Determination of Ethanol in Malt Beverages Using High-Performance Liquid Chromatography[1]

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ABSTRACT

The high-performance liquid chromatographic (HPLC) method using a hydrogen form cation exchange column to quantitate ethanol in malt beverages was evaluated. The method was fast and precise. Correlation with the reference distillation method was very good for ethanol levels in samples from low-calorie to imported beers. The HPLC method was more versatile than refractometry for the analysis of several samples.

Key words: *Analysis, Distillation, Ethanol, High-performance liquid chromatography, Refractometry.*

The brewing industry has three accepted methods for the quantitative determination of ethanol in malt beverages. These are refractometry (1), distillation (1), and gas chromatography (2,5).

The refractometry procedure is fast and precise for the routine analysis of products with known or similar composition. Numerous calibration curves may be required to account for changes in the angle of refraction due to the composition, not just the amount, of the extract (4).

The reference method for the quantitation of ethanol is distillation. This procedure is time-consuming and requires a relatively large volume of sample. When time and sample volumes are limited, such as in laboratory scale fermentations, this method is not applicable.

The gas chromatographic method requires a Chromosorb 103 column and a flame ionization detector. The method is applicable to a wide range of products. The results, which are obtained rapidly, are comparable to those of the distillation method (5); however, two potential drawbacks exist. First, the beer must be diluted one-to-one with an internal standard before injection. Second, direct injection of the sample, with its nonvolatile components, necessitates the use of a replaceable glass insert for the injection port. These inserts are not readily available for all gas chromatographs.

To date, few applications of HPLC to the quantitation of ethanol in beverages have been reported. Shimazu et al (6) used a calcium form cation exchange column with a water solvent and refractive index detector to quantitate ethanol in wine. The analysis, which required 25 min, did not achieve baseline separation of ethanol and glycerol.

We compared beer profiles from three different calcium form columns routinely used for carbohydrate analyses. The partial resolution of glycerol and ethanol, as seen in Fig. 1, made the accurate quantitation of ethanol difficult. These profiles were obtained from relatively new columns. With used columns, the two peaks merged completely, and quantitation was impossible.

The addition of an organic modifier, such as acetonitrile, to the water solvent gave baseline separation of components such as ethanol, propylene glycol, and glycerol (Fig. 2). Separation on a calcium form column requires about 60 min. The method lacks reproducibility because of excessive baseline noise.

An ion-exchange column for alcohol analysis was recently introduced by Bio-Rad Laboratories (3). The column is packed with a sulfonated polystyrene divinylbenzene resin in the hydrogen form. The separation (Fig. 3) is accomplished with a slightly acidic water solvent at an elevated temperature. In less than 10 min, ethanol is resolved at the baseline from all other eluting components. Wood et al (7) categorized the mechanism for the separation of aliphatic alcohols on this column as reverse-phase

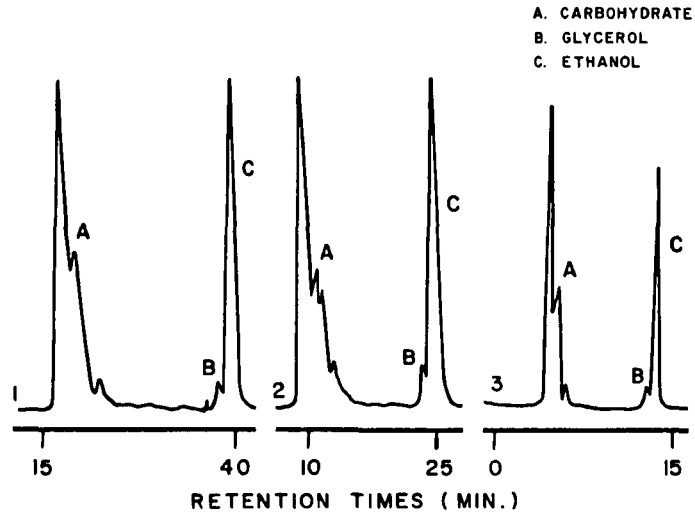

Fig. 1. Chromatographs demonstrating the separation of ethanol obtained with various carbohydrate columns: **1,** Aminex Q-15S (600 × 7.8 mm); **2,** HC-75 (300 × 7.8 mm); and **3,** HPX-87C (300 × 7.8 mm).

