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Note

Rapid high-performance liquid chromatography method for determination of ethanol and fusel oil in the alcoholic beverage industry

M. E. NEALE*
Gilby Canada Inc., 400 Kipling Avenue, Toronto, Ontario M8V 3L2 (Canada)
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Ethanol, and its homologues to pentanol have previously been analysed by high-performance liquid chromatography (HPLC)[1–4]. For ethanol, the methods[1,2] have utilised ion-exchange columns at elevated temperatures (*e.g.* 60–80°C). Such columns are expensive, fragile and lead to long elution times (*e.g.* 10–20 min). For the major components of fusel oil [ethanol, 1-propanol, isobutyl alcohol (2-methyl-1-propanol) and amyl alcohols (2-methyl- and 3-methyl-1-butanol)], similar columns have been used[3]. Similar ion-exchange columns have also been used for the separation of various alcohols, carbonyl compounds, acids and "carbohydrates"[4]. This comprehensive paper includes some of the compounds of fusel oil, but not the isomeric methyl-1-butanols which would elute in excess of 30 min by the given HPLC conditions. An on-line chemical abstracts computer search (key-words: fusel, ethanol, HPLC, etc.) was performed over the years 1967–1987. Some 80 references were returned, none related in any way to the composition of fusel oil, the subject of this paper.

In this study, a reversed-phase C_8 column with a methanol–water eluent has been used for both determinations, only the recorder chart speed has been changed. In the case of ethanol determination in distillation column "bottoms", ethanol is eluted after all other components of the fermentation process, such as citric acid, glycerol, methanol, lactic acid, glucose, etc.

In the case of fusel oil analysis, the isomeric amyl alcohols noted above co-elute [as is common with many gas chromatographic (GC) methods]. For most purposes this is not a disadvantage, since GC examination of fusel oils[5] in this laboratory show the five constituents listed above to constitute about 85% of the fusel oil; additionally it contains about 10% water, leaving a small percentage consisting of a mixture of some 40–50 other compounds.

* Present address: Mann Testing Laboratories Ltd., 5550 McAdam Road, Mississauga, Ontario L4Z 1P1, Canada.

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EXPERIMENTAL

Apparatus

The liquid chromatograph used was a Bio-Rad Labs. system consisting of a Model 1770 differential refractometer, a Model 1330 pump and a column oven (set at 25°C). The detector volume was 12 μl. The column was a C_8, 5 μm particle size, 220 mm × 4.6 mm I.D. cartridge (Brownlee) fitted with a Rheodyne 20-μl syringe-loading sample loop injector. Data acquisition was by a Shimadzu CR3-A recording integrator (Tekscience, Oakville, Canada).

Reagents

The mobile phase was 500 ml HPLC-grade methanol (Caladon Labs., Georgetown, Canada) diluted to 1 l with HPLC-grade water (Caledon Labs.). HPLC-grade methanol was used as solvent. The standards were anhydrous ethanol, 1-propanol, isobutanol and isoamyl alcohol (mixed isomers: 2-methyl and 3-methyl-1-butanol) of known apparent density (BDH Chemicals, Toronto, Canada).

Sample preparation

Samples containing yeast and/or unfermented grains, for ethanol determination were filtered through paper (Whatman grade 4). The analyte solutions were prepared by mixing equal volumes of prepared sample and HPLC-grade methanol and filtering through a 0.45-μm disposable filter and injected.

Analyte solutions for fusel oil determinations were prepared by mixing a 200 μl sample with 4.8 ml of methanol–water (60:40) (Socorex dispensing pipettes 821-200 and 831-5, respectively), filtering through a 0.45-μm disposable filter and injecting.

Chromatographic conditions

Ethanol is satisfactorily resolved from other components of the fermented grain (glucose, organic acid, glycerol, most of which co-elute) using the described solvent at a flow-rate of 1 ml/min (Fig. 1).

The four alcohols in fusel oil are satisfactorily resolved using the above conditions (Fig. 2).

RESULTS AND DISCUSSION

Ethanol in distillation column "bottoms"

The distillation column "bottoms" from the steam distillation of fermented grain or molasses contains residual ethanol, the lower the level, the higher the distillation efficiency.

Fig. 1 shows the chromatogram of a solution of ethanol in laboratory-prepared still "bottoms". The separation is shown to be adequate for quantitation purposes.

Quantifiable levels of ethanol were determined by diluting ethanol to 0.05–0.40% (v/v) (10 nl/20 μl–80 nl/20 μl) and chromatographing under the given conditions. The relationship between area-counts and nanolitres ethanol injected was found to be linear ($r = 0.999$). The limiting "detectable" concentration of ethanol detectable was 0.01% (based on a pre-set minimum area rejection of 1000 units).

