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DETERMINATION OF ALCOHOLS IN ALCOHOLIC BEVERAGES BY MICRO HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH INDIRECT PHOTOMETRIC DETECTION

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SUMMARY

Aliphatic alcohols in alcoholic beverages were determined by micro high-performance liquid chromatography with indirect photometric detection. Theophylline was added to the mobile phase as the visualization agent. Ethanol, propanols and butanols could be determined by this method.

INTRODUCTION

Indirect photometric detection allows the analysis of non-UV-absorbing species in high-performance liquid chromatography (HPLC) using an ultraviolet (UV) detector. This technique is capable of detecting both ionic and non-ionic species by incorporating a UV-absorbing species in the mobile phase. The detection principle of the former species has been elucidated, whereas that of the latter species is complex. Most types of indirect photometric detection of non-electrolytes involve disturbance of the partitioning process of the visualization agent due to the analyte, which leads to the generation of the induced peaks.

Schill and Crommen[1] derived an equation for calculating the change in the concentration of a mobile phase component k relative to an injected solute j (both uncharged) as follows:

$$\Delta C_k = \theta\alpha_s/(1 - \alpha_s) \tag{1}$$

where θ is the fractional coverage of the solid phase by k and $\alpha_s = k'_j/k'_k$, k' being the capacity factor.

We have proposed a detection principle for non-electrolytes in indirect photometry[2] in which the peak area of the induced peak is correlated with the capacity factors of the analyte and the visualization agent, as given by the following equation:

$$S = FC_{a0}W_{b0}|c| \cdot \frac{\varphi}{\varphi + 1} \cdot \frac{k'_a(k'_b + 1)}{|k'_a - k'_b|} \tag{2}$$

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where S is the peak area of the induced peak, F is the response factor of the detector, C_{a0} is the concentration of the mobile phase component in the eluent, W_{b0} is the amount of the analyte injected, c is a coefficient accounting for the degree of variation of the capacity factor of the visualization agent (mobile phase component) due to the analyte, φ is the phase ratio and k'_a and k'_b are the capacity factors of the visualization agent and the analyte, respectively.

Eqn. 1 can be rewritten by using the same parameters as used in eqn. 2:

$$\Delta C_a = \theta k'_b/(k'_a - k'_b) \tag{3}$$

ΔC_a in Eqn. 3 corresponds to the peak height of the induced peak.

In our simulation[2], dividing eqn. 2 by $(1 + k'_b)$ gives an equation representing the peak height, h, of the induced peak:

$$h = Ack'_a/(k'_b - k'_a) \tag{4}$$

where A is a constant and the direction of the induced peak is considered.

The right-hand side of eqn. 4 is not equivalent to that of eqn. 3, which was derived from eqn. 1. Eqn. 3 indicates that when the capacity factor of the analyte, k'_b, goes to infinity, ΔC_a is identical with $-\theta$. On the other hand, the peak height goes to zero when k'_b goes to infinity in eqn. 4. The latter phenomenon is usually observed in the indirect photometric detection of non-electrolytes. In addition, if α_s is defined as k'_k/k'_j, *i.e.*, k'_a/k'_b, the right-hand side of eqn. 1 becomes $\theta k'_a/(k'_b - k'_a)$, which seems to be equivalent to eqn. 4 derived from our simulation. Further discussion will be required in order to evaluate these equations.

It is clear from these equations that the signal intensity of the analyte of interest can be enhanced by careful selection of the visualization agent and the mobile phase composition.

A few applications of the indirect photometric detection of alcohols have been reported[3–7], including the determination of ethanol in alcoholic beverages[3]. This paper describes determination of lower alcohols in alcoholic beverages by micro HPLC with indirect photometric detection.

EXPERIMENTAL

The liquid chromatograph employed in previous work[6] was used, consisting of a Microfeeder pump (Azumadenki Kogyo, Tokyo, Japan) equipped with a gas-tight syringe, an ML-422 micro valve injector (0.02 μl; Japan Spectroscopic, Tokyo, Japan), a laboratory-made water-bath, a fused-silica micropacked separation column, a Uvidec-100V UV spectrophotometer (Japan Spectroscopic) and a recorder. Develosil ODS-3K chemically bonded silica packing (3 μm; Nomura Chemical, Seto, Japan) was packed in the laboratory by a slurry packing method. The separation column was immersed in the water-bath to avoid the effects of variations in the room temperature.