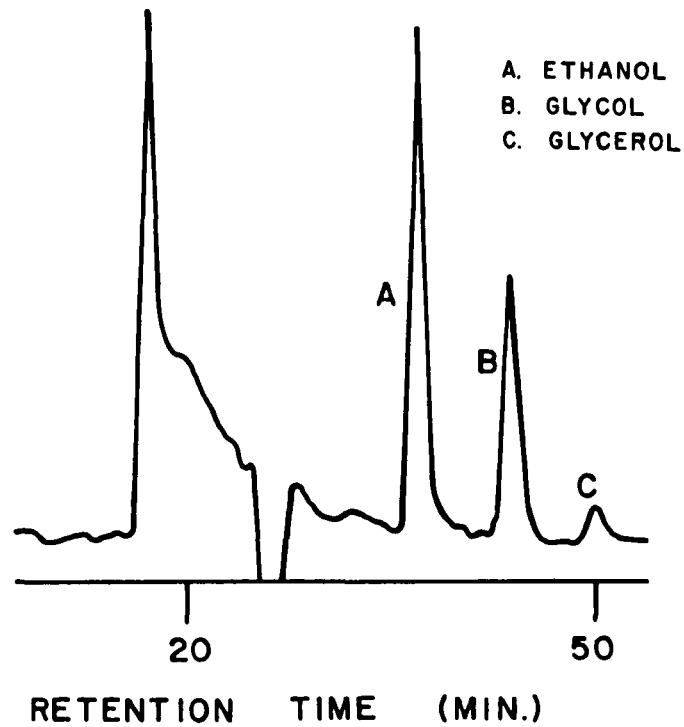

Fig. 2. Chromatogram from Aminex Q-15S column with 30% acetonitrile added to the water solvent.

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partitioning. They found that the elution order was an increasing carbon number with a log-normal relationship to capacity factor (k').

The ethanol data and the statistical analyses presented in this article demonstrate that HPLC with an HPX-85 alcohol column is a time-saving alternative to distillation and a versatile alternative to refractometry for the accurate quantitation of ethanol in various malt beverages.

EXPERIMENTAL

Apparatus

A Water's model ALC/GPC 244 liquid chromatograph equipped with an R401 refractive index detector and an M6000A pump was used, incorporating a Water's Intelligent Sample Processor for automatic sampling. Integration was by means of a Water's model 730 integrator. The heating block (Scientific Systems, Inc., model CH-20) was capable of handling a 250 × 6-mm column. Specific gravity values were obtained by using a Mettler/Paar model 55 densitometer.

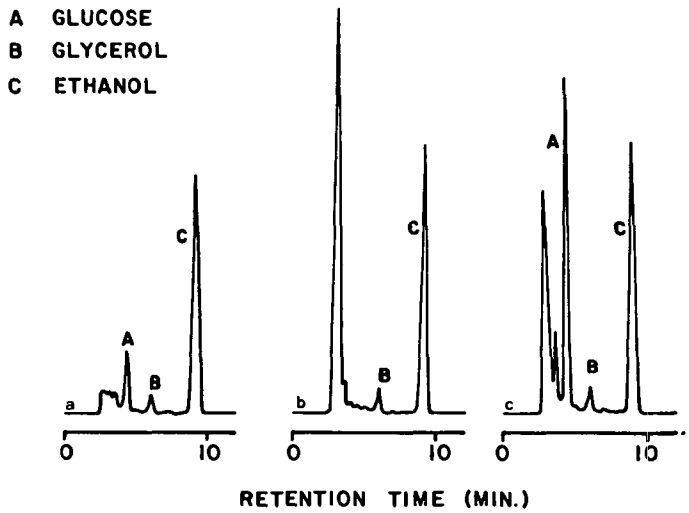

Fig. 3. Typical chromatograms of a variety of malt beverages obtained with the HPX-85 alcohol analysis column. Beverages are **(a)** low calorie, **(b)** premium, and **(c)** ale.