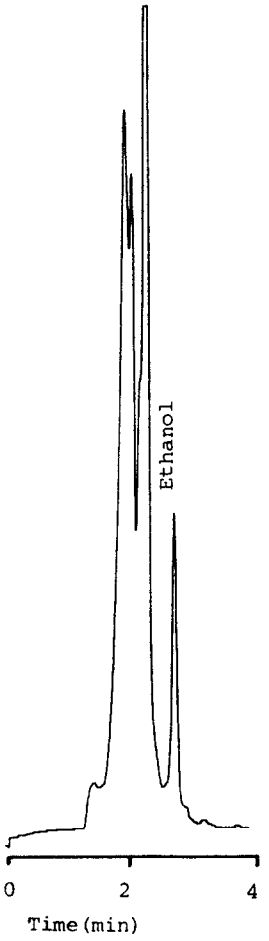

Fig. 1. Chromatogram showing separation of ethanol from other components in still-bottom samples.

TABLE I

RECOVERY OF ADDED ETHANOL (STILL-BOTTOM ANALYSIS)

Amount added (%, v/v)	*Amount found (%, v/v)*	*Recovery (%)*
0.077	0.075	97.4
0.179	0.178	99.4
0.278	0.271	97.5
0.376	0.360	95.7
0.463	0.468	101.1
	Mean	98.2

Recovery of added ethanol was found to be in the range 95.7–101.7% (Table I). Since levels of ethanol below 0.05% are difficult and expensive to obtain the conditions presented therefore provide for more than adequate sensitivity, together with rapid sample preparation and analysis time.

In the case of fusel oil analysis, Fig. 2 shows a chromatogram of the calibration mixture, and Fig. 3, that of a typical fusel oil.

The linearity of detection of the four components was determined for the ranges shown in Table II. For each case, the other three components were kept as constant as possible. All samples were weighed and converted to volume percentages via their appropriate densities. In all cases, the relationship between area counts and percentage (v/v) component was found to be linear (r = 0.999). The limiting detectable concentration of ethanol detectable was 0.2% (based on a preset minimum area rejection of 1000 units). Levels much below 0.5% are difficult and expensive to obtain. For all components, a comparison between the variances found for repeated injection of one sample mixture was compared with that for several sample mixtures. At the 0.05 probability level, there was no significant difference (F = 4.04, 1.13, 1.63 and

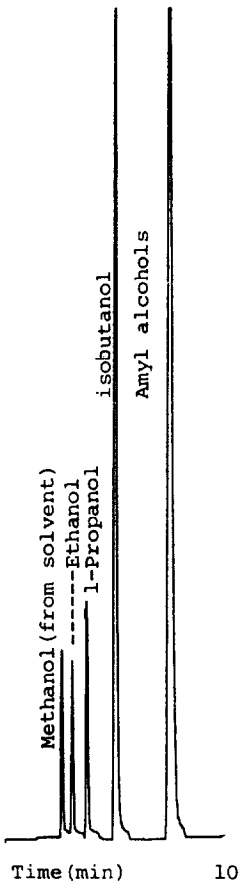

Fig. 2. Chromatogram showing fusel oil calibration mixture (used for fusel oil analysis).

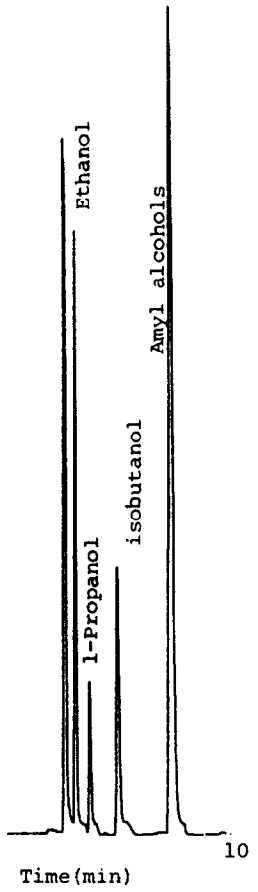

Fig. 3. Chromatogram showing typical fusel oil composition.

TABLE II

COMPONENT RANGES FOR LINEARITY TESTING (IN FUSEL OIL ANALYSIS)

Component	*Percentage range*
Ethanol	0, 2.5, 5, 7.5, 10
1-Propanol	0, 2.5, 5, 7.5, 10
Isobutanol	0, 10, 25, 50
Amyl alcohols	0, 40, 60, 80

