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Determination of ethanol in alcoholic beverages by high-performance liquid chromatography–flame ionization detection using pure water as mobile phase

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Abstract

A method for the determination of ethanol in alcoholic beverages by high-performance liquid chromatography–flame ionization detection (HPLC–FID) was developed. An FID system could be directly connected to an HPLC system using pure water as a mobile phase. In a durability test using triacontylsilyl (C_{30})-silica gel stationary phase for 96 h, no significant change in the retention time of four alcohol compounds was observed. So the HPLC separation of alcoholic beverages was carried out on the C_{30}-silica gel stationary phase. On application to the analysis of six kinds of alcoholic beverages, ethanol could be determined accurately by the proposed method.

Keywords: Wine; Food analysis; Detection, LC; Mobile phase composition; Flame ionization detection; Instrumentation; Ethanol

1. Introduction

The accurate determination of ethanol in alcoholic beverages is indispensable for economic reasons, particularly in relation to the taxes imposed on alcohol in different countries. The official methods for the determination of ethanol in alcoholic beverages are based on the measurement of specific gravity, carried out after a previous distillation of the samples [1]. However, some components, such as aromatic compounds, are retained in the distillates. Chromatographic techniques are expected to provide accurate methods for determining ethanol in alcoholic beverages.

Reversed-phase high-performance liquid chromatography (RP-HPLC) can produce higher resolution in the separation of organic compounds, and so is employed as an analytical technique in a wide variety of chemical laboratories. In the determination of ethanol in alcoholic beverages, the detection method of ethanol is a technical problem. A differential refractive index detector can be used for detecting ethanol [2], whereas its stability for detecting analytes is sensitive to the atmospheric temperature. The conductometric detection of ethanol in alcoholic beverages has also been developed, where an electrolyte solution was used as a mobile phase [3].

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Flame ionization detection (FID), one of the common detection methods in gas chromatography (GC), is a universal detection method for organic compounds. Earlier attempts to adapt FID for use in HPLC have been made using complicated interfaces. In the interface, for example, the mobile phase of HPLC was evaporated and the analytes were transported into the detector by using a moving wire [4]. However, these systems are hardly used nowadays.

In this study, a directly coupled HPLC–FID system was developed using pure water as a mobile phase. The stability of the retention of alcohols in the system during a long period was evaluated. The determination of ethanol in alcoholic beverages by the system was also carried out and was compared with that obtained by using GC–FID.

2. Experimental

2.1. Reagents

Water used in this study was prepared in our laboratory using a Millipore (San Jose, CA, USA) Milli-Q Gradient system at an output of 18.2 MΩ. Four alcohols (methanol, ethanol, 1-propanol and 1-butanol) were purchased from Wako Pure Chemical (Osaka, Japan). For chromatographic analysis, these alcohols, as well as commercially available alcoholic beverages, were dissolved in water so as the concentration of alcohols might be 1–2% (v/v).

2.2. HPLC–FID

A schematic diagram of the HPLC–FID system employed in this study is presented in Fig. 1. The HPLC–FID system consisted of a Shodex (Tokyo, Japan) Degas KT25 degasser, a Shimadzu (Kyoto, Japan) LC-6A pump, a Rheodyne (Cotati, CA, USA) Model 7725i sample injector with a 5-μl sample loop, a Nomura Chemical (Seto, Aichi, Japan) Develosil C30-UG-5 separation column (150 mm× 4.6 mm I.D., particle size: 5 μm), a Shimadzu CTO-6A column oven, an FID system from a Hitachi (Tokyo, Japan) G-3000 capillary GC system, and a restrictor (stainless steel, 50 mm×0.1 mm I.D.).

The coupling of the FID system to the HPLC system was carried out as follows. A portion of the effluent from the separation column in the HPLC system was split using a T-piece. Then the split effluent was introduced into the FID system via a capillary tube (fused-silica, 270 mm×40 μm I.D.), as shown in Fig. 2. The capillary tube was heated at 150 °C so as to maintain a stable vaporization of water in the FID system.

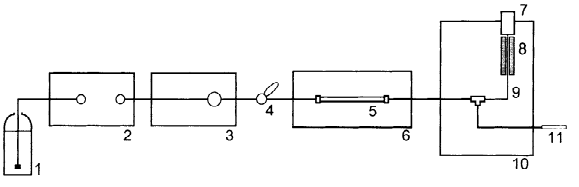

Fig. 1. Schematic diagram of the HPLC–FID system. 1, Water; 2, degasser; 3, pump; 4, injector; 5, separation column; 6, column oven; 7, FID; 8, heater; 9, fused-silica capillary tube; 10, GC oven; 11, restrictor.