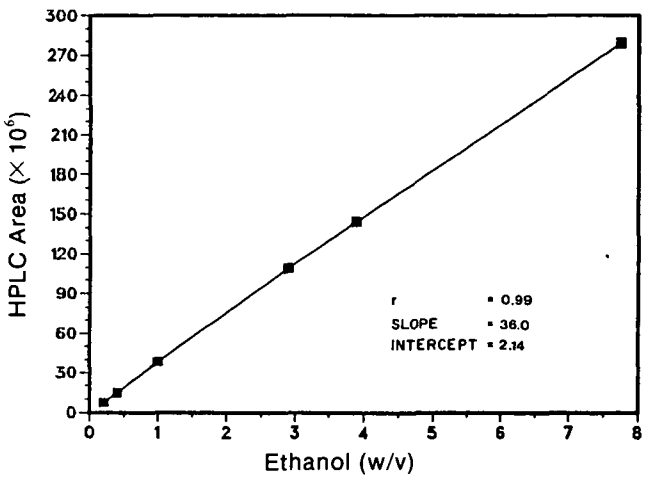

Fig. 4. Ethanol calibration curve: high-performance liquid chromatography vs ethanol (w/v).

HPLC Conditions

The column used was the HPX-85 (250 × 4 mm) introduced by Bio-Rad Laboratories for alcohol analysis. Operative temperature was 85° C, using 0.01*N* H_2SO_4 in Milli-Q water as a solvent. Flow rate was 0.4 ml/min under a pressure of 400 psi. Detector was ΔRI and attenuation ×32. Sample volume was 10 μl, and the guard column was Aminex A-15S (H^+ form).

Methodology

Beer samples were prepared for analysis by ultrasonic degassing in a covered flask. Quantitation was accomplished by means of a single external standard of 3.00% (w/v) absolute ethanol in Milli-Q™ water. The resulting ethanol values for beer were in terms of w/v. These were converted to w/w by using the specific gravity of the beer, previously determined by a Mettler/Paar densitometer.

Comparative Methods

Ethanol values were also determined by ASBC procedures for distillation and refractometry (1). For both methods, the specific gravities were again determined using a Mettler/Paar densitometer. The calibration equations used in the refractometry method were calculated for a limited sampling of beers with similar composition but were applied to all samples.

RESULTS AND DISCUSSION

Calibration Curve

Ethanol standards, the concentrations of which were verified by their specific gravities, were analyzed by the HPLC method. Figure 4 shows the regression line for the ethanol areas obtained. The correlation coefficient was almost one. The intercept was equivalent to an ethanol concentration of 0.5% (w/v). The detector response was linear over the range of ethanol levels analyzed, 0.20–8.00% (w/v). With the linear response, a single concentration near the midpoint of the expected ethanol values in beer, 3.00% (w/v), was chosen for the external standard. This standard was used for all of the HPLC analyses.

Determination of Precision

A series of 16 beers was analyzed in duplicate to estimate the precision of the HPLC method. The 95% confidence interval for the mean of duplicates was 0.04% (w/w). The standard deviation was 0.024% (w/w), giving a relative standard deviation of less than 1%.

The precision was also checked over time. Table I presents the ethanol data from a series of four beers analyzed during a three-month period. The mean, standard deviation, and coefficient of variation were determined for each sample. The statistical analysis showed that, for all samples, the coefficient of variation did not exceed 1.5, which was equivalent to that found for the same-day duplicates.

