The inlet of the Tee was connected to the outlet of the separation column. While one of the outlets of the Tee was connected to the FID system using a piece of stainless steel capillary tubing (80 cm×40 μm I.D., ISCO, Lincoln, NE, USA), the other outlet of the Tee was connected with a fused-silica capillary (93 cm×103 μm I.D., Polymicro Technologies, Phoenix, AZ, USA) whose outlet was directed to a waste water bottle. The ratio of the water flow to the FID

system over the flow-rate to the waste bottle varied depending on the dimension of capillary tubings and the total water flow-rate used.

2.2. Reagents

All of the carboxylic acids, carbohydrates, and amino acids were purchased from Aldrich (Milwaukee, WI, USA). HPLC-grade water was obtained from Fisher Scientific (Fair Lawn, NJ, USA) and used as the chromatographic eluent in this work. The solutions of analytes were prepared in pure water.

2.3. Columns

A ''graphitized'' carbon column (Hypercarb) was purchased from Alltech (Deerfield, IL, USA). The column dimension is 10 cm×2.1 mm and the particle size is 5 μm. A poly(styrene–divinylbenzene) column (PRP-1, 25 cm×4.1 mm) was obtained from Hamilton (Reno, NV, USA). The size of the PRP-1 packing particles is also 5 μm.

3. Results and discussion

3.1. FID conditions

Since the liquid water eluent becomes vapor once it enters the FID system, even a very small amount of liquid water results in a large volume of steam in the FID zone. This volume expansion of the effluent dramatically influences the stability of FID. This is the main reason why the FID system is not stable in the splitless mode. To ensure the FID flame remained lit, the hydrogen flow-rate was increased to 135 ml/min in this study, although the manufacturer-recommended hydrogen flow is 45 ml/min for the normal GC mode. The air flow-rate was 360 ml/min. The FID temperature was set at 350°C. In order to evaluate the influence of restrictor position on FID performance, the restrictor was placed inside the FID zone at different positions (0.5 to 6 cm, the distance between the restrictor outlet and the tip of the FID jet). Methylene chloride was injected and its peak areas were used for this evaluation. The total flow-rate of water eluent was 0.65 ml/min but only ~0.04 ml/min passed through the FID system for this experiment. Our results demonstrated that the peak area of methylene chloride was not significantly affected by restrictor positions. The restrictor position of 5 cm was used for the remainder of the work. Note that no column was used in this experiment.

3.2. The influence of the total water flow-rate on FID stability

The effect of flow-rate on FID performance was evaluated using the same setup as used in the restrictor position studies. Again, no column was used in this experiment. As shown in Table 1, the total water flow-rate ranged from 0.13 to 1.24 ml/min, while the flow to the FID system only had a range of 0.013 to 0.06 ml/min. It should be pointed out that the fraction of flow to the FID system decreased with increasing total flow-rate. For example, 11.5% of the lowest total flow went to the FID system (0.015 ml/min out of 0.13 ml/min) while only 4.8% of the highest flow-rate passed through the FID system (0.06 ml/min out of 1.24 ml/min). Since only a small fraction of water flowed to the FID system, the flame ionization detector was stable at all flow-rates used including the highest flow-rate of 1.24 ml/min, while the FID system in the splitless mode was not stable at flow-rates higher than 0.2 ml/min. The peak areas of methylene chloride were similar at flow-rates ranging from 0.36 to 1.24 ml/min. But the peak area obtained at 0.13 ml/min was about 20% lower than the peak areas resulted by other higher flow-rates.

3.3. Separation of test analytes

The Hypercarb column was used to separate carbohydrates in subcritical water chromatography.

Table 1
Comparison of the total flow-rate and the fraction to the FID system

Total flow-rate (ml/min)	Flow to FID system (ml/min)	% Flow to FID system
0.13	0.015	11.5
0.36	0.037	10.3
0.65	0.04	6.2
0.85	0.05	5.9
1.24	0.06	4.8

The total flow-rate used was 0.2 ml/min. Fig. 1 shows chromatograms of glucose and fructose at two different temperatures. As we can see, the carbohydrates were detected by FID without any derivatization and sharp peaks were obtained in subcritical water chromatography.

Separation of three carboxylic acids including acetic, propionic, and butyric acids was performed using the PRP-1 column at three temperatures. The flow-rate was 0.85 ml/min. Fig. 2 shows chromatograms of these three carboxylic acids obtained by subcritical water chromatography. We can easily see that the retention of carboxylic acids significantly decreased with increasing water temperature. For