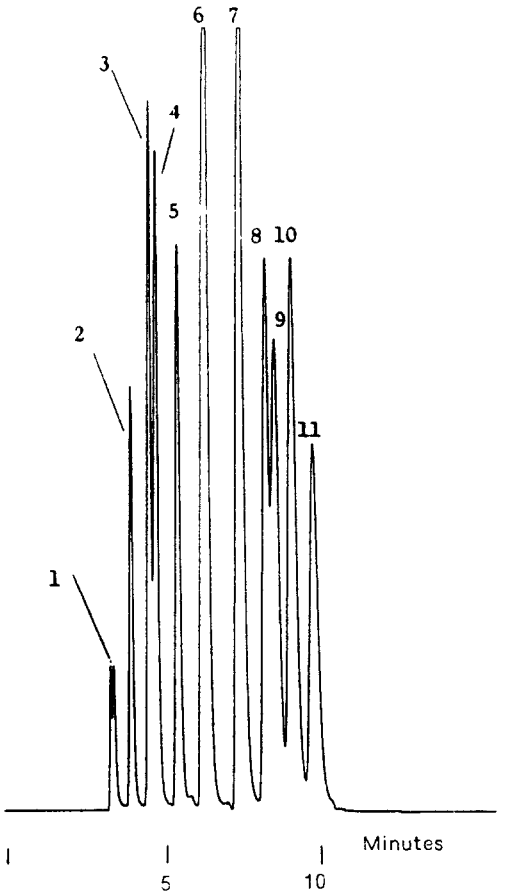

Fig. 4. Chromatogram showing elution of the major and some minor constituents of fused oil. Peaks: 1 = methanol; 2 = ethanol; 3 = 2-propanol; 4 = 1-propanol; 5 = 2-methyl-2-propanol; 6 = 1-butanol + isobutanol; 7 = 2-methyl-2-butanol + 2,2-dimethyl-2-propanol; 8 = 3-pentanol; 9 = 2-pentanol; 10 = 2-methyl + 3-methyl-1-butanol; 11 = 1-pentanol.

4.51 respectively; $n_1 = n_2 = 5$), indicating sufficient confidence in the repeatability of sample preparation.

Some of the more common constituents of fusel oil were chromatographed (Fig. 4). Their elution times, together with that of the solvent and the four major constituents are shown in Fig. 5. Ethanol is not resolved from acetyl methyl carbinol, and isobutanol is not resolved from 1-butanol and ethyl acetate. However, these constituents are found at much lower levels than the four major components and do not constitute significant interference.

The method has also been compared with a GC method developed by Canadian customs and excise designed to be used for the regulation of ethanol in fusel oils. These results are shown in Table III. Exact correspondence was not found between the methods. The difference may be due to the manner in which the GC calibration standards for the external method are prepared: further work to inves-

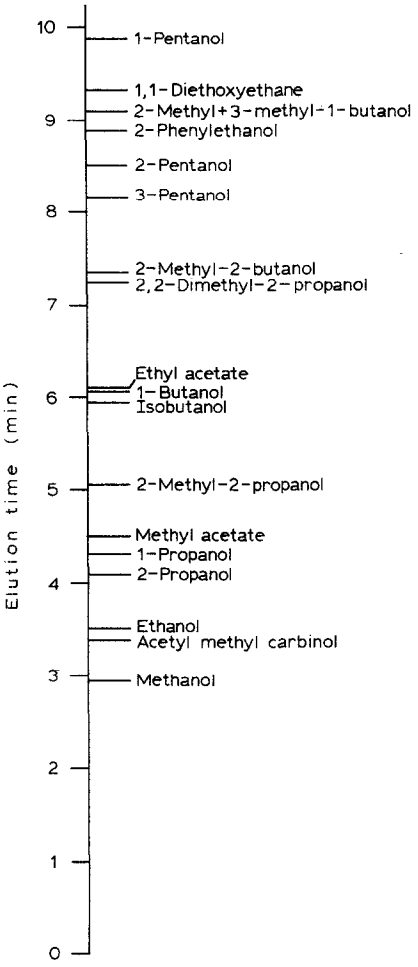

Fig. 5. Chart showing elution times of some fusel oil components.

TABLE III

COMPARISON OF RESULTS BETWEEN PROPOSED METHOD AND EXTERNAL STANDARD GC METHOD (REVENUE CANADA)

All results average of triplicate analyses.

Sample	*Ethanol (%)*		*1-Propanol (%)*		*Isobutanol (%)*		*Amyl alcohols (%)*	
	LC	*GC*	*LC*	*GC*	*LC*	*GC*	*LC*	*GC*
A	8.87	9.76	4.14	4.24	15.20	16.95	51.71	54.50
B	6.89	7.68	3.80	3.96	15.40	17.30	53.26	57.02
C	7.24	7.98	4.17	4.24	14.19	15.66	55.58	58.90
D	5.89	6.72	0.50	0.63	19.00	20.85	54.27	59.38

tigate the discrepancies is beyond the scope of this paper. However, the results by the proposed method will be sufficiently accurate for monitoring fusel oil by-product when many samples have to be analysed, and the simplicity of the method and equipment makes it amenable to use by unskilled operators.

ACKNOWLEDGEMENT

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