TABLE I
Precision of the High-Pressure Liquid Chromatography Method for Ethanol (w/w) over Time by Analysis of Variance

	Sample Beers			
	1	2	3	4
Month				
1	2.16	2.67	3.28	4.85
2	2.19	2.75	3.27	4.86
3	2.18	2.70	3.22	4.95
Mean	2.18	2.71	3.26	4.89
Standard deviation	0.02	0.04	0.03	0.06
Variation	0.0002	0.001	0.0006	0.002
Coefficient of variation	0.9	1.5	0.9	1.2

Beer Analyses

Thirty malt beverage samples, 10 each of low-calorie, premium, and super-premium or imported beers, were analyzed by distillation, refractometry, and HPLC. These products represent ethanol concentrations from 2.0 to 4.5% (w/w).

The first comparison was made between the HPLC method and the reference distillation method. The correlation between the two methods is illustrated in Fig. 5. The data was analyzed by a paired *t*-test. The two methods were found to be significantly different, even though all of the data points fell on or near the regression line. The HPLC method tended to give slightly higher ethanol levels than did distillation. This may have been caused by losses of ethanol during distillation or by the effect of the other volatiles on the specific gravity of the distillate. The fact that the methods were significantly different does not imply poor agreement between the methods. The regression analysis showed almost ideal agreement between the methods. The correlation coefficient and slope were 0.9956 and 1.01%, respectively. The intercept was 0.004% (w/w). The standard error of the regression line, which is analogous to a standard deviation, illustrates that the HPLC values deviated from the regression line by only 0.04% (w/w). The actual differences between the HPLC values and the distillation values ranged from −0.09 to 0.08% (w/w), the mean difference being −0.023 ± 0.008% (w/w). These results demonstrate that although a significant difference occurs between the methods, the HPLC method can closely predict the ethanol values obtained by distillation. The difference between methods appears to be caused by the narrow range of mean differences and the greater precision of the HPLC method compared to the distillation method. The actual differences are of little practical significance in the implementation of the HPLC method for routine analyses.

The second comparison was made between the HPLC method and refractometry. The correlation between the two methods is shown in Fig. 6. The data was again analyzed by a paired *t*-test. No significant difference was found between the two methods. The regression analysis indicates greater deviation between the actual ethanol values obtained by both methods. The correlation coefficient and slope were 0.9740 and 1.01%, respectively. The intercept was −0.02%. The standard error of the regression line was 0.11% (w/w). The difference between the actual ethanol values ranged from −0.20 to 0.29% (w/w). The mean difference was −0.01 ± 0.02% (w/w). These larger deviations showed that the samples were too varied in composition to be analyzed with the limited number of refractometry equations used for these analyses. Even though the slope was equal to one, and a paired *t*-test showed no significant difference between HPLC and refractometry, one method was not preferred over the other.

A third paired *t*-test was done with the distillation and refractometry data. No significant difference was found. However, the range of differences between the actual ethanol values was again quite large, −0.33 to 0.18% (w/w). The mean difference of −0.014 ± 0.019% (w/w) was very close to that found for the HPLC-refractometry comparison.

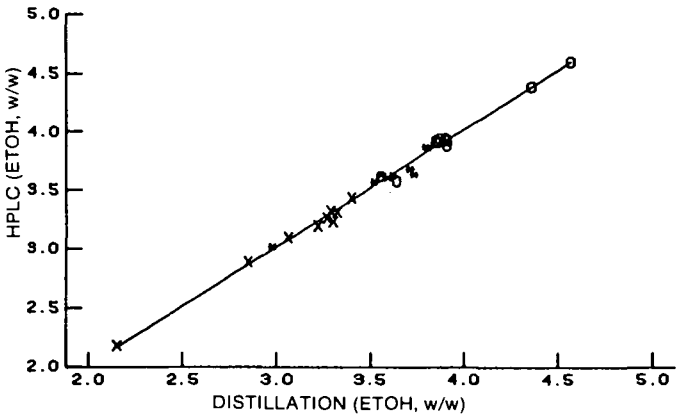

Fig. 5. Comparison of ethanol results obtained by high-performance liquid chromatography and by distillation. x = low calorie, * = premium, o = super-premium and imports.