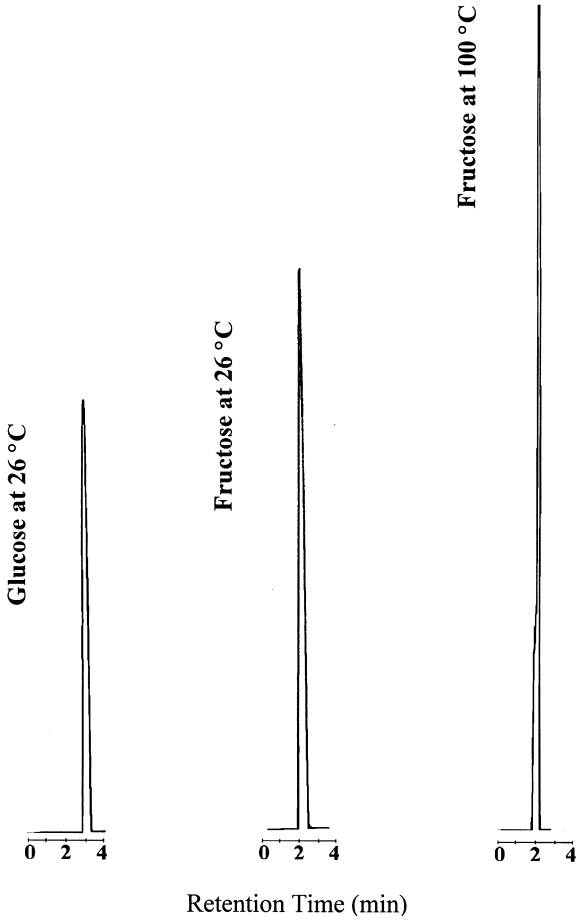

Fig. 1. Chromatograms of glucose and fructose (~3000 ng/μl each) obtained from the Hypercarb column. Total flow-rate was 0.2 ml/min.

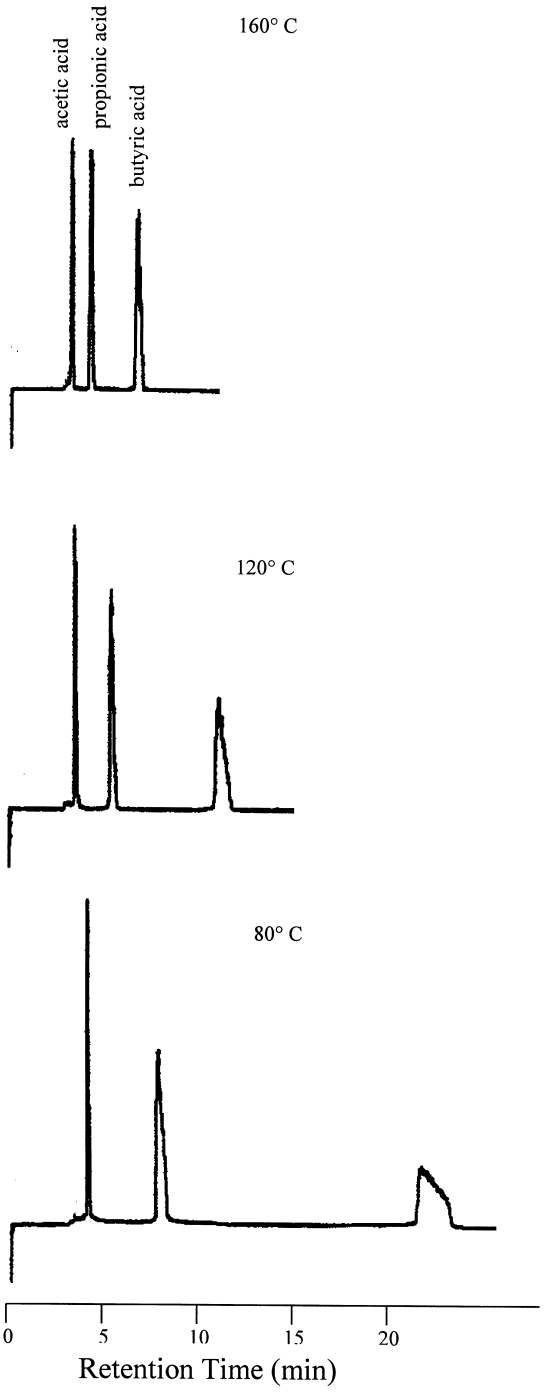

Fig. 2. Chromatograms of carboxylic acids (~2000 ng/μl each) obtained at 80°C (top), 120°C (middle), and 160°C (bottom) using the PRP-1 column. Total flow-rate was 0.85 ml/min.

example, butyric acid was not eluted until 23 min at 80°C. At this low temperature, the peak shape of butyric acid was very poor. However, when the temperature was raised to 160°C, the retention time of butyric acid was shortened to only 7 min. In addition, the peak shape of butyric acid was greatly improved at higher temperatures.

Separation of a mixture of five amino acids was also achieved using the PRP-1 column at 80°C. The total flow-rate was 0.85 ml/min. Fig. 3 shows the chromatogram of glycine, valine, leucine, phenylalanine, and tryptophan. These five free amino acids were well separated by pure water and detected by FID.