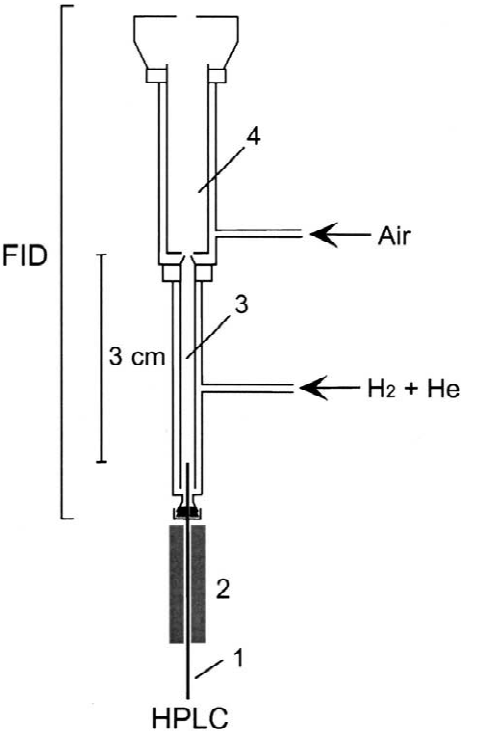

Fig. 2. Coupling of the FID system with the HPLC system. 1, Fused-silica capillary tube; 2, heater; 3, FID jet; 4, ion collector.

The chromatographic conditions were as follows: mobile phase, pure water; flow-rate, 1.0 ml min^{-1}; column temperature, 35 °C; FID temperature, 350 °C.

2.3. GC–FID

Ethanol in the alcoholic beverages was also determined by GC–FID using a Shimadzu GC-2010 system. The chromatographic conditions were as follows: column, Spelco (Bellefonte, PA, USA) Spelcowax 10 (15 m ×0.53 mm I.D., film thickness, 0.5 μm); injection mode, split (1:25); injection temperature, 250 °C; column temperature, 35 °C for 2 min, 1 °C min^{-1} to 70 °C, 10 °C min^{-1} to 240 °C; flow-rate, 30 cm s^{-1}, FID temperature, 350 °C.

3. Results and discussion

3.1. Coupling of FID and HPLC

Since liquid water changes into the vapor phase once it is introduced into an FID system, the flow-rate of the HPLC eluent introduced into the FID system was limited. Actually, the FID system could not be ignited when about 10 μl min^{-1} of water was introduced into it. Therefore, the capillary tube of 270 mm×40 μm I.D. in size was used to control the flow-rate of the split HPLC eluent. Moreover, the restrictor of 50 mm×0.1 mm I.D. in size had to be connected to the end of the HPLC system as a back-pressure regulator to maintain a stable flow of the split HPLC eluent introduced into the FID system, as shown in Fig. 1. Under these conditions, the back-pressure in the HPLC was 0.5 MPa and the flow-rate of the eluent introduced into the FID system was almost 7 μl min^{-1}.

In addition, the position of the capillary tube, the end of which was placed inside the FID jet, influenced the detection of alcohols. The influence was evaluated by placing the end of the capillary tube at different positions (0.5–3.5 cm, the distance between the end of the capillary tube and the tip of the FID jet). When the end of the capillary tube was placed closed to the tip of the FID jet (as well as the case of capillary GC), the sensitivity of FID system was poor. The best conditions for sensitive and stable detection were obtained when the end of the capillary tube was placed at 3 cm from the tip of the FID jet, as represented in Fig. 2. Fig. 3 shows a chromatogram of a standard alcohol solution obtained under these conditions. RSD values concerning peak area were almost 1% (n=8). The repeatability was fairly good. The position of the capillary tube was set in these conditions for the remainder of the work.

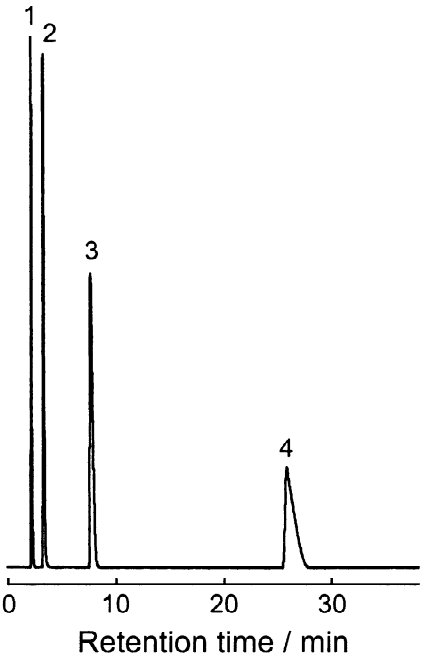

Fig. 3. HPLC–FID chromatogram of standard alcohol solution. Peaks: 1=methanol; 2=ethanol; 3=1-propanol; 4=1-butanol; conditions as described in text.

3.2. Stability of retention of alcohols

It is known that the retention of solute compounds on reversed-phase stationary phases decreases when pure water is used as a mobile phase. Nagae and Enami [5,6] investigated the influence of the length of the alkyl group of the silica gel packing materials on the degree of the decrease in the retention time of thymine and sodium nitrite. They found that triacontylsilyl (C_{30})-silica gels showed less decrease of the retention time in comparison with octadecylsilyl-silica gels and octylsilyl-silica gels. Therefore, we selected the Develosil C30-UG-5 as a separation column.