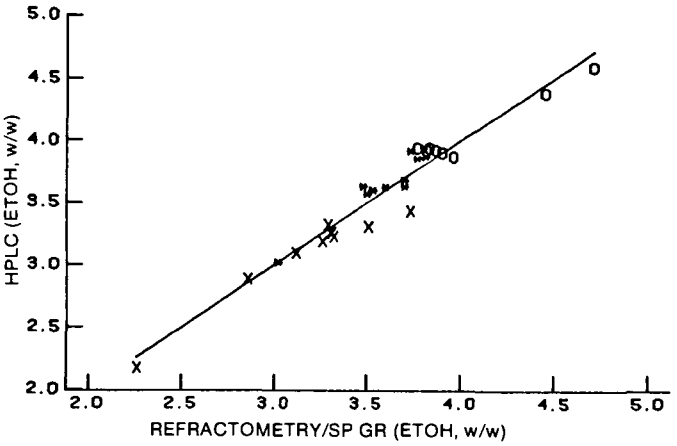

Fig. 6. Comparison of ethanol results obtained by high-performance liquid chromatography and by refractometry. x = low calorie, * = premium, and o = super-premium and imports.

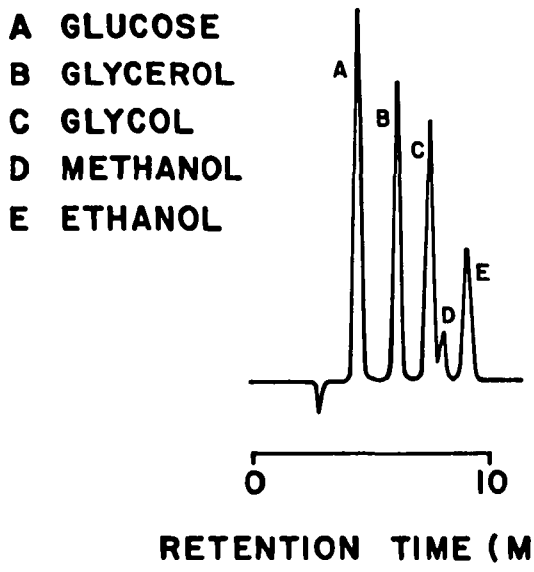

Fig. 7. Chromatogram of a standard mixture obtained with the HPX-85 alcohol column.

TABLE II
Comparison of High-Performance Liquid Chromatography to Accepted Methods for Ethanol Determinations

Method	Advantages	Disadvantages
Distillation	Reference method	Time consuming Large sample size
Refractometry	Fast Single product precision Small sample size	Manual No universal equation
High-performance liquid chromatography	Automatic Fast Comparable to distillation Small sample size	Cost factor if purchase of new equipment required

Further Applications

Ethanol is not the only component that can be quantitated with this column. Figure 7 is a profile of five components, at a concentration of 1% (w/v) each, that may also be of interest to brewers. These include glucose, glycerol, propylene glycol, and methanol. The resolution is near baseline for all of these components. Methanol and the other aliphatic alcohols are normally present in beer at concentrations below the detection limits of this method when using the conditions described.

CONCLUSION

Modern HPLC, with an HPX-85 alcohol analysis column, provides a fast and precise method for the quantitation of ethanol in malt beverages. The method can be completely automated for both sample analysis and data reduction. The ethanol values obtained by HPLC analyses were comparable to those found for the more time-consuming distillation method. Unlike the refractometry method, the HPLC method lends itself to the analysis of diverse samples, regardless of extract composition and without the need for numerous calibration curves. The advantages and disadvantages of all three methods are outlined in Table II.

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