3.4. Linear dynamic range and limit of detection

3.4.1. Without a column

The linear dynamic ranges and the detection limits of fructose, leucine, and phenol were determined. The injector was directly connected to the Tee union without a separation column. Therefore, no column was used in this experiment. The oven temperature was 30°C. The total flow-rate used was 0.36 ml/min. The concentration of the injected solutions ranged from 100 to 10 000 ng/μl for fructose and leucine, while the concentration range for phenol was 100 to 100 000 ng/μl. Since only a small fraction of the water eluent went to the FID system, the actual mass of analytes that reached the FID system only ranged from 0.021 to 21 μg. Therefore, the disadvantage of this split mode is its worsened sensitivity. The logarithmic relationship between the peak area and the mass detected by FID is shown in Fig. 4 for fructose, leucine, and phenol. The correlation coefficients are 0.9909, 0.9953, and 0.9991 for leucine, fructose, and phenol, respectively. The peak areas at each concentration were measured in the least by triplicates. The relative standard deviation was typically less than 5% for higher concentrations and 15% for the concentrations near the detection limit. The limit of detection (S/N=3:1, the mass that reached the FID system) was 63 ng or lower for all three analytes. Please note that the actual mass injected was much higher than this (612 ng or 306 ng/μl).

3.4.2. With the PRP-1 column

In this experiment, the PRP-1 column was connected between the injector and the Tee union. The oven temperature was set at 80°C. Since this PRP-1 column has a greater I.D. (4.1 mm), a total flow-rate of 0.94 ml/min was used. The dynamic range and detection limits of mannose, leucine, and phenylalanine were determined. The concentration range was 156 to 5000 ng/μl for analytes used in this experiment. The mass of analyte that reached the FID system ranged from 19 to 600 ng. The logarithmic relationship between the mass detected and the peak areas is illustrated in Fig. 5. The correlation coefficients ranged from 0.9934 to 0.9938 for the three analytes. The limit of detections (S/N=3:1) were 45, 38, and 111 ng (375, 317, and 925 ng/μl injected)

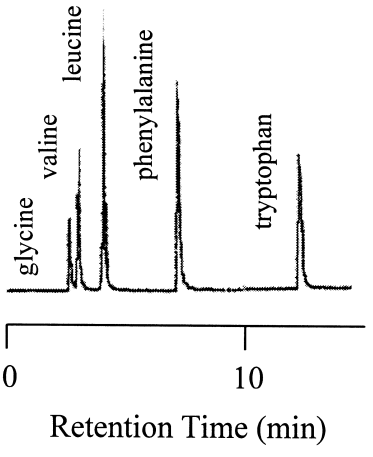

Fig. 3. Chromatograms of glycine, valine, leucine, phenylalanine, and tryptophan (~1500 ng/μl each) obtained at 80°C using the PRP-1 column. Total flow-rate was 0.85 ml/min.

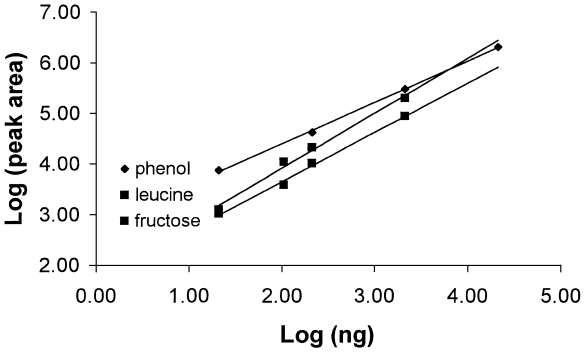

Fig. 4. The logarithmic relationship between the peak area and the mass detected by FID for fructose, leucine, and phenol.

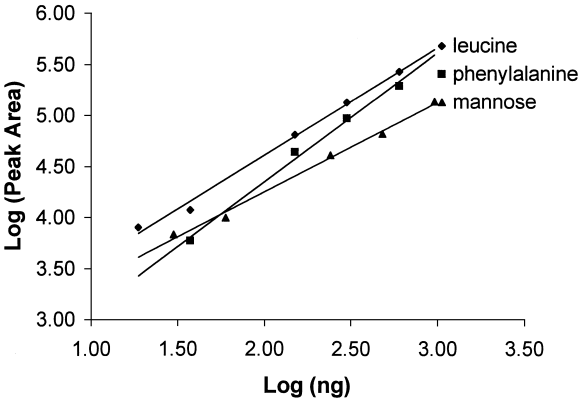

Fig. 5. The logarithmic relationship between the peak area and the mass detected by FID for mannose, leucine, and phenylalanine.

for mannose, leucine, and phenylalanine, respectively. The limit of detection was high because only a small fraction of the effluent went to the FID system.

4. Conclusions

A post-column split system for subcritical water chromatography–FID was constructed and tested in this study. The flame ionization detector was coupled to a subcritical water chromatograph via a Tee union and capillary restrictors. Since only 5–11% of the total flow went to the FID system in this split mode, the FID was very stable even at a total flow-rate of 1.24 ml/min. The fraction of the flow to the FID system can be adjusted by varying the dimension of the capillary restrictors to the FID system and to the waste bottle. Several carbohydrates, amino acids, and carboxylic acids were separated at different temperatures. The linear dynamic range was found to be up to three orders of magnitude and the limits of detection ranged from 38 to 111 ng (306–925 ng/μl injected) with split ratios of 1:10 to 1:17 (FID/waste bottle) for several analytes studied. Please note that the LOD can be greatly lowered by adjusting the dimension of restrictors to allow a higher percentage of the total flow to the FID system.

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