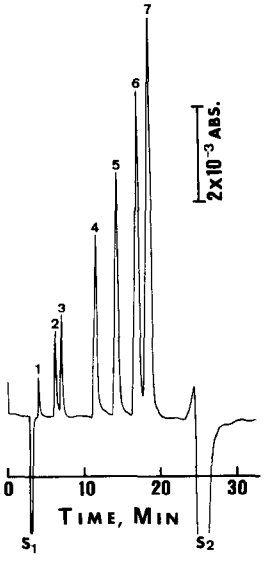
All the reagents were of analytical-reagent grade, except for HPLC-grade distilled water, and were supplied by Wako (Osaka, Japan).

RESULTS AND DISCUSSION

Most alcoholic beverages contain trace amounts of C_1–C_5 aliphatic alcohols in addition to ethanol. The determination of these alcohols can be achieved by various methods, including headspace gas chromatography. Aliphatic alcohols can be separated by reversed-phase HPLC and can be detected by the indirect method[3–7]. Betz and Nikelly[3] reported the determination of ethanol in alcoholic beverages using conventional HPLC with indirect photometric detection. The sensitivity achieved was 0.1%, which was low enough to determine ethanol.

We have previously examined the indirect photometric detection of aliphatic alcohols in micro HPLC[6], and found that the analyte eluting closer to the system peak gave the larger signal intensity and that the retention time of the system peak coincided with that of the visualization agent. This means that the sensitivity of the analyte of interest can be improved by careful selection of the visualization agent.

Fig. 1 demonstrates the indirect photometric detection of an artificial mixture of ethanol, propanols and butanols using theopylline as the visualization agent. All the propanol and butanol isomers were well separated in the reversed-phase mode. The peaks denoted by S_1 and S_2 are the system peaks. The former system peak is caused by a difference in composition between the sample solution and the mobile phase, and the retention time of the latter system peak coincides with that of the visualization agent (theophylline). A concentration of 0.5% (v/v) of each analyte was



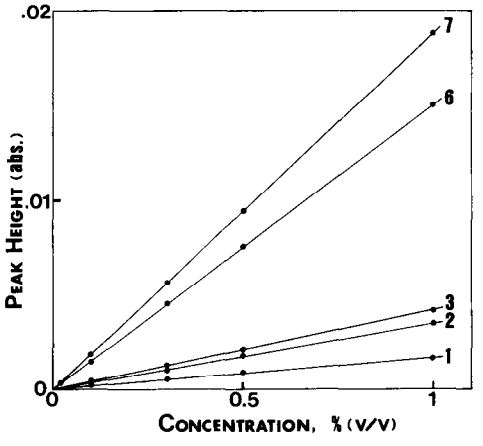


Fig. 1. Indirect photometric detection of the components of an artificial mixture of alcohols using theopylline as the visualization agent. Column, Develosil ODS-3K (100 × 0.35 mm I.D.); mobile phase, methanol–water (12:88) containing 0.4 m*M* theophylline; flow-rate, 2.8 μl/min; wavelength of UV detection, 210 nm. Samples: 1 = ethanol; 2 = 2-propanol; 3 = 1-propanol; 4 = 2-methyl-2-propanol; 5 = 2-butanol; 6 = 2-methyl-1-propanol; 7 = 1-butanol; 0.5% (v/v) of each was injected.

Fig. 2. Calibration graphs for alcohols. Operating conditions as in Fig. 1.

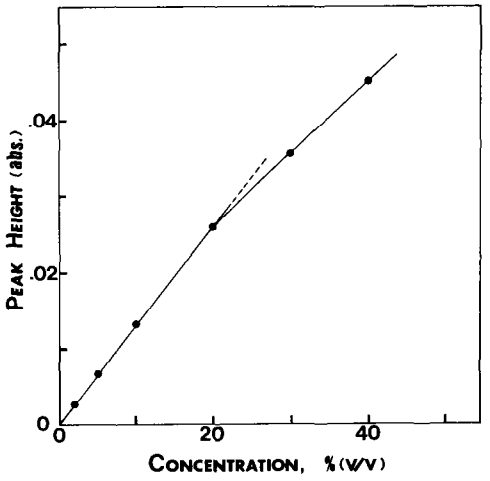


Fig. 3. Calibration graph for ethanol at higher concentrations. Operating conditions as in Fig. 1.

injected, indicating that the longer the retention time of the analyte (or the closer to the system peak), the higher peak was observed. The separation was carried out at room temperature (*ca.* 20°C), but the temperature of the water-bath should be controlled if the room temperature changes significantly, *e.g.*, a difference of 5°C requires a different calibration graph for the determination. Variation of the peak height due to variation of the column temperature is more significant for the analytes eluting close to the system peak.