The stability of the retention time of alcohols on C_{30}-silica gels was investigated by means of a following duration test: the separation column was preliminarily conditioned with water–methanol (50:50, v/v) for 1 h, and then pure water was passed though for 96 h. After the supply of pure water had begun, the retention time of methanol, ethanol, 1-propanol and 1-butanol was regularly measured, as shown in Fig. 4. Because no significant decrease in the retention time of alcohols was observed, the use of C_{30}-silica gel stationary phase could be considered to produce a stable separation of the alcohols. It seemed that the continuous determination of ethanol in alcoholic beverages could be performed under these conditions.

3.3. Determination of ethanol in alcoholic beverages

The proposed method was applied to determine ethanol in alcoholic beverages. Six kinds of diluted alcoholic beverages were injected into the chromatograph without sample treatment. As an example, an HPLC–FID chromatogram of white wine sample is represented in Fig. 5. Ethanol in alcoholic beverages could be separated from other components. The same sample solutions were analyzed by GC–FID in order to compare with that by HPLC–FID. The analytical results obtained by both methods are summarized in Table 1, where the values are expressed as the mean±expanded uncertainty. The mean values by HPLC–FID were comparable with those by GC–FID. The difference between the labeled value and the mean value by HPLC–FID was within 1% for each sample: it seemed that the labeled values of the sample tested were reliable.

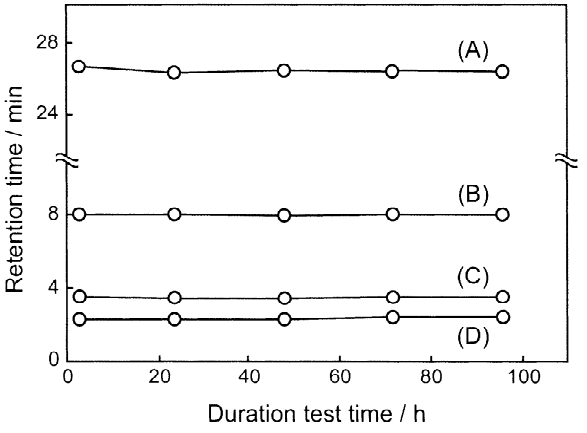

Fig. 4. Change of retention time of 1-butanol (A), 1-propanol (B), ethanol (C) and methanol (D) on C_{30}-silica gel stationary phase. Mobile phase: pure water, column temperature: 35 °C.

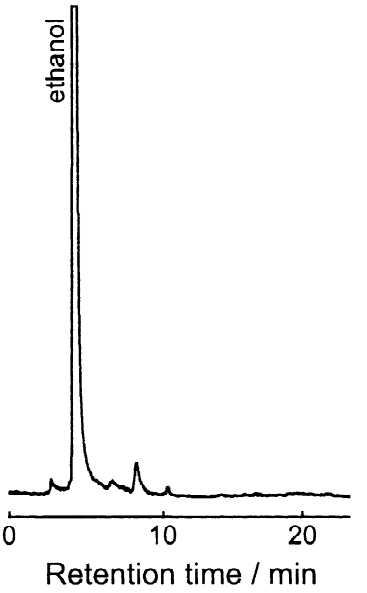

Fig. 5. HPLC–FID chromatogram of a white wine sample; conditions as described in text.

The uncertainty concerning the determination of ethanol in alcoholic beverages by both HPLC–FID and GC–FID was evaluated according to a literature [7] using uncertainties of the following sources: chromatographic determination of ethanol in the

Table 1
Comparison of ethanol concentrations[a] in alcoholic beverages determined by HPLC–FID with those determined by GC–FID and labeled values

Alcoholic beverage	Measurement value[b]		Labeled value
	HPLC–FID	GC–FID	
White wine	10.7±0.2	10.7±0.2	<11
Red wine	11.7±0.2	11.6±0.2	<12
Sake	13.8±0.2	13.8±0.3	13–14
Shochu	25.4±0.6	25.3±0.3	25
Brandy	37.6±0.8	37.8±0.5	37
Whisky	40.2±0.7	40.2±0.6	40

[a] Presented as % (v/v) at 15 °C.
[b] Mean±expanded uncertainty (coverage factor, $k=2$).

sample solution, chromatographic determination of ethanol in the calibration solution, preparation of the sample solution by diluting the alcoholic beverage in water, preparation of the calibration solution, and purity of ethanol used for preparing the calibration solution. Because the same sample solutions were chromatographed on HPLC–FID and GC–FID, the differences in the combined uncertainty were due to the differences of uncertainty concerning the chromatographic determination of ethanol in both the calibration solution and the sample solutions. Although the HPLC–FID system used was a laboratory-made system, the repeatability of the determination of ethanol by HPLC–FID was almost same as that by GC–FID.

4. Conclusion

In this study, we developed an HPLC–FID system using pure water as a mobile phase; its application to the determination of ethanol in alcoholic beverages was also investigated. The results obtained indicate that the proposed system produces an accurate determination of ethanol in alcoholic beverages.

In HPLC analysis, the detection of some organic compounds other than ethanol also present technical problems. The HPLC–FID system developed in this study is expected to be useful also for these analyses.

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