Fig. 2 illustrates the calibration graphs for ethanol, propanols and butanols, all of which are linear up to 1% (v/v). The signal intensity (peak height) of ethanol

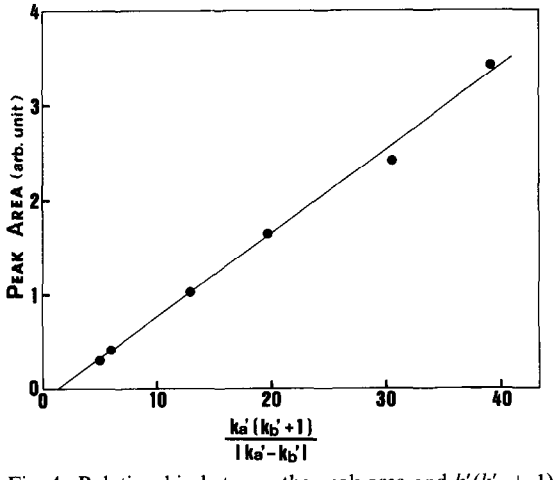


Fig. 4. Relationship between the peak area and $k'_a(k'_b + 1)/|k'_a - k'_b|$. Operating conditions as in Fig. 1.

was *ca.* one tenth of that of the butanols, which was advantageous for the simultaneous determination of the major component (ethanol) and trace amounts of the butanols. The concentration detection limits depended on the analyte, *e.g.*, several tens of 1 ppm for butanols and *ca.* 0.01% for propanols. The mass detection limits correspond to nanogram to subnanogram levels. The concentration detection limits of these alcohols will be improved by using a conventional HPLC system if the dynamic reserve (defined as the ratio of the background to its noise level) is maintained because the injection volume can be increased compared with the micro HPLC system, whereas the mass detection limits will not be improved by using the conventional HPLC system.

Fig. 3 illustrates the calibration graph for ethanol at concentrations up to 40% (v/v). The graph deviated from linearity at concentrations higher than 20%, which means that the determination of ethanol above 20% must be performed graphically or the sample must be diluted. This deviation from the linearity may arise because the amounts of ethanol injected were too large for the microcolumn, *e.g.*, *ca.* 6.4 μg in the injection of 40% ethanol. However, the peak shape was still good even when a sample containing 40% ethanol was injected.

The concentration of theophylline in the mobile phase was 0.4 m*M*. This concentration was established by considering the linearity of the detector and the sensitivity of the analyte as discussed below. The background of the mobile phase containing 0.4 m*M* theophylline was 0.17 absorbance unit at 210 nm. The detector employed in this work was linear up to 0.25 absorbance unit at this wavelength, which indicates that the deviation from linearity of the calibration graph is not due to the non-linearity of the detector at higher absorbance[6]. The background level of the mobile phase should be increased as much as possible until the dynamic reserve reaches the maximuim, because the peak height in indirect photometric detection increases with increasing background level[6].

Eqn. 2 indicates that the peak area increases with increasing capacity factor of the analyte and the visualization agent and/or with decreasing difference between the two capacity factors. Fig. 4 illustrates the correlation between the peak area and

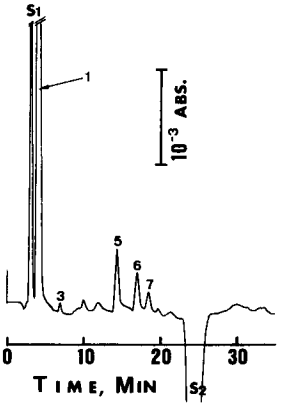


Fig. 5. Indirect photometric detection of the components of gin. Operating conditions as in Fig. 1 except for the sample (0.02 μl of gin).

TABLE I

DETERMINATION OF ALCOHOLS IN ALCOHOLIC BEVERAGES

Sample	*Concentration (%, v/v)*				
	Ethanol	*1-Propanol*	*2-Butanol*	*2-Methyl-1-propanol*	*1-Butanol*
Gin	36.5	0.027	0.064	0.031	0.014
Whisky	42.0			0.025	
Saké	16.3	0.29			
Beer	5.6				

$k'_a(k'_b + 1)/|k'_a - k'_b|$, in which c is not considered. Although each analyte is likely to possess its own c value, a good correlation is observed for these alcohols, *i.e.*, these alcohols have nearly the same c value. Eqn. 2 is very convenient for selecting the chromatographic conditions for the indirect detection of non-electrolytes.

Fig. 5 demonstrates the indirect photometric detection of the components of gin. Ethanol, 1-propanol, 2-butanol, 2-methyl-1-propanol and 1-butanol were detected and their concentrations could be determined.

The results of the determination of alcohols in various alcoholic beverages are shown in Table I. The determination of ethanol in gin and whisky was performed after diluting the sample four-fold with distilled water. The other samples were injected directly without any treatment.

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